

BD FACSDiva Software Reference Manual

For In Vitro Diagnostic Use

<http://www.bdbiosciences.com/>
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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. Refer to the ReadMe file for details.
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. Refer to the ReadMe file for details.
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. Refer to the ReadMe file for details.
337370 Rev A	1/04	Updated for version 4.0: user login, shared vs private Experiments, new Worksheet tools (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. Refer to “New Features” in the online help for details.
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. Refer to New Features in the <i>BD FACSDiva Software Quick Start Guide</i> for details.

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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 the instrument user's guide.

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 instrument 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, use the *BD FACSDiva Software Quick Start Guide*.

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 guide. 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
<i>Italics</i>	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, “choose File > Print” means to choose Print from the File menu.
Ctrl-X	When used with key names, a dash means to press two keys simultaneously. For example, Ctrl-P means to hold down the Control key while pressing the letter <i>p</i> .

Using Microsoft Windows

- Tip** To delete objects in the workspace, be sure to press the Delete key rather than the Backspace key.

You will notice that the Windows mouse has two selection buttons. Most actions are performed with the left button. Press the right mouse button while clicking an icon or object to open a contextual menu. Contextual menus contain common commands that apply to the selected item. For example, by clicking an open Experiment with your right mouse button, you can choose to copy, rename, or close the Experiment.

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.
- Refer to the Troubleshooting section in the Software or Instrument Help books.

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 instrument performance

For instrument support from within the US, call (877) 232-8995, prompt 2, 2.

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 (IVD) use when used with IVD reagents and instruments. Refer to the information supplied by the manufacturer for application-specific limitations.

Software Installation and Setup

The following topics are covered in this chapter:

- About BD FACSDiva Software on page 16
- Installing BD FACSDiva Software on page 19
- Starting the Software on page 34
- Administrative Options on page 36
- Quitting the Software on page 41

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, and biexponential display.

To simplify Experiment and data management, BD FACSDiva software uses a *Browser* view that allows users to easily organize Experiments, group Specimens and Tubes, design global or tube-specific analyses, and set independent instrument 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 the BD FACSCanto™, BD FACSAria™, BD™ LSR II, or BD FACSVantage™ SE upgraded with the digital electronics option, this PC-based software provides the user with all the setup, acquisition, control, and analysis features to quickly and efficiently generate quality data from a BD FACST™ brand digital flow cytometer.

What's Included

The BD FACSDiva installer installs the following applications:

- BD FACSDiva software for acquiring and analyzing data
- BD FACSDiva Data Management Utility program for backing up and restoring data
- Sentinel System Driver™ for using the security module
- Java™ 2 Runtime Environment (JRE) for running BD FACSDiva software
- Sybase™ SQL Anywhere™ Studio for running the database
- Adobe® Acrobat® Reader for viewing the PDF version of the reference manual and quick start guide

Documentation

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

- The *BD FACSDiva Software Quick Start Guide* contains tutorials to help new users get started using the software, or experienced users become familiar with new features. A PDF file of the quick start guide can be accessed from Start > Programs > BD FACSDiva Software.
- The online help system contains information on how to use BD FACSDiva software and your instrument. To access the online help, choose Help > Online Help within BD FACSDiva software.

The online 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 tab.

- 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.

To access the *BD FACSDiva Software Reference Manual*, choose Help > Software Manual or double-click the shortcut icon on the desktop.



System Requirements

Hardware

- BD FACS brand digital flow cytometer: BD FACSAria, BD FACSCanto, BD LSR II, or BD FACSVantage SE cytometer upgraded with the digital electronics option.
- (BD FACSVantage SE instruments only) BD FACSTM Accudrop option

- 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 the Windows 2000 Pro or Windows XP Pro operating system. 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 Make sure your operating system has been upgraded to Service Pack 4 (SP4) for Windows 2000 Pro, or SP1 or 1A for Windows XP. You can order or download service packs from the Microsoft website at <http://www.microsoft.com/Windows2000/downloads/servicepacks/sp4/>.

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 computer)

Software

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

- Sentinel System Driver
- Sybase SQL Anywhere Studio
- Java 2 Runtime Environment

Adobe Acrobat Reader is optional; it is needed to view the PDF version of the reference manual or quick start guide. You can install Acrobat Reader during BD FACSDiva software installation.

Compatibility

- BD FACSDiva software can import files in FCS 2.0 or 3.0 format, including files generated by BD CellQuest™, BD CellQuest™ Pro, or BD FACSDiva software.

NOTICE BD FACSDiva software cannot open BD CellQuest or BD CellQuest Pro Experiment documents.

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

NOTICE Use BD FACST™ Convert software to translate certain exported FCS 2.0 files into a Macintosh®-compatible format. BD CellQuest Pro software can analyze FCS 2.0 data files containing up to 16 parameters; it is provided with the BD FACStation™ data management system.

Installing BD FACSDiva Software

BD FACSDiva software has already been installed on workstations purchased from BD Biosciences. Use the following instructions for recovery purposes in case you need to reinstall the software, or when you are installing the software for the first time. An installation CD is included with the *BD FACSDiva Software Reference Manual*.

If you are upgrading the software, carefully follow the upgrade instructions provided with the CD.

NOTICE You must have Administrative access to install BD FACSDiva software.

Before Beginning This Procedure

Do the following before you begin installing the software.

If you are upgrading or reinstalling the software:

- 1** Make a backup copy of your current database.
See Backing Up the Database on page 212.
- 2** Uninstall the previous version of the software.
See Uninstalling the Current Software on page 29.
- 3** (Acquisition workstations, only) Reset the instrument electronics.
 - Turn on the instrument main power. (If the power is already on, turn it off, and then on again.)
 - Wait 5 minutes and restart the computer.



It takes approximately 5 minutes to fully boot up the cytometer electronics. To successfully complete the installation, you must restart the computer only after the cytometer electronics are fully booted.

All customers:

- Make sure your operating system has been upgraded to Service Pack 4 (SP4) for Windows 2000 Pro, or SP1 or 1A for Windows XP. You can order or download service packs from the Microsoft website at <http://www.microsoft.com/Windows2000/downloads/servicepacks/sp4/>.
- Close all open applications and windows.
- BD Biosciences recommends that you defragment the hard disk before you install new software.

Installing New Software

The installer adds the following components to the hard drive. When the correct version of a required component is not installed, the installer upgrades the component.

- BD FACSDiva software
- Data Management Utility
- Sentinel System Driver v5.41.1
- Java 2 Runtime Environment (JRE) v1.4.2_02
- Sybase SQL Anywhere v8.0.2
- Adobe Acrobat Reader v6.0

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

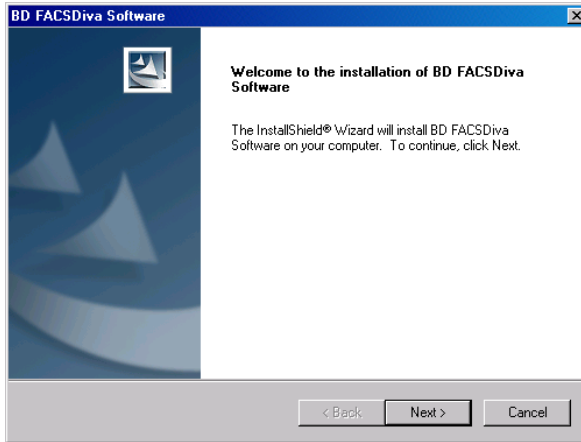
If the installer does not start automatically, use Windows Explorer to view the CD contents; find and double-click the Setup.exe icon.

2 Review the ReadMe file; click the close box to continue with installation.

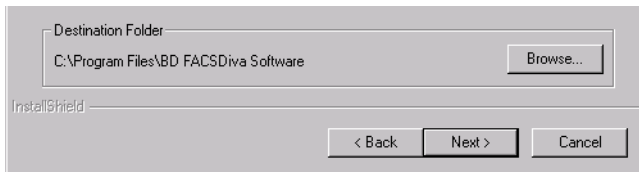
The ReadMe file contains important software information that is not included in this manual. Carefully review the file; then click the close box to return to Software Setup.

Tip BD Biosciences recommends that you print the ReadMe file and place the printed copy with your *BD FACSDiva Software Quick Start Guide* or instrument manual for reference.

- 3 When the welcome screen appears, click Next.



- 4 Click Yes to accept the license agreement and continue installation.
- 5 Review the installation instructions, and click Next.
- 6 Verify the destination folder; click Next.

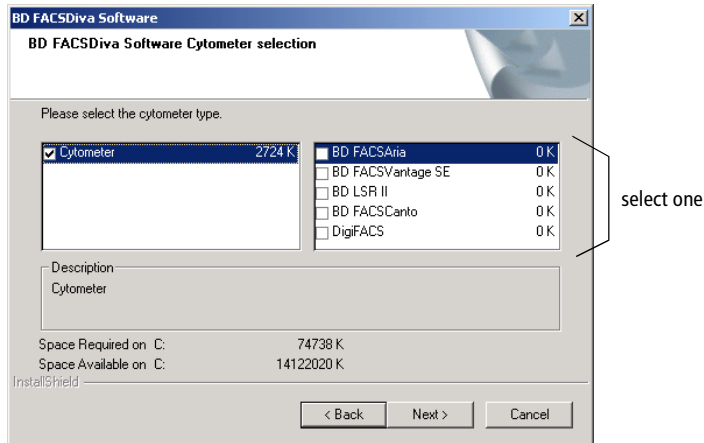


By default, the software is installed in the Program Files\BD FACSDiva Software folder on the C: drive.

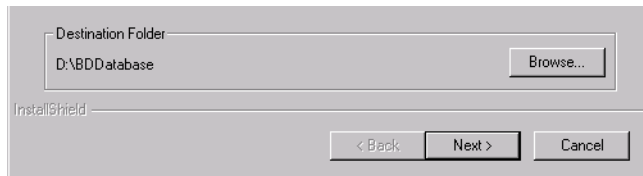
- 7 In the Cytometer Selection window, select the checkbox for your flow cytometer, and click Next.

The selection determines which firmware update and instrument online help files will be installed.

- Tip** For offline workstations, choose the instrument that is used most often in your laboratory.



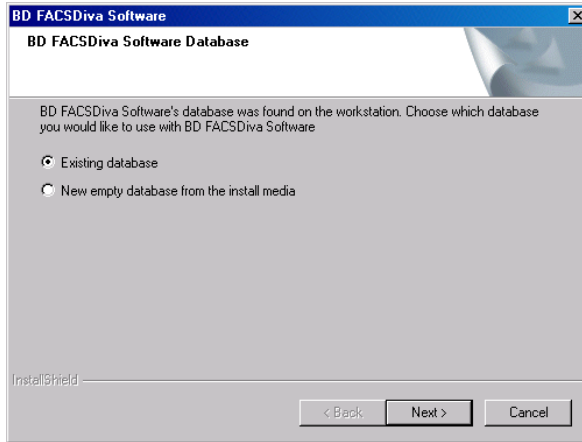
- 8 Verify the destination folder for the database; click Next.



By default, the database is installed in the BDDatabase folder on the D: drive. If there is no logical D drive on your system, database installation defaults to the C: drive.

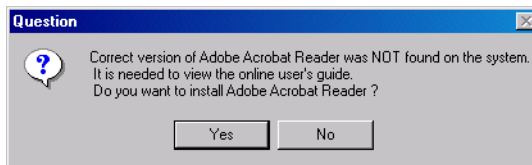
NOTICE Ensure that the destination folder does not reside on a removable media drive such as a Zip, CD, or DVD drive, or on a network drive. If you need to change the drive, keep the same folder name and path.

- 9 If you are prompted to do so, select a database option, and click Next.



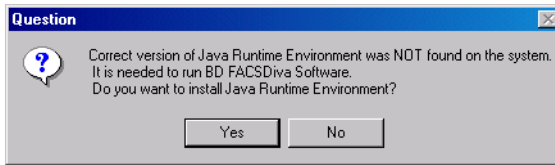
- (Recommended) Select *Existing database* to continue working with data in the current database. The database will be upgraded to work with the new software version.
- Select *New empty database...* to install an empty database. The existing database will be renamed BDFACS.dbx, where *x* is the next consecutive number.

- 10 (Optional) Click Yes to install or upgrade Adobe Acrobat Reader.



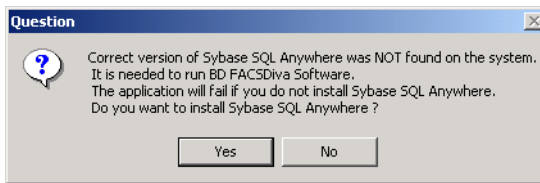
This version of BD FACSDiva software provides an updated version of Acrobat Reader (version 6.0), which is used to view the PDF version of the reference manual and quick start guide. To install or upgrade the software, follow the instructions in the dialog boxes and accept all default options.

- 11 Wait 15–20 seconds while the installer checks your version of JRE; click Yes to install it if prompted to do so.

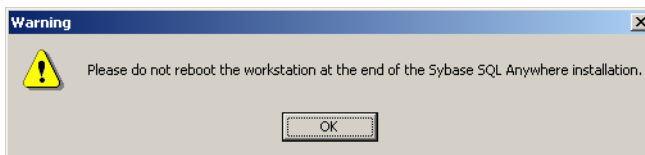


Follow the instructions in the dialog boxes and accept all default options to install JRE. If the correct version of JRE is already installed, the installer skips to the next installation.

- 12 Wait 15–20 seconds while the installer checks your version of Sybase SQL Anywhere; click Yes to install it if you are prompted to do so.



An information message appears; click OK to dismiss the message. The following dialog box appears:



Do not restart your computer after you install Sybase SQL Anywhere software. If you do, the BD FACSDiva software installation process will stop and you might have problems completing it later.

Click OK to dismiss the message. For instructions on installing Sybase SQL Anywhere, turn to page 30. When finished, return to this section and continue with step 13 to finish BD FACSDiva software installation.

13 Review the installation settings; click Next to begin copying files.

The installer loads BD FACSDiva software and its support files on the specified drives. If the workstation is connected to a cytometer, the installer downloads a new version of VxWorks (operating system that runs the cytometer).

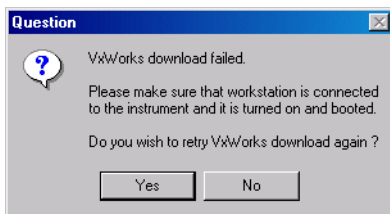
While the installer is checking to see if the workstation is connected to a cytometer, the following message appears on the screen:



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.

When all required files have been installed, a screen appears with the message “BD FACSDiva software has been installed.”

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 instrument is turned on and connected to the workstation, and then click Yes to try the VxWorks download again.

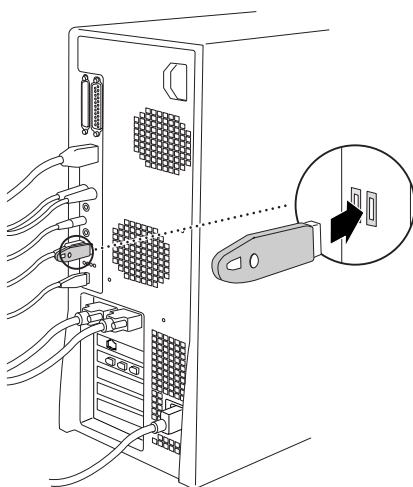
If the same message appears again, click No, finish the installation, and contact BD Biosciences technical support. Do not run your flow cytometer until VxWorks has been installed successfully.

- 14 If you have not already done so, click Next to review the ReadMe file; click the close box to continue with installation.

Tip BD Biosciences recommends that you print the ReadMe file and place the printed copy with your *BD FACSDiva Software Quick Start Guide* or instrument manual for reference.

- 15 Install the security module in the USB port at the back of the computer workstation, if needed.

The security module must be in place to run BD FACSDiva software.



NOTICE The location of your USB port and the cables on your workstation might be set up differently from those shown in this figure. The security module can be installed in any USB port.

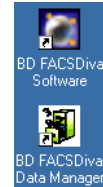
- 16 Click Finish to complete the installation.

It is recommended that you reboot the computer before starting the software for the first time.

NOTICE If you are reinstalling the software, turn the flow cytometer power off and then on, and then restart the computer.

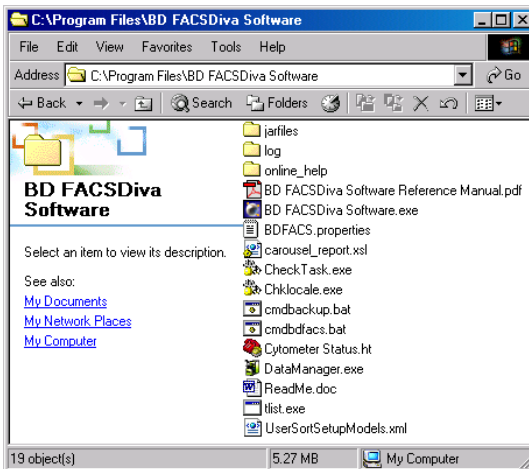
Files Installed

The installer places shortcuts to BD FACSDiva Software, BD FACSDiva Data Manager, the *BD FACSDiva Software Reference Manual* (PDF file), 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 specified drive. You will find a copy of the ReadMe file and supporting documentation, including a PDF file of the reference manual and quick start guide, in Program Files\BD FACSDiva Software as well as on the software CD.



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.

Uninstalling the Current Software

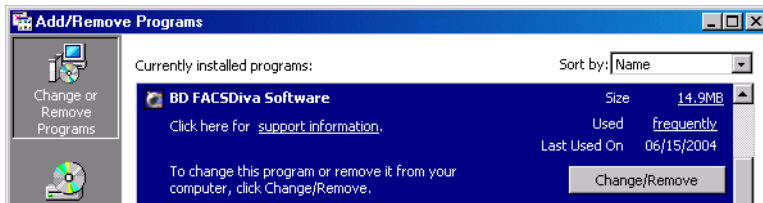
Follow the steps in this section only if you need to remove the current software version from your system. The uninstall process removes the current software and associated files while preserving the database and list-mode data files. When you reinstall the software, you will have the option to reuse your existing data files or install an empty database.



If you are reinstalling the software, DO NOT uninstall Sybase SQL Anywhere (there might be two versions on your system). If you uninstall Sybase, it will break the environment variable PATH and prevent many applications from running, including BD FACSDiva software. Reinstalling Sybase does NOT fix the problem.

NOTICE 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 reenter them later. To preserve these settings when you are running a BD FACSCanto flow cytometer, copy the existing cytometer.dat file in C:\Program Files\Common Files\BD into the D:\Service\ directory. When prompted, click Yes to overwrite the existing file. After the upgrade is finished, replace the new cytometer.dat file with the backed-up copy.

- 1 From the Windows Start menu, choose Settings > Control Panel.
- 2 Double-click the icon for Add/Remove Programs.
- 3 Locate and select BD FACSDiva software in the list of currently installed programs; click Change/Remove.



- 4 Follow the prompts on the screen to remove all installed components, and then click Finish.

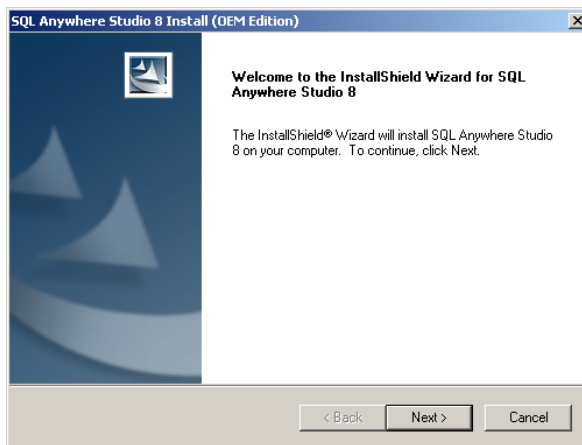
- 5 Restart your computer.
- 6 If you are reinstalling the software, go to Installing New Software on page 21 to complete the process.

Installing Sybase SQL Anywhere Studio

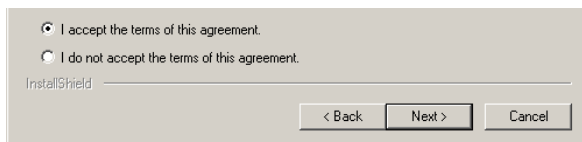
This version of BD FACSDiva software provides an updated version of Sybase SQL Anywhere, which is required to use BD FACSDiva software. Do the following to install the new version of Sybase SQL Anywhere.

The SQL Anywhere Studio Setup program is started automatically from within BD FACSDiva Setup.

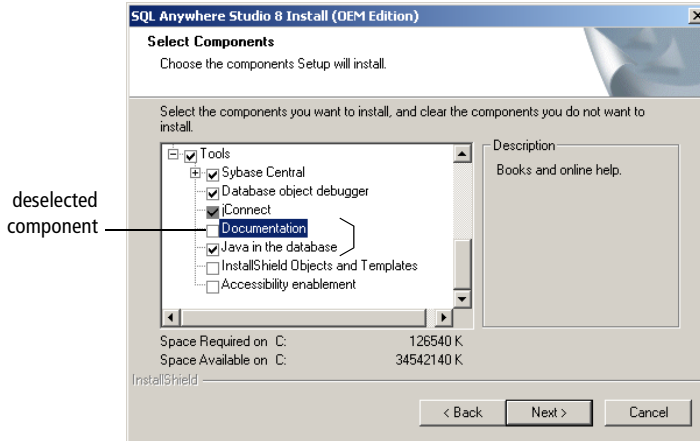
- 1 Click Next to begin installing SQL Anywhere.



- 2 Review the license agreement, select the radio button to accept the terms of the agreement, and then click Next.

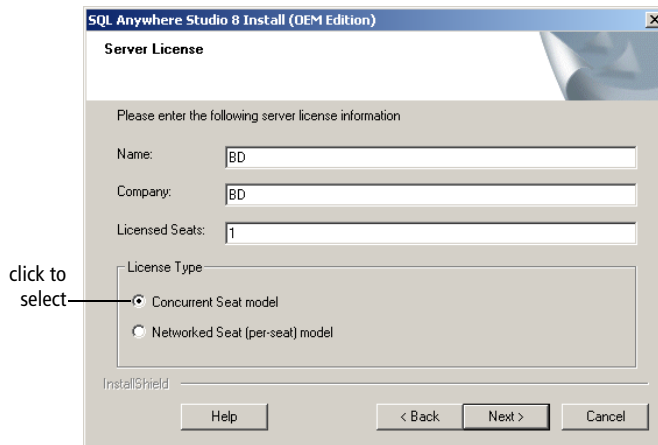


- 3 Verify the install location and click Next.
By default, SQL Anywhere is installed on the C: drive.
- 4 At the shared folder screen, click Next.
- 5 (Optional) At the Select Components screen, scroll to Tools. Deselect the Documentation component, and then click Next.



- Tip** Save disk space by installing the software without its accompanying documentation.

- At the Server License screen, change the License Type to Concurrent Seat model, and click Next.

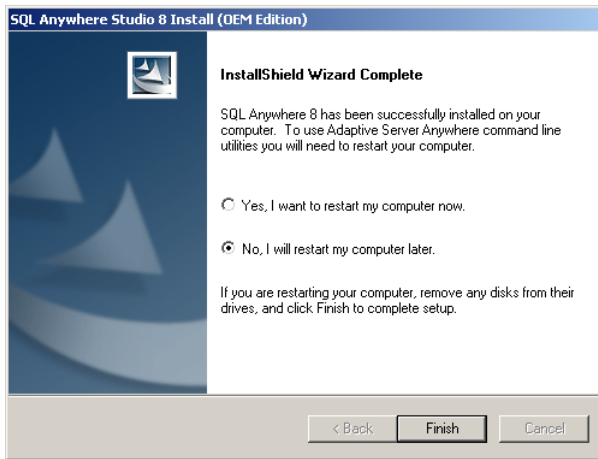


- At the Select Program Folder screen, click Next.
- Click Next to start copying files.
- When Setup is complete, deselect the checkbox to review the ReadMe file, and click Finish.
- At the Install Complete screen, select the radio button to restart the computer later, and click Finish (Figure 1-1 on page 33).



Do not restart your computer until you have finished installing BD FACSDiva software. If you restart the computer now, the installation process will stop and you might have problems completing it later.

Figure 1-1 Deferring restart until after BD FACSDiva installation is complete



- 11** Return to step 13 on page 26 to continue installing BD FACSDiva components.

Starting the Software

NOTICE If you are using the software for acquisition from the cytometer, start the software as described in your instrument 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.

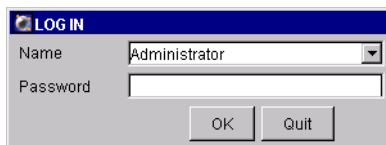
Alternatively, choose Start > Programs > BD FACSDiva Software > BD FACSDiva Software.



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

- 2 Leave the login name as Administrator, and click OK.

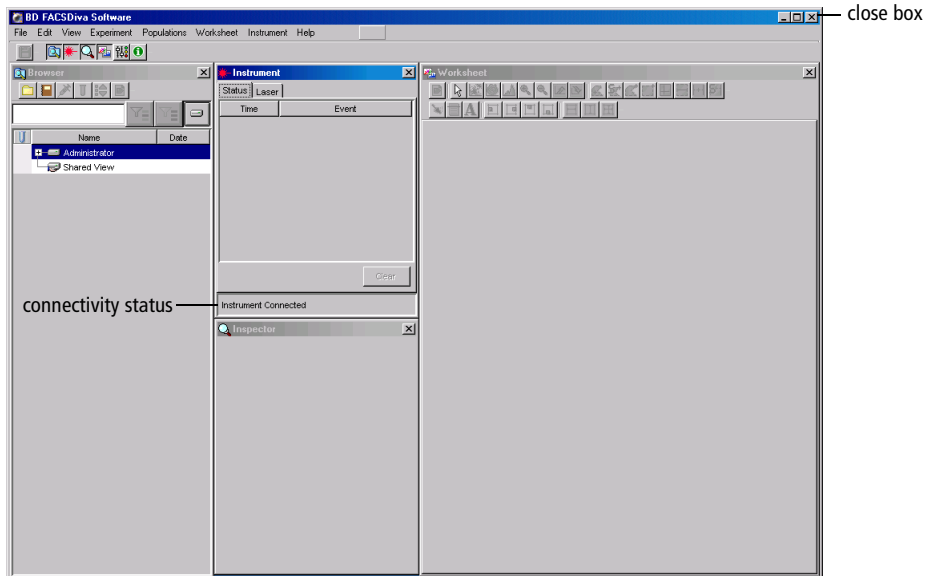
No password is required the first time you log into the software. You should assign a password to the administrator account as soon as possible. For instructions, see Adding or Modifying a Password on page 40.



To create additional user names, see Adding Users on page 36.

After a successful login, frames appear in the workspace (Figure 1-2 on page 35). (Your workspace might look slightly different from that shown in this example.) For a full description of workspace components, see Chapter 2.

Figure 1-2 BD FACSDiva workspace



NOTICE To verify the workstation has successfully connected to the cytometer, check that the Instrument frame displays the message “Instrument connected” or “The system is ready” at the bottom of the frame. If the message reads “Instrument Disconnected,” see Electronics Troubleshooting on page 219 for assistance.

Administrative Options

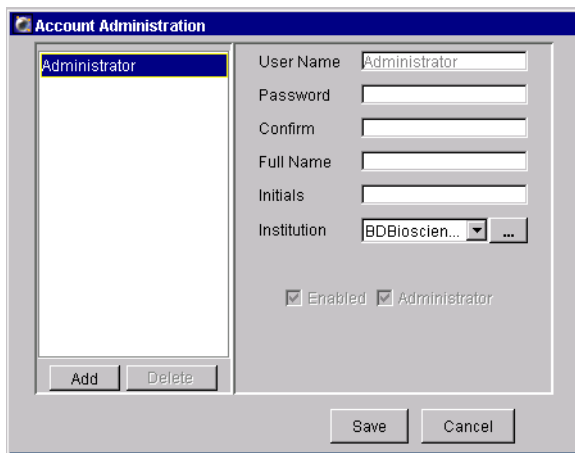
If you have administrator privileges in BD FACSDiva software, you can add or disable users as described in the following sections. For instructions on changing your password, see *Adding or Modifying a Password* on page 40. Note that all users can add or modify passwords; you do not need administrative access.

Adding Users

Only the system administrator or users with administrative access can add users.

- 1 Log into the software.
- 2 Choose File > Administration.

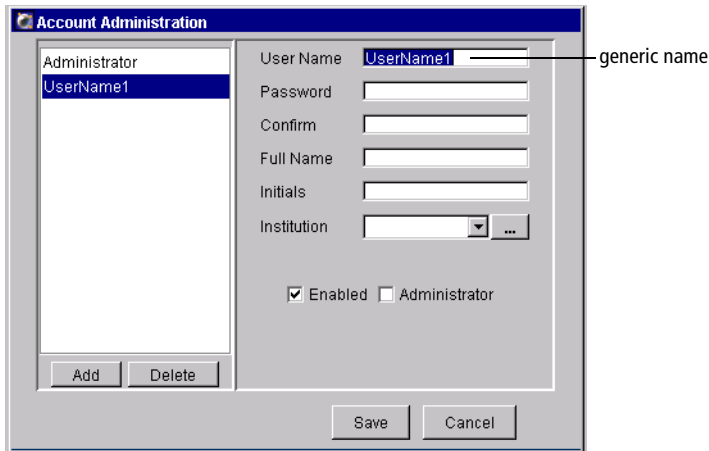
The Account Administration dialog box appears, where you can add or modify the attributes of a user, enable or disable users, or grant administrative access.



The screenshot shows the 'Account Administration' dialog box. It features a list box on the left with 'Administrator' selected. To the right, there are several input fields: 'User Name' (containing 'Administrator'), 'Password', 'Confirm', 'Full Name', 'Initials', and 'Institution' (a dropdown menu showing 'BD Bioscienc...' and a browse button). Below these fields are two checked checkboxes: 'Enabled' and 'Administrator'. At the bottom of the dialog are four buttons: 'Add', 'Delete', 'Save', and 'Cancel'.

- 3 Click Add to add a user.

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



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

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

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

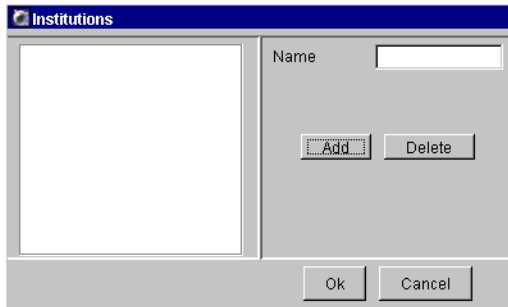
- 6 Confirm the password, if entered, by typing it again.

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

This information is not required; you can leave the fields blank, if you like. To add an institution, define choices by clicking on the “...” button next to the Institution drop-down menu:



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



- To add an institution, click Add. “Institutex” is added to the list of names. Change the name by selecting “Institutex” in the Name field and entering a new name. Press Enter to apply the change, or click OK to apply the change and dismiss the dialog box.
- To delete an institution, select the name in the list and click Delete. Click OK to dismiss the dialog box.

