BD Influx™ Cell Sorter User's Guide

For Research Use Only

bdbiosciences.com
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FCC information

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

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

Regulatory information

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

Class I (1) Laser Product

History

Revision	Date	Change made
23-11543-00 Rev. 01	4/2011	New document

Contents

1

About this guide

This section includes these topics:

Documentation overview

Introduction

This guide provides an introduction to the instrument and basic operation information for new operators, as well as a reference for continued use by experienced operators.

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

Using the guide

A familiarity with basic flow cytometry concepts is assumed throughout this manual. 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: System information.** This part includes information about system hardware and components, basic system procedures, and system maintenance. Some system procedures require the use of BD FACS Sortware sorter software.
- **Part 2: Software information.** This part includes information about BD FACS Sortware sorter software, general procedures for setting up and operating the instrument, and procedures for sorting samples. See Part 3: System workflow (page 221) for complete daily workflow procedures.
- **Part 3: System workflow.** This part includes the essential workflows for the setup and operation of the BD Influx system.
- **Part 4: Reference.** This part includes information about system options, system specifications, cytometer configurations, and troubleshooting.

The BD Influx Task Navigator provides links to workflow tasks and support information to help you quickly find information for common BD Influx tasks.

Other documentation

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

More information

- Conventions (page 11)
- BD FACS Sortware Help system (page 12)
- Technical assistance (page 14)
- Limitations (page 15)

Conventions

Introduction

The following tables list the safety symbols and document and Help content tools used throughout this manual.

Safety symbols

These 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
	Laser radiation hazard

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

Navigation and reference linkage tools

Convention	Use
Part	Parts identify different types of grouped information. For example, Part 1: Instrument information, Part 2: Software information, Part 3: System workflow, or Part 4: Reference.
Section menus	Each section menu includes links to topics within the section.
Introduction	Provides a basic summary of the topic so that you can quickly decide if the topic answers your specific questions.
More information	Provides links to additional concepts or procedures related to this information.

More information

- Documentation overview (page 10)
- BD FACS Sortware Help system (page 12)

BD FACS Sortware Help system

Introduction

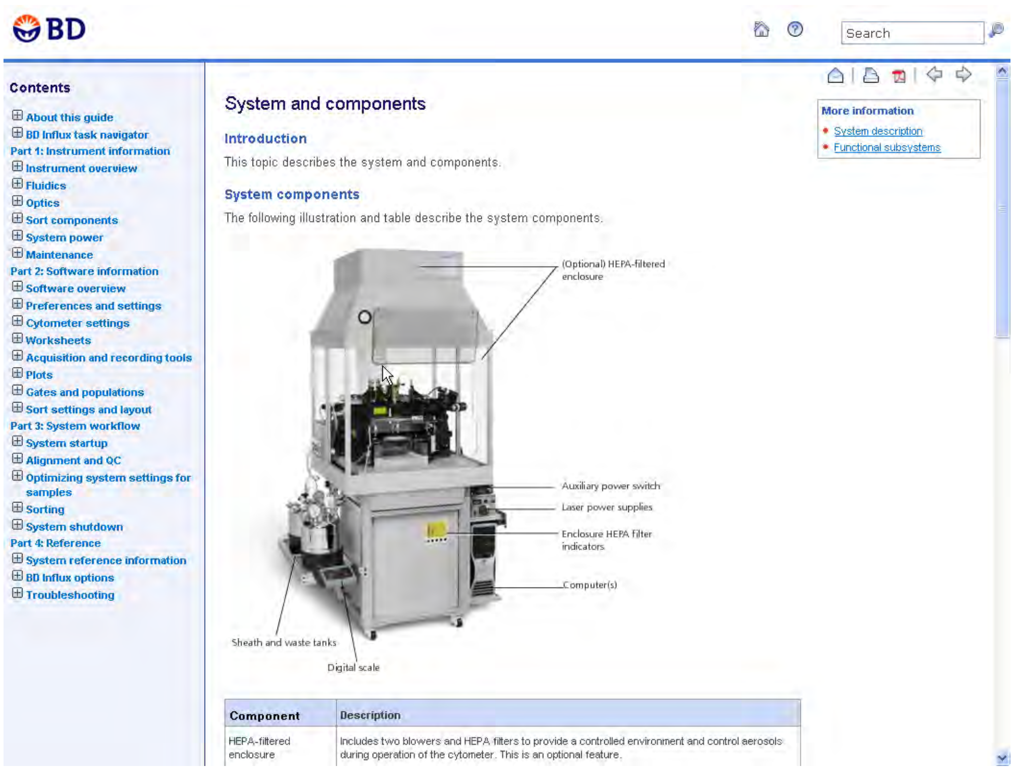
BD FACS Sortware sorter software includes a comprehensive Help system that includes all content from this user's guide. Internet access is not required to access this content.

Navigating the Help system

To access the BD FACS Sortware Help system:

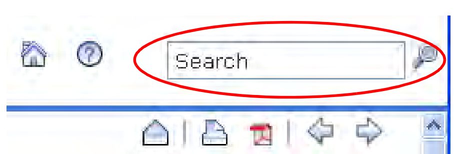
1. In the **BD FACS Sortware** window, select **Help > Contents.**

   The **Help** window opens.



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

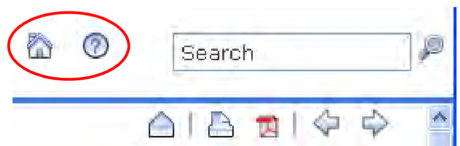
   – **Search.** Type words or phrases into the search field. Search results are displayed in a familiar web search format to help you find information quickly.



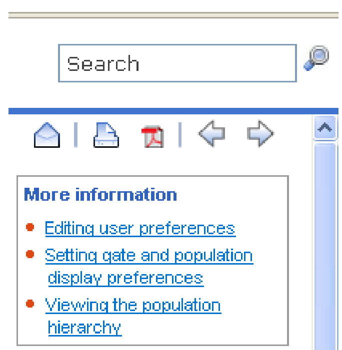
– **Print.** Use the print tools to print individual topics or to print entire sections as formatted PDF files.

– **Home.** Click the **Home** icon to return to the home page.

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



– **More Information.** Click links in the **More information** box to view additional related topics.



More information

- Documentation overview (page 10)
- Conventions (page 11)
- Technical assistance (page 14)

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 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 10)
- BD FACS Sortware Help system (page 12)
- Limitations (page 15)

Limitations

Intended use

This instrument is for Research Use Only (RUO). Not for use in diagnostic or therapeutic procedures.

The BD Influx system is intended for use in laboratory research. Any clinical use of the device requires approval of appropriate authorities, such as the FDA. Approval for use beyond laboratory research is the responsibility of the study sponsor, not BD Biosciences.

Warranty

BD Biosciences is providing software without warranty of any kind on an as-is basis. The software and workstations are intended for running the systems supplied by BD Biosciences. It is the responsibility of the buyer/user to ensure that all added electronic files including software and transport media are virus-free. If the workstation is used for Internet access or purposes other than those specified by BD Biosciences, it is the buyer/user's responsibility to install and maintain up-to-date virus protection software. BD Biosciences does not make any warranty with respect to the workstation remaining virus-free after installation.
BD Biosciences is not liable for any claims related to or resulting from the buyer/user's failure to install and maintain virus protection.

Safety

All devices, such as lasers, used in conjunction with the instrument must be used as indicated by the original manufacturer. Follow the precautions described in the *BD Influx Safety and Limitations Guide* included with your instrument.

More information

- Documentation overview (page 10)
- Technical assistance (page 14)

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2

BD Influx task navigator

This section provides navigation to common BD Influx tasks and includes links to useful information.

Common BD Influx tasks

System startup/setup

To...	Use this information.	What you might want to know...
Start up the BD Influx system	System startup (page 223)	Power distribution (page 64)
Load a sample tube	Introducing a sample into the system (page 237)	Sample introduction (page 33)
Perform daily QC	Alignment and QC (page 241)	• System startup (page 223) • Optimizing system settings for samples (page 263) • Optical detection (page 50)
Customize the window layout	Setting up your window layout (page 102)	BD FACS Sortware overview (page 94)
Optimize with compensation	Creating plots and gates for optimization (page 264)	• Optimizing with compensation controls (page 268) • Collecting data files for compensation (page 272) • Performing auto compensation (page 275)
Save and restore system settings	• Saving and restoring settings (page 112) • Saving and deleting settings (page 115)	Creating storage folders (page 117)

Sort setup

To...	Use this information.	What you might want to know...
Create sort trays	Using the Tray Control pane (page 210)	• Importing an existing BD Spigot sort device (page 209) • Modifying an existing sort device (page 214)
Set the drop delay with Accudrop	Determining the drop delay with BD FACS Accudrop (page 300)	• Estimating the drop delay (page 295) • Determining the drop delay using the calibration slides (page 304)
Create a user-defined sort mode	Creating a user-defined sort mode (page 207)	Using the Sort Settings pane (page 204)

Data visualization

To...	Use this information.	What you might want to know...
Create worksheets	Creating a worksheet layout (page 141)	Worksheet overview (page 138)
Create and modify plots	Creating plots in a worksheet (page 162)	• Modifying dot plot properties (page 166) • Customizing worksheet properties (page 143)
Create and modify gates and populations	Creating rectangle gates (page 187)	Gating overview (page 178)
View a sort report	Working with sort reports (page 312)	• Creating a worksheet layout (page 141) • Customizing worksheet properties (page 143)

Cleaning and maintenance

To...	Use this information.	What you might want to know...
Change or clean the nozzle tip	Cleaning the nozzle tip (page 234)	Removing and replacing the nozzle tip (page 81)
Flush the system	Flushing the system (page 233)	• Startup workflow (page 224) • System shutdown (page 319) • Backflushing the sample line for a different sample (page 78) • Decontaminating the fluidics (page 79)
Shut down the system	System shutdown workflow (page 320)	• Decontaminating the fluidics (page 79) • Replacing the nozzle assembly (page 89)

To...	Use this information.	What you might want to know...
Operate the HEPA enclosure	Enclosure operational checklist (page 354)	• About the HEPA-filtered enclosure option (page 348) • HEPA enclosure safety (page 352) • About the HEPA enclosure controls (page 355) • Setting up the HEPA enclosure digital display (page 357)
Perform regularly scheduled maintenance	• Daily maintenance overview (page 206) • Weekly maintenance overview (page 207) • Monthly maintenance procedures (page 208)	• Instrument cleaning and maintenance (page 71) • Component replacement (page 80)
Align the system after maintenance or service	Aligning and optimizing the optics workflow (page 242)	NA

Part 1

Instrument information

This part includes these sections:

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3

Instrument overview

This section includes these topics:

Other related information:

- About the HEPA-filtered enclosure option (page 348)

System description

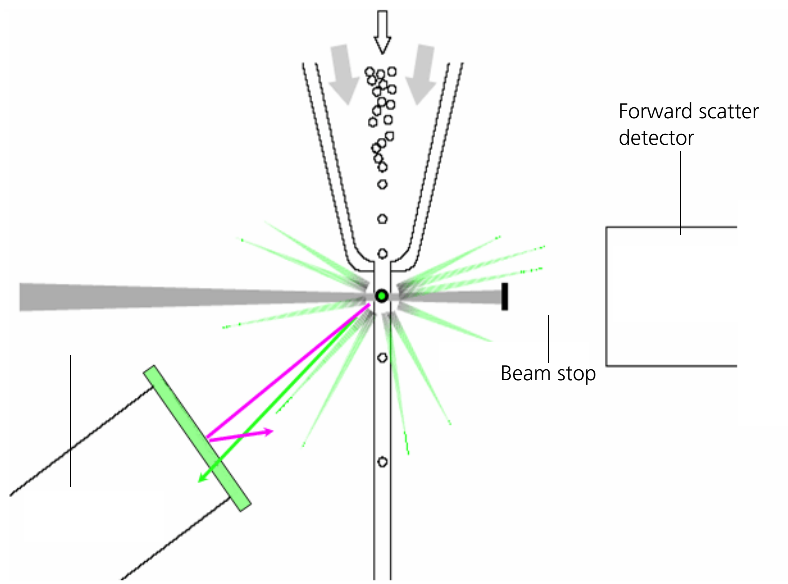
Introduction

This topic describes how the BD Influx high-speed cell sorter functions.

The BD Influx high-speed cell sorter is a research instrument that provides high-speed detection, sorting of particles excited by laser light, and analysis.

Detection

The BD Influx 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.

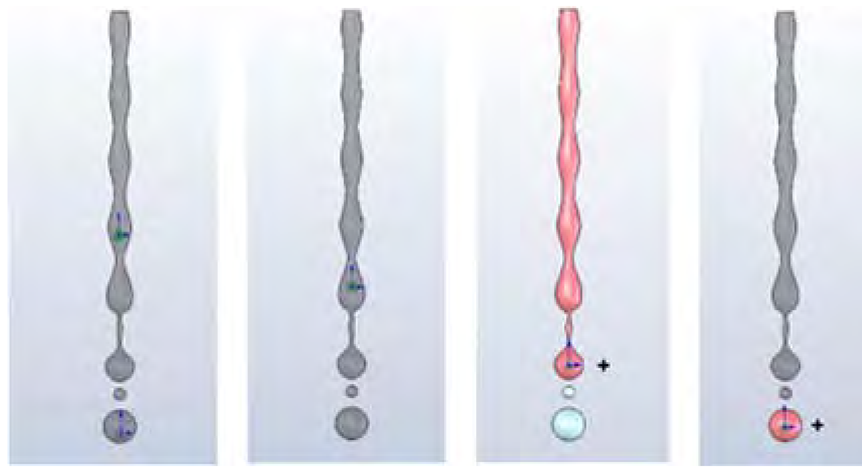


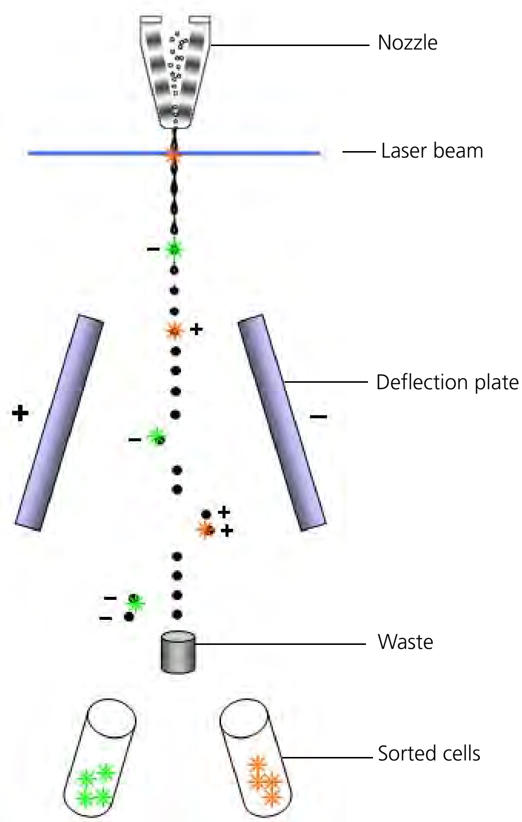
Sorting overview

The following steps describe the sorting process.

Stage	Description
1	Drop drive energy is applied to the stream to break it into highly uniform droplets.
2	Droplets detach from the stream a few millimeters downstream from the nozzle.
3	When a particle is detected and meets the predefined sorting criteria, an electrical charge is applied to the stream just as the droplet containing that particle breaks off from the stream.
4	Once broken off from the stream, the droplet—now surrounded by air—still retains its charge. The charged droplet passes by two strongly charged deflection plates.

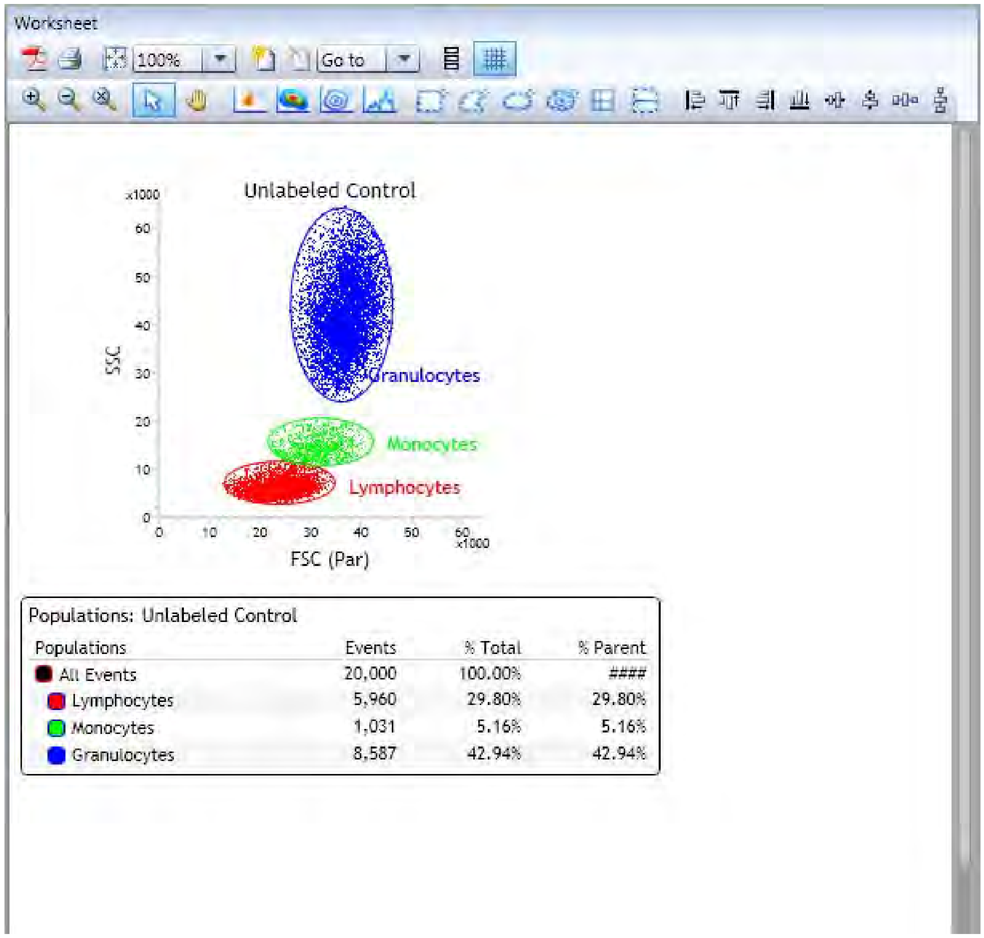
5	Electrostatic attraction and repulsion cause each charged droplet to be deflected to the left or right, depending on the droplet's charge polarity.
6	Uncharged droplets are not affected by the electric field and pass down the center to the waste drain.
7	Charged drops sort into the appropriate tube or well.





Analysis

Use worksheet tools to create plots, gates, and statistics views that you can use to visualize data, define populations, and create reports.



More information

- System and components (page 27)
- Functional subsystems (page 29)

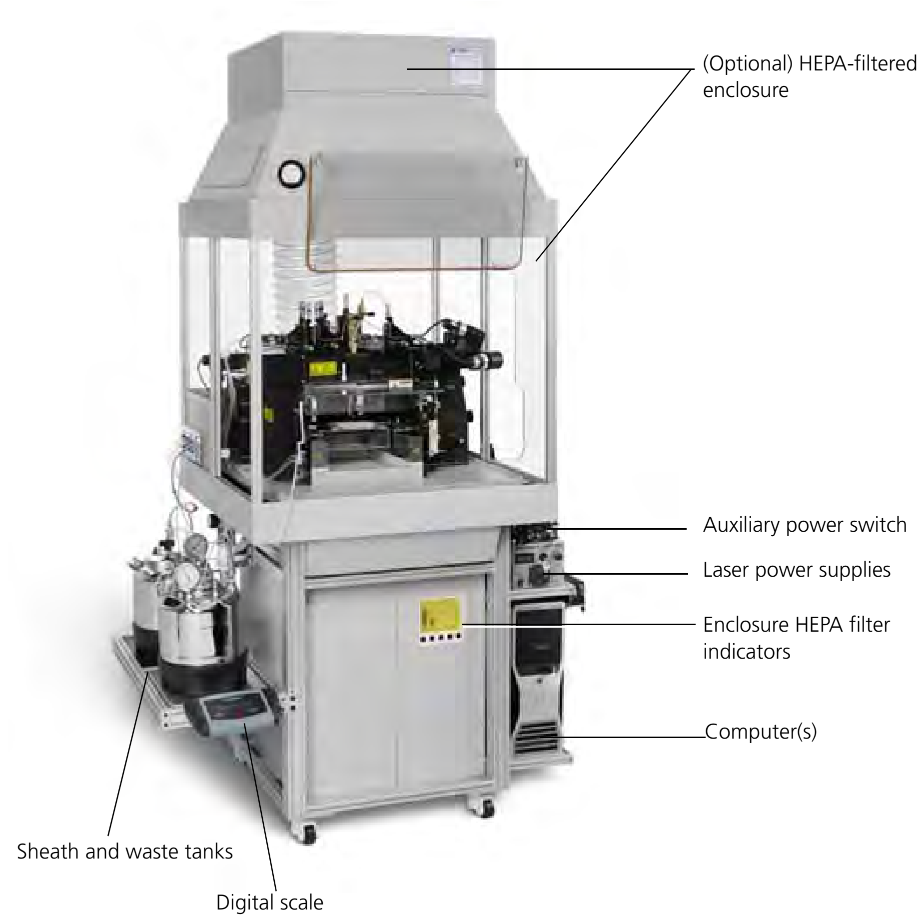
System and components

Introduction

This topic describes the system and components.

System components

The following illustration and table describe the system components.

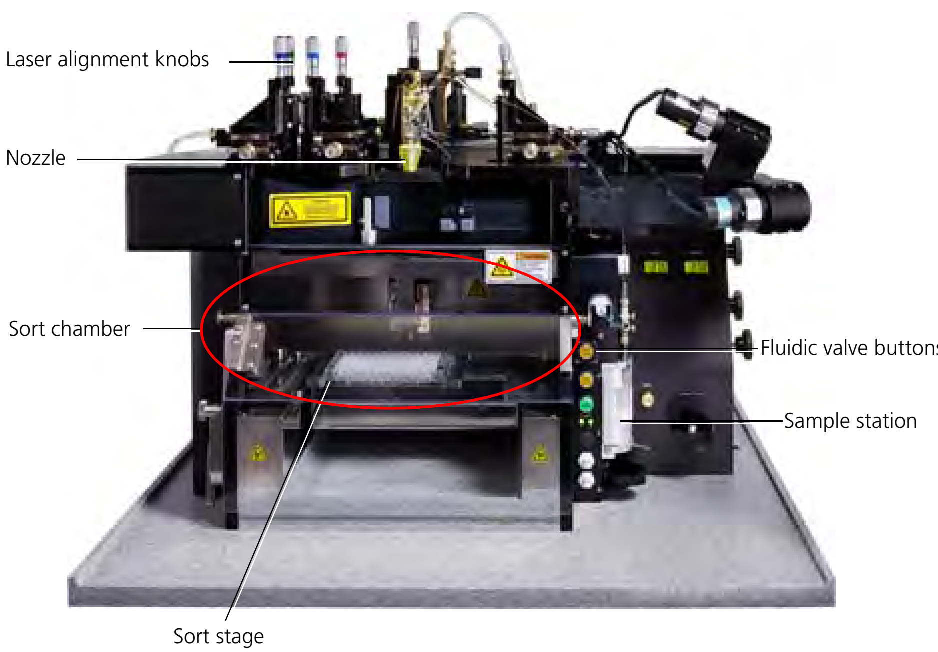


Component	Description
HEPA-filtered enclosure	Includes two blowers and HEPA filters to provide a controlled environment and control aerosols during operation of the cytometer. This is an optional feature.
Auxiliary power switch	Turns on the power for the electronics, oscilloscope, video cameras, and monitors.
Laser power supplies	Turn on each laser individually. You might have additional power supplies located on the back of the instrument or on the table next to the instrument, depending on the number and type of lasers on your system.
Enclosure HEPA filter indicators	Indicate the status of the HEPA filters.

Computer(s)	Runs BD FACS Sortware sorter software. Depending on your configuration, this computer might also serve as the cytometer interface, or the system might include a second computer to serve as the cytometer interface. The cytometer interface is a dedicated computer that interacts with BD Sortware software and controls the cytometer hardware.
Digital scale	Displays the sheath tank weight.
Sheath and waste tanks	Hold up to 7 liters of sheath or waste.

Instrument components

The following illustration and table describe the instrument components.



Component	Description
Laser alignment knobs	Control the beam alignment of each laser. Use these adjustments during signal optimization.
Nozzle	Hydrodynamically focuses the sample and sheath into a stream and directs it to the laser interrogation point. You can change the size of the nozzle tip depending on particle size.
Sort chamber	Contains the sort plate, sort stage, Accudrop laser, and the waste drain.

Component	Description
Sort stage	A moving mechanical assembly that holds and positions different sort devices. Includes inserts for slides, plates, multi-tube assemblies, and universal cooling assemblies for tubes.
Sample station	Holds and pressurizes the sample tube. Also includes valve buttons and a bubble detector.
Fluidic valve buttons	Controls the sample valves to run a stream or remove bubbles.

More information

- System description (page 24)
- Functional subsystems (page 29)

Functional subsystems

Introduction

This topic describes the primary functional subsystems of the instrument.

Instrument subsystems

The BD Influx system includes the following instrument subsystems:

- Fluidics (page 31)
- Optics (page 47)
- Sort components (page 57)
- System power (page 63)

More information

- System description (page 24)
- System and components (page 27)

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4

Fluidics

This section includes these topics:

Fluidic components

Introduction

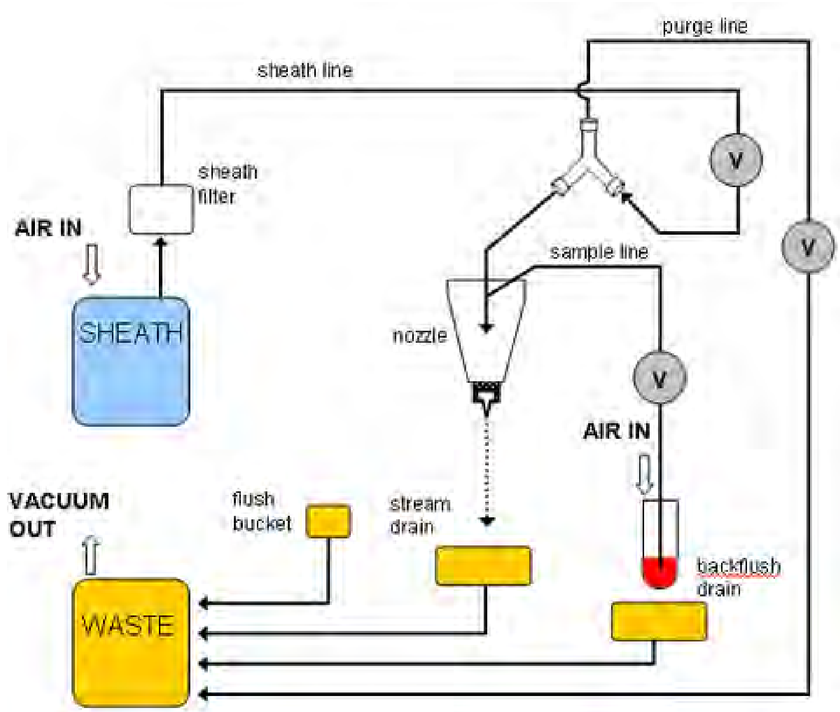
This topic describes the fluidics subsystem.

Components

The fluidic subsystem components are:

- Purge and sheath tanks
- Waste, sheath, and sample lines
- Flush bucket
- Stream drain and sample backflush drains
- Waste, sample, and sheath valves

The following figure shows the fluidics subsystem components.



More information

- System and components (page 27)
- Functional subsystems (page 29)
- Sample introduction (page 33)
- Pressure regulation and monitoring (page 37)
- Sheath tank and fluidic components (page 40)
- Waste fluidic components (page 43)

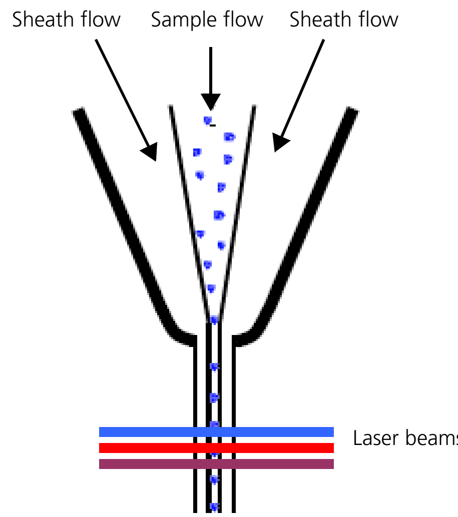
Sample introduction

Introduction

This topic describes the fluidic components that introduce the sample into the instrument.

Description

Sample introduction generates a stream of sample particles, typically suspended in saline, in the center of a larger stream of sheath fluid (saline).



The pressurized stream is forced through a tiny orifice in a nozzle. This produces a fine, fast-moving jet of fluid and sample particles.

Components

The main components of sample introduction include:

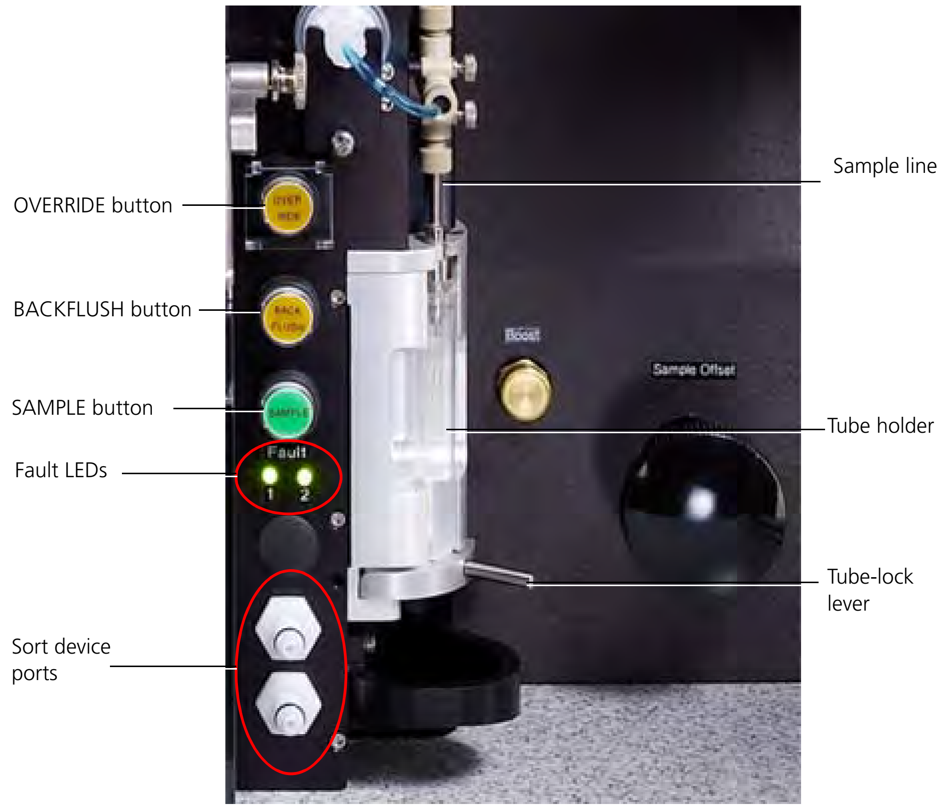
- Sample station
- Sample line
- Nozzle assembly

Sample station

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

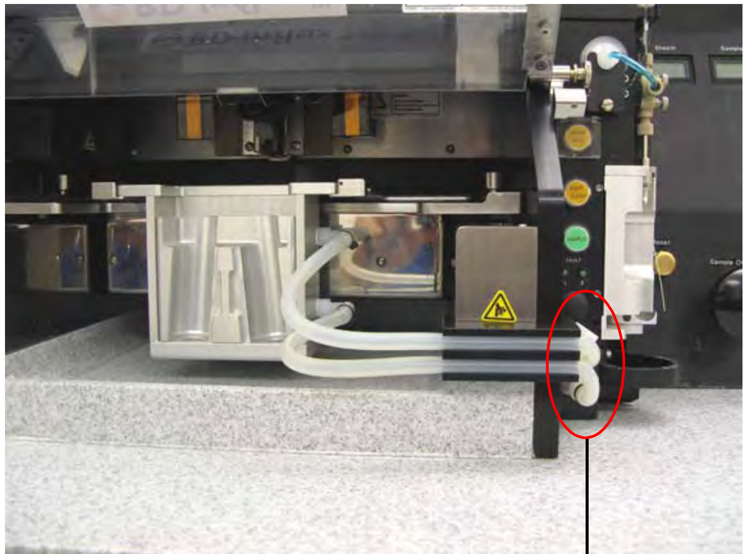
The sample station includes:

- Sample valve control buttons
- Sort device ports
- Sample line
- Tube holder for loading sample tubes
- Tube-lock lever



Component	Description
OVERRIDE button	Opens the pinch valve on the sample line during maintenance procedures.
BACKFLUSH button	Runs sheath fluid from the nozzle through the length of the sample line.
SAMPLE button	Pressurizes the sample tube and delivers sample to the nozzle.
Fault LEDs	LED 1 detects fluid in the air line. LED 2 detects air (bubbles) in the sample line. Both LEDs must be green to run samples.
Sort device ports	Connect to a universal sample cooling assembly (cooled sample assembly that inserts into the sort stage).
Sample line	Introduces the sample to the nozzle assembly.
Tube holder	Holds the tube in place.
Tube-lock lever	Locks the tube in place and ensures that the sample is sealed well with the top of the sample port. The tube is pressurized to allow the sample to travel to the nozzle.

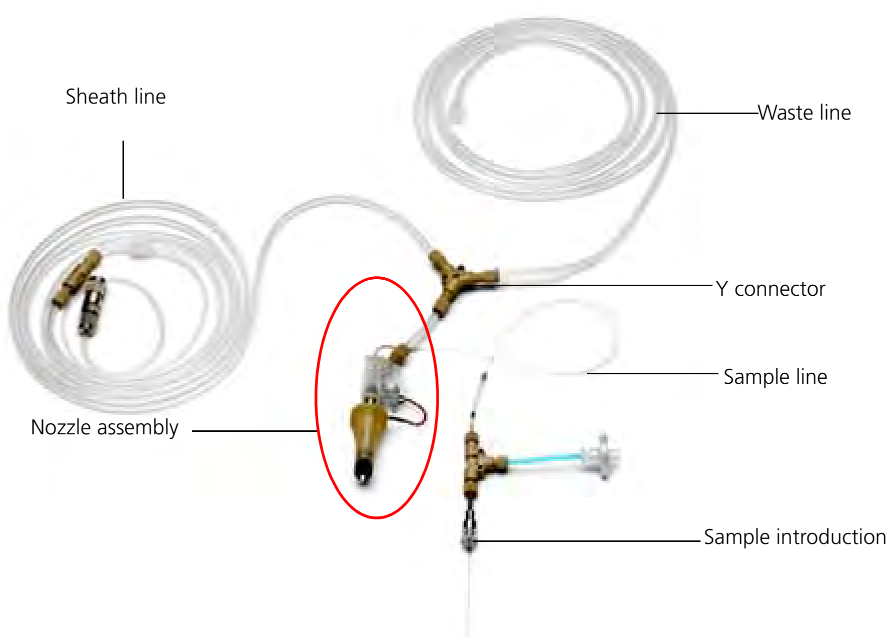
The sort device port connects to a universal sample cooling assembly (cooled sample assembly that inserts into the sort stage).



Tubing connecting the cooled sample plate to the sort device ports.

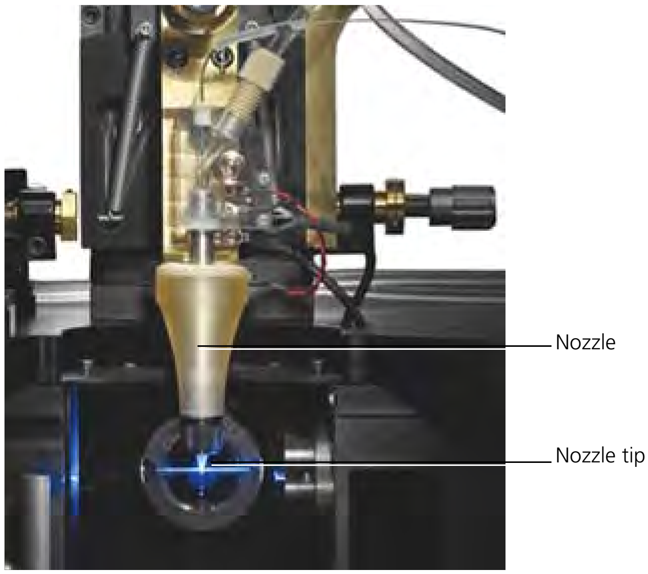
Sample line

The sample line delivers the sample from the sample station to the nozzle assembly. The sample line has an inner diameter of 254 µm. Backflush mode helps to minimize sample carryover by backflushing the sample line. This line can be easily replaced.



Nozzle assembly

The sample moves to the nozzle assembly where the sheath and the nozzle create hydrodynamic focusing on the sample so that the sample can be interrogated by the lasers. The assembly includes a piezoelectric element that converts the stream into controlled droplets.



More information

- Fluidic components (page 32)
- Pressure regulation and monitoring (page 37)
- Sheath tank and fluidic components (page 40)
- Removing the sample line (page 82)
- Installing a new sample line (page 83)
- Sorting troubleshooting (page 364)

Pressure regulation and monitoring

Introduction

This topic describes the fluidic components that regulate and monitor the fluidics pressure.

The fluidics subsystem applies air pressure to the sample tube and the sheath reservoir. It also monitors the sample and sheath pressures.

Components

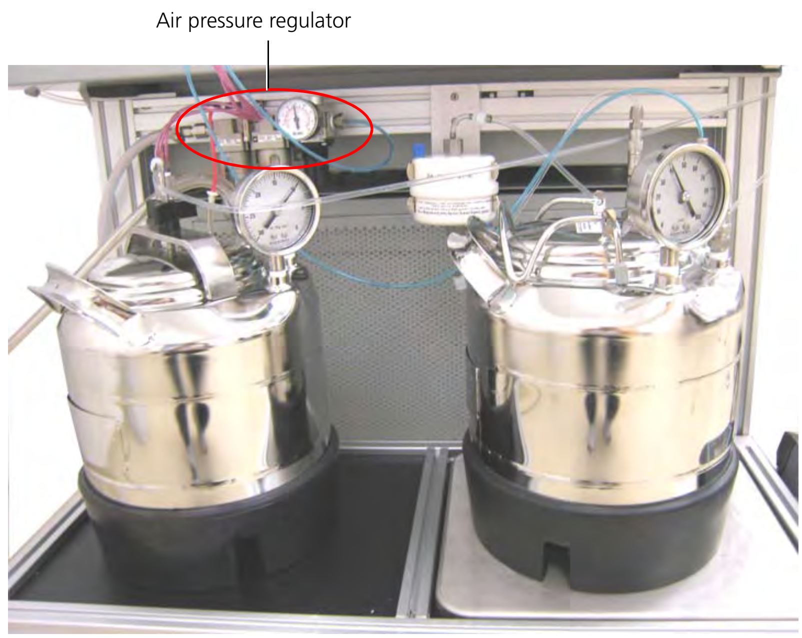
The main components of pressure regulation are:

- Air pressure (input) regulator
- Fluidic valve controls
- Pressure console

Air pressure regulator

Pressure is applied to the sheath tank and the sample tube to push sheath fluid and sample fluid through the nozzle. Sheath, sample, and boost pressure can be individually adjusted. An external air compressor is required if you do not have a compressed air supply in your laboratory.

See Aerosol evacuation unit and air compressor options (page 362) for more information.



Fluidic valve controls

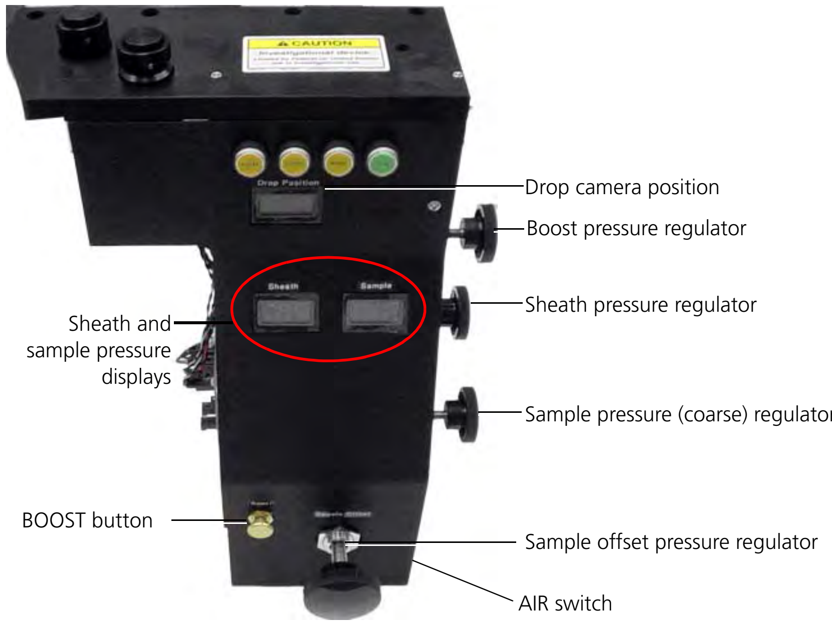
The fluidic valve control buttons (located at the top of the pressure console) control the valve mode for the sheath and purge valves.

The fluidic valve buttons are located above the sample station and the digital readouts. If your instrument has the enhanced forward scatter option installed (as shown), the fluidic valve buttons are obscured by the enhanced forward scatter collector.

Component	Description
PULSE button	Helps remove bubbles by quickly opening and closing the sheath valve.You must press PURGE before you can press PULSE.
PURGE button	Runs fluid from the nozzle to waste to flush bubbles from the nozzle area. In this mode, the sheath valve is closed and the waste valve is open.
RINSE button	Runs fluid through all fluidic lines and out the nozzle.
RUN button	Runs a sample stream through the nozzle. In this mode, the sheath valve is open and the waste valve is closed.

Pressure console

The pressure console includes all sample and sheath pressure adjustments and displays.



Component	Description
Drop camera position display	Displays the relative position of the drop camera. The drop camera is used to estimate the drop delay. The drop camera has a micrometer to adjust the vertical position of the drop camera in relation to the stream. This value is displayed in the drop camera display on the pressure console.
Boost pressure regulator	Adjusts the initial boost pressure (the amount of pressure above the sample pressure).
Sheath pressure regulator	Adjusts the sheath pressure. The sheath regulator can regulate input pressure from 3–100 PSI. Typical operating pressure is <80 PSI.
Sample pressure regulator	Used for coarse adjustment of the sample pressure. The sample pressure regulator can regulate input pressure from 3–100 PSI. Typical operating pressure is <80 PSI.
Sample offset pressure regulator	Provides fine-scale adjustment of the sample pressure. Used in combination with the sample pressure (coarse) adjustment. The sample offset regulator adds 0–5 PSI to the sample regulator.
AIR switch (toggle)	Located on the side of the pressure console. Used to toggle the air pressure on or off.
BOOST button	Located at the bottom of the pressure console. This button temporarily boosts sample pressure by the preset or the value you set with the Boost pressure regulator.
Sheath and sample pressure displays	Display the current sheath pressure and the current sample pressure.

More information

- Functional subsystems (page 29)
- Sheath tank and fluidic components (page 40)
- Aerosol evacuation unit and air compressor options (page 362)

Sheath tank and fluidic components

Introduction

This topic describes the sheath tank and the associated fluidic components.

Description

The sheath tank holds the sheath fluid. Pressure is applied to the sheath tank to push sheath fluid through the system.

Components

The main components of sheath tank and fluidics are:

- Sheath tank
- Digital scale (for sheath fluid level monitoring)
- Sheath line

Sheath tank

The sheath tank is an autoclavable 7-L tank.

The sheath tank has:

- A sheath filter to filter the sheath fluid.
- A sheath line to deliver the sheath to the nozzle.
- An air line to pressurize the tank.
- A pressure gauge to confirm the pressure.

- A release valve to remove the pressure from the tank.

Digital scale

The digital scale weighs the sheath tank and its fluid. The weight is used to determine the level of fluid in the tank.

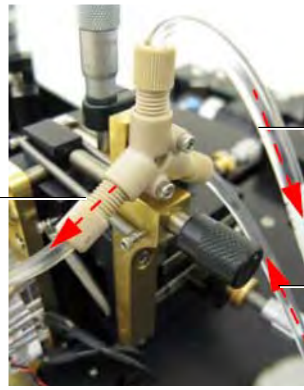
The digital scale must be zeroed to calibrate the scale and determine the weight of an empty tank each time you refill the sheath tank or whenever you shut down the system.

When you add sheath fluid to the tank, the additional weight is converted to a liquid volume value that determines the level of sheath in the tank (1 kg of measured weight is equal to 1 L of sheath fluid).

See Calibrating the digital scale with an empty tank (page 228) for more information about zeroing the scale. See Separate wall power source (page 66) for more information about power considerations for the digital scale.

Sheath line

The sheath line connects the output of the sheath filter with the Y-fitting on the nozzle stage assembly.



The sheath line delivers sheath fluid from the sheath filter, through the pressure console sheath pinch valve, to the nozzle.

More information

- System and components (page 27)
- Fluidic components (page 32)
- Pressure regulation and monitoring (page 37)

Waste fluidic components

Introduction

This topic describes the waste fluidic components.

Components

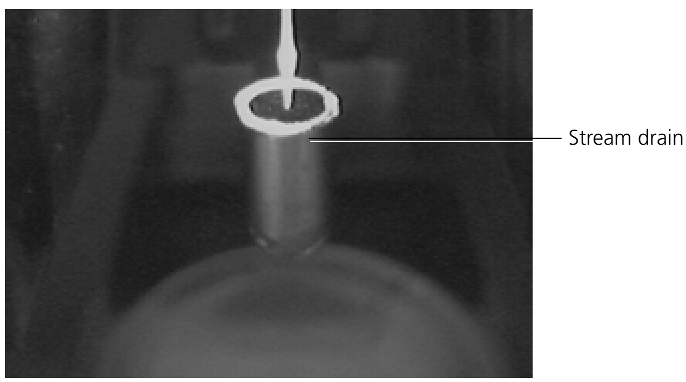
The main waste fluidic components are:

- Waste drains and bucket
- Waste tank
- Vacuum pump

Waste drains and bucket

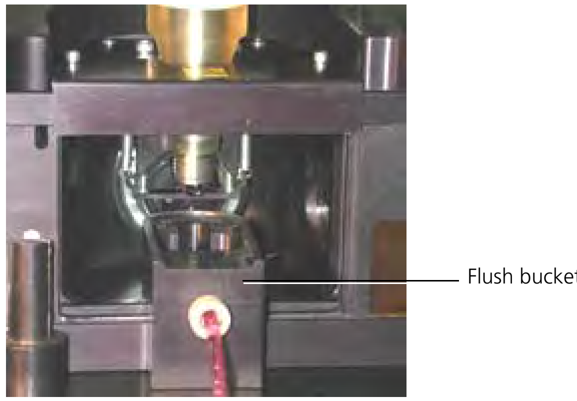
The waste drains consist of the stream drain, backflush drain, and the flush bucket.

- **Stream drain.** Located directly under the stream in the sort chamber. The non-sorted fluid from the nozzle falls into the stream drain and is vacuumed to the waste tank.



- **Backflush drain.** Located below the tube in the sample station. This drain is used to draw the backflushed fluid to the waste tank.

- **Flush bucket.** Used during a system flush or purge. It is placed directly under the nozzle. The fluid is vacuumed to the waste tank.



Waste tank

The waste tank is an autoclavable 7-L tank. It has one port for the vacuum line and one for the gauge. The gauge is used to monitor the amount of vacuum applied to the tank. The waste line carries the waste to the waste tank. The waste tank lid must have a complete seal with the tank, or else it will not have the vacuum needed to pull the fluids.

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.

Vacuum pump

The vacuum pump draws waste from the stream drain, backflush drain, and the flush bucket to the waste tank. The vacuum line connects directly to the vacuum source fitting on the waste tank.

The vacuum pump runs directly from the wall power source, independent from the instrument power circuitry.

Make sure that you run the system with the hydrophobic filter. Otherwise water can get into the vacuum lines and can cause damage to your pump.

More information

- Fluidic components (page 32)
- Sheath tank and fluidic components (page 40)

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5

Optics

This section includes these topics:

Optical illumination

Introduction

This topic describes the illumination subsystem.

The illumination subsystem includes all components necessary to apply a focused beam of laser light through the sample stream. All laser beams are deflected into the sort head and are aligned independently by adjustable mirrors. Each laser beam has a final focus lens that is mounted on a dedicated translational stage for fine adjustments.

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, beam shaping, and a focusing lens.
- Detection of the signal from the illuminated particle as well as the scatter light of the particle. This is covered in the next section.

Components

The main components of the illumination subsystem are:

- Lasers (up to seven)

Laser	Wavelength (nm)	Power (mW)
UV	355	100
Violet	405	50 and 150
Blue-violet	457	300
Blue	488	200
Green	532	150
Yellow-green	561	75 and 150
Red	640	50 and 150

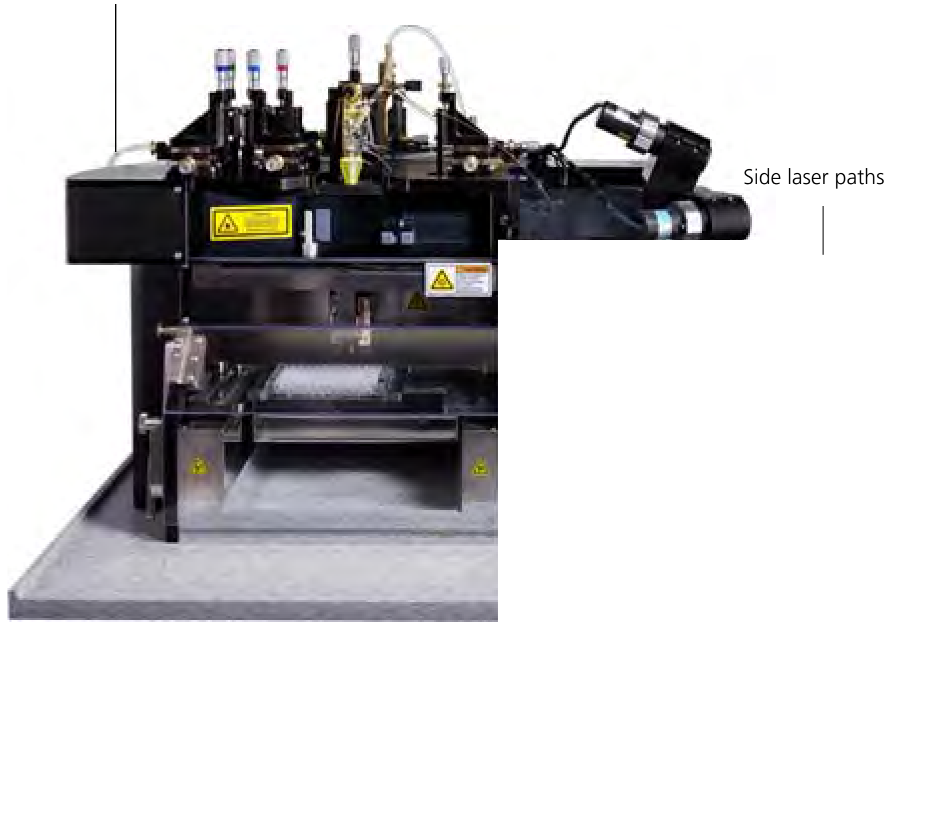
- Protective shields and guards
- Laser shutters and the shutter interlock system
- Steering optics and micropositioners
- Beam-shaping prisms and/or mirrors which create round or elliptical beam shapes (3:1 ratio with a typical beam height of 15–20 µm).
- Iris diaphragms
- Focusing lenses mounted on adjusting stages

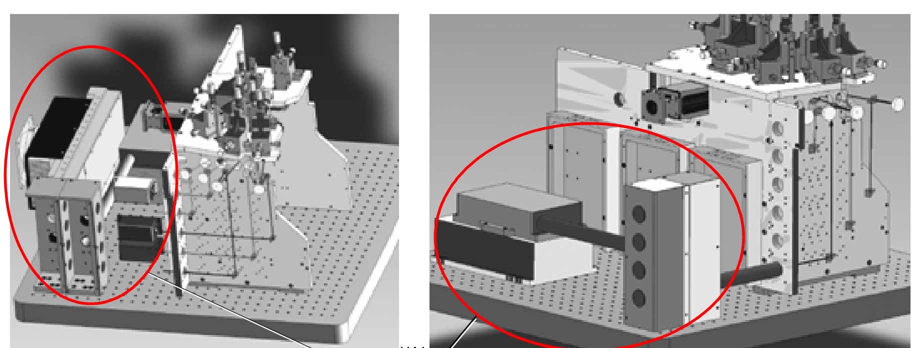
Location of lasers

The instrument includes air-cooled lasers in two locations:

- On the optical bench, behind the main sort head
- Attached directly to the rear of the sort head

All laser beams are deflected into the sort head and are aligned independently by adjustable mirrors. Each laser beam has a final focus lens that is mounted on a dedicated translational stage for fine adjustments.





More information

- Functional subsystems (page 29)
- Optical detection (page 50)
- Signal processing (page 55)

Optical detection

Introduction

This topic describes the optical detection components.

The optical detection system separates and quantifies the intensity of light and scattered light from the particle stream.

This detection system includes focusing lenses and iris diaphragms to help center the signal down the optical path, filters, and mirrors that route the signal to the appropriate detectors.

About light collection

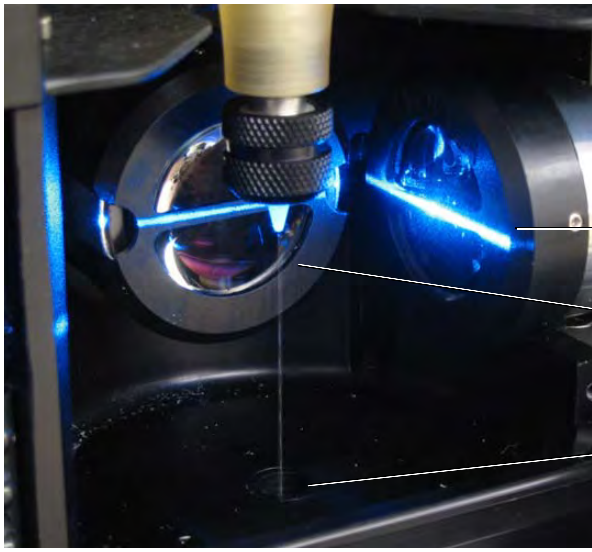
The collection lens collects the emitted light from the particle/laser interrogation. This signal then travels down the optical bench to the appropriate detectors. Emission light is collected through a 20X, 0.6 NA microscope objective. Light is focused on five spatially separated mirror pinholes. Modular detector blocks allow for a user-defined detection configuration.

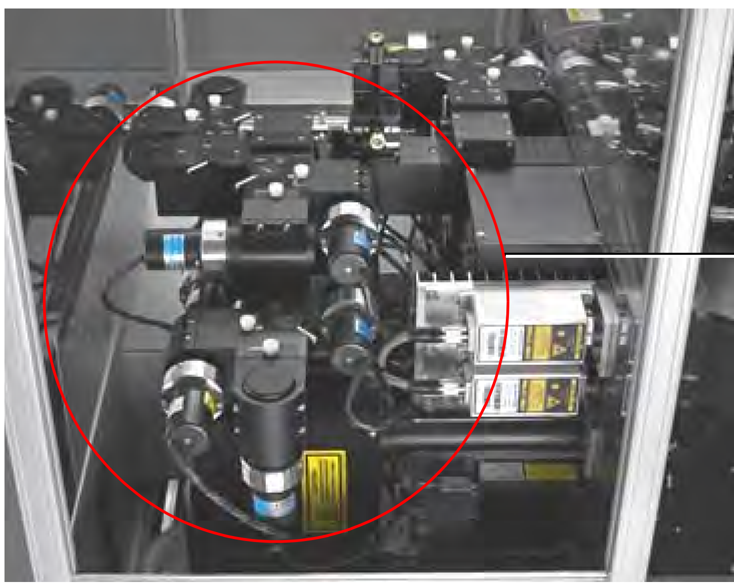
Detectors

There are three detectors in the system: forward scatter, small particle forward scatter, and side scatter.

