

BD LSR II User's Guide

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Patents

BD LSR II is covered by one or more of the following US patents and foreign equivalents: 4,745,285; 4,844,610; and 6,014,904.

PE and APC: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942

PerCP: US 4,876,190

Cy5.5 and Cy7: US 5,268,486; 5,486,616; 5,569,587; 5,569,766; and 5,627,027

Pe-Cy7: US 4,542,104

APC-Cy7: US 5,714,386

BD FACS lysing solution: US 4,654,312; 4,902,613; and 5,098,849

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 Règlement sur le matériel brouilleur du Canada.

History

Revision	Date	Change Made
334717 Rev. A	12/02	Initial release
338639 Rev. A	10/04	Updated software terminology and screen shots for BD FACSDiva software version 4.1
640752 Rev. A	5/06	Updated software terminology and screen shots for BD FACSDiva software version 5.0

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About This Guide





The *BD LSR II User's Guide* describes the procedures necessary to operate and maintain your BD™ LSR II flow cytometer. Because many instrument functions are controlled by BD FACSDiva™ software, this guide also contains information about software features required for basic instrument setup and operation.

For an annotated list of additional documentation for your BD LSR II flow cytometer, see BD LSR II Documentation on page xii.

Conventions


The following tables list conventions used throughout this guide. Table 1 lists the symbols that are used to alert you to a potential hazard. Text and keyboard conventions are shown in Table 2.

Table 1 Hazard symbols^a

Symbol	Meaning
	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death
	Electrical danger
	Laser radiation
	Biological risk

a. Although these symbols appear in color on the instrument, they are in black and white throughout this user's guide; their meaning remains unchanged.

Table 2 Text and keyboard conventions

Convention	Use
 Tip	Highlights features or hints that can save time and prevent difficulties
NOTICE	Describes important features or instructions
<i>Italics</i>	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, “choose File > Print” means to choose Print from the File menu.
Ctrl-X	When used with key names, a dash means to press two keys simultaneously. For example, Ctrl-P means to hold down the Control key while pressing the letter <i>p</i> .

BD LSR II Documentation

BD LSR II Online Help

The online help installed with your BD FACSDiva software contains the same text as that in the documents listed below, enhanced with features like full text search and related topic links. Access BD LSR II online help from the Help menu of BD FACSDiva software.

BD LSR II online help topics are compiled from the following sources:

- *BD FACSDiva Software Reference Manual*
- *BD LSR II User's Guide*
- *BD High Throughput Sampler User's Guide*

Printed Documentation

A printed copy of the following documents is distributed with the BD LSR II flow cytometer:

- *BD LSR II User's Guide* describes procedures necessary to operate and maintain your BD LSR II flow cytometer. Because many instrument functions are controlled by BD FACSDiva software, this guide also contains information about software features required for basic instrument setup and operation.
- *BD LSR II Safety and Limitations* booklet discusses the safety features of the BD LSR II flow cytometer. It lists precautions for the cytometer's laser, electrical, and biological hazards, and states limitations of use.
- *BD LSR II Facility Requirement Guide* contains specifications for:
 - instrument weight and size
 - temperature and other environmental requirements
 - electrical requirements
- *Getting Started with BD FACSDiva Software* contains tutorial exercises that familiarize you with key software procedures and concepts.
- A printed copy of the *BD High Throughput Sampler User's Guide* is distributed with the BD™ High Throughput Sampler (HTS) option. This document describes how to set up and operate the HTS. It also contains a description of BD FACSDiva software features specific to the HTS.

Electronic Documentation

PDF versions of the following documents can be found on the BD FACSDiva software installation disk or on your computer hard drive:

- The *BD FACSDiva Software Reference Manual* includes instructions or descriptions for installation and setup, workspace components, acquisition controls, analysis tools, and data management. It can be accessed from the BD FACSDiva Software Help menu (Help > Software Manual), or by double-clicking the shortcut on the desktop. In addition, a printed copy can be requested from BD Biosciences.
- *Getting Started with BD FACSDiva Software* can be accessed from Start > Programs > BD FACSDiva Software, or by double-clicking the shortcut on the desktop.
- The *BD LSR II User's Guide* and *BD High Throughput Sampler User's Guide* PDFs can be found on the BD FACSDiva software installation disk in the Instrument User Guides folder.
- The *BD FACSDiva Option White Paper* can be downloaded from the BD Biosciences website. This white paper contains an in-depth discussion of the digital electronics used in the BD LSR II cytometer.

Technical Assistance

For technical questions or assistance in solving a problem:

- Read sections of the documentation specific to the operation you are performing (see BD LSR II Documentation on page xii).
- See Chapter 7, Troubleshooting.

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.

When contacting BD Biosciences, have the following information available:

- product name, part number, and serial number
- version of BD FACSDiva software you are using
- any error messages
- details of recent system performance

For instrument support from within the US, call (877) 232-8995.

For support from within Canada, call (888) 259-0187.

Customers outside the US and Canada, contact your local BD representative or distributor.

Safety and Limitations

The BD LSR II flow cytometer and its accessories are equipped with safety features for your protection. Operate only as directed in the *BD LSR II User's Guide* and the *BD LSR II Safety and Limitations* booklet. Do not perform instrument maintenance or service except as specifically stated. Keep this safety information available for reference.

Laser Safety



Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. The main hazard of laser radiation is direct or indirect exposure of the eye to thermal radiation from the visible and near-infrared spectral regions (325–1400 nm). Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness.



There are other potentially serious hazards in other spectral regions. Excessive ultraviolet exposure produces an intolerance to light (photophobia) accompanied by redness, a tearing discharge from the mucous membrane lining the inner surface of the eyelid (conjunctiva), shedding of the corneal cell layer surface (exfoliation), and stromal haze. These symptoms are associated with photokeratitis, otherwise known as snow blindness or welder's flash, which results from radiant energy-induced damage to the outer epidermal cell layer of the cornea. These effects can be the result of laser exposure lasting only a fraction of a second.



Laser Product Classification

Laser hazard levels depend on laser energy content and the wavelengths used. Therefore, it is impossible to apply common safety measures to all lasers. A numbered system is used to categorize lasers according to different hazard levels. The higher the classification number, the greater the potential hazard. The BD LSR II flow cytometer is a Class I (1) laser product per 21 CFR Subchapter J and IEC/EN 60825-1:1994 + A1:2003 + A2:2001. The lasers and the laser energy are fully contained within the instrument structure and call for no special work area safety requirements except during service procedures. These procedures are to be carried out only by BD Biosciences service personnel.

Precautions for Safe Operation

-   Modification or removal of the optics covers or laser shielding could result in exposure to hazardous laser radiation. To prevent irreparable damage to human skin and eyes, do not remove the optics covers or laser shielding, adjust controls, or attempt to service the instrument any place where laser warning labels are attached (see Symbols and Labels on page xxi).

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

-   Keep all instrument doors closed during instrument operation. When operated under these conditions, the instrument poses no danger of exposure to hazardous laser radiation.

Electrical Safety



Lethal electrical hazards can be present in all lasers, particularly in laser power supplies. Every portion of the electrical system, including the printed circuit boards, should be considered to be at a dangerous voltage level. Avoid potential shock by following these guidelines.

- **Turn off the power switch and unplug the power cord** before servicing the instrument, unless otherwise noted.
- Connect the equipment only to an approved power source. Do not use extension cords. Have an electrician immediately replace any damaged cords, plugs, or cables. Refer to the *BD LSR II Facilities Requirement Guide* for specific information.
- Do not remove the grounding prong from the power plug. Have a qualified electrician replace any ungrounded receptacles with properly grounded receptacles in accordance with the local electrical code.
- For installation outside the US, use a power transformer or conditioner to convert the local power source to meet the BD LSR II power requirements (120 V \pm 10%, 50/60 Hz). Contact your local BD office for further information.

Biological Safety



All biological specimens and materials coming into contact with them are considered biohazardous. Avoid exposure to biohazardous material by following these guidelines.

- Handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.
- Expose waste container contents to bleach (10% of total volume) for 30 minutes before disposal. Dispose of waste in accordance with local regulations. Use proper precaution and wear suitable protective clothing, eyewear, and gloves.
- Prevent waste overflow by emptying the waste container frequently or whenever the waste management system alarms.

For information on laboratory safety, refer to the following guidelines. NCCLS documents can be ordered online at www.nccls.org.

- Schmid I, Nicholson JKA, Giorgi JV, et al. Biosafety guidelines for sorting of unfixed cells. *Cytometry*. 1997;28:99-117.
- *Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline*. Wayne, PA: National Committee for Clinical Laboratory Standards, 1997. NCCLS document M29-A.
- *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline*. Wayne, PA: National Committee for Clinical Laboratory Standards; 1990. NCCLS document H18-A.

General Safety



The instrument handles are for BD Biosciences authorized personnel only. Do not access them or attempt to lift the instrument with them, or you could injure yourself.



To avoid burns, do not touch the fan guards on the back of the instrument. The fan guards could be hot during and after instrument operation.

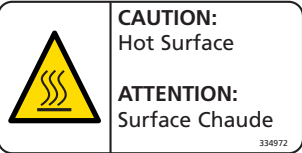
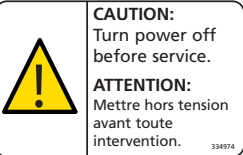
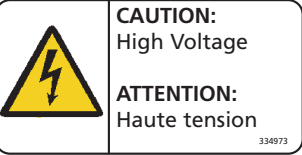





Movement of mechanical parts within the instrument can pinch or injure your hands or fingers. To prevent injury by moving parts, keep your hands and clothing away from the instrument during operation.

Symbols and Labels

The following symbols, warnings, or precaution labels appear on the BD LSR II flow cytometer or the waste and fluid tanks.

Symbol	Meaning	Location(s)
	Dangerous voltage	Rear instrument panel near power receptacle
	Laser radiation hazard	Near all removable covers and any place where the laser beam can emerge from the instrument
	Caution! Consult accompanying documents.	Near the instrument handles

Label	Meaning	Location(s)
 <p>CAUTION: Hot Surface ATTENTION: Surface Chaude</p> <p>334972</p>	<p>Caution Hot surface</p>	<p>Rear instrument panel adjacent to exhaust fans</p>
 <p>CAUTION: Turn power off before service. ATTENTION: Mettre hors tension avant toute intervention.</p> <p>334974</p>	<p>Caution Turn power off before service.</p>	<ul style="list-style-type: none"> • Rear instrument panel adjacent to power receptacle • Near internal power shield
 <p>CAUTION: High Voltage ATTENTION: Haute tension</p> <p>334973</p>	<p>Caution High voltage</p>	<p>Rear instrument panel adjacent to power receptacle</p>
 <p>Waste (A) 336325 Rev. A</p>	<p>Biological risk: Waste Risk of exposure to biologically transmissible disease</p>	<p>System waste tank</p>
 <p>DANGER VISIBLE AND/OR INVISIBLE LASER RADIATION WHEN REMOVED. AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION.</p>	<p>Danger Visible and/or invisible laser radiation when removed. Avoid eye or skin exposure to direct or scattered radiation.</p>	<p>Near all removable covers and any place where the laser beam can emerge from the instrument</p>
 <p>DANGER RAYONNEMENT LASER VISIBLE ET/OU INVISIBLE LORS DU RETRAIT EVITER L'EXPOSITION OCULAIRE OU CUTANEE AU RAYONNEMENT DIRECT OU DIFFUSE</p>		
<p>(B)</p>	<p>Sheath</p>	<p>Near BD FACSTFlow™ solution (sheath) port</p>

Limitations

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

BD Biosciences delivers software and workstations that are intended for running the instruments 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 buyer/user's failure to install and maintain virus protection.

Introduction

The following topics are covered in this chapter:

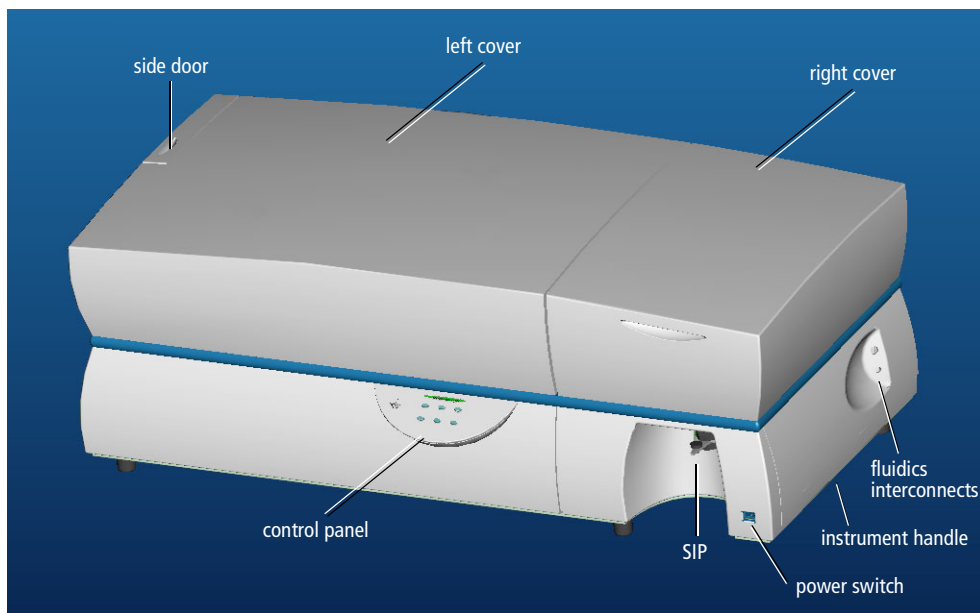
- Overview on page 26
- Fluidics on page 28
- Optics on page 32
- BD LSR II Workstation on page 36

Overview

The BD LSR II is an air-cooled multi-laser benchtop flow cytometer with the ability to acquire parameters for a large number of colors. It uses fixed-alignment lasers that transmit light reflected by mirrors through a flow cell to user-configurable octagon and trigon detector arrays. These detectors collect and translate fluorescence signals into electronic signals. Instrument electronics convert these signals into digital data.

Components

Figure 1-1 BD LSR II flow cytometer



Power Switch

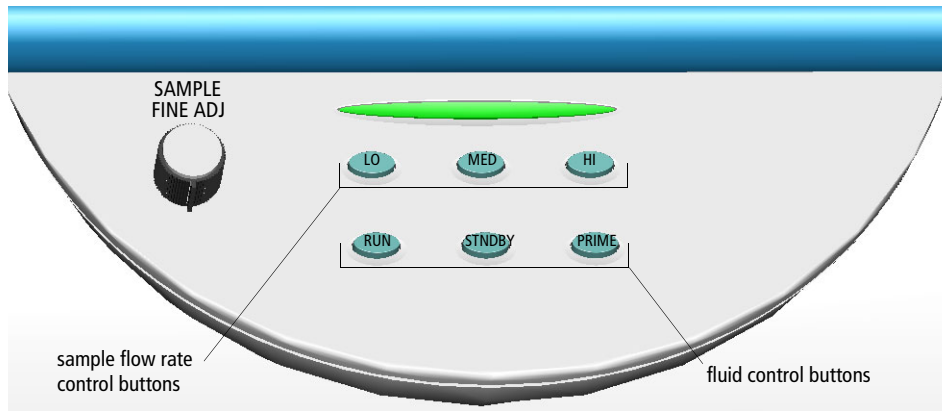
The power switch is located on the lower-right side of the BD LSR II instrument as shown in Figure 1-1.

Control Panel

The control panel contains fluidics controls (Figure 1-2). For a description of the controls, see:

- Sample Flow Rate Control on page 28
- Fluid Control on page 28

Figure 1-2 Control panel



Handles



The instrument handles (Figure 1-1 on page 26) are for BD Biosciences authorized personnel only. Do not access them or attempt to lift the instrument with them, or you could injure yourself.

Fluidics

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

Sample Flow Rate Control

Three flow rate control buttons—LO, MED, and HI—set the sample flow rate through the flow cell. The SAMPLE FINE ADJ knob allows you to adjust the rate to intermediate levels.

When the SAMPLE FINE ADJ knob is at its midpoint, the sample flow rates at the LO, MED, and HI settings are approximately 12, 35, and 60 $\mu\text{L}/\text{min}$ of sample, respectively. The knob turns five full revolutions in either direction from its midpoint, providing sample flow rates from 0.5–2X the midpoint value. For example, if the LO button is pressed, the knob will give flow rates from approximately 6–24 $\mu\text{L}/\text{min}$.

Fluid Control

Three fluid control buttons—RUN, STNDBY, and PRIME—set the instrument mode (Figure 1-2 on page 27).

- RUN pressurizes the sample tube to transport the sample through the sample injection tube and into the flow cell.

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

- STNDBY (standby) stops fluid flow to conserve sheath fluid.

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

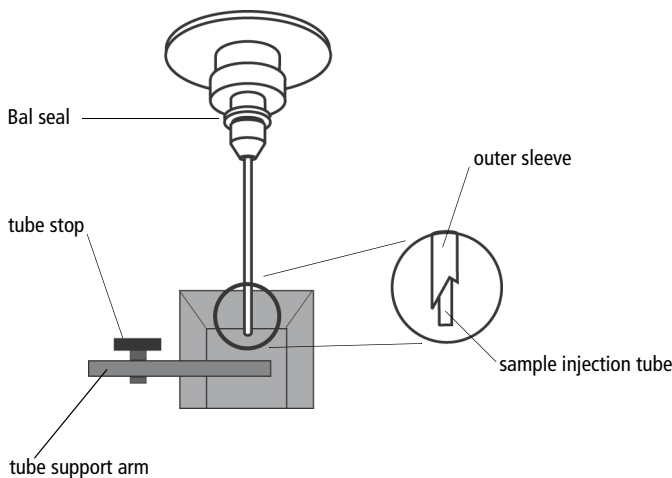
- PRIME prepares the fluidics system by draining and filling the flow cell with sheath fluid.

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

Sample Injection Port

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

Figure 1-3 Sample injection port (SIP)



- Sample injection tube—stainless steel tube that carries sample from the sample tube to the flow cell. This tube is covered with an outer sleeve that serves as part of the droplet containment system.
- Tube support arm—arm that supports the sample tube and activates the droplet containment system vacuum. The vacuum is on when the arm is positioned to the side and off when the arm is centered.

Droplet Containment System

The droplet containment system prevents sheath fluid from dripping from the SIP and provides biohazard protection.

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

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

Sheath and Waste Containers

The sheath and waste containers are outside the instrument and can be positioned on the floor.

Sheath Container



The sheath container has a capacity of 8 L. Sheath fluid is filtered through an in-line, interchangeable filter that prevents small particles from entering the sheath fluid lines.



Before opening the sheath container:


- 1 Put the instrument in STNDBY mode.
- 2 Disconnect the air line (green).
- 3 Depressurize the sheath container by lifting its vent cap.

Waste Container

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

  To avoid leakage of biohazardous waste, put the instrument in STNDBY mode before disconnecting the waste container.

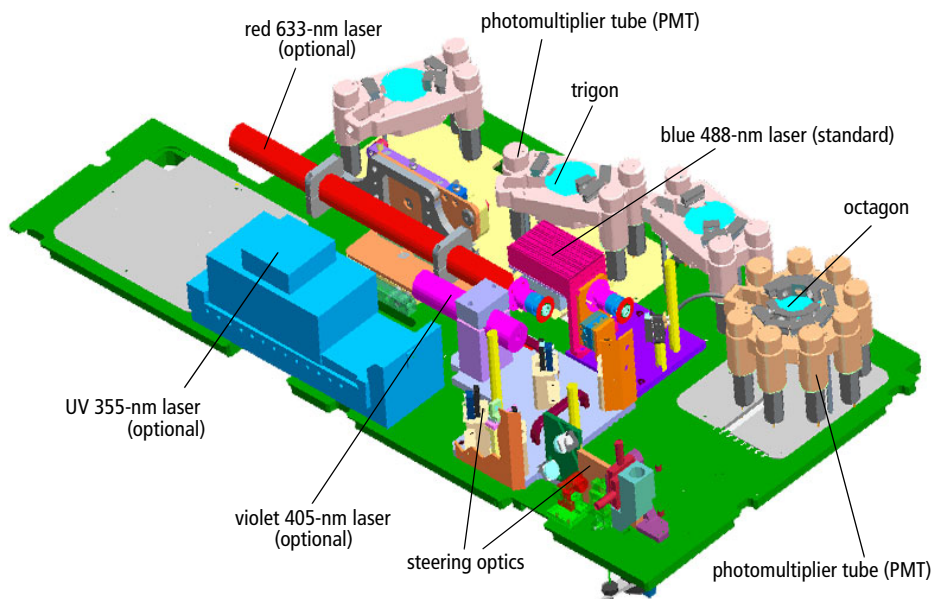
  The waste container contents might be biohazardous. Treat contents with bleach (10% of total volume). Dispose of waste with proper precautions in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

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

Optics

Figure 1-4 shows the optical bench components of the BD LSR II instrument.

Figure 1-4 Optical bench components (engineering model)



photomultiplier tubes (PMTs)	Devices that convert optical signals into electrical signals (see Detectors on page 35)
octagon	Array of PMTs and filters that can detect up to eight signals (Figure 1-5 on page 35)
trigon	Array of PMTs and filters that can detect up to three signals (Figure 1-6 on page 36)
lasers: blue 488 nm, red 633 nm, UV 355 nm, and violet 405 nm	See Lasers on page 33.
optics cover	Shielding that houses the flow cell, forward scatter (FSC) assembly (see Detectors on page 35), and the excitation optics

Lasers

The BD LSR II flow cytometer has a fixed-alignment 488-nm laser with the option of additional fixed-alignment lasers.

Table 1-1 BD LSR II flow cytometer laser options

	Lasers	Type	Wavelength (Color)	Power (mW)	Warm-Up Time (min)
Standard	Coherent Sapphire™	solid state	488 nm (blue)	20	30
Optional	JDS Uniphase 1344P	helium-neon (HeNe) gas	633 nm (red)	17	20
	Coherent Radius™ 405	solid state	405 nm (violet)	25	15
	Lightwave Xcyte™	solid state	355 nm (UV)	20	30

The primary blue 488-nm laser (Coherent Sapphire) generates forward scatter (FSC) and side scatter (SSC) signals and four fluorescence signals.

- The optional red 633-nm laser (JDS Uniphase 1344P) generates two fluorescence signals.
- The optional violet 405-nm laser (Coherent Radius 405) generates two fluorescence signals.
- The optional UV 355-nm laser (Lightwave Xcyte) generates two fluorescence signals.

Filters

Optical filters attenuate light or help direct it to the appropriate detectors. The BD LSR II instrument uses dichroic filters. Dichroic filters transmit light of a specific wavelength, while reflecting other wavelengths. The name and spectral characteristics of each filter appear on its holder.

There are three types of dichroic filters:

- Shortpass (SP) filters transmit wavelengths that are shorter than the specified value.
- Longpass (LP) filters transmit wavelengths that are longer than the specified value.
- Bandpass (BP) filters pass a narrow spectral band of light by combining the characteristics of shortpass filters, longpass filters, and absorbing layers. Discriminating filters (DF) and ALPHA™ filters (AF) are types of bandpass filters.

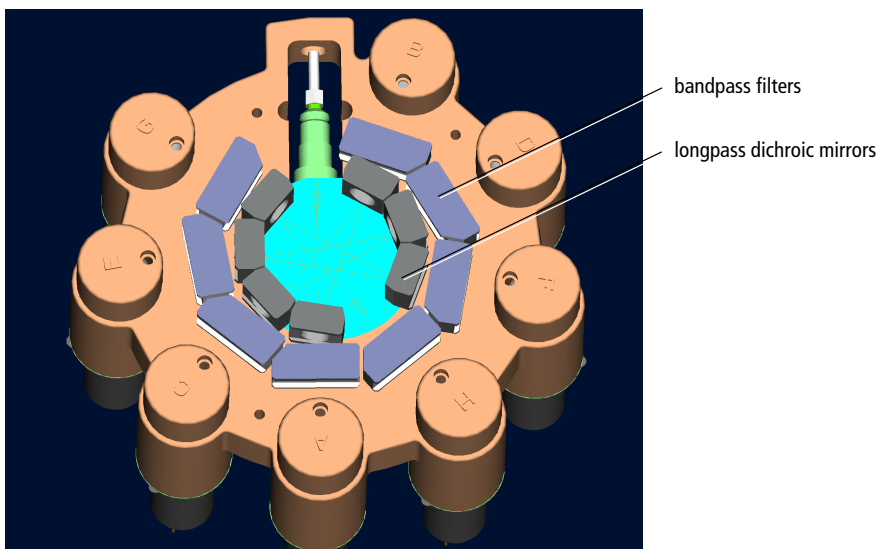
When dichroic filters are used as steering optics to direct different color light signals to different detectors, they are called dichroic mirrors or beam splitters.

- Shortpass dichroic mirrors transmit shorter wavelengths of light to one detector while reflecting longer wavelengths to a different detector.
- Longpass dichroic mirrors transmit longer wavelengths to one detector while reflecting shorter wavelengths to a different detector.

The BD LSR II instrument octagon and trigon detector arrays use dichroic longpass mirrors on their inner rings, and bandpass filters on their outer rings. However, you can customize the arrays with other types of filters and mirrors.

In Figure 1-5 on page 35, the inner ring is colored gray, and the outer is blue.

Figure 1-5 Dichroic filter types in octagon array



The steering optics and filters mounted on the BD LSR II instrument are listed in Table E-1 on page 154.

See Optical Filters on page 127 for a more detailed explanation of how filters work in the BD LSR II flow cytometer.

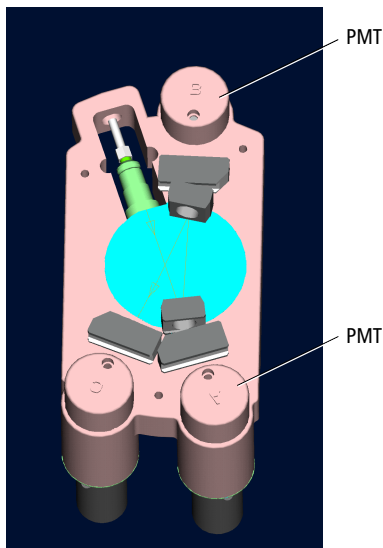
Detectors

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

There are two types of signal detectors in the BD LSR II flow cytometer: the photodiode and the photomultiplier tubes, or PMTs (Figure 1-6 on page 36). A photodiode is less sensitive to light signals than the PMTs. Therefore, a photodiode is used to detect the stronger forward scatter signal. PMTs are used to detect the weaker signals generated by side scatter and all fluorescence channels. These signals are amplified by applying a voltage to the PMTs. As the voltage is increased, the detector sensitivity increases, resulting in increased

signal. As the voltage is decreased, the detector sensitivity decreases, resulting in decreased signal. Detector voltages are adjusted in BD FACSDiva software.

Figure 1-6 PMT-type detectors in trigon array



The default locations of specific detectors and filters within BD LSR II instrument octagon and trigon arrays are shown in Table E-1 on page 154.

BD LSR II Workstation

Acquisition, analysis, and most BD LSR II instrument functions are controlled by the BD LSR II workstation. It includes a PC, one or two monitors, and a printer.

Your workstation is equipped with the following:

- a BD Biosciences–validated Microsoft® Windows® operating system
- BD FACSDiva software for data acquisition and analysis
- software documentation including an online help system

See BD LSR II Documentation on page xii for more information.