Once you click OK, all listed institutions can be chosen from the Institution drop-down menu in the Account Administration dialog box. Note that unassigned institutions are not saved from one login session to the next.

- 8** To grant the user administrative access, select the Administrator checkbox.



Users with administrative access can add or modify other user accounts.

- 9** Ensure all user information is correct, and click Save.

Deleting Users

Note that you must have administrative access to delete a user.

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

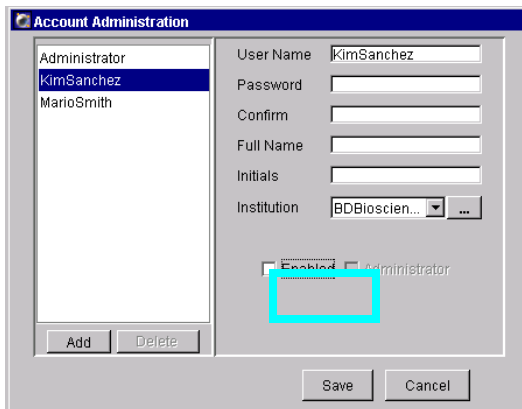
See Exporting Experiments on page 208 for instructions. Note that you can enable the option to automatically delete Experiments after export.

- 2 Choose File > Administration.
- 3 Select the user name in the list, click Delete, and then click Save.

Disabling a Saved User

When users have saved Experiments in the database, they cannot be deleted, but they can be disabled. Disabled users can no longer log into the software. However, their Experiments are saved in the database and their shared Experiments are available to all users.

- 1 Log into the software.
- 2 Choose File > Administration.
- 3 Select the user in the list of user names, and deselect the Enabled checkbox; click Save.

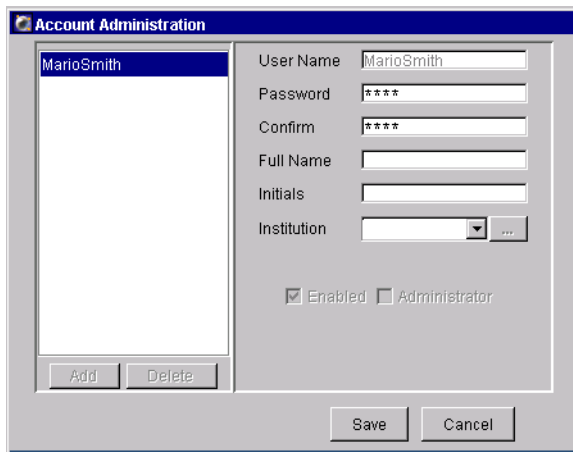


Adding or Modifying a Password

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 into the software.
- 2 Choose File > Administration.

The Account Administration dialog box 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.
- Tip** Keep a copy of your password in a secure location in case you forget it.

Quitting the Software

Do one of the following to quit the software:

- Choose File > Quit.
- Click the close box in the upper-right corner of the workspace (Figure 1-2 on page 35).

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.

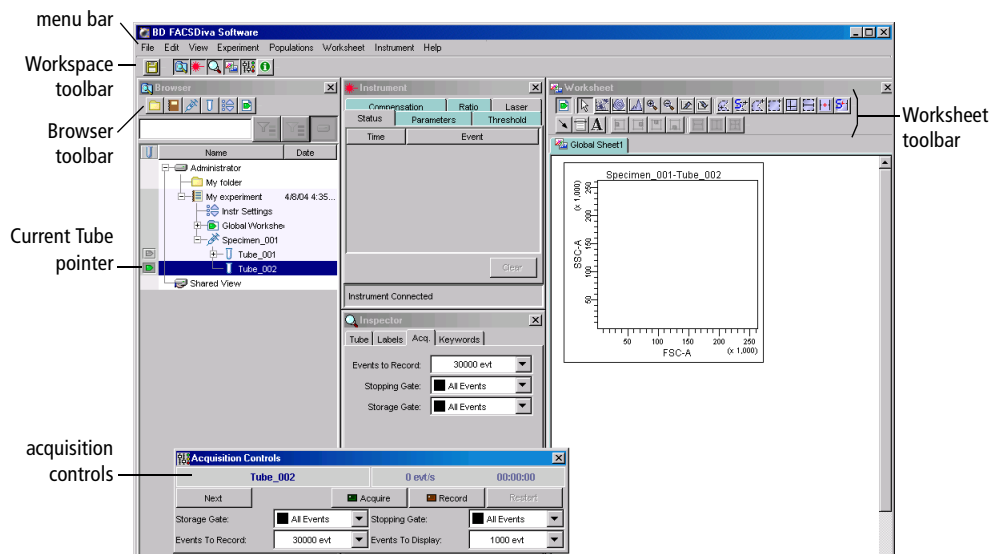
- Workspace Components on page 44
- Inspector on page 47
- Browser on page 48
- Experiments on page 58
- Specimens on page 70
- Tubes on page 74
- Instrument Settings on page 78
- Analysis Objects on page 79
- Keywords on page 82
- User Preferences on page 86

Workspace Components

When you start BD FACSDiva software, the workspace appears (Figure 2-1). *Frames* containing the main software components are displayed within the workspace. Display or close frames by clicking buttons in the Workspace toolbar.

Most software functions are accessed using the menu bar at the top of the workspace and toolbars within the Browser and Worksheet frames. Acquisition and data loading is controlled using the Current Tube pointer or buttons within the Acquisition Controls frame.

Figure 2-1 BD FACSDiva workspace




The following elements are displayed in the workspace:

- menu bar—contains pull-down menus with commands to operate the software
- Workspace toolbar—displays buttons used to save the current Experiment and to show or hide frames; see *Workspace Toolbar* on page 45.


Workspace Toolbar





The following buttons are displayed in the Workspace toolbar. Note that some buttons are shown only for certain instruments; refer to your instrument 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 frame; see Browser on page 48.


 Instrument—hides or shows the Instrument frame; see Instrument Controls on page 92.


 Inspector—hides or shows the Inspector frame; see Inspector on page 47.


 Worksheet—hides or shows the Worksheet frame; see Worksheets on page 138.

 Acquisition Controls—hides or shows the Acquisition Controls frame; see Acquisition Controls on page 104.

 Acquisition Status—hides or shows the Acquisition Status frame; see Acquisition Status on page 108.

 Sort Setup—hides or shows the Sort Setup frame
This button appears only if your instrument is equipped with sorting features.

 Carousel Controls—hides or shows the Carousel Controls frame
This button appears only if your instrument is equipped with the BD FACST[™] Loader option.

 Plate Layout—hides or shows the Plate Layout frame
This button appears only if your instrument is equipped with the BD[™] High Throughput Sampler (HTS) option.

View Options

The BD FACSDiva workspace can be rearranged to suit your needs. You can resize the workspace window, or reposition or resize frames within the workspace. Changes are user-specific, and are saved from one login session to the next.

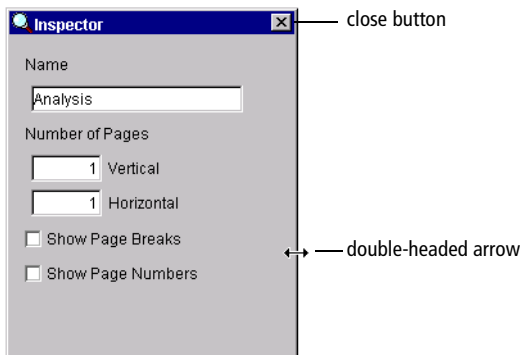
If you have a second monitor, the BD FACSDiva workspace can be viewed on both monitors. To maximize the workspace, click the maximize button. The Workspace will expand to fill the screen (or two screens, if applicable).



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

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

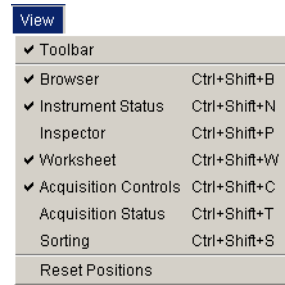
Figure 2-2 Resizing a workspace frame



- To view or hide different frames in the Workspace, choose an option from the View menu, or click the corresponding button in the Workspace toolbar.

You can also hide a frame by clicking the close button (Figure 2-2 on page 46).

- To restore frames to their default position and size, choose View > Reset Positions.

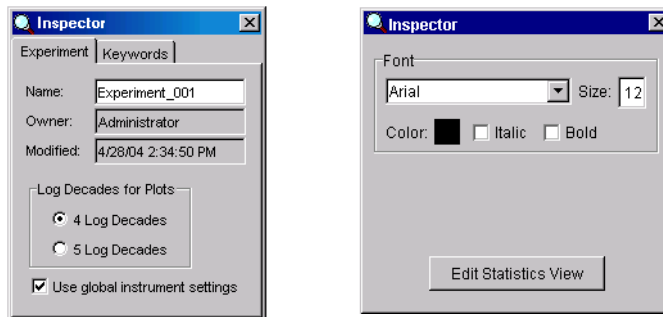


Inspector

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 frame, click the Inspector button in the Workspace toolbar (🔍). The contents of the Inspector frame 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

Unlike other BD software, BD FACSDiva software does not generate separate files for data display, instrument settings, and data. The software stores and accesses all experimental data from a single database. As you create Experiments and record data, the software writes Experiment components to the database. Stored elements are shown in the Browser.


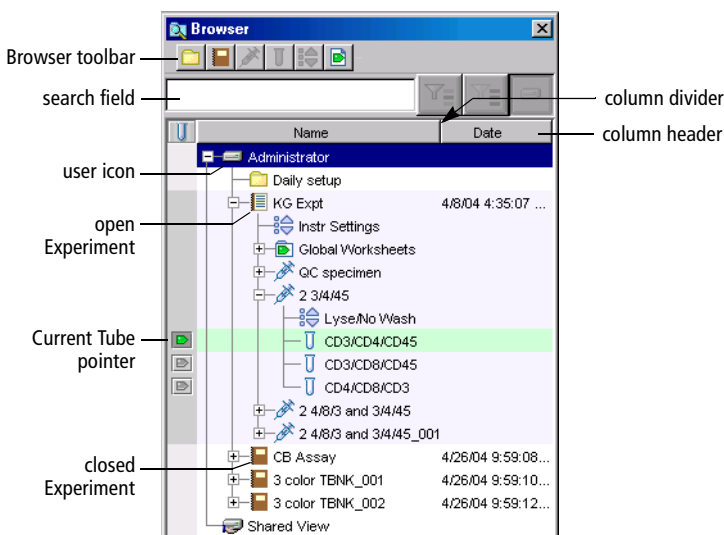
The Browser is where you create and access experimental data stored in the database. Data is listed by login name in a hierarchical view. You can hide or display the Browser by clicking the Browser button in the Workspace toolbar (.

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 about private vs shared Experiments, see Making Experiments Private or Shared on page 65.

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 56.

Use the search field above the Browser to enter criteria to restrict the number of listed Experiments. See *Using the Search Field* on page 50.

- 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 53.

- organizes experimental 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 selecting the element and entering a new name. (Alternatively, you can 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 contextual menu with options specific to that item. A summary of all contextual menus is provided in Contextual Menus on page 235.
- 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 52.

Using the Search Field

Use the search field in the Browser frame to find Experiments containing specific elements or to limit the number of Experiments listed in the Browser. For more search options, see Finding Saved Data on page 66.

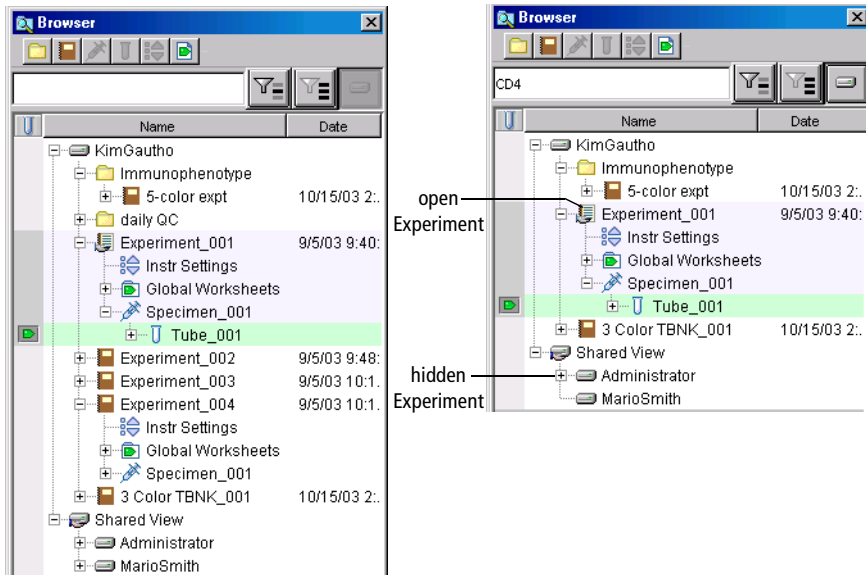
- 1 Enter a search criterion (element name, fluorochrome label, custom keyword name or value, or population name), and then click the Find button.




The Browser lists only Experiments containing the specified element, along with the currently open Experiment. In the example shown in Figure 2-5 on page 51, Experiment_001 is open at the time the search was performed, so it remains displayed even though it does not contain anything matching the search criteria.

Note that you might have to expand a folder or user icon to see all Experiments. In Figure 2-5, the user icon for Administrator has a plus sign (+) next to it, indicating that one or more Experiments containing the required element are stored under the administrator login name.


Figure 2-5 Browser before and after using Find



- 2** (Optional) To hide shared Experiments matching the search criterion, click the View Own button ().

Experiments under the Shared View icon are hidden.

Note that all Experiments must be closed before you can use this button. If an Experiment is open, the button is disabled.

- 3** To list all Experiments again, click the Display All button (.

NOTICE You cannot use the Search 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.

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 the Current Tube Pointer

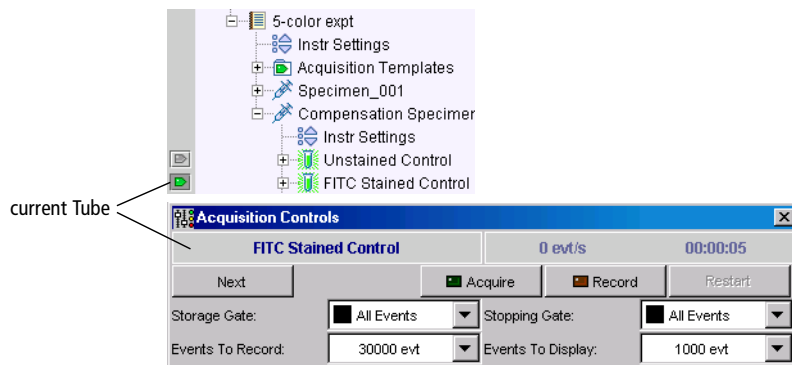
When an Experiment is open, a grey pointer or plot icon appears next to Tubes in the Browser (Figure 2-6). Click the icon next to a Tube to set the *Current Tube pointer*, which indicates the Tube currently selected for data acquisition, recording, or data display on a global worksheet. When the software is connected to the instrument, the pointer can also be used to control acquisition and recording.

During Acquisition

When the software is connected to the cytometer, a grey pointer icon is displayed next to Tubes in the open Experiment. Click on the grey 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 Controls and Acquisition Status frames (Figure 2-6).

For other pointer states, see Current Tube Pointer on page 106.

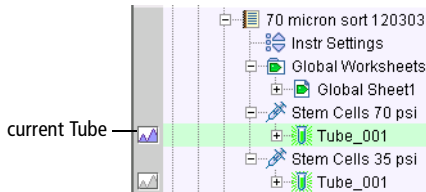
Figure 2-6 Current Tube pointer during acquisition



During Analysis

When the software is disconnected from the cytometer or a recorded Tube contains incompatible instrument settings, a plot icon is displayed next to Tubes with recorded data in the open Experiment. Click on the grey 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 move the Current Tube pointer.

Figure 2-7 Current Tube pointer during analysis



Adding New Elements to the Browser

Use buttons in 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.

shown only on sorting instruments





































shown only on instruments with a plate loader



Tip You can customize Browser toolbar buttons to add a predefined template to the Browser. See Template Preferences on page 89 for instructions.

To add...	First select or right-click...	And then choose one of these options...
Folders	<ul style="list-style-type: none"> (to create a folder inside a folder)	<ul style="list-style-type: none">Click the New Folder button ().Choose Experiment > New Folder.Choose New Folder from the contextual menu.Press Ctrl-N.

To add...	First select or right-click...	And then choose one of these options...
 Experiments or Experiment templates	<ul style="list-style-type: none">   (to create an Experiment inside a folder) 	<ul style="list-style-type: none"> Click the New Experiment button (). Choose New Experiment from the contextual menu. Choose Experiment > New Experiment or press Ctrl-E.
 Specimens or panel templates	<ul style="list-style-type: none">  	<ul style="list-style-type: none"> Click the New Specimen button (). Choose New Specimen from the contextual menu. Choose Experiment > New Specimen or press Ctrl-M.
 Tubes or analysis templates	<ul style="list-style-type: none">   or →    or →  	<ul style="list-style-type: none"> Click the New Tube button (). Choose New Tube from the contextual menu (available only when a Specimen is selected). Choose Experiment > New Tube or press Ctrl-T.
 Specimen-specific instrument settings	<ul style="list-style-type: none">   or →  	<ul style="list-style-type: none"> Click the New Instrument Settings button (). Choose Experiment > New Instrument Settings. Choose New Instrument Settings from the contextual menu.
 Tube-specific instrument settings	<ul style="list-style-type: none">   or →  	<ul style="list-style-type: none"> Click the New Instrument Settings button (). Choose Experiment > New Instrument Settings. Choose New Instrument Settings from the contextual menu.

To add...	First select or right-click...	And then choose one of these options...
 Sort Layouts (if appropriate)	<ul style="list-style-type: none">   	<ul style="list-style-type: none"> Click the New Sort Layout button (). Choose Sort > New Sort Layout. Choose New Sort Layout from the contextual menu.
 Global worksheets or analysis templates	<ul style="list-style-type: none">  	<ul style="list-style-type: none"> Click the New Global Worksheet button (). Choose New Global Worksheet from the contextual menu. Choose Experiment > New Global Worksheet.
 Plates or Plate templates (if appropriate)	<ul style="list-style-type: none">  	<ul style="list-style-type: none"> Click the arrow control and choose a plate type from the drop-down menu. Choose Experiment > New Plate.

NOTICE Each new Experiment starts with default instrument settings. After recording a Tube, a copy of the instrument settings in effect at the time of recording is stored with the Tube. User-defined instrument settings can be created at the Experiment, Specimen, or Tube level. See Instrument Settings on page 78.

Organizing the Browser

Experiments are set up hierarchically to help organize experimental data. You can use Tubes and Specimens to help organize your work, and use 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 a lot of 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 organization strategies for naming and grouping Experiments in BD FACSDiva software. Figure 2-8 shows Experiments grouped by studies or month.

Figure 2-8 Example folder organization

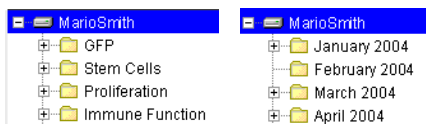


Figure 2-9 shows two strategies for organizing QC Experiments. In one, Experiments are organized by month at the Experiment level, by date at the Specimen level, and by samples run at the Tube level. The second example organizes QC work by month at the folder level, by date at the Experiment level, and by sample run at the Tube level.

Figure 2-9 Example organization of QC work

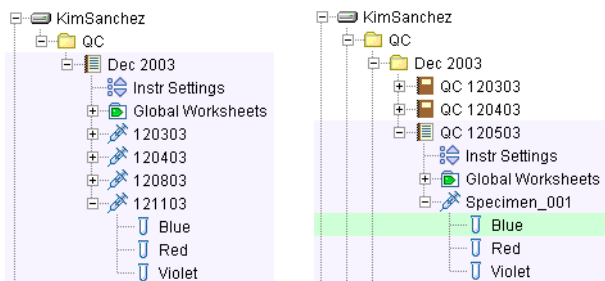
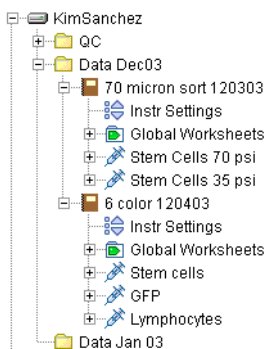


Figure 2-10 shows an example of how you can organize your daily work by month. By including both a descriptor (6 Color) and date in the Experiment name, you can easily search for an Experiment by the descriptor or date using the search field in the Browser or the Edit > Find feature.

Figure 2-10 Example organization of daily work



Tip To minimize the number of Experiments in the Browser, click the View Own button to see only your Experiments, or use the Find feature to limit the number of Experiments displayed. 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 nested inside outer folders for additional levels of organization.

Experiments

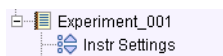
An *Experiment* is a group of elements used to acquire and analyze data from the flow cytometer. Experiments can include global worksheets, Specimens (material to be analyzed), Tubes (acquisition data and reagents used to analyze the specimen), Analysis objects (plots, gates, and statistics views), and Sort Layouts or Plates (if applicable). Instrument settings can be applied at the Experiment, Specimen, or Tube level.

You build Experiments as you acquire 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 start a new Experiment, click the New Experiment button .

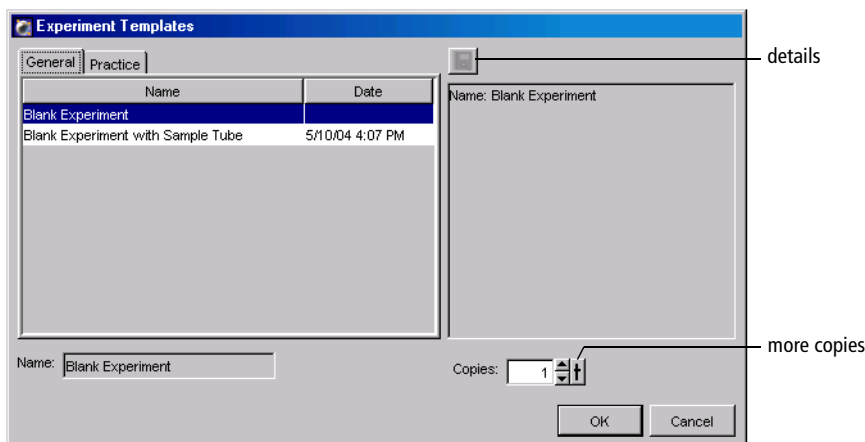
The currently open Experiment closes and a new, open Experiment is added to the Browser. (If you are recording data when the button is clicked, the current Experiment does not close. The new Experiment is added as a closed Experiment.)



By default, the New Experiment button adds an Experiment with default instrument settings and a blank worksheet, but the button can be customized to add a predefined Experiment template. For more information, see Template Preferences on page 89.

- To start an Experiment based on a saved template, choose Experiment > New Experiment or press Ctrl-E. The Experiment Templates dialog box appears where you can select the template type and number of experiments to create (Figure 2-11).

Figure 2-11 Selecting a template



To view details about the Experiment template, click the details button. The Experiment Layout dialog 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 1–50 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 61.

- 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 box that appears.

For more information, see Importing Experiments on page 210.

Opening Experiments

You can edit elements and acquire data only within an open Experiment, and 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 (Ctrl-O).
- Right-click an Experiment icon in the Browser and choose Open Experiment.

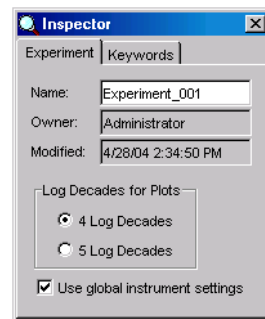
There might be a short delay while the software retrieves the Experiment from the database.

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 155)
- select whether to update global instrument settings automatically (see Using Global Instrument Settings on page 119)
- (on the Keywords tab) create or view Experiment-level keywords (see Keywords on page 82)



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 196.) 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. (Green highlighting indicates 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 all Specimens, Tubes, keywords, Sort Layouts, instrument 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 common functions such as instrument optimization or sorting. 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**.

An “Export Wizard” dialog box appears, with steps that show you how to create and export a template.

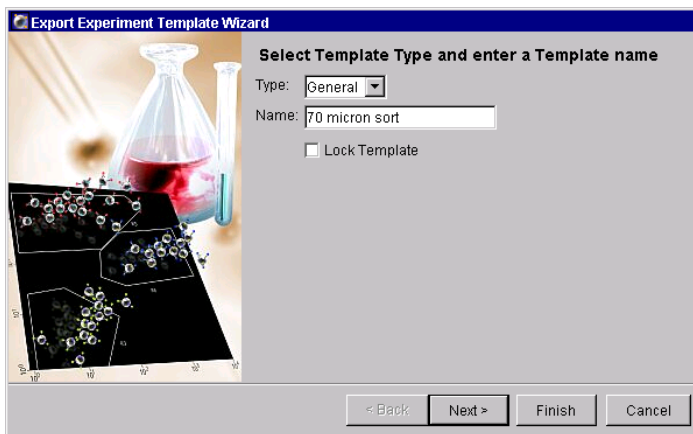
2 Choose the template type and verify the name; click Next.

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

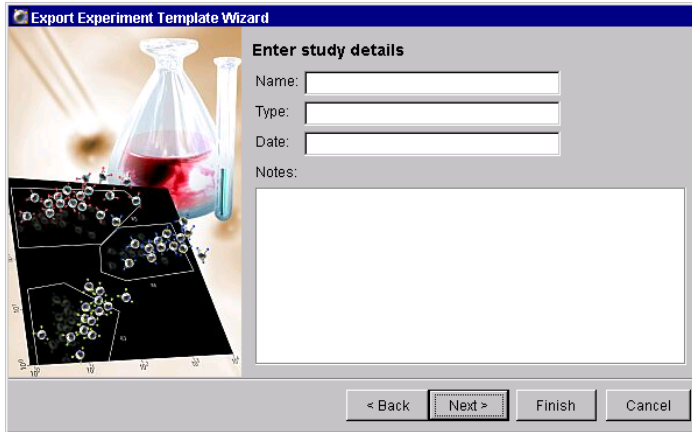
The template name is based on the name of the Experiment in the Browser. To change the name, enter a new name in the Name field. Note that Experiment names cannot include periods or commas.

Select the Lock Template checkbox to ensure 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.



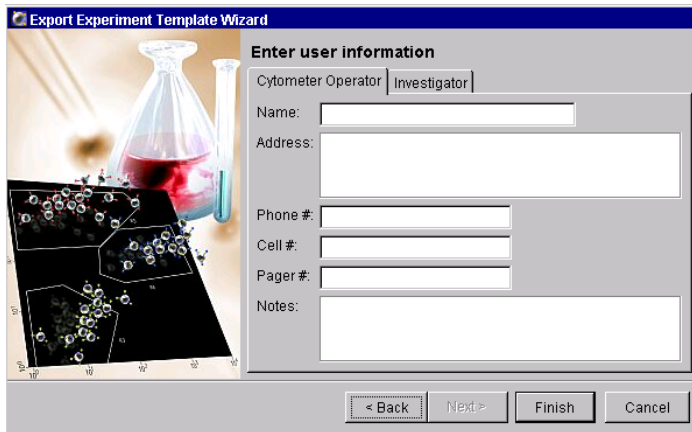
- 3 (Optional) Enter Study Details when prompted; click Next.



The screenshot shows a dialog box titled "Export Experiment Template Wizard" with a blue header bar. On the left is a graphic of laboratory glassware and a flow cytometry plot. The main area is titled "Enter study details" and contains four input fields: "Name:", "Type:", "Date:", and "Notes:". The "Notes:" field is a larger text area. At the bottom are four buttons: "< Back", "Next >" (highlighted with a dotted border), "Finish", and "Cancel".

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; click Finish.



The screenshot shows the same dialog box, now titled "Enter user information". It features two tabs: "Cytometer Operator" and "Investigator". Below the tabs are input fields for "Name:", "Address:", "Phone #:", "Cell #:", "Pager #:", and "Notes:". The "Notes:" field is a larger text area. At the bottom are four buttons: "< Back", "Next >" (highlighted with a dotted border), "Finish", and "Cancel".

Templates are saved in a folder in the D:\BDEExport\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 box. See Figure 2-11 on page 59.

Editing Templates

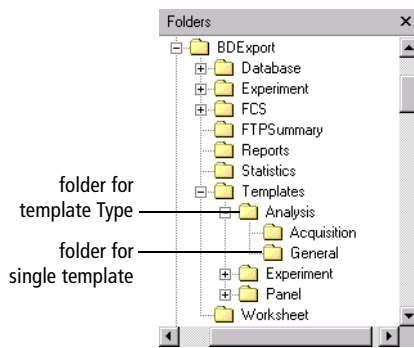
- To add or delete elements from a template, create an Experiment from the template, make the required changes, and then export the Experiment as a template. Save it with the same template type and name. When prompted, overwrite the previous template.

NOTICE A locked template cannot be overwritten.

- To rename a template, use Windows Explorer to navigate to the BDEExport\Templates folder, open the folder corresponding to the template type, and rename the *template.xml* file in the folder.

To rename a template type, rename the folder.

- To change the template type, locate the *template.xml* file in its corresponding type folder, and move it to a different folder at the same hierarchical level.
- To remove templates from the template directory, use Windows Explorer to navigate to the BDEExport\Templates folder.
 - To delete a template type and all templates within it, delete the corresponding folder in the Templates folder.
 - To delete a single template, open the folder corresponding to the template type, and delete the folder with the same name as the template.



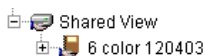
Making Experiments Private or Shared

When you log into BD FACSDiva software, all of your saved Experiments are listed under your user icon in the Browser. Other users will not be able to view your Experiments unless they have administrative access, or you have designated an Experiment as shared.

To make an Experiment available to other logged-in users, right-click the Experiment icon in the Browser, and choose Share Experiment. The Experiment icon changes to show that the Experiment is shared.

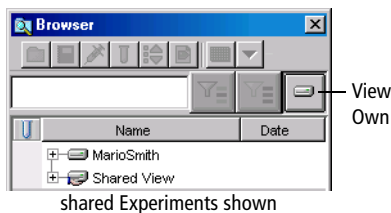


When other users log into the software, they will be able to view your shared Experiments under the Shared View icon in the Browser.



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.



Exporting and Importing Experiments

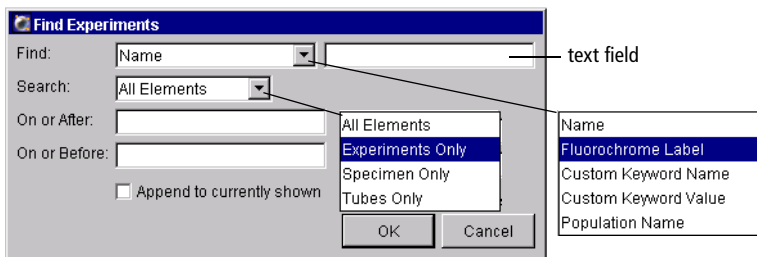
Experiments can be exported to the hard drive or an external storage device. See Exporting and Importing Experiments on page 208.

