BD FACSDiva™ Software Reference Manual

For Version 8.0

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History

Revision	Date	Change Made
23-11219-00 Rev. 01	5/2012	Initial release for BD FACSDiva software version 7.0.
23-14523-00	4/2013	Revised for BD FACSDiva software version 8.0.

Contents

Preface

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

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

First-time users of BD FACSDiva software should read:

- Chapter 1 for software requirements and compatibility, installation, and administrative options

- Chapters 2 and 3 to learn about basic software functions and cytometer controls

- Chapter 4 to learn about analysis tools like worksheets, plots, gates, and statistics

- Chapter 5 to learn how to manage data and import and export files

Conventions

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

Table 1 Hazard symbols

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

Table 2 Text and keyboard conventions

Convention	Use
NOTE	Describes important functions or instructions
Italics	Italics are used to highlight book titles.
	Italics are also used in describing software to indicate specific text typed into a window or dialog.
Bold	Bold text indicates software elements such as windows, menus, buttons, and tabs that are used to complete tasks.
>	The arrow indicates a menu choice. For example, "select **File > Print**" means to select **Print** from the **File** menu.
Ctrl+X	When used with key names, a plus sign means to press two keys simultaneously. For example, Ctrl+P means to hold down the Control key while pressing the letter *p*.

Technical Assistance

For technical questions or assistance in solving a problem:

- In BD FACSDiva software, select **Help > FACSDiva Help.** Locate and read the related topics.

- See the Troubleshooting section in the software or cytometer manuals.

- See the BD Biosciences website: bdbiosciences.com

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

When contacting BD Biosciences, have the following information available:

- Product name, part number, and serial number; software version and computer system specifications

- Error messages, if any

- Details of recent cytometer performance

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

For support in the US, call 877.232.8995.

For support in Canada, call 888.259.0187.

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

Limitations

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

1

Software Setup

The following topics are covered in this chapter:

About BD FACSDiva Software

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

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

What's Included

The BD FACSDiva installer includes the following applications:

- BD FACSDiva software for acquiring and analyzing data

- BD FACSDiva Data Manager utility for backing up and restoring the database

- Java™ 2 Runtime Environment for running BD FACSDiva software

- Sybase® SQL Anywhere® Studio for running the database

- Sentinel System Driver™ for using the security module

- Microsoft .NET 3.5 Framework

BD FACSDiva Software Version 8 in Windows 7 OS

BD FACSDiva software version 8.0 runs on the Microsoft Windows 7 32-bit operating system. It will not run on Windows XP.

The changes in the newer version are the default locations where some of the output files are saved. The basic functionality of the application remains the same as in the previous version.

BD FACSDiva software version 8.0 includes all of the new functions that were added to version 7.0. See the next section.

New Functions in BD FACSDiva Software Version 7.0

The new functions in BD FACSDiva software version 7 are summarized in the following sections.

Browser Functions

The following functions have been added to improve the experiment Browser.

- **Drag and Drop.** In the Browser, drag and drop specimens, tubes, cytometer settings, and analysis settings within open or closed experiments and folders, as long as the items are of the same object type. See Dragging and Dropping Elements in the Browser on page 47.

- **Experiment Size.** View experiment size in a Size column displayed in the browser.

- **Compensation Controls.** Right-click the **Experiment** icon in the **Browser** to access the compensation setup function.

- **Import CSV Files.** Import CSV (comma-separated value) files into an experiment to create browser elements. See Importing Experiment Elements on page 52.

- **FCS 3.1 File Format.** Import and export FCS 3.1 files.

Statistical Calculations

The following functions expand the statistical analysis capabilities.

- **Cumulative Statistics.** Use the cumulative display option to display all of the events that occur during acquisition or while recording data in plots, and median values in the statistics view. When in cumulative mode, a *C* is displayed in the upper left corner of the worksheet until acquisition is completed. See Display Options on page 94 and Setting Acquisition Display Options on page 188.

- **Save Settings to Profile.** When editing the statistics view, save your changes by selecting the **Save Settings to Profile** checkbox. See Selecting Statistics to Display on page 233.

Worksheet Functions

The following functions have been added to improve use and flexibility of worksheet elements.

- **Undo/Redo.** Undo and redo changes to worksheet elements (plots, gates, population hierarchies, and statistics). See Undo and Redo Functions on page 161.

- **Copy and Paste Multiple Worksheet Elements.** Cut, copy, and paste multiple worksheet elements to an external application in one operation. See Copying Worksheet Elements on page 171.

- **Auto-Scaling of the Time Parameter.** When selected, the time parameter is automatically scaled to maximize viewing of data in the plot. See Using the Time Parameter on page 188.

- **Dot Size Customizing.** In the Inspector, change the dot size displayed for a population. See Population Attributes on page 226.

- **Overlay Plots.** Compare tubes by creating overlay plots for histograms, dot plots, contour plots, or density plots. See Overlay Plots for Tube Data on page 197.

- **Expanded Export Formats.** Export worksheet elements (plots, statistics view, population hierarchy) in TIFF, PNG, JPEG, and BMP formats.

- **Export XML File During Batch Analysis.** Export the worksheet elements in XML format during a batch analysis.

Administrative Options for Managing Software Settings

- **Viewing Disk Space Usage.** Allows an administrator to view disk space usage for each experiment and user and manage disk space. See Viewing Disk Space Usage on page 32.

- **Current CS&T Settings.** Allows an administrator to set an option for the account of each user to always use current BD™ Cytometer Setup and Tracking (CS&T) settings.

Additional New Functions

Two new functions are noted here and discussed in detail in specific user's guides:

- **Automation**. Allows you to connect to BD FACSDiva software (via a server socket) and issue commands, such as creating experiments and recording plates, using an API (application programming interface). For instructions about using automation, see the *BD High Throughput Sampler User's Guide* (if applicable on your cytometer).

- **BD FACSLink and BD FACS Workflow Manager:** BD FACSLink™ software and the BD FACS™ Workflow Manager system allow you to transfer data generated in BD FACSDiva software to laboratory information system (LIS) software. For details about BD FACSLink, see the documentation that comes with the product.

Installation

See the *BD FACSDiva Software Installation Guide* for instructions on installing or upgrading BD FACSDiva software.

Software Documentation

The software package includes the following documentation.

- 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 PDF file, select **Help > Documentation > Reference Manual** or double-click the shortcut icon on the desktop.

- The online help system contains information on how to use BD FACSDiva software and your cytometer. The help system opens in a separate window so you can access the documentation while working in the software. You can quickly locate information using the **Search** function. Internet access is not required to access the help system.

  To access the online help, select **Help > FACSDiva Help** within BD FACSDiva software.

- A collection of BD FACSDiva tutorials is located in the Resource Library on the BD Biosciences website. These tutorials can help new users get started using the software or experienced users become familiar with new features. You can also find quick reference guides and other support materials at this location.

System Requirements

Hardware

- BD FACS brand digital flow cytometer: BD FACSCanto™, BD FACSCanto™ II, and BD FACSCanto™ 10-color configuration.

  See the user's guides for other cytometers that use BD FACSDiva software for research use only (RUO) purposes (BD FACSAria™ II, BD FACSAria™ III, BD FACSAria™ Fusion, BD™ LSR II, or BD LSRFortessa™).

- PC workstation configured to BD Biosciences specifications:

  - Acquisition workstations can be purchased only from BD Biosciences. The computer must have at least 4 GB of RAM.

  - Analysis-only workstations must be equipped with an Intel® i5-650 processor or higher with at least 4 GB of RAM and the Windows 7 operating system (US English only). For optimal performance and full analysis capability, we recommend that you purchase a workstation that has been validated by BD Biosciences. Contact your sales representative for more information.

  NOTE If a workstation does not have a D drive, then the default locations for output files will be created on the C drive.

  - Workstations must be HP Model Z200 or higher. Make sure the operating system is Windows 7 32-bit.

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

- Universal serial bus (USB) security module

Software

BD FACSDiva requires additional software applications and components, which are installed automatically when the BD FACSDiva software is installed. The software applications and components areas are as follows:

- Java 2 Runtime Environment

- Sybase SQL Anywhere Studio

- Sentinel System Driver

Compatibility

- **Importing.** BD FACSDiva software can import data files in FCS 2.0 or 3.0 format including files generated by BD CellQuest™, BD CellQuest™ Pro, or BD FACSDiva software, version 6.x or earlier.

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

- **Exporting.** BD FACSDiva software can export data files in FCS 2.0, 3.0, or 3.1 formats. The software defaults to version FCS 3.0 for data file exporting. FCS files can be analyzed by other software applications such as BD CellQuest, BD CellQuest Pro, FlowJo™, or ModFit *LT*™.

Starting the Software

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

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

To start the software:

1 Double-click the shortcut icon on the desktop.

Alternatively, select **Start > Programs > BD FACSDiva Software > BD FACSDiva Software.**

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

2 Leave the user name as Administrator, and click **OK.**
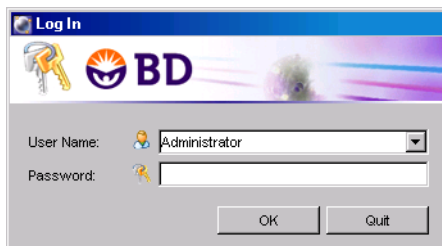
No password is required when you log in to the software. You should assign a password to the Administrator account as soon as possible. For instructions, see Adding or Modifying a Password on page 31.

After a successful login, the main application components appear in the workspace. Your workspace might look slightly different from that shown in this example.
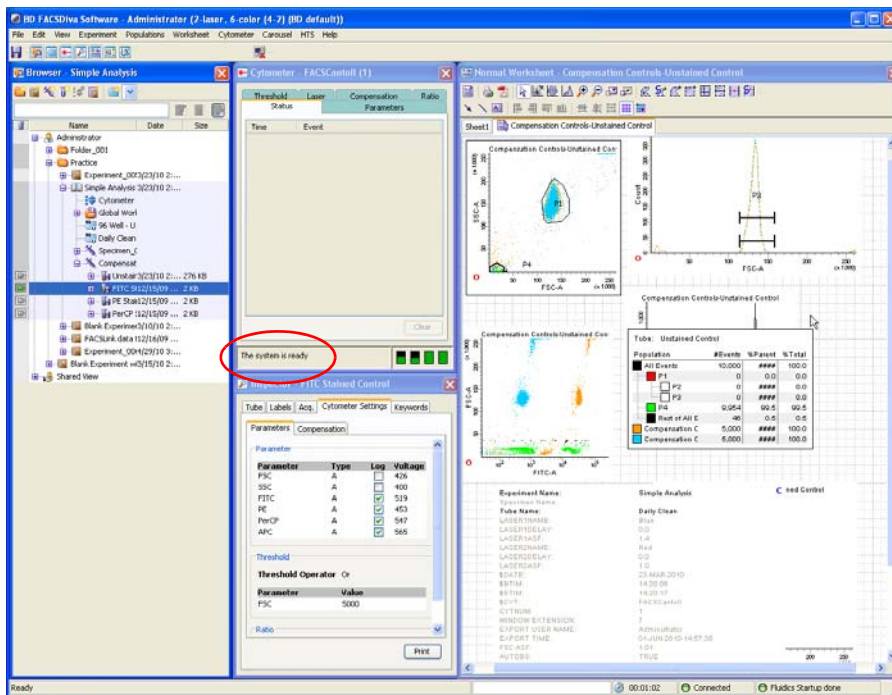
3 To verify that the workstation has successfully connected to the cytometer, check that the cytometer window displays the message *Cytometer Connected* or *The system is ready* at the bottom of the window. If the message is *Cytometer Disconnected*, see Electronics Troubleshooting on page 272 for assistance.

Administering Accounts

If you have administrator privileges in BD FACSDiva software, you can add, edit, or disable users, and export or import user profiles as described in the following sections. You do not need administrative access to change your password. See Adding or Modifying a Password on page 31.

Adding Users

To add a user:

1   Log in to the software as an administrator.

2   Select **File > Administration.**

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

3   Click **Add.**
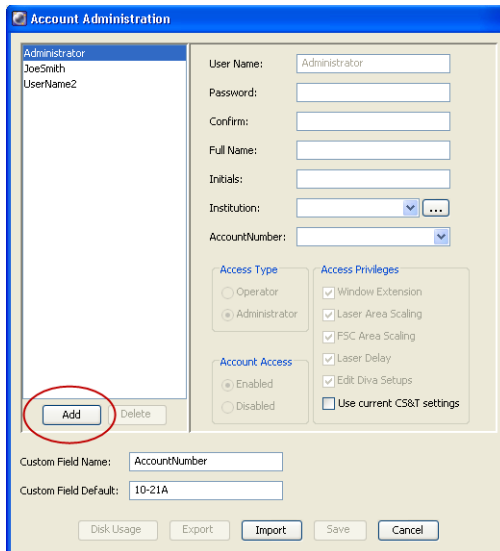
4 Select the name in the **User Name** field and enter a new name.

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

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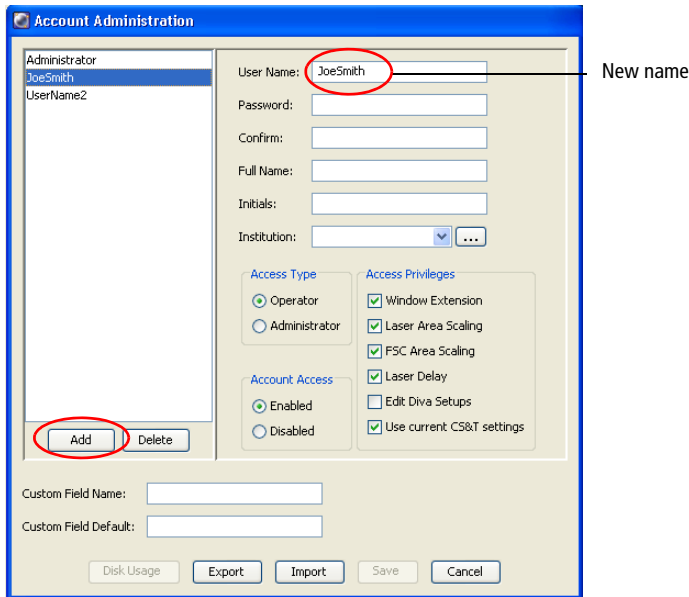
To create multiple new users quickly, click the **Add** button once for each new user, then select each new user and enter a name in the **User Name** field.

5   (Optional) Create a password.

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

        Passwords must be from 1–16 alphanumeric characters.

    b   Confirm the password by typing it again in the **Confirm** field.

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

    We recommend providing this information so it can be used as keywords and used in the User Tracking Log file. To add an institution, click the **Ellipsis** (…) button next to the **Institution** menu.
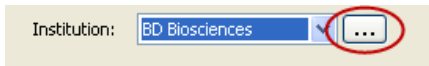
The following dialog opens for you to add or modify choices.
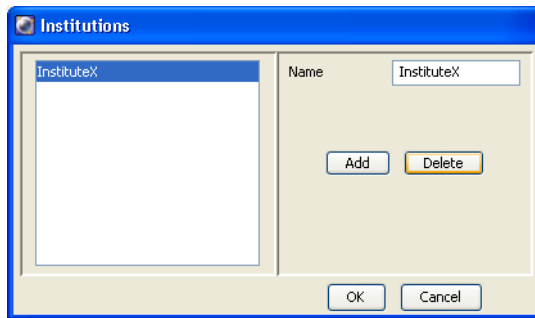
- To add an institution, click **Add.** *InstituteX* is added to the list of names. Change the name by selecting *InstituteX* in the **Name** field and entering a new name. Press Enter to apply the change, or click **OK** to apply the change and close the dialog.

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

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

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

7   Make selections under **Access Type, Access Privileges,** and **Account Access.**
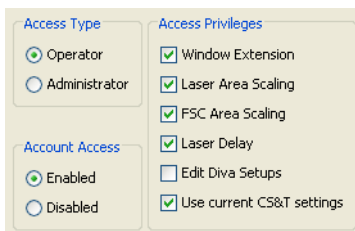
- **Access Type.** Select **Administrator** if you want to assign the user administrative privileges. Otherwise, select **Operator.** Administrators can add or modify user accounts, view all users' experiments, and edit cytometer configurations.

- **Account Access.** Select **Disabled** only when you want to disable a user. Otherwise, select **Enabled.** See Disabling Users on page 35.

- **Access Privileges.** Select the checkbox next to each setting the user is allowed to edit.

  For a description of the first four laser-related settings, see Laser Controls on page 111.

- Select the **Edit Diva Setups** checkbox to allow a user access to modify the setups saved in the Setup Catalog.

- **Use current CS&T settings**. Select this checkbox to always update the cytometer settings to the latest performance check.

8   (Optional) In the **Custom Field Name** field, enter a word or phrase to be associated with the user (for example, *Account Number* or *Department Name*).

A new menu is displayed under the Institution field with the custom field name you entered. We recommend providing this information so it can be used in keywords and in the User Tracking Log file.

NOTE   Keywords are limited to 20 characters.

Custom field name   Custom field value
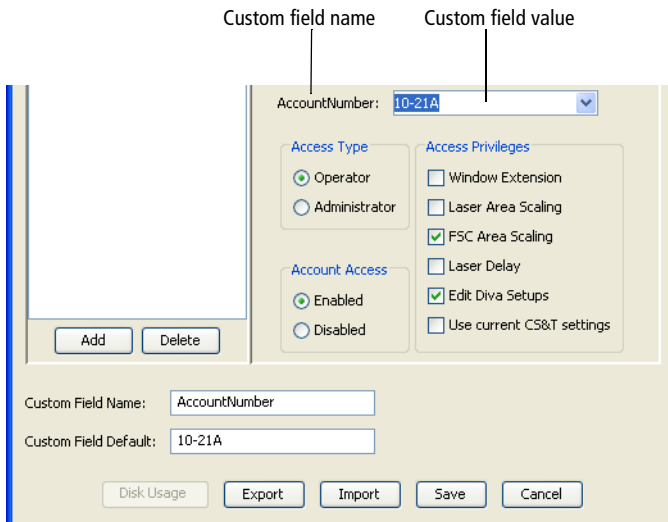
9 In the **Custom Field Default** field, enter the value associated with the custom field name you entered (for example, *10-21A* or *Finance Department*).

The value you entered is displayed in the new custom field you created in step 8.

If the custom field name is changed, the User Tracking Log header will not be updated until the new tracking log is created for the next month.

10 Ensure that all user information is correct and click **Save.**

Adding or Modifying a Password

We recommend 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.

To change the password:

1   Log in to the software.

2   Select **File > Administration.**

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

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

4   Confirm the password by re-entering it in the **Confirm** field.

5   Click **Save.**

Tracking User Logins

BD FACSDiva software automatically tracks user login information in a monthly tracking log. Access the user login information by logging in as an administrator and selecting **File > User Tracking Log** or looking in D:\BD\FACSDiva\log.

Logs are named *yyyy Month.csv* (for example, 2011 February.csv). Logs can be opened in a spreadsheet application such as Microsoft Excel®.

The following information is tracked in the monthly log:

- User name

- Full name

- Application name (BD FACSDiva, BD FACSCanto™ clinical software)

- Role (administrator, operator)

- Department (BD FACSCanto clinical software only)

- Institution

- Login time and date

- Logout time and date

- Build version

- Cytometer type

- Serial number

- Custom field

Viewing Disk Space Usage

BD FACSDiva software allows the administrator to view disk space usage for each user. The following information is displayed and can be sorted by category:

- User

- Experiment

- Specimen

- Tube

- File Name

- File Size

- Date Modified

To view disk space usage:

1 Select **File > Administration**.

2 Click **Disk Usage**.

Exporting User Profiles

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

1 Log in to the software as an administrator.

2 Select **File > Administration.**

3 From the list of user names, select those you want to export, and click **Export.**

- To select multiple contiguous names, click the first name in the series, then hold down the Shift key as you click the last name.

- To select multiple noncontiguous names, hold down the Ctrl key as you click each name.

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

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

Importing User Profiles

You must have administrative access to import user profiles.

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

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

2 Log in to the software as an administrator.

3 Select **File > Administration.**

4 Click **Import.**

5 In the **Import** window, select the file containing the names you want to import.
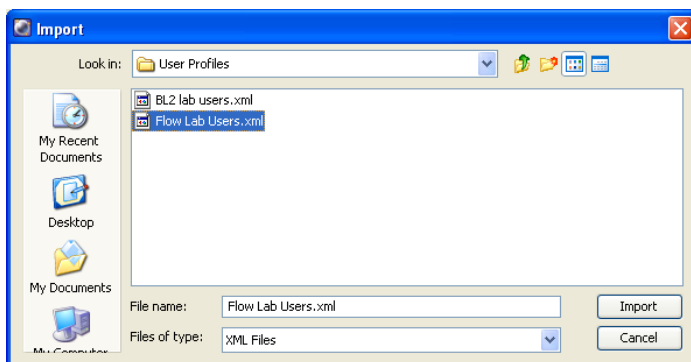
User names must be unique. If the file you are importing contains a duplicate of any existing user names, an error message appears displaying the names that are duplicates. Close the message, and either delete the duplicate user names from the file or select a different file to import.

6 Click **Import.**

7 Verify that all user names and passwords were imported.

Disabling Users

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

1   Log in to the software as an administrator.

2   Select **File > Administration.**

3   In the **Account Administration** dialog, select the user.

4   Select **Disabled** under **Account Access.**

5   Click **Save.**

Deleting Users

You must have administrative access to delete a user.

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

See Exporting Experiments on page 261. Enable the option to automatically delete experiments after export.

2   Select **File > Administration.**

3   Select the user name, then click **Delete.**

4   Click **Save.**

Quitting the Software

Do one of the following to quit the software:

- Select **File > Quit.**

- Click the **Close** button in the upper-right corner of the workspace window.

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

2

BD FACSDiva Workspace

This chapter describes the following BD FACSDiva workspace elements.

Workspace Components

When you start BD FACSDiva software, the workspace appears, as shown in the following figure. Hide or show windows by clicking buttons on the Workspace toolbar.
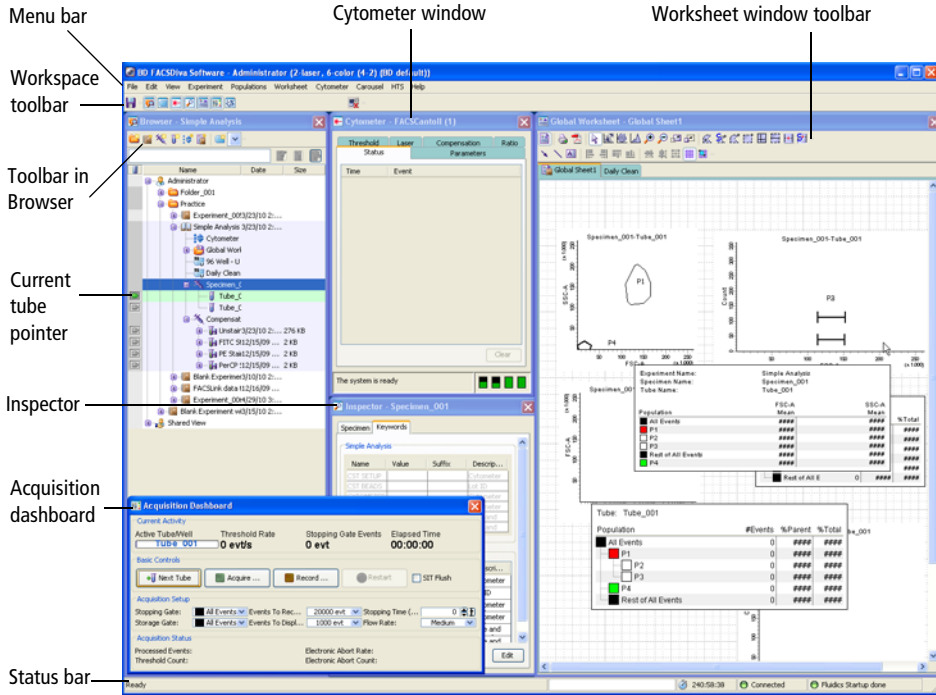
The following are brief descriptions of these components:

- Use the menu bar at the top of the workspace and the toolbars to control most software functions.

- Use the current tube pointer or buttons within the Acquisition Dashboard to control acquisition and data loading.

- Use the status bar at the bottom of the workspace to review cytometer connection status, fluidics information, and more.

Status Bar

The status bar at the bottom of the workspace provides the following
information:

- Application status (ready or not)

- Elapsed login time for the current user

- Cytometer connected or disconnected indicator

- Fluidics startup/shutdown state

The display of the status bar is selected by default. To close the status bar, clear
the **Status Bar** option in the **View** menu at the top of the workspace.

Workspace Toolbar

The Workspace toolbar appears near the top of the application window. It
includes the Save button and View/Hide buttons that show or hide various
windows within the application window.

Save   View/Hide buttons

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

Icon	Button Name	Description
	Save	Saves the current experiment to the database. Experiments are also saved when you close an experiment or quit the software.
	Browser	Hides or shows the Browser. See Browser on page 43.

Icon	Button Name	Description
	Plate	Hides or shows the Plate window. This button appears only if your cytometer is compatible with the BD™ High Throughput Sampler (HTS) option.
	Cytometer	Hides or shows the Cytometer window. See Cytometer Controls on page 108.
	Inspector	Hides or shows the Inspector. See Inspector on page 42.
	Worksheet	Hides or shows the Worksheet window. See Worksheets on page 156.
	Acquisition Controls	Hides or shows the Acquisition Dashboard. See Acquisition Dashboard on page 117.
	Biexponential Editor	Hides or shows the Biexponential Editor. See Working with the Biexponential Editor on page 203.
	Sorting	Hides or shows the Sorting window(s). This button appears only if your cytometer is equipped with sorting features.
	Automation Server	Turns the automation server connection on or off.

View Options

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

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

1   Click the **Restore** button in the upper right corner of the workspace to reduce it.

2   Drag the window border to fill the second monitor.

To return to one screen, select **View > Reset Positions**.

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

- To move a window, drag the title bar to a new position on the screen.

- To resize a window, position the cursor on the border. When the cursor changes to a double-headed arrow, drag the border.

Figure 2-1 Resizing a workspace window

Double-headed arrow
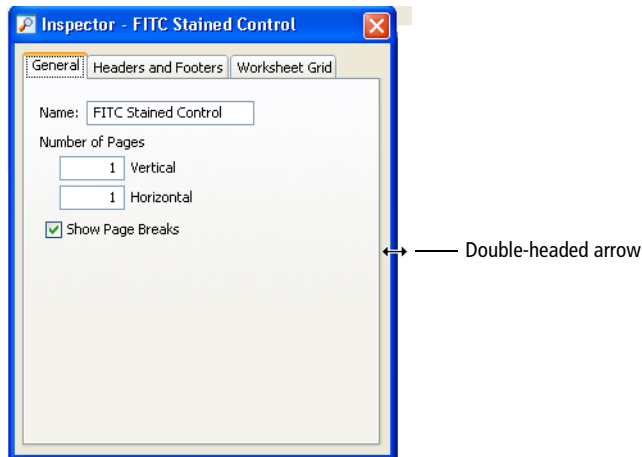
- To view or hide workspace windows, select an option from the **View** menu, or click the corresponding button on the **Workspace** toolbar.

- To restore windows to their default position and size, select **View > Reset Positions**.

Inspector

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

To display the Inspector, click the **Inspector** button on the **Workspace** toolbar. The contents of the Inspector vary depending on the object selected.

For example, compare the contents of an Experiment Inspector on the left (displayed when an experiment is selected in the Browser) with those of a Statistics Inspector on the right (displayed when a statistics view is selected on a worksheet).
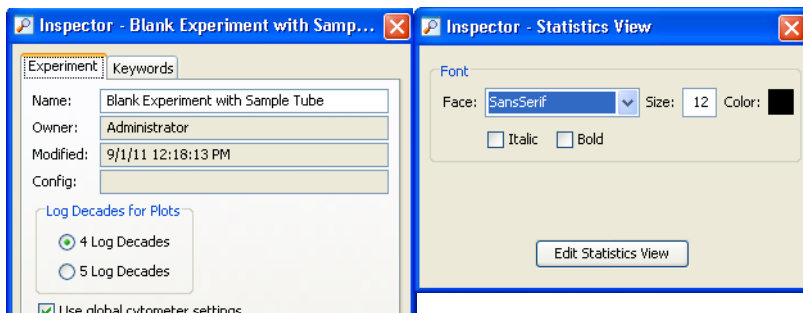
Browser

BD FACSDiva software stores and provides access to experiment data. Stored elements are shown in the Browser.

Browser toolbar
Search field
User icon
Open experiment
Current tube pointer
Closed experiment

Column divider
Column header
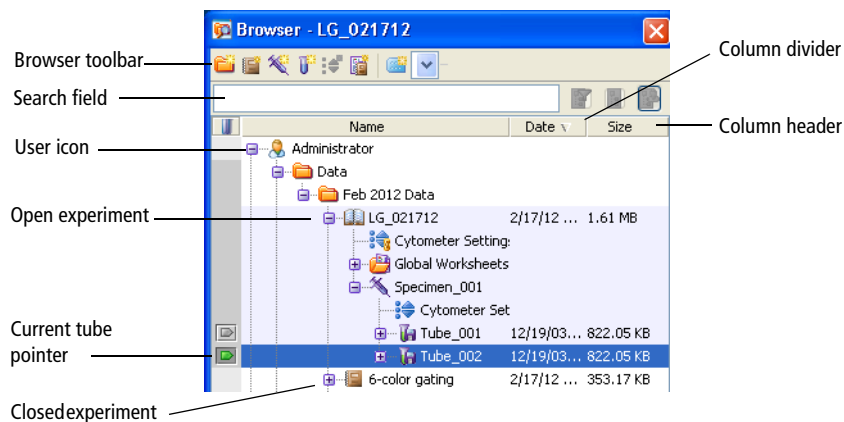
Create and access database elements in the Browser. Data is listed by login name in a hierarchical view. Hide or display the Browser by clicking the **Browser** button on the **Workspace** toolbar.

Users with administrative access can view all experiments in the database. Those without administrative access can only view their own experiments and any experiments that have been designated as shared. For more information, see Making Experiments Shared or Private on page 57.

Using the Browser

The Browser has the following functions:

- Lists experiments saved in the BD FACS 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 Using the Current Tube Pointer on page 48.

    - Use the search field above the Browser to find experiments or show fewer experiments in the Browser. See Using the Search Field on page 45.

    - View the file size of an experiment or tube in the size column.

- 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 46.

- Organizes experiment elements in a hierarchical view.

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

    - Sort experiments in the Browser by clicking inside the **Name** or **Date** column headers. Click in the same header again to reverse the sort order.

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

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

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

- Right-click any item in the Browser to display a shortcut menu with options specific to that item.

- Use the current tube pointer to start and stop data acquisition and recording and to load data. See Current Tube Pointer on page 123.

You can 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.

Using the Search Field

Use the Search field and associated buttons to find experiments or to show fewer experiments in the Browser.

Search field   Find button   Display All button   View Own button
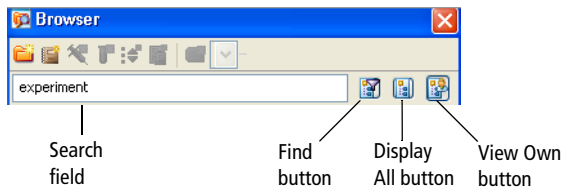
NOTE   You cannot use the find function to locate a folder. If a folder contains an experiment that meets the search criteria, it will have a plus sign (+) next to it.

Use the Search field and buttons in the following ways:

• To locate experiments by name, enter the name, and click the **Find** button.

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

• To hide other users' experiments, click the **View Own** button.

Experiments under the Shared View icon are hidden.

To enable the button, close all open experiments.

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

Adding New Elements to the Browser

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

NOTE You can customize the Browser toolbar buttons to add a predefined template to the Browser. See Template Preferences on page 100 for instructions.

Tool-bar Icon	Name (Icon or option)	New Element	Description
	New Folder		Adds a new folder as a child of the selected user or folder.
	New Experiment (icon)		Adds a new experiment (based on the blank experiment template) as a child of the selected user or folder. Note that this is slightly different behavior from selecting Experiment > New Experiment from the menu. See Adding Experiments on page 50.
	New Specimen		Adds a new specimen as a child of the selected experiment.
	New Tube		Adds a new tube as a child of the selected specimen.
	New Cytometer Settings		Adds new tube-specific cytometer settings as a child of the selected tube or new specimen-specific cytometer settings element as a child of the selected specimen.
	New Global Worksheet		Adds a new global worksheet as a child of the selected experiment.

Tool-bar Icon	Name (Icon or option)	New Element	Description
	New Sort Layout		Adds a new sort layout as a child of the selected tube or global worksheet. Note that the Sort Layout menu option is in the Sort menu, not the Experiment menu. This option is only available for a cytometer with sorting capability.
	New Plate (icon)		Allows you to select a plate type to add to the selected experiment. Note that this option is only available for a cytometer with a plate loader.

Dragging and Dropping Elements in the Browser

BD FACSDiva software lets you drag and drop specimens and tubes within open or closed experiments. Any previously associated analysis templates and cytometer settings are automatically moved with the tube or specimen.

To drag and drop Browser elements:

1   Select an item in the **Browser,** or hold down the Ctrl key to select multiple items of the same type, such as specimens or tubes.

2   Drag the items to the desired location in the **Browser.**

3   Drop the items by releasing the mouse button and the items will appear in the new location.

Rules for Moving Browser Elements

The drag and drop rules for folders, experiments, specimens, and tubes are as follows:

- The hierarchy in the Browser has to be followed when moving elements:

    -   Experiments can only be moved into or between folders.

- Specimens can only be moved within the experiment where they were created.

- Tubes can only be moved within the specimen where they were created.

- If a folder is moved, the entire hierarchy associated with that folder is moved.

• You can select multiple elements to drag and drop, but they must be the same object type (experiments, specimens, or tubes).

• Specimens and tubes associated with carousels or compensation controls cannot be moved.

Using the Current Tube Pointer

When an experiment is open, a gray pointer or plot icon appears next to tubes in the Browser.
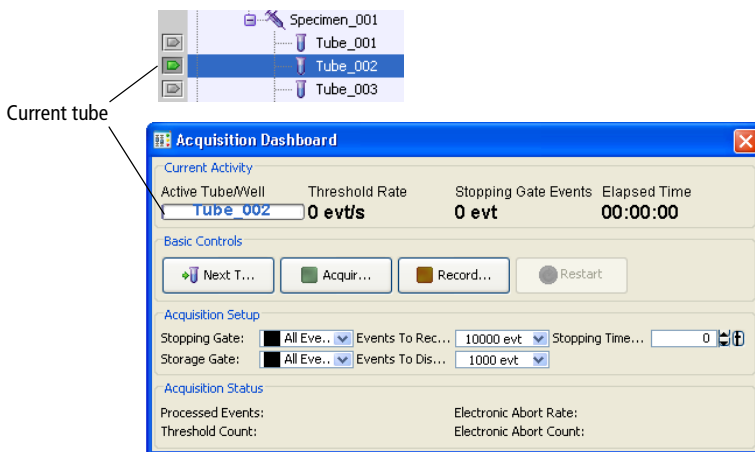
For more detailed information on current tube pointers and pointer states, see Current Tube Pointer on page 123.

During Acquisition

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

Offline

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

Current tube pointer

Experiments

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

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

Adding Experiments

You can add experiments by doing one of the following:

- Using the New Experiment Button on page 50

- Using the Duplicate without Data Function on page 50

- Creating an Experiment Based on a Saved Template on page 51

- Importing an Experiment on page 51

- Importing Experiment Elements on page 52

Using the New Experiment Button

To create a new experiment, click the **New Experiment** button on the **Browser** toolbar. The currently open experiment closes and a new, open experiment is added to the Browser.

NOTE If your cytometer configuration does not have a valid performance check, a warning from the software is displayed. Either click **OK** in the warning to continue, or run a performance check.

By default, the New Experiment button adds an experiment with default cytometer settings and a blank global worksheet (based on the blank experiment template), but the button can be customized to add a predefined experiment template. For more information, see Template Preferences on page 100.

Using the Duplicate without Data Function

You can duplicate an experiment by selecting **Edit > Duplicate without data.** The experiment is duplicated, but no data is saved and information about the application settings is not included. Application settings are associated with a cytometer configuration and include the parameters needed for the application, area scaling values, PMT voltages, and threshold values, but not compensation.

Creating an Experiment Based on a Saved Template

To create an experiment based on a saved template, select **Experiment > New Experiment.** After the Experiment Templates dialog opens, select the template type and number of experiments to create.
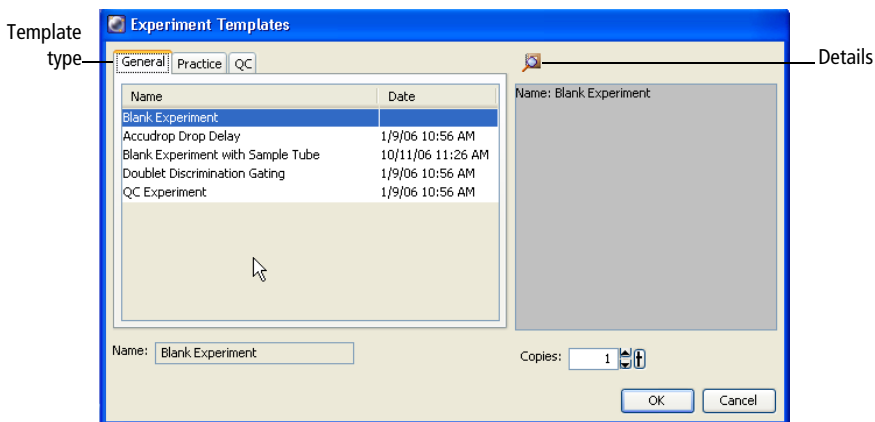
To view the experiment layout associated with the experiment template, click the **Details** button. The Experiment Layout opens, showing the specimens and tubes in the experiment, and any defined labels, keywords, and acquisition criteria. See Using the Experiment Layout on page 60.

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

Importing an Experiment

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

For more information, see Importing Experiments on page 263.

Importing Experiment Elements

Elements from a CSV file can be imported into an open experiment in
BD FACSDiva software. The experiment you are importing into must not contain
any recorded data. To be a valid file, the CSV file must be formatted as follows:

- The column headers across the top must be BD system defined keywords,
  starting with specimen name. See Keywords on page 83.