2

Instrument Setup

- Starting the Cytometer and Computer on page 38
- Setting Up the Optical Filters and Mirrors on page 39
- Preparing Sheath and Waste Containers on page 44
- Preparing the Fluidics on page 47
- Quality Control on page 49

Starting the Cytometer and Computer

- 1 Turn on the power to the flow cytometer.

Allow 60 minutes for lasers to warm up and stabilize.



Failure to warm up and stabilize the lasers could affect sample data.

- 2 Start up the BD LSR II workstation and log in to Windows.

Tip You can turn on the power to the flow cytometer and the workstation in any order.

- 3 Launch BD FACSDiva software by double-clicking the shortcut on the desktop, and log in to the software.

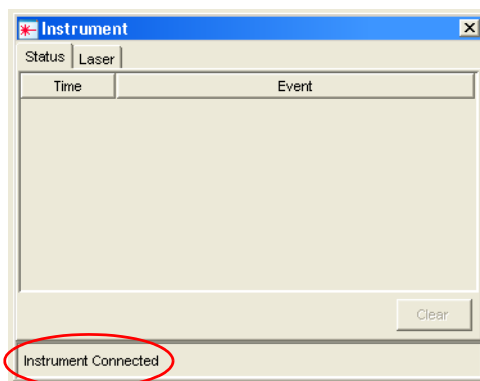
For details about user accounts in BD FACSDiva software, refer to the *BD FACSDiva Software Reference Manual*.

- 4 Check the Instrument window in BD FACSDiva software to ensure the cytometer is connected to the workstation.

The cytometer connects automatically. While connecting, the message *Instrument Connecting* is displayed in the window footer. When connection completes, the message changes to *Instrument Connected* (Figure 2-1 on page 39).

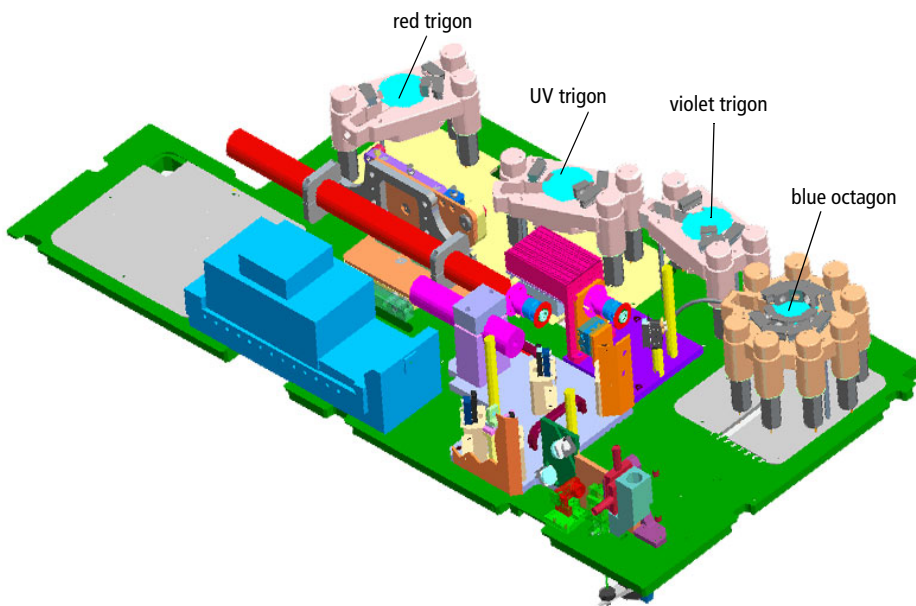
If the message *Instrument Disconnected* appears, refer to Electronics Troubleshooting in the *BD FACSDiva Software Reference Manual*.

Figure 2-1 Instrument Connected message



Setting Up the Optical Filters and Mirrors

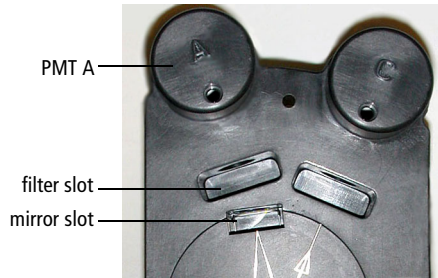
Before you run samples, set up the optical filters. The following figure shows the location of the detector arrays (beneath the instrument covers of the BD LSR II flow cytometer). Each detector array is labeled with its laser source.



Filter and Mirror Configurations

Each PMT (except the last PMT in every detector array) has two slots in front of it.

- The slot closest to the PMT holds a bandpass filter holder.
- The slot furthest from the PMT holds a longpass dichroic mirror holder.

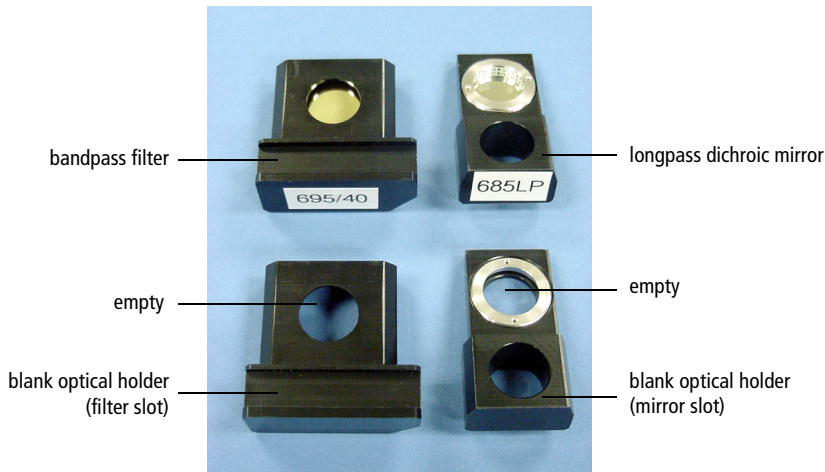


The last PMT in every detector array (PMT H in the octagon, and PMT C in all trigons) does not have a mirror slot.

Optical Holders, Filters, and Mirrors

Optical holders house filters and mirrors. Your instrument includes several blank (empty) optical holders.

Figure 2-2 Blank optical holders, filters, and mirrors



Tip To ensure data integrity, do not leave any slots unfilled in a detector array when you are using the associated laser. Always use a blank optical holder.

Default Configurations

Each BD LSR II instrument has a default filter and mirror configuration. The standard default configuration is specified for an instrument with an octagon detector array for the 488-nm blue laser, and trigon detector arrays for any optional lasers. See Appendix E for a detailed description of the standard default configuration, also called the 4-Blue 2-Violet 2-UV 2-Red default configuration.

Additional special-order configurations are specified for instruments with non-standard detector arrays. For example, there is a 6-Blue 6-Violet 2-UV 3-Red default configuration for an instrument that has octagon detector arrays for both the 488-nm blue laser and the 405-nm violet laser, and trigon detector arrays for both the UV laser and the 633-nm red laser. Detailed descriptions of the additional special-order configurations are in Appendix F.

BD FACSDiva Instrument Configuration

When you acquire data using BD FACSDiva software, you specify an instrument configuration. The instrument configuration defines which fluorochrome or scatter parameter is measured at each instrument detector.

BD FACSDiva software provides a default instrument configuration based on your BD LSR II. Use the Instrument Configuration dialog to modify, delete, and create new instrument configurations. (Refer to the Instrument and Acquisition Controls chapter of the *BD FACSDiva Software Reference Manual* for details.)

BD LSR II Configuration Upgrade

If you upgrade your BD LSR II cytometer with additional lasers or special order detector arrays, you will need to run the ICA software to update the BD FACSDiva database for your instrument. Instructions for installing and using the ICA software are in Appendix F.

Changing Optical Filters or Mirrors

  Follow the precautions outlined in Laser Safety on page xvii while changing optical filters or mirrors.

- 1** Lift the appropriate instrument cover.
 - The octagon array is located under the right instrument cover.
 - The three trigon arrays are located under the left instrument cover.
- Tip** To open the left instrument cover, you must first open the right cover and the side door.
- 2** Remove the appropriate filter holder or mirror holder.
- 3** Replace it with the new filter holder or mirror holder.
- Tip** The filters and mirrors fit easily into the optical holders in only one way.
- 4** Close the instrument cover(s).

Custom Configurations

See Custom Configurations in Appendix E for specifications of some common custom filter and mirror configurations. The custom filters and mirrors used in these configurations are included with your spares kit.

Filter and Mirror Specifications

Table 2-1 Longpass dichroic mirrors in octagon or trigon

Specification	Measurement
diameter	0.625 in. +0.000, -0.005
minimum clear aperture	0.562 in.
incident angle	11.25°
thickness	0.125 in \pm 0.005 in.

Table 2-2 Bandpass filters in octagon or trigon

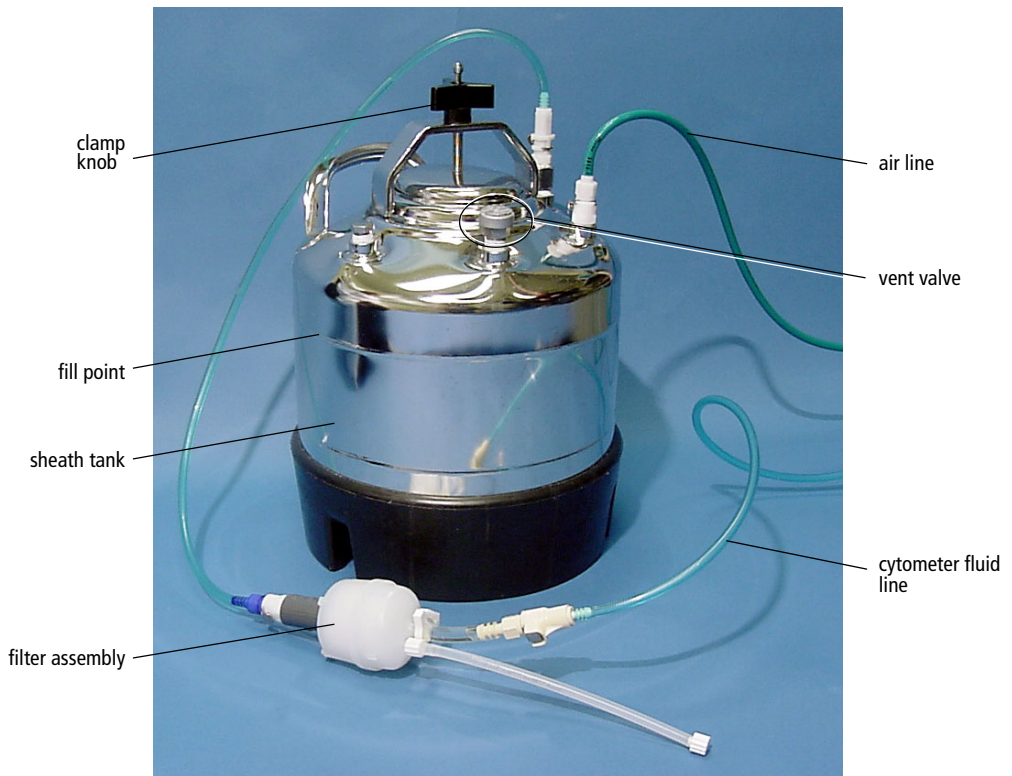
Specification	Measurement
diameter	1.00 in. \pm 0.010 in.
minimum clear aperture	0.85 in.
incident angle	0°
thickness	0.12–0.35 in.

Preparing Sheath and Waste Containers

- ☑ **Tip** Check the fluid levels in the sheath and waste containers every time you use the instrument. This ensures that you will not run out of sheath fluid during an experiment and that the waste container will not become too full.

Preparing the Sheath Container

Figure 2-3 Sheath container



- 1** Make sure that the flow cytometer is in STNDBY mode.
- 2** Disconnect the air line (green) from the sheath container (Figure 2-3 on page 44).
- 3** Depressurize the sheath container by pulling up on its vent valve.
- 4** Remove the sheath container lid.

Unscrew the clamp knob and lift.

- 5** Add 6 L of sheath fluid, such as BD FACSSlow™ solution, to the sheath container.

NOTICE Do not fill the sheath tank to its maximum capacity (8 L). When a full tank is pressurized, erratic instrument performance can result.

- Tip** For calcium-flux experiments, use a sheath fluid that does not contain preservatives. For example, use 1X phosphate-buffered saline (PBS).

- 6** Close the sheath container lid.
- 7** Reconnect the air line (green).
- 8** Make sure the lid is tightly sealed with the gasket in place, the clamp knob is finger-tight, and that the cytometer fluid line (blue) is not kinked.

- Tip** Inspect the sheath container periodically, as sheath fluid can cause corrosion.

Preparing the Waste Container



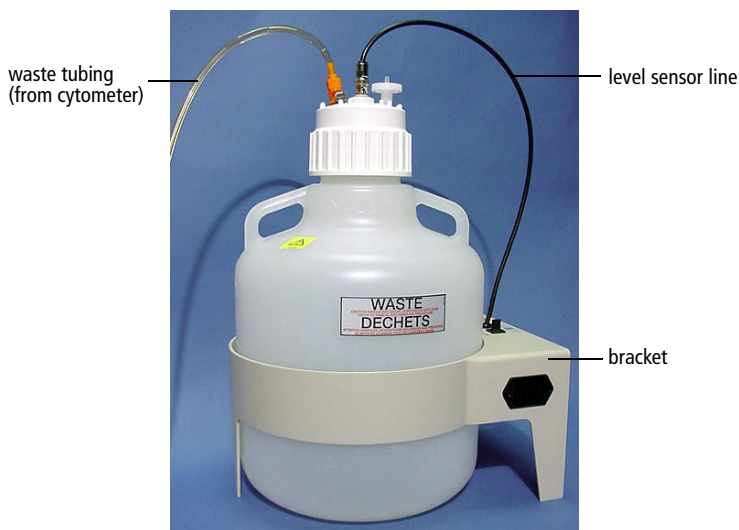
-   All biological specimens and materials coming into contact with them are considered biohazardous. Handle as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear,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



Figure 2-4 Waste container



- 1 Disconnect the orange waste tubing and the black level sensor line from the waste container.

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

- 2 Empty the waste container.

-   The waste container contents might be biohazardous. Treat contents with bleach (10% of total volume). Dispose of waste using proper precautions and in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

- 3 Add approximately 1 L of bleach to the waste container and close it.
- 4 Reconnect the orange waste tubing and make sure it is not kinked.
- 5 Reconnect the level sensor line.

Preparing the Fluidics

Next, make sure the fluidics system is ready. This section covers the following preparations:

- Removing Air Bubbles (below)
- Priming the Fluidics on page 49

Removing Air Bubbles

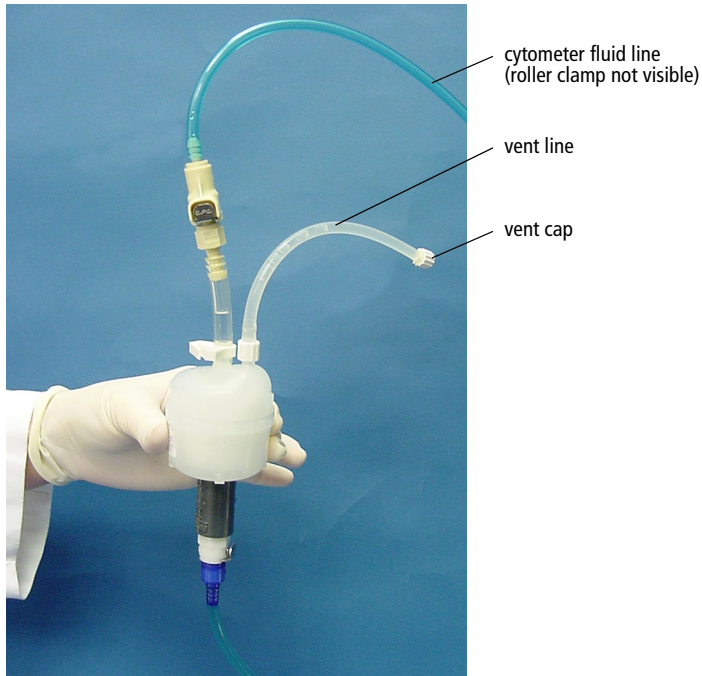
Trapped bubbles can occasionally dislodge and pass through the flow cell, resulting in inaccurate data.

NOTICE Do not vigorously shake, bend, or rattle the sheath filter or you might damage it.

- 1 Check the sheath filter for trapped air bubbles.
- 2 If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.

- 3 Pinch the vent line closed.

Figure 2-5 Sheath filter



- 4 Loosen the sheath filter vent cap to bleed off any air in the filter; collect the excess fluid in a container.
- 5 Replace the vent cap.
- 6 Check the fluid lines for air bubbles.
- 7 Open the roller clamp on the blue cytometer fluid line (if necessary) to bleed off any air in the line.
- 8 Close the roller clamp.

Priming the Fluidics

Sometimes, air bubbles and debris are stuck in the flow cell. This is indicated by excessive noise in the forward scatter parameter. In these cases, it is necessary to prime the fluidics system.

- 1** Remove the tube from the SIP.
- 2** Press the PRIME fluid control button to force the fluid out of the flow cell and into the waste container.

Once drained, the flow cell automatically fills with sheath fluid at a controlled rate to prevent bubble formation or entrapment. The STNDBY button is amber after completion.

- 3** Install a 12 x 75-mm tube with 1 mL of DI water on the SIP and place the support arm under the tube.
- 4** Repeat the priming procedure, if needed.

Quality Control

An instrument quality control (QC) procedure, performed on a regular basis, provides a standard for monitoring instrument performance. Instrument QC consists of running QC samples and recording parameter means and CVs.

QC data should be analyzed for trends involving 10 or more runs. By keeping the instrument settings and QC sample constant, changes in the recorded means and CVs will indicate how much instrument performance varies over time.

QC results are affected by laser and fluidics performance. We strongly recommend following the laser and fluidics maintenance procedures in Chapter 6.

See QC Particles on page 150 for a list of acceptable QC beads.

A sample QC log is provided in Appendix B. It can be photocopied or used as a guide in designing your own QC log.

3

Running Samples

This chapter describes procedures that use BD FACSDiva software to record and analyze sample data:

- Sample Optimization Using Instrument Setup on page 52
- Recording and Analyzing Data on page 65

Before You Begin

Before attempting to perform the procedures in this chapter, you should be familiar with:

- BD LSR II instrument startup, setup, and QC procedures (see Chapter 2, Instrument Setup on page 37)
- BD FACSDiva software concepts: workspace components, instrument and acquisition controls, tools for data analysis.

To become familiar with BD FACSDiva software, perform the tutorial exercises in *Getting Started with BD FACSDiva Software*.

For additional details, refer to the *BD FACSDiva Software Reference Manual*.

Sample Optimization Using Instrument Setup

Before you record data for a sample, optimize the following for the sample type and fluorochromes used:

- PMT voltages
- threshold setting
- compensation values

This section describes how to optimize samples using the Instrument Setup feature of BD FACSDiva software. In particular, instrument setup automatically calculates compensation settings. If you choose to perform compensation manually, not all of the instructions given below will apply. For detailed instructions, refer to the *BD FACSDiva Software Reference Manual*.

Sample optimization consists of several main steps, to be performed in the order listed below. Each of the steps is explained in greater detail in the sections that follow.

- 1** Verify instrument configuration and user preferences.
- 2** Create an experiment.
- 3** Adjust voltages and the threshold setting.
- 4** Record the compensation tubes.
- 5** Calculate compensation settings.

The data shown in this example is from a 4-color bead sample with the following fluorochromes:

- FITC
- PE
- PerCP
- APC

To perform the example exercise shown here, prepare an unstained control tube and single-stained tubes for each fluorochrome.

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

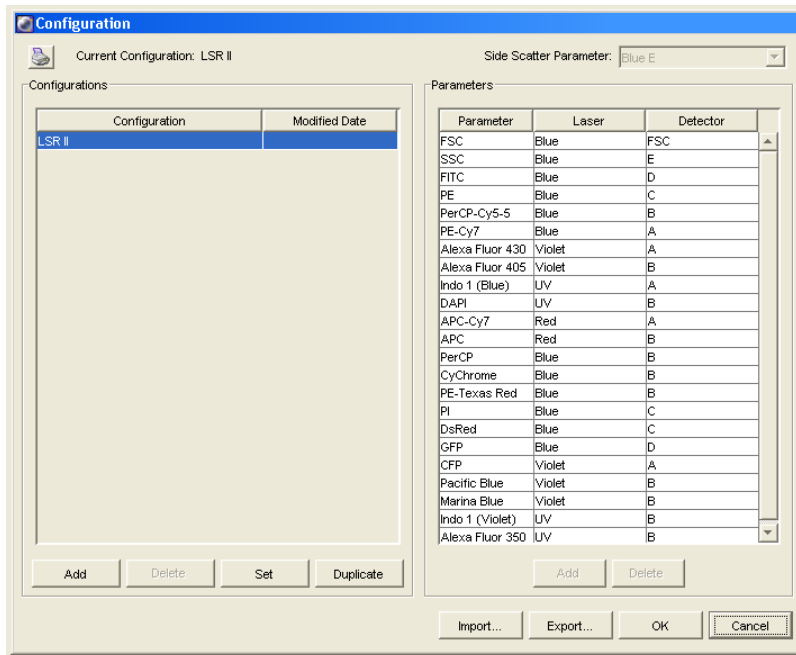
Verifying Instrument Configuration and User Preferences

To obtain accurate data results, the current BD FACSDiva software instrument configuration must reflect your BD LSR II instrument optics. Verify the configuration and preferences before you create an experiment.

- 1 Choose Instrument > Instrument Configuration and verify the current configuration.

Figure 3-1 shows an Instrument Configuration dialog whose current configuration is the standard default configuration.

Figure 3-1 Instrument Configuration dialog



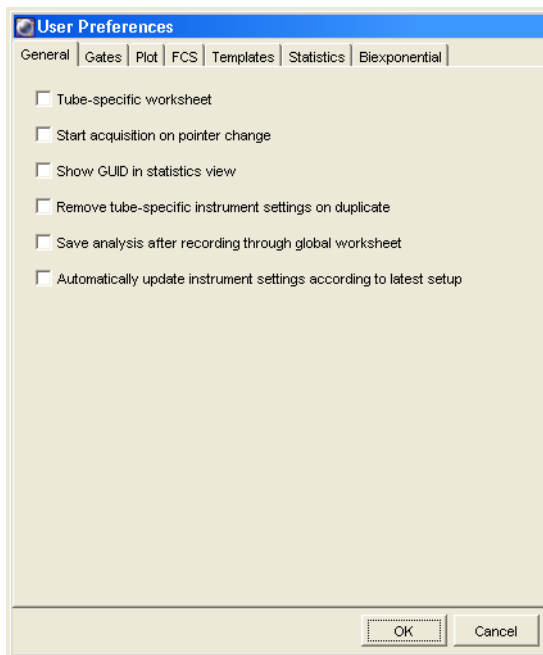
Check that the configuration includes the parameters to be measured and that the channels correspond to the optical mirrors and filters. For our bead

sample, the instrument configuration includes the following parameters: FITC, PE, PerCP, and APC.

2 Choose Edit > User Preferences.

- Verify that the settings under the General tab match those displayed in Figure 3-2 (all checkboxes deselected).

Figure 3-2 User Preferences dialog



- Under the Templates tab, verify that the *Default global worksheet* checkbox is enabled.

Refer to the *BD FACSDiva Software Reference Manual* for more information about instrument configuration and user preferences.

Creating an Experiment

In this section, you create an experiment in a new folder, specify the parameters for instrument setup, and add compensation tubes.







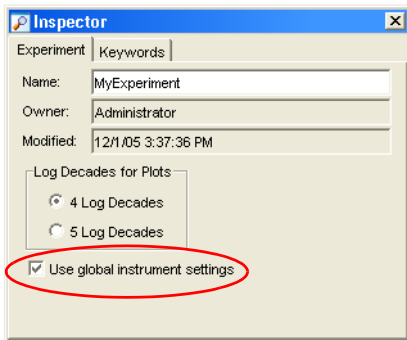
- 1 Click the corresponding buttons in the Workspace toolbar to display the Browser (, Instrument (, Inspector (, Worksheet (, and Acquisition Dashboard () windows, as needed.
- 2 Add a new folder to the Browser (); rename the folder *MyFolder*.
- ✓ **Tip** To place an experiment inside a folder, select the folder *before* you create the experiment.
- 3 Create an experiment inside *MyFolder*, and rename the experiment *MyExperiment*.
- 4 With the experiment selected in the Browser, verify in the Inspector that the *Use global instrument settings* checkbox is checked.

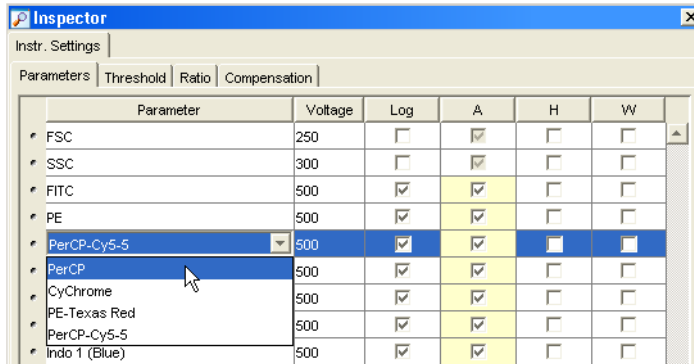
Figure 3-3 Use global instrument settings checkbox



- 5 Specify the parameters for instrument setup.
 - In the Browser, select the Instr Settings of *MyExperiment*.
 - Make sure the parameters you need appear on the Parameters tab in the Inspector.

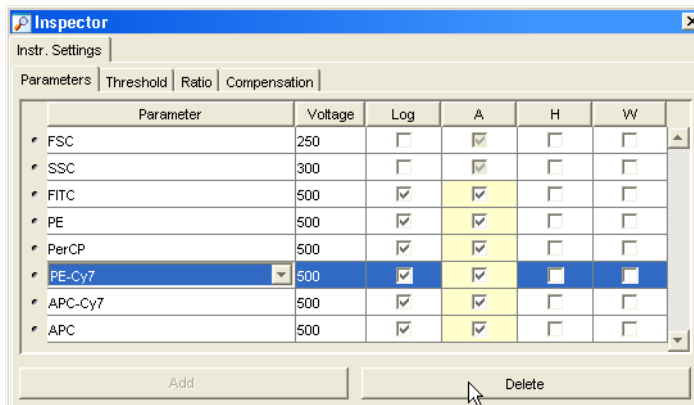
If more than one parameter is available for a particular PMT, you might have to select the one you need from a menu.

For this example, choose PerCP from the PerCP-Cy5-5 menu.



- Delete any unnecessary parameters.

To delete a parameter, select it (by clicking the selection button to the left of the parameter name), and then click the Delete button.



- Verify that Log is deselected for the FSC and SSC parameters, and is selected for each fluorescence parameter.

- Verify that A (area) is selected for every parameter, and that H (height) and W (width) are deselected for every parameter.