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 201 and Importing FCS Files on page 204.

Finding Saved Data

Use the search field at the top of the Browser frame to search the database for Experiments containing specific Browser elements, reagents, custom keyword names or values, or population names. See Using the Search Field on page 50.

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



- Use the Find drop-down 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 drop-down 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 (ie, 5/17/01 or May 17, 2001).

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

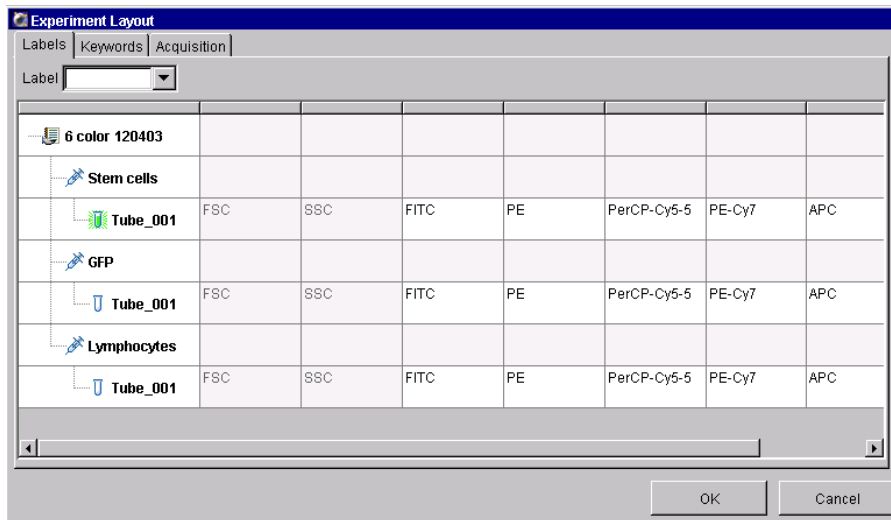
NOTICE You cannot use the Find function to locate a folder. If a folder contains an Experiment that meets the search criteria, it will have a plus sign (+) next to it, indicating Experiments are inside the folder.

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 the Experiment Layout dialog box to create fluorochrome labels, enter values for keywords, or enter acquisition criteria for each Tube in your Experiment. To access this feature, open an Experiment, and choose Experiment > Experiment Layout. A dialog box appears listing all Specimens and Tubes in the Experiment.

Figure 2-12 Experiment Layout dialog box



Editing Labels

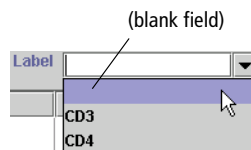
Use the Labels tab of the Experiment Layout dialog box (Figure 2-12 on page 67) to enter parameter labels for each fluorochrome in your experiment. Parameter labels will be displayed on plot axes and in statistics views.

- 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 Drag the mouse or hold down the Control key while clicking to select and label multiple fields at a time. For example, if all samples in the experiment were stained with CD3 FITC, select all the FITC fields in the table, and then enter CD3. All selected fields are labeled with CD3 at once.

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



Note that labels can also be entered on the Labels tab of the Tube Inspector. See Using the Tube Inspector on page 74.

Editing Keywords

All keywords currently defined for the Experiment are listed in the Keywords tab of the Experiment Layout dialog box. See Defining Keywords on page 82.

Enter a keyword value by selecting a cell and then entering a value in the Value field. If the keyword was set up with selectable choices, the Value field changes to a drop-down menu where you can choose an available value. Keyword changes are automatically updated in the Keywords tab of the corresponding Inspector.

If more than one cell is selected, the change will be made in all selected cells at the same time. Keywords must be of the same 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
 - dragging the mouse across a selection while holding down the left mouse button.
 - holding down the Shift key while clicking the first and last fields in a range.
- Select multiple non-contiguous fields by holding down the Ctrl key while clicking each selection.
- Cancel a value entered in a field or text box by pressing the Esc key before you click OK. This restores the previous value and returns you to the Experiment Layout dialog box.

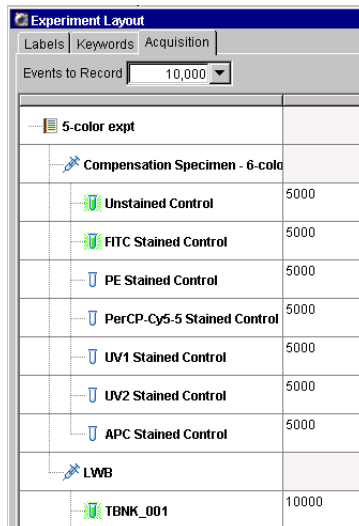
NOTICE An individual field cannot be deselected from a selected group.

Editing the Number of Events to Record

The Acquisition tab in the Experiment Layout dialog box 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 drop-down menu.

If more than one cell is selected, the change will be made in all selected cells at the same time.

Note that the number of Events to Record can also be entered on the Acq. tab of the Tube Inspector or in the Acquisition Controls frame. See Using the Tube Inspector on page 74 or Acquisition Controls on page 104 for more information.



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 instrument settings (see Creating Specimen- or Tube-Specific Settings on page 118).

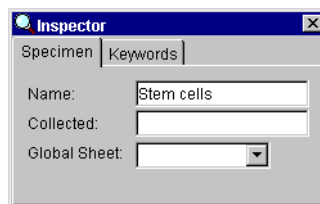
- To create a new Specimen, see Adding New Elements to the Browser on page 53.
- To save a Specimen as a panel, see Exporting a Specimen as a Panel on page 71.
- To create a new Specimen from a panel template, see Importing a Panel Template on page 73.
- To edit a panel template, see Editing Templates on page 64.

Using the Specimen Inspector

Use the Specimen Inspector for the following:

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

Note that 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 this Specimen. The menu lists all global worksheets in your Experiment.

The chosen worksheet will be displayed automatically when you select a Tube below this Specimen.

- Click the Keywords tab to view or edit keywords that will be stored with the Specimen. For more information, see Keywords on page 82.

Exporting a Specimen as a Panel

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 and their associated Analysis objects.

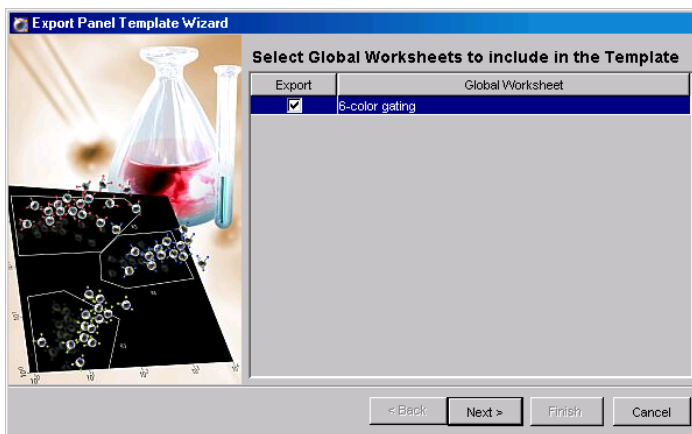
Panels are stored outside the Browser to simplify the Browser display. To export a Specimen as a panel, do the following. Note that Specimens can be exported as templates only from open Experiments.

- 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.

An “Export Wizard” dialog box appears, with steps that show you how to export a template.

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

All defined global worksheets are shown. Click to select the checkbox next to each worksheet you would like to include.



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



Panels can be grouped by category so they are easier to find later. To add a category to the Type drop-down menu, enter a name in the Type field. Your new type will be available from the drop-down 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 to ensure 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, and click Finish.

Comments can be viewed when you are importing a panel template. See *Importing a Panel Template* on page 73.

Panels are saved in a folder in the D:\BDEExport\Templates\Panel directory. A new folder is created for each panel type. When you create a new

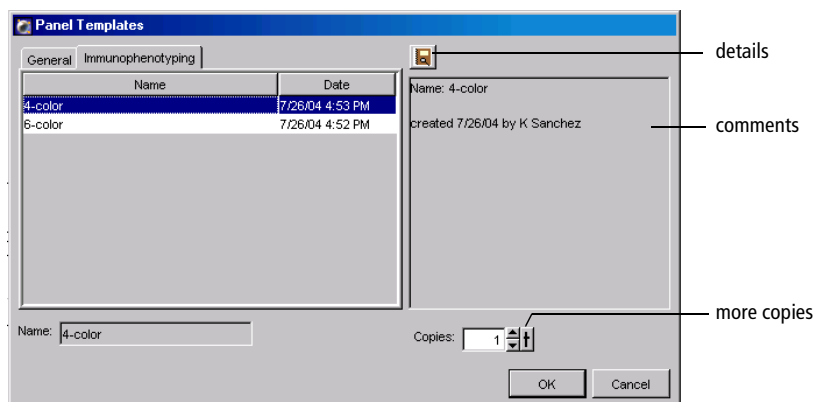
Specimen based on a panel, each type is represented by a tab in the Panel Templates dialog box. See Figure 2-13 on page 73.

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 box 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 2-13 Selecting a panel to import



To view details about the panel template, click the details button. The Experiment Layout dialog appears showing a list of Tubes in the panel, any defined labels, keywords, and acquisition criteria. See Using Experiment Layout on page 67.

Note that you can import 1–50 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 on page 71.

Tubes

A *Tube* can contain acquisition criteria, information about the reagents used to analyze the Specimen, the data for recorded events, Tube-specific instrument 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 53.
- To create a new Tube with a predefined analysis template, see Creating a Tube with a Predefined Analysis Template on page 77.
- To duplicate a Tube, right-click the Tube and choose Duplicate Without Data, or use Copy and Paste commands.

Using the Tube Inspector

There are four components to the Tube Inspector, accessed by clicking the tabs at the top of the Inspector: Tube, Labels, Acquisition, and Keywords. If a Tube contains instrument settings (ie, Tube-specific settings or settings copied during recording), an Instrument Settings tab is also shown.

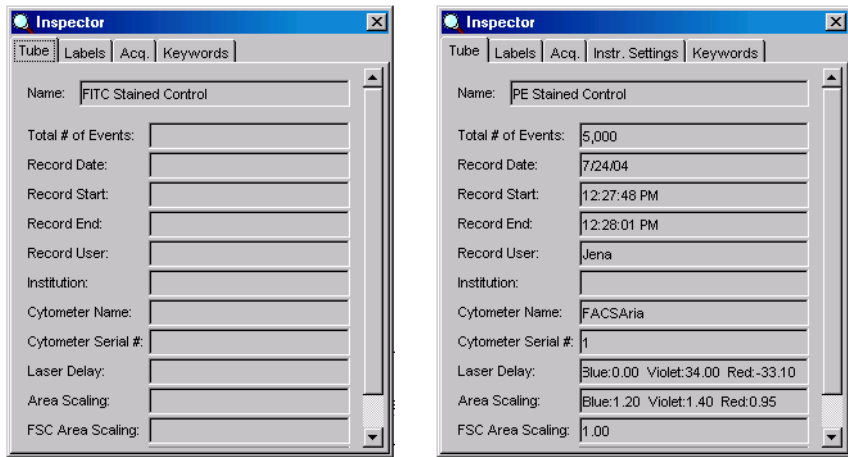
NOTICE Many values defined on the Labels, Acquisition, and Keywords tabs can be viewed and edited using the Experiment Layout dialog box. (See Using Experiment Layout on page 67.)

- Use the Tube tab to name the tube and to view certain keywords and settings saved with recorded data (Figure 2-14 on page 75).

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

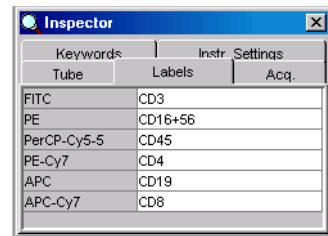
Note that keyword fields in the Tube tab cannot be edited.

Figure 2-14 Tube tab before (left) and after (right) recording data



- Use the Labels tab to enter parameter labels for each fluorochrome used by the Tube. Parameter labels will be displayed on plot axes and in statistics views.

Tip Labels can also be entered in the Experiment Layout dialog box. See Using Experiment Layout on page 67.



- 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)

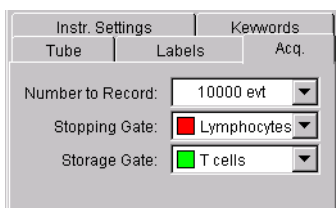
- whether you want to record only events within a predefined population (Storage Gate)



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, a tethered gate, or a gate drawn on a biexponential plot.

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

Your Acq. tab would look like the following:



The instrument would keep acquiring events until 10,000 events were collected in the Lymphocyte gate; however, only events that fell into the *T cells* 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 drop-down menus in the Acquisition Controls frame. (See Acquisition Controls on page 104.) Inspector values are updated if you change the settings from these menus.

- When available, use the Instr Settings tab to view or edit Tube-specific instrument settings.

Instrument settings can apply at the Tube, Specimen, or Experiment level. See Creating Specimen- or Tube-Specific Settings on page 118 for details.

During offline use, you can edit instrument settings for unrecorded Tubes in the Inspector. (Instrument settings for recorded Tubes cannot be edited.) When you are connected to the instrument, you can change voltages, thresholds, and ratios only in the Instrument frame; the Inspector shows a report of the settings for a selected Tube or Instr Settings icon. For more information, see Instrument Settings on page 109.

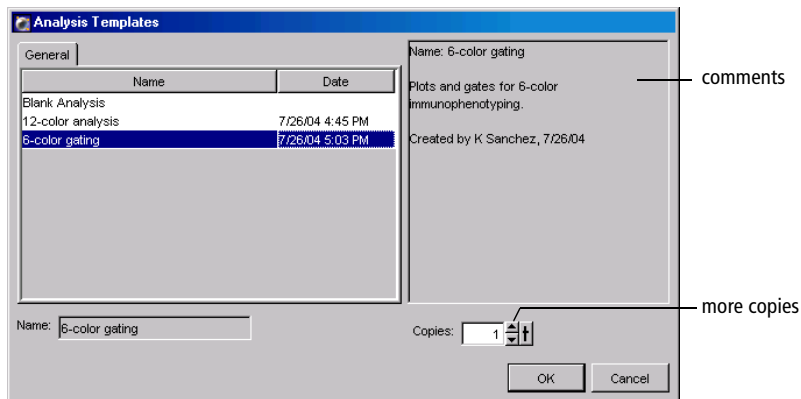
- Use the Keywords tab to view or edit keywords that will be stored with the Tube. See Keywords on page 82.

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 79.

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

The Analysis Templates dialog box 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 will be created for each specified copy. You can create 1–50 Tubes at a time with the selected analysis. To change the number of copies, click the up arrow next to the Copies field.

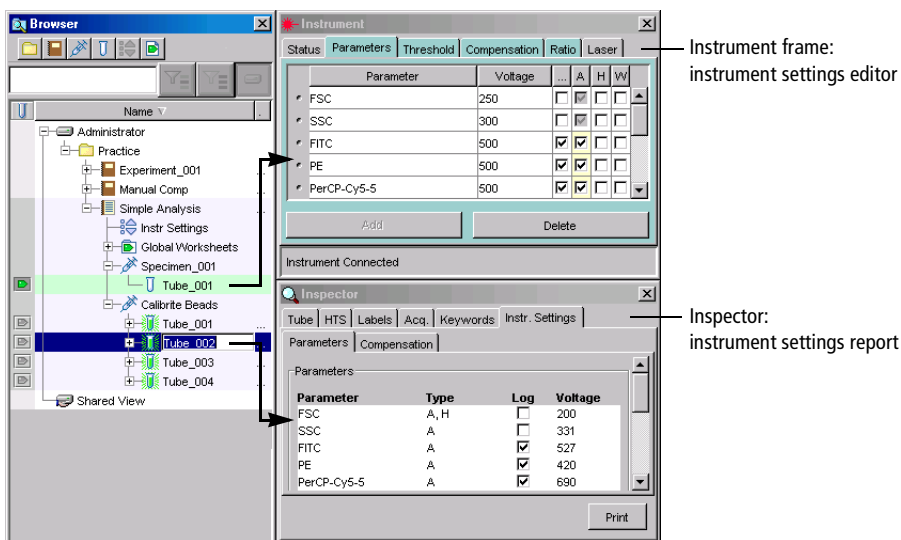
When you click OK, the designated number of Tubes are added to the Browser, with a copy of your templated Analysis object under each Tube. Plots, gates, and statistics views are added to the current worksheet unless the Tube-specific worksheet preference is enabled. In this case, Analysis objects are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

Instrument Settings

Instrument settings represent the collection of values for parameters measured, photomultiplier (PMT) voltages, threshold, compensation, and the calculated parameters Log and Ratio. Instrument settings can apply to Tubes, Specimens, or Experiments. When no Tube-specific instrument settings exist, Specimen settings apply; when no Specimen-specific settings exist, Experiment settings apply.

Every new Experiment starts with default instrument settings values. These values can be optimized manually or overwritten by copying optimized settings into the Experiment or applying a saved compensation Setup. Default parameters are determined by the current instrument configuration.

During offline use, you can edit instrument settings in the Inspector. When you are connected to the instrument, you can change voltages, thresholds, and ratios only in the Instrument frame; the Inspector shows a report of the settings for a selected Tube or Instr Settings icon. For more information, see *Instrument Settings* on page 109.



Instrument frame:
instrument settings editor

Inspector:
instrument settings report

Parameter	Voltage	A	H	W
FSC	250	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
SSC	300	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
FITC	500	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
PE	500	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
PerCP-Cy5-5	500	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Parameter	Type	Log	Voltage
FSC	A, H	<input type="checkbox"/>	200
SSC	A	<input type="checkbox"/>	331
FITC	A	<input checked="" type="checkbox"/>	527
PE	A	<input checked="" type="checkbox"/>	420
PerCP-Cy5-5	A	<input checked="" type="checkbox"/>	690

Analysis Objects

An *Analysis object* contains the plots, gates, and statistics used to analyze the event data in a Tube. An Analysis object appears under a Tube in the Browser after you create a plot, statistics view, or population hierarchy for that Tube. Analysis objects can also be associated with global worksheets, which are not tied to specific Tubes. Tools for data analysis are described in more detail in Chapter 4; analysis examples can be found in the *BD FACSDiva Software Quick Start Guide*.

Analysis objects are displayed only in open Experiments and are associated with a particular Tube or with a global worksheet. You can select an Analysis object and save it as a template, copy it from one Tube to another, or copy it from a global worksheet to a Tube. When an Analysis object is copied or saved as a template, all plots, gates, statistics views, and population hierarchies are included.

Saving an Analysis Template

Any Analysis object can be saved as a template, including plots, gates, statistics views, and population hierarchies. 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 object or a Tube with an Analysis object, and choose **Export > Analysis template**.

An “Export Wizard” dialog box appears, with steps that show you how to export a template.

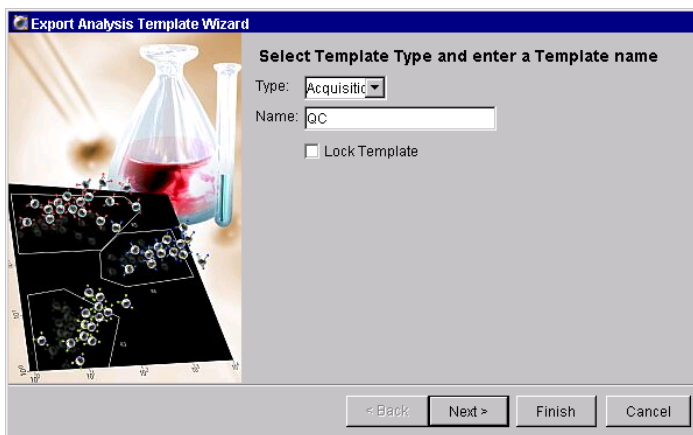
- 2 Choose the template type and verify the name; click **Next**.

Group templates by type so they are easier to find later. To add a new type to the drop-down menu, enter a name in the **Type** field. Your new type will be available from the drop-down 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 to ensure 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, and click Finish.

Comments can be viewed when you are importing an analysis template. See *Creating a Tube with a Predefined Analysis Template* on page 77.

Templates are saved in a folder in the D:\BDEExport\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 box.

Copying Analysis Objects

- 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 object and choose Copy.

Alternatively, select the Analysis object and press Ctrl-C.

- 3 Select the icons for 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 discontinuous icons in the Browser, hold down the Control key while clicking.

The new Analysis object overwrites any plots, gates, population hierarchies, and statistics that already exist. When pasted to a Tube, the new plot(s) and statistics are pasted into the active worksheet (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 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.
- Attach numerical data, such as cell count, to a Tube or Specimen.
- Attach labels to data, making it easier to find in the database. See Finding Saved Data on page 66.
- Display Tube, Specimen, or Experiment keywords in the headers of statistics views. Keywords are exported along with the statistics.

Defining Keywords

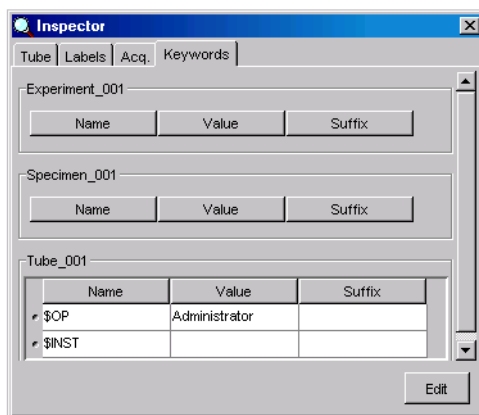
Keywords can be defined at the Experiment, Specimen, or Tube level.

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.

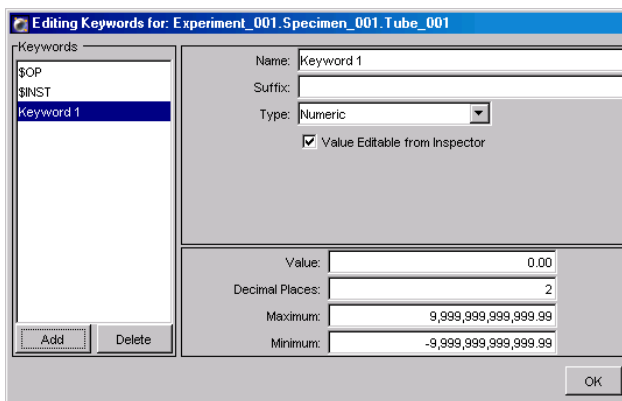
- 1** In an open Experiment, select an Experiment, Specimen, or Tube in the Browser.
- 2** Click the Keywords tab in the Inspector of the selected item.
- 3** Click the Edit button to add or change a keyword (Figure 2-15 on page 83).

You can create or edit keywords only for the item that was selected in the Browser. For example, if a Tube was selected, you cannot edit Specimen or Experiment-level keywords.

Figure 2-15 Creating custom keywords



4 Click the Add button.



5 Name the keyword and add any required suffix.

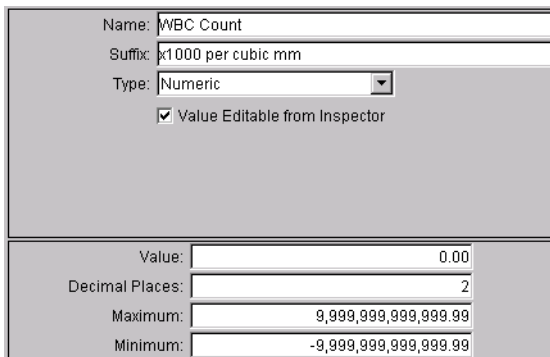
Select the generic name in the Name field and enter a new name. Each keyword name must be unique; use the suffix to define values, such as units of measure.

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

- Use *Numeric* for keywords defined by numerical values, such as numbers of cells (Figure 2-16). 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).

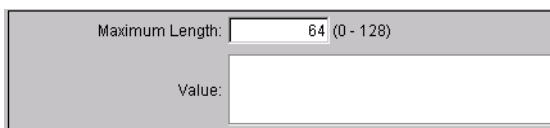
Specify a Value in the dialog box or check *Value Editable from Inspector* and enter the value there.

Figure 2-16 Defining a numeric keyword



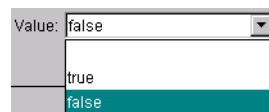
Name:	WBC Count
Suffix:	x1000 per cubic mm
Type:	Numeric
	<input checked="" type="checkbox"/> Value Editable from Inspector
Value:	0.00
Decimal Places:	2
Maximum:	9,999,999,999,999.99
Minimum:	-9,999,999,999,999.99

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



Maximum Length:	64 (0 - 128)
Value:	

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

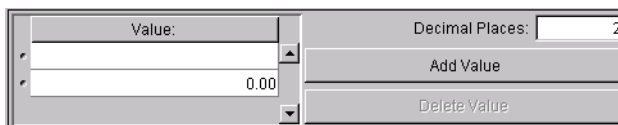


Value:	false
	true
	false

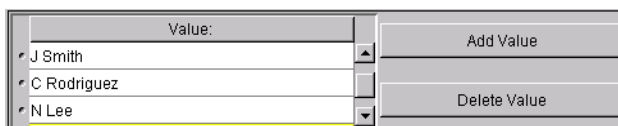
- Use *Selectable Numeric* to define a set of selectable numeric keywords, such as a list of values. Define the set of values by clicking Add Value, selecting the value in the Value field, and entering the required value.

All defined values will appear in a drop-down 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).

A screenshot of a dialog box titled 'Edit Keywords'. It features a 'Value:' field with a dropdown arrow and a 'Decimal Places:' field with a text input containing '2'. Below these fields are two buttons: 'Add Value' and 'Delete Value'. The 'Value:' field currently displays '0.00'.

- 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 will appear in a drop-down menu in the Value field of the Keywords Inspector.

A screenshot of the 'Edit Keywords' dialog box. The 'Value:' field is a list box containing three items: 'J Smith', 'C Rodriguez', and 'N Lee'. The 'N Lee' item is highlighted with a yellow background. To the right of the list box are 'Add Value' and 'Delete Value' buttons.

- 7 Click OK to dismiss the Edit Keywords dialog box.

Deleting Keywords

Only custom keywords can be deleted. BD-defined keywords (such as \$OP for operator or \$INST for instrument) cannot be edited or deleted.

- 1 In an open Experiment, select the Browser item containing the keyword you want to delete.
- 2 Click the Keywords tab in the Inspector of the selected item.
- 3 Click the Edit button to access the Edit Keywords dialog box.
- 4 Select a keyword, and click Delete.

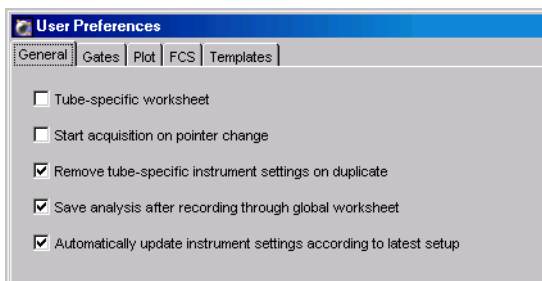
User Preferences

Certain default settings can be changed using the User Preferences command on the Edit menu. Preferences apply to all Experiments in the Browser; changes are saved with your login name and are retained from one session to the next. Note that additional preferences might be available for your instrument type; refer to your instrument manual for a description.

There are five types of user preferences available to all users: General Preferences, Gate Preferences, Plot Preferences, FCS Preferences, and Template Preferences.

General Preferences

General preferences apply to worksheets, acquisition controls, and instrument 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* 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.
- Start acquisition on pointer change—acquisition will begin each time the Current Tube pointer is moved to a new Tube that does not already contain recorded data. By default, this preference is not selected.

- Remove tube-specific instrument settings on duplicate—tube-specific instrument 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.
- Automatically update instrument settings according to the latest setup—when you open an Experiment, instrument settings that are linked to a Setup are automatically updated if Setup settings were changed. By default, this preference is selected.

Gate Preferences

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

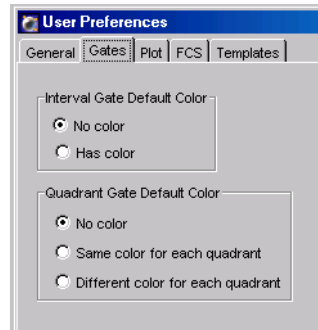
- Interval Gate Default Color—Select one of the two radio buttons to specify whether populations defined by an Interval gate should be assigned a color or retain the color of the parent population.

By default, populations are not colored.

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

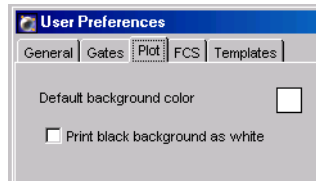
- no color used (color is determined by parent population)
- all quadrants (gated populations) assigned the same color
- each quadrant (gated population) shown in a different color

By default, Quadrant populations are not colored.



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.



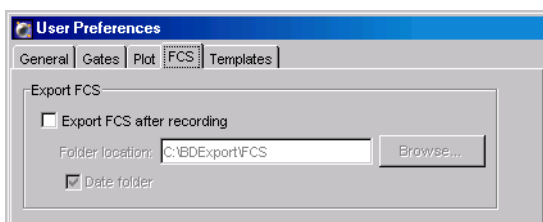
If you set the default background to black, select the checkbox to print plots with a white background; white gates and populations are then automatically printed in black.

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 201 for more information.

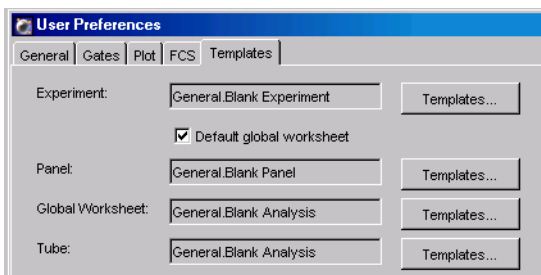
When the preference is selected, you can specify an export folder location by clicking the Browse button, or by entering a folder path in the Folder location field.

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



Template Preferences

Template preferences allow you to select which predefined 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), 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 and select a saved template in the dialog box 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, and by default, it is selected.

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.

3

Instrument and Acquisition Controls

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


Many instrument functions can be controlled within BD FACSDiva software. Most instrument controls are located in the Instrument frame; other instrument controls are accessed from the Instrument menu. Acquisition controls are available in the Acquisition Controls frame and the Browser.

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

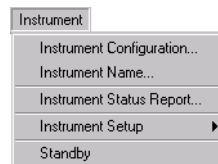
The following sections contain an overview of these controls:

- Instrument Controls on page 92
- Acquisition Controls on page 104
- Instrument Settings on page 109
- Controls for Compensation Correction on page 122

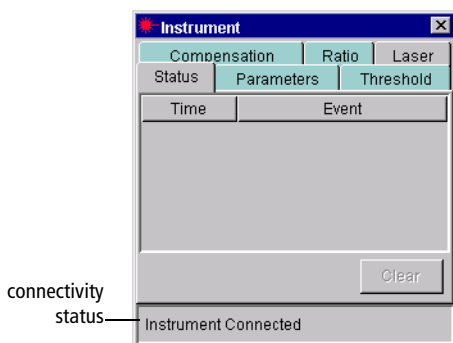
Instrument Controls

Instrument controls are accessed within the Instrument menu or the Instrument frame. To display the Instrument frame, click the Instrument button in the Workspace toolbar ()

- Use the Instrument menu to access instrument configuration options, identify the instrument name and serial number, display an Instrument Status report, perform instrument setup functions, and connect or disconnect communication between the workstation and the cytometer.