- The keywords must be typed in all capital letters (SPECIMEN NAME,
  TUBE NAME, SAMPLE ID, etc).

To import a CSV file:

1   Create a blank experiment for the imported files.

2   Select **File > Import > CSV files.**

3   Navigate to the files you want to import in the dialog that opens.

4   Select the file(s), then click **Import.**

Opening Experiments

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

Do one of the following to open a closed experiment:

- Double-click a closed experiment icon in the **Browser.**

- Select an experiment in the **Browser** and select **Experiment > Open
  Experiment** or press Ctrl+O.

- Right-click an experiment icon in the **Browser** and select **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.
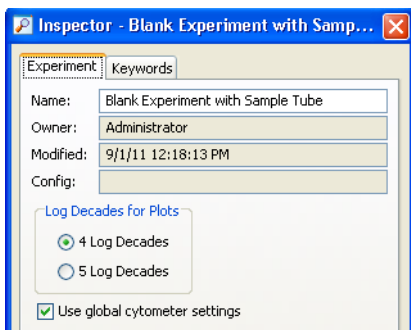
In the Inspector, you can:

- Name the experiment

- Specify the number of logs to display for all plots in the experiment (see Changing Log Display on page 181)

- Select whether or not to update global cytometer settings automatically (see Using Global Cytometer Settings on page 133)

- On the Keywords tab, create or view experiment-level keywords (see Keywords on page 83)

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. The configuration is the cytometer configuration associated with the experiment. These fields cannot be changed.

Saving Experiments

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

You can 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 can include any of the following components, but do not include recorded data. You can set up experiment templates for frequently used experiments. Templates are stored outside the database. Note that experiments can be exported as templates whether they are open or closed.

- Specimen names

- List of tube names

- Any parameter labels for each tube

- Keywords

- Acquisition criteria

- Normal worksheets and associated analysis objects

- Global worksheets and associated analysis objects, including all settings such as page breaks

- Experiment level cytometer settings

- Specimen- or tube-specific cytometer settings. These cytometer settings, including compensation values, are automatically included if an experiment template is created from a specimen with recorded data.

To export an experiment as a template:

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

The Export Wizard dialog opens, with steps that show you how to create and export a template.
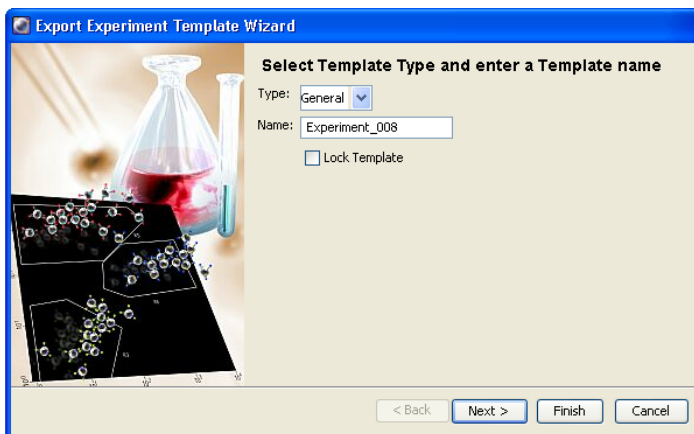
2 Enter values in this dialog as follows:

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

- **Name**. 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.

- **Lock Template**. Select the **Lock Template** checkbox so the template cannot be overwritten by a template with the same name. Locked

templates and default templates provided with the software cannot be overwritten.

3 Click **Next**.

4 (Optional) Enter study details in the next window and click **Next**.

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

5 (Optional) Enter operator and investigator information in the next window.

6 Click **Finish**.

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

Editing Templates

Template files are stored on your computer as XML files. They are organized in folders by type within BDExport\Templates. The name of each template file consists of the template name followed by the XML extension.
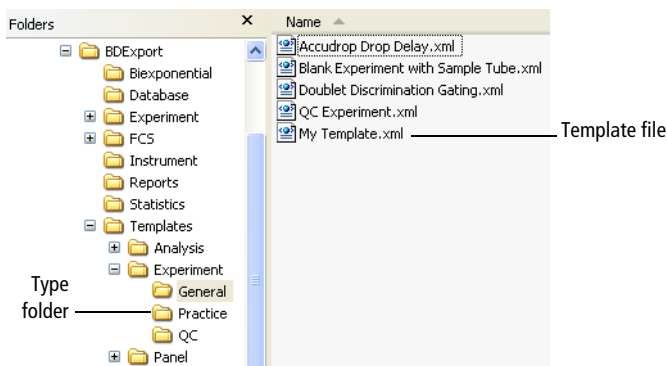
Do one of the following to edit a saved experiment template:

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

  NOTE A locked template cannot be overwritten.

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

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

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

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

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

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

Making Experiments Shared or Private

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

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

When other users log in to the software, they can add or delete elements within shared experiments under the Shared View icon in the Browser.
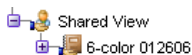
To remove the shared status, right-click a shared experiment and select **Make Private**.

You can view just your experiments (that is, 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.

 — View Own

 — View Shared
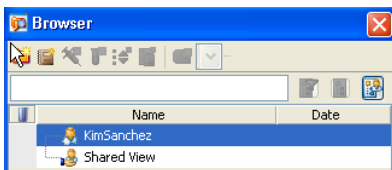
Shared experiments shown
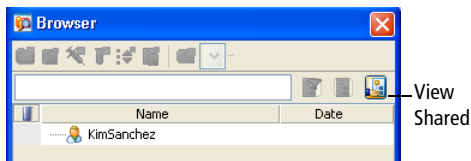
Shared experiments hidden

Exporting and Importing Experiments

Experiments can be exported to the hard drive or an external storage device. See Exporting and Importing Experiments on page 261.

Experiment data can be exported in FCS 2.0, 3.0, or 3.1 file format. You can also import FCS files from another BD application. See Exporting FCS Files on page 253 and Importing FCS Files from BD Biosciences Applications on page 256.

Using the Find Experiments Function

The Find Experiments dialog, like the search field on the Browser toolbar, enables you to search for experiments. However, the Find Experiments dialog provides greater specificity in your search, enabling you to search the child elements within experiments and use date ranges.For more information on the Search field in the Browser, see Using the Search Field on page 45.

To use the Find Experiments function, select **Edit > Find**. The Find Experiments dialog opens.
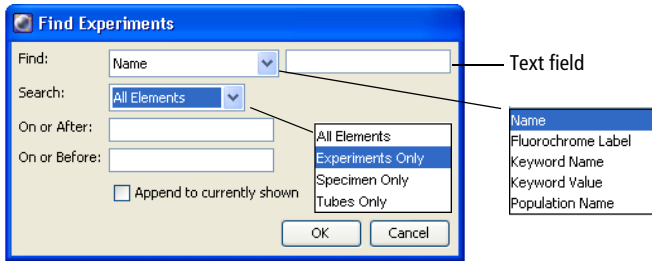
Use the functions in the Find Experiments dialog as follows:

- **Find menu and text field**. Search for a text string in a particular attribute of the experiment, such as fluorochrome label or keyword. Select a category from the menu, then enter specific information in the text field next to the menu. For example, select **Fluorochrome Label** and enter *CD4* in the text field.

- **Search menu**. Select a menu item other than All Elements to restrict the search to a certain type of data element (experiments, specimens, or tubes).

- **On or After, and On or Before**. Enter a date to restrict the search to a specified time period. Enter the month first, followed by the day and the year (for example, 5/17/11 or May 17, 2011).

- **Append to currently shown**. Add the results of the search to the experiments currently displayed in the Browser. Clear the checkbox to display only experiments meeting the specifications of the current search.

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

If no experiments contain 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 Experiment Layout

Use the Experiment Layout to create labels, enter values for keywords, or enter acquisition criteria for each tube in an experiment.

Open an experiment and select **Experiment > Experiment Layout**. The Experiment Layout dialog opens. It lists all specimens and tubes in the experiment. Use the layout list area to create lists of labels, keywords, and acquisition criteria. Click the arrows to display or hide the list function.
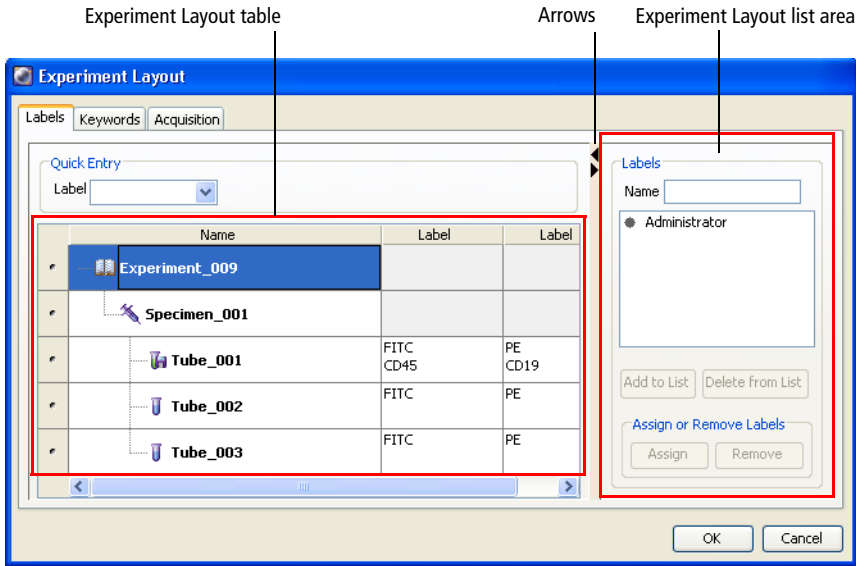
Using Experiment Layout Lists

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

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

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

For details on how to use the lists, see Using the Labels Tab on page 61, Using the Keywords Tab on page 63, and Using the Acquisition Criteria Tab on page 66.

Editing Element Names

1   Select any element in the **Name** column under any of the tabs in **Experiment Layout,** such as experiment, specimen, or tube.

2   Type over the name to change it, then press Enter.

   The new name is saved immediately, even if you click Cancel.

Using the Labels Tab

Use the Labels tab of the Experiment Layout dialog to enter parameter labels for each fluorochrome in your experiment. Parameter labels will be displayed on plot axes and in statistics views.

Using the Experiment Layout Table

You can use the Experiment Layout table to modify labels in the following ways:

- **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, select it from the **Label** menu. (The menu is blank until you have defined at least one label.)

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

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

- **Delete labels**. To delete a label, click the label cell and press the Delete key. Alternatively, click the **Label** menu and select the blank label field at the top of the list, then press Enter.
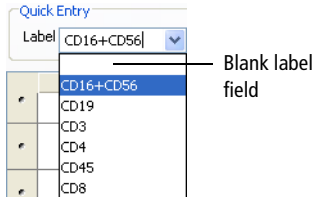
Using the Experiment Layout List

You can use the Experiment Layout list to modify or apply labels as follows:

- **Add a label.** Type a label in the **Name** field and click the **Add to List** button. (Labels are case sensitive.)

- **Delete a label.** Click the **Delete from List** button. Clicking **OK** saves labels to the database.

- **Apply a label.** Select a label field in the **Experiment Layout** table, select the label from the list, and click **Assign**. Click **Remove** to clear the label from the field.

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

Labels can also be entered for individual tubes on the Labels tab of the Tube Inspector. See Using the Tube Inspector on page 75.

Using the Keywords Tab

All keywords currently defined for the experiment are listed in the Keywords tab of Experiment Layout.
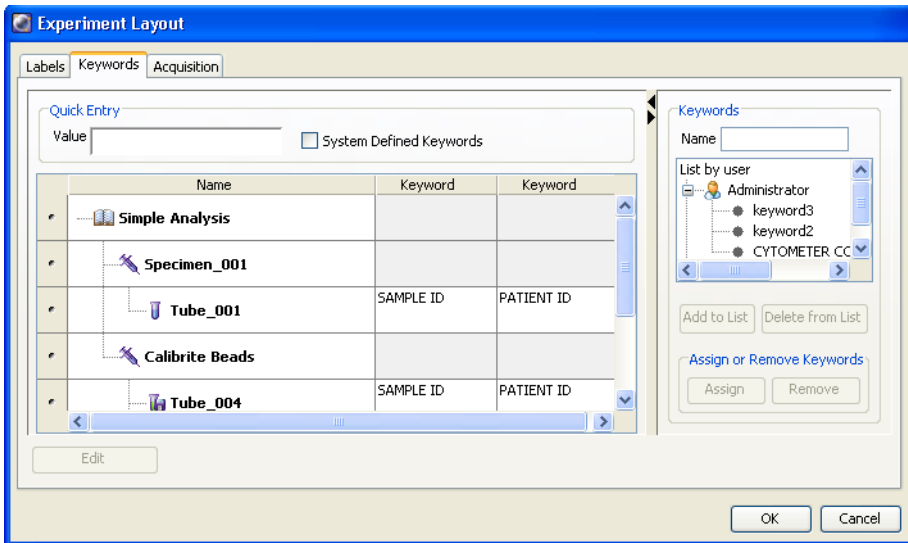
Keywords are used to identify a file or set of statistics. See Defining and Editing Keywords on page 84. Note that keywords are limited to 20 characters.

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

Entering a Keyword Value

Enter keyword values in one of the following ways:

- Enter a value in the **Value** field.

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

- Apply a keyword to multiple elements in the **Keywords** list.

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

- Apply a keyword to multiple specimens or tubes.

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

Selecting Multiple Keyword Fields

Use one of the following methods to select multiple keyword fields:

- Select multiple contiguous fields by doing one of the following:

  - Click the left mouse button and drag down a column.

  - Hold down the Shift key, then click on the first and last fields in a range. You can also click in a cell and press Shift+Ctrl+End to select the rest of the cells in the column.

- Select noncontiguous fields in a keyword column by holding down the Ctrl key while clicking each selection.

- Select a cell in the column or row to select the keyword type, then click the column or row header to select multiple keyword cells of that type. Enter a keyword or select a keyword from the **Value** menu to change the selected cells.

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

NOTE An individual field cannot be cleared from a selected group.

Using the Acquisition Criteria Tab

Use the Acquisition tab in the Experiment Layout to view and edit the acquisition parameters for multiple tubes, including the number of events to record, the preferred global worksheet, stopping and storage gates, and stopping time.
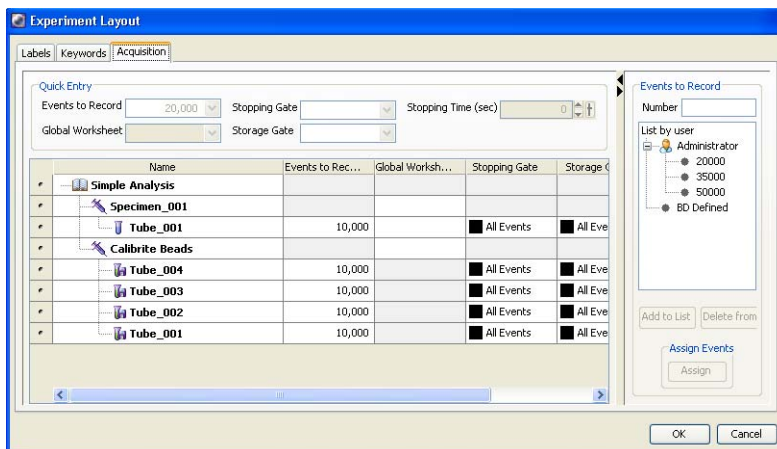
Events to Record

Change the events to record by selecting one or more fields and then entering a new number. You can also select a value from the **Events to Record** menu or the **Events to Record** list. If more than one cell is selected, the change is made in all selected cells at the same time.

To use the **Events to Record** list, type a number in the **Number** field and click the **Add to List** button to add the new item to the list.

Delete an item from the list by clicking the **Delete from List** button. Click **OK** to save items to the database.

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

Another way to add an events-to-record number using the list is to select the field you want to change and double-click the item in the list to apply it to the field. For example, select the **Events to Record** field for a particular tube, then double-click **50,000** in the **Events to Record** list to apply 50,000 to the selected tube. To change multiple cells at once, click the column header to select the entire column and assign values (of the same type) in the **Events to Record**, **Global Worksheet**, **Stopping/Storage Gates**, and **Stopping Time** fields.

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

Preferred Global Worksheet

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

Storage and Stopping Gate

To assign a storage or a stopping gate, select the tube, specimen, or well in the **Storage** or **Stopping Gate** column and select the desired gate from the menu in the column or the field in the **Quick Entry** area. The storage and stopping gates in the menus are based on the global worksheet that is selected.

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

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

When you set the current tube pointer to a tube, the global worksheet tab changes to the preferred global worksheet, and the preferred stopping and storage gates are applied. You can then change those stopping and storage gates, but not the preferred stopping and storage gates (because preferred gates can only be changed in the Experiment Layout). If a global worksheet is changed when a stopping gate or storage gate other than All Events is specified, then the gate selection is reset to All Events, provided that the selected gate name and type (for example, rectangle or polygon) drawn on the same coordinate system (for example, log, linear, or biexponential), using the same parameters, do not appear in the new global worksheet.

Stopping Time

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

Specimens

A specimen consists of the name of the material to be analyzed and a list of the tubes. Specimens can also contain cytometer settings (see Creating Specimen- or Tube-Specific Settings on page 132).

- To create a new specimen, see Adding New Elements to the Browser on page 46.

- To save a specimen as a panel, see Exporting a Specimen as a Panel Template on page 70.

- To create a new specimen from a panel template, see Importing a Panel Template on page 72.

- To edit a panel template, see Editing Templates on page 56.

Using the Specimen Inspector

The Inspector displays specimen options when you select a specimen in the Browser.
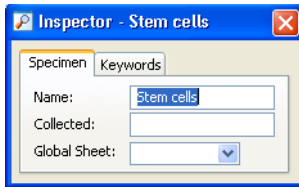
In the Specimen Inspector, you can:

- Enter the specimen name or sample type. Specimen names cannot contain commas or periods. Spaces at the beginning or end of the name are automatically removed.

- Specify the date your sample was collected.

- Select a default global worksheet for the specimen. The menu lists all global worksheets in your experiment. The selected worksheet is displayed automatically when you select a tube below this specimen.

- Click the **Keywords** tab to view or edit keywords stored with the specimen. For more information, see Keywords on page 83.

Exporting a Specimen as a Panel Template

A panel is a collection of tests, reagents, or markers commonly used together in the same experiment. Any specimen can be exported as a panel. A panel template can include any of the following components:

- Specimen name

- List of tube names

- Any parameter labels for each tube

- Keywords

- Acquisition criteria

- Normal worksheets and associated analysis objects

- Global worksheets and associated analysis objects. These are included by selecting the global worksheets you want to include using the Export Panel Template Wizard.

- Specimen- or tube-specific cytometer settings. These settings, including compensation values, are automatically included if a panel template is created from a specimen with recorded data.

Panels are stored outside the Browser to simplify the display. Note that specimens can be exported as templates only from open experiments.

To export a specimen as a panel:

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 select **Export > Panel Template**.

    The Export Panel Template Wizard dialog opens, and provides steps that show you how to export a template.

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

All defined global worksheets are shown.

4   Select the **Export** checkbox next to each global worksheet you want to include, then click **Next**.

The Select Template Type and enter a Template name dialog opens.
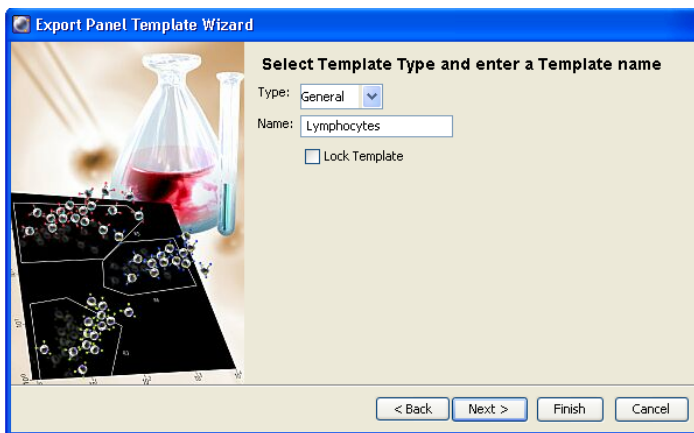
Enter values in this dialog as follows:

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

- **Name field**. 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.

- **Lock Template checkbox**. Select this checkbox to prevent the panel from being overwritten by a panel template with the same name.

Locked panels and any default panels provided with the software cannot be overwritten.

5 Click **Next**.

6 (Optional) Enter comments for the panel template in the **Study Template** dialog.

Comments can be viewed when you are importing a panel template. See Importing a Panel Template on page 72.

7 Click **Finish**.

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

Importing a Panel Template

To create a new specimen based on a panel template, select **Experiment > New Specimen** or press Ctrl+M.

The Panel Templates dialog opens. Select 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.
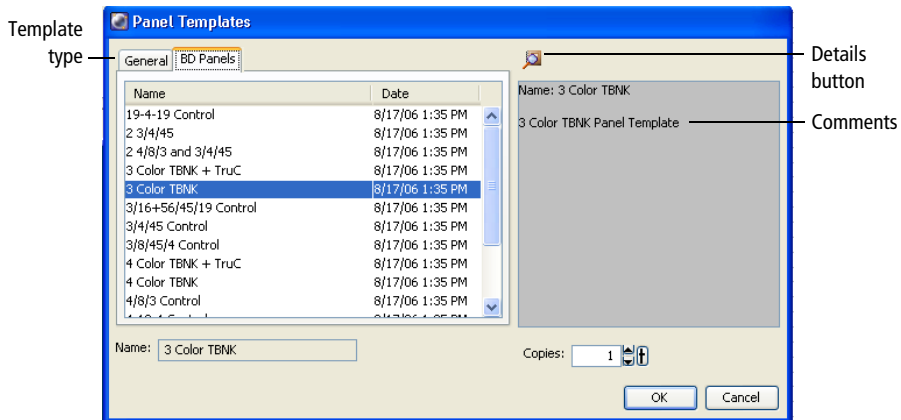
To view details about the panel template, click the **Details** button. The Experiment Layout appears showing a list of tubes in the panel, any defined labels, keywords, and acquisition criteria. You can view but not edit elements in Experiment Layout when importing a template. See Using the Experiment Layout on page 60.

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

For information about creating panels, see Exporting a Specimen as a Panel Template on page 70.

Applying a Panel Analysis

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

Panel templates can also be used as panel analysis templates. See Exporting a Specimen as a Panel Template on page 70. When applied to a specimen, a new normal worksheet is created containing all of its associated analysis objects and worksheet elements.

If a panel analysis includes global worksheets, the global worksheet information is not applied. See Worksheets on page 156 for details about normal and global worksheets.

To apply a normal worksheet panel analysis to a selected specimen:

1   Right-click the specimen, and then select **Apply Panel Analysis**.

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

2   Select a panel template, and then click **OK**.

The worksheet elements (such as plots, statistics, and population hierarchies) of the selected panel template are imported, subject to the following constraints:

•   The target specimen must have the same number of tubes as the panel analysis being applied.

•   If panel analysis tube names do not match the tube names in the Browser, click **Continue** to apply the analysis. If the tubes in the specimen already have existing analyses (gates and worksheet elements), you can overwrite the previous analysis or exit without overwriting.

•   If a panel analysis has only global worksheets, the elements are not imported.

Tubes

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

Most tube-specific information is entered using the Tube Inspector as described in the next section. For information on how to manipulate tube elements, see the following:

- To create a new tube, see Adding New Elements to the Browser on page 46.

- To create a new tube with a predefined analysis template, see Creating a Tube with a Predefined Analysis Template on page 79.

- To duplicate a tube, right-click the tube and select **Duplicate Without Data**, use the **Copy** and **Paste** commands, or click **Next** in the **Acquisition Dashboard**.

Using the Tube Inspector

When the current tube pointer is selected (green), the Tube Inspector opens. The Tube Inspector has four tabs: Tube, Labels, Acquisition, and Keywords. If a tube contains cytometer settings (that is, tube-specific settings or settings copied during recording), a Cytometer Settings tab is also shown.
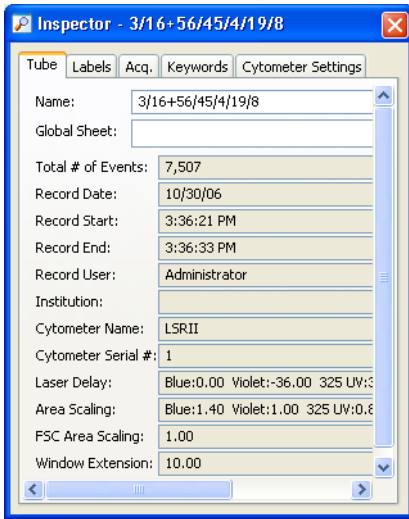
Many values defined on the Labels, Acquisition, and Keywords tabs can be viewed and edited using Experiment Layout. (See Using the Experiment Layout on page 60.)

In the Inspector, you can:

- Use the Tube tab to name the tube and to view certain keywords and settings saved with recorded data.

  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.

- Use the Labels tab to enter parameter labels for each fluorochrome. Labels are displayed on plot axes and in statistics views.
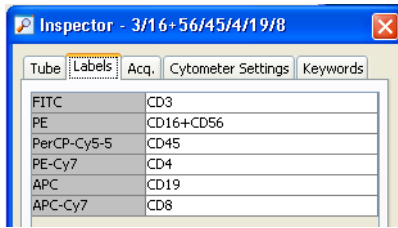
NOTE Label-specific tubes entered in the Tube Inspector are automatically displayed in the Create (or Modify) Compensation Controls dialog.

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

  - The number of events to record. Select the number from the menu or enter a value in the field.
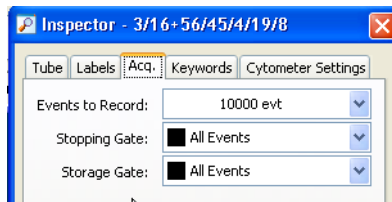
  - Whether you want to restrict the number of counted events to a predefined population.

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

The Acq. tab would look like the following.
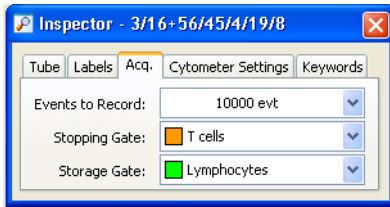
The cytometer would keep acquiring until 10,000 events were collected in the T-cell gate. However, only events that fell into the Lymphocytes gate would be saved to the database.

You can also specify the number of events to record, the stopping gate, and the storage gate using the menus in the Acquisition Setup section of the Dashboard (see Acquisition Dashboard on page 117) or the Experiment Layout (see Using the Experiment Layout on page 60). Inspector values are updated if you change the settings from these menus.

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

While working offline, you can use the Inspector to edit global cytometer settings or tubes with cytometer settings. (Cytometer settings for recorded tubes cannot be edited.) While connected to the cytometer, you can change voltages, thresholds, and ratios for tubes only in the Cytometer window. The Inspector shows a report of the settings for a selected tube. For more information, see Cytometer Settings on page 125.

Cytometer settings can apply at the tube, specimen, or experiment level. See Creating Specimen- or Tube-Specific Settings on page 132 for details.

• When available, use the Keywords tab to view or edit keywords stored with the tube. See Keywords on page 83.

Creating a Tube with a Predefined Analysis Template

If you have a predefined analysis template, you can create one or more tubes in a single step. For instructions on creating an analysis template, see Saving an Analysis Template on page 80.

To create a tube using a predefined analysis template:

1   Select **Experiment > New Tube.**

The Analysis Templates dialog opens.

2   Select 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.
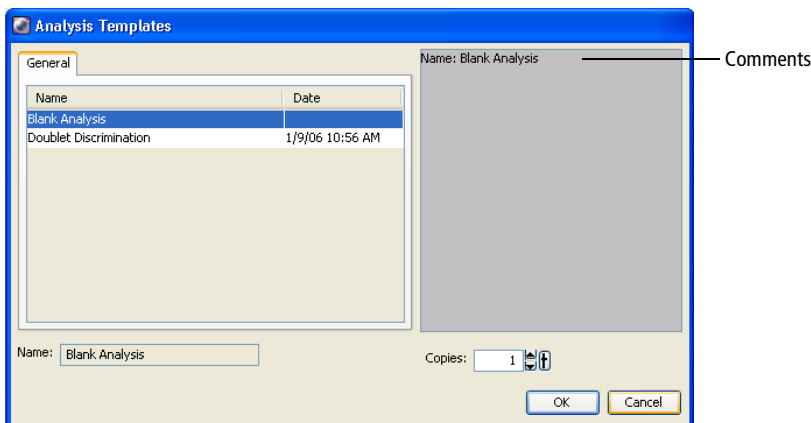
3   Specify the number of copies and click **OK**.

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

The designated number of tubes is added to the Browser, with a copy of your analysis template object under each tube.

Plots, gates, and statistics views are added to the current worksheet unless the tube-specific worksheet preference is enabled. In this case, analysis objects are placed on individual worksheets for each new tube.

Analysis Objects

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

Tools for data analysis are described in more detail in Chapter 4.

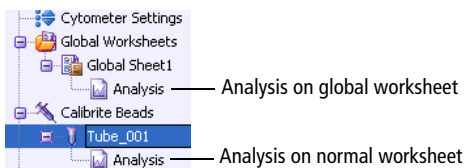An analysis can be saved as a template, or copied from any tube or worksheet in the Browser to another. When copied or saved as a template, the analysis includes all associated plots, gates, statistics views, population hierarchies, text boxes, lines, and arrows.

Saving an Analysis Template

You can create and save analysis templates for common functions such as acquisition or analysis. An analysis template can be assigned as a default worksheet or applied to one or more tubes at a time. Templates can be exported only from open experiments.

To create and save an analysis template:

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

The Export Wizard dialog opens, and displays the steps for exporting a template.
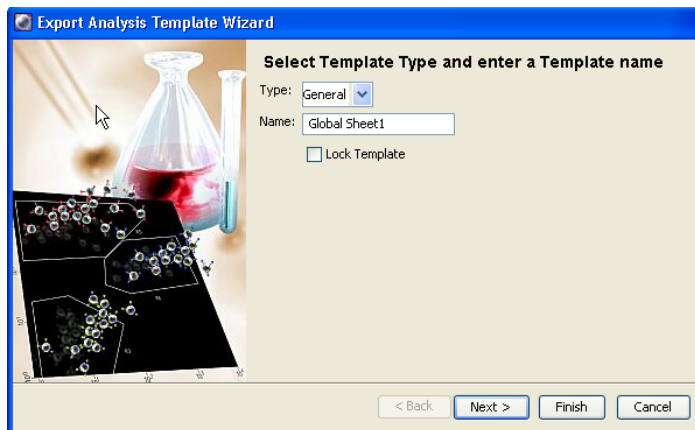
2 Enter values in this dialog as follows:

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

- **Name field**. 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.

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

3 Click **Next.**

4 (Optional) Enter comments for the analysis template.

Comments can be viewed when you are importing an analysis template. See Creating a Tube with a Predefined Analysis Template on page 79.

5   Click **Finish.**

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

Copying Analyses

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

To duplicate an existing analysis:

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

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

2   Right-click the analysis icon and select **Copy.**

Alternatively, select the icon and press Ctrl+C.

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

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

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

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

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

Keywords

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

Use keywords to do the following:

- Define a list of terms (selectable strings) to be stored with each experiment.

  Keywords are limited to 20 characters and up to 100 value choices of selectable numeric or string data types.

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

- Attach labels to data, making it easier to locate. See Using the Find Experiments Function on page 58.

- Display tube, specimen, or experiment keywords in the headers of statistics views. Keywords are exported along with the statistics.

- Share keywords with other cytometer users by adding them to the global keyword list.

Defining and Editing Keywords

Use the Inspector or the Experiment Layout to define keywords at the experiment, specimen, or tube level and to add keywords to the global keyword list so other cytometer users can access them. See Using the Keywords Tab on page 63 for more details.

To add a keyword to an experiment, specimen, or tube:

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

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

   The Keywords tab of the Experiment Layout dialog opens.

2   Click **Edit.**
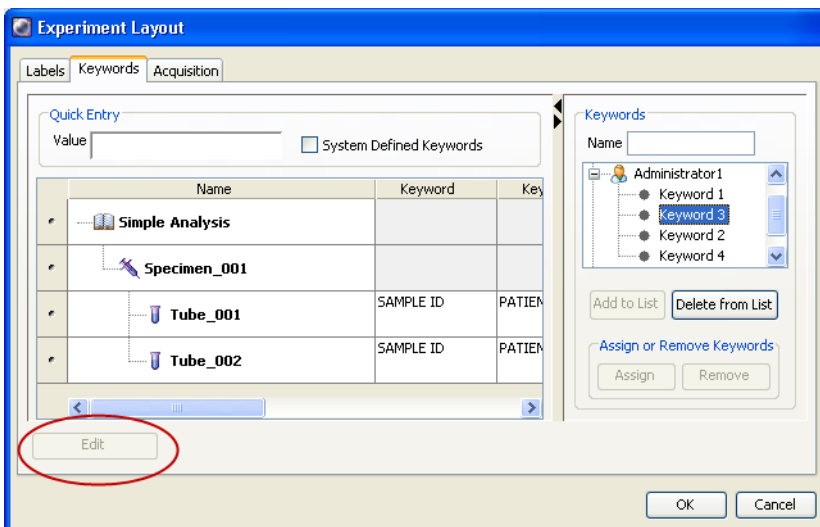
3   In the **Editing Keywords** dialog, click **Add.**

4   Name the keyword and add any required suffix.
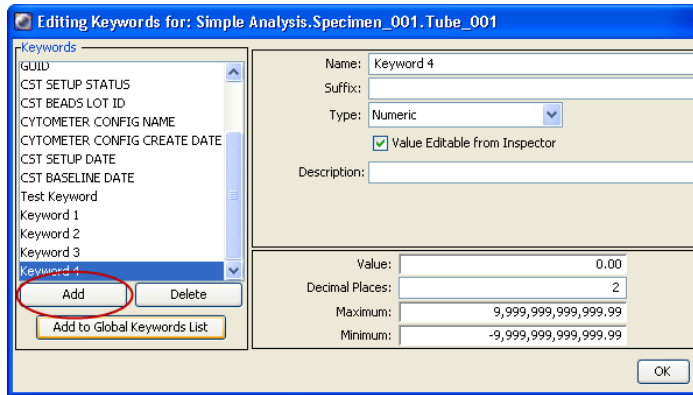
Select the generic name in the **Name** field and enter a new name. Each name must be unique. Use the suffix to define values, such as units of measure.

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

- **Numeric**. Use for keywords defined by numerical values, such as numbers of cells. Limit the range by entering minimum and maximum values. Enter a number to specify the number of digits to the right of the decimal place (maximum of 14).

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

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

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

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

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


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

6 (Optional) Use the **Description** field to enter text describing the keyword. A keyword with a description helps others to understand what the keyword is and how to use it.

7 (Optional) Select the keyword and click the **Add to Global Keywords List** button to add the keyword to the global list as displayed in the Experiment Layout. This allows other cytometer users to view and use those keywords.
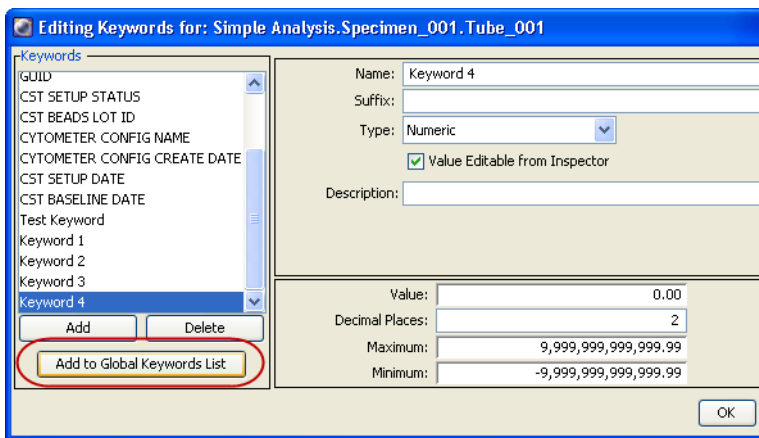
8 Click **OK** to close the **Editing Keywords** dialog.

View and maintain global keywords in the Keywords list on the right side of the dialog.
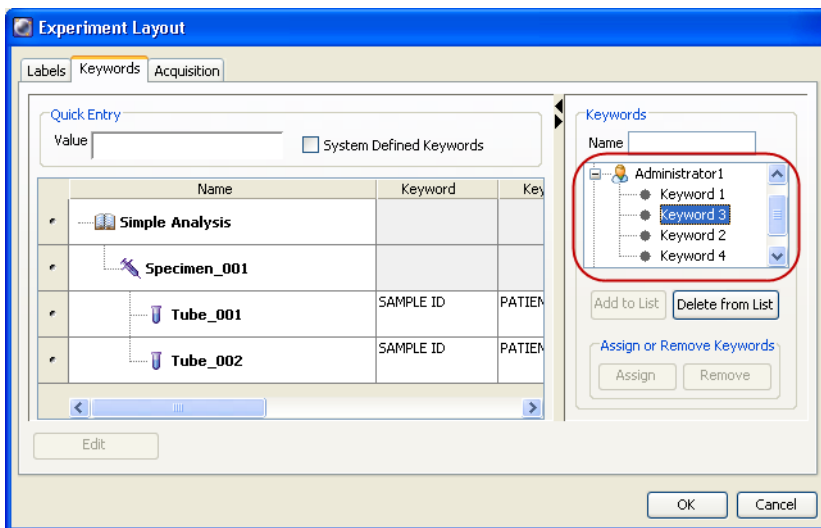
NOTE If custom keywords of the same name are defined for more than one level in an experiment hierarchy, the lower-level definition overwrites the one at a higher level.

Deleting Keywords

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

To delete a keyword:

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

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

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

User Preferences

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

The following user preferences are available:

- General Preferences on page 90

- Gate Preferences on page 92

- Worksheet Preferences on page 93

- Plot Preferences on page 96

- FCS Preferences on page 97

- Template Preferences on page 100

- Statistics Preferences on page 101

- Biexponential Preferences on page 103

- Carousel Preferences on page 103 (cytometer specific)

General Preferences

General preferences apply to worksheets, acquisition controls, and cytometer settings.
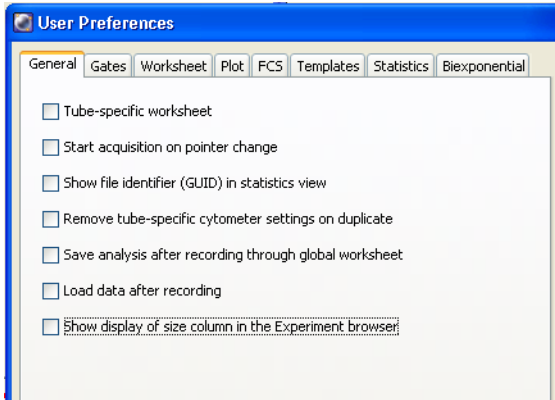
The checkboxes in the dialog are used as follows:

- **Tube-specific worksheet**. When selected (checked), a separate normal worksheet is automatically created for each tube when you do one of the following:

  - Duplicate the tube without data. (See Using the Duplicate without Data Function on page 50.)

