Getting Started with BD FACSDiva Software

For In Vitro Diagnostic Use

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History

Revision	Date	Change Made
337647 Rev A	01/04	Initial release for BD FACSDiva software version 4.0.
338007 Rev A	04/04	Updated for version 4.0.1: CE IVD marking
338574 Rev A	09/04	Updated for version 4.1; see the New Features section for details. **Modified:** Creating an Experiment, Working With Experiments in the Browser, Importing Data, Using Tethering and Batch Analysis, Gating Multicolor Experiments, Setting Up Compensation Controls. **New:** Changing Browser Button Defaults, Using Biexponential Display.
640744 Rev A	05/06	Updated for version 5.0; see the New Features section for details. **Modified:** Becoming Familiar with the Workspace, Using Administrative Options, Using Biexponential Display. **New:** Adjusting Biexponential Scaling, Copying and Pasting Gates, Publishing and Presenting Data. **Moved:** Calculating Compensation Manually (to *BD FACSDiva Software Reference Manual*).

Contents

About This Guide

This *Getting Started* guide provides tutorials designed to familiarize you with BD FACSDiva™ software. If you are a new user, use the tutorials to get started with the software. If you are an experienced user, try the updated tutorials to become familiar with new software features. Updated tutorials are highlighted in the New Features section.

New Features

This version of BD FACSDiva software includes support for the BD FACSCanto™ II flow cytometer, and includes features for plate-based acquisition using the BD™ High Throughput Sampler (HTS) on both the BD FACSCanto and BD FACSCanto II flow cytometer.

New features available to all instruments include the following.

New Look and Feel

The BD FACSDiva software interface has been updated to provide a more intuitive look and feel. If you are familiar with a previous version of BD FACSDiva software, you will notice that many of its icons have changed. In addition, acquisition controls and status are now located in the Acquisition Dashboard, which contains all the software elements used to acquire data and monitor acquisition. For an overview of the Dashboard, see Becoming Familiar with the Workspace on page 13. For more detailed information, refer to Acquisition Controls in the *BD FACSDiva Software Reference Manual*.

Administrative Options

- User profiles can be exported and imported to assist with sharing profiles between workstations.
- Administrators can control user access to certain instrument settings (window extension, area scaling, laser delay).
- The ability to create instrument configurations is restricted to users with administrative access.

To see the new administrative options, see Creating a New User on page 18 or refer to Administrative Options in the *BD FACSDiva Software Reference Manual*.

Enhanced Instrument Configuration Options

- BD-default instrument configurations can no longer be edited or deleted.
- Instrument configurations can be duplicated, exported, imported, and printed.

To see the new configuration options, refer to Instrument Controls in the *BD FACSDiva Software Reference Manual*.

Biexponential Display

The biexponential display feature has been enhanced as follows. Refer to the *BD FACSDiva Software Reference Manual* for details.

- Biexponential display is enabled by default, but can be disabled in user preferences, allowing more events to be recorded per experiment.
- Automatic biexponential scaling values can be manually adjusted in the Biexponential Editor. In addition, scaling values can be exported, imported, and applied to other tubes in the experiment.
- You can use the shortcut menu in a biexponential plot to optimize the display of a selected population. For an example, see Adjusting Biexponential Scaling on page 50.

- Plot scales are saved as keywords, so that scaling is preserved when you transfer the FCS file to another workstation equipped with BD FACSDiva software (version 5.0 or later).

- Gates are preserved when a plot is switched from log to biexponential scaling, and gates can be copied between log and biexponential plots. Gates are linked to parameter values, so they adapt as plot scaling changes.

- Gates in biexponential plots can be used as stopping gates.

Analysis Features

- You can now copy gates between plots, and copy gating hierarchies from one population hierarchy to another. Gates and hierarchies can be copied between tubes within the same experiment, or between experiments. For an example, see Gating Multicolor Experiments on page 57.

- New options in the Plot Inspector allow you to hide plot outlines, tick marks, and tick mark labels, and to show a full grid outline, half grid outline, or no grid outline at all. See Publishing and Presenting Data on page 66.

- In a population hierarchy, the population that will be used for subsetting is now highlighted. See Defining and Subsetting Populations on page 45.

- By default, dot plots are sized to fit three across a worksheet; histograms are sized to fit two across.

- The maximum number of global worksheets has been increased to 50, and the maximum number of gates per tube has been increased to 256.

- When global worksheets have been assigned to tubes or specimens, assignments can be maintained during a batch analysis. See Performing a Batch Analysis on page 53.

- For populations in statistics views, values for %Grandparent can be shown.

Carousel Improvements for the BD FACSCanto Loader

- There is a new Carousel menu containing options for setting up your carousels and running cleaning tubes on the Loader. All setup functions have been combined in the Carousel Setup window.
- Carousel controls (including mixing) are included in the Acquisition Dashboard.
- New carousel controls allow you to rerun an entire carousel, a selected specimen, or individual tubes on a carousel.
- There is an improved carousel report that highlights tube errors.

Other Features and Changes

- The software can be installed in fewer steps.
- The BD FACSCanto default FSC parameter is now area (A), not height (H).
- Optimized setups from BD FACSCanto clinical software are listed in the setup catalog for BD FACSDiva software, so they can be directly linked to experiments.
- In the Acquisition Dashboard, you can enter a stopping time for manual acquisition, and a counter is available for stopping gate events.
- Instrument settings can be imported and exported out of BD FACSDiva software.
- For the BD HTS option:
  - improved Plate window with a more intuitive interface
  - plate acquisition controls within the Acquisition Dashboard
  - plate ID saved with FCS files

Conventions

The following table lists conventions used in this guide.

Table 1 Text and keyboard conventions

Convention	Use
☑ **Tip**	Highlights features or hints that can save time and prevent difficulties
NOTICE	Describes important features or instructions
>	The arrow indicates a menu choice. For example, "choose File > Print" means to choose Print from the File menu.
Ctrl-*X*	When used with key names, a dash means to press two keys simultaneously. For example, Ctrl-P means to hold down the Control key while pressing the letter *p*.

Technical Assistance

For technical questions or assistance in solving a problem:

- In BD FACSDiva software, choose Help > Online Help. Locate topics specific to the operation you are performing.

  To quickly find information on any topic, enter search terms on the Search tab. You can search individual books, or search all books at once. You can also use the Contents or Index tab to find information. To find software information, click to expand topics in the Software Help book; to find instrument information, expand the Instrument Help book.

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

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

When contacting BD Biosciences, have the following information available:

- product name, part number, and serial number; software version and computer system specifications
- any error messages
- details of recent instrument performance

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

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

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

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

BD FACSDiva Tutorials

Use the following tutorials to learn how to use BD FACSDiva™ software if you are a new user, or practice using new features if you are an experienced user.

Before You Begin

- If you already have BD FACSDiva software on your system, upgrade it using the instructions provided with the installation CD. After the upgrade, restart your instrument and computer as described in the upgrade instructions.

- If you are new to BD FACSDiva software, start the software by double-clicking the shortcut icon on the desktop.

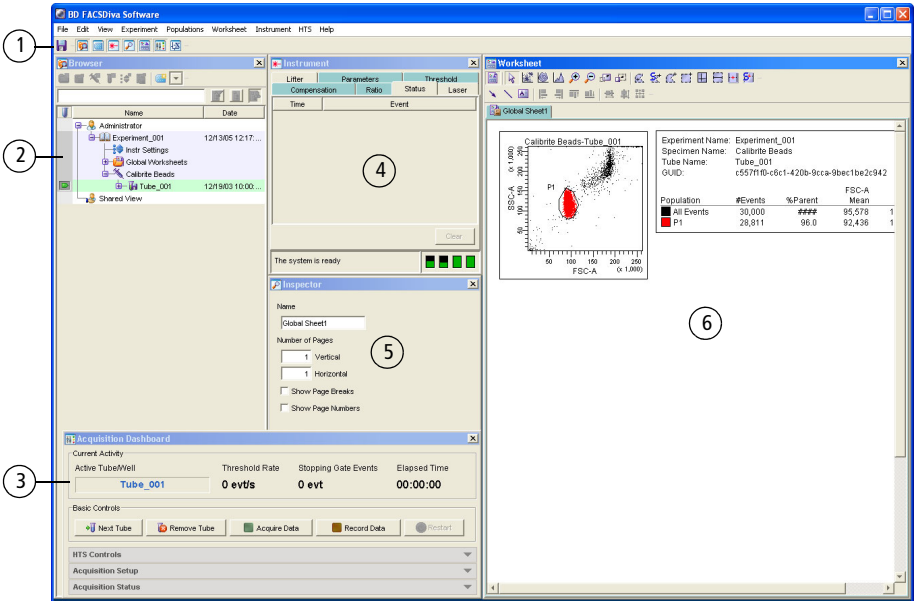
When the Log In dialog box appears, click OK. No password is required the first time you log into the software. For more information about user login, see Using Administrative Options on page 17.

Becoming Familiar with the Workspace

After a successful login, the BD FACSDiva workspace appears showing the main application windows (Figure 1). Hide or show windows by clicking buttons in the Workspace toolbar (① in the figure).

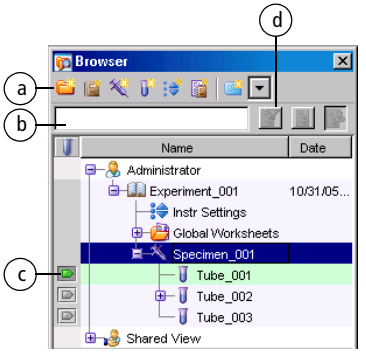
If you are new to BD FACSDiva software, take a minute to become familiar with the workspace. Some software features are instrument-specific, so your workspace components might look different from those shown in this example. For information about instrument-specific components, refer to your instrument manual.

Figure 1 BD FACSDiva workspace



①	Click a button in the **Workspace toolbar** to hide or show the corresponding window. Most windows can be resized by dragging a border or corner. 

2



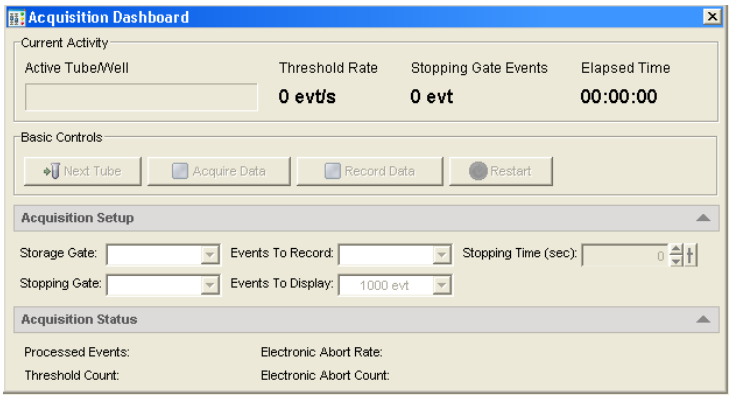
The **Browser** lists folders, experiments, and experimental elements in a hierarchical view, provides an interface for setting up experiments, and contains a **current tube pointer** (c) indicating the tube for which acquisition or analysis data will be shown.