- **Forward scatter (FSC) detector.** This detector collects the scattered light from the particle/laser interaction. Resolution for the standard forward scatter detector is >0.5 µm (measured using beads). The collection angle is 2–17°.
- **Small particle forward scatter detector (SPO).** This detector measures tiny particle applications (for example, marine biology). The scatter is detected by a 20X, 0.42 NA microscope objective. Resolution for the SPO is >0.2 µm (measured using beads and 0.1-µm filtered sheath fluid). The collection angle is 2–30°. See Small particle forward scatter detector option (page 361) for more information.

- **Side scatter (SSC) detector.** Side scatter is collected through the 90° collection lens and is measured using a PMT. Side scatter resolution is >0.2 µm (measured using beads and 0.1-µm filtered sheath fluid).

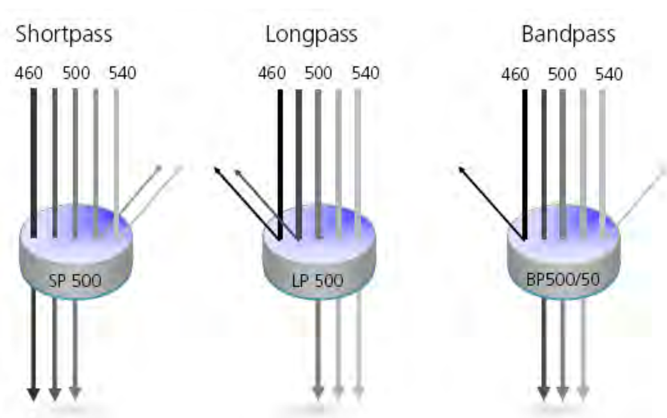


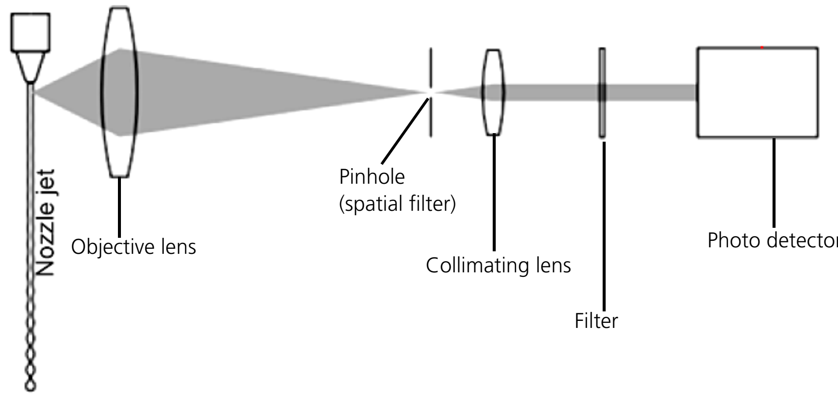


Filters

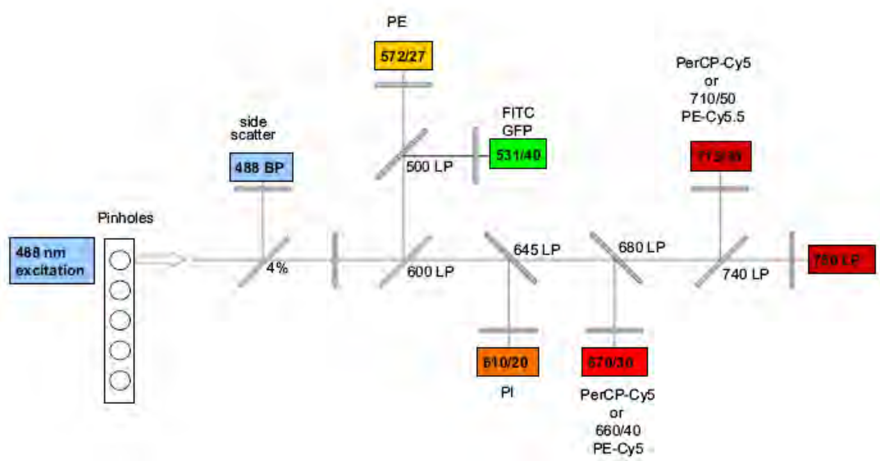
In addition to the PMTs, the optical detection modules include the following filters.

Component	Description
Longpass (LP) filters	These filters steer light between the detectors within a detector array. They reflect shorter wavelengths and transmit longer wavelengths. For example, a 505 nm LP filter allows wavelengths equal to or greater than 505 nm to pass through to the detector.
Bandpass (BP) filters	Allow a designated range of spectral wavelength to pass through to the PMTs. A BP filter is located in front of each PMT. For example, a 530/40 BP allows wavelengths between 510 and 550 nm through to the detector.
Neutral density (ND) filters	The neutral density filters attenuate (decrease) the amount of light that passes through.
Shortpass (SP) filters	These filters reflect longer wavelengths and transmit shorter wavelengths. For example, a 550 SP allows only wavelengths equal to or less than 550 nm to pass through.
Rejection (RP) filters	These filters are used with a longpass filter to pass most frequencies unaltered, but they attenuate a specific range of frequencies to very low levels. It is the opposite of a bandpass filter. For example, if a 532/20 rejection filter is set in front of a 530/20 BP, and the BP is set in front of a detector, then wavelengths between 522–542 nm are not allowed to pass through to the 530/20 BP. This is commonly used in situations to avoid another laser's signal from getting to the detector.
Par (parallel polarizer)	The parallel FSC measures the standard, polarized FSC signal.
Per (perpendicular polarizer)	The perpendicular FSC measures depolarized FSC light. This is used for specialized applications like marine biology.





The following illustration shows an optical bench layout that includes: 488 nm excitation, scatter, and FITC, PE, PI, PE-Cy™5, PerCP, and PE-Cy™5.5 detectors.

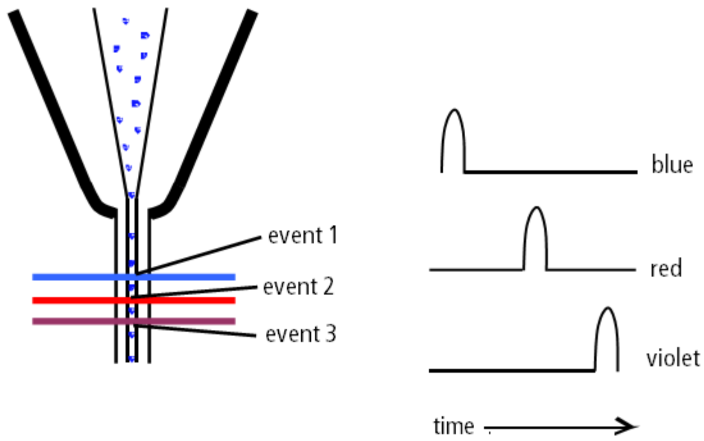


Laser delay

The lasers are spatially separated, causing a slight delay between the detection of each laser's signal. 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 set laser delay when you optimize multiple lasers. You typically set the laser delays during daily instrument QC and optimization.

The following table describes laser delay with three lasers.

Process	Description
1	A particle intercepts the blue laser first.
2	A signal is generated.
3	The same particle passes through the red laser in a given amount of time (red laser delay). The time between the blue laser and the red laser is measured when the particle hits the beam.
4	The same particle passes through the violet laser in a given amount of time (violet laser delay). The time between the red laser and the violet laser is measured when the particle hits the beam.



More information

- Functional subsystems (page 29)
- Signal processing (page 55)
- Setting laser delays (page 257)
- Optical detector modules (page 333)
- Small particle forward scatter detector option (page 361)

Signal processing

Introduction

This topic describes the signal processing components.

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

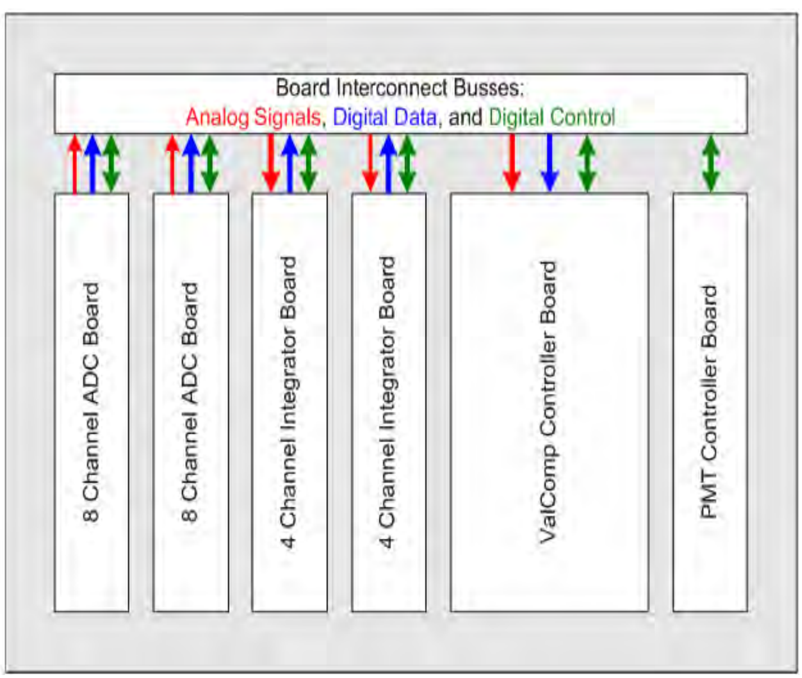
- Monitored by the operator
- Acquired and analyzed by BD FACS Sortware sorter software
- Used by the sort electronics subsystem

Components

The main signal processing components are:

- **Logarithmic and linear pre-amplifiers (log and lin preamps).** Modify signal input to produce an output voltage. Linear amplifiers check coefficients of variation (CVs) in scatter or fluorescence signals. Log amplifiers detect smaller particles in scatter or fluorescence signals.
- **Analog-to-digital converters (ADCs).** Perform 16-bit analog-to-digital conversion (65,536 channels). ADCs provide raw data to help determine compensation.
- **Digital signal processors (DSPs).** Perform a 16 x 16 digital compensation matrix. Compensated parameters are added as separate parameters.
- **Integrators.** Dedicated hardware components that measure the area and the width of a voltage pulse for selected parameters. They work in parallel with the height and peak measurement. Integrator boards are optional on the BD Influx instrument.

  The following illustration shows the signal processing components in the BD Influx electronics cabinet.



More information

- Functional subsystems (page 29)
- Optical detection (page 50)
- Sort electronics (page 58)

6

Sort components

This section includes these topics:

Sort electronics

Introduction

This topic describes the sort electronics components.

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

Components

The main components of sort electronics are:

- **Control circuitry.** Includes 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 droplets. You can adjust the piezo amplitude and frequency when you define the droplet breakoff and when you configure a sort.
- **Stream deflection.** High-voltage deflection plates deflect droplets from the main stream and direct droplets to specific tubes or wells in the sort tray.

More information

- Sort components (page 57)
- Sorted sample collection (page 59)
- Deflection plate power (page 67)

Sorted sample collection

Introduction

This topic describes the sorted sample collection components.

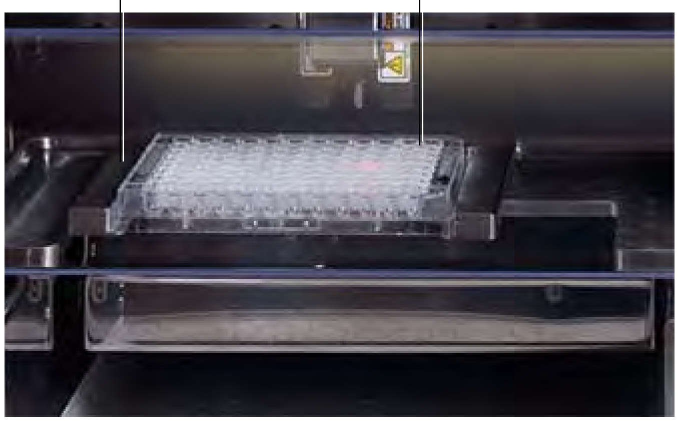
Sample collection devices

During sorting, the sample collection subsystem collects the charged droplets. You can sort droplets into the following sort devices:

- Multiple test tubes
- 96-well tray
- Standard microscope slides

Sort stage

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



More information

- Sort electronics (page 58)
- Sort monitoring rack (page 61)

Sort monitoring rack

Introduction

This topic describes the sort monitoring rack.

Use the sort monitoring rack to operate BD FACS Software sorter software, set up and adjust the drop frequency and alignment, and monitor a sort in real time as the BD Influx sorter acquires data.

Components

The sort monitoring rack includes the following components.

Component	Description
Pinhole monitor	Displays an image of the pinholes through the pinhole camera.
Drop monitor	Displays an image of the stream breakoff through the drop camera.
Sort stream monitor	Displays an image of the sidestreams and stream drain through the stream camera.

Component	Description
Deflection plate power	Includes the PLATE power button and +/- HV (high voltage) readouts. Deflection plate voltage is on when the PLATE button is illuminated.
Sortware user interface	Consists of a monitor, keyboard, and mouse.
Oscilloscope	Displays voltage pulses and laser delay settings as particles pass through the laser.

Other components

Other monitoring components are described in the fluidics section of this guide. These components include:

- Pressure readouts
- Sample station fault LED indicators
- Drop position indicator

More information

- Functional subsystems (page 29)
- Sample introduction (page 33)
- Pressure regulation and monitoring (page 37)
- Sorted sample collection (page 59)
- Power distribution (page 64)
- Deflection plate power (page 67)

7

System power

This section includes these topics:

Power distribution

Introduction

This topic describes the power distribution controls.

Power is distributed to the instrument components by switched and unswitched power strips. You can use the power distribution controls to turn on specific parts of the instrument as needed.

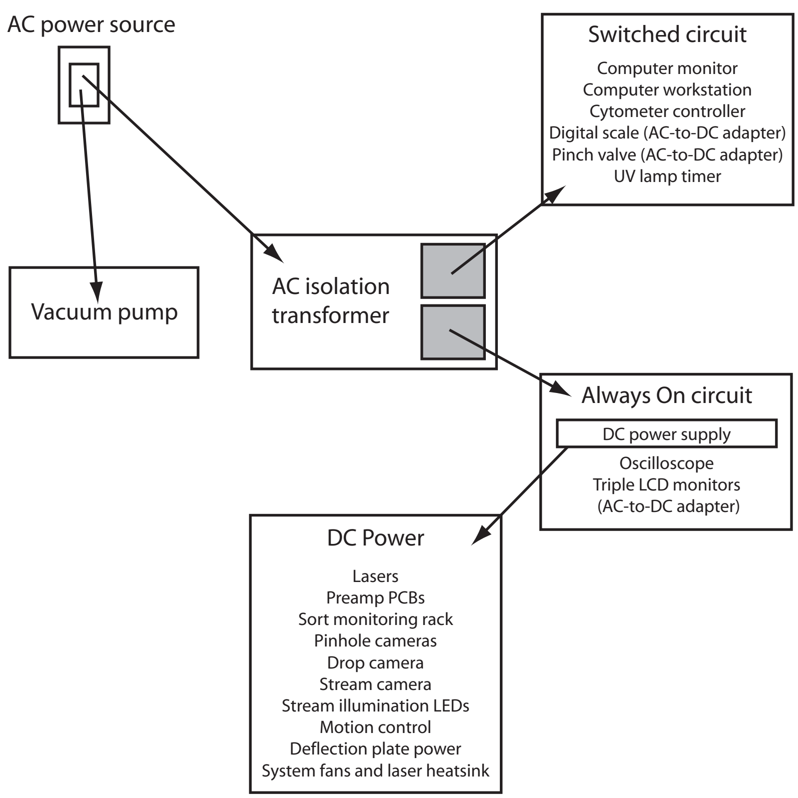
Components

The electronics and power subsystems consist of the following:

- AC power
- DC power

How power is distributed

This figure shows how power is distributed from the AC power source to the isolation transformer, from the isolation transformer to the switched circuit or the Always On circuit, and from the Always On circuit to the DC power.



More information

- AC power (page 65)
- DC power supply (page 67)

AC power

Introduction

This topic describes the AC power for the BD Influx system.

Main power switch and isolation transformer

The BD Influx electrical system uses a power source of 120 VAC/60 Hz/15 A. From the source, the instrument sends the AC power to an isolation transformer. The main system power switch (power source) is typically located in the back of the instrument or on the bench next to the instrument.

The isolation transformer sends 120 VAC to the *Always On* and *Switched* circuits. The components of these two circuits are plugged into circuit-specific power strips which are plugged into the back of the transformer.

Always On circuit

The Always On circuit components run as long as the instrument is connected to its power source and that component is switched on. This circuit sends 120 VAC to the protector power strip. This power strip supplies 120 VAC to the following components:

- Computer and monitor
- Digital scale
- UV lamp timer (controls when the UV lamp is turned on and off)

A separate 8-outlet power supply delivers 120 VAC to the following components:

- All lasers
- Pinch valve power supply (solenoid pinch valve controller PCB on the pressure console)
- An additional power strip (on the back of the DC power supply)
- Upper and lower plenum of the optional HEPA-filtered enclosure

Switched circuit

The Switched circuit is controlled by a 6-outlet power strip. The power strip receives 120 VAC from the isolation transformer. The main ON/OFF switch must be turned on to deliver 120 VAC to the following components:

- DC power supply (See DC power supply (page 67) for more information.)
- Oscilloscope
- Triple LCD (120 VAC converted to 12 VDC/2 A)

Separate wall power source

A vacuum pump runs directly from the wall power source, independent from the instrument power circuitry. The vacuum pump is supplied with the instrument, but any vacuum pump that meets BD specifications can be used.

You should consider powering the digital scale from a separate wall power source to avoid having to reset the digital scale each time the system is shut down.

Auxiliary power switch and laser power supply

The following figure shows the location of the power switches on the front of the system.

Emergency shutoff

Switch the main power switch off in emergency shutdown situations.

More information

- Power distribution (page 64)
- DC power supply (page 67)
- System startup (page 223)

DC power supply

Introduction

This topic describes the components that are powered by the DC power supply.

DC power

The DC power supply receives 120 VAC from the 8-outlet power strip. The system ON/OFF switch must be switched on for the DC power supply to supply power to its DC components.

The DC power supply distributes DC voltages to the following components:

- Preamp PCBs
- Sort monitoring rack
- Forward pinhole camera
- Head connect PCB, which delivers power to the following components:
  - Pinhole camera
  - Drop camera
  - Stream camera
  - Stream illumination laser
- Deflection plate voltage
- Main control, which controls the preamp and laser heatsink fans

Deflection plate power

The PLATES button turns the power to the deflection plates on and off. The button illuminates when the power is on. The button and the HV readouts are located below the pinhole monitors on the sort monitoring rack. The voltage should be 3.6 kV (+HV) and –3.6kV (–HV) (+/– 0.02kV).

More information

- Sort electronics (page 58)
- Power distribution (page 64)
- AC power (page 65)
- Calibrating the digital scale with an empty tank (page 228)

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8

Maintenance

This section includes these maintenance topics:

This section 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 your BD Influx system administrator. Contact your BD service representative if you do not have the proper tools or materials to perform these maintenance procedures.

Daily maintenance

Daily maintenance includes the following procedures.

Stage	Description
1	Cleaning and inspecting the instrument (page 72)
2	Inspecting and cleaning the deflection plates (page 72)
3	Bypassing the sheath filter (page 75)
4	Decontaminating the fluidics (page 79)
5	Cleaning the nozzle tip (page 234)

Weekly maintenance

Weekly maintenance includes the following procedures.

Stage	Description
1	Cleaning the fan guards (page 77)
2	Decontaminating the fluidics (page 79)

Monthly maintenance

In addition to daily and weekly maintenance procedures, perform a system flush with bleach each month. See Flushing the system (page 233) for more information.

Periodic maintenance

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

Stage	Description
1	Backflushing the sample line for a different sample (page 78)
2	Removing the sample line (page 82)
3	Installing a new sample line (page 83)
4	Removing the sheath and waste fluidics lines (page 85)
5	Installing new sheath and waste fluidics lines (page 87)

More information

- Instrument cleaning and maintenance (page 71)
- Component replacement (page 80)
- Flushing the system (page 233)

Instrument cleaning and maintenance

Introduction

This topic lists all the instrument cleaning and maintenance procedures that you can perform without assistance from BD service.

- If you are performing regular daily, weekly, or monthly maintenance, follow the specific maintenance workflows.
- If you need to clean or maintain the system due to accidents, spills, repairs, upgrades, or changes in performance, you can perform the cleaning and maintenance procedures in any order (as needed).

Individual cleaning and maintenance procedures

See the following topics for cleaning or maintenance (as needed):

- Cleaning and inspecting the instrument (page 72)
- Inspecting and cleaning the deflection plates (page 72)
- Bypassing the sheath filter (page 75)
- Cleaning the fan guards (page 77)
- Backflushing the sample line for a different sample (page 78)
- Decontaminating the fluidics (page 79)
- Component replacement (page 80)
- Flushing the system (page 233)
- Cleaning the nozzle tip (page 234)

More information

- Maintenance overview (page 70)
- Component replacement (page 80)

Cleaning and inspecting the instrument

Introduction

This topic describes the basic instrument cleaning and inspection procedures. Perform this procedure daily.

Required materials

- 10% bleach
- Deionized (DI) water
- Wipes

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.
3. Clean the sort tray with a solution of 10% bleach and DI water.
4. Clean the stream camera by wiping off any debris or spots.
5. Vacuum dust and lint from fan areas, such as the back of the sort electronics console, laser power supplies, and the computer.
6. Inspect the tubing and fluidics for leaks.

More information

- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Inspecting and cleaning the deflection plates (page 72)
- Component replacement (page 80)

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.

Required materials

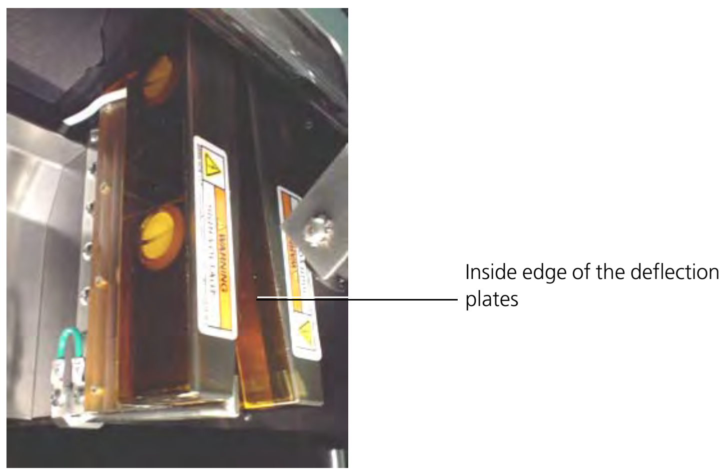
- Kimwipes® wipe or other lint-free towel
- DI water

Inspecting the deflection plates

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

To inspect the deflection plates:

1. Power off the deflection plates by pressing the PLATES button on the sort monitoring rack. The button should not be illuminated.
2. To view the plates, open them by swinging them away from the stream drain so that the protective tape is facing towards you.
3. Inspect the protective tape on the high-voltage deflection plates. Look for signs of wear or ripping. Check for wear or damage on the inside edge of the deflection plates.



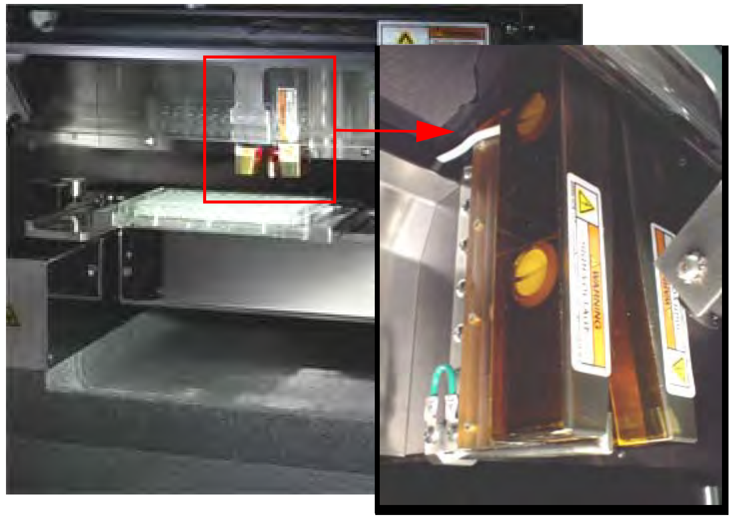
4. Inspect for signs of water leakage or wetness.
5. Clean the plates thoroughly if salt crystals are visible anywhere on the tape.

Cleaning the deflection plates

To clean the deflection plates:

1. Power off the deflection plates by pressing the PLATES button on the sort monitoring rack. The button should not be illuminated.
2. To view the plates, open them by swinging them away from the stream drain so that the protective tape is facing towards you.
3. Wet a Kimwipes wipe or other lint-free towel with DI water and wipe down the deflection plates.
4. Wipe the plates again with a dry wipe to ensure that there is no fluid on the plates before closing them.

5. Close the deflection plates by swinging them into their closed position.



The attached magnetic latches hold them in place.

More information

- Deflection plate power (page 67)
- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Bypassing the sheath filter (page 75)

Bypassing the sheath filter

Introduction

This topic describes how to remove and bypass the sheath filter when you want to perform a dry shutdown or when you want to replace the filter or tubing.

Procedure

To bypass the sheath filter:

1. Press RUN to turn off all fluidics.
2. Turn off the AIR switch on the pressure console.
3. Depressurize the sheath tank.
4. Remove the external sheath filter.
5. Disconnect the upper quick connect fitting from the instrument sheath line.

Upper quick connect

6. Disconnect the lower quick connect fitting from the tank sheath line.

Lower quick connect

7. Reconnect the sheath tank without the sheath filter.
   a. Close the pressure relief valve on the tank.
   b. Connect the external air line to the air input fitting on the tank.

c. Bypass the sheath filter by connecting the tank sheath line to the instrument sheath line.

The sheath filter is now bypassed. You can now flush the instrument fluidics with DI water, dry the fluidics tubing, or replace the sheath filter.

More information

- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Cleaning the fan guards (page 77)
- Component replacement (page 80)

Cleaning the fan guards

Introduction

This topic describes how to clean the fan guards on the system. Perform this procedure weekly.

The system includes fan guards in the following locations:

- Computer tower
- Main power supply
- Acquisition system grills

Procedure

To clean the fan guards:

1. Brush or vacuum the top of the fan guards to clear any particulates or debris.

More information

- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Backflushing the sample line for a different sample (page 78)

Backflushing the sample line for a different sample

Introduction

This topic describes how to prevent sample carryover when you run samples on the instrument. Perform this procedure as needed or when the sample line is clogged.

Required materials

Sheath fluid

Procedure

To backflush the sample line for a different sample:

1. Load a sample tube with sheath fluid.
2. Run clean sheath through the sample line at a high sample pressure (~3 PSI over the sheath pressure).
3. Hold the **BOOST** button to maintain the high sample pressure.
4. Run until no particles are detected.

   When you switch from one sample to another, make sure to backflush the sample line for approximately 10 seconds. This helps to minimize sample carryover.

More information

- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Removing and replacing the nozzle tip (page 81)
- Decontaminating the fluidics (page 79)
- Component replacement (page 80)

Decontaminating the fluidics

Introduction

This topic describes how to decontaminate the fluidics.

Required materials

- 10% bleach
- DI water (1L)

Procedure

To decontaminate the fluidics:

1. Load a tube of 10% bleach and press SAMPLE. Run it for 5 minutes.
2. Load a tube of DI water and press SAMPLE. Run it for 5 minutes.
3. Press RUN to turn off all fluidics.
4. Turn off the AIR switch on the pressure console.
5. Empty the sheath tank, rinse it, and fill it with about 500 mL of DI water.
6. Bypass the sheath filter.
7. Reattach the sheath tank and turn the air on.
8. Press RINSE, and then BACKFLUSH to rinse all fluid lines with DI water until the tank runs dry and the sample line is no longer dripping water.
9. Press RINSE to turn off the flow.
10. Remove the nozzle tip and place the flush bucket under the nozzle.

Caution! Make sure the piezo amplitude is turned off before removing the nozzle tip.

11. Disconnect the air and fluid lines from the sheath tank and connect them to each other.
12. Press RINSE, then BACKFLUSH.
13. Allow air to blow through the system for about 10–15 minutes to completely dry it out.
14. Turn off the air supply.
15. Empty all fluid from the waste and sheath tanks, rinse them with clean DI water, and allow them to dry overnight.

More information

- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Bypassing the sheath filter (page 75)
- Replacing the nozzle assembly (page 89)

Component replacement

Introduction

This topic lists the components you can remove and replace without the assistance of a service representative.

Before replacing components

Before beginning any maintenance procedures, do the following:

- Shutter or turn off the power to 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 appropriate biohazard precautions.

User-replaceable component procedures

You can perform the following component replacement procedures:

- Removing and replacing the nozzle tip (page 81)
- Removing the sample line (page 82)
- Installing a new sample line (page 83)
- Removing the sheath and waste fluidics lines (page 85)
- Installing new sheath and waste fluidics lines (page 87)
- Replacing the nozzle assembly (page 89)
- Replacing the sample stopper (page 90)

More information

- Power distribution (page 64)
- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Decontaminating the fluidics (page 79)
- Turning off the power (page 326)

Removing and replacing the nozzle tip

Introduction

This topic describes how to remove and replace the nozzle tip. You need to remove the nozzle tip to clean the nozzle or change nozzle sizes.

Removing the nozzle tip

To remove a nozzle tip:

1. Turn the system power off.

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

2. Loosen the nozzle nut by hand. Do not use a wrench.
3. Remove the nut and nozzle tip.
4. Place the nozzle tip, nut, and O-ring in a safe place.

Replacing the nozzle tip

To replace the nozzle tip:

1. Place the clean nozzle tip into the nozzle nut, ensuring that the O-ring is in place around the nozzle tip.
2. Screw the nozzle tip onto the nozzle, tightening by hand as much as possible. Do not use a wrench.

More information

- Maintenance overview (page 70)
- Instrument cleaning and maintenance (page 71)
- Backflushing the sample line for a different sample (page 78)
- Decontaminating the fluidics (page 79)
- Cleaning the nozzle tip (page 234)

Removing the sample line

Introduction

This topic describes how to remove the sample line.

Removing 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 clogged.

Before you begin

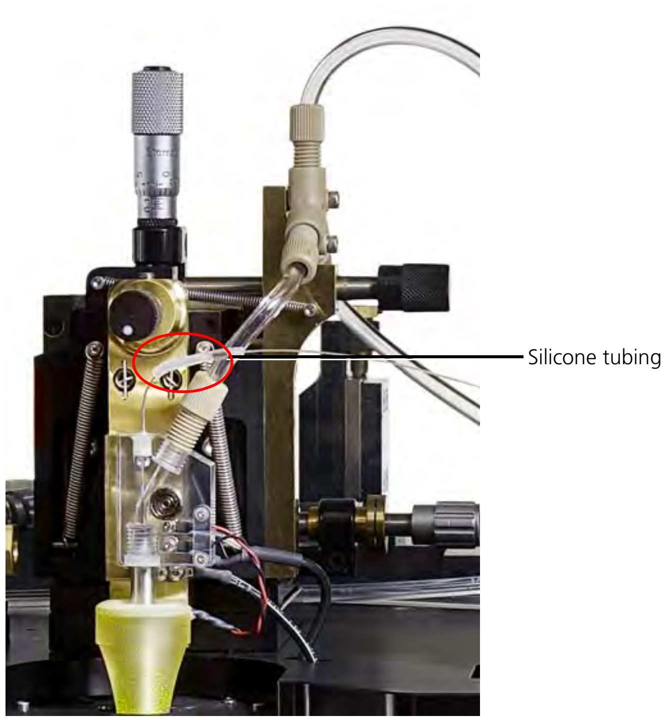
- See Before replacing components (page 80) for more information.
- Make sure that your system is dry.

Procedure

To remove the sample line:

1. Verify that the air switch is on.
2. Remove the sample line from the nozzle assembly.
3. Gently pull the silicone rubber seal where it attaches to the stainless steel tubing.

Caution! Do not bend the stainless steel fitting.



4. Press the OVERRIDE button to release the sample line pinch valve.
5. Remove the sample line from the sample line pinch valve.
6. Remove the sample line from the sample stopper column.

7. Gently pull on the silicone rubber seal where it attaches to the stainless steel tubing.

Caution! Do not bend the stainless steel fitting.

More information

- Maintenance overview (page 70)
- Decontaminating the fluidics (page 79)
- Component replacement (page 80)
- Installing a new sample line (page 83)

Installing a new sample line

Introduction

This topic describes how to install a new sample line to help minimize sample carryover. Perform this procedure every 4 to 6 months, or when decreased event rates indicate that the sample line is clogged.

Required materials

- Sheath sample line
- DI water (2–3 mL)
- Mirror
- Flashlight

Before you begin

- See Before replacing components (page 80) for more information.
- Remove the existing sample line.

Procedure

To install a new sample line:

1. Remove the nozzle tip.
2. 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.

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

3. Use a mirror and a flashlight to verify that the sample line is extending about 1 mm beyond the end of the stainless steel fitting inside the nozzle assembly.

Sample line installed incorrectly

Sample line installed correctly

Caution! Tighten the cap screw by hand. Do not use a wrench or you might crack the fitting.

4. Press OVERRIDE on the sample station, if it is not already on.
5. Install the sample line into the sample station by feeding it through the stainless steel tubing.
6. Insert the silicone section of the tubing into the pinch valve.
7. Verify that the sample line is not blocking the bubble detector just below the pinch valve.

   The bubble detector should have only silicone tubing in front of it. When the tubing is inserted properly, the bubble detector will register that there is air in the sample line, and the LED light will be off.

8. Install a tube and check the length of the sample line. Adjust if necessary.
9. Run 2–3 mL of clean DI water through the sample line before running samples.

More information

- Maintenance overview (page 70)
- Component replacement (page 80)
- Removing the sample line (page 82)

Removing the sheath and waste fluidics lines

Introduction

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

Before you begin

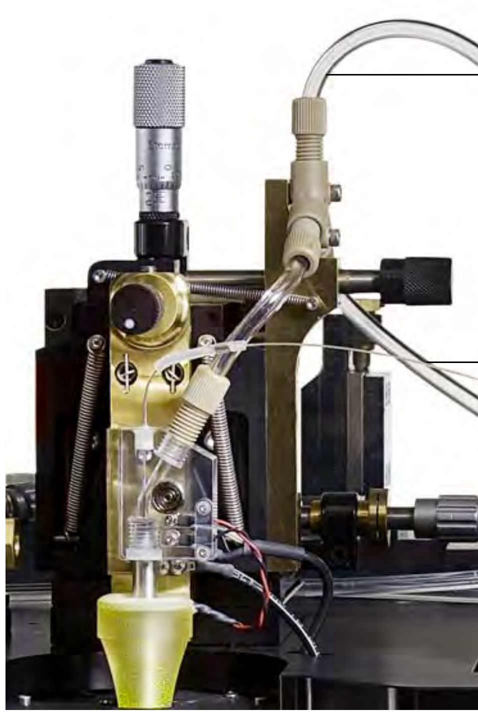
See Before replacing components (page 80) for more information.

Procedure

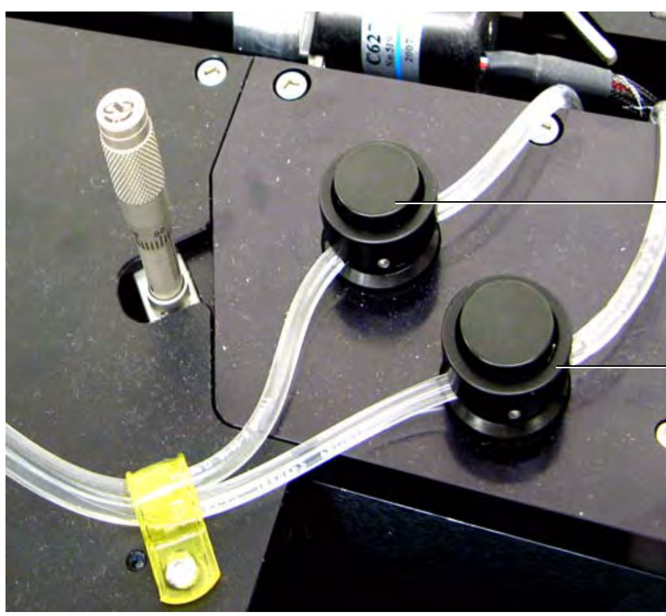
To remove the fluidics lines:

1. Verify that the air switch is on.
2. Depressurize the sheath tank.
   a. Disconnect the air line to the sheath tank.
   b. Open the pressure release valve.

3. Remove the sheath and purge lines from the top of the Y fitting above the nozzle.



4. Press RINSE to open both the sheath and purge valves.
5. Remove the tubing from the two valves.



6. Remove the hold-down covers that route the fluidic lines along the back of the instrument.

   If you have an instrument with a hood, you will have clips along the back of the instrument instead of a hold-down cover.

7. Undo the connections on the fluidics lines going to the sheath and waste tanks.
8. Remove the sheath filter.

More information

- Maintenance overview (page 70)
- Component replacement (page 80)
- Installing new sheath and waste fluidics lines (page 87)

Installing new sheath and waste fluidics 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)

Before you begin

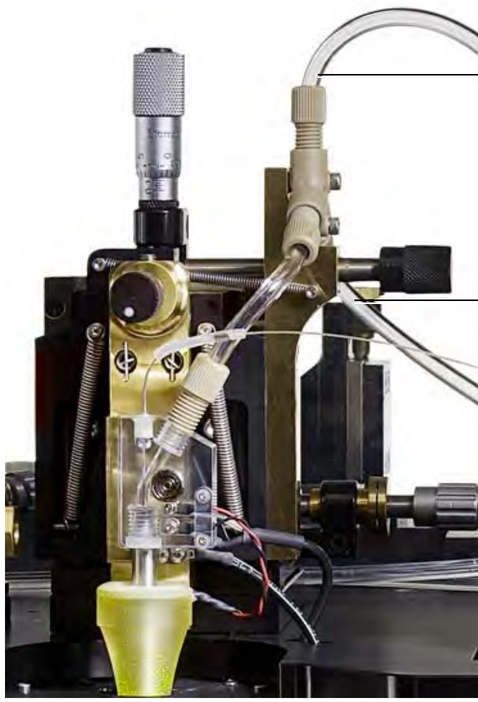
- See Before replacing components (page 80) for more information.
- Remove the existing fluidics lines.

Procedure

To install new fluidics lines:

1. Attach a new sheath filter.
2. Attach the new sheath and waste/purge lines and reroute them to the top of the instrument.
3. Attach the fittings to the Y fitting.

The waste/purge line connects to the top of the Y fitting and the sheath line connects to the back of the Y fitting.



4. Slide the lines into the pinch valves.

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

5. Run at least 200 mL of sterile sheath fluid through the tubes before running samples.

More information

- Maintenance overview (page 70)
- Component replacement (page 80)
- Removing the sheath and waste fluidics lines (page 85)

Replacing the nozzle assembly

Introduction

This topic describes how to replace the nozzle assembly.

Required materials

- Sterile sheath fluid (at least 200 mL)
- 7/64 in. Allen wrench

Before you begin

See Before replacing components (page 80) for more information.

Removing the nozzle assembly

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

To remove the nozzle assembly:

1. Remove the sample line from the top of the nozzle assembly by sliding the silicone off the stainless steel tubing.
2. Detach the nozzle line from the Y fitting.
3. Remove the screw using a 7/64 Allen wrench to hold the nozzle assembly in place.
4. Inspect the drop charge contact and the piezo drive connections for corrosion or bending.

Installing the new nozzle assembly

To install the new 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 is extending about 1 mm beyond the end of the stainless steel fitting inside the nozzle assembly.
3. Attach the new nozzle assembly using the thumbscrew removed earlier.

   The nozzle assembly will key into position automatically.

4. Tighten the thumbscrew by hand.

   Do not use a wrench or you might crack the fitting.

5. Reattach the sheath tubing to the Y fitting.
6. Before attaching a nozzle tip, run at least 200 mL of sterile sheath fluid through the new nozzle assembly.

More information

- Component replacement (page 80)

Replacing the sample stopper

Introduction

This topic describes how to remove the sample stopper.

Removing the sample stopper

To remove the sample stopper:

1. Turn off the power to the electronics and the lasers.

   The power to the pressure console can be left on.

2. Remove the sample line from the nozzle assembly.
3. Gently pull the silicone rubber seal where it attaches to the stainless steel tubing.

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

4. Press the OVERRIDE button to release the sample line pinch valve.
5. Remove the sample line from the sample line pinch valve.
6. Remove the sample line from the sample stopper column.

   There is no need to remove the sample line from the nozzle assembly.

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

Installing a new sample stopper

To install a new sample stopper:

1. Slide the new sample stopper onto the sample column.

   Do not force the sample stopper on. You can use a small amount of detergent to make it easier to slide the stopper onto the sample column.

2. Inspect the stopper for cracks and replace it if there are cracks.
3. Replace the sample line in the sample column.

More information

- Component replacement (page 80)

Part 2

Software information

This part includes these sections:

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9

Software overview

This section includes these topics:

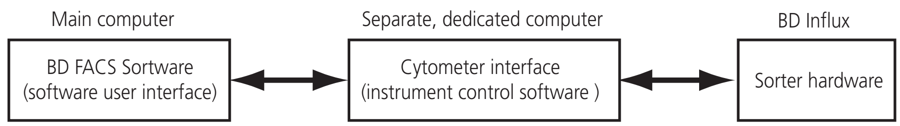
BD FACS Sortware overview

Introduction

This topic describes how BD FACS Sortware sorter software interacts with the instrument. It also describes the user interface and defines the panes that you can select from the main software window.

How the software interacts with the instrument

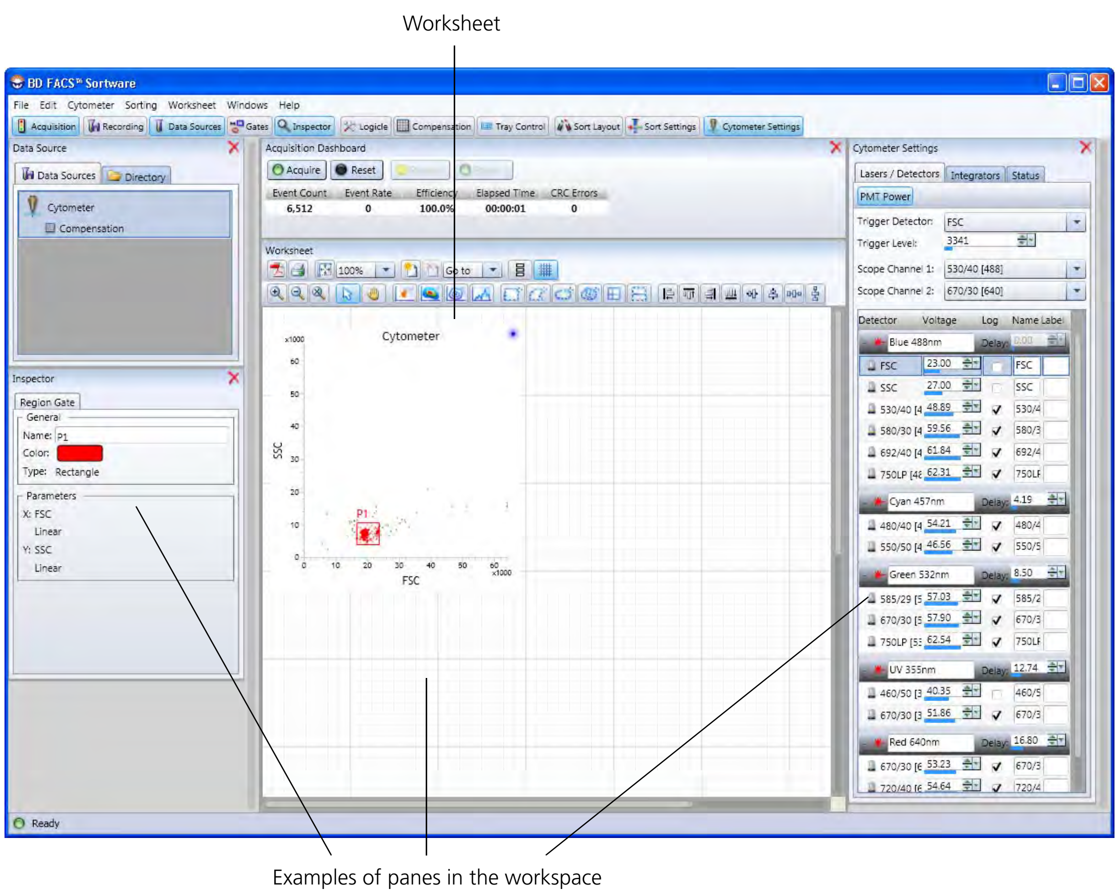
BD FACS Sortware sorter software serves as the user interface and works with the cytometer interface to control the instrument, acquisition, sorting, and analysis. The cytometer interface is a separate, dedicated computer that connects to the main computer and the sorter hardware.



User interface

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

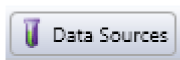
The worksheet is always open. Use the worksheet to create and modify plots, monitor sort data, and analyze sort results.



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 Software sorter software** toolbar to open panes in the workspace.

You can arrange individual panes to create specific window layouts. See Setting up your window layout (page 102) for more information.

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 155) for more information.
	Opens the **Recording Settings** pane. Use this pane to set details about the FSC file that is generated when you record data. See Using the Recording Settings pane (page 152) 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 (FSC files). See Using the Data Sources pane (page 148) 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 180) for more information.
	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 163) 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 133) 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 130) for more information.

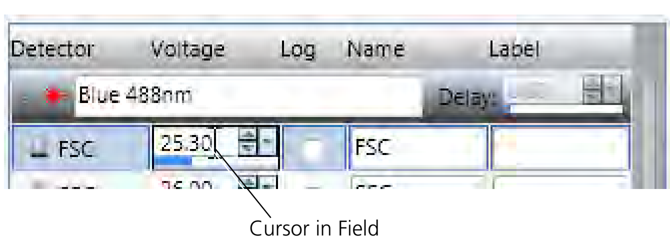
Control	Description
	Opens the **Tray Control** pane. Use this pane to view or modify the current sort device and adjust the offsets for the tray, or create a new sort device configuration. See Using the Tray Control pane (page 210) for more information.
	Opens the **Sort Layout** pane. Use this pane 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 217) for more information.
	Opens the **Sort Settings** pane. Use this dialog 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 204) 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 120) for more information.

Setting numeric values in panes and dialogs

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

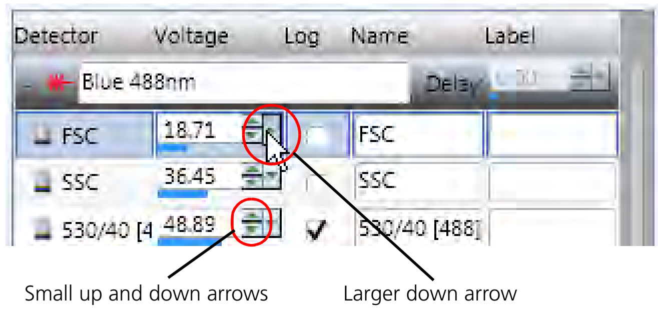
To set numeric values in fields with the mouse:

- Hold the mouse pointer over the field 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.

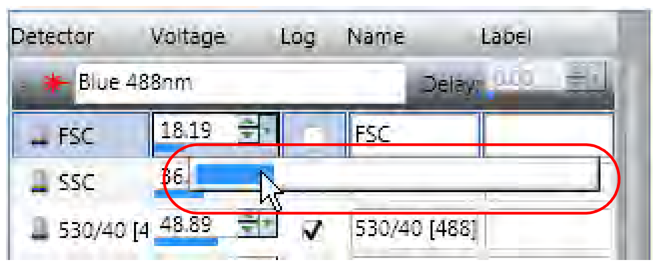


- Click the small up and down arrows to change the value.
- Click in the small blue/white data slider below the value to change the value.

- 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 dependant 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

- About lasers and pinholes (page 98)
- Preferences and settings (page 101)
- Worksheets (page 137)
- Saving and restoring settings (page 112)

About lasers and pinholes

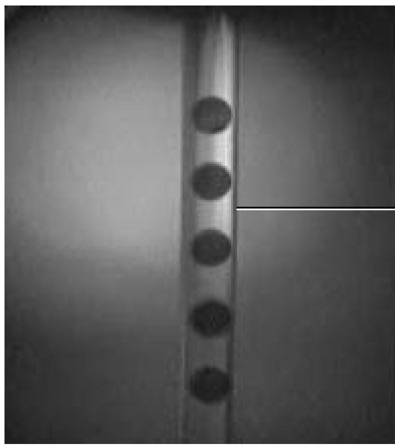
Introduction

This topic describes how lasers are assigned to pinholes.

About pinholes

Pinholes are used (along with the pinhole monitor) to provide a visual reference for aligning the sample stream and to help determine droplet breakoff points. One of the five pinholes is assigned as the "trigger," which serves as the reference for comparisons in alignment.

The following illustration shows a stream aligned with the pinholes as displayed through the pinhole monitor.



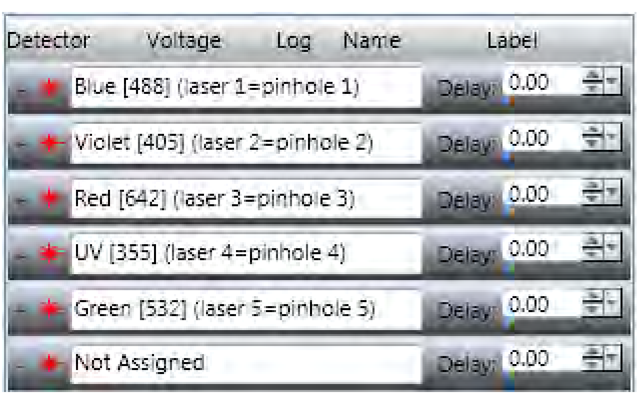
Aligned sample stream and pinholes

How lasers are assigned to pinholes

The pinholes are set to correspond to the physical laser setup of your instrument. Since lasers are assigned to pinholes during initial installation and setup, you do not need to change the laser-to-pinhole assignment unless you change or add lasers to your instrument.

The order of the lasers in the **Cytometer Setup** pane are based on the physical laser setup on your instrument.

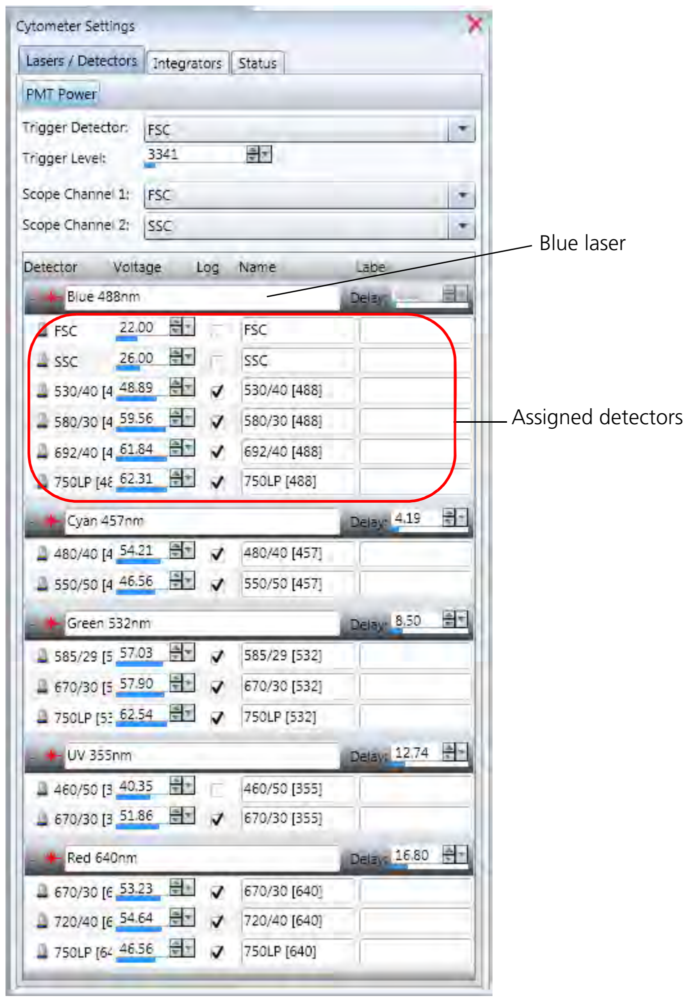
Note that laser 1 (as it appears in the list of lasers) corresponds to pinhole 1. The following figure shows descriptions added to the laser name to indicate how the lasers correspond to the pinholes.



How detectors are assigned to lasers

Detectors are assigned to lasers during initial installation and setup. Typically, you do not need to change the detector-to-laser assignment unless you make changes to the system optics or create a custom configuration.

In the following figure, FSC, SSC, and the 488-nm laser detectors are assigned to the Blue (488) laser (pinhole 1).



More information

- BD FACS Sortware overview (page 94)

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10

Preferences and settings

This section includes these topics:

Setting up your window layout

Introduction

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

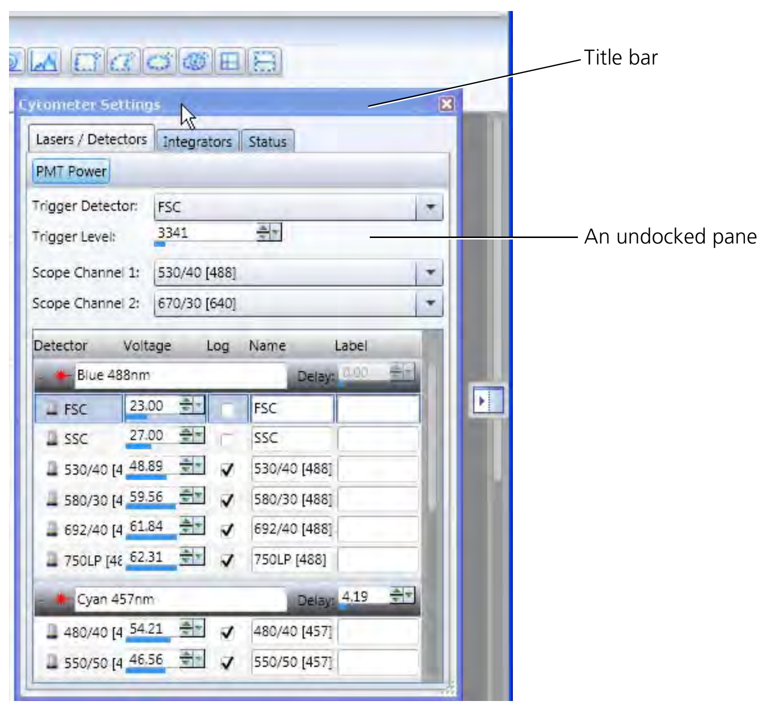
You can open different panes within the **BD FACS Sortware sorter software** window and drag and dock them to different locations, or create standalone (floating) panes and save the window layout.

Undocking and docking panes

To undock panes:

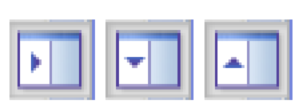
1. Click in the title bar area of any docked pane and drag the pane away from its docked location.

   The pane becomes a floating pane.



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

1. Drag the floating pane over one of the following vertical or horizontal docking targets.



   The floating pane is now a docked pane.

Changing themes

To change the window theme:

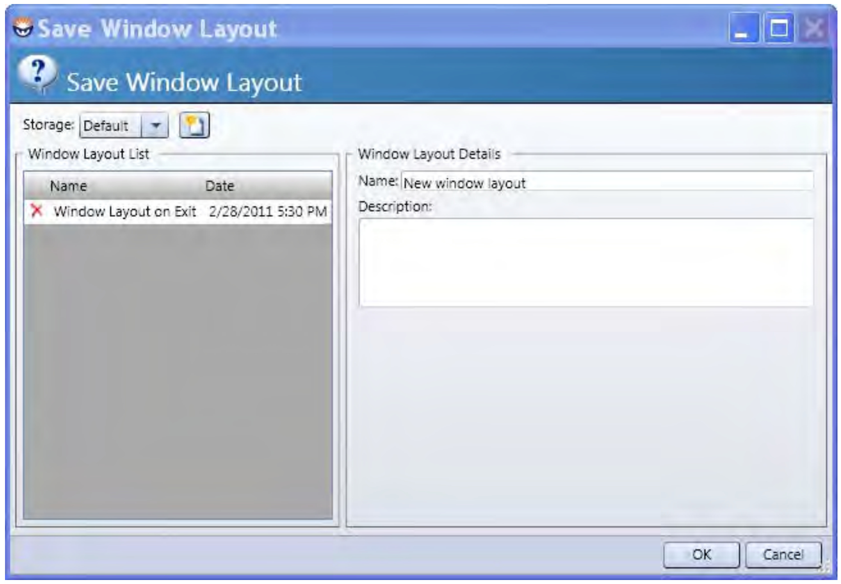
1. Select **Windows > Themes.**
2. Select the default or other available window theme.

