BD FACSDiva Software 6.0 Reference Manual

For In Vitro Diagnostic Use

bdbiosciences.com
Part No. 642218 Rev. A
June 2007

BD Biosciences
2350 Qume Drive
San Jose, CA 95131-1807
USA
Tel (877) 232-8995
Fax (800) 325-9637
facservice@bd.com

Asia Pacific
Tel (65) 6-861-0633
Fax (65) 6-860-1593

Europe
Tel (32) 2 400 98 95
Fax (32) 2 401 70 94
help.biosciences@europe.bd.com

Brazil
Tel (55) 11-5185-9995
Fax (55) 11-5185-9895

Japan
Nippon Becton Dickinson Company, Ltd.
Toll Free 0120-8555-90
Tel 81-24-593-5405
Fax 81-24-593-5761

Canada
Toll Free (888) 259-0187
Tel (905) 542-8028
Fax (888) 229-9918
canada@bd.com

Mexico
Toll Free 01-800-236-2543
Tel (52) 55 5999 8296
Fax (52) 55 5999 8288

Notice to Customers

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.

BD FACSDiva software contains VxWorks as embedded software ("Run-Time Module"). The Run-Time Module was developed by a third party and we are obligated to notify our customers about the limitations for use.

Regarding this Run-Time Module, you are prohibited from: (i) copying the Run-Time Module contained herein, except for archive purposes consistent with your archive procedures; (ii) transferring the Run-Time Module to a third party; (iii) modifying, decompiling, disassembling, reverse engineering or otherwise attempting to derive the Source Code of the Run-Time Module; (iv) exporting the Run-Time Module or underlying technology in contravention of applicable US and foreign export laws and regulations; and (v) using the Run-Time Module other than in connection with operation of BD instrumentation. The Run-Time Module is licensed, not sold and BD and its licensors retain ownership of all copies of the Run-Time Module. BD expressly disclaims all implied warranties, including without limitation the implied warranties of merchantability, fitness for particular purpose, title and noninfringement. Under no event shall BD or its licensors be subject to any liability for any special, indirect punitive incidental or consequential damages. Any further distribution of the Run-Time Module shall be subject to the same restrictions set forth herein.

History

Revision	Date	Change Made
341756 Rev A	8/01	Production release for BD FACSDiva™ software version 1.0.
330798 Rev A	1/02	Updated for version 2.0: enhanced performance, database redesign and data management utility, scalable data display, instrument settings features, Next button, more copy/paste ability, plot display features.
330802 Rev A	6/02	Updated for version 2.1: enhanced performance, workspace redesign with separable components, Browser-level folders, functioning Acquisition pointer, Sort Layout redesign, objects duplicated by dragging, drill-down gating, log decade gridlines on plots, view/hide gate boundaries, context-sensitive cursors, histogram smoothing, gate changes downloaded during sorting, automatic acquisition during record/sort, experiment import/export, Ratio Scaling factor per ratio, Area Scaling factor per laser.
333602 Rev A	11/02	Updated for version 2.2: Acquisition Templates, User Preferences, automated compensation calculation, copy/paste spectral overlap values, Instrument Status report, Sort report, Sort Layout counters, Contour plots, Auto-Interval gates, sticky buttons, Statistics View editor.
337370 Rev A	1/04	Updated for version 4.0: user login, shared vs private experiments, new Worksheet buttons (increase/decrease plot, snap-to interval gate), new User Preferences, experiment and specimen templates, batch analysis, adjustment controls for snap-to gates, instrument features for the BD FACSCanto™ instrument.
337999 Rev A	4/04	Updated for version 4.0.1: CE IVD release
338572 Rev A	9/04	Updated for version 4.1: biexponential plots, hinged quadrant gates, density plots, User Preferences for default templates and plot background color, global instrument settings, restrictions on where instrument settings are edited, new process for creating compensation control tubes, default QC templates, FSC area scaling, copy/paste worksheet elements to Microsoft® Office applications, support for the BD™ High Throughput Sampler (HTS) on the BD™ LSR II.
640749 Rev A	5/06	Updated for version 5.0: workflow improvements for the BD FACS™ Loader and support for the BD HTS option on BD FACSCanto instruments, new look and feel, ability to disable biexponential scaling, apply scaling values to other elements in experiments, scale to population, copy/paste gates, import/export user profiles, import/export, duplicate, and print instrument configurations. Refer to New Features in *Getting Started with BD FACSDiva Software* for details.
642218 Rev A	6/07	Updated for version 6.0: easier steps for cytometer configuration, workflow improvements for administration, browser usage, acquisition, import and export of files, improved look and feel, more robust statistics, new gating options, and support for the 375 Laser for the BD FACSAria. Refer to New Features in *Getting Started with BD FACSDiva Software* for details.

Contents

About This Manual

This manual describes how to use BD FACSDiva™ software. For information on how to operate and maintain your flow cytometer, refer to your cytometer manual.

The *BD FACSDiva Software Reference Manual* assumes you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, refer to the documentation provided with your computer.

First-time users of BD FACSDiva software should read:

- Chapter 1 for software requirements and compatibility, installation, and administrative options
- Chapter 2 and Chapter 3 to learn about basic software functions and cytometer controls
- Chapter 4 to learn about analysis tools like worksheets, plots, gates, and statistics
- Chapter 5 to learn how to manage data and import and export files

For practice tutorials to help you get started with the software, refer to *Getting Started with BD FACSDiva Software*.

Once you become familiar with routine operation and need only a quick reminder of the software menus or keyboard shortcuts, see Appendix A. For a review of digital theory, see Appendix B.

Conventions

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

Table 1 Hazard symbols

Symbol	Meaning
⚠	Caution: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death

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
Italics	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 *p*.

Technical Assistance

For technical questions or assistance in solving a problem:

- In BD FACSDiva software, choose Help > Online Help. Locate and read topics specific to the operation you are performing.
- In BD FACSDiva software, choose Help > Online Training.
- Refer to the Troubleshooting section in the Software or Cytometer books.
- Refer to the BD Biosciences website: bdbiosciences.com

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; software version and computer system specifications
- any error messages
- details of recent cytometer performance

BD Biosciences might also request the console.log and LogFile.xml files located in C:\Program Files\BD FACSDiva Software\log, as well as your exported experiment file.

For cytometer 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.

Limitations

For In Vitro Diagnostic Use (IVD) when used with IVD reagents and cytometers. Refer to the information supplied by the manufacturer for application-specific limitations.

1

Software Installation and Setup

The following topics are covered in this chapter:

About BD FACSDiva Software

BD FACSDiva software is a flexible data acquisition and analysis package specifically designed for digital-based flow cytometers. The software uses flexible features to simplify acquisition, including experiment templates, user-definable experiment layouts, and automated compensation calculation. The unique software also provides powerful analysis features including one-click snap-to gating tools, hierarchical gating, the ability to copy and paste gates, and biexponential display.

To simplify experiment and data management, BD FACSDiva software uses a *Browser* view that allows you to easily organize experiments, group specimens and tubes, design global or tube-specific analyses, and set independent cytometer settings. The Browser also allows you to manage and process recorded data in the context of a single tube or panel, as well as an entire experiment.

Supporting BD FACSCanto™, BD FACSAria™ or BD™ LSR II flow cytometers with the digital electronics option, this PC-based software provides you with all the setup, acquisition, control, and analysis features to quickly and efficiently generate quality data from a BD FACS™ brand digital flow cytometer.

What's Included

The BD FACSDiva installer installs the following applications:

- BD FACSDiva software 6.0 for acquiring and analyzing data
- BD FACSDiva Data Manager utility for backing up and restoring the database
- Java™ 2 Runtime Environment (JRE) v1.5.0_11 for running BD FACSDiva software
- Sybase® SQL Anywhere® Studio v9.0.2 for running the database
- Sentinel System Driver™ v5.41.1 for using the security module

- Adobe® Acrobat® Reader® v7.0 for viewing the PDF versions of the reference manual and *Getting Started* guide

- Microsoft .NET 2.0 Framework

Documentation

The software package includes online and paper documentation to help you learn how to use the application.

- The *BD FACSDiva Software Reference Manual* contains reference information on all software components. It is available as a PDF that can be opened, searched, and printed using Adobe Acrobat Reader, or a printed copy can be requested from BD Biosciences.

  To access the PDF file, choose Help > Literature > Reference Manual or double-click the shortcut icon on the desktop.

- *Getting Started with BD FACSDiva Software* contains tutorials to help new users get started using the software or experienced users become familiar with new features. A printed copy is provided with each BD FACSDiva software release.

  To access the PDF file, double-click the shortcut icon on the desktop or choose Help > Literature > Getting Started Guide.

- The online help system contains information on how to use BD FACSDiva software and your cytometer. Help opens in a separate window so you can access the documentation while working in the software. You can quickly locate information using the Search function.

  To access the online help, choose Help > Online Help within BD FACSDiva software.

- For online customer training on BD FACSDiva software, choose Help > Online Training.

System Requirements

Hardware

- BD FACS brand digital flow cytometer: BD FACSAria, BD FACSCanto, BD FACSCanto II, or BD LSR II.

- PC workstation configured to BD Biosciences specifications

  - Acquisition workstations can be purchased only from BD Biosciences. The computer must have at least 2 GB of RAM.

  - Analysis-only workstations must be equipped with a Pentium® III Xeon® 1 GHz processor or higher with at least 512 MB of RAM (2 GB for large data files), 10 GB of available hard-drive space, and Windows XP Pro operating system (US English only). For optimal performance and full analysis capability, we recommend that you purchase a workstation that has been validated by BD Biosciences. Contact your sales representative for more information.

  NOTICE Workstations must be XW4100 or later. Make sure your operating system has been upgraded to Service Pack 2 for Windows XP. To order or download service packs, refer to the Microsoft website (microsoft.com/downloads).

  Workstation requirements are subject to change. Contact your BD Biosciences sales representative for up-to-date requirements.

- Universal Serial Bus (USB) security module (provided with the *Getting Started* guide or BD FACSCanto system software)

Software

The following software is required to run BD FACSDiva software. The installer for each application is launched automatically during BD FACSDiva software installation.

- Java 2 Runtime Environment
- Sybase SQL Anywhere Studio
- Sentinel System Driver
- Microsoft Excel (for the User Tracking Log)
- Adobe Acrobat Reader (for viewing PDFs of the documentation)

Compatibility

- Importing—BD FACSDiva software can import data files in FCS 2.0 or 3.0 format including files generated by BD CellQuest™, BD CellQuest™ Pro, or BD FACSDiva software, version 5.0.2 or earlier.

  NOTICE BD FACSDiva software can only open FCS files from BD CellQuest or BD CellQuest Pro, not experiment documents.

- Exporting—BD FACSDiva software can export data files in FCS 2.0 or 3.0 default formats. FCS files can be analyzed by other software applications such as BD CellQuest, BD CellQuest Pro, FlowJo™, or ModFit *LT*™.

Installing BD FACSDiva Software

Use the following instructions to install BD FACSDiva™ software or upgrade to the latest version. The installation CD is packaged with the BD FACSDiva software *Getting Started* guide or BD FACSCanto™ system software.

NOTICE You must have Microsoft® Windows® Administrator access to install BD FACSDiva software. Please read all instructions before you proceed.

NOTICE Only the US English version of the Microsoft Windows XP operating system is supported by BD FACSDiva software version 6.0.

If you are installing the software for the first time, skip to Installing New Software on page 21. Otherwise, continue with the next section.

NOTICE Once installation is complete, see the Cytometer Setup and Tracking Application Guide to learn how to create base configurations.

Before upgrading the software, do the following:

1. In the current version of software, select the cytometer configuration that will be used as the base configuration for Cytometer Setup and Tracking. The number of lasers, detectors, and parameters that are associated with that configuration will be used to populate the new base configuration. For details, see the Cytometer Setup and Tracking Application Guide.

2. It is important that the delays for all available lasers be properly set prior to upgrading. Refer to the user's guide for your particular cytometer. Correct laser delays are used to determine the proper laser order for cytometer configuration in Cytometer Setup and Tracking.

3. Make sure you have a valid database backup stored off the computer hard disk (eg, on a server or CD/DVD). Refer to the *BD FACSDiva Software Reference Manual* for instructions.

4. Workstations must be XW4100 or later. Update your operating system to Windows XP, Service Pack 2 (SP2). Order or download service packs from the Microsoft website (www.microsoft.com/downloads).

5. BD recommends that you defragment the hard disk before you install new software (the C and D drives).

Installing New Software

The installer places the following components on the hard drive. If the correct version of a helper application (not including the main BD FACSDiva software) is already installed, the installer skips to the next installation step.

- BD FACSDiva software 6.0
- BD FACSDiva Data Manager
- Sentinel™ System Driver v5.41.1
- Microsoft .NET 2.0 Framework
- Java™ 2 Runtime Environment (JRE) v1.5.0_11
- Sybase SQL Anywhere® v9.0.2
- Adobe® Acrobat® Reader® v7.0

NOTICE For computers running the BD FACSAria™ or the BD™ LSR II cytometers: area scaling, window extension, and laser delay values are stored in the database. If you plan to install an empty database, record these values before uninstalling the software so you can re-enter them later.

1. Before installation, **turn the flow cytometer power off and then on again. Wait 5 minutes and restart the computer.**

2. Close all open applications and windows.

3. Insert the BD FACSDiva installation CD into the CD-ROM drive.

   NOTICE If a previous version of the BD FACSDiva software application is installed, the uninstall process removes that version and its associated files while preserving the database and list-mode data files.

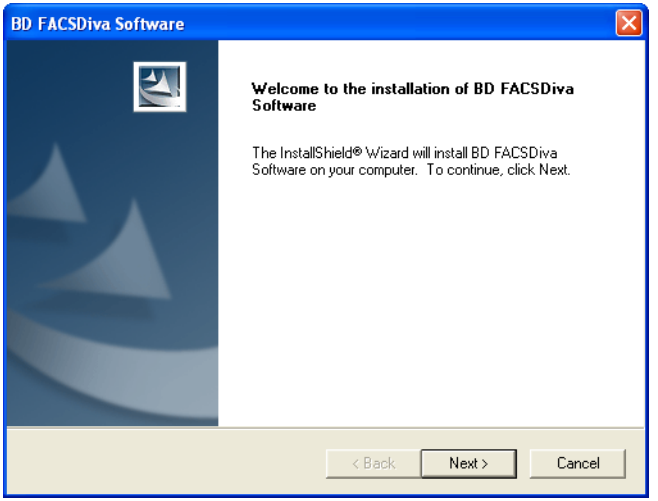
   If the installer does not start automatically after uninstalling the previous version, use Windows Explorer to view the CD contents, then find and double-click the Setup.exe icon or remove and reinsert the CD.

4. Carefully review the ReadMe file. Click  to continue with installation.

   NOTICE The ReadMe file contains important software information that is not included in the accompanying documentation.

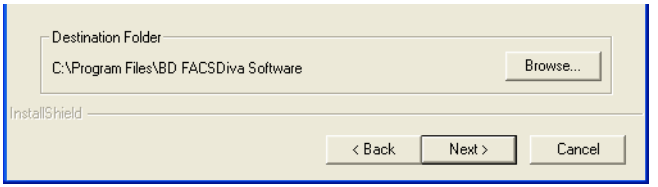
☑ **Tip** BD Biosciences recommends that you print the ReadMe file and place the printed copy with your *Getting Started* guide or cytometer manual for reference. To locate ReadMe information after software installation, double-click the shortcut on the desktop.

5 When the welcome screen appears, click Next.



6 Click Yes to accept the license agreement and continue installation.

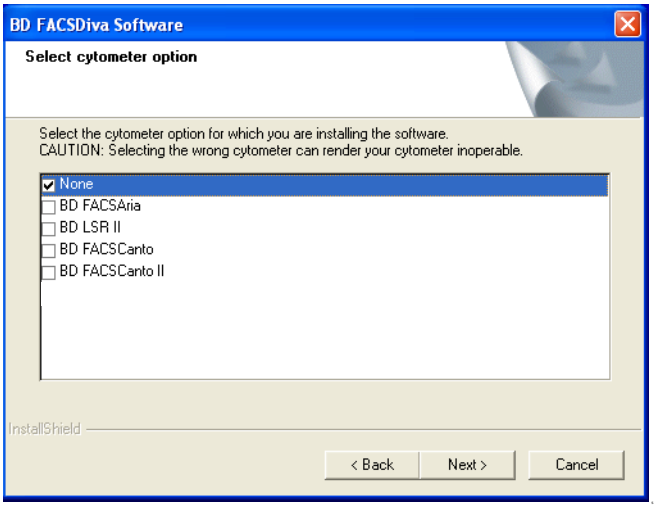
7 Verify the destination folder. Click Next.



By default, the software is installed in the Program Files\BD FACSDiva Software folder on the C drive.

8 In the cytometer selection window, select the checkbox for your flow cytometer and click Next (Figure 1-1).

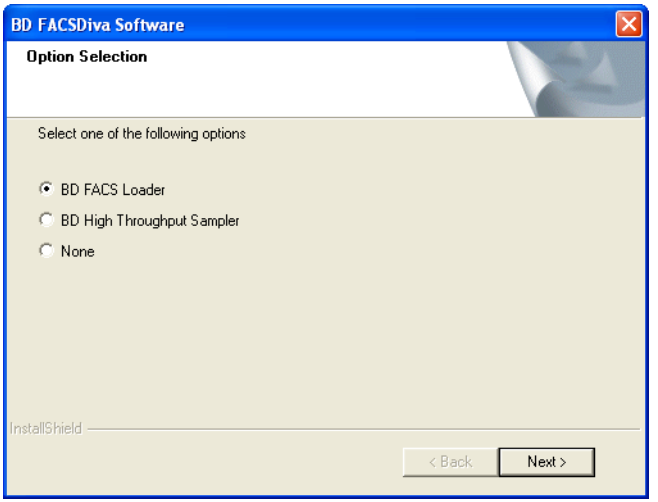
Figure 1-1 Selecting a cytometer option



For offline workstations, select the cytometer used most often in your laboratory.

9 For the BD LSR II or BD FACSCanto™ flow cytometer, select the option that corresponds to your cytometer, and click Next.

Figure 1-2 BD FACSCanto cytometer options (example)

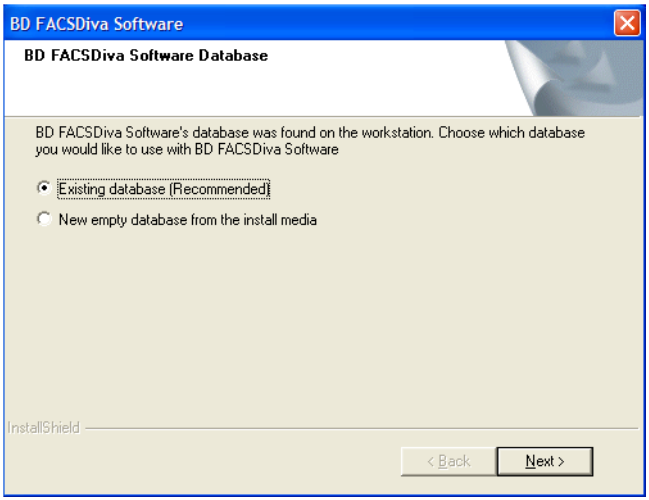


10 When prompted, select a database option, and click Next.

- Select *Existing database (Recommended)* to continue working with data in the current database. The database will be upgraded to work with the new software version.

- Only select *New empty database from the install media* if you want to install an **empty** database. The existing database will be renamed BDFACS.db*x*, where *x* is the next consecutive number.

NOTICE Contact BD Biosciences Customer Support before upgrading with a new empty database on an acquisition workstation.



11 Wait while the installer loads software. (This can take several minutes.)

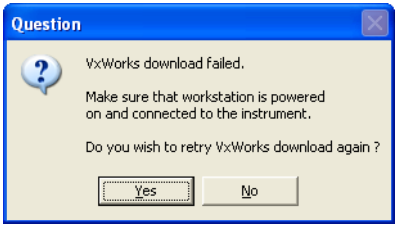
The installer loads BD FACSDiva software and its support files in the appropriate locations. If the workstation is connected to a cytometer, the installer uploads files to the cytometer.

While the installer is checking to see if the workstation is connected to a cytometer, the following message appears on the screen:

Figure 1-3 Message for BD LSR II (example)

Do not click the mouse or press any keys while the DownloadVxWorks message is displayed. Doing so could cause the installer to lock up and prevent installation from continuing.

If the VxWorks download is unsuccessful, the following message appears:



- If you are installing the software on an analysis-only workstation, click No. The VxWorks download is not required.

- If you are installing the software on an acquisition workstation, verify that the cytometer is turned on and connected to the workstation, and then click Yes to try the VxWorks download again.

  If the same message appears again, click No, finish the installation, and contact BD Biosciences Customer Support. Do not run your flow cytometer until VxWorks has been successfully installed.

12 The Reboot Cytometer reminder message appears. Click OK to close the message.

13 Select Yes to restart the computer immediately after installation. Click Finish to complete the installation.

14 Once the computer restarts, install the security module in the USB port of the computer workstation, if needed.

The security module must be in place to run BD FACSDiva software. The security module can be installed in any USB port.

15 Turn the cytometer power off and then on again. Wait 5 minutes before launching the BD FACSDiva software.

To finalize the download of cytometer files, you **must** restart the cytometer after the software is upgraded and the computer has been restarted. The update will be complete when you launch the new software version and establish connection with the cytometer. Do not interrupt the application during startup.

NOTICE If you plan to use the Cytometer Setup and Tracking features to create base configurations, see the Cytometer Setup and Tracking Application Guide.

Files Installed

The installer places shortcuts to BD FACSDiva Software, BD FACSDiva Data Manager, the BD FACSDiva software *Getting Started* guide and reference manual (PDF files), and the ReadMe file on the desktop. These shortcuts are also added to the Start menu (Start > Programs > BD FACSDiva Software).

shortcuts on desktop

The software and all supporting files are placed in the Program Files folder on the C drive (See Figure 1-4 on page 28). You will find a copy of the ReadMe file and supporting documentation, including a PDF file of the reference manual and *Getting Started* guide, in Program Files\BD FACSDiva Software as well as on the software CD.

Figure 1-4 Contents of BD FACSDiva Software folder

To ensure that data can be accessed by the software, do not move, rename, or delete the BDFACS.db file, BDFACS.log file, or BDData folder inside the BDDatabase folder on the D drive. Do not change the name of any file or folder within the BDData folder.

Starting the Software

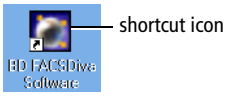
NOTICE If you are using the software for acquisition from the cytometer, follow the startup sequence in your cytometer manual.

Before starting the software for the first time, review the BD FACSDiva ReadMe file. A shortcut is copied to the Windows desktop during installation.

To start the software, do the following:

1. Double-click the shortcut icon on the desktop.

   Alternatively, choose Start > Programs > BD FACSDiva Software > BD FACSDiva Software.

   The BD FACSDiva workspace appears, showing the Log In dialog.

2. Leave the user name as Administrator, and click OK.

   No password is required when you log in to the software. You should assign a password to the administrator account as soon as possible. For instructions, see Adding or Modifying a Password on page 35.

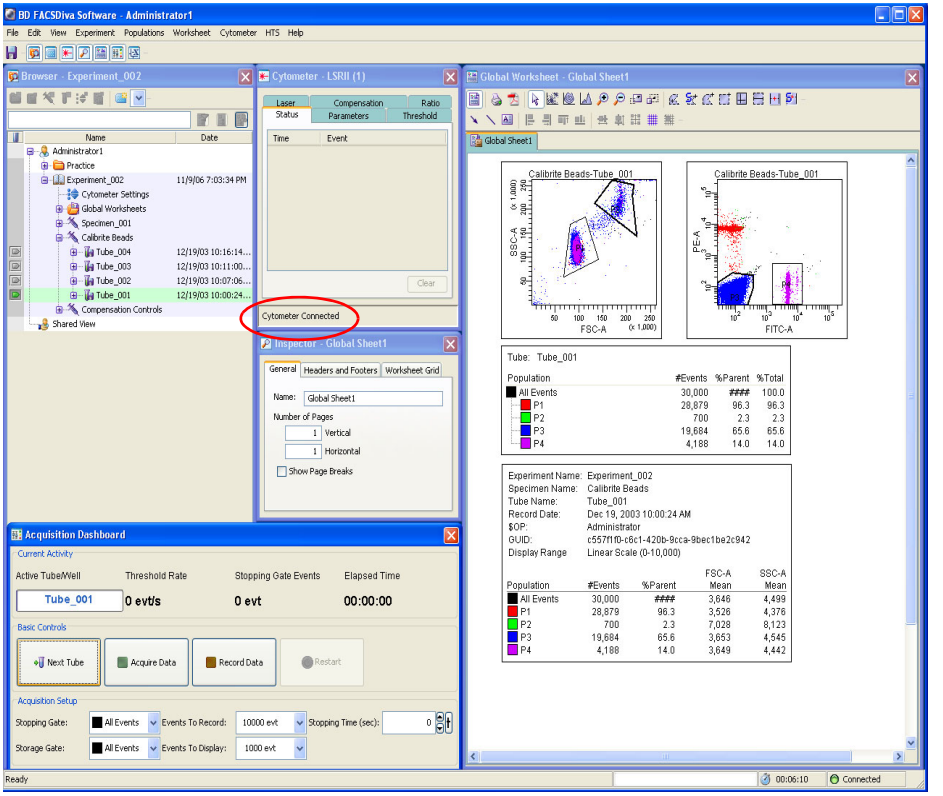
   

   NOTICE If a message is displayed regarding Windows Extensions that have been changed, select to change or not. Refer to the *Cytometer Setup and Tracking Application Guide* for details.

   To create additional user names, see Adding Users on page 31.

After a successful login, the main application components appear in the workspace (Figure 1-5). (Your workspace might look slightly different from that shown in this example.) For a full description of workspace components, see Chapter 2.

Figure 1-5 BD FACSDiva workspace



NOTICE To verify the workstation has successfully connected to the cytometer, check that the Cytometer window displays the message “Cytometer Connected” or “The system is ready” at the bottom of the window. If the message reads “Cytometer Disconnected,” see Electronics Troubleshooting on page 280 for assistance.

Administering Accounts

If you have administrator privileges in BD FACSDiva software, you can add, edit, or disable users, and export or import user profiles as described in the following sections. You do not need administrative access to change your password. See Adding or Modifying a Password on page 35.

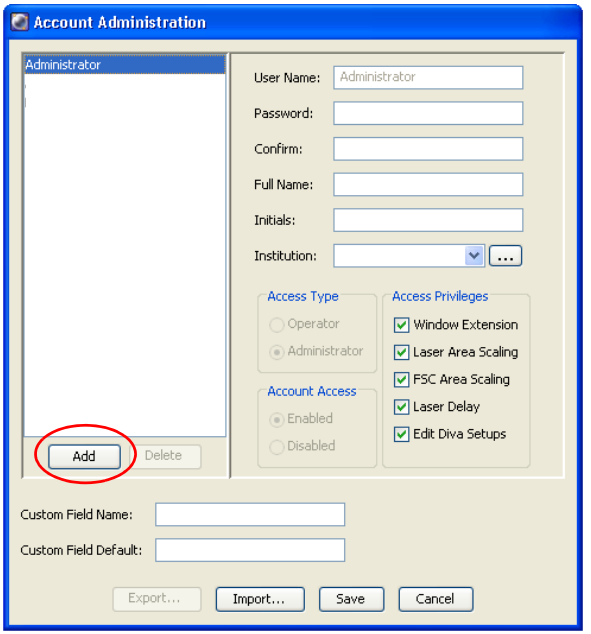
Adding Users

1 Log in to the software as Administrator.

2 Choose File > Administration.

The Account Administration dialog appears. In this dialog you can add or modify the attributes of a user, enable or disable users, or grant administrative access.

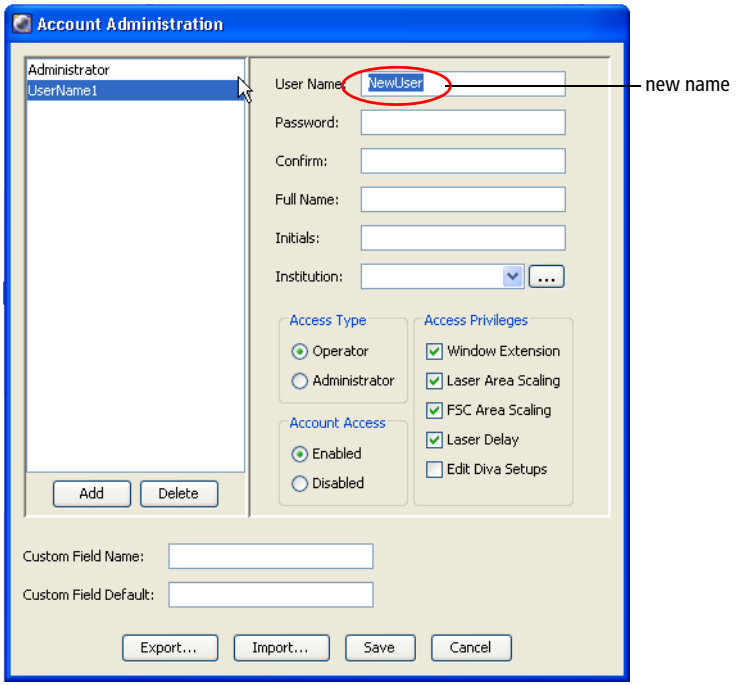
3 Click Add.



4 Select the name in the User Name field and enter a new name.

User names can consist of 4–20 alphanumeric characters. Spaces are not allowed.

☑ **Tip** To create multiple new users quickly, click the Add button once for each new user, then select each new user and name it in the User Name field.



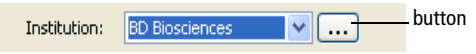
5 Press the Tab key or click in the Password field; enter a password, if needed.

Passwords are not required. If you want to add a password, enter from 1–16 alphanumeric characters.

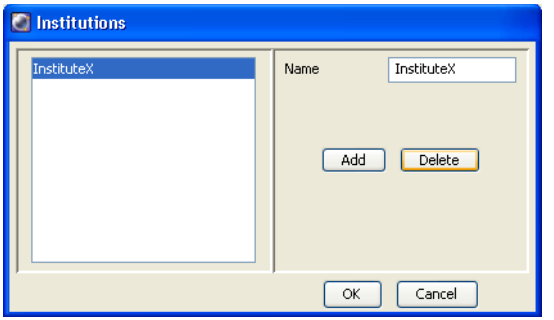
6 Confirm the password, if entered, by typing it again in the Confirm field.

7 (Optional) Enter the user's full name, initials, and institution in the remaining fields.

It is recommended to provide this information so it can be used as keywords and in the User Tracking Log file. To add an institution, click the "…" button next to the Institution menu:



The following dialog appears where you can add or modify choices:



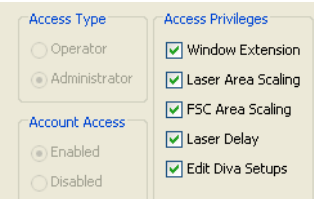
- To add an institution, click Add. "Institute*X*" is added to the list of names. Change the name by selecting "Institute*X*" in the Name field and entering a new name. Press Enter to apply the change, or click OK to apply the change and close the dialog.

- To delete an institution, select the name in the list and click Delete. Click OK to close the dialog.

Once you click OK, all listed institutions can be chosen from the Institution menu in the Account Administration dialog.

NOTICE If an institution is not assigned to a user, it is not saved from one login session to the next.

8 Make selections for Access Type, Access Privileges, and Account Access.



- Change the Access Type to Administrator if you want to assign the user administrative privileges. Administrators can add or modify user accounts, view all users' experiments, and edit cytometer configurations.

NOTICE For BD FACSAria, if users need to change sheath pressures, they must be given access to all privileges.

- Under Access Privileges, select the checkbox next to each setting the user is allowed to edit. For a description of the first four laser-related settings, see Laser Controls on page 122.

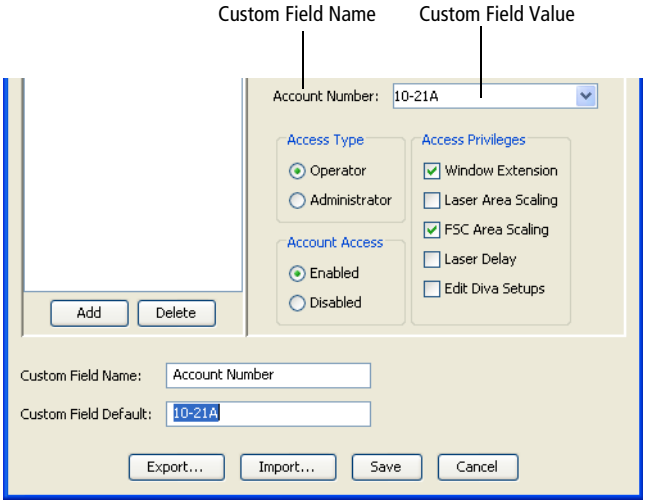
- Also under Access Privileges, select the Edit Diva Setups checkbox to allow a user access to modify the Diva setups saved in the Setup Catalog.

- Change the Account Access to Disabled only when you want to disable a user. See Disabling Users on page 40.

9 (Optional) In the Custom Field Name field, enter a word or phrase to be associated with the user (eg, Account Number or Department Name). See Figure 1-6 on page 35. A new menu is displayed under the Institution field with the Custom Field Name you entered. BD recommends providing this information so it can be used in keywords and in the User Tracking Log file.

NOTICE Keywords are limited to 20 characters.

Figure 1-6 Entering a value for the Custom Field Name



10 In the Custom Field Default field, enter the value associated with the Custom Field Name you entered (eg, 10-21A or Finance Department). The value you entered is displayed in the new custom field you created in step 9.

NOTICE If the Custom Field Name is changed, the User Tracking Log header will not be updated until the new Tracking Log is created for the next month.

11 Ensure all user information is correct and click Save.

Adding or Modifying a Password

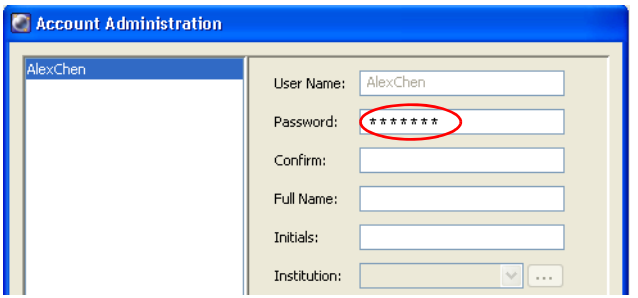
BD Biosciences recommends that you assign a password to the administrator account as soon as possible. If you are not an administrator but have an assigned password, you can change your password as follows.

1 Log in to the software.

2 Choose File > Administration.

The Account Administration dialog appears showing only your user name, unless you have administrative access.

3 Enter a new password of up to 16 alphanumeric characters.



4 Confirm the password by re-entering it in the Confirm field; click Save.

☑ **Tip** Keep a copy of your password in a secure location in case you forget it.

Tracking User Logins

BD FACSDiva software automatically tracks user login information in a monthly tracking log. Access the user login information by choosing File > User Tracking Log or looking in C:\Program Files\BD FACSDivaSoftware\log.

Logs are named *yyyy Month.csv* (for example, 2006 February.csv). Logs can be opened in a spreadsheet application such as Microsoft Excel.

The following information is tracked in the monthly log:

- user name
- full name
- application name (BD FACSDiva, BD FACSCanto clinical software)

- role (administrator, operator)
- department (BD FACSCanto clinical software only)
- institution
- login time and date
- logout time and date
- build version
- cytometer type
- serial number
- custom field

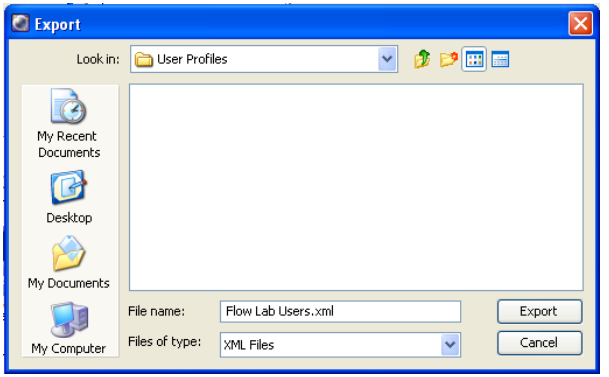
Exporting User Profiles

User profiles can be exported for use on another computer. To export and import user profiles, you must have administrative access.

1. Log in to the software as Administrator.
2. Choose File > Administration.
3. From the list of user names, select those you want to export, and click Export.
    - To select multiple contiguous names, click the first name in the series, then hold down the Shift key as you select the last name.
    - To select multiple noncontiguous names, hold down the Ctrl key as you click each name.

4 Enter a name for your exported file and click Export.

By default, exported user profiles are stored in D:\BDExport\User Profiles.



Importing User Profiles

You must have administrative access to import user profiles.

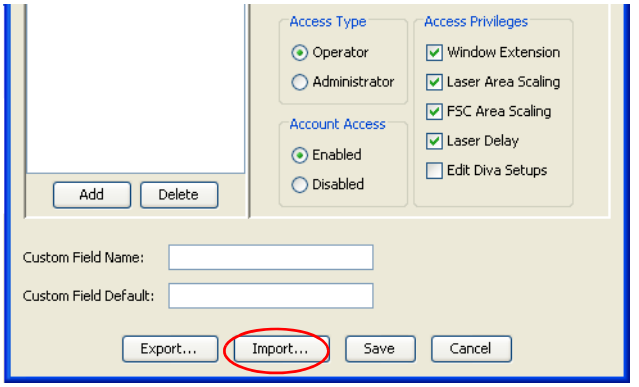
1 Transfer the electronic file containing the user profiles to the secondary computer.

Files can be transferred over a network or via a portable storage device such as a USB flash drive.

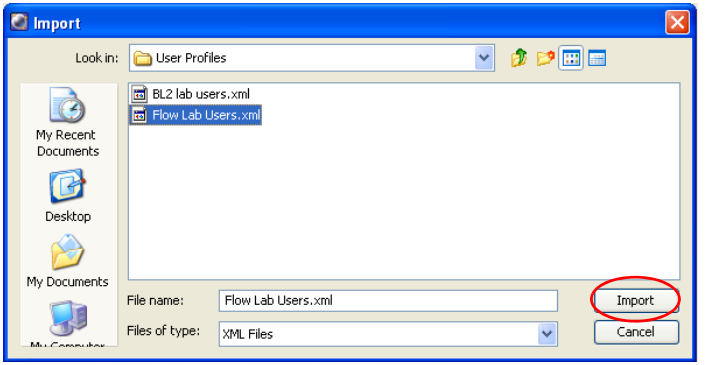
2 Log in to the software as Administrator.

3 Choose File > Administration.

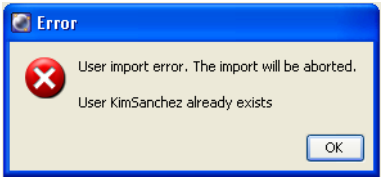
4 Click Import.



5 Select the file containing the names you want to import, and click Import.



NOTICE User names must be unique. If the file you are importing contains a duplicate of any existing user names, the following message appears displaying the names that are duplicates:



Click OK to close the message, and either delete the duplicate user names or choose a different file to import.

6 Verify that all user names and passwords were imported.

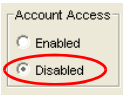
Disabling Users

When users have saved experiments in the Browser, they cannot be deleted, but they can be disabled. Disabled users can no longer log in to the software. However, their experiments are shown in the Browser (to Administrators) and their shared experiments are available to all users.

1 Log in to the software as Administrator.

2 Choose File > Administration.

3 In the Account Administration dialog, select the user, select Disabled under Account Access, and click Save.



Deleting Users

You must have administrative access to delete a user.

1 Export and then delete the user's experiments from the Browser.

See Exporting Experiments on page 268. Enable the option to automatically delete experiments after export.

2 Choose File > Administration.

3 Select the user name, click Delete, and then click Save.

Quitting the Software

Do one of the following to quit the software:

- Choose File > Quit.
- Click  in the upper-right corner of the workspace window.

All Browser and worksheet elements are automatically saved when you quit the software.

2

BD FACSDiva Workspace

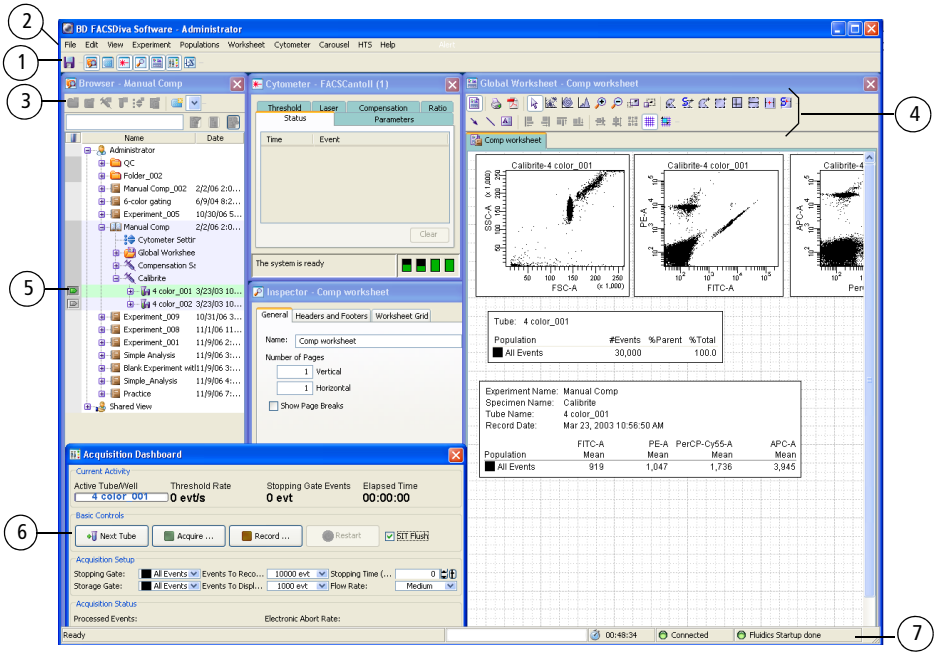
This chapter contains a description of the following BD FACSDiva workspace elements. Descriptions for other software components can be found in Chapter 3 and Chapter 4.

Workspace Components

When you start BD FACSDiva software, the workspace appears showing the main application windows (Figure 2-1). Hide or show windows by clicking buttons on the Workspace toolbar (1).

Most software functions are controlled using the menu bar at the top of the workspace (2) and toolbars within the Browser (3) and Worksheet (4) windows. Acquisition and data loading is controlled using the current tube pointer (5) or buttons within the Acquisition Dashboard (6). The Status bar (7) at the bottom of the workspace provides cytometer connection status, fluidics information, etc.

Figure 2-1 BD FACSDiva workspace



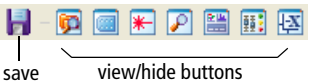
Status Bar

The Status Bar at the bottom of the workspace provides the following information:

- Application status, ready or not
- Elapsed login time for the current user
- Cytometer connected or disconnected
- Fluidics startup/shutdown state (for BD FACSAria and BD FACSCanto platforms)

The display of the Status Bar is selected by default. To close the Status Bar, deselect Status Bar in the View menu at the top of the workspace.

Workspace Toolbar



The following buttons are displayed on the Workspace toolbar. Note that some buttons are shown only for certain cytometers; refer to your cytometer manual for details.


Save—Saves the current experiment to the database. Experiments are also saved when you close an experiment or quit the software.


Browser—Hides or shows the Browser; see Browser on page 48.


Plate—Hides or shows the Plate window. This button appears only if your cytometer is compatible with the BD™ High Throughput Sampler (HTS) option.


Cytometer—Hides or shows the Cytometer window; see Cytometer Controls on page 106.

Inspector—Hides or shows the Inspector; see Inspector on page 48.

Worksheet—Hides or shows the Worksheet window; see Worksheets on page 176.

Acquisition Controls—Hides or shows the Acquisition Dashboard; see Acquisition Dashboard on page 129.

Biexponential Editor—Hides or shows the Biexponential Editor; see Working with the Biexponential Editor on page 212.

Sorting—Hides or shows the Sorting window(s). This button appears only if your cytometer is equipped with sorting features.

View Options

The BD FACSDiva workspace can be resized to suit your needs, and you can reposition or resize windows within the workspace. See Figure 2-2 on page 47. Changes are user-specific, and are saved from one login session to the next.

If you have a second monitor, do the following to view the BD FACSDiva workspace on both monitors.

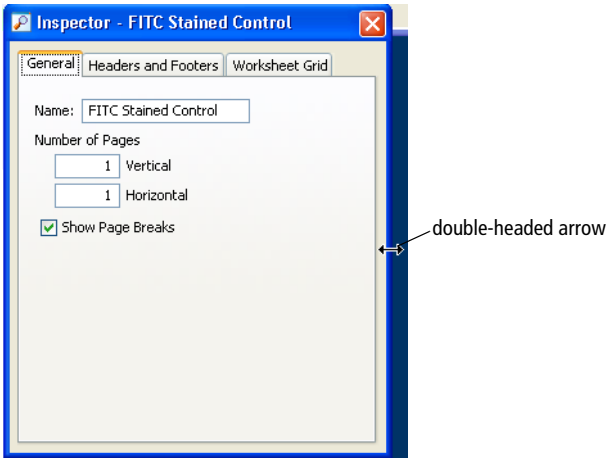
1. Click to reduce the workspace.
2. Drag the window border to fill the second monitor.

Tip To return to one screen, click or choose View > Reset Positions.

Whether viewed on one monitor or two, workspace windows can be resized and repositioned for the most efficient operator workflow.

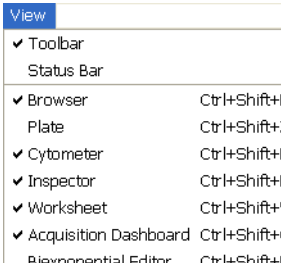
- To move a window, drag the title bar to a new position on the screen.
- To resize a window, position the cursor on the border. When the cursor changes to a double-headed arrow, drag the border.

Figure 2-2 Resizing a workspace window



- To view or hide workspace windows, choose an option from the View menu, or click the corresponding button on the Workspace toolbar.

  You can also hide a window by clicking ☒ to close it.



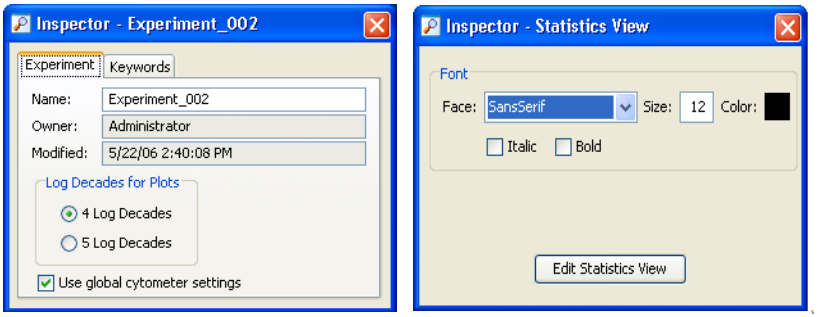
☑ **Tip** To restore windows to their default position and size, choose View > Reset Positions.

Inspector

The *Inspector* provides an easy-to-use interface for viewing or modifying the attributes of a single object or set of objects on the worksheet or in the Browser. For example, the Inspector can be used to change plot attributes like the background color, title, axes labels, and scale, or to enter the name of an experiment, specimen, or tube.

To display the Inspector, click the Inspector button ( ) on the Workspace toolbar. The contents of the Inspector vary depending on the object selected. For example, Figure 2-3 compares the contents of an Experiment Inspector (displayed when an experiment is selected in the Browser) with those of a Statistics Inspector (displayed when a statistics view is selected on a worksheet).

Figure 2-3 Experiment Inspector (left) vs Statistics Inspector (right)



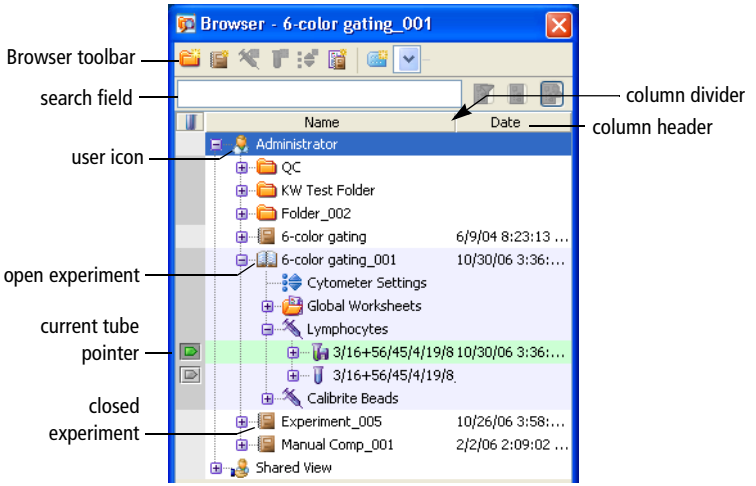
Different Inspectors are described in the following sections.

Browser

BD FACSDiva software stores and accesses all experiment data from a single database. Stored elements are shown in the Browser. See Figure 2-4 on page 49.

The Browser is where you create and access database elements. As you create experiments and record data, the software writes experiment components to the database. Data is listed by login name in a hierarchical view. Hide or display the Browser by clicking the Browser button ( ) on the Workspace toolbar.

Figure 2-4 Browser with representative experiments



Users with administrative access can view all experiments in the database. Those without administrative access can view only their own experiments and any experiments that have been designated as shared. For more information, see Making Experiments Shared or Private on page 64.

Using the Browser

The Browser has the following functions.

- lists experiments saved in the BDFACS database

  Adding or deleting elements from the Browser will add or delete elements from the database. Browser elements can be listed by name or date in ascending or descending order. Folders can be used to group experiments. See Organizing the Browser on page 55.

  Use the search field above the Browser to find experiments or show fewer experiments in the Browser. See Using the Search Field on page 51.

- provides an interface for setting up experiments

  You must select elements in the Browser to activate certain buttons. For example, you must select a specimen or tube to activate the New Tube button. See Adding New Elements to the Browser on page 51.

- organizes experiment elements in a hierarchical view

  - View elements listed under an experiment, specimen, or tube by clicking once on the plus sign (+) next to the corresponding icon.

  - Sort experiments in the Browser by clicking inside a column header. Click in the same header again to reverse the sort order.

  - Resize columns in the Browser by dragging the column dividers.

☑ **Tip** Use the arrow keys on your keyboard to move between elements in the Browser. Use the right arrow key to expand an element, or the left arrow key to collapse it.

- provides shortcuts for renaming database elements, accessing element-specific options, and acquiring and recording data

  - Rename any Browser element in an open experiment by clicking the element and entering a new name. (Alternatively, select the item and choose Edit > Rename, or right-click the item and choose Rename.)

  - Right-click any item in the Browser to display a shortcut menu with options specific to that item. A summary of menus is provided in Menus on page 295.

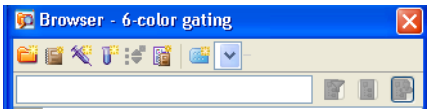
  - Use the current tube pointer to start and stop data acquisition and recording and to load data. See Using the Current Tube Pointer on page 54.

Using the Search Field

Use the search field to find experiments or show fewer experiments in the Browser. For more search options, see Finding Saved Data on page 65.

NOTICE You cannot use the Find function to locate a folder. If a folder contains an experiment that meets the search criteria, it will have a plus sign (+) next to it, indicating experiments are inside the folder.

- To locate experiments by name, enter the name, and click the Find button ( ).



  The Browser lists only experiments with that name, along with the currently open experiment. Click the plus sign (+) next to a folder or user icon to view any hidden experiments.

- To hide other users' experiments, click the View Own button ( ).

  Experiments under the Shared View icon are hidden.

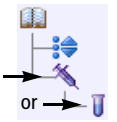
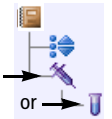
☑ **Tip** Close all open experiments to enable the button.

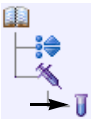
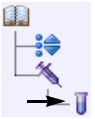
- To list all experiments again, click the Display All button ( ).

Adding New Elements to the Browser

Use buttons on the Browser toolbar to add new items to the Browser. You can also add items using menu commands or keyboard shortcuts. You must select elements in the Browser to activate certain buttons, as shown in the following table.

☑ **Tip** You can customize Browser toolbar buttons to add a predefined template to the Browser. See Templates Preferences on page 101 for instructions.

