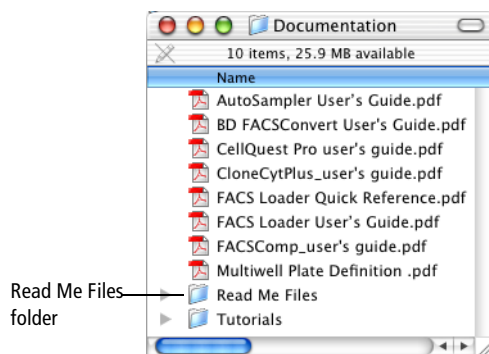


BD Multiset™ Software Tutorial

This tutorial guides you through a typical acquisition using BD Multiset software. If you are already familiar with previous versions of BD Multiset software on Mac® OS 9, refer to the BD Multiset software (version 2.1 or later) ReadMe file for a description of new features. ReadMe files are located in the Documentation folder/Read Me Files folder on your hard drive.



This tutorial covers:

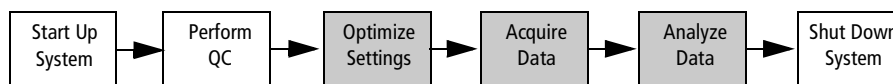
- Optimizing settings
- Acquiring and analyzing data
- Creating user-defined reagents

To perform the steps in this tutorial, you will need:

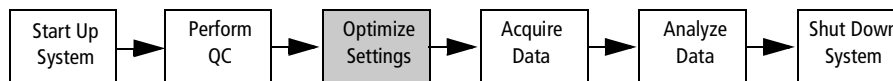
- A cytometer and a computer with BD FACStation™ software (version 5.1 or later) and BD Multiset software (version 2.1 or later).
- A tube or a panel of tubes stained with BD Tritest™ or BD Multitest™ reagent system. For calculation of absolute counts, you can use the BD Trucount™ absolute counting system or you can enter information from a cell counter.
- BD FACSCOMP™ software to set up the cytometer for the sample(s) you prepared. Refer to the *BD FACSCOMP Software Tutorial* for details.

BD Multiset software is the BD Biosciences Macintosh® application for analyzing multicolor flow cytometry data. BD Multiset software's automated acquisition and analysis interface is designed specifically for use with the three-color BD Tritest and four-color BD Multitest reagent system from BD Biosciences. These lyse/no-wash (LNW) reagents employ BD Multiset software's expert fluorescence gating. When the reagents are used with BD Trucount™ absolute counting system, BD Multiset software calculates absolute counts for each reagent subset.

Acquiring and analyzing flow cytometry data is a multistep process. For this tutorial, we will focus on optimizing settings, and acquiring and analyzing data as it pertains to samples acquired with BD Multiset software.



Optimizing Settings



Entering Information in BD Multiset Software

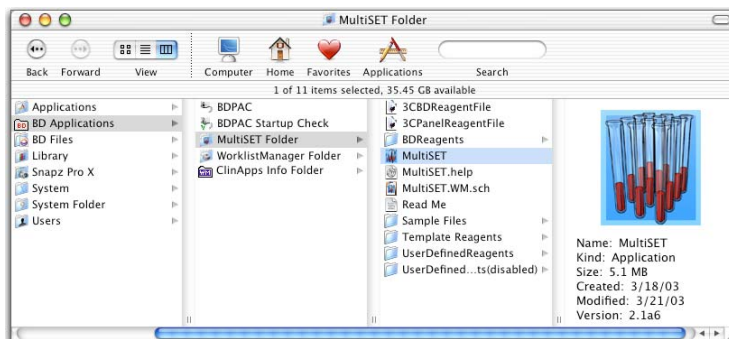
- 1 Launch BD Multiset software by clicking the application icon in the Dock.



Multiset icon in Dock

If the icon is not in the Dock, you can place it in the Dock as follows:

- Display the MultiSET Folder in the BD Applications folder.



- Drag the MultiSET icon to the Dock.

