BD FACSJazz™ Cell Sorter User's Guide

For Research Use Only

bdbiosciences.com
23-14339-00
7/2012

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FCC information

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Regulatory information

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

Class 1 Laser Product.

Caution: Use of controls or adjustments or performance of procedures other than those specified in the user's guide may result in hazardous radiation exposure.

History

Revision	Date	Change made
23-14339-00	7/2012	New document

Contents

Part 3: System workflow

Part 4: Reference

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About this guide

This chapter includes these topics:

Documentation overview

Introduction

The guide contains a basic description of the BD FACSJazz™ cell sorter, BD FACS™ Sortware sorter software, QC procedures, configuration, operating procedures, and maintenance information.

Using the guide

A familiarity with basic flow cytometry concepts is assumed throughout this guide. You should 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.

The information in this guide is organized into the following parts:

- **Part 1: Instrument information.** Includes information about system hardware and components, basic system procedures, and system maintenance. Some system procedures require the use of BD FACS Sortware software.
- **Part 2: Software information.** Includes information about BD FACS Sortware software, general procedures for setting up and operating the instrument, and procedures for sorting samples.
- **Part 3: System workflow.** Includes the essential workflows for the setup and operation of the BD FACSJazz system.
- **Part 4: Reference.** Includes information about the BD FACSJazz options and troubleshooting.

Other documentation

See the *BD FACSJazz Safety and Limitations Guide* for descriptions of safety and warning labels, general system hazards, and laser, electrical, and biological hazards.

More information

- Safety symbols (page 15)
- Technical assistance (page 17)
- BD FACS Sortware help system (page 16)

Safety symbols

Introduction

The following table lists the safety symbols used throughout this guide.

Safety symbols

The following safety symbols are used in this guide to alert you to potential hazards.

Symbol	Meaning
	General warning. Risk of personal injury to operator.
	Dangerous. High voltage. Risk of electrical shock.
	Biohazard

For a complete description of safety hazards, see the *BD FACSJazz Safety and Limitations Guide*.

More information

- Documentation overview (page 14)
- BD FACS Sortware help system (page 16)

BD FACS Sortware help system

Introduction

BD FACS Sortware software includes a comprehensive help system that includes all content from this user's guide. Internet access is not required to access this content.

Navigating the help system

To access the BD FACS Sortware software help system:

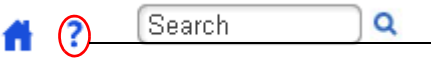
1. In the **BD FACS Sortware** window, select **Help > Contents.**

   The help window opens.

2. Use the table of contents, interactive links, related topics, or the search tool to locate topics of interest.

   – **Search.** Type words or phrases into the search field. Search results are displayed in a familiar web search format to help you find information quickly.

   – **Tips.** Click the **Help** icon to view tips for using the help system.



   – **Print.** Use the print tools to print individual topics or to print entire sections as formatted PDF files.

More information

- Documentation overview (page 14)
- Safety symbols (page 15)
- Technical assistance (page 17)

Technical assistance

Introduction

This topic describes how to get technical assistance.

Contacting technical support

If you require assistance, contact your local BD Biosciences technical support representative or supplier. For the current technical support phone and email contact information, go to bdbiosciences.com/support/technical.

When contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number
- Any error messages
- Details of recent system performance

More information

- Documentation overview (page 14)
- BD FACS Sortware help system (page 16)

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Part 1

Instrument information

This part includes these chapters:

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Instrument overview

This chapter includes these topics:

System description

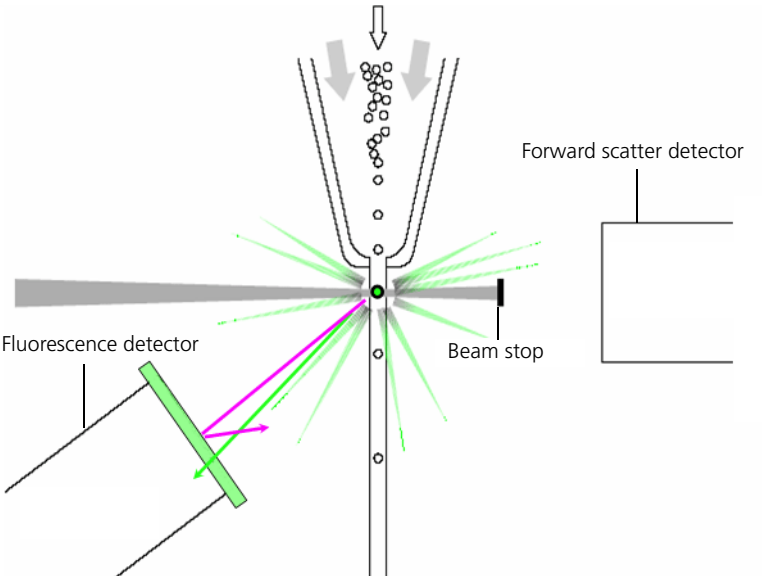
Introduction

This topic describes the BD FACSJazz cell sorter system.

The BD FACSJazz cell sorter system provides detection, analysis, and sorting of particles excited by laser light. The instrument has a fixed nozzle size (100 µm) and sheath pressure (27 psi).

Detection

The BD FACSJazz cell sorter focuses laser light on a fast-moving, thin stream of particles (for example, cells, chromosomes, or organisms). Sensitive photomultiplier tubes (PMTs) collect the fluorescence and light scatter emitted by the particles.

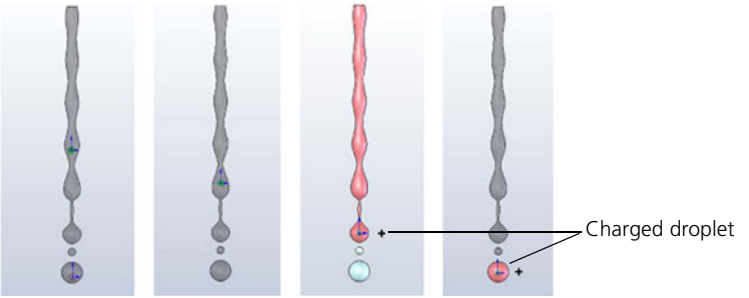


Sorting

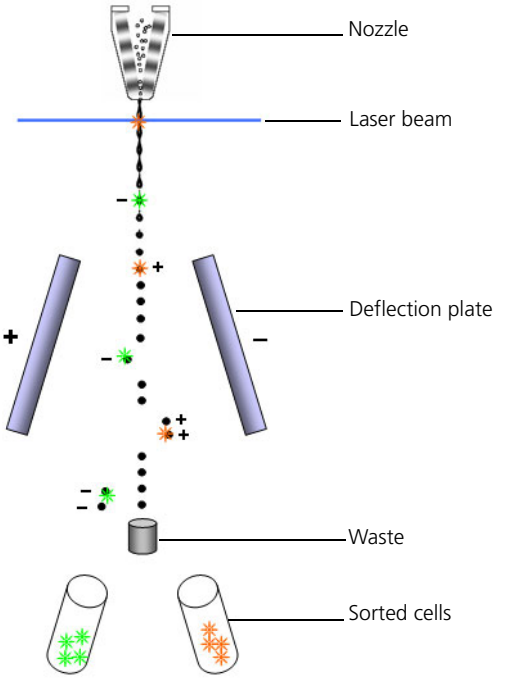
The following are the steps in the sorting process

Stage	Description
1	Drop-drive energy is applied to the stream to break it into highly uniform drops.
2	Droplets detach from the stream a few millimeters downstream from the nozzle. This is the breakoff point. The drop is surrounded by air.
3	When a particle that meets the sorting criteria is detected, an electrical charge is applied to the stream at the breakoff point. The drop acquires an electrical charge (positive or negative), depending on whether the particle has been gated to be sorted.
4	The charged drop passes between two high-voltage deflection plates.
5	Electrostatic attraction and repulsion deflect the charged drop into the left or right stream, depending on the drop's charge polarity.
6	Charged drops sort into a collection device (multiwell plate or tube).
7	Uncharged drops pass down the center stream to the waste drain.

The following figure shows stages 1 to 3 of the sorting process.

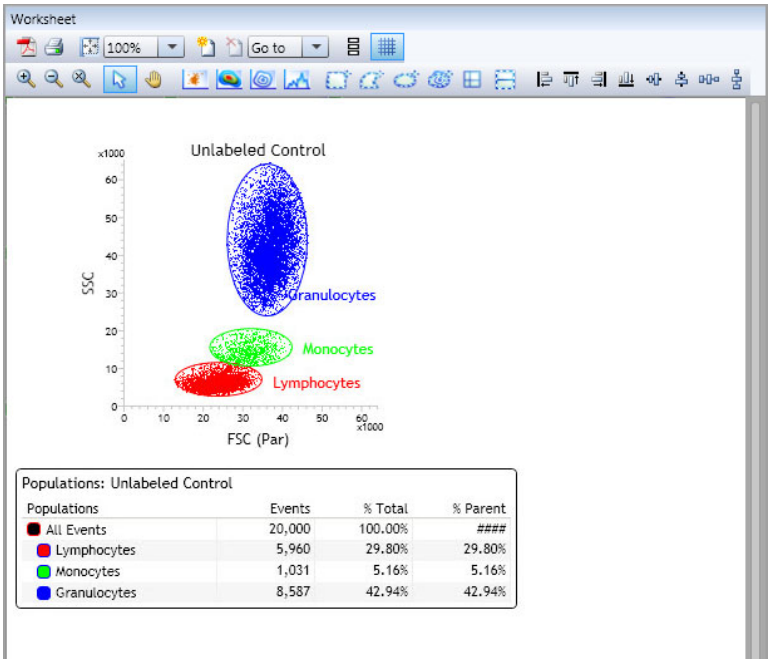


The following figure shows stages 4 to 7 of the sorting process.


Nozzle
Laser beam
Deflection plate
Waste
Sorted cells

Analysis

Use the BD FACS Sortware worksheet tools for sorting and data analysis. The following figure shows a worksheet.



More information

- System and components (page 26)
- Functional subsystems (page 28)

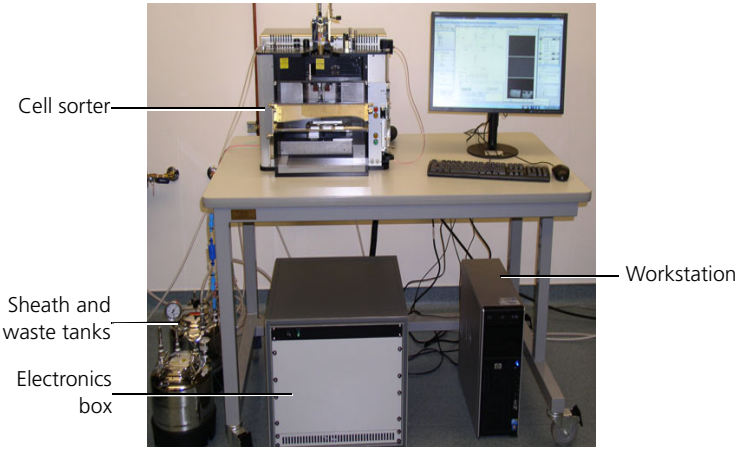
System and components

Introduction

This topic describes the BD FACSJazz system and components.

System components

The following figure shows the system components.

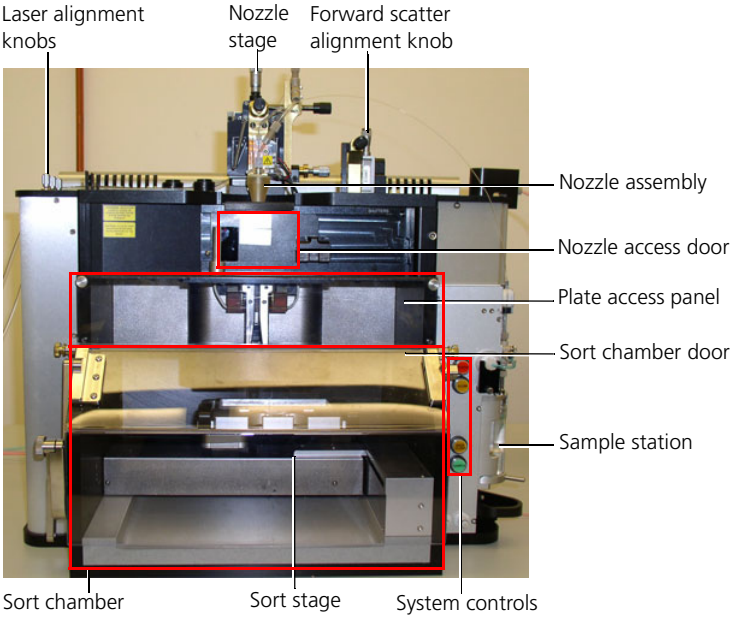


The following table describes the system components.

System Component	Description
Cell sorter	A compact research cell sorter that has a fixed nozzle size (100 µm) and sheath pressure (27 psi). Several hardware options and upgrades can be used to customize the system for different applications.
Sheath and waste tanks	7-L stainless steel tanks.
Electronics box	Contains the isolation transformer, DC power supply, and cytometer interface—a dedicated computer that interacts with the software and controls the cytometer hardware. **Note:** In an emergency, use the main power switch, located on the front of the electronics box, to power down the system.
Workstation	Runs BD FACS Sortware software.

Instrument components

The following figure shows the instrument components.



The following table describes the instrument components.

Instrument component	Description
Laser alignment knobs	Control the beam alignment of each laser.
Nozzle stage	Aligns the sheath stream to the collection lens and pinholes and to the BD FACS™ Accudrop laser and waste collector.
Forward scatter alignment knob	Used to align the forward scatter signal to the detector. (Intended for Service Use Only. Not for routine user adjustment.)
Nozzle assembly	Creates the hydrodynamic focusing of the sample stream in the sheath stream.
Nozzle access door	Sliding door that provides access to the nozzle and interrogation chamber.
Plate access panel	Provides access to the deflection plates. When open, the deflection plates and Accudrop laser are disabled.

Instrument component	Description
Sort chamber door	Provides access to the sort chamber. When open, the deflection plates and Accudrop laser are disabled.
Sample station	The sample station consists of the following: • **Sample tube holder.** Holds and pressurizes the sample tube. • **Bubble detector.** Detects air bubbles from the sample tube and stops the sample flow when the sample tube is empty, preventing air bubbles from reaching the nozzle assembly.
System controls	Includes the deflection plates power button (PLATES), illumination (ILLUM), and sample valve controls (BACKFLUSH and SAMPLE).
Sort stage	A moving mechanical assembly that holds and positions sort tray devices (for example, multiwell plates and tube holders).
Sort chamber	The sort chamber consists of the following: • **Deflection plates.** High-voltage plates that deflect droplets from the main stream into specific tubes or wells in the sort tray. • **Sort tray.** Plates or adapters that hold tubes or slides. • **Accudrop laser.** A laser that is used for determining the drop delay and to correctly set up the side and center streams. • **Waste drain.** Collects the uncharged droplets of the center stream.

Functional subsystems

The BD FACSJazz system includes the following functional subsystems:

- Fluidics (page 31)
- Optics and signal processing (page 45)
- Sort components (page 55)

More information

- System description (page 22)
- BD FACSJazz options (page 361)

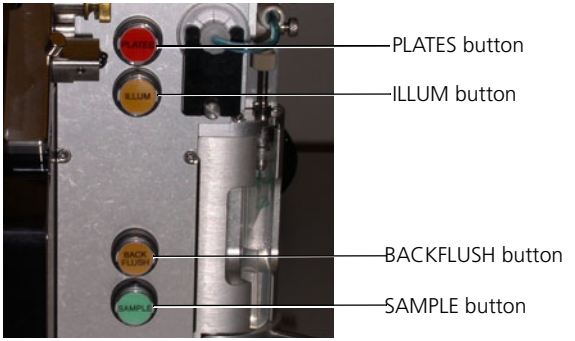
System controls

Introduction

This topic describes the BD FACSJazz system controls.

Components

The following figure shows the BD FACSJazz system controls.



The following table describes the BD FACSJazz system controls.

Component	Description
PLATES button	Turns the power to the plates on and off.
ILLUM button	Turns the light to view the lower section of the stream on and off.
BACKFLUSH button	Carries sheath fluid from the nozzle back to the sample station via the sample line.
SAMPLE button	Opens the sample valve when a tube is in place and the fluidics are in stream mode, which allows sample to be carried from the tube to the nozzle.

More information

- System description (page 22)
- System and components (page 26)
- Pressure Console pane (page 92)

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Fluidics

This chapter includes these topics:

Fluidics overview

Introduction

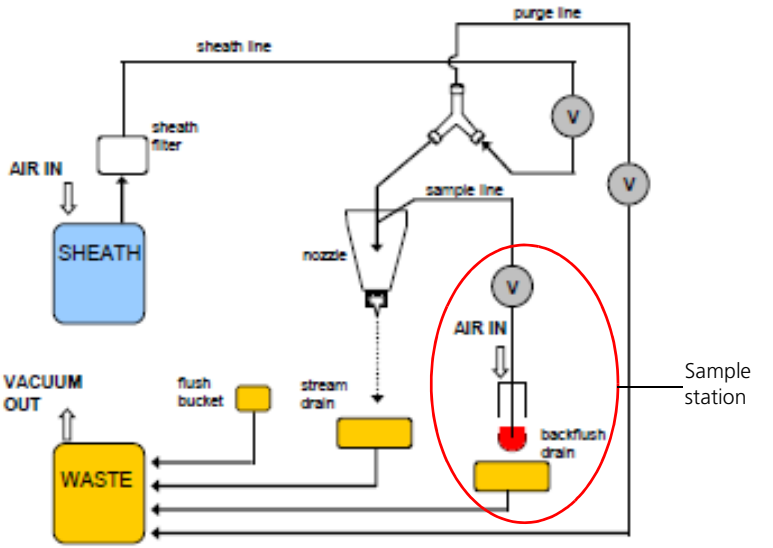
This topic describes the fluidics subsystem.

Components

The fluidic subsystem components are:

- System controls
- Sample introduction components
- Waste tank and associated components

The following figure shows the fluidics subsystem components.



More information

- System and components (page 26)
- System controls (page 29)
- Functional subsystems (page 28)
- Sheath tank components (page 34)
- Waste fluidic components (page 40)

Sample introduction

Introduction

This topic describes the fluidic components that move sample fluid from the sample tube to the sort chamber.

The purpose of the fluidics system is to present the sample to the laser intercept in a single-file stream in a uniform and reproducible fashion. The resulting laser/particle interaction generates characteristic light-scatter and fluorescence signatures enabling particles to be analyzed and selected for sorting.

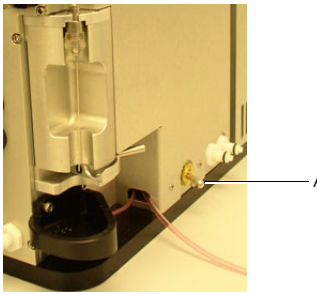
Components

The main components of sample introduction include:

- Air pressure source
- Sheath tank components
- Sheath line
- Nozzle assembly
- Sample line
- Sample station
- Pressure Console pane

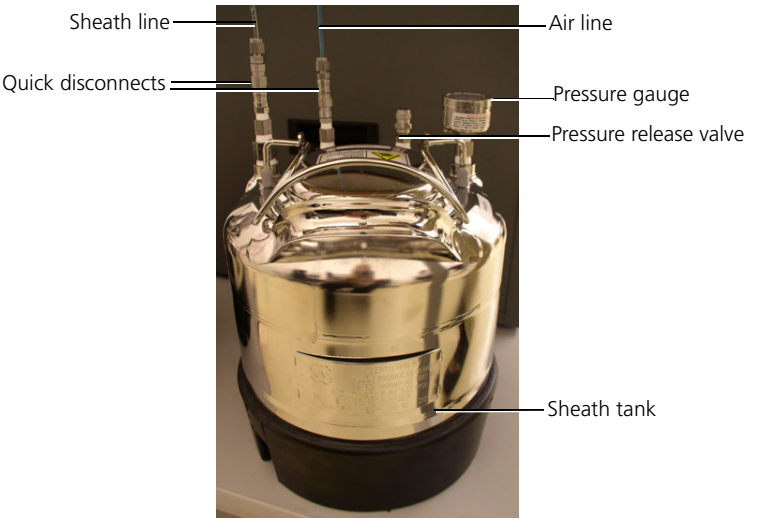
Air pressure source

A house air supply or air compressor (available as an option) is needed to provide air pressure to the system. The Air switch is a physical toggle on the system that is used to switch the air pressure on or off.



Sheath tank components

The sheath tank is a 7-L stainless steel vessel that holds the sheath fluid (saline) and is pressurized to force the sheath fluid into the system through the nozzle.



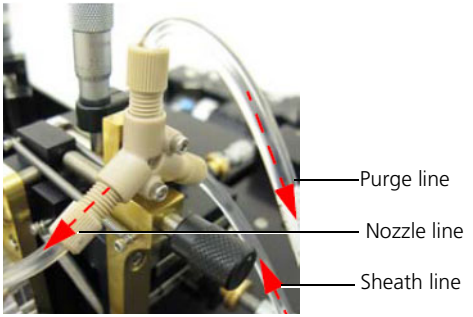
The associated components of the sheath tank and fluidics are:

- **Air line.** Provides pressure to the tank so that sheath fluid can flow.
- **Sheath line.** Conveys the sheath to the nozzle assembly.
- **Pressure gauge.** Displays the internal tank pressure.
- **Pressure release valve.** Removes pressure from the tank prior to removing the tank lid.

- **Sheath filter.** Removes possible contaminants from the sheath fluid. The following figure shows the sheath filter.

Sheath line

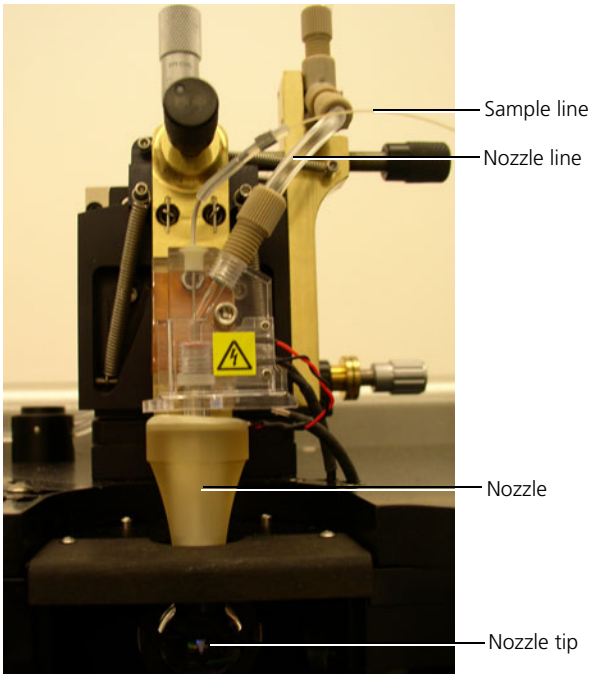
The sheath line connects the sheath filter with the Y-fitting on the nozzle stage assembly. It delivers sheath fluid from the sheath filter, through the sheath pinch valve to the nozzle.



Nozzle assembly

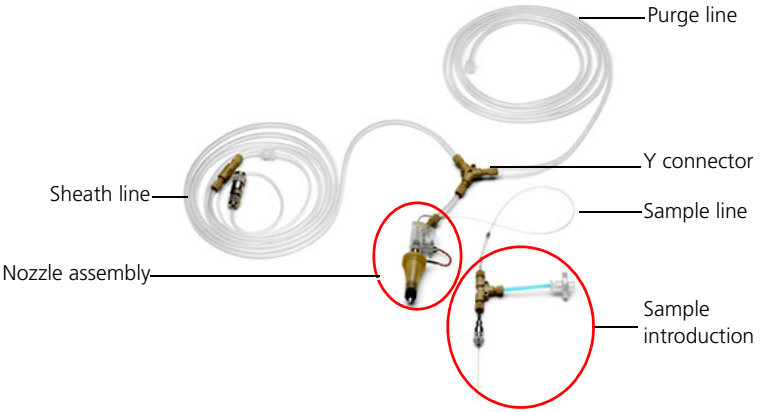
The nozzle assembly creates the hydrodynamic focusing of the sample stream in the sheath stream.

The assembly uses a piezoelectric element that applies drop-drive energy to the stream to break it into highly uniform drops. The nozzle assembly also charges the stream as needed to create charged drops for sorting.



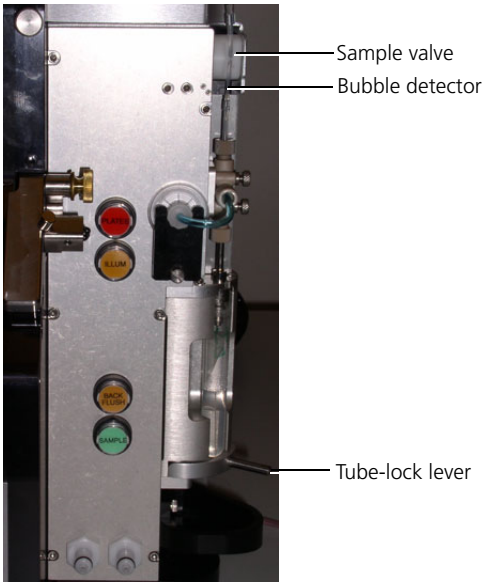
Sample line

The sample line carries the pressurized sample stream from the sample tube to the nozzle assembly. The sample line has an inner diameter of 0.01 inches. The sample line can easily be replaced either by itself or as part of the disposable fluidics kit.



Sample station

The sample station is where the sample tube is loaded and the sample is pressurized for sample introduction.



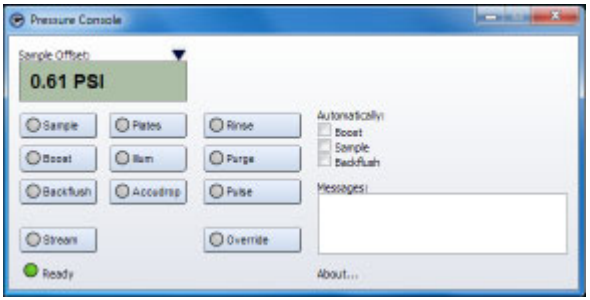
The following table describes the sample station components.

Component	Description
Sample valve	Opens and closes the sample line.
Bubble detector	Detects bubbles and closes the sample valve when a bubble is detected.
Tube-lock lever	Locks the sample tube in place and ensures that the sample tube is sealed with the top of the sample port.

Pressure Console pane

Pressure is applied to the sheath tank and the sample tube to push sheath fluid and sample fluid through the nozzle simultaneously. The Pressure Console pane is the part of BD FACS Sortware software that includes controls that regulate and monitor the sample and sheath fluidics pressures. The sheath pressure of the BD FACSJazz system is set to 27 psi. The sample offset pressure regulator displays the differential amount of pressure applied to the sample relative to the sheath.

The following figure shows the Pressure Console pane. See Pressure Console pane (page 92) for more information.



The Sample and Backflush buttons are also available in the system controls. See System controls (page 29) for more information.

More information

- Fluidics overview (page 32)
- System controls (page 29)
- Pressure Console pane (page 92)

Waste fluidic components

Introduction

This topic describes the waste fluidic components.

Components

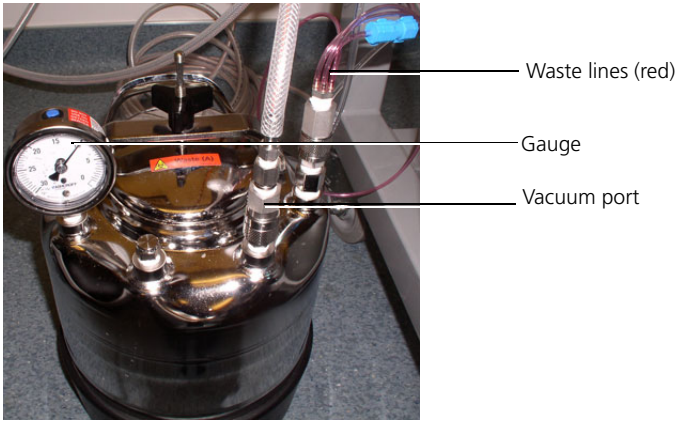
The main waste fluidic components are:

- Waste tank
- Purge line
- Waste drains and lines
- Vacuum source

Waste tank

The waste tank is an autoclavable stainless steel 7-L tank.

The waste tank lid seals with the tank to maintain the vacuum. It has one port for the vacuum line, one for the waste lines, and one for the gauge used to monitor the amount of vacuum applied to the tank.



See Maintenance (page 61) for information about system maintenance tasks involving the waste tank.

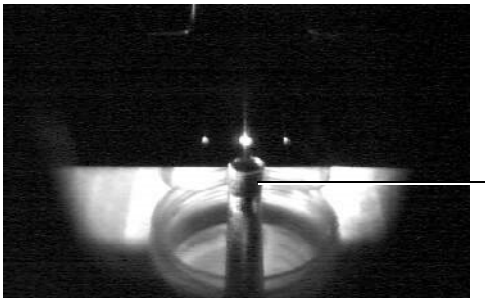
Purge line

The purge line connects the waste tank with the Y-fitting on the nozzle stage assembly. During rinse, purge, and pulse fluidic modes, the purge line valve is open and fluid travels through the purge line to the waste.

Waste drains and lines

The waste drains consist of the stream drain, backflush drain, and the flush bucket.

- **Stream drain.** Located directly under the stream in the sort chamber. The middle stream of uncharged drops falls into the stream drain and is aspirated to the waste tank.



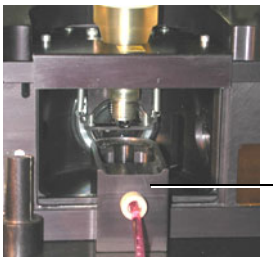
- **Backflush drain.** Located below the tube holder in the sample station. This drain is used to collect and aspirate the fluids flushed back through the sample line to the waste tank.

- **Flush bucket.** A movable receptacle that is used during startup and shutdown to catch the stream from the nozzle assembly and during a run to access the plates without stopping the stream.

  It is placed directly under the nozzle to capture and aspirate fluid to the waste tank during maintenance and troubleshooting.

  The flush bucket mounts in two places:

  – Under the nozzle assembly when in use
  – On top of the instrument above the sample station when not in use



Caution! The contents of the waste tank and waste tubing could be contaminated with biohazardous material. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.

- **Waste lines.** Carry the waste from the drains to the waste tank.

Vacuum source

A house vacuum supply or vacuum pump (available as an option) is needed to depressurize the waste tank to draw waste fluid from the waste drains to the waste tank. The vacuum line connects to the vacuum port on the waste tank. The vacuum pressure in the waste tank must be between 5 and 10'' Hg.

The hydrophobic waste filter is connected between the vacuum source and the waste tank to prevent water from getting into the vacuum source.

Caution! Do not run the system without the waste filter in place. Liquid contamination in the house vacuum supply or the dedicated air compressor can damage the vacuum system.

More information

- Fluidics overview (page 32)
- Sheath tank components (page 34)
- Air compressor and vacuum pump (page 364)

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4

Optics and signal processing

This chapter includes these topics:

Illumination optics

Introduction

This topic describes the illumination subsystem.

The illumination subsystem steers and focuses the main excitation laser beam (and additional excitation lasers if present) onto the sample at the intersection of the stream and the emission optics.

Functions of the optic system

The optic system is one of the three main systems on a cytometer. It has two main functions:

- Illumination of the particles using laser(s), steering optics, and a focusing lens.
- Detection of the signal from the illuminated particle as well as the scatter light of the particle (covered in the next chapter).

Components

The main components of the illumination subsystem are:

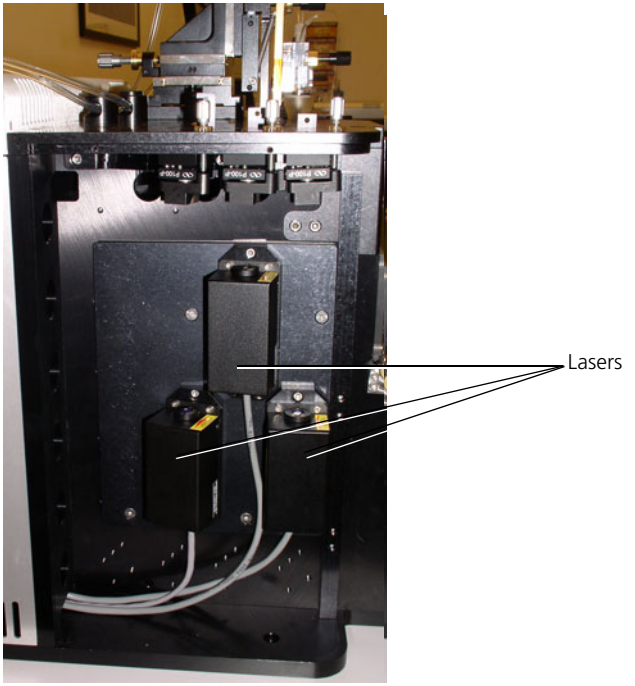
- Lasers (up to three sample excitation lasers)

Laser	Wavelength (nm)	Power (mW)
Blue	488	80
Red	640	50
Violet	405	50

- Laser shutters and the shutter interlock system
- Steering optics
- Protective shields and guards
- Beam-shaping lenses (within laser head) that create a final elliptical beam profile at the focus (3:1 ratio with a typical beam height of 15–20 µm)

Location of the lasers

The instrument includes air-cooled lasers mounted directly to the side of the sort head.

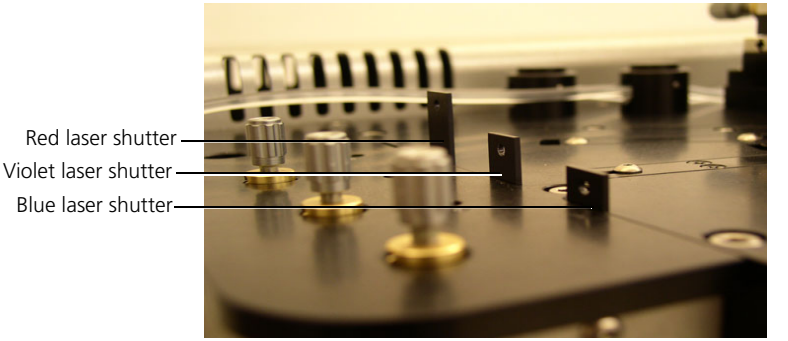


Laser shutter positions

For each laser there is a laser shutter tab that is used to block the laser or to allow the laser light to pass through. The laser shutter tab can be in one of three positions:

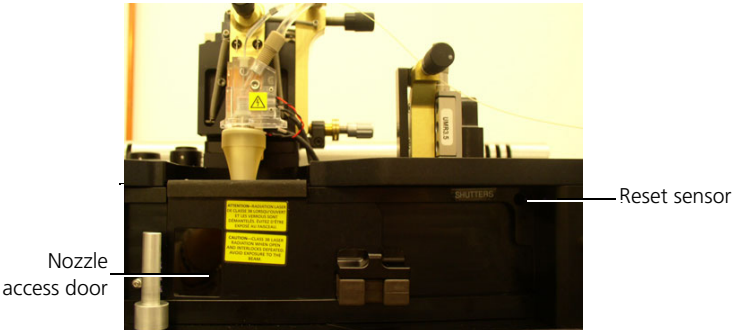
- **Top.** Open position. Laser light can pass through.
- **Bottom.** Closed position. Laser light is blocked.
- **Middle.** For service only. Do not use this position.

The following image shows an instrument with the three-laser configuration.



Laser interlock

The lasers are interlocked to the nozzle access door. The lasers are disabled when the nozzle access door is opened. To reset the interlock after closing the nozzle access door, place your finger over the reset sensor in the upper-right corner of the nozzle access door.



More information

- Functional subsystems (page 28)
- Collection optics (page 49)

Collection optics

Introduction

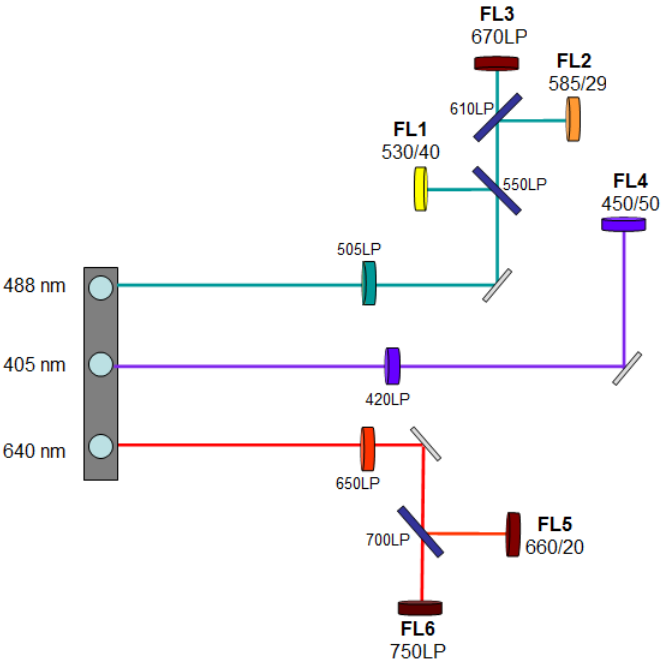
This topic describes the optical collection components.

About optical detection

The optical collection system collects scattered light and fluorescence from the laser/particle interaction.

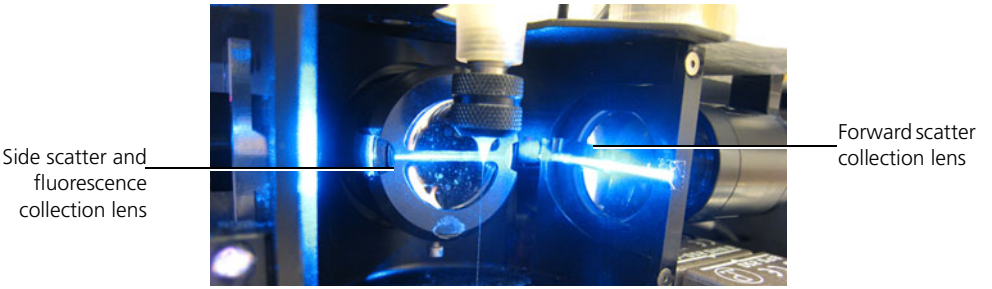
This detection system uses pinhole mirrors with the primary fluorescence objective lens to create spatial filters and separate fluorescence collected from the different laser intercepts with the sample stream. For each laser present, a separate series of dichroic mirrors and bandpass filters then precisely defines one or more wavebands of interest for quantification by the electronics.

The following illustration shows an example of an optical bench layout that includes: 488-nm excitation, scatter, and FITC, PE, PI, PE-Cy™5, and PE-Cy™7 detectors.



Light collection

The collection lens collects the light emitted from the particle during laser interrogation. Scattered and fluorescent light is collected through a 20x, 0.6 NA microscope objective and focused onto three spatially separated mirror pinholes.



Detectors

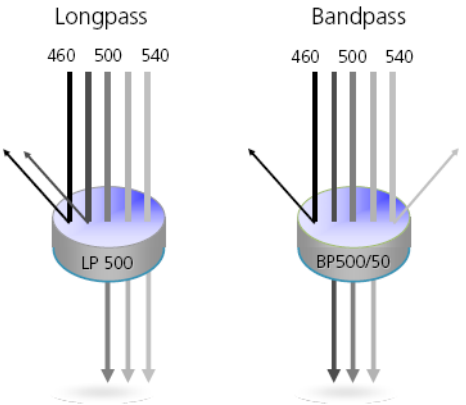
There are up to eight optical PMT detectors in the system:

- **Forward scatter (FSC) detector.** Collects forward-scattered light from the particle/laser interaction. Resolution for the standard forward scatter detector is 1 µm (measured using beads). The collection angle is 2° to 17°.
- **Side scatter (SSC) detector.** Collects side-scattered light through the microscope. Side scatter resolution is 1 µm (measured using beads).
- **Fluorescence detectors.** Either four or six PMTs assigned to the one, two, or three lasers in a number of fixed configurations. These detectors collect fluorescent light through the microscope objective and focus the light onto three spatially separated pinhole mirrors.

Filters

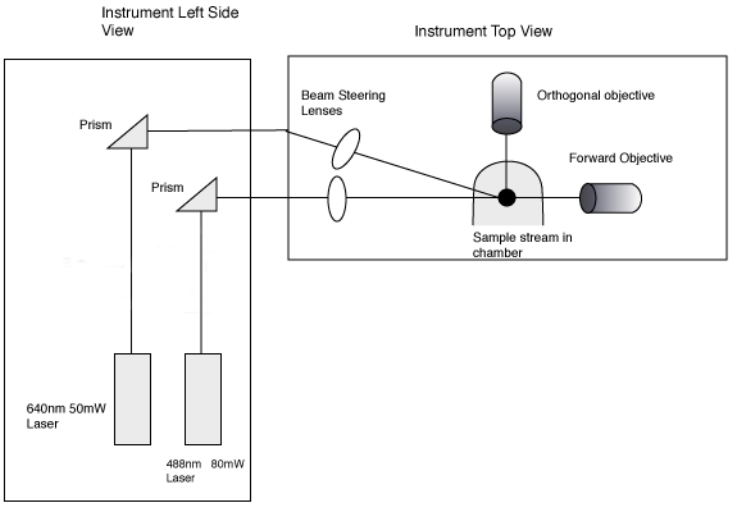
The emitted light from the particle/laser interrogation is directed to PMTs via optical filters. The dichroic mirrors steer certain wavelengths to detectors, while the bandpass filters allow specific wavelengths to be measured by the PMTs.

Filter type	Description
Longpass (LP) dichroic mirrors	The longpass dichroic mirrors reflect shorter wavelengths and transmit longer wavelengths. For example, a 505 LP dichroic mirror allows light of wavelengths equal to or greater than 505 nm to pass towards one detector, while reflecting light shorter than 550 nm to another. In this application, dichroic mirrors are characterized and used at 45° incidence.
Bandpass (BP) filters	A bandpass filter allows a specified range of spectral wavelengths to pass through to the PMTs. For example, a 530/40 BP filters allows wavelengths between 530 nm ±20 (510 and 550 nm) through to the detector. In a few specific instances, the final filter before a PMT is a longpass filter (characterized in this case for normally incident light).
Neutral density (ND) filters	Neutral density filters allow a certain percentage of light to pass through. A neutral density filter is typically used with the FSC PMT due to the magnitude of the collected scatter signal.



The following figure shows the layout of the BD FACSJazz excitation optics.

BD FACSJazz excitation optics



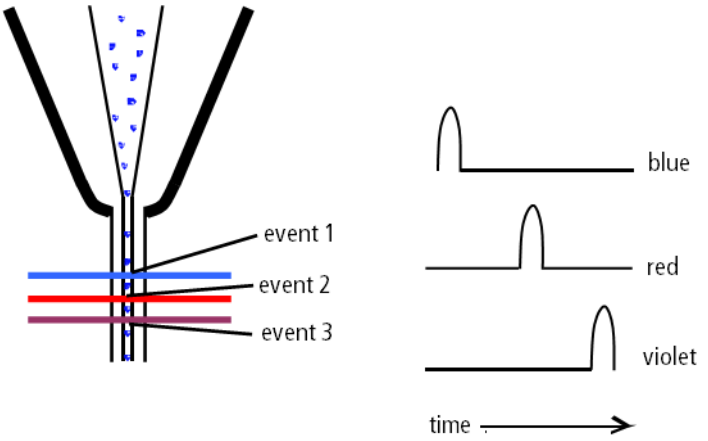
Laser delay

When more than one laser is installed, the lasers are spatially separated, causing timing differences between the light signals generated by each particle as it passes through the succession of lasers.

The calculated laser delay is a factor used to align all the signals so they can be measured and displayed on the same time scale. You typically optimize the laser delay(s) during daily instrument QC and optimization.

The following table describes laser delay with three lasers.

Process	Description
1	A particle intercepts the blue laser and generates signals.
2	A few microseconds later, the particle passes through the second laser, generating additional signals.
3	Moments later, the same particle passes through the third laser and generates a third batch of signals.



More information

- Functional subsystems (page 28)
- Signal processing components (page 54)

Signal processing components

Introduction

This topic describes the signal processing components.

Signal processing controls, amplifies, and processes PMT signals into data that can be:

- Monitored by the operator
- Acquired and analyzed by BD FACS Sortware
- Used by the sort electronics subsystem

Signal processing components

The main signal processing components are:

- **Logarithmic and linear preamplifiers (log and lin preamps).** Convert the current pulses generated by the PMTs to voltage pulses that can be quantified.
- **Analog-to-digital converters (ADCs).** Perform 16-bit analog-to-digital conversion (65,536 channels).
- **Digital signal processors (DSPs).** Perform an 8 x 8 digital compensation matrix. Compensated parameters are available to the user for display, separately from the raw (uncompensated) ADC output signals, as separate parameters.

More information

- Functional subsystems (page 28)
- Sort electronics (page 56)

5

Sort components

This chapter includes these topics:

Sort components overview

Introduction

This topic provides an overview of the BD FACSJazz sort components.

Components

The sort components are:

- Sort electronics
- Sorted sample collection
- Sort monitoring with the Sortview pane (page 95)

More information

- Functional subsystems (page 28)
- Sort electronics (page 56)
- Sample collection (page 58)

Sort electronics

Introduction

This topic describes the components of the sort electronics subsystem.

The sort electronics subsystem contains the electronics components necessary for processing signal data and for sorting particles.

Sort electronics components

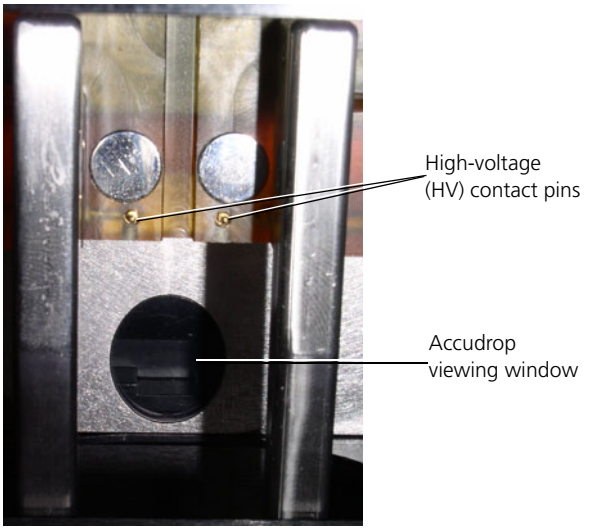
The main components of sort electronics are:

- **Control circuitry.** Circuit boards, counters, and other components that control sorting and deflection.
- **Piezo drive.** Powers the piezoelectric element in the nozzle assembly that converts the stream into controlled drops. You can optimize the piezo amplitude and frequency in the Sort Settings pane.

  The drop charge is disabled when the sort chamber door is open.

- **High-voltage deflection plates.** Pull drops from the main stream into left and right side streams, depending on their polarity, and direct them to specific tubes or wells in the sort tray.

Closed deflection plates



Open deflection plates

More information

- Functional subsystems (page 28)
- Sample collection (page 58)

Sample collection

Introduction

This topic describes the components for collecting sort stream drops.

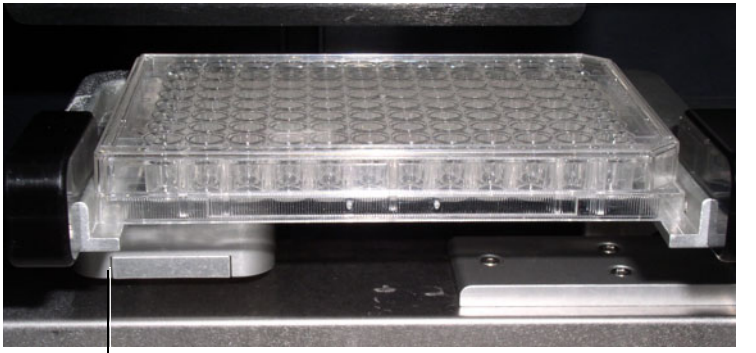
Sample collection devices

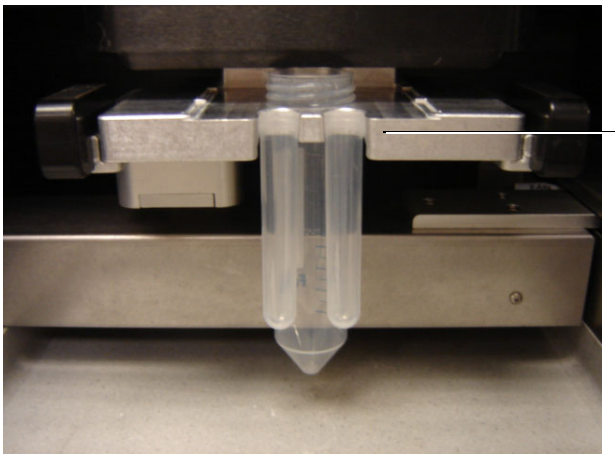
The sample collection subsystem collects the charged drops. You can collect sorted droplets into any device that will fit within the sort collection area, which include the following sort devices:

- 5-mL tubes (12 x 75 mm), 15-mL conical tubes
- Multiwell plates (6, 24, 48, 96, and 384-well plates)
- Standard microscope slides (1 x 3 in.; or 25 x 75 mm)
- 4 x 12 slides (1 x 3 in.)
- Petri dish (9-cm diameter)
- Terasaki plates (81 mm x 56 mm)
- Chilled 5-mL tubes (12 x 75 mm), 15-mL conical tubes (optional)

Sort stage

The main component of sample collection is the sort stage—a platform with an xy-axis mechanism that holds sort trays (plates, or adapters which hold tubes, slides, or other devices). You can control the position of the sort stage with BD FACS Sortware.