To add...	First select...	Then choose one of these options...
Folders	• (your user icon) • (to create a folder inside a folder)	• Click the New Folder button ( ) or Press Ctrl-N. • Choose Experiment > New Folder. • Right-click and choose New Folder from the menu.
Experiments	• • (to create an experiment inside a folder)	• Click the New Experiment button ( ). • Choose Experiment > New Experiment or press Ctrl-E. • Right-click and choose New Experiment from the menu.
Specimens	•	• Click the New Specimen button ( ). • Choose Experiment > New Specimen or press Ctrl-M. • Right-click and choose New Specimen from the menu.
Tubes	•  • 	• Click the New Tube button ( ). • Choose Experiment > New Tube or press Ctrl-T. • Right-click and choose New Tube from the menu (available only when a specimen is selected).
Specimen-specific cytometer settings	• 	• Click the New Cytometer Settings button ( ). • Choose Experiment > New Cytometer Settings. • Right-click and choose New Cytometer Settings or Import Cytometer Settings from the menu.

To add...	First select...	Then choose one of these options...
Tube-specific cytometer settings	• 	• Click the New Cytometer Settings button ( ). • Choose Experiment > New Cytometer Settings. • Right-click and choose New Cytometer Settings or Import Cytometer Settings from the menu.
Sort layouts (shown only on sorting cytometers)	•  • 	• Click the New Sort Layout button ( ). • Choose Sort > New Sort Layout. • For a normal worksheet, select the tube, right-click and choose New Sort Layout from the menu.
Global worksheets	•	• Click the New Global Worksheet button ( ). • Choose Experiment > New Global Worksheet. • Right-click and choose New Global Worksheet from the menu.
Plates (shown only on cytometers with a plate loader)	•	• Click the arrow control and choose a plate type from the menu, or click the New Plate button ( ). • Choose Experiment > New Plate.

NOTICE For information on analysis templates, see Creating a Tube with a Predefined Analysis Template on page 83; for experiment templates, see Exporting Experiments as Templates on page 60; for panel templates, see Exporting a Specimen as a Panel Template on page 75.

Using the Current Tube Pointer

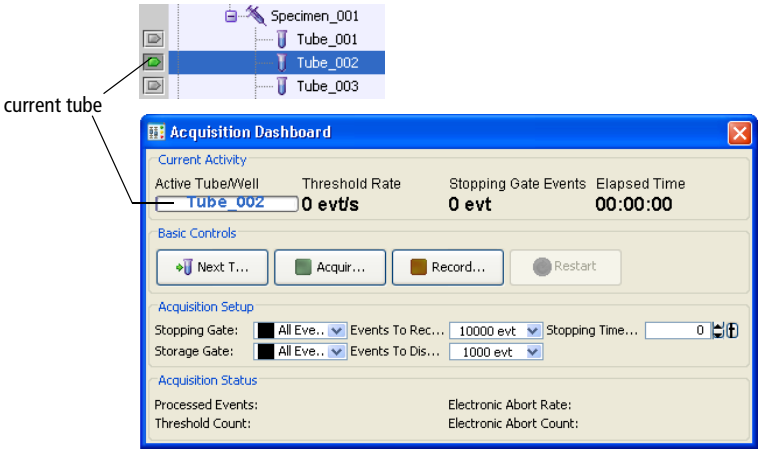
When an experiment is open, a gray pointer or plot icon appears next to tubes in the Browser (Figure 2-5). Click the icon next to a tube to set the *current tube pointer*, which indicates the tube currently selected for data acquisition, recording, or data display on a global worksheet. When the software is connected to the cytometer, the pointer can also be used to control acquisition and recording.

During Acquisition

When the software is connected to the cytometer, a gray pointer icon is displayed next to tubes in the open experiment. Click the gray pointer icon to select the next tube for acquisition or data display—the pointer turns green to indicate the currently selected tube and acquisition starts if specified in User Preferences. The name of the current tube is displayed in the Acquisition Dashboard (Figure 2-5).

For other pointer states, see Current Tube Pointer on page 135.

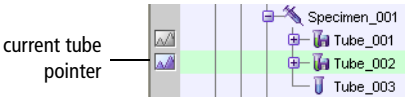
Figure 2-5 Current tube pointer for acquisition workstation



Offline

When the software is disconnected from the cytometer, or a recorded tube contains incompatible cytometer settings, a plot icon is displayed next to tubes with recorded data in the open experiment. Click the gray plot icon to select that tube for analysis—the plot icon is shaded and data for the selected tube is shown in the global worksheet. To display data for a different tube, click to set the current tube pointer.

Figure 2-6 Current tube pointer for offline workstation



Organizing the Browser

Experiments are set up hierarchically to help organize data. Use tubes and specimens to organize your work, and folders to group similar experiments in the Browser. It is important to name Browser elements with meaningful names to help you find the data later.

BD Biosciences recommends that you determine an organization strategy before you generate data. You can name experiments according to the nature of the analysis to be performed, such as *5-color analysis* or *Immunophenotyping*. Specimens can be named according to the type of cells to be analyzed, such as *LWB* (lysed whole blood) or *Hybridoma Line*. Tubes can be named according to the reagents used to stain the sample, such as *CD4 FITC* or *Multitest TBNK*.

The following examples show different organizing strategies in the Browser. Figure 2-7 shows experiments grouped by studies or date.

Figure 2-7 Example folder organization

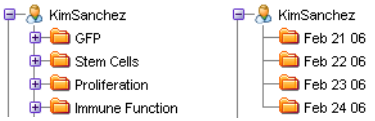


Figure 2-8 shows two strategies for organizing QC experiments. In one, experiments are organized by month at the experiment level, by date at the specimen level, and by samples run at the tube level. The second example organizes QC work by month at the experiment level, by sample type at the specimen level, and by date at the tube level.

Figure 2-8 Example organization of QC work

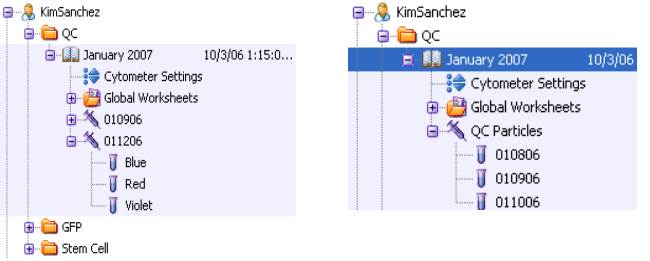
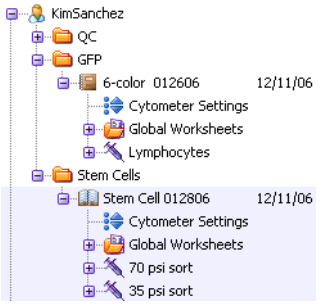


Figure 2-9 shows an example of how you can organize your daily work by study type. By including both a descriptor (eg, 6-color) and date in the experiment name, you can easily find your experiment once it has been exported.

Figure 2-9 Example organization of daily work



☑ **Tip** List experiments by date by clicking the Date column header in the Browser, or list experiments alphabetically by clicking in the Name column header.

NOTICE To move experiments between folders, use the Cut and Paste With Data commands. BD FACSDiva software does not support dragging experiments between folders.

☑ **Tip** Folders can be placed inside folders for additional levels of organization.

Experiments

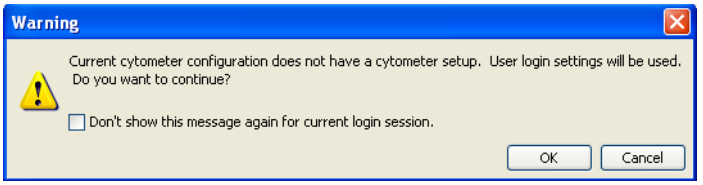
An *experiment* is a group of elements used to record and analyze data from the flow cytometer. Experiments can include global worksheets, specimens (material to be analyzed), tubes (acquisition data and reagents used to analyze the specimen), analysis objects (plots, gates, and statistics views), and Sort Layouts or plates (if applicable). Cytometer settings can be applied at the experiment, specimen, or tube level.

You build experiments as you record and analyze data. Each new experiment adds another group of objects to the Browser. Experiments can be private or shared, and can be exported with data for archival purposes or exported without data for use as a template.

Starting a New Experiment

- To create a new experiment, click the New Experiment button .

NOTICE If your cytometer configuration does not have a valid performance check, a warning from Cytometer Setup and Tracking is displayed. Either click OK in the warning to continue, or run a performance check.

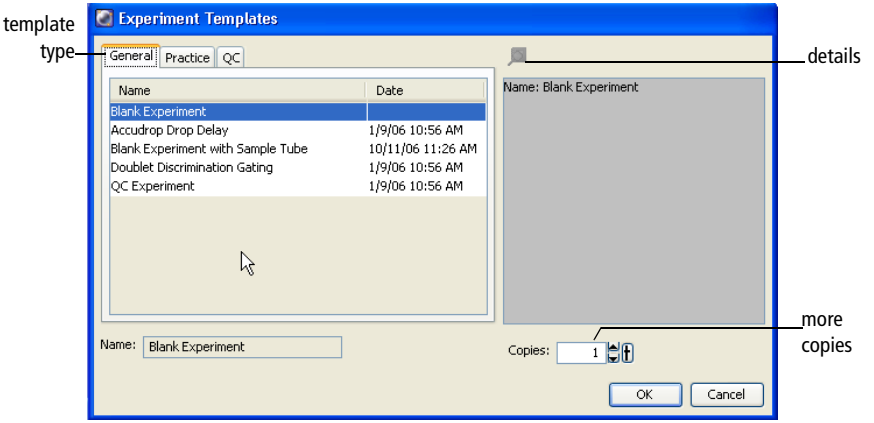


The currently open experiment closes and a new, open experiment is added to the Browser. (If you are recording data when the button is clicked, the current experiment does not close. The new experiment is added as a closed experiment.)

By default, the New Experiment button adds an experiment with default cytometer settings and a blank global worksheet, but the button can be customized to add a predefined experiment template. For more information, see Templates Preferences on page 101.

- To create an experiment based on a saved template, choose Experiment > New Experiment or press Ctrl-E. The Experiment Templates dialog appears where you can select the template type and number of experiments to create.

Figure 2-10 Selecting a template



To view the experiment layout associated with the experiment template, click the details button. Experiment Layout appears showing the specimens and tubes in the experiment, any defined labels, keywords, and acquisition criteria. See Using Experiment Layout on page 67.

Note that you can create up to 50 copies of an experiment template at a time. To change the number of copies, click the up arrow next to the Copies field.

For information about creating experiment templates, see Exporting Experiments as Templates on page 60.

- To import an experiment stored on the hard drive or an external storage device, choose File > Import > Experiments. Locate the experiment to import in the dialog that appears.

  For more information, see Importing Experiments on page 270.

Opening Experiments

You can edit elements and record data only within an open experiment. Only one experiment can be open at a time. An open experiment is indicated by an open-book icon ( ). You cannot close an open experiment during acquisition.

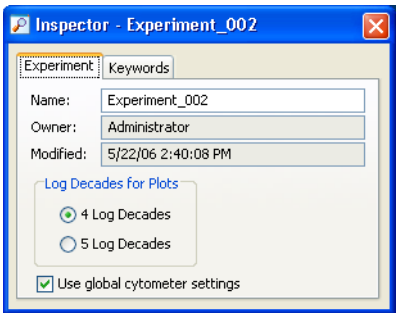
Do one of the following to open a closed experiment:

- Double-click a closed experiment icon ( ) in the Browser.
- Select an experiment in the Browser and choose Experiment > Open Experiment or press Ctrl-O.
- Right-click an experiment icon in the Browser and choose Open Experiment.

There might be a short delay while the software retrieves the experiment from the database.

Using the Experiment Inspector

The Inspector displays experiment options when you select an experiment in the Browser.



In the Inspector, you can:

- Name the experiment.
- Specify the number of logs to display for all plots in the experiment (see Changing Log Display on page 198).

- Select whether to update global cytometer settings automatically (see Using Global Cytometer Settings on page 147).

- On the Keywords tab, create or view experiment-level keywords (see Keywords on page 88).

Note that experiment names cannot contain commas or periods. Spaces at the beginning or end of the name are automatically removed. The experiment modification date is the date the experiment was created or the date data was last collected; the Owner name is the name of the logged-in user who created the experiment. These fields cannot be changed.

Saving Experiments

All experiments are stored in the BDFACS database. (See Working with BD FACSDiva Data on page 254.) Any changes to an open experiment, related Browser elements, and worksheet are saved when you close an experiment, quit the software, or click the Save button ( ) on the Workspace toolbar. List-mode data is saved after a tube is successfully recorded. (A disk is appended to the tube icon when data has been saved.) The experiment modification date is automatically updated each time data in the experiment changes.

☑ **Tip** Locate saved data more easily by naming experiments and experiment elements with meaningful names.

Exporting Experiments as Templates

Any experiment can be exported as a template. Experiment templates include specimens, tubes, keywords, Sort Layouts, cytometer settings, labels, worksheet elements, and worksheets (including all settings such as page breaks), but do not include recorded data. You can set up experiment templates for frequently used experiments. Templates are stored outside the Browser to simplify the Browser display.

To export an experiment as a template, do the following. Note that experiments can be exported as templates whether they are open or closed.

1 Right-click an experiment and choose Export > Experiment Template.

The Export Wizard dialog appears, with steps that show you how to create and export a template.

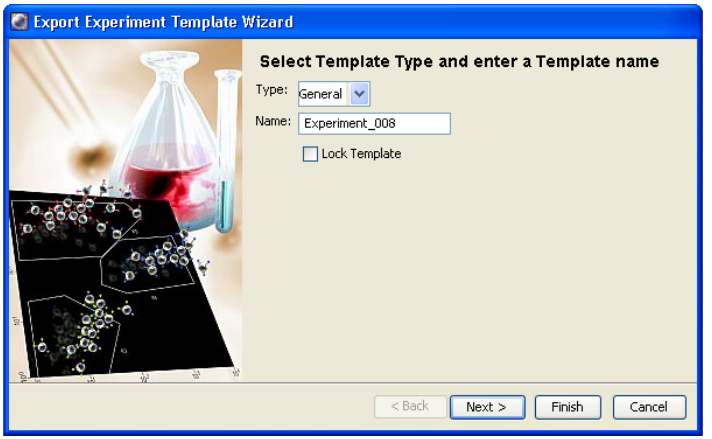
2 Enter the template type and verify the name. Click Next.

Templates can be grouped by category so they are easier to find later. To add a category to the Type menu, enter a name in the Type field. Your new type will be available from the menu the next time you create a template. Note that types cannot include any of the following characters: \ / : * ? " < > | , .

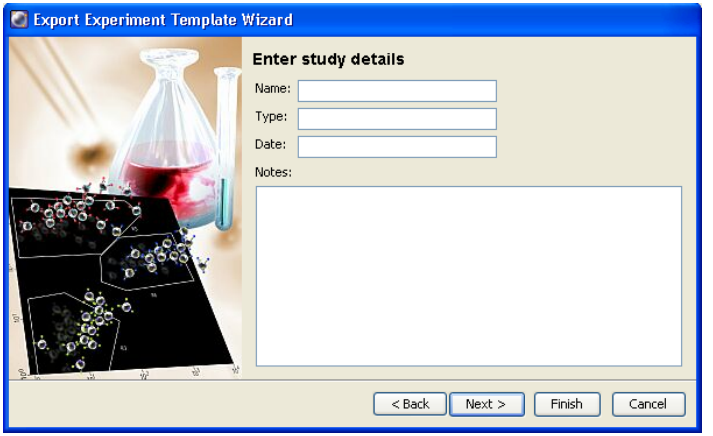
The template name is based on the name of the experiment in the Browser. To change the name, enter a new name in the Name field. Note that experiment names cannot include periods or commas.

Select the Lock Template checkbox to ensure that the template cannot be overwritten by a template with the same name. Locked templates and default templates provided with the software cannot be overwritten.

You must enter a template type and name to proceed.

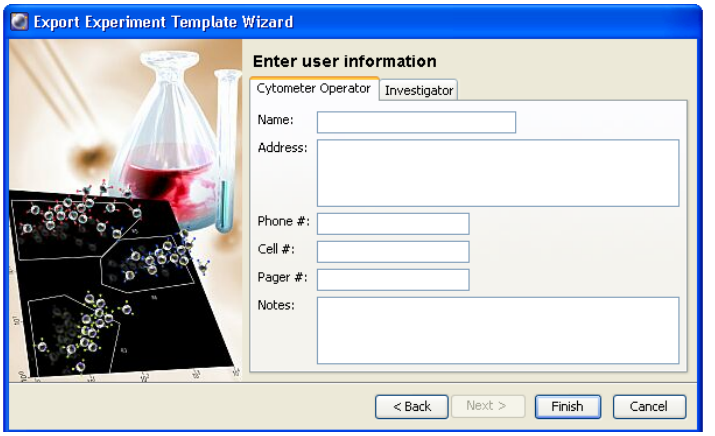


3 (Optional) Enter Study Details when prompted; click Next.



Study details are not required, but they can be used to distinguish between experiment templates with similar names when you have a lot of templates.

4 (Optional) Enter operator and investigator information.



5 Click Finish.

Experiment templates are saved in a folder in the D:\BDExport\Templates\Experiment directory. A new folder is created for each template type. When you create a new experiment based on a template, each type is represented by a tab in the Experiment Templates dialog. See Figure 2-10 on page 58.

Editing Templates

The following are ways to edit a saved experiment template:

- To add or delete elements from a template, create an experiment from the template, make the required changes, and then export the experiment as a template. Save it with the same template type and name. When prompted, overwrite the previous template.

  NOTICE A locked template cannot be overwritten.

- To rename a template, use Windows Explorer to navigate to the BDExport\Templates folder. Open the folder corresponding to the template type and rename the *template*.xml file in the folder. See Figure 2-11 on page 64.

  To rename a template category, rename the folder the template is stored in.

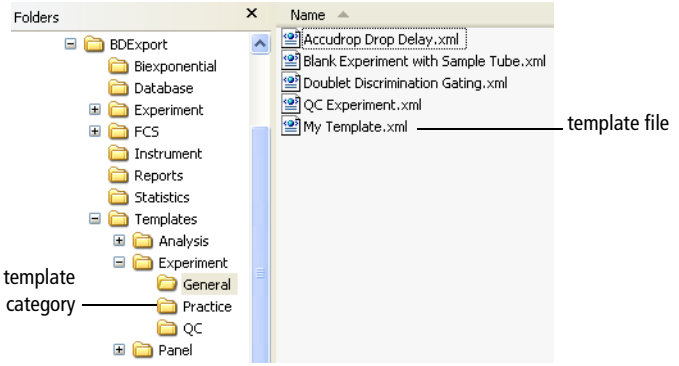
- To change a template's category, move the *template*.xml file to a different category folder at the same hierarchical level.

- To remove templates from the template directory, use Windows Explorer to navigate to the BDExport\Templates folder.

  - To delete a template category and all associated templates, delete the category folder in the Templates*template type* folder.

  - To delete a single template, locate and delete the template file.

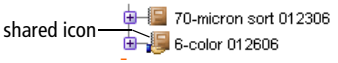
Figure 2-11 Editing a template



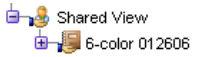
Making Experiments Shared or Private

When you log in to BD FACSDiva software, all your saved experiments are listed under your user icon in the Browser. Other users cannot access your experiments unless they are administrators or you have designated an experiment as shared.

To make an experiment accessible to other users, right-click the experiment icon and choose Share Experiment. The experiment icon changes to show that the experiment is shared.



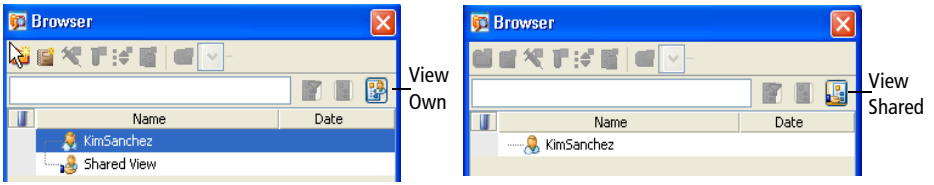
When other users log in to the software, they will be able to add or delete elements within shared experiments under the Shared View icon in the Browser.



To remove the shared status, right-click a shared experiment and choose Make Private.

You can view only your experiments (ie, hide all shared experiments) by clicking the View Own button in the Browser. (All experiments must be closed to enable the button.) Click the View Shared button to see all experiments again (Figure 2-12 on page 65).

Figure 2-12 Viewing and hiding shared experiments



shared experiments shown

shared experiments hidden

Exporting and Importing Experiments

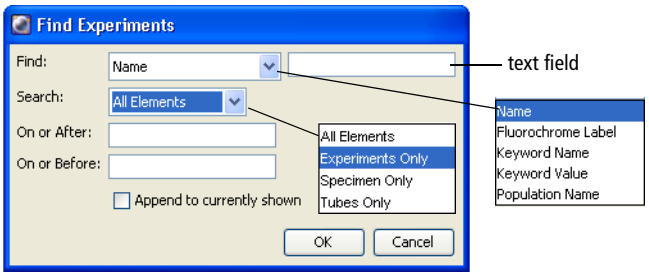
Experiments can be exported to the hard drive or an external storage device. See Exporting and Importing Experiments on page 268.

Experiment data can be exported in FCS 2.0 or 3.0 file format. You can also import FCS files from another BD application. See Exporting FCS Files on page 259 and Importing FCS Files from BD Biosciences Applications on page 263.

Finding Saved Data

Use the search field at the top of the Browser to search in the Browser for experiments containing specific Browser elements, reagents, keyword names or values, or population names. See Using the Search Field on page 51.

Alternatively, choose Edit > Find or press Ctrl-F and use the drop-down menus to restrict your search to predefined data categories (see the following figure).



- Use the Find menu to choose the type of information you are searching for. Select a category from the menu, then enter specific information in the text field next to the menu. For example, choose Fluorochrome Label and enter CD4 in the text field.

- Use the Search menu to search only within a certain type of data element (experiments, specimens, or tubes).

- Search within a specified time period by entering dates in the On or After and On or Before fields. Enter the month first, followed by the day and the year (eg, 5/17/07 or May 17, 2007).

- Select the *Append to currently shown* checkbox to list experiments containing the required information along with current information as shown in the Browser. Deselect the checkbox to display only experiments containing the required information.

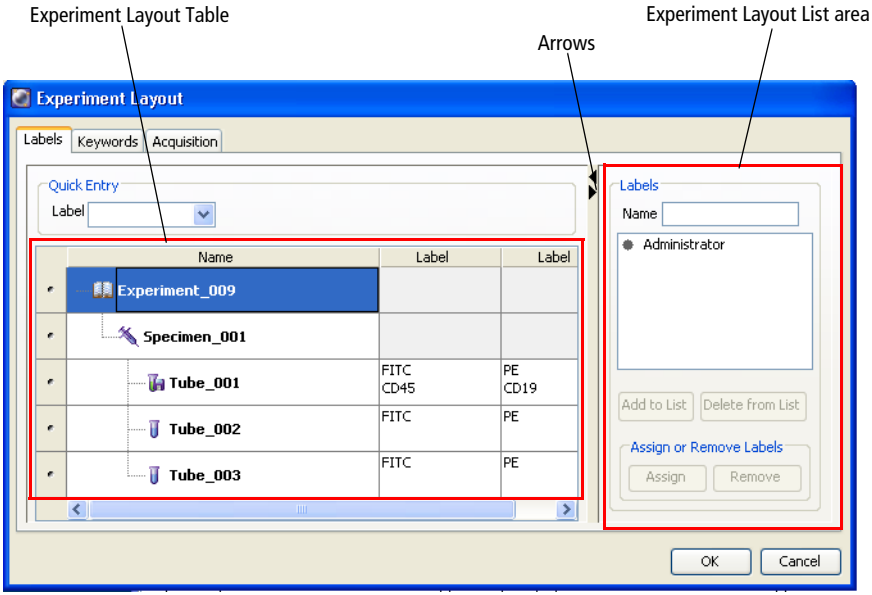
NOTICE You cannot use the Find function to locate a folder; however, if a folder contains an experiment that meets the search criteria, that folder is displayed with a plus sign (+) next to it, indicating experiments are inside the folder.

If there are no experiments containing the requested information, the Browser will list only the currently open experiment along with any existing folders. To display all Browser elements again, click Display All.

Using Experiment Layout

Use Experiment Layout to create labels, enter values for keywords, or enter acquisition criteria for each tube in an experiment. Open an experiment and choose Experiment > Experiment Layout. Experiment Layout is displayed listing all specimens and tubes in the experiment. Click the arrows to display or hide the list function for creating lists of labels, keywords, and acquisition criteria.

Figure 2-13 Experiment Layout



Using Experiment Layout Lists

Use Experiment Layout lists to manage your labels, keywords, and acquisition criteria. Click the arrow at the top right of the Experiment Layout window to show or hide the Experiment Layout List area. (See Figure 2-13). What is displayed in the list area changes depending on which tab you choose (Labels, Keywords, or Acquisition).

All labels, keywords, and events to record that you create in a list are entered under your login name. You can only delete items that you created. However, you can view and assign labels, keywords, and events to record that other users on your workstation have entered in their lists.

The Labels and Events to Record lists also display BD defined labels and events to record; BD defined labels and events to record cannot be deleted from the lists.

For specifics on how to use the lists, refer to the following sections: Labels on page 68, Keywords on page 69, and Acquisition Criteria on page 71.

Editing Element Names

Select any element in the Name column under any of the tabs in Experiment Layout, such as experiment, specimen, or tube. Type over the name to change it, then click Enter. The new name is saved immediately, even if you click Cancel.

Labels

Use the Labels tab of Experiment Layout (Figure 2-13 on page 67) to enter parameter labels for each fluorochrome in your experiment. Parameter labels will be displayed on plot axes and in statistics views.

Using the Experiment Layout Table

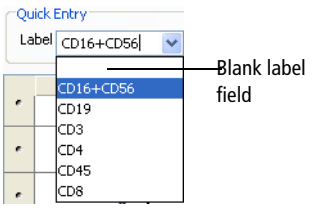
- To add or change labels, select the field(s) listing the fluorochromes to be labeled, and type to enter a label.

  If a label has been previously defined, choose it from the drop-down Label menu. (The menu is blank until you have defined at least one label.)

☑ **Tip** Click the column or row header to select and label multiple cells at a time. For example, if all samples in the experiment were stained with CD3 FITC, select the column header for all the FITC parameters in the table, and then enter CD3 or select CD3 from the drop-down Label menu. All selected cells are labeled with CD3 at once.

Multiple labels can also be entered by selecting the column or row header and using copy (Ctrl-C) and paste (Ctrl-V). In addition, copy and paste can be used to copy one label value to multiple label cells or to copy multiple label values to multiple label cells. However, the number of cells copied must be the same as the number of cells that are pasted.

- To delete a label, click the label cell and press the Delete key. Alternatively, click the Label menu and select the blank label field at the top of the list; then press Enter.



Using the Experiment Layout List

To add a label to the Labels list, type a label in the Name field and click the Add to List button. (Labels are case sensitive.) Delete a label from the list by clicking the Delete from List button. Clicking OK saves labels to the database.

To apply a label from the list, select a label field in the Experiment Layout table, select the label from the list, and click Assign. Click Remove to clear the label from the field.

☑ **Tip** You can also double-click a list item to quickly apply it to a selected field. For example, select the FITC parameter in the Label column, then double-click CD3 in the Labels list to apply CD3 to FITC.

Labels can also be entered on the Labels tab of the Tube Inspector. See Using the Tube Inspector on page 80.

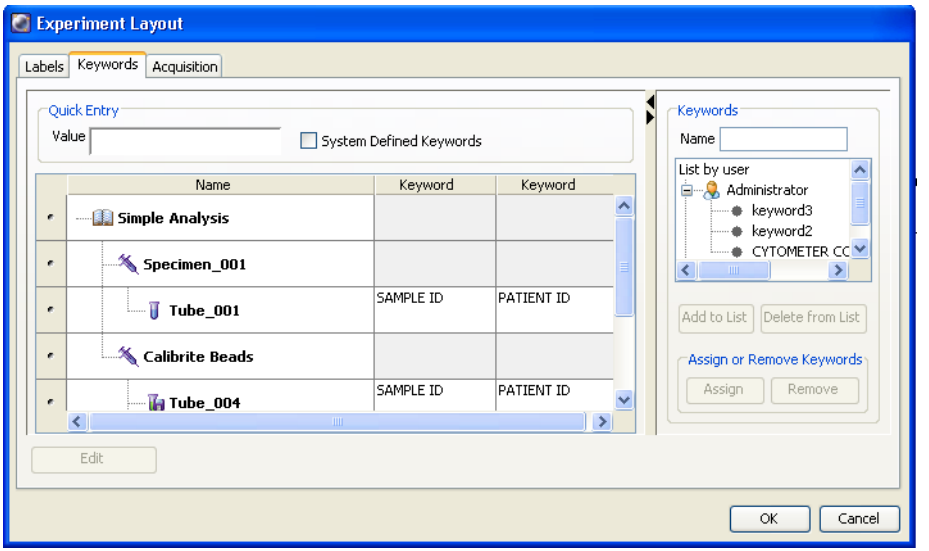
Keywords

All keywords currently defined for the experiment are listed in the Keywords tab of Experiment Layout. Keywords are used to identify a file or set of statistics. See Defining and Editing Keywords on page 89.

☑ **Tip** Select the System Defined Keywords checkbox to display the keywords that are automatically generated.

Enter a keyword value by selecting a cell and then entering a value in the Value field or directly in the selected cell. If the keyword was set up with selectable choices, the Value field changes to a menu where you can choose an available value. You can also select a keyword, right-click, and select Copy Keyword Data from the menu. Then select an element name, right-click, and select Paste Keyword Data to paste in the keyword. Keyword changes are automatically updated in the Keywords tab of the corresponding Inspector.

NOTICE Keywords are limited to 20 characters.



Alternatively, use the Keywords list to add keywords to the elements. Select an element (experiment, specimen, or tube) by clicking the element name in the table, then select the keyword from the list and click Assign. The keyword is added to the selected element(s) in any available column or in a new column to the right. (Keywords are case sensitive.) Click Remove to clear the keyword from the element.

Another way to add a new keyword is to select a keyword from the list, select the specimen or tube name in the Name column, and click Assign. If more than one cell is selected, the change is made in all selected cells at the same time. Keywords must be of the same type (such as all numeric or all string type) and range of values to be included in a multiple selection.

Use the following methods to select multiple keyword fields at the same time.

- Select multiple contiguous fields by:
  - Pressing the left mouse button and dragging down a column.
  - Holding down the Shift key while clicking the first and last fields in a range. You can also click in a cell and press Shift-Ctrl-End to select the rest of the cells in the column.
- Select noncontiguous fields in a keyword column by holding down the Ctrl key while clicking each selection.
- Select a cell in the column or row to choose the keyword type, then click the column or row header to select multiple keyword cells of that type. Enter a keyword or select a keyword from the Value menu to change the selected cells.

☑ **Tip** Cancel a value entered in a field or text box by pressing the Esc key before you click OK. This restores the previous value.

NOTICE An individual field cannot be deselected from a selected group.

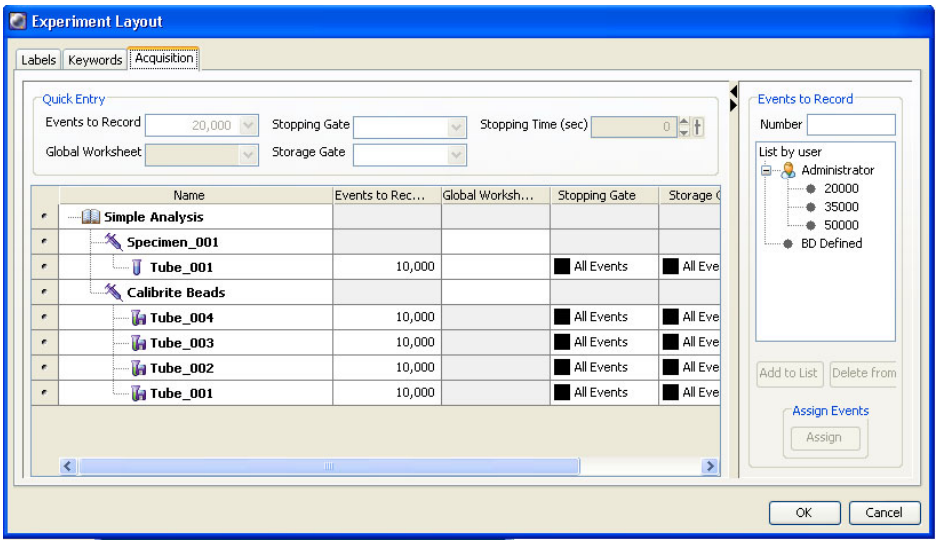
Acquisition Criteria

The Acquisition tab in Experiment Layout shows the number of events to record for each tube. Edit this number by selecting one or more fields and then entering a new number. You can also choose a value from the Events to Record menu or the Events to Record list. If more than one cell is selected, the change is made in all selected cells at the same time.

To use the Events to Record list, type a number in the Number field and click the Add to List button to add the new item to the list. Delete an item from the list by clicking the Delete from List button. Clicking OK saves items to the database.

To add new events to record to a tube, select an Events to Record field in the Experiment Layout table, select the events number from the list, and click Assign to assign the number to the selected tube. Click Remove to clear the number from the tube.

Tip Another way to add an events to record number using the list is to select the field you want to change and double-click the item in the list to apply it to the field. For example, select the Events to Record field for a particular tube, then double-click 50,000 in the Events to Record list to apply 50,000 to the selected tube.



To change multiple cells at once, click the column header to select the entire column and assign values (of the same type) for Events to Record, Global Worksheet, Stopping/Storage Gates, and Stopping Time.

Values can also be entered by selecting the column header or row button and using copy (Ctrl-C) and paste (Ctrl-V). In addition, copy and paste can be used to copy one value to multiple cells or to copy multiple values to multiple cells. However, the number of cells copied must be the same as the number of cells that are pasted.

To assign a preferred global worksheet, select the tube, specimen, or well in the Global Worksheet column and choose the desired worksheet from the menu in the column or in the Quick Entry Global Worksheet field. The menu displays the global worksheets that are in the currently open experiment.

To assign a storage or a stopping gate, select the tube, specimen, or well in the Storage or Stopping Gate column and choose the desired gate from the menu in

the column or the Quick Entry field. The storage and stopping gates in the menus are based on the global worksheet that is selected.

NOTICE When there is no preferred storage gate or stopping gate set for a tube, the gates in the Tube Inspector are based on the selected global worksheet. If changes are made to the storage and stopping gates, be sure to have the correct global worksheet open when checking the gates in the Tube Inspector.

The preferred stopping and storage gates can be set only in Experiment Layout. The stopping and storage gates in the Inspector and the Acquisition Dashboard interact with the currently selected worksheet to get the available gates and set them; they do not use the preferred global worksheet to set the gates.

When you set the current tube pointer to a tube, the global worksheet tab changes to the preferred global worksheet and the preferred stopping and storage gates are applied. You can then change those stopping and storage gates, but not the preferred stopping and storage gates (because preferred gates can only be changed in Experiment Layout).

NOTICE If a global worksheet is changed when a stopping gate or storage gate other than All Events is specified, then the gate selection is reset to All Events, provided that the selected gate name and type (eg, rectangle, polygon) drawn on the same coordinate system (eg, log, linear, biexponential), using the same parameters, do not appear in the new global worksheet.

To assign a stopping time to a tube, select the tube and in the Stopping Time column, click the arrows to increase or decrease the values in small increments, or click the pointer in the slider bar and drag it to a new value. You can define the stopping time in the same way using the Quick Entry Stopping Time field at the top of the window.

The number of Events to Record can also be entered on the Acq. tab of the Tube Inspector or in the Acquisition Setup section of the Dashboard. See Using the Tube Inspector on page 80 or Acquisition Dashboard on page 129.

Specimens

A *specimen* consists of the name of the material to be analyzed and a list of the tubes used to analyze the material. Specimens can also contain cytometer settings (see Creating Specimen- or Tube-Specific Settings on page 146).

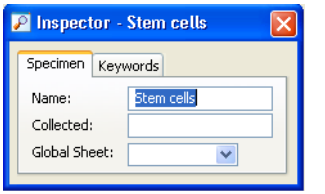
- To create a new specimen, see Adding New Elements to the Browser on page 51.
- To save a specimen as a panel, see Exporting a Specimen as a Panel Template on page 75.
- To create a new specimen from a panel template, see Importing a Panel Template on page 77.
- To edit a panel template, see Editing Templates on page 63.

Using the Specimen Inspector

In the Specimen Inspector, you can do the following:

- Use the Name field to enter the specimen name or sample type.

  Specimen names cannot contain commas or periods. Spaces at the beginning or end of the name are automatically removed.



- Use the Collected field to specify the date your sample was collected.
- Use the Global Sheet menu to choose a default global worksheet for the specimen. The menu lists all global worksheets in your experiment.

  The chosen worksheet is displayed automatically when you select a tube below this specimen.
- Click the Keywords tab to view or edit keywords stored with the specimen. For more information, see Keywords on page 88.

Exporting a Specimen as a Panel Template

A *panel* is a collection of tests, reagents, or markers commonly used together in the same experiment. Any specimen can be exported as a panel. Along with the specimen name and collection date, an exported panel contains a list of tubes and any parameter labels defined for each tube. Exported panels can also include global worksheets or normal worksheets and their associated analysis objects, and specimen- or tube-specific cytometer settings.

Panels are stored outside the Browser to simplify the Browser display. To export a specimen as a panel, do the following. Note that specimens can be exported as templates only from open experiments.

1. Set up your specimen with a list of tubes, define labels for each tube, and create analysis objects on a global worksheet.

2. Right-click the specimen and choose Export > Panel Template.

   The Export Wizard dialog appears, with steps that show you how to export a template.

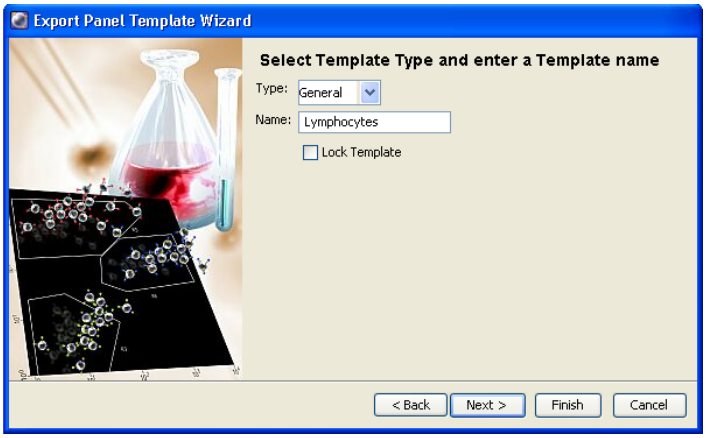
3. Select the global worksheet(s) you want to include in the panel, and click Next.

   All defined global worksheets are shown. Select the Export checkbox next to each worksheet you want to include.

4. Choose the template type and verify the name; click Next.

   See Figure 2-14 on page 76.

Figure 2-14 Choosing template type



Panels can be grouped by category so they are easier to find later. To add a category to the Type menu, enter a name in the Type field. Your new type will be available from the menu the next time you create a panel. Note that types cannot include any of the following characters: \ / : * ? " < > | , .

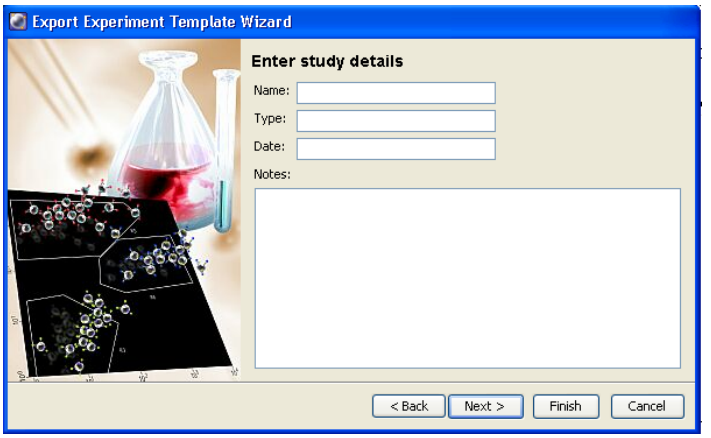
The panel name is based on the name of the specimen in the Browser. To change the name, enter a new name in the Name field. Note that panel names cannot include periods or commas.

Select the Lock Template checkbox so the panel cannot be overwritten by a panel template with the same name. Locked panels and any default panels provided with the software cannot be overwritten.

You must enter a template type and name to proceed.

5 (Optional) Enter comments for the panel template.

Comments can be viewed when you are importing a panel template. See Importing a Panel Template on page 77.



6 Click Finish.

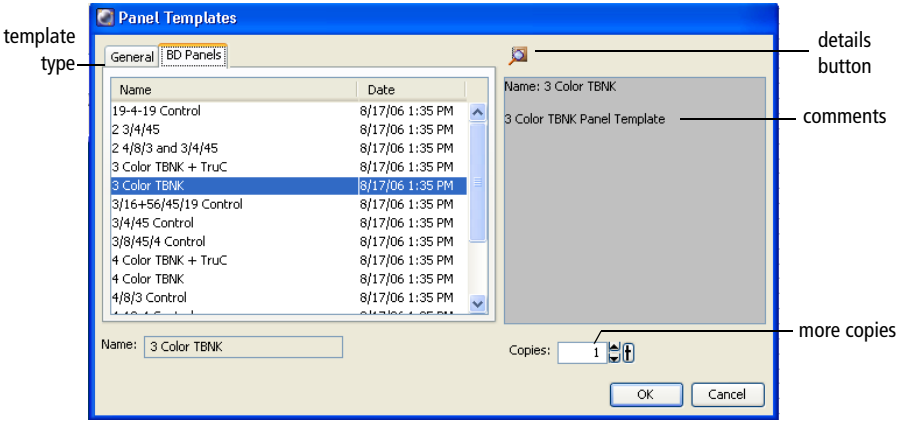
Panel Templates are saved in a folder in the D:\BDExport\Templates\Panel directory. A new folder is created for each panel type. When you create a new specimen based on a panel, each type is represented by a tab in the Panel Templates dialog. See Figure 2-15 on page 78.

Importing a Panel Template

To create a new specimen based on a panel template, choose Experiment > New Specimen or press Ctrl-M.

The Panel Templates dialog appears where you can choose a panel to import. If any comments were saved with the panel, they are shown in the box next to the list of panel templates.

Figure 2-15 Selecting a panel to import



To view details about the panel template, click the details button. Experiment Layout appears showing a list of tubes in the panel, any defined labels, keywords, and acquisition criteria. You can view but not edit elements in Experiment Layout when importing a template. See Using Experiment Layout on page 67.

Note that you can import up to 50 copies of a panel at a time. Each panel will be imported as a single specimen. To change the number of copies, click the up arrow next to the Copies field.

For information about creating panels, see Exporting a Specimen as a Panel Template on page 75.

Applying a Panel Analysis

A normal worksheet is for displaying analysis elements such as plots, gates, statistics, and custom text from multiple tubes. To add a normal worksheet to an open experiment, switch to the normal worksheet view (white tabs) and choose Worksheet > New Worksheet.

Panel templates can also be used as panel analysis templates. See Exporting a Specimen as a Panel Template on page 75. When applied to a specimen, a new normal worksheet is created containing all of its associated analysis objects and worksheet elements.

If a panel analysis includes global worksheets, the global worksheet information is not applied. See Worksheets on page 176 for details about normal and global worksheets.

1. To apply a normal worksheet panel analysis to a selected specimen, right-click the specimen and choose Apply Panel Analysis.

   The Panel Template dialog appears displaying the panel templates available in the application.

2. Select a panel template and click OK.

   The worksheet elements (such as plots, statistics, population hierarchies) of the selected panel template are imported.

   - The target specimen must have the same number of tubes as the panel analysis being applied or an error message is displayed.
   - If panel analysis tube names do not match the tube names in the Browser, choose Continue to apply the analysis. If the tubes in the specimen already have existing analyses (gates and worksheet elements), you can overwrite the previous analysis or exit without overwriting.
   - If a panel analysis has only global worksheets, the elements are not imported.

Tubes

A *tube* can contain acquisition criteria, information about the reagents used to analyze the specimen, the data for recorded events, tube-specific cytometer settings, analysis objects (plots, gates, and statistics views), and Sort Layouts (if applicable). Keywords can also be saved with tube data.

Most tube-specific information is entered using the Tube Inspector.

- To create a new tube, see Adding New Elements to the Browser on page 51.
- To create a new tube with a predefined analysis template, see Creating a Tube with a Predefined Analysis Template on page 83.
- To duplicate a tube, right-click the tube and choose Duplicate Without Data, or use the Copy and Paste commands.

Using the Tube Inspector

When the current tube pointer is selected (green), there are four components to the Tube Inspector, each accessed by clicking the tabs at the top of the Inspector: Tube, Labels, Acquisition, and Keywords. If a tube contains cytometer settings (ie, tube-specific settings or settings copied during recording), a Cytometer Settings tab is also shown.

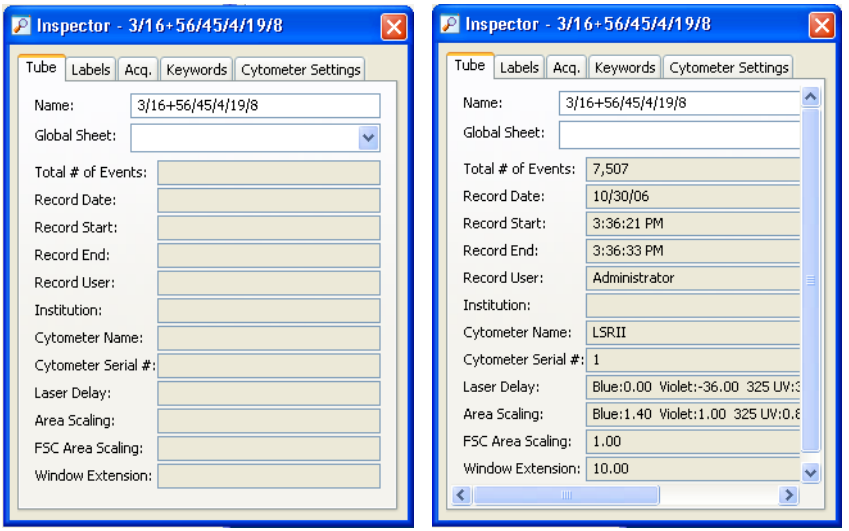
NOTICE Many values defined on the Labels, Acquisition, and Keywords tabs can be viewed and edited using Experiment Layout. (See Using Experiment Layout on page 67.)

- Use the Tube tab to name the tube and to view certain keywords and settings saved with recorded data (Figure 2-16 on page 81).

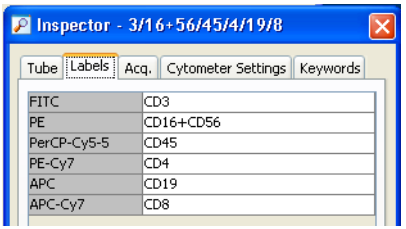
  Tube names cannot contain periods. Spaces at the beginning or end of the name are automatically removed.

  Note that keyword fields in the Tube tab cannot be edited.

Figure 2-16 Tube tab before recording data (left) and after (right)

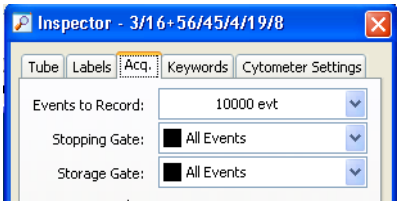


- Use the Labels tab to enter parameter labels for each fluorochrome. Labels are displayed on plot axes and in statistics views.



☑ **Tip** Label-specific tubes entered in the Tube Inspector are automatically displayed in the Create (or Modify) Compensation Controls dialog.

- Use the Acq. tab to specify the following acquisition criteria:



  - the number of events to record

    Choose a number from the drop-down menu or enter a value in the field.

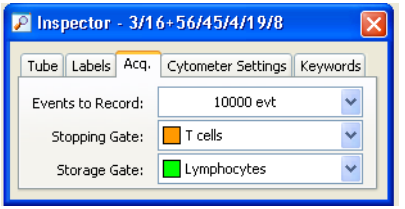
  - whether you want the number of counted events restricted to a predefined population (Stopping Gate)

- whether you want to record only events within a predefined population (Storage Gate)

The *Stopping Gate* and *Storage Gate* settings control the number of events collected and saved to the database. Any population can be used for a stopping or storage gate except one defined by a snap-to gate or a tethered gate.

For example, if you were performing an immunophenotyping experiment and wanted to collect data only for the lymphocytes, you could direct the software to collect 10,000 T-cell events for the stopping gate and record only events in the Lymphocyte storage gate.

Your Acq. tab would look like the following:



The cytometer would keep acquiring until 10,000 events were collected in the T-cell gate; however, only events that fell into the Lymphocytes gate would be saved to the database.

You can also specify the Number of Events to record, the Stopping Gate, and the Storage Gate using the menus in the Acquisition Setup section of the Dashboard (see Acquisition Dashboard on page 129) or Experiment Layout (see Using Experiment Layout on page 67). Inspector values are updated if you change the settings from these menus.

- When available, use the Cytometer Settings tab to view or edit tube-specific cytometer settings.

During offline use, you can edit global cytometer settings or tubes with cytometer settings in the Inspector. (Cytometer settings for recorded tubes cannot be edited.) When you are connected to the cytometer, you can change voltages, thresholds, and ratios for tubes only in the Cytometer window; the Inspector shows a report of the settings for a selected tube. For more information, see Cytometer Settings on page 137.

Cytometer settings can apply at the tube, specimen, or experiment level. See Creating Specimen- or Tube-Specific Settings on page 146 for details.

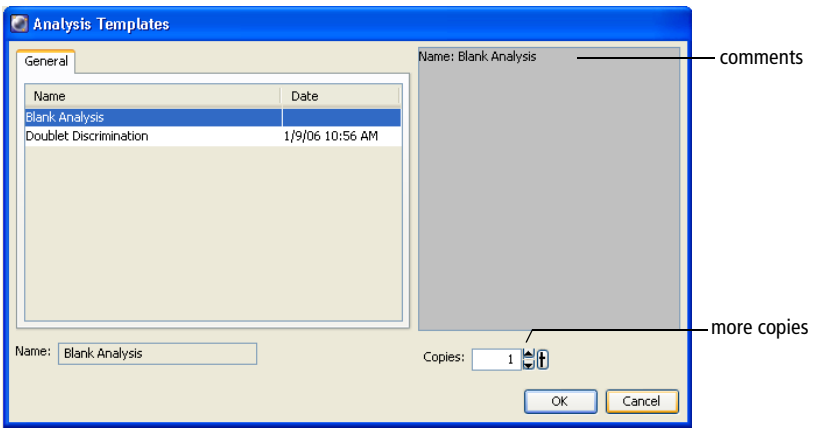
- When available, use the Keywords tab to view or edit keywords stored with the tube. See Keywords on page 88.

Creating a Tube with a Predefined Analysis Template

If you have an analysis template already defined, you can create one or more tubes using the predefined analysis in a single step. For instructions on creating an analysis template, see Saving an Analysis Template on page 86.

1 Choose Experiment > New Tube or press Ctrl-T.

The Analysis Templates dialog appears where you can choose an analysis template to import. If any comments were saved with the template, they are shown in the box next to the list of templates.



2 Specify the number of copies and click OK.

One tube is created per copy, up to 50 tubes. To change the number of copies, click the up arrow next to the Copies field.

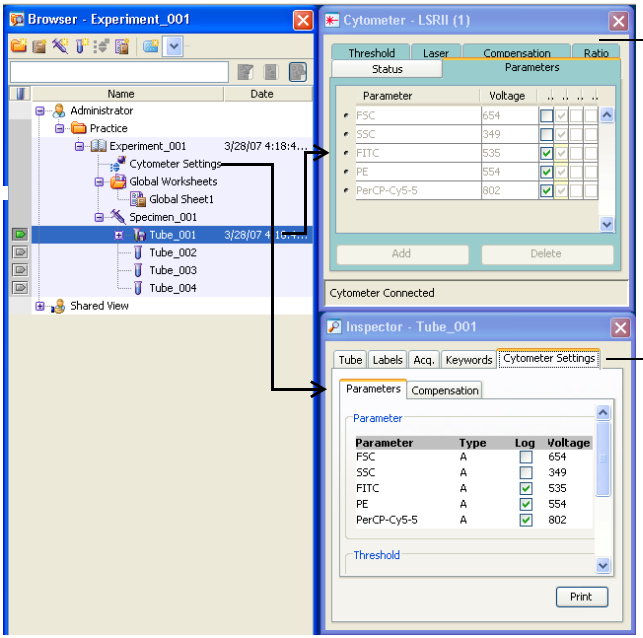
When you click OK, the designated number of tubes is added to the Browser, with a copy of your analysis template object under each tube. Plots, gates, and statistics views are added to the current worksheet unless the tube-specific worksheet preference is enabled. In this case, analysis objects are placed on individual worksheets for each new tube.