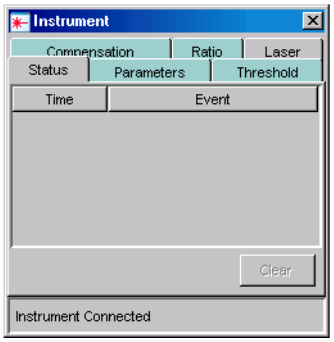
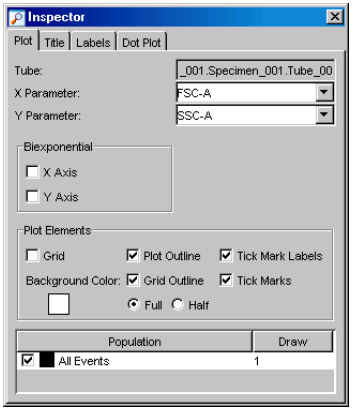
Only one experiment can be open at a time. In an open experiment, you can add, rename, or copy elements, and record or display data.

Click a button in the Browser toolbar (a) to add elements to the Browser. To display fewer experiments, enter a term in the search field (b) and click the Find button (d).

3

The **Acquisition Dashboard** contains controls for setting up, starting, and monitoring data acquisition and recording. Basic controls are always shown; Acquisition setup and status information can be hidden or shown by clicking the arrow in the title bar of each section. Your Dashboard might contain more controls than those shown depending on your instrument type and options.



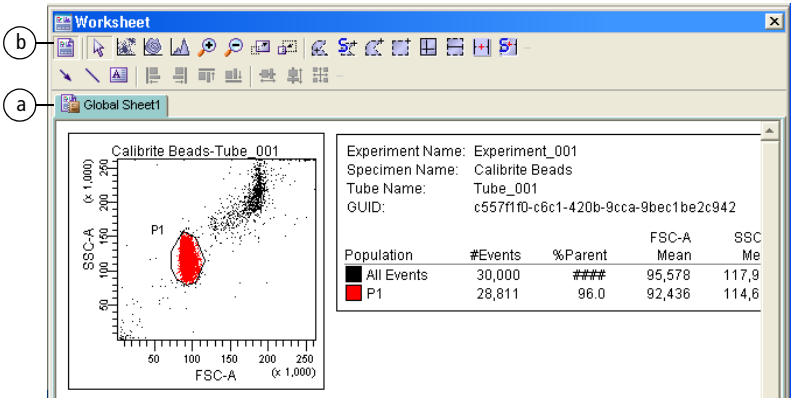
4		The **Instrument** window shows instrument connectivity status. When you are connected to an instrument, the window also shows status messages and laser controls. If an experiment is open and the current tube pointer is set, the window displays instrument settings for the current acquisition tube. Additional controls might be displayed in this window depending on the instrument you are running; refer to your instrument manual for a description.
5		Use the **Inspector** to view or modify the attributes of one or more objects in the worksheet or Browser. The contents of the Inspector change depending on what is selected. For example, this Inspector shows plot options.

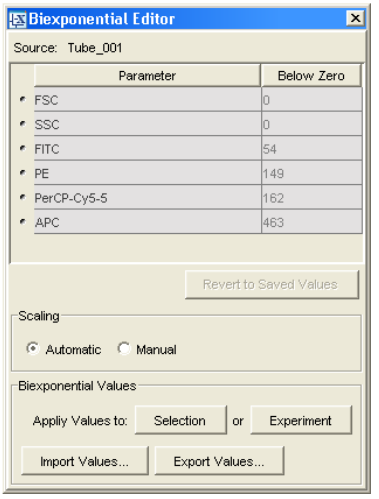
6

The **Worksheet** window is where you create global or normal worksheets containing plots, gates, statistics, and custom text.

Plots on a *global worksheet* show data for only one tube at a time, the tube that is selected with the current tube pointer. These worksheets are indicated by green-tinted tabs (a). Plots on a *normal worksheet* are tube-specific; these worksheets are indicated by gray-tinted tabs.

Click a button in the Worksheet toolbar (b) to switch between the global or normal worksheets view, create analysis objects or free text, or align objects on a worksheet.





Unlike other workspace windows, the **Biexponential Editor** is hidden by default. When you are using automatic scaling, there is no need to show the Biexponential Editor.

Use the Biexponential Editor to manually adjust biexponential scales, export and import scale values, and apply values to other elements in an experiment.

Using Administrative Options

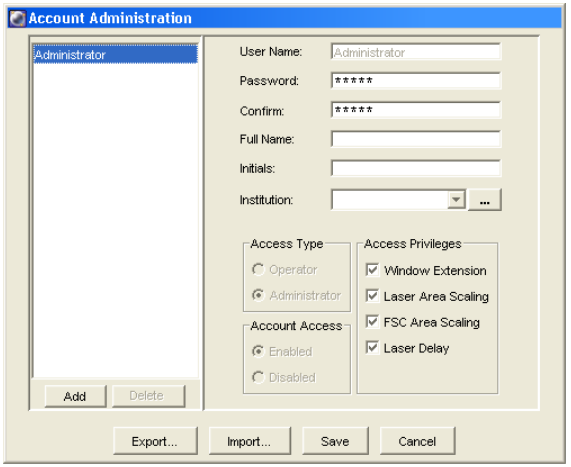
If you have administrator privileges, BD FACSDiva software allows you to create user names that can be password-protected. Experiments, workspace setup, and user preferences are saved with your login name. After login, other users cannot view your experiments unless they have administrative access, or you have designated an experiment as shared.

The following tutorials describe how to add an administrator password and create a new user, and show how workspace setup, user preferences, and the Browser view differ from one user to another.

Adding an Administrator Password

When you first log into the software, no administrator password is required. BD Biosciences recommends that you protect your administrator account by assigning a password.

1. Log into the software as Administrator, if necessary.
2. Choose File > Administration.
3. Enter a password of 1–16 alphanumeric characters in the Password field.
4. Confirm the password by typing it again.



5 Enter your name, initials, and institution if you would like.

For instructions on adding institution names to the menu, see step 8 under Creating a New User.

6 Click Save.

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

Creating a New User

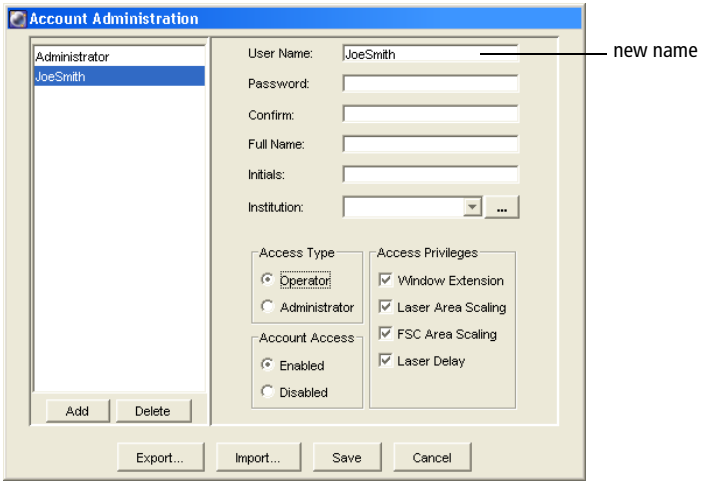
1 Log into the software as Administrator, if necessary.

Enter the password you defined in the previous section and click OK.

2 Choose File > Administration.

3 In the Account Administration dialog box, click Add.

4 Select the generic name in the User Name field, and enter *JoeSmith*.



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

5 Press the Tab key or click in the next field; enter *1234* for the password.

6 Confirm the password by typing *1234* in the Confirm field.

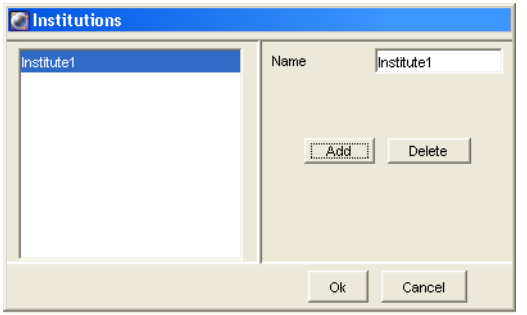
7 (Optional) Enter Joe Smith for Full Name, and JS for Initials.

This information is not required; you can leave the fields blank.

8 (Optional) To add an institution, click the "…" button next to the Institution menu:

The Institution dialog box appears.

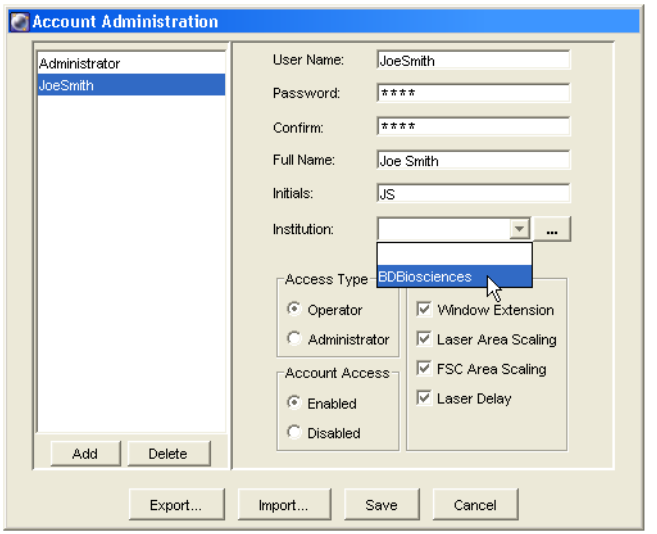
- In the Institutions dialog box, click Add.



- Select *Institute1* in the Name field, enter *BDBiosciences*, and press Return; click Ok to dismiss the Institutions dialog box.

  Institution names can be 1–20 alphanumeric characters. Spaces are not allowed.

- Choose BDBiosciences from the Institution menu in the Account Administration dialog box.

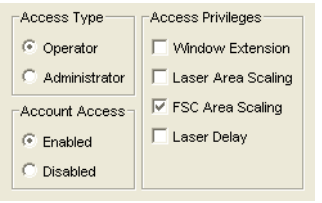


9 Leave the Access Type as Operator, and the Account Access as Enabled.

Change the Access Type to Administrator only when you want to provide the selected user with administrative privileges. Administrators can create and disable users, view all users' experiments, and edit instrument configurations.

Change the Account Access to Disabled only when you want to disable a user's login name.

10 Under Access Privileges, click to deselect all options except FSC Area Scaling.



You can limit which setting(s) a user can edit by clicking to deselect the corresponding checkbox. In this example, Joe Smith will be able to edit only FSC area scaling settings.

For more information about these settings, refer to the *BD FACSDiva Software Reference Manual* or your instrument manual.

11 Click Save to add the new user.

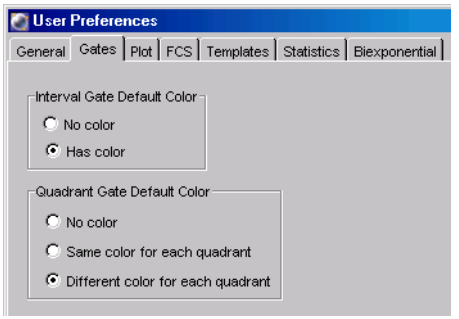
JoeSmith will be available as a login name the next time you log into the software, as you will see in the next section.