Entering Information in the Sign In and Set Up Views

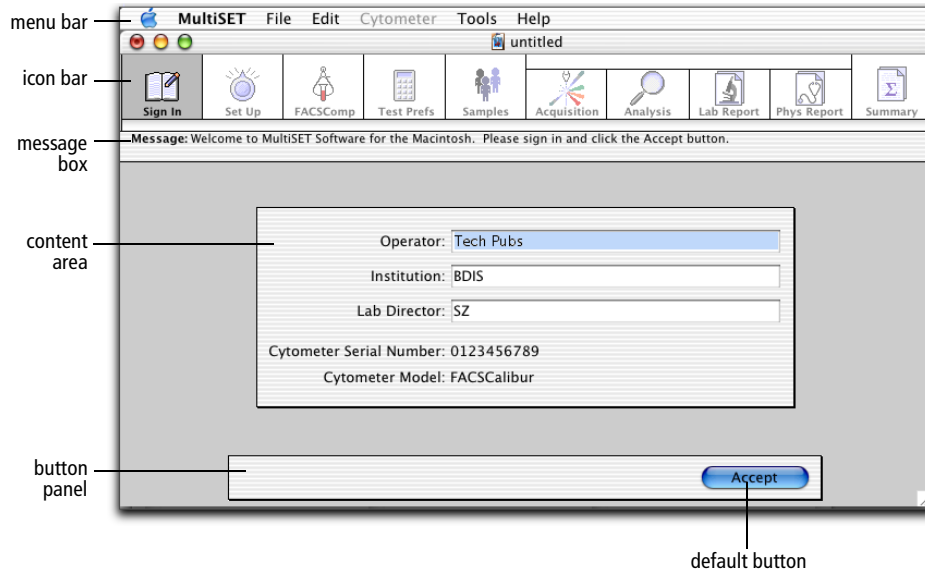


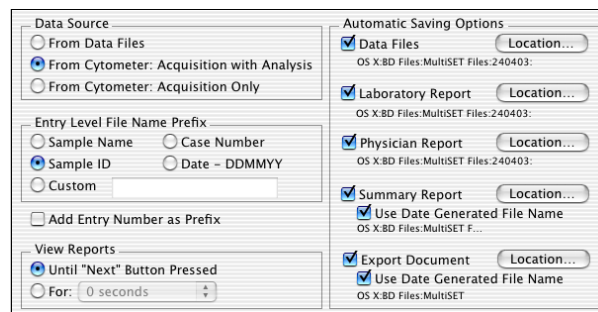
Figure 1-1 Main application window

1 Fill in the Operator field.

Only the Operator field is required. Entries in Institution and Laboratory are optional.

2 Click Accept or press Return after entering information.

3 Make the following selections in the Set Up View.



- Data is acquired and immediately analyzed when *Acquisition with Analysis* is chosen under Data Source.
- The selection made under Entry Level File Name Prefix determines the file name prefix for data files saved.

- Reports can be viewed as long as you want when *Until “Next” Button Pressed* is chosen under View Reports. Alternatively, you can choose a time limit.
- A selected checkbox Under Automatic Saving Options enables the Location button for that file so you can specify a storage location. The current file path appears below each file type. The default storage location is a dated folder located in the Multiset Files folder in the BD Files folder. A new dated folder (DDMMYY) is created each day when you start the program.

4 Click Accept to continue to the FACSCComp view.

Viewing the FACSCComp View

The FACSCComp view displays the name of the most recent Calib File and the date and time that it was created. Before following this tutorial you should have run BD FACSCComp software. On a daily basis you may find it easier to launch BD FACSCComp software at this view rather than running it separately.

1 Click Skip FACSCComp.

Making Selections in the Test Preferences View

1 Make the following selections.

Physicians Report Choices	
<input checked="" type="checkbox"/>	CD3+CD4+ %T Lymph
<input checked="" type="checkbox"/>	CD3+CD8+ %T Lymph
<input checked="" type="checkbox"/>	CD3+CD4+CD8+ %T Lymph
<input checked="" type="checkbox"/>	CD3+ %Lymph
<input checked="" type="checkbox"/>	CD3+ Abs Cnt
<input checked="" type="checkbox"/>	CD3+CD4+ %Lymph
<input checked="" type="checkbox"/>	CD3+CD4+ Abs Cnt
<input checked="" type="checkbox"/>	CD3+CD8+ %Lymph

<input checked="" type="checkbox"/>	Report Reference Ranges
<input checked="" type="checkbox"/>	QC Message for Out of Normal Range

Laboratory Report Choices	
<input checked="" type="checkbox"/>	Report Percents
<input checked="" type="checkbox"/>	Report Absolute Counts

Summary Report ID	
<input type="checkbox"/>	Sample Name
<input checked="" type="checkbox"/>	Sample ID
<input type="checkbox"/>	Case Number

- All subsets for BD-defined reagents are listed under Physicians Report Choices. Selected subsets will be included on the Physicians Report if reagents producing these results are part of the entry’s panel.
- Reference ranges appear on the Physicians Report when the Report Reference Ranges checkbox is selected. Reference ranges are defined within Subset Ranges. The range selected in Panel Tools is the range applied to the Laboratory and Physicians Reports.
- Percentages and absolute counts are reported on the Physician’s Report when Report Percents and Report Absolute Counts under Laboratory Report Choices are selected.
- The choices made under Summary Report ID determines what appears on the Summary Report for each entry.