Cytometer Settings 

Cytometer settings represent the collection of values for parameters measured, photomultiplier (PMT) voltages, threshold, compensation, and any ratio measurements collected. Cytometer settings can apply to tubes, specimens, or experiments. When no tube-specific cytometer settings exist, specimen settings apply; when no specimen-specific settings exist, experiment settings apply.

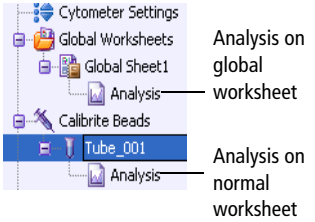
Every new experiment starts with default cytometer settings. These values can be optimized manually or overwritten by importing optimized settings or applying a saved setup. Default parameters are determined by the current cytometer configuration.

During offline use, you can edit cytometer settings for tubes in the Inspector. When you are connected to the cytometer and have the current tube pointer set, you can change voltages, thresholds, and ratios for tubes only in the Cytometer window; the Inspector shows a report of the settings for a selected tube. For more information, see Cytometer Settings on page 137.



Analysis Objects

An *Analysis* icon in the Browser represents elements that were created to analyze event data. The icon appears under a Global Sheet when you create a plot, statistics view, population hierarchy, text box, line, or arrow on a global worksheet; it appears under a tube after you create any of these items on a normal worksheet.



Tools for data analysis are described in more detail in Chapter 4; analysis examples can be found in *Getting Started with BD FACSDiva Software*.

An Analysis can be saved as a template, or copied from any tube or worksheet in the Browser to another. When copied or saved as a template, the Analysis includes all associated plots, gates, statistics views, population hierarchies, text boxes, lines, and arrows.

Saving an Analysis Template

You can set up analysis templates for common functions such as acquisition or analysis. Analysis templates can be assigned as a default worksheet or applied to one or more tubes at a time.

To create an analysis template, do the following. Note that templates can be exported only from open experiments.

1. Right-click an Analysis icon, or a tube or global worksheet that contains an Analysis icon, and choose Export > Analysis template.

   The Export Wizard dialog appears, with steps that show you how to export a template.

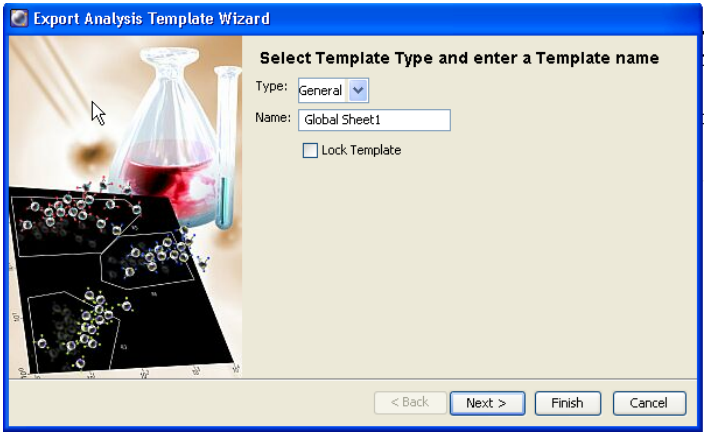
2. Choose the template type and verify the name; click Next.

   Group templates by type so they are easier to find later. To add a new category to the menu, enter a name in the Type field. Your new category will be available from the menu the next time you create a template. Types cannot include any of the following characters: \ / : * ? " < > | , .

   The template name is based on the name of the worksheet in the Browser, To change the name, enter a new name in the Name field. Template names cannot include periods or commas.

   Select the Lock Template checkbox so the template cannot be overwritten by a template with the same name. Locked templates and any default templates provided with the software cannot be overwritten.

   You must enter a template type and name to proceed.



3 (Optional) Enter comments for the analysis template.

Comments can be viewed when you are importing an analysis template. See Creating a Tube with a Predefined Analysis Template on page 83.

4 Click Finish.

Analysis Templates are saved in a folder in the D:\BDExport\Templates\Analysis directory. A new folder is created for each template type. When you create a new tube or worksheet based on a template, each type is represented by a tab in the Analysis Templates dialog.

Copying Analyses

☑ **Tip** When you copy and paste an Analysis icon or duplicate a tube with an Analysis, the duplicated worksheet elements are added to the available space on the existing worksheet. To make sure the duplicated analysis starts a new page, move or edit worksheet elements so they fit a full page *before* you duplicate the Analysis.

1 Open an experiment and expand the tube or global worksheet containing the analysis you want to copy.

☑ **Tip** Use the arrow keys on your keyboard to access and expand Browser elements. Use the down arrow key to locate an element, and use the right arrow key to expand it.

2 Right-click the Analysis icon and choose Copy.

Alternatively, select the icon and press Ctrl-C.

3 Select the tubes or global worksheets where you want to apply the analysis and press Ctrl-V.

You can also right-click the selected icons and choose Paste.

☑ **Tip** To select noncontiguous icons in the Browser, hold down the Ctrl key while clicking each icon.

The new analysis overwrites any analysis objects that already exist. When pasted to a tube, the new plot(s) and statistics are pasted into the active worksheet (the worksheet currently displayed).

NOTICE When pasting an analysis object from a global worksheet to a tube, some of the analysis can be lost if the tube uses a different set of parameters.

Keywords

Keywords are used to annotate files or sets of statistics. Keywords can be defined and saved in the database with experiments, specimens, or tubes. Experiment- and specimen-level keywords are also saved with tubes. When you export FCS data, user-defined keywords are included in the header of exported FCS files.

Use keywords for the following:

- Define a list of terms (Selectable Strings) that can be stored with each experiment.

  NOTICE Keywords are limited to 20 characters.

- Attach numerical data, such as cell count, to a tube or specimen.

- Attach labels to data, making it easier to locate. See Finding Saved Data on page 65.
- Display tube, specimen, or experiment keywords in the headers of statistics views. Keywords are exported along with the statistics.
- Share keywords with other cytometer users by adding them to the global keyword list.

Defining and Editing Keywords

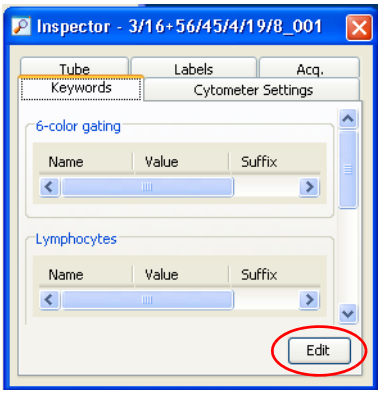
Use the Inspector or Experiment Layout to define keywords at the experiment, specimen, or tube level and to add keywords to the global keyword list, so other cytometer users can access them. See Keywords on page 69 for more details.

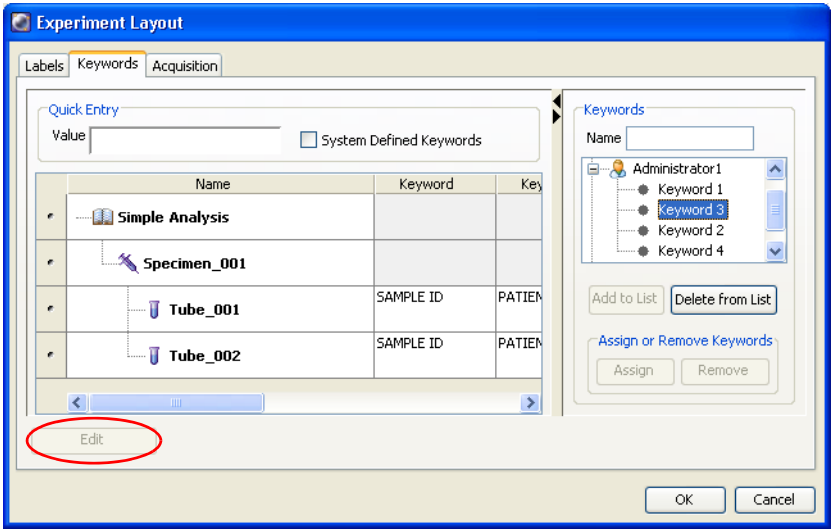
NOTICE If custom keywords of the same name are defined for more than one level in an experiment hierarchy, the lower-level definition overwrites the one at a higher level.

1. In an open experiment, select an experiment, specimen, or tube in the Browser. Click the Keywords tab in the Inspector and click Edit.

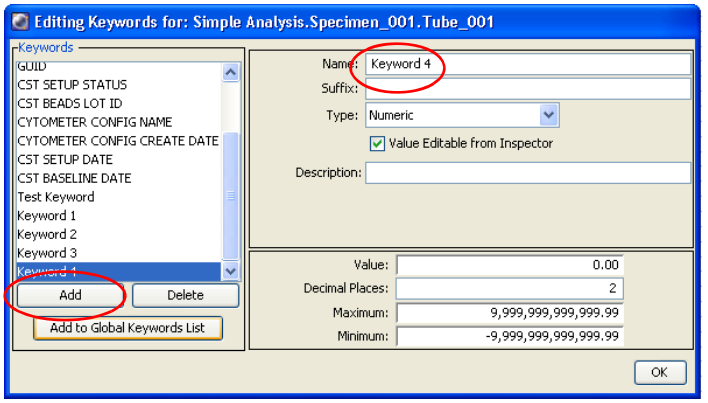
   Alternatively, choose Experiment > Experiment Layout, click the Keywords tab, then select an experiment, specimen, or tube in the first column. Click Edit.

Figure 2-17 Creating custom keywords in the Inspector or Experiment Layout





2 In the Editing Keywords dialog, click Add.



3 Name the keyword and add any required suffix.

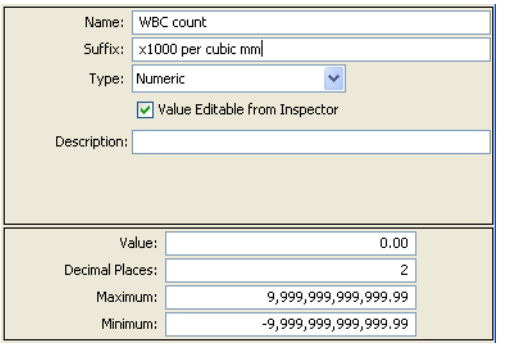
Select the generic name in the Name field and enter a new name. Each name must be unique; use the suffix to define values, such as units of measure.

4 Select the type and define the keyword(s).

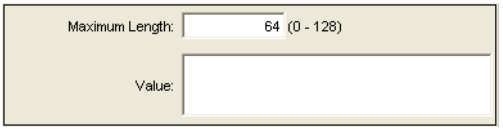
- Use *Numeric* for keywords defined by numerical values, such as numbers of cells (Figure 2-18). Limit the range by entering Minimum and Maximum values; enter a number to specify the number of digits to the right of the decimal place (maximum of 14).

  Specify a Value in the dialog or check *Value Editable from Inspector* and enter the value there.

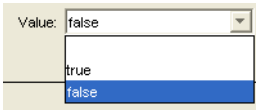
Figure 2-18 Defining a numeric keyword



- Use *String* for keywords defined by text, such as sample identifiers. In the Value field, enter up to 128 characters.

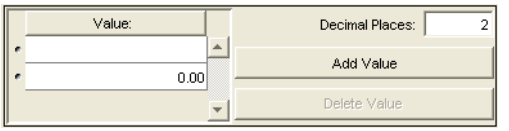


- Use *Boolean* for keywords that require a true or false answer. Select true or false from the Value menu in the dialog or the Keyword Inspector.

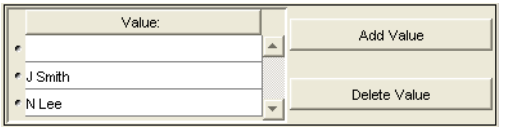


- Use *Selectable Numeric* to define a set of selectable numeric keywords, such as a list of values. Define the set of values by clicking Add Value, selecting the value in the Value field, and entering the required value. All defined values appear in a menu in the Value field of the Keyword Inspector.

Use the Decimal Places field to specify the number of digits to the right of the decimal place (maximum of 14).



- Use *Selectable String* to define a set of selectable text keywords. Define selections by clicking Add Value, selecting the value in the Value field, and entering the required text. All defined values appear in a menu in the Value field of the Keywords Inspector.

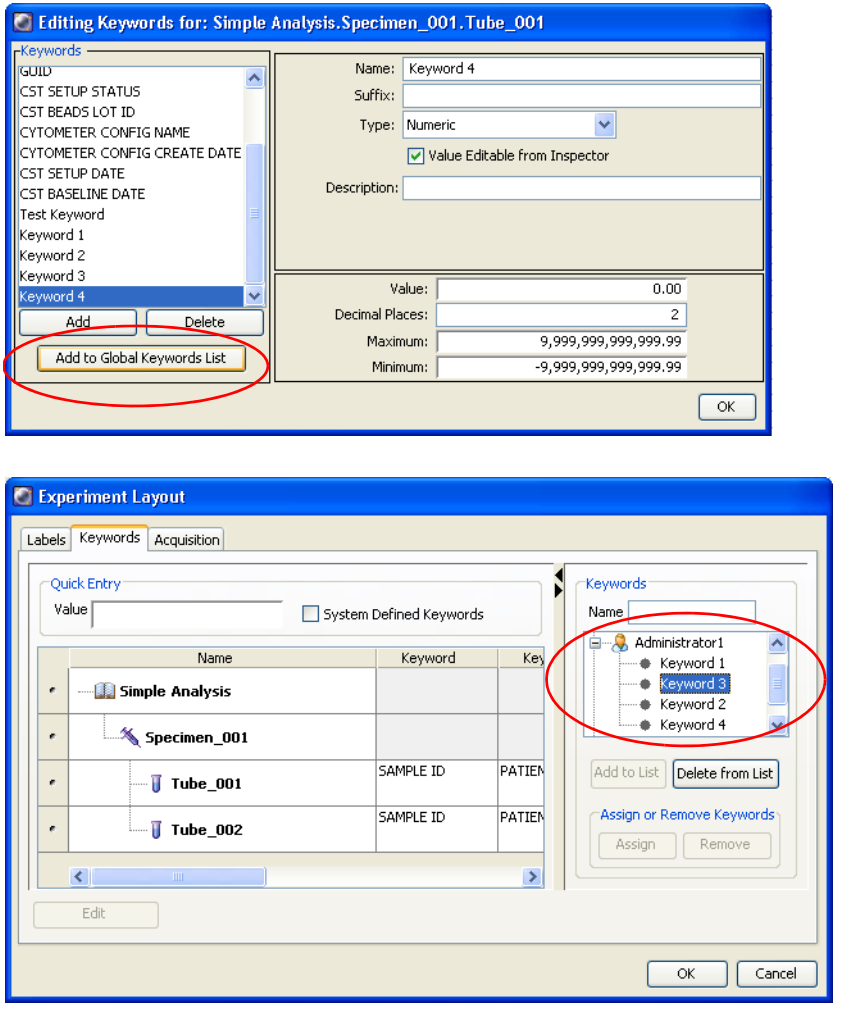


5 (Optional) Use the Description field to enter text describing the keyword.

☑ **Tip** A keyword with a description helps others to understand what the keyword is and use it.

6 (Optional) Select the keyword and click the Add to Global Keywords List button to add the keyword to the global list as displayed in Experiment Layout. See Figure 2-19 on page 94. This allows other cytometer users to view and use those keywords.

Figure 2-19 Editing Keywords dialog



7 Click OK to close the Edit Keywords dialog.

Deleting Keywords

Only custom keywords can be deleted. BD-defined keywords (such as $OP for operator or $CYT for the name of the cytometer used to collect data) cannot be edited or deleted.

1. In an open experiment, select the Browser item containing the keyword you want to delete. Click the Keywords tab in the Inspector and click Edit.

   Alternatively, open Experiment Layout, click the Keywords tab, select the experiment element containing the keyword you want to delete, then click Edit.

2. In the Editing Keywords dialog, select a keyword and click Delete.

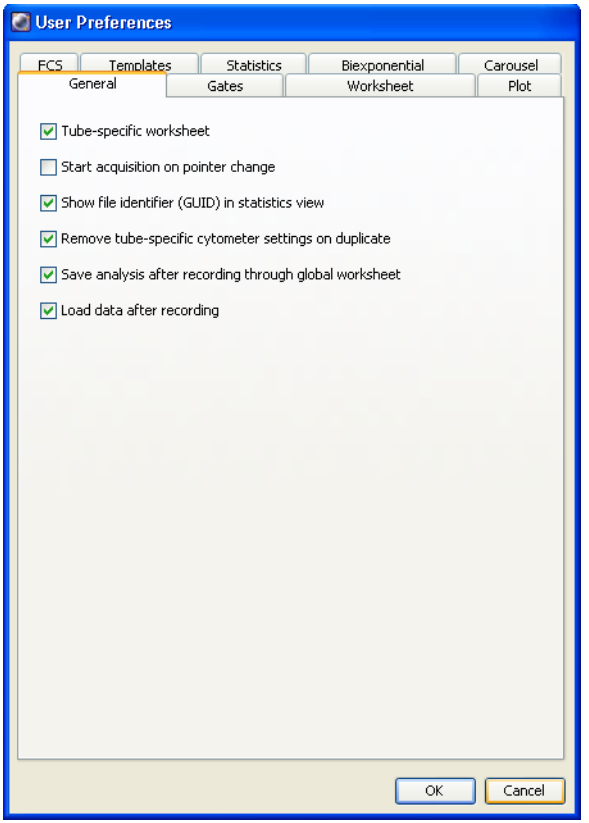
User Preferences

Certain default settings can be changed using the Edit > User Preferences command. Preferences apply to all experiments in the Browser. After you click OK in the User Preferences dialog, changes are saved with your login name and are retained from one session to the next.

The following User Preferences are available for all cytometers: General Preferences, Gates Preferences, Worksheet Preferences, Plot Preferences, FCS Preferences, Templates Preferences, Statistics Preferences, and Biexponential Preferences. Additional preferences might be available for your cytometer type; refer to your cytometer manual for a description.

General Preferences

General preferences apply to worksheets, acquisition controls, and cytometer settings.



- Tube-specific worksheet—When selected (checked), a new worksheet is automatically created for each tube when you duplicate without data or click the Next button, or when the *Save analysis after recording through global worksheet* preference is enabled for global worksheets. If a worksheet with the name of the specimen and tube already exists, the copied elements are pasted into the existing worksheet. By default, this preference is not selected.

NOTICE This preference does not apply when you are recording data from wells on a plate (eg, with the BD HTS option).

- Start acquisition on pointer change—Acquisition begins each time the current tube pointer is set to a new tube that does not already contain recorded data. By default, this preference is not selected.

- Show file identifier (GUID) in statistics view—Show the GUID keyword, the FCS file's unique identification number, in the header of statistics views. By default, this preference is selected.

- Remove tube-specific cytometer settings on duplicate—Tube-specific cytometer settings are not included when you duplicate a tube, or copy and paste a tube. Note that settings are included when you paste with data, even when the preference is enabled. By default, this preference is selected.

- Save analysis after recording through global worksheet—Analysis elements on the global worksheet are automatically copied to a tube-specific worksheet after recording. An analysis object is saved for each tube unless it already contains an analysis object. By default, this preference is selected.

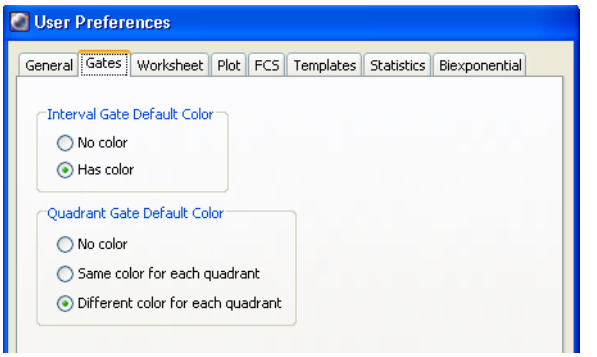
  NOTICE This preference does not apply when you are recording data from wells on a plate (eg, with the BD HTS option).

- Load data after recording— Data is loaded into plots automatically when recording is finished. By default, this preference is selected.

  Deselect the checkbox to *not* load data into plots when recording is done. If the checkbox is not selected and you want to load data for a tube, set the current tube pointer to load the data. The background of the tube pointer changes from black to gray, indicating the data is loading.

Gates Preferences

Gates preferences define how populations are colored within Interval and Quadrant gates.



- Interval Gate Default Color—Select one of the two options to specify whether populations defined by an Interval gate should be assigned a color or retain the color of the parent population.

  By default, populations are not colored.

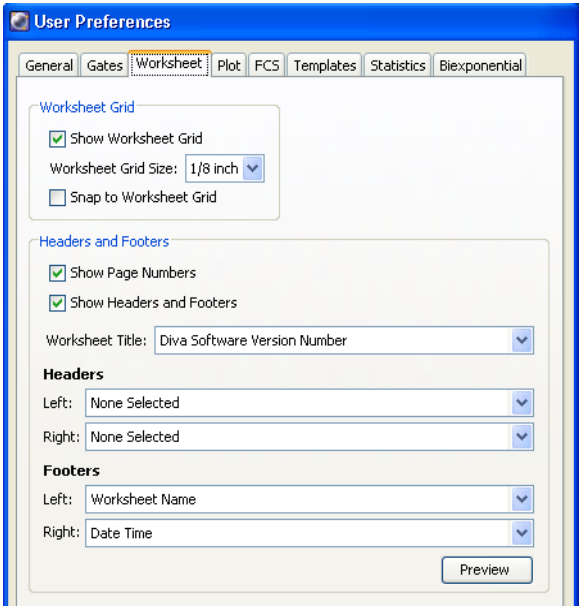
- Quadrant Gate Default Color—Select one of the three options to specify how populations defined by quadrant gates should be colored:

  - no color used (color is determined by parent population)
  - all quadrants (gated populations) assigned the same color
  - each quadrant (gated population) shown in a different color

  By default, quadrant populations are not colored.

Worksheet Preferences

Worksheet preferences allow you to show a grid display on the worksheet, to set the grid size, and to have elements in the worksheet snap to the grid. This is also where worksheet title, page number, and header and footer information can be defined.



Worksheet Grid

- Show Worksheet Grid—Select to have the grid displayed on the worksheets. This is selected by default.
- If the Show Worksheet Grid is selected, a default grid size of 1/8 inch is displayed. Use the grid size menu to change the size to 1/4 inch, 1/2 inch, or 1 inch. If the Show Worksheet Grid is not selected, the grid size menu is unavailable.
- Snap-To Worksheet Grid—Select to make all worksheet elements that are added, moved, or resized snap to the grid. By default, this is deselected.

☑ **Tip** The worksheet grid does not appear in printouts or in PDF files.

Headers and Footers

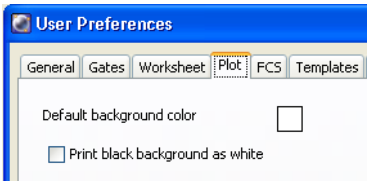
In the Headers and Footers section of the Worksheet tab, you can designate the information to be displayed on worksheet printouts or PDF files. Cytometer Name, Experiment Name, User Name, Date Time, and Printed by User Name are useful to identify and keep track of worksheets. Show Page Numbers and Show Headers and Footers are selected by default.

Define Worksheet Title, Headers, and Footers by selecting from the menus or typing a custom word in the menu field. Choose None Selected to leave blank.

Click Preview to see your selections displayed on a worksheet and make any changes needed. Worksheet title and header and footer information can also be entered, edited, and previewed in the Worksheet Inspector. The selections made in the Worksheet Inspector take precedence over those in User Preferences. See Using the Worksheet Inspector on page 183.

Plot Preferences

By default, plots are created with a white background. To change the default background color, click the color box on the Plot preference tab. A palette appears from which you can choose a new color.



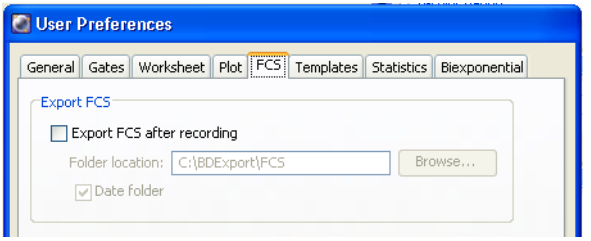
If you set the default background to black, select the checkbox to print plots with a white background. White gates and populations are then automatically printed in black.

FCS Preferences

Enable the Export FCS preference to automatically export an FCS 3.0 file after each tube is recorded. To export FCS 2.0 data, you need to export manually. See Exporting FCS Files on page 259 for more information.

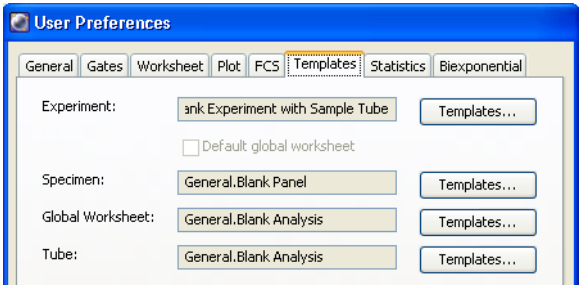
When the preference is selected, specify an export folder location by clicking the Browse button or by entering a folder path in the Folder location field.

☑ **Tip** Select the *Date folder* checkbox to automatically create a dated folder in the specified directory each day files are exported.



Templates Preferences

Templates preferences allow you to select which template will open when you click the corresponding button on the Browser toolbar. By default, the New Experiment, Specimen, Tube, and Global Worksheet buttons create a blank experiment, panel (specimen), tube, and worksheet, respectively.



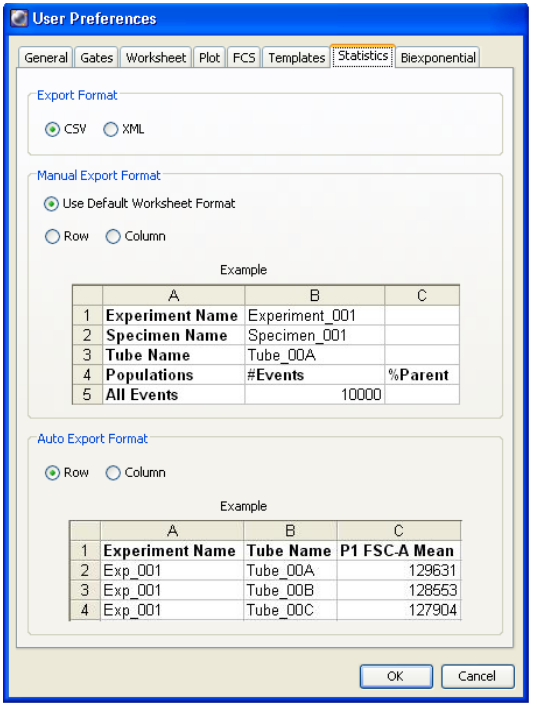
- To assign a saved template as a default experiment, specimen, tube, or global worksheet, click the Templates button next to the corresponding item. Then select a saved template in the dialog that appears. The selected template remains in effect for the current user until it is changed in User Preferences.

- To add a normal worksheet (instead of a global worksheet) to each new experiment, deselect the *Default global worksheet* checkbox. This checkbox is only available for the Blank Experiment template. It is selected by default.

  Note that when you assign an analysis template as the default global worksheet, the assigned template is added to each new blank experiment. To add a blank global worksheet, leave the global worksheet template as Blank Analysis and leave the *Default global worksheet* checkbox selected.

Statistics Preferences

Statistics preferences determine the format of exported statistics files.



- Export Format—Specifies the file type of exported statistics, including those generated during Carousel auto-export or batch analysis. Choose either CSV (comma-separated value) format or XML (extensible markup language) format. For more information, see Exporting Statistics on page 246.

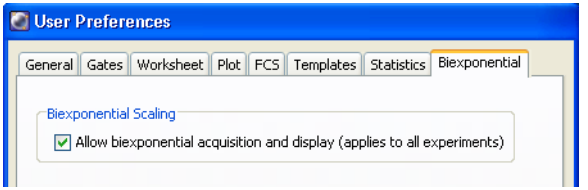
- Manual Export Format—Specifies the format of exported files when you select one or more statistics views and choose File > Export > Statistics. Choose from the default worksheet format, row, or column. For each option, an example of the exported file is shown.

- Auto Export Format—Specifies the format of exported files when you export statistics during a batch analysis or Loader carousel run (BD FACSCanto cytometers only). For more information, see Batch Analysis on page 248 or refer to your cytometer manual. Tubes can be exported in row or column format. For each option, an example of the exported file is shown.

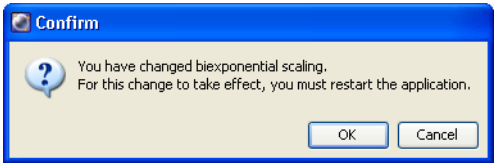
  NOTICE IF XML is selected as the export format, Auto Export Format choices (Row or Column) are unavailable because XML statistics are always displayed in rows.

Biexponential Preferences

To disable biexponential display for all experiments in the Browser, disable the biexponential scaling preference. Disabling the preference allows more events to be recorded per experiment; however, large data files can be truncated when you re-enable it.



BD FACSDiva software must be restarted for this preference change to take effect.



3

Cytometer and Acquisition Controls

BD FACSDiva software supports several different cytometers. This chapter contains information about cytometer and acquisition controls that are common to all cytometers. For cytometer-specific controls, consult your cytometer manual.

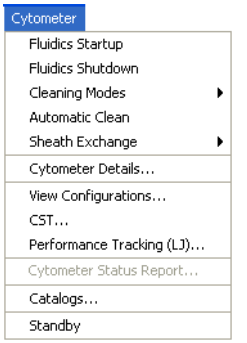
Many cytometer functions can be controlled within BD FACSDiva software, either within the Cytometer window or from the Cytometer menu. Acquisition controls are available in the Acquisition Dashboard and the Browser.

You must be connected to a cytometer (working from an acquisition workstation) to enable many of these functions.

The following sections contain an overview of these controls:

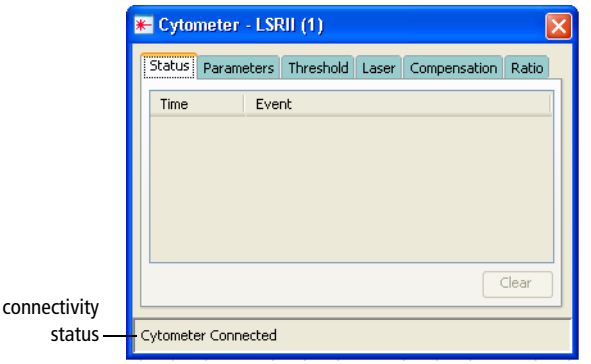
Cytometer Controls

Cytometer controls are accessed from the Cytometer menu or the Cytometer window. To display the Cytometer window, click the Cytometer button ( ) on the Workspace toolbar.



- Use the Cytometer menu to identify the cytometer; access cytometer configurations, performance tracking information, and settings catalogs; perform cytometer setup functions; display a Cytometer Status report; and connect to or disconnect from the cytometer. Cytometer menu commands vary depending on the cytometer connected to your workstation.

- Use the Cytometer window to view workstation connectivity status. When the software is connected to the cytometer, status messages and laser controls are also shown in the window. If an experiment is open and the current tube pointer is set, the window displays cytometer settings for the current acquisition tube.



The following sections contain descriptions of Cytometer menu commands, status messages, and laser controls. For a description of cytometer settings tabs, see Cytometer Settings on page 137.

Cytometer Configurations

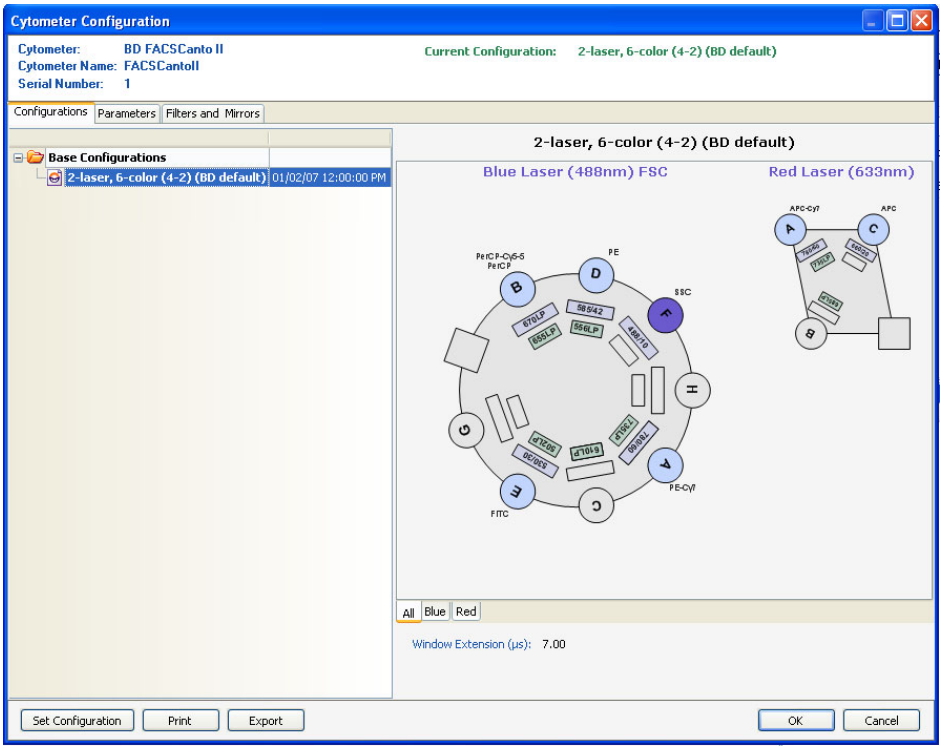
This section contains the following information:

The physical configuration of a cytometer is the combination of lasers, detector arrays, filters, and dichroic mirrors inside the cytometer. BD FACSDiva software refers to this as the *base configuration*. To begin using the Cytometer Setup and Tracking features, a configuration matching your cytometer must be created within the software. This is typically done by the BD Biosciences field service engineer during installation. This base configuration serves as the template from which custom configurations can be created.

Only users with administrative access can create, modify, or delete custom configurations. Custom configurations can be created for the different filter, mirror, and fluorophore combinations used in your lab. Custom configurations can also include other information (eg, cytometer-specific information, comments, etc). Any user can then set the appropriate configuration for a particular experiment. Once a configuration is set, it is listed as the *current configuration* in the Cytometer Configuration window. See Figure 3-1 on page 108.

NOTICE When upgrading from a previous version of BD FACSDiva software (v5.0.x or earlier), the base configuration is automatically set to what was used previously, minus filter and mirror information, which can then be added.

Figure 3-1 BD default cytometer configuration for BD FACSCanto II flow cytometer



The Cytometer Configuration window determines which parameters are listed in the Parameters tab of the Cytometer Settings Inspector or Cytometer window.

Verifying Appropriate Parameters, Filters, and Mirrors

Before creating a new configuration, verify that the necessary fluorophores, filters, and mirrors are defined.

☑ **Tip** To ensure that the correct parameters appear on your Parameters tab, set the configuration you want to use *before* you create a new experiment.

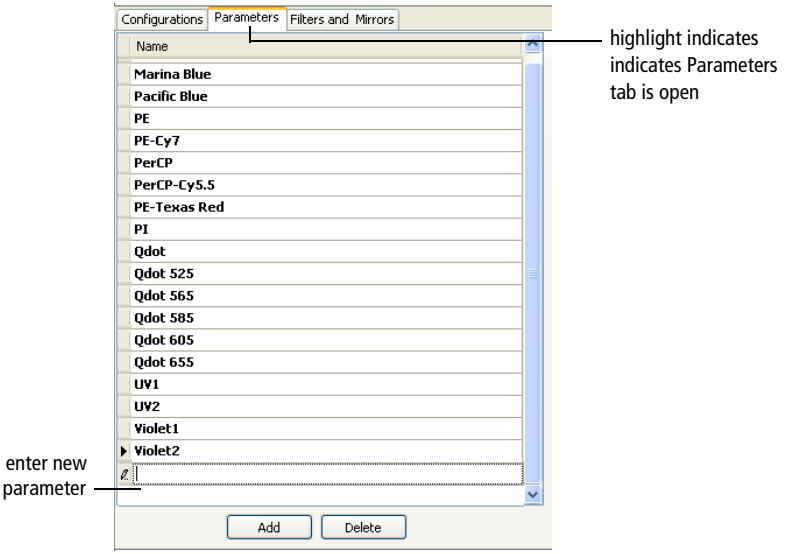
Adding to the Parameters List

The parameter names you enter are the names that will appear on the Parameters tab in the Cytometer Inspector or Cytometer window. Parameter names must be unique within the configuration, and cannot include commas or periods. Spaces at the beginning or end of the name are automatically removed.

To add new parameters, perform the following steps:

1. Log in to BD FACSDiva software as an administrator.
2. Choose Cytometer > View Configurations.
3. Click the Parameters tab to open the Parameters list.
4. Click Add, enter the new parameter name, and click OK to close the Cytometer Configuration window and save the changes.

NOTICE You cannot add SSC to the Parameters list. See Setting SSC on page 113.

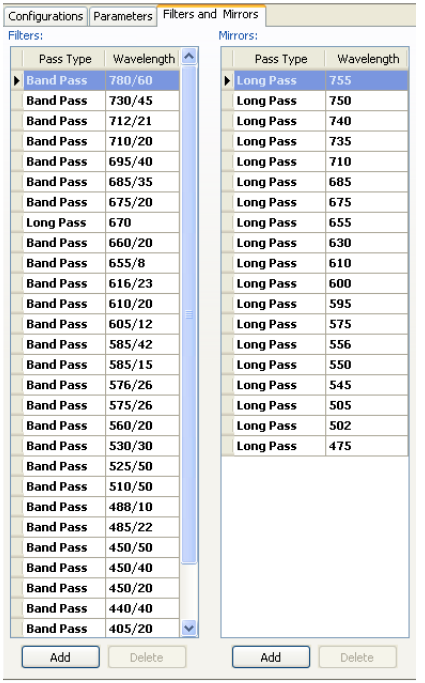


Adding to the Filters and Mirrors List

To add new filters or mirrors, perform the following steps:

1. Choose Cytometer > View Configurations, if necessary.

2. In the Cytometer Configurations window, click the Filters and Mirrors tab to open both the Filters list and the Mirrors list.



3. To add to either list, click Add, enter the new name, and click OK to close the Cytometer Configuration window and save the changes.

⚠ Bead lot information is not available for non-BD filters and mirrors.

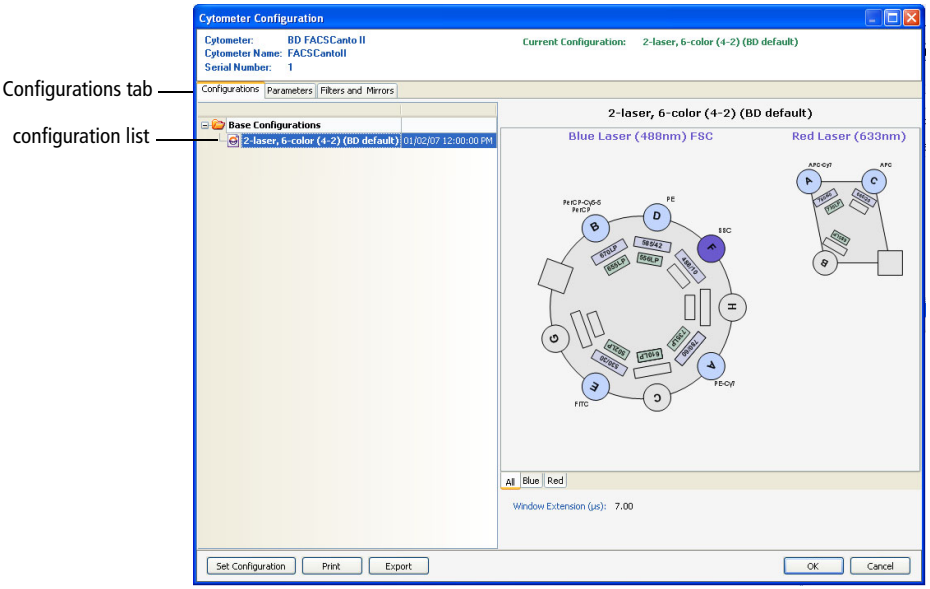
Creating Custom Configurations

Follow these steps to create a custom configuration. To modify an existing configuration, see Duplicating Existing Configurations on page 115.

1 Verify that you are logged in to the software as an administrator.

2 Choose Cytometer > View Configurations.

The Cytometer Configuration window appears.



NOTICE By default, FSC is triggered off of the blue laser. If you need to change this default, call your BD Biosciences service representative.

3 In the configuration list, perform the following steps:

- Right-click the Base Configurations folder, choose New Folder, rename the folder, and press Enter.

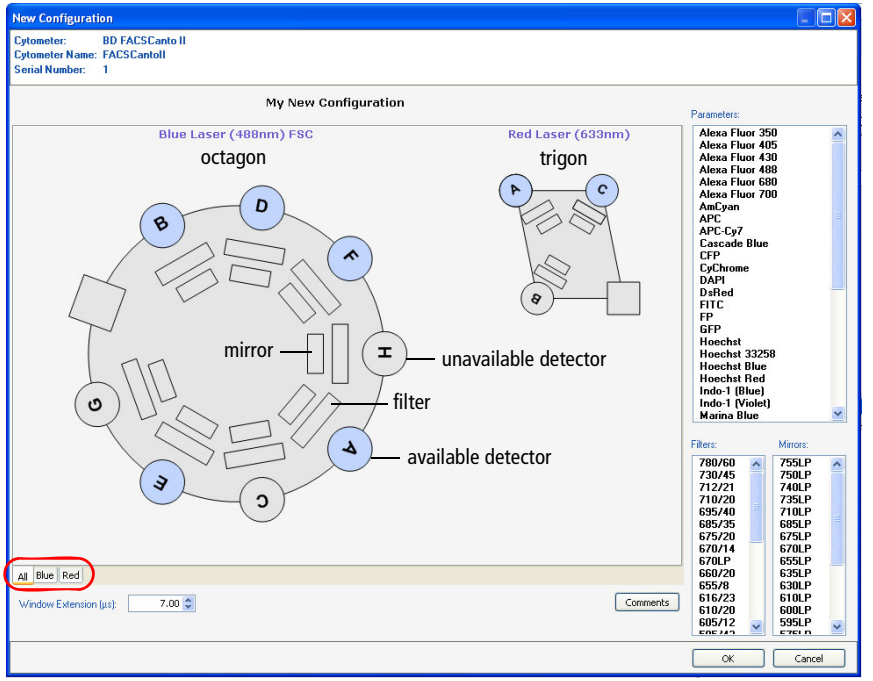
- Right-click the new folder, choose New Configuration, rename the configuration, and then press Enter.

  A blank configuration appears in the Cytometer Configuration window that includes the base configuration lasers, detector array, FSC position, and active detectors.

4 In the configuration list, double-click the new configuration.

The following window appears.

Figure 3-2 My New Configuration window



NOTICE Unavailable detectors are based on your cytometer's optics.

5 (Optional) Click a laser tab (circled in red in Figure 3-2) to see the laser detector array in an enlarged view.

Click the All tab to return to the default view.

Setting SSC

1 To set SSC, right-click the appropriate detector.

The position of SSC is based on your detector array. Refer to your cytometer manual for more information.

2 Choose Set Side Scatter from the menu.

☑ **Tip** Once SSC is set, the detector is locked. To unlock the detector, delete the parameter.

Labeling Parameters, Filters, and Mirrors

1 Perform the following steps:

- Label the detectors—Drag the parameter name from the Parameters list onto the appropriate detector. Available detectors are colored light blue. To add multiple contiguous parameters, Shift-click the parameter names and drag them onto the appropriate detector. To add multiple noncontiguous parameters, Ctrl-click the parameter names and drag them onto the appropriate detector.

  The parameter names you assign are the names that will appear on the Parameters tab in the Cytometer Inspector or Cytometer window. Parameter names are listed in alphabetical order. The parameter that shows by default is the first in the list.

- Label the filters—Drag the filter name from the Filters list onto the appropriate box.

- Label the mirrors—Drag the mirror name from the Mirrors list onto the appropriate box.

Adding Comments

1. Click the Comments button.
2. Enter text in the Cytometer Configuration Comments dialog; click OK to save the comments.

   When everything is labeled, click OK to save the changes.
3. To make the new configuration the current configuration, click Set Configuration. The selected configuration in the list is highlighted.

   The Current Configuration name changes at the top of the Cytometer Configuration window.

⚠ You must click Set Configuration for the new configuration to apply. For accurate data results, always verify that the cytometer optics match the current cytometer configuration.

Printing Configurations

The printout includes the user name, date and time printed, information about the cytometer, configuration name and details, and a graphic representation of the configuration.

1. To print the current cytometer configuration, click Print.

   The Print Preview window appears.
2. In the Print Preview window, choose from the following options:
   - Page Setup to change page setup options
   - Print... to choose print options
   - Print to print one copy

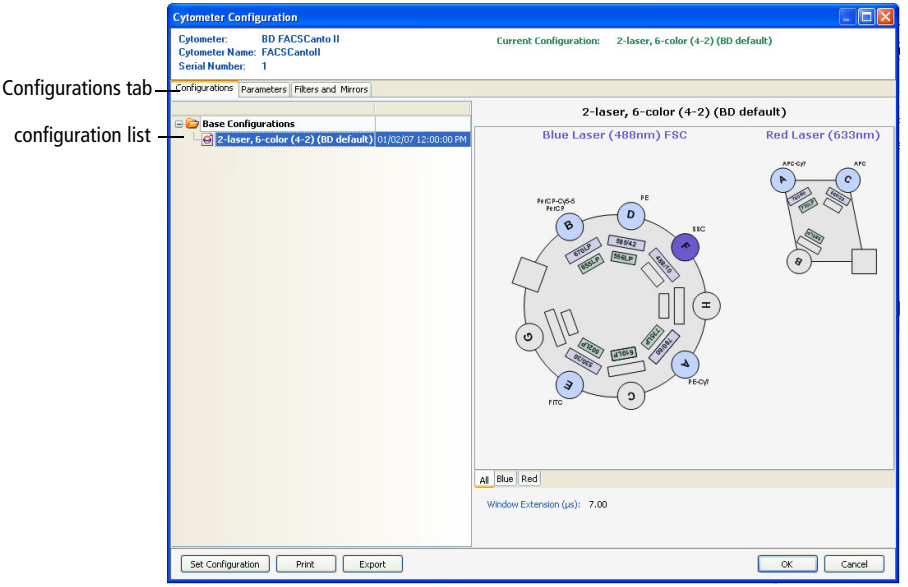
Duplicating Existing Configurations

You cannot edit or delete BD-defined cytometer configurations. However, you can duplicate them to use as a starting point to define your own configuration. To set up a new configuration, see Creating Custom Configurations on page 111.

NOTICE You cannot edit a configuration if a baseline has already been defined. The configuration is locked.

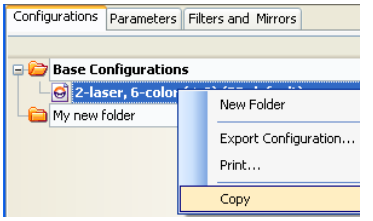
1 Verify that you are logged in to the software as an administrator.

2 Choose Cytometer > View Configurations.



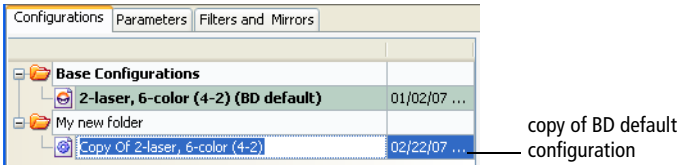
3 In the configuration list of the Cytometer tab, perform the following steps:

- Right-click the Base Configurations folder, choose New Folder, rename the folder, and press Enter.



- Right-click the BD default configuration listed under the Base Configuration folder, and choose Copy.
- Right-click the new folder and choose Paste.

  A copy of the BD configuration is created in the New Folder.

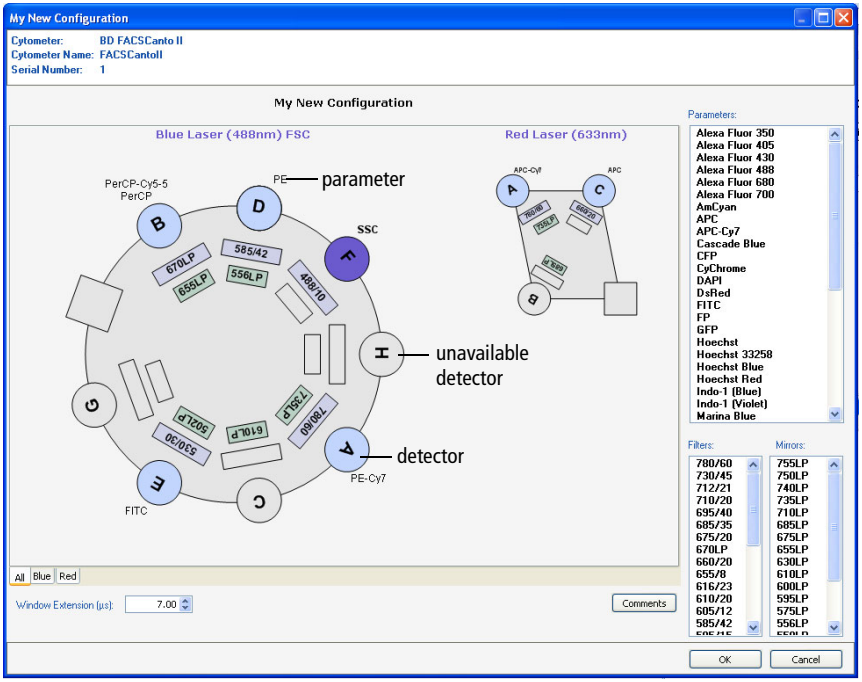


- Rename the configuration and press Enter.

  The following characters cannot be entered: \ / : * ? “ < > |

Editing Parameters

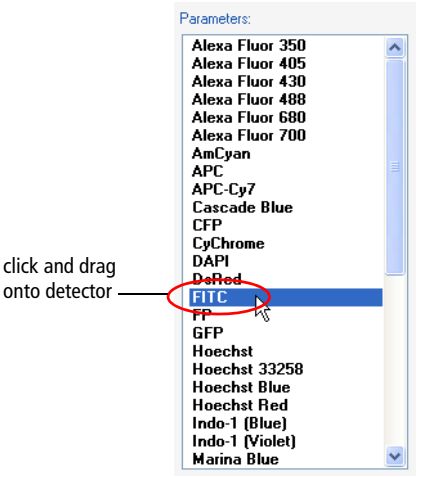
1 In the configuration list, double-click the new configuration.



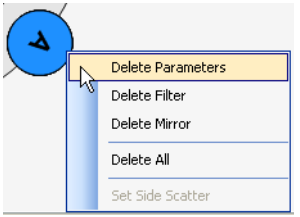
2 To rename a detector, drag the new name from the Parameter list to the available detector. See Figure 3-3 on page 118.

Available detectors are colored light blue. To add multiple contiguous parameters, Shift-click the parameter names and drag them onto the appropriate detector. To add multiple noncontiguous parameters, Ctrl-click the parameter names and drag them onto the appropriate detector.

Figure 3-3 Parameters list



☑ **Tip** If you want to leave a detector empty (inactive), right-click the detector (colored circle) of the parameter you want to delete, and choose Delete Parameters.



3 To add a new filter or mirror to the list, go to the Filter or Mirror list and repeat step 2.

4 Click OK.

5 Make sure the appropriate configuration is listed as the Current Configuration and click OK.

To use a different configuration, select the configuration name from the list and click Set Configuration. The selected configuration in the list is highlighted green.

⚠ You must click Set Configuration for the new configuration to apply. For accurate data results, always verify that the cytometer optics match the current cytometer configuration.

☑ **Tip** To ensure that the correct parameters appear on your Parameters tab, set the configuration you want to use *before* you create a new experiment.

Deleting Configurations

You cannot delete BD-default configurations or the Current Configuration.

To delete a user-defined configuration, right-click the configuration in the list, and choose Delete.

NOTICE If you delete a configuration with data associated, you will lose the ability to track that data.

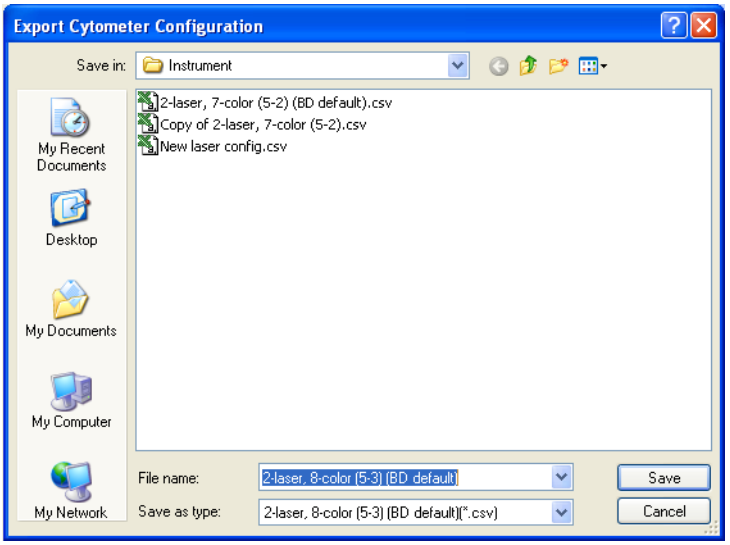
Exporting Configurations

You can export user-defined configurations for use on a different workstation (with the same base configuration) or to back up for storage.