More information

- Functional subsystems (page 28)
- Sort electronics (page 56)
- Creating a sort device layout (page 235)

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6

Maintenance

This chapter includes these maintenance topics:

This chapter also includes the following component replacement topics:

Maintenance overview

Introduction

This topic describes the periodic maintenance procedures required for proper system operation and optimal performance.

Who should perform maintenance

This maintenance should be performed by the BD FACSJazz system administrator or other trained person.

Contact your BD service representative for information about ordering the tools or materials needed to perform these maintenance procedures.

Daily maintenance

Daily maintenance includes the following procedures.

- Cleaning and inspecting the instrument (page 63)
- Inspecting and cleaning the deflection plates (page 64)
- Cleaning the nozzle tip (page 259)

Monthly maintenance

In addition to daily maintenance procedures, decontaminate the fluidics each month.

See Decontaminating the fluidics (page 66) for more information.

Periodic maintenance

Perform these maintenance procedures every 4 to 6 months or as needed.

Procedure	When
Replacing the sample line (page 72)	Every 4 to 6 months or as needed
Replacing the sheath and waste fluidic lines (page 75)	As needed
Vertically aligning the lasers (page 68)	As needed

More information

- Cleaning and inspecting the instrument (page 63)
- Component replacement (page 70)

Cleaning and inspecting the instrument

Introduction

This topic describes the basic instrument cleaning and inspection procedure. Perform this procedure daily to avoid contamination and degradation of the instrument.

Required materials

- 10% bleach
- Deionized (DI) water
- Kimwipes® wipe or other lint-free towel
- Small hand-held vacuum

Procedure

To clean and inspect the instrument:

1. Remove dust from all exposed surfaces.
2. Clean salt buildups from the parts and areas exposed to sheath or sample fluids.

   Caution! All biological specimens and materials can transmit potentially fatal disease. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.
3. Clean the sort tray with a solution of 10% bleach and then wipe down with DI water.
4. Vacuum dust and lint from fan areas, such as the back of the electronics box and the computer, if needed.
5. Inspect the tubing and fluidics for leaks.

More information

- Maintenance overview (page 62)
- Inspecting and cleaning the deflection plates (page 64)
- Component replacement (page 70)

Inspecting and cleaning the deflection plates

Introduction

This topic describes how to inspect the deflection plates for wear or damage and how to clean them. Perform this procedure twice daily, before starting the system and after shutting down the system, and as necessary.

Required materials

- Kimwipes wipe or other lint-free towel
- DI water

Inspecting the deflection plates

To inspect the deflection plates:

1. Inspect the deflection plates for signs of fluid or salt buildup.

   Clean the plates thoroughly if salt crystals are visible.

Cleaning the deflection plates

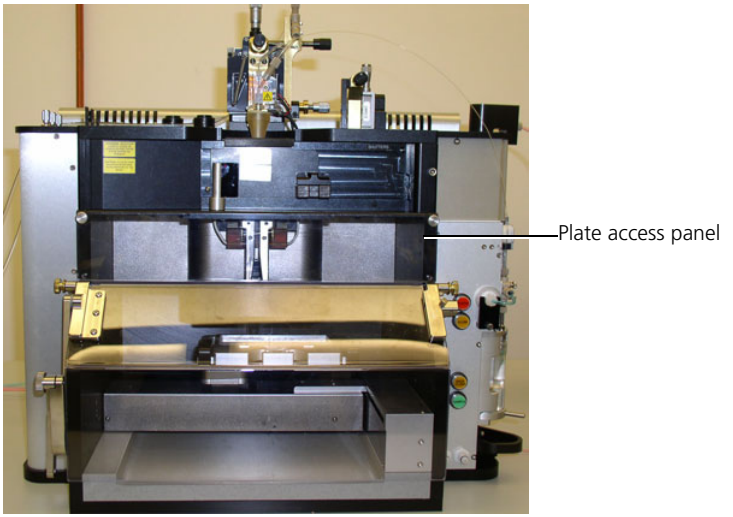
Caution: Electrical! To prevent electrical shock when cleaning the deflection plates, make sure that the plates are powered off.

To clean the deflection plates:

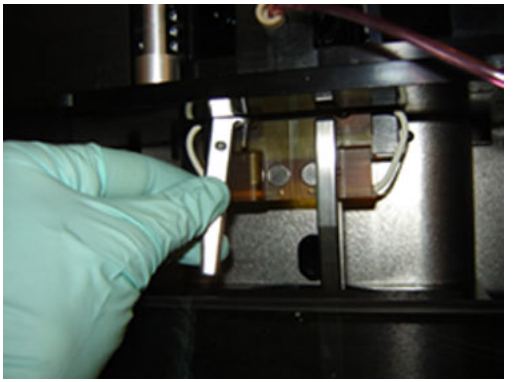
1. If you want to keep the sheath running, position the flush bucket under the nozzle to prevent the stream from wetting the plates during the procedure.
2. Power off the deflection plates by pressing the PLATES button.

   The PLATES button is not illuminated when the plates are powered off.
3. Remove the plate access panel.

When the plate access panel is removed, the deflection plates are automatically powered off as an additional safeguard.



4. Open and remove the plates by lifting each plate separately out of the holder assembly.



5. Wet a Kimwipes wipe or other lint-free towel with DI water and wipe down the deflection plates and the body of the deflection plate holder.

Caution! All biological specimens and materials can transmit potentially fatal disease. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

6. Wipe the plates and the plate holder assembly again with a dry wipe.
7. Re-install the deflection plates by sliding the pins on the plates into the deflection plate holders.
8. Close the deflection plates by swinging them into their closed position.
9. Restart the sheath stream or remove the flush bucket, if used.

   The attached magnetic latches hold them in place.
10. Replace the plate access panel.

More information

- Maintenance overview (page 62)
- Cleaning and inspecting the instrument (page 63)

Decontaminating the fluidics

Introduction

This topic describes how to decontaminate the fluidics.

This procedure is similar to the dry shutdown process. If your lab does not usually perform dry shutdowns, perform this procedure weekly or monthly to maintain a clean system.

Required materials

- 0.5–1 L of 10% bleach or 70% ethanol
- DI water

Procedure

To decontaminate the fluidics:

1. Install the nozzle tip.
2. Bypass the sheath filter.
3. Place the flush bucket under the nozzle.
4. Empty the waste tank.
5. Rinse the system with bleach or ethanol.
    a. Fill the sheath tank with 0.5 L to 1 L of 10% bleach or 70% ethanol.
    b. In the **Pressure Console** pane, click **Rinse** and **Backflush.**
    c. Once the system begins to run dry, click **Rinse** to stop the fluidics.
    d. Unload the tube from the sample station.
6. Rinse the system with DI water.
    a. Rinse out the sheath tank with DI water.
    b. Empty the waste tank.
    c. Fill the sheath tank with at least 2x volume of DI water (for example, if you used 1 L of bleach or ethanol, rinse with 2 L of DI water).
    d. Fill the debubble reservoir with DI water, submerge the nozzle tip in the reservoir, then click **Purge** to draw DI water in to the nozzle body. After 1 minute, click **Purge** to stop the process.
    e. In the **Pressure Console** pane, click **Rinse** and **Backflush.**
    f. Once the system begins to run dry, click **Rinse** to stop the fluidics.

7. Complete one of the following workflows according to your requirements:
   - Dry shutdown workflow (page 351)
   - Dry startup workflow (page 248)

More information

- Maintenance overview (page 62)
- Cleaning and inspecting the instrument (page 63)
- Replacing the nozzle assembly (page 77)

Vertically aligning the lasers

Introduction

This topic describes how to adjust the vertical alignment of the lasers. Perform this procedure as needed when the vertical laser alignment drifts (laser beams do not go through the pinholes).

Required materials

- 3-µm SPHERO™ Ultra Rainbow beads
- 0.5 mL of filtered sheath fluid
- 5/64'' Allen wrench

Before you begin

- Prepare a tube of 3-µm SPHERO Ultra Rainbow beads (1 drop per 0.5 mL of filtered sheath fluid).
- Ensure that the stream is aligned. See Aligning the stream (page 277).

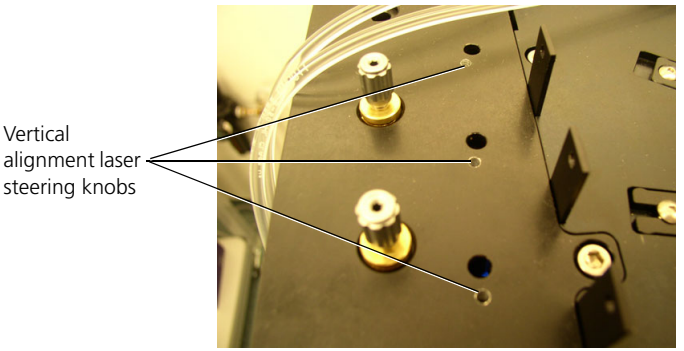
Adjusting the vertical alignment of the laser

To adjust the vertical alignment of the laser:

1. Load the tube of Ultra Rainbow beads onto the sample tube holder and close the tube-lock lever.
2. Verify that the sample pressure offset is about 1.0 psi.

   Use the Pressure Control pane to adjust the sample offset, if necessary.

   See Introducing a sample into the system (page 264).
3. In the **Pressure Console** pane, click **Sample** to start running beads.
4. Use a 5/64'' Allen wrench to adjust the appropriate vertical alignment laser steering knob so that the flash of beads in the core stream is in the center of the pinhole (as viewed in the pinhole image of the Sortview pane).



More information

- Sortview pane (page 95)
- Alignment and QC (page 267)
- Preparing beads for QC (page 276)

Component replacement

Introduction

This topic lists the typical component replacement procedures you perform without the assistance of a service representative.

Before replacing components

Before beginning any maintenance procedures, complete the following tasks:

- Shutter all lasers
- Turn off the piezo amplitude
- Turn off the deflection plates
- Turn off all PMTs
- Decontaminate any parts of the instrument that have been in contact with biohazardous samples, or use biohazard precautions

User-replaceable component procedures

You can perform the following component replacement procedures:

- Replacing the nozzle tip (page 71)
- Replacing the sample line (page 72)
- Replacing the sheath and waste fluidic lines (page 75)
- Replacing the nozzle assembly (page 77)
- Replacing the sample stopper (page 80)

More information

- Maintenance overview (page 62)
- Cleaning and inspecting the instrument (page 63)
- Decontaminating the fluidics (page 66)
- Cleaning the nozzle tip (page 259)
- Turning off the power (page 357)

Replacing the nozzle tip

Introduction

This topic describes how to remove and replace a nozzle tip. You need to remove the nozzle tip to clean the nozzle. Perform this procedure as needed.

Removing the nozzle tip

To remove the nozzle tip:

1. Open the nozzle access door.
2. Loosen the nozzle nut by hand.

   Do not use a wrench.
3. Remove the nut and nozzle tip.
4. Place the nozzle tip and nut in a safe place.

Cleaning the nozzle tip

See Cleaning the nozzle tip (page 259) for instructions.

Installing the nozzle tip

To attach the nozzle tip:

1. Place the clean nozzle tip into the nozzle nut, ensuring that the O-ring is in place in the nut.
2. Install the nozzle tip onto the nozzle, ensuring that the nozzle tip is seated in the nozzle body and not jammed or askew.
3. Tighten the nozzle tip by hand as much as possible.

   Do not use a wrench.

More information

- Maintenance overview (page 62)
- Decontaminating the fluidics (page 66)
- Component replacement (page 70)

Replacing the sample line

Introduction

This topic describes how to replace the sample line.

Replacing the sample line prevents any sample carryover or cross-contamination of cells. Perform this procedure every 4 to 6 months, or when decreased event rates indicate that the sample line is kinked or clogged and cannot be unclogged by backflushing or running 10% bleach through the sample line.

Required materials

- Sample line
- 7/64'' Allen wrench
- 1/16'' Allen wrench
- 2–3 mL of DI water

Before you begin

- See Before replacing components (page 70) for more information.
- Make sure the system is dry.

Removing the sample line

To remove the sample line:

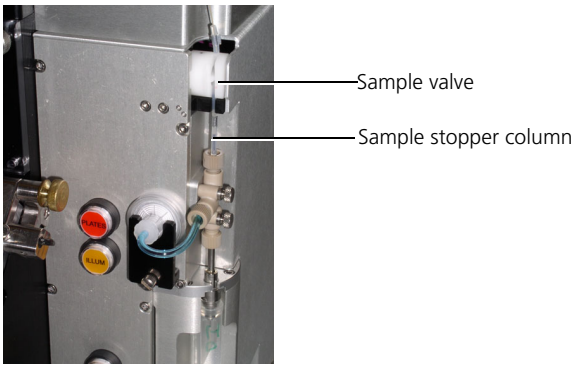
1. Verify that the Air switch is on and the fluidic modes are off.
2. Remove the nozzle line from the Y-fitting.
3. Remove the nozzle assembly using the 7/64'' Allen wrench to loosen the socket-head cap screw that holds the nozzle assembly in place.

4. Gently remove the sample line from the nozzle assembly while supporting the stainless steel tubing.

Caution! All biological specimens and materials can transmit potentially fatal disease. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Use proper precautions and wear suitable protective clothing, eyewear, and gloves.

Caution! Do not bend the stainless steel fitting.

5. In the **Pressure Console** pane, click **Override** to open the sample valve.



6. Remove the sample line from the sample valve.
7. Gently remove the sample line from the sample stopper column.

Installing a new sample line

To install a new sample line:

Caution! Do not bend the stainless steel fitting and be careful not to kink the sample line.

1. Attach the sample line into the sample stopper column.

2. Install a tube onto the sample station and check the depth of the sample line.

   The sample line should just touch the bottom of the tube without bending.

3. Verify that the fluidics are not running, then with the tube still locked in place, in the **Pressure Console** pane, click **Override** to open the sample valve.

4. Insert the silicone section of the tubing into the pinch valve using a 1/16'' Allen wrench to gently push the tube inside the valve, if necessary.

5. Verify that the sample line is not blocking the bubble detector just below the sample valve.

   The bubble detector should have only silicone tubing in front of it.

6. Remove the nozzle tip.

7. Gently feed the sample line through the stainless steel fitting on top of the nozzle assembly, sliding the silicone rubber tubing over the stainless steel fitting.

8. Verify that the sample line extends about 1 mm beyond the end of the stainless steel fitting inside the nozzle assembly.

Sample line installed incorrectly

Sample line installed correctly

9. Reattach the nozzle assembly using the socket-head cap screw removed earlier.

   The nozzle assembly keys into position automatically.

10. Perform the dry startup procedure. See Dry startup workflow (page 248).

More information

- Maintenance overview (page 62)
- Decontaminating the fluidics (page 66)
- Component replacement (page 70)

Replacing the sheath and waste fluidic lines

Introduction

This topic describes how to replace the fluidics lines that connect to the sheath and waste tanks. Perform this procedure as needed.

Required materials

- Sterile sheath fluid (at least 200 mL)
- 3/22'' Allen wrench

Before you begin

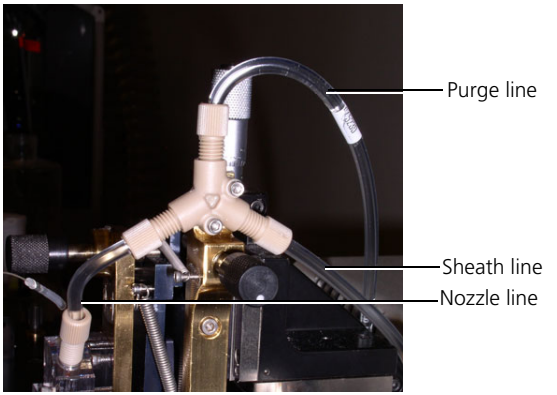
- See Before replacing components (page 70) for more information.
- Perform a dry shutdown (see Dry shutdown workflow (page 351)).

Removing the fluidic lines

To remove the fluidic lines:

1. Verify that the Air switch is on and the fluidic modes are off.
2. Depressurize the sheath tank.
   a. Disconnect the air line to the sheath tank.
   b. Open the pressure release valve.
3. Remove the nozzle line from the Y-fitting above the nozzle.

4. Remove the Y-fitting using a 3/22'' Allen wrench.



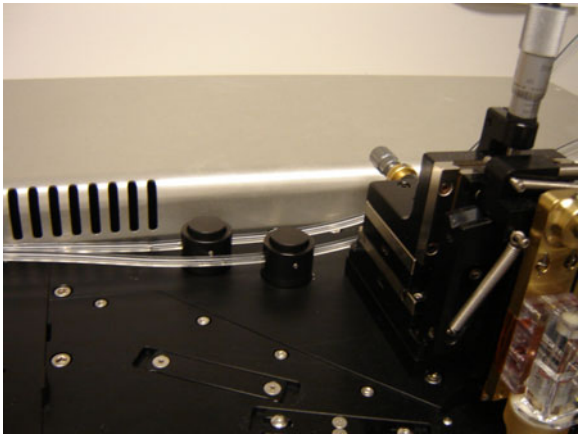
5. In the **Pressure Console** pane, click **Rinse** to open both the sheath and purge valves.
6. Remove the tubing from the two valves on the top of the instrument.
7. Undo the blue connections on the fluidics lines going to the sheath and waste tanks.

Installing new sheath and waste fluidic lines

To install new fluidic lines:

1. Attach a new sheath filter.
2. Attach the new sheath and purge lines and route them to the top of the instrument.
3. Attach the Y-fitting to the chassis.Attach the nozzle line to the Y-fitting.
4. Slide the lines into the pinch valves.

The purge line runs through the left pinch valve and the sheath line runs through the right pinch valve.



5. Perform a dry startup.

More information

- Maintenance overview (page 62)
- Dry startup workflow (page 248)

Replacing the nozzle assembly

Introduction

This topic describes how to replace the nozzle assembly.

Required materials

- 7/64'' Allen wrench
- 1–2 L of sterile sheath fluid

Before you begin

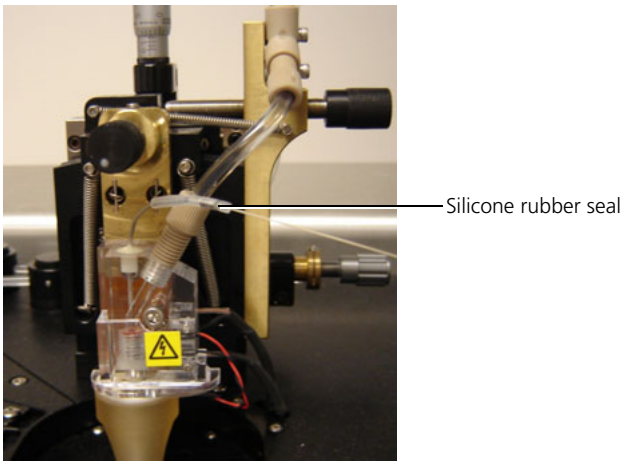
See Before replacing components (page 70) for more information.

Removing the nozzle assembly

Caution: Electrical! To prevent possible electrical shock when working with the nozzle assembly, make sure that the system power is off.

To remove the nozzle assembly:

1. Remove the sample line from the top of the nozzle assembly by sliding the silicone rubber tubing off the stainless steel tubing.



2. Detach the nozzle line from the Y-fitting.
3. Remove the nozzle assembly using the 7/64'' Allen wrench to loosen the socket-head cap screw that holds the nozzle assembly in place.
4. Inspect the drop charge contact and the piezo drive connections for corrosion or bending.

   Remove corrosion, if corrosion is present. Bend connections back into the proper orientation, if connections are bent.

Installing the nozzle assembly

To install the nozzle assembly:

1. Gently feed the sample line through the stainless steel fitting on the top of the new nozzle assembly, sliding the silicone rubber tubing over the stainless steel fitting.

   Caution! Do not bend the sample line or the stainless steel fitting.

2. Verify that the sample line extends about 1 mm beyond the end of the stainless steel fitting inside the nozzle assembly.

Sample line installed incorrectly

Sample line installed correctly

3. Attach the new nozzle assembly using the socket-head cap screw removed earlier.

   The nozzle assembly keys into position automatically.

4. Tighten the socket-head cap screw using an Allen wrench.
5. Reattach the sheath tubing to the Y-fitting.
6. Perform a dry start up.

More information

- Component replacement (page 70)
- Before replacing components (page 70)
- Dry startup workflow (page 248)

Replacing the sample stopper

Introduction

This topic describes how to replace the sample stopper. Replace the sample stopper when it is worn out or when there is a leak around the tube seal.

Removing the sample stopper

To remove the sample stopper:

1. Power off the electronics and the lasers.

   Caution! Do not bend the stainless steel fitting.

2. Remove the sample line from the pinch valve.

   a. In the **Pressure Console** pane, click **Override** to open the sample pinch valve.

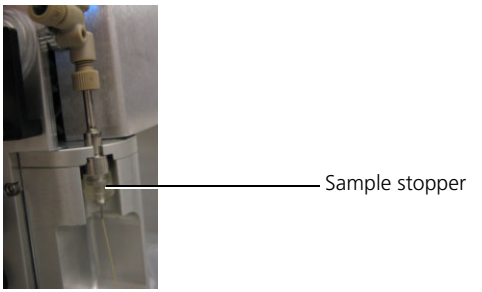
   b. Hold the sample line above and below the pinch valve and remove.

3. Remove the sample line from the sample-stopper column.

   Caution! Do not remove the sample line from the nozzle assembly.

4. Gently pull the sample stopper off the end of the sample column.

   Removing the sample station T from the chassis allows better access to the sample stopper.



Installing a new sample stopper

To install a new sample stopper:

1. Gently slide the new sample stopper onto the sample column.
2. Inspect the stopper for cracks, and replace it if there are any.
3. Replace the sample line in the sample column.
4. Replace the sample line in the sample valve.

More information

- Maintenance overview (page 62)
- Component replacement (page 70)
- Replacing the sample line (page 72)

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Part 2

Software information

This part includes these chapters:

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7

Software overview

This chapter includes these topics:

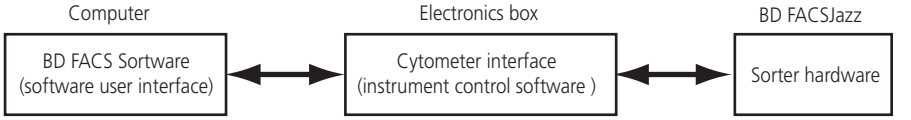
BD FACS Sortware overview

Introduction

This topic describes the BD FACS Sortware user interface, including the panes that you can select from the main window.

How the software interacts with the instrument

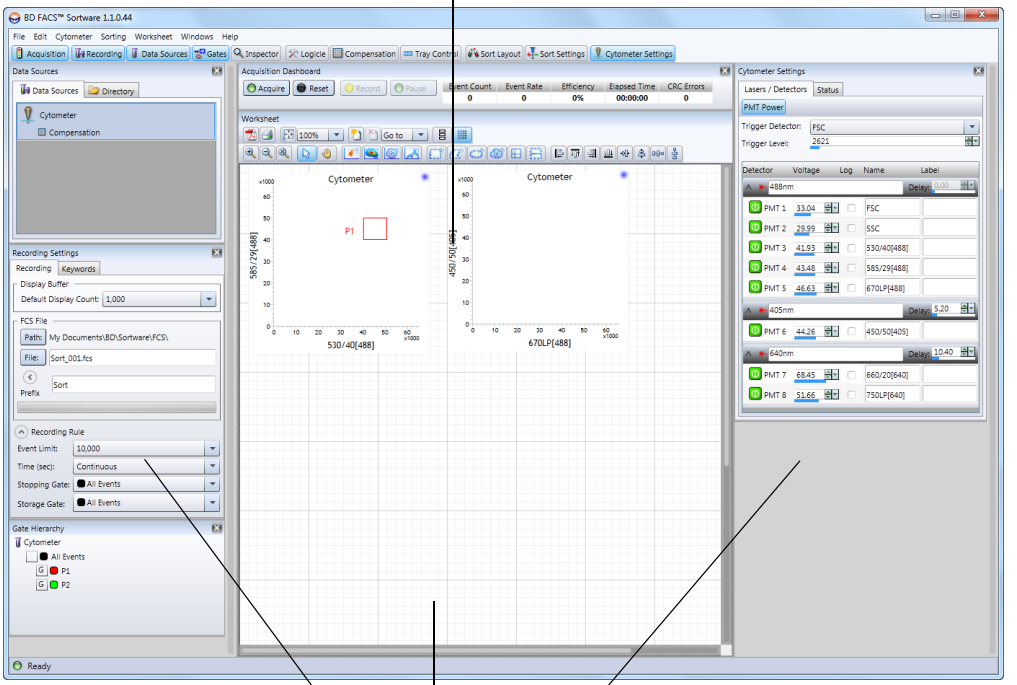
BD FACS Sortware serves as the user interface and works through the cytometer interface to control the instrument, acquisition, sorting, and analysis. The cytometer interface is the access point for controlling the cytometer and keeps track of the state of the instrument.



User interface

BD FACS Sortware serves as the user interface and provides all user control for the instrument, acquisition, sorting, and analysis.

The worksheet is always open. Use the worksheet to create and modify plots, monitor sort data, and analyze sort results.

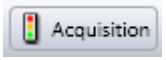
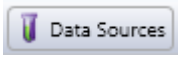

Worksheet
Examples of panes in the workspace

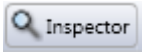
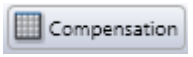
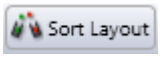
Panes

Panes can be opened, closed, resized, moved, and pinned within the workspace so that you can create your own window layout. Click the tools on the BD FACS Sortware toolbar to open panes in the workspace.

You can arrange individual panes to create specific window layouts. See Setting up your window layout (page 98) for more information.

The following table describes the toolbar controls and the panes they open.

Control	Description
	Opens the Acquisition Dashboard pane. Use this pane to start or stop acquisition, record events, and monitor data acquisition details. See Using the Acquisition Dashboard (page 166) for more information.
	Opens the Recording Settings pane. Use this pane to set details about the flow cytometry standard (FCS) file that is generated when you record data. See Using the Recording Settings pane (page 162) for more information.
	Opens the Data Sources pane. Use this pane to view data and information from the instrument or loaded or recorded data sources (FCS files). See Using the Data Sources pane (page 154) for more information.
	Opens the Gate Hierarchy pane. Use this pane to display and manipulate the hierarchy (tree) of the gates in an active plot. See Using the Gate Hierarchy pane (page 199) for more information.

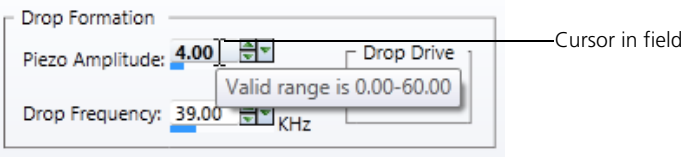
Control	Description
	Opens the Inspector pane. Use this pane to view and modify data source properties and properties for selected worksheet items. See Using the Inspector to view and modify plot properties (page 176) for more information.
	Opens the Logicle Scaling pane. Use the logicle scale after compensation to view events that have negative values. See Using the Logicle Scaling pane (page 136) for more information.
	Opens the Compensation pane. Use this pane to create a compensation matrix, select parameters for auto compensation, and calculate fluorescence spillover values. See Using the Compensation pane (page 131) for more information.
	Opens the Tray Control pane. Use this pane to view or modify the current sort device and adjust the offsets for the tray, or create a new sort device configuration. See Using the Tray Control pane (page 233) for more information.
	Opens the Sort Layout pane. Use this pane to select the sort device, set up populations to be sorted, and control the position and readiness of the sort device. See Using the Sort Layout pane (page 241) for more information.
	Opens the Sort Settings pane. Use this pane to set up the drop formation, drop delay, breakoff, and deflection parameters, and to select a sort mode. See Using the Sort Settings pane (page 226) for more information.
	Opens the Cytometer Settings pane. Use this pane to view the status or modify the laser and detector settings of the cytometer. See Using the Cytometer Settings pane (page 118) for more information.

Setting numeric values in panes and dialogs

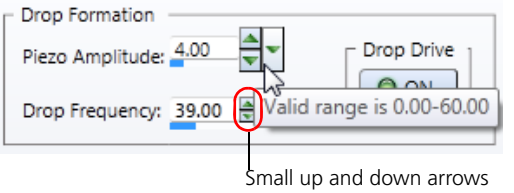
BD FACS Sortware panes and dialogs include fields with adjustable numeric values. You can adjust these numeric values using different mouse and keyboard options.

To set numeric values in fields with the mouse:

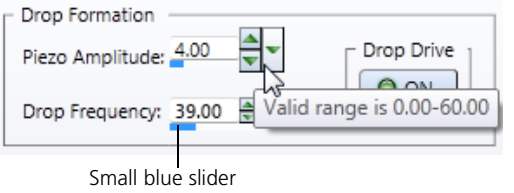
- Hold the mouse pointer over the field (the value will change from normal to bold) and roll the mouse scroll wheel up or down to change the value. You do not need to click in the field to change the values with the scroll wheel.



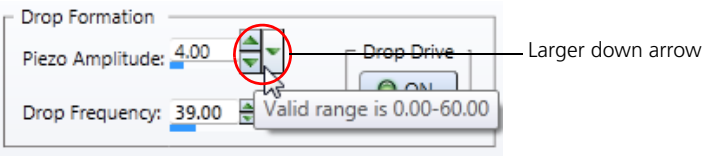
- Click the small up and down arrows to change the value.



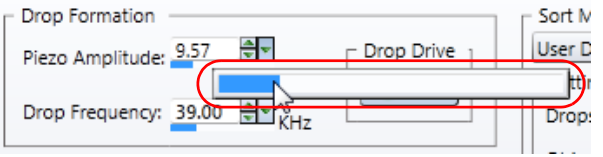
- Click in the small blue/white data slider below the value to change the value.



- In the field, click and hold the larger down arrow.



The large data slider appears.



Continue to hold the mouse button and slide the data slider left or right to adjust the values.

To set numeric values in fields with the keyboard:

- Click in the field and press the up or down arrow keys to adjust the values.
- Click in the field and press Ctrl and the up or down arrow keys to adjust the values in larger increments.

  The increment values are dependent on the field that is being adjusted. For example, the detector voltage is adjusted in increments of 0.1 with the arrow keys and 1.0 volts with Ctrl+arrow keys, while piezo amplitude is adjusted in increments of 0.01 and 0.1, respectively.

More information

- Preferences and settings (page 97)
- Worksheets (page 139)
- About settings files (page 110)
- Working with settings files (page 113)

Pressure Console pane

Introduction

This topic describes the Pressure Console pane in BD FACS Sortware.

About the Pressure Console pane

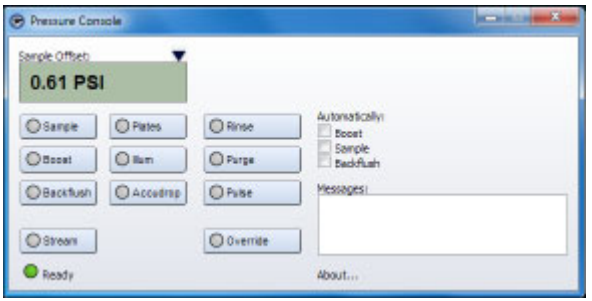
The Pressure Console pane has the software controls that regulate and monitor the sample and sheath fluidics pressures, and control the deflection plates and Accudrop laser. The Pressure Console pane starts when BD FACS Sortware software is started.

The following figure shows the collapsed view of the Pressure Console pane.

To change the offset pressure, select the pressure offset control and perform any of the following actions:

- Use the scroll wheel on the mouse.
- Use the arrow keys on the keyboard.
- Type a new value and press Enter.

The following figure shows the expanded view of the Pressure Console pane.



The following table describes the Pressure Console pane controls and displays.

Control	Description
Offset	Allows you to adjust and view the sample offset.
Boost	When this button is held down, the sample offset is increased an additional 3.0 psi over the current offset. Boost stops when the button is released. The shortcut key is F7.
Sample	Opens the sample valve when a tube is in place and the fluidics are in stream mode, allowing the sample to be carried from the sample tube to the nozzle. The shortcut key is F6.
Plates	Turns the power to the plates on and off.
Stream	Opens the sheath valve (turns the stream on) and closes the sheath valve (turns the stream off). The shortcut key is F8.
Backflush	Opens the sample valve when no tube is in place and fluidics are in stream mode, allowing sheath fluid to be carried from the nozzle to the sample station.
Illum	Turns the BD FACS Accudrop laser on and off.
Purge	Opens and closes the waste valve, allowing fluid to be carried from the debubbler up through the nozzle to the waste line.
Pulse	Pulses the fluidic valves to clear bubbles in the nozzle.

Control	Description
Accudrop	Moves the Accudrop filter in and out of position over the sort stream camera.
Rinse	Opens and closes the sheath and waste valves.
Override	Opens the sample valve regardless of the state of the other fluidics to allow for the removal or installation of the sample line or other maintenance procedures.
Automatically boost before sampling	Automatically boosts the sample briefly when Sample is clicked.
Start sampling when door is closed	Automatically starts sampling when the sample door is closed.
Backflush when door is opened	Automatically opens the sample valve when the door is opened, briefly backflushing the sample line.
Messages	Displays system messages.

More information

- System startup (page 247)
- System shutdown (page 349)

Sortview pane

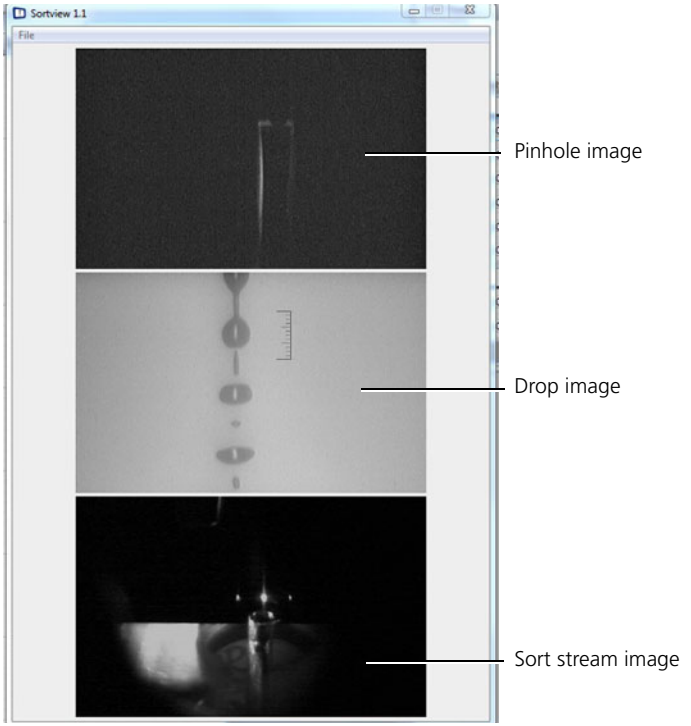
Introduction

This topic describes the Sortview pane and how to use it to monitor your sort.

About the Sortview pane

Sortview is a pane within BD FACS Sortware. Sortview displays three different images (pinhole, drop, and sort stream) on the computer screen that are taken from the cameras on the BD FACSJazz system. These images are used in sort monitoring as well as in system QC and alignment.

The Sortview pane opens when BD FACS Sortware software is started.



Monitoring sorts with the Sortview pane

To monitor sorts with Sortview:

1. View the images from the BD FACSJazz cameras displayed in the **Sortview** pane.
2. Resize the window as needed.

   The camera displays will arrange themselves horizontally if the window is stretched horizontally.

   The following table describes what each image displays.

Display	Description
Pinhole image	Displays an image of the pinholes.
Drop image	Displays an image of the stream breakoff.
Sort stream image	Displays an image of the side streams and the stream drain.

3. Click each image to zoom, if needed.

More information

- Sample introduction (page 33)
- Sort components overview (page 56)
- BD FACS Sortware overview (page 86)

8

Preferences and settings

This chapter includes these topics:

Setting up your window layout

Introduction

This topic describes how to set up and customize the BD FACS Sortware window layout.

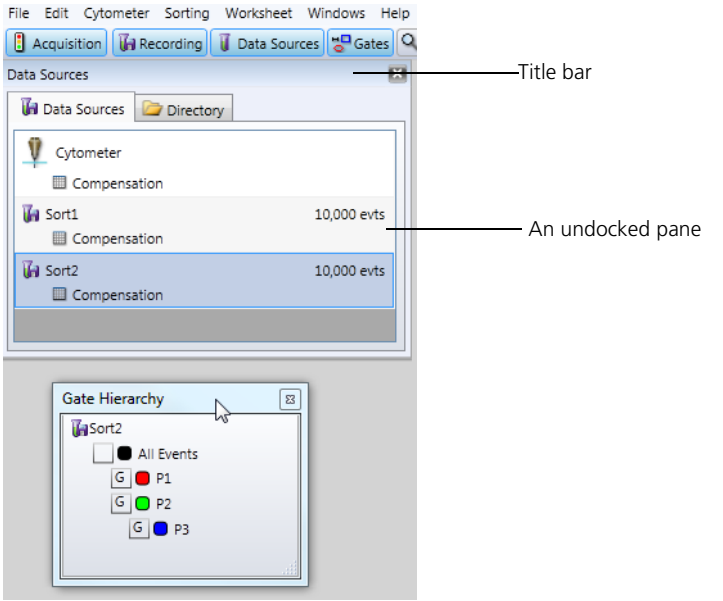
You can open different panes within the BD FACS Sortware window and drag and dock them to different locations, or create standalone (floating) panes.

Undocking and docking panes

To undock panes:

1. Click in the title bar area of any docked pane and drag the pane away from its docked location.

   The pane becomes a floating pane.



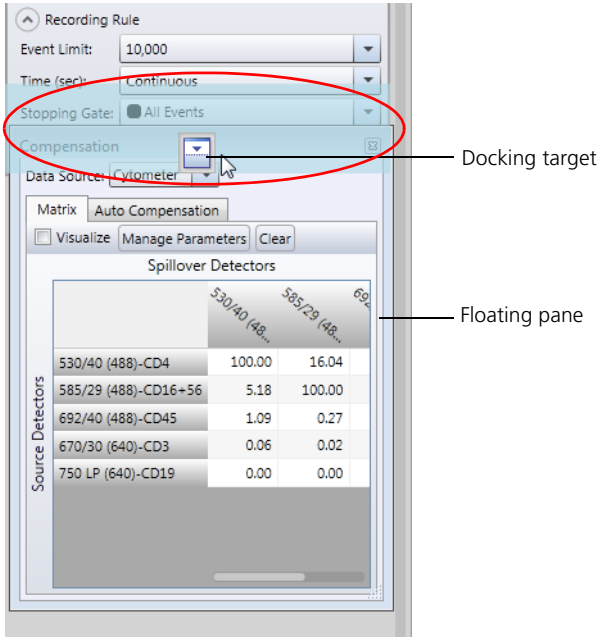
To dock a floating pane to a new location in the workspace:

1. Drag the floating pane to where you want to dock it.

   Docking targets appear when floating panes are dragged near a dockable location.

2. Drag the floating pane over the docking target.
3. When the area where you are docking highlights, release the mouse button.

   The floating pane docks in the direction of the arrow on the docking target.



Changing themes

To change the window theme:

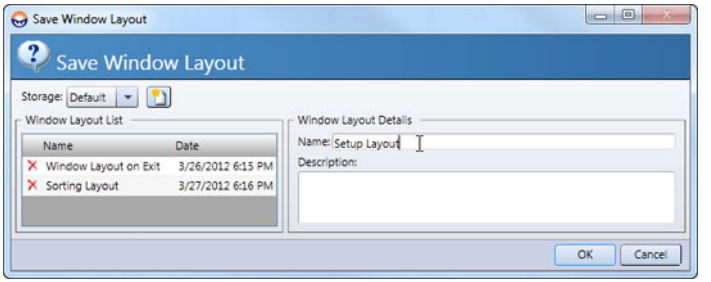
1. Select **Windows > Themes**.
2. Select the default or other available window theme.

Saving the window layout

To save the current window layout:

1. Select **Windows > Save Window Layout.**

   The Save Window Layout dialog opens.



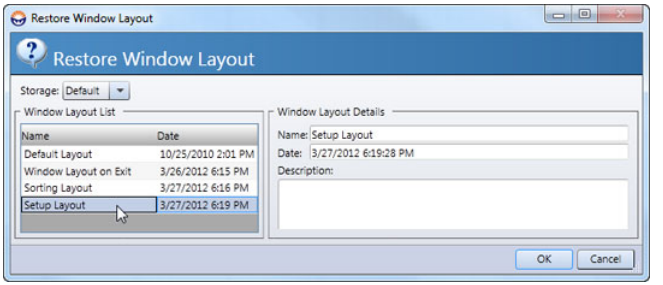
2. Select the storage folder where you want to save this layout from the **Storage** menu.
3. Under **Window Layout Details**, type a name for the new window layout in the **Name** field.
4. Click **OK** to save the window layout and close the dialog.

Restoring the window layout

To restore the window layout:

1. Select **Windows > Restore Window Layout.**

   The Restore Window Layout dialog opens.



2. Select the storage folder that contains the file you want to restore (default or a user-defined storage folder) from the **Storage** menu.
3. Under **Window Layout List,** select a layout to restore.
4. Click **OK.**

More information

- Preferences and settings (page 97)
- Editing user preferences (page 101)
- About settings files (page 110)
- Working with settings files (page 113)

Editing user preferences

Introduction

This topic describes how to edit user preferences for gates, plots, statistical displays, acquisition, and the cytometer using the Edit User Preferences dialog.

Preferences are not required. You can set preferences at any time and in any order. If you do not specify preferences, default settings are used.

User preferences

You can perform the following user preferences tasks:

- Setting cytometer preferences (page 102)
- Setting gate and population display preferences (page 105)
- Setting plot display preferences (page 107)
- Setting statistics view display preferences (page 108)

More information

- Preferences and settings (page 97)
- About settings files (page 110)
- Working with settings files (page 113)

Setting cytometer preferences

Introduction

This topic describes how to specify:

- The display of a parameter name
- How plots are used
- How the cytometer updates at startup
- The sort tray direction

These are global settings. You can use the default values or set custom preferences as needed.

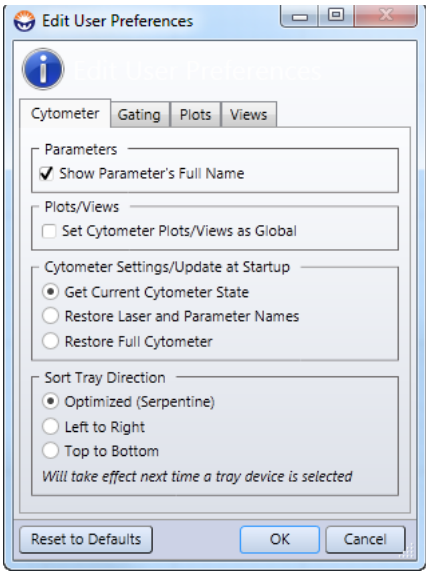
Procedure

To set cytometer preferences:

1. Select **Edit > Preferences**.

   The Edit User Preferences dialog opens.

2. Click the **Cytometer** tab.



3. Set cytometer preferences.

   - Under **Parameters,** select the **Show Parameter's Full Name** checkbox to display the full fluorochrome/parameter name in plots and statistics views.
   - Under **Plot/Views,** select **Set Cytometer Plots/Views as Global**.

     All worksheet elements (plots, population hierarchies, statistics views) with Cytometer as the data source show data from a single file (the file selected in the Data Sources pane). Use this to quickly view all the data from a file. Non-global worksheet elements (those showing data from a file other than the file selected in the Data Sources pane) can still be added to the worksheet when this option is selected.

     See Worksheet overview (page 140) for more information.

- Under **Cytometer Settings/Update at Startup**, select a startup option.

Option	Description
Get Current Cytometer State	Gets the current instrument state from the cytometer interface. This does not include parameter names or labels.
Restore Laser and Parameter Names	Gets the current instrument state from the cytometer interface and restores parameters and laser names.
Restore Full Cytometer	Restores the cytometer settings to the settings from the last time the system was used.

- Under **Sort Tray Direction**, select a tray sort direction.

Option	Description
Optimized (Serpentine)	Sets the sort tray sorting direction to move in a serpentine motion: A1 to A12, B12 to B1, etc.
Left to Right	Sets the sort tray sorting direction to move left to right.
Top to Bottom	Sets the sort tray sorting direction to move top to bottom.

4. Click **OK** to apply your preferences.

More information

- Preferences and settings (page 97)
- Setting gate and population display preferences (page 105)
- Setting plot display preferences (page 107)
- Setting statistics view display preferences (page 108)

Setting gate and population display preferences

Introduction

This topic describes how to set gate and population display preferences.

These are global settings. You can use the default values or set custom preferences whenever you want to modify the appearance of gates and populations.

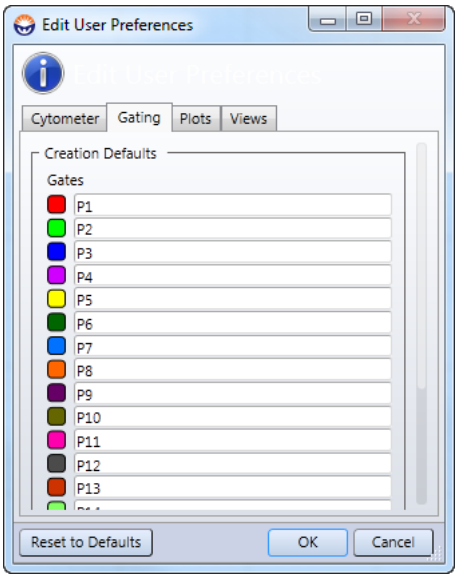
Procedure

To set the default gate and population colors:

1. Select **Edit > Preferences**.

   The Edit User Preferences dialog opens.

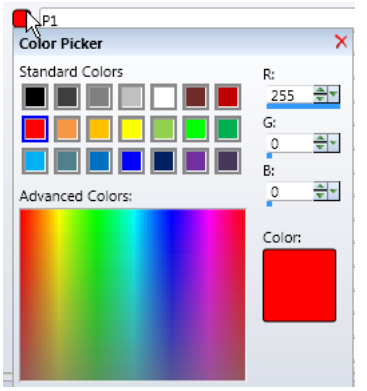
2. Click the **Gating** tab.



3. Click in the **Gates** field and enter a new default name for any of the gates (populations) in the list.

4. Right-click a colored box next to a gate (population) in the list (for example, P1).

The Color Picker dialog opens.



5. Click on a color sample in the Color Picker dialog or click **Advanced Colors** to create a custom color sample.

   The selected color appears in the Color sample at the bottom of the dialog.

6. Close the dialog to apply the color.

7. Click **OK** to apply your preferences.

More information

- Preferences and settings (page 97)
- Setting cytometer preferences (page 102)
- Setting plot display preferences (page 107)
- Setting statistics view display preferences (page 108)

Setting plot display preferences

Introduction

This topic describes how to set the plot background, display attributes, and label fonts.

These are global settings. You can use the default values or set custom preferences whenever you want to modify the appearance of plots.

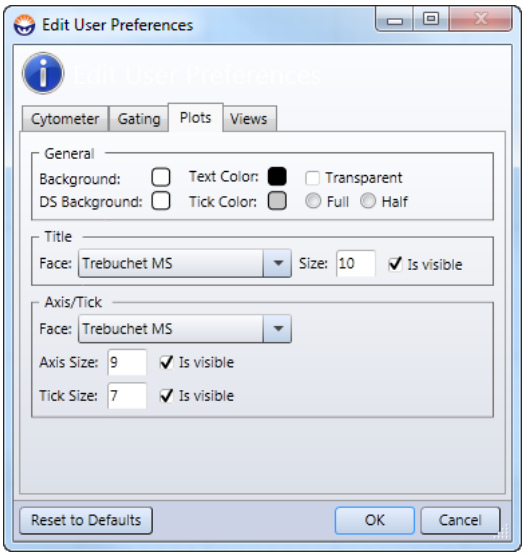
Procedure

To set the plot background and the plot label fonts:

1. Select **Edit > Preferences**.

   The Edit User Preferences dialog opens.

2. Click the **Plots** tab.



3. Make plot display selections:
   - Under **General,** right-click the color sample for one of the options to open the color picker and select colors for the plot frame background, the plot area background, text, and tick marks. You can also set the level of transparency and show the plot border as full or half.
   - Under **Title**, modify the plot title font face, font size, or make the title invisible.
   - Under **Axis/Tick**, modify the axis label font face, axis size, tick mark size, or make these elements invisible.
4. Click **OK** to apply your preferences.

More information

- Preferences and settings (page 97)
- Setting cytometer preferences (page 102)
- Setting gate and population display preferences (page 105)
- Setting statistics view display preferences (page 108)

Setting statistics view display preferences

Introduction

This topic describes how to set statistics view display preferences.

Statistics views contain summary data for plots and populations. These views include the population hierarchy and statistics box and appear in the worksheet. They can be saved or printed.

These are global settings. You can use the default values or set custom preferences whenever you want to modify the appearance of statistics views.

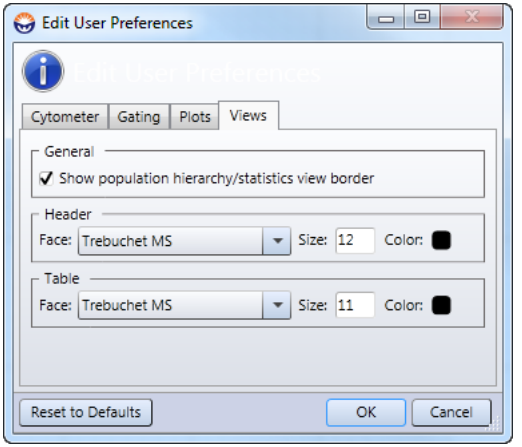
Procedure

To set statistics view display preferences:

1. Select **Edit > Preferences**.

   The Edit User Preferences dialog opens.