6 Choose Instrument > Instrument Setup > Create Compensation Controls.

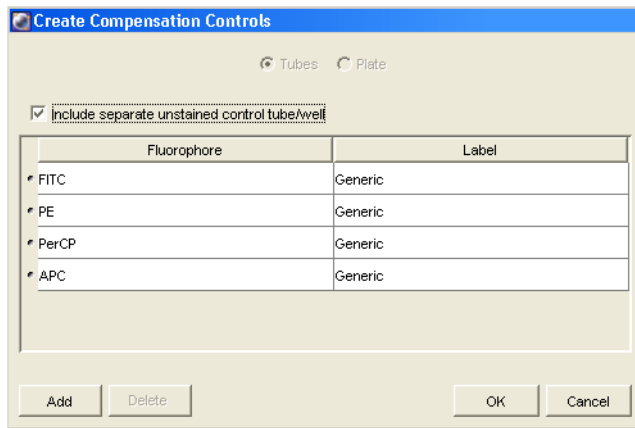
The Create Compensation Controls dialog is displayed (Figure 3-4).

- Verify that the *Include separate unstained control tube/well* checkbox is selected, and that the compensation control tube list matches the fluorescent parameters selected in step 5 above.

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

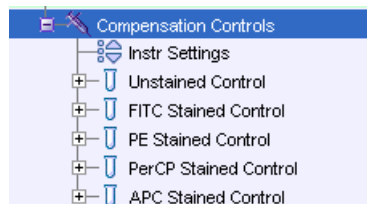
- Click OK to create the control tubes.

Figure 3-4 Create Compensation Controls dialog



Compensation controls are added to the experiment, along with a single stained control tube for each parameter in the experiment, and an unstained control tube (Figure 3-5). Worksheets containing appropriate plots and gates are added for each compensation tube.

Figure 3-5 Compensation tubes



Adjusting the Voltages and Threshold

In this section, you use the unstained control tube to adjust FSC and SSC voltages and FSC threshold, to gate the population of interest (bead singlets, in this case), and to adjust fluorescence PMT voltages.

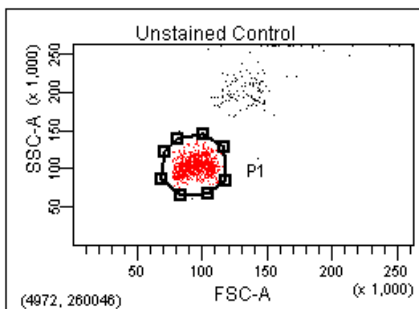
- 1 Install the unstained control tube on the cytometer.
- 2 Expand the Compensation Controls specimen in the Browser.
- 3 Click to set the current tube pointer next to the unstained control tube (it becomes green) and click Acquire Data.
- 4 Adjust the FSC and SSC voltages to appropriately position the singlet bead population for gating (Figure 3-6 on page 60).
 - Click the Parameters tab in the Instrument window.
 - Use the up and down arrows or drag the voltage sliders to adjust the voltage settings.
- 5 Click the Threshold tab and adjust the FSC threshold, if needed.

Adjust the FSC Threshold to remove most of the debris without clipping the singlet population (Figure 3-6 on page 60).

- 6 Adjust the P1 gate on the Unstained Control worksheet, as needed, to encompass only the singlet population (Figure 3-6).

Tip To quickly adjust the gate, right-click the gate boundary, and choose Recalculate.

Figure 3-6 P1 gate adjusted to singlet population

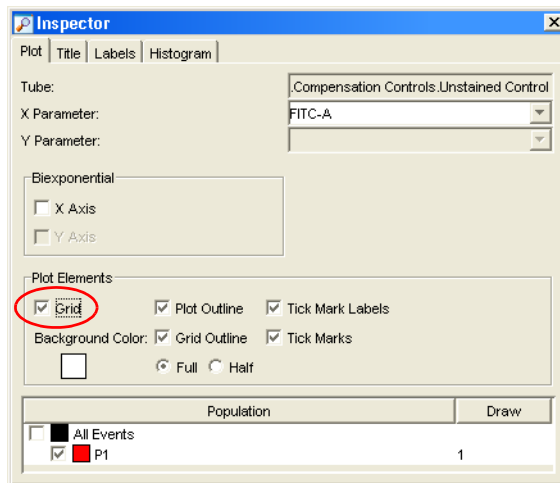


- 7 Right-click the gate and choose Apply to All Compensation Controls.

The P1 gate on each Stained Control worksheet is updated with your changes.

- 8 Display log decade gridlines on the fluorescence histograms.
 - Select all fluorescence histograms on the Unstained Control worksheet.
 - In the Plot Inspector, select the Grid checkbox (Figure 3-7).

Figure 3-7 Inspector for fluorescent plots

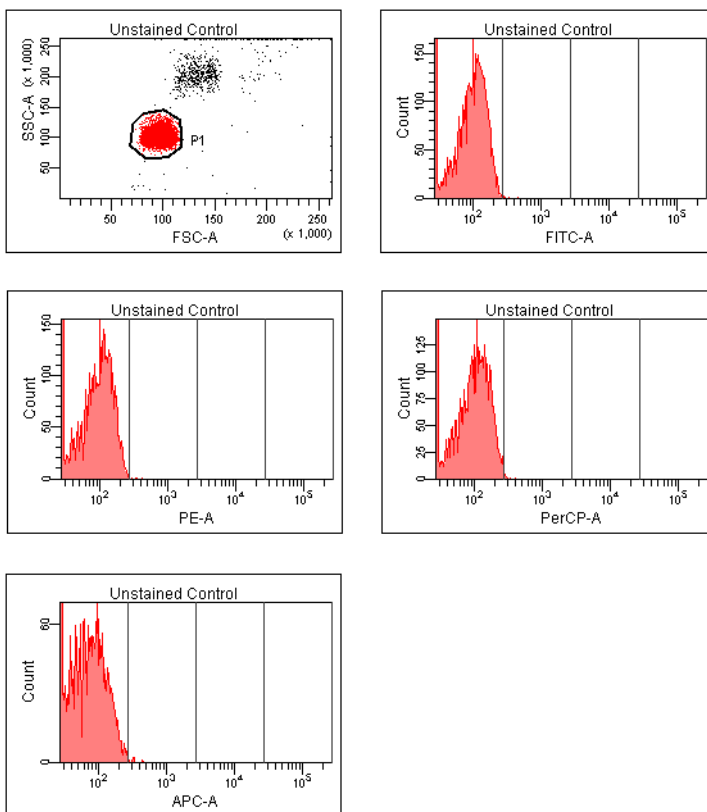


In a four decade log display, values are displayed from 26–262,143. The first log decade ranges from 26–262. Gridlines are used to delineate log decades on plots.

- 9 For each fluorescence parameter, adjust PMT voltages to place the negative population within the first log decade (Figure 3-8 on page 62).

To adjust voltage settings, use controls in the Parameters tab in the Instrument window. Refer to the *BD FACSDiva Software Reference Manual* for assistance.

Figure 3-8 Unstained Control worksheet plots after PMT adjustment



- 10 Click Record Data; when all events have been recorded, remove the unstained control tube from the cytometer.



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

Calculating Compensation

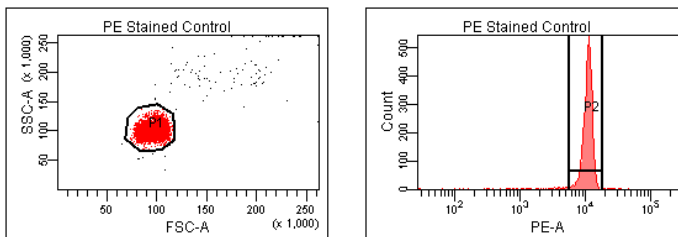
Before you can calculate compensation, you need to record data for each single-stained control.

- 1 Install the first stained control tube onto the cytometer.
- 2 In the Acquisition Dashboard, click Next Tube, and then Acquire Data.

Alternatively, move the current tube pointer to the next tube and click the pointer to start acquisition.
- 3 Click Record Data, or Alt-click the current tube pointer to record data.
- 4 When recording is finished, install the next stained control tube onto the cytometer.
- 5 Repeat steps 2 through 4 until data for all stained control tubes has been recorded.
- 6 Double-click the first stained control tube (FITC stained control) to display the corresponding worksheet.
- 7 Verify the snap-to interval gate surrounds the fluorescence-positive peak on the histogram (Figure 3-9).

Adjust the gate, if needed.

Figure 3-9 Gating the positive population

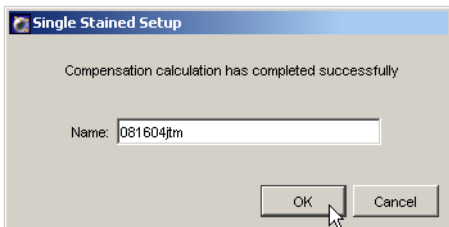


- 8 Repeat steps 6 and 7 for the remaining compensation tubes.

- 9 Choose Instrument > Instrument Setup > Calculate Compensation.

If the calculation is successful, a dialog is displayed where you can enter a name for the compensation setup.

- 10 Enter a setup name, and click OK.



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

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

NOTICE BD Biosciences recommends that you always visually and statistically inspect automatically calculated overlap values. The means of the positive controls should be aligned with the means of the negative.

Recording and Analyzing Data

This section outlines some basic acquisition and analysis tasks using BD FACSDiva software. The example shows data from two 4-color bead samples with the following fluorochromes:

- FITC
- PE
- PerCP
- APC

The procedure builds on the results obtained in the previous exercise: Sample Optimization Using Instrument Setup on page 52. To perform this procedure, prepare two tubes containing all four fluorochromes.

If you use a different sample type (or if you have skipped the optimization exercise), your software window content and your data plots and statistics may deviate from those shown here. You might also need to modify some of the instructions in the procedure.

For additional details on completing some of the steps below, refer to the *BD FACSDiva Software Reference Manual*.

Preparing the Workspace

In this section, you prepare your workspace before recording data.

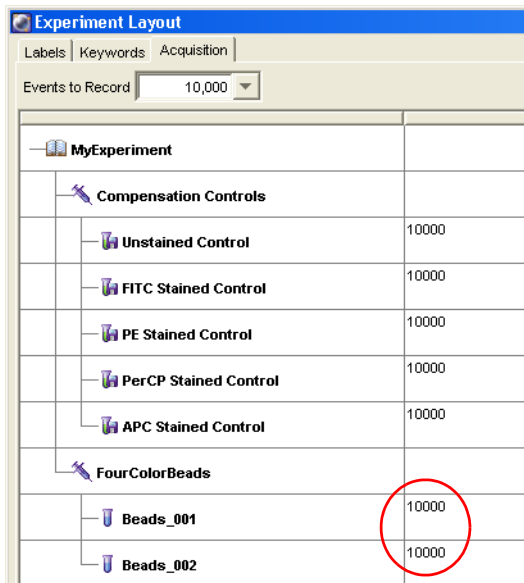
- 1** Using the Browser toolbar, create a new specimen in *MyExperiment* and rename it *FourColorBeads*.
- 2** Create two tubes for the *FourColorBeads* specimen. Rename the tubes *Beads_001* and *Beads_002*.
- 3** Expand the Global Worksheets folder in *MyExperiment* to access the default global worksheet, and rename the worksheet *MyData*.

- 4 Choose Experiment > Experiment Layout to display the Experiment Layout dialog.

Use this dialog to define parameter labels and to specify the number of events to record for each tube.

Parameter labels appear on the plot axes and in all statistics views. For these bead samples, you do not need to define additional parameter labels. When you record immunophenotyping data, use the Labels tab of the Experiment Layout dialog to provide reagent antibody labels.

- Click the Acquisition tab. Verify the number of events to acquire is set to 10,000 for both tubes.



- Click OK.

5 On the *MyData* worksheet, create some plots for previewing data:

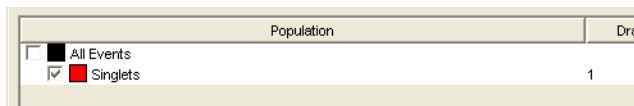
- FSC vs SSC
- FITC vs PE
- FITC vs PerCP
- FITC vs APC

Tip Double-click the Dot Plot button to keep the button selected until you create all plots.

Recording Data

In this section, you preview and record data for multiple samples.

- 1** Install the first sample tube on the cytometer.
- 2** Move the current tube pointer to *Beads_001*.
- 3** Click Acquire Data in the Acquisition Dashboard to begin acquisition.
- 4** While data is being acquired:
 - Draw a gate around the singlets on the FCS vs SSC plot.
 - Rename the P1 gate to *Singlets*.
 - Use the Inspector to set the other plots to show only the singlet population.

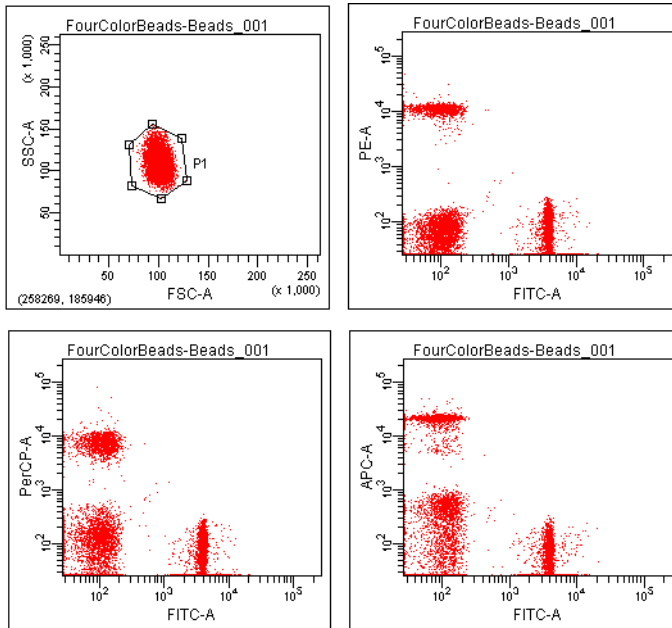


- 5** Click Record Data.

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

The *MyData* worksheet plots should resemble those in Figure 3-10.

Figure 3-10 Recorded data showing singlet population



- 7 Install the second sample tube on the cytometer.
- 8 Move the current tube pointer to *Beads_002*.
- 9 Click Acquire Data to begin acquisition.
Before recording, preview the data on the *MyData* worksheet.
- 10 Click Record Data.
- 11 When event recording has completed, remove the second tube from the cytometer.

- 12** If you are recording more than two tubes, repeat steps 7 through 11 for the additional tubes.
- 13** Print the experiment-level instrument settings.

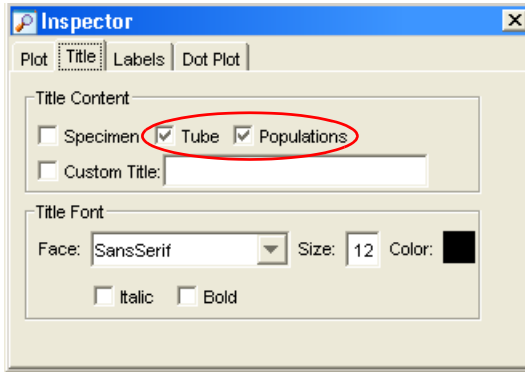
Right-click the Instr Settings icon and choose Print.

Analyzing Data

In this section, you analyze the recorded tubes by creating plots, gates, a population hierarchy, and statistics views on a new global worksheet. When complete, your new global worksheet should resemble that in Figure 3-11 on page 72.

- 1** Use the Browser toolbar to create a new global worksheet; name it *MyDataAnalysis*.
- 2** Create the following plots on the *MyDataAnalysis* worksheet:
 - FSC vs SSC
 - FITC vs PE
 - FITC vs PerCP
 - FITC vs APC
- 3** Create a population hierarchy and a statistics view, and move them below the plots on the worksheet.
 - Choose Populations > Show Population Hierarchy.
 - Choose Populations > Create Statistics View.
- 4** Move the current tube pointer to *Beads_001*.
- 5** Draw a gate around the singlets on the FSC vs SSC plot.
- 6** Use the population hierarchy to rename the population *Singlets*.

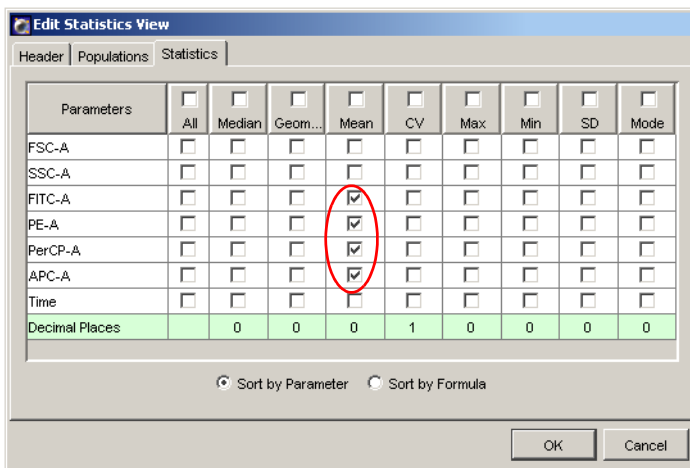
- 7 Select all plots except the FSC vs SSC plot, and use the Plot tab in the Inspector to specify to show only the singlet population.
- 8 Select all plots, and click the Title tab in the Inspector; select the Tubes and Populations checkboxes to display their names in plot titles.



- 9 On all fluorescence plots:
 - Draw a gate around the FITC-positive population, for the first plot only, and name the population *FITC positive*.
 - Draw a gate around the PE-positive population, and name the population *PE positive*.
 - Draw a gate around the PerCP-positive population, and name the population *PerCP positive*.
 - Draw a gate around the APC-positive population, and name the population *APC positive*.

10 Format the Statistics view.

- Right-click the Statistics view and choose Edit Statistics View.
- Click the Header tab, and select the Specimen Name and Tube Name checkboxes.
- Click the Populations tab, and select all populations except All Events; deselect the %Parent, %Total, and # Events checkboxes.
- Click the Statistics tab, and select the mean for each of the fluorescence parameters.

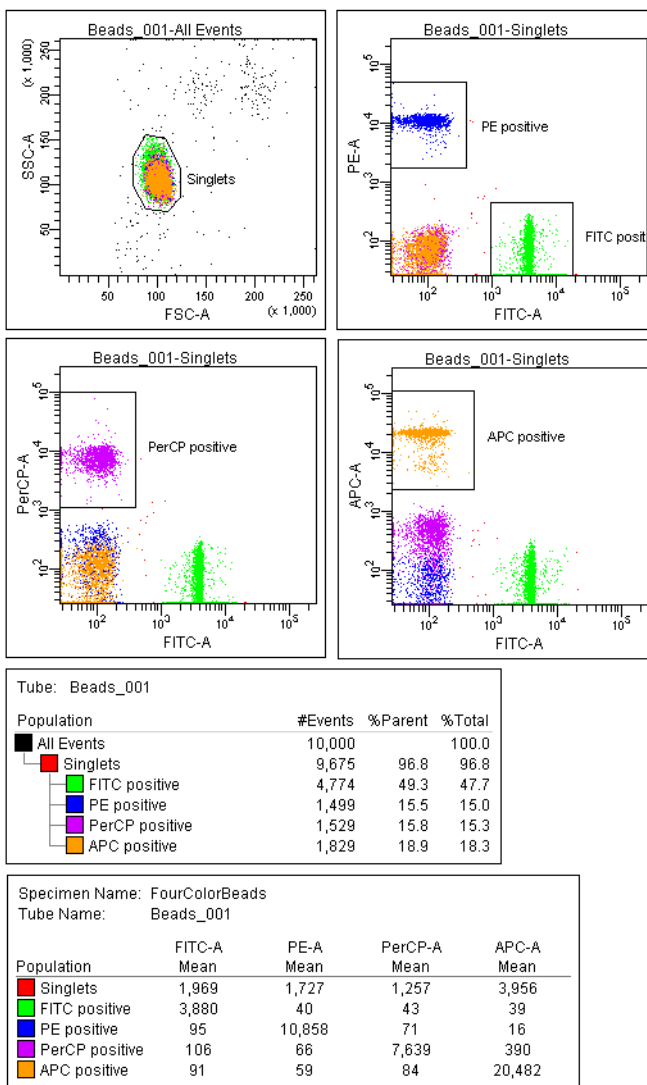


- Click OK.

11 Print the analysis.

Your global worksheet analysis objects should now resemble those in Figure 3-11 on page 72.

Figure 3-11 Bead analysis



Reusing the Analysis

Global worksheets allow you to apply the same analysis to a series of recorded tubes. Once you define an analysis for a tube, use it to analyze the remaining tubes in the experiment. After viewing the data, print the analysis or save it to a tube-specific worksheet (see Saving the Analysis).


- 1 Move the current tube pointer to the tube *Beads_002*.
 - 2 View the *Beads_002* data on your analysis worksheet. Adjust gates as needed.
- Tip** Adjustments will apply to subsequent tubes viewed on the worksheet. Avoid altering a global worksheet by saving an analysis to a tube-specific worksheet, and making adjustments on the tube-specific worksheet.
- 3 Print the analysis.

Saving the Analysis

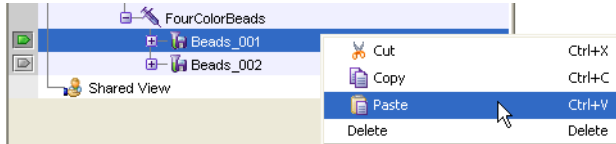
When an analysis is performed with a global worksheet, the analysis is not saved with the tube.

- Tip** If you define your analysis on a global worksheet before recording data, you can specify to automatically save the analysis after recording data. This option is set in user preferences.

To save a copy of the analysis of *Beads_001* with that tube:

- 1 Expand the *MyDataAnalysis* global worksheet icon in the Browser.
- 2 Right-click on its analysis and select Copy.
- 3 Click the Worksheets View button () on the Worksheet toolbar to switch to the normal (tube-specific) worksheet view.
- 4 Choose Worksheet > New Worksheet to create a new normal worksheet.

- 5 Click in the new worksheet; use the Worksheet Inspector to rename it *Beads_001_Analysis*.
- 6 Right-click the *Beads_001* tube icon in the Browser, and choose Paste.



The analysis objects from the *MyDataAnalysis* global worksheet are copied to the *Beads_001_Analysis* normal worksheet. View the analysis by double-clicking the *Beads_001* tube icon in the Browser.

- Tip** Apply the global worksheet analysis to multiple tubes (on a single normal worksheet) by selecting more than one tube before you paste the analysis.

4

DNA Analysis

The following topics are covered in this chapter:

- Criteria for DNA Experiments on page 76
- CEN Optimization on page 78
- CTN Resolution on page 84
- Optimization for Data Recording on page 86

Criteria for DNA Experiments

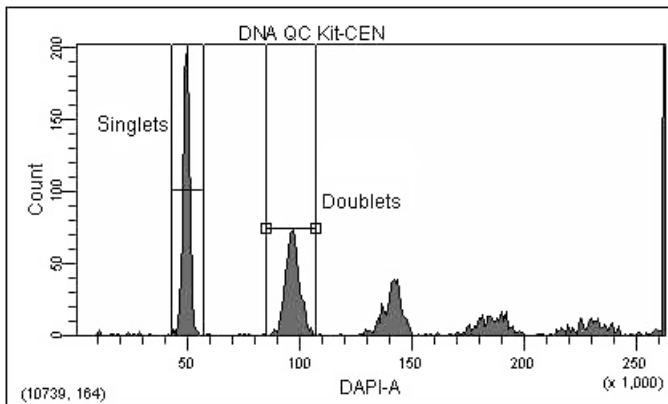
To support DNA experiments, a flow cytometer must provide:

- ability to resolve histogram peaks (populations)
- linearity of the DNA fluorescence signal
- ability to distinguish singlets from aggregates

While obtaining good peak resolution for DNA also depends on proper sample preparation, optimization of instrument optics and fluidics is critical. A flow cytometer's ability to resolve peaks can be assessed by measuring the CV of a reference particle: the lower the CV, the better the resolution.

Linearity is also critical for DNA experiments. To assess the linearity of DNA data, the pulse-area signal is used to measure the amount of DNA fluorescence detected from cells or nuclei. On an instrument with good linearity, the doublet peak should be located at twice the mean channel of the singlet peak (Figure 4-1).

Figure 4-1 Area signal and DNA fluorescence



Doublet discrimination, or the ability to distinguish singlets from aggregates, is also important for DNA experiments. Since doublets of G₀/G₁ cells have the same amount of DNA fluorescence as singlet G₂+M cells, they accumulate in the same fluorescence area channels (Figure 4-1 on page 76). Therefore, singlets and doublets must be distinguished to obtain cell-cycle analysis accuracy. Signal width vs area can be employed to accurately identify aggregate events.

DNA Setup

In this chapter, you use the BD™ DNA QC Particles kit to verify critical DNA analysis criteria, and optimize your cytometer for DNA experiments. The instructions given here assume that DAPI is being used as described in How to Use DAPI with DNA QC, below. If you are using PI for DNA QC, see How to Use PI with DNA QC, and substitute PI for DAPI in subsequent instructions.

How to Use DAPI with DNA QC

Before beginning this chapter, do the following.

- Prepare biological standards for instrument quality control using the BD DNA QC Particles kit. Substitute the PI solution in the BD DNA QC Particles kit with a 1.0-nanomolar (nM) DAPI solution prepared in 1% BSA.
- Prepare one tube each of chicken erythrocyte nuclei (CEN) and calf thymocyte nuclei (CTN) sample according to the kit instructions. Substitute the DAPI solution for the PI solution.
 - The CEN sample is used to check instrument resolution (CV) and linearity.
 - The CTN sample is used to verify the system's ability to resolve singlets from aggregates.

How to Use PI with DNA QC

You can use the PI that comes with the BD DNA QC Particles kit instead of DAPI. To do so, prepare the CEN and CTN samples as described in the kit instructions.

CEN Optimization

Use the following procedure to set up BD FACSDiva software for a DNA experiment that uses DAPI as the DNA-staining dye. If you are using another sample type, modify the steps accordingly. If you are using PI, substitute PI for DAPI throughout the procedure.

Preparing the Workspace

- 1 Choose Instrument > Instrument Configuration and verify the current configuration.

Figure 3-1 on page 54 shows an Instrument Configuration dialog whose current configuration is the standard default configuration.

Verify that DAPI appears in the current configuration.

For accurate data results, the Instrument Configuration dialog box must reflect the physical layout of the BD LSR II octagons and trigons. Modifications to the current configuration will not apply unless you click Set Configuration.

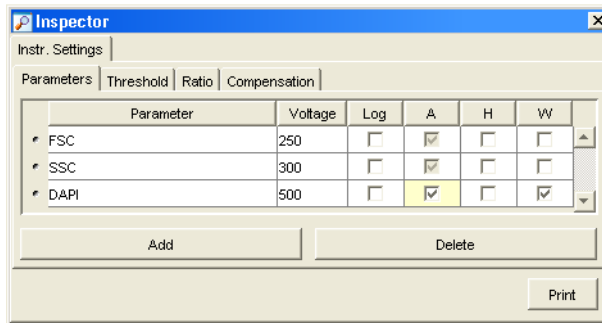
- 2 Choose Edit > User Preferences.

Verify that under the General tab, all checkboxes are deselected (Figure 3-2 on page 55).

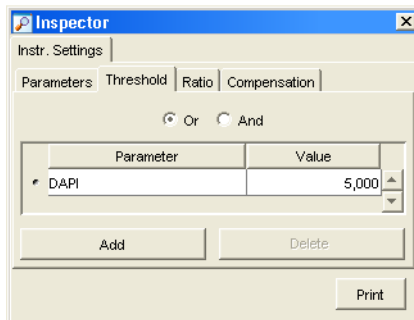
Refer to the *BD FACSDiva Software Reference Manual* for more information about the instrument configuration and user preferences.