  - Click the **Next** button in the **Acquisition Dashboard**.

  - Enable the **Save analysis after recording through global worksheet** preference.

  By default, this preference is not selected.

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

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

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

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

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

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

- **Show display of size column in the Experiment browser.** By default, this preference is not selected. You have to select the checkbox to enable this setting.

  NOTE   Enabling the display of the size column will noticeably slow down Browser performance.

Gate Preferences

Gate preferences define how populations are colored within interval and quadrant gates.
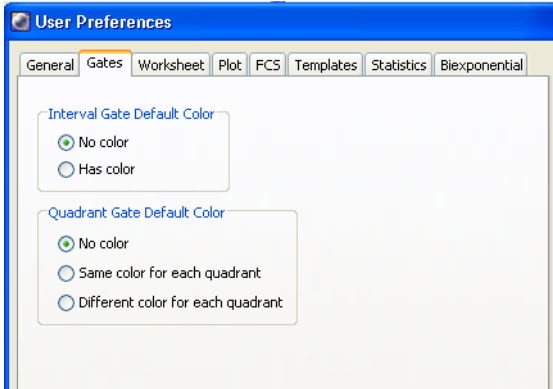
The Gates tab has the following features:

- **Interval Gate Default Color**. Select **No color** or **Has color** to specify whether populations defined by an interval gate should be assigned a color or retain the color of the parent population, respectively. By default, populations are not colored.

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

  - **No color**. No default color is specified, that is, the color is determined by the parent population.

  - **Same color for each quadrant**. All quadrants (gated populations) are assigned the same color.

  - **Different color for each quadrant**. Each quadrant (gated population) is shown in a different color.

 By default, quadrant populations are not colored.

Worksheet Preferences

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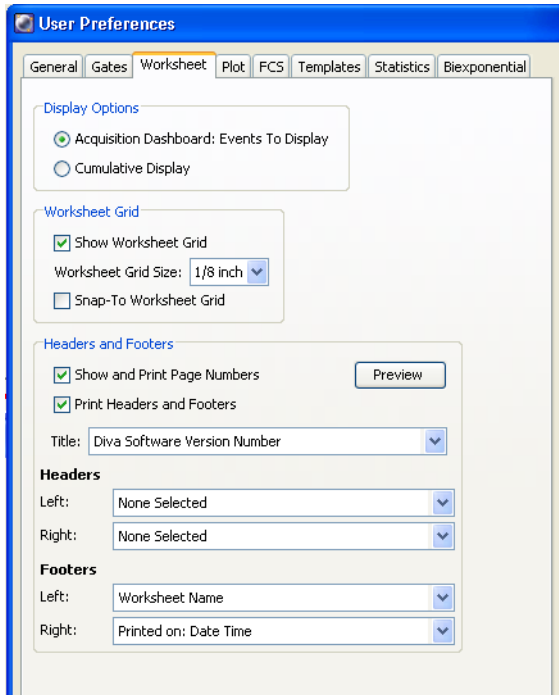
Worksheet preferences in this tab are described in the following subsections.

Display Options
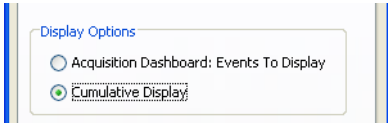
This group has the following options:

- **Acquisition Dashboard: Events to Display.** The number selected in the Events to Display field will be the number of events displayed in the plots and statistics.

  When selected, any newly created plot will remain in Acquisition Dashboard mode.

  Cumulative Display. This option enables display of all events from the beginning of acquisition or recording.

  - When selected, any new plot or statistics views you create will remain in cumulative display mode.

  - When a gate in a cumulative plot is moved or resized, the cumulative values in the display are replaced by #### until the acquisition is completed. Events acquired prior to the gate move or resize will not be lost but will display in total when acquisition is completed.

  - When a gate is deleted, all data is removed from the statistics views and population hierarchy.

  - When in cumulative display mode, the plots, statistics views, and population hierarchies will display a *C* in the upper left-hand corner during acquisition.

  This option can also be accessed from the Plot and Statistics Inspectors.

Worksheet Grid

This group has the following options:

- **Show Worksheet Grid**. Select this checkbox to have the grid displayed on the worksheets. This option is selected by default.

    If the Show Worksheet Grid checkbox is selected, a default grid size of 1/8 inch is displayed. Use the grid size menu to change the size to 1/4 inch, 1/2 inch, or 1 inch. If the Show Worksheet Grid checkbox is not selected, the grid size menu is unavailable.

- **Snap-To Worksheet Grid**. Select to make all worksheet elements that are added, moved, or resized snap to the grid. By default, this option is not selected.

    NOTE The worksheet grid does not appear in printouts or in PDF files.

Headers and Footers

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

Define worksheet title, headers, and footers by selecting from the menus or typing a custom word in the menu field. Select **None Selected** to leave blank.

Click **Preview** to see your selections displayed on a worksheet and make any changes needed. Worksheet title and header and footer information can also be entered, edited, and previewed in the Worksheet Inspector. The selections made in the Worksheet Inspector take precedence over those in User Preferences. See Using the Worksheet Inspector on page 165.

Plot Preferences

The plot preferences allow you to set the graphic parameters for your plot.

Default Background Color

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 select a new color.

Click to display the color palette.

Color palette with white selected
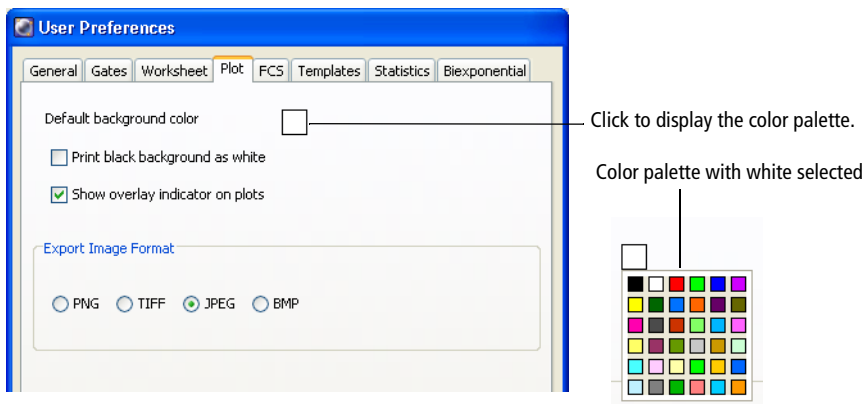
Print Black Background as White

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.
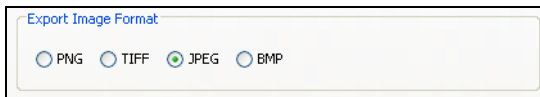
Show Overlay Indicator on Plots

If you select the checkbox to show an overlay indicator on the plot, the indicator appears as a green *O* in the top left corner. This indicates that the plot contains overlay data from another tube.
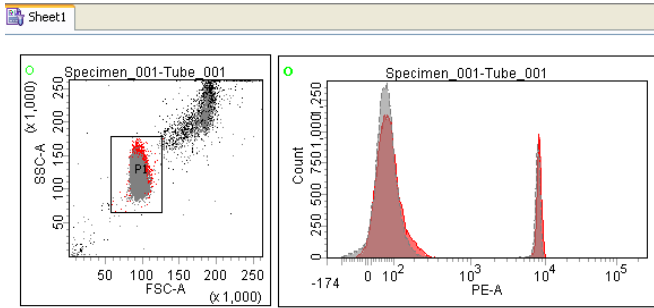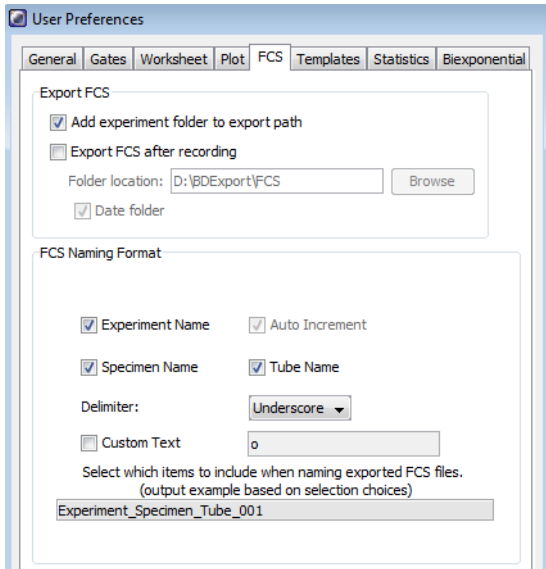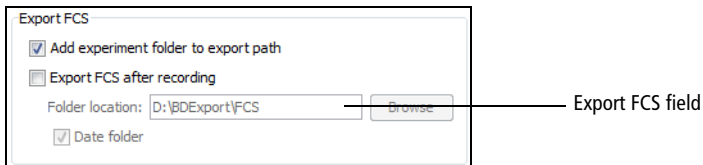
Export Image Format

When you select an export format, worksheet elements such as plots, statistics, and population hierarchies can be saved in PNG, TIFF, JPEG, and BMP image formats.

FCS Preferences

FCS preferences allow you to specify FCS file export and naming options.

FCS Export Options

Export FCS field

The Export FCS section has the following options:

- **Add experiment folder to export path.** Automatically adds the experiment folder to the same location as the FCS 3.0 file.

- **Export FCS after recording**. An FCS 3.0 file is automatically exported after each tube is recorded. For FCS 3.1 or 2.0 data, a manual export is required. See Exporting FCS Files on page 253 for more information.

- **Folder location**. Allows you to specify an export folder location by clicking the **Browse** button or by entering a folder path in the **Folder location** field.

• **Date folder.** Automatically creates a dated folder in the specified directory each day files are exported.

FCS File Names

Before exporting FCS files, you can define how the files will be named. Naming files prior to exporting prevents confusion when examining the data at a later time. The name of an FCS file can contain significant information that provides information about what is in the file without opening it.

In the FCS Naming Format section, you can specify the following FCS file names:

• **Experiment Name.** The name of the experiment is included in the FCS file name when exported.

• **Specimen Name.** The name of the specimen is included when the FCS file is exported.

• **Tube Name.** The name of the tube is included when the FCS file is exported.

• **Custom Text.** Allows you to enter a unique name as part of the FCS file name.

• **Auto Increment.** Makes file names unique while also indicating the order of FCS file processing. When processing multiple files with unique, derived names (for example, tube name), the auto increment function helps indicate

which files were exported as part of a batch process. This function is a system default that is always selected and cannot be cleared by the user.

- **Delimiter.** Select which delimiter (underscore, dash, space, comma, period, or semicolon) will separate each word within the FCS file name.

NOTE You can use these options in any combination for naming FCS files. All exported FCS files, whether by manual export, auto-export, or exported as part of an experiment, follow the defined format.

Template Preferences

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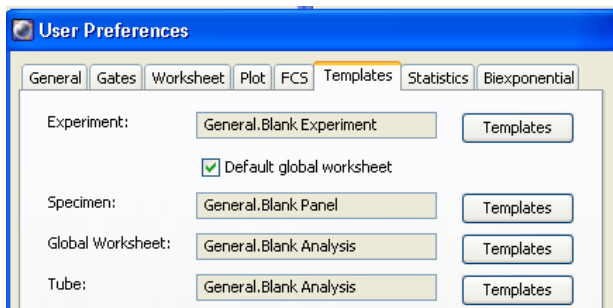

Use the options in this tab as follows:

- To assign a saved template as a default experiment, specimen, tube, or global worksheet, click the **Templates** button next to the corresponding item. Then select a saved template in the dialog that opens. 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, clear the **Default global worksheet** checkbox. This checkbox is only available for the Blank Experiment template. It is selected by default.

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

Statistics Preferences

Statistics preferences determine the format of exported statistics files. The statistics tab contains two sub-tabs: the General tab and the Export tab.
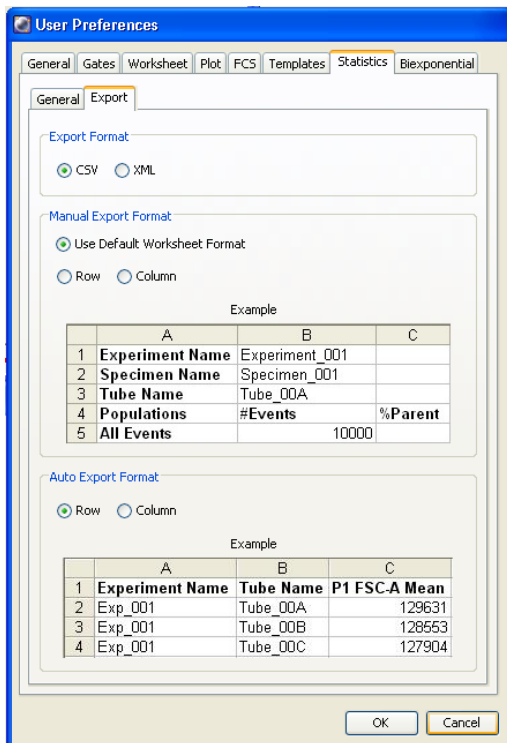
Statistics: General Tab
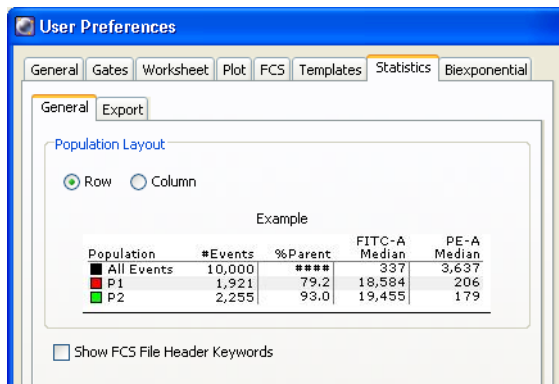
The General tab has the following options:

- **Population Layout**. You can select whether to arrange the population layout in a row or column.

- **Show FCS File Header Keywords**. Select this option to show the FCS file keywords associated with the tube in the Edit Statistics View header tab.

Statistics: Export Tab


The Export tab has the following options:

- **Export Format**. Specifies the file type of exported statistics, including those generated during carousel auto-export or batch analysis. Select either CSV format or XML format. For more information, see Exporting Statistics on page 241.

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

- **Auto Export Format**. Specifies the format of exported files when you export statistics during a batch analysis or BD FACS™ Loader carousel run (BD FACSCanto cytometers only). For more information, see Batch Analysis on page 242 or see your cytometer manual. Tubes can be exported in row or column format. For each option, an example of the exported file is shown.

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

Biexponential Preferences

The Biexponential tab enables you to configure the biexponential display of experiments in the Browser.
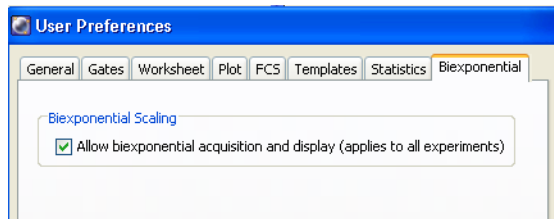

To disable biexponential display for all experiments in the Browser, disable the biexponential scaling preference. Disabling the preference allows more events to be recorded per experiment. However, large data files can be truncated when you re-enable it.

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

Carousel Preferences

If your cytometer uses a carousel and you select the carousel option upon starting the software, the Carousel tab appears under User Preferences. The Carousel tab contains two sub-tabs: the General tab and the Save Options tab.

Carousel: General Tab
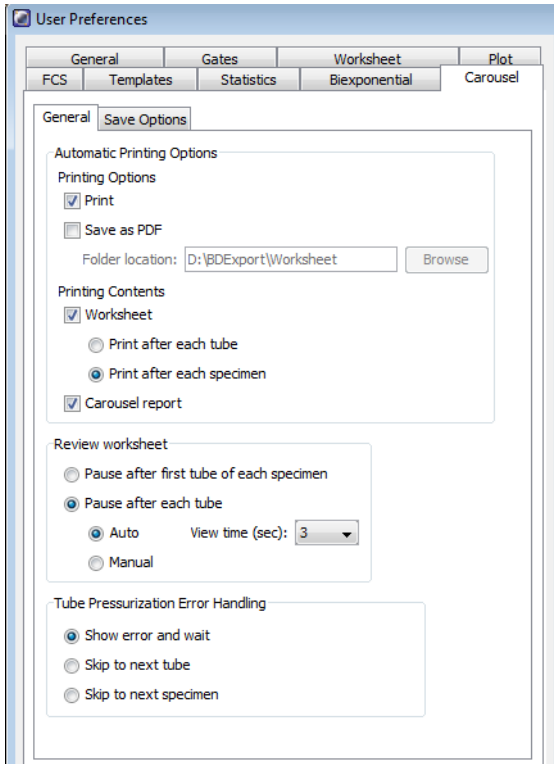
The General tab under Carousel preferences has the following options:

- **Automatic Printing Options**. Select printing options, contents, and reports.

- **Review Worksheet**. Select measurements of time (in seconds) to view each result as the data is gathered.

- **Tube Pressurization Error Handling**. Determine how the cytometer will handle tubes that display a pressurization error.

Carousel: Save Options Tab
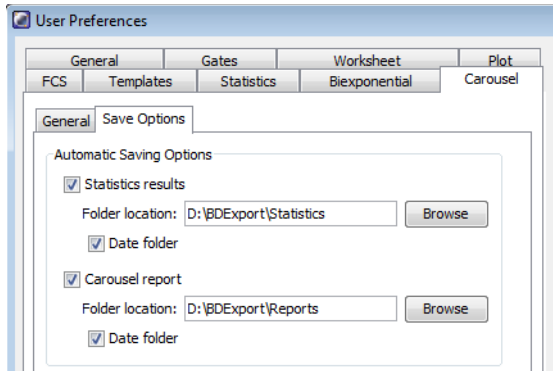
The Save Options tab under Carousel preferences has the following options:

- **Statistics results**. Select this option to save the statistics data to the folder you have indicated. You can also select the **Date folder** checkbox so that the statistics file will be saved in a folder labeled with the current date.

- **Carousel report.** Select this option to save the carousel report to the folder you have indicated. You can also select the **Date folder** checkbox so that the carousel report will be saved in a folder labeled with the current date.

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3

Cytometer and Acquisition Controls

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

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

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

The following sections contain an overview of these controls:

- Cytometer Controls on page 108

- Acquisition Dashboard on page 117

- Cytometer Settings on page 125

- Compensation Correction on page 138

Cytometer Controls

Cytometer controls are accessed from the Cytometer menu or the Cytometer window. To display the **Cytometer** window, click the **Cytometer** button on the **Workspace** toolbar or select **Cytometer** from the **View** menu.

- Use the Cytometer menu to identify the cytometer; access cytometer configurations, performance tracking information, and settings catalogs; perform cytometer setup functions; display a cytometer status report; and connect to or disconnect from the cytometer. Cytometer menu commands vary depending on the cytometer connected to your workstation.
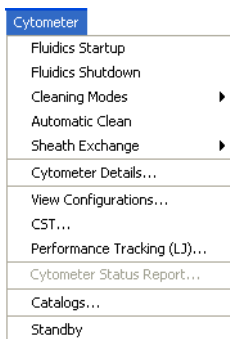
- Use the Cytometer window to view workstation connectivity status. When the software is connected to the cytometer, status messages are shown in the window. If an experiment is open and the current tube pointer is set, the window displays cytometer settings and laser controls for the current acquisition tube.

Connectivity status
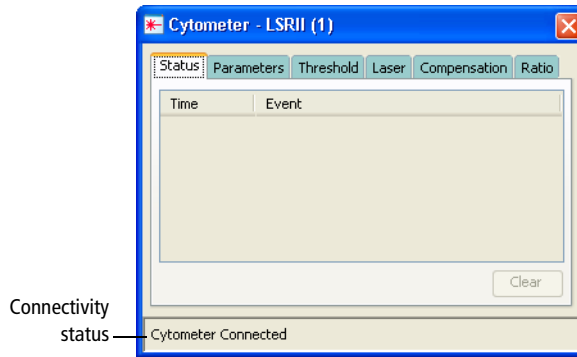
The following sections contain descriptions of Cytometer menu commands, status messages, and laser controls. For a description of cytometer settings tabs, see Cytometer Settings on page 125.

Cytometer Configurations

The physical configuration of a cytometer is the combination of lasers, detector arrays, filters, and dichroic mirrors inside the cytometer. This is referred to as the base configuration. A configuration matching your cytometer must be created within the software. This is typically done by the BD Biosciences field service engineer during installation. This base configuration serves as the template from which other configurations can be created.

Only users with administrative access can create, modify, or delete configurations. Configurations can be created for the different filter, mirror, and fluorophore combinations used in your lab. These configurations can also include other information (for example, cytometer-specific information and comments). Any user can then set the appropriate configuration for a particular experiment. Once a configuration is set, it is listed as the current configuration in the Cytometer Configuration window.

Cytometer Details

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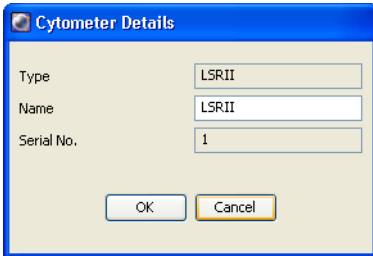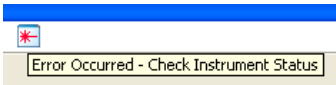

Status Messages

The Status tab of the Cytometer window lists status messages specific to your cytometer such as communication or fluidics errors. Messages are listed next to the time the event occurred. To view the whole message, resize the **Cytometer** window.
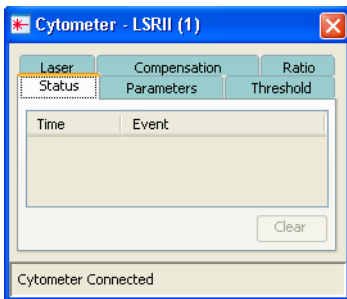
If the Status tab is hidden by another tab, it turns red to alert you when a message is sent from the cytometer. If the Status tab or Cytometer window is hidden when a message is sent, the window icon appears at the top of the workspace with a message alerting you to check the Status tab.

Error Occurred - Check Instrument Status

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

Click the **Clear** button to clear the current status messages.

Laser Controls

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

NOTE   Access to laser delay, window extension, and FSC area scaling settings are set by your administrator. See Adding Users on page 25. To adjust a setting that is disabled, consult your administrator.
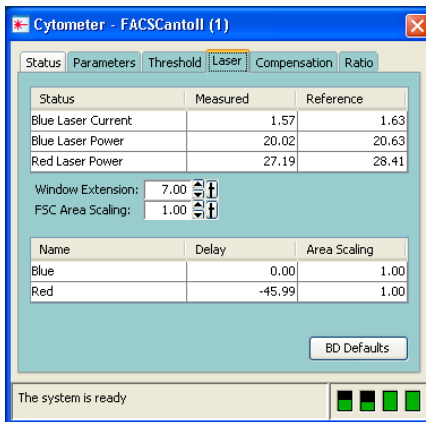
Values entered in the Laser tab apply globally to BD FACSDiva software. They are not saved with experiments or tubes. The values at startup are the last entered values.

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 75.

- **Name.** Displays the laser name.

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

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

- **Window Extension.** Extends the time in which area is measured by a value of 0–25 µsec. For more information, see See Using the Window Extension on page 114.

- **FSC Area Scaling.** Adjusts area measurements to be the same magnitude as height measurements for signals from the FSC detector.

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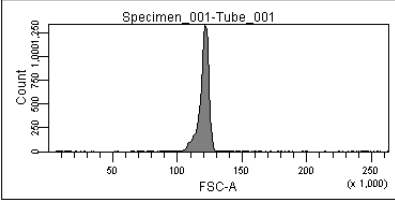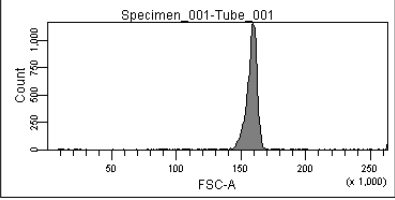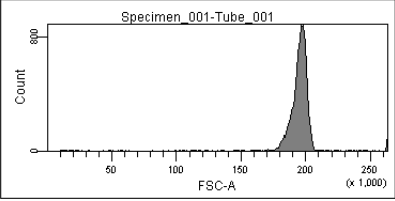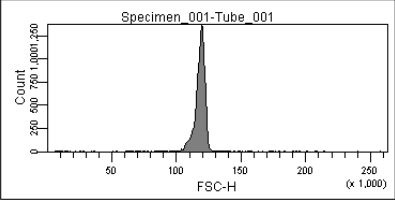
Using Area Scaling

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

While height measurements can be adjusted with voltages, area measurements can be changed by applying a scaling factor. To determine whether an adjustment is needed, area signal is usually compared to height signal for one parameter from each laser, as well as for FSC.

NOTE When FSC is detected using a photodiode, FSC area scaling might need a different area scaling factor than that applied to the other parameters for that laser.

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

Area Scaling	Sample Plot
0.75	
1.00	
1.25	
N/A	

See your cytometer manual for specific examples on when to adjust area scaling, if applicable.

NOTE   Area scaling does not change the height measurements in any way, nor does it affect the threshold.

Using the Window Extension

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

The window extension extends the detection time to allow a more complete recording of the pulse. When you increase the window extension (up to 25 µsec), half of the setting is applied to each side of the pulse so the entire pulse area is inside the gate (Figure 3-1).

NOTE   If the window extension is too wide, more noise is included and the coefficients of variation (CVs) increase. If the window is too narrow, pulses might be measured incompletely.

Figure 3-1 Setting a window extension

Threshold

Window gate

Window extension set to 0

Window gate + extension

Window extension set accurately
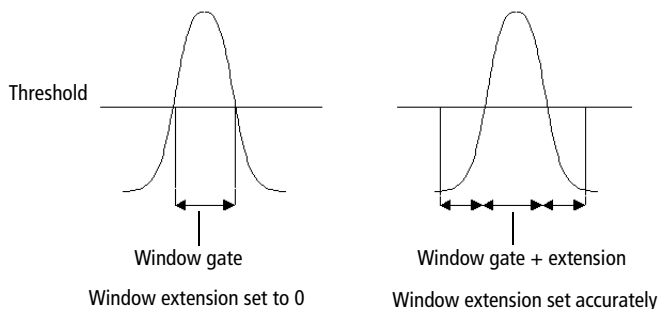
Cytometer Status Report

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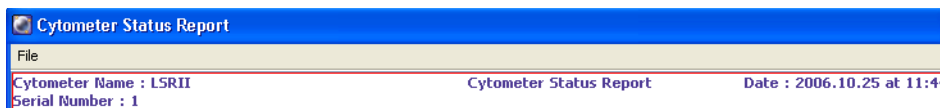
Five types of information are displayed on the report:

- User access privileges

- Cytometer information

- Cytometer settings

- Parameter labels

- Sorting settings (for cytometers equipped with sorting features)

Report Description

- The User Access Privileges section lists access settings for the current user.

- The Cytometer Info section lists values for laser delay, area scaling, window extension, and FSC area scaling. (These values are described in Laser Controls on page 111.) Some cytometers will show additional information. See your cytometer manual for details.

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

Parameters

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

Threshold

Parameter	Value
FSC	5000
Threshold Operator	Or

Spectral Overlap (%)

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

- The Sorting Settings section contains a list of all sort settings (if applicable on your cytometer). See your cytometer manual for more information.

Printing or Exporting the Cytometer Status Report

- To print the report, select **File > Print Report** in the **Cytometer Status Report** window. Preview the printed report or set up the page for printing by selecting the corresponding selections from the **File** menu.
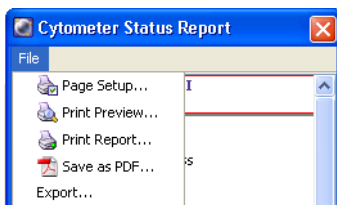
- To export the report, select **File > Export** in the **Cytometer Status Report** window. A dialog opens where you can specify the file storage location and select the export format.

  A CSV file is exported and can be opened with a spreadsheet application such as Microsoft Excel.

Standby and Connect

For acquisition workstations (computer connected to a cytometer), use the **Cytometer > Standby** command to interrupt communication between the software and the cytometer. The menu command is disabled during acquisition, recording, or fluidics procedures for cytometers with software-controlled fluidics modes. For information about working offline, see Working Offline on page 246.

NOTE If your cytometer has software-controlled fluidics, you cannot operate the fluidics when the cytometer is in standby.

When in standby, select **Cytometer > Connect** to re-establish communication.
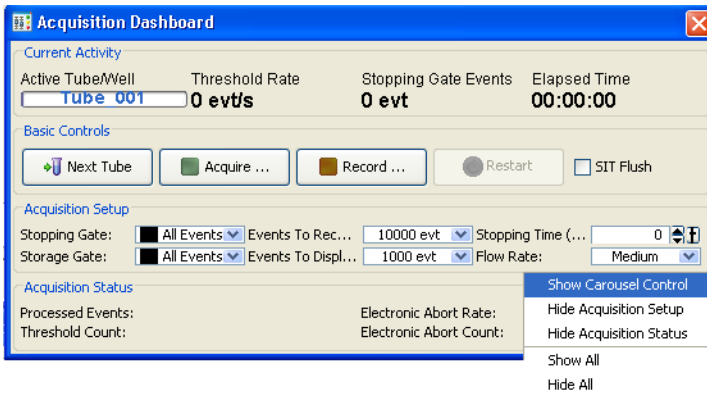
Acquisition Dashboard

The Acquisition Dashboard contains controls for setting up, starting, and monitoring data acquisition and recording. See Figure 3-2 on page 118.

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

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

Figure 3-2 Acquisition Dashboard

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

Current Activity and Basic Controls

The top of the Acquisition Dashboard displays activity for the current acquisition tube (indicated by the current tube pointer) along with acquisition controls. A progress bar appears behind the active tube name when data is being recorded.
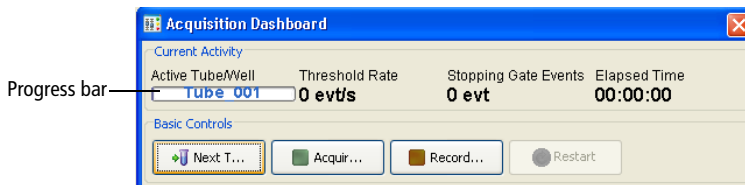
During acquisition or recording, the following are indicated:

- **Active Tube/Well name**. The current tube or well.

- **Threshold Rate**. Events per second for events that trigger the system threshold.

- **Stopping Gate Events**. Number of recorded events within the stopping gate.

- **Elapsed Time**. Amount of time passed since the **Acquire, Record, or Restart** button was clicked.

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

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

  If the tube-specific worksheet preference is selected (see General Preferences on page 90), clicking Next Tube automatically places analysis objects for the new tube onto a new blank normal worksheet.

  If the start acquisition on pointer change preference is selected, acquisition starts automatically when you click Next Tube.

- **Acquire Data**. Starts acquisition for the active tube/well. Events are displayed in plots but data is not saved to the database. Statistics are displayed in statistics views and the values are updated in accordance with the events to display setting.

  NOTE   You can also use the current tube pointer in the Browser to control acquisition and recording. See Current Tube Pointer on page 123.

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

- **Record Data**. Starts recording data for the active tube/well. The acquisition timer and all counters reset to zero when this button is clicked (except during a sort, if applicable on your cytometer). Events are recorded until the requested number of events to record has been saved or the stopping time (if entered) has elapsed, whichever comes first. The resulting data is saved in the database.

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

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

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

- **Restart.** Clears data from plots, resets the timer and counters to zero (except during a sort, if applicable on your cytometer), and restarts statistics. You can restart during acquisition or recording.

⚠ Clicking Restart during recording overwrites all previously recorded data.

Acquisition Setup

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

- **Stopping Gate**. Population for which events are to be counted.

- **Storage Gate**. Population for which events are to be recorded (saved).

- **Events to Record**. Number of events to be recorded for the current tube.

  Recording stops when the Stopping Gate Events counter reaches the entered value. If a stopping gate other than All Events is specified, recording stops when the number of events within that gate reaches the entered value. If a stopping time is entered, recording stops after the

entered number of events has been saved or the specified time has elapsed, whichever comes first.

NOTE   The number of events to record that is selected last in the Acquisition Dashboard remains in place for that experiment from one login session to another.

Use the Experiment Layout to set events to record for multiple tubes in an experiment. Also use the Experiment Layout to set storage gate, stopping gate, stopping time, and global worksheet values. See Using the Experiment Layout on page 60. You can also set events to record, storage gate, and stopping gate values in the **Acquisition** tab of the **Tube Inspector.** See Using the Tube Inspector on page 75.

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

  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.

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

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

  Do one of the following to change any value:

  - Select the value in the field and enter a new value.

  - Click the pointer on the slider bar and drag it to a new value.

- Use the mouse to click the up and down arrows or press the arrow keys on your keyboard to increase or decrease the values in small increments.

- Hold down the Ctrl key while clicking the arrows or pressing the keys to increase stepped values in increments of 10.

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

Acquisition Status

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

Acquisition Status			
Processed Events:		Electronic Abort Rate:	
Threshold Count:	58470 evt	Electronic Abort Count:	304 evt

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

  Statistics are calculated only on processed events.

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

- **Threshold Count**. Cumulative count of all events that trigger the system threshold (events that pass through the laser beam).

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

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

Current Tube Pointer

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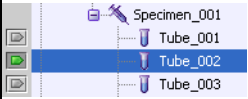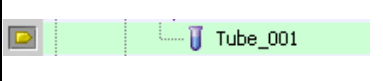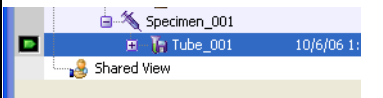
NOTE   Verify that the current tube pointer is set to the appropriate tube before you record data.

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

  NOTE   In User Preferences, you can specify that acquisition will begin automatically every time the current tube pointer is set to a new tube. See General Preferences on page 90 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.

Current Tube Pointer Appearance	Description
	A green pointer indicates the current acquisition tube (Tube_002 in this example). Events will be acquired for this tube when the pointer is clicked. Set the pointer to any other tube within the open experiment by clicking its associated gray pointer (such as next to Tube_003).
	A blue pointer indicates that the current acquisition tube is hidden within a collapsed specimen or experiment. Expand the specimen to see the current acquisition tube.
	A yellow pointer indicates that the tube is currently acquiring data.
	An orange pointer indicates that the tube is currently recording events.
	After events have been recorded, the pointer reverts to green and a disk icon is added to the tube. Cytometer settings in effect at the time the tube was recorded are saved with the tube.
	A green tube pointer with black background is displayed if the Load data after recording checkbox is cleared in User Preferences. Setting the current tube pointer changes the background to gray and loads the data.

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

Cytometer Settings

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

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

Figure 3-3 Viewing cytometer settings

Cytometer window: Cytometer settings editor

Inspector: Cytometer settings report
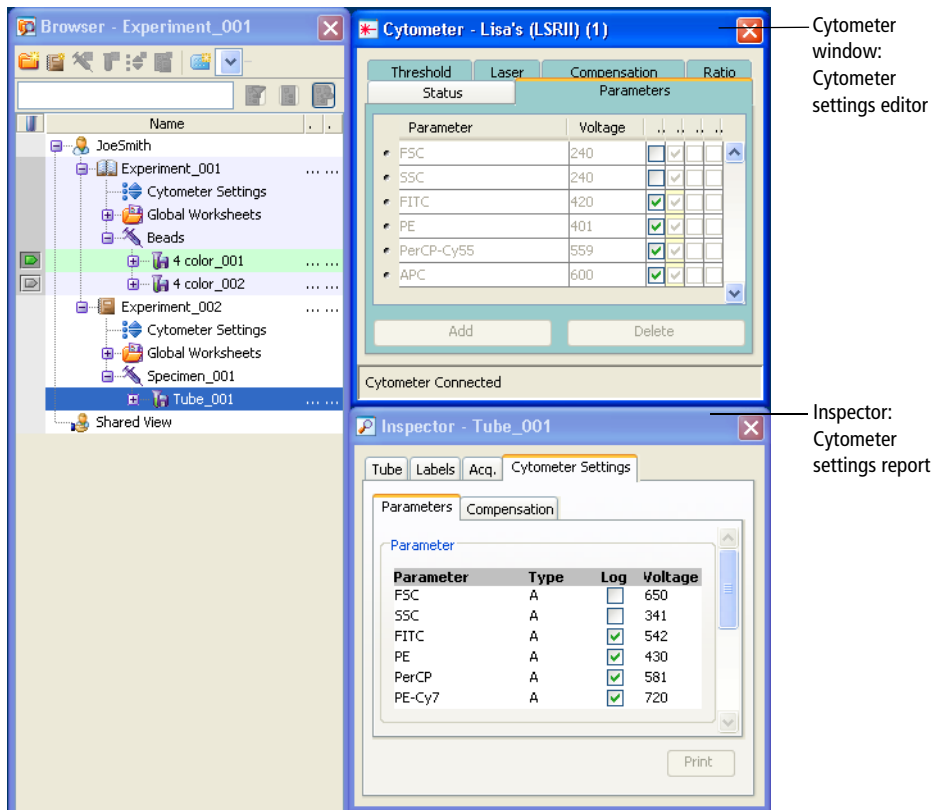
See the following sections for information about cytometer settings:

- Adjusting Cytometer Settings on page 126

- Creating Specimen- or Tube-Specific Settings on page 132

- Using Global Cytometer Settings on page 133

- Printing Cytometer Settings on page 136

- Exporting Cytometer Settings on page 137

Adjusting Cytometer Settings

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

To adjust a setting, select the field containing the value you want to change. Software controls, consisting of up and down arrows and a slider bar, appear next to the value as shown in Figure 3-4.