2. Click the **Views** tab.



3. Make statistics views selections:
   - Under **General,** select or clear the **Show population hierarchy/statistics view border** checkbox to show or hide the border for the statistics view.
   - Under **Header,** modify the statistics view header font face, size, or color.
   - Under **Table,** modify the table text display font face, size, or color.
4. Click **OK** to apply your preferences.

More information

- Preferences and settings (page 97)
- Setting cytometer preferences (page 102)
- Setting gate and population display preferences (page 105)
- Setting plot display preferences (page 107)

About settings files

Introduction

This topic describes settings files and the different settings that can be saved and restored in them.

Settings files

When you start BD FACS Sortware, all settings display default selections. To apply specific values or layouts, you can restore these settings from a saved settings file.

Settings that can be restored

The settings that can be restored are:

- Workspace
- Cytometer settings
- Analysis templates
- Sort layouts
- Window layouts
- Compensation settings

Workspace settings

The following elements are saved with a workspace:

- Cytometer settings
- Compensation
- Analysis template
- Sort layout
- Window layout
- Data sources
- Gates
- Fluidics setup

You can restore a previously saved workspace or the workspace that was automatically saved the last time you closed the software (Workspace on Exit).

Data sources, Gates, and Sort settings are only saved with the Workspace.

When you restore a workspace, you can select any of the following options.

If you select...	The following data is restored...
Restore Cytometer Settings: Laser and Parameter Names	• Laser names • Detector names • Detector labels
Restore Cytometer Settings: All Settings	All settings in the Cytometer Settings pane. Laser delays can be excluded from the cytometer settings restore, if desired, by clearing the Restore Laser Delays checkbox.
Restore Compensation	• Compensation matrix • Logicle scaling
Restore Data Sources	Any data sources recorded in a saved workspace
Restore Gates	Any gates (local and global) Note that gates are automatically restored if data sources are restored.
Restore Analysis Template	• Worksheet settings • Analysis elements
Restore Sort Layout	Sort Layout pane (except the piezo amplitude value)
Restore Window Layout	Pane presence, docking, and position
Restore Fluidics Setup	Piezo amplitude, drop frequency, stream focus, drop charge, drop delay, and laser intercept

Cytometer settings

When you save cytometer settings, you save all settings in the Cytometer Settings pane. These settings are also saved with a saved workspace. You can restore these cytometer settings separately or when you restore a workspace.

Analysis template settings

When you save an analysis template, you save the current worksheet settings and analysis elements (plots, population hierarchies, statistics views). When you restore an analysis template, you can select one of the following options.

If you select...	Then...
Replace Current Analysis	The current analysis is replaced by the saved analysis. If you clear the checkbox, the current analysis remains and the restored analysis is added to a new page.
Restore Worksheet Preferences	All worksheet settings in the Inspector pane except scale and number of pages are restored.

Sort layout settings

When you save or restore a Sort layout, you save or restore the current settings from the Sort Layout pane.

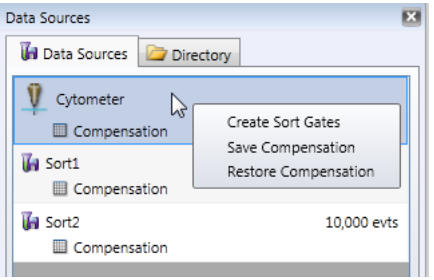
Window layout settings

When you save a window layout, you save the current window layout and pane locations. You can restore the saved layout, a default layout, or the window layout that was automatically saved the last time you closed the software (Window Layout on Exit).

See Setting up your window layout (page 98) for more information.

Compensation settings

After you have calculated compensation, you can save the compensation by right-clicking the Cytometer data source. You can restore the compensation matrix to other FCS files in the Data Sources pane by right-clicking Restore Compensation.



More information

- Working with settings files (page 113)
- Creating storage folders (page 116)

Working with settings files

Introduction

This topic describes how to save, overwrite, restore, and delete settings files.

Saving settings files

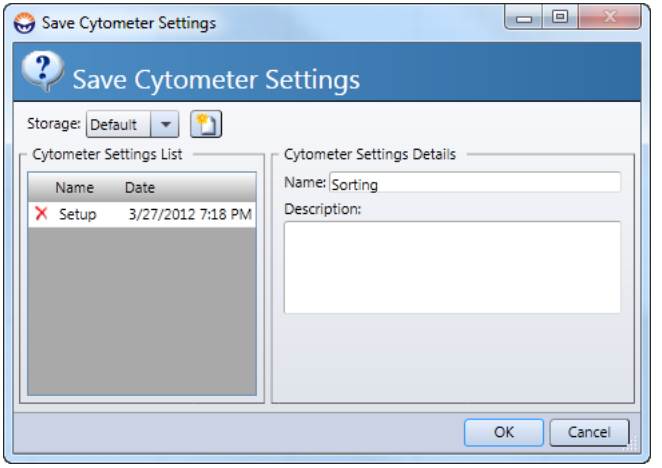
You can save settings at any time. Workspaces and window layouts are automatically saved when you exit BD FACS Sortware.

To save settings:

1. Select **File > Save**, then select a settings file type.

   The selected save dialog opens.

2. Select the storage folder where you want to save this settings file from the **Storage** menu.



See Creating storage folders (page 116) for more information about creating custom storage folders.

3. Type a name for the new settings file in the **Name** field.
4. Click **OK** to save the settings and close the dialog.

Overwriting a saved settings file

To overwrite a setting that you have already saved:

1. Select **File** > **Save**.
2. Select the name of the settings file and click **OK**.
3. Click **Yes** to confirm the overwrite.

Restoring settings files

To restore a settings file:

1. Select **File > Restore**, then select a settings file type.

   The selected restore dialog opens.

2. Select the storage folder that contains the file you want to restore (Default or a user-defined storage folder) from the **Storage** menu.

3. Select a file to restore.

4. Click **OK** to open the settings file and apply all settings.

Deleting saved settings files

To delete saved settings files:

1. In the list of settings files, click the red **X** for the settings file you want to delete.

   The Confirm Deletion dialog opens.

2. Click **Yes** to delete the cytometer settings file.

3. Click **OK** to close the dialog.

More information

- Setting up your window layout (page 98)
- About settings files (page 110)
- Creating storage folders (page 116)
- Preparing beads for QC (page 276)

Creating storage folders

Introduction

This topic describes how to create custom storage folders for settings files.

Procedure

To create new storage folders for user-defined settings:

1. Select **File > Save** and select a settings file.

   The selected save dialog opens.

2. Click **Add New Storage Location**.

   The Create Storage Location dialog opens.

3. In the **Name** field, type a name for the new storage folder.

4. Click **OK**.

   The new storage folder appears in the Storage field.

More information

- About settings files (page 110)
- Working with settings files (page 113)

9

Cytometer settings

This chapter describes the different adjustment, customization, and compensation tasks you can perform using the Cytometer Settings pane. These tasks are not typically performed as a part of the daily workflow and can be performed as needed.

This chapter includes these topics:

Other related information:

- Alignment and QC (page 267)
- Optimizing system settings for samples (page 295)

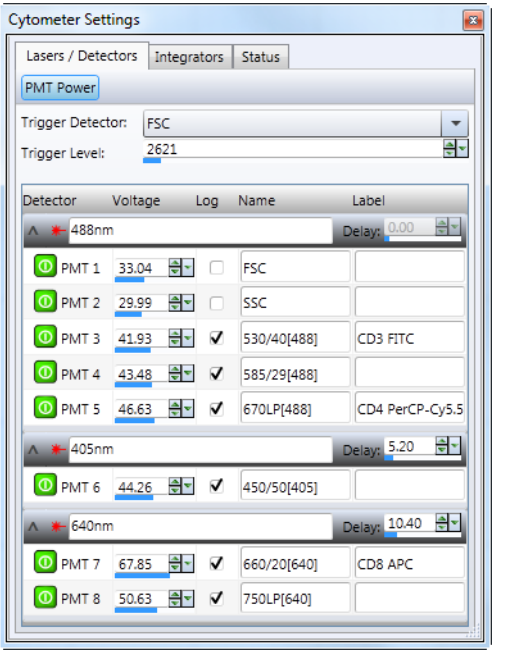
Using the Cytometer Settings pane

Introduction

This topic describes the Cytometer Settings pane and the functions of the different tabs.

Description

Use this pane to view the status or modify the laser and detector settings of the cytometer. You can also view the integrator board options.



This pane includes the following tabs:

Tab	Description
Lasers/Detectors	Use this tab to view and modify triggers, laser names and delays, detectors, power, voltage, scale, and labels.
Integrators	Use this tab to view any optional integrators. Integrators measure the area and the width of a voltage pulse for selected parameters.
Status	Use this tab to view the current status of the cytometer and any error conditions.

More information

- Viewing the cytometer status (page 119)
- Integrators (page 368)

Viewing the cytometer status

Introduction

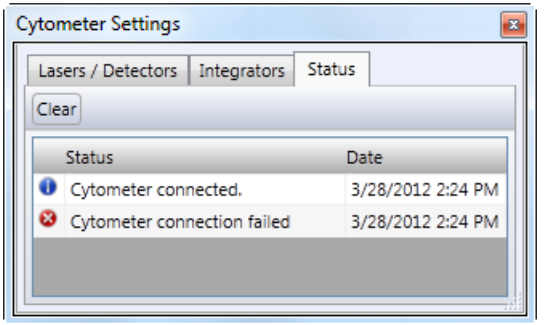
This topic describes how to view the current cytometer status and any cytometer error conditions, and how to clear the status logs.

Viewing the cytometer status

To view the cytometer status:

1. Click **Cytometer Settings** on the **BD FACS Sortware** toolbar to display the **Cytometer Settings** pane.

2. Click the **Status** tab.



The current cytometer status is listed by date and time.

Clearing the status logs

To clear the status logs:

1. In the **Status** tab, click **Clear**.

   The status screen refreshes and all status messages are cleared. New status messages appear in the Status tab if any new errors have occurred.

More information

- Using the Cytometer Settings pane (page 118)

Assigning fluorochrome labels to detectors

Introduction

This topic describes how to assign fluorochrome labels to specific detectors.

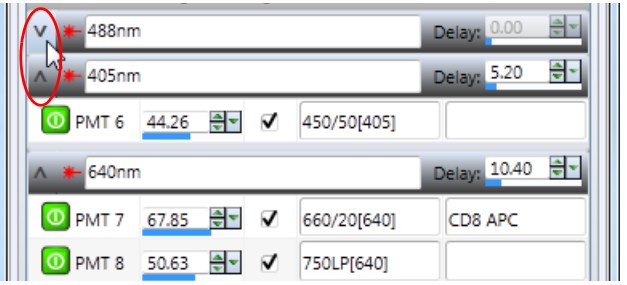
The label identifies how the detector is being used for a specific configuration and creates a more descriptive parameter label whenever the detector is used in a plot.

Procedure

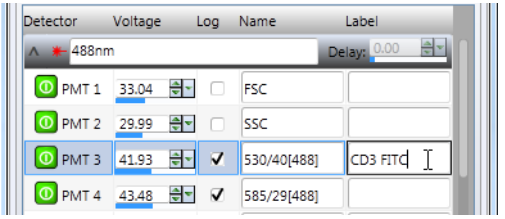
To assign a fluorochrome label to a detector:

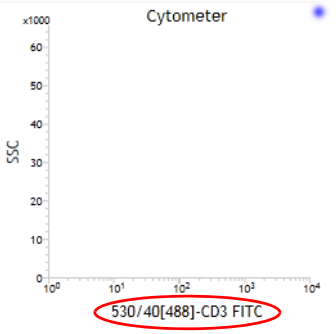
1. On the toolbar, click **Cytometer Settings** to display the **Cytometer Settings** pane.
2. In the **Lasers/Detectors** tab, locate the detector you want to configure in the **Detectors** list.

   You can click the down or up arrows in the laser bar to expand or collapse the list.



3. In the **Label** field, type a fluorochrome name.





The fluorochrome label appears in the parameter label in a plot.

More information

- Using the Cytometer Settings pane (page 118)

Selecting amplification preferences

Introduction

This topic describes how to set linear or logarithmic amplification.

About amplification preferences

The amplification preference sets the scale for plots. The instrument is pre-configured for linear (lin) signal amplification for scatter detectors and logarithmic (log) amplification for fluorescence detectors.

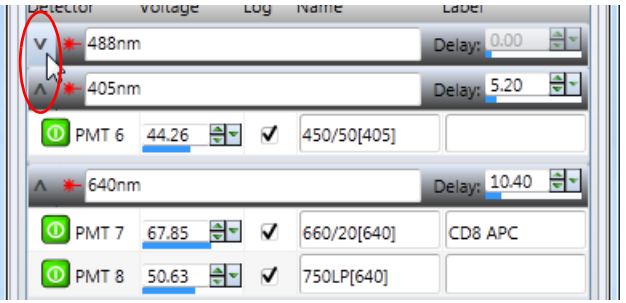
Lin amplification is typically used whenever you use the FSC and SSC detectors and log amplification is used for all other fluorescence channels to provide a wider range of intensities. However, depending on your application, you may want to use log amplification with the FSC and SSC detectors to achieve higher resolution on the lower end of the scale and/or lin amplification for other fluorescence channels.

Procedure

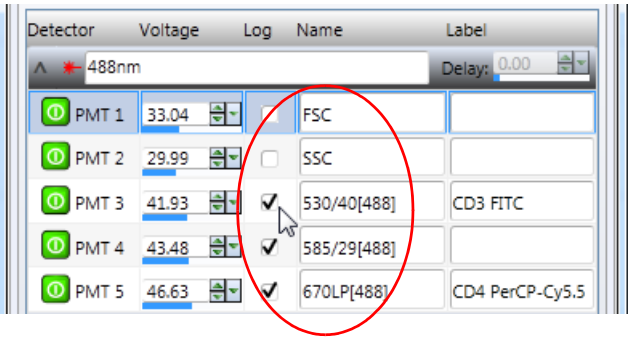
To change lin or log settings:

1. On the toolbar, click **Cytometer Settings** to display the **Cytometer Settings** pane.
2. In the **Lasers/Detectors** tab, locate the detector you want to configure in the **Detectors** list.

   You can click the down or up arrows in the laser bar to expand or collapse the list.



3. Select the **Log** checkbox to enable logarithmic amplification, or clear it to enable linear amplification.



More information

- Assigning fluorochrome labels to detectors (page 120)
- Adjusting PMT voltages and using optional integrators (page 125)

Powering the PMTs on and off

Introduction

This topic describes how to power all the PMTs on and off and how to power off individual PMTs.

PMTs are automatically powered on or off based on the user preferences selected when you open BD FACS Sortware. Power off all the PMTs for maintenance purposes.

Powering all PMTs on or off

To power all the PMTs on or off:

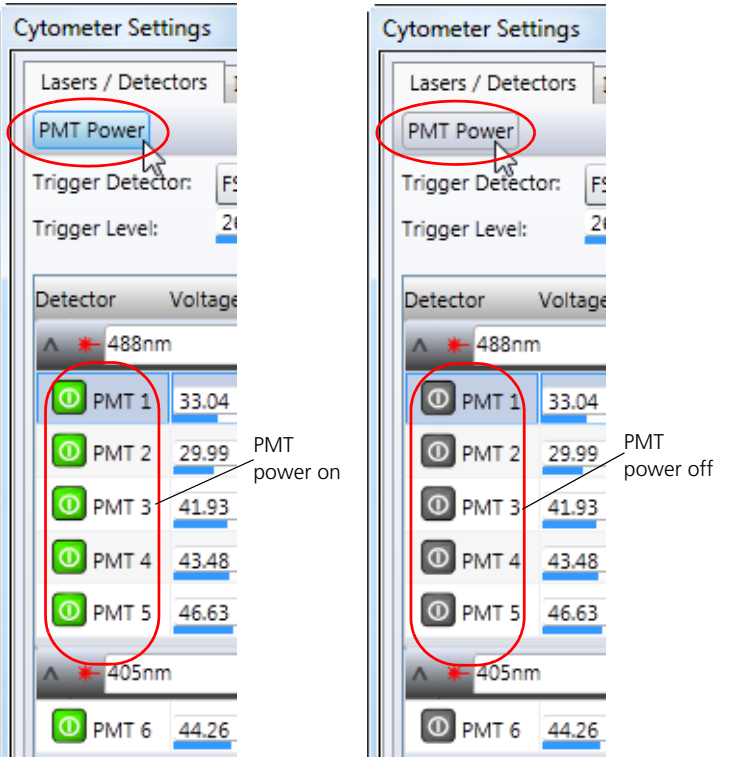
1. Click **Cytometer Settings** on the **BD FACS Sortware** toolbar.

   The Cytometer Settings pane opens.

2. Click the **Lasers/Detectors** tab.

3. Click **PMT Power** to toggle all the power buttons on or off.

When the power to a PMT is on, the PMT power button is green. When the power is off, the button is gray.

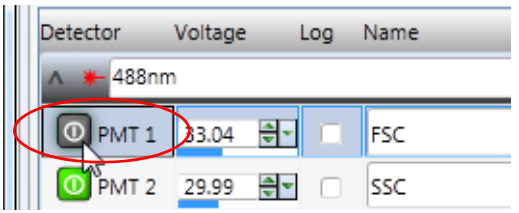


Powering off individual PMTs

If you do not want all PMTs on, you can selectively power off individual PMTs.

To power off individual PMTs:

1. In the **Lasers/Detectors** tab, click the power button for any individual detectors you want to power off.



   Click the button again to turn it back on.

More information

- Assigning fluorochrome labels to detectors (page 120)
- Adjusting PMT voltages and using optional integrators (page 125)

Adjusting PMT voltages and using optional integrators

Introduction

This topic describes how to adjust PMT voltages and set laser delays using the Cytometer Settings pane.

You can adjust voltages at any time. However, adjustment is typically performed after you create plots and while acquiring data.

Procedure

To adjust PMT voltages:

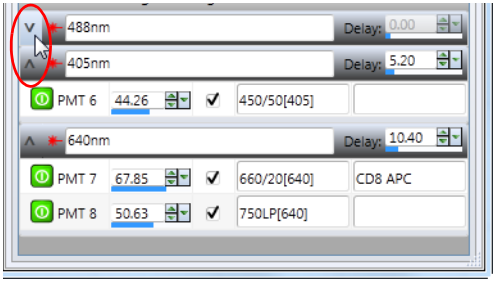
1. Click **Cytometer Settings** on the **BD FACS Sortware** toolbar.

   The Cytometer Settings pane opens.

2. Click the **Lasers/Detectors** tab.

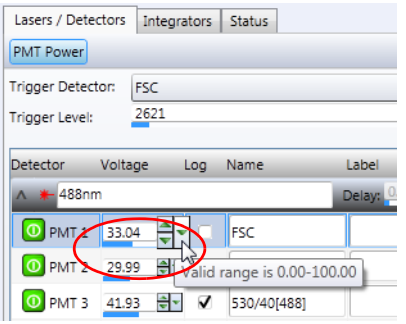
3. In the **Detectors** list, locate the detector you want to set.

   You can click the down or up arrows in the laser bar to expand or collapse the list.



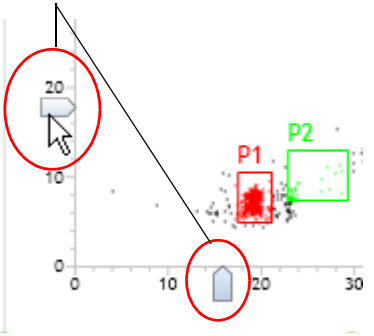
4. Adjust the values by using the mouse scroll wheel, small arrows, data slider, or keyboard keys.

   See Setting numeric values in panes and dialogs (page 90) for more information.



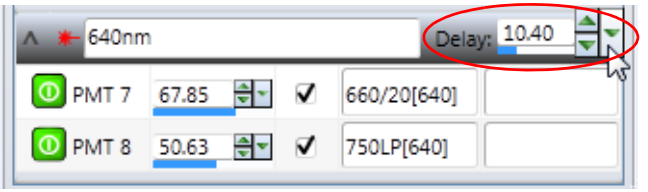
You can also use the data sliders in the plot to perform a coarse voltage adjustment. You need to place the mouse cursor over the plot axes to make the sliders visible.

Plot axis data sliders



5. Adjust the laser delay in the **Delay** field using the mouse scroll wheel, data slider, or keyboard keys.

   See Setting numeric values in panes and dialogs (page 90) for more information.



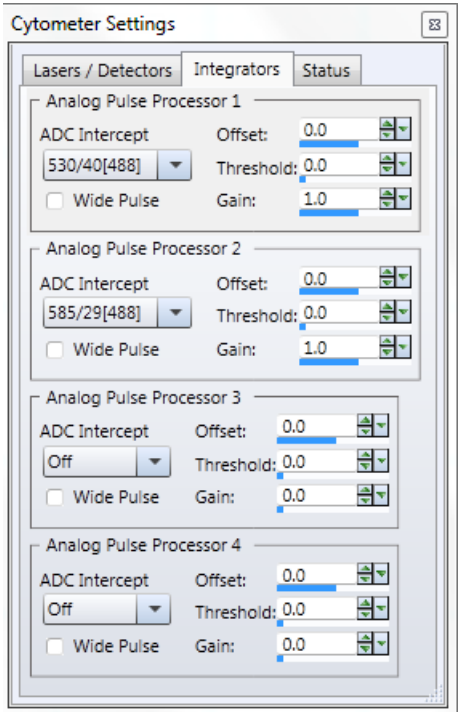
Integrators tab

Integrators are designed for use with linear events. They measure the area and the width of a voltage pulse for selected parameters and work in parallel with the height and peak measurements. You can select to enable or disable integrators as needed. The settings become the default but can be changed at any time. You can apply different integrators to different plots within the same worksheet.

Turning integrators on

To turn the integrators on:

1. Click the **Integrators** tab in the **Cytometer Settings** pane.



2. Select a parameter from the **ADC Intercept** menu to turn the integrators on.
3. Set the gain to *1.0* initially and adjust as needed depending on your sample.

   Area and width for the selected parameter are now available to view in a plot.

More information

- Collection optics (page 49)
- Integrators (page 368)

Selecting channels to capture

Introduction

This topic describes how to select channels in the Cytometer Configuration dialog.

The default channel configuration enables all available channels for every sort, even if channels are not being used for specific samples. This results in a long list of available parameters for each plot.

Select only specific channels each time you run samples to minimize the list of available parameters.

Procedure

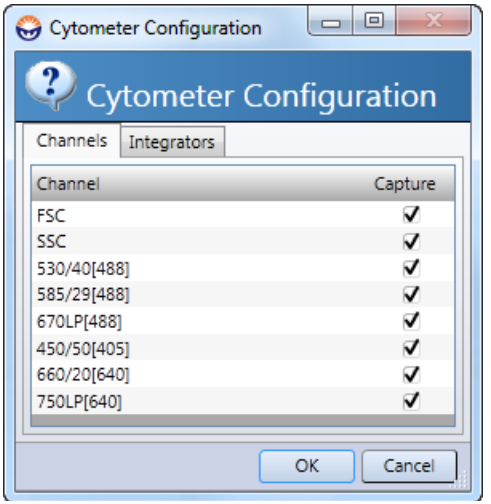
To select specific channels to capture:

1. Select **Cytometer > Configuration**.

   The Cytometer Configuration dialog opens.

2. In the **Channels tab**, select the appropriate checkboxes.

   Select the **Capture** checkboxes only for channels that you want to capture with the current set of samples.



3. If some integrators are turned on, click the **Integrators** tab and select the integrators that you want to capture.
4. Click **OK** to apply your preferences and close the dialog.

More information

- Viewing cytometer details (page 130)
- Resizing plots (page 189)

Viewing cytometer details

Introduction

This topic describes how to open the Cytometer Details dialog to view information about the cytometer configuration.

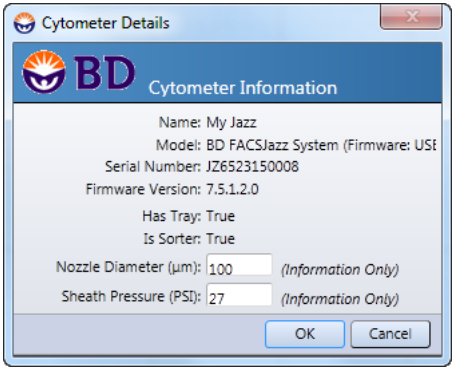
This information is also displayed on the sort report header.

Procedure

To open the Cytometer Details dialog:

1. Select **Cytometer > Details**.

   The Cytometer Details dialog opens.



   These values are for informational purposes only. The settings are displayed on the sort report.

2. Click **OK** to close the dialog.

More information

- Working with sort reports (page 340)

Using the Compensation pane

Introduction

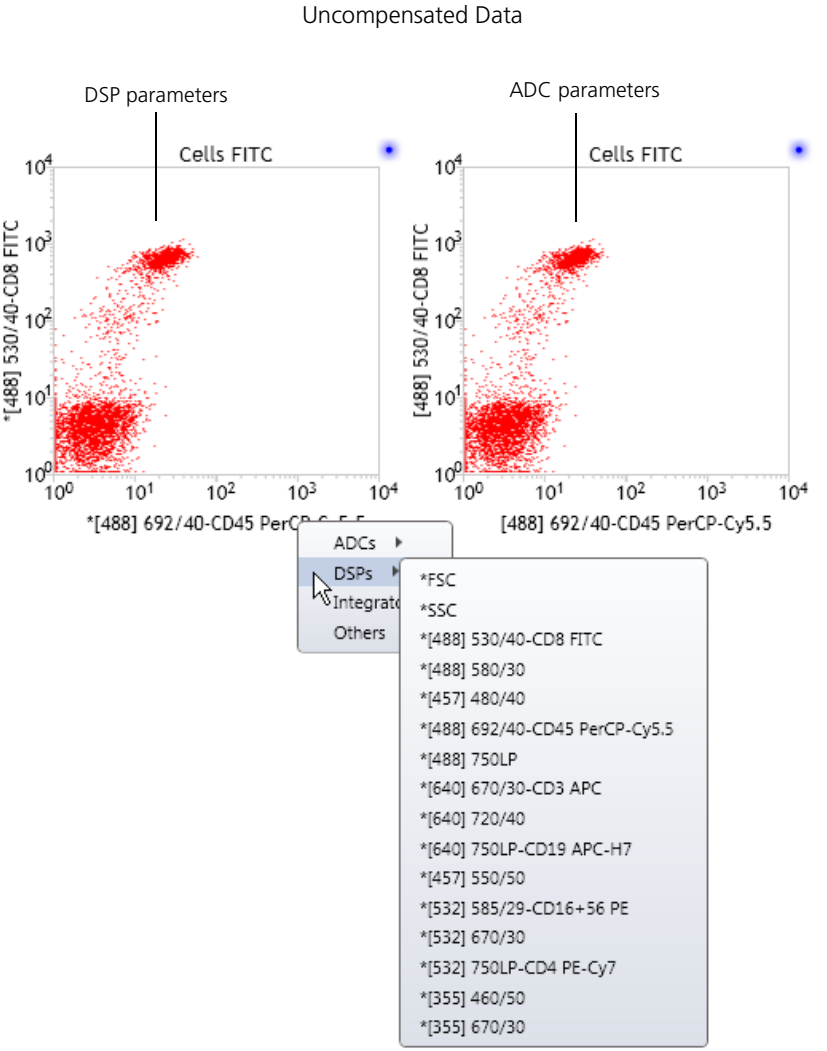
This topic describes the Compensation pane and related tabs and dialogs used to create a compensation matrix and calculate spillover values.

About compensation

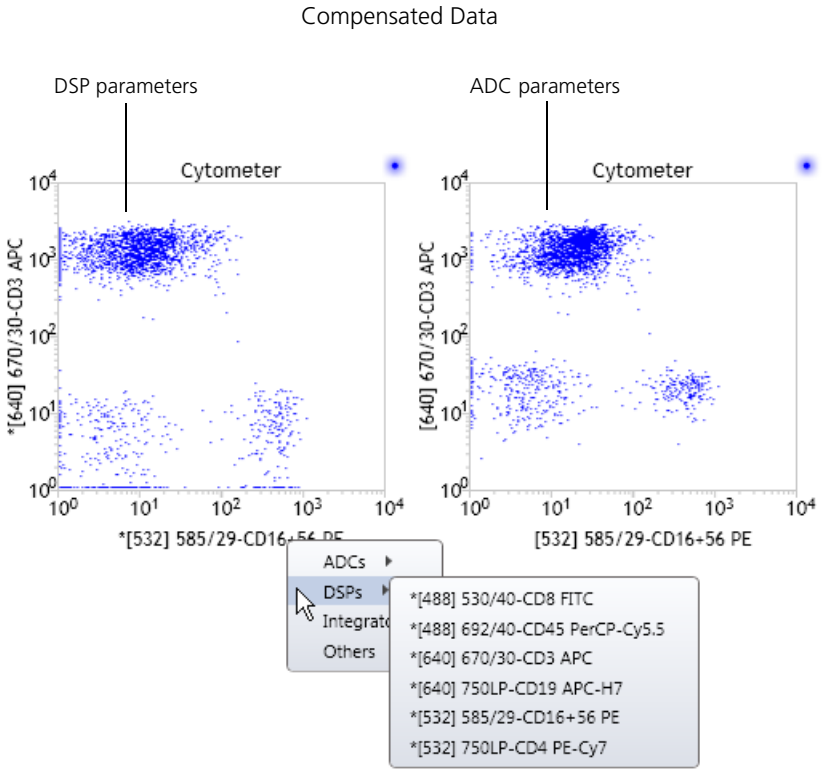
To create a compensation matrix and calculate compensated values, you need to collect single- or multicolor control data using ADCs, identify populations as positive or negative, and calculate the results to determine spillover values for each fluorochrome you plan to use. BD FACS Sortware uses the calculated values (matrix) and automatically applies compensation to minimize fluorescence spillover.

You can view your compensated data on ADC parameters, but to sort with these compensated values, you need to create sort gates using digital signal processors (DSPs) as your parameters. Altering or applying a new compensation matrix to previously recorded files will not change the compensated events in the DSP parameters (including files with no compensation matrix defined). However, you can view alternate compensation with ADC parameters.

If no compensation matrix was defined for a recorded file, all DSP parameters will be available to view (regardless of ADC capture status) and the DSP values for a given parameter will match the ADC values in the same parameter.



Once a compensation matrix is defined on the cytometer, DSP parameters in the subsequent recorded files will display only the compensation-defined parameters.

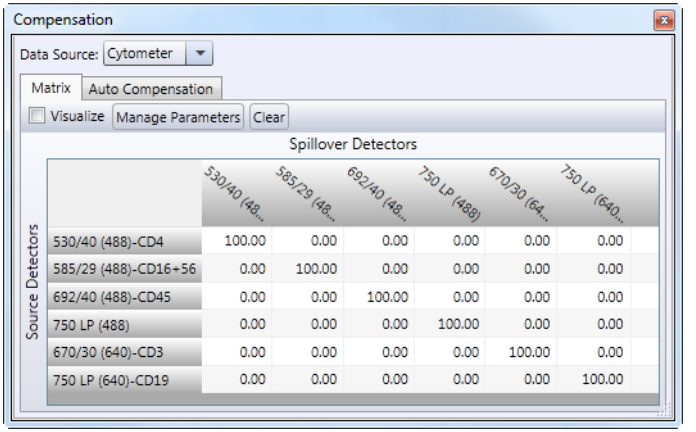


Creating a compensation matrix

To create a compensation matrix:

1. Click **Compensation** on the **BD FACS Sortware** toolbar.

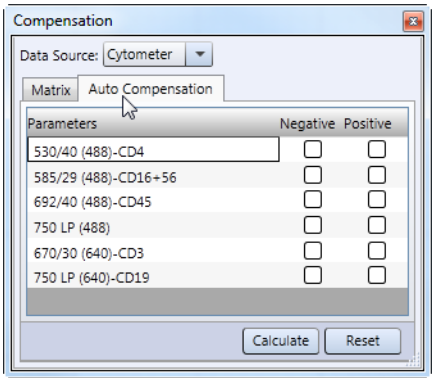
The compensation matrix displays the spillover values for each compensated parameter.



2. In the **Matrix** tab, set the following properties.

Properties	Description
Data Source	Select a data source for compensation.
Visualize	Select this checkbox to view the software compensation for ADCs in the plot.
Manage Parameters	Click to display the Select Compensation Parameters dialog.
Clear	Click to clear the values in the compensation matrix.
Values in the matrix	Manually adjust compensation values if needed.

3. In the **Auto Compensation** tab, set the following properties.



Properties	Description
Negative	Select and drag, or right-click and select the ADC parameters that correspond to a negative control. You can drag a universal negative population on top of the negative header to populate all negative boxes.
Positive	Select and drag, or right-click and select the ADC parameters that correspond to a positive control.
Calculate	Click to calculate compensation values after positive and negative populations have been assigned.
Reset	Clears all the selected populations.

More information

- BD FACS Sortware overview (page 86)

Using the Logicle Scaling pane

Introduction

This topic describes the Logicle Scaling pane.

Use the logicle scale after compensation has been applied to view any events with negative values. This is useful when evaluating the compensation matrix.

Description

After compensation, populations can include low mean values and negative values. Using a logarithmic scale, plots display a wide range of event data. However, they do not properly display events that have compensated low mean values, high variances, or negative values.

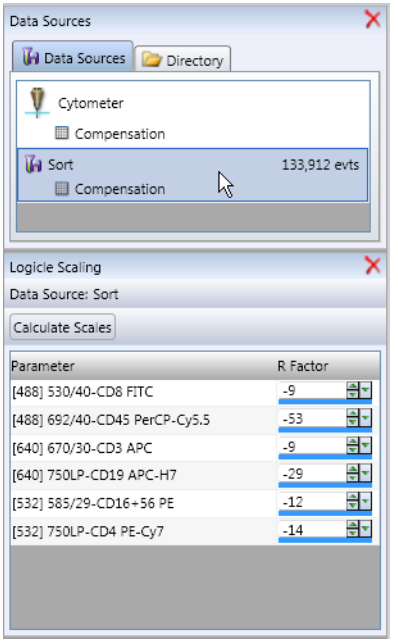
Logicle scaling is available only when viewing ADC-compensated data.

Procedure

To calculate logicle display for the data:

1. In the **Compensation** pane, select the data source that you want to view logicle scaling on (the data source must have a compensation matrix).
2. Select the **Visualize** checkbox.

3. In the **Data Sources** pane, select the data source that you want to perform logicle scaling on.

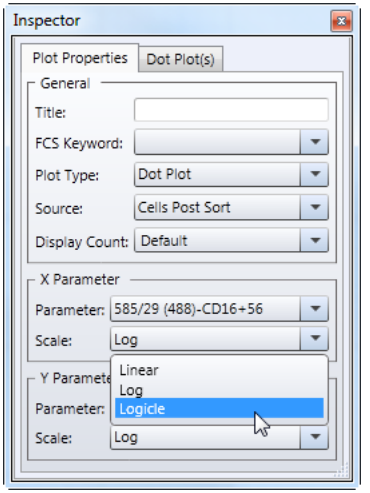


4. Click **Calculate Scales** to calculate the logicle display for the data.

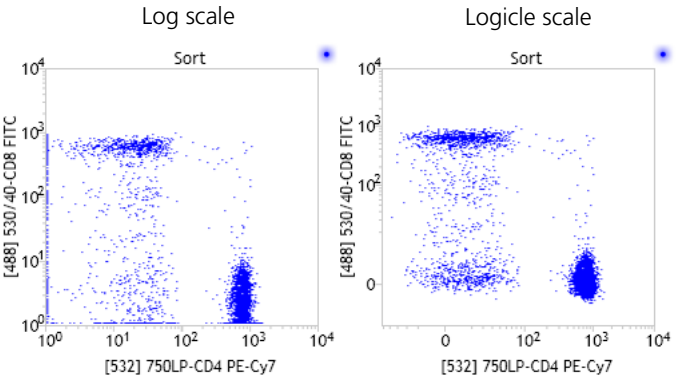
   You can adjust the R Factor (logical scale ratio) for each parameter to include negative or positive outliers in the plot to display data that conveys full and accurate information about the distributions of the events.

5. To view the logicle scale on a plot (which must be an ADC plot), select the plot and then open the **Inspector** pane.

6. Select **Logicle** from the **Scale** field under **X Parameter** or **Y Parameter.**



The following figure shows a plot in log scale and a plot in logicle scale.



More information

- Software overview (page 85)
- Setting numeric values in panes and dialogs (page 90)
- Using the Compensation pane (page 131)

10

Worksheets

This chapter includes these topics:

Worksheet overview

Introduction

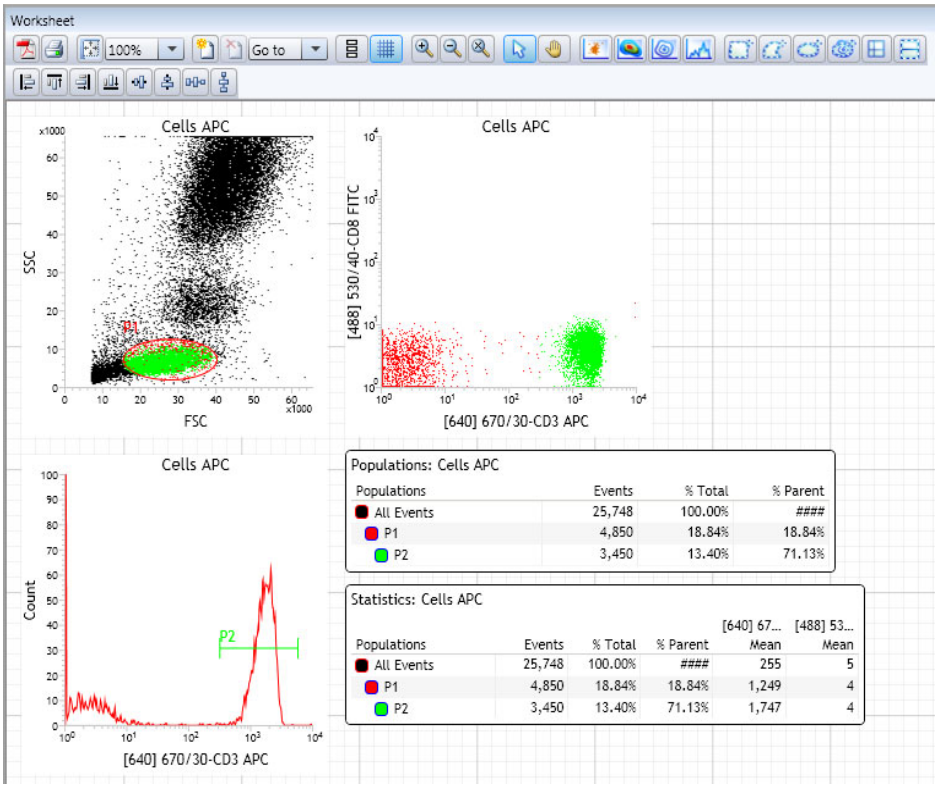
This topic provides an overview of worksheet elements, layouts, and how to manage multiple worksheets.

Description

A worksheet contains all analysis elements including plots, gates, and statistics views. A new (blank) worksheet opens each time you start BD FACS Sortware. You can save and restore a worksheet with its analysis elements as an analysis template.

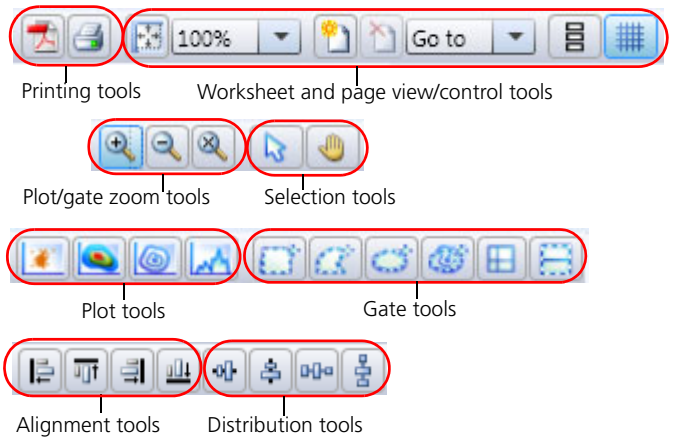
Worksheet layouts

A worksheet layout is created from a blank worksheet by adding plots, gates, population hierarchies, and statistics views that display your data.



Worksheet tools

The Worksheet toolbar provides tools for creating plots and gates, managing multiple worksheets, and organizing worksheet items.



See Creating plots in a worksheet (page 175) for more information about using worksheet tools to create plots. See Gating overview (page 196) for more information about using worksheet tools to create and modify gates.

Analysis templates

You can save and restore analysis templates, and then modify the plots, histograms, gates, and statistics views to suit your needs.

Worksheet pages

You can create multiple pages in each worksheet and modify the size, distribution, alignment, magnification, and properties details of all elements on each page of a worksheet.

Undo and redo functions

You can undo and redo most actions (for example, adding or deleting any worksheet elements or changing Inspector properties) when working with the worksheet.

To undo an action:

1. Select **Edit > Undo**.

To redo an action:

1. Select **Edit > Redo**.

You can perform multiple undos and redos.

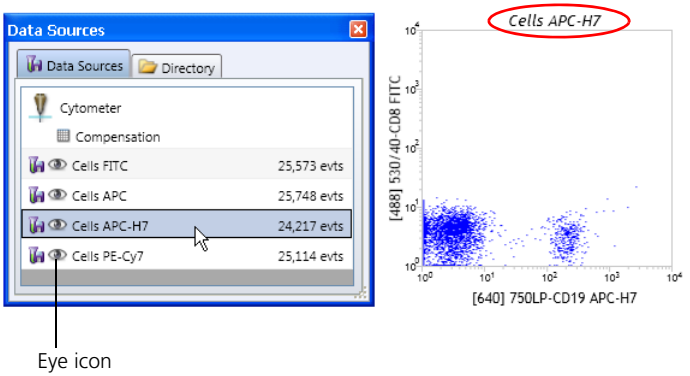
Defining a global worksheet

Individual worksheet elements (plots, hierarchies, and statistics views) are linked to one data source (cytometer for real-time data, or an FCS file). When you select Set Cytometer Plots/Views as Global in the Edit User Preferences dialog, all worksheet elements with cytometer as the data source display data from an FCS file. Use this preference to quickly view data from multiple FCS files.

To use cytometer plots/views as global worksheet elements:

1. Select **Edit** > **Preferences**.
2. Click the **Cytometer** tab.
3. Select the **Set Cytometer Plots/Views as Global** checkbox.
4. Create plots, hierarchies, or statistics view with **Cytometer** selected in the **Data Sources** pane.

   With this feature selected, the FCS files in the Data Sources pane will have an eye icon beside them if the files were collected using the same configuration as the cytometer data source. You can now select any FCS file with an eye icon and the data will be displayed in the worksheet.



Note that the title of the plot is italicized. This indicates that the Set Cytometer Plots/Views as Global option is selected.

If the Set Cytometer Plots/Views as Global is not selected, the plots are specific to the FCS file selected during plot creation.

To change the plots to the same FCS file:

1. Select all plots.
2. In the **Inspector** pane, select the FCS file from the **Source** field.

More information

- Creating a worksheet layout (page 144)
- Customizing worksheet properties (page 146)
- Aligning and distributing worklist items (page 149)
- Magnifying (scaling) analysis elements on a worksheet (page 150)
- Using the Inspector to view and modify plot properties (page 176)

Creating a worksheet layout

Introduction

This topic describes how to create a worksheet layout from a blank (default) worksheet.

A blank worksheet opens in the window each time you start BD FACS Sortware.

Creating a new worksheet layout

To create a new worksheet layout:

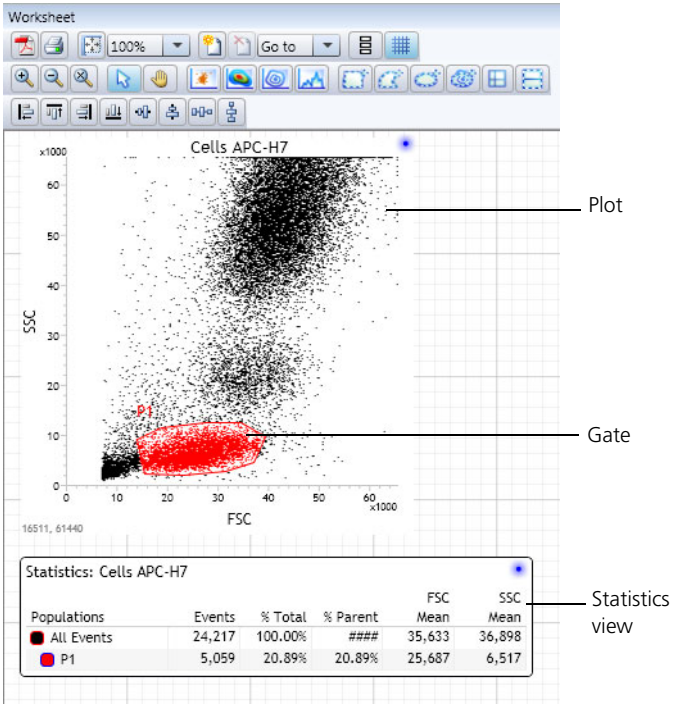
1. Add analysis elements to the worksheet:
   a. Click a plot tool on the **Worksheet** toolbar to add plots.

      You can add multiple plots to each worksheet.

   b. Click a gate tool on the **Worksheet** toolbar to add gates to the plots and define populations.

   c. Right-click the plot and select **Statistics View** to create a statistics view of the plot data.

d. Right-click the plot and select **Population Hierarchy** to create a population hierarchy of the plot data.



Adding pages to a worksheet

To add pages to a worksheet:

1. Click **Add Page** on the **Worksheet** toolbar.

The page is added after the last page in the worksheet.

Deleting pages from a worksheet

To delete pages from a worksheet:

1. In a multipage worksheet, scroll or click **Go** to locate the page you want to delete.
2. Right-click the page and select **Delete Page.**
3. Click **Delete Last Page** on the **Worksheet** toolbar to delete the last page from the worksheet.

Deleting analysis elements

To delete analysis elements (plots, gates, histograms, statistics views) from a worksheet:

1. Right-click an analysis element in the worksheet and select **Delete.**

   The analysis element is deleted. You can select multiple items at once to delete.

More information

- Creating plots in a worksheet (page 175)
- Gating overview (page 196)
- Displaying a statistics view (page 222)

Customizing worksheet properties

Introduction

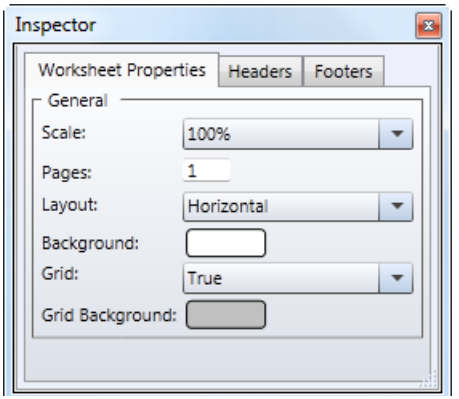
This topic describes how to customize worksheet properties.

Customizing worksheet properties using the Inspector or the Worksheet toolbar

To customize worksheet properties using the Inspector or the Worksheet toolbar:

1. Click on a blank area of the worksheet.
2. Click **Inspector** on the **BD FACS Sortware** toolbar.

The Inspector opens.



3. Under **General**, select a display scale from the **Scale** menu.

   The default is 100%.

4. In the **Pages** field, specify the number of pages in the worksheet.

5. Select a horizontal or vertical page layout from the **Layout** menu or use the worksheet toolbar.

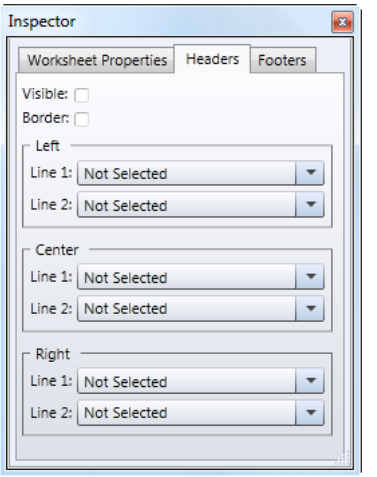
6. Select **True** from the **Grid** menu to display a grid on the worksheet pages or select **False** to display the worksheet pages without a grid.

   You can also use the toolbar menu to toggle the grid on or off.

Customizing worksheet headers and footers

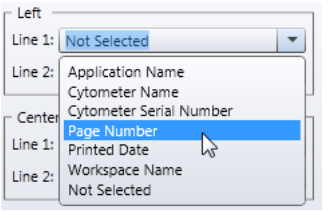
To customize worksheet headers and footers:

1. In the **Inspector** pane, click the **Headers** or **Footers** tab.



2. Select the header and footer properties.

   - Select the **Visible** checkbox to display a header or footer on the worksheet.
   - Select the **Border** checkbox to display a border around the header or footer.
   - Under **Left**, **Center**, and **Right**, select the elements you want to include in Line 1 and Line 2. You can also type in the field to enter information.



More information

- Worksheet overview (page 140)
- Aligning and distributing worklist items (page 149)

Aligning and distributing worklist items

Introduction

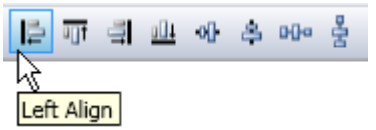
This topic describes how to align and distribute plots, histograms, statistics, and other items on a worksheet.

You can drag items to different locations within a worksheet.