- 3 Create a new experiment and rename it *DNA*.

- 4 With the *DNA* experiment icon selected, verify in the Inspector that the *Use global instrument settings* checkbox is checked (Figure 3-3 on page 56).
- 5 Select the Instr Settings icon in the *DNA* experiment, and use the Inspector to specify the parameters and threshold setting for your experiment.
 - Click the Parameters tab in the Inspector.
 - For this experiment, delete all parameters except FSC, SSC, and DAPI.
 - Verify that the Log checkbox is deselected for all parameters.
 - For DAPI, verify that the A (area) checkbox is selected, and select the W (width) checkbox.

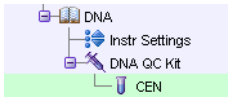


- Click the Threshold tab and change the threshold parameter to DAPI; verify that the threshold value is set to 5,000.



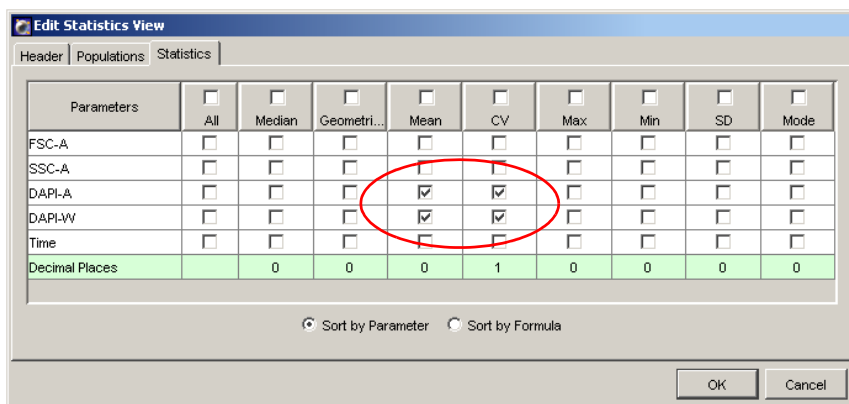
- 6 Create a new specimen in the *DNA* experiment and rename it *DNA QC Kit*.
- 7 Open the specimen to access its tube. Rename the tube *CEN*, and move the current tube pointer to *CEN*.

Your experiment should look similar to the figure below.



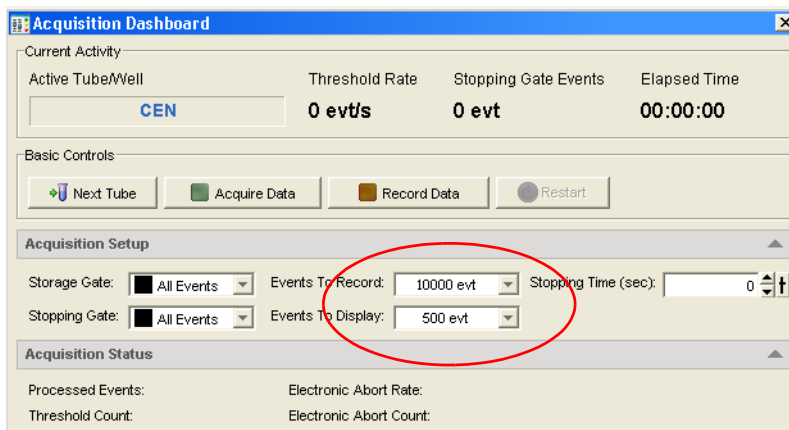
- 8 Create a normal worksheet for the *CEN* tube with the following plots:
 - FSC-A vs SSC-A dot plot
 - DAPI-A vs DAPI-W dot plot
 - DAPI-A histogram
- 9 Create a statistics view and display the mean and CV for DAPI-A and DAPI-W.
 - Choose Populations > Create Statistics View.
 - Right-click on the Statistics view and select Edit Statistics View.
 - On the Population tab, deselect #Events and %Parent.
 - On the Statistics tab, select the mean and CV for both DAPI-A and DAPI-W, and set Decimal Places to 1 for the CVs (Figure 4-2 on page 81).

Figure 4-2 Edit Statistics view



- Click OK.

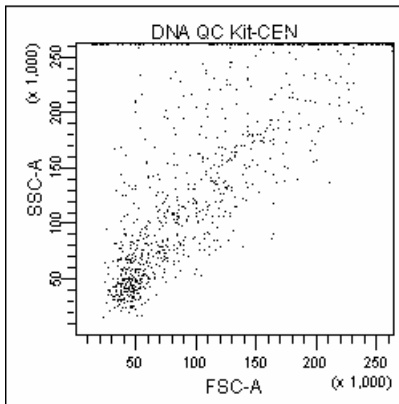
10 In the Acquisition Dashboard, set the Events to Record to 10,000 and the Events to Display to 500.



Running CEN

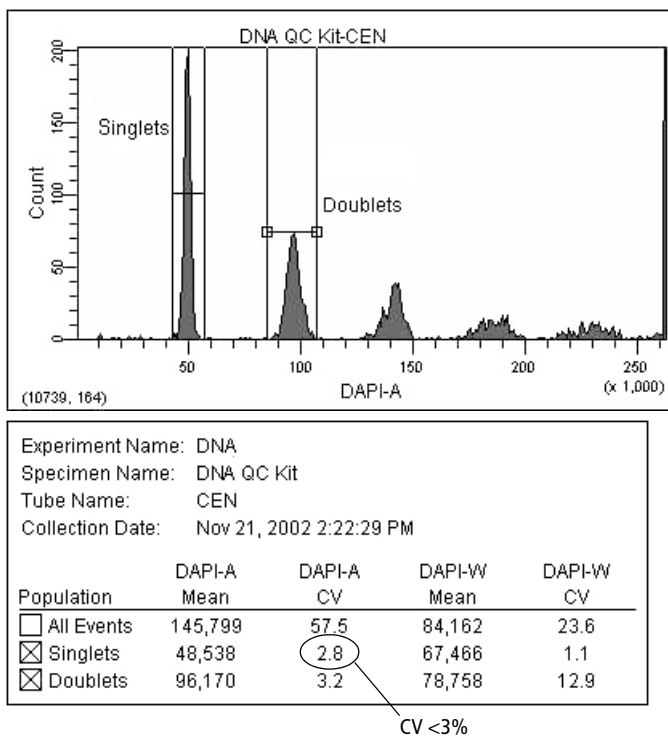
- 1 On the BD LSR II cytometer control panel, press RUN and LO.
- 2 Install the CEN sample tube on the SIP.
- 3 Verify that the green current tube pointer is in front of the CEN tube in the Browser.
- 4 In the Acquisition Dashboard, click Acquire Data.
- 5 Adjust the FSC and SSC voltages to place the CEN on scale in the FSC vs SSC dot plot.

Figure 4-3 FSC and SSC voltages adjusted



- 6 Adjust the DAPI voltage to place the singlet nuclei at approximately channel 50×10^3 on the DAPI-A axis in the DAPI-A histogram plot (Figure 4-4 on page 83).
- 7 Adjust the event rate (displayed in the Acquisition Dashboard) to approximately 200 events/second with the SAMPLE FINE ADJ knob.
- 8 Draw Interval gates around the first two peaks on the DAPI-A histogram. Name the populations *Singlets* and *Doublets* (Figure 4-4 on page 83).

Figure 4-4 Defining singlet and doublet populations



9 In the Acquisition Dashboard, click Record Data.

10 When recording finishes, note the CV of the Singlet population.

- If the CV is $\leq 3\%$, continue to step 11.
- If the CV is $> 3\%$, restart the acquisition. Decrease the flow rate with the SAMPLE FINE ADJ knob until the CV is $\leq 3\%$, and then re-record the data.

If the CV does not improve, see Troubleshooting on page 113.

- 11** Verify the linearity and print the worksheet.
 - Divide the mean of the Doublet population by the mean of the Singlet population. The mean ratio should be 2.00 +/-0.05. If you do not achieve a ratio between 1.95–2.05, contact BD Customer Support.
 - Copy the population means and CVs, and the calculated linearity result into your QC log.
- 12** Remove the CEN sample from the cytometer.

CTN Resolution

Singlets can be distinguished from aggregates based on size. With BD FACSDiva software, aggregates can be resolved from singlets on an area vs width plot. With this plot, singlets are distinguished from doublets by the width measurement; singlets have a smaller width measurement. Discriminating the singlets from the aggregates enhances the accuracy of cell-cycle analysis.

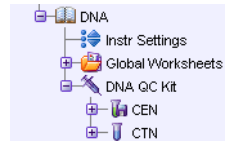
Running CTN

- 1** On the BD LSR II cytometer control panel, press RUN and LO.
- 2** Install the CTN sample tube on the cytometer.
- 3** Adjust the event rate to approximately 500 events/second.
- 4** Click Next Tube in the Acquisition Dashboard.

A new tube is created under the DNA QC Kit specimen.

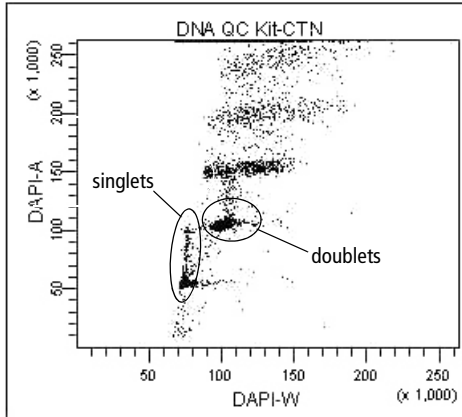
- 5** Change the name of the tube to *CTN*.

The Next Tube button duplicates the CEN tube and analysis objects. The new plots and statistics view appear below the previous objects on the worksheet. Acquisition for the CTN tube starts automatically.



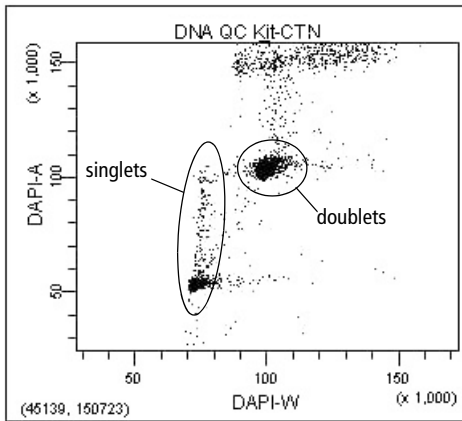
- Adjust the DAPI voltage to place the first peak at approximately channel 50×10^3 on the DAPI-A axis.

Figure 4-5 Doublet discrimination with unzoomed plot



- Use the Zoom-In button to magnify the area showing the singlets and doublets on the DAPI-A vs DAPI-W plot (Figure 4-6).

Figure 4-6 Doublet discrimination with zoomed plot



If you are unable to distinguish the singlet and doublet populations, see Chapter 7, Troubleshooting.

- 8** Draw a gate around the singlet CTN events.
- 9** Adjust the event rate to approximately 200 events/second with the SAMPLE FINE ADJ knob.
- 10** Click Record Data to save the data.
- 11** Print the worksheet.
- 12** Remove the CTN tube from the cytometer and put the instrument in standby mode.

Optimization for Data Recording

Optimize the instrument settings for the actual sample.

- 1** Install the sample tube and adjust the FSC and SSC voltages to place the data events on scale.
- 2** Adjust the DAPI voltage to place the singlet diploid events at about channel 50×10^3 .

After optimizing the instrument settings, record data for each sample tube. BD FACSDiva software does not include DNA analysis algorithms. Export your data files for analysis in a third-party application such as ModFit *LT*[™].

5

Calcium Flux

The following topics are covered in this chapter:

- Intracellular Calcium Concentration on page 88
- Calcium Flux Optimization on page 89
- Recording Calcium Flux Data on page 96

Intracellular Calcium Concentration

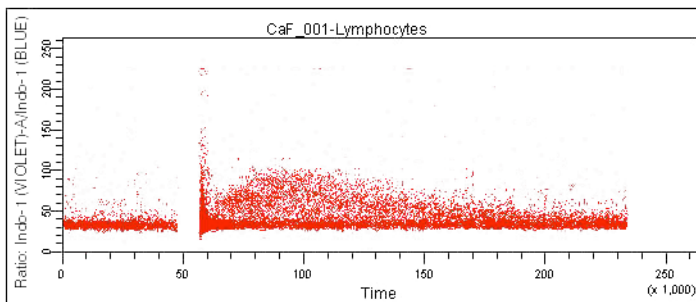
Flow cytometry can be used to measure the concentration of intracellular free calcium ions. Measurement of calcium ion (Ca^{++}) concentration can be made on large numbers of single cells, which provides information about the number of responding cells as well as the relative magnitude of the response to a given stimulus. Ca^{++} concentration can be correlated with other parameters, such as time, phenotype, and cell cycle.

In their resting state, eukaryotic cells maintain an internal Ca^{++} concentration far less than that of the extracellular environment. Elevation in intracellular Ca^{++} concentration is often used as an indicator of cellular activation in response to a stimulus. Calcium flux is also an indicator of whether the cells in a population remain functional after exposure to a drug or other compound.

Several fluorescent dyes measure intracellular Ca^{++} levels. For most of them, the amount of Ca^{++} entering a cell is indicated by a change in fluorescence emission. For example, the emission spectrum of indo-1 changes from blue to violet upon binding to Ca^{++} . The ratio of violet to blue fluorescence is independent of the amount of dye within the cell.

When normal cells are analyzed for calcium flux with indo-1 by flow cytometry, a shift in the violet/blue ratio is obtained (Figure 5-1). A break in data occurs when the stimulus is added to the sample tube. The increase in the ratio over time reflects the increase in intracellular Ca^{++} concentration.

Figure 5-1 Calcium flux data



Calcium Flux Optimization

Before beginning this section, do the following:

- Start up the instrument and perform QC.
- Ensure that the appropriate filters are installed. See Setting Up the Optical Filters and Mirrors on page 39.
- Review the following section, Using the Time Parameter.

Tip For calcium-flux experiments, use a sheath fluid that does not contain preservatives. For example, use 1X phosphate-buffered saline (PBS).

Using the Time Parameter

The Time parameter shows how events change over time. In calcium flux experiments, the Time parameter displays the rate at which the cells in the sample respond to a stimulus.

The Time parameter is a fixed scale and cannot be altered. The values for the Time parameter are in 10-ms increments; thus for a given event, a Time parameter of 123 represents 1,230 ms. Because a plot spans 2.6–262,143, tickmarks will span 2.6–2,621,430 ms (or 44 minutes). Therefore, it takes an event approximately 44 minutes to travel from one end of a plot to the other.

Tip Do not restart data recording during a calcium flux experiment; data will be lost.

Setting Up the Experiment

- 1** Choose Instrument > Instrument Configuration and verify the current configuration.

Figure 3-1 on page 54 shows an Instrument Configuration dialog whose current configuration is the standard default configuration.

Verify that Indo-1 (Violet) and Indo-1 (Blue) appear in the current configuration.

For accurate data results, the Instrument Configuration dialog must reflect the physical layout of the BD LSR II octagons and trigons. Modifications to the current configuration will not apply unless you click Set Configuration.

- 2** Choose Edit > User Preferences.

Verify that under the General tab, all checkboxes are deselected (Figure 3-2 on page 55).

Refer to the *BD FACSDiva Software Reference Manual* for more information about the instrument configuration and user preferences.

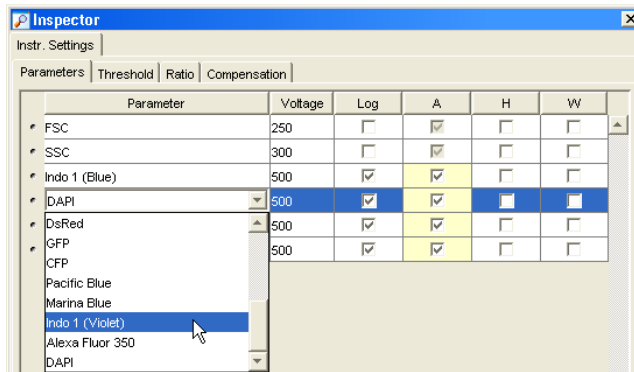
- 3** Create a new experiment and rename it *CalciumFlux*.
- 4** With the *CalciumFlux* experiment icon selected, verify in the Inspector that the *Use global instrument settings* checkbox is checked (Figure 3-3 on page 56).

5 Select the Instr Settings icon in the *CalciumFlux* experiment, and use the Inspector to specify parameters and settings for your experiment.

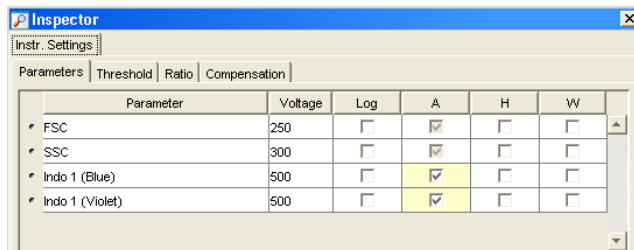
- Make sure the parameters you need appear on the Parameters tab.

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

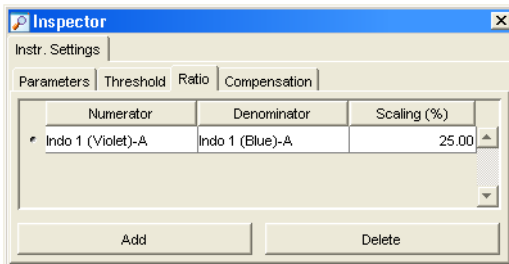
For the example shown here, choose Indo-1(Violet) from the DAPI menu.



- For this experiment, delete all parameters except FSC, SSC, Indo-1 (Blue), and Indo-1 (Violet).
- Verify that the Log checkbox is deselected for all parameters.
- Verify that the A (area) checkbox is selected for Indo-1 (Blue) and Indo-1 (Violet).

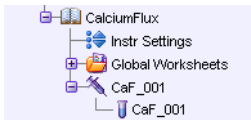


- Within the Ratio tab, click the Add button. Select Indo-1 (Violet)-A for the Numerator, and Indo-1 (Blue)-A for the Denominator.



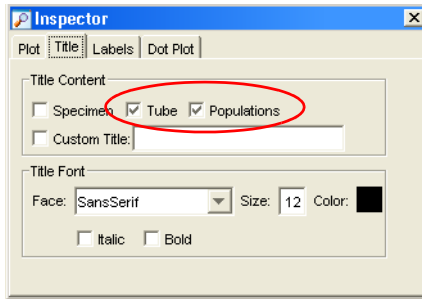
- 6 Create a new specimen in the *CalciumFlux* experiment.
- 7 Open the specimen to access its tube. Rename the tube *CaF_001*, and move the current tube pointer to *CaF_001*.

At this point, your experiment should look similar to this one.



- 8 Create a normal worksheet for the *CaF_001* tube with the following dot plots:
 - FSC-A vs SSC-A
 - Indo-1 (Blue)-A vs Indo-1 (Violet)-A
 - Time vs Ratio: Indo-1 (Violet)-A/Indo-1 (Blue)-A

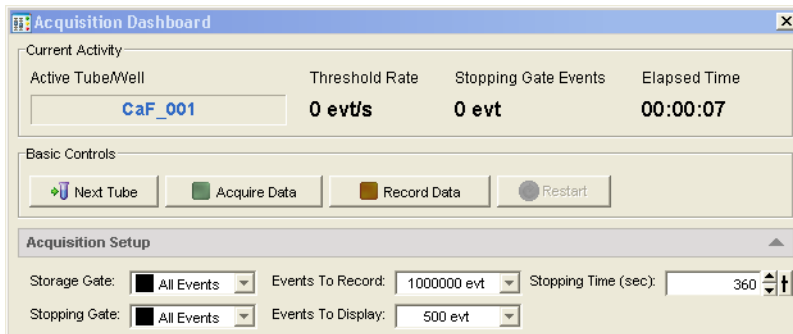
- 9 Select all plots. Click the Title tab in the Inspector and select the Tubes and Populations checkboxes to display their names in plot titles.



- 10 Create a statistics view and display the mean for Indo-1 (Blue)-A, Indo-1 (Violet)-A, and the ratio parameter.

- Choose Populations > Create Statistics View.
- Right-click on the Statistics view and select Edit Statistics View.
- Under the Population tab, deselect #Events and %Parent.
- Under the Statistics tab, select the mean for Indo-1 (Blue)-A, Indo-1 (Violet)-A, and the ratio parameter.
- Click OK.

- 11 In the Acquisition Dashboard, set the Events To Record to 1,000,000, the Events To Display to 500, and the Stopping Time to 360 seconds.

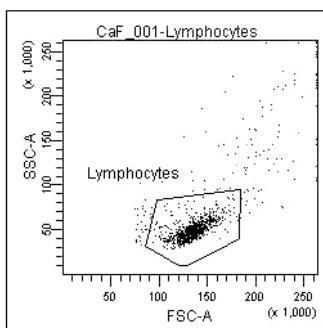


Only the specified number of Events to Display are shown during acquisition and recording. After data recording is complete, all recorded events will be displayed.

Optimizing for Calcium Flux

- 1 On the control panel, press RUN and LO.
- 2 Install the sample (not yet stimulated) on the SIP.
- 3 Verify that the green current tube pointer is in front of the *CaF_001* tube in the Browser. In the Acquisition Dashboard, click Acquire Data.
- 4 Adjust the FSC and SSC voltages to place the lymphocytes on scale in the FSC vs SSC dot plot.
- 5 Adjust the FSC threshold to remove debris without cutting into the lymphocyte population.
- 6 Draw a gate around the lymphocytes.
- 7 Create a population hierarchy view and rename the population *Lymphocytes*.

Figure 5-2 Lymphocyte gate



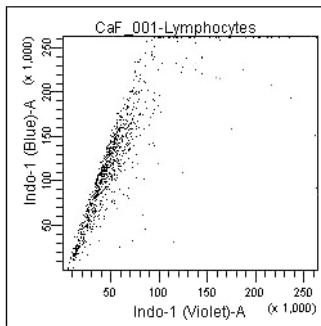
- 8 Display only the lymphocyte population in the remaining two dot plots.

Select the two plots, right-click inside one of the plots and choose Show Populations > Lymphocytes.

- 9 Adjust the Indo-1 (Violet)-A and the Indo-1 (Blue)-A voltages to optimize the signal.

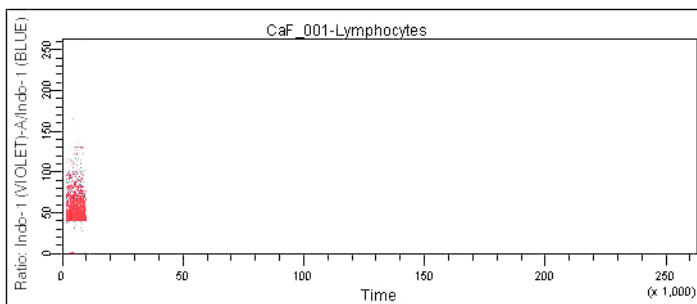
The signal, when displayed in the plot, should resemble that of Figure 5-3.

Figure 5-3 Optimized Indo-1 signal



- 10 Set the mean of the ratio parameter to about 50,000.
 - Select the *CaF_001* tube in the Browser and click the Ratio tab in the Inspector.
 - Adjust the value in the Scaling (%) field (a higher percentage will raise the mean) until the mean of the ratio parameter is about 50,000 (use the statistics view).

Your time vs ratio plot should resemble this:



Recording Calcium Flux Data

- 1 In the Acquisition Dashboard, change the Events to Display to 50,000 events.
- 2 Verify that the unstimulated sample is still running.
- 3 Use the cytometer's SAMPLE FINE ADJ knob to adjust the event rate to about 200 events/second.

The event rate is displayed in the Acquisition Dashboard.

- 4 Click Record Data.

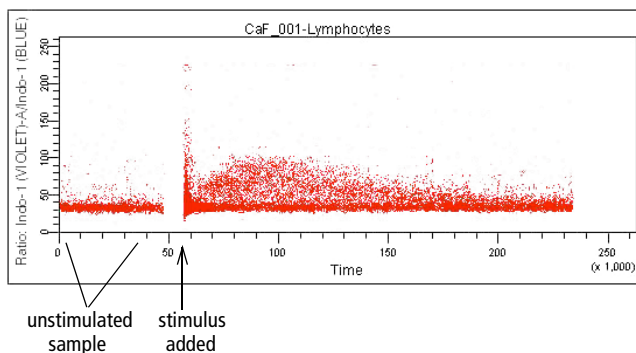
NOTICE Keep the instrument in RUN mode as you perform steps 5 through 7 below. Do not stop recording. The unstimulated sample data must be maintained. If recording is stopped, ensure that the subsequent data is appended to the unstimulated sample data.

- 5 When approximately 10,000 events have been recorded, remove the unstimulated sample tube from the cytometer.
- 6 Add the stimulus to the tube and mix thoroughly.

7 Reinstall the tube on the SIP.

After a few seconds, the Ca^{++} concentration begins to increase on the time vs ratio plot (Figure 5-4). Acquisition stops automatically when the Stopping Time is reached.

Figure 5-4 Cellular response to stimulus over time



8 Remove the tube from the cytometer.

9 Clean the fluidics system with 10% bleach for 5 minutes, and then with DI water for 5 minutes.

Tip Residual stimulus must be removed from the system. Any remaining stimulus would activate cells in subsequent samples.

10 To run another sample, install the next sample tube on the SIP.

11 In the Acquisition Dashboard, click Next Tube to create a new tube, and rename the new tube appropriately.

12 Repeat steps 3 through 11.

BD FACSDiva software does not include calcium flux analysis algorithms. Export your data files for analysis in a suitable third-party application.

6

Maintenance

- Daily Cleaning and Shutdown on page 100
- Scheduled Maintenance on page 103
- Periodic Maintenance on page 107

The BD LSR II instrument is designed to require minimum maintenance. However, to preserve the reliability of the instrument, you must regularly perform basic preventive maintenance procedures. This chapter explains routine cleaning procedures that will keep your instrument in good condition.



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

- Tip** A 5% solution of sodium hypochlorite can be substituted for undiluted bleach in the following cleaning procedures. However, higher concentrations of sodium hypochlorite and use of other cleaning agents might damage the instrument.

Daily Cleaning and Shutdown

Perform the following maintenance procedures every day:

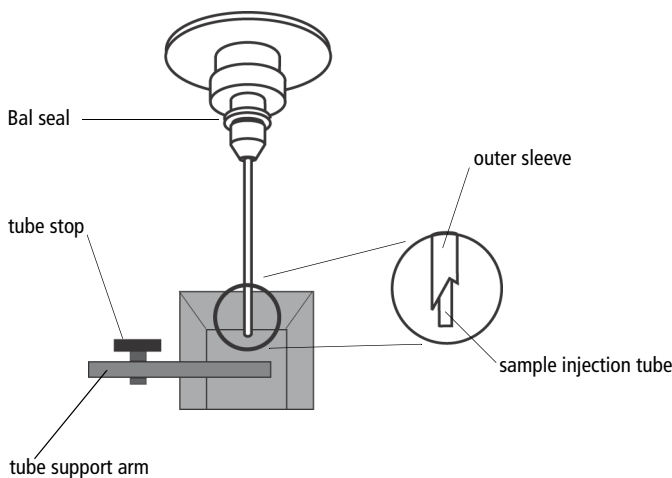
- Daily Fluidics Cleaning on page 100
- Daily Shutdown on page 102

Daily Fluidics Cleaning

Each time you shut down the instrument, clean the sample injection tube and the area between the injection tube and the outer sleeve (Figure 6-1 on page 101). This prevents the sample injection tube from becoming clogged and removes dyes that can remain in the tubing.