Saving the window layout

To save the current window layout:

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

   The **Save Window Layout** dialog opens.



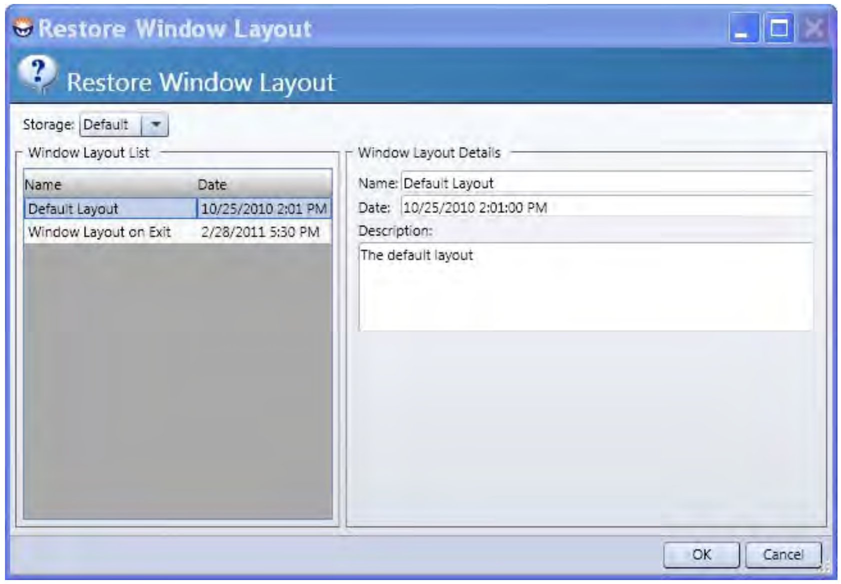
2. Select the storage folder where you want to save this layout from the **Storage** menu.
3. Under **Window Layout Details,** type a name for the new window layout in the **Name** field.
4. Click **OK** to save the window layout and close the dialog.

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 101)
- Editing user preferences (page 105)
- Saving and deleting settings (page 115)
- Restoring saved settings (page 118)

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 values are used.

User preferences

You can set the following user preferences:

- Setting cytometer preferences (page 105)
- Setting gate and population display preferences (page 108)
- Setting plot display preferences (page 110)
- Setting statistics views display preferences (page 111)

More information

- Preferences and settings (page 101)
- Saving and deleting settings (page 115)
- Restoring saved settings (page 118)

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 information:

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 fluorchrome/parameter name in plots and statistics views.
- Under **Plot/Views**, select plot and view options.

If you select...	Then...
Set Scope to Parameter(s)	Select a plot to view the signal on the oscilloscope using parameters from a plot.
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 138) for more information.

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

If you select...	Then...
Get Current Influx State from Server	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 labels.
Restore Full Cytometer	Restores the cytometer settings to the settings from the last time the system was used.

- Under **Sort Tray Direction**, select **Optimized** (for the plate to move in a serpentine motion: A1 to A12, B12 to B1), **Left to Right**, or **Top to Bottom** as the default sort tray sorting direction.

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

More information

- Preferences and settings (page 101)
- Setting gate and population display preferences (page 108)
- Setting plot display preferences (page 110)
- Setting statistics views display preferences (page 111)

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.

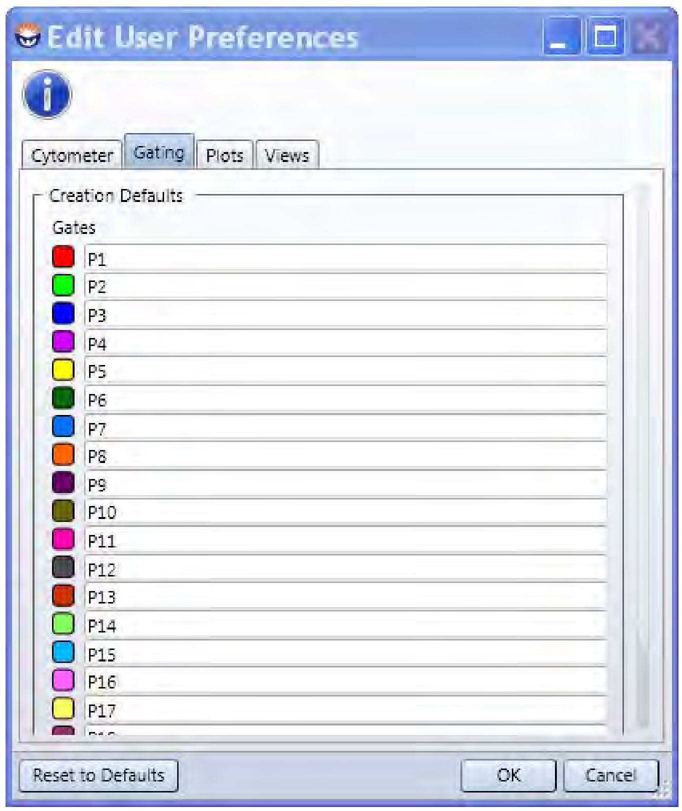
Procedure

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 101)
- Setting cytometer preferences (page 105)
- Setting plot display preferences (page 110)
- Setting statistics views display preferences (page 111)

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.

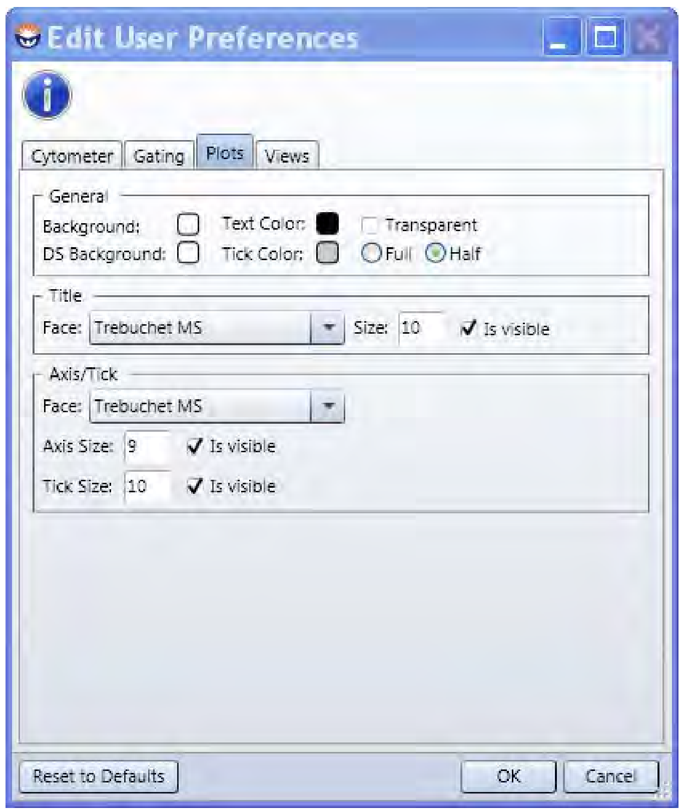
Procedure

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 these 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 or 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 plot 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 101)
- Setting cytometer preferences (page 105)
- Setting gate and population display preferences (page 108)
- Setting statistics views display preferences (page 111)

Setting statistics views display preferences

Introduction

This topic describes how to set statistical 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.

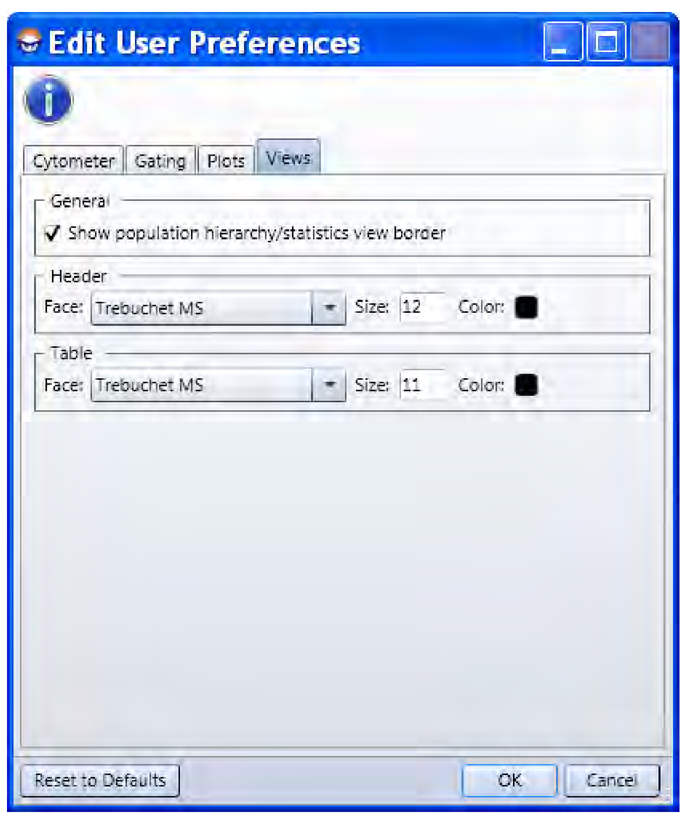
Procedure

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, and color.
   - Under **Table**, modify the table text display font face, size, and color.
4. Click **OK** to apply your preferences.

More information

- Preferences and settings (page 101)
- Setting gate and population display preferences (page 108)
- Setting plot display preferences (page 110)
- Setting cytometer preferences (page 105)

Saving and restoring settings

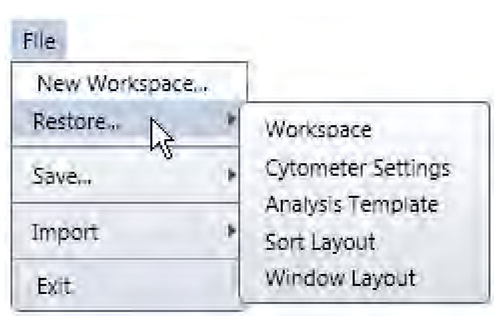
Introduction

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

Settings that can be restored

The settings that can be restored are:

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



Workspace

The following elements are saved with a workspace:

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

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

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
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	All settings in the following panes: • Sort Settings • Sort Layout • Tray Control
Restore Window Layout	Pane presence, docking, and position

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

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 any 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

When you save or restore a Sort layout, you save or restore the current settings from the **Sort Settings**, and **Sort Layout** dialogs, and the **Tray Control** pane.

Window layout

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 102) for more information.

Compensation

After you have calculated compensation, you can save the compensation by right-clicking and selecting **Compensation** below **Cytometer Data Source**. You can restore the compensation matrix to other FCS files in the **Data Sources** pane.

More information

- Saving and deleting settings (page 115)
- Creating storage folders (page 117)
- Restoring saved settings (page 118)

Saving and deleting settings

Introduction

This topic describes how to save new or modified settings. This topic also describes how to delete existing settings.

Settings include the workspace, cytometer settings, analysis templates, and sort layouts. You can save settings at any time. Note that workspaces and window layouts are automatically saved when you close the software.

Saving settings files

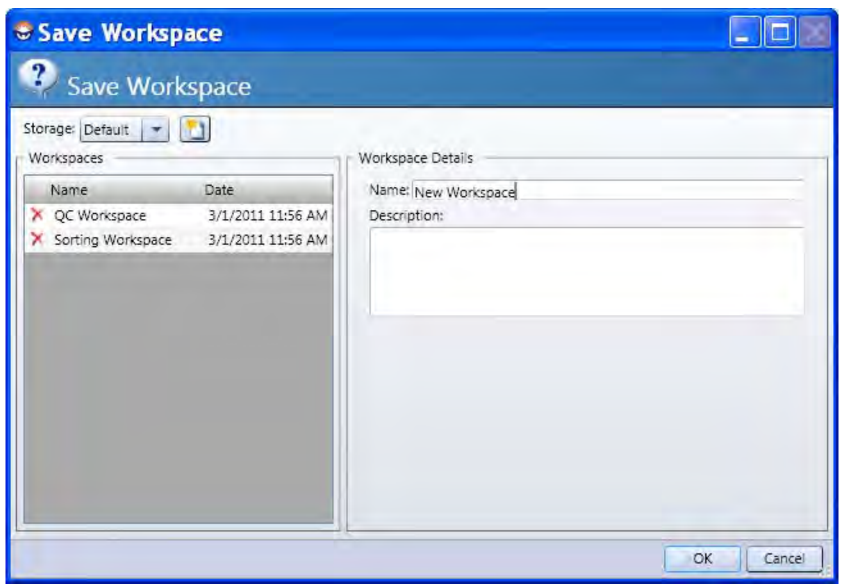
To save settings:

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

   The selected **Save** dialog opens.

   See Saving and restoring settings (page 112) for more information.

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

   See Creating storage folders (page 117) for more information about creating custom storage folders.

3. Under **Workspace Details**, type a name for the new settings file in the **Name** field.

4. Click **OK** to save the workspace and close the dialog.

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.

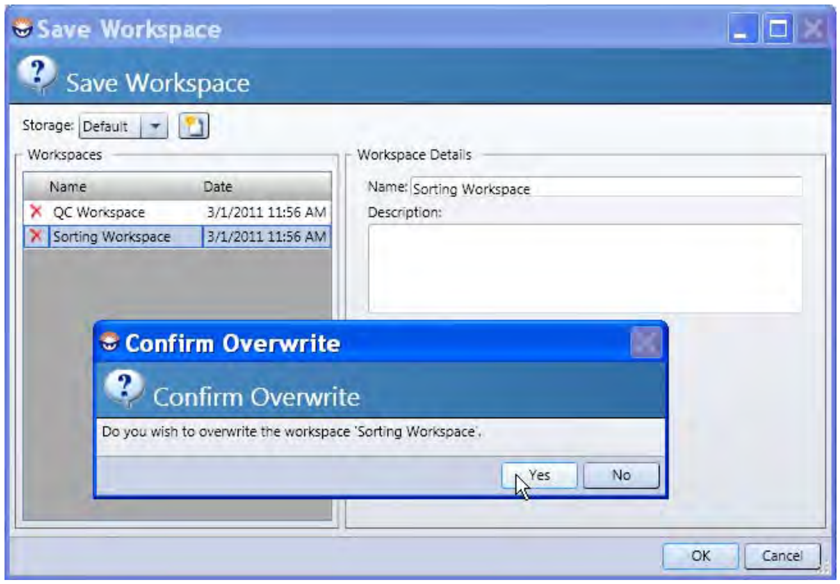
2. Click **Yes** to delete the cytometer settings file.

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

Overwriting a saved setting

To overwrite a setting that you have already saved:

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



More information

- Creating storage folders (page 117)
- Setting up your window layout (page 102)
- Restoring saved settings (page 118)
- Preparing beads for QC (page 248)

Creating storage folders

Introduction

This topic describes how to create custom storage folders for user-defined workspaces, cytometer settings, analysis templates, and sort layouts.

Procedure

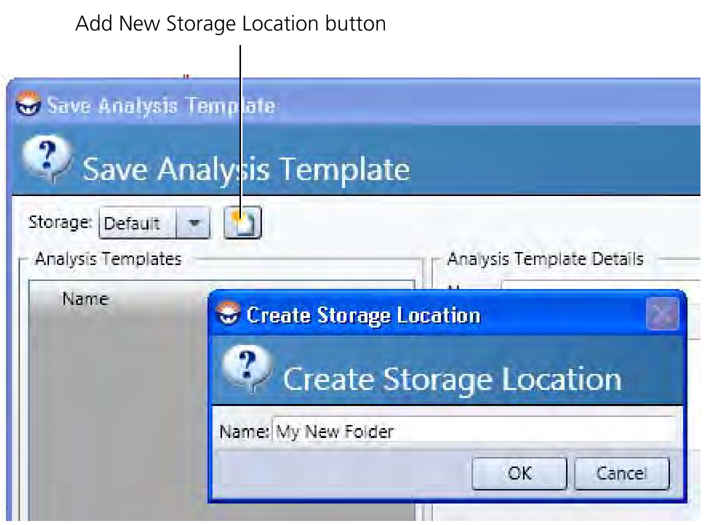
To create new storage folders for user-defined templates and settings:

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

- Saving and deleting settings (page 115)

Restoring saved settings

Introduction

This topic describes how to restore saved settings.

When you start the BD FACS Sortware sorter software, all settings display default values. If you want to apply specific values or layouts, you need to restore these settings from a saved settings file.

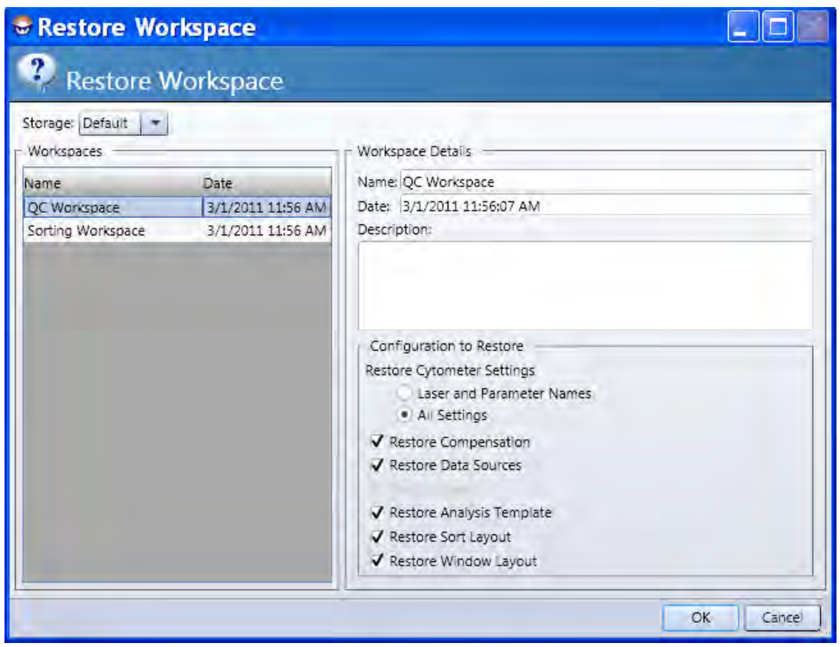
Procedure

To restore saved settings files:

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

   The selected **Restore** dialog opens.

   See Saving and restoring settings (page 112) for more information about what system settings files data is restored.



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 **Workspaces,** select a file to restore.
4. Click **OK** to open the settings file and apply all settings.

More information

- Saving and deleting settings (page 115)

11

Cytometer settings

This section 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 section includes these topics:

Other related information:

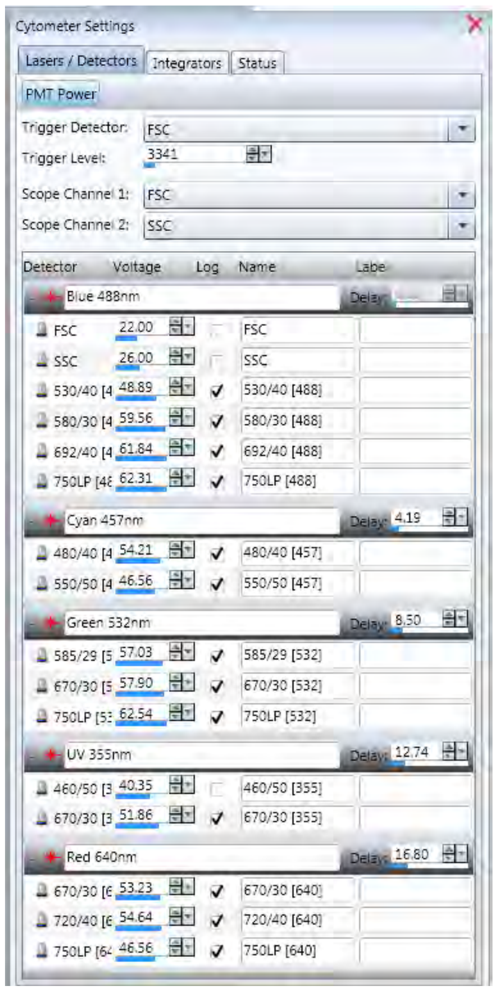
Using the Cytometer Settings pane

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 and scope details, 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 and any cytometer error conditions.

More information

- Cytometer settings (page 119)
- Viewing cytometer status (page 121)

Viewing cytometer status

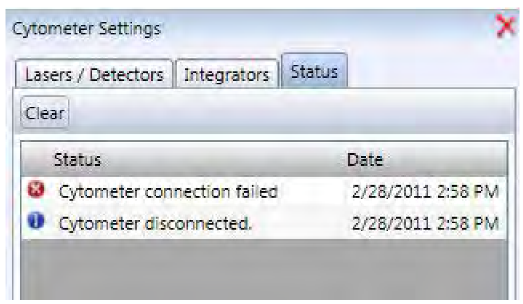
Introduction

This topic describes how to view the current 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 sorter software** toolbar to display the **Cytometer Settings** pane.
2. Click the **Status** tab.



The current cytometer status is listed by date.

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 occur

More information

- Using the Cytometer Settings pane (page 120)

Assigning fluorochrome labels to detectors

Introduction

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

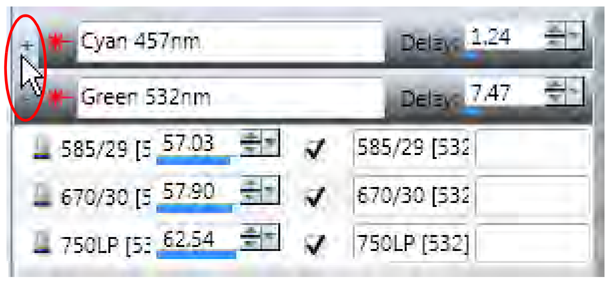
The label identifies how the detector is being used for a specific configuration and creates a more descriptive parameter label whenever the detector is used in a plot.

Procedure

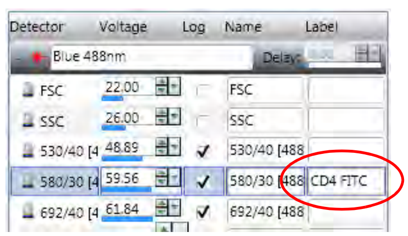
To assign a fluorochrome label to a detector:

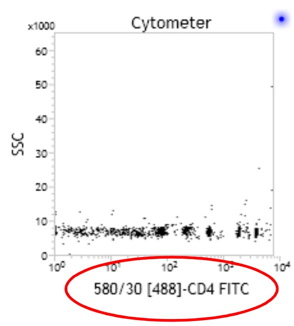
1. In the toolbar, click **Cytometer Settings** to display the **Cytometer Settings** pane.
2. In the **Lasers/Detectors** tab, locate the detector you want to configure in the **Detectors** list.

   You can click **+/-** 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

- Cytometer settings (page 119)
- Assigning fluorochrome labels to detectors (page 121)

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.

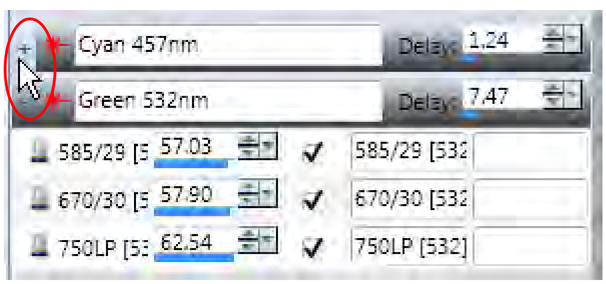
Lin amplification is typically used whenever you use the FSC and SSC detectors. Use log amplification for all other fluorescence channels to provide a wider range of intensities. In special applications (for example, distinguishing small particles on an FSC vs SSC plot), you can use log amplification with FSC and SSC detectors to achieve a higher resolution on the lower end of the scale.

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 **+/-** 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 121)
- Adjusting PMT voltages and using 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 the BD FACS Sortware sorter software. Power off all the PMTs for maintenance purposes.

Powering all PMTs on and off

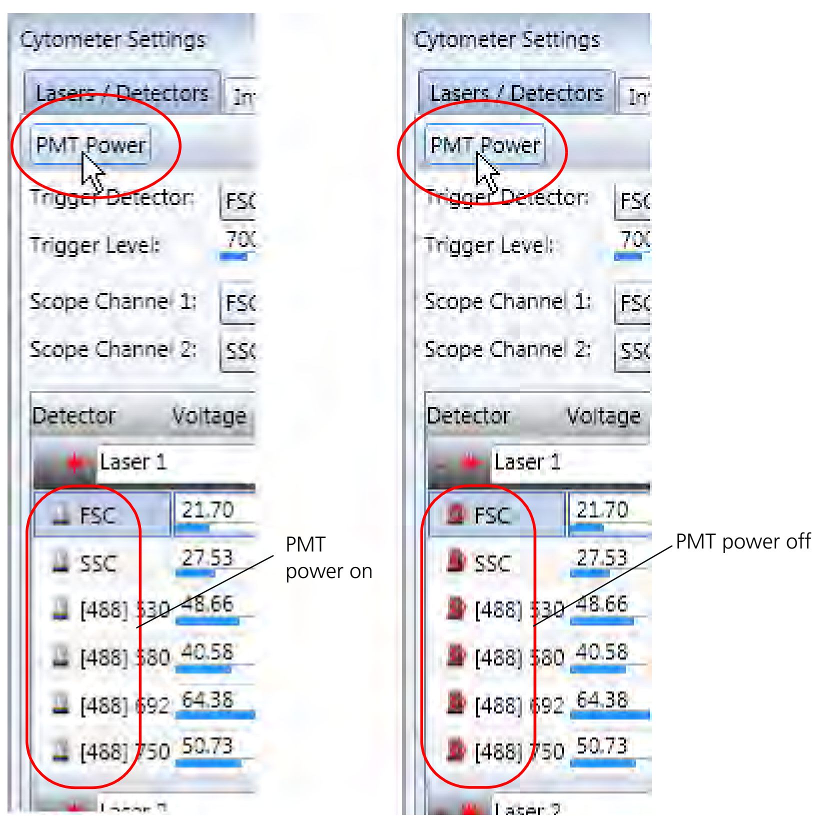
To power all the PMTs on and off:

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 turn on all the power buttons.



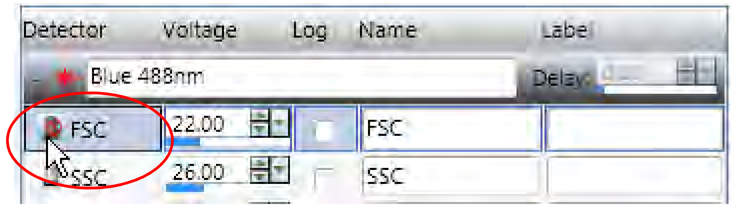
4. Click **PMT Power** again to turn off all the power buttons.

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.



More information

- Assigning fluorochrome labels to detectors (page 121)
- Adjusting PMT voltages and using integrators (page 125)

Adjusting PMT voltages and using 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 are acquiring data.

Before you begin

You need to use the oscilloscope to monitor your PMT voltage gain adjustments, so make sure that you set up the oscilloscope display first.

Procedure

To adjust PMT voltages:

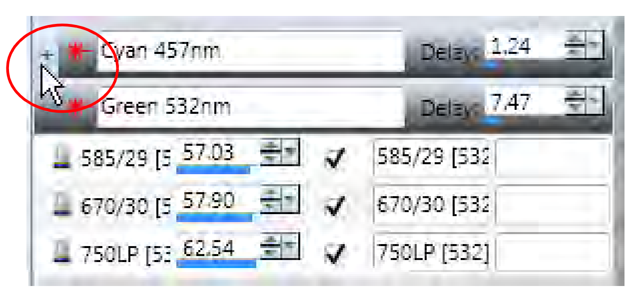
1. Click **Cytometer Settings** on the **BD FACS Software sorter software** toolbar.

   The **Cytometer Settings** pane opens.

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

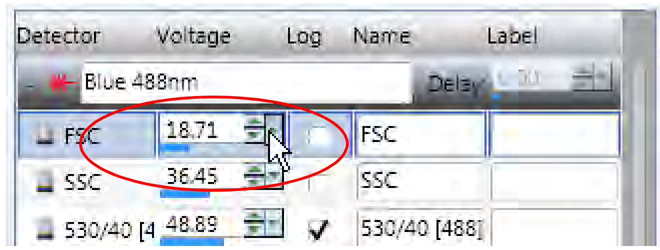
3. In the **Detectors** list, locate the detector you want to set.

   You can click **+/-** in the laser bar to expand or collapse the list.

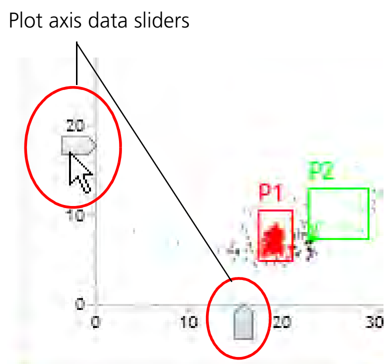


4. Adjust the values by using the mouse scroll wheel, small arrows, data slider, or keyboard keys.

   See Setting numeric values in panes and dialogs (page 96) for more information.

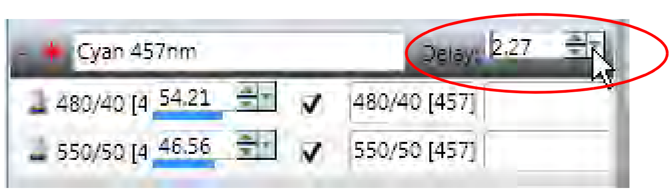


   You can also use the data sliders in the plot to perform a coarse voltage adjustment. You need to place the mouse cursor over the plot axes to make the sliders visible.



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 96) for more information.



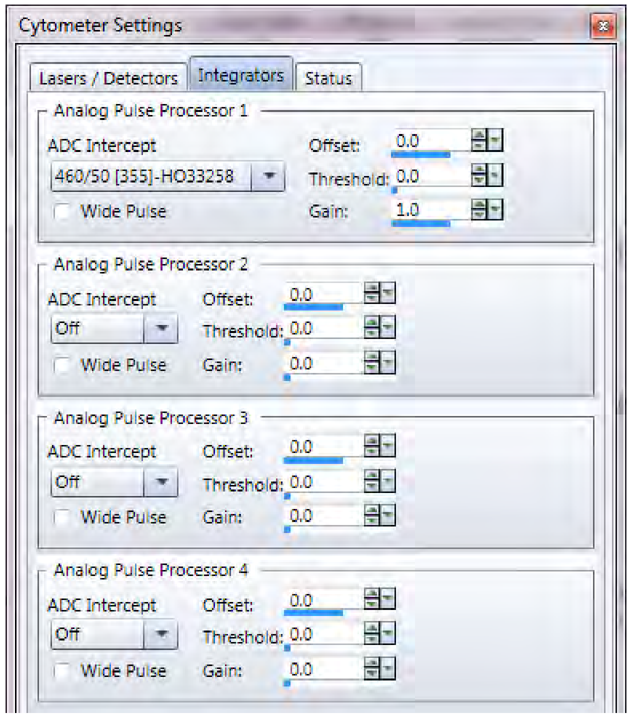
Integrators tab

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 measurement. 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 in 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

- Optical detection (page 50)
- Cytometer settings (page 119)

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

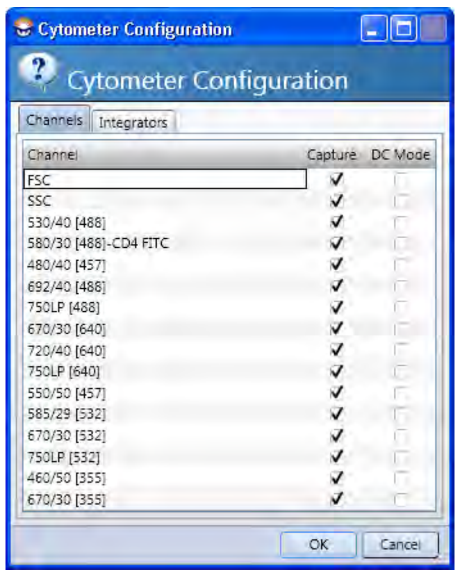
To select specific channels to capture:

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.
   - (Optional) Select the **DC Mode** checkboxes for channels when you want to plug an input into an ADC (other than a PMT) and use a direct current. When this mode is enabled, a PMT is not being used. This mode can be useful for some non-pulse measurements.



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

- Cytometer settings (page 119)
- Viewing and setting cytometer details (page 129)
- Resizing plots (page 172)

Viewing and setting cytometer details

Introduction

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

This information is useful as a quick reference when multiple users work with the same instrument and software. This information is also displayed on the sort report header. You should update these values whenever you change nozzles or sheath pressure settings.

Procedure

To open the Cytometer Details dialog:

1. Select **Cytometer > Details**.

   The **Cytometer Details** dialog opens.

2. Type in the appropriate values for the cytometer configuration:
   - Diameter of the installed nozzle tip
   - Current sheath pressure

   These values are for informational purposes only. The settings are not applied or used in other settings or configurations, but are saved and restored with the workspace and cytometer settings (system settings files).

3. Click **OK** to apply your preferences and close the dialog.

More information

- Cytometer settings (page 119)
- Assigning fluorochrome labels to detectors (page 121)

Using the Compensation pane

Introduction

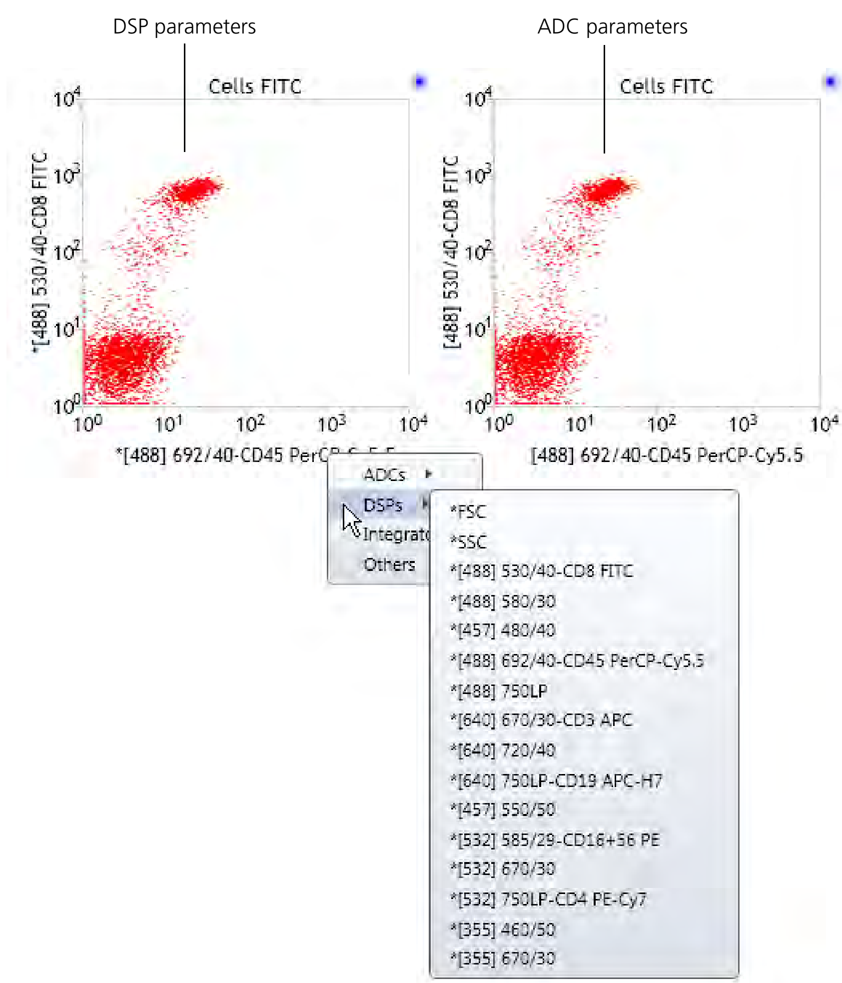
This topic describes the **Compensation** pane and related tabs and dialogs used to create a compensation matrix and calculate spillover values.

About compensation

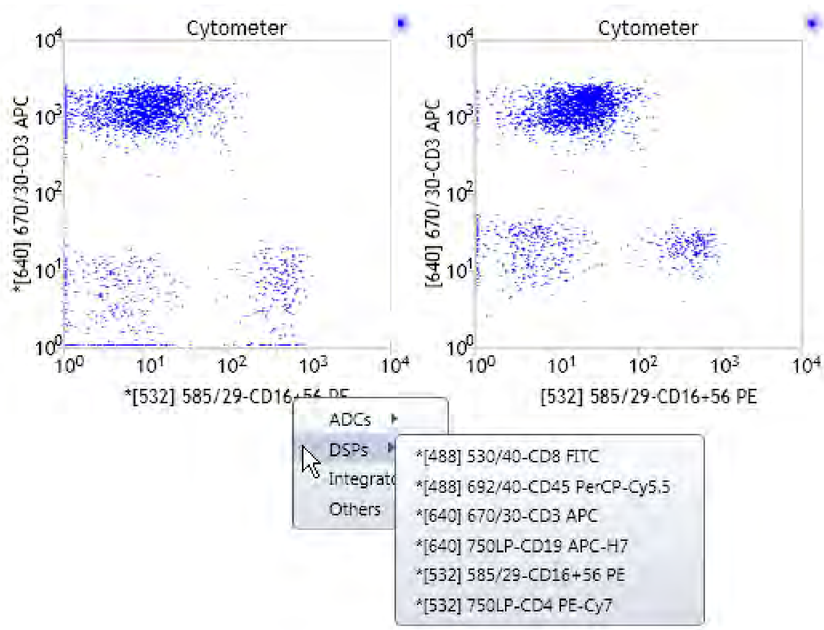
To create a compensation matrix and calculate compensated values, you need to collect single or multicolor control data using ADCs, identify populations as positive or negative, and calculate the results to determine spillover values for each fluorochrome you plan to use. BD FACS Sortware sorter software 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 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 only display the compensation-defined parameters.

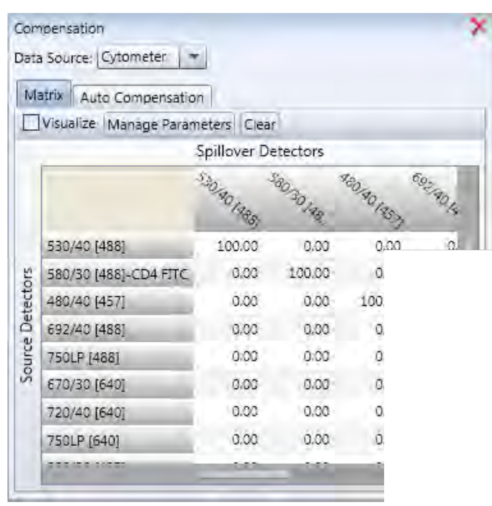


Creating a compensation matrix

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.
Value	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 the 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.

More information

- BD FACS Sortware overview (page 94)

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

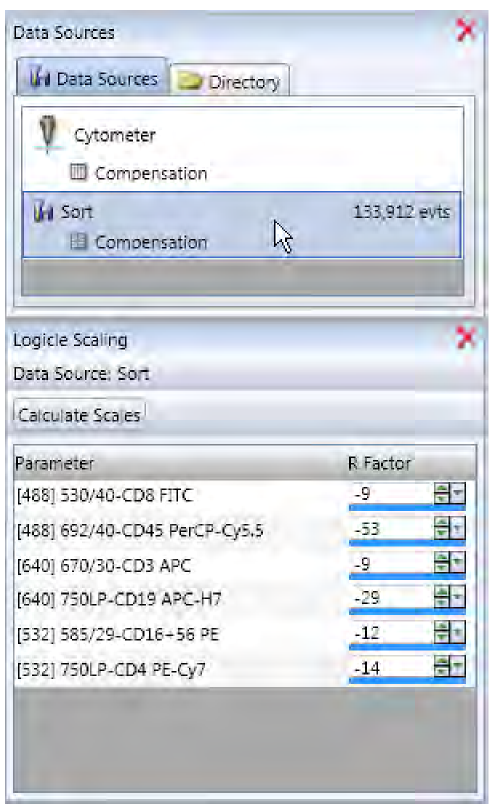
After compensation, populations can include low mean values and negative values. Using a logarithmic scale, plots display a wide range of event data. However, they do not properly display events that have compensated low mean values, high variances, or negative values.

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

Procedure

To calculate logicle display for the data:

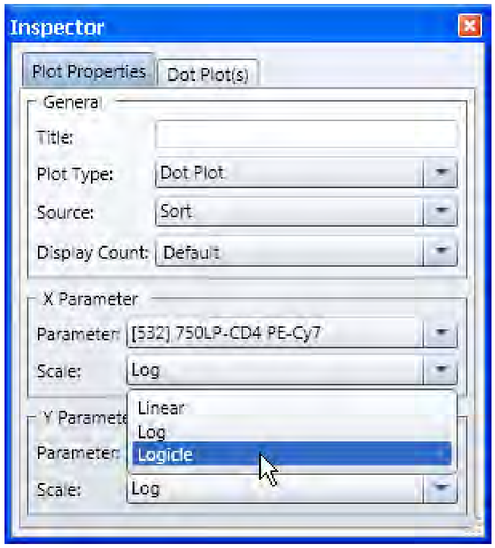
1. In the **Compensation** pane, select the data source that you want to view logicle scaling on (the data source must have a compensation matrix), then select the **Visualize** checkbox.
2. In the **Data Sources** pane, select the data source that you want to perform logicle scaling on.
3. Click **Calculate Scales** to calculate the logicle display for the data.



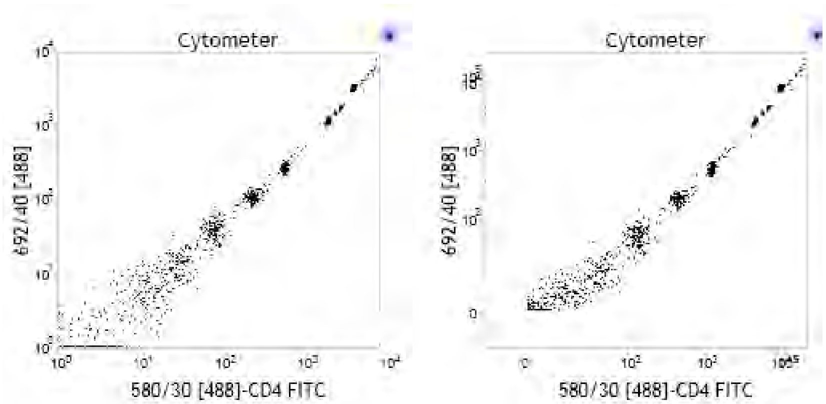
You can adjust the R Factor (logical scale ratio) for each parameter to include negative or positive outliers in the plot to display data that conveys full and accurate information about the distributions of the events.

4. To view the logicle scale on a plot, select the plot and then open the **Inspector** pane.

5. Select the **Logicle Scale** field and **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 93)
- Setting numeric values in panes and dialogs (page 96)
- Using the Compensation pane (page 130)

Importing cytometer settings from BD Spigot software

Introduction

This topic describes how to import existing cytometer settings from previous versions of BD Spigot software using BD FACS Sortware sorter software.

You must be running BD FACS Sortware sorter software to perform this procedure.

Requirements

You must have access to BD Spigot data files.

Procedure

To import cytometer settings from BD Spigot:

1. Select **Cytometer > Import Spigot Settings.**

   The **Select Cytometer File** dialog opens.

2. Navigate to a folder containing Spigot configuration files and select the desired configuration.

   Configuration files are the files that you loaded in Spigot using **Open Experimental Setup** or **Open Full Configuration.**

3. Click **Open.**

The configuration file imports and sets the BD Influx electronics with the following settings.

Category	Details
System settings	• PMT Power • PMT Log Amplification Enable/Disable • PMT Voltage • Laser Delay • Trigger Channel • Trigger Threshold • ADC DC Mode Enable/Disable • ADC Laser Select • Integrator Baseline • Integrator Threshold • Integrator Channel • Integrator Gain
Sort settings	• Drop Delay • Stream Focus • Deflection Gain • Drop Amplitude • Drop Phase • Sort Extra Window • Drop Frequency • Sort Mode

More information

• Importing an existing BD Spigot sort device (page 209)

12

Worksheets

This section includes these topics:

Worksheet overview

Introduction

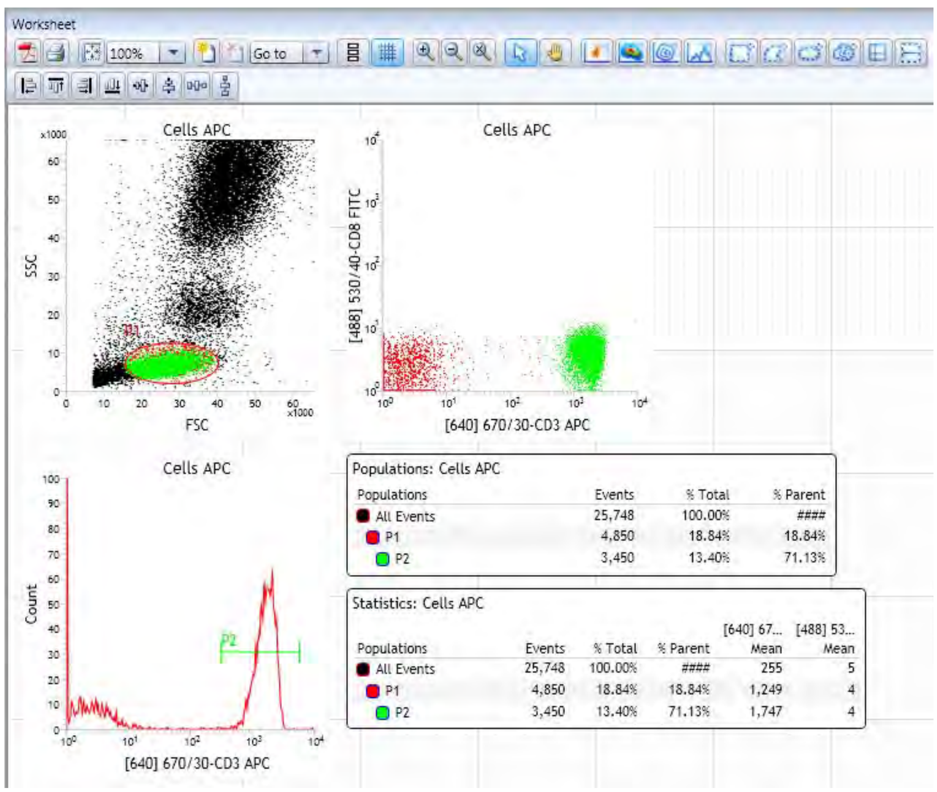
This topic provides an overview of worksheet elements, layouts, and how to manage multiple worksheets.

Description

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

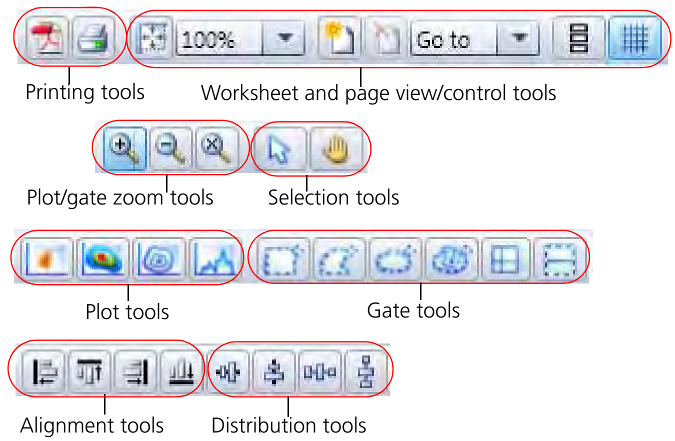
Worksheet layouts

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 162) for more information about using worksheet tools to create plots. See Gating overview (page 178) 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 or redo an action:

- To undo an action, press **Ctrl+Z** on your keyboard, or select **Edit > Undo.**
- To redo an action, select **Edit > Redo.**

You can perform multiple undos and redos.

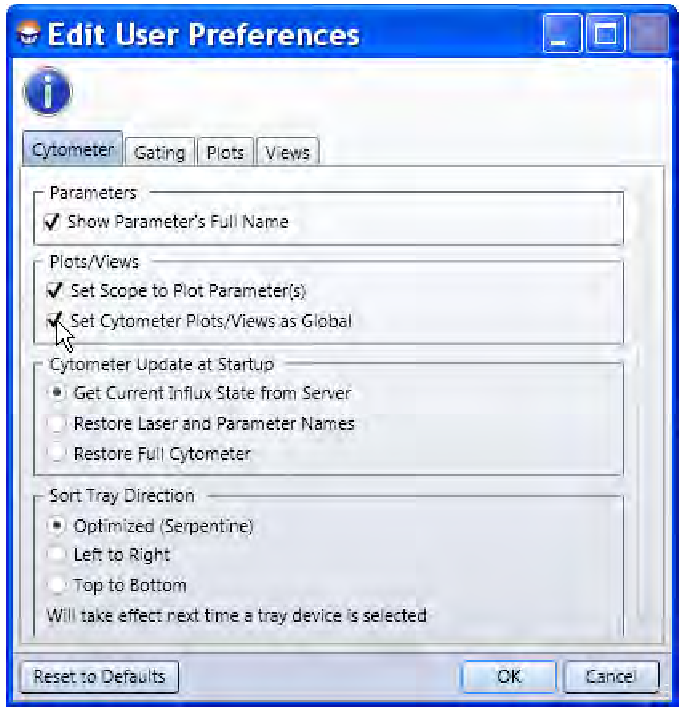
Defining a global worksheet

Individual worksheet elements (plots, hierarchies, 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 **Preference** dialog, all worksheet elements with cytometer as the data source show data from a single file. Use this to quickly view all data from a file.

To view live data:

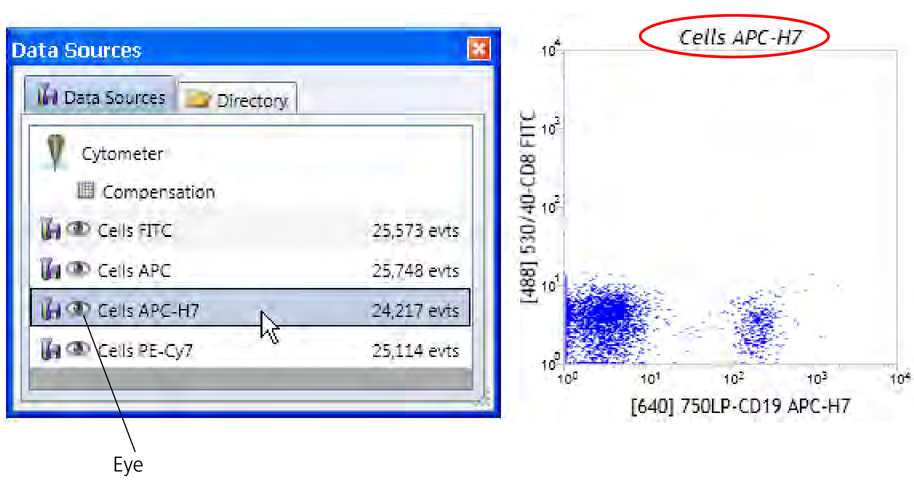
1. Select **Edit > Preferences.**
2. Click the **Cytometer** tab.

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, the FCS files in the **Data Sources** pane will have an eye icon beside it if the file was collected using the same configuration. The eye is an indicator that as the FCS file is selected, the data will show in the worksheet.



   Note that the title of the plot is italicized. This indicates that the option is selected.

   If the **Set Cytometer Plots/Views as Global** is not selected, the plots are specific to the FCS file selected when drawing.

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 141)
- Customizing worksheet properties (page 143)
- Aligning and distributing worklist items (page 145)
- Magnifying (scaling) analysis elements on a worksheet (page 146)
- Using the Inspector to view and modify plot properties (page 163)

Creating a worksheet layout

Introduction

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

A blank worksheet appears in the window each time you start BD FACS Sortware sorter software.

Creating a new worksheet layout

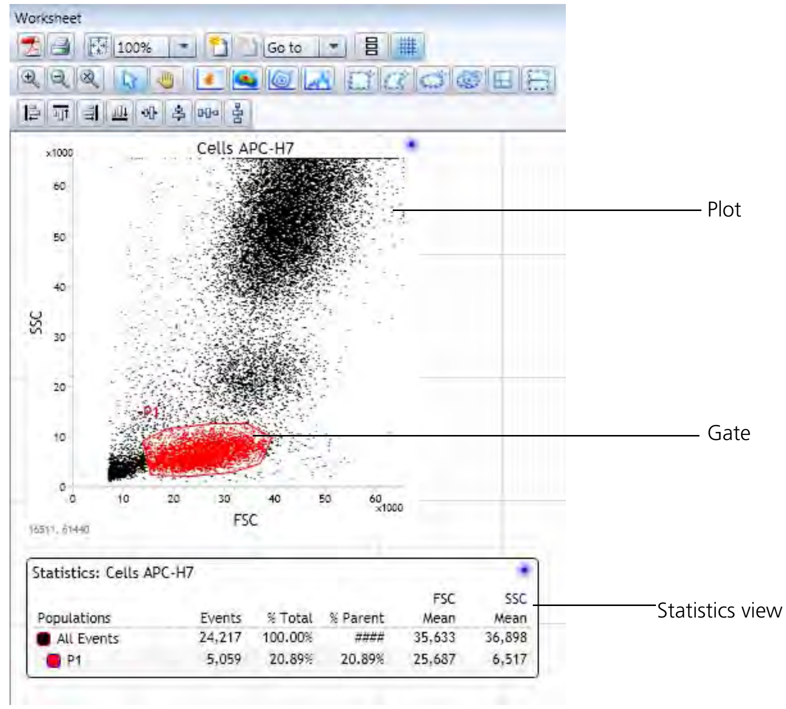
To create a new worksheet layout:

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.

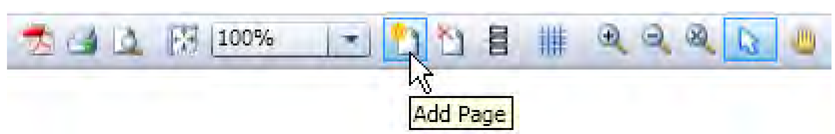
d. Right-click the plot and select **Population Hierarchy** to create a population hierarchy of the plot data.



Adding pages to a worksheet

To add pages to the 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** to locate the page you want to delete.
2. Right-click and select **Delete Page,** or press the Delete key.
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,** or press the Delete key.

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

More information

- Creating plots in a worksheet (page 162)
- Displaying a statistics view (page 200)
- Gating overview (page 178)

Customizing worksheet properties

Introduction

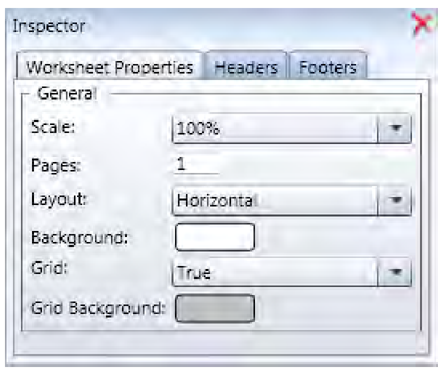
This topic describes how to customize worksheet properties.

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 sorter software** toolbar.

   The **Inspector** opens.

3. Under **General**, select a display scale from the **Scale** menu. You can also use the toolbar to define the scale. Press **Ctrl** and roll the mouse wheel to adjust the page scaling.

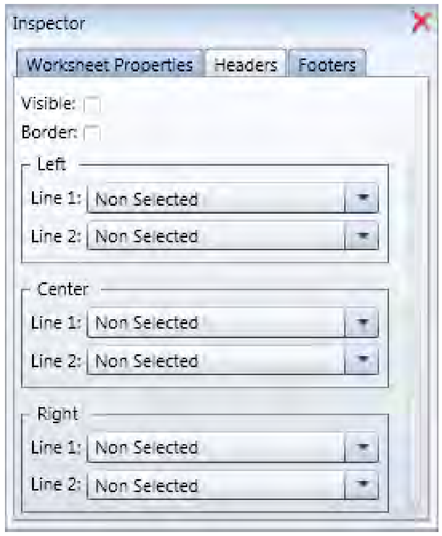
   The default is 100%.

   - In the **Pages** field, specify the number of pages in the worksheet.
   - Select a horizontal or vertical page layout from the **Layout** menu or use the worksheet toolbar.
   - 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.