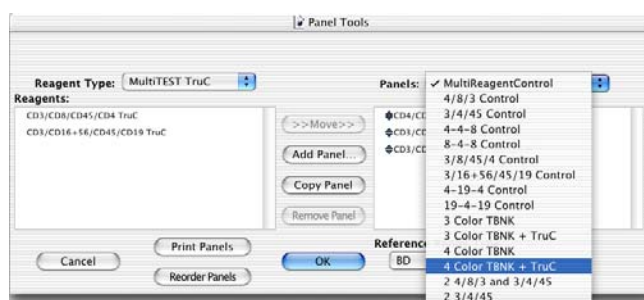
Panel Tools

Panel Tools allow you to create reagent combinations (panels) that you use regularly.

- 1 Click Panel Tools.
- 2 Click on the Panels pop-up menu and select the panel that corresponds to the reagents you used to prepare your samples.

The column on the left provides a list of reagents corresponding to the Reagent Type displayed. For example, the following graphic shows MultiTEST TruC as the Reagent Type and the reagents listed in the Reagents column are the four-color Multitest TruC (with Trucount tubes) reagents.

When you select a panel from the Panels pop-up menu, the reagents in the panel are listed below the panel name in the column on the right.



If the sample(s) you prepared is not one of the predefined panels provided, you will need to create a panel as described in the following steps.

- Click Add Panel.
- Enter a unique panel name and click Add.
- Click a reagent from the list on the left, click Move to add it to the panel.
- Continue until all reagents that you want in the panel have been added.
- Click OK.

Lot IDs

- 1 Click Lot IDs.
- 2 Click the Absolute Count Beads icon in the Lot IDs dialog.

- 3 Enter the lot ID number and the beads per pellet for the Trucount tubes.

These values are found on the BD Trucount tube foil pouch label. This information is critical for calculating absolute counts. Only one lot ID can be entered per schedule document.

- 4 Click Save.
- 5 Click Accept to advance from the Test Preferences view to the Samples view.

Entering Information in the Samples View

- 1 Enter the Sample ID.

Click to enter information in the first field. Press the tab key to move from field to field.

You must enter information in the ID column associated with the entry-level file name prefix, which you selected in the Set Up view. In this tutorial, that is the Sample ID. This prefix is used for naming data files, Laboratory Report files, and Physician Report files. Also, enter information in the field(s) selected in the Test Preferences view for the Summary Report ID. In this tutorial that is also the Sample ID. The message box at the top of the window will indicate which fields are required.

- 2 Select the panel you want to run for each sample.

Click on the panel field to open the pop-up menu, and select the panel.

- 3 Select one of the following choices and enter information in the appropriate fields if you want absolute counts and are NOT using BD Trucount tubes.

Enter one of the following:

- Enter a WBC count (x1000 cells/cubic mm) and Lymphs (%).

Enter a value from 0.0 to 999.9 in the WBC field. The white blood cell (WBC) count is obtained from a cell counter, independent of the flow cytometer.

Enter a value from 0 to 100 in the Lymphs (%) field. This value is obtained from a cell counter or differential count, independent of the flow cytometer.

- Enter Lymphs (x1000)

Enter a value from 0.00 to 999.99 in the Lymph (x1000/ μ L) field. This value is obtained from a cell counter, independent of the flow cytometer.

BD Multiset software will use these values to calculate an absolute count for each subset for which you request an absolute count.

Choosing Preferences

- 1 Choose MultiSET > Preferences.
- 2 Click the Printing icon.
- 3 Select to print for all report types.
- 4 Click Save.

Saving a Schedule Document

- 1 Choose File > Save to save the Schedule document.

A default name appears (ddmmyy.sch). Enter a different name if you want.

The Schedule document file contains all data entered at the Sign In, Set Up, and Samples views, as well as preferences. You can save a Schedule document and open it later to view, to complete processing, or to add more samples for processing. Only one Schedule document can be open at a time. You cannot print a Schedule document.

- 2 Click Save.

The default storage location for the Schedule document file is User/Your Name/Documents. To choose a different location do the following.