- Use the Instrument frame to view workstation connectivity status. When the software is connected to the cytometer, Status messages and Laser controls are also shown in the frame. If an Experiment is open and the Current Tube pointer is set, the frame displays instrument settings for the current acquisition Tube.



The following sections contain descriptions of Instrument menu commands, Status messages, and Laser controls. For a description of instrument settings tabs, see Instrument Settings on page 109.

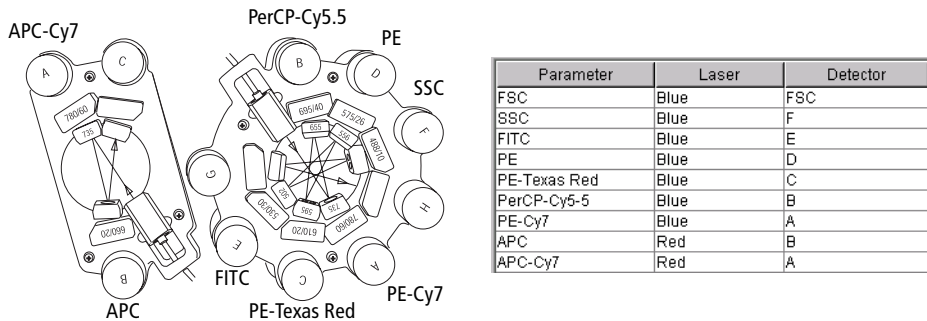
Instrument Configuration

Flow cytometers are equipped with specific sets of lasers, filters, and dichroic mirrors. The Instrument Configuration dialog box lets you define which fluorochromes or cell parameters will be measured at each photomultiplier tube (PMT) detector.

BD FACSDiva software is provided with default configurations specific to your instrument. In addition, you can define custom configurations, or add or modify labels in an existing configuration. As you change fluorochromes, filters, or mirrors, you can switch between predefined configurations that match your optics setup.

For example, Figure 3-1 shows how the Instrument Configuration dialog box corresponds to the optical stage for the BD FACSAria cell sorter. Refer to your instrument manual for instrument-specific configuration information.

Figure 3-1 Instrument Configuration dialog box corresponding to BD FACSAria detector arrays



Selections made in the Instrument Configuration dialog box determine which parameters are listed on the Parameters tab in the Inspector or the Instrument frame. If you commonly use one PMT detector to measure multiple parameters, you can list all possible parameters in the Instrument Configuration dialog box and choose the appropriate fluorochrome for your Experiment in the Parameters tab.



For accurate data results, the instrument optics setup must match the current instrument configuration.

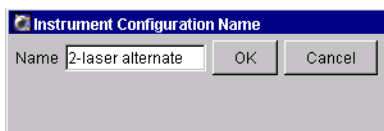
Defining a New Configuration

Follow these steps to create a custom configuration.

- 1 Choose Instrument > Instrument Configuration.

The Instrument Configuration dialog box appears.

- 2 Click the Add Configuration button.
- 3 Name the configuration, and click OK.



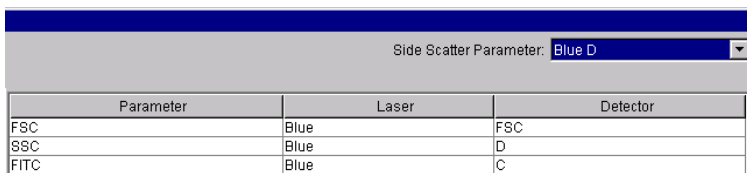
- 4 Select the new configuration in the list of configurations; click once on the Add Parameter button for each parameter to be added.
- 5 Specify the laser and detector, and enter a name for each parameter.

You must first assign a detector before you can enter a parameter name. To change the laser or detector, click the field in the corresponding column. A drop-down menu appears listing all available choices.

The parameter names you enter are the names that will appear on the Parameters tab in the Inspector or Instrument frame. When more than one fluorophore is entered for a given channel, the first listed fluorophore will be shown by default. Note that 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.

- 6 Use the drop-down menu at the top of the dialog box to assign the side-scatter parameter (all systems except the BD FACSVantage SE).

You must assign the side scatter parameter to ensure it is excluded from compensation calculations. Once it has been assigned, the SSC parameter name cannot be edited.



- 7 To make the new configuration the current configuration, click Set Configuration; then click OK.



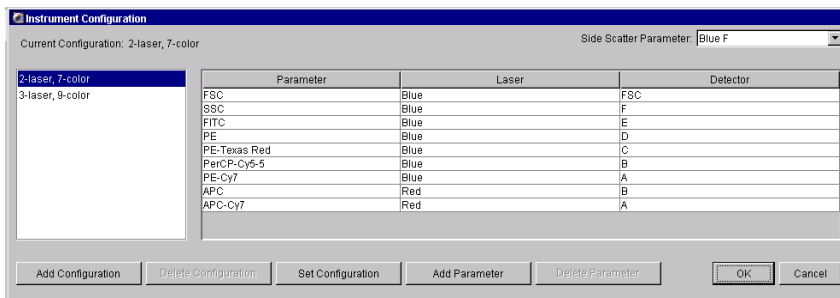
You must click Set Configuration for the new configuration to apply. For accurate data results, always verify that the instrument optics setup matches the current instrument configuration.

Adding or Modifying Parameters

To add or modify parameters in a configuration, do the following.

- 1 Choose Instrument > Instrument Configuration.
- 2 Select the configuration to be modified.

NOTICE For the BD FACSCanto instrument, only user-defined configurations can be modified. Default configurations cannot be changed.



- 3 Click Add Parameter to add a new parameter; choose the laser used to excite the fluorochrome and the detector used to detect it.

You must first assign a detector before you can change a parameter label.

- 4 Click in any parameter field except FSC or SSC and enter a new name.

FSC and SSC parameter names cannot be changed.

- 5 Continue adding or modifying parameters as needed.

- 6 Make sure the appropriate configuration is listed as the Current Configuration, and click OK.

To use a different configuration as the current one, select the name in the list of configurations, and click Set Configuration.



You must click Set Configuration for the new configuration to apply. For accurate data results, always verify that the instrument optics setup matches the current instrument configuration.

Changing Configurations

To switch between defined configurations, select the configuration name in the Instrument Configuration dialog box, and click Set Configuration.

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

Deleting a Parameter or Configuration

Use the buttons at the bottom of the Instrument Configuration dialog box to delete a parameter or defined configuration. Note that the current configuration cannot be deleted.



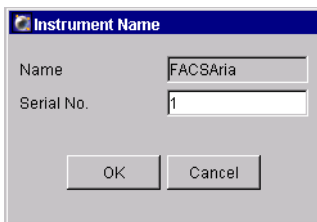
The software does not prevent you from deleting a predefined configuration. If you delete one of the default configurations, it will have to be re-created as if it were a new configuration. Refer to your instrument manual for examples if you need to re-create a default configuration.

- 1 Select the parameter or configuration to be deleted.
- 2 Press the Delete Parameter button or the Delete Configuration button.

Instrument Name

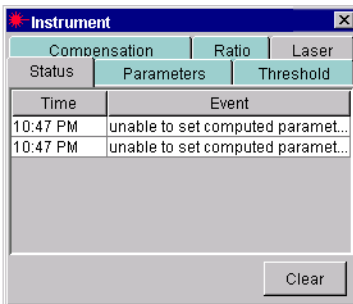
Each instrument is assigned a name and serial number, which are used to label FCS files and might also be needed during troubleshooting. Choose Instrument > Instrument Name to access a dialog box where you can enter or locate the unique serial number for the connected instrument. (The instrument name is specified during software installation and cannot be subsequently changed.)

If your software lists the default serial number of 1, BD Biosciences recommends that you input the actual serial number, found on the name plate of your instrument.

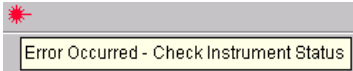


Status Messages

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



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

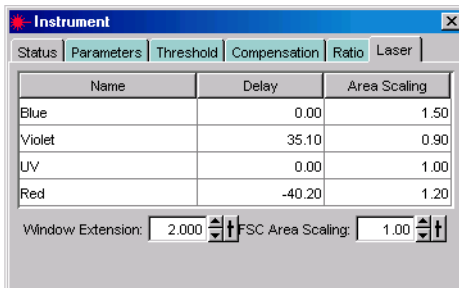


To resolve instrument errors, try shutting down the computer and the instrument, and then restarting them. If the message reappears, contact technical support for assistance. Provide the exact wording of the status message when you call for assistance.

Click the Clear button to clear the current status messages.

Laser Controls

Lasers are instrument-specific, thus laser controls for your instrument might be different from those shown in this section. If the following controls do not apply, consult your instrument user's guide. For the BD FACSCanto system, laser name, delay, and laser area scaling settings can be edited only by field service engineers.



The values entered in the Laser tab apply globally to BD FACSDiva software—values are not saved with Experiments or Tubes. The default values at startup are the last set of values used by the software.

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 Using the Tube Inspector on page 74.

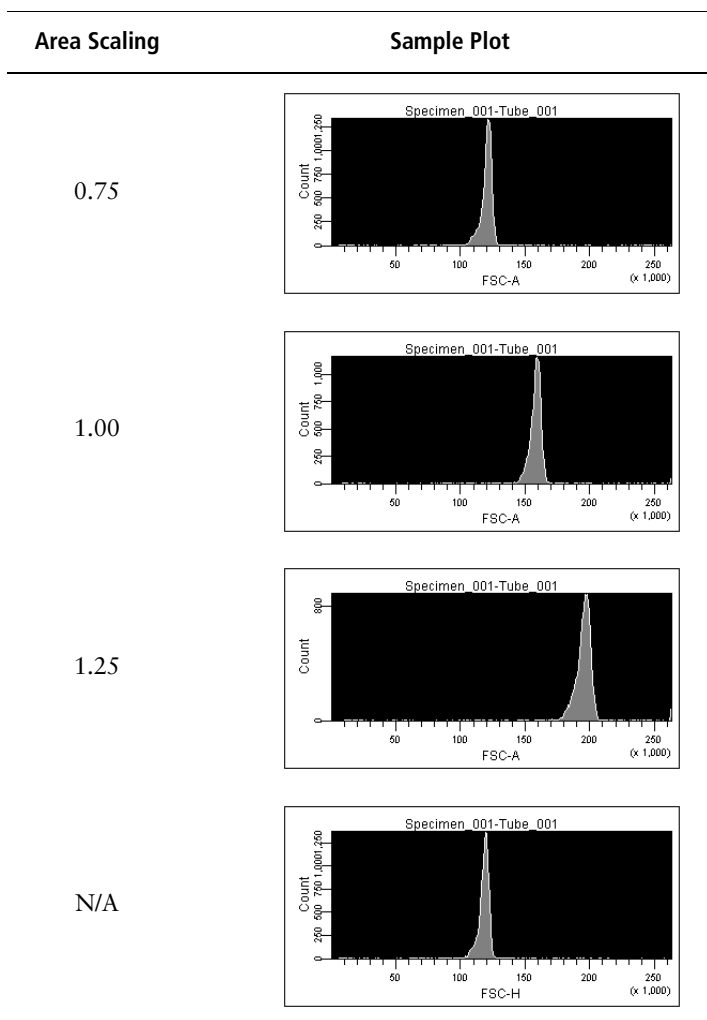
- **Name**—displays the laser name. Rename a laser by clicking in a field and entering a new name. The corresponding fields are updated in the Instrument Configuration dialog box.
- **Delay**—adjusts the amount of time between signals from different laser intercepts, from -200 to 200 μsec , to align signals from multiple lasers. Laser delay values are applied to all channels detected from their respective lasers, as specified in the current instrument 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 Using the Window Extension on page 101.
- **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.

Using Area Scaling

To ensure 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).



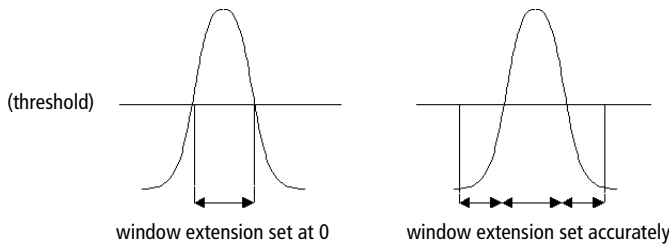
Refer to your instrument manual for specific examples on when to adjust area scaling, if applicable. Note that area scaling does not change the height measurements in any way, nor does it affect the threshold.

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 signal 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 3-2). 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 3-2 Setting a window extension



Instrument Status Report

The instrument status report provides a list of all instrument settings at the time the report was created. You must be connected to the instrument to create the report. To do so, click to set the Current Tube pointer, and choose Instrument > Instrument Status Report. The report is displayed in a separate window with a menu bar above the report header. The header lists the instrument, instrument serial number, and the date and time the report was prepared.



Three types of information are displayed on the report: instrument information, instrument settings, and sorting settings (for instruments equipped with sorting features). The report can be printed or exported.

- The Instrument Info section lists lasers used, with corresponding delay and area scaling values, along with the window extension. (These values are described in Laser Controls on page 98.) Some instruments will show additional information; refer to your instrument manual for information.

Instrument Info		
Lasers	Delay	Area Scaling
Blue	0.0	1.5
Violet	35.1	0.9
UV	0.0	1.0
Red	-40.2	1.2
Window Extension		2.0
FSC Area Scaling		0.2

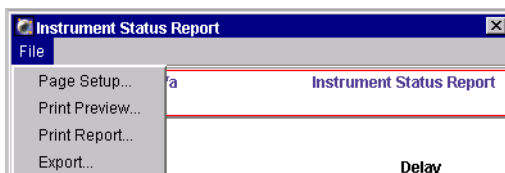
- The instrument settings section displays settings for the current acquisition Tube. All parameters collected for the Tube are listed, along with voltage settings and whether Log is on or off. Threshold and ratio settings are shown only if values have been entered in instrument settings. Compensation 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 instrument manual for more information.

Printing or Exporting the Report

- To print the report, choose File > Print Report within the Instrument Status Report window. You can 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 Instrument Status Report window. A dialog box appears where you can specify the file storage location and choose the export format.

The file is exported as a list of comma-separated values (CSV) that can be opened with a spreadsheet application such as Microsoft Excel.

Standby and Connect

For acquisition workstations (computer connected to a cytometer), use the Instrument > Standby command to interrupt communication between the software and the instrument. The command is disabled during acquisition, recording, or fluidics procedures (for instruments with software-controlled fluidics modes). For information about working offline, see Working Offline on page 194.

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

When in Standby, choose Instrument > Connect to reestablish communication.

Acquisition Controls

Acquisition and recording are controlled using buttons in the Acquisition Controls frame or the Current Tube pointer in the Browser. The Acquisition Controls frame is described in this section. For information about the Current Tube pointer, see Current Tube Pointer on page 106.


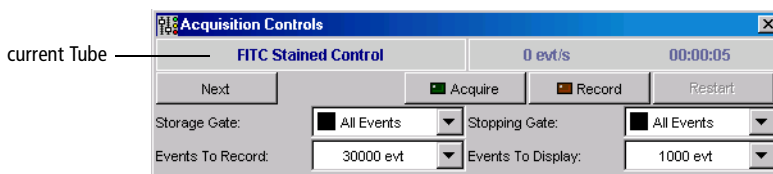
To display the Acquisition Controls frame, click the corresponding button on the Workspace toolbar (). Controls are displayed only when the workstation is connected to the cytometer. The top of the frame shows the current Tube (indicated by the Current Tube pointer), along with the threshold rate and elapsed time. See Acquisition Status on page 108 for a description of these counters.

Figure 3-3 Acquisition Controls frame




Other controls are as follows. Note that additional acquisition controls might be available for your instrument; refer to your instrument manual for information.

- **Next**—moves the Current Tube pointer to the next Tube in the Browser. If no Tube exists, clicking Next creates a new Tube by duplicating the previous Tube without data. If the Tube Specific Worksheets preference is enabled (see General Preferences on page 86) and you are viewing a normal worksheet, clicking Next automatically places Analysis objects for the new Tube onto a new, blank worksheet.
- **Acquire**—starts acquisition for the current Tube (indicated by the Current Tube pointer). Events are displayed in any plots created for the Tube but data is not saved to the database. Statistics are displayed in the statistics views and the values are updated in accordance with the Events to Display setting.

When acquisition is in progress, click the Acquire button to stop acquisition.

- **Record**—starts recording data for the current Tube. 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 reached. The resulting data is saved in the database.

 **Tip** Use the Processed Events counter in the Acquisition Status frame to view the number of events recorded to the data file as you record data.

When recording is in progress, click the Record button to stop recording data before reaching the specified number of events. If you click the Acquire button while data is being recorded, a confirmation dialog box appears where you can choose to stop or continue recording.

NOTICE If you click Record for a Tube that already has data, you can choose to Append, Overwrite or Cancel. Data can be appended only if the current instrument 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. Restart can be used during acquisition or recording.



Clicking Restart during recording overwrites all previously recorded data.

- **Storage Gate**—specifies the population for which events are to be recorded.
- **Stopping Gate**—specifies the population for which events are to be counted.
- **Events to Record**—specifies the number of events to be recorded for the current Tube. Recording will stop when the valid event counter reaches the specified value. If a Stopping Gate is specified, recording stops when the number of valid events within that gate reaches the specified value.

Note that the Events to Record, Storage Gate, and Stopping Gate are reflected in the Acquisition tab of the Tube Inspector. See Using the Tube Inspector on page 74.

- **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 choose 1,000, only the most recently acquired 1,000 events will be shown. Selecting a lower number will allow 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.

Current Tube Pointer

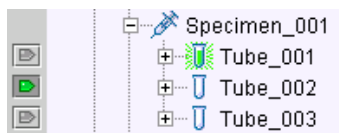
When the workstation is connected to the cytometer, a grey pointer icon is displayed next to Tubes in an open Experiment.

- To select a Tube for acquisition, click the grey pointer next to the Tube. The pointer changes to green and the name of the selected Tube is displayed in the Acquisition Controls and Acquisition Status frames.
- To start acquisition, click the green pointer. The pointer changes to yellow indicating that acquisition is in progress. Click the pointer again to stop acquisition.

Tip In User Preferences, you can specify that acquisition begin automatically every time the Current Tube pointer moves to a new Tube. See General Preferences on page 86 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. The pointer can be moved to any other Tube within the open Experiment by clicking its associated gray pointer (such as next to Tube_003).



A blue pointer indicates 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 the Tube is currently acquiring data.



An orange pointer indicates the Tube is currently recording events.



After events have been recorded, the pointer reverts to green and the Tube icon is highlighted with green. Instrument settings in effect at the time the Tube was recorded are saved with the Tube.



The pointer does not automatically advance to the next Tube after data has been recorded. To acquire the next Tube, you must click the gray pointer to the left of the subsequent Tube in the Browser, or click the Next button in the Acquisition Controls frame.

When you close and then open an Experiment, the green pointer disappears. You must click to set the Current Tube pointer. As a general rule, always verify that the appropriate Tube is indicated by the Current Tube pointer before you click Acquire.

Acquisition Status

The Acquisition Status frame provides ongoing status during acquisition or recording. The fields in the frame cannot be edited.


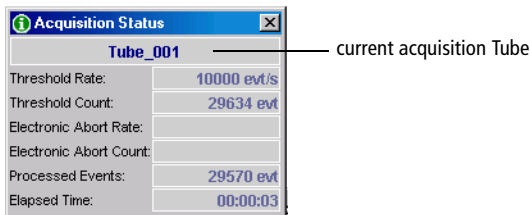
To display the frame, click the Acquisition Status button in the Workspace toolbar (). When the workstation is connected to the cytometer and the Current Tube pointer has been set, the name of the current Tube appears at the top of the frame. A progress bar appears behind the Tube name when data is being recorded.

Figure 3-4 Acquisition Status frame



The following information can be viewed in the Acquisition Status frame:

- Threshold Rate—events/second for events that trigger the system threshold
- Threshold Count—cumulative count of all events that trigger the system threshold (ie, events that pass through the laser beam)

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

- 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

- Processed Events—cumulative count of events processed by the software for the current Tube

Statistics are calculated only on processed events.

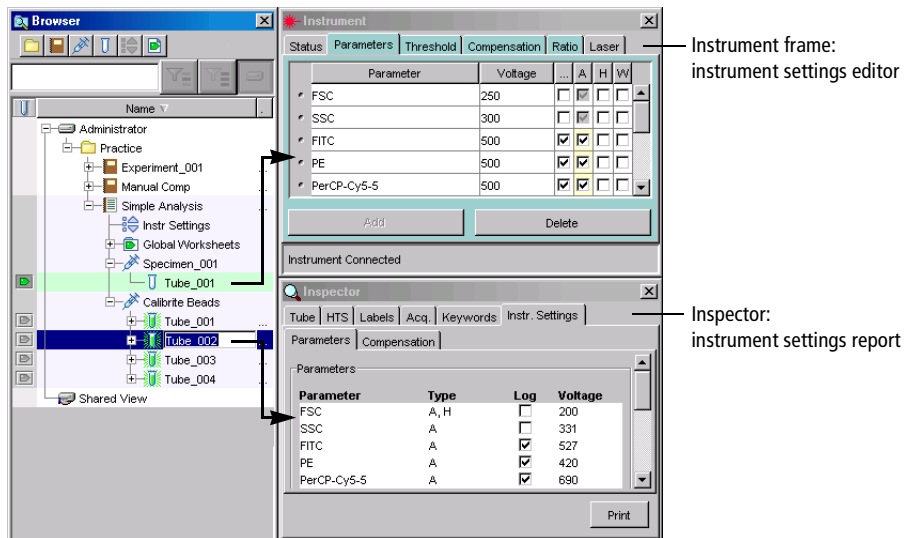
- Elapsed Time—amount of time that has passed since the Acquire, Record, or Restart button was clicked

Instrument Settings

Instrument settings represent the collection of values for parameters measured, PMT voltages, threshold, compensation, and the calculated parameters Log and Ratio. Instrument settings can apply to Tubes, Specimens, or Experiments.

During offline use, instrument settings are edited in the Inspector. When you are connected to the instrument, you can change voltages, thresholds, and ratios only in the Instrument frame; the Inspector shows a report of the settings for a selected Tube or Instr Settings icon. You can use this feature to compare settings for the current Tube with those from another Tube or Experiment (Figure 3-5).

Figure 3-5 Viewing instrument settings



See the following sections for information about instrument settings:

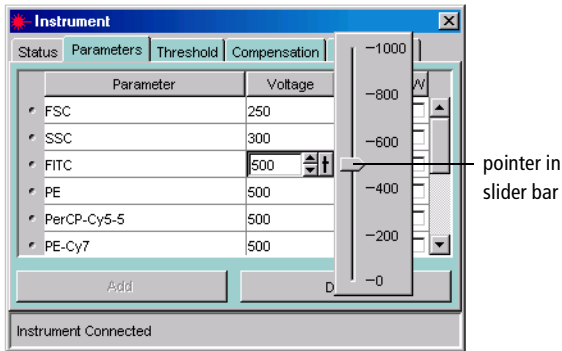
- Adjusting Instrument Settings on page 110
- Creating Specimen- or Tube-Specific Settings on page 118
- Printing Instrument Settings on page 121

Adjusting Instrument Settings

To edit instrument settings during acquisition, click to position the Current Tube pointer in an open Experiment. Once a Tube has been selected, four instrument settings tabs are shown in the Instrument frame. (These four tabs appear in the Inspector frame during offline use.) Use controls in each tab to edit or adjust instrument settings. Within certain tabs, you can adjust settings using software controls or your keyboard.

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 3-6.

Figure 3-6 Software controls for adjusting instrument 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 Control key while clicking the arrows or pressing the keys to increase stepped values ten-fold.

Using the Parameters Tab

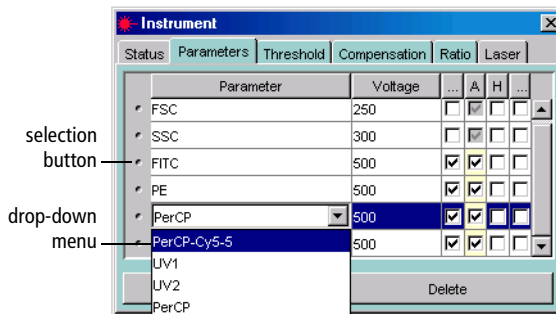
A *parameter* is a measurement of a cell property determined as the cell passes through the laser beam. Each parameter is the output of a single PMT or photodiode, measuring fluorescent or scattered light. During acquisition, parameter data is sent from the cytometer to the workstation. By default, data is acquired and stored for the parameters listed in the current instrument configuration (see Instrument Controls on page 92).

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 unlisted parameters by clicking the Add button.

Parameters are listed in the order they are defined in the Instrument Configuration dialog box. 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 channel by clicking the parameter field and choosing a different fluorophore from the drop-down 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 Control 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 241.

- 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 Instrument Settings on page 110.

- 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 60.

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, only 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 this reason, the lowest decade on a log plot is always empty for height. For more information, see Parameter Values on page 241.

You can convert to log display before or after acquisition because data is always measured and stored in linear. If you change from linear to log or biexponential during analysis, the data will be redisplayed.

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. Thus, after appending 5 minutes of data to a 10-minute data set, the Time parameter of the last event would appear at 90,000.

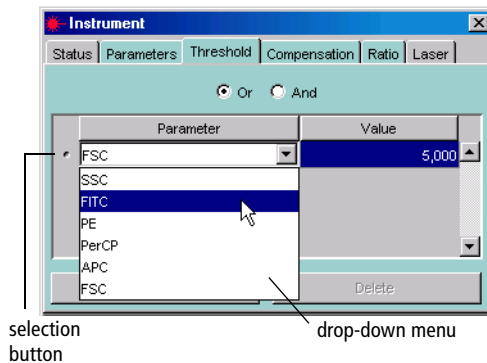
Tip To allow enough time for Ca^{++} flux response and resolution, enter a large value for the *Events to Record* before recording events for a calcium flux experiment. You cannot enter a specific time in which to record events or assign a time resolution.

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 drop-down menu that appears.



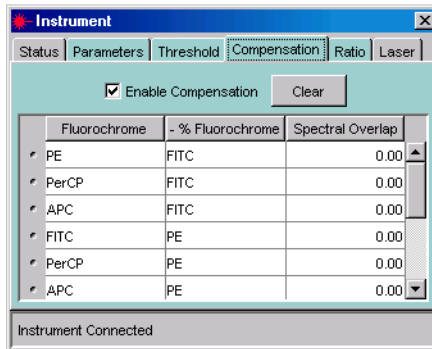
- Delete a Threshold parameter by clicking the selection button next to the row to delete, then clicking the Delete button.

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 122.



There are two ways to adjust compensation in BD FACSDiva software: automatically using the Instrument Setup feature, or manually. To practice using each of these methods, try the corresponding tutorials in the *BD FACSDiva Software Quick Start Guide*.

When compensation is calculated using Instrument Setup (see Calculating Compensation Using Instrument Setup on page 123), you should not need to adjust the values after calculation.

If you are adjusting compensation manually, 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. To clear all values, select all rows, and then 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 instrument settings object to another. To do so,

- 1 Right-click the Tube or Instr 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 instrument 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.
 - Choose Paste Spectral Overlap to update 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.
 - Choose Paste Spectral Overlap with Zeros to update existing values with the values from the copied settings, and to overwrite non-zero values in the target settings with zeros from the source. 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 instrument 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.

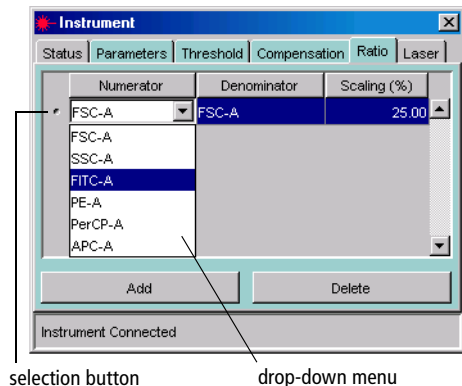
When a parameter exists in the target, but not in the source, the values for that parameter will not be changed. Conversely, if a parameter exists in the source but not the target, the values for that parameter are not added to the target.

Using the Ratio Tab

Ratios are most commonly used for calcium flux experiments. They are calculated by dividing the signal from one fluorescence detector by the signal from another fluorescence detector and then multiplying by a percentage value (25%, by default).

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 drop-down 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 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 instrument settings at the Specimen or Tube level:

- Select the Tube or Specimen in the Browser, and then click the New Instrument Settings button in the Browser toolbar ().
- Right-click the Specimen or Tube icon in the Browser and choose New Instrument Settings.
- Select the Specimen or Tube and choose Experiment > New Instrument Settings.
- Copy instrument settings from another Browser element (Experiment, Specimen, or Tube), and paste them to the target Tube or Specimen.

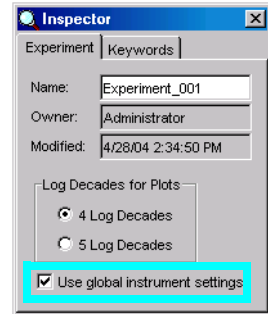
When you create new settings, initial values are copied from the closest parent settings. Further adjustments apply to the Experiment-level settings only when the *Use global instrument settings* preference is enabled (selected by default).

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 Instrument Settings on page 119.

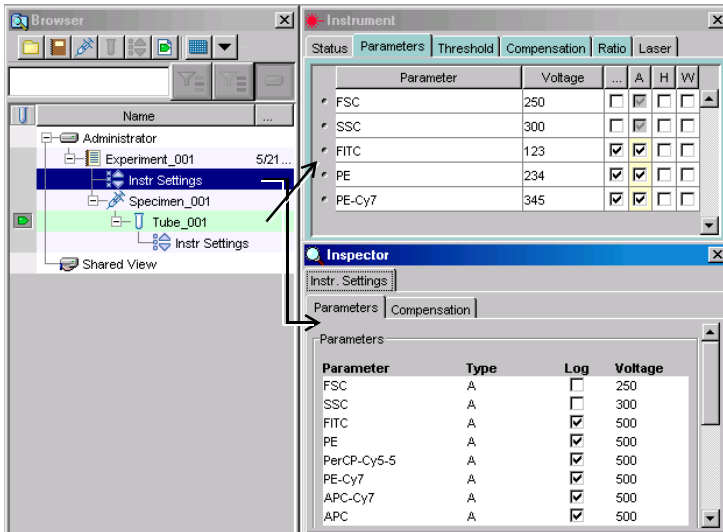
Using Global Instrument Settings

The *Use global instrument settings* option is available in the Experiment Inspector. When this preference is enabled, Experiment-level instrument 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.

