

BD CSampler™ Software User Guide

Science is hard. Flow cytometry should be easy.™

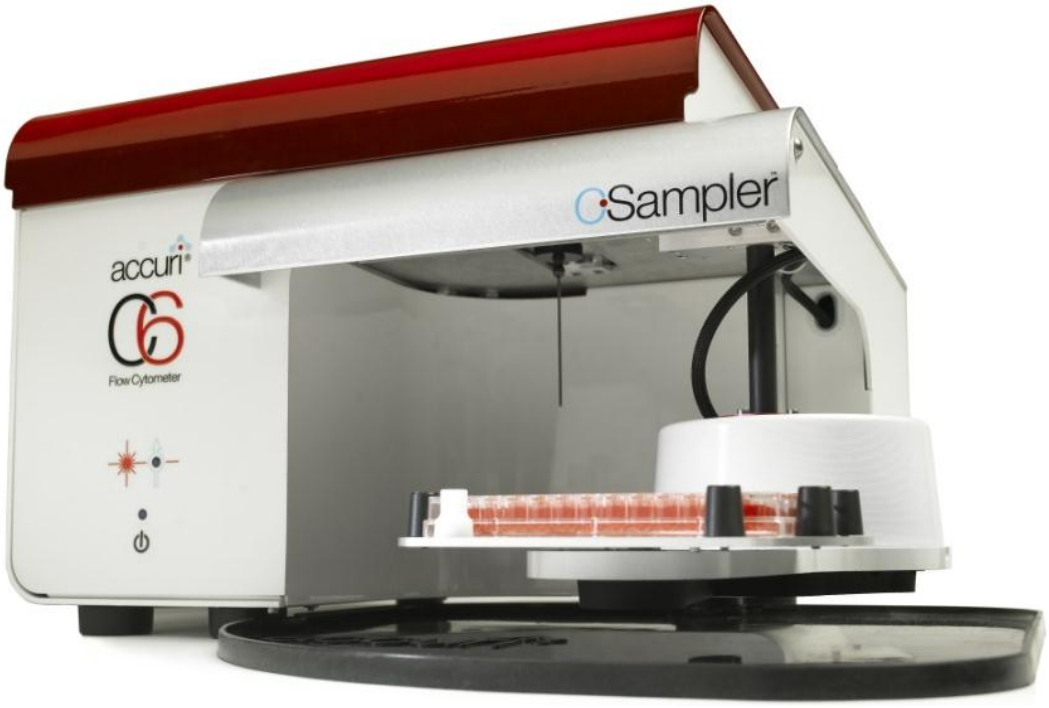


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1 INTRODUCTION TO BD CSAMPLER

The BD CSampler is an optional accessory for the BD Accuri C6 and C5 flow cytometers that allows for the collection of samples prepared in 48- and 96-well plates and standard 12 x 75 mm tubes in a 24-tube rack.

The BD CSampler is compatible with the following plates:

96-well plates (standard flat, u-, and v-bottom)

96-well deep well plates

48-well plates

24-tube rack for standard 12 x 75 mm tubes or microcentrifuge tubes

NOTE: For best results, only use the 24-tube rack supplied with the BD CSampler.

1.1 About this Manual

This manual describes the installation and operation of the BD CSampler in conjunction with the BD Accuri C6 flow cytometer. Installation and operation of the BD CSampler with the BD Accuri C5 flow cytometer is in all points identical to those with the C6 and every mention of the C6 instrument in the manual can be substituted by C5. The CSampler software user interface will also refer to the connected flow cytometer as ‘C6’ even when connected to a BD Accuri C5 flow cytometer.

1.2 Installing the BD CSampler

Install and validate the BD Accuri C6 flow cytometer with 6- and 8-peak beads before installing the BD CSampler. Inspect the BD CSampler Accessory Kit contents to ensure delivery of all components.

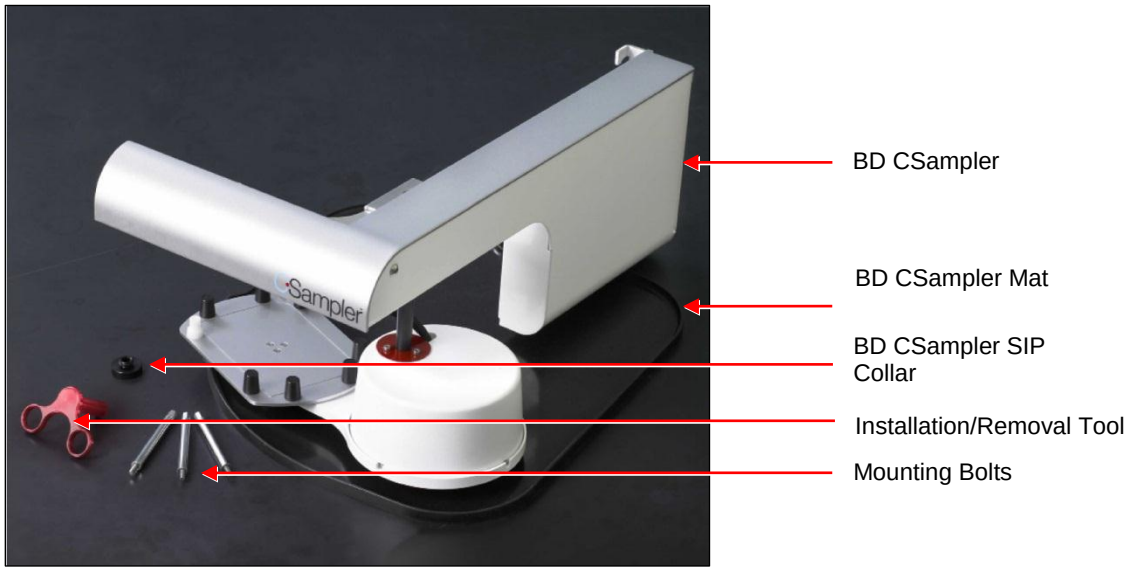


Figure 1-1. BD CSampler Accessory Kit

Table 1-1. BD CSampler Shipping Contents

Qty	*Item*
1	BD CSampler
1	BD CSampler Mat
3	Mounting Bolts
1	Installation and Removal Tool
1	BD CSampler Collar
1	24-Tube Rack (not shown)
1	BD CSampler Software (not shown)

To install the BD CSampler:

- Turn off the flow cytometer.

  CAUTION: Failure to shut down the flow cytometer during BD CSampler installation could result in damage to both the flow cytometer and BD CSampler electronic modules.

- Unscrew the SIP collar, remove the sample stage, and install the BD CSampler SIP collar.

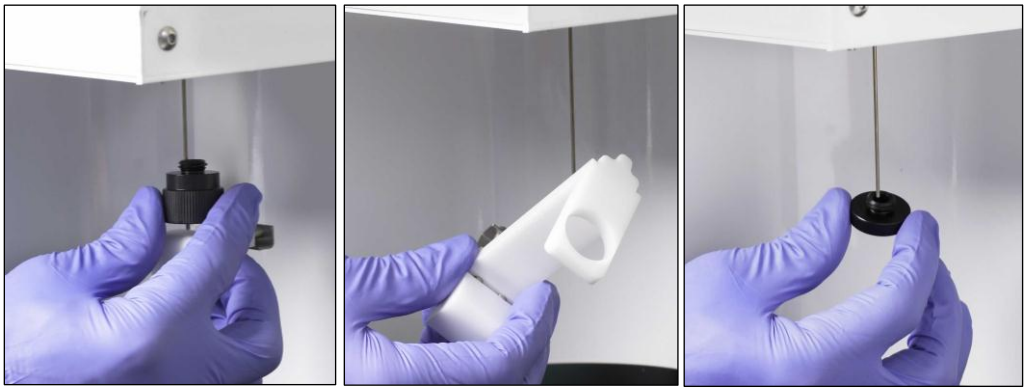


Figure 1-2. Installing the BD CSampler SIP Collar

- Open the lid of the flow cytometer and locate the bolt holes for mounting the BD CSampler.

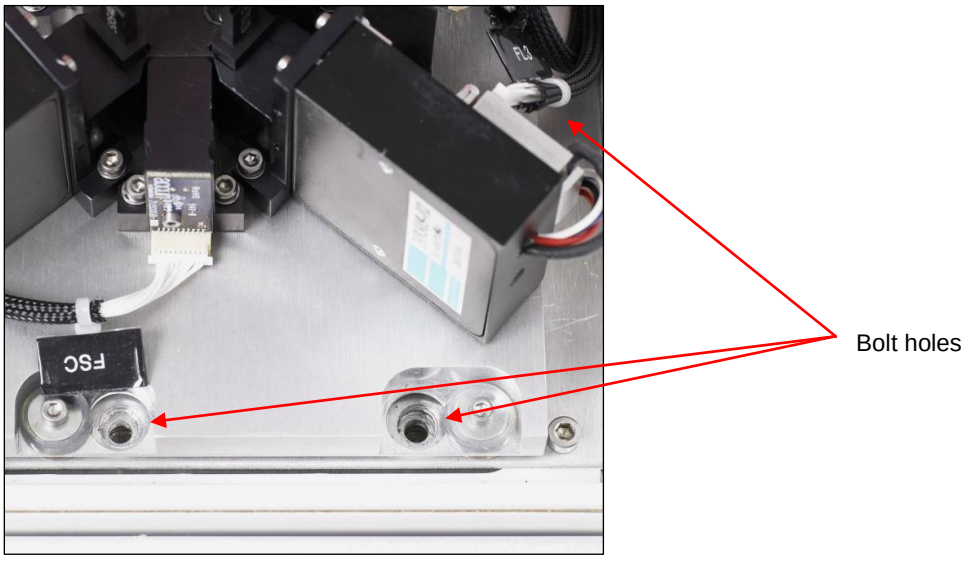


Figure 1-3. Location of Mounting Bolt Holes

- Hold the BD CSampler chassis with both hands and place the tab into the slot on the back of the flow cytometer.

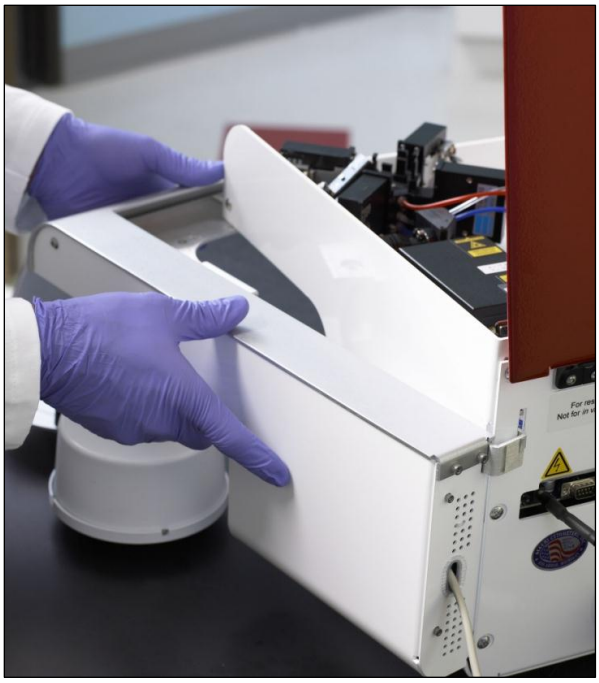


Figure 1-4. Insert BD CSampler Tab into Flow Cytometer

- Align the front of the BD CSampler so that the holes on the module are lined up with the three threaded holes in the flow cytometer.
- Hold the module with one hand and partially screw in the right mounting bolt to secure the BD CSampler to the flow cytometer. Tighten with the installation and removal tool.



Figure 1-5. Secure the BD CSampler to the Flow Cytometer

CAUTION: Do not completely tighten any bolt before all three bolts are partially screwed. Completely tightening a bolt too early can cause shearing stress on the materials.

- Partially screw in the remaining two mounting bolts.



Figure 1-6. Secure the BD CSampler to the Flow Cytometer

- Confirm that the module is lined up properly with the flow cytometer and completely tighten all three bolts.
- Connect the serial cable to the socket at the back of the flow cytometer.

Turn on the flow cytometer. The BD CSampler automatically aligns itself and rests in the home position.



Figure 1-7. Connect the BD CSampler Cable to the Flow Cytometer

- Place the mat on the bench, under the arm of the BD CSampler.



Figure 1-8. BD CSampler with Mat Installed

CAUTION: Failure to keep the mat clear of plates, tubes or any other materials may result in damage to the BD CSampler.

Run validation beads to ensure correct BD CSampler operation (see chapter 3, “Validating the Performance of the BD Accuri C6”).

2 BD CSAMPLER SOFTWARE OVERVIEW

BD CSampler Software controls the BD Accuri C6 flow cytometer system with BD CSampler in order to acquire data, generate statistics, and analyze results. BD CSampler Software provides the following features:

Tabbed views for collection, analysis, and statistics

Digital signal processing and color compensation at any time

Drag and drop plots

File export in FCS 3.0 format

Seamless data file importation into FCS Express

Batch Analysis of sample data

Enhanced Analysis upgrade adds:

- Drag and drop of publication-quality images
- Event coloring
- Live gating

2.1 Starting BD CSampler Software

Do not use BD CSampler Software until the BD CSampler has been completely set up (see chapter 1, “Introduction to BD CSampler”).

To open BD CSampler Software:

- Double-click on the BD CSampler Software icon on the computer desktop. BD CSampler Software opens a new, blank workspace.
- Select a plate type from the drop-down menu.

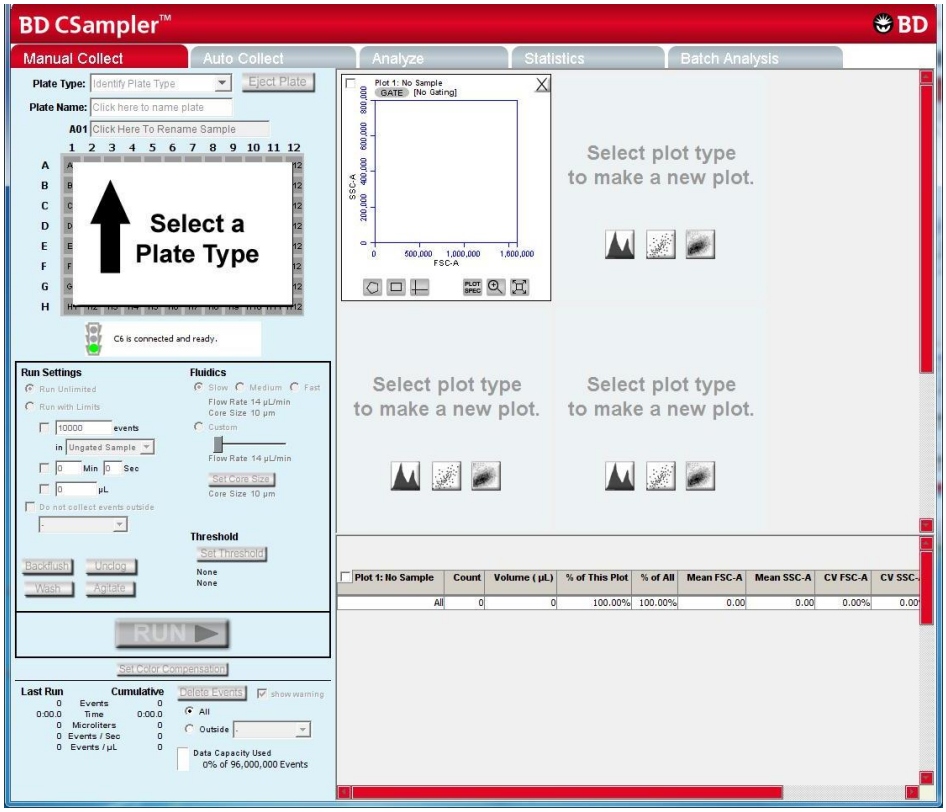


Figure 2-1. New BD CSampler Software Workspace

NOTE: If BD CSampler Software displays the message *Extra startup time needed due to cleaning or improper shutdown,* the flow cytometer will take several more minutes than usual to recover and return to the green-light ready state. This may occur on initial flow cytometer startup after installation. It will also occur after an interruption of power to the unit.

2.2 BD CSampler Software Workspace

The main BD CSampler Software window is called the BD CSampler workspace. The workspace contains controls and displays that provide access to all functions required for data acquisition and analysis. The workspace is organized on five separate tabs:

Manual Collect—Contains controls for setting up data collection and acquiring data in any order (see chapter 4 for details).

Auto Collect—Contains controls for automatically collecting data from several wells in order (horizontally or vertically), starting at a designated well (see chapter 5 for details).

Analyze—Allows analysis of multiple samples simultaneously (see chapter 6 for details).