NOTICE To calculate or re-calculate compensation on recorded files, export the configuration to an analysis-only workstation.

☑ **Tip** For offline experiment setup, export the configuration from an acquisition workstation, and then import the configuration to an offline workstation.

1 In the Cytometer Configuration window, select a configuration in the list, and click Export.



2 Verify the file name and click Save.

By default, exported configurations are saved in D:\BDExport\Instrument.

Importing Configurations

⚠ Verify that the imported configuration has the same number of lasers and parameters as the base configuration.

1 Transfer the saved cytometer configuration file to the secondary workstation.

2 Log into BD FACSDiva software as Administrator and choose Cytometer > Cytometer Configuration.

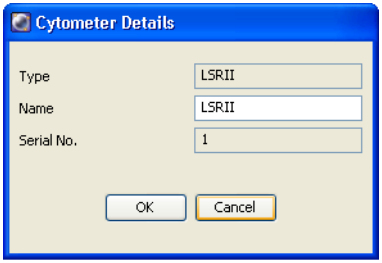
3 In the Cytometer Configuration window, right-click the folder you want to import the configuration into, and choose Import Configuration.

4 Navigate to and select your saved file and click Import.

5 Click Set Configuration to make the imported configuration the Current Configuration.

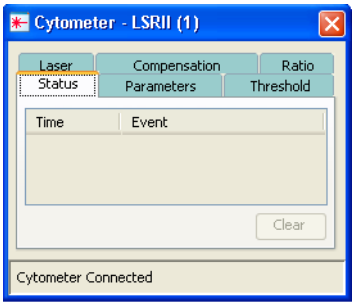
Cytometer Details

During software installation, each cytometer is assigned a name and serial number. This information is saved in FCS files and might also be needed during troubleshooting. To view the cytometer details and serial number, choose Cytometer > Cytometer Details. The Administrator can modify the Name field by clicking in the field and entering a new name.

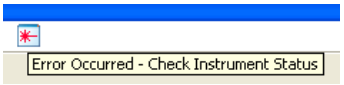


Status Messages

The Status tab of the Cytometer window lists status messages specific to your cytometer such as communication or fluidics errors. Messages are listed next to the time the event occurred. To view the whole message, resize the Cytometer window.



If the Status tab is hidden by another tab, it turns red to alert you when a message is sent from the cytometer. If the Status tab or Cytometer window is hidden when a message is sent, the window icon appears at the top of the workspace with a message alerting you to check the Status tab:



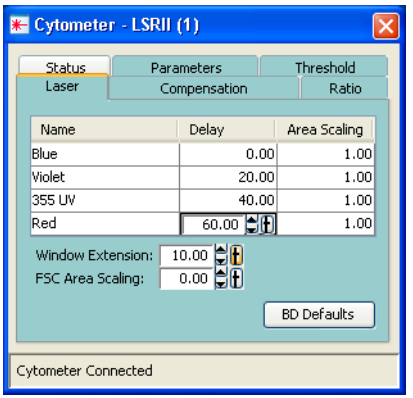
To resolve cytometer errors, refer to the Troubleshooting section in your cytometer user's guide. If the message persists, contact technical support for assistance. Provide the exact wording of the status message when you call.

Click the Clear button to clear the current status messages.

Laser Controls

Lasers are cytometer-specific; therefore, laser controls for your cytometer might be different from those shown in this section. If the following controls do not apply, consult your cytometer user's guide.

NOTICE Access to laser delay, area scaling, window extension, and FSC area scaling settings are set by your administrator. See Adding Users on page 31. To adjust a setting that is disabled, consult your administrator.



Values entered in the Laser tab apply globally to BD FACSDiva software—they are not saved with experiments or tubes. The values at startup are the last entered values.

☑ **Tip** Although delay and area scaling values are not saved with experiments or tubes, you can view the values used for a recorded tube by viewing tube information in the Inspector. See See Using the Tube Inspector on page 80.

- Name—Displays the laser name.

- Delay—Adjusts the amount of time between signals from different laser intercepts, from –200 to 200 µsec, to align signals from multiple lasers. Laser delay values are applied to all parameters detected from their respective lasers, as specified in the current cytometer configuration.

- Area Scaling—Adjusts area measurements to be the same magnitude as height measurements for signals from the corresponding laser. See the following section for more information.

- Window Extension—Extends the time in which area is measured by a value of 0–25 µsec. For more information, see See Using the Window Extension on page 125.

- FSC Area Scaling—Adjusts area measurements to be the same magnitude as height measurements for signals from the FSC detector. See the following section for more information.

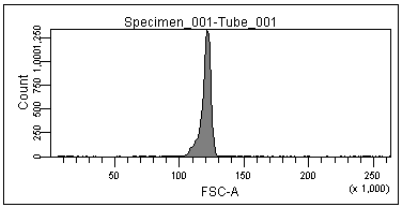
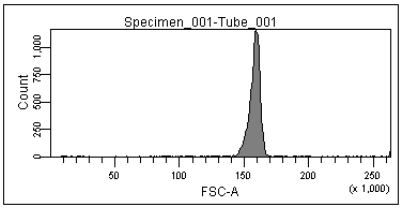
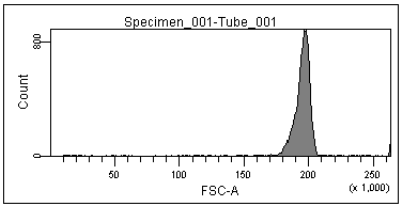
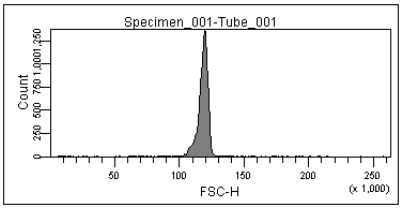
- BD Defaults—Click to restore all values in the Laser tab to their default settings as set by BD Field Service during installation or service.

Using Area Scaling

To ensure that detectors are working within a linear dynamic range, it is important to adjust height and area measurements to the same magnitude. The relationship between area and height is affected by sheath velocity, particle size, and the type of detector. For example, photodiode-generated pulses can be different from those generated by PMTs.

While height measurements can be adjusted with voltages, area measurements can be changed by applying a scaling factor. To determine whether an adjustment is needed, area signal is usually compared to height signal for one parameter from each laser, as well as for FSC. (When FSC is detected using a photodiode, FSC area scaling might need a different area scaling factor than that applied to the other parameters for that laser.)

By default, area scaling is set to 1. Notice how different scaling values affect the data display in the following plots. In this example, an area scaling value of 0.75 best matches the magnitude of the height (fourth plot).

Area Scaling	Sample Plot
0.75	
1.00	
1.25	
N/A	

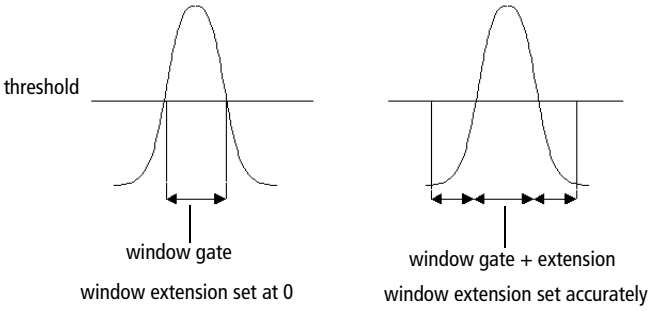
Refer to your cytometer manual for specific examples on when to adjust area scaling, if applicable. Note that area scaling does not change the height measurements in any way, nor does it affect the threshold.

Using the Window Extension

A sample pulse is the electronic representation of the amount of signal received at the detector from a single cell. The *window gate* is the amount of time during which the pulse is sampled. Depending on where the threshold is set, you can miss signals at the beginning and end of the pulse, especially if you have to raise the threshold to exclude debris.

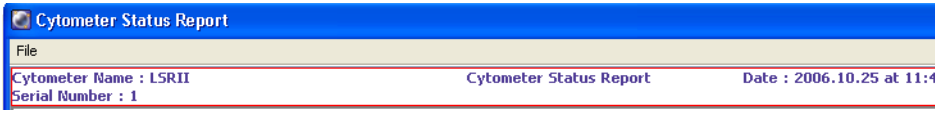
The *window extension* extends the detection time to allow a more complete recording of the pulse. When you increase the window extension (up to 25 µsec), half of the setting is applied to each side of the pulse so the entire pulse area is inside the gate (Figure 3-4). Note that if the window extension is too wide, more noise is included and CVs increase. If the window is too narrow, pulses might be measured incompletely.

Figure 3-4 Setting a window extension



Cytometer Status Report

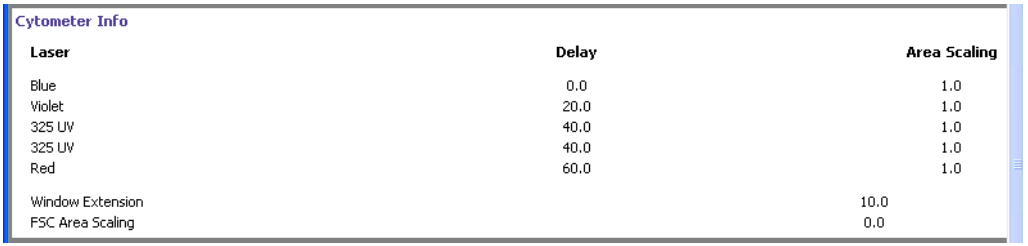
The cytometer status report provides a list of all cytometer settings at the time the report was created. You must be connected to the cytometer to create the report. In an open experiment, set the current tube pointer, then choose Cytometer > Cytometer Status Report. The report is displayed in a separate window with a menu bar above the report header. The header lists the cytometer name, type, serial number, and the date and time the report was prepared.



Five types of information are displayed on the report:

- User access privileges
- Cytometer information
- Cytometer settings
- Parameter labels
- Sorting settings (for cytometers equipped with sorting features)
- The User Access Privileges section lists access settings for the current user.

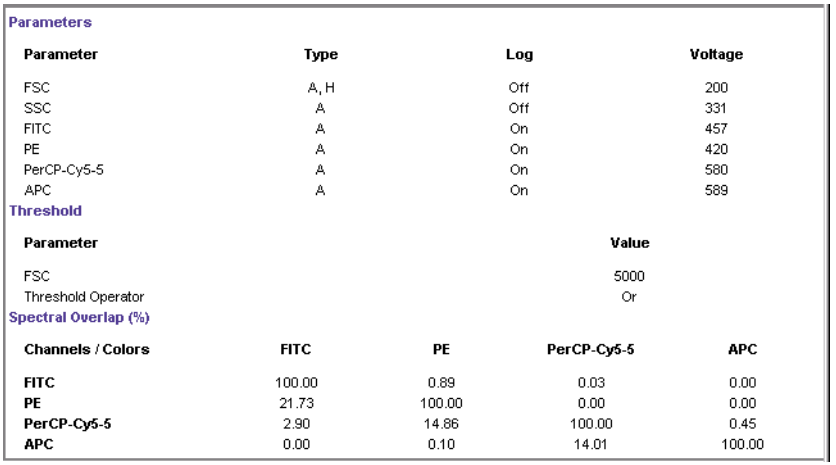
- The Cytometer Info section lists values for laser delay, area scaling, window extension, and FSC area scaling. (These values are described in Laser Controls on page 122.) Some cytometers will show additional information; refer to your cytometer manual for details.



Cytometer Info

Laser	Delay	Area Scaling
Blue	0.0	1.0
Violet	20.0	1.0
325 UV	40.0	1.0
325 UV	40.0	1.0
Red	60.0	1.0
Window Extension		10.0
FSC Area Scaling		0.0

- The cytometer settings section displays settings for the current acquisition tube. All parameters collected for the tube are listed, along with voltage settings and whether Log is on or off. Threshold and ratio settings are shown only if values have been entered in cytometer settings. Compensation is displayed in a table of spectral overlap values.



Parameters

Parameter	Type	Log	Voltage
FSC	A, H	Off	200
SSC	A	Off	331
FITC	A	On	457
PE	A	On	420
PerCP-Cy5-5	A	On	580
APC	A	On	589

Threshold

Parameter	Value
FSC	5000
Threshold Operator	Or

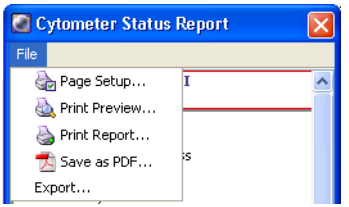
Spectral Overlap (%)

Channels / Colors	FITC	PE	PerCP-Cy5-5	APC
FITC	100.00	0.89	0.03	0.00
PE	21.73	100.00	0.00	0.00
PerCP-Cy5-5	2.90	14.86	100.00	0.45
APC	0.00	0.10	14.01	100.00

- The Sorting Settings section contains a list of all sort settings. Refer to your cytometer manual for more information.

Printing or Exporting the Report

- To print the report, choose File > Print Report from the Cytometer Status Report window. Preview the printed report or set up the page for printing by choosing the corresponding selections from the File menu.



- To export the report, choose File > Export within the Cytometer Status Report window. A dialog appears where you can specify the file storage location and choose the export format.

  A comma-separated values (CSV) file is exported and can be opened with a spreadsheet application such as Microsoft Excel.

Standby and Connect

For acquisition workstations (computer connected to a cytometer), use the Cytometer > Standby command to interrupt communication between the software and the cytometer. The menu command is disabled during acquisition, recording, or fluidics procedures (for cytometers with software-controlled fluidics modes). For information about working offline, see Working Offline on page 250.

NOTICE If your cytometer has software-controlled fluidics (eg, BD FACSCanto, BD FACSAria), you cannot operate the fluidics when the cytometer is in Standby.

When in Standby, choose Cytometer > Connect to re-establish communication.

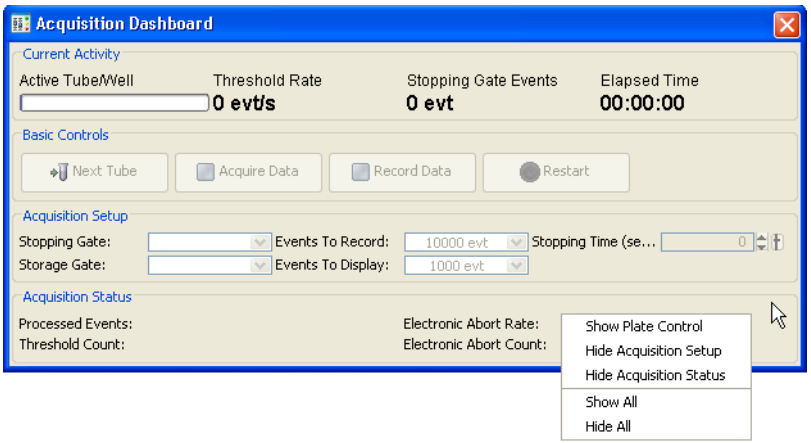
Acquisition Dashboard

The Acquisition Dashboard contains controls for setting up, starting, and monitoring data acquisition and recording. See Figure 3-5 on page 129.

To display the Acquisition Dashboard, click the corresponding button on the Workspace toolbar ( ). Controls are displayed only when the workstation is connected to the cytometer. The Acquisition Dashboard can be resized by dragging its border. After resizing the dashboard, the size remains the same from one login session to another.

NOTICE Your Dashboard might contain more controls than those shown depending on the options installed on your cytometer. Refer to your cytometer manual for details.

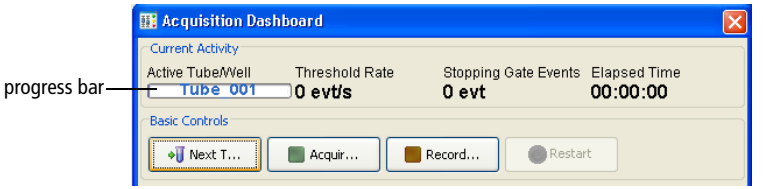
Figure 3-5 Acquisition Dashboard



To show or hide the optional sections in the Acquisition Dashboard, right-click in the Acquisition Dashboard in any blank area (except for Basic Controls). A menu is displayed where you can choose to show or hide different sections of the dashboard.

Current Activity and Basic Controls

The top of the Acquisition Dashboard displays activity for the current acquisition tube (indicated by the current tube pointer), along with acquisition controls. (When you are acquiring from a plate, this area shows current well activity.) A progress bar appears behind the Active Tube name when data is being recorded.



During acquisition or recording, the following are indicated:

- Active Tube/Well name
- Threshold Rate—Events per second for events that trigger the system threshold
- Stopping Gate Events—Number of recorded events within the stopping gate
- Elapsed Time—Amount of time passed since the Acquire, Record, or Restart button was clicked

Acquisition controls are as follows. If your Dashboard contains additional controls, refer to your cytometer manual for information.

- Next Tube—Sets the current tube pointer to the next tube in the Browser. If no tube exists, clicking Next Tube creates a new tube by duplicating the previous tube without data.

  If the *Tube-specific worksheet* preference is enabled (see General Preferences on page 96), clicking Next Tube automatically places analysis objects for the new tube onto a new blank normal worksheet.

  If the *Start acquisition on pointer change* preference is enabled, acquisition starts automatically when you click Next Tube.

- Acquire Data—Starts acquisition for the Active Tube/Well. Events are displayed in plots but data is not saved to the database. Statistics are displayed in statistics views and the values are updated in accordance with the Events to Display setting.

☑ **Tip** You can also use the current tube pointer in the Browser to control acquisition and recording. See Current Tube Pointer on page 135.

When acquisition is in progress, click Stop Acquiring to stop acquisition.

- Record Data—Starts recording data for the Active Tube/Well. The acquisition timer and all counters reset to zero when this button is clicked (except during a sort). Events are recorded until the requested number of Events to Record has been saved or the Stopping Time (if entered) has elapsed, whichever comes first. The resulting data is saved in the database.

☑ **Tip** Use the Stopping Gate Events counter to view the number of events saved to the data file as you record data.

When recording is in progress, click Stop Recording to stop recording data before you reach the specified number of events. If you click Stop Acquiring Data while data is being recorded, a confirmation dialog appears where you can choose to stop or continue recording.

NOTICE If you click Record Data for a tube that already has data, you can choose to Append (add the data in Events to Record to the original file), Overwrite, or Cancel. Data is appended only if the current cytometer settings are identical to the settings saved with the recorded tube. If the settings were changed, you can only overwrite or cancel.

- Restart—Clears data from plots, resets the timer and counters to zero (except during a sort), and restarts statistics. You can use Restart during acquisition or recording.

⚠ Clicking Restart during recording overwrites all previously recorded data.

Acquisition Setup

Use the Acquisition Setup fields to control the number of events to record and display.

- Storage Gate—Population for which events are to be recorded (saved)
- Stopping Gate—Population for which events are to be counted
- Events to Record—Number of events to be recorded for the current tube

  Recording stops when the Stopping Gate Events counter reaches the entered value. If a Stopping Gate other than All Events is specified, recording stops when the number of events within that gate reaches the entered value. If a Stopping Time is entered, recording stops after the entered number of events has been saved or the specified time has elapsed, whichever comes first.

  NOTICE The number of Events to Record that is selected last in the Acquisition Dashboard remains in place for that experiment from one login session to another.

☑ **Tip** Use Experiment Layout to set Events to Record for multiple tubes in an experiment. Also use Experiment Layout to set Storage Gate, Stopping Gate, Stopping Time, and Global Worksheet. See Using Experiment Layout on page 67.

☑ **Tip** You can also set Events to Record, Storage Gate, and Stopping Gate in the Acquisition tab of the Tube Inspector. See Using the Tube Inspector on page 80.

- Events to Display—Determines the number of events shown in plots during acquisition. Enter any value from 10–100,000 events. For example, if you enter 1,000, only the most recently acquired 1,000 events will be shown. Entering a lower number allows the display to update more quickly.

NOTICE During acquisition or recording, statistics are calculated only on the number of currently displayed events. Statistics are updated as the display changes. For this reason, responsiveness can decline as the software calculates more statistics on a greater number of displayed events. When recording is complete and acquisition is stopped, statistics are calculated on the total number of recorded events.

☑ **Tip** You have the option to not load data into plots when recording is finished. To choose this option, select Edit > User Preferences and deselect the *Load data after recording* checkbox in the General tab. If you want to load data for a tube, set the current tube pointer to load the data. The background of the tube pointer changes from black to gray, indicating that the data is loading.

- Stopping Time (sec)—Amount of time to record data

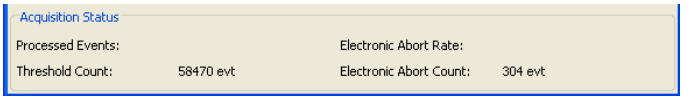
  Do one of the following to change any value:

  - Select the value in the field and enter a new value.
  - Click the pointer in the slider bar and drag it to a new value.
  - Use the mouse to click the up and down arrows or press the arrow keys on your keyboard to increase or decrease the values in small increments.
  - Hold down the Ctrl key while clicking the arrows or pressing the keys to increase stepped values in increments of 10.

  When a stopping time is entered, BD FACSDiva software will stop recording data after the specified number of events has been saved or the specified time has elapsed, whichever comes first. BD FACSDiva software saves the last stopping time that was entered when you log off and retains that setting when the software is restarted.

Acquisition Status

Use Acquisition Status fields for ongoing status during acquisition or recording. Show or hide Acquisition Status information by right-clicking in the Acquisition Dashboard (in any blank area except for Basic Controls). A menu is displayed where you can choose to show or hide different sections of the dashboard. These fields cannot be edited.



- Processed Events—Cumulative count of events processed by the software for the current tube

  Statistics are calculated only on processed events.

  Because processed events and electronic aborts are measured in different system locations, discrepancies between these counters can be observed during and after acquisition.

- Threshold Count—Cumulative count of all events that trigger the system threshold (ie, events that pass through the laser beam)

- Electronic Abort Rate—Aborted events/second, usually zero at recommended event rates or when the window extension is zero

- Electronic Abort Count—Cumulative count of events that are not processed by the system, including events that arrive too close together to be processed individually and events that arrive too fast for the system to process

Current Tube Pointer

When the workstation is connected to the cytometer and an experiment is open, a gray pointer icon is displayed next to tubes in the Browser. To activate acquisition controls, click the icon next to the tube you want to acquire to set the *current tube pointer*. The icon turns green and the tube becomes the Active Tube in the Acquisition Dashboard. You can also use a current tube pointer to activate acquisition and recording.

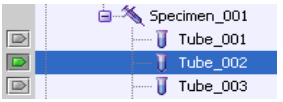
NOTICE Verify that the current tube pointer is set to the appropriate tube before you record data.

- To start acquisition, click the green pointer. The pointer changes to yellow, indicating that acquisition is in progress. Click the pointer again to stop acquiring.

☑ **Tip** In User Preferences, you can specify that acquisition will begin automatically every time the current tube pointer is set to a new tube. See General Preferences on page 96 for more information.

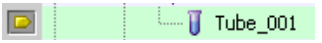
- To start recording data, hold down the Alt key while clicking the pointer. The pointer changes to orange, indicating that recording is in progress. While recording, Alt-click the pointer to switch from recording to acquisition; click the pointer without holding down the Alt key to stop acquisition and recording.

The following examples show how the pointer appearance changes depending on the acquisition status and the visibility of Browser elements.



A green pointer indicates the current acquisition tube (Tube_002 in this example). Events will be acquired for this tube when the pointer is clicked. Set the pointer to any other tube within the open experiment by clicking its associated gray pointer (such as next to Tube_003).

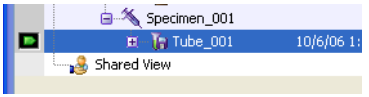
A blue pointer indicates that the current acquisition tube is hidden within a collapsed specimen or experiment. Expand the specimen to see the current acquisition tube.



A yellow pointer indicates that the tube is currently acquiring data.

An orange pointer indicates that the tube is currently recording events.

After events have been recorded, the pointer reverts to green and a disk icon is added to the tube. Cytometer settings in effect at the time the tube was recorded are saved with the tube.



A green tube pointer with black background is displayed if the *Load data after recording* checkbox is deselected in User Preferences. Setting the current tube pointer changes the background to gray and loads the data.

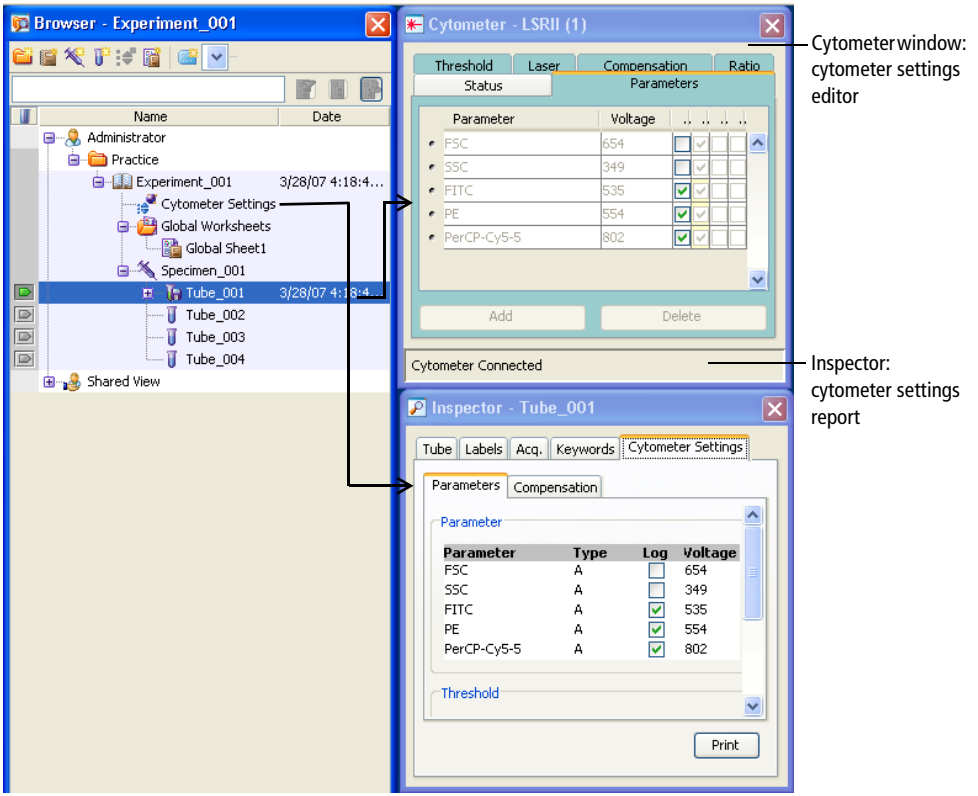
The pointer does not automatically advance to the next tube after data has been recorded. To record the next tube, click the gray pointer for the subsequent tube in the Browser, or click Next Tube in the Acquisition Dashboard.

Cytometer Settings

Cytometer settings represent the collection of values for parameters measured, PMT voltages, threshold, compensation, and any ratio measurement collected. Cytometer settings can apply to tubes, specimens, or experiments.

During offline use, cytometer settings for tubes are edited in the Inspector. When you are connected to the cytometer, you can change voltages, thresholds, and ratios for tubes only in the Cytometer window; the Inspector shows a report of the settings for a selected tube. You can use this feature to compare settings for the current tube with those from another tube or experiment (Figure 3-6).

Figure 3-6 Viewing cytometer settings



See the following sections for information about cytometer settings:

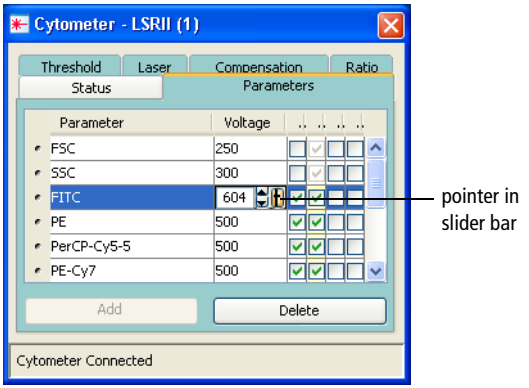
- Adjusting Cytometer Settings on page 138
- Creating Specimen- or Tube-Specific Settings on page 146
- Using Global Cytometer Settings on page 147
- Printing Cytometer Settings on page 149
- Exporting Cytometer Settings on page 150

Adjusting Cytometer Settings

To edit cytometer settings during acquisition, set the current tube pointer to a tube in an open experiment. Once the pointer has been set, cytometer settings tabs are shown in the Cytometer window. Use controls in each tab to edit or adjust cytometer settings.

To adjust a setting, select the field containing the value you want to change. Software controls, consisting of up and down arrows and a slider bar, appear next to the value as shown in Figure 3-7.

Figure 3-7 Software controls for adjusting cytometer settings



Do one of the following to change any value:

- Select the value in the field and enter a new value.
- Click the pointer in the slider bar and drag it to a new value.
- Use the mouse to click the up and down arrows or press the arrow keys on your keyboard to increase or decrease the values in small increments.
- Hold down the Ctrl key while clicking the arrows or pressing the keys to increase stepped values in increments of 10.

Using the Parameters Tab

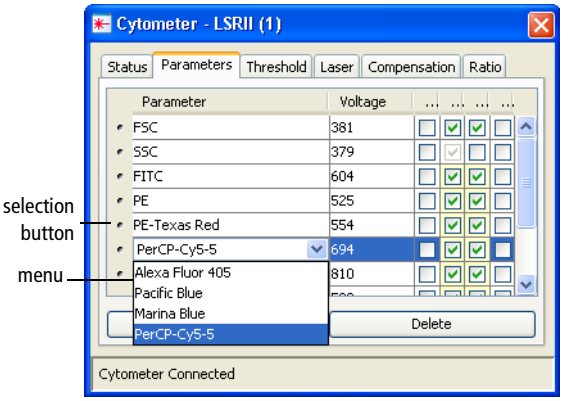
Each parameter is the output of a single PMT or photodiode, measuring fluorescent or scattered light. During acquisition, parameter data is sent from the cytometer to the workstation. By default, data is recorded for the parameters listed in the current cytometer configuration (see Cytometer Controls on page 106).

Use the Parameters tab to specify which parameter data should be sent and stored, to apply PMT amplification (or electronic gain for FSC), and to convert the parameter display to log.

- Add new parameters by clicking the Add button.

  Parameters are listed in the order they are defined in the Cytometer Configuration dialog. When more than one fluorophore is defined for a given channel, the first listed fluorophore is added by default.

- Change to an alternate fluorophore for any parameter by clicking the parameter field and choosing a different fluorophore from the menu that appears (see the following figure).



- Delete parameters by clicking the selection button next to the row to delete, and then clicking the Delete button.

☑ **Tip** To save space in the database, delete parameters that are not applicable for the corresponding tube.

☑ **Tip** Select multiple contiguous rows by holding down the Shift key as you click; select multiple non-contiguous rows by holding down the Ctrl key as you click. Click the Delete button to delete all selected rows.

- Measure signal height or width along with area by selecting the appropriate checkboxes. To measure height only, select Height and then deselect Area. (Either Area or Height must be selected for all listed parameters.) When Area or Height is selected, it will be measured for all fluorescent parameters.

  For more information about parameter measurements, see Parameter Values on page 301.

- Adjust the signal for events displayed in plots by changing PMT voltages (electronic gain for FSC). Higher voltages increase detector sensitivity, resulting in increased signal; lower voltages decrease detector sensitivity, resulting in decreased signal.

  Voltages can be adjusted from 0–1,000 V. To use the controls, see Adjusting Cytometer Settings on page 138.

- For any listed parameter, select the Log checkbox to convert the parameter display to a log scale. Log data can be displayed over four- or five-log decades by selecting the appropriate option in the Experiment Inspector. See Using the Experiment Inspector on page 59.

☑ **Tip** Select multiple rows before clicking the checkbox to turn log on or off for multiple parameters at once.

Considerations When Using the Log Display

All data originating from the digital electronics is linear data from 0–262,143 ($2^{18} - 1$). BD FACSDiva software does not use log values. It uses linear values that can be displayed on a linear, log, or biexponential scale. Changing the data display does not affect statistics because statistics are always calculated on linear data.

Linear plots have tickmarks on 0, 10,000, 20,000, and so on. Logarithmic plots show a range of 26–262,143 (four-log decades) or 2.6–262,143 (five-log decades). In order to display all height measurements on a similar scale, BD FACSDiva software multiplies height values by 16. For more information, see Parameter Values on page 301.

You can alter the display before or after recording because data is always measured and stored in linear. If you change from linear to log or biexponential during analysis, the data will be re-displayed.

Using the Time Parameter

The Time parameter can be used to show how events change over time. In calcium flux experiments, the Time parameter is used to display the rate at which the cells in the sample respond to a stimulus.

The Time parameter is displayed on a fixed scale of 0–262,143, where each tick represents 10 ms. Thus, an event that appears at position 50,000 on the Time scale is equal to 8 min 20 sec; an event that appears at 60,000 is equal to 10 minutes. A plot can display up to 43 minutes of Time data.

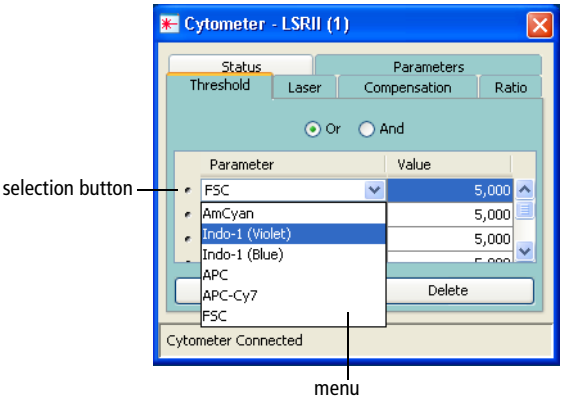
When you append data to a recorded tube, time is added to the existing data set. Therefore, after appending 5 minutes of data to a 10-minute data set, the Time parameter of the last event would appear at 90,000.

NOTICE If a plot displaying the Time parameter is hidden during acquisition or recording, no data will be shown for the time in which it was hidden.

Using the Threshold Tab

Use the Threshold tab to specify a boundary below which data will not be acquired. Threshold data consists of uncompensated linear signal height. Threshold values can be adjusted from 200 to approximately 262,143.

- Add a Threshold parameter by clicking the Add button.
- Change a listed parameter by clicking the parameter name and choosing a different item from the menu that appears.



- Delete a Threshold parameter by clicking the selection button next to the row to delete, then clicking the Delete button.

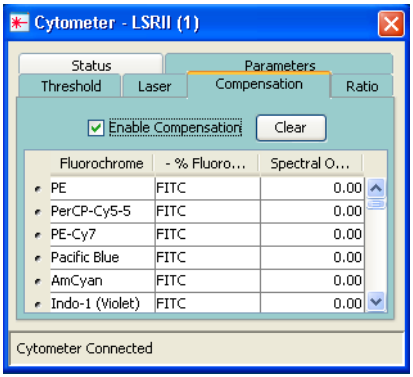
When more than one parameter is listed, use *Or/And* to define combined threshold values.

- *Or* Threshold—Signals must be equal to or greater than any one of the listed threshold values to be displayed and counted.

- *And* Threshold—Signals must be equal to or greater than all listed threshold values to be displayed and counted.

Using the Compensation Tab

The Compensation tab displays Spectral Overlap values for all parameter combinations in the experiment. For general information about compensation, see Controls for Compensation Correction on page 151.



Adjust compensation in BD FACSDiva software either automatically using the Compensation Setup feature, or manually. When compensation is calculated using Compensation Setup (see Using Compensation Setup on page 152), you should not need to adjust the values after calculation.

If you are adjusting compensation manually (see Calculating Compensation Manually on page 168), click in the Spectral Overlap field to access controls to adjust the values, or click to select the value in the field and enter a new value. To clear one or more values, select one or more rows and click Clear.

Compensation values range from 0–1,000%; the slider control displays increments of 100. Adjustments can be made during acquisition or on previously recorded data. View or record uncompensated data by deselecting the Enable Compensation checkbox.

Copying Spectral Overlap Values

If you are performing compensation manually, you can copy spectral overlap values from one set of cytometer settings to another.

NOTICE You cannot copy spectral overlap values to label-specific compensation values.

1. Right-click the tube or Cytometer Settings icon containing the values you want to copy and choose Copy Spectral Overlap.

   This command copies only the Spectral Overlap values from the current cytometer settings.

2. Select the Browser item(s) for which you want to update the Spectral Overlap values, right-click the selected items, and choose one of the Paste options.

   - Paste Spectral Overlap—Updates existing values with the values from the copied settings without overwriting non-zero values in the target settings with zeros from the source. This is useful when you are combining compensation settings from multiple tubes for a complete compensation matrix.

   - Paste Spectral Overlap with Zeros—Overwrites all existing values with the values from the copied settings. This is useful when you are copying compensation values to a tube that already has compensation values.

   For example, note how the two paste options affect the data for the following compensation tubes.

	Source Tube	Destination Tube	Result after	
			Paste	Paste with Zeros
FITC-% PerCP	7	5	7	7
PerCP-% FITC	0	2	2	0

NOTICE If the target cytometer settings use a set of parameters or PMT voltages that are different from the pasted object, a warning message is displayed. Only compensation values that use a matching set of parameters can be pasted.

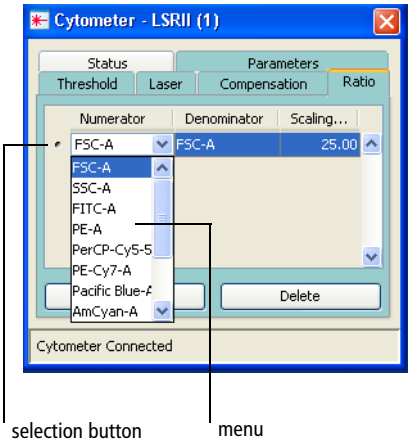
When a parameter exists in the target, but not in the source, the values for that parameter will not be changed. Conversely, if a parameter exists in the source but not the target, the values for that parameter are not added to the target.

Using the Ratio Tab

Ratios are most commonly used for calcium flux experiments. Ratios are calculated by dividing the signal from one fluorescence detector by the signal from another fluorescence detector and then multiplying by a percentage of the total resolution, which is 262,143 (eg, 25% of 262,143 = 64,000).

Ratios are calculated from uncompensated linear data and are always reported in linear. Ratios can be used for sorting just like any other parameter.

- Include up to 10 ratio calculations by clicking the Add button at the bottom of the Ratio tab.
- Specify the numerator and denominator by choosing parameters from the menus in each field. All parameters listed in the Parameters tab are available for ratio calculations.
- Adjust the ratio scaling factor by entering a value in the Scaling % column, from 0–200%.



- Delete a ratio calculation by clicking the selection button next to the row to delete, and clicking the Delete button.

NOTICE If a parameter used in a ratio calculation is subsequently removed from the Parameters tab, the ratio will be deleted.

Creating Specimen- or Tube-Specific Settings

Create specimen- or tube-specific settings when you need to collect data for a subset of your experiment using different settings than you are using in other parts of the experiment. For example, some tubes or specimens might use different scatter, fluorescent, or ratio parameters, or different measurement types or thresholds.

Do one of the following to add cytometer settings at the specimen or tube level:

- Select the tube or specimen in the Browser, then click the New Cytometer Settings button () on the Browser toolbar.

- Right-click the specimen or tube icon in the Browser and choose New Cytometer Settings.

  Alternatively, choose Import Cytometer Settings to import an existing settings file. See Importing Settings on page 151.

- Select the specimen or tube and choose Experiment > New Cytometer Settings.

- Copy cytometer settings from another Browser element (experiment, specimen, or tube) and paste them to the target tube or specimen.

When you create new settings, initial values are copied from the closest parent settings. Further adjustments apply to the experiment-level settings only when the *Use global Cytometer settings* preference is enabled (selected by default).

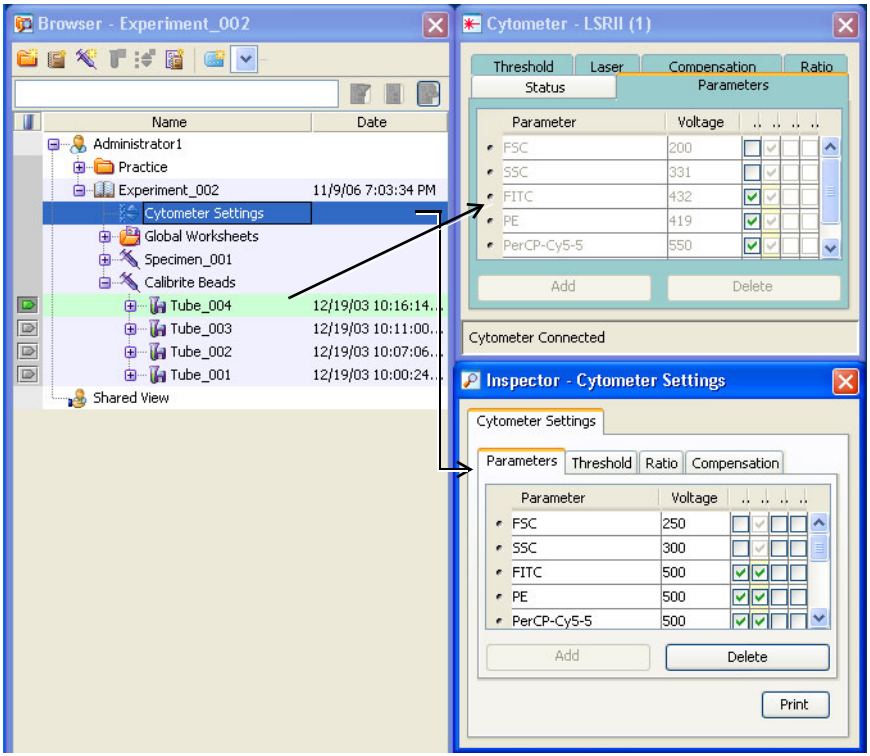
If you want to create specimens or tubes with varying settings that do not update with the latest settings changes in the experiment, deselect the preference. For more information, see Using Global Cytometer Settings below.

Using Global Cytometer Settings

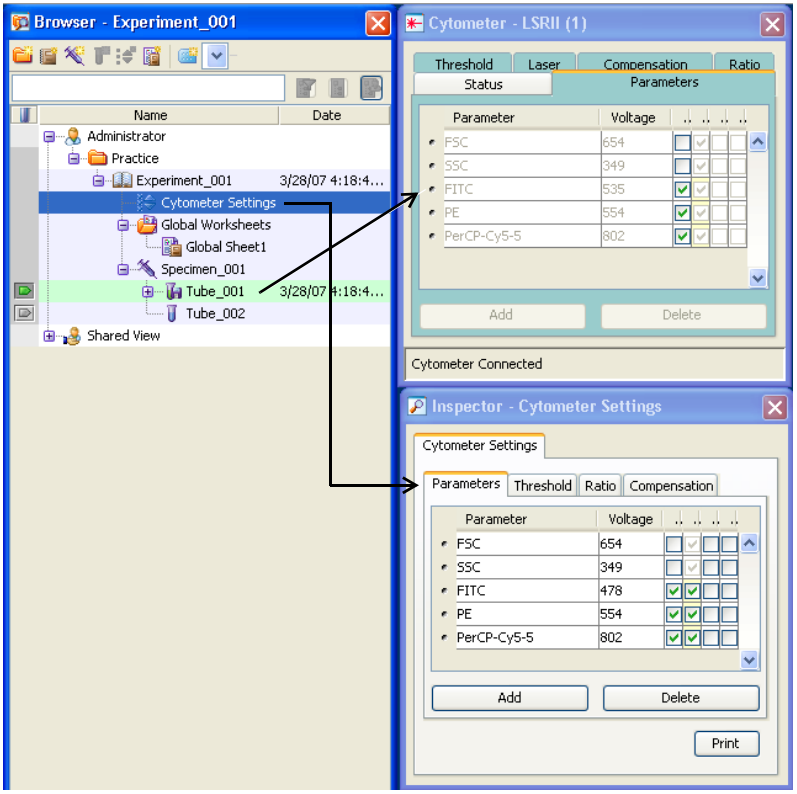
The *Use global cytometer settings* option is available in the Experiment Inspector. When this preference is enabled, experiment-level cytometer settings are automatically updated to reflect changes to tube- or specimen-specific settings, and subsequent tubes are automatically updated to use the latest experiment-level settings.

NOTICE If tube- or specimen-specific settings are linked to a setup (see Applying a Compensation Setup to Cytometer Settings on page 164), experiment-level settings are not updated automatically, even when this preference is enabled.

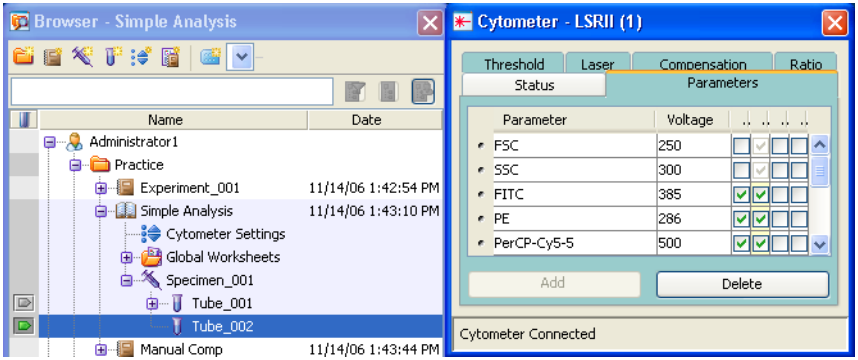
For example, if you disable the *Use global cytometer settings* option and make changes to tube-specific settings (Cytometer window), notice that the global settings (in the Inspector) do not change.



If you make the same changes to tube-specific settings with the global settings option enabled, the changes are automatically applied to the experiment-level settings.

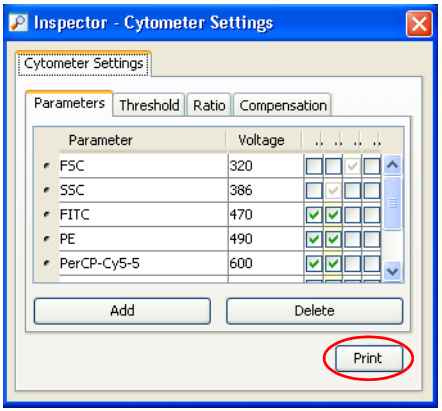


Additionally, updated settings are automatically applied to the remaining unrecorded tubes in the experiment, even when they have tube-specific settings. Updated settings are applied when you set the current tube pointer to a different tube.



Printing Cytometer Settings

To print cytometer settings for the current tube, select cytometer settings in the Browser and click the Print button in the Inspector, or right-click the Cytometer Settings icon and choose Print from the shortcut menu.



A printout will be generated that includes:

- name of experiment, specimen, and tube
- the date data was recorded
- list of parameters collected, voltage values, and whether log is on or off
- threshold parameter(s) and value(s)
- compensation state (enabled/disabled) and values
- ratio parameters and scaling

A more detailed, formatted report of cytometer settings can be printed, viewed, or exported using the Cytometer Status Report option on the Cytometer menu. See Cytometer Status Report on page 126 for more information.

Exporting Cytometer Settings

You can export cytometer settings as a CSV file stored outside the BD FACSDiva database, and import settings for use in another experiment.

When stored outside the BD FACSDiva database, settings are not modified by the *Use global cytometer settings* feature or by updates to the setup catalog. (See Using Global Cytometer Settings on page 147 or Using Compensation Setups on page 163.) Also, you can manually edit compensation and voltage settings while keeping a copy of your original settings outside the Browser.

NOTICE You can export global cytometer settings that contain label-specific compensation values. However, the exported file will only contain the generic compensation values for that fluorochrome, not the label-specific compensation values in the compensation matrix.

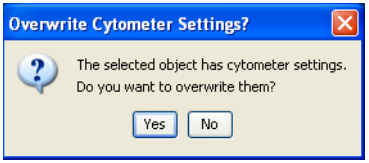
1. Right-click the Cytometer Settings icon in the Browser and choose Export (to the Instrument folder).
2. Enter a file name and click Export.

   By default, exported settings are stored in D:\BDExport\Instrument.

Importing Settings

1. Right-click an open experiment, specimen, or tube icon in the Browser and choose Import Cytometer Settings.

2. Click Yes to overwrite the current settings.

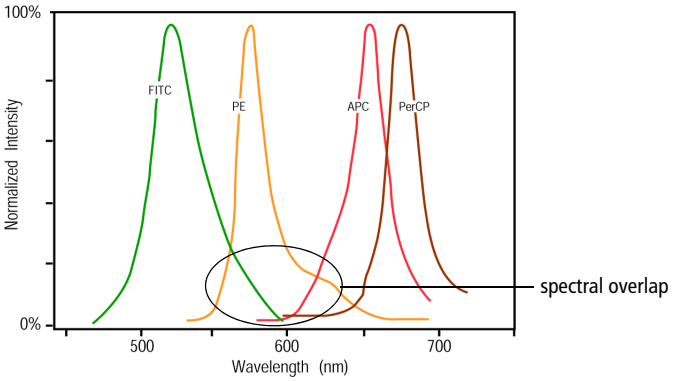


3. Select the settings file you want to import and click Import.

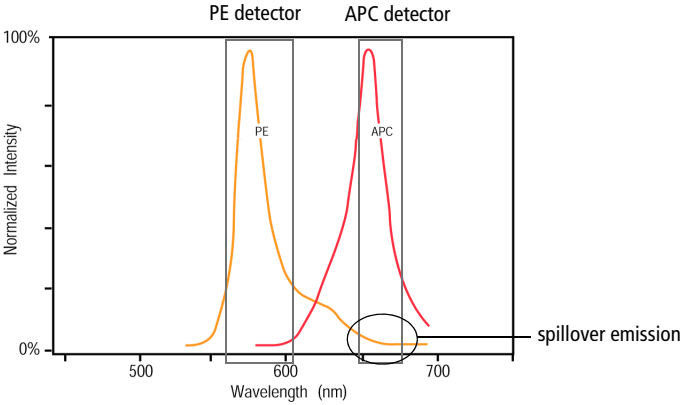
   The selected settings are added to the Browser.

Controls for Compensation Correction

Different fluorochromes can emit light over a common range of wavelengths. In the following example, FITC, PE, and APC all emit fluorescence in the 550–625 nm range.



When the emission of one fluorochrome is detected by a detector designated for another fluorochrome, it is impossible to separate the two signals optically. The following example illustrates PE spillover into the APC detector.



Compensation is the correction applied to the raw data to remove the effects of this spillover emission (ie, fluorescence). For example, when you are measuring APC fluorescence, compensation removes the PE fluorescence that is detected by the APC detector, or APC–%PE.

The following sections describe the compensation controls available in BD FACSDiva software.

Using Compensation Setup

As the number of fluorescence parameters in an experiment increases, compensation becomes increasingly difficult to set manually. For a six-color experiment, 30 spectral overlap values need to be adjusted, and for an eight-color experiment, 56 values need to be adjusted. The process of manually correcting spectral overlap values can take several hours and is very difficult to set accurately.

The Compensation Setup feature in BD FACSDiva software is designed to automatically calculate spectral overlap values for an experiment, saving time and eliminating the inaccuracies introduced with manual compensation.

Compensation Setup is designed to work with single-stained controls. These controls can consist of single-stained cells or capture beads. An unstained control is required as well, in a separate tube or in the same tube as the single-stained controls. Refer to your cytometer manual for specific examples.

Choose Experiment > Compensation Setup to access setup functions. For details on each function, see the following:

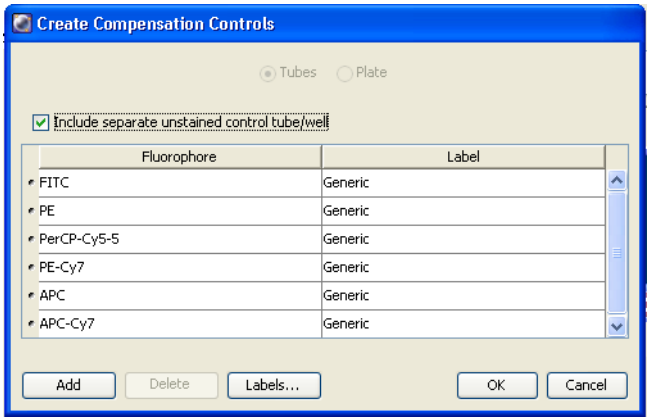
- Creating Compensation Controls on page 153
- Defining Label-Specific Controls on page 155
- Calculating Compensation on page 159
- Viewing the Compensation Setup Catalog on page 160

Creating Compensation Controls

Choose Experiment > Compensation Setup > Create Compensation Controls to automatically add compensation controls and analysis objects to your experiment. A dialog appears where you can add or delete controls, define label-specific controls, or change the order of the compensation controls (Figure 3-8 on page 154).