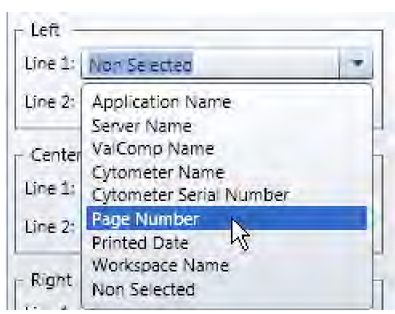
Customizing worksheet headers and footers

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 138)
- Aligning and distributing worklist items (page 145)

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. If you drag the item outside the viewing area, the viewing area automatically scrolls.

Aligning items

To align items on a worksheet:

1. Click the item you want to use as your 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 on the item you want to use as your anchor item.
2. **Ctrl+click** the other items you want to distribute evenly to 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 137)
- Magnifying (scaling) analysis elements on a worksheet (page 146)

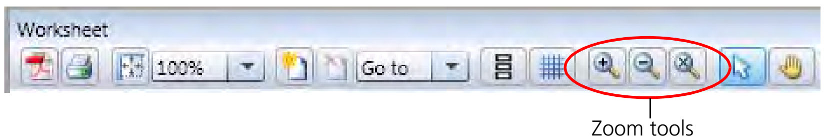
Magnifying (scaling) analysis elements on a worksheet

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. Tick marks on the axes of the plot adjust to reflect the zoomed view.



To zoom in or out:

1. 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	Click **Zoom Out** on the **Worksheet** toolbar.
Clear the zoom	Click **Clear Zoom** on the **Worksheet** toolbar.

More information

- Worksheet overview (page 138)
- Creating a worksheet layout (page 141)
- Resizing plots (page 172)

13

Acquisition and recording tools

This software reference section describes the tools you use to select a data source, set the data recording details, acquire, and record.

This section includes these topics:

- Using the Data Sources pane (page 148)
- Using the Recording Settings pane (page 152)
- Using the Acquisition Dashboard (page 155)

Other related information:

- Alignment and QC (page 241)
- Optimizing system settings for samples (page 263)
- Sorting (page 283)

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

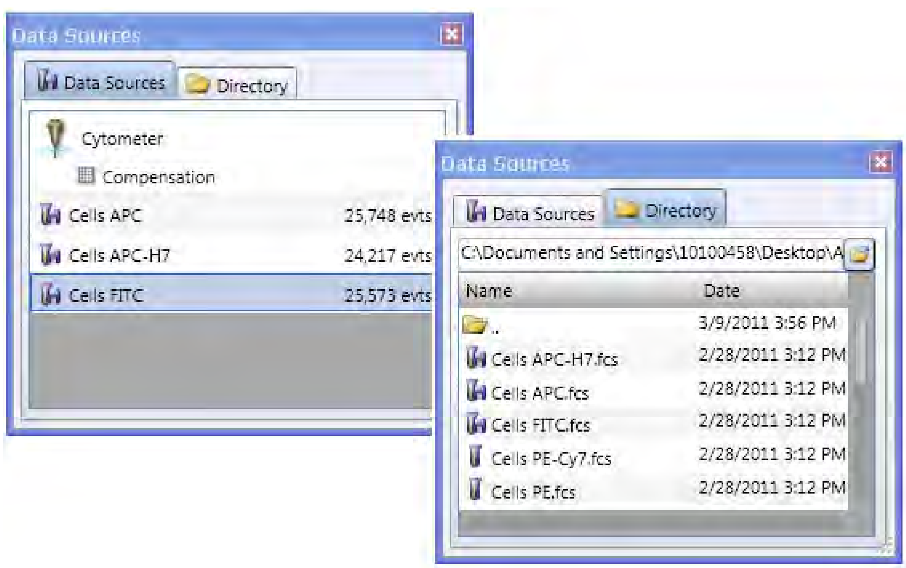
You can also right-click the data source in the **Data Sources** pane to:

- Create sort gates
- Save and restore compensation
- Export BD FACSDiva™ software files
- Export FCS files in comma separated values (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 sorter software 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.



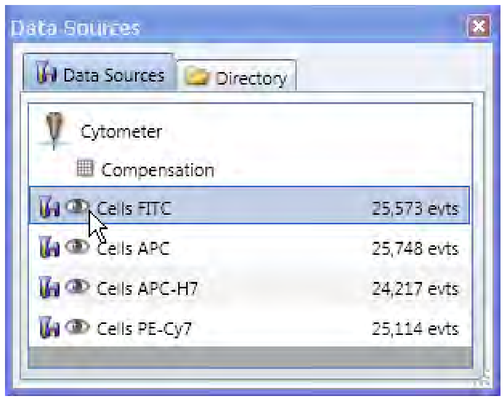
Selecting, importing, and deleting FCS files in the Data Sources pane

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.

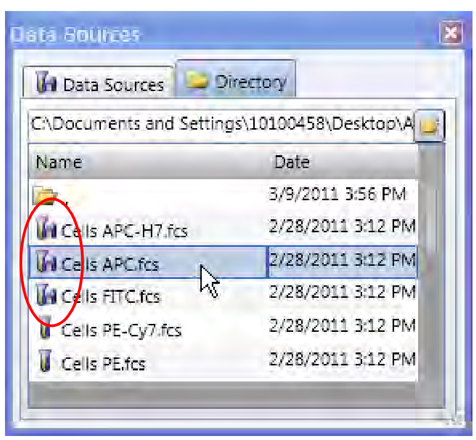


Importing FCS files as data sources

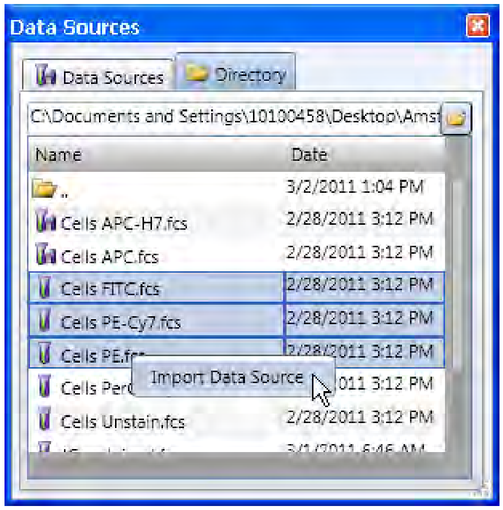
To import FCS files as data sources:

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 FCS file 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**, or select the file and press the Delete key.

Viewing data source details

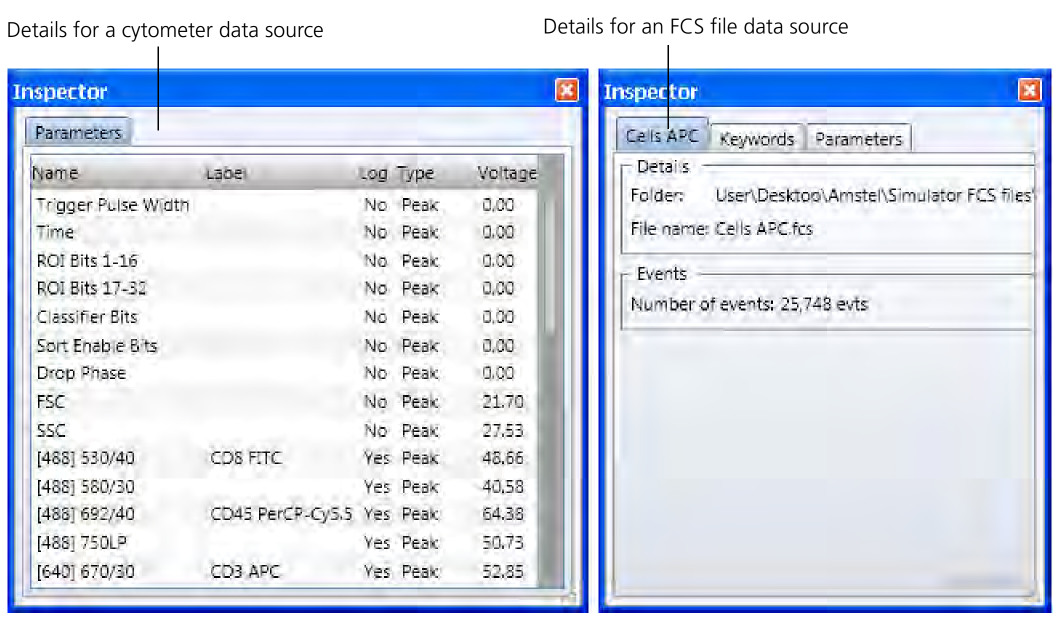
To view data source details using the Inspector:

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

   The **Inspector** opens in the left side of the workspace.

2. Select the cytometer or an FCS data source in the **Data Sources** pane.

   The **Inspector** displays the data source details.



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

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

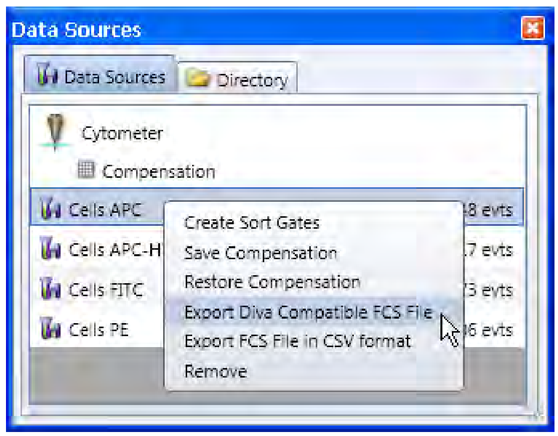
If you want to change the recording preferences or keywords before you record an FCS file, you need to use the **Recording Settings** pane.

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 152)
- Using the Acquisition Dashboard (page 155)

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 FSC 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.

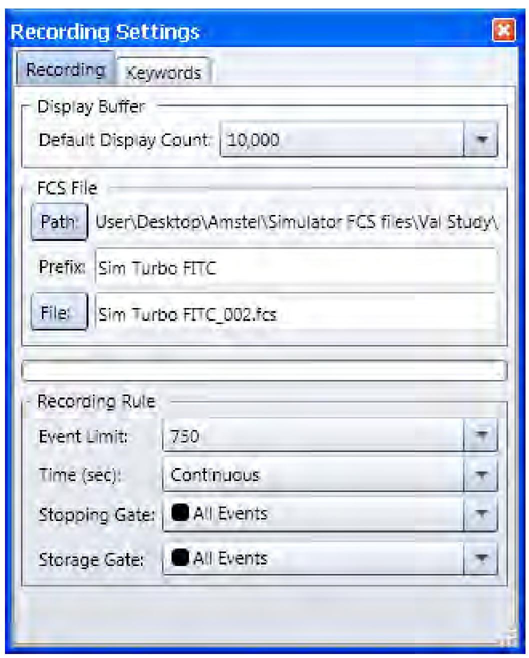
Setting recording properties

To set recording properties:

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

   The **Recording Settings** pane opens.

2. Click the **Recording** tab.



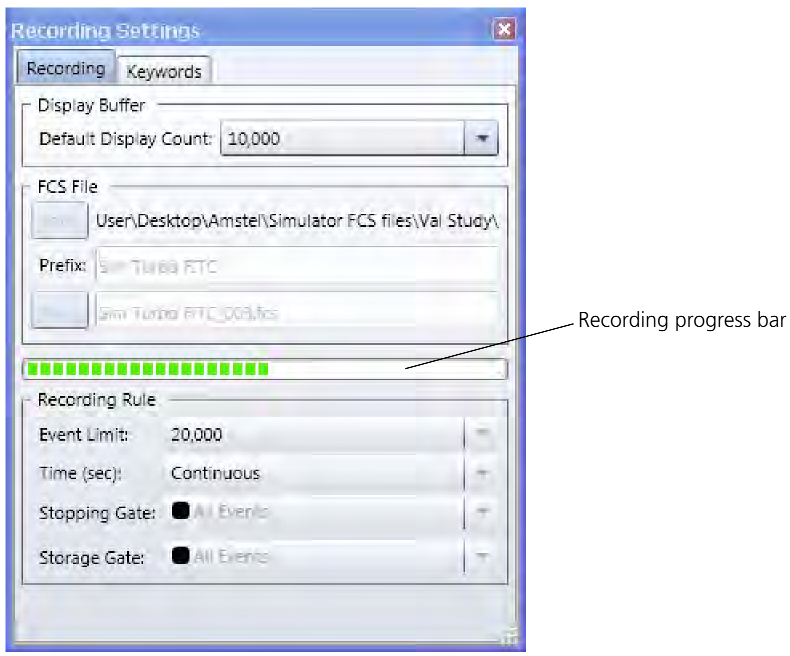
3. Under **Display Buffer**, select an event display count from the **Default Display Count** menu.

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

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



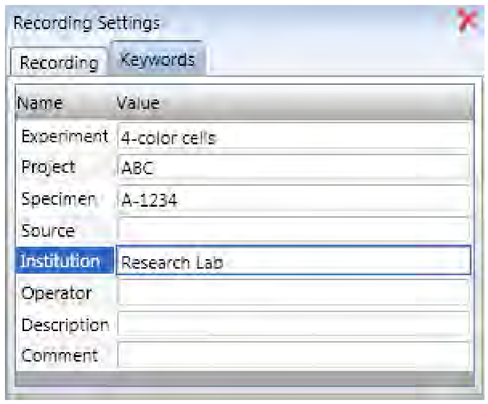
5. Under Recording Rule, set the following properties.

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.

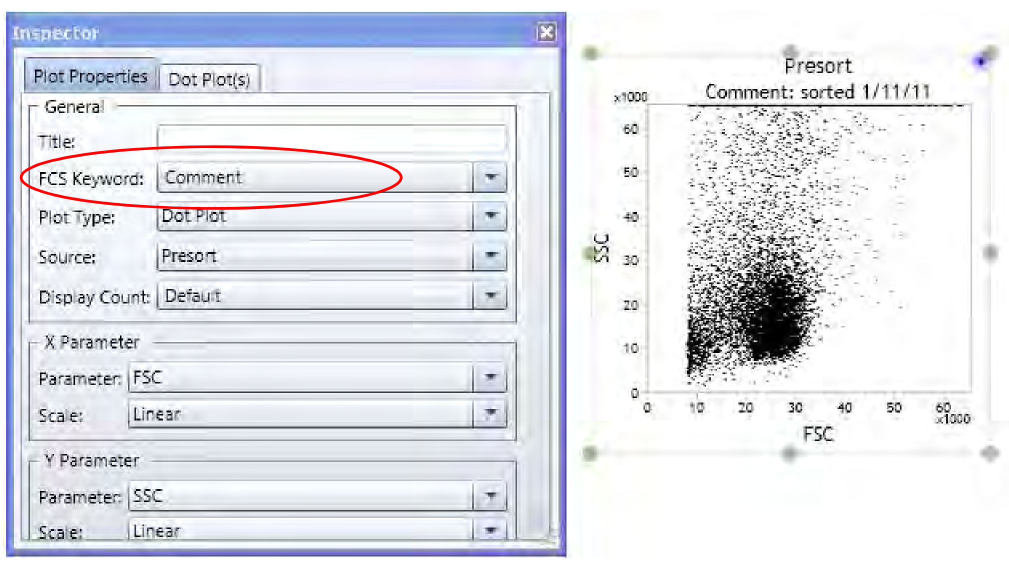


Viewing keywords with plots

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.



More information

- Software overview (page 93)
- Using the Acquisition Dashboard (page 155)

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 and acquisition modes.

Idle

During acquisition

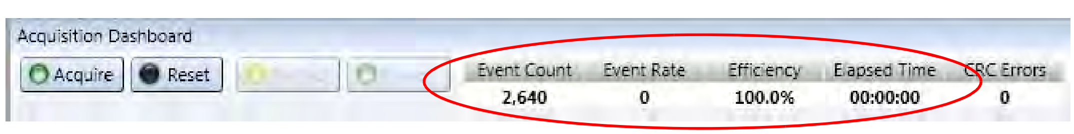
Acquisition controls

The **Acquisition Dashboard** has the following controls.

Control	Description
Acquire	Starts acquisition and populates plots with data. During acquisition or recording, 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 only available after you click **Acquire**.
Pause	Pauses the current acquisition or recording. This button is only available during acquisition or recording.
Resume	Resumes acquisition or recording after pausing.

Status display

The **Acquisition Dashboard** counters are located to the right of 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 (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 152)
- Sorting (page 283)

14

Plots

This section includes these topics:

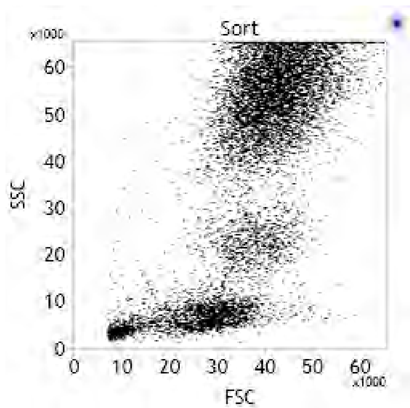
Plot overview

Introduction

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

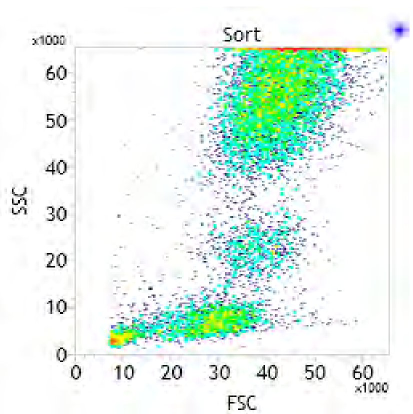
Dot plots

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.



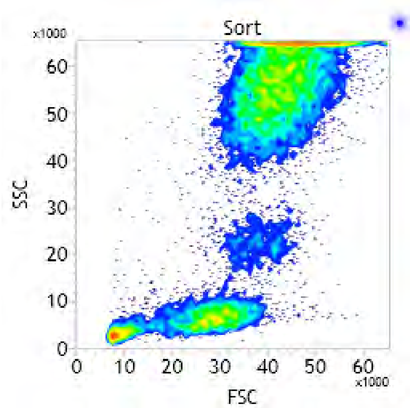
Density plots

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.



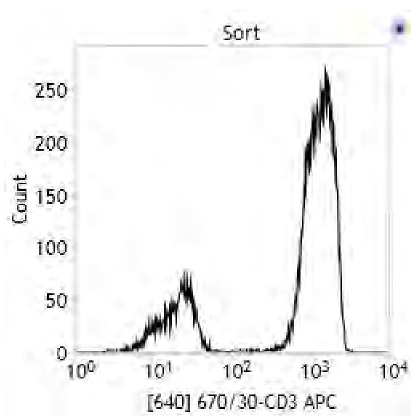
Contour plots

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

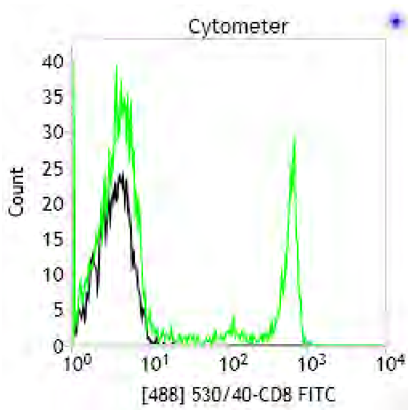
Histograms are graphical representations of a single parameter of data. The horizontal axis of the graph represents the signal intensity of the parameter, and the vertical axis represents the number of events (counts) or percentage of events.



Plot overlays

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

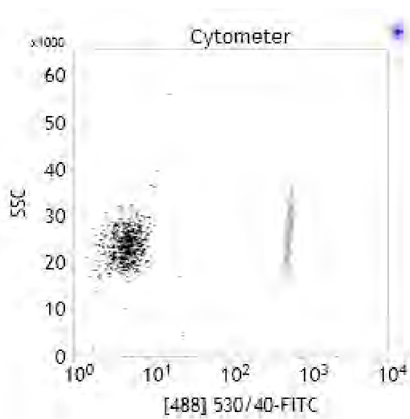
The following example shows a stained sample from a recorded FCS file (green) layered on an unstained sample from the cytometer (black).



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 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 Preference** dialog.

Histograms display all events as black lines or filled areas by default. You can modify the color of the events and overlay events in the **Histogram** properties.

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).
- **Gray scale.** Displays darker gray at lower levels and fades to white as the levels rise.

More information

- Creating plots in a worksheet (page 162)
- Modifying dot plot properties (page 166)
- Creating histogram overlays (page 170)
- Modifying density plot properties (page 166)
- Modifying contour plot properties (page 168)

Creating plots in a worksheet

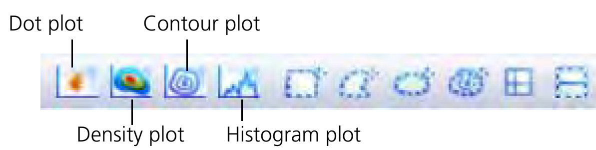
Introduction

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 move a plot, click and drag the plot.
   - To make the plot tool sticky, double-click it. This allows you to create many plots quickly on a worksheet.
   - To unstick the plot tool, click a different worksheet tool.

More information

- Plot overview (page 158)
- Creating a worksheet layout (page 141)
- Modifying dot plot properties (page 166)

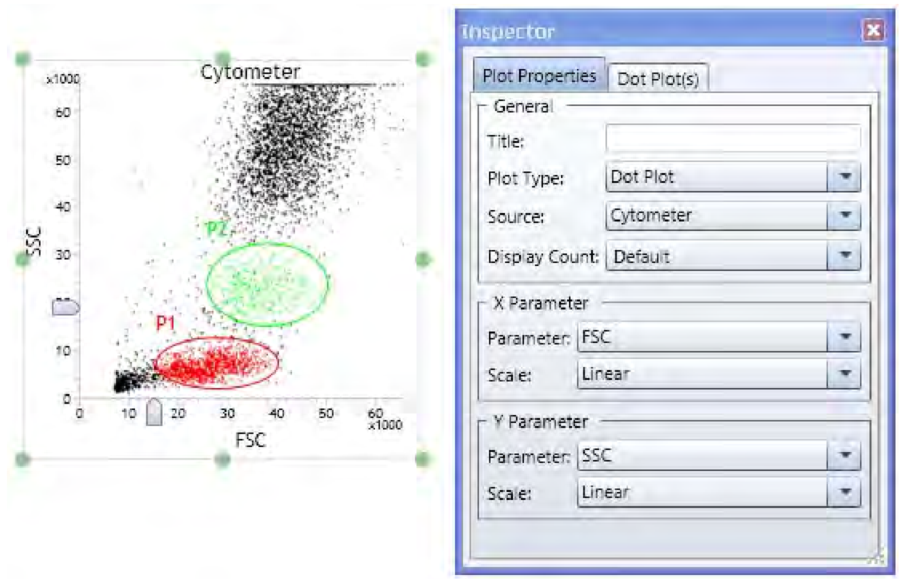
Using the Inspector to view and modify plot properties

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, type, source, and X/Y parameters and scales.



Guidelines

- The axis parameter name includes the measurement type.
- 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 sorter software** 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.
Plot Type	Select a different plot type.
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.
Integrators	The **Integrators** subgroup is only available if 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 110)
- Viewing cytometer status (page 121)
- Adjusting PMT voltages and using integrators (page 125)
- Creating plots in a worksheet (page 162)
- Modifying dot plot properties (page 166)
- Resizing plots (page 172)

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 158) for more information about plots using a target source.

More information

- Setting plot display preferences (page 110)
- Setting statistics views display preferences (page 111)

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.

Property	Action
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 smoothing or select *False* to disable the smoothing of the edge.
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 110)
- Setting statistics views display preferences (page 111)

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 110)
- Setting statistics views display preferences (page 111)

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.

Property	Action
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 110)

Creating histogram overlays

Introduction

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

Before you begin

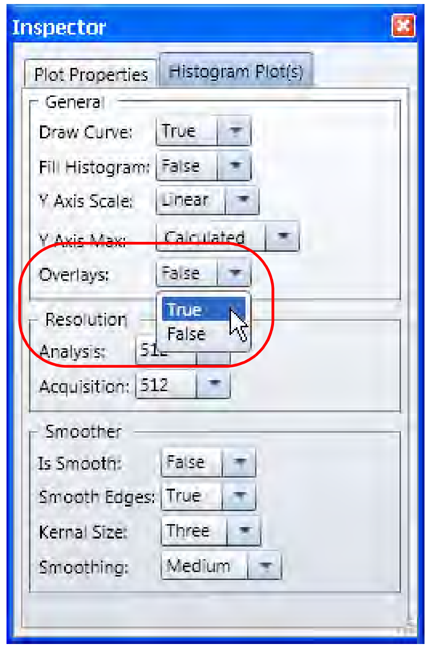
- Create a histogram on a worksheet and open the **Inspector** pane.
- If you want to make an overlay that includes the cytometer, the original histogram needs to have the cytometer as its data source.
- You need to have at least one FCS file in the **Data Sources** pane to create an overlay.

Procedure

To create overlays:

1. Click a histogram on the worksheet.
2. In the **Inspector** pane, click the **Histogram Plot(s)** tab.

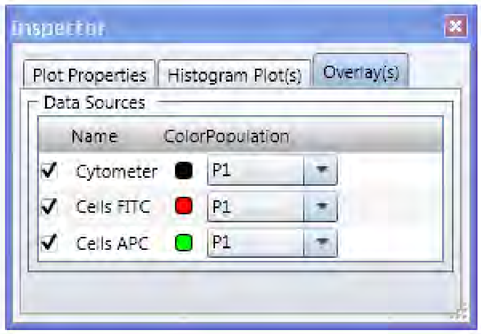
3. In the **Overlays** menu, select *True*.

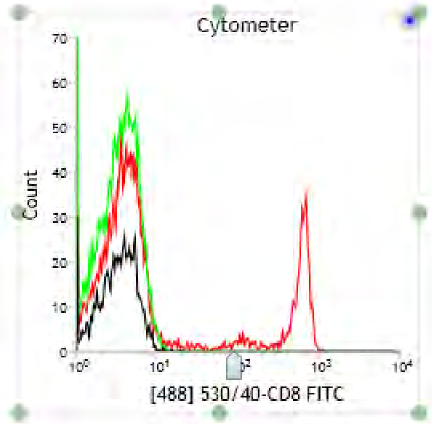


The **Overlays** tab appears.

4. Click **Overlays** and select 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 162)
- Resizing plots (page 172)

Resizing plots

Introduction

This topic describes how to resize plots.

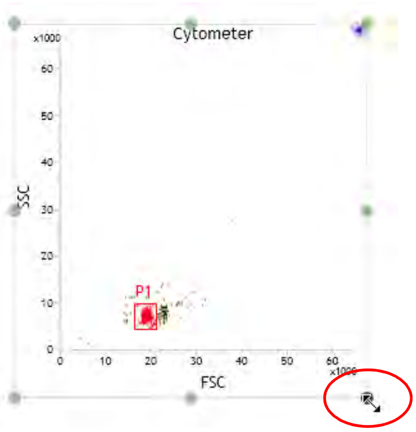
You can manually resize plots and plot events to any size within the worksheet, or expand, collapse, or reshape them based on predefined increments.

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:

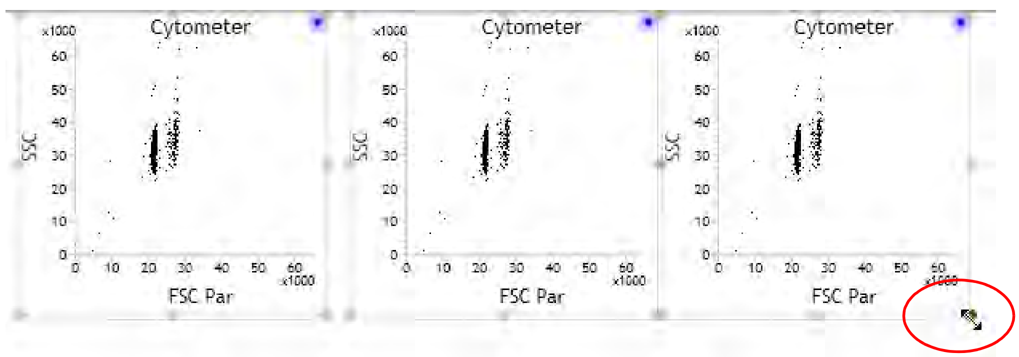
   - To proportionally size the plot, drag a corner handle in or out.
   - To stretch the plot in a specific direction, drag a top, bottom, left, or right handle.

Proportionally resizing multiple plots

To proportionally resize multiple plots (simultaneously):

1. Click on the first plot you want to resize.
2. Press Ctrl and 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 of resizing a plot

Here are some additional ways of resizing a plot.

Method	Procedure
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.**
To make a plot square (reshape a modified plot)	Right-click a plot and select **Resize Plot > Make Square**.

More information

- Magnifying (scaling) analysis elements on a worksheet (page 146)

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 174)

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.

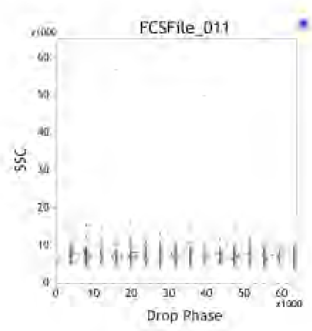
Copying and pasting plots into third-party software

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

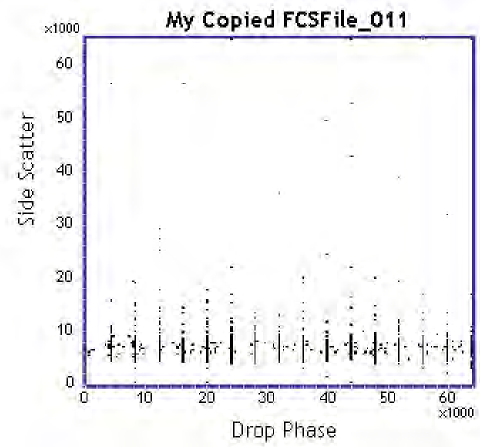
1. Right-click a plot and select **Copy**.
2. Open Microsoft PowerPoint and click on the target slide.
3. Select **Edit > Paste Special > Picture** (**Enhanced Metafile**).
4. Click **OK**.

   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 110)

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15

Gates and populations

This section 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 view

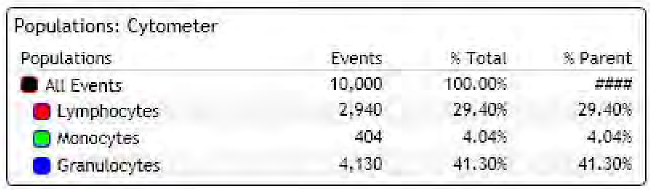
A population is a gated set of data. Populations can be viewed in the **Gate Hierarchy** pane, population hierarchy, or statistics view. Population hierarchies and statistics views are elements in a worksheet.

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.

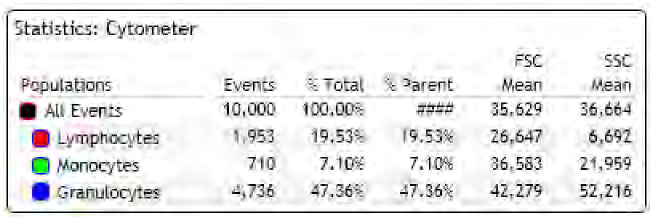
Comparison between gate hierarchies, population hierarchies, and statistics views

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

Gate hierarchy



Population hierarchy



Statistics view

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 in **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

Displays and actions	Gate hierarchy	Population hierarchy	Statistics view
Allows creation of AND or OR gates	No	Yes	No
Displays limited number of statistical details about selected populations	No	Yes	No
Displays broad choice of statistical details about selected populations and parameters	No	No	Yes

More information

- Editing user preferences (page 105)
- Setting gate and population display preferences (page 108)
- Viewing the population hierarchy (page 184)

Using the Gate Hierarchy pane

Introduction

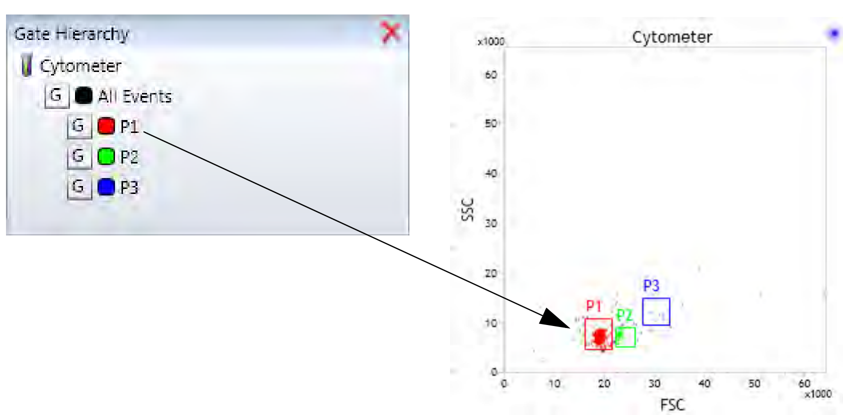
This topic describes how to open and use the **Gate Hierarchy** pane to view and manipulate the relationship of populations, and show and hide populations.

Opening the Gate Hierarchy pane

To open the Gate Hierarchy pane:

1. On the **BD FACS Sortware sorter software** 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 plot.



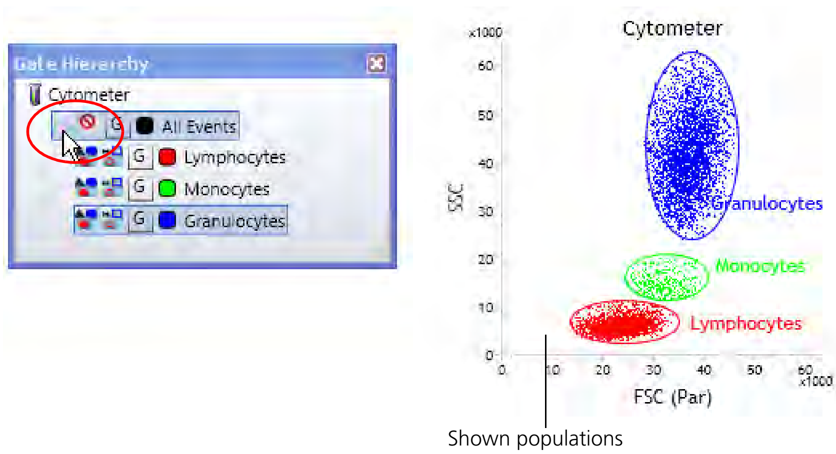
   The gate hierarchy corresponds to gates in the selected plots, population hierarchy, statistics view, or data source. When a data source, population hierarchy, or statistics view is selected, the gate hierarchy displays only the global/local status.

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:

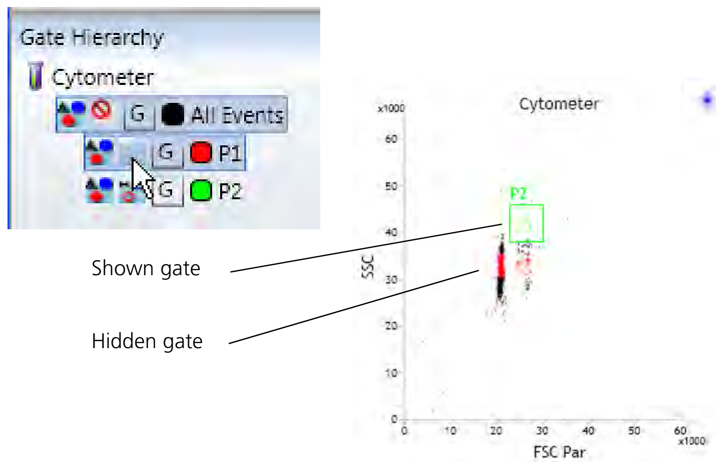
1. Click on a plot with gated populations.
2. In the **Gate Hierarchy** pane, click the **Show/Hide** icon for the population you want to show or hide.



Showing or hiding gates (gate frames)

To show or hide gates (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.

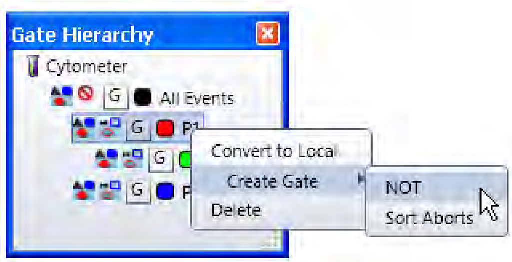


Creating Boolean gates using the Gate Hierarchy pane

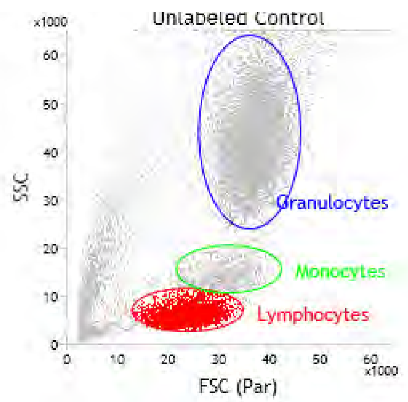
To create Boolean gates using the Gate Hierarchy pane:

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: Unlabeled Control

Populations	Events	% Total	% Parent
All Events	20,000	100.00%	####
Lymphocytes	5,960	29.80%	29.80%
Monocytes	1,031	5.16%	5.16%
Granulocytes	8,587	42.94%	42.94%
NOT(Lymphocytes)	14,040	70.20%	70.20%

More information

- Viewing the population hierarchy (page 184)
- Converting global and local gates (page 197)

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 view can display the following:

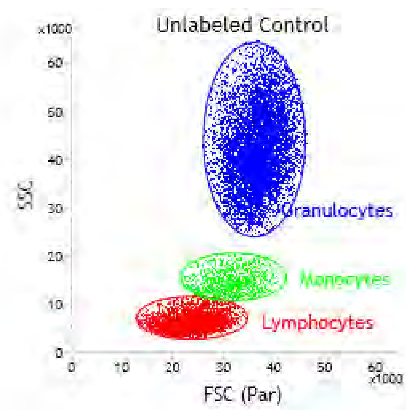
- Events
- %Total
- %Parent
- %Grandparent

Displaying a population hierarchy

To display a population hierarchy:

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

   The population hierarchy appears.



Populations: Unlabeled Control

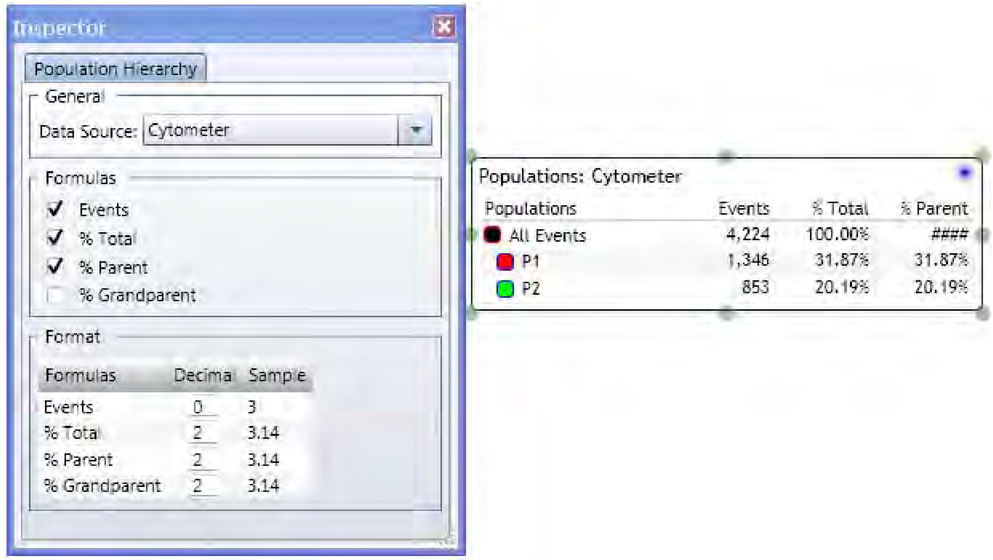
Populations	Events	% Total	% Parent
All Events	20,000	100.00%	####
Lymphocytes	5,960	29.80%	29.80%
Monocytes	1,031	5.16%	5.16%
Granulocytes	8,587	42.94%	42.94%

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. 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.

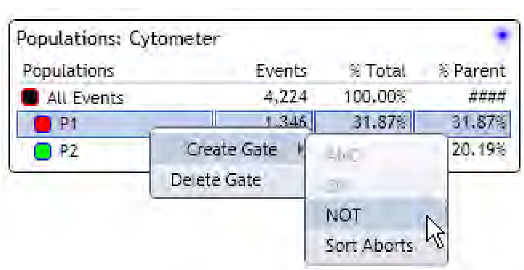
5. Under **Format**, change the value in the **Decimal** field to change significant figures for the numbers displayed.

Creating Boolean gates using the population hierarchy

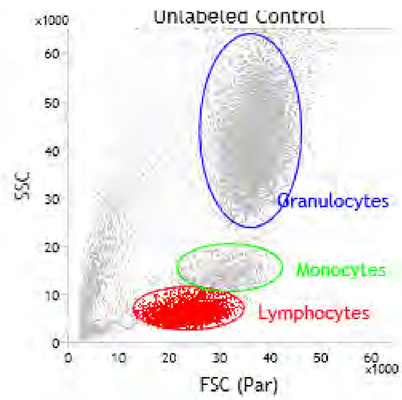
To create Boolean gates using the population hierarchy:

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: Unlabeled Control

Populations	Events	% Total	% Parent
All Events	20,000	100.00%	####
Lymphocytes	5,960	29.80%	29.80%
Monocytes	1,031	5.16%	5.16%
Granulocytes	8,587	42.94%	42.94%
NOT(Lymphocytes)	14,040	70.20%	70.20%

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 180)
- Creating rectangle gates (page 187)

Creating rectangle gates

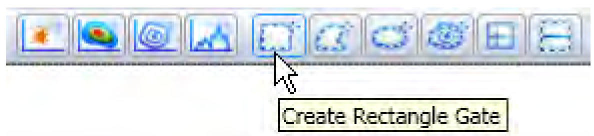
Introduction

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

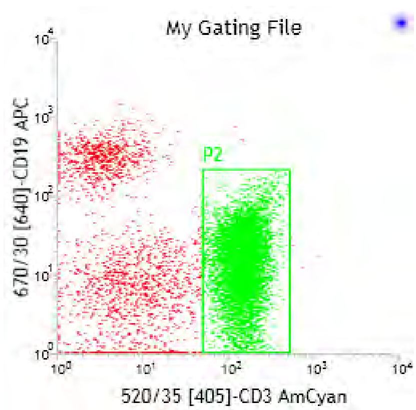
Procedure

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 180)
- Creating polygon gates (page 188)

Creating polygon gates

Introduction

This topic describes how to create polygon gates.

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

Limitations

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

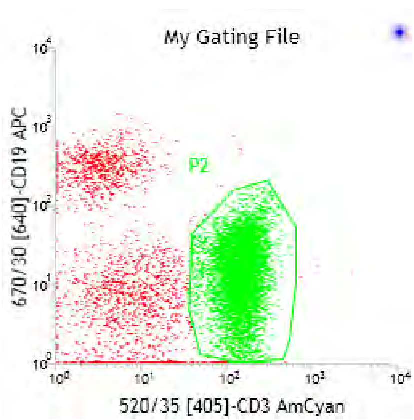
Procedure

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 187)
- Creating ellipse gates (page 189)

Creating ellipse gates

Introduction

This topic describes how to create ellipse gates.

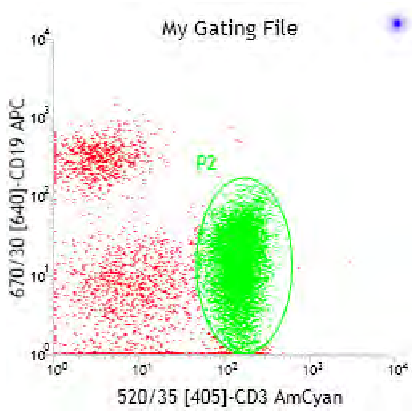
You can draw an elliptical gate on dot, density, or contour plots.

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 188)
- Creating contour gates (page 190)

Creating contour gates

Introduction

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

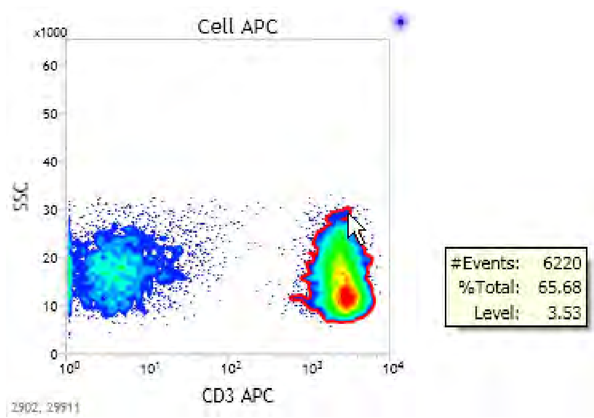
You can only draw a contour gate on a contour plot.

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 (in zoom view).

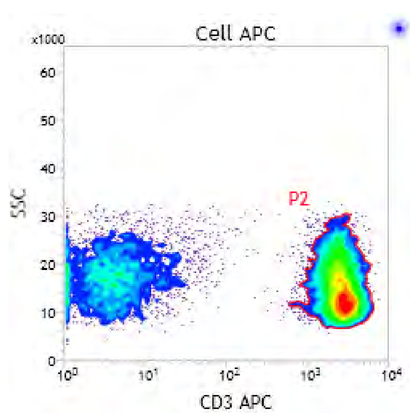


Creating contour gates

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 188)
- Creating interval gates (page 192)

Creating interval gates

Introduction

This topic describes how to create an interval gate.

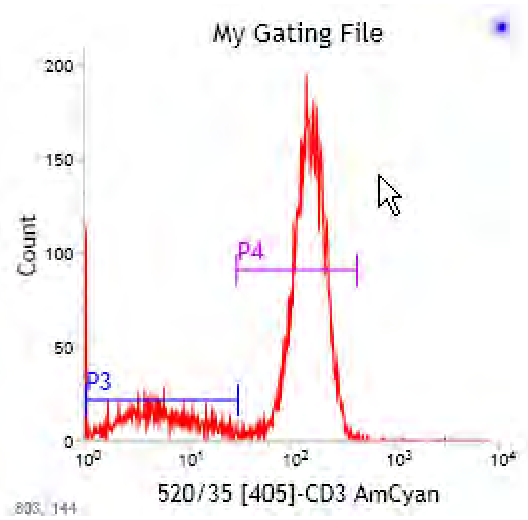
Intervals gates are gates between left and right endpoints on the horizontal axis. You can only draw an interval gate on a histogram.

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 189)
- Creating quadrant gates (page 193)

Creating quadrant gates

Introduction

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

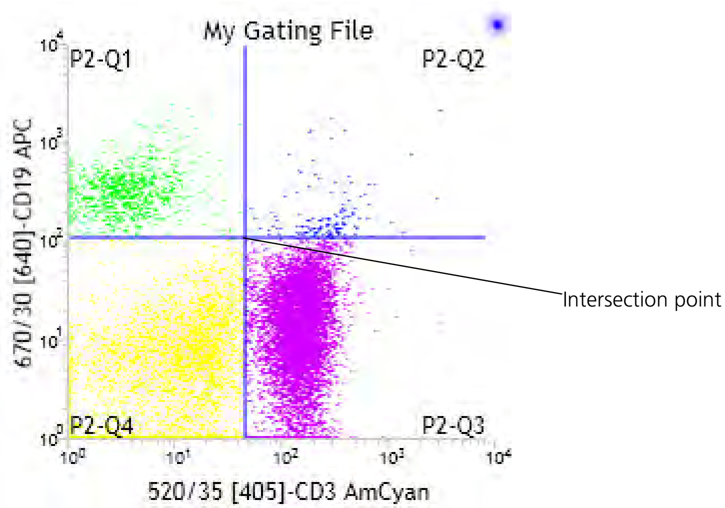
Quad gates divide a plot into four quadrants. Each quadrant has its own population statistics. You can name and color the population in each quadrant individually.

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 192)
- Converting global and local gates (page 197)

Working with gates

Introduction

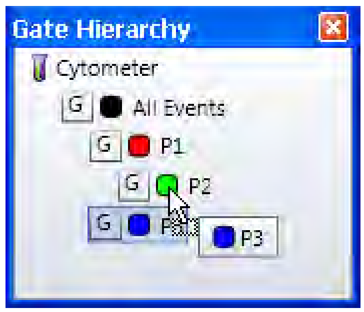
This topic describes how to modify gate names and colors and manipulate the gate size and display.

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.

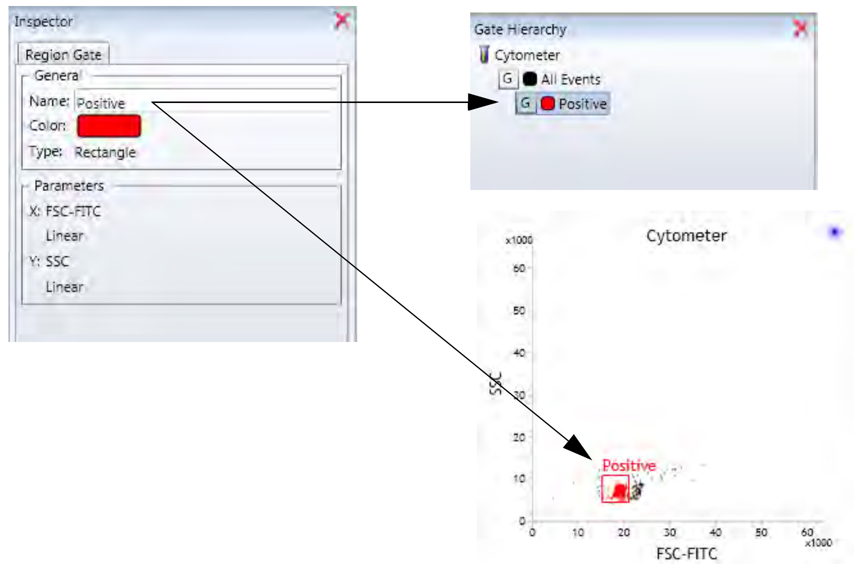
To rename a gate:

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, Positive).

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



Changing a gate color

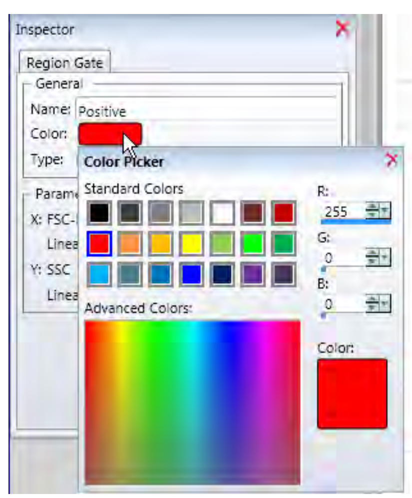
This procedure only affects the color of individual gates (global or local). To set the default preferences for all gates, use the **Edit Preferences** dialog. Colors are unique to gates. You cannot use the same color for two different gates.

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 a color picker, then select a color.



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

Resizing gates proportionately

To 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 cursor over a vertex.

   The cursor will show cross hairs.
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**.

   Alternatively, click the gate in a plot and press Delete on your keyboard.

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

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

   Alternatively, click the population and press Delete on your keyboard.

More information

- Setting gate and population display preferences (page 108)
- Viewing the population hierarchy (page 184)
- Converting global and local gates (page 197)

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 all the cytometer FCS files imported in the **Data Sources** pane that use the same plot parameters.

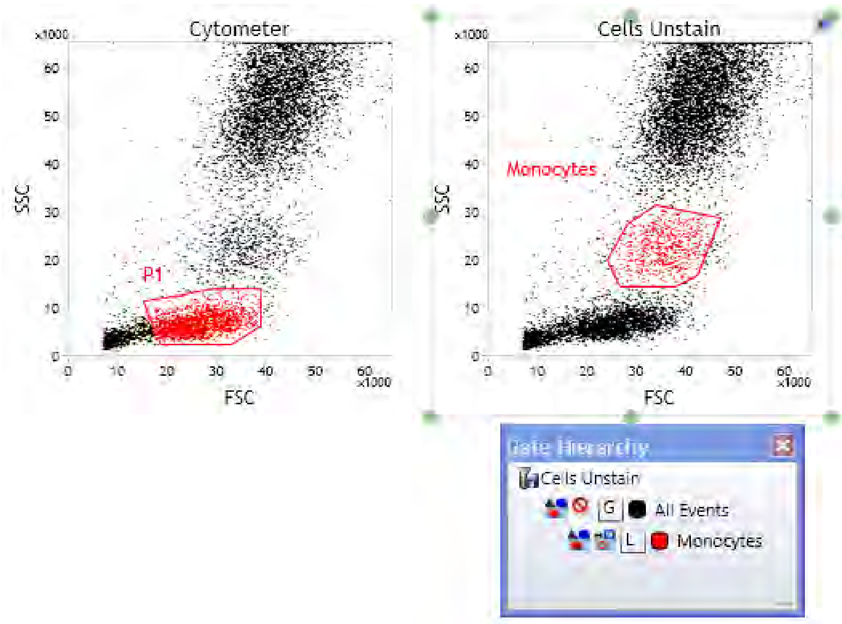
- **Local gates.** You can make global gates unique by converting them to local gates. Local gates are indicated by an *L* in the **Gate Hierarchy** pane. These gates only apply to a specific data source. When a gate is converted from global to local, the original global gate remains on all other data 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

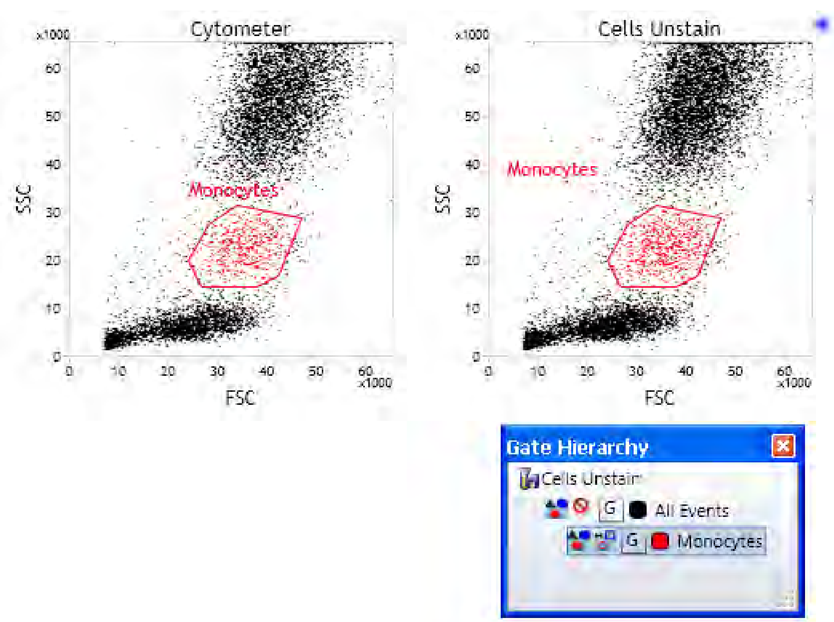
The following rules apply when you convert a global gate to a local gate or a local gate to a global gate:

- If a global gate has child gates and is converted to local, all child gates will also be converted to local gates.
- Gates created with a local gate as a parent will be local (including Boolean gates).
- 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 this 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 global 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 or a local gate to global 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 gates

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 178)

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:

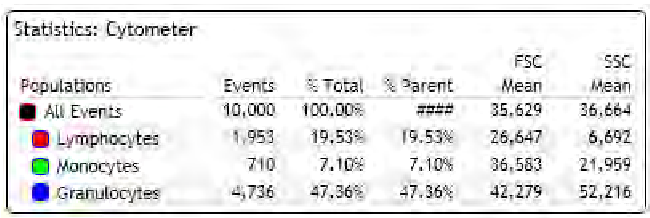
- Population
- Events
- %Total
- %Parent
- X-axis parameter mean events
- Y-axis parameter mean events

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

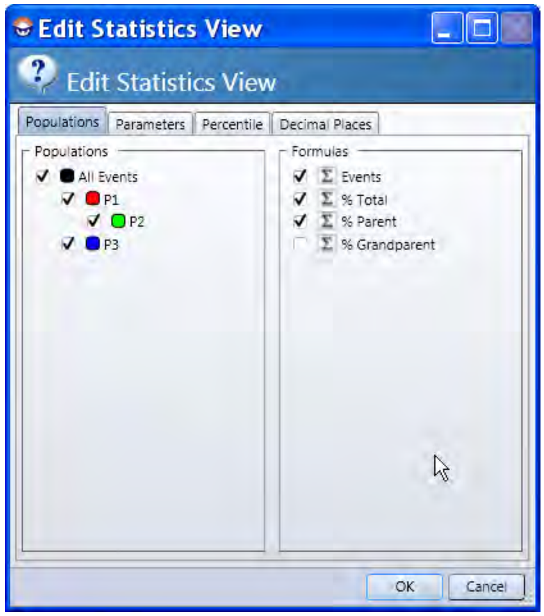
   If your plot has gates and defined populations, the data for each population and All Events appear in their hierarchical order.