Statistics—Displays statistical information (see chapter 7 for details).

Batch Analysis—Contains controls for analyzing batches of sample data (see APPENDIX F for details).

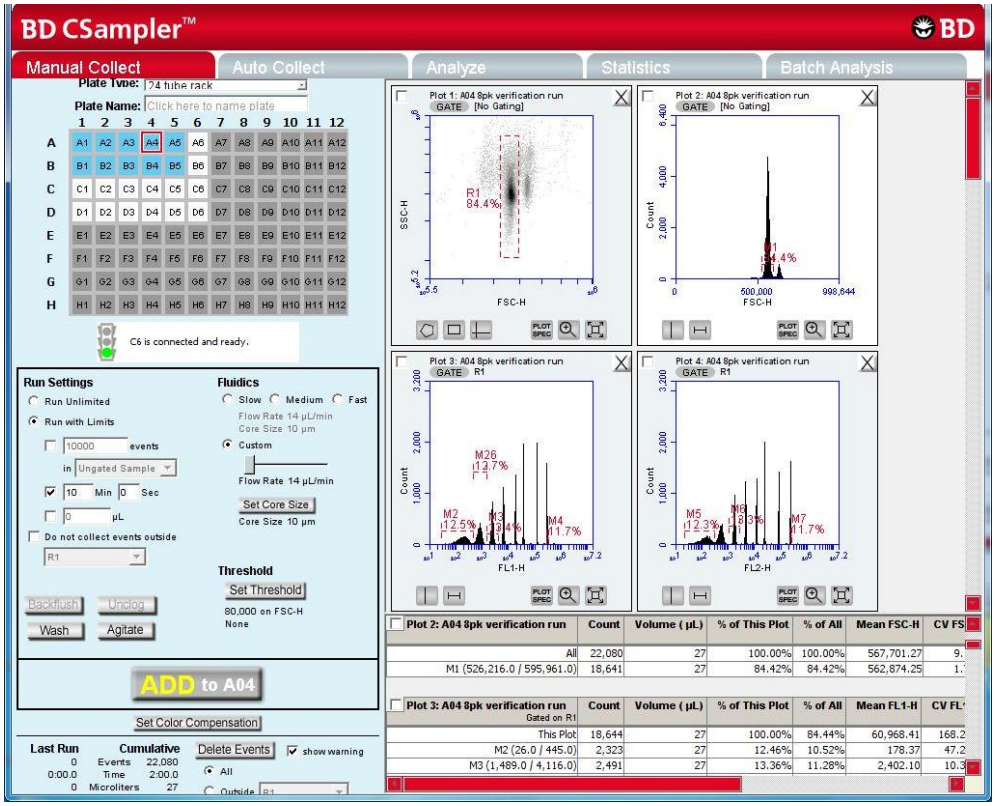


Figure 2-2. BD CSampler Software *Manual Collect* Tab Workspace

2.3 Opening a New BD CSampler Software Workspace

A new BD CSampler Software workspace (Figure 2-2) displays a single FSC-A versus SSC-A density plot that is pre-zoomed to channel values of 1,600,000 and 800,000 respectively. Run Settings will be set to *Run Unlimited*, the Threshold will be set to channel 80,000 on the FSC-H signal, and no color compensation values will be set. A new workspace can be used to create an analysis template and to collect a new dataset.

To open a new workspace:

- Do one of the following:
  - If BD CSampler Software is not already open, double-click on the BD CSampler Software icon on the computer desktop.
  - If BD CSampler Software is already open, select *File > New Workspace File*. If desired, save any unsaved changes to the previous workspace when prompted.

2.4 Loading and Ejecting a Plate

BD CSampler controls movement of the plate platform into position for efficient loading or ejecting racks and plates.

To move the BD CSampler arm to the load or eject position click on the *Load Plate/Eject Plate* toggle button.

NOTE: After 15 minutes in the eject position, the arm and plate platform automatically return to the home position with the SIP in the wash station (the white receptacle on the plate platform).

2.5 Aligning the BD CSampler after a Collision

The BD CSampler performs an alignment to verify that the plate is aligned to the SIP every time the flow cytometer is powered up or if the BD CSampler arm collides into an object. A manual alignment can be performed in BD CSampler Software at any time.

If there is an obstruction in the path of the BD CSampler arm, BD CSampler Software displays a red Traffic Light and opens a message box indicating that a collision has occurred.

To perform an alignment:

- Remove any objects from the BD CSampler mat.

Do one of the following:

- If a collision has occurred, click on the *Align* button in the Collision Detected dialog box.
- If a collision did not occur, select *Instrument* > *Align BD CSampler*.



Figure 2-2. Collision Detected Window

If a second collision occurs, BD CSampler Software automatically performs a second alignment. If the second alignment fails, contact Technical Support. Samples left on the sample stage can be recovered by gently pushing down on the white cylindrical motor housing.

2.6 Exiting BD CSampler Software

- Select *File* > *Quit.*
- If prompted to save changes to the BD CSampler Software workspace, do one of the following:
  a. Click on the *Yes* button to save changes.

b. Click on the *No* button to close BD CSampler Software without saving changes.

c. Click on the *Cancel* button to cancel the exit and keep BD CSampler Software open.

2.7 Using the Example BD CSampler Software File

An example BD CSampler Software file of a four-color analysis of human peripheral blood (HPB 4 Color Example.c6) can be downloaded from the BD Accuri website (http://www.bdbiosciences.com/support/resources/accuri/index.jsp). This data file can be used to explore various tools in BD CSampler Software without the worry of corruption or loss of experimental data. Figures throughout this user guide show data from the example file.

To create the example file, four sample tubes were used to assess the $CD3^+CD4^+$ and $CD3^+CD8^+$ cell populations. These samples were prepared by staining peripheral blood with directly conjugated antibodies, followed by red cell lysis, according to standard methods.

The following table describes the experimental design:

- Tube 1—Background control (unstained)
- Tube 2—White blood cell gating control ($CD45^+$)
- Tube 3—T-cell gating control
- Tube 4—Experimental sample

Table 2-1. Experimental Design for HPB 4-Color Tutorial File

Fluorochrome	*Sample Tube*			
	Tube 1	Tube 2	Tube 3	Tube 4
FITC	Isotype	Isotype	**CD3**	**CD3**
PE	Isotype	Isotype	Isotype	**CD4**
PE-Cy7	Isotype	**CD45**	**CD45**	**CD45**
APC	Isotype	Isotype	Isotype	**CD8**

This experimental design does not contain all single-stained fluorescence controls, but only those required for determining color compensation settings to correct fluorescence spillover. For details on correcting fluorescence spillover, see section 4.14.

3 VALIDATING THE PERFORMANCE OF THE BD ACCURI C6 FLOW CYTOMETER

Perform a validation of the system at least once each day of use. This ensures that the flow cytometer is working properly before running experimental samples.

Using the same BD CSampler Software file each day allows identification of trends over time. When collecting validation data, BD CSampler Software advances to the next empty well in row A-B (for 8-peak beads) or C-D (for 6-peak beads). Include the date in the *Sample Naming Field* for each day of validation. Start a new validation bead file when all data wells are full.

Reagents required:

Spherotech 8-Peak Validation Beads (PN 653144, supplied with the initial flow cytometer shipment)

Spherotech 6-Peak Validation Beads (PN 653145, supplied with the initial flow cytometer shipment)

- Sheath fluid: Deionized, filtered water (0.2 µm filter) plus Bacteriostatic Concentration Solution (PN 653156, supplied with the initial flow cytometer shipment)

3.1 Running Validation Beads (Daily Start-Up)

Setup:

- Verify that the Bead Template has been copied to the BD CSampler Software computer. The file is located on the BD CSampler Software CD or flash drive and is on the BD Accuri website (http://www.bdbiosciences.com/support/resources/accuri/index.jsp/).

  NOTE: The template is set up for the 24-tube rack only.

- Open BD CSampler Software.
- Select *File* > *Open Workspace or Template*.
- In the Open dialog box, browse to the location of the bead template file and open the file. If adding to an established BD CSampler Software bead file, browse to the location of the file.

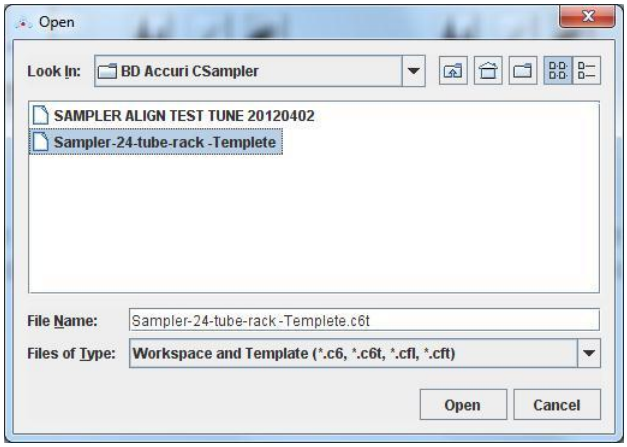


Figure 3-1. Open Bead Template

- Place the following tubes on the 24-tube rack:

Table 3-1. 24-Tube Rack Setup for Validation

Type of Sample	*Location in BD CSampler Software Sample Grid/Tube Rack*
2 mL of filtered, deionized water	A1
8-peak validation beads, prepared according to package instructions	A2
6-peak validation beads, prepared according to package instructions	C1

- Select well A1 in BD CSampler Software.

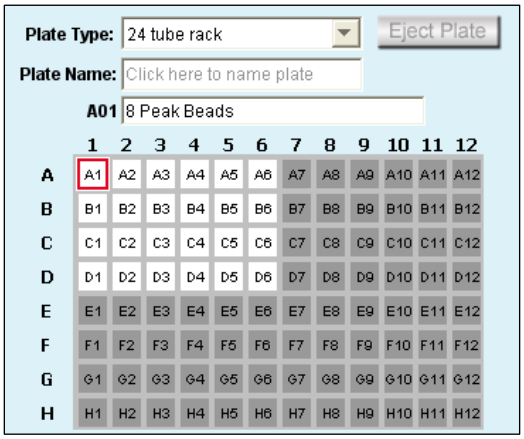


Figure 3-2. Select Well A1

- Enable the *Run with Limits* radio button in the Instrument Control Panel.

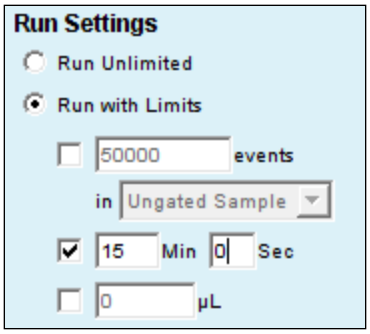


Figure 3-3. Run Limits: Disable Run Unlimited

- Enable the *Time* check box next to the *Min* and *Sec* fields in the Instrument Control Panel and type in a run time of fifteen minutes.
- Select the *Fast* radio button in the Fluidics section of the Control Panel.
- Click on the *RUN* button to rinse the SIP.
- When prompted, save the file.
- Once the run is finished, click on the *Delete Sample Data* button to delete data collected during the rinse.

Run 8-Peak Validation Beads:

- Select the first empty data well in rows A-B. This should correspond to the tube containing 8-peak beads.
- Disable the *Time* check box next to the *Min* and *Sec* fields, enable the *Events* check box and type *50,000* into the *Events* field.
  - Select *Ungated Sample* from the associated drop-down list.

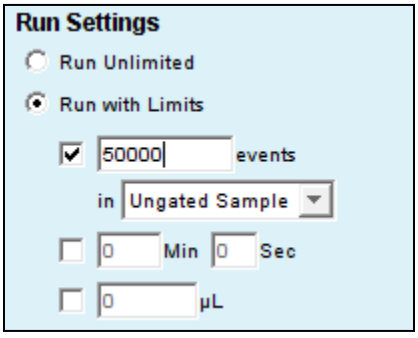


Figure 3-4. Run Limits: 50000 Events

- If desired, click on the *Agitate* button to resuspend the beads in the 24-tube rack.
- Select the Slow radio button in the Fluidics section of the Control Panel.
- Click on the *RUN* button to start acquisition. Acquisition automatically stops after 50,000 total events are acquired.

  CAUTION: Make sure the well in BD CSampler Software is empty before starting the run. If the button displays *ADD TO,* the well already contains data.

  NOTE: The R1 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

- Name the sample by typing a name in the text box just above the Sample Grid. Include the date in the sample name to differentiate it from samples collected on other dates.

  NOTE: Samples can be named before, during, or after collection.

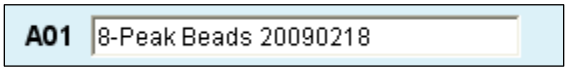


Figure 3-5. Sample Name: 8-Peak Beads

- When the collection is finished, click on the *Wash* button to minimize sample carryover.

Run 6-Peak Validation Beads:

- If desired, click on the *Agitate* button to resuspend the beads in the 24-tube rack.
- Click on the well corresponding to the tube containing 6-peak beads.

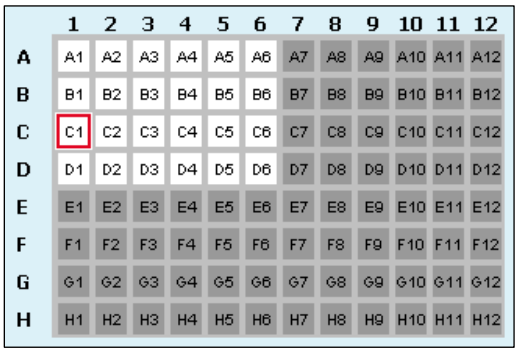


Figure 3-6. Select Well for 6-Peak Beads

- Verify that the check box by *Events* is still enabled and set at 50000 and that *Ungated Sample* is still selected from the drop-down list.

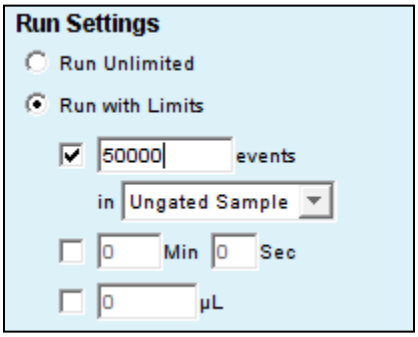


Figure 3-7. Run Limits: 50000 Events

- Click on the *RUN* button.

  NOTE: The R2 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

- Name the sample with a name that includes the date processed.

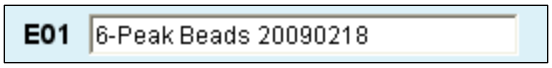


Figure 3-8. Sample Name: 6-Peak Beads

- When the collection is finished, click on the *Wash* button to minimize sample carryover.

End the Procedure:

- Place a tube with 2 mL of filtered, deionized water in the A1 position of the 24-tube rack and advance to the data well in BD CSampler Software.
- Select the *Time* check box (*Min Sec*) in the Instrument Control Panel and set it for two minutes.

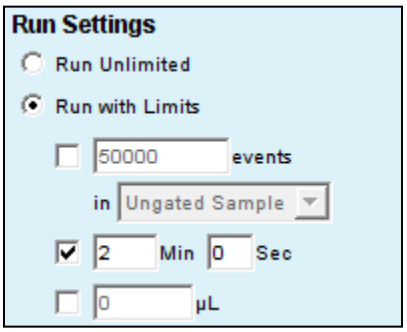


Figure 3-9. Run Limits: 2 Minutes

- Click on the *RUN* button.
- When the run is finished, leave the tube on the SIP. The BD CSampler will automatically place the SIP in the wash station after 15 minutes in the idle position.

3.2 Saving Validation Bead Data

By default, BD CSampler Software automatically saves data at the end of each sample run. Data can also be saved manually at any time. For information on saving data, see section 4.12.

3.3 Analyzing and Recording Validation Bead Data

After the bead data is collected, analyze the data using the *Manual Collect* tab of BD CSampler Software to ensure that the flow cytometer is functioning properly.

- Click on the well that contains the most recent 8-peak bead data (rows A and B).

- On the first FSC-H vs. SSC-H plot (scatter plot) in the bead file, adjust the pre-drawn region (R1) to encompass the main population by dragging the border of the region (see Figure 3-10). R1 should contain at least 80% of total events.

  NOTE: There is usually a "shadow" population (called *bead doublets* or *clumps*) that is slightly higher in FSC-H than the main cluster of beads; this is normal for these beads. Do not include the shadow group in R1.