- Tip** Follow this procedure immediately after running viscous samples or nucleic acid dyes such as Hoechst, DAPI, propidium iodide (PI), acridine orange (AO), or thiazole orange (TO).

Figure 6-1 Sample injection port (SIP)



- 1** Set the fluid control to RUN.
- 2** Install a tube containing 3 mL of a bleach solution on the SIP with the support arm to the side (vacuum on) and let it run for 1 minute.

Tip For the bleach solution, use BD™ FACSClean solution or a 1:10 dilution of bleach in DI water.

BD FACSClean solution is a bleach-based cleaning agent designed for daily use in cytometer maintenance.

- 3** Move the support arm under the tube (vacuum off) and allow the bleach solution to run for 5 minutes with the event rate set to HI.
- 4** Repeat steps 2 and 3 with BD™ FACSRinse solution.
BD FACSRinse solution is a detergent-based cleaning solution.
- 5** Repeat steps 2 and 3 with DI water.
- 6** Set the fluid control to STNDBY.

- 7 Place a tube containing no more than 1 mL of DI water on the SIP.

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

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

- 8 Set the cytometer to STNDBY mode.

Daily Shutdown

- 1 Turn off the flow cytometer.
- 2 Choose Start > Shutdown to turn off the computer, if needed.

- Tip** If the instrument will not be used for a week or longer, perform a system flush (see System Flush on page 103) and leave the fluidics system filled with DI water to prevent saline crystals from clogging the fluidics.

Scheduled Maintenance

Perform the following maintenance procedures every 2 weeks:

- System Flush on page 103
- Waste Management System Maintenance on page 105

System Flush

An overall fluidics cleaning is required to remove debris and contaminants from the sheath tubing, waste tubing, and flow cell. Perform the system flush at least every 2 weeks.



Instrument hardware might be contaminated with biohazardous material. Use 10% bleach to decontaminate the BD LSR II flow cytometer. Flushing with 10% bleach is the only procedure recommended by BD Biosciences for decontaminating the instrument.

- 1** Remove the sheath filter.
 - Press the quick-disconnects on both sides of the filter assembly.
 - Remove the filter assembly.
 - Connect the two fluid lines.
- Tip** Do not run detergent, bleach, or ethanol through the sheath filter. They can break down the filter paper within the filter body, causing particles to escape into the sheath fluid, possibly clogging the flow cell.
- 2** Empty the sheath container and rinse it with DI water.
- 3** Fill the sheath container with at least 1 L of a 1:10 dilution of bleach or full-strength BD FACSClean solution.
- 4** Empty the waste container if needed.

- 5** Open the roller clamp by the fluidics interconnect, and drain the fluid into a beaker for 5 seconds.
- 6** Remove the DI water tube from the SIP.
- 7** Prime twice (perform the following twice):
 - Press the PRIME button on the fluidics control panel.
 - When the STNDBY button lights (amber), press the PRIME button again.
- 8** Install a tube with 3 mL of a 1:10 dilution of bleach or full-strength BD FACSClean solution on the SIP.
- 9** Press the RUN fluid control button; run the cytometer on HI for 30 minutes.
- 10** Press the STNDBY fluid control button and depressurize the sheath container by lifting the vent valve.
- 11** Repeat steps 2 through 10 with BD FACSRinse solution in place of the bleach solution.
- 12** Repeat steps 2 through 10 with DI water in place of the BD FACSRinse solution.
- 13** Replace the sheath filter and refill the sheath container with sheath fluid.

Waste Management System Maintenance

The waste management system for the BD LSR II instrument has an alarm powered by a 9-volt battery that must be tested and changed regularly to ensure its continued operation. Test the battery every 2 weeks after you flush the system; see the following section. Change the battery as needed; see Changing the Battery on page 106

Testing the Battery and Alarm

- 1 Locate the Battery Test switch on the waste container bracket (Figure 6-2).

Figure 6-2 Battery Test switch



- 2 Toggle the switch.

If the battery and the alarm are working properly, you should hear buzzing. If you do not hear any sound, change the battery as described in the following section.

- 3 Release the switch.

Changing the Battery

You will need the following supplies to change the battery:

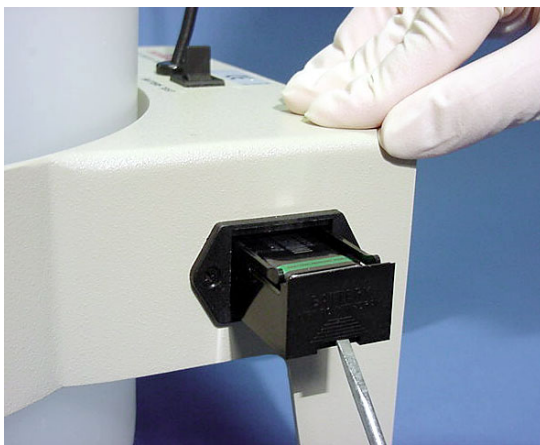
- small flat-head screwdriver
- 9-volt battery

Perform the following steps.

- 1** Insert the tip of a flat-head screwdriver into the slot as shown in Figure 6-3 and pry it out.

A small drawer will slide out.

Figure 6-3 Opening drawer



- 2** Remove the drawer.
- 3** Remove the battery from the drawer (Figure 6-4 on page 107).
- 4** Replace a new 9-volt battery into the drawer, making sure that it is in the correct orientation.

Markings in the drawer will guide you.

Figure 6-4 Changing battery



- 5 Slide the drawer into the bracket until you feel a click.
- 6 Test the new battery.

See Testing the Battery and Alarm on page 105.

Periodic Maintenance

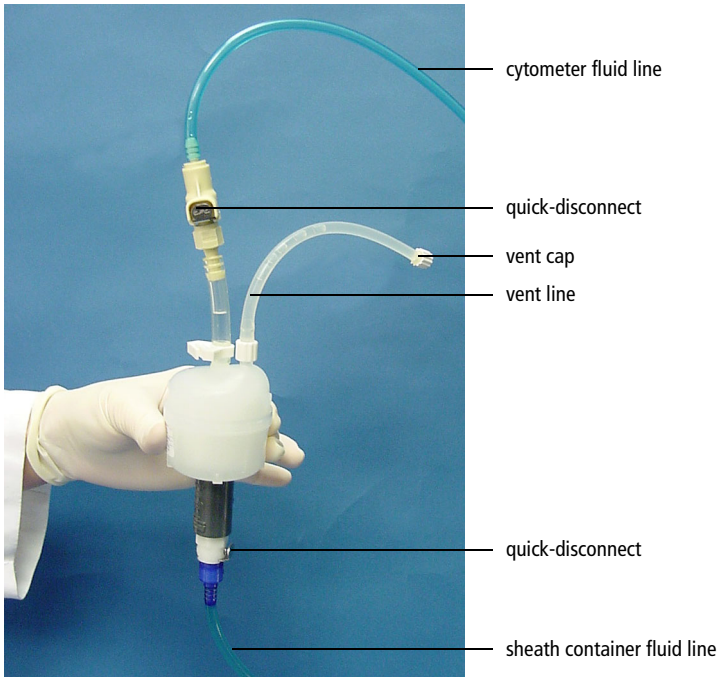
The following instrument components should be checked occasionally and cleaned as necessary. The frequency will depend on how often the instrument is run. Other components should be checked periodically for wear and replaced if necessary.

- Changing the Sheath Filter on page 108
- Changing the Bal Seal on page 110
- Changing the Sample Tube O-Ring on page 112

Changing the Sheath Filter

The sheath filter (Figure 6-5), connected to the top of the sheath container, filters the sheath fluid as it comes from the sheath container. Increased debris appearing in an FSC vs SSC plot can indicate that the sheath filter needs to be replaced. We recommend changing the sheath filter assembly every 3–6 months.

Figure 6-5 Sheath filter



- Tip** To avoid spraying sheath fluid, depressurize the sheath container before opening it.

Remove the Old Filter

- 1** Place the instrument in STNDBY.
- 2** Disconnect the air line (green).
- 3** Depressurize the sheath container by lifting the vent valve.

For a description of the vent valve, see Figure 2-3 on page 44.

- 4** Detach the cytometer fluid line from the filter assembly by squeezing the quick-disconnect.
- 5** Detach the sheath container fluid line from the filter assembly by squeezing the quick-disconnect.
- 6** Remove the vent line from the filter; set aside.

Twist to remove.

- 7** Discard the used filter assembly.

Attach the New Filter

- 1** Connect the vent line to the filter. Twist to attach.
- 2** Connect the sheath container fluid line to the filter assembly via the quick-disconnect.
- 3** Attach the cytometer fluid line to the filter assembly via the quick-disconnect.
- 4** Turn on the instrument to pressurize the sheath container.
- 5** Loosen the filter's vent cap to bleed off any air in the sheath filter.
- 6** Carefully tap the filter assembly to dislodge any air trapped in the filter element.
- 7** Loosen the filter's vent cap again to bleed off any air in the sheath filter.

Changing the Bal Seal

The sample injection tube Bal seal is a Teflon ring that forms a seal with the sample tube and ensures proper tube pressurization. Over time, this seal becomes worn or cracked and requires replacement. Replacement is necessary if a proper seal is not formed when a sample tube is installed on the SIP.

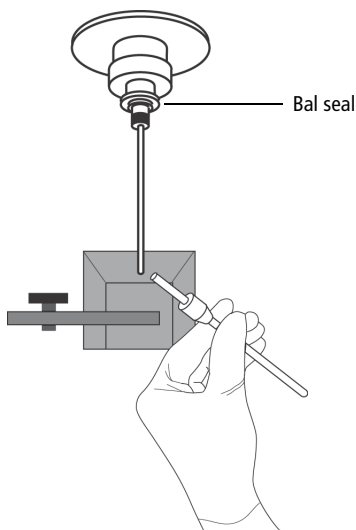
☑ **Tip** Indications that a proper seal has not formed include:

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

To replace the Bal seal:

- 1** Remove the outer droplet sleeve from the sample injection tube by turning the retainer counterclockwise.

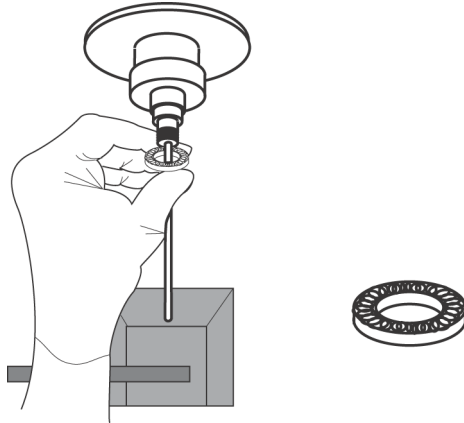
Figure 6-6 Removing the outer sleeve



☑ **Tip** Work carefully—the outer sleeve can fall out as you loosen the retainer.

- 2 Remove the Bal seal by gripping it between your thumb and index finger and pulling (Figure 6-7).

Figure 6-7 Removing the Bal seal



- 3 Install the new Bal seal spring-side up.
Gently push the seal in place to seat it.
- 4 Reinstall the retainer and outer sleeve over the sample injection tube.
Tighten the retainer just enough to hold it in place.
- 5 Install a sample tube on the SIP to ensure that the outer sleeve has been properly installed.

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

Changing the Sample Tube O-Ring

The sample tube O-ring, located within the retainer, forms a seal that allows the droplet containment vacuum to function properly. Replace the O-ring when droplets form at the end of the sample injection tube while the vacuum is operating.



Instrument hardware might be contaminated with biohazardous material. Wear suitable protective clothing, eyewear, and gloves whenever cleaning the instrument or replacing parts.

- 1** Remove the outer droplet sleeve from the sample injection tube.

Turn the retainer counterclockwise and pull the outer sleeve from the retainer (Figure 6-6 on page 110).

- 2** Invert the retainer and allow the O-ring to fall onto the benchtop.

If the O-ring does not fall out initially, tap the retainer on the benchtop to dislodge the O-ring.

- 3** Drop the new O-ring into the retainer.

Make sure the O-ring is seated properly in the bottom of the retainer.

- 4** Reinstall the retainer and the outer sleeve.

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

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

7

Troubleshooting

The tips in this section are designed to help you troubleshoot your experiments. You can find additional troubleshooting information in the *BD FACSDiva Software Reference Manual*.

If additional assistance is required, contact your local BD Biosciences technical support representative. See Technical Assistance on page xv.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Droplet containment vacuum not functioning	Worn O-ring in retainer	Replace the O-ring. See Changing the Sample Tube O-Ring on page 112.
	Outer sleeve not seated in the retainer	<ol style="list-style-type: none"> 1 Loosen the retainer (Figure 6-6 on page 110). 2 Push the outer sleeve up into the retainer until seated. 3 Tighten the retainer.
	Outer sleeve not on the sample injection tube	<p>Replace the outer sleeve.</p> <ol style="list-style-type: none"> 1 Loosen the retainer. 2 Slide the outer sleeve over the sample injection tube until it is seated. 3 Tighten the retainer.
	Waste line pinched, preventing proper aspiration	Check the waste line.
	Waste tank full	Empty the waste tank.
Sample tube not fitting on SIP	Sample tube other than BD Falcon tubes used	Use BD Falcon 12 x 75-mm sample tubes. See Equipment on page 152.
	Worn Bal seal	Replace the Bal seal. See Changing the Bal Seal on page 110.
Rapid sample aspiration	Support arm to the side	Place the support arm under the sample tube.
	Droplet containment module failing	Call your service representative.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in acquisition display and green RUN button	Threshold not set to correct parameter (usually FSC)	Set the threshold to the correct parameter for your application.
	Threshold level too high	Lower the threshold level.
	PMT voltage for threshold parameter set too low	Set the PMT voltage higher for the threshold parameter.
	Gating issue	Refer to the <i>BD FACSDiva Software Reference Manual</i> for information on setting gates.
	Air in sheath filter	Purge the filter. See Removing Air Bubbles on page 47.
	No sample in tube	Add sample to tube or install new sample tube.
	Sample not mixed properly	Mix sample to suspend cells.
	Waste tank full	Empty the waste tank.
	PMT voltages set too low or too high for display parameter	Reset PMT voltages.
	Too few events displayed	Increase the number of events to display.
Sample injection tube clogged		Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes.
		If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 100.
Bal seal worn		Replace the Bal seal. See Changing the Bal Seal on page 110.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in acquisition display and green RUN button (continued)	Laser not warmed up	<p>Wait the recommended amount of time for the laser to warm up.</p> <ul style="list-style-type: none"> • 30 min for the 488-nm (blue) • 30 min for the 355-nm (UV) • 15 min for the 405-nm (violet) • 20 min for the 633-nm (red)
	Laser delay set incorrectly	<p>Adjust the laser delay settings.</p> <p>See Setting Laser Delay on page 193.</p>
	Laser not functioning	<p>Verify malfunction by changing the threshold to an alternative laser while running appropriate QC particles. If not successful, contact BD Biosciences.</p>
No events in acquisition display and orange RUN button	RUN not activated	<p>Press the RUN button.</p>
	Sample tube not installed or not properly seated	<p>Install the sample tube correctly on the SIP.</p>
	Sample tube cracked	<p>Replace the sample tube.</p>
	Sheath container not pressurized	<ul style="list-style-type: none"> • Ensure that the sheath container lid and all connectors are securely seated. • Inspect the O-ring and replace if necessary. See Changing the Sample Tube O-Ring on page 112.
	Bal seal worn	<p>Replace the Bal seal. See Changing the Bal Seal on page 110.</p>
Air leak at sheath container	<p>Ensure that the sheath container lid and all connectors are securely seated.</p>	

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No events in acquisition display and orange RUN button (continued)	Sheath container empty	Fill the sheath container.
	Air in sheath filter	Purge the filter. See Removing Air Bubbles on page 47.
No fluorescent signal	Incorrect fluorochrome assignment	Make sure the instrument configuration in the software matches the optical filters in the instrument.
	Wrong filter installed	Make sure the appropriate filter is installed for each fluorochrome. See Changing Optical Filters or Mirrors on page 42.
	Laser not functioning	Verify laser malfunction by changing the threshold to an alternative laser while running appropriate QC particles. If not successful, contact BD Biosciences.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
High event rate	Air bubble in sheath filter or flow cell	Remove the air bubble. See Removing Air Bubbles on page 47.
	Threshold level too low	Increase the threshold level. Refer to the <i>BD FACSDiva Software Reference Manual</i> for instructions.
	PMT voltage for threshold parameter set too high	Set the PMT voltage lower for the threshold parameter. Refer to the <i>BD FACSDiva Software Reference Manual</i> for instructions.
	Sample too concentrated	Dilute the sample.
	Sample flow rate set on HI	Set the sample flow rate to MED or LO.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Low event rate	Threshold level too high	Lower the threshold level. Refer to the <i>BD FACSDiva Software Reference Manual</i> for instructions.
	PMT voltage for threshold parameter set too low	Set the PMT voltage higher for the threshold parameter. Refer to the <i>BD FACSDiva Software Reference Manual</i> for instructions.
	Sample not adequately mixed	Mix the sample to suspend cells.
	Sample too dilute	Concentrate the sample. If the flow rate setting is not critical to the application, set the flow rate switch to MED or HI.
	Sample injection tube clogged	Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes. If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 100.
Erratic event rate	Sample tube cracked	Replace the sample tube.
	Bal seal worn	Replace the Bal seal. See Changing the Bal Seal on page 110.
	Sample injection tube clogged	Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes. If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 100.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Erratic event rate (continued)	Sample injection tube clogged	Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes. If the event rate is still erratic, clean the sample injection tube. See Daily Cleaning and Shutdown on page 100.
	Contaminated sample	Prepare the specimen again. Ensure that the tube is clean.
	Worn sheath filter	Replace the filter. See Changing the Sheath Filter on page 108.
Distorted scatter parameters	Instrument settings maladjusted	Optimize the scatter parameters. Refer to the <i>BD FACSDiva Software Reference Manual</i> for instructions.
	Air bubble in sheath filter or flow cell	Purge the air from the filter. See Removing Air Bubbles on page 47.
	Flow cell dirty	Perform the system flush procedure. See System Flush on page 103.
	Air leak at sheath container	Ensure that the sheath container lid is tight and all connectors are secure.
	Hypertonic buffers or fixative	Replace the buffers and fixative.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Excessive amount of debris in display	Threshold level too low	Increase the threshold level.
	Sheath filter dirty	Replace the filter. See Changing the Sheath Filter on page 108.
	Flow cell dirty	Flush the system. See System Flush on page 103.
	Dead cells or debris in sample	Examine the sample under a microscope.
	Sample contaminated	Re-stain the sample, ensure tube is clean.
	Stock sheath fluid contaminated	Rinse the sheath container with DI water; then fill with sheath fluid from another (or new lot) bulk container.
High CV	Air bubble in sheath filter or flow cell	Purge the filter. See Removing Air Bubbles on page 47.
	Sample flow rate set too high	Set the sample flow rate lower.
	Air leak at sheath container	Ensure that the sheath container lid is tight and all connectors are secure.
	Flow cell dirty	Flush the system. See System Flush on page 103.
	Poor sample preparation	Repeat sample preparation.
	Sample not diluted in same fluid as sheath fluid	Dilute the sample in the same fluid as you are using for sheath.
	Old or contaminated quality control (QC) particles	Make new QC samples and perform the quality control procedure again.

Instrument Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Poor QC results	Air bubble or debris in flow cell	Prime the fluidics system. See Priming the Fluidics on page 49.
	Old or contaminated QC particles	Make new QC samples and perform the quality control procedure again.
	Sample not diluted in same fluid as sheath fluid	Dilute the sample in the same fluid as you are using for sheath. If you are running BD CaliBRITE™ beads, dilute them in BD FACSTflow solution and use BD FACSTflow solution for sheath fluid.
	Laser not warmed up	Wait the recommended amount of time for the laser to warm up. <ul style="list-style-type: none"> • 30 min for the 488-nm (blue) • 30 min for the 355-nm (UV) • 15 min for the 405-nm (violet) • 20 min for the 633-nm (red)
	Laser not functioning	Contact BD Biosciences.
Optical alignment problem	Contact BD Biosciences.	

Appendix A

Technical Overview

This appendix contains a technical overview of the following topics:

- Fluidics on page 124
- Optics on page 125
- Electronics on page 134

Fluidics

The fluidics system in the BD LSR II flow cytometer is pressure driven—a built-in air pump provides a sheath pressure of 5.5 psi. After passing through the sheath filter, sheath fluid is introduced into the lower chamber of the quartz flow cell.

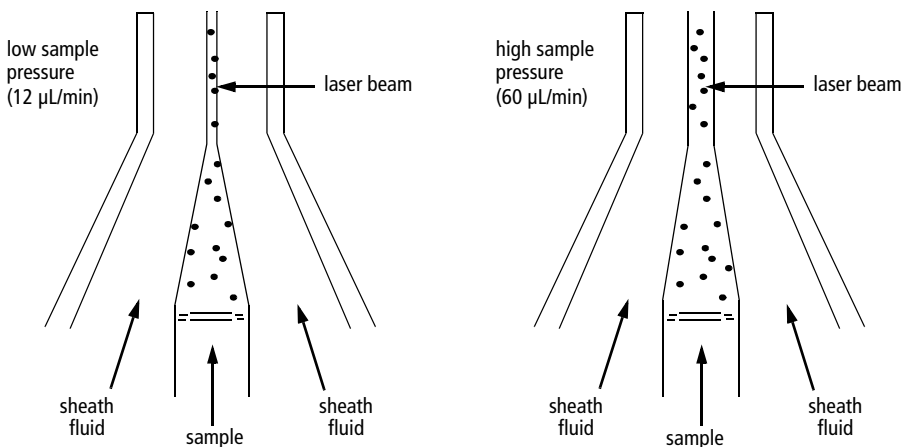
The sample to be analyzed arrives in a separate pressurized stream. When a sample tube is placed on the sample injection port (SIP), the sample is forced up and injected into the lower chamber of the flow cell by a slight overpressure relative to the sheath fluid. The conical shape of the lower chamber creates a laminar sheath flow that carries the sample core upward through the center of the flow cell, where the particles to be measured are intercepted by the laser beam (Figure A-1 on page 125). This process is known as hydrodynamic focusing.

The objective in flow cytometric analysis is to have at most one cell or particle moving through a laser beam at a given time. The difference in pressure between the sample stream and sheath fluid stream can be used to vary the diameter of the sample core. Increasing the sample pressure increases the core diameter and therefore the flow rate (Figure A-1 on page 125).

- A higher flow rate is generally used for qualitative measurements such as immunophenotyping. The data is less resolved but is acquired more quickly.
- A lower flow rate is generally used in applications where greater resolution and quantitative measurements are critical, such as DNA analysis.

Proper operation of fluidic components is critical for particles to intercept the laser beam properly. Always ensure that the fluidics system is free of air bubbles and debris and is properly pressurized.

Figure A-1 Hydrodynamic focusing of the sample core through the flow cell



Optics

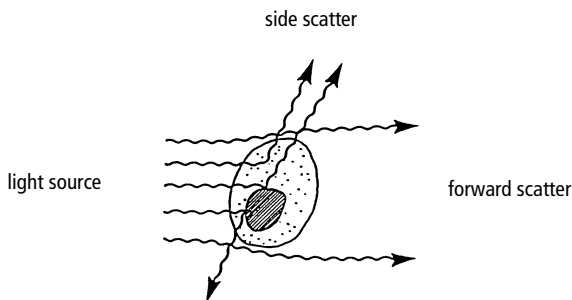
The optics system consists of lasers, optical filters, and detectors. Lasers illuminate the cells or particles in the sample and optical filters direct the resulting light scatter and fluorescence signals to the appropriate detectors.

Light Scatter

When a cell or particle passes through a focused laser beam, laser light is scattered in all directions (Figure A-2 on page 126). Light that scatters axial to the laser beam is called forward scatter (FSC); light that scatters perpendicular to the laser beam is called side scatter (SSC). FSC and SSC are related to certain physical properties of cells:

- FSC—indicates relative differences in the size of the cells or particles
- SSC—indicates relative differences in the internal complexity or granularity of the cells or particles

Figure A-2 Forward scatter (FSC) and side scatter (SSC)



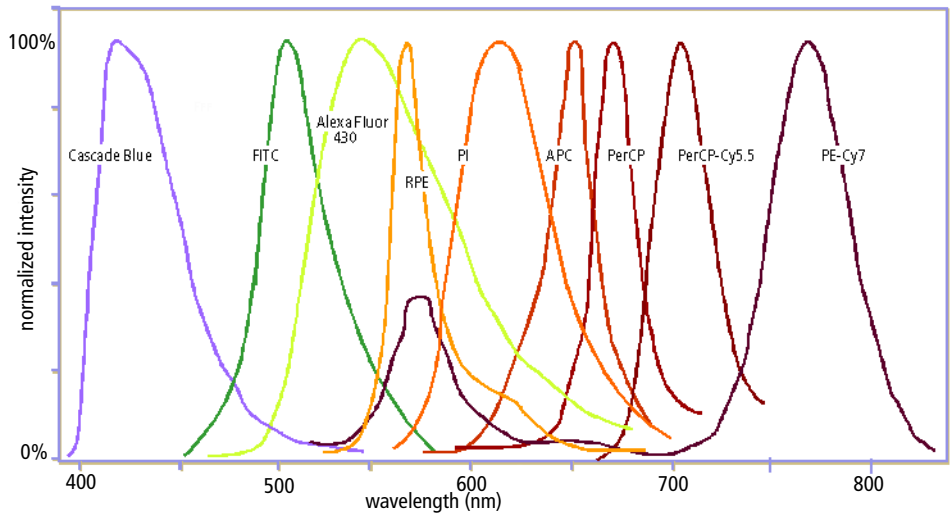
Fluorescence

When cells or particles stained with fluorochrome-conjugated antibodies or other dyes pass through a laser beam, the dyes can absorb photons (energy) and be promoted to an excited electronic state. In returning to their ground state, the dyes release energy, most of which is emitted as light. This light emission is known as fluorescence.

Fluorescence is always a longer wavelength (lower-energy photon) than the excitation wavelength. The difference between the excitation wavelength and the emission wavelength is known as the Stokes shift. Some fluorescent compounds such as PerCP exhibit a large Stokes shift, absorbing blue light (488 nm) and emitting red light (675 nm), while other fluorochromes such as FITC have a smaller Stokes shift, absorbing blue light and emitting green light (530 nm).

The emission spectra for some commonly used fluorochromes are shown in Figure A-3 on page 127.

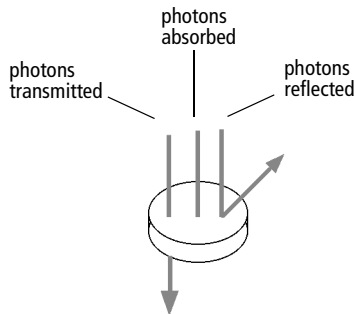
Figure A-3 Emission spectra of commonly used fluorochromes



Optical Filters

Optical filters modify the spectral distribution of light scatter and fluorescence directed to the detectors. When photons encounter an optical filter, they are either transmitted, absorbed, or reflected (Figure A-4).