Editing the statistics view

To edit 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 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 197)

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16

Sort settings and layout

This section includes these topics:

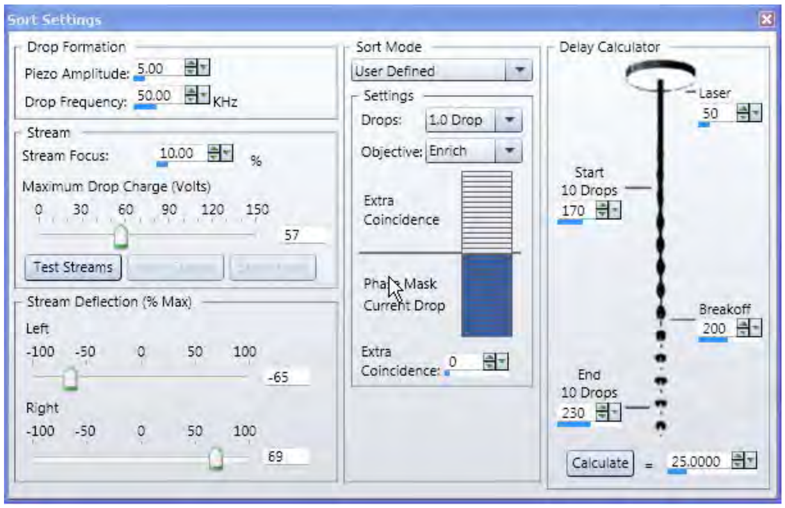
Using the Sort Settings pane

Introduction

This topic describes the **Sort Settings** pane and the different functions it provides.

Description

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



This pane includes the following tools.

Tool	Description
Drop Formation	Contains controls for determining the stream breakoff.
Stream	Contains controls for optimizing the side streams.
Stream Deflection	Contains controls for adjusting of the side streams individually.
Sort Mode and Settings	Shows the representation of each sort mode.
Delay Calculator	Contains fields used to calculate the approximate drop delay.

More information

- Sort modes (page 205)

Sort modes

Introduction

This topic describes the different sort mode drop count options and describes phase gates and coincidence.

About sort mode drop count

When you select a sort mode, you need to decide on a drop count. The following table describes each drop count option.

Drop count	Description
1.0 or 2.0 drops	Use 1.0 or 2.0 drops when the exact count of a sort is important. For example, when you are sorting one particle into each of 96 wells, the position of the particle inside the droplet must be within the boundary of the droplet. Particles that are too close to the droplet boundaries might end up in either droplet, and empty droplets might inadvertently be sorted.
1.5 drop	This sort mode sorts two drops when one particle is on a droplet boundary and one particle is in the center of a drop. This ensures an accurate sort count.

Drop count attributes

Each drop count is paired with one of the following attributes.

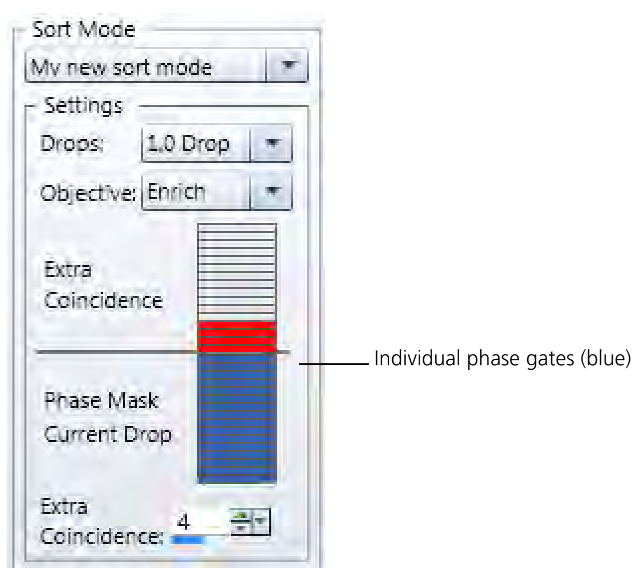
Drop attribute	Description
Enrich	The purity of a sort with coincidence disabled is completely determined by the sample-to-droplet rate ratio and might be compromised at high sample rates. This mode is most often used for collecting rare populations when sort yield is more important than purity. This mode provides low throughput, lower purity, high yield, and high recovery.
Pure	This is the number of particles sorted out of the number requested to be sorted. Use this setting when sample rates are low compared to droplet rates, and when the count of the sorted particles is important. This mode is used for single-cell sorting into multiwell plates, and is useful when sorting large particles. This mode provides low throughput, high purity, lower yield, and high recovery.

Drop attribute	Description
Single	This setting is available for the 1.0 drop mode only. It applies a phase gate that allows only particles in the center of droplets to be sorted. This is useful when recovery of large particles (greater than ¼ of the nozzle diameter) is important. Large particles near droplet boundaries cause problems in the breakoff point and can cause sort streams to spray. This mode provides high purity, lower yield, and higher recovery for large particles.
Yield	This is the number of desired particles sorted out of the total number of desired particles in the entire sample aliquot. Use this setting when sample rates are high compared to droplet rates, and when the count (recovery) of the sorted particles is less important. This mode provides high throughput, high purity, high yield, and lower recovery.
Recovery	This is the number of cells sorted out of the final sort counter of the instrument. Use this setting for higher recovery of sorted particles.

Phase gates and coincidence

When selecting a pre-defined sort mode or creating a user-defined sort mode, consider both the position of particles inside droplets and coincident particles.

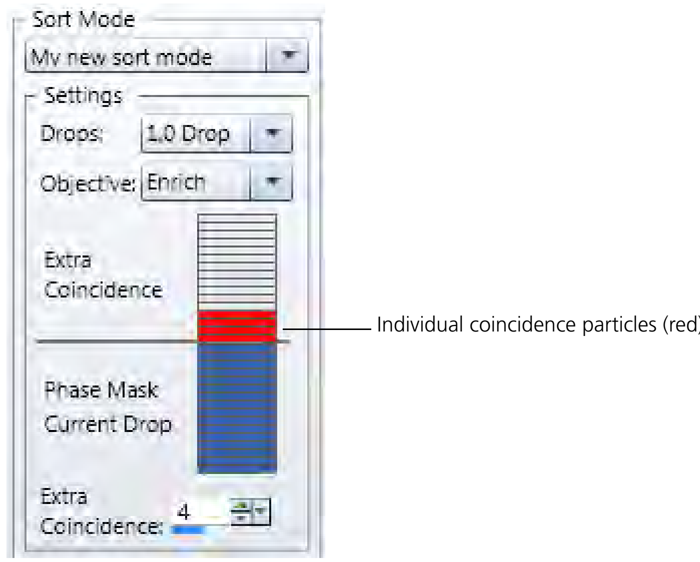
- **Phase gates.** Allow you to sort only particles that are in a certain position in the droplet, such as the middle.

  You can click to select individual phase gates in the **Phase Mask** area of the coincidence scale.

- **Coincidence.** This occurs when two or more particles are closer than the spacing of the droplets. This results in more than one particle in a droplet.

Coincidence is higher when sample rates are higher and when droplet rates are lower.



To increase throughput or sort with additional particles, click to add extra coincidence in the **Extra Coincidence** area of the coincidence scale.

More information

- Selecting a pre-defined sort mode (page 287)
- Creating a user-defined sort mode (page 207)

Creating a user-defined sort mode

Introduction

This topic describes how to create a user-defined sort mode.

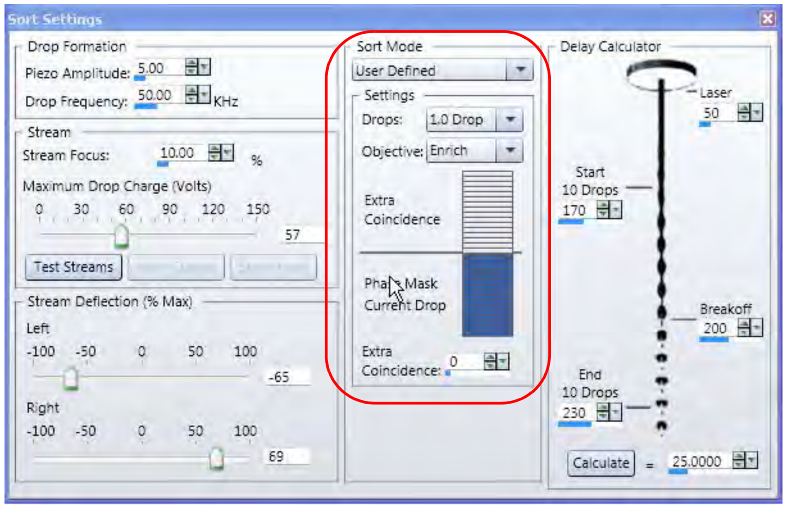
Procedure

To create a user-defined sort mode:

1. Click **Sort Settings** on the **BD FACS Sortware sorter software** toolbar.

   The **Sort Settings** pane opens.

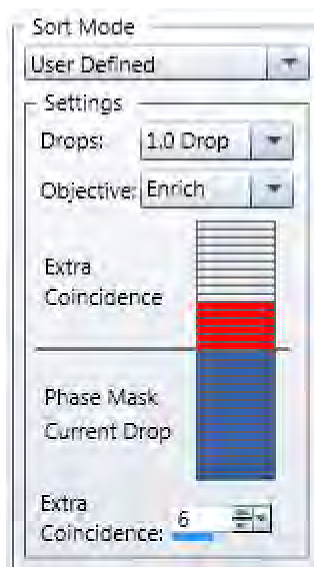
2. Under **Sort Mode**, select the **User Defined** sort mode from the menu.



3. Under **Settings**, select the number of drops from the **Drops** menu.

4. Select the target result you want from the **Objective** menu.

5. In the coincidence scale, click to add individual phase gates that determine the threshold and sort only particles that are in a certain position in the droplet.



6. In the **Extra Coincidence** field, click to add or remove extra coincidence particles.

   - To ensure maximum purity, reduce or eliminate individual coincidence particles.
   - To increase throughput or sort with additional particles, add extra coincidence particles.

   See Setting numeric values in panes and dialogs (page 96) for more information.

Saving a user-defined sort mode

To save a user-defined sort mode:

1. Select **Sorting > Save Sort Mode**.

   The **Save Sort Mode** dialog opens.

2. Under **New Sort Mode**, type a new name for the user-defined sort mode in the **Name** field.
3. Click **OK**.

More information

- About sort mode drop count (page 205)
- Importing an existing BD Spigot sort device (page 209)

Importing an existing BD Spigot sort device

Introduction

This topic describes how to import an existing sort device into BD Spigot software.

Requirements

You must have BD Spigot tray files available in a folder on your computer or on a portable storage device to access them and perform this procedure.

Procedure

To import an existing sort device:

1. Select **Sorting > Import Spigot Device.**

   The **Select Spigot Sort Tray File** dialog opens.

2. Navigate to the folder that contains your Spigot tray files (.tray).
3. Select the file, then click **Open**.

   The tray file saves as an available sort device. You can select the sort device in the **Sort Layout** dialog, then modify it in the **Tray Control** pane.

More information

- Using the Tray Control pane (page 210)

Using the Tray Control pane

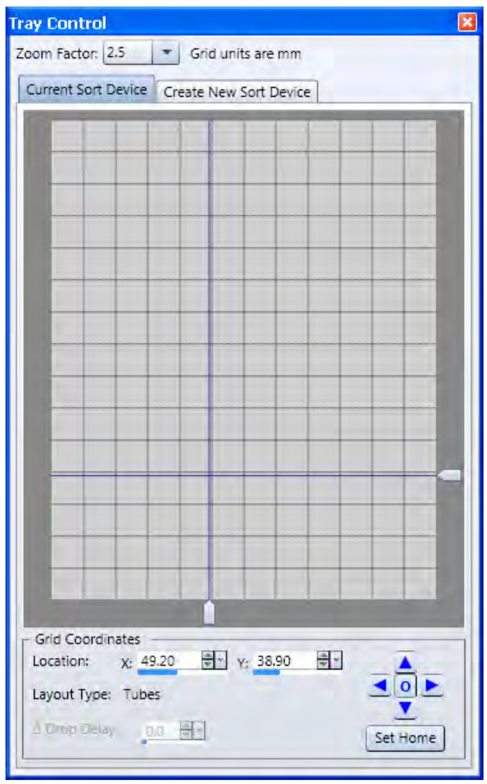
Introduction

This topic describes the **Tray Control** pane which is used to view or modify the sort device.

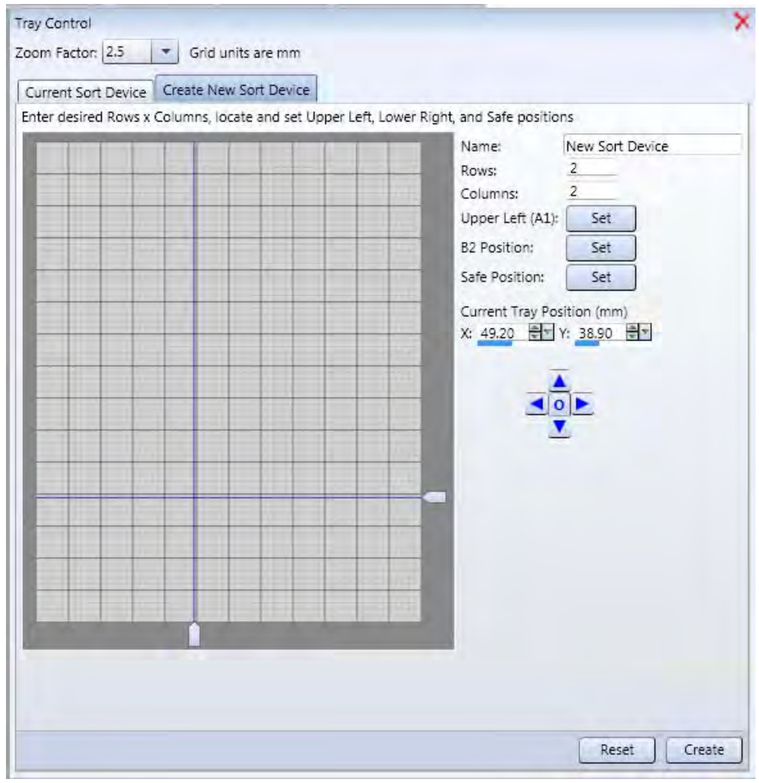
Description

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. This pane includes two tabs.

- **Current Sort Device** tab. This tab displays the layout for the current sort device. You can modify the layout using the offset markers and grid coordinate controls.



- **Create New Sort Device** tab. Use this tab to create a new sort device layout by setting the number of rows and columns in the layout, selecting top (A1), bottom (B1), and Safe positions, and the current tray position.



Creating a new sort device

Introduction

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

Sort devices are plates or multi-tube assemblies. You need to create plate or sort device layouts using the **Tray Control** pane to map the locations of wells or tubes. You can save sort devices for future sorts and select them in the **Sort Layout** pane when you are ready to start a sort.

You can restore and modify sort device layouts using the **Tray Control** pane. You can manage a list of sort devices using the **Manage Sort Device** dialog.

Before you begin

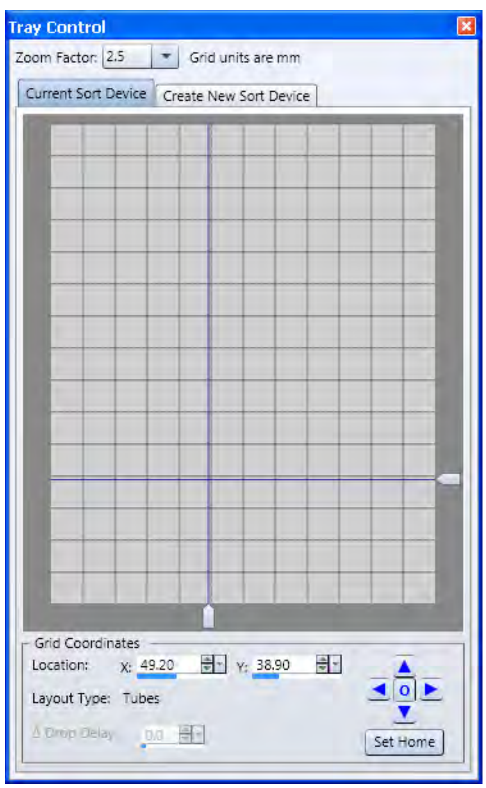
Standard tray sort device dimensions and well layouts are based on BD Falcon™ plates. If you plan to use small well plates (96-well) from other manufacturers, you need to create a custom tray sort device to match the plate.

- Set up for a sort.
- Place a sort device in the sort chamber.

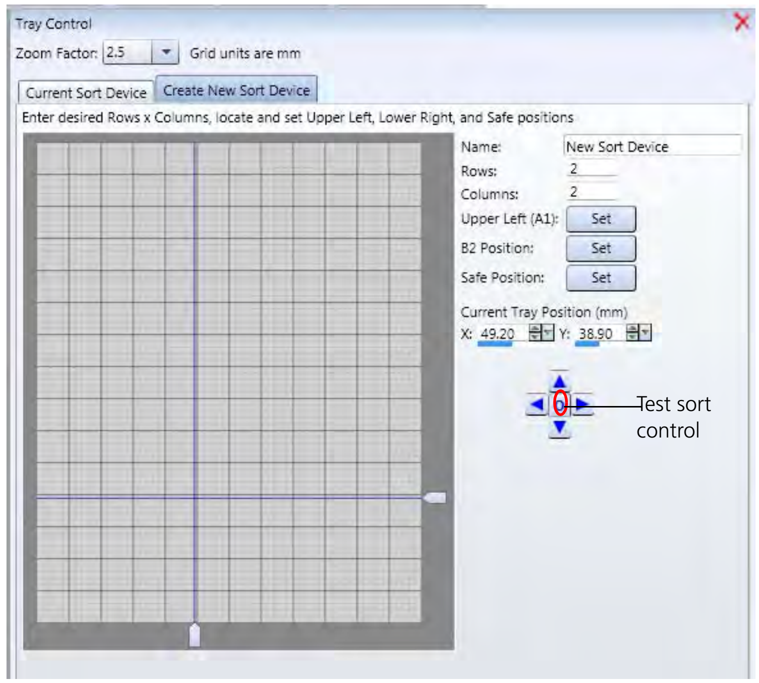
Procedure

To create a new sort device:

1. Select **Tray Control** from the **BD FACS Sortware sorter software** menu to display the **Tray Control** pane.



2. Click the **Create New Sort Device** tab.



3. Type a name for the new sort device in the **Name** field.
4. Enter the number of rows and columns in the **Rows and Columns** field.

   The number of rows for the device appears in the **Rows** field. The number of columns for the device appears in the **Columns** field.
5. On the map, move the cursor to set the A1 (first well) position (upper left).
   a. Note where the sort device is in relation to the stream drain. Move the cursor on the map to bring the device A1 position close to the stream.
   b. Click **Test Sort** to sort a drop on the sort device.
   c. Open the **Sort Layout** pane.
   d. Click **Eject** to move the device forward.
   e. Check the position of the test sort drop.
   f. Repeat steps a to e until the test sort drop hits the center of the well.
   g. Click the **Set** button next to **Upper Left (A1).**
6. Move the cursor to set the B1 (last well) position (bottom right).
7. Repeat step 5 for the B1 position.
8. Move the cursor to set the safe position.

   When you use the **Sort Layout** pane to set up or run a sort, you can click **Safe** to move the sort tray to this mapped safe position away from the sort chamber. Make sure to allow for enough space between the wells and the safe position (approximately equal to the number of rows in the plate) so that the sort tray can clear the sort chamber.

9. Click the **Set** button next to **Safe Position**.
10. (Optional) Adjust the X or Y offsets (indicated by the blue line in the sort device map) by clicking the position up/down and left/right arrows, or by dragging the position markers in the sort device map.
11. Click **Create** to create the new sort device.

More information

- Modifying an existing sort device (page 214)
- Deleting sort devices (page 216)

Modifying an existing sort device

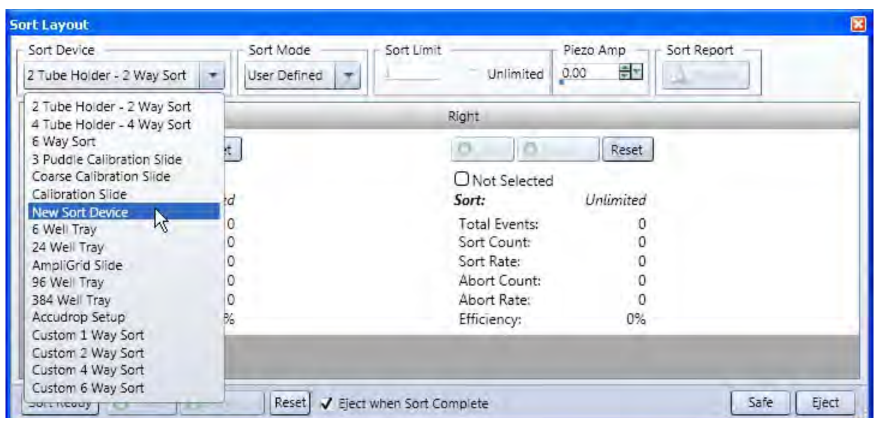
Introduction

This topic describes how to modify an existing sort device using the **Tray Control** pane.

Procedure

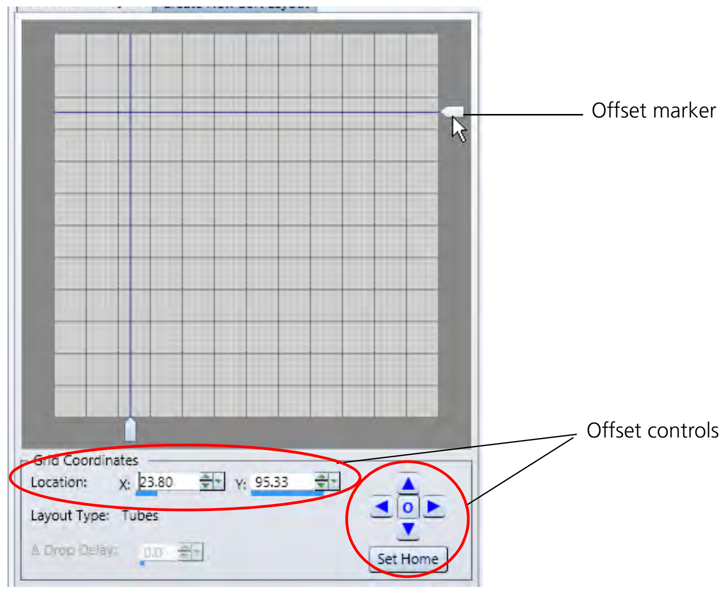
To modify a sort device:

1. In the **Sorting 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 A1, B1, and Safe positions to the new locations. The new positions are saved with the selected sort device.

More information

- Using the Tray Control pane (page 210)

Deleting sort devices

Introduction

This topic describes how to delete existing sort devices using the **Manage Sort Devices** dialog.

Procedure

To delete existing sort devices:

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

   The sort device is deleted.

More information

- Using the Tray Control pane (page 210)

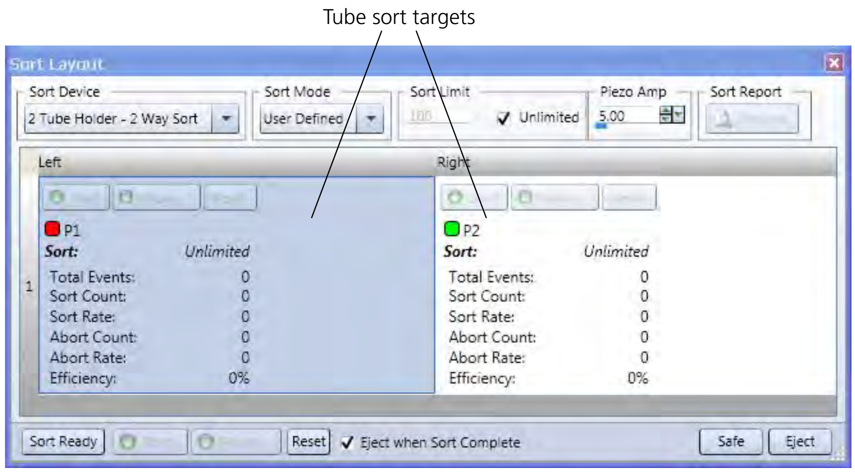
Using the Sort Layout pane

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 tools.

Tool	Description
Sort Device	Lists the available sort devices.
Sort Mode	Lists the available preset sort modes or user-defined modes.
Sort Limit	Displays the number of events. Modify this setting by typing a value in the field.
Unlimited	Enables or disables a continuous sort.
Piezo Amp	Displays the current piezo amplitude. Modify this setting by typing a value in the field or using the data slider.
Sort Report	Displays a preview of the sort report after the sort completes.

This 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 (P1, P2, etc.)	Right-click in a sort target to select a population for the tube or well.
Start, Stop, Pause, Reset	Click to control the sort operation.

Control or action	Procedure
Eject when sort complete (checkbox)	Select to eject the sort tray at the end of a sort.
Sort Ready	Click to move the sort tray to the sort ready position.
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 93)
- Using the Tray Control pane (page 210)
- Sort setup workflow (page 284)

Controlling the sort tray 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.

Moving the sort tray to predefined positions

To move the sort tray to predefined positions:

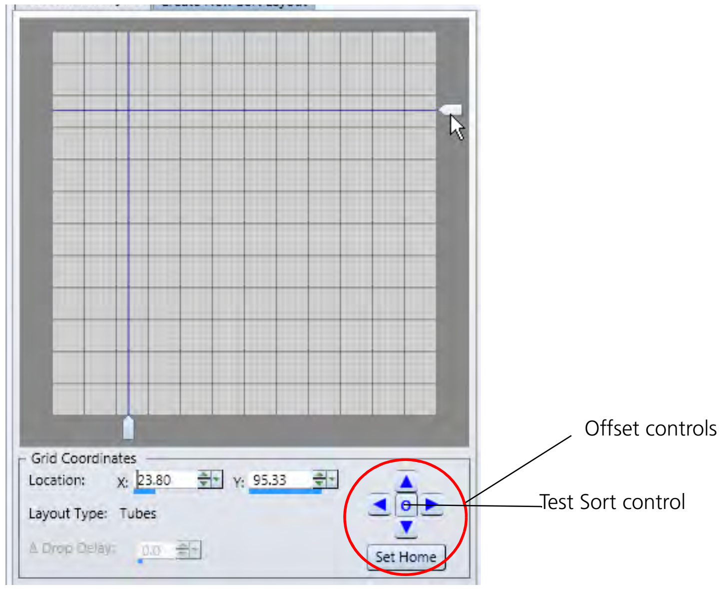
- To move the sort tray to the Home position (ready for sorting), click **Sort Ready.**
- To move the sort tray to the safe position (away from the sample stream), click **Safe.**
- To move the sort tray to the eject position so you can remove the sort device, Click **Eject.**

Manually moving the sort tray position

To manually move the sort tray position:

1. Open the tray control.
2. Click the **Test Sort** button to determine the current setting.

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



4. Click **Set Home** to save setting for the current sort device.

More information

- Using the Tray Control pane (page 210)
- Using the Sort Layout pane (page 217)

Part 3

System workflow

This part includes these sections:

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17

System startup

This section includes these topics:

Startup workflow

Introduction

This topic describes the workflow steps for starting up the Influx system.

The workflow you perform depends on how the BD Influx instrument was shut down.

Workflow (dry startup)

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

Stage	Description
1	Powering up the system (page 226)
2	Preparing the fluidics tanks (page 228)
3	Flushing the system (page 233)
4	Cleaning the nozzle tip (page 234)
5	Removing bubbles from the sample line (page 235)
6	Backflushing the sample line (page 236)
7	Introducing a sample into the system (page 237)

Workflow (wet startup)

Use this workflow if you are re-starting the Influx instrument after a wet shutdown (DI water in the instrument).

Stage	Description
1	Powering up the system (page 226)
2	Preparing the fluidics tanks (page 228)
3	(If needed) Flushing the system (page 233)
4	(If needed) Cleaning the nozzle tip (page 234)
5	Removing bubbles from the sample line (page 235)
6	Backflushing the sample line (page 236)
7	Introducing a sample into the system (page 237)

More information

- Startup and troubleshooting components (page 225)
- System shutdown (page 319)
- System shutdown workflow (page 320)

Startup and troubleshooting components

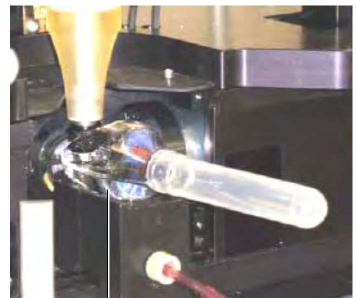
Introduction

This topic describes the tools that are used during BD Influx 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.

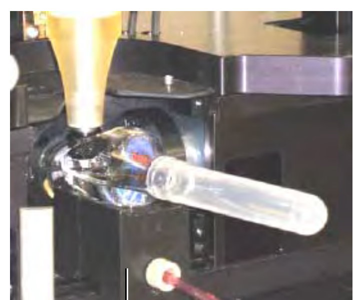
Debubble reservoir attached to a tube to hold fluid



Debubble reservoir with DI water used to submerge a nozzle tip during a wet shutdown

- **Flush bucket.** This is a moveable receptacle that is attached to the waste line. Use this tool during startup and shutdown to catch the sample stream from the nozzle assembly (when you bypass the flow to the sort stage and the drain).

Flush bucket under the nozzle assembly during a flush procedure



Debubble reservoir located on the flush bucket during a wet shutdown

The flush bucket mounts in two places:

– Under the nozzle assembly when in use

– On top of the pressure console when not in use

Use the flush bucket during startup whenever 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 whenever you:

– Dry the fluidics system in a dry shutdown
– Submerge the nozzle tip in DI water for a wet shutdown

More information

- System startup (page 223)
- Startup workflow (page 224)

Powering up the system

Introduction

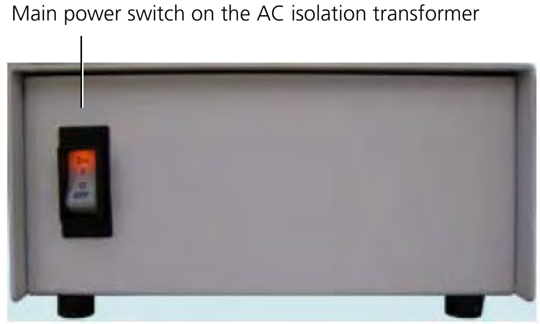
This topic describes how to power up the BD Influx system.

Procedure

To power up the system:

1. Turn on the main power.

   The power source is typically located on the back of the instrument or on the bench next to the instrument.

   The main AC isolation transformer is included with US instruments. This component and procedure might be different based on the transformer used for your specific installation. If you are not sure about the location of your power conditioner, contact your BD Service representative.

2. Turn on the electronics using the auxiliary power switch on the front of the instrument table.

This powers the system electronics.

Caution: Laser! Close all laser shutters and power up all lasers in accordance with the manufacturer's procedures.

3. Turn on the power to the cytometer interface computer.

   The cytometer interface powers up in approximately 90 seconds.

4. Start up the main computer.
5. Turn the key on each laser to turn them on. Allow at least 30 minutes of warmup time.
6. Double-click the BD FACS Sortware sorter software icon on the desktop to start the software.

More information

- System startup (page 223)
- Power distribution (page 64)
- Preparing the fluidics tanks (page 228)

Preparing the fluidics tanks

Introduction

This topic describes how to prepare the fluidics tanks and balance the sample-to-sheath pressure. This topic includes procedures for calibrating the digital scale for an empty tank, and measuring a full or partially filled tank.

Required materials

- 1 empty sheath 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.

Calibrating the digital scale with an empty tank

Perform this procedure only if the digital scale power has been turned off and you have an empty sheath tank, or if you need to refill an empty sheath tank.

To calibrate (zero) the digital scale and determine the weight of an empty sheath tank:

1. Turn on the digital scale.
2. Place an empty sheath tank (with its cover on and the hoses and tubing connected) on the scale.

3. Note the weight of the empty tank before you zero the scale. Save the note as a reference.

4. On the digital scale control panel, press On/Off Zero.

The empty tank weight is now equal to zero on the digital scale.

When you add sheath fluid to the tank, the additional weight is converted to a liquid volume value that determines the sheath level in the tank (1 kg of measured weight is equal to 1 L of sheath fluid).

Measuring a full or partial tank

If the digital scale power is turned off, it does not retain the "empty tank" calibration. The next time it powers up, the digital scale only reflects the current, combined weight of the tank and fluid.

If you have a full or partially full tank, you should not recalibrate the scale. Instead, use the empty tank measurement from step 3 and subtract the weight of the empty tank from the weight that displays on the digital scale to get an accurate measurement.

This eliminates the need for multiple sheath tanks and helps you avoid transferring sheath from one tank to another.

When the tank is empty, you can recalibrate the digital scale before you refill the sheath.

Filling the sheath tank

Check the sheath level during startup (each day) and refill the tank as needed.

To fill the sheath tank:

1. After you calibrate the digital scale with an empty sheath tank, place the empty tank on the floor before refilling it.

   This helps to prevent accidental spillage of sheath fluid onto the digital scale.

2. Fill the sheath tank with up to 7 L of sheath fluid.

   Do not fill past the weld line on the tank. This ensures that there is adequate space for the tank to pressurize.

3. Attach the sheath tank lid.

4. Place the full tank into position on the digital scale.
5. If you are starting the instrument from a dry shutdown, disconnect the blue quick connect sheath and air lines from each other, and attach the sheath line to a 0.2-µm sheath filter.
6. Attach the air line to the tank and the sheath line (with the filter attached) to the sheath tank.

   If you are starting the instrument from a wet shutdown, the filter should already be attached.

Preparing the waste tank

Check the waste tank level during startup (each day) and empty it each time you fill the sheath tank.

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.

To prepare the waste tank:

1. Disconnect the quick connect fittings.
2. Remove the sheath tank lid near the sink.
3. Empty the waste tank carefully so that you do not spill waste fluid on the pressure gauge.
4. Add bleach if sorting biohazardous samples. Add 0.7 L of bleach to achieve a 10% concentration of bleach.
5. Attach the sheath tank lid.

6. Place the waste tank next to the sheath tank, and then attach the quick connect fittings for the waste line input and the vacuum source.

7. Ensure that the hydrophobic waste filter is connected between the vacuum source and the waste tank.

8. If the waste tank overfills and wets the filter, empty the waste tank and replace the filter.

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

Pressurizing the waste tank

To pressurize the waste tank:

1. Turn on the house vacuum supply or vacuum pump.
2. Check the waste gauge and ensure that it reads between 5" Hg and 10" Hg.
   - If the gauge reads less than 5" Hg after a few seconds, tighten the waste tank lid to ensure that it is tightly sealed. If the waste lid is sealed but the pressure does not increase, see topics about pressurizing tanks in Sorting troubleshooting (page 364) for more information.
   - If the gauge reads more 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. Switch the pressure console Air switch on.

4. Verify that the sheath tank is sealed by checking the pressure gauge for the appropriate pressure. The pressure should be between 5–65 PSI depending on the nozzle size (use more pressure for larger nozzles).

   If the tank is not pressurizing, verify that the release valve is closed, or re-seat the sheath reservoir lid.

5. Use the SHEATH pressure regulator knob on the right side of the pressure console to adjust the pressure level.

Setting the sample pressure

To set the sample pressure:

1. Set the sample pressure to at least 1 PSI more than the sheath pressure with the SAMPLE pressure regulator knob.

   The sample flow rate is determined by the sample pressure setting. You can typically achieve a low flow rate when the sample pressure is 1 PSI higher than the sheath pressure.

   You can adjust the sample pressure to achieve a specific sample flow rate after you introduce a sample into the system.

More information

- System startup (page 223)
- Flushing the system (page 233)
- Introducing a sample into the system (page 237)
- Sorting troubleshooting (page 364)

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.

You should flush the system:

- After you start up the instrument from a dry shutdown
- Whenever you change sample types
- After you change the fluidic tubing

Required materials

Flush bucket

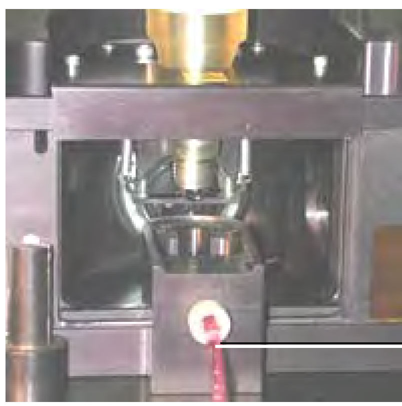
Flushing the fluidics

To flush the system:

1. Remove the nozzle from the sort head if you are performing a wet shutdown.

   For a dry shutdown, the nozzle is already removed from the sort head.

2. Place the flush bucket under the nozzle.



Flush bucket

3. Press 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 running at a high pressure, the flush bucket can easily overfill.

4. If you are using the 0.2-µm sheath filter, remove it from the metal bracket and tap it gently to dislodge any air bubbles.

5. Press RINSE again to stop the flow.

More information

- System startup (page 223)
- Preparing the fluidics tanks (page 228)
- Removing and replacing the nozzle tip (page 81)
- Cleaning the nozzle tip (page 234)
- Startup and troubleshooting components (page 225)
- Sorting troubleshooting (page 364)

Cleaning the nozzle tip

Introduction

This topic describes how to clean the nozzle tip.

Clean the nozzle tip when the stream is diverted by an apparent obstruction or clog. You should also clean the nozzle tip daily as part of the startup workflow.

Required materials

Syringe with 0.2-µm filtered water, sheath fluid, or a mild detergent

Before you begin

Remove the nozzle if it is still installed.

Procedure

To clean the nozzle tip:

1. Sonicate the nozzle for 1–2 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. Flush the nozzle again in the direction of normal sheath flow.
5. Install the nozzle tip.

More information

- System startup (page 223)
- Backflushing the sample line (page 236)
- Removing and replacing the nozzle tip (page 81)

Removing bubbles from the sample line

Introduction

This topic describes how to remove bubbles from the sample line.

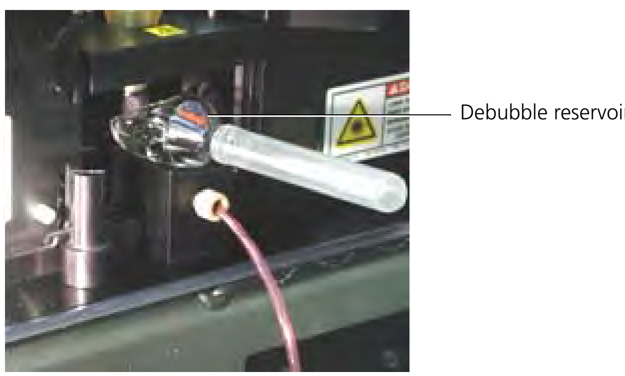
Required materials

- Debubble reservoir
- Flush bucket

Procedure

To remove bubbles from the sample line:

1. Fill the debubble reservoir with sheath fluid and place it on top of the flush bucket.
2. Ensure that the tip of the nozzle is submerged in fluid.



3. Press PURGE to pull fluid up through the nozzle tip and remove air from the system.
4. When all air has traveled past the purge valve, press PULSE to free additional bubbles that might be trapped in the nozzle.
5. Repeat steps 3 and 4 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, refilling the debubble reservoir throughout the process as needed.
   - Use ethanol instead of sheath fluid in the debubble reservoir to dislodge bubbles.
   - 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 and then reinsert the debubble reservoir briefly to introduce a large bubble into the nozzle. The large bubble will often dislodge the smaller bubbles.

6. Press RUN to start a stream with the debubble reservoir still in place.
7. Remove the debubble reservoir and remove excess fluid from the nozzle tip with a cotton swab, if needed.
8. 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.

More information

- System startup (page 223)
- Cleaning the nozzle tip (page 234)
- Startup and troubleshooting components (page 225)

Backflushing the sample line

Introduction

This topic describes how to backflush the sample line to remove any fluid in the sample line.

Procedure

To backflush the sample line:

1. Press RUN to start the stream.
2. Remove the sample tube and move the sample tube lever to the open position.
3. Press BACKFLUSH to backflush the sample line.
4. After about 30 seconds, press BACKFLUSH again to turn backflushing off.

More information

- Startup and troubleshooting components (page 225)
- System startup (page 223)
- Cleaning the nozzle tip (page 234)
- Introducing a sample into the system (page 237)

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 workflow sections.

See Part 3: System workflow (page 221) 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 pressure so that the sample core stream is as narrow as possible. When sample pressure is high, the sample core stream widens and the particles are dispersed into the wider stream. This causes defocused laser light during sample interrogation and a less accurate event count.

If you want to increase the event rate, the sample concentration is important. If the sample is diluted, the sample pressure must remain low. If the concentration is high, then you can increase the sample pressure without causing substantial event loss.

Typically, you can achieve a low flow rate when the sample pressure is less than 1 PSI higher than the sheath pressure on some instruments. This setting can be different for each instrument and depends on the concentration of the sample.

Requirements

- Use only BD Falcon 5-mL polypropylene sample tubes.
- Filter all samples to 40 µm, or about half the nozzle tip size, to prevent nozzle clogs and ensure optimal sorting.
- Use the flush bucket and Erlenmeyer flask to catch the stream during the stream testing procedure.

Before you begin

- Make sure that the sample pressure is set to approximately 1 PSI over the sheath pressure.
- Make sure that the fault LEDs are both green. If the LEDs do not light up, see the fluidics troubleshooting section for more information.

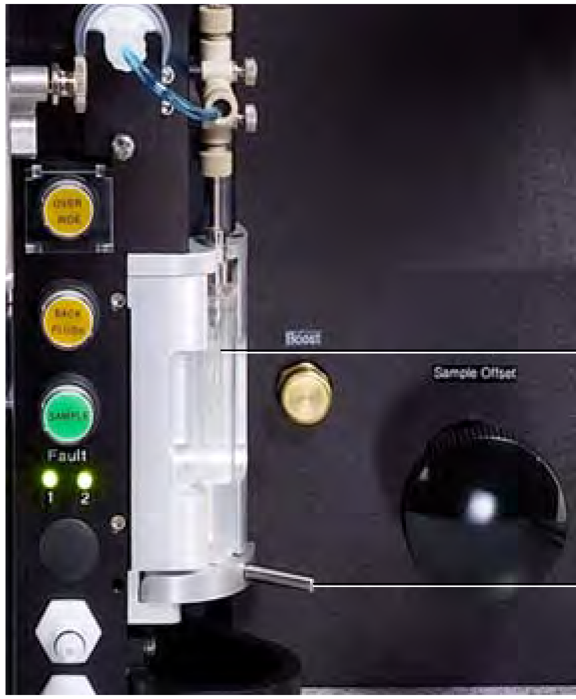
Loading a tube

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 not properly installed and locked in place.

To load a sample tube:

1. Fill 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

You can run a sample at a low rate to help preserve the sample during alignment, optimization, or setting droplet breakoff.

To run a sample:

1. Press SAMPLE to open the sample valve and begin running the sample.
2. Press BOOST for a few seconds to temporarily boost the sample to 3 to 5 PSI higher than the sheath pressure and introduce the sample into the sample line quickly.
3. Create an FSC vs SSC dot plot to view the event scatter.
4. Monitor the event scatter in the plot.
    a. Increase the sample pressure with the SAMPLE pressure regulator knob while you view events on the dot plot until the events begin to scatter.
    b. Adjust the pressure to keep the events cluster as tight as possible.
5. Monitor the sample flow in the pinhole monitor.
    - If you are running bright calibration beads or cells at a low flow rate, a narrow sample core appears.
    - If the flow rate is too high, the sample core appears very large.
    - If no beads are observed flashing in the stream, then the flow rate is too low.

6. Adjust the sample pressure with the SAMPLE pressure regulator knob until you observe a low flow rate.

Removing a sample tube

Caution: Biohazard! Use care when removing the sample tube. The sample tube is usually pressurized and can include aerosolized cells, spills, or splatterings of sample fluid.

To remove the sample tube:

1. Press SAMPLE to stop the sample run.
2. Push the tube-lock lever slowly backward until the tube is pushed down into the recessed area of the tube-lock lever.
3. Remove the tube and then press **BACKFLUSH** to allow the residual sample to backflush.
4. After the pressure is released you can remove the tube safely.

More information

- Sample introduction (page 33)
- Pressure regulation and monitoring (page 37)
- System startup (page 223)

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18

Alignment and QC

This section 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

Before running samples each day, the system needs to be aligned and optimized. Tracking the results of alignment ensures that the system is performing consistently over time and can help in troubleshooting instrument problems.

While most alignment and optimization involves the mechanical adjustments of optics and fluidics, some alignment and optimization requires the use of BD FACS Sortware sorter software.

Fine-tuning the instrument involves positioning the sample core at the focal point of the objective lens in the pinhole, illuminating the sample core optimally, and aligning the fluorescence and forward scatter (FSC). You might need to adjust PMT gains as you tune the system to keep the data on scale.

Workflow

Stage	Description
1	Create a QC workspace that includes all required scatter and fluorescent parameters, worksheet elements, and voltage settings. See Creating a QC workspace (page 243).
2	Prepare beads that you can use for alignment and QC. See Preparing beads for QC (page 248).
3	Introduce your sample into the system. See Aligning the stream (page 249).
4	Align the stream to the pinholes and the center of the drain, or use BD Accudrop to align the side and center streams. See Aligning the stream (page 249).
5	Optimize a fluorescence channel by aligning the primary laser into the sample stream. See Aligning the primary laser to the core stream (page 253).
6	Optimize a fluorescence signal by aligning to the fluorescence detectors. See Optimizing the fluorescence signal for the primary laser (page 255).
7	Adjust the forward scatter stage to maximize the signal from the standard and small particle forward scatter detectors. See Optimizing the forward scatter signal (page 256).
8	Optimize additional lasers that your system might include. See Optimizing additional lasers (page 257).
9	Save the QC workspace to store all alignment settings. See Saving the QC workspace (page 260).

More information

- Creating a QC workspace (page 243)

Creating a QC workspace

Introduction

This topic describes how to create a QC workspace for a specific sorting nozzle size and sheath pressure.

The QC workspace includes a worksheet with plots to measure and track all scatter and fluorescent parameters that are required for QC. Once you assign lasers, create a worksheet, and adjust voltages, you can save the QC workspace and re-use it for daily QC or modify it for use with different nozzle sizes.

About the QC workspace

The QC workspace is designed to simplify alignment by presenting the correct alignment procedure. The procedure typically includes the following:

1. Aligning the stream to pinholes and the drain
2. Aligning the primary fluorescence parameter
3. Aligning the forward scatter
4. Aligning additional lasers

After alignment and QC, the resulting cytometer settings can be saved to be used as the base daily cytometer settings.

Procedure summary

This example shows how to create a new 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 only show the essential categories for events, median or mean, and CV or RCV.

Stage	Description
1	Start with a QC workspace. See Creating a QC workspace (page 243)
2	Add plots for the primary laser fluorescence parameters. See Adding plots for primary laser fluorescence parameters (page 245)
3	Add and customize statistics views for the primary laser fluorescence parameters and create a gate for QC population. See Adding and customizing a statistics view (page 245)

Stage	Description
4	Add an FSC vs SSC plot and statistics view. See Adding an FSC vs SSC plot and a statistics view (page 247)
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 247)
6	Save the workspace and an FCS file (after you perform the alignment procedures). See Saving the QC workspace (page 260)

Creating a QC workspace

To create a QC workspace:

1. Select **File > New Workspace**.

   A confirmation dialog opens.

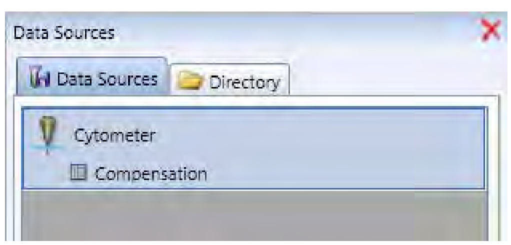
2. Click **OK**.

   The worksheet clears and all plots, gates, data sources, and statistics are deleted.

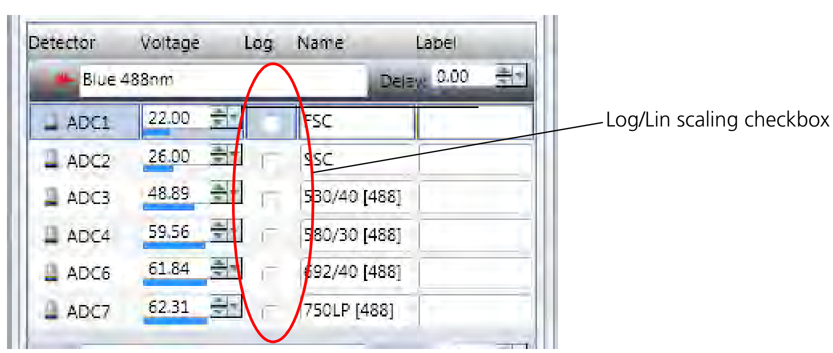
3. (Optional) Add header or footer information to customize the worksheet.

   See Customizing worksheet properties (page 143) for more information.

4. In the **Data Sources** tab, in the **Data Sources** pane, select **Cytometer** as the source.



5. In the **Cytometer Settings** pane, clear the **Log** checkbox for each detector to ensure that all parameters use the linear amplifier.



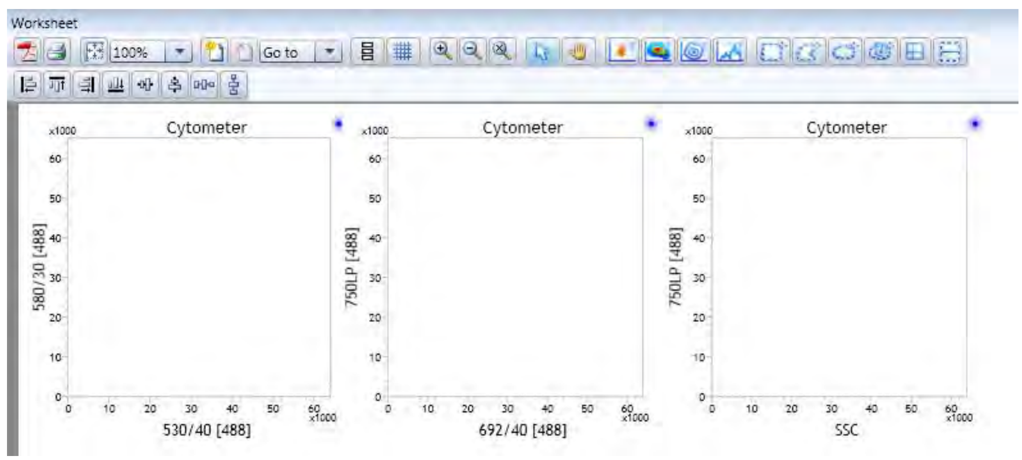
Adding plots for primary laser fluorescence parameters

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 parameter and select different ADC parameters.

   Add dot plots for the primary (blue) laser fluorescence parameters and one for side scatter. For example, create the following fluorescence parameters:

   - 530/40 [488] vs 580/30 [488]
   - 692/40 [488] vs 750 LP [488}
   - SSC vs 750 LP [488}



Adding and customizing a statistics view

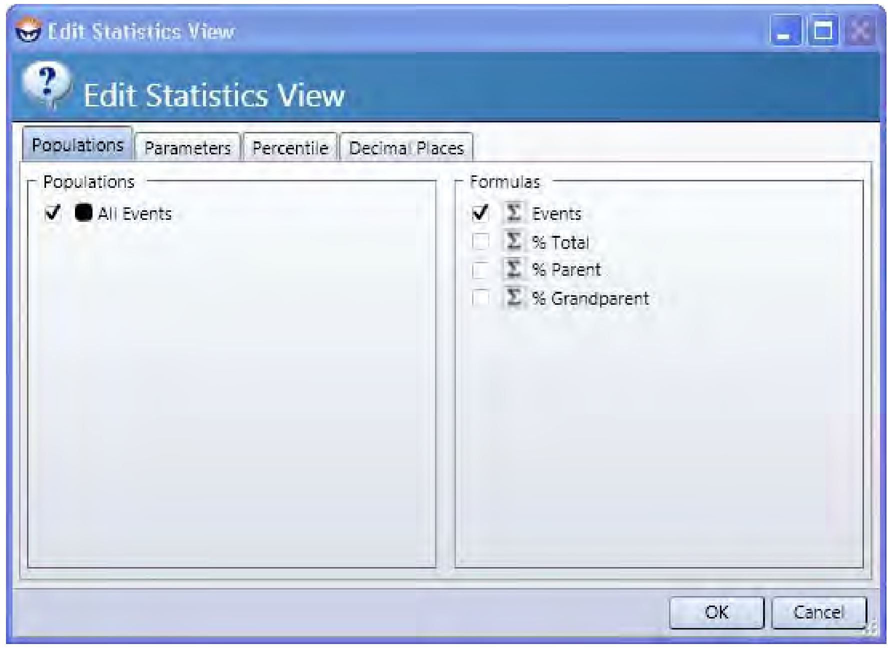
To add and customize a statistics view for the primary laser fluorescence parameters:

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**.

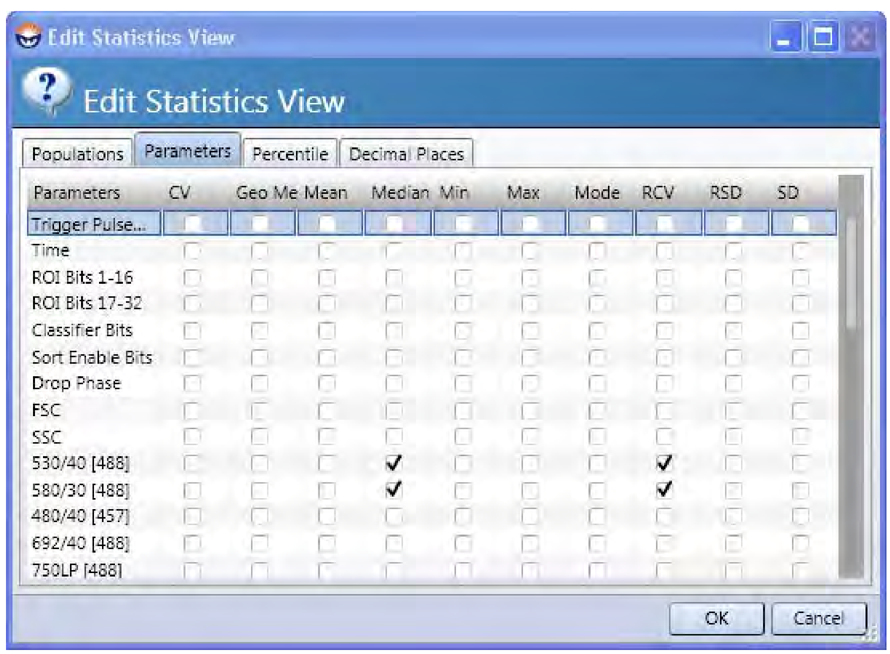
   The **Edit Statistics View** dialog opens.

3. In the **Populations** tab, under **Formulas**, select only the **Events** checkbox.



4. Click the **Parameters** tab.

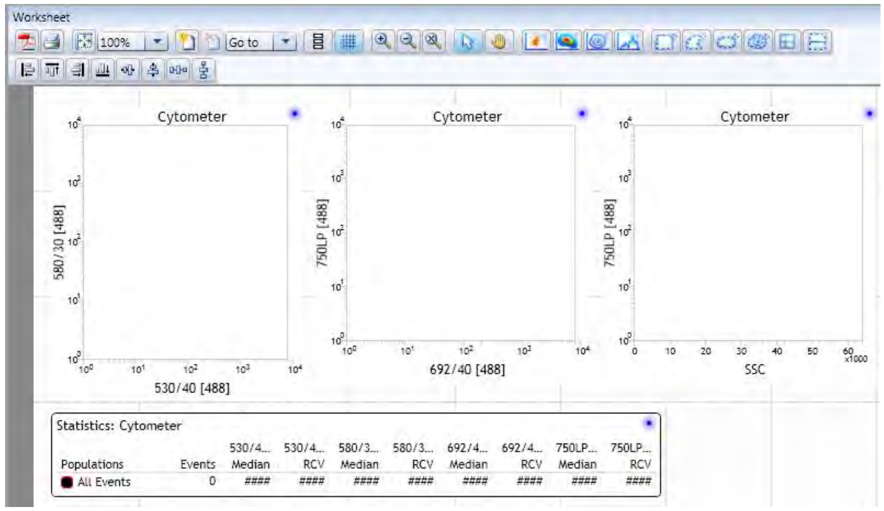
5. For each primary laser fluorescence parameter (488), select the **Median** and **RCV** checkboxes.