☑ **Tip** To transfer user profiles to another computer, select the user(s) you want to export and click Export. Save the exported file. Copy the saved file to the other computer and click Import in the Account Administration dialog box. All defined profiles will be imported.

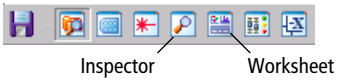
Viewing User-Specific Changes

Along with experiments, workspace setup and user preferences are saved with your login name, as you will see in this section.

1. Choose File > Log Out.
2. Log into the software as JoeSmith.
3. Choose Edit > User Preferences.
4. Click the Gates tab and change the default preferences to the following:



5. Click OK to dismiss the User Preferences dialog box.
6. Click buttons in the Workspace toolbar to hide the Worksheet window and the Inspector.

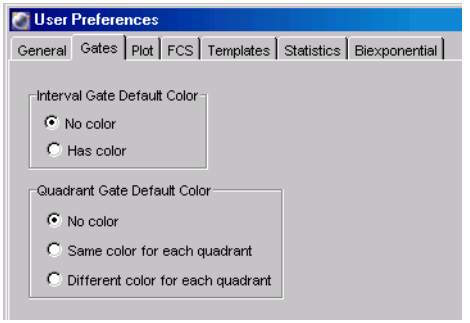


7. Drag the Instrument window on top of the Browser, and resize the BD FACSDiva workspace so it is half the width it is now.
8. Choose File > Log Out.

9 Log into the software as Administrator.

As the instrument connects, notice that the window changes to the size it was previously, and that the five main workspace windows are now shown.

10 Choose Edit > User Preferences, click the Gates tab, and note that the preferences are back to the default options.



11 Click OK to dismiss the User Preferences dialog box.

12 Log out and then back into the software as JoeSmith.

Notice how your workspace setup and preferences revert to how they were previously set when you were logged in as JoeSmith.

13 To restore the workspace to its default size and windows to their default positions, choose View > Reset Positions.

Click the Inspector and Worksheet buttons to show their windows, if you like.

14 Log out and then back into the software as Administrator.

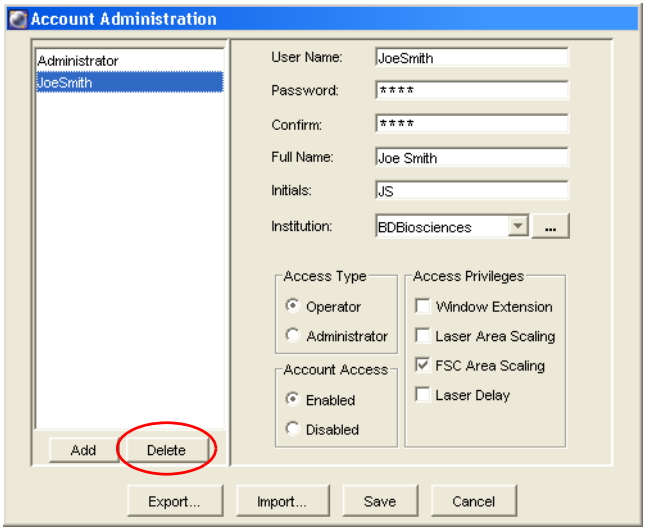
Deleting a User

Next, we will delete JoeSmith as a user name. User names can be deleted as long as they do not have saved experiments in the database. Once a user has saved experiments, you can either disable the user name, or archive their experiments and then delete the user name. Refer to the *BD FACSDiva Software Reference Manual* for details.

Only Administrators or those with administrative access can delete or disable other users.

1. Choose File > Administration.

2. Select JoeSmith in the list of users, and click Delete.



3. Click Save to save your changes and dismiss the dialog box.

4. (Optional) Log out and then back into the software and verify that JoeSmith is not available as a login name.

Creating and Working with Experiments

An *experiment* is a group of elements used to acquire and analyze data from the flow cytometer. You build experiments as you acquire and analyze data. The Browser is where you create experiments and access stored data.

There are three ways to add experiments to the Browser.

- Use the New Experiment button ( ) in the Browser toolbar to create a new, empty experiment with default instrument settings. The button can be customized to add any experiment template, as you will see in Changing Browser Button Defaults on page 38.

- Use the Experiment > New Experiment command to create a new experiment based on a saved template with customized elements such as plots, gates, statistics views, and instrument settings.

- Use the File > Import > Experiments command to access experiment elements and data from an archived experiment.

The following tutorials describe how to use each of these commands and how to work with the resulting experiments in the Browser. You must be connected to the instrument to use some of the features in this section. If needed, start up your instrument and launch and log into the software before you proceed.

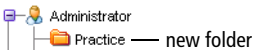
Creating a Folder and an Experiment

This section describes how to create a practice folder and an experiment containing basic analysis options.

1 Click the New Folder button ( ) in the Browser toolbar.

2 Rename the folder *Practice*.

To rename any Browser object, select it in the Browser and start typing. Press Enter to apply the new name.

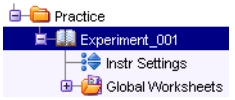


Notice that the folder is placed at the top of the Browser. Folders are listed before experiments in the order they are created.

3 Select the Practice folder and click New Experiment ( ) in the Browser toolbar.

☑ **Tip** To place an experiment in a folder, select the folder *before* you create the experiment.

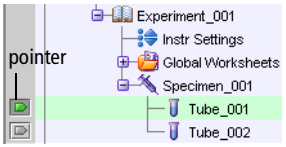
A new, open experiment is added below the folder in the Browser. The experiment contains default instrument settings and a global worksheet. The Worksheet window shows a blank global worksheet (indicated by a green-tinted tab).



4 Click New Specimen ( ) to add a specimen and tube to the experiment; click New Tube ( ) to add a second tube.

5 Click to set the current tube pointer next to Tube_001 in the Browser.

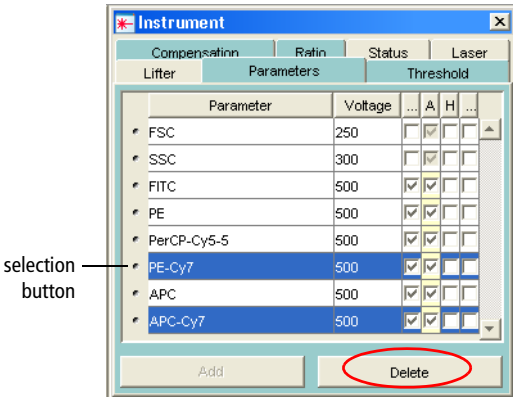
The pointer changes to green, and four green-tinted tabs appear in the Instrument window. The current tube pointer indicates the tube for which instrument settings adjustments will apply and for which acquisition data will be shown.



6 Click the Parameters tab in the Instrument window, and delete all parameters except FSC, SSC, FITC, PE, PerCP-Cy5-5, and APC.

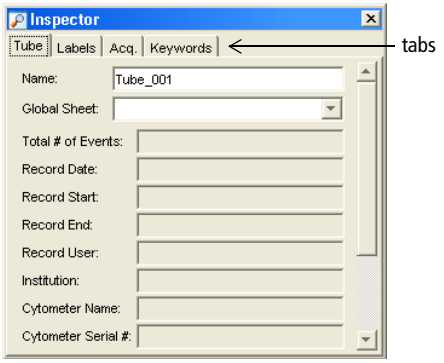
☑ **Tip** Save space in your database by deleting unneeded parameters.

To delete parameters, click the selection button next to each unneeded parameter. Hold down the Ctrl key to select more than one. When you are finished selecting, click Delete.



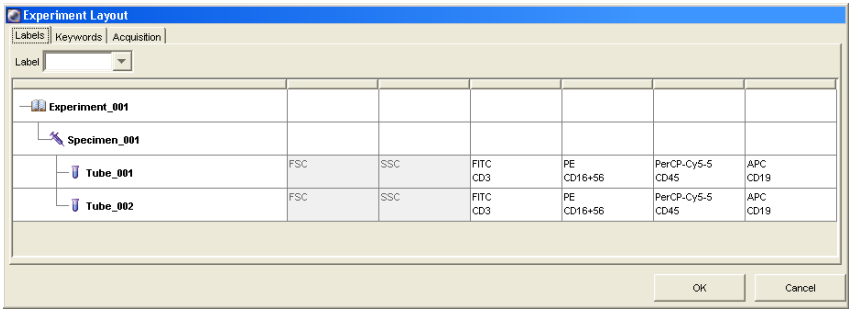
7 Select each item in the Browser and notice how the Inspector changes.

When you select the tube, notice there are four tabs in the Inspector. Click each tab in turn to view the different tube options.



8 Choose Experiment > Experiment Layout, and define labels for each parameter.

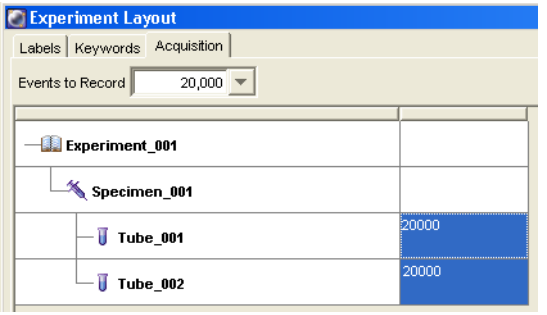
Select the two FITC fields and enter CD3, select the two PE fields and enter CD16+56. Enter CD45 and CD19 for the PerCP-Cy5-5 and APC fields, respectively.



☑ **Tip** When your experiment contains many tubes that share the same parameter labels, use Experiment Layout to enter all labels at once.

9 In the Experiment Layout dialog box, click the Acquisition tab, select the fields labeled 10000, and change Events to Record to 20,000 events; click OK.

Choose 20,000 from the Events to Record menu, or enter the value.



Notice that when you select Tube_001 in the Browser, the Labels and Acq. tab in the Inspector are updated with your changes.

10 Create an FSC vs SSC plot on the global worksheet.

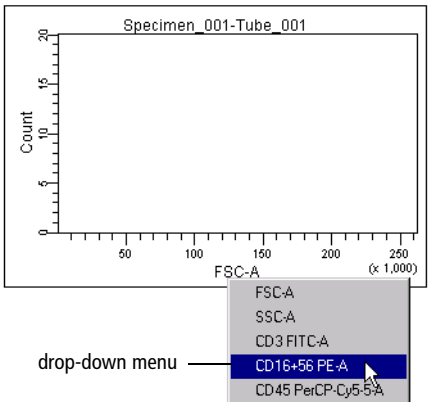
Select Dot Plot ( ) on the Worksheet toolbar and click in the worksheet. A plot of a default size is drawn.

11 Create a PE histogram next to the FSC vs SSC dot plot.

Select Histogram ( ) and click in the worksheet. A histogram of a default size is drawn.

To change the plot parameter, click the x-axis label and choose PE-A from the menu that appears (Figure 2). Notice that the parameter names include the labels you defined previously.

Figure 2 Changing plot parameters



☑ **Tip** If the parameter names do not appear, it might be because the current tube pointer is not active (green). When working on global worksheets, you must activate the current tube pointer to associate labels with parameters.