Aligning items

To align items on a worksheet:

1. Click the item you want to use as the anchor item.
2. Ctrl+click the other items you want to align to the anchor item.
3. Click an alignment tool on the **Worksheet** toolbar (left, top, right, bottom, center horizontal, or center vertical).

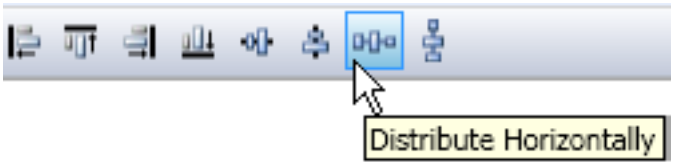


Distributing items

To evenly distribute items on a worksheet:

1. Click the item you want to use as the anchor item.
2. Ctrl+click the other items you want to distribute evenly around the anchor item.
3. Click a distribute tool on the **Worksheet** toolbar.

You can distribute selected objects horizontally, vertically, or both.



When distributing in both directions, the selected objects are evenly spaced. The objects are not resized.

More information

- Worksheets (page 139)
- Magnifying (scaling) analysis elements on a worksheet (page 150)

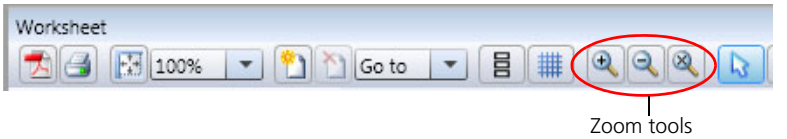
Magnifying (scaling) analysis elements on a worksheet

Introduction

This topic describes how to enlarge or shrink the size of analysis elements on the worksheet.

Zooming in and out

You can select an area within a plot and enlarge the image. You can zoom multiple times. Tick marks on the axes of the plot adjust to reflect the zoomed view.



1. To zoom in or out:
2. Complete one of the actions in the following table.

   You must have the plot selected to use the zoom out or clear buttons.

To...	Do this...
Zoom in on an area in a plot	1. Click **Zoom In** on the **Worksheet** toolbar. 2. Select an area in the plot that you want to zoom in on using the mouse.
Zoom out	1. Click **Zoom Out** on the **Worksheet** toolbar.
Clear the zoom	1. Click **Clear Zoom** on the **Worksheet** toolbar.

More information

- Worksheet overview (page 140)
- Creating a worksheet layout (page 144)
- Resizing plots (page 189)

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11

Acquisition and recording tools

This software reference chapter describes the tools used to select a data source, set the data recording details, acquire, and record.

This chapter includes these topics:

Other related information:

Using the Data Sources pane

Introduction

This topic describes how to use the Data Sources pane to view data and information from the cytometer or recorded data sources (FCS files).

The Data Sources pane also allows you to browse and import FCS files.

About the Data Sources pane

From the Data Sources pane, you can double-click the data source and open the following:

- FCS keyword browser
- Compensation matrix
- Index sort analysis

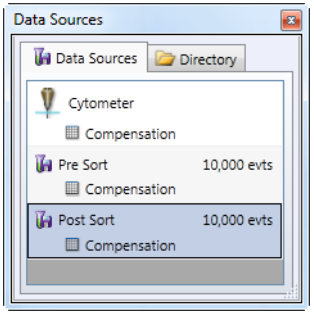
You can also right-click the data source in the Data Sources pane to:

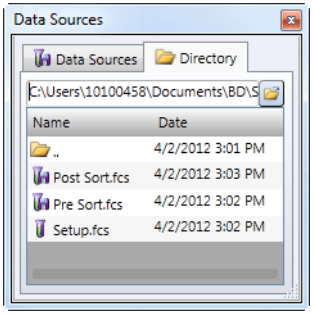
- Create sort gates, which are gates that identify events that were or are being sorted
- Save and restore compensation
- Export BD FACSDiva™–compatible software files
- Export FCS files in comma separated value (CSV) format
- Remove (delete) the data source from the Data Sources pane

Data sources tabs

This pane has two tabs:

- **Data Sources.** This tab displays a cytometer (real-time) source and the current FCS (recorded) file or other saved FCS files that you add to the list. BD FACS Sortware supports FCS v3.0 format.



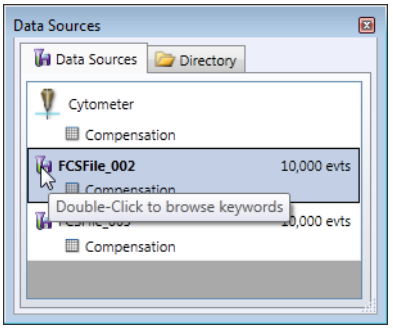


- **Directory.** This tab displays the directory you can use to locate recorded FCS data files and to select FCS files to add to the Data Sources list.

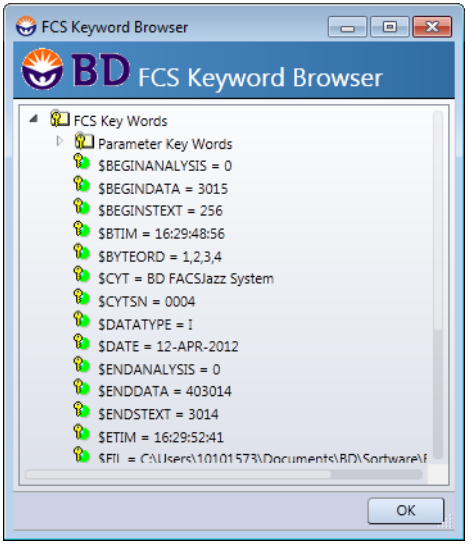
Viewing cytometer and FCS file information

To open the FCS keyword browser:

1. In the **Data Sources** tab, double-click the tube icon or FCS file.

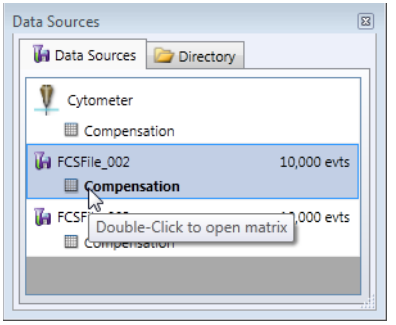


The FCS Keyword Browser opens.

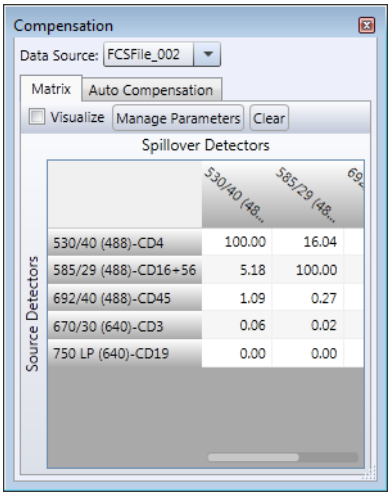


To open the compensation matrix:

1. In the **Data Sources** tab, double-click **Compensation** beneath the FCS file.

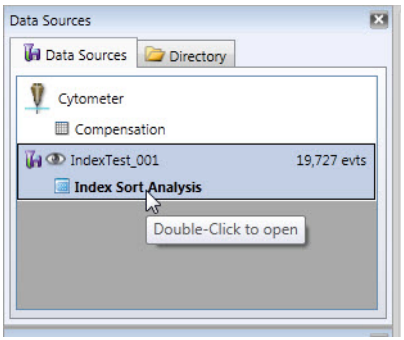


The compensation matrix opens.

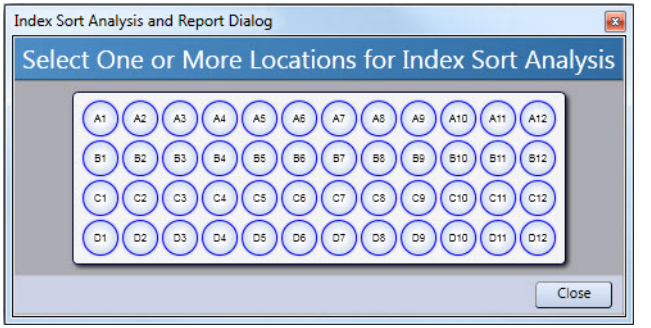


To open the Index Sort Analysis and Report dialog:

1. In the **Data Sources** tab, double-click the **Index Sort Analysis** beneath the FCS file.



The Index Sort Analysis and Report dialog opens.



Selecting, importing, and deleting FCS files in the Data Sources pane

When you first open the software or restore a settings file, Cytometer is selected as the default data source. At any time, one data source is always selected in the Data Sources pane.

To select an FCS file as the data source:

1. Click an FCS file in the list to display collected (recorded) data for analysis.

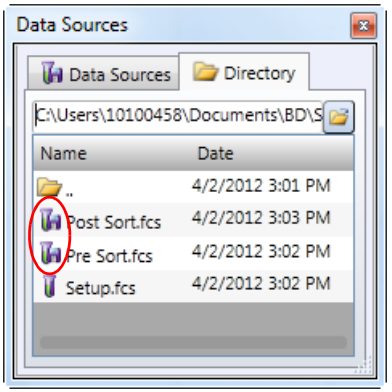
   FCS files only appear in the list when you record acquired data or when you import an FCS file.

Importing FCS files as data sources

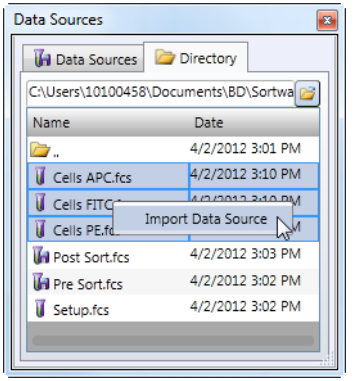
To import FCS files as data sources:

1. If the FCS file you want to use is not in the list, click the **Directory** tab and navigate to the folder that contains your FCS files.
2. Double-click the file you want to add to the **Data Sources** tab.

A disk icon appears next to the FCS files that were added to the list.



3. To import multiple FCS files at the same time, select the files, right-click, and then select **Import Data Source**.



Removing an FCS file from the Data Sources tab

To remove an FCS file from the Data Sources tab:

1. Right-click the file name and select **Remove**.

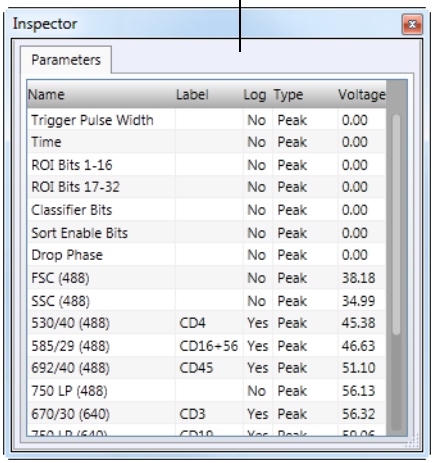
Viewing data source details

To view data source details using the Inspector:

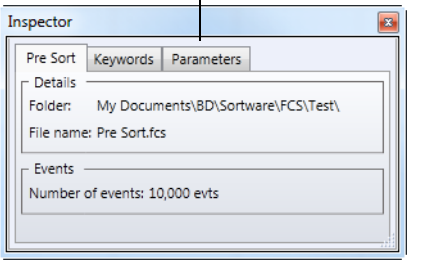
1. Click **Inspector** on the **BD FACS Sortware** toolbar.
2. Select the cytometer or an FCS data source in the **Data Sources** pane.

   The Inspector displays the data source details.

Details for the Cytometer data source



Details for an FCS file data source



Cytometer data sources details display the name, label, scale, data type, and voltage values for all current parameters for this cytometer.

FCS data source details display the name, location, and number of recorded events, keywords, keyword values, and the parameter settings. The parameter settings are the settings used during recording.

If you want to change the recording preferences or keywords before you record an FCS file, you need to use the Recording Settings pane.

Exporting FCS files

FCS files can be exported as BD FACSDiva–compatible files or as CSV files.

To export FCS files compatible with BD FACSDiva software:

1. In the **Data Sources** tab, right-click the FCS file you want to export.
2. Select **Export Diva Compatible FCS File**.

   The Save Diva Compatible FCS dialog opens.
3. Navigate to the target export folder, then click **Save**.

To export FCS files in CSV file format:

1. In the **Data Sources** tab, right-click the FCS file you want to export.
2. Select **Export FCS File in CSV format**.

   The Save CSV File dialog opens.
3. Navigate to the target export folder, then click **Save**.

More information

- Using the Recording Settings pane (page 162)
- Using the Acquisition Dashboard (page 166)

Using the Recording Settings pane

Introduction

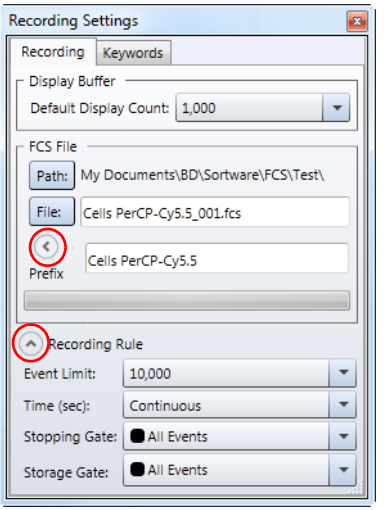
This topic describes the Recording Settings pane and how to set recording properties.

Description

Use the Recording Settings pane to set specific recording and keyword properties before you record data to set details about the FCS file that is generated during recording.

This pane has two tabs. Use the Recording tab to set the default display count (total events to display), name the FCS file, and set the default location for the FCS files. Use the Keywords tab to define values for keywords in the FCS file.

Certain elements of the Recording Settings pane can be minimized. Click the arrow button to hide or show these elements.



Setting recording properties

To set recording properties:

1. Click **Recording** on the **BD FACS Sortware** toolbar.

   The Recording Settings pane opens.

2. Click the **Recording** tab.

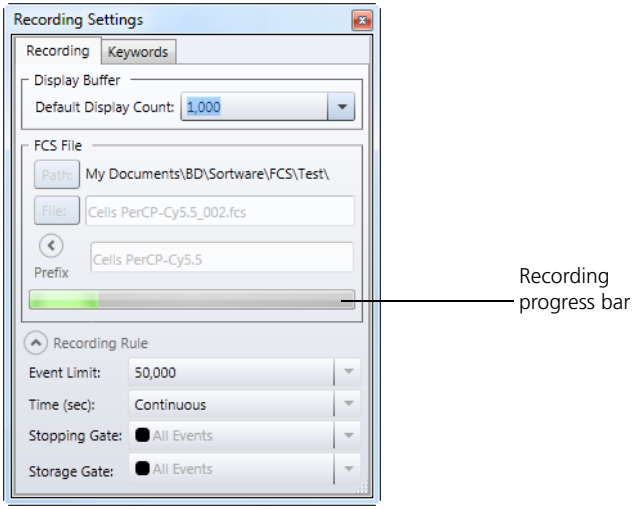
3. Under **Display Buffer**, select an event display count from the **Default Display Count** menu.

   The default display count determines the number of events to display in cytometer plots. You can modify the display count for individual plots in the Plot Properties Inspector. However, the default display count setting determines the maximum display count that can be selected (up to 1,000,000 events). The default display count does not apply to plots made with existing FCS files.

4. Under **FCS File**, set the following properties for the FCS file that is generated when you record data.

Property	Description
Path	Click **Path** to select a storage location for the generated FCS file.
File	Click **File** to view which FCS files have been recorded or to select an existing FCS file to append or overwrite.
File field	Enter a file name for an FCS file.
Prefix	Enter a prefix for the FCS files (for example, Presort).

The recording progress bar appears at the bottom of the pane and displays the current recording progress.



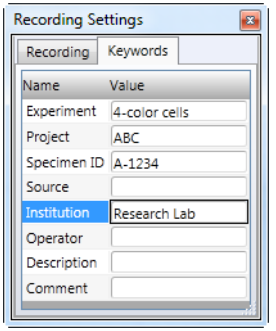
5. Under **Recording Rule**, set the following properties.

Property	Description
Event Limit	Select the event limit (total events collected) as a stopping rule.
Time (sec)	Select the time-based stopping rule.
Stopping Gate	Select an available gate as a stopping gate. This indicates which gate to use to fulfill the event limit or time stopping rules.
Storage Gate	Select an available gate as the storage gate. Indicates which gated data is saved in the FCS data file.

Setting keyword values

To set keyword values that appear in the generated FCS file:

1. Click the **Keywords** tab.
2. Enter a value for each keyword name you want to include in the generated FCS file.



More information

- Software overview (page 85)
- Using the Acquisition Dashboard (page 166)
- Viewing keywords with plots (page 193)

Using the Acquisition Dashboard

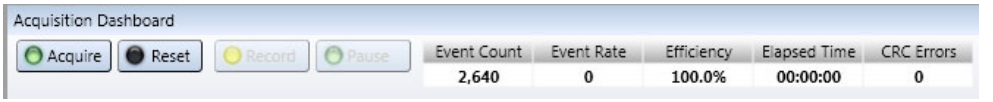
Introduction

This topic describes the Acquisition Dashboard and what data it displays.

Description

Use the Acquisition Dashboard to start or stop acquisition, record events, and monitor data acquisition details. The following figures show the controls and how they toggle between idle, acquisition, and recording modes.

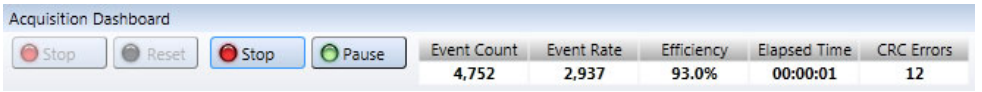
Idle



During acquisition

Acquisition controls

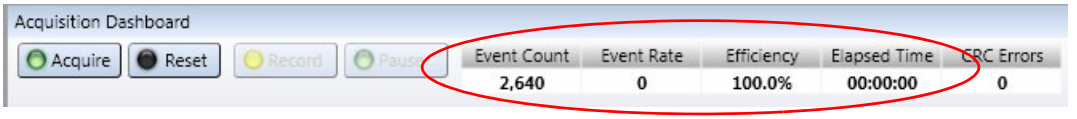
During recording the Acquisition Dashboard has the following controls.



Control	Description
Acquire	Starts acquisition and populates plots with data. During acquisition this button becomes the Stop button.
Stop	Stops the current acquisition or recording.
Reset	Clears the current acquisition data and status display. You can also press the F5 key to reset when acquiring with the cytometer selected in the data source.
Record	Records the current acquisition data in an FCS file. After you record data, the FCS file appears in the Data Sources pane. This button is available only after you click Acquire.
Pause	Pauses the current acquisition or recording. This button is available only during acquisition or recording.
Resume	Resumes acquisition or recording after pausing.

Status display

The Acquisition Dashboard counters are located to the right of or below the acquisition buttons.



The counters display the following acquisition status.

Status	Description
Event Count	Displays the total event count.
Event Rate	Displays the current event rate.
Efficiency	Displays the efficiency (accuracy) percentage of the acquisition: (total events – electronic aborts)/total events x 100.
Elapsed Time	Displays the total elapsed time for acquisition.
CRC Errors	Indicates cyclic redundancy check (CRC) errors. CRC notes any errors that occur during the data transmission process.

More information

- Using the Recording Settings pane (page 162)
- Sorting (page 319)

12

Plots

This chapter includes these topics:

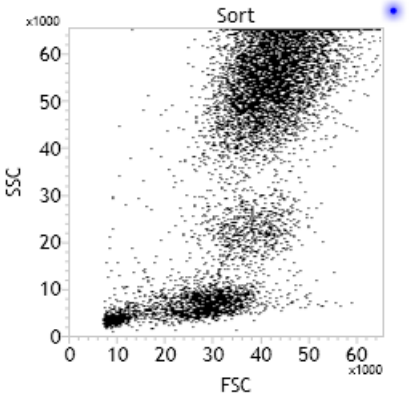
Plot overview

Introduction

This topic describes the types of plots you can create and the color display options.

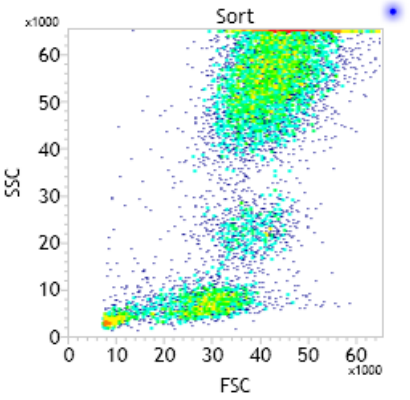
Dot plots

Dot plots display two-parameter data. Each dot in a dot plot represents one or more events. The dot location is defined by two values—one for each parameter.



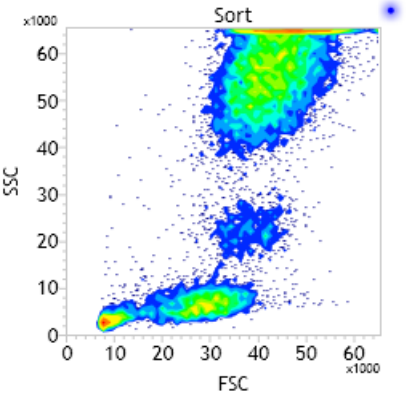
Density plots

Density plots display simulated three-dimensional events. They are similar to dot plots except that they use different colors to show the number of events. The position of each event on the X and Y axes reflects its parameter values. The color shows how many events fall at each position.



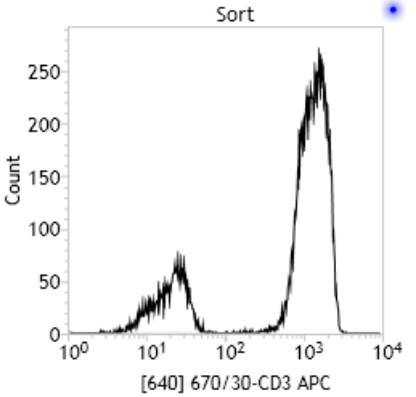
Contour plots

Contour plots are graphical representations of two-parameter data in which contour lines show the distribution of events. Similar to a topographical map, contour lines show event frequencies as peaks and valleys.



Histograms

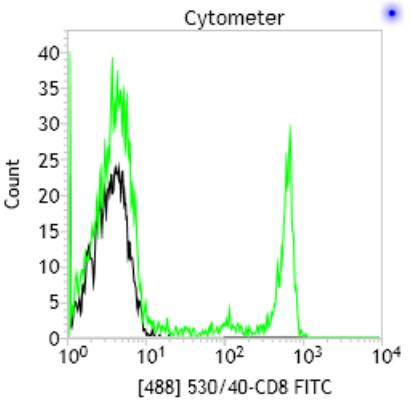
Histograms are graphical representations of a single parameter of data. The horizontal axis of the graph represents the signal intensity of the parameter, and the vertical axis represents the number of events (counts) or percentage of events.



Plot overlays

Plot overlays display gated populations or All Events of two or more FCS files (or the cytometer) as histogram layers. This allows direct comparison of events. Plot overlays are available only with histograms.

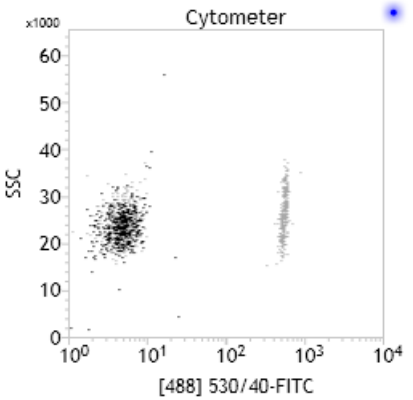
The following example shows a stained sample from a recorded FCS file (green) layered on an unstained sample from the cytometer (black).



Cytometer plot with a target source

You can add a target source to a cytometer plot to compare live data to a specific target FCS file with the same parameters and sample type. The targets can provide an acceptable range of data or specific event clusters that you want to approximate with live data. The target source appears in gray as an overlay (bottom layer) in the plot. This is particularly useful for quality control (QC). Target sources are available only with cytometer dot plots.

The following figure shows live (black) events on top of FCS (gray) target events.



Dot plot and histogram color display

Dot plots display all events as black dots by default. Gated populations in the dot plot appear in different colors. You can modify the color of the events in the Inspector pane or select different default colors in the Edit User Preferences dialog.

Histograms display all events as black lines by default. You can modify the color of the events and overlay events in the Histogram properties tab of the Inspector pane.

Density and contour plot color display

Density and contour plots use the following color type options:

- **Single color.** The lowest level is the original population color and the colors fade toward white as levels increase.
- **Multiple colors.** The color shows how many events fall at each position by using a different color for each level. Colors range from dark blue (representing the lowest number of events) through the spectrum to red (representing the highest number of events).
- **Gray scale.** Displays darker gray at lower levels and fades to white as the levels rise.

More information

- Creating plots in a worksheet (page 175)
- Modifying dot plot properties (page 180)
- Modifying contour plot properties (page 183)
- Creating histogram overlays (page 186)
- Modifying density plot properties (page 181)

Creating plots in a worksheet

Introduction

This topic describes how to create plots using the plot tools on the Worksheet toolbar. All plot types use the same procedure.

Procedure

To create plots:

1. Open a worksheet or restore an analysis template.
2. Click a plot tool on the **Worksheet** toolbar.

3. Add the plot to the worksheet by doing one of the following:
   - To insert a new plot using the default size, click on the worksheet.
   - To draw a plot of any size, drag on the worksheet.
   - To create many plots quickly on a worksheet, double-click the plot tool on the toolbar, then click on the worksheet where you want to add each plot. To unstick the plot tool, click a different worksheet tool.

More information

- Creating a worksheet layout (page 144)
- Plot overview (page 170)
- Modifying dot plot properties (page 180)

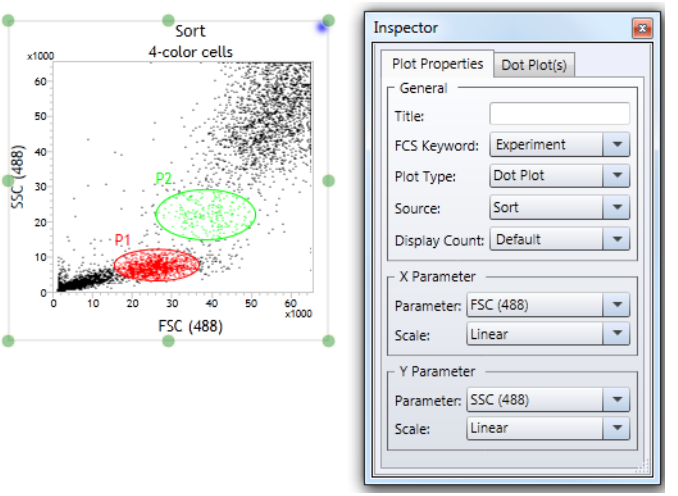
Using the Inspector to view and modify plot properties

Introduction

This topic describes how to use the Inspector to view and modify plot and parameter properties after you create a plot.

Description

You can click a plot on a worksheet to display plot properties in the Inspector pane. Use the Plot Properties tab to modify the plot title, displayed keyword, type, source, display count, and X/Y parameters and scales.



Guidelines

- If you select Cytometer as the data source, you can manually assign x- and y- axis parameters to plots from a list of available parameters.
- If you select an FCS file as the data source, the parameter names come from the FCS file, and labels are not applied unless they are in the FCS file.
- If you select multiple plots on a worksheet, the parameter list includes the common parameter names and labels for each selected plot.

Modifying plot and parameter properties

To modify plot and parameter properties:

1. Click **Inspector** on the **BD FACS Sortware** toolbar.

   The Inspector opens.

2. Click a plot on the worksheet.

3. In the **Inspector,** click the **Plot Properties** tab.

4. Set the following properties under **General** as needed.

Property	Action
Title	Enter a name for the plot. If this field is blank, the title defaults to the FCS file name.
FCS Keyword	Select a keyword to display under the plot title. Keywords will display on the plot only if a keyword was recorded. Only one keyword can be selected.
Plot Type	Select a different plot type (for example, dot, density, or contour).
Source	Select Cytometer or an FCS file data source for the plot.
Display Count	Select the maximum number of events to display. The default for cytometer plots is the default display count in the Recording Settings pane. The default for FCS file plots is the total number of events in the file.

5. Set the following properties under **X Parameter** as needed.

Property	Action
Parameter	Select an x-axis parameter for the plot.
Scale	Select linear, log, or logicle (biexponential) scale. Different parameters will have different scales available to them.

6. Set the following properties under **Y Parameter** as needed.

Property	Action
Parameter	Select a y-axis parameter for this plot.
Scale	Select linear, log, or logicle (biexponential) scale. Different parameters will have different scales available to them.

Modifying plot axis parameters

Introduction

This topic describes how to modify the axis parameters for existing plots.

Modifying plot axis parameters in a plot

To modify the plot axis parameters in a plot:

1. In a worksheet, right-click on an x- or y- parameter label in a plot and select a parameter subgroup from the menu.
2. Select a parameter.

Parameter type	Description
ADC	Includes all available detectors for raw data collection.
DSP	Includes all available detectors for compensated data collection (must be used for compensated data when sorting).

Parameter type	Description
Integrators (optional)	The Integrators subgroup is available only if the system has the integrators option installed and integrators are turned on for real-time data (or integrators were recorded for FCS files). Integrators are available for FSC, SSC, and any ADC parameter. • Measures the area. • Measures pulse width at threshold.
Others	• **Trigger Pulse Width.** Signal width of the pulse above the threshold. • **Time.** Time stamp that occurs every 100 ms. • **ROI Bits 1-16.** Bits 1–16 of the 32-bit field. Computed results from lookup tables, one bit per ROI (high = in, low = out). • **ROI Bits 17-32.** Bits 17–32 of the 32-bit field. • **Classifier Bits.** Result from classification hardware based on lookup tables, one bit per sort direction. • **Sort Enable Bits.** Includes information about sort decisions and counters associated with a sort direction. • **Drop Phase.** Measured location of an event center within the drop phase and distances out to 4 drops to the nearest events before and after, in 1/16 of a drop.

More information

- Setting plot display preferences (page 107)
- Viewing the cytometer status (page 119)
- Adjusting PMT voltages and using optional integrators (page 125)
- Creating plots in a worksheet (page 175)
- Modifying dot plot properties (page 180)
- Resizing plots (page 189)
- Integrators (page 368)

Modifying dot plot properties

Introduction

This topic describes how to modify dot plot properties using the Inspector pane.

You can also modify the plot display preferences using the Edit User Preferences dialog.

Procedure

To modify dot plot display properties:

1. In the **Inspector**, click the **Dot Plot(s)** tab.
2. Set the following properties under **General** as needed.

Property	Action
Dot Size	Select a small, medium, or large dot size to represent events in the plot.
Target Source	Select an available FCS file as the target source. See Plot overview (page 170) for more information about plots using a target source.

More information

- Setting plot display preferences (page 107)
- Setting statistics view display preferences (page 108)

Modifying density plot properties

Introduction

This topic describes how to modify density plots using the Inspector pane.

You can also modify the plot display preferences using the Edit User Preferences dialog.

Modifying density plot display properties

To modify density plot display properties:

1. In the **Inspector**, click the **Density Plot(s)** tab.
2. Set the following properties under **General** as needed.

Property	Action
Dot Size	Select a small, medium, or large dot size to represent events in the plot.
Color Type	Select a color scheme for the event data.
Percentage	Type a percentage or use the data slider. This value determines the peak height and spacing between density levels.
Scale Mode	Select a linear, probability density, or logarithmic scale.
Show Density Lines	Select **True** to outline the density area or select **False** for no outlines.
Show Outliers	Select **True** to show outliers (events that are below the lowest level) or select **False** to hide outliers.

3. Set the following properties under **Resolution** as needed.

Property	Action
Analysis	Select a display resolution for analysis.
Acquisition	Select a display resolution for acquisition.

4. Set the following properties under **Smoother** as needed.

Property	Action
Is Smooth	Select **True** to enable smoothing or select **False** to disable smoothing.
Smooth Edges	Select **True** to enable edge smoothing or select **False** to disable edge smoothing.
Kernel Size	Select a kernel size of 3, 5, or 7.
Smoothing	Select a smoothing level.

The density plot is drawn without smoothed data by default. Smoothing data does not affect the calculation of statistics or the display of outliers.

More information

- Setting plot display preferences (page 107)
- Setting statistics view display preferences (page 108)

Modifying contour plot properties

Introduction

This topic describes how to modify contour plots using the Inspector pane.

You can also modify the plot display preferences using the Edit User Preferences dialog.

Modifying contour plot display properties

To modify contour plot display properties:

1. In the **Inspector**, click the **Contour Plot(s)** tab.
2. Set the following properties under **General** as needed.

Property	Action
Dot Size	Select a small, medium, or large dot size to represent events in the plot.
Color Type	Select a color scheme for the event data.
Percentage	Type a percentage or use the data slider. This value determines the peak height and spacing between contour levels.
Scale Mode	Select a linear, probability density, or logarithmic scale.
Fill Contour	Select **True** to fill the contour area or select **False** to leave the contour area empty.
Show Contour Lines	Select **True** to outline the contour area or select **False** for no outlines.
Contour Lines Colored	Select **True** to color the contour area or select **False** for no color.
Show Outliers	Select **True** to show outliers (events that are below the lowest level) or select **False** to hide outliers.

3. Set the following properties under **Resolution** as needed.

Property	Action
Analysis	Select a display resolution for analysis.
Acquisition	Select a display resolution for acquisition.

4. Set the following properties under **Smoother** as needed.

Property	Action
Is Smooth	Select **True** to enable smoothing or select **False** to disable smoothing.
Smooth Edges	Select **True** to enable edge smoothing or select **False** to disable edge smoothing.
Kernel Size	Select a kernel size of 3, 5, or 7.
Smoothing	Select a smoothing level.

The contour plot is drawn without smoothed data by default. Smoothing data does not affect the calculation of statistics or the display of outliers.

More information

- Setting plot display preferences (page 107)
- Setting statistics view display preferences (page 108)

Modifying histogram properties

Introduction

This topic describes how to modify histograms using the Inspector pane.

You can also modify the plot display preferences using the Edit User Preferences dialog.

Modifying histogram display properties

To modify histogram display properties:

1. In the **Inspector**, click the **Histogram Plot(s)** tab.
2. Set the following properties under **General** as needed.

Property	Action
Draw Curve	Select **True** to draw curves between data points on the histogram or select **False** to draw vertical bars of data.
Fill Histogram	Select **True** to fill the histogram area or select **False** to leave the histogram area empty.
Y Axis Scale	Select linear, logarithmic, percent, or normalized percent.
Y Axis Max	Select or type in a y-axis maximum value or select **Calculated** for an auto-calculated scaling.
Overlays	Select **True** to enable an overlay or **False** to disable an overlay.

3. Set the following properties under **Resolution** as needed.

Property	Action
Analysis	Select a display resolution for analysis.
Acquisition	Select a display resolution for acquisition.

4. Set the following properties under **Smoother** as needed.

Property	Action
Is Smooth	Select **True** to enable smoothing or select **False** to disable smoothing.
Smooth Edges	Select **True** to enable edge smoothing or select **False** to disable edge smoothing.
Kernel Size	Select a kernel size of 3, 5, or 7.
Smoothing	Select a smoothing level.

The histogram is drawn without smoothed data by default. Smoothing data does not affect the calculation of statistics or the display of outliers.

More information

- Setting plot display preferences (page 107)

Creating histogram overlays

Introduction

This topic describes how to create overlays on a histogram using the Inspector pane.

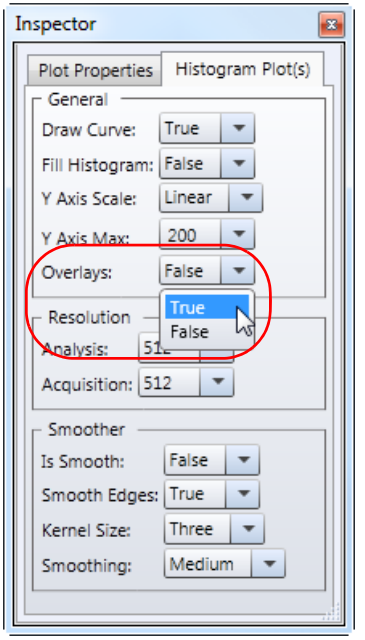
Before you begin

- Create a histogram on a worksheet and open the Inspector pane.
- If you want to make an overlay that includes the cytometer data, the original histogram needs to have the cytometer as its data source.
- You need to have at least one FCS file in the Data Sources pane to create an overlay.

Procedure

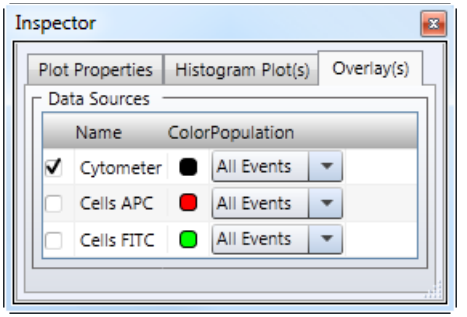
To create overlays:

1. Click a histogram on the worksheet.
2. In the **Inspector** pane, click the **Histogram Plot(s)** tab.
3. From the **Overlays** menu, select **True**.

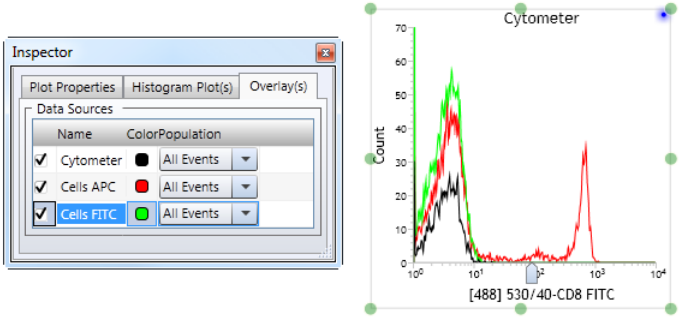


The **Overlays** tab appears.

4. Click the **Overlays** tab and select the checkboxes for the FCS files to overlay.



5. Select the population that you want to overlay and the color of each overlay.



More information

- Creating plots in a worksheet (page 175)
- Resizing plots (page 189)

Resizing plots

Introduction

This topic describes how to resize plots.

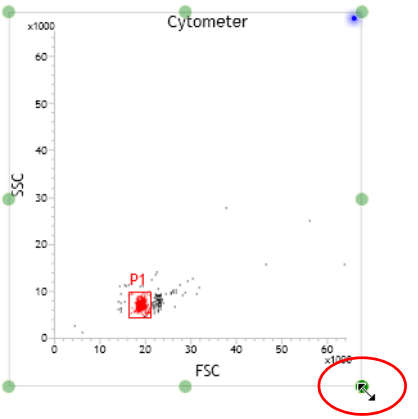
You can manually resize plots and plot events to any size within the worksheet, or expand, collapse, or reshape them based on predefined increments.

Manually resizing a plot

To manually resize a plot:

1. Click on a plot in the worksheet.

   The plot frame and sizing handles are enabled.



2. Drag a handle to size the plot by doing one of the following:

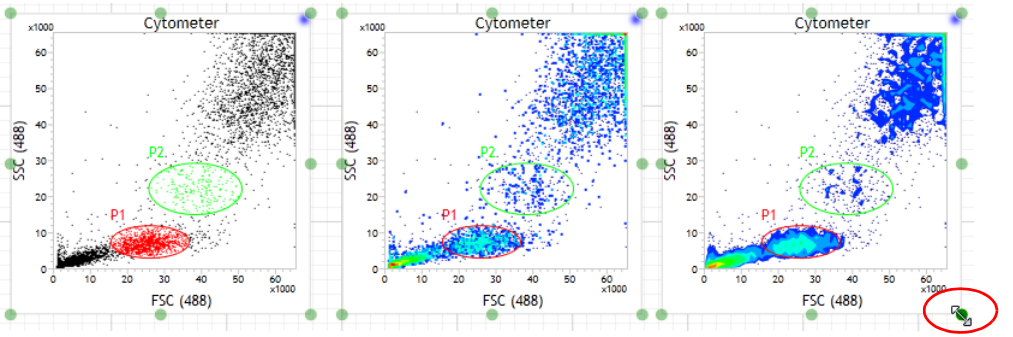
   - To proportionally size the plot, drag a corner handle in or out.
   - To stretch the plot in a specific direction, drag a top, bottom, left, or right handle.

Proportionally resizing multiple plots

To proportionally resize multiple plots (simultaneously):

1. Click on the first plot you want to resize.
2. Ctrl+click on all other plots you want to resize.

   The plot frames and sizing handles are enabled.



3. Drag a handle to size the plots by doing one of the following:
   - To proportionally size the plot, drag a corner handle in or out.
   - To stretch the plot in a specific direction, drag a top, bottom, left, or right handle.

Additional ways to resize a plot

The following table describes additional ways of resizing a plot.

Method	Procedure
To make a plot square (reshape a modified plot)	Right-click a plot and select **Resize Plot > Make Square.**
To expand a single plot by predefined increments	Right-click the plot and select **Resize Plot > Expand.**
To contract (collapse) a single plot by predefined increments	Right-click a plot and select **Resize Plot > Contract.**

More information

- Magnifying (scaling) analysis elements on a worksheet (page 150)

Saving plots as images

Introduction

This topic describes how to save a plot as a PNG image file.

Procedure

To save a plot as an image file:

1. In a worksheet, right-click a plot and select **Save As**.

   The Save as PNG image dialog opens.

2. Navigate to a target folder.
3. In the **File name** field, type a name for the image.
4. Click **Save**.

More information

- Copying and duplicating plots (page 191)

Copying and duplicating plots

Introduction

This topic describes how to copy and paste plots into third-party software as an editable metafile, and how to duplicate plots within a worksheet.

About copying and pasting plots

You can copy plots and associated metadata from a worksheet and paste them into third-party software (for example, Microsoft Word, PowerPoint®). After you paste the plot, you can modify the plot name, axis labels, font, graphic border colors and thickness, and delete elements from the image.

The following example describes how to copy and paste into a Microsoft PowerPoint slide.

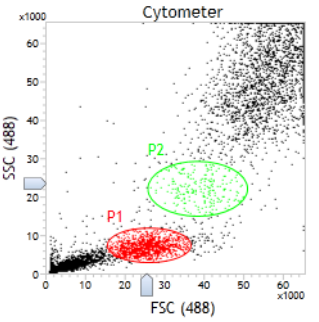
Copying and pasting plots into third-party software

To copy and paste a plot and the associated metadata into third-party software:

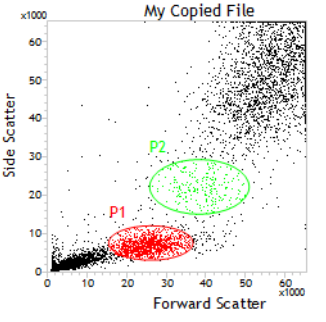
1. Right-click a plot and select **Copy**.
2. Open Microsoft PowerPoint and click on the target slide.
3. Select **Edit > Paste Special > Picture (Enhanced Metafile)**.
4. Click **OK**.

   The plot appears on the PowerPoint slide. The image pastes as a single, grouped object. If you want to modify the image, right-click the image and select **Grouping > Ungroup.** Once the element you want to modify is ungrouped, you can customize the image.

Copied plot before modifications



Copied plot after ungrouping and modifications



Duplicating plots

To create an exact duplicate of a plot within a worksheet:

1. Right-click the plot and select **Duplicate**.

   A duplicate plot appears.

More information

- Setting plot display preferences (page 107)

Viewing keywords with plots

Introduction

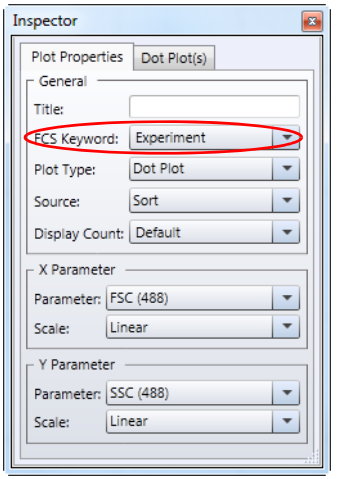
This topic describes how to view keywords with plots.

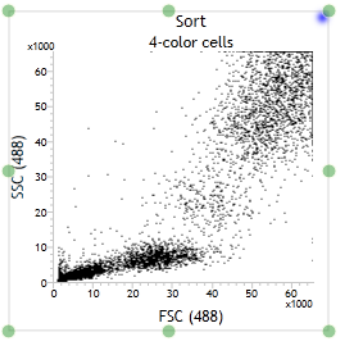
Viewing keywords with plots

To view keywords with plots:

1. Save an FCS file with at least one keyword.
2. Select the FCS file in the **Data Sources** pane and create plots if required.
3. Select the plots.
4. Select the FCS keyword from the **FCS Keyword** menu in the **Inspector** pane.

   You can select only one keyword to view in the plot title.





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13

Gates and populations

This chapter includes these topics:

Gating overview

Introduction

This topic describes the gating tools you can use to create gates and define populations.

Gating allows you to identify events of interest, classify events in populations, display them in plots, and calculate population statistics for display as statistics views. Gates are organized in a gate hierarchy based on parent and child (subpopulation) relationships.

Types of gates

You can draw the following types of gates on a plot:

- Rectangle
- Polygon
- Ellipse
- Contour
- Quadrant (Quad)
- Interval

About drawing gates

- The minimum and maximum size of a gate is determined by the size of the plot.
- You cannot drag the vertex outside the plot.
- All gate types can be used for sorting.

Gate and population hierarchies and statistics views

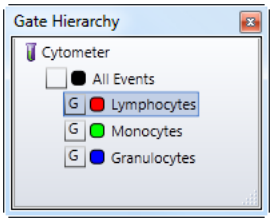
A population is a gated set of data. Populations can be viewed in the Gate Hierarchy pane, population hierarchy, or statistics view. Population hierarchies and statistics views are elements in a worksheet.

Populations are assigned in the hierarchy based on the selected plot. When you draw a gate on a plot, the population is identified by a population number and a color. You can define default colors and names for each population in the Edit User Preferences dialog.

Comparison between gate hierarchies, population hierarchies, and statistics views

The following figures are examples of a gate hierarchy, population hierarchy, and statistics view.

Gate hierarchy



Population hierarchy

Populations: Cytometer

Populations	Events	% Total	% Parent
All Events	20,592	100.00%	####
Lymphocytes	2,440	11.85%	11.85%
Monocytes	446	2.17%	2.17%
Granulocytes	5,659	27.48%	27.48%

Statistics view

Statistics: Cytometer

Populations	Events	% Total	% Parent	FSC (488) Mean	SSC (488) Mean
All Events	20,592	100.00%	####	23,389	17,690
Lymphocytes	2,440	11.85%	11.85%	25,730	6,776
Monocytes	446	2.17%	2.17%	40,072	21,948
Granulocytes	5,659	27.48%	27.48%	58,450	54,426

The following table describes the similarities and differences in displays and actions of the Gate Hierarchy pane, population hierarchy, and statistics view.

Displays and actions	Gate hierarchy	Population hierarchy	Statistics view
Displays the hierarchical relationship of gated populations	Yes	Yes	Yes
Interacts with the Inspector pane to modify gate name or color	Yes	Yes	Yes
Allows manipulation of parent/child populations by drag and drop	Yes	Yes	No
Allows drag and drop of populations into Compensation or Sort Layout panes	Yes	Yes	No
Allows creation of Sort Abort or NOT gates	Yes	Yes	No
Controls the show/hide display of gates and populations	Yes	No	No
Displays and allows the manipulation of global or local status	Yes	No	No
Allows creation of AND or OR gates	No	Yes	No
Displays a limited number of statistical details about selected populations	No	Yes	No
Displays a broad choice of statistical details about selected populations and parameters	No	No	Yes

More information

- Editing user preferences (page 101)
- Setting gate and population display preferences (page 105)
- Viewing the population hierarchy (page 203)

Using the Gate Hierarchy pane

Introduction

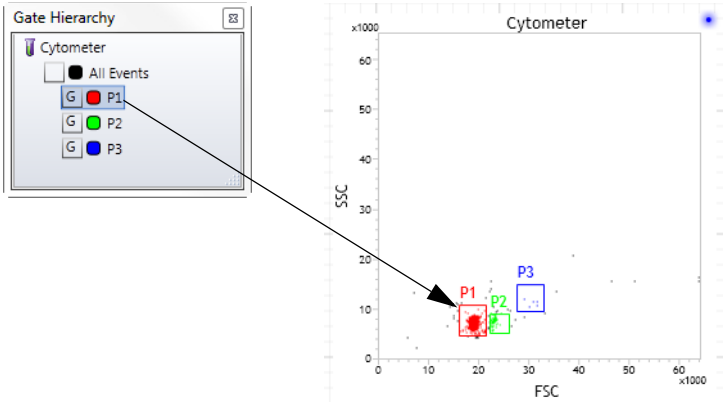
This topic describes how to open and use the Gate Hierarchy pane to view and manipulate the relationship of populations, and show and hide populations.

Opening the Gate Hierarchy pane

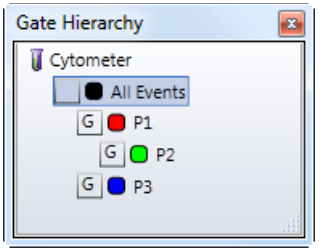
To open the Gate Hierarchy pane:

1. On the **BD FACS Sortware** toolbar, click **Gates** to display the hierarchy (tree) of the gates in an active plot.

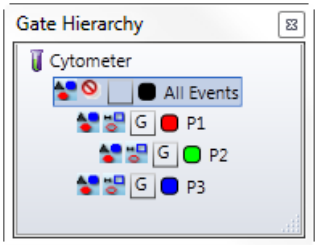
   The Gate Hierarchy pane opens. The gate hierarchy corresponds to gates in the selected plots, population hierarchy, statistics view, or data source.