- Make a selection from the Where pop-up menu.
- Click on the arrow next to the pop-up to display the bottom half of the dialog.
- Make a selection from the left and right columns.

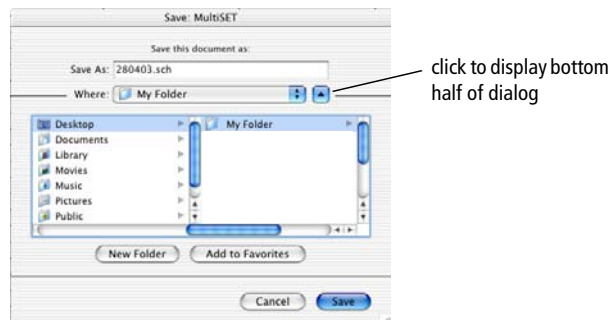


Figure 1-2 Save MultiSET dialog

In the example shown in Figure 1-2, the Schedule document will be saved in *My Folder* on the Desktop.

NOTICE If you want to routinely use the same Sign In, Set Up, and Test Preferences information, create a template Schedule document to save time. To create a template Schedule document, save the Schedule before entering sample-specific information at the Samples view. When using the template Schedule document, select File > Save As instead of Save. This enables you to save a copy of the template with a different name, in a different folder, or on a different disk.

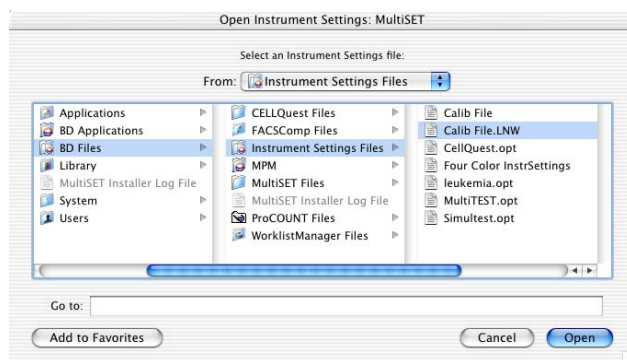
Retrieving Instrument Settings

For this exercise, you will retrieve the Calib File.LNW instrument settings generated from the QC run performed by BD FACSComp software. These settings are appropriate for human peripheral blood samples prepared with Tritest or Multitest reagents.

- 1 Choose Cytometer > Instrument Settings.

Instrument Settings can be accessed from the Samples view or while viewing reports.

- 2 Click Open.
- 3 Navigate to the Calib File.LNW instrument settings file located in the BD Files/Instrument Settings Files folder, then click Open.



If you have not created an optimized instrument settings file, run FACSComp to set up the cytometer for the sample(s) you prepared. Refer to the *BD FACSComp Software Tutorial* for more information.

- 4 Click Set to send these settings to the cytometer.
- 5 Click Done.

Optimizing Instrument Settings

It is necessary to view your samples and optimize, the SSC and FL3 threshold instrument settings before saving data.

- 1 Click Run Tests from the Samples view to advance to the Acquisition view.

The Acquisition view allows you to view a live display of samples before and during acquisition. Data is not saved until you click Acquire.

- 2 Display the Detectors/Amps and Threshold windows.

- Choose Cytometer > Detectors/Amps.
- Choose Cytometer > Threshold.

- 3 Set the fluidic mode to RUN and install the stained sample on the cytometer.

The tube to be installed on the cytometer is indicated at the top of the Acquisition view.

- 4 If necessary, while viewing a plot with SSC displayed, optimize the SSC voltage or Amp Gain settings.

An example of optimized SSC settings is shown in Figure 1-3.

- 5 If necessary, while viewing a plot with FL3 displayed, optimize the FL3 threshold setting to exclude most of the debris.

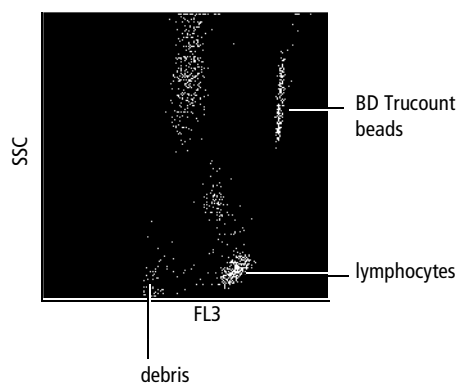
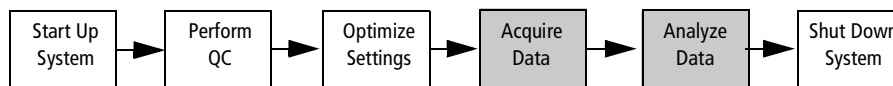


Figure 1-3 Optimized SSC and FL3 threshold settings

- 6 Close the Detectors/ Amps and Threshold windows.