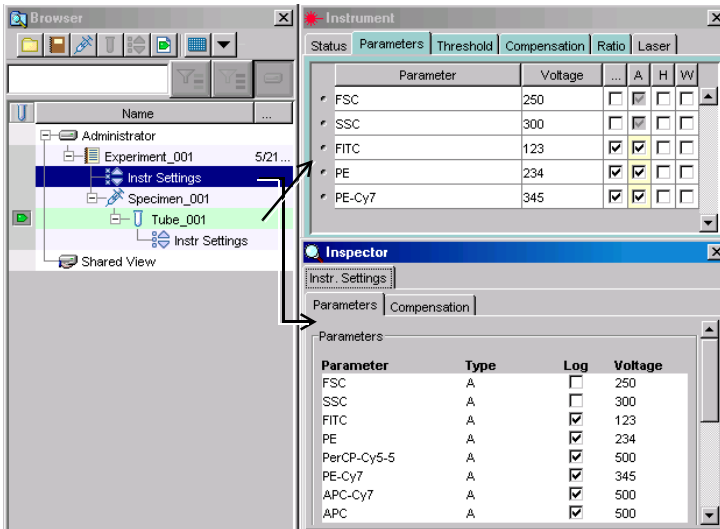
NOTICE If Tube- or Specimen-specific settings are linked to a Setup (see Applying a Setup to Instrument Settings on page 131), Experiment-level settings are not updated automatically, even when this preference is enabled.



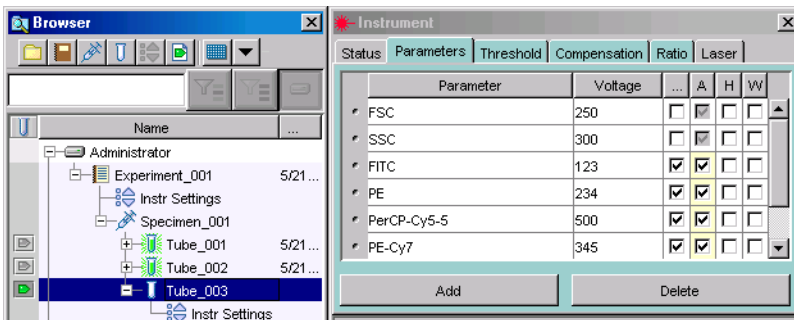
For example, if you disable the *Use global instrument settings* option and make changes to Tube-specific settings (Instrument frame), notice that the global settings (in the Inspector) do not change.



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:

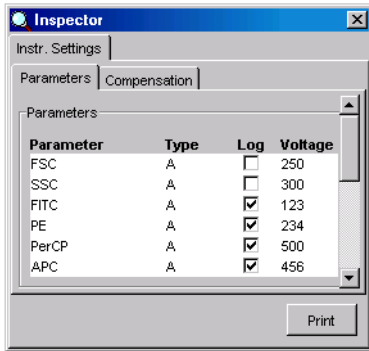


Additionally, updated settings are automatically applied to the remaining unrecorded Tubes in the Experiment, even when they have Tube-specific settings. Updated settings are applied when you move the Current Tube pointer:



Printing Instrument Settings

To print instrument settings for the current Experiment, Specimen, or Tube, select the item in the Browser and click the Print button in the Instrument Settings Inspector (report view), or right-click the selected item and choose Print from the contextual menu.



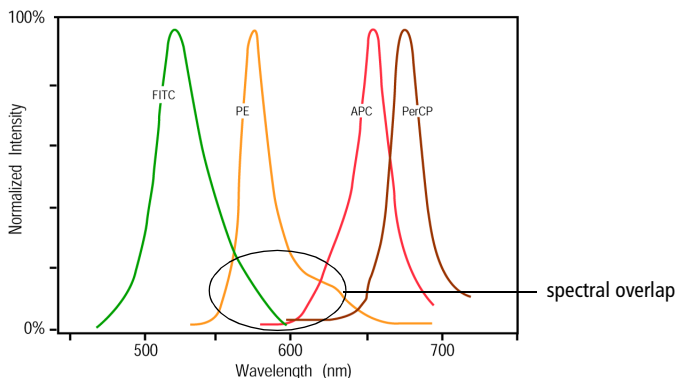
A printout will be generated that includes:

- name of Experiment, Specimen, and Tube
- collection date
- list of parameters collected, voltage values, and whether log is on or off
- threshold parameter(s) and value(s)
- compensation state and values
- ratio parameters

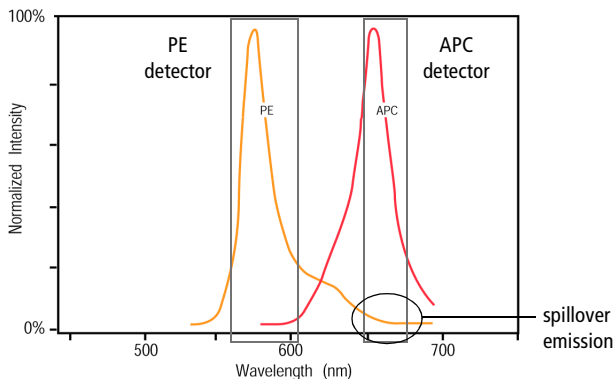
A more detailed, formatted report of instrument settings can be printed, viewed, or exported using the Instrument Status Report option on the Instrument menu. See Instrument Status Report on page 101 for more information.

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 $APC - \%PE$.

Generally, in analog systems, compensation correction is applied electronically before the data is stored, while in digital systems, the software applies the correction real time or after the data is stored. This section describes the compensation controls available in BD FACSDiva software.

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

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

Instrument Setup features are accessed from the Instrument menu. Choose Instrument > Instrument Setup to access setup functions. For details on each function, see the following:

- Creating Compensation Controls on page 124
- Defining Label-Specific Controls on page 125
- Calculating Compensation on page 128
- Viewing the Setup Catalog on page 128

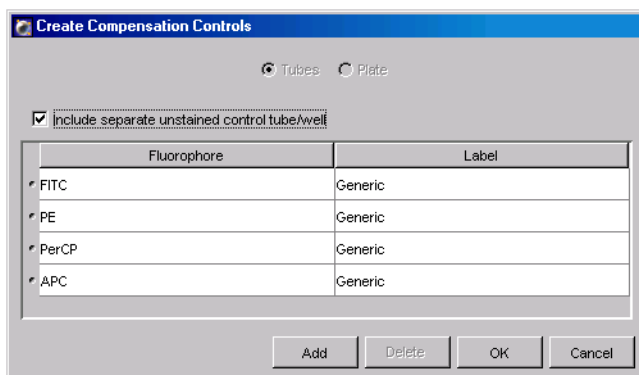
To practice using the Instrument Setup feature, do the corresponding tutorial in the *BD FACSDiva Software Quick Start Guide*.

Creating Compensation Controls

Choose Create Compensation Controls to automatically add compensation controls and Analysis objects to your Experiment. A dialog box appears where you can add or delete controls, define label-specific controls, or change the order of the compensation controls (Figure 3-7).

NOTICE In the Create Compensation Controls dialog, you can add only parameters that are listed in the Parameters tab. To change to a secondary fluorophore for any channel, edit instrument settings in the Inspector or Instrument frame *before* you create compensation controls.

Figure 3-7 Creating compensation controls



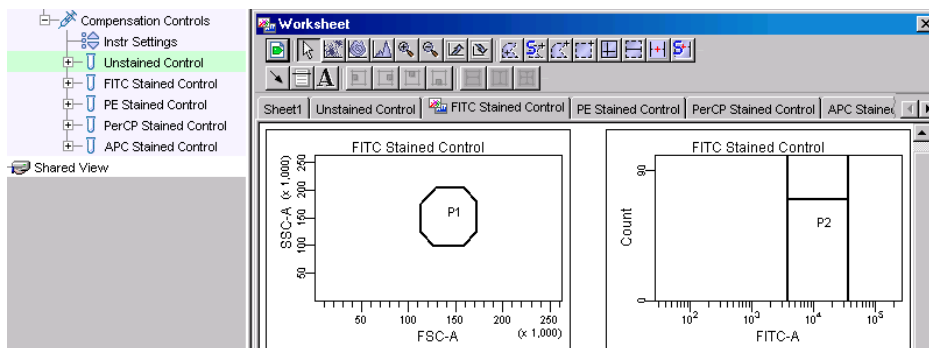
- Leave the *Include separate unstained control tube/well* checkbox selected when you are running unstained sample as one of your compensation controls.
- Deselect the checkbox when you are including unstained sample in each of your stained control tubes or wells. In this case, you will need to gate the unstained population in each fluorescence histogram before you calculate compensation.
- For information about label-specific controls, see Defining Label-Specific Controls on page 125.

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 fluorophore, along with an unstained control worksheet if the checkbox was selected (Figure 3-8). The Experiment's instrument settings are copied to the Specimen, 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 3-8 Compensation controls and worksheets



Defining Label-Specific Controls

Create label-specific controls if your experiment contains samples stained with the same fluorophore 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.

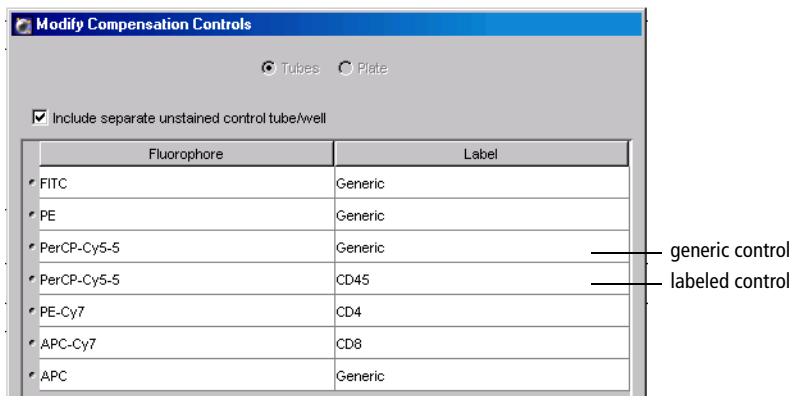
- 1 Choose Instrument > Instrument Setup > Create Compensation Controls or Modify Compensation Controls.

The Create (or Modify) Compensation Controls dialog box appears (Figure 3-9).

Note that some of the parameters already contain labels. When a parameter name includes a hyphen (indicating it is a fluorophore conjugate) and a label has already been assigned in the Experiment Layout dialog box or the Inspector, BD FACSDiva 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 drop-down 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 3-9 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 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 frame, 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.

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 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.

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

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

- 3 If needed, adjust PMT voltages to place populations at the required locations.

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

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

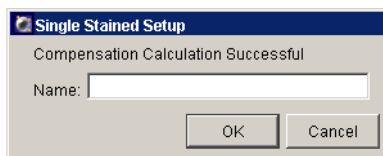
- 5 If you *do not* have a separate unstained control, create an Autointerval gate around the negative population in each fluorescence histogram.

If you have an Unstained Control Tube (or Well), you can skip this step.

Calculating Compensation

After gates have been adjusted, you are ready to calculate compensation. Choose Instrument > Instrument 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; otherwise, a dialog box appears where you can enter a name for the compensation Setup:



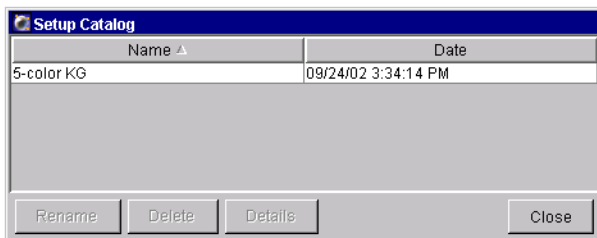
Enter a name, and click OK to dismiss the dialog box.

- Tip** Include the Experiment name or date to keep track of compensation Setups.

Viewing the Setup Catalog

After a successful compensation calculation is named, all instrument settings associated with the compensation calculation are saved in a Setup catalog. A saved Setup contains parameter and label information, the 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 Instrument Setup sessions by choosing Instrument > Instrument Setup > Setup Catalog.



- To edit a Setup session, select the session and click Rename or Delete.
- To view the details of a Setup session, select the session and click Details.

A window appears listing all instrument settings and labels associated with the Setup.

Fluorophore	Labels	A	H	W	Log	Threshold (Or)	PMT Voltage
FSC		<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	5000	250
SSC		<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		300
FITC	Generic	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>		525
PE	Generic	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>		496
PerCP	Generic	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>		591
APC	Generic	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>		680

- Click the Spectral Overlap tab to view calculated overlap values in the form of an MFI table.
- Click the Lasers tab to view the laser delay setting and area scaling value for each laser.
- Click Close to return to the Setup Catalog.

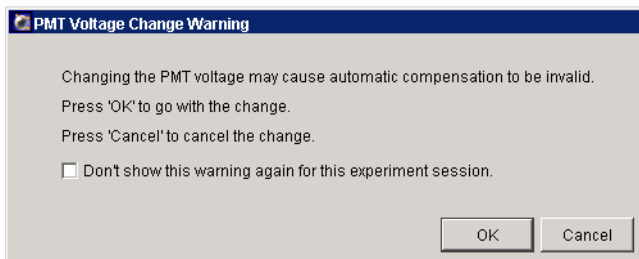
Note that much of the information displayed in the Setup Catalog can be printed or exported by printing or exporting a set of linked instrument settings. See Printing Instrument Settings on page 121 for more information.

Using Setups

When compensation is calculated by the software, the Experiment is linked to the resulting compensation Setup. When the *Use global instrument settings* preference is enabled for the Experiment, the Setup is applied to Tubes in the Experiment as the Current Tube pointer is moved. To further optimize settings based on a Setup, be sure to unlink the Experiment from the Setup before you optimize the settings. See Unlinking a Setup from Instrument Settings on page 130.

A Setup can also be applied to a new Experiment, as described in Applying a Setup to Instrument Settings on page 131. 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 133.

NOTICE Changes to PMT voltages for a Tube that is linked to a Setup could invalidate the linked compensation settings. The software warns you when you change voltages for a Tube that is linked to a calculated Setup:



- To disregard this message and apply the change, click OK. Click the checkbox if you do not want the warning message to appear again.
- To keep the same PMT settings, click Cancel.

Unlinking a Setup from Instrument Settings

You can unlink instrument settings from a calculated Setup if you need to make adjustments to the settings. To do so, right-click an Instr Settings icon and choose Unlink From *SetupName*; click Yes in the dialog box that appears.

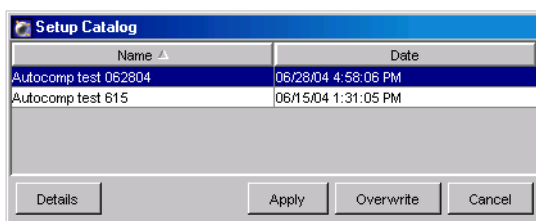
NOTICE If you re-link the settings and the preference is enabled to *Automatically update instrument settings according to latest setup*, your adjustments will be overwritten by the previous settings.

Applying a Setup to Instrument Settings

Saved Setup values (spectral overlap, threshold, and PMT voltages) can be applied to an Experiment, and spectral overlap values in a Setup can be applied to a recorded Tube.

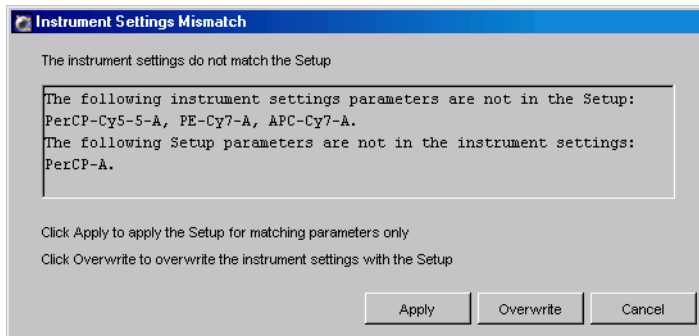
- To apply a Setup, right-click an Instr Settings icon in the Browser and choose Apply Setup.
- To apply spectral overlap values, right-click an Instr Settings icon under a recorded Tube and choose Apply Compensation.

In either case, a Setup Catalog appears where you can select a Setup to apply:



- Click Details to view values associated with the Setup.
- Click Apply to apply values to matching parameters in the instrument settings. Only parameters that match those in the Setup are updated. Settings are then linked to the Setup, so if you add a parameter that matches one in the Setup, values for the matching parameter are updated automatically.
- Click Overwrite to replace the existing settings with settings based on the selected Setup. (This option is not available for recorded Tubes.) If parameters and PMT voltages match, the Setup Catalog is dismissed. If the parameters or PMT voltages do not match, the following message appears (Figure 3-10 on page 132).

Figure 3-10 Instrument Settings Mismatch message



- Click Apply to apply PMT voltages, spectral overlap values, and threshold values to parameters in the instrument settings 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, spectral overlap and PMT values are updated automatically.
- Click Overwrite to replace the existing settings with settings based on the selected Setup. Parameters that did not match are removed.

NOTICE If the Setup or instrument 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 133.

Once an instrument settings object has been linked to a Setup, you cannot edit compensation values manually. The compensation editor of the instrument settings is locked, and the Clear button and Paste Spectral Overlap commands are disabled.

Automatically Updating Linked Instrument Settings

The General tab of User Preferences contains a checkbox to *Automatically update instrument settings according to the latest setup*. When this preference is selected (which is the default setting), instrument settings for unrecorded Tubes that are linked to a named Setup are automatically updated when the Setup changes.

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:

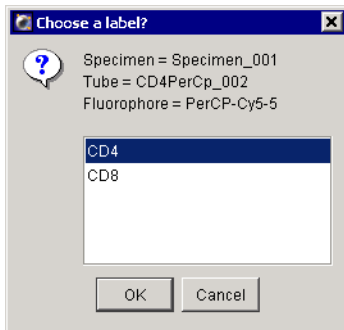
- Use the Experiment Layout dialog box to label all Tubes in the Experiment at once.
- Use the Labels tab in the Inspector to label one Tube at a time.

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 box that appears.
Label-specific	Labeled	Label-specific, if label of Control Tube matches the parameter label. Otherwise, label must be chosen in dialog box 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 box appears where you can choose which control to apply. See Figure 3-11 on page 134.

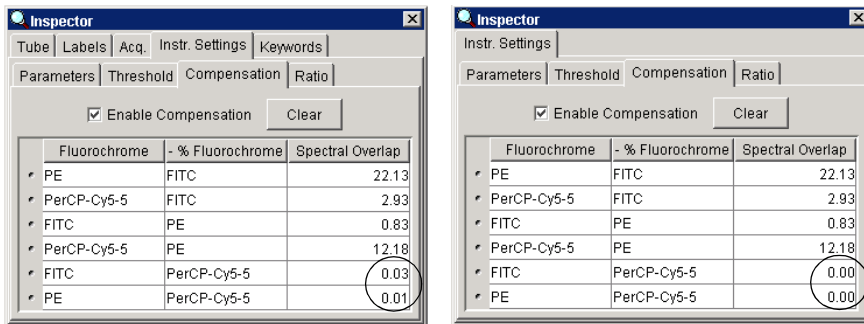
Figure 3-11 Choosing a control label



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 box without choosing a control, the software will not apply a complete compensation matrix resulting in uncompensated channels, as shown in the following figure.

Figure 3-12 Complete (left) vs incomplete (right) compensation matrix using label-specific controls



Calculating Compensation Manually

When you adjust compensation settings manually, 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.

Compensation settings for single-color Tubes are combined for a mixed-color Tube using the Copy Spectral Overlap and Paste Spectral Overlap commands. See Copying Spectral Overlap Values on page 116 for a description of these commands. For a demonstration of this feature, practice the tutorial on Calculating Compensation Manually in the *BD FACSDiva Software Quick Start Guide*.

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

NOTICE If you paste compensation values to a Tube with different Voltage settings than the source Tube, a warning message will appear.

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 that you can print or export.

This chapter provides an overview of data analysis. Application-specific examples of data analysis can be found in your instrument manual.



The following topics are covered in this chapter:

- Worksheets on page 138
- Plots on page 150
- Gates on page 167
- Population Hierarchy on page 178
- Statistics on page 184
- Batch Analysis on page 192
- Working Offline on page 194

Worksheets

A *worksheet* is the main work area of BD FACSDiva software—it is where you create plots, define gates, show statistics and population hierarchies, and enter custom text. Because plots and statistics for a Tube can be on different worksheets, multiple worksheets can be used to organize your workflow. For example, use one worksheet for instrument QC and sample optimization, and use a second worksheet for analysis. Worksheets are shown in the Worksheet frame, and can be normal or global.

Normal worksheets have grey-tinted tabs and contain Tube-specific analysis elements, while *global worksheets* have green-tinted tabs and contain elements that can show data from any Tube. Select a Tube's data for display by moving the Current Tube pointer. For more information, see the following section, Normal Worksheets, or Global Worksheets on page 139.

To display the Worksheet frame, click the Worksheet button in the Workspace toolbar (). To toggle between the worksheet and global worksheet view, click the Worksheets view button in the Worksheet toolbar (.

Normal Worksheets

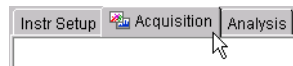
- To add a new worksheet to an open Experiment, choose Worksheet > New Worksheet. A maximum of 30 worksheets is allowed. By default, new worksheets are sized to fill the open frame.
- To expand the size of a worksheet, use the Worksheet Inspector to increase the number of pages. A worksheet can have up to 250 pages. Page breaks are indicated by a dotted line when the Show Page Breaks option is enabled. See Using the Worksheet Inspector on page 144.

Tip Resize the Worksheet frame to view horizontal or vertical scroll bars.

- To find a Tube-specific object on a worksheet, double-click its associated Tube in the Browser. The first object associated with the Tube will be displayed at the top of the Worksheet frame. (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**.

Switch between multiple worksheets by clicking the tabs at the top of the worksheet (see figure). You can work within only one worksheet, the active worksheet, at a time. (The active worksheet is indicated by a plot icon next to the worksheet name.)



NOTICE While there is no impact on data collection or instrument 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 frame.

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. You can display data for any Tube by moving the Current Tube pointer.


A maximum of ten 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 available for normal worksheets are available for global worksheets. To move objects between worksheets and global worksheets, you must 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 worksheet from a global worksheet by the color and title on the worksheet tabs. Normal worksheets are titled *SheetN* when created, and they have the same neutral color background as most other tabs. Global worksheets are titled *Global SheetN* when created, and have colored tabs.

Create a global worksheet in any of the following ways:

- Click the New Global Worksheet button in the Browser toolbar ().
- Choose Experiment > New Global Worksheet.
- Right-click an open Experiment or a Global Worksheets folder and choose New Global Worksheet from the menu.

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.

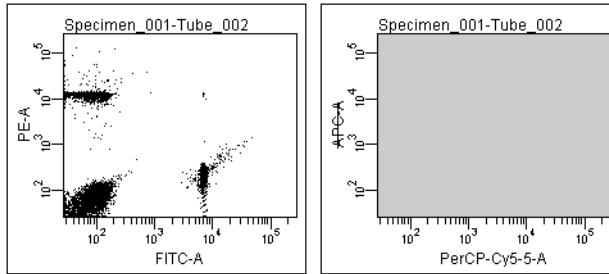
Using Global Worksheets

The following example shows one way in which global worksheets can be used. For more examples, see Using Gating Features in the *BD FACSDiva Software Quick Start Guide*, or refer to your instrument manual.

- 1** Create a global worksheet and generate all required plots.
- 2** Move 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, and population hierarchies as needed.
- 5** Move 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 greyed out (the missing parameter is crossed out). See Figure 4-1 on page 141.

Figure 4-1 Plot displaying all parameters (left) and missing parameter (right)



6 Edit the gates to reflect the data from the second Tube.

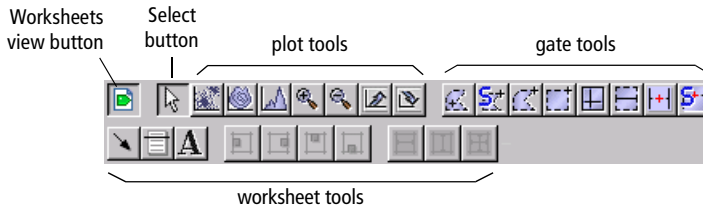
NOTICE Once edited, the gates in step 6 remain as edited, even if you return to Tube 1 by moving the Current Tube pointer. Gates are global and attached to the global worksheet, not the Tube.

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 86.

Using the Worksheet Toolbar

There are five sets of tools on the Worksheet toolbar: the Worksheets view button, a Select button, plot tools, gate tools, and worksheet tools. Certain tools are enabled only when elements appropriate for the tool are selected. To use any enabled tool, click once to select it. To reuse a tool multiple times without reselecting it, double-click the tool. It will remain selected until you select another tool or press the Esc key.



A summary of tool functions follows.



Worksheets view button—toggles the Worksheet frame between the global and normal worksheet view.



Select button—selects (by clicking), moves (by dragging), or resizes (by dragging a selection handle of) objects in a worksheet.

Plot Tools

Use Plot tools to create plots, zoom in and out on plot data, and resize plots.



Plot tools—create dot (D), contour (C) or histogram (H) plots. Select a Tube, select the tool, and then click in the worksheet to create a plot of a default size. For other options, see *Creating Plots* on page 151.



Zoom-In tool—magnifies an area of a plot. Select the tool, 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 tool—undoes the last zoom-in action. Select the tool and click once in the plot. Each click with the tool undoes the last zoom-in action. To return to the originally sized plot in one click, hold down the Control key while clicking in the plot.



Increase Plot Size tool—increases the size of plots on the worksheet. Select the tool and click once in a plot. The length of the x- and y-axes doubles each time you use the tool.



Decrease Plot Size tool—decreases the size of plots on the worksheet. Select the tool and click an increased plot. The length of the x- and y-axes halves each time you use the tool. Plots cannot be resized below their original size.

Gate Tools

Use gate tools to define population subsets on plots. The Interval Gate tools are the only tools that can be used to gate data on a histogram. For more information about gate tools, see *Gates* on page 167.



Autopolygon-Gate tool—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 tool—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 tool—allows a polygonal gate to be drawn around a population on a dot or contour plot.



Rectangle Gate tool—creates a rectangular gate around a population on a dot or contour plot.



Quadrant Gate tool—divides a plot into four quadrant gates. Each quadrant has its own population statistics.



Interval Gate tool—allows a range of events to be selected on a dot or contour plot or histogram.



Autointerval Gate tool—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 tool—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 Tools

Use Worksheet tools to customize worksheets. See *Editing Worksheets* on page 145 and *Aligning and Resizing Worksheet Elements* on page 148.



Customizing tools—allow you to personalize worksheets with arrows (A), lines (L), and text (T).



Align tools—align selected objects in a worksheet. A minimum of two objects must be selected for the tools to be active.

Use these tools to align worksheet elements at their left, right, top, and bottom edges, respectively, with the last-selected object (object with yellow selection handles).



Distribute tools—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 tools to be active.

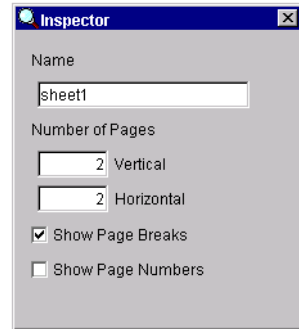


Make Same Size tool—makes selected objects in a worksheet the same size as the last-selected object. A minimum of two objects must be selected for the tool to be active.

Using the Worksheet Inspector

Use the Worksheet Inspector to name the worksheet or global worksheet, increase the number of pages in the worksheet, and hide or show page breaks and page numbers.

To view worksheet options in the Inspector frame, click the background area of a worksheet. (If you click an object on the worksheet, the Inspector shows the properties of that object.)



- To change the name of the worksheet, select the text in the Name field, enter the new name, and then press Enter.
- To enlarge the worksheet area, 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 10 global worksheets. The product of Horizontal x Vertical cannot exceed 250 or 10, 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.

- To show numbers on the lower-right corner of each printable page, select the Show Page Numbers checkbox. Pages are numbered in the order in which they will be printed. When page numbers are shown, they will also appear on each printed page.



Editing Worksheets

Worksheets or global worksheets are used to display plots, gates, population hierarchies, and statistics. For information on creating these elements, see the corresponding sections in this chapter. Additionally, worksheets can be customized with lines, arrows, and text.

NOTICE Lines, arrows, and text are not saved with Analysis objects, thus they are not included when you copy an Analysis object to another Tube or worksheet, or when an Analysis object is saved automatically via user preferences (*Save analysis after recording through global worksheet*).

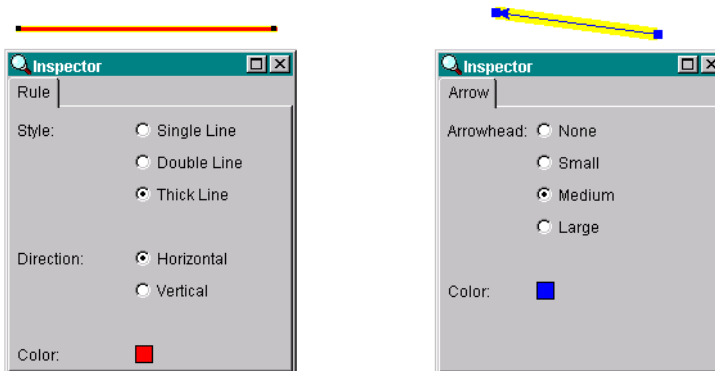
Adding Lines or Arrows

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 tool () and then 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)

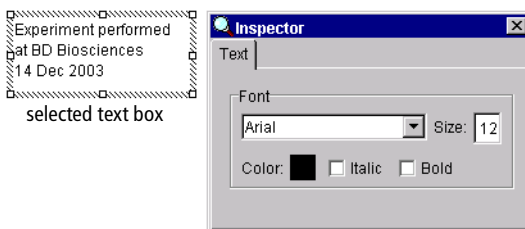


- Specify the line style, direction, and color by making appropriate selections in the Rule Inspector. Resize a line on the worksheet by dragging one of the black handles on either end of the selected line; move the line by selecting the line and dragging.
- Change the style of the arrowhead and the 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

You can add text anywhere on a worksheet. Click the Text tool () and then click in the worksheet to insert a text box; 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.



- 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 then pressing Delete.

Duplicating Worksheet Elements

Duplicate an element on a worksheet in one of the following ways:

- Hold down the Control key and drag a worksheet element to a new location. The element is duplicated when the mouse button is released.
- Press Ctrl-C to copy an element; press Ctrl-V to paste it in a new location, such as on another worksheet. Copied elements can also be pasted to another Tube, or to any Microsoft Office application.
- Right-click a plot and choose Duplicate, or select the plot and press Ctrl-D.

Exporting Worksheet Elements

Individual worksheet elements such as plots, population hierarchies, and statistics views can be copied to any Microsoft Office application.

- 1** Select the worksheet element and press Ctrl-C.
- 2** Open a Microsoft Office document (such as a Word document or PowerPoint® slide), and press Ctrl-V.