When a data source, population hierarchy, or statistics view is selected, the gate hierarchy displays only the global/local status.



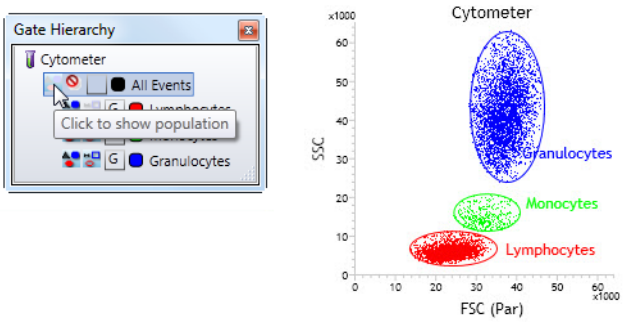
When a plot is selected, the Gate Hierarchy pane displays the population and gate frame controllers as well as the global/local status.



Showing or hiding populations

To show or hide populations in a plot:

1. Click on a plot with gated populations.
2. In the **Gate Hierarchy** pane, click the **Show/Hide** icon for the population you want to show or hide.

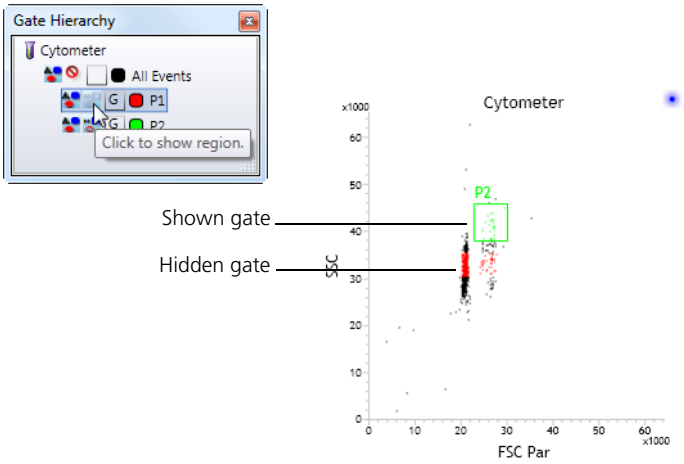


Shown populations

Showing or hiding gates (gate frames)

To show or hide gate frames in a plot:

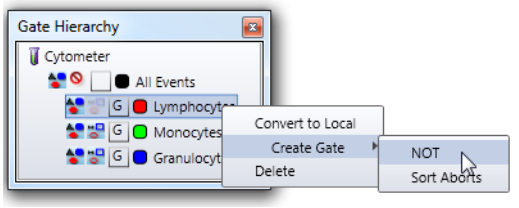
1. In the **Gate Hierarchy** pane, click the **Show/Hide** icon for the gate you want to show or hide in the plot.



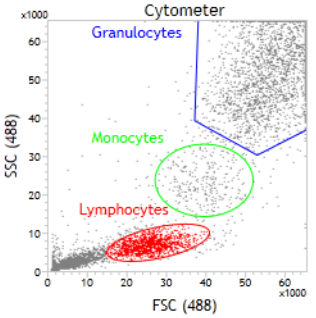
Creating Boolean gates using the Gate Hierarchy pane

To create Boolean gates using the Gate Hierarchy pane:

1. In the **Gate Hierarchy** pane, right-click a population.
2. Select **Create Gate**, then select a Boolean gate type: **NOT** or **Sort Aborts**.



The new gate appears. In the following figure, the NOT events appear as the new population color.



Populations: Cytometer

Populations	Events	% Total	% Parent
All Events	41,536	100.00%	####
Lymphocytes	4,935	11.88%	11.88%
Monocytes	1,122	2.70%	2.70%
Granulocytes	11,423	27.50%	27.50%
NOT(Lymphocytes)	36,601	88.12%	88.12%

More information

- Viewing the population hierarchy (page 203)
- Converting global and local gates (page 218)

Viewing the population hierarchy

Introduction

This topic describes how to view population hierarchy information using the population hierarchy and the Inspector.

Population hierarchy components

A population hierarchy can display the following:

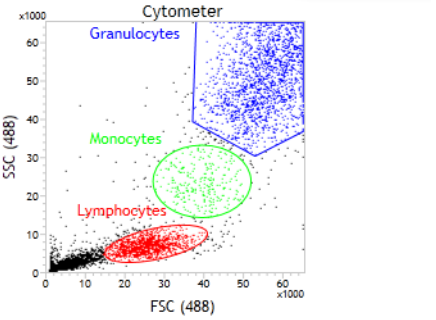
- Events
- %Total
- %Parent
- %Grandparent

Displaying a population hierarchy

To display a population hierarchy:

1. Right-click a plot and select **Population Hierarchy**.

   The population hierarchy appears.



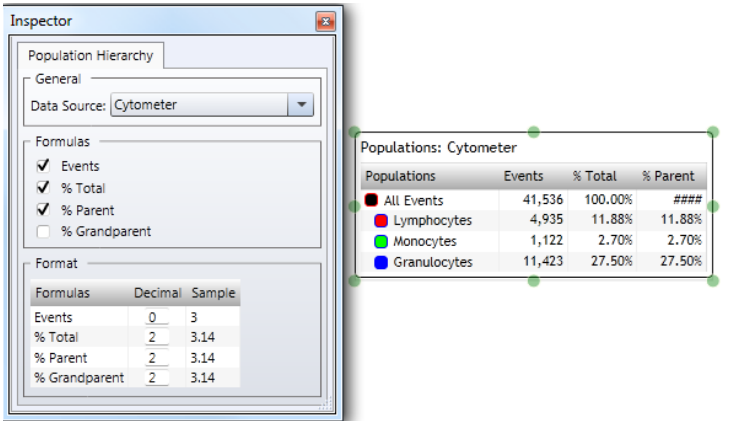
Populations: Cytometer

Populations	Events	% Total	% Parent
All Events	41,536	100.00%	####
Lymphocytes	4,935	11.88%	11.88%
Monocytes	1,122	2.70%	2.70%
Granulocytes	11,423	27.50%	27.50%

Modifying the population hierarchy

To modify the population hierarchy:

1. In the worksheet, click the header of the population hierarchy.
2. The **Inspector** refreshes and displays the **Population Hierarchy** tab.

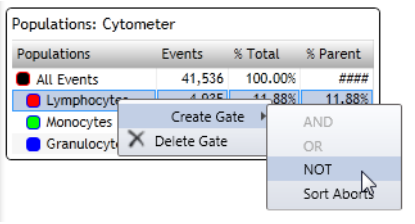


3. Under **General**, select an FCS file as a data source if needed.
4. Under **Formulas**, select the checkboxes for the statistical categories you want to include in the population hierarchy.
5. Under **Format**, change the value in the **Decimal** field to change the significant figures for the numbers displayed.

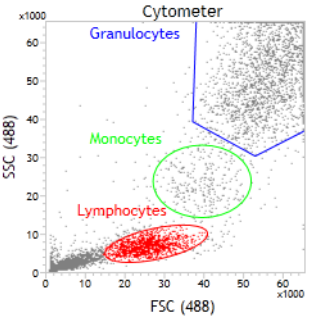
Creating Boolean gates using the population hierarchy

To create Boolean gates using the population hierarchy:

1. In the population hierarchy, right-click one or more populations.
2. Select **Create Gate**, then select a Boolean gate type (AND, OR, NOT, or Sort Aborts).



The new gate appears. In the following figure, the NOT events appear as the new population color.



Populations: Cytometer

Populations	Events	% Total	% Parent
All Events	41,536	100.00%	####
Lymphocytes	4,935	11.88%	11.88%
Monocytes	1,122	2.70%	2.70%
Granulocytes	11,423	27.50%	27.50%
NOT(Lymphocytes)	36,601	88.12%	88.12%

Deleting a population hierarchy

To delete a population hierarchy from a worksheet:

1. Right-click on the header of the population hierarchy in the worksheet and select **Delete**.

More information

- Using the Gate Hierarchy pane (page 199)
- Creating rectangle gates (page 206)

Creating rectangle gates

Introduction

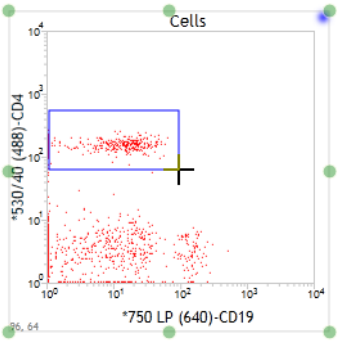
This topic describes how to create rectangle gates. You can draw rectangle gates on dot, density, or contour plots.

Procedure

To create a rectangular gate:

1. Click the **Rectangle** gate tool on the **Worksheet** toolbar.

2. Click inside a plot to position the first corner, then drag diagonally to the opposite corner point, then release the mouse button to set the gate.



The Gate Hierarchy pane updates to include the new gated population.

More information

- Using the Gate Hierarchy pane (page 199)
- Creating polygon gates (page 207)

Creating polygon gates

Introduction

This topic describes how to create polygon gates.

You can draw polygon gates on dot, density, or contour plots.

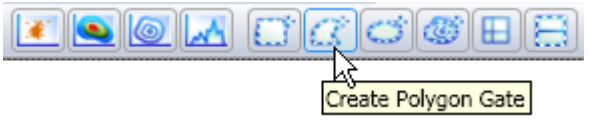
Limitations

Polygon gates require a minimum of 3 vertices and allow a maximum of 40.

Procedure

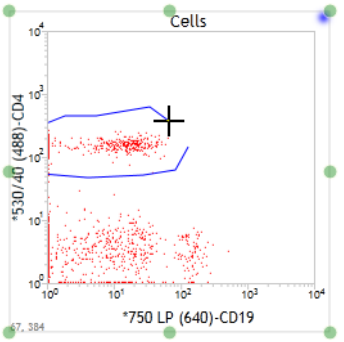
To create a polygon gate:

1. Click the **Polygon** gate tool on the **Worksheet** toolbar.



2. Click inside a plot to position the first vertex, then click to position each vertex.
3. Double-click to complete the polygon to close the gate.

   This draws lines between vertices and creates the polygon shape.



   The Gate Hierarchy pane updates to include the new gated population.

More information

- Creating rectangle gates (page 206)
- Creating ellipse gates (page 209)

Creating ellipse gates

Introduction

This topic describes how to create ellipse gates.

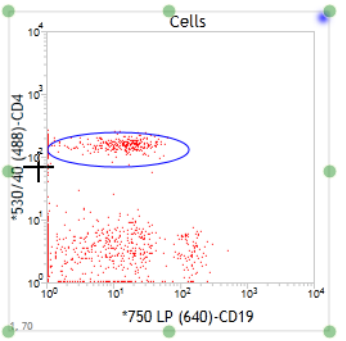
You can draw ellipse gates on dot, density, or contour plots.

Procedure

To create an ellipse gate:

1. Click the **Ellipse** gate tool on the **Worksheet** toolbar.

2. Click on a plot, then drag the cursor to create an ellipse of the desired shape. Release the mouse button to set the gate.



The Gate Hierarchy pane updates to include the new gated population.

More information

- Creating polygon gates (page 207)
- Creating contour gates (page 210)

Creating contour gates

Introduction

This topic describes how to view contour levels and create contour gates.

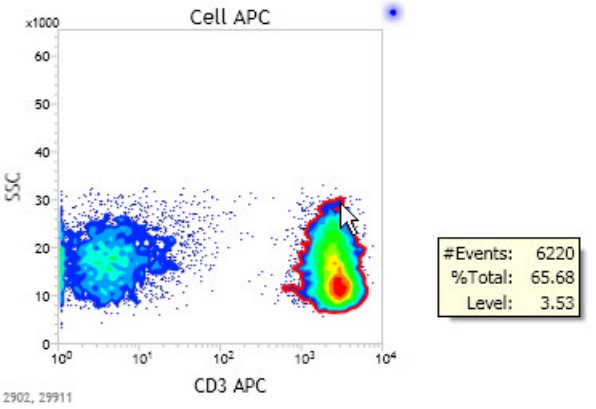
You can only draw contour gates on contour plots.

Viewing contour levels

To view contour levels:

1. Move the mouse cursor over any population (contour level) in the plot to display a data box.

   The following figure shows a contour plot and the data box for the contour level.

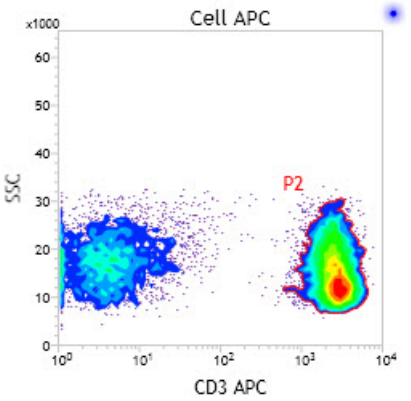


Creating contour gates

To create a contour gate:

1. Click the **Contour** gate tool on the **Worksheet** toolbar.

2. Click the population to automatically set a gate on the population (contour level).



The Gate Hierarchy pane updates to include the new gated population.

More information

- Creating polygon gates (page 207)
- Creating interval gates (page 212)

Creating interval gates

Introduction

This topic describes how to create an interval gate.

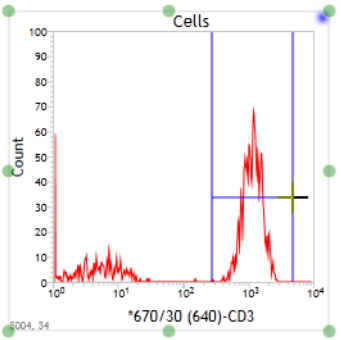
Interval gates are gates between the left and right endpoints on the horizontal axis. You can draw interval gates only on histograms.

Procedure

To create an interval gate:

1. Click the **Interval Gate** tool on the **Worksheet** toolbar.

2. Click in the plot to set the start point (right or left), drag horizontally, then release the mouse button to set an end point (right or left).



The Gate Hierarchy pane updates to include the new gated population.

More information

- Creating ellipse gates (page 209)
- Creating quadrant gates (page 213)

Creating quadrant gates

Introduction

This topic describes how to create quadrant (quad) gates. You can draw quadrant gates on dot, density, or contour plots.

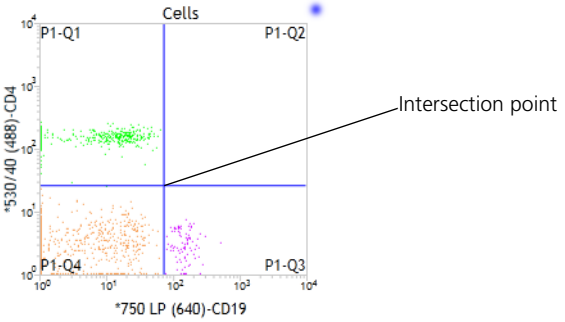
Quadrant gates divide a plot into four quadrants. Each quadrant has its own population statistics. You can name and color the population in each quadrant individually.

Procedure

To create a quadrant gate:

1. Click the **Quadrant Gate** tool on the **Worksheet** toolbar.

2. Click in the plot to position the quadrant intersection point.



The Gate Hierarchy pane updates to include the new gated population.

More information

- Creating interval gates (page 212)
- Converting global and local gates (page 218)

Working with gates

Introduction

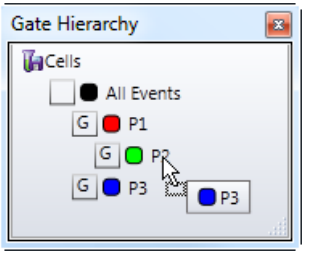
This topic describes how to modify gate names and colors and manipulate the gate size and display.

This topic also describes how to reprioritize gated populations and how to delete gates in a plot.

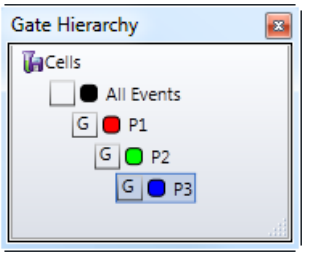
Changing the hierarchical position of gates

To change the hierarchical position of gates:

1. In the **Gate Hierarchy** pane, click and drag a gate to the new desired parent.



In this example, the P3 gate is now a child of P2. You can also perform the same action using the population hierarchy.



Renaming gates

Names are unique to gates. You cannot use the same name for two different gates. You cannot rename all events.

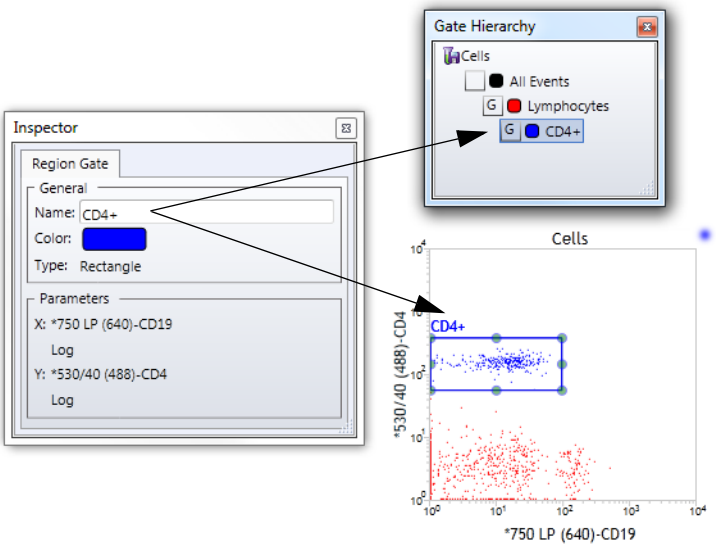
To rename a gate:

1. In the **Gate Hierarchy** pane, click a population.

   Alternatively, click a gate in a plot.

2. In the **Inspector**, in the **Name** field, type a name for the gate (for example, *CD4+*).

   The new gate name appears in the Gate Hierarchy pane and in the plot.



Changing a gate color

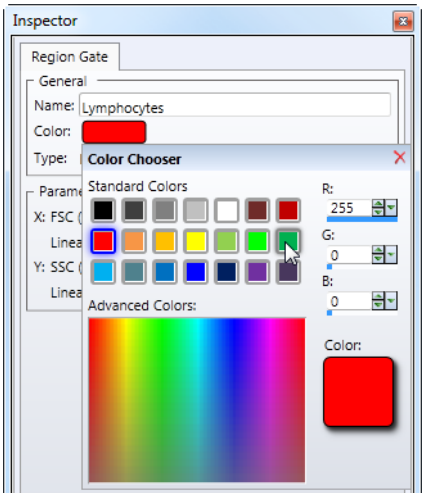
This procedure only affects the color of individual gates (global or local). To set the default preferences for all gates, use the Edit Preferences dialog. Colors are unique to gates. You cannot use the same color for two different gates.

To change a gate color:

1. In the **Gate Hierarchy** pane, click a population.

   Alternatively, click a gate in a plot.

2. In the **Inspector**, right-click the color box to display the color picker, then select a color.



   The new gate color appears in the Gate Hierarchy pane and in the plot.

3. Close the color box to apply the new color.

Resizing gates proportionately

To proportionally resize an existing gate:

1. Click a gate in a plot.

   The outline and handles are enabled.

2. Drag a corner handle in or out to resize the gate.

   If the gate is too small to view or drag a handle, zoom in on the gate, then resize it.

Resizing gates using vertices

To resize gates using vertices:

1. Double-click a gate.
2. Move the cursor over a vertex.

   The cursor will show crosshairs.

3. Click and move the vertex to desired location.

Rotating gates

To rotate a gate:

1. Click a gate in a plot.

   The outline and handles are enabled.

2. Move the cursor over a corner handle to display a rotation handle.
3. Click and drag the rotation handle to the right or left to rotate the gate.

Prioritizing gated populations in plots

You can prioritize the gate display in a plot and move gated populations forward or backward in the display.

To prioritize the gate display:

- To move a gate back in the display, right-click the gate, select **Send to Back**, then select the population you want to move.
- To move a gate forward in the display, right-click the gate, select **Bring Forward,** then select the population you want to move.

Deleting gates

To delete a gate in a plot:

1. Right-click a gate in a plot and select **Delete**.

To delete a gate in the Gate Hierarchy pane or Population Hierarchy view:

1. Right-click the population and select **Delete**.

More information

- Setting gate and population display preferences (page 105)
- Viewing the population hierarchy (page 203)
- Converting global and local gates (page 218)

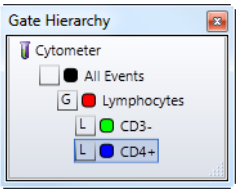
Converting global and local gates

Introduction

This topic describes how to convert gates from global to local and from local to global.

About global and local gates

- **Global gates.** By default, when you create a new gate in a plot, the gate is global. Global gates are indicated by a *G* in the Gate Hierarchy pane. These gates apply to the cytometer data source and all FCS files imported in the Data Sources pane that have the same configuration as the cytometer.



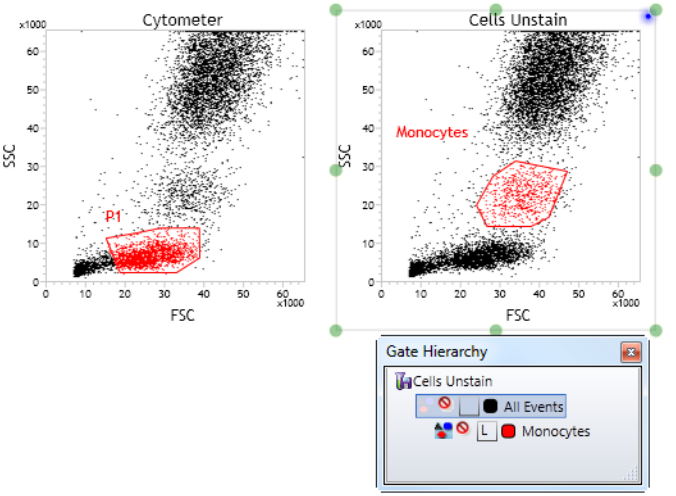
- **Local gates.** You can make global gates unique by converting them to local gates. Local gates are indicated by an *L* in the Gate Hierarchy pane. These gates only apply to a specific data source. When a gate is converted from global to local, the original global gate remains on all other data sources. This allows you to change the position, color, shape, and name of a local gate without affecting the corresponding global gate on other data sources.

Rules for converting gates

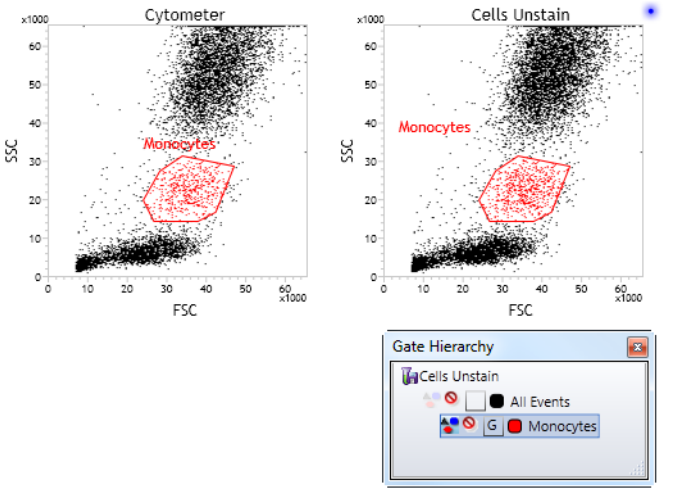
The following rules apply when you convert a global gate to a local gate or a local gate to a global gate:

- If a global gate has child gates and is converted to local, all child gates will also be converted to local gates.
- Gates (including Boolean gates) created with a local gate as a parent will be local.
- You cannot drag a global gate onto a local gate.
- Gates converted from global to local retain a link to their original global gate. When a local gate is converted back to global, it will update (overwrite) the original global gate with the local gate's position, size, name, and color.

In the following example, the global red P1 gate was converted to a local gate in the data source *Cells Unstain*. The gate was moved and renamed *Monocytes*.



The local gate *Monocytes* was converted back to global. The P1 gate was overwritten with the *Monocytes* gate properties.



- If a local gate was created without a link to a global gate (for example, you created a child of a local gate), the gate name must be unique in order to convert to global.
- If a local gate is converted to global and has child gates, the child gates will also be converted to global.
- You cannot convert a local gate to a global gate if its parent gate is local.
- Sort gates are always local gates (you cannot convert them to global).
- You cannot convert a global gate to a local gate or a local gate to a global gate if Boolean or sort abort gates are dependent on those gates.
- You cannot convert the global/local status of Boolean or sort abort gates.

Converting a global gate into a local gate

To convert a global gate into a local gate:

1. Right-click a gate in the **Gate Hierarchy** pane and select **Convert to Local.**

   Alternatively, right-click a gate in a plot and select **Convert to Local.**

Converting a local gate into a global gate

You can convert a local gate (in one data source) into a global gate (for all data sources with matching parameters).

To convert a local gate to a global gate:

1. Right-click a gate in the **Gate Hierarchy** pane and select **Convert to Global.**

   Alternatively, right-click a gate in a plot and select **Convert to Global.**

   The gate is now associated to all plots with matching parameters.

More information

- Gating overview (page 196)

Displaying a statistics view

Introduction

This topic describes how to display a statistics view for a plot in a worksheet.

Default statistics view components

The default statistics view displays the following:

- Population
- Events
- % Total
- % Parent
- X-axis parameter mean events
- Y-axis parameter mean events

Displaying statistics for a plot

To display a statistics view for a plot:

1. In a worksheet, right-click a plot and select **Statistics View**.

   A statistics view appears.

Statistics: Cytometer

Populations	Events	% Total	% Parent	FSC Mean	SSC Mean
All Events	10,000	100.00%	####	35,629	36,664
Lymphocytes	1,953	19.53%	19.53%	26,647	6,692
Monocytes	710	7.10%	7.10%	36,583	21,959
Granulocytes	4,736	47.36%	47.36%	42,279	52,216

   If the plot does not have gates, only data for All Events appears.

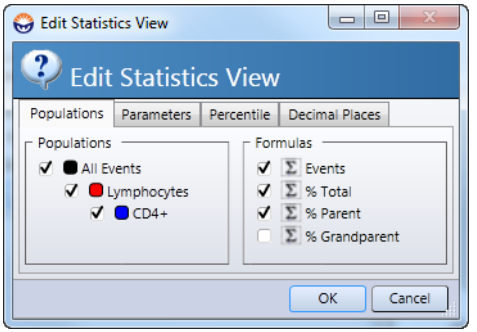
   If your plot has gates and defined populations, the data for each population and All Events appear in their hierarchical order.

Editing the statistics view

To edit a statistics view:

1. Click the header of the statistics view.
2. In the **Inspector** pane, change the data source (if needed) and click **Edit Statistics View**.

   The Edit Statistics View dialog opens.



3. Use the tabs in this dialog to edit the population, parameters, percentiles, and decimal values.
4. Click **OK** to apply the changes.

Moving a statistics view

To move a statistics view within a worksheet:

1. Click the header of the statistics view and drag it.

Deleting a statistics view

To delete a statistics view from the worksheet:

1. Right-click the header of a statistics view box and select **Delete**.

More information

- Converting global and local gates (page 218)

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14

Sort settings and layout

This chapter includes these topics:

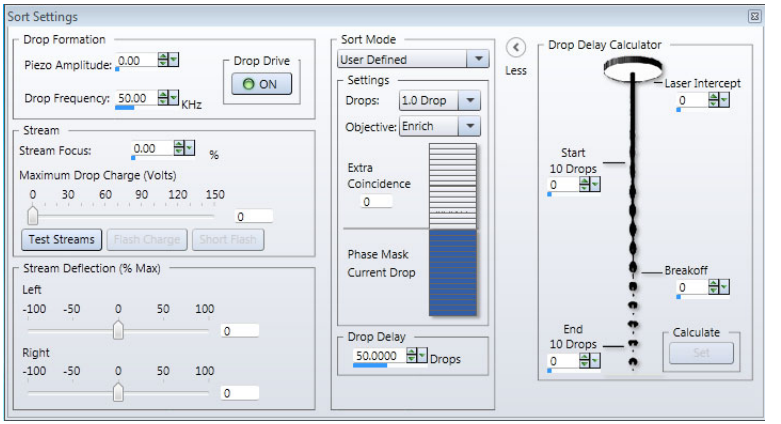
Using the Sort Settings pane

Introduction

This topic describes the Sort Settings pane.

Description

Use this pane to set the drop formation, delay, breakoff, and deflection parameters, and to select a sort mode.



This pane includes the following sections.

Section	Description
Drop Formation	Controls for determining the stream breakoff.
Stream	Controls for optimizing the side streams.
Stream Deflection	Controls for adjusting the side streams individually.
Sort Mode	Select a defined sort mode. Configure and save a new custom sort mode.
Delay Calculator	Tools to calculate the approximate drop delay.

More information

- Sort mode settings (page 227)

Sort mode settings

Introduction

This topic describe sort mode settings.

Sort Mode panel

The Sort Mode panel of the Sort Settings pane contains controls for selecting a defined sort mode, for modifying a defined sort mode, and for defining a new sort mode.

About sort mode

A sort mode is a configuration of settings that define the logic by which a gated event assigned for sorting will be accepted or rejected. The following factors are relevant to sort mode configuration.

- **Sort Efficiency.** The percentage of target events in the sample that has been accepted for sorting. Sort efficiency can be less than 100% because target events can be lost when sorting due to coincidence aborts and phase-mask aborts. Electronic aborts or hardware aborts affect the acquisition efficiency, which is reported in the Acquisition Dashboard pane. These events are not processed, cannot be targeted to sort, and do not affect sort efficiency (since they cannot be targeted).
- **Recovery.** The percentage of target sort events (the yield) that is collected in the sort device. Recovery can be less than 100% because target sort events are lost due to incorrect drop delay, incorrect flash charge setting, and migration of target events out of their drop between the interrogation point and the drop breakoff.

- **Coincidence.** The presence of another event within a specified distance (the "coincidence zone") of a sort event. Coincidence causes a sort event to be rejected whenever a nontarget event is located within the coincidence zone, thus reducing yield. Coincidence decreases the probability that a nontarget "passenger" will sort along with a target sort event, thus enhancing sort purity. Coincidence is enabled or disabled using the Objective setting. Coincidence override allows a sort to proceed when a sort target is located within the coincidence zone of another sort target for the same stream. Increase the magnitude of the coincidence zone using the Extra Coincidence setting or the Drops setting.
- **Phase Mask.** Sets the region within its drop that a target event can occupy and be accepted for sorting.

Drops setting

Selects the number of drops to be sorted per target sort event.

The Drops tool is a selection list with the following options: 1, 1.5, and 2. The higher the Drops setting, the greater the potential recovery. However, increasing the Drops setting expands the coincidence zone, which may reduce sort efficiency. A Drops setting greater than 1 reduces the sort-stream precision because of the repulsion of the second drop.

- **1.0 Drop.** Sort one drop per sort event.
- **1.5 Drops.** Sort one drop per sort event if the event is in the middle 50% of a drop. Sort two drops if the event is in outer (leading or lagging) 25% of the drop. Probability dictates that in half of the events, one drop is sorted. In the other half of events, two drops are sorted. The second drop is either the leading or the lagging drop, depending on the position of the event within its drop.
- **2.0 Drops.** Sort two drops per sort event. The second drop is either the leading or the lagging drop, depending on the position of the event within its drop.

Objective setting

Selects how coincidence is applied to the sort decision.

The Objective setting influences sort purity and sort efficiency. The Objective tool includes the following options:

- **Enrich.** Disables all coincidence.
- **Purify.** Enables coincidence and coincidence override.
- **Single.** Enables coincidence and disables coincidence override to ensure that only one target event can be sorted.

Extra Coincidence setting

Sets how far the coincidence zone of a drop extends into the adjacent drops. Every sorted drop is subject to the same coincidence settings.

Between the interrogation point and drop breakoff, a nontarget event on the edge of an adjacent drop can migrate into the drop containing a target sort event.

The Extra Coincidence scale is a numeric scale with values from 0 to 16, each higher number representing an increment of 1/16 drop. The Extra Coincidence setting is applied to both the leading and the lagging drop. Increasing the magnitude of the Extra Coincidence setting can increase the purity of a sort, but might decrease the sort efficiency.

Phase Mask setting

Sets the region within its drop that a target event can occupy and be accepted for sorting.

Between the interrogation point and drop breakoff, a target sort event on the edge of its drop can migrate out of the target drop. A phase mask is typically used to sort only those events that occupy the central region of the drop and reject those events on the leading or lagging edge and to increase the precision of the side stream. However, the user can define a phase mask to select a different region within a drop that a target event must occupy. A phase mask decreases sort efficiency and might increase recovery.

Default sort modes

Four configurations of sort settings have been programmed in BD FACS Sortware for your convenience. Any of the following configurations can be added to a Drops setting to create a default sort mode.

- **Pure.** Purify objective with extra coincidence setting 4 and no phase mask.
- **Yield.** Purify objective with no extra coincidence and no phase mask.
- **Enrich.** Enrich objective with no extra coincidence and no phase mask.
- **Single.** Single objective with 4/16th extra coincidence and a 10/16th phase mask.

The following table lists the default sort modes and their attributes.

Name/Setting	Drops	Objective	Extra Coincidence	Phase Mask
Pure	1.0 1.5 2.0	Purify	4/16	No
Yield	1.0 1.5 2.0	Purify	0	No
Enrich	1.0 1.5 2.0	Enrich	0	No
Single	1.0	Single	4/16	10/16

More information

- Creating a user-defined sort mode (page 231)

Creating a user-defined sort mode

Introduction

This topic describes how to define (configure, name, and save) a user-defined (custom) sort mode. User-defined sort modes are fully modifiable and can be saved for future use.

Procedure

To define a sort mode:

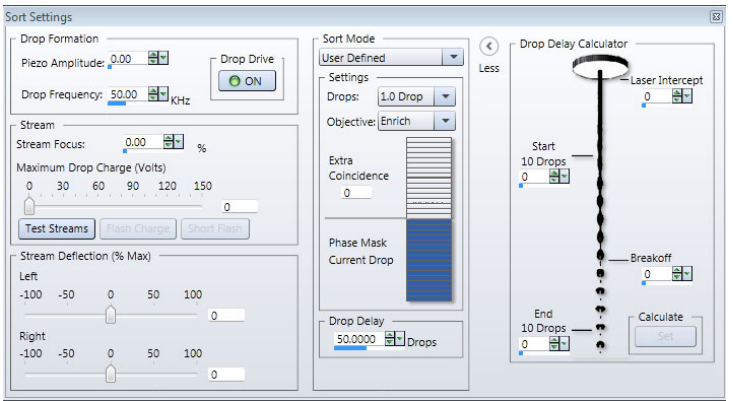
1. Open the **Sort Settings** pane.

   On the **BD FACS Sortware** toolbar, click **Sort Settings**.

   If the Sort Settings pane is already open, make sure that the flash charge is off before selecting User Defined in the Sort Mode menu. To turn off flash, under **Stream**, click **Flash Charge.**

   Note: Do not select the user-defined sort mode when flash is on.

2. In the **Sort Mode** panel, select **User Defined** from the menu at the top.



3. Under **Settings**:

   a. Make a selection from the **Drops** menu.

   b. Make a selection from the **Objective** menu.

   c. Select the extra coincidence value.

      Use either the graphic scale or the numeric selection list to choose a value between 0 and 16.

   d. Select a phase mask by clicking on the graphic.

   See Setting numeric values in panes and dialogs (page 90) for more information.

Saving a user-defined sort mode

To save a user-defined sort mode:

Note: Do not save a user-defined sort mode when flash is on.

1. Under **Stream**, click **Flash Charge** to turn off flash, if necessary.
2. Select **Sorting > Save Sort Mode**.

   The Save Sort Mode dialog opens.

3. Under **New Sort Mode**, type a descriptive name in the **Name** field and add a description in the **Description** field.
4. Click **OK** to save the new sort mode and close the dialog.

   The new sort mode configuration is available in the Sort Mode menu.

More information

- Sort mode settings (page 227)

Using the Tray Control pane

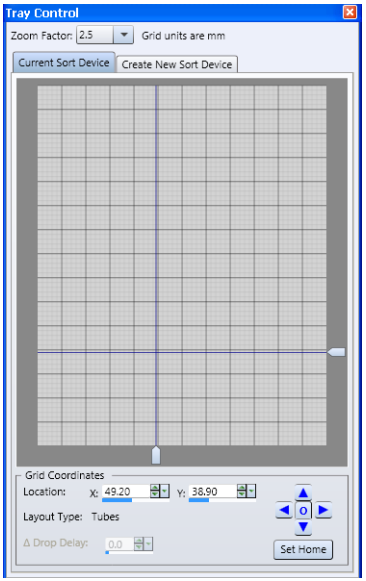
Introduction

This topic describes how to use the Tray Control pane to view or modify the sort device configuration.

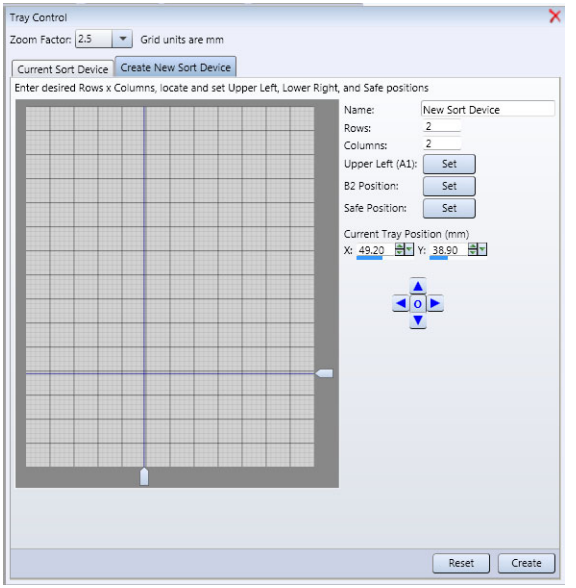
Description

Use this pane to view and adjust the offsets for the sort device or to configure a new sort device layout. This pane includes the following tabs.

- **Current Sort Device.** This tab displays the position of the tray. Modify the position of the sort device using the offset markers or grid coordinate controls and click **Set Home** to set a new sort ready position for the current sort device.



- **Create New Sort Device.** Use this tab to create a new sort device layout.



More information

- Creating a sort device layout (page 235)

Creating a sort device layout

Introduction

This topic describes how to create a new sort device layout using the Tray Control pane.

About creating new sort device layouts

In BD FACS Sortware, default sort device dimensions and well layouts are based on BD Falcon™ plates. When other sort devices are used, it might be necessary to create a custom sort device layout or custom devices.

When you create a new sort device layout, it appears as a selection in the Sort Device menu in the Sort Layout pane. You can manage the list of user-created sort devices using the Manage Sort Device dialog.

Before you begin

- Set up for a sort.
- Install a sort device on the tray.

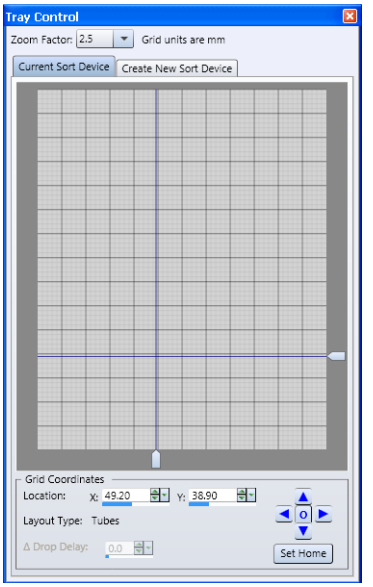
Procedure

To create a new sort device layout:

1. Open the **Sort Settings** pane.

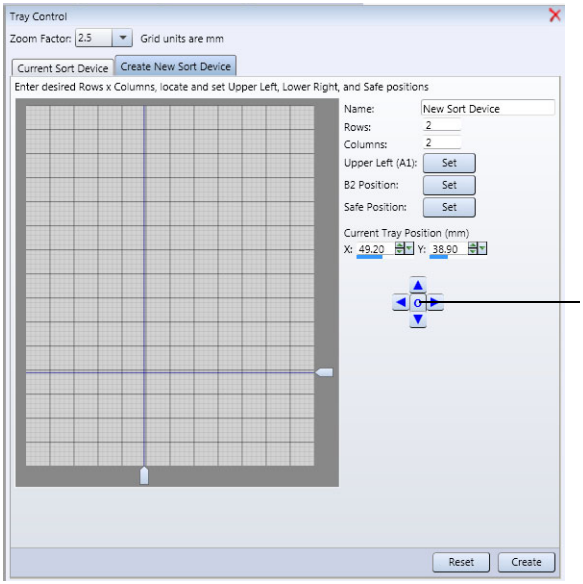
   On the **BD FACS Sortware** toolbar, click **Sort Settings**.

2. Click the **Tray Control** pane.



3. Click the **Create New Sort Device** tab.
4. Type a name for the new sort device layout in the **Name** field.
5. Enter the number of rows and columns in the **Rows** and **Columns** fields.
6. Set the A1 (first well) position.
    a. Use the tray position controls to bring the A1 position close to the stream.

b. Click the **Test Sort** button to sort a few drops onto the sort device.



c. Open the **Sort Layout** pane.

d. Click **Eject** to eject the tray.

e. Check the position of the test sort drop on the sort device.

f. Repeat steps a to e to place the test sort drop in the center of the well.

g. Click the **Set** button next to **Upper Left (A1).**

7. Move the cursor to set the last well position (bottom right).
8. Repeat step 6 for the last well position.
9. Move the cursor to set the safe position.
10. Click the **Set** button next to **Safe Position**.
11. Click **Create** to create the new sort device.

More information

- Modifying an existing sort device (page 238)
- Deleting a sort device (page 240)

Modifying an existing sort device

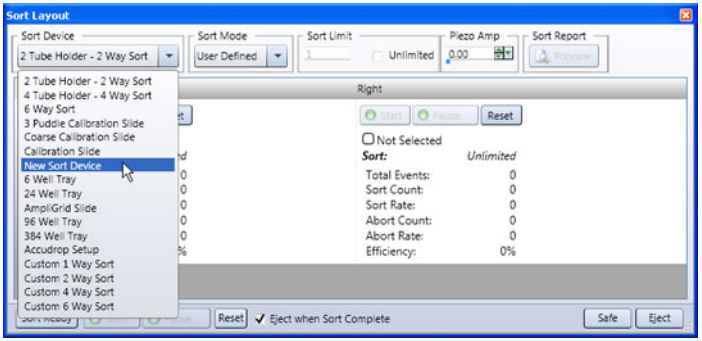
Introduction

This topic describes how to modify the home position of an existing sort device layout using the Tray Control pane.

Procedure

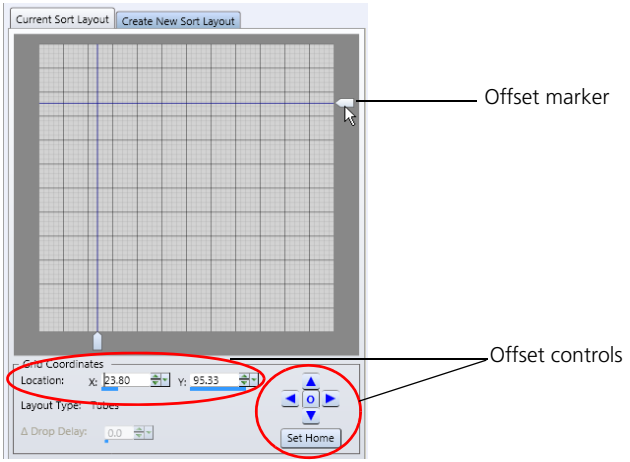
To modify a sort device:

1. In the **Sort Layout** dialog, under **Sort Device**, select a sort device.



The selected sort device appears in the Current Sort Device tab in the Tray Control pane.

2. Drag the vertical or horizontal offset markers to a new position in the map, or adjust the X and Y locations by clicking the up and down arrows in the **Location** fields.



3. Click **Set Home** to shift the sort ready position to the new location.

   The new position is saved for the current sort device.

More information

- Using the Tray Control pane (page 233)

Deleting a sort device

Introduction

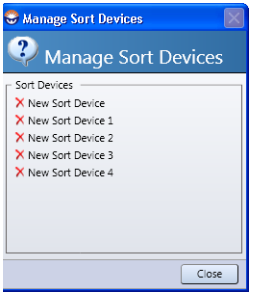
This topic describes how to delete a saved (named) sort device layout using the Manage Sort Devices dialog.

Procedure

To delete a saved sort device:

1. Select **Sorting > Manage Sort Devices** from the **BD FACS Sortware** menu.

   The Manage Sort Devices dialog opens.



2. Under **Sort Devices**, click the **X** next to the sort device you want to delete.

   A confirmation dialog opens.

3. Click **Yes** to delete the sort device.

More information

- Using the Tray Control pane (page 233)

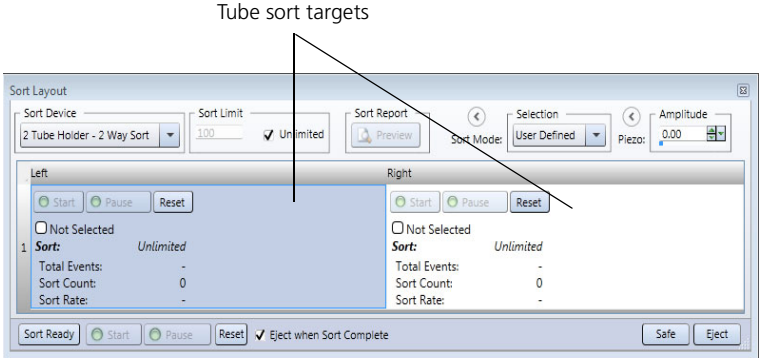
Using the Sort Layout pane

Introduction

This topic describes the Sort Layout pane tools, options, and sort controls.

Description

Use this pane to select the sort tray or tube, select the sort mode, define the population for the sort target, and control the position and readiness of the sort tray or tube.



Tools and controls

This dialog includes the following sections.

Section	Description
Sort Device	Lists the available sort devices.
Sort Limit	Displays the number of events to sort. Modify this setting by typing a value in the field. Select the Unlimited checkbox to enable a continuous sort.
Sort Report	Displays a preview of the sort report after the sort completes. Sort reports save to C:\Users\My User Name\Documents\BD\Sortware\Sort Reports.
Sort Mode	Lists the available preset sort modes and any user-defined modes.
Piezo	Displays the current piezo amplitude. Modify this setting by typing a value in the field or by using the data slider.

The Sort Layout dialog includes the following sort controls and actions.

Control or action	Procedure
Selecting individual sort targets	Click inside the sort target to select only that target.
Selecting all sort targets	Click the upper-left corner of the sort target table to select sort targets.
Selected population	Right-click in a sort target to select a population gate for the tube or well.
Start, Stop, Pause, Reset	Click to control the sort operation.
Eject when sort complete (checkbox)	Select to eject the sort tray at the end of a sort. Clear the checkbox to keep the tray in position.
Sort Ready	Click to move the sort tray to the sort ready position and to ready the system for a sort.
Safe	Click to move the sort tray to the safe position, away from the sort head and deflection plates.
Eject	Click to eject the sort tray.

More information

- Software overview (page 85)
- Using the Tray Control pane (page 233)
- Sort setup workflow (page 320)

Setting a new Sort Ready or Home position

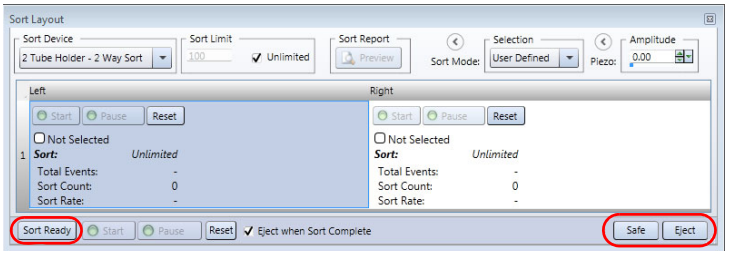
Introduction

This topic describes how to move the sort tray position to optimize tube or well alignment with the sample stream. Use the Sort Layout dialog and the Tray Control pane to perform this procedure as needed.

Defining a new sort ready position for a sort device

To move the sort tray to predefined positions:

- To move the sort tray to the home position (ready for sorting), click **Sort Ready.**
- To move the sort tray to the safe position (away from the sample stream), click **Safe.**
- To move the sort tray to the eject position so you can remove the sort device, click **Eject.**

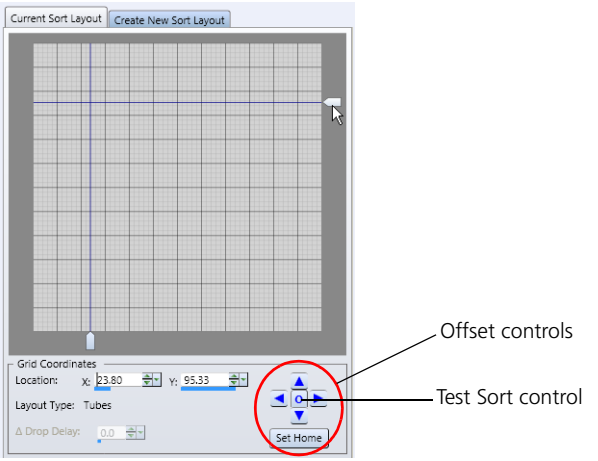


Manually moving the sort tray position

To manually move the sort tray position:

1. Open the **Tray Control** pane.
2. Click **Sort Ready** in the Sort Layout dialog.
3. Click the **Test Sort** button to determine the current setting.

4. Move the tray by clicking the offset control arrows or by adjusting the values in the X or Y location fields by using the mouse scroll wheel, small arrows, data slider, or keyboard keys.