NOTICE In the Create Compensation Controls dialog, you can add only parameters that are listed in the Parameters tab. To change to another fluorophore for any parameter, edit cytometer settings in the Cytometer window or Inspector *before* you create compensation controls.

Figure 3-8 Creating compensation controls



- Leave the *Include separate unstained control tube/well* checkbox selected when you are running unstained sample as one of your compensation controls.

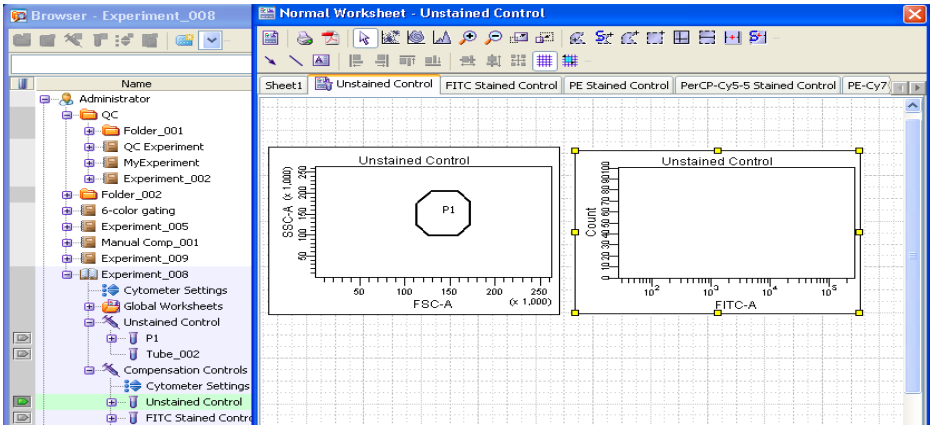
- Deselect the checkbox when you are including unstained sample in each of your stained control tubes or wells. In this case, you will need to gate the unstained population in each fluorescence histogram before you calculate compensation.

When you click OK, a new specimen named *Compensation Controls* is added to the open experiment in the Browser, with tubes for each specified parameter. A tube-specific worksheet is added for each specificity of a given fluorophore, along with an unstained control worksheet if the checkbox was selected (Figure 3-9 on page 155). The experiment's cytometer settings are copied to the controls, all compensation values are set to zero, and the Enable Compensation checkbox is deselected.

These tubes and plots are used to record data prior to calculating compensation. PMTs and laser settings can be set before the compensation tubes are created or any time before the first compensation tube is recorded.

Only one set of compensation tubes can be created per experiment. Compensation cannot be calculated if PMT voltage settings are changed while recording compensation tubes. All tubes must be recorded with consistent PMT voltages.

Figure 3-9 Compensation controls and worksheets



Defining Label-Specific Controls

Create label-specific controls if your experiment contains samples stained with the same fluorophores conjugated to different antibodies (labels) that require different compensation values. This is especially noticeable in tandem conjugates due to lot-to-lot variation. Label-specific controls can be defined during the creation of compensation controls (described in the previous section) or you can modify existing controls.

1 Choose Experiment > Compensation Setup > Create Compensation Controls or Modify Compensation Controls.

The Create (or Modify) Compensation Controls dialog appears (Figure 3-10).

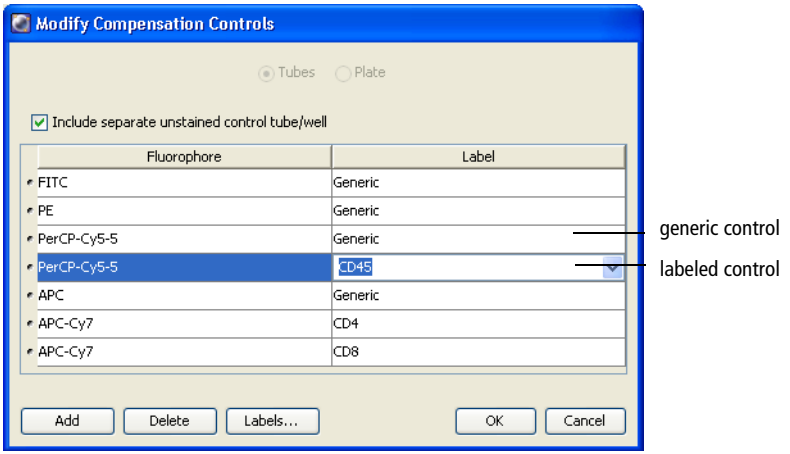
Some of the parameters may already contain labels. If a label has already been created in Experiment Layout or the Inspector, the software automatically assigns the label to the corresponding compensation control.

2 To add a generic control or a control with a different label, click Add.

- Choose the appropriate fluorophore from the menu.

- Use the selection button next to the fluorophore name to drag the new fluorophore to the required position in the list.
- Enter a label.

Figure 3-10 Keeping a generic control while adding label-specific controls



3 To edit a control, choose or enter a different label.

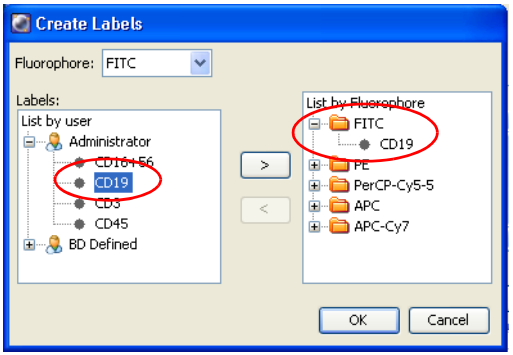
4 To delete a control, click the selection button next to the fluorophore and click Delete.

5 To define label-specific controls while creating or modifying compensation controls:

- Click the Labels button in the Create (or Modify) Compensation Controls window and the Labels dialog is displayed.

  BD FACSDiva software displays a list of default labels and any user-defined custom labels. Any label from this list can be added to or removed from compensation controls, whether used in an experiment or not. Use the Shift or Ctrl keys to select multiple labels at once.

- Choose the appropriate fluorophore from the Fluorophore menu or select it from the right pane.



- Choose the appropriate label either from the custom BD Defined labels list or from the labels you defined in Experiment Layout.

- Click the right arrow (>) to move the label into the appropriate fluorophore folder. To delete a label from a fluorophore folder, click the left arrow (<).

- Click OK. The Labels dialog closes and the newly-labeled fluorophore is displayed in the Create (or Modify) Compensation Controls window.

6 Click OK to add the controls to your experiment.

Label-specific controls and analysis objects are created automatically.

- If controls were added, the corresponding controls and worksheets are added to the open experiment and Worksheet window, respectively.

- If controls were edited, the corresponding controls and worksheets are renamed.

The labels appear in the Browser, the Labels tab of the Inspector, and on associated worksheets.

Adding Compensation Controls to an Existing Compensation Setup

Before or after you calculate compensation and create a compensation setup, you can add a new compensation control, record and gate the newly added control, and then recalculate compensation to create a new setup.

1. Verify that the parameter(s) you want to add to the Compensation Controls are listed on the Parameters tab in the Cytometer window for the experiment's cytometer settings.

   NOTICE In the Modify Compensation Controls dialog, you can add only parameters that are listed in the Parameters tab. To change to another fluorophore for any parameter, edit cytometer settings in the Cytometer window or Inspector *before* you modify compensation controls.

2. Choose Experiment > Compensation Setup > Modify Compensation Controls.

3. In the Modify Compensation Controls dialog, click Add.

4. Click the fluorophore that was added, and choose the appropriate parameter from the menu.

5. Repeat steps 3 and 4 for additional controls, if needed; click OK to dismiss the dialog.

6. Record data for the new controls.

7. Choose Experiment > Compensation Setup > Calculate Compensation.

8. Enter a compensation setup name, click Link and Save, and click OK.

   To apply compensation values to that experiment's cytometer settings, click Apply Only or Cancel to exit the dialog.

Editing Gates

After you create appropriate compensation controls, you need to verify gates before you calculate compensation. Gates in the software-defined analysis objects are snap-to gates, so minimal editing is required.

1. Record data for all controls.

2. On any plot, move the P1 gate to the required population.

   If the gate does not contain all required events, edit the gate or right-click it and choose Recalculate.

3. Right-click the P1 gate and choose Apply to All Compensation Controls.

   This applies the P1 gate changes to all worksheets at one time.

4. Verify that the P2 gate encompasses the positive population on each fluorescence histogram.

   If needed, edit the gate or right-click the gate and choose Recalculate.

5. If you *do not* have a separate unstained control, create an autointerval gate around the negative population in each fluorescence histogram.

   If you have an unstained control tube (or well), you can skip this step.

Calculating Compensation

After data has been recorded and gates have been adjusted, you are ready to calculate compensation. Choose Experiment > Compensation Setup > Calculate Compensation. The software calculates the overlap as the median fluorescence intensity (MFI) of the positive stained control minus the MFI of the negative stained control for each control in all channels. If there is a gated unstained population in the Unstained Control tube and a gated unstained population in the Stained Control tube, the population in the Stained Control tube will be used in the calculation.

- If the compensation calculation is not successful, an error message will be displayed. Click Cancel, make necessary adjustments, and then recalculate.

- If the compensation calculation is successful, a dialog appears prompting you for the name for the compensation setup. Enter a name, and click:

  - Link & Save—Links cytometer settings to the experiment and save the setup to the compensation setup catalog.

  - Apply Only—Applies the cytometer settings to the experiment without saving the settings to the compensation setup catalog.

  - Cancel—Dismisses the dialog without saving the setup.

☑ **Tip** Include the experiment name or date when saving to keep track of compensation setups.

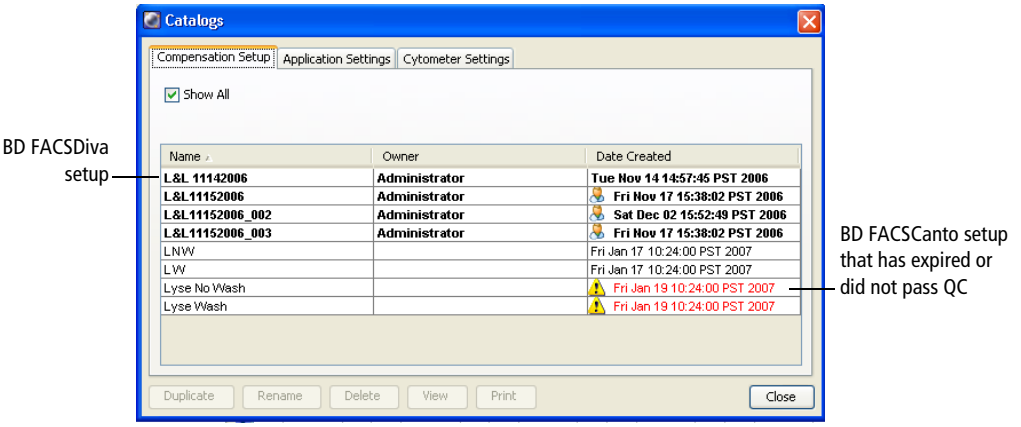
- Click OK.

Viewing the Compensation Setup Catalog

After a successful compensation calculation is named, all cytometer settings associated with the compensation calculation are saved as a compensation setup. A compensation setup contains parameter and label information, threshold and PMT voltages for each parameter, and calculated spectral overlap values in the form of an MFI table.

View a list of all saved compensation setups by choosing Cytometer > Catalogs and clicking the Compensation Setup tab. The tab lists all saved BD FACSDiva compensation setups, as well as compensation setups from BD FACSCanto clinical software (if applicable). BD FACSDiva compensation setups are always shown in bold. BD FACSCanto compensation setups that are >24 hours old or did not pass quality control checks are shown in red with a yellow triangle. See Figure 3-11 on page 161.

Figure 3-11 Compensation Setup catalog with BD FACSDiva and BD FACSCanto setups



If you have Administrator privileges, you can do the following:

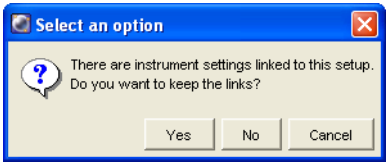
- To make a copy of any compensation setup, select the name and click Duplicate. Enter a new name, and click OK.

  If you duplicate, but do not rename, the compensation setup, the user icon ( ) appears in the Date Created column.

  The new compensation setup is always a BD FACSDiva setup, even if it was derived from a BD FACSCanto setup.

- To rename a BD FACSDiva compensation setup, select the name and click Rename (for setups that are linked but have no data). BD FACSCanto compensation setups cannot be renamed.

  If the selected compensation setup is linked to any cytometer settings in the Browser, a message appears where you can choose to keep or discard existing links.



- Click Yes to rename the compensation setup in all cases where it is linked (existing links are maintained).

- Click No to rename the compensation setup and remove existing links.

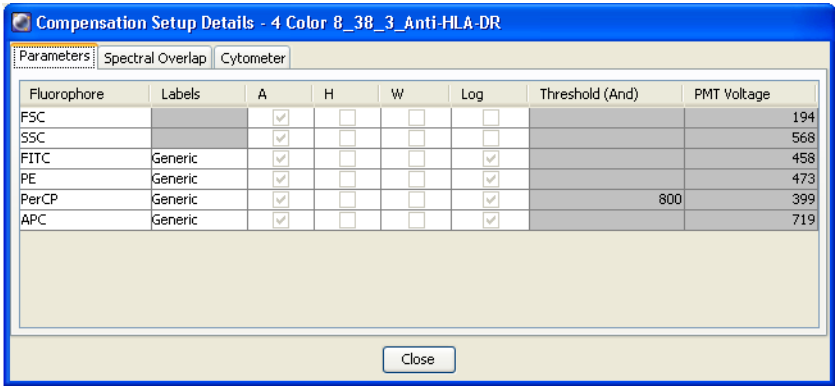
- Click Cancel to keep the existing name and links.

- To remove a BD FACSDiva compensation setup, select the name and click Delete. (BD FACSCanto compensation setups cannot be deleted.)

  If the selected compensation setup is linked to any cytometer settings in the Browser, a confirmation dialog appears informing you that the linked settings will become unlinked. Click OK to confirm, or Cancel to keep the setup and links.

- To edit a compensation setup, select the name and click Edit.

  A window appears listing the compensation setup's parameter settings and labels.



- Click the Spectral Overlap tab to edit calculated overlap values in the form of a table.

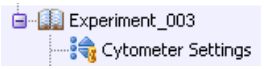
- Click the Cytometer tab to view the laser delay setting, window extension, and area scaling values in effect at the time the compensation setup was created. (These settings are *not* downloaded when you apply the setup.)

- Click Close to return to the Compensation Setup tab of the Catalogs dialog.

- To print a compensation setup, select the name and click Print.

☑ **Tip** Compensation setup information can also be printed or exported by printing or exporting a cytometer status report. See Printing or Exporting the Report on page 128.

Using Compensation Setups



After you calculate compensation and name the resulting setup, the experiment's cytometer settings are linked to the named setup. This is indicated by a chain-link icon in the Browser. After settings have been linked, you cannot edit spectral overlap values or change PMT voltages.

When the *Use global cytometer settings* preference is enabled for the experiment, the setup is applied to unrecorded tubes as the current tube pointer is set. To change voltages without triggering the error message, you can unlink from the setup. See Unlinking a Setup from Cytometer Settings on page 164.

NOTICE If tube- or specimen-specific settings are linked to a setup (see Applying a Compensation Setup to Cytometer Settings on page 164), experiment-level settings are not updated automatically, even when the global cytometer settings preference is enabled.

A setup can also be applied to cytometer settings in a new experiment, as described in Applying a Compensation Setup to Cytometer Settings on page 164. When you apply a setup, if the setup contains label-specific tubes and tubes in the experiment are not labeled, you are prompted to choose a compensation value, as described in Applying Label-Specific Compensation Settings to Tubes on page 166.

Unlinking a Setup from Cytometer Settings

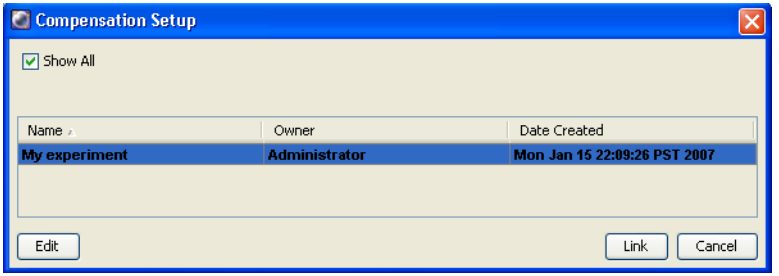
To unlink cytometer settings from a setup, right-click a Cytometer Settings icon and choose Unlink From *SetupName*; click OK to confirm.

Applying a Compensation Setup to Cytometer Settings

Saved compensation setup values (spectral overlap, threshold, and PMT voltages) can be applied to an experiment, specimen, or tube, and spectral overlap values in a compensation setup can be applied to a recorded tube.

- To apply a setup, right-click the Cytometer Settings icon in the Browser and choose Link Setup.
- To apply spectral overlap values, right-click a Cytometer Settings icon under a recorded tube and choose Apply Compensation.

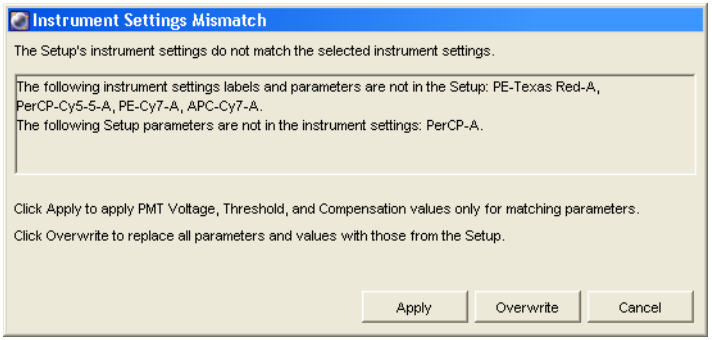
In either case, the compensation setup catalog appears where you select a setup to link:



- For BD FACSDiva setups, select the setup and click Edit to edit values associated with the compensation setup.
- For BD FACSCanto setups, select the setup and click View to view values associated with the compensation setup.

- Click Link to link compensation setup values to matching parameters in the cytometer settings. Only parameters that match those in the compensation setup are updated. Settings are then linked to the compensation setup, so if you add a parameter that matches one in the compensation setup, values for the matching parameter are updated automatically.

  If parameters do not match, a message appears:



  - Click Apply to apply PMT voltages, threshold, and spectral overlap values to cytometer settings parameters that match those in the setup. Only matching parameters are updated. Settings are then linked to the setup, so if you subsequently add a parameter that matches one in the setup, values are updated automatically.

  - Click Overwrite to replace the existing settings with settings from the setup. Parameters that did not match are removed.

NOTICE If the setup or cytometer settings contain label-specific controls, you will be prompted to choose which spectral overlap value to use. For more information, see Applying Label-Specific Compensation Settings to Tubes on page 166.

Once cytometer settings have been linked to a setup, you cannot edit compensation values manually. The compensation editor of the cytometer settings is locked, and the Clear button and Paste Spectral Overlap commands are unavailable.

Applying Label-Specific Compensation Settings to Tubes

If your experiment contains label-specific compensation controls, you will need to specify which spectral overlap value to use for each tube in the experiment by assigning appropriate labels. Label tubes in one of the following ways:

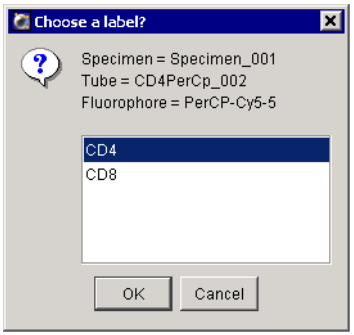
- Use Experiment Layout to label all tubes in the experiment at once.
- Use the Labels tab in the Inspector to label one tube at a time.

The software determines whether to use compensation values from generic or label-specific controls based on the following criteria.

Setup Control	Tube Parameters	Spectral Overlap Value Applied
Generic	Not labeled	Generic
Generic	Labeled	Generic
Generic and label-specific	Not labeled	Generic
Generic and label-specific	Labeled	Label-specific, if control label matches the parameter label. Otherwise, generic value is applied.
Label-specific	Not labeled	Label must be chosen in dialog that appears.
Label-specific	Labeled	Label-specific, if label of Control tube matches the parameter label. Otherwise, label must be chosen in dialog that appears.

If a compensation control is label-specific and you record a control that hasn't been assigned a label or whose label does not match the control, a dialog appears where you can choose which control to apply. See Figure 3-12 on page 167.

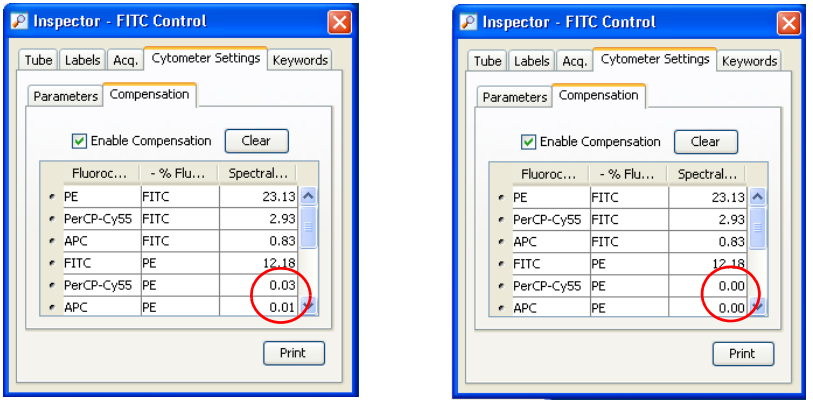
Figure 3-12 Choosing a control label



After you choose a control, spectral overlap values are applied using the value for the chosen label.

NOTICE If you click Cancel and dismiss the dialog without choosing a control, the software will not apply a complete compensation matrix, which will result in uncompensated channels, as shown in the following figure.

Figure 3-13 Complete (left) vs incomplete (right) compensation matrix using label-specific controls



Calculating Compensation Manually

The following tutorial describes how to determine compensation settings and manually construct a compensation matrix using the Copy/Paste Spectral Overlap commands. To determine settings, the means for each fluorescence-positive population are compared to the means for its negative population. Appropriate compensation networks are adjusted to align the means.

☑ **Tip** Compare medians for populations with many outlying events. Compare means when cells are clustered more closely together with few outlying events.

Note that compensation calculation is part of cytometer settings optimization that typically occurs after you have performed daily quality control and adjusted the voltages and threshold. Refer to your cytometer manual for instructions.

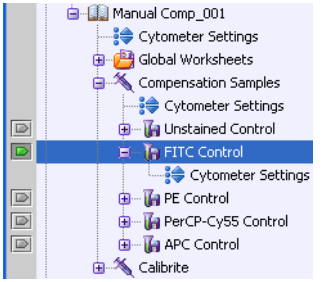
Adjusting FITC Compensation

This section describes how to determine spillover coefficients for the FITC fluorochrome. The same procedure is used for the remaining fluorochromes in the experiment.

1 Choose File > Import > Experiments, and choose Manual Comp.

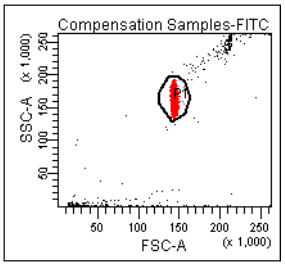
This experiment is installed in your D:\BDExport\Experiments folder during software installation.

2 Open the Manual Comp experiment and set the current tube pointer to the FITC Control under the Compensation Samples specimen.



Data for the FITC tube is shown on the worksheet.

3 Create a snap-to gate around the singlet events on the FSC vs SSC dot plot.

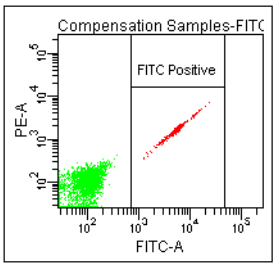


4 Select the fluorescence plots on the worksheet, right-click either plot and choose Show Populations > P1.

Now only singlet events are shown in the plots.

5 Draw an interval gate around the FITC-positive events on the FITC-A vs PE-A plot. In the population hierarchy, rename the population *FITC Positive*.

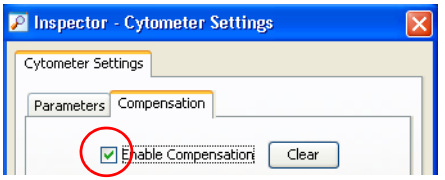
6 Right-click the FITC Positive interval gate and choose Invert Gate. Rename the NOT(FITC Positive) population *Negative*.



Tube: FITC Control

Population	#Events	%Parent	%Total
All Events	5,000		100.0
P1	4,632	92.6	92.6
FITC Positive	1,787	38.6	35.7
Negative	2,845	61.4	56.9

7 Select the FITC tube in the Browser, then click the Compensation tab in the Inspector. Select the Enable Compensation checkbox.



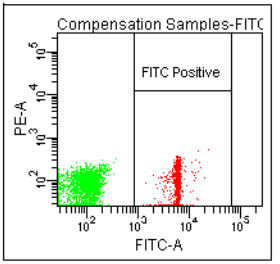
8 Adjust the PE–%FITC spectral overlap until the mean of the FITC-positive population matches the mean of the negative (Figure 3-14 on page 171).

To adjust the setting, click in the Spectral Overlap field. Software controls, consisting of up and down arrows and a slider bar, appear next to the value.

Do one of the following to change the value:

- Click the pointer in the slider bar and drag it to a new value.
- Use the mouse to click the up and down arrows or press the arrow keys on your keyboard to increase or decrease the values in small increments.
- Hold down the Ctrl key while clicking the arrows or pressing the keys to increase stepped values in increments of 10.
- Select the value in the field and enter a new value.

Figure 3-14 Adjusting for FITC spectral overlap



Experiment Name: Manual Comp
Specimen Name: Compensation Samples
Tube Name: FITC Stained Control
Record Date: Mar 23, 2003 10:31:54 AM

Population	FITC-A Mean	PE-A Mean	PerCP-Cy55-A Mean	APC-A Mean
All Events	2,367	77	171	89
P1	2,354	76	169	88
FITC Positive	5,947	76	297	92
Negative	98	76	89	86

means aligned

9 Adjust PerCP-Cy55–%FITC spectral overlap until the means of the respective positive populations match those of the negative.

10 Slightly adjust the APC–%FITC setting until the population means match.

Your statistics view should look similar to the following:

Experiment Name: Manual Comp
Specimen Name: Compensation Samples
Tube Name: FITC Stained Control
Record Date: Mar 23, 2003 10:31:54 AM

Population	FITC-A Mean	PE-A Mean	PerCP-Cy55-A Mean	APC-A Mean
All Events	2,367	77	88	85
P1	2,354	76	87	83
FITC Positive	5,947	76	88	80
Negative	98	76	85	86

Adjusting Compensation for the Remaining Tubes

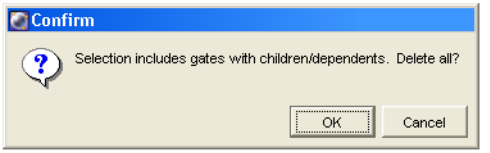
Repeat the following procedure for each remaining compensation control.

1 Set the current tube pointer to the next tube in the Browser to display its data.

2 Change the plot axes to display the appropriate fluorochromes.

For example, when you are compensating the PE tube, change the x-axis to PE-A, and the y-axis to FITC-A.

3 Right-click the interval gate and press the Delete key. Click OK to confirm.



4 Draw an interval gate around the fluorescence-positive cells. Rename the population *(fluorochrome name) Positive*.

5 Right-click the Positive population in the population hierarchy and choose Invert Gate. Rename the NOT(Positive) population *Negative*.

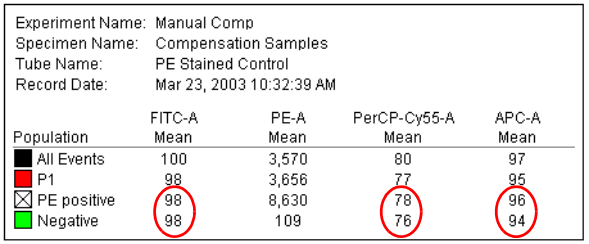
Your worksheet should contain objects similar to those shown in Figure 3-12 on page 167.

6 Verify that the Enable Compensation checkbox is selected and adjust the appropriate compensation networks to remove spectral overlap from the respective detectors.

For example, for PE compensation, adjust FITC–%PE, PerCP-Cy55–%PE, and APC–%PE compensation until the means for each fluorescent parameter match as closely as possible (Figure 3-15 on page 173).

☑ **Tip** Use the Ctrl key and the arrow keys on the keyboard to quickly change the settings.

Figure 3-15 Adjusting for PE spectral overlap



Experiment Name: Manual Comp
Specimen Name: Compensation Samples
Tube Name: PE Stained Control
Record Date: Mar 23, 2003 10:32:39 AM

Population	FITC-A Mean	PE-A Mean	PerCP-Cy55-A Mean	APC-A Mean
All Events	100	3,570	80	97
P1	98	3,656	77	95
PE positive	98	8,630	78	96
Negative	98	109	76	94

7 Proceed with adjustments for the next tube.

Manually Constructing a Compensation Matrix

Now that you have determined the spectral overlap setting for each fluorochrome, you can copy the settings to a test sample.

NOTICE If you paste compensation values to a tube with different voltage settings than those for the source tube, a warning message appears.

1 Enlarge the Inspector so you can see all compensation networks.

2 Right-click the FITC Stained Control tube in the Browser and choose Copy Spectral Overlap.

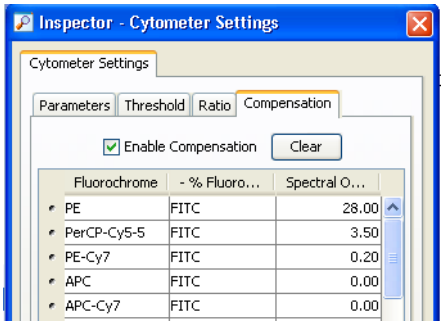
3 Expand the Calibrite specimen below the Compensation Samples specimen in the Browser.

Notice that there are two tubes below the specimen.

4 Select the tubes under the Calibrite specimen, right-click the tubes and choose Paste Spectral Overlap.

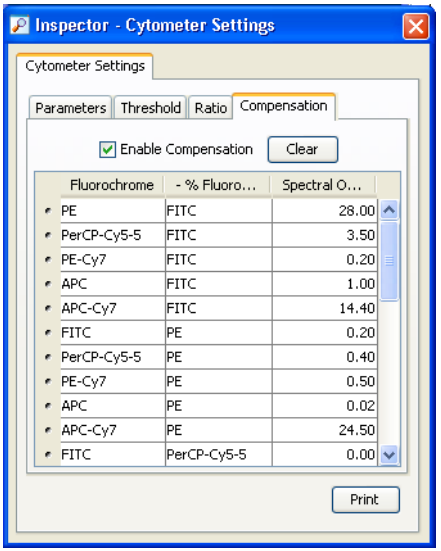
After data has been pasted, select one of the Calibrite tubes in the Browser. In the Inspector, notice that the FITC compensation networks now have spectral overlap settings (Figure 3-16 on page 174).

Figure 3-16 Pasting overlap settings for the FITC tube



5 Copy Spectral Overlap settings for each compensation control in turn, and paste the settings to the tubes under the Calibrite specimen.

When you finish pasting settings for the APC Stained Control, the compensation matrix should be complete.



☑ **Tip** Use the Paste Spectral Overlap with Zeros command in cases where you are pasting spectral overlap settings to a tube that already has a complete set of spectral overlap values. See Copying Spectral Overlap Values on page 144.

4

Tools for Data Analysis

During analysis, recorded data is displayed in plots while gates are used to define populations of interest. BD FACSDiva software analyzes the data and calculates statistics for print or export.

This chapter provides an overview of data analysis. Application-specific examples of data analysis are found in your cytometer manual.

The following topics are covered in this chapter:

Worksheets

A *worksheet* is the main work area of BD FACSDiva software—it is where you create plots, define gates, show statistics views and population hierarchies, and enter custom text. You can use multiple worksheets to organize your workflow. For example, use one worksheet for cytometer QC and sample optimization, and use a second worksheet for analysis. Two types of worksheets can be shown in the Worksheet window: normal and global.

Normal worksheets have white tabs and contain tube-specific analysis elements, while *global worksheets* have green tabs and contain elements that show data from any tube. You display a tube's data in a global worksheet by clicking to set the current tube pointer.

To display the Worksheet window, click the Worksheet button () on the Workspace toolbar. To toggle between the normal and global worksheet view, click the Worksheets View button () on the Worksheet toolbar.

Normal Worksheets 

Normal worksheets display analysis elements such as plots, gates, statistics, and custom text from multiple tubes. The analysis elements are tube-specific. Once a plot or statistics view is created for a tube, an analysis object is associated with it in the Browser.

- To add a normal worksheet to an open experiment, switch to the normal worksheet view (white tabs) and choose Worksheet > New Worksheet. A maximum of 30 worksheets is allowed. By default, new worksheets are sized to fill the open window.

- To expand the size of a worksheet, use the Worksheet Inspector to increase the number of pages. A worksheet can have up to 250 pages. Page breaks are indicated by a dotted line when the Show Page Breaks option is selected. See Using the Worksheet Inspector on page 183.

- To find a tube-specific object on a worksheet, double-click its associated tube in the Browser. The first object associated with the tube is displayed at

the top of the Worksheet window. (Alternatively, double-click any worksheet element to locate the corresponding tube in the Browser.)

- To delete a worksheet, click the worksheet tab and choose Worksheet > Delete Worksheet. A worksheet is automatically deleted if its analysis objects are deleted.

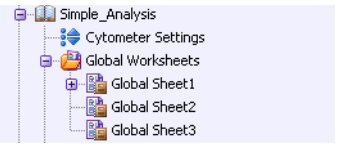
Switch between multiple worksheets by clicking the tabs at the top of the worksheets (see figure). You can work within only one worksheet, the active worksheet, at a time.

☑ **Tip** The active worksheet is indicated by an icon next to the worksheet name.

NOTICE While there is no impact on data collection or cytometer performance, responsiveness can decline as more plots, statistics, gates, and events are displayed for each tube. To improve system response time, limit the number of plots displayed in the viewable area of the Worksheet window.

Global Worksheets

Global worksheets allow you to create a single analysis object for acquiring or recording data from a set of tubes. Analysis objects are part of the global worksheet, and are not tied to a specific tube. Display data for any tube by clicking its current tube pointer. By default, each time a new experiment is created, a global worksheet is created.



A maximum of 50 global worksheets can be set up for each experiment, and each global worksheet can contain up to 10 pages. When the first global worksheet is added to an experiment, a Global Worksheets folder is created in which all global worksheets for that experiment will be stored. Global worksheets are displayed in this folder in the order they were created.

All data analysis and Worksheet tools can be used on both normal and global worksheets. To move objects between worksheets, use the copy and paste functions; objects cannot be dragged.

NOTICE If you copy an analysis object that spans more than 10 pages from a normal worksheet to a global worksheet, only objects that fit on the first 10 pages will be copied.

☑ **Tip** Differentiate a normal worksheet from a global worksheet by the color and title of the worksheet tabs. Normal worksheets are titled *SheetN* by default, and they have white tabs. Global worksheets are titled *Global SheetN*, and have green tabs.

Create a global worksheet in any of the following ways:

- Click the New Global Worksheet button () on the Browser toolbar.
- Choose Experiment > New Global Worksheet.
- Right-click an open experiment or a Global Worksheets folder and choose New Global Worksheet.

NOTICE Analysis objects on global worksheets derive their titles and headers from the current tube. Sort layouts on global worksheets use the population hierarchy of the global worksheet, not the tube. Tube-specific plots cannot be made on a global worksheet, and non–tube-specific plots cannot be made on a normal worksheet.

Using Global Worksheets

The following example shows one way in which global worksheets can be used. For more examples, refer to Using Gating Features in *Getting Started with BD FACSDiva Software*, or to your cytometer manual.

1. Create a global worksheet and generate all required plots.
2. Set the current tube pointer to the first tube for which you are acquiring data.

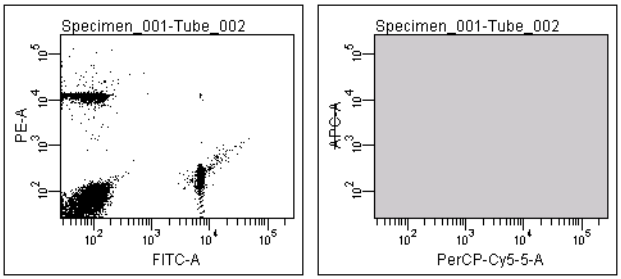
3 Start acquisition or recording. Data will appear on the global worksheet.

4 Create gates, statistics views, and population hierarchies as needed.

5 Set the current tube pointer to the next tube and acquire or record data.

Data will appear on the global worksheet using the gates created in step 4. If the new tube uses fewer parameters than the previous tube, data might not be displayed in all plots. Any plots that use a missing parameter will appear grayed out (the missing parameter is crossed out). See Figure 4-1.

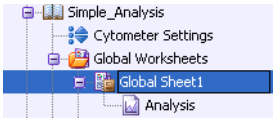
Figure 4-1 Plot displaying all parameters (left) and missing parameter (right)



6 Edit the gates to reflect the data from the second tube.

NOTICE Once edited, the gates in step 6 remain as edited, even if you return to the first tube by moving the current tube pointer. Gates are global and attached to the global worksheet, not to the tube.

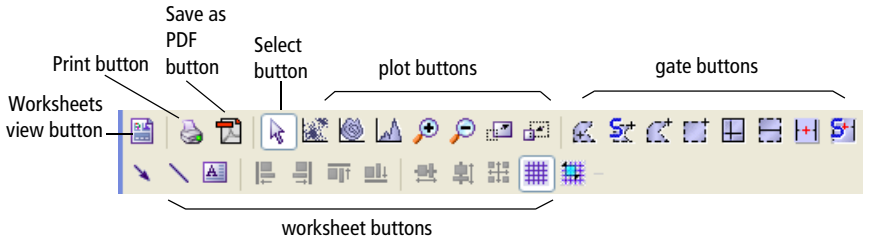
7 To save the analysis with a tube, copy the analysis object to the tube.



You can also enable the user preference to automatically save a copy after recording; see General Preferences on page 96.

Using the Worksheet Toolbar

There are five sets of buttons on the Worksheet toolbar: the Worksheets View button, a Select button, and plot, gate, and worksheet buttons. Certain buttons are enabled only when elements appropriate for the button are selected. To use any enabled button, click once to select it. To reuse a button multiple times without reselecting it, double-click the button. It will remain selected until you select another button or press the Esc key.



A summary of button functions follows.

Worksheets view button—Toggles the Worksheet window between the global and normal worksheet views.

Print button—Displays the Print dialog to print the worksheet.

Save as PDF button—Saves the worksheet as a PDF file. This button is displayed when there is an open experiment.

Select button—Selects (by clicking), moves (by dragging), or resizes (by dragging a selection handle of) objects in a worksheet.

Plot Buttons

Use Plot buttons to create plots, zoom in and out on plot data, and resize plots.

Plot buttons—Create dot (D), contour (C), or histogram (H) plots. For a normal worksheet, select a tube, select a plot button, and then click in the worksheet to create a plot of a default size. For a global worksheet, select a plot button and click in the worksheet. For other options, see Creating Plots on page 194.

Zoom-In button—Magnifies an area of a plot. Select the button, click in the plot, and drag to define the area of the plot to be enlarged. The tickmarks on the axes of the plot adjust to reflect the magnified view. A zoomed plot has a magnifying icon in its lower-left corner.

Zoom-Out button—Undoes the last zoom-in action. Select the button and click once in the plot. Each click with the button undoes the last zoom-in action. To return to the originally sized plot in one click, hold down the Ctrl key while clicking in the plot.

Increase Plot Size button—Increases the size of plots by two on the worksheet. Select the button and click once in a plot. The length of the x- and y-axes doubles each time you use the button.

Decrease Plot Size button—Decreases the size of plots on the worksheet. Select the button and click an increased plot. The length of the x- and y-axes halves each time you use the button. Plots cannot be resized below their original size.

Gate Buttons

Use gate buttons to define population subsets on plots. The Interval Gate buttons are the only buttons that can be used to gate data on a histogram. For more information, see Gates on page 217.

Autopolygon-Gate button—Draws a gate automatically around a distinct cluster on a dot or contour plot. Once the gate has been drawn, its shape and size remain constant, even as new data is provided to the gate.

Snap-To Gate button—Draws a gate automatically around a distinct cluster on a dot or contour plot. Unlike an autopolygon gate, a snap-to gate recalculates as new data is provided to the gate.

Polygon Gate button—Allows a polygonal gate to be drawn around a population on a dot or contour plot.

Rectangle Gate button—Creates a rectangular gate around a population on a dot or contour plot.

Quadrant Gate button—Divides a plot into four quadrants. Each quadrant has its own population statistics.

Interval Gate button—Allows a range of events to be selected on a dot, contour, or histogram plot.

Autointerval Gate button—Draws an Interval gate automatically around a range of events on a dot or contour plot or histogram. Once the gate has been drawn, its shape and size remain constant, even as new data is provided to the gate.

Snap-To Interval Gate button—Draws an Interval gate automatically around a range of events on a dot or contour plot or histogram. Unlike an autointerval gate, a snap-to interval gate automatically recalculates as new data is provided to the gate.

Worksheet Buttons

Use Worksheet buttons to customize worksheets. See Editing Worksheets on page 185 and Aligning and Resizing Worksheet Elements on page 189.

Customizing buttons—Allow you to personalize worksheets with arrows (A), lines (L), and text (T).

Align buttons—Align selected objects in a worksheet. A minimum of two objects must be selected for the buttons to be active.

Use these buttons to align worksheet elements at their left, right, top, and bottom edges, respectively, with the last-selected object (object with yellow selection handles).

Distribute buttons—Put the same amount of horizontal or vertical space between selected objects in a worksheet. A minimum of three objects must be selected for the buttons to be active.

Make Same Size button—Makes selected objects in a worksheet the same size as the last-selected object. A minimum of two objects must be selected for the button to be active.

Show Grid button—Makes a grid display in a worksheet. The grid does not display in printed worksheets or in PDF files.

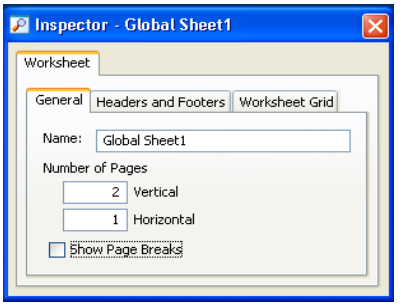
Snap-To Grid button—Makes elements in the worksheet (such as plots, statistics views, population hierarchies, text boxes, and arrows) that are added, moved, or resized, snap to the grid. This button is only active if the Show Grid button is active.

Using the Worksheet Inspector

Use the Worksheet Inspector to name a worksheet or global worksheet, increase the number of pages in the worksheet, and hide or show page breaks and page numbers. Also use the Worksheet Inspector to show headers and footers and define what elements headers and footers will display. In the Worksheet Grid tab, choose to show or hide the grid, define the spacing of the grid bars, and enable or disable the Snap-To Grid option.

To view worksheet options in the Inspector, click the background area of a worksheet. (If you click an object on the worksheet, the Inspector shows the properties of that object.)

Using the General Tab



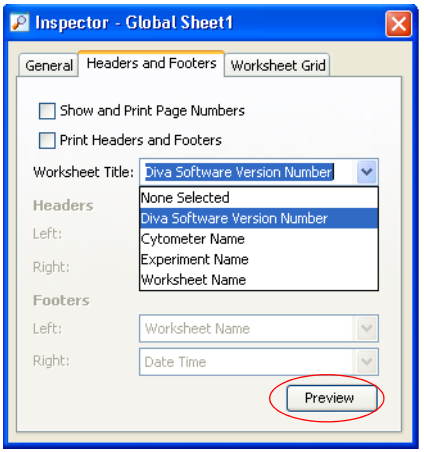
- To change the name of the worksheet, select the text in the Name field, enter the new name, and press Enter.
- To add pages to a worksheet, change the values in the Number of Pages fields. Additional pages are added in the Vertical or Horizontal direction, to a maximum of 250 normal worksheets or 50 global worksheets. (The product of Horizontal x Vertical cannot exceed 250 or 50, respectively.)
- To hide page breaks, deselect the Show Page Breaks checkbox.

  NOTICE Do not place worksheet elements on the dotted line representing a page break. Objects that straddle a page break are split between two printed sheets.

Using the Headers and Footers Tab

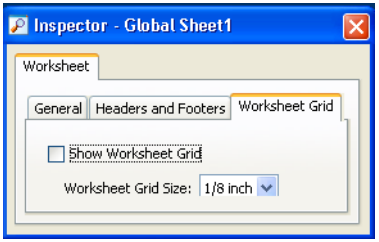
- To show page numbers, select the Show and Print Page Numbers checkbox. Pages are numbered in the order in which they will be printed and will appear on each printed page.
- To print headers and footers, select the Print Headers and Footers checkbox.
- For the worksheet title, select from the choices in the menu or type a custom title. To leave the title blank, choose None Selected.
- Under Headers and Footers, select what you want to display on the left and right sides of the worksheet page from the menus, or type a custom header or footer. To leave the headers or footers blank, choose None Selected.

  Click the Preview button to see the selections made.



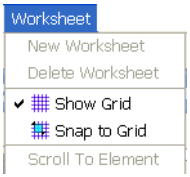
NOTICE Worksheet grid, title, and header and footer information can be entered, changed, and previewed in the User Preferences dialog; however, selections made in the Worksheet Inspector take precedence over selections in User Preferences. See Headers and Footers on page 100.

Using the Worksheet Grid Tab



- To show the grid on the worksheet, select the Show Worksheet Grid checkbox. By default, this checkbox is selected.
- To change the spacing between bars of the worksheet grid, select a value from the Worksheet Grid Size menu.
- To make elements in the worksheet that are added, moved, or resized snap to the worksheet grid, select the Snap to Worksheet Grid checkbox. By default, this checkbox is not selected.

☑ **Tip** Another way to select the Worksheet Grid or the Snap to Grid is by using the Worksheet menu in the menu bar at the top of the workspace.



Editing Worksheets

Worksheets can display plots, gates, population hierarchies, and statistics views. For information on creating these elements, see the corresponding sections in this chapter. Additionally, worksheets can be customized with lines, arrows, and text.

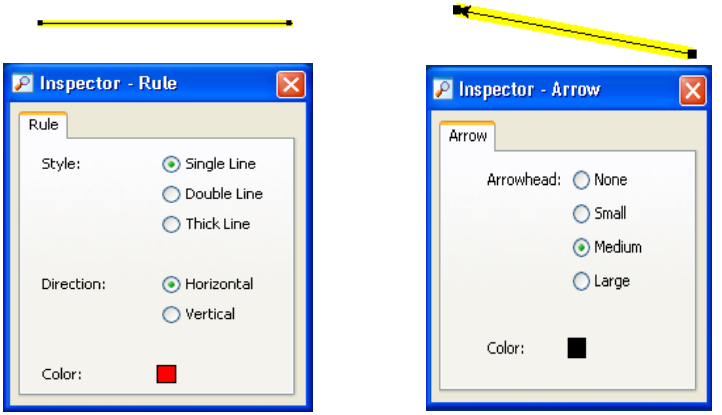
Adding Lines or Arrows

Use lines to separate header information from the rest of your worksheet, or to delineate areas of your worksheet. Use arrows to point to an area of interest.

To add a line or arrow, select the Line ( ) or Arrow ( ) button and click in the worksheet. Use the Inspector to change the properties of the line or arrow.

The appropriate Inspector is displayed when the line or arrow is selected in the worksheet. A selected object is highlighted in yellow (Figure 4-2).

Figure 4-2 Rule Inspector for selected line (left) and Arrow Inspector for selected arrow (right)



- Specify the line style, direction, and color by making appropriate selections in the Rule Inspector. Resize a line on the worksheet by dragging one of the black handles on either end of the selected line; move the line by selecting the line and dragging.

- Change the style of the arrowhead and the color of the arrow in the Arrow Inspector. Change the length or angle of the arrow by dragging one of the black handles on either end of the selected arrow; move the arrow by selecting the arrow and dragging.

- Delete a line or arrow by selecting it, and then pressing Delete.

Adding Text

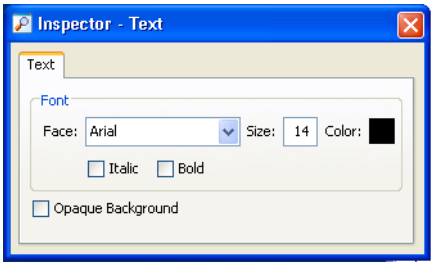
To insert a text box on a worksheet, click the Text button ( ) and click in the worksheet. Use the Inspector to change the text properties.

- Edit the text in a text box by selecting the current text, then entering new text. Click anywhere outside the text box to complete the entry.

- Change the text properties by making selections in the Text Inspector (text does not need to be selected). Changes apply to all text within the selected text box.

- For a text box with an opaque background, select the Opaque Background checkbox. For a transparent text box (with grid lines showing), leave this checkbox deselected.

selected text box showing opaque background



- Move a text box by selecting it and dragging the border to a new location.
- Resize a text box by selecting it and dragging one of the selection handles in or out.
- Delete a text box by selecting it and pressing Delete.

Copying Worksheet Elements

Individual worksheet elements such as plots, population hierarchies, and statistics views can be duplicated within a worksheet or copied to any Microsoft Office application by the following methods:

- Hold down the Ctrl key and drag a worksheet element within a worksheet or to a Microsoft Office document. The element is duplicated when the mouse button is released.
- Press Ctrl-C to copy an element and press Ctrl-V to paste it in a new location, such as on another worksheet or to any Microsoft Office document.
- To create a duplicate of a plot, right-click a plot and choose Duplicate, or select the plot and press Ctrl-D.

Copying the Workspace

You can copy an image of the BD FACSDiva workspace—including the worksheet, Browser, and Inspector—and resize it or edit it in a word processing or graphics application.

1 Copy the workspace to the clipboard.

- To copy an image of the BD FACSDiva software workspace, press Alt-Print Screen.
- To copy an image of the entire screen, press Print Screen.

2 Paste the image into an open window in the word processing or graphics application.

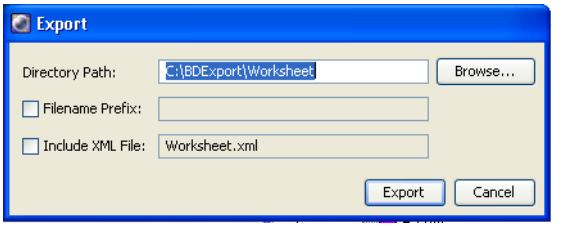
The image is stored in memory until it is pasted into another application.

Exporting Worksheet Elements

Plots or other worksheet elements can be exported as graphics for word processing applications or to send electronically. Worksheet elements can also be exported as an XML file. In addition, file names can display a prefix useful for identifying a group of elements as part of a specific worksheet (eg, 3-color).

1 Select one or more worksheet elements.

2 Choose File > Export > Worksheet Elements.



3 Specify the Directory Path (folder) where the elements will be stored. By default, elements are stored in D:\BDExport\Worksheet.

   Click Browse to select a different folder than what appears by default.

4 Select the Filename Prefix checkbox and enter text. The prefix is displayed at the beginning of each element file name that is exported.

5 Select the Include XML File checkbox to export the worksheet in XML format. The worksheet file includes all the selected elements. The prefix that was entered is displayed as the XML worksheet name.

6 Click Export.

   On export, if an XML file of the same name already exists, a dialog is displayed with the option to overwrite the file, append (add the file using the next sequential number to name it), or to cancel the export.

Each selected element will be stored as a separate JPEG file or XML file. File names are determined by the type of object selected, for example, DOTPLOT_1.jpeg, HISTOGRAM_1.jpeg, or Worksheet.xml.

NOTICE Exported elements cannot be imported back into BD FACSDiva software.

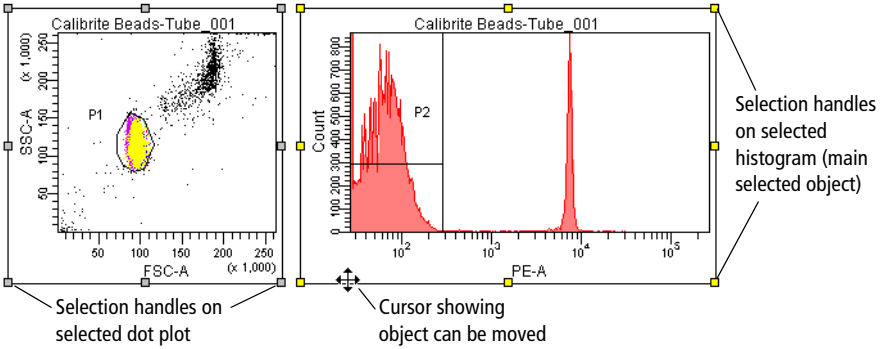
Aligning and Resizing Worksheet Elements

Use worksheet buttons to align or resize one or more elements on a worksheet.