You have just completed optimization of instrument settings for the sample you are running.

Acquiring and Analyzing Data



You can acquire data files and immediately analyze the data, if you want. Alternatively, you can acquire data files and perform batch data analysis at a later time. For this tutorial, we chose to do Acquisition with Analysis in the Set Up view.

For information about performing batch data analysis, refer to the *BD Multiset Software User's Guide*.

1 Click Acquire.

Acquisition continues until the designated number of events to acquire is reached. Multiset performs an initial acquisition by acquiring the number of events set in the Events Preferences. The software then checks to see if the minimum target population events have been collected. The acquisition target events are set in Reagent Tools. If the target population event numbers have been reached, Multiset proceeds to analysis. If the target population event numbers have not been reached, Multiset calculates the additional number of events needed to satisfy the target population requirement.

2 Remove the sample from the cytometer when acquisition is complete.

3 Install a tube of DI water on the SIP and place the cytometer in standby.

Analyzing Data

The Laboratory Report view appears when acquisition for a single tube is complete. As each tube within a panel is acquired, the information is added to the Laboratory Report. When appropriate, QC Messages are reported. At the bottom of the report, Entry Level QC results are summarized. The Laboratory Report file is a PDF file that can be opened and printed by Adobe® Acrobat Reader or any software that reads PDF files.

You will adjust the Expert gate and other gates to practice manual gating.

1 Use the scroll bar to view all the information on the Laboratory Report. See Figure 1-4 on page 11.

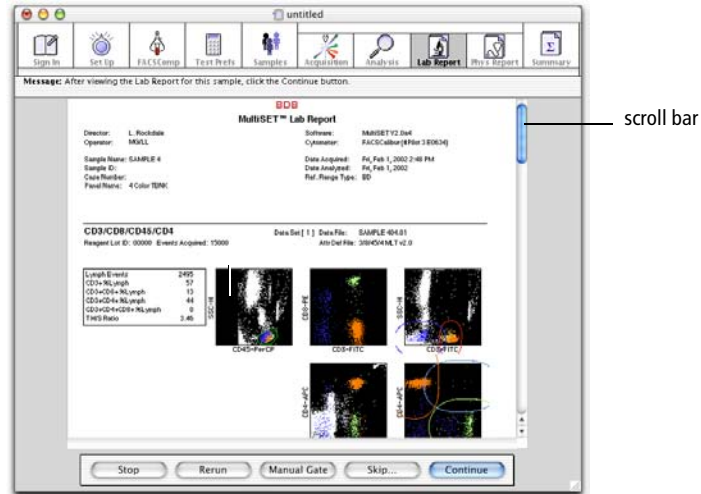


Figure 1-4 Laboratory Report

- 2 Click Manual Gate at the bottom of the Lab Report view.
- 3 Adjust the Expert gate. See Figure 1-5.

BD MultiSET software's expert lymph gate identifies the lymphocyte population from a CD45 vs side scatter (SSC) or CD3 vs SSC display in any parameter. However, BD Biosciences has validated performance of reagents containing CD45 PerCP or CD3 PerCP only.

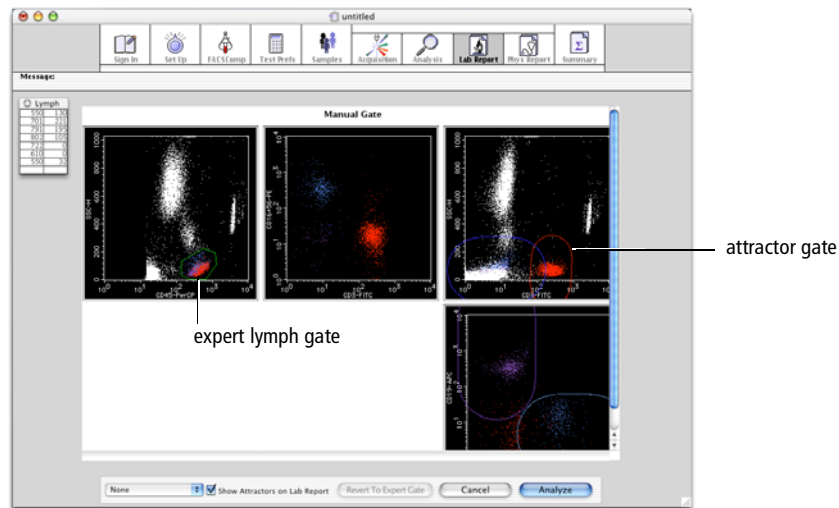


Figure 1-5 Manual Gating view

To adjust the expert gate, do the following.