You can also 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.

Aligning and Resizing Worksheet Elements

Use worksheet tools to align or resize one or more elements on a worksheet. The following tools can be used with any worksheet object.

Tip When you duplicate an Analysis object (eg, by copying and pasting an Analysis object or duplicating a Tube with an Analysis object), elements in the new Analysis object are added to the available space on the existing worksheet. To contain an Analysis object on one page, edit the size or position of worksheet elements so they fill a page *before* the Analysis object is duplicated.


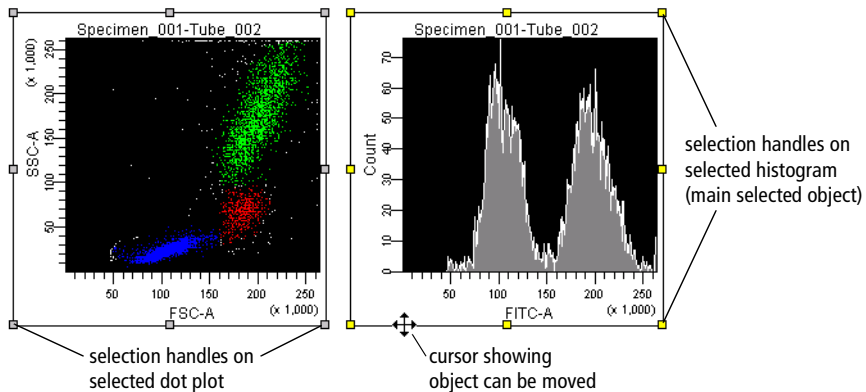




- To move a selected object, position the cursor on the border of the object. When the cursor changes to two double-headed arrows, drag the object to move it. See Figure 4-3.
- To make objects the same size, hold down the Shift key while selecting two or more objects on a worksheet. The object selected last becomes the main selected object, indicated by yellow selection handles (Figure 4-3). Click the Make Same Size tool (); all objects are resized to the same size as the main selected object.

Figure 4-3 Selecting multiple objects



Note that 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 tool () . Use these tools 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 tool (); the objects are distributed equally in a horizontal or vertical direction.
- To increase the size of a plot, select the Increase Plot Size tool () and then click a plot on the worksheet. The length of the x- and y-axes doubles each time you use the tool. Use the Decrease Plot Size tool () to return the plot to its previous size. Note that plots cannot be reduced below their original size.

Tip You can also use the Zoom In tool to magnify an area in a plot.

Printing Worksheets

Choose one of the following commands from the File menu to set up for printing or to print worksheets.

- Choose Page Setup to set the size of the printed page (eg, A4 vs letter), the orientation (portrait or landscape), and the margin size. Your options will vary depending on the printer configured with your workstation.

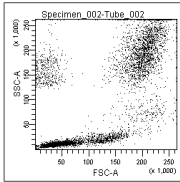
If there are multiple worksheets in the Experiment, options apply only to the active worksheet.

- Choose Print Preview to view a thumbnail of all printable pages at 10, 30, 50, or 100%. Click the close box to return to the worksheet.
- Choose Print to print the active worksheet.

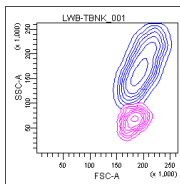
NOTICE Do not place worksheet elements on the dotted line representing a page break. Objects that straddle a page break will be split between two printed sheets.

Plots

Multiparameter data events can be displayed in dot, contour, density, or histogram plots.

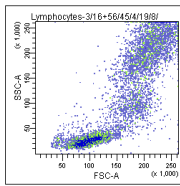


Dot Plot—graphical representation of two-parameter data, where each axis displays the signal intensity of one parameter and each dot represents one or more events (cells or particles)



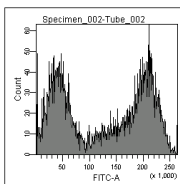
Contour Plot—graphical representation of two-parameter data, where each event has a position in the plot according to its intensity values for both parameters

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 tools 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 162.

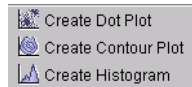
- To create a plot using a plot tool, select a plot tool from the Worksheet toolbar, and click once on the worksheet to draw a plot of default size.



If you prefer to define the plot size, click and drag on the worksheet to create a rectangle of the required size.

- Tip** To create multiple plots, double-click the Plot tool. The tool will remain selected until another tool 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 create a plot on a normal worksheet using menu commands, select one or more Tubes in the Browser, right-click the selected Tubes, and choose an option from the menu.



If you create plots while the global worksheet view is displayed, plots are part of the Analysis object for the current global worksheet.

If you create plots while the normal worksheet view is displayed, plots are associated with the Analysis object of the selected Tube(s), or with the current acquisition Tube if no Tube is selected. If more than one Tube is selected before you create a plot, plots are drawn for each Tube and staggered on the worksheet.

Creating Gated Plots

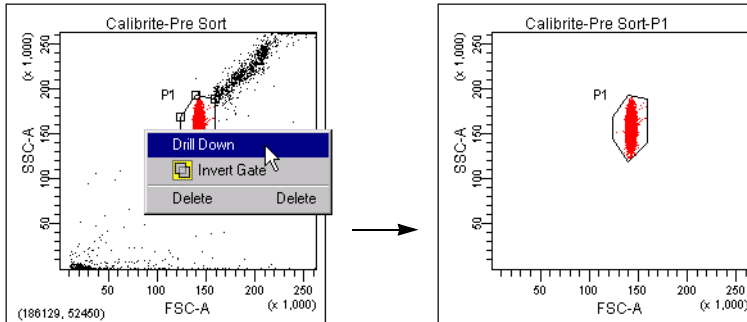
After gated populations have been defined, use the Drill Down feature to easily create a plot showing data from only one population.

1 In a plot showing one or more gated populations, right-click the gate border.

Tip If the gate border is not shown, first right-click the plot border and choose Show Gate > *population name*.

2 Choose Drill Down from the menu.

A new plot appears with the same plot parameters and axis scale settings, showing only data from the selected population.



Duplicating Plots

Duplicate a plot in one of the following ways:

- While holding down the Control 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; 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 148 or *Resizing Plots* on page 154.
- 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 147.
- Duplicate a plot on the same worksheet: see *Duplicating Plots* on page 152.
- Change plot parameters: see *Changing Plot Parameters* on page 154.
- Zoom in and out on plot data, or alter plot size: see *Plot Tools* on page 142 or *Resizing Plots* on page 154.
- Change between four- and five-log decade displays for a plot: see *Changing Log Display* on page 155.
- Select and sequence the order of populations to display: see *Choosing Populations to Display* on page 156.
- Show or hide plot grids: see *Formatting Plots* on page 157.
- Add, remove, or change plot titles and axis labels: see *Formatting Plots* on page 157.
- Display biexponential scales: see *Using Biexponential Scaling* on page 159.


For each type of plot (Dot, Contour, Density, and Histogram) there are also specific formatting and editing features available. See *Using the Plot Inspector* on page 157.

Resizing Plots

To enlarge a plot, select the plot and drag one of the selection handles. (See *Aligning and Resizing Worksheet Elements* on page 148.) You can also use the Increase Plot Size tool to double the size of a plot on a worksheet, and the Decrease Plot Size tool to return the plot to its original size.

- 1 Select the Increase Plot Size tool () and click a plot on the worksheet.

The size of the plot doubles, making it easier to view individual events.

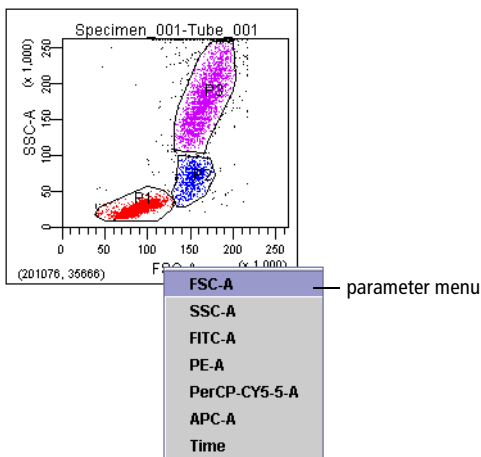
- 2 Select the Decrease Plot Size tool () and click the doubled plot.

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



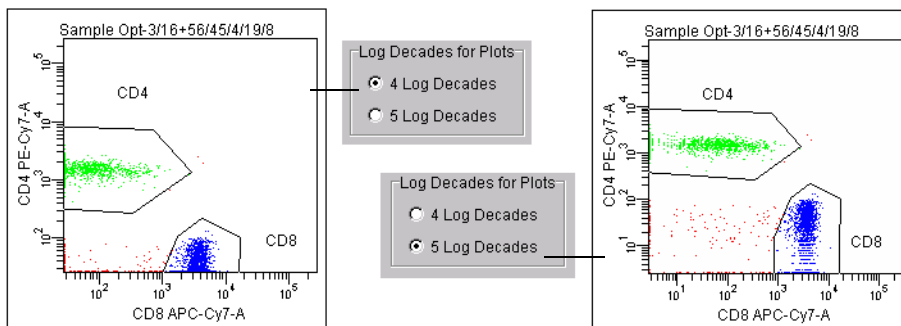
All parameters specified in the Parameters tab are available. Depending on which checkboxes are selected in the Parameters tab, parameters will be listed as *parameter-A*, *parameter-H*, or *parameter-W*.

You can also choose Time as a parameter. For more information, see Using the Time Parameter on page 113.

Changing Log Display

Log data can be 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.

Tip Use the Show Grid feature to delineate log decades on plots. See Formatting Plots on page 157.

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 View on page 179.
- Create Statistics View—displays statistics for populations in the plot. See Statistics on page 184.
- Show Populations—allows you to select which population(s) to display in the plot by selecting the checkbox(es). (Populations can also be selected in the Plot Inspector; see Formatting Plots on page 157.)

Show Population Hierarchy	Ctrl+G
Create Statistics View	Ctrl+R
Show Populations	▶
Show Gate	▶
Bring to Front	▶
Send to Back	▶
Order Populations by Count	
Duplicate	Ctrl+D
 Cut	Ctrl+X
 Copy	Ctrl+C
Delete	Delete



If you gate on a population that has been assigned No Color (☒), no events appear in the plot.

- Show Gate—shows or hides the gate outline for selected populations in the plot. See Hiding and Showing Gates on page 177. 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 on the current worksheet.
- Delete—deletes the plot (Alternatively, select the plot and press the Delete key.)

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 changes in the Inspector 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).

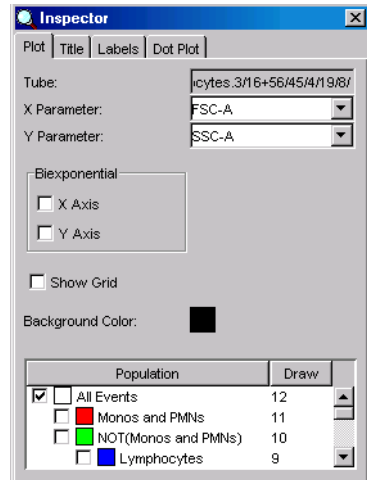
Formatting Plots

Use the Plot tab of the Inspector to view the Tube associated with the plot, to view or change the plot parameters and the background color, to turn on Biexponential scaling, and to specify the populations to show in the plot.

- Change the plot parameters by choosing a parameter from the X or Y Parameter drop-down menu (only the X parameter can be changed for histograms). All parameters specified in the Parameters tab are available. In addition, you can choose Time.

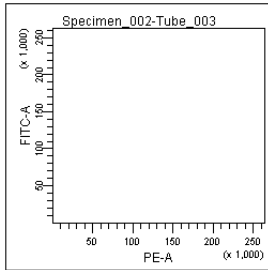
NOTICE Parameters can also be changed directly on a plot by clicking the axis label and choosing a different parameter from the menu that appears. See Changing Plot Parameters on page 154.

- Turn on Biexponential scaling for the x-axis, y-axis, or both by selecting the corresponding checkbox(es). For more information, see Using Biexponential Scaling on page 159.

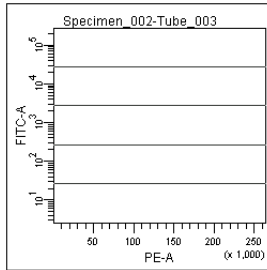


- Select the Show Grid checkbox to display gridlines at each log decade in a log plot.

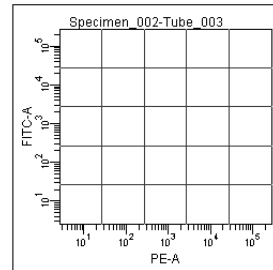
Note that gridlines are shown only if the Log checkbox is selected for the parameter shown in the plot. See the following examples.



linear fluorescent plot

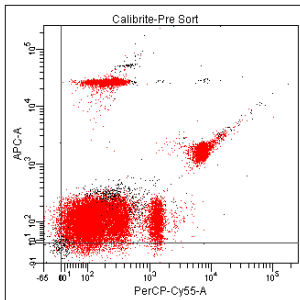


fluorescent plot with one log parameter



fluorescent plot with two log parameters

When biexponential scaling is in effect, gridlines delineate the zero-point on each biexponential axis:



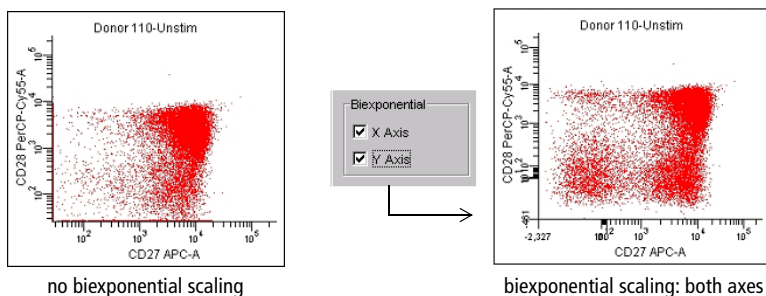
- Change the plot background color by clicking on the Background Color button. A palette appears from which you can choose a new color.
- Tip** Use Plot Preferences to change the default background color for all new plots. See Plot Preferences on page 88.
- Select the checkbox for each population to be displayed in the plot. Alternatively, deselect a checkbox to hide the population. You can also

specify populations using the plot contextual menu. See Choosing Populations to Display on page 156.

- 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.

Using Biexponential Scaling

Digital data can have events with negative values. Biexponential scaling is used to show these events on plots and to 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 scaling reveals the hidden double-negative population in the following example:



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 anytime instrument settings are changed. If you change a compensation coefficient, new scales are calculated.

NOTICE When scales change due to compensation adjustment or processing of a new data file, non-adaptive gates do not change their size or position. Events that were inside a gate can fall out of the gate boundary when the biexponential scale changes. If you are using non-adaptive gates on a biexponential plot, always check gated populations after the scale changes.

Note that width, ratios, and time are always shown on a linear scale; biexponential scaling 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 the following:

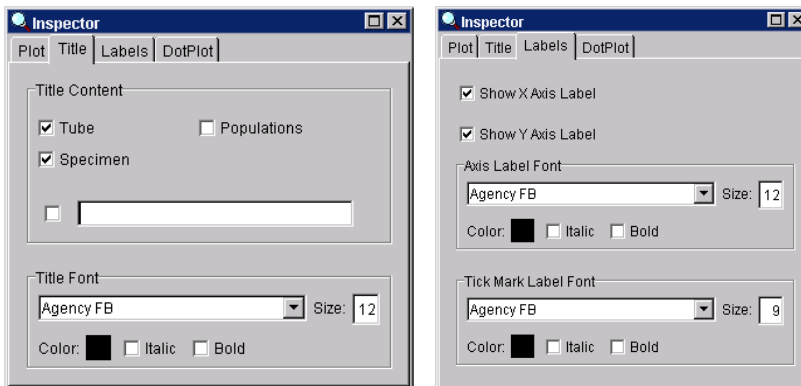
- Formatting Plots on page 157 describes how gridlines are displayed on biexponential plots.
- Hiding and Showing Gates on page 177 describes gating limitations.
- Batch Analysis on page 192 describes how to batch-analyze data files using the same biexponential scales.

To practice using this feature, try the batch analysis tutorial in the *BD FACSDiva Software Quick Start Guide*.

Changing the Plot Title and Axes Label Fonts

Use the Title tab of the Plot Inspector to specify and format the plot title. Use the Labels tab to specify whether to hide or show axes labels and to format the axes labels.

Figure 4-6 Inspector tabs for formatting plot titles and labels



- Within the Title tab, click the Title Content checkboxes to add Tube, Specimen, or Population names to the plot title. Each checked field will appear in the plot title separated by a hyphen (eg, Specimen_001-Tube_003).

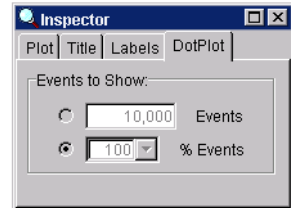
To create a custom title, click the checkbox next to the blank field and enter a title in the field.

- Within the Labels tab, choose to 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, axes labels, and tick-mark labels by making selections in the appropriate Font formatting box.

Formatting Dot Plots

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 radio button and enter any number of events, from 1% of the total events to the total number of events acquired.
- Select the lower radio button and choose a percentage of the total events from the drop-down 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 first 1,000 events.

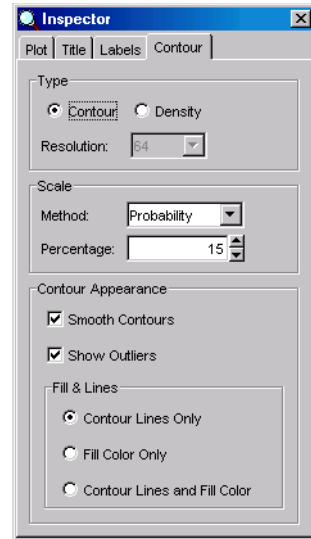


The minimum number of events to display is 1% of the total number of events.

Formatting Contour Plots

Contour plots are not available during acquisition. During analysis, use the Contour tab of the Plot Inspector to specify the type, scale, and appearance of the Contour plot.

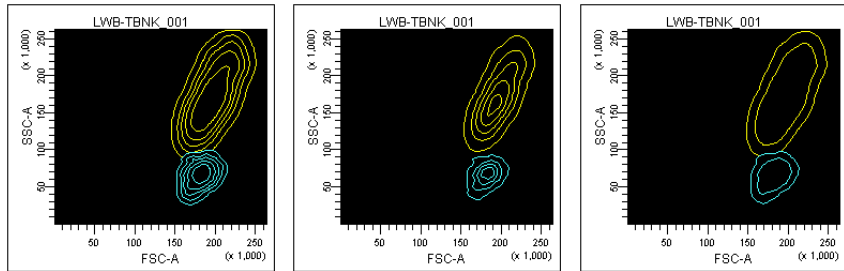
- Choose a scale method from the drop-down menu. The default method is probability.
- Choose Probability to calculate contour levels as a percentage of the total event number. With this method, contour levels are not based on the maximum number of events. Instead, the area between each pair of contour 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%.
- Choose Linear Density to calculate 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.
- Choose Log Density to calculate 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.

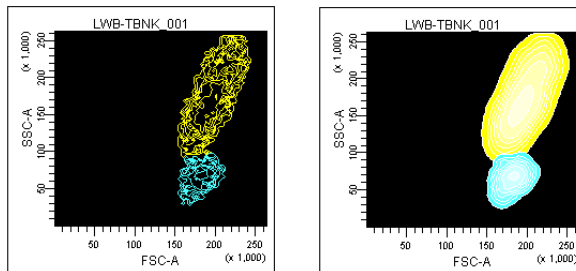
For an example showing each scale method, see the following figure.

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 radio button under Appearance 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).

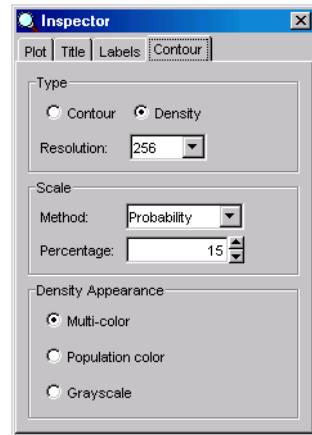
Figure 4-8 Contour plot with smoothing deselected (left); contour lines and fill color (right)



Formatting Density Plots

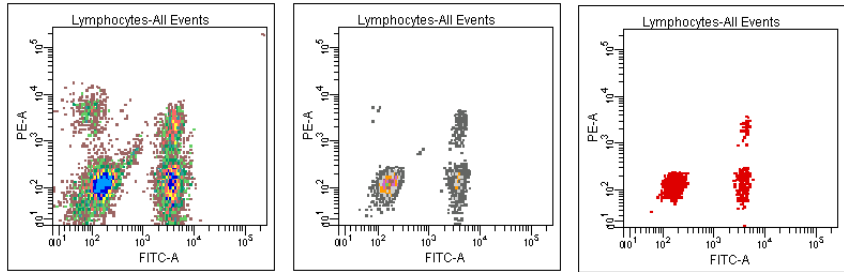
Use the Contour tab of the Plot Inspector to change a Contour plot to a Density plot, and to specify the scale and appearance of the Density plot.

- Choose the plot resolution, from 128, 256, or 512 bins. Data from adjacent bins are added to condense higher resolution data (more bins) into the chosen number of bins.
- Choose a scale method from the drop-down menu. The default method is probability.
 - Choose Probability to calculate 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.
 - Choose Linear Density to calculate 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.
 - Choose Log Density to calculate 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.



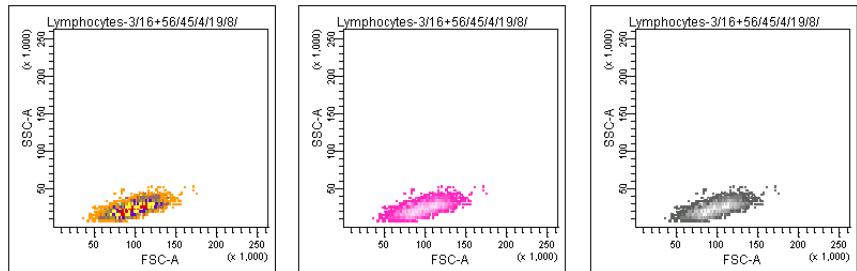
For an example showing each scale method, see the following figure.

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

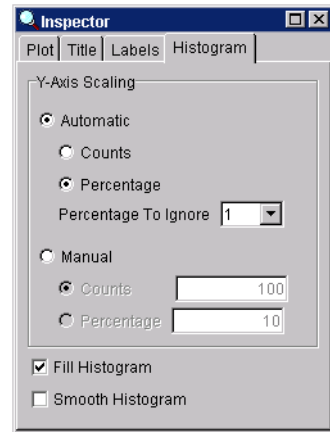


Formatting Histograms

Use the Histogram tab of the Inspector to format histograms. The y-axis scale can show either event counts or percentage of events in the histogram. For either method, you can 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.

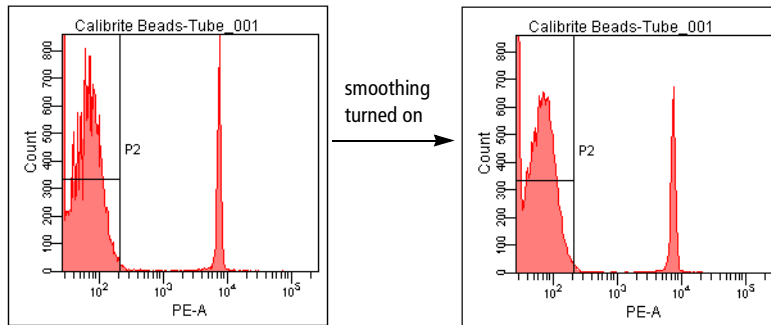


- Select a value from the Percentage to Ignore menu to disregard outlying events when calculating the y-axis scale.

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 events 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.



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 can be 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 view to create subsets within defined populations.

Gates are defined using tools on the Worksheet toolbar (). There are three types of gating tools:

- Manual gating tools such as the Polygon or Rectangle Gate tool allow you to define gate boundaries. See Drawing Manual Gates on page 168.
- Automatic gating tools such as the Autopolygon or Autointerval Gate tool automatically define gate boundaries for a selected population. Gate boundaries remain static after they are defined. See Creating Automatic Gates on page 170.
- Snap-To gating tools such as the Snap-To or Snap-To Interval Gate tool automatically define gate boundaries, but the boundaries change when data in the gate changes. See Working With Snap-To Gates on page 171.

To practice using gate tools, try the gating exercises in the *BD FACSDiva Software Quick Start Guide*.

Tip To create multiple gates, double-click a gate tool. The tool remains selected until you press the Escape key, select another tool, or click the same tool again.



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 visualize all events.

NOTICE If you change the number of log decades for plots after gates have been defined, gated populations might be affected.

Drawing Manual Gates

With manual gating tools, 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 Gate Preferences on page 87). You can also change the color of a population using the Population Hierarchy Inspector; see Changing the Color of Events on page 180.

	<p>Use the Polygon Gate tool to create a Polygon gate on a dot, density, or contour plot.</p> <ol style="list-style-type: none">1 Select the tool 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.
	<p>Use the Rectangle Gate tool to create a Rectangular gate on a dot, density, or contour plot.</p> <ol style="list-style-type: none">1 Select the tool and click in the plot to position the gate.2 Drag diagonally until the gate outline is the required size.

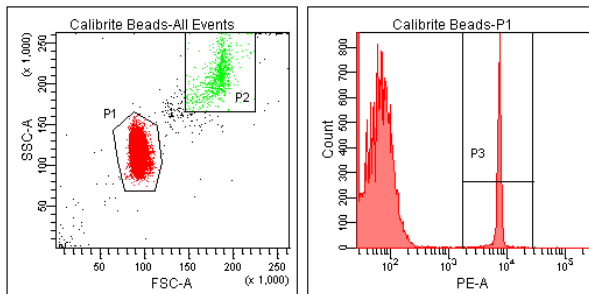


Use the Interval Gate tool to select a range of events in a plot. Interval gates can be used on dot, density, or contour plots, as well as histograms.

- 1 Select the tool 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 4-11 Polygon (P1), Rectangle (P2), and Interval (P3) gates



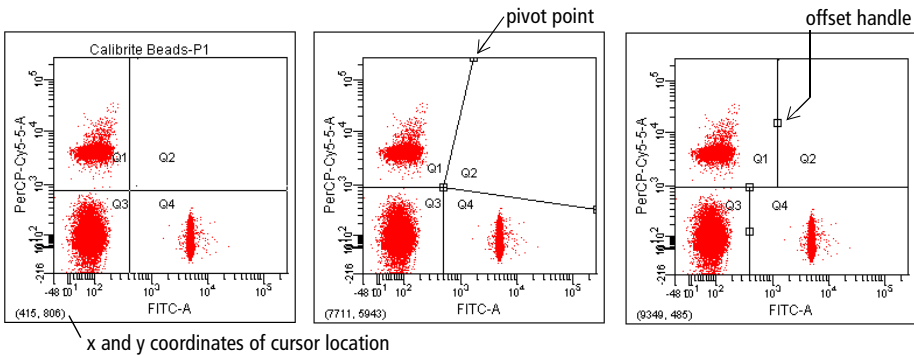
Use the Quadrant Gate tool to divide a dot or contour plot into four separate populations. Each quadrant population can be named and colored individually. Quadrant populations can be used for subsetting or sorting.

- 1 Select the tool and click in the plot.
- 2 Drag the mouse to position the intersection of the quadrant markers; the cursor location is indicated by the coordinates at the bottom of the plot (Figure 4-12 on page 170).
- 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 on 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 the population color does not change when you draw a Quadrant gate.

Figure 4-12 Rectilinear, pivoted, and offset quadrant segments





Creating Automatic Gates

With automatic gating tools, gate boundaries are defined when you click on a population in a plot. Unlike Snap-To gates, gate boundaries remain static after they are defined. Automatic gates include Autopolygon and Autointerval gates.



Always inspect populations defined by automatic gates to ensure all required events have been included.

	<p>Use the Autopolygon Gate tool to automatically create a Polygon gate around a population in a dot, density, or contour plot.</p> <ol style="list-style-type: none"> 1 Select the tool and click a distinct population in the plot. 2 Verify that the gate includes required events.
	<p>Use the Autointerval Gate tool to automatically select a range of events in a dot, density, contour, or histogram plot.</p> <ol style="list-style-type: none"> 1 Select the tool and click on 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.



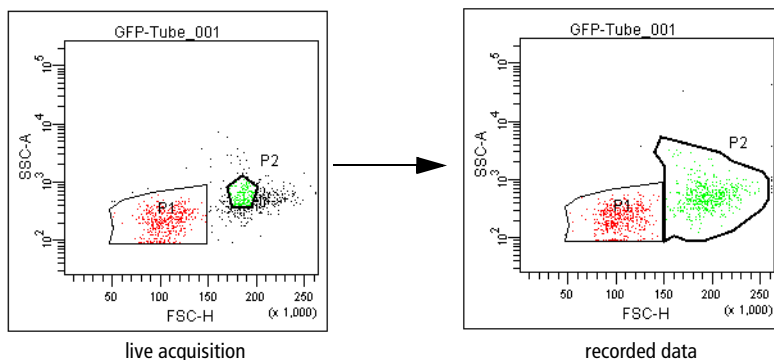
	<p>Use the Snap-To Gate tool to automatically create a Snap-To polygon gate around a population in a dot, density, or contour plot.</p> <ol style="list-style-type: none">1 Select the tool and click a distinct population in the plot.2 Verify that the gate includes required events.
	<p>Use the Snap-To Interval Gate tool to automatically select a range of events in a dot, density, contour, or histogram plot.</p> <ol style="list-style-type: none">1 Select the tool and click on a peak in the plot.2 Verify that the gate includes required events.

Figure 4-13 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 4-13 Autopolygon (P1) vs Snap-To gate (P2)

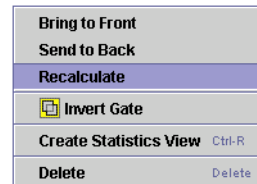


NOTICE Snap-To gates do not work well with diffuse clusters or rare events. Also, populations defined by Snap-To gates (and any populations derived from them) cannot be sorted.

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. You can force the gate to readjust by right-clicking on the gate boundary or on the population in the Population Hierarchy view (see Using the Population Hierarchy View on page 179) and choosing Recalculate from the menu that appears.



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 can be found, the system beeps and the Snap-To gate maintains its original position.

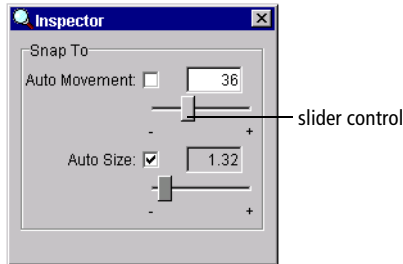
You can change how far the gate moves to find a new cluster by adjusting the Movement value. The Movement range is a percentage of the plot width, or resolution, from 0–100%. A higher Movement value allows the Snap-To gate to travel greater distances to locate a cluster. The Snap-To gate retains this 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 Return.