- To move a selected object, position the cursor on the border of the object. When the cursor changes to two double-headed arrows, drag the object to move it. See Figure 4-3.

- To make objects the same size, hold down the Shift key while selecting two or more objects on a worksheet. The object selected last becomes the main selected object, indicated by yellow selection handles (Figure 4-3). Click the Make Same Size button ( ). All objects are resized to the same size as the main selected object.

Figure 4-3 Selecting multiple objects



Individual selected objects can also be resized by dragging a selection handle. Position the cursor over the selection handle. When the cursor changes to a double-headed arrow, drag the border in the direction of the arrow.

- To align multiple objects, select two or more objects, and click the appropriate button ( ). Use these buttons to align to the left, right, top, and bottom edges, respectively. Objects are aligned in relation to the last selected object.

- To put equal amounts of space between objects, select three or more objects on a worksheet. Click the appropriate Distribute button ( ). The objects are distributed equally in a horizontal or vertical direction.

- To increase the size of a plot, click the Increase Plot Size button ( ) and then click a plot on the worksheet. The length of the x- and y-axes doubles each time you use the button. Use the Decrease Plot Size button ( ) to return the plot to its previous size. Plots cannot be reduced below their original size.

☑ **Tip** You can also use the Zoom In button to magnify an area in a plot.

Printing Worksheets

Designate the information to print in the worksheet headers and footers, either in the Worksheet Inspector or the User Preferences dialog. Then choose one of the following commands from the File menu to set up for printing or to print worksheets.

- Choose File > Page Setup to set the size of the printed page (eg, A4 or letter), the orientation (portrait or landscape), and the margin size. Your options will vary depending on the printer configured with your workstation.

  If there are multiple worksheets in the experiment, options apply only to the active worksheet.

- Choose File > Print Preview to view a thumbnail of all printable pages at 10, 30, 50, or 100%. Click  to return to the worksheet.

- Choose File > Print to print the active worksheet. You can also click the printer button on the worksheet toolbar to print the active worksheet.

NOTICE Do not place worksheet elements on the dotted line representing a page break. Objects that straddle a page break are split between two printed sheets.

Saving Worksheets as PDF Files

To save a worksheet as a PDF file:

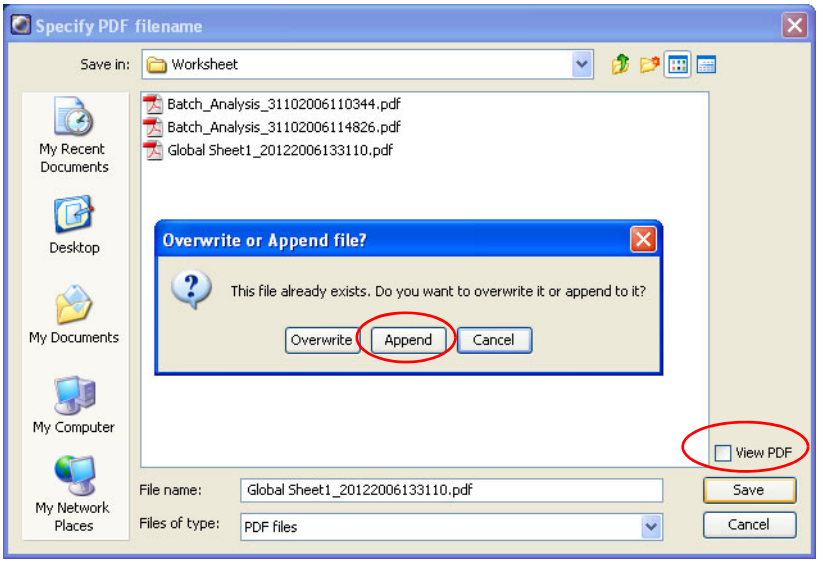
1. Either choose File > Save as PDF or click the Save as PDF icon () in the Worksheet toolbar.

   Worksheets are saved by default in the BDExport\Worksheet folder.

2. To append a worksheet PDF file to an already existing PDF file, select the file you want to append to in the Save as PDF dialog and click Save.

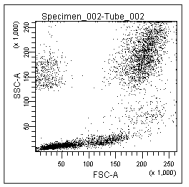
   A new dialog is displayed where you can select Append (or Overwrite to replace the existing PDF file).

☑ **Tip** To view the worksheet you are saving as a PDF file, click the View PDF checkbox, then click Save, and the PDF is displayed.

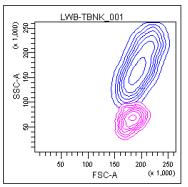


Plots

Multiparameter data events can be displayed in dot, contour, density, or histogram plots.

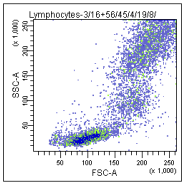


Dot Plot—Graphical representation of two-parameter data, where each axis displays the signal intensity of one parameter and each dot represents one or more events (cells or particles).



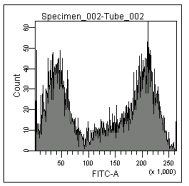
Contour Plot—Graphical representation of two-parameter data, where each event has a position in the plot according to its intensity values for both parameters.

Contour lines provide a third dimension by joining x- and y-coordinates with similar event counts. These plots are similar to topographic maps which use contour lines to show points at the same elevation.



Density Plot—Graphical representation of two-parameter data where each axis displays the signal intensity of one parameter and colors indicate the number of events.

Density plots are similar to dot plots, except colors are used to represent the accumulation of events (density) for events with the same signal intensity. A density plot simulates three-dimensional event display.



Histogram—Graphical representation of single-parameter data, where the horizontal axis represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).

Creating Plots

Use menu commands or plot buttons to create dot plots, contour plots, or histograms. Note that contour plots are not available during acquisition. When you create a contour plot during acquisition, a density plot appears by default. During analysis, a contour plot appears. To create a density plot during acquisition, first create a contour plot, then use the Inspector to change the plot type to density. See Formatting Contour Plots on page 205.

- To create a plot using a plot button, click the appropriate button on the Worksheet toolbar, and click once on the worksheet to draw a plot of default size. The default size of the plot is based on the paper size so that three dot or contour plots or two histograms can fit in the width of the page.

  If you prefer to define the plot size, click the plot and drag the border to create a plot of the preferred size.

☑ **Tip** To create multiple plots, double-click the Plot button. The button will remain selected until another button is selected, or until you press the Esc key. You can then repeatedly click in the worksheet and the same plot type will be created each time.

- To use menu commands to create a plot in a normal worksheet (normal worksheet must be active), select one or more tubes in the Browser, right-click and choose a plot option from the menu, then click in the normal worksheet to display the plot.

  To create plots in a global worksheet (global worksheet must be active), click the icon for the global sheet that is active, right-click and choose a plot option from the menu, then click in the worksheet to display the plot.

Creating Gated Plots

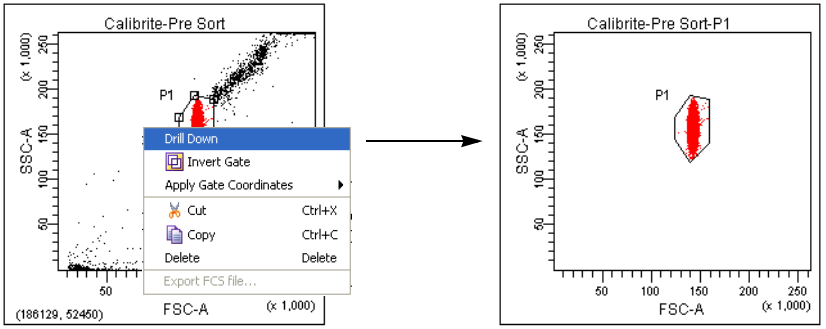
After gated populations have been defined, use the Drill Down feature to create a new plot showing data from only one population.

1 In a plot showing one or more gated populations, right-click the gate border.

☑ **Tip** If the gate border is not shown, right-click the plot border, choose Show Gate, and select the appropriate gate.

2 Choose Drill Down from the menu.

A new plot appears with the same plot parameters and axis scale settings, showing data only from the selected population.



Duplicating Plots

Duplicate a plot in one of the following ways:

- While holding down the Ctrl key, drag the plot to a new location. The plot is duplicated when you release the mouse button.
- Press Ctrl-C to copy a plot and press Ctrl-V to paste it in a new location, such as on another worksheet.
- Right-click the plot border and choose Duplicate.

Editing Plots

You can perform any of the following operations on a plot:

- Move or resize a plot. See Aligning and Resizing Worksheet Elements on page 189 or Resizing Plots on page 196.

- Cut or copy a plot and paste it to another worksheet or global worksheet, or to any Microsoft Office document. See Exporting Worksheet Elements on page 188 or Copying Worksheet Elements on page 187.
- Duplicate a plot on the same worksheet. See Duplicating Plots on page 195.
- Change plot parameters. See Changing Plot Parameters on page 197.
- Zoom in and out on plot data, or alter plot size. See Plot Buttons on page 180 or Resizing Plots on page 196.
- Change between four- and five-log decade displays for a plot. See Changing Log Display on page 198.
- Select and sequence the order of populations to display. See Choosing Populations to Display on page 199.
- Show or hide plot grids, outlines, tick marks, and tick labels. See Formatting Plots on page 200.
- Add, remove, or change plot titles and axis labels. See Editing Plot Titles and Axes Labels on page 203.
- Display biexponential scales. See Using Biexponential Display on page 211.

For each type of plot (dot, contour, density, and histogram), specific formatting and editing features are available. See Using the Plot Inspector on page 200.

Resizing Plots

To resize a plot, select it and drag one of its selection handles. See Aligning and Resizing Worksheet Elements on page 189. You can also use the Increase Plot Size button to double the size of a plot on a worksheet, and the Decrease Plot Size button to return the plot to its original size.

1 Click the Increase Plot Size button () and click a plot on the worksheet.

The size of the plot doubles, making it easier to view individual events.

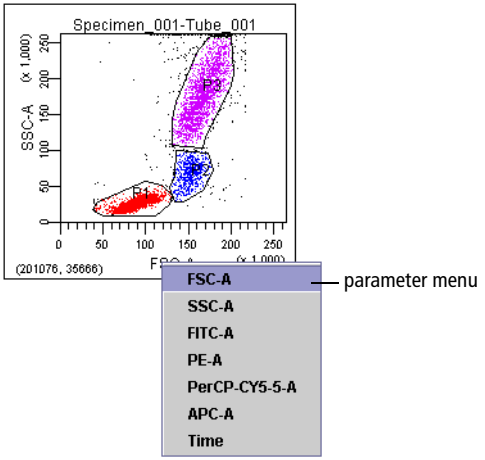
2 Click the Decrease Plot Size button ( ) and click the doubled plot.

The plot returns to its original size. Plots cannot be reduced below their original size.

Changing Plot Parameters

To change plot parameters, click the axis label in a plot and choose a parameter from the menu that appears (Figure 4-4). You can also change plot parameters using the Plot Inspector.

Figure 4-4 Changing plot parameters



All parameters specified in the Parameters tab are available. Depending on which checkboxes are selected in the Parameters tab, parameters will be listed as *parameter*-A, *parameter*-H, or *parameter*-W.

You can also choose Time as a parameter. For more information, see Using the Time Parameter on page 141.

Changing Log Display

Log data is displayed in four- or five-log decade plots. To change the display, select the experiment in the Browser and make the appropriate selection in the Experiment Inspector. Log display properties apply to all plots in the experiment.

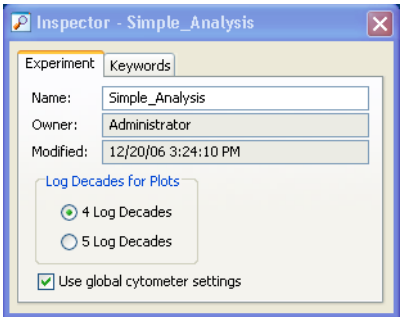
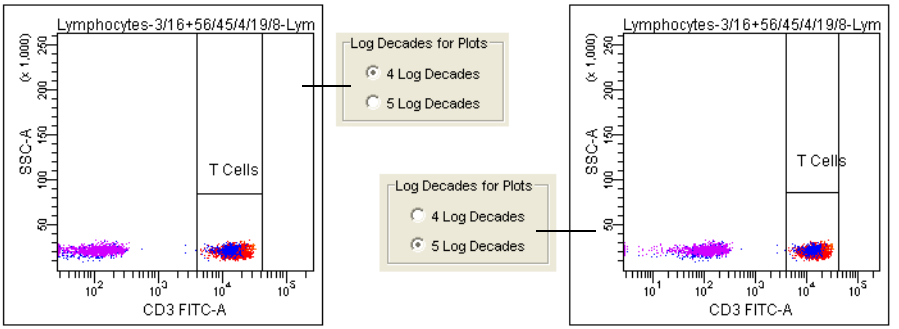


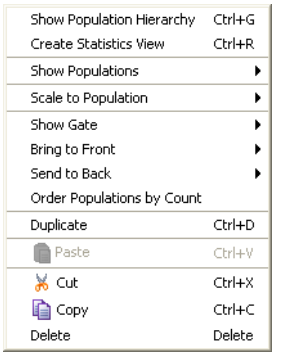
Figure 4-5 Plot displaying log data in four- and five-log decades



Four-log plots display values from 26–262,143; five-log plots display values from 2.6–262,143. Thus, the first log decade ranges from 2.6–26 or 26–262, depending on the selected scale.

☑ Select the Grid checkbox in the Plot Inspector to delineate log decades on plots.

Choosing Populations to Display



Right-click the border of any plot to access a menu where you can choose from the following:

- Show Population Hierarchy—Displays the population hierarchy for the associated tube. See Using the Population Hierarchy on page 232.
- Create Statistics View—Displays statistics for populations in the plot. See Statistics on page 238.
- Show Populations—Allows you to select which population(s) to display in the plot. (Populations can also be selected in the Plot Inspector. See Formatting Plots on page 200.)

If you display a population that has been assigned No Color (⊠), no events appear in the plot.

- Scale to Population—Adjusts biexponential scales to fit the selected population. See Scaling to a Population on page 215.
- Show Gate—Shows or hides the gate outline for selected populations in the plot. See Hiding and Showing Gates on page 229. This option appears only after a gate has been created.
- Bring to Front—Allows you to specify which population to display in front of the other populations (useful when the events of interest are obscured behind another population).
- Send to Back—Displays a selected population behind other populations in the plot (useful for uncovering events of interest).
- Order Populations by Count—Displays smaller populations in front of larger populations.
- Duplicate—Makes a copy of the plot.

- Delete—Deletes the plot.

Using the Plot Inspector

The Plot Inspector is used to format plots. Different options are displayed in the Inspector when you select one or more plots on the worksheet.

☑ **Tip** Select multiple plots to make Inspector selections apply to all plots at once. Only options available to all selected plots are enabled.

There are four components to the Plot Inspector, accessed by clicking the tabs at the top of the Inspector: Plot, Title, Labels, and Dot Plot (or Histogram or Contour, depending on the type of plot selected).

Formatting Plots

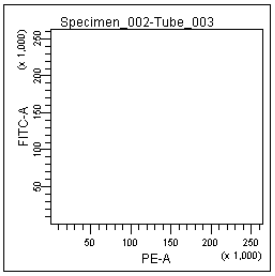
Use the Plot tab of the Inspector to view or change plot parameters, turn on Biexponential scaling, edit the plot appearance, and specify populations to show.

- Change the plot parameters by choosing a parameter from the X Parameter or Y Parameter menus (only the X parameter can be changed for histograms). All parameters specified in the Parameters tab are available. In addition, you can choose Time.

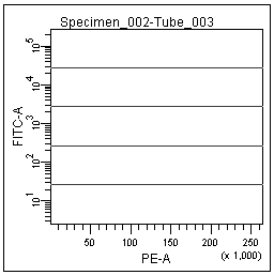
- Turn on Biexponential scaling for the x-axis, y-axis, or both by selecting the corresponding checkbox(es). For more information, see Using Biexponential Display on page 211.

- Select the Grid checkbox to show gridlines at each log decade in a log plot, or to show the zero point for biexponential scaling.

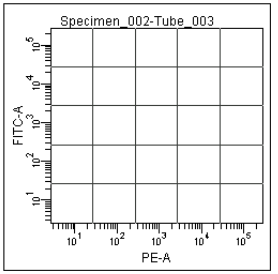
Gridlines are shown only if the Log checkbox in the Parameter tab of the Cytometer window is selected for the parameter displayed in the plot.



linear fluorescent plot

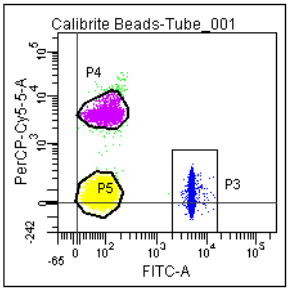


fluorescent plot with one log parameter



fluorescent plot with two log parameters

When biexponential scaling is in effect, gridlines delineate the zero point.



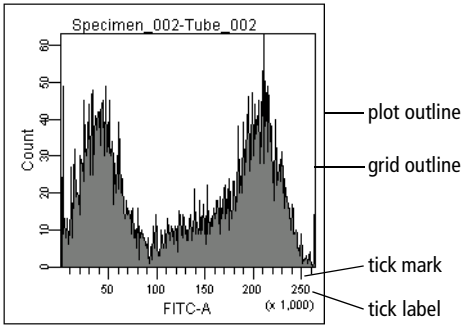
- Change the plot background color by clicking the Background Color box. A palette appears from which you can choose a new color.

☑ **Tip** Use Plot Preferences to change the default background color for all new plots. See Plot Preferences on page 100.

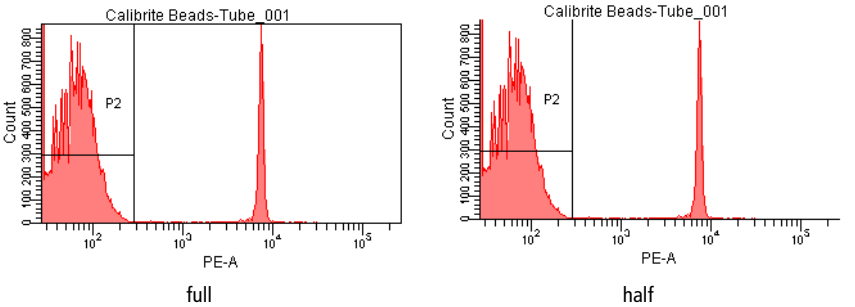
- Change the plot appearance by deselecting checkboxes for the following:
  - Plot outline—Hides the black line around the outside of a plot.
  - Grid outline—Hides the black outline within a plot.

- Tick labels—Hides tick numbers. (To hide axes labels, click the Labels tab.)

- Tick marks—Hides tick lines.



- Show a full or half grid outline by selecting the appropriate option.



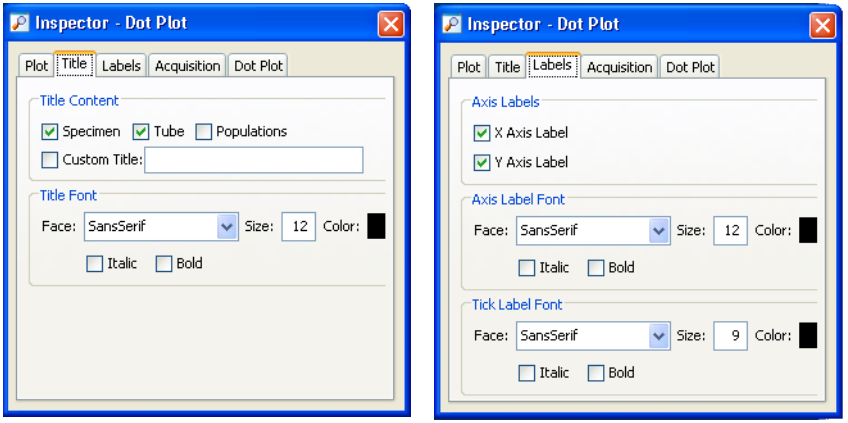
- Select the checkbox for each population to be displayed in the plot. Deselect a checkbox to hide the population.

- Change the population drawing order by deselecting all populations, and then reselecting them in the reverse order of how you want them displayed. Populations with a drawing order of 1 are displayed in front of populations with a higher drawing order.

Editing Plot Titles and Axes Labels

Use the Title tab of the Plot Inspector to specify and format the plot title. Use the Labels tab to hide, show, or format axis labels.

Figure 4-6 Inspector tabs for formatting plot titles and labels



- Within the Title tab, click the Title Content checkboxes to add specimen, tube, or population names to the plot title. Each checked field will appear in the plot title separated by a hyphen (eg, Calibrite Beads-Tube_003).

  To create a custom title, click the Custom Title checkbox and enter a title.

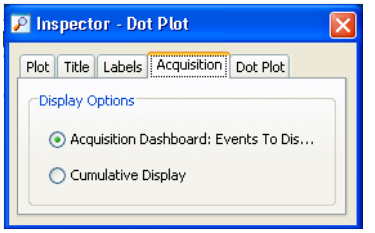
- Within the Labels tab, show or hide x- and y-axis labels by selecting or deselecting the appropriate checkbox.

- Within the Title and Labels tabs, format the plot title, axis labels, and tick-mark labels in the Font formatting boxes.

Setting Acquisition Display Options

Use the Acquisition tab of the Plot Inspector to specify the number of events to display during acquisition, on a per plot basis.

- Click the Acquisition Dashboard: Events to Display button to show the number of events based on the number in the Acquisition Dashboard. This option is selected by default.



- Click the Cumulative Display button to show all events from the beginning of acquisition, recording, restarting, or the addition, movement, or deletion of a gate. Because adding, moving, or deleting a gate impacts the display through the different gate structure, the plot is cleared and then starts to display cumulative events again from that point.

  NOTICE Statistics during acquisition are always calculated on the number of events selected in the Acquisition Dashboard.

  Since cumulative event display only applies to events during acquisition, when acquisition stops, the plot or histogram shows the number of events selected in the Acquisition Dashboard.

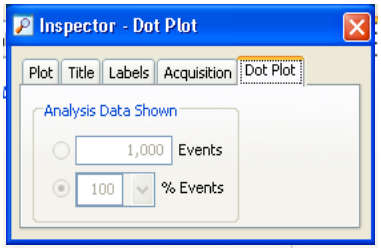
  NOTICE If a dot plot is not visible during acquisition, the plot is not updated. So if a cumulative plot is scrolled into view, the plot displays as much data as possible, up to the last 250,000 events.

☑ When data in a dot plot is displayed as cumulative, there is a “C” shown in the upper left corner of the dot plot.

Formatting Dot Plots for Analysis

Use the Dot Plot tab of the Inspector to specify how many events to show in the plot during analysis. Choose from the following:

- Select the upper button and enter any number of events, from 1% of the total events to the total number of events acquired. When fewer than the total number of events is shown, the plot shows the last recorded events (eg, if 1,000 is selected, it will show the last 1,000 events recorded).



- Select the lower button and choose a percentage of the total events from the menu, or enter a percentage value in the field. The percentage is determined from the total number of events (eg, displaying 25% of events for a 4,000-event file will show every fourth event, not the last 1,000 events).

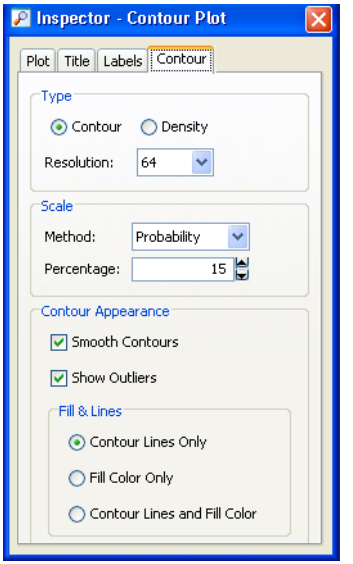
  The minimum number of events to show is 1% of the total number.

Formatting Contour Plots

Use the Contour tab of the Plot Inspector to specify the type, scale, and appearance of the Contour plot.

NOTICE During acquisition, data in a contour plot is shown in density mode, but reverts to contour data when acquisition is paused or data is recorded.

- Choose a scale method from the menu. The default method is probability.



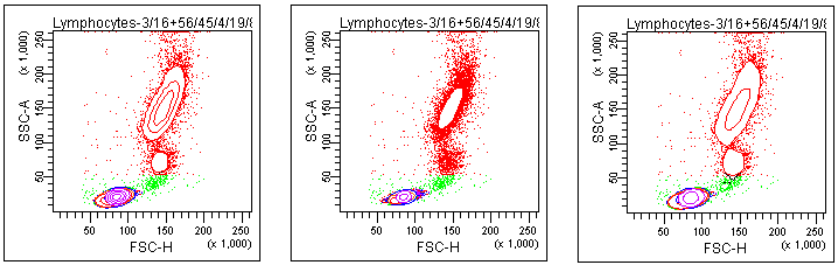
  - Probability—Calculates contour levels as a percentage of the total event number. With this method, contour levels are not based on the maximum number of events. Instead, the area between each pair of contour lines contains an equal percentage of the total events. The starting value (the outermost contour) is half the percentage value entered. For example, with 20% probability the outermost contour represents 10% of the total number of events, the next contour represents 30%, then 50%, 70%, and 90%.

  - Linear Density—Calculates contour levels as a percentage of the maximum event number (peak height), with equal spacing between contour lines. The starting value (the outermost contour) is half the value entered. For example, with 20% linear density, the outermost contour represents 10% of the peak height, the next contour represents 30%, then 50%, 70%, and 90%. Equal spacing tends to put most of the contour lines on the higher peaks (representing larger numbers of events) and might not show lower features.

  - Log Density—Calculates contour levels as a percentage of the maximum event number (peak height), with logarithmic spacing between contour lines. Log-density contours begin at the innermost contour using the peak height percentage you entered, and continue until they reach a threshold value of 1. For example, with 50% log density, the innermost contour represents 50% of the peak height. Each successive contour line represents 50% of the preceding contour, so the next contour represents 25% of peak height, then 12%, 6%, 3%, and 1%. This method shows more detail in the lower regions, while still showing peak heights.

For an example of each scale method, see the following figure.

Figure 4-7 Contour plot at 20% probability (left), linear density (middle), log density (right)

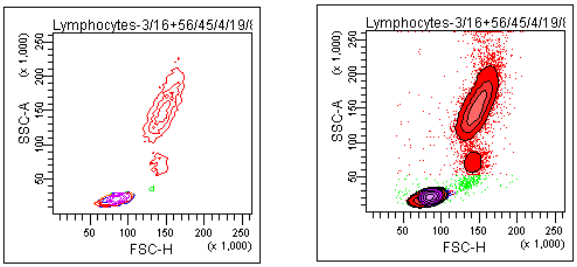


- Select the Smooth Contours checkbox to reduce irregularities in the contour lines. Smoothing does not affect statistics calculated for contour plots. However, when contours are smoothed, population colors can appear outside the gates that define them. Deselecting smoothing will disable other options for formatting contour plots. See Figure 4-8 for an example of an unsmoothed plot.

- Select the Show Outliers checkbox to display data (points) that fall outside the lowest contour level.

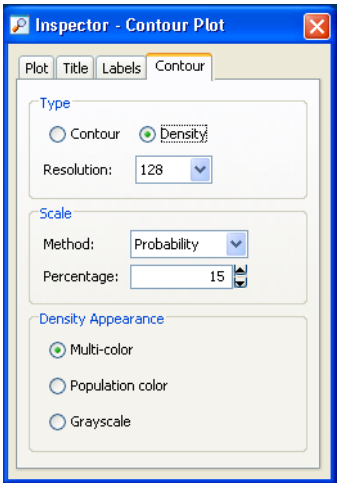
- Select the appropriate button under Fill & Lines to change the look of the contour plot using contour lines only (in their population colors), fill color only, or contour lines and fill color. When fill color is used, color shading lightens as contour levels increase (Figure 4-8).

Figure 4-8 Contour plot with smoothing deselected (left); contour lines and fill color (right)



Formatting Density Plots

Select Density button in the Contour tab of the Plot Inspector to change a Contour plot to a Density plot. To specify the scale and appearance of the Density plot:



- Choose the plot resolution, from 128, 256, or 512 bins. Data from adjacent bins is added to condense higher resolution data (more bins) into the chosen number of bins.

- Choose a scale method from the menu. The default method is probability.

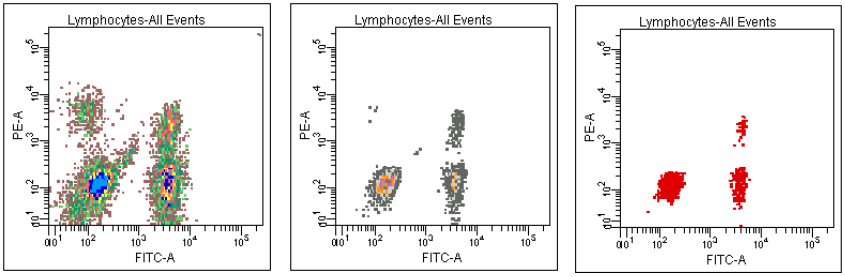
  - Probability—Calculates density levels as a percentage of the total event number. With this method, density levels are not based on the maximum number of events. Instead, the number of events between each level contains an equal percentage of the total events. The starting level (outermost color) is half the percentage value entered. For example, with 10% probability the lowest level represents 5% of the total number of events, the next level represents 15%, then 25%, 35%, and so on.

  - Linear Density—Calculates density levels as a percentage of the maximum event number (peak height), with equal spacing between density levels. The starting value (the lowest level) is half the value entered.

  - Log Density—Calculates density levels as a percentage of the maximum event number (peak height), with logarithmic spacing between density levels. Log-density levels begin at the innermost contour using the peak height percentage you entered, and continue until they reach a threshold value of 1.

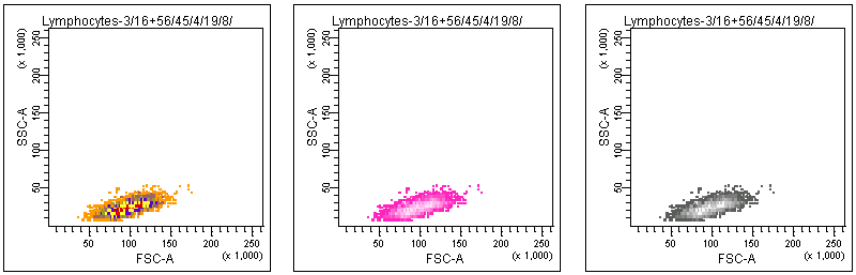
For an example showing each scale method, see the following figure.

Figure 4-9 Density plots at 5% probability, linear density, and log density



- Select an option under Density Appearance to display density levels in multiple colors, with a different color for each level; in the population color, where the color starts from the population color and fades to white as the levels rise; or in grayscale, where the color starts from gray and lightens to white as the levels rise (Figure 4-10).

Figure 4-10 Density plots with multicolor, population color, and grayscale appearance



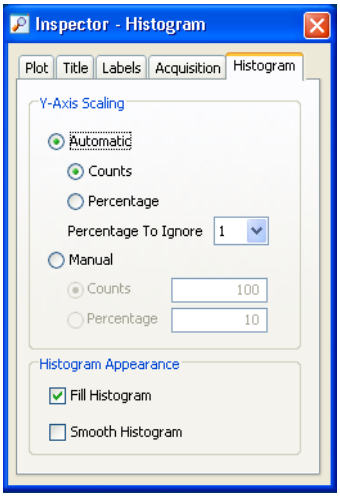
Formatting Histograms

Use the Histogram tab of the Inspector to format histograms. The y-axis scale shows either event counts or percentage of events in the histogram. For either method, set the maximum value or have it automatically calculated by the software.

- Select Automatic Counts if you want the y-axis to scale automatically to the highest peak in the histogram.

- Select Automatic Percentage if you want the histogram to scale automatically to the highest percentage of the histogram data.

  The software finds the highest peak in each histogram and divides the number of events in the highest peak by the total number of events in the histogram. This percentage is used as the maximum value for the y-axis, and changes automatically as the data displayed in the plot changes.



- Select a value from the Percentage to Ignore menu to disregard outlying events when calculating the y-axis scale.

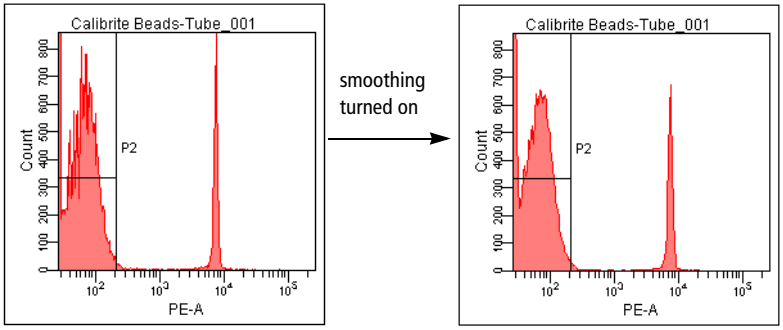
  A high number of events at either end of the x-axis can skew the maximum value. When a value is specified, the software disregards the selected percentage of bins at each end of the x-axis when automatically calculating the y-axis scale.

- Select Manual Counts to display a fixed count on the y-axis. Enter an integer between 1 and 50,000 in the numeric field.

- Select Manual Percentage to display a fixed percentage value on the y-axis. Enter an integer between 1 and 100 in the numeric field.

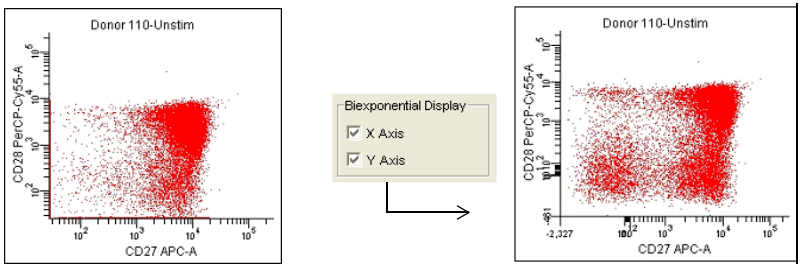
- Select the Fill Histogram checkbox to fill in the area between histogram peaks. Deselecting the checkbox will show the individual bins. (Individual channel bins are more apparent on a zoomed-in histogram.)

- Select the Smooth Histogram checkbox to display smaller spikes around the histogram peaks. Smoothing does not affect histogram statistics.



Using Biexponential Display

Digital data can have events with negative values. Biexponential display is used to show these events on plots and improve resolution between poorly resolved populations. To activate the feature for either plot axis, select the corresponding checkbox in the Plot Inspector. Note how biexponential display reveals the hidden double-negative population in the following example.



no biexponential scaling

biexponential scaling (both axes)

The linear range of the biexponential scale is determined by the extent of negative data for each parameter. During acquisition, data is periodically sampled to determine the scaling point. Once recording begins, periodic sampling stops if scales have already been determined, or after the first time the scaling point is determined during recording. Scales are recalculated after recording is finished, and any time cytometer settings are changed. If you change a compensation coefficient, new scales are calculated.

Width, ratios, and time are always shown on a linear scale; biexponential display does not apply for these parameters. In addition, original event data is maintained as the basis for statistical calculation and FCS export regardless of the data display.

For more information about working with biexponential plots see:

- Formatting Plots on page 200 to show gridlines on biexponential plots
- Working with the Biexponential Editor (next section) for instructions on how to adjust scales manually, apply scaling values to other tubes in an experiment, and import or export biexponential scale values
- Scaling to a Population on page 215 to optimize the display for a selected population
- Disabling Biexponential Display on page 216 to disable the feature
- Hiding and Showing Gates on page 229 for potential gating limitations
- Batch Analysis on page 248 to batch-analyze data files using the same biexponential scales

To practice using this feature, try the batch analysis tutorial in *Getting Started with BD FACSDiva Software*.

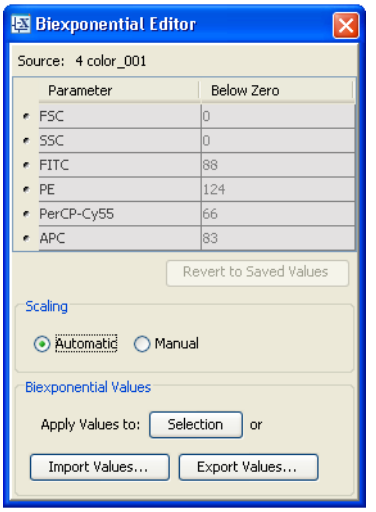
Working with the Biexponential Editor

When data is displayed on a biexponential scale, the software determines the extent of negativity based on the range of compensated data for all events in the FCS file. This is known as automatic scaling. Use the Biexponential Editor to

manually adjust the range of the negative scale (Figure 4-11). To display the Biexponential Editor, click the corresponding button on the Workspace toolbar ( ).

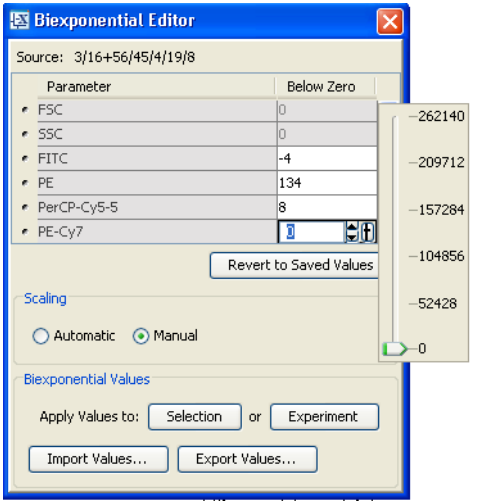
Tip Another way to adjust biexponential scales is with the Scale to Population feature. See Scaling to a Population on page 215.

Figure 4-11 Using the Biexponential Editor

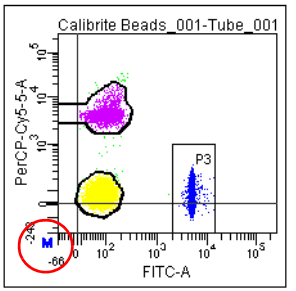


- To adjust scales manually, click Manual. The Below Zero fields are enabled for all listed fluorescent parameters. Click in any field to change the below zero value. Manual scaling values are saved with cytometer settings.

  To change values, enter a new value, click the up or down arrows, or drag the slider control.



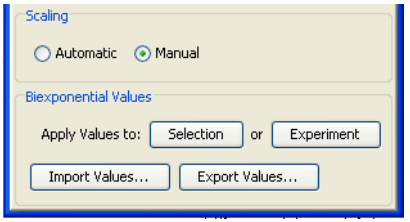
NOTICE Manual scaling values apply to all biexponential plots that show the associated parameter. Plots for which manual scaling are in effect are marked with an "M".



Click Revert to Saved Values to return to the original scaling values or click Automatic under Scaling.

Along with manual scaling, use the Biexponential Editor to export and import scale values, and apply values to other elements in an experiment.

- To apply biexponential values (automatic or manual) to other elements, click the Selection or Experiment button.



  Apply Values to Selection applies the biexponential values to only those tubes or specimens selected in the Browser; Apply Values to Experiment applies the values to all tubes.

- To save a set of biexponential values as an XML file, click Export Values.

  Enter the file name and specify the saving location in the dialog that appears. By default, files are saved in BDExport\Biexponential.

- To import a set of biexponential values, click Import Values.

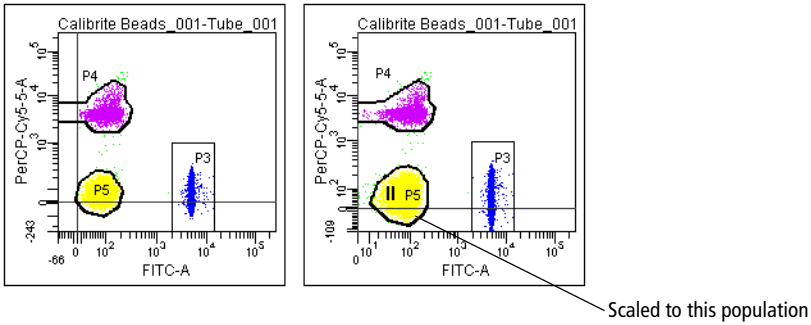
  Navigate to the XML file you want to import in the dialog that appears and click Import.

Scaling to a Population

The Scale to Population feature allows you to adjust biexponential scaling to fit a selected population. To use the feature, do the following:

1. Right-click a biexponential plot.
2. Choose Scale to Population, and select the population you want to scale to.

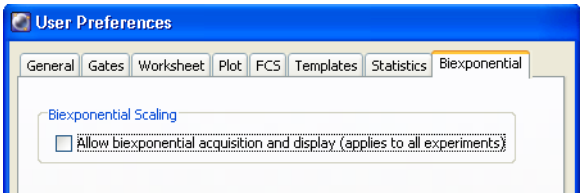
All of the plot's scaling adjusts to fit the selected population. The gate label is marked with two vertical bars (P2, in this example), indicating it is the population for which scaling is in effect.



☑ **Tip** To return to automatic scaling, right-click the plot and choose Scale to Population > All Events.

Disabling Biexponential Display

In User Preferences, you can elect to disable biexponential display by deselecting the checkbox to *Allow biexponential acquisition and display*. Disabling scaling allows more events to be recorded per experiment; however, large data files can be truncated when you re-enable it.



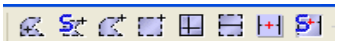
When you click OK, a message appears reminding you to restart BD FACSDiva software to make the change effective.

After you restart, plots that were set to biexponential switch to log, and biexponential features such as the Biexponential Editor and scale to population are no longer available.

Gates

Gates are used to identify and define subsets of data, or *populations*, on plots with linear, logarithmic, or biexponential scales. After defining gates, you can combine them to create joined, intersected, or inverted gates. Gated populations are used to generate statistics and limit the number of events collected or stored in the database. You can restrict a plot to display one or more populations, display populations in a hierarchical view, and use the population hierarchy to create subsets within defined populations.

Gates are defined using buttons on the Worksheet toolbar. There are three types of gating buttons:



- Manual gating buttons such as the Polygon or Rectangle Gate button allow you to define gate boundaries. See Drawing Manual Gates on page 219.
- Automatic gating buttons such as the Autopolygon or Autointerval Gate button automatically define gate boundaries for a selected population. Gate boundaries remain static after they are defined. See Creating Automatic Gates on page 221.
- Snap-To gating buttons such as the Snap-To or Snap-To Interval Gate button automatically define gate boundaries, but the boundaries change when data in the gate changes. See Working with Snap-To Gates on page 222.

To practice using gating features, try the gating exercises in *Getting Started with BD FACSDiva Software*.

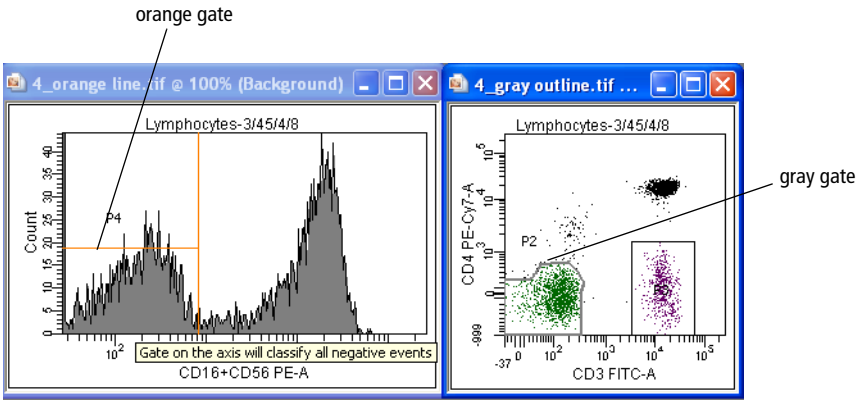
☑ **Tip** To create multiple gates, double-click a gate button. The button remains selected until you press the Esc key, select another button, or click the same button again.

NOTICE If you change the number of log decades for an experiment after gates have been defined, gated populations might be affected.

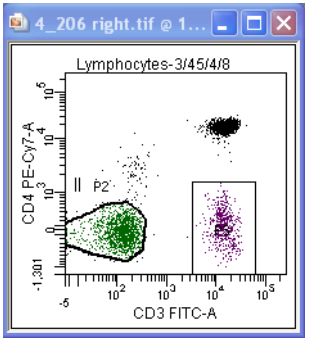
NOTICE Because of how digital data is displayed on a log scale, populations can be split or events can be hidden next to the plot axis. When you are drawing a gate, make sure to include all events. When events are on the axis, extend the gate boundary past the axis to capture all events, or use biexponential scaling to view all events.

When a gate crosses the axis on a log or linear plot, all events below zero (to negative infinity) will be part of the population it identifies. When a gate crosses an axis in a biexponential plot, the gate color turns orange to indicate that all events below the axis are included in the gate. This color change does not affect gates that are drawn below zero on a biexponential plot.

Figure 4-12 Gates for populations with below-axis events



A gate created on a log plot can now be shown on a biexponential plot and vice versa. Because gate geometry and movement might be different for a gate out of its home coordinates, this gate is indicated in gray.



Editing a gray gate will change it to black, indicating it has switched home coordinate systems.

Drawing Manual Gates

With manual gating buttons, the user defines gate boundaries. Manual gates include polygon, rectangle, quadrant, or interval gates.

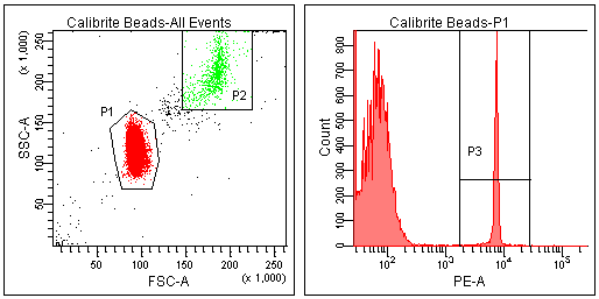
Note that populations defined by intervals and quadrants retain the color of their parent unless you have specified otherwise in User Preferences (see Gates Preferences on page 98). You can also change the color of a population using the Population Hierarchy Inspector. See Changing the Color of Populations on page 234.

	Use the Polygon Gate button to create a polygon gate on a dot, density, or contour plot. **1** Click the button and click in the plot to establish the starting point (first vertex). **2** Move the cursor to create the next vertex and click. **3** Continue moving the cursor and setting vertices; double-click the last vertex to complete the gate.
	Use the Rectangle Gate button to create a rectangle gate on a dot, density, or contour plot. **1** Click the button and click in the plot to position the gate. **2** Drag diagonally until the gate outline is the required size.

	Use the Interval Gate button to select a range of events in a plot. Interval gates can be used on dot, density, or contour plots, as well as histograms. **1** Click the button and click in the plot to position the left edge of the interval. **2** Drag the mouse to position the right edge.

Examples of each type of gate are shown in the following figure. The histogram shows events from the polygon gate. Notice how the population color does not change when you draw an interval gate.

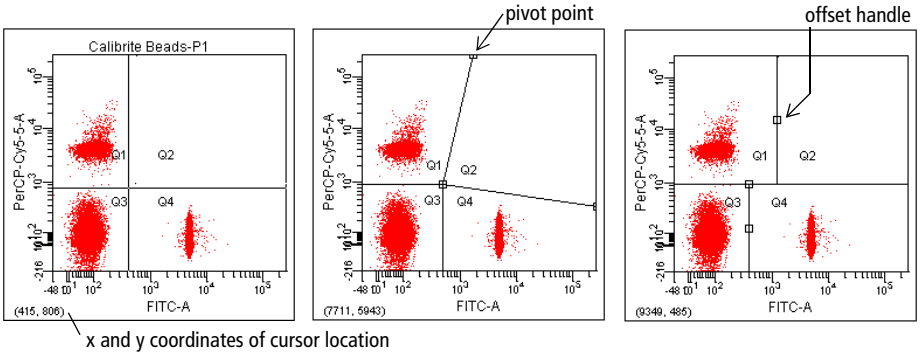
Figure 4-13 Polygon (P1), rectangle (P2), and interval (P3) gates



	Use the Quadrant Gate button to divide a dot or contour plot into four separate populations. Each quadrant population can be named and colored individually. Quadrant populations can be used for subsetting or sorting. **1** Click the button and click in the plot. **2** Drag the intersection of the quadrant markers to position the gate. The cursor location is indicated by the coordinates at the bottom of the plot (Figure 4-14 on page 221). **3** (Optional) Drag a pivot point to rotate the top or right segment, or drag an offset handle to offset a segment from the center point. For pivoted or offset segments, Shift-click the quadrant boundary to return the gate to its rectilinear form.

Examples of each type of quadrant gate are shown in the following figure. Each plot shows events from the polygon gate in the previous example. Notice how population color does not change when you draw a quadrant gate.

Figure 4-14 Rectilinear, pivoted, and offset quadrant segments



Creating Automatic Gates

With automatic gating buttons, gate boundaries are defined when you click a population in a plot. Unlike snap-to gates, gate boundaries remain static after they are defined. Automatic gates include autopolygon and autointerval gates.

☑ **Tip** Always inspect populations defined by automatic gates to ensure all required events have been included.

	Use the Autopolygon Gate button to automatically create a polygon gate around a population in a dot, density, or contour plot. **1** Click the button and click a distinct population in the plot. **2** Verify that the gate includes required events.
	Use the Autointerval Gate button to automatically select a range of events in a dot, density, contour, or histogram plot. **1** Click the button and click a peak in the plot. **2** Verify that the gate includes required events.

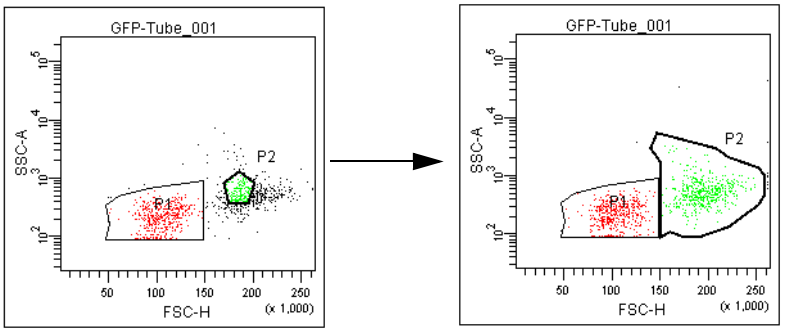
Working with Snap-To Gates

Two types of snap-to gates are available: snap-to polygon gates and snap-to interval gates. Snap-to gates are like autopolygon and autointerval gates in that a gate is drawn automatically when you click on events in a plot. Unlike their respective counterparts, however, snap-to gates are automatically redrawn when data in the gate changes.

	Use the Snap-To Gate button to automatically create a snap-to polygon gate around a population in a dot, density, or contour plot. **1** Click the button and click a distinct population in the plot. **2** Verify that the gate includes required events.
	Use the Snap-To Interval Gate button to automatically select a range of events in a dot, density, contour, or histogram plot. **1** Click the button and click a peak in the plot. **2** Verify that the gate includes required events.

Figure 4-15 illustrates the difference between an autopolygon gate and a snap-to polygon gate. Notice that the snap-to gate outline appears thicker to differentiate the snap-to feature. The snap-to gate changes after data is recorded while the autopolygon gate remains the same.

Figure 4-15 Autopolygon (P1) vs snap-to gate (P2)



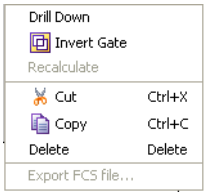
live acquisition recorded data

NOTICE Snap-to gates do not work well with diffuse clusters or rare events. Also, populations defined by snap-to gates (and any populations derived from them) cannot be sorted.

A snap-to gate will change under the following circumstances:

- after recording data, if the snap-to gate was created during live acquisition or on a plot without any data displayed
- if a change to the gate hierarchy results in a change to the data appearing in the snap-to gate
- if you edit one of the polygon vertices

NOTICE After moving a vertex, the snap-to gate does not readjust automatically. To force the gate to readjust, right-click the gate boundary or population name in the population hierarchy (see Using the Population Hierarchy on page 232), and choose Recalculate from the menu.



Adjusting the Movement of Snap-To Gates

A snap-to gate is automatically redrawn when the data in the plot changes, such as when live acquisition is finished or new data is read into the plot. When updating, the snap-to gate searches for a cluster closest to where it was originally placed. If no cluster is found, the system beeps and the snap-to gate maintains its original position.

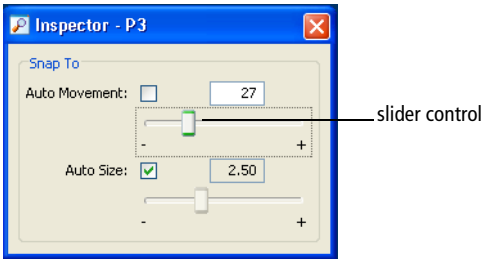
You can change how far the gate moves to find a new cluster by adjusting the Auto Movement value in the Inspector. The Auto Movement range is a percentage of the plot width, or resolution, from 0–100%. A higher Auto Movement value allows the snap-to gate to travel greater distances to locate a cluster. The snap-to gate retains this setting if another data file is read into the plot or the gate is applied to a new data file.