12 Create a FITC vs PerCP-Cy5-5 plot below the FSC vs SSC plot.

☑ **Tip** Hold down the Ctrl key, click the border of the FSC vs SSC plot, and drag it down. A duplicate plot is created as you drag. Release the mouse when the plot is in an appropriate location. Any plot can be duplicated in this manner.

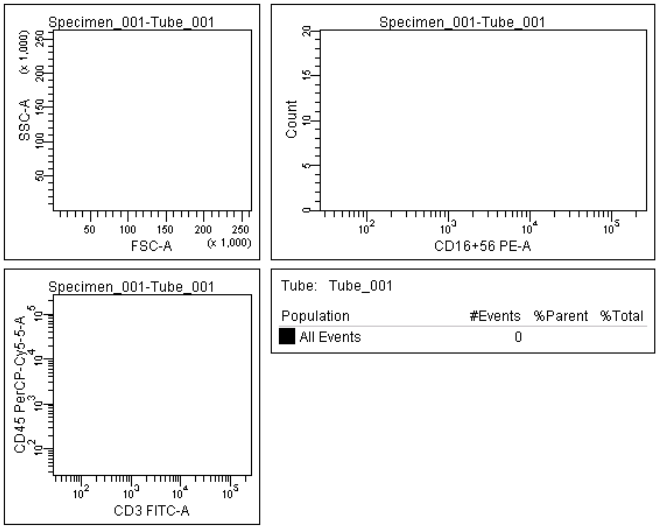
Click each axis label in turn and choose the appropriate parameter.

13 Create a population hierarchy view.

Right-click any plot and choose Show Population Hierarchy. Drag the resulting view to the right of the FITC vs PerCP-Cy5-5 dot plot. Resize it so it fits on the worksheet.

The population hierarchy is used to list and subset defined populations, as you will see in the tutorial on Subsetting Populations on page 43.

Your worksheet should look similar to the following figure:



Exporting an Experiment as a Template

Now that you have created a simple experiment, you can export it as a template. Experiment templates include specimens, tubes, keywords, instrument settings, labels, worksheet elements, and worksheets (including all settings such as page breaks), but do not include recorded data. They can also include Sort Layouts, if you are running a sorter.

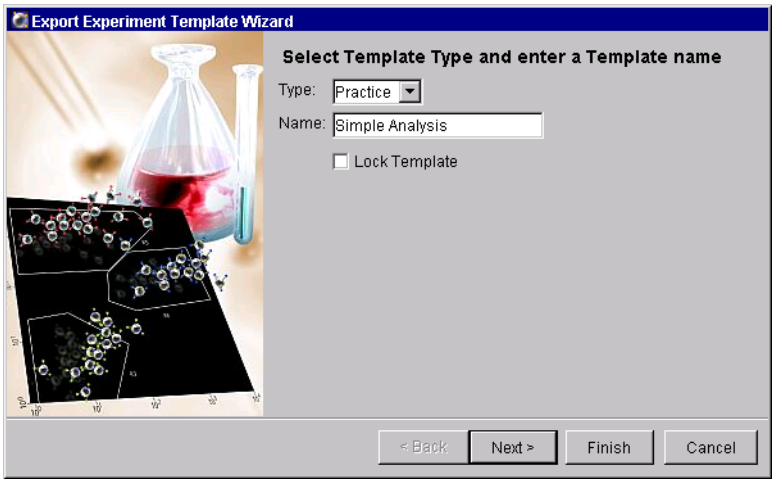
Any experiment can be saved as a template. Templates are stored outside the Browser to simplify the Browser display.

1 Select Experiment_001 in the Browser, and choose File > Export > Experiment Template.

☑ **Tip** Make sure you choose the Experiment Template command, and not the Experiments command.

The Export Experiment Template Wizard appears. The bold text at the top of the dialog tells you what to do at each screen.

2 Enter *Practice* for the template Type, and *Simple Analysis* for the template Name.



The template Type is used to group similar templates so they are easy to find. At a minimum, you need to enter only the Type and Name when you are creating a template; the remaining screens are optional.

☑ **Tip** Select the Lock Template checkbox when you want to prevent other users from overwriting your template.

3 Click Next to view the remaining screens, and then Finish.

You can enter information into any field.

Creating an Experiment from a Template

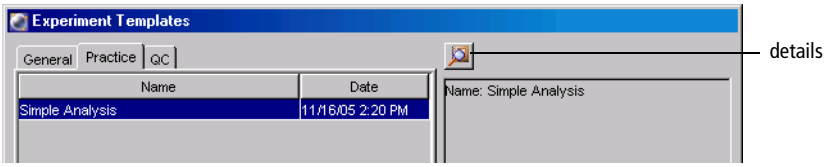
Next, you will create a new experiment from the template you just created. Remember that to place an experiment in a folder, you need to select the folder *before* you create the experiment.

1 Select the Practice folder, and choose Experiment > New Experiment.

☑ **Tip** Use the menu command rather than the New Experiment button when you want to use an experiment template. You can also press Ctrl-E.

The Experiment Templates dialog box appears.

2 Click the Practice tab in the dialog box, select the Simple Analysis template, and click the details button.



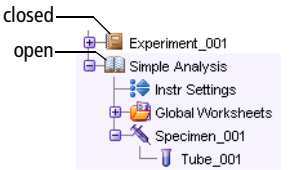
The Experiment Layout dialog box appears, showing tube labels, keywords, and acquisition settings for the template.

3 Click the tabs in the Experiment Layout dialog box and review the settings, and then click Cancel.

The settings are those you entered when you set up the template. These details are read-only—you cannot make changes to a predefined template.

4 Click OK to create the new experiment.

The previous experiment closes and a new experiment named Simple Analysis is added to the Browser. When you are working with experiments, only one experiment can be open at a time.



Notice that the global worksheet includes the plots and population hierarchy you set up previously.

Importing an Experiment

Next, you will import an experiment into the Practice folder. Imported experiments are different from templates in that they can contain recorded data.

1 Select the Practice folder in the Browser, and choose File > Import > Experiments.

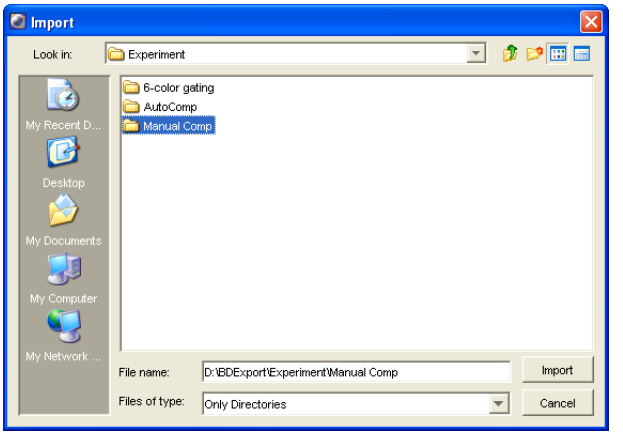
Remember that to place an experiment in a folder, select the folder *before* you create the experiment.

☑ **Tip** If you forget to select the folder first, you can move an experiment using Cut and Paste With Data commands.

By default, the D:\BDExport\Experiment folder appears when you import or export an experiment, unless you previously navigated to a different folder.

2 Select the folder labeled Manual Comp, and click Import.

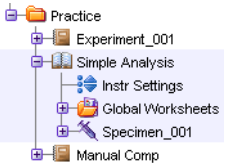
Experiments to import are always represented by a folder. The folder will be converted to an experiment icon after it is imported into the Browser. To select the folder, click once (do not double-click), and click Import.



Notice that the previous experiment does not close. The new experiment is added below the Simple Analysis experiment in the Browser.

Working with Experiments in the Browser

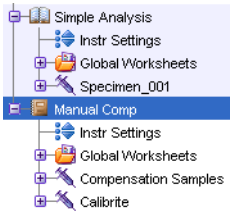
You should now have three experiments in the Browser: a closed default experiment (Experiment_001), an open experiment template (Simple Analysis), and a closed imported experiment (Manual Comp).



The following exercise demonstrates the difference between closed and open experiments, and how to use the windows to compare instrument settings.

1 Click once on the plus sign (+) next to the Manual Comp experiment to expand it.

Although the experiment's contents are shown in the Browser, the plots are still blank. You must open an experiment to access its stored data.



NOTICE Expanding an experiment is not the same as opening it by double-clicking.

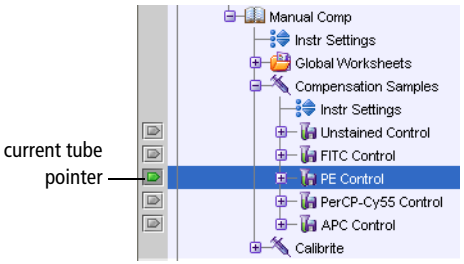
2 Double-click the Manual Comp experiment to open it.

The Simple Analysis experiment closes. Analysis objects for the Manual Comp experiment are shown on the worksheet.

3 Click the (+) to expand one of the experiment's specimens.

The Manual Comp experiment contains multiple tubes under each specimen. Notice that each tube has a disk icon, indicating it contains saved data.

4 Click to set the current tube pointer next to any tube in the Browser.



Data is shown in the worksheet. When you are viewing data on a global worksheet, use the current tube pointer to select the tube for which data will be shown.

5 Select a different tube in the Browser.

Notice that the data display does not change. To change the data displayed on a global worksheet, you must reset the current tube pointer. Try setting the pointer next to different tubes in the Browser and notice how data in the plots changes.

6 Expand the Simple Analysis experiment, and select the Instr Settings icon.

The Inspector displays instrument settings for the selected object even though the experiment is closed.

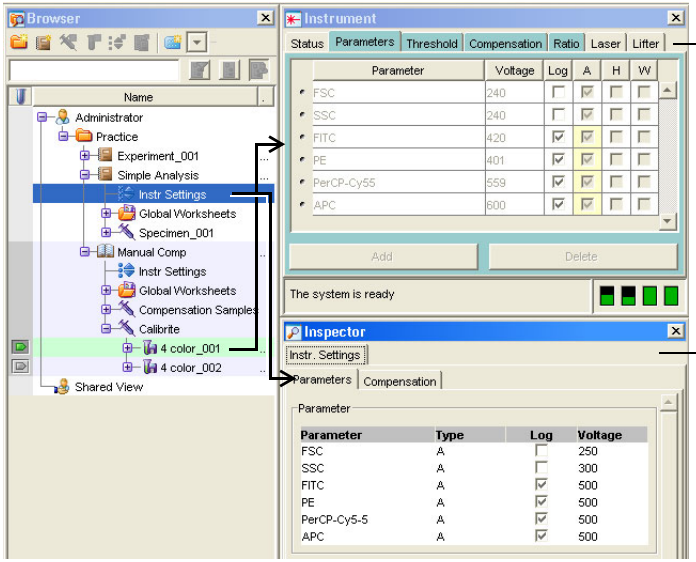
☑ **Tip** Use the Inspector to get information about any selected object in the Browser, even when its associated experiment is closed.

7 Click the Parameters tab in the Instrument window (Figure 3 on page 37).

The Instrument window shows instrument settings for the tube with the current tube pointer (the tube within the Manual Comp experiment). The four instrument settings tabs are tinted green to remind you that they show settings for the current tube. (The tube's name in the Browser is also highlighted with green.)