Figure A-4 Effect of an optical filter on incident photons



Even though an optical filter is rated at its 50% transmission point, the filter passes—or lets through—a minimal amount of light outside of this indicated rating.

The slope of an optical filter transmission curve indicates filter performance. A relatively steep slope indicates a high-performance, high-quality optical filter that provides deep attenuation of out-of-band wavelengths. A less steep slope indicates that more light outside the rated bandwidth is being transmitted.

Two kinds of filters are used on the BD LSR II flow cytometer:

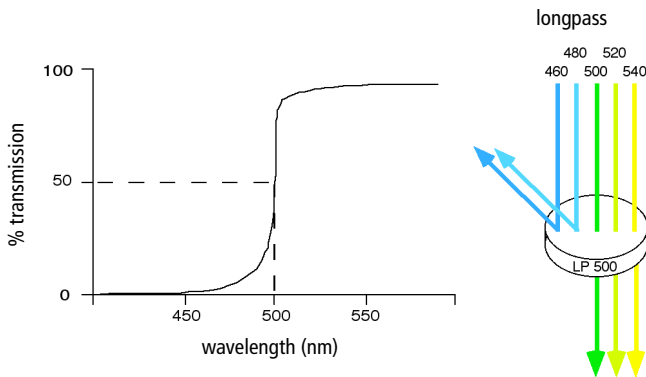
- longpass (LP)
- bandpass (BP), including discriminating filters (DF) and ALPHA filters (AF)

A third filter type, the shortpass (SP), is not recommended, but can be used in some custom configurations. See Shortpass Filter on page 129.

LP, BP, and SP filters are referred to as dichroic filters. See Dichroic Mirrors on page 131.

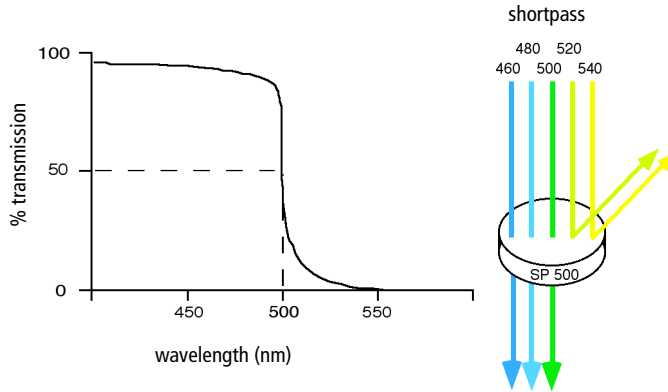
Longpass Filter

LP filters pass wavelengths longer than the filter rating. For example, a 500-LP filter permits wavelengths longer than 500 nm to pass through it and either absorbs or reflects wavelengths shorter than 500 nm.



Shortpass Filter

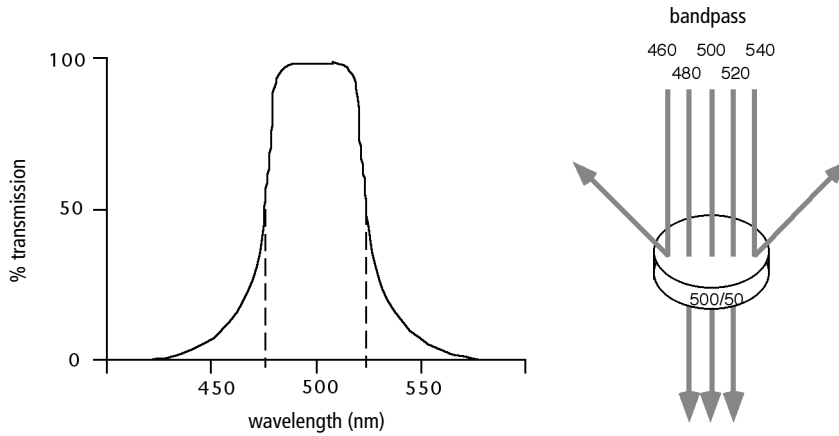
An SP filter has the opposite properties of a longpass filter. An SP filter passes light with a shorter wavelength than the filter rating.



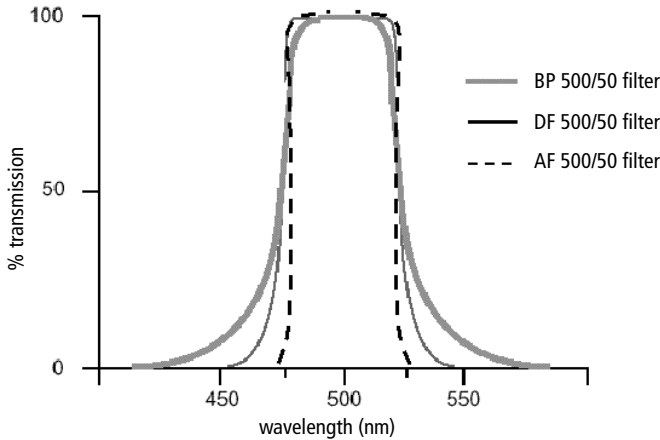
Bandpass Filter

A BP filter transmits a relatively narrow range or band of light. Bandpass filters are typically designated by two numbers. The first number indicates the center wavelength and the second refers to the width of the band of light that is passed. For example, a 500/50 BP filter transmits light that is centered at 500 nm and has a total bandwidth of 50 nm. Therefore, this filter transmits light between 475 and 525 nm (Figure A-5 on page 130).

Figure A-5 Bandpass filter



BP and DF filters have the same general function—they transmit a relatively narrow band of light. The principal difference between them is their construction. DF filters have more cavities or layers of optical coatings, resulting in a steeper transmission curve than the curve for a BP filter. This steep slope means that a DF filter is better at blocking light outside the rated bandwidth of the filter.



Dichroic Mirrors

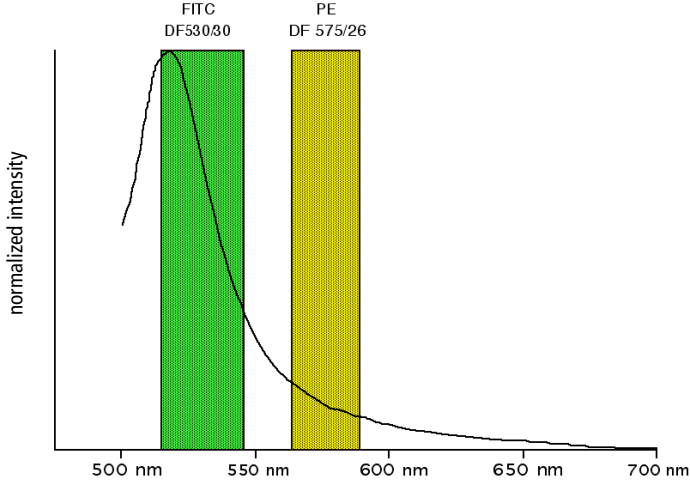
Dichroic filters that are used to direct different color light signals to different detectors are called dichroic mirrors or beam splitters.

Although dichroic mirrors have the properties of LP or SP optical filters, you can not necessarily use any type of LP or SP filter as a beam splitter. A beam splitter must have a surface coating that reflects certain wavelengths, but many LP or SP filters are absorbance filters that do not have any specific reflective characteristics. Also, optical filters and beam splitters are rated at a specific angle of incidence. When used in front of the fluorescence detectors, they are perpendicular to the incident light, and when used as a beam splitter, they are placed at an angle relative to the light source. Their optical properties are therefore designed for that angle of incidence.

Compensation Theory

Fluorochromes emit light over a range of wavelengths (Figure A-3 on page 127). Optical filters are used to limit the range of frequencies measured by a given detector. However, when two or more fluorochromes are used, the overlap in wavelength ranges often makes it impossible for optical filters to isolate light from a given fluorochrome. As a result, light emitted from one fluorochrome appears in a detector intended for another (Figure A-6). This is referred to as spillover. Spillover can be corrected mathematically by using a method called compensation.

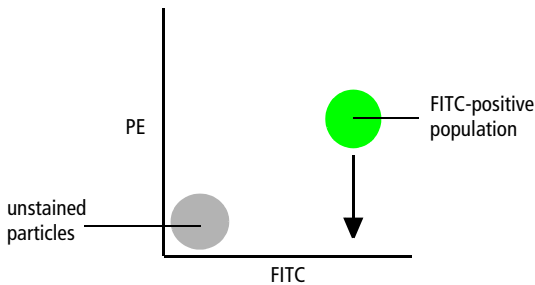
Figure A-6 Spillover from the FITC fluorochrome to the PE detector



For example, FITC emission appears primarily in the FITC detector, but some of its fluorescence spills over into the PE detector. The spillover is corrected or compensated for: hence the term fluorescence compensation.

Figure A-6 shows that some of the FITC emission appears in the PE detector. This can be seen in a dot plot of FITC vs PE (Figure A-7).

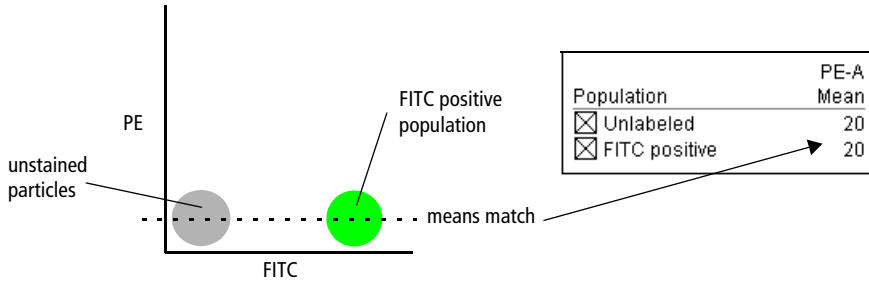
Figure A-7 Theoretical display of FITC vs PE without compensation



This FITC spillover in the PE detector is to be corrected as indicated by the arrow in Figure A-7. Using the Compensation tab of the Instrument window in BD FACSDiva software, adjust the PE-%FITC spectral overlap value.

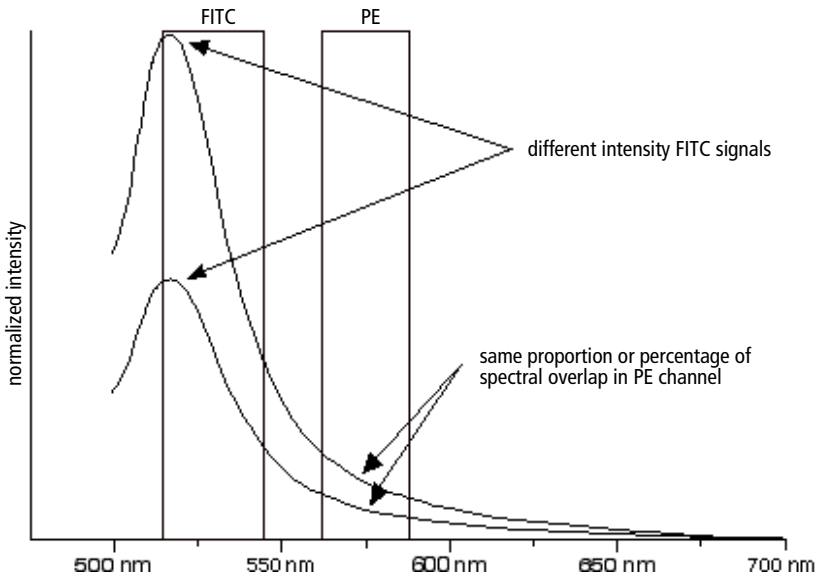
Compensation is optimal when the positive and negative FITC populations have the same means or medians in the PE parameter statistics (Figure A-8).

Figure A-8 FITC spillover optimally compensated out of the PE parameter



Once fluorescence compensation has been set for any sample, the compensation setting remains valid for a subsequent dim or bright sample, because compensation subtracts a percentage of the fluorescence intensity. Figure A-9 illustrates this principle. Although the signals differ in intensity, the percentage of the FITC spillover into the PE detector remains constant.

Figure A-9 Two FITC signals of different intensity

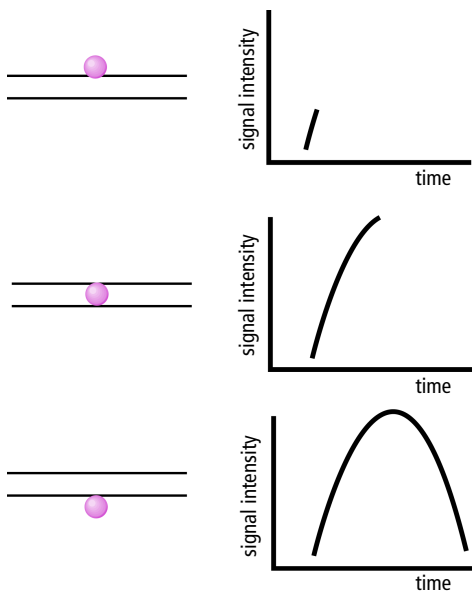


Electronics

As cells or other particles pass through a focused laser beam, they scatter the laser light and can emit fluorescence. Because the laser beam is focused on a small spot and particles move rapidly through the flow cell, the scatter or fluorescence emission has a very brief duration—only a few microseconds. This brief flash of light is converted into an electrical signal by the detectors. The electrical signal is called a pulse (Figure A-10).

- 1 A pulse begins when a particle enters the laser beam. At this point, both the beam intensity and signal intensity are low.
- 2 The pulse reaches a maximum intensity or height when the particle reaches the middle of the beam, where the beam and signal intensity are the brightest. The peak intensity, or height of the pulse, is measured at this point.
- 3 As the particle leaves the beam, the pulse trails off below the threshold.

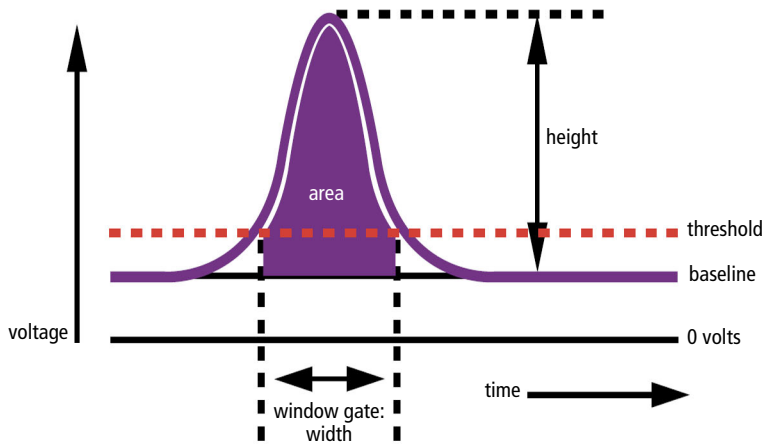
Figure A-10 Anatomy of a pulse



Pulse Measurements

The pulse processors measure pulses by three characteristics: height, area, and width (Figure A-11).

Figure A-11 Pulse measurements



- pulse height is the maximum digitized intensity measured for the pulse
- pulse area is an integration of the digitized measures over time
- pulse width calculates: $\frac{\text{area}}{\text{height}} \times 64000$

Digital Electronics

BD LSR II flow cytometer electronics digitize the signal intensity produced by a detector. The digitized data is stored in memory and further processed by the electronics to calculate

- pulse height, area, and width
- compensation
- parameter ratios

These results are transferred to your workstation computer for further processing by BD FACSDiva software. For more information about digital theory, refer to Digital Theory in the *BD FACSDiva Software Reference Manual*.

BD FACSDiva Option White Paper

For an in-depth discussion of digital electronics, visit our website at bdbiosciences.com/immunocytometry_systems/ and download the *BD FACSDiva Option White Paper*.

If you have difficulty downloading this white paper from the web, contact your local BD Biosciences technical support representative or supplier for updated instructions (see Technical Assistance on page xv).

Threshold

The threshold is the level at which the system starts to measure signal pulses. A threshold is defined for a specific detector signal. The system continuously samples the digitized signal data and calculates pulse area, height, and width for all channels based on the time interval during which the threshold is exceeded.

Thresholds can also be set for more than one parameter, and pulse measures are based on either of the following:

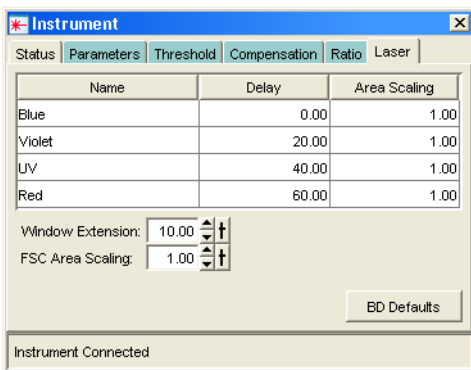
- intervals during which ALL signals exceed their threshold value
- intervals during which ANY signal exceeds its threshold value

Laser Controls

Controls in the Laser tab of the Instrument window are used to set the (laser) delay, area scaling, and window extension values.

These parameters are set by BD Biosciences service personnel when the BD LSR II flow cytometer is installed, and they rarely need to be changed. Record and save these parameter values for future reference.

If needed, see Appendix G for instructions on optimizing laser delay settings. Do not otherwise change the settings in the Laser tab unless instructed to do so by BD Biosciences. Changing the settings will affect your data.



Appendix B

BD LSR II QC Log

This sample quality control (QC) log can be photocopied or used as a guide in designing your own QC log.

Instrument Serial Number/Name		Calibration Particle				Lot #
Date						
FSC	Mean					
	CV					
SSC	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
	Mean					
	CV					
Operator Initials						

Appendix C

Filter Templates

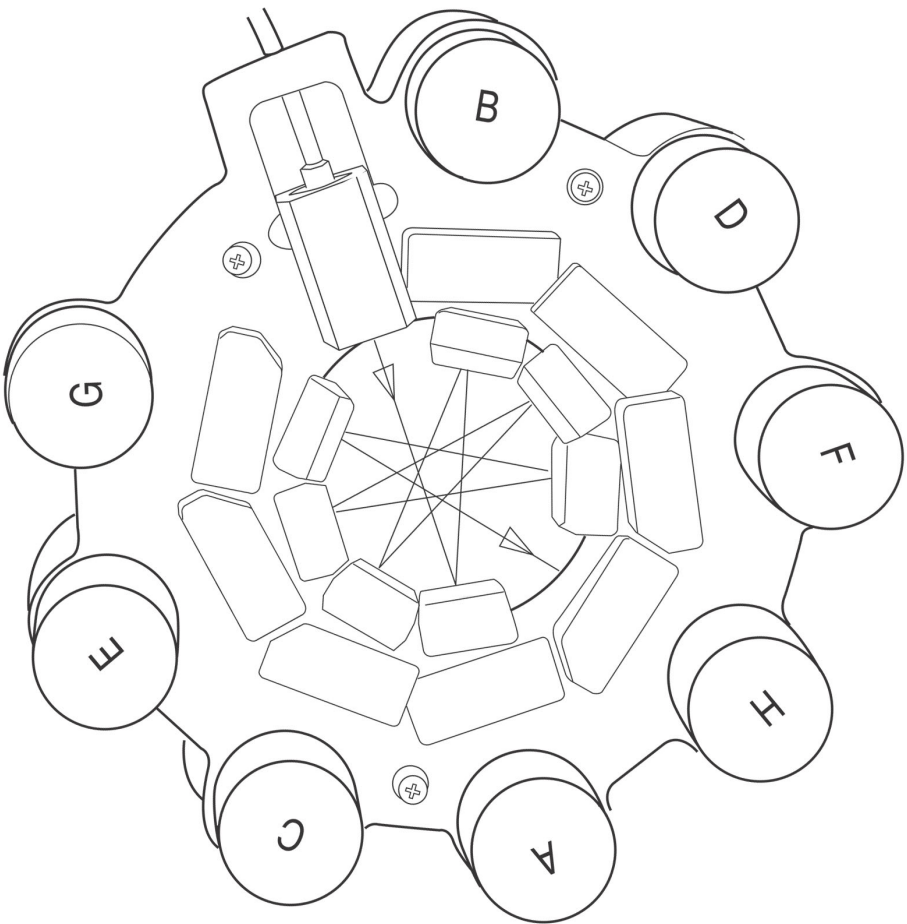
You can use these templates to note your custom filter configurations for the BD LSR II instrument.

- Octagon Template on page 145
- Trigon Template on page 147

Octagon Template

Laser _____

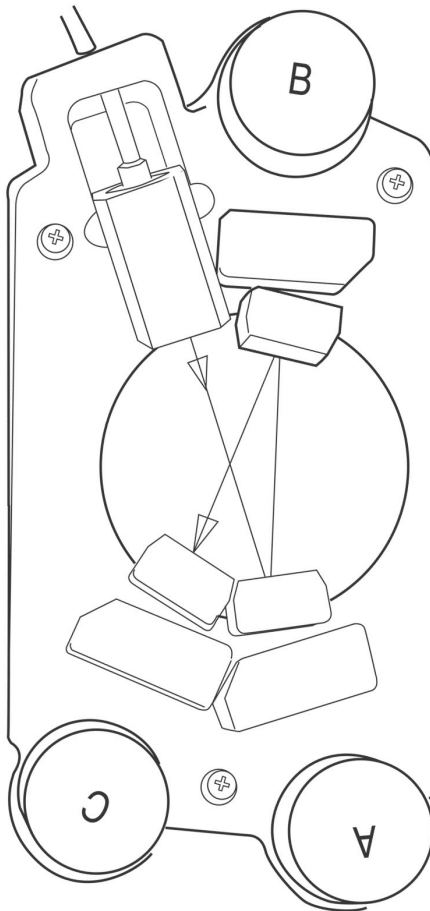
Experiment _____



Trigon Template

Laser _____

Experiment _____



Appendix D

Supplies and Consumables

To order spare parts and consumables, such as bulk fluids, from BD Biosciences:

- within the US, call: (877) 232-8995.
- outside the US, contact your local BD Biosciences customer service representative.

Worldwide contact information can be found at bdbiosciences.com.

Use the following part numbers to order supplies for your BD LSR II system:

- QC Particles on page 150
- Reagents on page 151
- Equipment on page 152

QC Particles

Particle	Laser	Supplier	Catalog No.
<ul style="list-style-type: none"> • BD Calibrite 3 beads (unlabeled, FITC, PE, PerCP) • BD Calibrite APC beads 	<ul style="list-style-type: none"> • blue 488 nm • red 633 nm 	BD Biosciences	<ul style="list-style-type: none"> • 340486 • 340487
<ul style="list-style-type: none"> • SPHERO™ Rainbow Calibration Particles (8 peak) • SPHERO Ultra Rainbow Fluorescent Particles (single peak) 	<ul style="list-style-type: none"> • all • all 	<ul style="list-style-type: none"> • BD Biosciences • Spherotech, Inc. 	<ul style="list-style-type: none"> • 559123 • URFP-30-2
DNA QC Particles kit	blue 488 nm	BD Biosciences	349523

Reagents

Reagent	Supplier	Catalog No.
BD FACSF _{low} sheath fluid	BD Biosciences	340398 (US and Latin America) 342003 (Europe)
Monoclonal antibodies	BD Biosciences	— ^a
BD FACST [™] lysing solution	BD Biosciences	349202
BD FACSRinse solution	BD Biosciences	340346
BD FACSClean solution	BD Biosciences	340345
Dyes and fluorochromes	BD Biosciences Molecular Probes Sigma	—
Chlorine bleach (5% sodium hypochlorite)	Clorox or other major supplier (to ensure that the bleach is at the correct concentration and free of particulate matter)	—

a. Refer to the BD Biosciences Product Catalog or the BD Biosciences website (bdbiosciences.com).

Equipment

Equipment Item	Supplier	Catalog No.
Bal seal	BD Biosciences	343509
O-ring, sample tube		343615
Sheath filter assembly		344678
BD Falcon polystyrene test tubes, 12 x 75-mm	BD Labware	352052 352054 352058

Appendix E

Standard Default Configuration

The standard default configuration for a BD LSR II cytometer supports detectors, filters, and mirrors for one to four lasers. This appendix describes how to set up the instrument optics using standard default configuration components.

- Standard (4-Blue 2-Violet 2-UV 2-Red) Configuration Specification on page 154
- Custom Configurations on page 159

The BD LSR II cytometer can also be ordered with one of several optional configurations, which are described in Appendix F.

Standard (4-Blue 2-Violet 2-UV 2-Red) Configuration Specification

The standard configuration supports a blue octagon, and violet, UV, and red trigons. Table E-1 shows the detectors, filters, and mirrors used in the standard default configuration, and recommended fluorochromes per detector. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

Table E-1 Default filters and fluorochromes

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	735 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	550 LP	575/26	PE, PI
	D	505 LP	530/30	FITC, GFP
	E	blank	488/10	SSC
	F	blank	blank	none
	G	blank	blank	none
	H	—	blank	none
violet trigon (405-nm laser)	A	505 LP	525/50	AmCyan
	B	blank	440/40	Pacific Blue™
	C	—	blank	none
UV trigon (355-nm laser)	A	505 LP	530/30	Indo-1 (Blue)
	B	blank	450/50	Indo-1 (Violet), DAPI
	C	—	blank	none

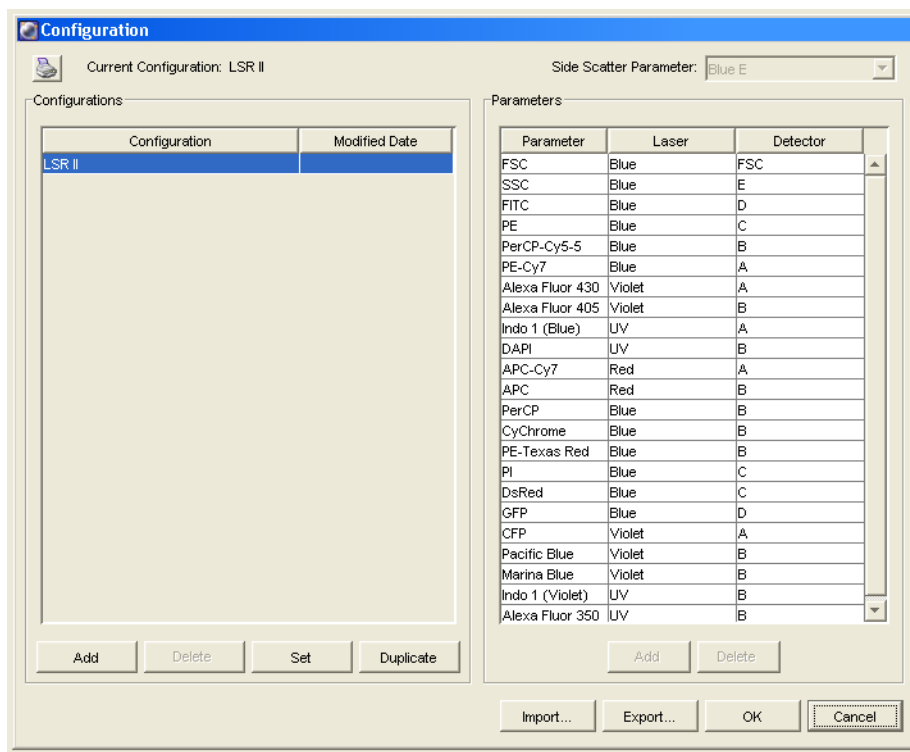
Table E-1 Default filters and fluorochromes (continued)

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
red trigon (633-nm laser)	A	735 LP	780/60	APC-Cy7
	B	blank	660/20	APC
	C	—	blank	none

Default Configuration

Figure E-1 shows the standard default instrument configuration.