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. Do one of the following:
   - If you do not have the FSC depolarizer option, create an FSC vs SSC plot.
   - If you have the FSC depolarizer option, create plots for FSC (Par) vs SSC, and FSC (Par) vs FSC (Per).
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.**

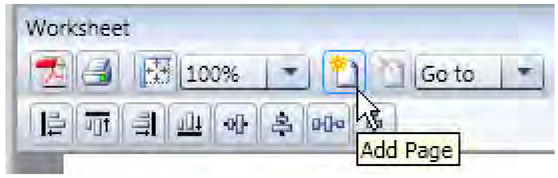
   The **Edit Statistics View** dialog opens.
4. In the **Populations** tab, under **Formulas**, select only the **Events** checkbox.
5. Click the **Parameters** tab.
6. For FSC and SSC, select the **Median** and **RCV** checkboxes. If you have the depolarizer option, also select **FCS (Per)**.
7. Click **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** toolbar to add pages.



More information

- Saving and restoring settings (page 112)
- Saving and deleting settings (page 115)
- Viewing cytometer status (page 121)

Preparing beads for QC

Introduction

This topic describes how to prepare beads for alignment and QC.

Required materials

- BD Falcon™ 35-2063 5-mL polypropylene sample tubes
- 3 µm SPHERO™ Ultra Rainbow beads
- 1 mL sheath fluid

Preparing beads

To prepare a tube of beads:

1. Add at least 2 to 3 drops of beads and 1 mL of sheath fluid to a tube.
2. Load the tube of Ultra Rainbow beads onto the sample tube holder and close the sample lever to lock the tube into place.
3. Press SAMPLE to start the sample flow.

More information

- Introducing a sample into the system (page 237)
- Aligning the stream (page 249)

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. If you have the BD FACS Accudrop feature, this is where you would also ensure that the Accudrop laser is hitting the side and center streams equally.

Required materials

Erlenmeyer flask

Before you begin

Caution: Shock hazard. You must power off the deflection plates before manually opening or adjusting them.

1. Ensure that the deflection plate power is off.

   See Deflection plate power (page 67) for more information.

2. Manually open the deflection plates to prevent the sheath fluid from splashing on the plates and spraying.

Testing the stream alignment

1. Place an Erlenmeyer flask is placed onto the sort stage under the stream drain.

2. Remove the flush bucket so that the stream passes through the illumination chamber into the sort chamber.

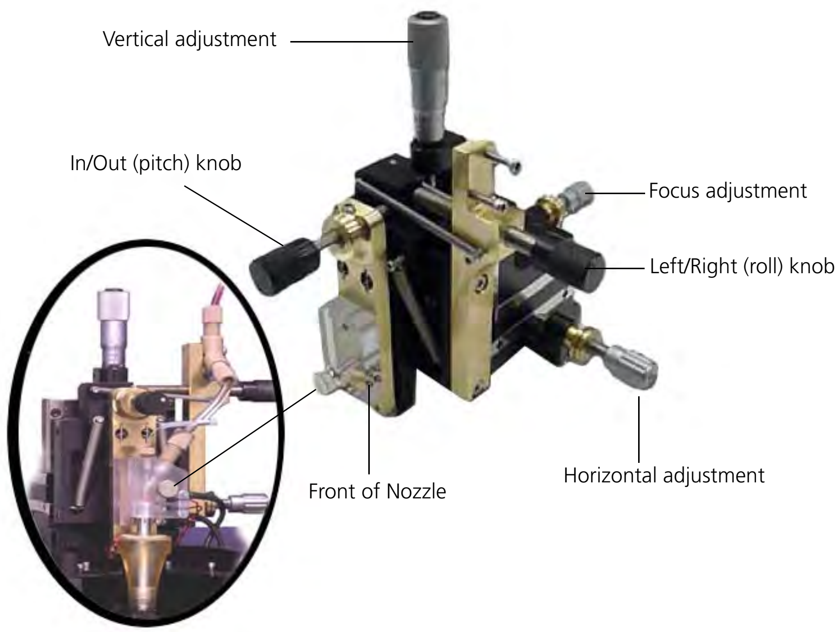
Flush bucket under the nozzle assembly

Caution! The stream might splash and spray out of the drain if the system is running at high pressures and droplets are not being generated.

3. Press **ILLUM** on the monitor stand to illuminate the stream just above the drain.

How to adjust 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 (perpendicular to the light path). Set this above the first pinhole.
Focus (silver knob at the back of nozzle stage): Pinhole alignment	Moves the stage perpendicular to the light path (either to focus the illumination beam or focus the fluorescent/scattered light spot).
Horizontal (bottom silver knob): Pinhole alignment	Moves the stage in and out, parallel to the light path.
In/Out (pitch) black knob: Drain alignment	Moves the nozzle pitch (in or out) and adjusts the stream alignment with the center of the stream drain.
Left/Right (roll) black knob: Drain alignment	Moves the nozzle roll (left or right) and adjusts the stream alignment with the center of the stream drain.

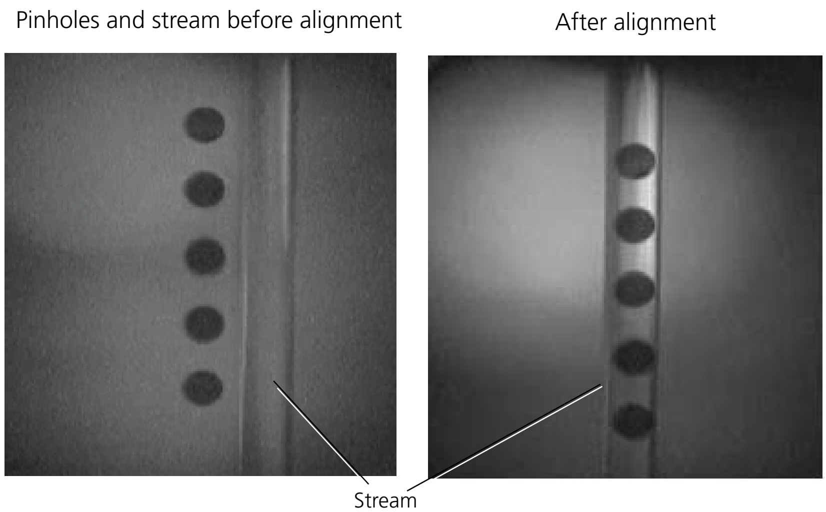
Adjusting the nozzle stage to align the stream

To adjust the nozzle stage to align the stream:

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.

   View the stream on the pinhole monitor.



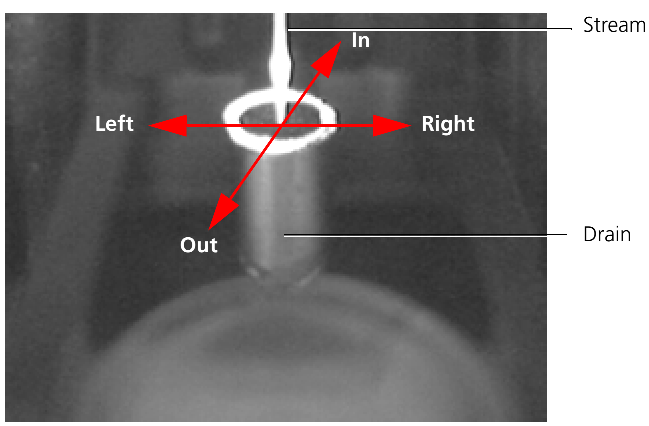
Aligning the stream with the drain

To align the stream with the drain:

1. Adjust the In/Out (pitch) and Left/Right (roll) 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 go back and forth between aligning the stream to the pinhole and to the drain to get an accurate alignment.



Aligning the side and center streams with Accudrop

After you align the stream with the pinholes and the drain, you can use Accudrop to align the side and center streams (the stream is brightest when the laser is aligned with the stream). Ensuring that the Accudrop laser is illuminating the streams equally is important for determining the most accurate drop delay before sorting.

To align the side and center streams with Accudrop:

1. Determine the stream breakoff.
2. Move the deflection plates into the sort position.
3. Turn on the deflection plates.
4. Test the side streams.
5. View the side streams in the stream drain monitor.
6. Ensure that both the side and center streams are illuminated by the Accudrop laser. Adjust the nozzle stage focus knob if necessary.
7. Adjust the In/Out pitch knob only if absolutely necessary.
8. Confirm that the stream is still focused on the pinholes. If not, repeat steps 1 through 7.
9. Turn off the test streams.
10. Turn off the deflection plates.

More information

- Aligning and optimizing the optics workflow (page 242)
- Preparing beads for QC (page 248)
- Aligning the primary laser to the core stream (page 253)
- Optimizing the droplet breakoff (page 285)
- Optimizing the side streams (page 289)

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.

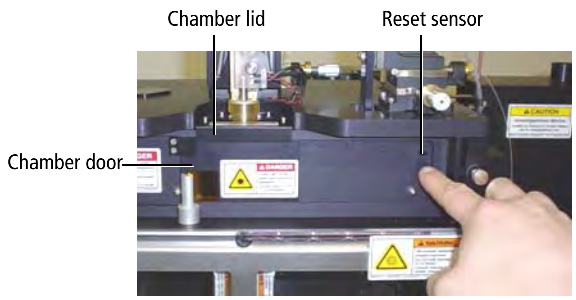
Before you begin

- Prepare a tube of SPHERO Ultra Rainbow beads
- Verify that the fault LEDs are both green.

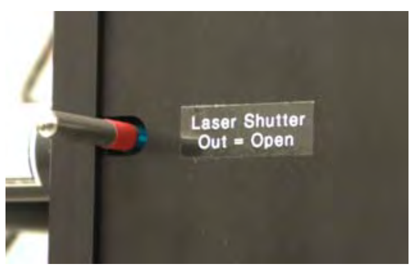
Procedure

To align the primary laser to the core stream:

1. Close the nozzle chamber door and put the chamber lid 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 on the left side of the sort head by pulling the laser shutter interlock pin out.



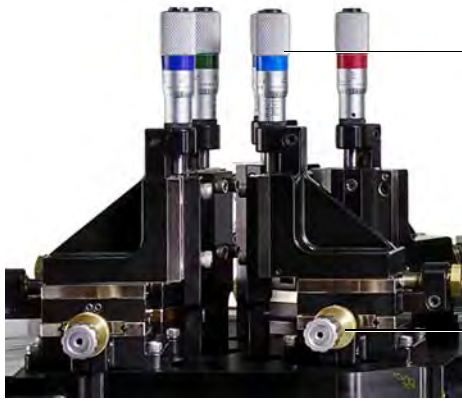
   You will see a glow in the pinhole monitor when the blue laser hits the stream.

4. Load the tube of Ultra Rainbow beads onto the sample tube holder and close the sample lever to lock the tube into place.
5. Check the sample pressure gauge to verify that the sample pressure is about 1 PSI above the sheath pressure. Adjust the sample offset knob to correct the sample pressure, if necessary.

   See Introducing a sample into the system (page 237).

6. Press SAMPLE to start running beads.
7. Press **BOOST** for a few seconds to deliver sample to the laser more quickly, if needed.

8. Adjust the blue laser Vertical adjustment knob so that the laser glow appears just above the top pinhole and look for the flash of beads in the core stream.



9. Adjust the sample offset to change the flow rate until you see a narrow core stream in the pinhole monitor.
   - If the sample core stream is very wide, the flow rate is too high.
   - If the sample core stream is not visible, the flow rate is too low.

   When an optimal low flow rate is achieved, the sample pressure is typically slightly higher than the sheath pressure, usually within 1 PSI.
10. Adjust the blue laser Horizontal adjustment knob until you see the brightest signal in the pinhole monitor.
11. Adjust the Focus knob on the nozzle stage (back of nozzle stage) to focus the signal.
12. Repeat steps 10 and 11 until the sample core is as narrow and bright as possible.
13. Adjust the blue laser Vertical adjustment knob to place the sample core back on the top of the pinhole.

More information

- Optimizing the fluorescence signal for the primary laser (page 255)
- Sorting troubleshooting (page 364)

Optimizing the fluorescence signal for the primary laser

Introduction

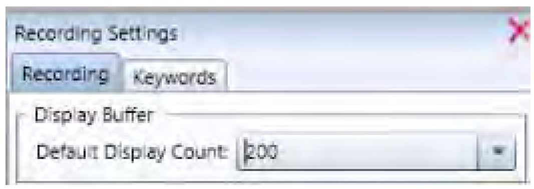
This topic describes how to use BD FACS Sortware sorter software to optimize the fluorescence signal for the primary laser.

Procedure

To optimize the fluorescence signal for the primary laser:

1. Restore the user-defined QC workspace if needed.
2. Click **Recording** on the **BD FACSortware** toolbar.

   The **Recording Settings** pane opens.
3. Under **Display Buffer,** select a default display count of *200*.

   The default display count dictates the default refresh for this session. While making adjustments, it is helpful to have a quick refresh.
4. Click a plot that has the primary (typically blue) laser parameters.
5. Use the vertical and horizontal adjustment knobs to maximize the bead fluorescence signal in the plots associated with the laser you are optimizing.
6. View the oscilloscope pulse monitor and the plots in the worksheet while making adjustments to achieve the maximum signal.

   When a plot is selected, the oscilloscope (channels 1 and 2) update to reflect the signal from the detector (parameters).
7. Once the signal has been optimized, adjust the fluorescence parameter PMTVs so that the bead population is at a mean of approximately 45,000 for each blue laser parameter.
8. Draw a gate around the primary laser signal.

More information

- Saving and restoring settings (page 112)
- Worksheet overview (page 138)
- Viewing and setting cytometer details (page 129)

Optimizing the forward scatter signal

Introduction

This topic describes how to adjust the forward scatter stage to maximize the signal from the standard and small particle forward scatter detectors.

Before you begin

- Open your QC workspace (including plots and statistics views).
- Align the fluorescence detectors.

Optimizing the forward scatter signal

To optimize the forward scatter signal:

1. In the worksheet, click a plot that has the FSC parameter.

   This updates the scope to monitor the forward scatter detector.

2. Adjust the Horizontal and Vertical knobs on the forward scatter stage to maximize the signal from the detector.

   View the oscilloscope pulse monitor and the plot in the worksheet while adjusting the focus, vertical, and horizontal positions of the forward scatter stage to further optimize the forward scatter signal.

3. Adjust the FSC and SSC voltages to place the bead population at a mean of approximately 25,000.

Optimizing for the small particle detector

To optimize the forward scatter for the small particle detector or polarized forward scatter options:

1. Flip the switch on the pinhole monitor to Video 2.

   Video 2 displays the small particle detector forward scatter pinhole.

2. Adjust the focus, vertical, and horizontal positions of the FSC assembly.

   View the pinhole monitor and the plot in the worksheet while adjusting the three knobs on the forward scatter stage until the signal is maximized.

3. Adjust the voltages to place the bead population at a mean of about 25,000.

More information

- Optimizing the fluorescence signal for the primary laser (page 255)
- Optimizing additional lasers (page 257)

Optimizing additional lasers

Introduction

This topic describes how to optimize additional lasers that your system might include.

About additional lasers

Additional lasers must be aligned to the correct pinhole using the pinhole monitor. Fine-tune the laser using the oscilloscope and the signals viewed in BD FACS Sortware sorter software.

The primary laser (through the top pinhole) is the system trigger. With multi-laser systems, you need adjust the laser delay to synchronize the signals from additional lasers.

Before you begin

- View the **Cytometer Settings** pane to verify that the lasers are listed in the proper order.
- Ensure that the primary laser signal and forward scatter signals are optimized.
- Make sure you have your QC log sheet.

Aligning additional lasers

To align additional lasers:

1. Open the shutter for the laser that you want to align.
2. Verify that the laser is aligned with the correct pinhole by looking for beads in the core stream on the pinhole monitor.

   To optimize the laser, see Aligning the primary laser to the core stream (page 253).

Setting laser delays

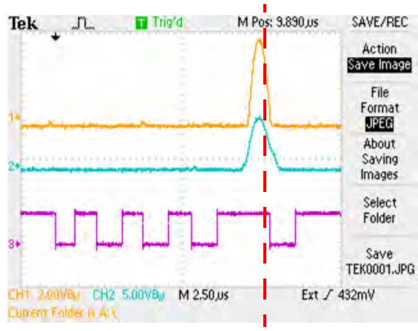
To set laser delays:

1. In the QC workspace worksheet, click a plot that has the parameters for the laser you are aligning.

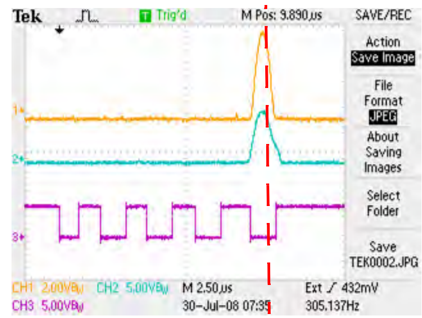
   If the plot does not exist on the worksheet, create a new dot plot and statistics view. The statistics view should include total events, median, and rCV for the laser you are aligning.
2. View the oscilloscope and note where the voltage pulse is in relationship to the laser delay bucket.
3. In the **Cytometer Settings** pane, locate the laser you want to adjust in the list.
4. Adjust the delay in the **Delay** field using the mouse scroll wheel, data slider, or keyboard keys.

   See Setting numeric values in panes and dialogs (page 96) for more information.

Continue to adjust the value until the signal from the laser lines up with the appropriate bin on the third oscilloscope trace.



Laser delay unadjusted



Laser delay adjusted

5. Use the Vertical and Horizontal adjustment knobs to maximize the bead fluorescence signal in the plots associated with the laser you are optimizing. View the oscilloscope pulse monitor and the plots in the worksheet while making adjustments. The goal is to achieve the maximum signal.

6. Repeat steps 4 and 5 until the laser signal is maximized and the laser delay is optimal based on the voltage pulse in the oscilloscope.

7. Adjust the PMTV to place the bead population at a mean of about 45,000 for each parameter of the laser you are aligning.

   You may need to adjust the PMTVs as you are optimizing the signal if the bead population is off scale.

More information

- Optimizing the forward scatter signal (page 256)
- Creating an FCS file to record laser alignment (page 259)

Creating an FCS file to record laser alignment

Introduction

This topic describes how to create an FCS file to record laser alignment.

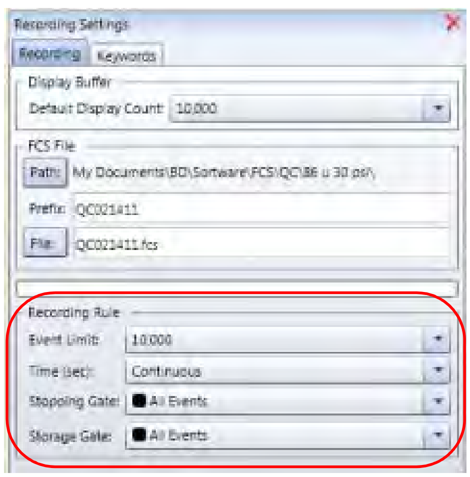
Procedure

After the instrument alignment is optimized for all lasers, create 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 FACSortware** toolbar.

   The **Recording Settings** pane opens.
3. Under **FCS File**, click **Path**.
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. Lower the event rate so that you have a small core stream.
8. In the **Acquisition Dashboard**, click **Acquire**, then click **Record** to record the data file.
9. Note the RCV, Median, and PMTV in your QC log.

More information

- Laser delay (page 53)
- Adjusting PMT voltages and using integrators (page 125)
- Optimizing the forward scatter signal (page 256)
- Saving the QC workspace (page 260)
- Verifying alignment using the target source (page 261)

Saving the QC workspace

Introduction

This topic describes how to save the QC workspace.

Procedure

To save the QC workspace:

1. (Optional) Click **Cytometer > Details**.

   The **Cytometer Details** dialog opens.

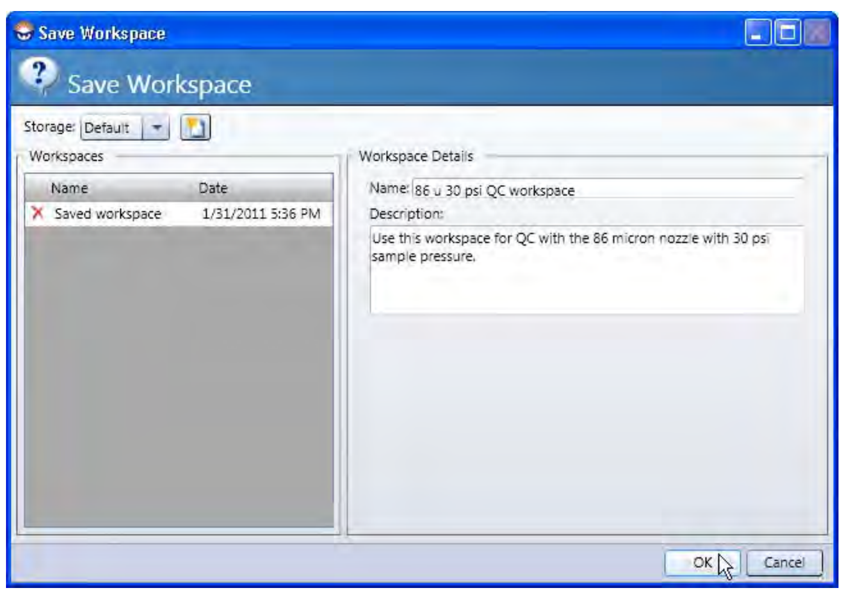
   a. Type the nozzle and pressure that is used for this QC workspace.

   b. Click **OK** to apply your preferences and close the dialog.

2. Select **File > Save > Workspace.**

   The **Save Workspace** dialog opens.

3. Under **Workspace Details**, type a useful name for the QC workspace in the **Name** field (for example, *86 u 30 psi QC Workspace)*.

4. (Optional) Type a description of this workspace template in the **Description** field.

5. Click **OK.**

You can restore the QC workspace whenever you want to perform QC.

More information

- Aligning the stream (page 249)
- Optimizing additional lasers (page 257)
- Verifying alignment using the target source (page 261)

Verifying alignment using the target source

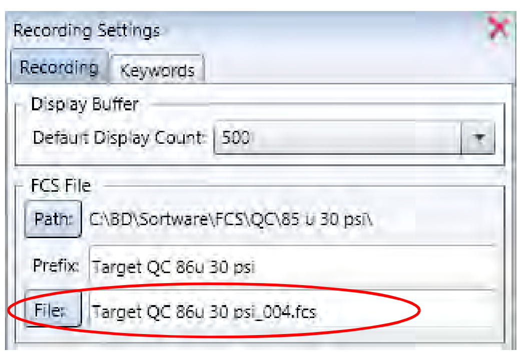
Introduction

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 must match the parameters and the (log/lin) of the plots.

Recording a target source FCS file

To record a target source FCS file:

1. In the **Recording Settings** pane, create a QC FCS file that contains a small number of events (for example, 500 to 750).
2. In the **File** field, name the file (for example, *Target QC 86u 30 psi*).



See Creating an FCS file to record laser alignment (page 259) for instructions on creating an FCS file using the **Recording Settings** pane.

3. In the **Acquisition Dashboard**, click **Acquire**, then click **Record** to record the data file.

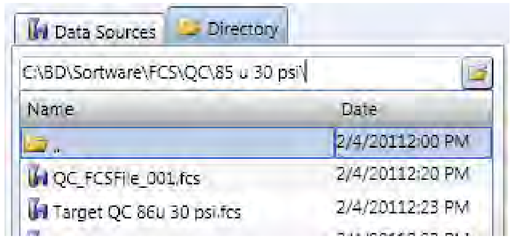
Applying the target source

You can add a target source to your plots to show what your optimized alignment looks like.

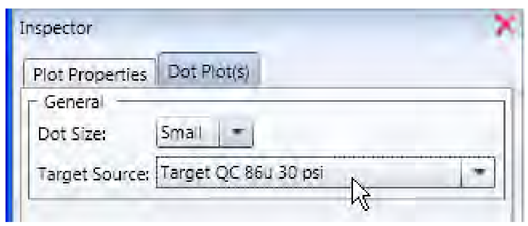
To apply the target source:

1. Open the appropriate QC workspace.
2. In the **Data Source** pane, click the **Directory** tab.

3. Click the **Open Folder** button and navigate the folder that contains the new target FCS file.



4. Click **OK**.
5. Double-click the FCS file to add it to the **Data Sources** tab.
6. In the worksheet, select all plots.
7. In the **Inspector**, click the **Dot Plot(s)** tab.
8. In **Target Source** field, select the target QC FCS file.



The target source appears in gray as an overlay (bottom layer) in the plot.

9. Acquire the sample and view where the live events are in relationship to the gray target data. If the live cells are close to the target data, then the alignment is still good. If not, the lasers alignment needs adjustment.

More information

- Optimizing the forward scatter signal (page 256)
- Laser delay (page 53)
- Adjusting PMT voltages and using integrators (page 125)
- Saving the QC workspace (page 260)

19

Optimizing system settings for samples

This section includes these topics:

Creating plots and gates for optimization

Introduction

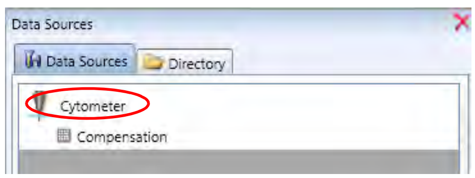
This topic describes how to create plots and gates that you can use to optimize the fluorescence settings for a sample. This describes basic optimization tasks and does not include compensation.

The following procedure uses 8-peak beads to illustrate the example.

Creating plots for optimization

To create plots to optimize settings for your sample:

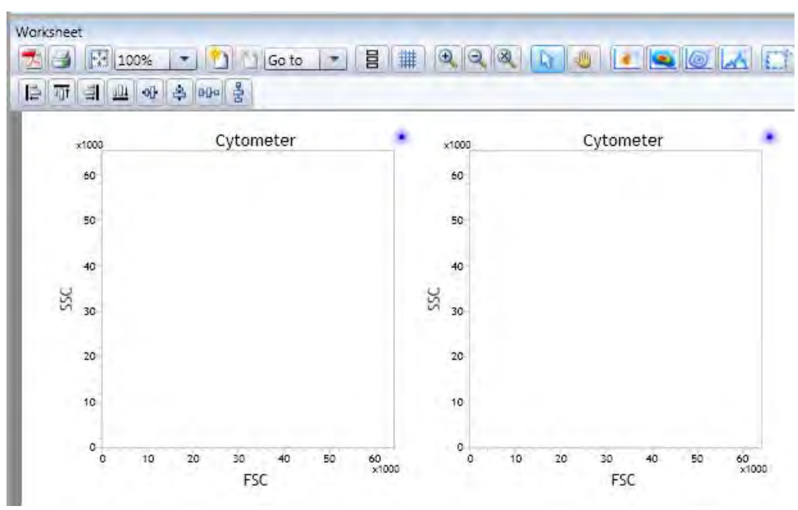
1. Load a tube of your sample and press SAMPLE.
2. In the **Data Sources** pane, select **Cytometer** as the data source.



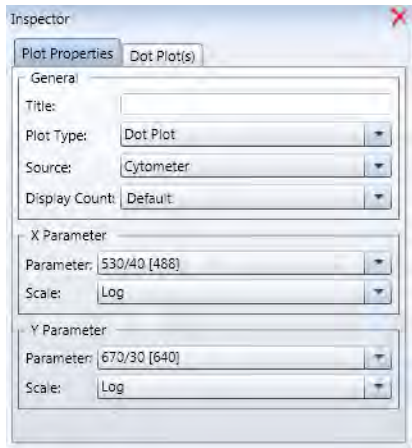
3. Double-click the **Dot Plot** tool on the **Worksheet** toolbar, then click twice in the worksheet to create two plots (scatter and fluorescence).

   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.
5. Verify that the first plot (scatter) displays FSC vs SSC for the x- and -y axis labels.

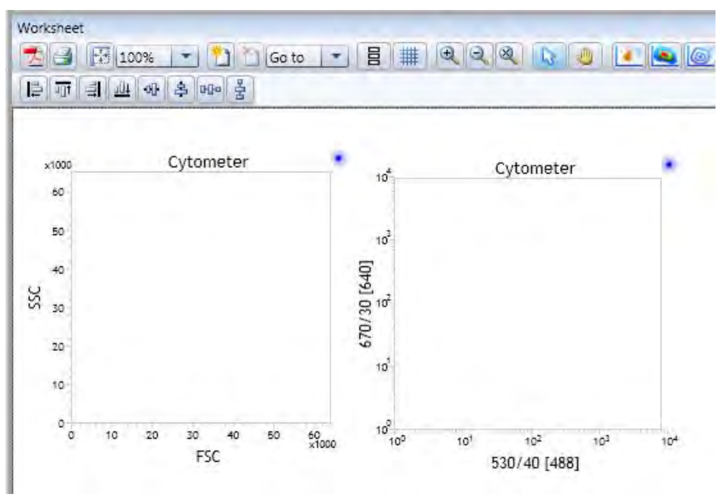


6. Click the second plot (fluorescence) to enable the **Plot Properties** tab in the **Inspector.**



7. Under **X Parameter,** select *530/40* from the **Parameter** menu.
8. Under **Y Parameter,** select 670/30 from the **Parameter** menu.

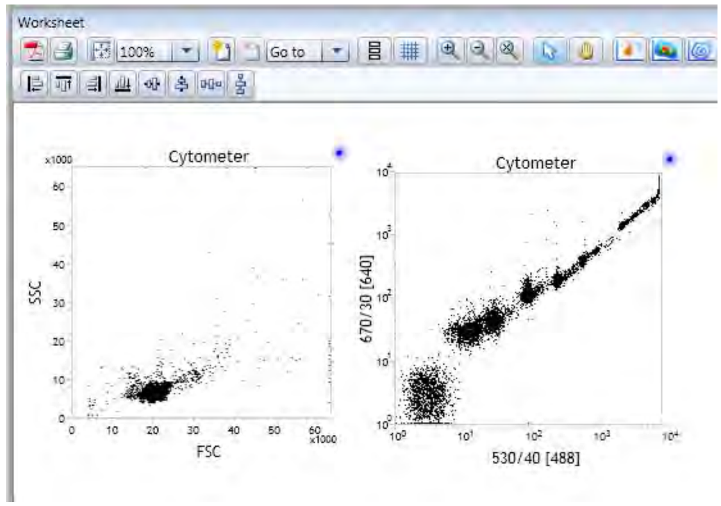
   You can substitute different fluorescence parameters as needed.



9. Click **Acquire** in the **Acquisition Dashboard** to populate the plots with data.

10. Adjust the voltages for the scatter plot to place the population on scale, if needed.

11. Adjust the voltages for the fluorescence to place all peaks on scale.

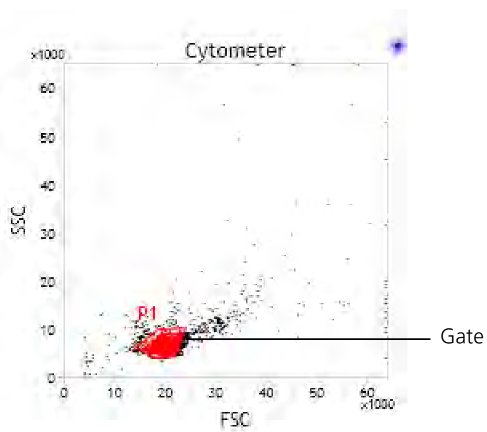


Creating gates for optimization

To create gates to optimize settings for your sample:

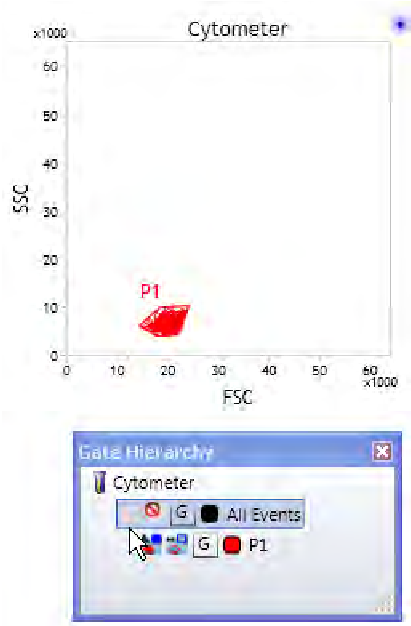
1. In the worksheet, click the scatter (FSC vs SSC) plot.

2. Create a gate on the singlet bead population.



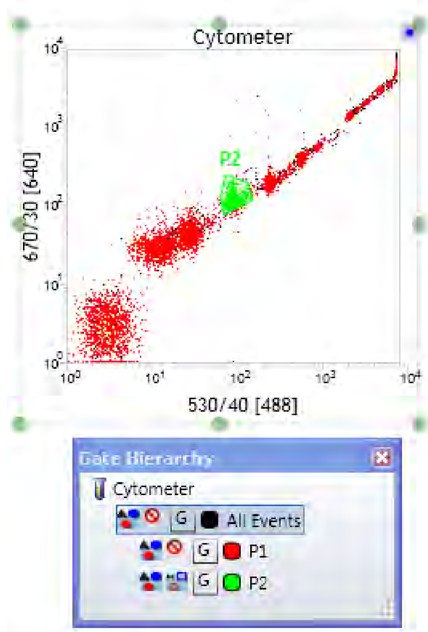
3. In the worksheet, click the scatter plot to enable the gating controls in the **Gate Hierarchy** pane.

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 187)
- Creating polygon gates (page 188)
- Creating ellipse gates (page 189)
- Creating interval gates (page 192)

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

Stage	Description
1	Optimizing scatter parameters (page 269)
2	Optimizing fluorescence detector voltages (page 271)
3	Collecting data files for compensation (page 272)
4	Defining populations for compensation (page 273)
5	Performing auto compensation (page 275)
6	Saving a compensation matrix (page 279)

More information

- Creating plots and gates for optimization (page 264)
- Restoring (importing) a compensation matrix (page 280)

Optimizing scatter parameters

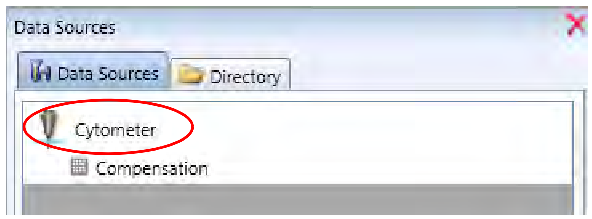
Introduction

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

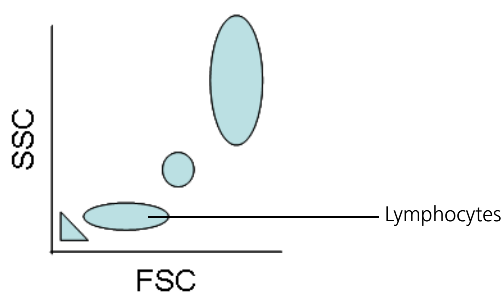
To optimize scatter parameters:

1. Load the tube of unstained cells and press SAMPLE.
2. In the **Data Sources** pane, select **Cytometer** as the data source.



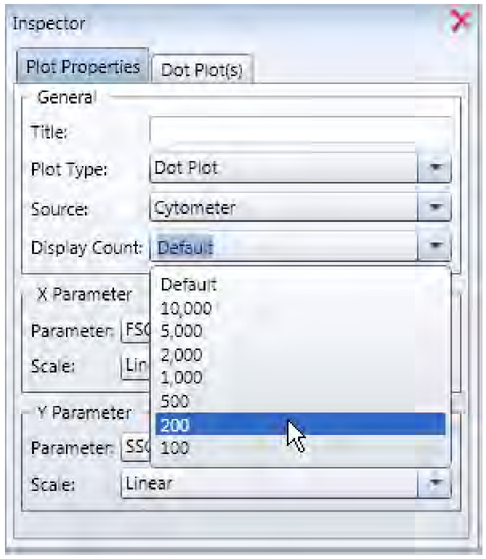
3. Create an FSC vs SSC dot plot in the worksheet.
4. Click **Acquire** in the **Acquisition Dashboard** to populate the plots with data.

The plot populates.



5. Click the FSC vs SSC plot to enable the **Plot Properties** tab in the **Inspector.**

6. In the **Inspector**, select *200* from the **Display Count** menu.



A low display count is useful for setup and compensation. Use a larger display count when you acquire a data file.

7. Adjust the FSC and SSC voltages so that sample is on scale.

8. Continue to adjust the following as needed:

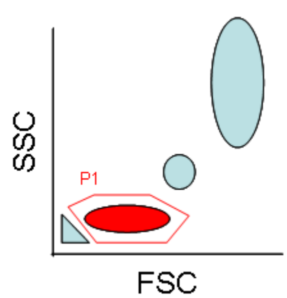
   – Sample pressure (on the BD Influx)

   – Display count (in the **Inspector** pane)

9. In the **Cytometer Settings** pane, adjust the trigger level.

   If you set the trigger level too high, the population does not appear in the plot. If you adjust the trigger too low, you might display debris and less of the actual sample.

10. 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.

11. Press SAMPLE to stop sample introduction.

12. Remove the sample tube and press BACKFLUSH for approximately 10 seconds.

More information

- Optimizing with compensation controls (page 268)
- Optimizing fluorescence detector voltages (page 271)

Optimizing fluorescence detector voltages

Introduction

This topic describes how to optimize the fluorescence detector voltages using the fully stained sample or the single-color cellular compensation controls. This procedure uses the single-color compensation controls.

- For cell-based compensation, adjust the voltages for the detectors before recording samples.
- For bead-based compensation, adjust the voltages with a sample that contains fully stained (positive) and unstained (negative) cells, and verify that both stained and unstained cells and beads appear onscale.

Procedure

To optimize the fluorescence detector voltages:

1. Create dot plots so that each parameter in the experiment is represented.
2. Load a tube containing fully stained positive control (mixed with negative control) on the tube holder and press SAMPLE.
3. Adjust the voltage as necessary so that the negative and positive populations are on scale for each parameter.
4. In the **Acquisition Dashboard**, click **Reset** to refresh all plots.

More information

- Optimizing with compensation controls (page 268)
- Optimizing scatter parameters (page 269)
- Collecting data files for compensation (page 272)

Collecting data files for compensation

Introduction

This topic describes how to collect data files for compensation using cell controls.

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 Capture** tab, select the detectors you want to record for this experiment.
3. Select **Cytometer** as the data source.
4. In the **Recording Settings** pane, under **Recording Rule**, set the event limit value for your sample.
5. Load the tube of unstained cells and press **SAMPLE.**
6. In the **Recording Settings** pane, click **Path** to select where you want to save the FCS file for the unstained control.
7. 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.
8. In the **Acquisition Dashboard**, click **Acquire**, then click **Record** to create an FCS file for the unstained sample tube.
9. Press **SAMPLE** and remove the sample tube.
10. Press **BACKFLUSH** for 30–60 seconds.
11. Load a tube of single-color stained cells and press **SAMPLE.**
12. Repeat steps 7 to 11 to record the remaining fluorescence controls.

Next step

Defining populations for compensation (page 273)

More information

- Optimizing with compensation controls (page 268)

Defining populations for compensation

Introduction

This topic describes how to define populations for compensation. 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).

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

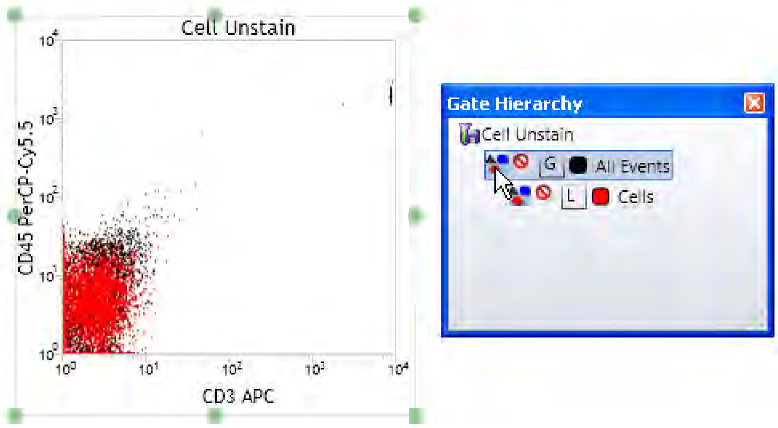
- For each single-color tube, make the FSC vs SSC gate a local gate.
- Draw child gates for the positive population specific to the source for a single color.
- The negative population will be the local FSC vs SSC gate applied from the unstained control sample.

Selecting the negative control population

To identify and select a negative control population:

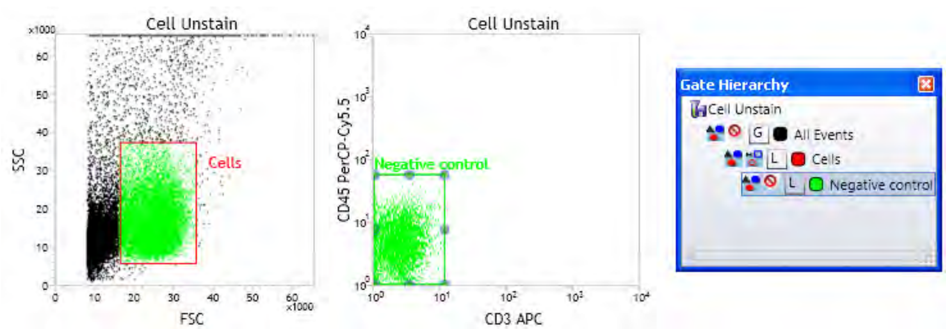
1. Add a new page to the worksheet.
2. In the **Data Sources** pane, select the unstained control.
3. Create two FSC vs SSC plots.
4. Draw a gate around the population of interest in the first plot.
5. Rename the gate (if needed), by typing in the **Name** field in the **Inspector** pane when the gate 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, PerCP-Cy5.5 vs APC.
7. In the **Gate Hierarchy** pane, right-click the newly created gate (cells) and select **Convert to Local**.
8. Select the second plot.

9. In the **Gate Hierarchy** pane, select the **Hide/Show** button for **All Events** to hide all events.



10. In the **Gate Hierarchy** pane, 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 an APC positive control, select **APC** as one of the axes on the plot).

17. Select the gate (cells) in the **Gate Hierarchy** pane, and then draw a gate around the positive population.

18. Rename the new population (for example, *APC Control).*

19. Repeat steps 13 to 18 for the remaining compensation controls.

More information

- Working with gates (page 194)
- Optimizing with compensation controls (page 268)
- Collecting data files for compensation (page 272)
- Performing auto compensation (page 275)

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.

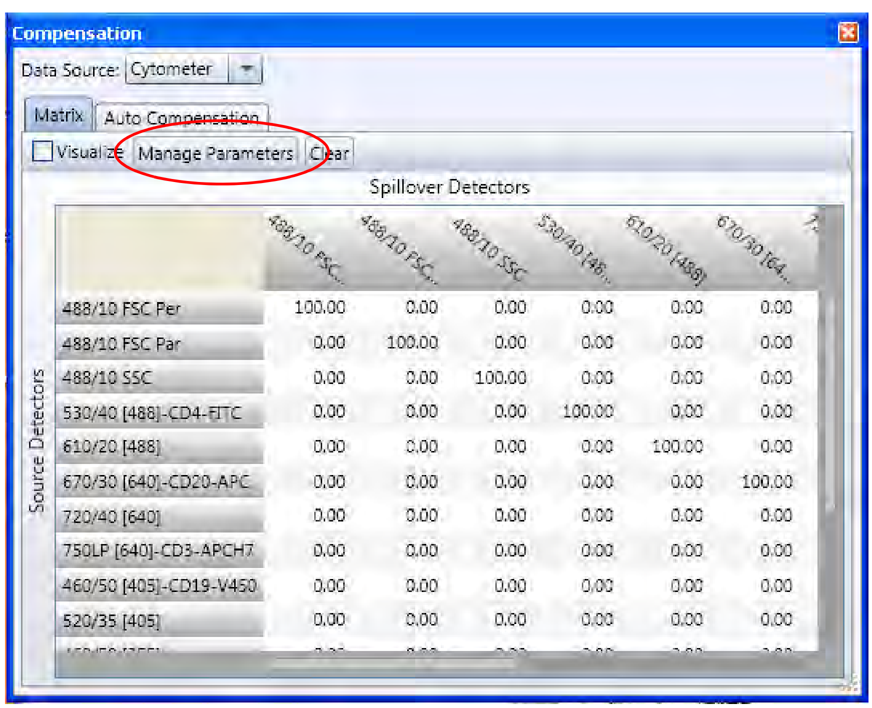
Selecting populations for the negative compensation control

To select the population for the negative compensation control:

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

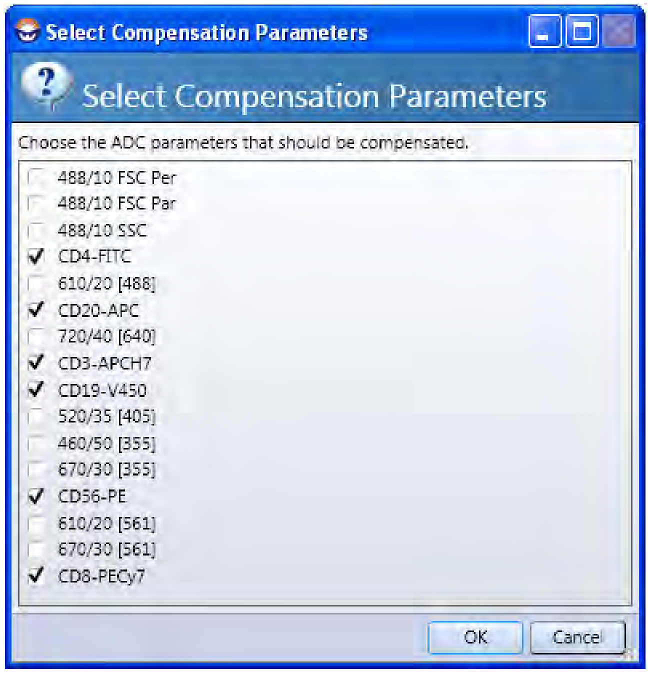
   The **Compensation** pane opens.

2. Click the **Matrix** tab, then click **Manage Parameters**.



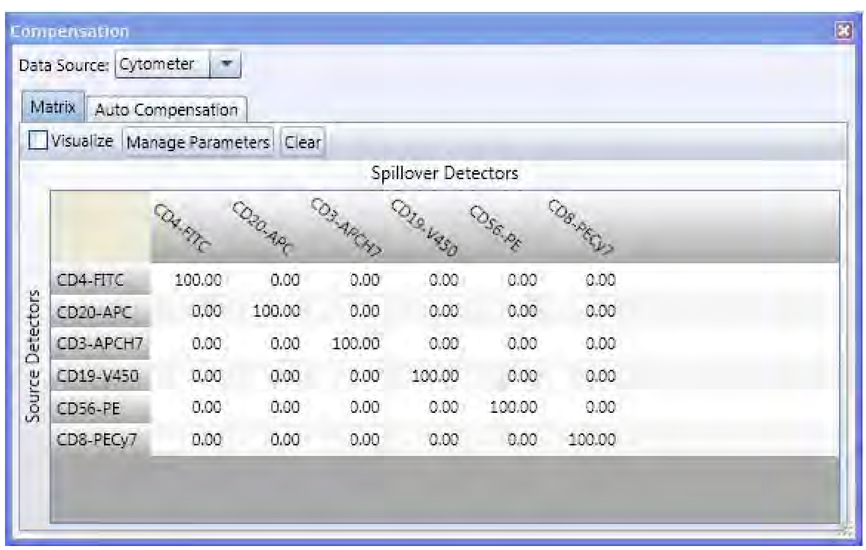
The **Select Compensation Parameters** dialog opens.

3. Select only the checkboxes for the ADC parameters that should be compensated.



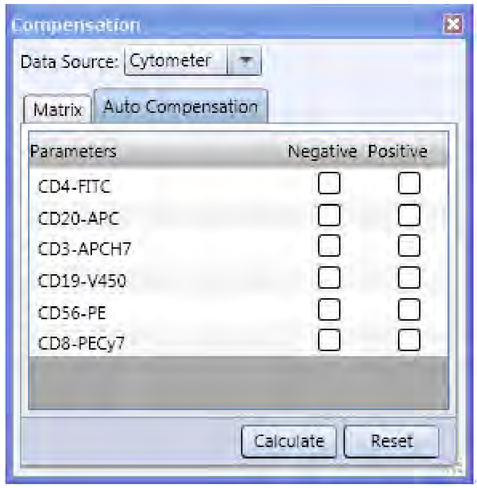
4. Click **OK** to apply the selections and close the dialog.

   The **Matrix** tab refreshes and displays the compensation matrix for the selected parameters.



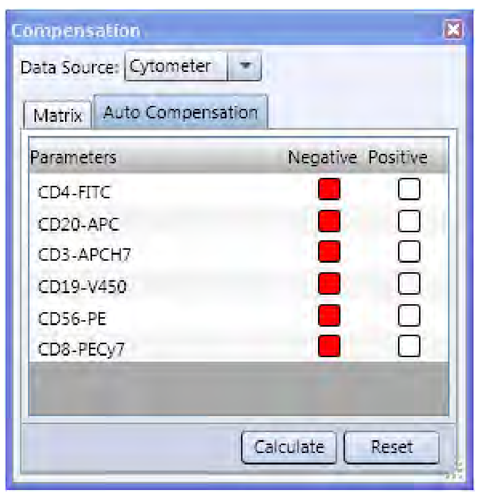
5. Click the **Auto Compensation** tab.

6. Make sure 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.



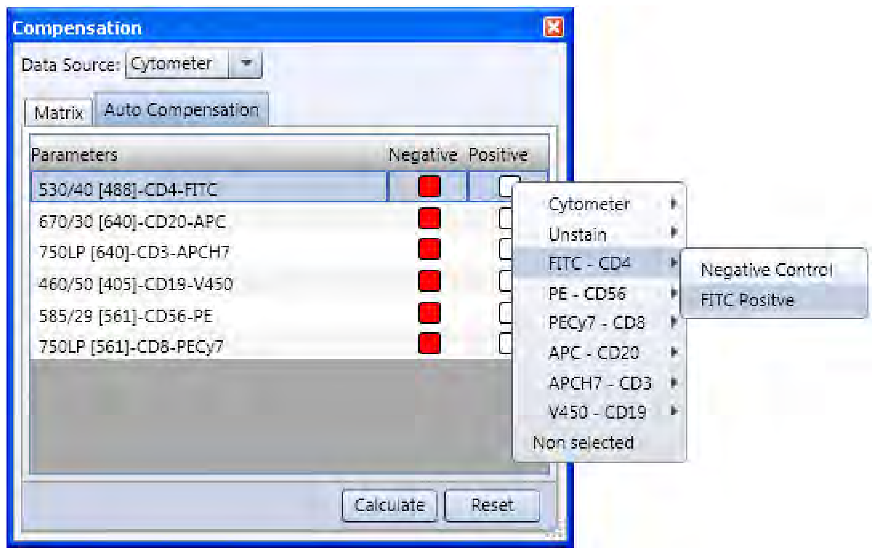
Selecting populations for the positive compensation control

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 uploads it into the Influx 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.

More information

- Optimizing with compensation controls (page 268)
- Defining populations for compensation (page 273)
- Saving a compensation matrix (page 279)
- Restoring (importing) a compensation matrix (page 280)

Saving a compensation matrix

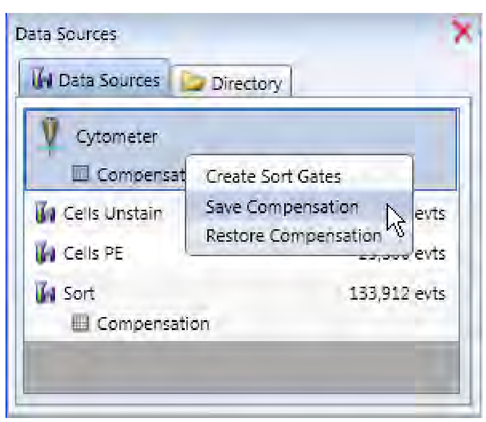
Introduction

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 268)
- Performing auto compensation (page 275)
- Restoring (importing) a compensation matrix (page 280)

Restoring (importing) a compensation matrix

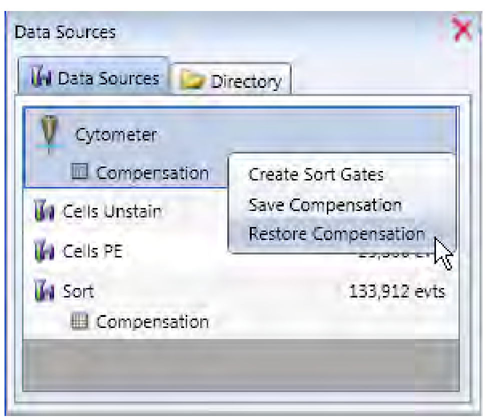
Introduction

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 275)
- Saving a compensation matrix (page 279)

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20

Sorting

This section includes these topics:

Sort setup workflow

Introduction

This topic describes the key workflow steps for setting up and performing a sort.

Before you begin

- Perform QC.
- Optimize system settings for your samples.

Workflow

Stage	Description
1	Optimizing the droplet breakoff (page 285)
2	Optimizing the side streams (page 289)
3	Estimating the drop delay (page 295)
4	Determining an accurate drop delay (page 300)
5	Monitoring the sort (page 311)

More information

- Creating plots and gates for optimization (page 264)
- Selecting a pre-defined sort mode (page 287)
- Aligning the side streams with the sort tubes (page 292)
- Saving and restoring sort layouts (page 310)
- Working with sort reports (page 312)
- Index sorting (page 314)
- Fluidics pressure and drop frequency settings (page 343)

Optimizing the droplet breakoff

Introduction

This topic describes how to optimize the droplet breakoff.

Droplet formation and breakoff are achieved by applying piezoelectric element to focus an acoustic wave into the stream at the nozzle tip.

Procedure

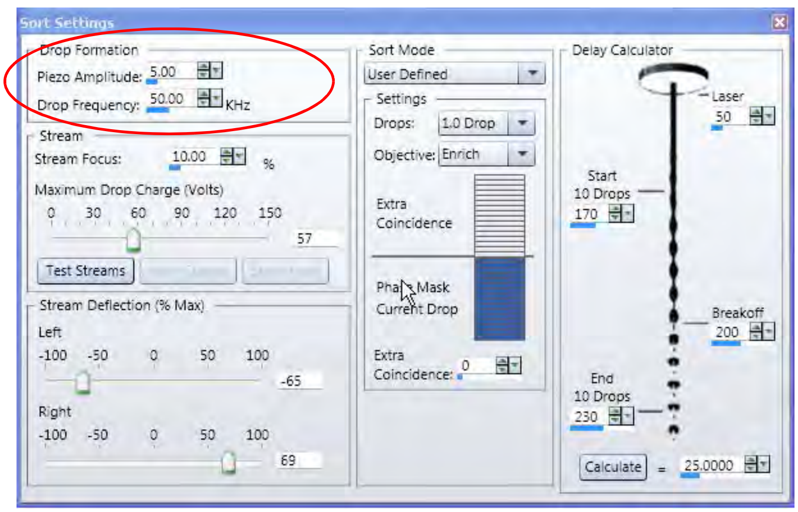
To optimize the droplet breakoff:

1. Click **Sort Settings** on the **BD FACS Sortware sorter software** toolbar.

   The **Sort Settings** dialog opens.

2. Under **Drop Formation**, enter values in the **Piezo Amplitude** and **Drop Frequency** fields using the mouse wheel, data slider, or keyboard keys.

   See Fluidics pressure and drop frequency settings (page 343) for starting values for various nozzle sizes and sheath pressure combinations.



3. For optimal breakoff stability, adjust the piezo amplitude to a wavelength equal to approximately 4.5 stream diameters (approximately 0.88 times the nozzle size).

   Find the optimal setting for each nozzle and sheath pressure combination by adjusting the piezo amplitude and drop frequency. Typically, the breakoff in drops will be approximately half the frequency in kHz.

   - If forward scatter CVs and noise increase, the amplitude might be too high.
   - If the side streams spray without particles running, the amplitude might be too low.
   - If the side streams do not spray without particles running and start to spray when the particles are running, the particle might be too large for the nozzle size.