Figure 3-4 Software controls for adjusting cytometer settings
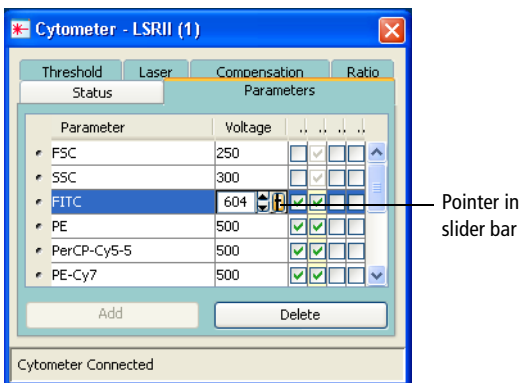
Pointer in slider bar

Do one of the following to change any value:

- Select the value in the field and enter a new value.

- Click the pointer on the slider bar and drag it to a new value.

- Use the mouse to click the up and down arrows or press the arrow keys on your keyboard to increase or decrease the values in small increments.

- Hold down the Ctrl key while clicking the arrows or pressing the keys to increase stepped values in increments of 10.

Using the Parameters Tab

Each parameter is the output of a single PMT or photodiode, measuring fluorescent or scattered light. During acquisition, parameter data is sent from the cytometer to the workstation. By default, data is recorded for the parameters listed in the current cytometer configuration (see Cytometer Controls on page 108).

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 scaling.

- Add new parameters by clicking the **Add** button.

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

- Change to an alternate fluorophore for any parameter by clicking the parameter field and selecting a different fluorophore from the menu that opens (see the following figure).
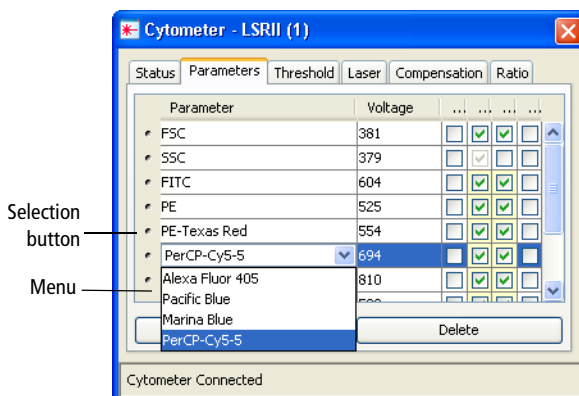
- Delete parameters by clicking the selection button next to the row to delete, and then clicking the **Delete** button. Select multiple contiguous rows by holding down the Shift key as you click. Select multiple non-contiguous rows by holding down the Ctrl key as you click.

   NOTE To reduce FCS file size and save space in the database, delete parameters that are not applicable for the corresponding tube.

- Measure signal height or width along with area by selecting the appropriate checkboxes. To measure height only, select **Height** and then clear **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.

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

   Voltages can be adjusted from 0–1,000 V. To use the controls, see Adjusting Cytometer Settings on page 126.

- 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 53.

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

Data Display

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

Linear plots have tickmarks at 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.

Using the Threshold Tab

Use the threshold tab to specify a boundary below which data will not be processed. 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 selecting a different item from the menu that appears.

Selection button — (label pointing to row in dialog)

Menu — (label below dialog)
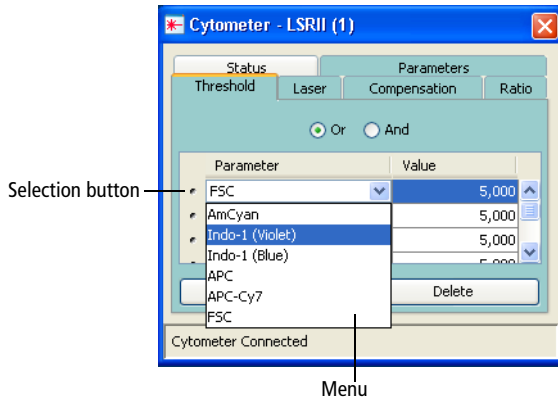
- Delete a threshold parameter by clicking the selection button next to the row to delete, then clicking the **Delete** button.

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

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

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

Using the Compensation Tab

The Compensation tab displays spectral overlap values for all parameter combinations in the experiment. For general information about compensation, see Compensation Correction on page 138.
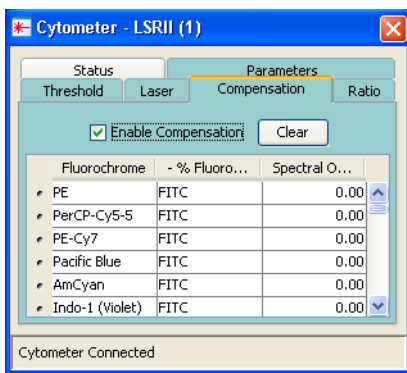
Adjust compensation in BD FACSDiva software either automatically using the compensation setup feature, or manually. When compensation is calculated using compensation setup (see Using Compensation Setup on page 139), you should not need to adjust the values after calculation.

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.**

Compensation values range from 0–1,000%. Adjustments can be made during acquisition or on previously recorded data. View or record uncompensated data by clearing the **Enable Compensation** checkbox.

Using the Ratio Tab

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

Ratios are calculated from uncompensated linear data and are always reported as linear data. 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 selecting parameters from the menus in each field. All parameters listed in the Parameters tab are available for ratio calculations.

- Adjust the ratio scaling factor by entering a value in the **Scaling %** column, from 0–200%.

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

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

Creating Specimen- or Tube-Specific Settings

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

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

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

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

  Alternatively, select **Import Cytometer Settings** to import an existing settings file. See Importing Settings on page 137.

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

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

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

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

Using Global Cytometer Settings

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

NOTE If tube- or specimen-specific settings are linked to a setup (see Applying a Compensation Setup to Cytometer Settings on page 151), experiment-level settings are not updated automatically, even when this preference is enabled.

For example, if you disable the option and make changes to tube-specific settings in the Cytometer window, the global settings in the Inspector do not change.
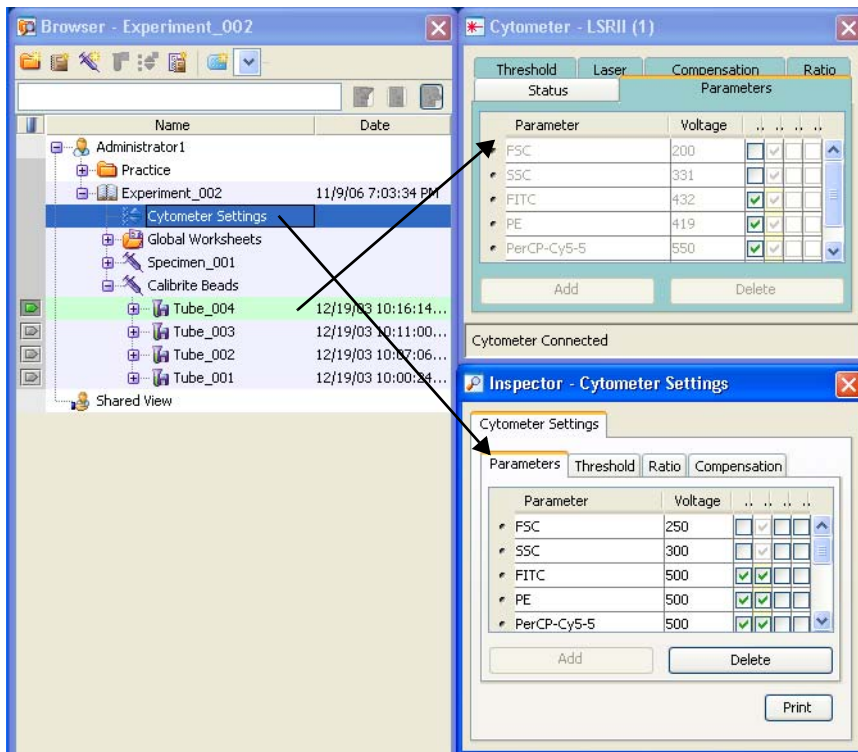
If you make the same changes to tube-specific settings with the global settings option enabled, the changes are automatically applied to the experiment-level settings.
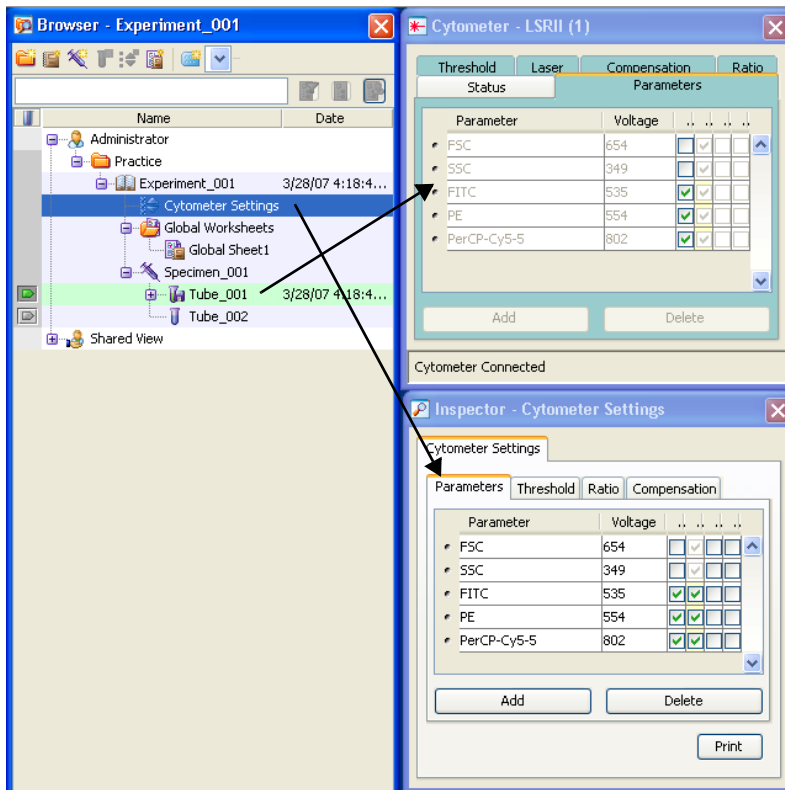
Additionally, updated settings are automatically applied to the remaining unrecorded tubes in the experiment, even when they have tube-specific settings. Updated settings are applied when you set the current tube pointer to a different tube.

Printing Cytometer Settings

To print cytometer settings for the current tube, select cytometer settings in the **Browser** and click the **Print** button in the **Inspector,** or right-click the **Cytometer Settings** icon and select **Print** from the shortcut menu.
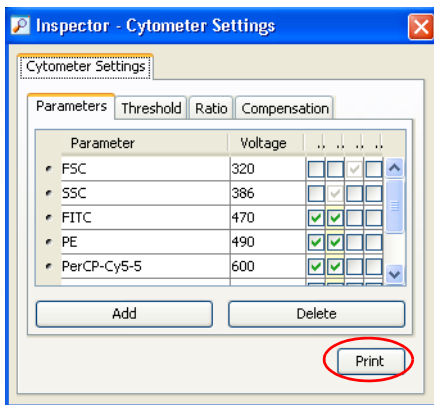
A printout will be generated that includes:

- Name of experiment, specimen, and tube

- The date data was recorded

- List of parameters collected, voltage values, and whether log is on or off

- Threshold parameter(s) and value(s)

- Compensation state (enabled/disabled) and values

- Ratio parameters and scaling

A more detailed, formatted report of cytometer settings can be printed, viewed, or exported using the Cytometer Status Report option on the Cytometer menu. See Cytometer Status Report on page 115 for more information.

Exporting Cytometer Settings

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

When stored outside the BD FACSDiva database, settings are not modified by the Use global cytometer settings feature or by updates to the setup catalog. (See Using Global Cytometer Settings on page 133 or Using Compensation Setups on page 150.) Also, you can manually edit compensation and voltage settings while keeping a copy of your original settings outside the Browser.

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

1 Right-click the **Cytometer Settings** icon in the **Browser** and select **Export**.

2 Enter a file name and click **Export**.

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

Importing Settings

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

2 Click **Yes** to overwrite the current settings.

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

The selected settings are added to the Browser.

Compensation Correction

Different fluorochromes can emit light over a common range of wavelengths. In the following example, FITC, PE, and APC all emit fluorescence in the 550–625-nm range.
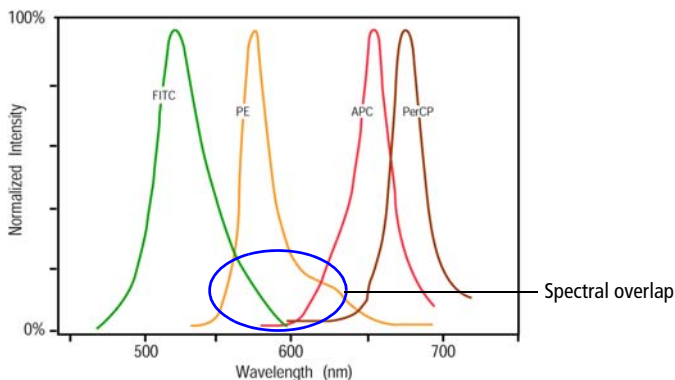
When the emission of one fluorochrome is detected by a detector designated for another fluorochrome, it is impossible to separate the two signals optically. The following example illustrates PE spillover into the APC detector.
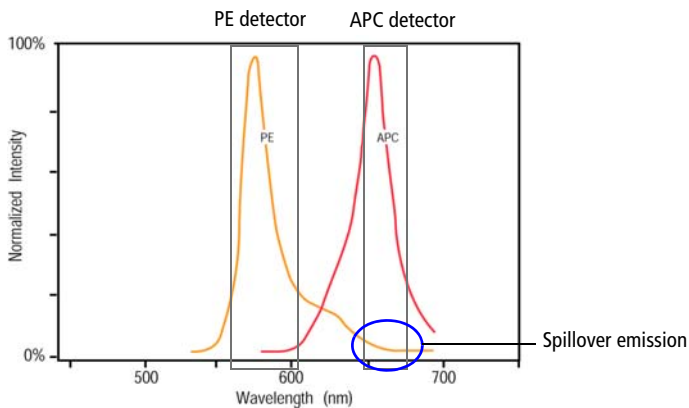
Compensation is the correction applied to the raw data to remove the effects of this spillover emission (ie, fluorescence). For example, when you are measuring

APC fluorescence, compensation removes the PE fluorescence that is detected by the APC detector, or APC–%PE.

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

Using Compensation Setup

The compensation setup function in BD FACSDiva software is designed to automatically calculate spectral overlap values for an experiment.

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

Select **Experiment > Compensation Setup** to access setup functions.

For details on each function, see the following:

- Creating Compensation Controls on page 139

- Defining Label-Specific Controls on page 141

- Calculating Compensation on page 146

- Viewing the Compensation Setup Catalog on page 147

Creating Compensation Controls

Once you have created your experiment, you are ready to create compensation controls.

Select **Experiment > Compensation Setup > Create Compensation Controls**. Alternatively, right-click the experiment icon and select **Compensation Setup > Create Compensation Controls**.

A dialog appears where you can add or delete controls, define label-specific controls, or change the order of the compensation controls (Figure 3-5 on page 140).

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

Figure 3-5 Creating compensation controls
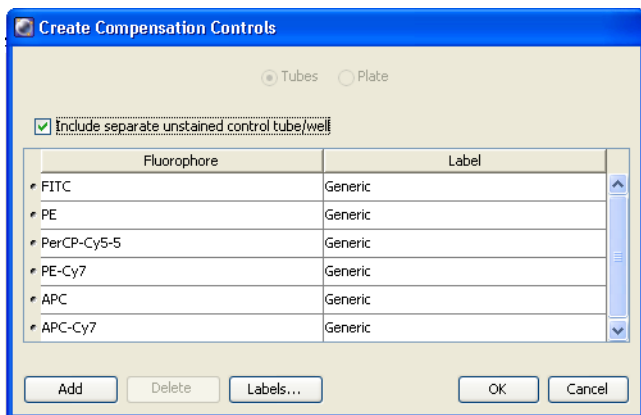
- Leave the Include separate unstained control tube/well checkbox selected when you are running unstained sample as one of your compensation controls.

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

When you click OK, a new specimen named *Compensation Controls* is added to the open experiment in the Browser, with tubes for each specified parameter. A tube-specific normal worksheet is added for each specificity of a given fluorophore, along with an unstained control worksheet if the checkbox was selected (Figure 3-6 on page 141). The experiment's cytometer settings are copied to the controls, all compensation values are set to zero, and the Enable Compensation checkbox is cleared.

These tubes and plots are used to record data prior to calculating compensation. PMTs and laser settings can be changed 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 fluorescence PMT voltage settings are changed while recording compensation tubes. All tubes must be recorded with consistent fluorescence PMT voltages.

Figure 3-6 Compensation controls and worksheets
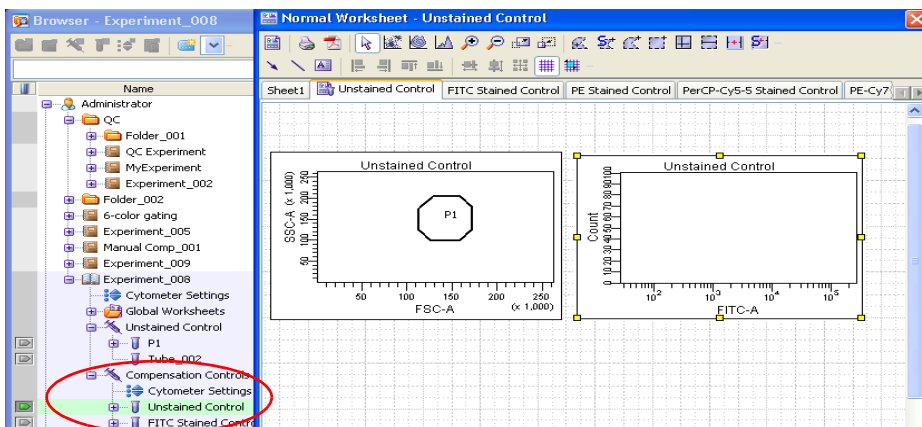
Defining Label-Specific Controls

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

1 Select **Experiment > Compensation Setup > Create Compensation Controls** or **Modify Compensation Controls.**

Alternatively, right-click the **Experiment** icon and select **Compensation Setup > Create Compensation Controls**.

The Create (or Modify) Compensation Controls dialog opens (Figure 3-7).

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

2 To add a generic control or a control with a different label, click **Add** and do the following:

a Select the appropriate fluorophore from the menu.

b Use the selection button next to the fluorophore name to drag the new fluorophore to the required position in the list.

c Enter a label.

Figure 3-7 Keeping a generic control while adding label-specific controls
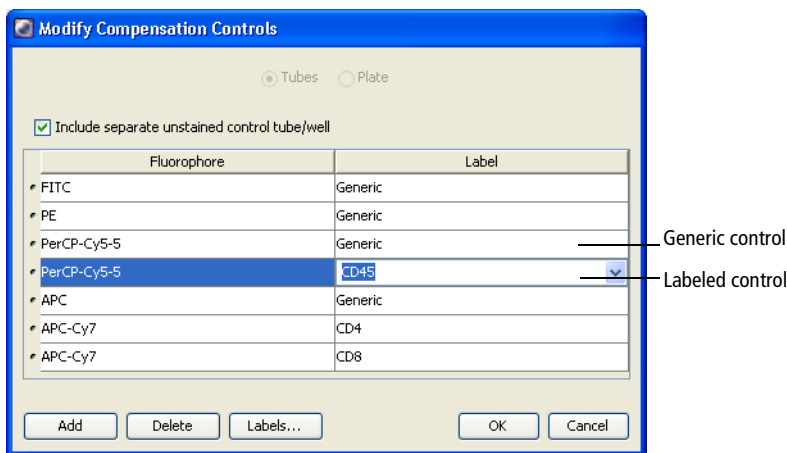
3 To edit a control, select or enter a different label.

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

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

a Click the **Labels** button in the **Create** (or **Modify**) **Compensation Controls** dialog**.**

The Labels dialog opens. The software displays a list of default labels and any other labels. Any label from this list can be added to or removed from compensation controls, whether used in an experiment or not. Use the Shift or Ctrl keys to select multiple labels at once.

b Select the appropriate fluorophore from the **Fluorophore** menu or select it from the right pane.
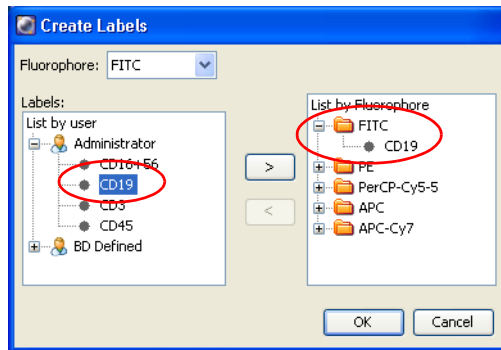
c Select the appropriate label either from the custom **BD Defined** labels list or from the labels you defined in the **Experiment Layout**.

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

e Click **OK**.

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

6 Click **OK** to add the controls to your experiment.

Label-specific controls and analysis objects are created automatically.

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

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

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

Adding Compensation Controls to an Existing Compensation Setup

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

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

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

2 Select **Experiment > Compensation Setup > Modify Compensation Controls.**

Alternatively, right-click the **Experiment** icon and select **Compensation Setup > Modify Compensation Controls**.

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

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

5 Repeat steps 3 and 4 for additional controls, if needed. Click **OK** to close the dialog.

6   Record data for the new controls.

7   Select **Experiment > Compensation Setup > Calculate Compensation.**

Alternatively, right-click the **Experiment** icon and select **Compensation Setup > Calculate Compensation**.

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

To apply compensation values to that experiment's cytometer settings, click **Apply Only**.

Recording Compensation Controls

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

1   Record data for all controls.

2   On any worksheet, 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 select **Recalculate**. Do not delete the P1 gate.

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

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

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

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

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

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

Calculating Compensation

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

If the compensation calculation is not successful, an error message displays. Make necessary adjustments, and then recalculate.

If the compensation calculation is successful, a dialog appears prompting you for the name for the compensation setup.

1   Enter a name, then click one of the following:

- **Link & Save.** Links cytometer settings to the experiment and saves the setup to the compensation setup catalog.

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

- **Cancel.** Closes the dialog.

    NOTE   Include the experiment name or date when saving to keep track of compensation setups.

2   Click **OK**.

Viewing the Compensation Setup Catalog

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

View a list of all saved compensation setups by selecting **Cytometer > Catalogs** and clicking the **Compensation Setup** tab. The tab lists all saved BD FACSDiva compensation setups, as well as compensation setups from BD FACSCanto clinical software (if applicable). BD FACSDiva compensation setups are always shown in bold. Compensation setups from BD FACSCanto software that are >24 hours old or did not pass quality control checks are shown in red with a yellow triangle. See Figure 3-8 on page 147.

Figure 3-8 Compensation Setup catalog with BD FACSDiva and BD FACSCanto setups
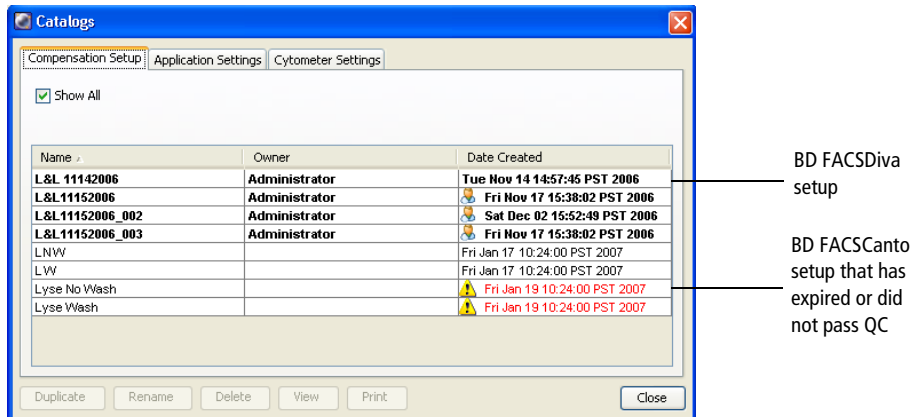
- To make a copy of any compensation setup:

  a Select the name and click **Duplicate**.

  b Enter a new name, and click **OK**.

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

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

  If the selected compensation setup is linked to any cytometer settings in the Browser, a dialog is displayed where you can select to keep or discard existing links.
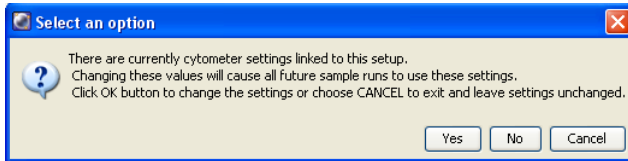
  - Click **Yes** to rename the compensation setup in all cases where it is linked (existing links are maintained).

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

  - Click **Cancel** to keep the existing name and links.

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

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

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

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

Fluorophore	Labels	A	H	W	Log	Threshold (Or)	PMT Voltage
FSC		✓	☐	☐	☐	5000	465
SSC		✓	☐	☐	☐		515
FITC	Generic	✓	☐	☐	✓		540
PE	Generic	✓	☐	☐	✓		430
PerCP-Cy5-5	Generic	✓	☐	☐	✓		586
PE-Cy7	Generic	✓	☐	☐	✓		722
Pacific Blue	Generic	✓	☐	☐	✓		406
APC	Generic	✓	☐	☐	✓		509
APC-Cy7	Generic	✓	☐	☐	✓		462
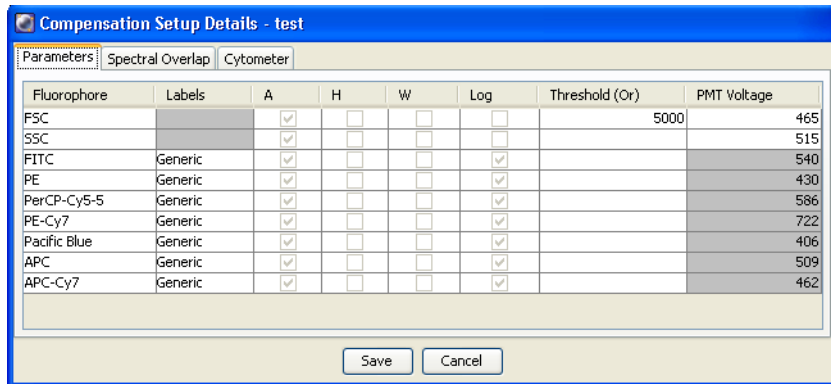
- Click the **Parameters** tab to edit the FSC and SSC voltages or threshold value.

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

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

- Click **Save** to save the edits and then **Cancel** to exit the dialog.

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

NOTE Compensation setup information can also be printed or exported by printing or exporting a cytometer status report. See Printing or Exporting the Cytometer Status Report on page 116.

Using Compensation Setups

If Link & Save is selected during compensation calculation, the experiment's cytometer settings are linked to the named setup. This is indicated by a chain-link icon in the Browser. After settings have been linked, you cannot edit spectral overlap values or change PMT voltages.

When the Use global cytometer settings preference is enabled for the experiment, the setup is applied to unrecorded tubes as the current tube pointer is set. To change voltages without triggering the error message, you can unlink from the setup. See Unlinking a Setup from Cytometer Settings on page 150.

NOTE If tube- or specimen-specific settings are linked to a setup (see Applying a Compensation Setup to Cytometer Settings on page 151), experiment-level settings are not updated automatically, even when the global cytometer settings preference is enabled.

A setup can also be applied to cytometer settings in a new experiment, as described in Applying a Compensation Setup to Cytometer Settings on page 151. When you apply a setup, if the setup contains label-specific tubes and tubes in the experiment are not labeled, you are prompted to select a compensation value, as described in Applying Label-Specific Compensation Settings to Tubes on page 152.

Unlinking a Setup from Cytometer Settings

To unlink cytometer settings from a setup:

1   Right-click a **Cytometer Settings** icon and select **Unlink From** *SetupName*.

2   Click **OK**.

Applying a Compensation Setup to Cytometer Settings

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

- To apply a setup, right-click the **Cytometer Settings** icon in the **Browser** and select **Link Setup**.

- To apply spectral overlap values, right-click a **Cytometer Settings** icon under a recorded tube and select **Apply Compensation**.

In either case, the Compensation Setup catalog opens where you select a setup to link.
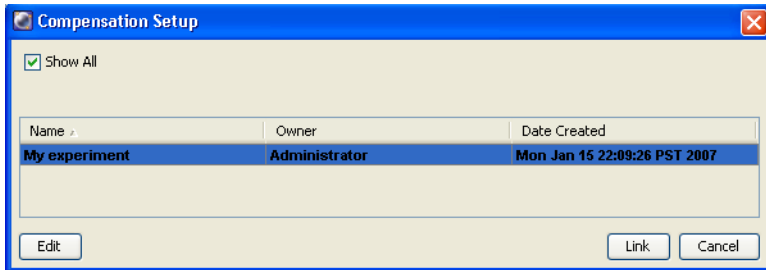
- For BD FACSDiva setups, select the setup and click **Edit** to edit values associated with the compensation setup.

- For BD FACSCanto software setups, select the setup and click **View** to view values associated with the compensation setup.

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

  If parameters do not match, the following dialog opens.

Cytometer Settings Mismatch

The following cytometer settings labels and parameters are not in the Setup: PerCP-A, AmCyan-A, Indo-1 (Violet)-A, Indo-1 (Blue)-A.
The following Setup parameters are not in the cytometer settings: PerCP-Cy5-5-A.

Click Apply to apply PMT Voltage, Threshold, and Compensation values only for matching parameters.
Click Overwrite to replace all parameters and values with those from the Setup.

[Apply] [Overwrite] [Cancel]

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

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

NOTE If the setup or cytometer settings contain label-specific controls, you are prompted to select which spectral overlap value to use. For more information, see Applying Label-Specific Compensation Settings to Tubes on page 152.

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

Applying Label-Specific Compensation Settings to Tubes

If your experiment contains label-specific compensation controls, you 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 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
	Labeled	Generic
Generic and label-specific	Not labeled	Generic
	Labeled	Label-specific, if the control label matches the parameter label. Otherwise, the generic value is applied.
Label-specific	Not labeled	Label must be selected in the dialog that opens.
	Labeled	Label-specific, if the label of the control tube matches the parameter label. Otherwise, the label must be selected in dialog that opens.

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 opens where you can select which control to apply.

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

NOTE   If you click **Cancel** and close the dialog without selecting a control, the software does not apply a complete compensation matrix, which results in uncompensated channels.

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4

Tools for Data Analysis

During analysis, recorded data is displayed in plots while gates are used to define populations of interest. BD FACSDiva software analyzes the data and calculates statistics for print or export.

This chapter provides an overview of data analysis. Application-specific examples of data analysis are found in your cytometer manual.

The following topics are covered in this chapter:

Worksheets

A worksheet is the main work area of BD FACSDiva software. It is where you create plots, define gates, show statistics views and population hierarchies, and enter custom text. You can use multiple worksheets to organize your workflow. For example, use one worksheet for sample optimization, and use a second worksheet for analysis. Two types of worksheets can be shown in the Worksheet window: normal and global.

Normal worksheets have white tabs and contain tube-specific analysis elements, while global worksheets have green tabs and contain elements that show data from any tube. You display a tube's data in a global worksheet by clicking to set the current tube pointer.

Some worksheet elements have an undo/redo function that allows you to revert back to a previous version of an edited worksheet, but only if you haven't already saved the changes. If you change your mind after undoing the edit, you can also redo the edit if you haven't already saved the change.

To display the **Worksheet** window, click the **Worksheet** button on the **Workspace** toolbar. To toggle between the normal and global worksheet view, click the **Worksheets View** button on the **Worksheet** toolbar.

Using the Worksheet Toolbar

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

Labels on the toolbar image:
- Save as PDF icon
- Print icon
- Select icon
- Plot icons
- Gate icons
- Worksheets view icon
- Worksheet icons
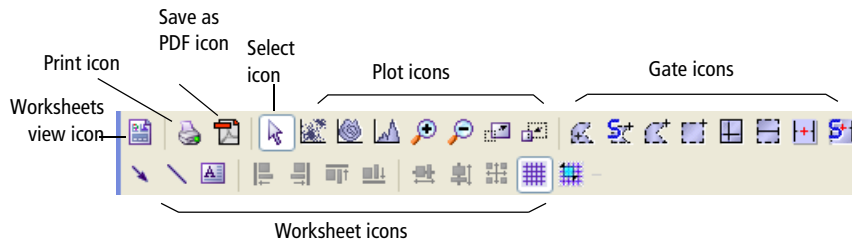
General Icons

Icon	Name	Description
	Worksheets view	Toggles the Worksheet window between the global and normal worksheet views.
	Print	Displays the Print dialog to print the worksheet.
	Save as PDF	Saves the worksheet as a PDF file. This button is displayed when there is an open experiment.
	Select	Selects (by clicking), moves (by dragging), or resizes (by dragging a selection handle of) objects in a worksheet.

Plot Icons

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

Icon	Name	Description
D C H	Plot	Create dot (D), contour (C), or histogram (H) plots. For a normal worksheet, select a tube, click a plot button, and then click in the worksheet to create a plot of a default size. For a global worksheet, click a plot button and click in the worksheet. For other options, see Creating Plots on page 177.
	Zoom-In	Magnifies an area of a plot. Click the button, click in the plot, and drag to define the area of the plot to be enlarged. The tickmarks on the axes of the plot adjust to reflect the magnified view. A zoomed plot has a magnifying icon in its lower-left corner.
	Zoom-Out	Reverses the last zoom-in action. Click the button and click once in the plot. Each click with the button undoes the last zoom-in action. To return to the originally sized plot in one click, hold down the Ctrl key while clicking in the plot.
	Increase Plot Size	Increases the size of plots by two on the worksheet. Click the button and click once in a plot. The length of the x- and y-axes doubles each time you use the button.
	Decrease Plot Size	Decreases the size of plots on the worksheet. Click the button and click an increased plot. The length of the x- and y-axes halves each time you use the button. Plots cannot be resized below their original size.

Gate Icons

Use gate icons to define population subsets on plots. The Interval Gate icons are the only buttons that can be used to gate data on a histogram. For more

information, see Gates on page 207.

Icon	Name	Description
	Autopolygon Gate	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 plot.
	Snap-To Gate	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	Allows a polygonal gate to be drawn manually around a population on a dot or contour plot.
	Rectangle Gate	Creates a rectangular gate around a population on a dot or contour plot.
	Quadrant Gate	Divides a plot into four quadrants. Each quadrant has its own population statistics.
	Interval Gate	Allows a range of events to be selected on a histogram or on the x-axis of a dot or contour plot.
	Autointerval Gate	Draws an interval gate automatically around a range of events on a histogram or on the x-axis of 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 Interval Gate	Draws an interval gate automatically around a range of events on a histogram or on the x-axis of a dot or contour plot. Unlike an autointerval gate, a snap-to interval gate automatically recalculates as new data is provided to the gate.

Worksheet Icons

Use Worksheet icons to customize worksheets. See Editing Worksheets on page 168 and Aligning and Resizing Worksheet Elements on page 173.

Icon	Name	Description
↘ ╲ A̲ A L T	Customize	Allows you to personalize worksheets with arrows (A), lines (L), and text (T).
⫧ ⫧ ⫧ ⫧	Align	Align selected objects in a worksheet. A minimum of two objects must be selected for the buttons to be active. Use these buttons to align worksheet elements at their left, right, top, and bottom edges, respectively, with the last-selected object (object with yellow selection handles).
⫧ ⫧	Distribute	Put the same amount of horizontal or vertical space between selected objects in a worksheet. A minimum of three objects must be selected for the buttons to be active.
⊞	Make Same Size	Makes selected objects in a worksheet the same size as the last-selected object. A minimum of two objects must be selected for the button to be active.
▦	Show Grid	Makes a grid display in a worksheet. The grid does not display in printed worksheets or in PDF files.
▦	Snap-To Grid	Makes elements in the worksheet (such as plots, statistics views, population hierarchies, text boxes, and arrows) that are added, moved, or resized, snap to the grid. This button is only active if the Show Grid button is active.

Undo and Redo Functions

BD FACSDiva software provides one level of undo and redo functionality for many actions in worksheets. The specific actions that have the undo and redo capability include:

- Creating or pasting gates

- Cutting or deleting gates

- Creating, pasting. or moving worksheet elements

- Cutting or deleting worksheet elements

- Duplicating worksheet elements

- Resizing worksheet elements

- Drilling down from a gate

- Deleting normal worksheets

The following functions are not supported by the undo and redo functions.

- Moving or resizing gates

- Duplicating plots using the Ctrl key

- Aligning plots on worksheets

Normal Worksheets

Normal worksheets display analysis elements such as plots, gates, statistics, and custom text from multiple tubes. The analysis elements are tube-specific. Once a plot or statistics view is created for a tube, an analysis object is associated with the tube in the Browser.

- To add a normal worksheet to an open experiment, switch to the normal worksheet view (white tabs) and select **Worksheet > New Worksheet**. A maximum of 30 worksheets is allowed. By default, new worksheets are sized to fill the open window.

- To expand the size of a worksheet, use the Worksheet Inspector to increase the number of pages. A worksheet can have up to 250 pages. Page breaks are indicated by a dotted line when the Show Page Breaks option is selected. See Using the Worksheet Inspector on page 165.

- To find a tube-specific object on a worksheet, double-click its associated tube in the **Browser**. The first object associated with the tube is displayed at the top of the Worksheet window. (Alternatively, double-click any worksheet element to locate the corresponding tube in the **Browser**.)

- To delete a worksheet, click the worksheet tab and select **Worksheet > Delete Worksheet**. A worksheet is automatically deleted if its analysis objects are deleted.

  To undo deletion of a normal worksheet, select **Edit > Undo Delete Worksheet.**

Switch between multiple worksheets by clicking the tabs at the top of the worksheets (see the following figure). You can work within only one worksheet, the active worksheet, at a time. The active worksheet is indicated by an icon next to the worksheet name.
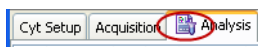
NOTE   While there is no impact on data collection or cytometer performance, responsiveness can decline as more plots, statistics, gates, and events are displayed for each tube. To improve system response time, limit the number of plots displayed in the viewable area of the Worksheet window.

Global Worksheets

Global worksheets allow you to create plots, gate, and statistics for acquiring or recording data from a set of tubes. Analysis objects are part of the global worksheet, and are not tied to a specific tube. Display data for any tube by clicking its current tube pointer. By default, each time a new experiment is created, a global worksheet is created.