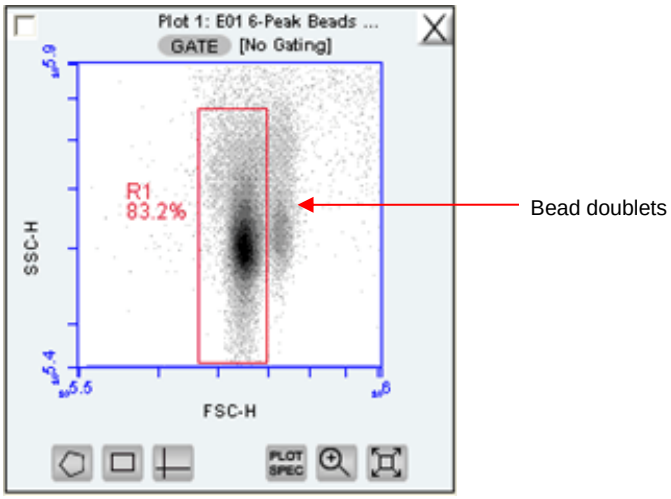


Figure 3-10. Plot with Bead Doublets

- Verify that the next three plots (FL1-H, FL2-H, and FL3-H) are gated on scatter region R1 and that the plots display the message *R1 in all* next to the *GATE* button (Figure 3-11). If it is not displayed, click on the *GATE* button and select *R1 on all events* from the pop-up dialog box (for information about gates, see section 4.7).

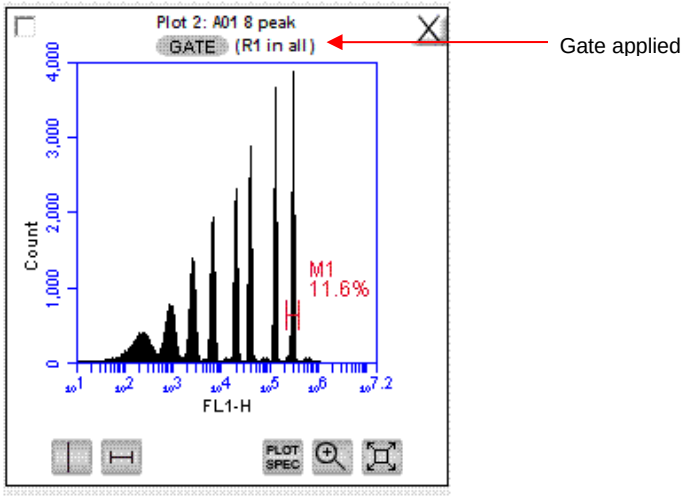


Figure 3-11. Gate Applied to 8-Peak Bead Plot

- Measure the CV of the top (brightest, far right) peak on each of the three fluorescence plots. Do the following to place the pre-drawn horizontal marker tightly around the peaks:
  - Use the Zoom Tool in the plot to zoom in on the top peak (see section 4.11).
  - Adjust the marker tightly around the peak by clicking on the marker and dragging its edges.
  - Click on the Expand Tool to zoom back out.

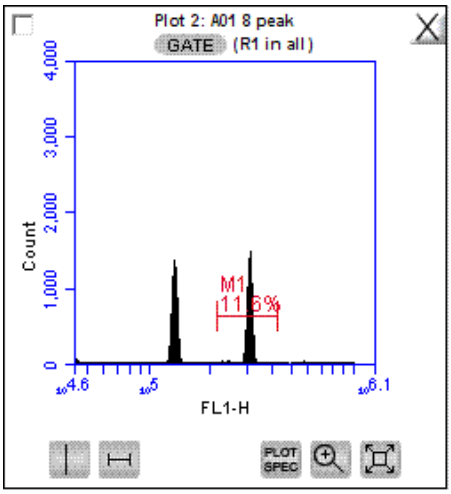


Figure 3-12. Zoomed View of Plot

- Compare the bead run to the manufacturing results sent with the flow cytometer. If the flow cytometer is performing properly, the data plots should look similar to the 8-peak bead plots in Figure 3-13. Look for the following:
  - One main population of beads on FSC-H vs. SSC-H (a shadow population is acceptable)
  - Eight discernable peaks on FL1-H and FL2-H
  - At least six peaks on FL3
  - Top peak CVs are less than 5%

  NOTE: FL4-H performance is validated using the 6-peak beads described in steps 6-9.

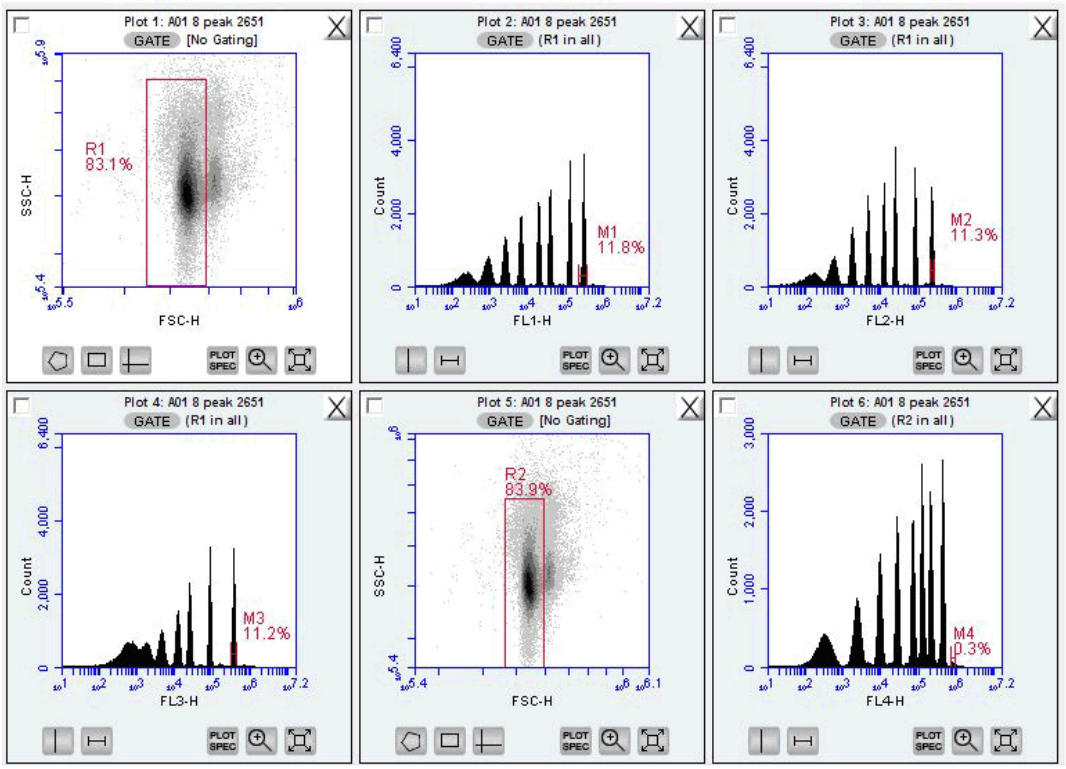


Figure 3-13. 8-Peak Validation Bead Data

- Select the well containing the most recent 6-peak bead data.
- Adjust the pre-drawn region R2 in the 6-peak scatter plot FSC-H vs. SSC-H to encompass the main population (similar to the procedure for the 8-peak beads). This population should look like an exclamation point. The R2 region should encompass the entire exclamation point (Figure 3-14).

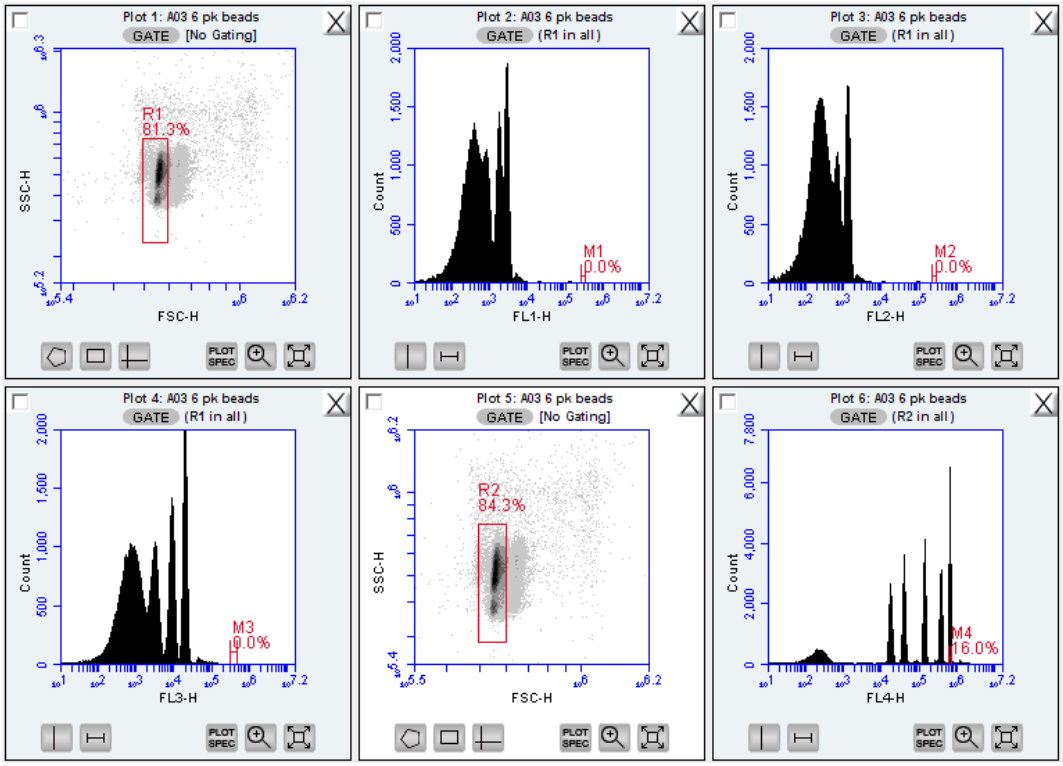


Figure 3-14. 6-Peak Validation Bead Data

- Verify that the FL4-H plot is gated on region R2. If it is not, click on the *GATE* button and select *R2 on all events* from the pop-up dialog box.
- Measure the CV of the top (brightest, far right) peak by adjusting the marker in the FL4-H plot so that it is placed tightly around the peak. See the plots in Figure 3-14 for an example of acceptable 6-peak bead data. Look for the following:
  - One main population of beads on FSC-H vs. SSC-H
  - Six peaks on FL4-H
- If desired, record the following information for each parameter in the BD Accuri C6 Cytometer Log (available on the BD CSampler Software CD or flash drive):
  - Number of peaks
  - Mean channel numbers for the top peaks and forward scatter
  - CVs for the top peaks and forward scatter
- If this is the first validation run, send a copy of the file to Accuri Technical Support for analysis. See Section 8.6 for email address.

3.4 Monitoring Validation Bead Data

If a single BD CSampler Software file is used to collect validation bead data, it is easy to monitor flow cytometer performance over time using the *Statistics* tab.

To monitor the bead data:

- Save the 8- and 6-peak validation bead data from each day in separate wells. Grouping the 8-peak data runs separately from the 6-peak runs in the 24-well grid simplifies use of the *Statistics* tab.
- Create a table in the *Statistics* tab that contains the mean channel numbers and CVs for the top peaks on each detector and the forward scatter (see chapter 7 for details on creating tables in the *Statistics* tab).

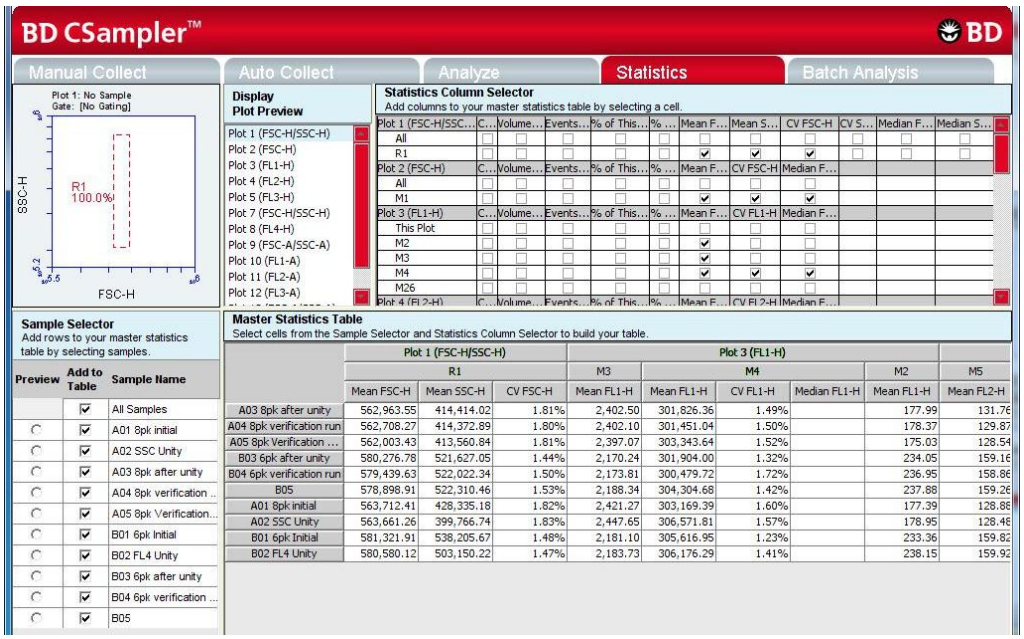


Figure 3-15. *Statistics* Tab: 8-Peak Bead Data from Successive Days

- Compare statistics over time for trends or sudden changes in mean values to assess the cytometer's performance. The BD Accuri C6 flow cytometer reports arithmetic means.

3.5 Troubleshooting Validation

The following conditions may indicate a problem with the flow cytometer or the validation beads:

- Very broad CV (> 5.0%) or multiple populations for FSC-H on the 8- or 6-peak beads (excluding the doublet population).
- Fewer than eight peaks for FL1 or FL2.
- Fewer than six peaks for FL3 or FL4.

To troubleshoot validation:

- If bead data were not acquired using the slow rate, select *Slow*, resuspend the beads, and recollect the data.
- If the beads have been diluted for more than one week, kept at room temperature or warmer, or exposed to light for long periods of time, performance may be substandard. Make new bead suspensions and run the bead sample again.

- There may be a bubble or clog in the flow cell. Do one or more of the following:
    - Run the bead sample again.
    - In BD CSampler Software select the *Unclog* button and follow the software prompts. When the cycle is finished (a green Traffic Light will be displayed), run the bead sample again.
    - In BD CSampler Software select the *Backflush* button and follow the software prompts. When the cycle is finished (a green Traffic Light will be displayed), run the bead sample again.
    - Contact Accuri Technical Support.

4 MANUAL DATA ACQUISITION

The *Manual Collect* tab is used to set the data collection criteria, start and stop data acquisition, and view data on collected samples. The tab contains buttons and controls for performing the following functions:

Run individual samples from a plate or tube rack. Settings such as thresholds and color compensation can be applied to either the current sample being viewed or to all the samples in the sample grid.

Set up experiments to be collected in the *Auto Collect* tab. The following can be set up in the *Manual Collect* tab before switching to the *Auto Collect* tab:

1. Create plots (histogram, dot, or density) for viewing data.
2. Set up gating strategies.
3. Set threshold values.

NOTE: Run Limit settings are not carried over into the *Auto Collect* tab.

Collect data from a plate if the plate has been interrupted or aborted in the *Auto Collect* tab.

View sample data in plots and in the Statistics Table.

Print plots and statistics.

Perform color compensation.

Import and export data.

4.1 Viewing the *Manual Collect* Tab

The *Manual Collect* tab is organized into two major sections:

Instrument Control Panel—Panel on the left side of the window that contains controls for collecting data.

Data display—Large area on the right side of the window that shows the sample data in plots and in a Statistics Table.