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 Size feature to adjust automatic sizing of the gate. A higher 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.

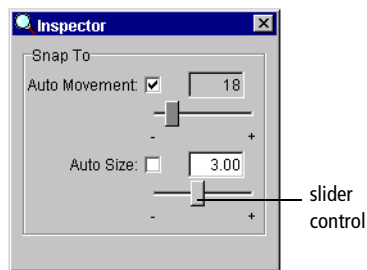
- 1 Select one or more Snap-To gates in a plot.
- 2 In the Inspector, deselect the Auto Size checkbox.

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.

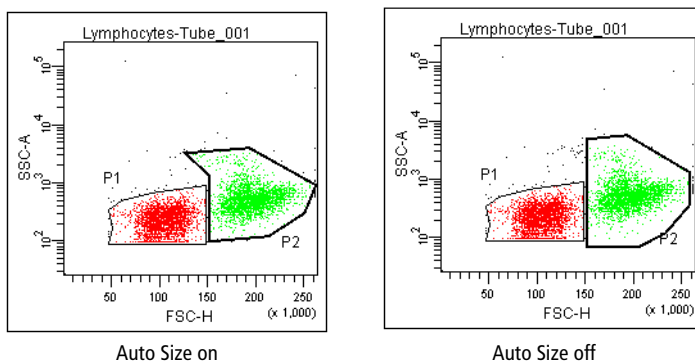
Alternatively, enter a value in the Auto Size field and press Return.

- Tip** Display gate statistics to view the effects of the gate changes.



The following figure shows how adjusting Auto Size affects the Snap-To gate in the plot.

Figure 4-14 Snap-To gate with automatic sizing (left) and user-adjusted sizing (right)



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 remembered and used when reading in the next or previous file.
- A tethered one-dimensional gate can move only on the X axis (horizontally).

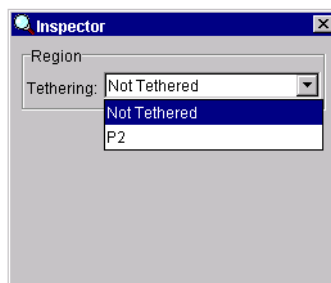
Follow these steps to create a tethered gate. For an example showing how tethered gates adjust for subsequent data files, try the batch analysis tutorial in the *BD FACSDiva Software Quick Start Guide*.

- 1** Create a polygon gate (P1) and a Snap-To gate (P2) in an appropriate plot.
- 2** Select P1 in the plot.
- 3** In the Gate Inspector, select the value in the Tethering field.

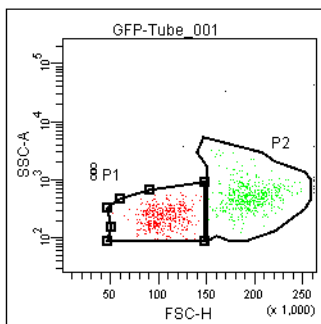
A menu appears listing all Snap-To gates in the current plot and other plots that share the current plot's parameters.

- 4** Choose P2 from the Tethering menu.

The P1 gate boundary changes and a chain-link icon appears next to the gate label to indicate that the gate is tethered, as shown



in the following 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 178).

- 1 Click once on a gate to select it.

Selection handles appear on the gate.

- 2 Make changes to the selected gate; 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.

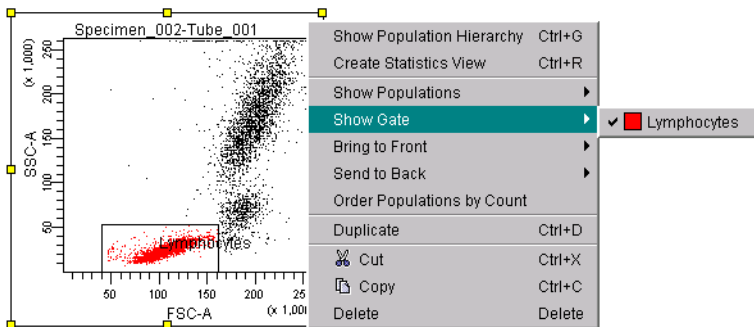
Note that non-adaptive gates on biexponential plots keep their on-screen size and shape regardless of the plot's scaling. You might have to move gates in response to new event positions as the scale changes.

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 do so, right-click the border of the plot and choose Show Gate from the menu (Figure 4-15).

- 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 4-15 Hiding a gate boundary and label



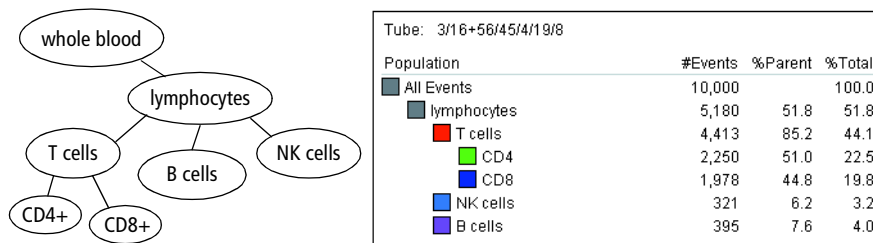
NOTICE You cannot show a gate in a lin/log plot if the gate was created on a biexponential plot, and you cannot show a gate in a biexponential plot if the gate was created on a lin/log plot.

Population Hierarchy

All gates and their defined populations are listed in a Population Hierarchy view. 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 view 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 4-16.

Figure 4-16 Immunophenotyping hierarchy and corresponding Population Hierarchy view



Population	#Events	%Parent	%Total
All Events	10,000		100.0
lymphocytes	5,180	51.8	51.8
T cells	4,413	85.2	44.1
CD4	2,250	51.0	22.5
CD8	1,978	44.8	19.8
NK cells	321	6.2	3.2
B cells	395	7.6	4.0

Tip To avoid errors when defining population subsets, always keep the Population Hierarchy in view. A population can remain selected after you change its name or color, and you can accidentally create a subset with the next defined population when you did not intend to. This is immediately apparent when the Population Hierarchy is in view.

To show the Population Hierarchy view, 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.

You can move or resize a Population Hierarchy view just like any other worksheet element; see *Aligning and Resizing Worksheet Elements* on page 148.

Using the Population Hierarchy View

Use the Population Hierarchy view 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 the *BD FACSDiva Software Quick Start Guide*.
- 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 Events on page 180.
- 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.

Tube: 3/16+56/45/4/19/8

Population	#Events	%Parent	%Total
All Events	10,000		100.0
MNEs	6,309	63.1	63.1
NOT(MNEs)	3,691	36.9	36.9
Lymphocytes	3,379	91.5	33.8
T Cells	1,836	54.3	18.4
B Cells	189	5.6	1.9
NK Cells	1,238	36.6	12.4

gate properties — FITC-A vs PE-A

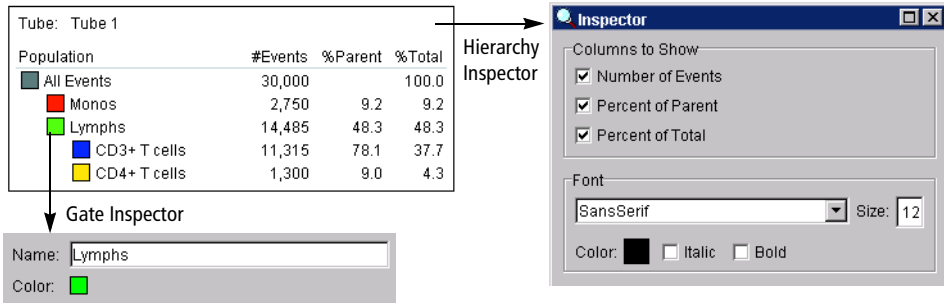
- view the relative percentages of different populations—To see how these numbers are calculated, see Calculating Statistics on page 189.

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 view and choose Create Statistics View from the menu that appears.
- delete populations—Select any population and press the Delete key.

Using Population Hierarchy Inspectors

Click anywhere inside the Population Hierarchy header to see the 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 view.)
- Use the Hierarchy Inspector to select which information should appear in the Hierarchy view and to change the font.

Changing the Color of Events

By default, populations defined by quadrants and intervals are not assigned a color (☒) in the Population Hierarchy view; they retain the color of their parent gate. The default settings can be changed using User Preferences (see Gate Preferences on page 87). 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 view or click the Color box in the Gate Inspector.
- 2 Choose a new color from the menu that appears or click the No Color box (☒) to leave the gated events uncolored.





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:

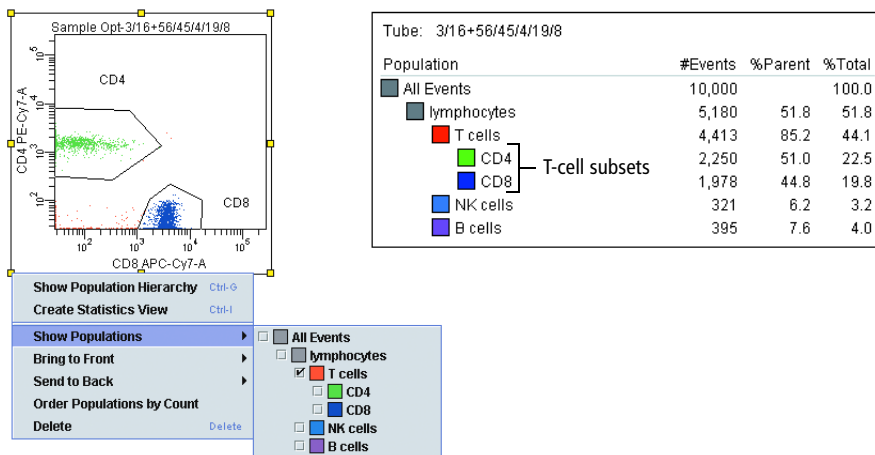
- 1 Select a population in the Population Hierarchy view.
- 2 Select the appropriate gate tool.
- 3 Draw a gate in a plot.

Alternatively,

- 1 Create a new plot, right-click inside the white border of the plot, and choose Show Population > *Population name* (Figure 4-17).
- 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 view (Figure 4-17).

Figure 4-17 Creating population subsets

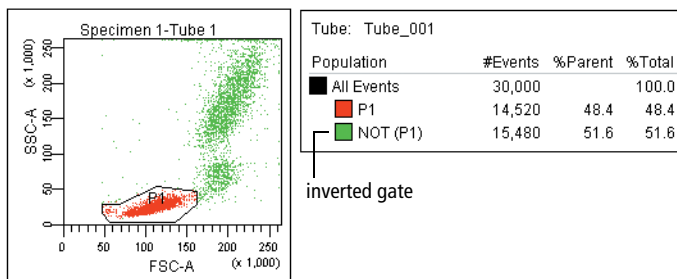


Defining a Derived Gate

You can use one or more previously defined gates to create a *derived* (Boolean) gate. Derived gates consist of the following:

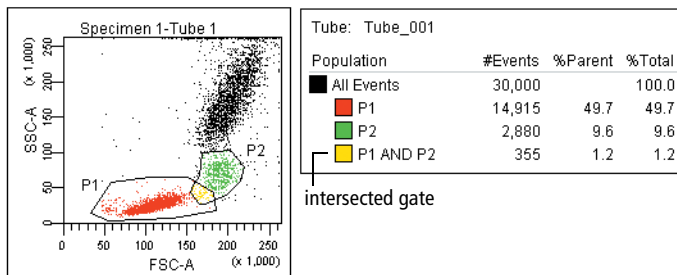
- Inverted gates use the NOT operator to select events outside a defined gate. Any event outside the specified gate satisfies the inverted gate.

To define an inverted gate, right-click an existing population in the Population Hierarchy view and choose Invert Gate.



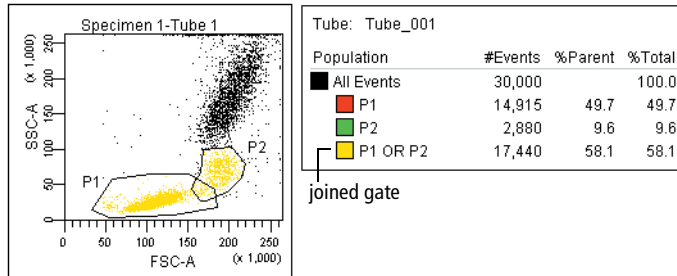
- Intersected gates use the AND operator to combine two or more individual gates. Only events that are in the specified gates satisfy an intersected gate.

To define an intersected gate, select two or more populations in the Population Hierarchy view. Right-click the selected populations and choose Intersect Gates.



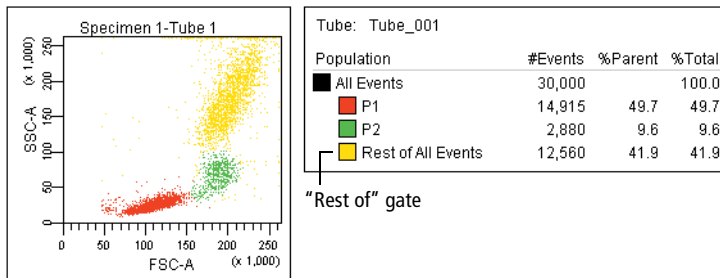
- Joined gates use the OR operator to combine two or more individual gates. An event that falls in any of the specified gates satisfies the joined gate.

To define a joined gate, select two or more populations in the Population Hierarchy view. 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 contains children.

To define a *Rest of* gate, right-click a parent population in the Population Hierarchy view 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.



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 on the number of currently displayed events. Statistics 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 worksheet icon (global worksheet view) in the Browser and choose Create Statistics View (or press Ctrl-R). 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 view and choose Create Statistics View. The resulting statistics view lists the number of events and %Parent for the selected population.

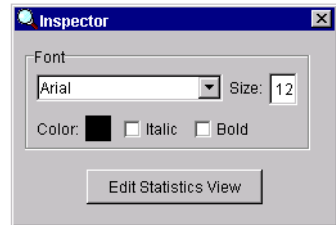
You can move or resize a statistics view like any other worksheet element. See [Aligning and Resizing Worksheet Elements](#) on page 148.

Selecting Statistics to Display

Use the Statistics Inspector to change the font of the statistics view; use the Edit Statistics View dialog box to specify the statistics to display. To access this dialog box, 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.

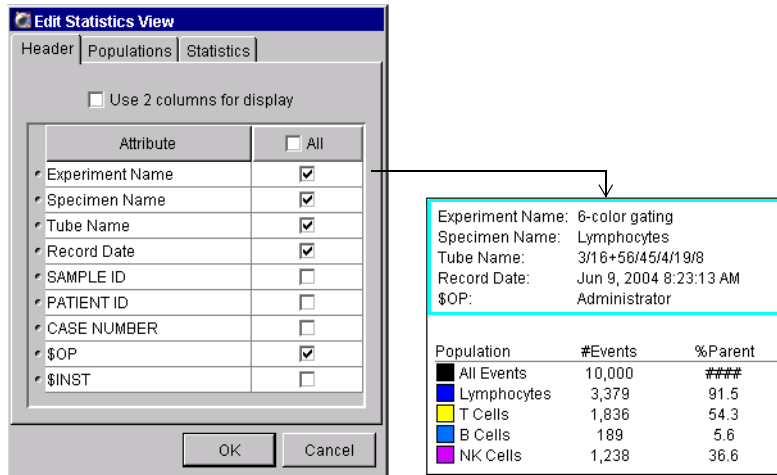
There are three components to the dialog box, accessed by clicking the tabs labeled Header, Populations, and Statistics. See Figure 4-18.



Editing Header Information

Use the Header tab to specify information to be included in the header of the statistics view.

Figure 4-18 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 Specimen and Tube name 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.

<input type="checkbox"/> Show Population	Populations	<input type="checkbox"/> All	<input type="checkbox"/> Parent Name	<input checked="" type="checkbox"/> #Events	<input checked="" type="checkbox"/> %Parent	<input type="checkbox"/> %Total
<input checked="" type="checkbox"/>	All Events	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	MNEs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	NOT(MNEs)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	Lymphocytes	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	T Cells	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	B Cells	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	NK Cells	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Decimal Places				1	1

- 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 and %Total Population 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.

Parameters	All	Median	Geometri...	Mean	CV	Max	Min	SD	Mode
FSC-H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
SSC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CD3 FITC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CD16+CD56 PE-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CD45 PerCP-Cy5-5-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CD4 PE-Cy7-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CD19 APC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CD8 APC-Cy7-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Time	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Decimal Places		0	0	0	1	0	0	0	0

Sort by Parameter Sort by Formula

OK Cancel

- 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 radio 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).

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.

For an explanation of how statistics are calculated, see the following section.

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
- % 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

$$\bar{x} = \left(\sum_{i=1}^n X_i \right) / n \quad \text{where } n = \text{number of events in the population, and } X_i \text{ is a value for a particular parameter, where } i = 1 \text{ to } n$$

- 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

$$\bar{X}_{\text{geo}} = 10^{\left(\sum_{i=1}^n \log X_i\right) / n} \quad \text{where } n = \text{number of events in the population, and } X_i \text{ is a value for a particular parameter, where } i = 1 \text{ to } n$$

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 (####).

- Standard deviation (SD)—a measure of the spread around the mean for events within a defined population, defined as

$$SD = \sqrt{\sum_{i=1}^n (X_i - \bar{X})^2 / (n - 1)}$$

- Coefficient of variation (CV)—the SD divided by the mean within a defined population, expressed as a percentage

The CV is defined as

$$\text{Percent CV} = (SD / \bar{X}) \times 100$$

- Median—linear value with an equal number of values above and below it

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

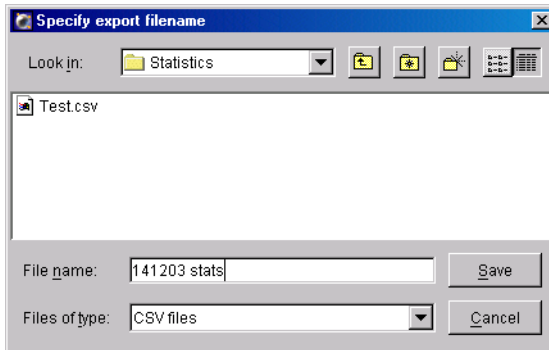
Exporting Statistics

You can export statistics for use in a spreadsheet, word processing, or other third-party application. Statistics information (including header text) is exported to a specified file type. To export statistics, do the following.

- 1 Select one or more statistics views.
- 2 Choose File > Export > Statistics.
- 3 Enter a name for the statistics file and specify a storage location in the dialog box that appears (Figure 4-19).

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 4-19 Exporting statistics



- 4 Specify the file type (CSV 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 Text for word-processing applications such as Microsoft Word.

Batch Analysis

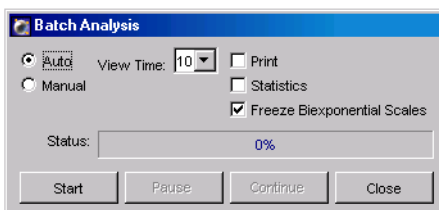
Use the *batch analysis* feature to automatically advance a selected set of Tube data through an analysis template on a global worksheet. To practice using this feature, try the tutorial in the *BD FACSDiva Software Quick Start Guide*.

- 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. Note that Tubes without data are skipped during a batch analysis.

- 2 Right-click the selected item(s) and choose Batch Analysis.

The following dialog box 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. You can 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 (from 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 Print 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.

Each Tube adds a new row 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 statistics 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 For exported statistics, specify the file name and storage location in the dialog box that appears.

By default, files are exported to D:\BDEExport\Statistics.

- Tip** To append data to an existing file, select the file in the export file dialog box, and click Save. When prompted, click Append. Results will be appended to the selected file.

- 7 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.

NOTICE If you are using non-adaptive gates on a biexponential plot, verify that populations are within the intended gate after the scale changes.

- 8 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 an instrument), most instrument, 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 52. Analysis objects can be edited, moved, copied, and fine-tuned using gates and drill-down functionality. Population hierarchies can be derived and statistics can be calculated and exported based on data previously acquired.

- Tip** When you set up an Experiment offline, you might need to create Tube-specific instrument settings and disable the *Use global instrument settings* preference in order to maintain the settings when you go online. For example, when setting up an Experiment for instrument optimization, if your first Tube uses a different set of parameters than your second Tube and the global instrument settings option is checked, Experiment-level instrument 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 on page 196
- Exporting and Importing Data on page 201
- Exporting and Importing Experiments on page 208
- Using the Data Manager Utility on page 211

Working with BD FACSDiva Data

Unlike other BD software, BD FACSDiva software does not generate separate files for data display, instrument settings, and data. The software stores and accesses all experimental data from a single database. As you create Experiments in the Browser (see Experiments on page 58), 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 tool on the Workspace toolbar (). The database contains a record of all Browser items, worksheet elements, Experiment settings, and instrument control settings.

Recorded event data is saved separately in FCS 3.0 floating-point format.* Green highlighting on a Tube icon in the Browser signifies 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 201).



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 <http://www.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, backing up the database on a regular basis, and deleting Experiments, Specimens, or Tubes that are no longer needed.



To preserve the integrity of your data, 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 199). 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 back up Experiments and store them in an offsite storage location.
- Back up the database weekly.
- Be sure you have successfully completed a copy, record, duplicate, print, save, or delete operation before closing BD FACSDiva software. Because some operations take time to complete, you risk losing unsaved data if you terminate the software prematurely.

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.

Table 5-1 Data management options

Option	Function	When to Do	See...
Export FCS	Preserves FCS 2.0 or 3.0 list-mode data ^a for analysis by a compatible software application or reanalysis (In case of data loss, files can be imported back into BD FACSDiva software.)	After each significant Experiment	page 201
Export Experiments	Relocates Experiments to a specified drive with the option to remove them from the current database on completion of export	When necessary to free up disk space	page 208
Delete Experiments	Frees up space in the database and hard drive by removing unnecessary or outdated components	When necessary to free up disk space	page 200
Back up database	Copies the current database to a specified drive	Weekly	page 212

a. Analysis objects are not retained; only FCS files, Browser hierarchy, and instrument settings are included.

Optimizing Data Processing

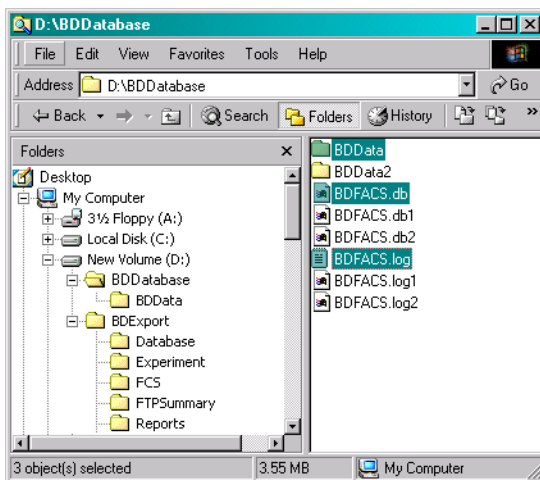
To save memory (and disk space for permanent storage), it is recommended that you save only parameters that are being used (for example, by deleting UV parameters when not running a UV experiment).

While there is no impact on data collection or instrument 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 frame.

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.
- 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 the disk in Windows Explorer. The disk capacity and space used is shown.

Deleting Experiments

Do the following to delete unneeded Experiments.



Deleting Experiments also deletes any associated event data. Back up the database or export Experiments or FCS files before you delete Experiments.

1 Export FCS files or Experiments or back up the database 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 BD FACSDiva 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 discontinuous elements, hold down the Control key while you select each element to be removed.

3 Press the Delete key.

Alternatively, right-click selected elements and choose Delete from the contextual menu.

Exporting and Importing Data

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 Instrument settings are written to the FCS file but cannot be opened as a separate file in BD CellQuest Pro or BD CellQuest software. Instrument settings are not transferable between different cytometers. To include Analysis objects in the export (plots, gates, and statistics views), export the entire Experiment. See Exporting and Importing Experiments on page 208.

Use commands in the File menu to export and import Experiments and FCS files.

Exporting FCS Files

Note that you can enable a user preference to export FCS files automatically after each Tube is recorded. See FCS Preferences on page 88. When files are exported automatically, they are exported as FCS 3.0 with all parameters included. The FCS 3.0 format allows a floating point average to be maintained.

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 (this is possible in BD FACSDiva software; see Using the Compensation Tab on page 115) 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.

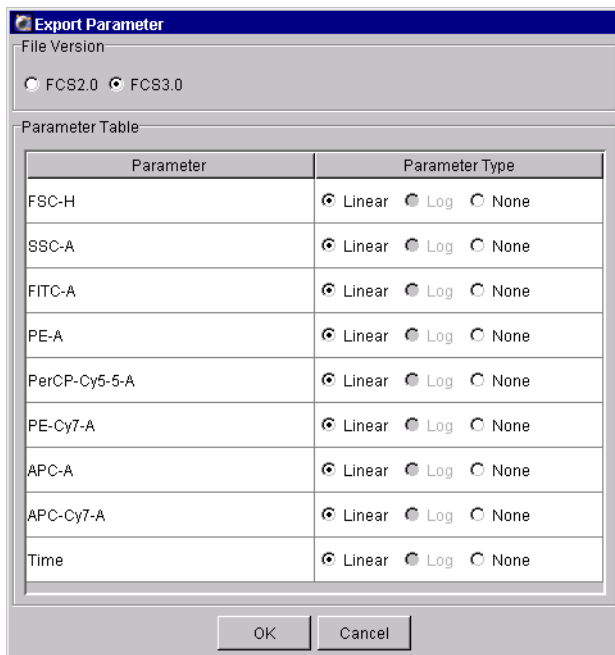
Note that Experiment- and Specimen-level keywords are exported with Tubes. User-defined keywords are included in the header of FCS files.

- 2 Choose File > Export > Export FCS.

Alternatively, right-click the selected item(s) and choose Export FCS.

- 3 Specify the FCS file version and parameters to export in the dialog box that appears (Figure 5-1).

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; the Log option is disabled for all parameters.

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). BD recommends exporting only Area measurements, not Height. 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 Pro or BD CellQuest software. Refer to the *BD FACSConvert User's Guide* for instructions.

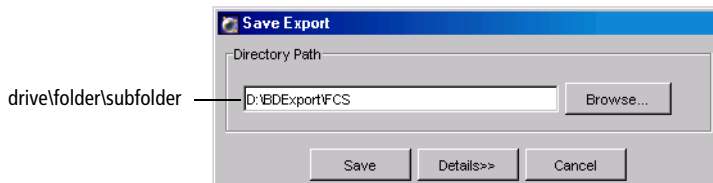
- Select Linear or Log for each parameter to be included in the data file; click None for parameters to exclude. (Log is available only for FCS 2.0 files.)

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 radio button). For FCS 3.0 files, make sure to include all parameters included in compensation calculations. See Important Considerations on page 206.

4 Click OK.

5 In the Save Export dialog box, verify the file storage location.



- Click the Browse button to change the file storage location. Navigate to a different directory in the dialog box 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.

Importing FCS Files

To convert imported data to the 18-bit linear format read by BD FACSDiva software, the software performs the following calculations.

- 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)

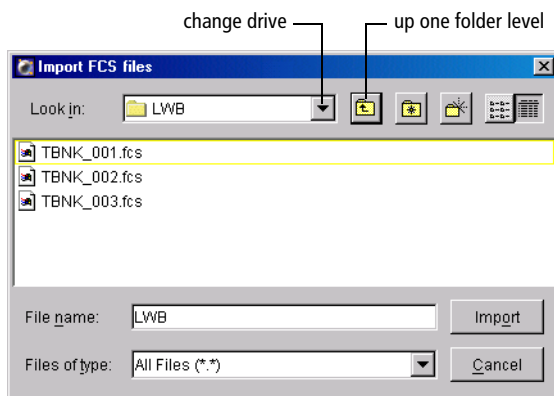
This places the data on the same scale as BD FACSDiva four-log data, from 26–262,144.

Follow these steps to import an exported file. The same procedure can be used 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. You can 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 box that appears.



- Use the buttons in the dialog box to find the files to be imported.
- Select multiple files by holding down the Control key as you click the file names.

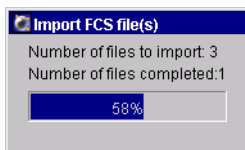
NOTICE You can select any type of file, but only those files saved in a valid FCS 2.0 or 3.0 format will be imported.



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.

4 Click Import.

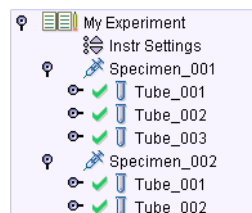
A progress bar appears showing the status of the import.



For each valid FCS file, a Tube is created in a Specimen in the currently 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_00x is used.

In the example shown to the right, the new Specimen is appended with _002 since the Experiment already contains a Specimen_001.

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.



Important Considerations

Note the following behavior when exporting or 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 acquired in 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 instrument settings from BD FACSDiva software v2.0 or later, the instrument settings cannot be copied, and the Duplicate Without Data option is disabled 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 (not all parameters chosen in the Export Parameters dialog box). 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 (this is possible in BD FACSDiva software; see Using the Compensation Tab on page 115) 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.

Exporting and Importing Experiments

Experiments can be exported to the hard drive or an offsite storage location using the Export option on the File menu. When the export is complete, you can choose to have the software remove the exported Experiments from the Browser, which 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). Experimental elements are exported as an XML file, while experimental 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 BDEExport\Experiments folder that is set up for you during software installation.

Exporting Experiments

- 1 Select one or more Experiment icons in the Browser.

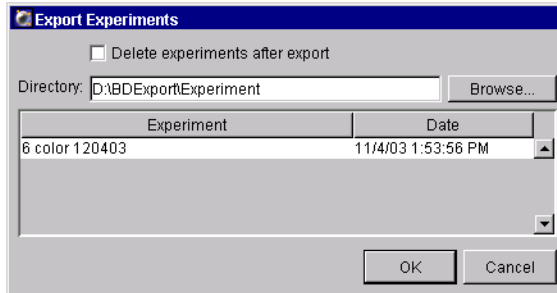
Tip If one of the Experiments is open or expanded, use the Ctrl key to select only Experiment icons.

- 2 Choose File > Export > Experiments.

-  Make sure you choose the Experiments command, rather than 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 box (Figure 5-2).

Figure 5-2 Exporting Experiments



- Verify the Experiments listed are those you intended to export. If 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. Enter the directory path by typing, or click the Browse button and select the storage location in the location dialog box 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 previous versions of BD FACSDiva software.

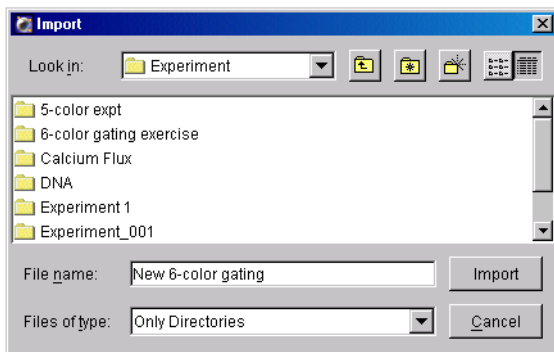
Importing Experiments

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 `_00x`, 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, use the Import FCS files command as described in Importing FCS Files on page 204.