The Inspector shows settings for the tube or Instr Settings icon that is selected in the Browser. In this case, the Inspector shows settings for the Simple Analysis experiment's instrument settings. You can use the Instrument window and Inspector to compare instrument settings for any two objects in the Browser.

Figure 3 Comparing instrument settings



8 In the Manual Comp experiment, expand one of the tubes under the Compensation Samples specimen and compare instrument settings to one of the Calibrite tubes.

- Click to place the current tube pointer next to any tube under Compensation Samples.
- Click the Parameters tab in the Instrument window.
- Expand the Calibrite specimen, if needed, and expand any tube under the specimen.
- Select the Instr Settings icon under the tube. Settings are shown in the Inspector.

The settings should be the same.

9 Double-click the Manual Comp experiment to close it.

Changing Browser Button Defaults

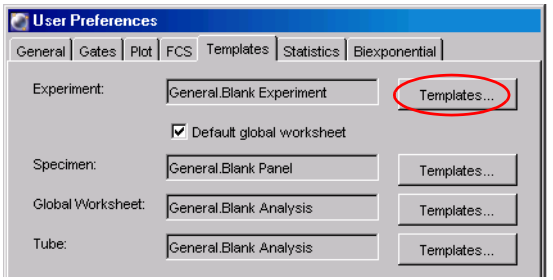
You can assign any saved template to a button in the Browser toolbar. For example, experiment templates can be assigned to the New Experiment button, panel templates can be assigned to the New Specimen button, and analysis templates can be assigned to the New Tube or New Global Worksheet button. The corresponding template is added to the Browser when you click the button in the Browser toolbar.

The following tutorial describes how to change template assignments for the New Experiment button.

1 Choose Edit > User Preferences.

2 Click the Templates tab.

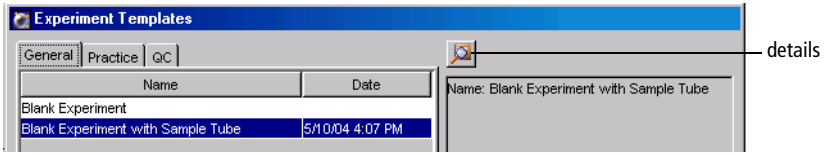
3 Click the Experiment Templates button:



The Experiment Templates dialog box opens.

4 On the General tab, select Blank Experiment with Sample Tube.

5 (Optional) Click the details button, view experiment details, and click Cancel.



The details button opens the Experiment Layout dialog box, where you can view the number of specimens and tubes in the experiment.

6 Click OK to assign the selected template and OK to close the User Preferences dialog box.

7 Select the Practice folder in the Browser, and click the New Experiment button.

Now, the button adds an experiment with a global worksheet, specimen, and tube.

8 (Optional) Assign the Simple Analysis template as the default experiment, and verify that the New Experiment button creates a Simple Analysis experiment.

9 Assign the New Experiment button to the template you prefer.

Using Gating Features

You can create four types of gates in BD FACSDiva software: polygon, rectangle, quadrant, and interval. Automatic and snap-to gating tools are available to create polygon and interval gates. The following exercises demonstrate the use of gating tools in BD FACSDiva software.

For this section, you will need both the Simple Analysis and Manual Comp experiments from the previous section. If you are starting here, create the Simple Analysis experiment as described in Creating a Folder and an Experiment on page 25, and import the Manual Comp experiment as described in Importing an Experiment on page 33.

You do not need to be connected to the instrument to perform these exercises. If you are not connected, you will see a plot icon ( ) for the current tube pointer in the Browser rather than a pointer icon.

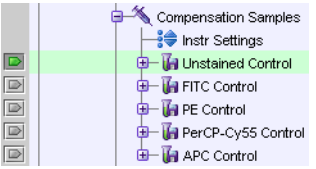
Creating Gates

This section describes how to use tools in the Worksheet toolbar to create different types of gates around populations in the Manual Comp experiment.

1. Double-click the Manual Comp experiment to open it.

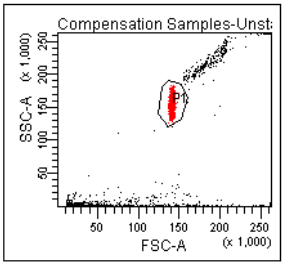
2. Set the current tube pointer next to the Unstained Control tube under the Compensation Samples specimen.

   The plots show data for that tube.



3. Create an autopolygon gate around the singlet events in the FSC vs SSC dot plot.

   - Select Autopolygon ( ) in the Worksheet toolbar.
   - Click the singlet events in the plot. A gate is drawn automatically.



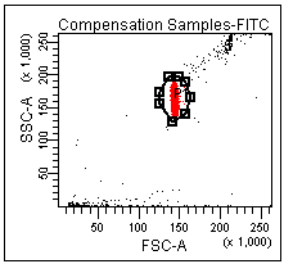
4. Move the pointer to the next tube in the Browser; notice that some of the events are outside the gate.

5. Select the autopolygon gate in the FSC vs SSC plot, and press Delete.

☑ **Tip** Press the Delete key, not the Backspace key. If the Delete key doesn't work, right-click the gate and choose Delete. This will activate the Delete key for the next time you use it.

6 Create a snap-to polygon gate ( ) around the singlet events in the FSC vs SSC dot plot.

Unlike an autopolygon gate, a snap-to gate automatically recalculates as new data is provided to the gate. The gate boundary is thicker to differentiate the snap-to feature.



7 Move the pointer back to the Unstained Control tube and notice how the gate changes.

8 (Optional) To practice using the other gating tools, delete the snap-to gate and create a rectangle gate ( ) and then a polygon gate ( ) around the singlet events in the plot.

To use the Polygon Gate button, do the following:

- Select Polygon ( ) in the Worksheet toolbar.
- Move the cursor over the plot: it changes to a crosshair.
- Click to set the first vertex.
- Release the mouse button and move the cursor to where you want the second vertex and click again.
- Continue moving the cursor around the population and clicking until the population is completely encompassed by the gate.

- Close the region by double-clicking or by clicking on the first vertex.

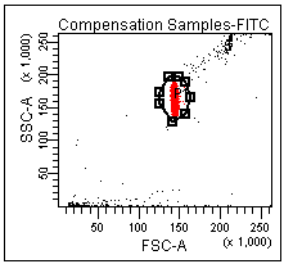
For more practice creating gates, go to Subsetting Populations on page 43 and Gating Multicolor Experiments on page 57.

Editing Gates

This section describes how to move and resize gates.

1 Select the gate you created in the FSC vs SSC dot plot.

Handles appear at each vertex.



2 Drag the selected gate to move it.

To move a gate, select the gate border, not one of the vertices.

Notice that the gate label moves with the gate.

3 Drag one of the selection handles to resize the gate.

After you move or resize a snap-to gate, the gate no longer adjusts automatically. You can force the gate to readjust by right-clicking on the gate boundary and choosing Recalculate from the shortcut menu.

4 Move the gate label by dragging it.

5 Click outside the gate to deselect it.

6 Close the Manual Comp experiment.

Subsetting Populations

The Simple Analysis experiment you set up previously includes three plots and a population hierarchy. The population hierarchy is used to view defined populations and display the relationship between different gated populations. This section describes how to subset populations using the population hierarchy.

For this tutorial, you will need the Simple Analysis experiment that was created in Creating a Folder and an Experiment on page 25, and sample files that were installed in your BDExport\FCS folder during software installation.

Importing Data

Before you can gate populations, you need to display data in the plots. This section describes how to import FCS files from the BDExport folder into your Simple Analysis experiment.

1 Open the Simple Analysis experiment by double-clicking it.

You can import data only into an open experiment.

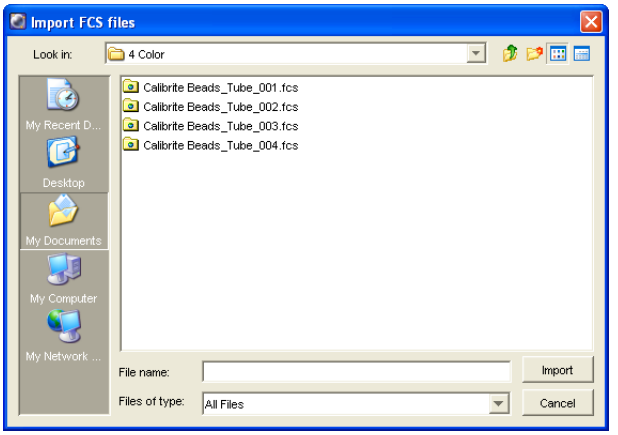
2 Choose File > Import > FCS files.

By default, the D:\BDExport\FCS folder appears, unless you previously navigated to a different folder.

3 Double-click the *4 color* folder, select all four FCS files in the folder, and click Import. See Figure 4 on page 44.

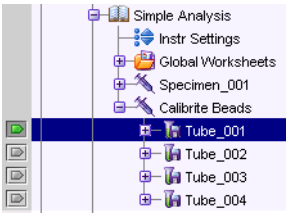
☑ **Tip** Hold down the Shift or Ctrl key to select more than one file at a time.

Figure 4 Selecting files to import



A new specimen named *Calibrite Beads* is added to the Browser, along with four tubes containing recorded data. Expand the specimen to see the tubes.

4 Set the current tube pointer to Tube_001 under the Calibrite Beads specimen.

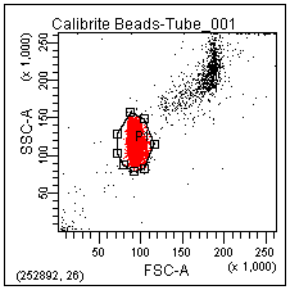


Data is displayed in the plots.

Defining and Subsetting Populations

1 Create an autopolygon gate around the singlet events on the FSC vs SSC dot plot for Tube_001.

- Select Autopolygon ( ) in the Worksheet toolbar.
- Click the singlet events in the plot; a gate is drawn automatically.



The new population, P1, is added to the population hierarchy.

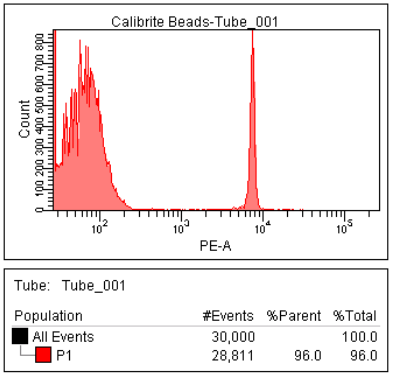
2 Drag the P1 label out of the center of the Autopolygon gate.

This prevents it from being obscured by data.

3 Set up the PE histogram to display data from only the P1 population.

Right-click inside the plot and choose Show Populations > P1.

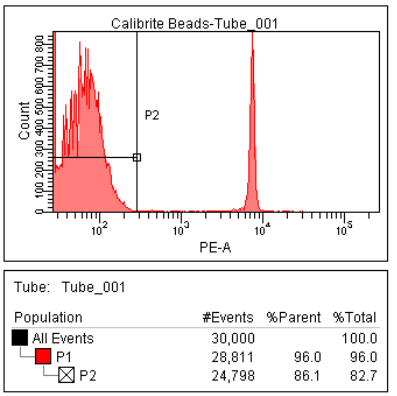
Only events within the P1 gate are shown in the histogram. Notice that all events in the histogram are now colored red, the color of the P1 population.