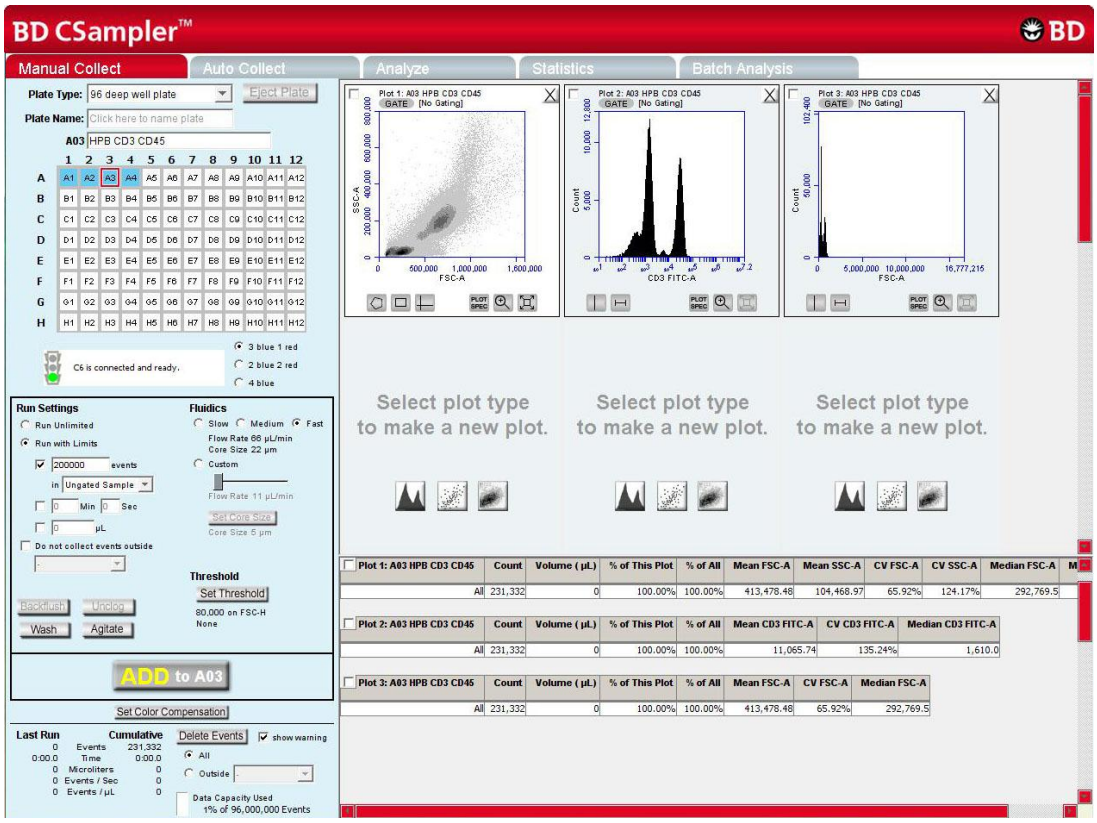


Figure 4-1. BD CSampler Software *Manual Collect* Tab

The following table describes each of the controls and indicators in the *Manual Collect* tab:

Table 4-1. *Manual Collect* Tab Controls

Control	*Description*
Plate Type	Drop-down list for setting plate type.
Load Plate/Eject Plate	Moves the sample plate into position to be loaded onto or ejected from the flow cytometer.
Plate Name	Text box for naming the plate.
Sample Naming Field	Text box for naming the sample.
Sample Grid	Matrix laid out in the configuration of a 96 or 48-well plate, or a 24-tube rack to correspond to sample vessel. BD CSampler Software acquires each sample into its own well in the Sample Grid. The wells can be filled with data in any order. The wells are color-coded: • White—Does not contain data. • Blue—Contains data. • Red outline—Currently selected for viewing or collecting data.

Control	*Description*
Traffic Light and Message	Indicator that displays BD CSampler Software's readiness and system messages. Before data collection can begin, the software must display a green Traffic Light  with the message *C6 is connected and ready.* The Traffic Light status is color-coded: • Green—BD CSampler Software is ready to collect data or is collecting data. • Yellow—The flow cytometer is preparing to perform an action or a non-critical error has occurred. • Red—A critical error has occurred.
Run Settings	Contains a set of controls that allow criteria definition for automatically stopping data collection. See section 4.2.6 for details.
Fluidics	Contains a set of controls for defining flow rate and core size. See section 4.2.1 for details.
Backflush	Performs a backflush to clear the SIP. See section 8.1 for details.
Unclog	Performs an unclog cycle to purge the flow cell of debris. See section 8.2.1 for details.
Wash	Performs a wash cycle to keep the SIP clean between samples. See section 4.2.8 for details.
Agitate	Performs an agitate cycle to keep particles in suspension. See section 4.15 for details.
Set Threshold	Sets the event threshold to gate out debris and noise from cell samples. The default value is 80,000 on FSC-H. See section 4.2.2 for details on setting threshold values.
RUN/PAUSE/ADD TO	Toggle button that performs the following functions: • *RUN*—Starts the sample acquisition. • *PAUSE*—Pauses the acquisition. Click on *ADD TO* to resume data collection. • *ADD TO*—Allows additional sample collection into a well that already contains data.
Set Color Compensation	Opens the Color Compensation dialog box for correcting fluorescence spillover. See section 4.14 for details.
Acquisition Counters	Displays the following information about the most recent acquisition for the selected well *(Last Run)* and all acquisitions for the selected well *(Cumulative)* in real-time: • *Events*—Number of events sampled. • *Time*—Elapsed acquisition time. • *Microliters*—Volume of acquired sample. • *Events/sec*—Events acquired per second. When the run is completed, this is the average value. • *Events/µL*—Events acquired per microliter. When the run is completed, this is the average value.

Control	Description
Delete Events	Permanently deletes all events from the current sample. Enable the *Show warning* check box to display a warning message before deleting sample data. Also contains a *Data Capacity Used* meter that displays the amount of data storage capacity currently used in BD CSampler Software. Gated events can be deleted with the *Enhanced Analysis Features.*
Plots Pane	Area displaying two rows of plot corrals for graphically viewing data on the selected sample. Scroll up or down to view additional plots. Each plot corral contains buttons for creating histogram, density, and dot plots. For information on creating and using plots, see section 4.4.
Statistics Table	Table below the plots that displays statistical information on individual plots.

4.2 Collecting Sample Data

Events can be added to a well containing data. When a data well already contains data, the *RUN* button displays *ADD TO*. Run limits may need to be adjusted to accommodate additional data. Each data well holds a maximum of 1 million events. BD CSampler Software must display a green Traffic Light and the message *C6 is connected and ready* to collect data.

The figure below (Figure 4-2) shows a new workspace. Only a density plot of linear FSC-A vs. linear SSC-A is displayed. The plot is already zoomed to show channels 0 to 1,600,000 on FSC-A and 0 to 800,000 on SSC-A.

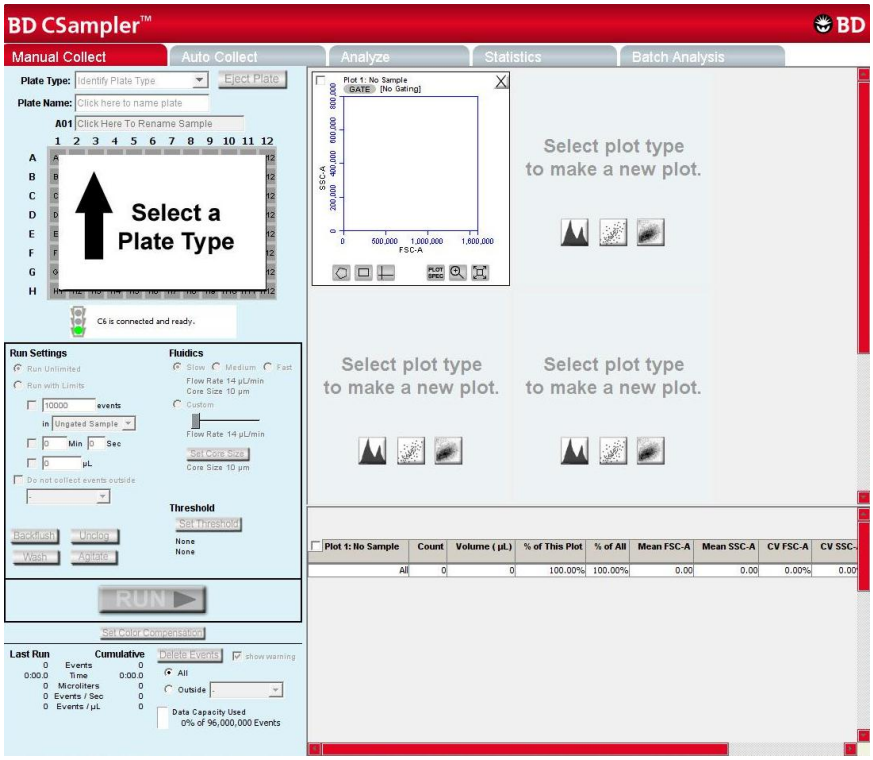


Figure 4-2. New BD CSampler Software Workspace

The following sections describe how to set up a sample acquisition and use plots in the *Manual Collect* tab.

To collect sample data:

- In the *Manual Collect* tab, do one of the following:

Select a plate type and name the plate (optional).

Open a template.

Create new plots (optional, see section 4.4).

Define acquisition settings (see sections 4.2.1 and 4.2.2).

If necessary, acquire some events to define regions, gates, and other settings.

Perform analysis in the *Manual Collect* or *Analyze* tab.

4.2.1 Setting the Fluidics Rate

The system can accommodate an upper limit of 10,000 events per second, but it is recommended to acquire samples at a rate of 2,500 events per second or less to ensure the best data resolution.

To set the fluidics rate:

- Click on the *Slow, Medium,* or *Fast* radio button in the *Fluidics* section of the *Manual Collect* tab.

  NOTE: It is recommended to start data collection on slow and observe the data rate. The setting can then be adjusted to medium or fast, if necessary. If the data rate is near or above 10,000 total events per second on the *Slow* setting, there are several possible solutions:

  - Increase the primary threshold channel, taking care that the increase does not remove cells of interest from the data set.
  - Include a secondary threshold, taking care not to exclude cells of interest.
  - Dilute the sample.

Fluidics rate settings and sample core size can be adjusted to accommodate very small or very large particles. For information on customizing these settings, see APPENDIX B.

4.2.2 Setting the Threshold

Use thresholds to gate out light scatter and/or fluorescence signals caused by debris in cell samples and electronic noise inherent in the system. When set correctly for any given sample set, resolution of particle or cell light scatter and fluorescence signals is greatly improved and data set size often can be reduced. By default, BD CSampler Software is set to a primary threshold of channel 80,000 on FSC-H.

Notes on setting thresholds:

Threshold settings can be changed before, during, or after data acquisition, but the most consistent, predictable results will be obtained if threshold settings are chosen before *final* data collection for any given experiment. The primary threshold is the parameter that triggers data collection. Optional secondary thresholds can be applied to filter out additional data.

All thresholds are set on the Height signal for any given parameter. For best results when setting or changing thresholds, create a plot that displays the Height signal for the threshold channel and observe the effect on data as the threshold is raised or lowered.

Table 4-2. Suggested Starting FSC-H Threshold Settings for Various Cell Types

Cell Type	*Start with FSC-H Threshold of:*	*Notes*
Cell Lines: Large (>20 µm)	500,000 to 1,000,000	Displaying FSC and SSC signals on Log scale is helpful
Cell Lines: Small (<20 µm)	200,000 to 500,000	Displaying FSC and SSC signals on Log scale is helpful
Unfixed, freshly isolated cells: white blood cells, spleen, thymus	200,000 to 500,000	Linear or log scale display of FSC, SSC
Fixed cell suspensions: white blood cells, spleen, thymus	200,000 to 500,000	Linear or log scale display of FSC, SSC
Platelets	10,000 to 30,000	Linear or log scale display of FSC, SSC
Bacteria, microparticles	Dual thresholds suggested	See document titled "Threshold and Analysis of Small Particles" on the BD Accuri website

CAUTION: Take care when setting the thresholds before or during data collection. Any event not meeting the threshold criteria will be not be acquired or saved. When changes are made to the threshold values after data collection, BD CSampler Software displays a warning message if the new threshold value will result in permanent data loss.

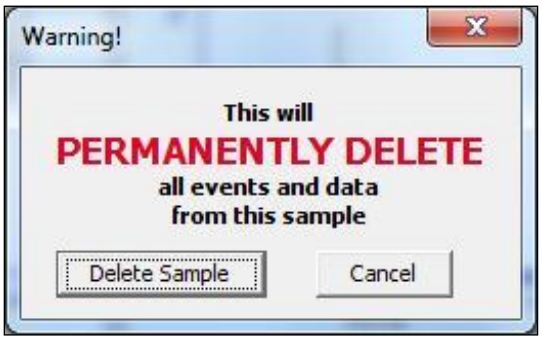


Figure 4-3. Threshold Settings Warning Message

To set the threshold:

- Do one of the following:

Select *Instrument > Set threshold*.

Click on the *Set Threshold* button in the Instrument Control Panel.

- Select the primary threshold parameter from the *Primary Threshold* drop-down list in the Threshold Settings dialog box.

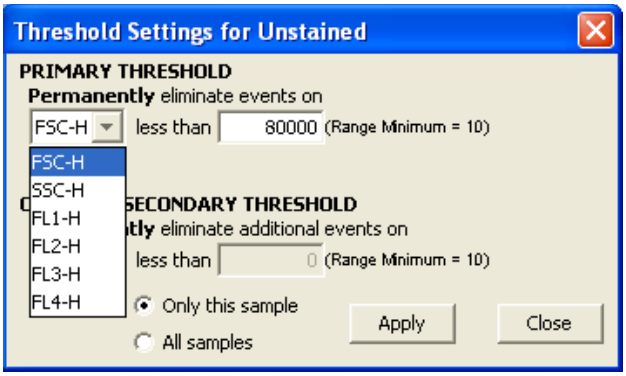


Figure 4-4. Primary Threshold Drop-Down List

- Type *80000* in the *less than* edit box to set the threshold minimum to channel 80,000.

  NOTE: A lower or higher FSC-H threshold may be required when working with small cells (such as platelets or bacteria) or large cells (such as cell lines), respectively. Refer to Technical Note: Threshold and Analysis of Small Particles on the BD Accuri C6 Flow Cytometer available at http://www.bdbiosciences.com/support/resources/accuri/index.jsp or Table 4-2.

To apply a secondary threshold:

Select the threshold parameter from the *Secondary Threshold* drop-down list.

Type a value in the *less than* edit box to set the threshold minimum.

- Do one of the following:
  - Select the *Apply to All samples* radio button to apply settings to all samples, including all previously collected data in other data wells.
  - Select the *Apply to Only this sample* radio button to apply settings to the current sample only.

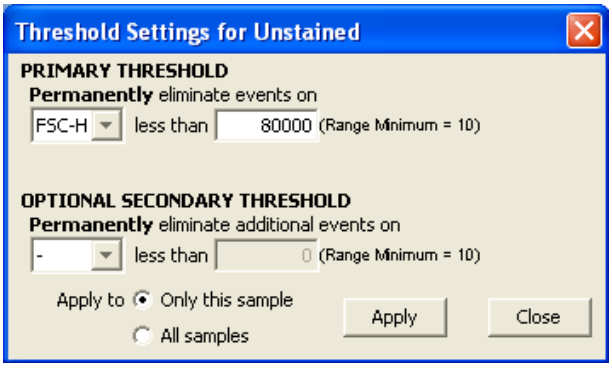


Figure 4-5. Threshold Settings Dialog Box

- Click on the *Apply* button to apply the threshold settings.
- Click on the *Close* button to close the dialog box.

4.2.3 Assigning a Plate Type

- Select one of the following options from the *Plate Type* drop-down list:
  - 96-well plate: U-bottom
  - 96-well plate: flat bottom
  - 96-well plate: V-bottom
  - 96 deep-well plate
  - 48-well plate
  - 24-tube rack

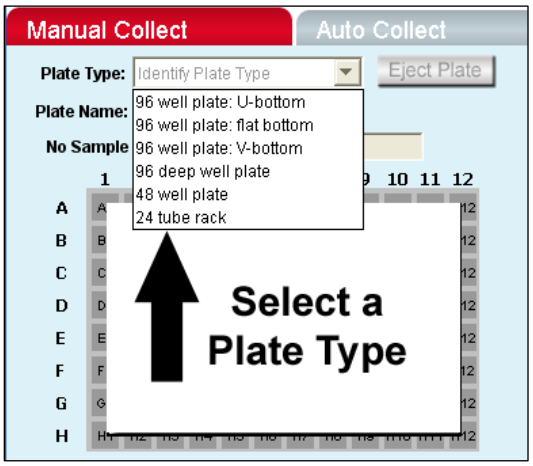


Figure 4-6. Plate Type Drop-Down Menu

The sample grid displays the available wells for data collection, based on the assigned plate type:

96-well plate assignment—Displays all 96 wells

48-well plate assignment—Displays wells rows A-F, columns 1-8

24-tube rack assignment—Displays wells rows A-D, columns 1-6

NOTE: Changing the plate type automatically opens a BD CSampler Software new blank workspace.

4.2.4 Naming the Plate

- Name the plate by typing a label in the *Plate Name* field (optional).