Figure E-1 Default configuration



Octagon and Trigon Maps

This section shows how to install mirrors and filters in your octagon and trigons for the standard default configuration.

If a slot is filled with a filter or mirror, an identifying number appears in that position on the configuration map. If a slot is filled with a blank optical holder, that position on the configuration map is unlabeled.

Figure E-2 Standard default configuration: blue octagon

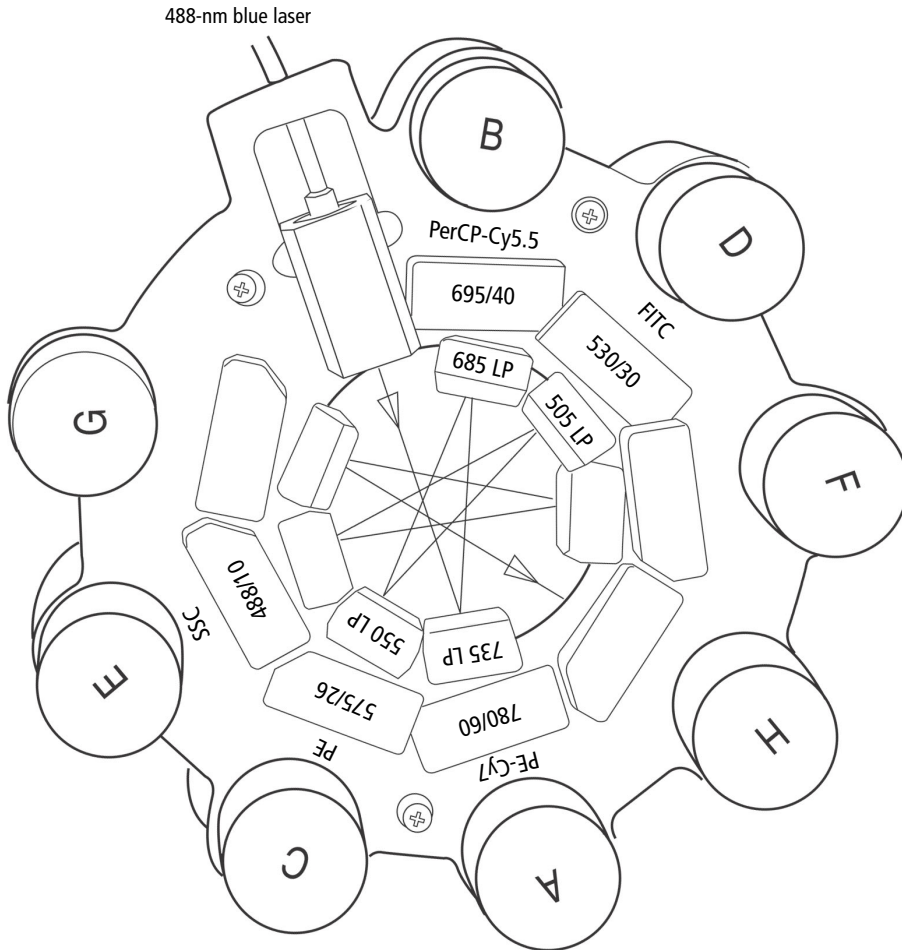
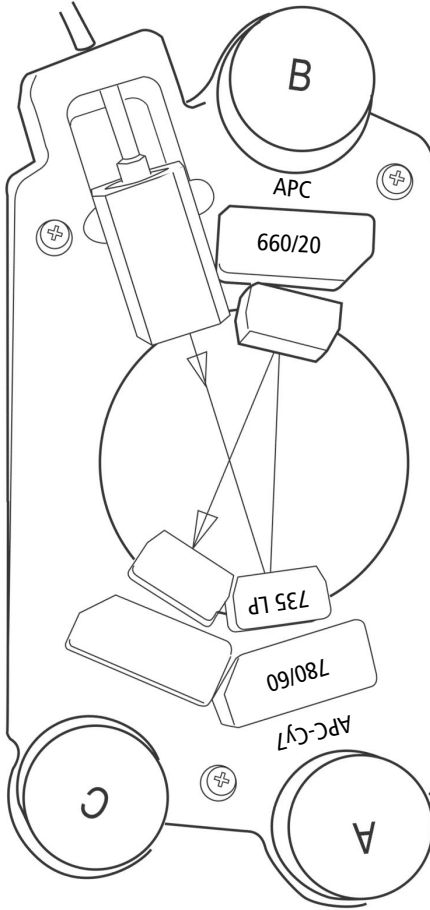


Figure E-3 Standard default configuration: red and violet trigons

633-nm red laser



405-nm violet laser

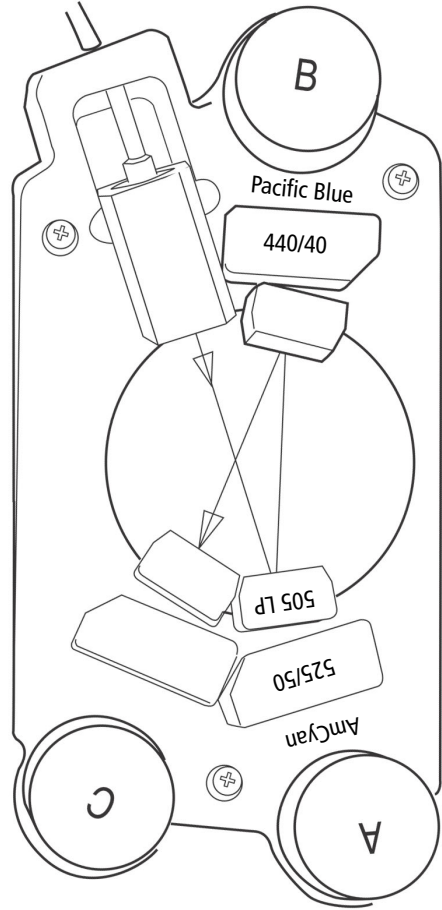
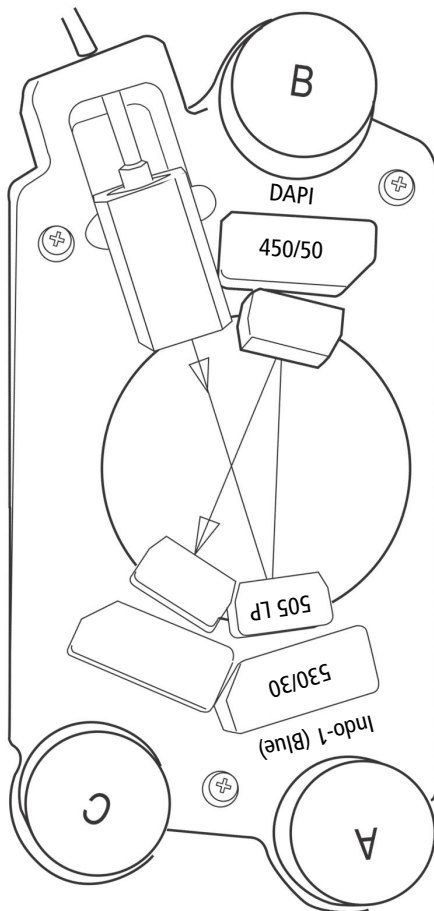


Figure E-4 Standard default configuration: UV trigon
355-nm UV laser



Custom Configurations

This section describes some common custom filter and mirror configurations. Table E-2 shows the detector arrays, mirrors, and filters used in the custom configurations, and recommended fluorochromes per detector. The mirrors and filters used in these custom configurations are contained in the BD LSR II instrument spares kit.

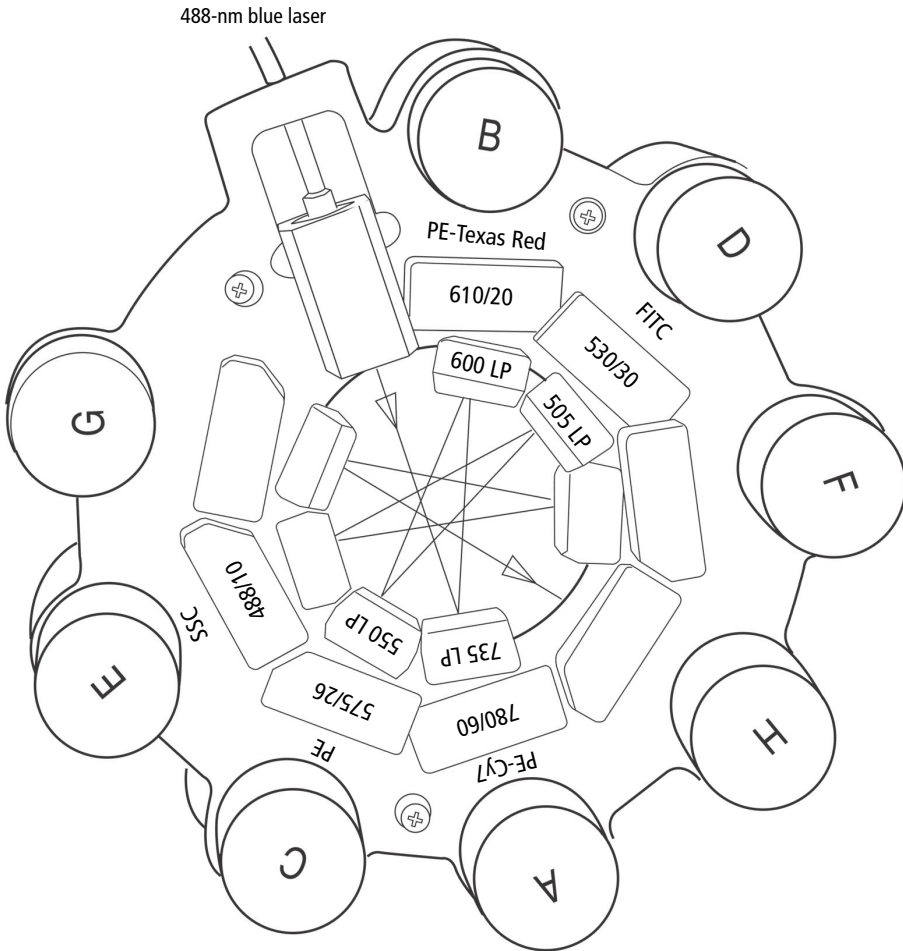
Table E-2 Spare filters and fluorochromes

Detector Array (Laser)	Mirror	Filter	Fluorochrome
blue octagon standard 488-nm blue laser	600 LP	610/20	PE-Texas Red™
	635 LP	670/14	PerCP BD Cy-Chrome™ reagent
		585/42	DsRed
violet trigon optional 405-nm violet laser	none	none	none
UV trigon optional 355-nm UV laser	450 LP		Indo-1 (Blue)
		405/20	Indo-1 (Violet)
red trigon optional 633-nm red laser	none	none	none

Maps on the following pages show how to install mirrors and filters in your octagon and trigons for common custom configurations.

PE-Texas Red™

To use PE-Texas Red™, replace the mirror and filter for the B PMT of the blue octagon as shown below.

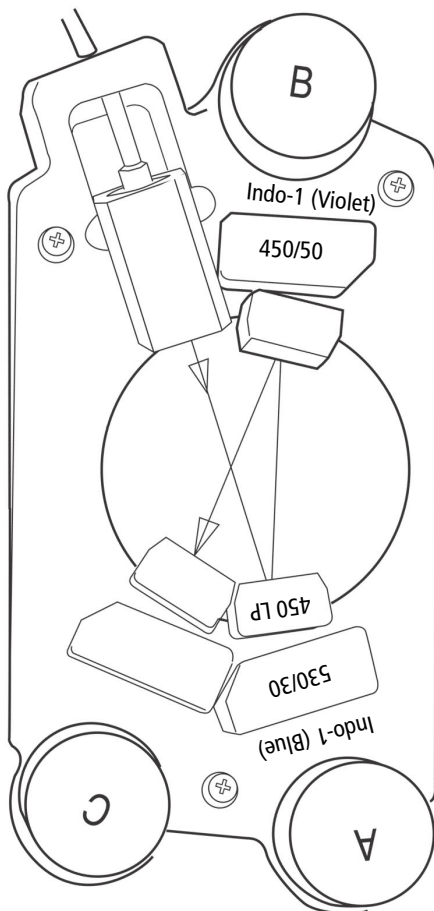


Indo-1

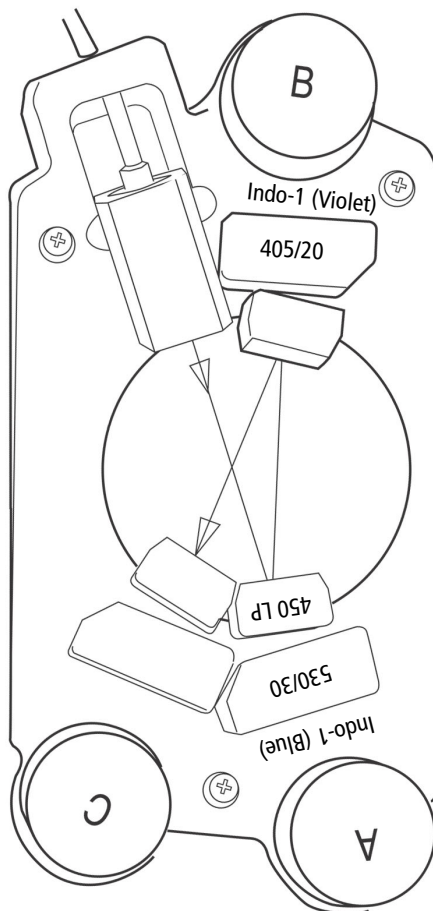
If you have a violet laser, replace the mirror for the A PMT of the UV trigon as shown on the left below.

If you do not have a violet laser, replace both the mirror for the A PMT and the filter for the B PMT of the UV trigon as shown on the right below.

355-nm UV laser
(with violet laser)

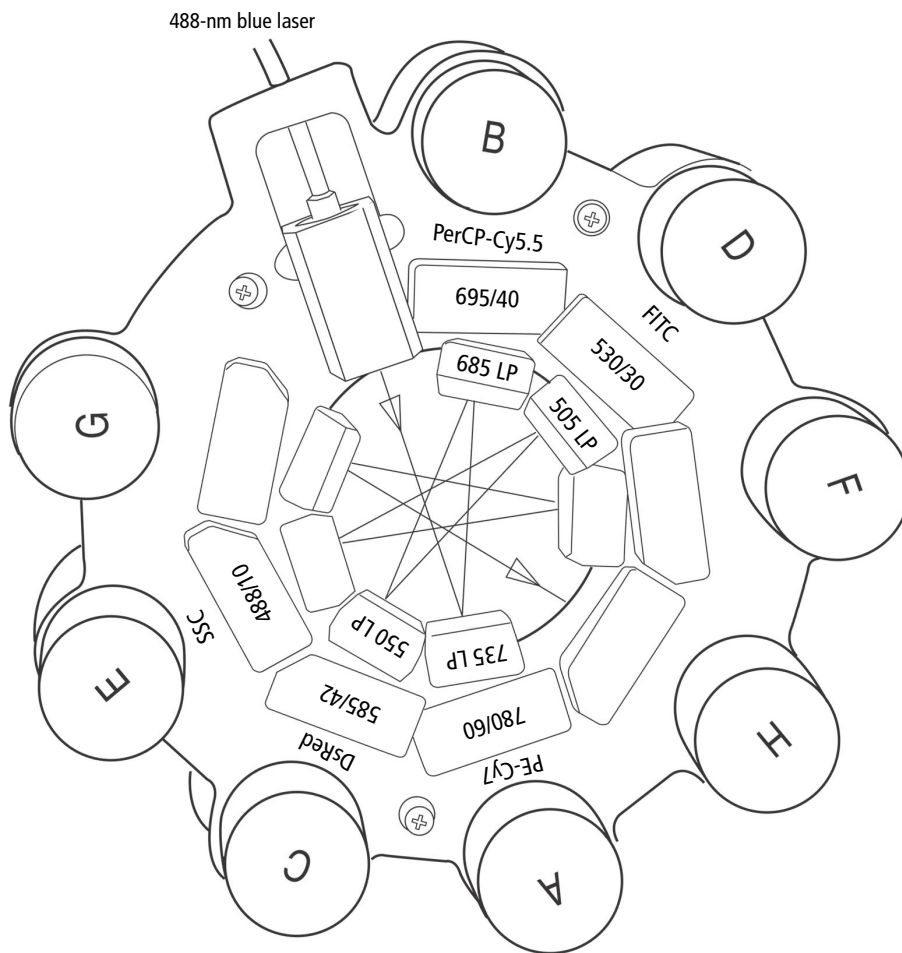


355-nm UV laser
(no violet laser)



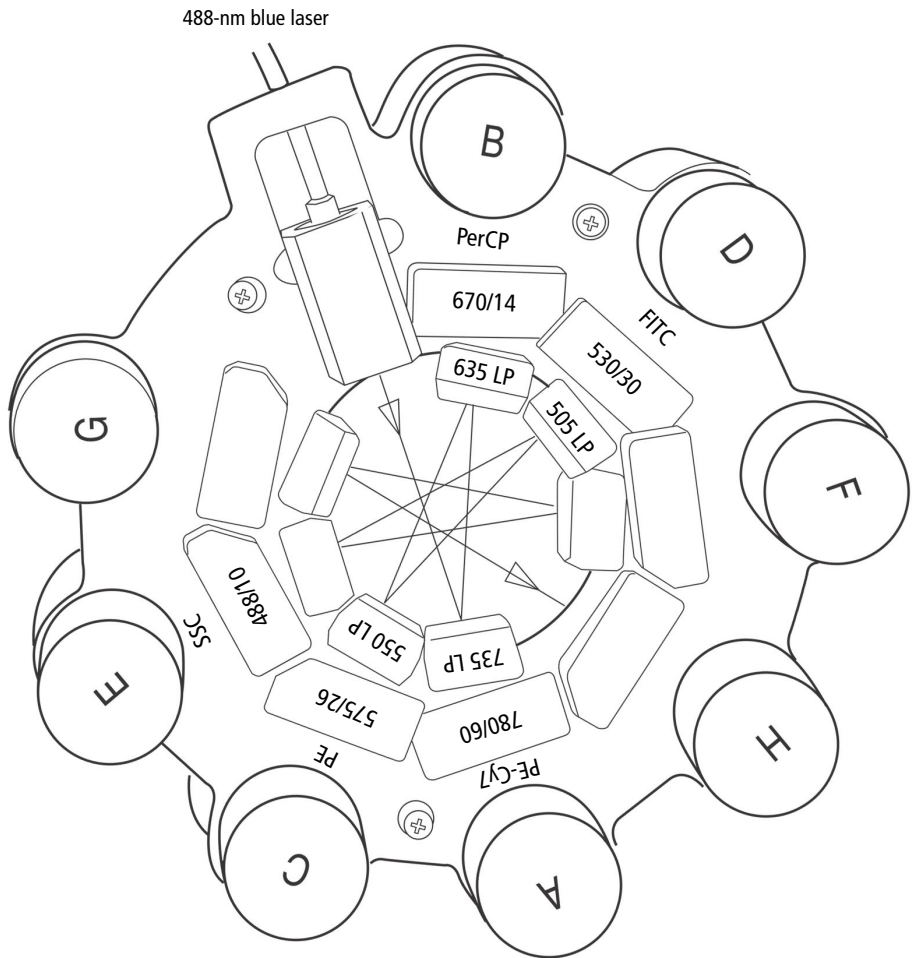
DsRed

To use DsRed, replace the filter for the C PMT of the blue octagon as shown below.



PerCP or BD Cy-Chrome Reagent

To use PerCP or BD Cy-Chrome reagent, replace the mirror and filter for the B PMT of the blue octagon as shown below.



Appendix F

Special Order Configurations

The BD LSR II cytometer can be ordered with, or upgraded to one of several laser and detector array options. If you upgrade your configuration, you must use BD FACS Instrument Configuration Application (ICA) to update your BD FACSDiva software default instrument configuration (database object).

This appendix contains the following information:

- Common Special Order Configurations on page 166
- Special Order Configuration Trigon and Octagon Maps on page 180
- BD FACS Instrument Configuration Application on page 187

Common Special Order Configurations

The following are commonly used configurations.

- 6-Blue 0-Violet 0-UV 3-Red Configuration on page 167
- 6-Blue 2-Violet 0-UV 3-Red Configuration on page 168
- 6-Blue 0-Violet 2-UV 3-Red Configuration on page 169
- 6-Blue 2-Violet 2-UV 3-Red Configuration on page 171
- 6-Blue 6-Violet 0-UV 3-Red Configuration on page 172
- 6-Blue 6-Violet 0-UV 4-Red Configuration on page 174
- 6-Blue 6-Violet 2-UV 3-Red Configuration on page 176
- 6-Blue 6-Violet 2-UV 4-Red Configuration on page 178

6-Blue 0-Violet 0-UV 3-Red Configuration

6-Blue 0-Violet 0-UV 3-Red supports a blue octagon, and a red trigon. Table F-1 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 0-Violet 0-UV 3-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 3-Color Red Trigon Default Configuration Map on page 185

Table F-1 6-Blue 0-Violet 0-UV 3-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none
red trigon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	—	660/20	APC

6-Blue 2-Violet 0-UV 3-Red Configuration

6-Blue 2-Violet 0-UV 3-Red supports a blue octagon, and violet and red trigons. Table F-2 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 2-Violet 0-UV 3-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 2-Color Violet Trigon Default Configuration Map on page 182
- 3-Color Red Trigon Default Configuration Map on page 185

Table F-2 6-Blue 2-Violet 0-UV 3-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none

Table F-2 6-Blue 2-Violet 0-UV 3-Red default mirror and filter configuration (continued)

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
violet trigon (405-nm laser)	A	505 LP	525/50	AmCyan
	B	blank	450/50	Pacific Blue™
	C	—	blank	none
red trigon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	—	660/20	APC

6-Blue 0-Violet 2-UV 3-Red Configuration

6-Blue 0-Violet 2-UV 3-Red supports a blue octagon, and UV and red trigons. Table F-3 on page 170 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 0-Violet 2-UV 3-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 2-Color UV Trigon Default Configuration Map on page 184
- 3-Color Red Trigon Default Configuration Map on page 185

Table F-3 6-Blue 0-Violet 2-UV 3-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none
UV trigon (355-nm laser)	A	505 LP	530/30	Indo-1 (Blue)
	B	blank	450/50	Indo-1 (Violet), DAPI
	C	—	blank	none
red trigon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	—	660/20	APC

6-Blue 2-Violet 2-UV 3-Red Configuration

6-Blue 2-Violet 2-UV 3-Red supports a blue octagon, and violet, UV, and red trigons. Table F-4 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 2-Violet 2-UV 3-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 2-Color Violet Trigon Default Configuration Map on page 182
- 2-Color UV Trigon Default Configuration Map on page 184
- 3-Color Red Trigon Default Configuration Map on page 185

Table F-4 6-Blue 2-Violet 2-UV 3-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none

Table F-4 6-Blue 2-Violet 2-UV 3-Red default mirror and filter configuration (continued)

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
violet trigon (405-nm laser)	A	505 LP	525/50	AmCyan
	B	blank	450/50	Pacific Blue™
	C	—	blank	none
UV trigon (355-nm laser)	A	505 LP	530/30	Indo-1 (Blue)
	B	blank	450/50	Indo-1 (Violet), DAPI
	C	—	blank	none
red trigon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	—	660/20	APC

6-Blue 6-Violet 0-UV 3-Red Configuration

6-Blue 6-Violet 0-UV 3-Red supports blue violet octagons, and a red trigon. Table F-5 on page 173 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 6-Violet 0-UV 3-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 6-Color Violet Octagon Default Configuration Map on page 183
- 3-Color Red Trigon Default Configuration Map on page 185

Table F-5 6-Blue 6-Violet 0-UV 3-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none
violet octagon (405-nm laser)	A	630 LP	655/8	Qdot 655
	B	595 LP	605/12	Qdot 605
	C	575 LP	585/15	Qdot 585
	D	545 LP	560/20	Qdot 565
	E	475 LP	525/50	AmCyan, Qdot 525
	F	blank	450/50	Pacific Blue™
	G	blank	blank	none
	H	—	blank	none
red trigon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	—	660/20	APC

6-Blue 6-Violet 0-UV 4-Red Configuration

6-Blue 6-Violet 0-UV 4-Red supports blue, violet, and red octagons. Table F-6 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 6-Violet 0-UV 4-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 6-Color Violet Octagon Default Configuration Map on page 183
- 4-Color Red Octagon Default Configuration Map on page 186

Table F-6 6-Blue 6-Violet 0-UV 4-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none

Table F-6 6-Blue 6-Violet 0-UV 4-Red default mirror and filter configuration (continued)

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
violet octagon (405-nm laser)	A	630 LP	655/8	Qdot 655
	B	595 LP	605/12	Qdot 605
	C	575 LP	585/15	Qdot 585
	D	545 LP	560/20	Qdot 565
	E	475 LP	525/50	AmCyan, Qdot 525
	F	blank	450/50	Pacific Blue™
	G	blank	blank	none
	H	—	blank	none
red octagon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	675 LP	685/35	Alexa Fluor® 680
	D	—	660/20	APC
	E	blank	blank	none
	F	blank	blank	none
	G	blank	blank	none
	H	—	blank	none

6-Blue 6-Violet 2-UV 3-Red Configuration

6-Blue 6-Violet 2-UV 3-Red supports blue and violet octagons, and UV and red trigons. Table F-7 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 6-Violet 2-UV 3-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 6-Color Violet Octagon Default Configuration Map on page 183
- 2-Color UV Trigon Default Configuration Map on page 184
- 3-Color Red Trigon Default Configuration Map on page 185

Table F-7 6-Blue 6-Violet 2-UV 3-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none

Table F-7 6-Blue 6-Violet 2-UV 3-Red default mirror and filter configuration (continued)

violet octagon (405-nm laser)	A	630 LP	655/8	Qdot 655
	B	595 LP	605/12	Qdot 605
	C	575 LP	585/15	Qdot 585
	D	545 LP	560/20	Qdot 565
	E	475 LP	525/50	AmCyan, Qdot 525
	F	blank	450/50	Pacific Blue™
	G	blank	blank	none
	H	—	blank	none
UV trigon (355-nm laser)	A	505 LP	530/30	Indo-1 (Blue)
	B	blank	450/50	Indo-1 (Violet), DAPI
	C	—	blank	none
red trigon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	—	660/20	APC

6-Blue 6-Violet 2-UV 4-Red Configuration

6-Blue 6-Violet 2-UV 4-Red supports blue, violet, and red octagons, and a UV trigon. Table F-8 shows the detectors, filters, and mirrors used in the default configuration. The word “blank” indicates that a blank optical holder should be used instead of a mirror or filter. A dash (—) indicates that no slot exists for a mirror in that PMT position.