4 Create an autointerval gate around the PE-negative peak in the PE-A histogram.

Select Autointerval Gate ( ) and click the negative peak in the plot. An interval gate is drawn automatically.

The new population, P2, is indented under the P1 population in the population hierarchy view. When you define a population in a gated plot (a plot showing a subset of All Events), the new population is automatically a subset of the population in the plot.



Notice also that the color box next to the P2 population is crossed out, meaning that the new population retains the color of its parent population. By default, populations defined by Interval and Quadrant gates are not assigned a color.

5 Set the FITC vs PerCP-Cy5-5 dot plot to show data from the P2 population.

Right-click inside the plot and choose Show Populations > P2.

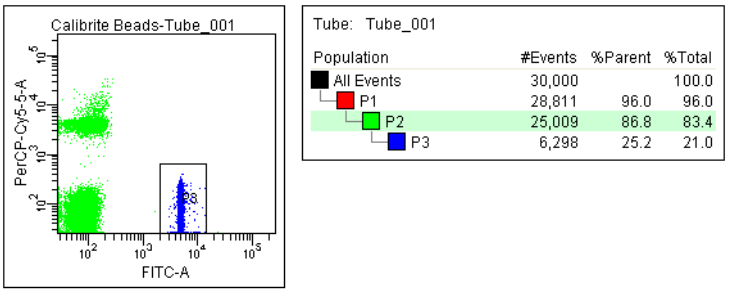
The plot is blank because the P2 population has no color assigned to it. To assign the population a color:

- Double-click the box next to P2 in the population hierarchy view.
- Choose a color from the menu.

Events inside the gated dot plot change to the selected color.

6 Create a rectangle gate around the FITC-positive events in the FITC vs PerCP-Cy5-5 dot plot.

☑ **Tip** Extend the gate past the x-axis to capture events on the axis.

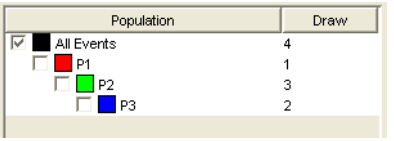


Because the plot is showing only P2 events, the new population, P3, is a subset of P2 events in the population hierarchy view. When a plot shows only a gated population, any new populations will be a subset of the gated population that is shown.

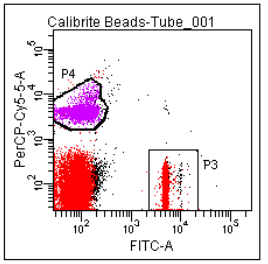
The following steps illustrate this feature in greater detail.

7 Set up the FITC vs PerCP-Cy5-5 dot plot to show all events.

☑ **Tip** You can also use the Plot Inspector to show populations in a plot. Select the plot; at the bottom of the Inspector, you will see a list of all defined populations. Select the checkbox next to All Events.



8 With P2 selected in the population hierarchy, create a snap-to polygon gate ( ) around the PerCP-Cy5-5–positive events.



Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	30,000		100.0
P1	28,811	96.0	96.0
P2	24,798	86.1	82.7
P3	5,962	24.0	19.9
P4	5,722	23.1	19.1

When a population is highlighted in the population hierarchy view, any new populations will be a subset of the population that is highlighted. In this case, the new population is a subset of P2 because it is highlighted, even though the plot is showing All Events.

☑ **Tip** To avoid errors when subsetting populations, always keep the population hierarchy in view. Make sure the appropriate population is highlighted before you create a gate.

9 Set up the FITC vs PerCP-Cy5-5 plot to show P2 events.

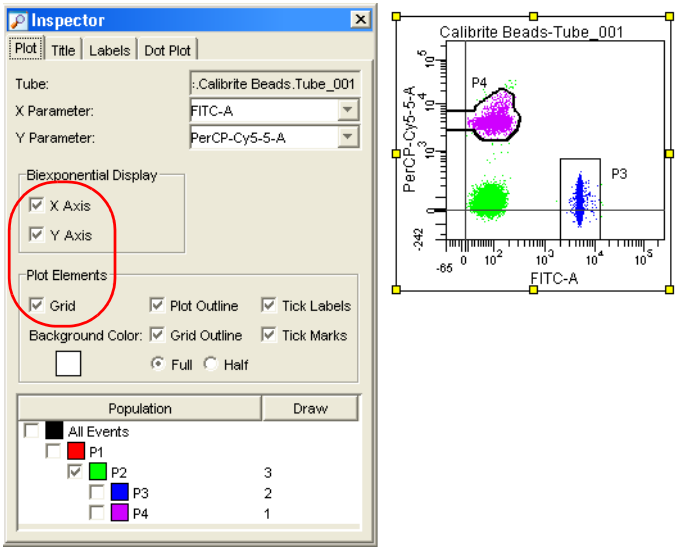
Using Biexponential Display

In the FITC vs PerCP-Cy5-5 plot, all populations have negative events below the plot axes. Use biexponential display to view populations with negative events.

☑ **Tip** Use Increase Plot Size ( ) to better view events on the axes. Click the button, and then click the plot on the worksheet. The plot expands to twice its size. Use Decrease Plot Size ( ) to return the plot to its original size.

1. Select the FITC vs PerCP-Cy5-5 plot on the worksheet.

2. In the Plot Inspector, select the Biexponential Display checkboxes for the x and y axes; click the Grid checkbox to view the plot's zero point.



Each population now appears in its entirety. Because the P4 population contains negative events, its gate boundary extends to the axis to capture any outliers.

Adjusting Biexponential Scaling

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. Advanced users can switch to manual scaling and adjust the range of the negative scale using the Biexponential Editor. This feature is described in the *BD FACSDiva Software Reference Manual*.

However, when automatic scaling is in effect, you can use the *scale to population* feature to reset the range of the negative scale for a selected population. To try this feature, do the following.

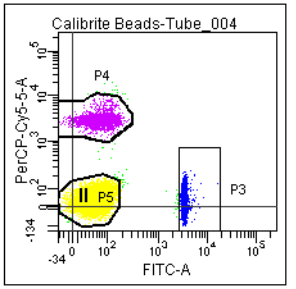
1. Create a snap-to polygon gate around the double-negative events in the FITC vs PerCP-Cy5-5 plot.

2. Move the current tube pointer to Tube_004.

   The y-axis scale starts at –1,242.

3. Right-click inside the FITC vs PerCP-Cy5-5 plot and choose Scale to Population > P5.

   The plot's scales adjust to fit the P5 population: the y-axis scale now starts at –134. The P5 gate label is marked with two vertical bars, indicating that it is the population for which scaling is in effect.



4. Scale to the P4 population to see how this changes the plot's scaling.

   There might be a short delay as the software recalculates the plot scaling.

5 Return the plot scaling to All Events.

   Right-click the plot and choose Scale to Population > All Events.

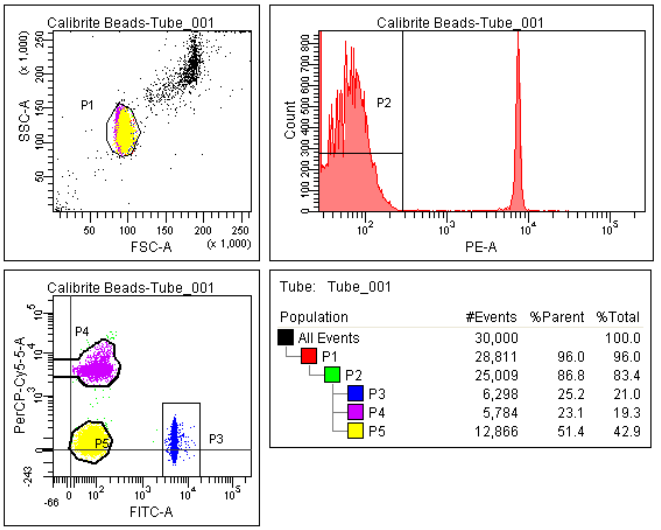
6 Move the current tube pointer back to Tube_001.

Using Tethering and Batch Analysis

When you want 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 move relative to a snap-to gate. This feature is useful when you expect changes in the population of interest in relation to another population; it can help automate the analysis of rare events.

Once you have set up an analysis with plots, gates, population subsets, tethered gates, and statistics, you can use batch analysis to automatically process a group of data files through your analysis and save or print results as you go.

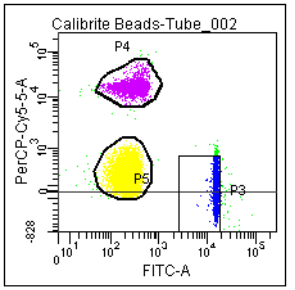
This tutorial demonstrates how to use biexponential display and the Snap-To tethering feature in a batch analysis. To perform the steps in this section, you will need the data and populations that were set up in the previous sections. At the start of this tutorial, your worksheet should look similar to the following:



Setting Up Tethered Gates

1 Move the current tube pointer to Tube_002 in the Browser.

The snap-to gates adjust to encompass their respective populations. However, the P3 gate is now missing some events.



We will use the tethering feature to ensure the gate does not miss events when data in the plot changes.

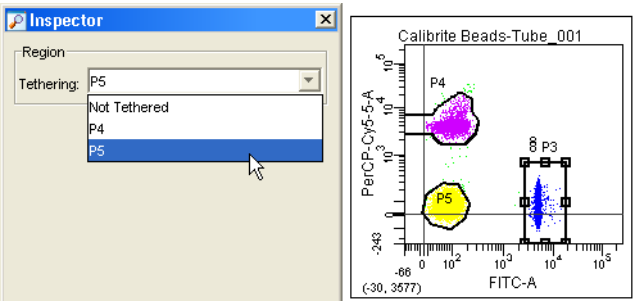
2 Move the current tube pointer back to Tube_001.

3 Select the P3 gate in the plot.

4 In the Inspector, choose P5 from the Tethering menu.

This tethers the rectangle gate to the snap-to gate, so it will move in relation to the gate around the double-negative population.

The boundary of the tethered gate becomes thicker, and a chain-link icon appears next to the gate label, indicating the gate is tethered.



5 Move the pointer to Tube_002 in the Browser.

Notice how the tethered gate moves in relation to the snap-to gate.

6 If necessary, adjust the rectangle gate so it captures all events.

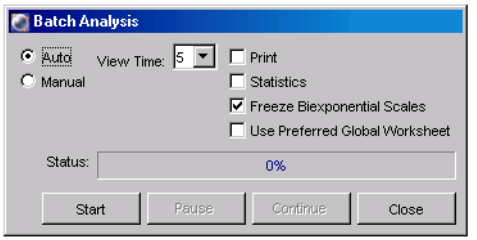
Performing a Batch Analysis

Now that you have defined and tested your analysis strategy to ensure it works with your data, you are ready to perform a batch analysis. When an analysis includes a plot with biexponential scales, you can process the batch with the same biexponential scales, or change the scaling in relation to the negative events in the data file. The following example shows both options.

1 Right-click the Calibrite Beads specimen in the Browser, and choose Batch Analysis.

The Batch Analysis dialog box appears.