- Click once to select the FL3 vs SSC plot with the expert gate (polygon-shaped gate). Double-click to enlarge a plot if needed.

- To redraw the lymph gate, position the cursor at the starting point you want on the plot and click. Continue until a polygon surrounds the lymphocytes.
- To revert to the original gate and display set by the software, click Cancel or Revert to Expert Gate.

NOTICE Changes to the gate and attractors are in effect for the current tube and are not saved.

4 Adjust an attractor gate.

Attractor gates identify lymphocyte subsets from fluorescence parameter combinations. Attractors are able to automatically adapt themselves if a population shifts.

- To move an attractor, click near its center and drag into position.
- To uniformly scale the size of an attractor, drag an edge.
- To change the orientation and length, drag one of the two endpoints.
- To revert to the original gate and display set by the software, click Cancel or Revert to Expert Gate.

5 Click Analyze to confirm the changes and recalculate the results.

Acquiring the Remaining Tubes

- 1** Click Continue to acquire the remaining tubes in your panel.
- 2** When you have acquired the last tube in the panel, click Continue from the Laboratory Report view to advance to the Physician Report view.

The Laboratory Report will automatically print.

Viewing the Physician Report

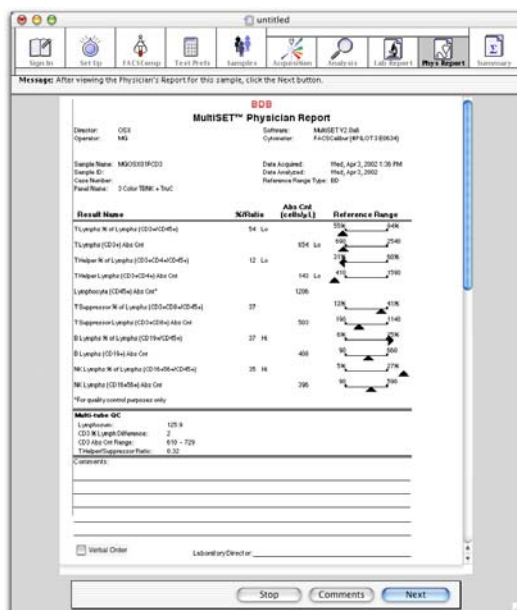
The Physician Report is a summary of information for one sample. It contains all results that were selected for each reagent in a panel. If more than one reagent in a panel provides a particular subset result, the average subset result is reported.

BD Multiset software reports multitube results, including lymphosum, two CD3 consistency results (% CD3 and CD3 Abs Ct Range), and a T-helper/suppressor (T H/S) ratio determined from more than one tube.

The report also contains a Comments section where you can enter the lab director's name as well as any additional sample information. The Verbal Order checkbox allows you to indicate when a test was verbally requested by a physician. The Physician Report

file is a PDF file that can be opened and printed by Adobe Acrobat Reader or any software that reads PDF files.

- 1 Use the scroll bar to view all the information on the Physicians Report.



- 2 Click Next.

The Physicians Report will automatically print.

Viewing the Summary Report View

The Summary view appears after the last sample in the schedule has been run. The Summary Report contains a list of all entries in the Schedule document.

The Summary Report file is a PDF file that can be opened and printed by Adobe Acrobat Reader or any software that reads PDF files.

- 1 Use the scroll bar to view all the information on the Summary Report. See Figure 1-6 on page 14.