The 6-Blue 6-Violet 2-UV 4-Red maps are:

- 6-Color Blue Octagon Default Configuration Map on page 181
- 6-Color Violet Octagon Default Configuration Map on page 183
- 2-Color UV Trigon Default Configuration Map on page 184
- 4-Color Red Octagon Default Configuration Map on page 186

Table F-8 6-Blue 6-Violet 2-UV 4-Red default mirror and filter configuration

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
blue octagon (488-nm laser)	A	755 LP	780/60	PE-Cy7
	B	685 LP	695/40	PerCP-Cy5.5
	C	655 LP	660/20	PE-Cy5
	D	600 LP	610/20	PE-Texas Red™
	E	550 LP	575/26	PE
	F	505 LP	530/30	FITC, Alexa Fluor® 488
	G	blank	488/10	SSC
	H	—	blank	none

Table F-8 6-Blue 6-Violet 2-UV 4-Red default mirror and filter configuration (continued)

Detector Array (Laser)	PMT (Detector)	Longpass Dichroic Mirror	Bandpass Filter	Fluorochrome or Scatter Parameter
violet octagon (405-nm laser)	A	630 LP	655/8	Qdot 655
	B	595 LP	605/12	Qdot 605
	C	575 LP	585/15	Qdot 585
	D	545 LP	560/20	Qdot 565
	E	475 LP	525/50	AmCyan, Qdot 525
	F	blank	450/50	Pacific Blue™
	G	blank	blank	none
	H	—	blank	none
UV trigon (355-nm laser)	A	505 LP	530/30	Indo-1 (Blue)
	B	blank	450/50	Indo-1 (Violet), DAPI
	C	—	blank	none
red octagon (633-nm laser)	A	755 LP	780/60	APC-Cy7
	B	710 LP	730/45	Alexa Fluor® 700
	C	675 LP	685/35	Alexa Fluor® 680
	D	—	660/20	APC
	E	blank	blank	none
	F	blank	blank	none
	G	blank	blank	none
	H	—	blank	none

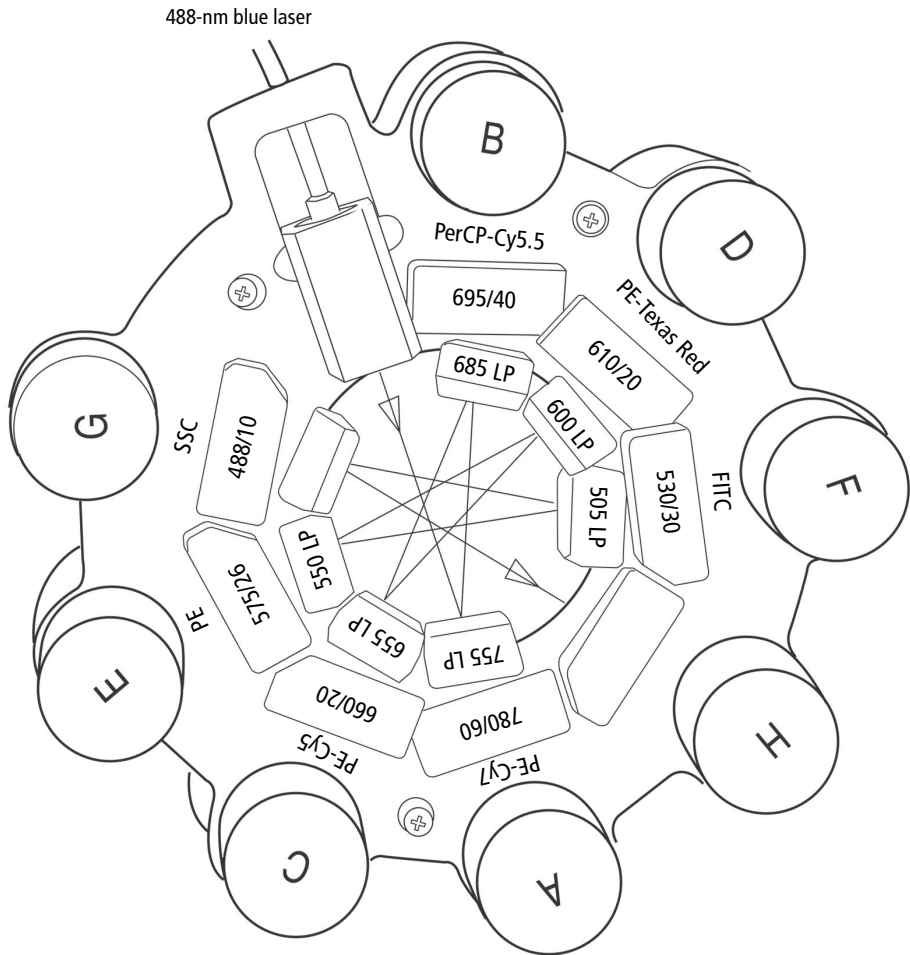
Special Order Configuration Trigon and Octagon Maps

If a slot contains a filter or mirror, a number appears in the corresponding position on the configuration map. If a slot contains a blank optical holder, nothing is written in the corresponding position on the configuration map.

The default configuration maps are:

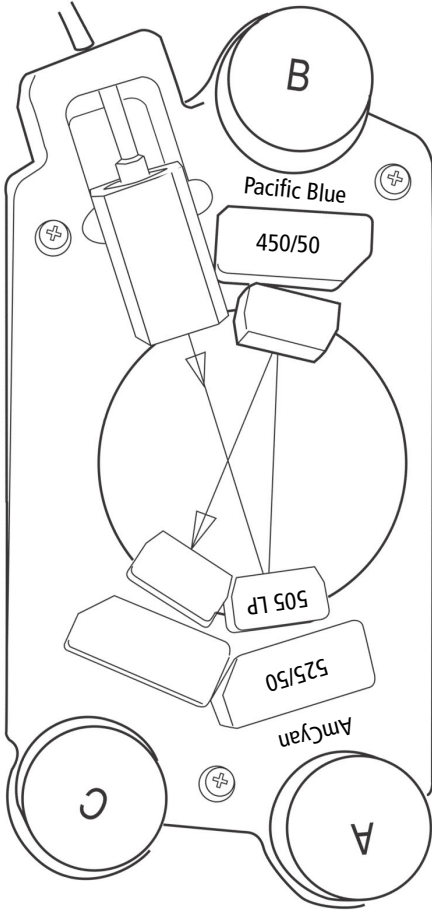
- 6-Color Blue Octagon Default Configuration Map on page 181
- 2-Color Violet Trigon Default Configuration Map on page 182
- 6-Color Violet Octagon Default Configuration Map on page 183
- 2-Color UV Trigon Default Configuration Map on page 184
- 3-Color Red Trigon Default Configuration Map on page 185
- 4-Color Red Octagon Default Configuration Map on page 186

6-Color Blue Octagon Default Configuration Map

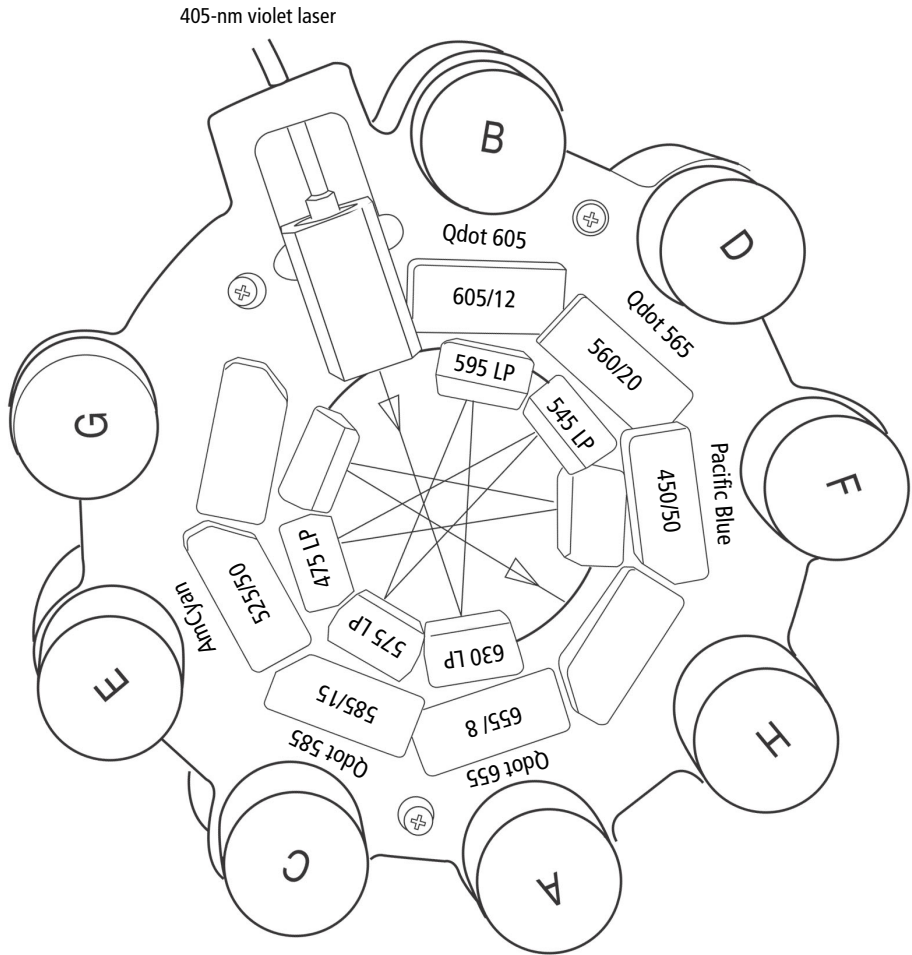


2-Color Violet Trigon Default Configuration Map

405-nm violet laser

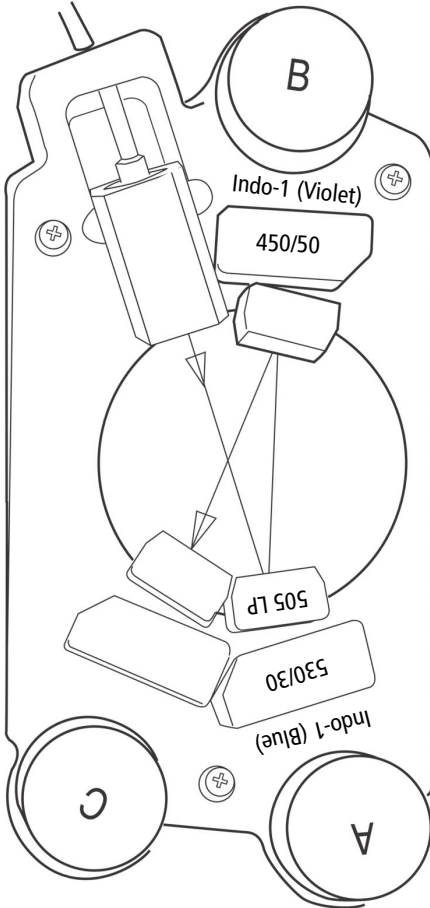


6-Color Violet Octagon Default Configuration Map



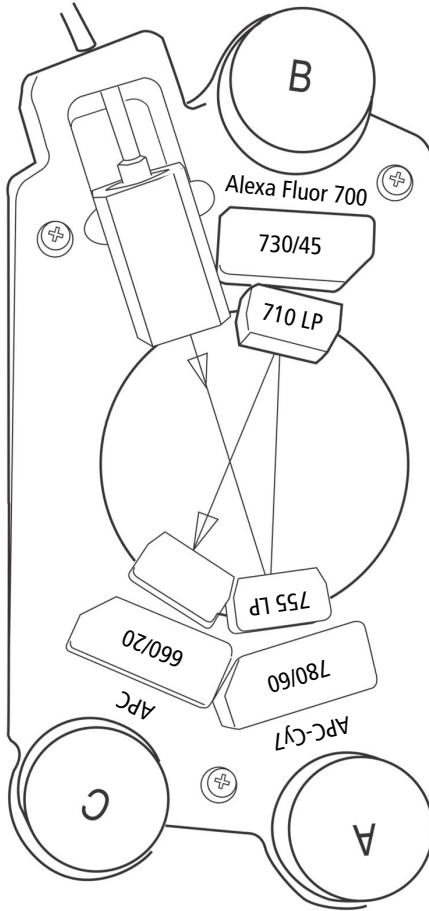
2-Color UV Trigon Default Configuration Map

355-nm UV laser

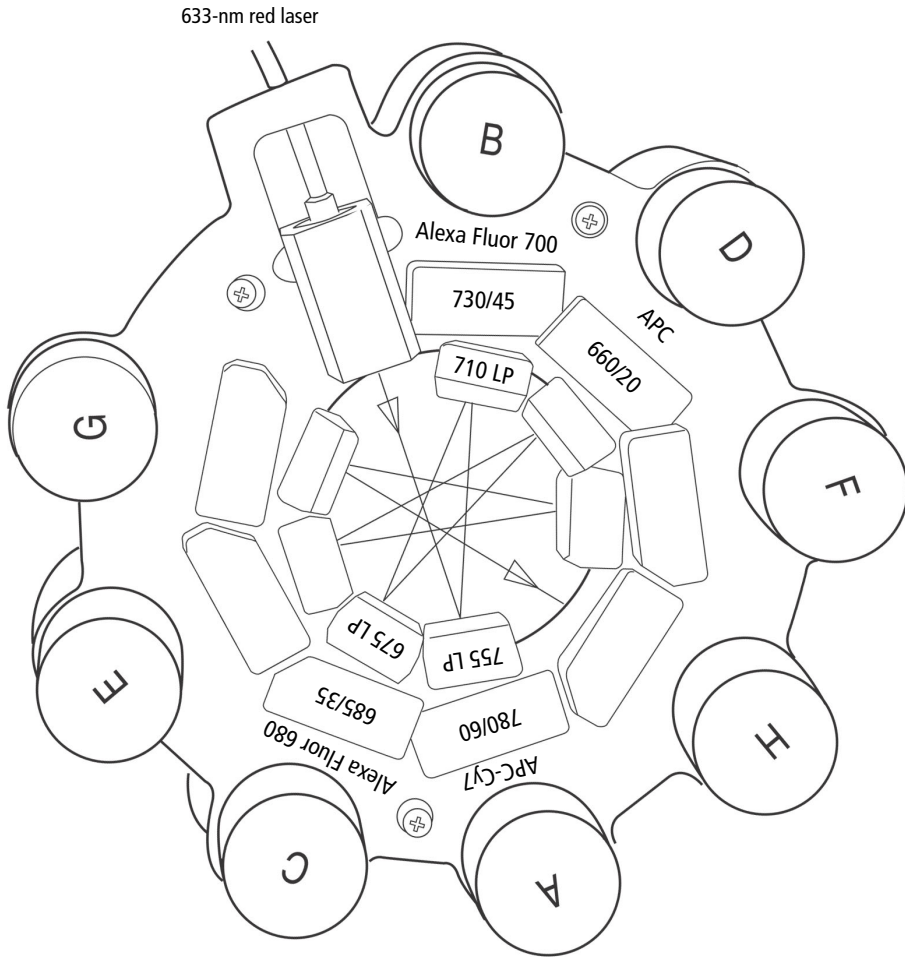


3-Color Red Trigon Default Configuration Map

633-nm red laser



4-Color Red Octagon Default Configuration Map



BD FACS Instrument Configuration Application

This section contains the following information:

- Installing ICA on this page
- Updating Your Default Configuration on page 188

Installing ICA

To install the BD FACS Instrument Configuration Application:

- 1 Mount your ICA CD on an available drive.

If the installer setup program does not start automatically, launch setup.exe from the CD root folder. After the setup runs, the InstallShield Wizard will lead you through several installation screens.

- 2 On the Setup Type screen, select *Complete installation*.
- 3 When the installation is complete, click Finish and eject the CD.

Re-installing ICA

If ICA is already installed on your computer, the InstallShield Wizard welcome screen asks you if you want to modify, repair, or remove the program. To re-install ICA, first remove it, then rerun the installer.

Uninstalling ICA

To remove ICA from your computer, you can either:

- Run the ICA installer and remove the program as described above.
- Use your operating system's *Add or Remove Programs* control panel.

Updating Your Default Configuration

When you upgrade your BD LSR II instrument configuration, your BD FACSDiva software default instrument configuration must be updated.

- 1** Click Start > Programs > ICA.

The ICA activity window is displayed (Figure F-1 on page 189 shows the ICA activity window as it would appear after step 3 below).

- 2** Choose LSRII from the Select Instrument pull-down menu.

The Select Configuration scroll view lists the available BD LSR II instrument configurations.

- 3** Select the BD LSR II instrument configuration that matches your configuration upgrade.

The main text area displays parameter, detector arrays, and channel information for the selected configuration (Figure F-1 on page 189).

- 4** Click Upgrade to update your BD FACSDiva software default instrument configuration.

In the main text area, a log shows an update of actions performed on the instrument configuration database (Figure F-2 on page 190).

Figure F-1 ICA: Select Configuration field

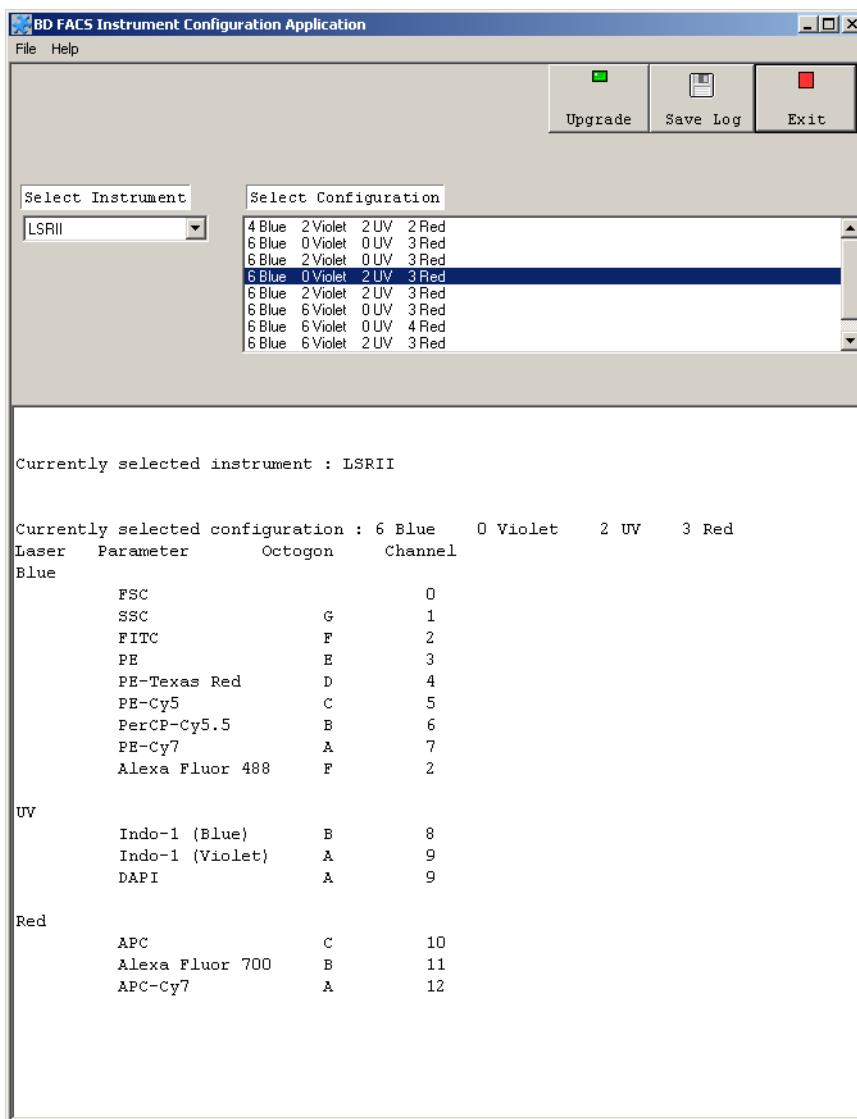
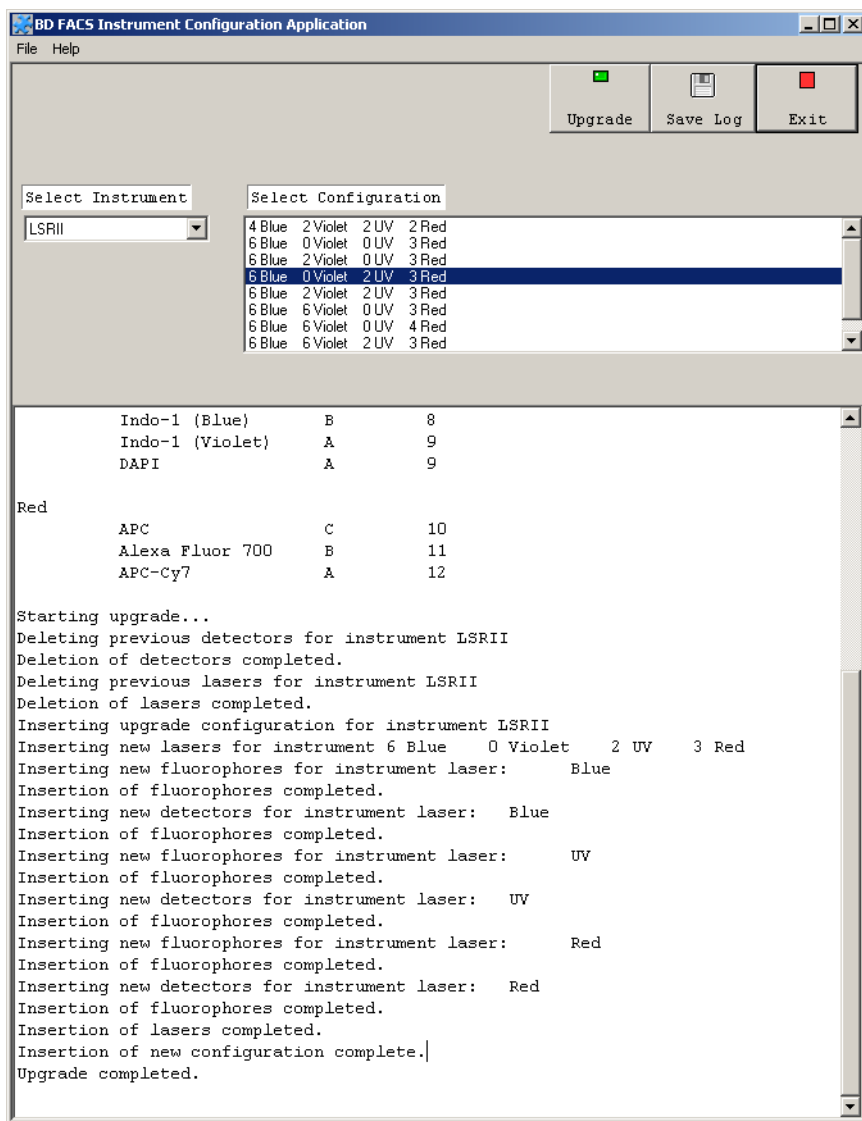


Figure F-2 ICA: upgrade log

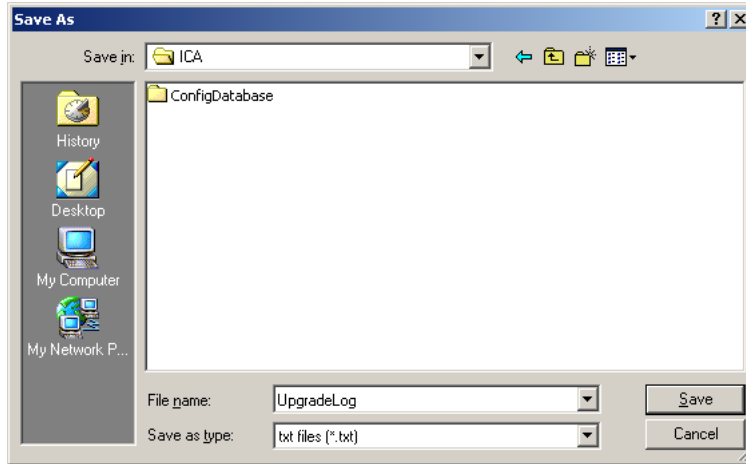


- 5 Click Save Log to save a file containing the current text of the ICA main text view.

A Save As dialog is displayed (Figure F-3).

- 6 Choose a location and name for your log file, and click Save.

Figure F-3 Select configuration



- 7 Click Exit to exit the program.

Appendix G

Setting Laser Delay

This appendix describes how to optimize laser delay settings in a multiple laser system.

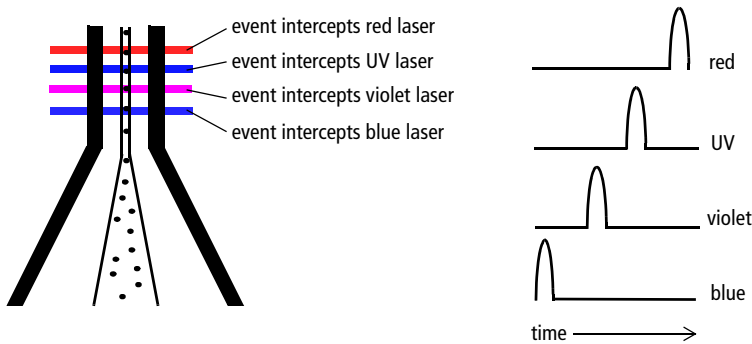
- About Laser Delay on page 194
- Optimizing Laser Delay on page 195

About Laser Delay

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

In the BD LSR II four-laser system, the blue laser intercepts the stream first, followed by the violet, UV, and red lasers. Because the laser signals are spatially separated, there is a slight delay between the detection of each laser's signal (Figure G-1).

Figure G-1 Signal separation over time



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

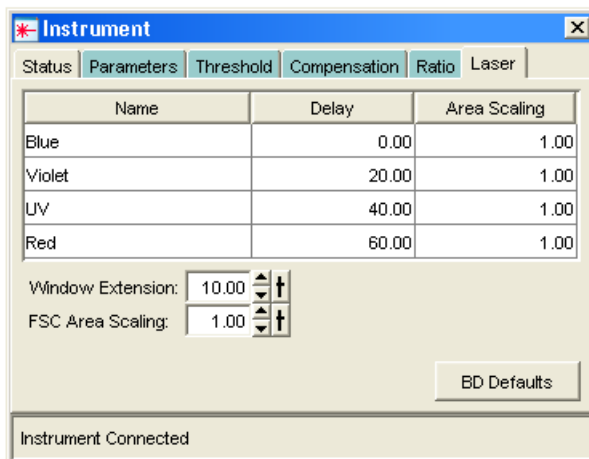
Optimizing Laser Delay

Laser delay is set using BD FACSDiva software. To optimize the delay for a given laser, you acquire events from a sample with a fluorescence signal excited by that laser. Follow the procedures in Chapter 3, Running Samples, for sample optimization and acquiring data.

- 1 While acquiring data from your sample, create a histogram plot to show the fluorescence signal excited by the laser in which the delay is to be optimized.
- 2 In the Acquisition Dashboard, set the Events to Display to 500 evt.
- 3 Select the Laser tab in the Instrument window (Figure G-2).

Window extension and laser delay values are displayed in μsec .

Figure G-2 Laser tab of the Instrument window



- 4 Set the window extension value to 0 μsec .

- 5** Set an initial laser delay value **ONLY** for the laser whose delay is to be optimized.
 - If you are optimizing the violet laser, set its delay to 20 μsec .
 - If you are optimizing the UV laser, set its delay to 40 μsec .
 - If you are optimizing the red laser, set its delay to 60 μsec .
- 6** While observing the positive events on the histogram plot, adjust the laser delay in 1 μsec increments within a range of 10 μsec of the initial setting.

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

You should also stay within a range of 10 μsec of the initial setting (step 5).

Preserve the setting that maximizes the fluorescence intensity.
- 10** Reset the window extension to 10 μsec .

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