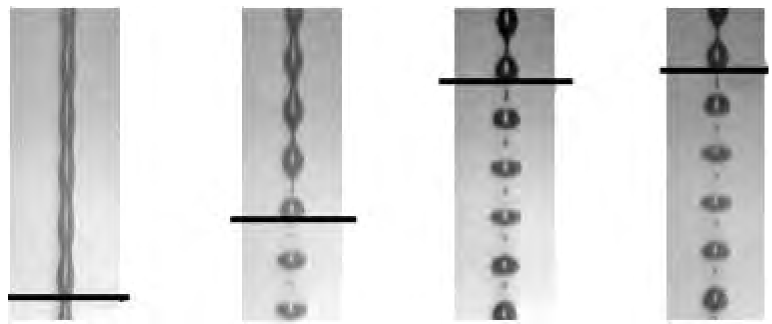
Warning! Do not touch the nozzle when the piezo amplitude is on. If you have a clog, turn off the piezo amplitude before removing the nozzle.

4. Adjust the drop camera micrometer so that you can see the breakoff in the camera image.

   The drop camera micrometer is the silver knob located behind the forward scatter detector stage.

5. In the **Drop Frequency** field, adjust the value to find the optimal breakoff.

   As you adjust the drop frequency, adjust the camera position as needed to view the breakoff. The optimal frequency has the highest breakoff while maintaining the proper drop spacing.



No breakoff | Initial breakoff | Adjusted breakoff | Highest breakoff and optimal frequency

Next step

- Optimizing the side streams (page 289)

More information

- Sort setup workflow (page 284)
- Fluidics pressure and drop frequency settings (page 343)

Selecting a pre-defined sort mode

Introduction

This topic describes how to select a pre-defined sort mode.

You can select sort modes in the **Sort Settings** pane in the **Sort Layout** pane.

Procedure

To select a sort mode:

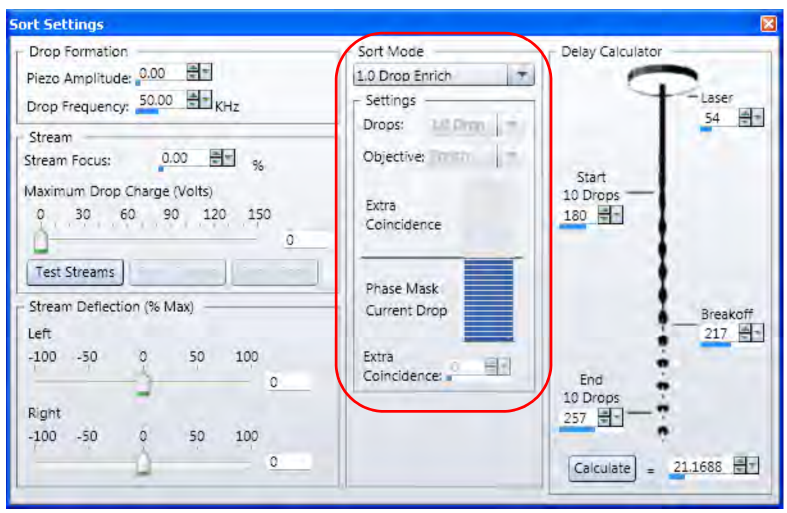
1. Click **Sort Settings** on the **BD FACS Sortware** toolbar.

   The **Sort Settings** dialog opens.

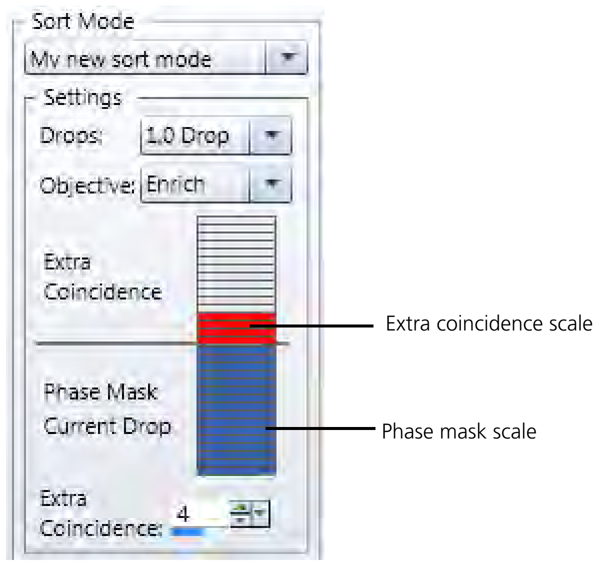
2. Under **Sort Mode**, select a pre-defined sort mode from the menu:

   - 1.0 Enrich, Pure, Single, or Yield
   - 1.5 Enrich, Pure, or Yield
   - 2.0 Enrich, Pure, or Yield

   See Sort modes (page 205) for more information about sort modes and coincidence.



The coincidence scale is a graphical rendering of a drop divided into 16 time slices. Extra coincidence is the number of time slices to add to the default one drop. The **Extra Coincidence** scale automatically displays the pre-defined phase mask and any extra coincidence for the selected mode.



More information

- Using the Sort Settings pane (page 204)
- Sort setup workflow (page 284)
- Optimizing the droplet breakoff (page 285)

Optimizing the side streams

Introduction

This topic describes how to optimize the side stream deflection by flash charging the stream.

Optimizing the side streams ensures that the drop charge is in phase with the drop formation, which provides the highest recovery of cells when sorting.

Before you begin

Warning: Electrical! Do not touch the plates when the voltage is on. The PLATES button illuminates when the voltage is on.

- Power down the deflection plates, clean them with DI water, and dry them completely.
- Make sure that a flask is in place on the sort tray in the Safe position to catch the test stream.
- Make sure that stream breakoff has been optimized.

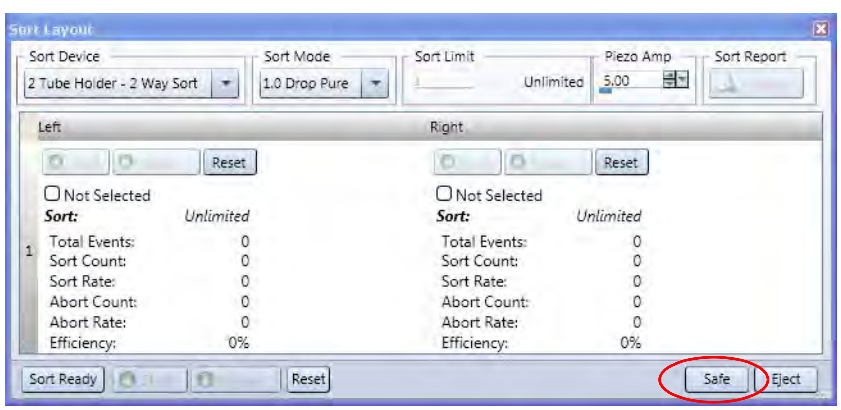
Adjusting the side streams with a flash charge

To adjust the side stream deflection:

1. Verify that the deflector plates voltage is turned off.
2. Close the deflections plates (by hand).
3. Press the PLATES button to turn on the high-voltage plates.

   The PLATES button illuminates when the voltage is on.
4. Click **Sort Layout** on the **BD FACS Sortware sorter software** toolbar.

   The **Sort Layout** dialog opens.
5. Place a flask on the sort tray and make sure it is centered.
6. Click **Safe** to move the sort tray so that the flask is centered with the stream.



7. Under **Sort Device,** select your sort device (**2 Tube Holder - 2 Way Sort** in this example).

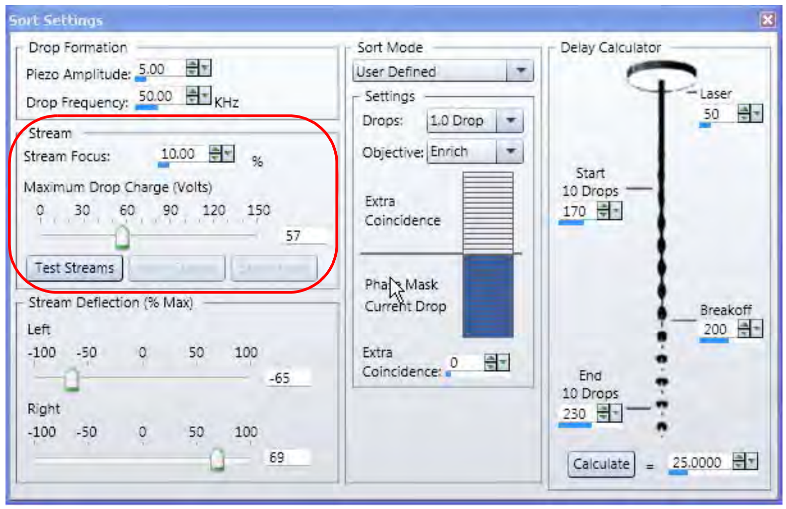
   The left and right sort streams appear in the **Sort Layout** pane.

8. Click **Sort Settings** on the **BD FACS Sortware sorter software** toolbar.

   The **Sort Settings** pane opens.

9. Under **Stream**, click **Test Streams** to charge the droplets and charge the side sort stream.

   Sort streams should now be visible in the sort stream monitor.

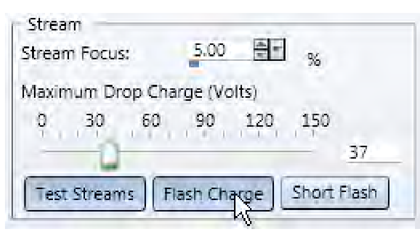


10. If the streams are fanning or not visible, adjust the piezo amplitude.

11. Under **Stream**, adjust the **Maximum Drop Charge** (**Volts**) so that the sort streams flow past the drain.

    - If the sort streams hit the plates, the gain is too high.
    - If the sort streams do not flow past the drain, the gain is too low.
    - If the side streams are not visible, make sure that they are not hitting the deflection plates by reducing the **Maximum Drop Charge** (**Volts**) value.

12. Click **Flash Charge** to test the droplet charge.

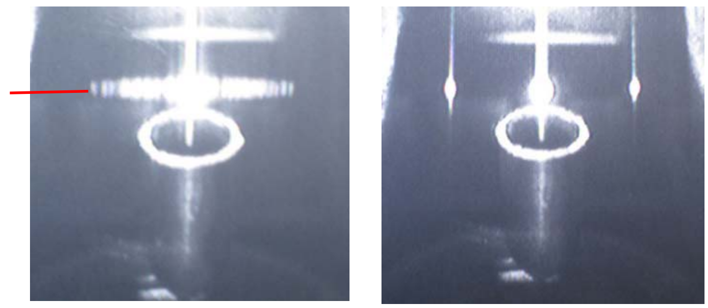


    If the charge is not in phase with the droplets, the side streams are not optimally deflected.

13. Under **Drop Formation**, in the **Piezo Amplitude** field, adjust the amplitude until the side streams are deflected.

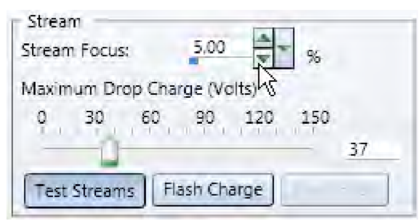
Before amplitude adjustment After amplitude adjustment

Fanning of main stream



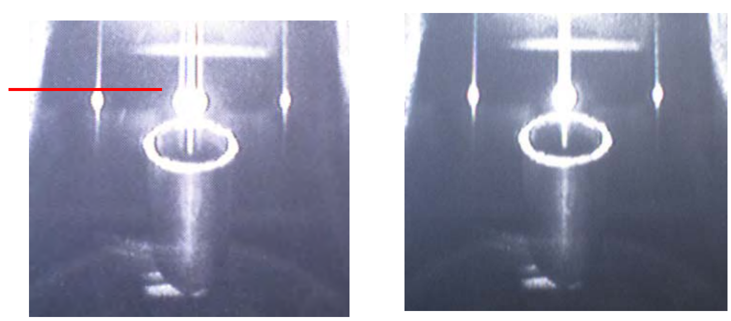
14. Under **Stream**, click **Flash Charge** again to turn it off.

15. Under **Stream**, adjust the **Stream Focus** value to minimize the center stream fanning.



Before stream focus adjustment After stream focus adjustment

Residual charge on drops



16. Click **Test Streams** to turn off the side stream deflection.

17. Use the BD Influx drop monitor to visually verify that the piezo amplitude can be increased or decreased without drastic change to the breakoff.

18. View and note how the drops are connected and note the size of the small detached droplet after the last connected drop.

    The droplet shape must remain constant while sorting. You might need to adjust the piezo amplitude while sorting to keep the shape of the drop at the breakoff constant.

Next step

- Estimating the drop delay (page 295)

More information

- Sort setup workflow (page 284)
- Selecting a pre-defined sort mode (page 287)

Aligning the side streams with the sort tubes

Introduction

This topic describes how to align the side streams with the sort tubes.

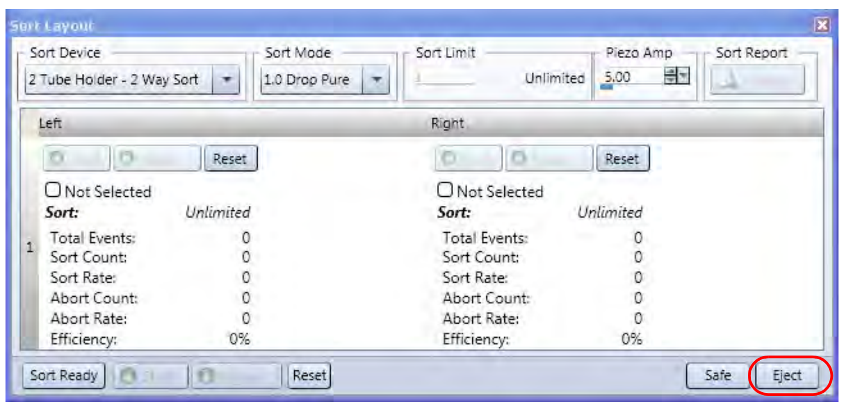
Procedure

To align the side streams with the sort tubes:

1. Click **Sort Layout** on the **BD FACS Sortware sorter software** toolbar.

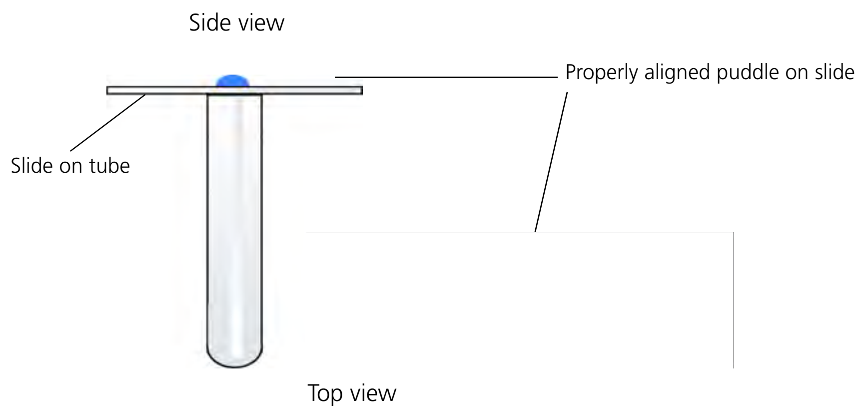
   The **Sort Layout** pane opens.

2. Click **Eject** to move the sort tray into the loading position.



3. Load the tube or sort device you are planning to sort with.
4. Under **Sort Device,** select the corresponding sort device.
5. Place the appropriate number of tubes in the desired positions in the sort device.
6. Place a microscope slide over the tubes.
7. Click **Sort Ready** to move the sort tray to the sort ready position.
8. Click **Test Streams** (two times quickly) to deposit a small puddle over the tubes.

9. Click **Eject** to access the sort tray and verify that the drops are aligned with the center of the tubes.

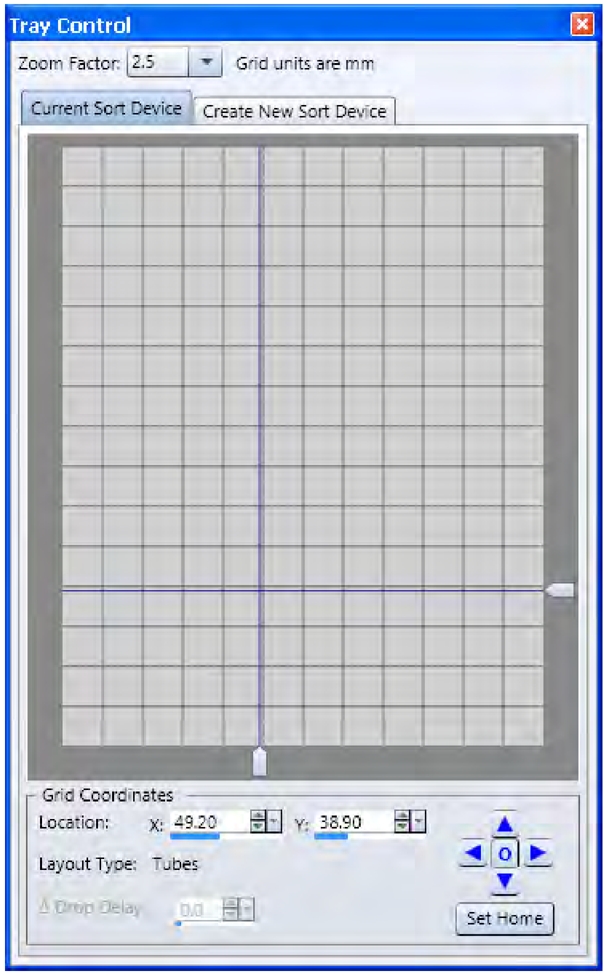


10. If the puddles are not aligned over the tubes, adjust the deflection gain and repeat steps 2 through 9 until the side streams are aligned with the sort tubes.

11. Adjust your tray control position if needed.

    - If the streams are hitting too far forward or backward, open the tray control pane and adjust the Y position to align the sort device with the streams.

- Click **Set Home** to save the X and Y coordinates for the chosen sort device.



More information

- Sorting (page 283)
- Sorted sample collection (page 59)
- Sort setup workflow (page 284)
- Optimizing the side streams (page 289)

Estimating the drop delay

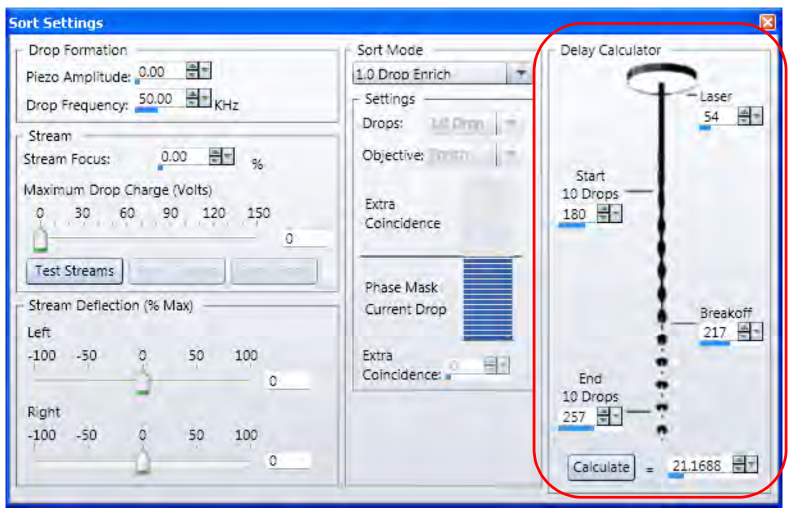
Introduction

This topic describes the delay calculator and the drop camera and provides a procedure for estimating the drop delay. The drop delay is usually about half the value of the drop frequency.

Use the delay calculator to approximate the delay and set the course adjustments for the starting position of the laser, where the drop starts, where the drop breaks from the stream, and where the drop ends. After determining the approximate drop delay using the camera, you can use BD FACS Accudrop or sort onto slides to determine the drop delay more accurately.

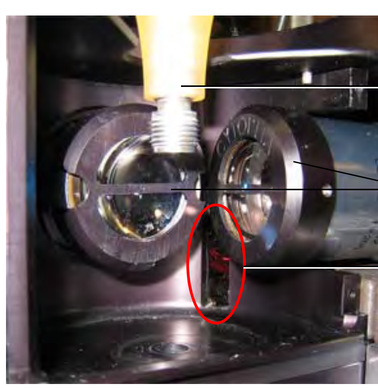
About the delay calculator

Use the delay calculator to approximate the drop delay. You need to enter the drop camera position at each of the four points indicated in the delay calculator to estimate the drop delay.



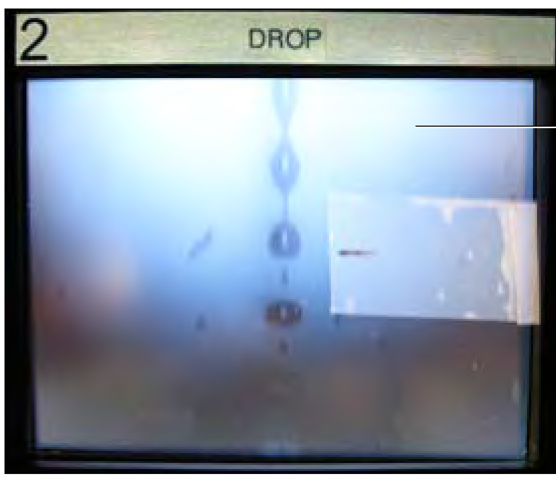
About the drop camera

The drop camera is located behind the back plate and between the two objective lenses.



Use the drop camera micrometer (the silver adjustment knob located behind the nozzle assembly) to adjust the vertical position of the drop camera in relationship to the stream. The resulting position value is displayed in the drop position display on the pressure console.

You can view the drop camera live image on the drop monitor (the middle LCD monitor on the sort monitoring rack).



Before you begin

Optimize the droplet breakoff and frequency.

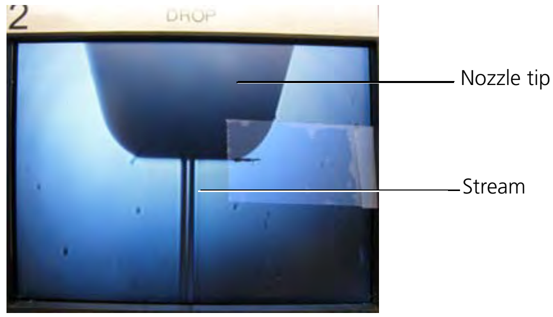
Finding the location of the first laser

To find the location of the first laser:

1. Open the nozzle chamber door.
2. Adjust the micrometer for the camera to move it up to the top of the stream.

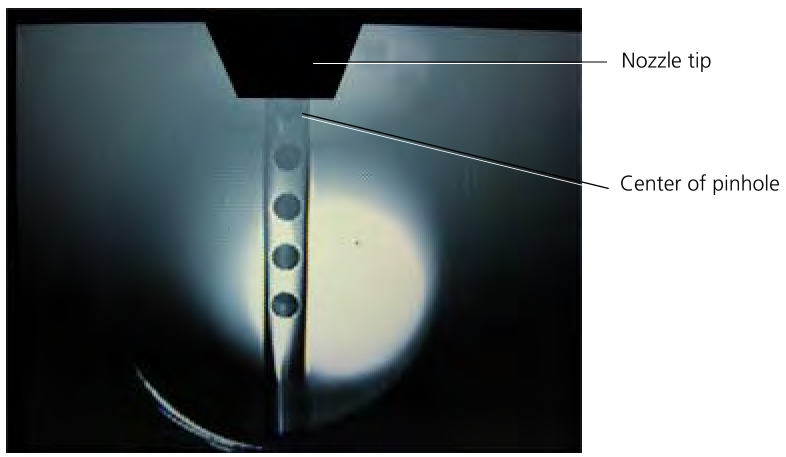
   The camera is at the top when you see the nozzle tip in the drop monitor.

3. Mark the bottom of the nozzle with a piece of tape as shown in the following figure.



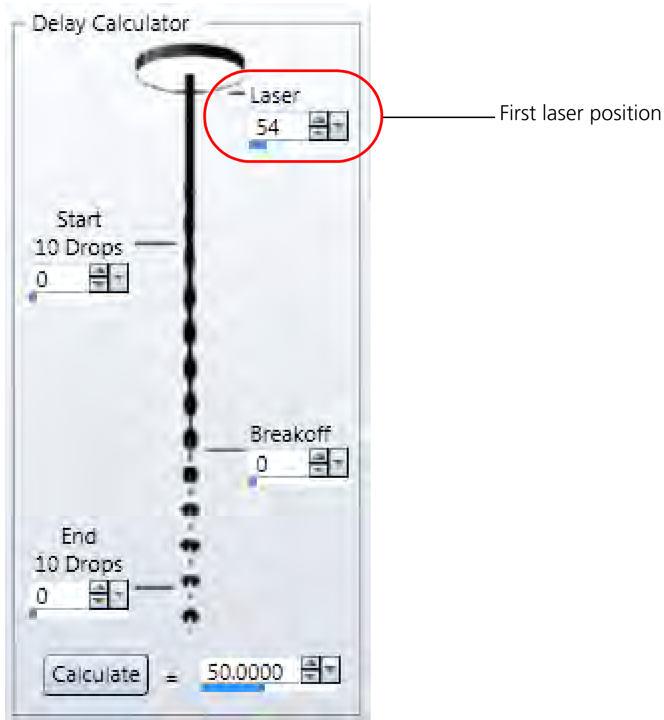
4. Adjust the silver vertical adjustment knob to move the nozzle stage down to the first pinhole.

   The nozzle is visible as a dark shadow in the pinhole monitor.



5. Enter the drop camera position in the **Laser** field.

This is the position of your first laser or first pinhole.



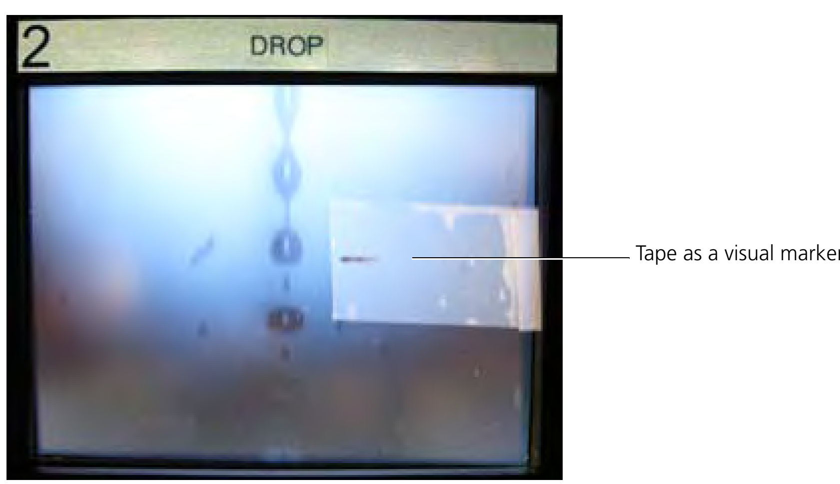
This number is constant for each instrument. Save this number for future use.

6. Move the nozzle back to its original position just out of the view of the pinhole monitor.
7. Close the nozzle chamber door, and reset the laser safety interlock by placing your finger on the reset sensor.

Locating the breakoff position

To locate the breakoff position:

1. Adjust the camera micrometer so that the breakoff is in view on the drop camera monitor.
2. On the drop monitor, use a piece of tape to create a visual marker that indicates the position of the breakoff.



3. Under **Delay Calculator**, enter the drop camera position in the **Breakoff** field.

   This converts the distance from the laser to the breakoff into the number of drops.

Finding the Start 10 Drops position

To find the Start 10 Drops position:

1. Move the drop camera micrometer up five drops.
2. Under **Delay Calculator**, enter the drop camera position in the **Start 10 Drops** field.

Determining the End 10 Drops position

To determine the End 10 drops position:

1. Adjust the micrometer for the camera to move the camera down, counting ten drops from the Start 10 Drops position in the drop monitor.
2. Position the drop camera so that the marker on your screen is at the end of ten drops, halfway between drops.
3. Under **Delay Calculator**, enter the drop camera position in the **End 10 Drops** field.

   This measures 10 drops in camera units.

4. Click **Calculate** to get the estimated drop delay.

   The calculator converts the number of drops into time using the frequency (drops/second). The estimated drop delay appears and is automatically set.

Next step

- Determining an accurate drop delay (page 300)

More information

- Sort setup workflow (page 284)
- Aligning the side streams with the sort tubes (page 292)

Determining an accurate drop delay

Introduction

This topic list the different options for determining a more accurate drop delay.

After you approximate the drop delay using the drop camera and the Drop Calculator, you can use BD FACS Accudrop or sort onto slides to determine the drop delay more accurately.

Drop delay workflow options

You can perform any of the following drop delay workflow options.

- Determining the drop delay with BD FACS Accudrop (page 300)
- Determining the drop delay using the calibration slides (page 304)
- Determining the drop delay using the three-puddle calibration slide (page 307)

More information

- Sort setup workflow (page 284)
- Aligning the side streams with the sort tubes (page 292)
- Estimating the drop delay (page 295)

Determining the drop delay with BD FACS Accudrop

Introduction

This topic describes how to determine a drop delay with BD FACS Accudrop.

BD FACS Accudrop technology is a method for determining precise drop delay on the BD Influx cell sorter.

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 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

- Determine the course drop delay value using the drop camera.
- In a tube, mix approximately one drop of BD FACS Accudrop beads (BD Part No. 345249) in 0.5 mL of filtered phosphate buffered saline (PBS) or sheath fluid.
- Use the delay calculator to estimate a starting point for Accudrop.

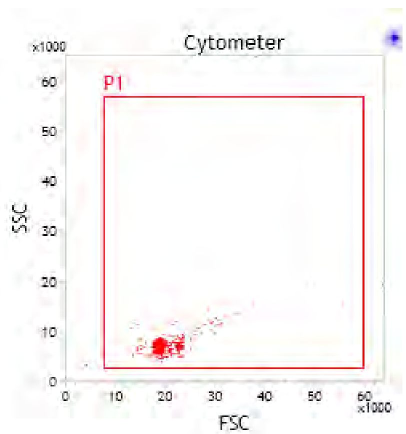
  See Estimating the drop delay (page 295) for more information

Determining the drop delay

To determine the drop delay:

1. Load the tube of Accudrop beads onto the cytometer.
2. Adjust the sample offset knob to achieve an event rate between 1,000 and 3,000.

   You might need to further dilute the beads to reduce coincidence abort rates when setting up the system at lower pressures or when using a large nozzle tip size. Larger nozzles and/or lower pressure produces larger drops, so with the same event rates there is higher chance of two cells ending up in one drop and, therefore, being aborted.
3. In the worksheet, create a large gate encompassing all events except debris of an FSC vs SSC plot.

   This gate should include all bead populations (including aggregates).

Selecting the sort mode

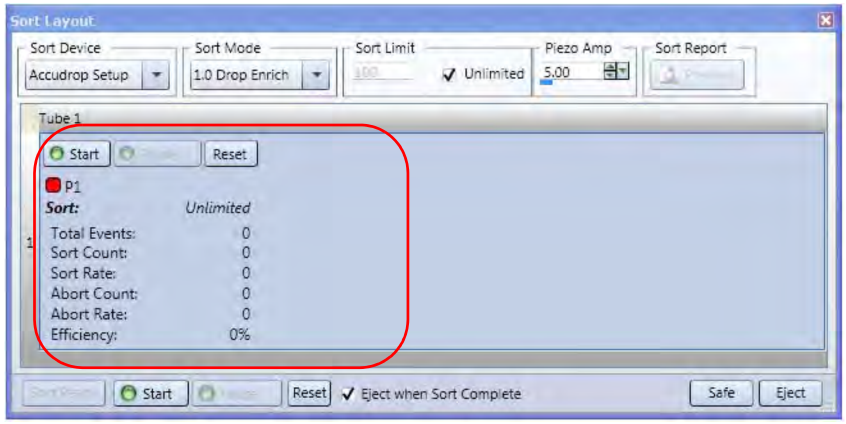
To select the sort mode:

1. Click **Sort Layout** on the **BD FACS Software sorter software** 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. Right-click the sort target and select a population (for example, *P1)*.

Alternatively, drag a population from a gate hierarchy or population hierarchy.

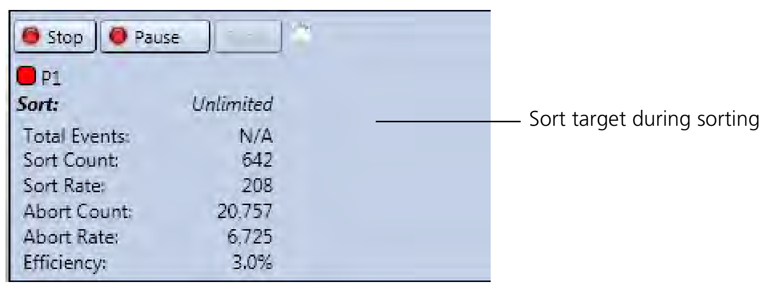


Starting the sort

To start the sort:

1. Click **Sort Ready**.
2. In the **Sort Layout** pane, click **Start**.

   You can monitor the sort data in the sort target.



   With the Accudrop filter in place, you can view the stream using the sort stream monitor.

Setting up the Accudrop filter

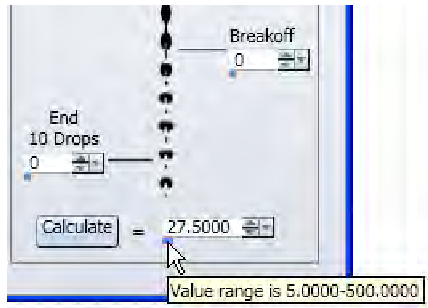
To set up the Accudrop filter:

1. On the Accudrop assembly, turn the Accudrop filter selection knob to the Accudrop optical filter position.
2. Ensure that the laser safety cover is closed.

Determining the drop delay

To determine the drop delay:

1. Locate the drop delay field in the **Sort Settings** dialog, under **Delay Calculator**, next to the **Calculate** button.

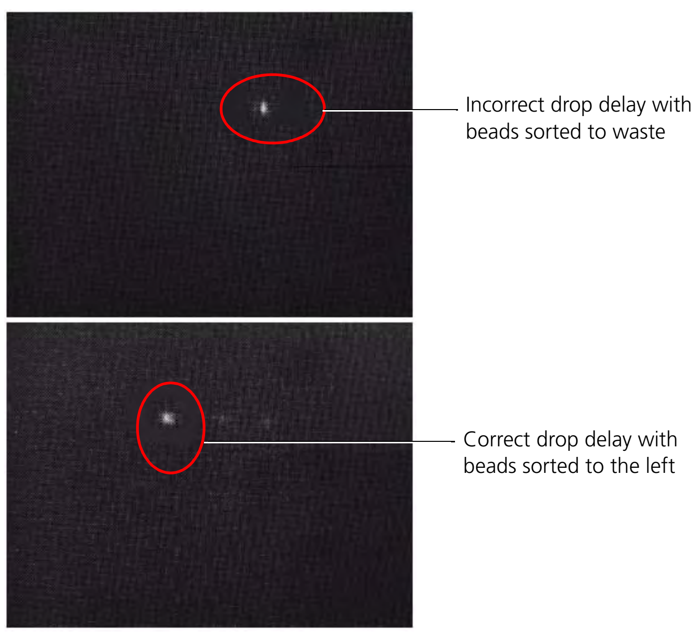


2. Notice the brightness of the beads being sorted to the left and to the center (waste bucket) in the drop camera.
3. Adjust the drop delay value while monitoring the center and left sort streams.

   The correct drop delay is set when center stream is dark (or not present) and the left stream is bright.

   The following figure illustrates 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 311)

More information

- Aligning and optimizing the optics workflow (page 242)
- Sort setup workflow (page 284)

Determining the drop delay using the calibration slides

Introduction

This topic describes how to test the estimated drop delay value to determine the precise drop delay by distributing multiple drops onto a coarse calibration microscope slide.

A precise drop delay setting gives you the best sort results. There are multiple ways to sort onto a slide to determine the delay. This section describes the calibration slide method.

Required materials

- Fluorescent beads (larger beads are easier to see and count on the slide)
- Slides
- A fluorescence microscope

Before you begin

Prepare a tube of fluorescent beads.

Preparing a calibration slide

To prepare a calibration slide:

1. Run a tube of fluorescent beads.
2. Create an FSC vs FL1 plot.
3. Draw a gate around the main bead population in the plot.
4. Adjust the Sample Offset knob to achieve an event rate between 100 and 1,000 beads per second.
5. Click **Sort Layout** on the **BD FACS Sortware sorter software** toolbar.

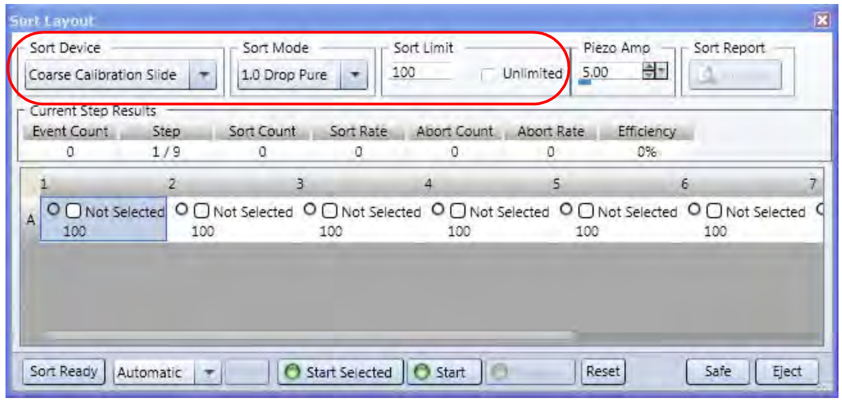
   The **Sort Layout** pane opens.
6. Click **Eject** to access the sort tray.
7. Place a microscope slide in the front position of the sort tray insert.

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 and reflect pre-defined values for the coarse calibration slide.



2. Select all wells and select the population to sort. For example, *P1*.

   This is the population you created for the main bead population.

A1 sort target with the P1 population selected.

3. Ensure that the left side stream is aligned with the A1 location of the slide.

   a. Click **Sort Ready.**

   b. Click **Start**, then click **Stop** quickly to deposit a single drop onto the slide.

   c. If the test drop is not aligning with the A1 location of the slide, adjust the sort tray position settings.

   See Controlling the sort tray position (page 219) for more information.

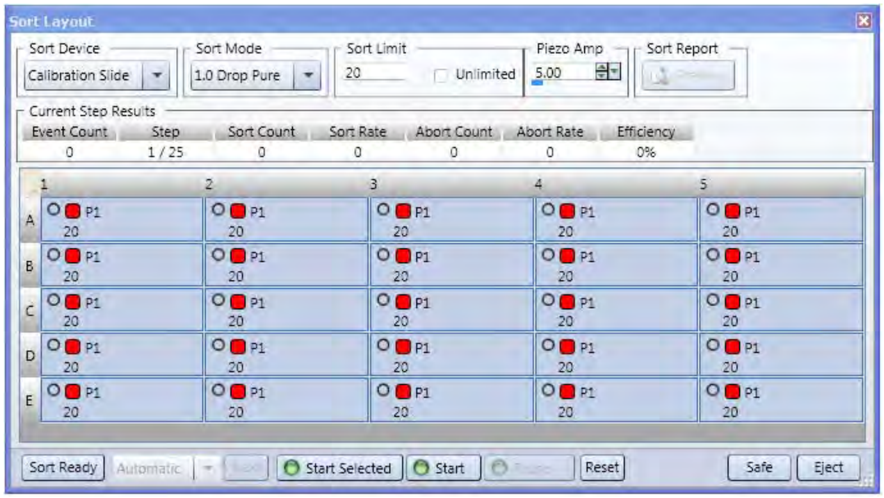
4. Click **Sort Ready.**

5. 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.

6. Using a fluorescence microscope, count the number of beads in each puddle and identify which well contains the most events. Optimal delay is the drop delay in the well containing the most events.

7. In the **Sort Drop Delay** dialog, click the drop delay setting that corresponds to the drop with the highest bead counts.
8. Click **OK** to set the drop delay.
9. In the **Sort Layout** pane, select **Sort Device > Coarse Calibration Slide**, and repeat steps 1 through 8.



Next step

- Monitoring the sort (page 311)

More information

- Saving and deleting settings (page 115)
- Sort setup workflow (page 284)
- Determining an accurate drop delay (page 300)

Determining the drop delay using the three-puddle calibration slide

Introduction

This topic describes how to determine the drop delay using the optional three-puddle calibration slide. This procedure provides an alternate method for determining the precise drop delay.

In this method, rather than counting the events, the perceived density of events in the puddles is used to determine the delay.

Before you begin

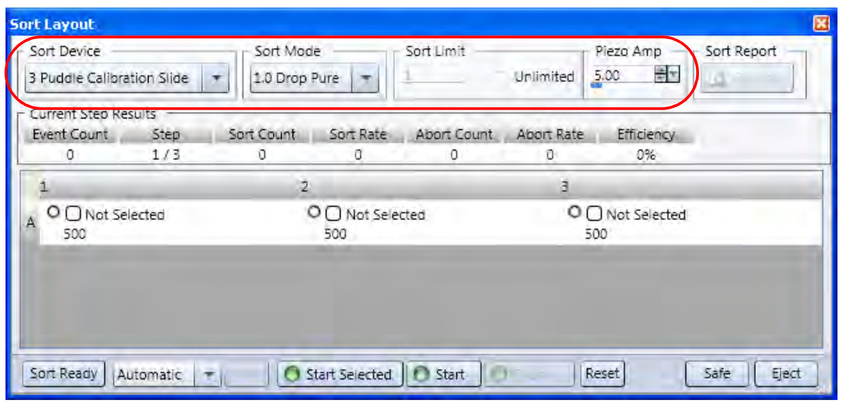
Prepare the slide as described in Preparing a calibration slide (page 304).

Procedure

To determine a precise drop delay using the three-puddle method:

1. In the **Sort Layout** pane, under **Sort Device**, select **3 Puddle Calibration Slide**.

   The **Sort Mode** and **Sort Limit** settings update automatically and reflect pre-defined values for the three-puddle calibration slide.



2. Select all wells and select the population to sort. For example, *P1*.

   This is the population you created for the main bead population.

A1 sort target with the P1 population selected

3. Ensure that the left side stream is aligned with the A1 location of the slide.

   a. Click **Sort Ready.**

b. Click **Start**, then click **Stop** (quickly) to deposit a single drop onto the slide.

c. If the test drop is not aligning with the A1 location of the slide, adjust the sort tray position settings.

See Controlling the sort tray position (page 219) for more information.

4. Click **Sort Ready**.

5. 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.

6. Using a fluorescence microscope, view the slide with the three sorted puddles to visually determine which puddle contains the most events.

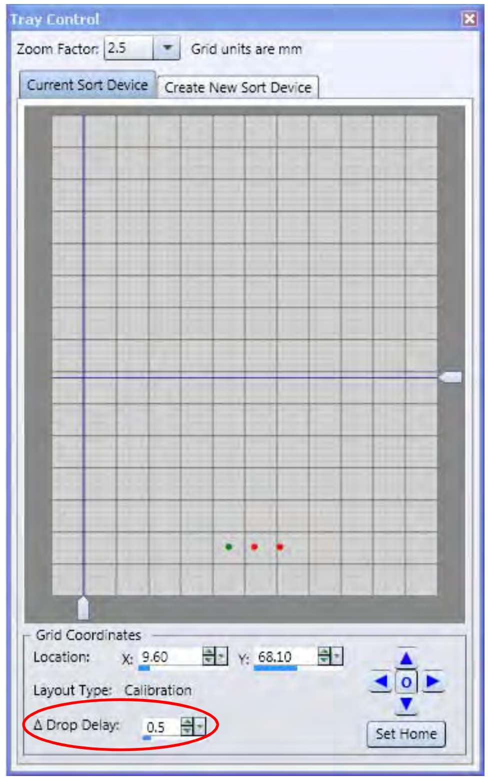
7. In the **Sort Drop Delay** dialog, click the drop delay setting that corresponds to the puddle with the highest bead counts.

8. Click **OK** to set the drop delay.

9. Repeat steps 1 through 8 until the first and third puddles contain an even amount of events.

10. If the first and third puddles continue to have uneven event density, perform a sort in smaller increments.

11. Open the sort tray and change the delta drop delay value to less than 1.



12. Click **Start** to sort.

13. Repeat steps 5 through 9 until the first and third puddles contain approximately the same amount of events.

Next step

- Monitoring the sort (page 311)

More information

- Determining an accurate drop delay (page 300)

Saving and restoring sort layouts

Introduction

After you have set up your sort, you can save all sort settings in the **Sort Settings**, **Sort Layout**, and **Tray Control** panes.

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 Saving and restoring settings (page 112) 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 this settings file from the **Storage** menu.

   See Creating storage folders (page 117) 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.

3. 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. It is important to monitor the sort to ensure that the breakoff does not change. It is also important to maintain the same scatter and population percentages during the sort.

This section provides useful 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 mm (about half of the nozzle tip size) to prevent nozzle clogs and ensure optimal sorting.
- Load a sample tube.
- Record data to create pre-sort FSC files.

Monitoring the sort

To monitor the sort:

1. View the scatter plot in the worksheet and slowly adjust the flow rate.

   Stop increasing the sample flow rate 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
Efficiency counter	Efficiency varies depending on population percentages and sample concentration.	Decrease the flow rate to improve sorting efficiency.
Gates and populations	Populations should stay within the gates and should not move during the sort.	Decrease the flow rate or adjust the gates. Refresh the plot display rate often to confirm that populations have not moved out of the gate.
Drop monitor	Breakoff should be stable and remain at the same drop.	Adjust the piezo amplitude to keep the breakoff at the same drop.
Stream monitor	Side streams should not fan or spray out of the drain.	• Verify that the nozzle is large enough for the cells being sorted. • Adjust the piezo amplitude slightly. • Filter the cells before sorting.

Saving a sort

To save a sort if a clog appears during a sort:

1. Quickly turn off the deflection plates, stop the sample flow, and stop the stream.

2. If you are working with nonhazardous materials, remove the collection device to avoid contamination with unwanted cells.

More information

- Sort setup workflow (page 284)
- Working with sort reports (page 312)

Working with sort reports

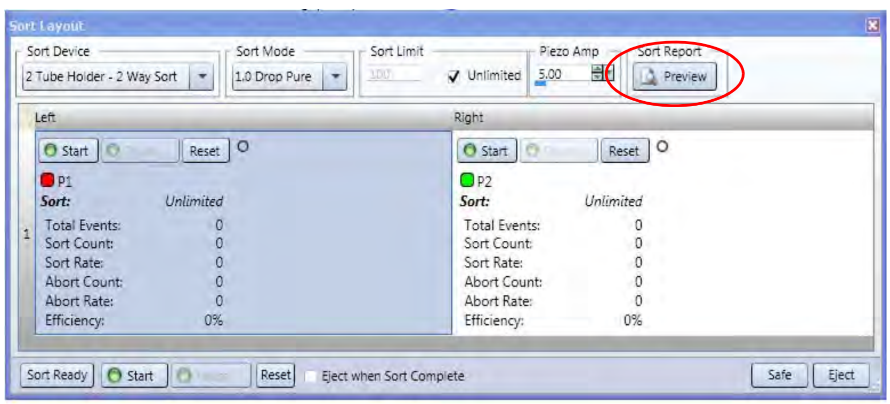
Introduction

This topic describes how to preview and print sort reports after a sort completes and how to save them as PDF files.

Viewing a report

To view a sort report:

1. Under **Sort Report**, click **Preview**.



The **Report Dialog** opens.

Sort Report

System

Sort Start:	2/17/2011 5:03:50 PM	Server:	Utopex
Application:	BD FACS™ Sortware	Build:	1.0.0.593
Version:	1.0.0.594	Cytometer Model:	inFlux v7 Sorter
ValComp:	1.4	Cytometer Serial #:	x5000002

Details

Data Source:	Cytometer	Sort Mode:	User Defined
Nozzle Diameter (μm):	86.00	Drop Envelope:	1.0 Drop
Sheath Pressure (PSI):	30.00	Sort Objective:	Enrich
Sort Device:	4 Tube Holder - 4 Way Sort	Phase Mask:	16/16
Piezo Amplitude:	4.50	Extra Coincidence Bits:	0
Drop Delay:	25.0007	Drop Frequency (kHz):	48.30

Sort Details

Name	Population	Event Limit	Event Count	Sort Count	Sort Rate	Aborts	Abort Rate	Time (sec)
Far Left	P1	10,000	31,622	10,000	163	85	1	61
Left	P2	10,000	59,764	10,000	82	57	0	121
Right	P3	10,000	32,708	10,000	156	89	1	64
Far Right	P4	10,000	62,548	4,165	33	44	0	125

Printing a sort report

To print a sort report:

1. View a sort report.
2. In the **Report Dialog**, click the **Print** button on the toolbar.
3. Complete your typical printing procedure.

Creating a PDF version of a sort report

To create a PDF sort report:

1. View a sort report.
2. In the **Report Dialog**, click the **PDF** button on the toolbar.

   The **Select PDF Output File** dialog opens.
3. In the **Save in** field, select a folder.
4. In the **File name** field, type a new name for the report.
5. Click **Save**.

More information

- Sort setup workflow (page 284)
- Monitoring the sort (page 311)

Index sorting

Introduction

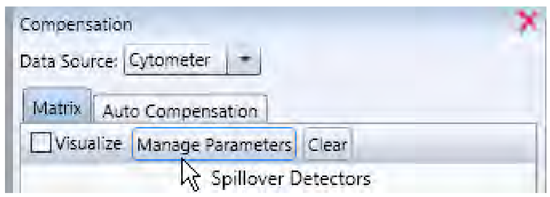
This topic describes index sorting.

Index sorting places cells from sort regions into wells on a plate or on a slide. You can use this feature to ensure that a sorted cell with a specific phenotype has been sorted. Index sorting is useful in characterizing subpopulations of phenotypically similar events using post-sort genetic, chemical, and/or metabolic applications.

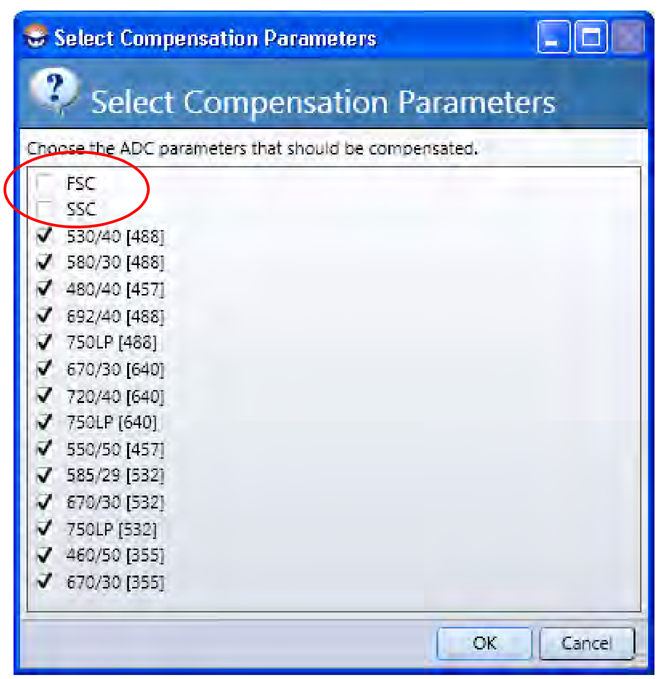
Index sorting onto a plate or slide

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.



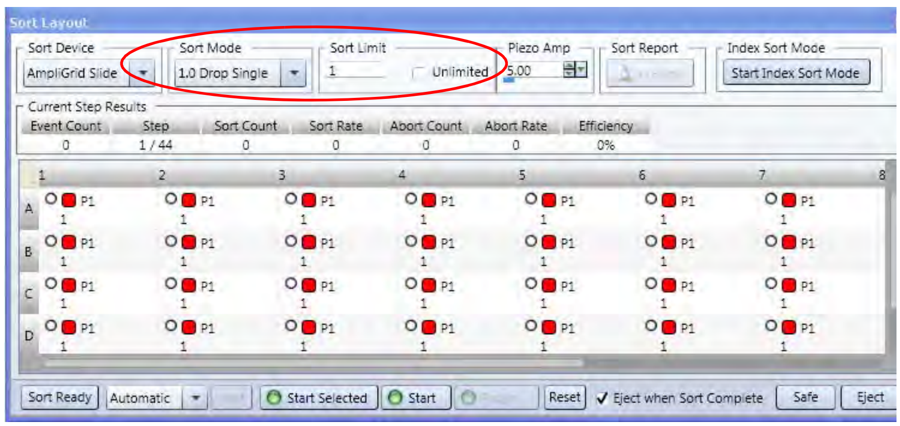
2. On the **BD FACS Sortware sorter software** toolbar, click **Sort Layout**.

3. In the **Sort Layout** dialog, select a multi-position sort device:

   - Default multi-position formats including: 6 Well Tray, 24 Well Tray, 96 Well Tray, 384 Well Tray, and Ampligrid Slide.
   - Custom multi-position formats.

4. Under **Sort Mode,** select *1.0 Drop Single Sort* mode.
5. Under **Sort Limit,** set the sort limit to *1*.
6. Prepare the sort chamber for sorting by cleaning the deposition area and installing a sort device onto the sort tray.
7. Align the side streams to the sort device using the **Sort Settings** dialog and the **Tray Control** pane.
8. Verify that the software is not currently acquiring data.
9. Install the sample tube onto the sample station and press the SAMPLE button.
10. (Optional) Run some sample and view the plots to ensure that the voltages, compensation, sort gates, and event rate are appropriate.
11. In the **Sort Layout** dialog, assign populations to each well sort target.

    See Index sorting onto a plate or slide (page 314) for more information.



12. Add a statistics view that has the medians of the parameters of interest.

Starting an index sort

To start an index sort:

1. Make sure the software is not currently acquiring data.

   Acquisition must be stopped so that Index Sort Mode acquisition can start.
2. In the **Sort Layout** pane, click **Sort Ready.**

3. In the **Sort Layout** pane, click **Start Index Sort Mode**.

Data is continually recorded and displayed while index sort mode is running.

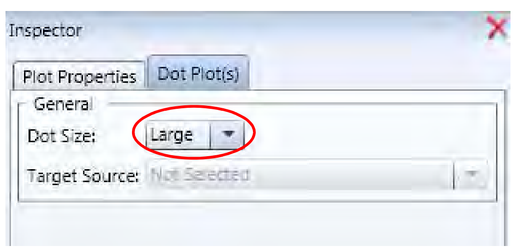
Index sort mode creates a CSV file where all the sort deposition information and tray position information is stored on an event-by-event basis. All the events are stored in the file, including those not actually sorted. You can import this CSV file into a spreadsheet to review the results.

Once the sort is complete, you must manually end index sort mode.

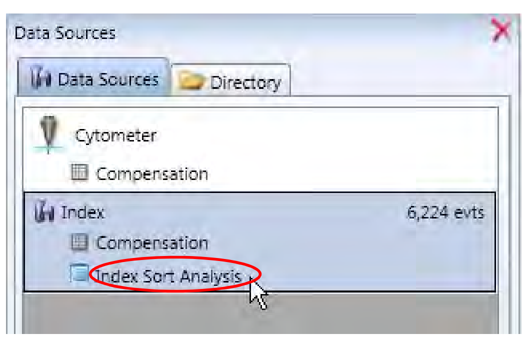
Analyzing the index sort

To analyze the index sort:

1. In the **Inspector** for plots, select *Large* from the **Dot Size** menu.

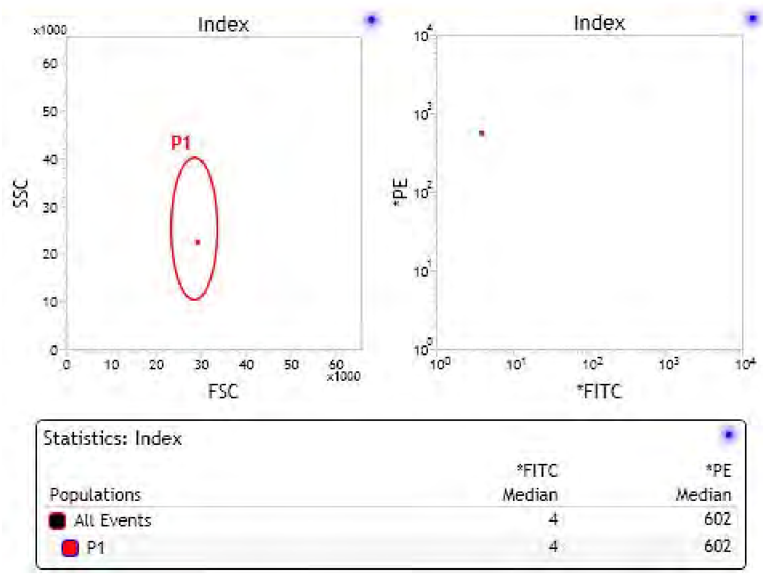


2. In the **Data Sources** pane, double-click the **Index Sort Analysis** for the index sort data source of interest.



3. In the **Index Sort Analysis and Report** dialog, select one or more locations to display on plot(s).

To select multiple locations, wait for the crosshairs to appear, then drag your mouse to include 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. 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 appears.