A maximum of 50 global worksheets can be set up for each experiment, and each global worksheet can contain up to 10 pages. When the first global worksheet is added to an experiment, a Global Worksheets folder is created in which all global worksheets for that experiment are stored. Global worksheets are displayed in this folder in the order they are created.
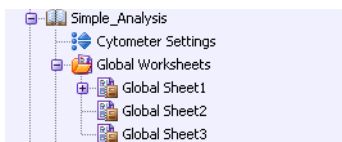
All data analysis and worksheet tools can be used on both normal and global worksheets. To move objects between worksheets, use the copy and paste functions. Objects cannot be dragged.

NOTE   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.

Differentiate a normal worksheet from a global worksheet by the color and title of the worksheet tabs. Normal worksheets are titled *SheetN* by default, and they have white tabs. Global worksheets are titled *Global SheetN*, and have green tabs.

Create a global worksheet in any of the following ways:

- Click the **New Global Worksheet** button on the **Browser** toolbar.

- Select **Experiment > New Global Worksheet.**

- Right-click an open experiment or a **Global Worksheets** folder and select **New Global Worksheet.**

Analysis objects on global worksheets derive their titles and headers from the current tube. Sort layouts (if applicable on your cytometer) 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 your cytometer manual.

1   Create a global worksheet and generate all required plots.

2   Set the current tube pointer to the first tube for which you are acquiring data.

3   Start acquisition or recording.

Data appears on the global worksheet.

4   Create gates, statistics views, and population hierarchies as needed.

5   Set the current tube pointer to the next tube and acquire or record data.

Data appears 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 appear grayed out (the missing parameter is crossed out).
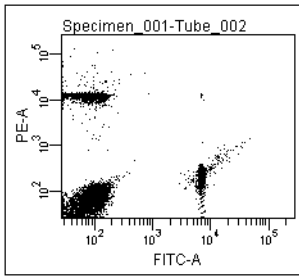
Plot displaying all parameters   Plot with missing parameters
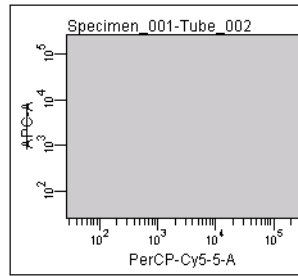
6 Edit the gates to reflect the data from the second tube.

Once edited, the gates remain as edited, even if you return to the first tube by moving the current tube pointer. Gates are global and attached to the global worksheet, not to the tube.

To save the analysis with a normal worksheet, copy the analysis object to a tube.

You can also enable the user preference to automatically save a copy after recording. See General Preferences on page 90.

Using the Worksheet Inspector

Use the Worksheet Inspector to name a worksheet or global worksheet, increase the number of pages in the worksheet, and hide or show page breaks and page numbers. Also use the Worksheet Inspector to show headers and footers and define what elements headers and footers will display. In the Worksheet Grid tab, select to show or hide the grid, define the spacing of the grid bars, and enable or disable the snap-to grid option.

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

Using the General Tab
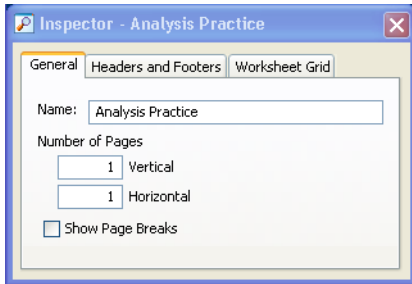
- To change the name of the worksheet, select the text in the **Name** field, enter the new name, and press **Enter.**

- To add pages to a worksheet, change the values in the **Number of Pages** fields. Additional pages are added in the vertical or horizontal direction, to a maximum of 250 normal worksheets or 50 global worksheets. (The product of horizontal x vertical cannot exceed 250 or 50, respectively.)

- To hide page breaks, clear the **Show Page Breaks** checkbox.

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

Using the Headers and Footers Tab
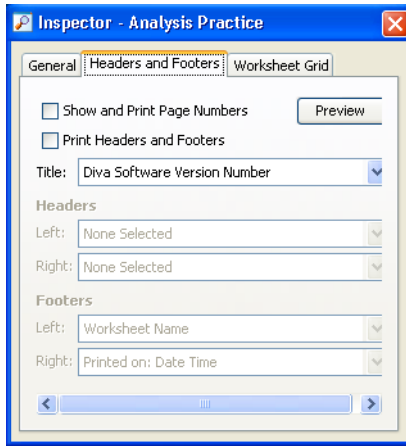
- To show page numbers, select the **Show and Print Page Numbers** checkbox. Pages are numbered in the order in which they are printed and numbers appear on each printed page.

- To print headers and footers, select the **Print Headers and Footers** checkbox.

- For the worksheet title, select from the choices in the menu or type a custom title. To leave the title blank, select **None Selected.**

- Under **Headers and Footers,** select what you want to display on the left and right sides of the worksheet page from the menus, or type a custom header or footer. To leave the headers or footers blank, select **None Selected.**

  Click the **Preview** button to see the selections made. Worksheet grid, title, and header and footer information can be entered, changed, and previewed in User Preferences. However, selections made in the Worksheet Inspector take precedence over selections in User Preferences**.** See Headers and Footers on page 95.

Using the Worksheet Grid Tab
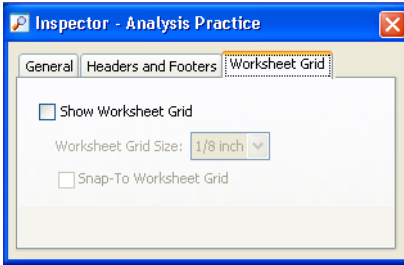
- To show the grid on the worksheet, select the **Show Worksheet Grid** checkbox. By default, this checkbox is selected. To change the spacing between bars of the worksheet grid, select a value from the **Worksheet Grid Size** menu.

- To make elements in the worksheet that are added, moved, or resized snap to the worksheet grid, select the **Snap-To Worksheet Grid** checkbox. By default, this checkbox is not selected.

- Another way to select the worksheet grid or the snap-to grid is by using the Worksheet menu in the menu bar at the top of the workspace.

Editing Worksheets

Worksheets can display plots, gates, population hierarchies, and statistics views. Additionally, worksheets can be customized with lines, arrows, and text.

Worksheet elements have an undo/redo function that allows you to revert back to a previous version of an edited worksheet if you haven't already saved the changes. If you change your mind and haven't already saved your changes, you can redo the edit.

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** button or **Arrow** button and click in the worksheet. Use the **Inspector** to change the properties of the line or arrow.

The appropriate Inspector is displayed when the line or arrow is selected in the worksheet. A selected object is highlighted in yellow (Figure 4-1).

Figure 4-1 Rule Inspector for selected line (left) and Arrow Inspector for selected arrow (right)
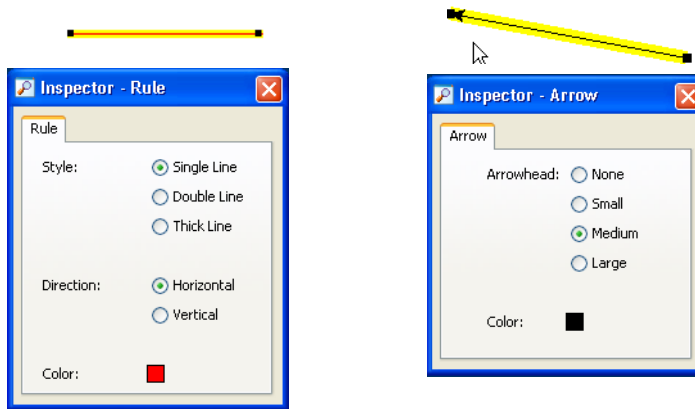
- Specify the line style, direction, and color by making appropriate selections in the **Rule Inspector.** Resize a line on the worksheet by dragging one of the black handles on either end of the selected line. Move the line by selecting the line and dragging.

- Change the size of the arrowhead and the color of the arrow in the **Arrow Inspector.** Change the length or angle of the arrow by dragging one of the black handles on either end of the selected arrow. Move the arrow by selecting the arrow and dragging.

- Delete a line or arrow by selecting it, and then pressing Delete**.**

Adding Text

To insert a text box on a worksheet, click the **Text** button and click in the worksheet. Use the **Inspector** to change the text properties.

- Edit the text in a text box by selecting the current text, then entering new text. Click anywhere outside the text box to complete the entry.

- Change the text properties by making selections in the **Text Inspector** (text does not need to be selected). Changes apply to all text within the selected text box.

- For a text box with an opaque background, select the **Opaque Background** checkbox. For a transparent text box (with grid lines showing), leave this checkbox cleared.
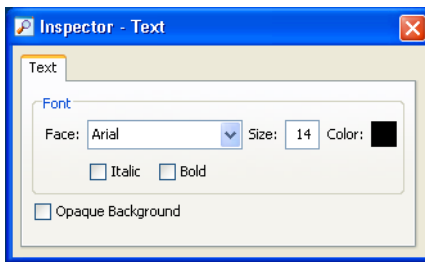
- Move a text box by selecting it and dragging the border to a new location.

- Resize a text box by selecting it and dragging one of the selection handles in or out.

- Delete a text box by selecting it and pressing Delete.

Copying Worksheet Elements

Worksheet elements such as plots, population hierarchies, and statistics views can be duplicated within a worksheet or copied to any Microsoft Office application by the following methods.

NOTE You must scroll through the entire worksheet to view all of the elements before copying and pasting.

- Hold down the Ctrl key and drag one or more worksheet elements within a worksheet to an open Microsoft Office document. The elements are duplicated when the mouse button is released.

- Press Ctrl+C to copy elements and press Ctrl+V to paste them in a new location, such as on another worksheet or to any Microsoft Office document.

- To create duplicates of plots, right-click the plots and select **Duplicate,** or select the plots and press Ctrl+D.

Exporting Worksheet Elements

Plots or other worksheet elements can be exported for use in other applications. Prior to exporting worksheet elements as images, you can define the file type for export in User Preferences. These worksheet elements can be saved as PNG, TIFF, JPEG, and BMP file types. For more information, see Export Image Format on page 97.

Worksheet elements can also be exported as XML files. In addition, file names can display a prefix useful for identifying a group of elements as part of a specific worksheet (for example, 3-color).

1 Select one or more worksheet elements.

2 Select **File > Export > Worksheet Elements.**

3 Specify the Directory Path (folder) where the elements will be stored. By default, elements are stored in D:\BDExport\Worksheet.

Click **Browse** to select a different folder than the default.

4   Select the **Filename Prefix** checkbox and enter text.

The prefix is displayed at the beginning of each element file name that is exported.

5   Select the **Include XML File** checkbox to export the worksheet in XML format.

The worksheet file includes all the selected elements. The prefix that was entered is displayed as the XML worksheet name.

6   Click **Export Worksheet Elements.**

On export, if an XML file of the same name already exists, a dialog opens with the option to overwrite the file, append (add the file using the next sequential number to name it), or to cancel the export.

Each selected element will be stored as a separate file of the type you selected. File names are determined by the type of object selected (for example, DOTPLOT_1.jpeg, HISTOGRAM_1.jpeg, or Worksheet.xml).

NOTE   Exported elements cannot be imported back into BD FACSDiva software.

Aligning and Resizing Worksheet Elements

Use worksheet buttons to align or resize one or more elements on a worksheet.

- To move a selected object, position the cursor on the border of the object. When the cursor changes to two double-headed arrows, drag the object to move it. See Figure 4-2.

- 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 4-2). Click the **Make Same Size** button (🔲). All objects are resized to the same size as the main selected object.

Figure 4-2 Selecting multiple objects

Selection handles on selected histogram (main selected object)

Selection handles on selected dot plot

Cursor showing object can be moved
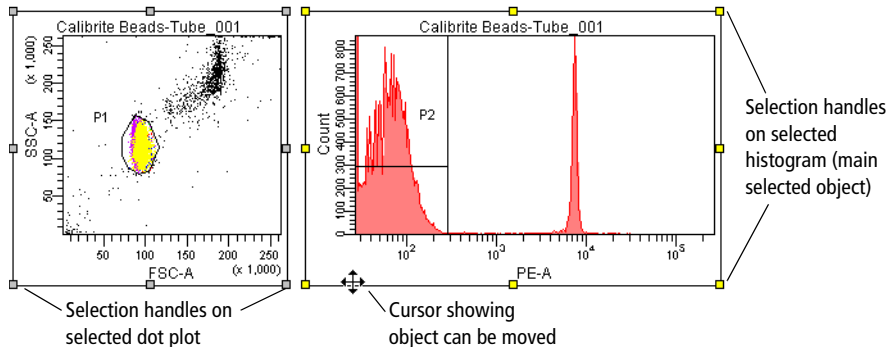
Individual selected objects can also be resized by dragging a selection handle. Position the cursor over the selection handle. When the cursor changes to a double-headed arrow, drag the border in the direction of the arrow.

- To align multiple objects, select two or more objects, and click the appropriate button (▐ ▐ ▜ ▟). Use these buttons to align to the left, right, top, and bottom edges, respectively. Objects are aligned in relation to the last selected object.

- To put equal amounts of space between objects, select three or more objects on a worksheet. Click the appropriate **Distribute** button ( ). The objects are distributed equally in a horizontal or vertical direction.

- To increase the size of a plot, click the **Increase Plot Size** button ( ) and then click a plot on the worksheet. The length of the x- and y-axes doubles each time you click the button. Click the **Decrease Plot Size** button ( ) to return the plot to its previous size. Plots cannot be reduced below their original size.

     NOTE   You can also use the Zoom In button to magnify an area in a plot.

Printing Worksheets

Designate the information to print in the worksheet headers and footers, either in the Worksheet Inspector or User Preferences. Then select one of the following commands from the File menu to set up for printing or to print worksheets.

- Select **File > Page Setup** to set the size of the printed page (for example, A4 or letter), the orientation (portrait or landscape), and the margin size. Your options will vary depending on the printer configured with your workstation.

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

- Select **File > Print Preview** to view a thumbnail of all printable pages at 10, 30, 50, or 100%. Click ❌ to return to the worksheet.

- Select **File > Print** to print the active worksheet. You can also click the printer button on the worksheet toolbar to print the active worksheet.

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

Saving Worksheets as PDF Files

To save a worksheet as a PDF file, either select **File > Save as PDF** or click the **Save as PDF** icon in the **Worksheet** toolbar.

Worksheets are saved by default in the BDExport\Worksheet folder.

To append a worksheet PDF file to an existing PDF file, select the file you want to append to in the **Save as PDF** dialog and click **Save.**
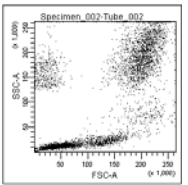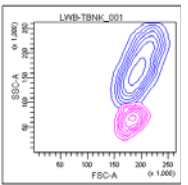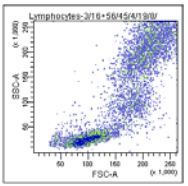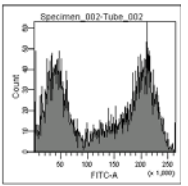
A new dialog opens where you can select **Append** (or **Overwrite** to replace the existing PDF file).

NOTE To view the worksheet you are saving as a PDF file, select the **View PDF** checkbox, then click **Save,** and the PDF is displayed.
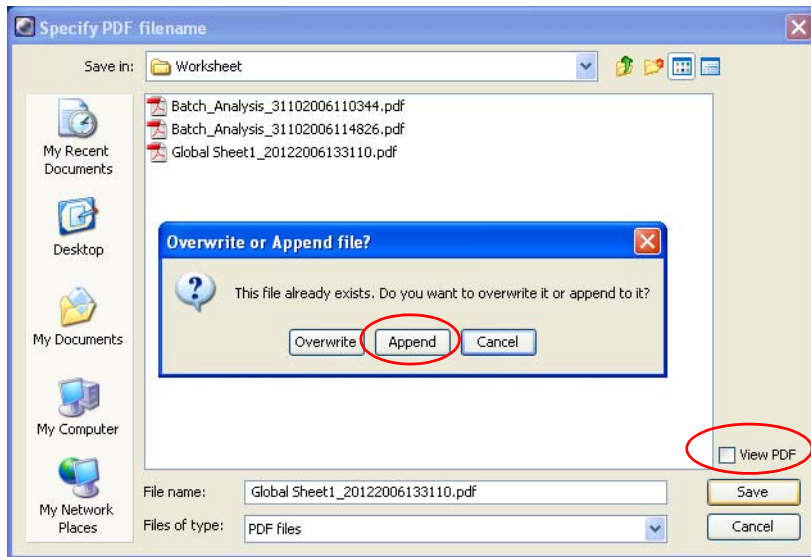
Plots

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

Plot Example	Plot Type	Description
	Dot	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	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	Graphical representation of two-parameter data where each axis displays the signal intensity of one parameter and colors indicate the number of events. Density plots are similar to dot plots, except colors are used to represent the accumulation of events (density) for events with the same signal intensity. A density plot simulates three-dimensional event display.
	Histogram	Graphical representation of single-parameter data, where the horizontal axis represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).

Creating Plots

Use menu commands or plot buttons to create dot plots, contour plots, or histograms. Note that contour plots are not available during acquisition. When you create a contour plot during acquisition, a density plot appears by default. During analysis, a contour plot appears. To create a density plot during acquisition, first create a contour plot, then use the Inspector to change the plot type to density. See Formatting Contour Plots on page 190.

• To create a plot using a plot button, click the appropriate button on the **Worksheet** toolbar, and click once on the worksheet to draw a plot of default size.

> **NOTE** To create multiple plots, double-click the **Plot** button. The button will remain selected until another button is selected, or until you press the Esc key. You can then repeatedly click in the worksheet and the same plot type will be created each time.

• To use menu commands to create a plot in a normal worksheet (normal worksheet must be active), select one or more tubes in the **Browser,** right-click and select a plot option from the menu, then click in the normal worksheet to display the plot.

• To create plots in a global worksheet (global worksheet must be active), click the icon for the global sheet that is active in the **Browser,** right-click and select a plot option from the menu, then click in the worksheet to display the plot.

Creating Gated Plots

Define the gated populations. Then use the drill-down function to create a new plot showing data from one population.

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

If the gate border is not shown, right-click the plot border, select **Show Gate,** and select the appropriate gate.

2   Select **Drill Down** from the menu.

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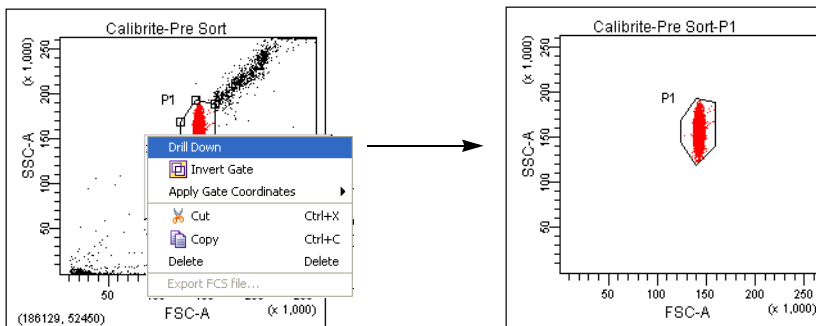
Duplicating Plots

Duplicate a plot in one of the following ways:

• While holding down the Ctrl key, drag the plot to a new location. The plot is duplicated when you release the mouse button.

• Press Ctrl+C to copy a plot and press Ctrl+V to paste it in a new location, such as on another worksheet.

• Right-click the plot border and select **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 173 or Resizing Plots on page 180.

- 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 171 or Copying Worksheet Elements on page 171.

- Duplicate a plot on the same worksheet. See Duplicating Plots on page 178.

- Change plot parameters. See Changing Plot Parameters on page 180.

- Zoom in and out on plot data, or alter plot size. See Plot Icons on page 158 or Resizing Plots on page 180.

- Change between four- and five-log decade displays for a plot. See Changing Log Display on page 181.

- Select and sequence the order of populations to display. See Selecting Populations to Display on page 182.

- Show or hide plot grids, outlines, tick marks, and tick labels. See Formatting Plots on page 184.

- Add, remove, or change plot titles and axis labels. See Editing Plot Titles and Axis Labels on page 187.

- Display biexponential scales. See Using Biexponential Display on page 201.

For each type of plot (dot, contour, density, and histogram), specific formatting and editing features are available. See Using the Plot Inspector on page 183.

Resizing Plots

To resize a plot, select it and drag one of its selection handles. See Aligning and Resizing Worksheet Elements on page 173. You can also use the **Increase Plot Size** button to double the size of a plot on a worksheet, and the **Decrease Plot Size** button to return the plot to its original size.

To increase the plot size, click the **Increase Plot Size** button ( ) and click a plot on the worksheet.

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

To decrease the plot size, click the **Decrease Plot Size** button ( ) and click the doubled plot.

The plot returns to its original size. Plots cannot be reduced below their original size.

NOTE   The undo/redo function is not available for resizing plots.

Changing Plot Parameters

To change plot parameters, click the axis label in a plot and select a parameter from the menu that appears (Figure 4-3). You can also change plot parameters using the Plot Inspector.

Figure 4-3 Changing plot parameters
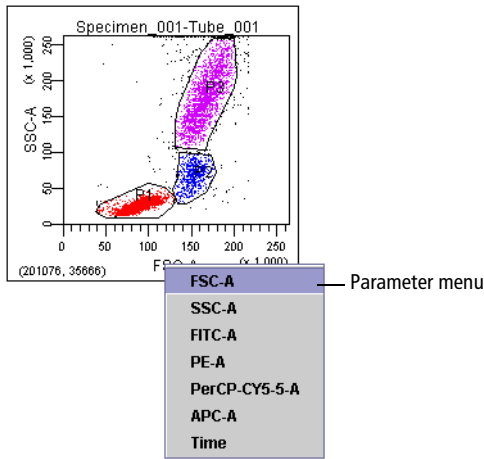
All parameters specified in the Parameters tab are available. Depending on which checkboxes are selected in the Parameters tab, parameters will be listed as *parameter*-A, *parameter*-H, or *parameter*-W.

Changing Log Display

Log data is displayed in four- or five-log decade plots. To change the display, select the experiment in the **Browser** and make the appropriate selection in the **Experiment Inspector.** Log display properties apply to all plots in the experiment.
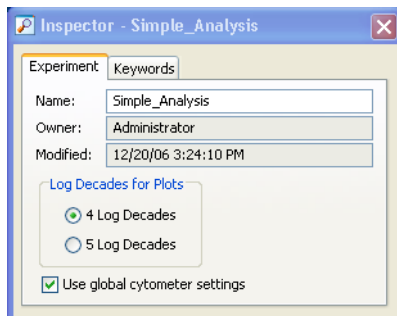
Figure 4-4 Plot displaying log data in four- and five-log decades
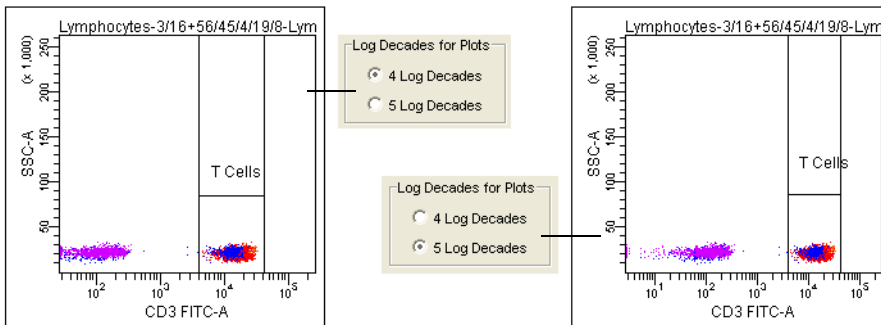
Four-log plots display values from 26–262,143. Five-log plots display values from 2.6–262,143. Thus, the first log decade ranges from 2.6–26 or 26–262, depending on the selected scale.

NOTE Select the **Grid** checkbox in the **Plot Inspector** to delineate log decades on plots.

Selecting Populations to Display

Right-click the border of any plot to access a menu where you can select from the following.

Menu selection	Description
Show Population Hierarchy	Displays the population hierarchy for the associated tube. See Using the Population Hierarchy on page 223.
Create Statistics View	Displays statistics for populations in the plot. See Statistics on page 231.
Show Populations	Allows you to select which population(s) to display in the plot. (Populations can also be selected in the Plot Inspector. See Formatting Plots on page 184.)
	If you display a population that has been assigned No Color (⊠), no events appear in the plot.

Menu selection	Description
Show Gate	Shows or hides the gate outline for selected populations in the plot. See Hiding and Showing Gates on page 219. 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.
Scale to Population	Adjusts biexponential scales to fit the selected population. See Scaling to a Population on page 205.
Duplicate	Makes a copy of the plot.
Delete	Deletes the plot.

Using the Plot Inspector

The Plot Inspector is used to format plots. Different options are displayed in the Inspector when you select one or more plots on the worksheet.

NOTE   Select multiple plots to make Inspector selections apply to all plots at once. Only options available to all selected plots are enabled.

There are four components to the Plot Inspector, accessed by clicking the tabs at the top of the Inspector: Plot, Title, Labels, and Dot Plot (or Histogram or Contour, depending on the type of plot selected).

Formatting Plots

Use the Plot tab of the Inspector to view or change plot parameters, turn on biexponential scaling, edit the plot appearance, and specify populations to show.

- Change the plot parameters by selecting a parameter from the **X Parameter** or **Y Parameter** menus (only the X parameter can be changed for histograms). All parameters specified in the Parameters tab are available. In addition, you can select time.

- Turn on biexponential scaling for the x-axis, y-axis, or both by selecting the corresponding checkbox(es). For more information, see Using Biexponential Display on page 201.

- Select the **Grid** checkbox to show gridlines at each log decade in a log plot, or to show the zero point for biexponential scaling.

  Gridlines are shown only if the Log checkbox in the Parameter tab of the Cytometer window is selected for the parameter displayed in the plot.
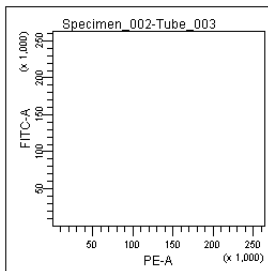
| Linear fluorescence plot | Fluorescence plot with one log parameter | Fluorescence plot with two log parameters |
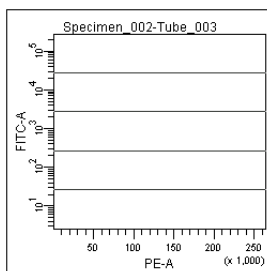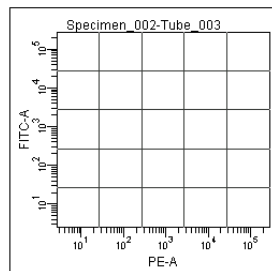
When biexponential scaling is in effect, gridlines delineate the zero point.
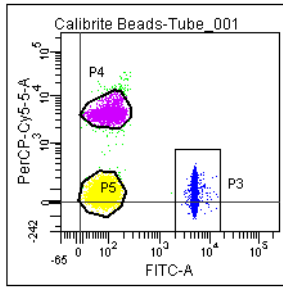
- Change the plot background color by clicking the **Background Color** box. A palette appears from which you can select a new color.

  NOTE Use **Plot Preferences** to change the default background color for all new plots. See Plot Preferences on page 96.

- Change the plot appearance by clearing checkboxes for the following:

  - **Transparent**. Makes the background transparent so that the grid lines are visible.

  - **Plot outline.** Hides the black line around the outside of a plot.

  - **Grid outline.** Hides the black outline within a plot.

  - **Tick labels.** Hides tick numbers. (To hide axis labels, click the **Labels** tab.)

  - **Tick marks.** Hides tick lines.
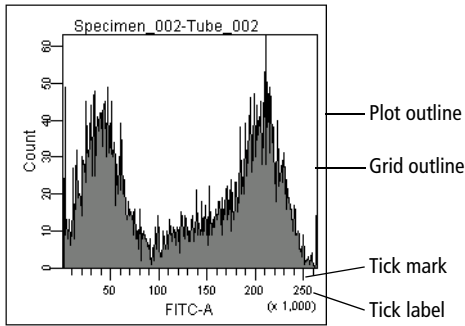
- Show a full or half grid outline by selecting the appropriate option.

Full   Half
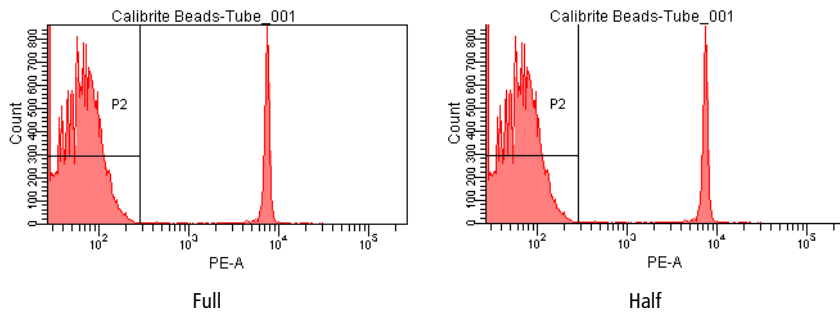
- Select the checkbox for each population to be displayed in the plot. Clear a checkbox to hide the population.

- Change the population drawing order by clearing all populations, and then reselecting them in the reverse order of how you want them displayed. Populations with a drawing order of 1 are displayed in front of populations with a higher drawing order.

Editing Plot Titles and Axis Labels

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

Figure 4-5 Inspector tabs for formatting plot titles and labels
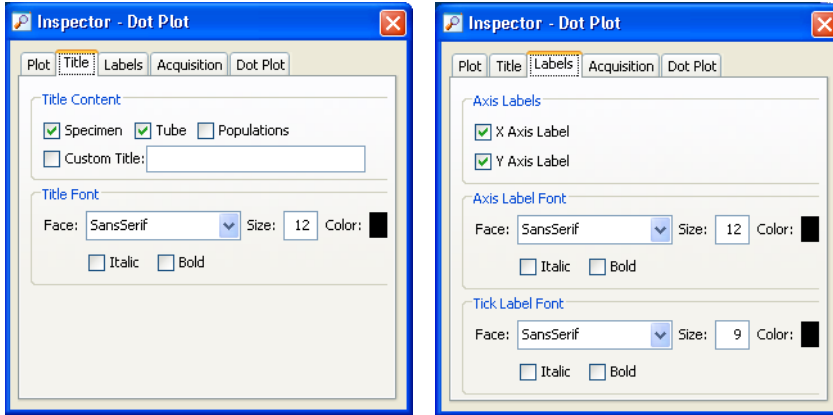
- Within the **Title** tab, select the **Title Content** checkboxes to add specimen, tube, or population names to the plot title. Each selected field will appear in the plot title separated by a hyphen (for example, Specimen Name-Tube_003).

  To create a custom title, select the **Custom Title** checkbox and enter a title.

- Within the **Labels** tab, show or hide x- and y-axis labels by selecting or clearing the appropriate checkbox.

- Within the **Title** and **Labels** tabs, format the plot title, axis labels, and tick-mark labels in the **Font** formatting boxes.

Setting Acquisition Display Options

Use the Acquisition tab of the Plot Inspector to specify the number of events to display during acquisition, on a per plot basis.

- Select the **Acquisition Dashboard: Events to Display** option to show the number of events based on the number in the **Acquisition Dashboard.** This option is selected by default.
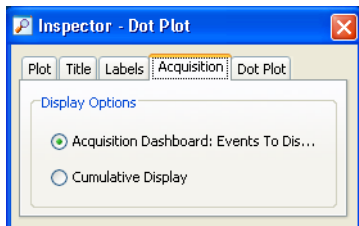
- Select the **Cumulative Display** option to show all events from the beginning of acquisition, recording, restarting, or the addition, movement, or deletion of a gate.

  Since cumulative event display only applies to events during acquisition and recording, when acquisition stops, the plot or histogram shows the number of events selected in the Acquisition Dashboard.

  NOTE If a dot plot is not visible during acquisition, the plot is not updated. So if a cumulative plot is scrolled into view, the plot displays as much data as possible, up to the last 250,000 events.

  When data in a dot plot is displayed as cumulative, there is a *C* shown in the upper left corner of the dot plot.

Using the Time Parameter

The time parameter can be used to show how events change over time. (Note that this is a research use only (RUO) function.) In calcium flux experiments, the time parameter is used to display the rate at which the cells in the sample respond to a stimulus.

When selected, the auto-scaling time parameter option automatically scales the x-axis during acquisition, maximizing the view of time parameter data when you select time as the x-axis parameter within a dot plot. The time parameter continues to change as time progresses so that the data is displayed in the dot plot. By default, this option is disabled.

You might want to enable cumulative display for plots when using the auto-scaling time parameter function. See Setting Acquisition Display Options on page 188.
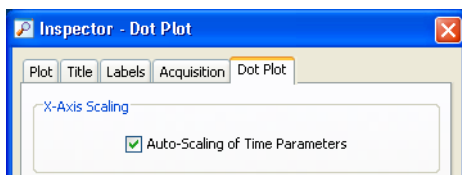
Manual zoom in and zoom out features are retained during autoscaling. The auto-scaling value is placed within the zoom history so that it can be reinstated by the user.

If manual zooming or auto-scaling have caused gates on the plot to become odd shapes or disappear, reselect the gates from the **Show Gate** menu item. See Hiding and Showing Gates on page 219.

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, and an event that appears at 60,000 is equal to 10 minutes. A plot can display up to 43 minutes of time data.

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

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.

Formatting Dot Plots for Analysis

Use the Dot Plot tab of the Inspector to specify how many events to show in the plot during analysis. Select from the following:

- Select the upper option under **Analysis Data Shown** and enter any number of events, from 1% of the total events to the total number of events acquired. When fewer than the total number of events is shown, the plot shows the last recorded events (for example, if 1,000 is selected, it will show the last 1,000 events recorded).
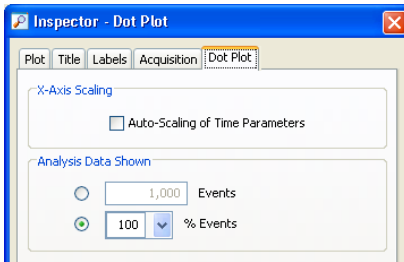
- Select the lower option and select a percentage of the total events from the menu, or enter a percentage value in the field. The percentage is determined from the total number of events (eg, displaying 25% of events for a 4,000-event file will show every fourth event, not the last 1,000 events).

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

Formatting Contour Plots

Use the Contour tab of the Plot Inspector to specify the type, scale, and appearance of the Contour plot.

NOTE During acquisition, data in a contour plot is shown in density mode, but reverts to contour data when acquisition is paused or data is recorded.

- Select a scale method from the menu. The default method is probability.
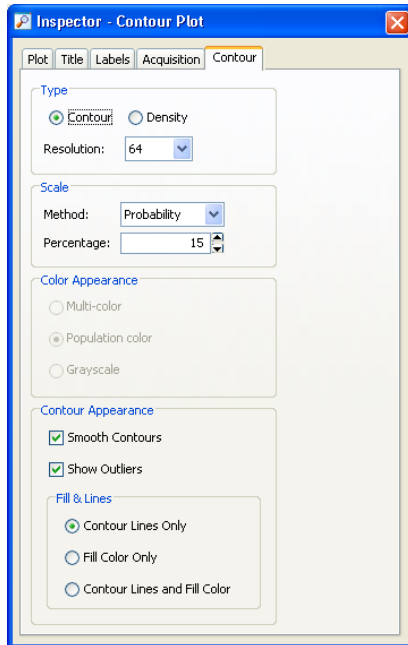
- **Probability.** Calculates contour levels as a percentage of the total event number. With this method, contour levels are not based on the maximum number of events. Instead, the area between each pair of contour lines contains an equal percentage of the total events. The starting value (the outermost contour) is half the percentage value entered. For example, with 20% probability the outermost contour represents 10% of the total number of events, the next contour represents 30%, then 50%, 70%, and 90%.

- **Linear Density.** Calculates contour levels as a percentage of the maximum event number (peak height), with equal spacing between contour lines. The starting value (the outermost contour) is half the value entered. For example, with 20% linear density, the outermost contour represents 10% of the peak height, the next contour represents 30%, then 50%, 70%, and 90%. Equal spacing tends to put most of the contour lines on the higher peaks (representing larger numbers of events) and might not show lower features.

- **Log Density.** Calculates contour levels as a percentage of the maximum event number (peak height), with logarithmic spacing between contour lines. Log-density contours begin at the innermost contour using the peak height percentage you entered, and continue until they reach a threshold value of 1. For example, with 50% log density, the innermost contour represents 50% of the peak height. Each successive contour line represents 50% of the preceding contour, so the next contour represents 25% of peak height, then 12%, 6%, 3%, and 1%. This method shows more detail in the lower regions, while still showing peak heights.

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

Figure 4-6 Contour plot at 20% probability (left), linear density (middle), log density (right)
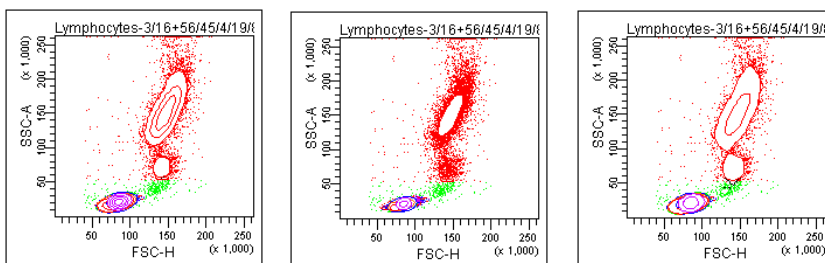
- Select the **Smooth Contours** checkbox to reduce irregularities in the contour lines. Smoothing does not affect statistics calculated for contour plots. However, when contours are smoothed, population colors can appear outside the gates that define them. Clearing the smoothing option disables other options for formatting contour plots. See Figure 4-7 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 option under **Fill & Lines** to change the look of the contour plot using contour lines only (in their population colors), fill color only, or contour lines and fill color. When fill color is used, color shading lightens as contour levels increase (Figure 4-7).