2 Set the batch analysis options as follows:



- Verify the Auto option is selected.
- Set the View Time to 5 seconds.
- Deselect the Print and Statistics checkboxes. When selected, the Print checkbox prints a copy of the analysis after each tube is processed; the Statistics checkbox exports statistics to a single file for the batch.
- Verify the Freeze Biexponential Scales checkbox is selected. This option processes all files with the same biexponential scales.
- Deselect the Use Preferred Global Worksheet checkbox. When an experiment contains multiple global worksheets, each assigned to a different tube, check this box to maintain worksheet assignments during a batch analysis.

3 Click Start.

Data for each tube is shown in the analysis template for 5 seconds before processing of the next tube begins. All tubes under the specimen are processed using the same biexponential scales. When the batch is finished, a message is displayed.

4 Click OK to dismiss the batch completion message.

☑ **Tip** You can process the batch using scale values from any tube in the batch. To process the batch with different scale values, do the following.

- Move the current tube pointer to Tube_003.

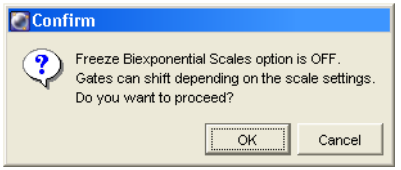
- Right-click the specimen and choose Batch Analysis.
- Click Start.

Now, all files are processed using scaling from Tube_003.

NOTICE The scaling for the data files in this example vary extensively for effect. Normally, all samples in a batch are more similar. When performing a batch analysis with biexponential scales, start with the current tube pointer next to the tube with the negative range that best meets your laboratory's criteria.

5 (Optional) Repeat the batch analysis with the Freeze Biexponential Scales option deselected.

When you click Start, the following message appears:



NOTICE When a batch analysis includes non-adaptive or non-tethered gates, unfreezing the biexponential scales can cause gated events to fall outside their population boundaries.

Click OK to proceed. Notice that the plot's scaling fluctuates for each subsequent tube.

Adjusting the Size of Snap-To Gates

When needed, you can adjust the size of a snap-to gate to capture a population's outlying events. Use the results from the previous exercise to try out this feature.

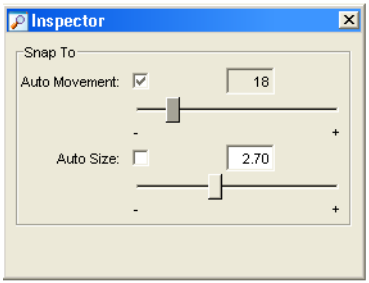
1 Move the current tube pointer to Tube_004.

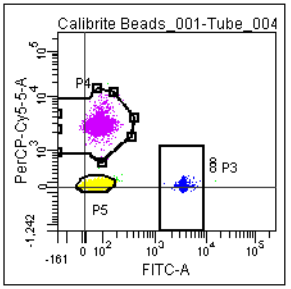
Notice that the P4 population has many outlying events.

2 Select the P4 gate in the plot.

3 In the Inspector, deselect Auto Size, and move the slider control to the right until the gate captures all events.

When the checkbox is selected, the software determines the size of a snap-to gate. The Inspector provides controls for overriding the software-determined value.





4 (Optional) Move the current tube pointer to another tube and verify that the Auto Size value is retained.

5 Close the Simple Analysis experiment.

Gating Multicolor Experiments

The following is an advanced gating tutorial that demonstrates derived, interval, and quadrant gating features through the analysis of lymphocyte subsets in a human peripheral blood sample. In this example, cells were stained with CD3 FITC, CD16+CD56 PE, CD45 PerCP-Cy5.5, CD4 PE-Cy7, CD19 APC, and CD8 APC-Cy7.

If you prefer to skip this tutorial, proceed to Publishing and Presenting Data on page 66.

Identifying Lymphocytes

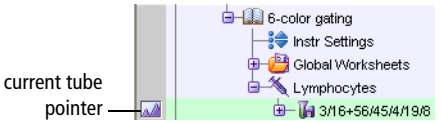
1 Import the experiment named *6-color gating* into your Practice folder.

Select the folder, choose File > Import > Experiments, and locate *6-color gating* in the BDExport\Experiment folder. Select the folder and click Import.

2 Double-click the new experiment to open it.

3 Expand the Lymphocytes specimen, and set the current tube pointer next to the 3/16+56/45/4/19/8 tube in the Browser.

The following figure shows what the current tube pointer looks like when you are working offline:

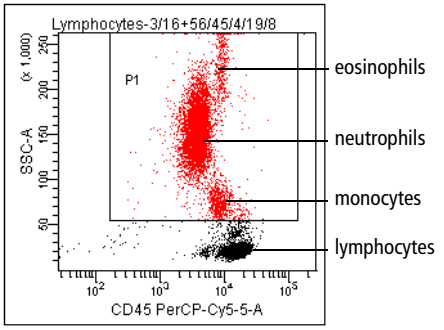


Six-color data is displayed in the plots.

4 Draw a rectangle gate ( ) around the monocytes, neutrophils, and eosinophils in the CD45 vs SSC dot plot (Figure 5 on page 58).

☑ **Tip** Extend the gate past the plot axes to capture events on the axis.

Figure 5 Gating non-lymphocyte events



5 Use the population hierarchy to rename the population *MNEs*.

The population hierarchy is part of the imported experiment. To rename the population, select P1 in the population hierarchy, enter *MNEs*, and press Return.

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

Population	#Events	%Parent	%Total
All Events	10,000		100.0
MNEs	6,347	63.5	63.5

Move the gate label off the population in the plot, if needed.

6 Right-click the *MNEs* population in the population hierarchy and choose Invert Gate.

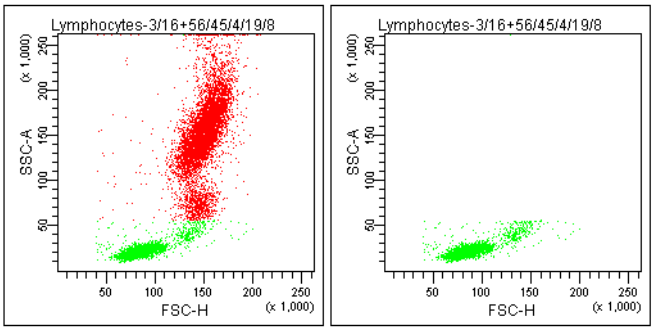
Inverted gates use the NOT operator to select events outside a defined gate. A new population containing all events except the monocytes, neutrophils, and eosinophils, NOT(MNEs), is added to the population hierarchy. All events in the plot that are not MNEs are colored green.

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

Population	#Events	%Parent	%Total
All Events	10,000		100.0
MNEs	6,319	63.2	63.2
NOT(MNEs)	3,681	36.8	36.8

7 Right-click the FSC vs SSC dot plot and choose Show Populations > NOT(MNEs).

The monocytes and neutrophils disappear from the plot, making it much easier to view the lymphocytes.

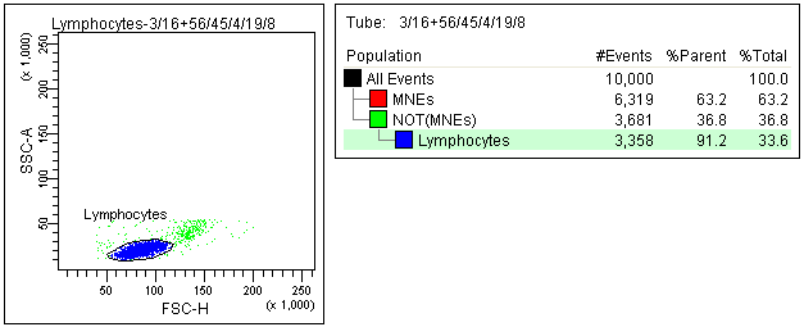


plot showing All Events

same plot showing NOT (MNEs)

8 Create an autopolygon gate ( ) around the lymphocytes; change the name of the population to *Lymphocytes* in the population hierarchy.

Since the lymphocyte population was defined in a plot showing only the NOT(MNEs) population, the new population becomes a subset of NOT(MNEs).



Identifying Lymphocyte Subsets

Now, you will create gates to identify CD3+, CD19+, and CD16+56+ populations (T, B, and NK cells, respectively).

☑ **Tip** To avoid errors when subsetting populations, always keep the population hierarchy in view. The last-selected population remains highlighted in the view, indicating that it will be the parent population of the next subset that is defined. Make sure the appropriate population is highlighted before you create a gate.

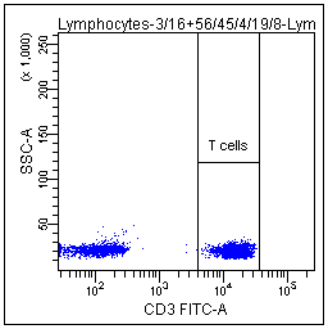
1 Right-click the Lymphocytes gate in the FSC vs SSC plot and choose Drill Down.

A new FSC vs SSC plot appears, displaying only Lymphocyte events. After gated populations have been defined in a plot, use the Drill Down feature to easily create a plot showing data from only one population.

2 Move the new plot to a clear area on the worksheet.

3 Change the FSC label on the new plot to CD3.

4 Draw an interval gate ( ) around the CD3+ cells and name the new population *T cells*.



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

Population	#Events	%Parent	%Total
All Events	10,000		100.0
MNEs	6,310	63.1	63.1
NOT(MNEs)	3,690	36.9	36.9
Lymphocytes	3,388	91.8	33.9
T cells	1,825	53.9	18.2

Interval gates, typically used on histograms, work equally well on dot plots (in a vertical direction).

The newly created population has a checked color box, indicating the population has no color. Events within an interval gate retain the color of their parent population (lymphocytes, in this case).

5 Use the Histogram button ( ) to create a CD19 histogram.

Change the x-axis parameter to CD19.

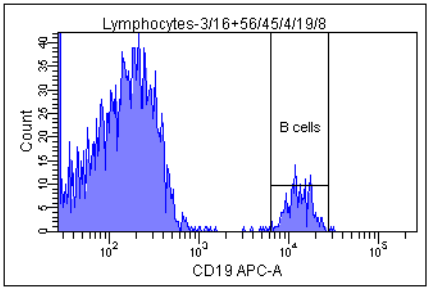
6 Set the histogram to show the Lymphocytes population.

The CD19 histogram will be used to define B cells. (You could also use a CD19 vs SSC dot plot.)

7 Select Lymphocytes in the population hierarchy view, and create an autointerval gate ( ) around the CD19+ cells.

An autointerval gate is like an autopolygon gate in that a gate is drawn automatically.

8 Name the new population *B cells*.



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

Population	#Events	%Parent	%Total
All Events	10,000		100.0
MNEs	6,319	63.2	63.2
NOT(MNEs)	3,681	36.8	36.8
Lymphocytes	3,358	91.2	33.6
T cells	1,824	54.3	18.2
B cells	189	5.6	1.9

9 Right-click within the CD3 vs SSC plot and choose Duplicate.

10 Move the new plot to a clear area on the worksheet.

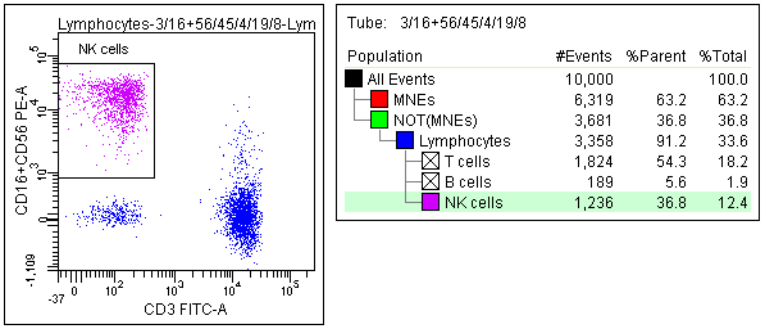
11 Change the y-axis to CD16+56.