2. Navigate to the folder where you want to save index sort reports, then select the CSV file type.

3. Click **Save**.

4. 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 an AmpliGrid 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

Positive sorts are typically indicated by numbers of 100 or higher. Index sorting may occasionally report additional sort events in a well even though the correct number was sorted.

More information

- Sort setup workflow (page 284)

21

System shutdown

This section 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 224) for more information.

About wet shutdown

Wet shutdown is useful when you plan to use the BD Influx 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

Stage	Description
1	Cleaning the sample line (page 321)
2	Installing the debubble reservoir (page 322)
3	Turning off the power (page 326)

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 321)
2	Rinsing the system (page 323)
3	Drying the fluidics lines (page 324)
4	Cleaning the sort chamber (page 325)
5	Turning off the power (page 326)

More information

- Instrument cleaning and maintenance (page 71)
- Startup workflow (page 224)

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
- Deionized (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 the following steps for the wet or dry shutdown workflow:

Shutdown workflow	Next steps
Wet shutdown	• Leave the tube of DI water on the sample holder. • Continue with Installing the debubble reservoir (page 322)
Dry shutdown	• Remove the tube from the sample holder. • Continue with Rinsing the system (page 323)

Next step

- Installing the debubble reservoir (page 322)

More information

- System shutdown workflow (page 320)

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. Fill the debubble reservoir with DI water.
2. Place it on top of the flush bucket.
3. Verify that the nozzle tip is submerged in the water in the dubble reservoir.
4. Press RUN to stop running the stream.

Next step

Turning off the power (page 326)

More information

- Pressure regulation and monitoring (page 37)
- Startup and troubleshooting components (page 225)
- System shutdown workflow (page 320)

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 dry shutdown:

1. Press RUN to turn off the stream.
2. Remove the DI water tube.
3. Turn off the air supply by switching the AIR switch on the pressure console off.
4. Empty the sheath tank and fill it with 0.5–1.0 L of DI water.
5. Reattach the sheath tank and turn the air on.
6. Install the flush bucket.
7. Turn on the air supply.
8. Press RINSE and then BACKFLUSH to rinse all fluid lines with DI water until the tank runs dry and the sample line is no longer dripping water.
9. Press RINSE to turn off the flow.

Next step

- Drying the fluidics lines (page 324)

More information

- System shutdown workflow (page 320)

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. Press RINSE, then press BACKFLUSH.
6. Allow air to blow through the system for about 10–15 minutes to completely dry it.
7. Turn off the system AIR switch on the side of the pressure console.
8. Turn off the house air and vacuum supply.
9. 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 325)

More information

- Removing and replacing the nozzle tip (page 81)
- Bypassing the sheath filter (page 75)
- System shutdown workflow (page 320)
- Rinsing the system (page 323)

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. Open and clean the deflection plates with DI water.
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 326)

More information

- Deflection plate power (page 67)
- Cleaning and inspecting the instrument (page 72)
- Bypassing the sheath filter (page 75)
- System shutdown workflow (page 320)

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 Sortware software.
4. Exit Windows and turn off the computer.
5. Turn off the system auxiliary power.
6. Turn off the air supply by turning off the pressure console, if needed.
7. Turn off all lasers.
8. Turn off the main power.
9. Turn off the house vacuum and air supply.
10. Depressurize the sheath tank by opening the release valve.

More information

- Power distribution (page 64)
- Cleaning and inspecting the instrument (page 72)
- System shutdown workflow (page 320)
- Cleaning the sort chamber (page 325)

Part 4

Reference

This part includes these sections:

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22

System reference information

This section includes these topics:

System specifications

Introduction

This topic describes the power and environment requirements, and provides dimensions for the BD Influx HEPA-filtered enclosure, table, and cytocart.

Operating environment and requirements

Item	Requirement
Main voltage (North American models)	No HEPA-filtered enclosure: 115–120 VAC/60 Hz, 8 A
	With HEPA-filtered enclosure: 115–120 VAC/60 Hz, 12 A
Main voltage (European models)	No HEPA-filtered enclosure: 220–240 VAC/50 Hz, 5A
	With HEPA-filtered enclosure: 220–240 VAC/50 Hz, 8 A
Temperature	18–25°C (65–77°F)
Humidity	5–80% non-condensing
Air pressure	90 PSI/6.2 bar/183 in. Hg, Max. 0.2 L/min @ 60 PSI
Room noise	<80 dBA, accumulated noise from all running equipment (without the HEPA enclosure)
Heat output	Maximum output estimated to be 6,200 BTU/h, laser choice dependent
Vacuum	2.5 PSI/0.17 bar/5.09 in. Hg or greater, 50 L/min recommended
Electrical requirements for hood enclosure	One 110 V/15 A circuit

Table dimensions

There are two tables available: the standard table and the extended table.

Item	Dimensions (W x D x H)
Standard table	77 x 77 x 89 cm (30 x 30 x 35 in.)
Extended table	77 x 102 x 89 cm (30 x 40 x 35 in.)

Cytocart dimensions

There are two cytocarts available: the standard cart and the HEPA-filtered cart.

Item	Dimensions (W x D x H)
Standard cytocart	117 x 89 x 82 cm (46 x 35 x 32 in.)
HEPA-filtered cytocart	See HEPA enclosure specifications (page 388) for more information.

More information

- System reference information (page 329)
- Sort performance (page 331)
- Optical detector modules (page 333)
- HEPA enclosure specifications (page 351)

Sort performance

Introduction

This topic describes sort performance characteristics.

Drop drive frequency

Adjustable 9–180 kHz

Purity and yield

At 60 PSI and 100 kHz with an average threshold rate of 25,000 events per second, a four-way sort achieves a purity of 98% and a yield >80% of Poisson's expected yield for all four populations. Higher threshold rates, even rates exceeding the droplet formation rate, can be achieved without affecting purity. However, yield will decrease based on Poisson statistics.

Viability

As shown in published literature, sorts performed using murine and human cells and/or cell lines demonstrated good recovery and viability in several experimental systems. Optimal sort conditions need to be established for different cell types.

Sort collection devices

All collection devices are designed to fit on the Computerized Cell Deposition Unit (CCDU). The CCDU is standard on all instruments.

The following sort collection devices can be used with CCDU:

- **Two-way sorting.** Microtubes, 12 x 75-mm, 15-mL, and 50-mL tubes
- **Three-way sorting.** One 50-mL tube and two 12 x 75-mm tubes
- **Four-way sorting.** Microtubes, 12 x 75-mm tubes
- **Plates and slides.** 6, 24, 48, 96, and 384-well plates; slides; and user-defined collection devices

Nozzles

- 70 µm
- 86 µm
- 100 µm
- 140 µm
- 200 µm (optional)

Sort monitoring

- Live video feed of waste collection and side streams.
- Live video feed of the breakoff point.
- Drop delay determination through a semi-automated sort protocol on microscope slides using the CCDU.

More information

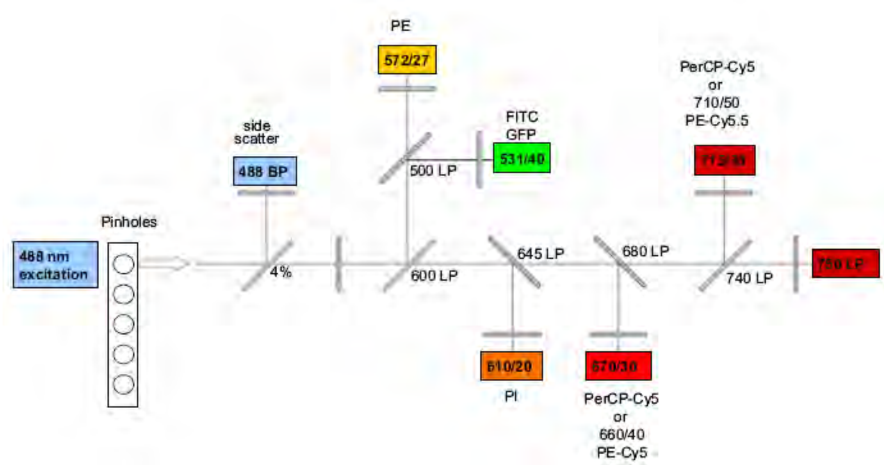
- System reference information (page 329)
- System specifications (page 330)
- Optical detector modules (page 333)

Optical detector modules

Introduction

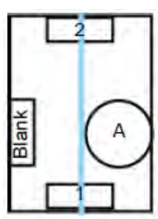
This topic provides reference information for optic placement in the PMT modules.

The following illustration shows an optical bench layout that includes: 488 nm excitation, scatter, and FITC, PE, PI, PE-Cy5, PerCP, and PE-Cy5.5 detectors.

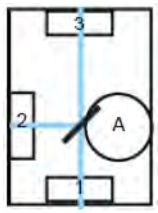


355-nm module (1 PMT)

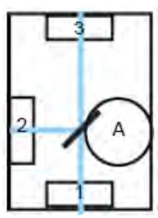
The following tables are grouped by wavelength.

Layout	PMT module	Longpass filter	Bandpass filter
	355-A	1: 400 LP	2: 460/50 BP
	Filters (LP and BP): 1. Lowest wavelength filter 2. Highest wavelength filter **Dichroics:** A: Dichroic empty		

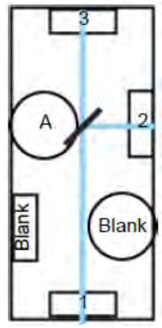
355-nm module (2 PMTs)

<table>
<tr><th>Layout</th><th>PMT module</th><th>Longpass filter</th><th>Bandpass filter</th></tr>
<tr><td rowspan="3">
</td><td>355-A</td><td>1: 400 LP
A (dichroic): 550 LP</td><td>2: 460/50 BP
3: 670/30 BP</td></tr>
<tr><td>355-B</td><td>1: 364 LP
A (dichroic): 440 LP</td><td>2: 406/15 BP
3: 530/40 BP</td></tr>
<tr><td colspan="3">Filters (LP and BP):
1. Lowest wavelength filter
2. Middle wavelength filter
3. Highest wavelength filter
Dichroics:
A: Dichroic</td></tr>
</table>

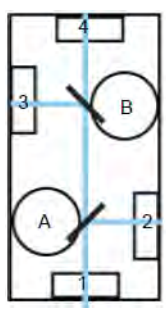
405-nm module (2 PMTs)

<table>
<tr><th>Layout</th><th>PMT module</th><th>Longpass filter</th><th>Bandpass filter</th></tr>
<tr><td rowspan="2">
</td><td>405-A</td><td>1: 420 LP
A (dichroic): 480 LP</td><td>2: 460/50 BP
3: 550/50 BP</td></tr>
<tr><td colspan="3">Filters (LP and BP):
1. Lowest wavelength filter
2. Middle wavelength filter
3. Highest wavelength filter
Dichroics:
A: Dichroic</td></tr>
</table>

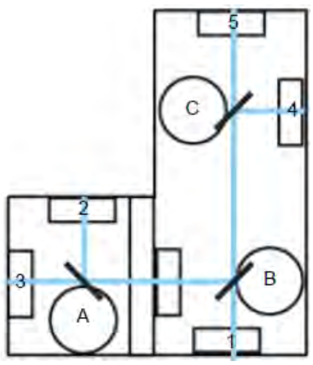
488-nm module (2 PMTs)

<table>
<tr><th>Layout</th><th>PMT module</th><th>Longpass filter</th><th>Bandpass filter</th></tr>
<tr><td rowspan="5">
</td><td>488-A</td><td>1: 505 LP
A (dichroic): 550 LP</td><td>2: 530/40 BP
3: 580/30 BP</td></tr>
<tr><td>488-B</td><td>1: 505 LP
A (dichroic): 527 LP</td><td>2: 513/17 BP
3: 542/27 BP</td></tr>
<tr><td>488-C</td><td>1: 505 LP
A (dichroic): 600 LP</td><td>2: 530/40 BP
3: 692/40 BP</td></tr>
<tr><td>488-D</td><td>1: 505 LP
A (dichroic): 550 LP</td><td>2: 530/40 BP
3: 610/20 BP</td></tr>
<tr><td colspan="3">Filters (LP and BP):
1. Lowest wavelength filter
2. Middle wavelength filter
3. Highest wavelength filter
Dichroics:
A: Dichroic</td></tr>
</table>

488-nm module (3 PMTs)

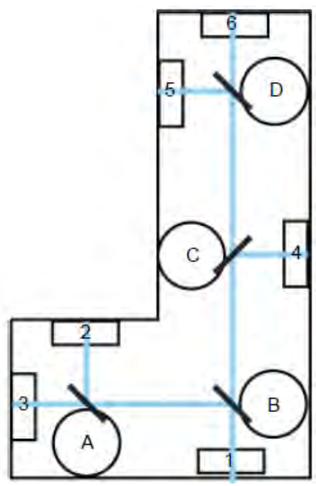
<table>
<tr><th>Layout</th><th>PMT module</th><th>Longpass filter</th><th>Bandpass filter</th></tr>
<tr><td rowspan="2">
</td><td>488-A</td><td>1: 505 LP
A (dichroic): 550 LP
B (dichroic): 685 LP</td><td>2: 530/40 BP
3: 670/30 BP
4: 710/50 BP</td></tr>
<tr><td colspan="3">Filters (LP and BP):
1. Lowest wavelength filter
2. Second lowest wavelength filter
3. Second highest wavelength filter
4. Highest wavelength filter
Dichroics:
A: Lowest wavelength dichroic
B: Highest wavelength dichroic</td></tr>
</table>

488-nm module (4 PMTs)

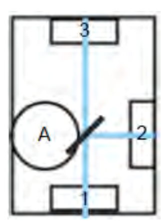
Layout	PMT module	Longpass filter	Bandpass filter	Rejection filter
	488-A	1: 505 LP A (dichroic): 550 LP B (dichroic): 600 LP C (dichroic): 645 LP	2: 530/40 BP 3: 580/30 BP 4: 610/20 BP 5: 670/30 BP	N/A
	488-B	1: 505 LP A (dichroic): 550 LP B (dichroic): 610 LP C (dichroic): 710 LP	2: 530/40 BP 3: 580/30 BP 4: 610/20 BP 5: 670/30 BP	N/A
	488-C	1: 505 LP A (dichroic): 550 LP B (dichroic): 600 LP C (dichroic): 645 LP	2: 530/40 BP[a] 3: 580/30 BP 4: 610/20 BP 5: 692/40 BP	2: 532/20 BP[b]
	488-D	1: 505 LP A (dichroic): 527 LP B (dichroic): 560 LP C (dichroic): 610 LP	2: 520/15 BP 3: 542/27 BP 4: 585/40 BP 5: 692/40 BP	N/A
	Filters (LP and BP): 1. Lowest wavelength filter 2. Second lowest wavelength filter 3. Middle wavelength filter 4. Second highest wavelength filter 5. Highest wavelength filter **Rejection filter:** In front of the 530/40 BP filter **Dichroics:** A: Lowest wavelength dichroic B: Middle wavelength dichroic C: Highest wavelength dichroic			

a. The 530/40 BP is mounted behind the 532/20 BP filter.
b. The 532/20 BP is mounted in front of the 530/40 BP filter.

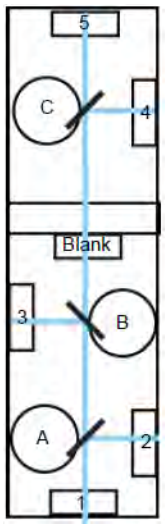
488-nm module (5 PMTs)

Layout	PMT module	Longpass filter	Bandpass filter
	488-nm	1: 505 LP A (dichroic): 550 LP B (dichroic): 600 LP C (dichroic): 645 LP D (dichroic): 740 LP 6: 750 LP	2: 530/40 BP 3: 580/30 BP 4: 610/20 BP 5: 692/40 BP
	Filters (LP and BP): 1. Lowest wavelength filter 2. Second lowest wavelength filter 3. Middle wavelength filter 4. Second highest wavelength filter 5. Highest wavelength filter **Dichroics:** A: Lowest wavelength dichroic B: Second lowest wavelength dichroic C: Second highest wavelength dichroic D: Highest wavelength dichroic		

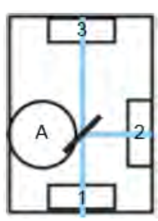
532-nm module (2 PMTs)

Layout	PMT module	Longpass filter	Bandpass filter
	532-A	1: 532 LP A (dichroic): 645 LP	2: 585/40 BP 3: 670/30 BP
	Filters (LP and BP): 1. Lowest wavelength filter 2. Middle wavelength filter 3. Highest wavelength filter **Dichroics:** A: Dichroic		

532-nm module (4 PMTs)

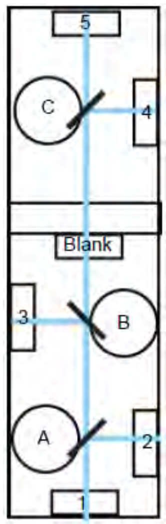
<table>
<tr><th>Layout</th><th>PMT module</th><th>Longpass filter</th><th>Bandpass filter</th></tr>
<tr><td rowspan="2">
</td><td>532-A</td><td>1: 532 LP
A (dichroic): 600 LP
B (dichroic): 645 LP
C (dichroic): 700 LP
5: 750 LP</td><td>2: 585/29 BP
3: 610/20 BP
4: 670/30 BP</td></tr>
<tr><td colspan="3">Filters (LP and BP):
1. Lowest wavelength filter
2. Second lowest wavelength filter
3. Middle wavelength filter
4. Second highest wavelength filter
5. Highest wavelength filter
Dichroics:
A: Lowest wavelength dichroic
B: Middle wavelength dichroic
C: Highest wavelength dichroic</td></tr>
</table>

561-nm module (2 PMTs)

<table>
<tr><th>Layout</th><th>PMT module</th><th>Longpass filter</th><th>Bandpass filter</th><th>Rejection filter</th></tr>
<tr><td rowspan="3">
</td><td>561-A</td><td>1: 570 LP[a]
A (dichroic): 610 LP</td><td>2:593/40 BP
3: 624/30 BP</td><td>1: 561 RB[b]</td></tr>
<tr><td>561-B</td><td>1: 570 LP
A (dichroic): 645 LP</td><td>2: 593/40 BP
3: 670/30 BP</td><td>1: 561 RB</td></tr>
<tr><td colspan="3">Filters LP and BP:
1. Lowest wavelength filter
2. Middle wavelength filter
3. Highest wavelength filter
Dichroics:
A: Dichroic</td><td></td></tr>
</table>

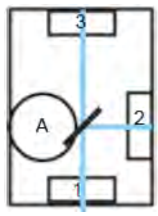
a. The 570 LP is mounted behind the 561 RB filter.
b. The 561 RB is mounted in front of the 570 LP filter.

561-nm module (4 PMTs)

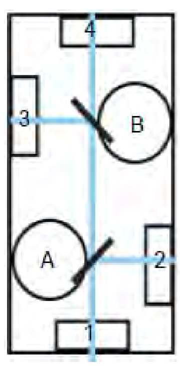
Layout	PMT module	Longpass filter	Bandpass filter	Rejection filter
	561-A	1: 570 LP[a] A (dichroic): 600 LP B (dichroic): 645 LP C (dichroic): 700 LP 5: 750 LP	2: 585/29 BP 3: 610/20 BP 4: 670/30 BP	1: 561 RB[b]
	561-B	1: 570 LP[a] A (dichroic): 645 LP B (dichroic): 685 LP C (dichroic): 740 LP 5: 750 LP	2: 585/29 BP 3: 610/20 BP 4: 670/30 BP or 670/30 BP	1: 561 RB
	Filters (LP and BP): 1. Lowest wavelength filter 2. Second lowest wavelength filter 3. Middle wavelength filter 4. Second highest wavelength filter 5. Highest wavelength filter **Dichroics:** A: Lowest wavelength dichroic B: Middle wavelength dichroic C: Highest wavelength dichroic			

a. The 570 LP is mounted behind the 561 RB filter.
b. The 561 RB is mounted in front of the 570 LP filter.

635-nm module (2 PMTs)

Layout	PMT module	Longpass filter	Bandpass filter
	635-A	1: 650 LP A (dichroic): 700 LP 3: 750 LP	2: 670/30 BP
	635-B	1: 650 LP A (dichroic): 740 LP 3: 750 LP	2: 692/40 BP
	Filters (LP and BP): 1. Lowest wavelength filter 2. Middle wavelength filter 3. Highest wavelength filter **Dichroics:** A: Dichroic		

635-nm module (3 PMTs)

Layout	PMT module	Longpass filter	Bandpass filter
	635-A	1: 650 LP A (dichroic): 680 LP B (dichroic): 740 LP 4: 750 LP	2: 660/20 BP 3: 720/40 BP
	635-B	1: 650 LP A (dichroic): 700 LP B (dichroic): 740 LP 4: 750 LP	2: 660/20 BP 3: 720/40 BP
	635-B_a	1: 650 LP A (Dichroic): 700 LP B (Dichroic): 740 LP 4: 750 LP	2: 670/30 BP 3: 720/40 BP
	Filters (LP and BP): 1. Lowest wavelength filter 2. Second lowest wavelength filter 3. Second highest wavelength filter 4. Highest wavelength filter **Dichroics:** A: Lowest wavelength dichroic B: Highest wavelength dichroic		

More information

- System reference information (page 329)
- Sort performance (page 331)
- Fluorescence specifications (page 341)

Fluorescence specifications

Introduction

This topic describes fluorescence sensitivity, resolution, and linearity of the emission optics.

Sensitivity

Fluorescence sensitivity was measured using SPHERO Rainbow Calibration Particles (RCP-30-5A) according to the manufacturer's specifications.

Setting	Value
Sheath pressure	33 PSI
Drop drive	~68 kHz
Excitation	488 nm, 200 mW
Emission	FITC: 530/40 nm PE: 580/30 nm

FITC: 125 molecules of equivalent soluble fluorochrome (MESF-FITC)

PE: 125 molecules of equivalent soluble fluorochrome (MESF-PE)

Resolution

Fluorescence resolution was measured using propidium iodide (PI)-stained chicken erythrocyte nuclei (CEN).

Setting	Value
Sheath pressure	33 PSI
Drop drive	~68 kHz
Excitation	488 nm, 200 mW
Emission	PI: 610/20 nm

Coefficient of variation (CV) of PI: <3%, full G_0/G_1 peak

Linearity

Fluorescence linearity was measured using PI-stained chicken erythrocyte nuclei (CEN).

Setting	Value
Sheath pressure	33 PSI
Drop drive	~68 kHz
Excitation	488 nm, 200 mW
Emission	PI: 610/20 nm

Doublet/singlet ratio: 1.95–2.05

More information

- System reference information (page 329)
- Optical detector modules (page 333)
- Fluidics pressure and drop frequency settings (page 343)

Fluidics pressure and drop frequency settings

Introduction

This topic describes fluidics pressure and drop frequency settings.

About fluidics pressure and drop frequency settings

For each nozzle size, there are several optional sheath pressures that can be used, depending on the requirements of your application and the resonant frequencies of the system.

The values in the following table are a sampling of theoretical optimal nozzle size/ sheath pressure/drop frequency combinations. These values are to be used as a starting place only and are based on typical Influx resonant frequencies. Every instrument requires droplet breakoff optimization.

Piezo amplitude. In general, piezo amplitudes will be between 2 and 20, though higher values may be needed for high sheath pressures and drop frequencies.

Drop delay. In general, the expected drop delay from most nozzles (drops to breakoff) is approximately half the drop frequency in kHz for optimized pressure/ frequency combinations. For example, a 70-µm nozzle at 33 PSI and 72 kHz will have an expected drop delay of about 32 to 40 drops when properly adjusted. Fewer drops increase the chances of optical interference and more drops increase the chance of less than optimal voltage being applied to the piezo drive.

Settings

Nozzle size (µm)	Sheath pressure (PSI)	Drop frequency (kHz)
70	22	59
	33	72
	65	101
86	15	39
	22	48
	33	58
100	7	23
	17	36
140	5	14
200	3	8

More information

- System reference information (page 329)
- Optimizing the droplet breakoff (page 285)
- Fluorescence specifications (page 341)
- Fluidics specifications (page 344)

Fluidics specifications

Introduction

This topic describes the system fluidics specifications.

General operation

- Laboratory air pressure and/or vacuum can be used for operation (regulated at 90 PSI, 6.2 bar).
- An optional air pressure supply and vacuum pump are available.
- Sheath pressure is adjustable from 1–90 PSI (0.07–6.2 bar).

Fluidics reservoirs

Autoclavable 7-L sheath and waste containers, equipped with pressure and vacuum readout, are provided. Remove the gauge before autoclaving.

Fluidics control

- Sheath, sample, and boost pressure can be individually adjusted.
- A sample flow fine adjustment is provided for precise regulation of sample flow.
- Purge, pulse, rinse, and run buttons are provided for quick stream startup and bubble removal.

Replaceable fluidics path

- The fluidics path, including the nozzle assembly can be exchanged. There are no inline valves. Only pinch valves are used.
- The sample line can also be exchanged.

Bubble detector

A bubble detector in the sample line detects air bubbles from the sample tube and stops sample flow when the sample tube is empty, preventing air bubbles from reaching the nozzle assembly.

Sample input

12 x 75-mm polypropylene tubes

More information

- System reference information (page 329)
- Electronics and signal processing specifications (page 345)

Electronics and signal processing specifications

Introduction

This topic describes electronics and signal processing specifications.

Data acquisition channels

16 channels, usually 14 colors plus forward and side scatter

Signal processing

- 16-bit analog-to-digital conversion, 65,536 channels
- Parallel data stream with channel ID and integrity check
- Less than 1 correlation error per 108 events

Acquisition rate

The system dead time is 5 µs. This leads to a maximum theoretical processing capability of 200,000 events/second, independent of the number of parameters.

Fluorescence compensation

16 x 16 digital compensation matrix (DSP). Compensated parameters are added to the bus as separate parameters.

Pulse processing electronics

- All signals are height (peak) by default.
- Optional pulse processor electronics add area and width measurements for a maximum of 8 parameters to the bus.
- Width measurement on the trigger parameter is standard.

Time

Time can be correlated to any parameter for kinetic experiments or other applications.

Threshold channel

- Any parameter can be used as the threshold from the primary laser.
- Lasers and detectors can easily be switched to change laser sequence.

More information

- System reference information (page 329)
- System specifications (page 330)

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23

BD Influx options

This section includes these topics:

About the HEPA-filtered enclosure option

Introduction

This topic provides a functional description of the BD Influx HEPA-filtered enclosure option.

Description

The HEPA (high efficiency particulate air) filtered enclosure is specifically designed for the BD Influx system. It provides protection from biological agents approved for use in Biosafety Level 1 or 2 environments.

The enclosure features:

- Recirculating HEPA filtration
- Variable speed blowers
- Solid state, automated speed control
- Analog and digital pressure indicators
- Clean room–compatible epoxy exterior

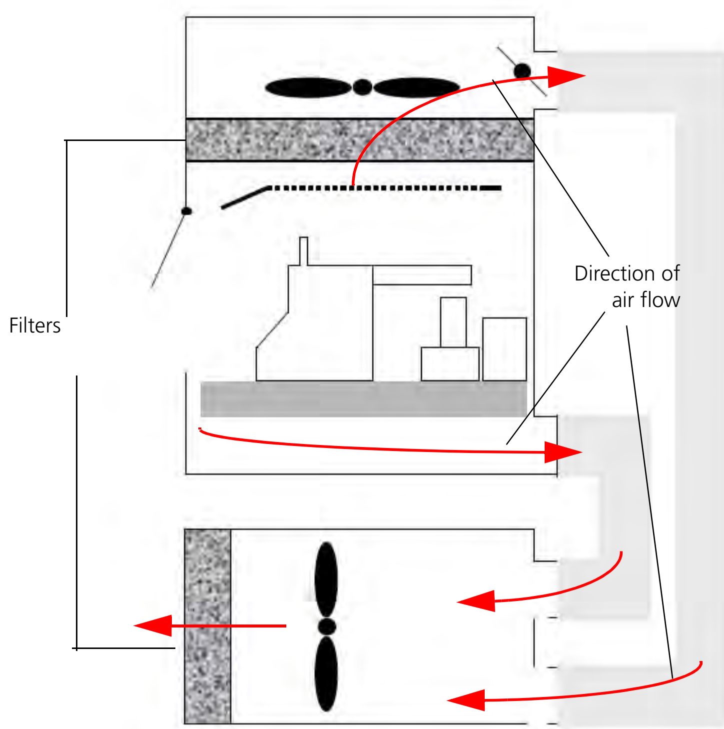
How it works

The stainless steel diffuser is located below the upper HEPA filter and ensures that the work area is supplied with HEPA-filtered air.

The filtered air flows around the instrument and the optical bench, then down into a common lower plenum.

A recirculated volume of air is returned to an upper plenum for refiltering, while the remaining air is exhausted to the room via a lower HEPA filter.

The arrows in the following figure indicate the direction of air flow.



A manual damper in the upper plenum maintains the balance between the two fan units. Another damper in the diffuser ensures a proper downflow velocity over the work area.

All areas of the enclosure are under negative pressure relative to the ambient external laboratory. Air that is handled by the enclosure is HEPA filtered before being released into the room.

If a leak occurs in a contaminated pressure plenum, the negative pressure creates suction and pulls air in so it cannot escape into the laboratory space.

If a leak occurs from one of the two positive pressure plenums (between a fan and a HEPA filter), the surrounding negative pressure area recaptures the contaminated air and recirculates it through the filter.

Components

The HEPA-filtered enclosure option includes the following components.

Component	Description
Upper plenum	Recirculates air. Provides access to the plenum electronics.
Top access panel	Provides access to the upper HEPA filter.
Pressure gauge	Displays the enclosure air pressure.

Component	Description
Enclosure cage	Provides a skeletal framework for the sliding polycarbonate panels that surround the instrument work area. All sliding panels are fully removable.
Enclosure tray	The laser optical bench has an air gap around it. Small drop-in gap trays are used in the rear optical area to restrict and balance the free flow of air around the bench. A front access panel provides for access to the trays and air gap.
Exhaust HEPA	Exhausts filtered air.
Enclosure top	Sits on top of the enclosure cage. Two removable panels on the right and left sides provide access to any internal pressure tubing or electrical wiring.
Front sash door	Provides access to the instrument.
Tray access panel	Provides access to the catch tray, below the instrument.
Digital display	Displays the HEPA filter status.
Lower plenum	A common plenum for both the recirculating and exhausting air supplies. The lower plenum includes a variable speed fan motor and replaceable exhaust HEPA filter, as well as a digital pressure display and a lower differential pressure gauge. A front access panel provides non-contaminated access to the plenum electronics.

More information

- BD Influx options (page 347)
- HEPA enclosure specifications (page 351)
- HEPA enclosure safety (page 352)
- About the HEPA enclosure controls (page 355)
- Setting up the HEPA enclosure digital display (page 357)

HEPA enclosure specifications

Introduction

This topic provides the HEPA enclosure and site specifications.

Dimensions

- Outside (W x D x H): 46 x 34 x 85 in. (117 x 86 x 216 cm)
- Height: 85.3 in. (216.7 cm)
- Width: 46 in. (116.8 cm)
- Depth: 34 in. (86.4 cm)
- Filtered work area (W x D): 32 x 17 in. (81.3 x 43.2 cm)
- Work surface height: 37 in. (94 cm)
- Sash dimensions (W x H): 25.5 x 8 in. (64.8 x 20.3 cm)
- Weight: 210 lb. (95.2 kg)

Airflow

- Inflow method: indirect exhaust
- Average inflow: 85–130 FPM
- Average downflow: 40–60 FPM

Filtration

- Efficiency: 99.9995% at 0.3
- Leak detection: >10 Jg/L
- Resistance: 0.48'' wg (inches of water gauge)
- Filter size: 559 x 559 x 90 mm
- Max flow: 320 CFM

Laboratory requirements

- Room height (floor-to-ceiling): 8 ft (2.44 m)
- Operational footprint (W x D): 7 x 7 ft (2.13 x 2.13 m)
- Electrical: 115 V at 50/60 Hz, 11 A peak, 5 A nominal

Enclosure location

Proper placement of the BD Influx system within the laboratory is essential.

Warning! A strong, disruptive air current (exceeding the intake velocity of the enclosure) might enable contamination to escape the enclosure.

To prevent exposure to disruptive air currents, place the BD Influx enclosure in a dead-end corner of the laboratory or cleanroom. The instrument should be away from personnel traffic, vents, doors, windows, or any other sources of disruptive air currents.

More information

- BD Influx options (page 347)
- About the HEPA-filtered enclosure option (page 348)
- HEPA enclosure safety (page 352)

HEPA enclosure safety

Introduction

This topic describes the official use, cautions, and limitations of the HEPA enclosure.

Intended use

The HEPA enclosure has been designed as a containment device to protect users from any aerosols generated while sorting with the BD Influx cell sorter.

It is designed for work with Biosafety Level (BSL) 1 and 2 agents as listed in The Centers for Disease Control HHS publication number 84-8395: *Biosafety in Microbiological and Biomedical Laboratories*.

U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, and National Institutes of Health, U.S. Government Printing Office, Washington, D.C. 20402

Biological safety

Caution! Biosafety Level 3 (BSL-3) agents should not be used unless other containment measures approved for work with BSL-3 agents encapsulate the entire enclosure or unless the enclosure is located within an existing BSL-3 rated facility.

Biosafety Level 4 or extremely high risk agents should never be used in the enclosure. Please consult your safety professional for a proper risk assessment.

Caution! The use of any hazardous material in the enclosure requires that it be monitored by a safety officer or other qualified individual.

Do not use explosive or flammable substances in the enclosure.

If chemical, radiological, or other non-microbiological hazards are present, be sure to employ appropriate protective measures in addition to formaldehyde decontamination.

Using proper technique

The BD Influx HEPA enclosure must be used properly in combination with proper sterile techniques to provide an adequate protective barrier.

Enclosure fluid spills

Instrument administrators and safety personal should create an emergency spill procedure. If you work with hazardous agents, you should also assemble a kit that includes:

- Respirator
- Chemical splash goggles
- Two pairs of gloves
- Two sheets of absorbent material
- Spill control pillows
- Waste disposal bags

Any fluid that spills inside the enclosure is primarily contained to the work area. If a large volume of fluid spills, some spilled fluid might drip into the tray area below the optical table. No drain is provided for the tray. You must manually remove any spilled fluid in the tray by removing the tray access panel. If the spill is large enough to create puddles of liquid in the tray area, then you should follow an emergency spill procedure.

If an accident causes spills and splatters in the work area, you must decontaminate all items and surfaces before any items are removed.

Disinfecting after a spill

If a biological spill drips down into the tray area:

1. Flood the area with up to 1 L of an appropriate disinfectant.
2. After the disinfectant has pooled in the tray, remove any fluid in the tray by removing the tray access panel. If the spill involves a hazardous Biosafety Level 2 or 3 agent:
    - Leave the enclosure running to permit aerosols to settle before you start cleanup procedures.
    - With some spills, you might need to decontaminate the room with an agent such as formaldehyde gas. Consult a safety professional for proper procedures and treatment of the specific agent.

Caution! Never use Biosafety Level 4 agents in the BD Influx enclosure.

If the spill contains volatile liquids that generate flammable or explosive vapors:

- Turn off the enclosure and other electrical devices.
- Evacuate and seal the room.
- Call for immediate help from a safety professional for proper procedures and treatment of the specific agent.

More information

- BD Influx options (page 347)
- HEPA enclosure specifications (page 351)
- Enclosure operational checklist (page 354)

Enclosure operational checklist

Introduction

This topic provides a list of steps you must complete before you begin using the HEPA enclosure.

You must perform these steps to ensure safe and effective operation.

Before you begin

A qualified professional or safety officer must approve the use of any hazardous agents with the BD Influx HEPA enclosure. This person should monitor the system and operating personnel at regular intervals to ensure appropriate use.

Checklist

Before introducing any biological agents into the enclosure:

1. Power up the enclosure.

   The digital display lights up.

2. Make sure all four enclosure panels are in place and are completely closed.

3. Pressurize both HEPA filters.

   Check the pressure on both analog gauges and the digital display. If the analog gauges and digital display show different values, the enclosure might require service.

4. Close and latch the enclosure sash door.

5. Follow all BD Influx instrument startup procedures.

   Warning! If a low-flow alarm condition occurs, stop all work and service the enclosure.

6. If you introduce any biological agents into the enclosure, continuously monitor the sample and work area for any fluid spills.

   Warning! The enclosure and blowers are designed for continuous operation. To minimize the chance of contamination, do not turn off the blowers unless you are shutting down the system for a period of non-use or for servicing the instrument or enclosure.

More information

- BD Influx options (page 347)
- System startup (page 223)
- HEPA enclosure safety (page 352)
- Setting up the HEPA enclosure digital display (page 357)

About the HEPA enclosure controls

Introduction

This topic describes the HEPA enclosure controls.

Controls

- **Blower power switch.** This controls the main power to the blowers and to the enclosure. It is located to the rear of the lower plenum assembly, near the floor. When you power on the switch, a brief calibration alarm sounds.
- **Fluorescent light switch.** This controls the operation of the fluorescent light inside the enclosure.
- **Digital display.** The digital display monitors the incoming signal from both upper and lower plenum sensors. These sensors sample the differential pressure between the pressurized side of the HEPA filters and the room. If certain threshold levels are exceeded, the display triggers a relay and alarm.

- **Alarm Acknowledge Button (ACK).** This temporarily mutes any audible alarm. The button is located on the lower plenum digital display. If a fault condition exists, the alarm resumes after a preset time until the fault is cleared.

- **Pressure indicators.** The enclosure provides both digital pressure indicators and analog pressure gauges.

Both indicators monitor the air pressure generated by the fans against the HEPA filter surfaces. The digital indicators are measured in mA, and appear as data bars.

More information

- BD Influx options (page 347)
- Enclosure operational checklist (page 354)
- Setting up the HEPA enclosure digital display (page 357)

Setting up the HEPA enclosure digital display

Introduction

This topic describes how to set up the digital display on the lower plenum of the HEPA enclosure.

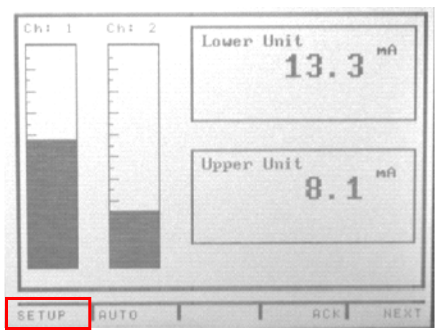
Make selections by pressing one of the control buttons at the bottom of the digital display which correspond to the task. The display is set at the factory. However, you can change the trigger levels.

Procedure

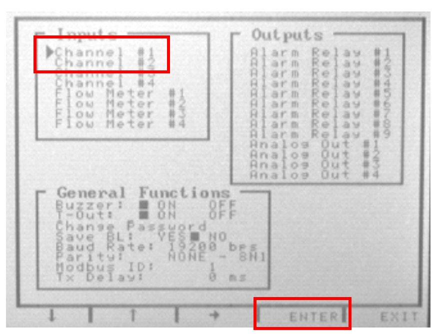
The **Display Main** screen displays a summary of both sensor channels.

To set up the HEPA enclosure digital display:

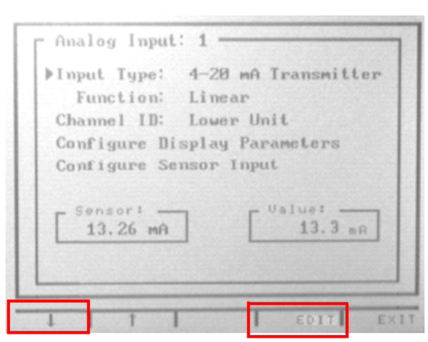
1. Press **SETUP**.



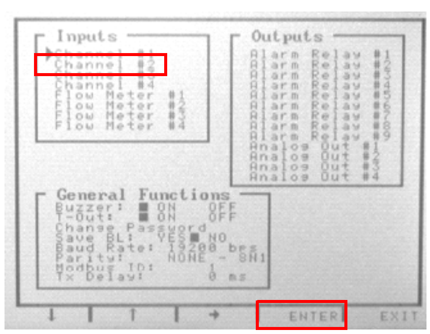
2. In the **Display Setup** screen, use the arrow to select **CHANNEL #1**, then press **ENTER**.



The **Analog Input #1** screen displays the parameters for the lower plenum sensor.



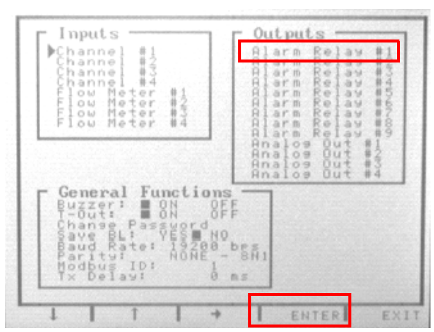
3. To select an item, press the arrows, then press **EDIT**. Make sure that the following parameters are set:
    - Input Type: 4–20 mA Transmitter
    - Function: Linear
    - Channel ID: Lower Unit
4. Select **Configure Display Parameters,** then press **EDIT**.
5. Make sure that the following parameters are set:
    - Max Value: 20.0 mA
    - Min Value: 4.8 mA
    - Format: 99999.9 mA
    - Units: mA
6. Press **EXIT**.
7. Select **Configure Sensor Input**, then press **EDIT.**
8. Make sure that the following parameters are set:
    - High Value: 20.0 mA
    - Sensor: 20.00 mA
    - Low Value: 4.0 mA
    - Sensor: 4.00 mA
9. Press **EXIT** to return to the **Display Setup** screen.
10. Use the arrow to select **CHANNEL #2**, then press **ENTER**.



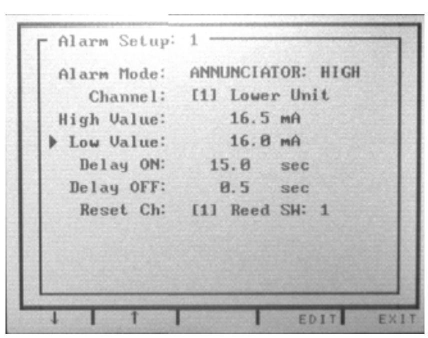
The **Analog Input #2** screen displays the parameters for the upper plenum sensor.

11. Press the arrows to select an item, then press **EDIT**.
12. Make sure that the following parameters are set:
    - Input Type: 4–20 mA Transmitter
    - Function: Linear
    - Channel ID: Upper Unit

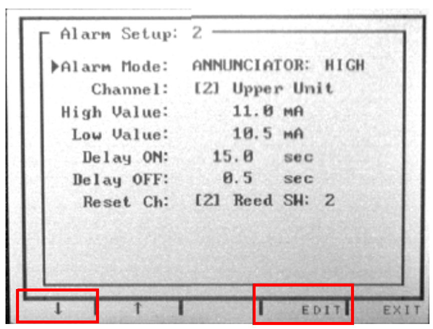
13. Select **Configure Display Parameters**, then press **EDIT.**
14. Make sure that the following parameters are set:
    - Max Value: 20.0 mA
    - Min Value: 4.0 mA
    - Format: 99999.9 mA
    - Units: mA
15. Press **EXIT**.
16. Select **Configure Sensor Input**, then press **EDIT**.
17. Make sure that the following parameters are set:
    - High Value: 20.0 mA
    - Sensor: 20.00 mA
    - Low Value: 4.0 mA
    - Sensor: 4.00 mA
18. Press **EXIT** to return to the **Display Setup** screen.
19. Use the arrow to select **ALARM RELAY #1**, then press **ENTER**.



The **Alarm Setup** screen allows a mechanical relay to be triggered by a current level from the lower plenum sensor.



20. Make sure that the following parameters are set:
    - Alarm Mode: Annunciator: High
    - Channel: [1] Lower Unit
    - High Value: 16.5 mA
    - Low Value: 16.0 mA
    - Delay ON: 15.0 sec
    - Delay OFF: 8.5 sec
    - Reset Ch: [1] Read SW: 1
21. Press **EXIT** to return to the **Display Setup** screen.
22. Use the arrow to select **ALARM RELAY #2**, then press **ENTER**.



23. Make sure that the following parameters are set:
    - Alarm Mode: Annunciator: High
    - Channel: [2] Upper Unit
    - High Value: 11.0 mA
    - Low Value: 10.5 mA
    - Delay ON: 15.0 sec
    - Delay OFF: 0.5 sec
    - Reset Ch: [2] Read SW: 2
24. Press **EXIT** twice to return to the **Display Main** screen.

    The digital display is now set up.

More information

- BD Influx options (page 347)
- About the HEPA enclosure controls (page 355)
- About the HEPA-filtered enclosure option (page 348)

Small particle forward scatter detector option

Introduction

This topic describes the Small Particle Detector Option (SPO).

Description

The SPO incorporates a 20X, 0.42 NA microscope objective, a mirror pinhole, and a pinhole camera. Resolution for the SPO is >0.2 µm (measured using beads and 0.1-µm filtered sheath fluid). The collection angle is 2–30°.

The small particle detection module, forward pinhole, and forward pinhole camera are optional devices that come with the SPO.

The forward image block assembly includes the following components:

- **Forward objective microscope lens.** Focuses the forward scatter light into the pinhole.
- **Forward pinhole.** Focuses the forward scatter beam profile.
- **Forward pinhole camera.** Monitors and displays (on the pinhole LCD) the forward scatter beam on the pinhole.

Contact your BD representative for more information about ordering and installing this option.

More information

- BD Influx options (page 347)
- About the HEPA-filtered enclosure option (page 348)
- Aerosol evacuation unit and air compressor options (page 362)

Aerosol evacuation unit and air compressor options

Introduction

This topic describes the aerosol evacuation unit option for the BD Influx HEPA-filtered enclosure. It also describes the air compressor option for the system.

Aerosol evacuation unit

The Surgifresh® evacuation unit is an aerosol management option for the HEPA-filtered enclosure. This unit evacuates aerosols from the lower chamber of the HEPA-filtered enclosure, preventing the aerosols from being circulated back through the enclosure.

Contact your BD representative for more information about ordering and installing this option.

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, pressurized air at 60–90 PSI to the cytometer during operation.

More information

- BD Influx options (page 347)

24

Troubleshooting

This section includes these topics:

Sorting troubleshooting

Introduction

This topic describes sorting problems and possible solutions.

Stream 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 Aligning the stream (page 249) 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 and O-ring are seated properly and aligned.
Clogged or damaged nozzle	Remove the nozzle and sonicate it in DI water or detergent to remove the clog. Examine the nozzle under a microscope to check for damage. If the nozzle is damaged, replace it.

No stream 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	Remove the nozzle and sonicate it in DI water or detergent to remove the clog. Examine the nozzle under a microscope to check for damage. If the nozzle is damaged, replace it.
Nozzle was not installed properly, seal is leaking	Reinstall the nozzle and ensure that the O-ring is properly installed.
Air in sheath filter	Purge the sheath filter.

Side streams 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 sort

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 min to 1 hr 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 personnel to check the pressure console for leaks.
Chemical residue in sheath line	Perform a system flush or replace the sheath line.
Sheath tank low or empty	Refill the sheath tank.
Air in sheath filter	Purge the sheath filter.
Clogged sheath filter	Replace the sheath filter.
Large cells or clumpy sample	• Install a larger nozzle. The nozzle should be at least 5–6 times the size of the particles being run. • Filter the sample.
Frequency setting is not optimal	1. See the Fluidics pressure and drop frequency settings (page 343) to select an approximate frequency. 2. Find the frequency setting that will give you 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 or use a larger nozzle.

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 not turned on	Turn on the deflection plates.
Deflection plates are open	1. Turn 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 Piezo amplitude to appropriate settings. 2. Optimize the drop drive frequency.
DI water is in the sheath lines	Use a sheath fluid with the appropriate salinity for drop charging.
Pressure and frequency settings are not optimized	1. See Fluidics pressure and drop frequency settings (page 343) to select an approximate frequency. 2. Find the frequency setting that will give you the shortest breakoff and most stable stream.
Bad contact point for drop charge connection	Make sure the piezo amplitude is zero and 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.
Arcing between deflection plates	1. Turn off the deflection plates. 2. Clean any salt buildup off the plates and dry them thoroughly.
Side streams are not aligned to the Accudrop laser	Realign the side streams.
Wet, dirty, or salty plates	1. Turn off the plates. 2. Clean and dry the plates.
Nozzle chamber door is open	Close the nozzle chamber door.

Fanning around center or side streams when deflection plates are on

Possible causes	Possible solutions
Clogged or damaged nozzle	Remove the nozzle and sonicate it in DI water or detergent to remove the clog. Examine the nozzle under a microscope to check for damage. If the nozzle is damaged, replace it.
Large cells or clumpy sample	• Install a larger nozzle. The nozzle should be at least 3–4 times the size of the particles being run. • Filter the sample.
Pressure and frequency settings are not optimized	1. See Fluidics pressure and drop frequency settings (page 343) to select an approximate frequency. 2. Scan the frequency settings around the recommended frequency to find the setting that will give you 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 precision mode is appropriate for your sorting requirements.

Low sort efficiency

Possible causes	Possible solutions
Event rate is too high for drop drive frequency	Decrease the event rate.
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	Set the laser knob to Accudrop.
Event rate is too low	• Add more beads to the tube. • Increase the event rate. • Increase the sample offset.

Erratic sort rate

Possible causes	Possible solutions
Event rate is too high	Decrease the event rate.
Clogged or kinked sample	• 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 precision mode is appropriate for your sorting requirements.
Breakoff is not stable during the sort	Watch the breakoff during the sort and adjust the amplitude if necessary.
Incorrect logic in sort gating	Verify the sorting gate strategy and check for any yellow populations (conflicts).
Side streams fanning during the sort	See Fanning around center or side streams when deflection plates are on (page 367).
Pressure is too high for cell viability	Use a larger nozzle and lower sheath pressure for fragile cells.
Collection tube or plate is not aligned correctly	Check the tubes and side streams for proper alignment before sorting.
Event rate is too high	Decrease the event rate.

Index sorting button is grayed out or cannot start

Possible causes	Possible solutions
Cannot perform index sort while acquiring data	Stop acquiring data.
No available DSP parameters	Manage parameters in the compensation window and deselect 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	Turn off the plates and clean them.
High speed or large nozzle sort	Modify the sort setup.

More information

Acquisition troubleshooting

Introduction

This topic describes acquisition problems and possible solutions.

No sample events

Possible causes	Possible solutions
Laser shutter is engaged	Open the laser shutter.
Laser power is off	Turn on the laser power supply and turn the key to fire the laser.
Laser safety interlock is engaged	Close the nozzle chamber door and place your finger over the optical sensor in the upper right corner to reset the safety interlock.
No sample in the tube	Add sample to the tube or install a new sample tube.
Sample is not mixed properly	Mix the sample thoroughly and re-install the tube on the instrument.
Sample line is clogged	Clean or replace the sample line.
Bubble detector triggered	• Press BACKFLUSH to reset the bubble detector. • Verify that the sample line is long enough to reach the sample.
Air line sensor was triggered	Contact your BD service representative.
Tube is not properly installed	1. Install the tube onto the sample station. 2. Close the sample lever under the tube. 3. Press SAMPLE to pressurize the tube.
Laser or stream 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 trigger detector.

Possible causes	Possible solutions
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 knob to increase the sample pressure.

No fluorescent or scatter signal

Possible causes	Possible solutions
Wrong optical filter is installed or optical filter is missing	Make sure that you have the correct optical filters installed for your application.
Laser delay or trigger delay is set incorrectly	1. Verify that you have the correct event number assigned for each channel. 2. Adjust the laser delay.
PMT is labeled incorrectly	Check your 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.
Excitation laser is in the wrong pinhole	Align the excitation laser to the correct pinhole.
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 cytometer interface.
Faulty connection between Sortware and the cytometer interface	• Make sure that the network cable is connected between the Sortware workstation and the cytometer interface. • Select **Cytometer > Connect to Cytometer.** • Do the following: – Turn off the auxiliary power. – Turn off the cytometer interface by pressing and holding the power button. – Turn on the auxiliary power. – Turn on the cytometer interface and wait for two beeps. – (If needed) Select **Cytometer > Connect to Cytometer.**

Distorted populations or high CVs

Possible causes	Possible solutions
Lasers are not properly aligned	Align and focus the lasers.
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	• Turn off the hood light. • Close all safety covers on the instrument. • 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.
Sheath filter is dirty or contaminated	1. Remove the sheath filter. 2. Perform a system flush or replace 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	Adjust the sort settings.
Misaligned laser	Realign the laser.
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.
Backwards or missing bandpass filter	Verify that all optical filters are correctly installed.
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 pressure.
PMT voltage is set too high	Decrease the PMT voltage.

Low acquisition efficiency

Possible causes	Possible solutions
Drop drive noise	Adjust the sort settings.
High sample event rate	Lower the sample offset.
Clumps or filaments in the sample	Filter the sample.
Poor laser alignment	Align the laser.

More information

- Sorting troubleshooting (page 364)
- Fluidics troubleshooting (page 373)
- Alignment troubleshooting (page 375)
- Applications troubleshooting (page 377)
- Electronics troubleshooting (page 378)

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 373).
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 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	Remove the nozzle and sonicate it in DI water or detergent to remove the clog. 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 pressure.
Sample concentration is too low	Concentrate the sample.
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
Sample lever was opened too quickly	Open the sample lever slowly, especially when running at high pressures.
The filter on the sample tube air line is wet	Replace the air filter.
The sensor on the sample lever is not responding	Contact your BD service representative.

More information

- Sorting troubleshooting (page 364)
- Acquisition troubleshooting (page 369)
- Alignment troubleshooting (page 375)
- Applications troubleshooting (page 377)
- Electronics troubleshooting (page 378)

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 turned is off	Turn on the laser power supply and turn the key to fire the laser.
Laser safety interlock is engaged	Close the nozzle chamber 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.
Laser is not emitting visible light	When aligning a UV laser, run beads that are excited by UV wavelengths.
Bad pinhole monitor settings	Adjust the brightness and contrast on the pinhole monitor.

Core stream not visible on pinhole monitor when running alignment beads

Possible causes	Possible solutions
Laser shutter is closed	Open the laser shutter.
Laser turned is off	Turn on the laser power supply and turn the key to fire the laser.
Laser safety interlock is engaged	Close the nozzle chamber door and place your finger over the optical sensor in the upper right corner to reset the safety interlock.
Nozzle is too low	Raise the nozzle above the pinhole.
Stream is not aligned	Align the stream before aligning the lasers.
Laser is not aligned	Align the lasers.
Laser is not emitting visible light	When aligning a UV laser, run beads that are excited by UV wavelengths.
Bad pinhole monitor settings	Adjust the brightness and contrast on the pinhole monitor.
Sample pressure is too low	Increase the sample pressure.
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 laser	Use the appropriate alignment beads.

Poor signal, hockey stick shape

Possible causes	Possible solutions
Dichoric filter is misaligned, backwards, or missing	Verify that all optical filters are properly installed.
Sample pressure is too high	Decrease the sample pressure.
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.

More information

- Sorting troubleshooting (page 364)
- Acquisition troubleshooting (page 369)
- Fluidics troubleshooting (page 373)
- Applications troubleshooting (page 377)
- Electronics troubleshooting (page 378)

Applications 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 364)
- Acquisition troubleshooting (page 369)
- Fluidics troubleshooting (page 373)
- Alignment troubleshooting (page 375)
- Electronics troubleshooting (page 378)

Electronics troubleshooting

Introduction

This topic describes electronic problems and suggests possible solutions.

Cytometer error when opening the Sortware software

Possible causes	Possible solutions
Sortware controller is not connected to the instrument	1. From the Cytometer menu, click **Connect to Cytometer.** 2. Turn off the Sortware controller by holding the power button. 3. Turn off the auxiliary power, then turn it back on. 4. Turn on the Sortware controller. When you hear two beeps, the controller is fully turned on.

Hear three continuous beeps from the Sortware controller

Possible causes	Possible solutions
Sortware controller is not connected to the instrument	1. Turn off the Sortware controller by holding the power button. 2. Turn off the auxiliary power and then turn it back on. 3. Turn on the Sortware controller. When you hear two beeps, the controller is fully turned on.

More information

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- Acquisition troubleshooting (page 369)
- Fluidics troubleshooting (page 373)
- Alignment troubleshooting (page 375)
- Applications troubleshooting (page 377)