Figure 4-7 Contour plot with smoothing off (left), contour lines and fill color (right)
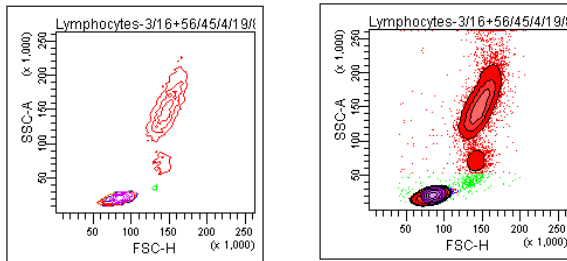
Formatting Density Plots

Select the **Density** option in the **Contour** tab of the **Plot Inspector** to change a contour plot to a density plot.
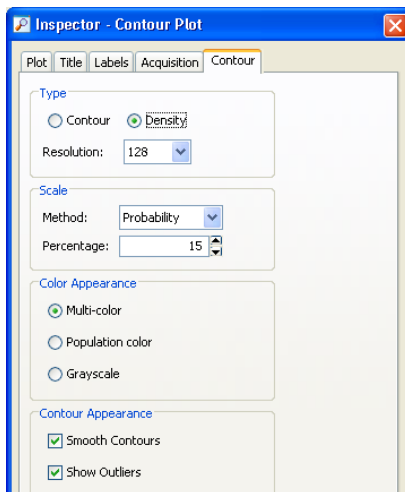
To specify the scale and appearance of the density plot:

- Select the plot resolution: from 128, 256, or 512 bins. Data from adjacent bins is added to condense higher resolution data (more bins) into the selected number of bins.

- Select a scale method from the menu. The default method is probability.

- **Probability.** Calculates density levels as a percentage of the total event number. With this method, density levels are not based on the maximum number of events. Instead, the number of events between each level contains an equal percentage of the total events. The starting level (outermost color) is half the percentage value entered. For example, with 10% probability the lowest level represents 5% of the total number of events, the next level represents 15%, then 25%, 35%, and so on.

- **Linear Density.** Calculates density levels as a percentage of the maximum event number (peak height), with equal spacing between density levels. The starting value (the lowest level) is half the value entered.

- **Log Density.** Calculates density levels as a percentage of the maximum event number (peak height), with logarithmic spacing between density levels. Log-density levels begin at the innermost contour using the peak height percentage you entered, and continue until they reach a threshold value of 1.

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

Figure 4-8 Density plots at 5% probability, linear density, and log density
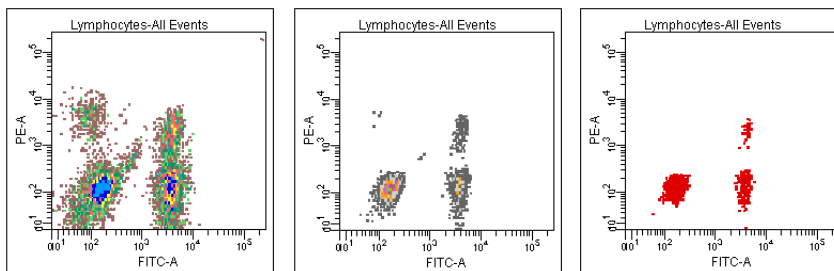
- Select an option under **Color Appearance** to display density levels in multiple colors, with a different color for each level; in the population color, where the color starts from the population color and fades to white as the levels rise; or in grayscale, where the color starts from gray and lightens to white as the levels rise (Figure 4-9).

Figure 4-9 Density plots with multicolor, population color, and grayscale appearance
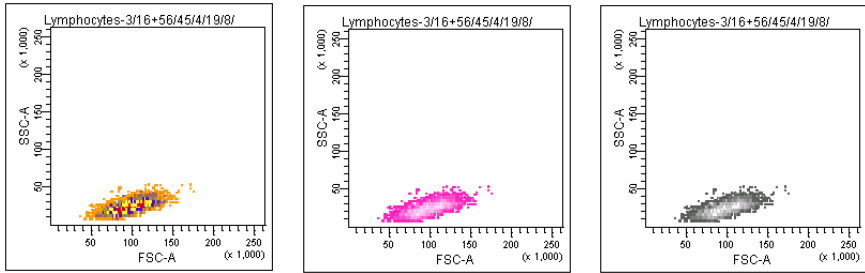
Formatting Histograms

Use the Histogram tab of the Inspector to format histograms. The y-axis scale shows either event counts or percentage of events in the histogram. For either method, set the maximum value or have it automatically calculated by the software.

- **Automatic Counts.** Select if you want the y-axis to scale automatically to the highest peak in the histogram.
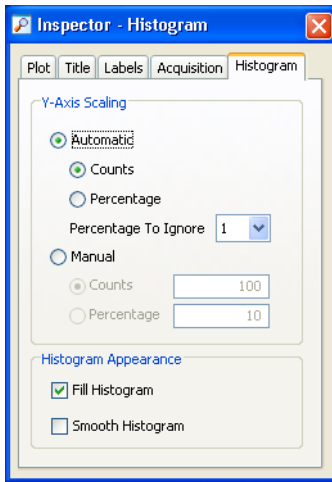
- **Automatic Percentage.** Select if you want the histogram to scale automatically to the highest percentage of the histogram data.

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.

- **Percentage to Ignore.** Select a value from the 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.

- **Manual Counts.** Select to display a fixed count on the y-axis. Enter an integer between 1 and 50,000 in the numeric field.

- **Manual Percentage.** Select to display a fixed percentage value on the y-axis. Enter an integer between 1 and 100 in the numeric field.

- **Fill Histogram.** Select to fill in the area between histogram peaks. Clearing the checkbox will show the individual bins. (Individual channel bins are more apparent on a zoomed-in histogram.) See Histogram Attributes on page 226 for more information.

- **Smooth Histogram.** Select to display smaller spikes around the histogram peaks. Smoothing does not affect histogram statistics.
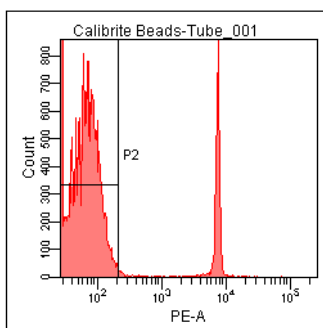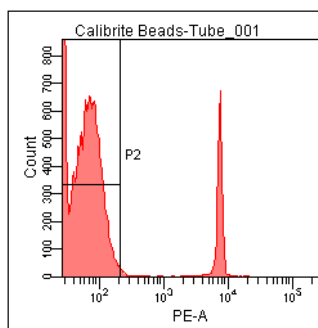
Smoothing off   Smoothing on

Overlay Plots for Tube Data

In normal worksheets, you can compare information between sample tubes by creating overlay plots for histograms, dot plots, contour plots, or density plots. You can drag one or more tubes from the Experiment Browser and drop them into an existing plot. The overlay appears in all plots that contain that tube.

You can perform these tasks by:

- Overlaying a plot created on a normal worksheet only, with additional tube data.

- Using any tube as a data overlay, provided it is part of the open experiment and contains recorded data.

Creating an Overlay Plot

To add an overlay plot:

1   Open an experiment.

2   Select and drag one or more tubes from the **Browser** and release them over an existing plot in a normal worksheet.

The data appears in a different color and line style. The overlay indicator, a green *O*, appears in the top left corner indicating that the plot contains data from another tube. The newly added overlay is added to all plots associated with the specified target tubes.
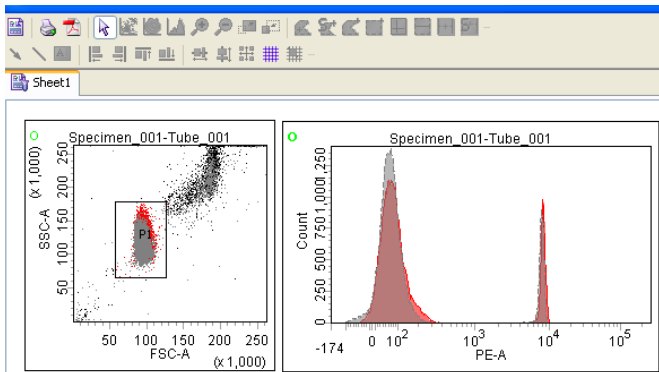
3 To display gated information, go to the **Inspector** and select the population you want to display.
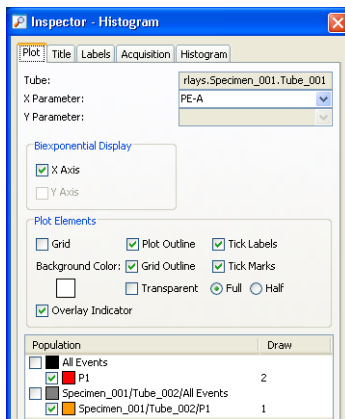
NOTE To delete a gated population from an overlaid tube, you have to select the population hierarchy for the specific tube and delete it there.

Modifying Overlay Plots

To suppress the display of a population in an overlay plot:

1 Select the plot.

2 Clear the checkbox corresponding to the population in the **Plot Inspector**.

To suppress the display of the overlay indicator on a specific plot:

1 Select the plot.

2 In the **Plot Inspector,** clear the **Overlay Indicator** option.

To suppress the display of the overlay indicator on all plots:

1 Select **Edit > User Preferences** and click the **Plots** tab.

2 Clear the **Show overlay indicator on plots** option.

Any overlay plots created after that will not display the green *O*.

To modify the look of an overlay plot, do one of the following:

• Select the overlay name in the population hierarchy, then modify the name or color in the **Plot Inspector**.

• Modify new display options such as dot size (dot plots and contour plots), line style, and line size (for histogram plots) by selecting the overlay name in the population hierarchy, then making modifications in the **Plot Inspector**.

To change the population display order for overlay plots:

1 Right-click any plot previously overlaid with data.

2 Select either **Bring to Front** or **Send to Back**.

3 Select the name of the overlay you want to move.

Or

1 Select the appropriate population in the population hierarchy associated with the overlaid plot.

2 Right-click the population.

3 Select either **Bring to Front** or **Send to Back**.

To remove the overlay plot:

1 Right-click any of the plots overlaid with tube data.

2 Do one of the following:

- Select **Remove Overlay**, then select the name of the overlay to remove.

- Select **Remove All Overlays**.

Or

1 Select the appropriate population in the population hierarchy.

2 Right-click the population.

3 Select **Remove Overlay(s)**.

Using the Legend

You can display a legend on a plot to show the tube with overlaid data. This function is turned off by default.

To turn on the legend:

1 Right-click in a plot.

2 Select **Show Legend**, then select the tube that you want to display in the legend.
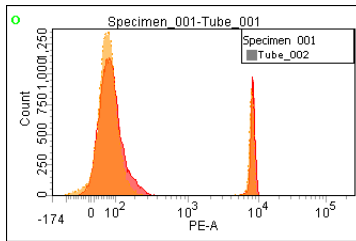
3 To display additional population information beyond what is shown in the legend, use the population hierarchy and modify the view.

An example is shown in the following figure.
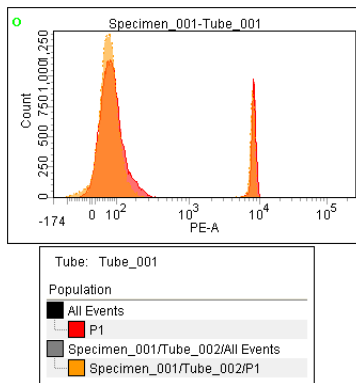
Using Biexponential Display

Digital data can have events with negative values. Biexponential display is used to show these events on plots and improve resolution between poorly resolved populations. To activate the feature for either plot axis, select the corresponding checkbox in the **Plot Inspector.** Note how biexponential display reveals the hidden double-negative population in the following example.
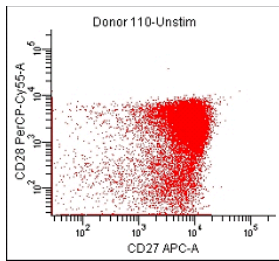
No biexponential scaling   Biexponential scaling (both axes)
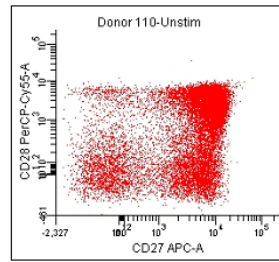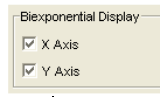
The linear range of the biexponential scale is determined by the extent of negative data for each parameter. During acquisition, data is periodically sampled to determine the scaling point. Once recording begins, periodic sampling stops if scales have already been determined, or after the first time the scaling point is determined during recording. Scales are recalculated after recording is finished, and any time cytometer settings are changed. If you change a compensation coefficient, new scales are calculated.

Width, ratios, and time are always shown on a linear scale. Biexponential display does not apply for these parameters. In addition, original event data is maintained as the basis for statistical calculation and FCS export regardless of the data display.

For more information about working with biexponential plots see:

- Formatting Plots on page 184 to show gridlines on biexponential plots.

- Working with the Biexponential Editor (next section) for instructions on how to adjust scales manually, apply scaling values to other tubes in an experiment, and import or export biexponential scale values.

- Scaling to a Population on page 205 to optimize the display for a selected population.

- Disabling Biexponential Display on page 206 to disable the feature.

- Hiding and Showing Gates on page 219 for potential gating limitations.

- Batch Analysis on page 242 to batch-analyze data files using the same biexponential scales.

Working with the Biexponential Editor

When data is displayed on a biexponential scale, the software determines the extent of negativity based on the range of compensated data for all events in the FCS file. This is known as automatic scaling. Use the Biexponential Editor to manually adjust the range of the negative scale (Figure 4-10). To display the **Biexponential Editor,** click the corresponding button on the **Workspace** toolbar.

Another way to adjust biexponential scales is with the scale to population function. See Scaling to a Population on page 205.

Figure 4-10 Using the Biexponential Editor
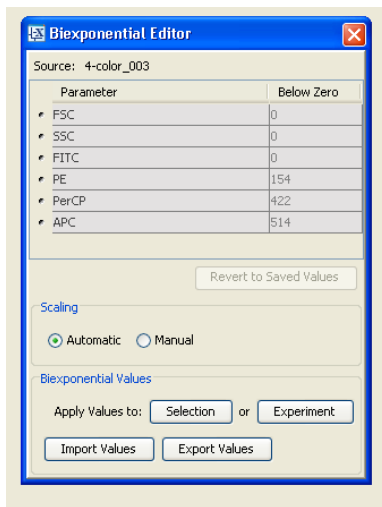
- To adjust scales manually, select the **Manual** option. The Below Zero fields are enabled for all listed fluorescence parameters. Click in any field to change the below zero value. Manual scaling values are saved with cytometer settings.

  To change values, enter a new value, click the up or down arrows, or drag the slider control.
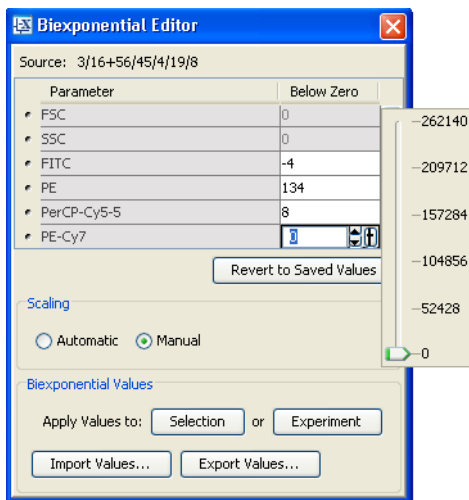
Manual scaling values apply to all biexponential plots that show the associated parameter. Plots for which manual scaling are in effect are marked with an *M* in the lower left-hand corner.
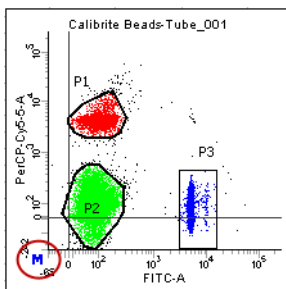
Click **Revert to Saved Values** to return to the original scaling values or select the **Automatic** option under **Scaling.**

Along with manual scaling, use the Biexponential Editor to export and import scale values, and apply values to other elements in an experiment.

- To apply biexponential values (automatic or manual) to other elements, click the **Selection** or **Experiment** button.
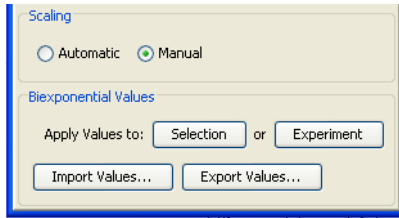
Clicking Selection applies the biexponential values to only those tubes or specimens selected in the Browser. Clicking Experiment applies the values to all tubes.

- To save a set of biexponential values as an XML file, click **Export Values.**

  Enter the file name and specify the saving location in the dialog that opens. By default, files are saved in BDExport\Biexponential.

- To import a set of biexponential values, click **Import Values.**

  Navigate to the XML file you want to import in the dialog that appears and click **Import.**

Scaling to a Population

The scale to population function allows you to adjust biexponential scaling to fit a selected population. To use the feature:

1   Right-click a biexponential plot.

2   Select **Scale to Population,** and select the population you want to scale to.

  All of the plot's scaling adjusts to fit the selected population. The gate label is marked with two vertical bars (P4, in this example), indicating that it is the population for which scaling is in effect.

Scaled to this population
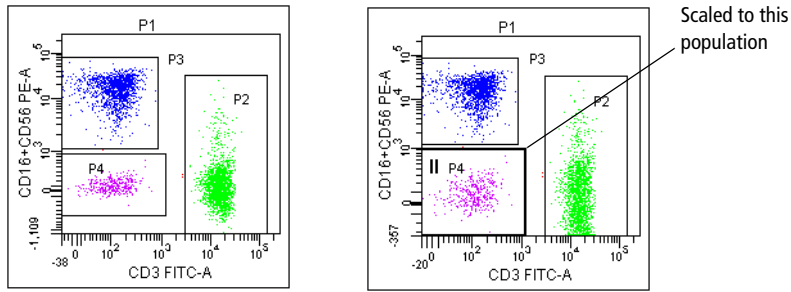
To return to automatic scaling, right-click the plot and select **Scale to Population > All Events.**

Disabling Biexponential Display

In **User Preferences,** you can elect to disable biexponential display by clearing the **Allow biexponential acquisition and display** checkbox. Disabling scaling allows more events to be recorded per experiment. However, large data files can be truncated when you re-enable it.
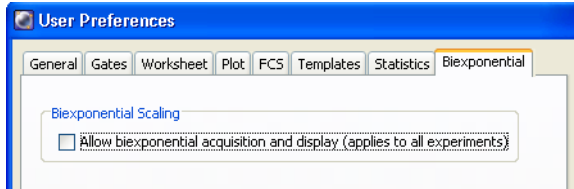
When you click **OK,** a message appears reminding you to restart BD FACSDiva software to make the change effective.

After you restart, plots that were set to biexponential switch to log, and biexponential functions such as the Biexponential Editor and scale to population are no longer available.

Gates

Gates are used to identify and define subsets of data (populations) on plots with linear, logarithmic, or biexponential scales. After defining gates, you can combine them to create joined, intersected, or inverted gates. Gated populations are used to generate statistics and limit the number of events collected or stored in the database. You can restrict a plot to display one or more populations, display populations in a hierarchical view, and use the population hierarchy to create subsets within defined populations.

Worksheet elements have an undo/redo function that allows you to revert back to a previous version of an edited worksheet if you haven't already saved the changes. If you change your mind and haven't already saved your changes, you can redo the edit.

Gates are defined using buttons on the Worksheet toolbar.
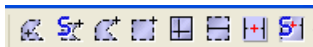
There are three types of gating buttons:

- Manual gating buttons such as the Polygon or Rectangle Gate buttons allow you to define gate boundaries. See Drawing Manual Gates on page 209.

- Automatic gating buttons such as the Autopolygon or Autointerval Gate buttons automatically define gate boundaries for a selected population. Gate boundaries remain static after they are defined. See Creating Automatic Gates on page 211.

- Snap-To gating buttons such as the Snap-To or Snap-To Interval Gate buttons automatically define gate boundaries, but the boundaries change when data in the gate changes. See Working with Snap-To Gates on page 212.

The following are tips for creating gates:

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

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

- Because of the way digital data is displayed on a log scale, populations can be split or events can be hidden next to the plot axis. When you are drawing a gate, make sure to include all events. When events are on the axis, extend the gate boundary past the axis to capture all events, or use biexponential scaling to view all events.

When a gate crosses the axis on a log or linear plot, all events below zero (to negative infinity) will be part of the population it identifies. When a gate crosses an axis, the gate color turns orange to indicate that all events below the axis are included in the gate.

Figure 4-11 Gates for populations with below-axis events
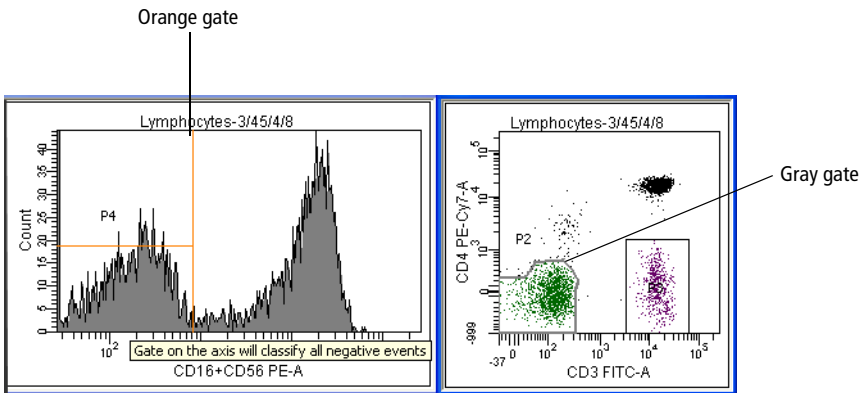
A gate created on a log plot can now be shown on a biexponential plot and vice versa. Because gate geometry and movement might be different for a gate out of its home coordinates, this gate is indicated in gray.

Editing a gray gate will change it to black, indicating that it has switched home coordinate systems.

Drawing Manual Gates

With manual gating buttons, the user defines gate boundaries. Manual gates include polygon, rectangle, quadrant, or interval gates.

Note that populations defined by intervals and quadrants retain the color of their parent unless you have specified otherwise in User Preferences (see Gate Preferences on page 92). You can also change the color of a population using the Population Hierarchy Inspector. See Changing the Color of Populations on page 226.

Button	How to use
	Use the Polygon Gate button to create a polygon gate on a dot, density, or contour plot.
	1 Click the button and click in the plot to establish the starting point (first vertex).
	2 Move the cursor to create the next vertex and click.
	3 Continue moving the cursor and setting vertices.
	4 Double-click the last vertex to complete the gate.
	Use the Rectangle Gate button to create a rectangle gate on a dot, density, or contour plot.
	1 Click the button.
	2 Click and hold in the plot to position the gate.
	3 Drag diagonally until the gate outline is the required size.
	Use the Interval Gate button to select a range of events in a plot. Interval gates can be used on dot, density, or contour plots, as well as histograms.
	1 Click the button.
	2 Click and hold in the plot to position the left edge of the interval.
	3 Drag the mouse to position the right edge.

Button	How to use
⊞	Use the Quadrant Gate button to divide a dot or contour plot into four separate populations. Each quadrant population can be named and colored individually. Quadrant populations can be used for subsetting or sorting. **1** Click the button and click in the plot. **2** Drag the intersection of the quadrant markers to position the gate. The cursor location is indicated by the coordinates at the bottom of the plot (Figure 4-13 on page 211). **3** (Optional) Drag a pivot point to rotate the top or right segment, or drag an offset handle to offset a segment from the center point. For pivoted or offset segments, Shift+click the quadrant boundary to return the gate to its rectilinear form.

Examples of polygon, rectangle, and interval gates 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 4-12 Polygon (P1), rectangle (P2), and interval (P3) gates
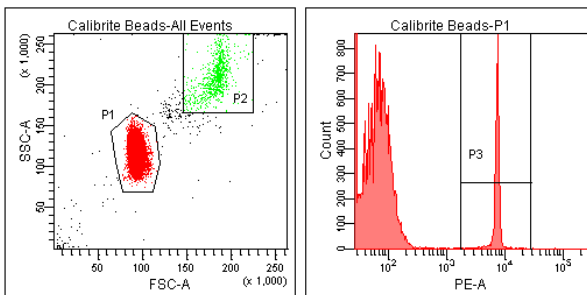
Examples of each type of quadrant gate are shown in the following figure. Each plot shows events from the polygon gate in the previous example. Notice how population color does not change when you draw a quadrant gate.

Figure 4-13 Rectilinear, pivoted, and offset quadrant segments

Creating Automatic Gates
========================
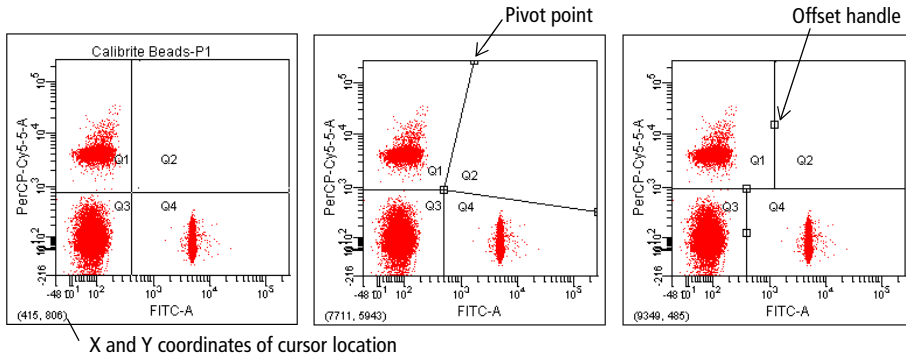
Creating Automatic Gates

With automatic gating buttons, gate boundaries are defined when you click a population in a plot. Unlike snap-to gates, gate boundaries remain static after they are defined. Automatic gates include autopolygon and autointerval gates.

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

Button	How to use
![autopolygon]	Use the Autopolygon Gate button to automatically create a polygon gate around a population in a dot, density, or contour plot.
	1 Click the button and click a distinct population in the plot.
	2 Verify that the gate includes the required events.
![autointerval]	Use the Autointerval Gate button to automatically select a range of events in a dot, density, contour, or histogram plot.
	1 Click the button and click a peak in the plot.
	2 Verify that the gate includes the 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.

Button	How to use
![Snap-To Gate button]	Use the Snap-To Gate button to automatically create a snap-to polygon gate around a population in a dot, density, or contour plot. **1** Click the button and click a distinct population in the plot. **2** Verify that the gate includes the required events.
![Snap-To Interval Gate button]	Use the **Snap-To Interval Gate** button to automatically select a range of events in a dot, density, contour, or histogram plot. **1** Click the button and click a peak in the plot. **2** Verify that the gate includes the required events.

Figure 4-14 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 function. The snap-to gate changes after data is recorded while the autopolygon gate remains the same.

Figure 4-14 Autopolygon (P1) vs snap-to gate (P2)
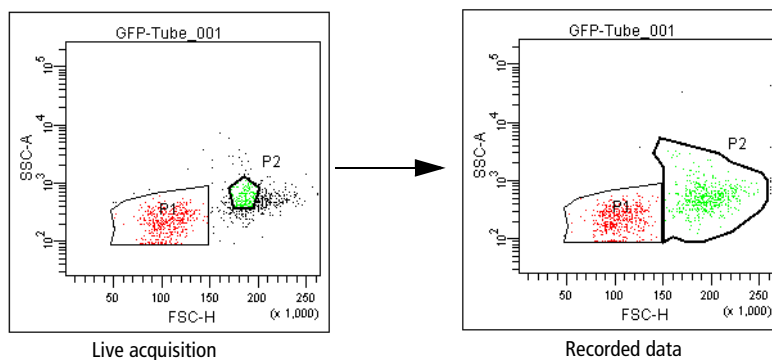
NOTE Snap-to gates do not work well with diffuse clusters or rare events. Also, populations defined by snap-to gates (and any populations derived from them) cannot be sorted.

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

NOTE After moving a vertex, the snap-to gate does not re-adjust automatically. To force the gate to re-adjust, right-click the gate boundary or population name in the population hierarchy (see Using the Population Hierarchy on page 223), and select **Recalculate** from the menu.

Adjusting the Movement of Snap-To Gates

A snap-to gate is automatically redrawn when the data in the plot changes, such as when live acquisition is finished or new data is read into the plot. When updating, the snap-to gate searches for a cluster closest to where it was originally placed. If no cluster is found, the system beeps and the snap-to gate maintains its original position.

You can change how far the gate moves to find a new cluster by adjusting the Auto Movement value in the Inspector. The auto movement range is a percentage of the plot width, or resolution, from 0–100%. A higher auto movement value allows the snap-to gate to travel greater distances to locate a cluster. The snap-to gate retains this setting if another data file is read into the plot or the gate is applied to a new data file.

1   Select one or more snap-to gates in a plot.

2   In the **Inspector,** clear the **Auto Movement** checkbox.

When the checkbox is selected, movement of the snap-to gate is limited to the software default value of 18 (not much movement).

3   Adjust the slider control toward the right until the gate encompasses the population of interest.

Alternatively, enter a value in the **Movement** field and press Enter**.**

Slider control
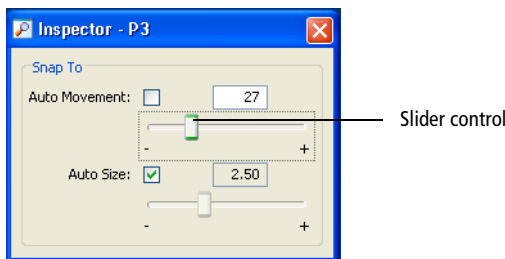
Adjusting the Size of Snap-To Gates

Cluster variability can cause BD FACSDiva software to draw a snap-to gate around only a portion of a population. Use the Auto Size function to adjust automatic sizing of the gate. A higher auto size value allows the snap-to gate to encompass a greater number of outlying events. A lower value restricts the gate to fewer outlying events. The snap-to gate retains this setting if another data file is read into the plot or the gate is applied to a new data file.

1   Select one or more snap-to gates in a plot.

2   In the **Inspector,** clear the **Auto Size** checkbox.

When the checkbox is selected, the software automatically determines the population size.

3   Adjust the slider control toward the right until the gate encompasses the entire population.

Slider control
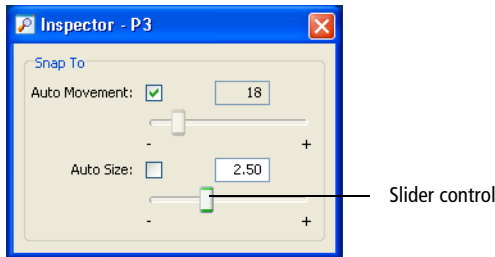
Alternatively, enter a value in the **Size** field and press Enter.

Display the statistics view to see the effects of the gate changes.

The following figure shows how adjusting the size affects the snap-to gate in the plot.

Figure 4-15 Snap-to gate with automatic sizing (left) and user-adjusted sizing (right)

Auto size on   Auto size off
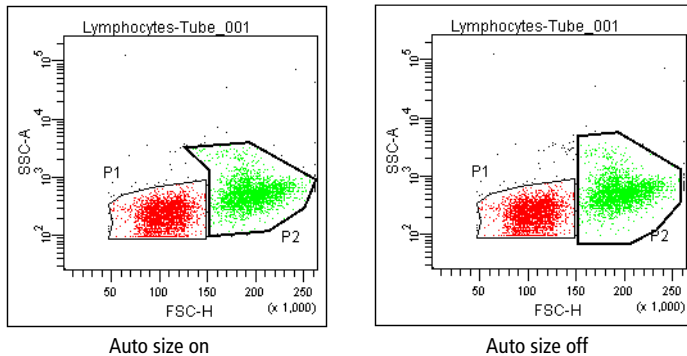
Tethering Snap-To Gates

A snap-to gate requires a minimum number of events in order to find a cluster. To automatically gate a small number of events or analyze an area of a plot that might or might not contain events, you can tether one or more manual gates to a snap-to gate. Tethered gate(s) move relative to the snap-to gate.

This function is useful when you expect changes in the population of interest in relation to another population. You can use tethered gates to help automate rare

event analysis. Tethered gates have the same properties as regular gates. For example, a plot can be gated and statistics can be generated from a tethered gate.

The following restrictions apply to tethered gates:

- For snap-to polygon gates, only interval gates with the same X parameter, or two-dimensional gates with the same X and Y parameters, can be tethered. For snap-to interval gates, only one-dimensional gates with the same X parameter can be tethered.

- Only manually drawn gates can be tethered to snap-to gates.

- Only one snap-to gate can be tethered to a manual gate. However, one snap-to gate can be tethered to many manually drawn gates.

- If you move, resize, or reshape the snap-to gate, the tethered gates remain the same. When you read in the next or previous file, the snap-to gate reverts to its previous position, size, or shape.

- If you move the tethered gate, the relative position is stored and used when reading in the next or previous file.

- A tethered one-dimensional gate can move only on the x-axis (horizontally).

Follow these steps to create a tethered gate:

1   Create a polygon (P1) and a snap-to (P2) gate in an appropriate plot.

2   Select **P1** in the plot.

3   In the **Gate Inspector,** select **P2** from the **Tethering** menu.

The menu lists all snap-to gates in the current plot and other plots that share the current plot's parameters.

Chain link icon   Tethered gate
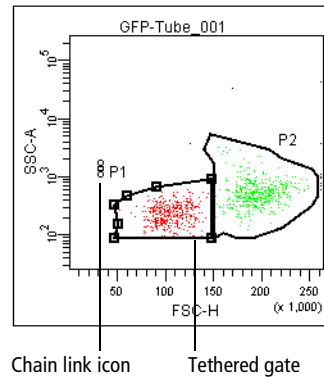
When the gate is tethered, its boundary changes and a chain-link icon appears next to the gate label, as shown in the previous figure. Note that a tethered gate has a bold outline similar to a snap-to gate.

Editing Gates

Any gate can be moved or resized, but you cannot add a vertex to or delete a vertex from an existing gate. Statistics are automatically updated after a gate is edited. Changes to a parent gate will affect all populations derived from that gate (see Population Hierarchies on page 222).

1   Click once on a gate to select it.

    Selection handles appear on the gate.

2   Make changes to the selected gate, then click outside the plot to deselect it.

- To resize a gate, drag any of the selection handles to a new location.

- To delete a gate, select the gate and select **Edit > Delete.**

  Alternatively, right-click the gate and select **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.

NOTE To avoid confusion, keep gate labels close to the populations they identify. Labels for quadrant gates in rectilinear or offset mode cannot be moved outside their respective quadrants; however, labels for pivoted quadrant gates can be moved past their respective segments.

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

Proportional Resizing of Gates

Use the proportional resize feature to resize all types of gates except quadrant gates. This feature only works on one gate at a time. There are two ways to proportionally resize a gate:

- To resize according to the dragging direction, Shift+drag a gate vertex.

  Select a gate boundary from a plot, hold down the Shift key, and drag the vertex of the gate boundary. The gate boundary is resized proportionally according to the direction in which it is dragged.

- To resize maintaining the original gate shape, Control+Shift+drag a gate vertex.

  Select a gate boundary from a plot, hold down both the Ctrl and Shift keys, and drag the vertex of the gate boundary. The gate boundary is resized proportionally according to its original shape.

  NOTE Proportional resizing does not allow dragging the vertex you've selected outside of the plot area. When the vertex reaches the edge of a plot, the gate boundary retains that shape and cannot be enlarged beyond the plot.

Hiding and Showing Gates

The boundaries of defined gates and their gate labels can be hidden or shown in any plot that shares the same parameters as the plot containing the original gate. To hide or show gates and their labels, right-click the border of the plot and select **Show Gate** from the menu (Figure 4-16).

- 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 4-16 Showing a gate boundary and label
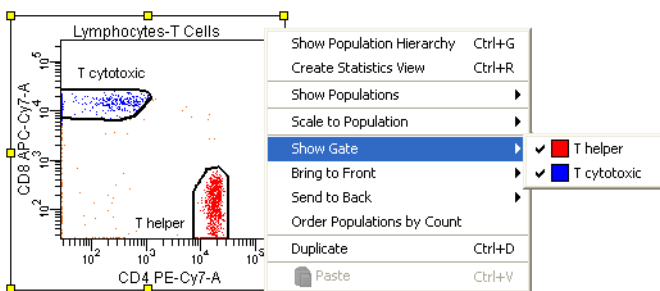
NOTE When you create a gate on a log plot and show it in a biexponential plot (or vice versa), the gate geometry can change. In addition, when you adjust biexponential scaling, gates might move in unexpected ways if they were created on a log plot.

To remind you that a gate was created on a log plot when it is shown in biexponential, the gate changes color when you drag it.

Copying and Pasting Gates

Gates can be copied from one plot to another or from one population hierarchy to another. When you copy a population from a hierarchy and it contains subsets, the subsets are included when you paste the population to another hierarchy.

A gate can be pasted on plots with the same or different parameters. The pasted gate takes on the parameters of the destination plot and is proportionally resized based on the destination plot size and coordinate system.

Dragging and Dropping Gates into Plots

Another way to create a copy of a gate is to use the drag and drop feature. Gates can be dragged and dropped in the same plot or to a different plot within the same worksheet. A gate can be pasted on plots with the same or different parameters. The pasted gate takes on the parameters of the destination plot and is proportionally resized based on the destination plot size and coordinate system. Drag and drop can also be used to drag gates into the population hierarchy. See Applying Gate Coordinates on page 230.

To drag and drop a gate:

1 Select a gate.

2 Hold down the Ctrl key, then select and drag the gate to the new destination.

A dialog opens if the same gate color exists in the new destination.

3 Select to use a new color or the same color.

The population hierarchy is updated to reflect the new gate.

To select multiple gates on a plot, hold down the Ctrl key while clicking, then drag the gates to the destination plot.

If a tethered gate is dropped onto a new area or plot, the copy is not tethered. All types of interval gates can be dragged and dropped to histograms.

Dragging Gates into the Population Hierarchy

The drag and drop feature can be used in the population hierarchy to drag and drop gates within the same worksheet, either in the same population hierarchy or a different one. See Applying Gate Coordinates on page 230.

To use the drag and drop feature:

1   Select a gate from a population hierarchy.

2   Hold down the Ctrl key and drag the gate under another gate in the same or a different population hierarchy.

A dialog opens if the same gate color exists in the new destination.

3   Select to use a new color or the same color.

The population hierarchy is updated to reflect the new gate.

You can only drag and drop one gate at a time. Dragging and dropping has the following conditions:

- Copying a parent copies all children under the parent.

- Copying a tethered gate creates a tethered copy only if its source is copied at the same time.

- When copying derived gates (AND, OR, NOT, Rest of), all of their source gates must be copied at the same time.

Population Hierarchies

All gates and their defined populations can be shown in a population hierarchy. Use the population hierarchy to see all populations defined for a tube and to view the relationship between gated populations.