5. Click **Set Home** to save new sort ready position for the current sort device.

More information

- Using the Tray Control pane (page 233)
- Using the Sort Layout pane (page 241)

Part 3

System workflow

This part includes these chapters:

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15

System startup

This chapter includes these topics:

Startup workflow

Introduction

This topic describes the workflow steps for starting up the BD FACSJazz system.

The workflow you perform depends on how the BD FACSJazz system was shut down.

Caution: Electrical! Do not place fluids on top of the instrument. Fluid spills on the instrument can lead to electric shock.

Dry startup workflow

Use this workflow if you are starting the BD FACSJazz instrument for the first time, or if you are starting up after a dry shutdown (no fluids in the instrument).

Step	Description
1	Powering up the system (page 252)
2	Preparing the fluidics tanks (page 253)
3	Flushing the system (page 257)
4	Cleaning the nozzle tip (page 259)
5	Backflushing the sample line (page 261)
6	Removing bubbles from the nozzle tip (page 262)
7	Introducing a sample into the system (page 264)

Wet startup workflow

Use this workflow if you are starting up the BD FACSJazz instrument after a wet shutdown (DI water in the instrument).

Step	Description
1	Powering up the system (page 252)
2	Preparing the fluidics tanks (page 253)
3	Cleaning the nozzle tip (page 259) (If needed)
4	Removing bubbles from the nozzle tip (page 262)

More information

- Startup, shutdown, and troubleshooting components (page 250)
- System shutdown (page 349)
- System shutdown workflow (page 350)

Startup, shutdown, and troubleshooting components

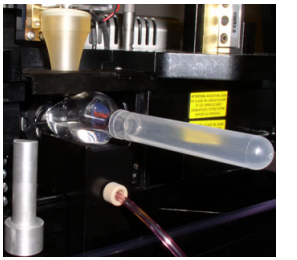
Introduction

This topic describes the tools that are used during BD FACSJazz startup, shutdown, and troubleshooting.

Tools

- **Debubble reservoir.** Use this tool when you remove bubbles from the fluidics during startup or as needed. This reservoir is also used during wet shutdowns to submerge the nozzle tip in fluid to prevent air from entering the fluidics.

The debubble reservoir is attached to a tube to hold fluid.

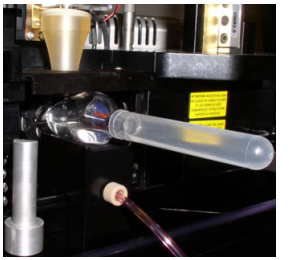


The debubble reservoir with DI water is used to submerge a nozzle tip during a wet shutdown.

- **Flush bucket.** This is a moveable receptacle that is attached to a waste line. Use this tool during startup and shutdown to hold the debubble reservoir or catch the stream directly under the

nozzle assembly (which prevents the stream from reaching the stream drain in the sort stage).

The flush bucket under the nozzle assembly during a flush procedure



The flush bucket under the debubble reservoir during a wet shutdown

The flush bucket mounts in two places:

- Under the nozzle assembly when in use
- On top of the instrument above the sample station when not in use

Use the flush bucket during startup when you:

- Rinse without a nozzle tip to fill the fluidic lines
- Purge with a nozzle tip to remove bubbles from the tip

Use the flush bucket during shutdown when you:

- Dry the fluidics system in a dry shutdown
- Submerge the nozzle tip in DI water using the debubble reservoir for a wet shutdown

More information

- System startup (page 247)
- Startup workflow (page 248)

Powering up the system

Introduction

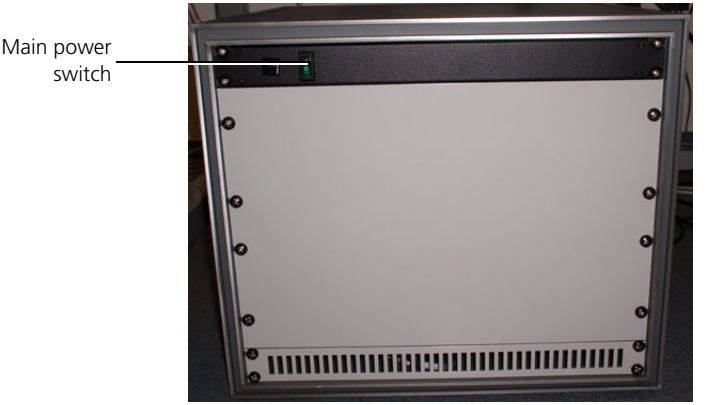
This topic describes how to power up the BD FACSJazz system.

Procedure

To power up the system:

Caution: Laser! Close all laser shutters.

1. Close all laser shutters.
2. Turn on the main power.



   This powers the system lasers and electronics. Wait at least 15 minutes for the lasers to warm up before performing QC. You can continue fluidics startup during this time.

3. Turn on the computer.
4. Start the software programs by double-clicking the BD FACS Sortware icon.

   The Sortview and Pressure Console panes start at the same time.

Next step

Preparing the fluidics tanks (page 253)

More information

- Pressure Console pane (page 92)
- Sortview pane (page 95)
- System startup (page 247)

Preparing the fluidics tanks

Introduction

This topic describes how to prepare the fluidics tanks during startup and how to balance the sample-to-sheath pressure.

Required materials

- Empty sheath tank and waste tank
- 7 L of sheath fluid
- 0.7 L of bleach (if sorting biohazardous samples)
- 0.2-µm sheath filter (provided). If you are not using a sheath filter, pre-filter your sheath fluid to 0.2 µm or smaller to avoid excess noise in the system.

Filling the sheath tank

Check the sheath level during startup (each day) and refill the tank as needed.

Always empty the waste tank when you refill the sheath tank.

To fill the sheath tank:

1. Disconnect the air and sheath line quick-connect fittings (when performing a wet startup).

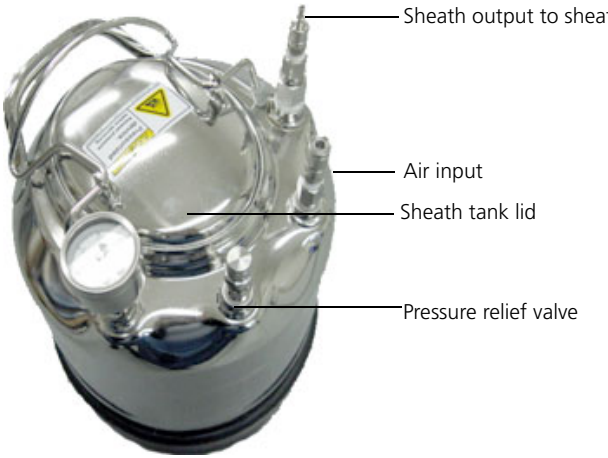
   Disconnect the sheath and air line from each other (when performing a dry startup).

2. Depressurize the sheath tank by opening the pressure relief valve (when performing a wet startup).

3. Place the empty tank on the floor or designated spot (from where the BD Service representative calibrated the tank) before refilling it.
4. Fill the sheath tank with up to 7 L of sheath fluid.

   Do not fill past the weld line on the sheath fluid tank to ensure that there is adequate space for the tank to pressurize.
5. Attach the sheath tank lid.

   Make sure that the O-ring is properly installed around the tank lid and that the lid is properly seated.



6. Attach the air line (blue) and the sheath line (clear) with the filter attached to the sheath tank.

   Make sure the sheath filter is inline (not bypassed).

Preparing the waste tank

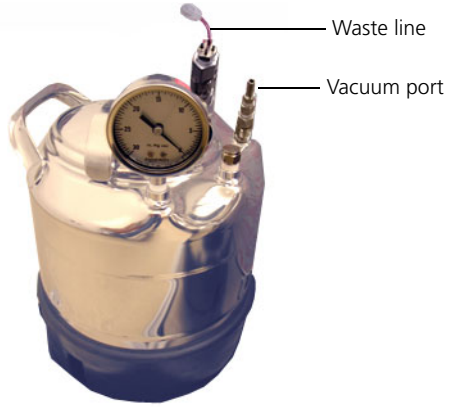
Empty the waste tank each time you refill the sheath tank.

Caution! The contents of the waste tank and waste tubing could be contaminated with biohazardous material. Follow standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.

To prepare the waste tank:

1. Disconnect the vacuum and the waste line quick-connect fittings.
2. Carry the waste tank to the waste disposal area, then remove the lid.
3. Empty the waste tank, taking care not to spill waste fluid over the pressure gauge.
4. Add enough bleach to achieve a 10% concentration of bleach.
5. Attach the waste tank lid.
6. Place the waste tank next to the sheath tank.

7. Attach the quick-connect fittings for the waste line (red) input and the vacuum source.



Caution! Do not run the system without the waste filter in place. Liquid contamination in the house vacuum supply or the dedicated air compressor can damage the vacuum system.

8. Ensure that the hydrophobic waste filter is connected between the vacuum source and the waste tank.
9. If the waste tank overfills and wets the filter, empty the waste tank and replace the filter.

Pressurizing the waste tank

To pressurize the waste tank:

1. Turn on the house vacuum supply or vacuum pump.
2. Verify that the waste pressure gauge reads between 5" Hg and 10" Hg.
   - If the gauge reads lower than 5" Hg, tighten the waste tank lid.
   - If the gauge reads higher than 10" Hg, reduce the vacuum applied to the waste tank.

Pressurizing the sheath tank

To pressurize the sheath tank:

1. Close the pressure relief valve on the sheath tank.
2. Verify that the house air pressure supply or dedicated air compressor is on.
3. Turn on the air supply by switching the Air switch on the right side of the cytometer near the sample station.
4. Verify that the sheath tank is sealed by checking the pressure gauge.

   The pressure should be about 27 to 29 psi.

   If the tank is not pressurizing, verify that the release valve is closed, or re-seat the sheath tank lid.

Next step

Flushing the system (page 257)

More information

- System startup (page 247)
- Introducing a sample into the system (page 264)
- About sample rate, sample pressure, and concentration (page 264)

Flushing the system

Introduction

This topic describes how to flush the fluidics system to prime the system with fluid, forcing air out of the fluidic lines.

Flush the system:

- After starting up the instrument from a dry shutdown
- After changing the fluidic tubing

Required materials

Flush bucket

Flushing the fluidics

To flush the system:

1. Make sure that the nozzle and nozzle nut are removed.
2. Place the flush bucket under the nozzle.
3. In the **Pressure Console** pane, click **Rinse,** and run for at least 30 seconds, checking that the lines are full of fluid and that the flush bucket is not overfilling.

   If the flush bucket is overfilling, check for low vacuum pressure, clogged lines, or possible pinches or kinks in the fluid waste line.

   Caution! If the vacuum pressure is low or the waste lines are clogged, the flush bucket can overfill.
4. Remove the sheath filter from the velcro attachment and tap it gently to dislodge any air bubbles.
5. In the **Pressure Console** pane, click **Rinse** again to stop the flow.

Next step

Cleaning the nozzle tip (page 259)

More information

- Replacing the nozzle tip (page 71)
- System startup (page 247)
- Startup, shutdown, and troubleshooting components (page 250)
- Preparing the fluidics tanks (page 253)

Cleaning the nozzle tip

Introduction

This topic describes how to clean the nozzle tip.

Clean the nozzle tip daily as part of the startup workflow. Also, clean the nozzle tip when the stream is diverted by an apparent obstruction or clog.

Required materials

- Syringe with 0.2-µm filtered water, sheath fluid, or a mild detergent
- Sonicator

Before you begin

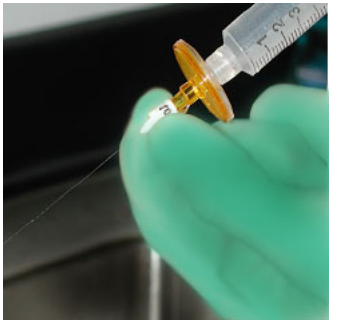
Remove the nozzle if it is still installed. See Replacing the nozzle tip (page 71) for more information.

Procedure

To clean the nozzle tip:

1. Sonicate the nozzle for 1 to 2 minutes.
2. Prepare a syringe with 0.2-µm filtered water, sheath fluid, or a mild detergent.

3. Flush the nozzle in the opposite direction of normal sheath flow.



4. Install the nozzle tip.

Next step

Backflushing the sample line (page 261)

More information

- Replacing the nozzle tip (page 71)
- System startup (page 247)
- Introducing a sample into the system (page 264)

Backflushing the sample line

Introduction

This topic describes how to backflush the sample line to remove air from the sample line.

Procedure

To backflush the sample line:

1. In the **Pressure Console** pane, click **Stream** to start the stream.
2. Remove the sample tube and move the tube-lock lever to the open position.
3. In the **Pressure Console** pane, click **Backflush**.
4. Once sheath fluid drips from the sample line, click **Backflush** again to turn backflushing off.

Next step

Removing bubbles from the nozzle tip (page 262)

More information

- System startup (page 247)
- Startup, shutdown, and troubleshooting components (page 250)
- Cleaning the nozzle tip (page 259)
- Introducing a sample into the system (page 264)

Removing bubbles from the nozzle tip

Introduction

This topic describes how to remove bubbles from the nozzle tip.

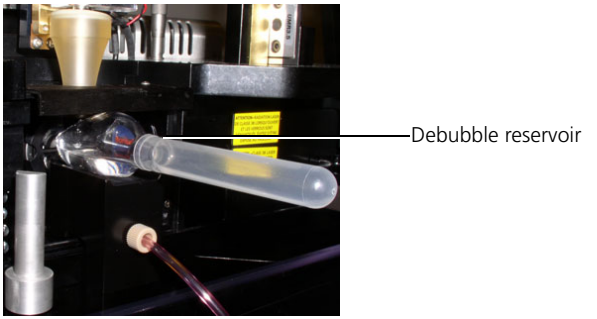
Required materials

- Debubble reservoir
- Flush bucket

Procedure

To remove bubbles from the nozzle tip:

1. Place the flush bucket under the nozzle assembly.
2. Place the empty debubble reservoir in the flush bucket.
3. In the **Pressure Console** pane, click **Stream** to fill the debubble reservoir with sheath fluid.
4. Ensure that the nozzle tip is submerged in the sheath fluid.



5. In the **Pressure Console** pane, click **Purge** to pull fluid up through the nozzle tip.

   This removes air from the tip.
6. When all air has traveled past the Y-fitting, click **Pulse** to pulse the fluidics lines to free additional bubbles that might be trapped in the nozzle.
7. Repeat steps 3 and 6 and until no additional bubbles are released from the nozzle.

If you are having difficulty removing all the bubbles, try the following:

- Make sure that the nozzle tip is submerged in fluid throughout the process, refilling the debubble reservoir in place as needed.
- Use ethanol instead of sheath fluid in the debubble reservoir. Follow with a sheath fluid purge to remove the ethanol.
- Verify that the O-ring is installed in the nozzle nut and that it is as tight as you can get it without using a wrench.
- Remove the debubble reservoir from the flush bucker, then reinsert it to introduce a large bubble into the nozzle. The large bubble may dislodge the smaller bubbles.

8. In the **Pressure Console** pane, click **Stream** to start the stream with the debubble reservoir still in place and the nozzle still submerged.
9. Remove the debubble reservoir carefully so that any fluid that spills out of the reservoir is caught by the flush bucket. Remove excess fluid from the nozzle tip with a cotton swab.
10. Verify that the stream is flowing straight out of the nozzle tip.

    If the stream is crooked or unstable, clean the nozzle and continue purging air from the lines.

Next step

Introducing a sample into the system (page 264)

More information

- System startup (page 247)
- Startup, shutdown, and troubleshooting components (page 250)
- Cleaning the nozzle tip (page 259)
- System shutdown (page 349)

Introducing a sample into the system

Introduction

This topic provides basic information about how to load a sample tube into the sample station, then run a sample and test the stream alignment.

Detailed information about aligning and optimizing the streams and lasers is included in the Alignment and QC workflow chapter.

See Alignment and QC (page 267) for more information.

About sample rate, sample pressure, and concentration

Sample rate or event rate is determined by the combination of the sample pressure, the concentration of the sample, and ultimately, the width of the core stream.

Adjust the sample pressure offset so that the sample core stream is as narrow as possible. When the sample pressure offset is raised, the sample core stream widens and the particles are dispersed into the wider stream. This increases the CV.

Typically, a low flow rate can be achieved when the sample pressure offset is about 1 psi. This setting varies between instruments and depends on the concentration of the sample.

Requirements

- Use only BD Falcon 12 x 75-mm polypropylene sample tubes.
- (Optional) Filter all samples to 40 µm (about half the nozzle tip diameter).

Before you begin

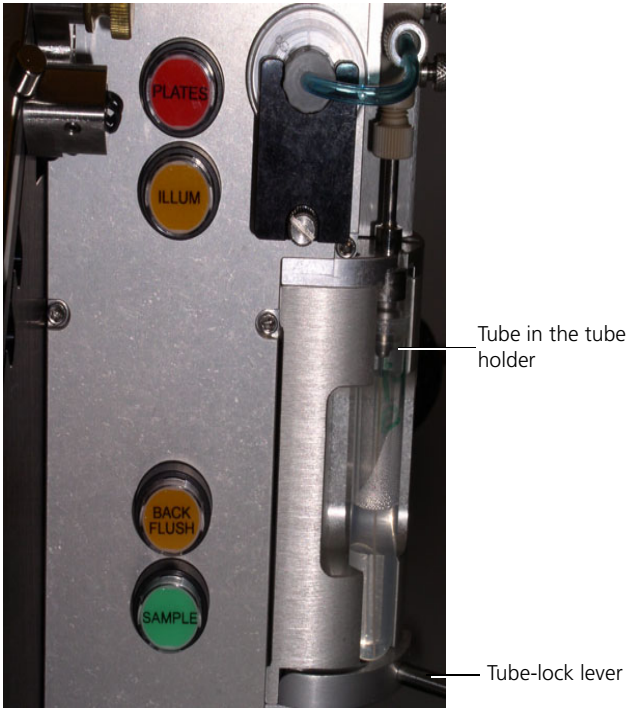
- Set the sample pressure offset to approximately 1 psi.
- Check the Status box for any messages regarding the system state and its ability to run samples.

Loading a tube

Caution: Biohazard! Use care when installing the sample tube. Once the tube is installed, the sample tube is pressurized. Sample fluid can spill or splatter if the sample tube is not properly installed and locked in place.

To load a sample tube:

1. Fill a sample tube with up to 3 mL of sample.
2. Load the sample tube into the sample tube holder and pull the sample tube-lock lever forward to lock the tube in place over the stopper. Do not damage or bend the sample line.



Running the sample

To run a sample:

1. In the **Pressure Console** pane, click **Sample** to open the sample valve and begin running the sample.
2. In the **Pressure Console** pane, click **Boost** and hold for a few seconds to temporarily boost the sample offset to 3 psi higher than the current offset and introduce the sample into the nozzle quickly.
3. Monitor the sample flow by looking at the event rate.

4. Adjust the sample pressure to achieve the desired event rate.

   Higher sample pressure offsets result in lower resolution (higher CV). While adjusting the sample offset, ensure that the acquisition efficiency percentage (located in the Acquisition Dashboard) is high. If the sample offset is too high relative to the sample concentration, the acquisition efficiency will be lower.

Removing a sample tube

Caution: Biohazard! Use care when removing the sample tube. The sample tube is usually pressurized and can include aerosolized cells.

To remove the sample tube:

1. In the **Pressure Console** pane, click **Sample** to stop the sample run.
2. Push the tube-lock lever slowly backward while holding the tube in place with your thumb until the tube is pushed down into the recessed area of the tube-lock lever and tube-lock lever is all the way back.
3. Remove the tube, being careful not to kink or bend the sample line.
4. In the **Pressure Console** pane, click **Backflush** to allow the residual sample to backflush.
5. In the **Pressure Console** pane, click **Backflush** to stop the backflush process.

More information

- Sample introduction (page 33)
- System startup (page 247)

16

Alignment and QC

This chapter includes these topics:

Aligning and optimizing the optics workflow

Introduction

This topic provides a workflow for aligning and optimizing the optics for instrument QC.

Purpose of the workflow

Each day before running samples, the system must be aligned and optimized. Track the results of alignment to confirm that the system is performing consistently over time. Tracking data can be useful for troubleshooting.

Alignment and optimization tasks involve mechanically adjusting the optics and fluidics, and setting values and recording data with BD FACS Sortware.

Daily alignment and optimization mean aligning the stream to the pinholes and the drain, aligning the primary fluorescence parameter, aligning the forward scatter, aligning additional lasers, positioning the sample core at the focal point of the objective lens in the pinhole, illuminating the sample core optimally, aligning the fluorescence and forward scatter, then adjusting PMT gains to place the QC particles at a target location.

Workflow

Step	Description
1	Create or load a saved QC workspace that includes appropriate cytometer settings, sort settings, and worksheet elements. See Creating a QC workspace (page 270).
2	Prepare the beads to be used for alignment and QC. See Preparing beads for QC (page 276).
3	Align the stream to the pinholes and the BD Accudrop laser, and introduce a sample.See Aligning the stream (page 277).
4	Align the primary laser to the core stream. See Aligning the primary laser to the core stream (page 283).

Step	Description
5	Optimize the fluorescence signal for the primary laser. See Optimizing the fluorescence signal for the primary laser (page 285).
6	Optimize the forward scatter signal. See Optimizing the forward scatter signal (page 286).
7	Optimize additional lasers. See Optimizing additional lasers (page 288).
8	Create an FCS file to record laser alignment. See Creating an FCS file to record laser alignment (page 290).
9	Save the QC workspace to save alignment settings. See Saving the QC workspace (page 292).
10	Verify alignment using the target source. See Verifying alignment using the target source (page 293).

More information

- Creating a QC workspace (page 270)

Creating a QC workspace

Introduction

This topic describes how to customize a QC workspace to be saved and used for daily QC.

The QC workspace includes a worksheet with plots to measure and track all scatter and fluorescence parameters required for QC.

About the QC workspace

The QC workspace simplifies alignment by presenting the correct alignment plots, gates, and statistic boxes.

After alignment and QC, the cytometer settings and sort settings can be saved to be used as the base daily cytometer settings.

Procedure summary

This example shows how to create a custom QC workspace by adding plots and statistics views that display data for the primary laser fluorescence parameters, FSC vs SSC, and all other configured lasers and included parameters. Statistics should show only the essential categories for events, such as median and rCV.

Stage	Description
1	Start with a new workspace. See Creating a QC workspace (page 270).
2	Add plots for the primary laser fluorescence parameters. See Adding plots for primary laser fluorescence parameters (page 272).
3	Add and customize statistics views for the primary laser fluorescence parameters and create a gate for the QC population. See Adding and customizing a statistics view (page 273).

Stage	Description
4	Add an FSC vs SSC plot and statistics view. See Adding an FSC vs SSC plot and a statistics view (page 275).
5	Add plots and statistics views for all other lasers and fluorescence parameters. See Adding plots and statistics for all other configured lasers and parameters (page 275).
6	Save the workspace and an FCS file (after you perform the alignment procedures). See Saving the QC workspace (page 292).

Creating a QC workspace

To create a QC workspace:

1. Select **File > New Workspace**.

   A confirmation dialog opens.

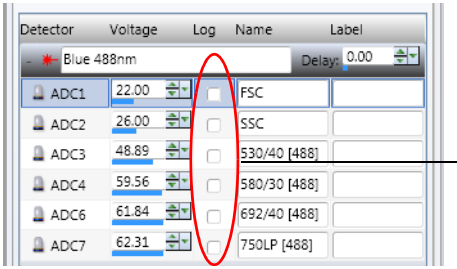
2. Click **OK**.

   The worksheet clears and all plots, gates, data sources, and statistics are deleted.

3. (Optional) Add header or footer information to customize the worksheet.

   See Customizing worksheet properties (page 146) for more information.

4. In the **Cytometer Settings** pane, clear the **Log** checkbox for each detector to ensure that all parameters use the linear amplifier.



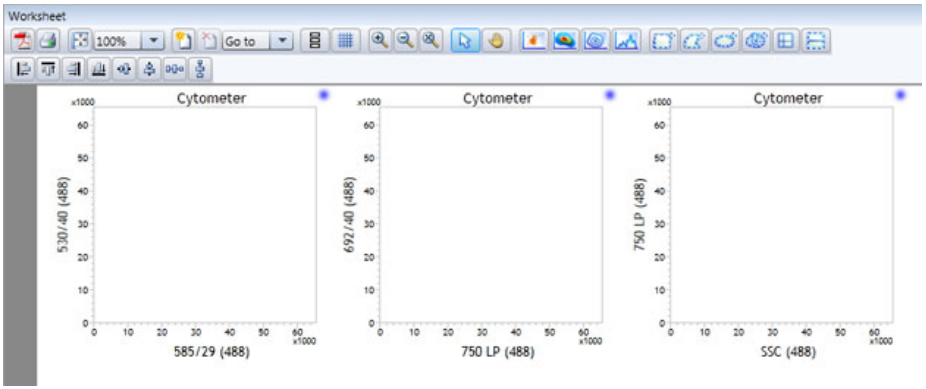
Adding plots for primary laser fluorescence parameters

To add plots for the primary laser fluorescence parameters:

1. On the worksheet, double-click the **Dot Plot** tool on the **Worksheet** toolbar, then click in the worksheet to create several plots.
2. To stop creating multiple dot plots, select a new tool such as the pointer icon used to select worksheet elements.
3. Use the alignment tools to align the plots on the worksheet, then right-click the x- and y-axis parameters and select different ADC parameters.

   Modify the dot plots for the primary (blue) laser fluorescence parameters and one plot for side scatter. For example, for an instrument detecting four fluorescence parameters using the blue laser, create the following dot plots:

   - 530/40 (488)vs 585/29 (488)
   - 692/40 (488) vs 750 LP (488)
   - SSC (488) vs 750 LP (488)



Adding and customizing a statistics view

To add and customize a statistics view for the primary laser fluorescence parameters:

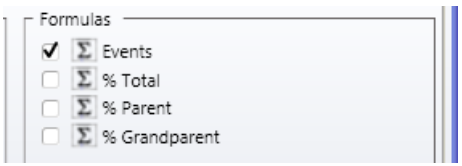
1. Right-click a plot, then select **Statistics View**.

   A statistics view appears in the worksheet.

2. Right-click the statistics view, then select **Edit Statistics View**.

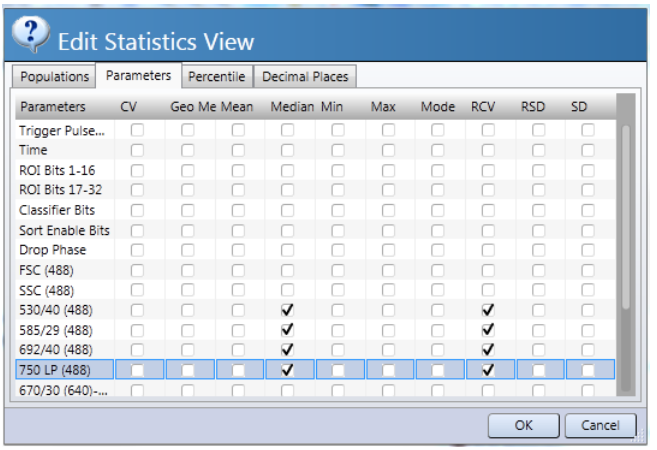
   The Edit Statistics View dialog opens.

3. In the **Populations** tab, under **Formulas**, select only the **Events** checkbox.



4. Click the **Parameters** tab.

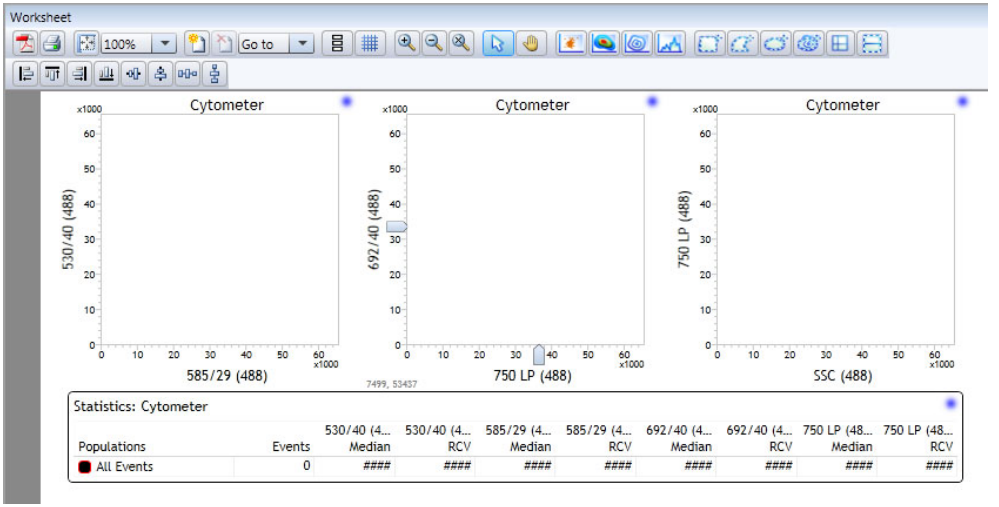
5. For each primary laser fluorescence parameter (488), select the **Median** and **RCV** checkboxes.



6. Click **OK**.

7. Drag the corners of the statistics view to size it to fit the width of the worksheet.

   The worksheet should look like the following figure.



Note that if you need more viewing or printing space for the statistics view, you can create separate statistics views for each plot, or any combination of plots.

Adding an FSC vs SSC plot and a statistics view

To add an FSC vs SSC plot and a statistics view:

1. Create a dot plot for FSC vs SSC.
2. Right-click the dot plot(s) and select **Statistics View**.

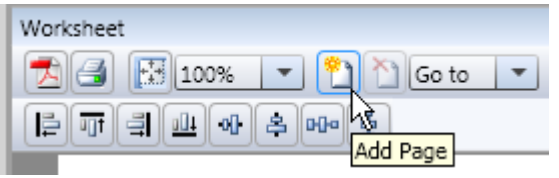
   A statistics view appears in the worksheet.
3. Right-click the statistics view and select **Edit Statistics View**.

   The Edit Statistics View dialog opens.
4. In the **Populations** tab, under **Formulas**, select only the **Events** checkbox.
5. Click the **Parameters** tab.
6. For FSC and SSC, select the **Median** and **RCV** checkboxes.
7. Click **OK**.
8. Drag the corners of the statistics view to size it to fit the width of the worksheet.

Adding plots and statistics for all other configured lasers and parameters

To add plots and statistics for all other configured lasers and parameters:

1. Create plots, select parameters, and add a statistics view for each laser.
2. If you require more space for your additional plots, click the **Add Page** tool on the **Worksheet** tool bar to add pages.



Next step Preparing beads for QC (page 276)

More information

- About settings files (page 110)
- Working with settings files (page 113)
- Viewing the cytometer status (page 119)

Preparing beads for QC

Introduction This topic describes how to prepare beads for alignment and QC.

Required materials

- BD Falcon 5-mL polypropylene sample tubes
- 3-µm SPHERO Ultra Rainbow beads
- 1 mL of sheath fluid

Preparing beads

To prepare a tube of beads:

1. Add at least 1 drop per 0.5 mL of sheath fluid to a tube.
2. Load the tube of Ultra Rainbow beads onto the sample tube holder and close the tube-lock lever.
3. In the **Pressure Console** pane, click **Sample** to start the sample flow.

Next step Aligning the stream (page 277)

More information

- Introducing a sample into the system (page 264)

Aligning the stream

Introduction

This topic describes the method for aligning the stream to the pinholes, as well as centering the stream to hit the center of the stream drain and ensuring that the Accudrop laser is intercepting the side and center streams equally.

Required materials

50-mL conical tube

Before you begin

Caution: Shock hazard. You must power off the deflection plates before manually opening or adjusting them.

1. Start up the system.
2. Ensure that the deflection plate power is off.

   See System controls (page 29) for more information.
3. Remove the plate access panel.
4. Manually open the deflection plates to prevent the sheath fluid from splashing on the plates and spraying.

   To remove the plates, lift up slightly and out (towards you).

Testing the stream alignment

To test the stream alignment:

1. Place a 50-mL conical tube in the two-way sort device installed on the sort stage so that the tube is centered below the stream drain.

2. Remove the flush bucket so that the stream passes through the illumination chamber into the sort chamber.

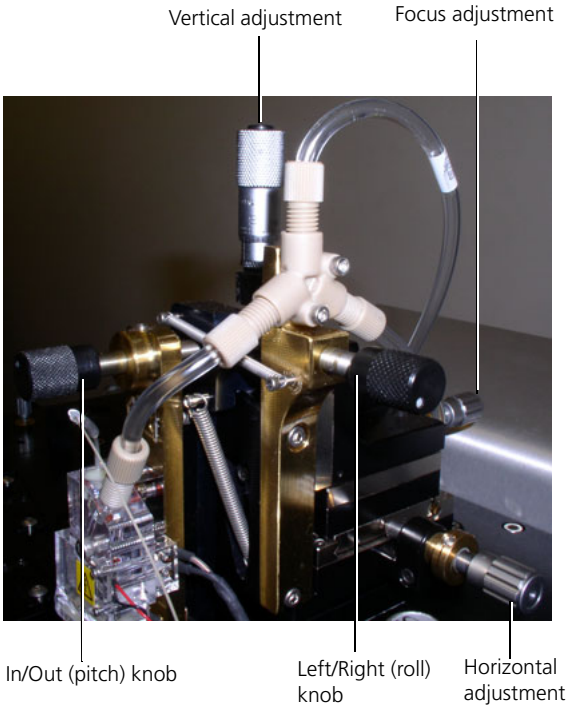
Caution! The stream might splash and spray out of the stream drain if the drain is clogged or vacuum is not applied.

Note: The plate access panel and Sort Chamber door must be closed for the Accudrop laser to illuminate.

3. In the **Pressure Console** pane, click **Illum** to illuminate the stream just above the drain.

Adjusting the nozzle stage

The nozzle assembly provides adjustment knobs used to center the stream on the pinhole and to center the stream into the drain.



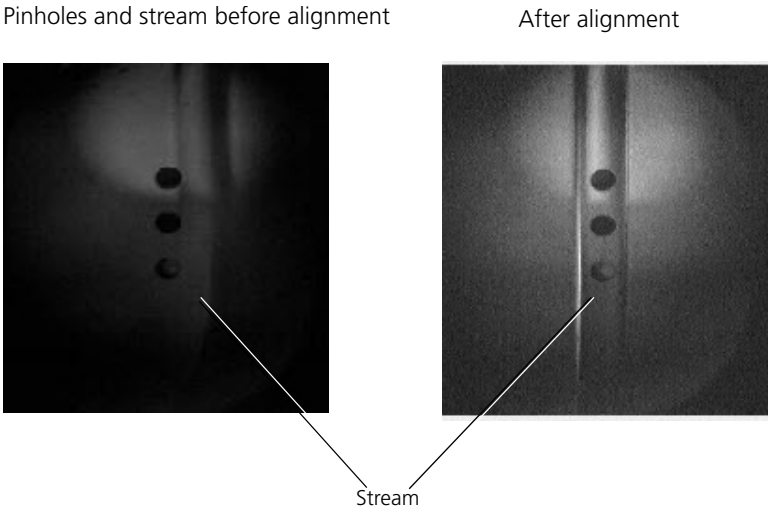
Adjustment	Description
Vertical (top silver knob). Pinhole alignment.	Moves the stage vertically.
Focus (silver knob at the back of nozzle stage). Pinhole alignment.	Moves the stage in and out perpendicular to the laser light path.

Adjustment	Description
Horizontal (silver knob on right side of nozzle). Pinhole alignment.	Moves the stage side to side, parallel to the laser light path.
Pitch (black knob front of nozzle stage). Drain/ Accudrop alignment.	Moves the nozzle pitch back and forth to the stream drain.
Left/Right (roll) black knob. Drain alignment.	Moves the nozzle roll left and right to the stream drain.

Aligning the stream to the pinholes

To align the stream to the pinholes:

1. Adjust the horizontal adjustment knob so that the stream is placed over the pinholes.
2. Adjust the focus adjustment knob so that the stream is in focus with crisp stream edges.

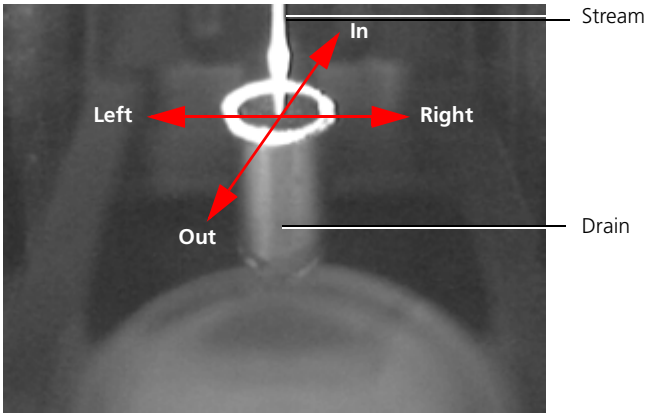


Aligning the stream with the drain

To align the stream with the drain:

1. Adjust the In/Out and Left/Right knobs on the nozzle stage to move the stream to the center of the drain, if needed.
2. Ensure that the stream is still focused on the pinholes.

   You might need to make small adjustments and go between aligning the stream to the pinhole and to the drain to get an accurate alignment.



Aligning the side and center streams to the Accudrop laser (optional)

After you align the stream with the pinholes and the drain, you can use the Accudrop laser to optimize the alignment of the side and center streams to the Accudrop laser (the stream is brightest when the laser is aligned with the stream). Ensuring that the Accudrop laser is illuminating the side and center streams equally is important for determining the most accurate drop delay before sorting.

To align the side and center streams with Accudrop:

1. Optimize the stream breakoff.
2. Move the deflection plates into the sort position.
3. Turn on the deflection plates.
4. Test and then optimize the side streams.

5. View the side streams in the stream drain image.
6. Ensure that both the side and center streams are illuminated by the Accudrop laser. Adjust the In/Out pitch and Left/Right roll knobs if necessary.
7. Confirm that the stream is still focused and aligned on the pinholes.

   If the stream is not focused and aligned on the pinholes, adjust the nozzle stage focus and horizontal adjustment. Then repeat steps 5 through 7 until the Accudrop laser is aligned with the side and center streams and the stream is centered and focused on the pinholes.
8. Turn off the test streams.
9. Turn off the deflection plates.

Next step

Aligning the primary laser to the core stream (page 283)

More information

- Aligning and optimizing the optics workflow (page 268)
- Preparing beads for QC (page 276)
- Optimizing the drop breakoff (page 321)
- Optimizing the side streams (page 322)

Aligning the primary laser to the core stream

Introduction

This topic describes how to optimize a fluorescence channel by aligning the primary laser into the sample stream. In this procedure, the blue (488-nm) laser is the primary laser.

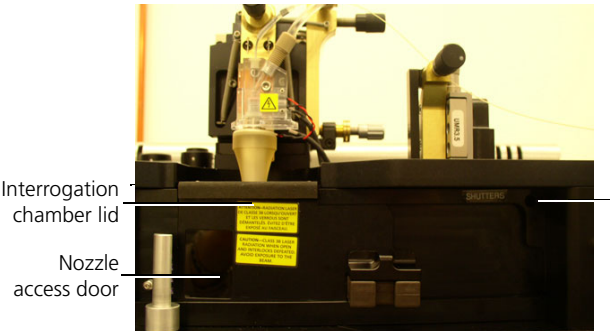
Before you begin

Prepare a tube of 3-µm SPHERO Ultra Rainbow beads (1 drop per 0.5 mL of filtered sheath fluid).

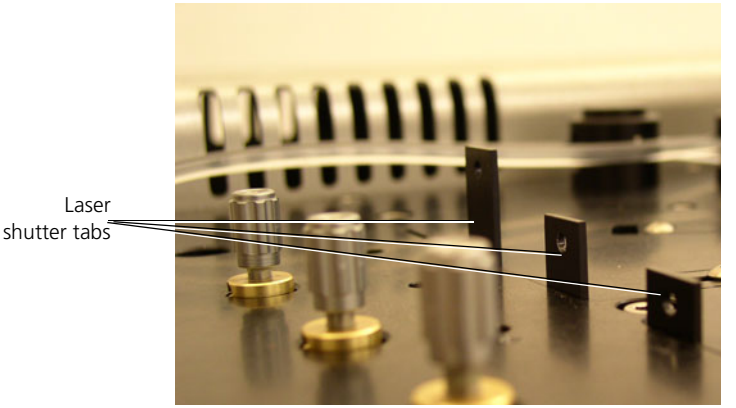
Procedure

To align the primary laser to the core stream:

1. Close the nozzle access door and ensure that the chamber lid is in place.
2. Place your finger over the reset sensor in the upper-right corner of the chamber door to reset the safety interlock.



3. Open the shutter for the 488-nm (primary) laser by pulling the laser shutter tab to the top position.



4. Load the tube of Ultra Rainbow beads onto the sample tube holder and close the tube-lock lever.
5. Verify that the sample pressure offset is about 1.0 psi.

   Use the Pressure Control pane to adjust the sample offset, if necessary.

   See Introducing a sample into the system (page 264).
6. In the **Pressure Console** pane, click **Sample** to start running beads.
7. Lower the offset as necessary to produce a narrow core stream which will reduce the sample CVs.

   If the event rate is too low, add more beads to your sample.
8. In the **Pressure Console** pane, click **Boost** for a few seconds to deliver sample to the laser more quickly, if needed.

Next step

Optimizing the fluorescence signal for the primary laser (page 285)

More information

- Aligning and optimizing the optics workflow (page 268)

Optimizing the fluorescence signal for the primary laser

Introduction

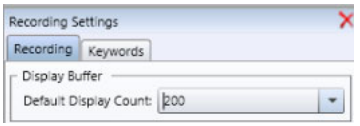
This topic describes how to use BD FACS Sortware to optimize the fluorescence signal for the primary laser.

Procedure

To optimize the fluorescence signal for the primary laser:

1. Restore the user-defined QC workspace.
2. Click **Recording** on the **BD FACS Sortware** toolbar.

   The Recording Settings pane opens.
3. Change the **Default Display Count** to *200*.



   The default display count dictates the default refresh rate. While making adjustments, it is helpful to have a low display count, which gives a quick refresh rate.
4. Click a plot that has the primary (typically blue) laser parameters.
5. Use the blue laser alignment knob to maximize the bead fluorescence signal in the plots associated with the laser you are optimizing.

6. Use the stream focus to optimize the signal.
7. Repeat steps 5 and 6 until the primary laser signals are at maximum intensity.
8. Once the signal has been optimized, adjust the fluorescence parameter photomultiplier tube voltages (PMTVs) so that the bead population is at a median of approximately 45,000 for each blue laser parameter.
9. Draw a gate around the primary laser signal.

Next step

Optimizing the forward scatter signal (page 286)

More information

- About settings files (page 110)
- Working with settings files (page 113)
- Viewing cytometer details (page 130)
- Worksheet overview (page 140)

Optimizing the forward scatter signal

Introduction

This topic describes how to adjust the forward scatter stage to maximize the signal from the forward scatter detectors.

Before you begin

- Open your QC workspace.
- Align the fluorescence detectors.

Optimizing the forward scatter signal

To optimize the forward scatter signal:

1. Adjust the horizontal and vertical knobs on the forward scatter stage to maximize the signal from the detector in the plot, if needed.

2. Adjust the FSC and SSC voltages to place the bead population at a mean of approximately 25,000.

Next step

Optimizing additional lasers (page 288)

More information

- Optimizing the fluorescence signal for the primary laser (page 285)

Optimizing additional lasers

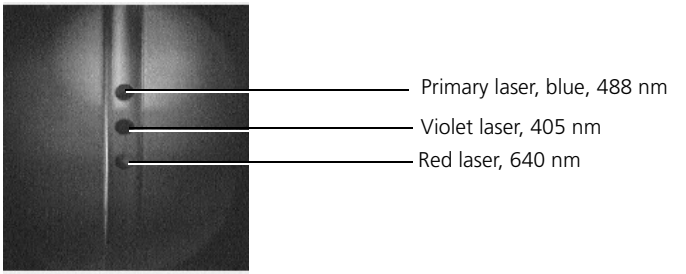
Introduction

This topic describes how to optimize additional lasers that your system might include.

About additional lasers

The primary laser (through the top pinhole) is the system trigger. With multi-laser systems, you need to adjust the laser delay to synchronize the signals from additional lasers.

When a system has more than one laser, the signal from the optional lasers need to be optimized also, which includes adjusting the laser to maximize the signal and adjusting the laser delay.



Before you begin

Ensure that the primary laser signal and the forward scatter signals are optimized.

Optimizing additional lasers

To optimize additional lasers:

1. In the QC workspace worksheet, view a plot that has the parameters for the laser that you are aligning.

   If the plot does not exist on the worksheet, create a new dot plot and statistics view. The statistics view should include total events, median, and rCV for the laser that you are aligning.

2. Use the laser alignment knob to maximize the bead fluorescence signal in the plots associated with the laser that you are optimizing.

The goal is to achieve the maximum signal.

3. In the **Cytometer Settings** pane, locate the laser that you want to adjust in the list.
4. Adjust the laser delay in small increments in the **Delay** field using the mouse scroll wheel, data slider, or keyboard keys.

   Adjust the delay above and below the current value to find the delay that gives the highest fluorescent signal in the plot.

   See Setting numeric values in panes and dialogs (page 90) for more information.
5. Repeat steps 2 and 4 until the signal is maximized in the plot.
6. Adjust the PMTV to place the bead population at a median of about 45,000 for each fluorescent parameter of the laser that you are aligning.

   You need to adjust the PMTVs as you are optimizing the signal if the bead population is off-scale.

Next step

Creating an FCS file to record laser alignment (page 290)

More information

- Optimizing the forward scatter signal (page 286)

Creating an FCS file to record laser alignment

Introduction

This topic describes how to create an FCS file to record laser alignment.

Procedure

After the instrument alignment is optimized for all lasers, record an FCS file for future reference.

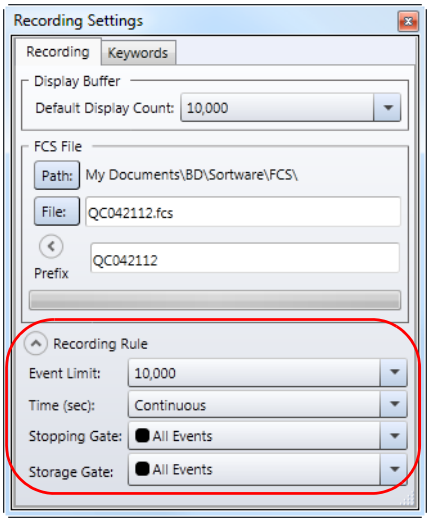
To save a data file to record laser alignment:

1. Ensure that the laser shutters for all applicable lasers are open.
2. Click **Recording** on the **BD FACS Sortware** toolbar.

   The Recording Settings pane opens.
3. Under **FCS File**, click **Path**.

   The Browse For Folder dialog opens.
4. Navigate to the folder where you want to store QC data, and click **OK**.
5. In the **File** field, name the new QC data file.

6. Under **Recording Rule**, modify the recording rules as needed.



7. Decrease the sample offset if needed so that you have a small core stream.
8. In the **Acquisition Dashboard**, click **Acquire**, then click **Record** to record the data file.

Next step

Saving the QC workspace (page 292)

More information

- Laser delay (page 52)
- Adjusting PMT voltages and using optional integrators (page 125)
- Optimizing the forward scatter signal (page 286)
- Verifying alignment using the target source (page 293)

Saving the QC workspace

Introduction

This topic describes how to save the QC workspace.

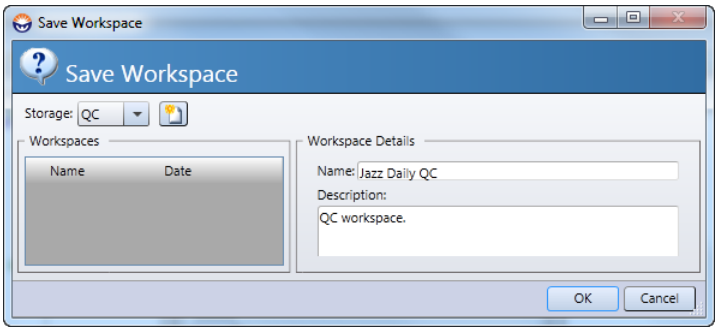
Procedure

To save the QC workspace:

1. Select **File > Save > Workspace.**

   The Save Workspace dialog opens.

2. Under **Workspace Details**, type a useful name for the QC workspace in the **Name** field (for example, *Jazz daily QC)*.



3. (Optional) Type a description of this workspace template in the **Description** field.

4. Click **OK**.

   You can restore the QC workspace whenever you want to perform QC.

Next step

Verifying alignment using the target source (page 293)

More information

- Aligning the stream (page 277)
- Optimizing additional lasers (page 288)

Verifying alignment using the target source

Introduction

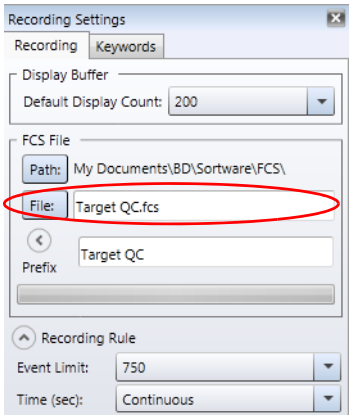
This topic describes how to use a target data source (FCS file) to verify instrument alignment.

The following procedures describe how to create a QC target FCS file specifically for this purpose and apply it as a target source. To use the target source feature, the target source FCS file configuration must match the same configuration as the cytometer data source.

Recording a target source FCS file

To record a target source FCS file:

1. In the **Recording Settings** pane, create a file which contains a few specific events (for example, set the event limit to *500*, and set a stopping gate on singlets). In the **File** field, name the file (for example, *Target QC*).