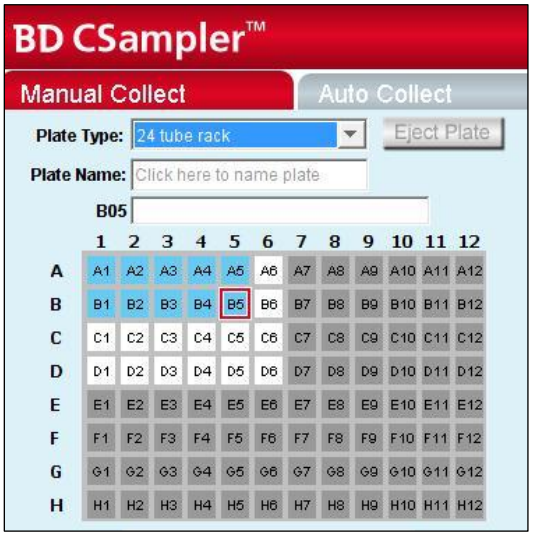


Figure 4-7. Plate Name Field

4.2.5 Naming the Sample

Samples can be named at any time. If no text is entered in the naming field, BD CSampler Software names the sample according to the well location (for example, `A01`).

To name the sample:

- Type the sample name into the text box above the 96-well grid.

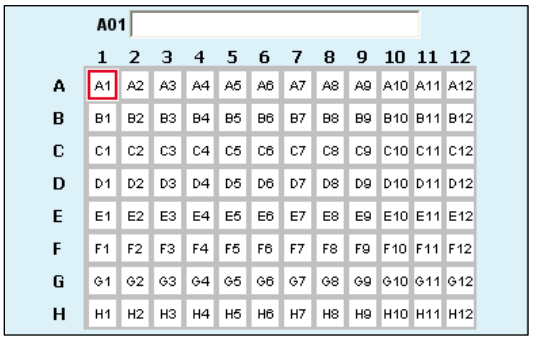


Figure 4-8. Sample Name Field

4.2.6 Setting a Run Limit

The run limit defines when data acquisition will stop. The following parameters can be used individually or in combination to set a run limit.

- Time
- Volume
- Number of events (in a specified gate)

If multiple run limits are set, data collection stops on whichever limit is reached first.

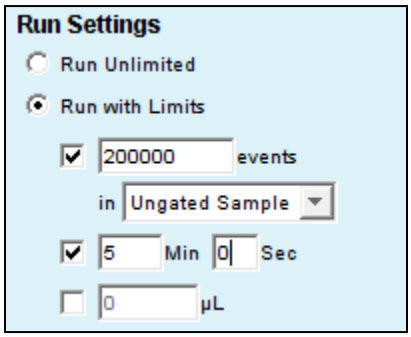


Figure 4-9. Run Limits Controls

To collect samples without setting a run limit:

- Enable the *Run Unlimited* check box. This requires a manual stop.

To stop the run after a specified number of events have been sampled:

- Enable the *Run with Limits* check box.
- Enable the check box next to the *events* field.
- In the associated text box, type the number of events at which to stop the run.
- Do one of the following in the drop-down list below the text box:
  - Select *Ungated Sample*.
  - Select a gating strategy (if one exists) to stop the run when the assigned number of events has been collected in the gated region.

To stop the run after a time has expired:

- Enable the *Run with Limits* check box.
- Enable the check box next to the *Min* and *Sec* fields.
- Type the number of minutes *(Min)* and seconds *(Sec)* at which to stop the run.

To stop the run after a specified volume has been sampled:

- Enable the *Run with Limits* box.
- Enable the check box next to the *µL* field.
- Type the volume in microliters (µL) at which to stop the run.

4.2.7 Running the Sample

- Gently resuspend the cells in the sample tube or plate, and load the tube rack or plate.
- Select a well in the sample grid that corresponds to the sample location on the plate or tube rack.
- Click on the *RUN* button to start the sample collection.

  BD CSampler Software begins fluidics initialization. During this time the Traffic Light turns yellow and the software displays the message *Preparing to analyze sample*. Once initialization is complete, the Traffic Light turns green and the software displays the message *Events are being recorded*. The well flashes blue during data collection. After the run limit is reached, the well stops flashing and remains blue, indicating that the well contains data.
- Additional data can be collected into a well that contains data at any time by clicking on the *ADD TO* button. Note that run limits may need to be adjusted when adding data to a well.

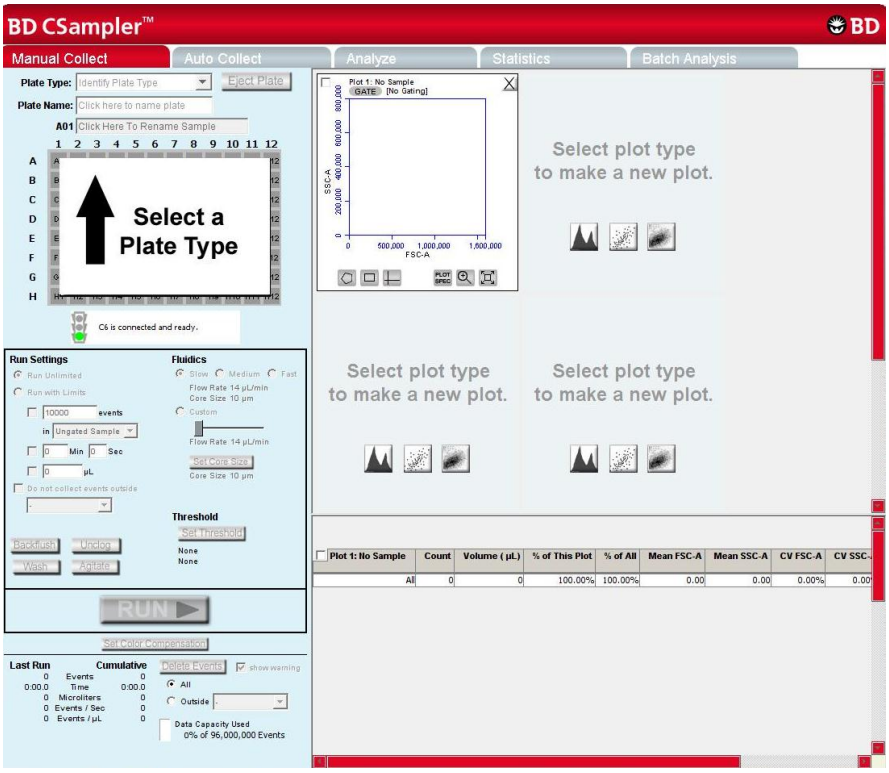


Figure 4-10. BD CSampler Software Workspace after Collecting Samples

4.2.8 Washing the SIP Between Samples

A wash cycle can be used to thoroughly clean the SIP between samples. The flow cytometer aspirates the contents of the SIP up and out of the SIP, then rinses the SIP and wash station with clean sheath fluid.

To wash the SIP:

- Click on the *Wash* button in the *Manual Collect* tab.

4.2.9 Adding New Sample Data to a BD CSampler Software File

Additional sample data can be added to a BD CSampler Software file that already contains data, either by moving to an empty data well before acquisition or by adding to a well which already contains data.

To add data to a BD CSampler Software file:

- Gently resuspend the sample and load the plate or tube rack.

NOTE: Generally, there is no need to perform a backflush between samples.

- Click on a data well in the sample grid. If an empty well is selected, any plots and gates created previously are still displayed, but they do not contain any data, as shown in the following figure.

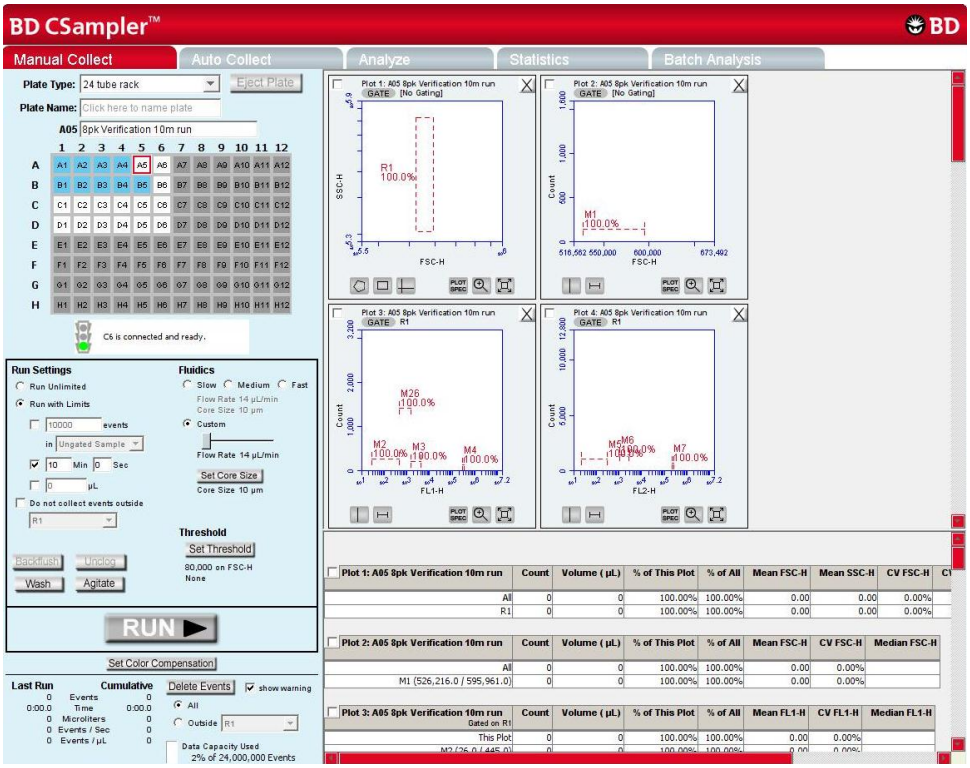


Figure 4-11. BD CSampler Software Workspace with Empty Sample Well and Empty Plots

- Click on the *RUN* (or *ADD TO*) button to start a sample collection. BD CSampler Software displays and updates data in real time during the collection. The flow cytometer stops sampling from the tube or well when the run limit is reached.

CAUTION: If the *ADD TO* button is selected, BD CSampler Software will collect data into a well that already contains data.

4.2.10 Pausing Data Collection

Sample acquisition can be interrupted any time during a run. To stop a run:

- Click on the *PAUSE* button.

To restart the run:

- Click on the *ADD TO* button. BD CSampler Software resumes data collection in the current well.

4.3 Ending a Data Collection Session

When sample collection is completed, clean the SIP and fluidics lines by following the procedure below:

- Load a 24-tube rack containing the following tubes:

Table 4-3. Plate Setup for Ending a Data Collection Session

Type of Sample	*Location in BD CSampler Software Sample Grid*
2 mL of filtered, deionized water	Empty data well (for example, D4)
2 mL of decontamination solution (PN 653154)	Empty data well (for example, D5)
2 mL of filtered, deionized water	Empty data well (for example, D6)

- In BD CSampler Software, select the well corresponding to the first tube of water. Set the time limit for two minutes and the fluidics speed to fast.
- Click on the *RUN* button.
- In BD CSampler Software, select the well corresponding to the decontamination solution.
- Set the time limit for two minutes and the fluidics speed to fast.
- Click on the *RUN* button.
- In BD CSampler Software, select the well corresponding to the second tube of water.
- Set the time limit for two minutes and the fluidics speed to fast.
- Click on the *RUN* button.
- When the run is finished, eject the plate/tube rack.

4.4 Creating Plots

Three plot types are available for viewing data: histogram, density, and dot plots.

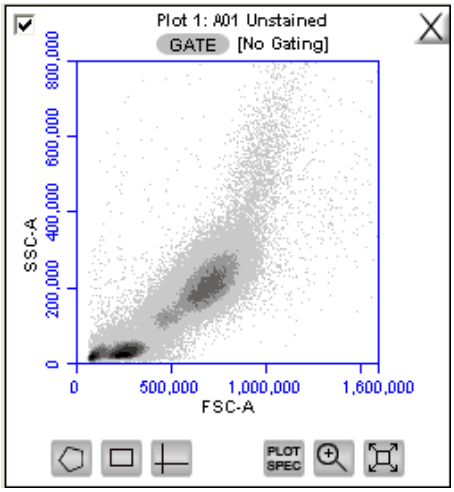


Figure 4-12. View of a Plot

Each plot contains a set of gating and marker tools, and a set of viewing tools:

- Gating and marker tools:
  - *Gate* button GATE—Opens the Change Gating dialog box for applying gates to a plot.
  - Polygonal Gating Tool — For drawing irregularly shaped gates around a population of events.
  - Rectilinear Gating Tool — For drawing a rectilinear gate around a population of events.
  - Quadrant Gating Tool — For gating the plot in quadrants.
  - Vertical Marker Tool — For gating histograms to the right or left of a vertical marker.
  - Horizontal Marker Tool — For gating histograms within a horizontal marker.
- Viewing tools:
  - Plot Spec Tool —Opens the Set Plot Specs dialog box for changing the x- and y-axis parameters, scaling the plot, and setting log or linear view.
  - Zoom Tool —Defines the Zoom range.
  - Expand Tool —Undoes one Zoom level.

To create a new plot:

- Click on one of the following icons in an empty plot corral:
  - *Density Plot*
  - *Dot Plot*
  - *Histogram Plot*

BD CSampler Software displays an FSC-A vs. SSC-A plot by default.

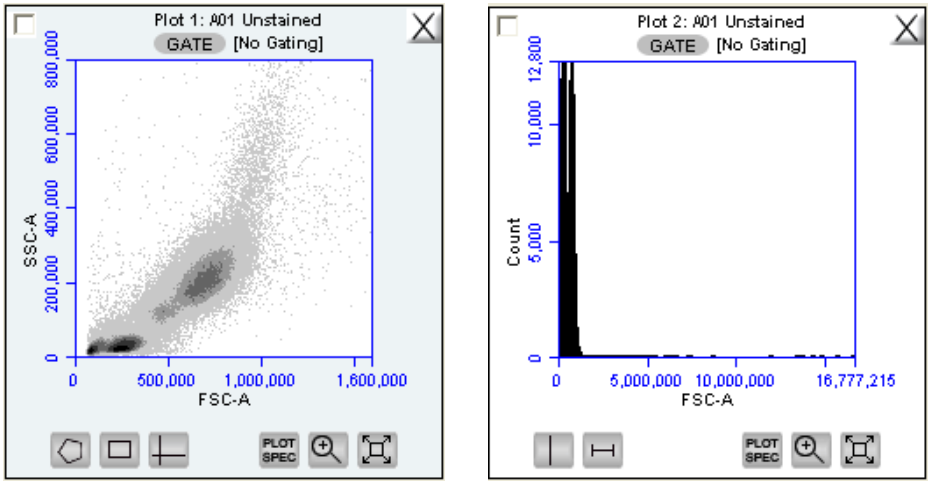


Figure 4-13. New Density and Histogram Plots

- Configure plot specifications as needed (see section 4.5 for details).

4.5 Changing Plot Specifications

The Plot Spec Tool allows manipulation of the data display in a plot, including axis parameter selection, channel range specification, and selection of linear or logarithmic axis scale. The Plot Spec Tool is available in the *Manual Collect* and *Analyze* tabs.

Set up or modify plot specifications at any time before or after collecting data.

To change the plot specifications:

- Click on the *Plot Spec Tool* icon.

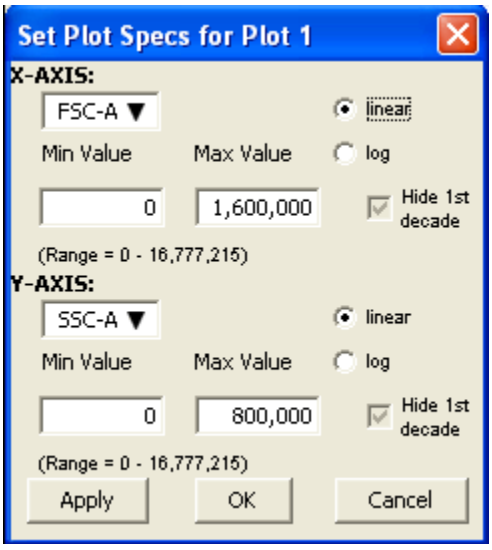


Figure 4-14. Set Plot Specs Dialog Box

- In the Set Plot Specs dialog box, do the following for each axis:
  - Select the desired parameter for display from the drop-down list.
  - Select the *linear* or *log* radio button to specify how data are scaled. (All parameters are collected with linear amplifiers on the flow cytometer, and therefore all channel values are linear. Selecting the log view of a parameter simply means the linear channel values are graphed on a logarithmic scale.)
  - Type in the minimum and maximum channels to set the channel range to view. Enable or disable the *Hide 1st decade* check box. It is often beneficial to unhide the first deca*de of a given p*arameter before applying fluorescence compensation. See section 4.14.
- Do one of the following:
  - Click on the *Apply* button to apply the changes without closing the dialog box.
  - Click on the *OK* button to apply the changes and close the dialog box.
  - Click on the *Cancel* button to close the dialog box without applying the changes.