For example, to define cell subsets during immunophenotyping of a whole blood sample, you first identify the lymphocytes, and then individual cell populations. The population hierarchy shows how these populations are identified by first defining a subset of lymphocytes from the whole blood sample (All Events), and then separating the lymphocytes into T, B, and NK cells. See Figure 4-17.

Figure 4-17 Immunophenotyping hierarchy and corresponding population hierarchy
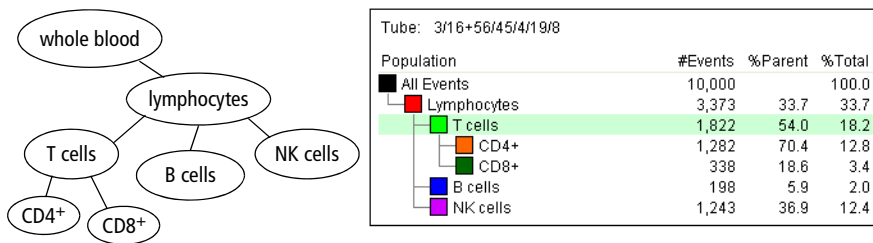
NOTE   To avoid errors when subsetting populations, always keep the population hierarchy in view. The last-selected population remains highlighted in the view, indicating that it will be the parent population of the next subset defined. Make sure the appropriate population is highlighted before you create a gate.

To show the population hierarchy:

- Right-click a tube or plot and select **Show Population Hierarchy.**

- Select a tube or plot and then press Ctrl+G.

- Select a tube or plot and select **Populations > Show Population Hierarchy.**

Move or resize a population hierarchy just like any other worksheet element. See Aligning and Resizing Worksheet Elements on page 173.

Using the Population Hierarchy

Use the population hierarchy to:

- **Rename populations.** Select any population and enter text to change its name. The new name will appear on all plots displaying that population.

- **Define population subsets.** Keep the population hierarchy in view so you can see the relationships between populations and their subsets. See Creating Population Subsets on page 227.

- **Copy gating hierarchies.** When you copy a population from a hierarchy and it contains subsets, the subsets are included when you paste the population to another hierarchy.

- **Change the color of defined populations.** Double-click the color box next to the population name and select a new color from the menu. See Changing the Color of Populations on page 226.

- **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 238.

NOTE Because some events reside in more than one population, sibling percentages do not always add up to 100%. Invalid values are represented by ####.

- **Display statistics for a single population.** Right-click any population in the population hierarchy and select **Create Statistics View** from the menu.

- **Delete gates.** Right-click any population and select **Delete,** or select the population and press the Delete key.

Using Population Hierarchy and Gate Inspectors

Click anywhere inside the population hierarchy header to see the **Population Hierarchy Inspector.** Click a population in the view to see the **Gate Inspector.**

Population Hierarchy Inspector

Gate Inspector

Population Hierarchy Inspector

Use the Population Hierarchy Inspector to select which information should appear in the population hierarchy and to change the font.
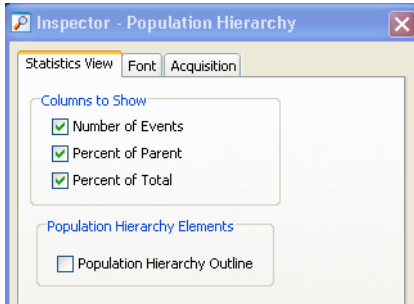
Gate Inspector
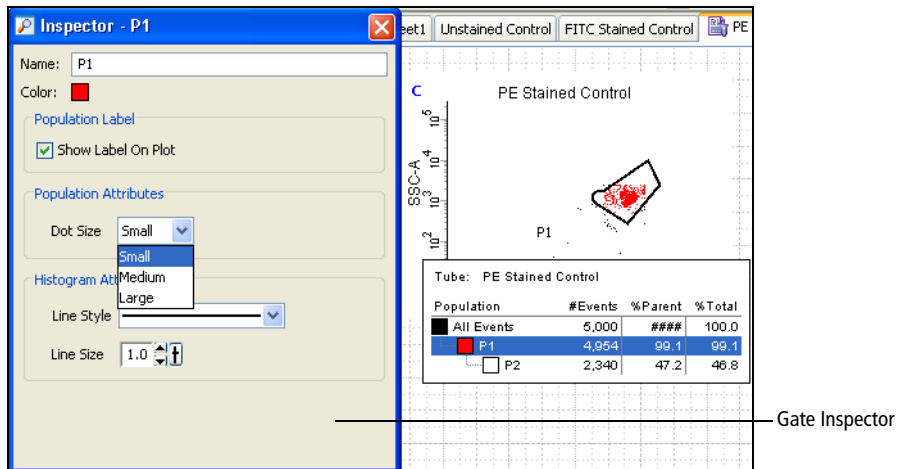
Gate Inspector

Use the Gate Inspector to change the population name and color, change dot size and line attributes, and show the population label.

You can also change a population name or population color directly in the population hierarchy.

Population Label

Select the checkbox under **Population Label** to show the population label on the plot.

Population Attributes

You can customize the dot size that is displayed on dot and contour plots. If the individual cells are low in volume, you can increase the size of the dots within a gate to obtain a viewable result.

To change the plot's dot size on dot and contour plots:

1   Select a population in the population hierarchy.

2   In the **Inspector,** select either **Small, Medium,** or **Large** under **Population Attributes**.

Histogram Attributes

Use the Histogram Attributes field to select a line style and thickness for a population when you are not using the fill histogram function or adding overlay information to an overlaid section of the plot:

1   Select a style from the **Line Style** menu.

2   Select a size from the **Line Size** menu.

Changing the Color of Populations

By default, populations defined by quadrants and intervals are not assigned a color in the population hierarchy. They retain the color of their parent population. Default settings can be changed using User Preferences (see Gate Preferences on page 92). For all other gate types, populations are assigned the color of the last gate they satisfy.

Population colors can also be changed manually after a gate is defined.

1   Double-click the color box in a population hierarchy or click the color box in the **Gate Inspector.**

Name: Lymphs
Color: ⬛
　　　Color box
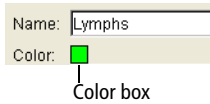
2　Select a new color from the palette that appears or click the **No Color** box (⊠) to leave the gated events uncolored.

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:

1　Select a population in the population hierarchy.

The population is highlighted.

2　Click the appropriate gate button.

3　Draw a gate in a plot.

Alternatively:

1　Create a new plot, then select **Show Population** and select the appropriate population (Figure 4-18).

2　Draw a gate around a subpopulation of the displayed events.

In either case, the new population appears indented below the selected population in the population hierarchy (Figure 4-18).

Figure 4-18 Creating population subsets
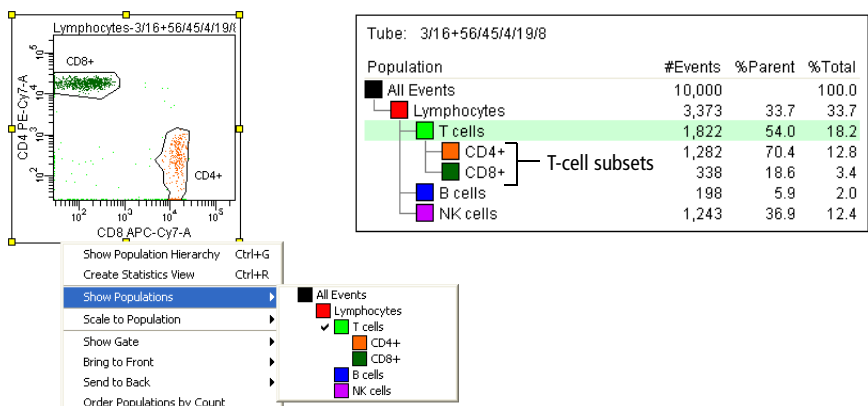
Defining a Derived Gate

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

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

  To define an inverted gate, right-click an existing population in the population hierarchy and select **Invert Gate.**
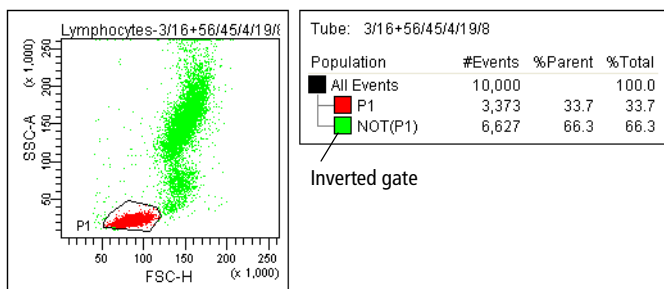
- Intersected gates use the AND operator to combine two or more individual gates. Only events that are in all of the specified gates satisfy an intersected gate.

To define an intersected gate, select two or more populations in the population hierarchy. Right-click the selected populations and select **Intersect Gates.**

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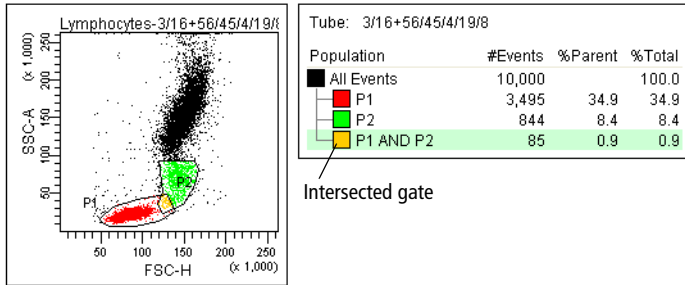
- Joined gates use the OR operator to combine two or more individual gates. An event that falls in any of the specified gates satisfies the joined gate.

  To define a joined gate, select two or more populations in the population hierarchy. Right-click the selected populations and select **Join Gates.**

Joined gate
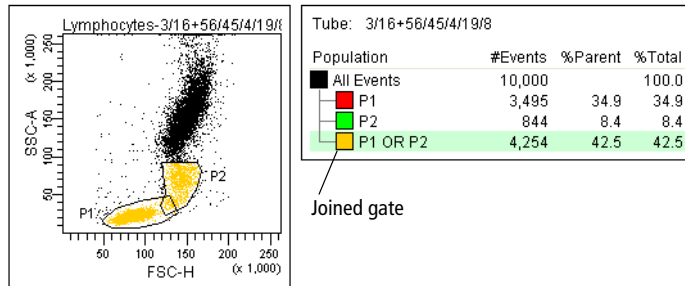
- *Rest of* gates select all remaining events that do not fall into any of the child gates of a parent gate. Thus, you can only access the *Rest of* option when you select a gate that already has subsets (children).

  To define a *Rest of* gate, right-click a parent population in the population hierarchy and select **Rest of.** Events in the *Rest of* population retain the coloring of their parent unless you select to change the color. The color was changed in the following example to illustrate the events.
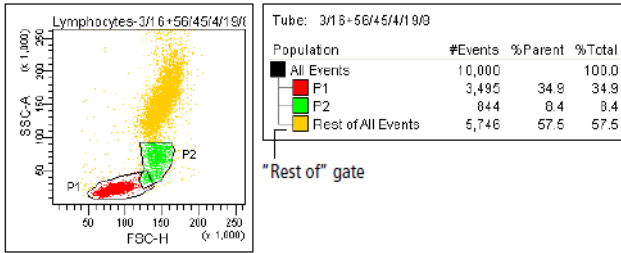
Applying Gate Coordinates

Use the apply gate coordinates function to apply the shape and size of a gate to other gates. The gate coordinates are applied to gate(s) of the same name and type (rectangle, polygon, etc) drawn on the same coordinate system as the selected gate(s), using the same parameters.

1 Right-click a gate on a plot or in a population hierarchy to select the gate whose coordinates you want to apply.

2 Select **Apply Gate Coordinates,** then select either **Current Experiment, Current Specimen,** or **Tube(s)/Well(s)** from the menu.
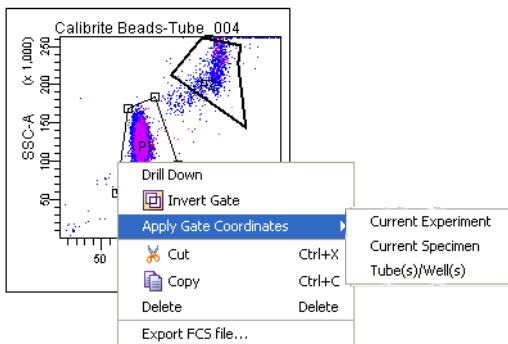
If Tube(s)/Well(s) is selected, a dialog opens. Select the tube or well to which the gate coordinates should be applied.

If Current Experiment or Current Specimen is selected, the apply gate coordinates function finds gates of the same name and type drawn on the same coordinate system as the selected gates that are using the same parameters, and adjusts the coordinates to match the selected gate(s).

NOTE The apply gate coordinates function is not available for snap-to gates or snap-to interval gates. This function can be used for normal worksheets as well as preferred global worksheets. This function cannot be used for a global worksheet unless it is the preferred global worksheet of the specimen or tubes of the target selection.

Statistics

BD FACSDiva software generates statistics from the linear values of acquired events. Statistics can be displayed for any parameter and calculated for any defined population. Statistics are displayed on the worksheet, like a plot, and can be exported to a file.

NOTE During acquisition, statistics are calculated for the number of currently displayed events and are updated as the display changes. For this reason, responsiveness can decline as more statistics are calculated on a greater number of displayed events. After recording, statistics are recalculated on the total number of recorded events.

To display a statistics view, do one of the following:

- Right-click a tube (normal worksheet view) or global sheet icon (global worksheet view) in the **Browser** and select **Create Statistics View.** The resulting statistics view lists the number of events and %Parent for all populations defined for the tube.

- Right-click any plot and select **Create Statistics View.** The resulting statistics view lists the number of events, %Parent, and means of the plot parameters for all populations displayed in the plot.

- Right-click a population in the population hierarchy and select **Create Statistics View.** The resulting statistics view lists the number of events and %Parent for the selected population.

Move or resize a statistics view like any other worksheet element. See Aligning and Resizing Worksheet Elements on page 173.

Using the Statistics Inspector

Click in a statistics view to open the **Statistics Inspector.** Use it to select the layout of the Statistics View and to change the font.

Cumulative Display for Statistics
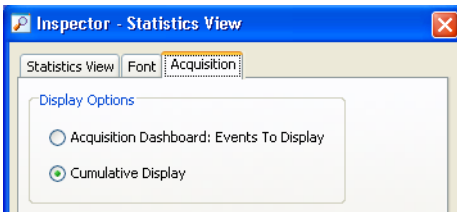
Select the **Acquisition** tab, then select the **Cumulative Display** option to display the median values in the statistic view from the beginning of acquisition, recording, restarting, or the addition, movement, or deletion of a gate. Because adding, moving, or deleting a gate impacts the display through the different gate structure, the cumulative values in the display are replaced by #### until the acquisition is completed.

When data in a statistics view is displayed as cumulative, there is a C shown in the upper left corner of the statistic view.

Selecting Statistics to Display

Use the Edit Statistics View dialog to specify the statistics to display. To open this dialog, do one of the following:

• Click the **Edit Statistics View** button in the **Statistics Inspector.**

• Right-click a statistics view and select **Edit Statistics View** from the menu.
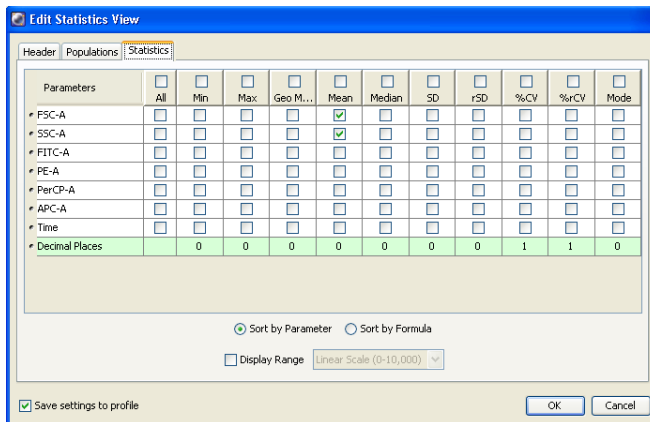
Select the **Save settings to profile** checkbox in the lower left-hand corner of the **Edit Statistics View** dialog to save updated or new settings as the default settings. After you click OK, changes are saved upon login, and are retained from one session to the next.

There are three components to the dialog, accessed by clicking the tabs labeled Header, Populations, and Statistics. See Figure 4-19 on page 234.

Editing Header Information

Use the Header tab to specify information to be included in the header of the statistics view, and to reorder headers and available keywords.

Figure 4-19 Statistics editor
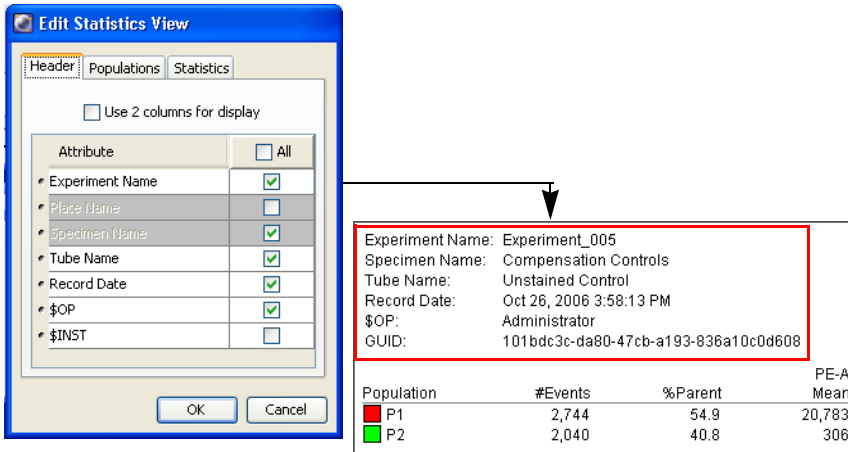
- Select the **Use 2 columns for display** checkbox to display header information in two columns.

- Display a header item by selecting its checkbox. Delete an item from the header by clearing its checkbox.

- Select the **All** checkbox to display all header items.

- Reorder header items by selecting any row and dragging it to a new position in the list.

⚠️ To ensure that statistics views include a tube identifier, always include the GUID (globally unique identifier) in the header of statistics views.

- Click **OK** to save your changes.

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.
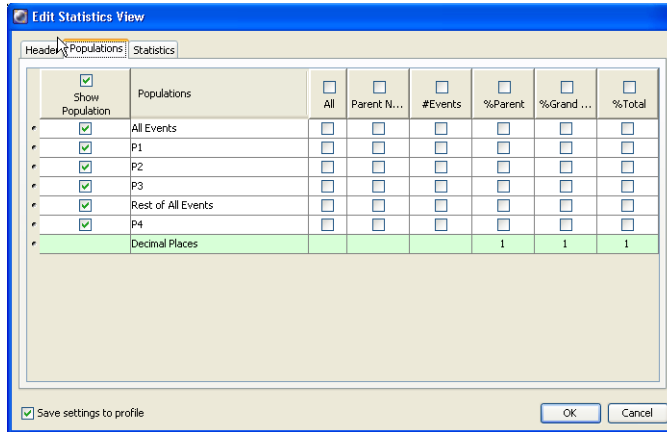
- To include a population in the statistics view, select the checkbox next to the population name in the **Show Population** column. To include all populations, select the checkbox at the top of the column.

- Specify additional population information to display by selecting the corresponding checkboxes.

  - Select the **All** checkbox in the population row to display all information for that population.

  - Select the checkbox in a column header to display that information for all selected populations.

  - Select individual checkboxes in a population row to display a subset of the listed information.

- Enter the number of integers (0 through 4) to display to the right of the decimal point for the %Parent, %Grandparent, and %Total statistics.

- Delete a population or statistic from the statistics view by clearing its checkbox.

- Click **OK** to save your changes.

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.
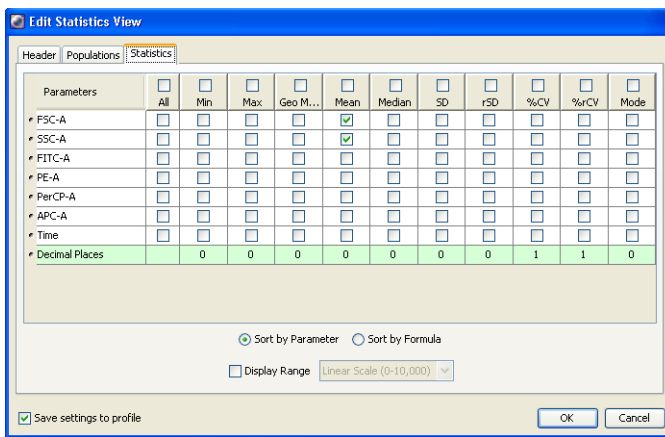
- Within each row, select checkboxes for each statistic to display for that parameter, or select the **All** checkbox to display all statistics for that parameter.

- 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 an option to specify how statistics should be sorted: by parameter (for example, FITC Mean, FITC CV, PE Mean, PE CV) or by formula (for example, FITC Mean, PE Mean, FITC CV, PE CV).

- At the bottom of the editor, select the **Display Range** checkbox to provide cross-matching scaling. In the menu, select the scaling.
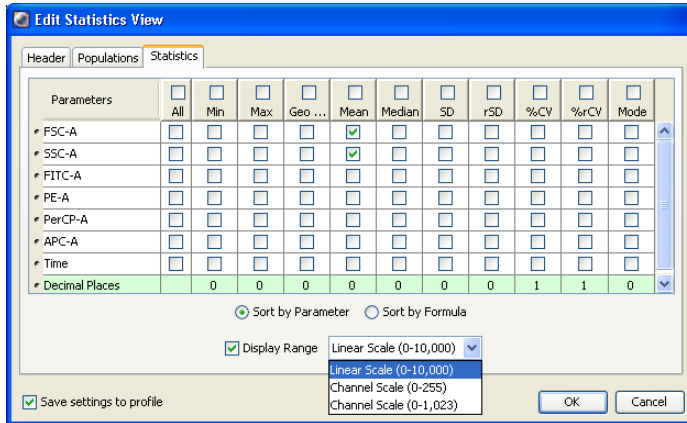
When Display Range is selected and the statistics view has two populations displayed (selected under the Populations tab in the Edit Statistics View dialog), delta values are displayed. The delta values show the absolute difference between two statistics (for example, between the P1 mean and P2 mean.)

When channel scales are selected, decimal places are not displayed in the statistics view and the Decimal Places row in the Edit Statistics View dialog is unavailable.

NOTE 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 appears before the parameter name.

- Click **OK** to save your changes.

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.

NOTE Event data can be out of range when the compensation matrix is applied. In plots, the out-of-range data stacks at the margins of the plot but statistics are calculated on the out-of-range data.

The following statistics can be calculated:

- **Number of events.** Total number of events in the defined population.

- **Parent.** Name of the next population up in the hierarchy.

- **%Parent.** Number of events in the defined population divided by the number of events in the parent gate (next population up in the hierarchy), expressed as a percentage.

- **%Grandparent.** Number of events in the defined population divided by the number of events in the grandparent gate (two populations up in the hierarchy), expressed as a percentage.

- **%Total.** Number of events in the defined population divided by the total number of events in the tube (all events), expressed as a percentage.

- **Mean.** Average linear value for events in the defined population, defined as:

$$\bar{X} = \left(\sum_{i=1}^{n} X_i\right)/n$$

   Where n = number of events in the population, and X_i is a value for a particular parameter, where i = 1 to n

- **Geometric mean.** Logarithmic average of the events in the defined population.

   This mean is less sensitive to outliers than the regular mean. The geometric mean is defined as:

$$\bar{X}geo = 10^{\left(\sum_{i=1}^{n} \log X_i\right)/n}$$

   Where n = number of events in the population, and X_i is a value for a particular parameter, where i = 1 to n

   NOTE The geometric mean cannot be calculated for events with negative values. If you include the geometric mean for populations with negative values, the resulting statistics will be invalid (####).

- **Median.** Linear value with an equal number of values above and below it.

   If the median of the data occurs between two values, those two values are added and divided by two to get the median.

   During acquisition, BD FACSDiva software uses a faster but less accurate method to calculate the median. Thus, after analysis, the median value can differ slightly from what is observed during acquisition.

- **Min.** Minimum linear value within a defined population.

- **Max.** Maximum linear value within a defined population.

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

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

- **Robust standard deviation (rSD).** The rSD is calculated as follows:

  The median of the data sample is computed:

  $$\theta_{median} = med_i\{x_i\}$$

  From that, the median absolute deviation is computed:

  $$\sigma_{median} = med_i\{(|x_i - \theta_{median}|)\}$$

  Then the robust standard deviation is computed:

  The constant is: $\sigma_{median} = \sigma_{median} / \phi^{-1}(0.75)$

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

  The CV is defined as:

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

- **%Robust coefficient of variation (%rCV).** The robust SD divided by the median within a defined population, expressed as a percentage.

  The rCV is defined as:

  $$Percent\ rCV = ((rSD) / median) \times 100$$

Exporting Statistics

Export statistics for use in a spreadsheet, word processing, or other third-party application. Statistics information (including header text) is exported to a file. Data from multiple tubes can be automatically exported to a single statistics file during a batch analysis or a BD FACS™ Loader run (BD FACSCanto cytometers only). For more information, see Batch Analysis on page 242.

To manually export statistics:

1   Select **Edit > User Preferences** and select a manual export format.

- Use CSV files for spreadsheet applications such as Microsoft Excel. Note that commas in the text of exported fields will cause the text to be split into two cells in the spreadsheet application.

- Use XML for text that can be used on any platform, including the Internet.

- Use text for word processing applications such as Microsoft Word.

    See Statistics Preferences on page 101 for more information.

2   Select one or more statistics views.

3   Select **File > Export > Statistics.**

4   Enter a name for the statistics file and specify a storage location in the dialog that opens (Figure 4-20).
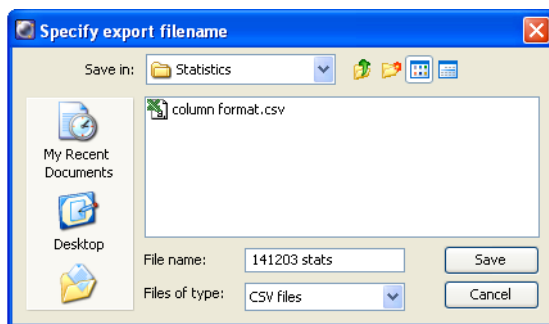
To append statistics to an existing file, locate and select the file, and click **Save.** When prompted, click **Append.** Results will be appended to the selected file.

5   Click **Save.**

Batch Analysis

Use the batch analysis function to automatically advance a selected set of tube data through an analysis on a global worksheet.

NOTE   If you plan to export statistics during the batch analysis, select your preferred auto export format in User Preferences before you begin. See Statistics Preferences on page 101 for more information.

1   Select an experiment, specimens, or tubes in the **Browser**.

If you select an experiment, all available data will be processed. If you select a specimen, only tubes under the selected specimen will be processed. Tubes without data are skipped during a batch analysis.

2   Right-click the selected item(s) in the browser and select **Batch Analysis**.

The following dialog opens.
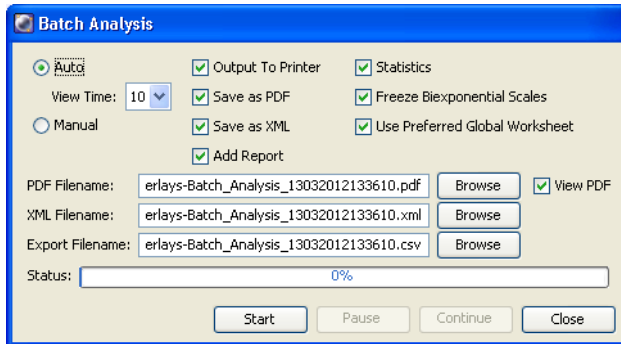
3 Select the type of analysis to be performed.

- Select **Auto** to analyze all files with no user intervention. Data is displayed in the global worksheet for the amount of time specified in the View Time field (in seconds) before analysis of the next tube begins. Make adjustments to your analysis during this pause, or let analysis proceed automatically.

- Select **Manual** to pause the batch after data is loaded for each tube. Click the **Continue** button to proceed with analysis of the next tube. The View Time field is disabled when you select manual analysis.

NOTE 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, select the amount of time to pause (0–60 seconds) after each tube's data is loaded.

Select zero only if you want to process the batch without reviewing the data between tubes.

5 Specify whether to print worksheets or export statistics before data for the next tube is loaded.

- Select the **Output To Printer** checkbox to print a copy of the analysis for each tube.

- Select the **Statistics** checkbox to export statistics to a single CSV file for the batch. The resulting file can be opened with a spreadsheet application such as Microsoft Excel.

  NOTE Depending on the auto export format selected in User Preferences, each tube adds a new row or column of results to the file. For each population in the statistics view, the software adds parameter statistics in the order in which they appear in the view. A new header row is added if you add or delete statistics or parameters during batch analysis. You cannot add, remove, or edit statistics views while the batch is running.

6 To save the worksheet as a PDF file, select the **Save as PDF** checkbox.

- When you select Save as PDF, the Add Report and View PDF checkboxes are selected by default.

- If you keep Add Report selected, the batch analysis report is added at the top of the worksheet's PDF file.

- If you keep View PDF selected, the PDF is automatically displayed at the completion of the batch analysis.

7 To save the worksheet as an XML file, select the **Save as XML** checkbox.

8 For exported statistics, specify the file name and storage location in the **Export Filename** field or click **Browse** to select the location.

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

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

9 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, clear the checkbox.

10 If you have different global worksheets assigned to tubes, select the **Use Preferred Global Worksheet** checkbox to preserve worksheet assignments during the analysis.

When the checkbox is cleared, all tubes will be processed using the same global worksheet even if different worksheets were assigned.

11 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.

Creating Specimen Reports

You can create specimen reports under the following conditions. If you select:

- An experiment listed in the Browser, the report will include data on all specimens.

- A specimen listed in the Browser, the report will include data on only that specimen.

- A tube listed in the Browser, the report will include data on the specimen associated with that tube.

To create a specimen report:

1 Select an experiment, specimen, or tube from the **Browser**.

2 Right-click and select **Create Specimen Reports**.

For each specimen, the software creates:

- One PDF report that contains all worksheet elements from either the preferred global worksheet or the active global worksheet (if the preferred worksheet is not specified), for all tubes in that specimen.

- One PDF report that contains all worksheet elements from each normal worksheet that contains data on that specimen. The PDF report file name consists of the specimen name and a date time stamp of the file creation.

- One CSV file that contains statistics for all the tubes of all the specimens selected. The CSV report file name consists of the specimen name and a date time stamp of the file creation.

Working Offline

When using BD FACSDiva software for offline analysis (the software is not connected to a cytometer), most cytometer, acquisition, and sorting controls are unavailable.

During offline operation, you can set up experiments or analyze recorded data. Display data for a recorded tube by clicking the current tube pointer in an open experiment. See Current Tube Pointer on page 123. Analysis objects can be edited, moved, copied, and fine-tuned using gates and the drill-down function. Population hierarchies can be derived and statistics can be calculated and exported based on data previously recorded.

When you set up an experiment offline, you might need to create tube-specific cytometer settings and disable the Use global cytometer settings preference in the Experiment Inspector to maintain the settings when you connect to the cytometer. For example, when setting up an experiment for cytometer optimization, if your first tube uses a different set of parameters than your second tube and the global cytometer settings option is selected, experiment-level cytometer settings will be updated to match the first set of parameters when that tube is recorded, and these settings will remain in effect when you record the next tube. By disabling the preference, individual tube-specific settings are maintained.

5

Data Management

The following topics are covered in this chapter:

- Working with BD FACSDiva Data on page 248

- Exporting and Importing FCS Files on page 252

- Exporting and Importing Experiments on page 261

- Using the Data Manager Utility on page 265

Working with BD FACSDiva Data

BD FACSDiva software stores and accesses all experiment data from a single database. As you create experiments in the Browser, the software writes experiment components to the database.

Any changes to an open experiment, related Browser elements, or worksheet are automatically saved when you close an experiment, quit the software, or click the **Save** button on the **Workspace** toolbar. The database contains a record of all Browser items, worksheet elements, experiment settings, and cytometer control settings.

Recorded event data is saved separately in FCS 3.0 floating-point format. A disk icon is added to a tube in the Browser when data has been saved for that tube. To analyze data in another software application or on a different BD FACSDiva workstation, data must be exported using the FCS export option in the software (see Exporting FCS Files on page 253).

⚠️ 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.

Maintaining Data

Because all data is saved in a database, the database can fill up the hard drive. It is important to maintain the database by keeping the size below recommended limits, exporting and archiving data from the Browser on a regular basis, and deleting experiments, specimens, or tubes that are no longer needed.

⚠️ For optimal performance, follow these precautions:

- The number of events that can be recorded for a single tube varies inversely with the number of gates and parameters (scatter parameters, fluorophores, area, height, width, and ratios). For optimal performance, limit the number of parameters to only those that are required.

- Monitor the disk capacity (see Verifying Database Size on page 251). The amount of hard disk space required for the database must not exceed 40–45% of the available disk space (the remaining disk space is reserved for a backup copy). When you are approaching the limit, delete unneeded experiments or export experiments and store them in an offsite storage location.

- The software allows the administrator to view and manage disk space usage for each experiment and each user on the system. See Viewing Disk Space Usage on page 32.

- Defragment the hard disk on a regular basis (for example, weekly). Use the defragmentation program in Windows. To run the program, select **Start > Programs > Accessories > System Tools > Disk Defragmenter.**

⚠ Data loss can occur if the defragmentation process is interrupted. We recommend that you back up your data before running the defragmentation procedure and allow sufficient time to defragment the drive.

- We recommend 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.

The following table summarizes the options for maintaining the BD FACSDiva database using the tools provided with the software. Follow the procedures established in your laboratory for scheduling data backups. General guidelines are provided in the table and a recommended weekly maintenance procedure is also provided.

Option	Function	When to Perform	See...
Export experiments	Copies experiments to a specified drive for data archiving	After each significant experiment	Exporting and Importing Experiments on page 261
Export FCS files[a]	Exports FCS 2.0, 3.0, or 3.1 list-mode data files	• After each significant experiment • For analysis in other software analysis applications, such as BD CellQuest Pro • After each recording, if selected in User Preferences, FCS tab	Exporting FCS Files on page 253
Delete experiments	Removes unnecessary or outdated components	As needed	Deleting Experiments on page 251
Back up the database	Copies the current database to a specified drive using the Data Manager utility	Weekly	Backing Up the Database on page 266

a. Enable the Export FCS preference to automatically export an FCS 3.0 file after each tube is recorded. To export FCS 2.0 or 3.1 data, you need to export manually.

Optimizing Data Processing

To save memory (and disk space for permanent storage), it is recommended that you save only parameters that are being used.

While there is no impact on data collection or cytometer performance, responsiveness can decline as more plots, statistics, gates, and events are displayed for each tube. To improve system response time, limit the number of plots displayed in the viewable area of the Worksheet window.

Verifying Database Size

The database should not exceed 40–45% of the available disk space. (The remaining space is reserved for a backup copy of the database.)

To determine the size of the database:

1   Use Windows Explorer to view the contents of the BDDatabase folder, at D:\BDDatabase.

2   Select the *BDData* folder, the *BDFACS.db* file, and the *BDFACS.log* file.

3   Right-click the selected items and select **Properties.**

A window opens showing the size of the selected components.

⚠️   If the sum of all data files is approaching 40–45% of the available disk space, delete or archive (export and delete) experiments to free up space on the drive.

NOTE   Another way to determine the amount of disk space being used is to open My Computer and select the disk in Windows Explorer. The total size and amount of free space are shown.

Deleting Experiments

⚠️   Deleting experiments also deletes any associated event data. Export experiments or FCS files before you delete them, if needed.

To delete unneeded experiments:

1   Export FCS files or experiments to safeguard important data.

When exporting experiments, you can select the checkbox to automatically delete the experiments after export. Then the remaining steps are not needed.

2   In the **Browser,** select the folders or experiments you want to remove.

> **NOTE**   When you delete a folder, you delete all experiments within the folder.

- To select multiple contiguous elements, click the first element, then hold down the Shift key while you select the last element.

- To select multiple noncontiguous elements, hold down the Ctrl key while you select each element to be removed.

3   Press the Delete key.

Alternatively, right-click the selected elements and select **Delete** from the menu.

Exporting and Importing FCS Files

Data can be exported as an FCS file that can be read by other BD software and third-party applications. Files can be exported in FCS 2.0, 3.0, or 3.1 format. To accommodate other software applications, FCS 2.0 log data is scaled down to four log decades during export. For formatting and naming preferences, see FCS Preferences on page 97.

Cytometer settings are written to the FCS file but cannot be opened as a separate file in BD CellQuest or BD CellQuest Pro software or any other software application. Cytometer settings are not transferable between different cytometers.

Exporting FCS Files

You can enable a user preference to export FCS files automatically after each tube is recorded. See FCS Preferences on page 97. When files are exported automatically, they are exported as FCS 3.0 with all parameters included.

NOTE   The FCS 2.0 standard limits the size of the data section to 99,999,999 bytes (roughly 2.5 million events with 7 parameters at 4 bytes per parameter per event). To export larger files, export as FCS 3.0 or limit the file size by exporting fewer parameters.

Follow the steps in this section to export FCS files when the automatic export preference is disabled.

⚠️   When you export compensated data with negative values (see Using the Compensation Tab on page 131) as FCS 2.0, the negative values are set to zero in the exported file. When the file is imported and analyzed in another software application or in BD FACSDiva software, statistical results can be different from the original file.

1   Select experiments, specimens, or tubes to export.

Data can be exported from closed or open experiments. Multiple items can be selected for exporting at one time. Individual FCS files will be generated for each tube in a selected specimen or experiment.

Experiment- and specimen-level keywords are exported with tubes. User-defined keywords are included in the header of FCS files.

2   Select **File > Export > FCS files.**

Alternatively, right-click the selected item(s) and select **Export > FCS files.**

3   Specify the FCS file version: FCS 2.0, FCS 3.0, or FCS 3.1.

4   Specify the FCS parameters to export in the dialog that opens.

When multiple files are selected, options apply to all exported files.

- Select the file format: FCS 2.0 (1,024 or 10-bit resolution), 3.0 (262,144 or 18-bit resolution), or 3.1 (262,144 or 18-bit resolution). When FCS 3.0 or 3.1 is selected, only linear data can be exported.