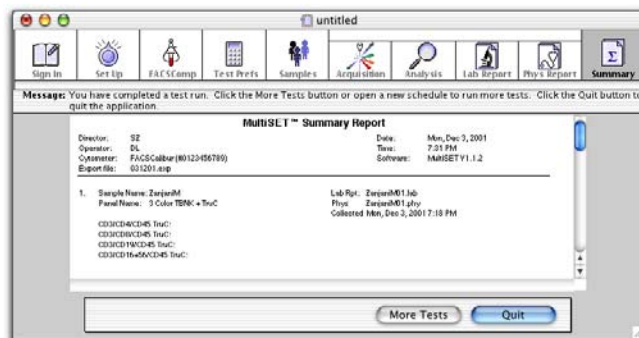


Figure 1-6 Summary Report

2 Click Quit.

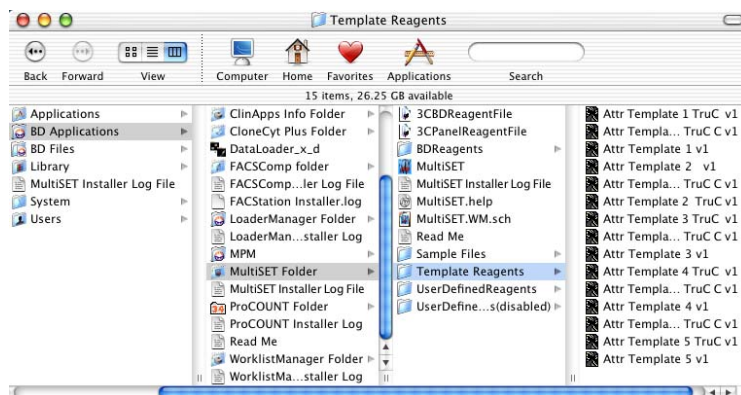
The Summary Report will automatically print and a message appears confirming you want to quit the software.

You have completed the optimization and data acquisition and analysis tutorials. To practice using the user-defined reagents feature, complete the following tutorial.

Using User-Defined Reagents

You can create user-defined reagents in the following ways.

- Copying and then customizing one of the predefined reagents that comes with the software to analyze BD Tritest and BD Multitest reagent combinations.
- Using any of the fifteen predefined user-defined templates provided in the Template Reagents folder in the Multiset Folder.



- Creating attractor sets for use with user-defined reagents in BD Attractors software.

Practice creating a user-defined reagent for a fictitious reagent with the following tutorial. The tutorial provides step-by-step instructions for modifying the predefined BD reagent CD3/CD8/CD45/CD4.

Copying a Predefined Reagent

- 1 Launch BD Multiset software, if needed.
- 2 Choose Tools > Reagent Tools.
- 3 Choose MultiTEST from the Reagent Type pop-up menu.
- 4 Choose CD3/CD8/CD45/CD4 from the Reagent pop-up menu.
- 5 Click Copy Reagent at the bottom of the Reagent Tools dialog.
- 6 Enter 3/X/45/4 as the reagent name; click OK.

UD is automatically appended to the name entered. The Reagent type changes to User Defined and the Reagent displayed is the copied reagent.

Customizing a User-Defined Reagent

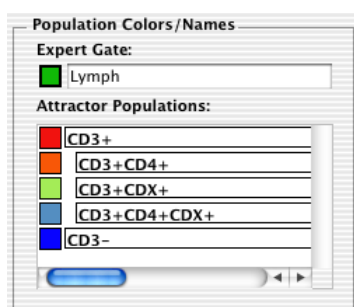
To practice customizing a user-defined reagent, you will make several changes to the reagent you just copied.

- 1 Change the FL2 antigen name to CDX.

Under the FL Parameter Info section, highlight the Antigen field and enter CDX in place of CD8.

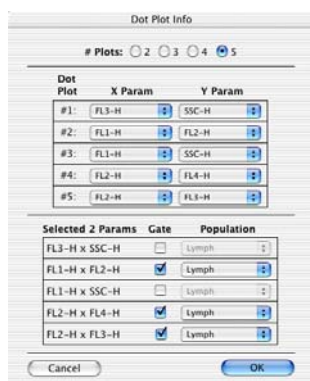
- 2 Under Acquisition Target Info, change the value in the *Min Events to Acquire* field to 2500.

- 3 Under population Colors/Names, replace the number 8 with X.



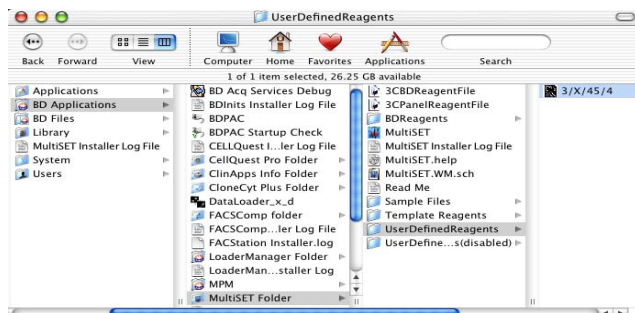
- 4 Click Dot Plot Info and make the following selections.