4.6 Changing the Number of Events in a Plot

The number of events displayed in all plots across all samples can be changed in order to improve data visualization or to normalize data sets. This option allows visual removal of a number of events from the plot without deleting data.

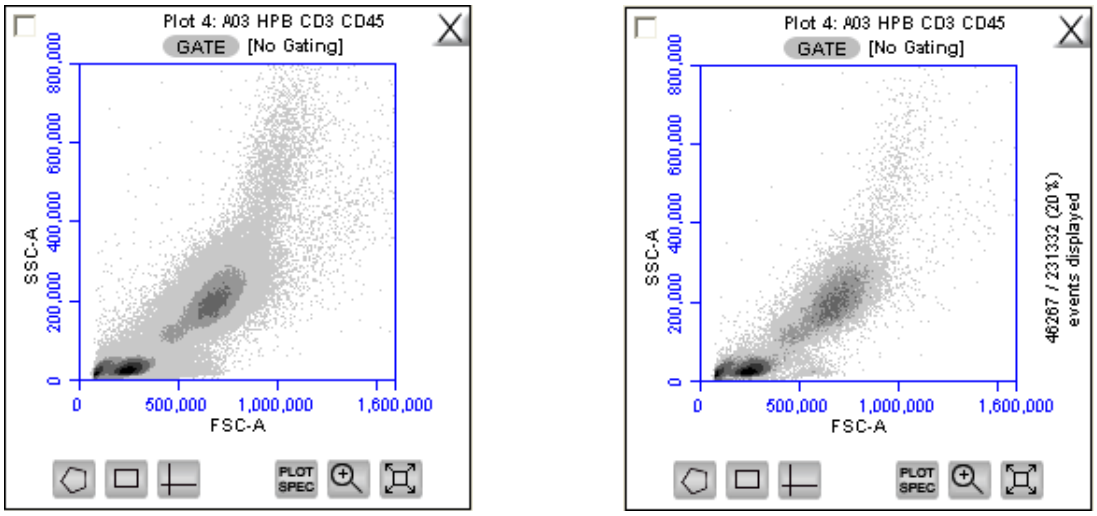


Figure 4-15. Before and After Changing Events Displayed

To change the events displayed in a plot:

- Select *Display > Events Display Settings.*

- Do one of the following in the Events Display Settings dialog box:
  - To view all collected events, select the *Show all events* radio button.
  - To view the first N events of a sample, select the *Display first* radio button and type a number in the *events collected* field.
  - To view a specified percentage of the whole in a pseudo-random manner, select the *Display* radio button and type a percentage to view (for example, if 20% is selected, every fifth event is displayed).

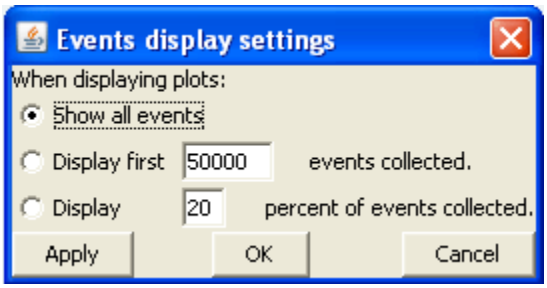


Figure 4-16. Events Display Settings Dialog Box

- Do one of the following:
  - Click on the *Apply* button to apply settings without closing the dialog box.
  - Click on the *OK* button to apply settings and close the dialog box.
  - Click on the *Cancel* button to close the dialog box without applying settings.

BD CSampler Software updates the display and shows a message in the plot that some events are not being displayed.

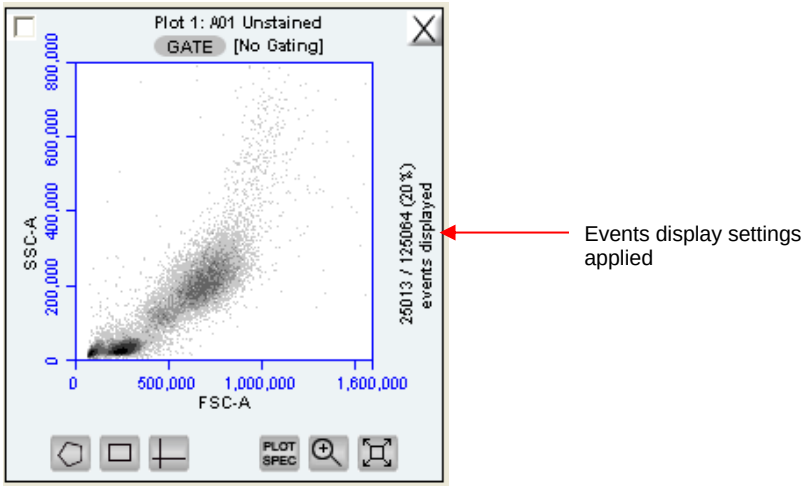


Figure 4-17. Plot with Events Display Settings Applied

4.7 Using Gates and Markers

A gate is a specified area within a plot that is used to designate a set of events for analysis. BD CSampler Software allows creation of any of the following types of gates:

- Polygonal gate—Gates an irregularly shaped area around a population of events.
- Rectilinear gate—Gates a rectilinear area around a population of events.
- Quadrant gate—Gates the plot in quadrants.
- Vertical marker—Gates a histogram plot to the right or left of a vertical marker.
- Horizontal marker—Gates a histogram plot within the upper and lower boundary of a horizontal marker.

4.7.1 Creating a New Gate

To create a gate in a density or dot plot:

- Click on one of the following gating tools:
  - *Polygonal Gating Tool* —typically used for irregularly shaped populations.
  - *Rectilinear Gating Tool* —typically used for evenly shaped populations.
  - *Quadrant Gating Tool* —typically used for analyzing fluorescence plots.
- Use the mouse to draw a region (labeled *P1* for a polygonal gate, *R1* for a rectilinear gate, or *Q1* for a quadrant gate). To draw a polygon, click on the mouse to anchor each vertex and double-click to close the polygon.

  NOTE: Gate labels can be changed by double-clicking on the label and typing a new gate name in the dialog box.

BD CSampler Software automatically displays the percentage of events within the region.

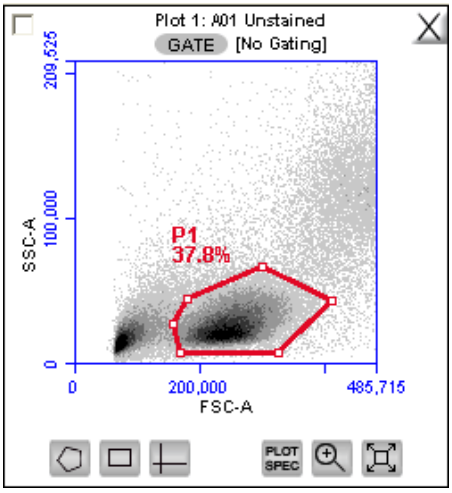


Figure 4-18. Using Polygonal Gating Tool

To create a vertical marker in a histogram plot:

- Click on the *Vertical Marker Tool* .

- Click the cursor at the point along the x-axis on which to place the marker. BD CSampler Software automatically displays the percentage of events to the left (*V1-L*) and right (*V1-R*) of the marker.

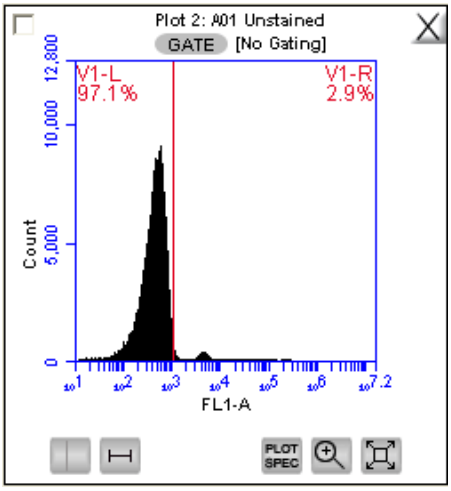


Figure 4-19. Using the Vertical Marker

NOTE: Only a single vertical marker can be set per histogram.

To create a horizontal marker in a histogram plot:

- Click on the *Horizontal Marker Tool* .
- Click and drag the cursor horizontally across the area to apply the gate. BD CSampler Software automatically displays the percentage of events within the margins of the marker (labeled *M1*).

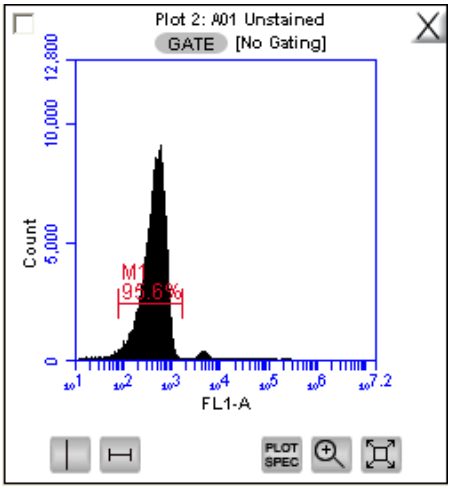


Figure 4-20. Using the Horizontal Marker

4.7.2 Applying a Gate to a Plot

- Click on the *GATE* button at the top of the plot. Only polygon (P), rectilinear (R), and marker (M) gating regions automatically appear in the Gating dialog box list of options.
- To include vertical markers or quadrant markers in the list of gates, enable the associated check box(es) in the Change Gating dialog box.

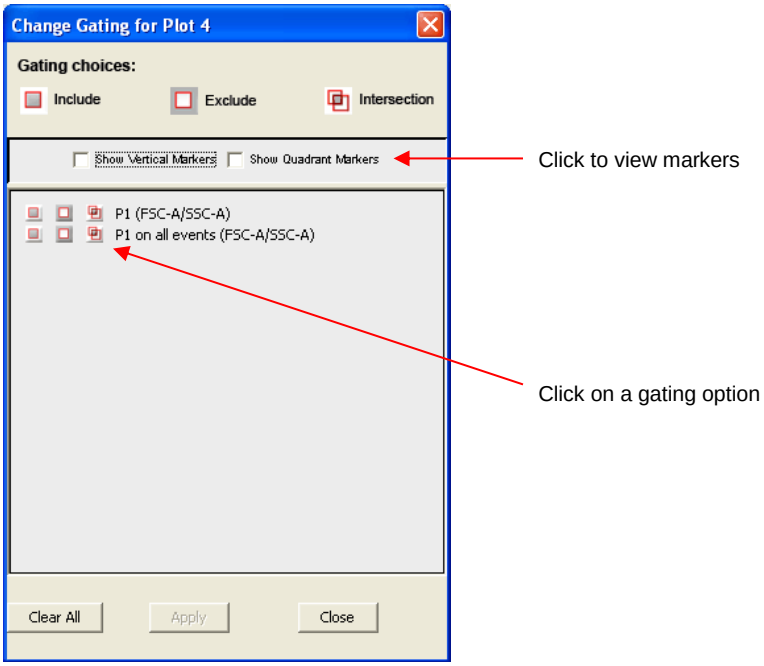


Figure 4-21. Selecting a Gating Option

- Select one of the following gating icons:
  - *Include* icon —to analyze the events within the region. Multiple gates can be included in a single plot.
  - *Exclude* icon —to analyze the events outside of the region.
  - *Intersection* icon —to analyze the events within the intersection of two or more regions.

- Click on the *Apply* button. BD CSampler Software displays the type of gate that is applied next to the *GATE* button in the plot.

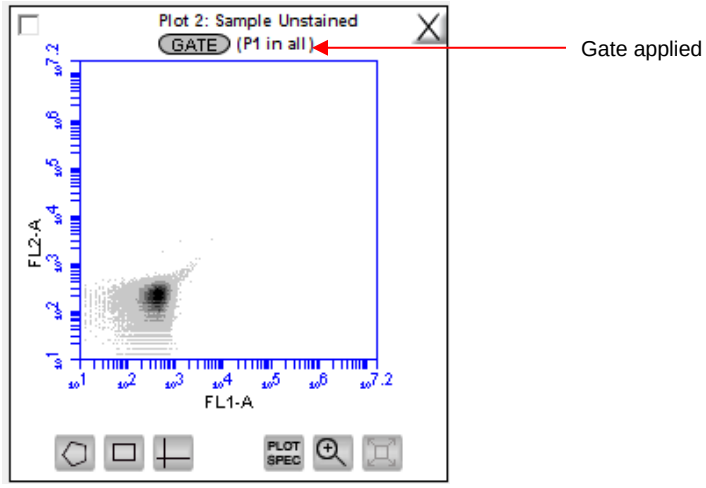


Figure 4-22. Plot Gated to Include P1

4.7.3 Creating and Applying Nested Gates

A series of nested gates can be created in which each gate is a subset of the previous one. Complex and informative gating strategies can be devised by the appropriate combination of nested gates using the Include, Exclude and Intersection gating tools.

To create nested gates:

- Draw any region or marker around a population of events (for example, P1).

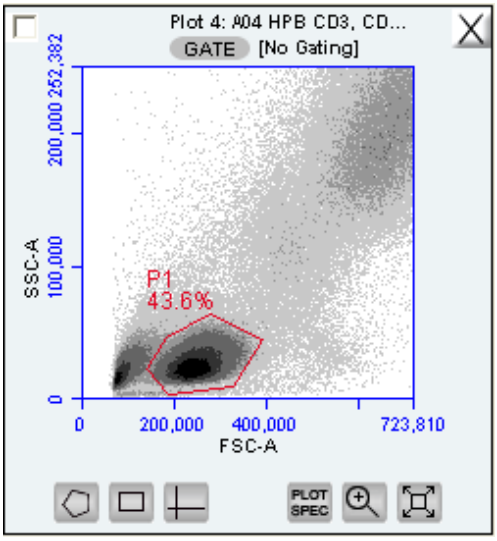


Figure 4-23. First Gate for Creating Nested Gates, Drawn in Plot 4

- Create a new plot and click on the *GATE* button to apply the P1 (parent) gate

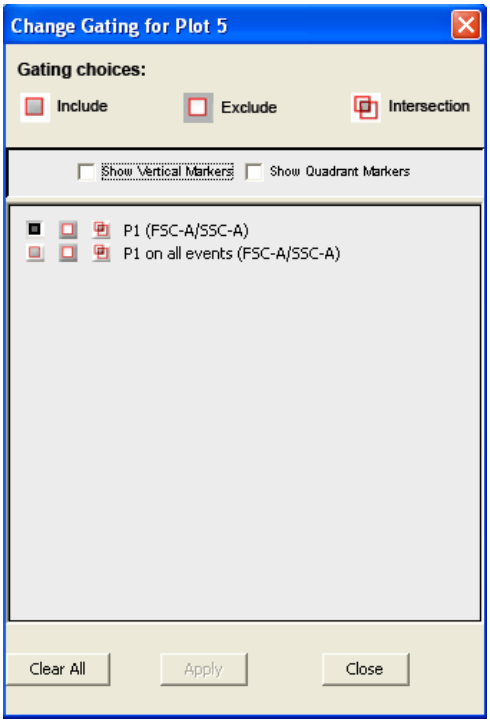


Figure 4-24. Applying the Parent Gate to Plot 5

- Close the dialog box. The plot displays only the populations within the parent gate.

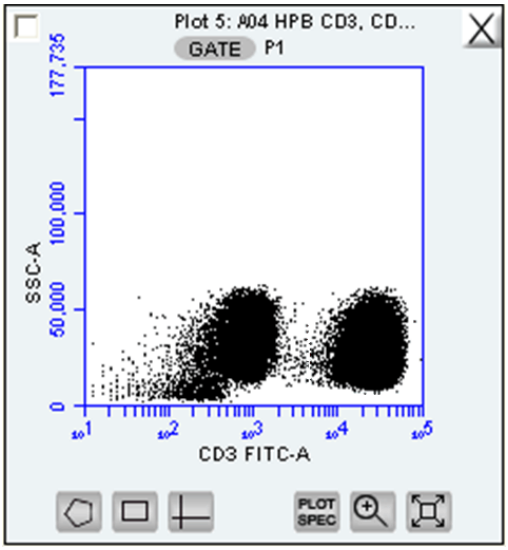


Figure 4-25. Parent Gate Applied to Dot Plot

- In the plot that is gated on the parent gate (plot 5 in this example), draw a second region or marker around a subset of the population displayed in the plot (R1).