  To make an exported file compatible with BD CellQuest Pro software, export it as FCS 2.0 with a maximum of 16 parameters (8 parameters for BD CellQuest software). To analyze compensated data, make sure the **Enable Compensation** checkbox is selected for each exported tube. Remember to run files through BD FACS™ Convert software before attempting to open them in BD CellQuest or BD CellQuest Pro software. See the *BD FACSConvert User's Guide* for instructions.

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

  FCS 2.0 log data is always exported in four decades. If the data spans five decades, only the upper four decades are included. You cannot select log for the time parameter. It is always exported as linear.

NOTE Minimize the file size by excluding unnecessary parameters (click the **None** button). For FCS 3.0 and 3.1 files, make sure to include all parameters included in compensation calculations. See Important Considerations on page 259.

5 Click **OK.**

6 In the **Save Export** dialog, verify the file storage location.

Drive\folder\subfolder —

Save Export
Directory Path
D:\BDExport\FCS   Browse
Save   Details>>   Cancel
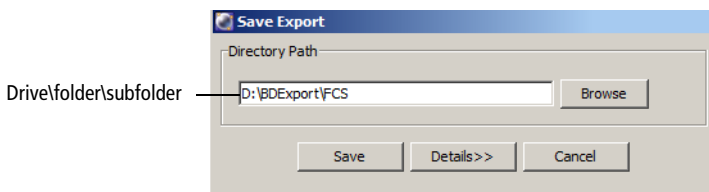
- Click the **Browse** button to change the file storage location. Navigate to a different directory in the dialog that opens.

NOTE   If you select Add experiment folder to export path in User Preferences, your FCS files will be placed in an experiment folder. See FCS Export Options on page 98.
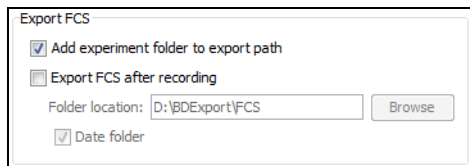
- Click the **Details** button to view the relative directory path and file name for each exported tube. Note that the file names are taken from the *specimen_tube* names in the Browser and cannot be changed.

NOTE   To avoid confusion, store exported FCS files in a folder different from exported experiments. We recommend that you store exported FCS files in the BDExport\FCS folder that is set up for you during software installation.

7   Click **Save** to export the files.

Exporting Gated Events as FCS Files

Gated events can be exported as FCS files. Keywords are created for the tubes, as well as a keyword that gives the full gate name of the selected gate. For exporting multiple FCS files, a menu of common gates in the selected tube is displayed in the Export FCS Files dialog.

Common gates are gates with the same name (for example, P1) and type (rectangle, polygon, etc) that are drawn on the same coordinate system (log, linear, biexponential) in the selected tubes.

NOTE   If a gate is the same name but not the same type (for example, events are all P1 gates in rectangles except for a P1 in a polygon), the gate that is a different type is not displayed in the menu of common gates.

The default FCS file includes the specimen name, the tube name, and the gate name.

To export gated events from one tube:

1 Select the gated events for export either by clicking on the gate in the plots or by selecting the gate in the population hierarchy (you can only export one).

2 Right-click and select **Export FCS file.**

3 Specify the FCS file version and parameters to export in the dialog that opens.

4 Click **OK.**

5 In the **Save Export** dialog, click **Save.**

To export gated event from multiple tubes:

1 Select **File > Export > FCS files.**

2 Under **Gated Events,** select from the menu of common gates to export.

When importing any FCS file that was exported using this function, only the gated area of events is displayed, with the drawn gate no longer shown.

NOTE Since exported gated events are no longer linked to the original file, if the file is deleted, events outside the gate are no longer accessible.

Importing FCS Files from BD Biosciences Applications

Follow these steps to import an FCS 2.0 or 3.0 file from any BD Biosciences software application.

1 Open the experiment that will contain the imported files.

Files can be imported into an open experiment only. Open an existing experiment or create a new one.

2 Select **File > Import > FCS files.**

3 Locate the files you want to import in the dialog that opens.

Change drive   Up one folder level
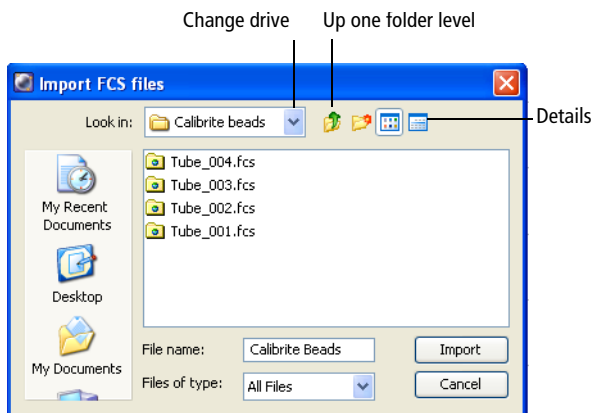
Details

- Use the buttons in the dialog to find the files to be imported.

- Select multiple files by holding down the Ctrl key as you click the file names.

To order imported files by date in the Browser, click the **Details** icon in the dialog and select the files in the date order you want. You can select any type of file, but only those files saved in a valid FCS 2.0, 3.0, or 3.1 format will be imported.

4 Click **Import.**

⚠ FCS files within an exported experiment do not contain the same information as FCS files exported using the export FCS command. Do not import FCS files within an exported experiment using the import FCS files command.

A progress bar appears showing the status of the import.

For each valid FCS file, a tube is created in a specimen in the open experiment. The specimen name is determined by keywords in the FCS file.

If any of the following keywords is defined, the first defined keyword is used as the specimen name: $SRC, SAMPLE ID, PATIENT ID. Otherwise, a default name of Specimen_00*x* is used.

In the following example, the new specimen is appended with *_001* since the experiment already contains a specimen with the same name.
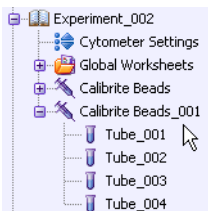
If the FCS file contains the *TUBE NAME* keyword, the value for that keyword is used as the tube name. If no *TUBE NAME* keyword exists, the FCS file name is used as the tube name.

Importing FCS Files from Other Applications

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

NOTE Linear data imported from other applications in FCS 2.0 or FCS 3.0 file format is normalized to the range of BD FACSDiva linear data. The conversion of 256 or 1,024 resolution data to 262,144 resolutions causes a binning effect in the plot displays.

- Linear data saved in FCS format from another software application is normalized to the range of BD FACSDiva linear data using the following formula:

$$X_{LIN} = X_{FCS} \times \frac{2^{18}}{\text{resolution}}$$

Where X_{LIN} = linear BD FACSDiva data, X_{FCS} = linear data in the FCS file, resolution = resolution in the FCS file (256, 1,024, 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 the FCS file, #dec = number of decades in the FCS file (usually four), res = resolution in the FCS file (256 or 1,024)

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

Important Considerations

Note the following behavior when exporting or importing FCS files:

- FCS files that begin with a space cannot be imported. Remove any leading spaces before importing FCS files.

- Any plots, gates, and statistics views associated with a tube are not included with an FCS file. To include analysis objects, export or import the experiment rather than the FCS file.

- While importing an FCS file, if the software determines that the file is too large to fit into memory, the data will be truncated and a warning message displayed.

- Data cannot be appended to an imported tube unless the data was imported as FCS 3.0 or FCS 3.1, and the file was generated using BD FACSDiva software v2.0 or later.

- If an imported tube does not contain cytometer settings from BD FACSDiva software v2.0 or later, the cytometer settings cannot be copied and the Duplicate Without Data option is unavailable when the tube is selected in the Browser.

- When you export FCS 2.0 files, the Enable Compensation checkbox must be selected for each tube in order to export compensated data. If the files are exported as uncompensated data (the Enable Compensation checkbox is cleared), 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 (the Enable Compensation checkbox is 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 or FCS 3.1 files, a valid compensation matrix might not be created if a tube is exported with only a subset of its recorded parameters (that is, not all parameters selected in the Export Parameters dialog). In this case, only compensated data is exported.

  If the tube contains enough parameters to construct a valid compensation matrix, uncompensated data is exported with the corresponding compensation matrix.

⚠ When you export compensated data with negative values (see Using the Compensation Tab on page 131) 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

Select **File > Export > Experiments** to export experiments to the hard drive. During the export, you can select to have the software remove exported experiments from the Browser. This removes them from the database, frees disk space, and can improve computer performance.

An exported experiment contains all Browser elements and their hierarchical structure, as well as worksheets and associated analysis objects (plots, gates, statistics views). Experiment elements are exported as an XML file, while data is exported in FCS 3.0 file format. To read the contents of the experiment, import it back into BD FACSDiva software using the import experiments command.

⚠ FCS files within an exported experiment do not contain the same information as FCS files exported using the export FCS command. Do not import FCS files within an exported experiment using the import FCS files command. To prevent confusion, we recommend that you store exported experiments in the BDExport\Experiments folder that is set up for you during software installation.

Exporting Experiments

1   Select one or more experiments in the **Browser.**

    If one of the experiments is open or expanded, use the Ctrl key to select only experiments.

2   Select **File > Export > Experiments.**

⚠ Make sure you select the **Experiments** command, not the **Experiment Template** command. Data is not included when you export an experiment as a template.

3   Make appropriate selections in the **Export Experiments** dialog (Figure 5-1).

    You can rename the experiment in this dialog before exporting.

Figure 5-1 Exporting experiments
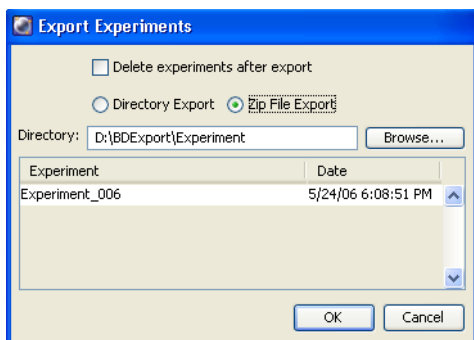
- Select **Directory Export** to export the experiment into a folder. Select **Zip File Export** to export the experiment as a Zip file (with FCS files.)

- Verify that the experiments listed are those you intended to export. If they are not, click **Cancel** and repeat steps 1 through 3.

- Select the **Delete experiments after export** checkbox if you want to delete the listed experiments from the Browser after exporting.

- Specify the directory where the experiments will be stored. By default, experiments are exported to BDExport\Experiment. Enter the directory path in the field or click the **Browse** button and select the storage location in the location dialog that appears.

  NOTE 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.

NOTE Exported experiments cannot be imported into older versions of BD FACSDiva software.

Exporting an Experiment Without Data

For third-party applications, an experiment can be exported without any data being associated with it. The experiment is saved as an XML file without any associated FCS files. The XML file is saved in a predefined, fixed folder location.

To export an experiment without data:

1   Select an experiment in the **Browser**.

2   Select **File > Export**.

3   Select **Experiment To Automation Template**.

The XML file is saved to D:\BDExport\Templates\Experiment\Automation.
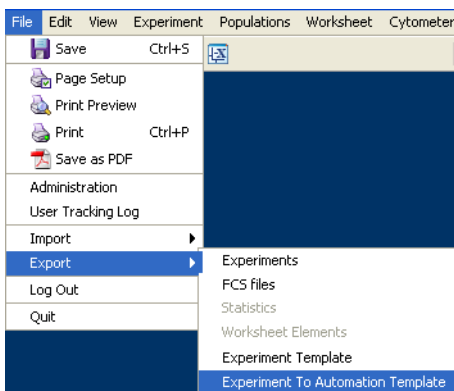
Importing Experiments

Experiments from the current or previous versions of BD FACSDiva software can be imported using the import experiments command. When an experiment is imported, its components are listed in the same order as when the experiment was originally exported. If an identically named experiment already exists in the Browser, the imported experiment is appended with _00*x*, where *x* is the next consecutive number for experiments of the same name.

To import an experiment:

1   Select **File > Import > Experiments.**

2   Locate the experiment or Zip file to be imported in the dialog that opens (Figure 5-2).

Figure 5-2 Importing experiments
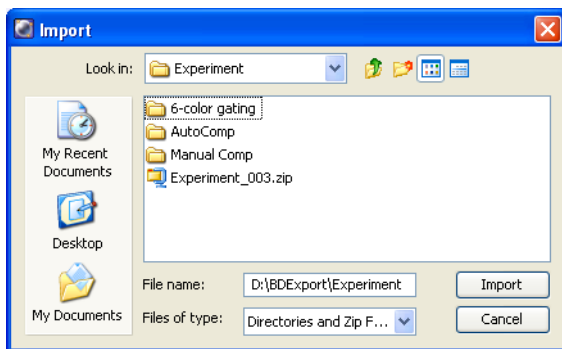
NOTE   Only BD FACSDiva experiments can be imported using this command. To import data from another application, see Importing FCS Files from Other Applications on page 258.

3   Select the folder containing the required experiment and click **Import.**

NOTE   If you select a folder that contains only data, a warning dialog opens. Select only folders containing valid BD FACSDiva experiments.

Using the Data Manager Utility

BD FACSDiva Data Manager is installed during BD FACSDiva software installation. It can be used to back up the current database to disk or a mapped network drive and to replace the current database with a stored copy. To back up your database to another storage medium (CD or tape), see the documentation provided with your computer.

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

To start the Data Manager:

1 Exit BD FACSDiva software, if necessary.

Data Manager cannot start when BD FACSDiva software is running.

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

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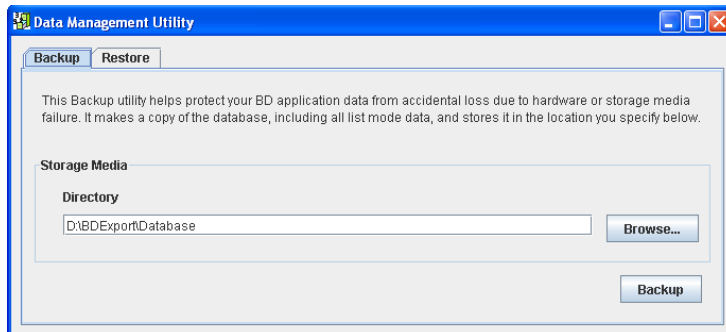
The Data Manager window has two tabs. Click the appropriate tab to initiate the following actions.

- **Backup.** Creates a backup copy of the BDFACS database on a specified drive. See the following section.

- **Restore.** Replaces the current database with a backup copy from a specified drive. See page 268.

Backing Up the Database

During a backup, the Data Manager copies the current database and associated list-mode data to a specified location. Backup files can be stored in any location on the hard drive as long as sufficient memory is available.

It is strongly recommended that you back up files to a hard disk or network device other than the one containing the database. If you back up to the disk containing the database and the hard disk crashes, both the BD FACSDiva database and the backup copy will be lost.

Before backing up the database, make sure you have adequate storage space on the backup medium. Exceeding the capacity of the hard disk can result in system errors and potential data loss.

If the workstation is connected to a network, files can be backed up directly to a mapped network drive. See Mapping a Network Drive on page 268.

NOTE Backing up does not free up space on the hard drive because the original files are retained. To back up directly to a DVD, use the Data Manager to direct-format the DVD and then back up to the DVD. Otherwise, back up to the drive and use DVD Writer to copy the backup onto the DVD.

1 Start the Data Manager utility.

See Using the Data Manager Utility on page 265. The Data Manager window appears with the Backup tab displayed.

2   Verify or change the path of the backup folder in the **Directory** field.
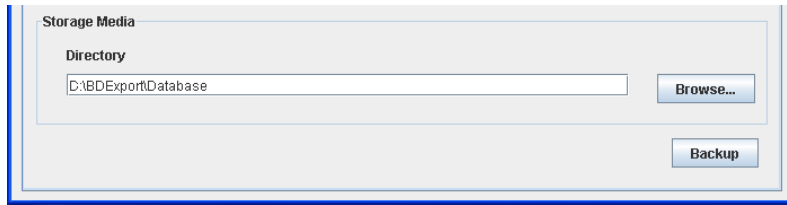
To change the path, click the **Browse** button. After mapping a network drive, files can be backed up directly to the network. See Mapping a Network Drive on page 268.

NOTE   To keep track of backups, we recommend that you save each backup in a separate folder. Click the **Browse** button and select a new folder. Make sure your most recent backup is valid before you discard or overwrite a previous version.

3   Click **Backup.**

A progress box opens, and a message is shown when the backup is complete.

4   Use Windows Explorer to verify the presence of the backup files in the specified location.
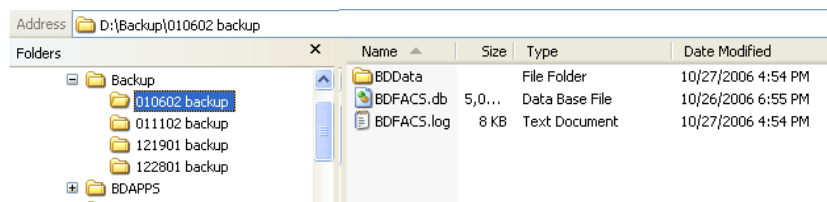
Data Manager adds three items to the specified folder during a backup:

•   A copy of the BDFACS.db database

•   A copy of the BDFacs.log

- A copy of the BDData folder containing all FCS files in the database at the time of backup

Mapping a Network Drive

If your workstation is connected to a network, use the following instructions to create a network drive. Once the network is mapped, you can view the contents of the drive by clicking its icon in Windows Explorer or in My Computer. Files can be backed up directly to a mapped network drive using the Data Manager.

1 Start Windows Explorer.

Select **Start > Programs > Accessories > Windows Explorer.**

2 Select **Tools > Map Network Drive.**

3 Select a drive letter from the **Drive** menu.

4 Enter the path of the network backup folder in the **Folder** field (*\\servername\foldername*).

Alternatively, click the **Browse** button and navigate to the required folder.

5 Click **Finish.**

Restoring a Database

Use the Restore tab of Data Manager to replace the existing database with a backup copy. Note that when you restore data from a backup, Data Manager automatically stops the Sybase Adaptive Server® Anywhere service. Therefore, make sure you have privileges to stop and restart system services before you begin the restore.

⚠ During a data restore, the current database is overwritten by the backup copy. Once the restore is in progress, it cannot be stopped or cancelled. To save your current data, export all experiments before you begin the restore.

1 Start the Data Manager utility.

See Using the Data Manager Utility on page 265. The Data Manager window opens with the Backup tab displayed.

2 Click the **Restore** tab.

3 Locate your database backup.
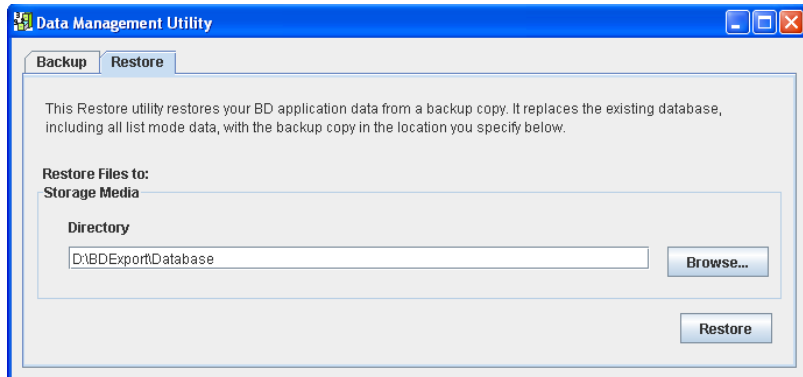
To change the path, click the **Browse** button or enter the path in the **Directory** field.

4 Click **Restore.**

The following dialog opens.
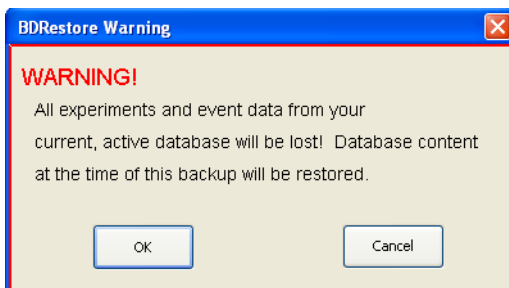
5 Click **OK** to continue.

The Data Manager utility verifies that the user has logged in as the administrator. If the verification succeeds, the Data Manager restores the backup files to D:\BDDatabase.

A progress box appears, followed by a message that the backup is complete.

NOTE　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 273 for instructions.

6　Click **OK** to close the message.

6

Troubleshooting

The tips in this section are provided to help you troubleshoot issues that might arise when using BD FACSDiva software. For cytometer-specific troubleshooting, see your cytometer manual.

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

Troubleshooting suggestions in this chapter are grouped under the following headings:

- Electronics Troubleshooting on page 272

- General Software Troubleshooting on page 273

- Compensation Setup Troubleshooting on page 276

- Analysis Troubleshooting on page 279

- Data Manager Troubleshooting on page 280

- Printing Troubleshooting on page 282

Electronics Troubleshooting

Observation	Possible Causes	Recommended Solutions
"Cytometer Disconnected" is in Cytometer window	Cytometer power is switched off.	Turn the cytometer power on.
	Communication failure between workstation and cytometer.	• Exit the software and then restart it. • If restarting does not work, reset the cytometer electronics: switch the power off, wait 10 seconds, and then switch the power on. Restart the computer.
	Ethernet cable between workstation and cytometer is disconnected.	Unplug and then plug in the cable connectors and make sure they are secure.
	IP address has changed.	Enter the correct IP address. Call BD Biosciences for assistance.
"Upgrading firmware…" is in Cytometer window	Firmware loading is incomplete.	Wait two minutes. If the message remains, restart the computer.
"Master DAQ Overflow" is in Cytometer window	Event rate is too high.	Decrease the event rate or verify the threshold.
	Too many analysis objects are on the worksheet or too many events are displayed.	Delete analysis objects, decrease the **Display** value, or delete parameters from the cytometer settings **Inspector.**
"Cytometer not responding" status message	Various possible causes.	Reset the cytometer electronics: switch the power off, wait 10 seconds, and then switch the power on. Restart the computer. ⚠ If this occurs during sorting, turn off the deflection plates before resetting the electronics.

General Software Troubleshooting

Observation	Possible Causes	Recommended Solutions
Software is not starting	FTP service was started by another application.	Stop the FTP service for the other application. **1** Select **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** Start BD FACSDiva software.
	Database is loading.	Verify that the **Start Services** buttons are available. If the buttons are disabled, the database is still loading. A large database can take 30 minutes or more to load. Delete or export experiments to reduce the database size.
	Conflicting application, driver, or security update was installed.	Contact your service representative for assistance.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Unable to access the database	Adaptive Server Anywhere is not running.	Verify that the database server has been started.
		1 Select **Start > Settings > Control Panel** and double-click the **Administrative Tools** icon.
		2 Double-click the **Services** icon.
		3 Select **Adaptive Server Anywhere.** If the **Start** button is enabled, click the button to start the database server. If the buttons are unavailable, the database could be loading.
Software is not responding	Other applications are running in the background.	Exit all other applications. Do not run scheduled tasks such as virus scans or disk defragmentation in the background when you are running BD FACSDiva software.
	Software is saving or loading a large data file.	Look for activity on the screen. If there is no activity, restart BD FACSDiva software.
	Too many histograms are displayed.	If screen updating is slow, move some histograms down on the worksheet and scroll up.
	Too many plots or gates are in the worksheet.	Reduce the number of plots or gates.
	Large number of statistics are being calculated.	Calculating statistics is a memory-intensive operation. If you are calculating many statistics on a large number of displayed events, wait 1–2 minutes before you use the software.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Software message "Hardware key not accessible…"	Security module is disconnected.	Verify that the security module is securely connected in the USB port, and then restart BD FACSDiva software.
Error message is in Status tab	Communication, fluidics, or sorting error.	Shut down the computer and the cytometer, and then restart them. If the message reappears, contact technical support. Make sure you provide the exact wording of the status message.
Shortcut keys (Ctrl+X) or Delete key are not functioning	Keys are not activated.	Use the menu selections to activate the keys. After the initial activation, the shortcut keys and Delete key can be used.
Numeric keypad is not functioning	Num Lock key was reset.	Press the Num Lock key on the keyboard and try the keypad again.
No wireless keyboard or mouse response	Keyboard or mouse is too far away from workstation.	Move the keyboard or mouse closer to the workstation.
	Obstruction between the keyboard or mouse and the workstation.	Remove the obstructing object.
	Batteries are low.	1 Replace the batteries. (See the documentation provided with the keyboard or mouse.) 2 Restart the computer.
"Error 12" software message	Driver is not installed.	Reinstall the software.
Plot button is disabled	No tube is selected in the Browser (normal worksheets only).	Select a tube to enable the button.

General Software Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
No cytometer settings are in Cytometer window	Current tube pointer is not set.	Click to set the current tube pointer.
	No experiment is open.	Open an experiment.
Faulty screen display or slow user interface response	Another program is running.	Close all other open programs.
	Pointer shadow is enabled.	Select **Start > Settings > Control Panel.** Double-click the **Mouse** icon and click the **Pointers** tab. Clear the **Enable pointer shadow** checkbox, then click **OK.**

Compensation Setup Troubleshooting

Observation	Possible Causes	Recommended Solutions
Error creating compensation controls	Naming conflict with existing control or worksheet.	**1** Locate the control or worksheet that is named *(ParameterName)* Stained Control, and change the name.
		2 Create the compensation controls again.

Compensation Setup Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Error calculating compensation	PMT voltages are not consistent between compensation controls.	Re-record all compensation controls with the same PMT settings.
	Insufficient single-stained controls for a setup.	Calculate compensation manually.
	Wrong number of gates for control plot(s).	• Display a population hierarchy and remove extra gates on control plots, if needed. • When no unstained control is included, create a gate around the negative population for each single-stained control.
	No data appears for one or more controls.	Record data for all controls.
	No root gate appears for the first control.	Create a P1 gate in the FSC vs SSC plot for the appropriate control.
	Wrong fluorochrome run for the stained control.	Re-record all compensation controls and recalculate.
	Insufficient events appear in the gated population.	Append data to the tube.
		Verify that the gate is set appropriately for the corresponding control.

Compensation Setup Troubleshooting (continued)

Observation	Possible Causes	Recommended Solutions
Error calculating compensation (continued)	Insufficient separation between positive and negative populations.	See your cytometer manual for suggestions on how to optimize the fluorescence signal.
		Re-record the compensation controls, draw new gates, and calculate the compensation again.
BD FACSCanto setup file cannot be imported	Setup file is missing or has errors.	Exit from BD FACSDiva software, re-run setup in BD FACSCanto clinical software, and open BD FACSDiva software again.

Analysis Troubleshooting

Observation	Possible Causes	Recommended Solutions
Fewer events than expected are in the gated population	Current tube pointer is set to the wrong tube.	Set the pointer to the correct tube.
	On-axis events are left out of the gate.	Redraw the gate to make sure events on the axis are included, or format the plot with biexponential display so that all events are shown.
	Plot is zoomed in.	Click the **Zoom Out** button for the plot or make the gate bigger.
	Laser delay is set incorrectly.	Adjust the laser delay settings. See your cytometer manual for instructions.
	Window extension is set incorrectly.	Adjust the window extension. See Using the Window Extension on page 114.
Missing analysis objects on worksheet	Analysis objects are obscured by other objects.	Double-click the tube containing the analysis objects of interest. Select the objects on the worksheet and move them to another location.
No events are in plots	Current tube pointer is not set.	Set the current tube pointer next to the appropriate tube.
	Data is no longer linked to the experiment.	Contact BD Biosciences for assistance in locating the missing data.
Differing statistics between exported and imported file	Compensated data with negative values is set to zero during FCS 2.0 export.	• Export the original data as FCS 3.0. • Export FCS 2.0 data with compensation disabled. • If statistical differences are significant, re-record the file.

Data Manager Troubleshooting

Observation	Possible Causes	Recommended Solution
Data Manager is not starting	BD FACSDiva software is in use.	Exit BD FACSDiva software before starting Data Manager.
Message during backup: "Unable to complete task. Exit value is: x."	Attempted to back up database into BD FACSDiva directory.	Do not back up the database into the Program Files\BD FACSDiva Software folder. Back up the database to a different disk location.
Software message: "An error occurred while attempting to restore the database."	No valid backup is in the specified directory.	Verify that the directory path is entered correctly.
Unable to read or write FCS file when attempting import or export	Incorrect file name or file path.	Verify the source and target file names and paths.
	File is in use by another program.	Verify that the file is not in use by another program.
	File was created with an unsupported FCS version.	Files for import or export must be created with a supported FCS version (2.0 or 3.0).
	Errors in file.	If the FCS file is corrupt, regenerate the file before importing or exporting.
	Incorrect data in file.	FCS files must contain list-mode data and must have the correct byte order.
	Data limit exceeded (FCS 2.0 only).	FCS 2.0 file format can write up to 99,999,999 bytes of list-mode data.

Data Manager Troubleshooting (continued)

Observation	Possible Causes	Recommended Solution
Unable to read or write FCS file when attempting import or export (continued)	File is read-only or hidden.	Verify that the file is not set as a read-only or hidden file.
	Data type within file is other than Integer or Float.	When creating the FCS file, the data type must be either Integer or Float.
	Incorrect resolution for one or more log parameters.	Log parameters must have 1,024 resolution.
	Incorrect keyword value.	Verify that all keywords are correctly specified.
Unable to append data to an existing FCS file	Cytometer settings changed since original data was collected.	When appending data, make sure the cytometer settings match those of the data already recorded.
	File is FCS 2.0.	You cannot append data to an FCS 2.0 file. Select an FCS 3.0 file instead.
One or more tubes were not exported	No data in the tubes selected for export.	Verify that the tubes selected for export contain data. Tubes with no data will be skipped.
	Incorrect parameters in tubes selected for export.	Verify that the tubes selected for export contain the required parameters.
Data is truncated in the imported file	File is too large to fit in available memory.	Move the file to a workstation with more RAM to maximize available import space.

Printing Troubleshooting

Not all print drivers are compatible with BD FACSDiva software. For optimal printing results, we recommend that you use only PCL-native print drivers. Emulated or postscript printer drivers are not recommended. Many printers have multiple print drivers available. Check with your printer manufacturer to obtain an appropriate driver.

Print spooling problems can occur on some Tektronix Phaser printers, causing excessive amounts of data to be spooled. When this occurs, the print spooler can corrupt the data. If you experience problems printing through the print spooler, try one of the following:

- Print directly to the printer. See the following section.

- Use a third-party application to print to a PDF file, and then print the PDF file.

Printing Directly to the Printer

To set up printing directly to the printer:

1 Select **Start > Devices and Printers.**

2 Right-click the appropriate printer icon and select **Printer properties.**

3 Click the **Advanced** tab.

4 Select the **Print directly to the printer** option.

> **NOTE** When using this print option, BD FACSDiva software is not available during printing.

Appendix A

Keyboard Shortcuts

This appendix provides a list of the available keyboard shortcuts.

- Keyboard Shortcuts on page 284

Keyboard Shortcuts

Keyboard shortcuts are provided for the following functions.

Objective	Key Combination	Condition to Activate Shortcut
Start/stop acquisition	Click the Browser pointer	To start, the current tube pointer must be set (green). To stop, acquisition or recording must be in progress (yellow or orange pointer).
Start/stop recording	Alt+click the Browser pointer	To start, the current tube pointer must be green or yellow. To stop, recording must be in progress (orange pointer).
Start batch analysis	Alt+S	Batch Analysis dialog must be active.
Pause batch analysis	Alt+P	Batch Analysis dialog must be active.
Continue batch analysis	Alt+N	Batch Analysis dialog must be active.
New folder	Ctrl+N	User icon or folder must be selected.
New experiment from a template	Ctrl+E	Item must be selected in the Browser.
Open experiment	Ctrl+O	Closed experiment must be selected.
Close experiment	Ctrl+W	Open experiment must be selected.
Find experiment	Ctrl+F	Item must be selected in the Browser.
Save experiment	Ctrl+S	
New specimen from a panel template	Ctrl+M	Item must be selected in an open experiment.
New tube with an analysis template	Ctrl+T	Specimen or tube must be selected in an open experiment.

Objective	Key Combination	Condition to Activate Shortcut
Duplicate (without data)	Ctrl+D	• Specimen or tube must be selected in an open experiment (not available for imported tubes). • Plot must be selected on the worksheet.
Cut	Ctrl+X	Item must be selected in the Browser or on the worksheet.
Copy	Ctrl+C	Item must be selected in the Browser or on the worksheet.
Paste	Ctrl+V	Appropriate recipient must be selected in the Browser, or nothing must be selected for a worksheet element.
Show population hierarchy	Ctrl+G	A tube must be selected in an open experiment or plot must be selected on the worksheet.
Create statistics view	Ctrl+R	Tube must be selected in an open experiment or plot must be selected on the worksheet.
Print active worksheet	Ctrl+P	Experiment must be open.

The following table has key combinations to show or hide specific windows.

Window	Key Combination
Browser	Ctrl+Shift+B
Cytometer	Ctrl+Shift+N
Inspector	Ctrl+Shift+P
Worksheet	Ctrl+Shift+W
Acquisition Controls	Ctrl+Shift+C
Acquisition Status	Ctrl+Shift+T

Window	Key Combination
Sorting	Ctrl+Shift+S
Carousel Controls	Ctrl+Shift+L

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. Include 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 function that automatically advances a selected set of tube data through an analysis template on a global worksheet.

Browser   List of all experiment data in a hierarchical view. Interface for setting up experiments and recording data.

channel   Output from the channel DAQ board which measures the input of a single detector. Bins on a histogram are also referred to as channels.

coefficient of variation (CV)   The standard deviation of the data divided by the mean of the data. Typically expressed as a percentage (also known as relative standard deviation).

When applied to channel data measured on a population of cells, the CV is a measure of variation independent of the population mean.

compensation   Process by which spillover fluorescence is removed from secondary parameters so that fluorescence values for a parameter reflect only the fluorescence of the primary fluorophore.

contour plot Graphical representation of two-parameter data in which contour lines show the distribution of events.

Similar to a topographical map, contour lines show event frequencies as peaks and valleys.

current tube pointer Pointer or plot icon next to tubes in an open experiment in the Browser. Indicates the tube currently selected for data acquisition, recording, or data display on a global worksheet.

When the software is connected to the cytometer, the pointer can also be used to control acquisition.

cytometer settings Collection of values for parameters measured, photomultiplier tube (PMT) voltages, threshold, compensation, and any ratio measurements collected.

cytometer setup and tracking Setup function in the BD FACSDiva software workspace.

Cytometer Setup and Tracking beads Beads used to automatically perform basic cytometer setup and performance tracking.

data file A collection of measured values from a single tube combined with text describing the data that has been stored to disk.

density plot Graphical representation of two-parameter data in which colored dots show density for events with the same signal intensity.

A density plot simulates three-dimensional event display.

derived gate Combination of one or more defined populations using the Boolean operators AND (intersected gate), OR (joined gate), or NOT (inverted gate).

dot plot Graphical representation of two-parameter data.

Each axis of the plot displays values of one parameter. A dot represents an event (particle).

Events to Record list The Events to Record list is one method for entering numbers of events in the Events to Record fields. This list is in the Experiment Layout.

experiment	Group of elements used to record and analyze data from the flow cytometer.
	An experiment can include any combination of the following: global worksheets, specimens, tubes, FCS data files, keywords, plots, gates, statistics, population hierarchies, worksheets, text, lines, or arrows.
flow cytometry standard (FCS)	Standard format for flow cytometer data files.
gate	Two-dimensional boundary defining a subset of the total sample population.
	See also derived gate, interval, population.
global worksheet	Worksheet for which elements can be used to display multiple data sets by moving the current tube pointer.
	See also worksheet, Worksheet window.
grid	Cross-hatched lines displayed on the worksheet used to align and resize analysis elements. Analysis elements can be snapped to the grid.
histogram	Graphical representation of single-parameter data.
	The horizontal axis of the graph represents the increasing signal intensity of the parameter, and the vertical axis represents the number of events (count).
Inspector	Software interface for viewing or modifying the attributes of a single object or set of objects on the worksheet or in the Browser.
interval	One-dimensional boundary defining a subset of the total sample population.
	See also gate, population.
laser delay	Amount of time between signals from different laser intercepts.
panel analysis	A panel template that is used to apply changes to the normal worksheet elements of a selected specimen.

panel template	Group of labeled tubes commonly used together in the same experiment.
	Any specimen can be exported as a panel. Along with the specimen name and collection date, an exported panel contains a group of tubes and any parameter labels defined for each tube. Exported specimen panels can also include global worksheets and their associated analysis objects.
parameter	Measurement of a cell property that is ascertained as the cell passes through the laser beam.
	Each parameter is the output of a single photomultiplier tube or photodiode, measuring fluorescent or scattered light.
population	Data subset defined by a gate or interval.
robust co-efficient of variation (rCV)	Robust coefficient of variation is calculated as follows:
	$\%rCV = ((rSD)/median) * 100)$
robust standard deviation (rSD)	Robust standard deviation is calculated as follows.
	The median of the data sample is computed:
	$\theta_{median} = med_i\{x_i\}$
	From that, the median absolute deviation is computed:
	$\sigma_{median} = med_i\{(|x_i - \theta_{median}|)\}$
	Then the robust standard deviation is computed:
	The constant is: $\sigma_{median} = \sigma_{median}/\phi^{-1}(0.75)$
snap-to gate	Gate drawn automatically when you select a peak or cluster of events in a plot.
	Unlike static gates, snap-to gates are automatically redrawn when data in the gate changes.
specimen	Browser object representing the type of material to be analyzed, the tubes used to analyze the material, the collection date, and user-defined keywords.

spectral overlap   Fluorescence detected in a channel other than the one for which it is intended.

standard deviation (SD)   A measure of the spread around the mean for events within a defined population, defined as:

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

stopping gate   Population for which events are to be counted.

storage gate   Population for which events are to be recorded.

tethered gate   Static gate linked to move relative to a snap-to gate.

threshold   A trigger signal and level of discrimination to eliminate unwanted events.

Only events with parameter values above the threshold will be analyzed.

tube   Browser object representing cytometer settings, acquisition criteria, parameter labels, recorded event data, analysis objects, keywords, and sort layouts (if applicable for your cytometer) 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.

worksheet   Tabbed area within Worksheet window for displaying data for a specific tube. All objects on a worksheet are printed with a single Print command.

See also global worksheet, Worksheet window.

Worksheet window   Window for viewing analysis objects on normal worksheets or global worksheets.

workspace window Component in BD FACSDiva software workspace.

Workspace windows can be hidden or shown, resized, and closed. The visibility, size, and position are saved when you quit the software and are restored when you start the software the next time.

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