You are adding an additional plot (FL2 vs FL3) and choosing a Lymph Gate to apply to the new plot.



- 5 Click OK to exit the Dot Plot Info dialog. Click OK to exit the Reagent Tools dialog.

A message dialog appears. The user-defined reagent is automatically saved in the UserDefinedReagents folder in MultiSET Folder.



Creating a New Panel

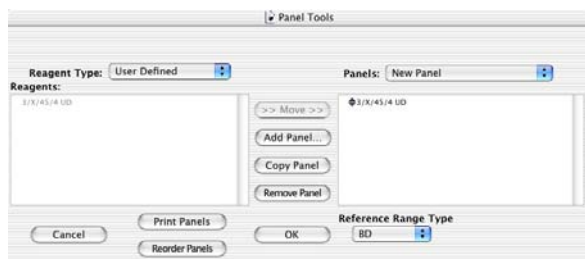
Before you can use the new reagent that you just defined to acquire and analyze data, you need to create a new panel with the new reagent added.

- 1 Choose Tools > Panel Tools.
- 2 Click Add Panel in the Panel Tools dialog.
- 3 Enter a name in the dialog that appears; click Add.
- 4 Choose User Defined from the Reagent Type pop-up menu.

The 3/X/45/4 UD reagent is listed under Reagents.

- 5 Select the reagent from the Reagents column and click Move.

The reagent appears beneath Panels.



- 6 Choose a different reference range type from the Reference Range Type pop-up menu, if you want.

You can define your own reference ranges in Tools > Subset Ranges. Up to six user-defined ranges can be entered for each subset. Refer to the *BD Multiset Software User's Guide* for details.

- 7 Click OK to exit the Panel Tools dialog.

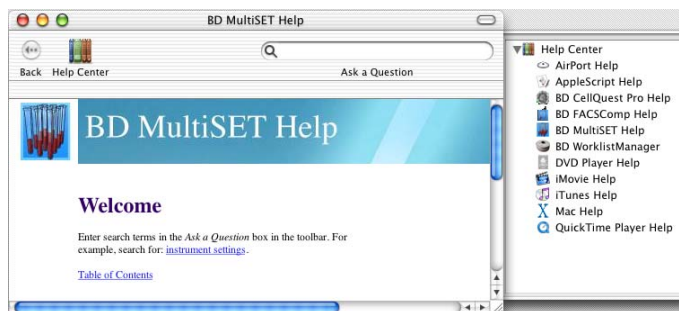
You have just completed the tutorial for creating user-defined reagents. To use the panel containing user-defined reagents, simply choose the panel in the Samples view from the Panel pop-up menu for a particular sample.

To practice using the Help feature, do the following tutorial.

Using Help

When you need assistance with BD Multiset software, use the Help viewer to quickly locate the information.

- 1 From the Help menu, choose Multiset Help.



The fastest way to search for help is to use the Ask a Question feature. You can also search by looking through the table of contents or index.

- 2 Enter *expert lymph gate* in the Search field; press Return.

A list of topics appears in the lower portion of the Help view. See Figure 1-7 on page 19. Double-click on a topic to go to that section of Help.

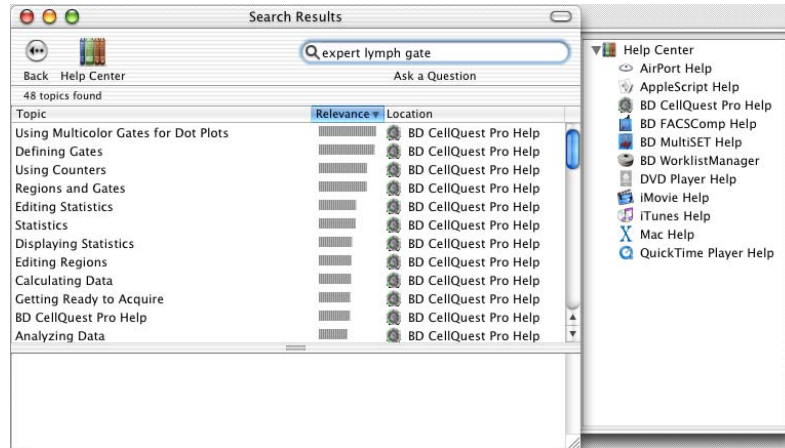


Figure 1-7 Topics listed in Search Results Window

- 3** When you are finished, close the Help viewer by clicking the red close button.
- 4** Choose MultiSET > Quit MultiSET; click Quit.