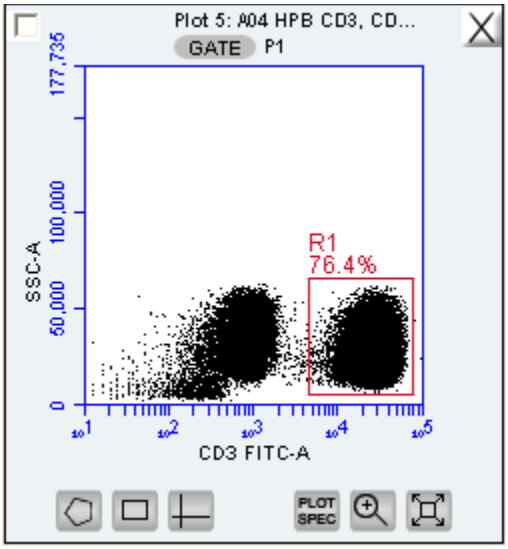


Figure 4-26. Second Gate for Creating Nested Gates

- Create a third plot and click on the *GATE* button.
- In the Change Gating dialog box, select the option in which the second gate is "*in"* the parent gate (e.g., R1 in P1; see Figure 4-27). Alternatively, selecting the *on all events* option (e.g. R1 on all events) "un-nests" the gates.

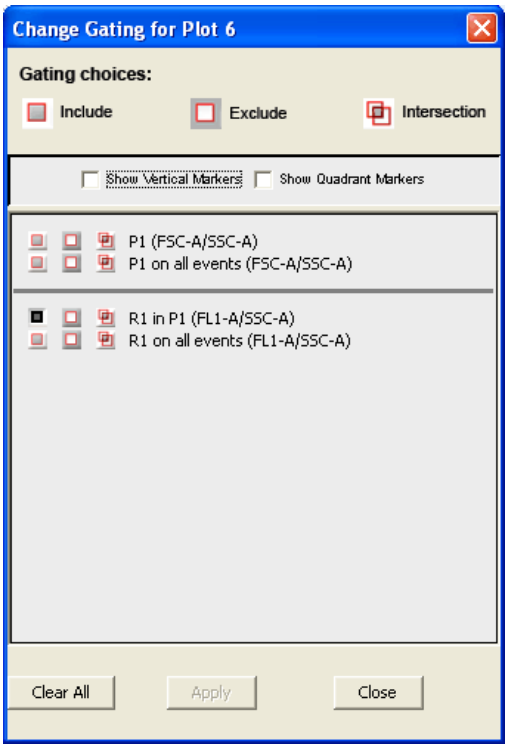


Figure 4-27. Applying the Child Gate

- Apply the gate. This is the *child gate.*

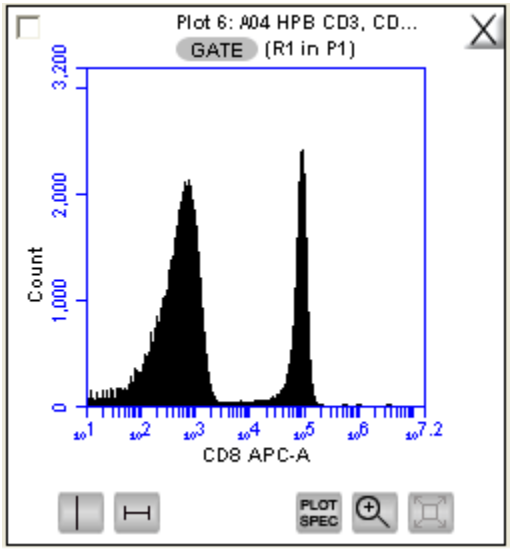


Figure 4-28. Third Plot with Nested Gate Applied (R1 in P1)

Statistics that reflect the nested gates can be viewed in the Statistics Table. .

Plot 6: A04 HPB CD3, CD4, CD45, CD8 Gated on (R1 in P1)	Count	Volume (µL)	% of This Plot	% of All	Mean CD8 APC-A	CV CD8 APC-A	Median CD8 APC-A
This Plot	76,415	0.0	100.00%	33.32%	18,572.85	211.93%	526.0

Figure 4-29. Statistics of Plot with Nested Gate

- Close the dialog box.

For instructions on renaming plots and regions, and event coloring see APPENDIX F, “Enhanced Analysis Features.”

4.8 Moving and Resizing Regions

Region Labels can be moved by clicking and dragging the label. To move or resize a region:

- Click on the border of the region.

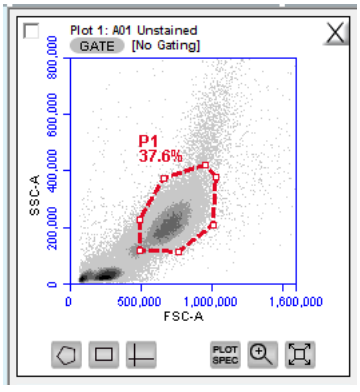


Figure 4-30. Selected Region in the *Batch Analysis* Tab

- Drag the region to the desired position.

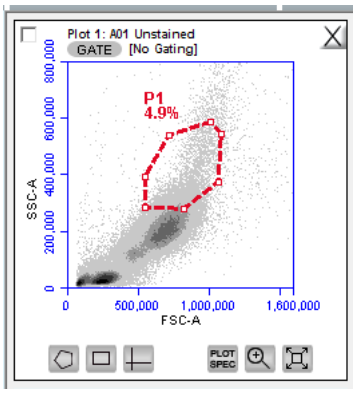


Figure 4-31. Moved Region in the *Batch Analysis* Tab

4.9 Changing the Number of Events in a Plot

The number of events displayed in all plots across all samples can be changed in order to improve data visualization or to normalize data sets. This option allows visual removal of a number of events from the plot without deleting data.

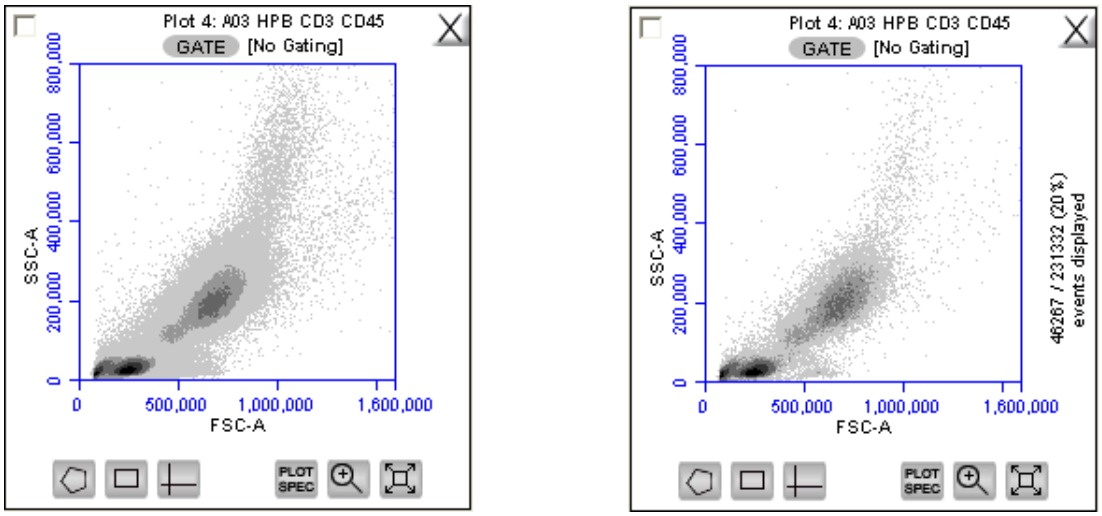


Figure 4-32. Before and After Changing Events Displayed

To change the number of events displayed in a plot:

- Select *Display* > *Events Display Settings.*
- Do one of the following in the Events Display Settings dialog box:
- To view all collected events, select the *Show all events* radio button.
- To view the first N events of a sample, select the *Display first* radio button and type a number in the *events collected* field. (Use this option for data normalization.)
- To view a specified percentage of the whole in a pseudo-random selection, select the *Display* radio button and type a percentage to view (for example, if 20% is selected, every fifth event is displayed).

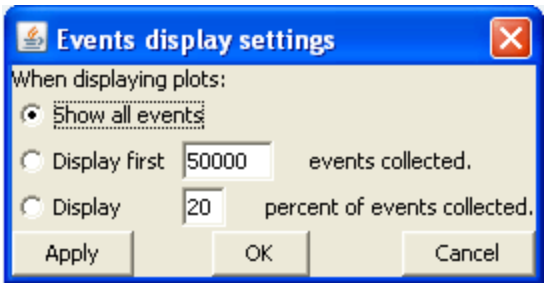


Figure 4-33. Events Display Settings Dialog Box

Do one of the following:

- Click on the *Apply* button to apply settings without closing the dialog box.
- Click on the *OK* button to apply settings and close the dialog box.
- Click on the *Cancel* button to close the dialog box without applying settings.

BD CSampler Software displays the plot with a message that some events are not being displayed.

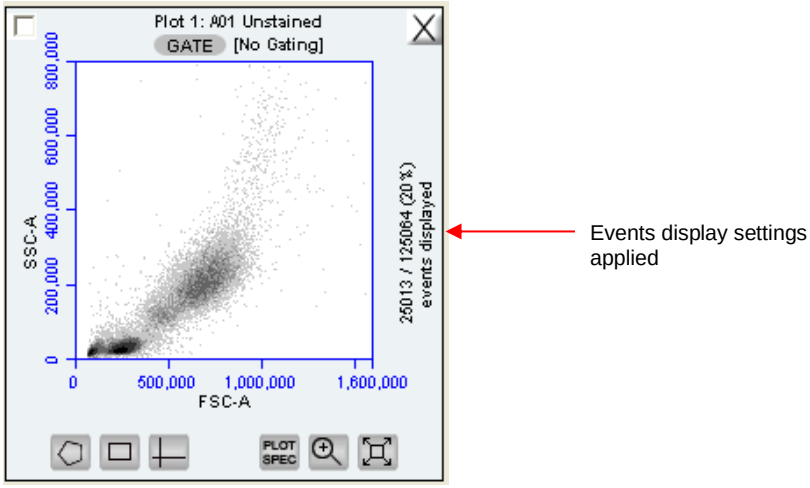


Figure 4-34. Plot with Events Display Settings Applied

4.10 Naming Plot Axes

The axis labels can be renamed in any plot from the *Manual Collect* or *Analyze* tab to identify the antibody staining or fluorochrome used in the sample.

To name a plot axis:

- Click on an axis label and select *Rename Parameters* from the drop-down menu. *Rename Parameters* can also be accessed in the *Edit* drop-down menu.

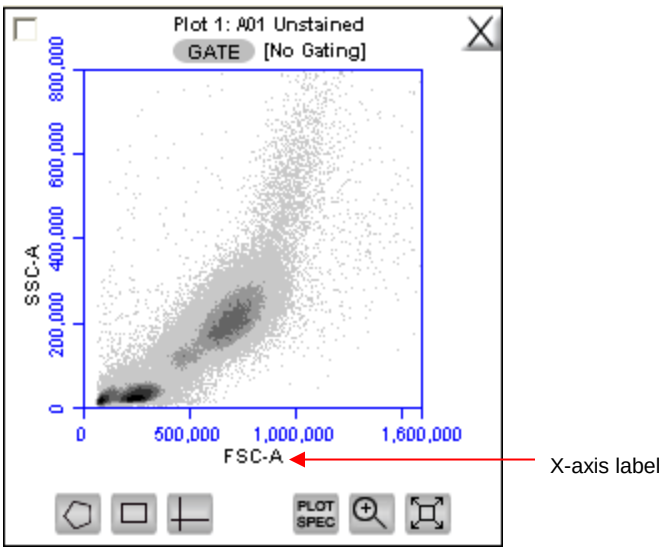


Figure 4-35. X-Axis Label

- In the Rename Parameters dialog box, type the new label in the edit box of the parameter. Any parameter can be renamed from this dialog box.

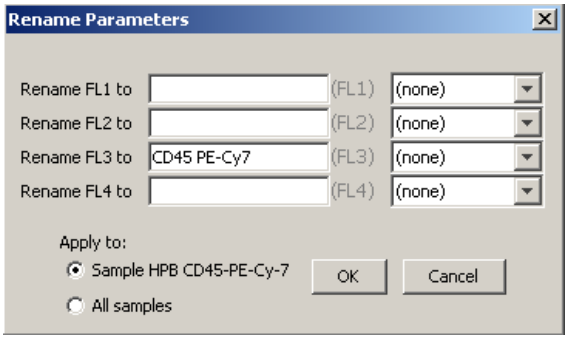


Figure 4-36. Rename Parameters Dialog Box

- Do one of the following:
  - Select the *Sample XXX* radio button (where *XXX* refers to the current sample) to apply the label to the current sample only.
  - Select the *All Samples* radio button to apply the label to all samples. Click OK.

To assign the name to the same parameter in another sample:

- Select another well in the sample grid.
- Click on an axis label in a plot and select *Rename Parameters* from the drop-down menu.
- Select the name from the drop-down list associated with the parameter.

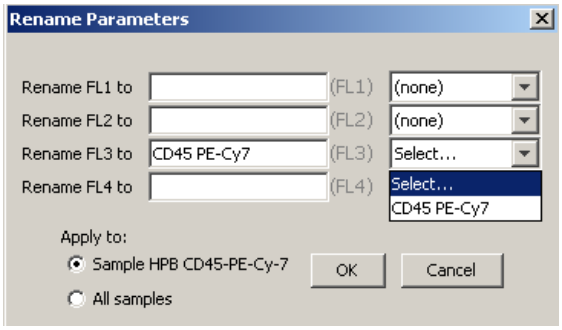


Figure 4-37. Rename Parameters Dialog Box with Axis Label Drop-Down List

4.11 Zooming on a Plot

BD CSampler Software automatically zooms the initial display of any parameter on a logarithmic scale from channel 10 to 16.7 x10^6. For most analyses, very few events fall into channels 0 to 10, so automatic zooming saves time by reducing the number of zoom steps. However, take care when setting markers (M), regions (R), or polygons (P) that require channels lower than 10 on a zoomed plot. It is recommended to unhide the first decade of data (see section 4.5) to prevent events from being excluded from gated regions, especially when setting fluorescence compensation (see section 4.14).

4.11.1 Basic Zoom

- Click on the *Zoom Tool* .
- Draw an area to zoom on by clicking and dragging the mouse in the plot.

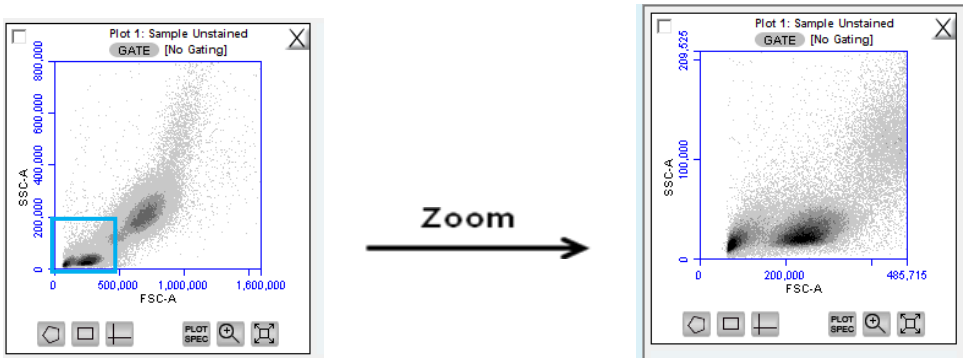


Figure 4-38. Before and After Using Zoom Tool

- Repeat steps 1-2 as needed to zoom in.

To zoom out:

- Click on the *Expand Tool* .
- Repeat step 1 as needed.

4.11.2 Zooming to a Specified Channel Range

Sometimes it can be helpful to view a plot in a specified channel range.

To view a specified channel range in a plot:

- Click on the Plot Spec Tool .

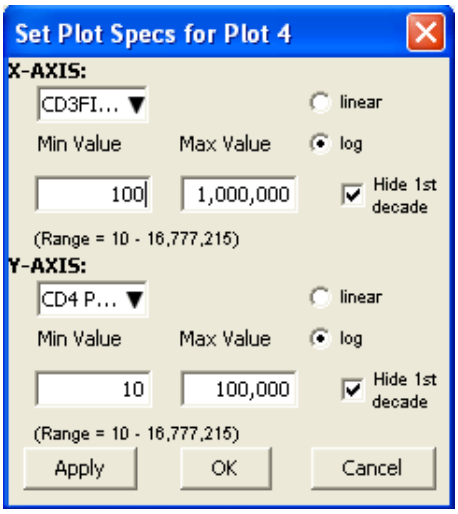


Figure 4-39. Plot Spec Dialog Box: Set Min and Max Channel Values for the X- and Y-Axes

- Specify the x-axis channel range by typing a minimum (*Min Value*) and maximum (*Max Value*) value under *X-Axis* in the Set Plot Specs dialog box.
- Specify the y-axis channel range by typing a minimum and maximum value under *Y-Axis*.
- Click on the *Apply* button to apply the changes and click on the *OK* button to close the Plot Spec dialog box.