1. Select one or more snap-to gates in a plot.
2. In the Inspector, deselect the Auto Movement checkbox.

When the checkbox is selected, movement of the snap-to gate is limited to the software default value of 18 (not much movement).

3 Adjust the slider control toward the right until the gate encompasses the population of interest.

Alternatively, enter a value in the Movement field and press Enter.



Adjusting the Size of Snap-To Gates

Cluster variability can cause BD FACSDiva software to draw a snap-to gate around only a portion of a population. Use the Auto Size feature to adjust automatic sizing of the gate. A higher Auto Size value allows the snap-to gate to encompass a greater number of outlying events; a lower value restricts the gate to fewer outlying events. The snap-to gate retains this setting if another data file is read into the plot or the gate is applied to a new data file.

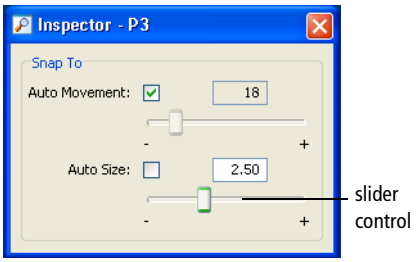
1 Select one or more snap-to gates in a plot.

2 In the Inspector, deselect the Auto Size checkbox.

When the checkbox is selected, the software automatically determines population size.

3 Adjust the slider control toward the right until the gate encompasses the entire population.

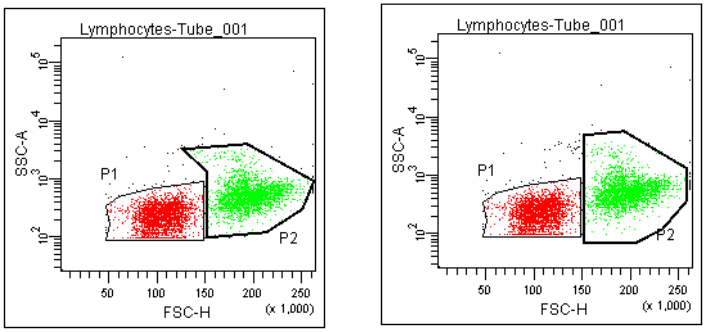
Alternatively, enter a value in the Size field and press Enter.



☑ **Tip** Display the statistics view to see the effects of the gate changes.

The following figure shows how adjusting the Size affects the snap-to gate in the plot.

Figure 4-16 Snap-to gate with automatic sizing (left) and user-adjusted sizing (right)



Auto Size on

Auto Size off

Tethering Snap-To Gates

A snap-to gate requires a minimum number of events in order to find a cluster. To automatically gate a small number of events or analyze an area of a plot that might or might not contain events, you can *tether* one or more manual gates to a snap-to gate. Tethered gate(s) move relative to the snap-to gate.

This feature is useful when you expect changes in the population of interest in relation to another population. You can use tethered gates to help automate rare event analysis. Tethered gates have the same properties as regular gates. For example, a plot can be gated and statistics can be generated from a tethered gate.

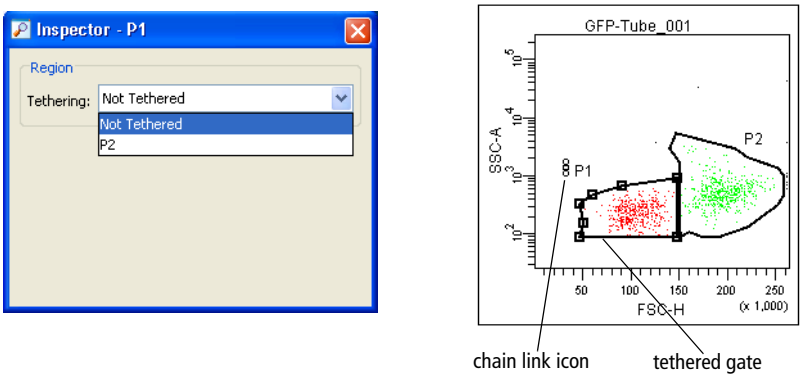
The following restrictions apply to tethered gates.

- For snap-to polygon gates, only interval gates with the same X parameter, or two-dimensional gates with the same X and Y parameters, can be tethered. For snap-to interval gates, only one-dimensional gates with the same X parameter can be tethered.
- Only manually drawn gates can be tethered to snap-to gates.
- Only one snap-to gate can be tethered to a manual gate; however, one snap-to gate can be tethered to many manually drawn gates.
- If you move, resize, or reshape the snap-to gate, the tethered gates remain the same. When you read in the next or previous file, the snap-to gate reverts to its previous position, size, or shape.
- If you move the tethered gate, the relative position is stored and used when reading in the next or previous file.
- A tethered one-dimensional gate can move only on the x-axis (horizontally).

Follow these steps to create a tethered gate. For an example showing how tethered gates adjust for subsequent data files, try the batch analysis tutorial in *Getting Started with BD FACSDiva Software*.

1. Create a polygon (P1) and a snap-to (P2) gate in an appropriate plot.
2. Select P1 in the plot.
3. In the Gate Inspector, select P2 from the Tethering menu.

   The menu lists all snap-to gates in the current plot and other plots that share the current plot's parameters.



When the gate is tethered, its boundary changes and a chain-link icon appears next to the gate label, as shown in the previous figure. Note that a tethered gate has a bold outline similar to a snap-to gate.

Editing Gates

Any gate can be moved or resized, but you cannot add a vertex to or delete a vertex from an existing gate. Statistics are automatically updated after a gate is edited. Changes to a parent gate will affect all populations derived from that gate (see Population Hierarchy on page 231).

1 Click once on a gate to select it.

Selection handles appear on the gate.

2 Make changes to the selected gate, then click outside the plot to deselect it.

- To resize a gate, drag any of the selection handles to a new location.To delete a gate, select the gate and choose Edit > Delete.

  Alternatively, right-click the gate and choose Delete from the menu. Gates derived from a deleted gate are also removed. For snap-to gates, any gates tethered to a deleted gate are untethered.

- To move a gate, drag the border of the gate. Note that the label moves with it. You can move the gate label independently by dragging just the label.

NOTICE To avoid confusion, keep gate labels close to the populations they identify. Labels for quadrant gates in rectilinear or offset mode cannot be moved outside their respective quadrants; however, labels for pivoted quadrant gates can be moved past their respective segments.

Note that non-adaptive gates on biexponential plots keep their on-screen size and shape regardless of the plot's scaling. You might have to move gates in response to new event positions as the scale changes.

Proportional Resizing of Gates

Use the proportional resize feature to resize all types of gates except quadrant gates. This feature only works on one gate at a time. There are two ways to proportionally resize a gate:

- To resize according to dragging direction, Shift-drag a gate vertex.

  Select a gate boundary from a plot, hold down the Shift key, and drag the vertex of the gate boundary. The gate boundary is resized proportionally according to the direction in which it is dragged.

- To resize maintaining original gate shape, Control-Shift-drag a gate vertex.

  Select a gate boundary from a plot, hold down both the Ctrl and Shift keys, and drag the vertex of the gate boundary. The gate boundary is resized proportionally according to its original shape.

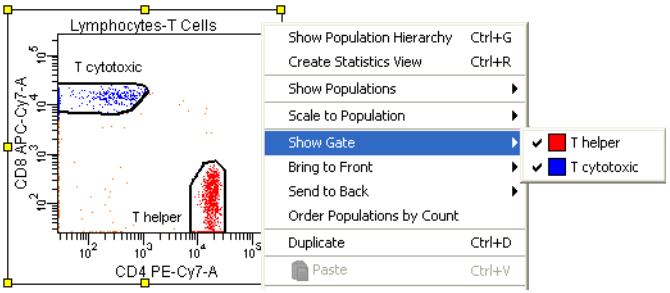
  NOTICE Proportional resizing does not allow dragging the vertex you've selected outside of the plot area. When the vertex reaches the edge of a plot, the gate boundary retains that shape and cannot be enlarged beyond the plot.

Hiding and Showing Gates

The boundaries of defined gates and their gate labels can be hidden or shown in any plot that shares the same parameters as the plot containing the original gate. To hide or show gates and their labels, right-click the border of the plot and choose Show Gate from the menu (Figure 4-17).

- If the gate boundary and label are currently showing, they are hidden.
- If the gate boundary and label are currently hidden, they are shown.

Figure 4-17 Showing a gate boundary and label



NOTICE When you create a gate on a log plot and show it in a biexponential plot (or vice versa), the gate geometry can change. In addition, when you adjust biexponential scaling, gates might move in unexpected ways if they were created on a log plot.

To remind you that a gate was created on a log plot when it is shown in biexponential, the gate changes color when you drag it.

Copying and Pasting Gates

Gates can be copied from one plot to another or from one population hierarchy to another. When you copy a population from a hierarchy and it contains subsets, the subsets are included when you paste the population to another hierarchy.

To practice working with this feature, refer to *Getting Started with BD FACSDiva Software*.

Dragging and Dropping Gates into Plots

Another way to create a copy of a gate is to use the drag and drop feature. Gates can be dragged and dropped in the same plot or to a different plot within the same worksheet. A gate can be pasted on plots with the same or different parameters. The pasted gate takes on the parameters of the destination plot and is proportionally resized based on the destination plot size and coordinate system. Drag and drop can also be used to drag gates into the population hierarchy. See Applying Gate Coordinates on page 237.

Drag and drop a gate by doing the following:

1. Select a gate.
2. Hold down the Ctrl key, then select and drag the gate to the new destination. A dialog is displayed if the same gate color exists in the new destination. Choose to use a new color or the same color.

   The population hierarchy is updated to reflect the new gate.

☑ **Tip** To select multiple gates on a plot, hold down the Ctrl key while clicking, then drag the gates to the destination plot.

If a tethered gate is dropped onto a new area or plot, the copy is not tethered. All types of interval gates can be dragged and dropped to histograms.

Dragging Gates into the Population Hierarchy

The drag and drop feature can be used in the population hierarchy to drag and drop gates within the same worksheet, either in the same population hierarchy or a different one.

1. Select a gate from a population hierarchy.
2. Hold down the Ctrl key and drag the gate under another gate in the same or a different population hierarchy. A dialog is displayed if the same gate color exists in the new destination. Choose to use a new color or the same color.

NOTICE You can only drag and drop one gate at a time.

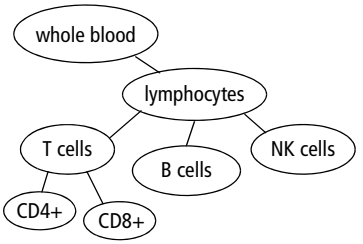
- Copying a parent copies all children under the parent.
- Copying a tethered gate creates a tethered copy only if its source is copied at the same time.
- When copying derived gates (AND, OR, NOT, Rest of), all of their source gates must be copied at the same time.

Population Hierarchy

All gates and their defined populations can be shown in a population hierarchy. Use the population hierarchy to see all populations defined for a tube and to view the relationship between gated populations.

For example, to define cell subsets during immunophenotyping of a whole blood sample, you first identify the lymphocytes, and then individual cell populations. The population hierarchy shows how these populations are identified by first defining a subset of lymphocytes from the whole blood sample (All Events), and then separating the lymphocytes into T, B, and NK cells. See Figure 4-18.

Figure 4-18 Immunophenotyping hierarchy and corresponding population hierarchy



Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
Lymphocytes	3,373	33.7	33.7
T cells	1,822	54.0	18.2
CD4+	1,282	70.4	12.8
CD8+	338	18.6	3.4
B cells	198	5.9	2.0
NK cells	1,243	36.9	12.4

☑ **Tip** To avoid errors when subsetting populations, always keep the population hierarchy in view. The last-selected population remains highlighted in the view, indicating that it will be the parent population of the next subset defined. Make sure the appropriate population is highlighted before you create a gate.

To show the population hierarchy, do one of the following:

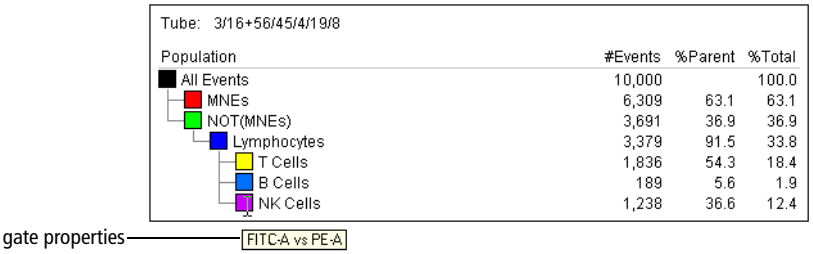
- Right-click a tube or plot and choose Show Population Hierarchy.
- Select a tube or plot and then press Ctrl-G.
- Select a tube or plot and choose Populations > Show Population Hierarchy.

Move or resize a population hierarchy just like any other worksheet element. See Aligning and Resizing Worksheet Elements on page 189.

Using the Population Hierarchy

Use the population hierarchy to:

- Rename populations—Select any population and enter text to change its name. The new name will appear on all plots displaying that population.
- Define population subsets—For an example, try the gating tutorial in *Getting Started with BD FACSDiva Software*.
- Copy gating hierarchies—When you copy a population from a hierarchy and it contains subsets, the subsets are included when you paste the population to another hierarchy.
- Change the color of defined populations—Double-click the color box next to the population name and choose a new color from the menu. See Changing the Color of Populations on page 234.
- View gate properties for any population—Place your cursor over any population to see the plot parameters its gate was drawn on, or its relationship to other gated populations.



- View the relative percentages of different populations—To see how these numbers are calculated, see Calculating Statistics on page 244.

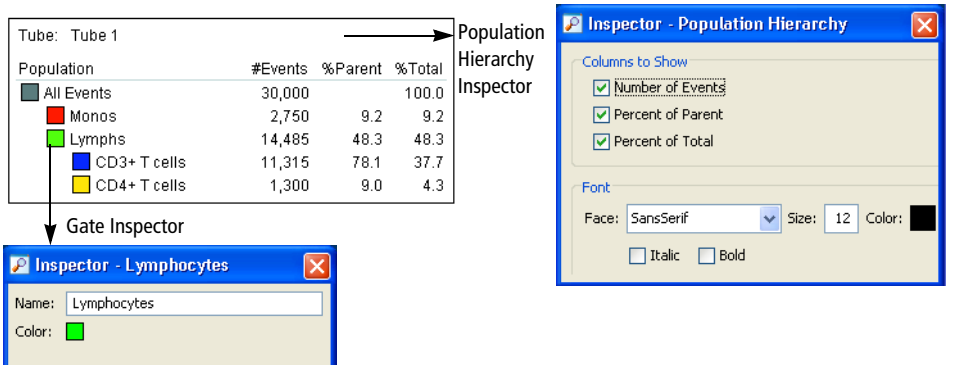
  NOTICE Because some events reside in more than one population, sibling percentages do not always add up to 100%. Invalid values are represented by ####.

- Display statistics for a single population—Right-click any population in the population hierarchy and choose Create Statistics View from the menu.

- Delete gates—Right-click any population and choose Delete, or select the population and press the Delete key.

Using Population Hierarchy Inspectors

Click anywhere inside the population hierarchy header to see the Population Hierarchy Inspector; click a population in the view to see the Gate Inspector.



- Use the Gate Inspector to change the population name and the color of events in a gated population. (You can also change a population name or event color directly in the population hierarchy.)

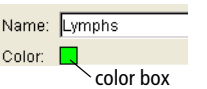
- Use the Population Hierarchy Inspector to select which information should appear in the population hierarchy and to change the font.

Changing the Color of Populations

By default, populations defined by quadrants and intervals are not assigned a color (☒) in the population hierarchy; they retain the color of their parent population. Default settings can be changed using User Preferences (see Gates Preferences on page 98). For all other gate types, populations are assigned the color of the last gate they satisfy.

Population colors can also be changed manually after a gate is defined.

1 Double-click the color box in a population hierarchy or click the Color box in the Gate Inspector.



2 Choose a new color from the palette that appears or click the No Color box (☒) to leave the gated events uncolored.

☑ **Tip** If you display a population that has been assigned No Color in a subsequent plot, no events will appear in the plot. If you plan to further subdivide any population, first assign it a color.

Creating Population Subsets

To restrict a subset to a certain population of events, do the following:

1 Select a population in the population hierarchy.

The population is highlighted.

2 Click the appropriate gate button.

3 Draw a gate in a plot.

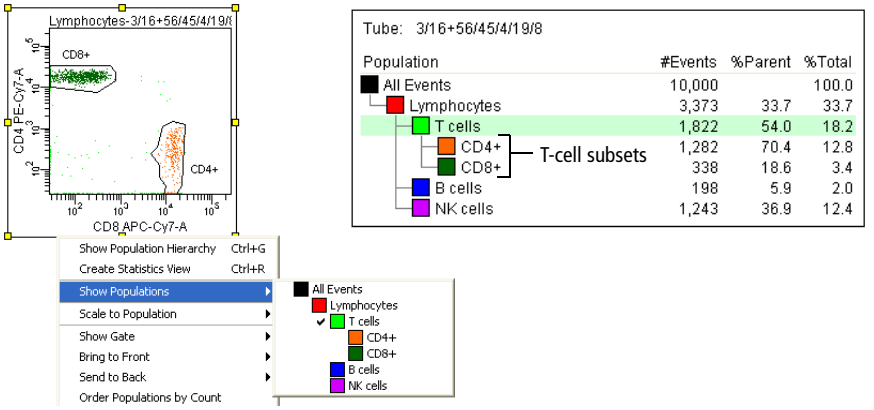
Alternatively,

1. Create a new plot, then choose Show Population and select the appropriate population (Figure 4-19).

2. Draw a gate around a subpopulation of the displayed events.

In either case, the new population appears indented below the selected population in the population hierarchy (Figure 4-19).

Figure 4-19 Creating population subsets

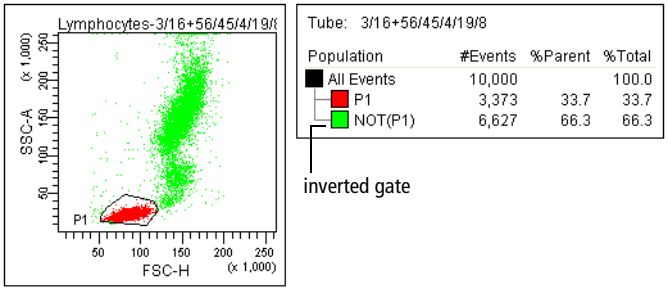


Defining a Derived Gate

Use one or more previously defined gates to create a *derived* (Boolean) gate. Derived gates consist of the following:

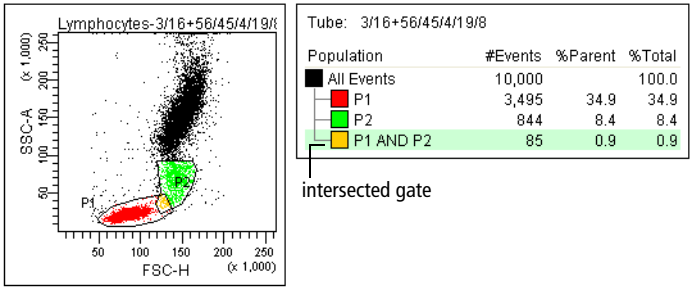
- Inverted gates use the NOT operator to select events outside a defined gate. Any event outside the specified gate satisfies the inverted gate.

  To define an inverted gate, right-click an existing population in the population hierarchy and choose Invert Gate.



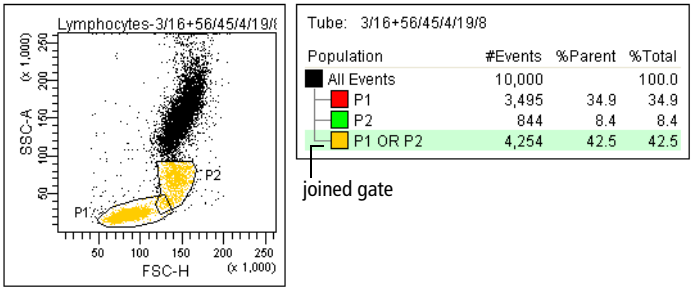
- Intersected gates use the AND operator to combine two or more individual gates. Only events that are in all of the specified gates satisfy an intersected gate.

  To define an intersected gate, select two or more populations in the population hierarchy. Right-click the selected populations and choose Intersect Gates.



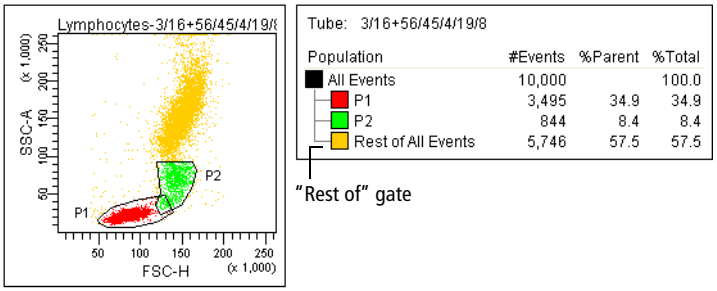
- Joined gates use the OR operator to combine two or more individual gates. An event that falls in any of the specified gates satisfies the joined gate.

  To define a joined gate, select two or more populations in the population hierarchy. Right-click the selected populations and choose Join Gates.



- *Rest of* gates select all remaining events that do not fall into any of the child gates of a parent gate. Thus, you can only access the *Rest of* option when you select a gate that already has subsets (children).

  To define a *Rest of* gate, right-click a parent population in the population hierarchy and choose *Rest of*. Events in the *Rest of* population retain the coloring of their parent unless you choose to change the color. The color was changed in the following example to illustrate the events.

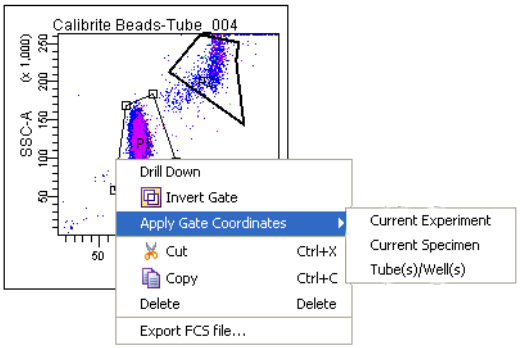


Applying Gate Coordinates

Use the Apply Gate Coordinates feature to apply the shape and size of a gate to other gates. The gate coordinates are applied to gate(s) of the same name and type (rectangle, polygon, etc) drawn on the same coordinate system as the selected gate(s), using the same parameters.

1 Right-click a gate on a plot or in a population hierarchy to select the gate whose coordinates you want to apply.

2 Select Apply Gate Coordinates, then select either Current Experiment, Current Specimen, or Tube(s)/Well(s) from the menu.



3 If Tube(s)/Well(s) is selected, a dialog is displayed. Select the tube or well to which the gate coordinates should be applied.

If Current Experiment or Current Specimen is selected, the Apply Gate Coordinates feature finds gates of the same name and type drawn on the same coordinate system as the selected gates that are using the same parameters, and adjusts the coordinates to match the selected gate(s).

NOTICE The Apply Gate Coordinates feature is not available for Snap-To gates or Snap-To interval gates. This feature can be used for normal worksheets as well as preferred global worksheets. This feature cannot be used for a global worksheet unless it is the preferred global worksheet of the specimen or tubes of the target selection.

Statistics

BD FACSDiva software generates statistics from the linear values of acquired events. Statistics can be displayed for any parameter and calculated for any defined population. Statistics are displayed on the worksheet, like a plot, and can be exported to a file.

NOTICE During acquisition, statistics are calculated for the number of currently displayed events and are updated as the display changes. For this reason,

responsiveness can decline as more statistics are calculated on a greater number of displayed events. After recording, statistics are recalculated on the total number of recorded events.

To display a statistics view, do one of the following:

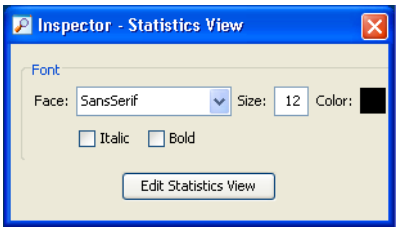
- Right-click a tube (normal worksheet view) or global sheet icon (global worksheet view) in the Browser and choose Create Statistics View. The resulting statistics view lists the number of events and %Parent for all populations defined for the tube.
- Right-click any plot and choose Create Statistics View. The resulting statistics view lists the number of events, %Parent, and means of the plot parameters for all populations displayed in the plot.
- Right-click a population in the population hierarchy and choose Create Statistics View. The resulting statistics view lists the number of events and %Parent for the selected population.

Move or resize a statistics view like any other worksheet element. See Aligning and Resizing Worksheet Elements on page 189.

Selecting Statistics to Display

Use the Statistics Inspector to change the font of the statistics view; use the Edit Statistics View dialog to specify the statistics to display. To access this dialog, do one of the following:

- Click the Edit Statistics View button in the Statistics Inspector.
- Right-click a statistics view and choose Edit Statistics View from the menu.

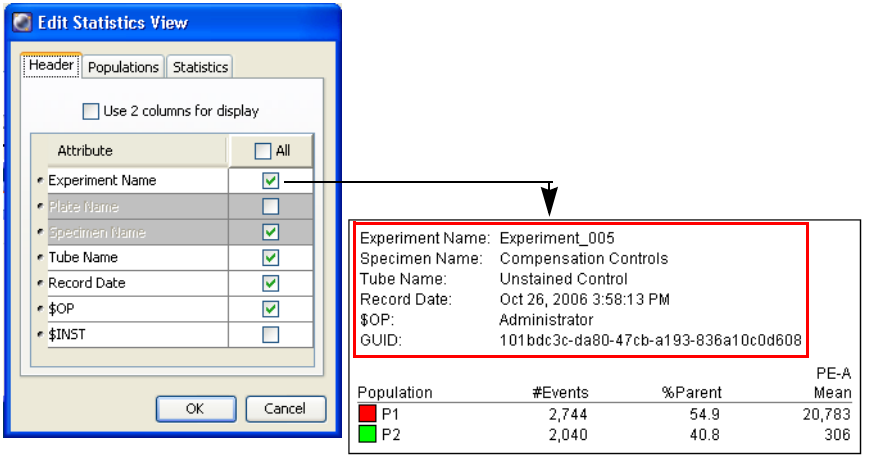


There are three components to the dialog, accessed by clicking the tabs labeled Header, Populations, and Statistics. See Figure 4-20.

Editing Header Information

Use the Header tab to specify information to be included in the header of the statistics view.

Figure 4-20 Statistics editor

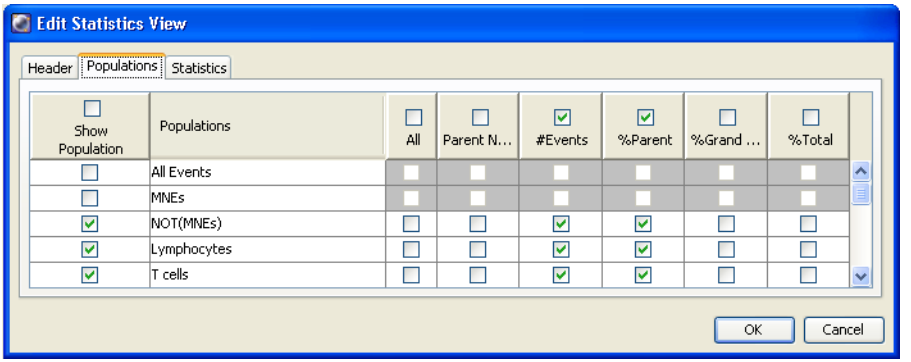


- Select the *Use 2 columns for display* checkbox to display header information in two columns.
- Display a header item by selecting its checkbox; delete an item from the header by deselecting its checkbox.
- Select the All checkbox to display all header items.
- Reorder header items by selecting any row and dragging it to a new position in the list.

To ensure that statistics views include a tube identifier, always include the GUID (globally unique identifier) in the header of statistics views.

Editing Population Statistics

Use the Populations tab to select the populations and types of population statistics to be displayed in each row of the statistics view. For a description of how statistics are calculated, see Calculating Statistics on page 244.

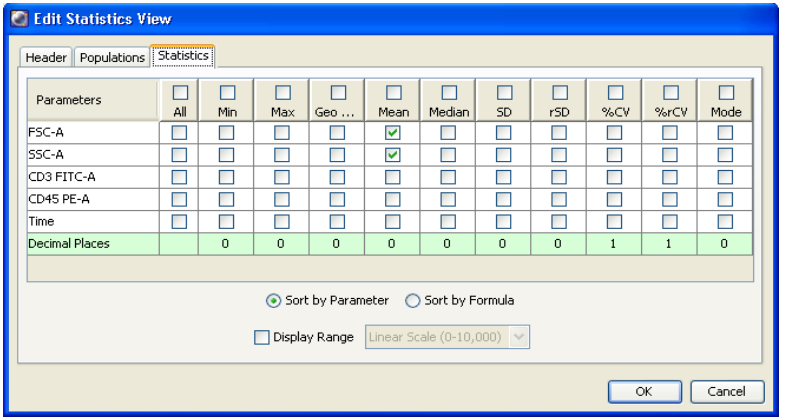


- To include a population in the statistics view, select the checkbox next to the population name in the Show Population column. To include all populations, select the checkbox at the top of the column.
- Specify additional population information to display by selecting the corresponding checkboxes.
  - Select the All checkbox in the population row to display all information for that population.
  - Select the checkbox in a column header to display that information for all selected populations.
  - Select individual checkboxes in a population row to display a subset of the listed information.
- Enter the number of integers (0 through 4) to display to the right of the decimal point for the %Parent, %Grandparent, and %Total statistics.
- Delete a population or statistic from the statistics view by deselecting its checkbox.

Editing Parameter Statistics

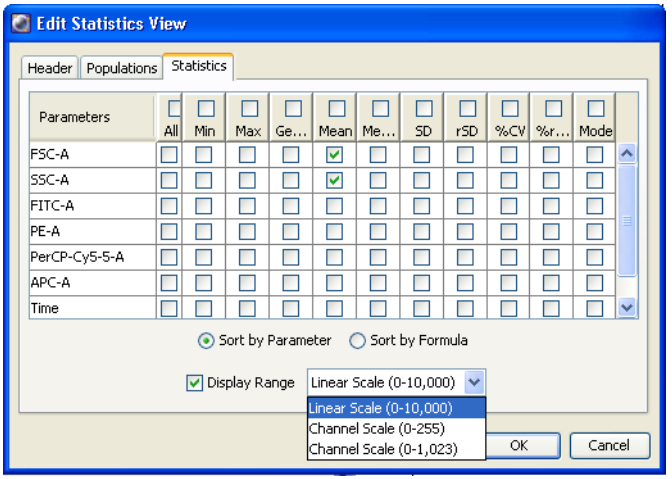
Use the Statistics tab to specify which parameter statistics are to be calculated and displayed in each column of the statistics view. Note that responsiveness can decline as you calculate more statistics on a greater number of displayed events.

For a description of how statistics are calculated, see Calculating Statistics on page 244.



- Within each row, select checkboxes for each statistic to display for that parameter, or select the All checkbox to display all statistics for that parameter.
- Within each column, select the checkbox in a column header to display that statistic for all parameters.
- In the All column, select the checkbox above the column header to display all statistics for all parameters.
- In the Decimal Places row, enter the number of integers (0 through 4) to display to the right of the decimal point for the statistic in the column header.
- At the bottom of the editor, select a button to specify how statistics should be sorted: by parameter (eg, FITC Mean, FITC CV, PE Mean, PE CV) or by formula (eg, FITC Mean, PE Mean, FITC CV, PE CV).

- At the bottom of the editor, select the Display Range checkbox to provide cross-matching scaling. In the menu, select the scaling.



When Display Range is selected and the Statistics View has two populations displayed (select under the Populations tab in Edit Statistics View), delta values are displayed. The delta values show the absolute difference between two statistics (eg, between the P1 mean and P2 mean.)

Experiment Name:	Blank Experiment with Sample Tube
Specimen Name:	Specimen_001
Tube Name:	Tube_001
Record Date:	Oct 31, 2006 3:31:33 PM
$OP:	Administrator
GUID:	e41a8ebe-9ffc-487d-9fbd-4426cab43dbd
Display Range	Channel Scale (0-1,023)

Population	#Events	%Parent	FITC-A Mean
P1	5,035	50	375
P2	4,965	50	887
Delta			512

When channel scales are selected, decimal places are not displayed in the Statistics View and the Decimal Places row in Edit Statistics View is unavailable.

NOTICE Statistics can be displayed for any tube parameter, as well as for the Time parameter. If a label has been specified for a parameter, it will appear before the parameter name.

Calculating Statistics

During acquisition, statistics are calculated on the number of currently displayed events. Recorded statistics are calculated on the total number of recorded events. All data originating from the digital electronics is linear and statistics are always calculated on linear data. When compensation is turned on, statistics are calculated on the compensated data.

Invalid values are represented by #### in a statistics view.

NOTICE Event data can be out of range when the compensation matrix is applied. In plots, the out-of-range data stacks at the margins of the plot but statistics are calculated on the out-of-range data.

The following statistics can be calculated:

- Number of events—total number of events in the defined population
- Parent—name of the next population up in the hierarchy
- %Parent—number of events in the defined population divided by the number of events in the parent gate (next population up in the hierarchy), expressed as a percentage
- %Grandparent—number of events in the defined population divided by the number of events in the grandparent gate (two populations up in the hierarchy), expressed as a percentage
- %Total—number of events in the defined population divided by the total number of events in the tube (all events), expressed as a percentage
- Mean—average linear value for events in the defined population, defined as

$$\bar{X} = \left(\sum_{i=1}^{n} X_i \right) / n$$

where n = number of events in the population, and X_i is a value for a particular parameter, where i = 1 to n

- Geometric mean—logarithmic average of the events in the defined population

  This mean is less sensitive to outliers than the regular mean. The geometric mean is defined as

$$\bar{X}geo = 10^{\left(\sum_{i=1}^{n} \log X_i \right) / n}$$

where n = number of events in the population, and X_i is a value for a particular parameter, where i = 1 to n

  NOTICE The geometric mean cannot be calculated for events with negative values. If you include the geometric mean for populations with negative values, the resulting statistics will be invalid (####).

- Median—linear value with an equal number of values above and below it

  NOTICE If the median of the data occurs between two values, those two values are added and divided by two to get the median.

  NOTICE During acquisition, BD FACSDiva software uses a faster but less accurate method to calculate the median. Thus, after analysis, the median value can differ slightly from what is observed during acquisition.

- Min—minimum linear value within a defined population

- Max—maximum linear value within a defined population

- Standard Deviation (SD)—a measure of the spread around the mean for events within a defined population, defined as

$$SD = \sqrt{\sum_{i=1}^{n} (X_i - \bar{X})^2 / (n-1)}$$

- Robust Standard Deviation (rSD)—The rSD is calculated as follows.

The median of the data sample is computed:

$$\theta_{median} = med_i\{x_i\}$$

From that, the median absolute deviation is computed:

$$\sigma_{median} = med_i\{(|x_i - \theta_{median}|)\}$$

Then the robust standard deviation is computed:

The constant is: $\sigma_{median} = \sigma_{median} / \phi^{-1}(0.75)$

- %Coefficient of Variation (%CV)—the SD divided by the mean within a defined population, expressed as a percentage

  The CV is defined as

  $$\text{Percent CV} = (SD/\bar{X}) \times 100$$

- %Robust Coefficient of Variation (%rCV)—the robust SD divided by the median within a defined population, expressed as a percentage

  The rCV is defined as

  $$\text{Percent rCV} = ((rSD)/median) \times 100$$

Exporting Statistics

Export statistics for use in a spreadsheet, word processing, or other third-party application. Statistics information (including header text) is exported to a file. Data from multiple tubes can be automatically exported to a single statistics file during a batch analysis or a BD FACS Loader run (BD FACSCanto cytometers only). For more information, see Batch Analysis on page 248 or refer to the *BD FACSCanto Flow Cytometer Reference Manual*.

To manually export statistics, do the following:

1 Choose Edit > User Preferences and select a manual export format.

See Statistics Preferences on page 103 for more information.

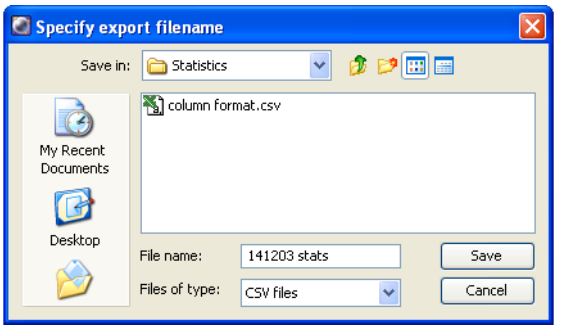
2 Select one or more statistics views.

3 Choose File > Export > Statistics.

4 Enter a name for the statistics file and specify a storage location in the dialog that appears (Figure 4-21).

☑ **Tip** To append statistics to an existing file, locate and select the file, and click Save. When prompted, click Append. Results will be appended to the selected file.

Figure 4-21 Exporting statistics



5 Specify the file type (CSV, XML, or Text) and click Save.

- Use CSV (comma-separated value) files for spreadsheet applications such as Microsoft Excel. Note that commas in the text of exported fields will cause the text to be split into two cells in the spreadsheet application.
- Use XML for text that can be used on any platform, including on the Internet.
- Use Text for word processing applications such as Microsoft Word.

Batch Analysis

Use the *batch analysis* feature to automatically advance a selected set of tube data through an analysis on a global worksheet. To practice using this feature, try the tutorial in *Getting Started with BD FACSDiva Software*.

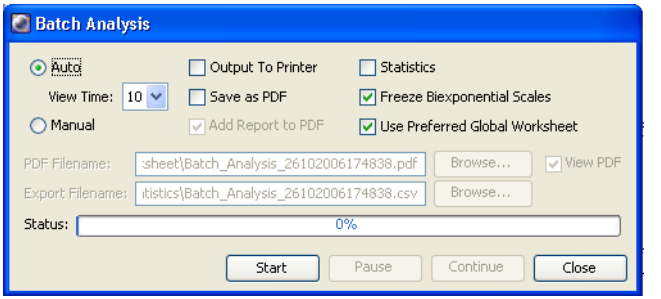
☑ **Tip** If you plan to export statistics during the batch analysis, choose your preferred auto export format in User Preferences before you begin. See Statistics Preferences on page 103 for more information.

1 Select an experiment, specimens, or tubes in the Browser.

If you select an experiment, all available data will be processed; if you select a specimen, only tubes under the selected specimen will be processed. Tubes without data are skipped during a batch analysis.

2 Right-click the selected item(s) and choose Batch Analysis.

The following dialog appears.



3 Select the type of analysis to be done.

- Select Auto to analyze all files with no user intervention. Data is displayed in the global worksheet for the amount of time specified in the View Time field (in seconds) before analysis of the next tube begins. Make adjustments to your analysis during this pause, or let analysis proceed automatically.

- Select Manual to pause the batch after data is loaded for each tube. Click the Continue button to proceed with analysis of the next tube. The View Time field is disabled when you select Manual analysis.

NOTICE If you make changes to the analysis during a pause, the changes remain in effect for the remainder of the tubes in the batch.

4 For an automatic batch analysis, choose the amount of time to pause (0–60 seconds) after each tube's data is loaded.

NOTICE Choose zero only if you want to process the batch without reviewing the data between tubes.

5 Specify whether to print worksheets or export statistics before data for the next tube is loaded.

- Select the Output to Printer checkbox to print a copy of the analysis for each tube.

- Select the Statistics checkbox to export statistics to a single CSV file for the batch. The resulting file can be opened with a spreadsheet application such as Microsoft Excel.

  Depending on the auto export format selected in User Preferences, each tube adds a new row or column of results to the file. For each population in the statistics view, the software adds parameter statistics in the order in which they appear in the view. A new header row is added if you add or delete statistics or parameters during batch analysis. You cannot add, remove, or edit statistics views while the batch is running.

6 To save the worksheets as a PDF file, select the Save as PDF checkbox. When Save as PDF is selected, the Add Report to PDF and View PDF checkboxes are selected by default. If you keep Add Report to PDF selected, the Batch Analysis Report is added at the top of the worksheets' PDF file. If you keep View PDF selected, the PDF is automatically displayed at the completion of the batch analysis.

7 For exported statistics, specify the file name and storage location in the Export Filename field or click Browse to select the location.

By default, files are exported to D:\BDExport\Statistics.

☑ **Tip** To append data to an existing file, select the file in the export file dialog and click Save. When prompted, click Append. Results will be appended to the selected file.

8 Specify whether to let biexponential scales fluctuate, if applicable.

When the Freeze Biexponential Scales checkbox is selected (default option), biexponential scaling does not change during batch analysis—all data is processed using scales from the tube with the current tube pointer. To allow scaling to change for each tube, deselect the checkbox.

9 If you have different global worksheets assigned to tubes, select the Use Preferred Global Worksheet checkbox to preserve worksheet assignments during the analysis.

When the checkbox is deselected, all tubes will be processed using the same global worksheet even if different worksheets were assigned.

10 Click Start to begin batch analysis.

A progress bar appears in the Status field showing the batch progress as a percentage (number of tubes completed vs total number of tubes in the batch). A message informs you when batch analysis is complete.

Working Offline

When using BD FACSDiva software for offline analysis (ie, the software is not connected to a cytometer), most cytometer, acquisition, and sorting controls are unavailable.

During offline operation, you can set up experiments or analyze recorded data. Display data for a recorded tube by clicking the current tube pointer in an open experiment; see Using the Current Tube Pointer on page 54. Analysis objects can be edited, moved, copied, and fine-tuned using gates and the drill-down function. Population hierarchies can be derived and statistics can be calculated and exported based on data previously recorded.

☑ **Tip** When you set up an experiment offline, you might need to create tube-specific cytometer settings and disable the *Use global cytometer settings* preference in the Experiment Inspector in order to maintain the settings when you connect to the cytometer. For example, when setting up an experiment for cytometer optimization, if your first tube uses a different set of parameters than your second tube and the global cytometer settings option is checked, experiment-level cytometer settings will be updated to match the first set of parameters when that tube is recorded, and these settings will remain in effect when you record the next tube. By disabling the preference, individual tube-specific settings are maintained.

5

Data Management

The following topics are covered in this chapter:

Working with BD FACSDiva Data

BD FACSDiva software stores and accesses all experiment data from a single database. As you create experiments in the Browser, the software writes experiment components to the database.

Any changes to an open experiment, related Browser elements, or worksheet are automatically saved when you close an experiment, quit the software, or click the Save button on the Workspace toolbar (). The database contains a record of all Browser items, worksheet elements, experiment settings, and cytometer control settings.

Recorded event data is saved separately in FCS 3.0 floating-point format.* A disk icon is added to a tube in the Browser when data has been saved for that tube. To analyze data in another software application or on a different BD FACSDiva workstation, data must be exported using the FCS Export option in the software (see Exporting FCS Files on page 259).

To ensure that data can be accessed by the software, do not move, rename, or delete the BDFACS.db file, BDFACS.log file, or BDData folder inside the BDDatabase folder. Do not change the name of any file or folder within the BDData folder.

* For more information, see isac-net.org.

Maintaining Data

Because all data is saved in a database, the database can fill up the hard drive. It is important to maintain the database by keeping the size below recommended limits, exporting and archiving data from the Browser on a regular basis, and deleting experiments, specimens, or tubes that are no longer needed.

For optimal performance, follow these precautions.

- The number of events that can be recorded for a single tube varies inversely with the number of gates and parameters (scatter parameters, fluorophores, area, height, width, and ratios). For optimal performance, limit the number of parameters to only those that are required.

- Monitor the disk capacity (see Verifying Database Size on page 257). The amount of hard disk space required for the database must not exceed 15 GB (the remaining disk space is reserved for a backup copy). When you are approaching the limit, delete unneeded experiments or export experiments and store them in an offsite storage location.

- Defragment the hard disk on a regular basis (eg, weekly). Diskeeper® defragmentation software is installed on workstations purchased through BD Biosciences. To run the program, choose Start > Programs > Executive Software Diskeeper. You can program the software to defragment the disk automatically on a preset schedule.

Data loss can occur if the defragmentation process is interrupted. BD recommends that you back up your data before running the defragmentation procedure and allow sufficient time to defragment the drive.

- BD Biosciences recommends that you disable sleep mode on your computer monitor when running BD FACSDiva software. Pressing keys while the system is waking up could execute an unwanted command that might result in loss of data without your knowledge.

Table 5-1 summarizes options for maintaining the BD FACSDiva database using the tools provided with the software. Follow the procedures established in your

laboratory for scheduling data backups. General guidelines are provided in the table and a recommended Weekly Maintenance Procedure is provided below.

Table 5-1 Data management options

Option	Function	When to Perform	See...
Export experiments	Copies experiments to a specified drive for data archiving	After each significant experiment	page 268
Export FCS[a] files	Exports FCS 2.0 or 3.0 list-mode data files	• After each significant experiment • For analysis in other software analysis applications, such as BD CellQuest Pro • After each recording, if selected in User Preferences, FCS tab	page 259
Delete experiments	Removes unnecessary or outdated components	As needed	page 258
Back up the database	Copies the current database to a specified drive using the Data Manager utility	Weekly	page 272

a. Enable the Export FCS preference to automatically export an FCS 3.0 file after each tube is recorded. To export FCS 2.0 data, you need to export manually.

Optimizing Data Processing

To save memory (and disk space for permanent storage), it is recommended that you save only parameters that are being used (for example, by deleting UV parameters when not running a UV experiment).

While there is no impact on data collection or cytometer performance, responsiveness can decline as more plots, statistics, gates, and events are

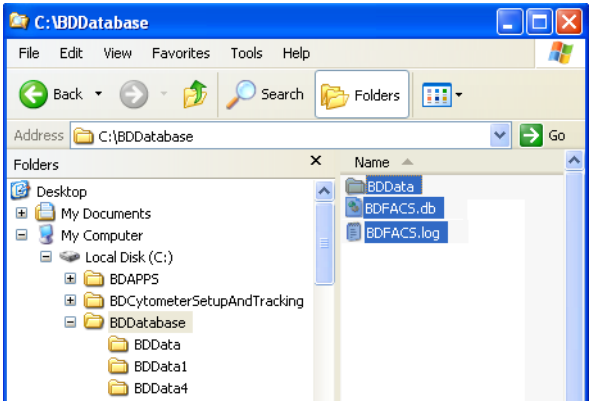
displayed for each tube. To improve system response time, limit the number of plots displayed in the viewable area of the Worksheet window.

Verifying Database Size

The database should not exceed 40–45% of the available disk space. (The remaining space is reserved for a backup copy of the database.) To determine the size of the database, do the following:

1. Use Windows Explorer to view the contents of the BDDatabase folder in the D drive.

2. Select the BDData folder, the BDFACS.db file, and the BDFACS.log file.



3. Right-click the selected items and choose Properties.

   A window appears showing the size of the selected components.

⚠ If the sum of all data files is approaching 15 GB (or 40–45% of the total disk space), delete or archive (export and delete) experiments to free up space on the drive.

☑ **Tip** To determine the amount of disk space being used, open My Computer and select the disk in Windows Explorer. The total size and amount of free space are shown.

Deleting Experiments

Do the following to delete unneeded experiments.

⚠ Deleting experiments also deletes any associated event data. Export experiments or FCS files before you delete.

1 Export FCS files or experiments to safeguard important data.

☑ **Tip** When exporting experiments, select the checkbox to automatically delete the experiments after export; the remaining steps are not needed.

2 In the Browser, select the folders or experiments you want to remove.

NOTICE When you delete a folder, you delete all experiments within the folder.

- To select multiple contiguous elements, click the first element; then hold down the Shift key while you select the last element.
- To select multiple noncontiguous elements, hold down the Ctrl key while you select each element to be removed.

3 Press the Delete key.

Alternatively, right-click selected elements and choose Delete from the menu.

Exporting and Importing FCS Files

Data for one or more tubes can be exported to an FCS file that can be read by other BD software and third-party applications. Files can be exported in FCS 2.0 or 3.0 format. To accommodate other software applications, FCS 2.0 log data is scaled down to four log decades during export.

NOTICE Cytometer settings are written to the FCS file but cannot be opened as a separate file in BD CellQuest or BD CellQuest Pro software or any other software application. Cytometer settings are not transferable between different cytometers.

Exporting FCS Files

You can enable a user preference to export FCS files automatically after each tube is recorded. See FCS Preferences on page 101. When files are exported automatically, they are exported as FCS 3.0 with all parameters included.

☑ **Tip** The FCS 2.0 standard limits the size of the data section to 99,999,999 bytes (roughly 2.5 million events with 7 parameters at 4 bytes per parameter per event). To export larger files, export as FCS 3.0 or limit the file size by exporting fewer parameters.

Follow the steps in this section to export FCS files when the automatic export preference is disabled.

⚠ When you export compensated data with negative values (see Using the Compensation Tab on page 143) as FCS 2.0, the negative values are set to zero in the exported file. When the file is imported and analyzed in another software application or in BD FACSDiva software, statistical results can be different from the original file.

1 Select experiments, specimens, or tubes to export.

Data can be exported from closed or open experiments; multiple items can be selected for exporting at one time. Individual FCS files will be generated for each tube in a selected specimen or experiment.

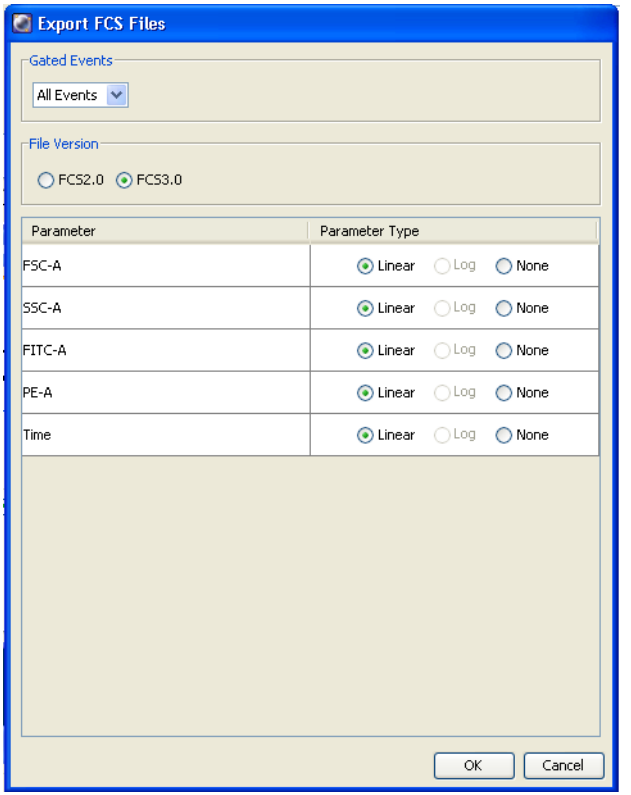
Experiment- and specimen-level keywords are exported with tubes. User-defined keywords are included in the header of FCS files.

2 Choose File > Export > FCS files.

Alternatively, right-click the selected item(s) and choose Export > FCS files.

3 Specify the FCS file version and parameters to export in the dialog that appears.

Figure 5-1 Export parameter options



When multiple files are selected, options apply to all exported files.

- Select the file format: FCS 2.0 (1024 or 10-bit resolution) or 3.0 (262,144 or 18-bit resolution). When FCS 3.0 is selected, only linear data can be exported.

  To make an exported file compatible with BD CellQuest Pro software, export it as FCS 2.0 with a maximum of 16 parameters (8 parameters for BD CellQuest software). To analyze compensated data, make sure the Enable Compensation checkbox is selected for each exported tube. Remember to run files through BD FACS™ Convert software before attempting to open them in BD CellQuest or BD CellQuest Pro software. Refer to the *BD FACSConvert User's Guide* for instructions.

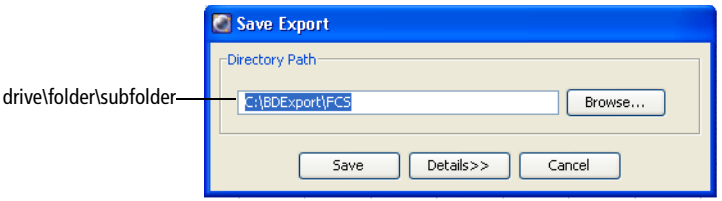
- Select Linear or Log for each parameter to be included in the data file; click None to exclude a parameter. (Log is available only for FCS 2.0 files.)

  FCS 2.0 log data is always exported in four decades; if the data spans five decades, only the upper four decades are included. You cannot select Log for the Time parameter; it is always exported as Linear.

☑ **Tip** Minimize the file size by excluding unnecessary parameters (select the None button). For FCS 3.0 files, make sure to include all parameters included in compensation calculations. See Important Considerations on page 266.