- 1 Choose File > Import > Experiments.
- 2 Locate the Experiment to be imported in the dialog box that appears (Figure 5-3).

Figure 5-3 Location dialog box for importing Experiments



- 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

The BD FACSDiva Data Manager utility 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 backed-up copy. To back up your database to another storage media (CD or tape), refer to the documentation provided with your computer.



Do not relocate the Data Manager utility or run it from a batch file located outside the BD FACSDiva application directory.

To launch Data Manager, do the following.

- 1 Quit BD FACSDiva software, if necessary.

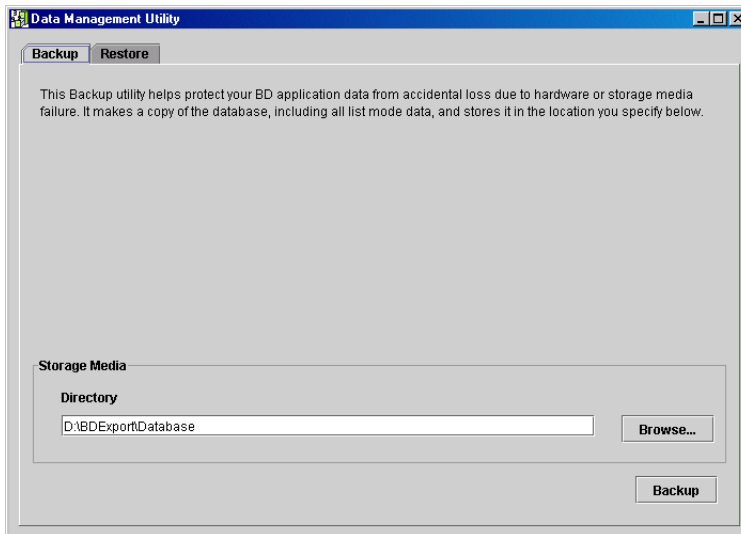
Data Manager cannot launch when BD FACSDiva software is running.

- 2 Launch BD FACSDiva Data Manager by double-clicking the shortcut on the desktop.



shortcut icon
on desktop

The following window appears:



The Data Manager window has two tabs. Click the appropriate tab to initiate the following actions.

Tab	Action	See...
Backup	Create a backup copy of the BDFACS database on a specified drive.	the following section
Restore	Replace the current database with a backup copy from a specified drive.	page 215

Backing Up the Database

During a backup, the Data Manager utility 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 provided 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 media. 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 214.

NOTICE Backing up does not free up space on the hard drive as the original files are retained.

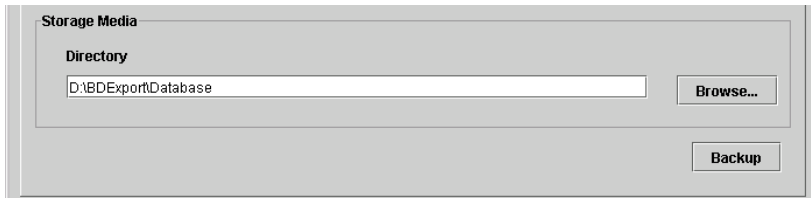


Tip To back up directly to a DVD, use the Data Manager utility to direct-format the DVD and then back up to the DVD. Otherwise, back up to the drive and use DVD Writer to copy the backup onto the DVD.

1 Launch Data Manager.

See Using the Data Manager Utility on page 211. The Data Manager window appears with the Backup tab displayed.

2 Verify or change the path of the backup folder in the Directory field.



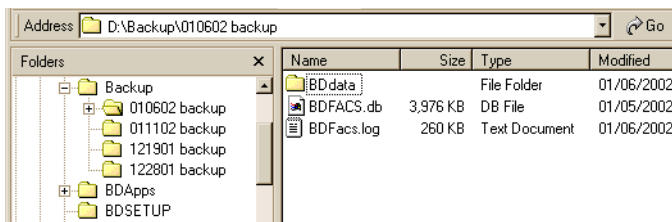
To change the path, click the Browse button. After mapping a network drive, files can be backed up directly to the network. See Mapping a Network Drive on page 214.

Tip BD Biosciences recommends saving each backup in a separate folder and not replacing a previous backup. To prevent confusion, create a new, dated folder for each database backup. Click the Browse button and use buttons in the Select Directory dialog box to create and select a new folder.

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 utility.

1 Launch Windows Explorer.

Choose Start > Programs > Accessories > Windows Explorer.

2 Choose Tools > Map Network Drive.

3 Choose a Drive letter from the Drive drop-down 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 the Data Manager utility 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.



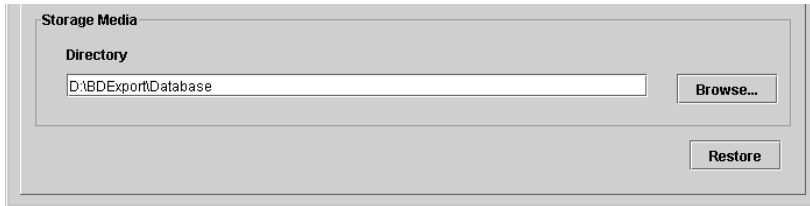
During a data restore, the current database is overwritten by the backup copy. Once the restore is in progress, it cannot be stopped or cancelled. To save your current data, make a backup before restoring a previous database.

1 Launch Data Manager.

See Using the Data Manager Utility on page 211. The Data Manager window appears with the Backup tab displayed.

2 Click the Restore tab.

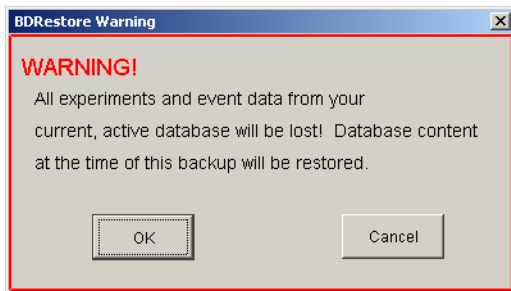
3 Locate your database backup.



To change the path, click the Browse button, or enter the path in the Directory field.

4 Click Restore.

The following message appears:



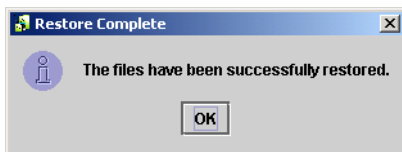
- 5 Click OK to continue.

The Data Manager utility verifies that the user has logged in as the administrator. If the verification passes, 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 220 for instructions.

- 6 Click OK to dismiss 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 instrument-specific troubleshooting, refer to your instrument 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 on page 218
- Electronics Troubleshooting on page 219
- General Software Troubleshooting on page 220
- Instrument Setup Troubleshooting on page 224
- Analysis Troubleshooting on page 225
- Data Manager Troubleshooting on page 227
- Printing Troubleshooting on page 229

Installation Troubleshooting

Observation	Possible Causes	Recommended Solutions
VxWorks download failure	Instrument power switched off	Verify the instrument power is on.
	Electronics not fully booted	Reset the instrument main power and restart the computer. After turning on the instrument main power, wait 5 minutes before beginning the software installation.
	Communication failure between workstation and instrument	<ul style="list-style-type: none"> Quit the software and then restart it. If restarting does not work, reset the instrument electronics by switching the power off, and then on. Restart the computer.
	Ethernet cable disconnected between workstation and instrument	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address changed	Enter the correct IP address. Call BD Biosciences for assistance.
	Software installed for 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 not recognized by installer as a logical drive	Copy contents of CD to C: drive (Temp folder) and then click the Setup.exe icon.


Electronics Troubleshooting

Observation	Possible Causes	Recommended Solutions
“Instrument Disconnected” in Instrument frame	Instrument power switched off	Verify the instrument power is on.
	Communication failure between workstation and instrument	<ul style="list-style-type: none"> • Quit the software and then restart it. • If restarting does not work, reset the instrument electronics by switching the power off, and then on. Restart the computer.
	Ethernet cable disconnected between workstation and instrument	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address changed	Enter the correct IP address. Call BD Biosciences for assistance.
“Upgrading firmware...” in Instrument frame	Firmware loading incomplete	Wait two minutes. If the message remains, restart the computer.
“Master DAQ Overflow” in Instrument frame	Event rate too high	Decrease the event rate or verify the threshold.
	Too many Analysis objects on worksheet or too many events displayed	Delete Analysis objects, decrease the Display value, or delete parameters from the instrument settings Inspector.
“Instrument not responding” Status message	Unknown	<p>Reset the instrument electronics by switching the power off, and then on. Restart the computer.</p> <p>NOTICE If this occurs during sorting, turn off the deflection plates before resetting the electronics.</p>

General Software Troubleshooting

Observation	Possible Causes	Recommended Solutions
Software not launching	FTP service started by another application	Stop the FTP service for the other application. <ol style="list-style-type: none">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 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.
	Conflicting application, driver, or security update installed	Contact your service representative for assistance.
Unable to access database	Adaptive Server Anywhere not running	Verify that the database server has been started. <ol style="list-style-type: none">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 greyed out, the database could be loading.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Software not responding	Saving or loading large data file	Look for activity on the screen. If there is no activity, restart BD FACSDiva software.
	Too many histograms displayed	If you have more than six histograms displayed in the viewable area of the worksheet, move them down on the worksheet and scroll up.
	Too many plots or gates	Reduce the number of plots or streamline your gating strategy.
	Other applications 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.
	Calculating large number of statistics	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. Use the Processes tab in the Windows Task Manager to verify memory usage.
Software frozen		Press Ctrl-Alt-Delete; then click the Task Manager button. Locate BD FACSDiva software in the Windows Task Manager, and click End Task.  Unsaved data will be lost.
Software message “Hardware key not accessible...”	Security module disconnected	Verify that the security module is securely connected in the USB port (see step 15 on page 27), and then restart BD FACSDiva software.

General Software Troubleshooting (continued)


Observation	Possible Causes	Recommended Solutions
Error message in Status tab	Communication, fluidics, or sorting error	Shut down the computer and the instrument, 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 not functioning	Keys 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 not functioning	Num Lock key reset	Press the Num Lock key on the keyboard and try the keypad again.
No wireless keyboard or mouse response	Keyboard or mouse too far away from workstation	Move the keyboard or mouse closer to the workstation.
	Obstruction between keyboard or mouse and workstation	Remove the obstructing object.
	Batteries low	<ol style="list-style-type: none"> 1 Replace the batteries. (Refer to the documentation provided with the keyboard or mouse.) 2 Restart the computer.
Error 12 software message	Driver not installed	Reinstall the software.
Plot tool disabled	No Tube selected in Browser (normal worksheets only)	Select a Tube to enable the tool.
No instrument settings editor	Current Tube pointer not set	Click to set the Current Tube pointer.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Faulty screen display or slow user interface response	Other program running	Close all other open programs.
	Pointer shadow enabled	Choose Start > Settings > Control Panel. Double-click the Mouse icon and click the Pointers tab. Deselect the checkbox to Enable pointer shadow; then click OK.
	Graphics hardware acceleration too fast	<p>Decrease the hardware acceleration setting.</p> <ol style="list-style-type: none">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 left.4 Repeat all steps for the second video card, if applicable.

Hardware acceleration

Manually control the level of acceleration and performance supplied by your graphics hardware. Use the Display Troubleshooter to assist you in making the change.

Hardware acceleration: None  Full

Disable cursor and bitmap accelerations. Use this setting to correct problems with the mouse pointer, or to correct problems with corrupt images.

pointer

Instrument Setup Troubleshooting

Observation	Possible Causes	Recommended Solutions
Error creating compensation controls	Naming conflict with existing control or worksheet	<ol style="list-style-type: none"> 1 Locate the control or worksheet that is named (<i>ParameterName</i>) Stained Control, and change the name. 2 Create the compensation controls again.
Error calculating compensation	PMT voltages 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 135.
	Wrong number of gates for control plot(s)	<ul style="list-style-type: none"> • Display a Population Hierarchy view 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 for one or more controls	Record data for all controls.
	No root gate for first control	Create a P1 gate in the FSC vs SSC plot for the appropriate control.
	Wrong fluorochrome run for Stained Control	Rerecord all compensation controls and recalculate.
	Insufficient events in gated population	Adjust the gate to encompass more events for the appropriate control.

Instrument Setup Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Error calculating compensation (continued)	Insufficient separation between positive and negative populations	Refer to your instrument manual for suggestions on how to optimize fluorescent signal. Rerecord the compensation controls, draw new gates, and calculate the compensation again. If automatic compensation fails, perform compensation manually. See Calculating Compensation Manually on page 135.

Analysis Troubleshooting

Observation	Possible Causes	Recommended Solutions
Fewer events than expected in gated population	Current Tube pointer on wrong Tube	Move the pointer to the correct Tube.
	On-axis events left out of gate	When drawing a gate, make sure events on the axis are included.
	Plot zoomed	Unzoom the plot or make the gate bigger.
	Laser delay set incorrectly	Adjust the laser delay settings. Refer to your instrument manual for instructions.
	Window extension set incorrectly	Adjust the window extension. See Using the Window Extension on page 101.

Analysis Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Missing Analysis objects on worksheet	Analysis objects 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 in plots	Current Tube pointer not set	Click to set the Current Tube pointer next to the appropriate Tube.
	Data no longer linked to Experiment	Contact BD Biosciences for assistance in locating the missing data.
Duplicated plots out of the page boundary	Page positioning lost during duplication	Before copying an Analysis object, arrange the plots and statistics views at the upper and lower edges of the page. Use the Next button to duplicate Tubes; page positioning is maintained.
Differing statistics between exported and imported file	Compensated data with negative values set to zero during FCS 2.0 export	<ul style="list-style-type: none">• Export the original data as FCS 3.0.• Export FCS 2.0 data with compensation disabled.• If statistical differences are significant, rerecord the file.

Data Manager Troubleshooting

Observation	Possible Causes	Recommended Solution
Data Manager utility not launching	BD FACSDiva software 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 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 source and target file names and paths.
	File in use by another program	Verify that the file is not in use by another program.
	File 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.
	Multiple parameters with same name in file	Make sure all parameters for an FCS file have unique names.

Data Manager Troubleshooting (continued)

Observation	Possible Causes	Recommended Solution
Unable to read or write FCS file when attempting import or export (continued)	File read-only or hidden	Verify that the file is not set as a read-only or hidden file.
	Data type within file 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	Instrument settings changed since original data collected	When appending data, make sure the instrument 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 not exported	No data in 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 truncated in imported file	File 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.
- Print to file and print the resulting file. See Printing to File on page 230.
- Use a third-party application to print to a PDF file, and then print the PDF file.

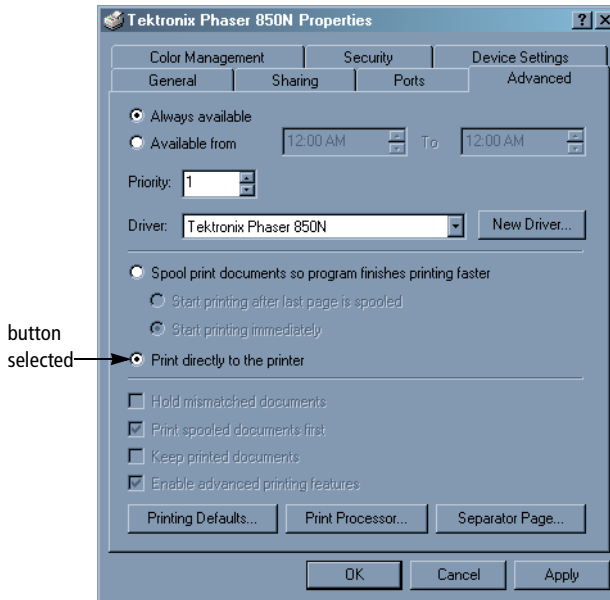
Printing Directly to the Printer

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* radio button (Figure 6-1 on page 230).

NOTICE When using this print option, BD FACSDiva software is not available during printing.

Figure 6-1 Printing directly to the printer



Printing to File

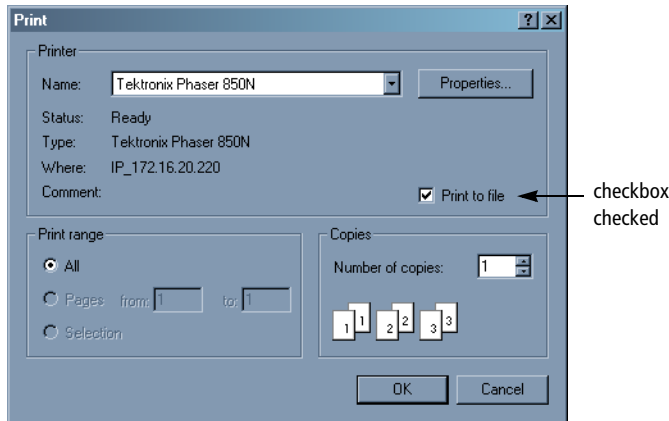
PrintFile is a utility that prints .prn files. Your field service engineer installed it during instrument installation. To print files via the PrintFile utility, follow these steps.

Enabling the PrintFile Utility

- 1 Choose Start > Programs > PrintFile > PrintFile and click Settings.
- 2 Click to deselect the *Enable spooler function* checkbox and click Save.
- 3 Click Exit in the PrintFile window.

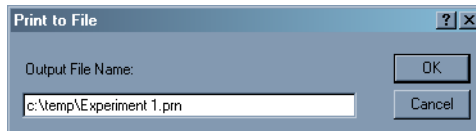
Printing

- 1 In BD FACSDiva software, choose File > Print.
- 2 Choose the correct printer from the Name drop-down menu.
- 3 Click to select the *Print to file* checkbox; click OK.



- 4 Enter the directory path and file name in the Print to File dialog box; click OK.

NOTICE Always include the *.prn* file extension at the end of the file name.



The file is saved to the specified directory.

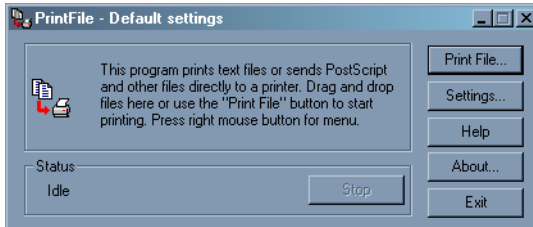
- 5 Use Windows Explorer to locate the resulting *.prn* file; double-click the icon to open the file.



A Print dialog appears. The PrintFile utility is launched and its window appears in the background.

- 6 In the Print dialog box, click OK.

The file prints on the selected printer. The PrintFile utility remains open. Follow the instructions in the window to print subsequent files.



- 7 Click Exit to quit the PrintFile utility program.

Appendix A

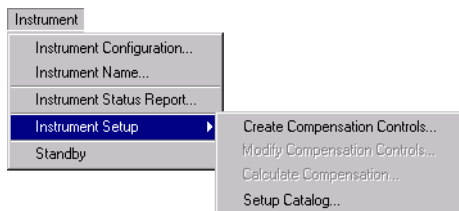
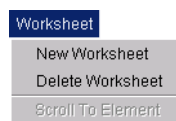
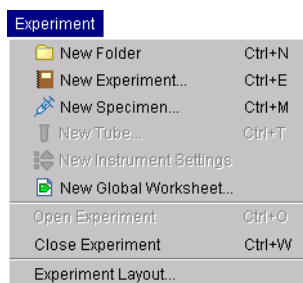
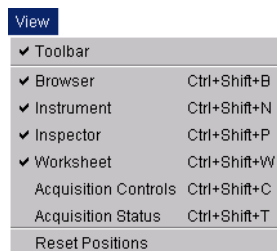
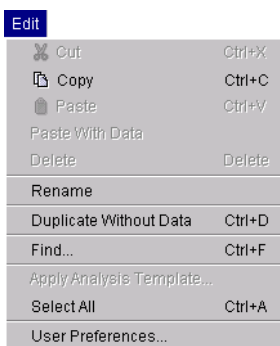
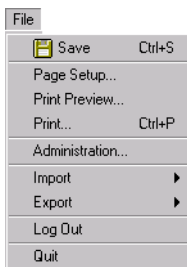
Menus and Keyboard Shortcuts

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 on page 234
- Contextual Menus on page 235
- Keyboard Shortcuts on page 236

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.



Contextual Menus

Right-click the indicated object to access the following menus.







Experiment

 Cut	Ctrl+X
 Copy	Ctrl+C
 Paste	Ctrl+V
Paste With Data	
Delete	Delete
Rename	
Duplicate Without Data	Ctrl+D
Open Experiment	Ctrl+O
Close Experiment	Ctrl+W
Batch Analysis...	
 New Global Worksheet	
 New Specimen	
 New Instrument Settings	
Share Experiment	
Make Private	
Export	






Instrument Settings

 Cut	Ctrl+X
 Copy	Ctrl+C
 Paste	Ctrl+V
Delete	Delete
Copy Spectral Overlap	
Paste Spectral Overlap	
Paste Spectral Overlap with Zeros	
Print	
Export	
Apply Setup...	
Unlink From...	





Global Worksheet

 Cut	Ctrl+X
 Copy	Ctrl+C
 Paste	Ctrl+V
Delete	Delete
Apply Analysis Template...	
Rename	
 Create Dot Plot	
 Create Contour Plot	
 Create Histogram	
Show Populations	
Show Population Hierarchy	
Create Statistics View	
Export	




Specimen

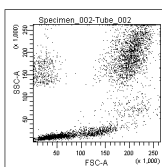
 Cut	Ctrl+X
 Copy	Ctrl+C
 Paste	Ctrl+V
Paste With Data	
Delete	Delete
Rename	
Duplicate Without Data	Ctrl+D
Batch Analysis...	
 New Tube	
 New Instrument Settings	
Export	

Tube

 Cut	Ctrl+X
 Copy	Ctrl+C
 Paste	Ctrl+V
Delete	Delete
Apply Analysis Template...	
Copy Spectral Overlap	
Paste Spectral Overlap	
Paste Spectral Overlap with Zeros	
Rename	
Duplicate Without Data	Ctrl+D
Batch Analysis...	
 New Instrument Settings	
Export	

Analysis

 Cut	Ctrl+X
 Copy	Ctrl+C
 Paste	Ctrl+V
Delete	Delete
Apply Analysis Template...	
Export	
Rename	



Plot



Show Population Hierarchy	Ctrl+G
Create Statistics View	Ctrl+R
Show Populations	▶
Show Gate	▶
Bring to Front	▶
Send to Back	▶
Order Populations by Count	
Duplicate	Ctrl+D
Cut	Ctrl+X
Copy	Ctrl+C
Delete	Delete

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, 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, 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 Browser.
Open Experiment	Ctrl-O	Closed Experiment must be selected.
Close Experiment	Ctrl-W	Open Experiment must be selected.

Objective	Key Combination	Condition to Activate Shortcut
Find Experiment	Ctrl-F	Item must be selected in 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	<ul style="list-style-type: none"> • Specimen or Tube must be selected in an open Experiment (not available for imported Tubes). • Plot must be selected on worksheet.
Cut	Ctrl-X	Item must be selected in Browser or on worksheet.
Copy	Ctrl-C	Item must be selected in Browser or on 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 worksheet.
Create statistics view	Ctrl-R	Tube must be selected in an open Experiment or plot must be selected on worksheet.
Print active worksheet	Ctrl-P	Experiment must be open.
To show or hide the following frames:		
Browser	Ctrl-Shift-B	
Instrument	Ctrl-Shift-N	
Inspector	Ctrl-Shift-P	
Worksheet	Ctrl-Shift-W	

Objective	Key Combination	Condition to Activate Shortcut
Acquisition Controls	Ctrl-Shift-C	
Acquisition Status	Ctrl-Shift-T	
Sorting	Ctrl-Shift-S	
Carousel Controls	Ctrl-Shift-L	

Appendix B

Digital Theory

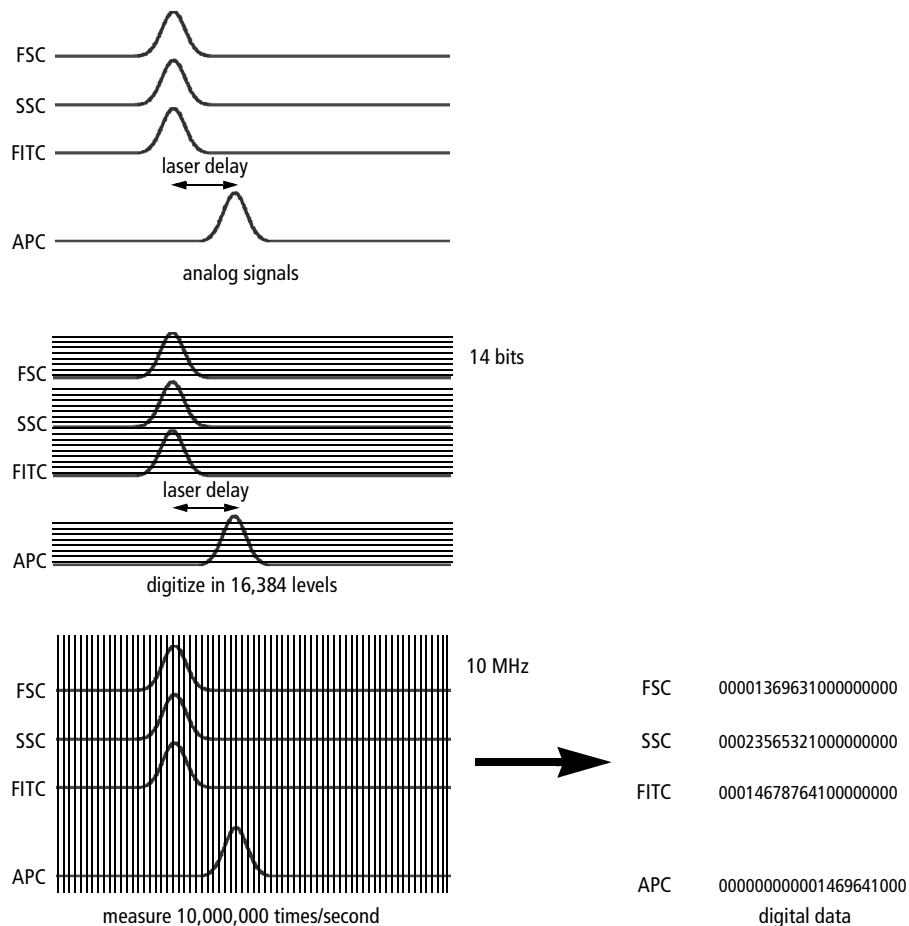
This chapter gives a brief overview on the following:

- How Digital Signals are Measured on page 240
- Threshold on page 241
- Parameter Values on page 241
- Ratios on page 242
- Compensation on page 242
- Electronic Aborts on page 242

How Digital Signals are Measured

In analog mode, pulses are sampled once per event. In digital mode, instrument 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 that is 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 can measure area, height, and width for up to 16 channels (depending on your instrument and installed options). Time is also a recorded parameter (see Using the Time Parameter on page 113).

- 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/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 99.

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 frame (see Acquisition Status on page 108).

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.

If the abort rate exceeds 10–20% of the total event rate, slow the sample differential or exclude debris by raising the threshold. The abort rate plateaus at half the event rate, and any additional events will be ignored, except during a purity sort. In that case, the total event rate will decrease rapidly as the system tries to sort. In no case will the system compromise the purity of sorted samples.

For more information about the window extension, see *Using the Window Extension* on page 101.

Glossary

acquisition Tube	Tube selected for acquisition or recording, as indicated by a green pointer
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 experimental 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)	<p>The standard deviation of the data divided by the mean of the data; typically expressed as a percentage (also known as Relative Standard Deviation)</p> <p>When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.</p>
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	<p>Graphical representation of two-parameter data in which contour lines show the distribution of events</p> <p>Similar to a topographical map, contour lines show event frequencies as peaks and valleys.</p>
Current Tube pointer	<p>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</p> <p>When the software is connected to the instrument, the pointer can also be used to control acquisition.</p>
data file	<p>A collection of measured values from a single Tube combined with text describing the data that has been stored to disk</p>
density plot	<p>Graphical representation of two-parameter data in which colored dots show density for events with the same signal intensity</p> <p>A density plot simulates three-dimensional event display.</p>
derived gate	<p>Combination of one or more defined populations using the Boolean operators AND (intersected gate), OR (joined gate), or NOT (inverted gate)</p>
dot plot	<p>Graphical representation of two-parameter data</p> <p>Each axis of the plot displays values of one parameter; a dot represents an event (particle).</p>
Experiment	<p>Group of elements used to acquire and analyze data from the flow cytometer</p> <p>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.</p>
flow cytometry standard (FCS)	<p>Standard format for flow cytometer data files</p>

frame	<p>Software element displaying main software components</p> <p>Frames 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.</p>
gate	<p>Two-dimensional boundary defining a subset of the total sample population</p> <p><i>See also</i> derived gate, interval, population.</p>
global worksheet	<p>Worksheet for which elements can be used to display multiple data sets by moving the Current Tube pointer</p> <p><i>See also</i> worksheet, Worksheet frame.</p>
histogram	<p>Graphical representation of single-parameter data</p> <p>The horizontal axis of the graph represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).</p>
Inspector	<p>Software interface for viewing or modifying the attributes of a single object or set of objects on the worksheet or in the Browser</p>
instrument settings	<p>Collection of values for parameters measured, photomultiplier (PMT) voltages, threshold, compensation, and the calculated parameters Log and Ratio</p>
interval	<p>One-dimensional boundary defining a subset of the total sample population</p> <p><i>See also</i> gate, population.</p>
laser delay	<p>Amount of time between signals from different laser intercepts</p>
panel template	<p>Group of labeled Tubes commonly used together in the same experiment</p> <p>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.</p>

parameter	<p>Measurement of a cell property that is ascertained as the cell passes through the laser beam</p> <p>Each parameter is the output of a single photomultiplier tube or photodiode, measuring fluorescent or scattered light.</p>
population	Data subset defined by a gate or interval
Snap-To gate	<p>Gate drawn automatically when you select a peak or cluster of events in a plot</p> <p>Unlike static gates, Snap-To gates are automatically redrawn when data in the gate changes.</p>
Sort Layout	<p>Floating window containing sorting instructions and controls (available only on sorting instruments)</p> <p>The Sort Layout designates which device will be used to collect sorted particles and which particles will be sorted into each sort location. Up to four sort counters can be displayed in the window to give ongoing status during a sort.</p>
Specimen	Browser object representing the type of material to be analyzed, the collection date, and user-defined keywords
spectral overlap	Fluorescence detected in a channel other than the one for which it is intended
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	<p>A trigger signal and level of discrimination to eliminate unwanted events</p> <p>Only events with parameter values above the threshold will be analyzed.</p>
Tube	Browser object representing instrument 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 frame for displaying data for a specific Tube. All objects on a worksheet are printed with a single Print command. <i>See also</i> global worksheet, Worksheet frame.
Worksheet frame	Window for viewing Analysis objects on worksheets or global worksheets

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