   See Creating an FCS file to record laser alignment (page 290) for instructions on creating an FCS file using the Recording Settings pane.

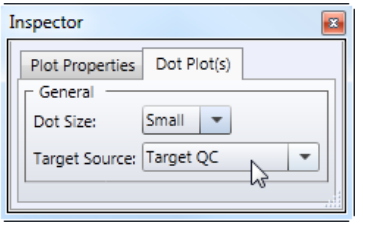
2. In the **Acquisition Dashboard**, click **Acquire,** then click **Record** to record the data file.

Applying the target source

You can add a target source to your plots to show what your optimized alignment looks like.

To apply the target source:

1. Select the plot(s) that you want to apply the target source to.
2. In the **Inspector**, click the **Dot Plot(s)** tab.
3. In **Target Source** field, select the target QC FCS file.



The target source appears in gray as an overlay (bottom layer) in the plot.

4. (Optional) Save the QC workspace with the target file displayed.
5. Acquire the sample and view where the live events are in relationship to the gray target data.

   If the live events are close to the target data, the alignment is consistent. If not, the lasers alignment needs adjustment or the PMTV settings need adjustments to match medians of 45,000 (fluorescent parameters) and 25,000 (scatter parameters).

More information

- Optimizing the forward scatter signal (page 286)
- Laser delay (page 52)
- Adjusting PMT voltages and using optional integrators (page 125)
- Saving the QC workspace (page 292)

17

Optimizing system settings for samples

This chapter includes these topics:

Creating plots and gates for optimization

Introduction

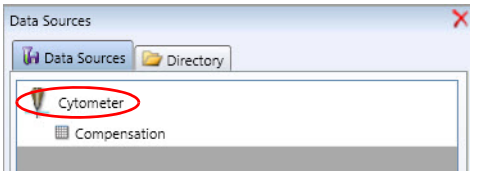
This topic describes how to create plots and gates that you can use to optimize the fluorescence settings for a sample. This topic describes basic optimization tasks and does not include compensation.

The following procedure uses 8-peak beads to illustrate the example.

Creating plots for optimization

To create plots to optimize settings for your sample:

1. Select **File > New Workspace** to start with a new workspace, if needed.
2. In the **Data Sources** pane, select **Cytometer** as the data source.

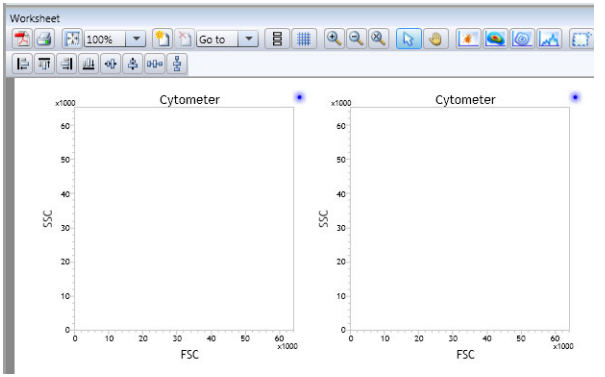


3. Double-click the **Dot Plot** tool on the **Worksheet** toolbar, then click twice in the worksheet to create two plots (scatter and fluorescence).

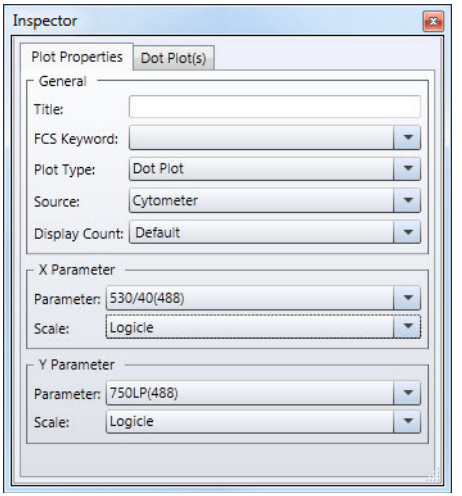
   To stop creating multiple plots, click the pointer icon (used to select other worksheet elements).

4. Use the alignment tools on the **Worksheet** toolbar to align the two plots.

Verify that the first plot (scatter) displays FSC vs SSC for the x- and y-axis labels.

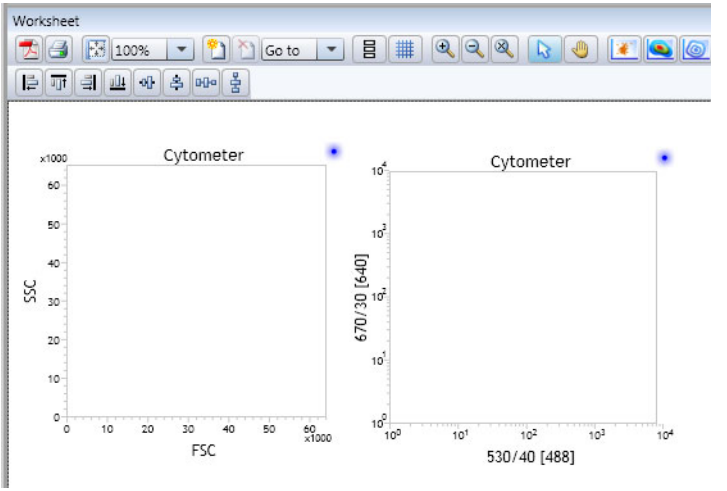


5. Click the second plot (fluorescence) to enable the **Plot Properties** tab in the **Inspector.**



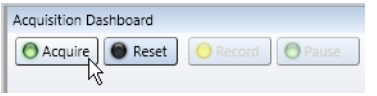
6. Under **X Parameter,** select *530/40(488)* from the **Parameter** menu.

7. Under **Y Parameter,** select *670/30(488)* from the **Parameter** menu.



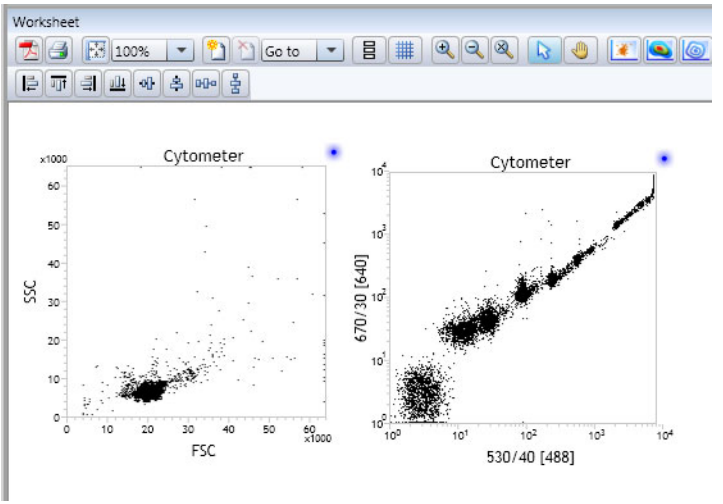
You can substitute different fluorescence parameters as needed.

8. Click **Acquire** in the **Acquisition Dashboard** to populate the plots with data.



9. Load a tube of the 8-peak beads sample.
10. In the **Pressure Console** pane, click **Sample**.
11. In the **Recording Settings** pane, select *200* from the **Default Display Count** menu.
12. Adjust the voltages for the scatter plot to place the population onscale, if needed.

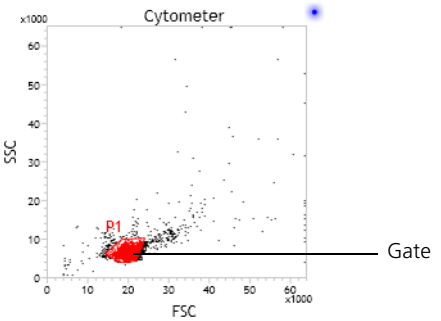
13. Adjust the voltages for the fluorescence to place all peaks onscale.



Creating gates for optimization

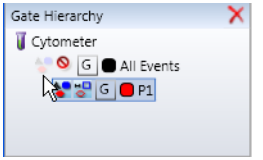
To create gates to optimize settings for your sample:

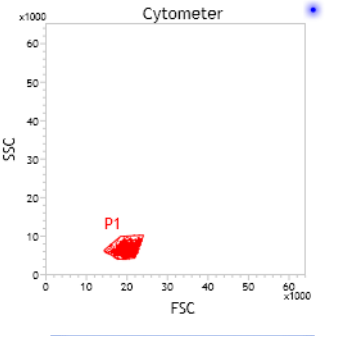
1. In the worksheet, click the scatter (FSC vs SSC) plot.
2. Create a gate on the singlet bead population.



3. In the worksheet, click the scatter plot to enable the gating controls in the **Gate Hierarchy** pane.

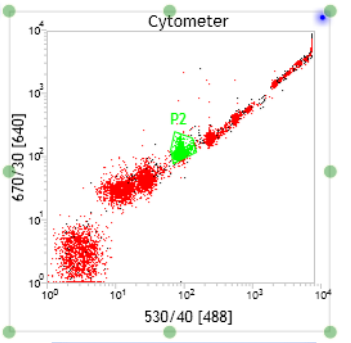
4. In the **Gate Hierarchy** pane, click the **Show/Hide** icon for the All Events population to show only the P1 population in the plot.





5. In the worksheet, click the fluorescence plot (530/40 vs 670/30).

6. Create a gate around one of the bead populations.



More information

- Creating rectangle gates (page 206)
- Creating polygon gates (page 207)
- Creating ellipse gates (page 209)
- Creating interval gates (page 212)

Optimizing with compensation controls

Introduction

This topic describes how to optimize the system using unstained and single-color controls to determine fluorescence spillover values and calculate compensation.

Examples and figures show the application of this workflow using a generic immunophenotyping assay. You should adapt the procedure steps for your specific application.

This procedure assumes that single-color controls are cells. If you plan to perform compensation with beads, your control tubes should include separate tubes with stained and unstained beads. Note that you must use ADCs to generate the raw data that you use to calculate compensation.

Workflow

Perform the following workflow steps to optimize the system with compensation controls.

Step	Description
1	Optimizing scatter parameters (page 302)
2	Optimizing fluorescence detector voltages (page 305)
3	Collecting data files for compensation (page 306)
4	Defining populations for compensation (page 308)
5	Performing auto compensation (page 311)
6	Saving a compensation matrix (page 315)

More information

- Creating plots and gates for optimization (page 296)
- Restoring (importing) a compensation matrix (page 316)

Optimizing scatter parameters

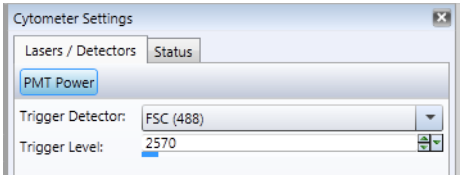
Introduction

This topic describes how to optimize scatter parameters by creating a plot, populating the plot with data, drawing a gate, and adjusting the PMTVs and trigger levels.

Procedure

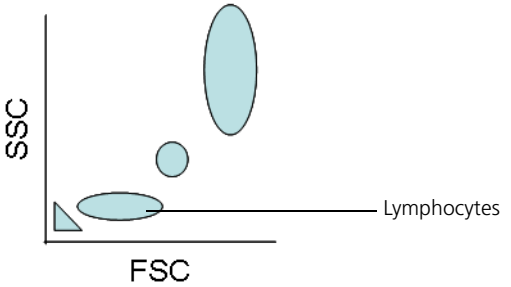
To optimize scatter parameters:

1. Select **File > New Workspace**, if needed.
2. In the **Data Sources** pane, select **Cytometer** as the data source.
3. In the **Cytometer Settings** pane, select **FSC** from the **Detection Trigger** menu.

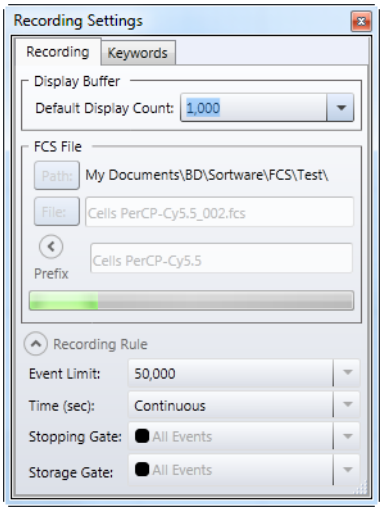


4. Create an FSC vs SSC dot plot in the worksheet.
5. Load the tube of unstained cells.
6. In the **Pressure Console** pane, click **Sample**.
7. Click **Acquire** in the **Acquisition Dashboard** to populate the plots with data.

   The plot populates.



8. Click the FSC vs SSC plot to enable the **Plot Properties** tab in the **Inspector.**
9. In the **Recording Settings** pane, select **200** from the **Default Display Count** menu.

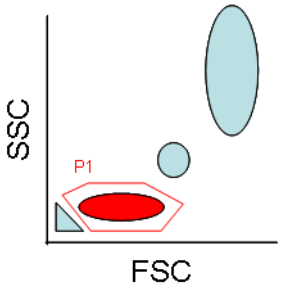


   A low display count is useful for setup and compensation. Use a higher display count when you acquire a data file.

10. Adjust the FSC and SSC voltages so that the sample is onscale.
11. In the **Cytometer Settings** pane, adjust the trigger level.

    If you set the trigger level too high, the population might not appear in the plot. If you adjust the trigger too low, you might display debris and less of the actual sample.

12. Draw a gate around the population of interest on the FSC vs SSC plot.



    You can rename or change the color of the gate if needed.

13. In the **Gate Hierarchy** pane, click the **Hide/Show** button for **All Events** to hide all events.
14. In the **Pressure Console** pane, click **Sample** to stop sample introduction.
15. Remove the sample tube.
16. In the **Pressure Console** pane, click **Backflush.** Then after approximately 10 seconds, click **Backflush** again to stop the backflush.

More information

- Optimizing with compensation controls (page 301)

Optimizing fluorescence detector voltages

Introduction

This topic describes how to optimize the fluorescence detector voltages using the single-color cellular compensation controls.

Procedure

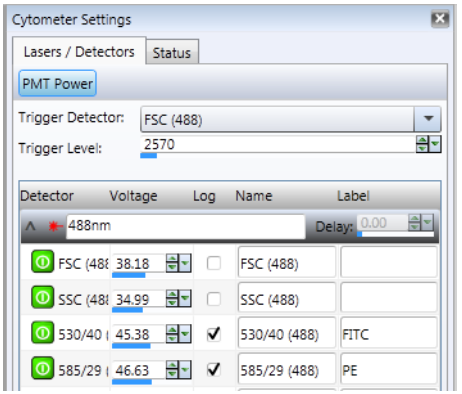
To optimize the fluorescence detector voltages:

1. In the **Cytometer Settings** pane, select **Log** for the fluorescence parameters that you will be using in your sample.
2. In the **Cytometer Settings** pane, add labels to the detectors.

   For example:

   a. Enter *FITC* for 530/40 (488).

   b. (Optional) Select preferences from the **Edit** menu and clear the **Show Parameter's Full Name** checkbox to show only the label for each parameter.



3. Create dot plots so that each parameter in the experiment is represented.
4. Load a tube containing the fully stained positive control (mixed with the negative control) on the tube holder.
5. In the **Pressure Console** pane, click **Sample**.

6. Adjust the voltage as necessary so that the negative and positive populations are onscale for each parameter.
7. In the **Acquisition Dashboard**, click **Reset** to refresh all plots.
8. In the **Pressure Console** pane, click **Sample** to stop sample introduction.
9. Remove the sample tube, and in the **Pressure Console** pane, click **Backflush.** Then after approximately 10 seconds, click **Backflush** again to stop the backflush.
10. Repeat steps 6 to 9 as necessary.

Next step

Collecting data files for compensation (page 306)

More information

- Optimizing with compensation controls (page 301)
- Optimizing scatter parameters (page 302)

Collecting data files for compensation

Introduction

This topic describes how to collect data files for compensation using cell controls. Compensation is performed by sampling a positive and negative population for each fluorescence parameter. The software uses these representative populations to compute the amount of dye spillover from each primary detector reaching other detectors. Compensation is computed using a compensation matrix (the inverted spillover matrix).

Before you begin

Optimize settings for the sample. All voltages should be verified before recording the first compensation control. Changes to fluorescence parameter voltages during or after this step can result in inaccurate spillover estimates.

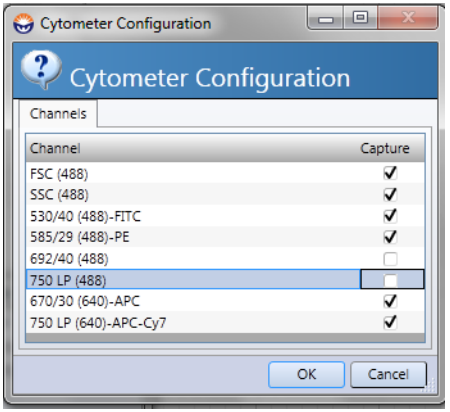
Recording controls

To record the unstained control:

1. Select **Cytometer > Configuration**.

   The Cytometer Configuration dialog opens.

2. In the **Channels** tab, select the detectors you want to record for this experiment.



3. Select **Cytometer** as the data source.

4. In the **Recording Settings** pane, click **Path** to select where you want to save the FCS file for the unstained control.

5. In the **Prefix** field, type a prefix (name) or type a name in the **File** field for the unstained control sample before you record data.

6. In the **Recording Settings** pane, under **Recording Rule**, set the event limit value for your sample (for example, *1,000* events).

7. Load the tube of unstained cells.

8. In the **Pressure Console** pane, click **Sample**.

9. In the **Acquisition Dashboard**, click **Acquire**, then click **Record** to create an FCS file for the unstained sample tube.

10. In the **Pressure Console** pane, click **Sample** and remove the sample tube.

11. In the **Pressure Console** pane, click **Backflush**. Then after approximately 30 seconds, click **Backflush** again to stop the backflush.
12. Load a tube of single-color stained cells.
13. In the **Pressure Console** pane, click **Sample**.
14. Repeat steps 6 to 13 to record the remaining fluorescence controls.

Next step

Defining populations for compensation (page 308)

More information

- Optimizing with compensation controls (page 301)
- Collecting data files for compensation (page 306)

Defining populations for compensation

Introduction

This topic describes how to define populations for compensation. The workflow provided is an example of performing compensation when each color control is in a separate tube. The same principles can be used when more than one color is in a tube.

Before you begin

Collect files for compensation for using cell controls.

Procedure summary

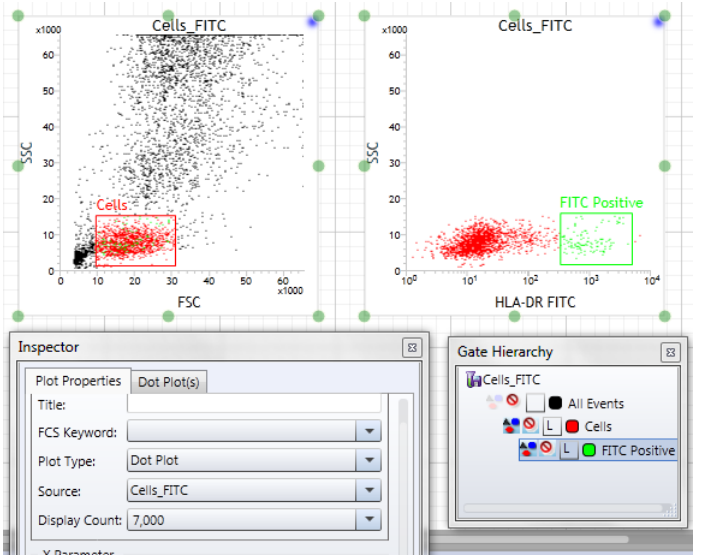
1. For each single-color tube, make the FSC vs SSC gate a local gate.
2. Draw child gates for the positive population specific to the source for a single color.
3. The negative population will be the local FSC vs SSC gate applied from the unstained control sample.

Selecting the negative control population

To identify and select a negative control population:

1. Add a new page to the worksheet.
2. In the **Data Sources** pane, select the unstained control.
3. Create two FSC vs SSC plots.
4. Draw a gate around the population of interest in the first plot, if needed.
5. Rename the gate (if needed), by typing in the **Name** field in the **Inspector** pane when the gate is selected (for example, *Cells*).
6. In the second dot plot, change the axes to fluorescence parameters using the **Inspector** pane or by right-clicking the axes labels (for example, FITC vs SSC).
7. Select the second plot.
8. In the **Gate Hierarchy** pane, right-click the created gate (*Cells*) and select **Convert to Local**.
9. In the **Gate Hierarchy** pane, click the **Hide/Show** button for **All Events** to hide all events.
10. In the **Gate Hierarchy** pane, ensure that the converted-to-local scatter gate is selected, then select the gate that you previously created (for example, *Cells*).
11. Draw a gate around the negative events in the second plot and rename the gate (for example, *Negative Control*).
12. (Optional) Change the axes in the second plot to other fluorescence parameters and make sure that your gate for negative cells is negative in all the relevant parameters.
13. Select both plots.
14. In the **Inspector** pane, change the source to one of your single-color compensation controls.
15. In the **Gate Hierarchy** pane, right-click the gate of interest (for example, *Cells*) and select **Convert to Local**.

16. Change the axes in the second plot so that one of the axes displays the parameter that matches your single-color sample (for example, if your sample is a FITC positive control, select *FITC* as one of the axes on the plot).
17. Select the gate (*Cells*) in the **Gate Hierarchy** pane, then draw a gate around the positive population.
18. Rename the new population (for example, *FITC Control)*.



19. Repeat steps 13 to 18 for the remaining compensation controls.

Next step

Performing auto compensation (page 311)

More information

- Working with gates (page 214)
- Optimizing with compensation controls (page 301)
- Collecting data files for compensation (page 306)

Performing auto compensation

Introduction

This topic describes how to select auto compensation parameters to build a compensation matrix, then select positive and negative controls and calculate compensation.

About applying compensation

Perform compensation using ADC parameters. However, when the plots that use compensation are created for sorting, create plots and gates using DSP parameters.

Before you begin

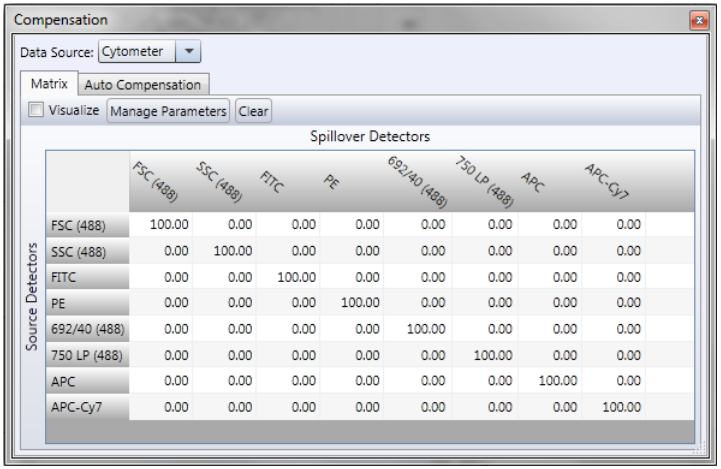
Gate populations for compensation using cells.

Selecting populations for the negative compensation control

To select the population for the negative compensation control:

1. Click **Compensation** on the **BD FACS Sortware** toolbar.

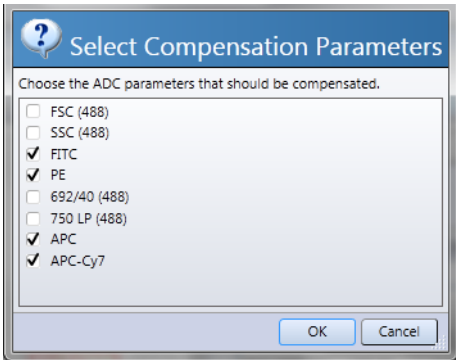
   The Compensation pane opens.



2. Click the **Matrix** tab, then click **Manage Parameters**.

   The Select Compensation Parameters dialog opens.

3. Select only the checkboxes for the ADC parameters that should be compensated.



4. Click **OK** to apply the selections and close the dialog.

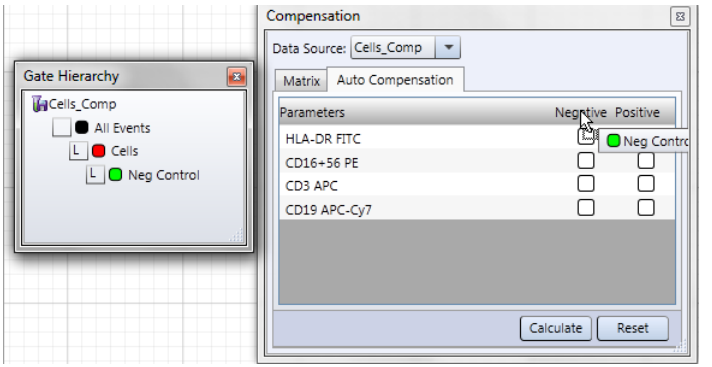
   The Matrix tab refreshes and displays the compensation matrix for the selected parameters.

5. Click the **Auto Compensation** tab.

6. Make sure that the list of parameters includes all parameters you want to include for compensation.

7. In the **Gate Hierarchy**, drag the negative control gate to the **Auto Compensation** tab and into the **Negative** column header.

   This applies the negative control to all parameters in the Auto Compensation tab.



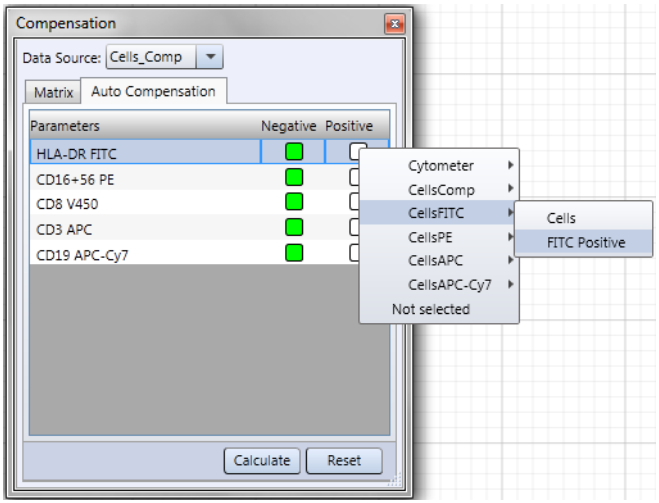
Selecting populations for the positive compensation control

To select populations for the positive compensation control:

1. In the **Positive** column, right-click the first parameter box, select the corresponding data source (FCS file) that contains the positive control, and then select the positive population.

   In the following example, the gate (FITC Positive) was created with the FITC-positive sample and is being assigned to the FITC-positive box. You can also drag the required populations from the Gate Hierarchy pane or the population hierarchy.

   The box changes color to reflect the assigned population. You can place the mouse cursor over each box to view the name of the population.



2. Repeat the assignments for the remaining parameters.

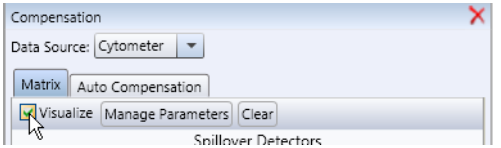
3. Click **Calculate**.

   A compensation matrix is computed from the sampled spillover values (population medians) defined in the selected populations and is uploaded into the BD FACSJazz firmware. Any unused DSP parameters (ADC parameters that are not compensated) are then removed from the list of available DSP parameters.

4. Click the **Matrix** tab, then select the **Visualize** checkbox.

   This turns on software compensation for the ADC parameters, which mimics the output of the DSPs. You can visually compare uncompensated data to compensated data by toggling the Visualize checkbox using the ADC data in memory, which is easier and more efficient than reloading matrixes into the firmware and recollecting data.

   The Visualize checkbox applies to the selected data source in the Compensation pane. If you are having trouble visualizing ADC compensation with the checkbox, verify that a spillover matrix exists for your data source (a compensation icon will be visible under the FCS file name in the Data Sources pane) and that the data source in the Compensation pane matches the data source of your plots.



   Compensation is applied through the software to the ADC (raw) data of the data source selected for visualization only. The DSP data is the actual compensated data used for classifying sort decisions. You must sort on DSP-gated parameters (hardware compensation), not on compensation-enabled ADC parameters (software compensation). DSP parameters are indicated by an asterisk (*) in front of the parameter name on the axis parameters.

Next step

Saving a compensation matrix (page 315)

More information

- Optimizing with compensation controls (page 301)
- Defining populations for compensation (page 308)
- Restoring (importing) a compensation matrix (page 316)

Saving a compensation matrix

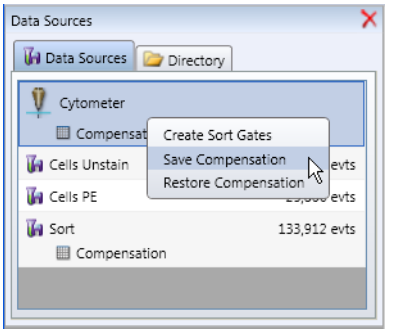
Introduction

This topic describes how to save a compensation matrix file so that you can restore specific compensation settings at any time.

Procedure

To save a compensation matrix file:

1. In **Data Sources** pane, under **Cytometer**, right-click **Compensation**, then select **Save Compensation**.



   The **Save Compensation Matrix** dialog opens.

2. In the **Name** field, type a new file name.
3. In the **Description** field, add a description about when or how this compensation matrix should be used.
4. Click **OK**.

   The compensation matrix file is saved. You can restore (import) this compensation matrix for other configurations.

More information

- Optimizing with compensation controls (page 301)
- Performing auto compensation (page 311)
- Restoring (importing) a compensation matrix (page 316)

Restoring (importing) a compensation matrix

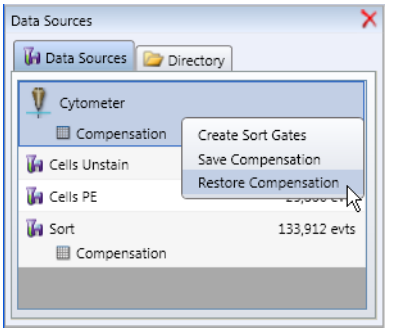
Introduction

This topic describes how to load a pre-defined compensation matrix for your configuration. Compensation matrixes can be restored on the cytometer (subsequent acquired or recorded data will have ADC visualized compensation as well as DSP compensation) or already recorded FCS files (only ADC visualized compensation will be available).

Restoring a compensation matrix file

To restore (import) a compensation matrix file:

1. In **Data Sources** pane, right-click **Cytometer** or another data source (FCS file), then select **Restore Compensation**.



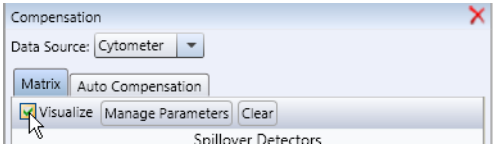
   The Restore Compensation Matrix dialog opens.

2. Under **Compensation List**, select a compensation matrix.

3. Click **OK**.

   The compensation matrix file imports into the current configuration.

4. In the **Compensation** pane, click the **Matrix** tab, select the desired data source, and then select the **Visualize** checkbox.



More information

- Performing auto compensation (page 311)
- Saving a compensation matrix (page 315)

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18

Sorting

This chapter includes these topics:

Sort setup workflow

Introduction

This topic describes the workflow steps for setting up and performing a sort.

Before you begin

- Perform QC.
- Optimize system settings for your samples.

Workflow

Step	Description
1	Optimizing the drop breakoff (page 321)
2	Optimizing the side streams (page 322)
3	Aligning the side streams with the sort tubes (page 327)
4	Determining an accurate drop delay (page 329)
5	Monitoring the sort (page 338)

More information

- Creating plots and gates for optimization (page 296)
- Saving and restoring sort layouts (page 337)
- Working with sort reports (page 340)
- Index sorting (page 341)

Optimizing the drop breakoff

Introduction

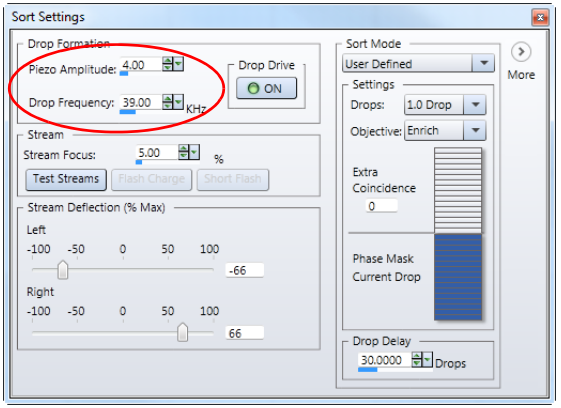
This topic describes how to optimize the drop breakoff.

Drop formation and breakoff are achieved by applying an acoustic wave to the stream. The stability of the drop breakoff is a function of nozzle size, sheath pressure, and drop frequency. The instrument has a fixed nozzle size (100 µm) and sheath pressure (27 psi). Minor adjustments can be made to the drop frequency (~39 kHz) for breakoff optimization. The optimal frequency has the highest breakoff (closest to the nozzle).

Procedure

To optimize the drop breakoff:

1. Click **Sort Settings** on the **BD FACS Sortware** toolbar.
2. The Sort Settings pane opens.



3. Set the piezo amplitude value.

   In the **Drop Formation** panel:

   a. Make sure the piezo amplitude is on.

   b. Set the piezo amplitude to *4.0*.

4. Adjust the drop frequency to achieve the highest breakoff.

If the breakoff is too high to visualize, lower the piezo amplitude to keep it on screen.

No breakoff

Initial breakoff

Adjusted breakoff

Highest breakoff and optimal frequency

Next step

Optimizing the side streams (page 322)

More information

- Sort setup workflow (page 320)

Optimizing the side streams

Introduction

This topic describes how to bring the breakoff into phase with the charge cycle, thus optimizing side-stream deflection, which helps maximize sort recovery.

Before you begin

- Verify that the deflector plates voltage is turned off. Make sure that they are clean and dry.
- Make sure that a waste collection device is in place to catch the test stream.
- Make sure that the stream breakoff has been optimized.
- Verify that the deflections plates are in the closed position, replace the plate access panel (interlocked to the Accudrop laser and deflection plates), and close the sort chamber door (interlocked to the Accudrop laser and deflection plates).

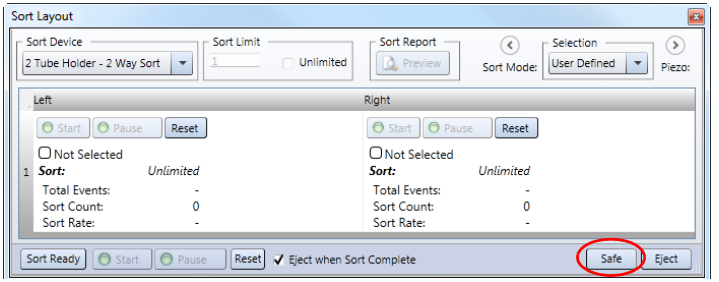
Optimizing the side streams with a flash charge

To optimize the side streams with a flash charge:

1. Close the nozzle access door (interlocked to the drop charge).
2. Turn on the plates by pressing the PLATES button (or clicking **Plates** in the **Pressure Console** pane).

   The PLATES button illuminates when the voltage is on.
3. Open the **Sort Layout** pane.
4. Under **Sort Device**, select **2 Tube Holder - 2 Way Sort.**

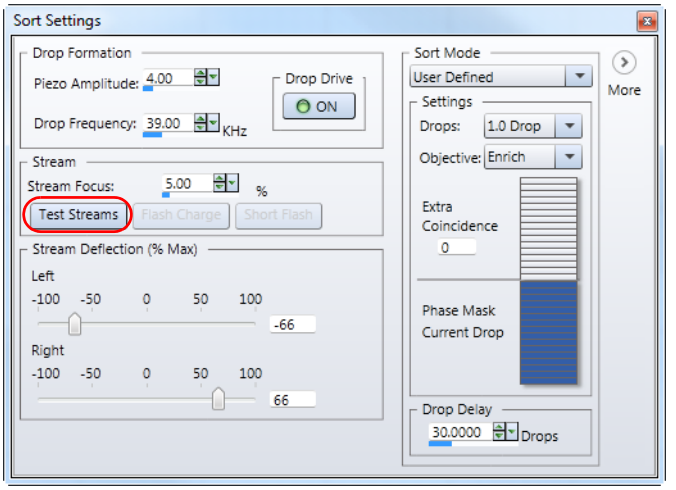
   The left and right sort positions appear.
5. Click **Safe** to move the sort tray so that the center of the waste receptacle aligns under the stream drain.



6. Click **Sort Settings** on the BD FACS Sortware toolbar.

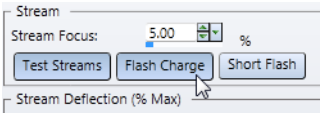
   The Sort Settings pane opens.
7. Under **Stream**, click **Test Streams** to charge the drops.

Sort streams should now be visible in the sort stream image.



If the streams are fanning or not visible, adjust the piezo amplitude.

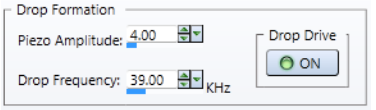
8. Click **Flash Charge** to test the synchronization of the drop formation with the charge cycle.



If the side streams are not optimally deflected, the drop formation is not in phase with the charge cycle.

9. Under **Drop Formation**, in the **Piezo Amplitude** field, adjust the amplitude until the side streams are optimally deflected. Keep the breakoff in camera view.

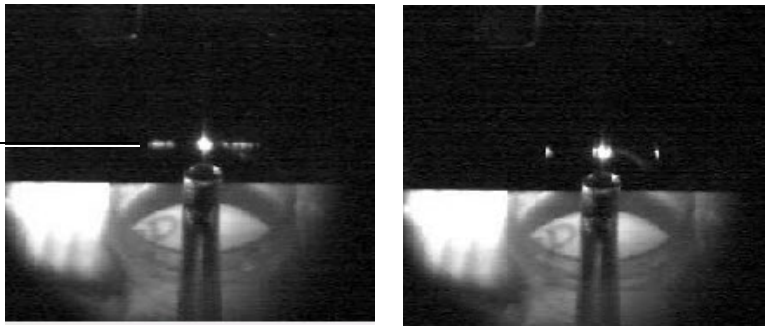
There should be little to no difference between test streams with flash charge on or off. Short flash can be used to further optimize side streams.



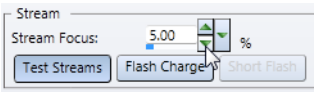
Before amplitude adjustment

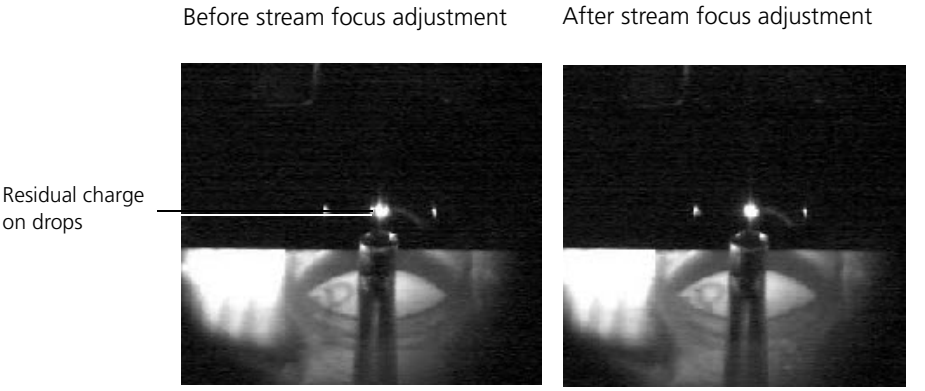
After amplitude adjustment

Fanning of main stream



10. Under **Stream**, click **Flash Charge** again to turn it off.
11. Under **Stream**, adjust the **Stream Focus** value to minimize the center stream fanning.





12. Click **Test Streams** to turn off the side stream deflection.
13. View and note how the drops are connected and note the size of the small detached drop after the last connected drop.

    The shape of the drop at the breakoff must remain constant during the sort. It may be necessary to adjust the piezo amplitude while sorting to maintain the drop shape.

Next step

Aligning the side streams with the sort tubes (page 327)

More information

- Sort setup workflow (page 320)

Aligning the side streams with the sort tubes

Introduction

This topic describes how to align the side streams with the sort tubes.

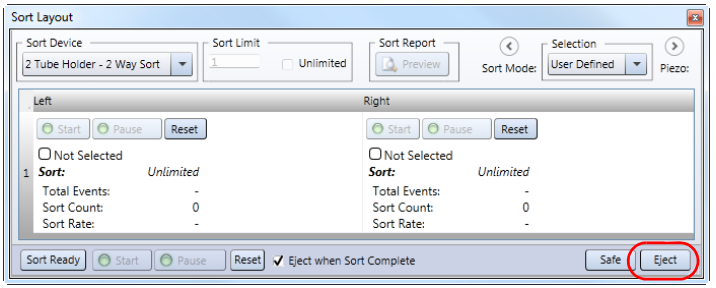
Procedure

To align the side streams with the sort tubes:

1. Click **Sort Layout** on the **BD FACS Software** toolbar.

   The Sort Layout pane opens.

2. Click **Eject** to move the sort tray into the loading position.



3. Load the tube or sort device.
4. Under **Sort Device**, select the sort device from the list.
5. Place the appropriate number of tubes in the desired positions in the sort device.
6. Click **Sort Ready** to move the sort tray to the sort ready position.
7. In the **Sort Settings** pane, click **Test Streams** to deposit sheath fluid into the tubes.
8. Verify that the side streams are aligned with the center of each tube.

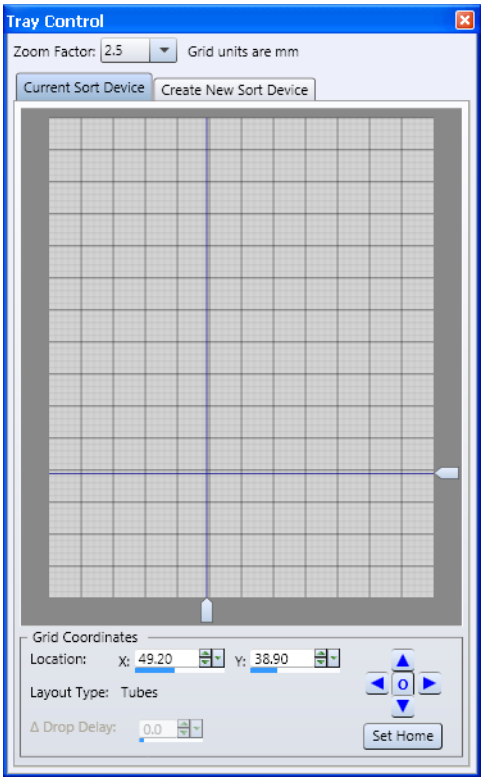
9. If the streams are too narrow or too wide, adjust the maximum stream deflection for the appropriate stream position. Repeat steps 2 through 8 until the side streams are aligned with the center of the sort tubes.

10. Adjust the tray control position if necessary.

    a. Click the **Tray Control** pane on the **BD FACS Sortware** toolbar to show the pane.

    b. If the streams are hitting too far forward or backward, adjust the Y position to align the sort device with the streams.

    c. Once the tray position has been optimized, click **Set Home** to save the x- and y-coordinates for the sort device.



Next step

Determining an accurate drop delay (page 329)

More information

- Sorting (page 319)
- Sample collection (page 58)
- Sort setup workflow (page 320)
- Optimizing the side streams (page 322)

Determining an accurate drop delay

Introduction

This topic list the options for determining an accurate drop delay, which will help ensure that you sort the correct target events.

Drop delay workflow options

The following drop delay workflows are available.

- Determining the drop delay with BD FACS Accudrop (page 330)
- Determining the drop delay using the calibration slides (page 334)
- Saving and restoring sort layouts (page 337)

More information

- Sort setup workflow (page 320)
- Aligning the side streams with the sort tubes (page 327)

Determining the drop delay with BD FACS Accudrop

Introduction

This topic describes how to determine the drop delay with BD FACS Accudrop.

How drop delay is determined

To determine the drop delay, the streams are illuminated by the Accudrop laser just below the deflection plates. When the Accudrop optical filter is in place, Accudrop beads can be viewed in the center and in the side streams as the drop-delay value is adjusted. The most precise drop-delay value yields the most particles in the side streams and the fewest in the center stream.

Before you begin

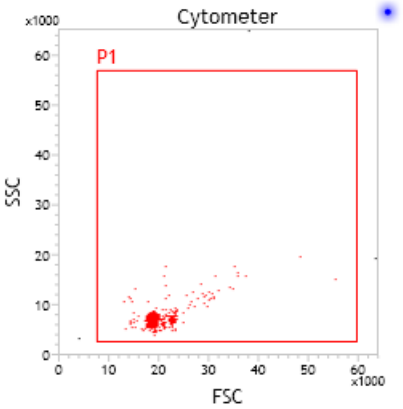
In a tube, mix one drop of BD FACS Accudrop beads (Part No. 345249) in 0.5 mL of filtered phosphate buffered saline (PBS) or sheath fluid.

Determining the drop delay

To determine the drop delay:

1. Load the tube of Accudrop beads.
2. In the **Cytometer Settings** pane, select **FSC** from the **Trigger Detector** menu.
3. Adjust the sample offset (in the **Pressure Console** pane) to achieve an event rate near 1,000.

4. In the worksheet, create a large gate encompassing all events of an FSC vs SSC plot.



This gate should include all bead populations, including aggregates.

Selecting the sort mode and starting the sort

To select the sort mode and start the sort:

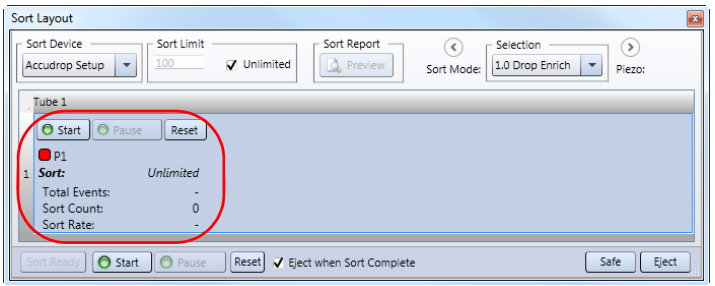
1. Click **Sort Layout** on the **BD FACS Sortware** toolbar.

   The Sort Layout pane opens.

2. Under **Sort Device**, select **Accudrop Setup**.

   The sort mode defaults to *1.0 Drop Enrich*, and the **Unlimited** checkbox is checked.

3. Assign the Accudrop gate as the target sort population.



4. Click **Sort Ready.**
5. Click **Start.**

Setting up the Accudrop filter

To set up the Accudrop filter:

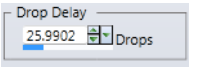
1. In the **Pressure Console** pane, click **Accudrop** to move the Accudrop filter in place.
2. Ensure that the sort chamber door is closed.

   The side stream camera should show the location of the Accudrop beads either in the center stream, the side stream, or both.

Determining the drop delay

To determine the drop delay:

1. Locate the **Drop delay** field in the **Sort Settings** dialog.



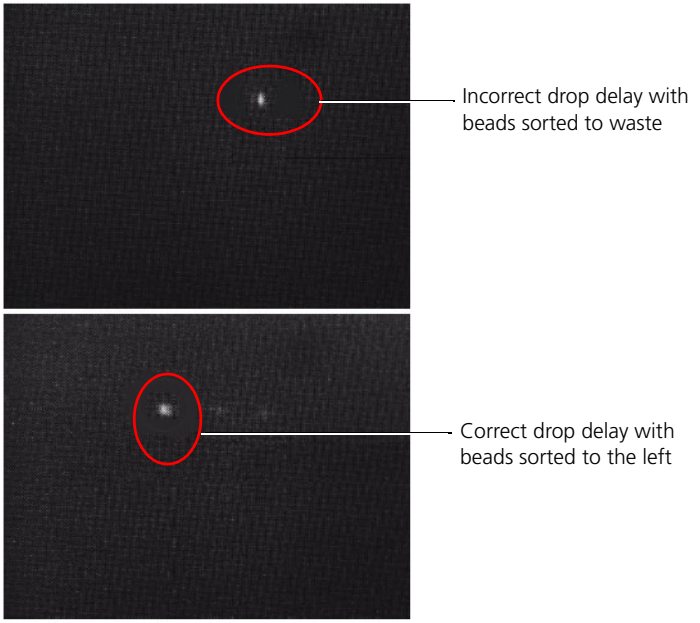
2. In the stream camera, note the brightness of the beads being sorted to the left and to the center (waste bucket).
3. Adjust the drop-delay value while monitoring the center and left sort streams.

   The correct drop delay is set when the center stream is dark (or not present) and the left stream is bright.

   The following figures illustrate correct and incorrect drop delays with the Accudrop filter in place. The image background color has been altered for illustrative purposes.

   The incorrect drop delay image shows that the illuminated beads are going to the waste bucket. The correct drop delay

image shows that the illuminated beads are being sorted to the left.



Next step

Monitoring the sort (page 338)

More information

- Aligning and optimizing the optics workflow (page 268)
- Sort setup workflow (page 320)

Determining the drop delay using the calibration slides

Introduction

This topic describes how to determine an accurate drop-delay value by sorting multiple drops forming puddles onto a microscope slide, each with a different drop-delay value.

A precise drop-delay value results in more beads in a target puddle. There are multiple ways to sort onto a slide to determine the delay. This topic describes the calibration slide method.

Required materials

- Fluorescent beads (larger beads are easier to see and count on the slide but may cause side stream fanning)
- 0.5 mL of filtered sheath fluid
- 12 x 75-mm glass slides
- A fluorescence microscope

Before you begin

- Prepare a tube of fluorescent beads (1 drop per 0.5 mL of filtered sheath fluid).
- Optimize the stream breakoff.