4 Click OK.

5 In the Save Export dialog, verify the file storage location.



- Click the Browse button to change the file storage location. Navigate to a different directory in the dialog that appears.

- Click the Details button to view the relative directory path and file name for each exported tube. Note that the file names are taken from the *specimen_tube* names in the Browser and cannot be changed.

☑ **Tip** To avoid confusion, store exported FCS files in a folder different from exported experiments. BD Biosciences recommends that you store exported FCS files in the BDExport\FCS folder that is set up for you during software installation.

6 Click Save to export the files.

Exporting Gated Events as FCS Files

Gated events can be exported as FCS files. Keywords are created for the tubes, as well as a keyword that gives the full gate name of the selected gate. For exporting multiple FCS files, a menu of common gates in the selected tube is displayed in the Export FCS Files dialog.

Common gates are gates with the same name (eg, P1) and type (rectangle, polygon, etc) that are drawn on the same coordinate system (log, linear, biexponential) in the selected tubes.

NOTICE If a gate is the same name but not the same type (eg, events are all P1 gates in rectangles except for a P1 in a polygon), the gate that is a different type is not displayed in the menu of common gates.

The default FCS file includes the specimen name, the tube name, and the gate name.

To export gated events from one tube:

1 Select the gated events for export either by clicking on the gate in the plots or by selecting the gate in the population hierarchy (you can only export one).

2 Right-click and choose Export FCS file.

3 Specify the FCS file version and parameters to export in the dialog that appears (Figure 5-1 on page 260).

4 Click OK.

5 In the Save Export dialog, click Save.

To export gated event from multiple tubes:

1 Choose File > Export > FCS files.

2 Under Gated Events, select from the menu of common gates to export.

When importing any FCS file that was exported using this feature, only the gated area of events is displayed, with the drawn gate no longer shown.

NOTICE Since exported gated events are no longer linked to the original file, if the file is deleted, events outside the gate are no longer accessible.

Importing FCS Files from BD Biosciences Applications

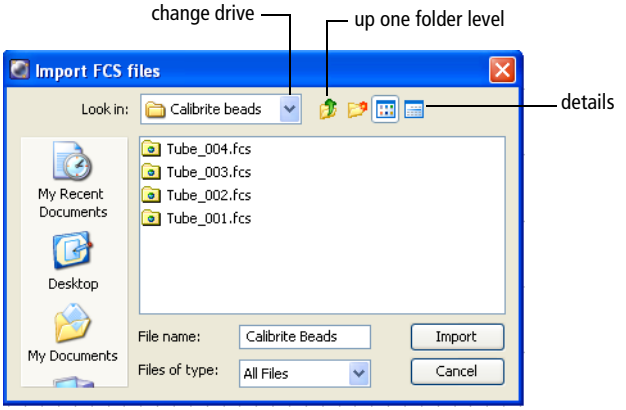
Follow these steps to import an FCS 2.0 or 3.0 file from any BD Biosciences software application.

1 Open the experiment that will contain the imported files.

Files can be imported into an open experiment only. Open an existing experiment or create a new one.

2 Choose File > Import > FCS files.

3 Locate the files you want to import in the dialog that appears.



- Use the buttons in the dialog to find the files to be imported.
- Select multiple files by holding down the Ctrl key as you click the file names.

☑ **Tip** To order imported files by date in the Browser, click the Details icon in the dialog and select the files in the date order you want.

NOTICE You can select any type of file, but only those files saved in a valid FCS 2.0 or 3.0 format will be imported.

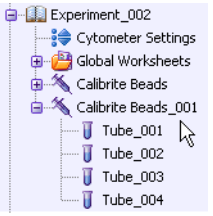
4 Click Import.

⚠ FCS files within an exported experiment do not contain the same information as FCS files exported using the Export FCS command. Do not import FCS files within an exported experiment using the Import FCS files command.

A progress bar appears showing the status of the import.

For each valid FCS file, a tube is created in a specimen in the open experiment. The specimen name is determined by keywords in the FCS file. If any of the following keywords is defined, the first defined keyword is used as the specimen name: $SRC, SAMPLE ID, PATIENT ID. Otherwise, a default name of Specimen_00*x* is used.

In the example shown to the right, the new specimen is appended with *_001* since the experiment already contains a specimen with the same name.



If the FCS file contains the TUBE NAME keyword, the value for that keyword is used as the tube name. If no TUBE NAME keyword exists, the FCS file name is used as the tube name.

Importing FCS Files from Other Applications

To convert imported data to the 18-bit linear format read by BD FACSDiva software, the software performs the following calculations.

NOTICE Linear data imported from other application in FCS 2.0 or FCS 3.0 file format is normalized to the range of BD FACSDiva linear data. The conversion of 256 or 1024 resolution data to 262,144 resolutions causes a binning effect in the plot displays.

- Linear data saved in FCS format from another software application is normalized to the range of BD FACSDiva linear data using the following formula:

$$X_{LIN} = X_{FCS} \times \frac{2^{18}}{\text{resolution}}$$

where X_{LIN} = linear BD FACSDiva data; X_{FCS} = linear data in FCS file; resolution = resolution in FCS file (256; 1024; or 262,144)

- Log data imported from FCS files is converted to linear data using the following formula:

$$X_{LIN} = 10^{\frac{x_{FCS} \times \#dec}{res}} \times \frac{2^{18}}{10^{\#dec}}$$

where X_{LIN} = linear BD FACSDiva data; X_{FCS} = log data in FCS file; #dec = number of decades in FCS file (usually four); res = resolution in FCS file (256 or 1024)

This places the data on the same scale as BD FACSDiva four-log data, from 26–262,144.

Important Considerations

Note the following behavior when exporting or importing FCS files.

- FCS files that begin with a space cannot be imported. Remove any leading spaces before importing FCS files.
- Any plots, gates, and statistics views associated with a tube are not included with an FCS file. To include analysis objects, export or import the experiment rather than the FCS file.
- While importing an FCS file, if the software determines that the file is too large to fit into memory, the data will be truncated and a warning message displayed.
- Data cannot be appended to an imported tube unless the data was imported as FCS 3.0, and the file was generated using BD FACSDiva software v2.0 or later.
- If an imported tube does not contain cytometer settings from BD FACSDiva software v2.0 or later, the cytometer settings cannot be copied and the Duplicate Without Data option is unavailable when the tube is selected in the Browser.
- When you export FCS 2.0 files, the Enable Compensation checkbox must be selected for each tube in order to export compensated data. If the files are exported as uncompensated data (Enable Compensation checkbox deselected), data can be recompensated after importing.

When you import FCS 2.0 files, the files cannot subsequently be uncompensated after importing if the files were exported as compensated data (Enable Compensation checkbox selected). Compensation values will be set to zero in the Inspector although the data appears compensated in the plots. Data can be further compensated by increasing the Compensation values.

- When you export FCS 3.0 files, a valid compensation matrix might not be created if a tube is exported with only a subset of its recorded parameters (that is, not all parameters chosen in the Export Parameters dialog). In this case, only compensated data is exported.

  If the tube contains enough parameters to construct a valid compensation matrix, uncompensated data is exported with the corresponding compensation matrix.

⚠ When you export compensated data with negative values (see Using the Compensation Tab on page 143) as FCS 2.0, the negative values are set to zero in the exported file. When the file is imported and analyzed in another software application or in BD FACSDiva software, statistical results can be different from the original file.

FCS 2.0 log data is scaled down to four log decades during export, which can also impact statistical results. After importing an FCS 2.0 file, any events that were below channel 26 are placed at 26, which can change statistics.

NOTICE Imported FCS files that were automatically created by Cytometer Setup and Tracking cannot be overwritten or appended.

Exporting and Importing Experiments

Use the File > Export > Experiments command to export experiments to the hard drive. During the export, you can choose to have the software remove exported experiments from the Browser. This removes them from the database, frees disk space, and can improve computer performance.

An exported experiment contains all Browser elements and their hierarchical structure, as well as worksheets and associated analysis objects (plots, gates, statistics views). Experiment elements are exported as an XML file, while data is exported in FCS 3.0 file format. To read the contents of the experiment, import it back into BD FACSDiva software using the Import Experiments command.

⚠ FCS files within an exported experiment do not contain the same information as FCS files exported using the Export FCS command. Do not import FCS files within an exported experiment using the Import FCS files command. To prevent confusion, BD Biosciences recommends that you store exported experiments in the BDExport\Experiments folder that is set up for you during software installation.

Exporting Experiments

1 Select one or more experiments in the Browser.

☑ **Tip** If one of the experiments is open or expanded, use the Ctrl key to select only experiments.

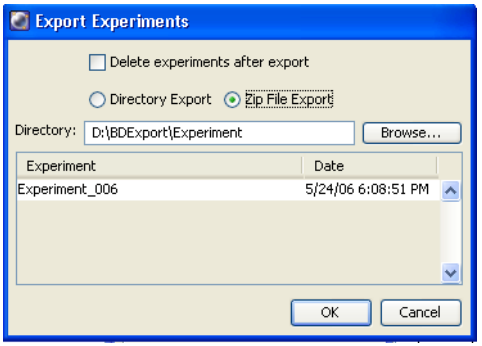
2 Choose File > Export > Experiments.

⚠ Make sure you choose the Experiments command, not the Experiment Template command. Data is not included when you export an experiment as a template.

3 Make appropriate selections in the Export Experiments dialog (Figure 5-2).

☑ **Tip** You can rename the experiment in this dialog before exporting.

Figure 5-2 Exporting experiments



- Select Directory Export to export the experiment into a folder. Select Zip File Export to export the experiment as a Zip file (with FCS files.)
- Verify that the experiments listed are those you intended to export. If they are not, click Cancel and repeat steps 1 through 3.
- Select the *Delete experiments after export* checkbox if you want to delete the listed experiments from the Browser after export.
- Specify the directory where the experiments will be stored. By default, experiments are exported to BDExport\Experiment. Enter the directory path in the field or click the Browse button and select the storage location in the location dialog that appears.

☑ **Tip** To avoid confusion, store exported experiments in a folder separate from exported FCS files.

4 Click OK.

The export process begins. If the *Delete experiments after export* checkbox was selected, each experiment will be deleted from the Browser immediately after its successful export.

NOTICE Exported experiments cannot be imported into older versions of BD FACSDiva software.

Importing Experiments

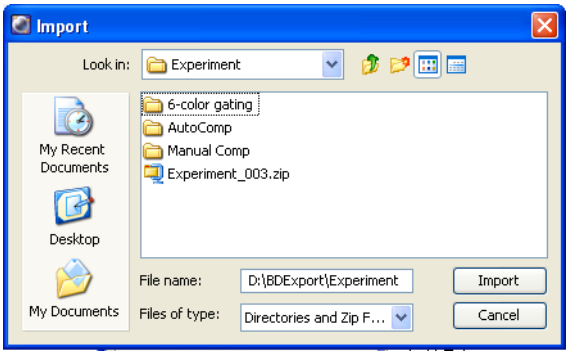
Experiments from the current or previous versions of BD FACSDiva software can be imported using the Import Experiments command. If an identically named experiment already exists in the Browser, the imported experiment is appended with _00*x*, where *x* is the next consecutive number for experiments of the same name.

NOTICE Only BD FACSDiva experiments can be imported using this command. To import data from another application, see Importing FCS Files from Other Applications on page 265.

1 Choose File > Import > Experiments.

2 Locate the experiment or Zip file to be imported in the dialog that appears (Figure 5-3).

Figure 5-3 Import dialog



3 Select the folder containing the required experiment and click Import.

NOTICE If you select a folder that contains only data, a warning message appears. Select only folders containing valid BD FACSDiva experiments.

Using the Data Manager Utility

BD FACSDiva Data Manager is installed during BD FACSDiva software installation. It can be used to back up the current database to disk or a mapped network drive and to replace the current database with a stored copy. To back up your database to another storage medium (CD or tape), refer to the documentation provided with your computer.

⚠ Do not move the Data Manager or run it from a batch file located outside the BD FACSDiva application directory.

To launch the Data Manager, do the following:

1. Quit BD FACSDiva software, if necessary.

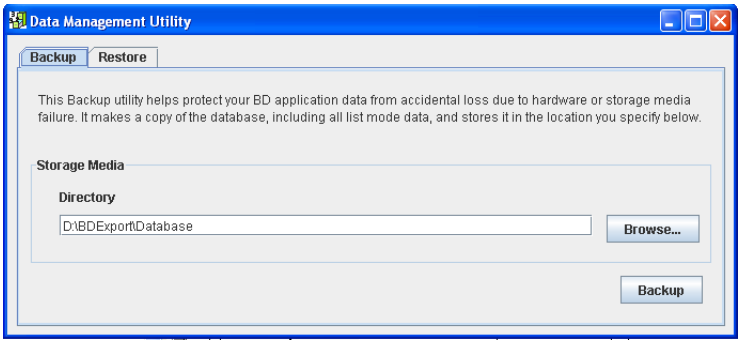
   NOTICE Data Manager cannot launch when BD FACSDiva software is running.

2. Launch BD FACSDiva Data Manager by double-clicking the shortcut on the desktop.

   shortcut icon on desktop

   The following dialog appears.



The Data Manager window has two tabs. Click the appropriate tab to initiate the following actions.

Backup—Creates a backup copy of the BDFACS database on a specified drive. See the following section.

Restore—Replaces the current database with a backup copy from a specified drive. See page 274.

Backing Up the Database

During a backup, the Data Manager copies the current database and associated list-mode data to a specified location. Backup files can be stored in any location on the hard drive as long as sufficient memory is available.

It is strongly recommended that you back up files to a hard disk or network device other than the one containing the database. If you back up to the disk containing the database and the hard disk crashes, both the BD FACSDiva database and the backup copy will be lost.

Before backing up the database, make sure you have adequate storage space on the backup medium. Exceeding the capacity of the hard disk can result in system errors and potential data loss.

If the workstation is connected to a network, files can be backed up directly to a mapped network drive. See Mapping a Network Drive on page 274.

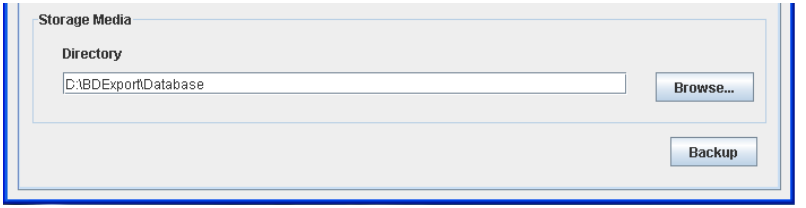
NOTICE Backing up does not free up space on the hard drive because the original files are retained.

☑ **Tip** To back up directly to a DVD, use the Data Manager to direct-format the DVD and then back up to the DVD. Otherwise, back up to the drive and use DVD Writer to copy the backup onto the DVD.

1. Launch Data Manager.

   See Using the Data Manager Utility on page 271. The Data Manager window appears with the Backup tab displayed.

2. Verify or change the path of the backup folder in the Directory field.



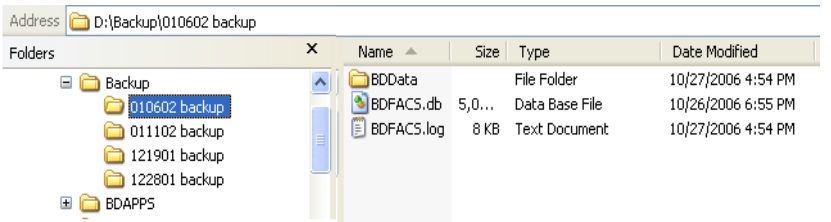
To change the path, click the Browse button. After mapping a network drive, files can be backed up directly to the network. See Mapping a Network Drive on page 274.

☑ **Tip** To keep track of backups, BD Biosciences recommends that you save each backup in a separate folder. Click the Browse button and select a new folder. Make sure your most recent backup is valid before you discard or overwrite a previous version.

3 Click Backup.

A progress box appears, and a message is shown when the backup is complete.

4 Use Windows Explorer to verify the presence of the backup files in the specified location.



Data Manager adds three items to the specified folder during a backup:

- a copy of the BDFACS.db database
- a copy of the BDFacs.log

- a copy of the BDData folder containing all FCS files in the database at the time of backup

Mapping a Network Drive

If your workstation is connected to a network, use the following instructions to create a network drive. Once the network is mapped, you can view the contents of the drive by clicking its icon in Windows Explorer or in My Computer. Files can be backed up directly to a mapped network drive using the Data Manager.

1. Launch Windows Explorer.

   Choose Start > Programs > Accessories > Windows Explorer.

2. Choose Tools > Map Network Drive.

3. Choose a Drive letter from the Drive menu.

4. Enter the path of the network backup folder in the Folder field (ie, *servername**foldername*).

   Alternatively, click the Browse button and navigate to the required folder.

5. Click Finish.

Restoring a Database

Use the Restore tab of Data Manager to replace the existing database with a backup copy. Note that when you restore data from a backup, Data Manager automatically stops the Sybase Adaptive Server® Anywhere service. Therefore, make sure you have privileges to stop and restart system services before you begin the restore.

NOTICE When you restore data from a previous release of the software, you need to reinstall the software after restoring the database. This will upgrade the

database to the latest compatible format. Any BD FACSDiva database from 4.2.1 or higher is supported.

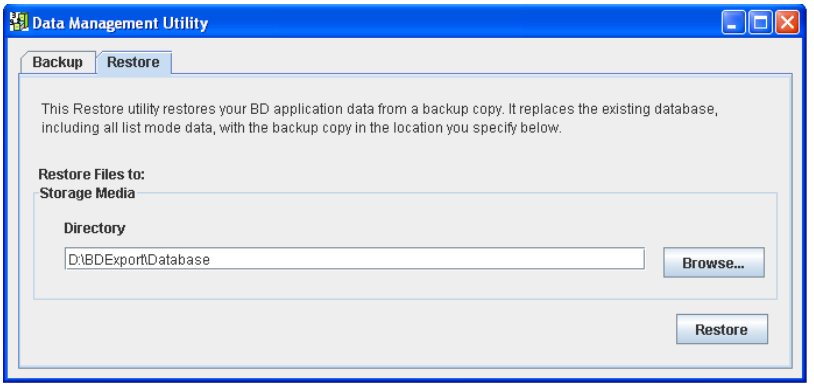
During a data restore, the current database is overwritten by the backup copy. Once the restore is in progress, it cannot be stopped or cancelled. To save your current data, export all experiments before you begin the restore.

1 Launch Data Manager.

See Using the Data Manager Utility on page 271. The Data Manager window appears with the Backup tab displayed.

2 Click the Restore tab.

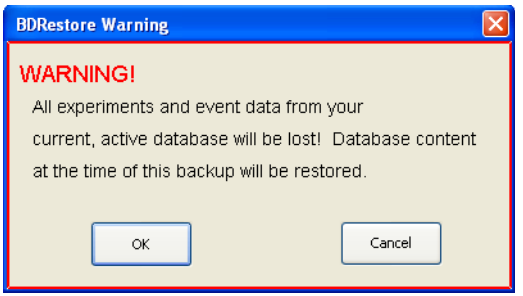
3 Locate your database backup.



To change the path, click the Browse button or enter the path in the Directory field.

4 Click Restore.

The following message appears:



5 Click OK to continue.

The Data Manager utility verifies that the user has logged in as the administrator. If the verification succeeds, the Data Manager restores the backup files to the D:\BDDatabase directory.

A progress box appears, followed by a message that the backup is complete.

NOTICE If an error occurs during this phase of the Data Manager Restore process, Sybase Adaptive Server Anywhere might have to be restarted manually. See General Software Troubleshooting on page 281 for instructions.

6 Click OK to close the message.

6

Troubleshooting

The tips in this section are provided to help you troubleshoot issues that might arise when using BD FACSDiva software. For cytometer-specific troubleshooting, refer to your cytometer manual.

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

Troubleshooting suggestions in this chapter are grouped under the following headings:

Installation Troubleshooting

Observation	Possible Causes	Recommended Solutions
VxWorks download failed.	Cytometer power is switched off.	Turn the cytometer power on.
	Electronics are not fully booted.	Reset the cytometer main power and restart the computer. After turning on the cytometer main power, wait 5 minutes before beginning the software installation.
	Communication failure between workstation and cytometer	• Quit the software and then restart it. • If restarting does not work, reset the cytometer electronics by switching the power off, and then on. Restart the computer.
	Ethernet cable between workstation and cytometer is disconnected.	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address has changed.	Enter the correct IP address. Call BD Biosciences for assistance.
	Software was installed for the wrong cytometer.	Uninstall the software, and then reinstall it. Make sure you select the correct cytometer at the Cytometer Selection screen.
Software message “Error 1301: Source file not found for Data1.cab”	D drive is not recognized by the installer as a logical drive.	Copy the CD contents to the C drive (Temp folder) and then click the Setup.exe icon.

Installation Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Saved databases from previous versions of the software cannot be restored into a new version of software.	Previous version of the software cannot be restored directly.	• Back up the current database. • Install the previous version of software and restore the database from that version. • Install the latest version of the software. Choose Existing Database during installation.

Electronics Troubleshooting

Observation	Possible Causes	Recommended Solutions
"Cytometer Disconnected" is in Cytometer window.	Cytometer power is switched off.	Turn the cytometer power on.
	Communication failure between workstation and cytometer	• Quit the software and then restart it. • If restarting does not work, reset the cytometer electronics: switch the power off, wait 10 seconds, and then switch the power on. Restart the computer.
	Ethernet cable between workstation and cytometer is disconnected.	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address has changed.	Enter the correct IP address. Call BD Biosciences for assistance.
"Upgrading firmware…" is in Cytometer window.	Firmware loading is incomplete.	Wait two minutes. If the message remains, restart the computer.
"Master DAQ Overflow" is in Cytometer window.	Event rate is too high.	Decrease the event rate or verify the threshold.
	Too many analysis objects are on the worksheet or too many events are displayed.	Delete analysis objects, decrease the Display value, or delete parameters from the cytometer settings Inspector.
"Cytometer not responding" status message	Various possible causes	Reset the cytometer electronics: switch the power off, wait 10 seconds, and then switch the power on. Restart the computer. ⚠ If this occurs during sorting, turn off the deflection plates before resetting the electronics.

General Software Troubleshooting

Observation	Possible Causes	Recommended Solutions
Software is not launching.	FTP service was started by another application.	Stop the FTP service for the other application. **1** Choose Start > Settings > Control Panel. **2** Double-click the Administrative Tools icon, and then the Services icon. **3** Select the conflicting FTP service, then click the Stop button. **4** Launch BD FACSDiva software.
	Database is loading.	Verify that the Start Services buttons are available. If the buttons are disabled, the database is still loading. A large database can take 30 minutes or more to load. ☑**Tip** Delete or export experiments to reduce the database size.
	Conflicting application, driver, or security update was installed.	Contact your service representative for assistance.

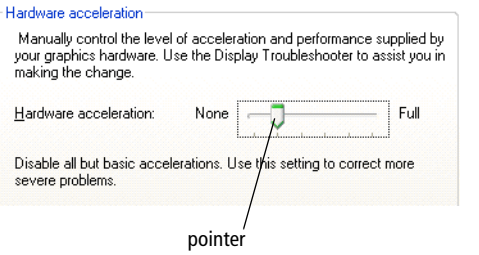
General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unable to access the database	Adaptive Server Anywhere is not running.	Verify that the database server has been started. **1** Choose Start > Settings > Control Panel and double-click the Administrative Tools icon. **2** Double-click the Services icon. **3** Select Adaptive Server Anywhere. If the Start button is enabled, click the button to start the database server. If the buttons are unavailable, the database could be loading.
Software is not responding.	Other applications are running in the background.	Quit all other applications. Do not run scheduled tasks such as virus scans or disk defragmentation in the background when you are running BD FACSDiva software.
	Software is saving or loading a large data file.	Look for activity on the screen. If there is no activity, restart BD FACSDiva software.
	Too many histograms are displayed.	If screen updating is slow, move some histograms down on the worksheet and scroll up.
	Too many plots or gates are in the worksheet.	Reduce the number of plots or gates.
	Large number of statistics are being calculated.	Calculating statistics is a memory-intensive operation. If you are calculating many statistics on a large number of displayed events, wait 1–2 minutes before you use the software.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Software message "Hardware key not accessible..."	Security module is disconnected.	Verify that the security module is securely connected in the USB port, and then restart BD FACSDiva software.
Error message is in Status tab.	Communication, fluidics, or sorting error	Shut down the computer and the cytometer, and then restart them. If the message reappears, contact technical support. Make sure you provide the exact wording of the status message.
Shortcut keys (Ctrl-*X*) or Delete key are not functioning.	Keys are not activated.	Use the menu selections to activate the keys. After the initial activation, the shortcut keys and Delete key can be used.
Numeric keypad is not functioning.	Num Lock key was reset.	Press the Num Lock key on the keyboard and try the keypad again.
No wireless keyboard or mouse response	Keyboard or mouse is too far away from workstation.	Move the keyboard or mouse closer to the workstation.
	Obstruction between the keyboard or mouse and the workstation.	Remove the obstructing object.
	Batteries are low.	**1** Replace the batteries. (Refer to the documentation provided with the keyboard or mouse.) **2** Restart the computer.
"Error 12" software message	Driver is not installed.	Reinstall the software.
Plot button is disabled.	No tube is selected in the Browser (normal worksheets only).	Select a tube to enable the button.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No cytometer settings are in Cytometer window.	Current tube pointer is not set.	Click to set the current tube pointer.
	No experiment is open.	Open an experiment.
Faulty screen display or slow user interface response	Another program is running.	Close all other open programs.
	Pointer shadow is enabled.	Choose Start > Settings > Control Panel. Double-click the Mouse icon and click the Pointers tab. Deselect the Enable pointer shadow checkbox, then click OK.
	Graphics hardware acceleration is too fast.	Decrease the hardware acceleration setting. **1** Right-click the desktop and choose Properties. **2** Click the Settings tab, click the Advanced button, then click the Troubleshooting tab. **3** Move the hardware acceleration pointer to the fourth tick mark down from the Full setting. **4** Repeat all steps for the second video card, if applicable. 

Compensation Setup Troubleshooting

Observation	Possible Causes	Recommended Solutions
Error creating compensation controls	Naming conflict with existing control or worksheet	**1** Locate the control or worksheet that is named *(ParameterName)* Stained Control, and change the name. **2** Create the compensation controls again.
Error calculating compensation	PMT voltages are not consistent between compensation controls.	Re-record all compensation controls with the same PMT settings.
	Insufficient single-stained controls for a setup	Calculate compensation manually. See Calculating Compensation Manually on page 168.
	Wrong number of gates for control plot(s)	• Display a population hierarchy and remove extra gates on control plots, if needed. • When no unstained control is included, create a gate around the negative population for each single-stained control.
	No data appears for one or more controls.	Record data for all controls.
	No root gate appears for first control.	Create a P1 gate in the FSC vs SSC plot for the appropriate control.
	Wrong fluorochrome run for the Stained Control	Re-record all compensation controls and recalculate.
	Insufficient events appear in the gated population.	Append data to the tube. Verify that the gate is set appropriately for the corresponding control.

Compensation Setup Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Error calculating compensation (continued)	Insufficient separation between positive and negative populations	Refer to your cytometer manual for suggestions on how to optimize the fluorescent signal. Re-record the compensation controls, draw new gates, and calculate the compensation again. If automatic compensation fails, perform compensation manually. See Calculating Compensation Manually on page 168.
BD FACSCanto setup file cannot be imported.	Setup file is missing or has errors.	Exit from BD FACSDiva software, re-run setup in BD FACSCanto clinical software, and open BD FACSDiva software again.

Analysis Troubleshooting

Observation	Possible Causes	Recommended Solutions
Fewer events than expected are in the gated population.	Current tube pointer is set to the wrong tube.	Set the pointer to the correct tube.
	On-axis events are left out of the gate.	Redraw the gate to make sure events on the axis are included.
	Plot is zoomed in.	Click the Zoom Out button for the plot or make the gate bigger.
	Laser delay is set incorrectly.	Adjust the laser delay settings. Refer to your cytometer manual for instructions.
	Window extension is set incorrectly.	Adjust the window extension. See Using the Window Extension on page 125.
Missing analysis objects on worksheet	Analysis objects are obscured by other objects.	Double-click the tube containing the analysis objects of interest. Select the objects on the worksheet and move them to another location.
No events are in plots.	Current tube pointer is not set.	Click to set the current tube pointer next to the appropriate tube.
	Data is no longer linked to the experiment.	Contact BD Biosciences for assistance in locating the missing data.
Differing statistics between exported and imported file	Compensated data with negative values is set to zero during FCS 2.0 export.	• Export the original data as FCS 3.0. • Export FCS 2.0 data with compensation disabled. • If statistical differences are significant, re-record the file.

Data Manager Troubleshooting

Observation	Possible Causes	Recommended Solution
Data Manager is not launching.	BD FACSDiva software is in use.	Quit BD FACSDiva software before launching Data Manager.
Message during Backup: "Unable to complete task. Exit value is: *x*."	Attempted to back up database into BD FACSDiva directory	Do not back up the database into the Program Files\BD FACSDiva Software folder. Back up the database to a different disk location.
Software message: "An error occurred while attempting to restore the database."	No valid backup is in specified directory	Verify that the directory path is entered correctly.
Unable to read or write FCS file when attempting import or export	Incorrect file name or file path	Verify the source and target file names and paths.
	File is in use by another program	Verify that the file is not in use by another program.
	File was created with an unsupported FCS version	Files for import or export must be created with a supported FCS version (2.0 or 3.0).
	Errors in file	If the FCS file is corrupt, regenerate the file before import or export.
	Incorrect data in file	FCS files must contain list-mode data and must have the correct byte order.
	Data limit exceeded (FCS 2.0 only)	FCS 2.0 file format can write up to 99,999,999 bytes of list-mode data.

Data Manager Troubleshooting (continued)

Observation	Possible Causes	Recommended Solution
Unable to read or write FCS file when attempting import or export (continued)	File is read-only or hidden.	Verify that the file is not set as a read-only or hidden file.
	Data type within file is other than Integer or Float.	When creating the FCS file, data type must be either Integer or Float.
	Incorrect resolution for one or more log parameters	Log parameters must have 1024 resolution.
	Incorrect keyword value	Verify that all keywords are correctly specified.
Unable to append data to an existing FCS file	Cytometer settings changed since original data was collected.	When appending data, make sure the cytometer settings match those of the data already recorded.
	File is FCS 2.0	You cannot append data to an FCS 2.0 file. Select an FCS 3.0 file instead.
One or more tubes were not exported.	No data in the tubes selected for export	Verify that the tubes selected for export contain data. Tubes with no data will be skipped.
	Incorrect parameters in tubes selected for export	Verify that the tubes selected for export contain the required parameters.
Data is truncated in the imported file.	File is too large to fit in available memory.	Move the file to a workstation with 2 GB of RAM to maximize available import space.

Printing Troubleshooting

Not all print drivers are compatible with BD FACSDiva software. For optimal printing results, BD Biosciences recommends that you use only PCL-native print drivers. Emulated or postscript printer drivers are not recommended. Many printers have multiple print drivers available. Check with your printer manufacturer to obtain an appropriate driver.

Print spooling problems can occur on some Tektronix Phaser printers, causing excessive amounts of data to be spooled. When this occurs, the print spooler can corrupt the data. If you experience problems printing through the print spooler, try one of the following.

- Print directly to the printer. See the following section.
- Use a third-party application to print to a PDF file, and then print the PDF file.

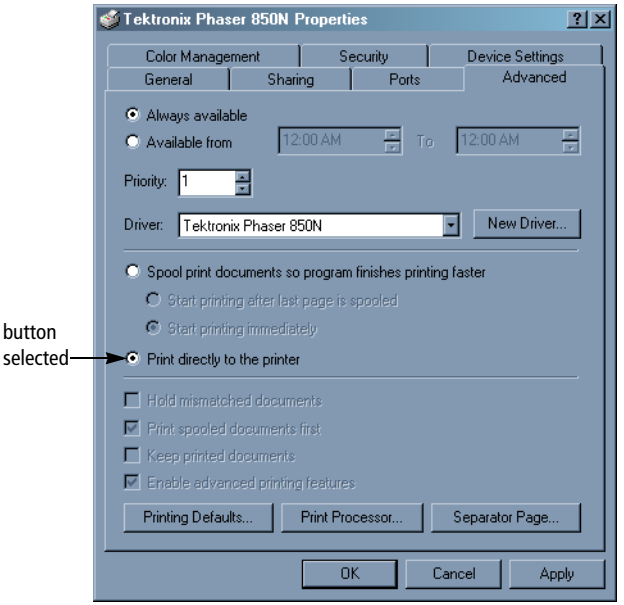
Printing Directly to the Printer

To set up printing directly to the printer, follow these steps.

1. Choose Start > Settings > Printers.
2. Right-click the appropriate printer icon and choose Properties.
3. Click the Advanced tab.
4. Select the *Print directly to the printer* button (Figure 6-1 on page 291).

   NOTICE When using this print option, BD FACSDiva software is not available during printing.

Figure 6-1 Printing directly to the printer



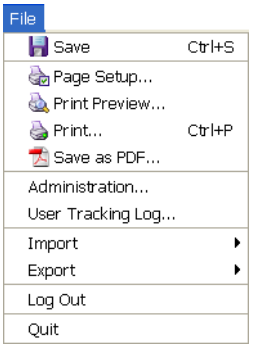
Appendix A

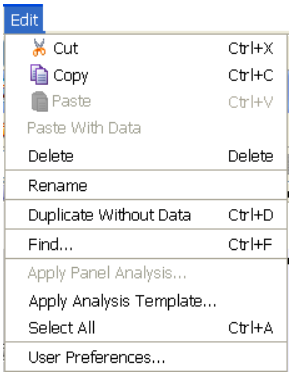
Menus and Keyboard Shortcuts

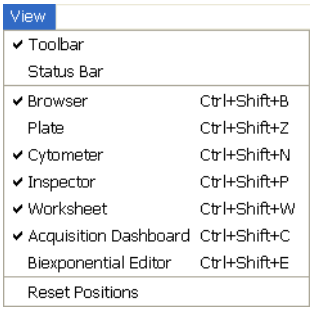
This appendix provides a guide to all software menus and a list of the available keyboard shortcuts. For more information, see the following:

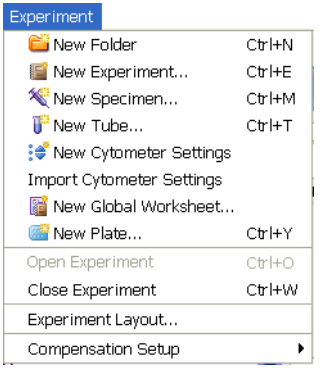
Software Menus

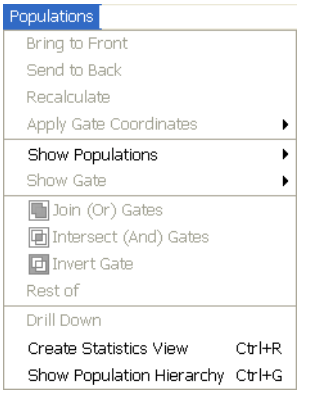
Choose a menu command in order to perform the corresponding task. When keyboard shortcuts are available, they are listed next to the command.

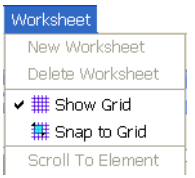


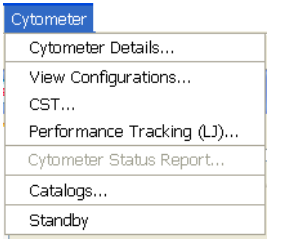


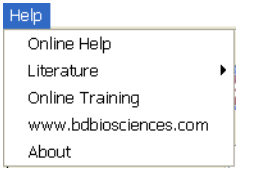








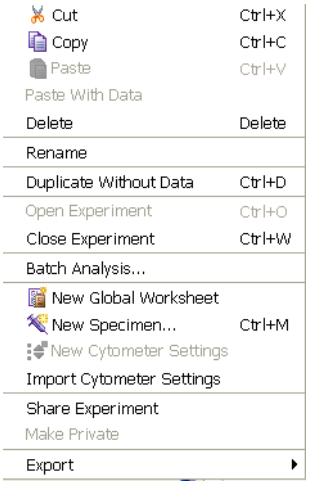




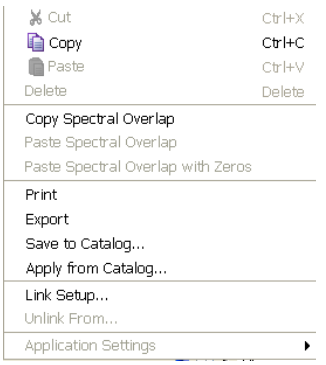
Menus

Right-click the indicated object to access the following menus.

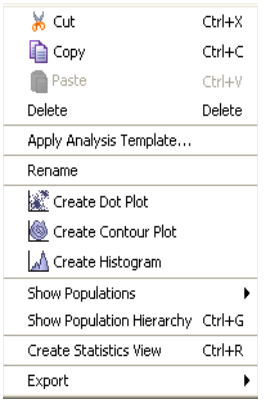
Experiment



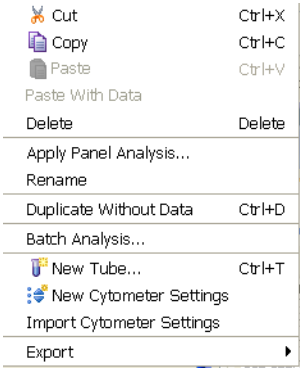
Cytometer Settings



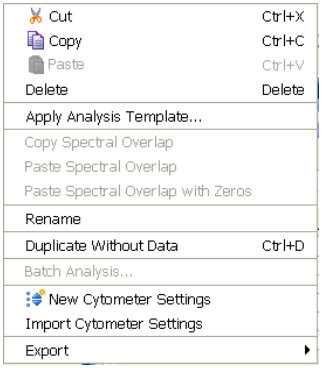
Global Worksheet
(analysis object existing)



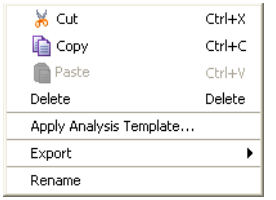
Specimen

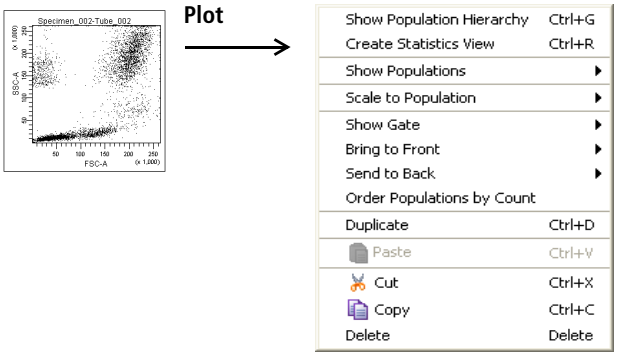


Tube



Analysis
(no analysis object set)





Keyboard Shortcuts

Keyboard shortcuts are provided for the following functions.

Objective	Key Combination	Condition to Activate Shortcut
Start/stop acquisition	Click Browser pointer	To start, the current tube pointer must be set (green); to stop, acquisition or recording must be in progress (yellow or orange pointer).
Start/stop recording	Alt-click Browser pointer	To start, the current tube pointer must be green or yellow; to stop, recording must be in progress (orange pointer).
Start batch analysis	Alt-S	Batch Analysis dialog must be active.
Pause batch analysis	Alt-P	Batch Analysis dialog must be active.
Continue batch analysis	Alt-N	Batch Analysis dialog must be active.
New Folder	Ctrl-N	User icon or folder must be selected.
New experiment from a template	Ctrl-E	Item must be selected in the Browser.
Open experiment	Ctrl-O	Closed experiment must be selected.

Objective	Key Combination	Condition to Activate Shortcut
Close experiment	Ctrl-W	Open experiment must be selected.
Find experiment	Ctrl-F	Item must be selected in the Browser.
Save experiment	Ctrl-S	
New specimen from a panel template	Ctrl-M	Item must be selected in an open experiment.
New tube with an analysis template	Ctrl-T	Specimen or tube must be selected in an open experiment.
Duplicate (Without Data)	Ctrl-D	• Specimen or tube must be selected in an open experiment (not available for imported tubes). • Plot must be selected on the worksheet.
Cut	Ctrl-X	Item must be selected in the Browser or on the worksheet.
Copy	Ctrl-C	Item must be selected in the Browser or on the worksheet.
Paste	Ctrl-V	Appropriate recipient must be selected in the Browser, or nothing must be selected for a worksheet element.
Show population hierarchy	Ctrl-G	Tube must be selected in an open experiment or plot must be selected on the worksheet.
Create statistics view	Ctrl-R	Tube must be selected in an open experiment or plot must be selected on the worksheet.
Print active worksheet	Ctrl-P	Experiment must be open.
To show or hide the following windows:		
Browser	Ctrl-Shift-B	
Cytometer	Ctrl-Shift-N	
Inspector	Ctrl-Shift-P	

Objective	Key Combination	Condition to Activate Shortcut
Worksheet	Ctrl-Shift-W	
Acquisition Controls	Ctrl-Shift-C	
Acquisition Status	Ctrl-Shift-T	
Sorting	Ctrl-Shift-S	
Carousel Controls	Ctrl-Shift-L	

Appendix B

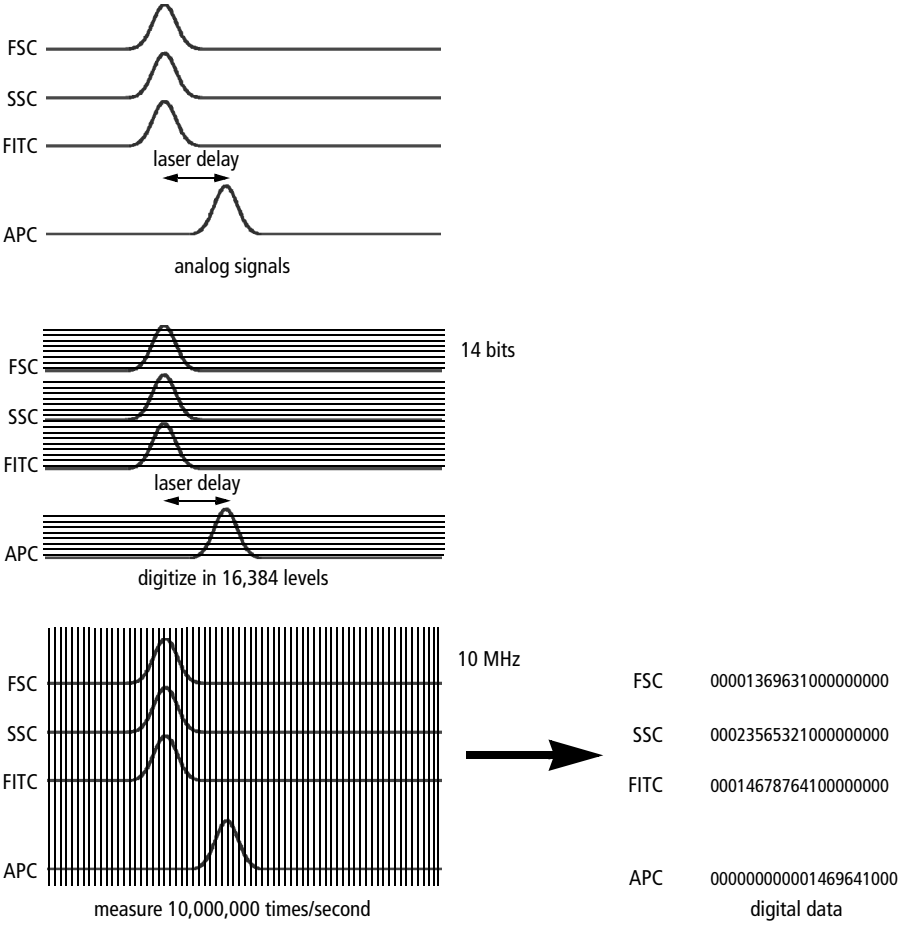
Digital Theory

This chapter gives a brief overview on the following:

How Digital Signals are Measured

In analog mode, pulses are sampled once per event. In digital mode, cytometer electronics continuously digitize signals to measure the light from the PMTs and FSC diode at a speed of 10 MHz, or 10 million times per second. This is done with 14-bit analog-to-digital converters that measure light in 16,384 discrete intervals. See Figure B-1.

Figure B-1 Generation of digital data



Threshold

Because BD FACSDiva data is digital, thresholds can be set as numerical values including logical *and/or* expressions. A threshold can be set on any signal from any laser.

Parameter Values

A *parameter* is a measurement of a cell property determined as the cell passes through the laser beam. Each parameter is the output of a single photomultiplier tube or photodiode, measuring fluorescent or scattered light.

Digital data can be measured for different pulse parameters. BD FACSDiva software measures area, height, and width for the number of channels available for your cytometer (depending on its configuration and installed options). Time is also a recorded parameter (see Using the Time Parameter on page 141).

- area = sum of all pulse heights (out of 262,144 for a typical pulse)
- peak (or height) = maximum digitized value of the pulse (out of 16,384)
- width = (scaled area divided by height) x 64K

Digital data is displayed on a 262,144 scale.

Area vs Height

Collecting area measurements for digital data provides greater resolution and scalability.

- When measuring area, the electronics add all measurements under the pulse, in effect increasing the resolution from 16,384 levels to close to 300,000 (for most practical applications). This is equivalent to approximately 18 bits (2^{18} = 262,144).

  To place BD FACSDiva data on the same scale (18-bit resolution), the software multiplies all height measurements by 16.

- When sheath pressure is low, events remain in the laser beam for a longer period, thus increasing the area measurement. In this case, a user-defined area scaling factor is used to bring area measurements back on scale. For example, area scaling can be used to align area measurements to height measurements. See Using Area Scaling on page 123.

Ratios

When working with digital data, ratios are not measured parameters. Ratios are calculated mathematically from uncompensated linear data and can be calculated between any two parameters, including area vs height.

Compensation

BD FACSDiva compensation is based on mathematical calculations performed by real-time digital signal processors, rather than on the hardware calculations performed by analog electronics. All data is collected as uncompensated linear data; thus, compensation can be adjusted during acquisition (ie, during setup mode) or during the analysis of recorded data. During analysis, data can also be converted to log.

Electronic Aborts

Under recommended operating conditions, all events are characterized, regardless of how closely they follow previous events. Thus, the rate of electronic aborts should be close to zero.

A small number of electronic aborts can be observed when a window extension is used. The window extension increases the amount of time during which the signal is measured. When two events pass closer together than the window extension allows, the system cannot precisely determine to which event the measured signal should be allocated. Therefore, it aborts both events. The number of electronic aborts is displayed in the Acquisition Status area of the Dashboard (see Acquisition Status on page 134).

Because aborted events are excluded from compensation calculations and are not passed on to the host computer, the system attempts to keep up with the data rate by aborting events as it approaches its throughput limit. The rate at which these aborts start to be generated depends on how many parameters you acquire and how much compensation needs to be calculated.

☑ **Tip** If the abort rate exceeds 10–20% of the total event rate, reduce the flow rate or exclude debris by raising the threshold.

For more information about the window extension, see Using the Window Extension on page 125.

Glossary

acquisition tube	Tube selected for acquisition or recording, as indicated by a green pointer
application settings	Cytometer settings that are linked with a specific cytometer configuration and automatically adjusted according to results obtained during the Cytometer Setup and Tracking daily setup
analysis	Numerical or graphical examination of data
analysis object	Worksheet elements used to analyze a tube; includes plots, gates, population hierarchies, and statistics views
area scaling	Correction factor to place area measurements on the same scale as height measurements
batch analysis	Software feature that automatically advances a selected set of tube data through an analysis template on a global worksheet
Browser	List of all experiment data in a hierarchical view; interface for setting up experiments and recording data
channel	Output from the channel DAQ board which measures the input of a single detector. Bins on a histogram are also referred to as channels.
coefficient of variation (CV)	The standard deviation of the data divided by the mean of the data; typically expressed as a percentage (also known as Relative Standard Deviation) When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.
compensation	Process by which spillover fluorescence is removed from secondary parameters so that fluorescence values for a parameter reflect only the fluorescence of the primary fluorophore

contour plot	Graphical representation of two-parameter data in which contour lines show the distribution of events Similar to a topographical map, contour lines show event frequencies as peaks and valleys.
current tube pointer	Pointer or plot icon next to tubes in an open experiment in the Browser. Indicates the tube currently selected for data acquisition, recording, or data display on a global worksheet When the software is connected to the cytometer, the pointer can also be used to control acquisition.
cytometer settings	Collection of values for parameters measured, photomultiplier (PMT) voltages, threshold, compensation, and any ratio measurement collected
cytometer setup and tracking	Setup feature in BD FACSDiva software workspace
Cytometer Setup and Tracking beads	Beads used to automatically perform basic cytometer setup and performance tracking
data file	A collection of measured values from a single tube combined with text describing the data that has been stored to disk
density plot	Graphical representation of two-parameter data in which colored dots show density for events with the same signal intensity A density plot simulates three-dimensional event display.
derived gate	Combination of one or more defined populations using the Boolean operators AND (intersected gate), OR (joined gate), or NOT (inverted gate)
dot plot	Graphical representation of two-parameter data Each axis of the plot displays values of one parameter; a dot represents an event (particle).
Events to Record List	The Events to Record List is one method for entering numbers of events in the Events to Record fields. This list is in Experiment Layout.

experiment Group of elements used to record and analyze data from the flow cytometer

An experiment can include any combination of the following: global worksheets, specimens, tubes, FCS data files, keywords, plots, gates, statistics, population hierarchies, worksheets, text, lines, or arrows.

flow cytometry standard (FCS) Standard format for flow cytometer data files

gate Two-dimensional boundary defining a subset of the total sample population

See also derived gate, interval, population.

global worksheet Worksheet for which elements can be used to display multiple data sets by moving the current tube pointer

See also worksheet, Worksheet window.

grid Cross-hatched lines displayed on the worksheet used to align and resize analysis elements. Analysis elements can be snapped to the grid.

histogram Graphical representation of single-parameter data

The horizontal axis of the graph represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).

Inspector Software interface for viewing or modifying the attributes of a single object or set of objects on the worksheet or in the Browser

interval One-dimensional boundary defining a subset of the total sample population

See also gate, population.

laser delay Amount of time between signals from different laser intercepts

panel analysis A panel template that is used to apply changes to the normal worksheet elements of a selected specimen

panel template Group of labeled tubes commonly used together in the same experiment

Any specimen can be exported as a panel. Along with the specimen name and collection date, an exported panel contains a group of tubes and any parameter labels defined for each tube. Exported specimen panels can also include global worksheets and their associated analysis objects.

parameter Measurement of a cell property that is ascertained as the cell passes through the laser beam

Each parameter is the output of a single photomultiplier tube or photodiode, measuring fluorescent or scattered light.

population Data subset defined by a gate or interval

robust co-efficient of variation (rCV) Robust coefficient of variation is calculated as follows:

%rCV = ((rSD)/median)*100)

robust standard deviation (rSD) Robust standard deviation is calculated as follows.

The median of the data sample is computed:

$$\theta_{median} = med_i\{x_i\}$$

From that, the median absolute deviation is computed:

$$\sigma_{median} = med_i\{(|x_i - \theta_{median}|)\}$$

Then the robust standard deviation is computed:

The constant is: $\sigma_{median} = \sigma_{median} / \phi^{-1}(0.75)$

snap-to gate Gate drawn automatically when you select a peak or cluster of events in a plot

Unlike static gates, snap-to gates are automatically redrawn when data in the gate changes.

specimen Browser object representing the type of material to be analyzed, the tubes used to analyze the material, the collection date, and user-defined keywords

spectral overlap Fluorescence detected in a channel other than the one for which it is intended

standard deviation (SD) A measure of the spread around the mean for events within a defined population, defined as

$$SD = \sqrt{\sum_{i=1}^{n} (X_i - \bar{X})^2 / (n-1)}$$

stopping gate Population for which events are to be counted

storage gate Population for which events are to be recorded

tethered gate Static gate linked to move relative to a snap-to gate

threshold A trigger signal and level of discrimination to eliminate unwanted events

Only events with parameter values above the threshold will be analyzed.

tube Browser object representing cytometer settings, acquisition criteria, parameter labels, recorded event data, analysis objects, user-defined keywords, and Sort Layouts for a single sample

window extension Extension of the time during which a pulse is sampled

window gate Time during which a pulse is sampled, based on the threshold value and window extension

worksheet Tabbed area within Worksheet window for displaying data for a specific tube. All objects on a worksheet are printed with a single Print command.

See also global worksheet, Worksheet window.

Worksheet window Window for viewing analysis objects on worksheets or global worksheets

workspace window Component in BD FACSDiva software workspace

Workspace windows can be hidden or shown, resized, and closed. The visibility, size, and position are saved when you quit the software and are restored when you start the software the next time.

Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z