12 Select Lymphocytes in the population hierarchy view, and draw a rectangle gate ( ) around the CD16+56+ cells.

13 Name the new population *NK cells* (Figure 6 on page 62).

☑ **Tip** Use biexponential display to show events on the plot axes. To turn on biexponential display, select the plot, and select the X Axis and Y Axis checkboxes in the Inspector.

Figure 6 Gating the NK cells in a biexponential plot



14 Create a joined gate to determine the percentage of T, B, and NK cells.

Joined gates use the OR operator to combine two or more individual gates.

- While pressing the Ctrl key, select the T, B, and NK Cells populations in the population hierarchy view.
- Right-click the selected populations and choose Join (Or) Gates.

A new population appears in the population hierarchy. The %Parent value indicates what percentage of the lymphocytes are T, B, or NK cells.

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

Population	#Events	%Parent	%Total
All Events	10,000		100.0
MNEs	6,310	63.1	63.1
NOT(MNEs)	3,690	36.9	36.9
Lymphocytes	3,388	91.8	33.9
T cells	1,825	53.9	18.2
B cells	188	5.5	1.9
NK cells	1,237	36.5	12.4
T cells OR B cells OR NK cells	3,250	95.9	32.5

Identifying T-Cell Subsets

This section describes how to use quadrant gates to identify T-helper and T-cytotoxic subsets.

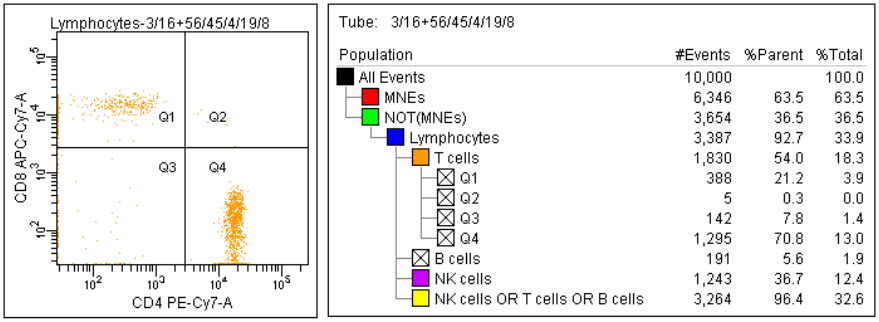
1 Change the color of the T cells in the population hierarchy.

Double-click the checked box in front of the T cell population and select the orange color box. The T cells now change to orange in all plots.

☑ **Tip** To further subset a population that has no color assigned to it (☒), you must first change the color.

2 Create a CD4 vs CD8 dot plot showing only the T cells.

3 Draw a quadrant gate to subset the events.



After you draw a quadrant gate, the population hierarchy lists each quadrant as a subset of the T cells. Again, you can change the color of any subset by double-clicking the box in front of the quadrant name, and selecting a color.

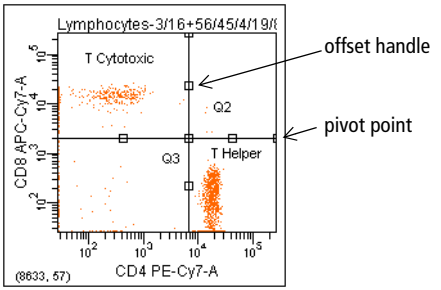
☑ **Tip** Any quadrant population can be used for additional subsetting or sorting. For example, sorting the Q1 cells would result in the following expression being sent to the cytometer: NOT(MNEs) in CD45/SSC AND Lymphocytes in FSC/SSC AND T cells in CD3/SSC AND Q1 in CD4/CD8. Also note that populations can share gates, as demonstrated by the T cells and B cells, which share the Lymphocytes gate as well as the NOT(MNEs) gate.

4 Rename the CD8⁺ population *T Cytotoxic*, and the CD4⁺ population *T Helper*.

5 Practice adjusting the quadrant gates.

- Click the intersection of the quadrant markers.

  Square handles appear:



- Drag the handle at the intersection—notice that the gate labels move with the quadrant markers.

- Drag an offset handle to offset a segment from the center point; Shift-click a quadrant marker to return the gate to its rectilinear form and return gate labels to their default position.

- Drag a pivot point to rotate the top or right segment; Shift-click a quadrant marker to return the gate to its rectilinear form and return gate labels to their default position.

The pivot and offset features cannot be used simultaneously.

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.

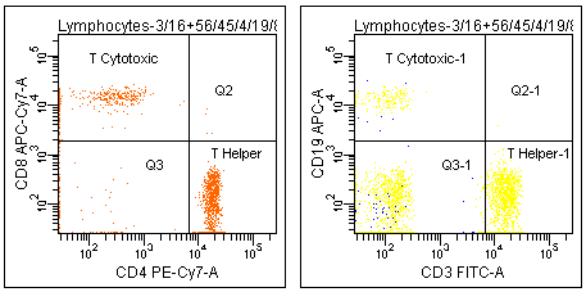
The following exercise demonstrates how to copy and paste gates.

1 Create a CD3 vs CD19 dot plot showing the Lymphocytes population.

2 Right-click the quadrant gate in the CD4 vs CD8 plot, and choose Copy.

3 Right-click the CD3 vs CD19 plot, and choose Paste.

The quadrant appears at the exact same coordinates as in the CD3 vs CD19 plot. Populations appear with the same name, appended with *-1*.



4 Adjust the quadrant gate as needed, and edit the names of the populations.

Change the name of the $CD19^+$ population to *CD19 positive Bs*, and the $CD3^+$ population to *CD3 positive Ts*.

5 Close the 6-color gating experiment.

Publishing and Presenting Data

After acquiring and analyzing data in BD FACSDiva software, you can export plots or gating hierarchies as graphical objects to use with a word-processing or graphics application, send by electronic mail, or publish on a website. You can also copy and paste objects between BD FACSDiva software and a third-party application such as Microsoft® Word.

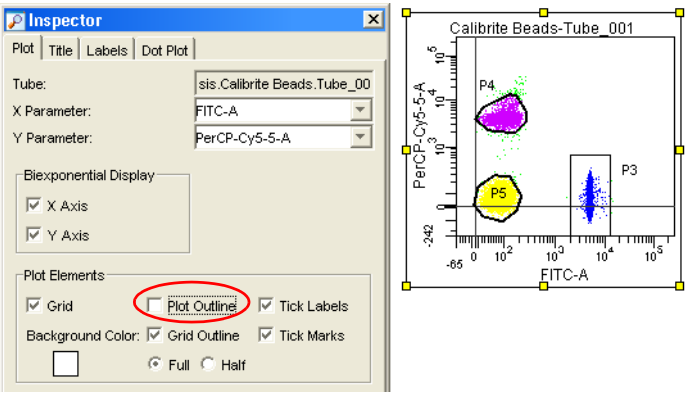
This section describes how to create publication-ready plots and export or copy them. For this exercise, you will need the 6-color gating experiment from Gating Multicolor Experiments on page 57.

1. Open the Simple Analysis experiment, and set the current tube pointer next to Tube_001 under the Calibrite Beads specimen.

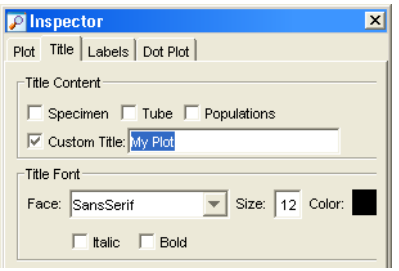
2. Select the FITC vs PerCP-Cy5-5 plot.

3. On the Plot tab of the Inspector, deselect the Plot Outline checkbox.

   The plot outline will not appear on the exported plot. (It shows only when the plot is selected.)

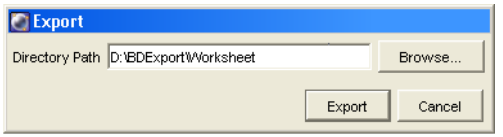


4 On the Title tab, deselect the Specimen and Tube checkboxes, and select the Custom Title checkbox. Enter *My Plot* as the plot title.



5 With the plot selected, choose File > Export > Worksheet Elements.

6 Click Export to save the plot in the BDExport\Worksheet folder.



The plot is saved as a JPEG file.

☑ **Tip** Multiple worksheet objects can be selected for export. When you choose Export > Worksheet Elements, all objects are exported at once. Each object is saved as a separate JPEG file.

7 To copy the plot, right-click the FITC vs PerCP-Cy5-5 plot and choose Copy.

8 Open a Microsoft Word document; right-click within the document and choose Paste.

Only one object can be copied and pasted at a time.

Maintaining the Database

Now that you have finished these tutorials, save space in your database by either archiving or deleting the experiments in your Practice folder. In either case, the experiments will no longer appear in the Browser the next time you use the software.

Archiving Experiments

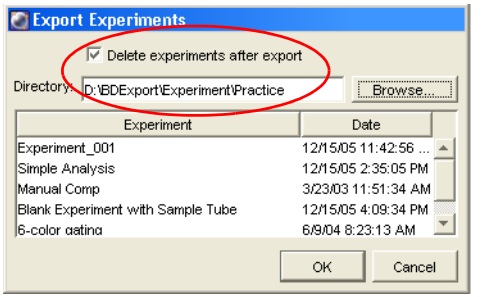
There are two ways to archive experiments. This section describes how to export experiments and delete them after export; deleting experiments is described on page 69. You can also use BD FACSDiva Data Manager to archive your entire database, as described in the *BD FACSDiva Software Reference Manual*.

1 Select all experiments within the Practice folder.

Select the first experiment in the folder. Hold down the Shift key while you select the last experiment in the folder.

2 Choose File > Export > Experiments.

3 Select the checkbox to *Delete experiments after export*; in the Directory field, append \Practice to D:\BDExport\Experiments:



This creates a Practice folder within D:\BDExport\Experiment where your archived experiments will be stored.

4 Verify that all your experiments are listed, and click OK.

Experiments are deleted from the Browser after export.

5 Select the Practice folder in the Browser, and press the Delete key; click OK to delete the folder.

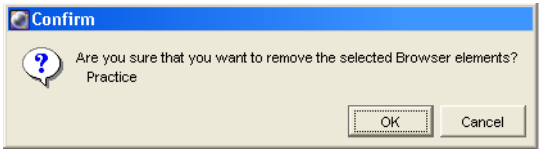
Deleting Experiments

1 Close any open experiment.

All experiments must be closed before you can delete the folder containing them.

2 Select the Practice folder and press the Delete key.

3 Click OK in the dialog that appears.



Deleted experiments are removed from the Browser and from the database on the D:\ drive (or C:\ drive, if no D:\ drive exists).