Preparing a calibration slide

To prepare a calibration slide:

1. Run a tube of fluorescent beads.
2. Create an FSC vs SSC plot.
3. Draw a gate around the singlet bead population in the plot.
4. Adjust the **Sample Offset** value to achieve an event rate of 500 beads per second.
5. Click **Sort Layout** on the BD FACS Sortware toolbar.

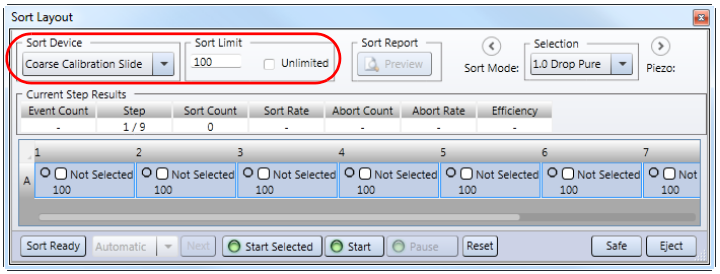
   The Sort Layout pane opens.
6. Click **Eject** to access the sort tray.
7. Place a microscope slide in the front position of the universal sort device.

Sorting onto a calibration slide

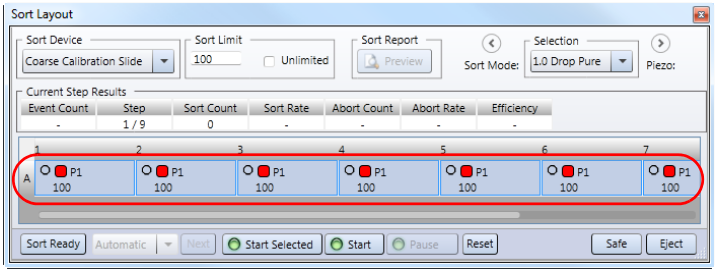
To sort onto a calibration slide:

1. In the **Sort Layout** pane, under **Sort Device**, select **Coarse Calibration Slide**.

   The sort mode and sort limit settings update automatically with the defined values for the coarse calibration slide.



2. Select all wells and assign the singlet bead gate for the fluorescent beads as the target sort population.



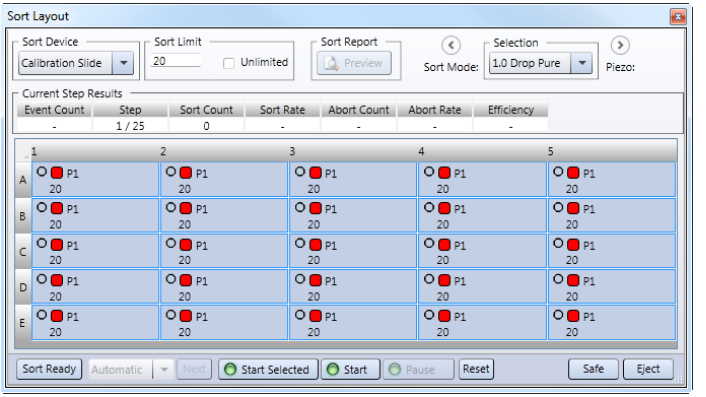
3. Click **Sort Ready** and ensure that the left side stream is aligned with the slide.

4. Click **Start** to start the sort.

   The Sort Drop Delay dialog opens once the sort is complete. Do not close this dialog until you have determined the proper delay in the next step.

5. Using a fluorescence microscope, count the number of beads in each puddle and identify which well contains the most beads.

6. In the **Sort Drop Delay** dialog, click the drop-delay value that corresponds to the drop with the highest bead counts.
7. Click **OK** to set the drop delay value.
8. In the **Sort Layout** pane, under **Sort Device,** select **Calibration Slide,** and repeat steps 1 through 7 to determine a precise drop delay.



Next step

Monitoring the sort (page 338)

More information

- About settings files (page 110)
- Working with settings files (page 113)
- Sort setup workflow (page 320)
- Determining an accurate drop delay (page 329)

Saving and restoring sort layouts

Introduction

After you have set up your sort, you can save all settings in the Sort Layout pane except the piezo amplitude.

About saving and restoring sort layouts

You can save a sort layout and restore it at any time. Note that the sort layout is also saved when you save a workspace. If you save a specific sort layout separately, you can restore it and apply it to any workspace.

See About settings files (page 110) for more information about what information is saved.

Saving a sort layout

To save a sort layout:

1. Select **File > Save > Sort Layout**.

   The selected Save Sort Layout dialog opens.

2. Select the storage folder where you want to save the settings file from the **Storage** menu.

   See Creating storage folders (page 116) for more information about creating custom storage folders.

3. Under **Sort Layout Details**, type a name for the new settings file in the **Name** field.

4. Click **OK** to save the workspace and close the dialog.

Restoring a saved sort layout

To restore a saved sort layout:

1. Select **File > Restore > Sort Layout**.

   The selected Restore Sort Layout dialog opens.

2. Under **Sort Layouts**, select the layout you want to restore, then click **OK**.

   The Sort Layout dialog reflects the restored layout.

Monitoring the sort

Introduction

This topic describes how to monitor the sort for best results.

The timing of when a drop is charged is defined during the sort setup and drop-delay optimization. It is important to monitor the sort to ensure that the breakoff does not change, if necessary making small adjustments to the piezo amplitude. It is also important to maintain the same scatter and population percentages during the sort.

This chapter provides tips to maintain a successful sort.

Before you begin

- Make sure that you have removed bubbles from the fluidics tubing.
- Turn on the deflection plates.
- Filter all samples to 40 µm (about half the nozzle tip size) to prevent nozzle clogs and ensure optimal sorting.
- Load a sample tube.
- Record data to create pre-sort FCS files.

Monitoring the sort

To monitor the sort:

1. View the scatter plot in the worksheet and slowly increase the sample offset (if needed).

   Stop increasing the sample offset when:

   - The scatter populations shift or change shape.
   - The sorting efficiency counter is unacceptably low.

2. Monitor the following.

Category	What to look for	Troubleshooting
Sort efficiency counter (in the Sort Layout pane)	Efficiency varies depending on population percentages and sample concentration.	Decrease the sample offset to improve sorting efficiency.
Acquisition efficiency (in the Acquisition Dashboard pane)	Efficiency becomes unacceptably low.	Reduce the sample offset, or filter, or dilute the samples.
Gates and populations (in worksheets)	Populations should stay within the gates and should not move during the sort.	Decrease the sample offset or adjust the gates. Refresh the plot display rate often to confirm that populations have not moved out of the gate.
Drop image	Breakoff should be stable and remain at the same drop.	Adjust the piezo amplitude to keep the breakoff at the same drop and maintain the breakoff profile.
Stream image	Side streams should not fan or spray out of the drain.	• Verify that the 100-µm nozzle is large enough for the cells being sorted. • Adjust the piezo amplitude slightly. • Filter the cells before sorting.

3. (Optional) Save an FCS file during a long sort to maintain a record of the sort.

Saving a sort

To save a sort if a clog forms during a sort:

1. Quickly turn off the deflection plates, stop the sample flow, stop the stream, and install the flush bucket under the nozzle.
2. If you are working with nonhazardous materials, remove the collection device to avoid contamination with unwanted cells.

More information

- Sort setup workflow (page 320)
- Working with sort reports (page 340)

Working with sort reports

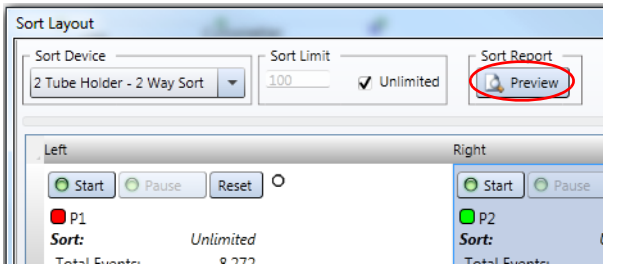
Introduction

This topic describes how to preview and print sort reports after a sort completes and how to save them as PDF files. Sort reports are automatically stored to the default location (C:\Users\My User Name\Documents\BD\Sortware\Sort Reports).

Viewing a sort report

To view a sort report:

1. Under **Sort Report**, click **Preview**.



The Report Dialog opens.

Printing a sort report

To print a sort report:

1. In the **Sort Layout**, click **Preview**, if needed.
2. In the **Report Dialog**, click the **Print** button on the toolbar.

Creating a PDF version of a sort report

To create a PDF sort report:

1. In the **Sort Layout**, click **Preview**, if needed.
2. In the **Report Dialog**, click the **PDF** button on the toolbar.

   The Select PDF Output File dialog opens.

   PDFs are created by default. This process creates a PDF to a user-specified location using the preview.
3. In the **Save in** field, select a folder.
4. In the **File name** field, type a new name for the report, then click **Save**.

More information

- Sort setup workflow (page 320)
- Monitoring the sort (page 338)

Index sorting

Introduction

This topic describes index sorting.

About index sorting

Index sorting is useful in characterizing subpopulations of phenotypically similar events using post-sort genetic, chemical, and/or metabolic applications.

Index sort mode is a special acquisition mode which records the x and y positions of the sort stage in the FCS file, allowing you to trace the events sorted to a particular location (for example, a well on a plate tray device) to the specific event that was sorted in BD FACS Sortware.

Before you begin

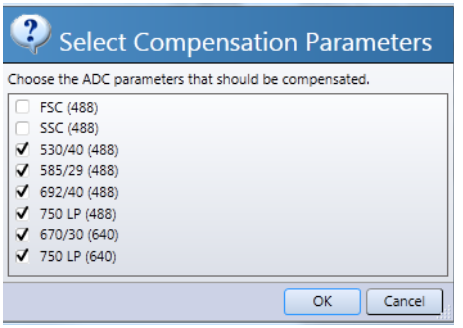
- Prepare the sort chamber for sorting by cleaning the deposition area and installing a sort device onto the sort tray.
- Optimize the stream breakoff.
- Determine the drop delay.

Setting up for index sorting

To set up for index sorting:

1. In the **Compensation** pane, click **Manage Parameters** and verify that there are at least two unassigned parameters.

   If all parameters are assigned, clear at least two compensation parameters from the ADC list before starting index sort mode. The FSC and SSC DSP channels are typically not needed for compensation and can be used for index sorting.



2. On the **BD FACS Sortware** toolbar, click **Sort Layout**.
3. In the **Sort Layout** pane, select a multi-position sort device:
   - Default multi-position formats include: 6 Well Tray, 24 Well Tray, 96 Well Tray, 384 Well Tray, and 4 x 12 Slide.
   - Custom multi-position formats.
4. Select a sort mode and sort limit.

   See Selecting the sort mode and starting the sort (page 331).

   Note: The 1-Drop Single sort mode is typically used for single-cell deposition.

5. Align the side streams to the sort device using the **Sort Settings** dialog and the **Tray Control** pane.
6. Load a sample and acquire data.
7. Ensure that the voltages, compensation, sort gates, and event rate are appropriate.
8. In the **Sort Layout** dialog, assign populations to each well sort target.
9. Stop the sample from running.

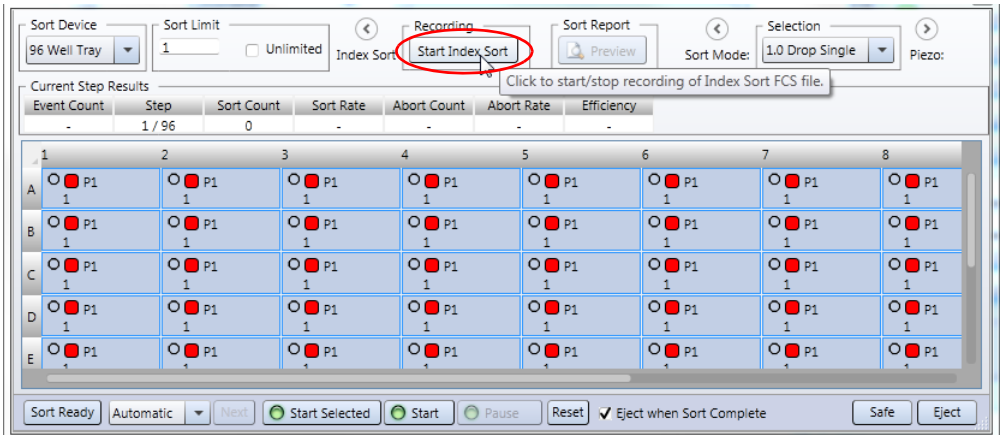
Starting an index sort

To start an index sort:

1. Make sure that the software is not currently acquiring data.

   Acquisition must be stopped so that index sort mode acquisition can start.

2. In the **Recording Settings** pane, click **Path** to select the path and file name for your index sort file.In the **Sort Layout** pane, click **Sort Ready**.
3. In the **Pressure Console** pane, click **Sample**.
4. In the **Sort Layout** pane, under **Recording**, click **Start Index Sort**.



Data is continually recorded and displayed while index sort mode is running.

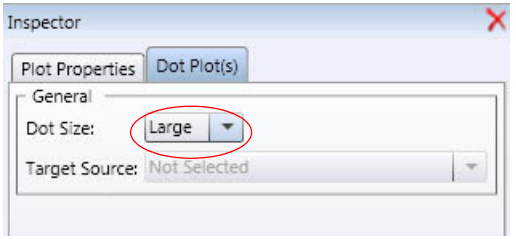
Index sort mode creates an FCS file. Using the Data Sources functions, you can generate a CSV file where all the sort deposition information and tray position information is stored on an event-by-event basis. All the events are stored in the file, including those not actually sorted. You can import this CSV file into a spreadsheet to review the results.

5. Once the sort is complete, click the **Stop Index Sort** button to stop the index sort mode.

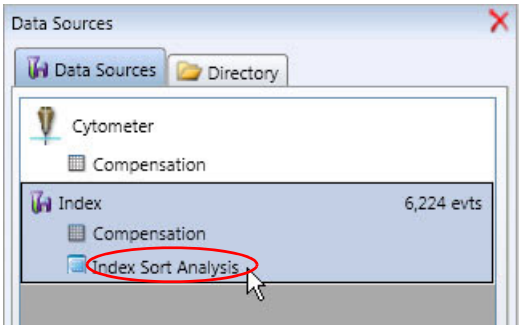
Analyzing the index sort

To analyze the index sort:

1. Select the index sort FCS file in the **Data Sources** pane.
2. Create dot plot(s) in the worksheet.
3. Select the plot(s).
4. In the **Inspector** dot plot(s) tab, select **Large** from the **Dot Size** menu.

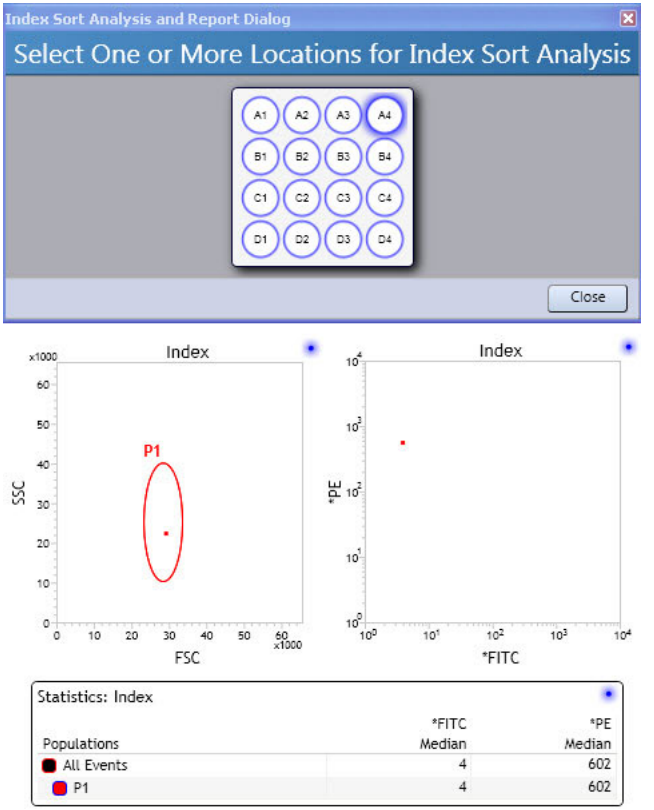


5. In the **Data Sources** pane, double-click **Index Sort Analysis** for the index sort data source of interest.



6. In the **Index Sort Analysis and Report** dialog, select one or more locations to display on plot(s).

To select multiple locations, drag the cross hair to include all locations of interest.



Viewing index sort results in a spreadsheet

You can generate an index sort report CSV file that you can import into a spreadsheet to view the results for each sort.

To generate the index sort report CSV file:

1. Create the statistics view for the index sort data source.
2. Right-click the statistics view associated with the plot you used for index sorting and select **Export Index Sort Statistics**.

   The Select Output File dialog opens.

3. Navigate to the folder where you want to save index sort reports, then select the CSV file type.
4. Click **Save**.
5. Open your spreadsheet application and open the index sort report as a CSV file.

   The following figure shows an example an index sort report for a 4 x 12 slide. To add Tray X and Tray Y medians, include them in your statistics view.

Data Source	Calibrite Mix Index_002							
			Tray X	Tray Y	530/40 blue	670/30 red	585/29 yellow-green	670/30 yellow-green
Well	Population	Events	Median	Median	Median	Median	Median	Median
A1	Scatter	1	3319	2128	9	65	4	138
A2	Scatter	1	3769	2128	9	4	2	1
A3	Scatter	1	4219	2128	8	3	5	9
A4	Scatter	1	4669	2128	7	6	2	3
A5	Scatter	1	5119	2128	11	5	314	54
A6	Scatter	1	5569	2128	12	83	1	152
A7	Scatter	1	6019	2128	17	2	877	108
A8	Scatter	1	6469	2128	8	110	4	129
A9	Scatter	1	6919	2128	10	1	1	4
A10	Scatter	1	7369	2128	8	86	2	151
A11	Scatter	1	7819	2128	9	67	1	116
B1	Scatter	1	3319	1679	20	2	978	86
B2	Scatter	1	3769	1679	12	73	5	181
B3	Scatter	1	4219	1679	11	1	1	1
B4	Scatter	1	4669	1679	11	1121	7	265
B5	Scatter	1	5119	1679	12	82	2	133
B6	Scatter	1	5569	1679	11	89	1	105
B7	Scatter	1	6019	1679	7	1	3	8
B8	Scatter	1	6469	1679	12	70	2	113
B9	Scatter	1	6919	1679	8	2	1	6
B10	Scatter	1	7369	1679	10	78	8	134
B11	Scatter	1	7819	1679	8	4	3	1
C1	Scatter	1	3319	1228	9	81	4	133
C2	Scatter	1	3769	1228	13	85	1	130
C3	Scatter	1	4219	1228	11	63	6	140
C4	Scatter	1	4669	1228	24	3	1032	127
C5	Scatter	1	5119	1228	13	92	4	137

More information

- Sort setup workflow (page 320)

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19

System shutdown

This chapter includes these topics:

System shutdown workflow

Introduction

This topic describes the workflows for performing a wet or dry shutdown of the system.

If you perform a wet shutdown, your startup workflow (the next day) is different than if you perform a dry shutdown. See Startup workflow (page 248) for more information.

About wet shutdown

Wet shutdown is useful when you plan to use the BD FACSJazz sorter the next day and you do not need to run a specific cleaning or decontamination protocol as a part of your daily maintenance.

Wet shutdown workflow

Step	Description
1	Cleaning the sample line (page 351)
2	Installing the debubble reservoir (page 352)
3	Turning off the power (page 357)

About dry shutdown

Dry shutdown is required when you need to prepare the system for a period of non-use, or when you want to run a specific cleaning or decontamination protocol in preparation for instrument service or maintenance.

Dry shutdown workflow

Stage	Description
1	Cleaning the sample line (page 351)
2	Rinsing the system (page 353)
3	Drying the fluidics lines (page 355)
4	Cleaning the sort chamber (page 356)
5	Turning off the power (page 357)

More information

- Cleaning and inspecting the instrument (page 63)
- Startup workflow (page 248)

Cleaning the sample line

Introduction

This topic describes how to clean the sample line for a wet or dry shutdown.

Required materials

- Sample tubes
- 10% bleach solution
- DI water

Procedure

To clean the sample line for a shutdown:

1. Load a tube of 10% bleach and run it for 5 minutes.
2. Load a tube of DI water and run it for 5 minutes.
3. Perform one of the following steps depending on the type of shutdown you are performing:
   - If you are performing a wet shutdown, leave the tube of DI water on the sample holder.
   - If you are performing a dry shutdown, remove the tube of DI water from the sample holder.

Next steps

- If you are performing a wet shutdown, see Installing the debubble reservoir (page 352).
- If you are performing a dry shutdown, see Rinsing the system (page 353).

More information

- System shutdown workflow (page 350)
- Startup, shutdown, and troubleshooting components (page 250)

Installing the debubble reservoir

Introduction

This topic describes how to use the debubble reservoir for a wet shutdown.

Required materials

- Debubble reservoir
- DI water
- Flush bucket

Procedure

To install the debubble reservoir:

1. While the stream is still running, place the flush bucket under the nozzle.
2. Fill the debubble reservoir with DI water.
3. Place it on top of the flush bucket.
4. Verify that the nozzle tip is submerged in the water in the debubble reservoir.
5. In the **Pressure Console** pane, click **Stream** to stop running the stream.
6. Disconnect the sheath line from the sheath tank.
7. Turn off the house vacuum and air supply.

Next step

Turning off the power (page 357)

More information

- Fluidics overview (page 32)
- Startup, shutdown, and troubleshooting components (page 250)
- System shutdown workflow (page 350)

Rinsing the system

Introduction

This topic describes the cleaning procedure to complete a dry shutdown.

Required materials

- 1 L of DI water for the sheath tank
- Flush bucket

Before you begin

Clean the sample line.

Procedure

To clean the system for a dry shutdown:

1. Install the flush bucket.
2. In the **Pressure Console** pane, click **Stream** to turn off the stream.
3. Remove the tube of DI water.
4. Turn off the air supply by switching the Air switch off.
5. Empty the sheath tank and fill it with 0.5–1.0 L of DI water.
6. Empty the waste tank.
7. Reattach the sheath tank and waste tank.
8. Turn on the air supply by switching the Air switch on.
9. In the **Pressure Console** pane, click **Rinse**, then click **Backflush** to rinse all fluid lines with DI water. Then after approximately 2 minutes, click **Backflush** again to stop the backflush.
10. Release the pressure in the sheath tank by opening the pressure release valve.
11. Bypass the sheath filter by disconnecting the sheath lines from the sheath filter and reconnecting the sheath lines together without the sheath filter.
12. Click **Rinse**, then click **Backflush** to rinse the tubing with the rest of the DI water until the tank runs dry and the sample line is not longer dripping water.
13. In the **Pressure Console** pane, click **Rinse** to turn off the flow.

Next step

Drying the fluidics lines (page 355)

More information

- System shutdown workflow (page 350)

Drying the fluidics lines

Introduction

This topic describes how to dry the fluidics lines for a dry shutdown.

Required materials

- DI water
- Flush bucket

Procedure

To dry the fluidics lines:

1. Remove the nozzle tip.
2. Place the flush bucket under the nozzle.
3. Release the pressure in the sheath tank by opening the pressure release valve.
4. Bypass the sheath filter by disconnecting the air and fluid lines from the sheath tank and connecting them to each other.
5. In the **Pressure Console** pane, click **Rinse**, then click **Backflush**.
6. Allow air to blow through the system for about 10–15 minutes to completely dry it.
7. In the **Pressure Console** pane, click **Rinse** to stop drying the system.
8. Turn off the air supply by switching the Air switch off.
9. Turn off the house air supply or air compressor and vacuum supply.
10. Empty all fluid from the waste and sheath tanks, rinse them with clean DI water, and allow them to dry overnight.

Next step

Cleaning the sort chamber (page 356)

More information

- Replacing the nozzle tip (page 71)
- Decontaminating the fluidics (page 66)
- System shutdown workflow (page 350)
- Rinsing the system (page 353)

Cleaning the sort chamber

Introduction

This topic describes how to clean the sort chamber for a dry shutdown.

Required materials

- DI water
- Kimwipes or other lint-free towel

Procedure

Caution: Biohazard! Wear protective clothing before cleaning the sort chamber.

To clean the sort chamber:

1. Verify that the deflection plates are off.
2. Clean the deflection plates with DI water. See Cleaning the deflection plates (page 64) for more information.
3. Wet a Kimwipes wipe or other lint-free towel and wipe any spills in the sort chamber or sample port area.
4. Wet a Kimwipes wipe or other lint-free towel with DI water and wipe again.

Next step

Turning off the power (page 357)

More information

- System controls (page 29)
- Cleaning and inspecting the instrument (page 63)
- Decontaminating the fluidics (page 66)
- System shutdown workflow (page 350)

Turning off the power

Introduction

This topic describes how to turn off the system power for a wet or dry shutdown.

Procedure

To turn off power for shutdown:

1. Save your workspace, if needed.
2. Select **Cytometer > Shutdown Cytometer,** then click **OK.**

   The cytometer interface is now disconnected from the instrument electronics and will turn off automatically.
3. Exit BD FACS Sortware software.
4. Turn off the computer.
5. Turn off the air supply by switching the Air switch off.
6. Turn off the main power.

More information

- Cleaning and inspecting the instrument (page 63)
- System shutdown workflow (page 350)
- Cleaning the sort chamber (page 356)

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Part 4

Reference

This part includes these chapters:

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BD FACSJazz options

This chapter includes these topics:

BD FACSJazz system options overview

Introduction

This topic lists the options and upgrades available for the BD FACSJazz system.

About options

System options described in this chapter have been validated and qualified for use with the BD FACSJazz system. If you plan to use other third-party hardware, you are responsible for validating the system and verifying the results.

Options

The following table lists the available options.

Category	Option	More information
System hardware	Aerosol evacuation unit	Aerosol management option (page 363)
	Air compressor and vacuum pump	Air compressor and vacuum pump (page 364)
	Biological safety cabinet	Biological safety cabinet (page 365)
	Temperature control	Temperature control option (page 367)
	Integrators	Integrators (page 368)
Optics	Laser upgrade	Contact your BD representative.

Ordering and installing options

Contact your BD representative for more information about ordering and installing these options.

Aerosol management option

Introduction

This topic describes the aerosol management option for the BD FACSJazz system.

Aerosol management option

The BD FACSJazz aerosol management option (AMO) uses a vacuum source to rapidly evacuate aerosolized particles through an ultra-low penetration air (ULPA) filter during routine sorting or acquisition. This unit evacuates aerosols from the sort chamber, preventing the aerosols from being circulated back through the chamber.

Using the AMO with the BD FACSJazz system

The following are some guidelines for using the AMO with the BD FACSJazz system:

- Operate the AMO at 20% during sorting.
- Operate the AMO at 100% for 1 minute after a clog, before opening the sort chamber door.
- Change the filter according to the manufacturer's instructions.

Installation

BD Service performs installation.

More information

- BD FACSJazz system options overview (page 362)

Air compressor and vacuum pump

Introduction

This topic describes the air compressor and vacuum pump options for the BD FACSJazz system.

Air compressor option

An external air compressor is necessary if you do not have a compressed air supply in your laboratory. The external air compressor provides a constant supply of clean, dirt- and oil-free air at sufficient pressure to achieve the needed 27 psi for the BD FACSJazz system during operation.

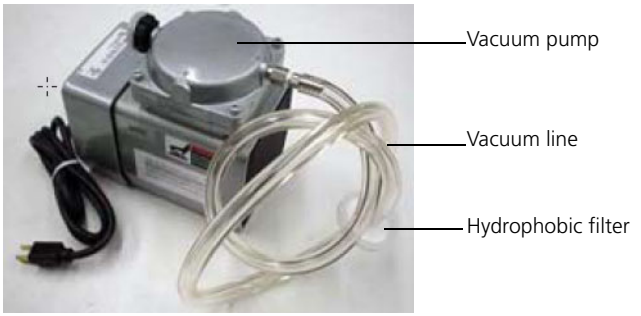
The air compressor runs directly from the wall power source, independent from the instrument power circuitry. Make sure that you run the system with clean, dry air.

Air compressor installation

Attach the air line to the sheath tank valve.

Vacuum pump option

An external vacuum pump is necessary if you do not have a vacuum supply in your laboratory. The vacuum pump runs directly from the wall power source, independent of the instrument power circuitry. Make sure that you run the system with a moisture trap. Otherwise, water can get into the vacuum lines and can cause damage to your pump.



More information

- BD FACSJazz system options overview (page 362)

Biological safety cabinet

Introduction

This topic describes the biosafety cabinet option for the BD FACSJazz system.

About the biosafety cabinet (BSC)

The biosafety cabinet (BSC) that is available as an option with the BD FACSJazz system is a Baker SterilGARD Class II A2 biological safety cabinet that separates the work space and air flow from the laboratory. It has a separate high efficiency particulate air (HEPA)-filtered air supply and exhaust that protects laboratory personnel from materials in the biosafety cabinet, and also protects the materials in the cabinet from outside contaminating agents.

See the BSC operator's manual for details on the structure and operation of the cabinet.

Using the BSC with the BD FACSJazz system

During a sort, the system may overheat in the BSC, resulting in poor sorting. Monitor the sort and adjust the piezo amplitude as needed.

Installation

The BSC is installed by the customer. BD Service installs the BD FACSJazz system. The BD FACSJazz system is placed in the indicated location in the BSC. Sheath and waste tanks are placed outside the BSC. If you have the temperature control option, cables and tubing exit the cable port on the right side wall.

More information

- BD FACSJazz system options overview (page 362)

Temperature control option

Introduction

This topic describes the BD™ temperature control option for the BD FACSJazz system.

Temperature control option

The BD temperature control option can be used to control the temperature of sorted samples in the BD FACSJazz system.

The temperature control option comes with a recirculating water bath, 15 x 75-mm tube and 15-mL tube temperature-controlled collection devices, and connecting tubing.

Using the temperature control option

To use the temperature control option:

1. Turn on the temperature control option.
2. Set the desired temperature.

   The temperature control option detects temperature changes in the sample input tube and the sample collection device and adjusts the temperature accordingly.

More information

- BD FACSJazz system options overview (page 362)

Integrators

Introduction

This topic describes the integrator option for the BD FACSJazz system.

About integrators

Integrators are dedicated hardware components that measure the area and the width of a voltage pulse for selected parameters. They work in parallel with the height measurement.

You can select to enable or disable integrators as needed. Each parameter is available for integration. You can apply different integrators to different plots within the same worksheet.

Installation

BD Service performs installation.

More information

- Adjusting PMT voltages and using optional integrators (page 125)
- Integrators tab (page 127)
- Turning integrators on (page 128)
- BD FACSJazz system options overview (page 362)

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Troubleshooting

This chapter includes these topics:

Sorting troubleshooting

Introduction

This topic describes sorting problems and their possible solutions.

Stream is out of alignment

Possible causes	Possible solutions
Nozzle is not aligned	Adjust the silver knobs to align the stream to the pinholes and adjust the black knobs to align the stream to the drain. See Optimizing the side streams (page 322) for more information.
Bubbles in the nozzle	• Install the debubble reservoir filled with clean sheath or ethanol and purge the nozzle. • Verify that the nozzle tip and O-ring are seated properly and are aligned.
Clogged or damaged nozzle	1. Remove the nozzle tip and sonicate it in DI water or detergent to remove the clog. 2. Examine the nozzle under a microscope to check for damage. If the nozzle is damaged, replace it.

No stream is visible

Possible causes	Possible solutions
Empty sheath tank	Refill the sheath tank.
Sheath tank is not pressurized	• Check the fittings for leaks and tighten the pressure release valve, using a wrench if necessary. • Make sure that the O-ring is properly installed around the tank lid and that the lid is properly seated. • Check the air compressor or the house air supply. Make sure that the Air switch is on.
Clogged or damaged nozzle	1. Remove the nozzle and sonicate it in DI water or detergent to remove the clog. 2. Examine the nozzle under a microscope to check for damage. If the nozzle is damaged, replace it.
Leaking nozzle seal	Reinstall the nozzle, ensuring that the O-ring is properly installed.
Air in the sheath filter	1. Use the RINSE fluidics mode and gently tap the sheath filter to remove air from the filter. 2. Purge the sheath filter.

Side streams are not aligned

Possible causes	Possible solutions
Stream deflection is incorrect	Start the stream and adjust the stream deflection so that the stream is aligned to the tube.
Tray position is incorrect	Use the Tray Control pane to move the tray to its appropriate location.

Last column is unreachable during plate sorting

Possible causes	Possible solutions
Side stream is deflected too far to the left and unable to reach the far right column	Decrease the side stream deflection and reinsert the tray.

Unstable stream or stream breakoff

Possible causes	Possible solutions
Debris or bubbles in the nozzle	• Remove the nozzle and sonicate it in DI water or detergent to remove the clog. • Purge the nozzle using the debubble reservoir and clean sheath fluid or ethanol. • Verify that the nozzle and O-ring are seated properly and tightened.
Fluidics are not warmed up	Run the stream for 30 minutes to 1 hour before sorting.
Air currents	• Close the sort chamber door. • Check the room for drafts.
Air or fluid leak	• Check the sheath tank for leaks. • Check the fluidic lines for leaks. • Contact BD service.
Chemical residue in the sheath line	Perform a system flush or replace the sheath line.
Sheath tank is low or empty	Refill the sheath tank.
Air in the sheath filter	Purge the sheath filter.
Clogged sheath filter	Replace the sheath filter.
Large cells or clumpy sample	Filter the sample.

Possible causes	Possible solutions
Frequency setting is not optimal	Find the frequency setting that gives the shortest breakoff and the most stable stream.
Sample line was not installed correctly in the nozzle	Adjust the sample line position in the nozzle.
Debris in the sample	Prepare a new sample.

No side streams are visible

Possible causes	Possible solutions
Drop charge is not turned on	1. Turn on the test deflection. 2. Start the drop charge amplitude at 0 and increase it while watching for side streams.
Deflection plates are powered off	Turn on the deflection plates.
Deflection plates are open	1. Power off the deflection plates. 2. Close the deflection plates.
Drop charge is too high	Start the drop charge amplitude at 0 and increase it while watching for side streams.
Drop drive is not turned on	1. Increase the piezo amplitude. 2. Optimize the drop-drive frequency.
DI water is in the sheath lines	Use sheath fluid for drop charging.
Pressure and frequency settings are not optimized	Find the frequency setting that gives the shortest breakoff and most stable stream.

Possible causes	Possible solutions
Bad contact point for drop charge connection	Make sure the piezo amplitude is set to 0, then remove the nozzle assembly to inspect the charge contacts. • If there is corrosion, clean the contact points. • If the contact points are damaged, replace them.
Contact point is stuck due to salt buildup	1. Power off the deflection plates. 2. Remove the deflection chamber access cover and deflection plates. 3. Remove the plate carrier and use a stream of warm water to clean the contact points. 4. Dry thoroughly before replacing and reassembling.
Arcing between deflection plates	1. Power off the deflection plates. 2. Clean and dry the plates.
Side streams are not aligned to the Accudrop laser	Realign the side streams.
Wet, dirty, or salty plates	1. Power off the plates. 2. Clean and dry the plates.
Nozzle access door is open	Close the nozzle access door.
Sort chamber door is open	Close the sort chamber door.
Plate access panel is open	Close the plate access panel.

Fanning around center or side streams when deflection plates are on

Possible causes	Possible solutions
Clogged or damaged nozzle	1. Remove the nozzle and sonicate it in DI water or detergent to remove the clog. 2. Examine the nozzle under a microscope to check for damage. 3. If the nozzle is damaged, replace it.
Clumpy sample	Filter the sample.
Frequency settings are not optimized	Scan the frequency settings around the recommended frequency to find the setting that gives the shortest breakoff and most stable stream.
Piezo amplitude is too low	Increase the piezo amplitude.
Stream focus is not set correctly	Adjust the stream focus to tighten the center stream.
Drop charging is out of phase with drop formation	1. Turn on test deflection and flash charge. 2. Adjust the piezo amplitude until the side streams are deflected as far as possible with minimum spraying.
Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.

Fluttering side streams, especially during Flash Charge and Short Flash modes

Possible causes	Possible solutions
Salt bridging that leads to partial discharge of the deflection plates	1. Power off the deflection plates. 2. Remove the deflection chamber access cover. 3. Remove and clean each plate. 4. Dry each plate and plate carrier thoroughly before reassembly. If the problem persists, also remove, clean and dry the plate carrier.

Fluttering side streams with AMO on

Possible causes	Possible solutions
AMO causes excessive air currents within the sort chamber	1. Ensure that the AMO is operating at the minimum level (20%) during the sort. 2. Run the sort with the sort chamber door slightly ajar (approximately 1 cm or 1/4–1/2 in. maximum at the bottom).

Low sort efficiency

Possible causes	Possible solutions
Event rate is too high for drop-drive frequency	Decrease the sample pressure.
Gating conflicts	Verify the sorting gate strategy.
Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.
Rare sort populations	Enrich the sample for the population of interest before sorting.
Sorted on ADC parameters, not on DSP parameters	Create plots with DSP parameters.

Cannot see excited beads in the Accudrop laser

Possible causes	Possible solutions
Accudrop filter is not in place	In the Pressure Console pane, click Accudrop to move the Accudrop filter in front of the sort stream camera.
Event rate is too low	• Add more beads to the tube. • Increase the event rate. • Increase the sample offset.

Can see excited Accudrop beads but not sorted Accudrop beads

Possible causes	Possible solutions
Deflected events are out of range of the Accudrop laser	1. Reduce the drop charge voltage until deflected drops just clear the drain. 2. Restore the deflection voltage after performing the Accudrop.

Erratic sort rate

Possible causes	Possible solutions
Event rate is too high	Decrease the event rate.
Clogged or kinked sample line	• Filter the sample. • Clean or replace the sample line.

Unexpected sort results

Possible causes	Possible solutions
Incorrect drop delay	Check the drop delay.
Incorrect sort mode	Verify that the sort mode is appropriate for your sorting requirements.
Breakoff is not stable during the sort	• Watch the breakoff during the sort and adjust the piezo amplitude if necessary. • Remove the nozzle and sonicate it.

Possible causes	Possible solutions
Incorrect logic in sort gating	Verify the sorting gate strategy and check for any yellow populations (conflicts).
Side streams are fanning during the sort	See Fanning around center or side streams when deflection plates are on (page 375).
Sort device is not aligned correctly	Check the tubes and side streams for proper alignment before sorting.
Event rate is too high	Decrease the sample pressure.

Index sorting button is not available or cannot start

Possible causes	Possible solutions
Cannot perform an index sort while acquiring data	Stop acquiring data.
No available DSP parameters	Manage parameters in the Compensation window and clear the parameters that you are not using.

Side streams are not hitting the target

Possible causes	Possible solutions
Deflection rate is too low or too high	• Increase the maximum drop charge. • Increase the maximum stream deflection.
Droplet breakoff is too low	Adjust the sort settings.
Wet or dirty plates	Power off the plates and clean them.

More information

- Startup, shutdown, and troubleshooting components (page 250)
- Acquisition troubleshooting (page 379)
- Fluidics troubleshooting (page 385)
- Alignment troubleshooting (page 389)
- Application troubleshooting (page 391)
- Electronics troubleshooting (page 392)
- Equipment troubleshooting (page 393)

Acquisition troubleshooting

Introduction

This topic describes acquisition problems and possible solutions.

No sample events

Possible causes	Possible solutions
Laser(s) are shuttered	Move the laser shutter tab to the open position.
Laser power is off	Turn on the laser power supply and turn the key to start the laser.
Laser safety interlock is engaged	Close the nozzle access door and place your finger over the optical sensor in the upper-right corner to reset the safety interlock.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).
No sample in the tube	Install a new sample tube.
Sample is not mixed properly	Mix the sample thoroughly and re-install the tube.
Sample line is clogged	Clean or replace the sample line.

Possible causes	Possible solutions
Bubble detector triggered	• In the Pressure Console pane, click Backflush to reset the bubble detector. • Verify that the sample line is long enough to reach the sample.
Air line sensor was triggered	Contact BD Service.
Tube is not properly installed	1. Re-install the tube. 2. Close the tube-lock lever under the tube. 3. In the Pressure Console pane, click Sample to pressurize the tube.
Laser or stream is not properly aligned	Align the stream and lasers.
Trigger is not set correctly	• Set the trigger detector or level according to the needs of your sample. • Check the PMT voltage and the linear/log setting for the trigger detector.
Tube is not pressurized	• Check the sample stopper for damage and replace it if necessary. • Check the tube for damage and replace it if necessary.
PMT power is off	Turn on the PMT power and adjust the voltage.
Sample pressure is too low	Adjust the sample offset to increase the sample pressure.

No fluorescent or scatter signal

Possible causes	Possible solutions
Fluorochrome is not optimized for detection filters	Make sure that you use the appropriate fluorochromes that can be detected by the BD FACSJazz system for your application.
Laser delay or trigger delay is set incorrectly	Adjust the laser delay.
PMT is labeled incorrectly	Check the instrument configuration to verify that you are looking at the correct channel.
Sample was not appropriately stained	Verify that the appropriate antibody or dye was added to the sample tube.
Forward scatter detector is not aligned	Align the forward scatter detector.
Wrong excitation laser wavelength	Use the correct excitation wavelength.
Wrong trigger channel is selected	Change the trigger detector and trigger level.

Sortware not connecting to the cytometer interface

Possible causes	Possible solutions
The cytometer interface is not turned on	Turn on the main power.
Faulty connection between BD FACS Sortware and the cytometer interface	1. Turn the main power off, then on, and wait for two beeps that indicate the cytometer interface is started. 2. Restart the computer. 3. Start BD FACS Sortware.

Distorted populations or high CVs

Possible causes	Possible solutions
Lasers are not properly aligned	Align the lasers.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).
Instrument settings were adjusted incorrectly	Optimize the cytometer settings.
Sample pressure is too high	Decrease the sample pressure.
Debris or bubbles in the nozzle	• Remove the nozzle and sonicate it in DI water or detergent to remove the clog. • Purge the nozzle using the debubble reservoir and clean sheath or ethanol. • Verify that the nozzle and O-ring are seated properly and tightened.
Poor sample preparation	Repeat sample preparation.
Excess background light	Dim the lights in the room.
Stream is not aligned	Align and focus the stream.
Sample/sheath index of refraction mismatch	Verify that you are using the appropriate sheath fluid and resuspension fluid for your sample.

Excessive amount of debris in plots

Possible causes	Possible solutions
Trigger level is set too low	Increase the trigger level.
Dead cells or debris in the sample.	Examine the sample under a microscope and prepare a new sample, if necessary.
Sheath filter is dirty or contaminated	1. Remove the sheath filter. 2. Perform a system flush or replace the sheath line. 3. Replace the sheath filter.
Sample is contaminated	Prepare a new sample.
Carryover from a previous sample	1. Remove the sample line. 2. Backflush the sample line. 3. Reset the plot display.

Noisy FSC or fluorescence signal

Possible causes	Possible solutions
Noise from the drop formation	Lower the piezo amplitude.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).
Ambient light leak	• Close all safety covers on the instrument. • Verify that all optical filters are correctly installed and the O-rings are in place.
Piezo amplitude is set too high	Lower the piezo amplitude.
Laser or stream is not properly aligned	Align the stream and lasers.
Sample pressure is too high	Decrease the sample offset.
PMT voltage is set too high	Decrease the PMT voltage.

Low acquisition efficiency

Possible causes	Possible solutions
Drop-drive noise	Lower the piezo amplitude.
High sample event rate	Lower the sample offset.
Clumps or filaments in the sample	Filter the sample.
Poor laser alignment	Align the laser.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).

More information

- Startup, shutdown, and troubleshooting components (page 250)
- Sorting troubleshooting (page 370)
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Fluidics troubleshooting

Introduction

This topic describes fluidics problems and suggests possible solutions.

Cannot pressurize the sheath tank

Possible causes	Possible solutions
Bad or missing O-ring	Replace the O-ring around the tank lid.
Tank is leaking pressure	• Check the fittings for leaks. • Tighten the pressure release valve, using a wrench if necessary. • Make sure that the lid is properly seated.
No air supply	Check the air compressor or house air supply.

Waste tank has no vacuum

Possible causes	Possible solutions
Tank is not sealed properly	• Connect all fittings. • Verify that the O-ring is installed around the lid and replace the O-ring if necessary. • Make sure that the lid is properly seated. • Tighten the lid.
No vacuum supply	Verify that the vacuum pump or supply is turned on.
Air filter is wet or clogged	Replace the air filter on the vacuum line.
Waste tank is full	Empty the waste tank.

Flush bucket, backflush drain, or stream drain does not empty

Possible causes	Possible solutions
No vacuum	See Waste tank has no vacuum (page 386).
Debris is clogging the drain	Remove the flush bucket and clean it with detergent.
Pinched tubing	Check the tubing for kinks.

Wide sample core

Possible causes	Possible solutions
Damaged sample line	Replace the sample line.
Bubbles in the nozzle	• Install the debubble reservoir filled with clean sheath or ethanol and purge the nozzle. • Verify that the nozzle and O-ring are seated properly and tightened.
Clogged or damaged nozzle	1. Remove the nozzle tip and sonicate it in DI water or detergent to remove the clog. 2. Examine the nozzle under a microscope to check for damage. If the nozzle is damaged, replace it.
Sample pressure is too high	Decrease the sample offset.
Sample concentration is too low	• Concentrate the sample. • Prepare a new sample with higher concentration.
Sample/sheath index of refraction mismatch	Verify that you are using the appropriate sheath fluid and resuspension fluid for your sample.
Sheath tank is empty or low	Fill the sheath tank.
Incorrect laser or stream alignment	Realign the stream and laser.

Sample tube makes popping sound when removed

Possible causes	Possible solutions
Tube-lock lever was opened too quickly	Open the tube-lock lever slowly.
The filter on the sample tube air line is wet	Replace the air filter.
The sensor on the tube-lock lever is not responding	Contact BD service.

Cannot open pinch valve to replace sample line

Possible causes	Possible solutions
Pinch valve does not spring open when tubing is not in place	1. Press the Override button on the Pressure Console to deactivate the pinch valve. 2. Use a tool to push the sample line behind the pinch valve.

More information

- Startup, shutdown, and troubleshooting components (page 250)
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Alignment troubleshooting

Introduction

This topic describes alignment problems and possible solutions.

Laser is not visible on the pinhole monitor

Possible causes	Possible solutions
Laser shutter is closed	Open the laser shutter.
Laser safety interlock is engaged	Close the nozzle access door and place your finger over the optical sensor in the upper-right corner to reset the safety interlock.
Stream is not aligned	Align the stream before aligning the lasers.
Laser is not aligned	Align the lasers.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).
Laser did not start up	Restart the main power.

Core stream not visible on pinhole monitor when running alignment beads

Possible causes	Possible solutions
Laser shutter is closed	Open the laser shutter.
Laser did not start	Restart the main power.
Laser safety interlock is engaged	Close the nozzle access door and place your finger over the optical sensor in the upper-right corner to reset the safety interlock.
Stream is not aligned	Align the stream before aligning the lasers.
Laser is not aligned	Align the lasers.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).

Possible causes	Possible solutions
Sample pressure is too low	Increase the sample offset.
Bubbles in the nozzle	• Install the debubble reservoir filled with clean sheath or ethanol and purge the nozzle. • Verify that the nozzle and O-ring are seated properly and tightened.
Beads are not excited by the laser	Use the appropriate alignment beads.

Poor signal, hockey stick shape

Possible causes	Possible solutions
Sample pressure is too high	Decrease the sample offset.
Sample carryover of bright particles	1. Rinse the sample line with detergent. 2. Backflush the sample line.
Stream or laser is not aligned	Align the stream or laser.
Lasers are vertically misaligned	Vertically align the lasers. See Vertically aligning the lasers (page 68).

More information

- Startup, shutdown, and troubleshooting components (page 250)
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Application troubleshooting

Introduction

This topic describes application problems and possible solutions.

Incorrect compensation

Possible causes	Possible solutions
Error with staining	Manually adjust the spillover values.

Unable to perform autocompensation

Possible causes	Possible solutions
Too few positive or negative events	Collect more events.

Unable to sort on compensation

Possible causes	Possible solutions
ADC parameters are being used instead of DSP parameters	Recreate plots using DSP parameters.

More information

- Sorting troubleshooting (page 370)
- Acquisition troubleshooting (page 379)
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Electronics troubleshooting

Introduction

This topic describes electronics problems and suggests possible solutions.

Cytometer error when opening BD FACS Sortware software

Possible causes	Possible solutions
Cytometer interface is not connected to the instrument	1. Turn the main power off, then on, and wait for two beeps that indicate the cytometer interface is started. 2. Restart the computer. 3. Open BD FACS Sortware.

Hear three repeating beeps from the electronics box

Possible causes	Possible solutions
Sortware controller is not connected to the instrument	1. Turn off the main power button on the front of the electronics box. 2. Turn on the main power button. When you hear two beeps from the electronics box, the controller is fully turned on. It may take up to two minutes for BD FACS Sortware to establish a connection after two beeps are heard.

More information

- Sorting troubleshooting (page 370)
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Equipment troubleshooting

No indication whether the Buffalo Aerosol Management System is running

Possible causes	Possible solutions
The Power button on the front of the unit does not indicate status.	1. Turn on the main switch at the back of the unit. The LED indicators on the control panel are illuminated. 2. Press the Power button on the front of the unit to turn the suction on and off. 3. View the filter flow gauge on the right side of the unit to verify that the suction is on or off.

Cannot reach the back of the sort chamber tray to clean spill

Possible causes	Possible solutions
The stepper table arm makes tray access difficult	Slide the tray forward until the back of the tray is in line with the front of the stepper arm. Do not remove the tray completely.

More information

- Sorting troubleshooting (page 370)
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