4.12 Saving a BD CSampler Software File

A BD CSampler Software file is a comprehensive (and often large) data file that contains instrument settings, FCS files, and plot layouts.

The file contains the entire workspace, including the following elements:

Sample data

Plot layouts

Zoom levels

Gating

Color compensation

Threshold settings

Collect tab settings

Changes made in the *Analysis* or *Statistics* tabs

By default, BD CSampler software automatically saves data at the end of each sample run. Data can also be saved manually. To save the entire BD CSampler file, save the file manually (see section 4.12.2).

When a file is saved, the software displays the file name in the upper left corner of the workspace (Figure 4-40).



Figure 4-40. Title Bar with File Name

4.12.1 Auto-Saving Files

By default, BD CSampler Software automatically saves the event data any time the flow cytometer reaches a run limit or if a run is paused. Auto-save *does not* save changes to acquisition settings, plots, or gating strategies that occur after the initial save when naming the BD CSampler Software file.

CAUTION: If changes are made after a run is paused or completed, BD CSampler Software *does not* automatically save the file. Save these changes manually (see section 4.12.2).

To enable or disable auto-save:

- Select *File* > *Auto-save Settings*.
- Do one of the following in the Auto-save Settings dialog box:
  - Select the *Auto-save Enabled* radio button to enable auto-save.
  - Select the *Auto-save Disabled* radio button to disable auto-save.

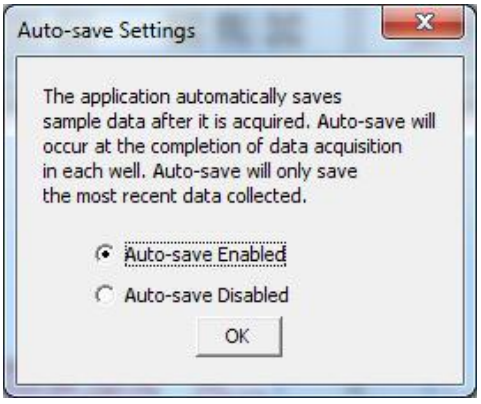


Figure 4-41. Auto-Save Settings Dialog Box

- Click on the *OK* button to accept the change and close the dialog box.
- If prompted to save the workspace before closing, do one of the following:
  - Click on the *Yes* button to save the entire workspace.

- Click on the *No* button to exit the dialog box without saving the workspace.

4.12.2 Manually Saving Files

To manually save a BD CSampler Software file:

- Select *File* > *Save.*

To manually save a BD CSampler Software file with a new name:

- Select *File* > *Save File As.*

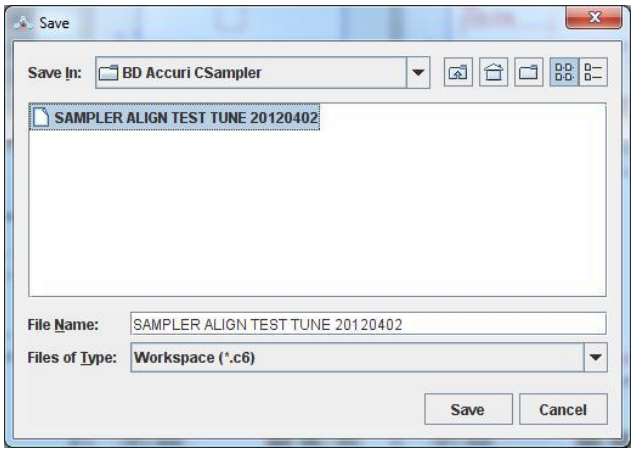


Figure 4-42. Save BD CSampler Software File

- If necessary, navigate to the location to save the file.
- In the Save dialog box, enter the file name and click on the *Save* button. The file is saved with the extension .c6.

4.13 Creating a BD CSampler Template

A BD CSampler template contains a predefined workspace for quick and easy setup and analysis. All markers, regions, gates, parameter names, and sample names are saved without any data points. BD Accuri provides several templates (see the BD CSampler Software CD, flash drive or the BD Accuri website at http://www.bdbiosciences.com/support/resources/accuri/index.jsp) or create custom templates.

To create a template:

- Define plot, gating, and acquisition settings in a blank workspace, or use the current .c6 file.
- Select *File* > *Save template as*.

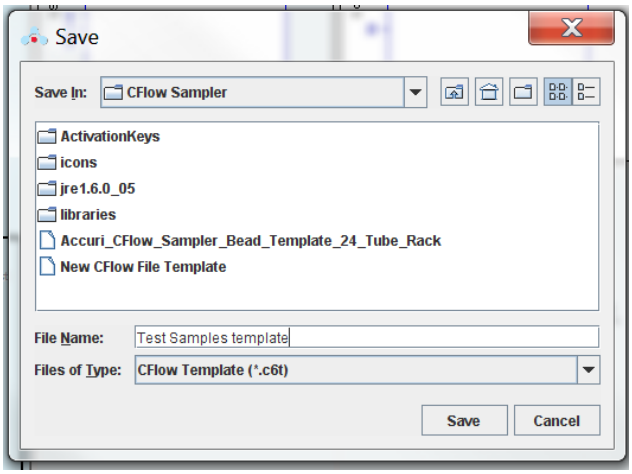


Figure 4-43. Save BD CSampler SoftwareTemplate

- If necessary, navigate to the location to save the template file.
- In the Save dialog box, enter the file name and click on the *Save* button. BD CSampler Software saves the file with the extension .c6t.

  NOTE: Acquisition settings are saved based on the currently selected sample.

4.14 Understanding Fluorescence Spillover

Fluorochromes typically emit light over a broad range of wavelengths, resulting in the fluorescence signal appearing not only in the expected, primary detector for that fluorochrome but in other detectors as well. This phenomenon is often called fluorescence “spillover,” and can be a source of confusion when interpreting multi-color flow cytometric data.

4.14.1 Recognizing Fluorescence Spillover

When performing a multi-color experiment, prepare a set of control samples stained with individual fluorochromes used in the experiment. These single-stained controls will allow determination of the extent of fluorescence spillover from each fluorochrome. The example shown in the figure below shows data collected for a PE-Cy7 single-stained control. Most of the fluorescence signal from PE-Cy7 positive cells is detected in the FL3 (670 LP), as expected. However, there is also PE-Cy7 signal detected in FL1 (530 or 533 BP) and FL2 (585 BP), so that plots of data for those detectors appear to have positively fluorescent cells. No signal from PE-Cy7 appears in detector FL4.

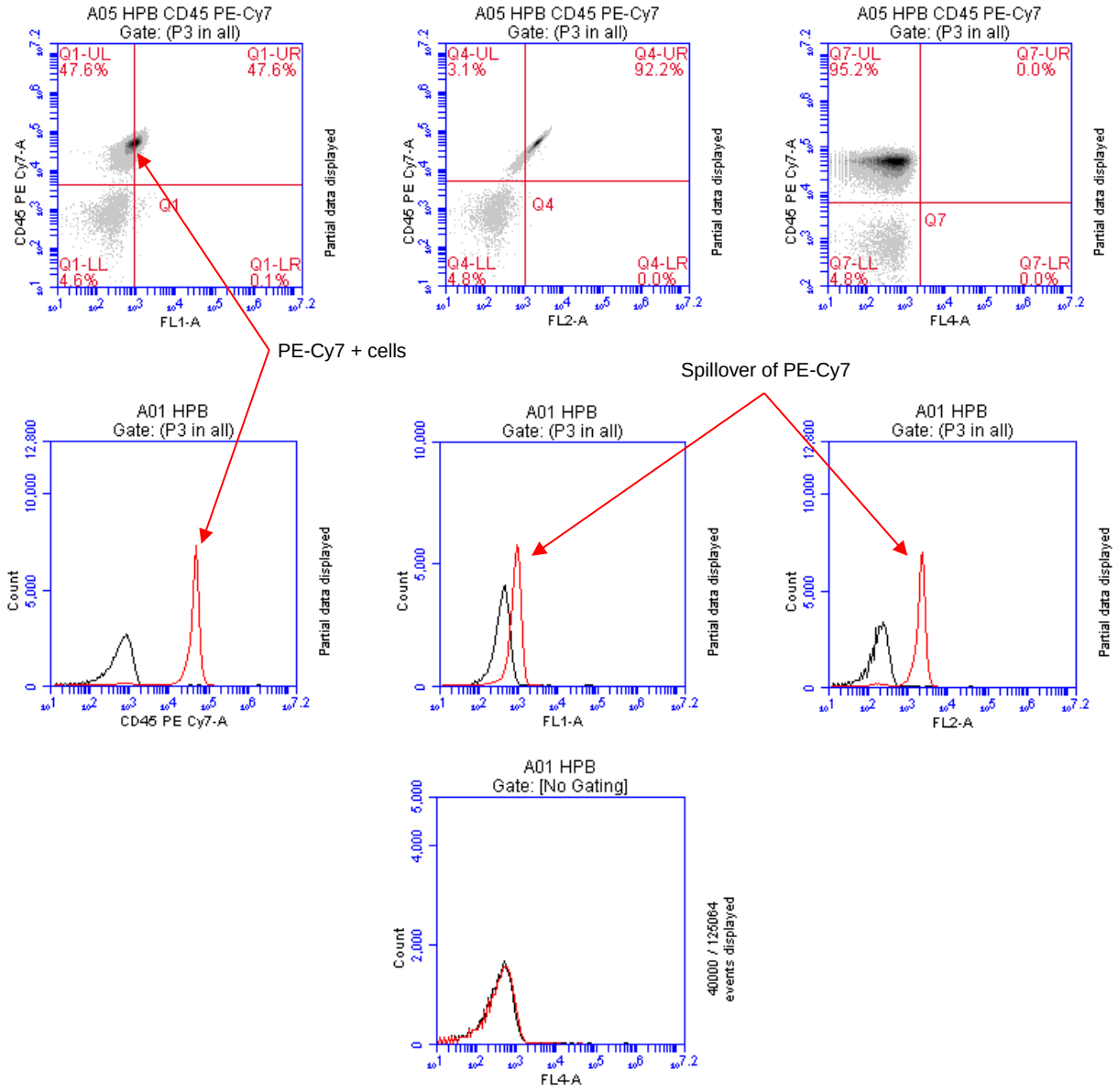


Figure 4-44. Fluorescence Spillover in Different Plots

4.14.2 Correcting Fluorescence Spillover

Fluorescence spillover can be removed from plots by applying a mathematical algorithm to collected data. This process is often called *color compensation* (or *fluorescence subtraction*). Because data collection on the flow cytometer is digital, color compensation can be applied or removed before, during, or after data collection. The color compensation algorithm subtracts a user-defined percentage of fluorescence signal from every event, thereby redistributing data to lower channels on the fluorescence scale and removing the apparent fluorescence spillover. When color compensation has been properly applied to a data set, the median fluorescence channel value in non-primary detectors for any given single-stained control sample should be the same as that of an unstained control sample.

The following figure shows the data after proper color compensation has been applied to the PE-CY7 example in section 4.14.1.

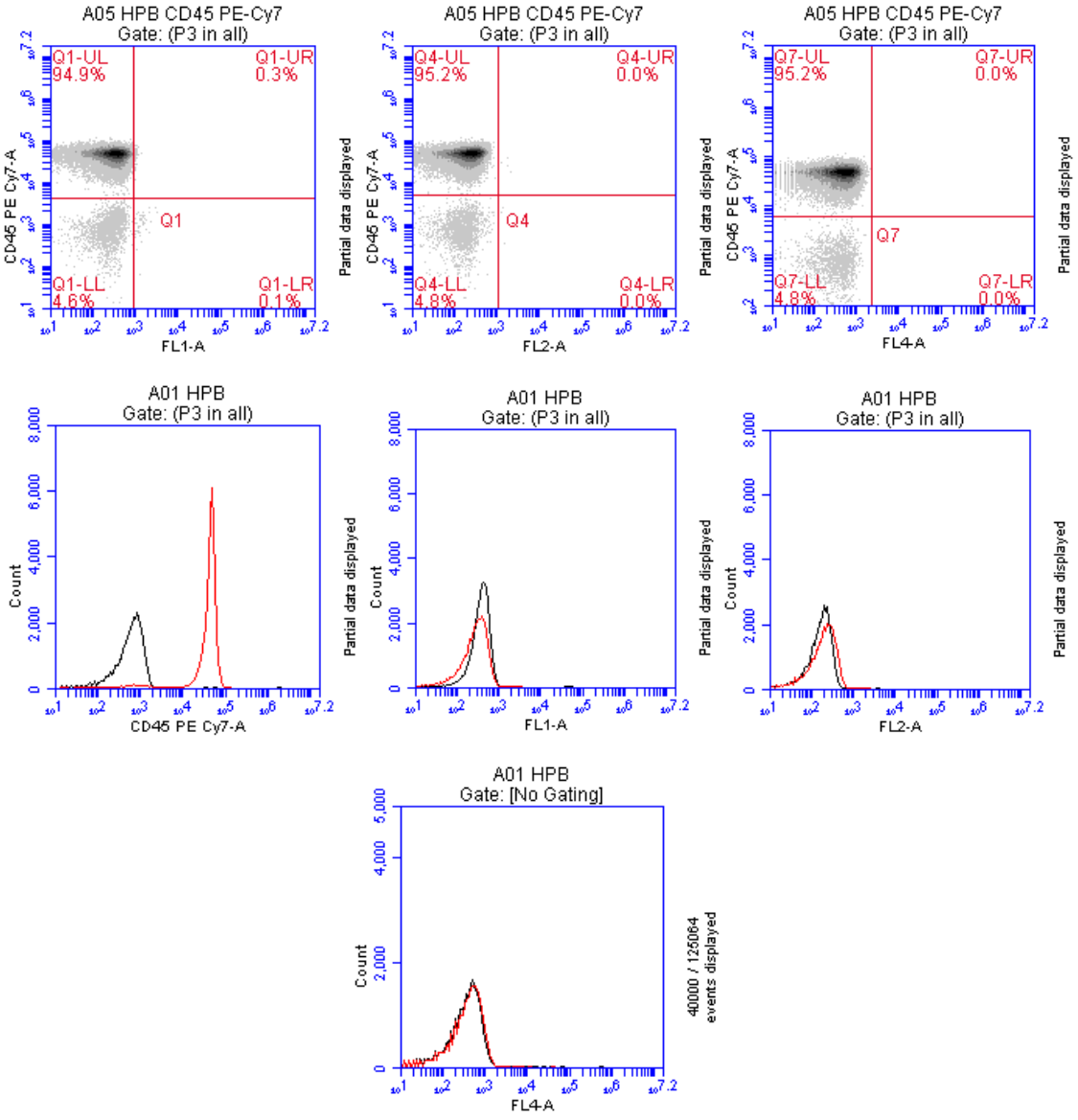


Figure 4-45. Corrected Fluorescence Spillover

The PE-CY7 fluorescence is now confined to the FL3 detector, and no longer spills into FL2 or FL1.

To correct fluorescence spillover:

- Click on the Quadrant Tool of a plot and click inside the plot.

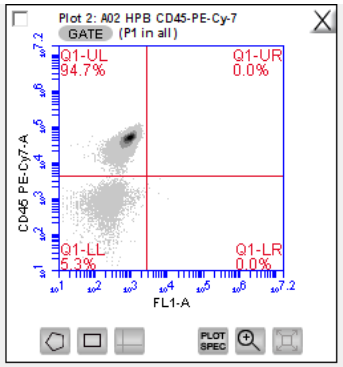


Figure 4-46. Placing a Quadrant Tool

- Adjust the quadrant marker position so that all positive populations are cleanly contained in individual quadrants.

  BD CSampler displays the median fluorescence channel value for the events in each quadrant in the Statistics Table (shown below).

Plot 3: Sample A2 Gated on (P1 in all)	Count		Median FL1-A	Median CD45 PE-Cy7-A
This Plot	101,660	%	NA	NA
Q1-UL	96,737	%	841.0	45,399.0
Q1-UR	0	%	0.0	0.0
Q1-LL	4,921	%	240.0	771.0
Q1-LR	2	%	10,200.0	1,322.0

Figure 4-47. Statistics Table Displaying Median Values

- Compare the median values of the affected channel. If the median value of the UL or LR quadrant is not equal to the median value of the negative population (LL), fluorescence compensation should be applied.

- Click on the *Set Color Compensation* button in the *Manual Collect* or *Analyze* tab to open the *Compensation Settings* dialog box. The dialog box contains four rows of *FL* buttons, one row for each fluorescence channel.

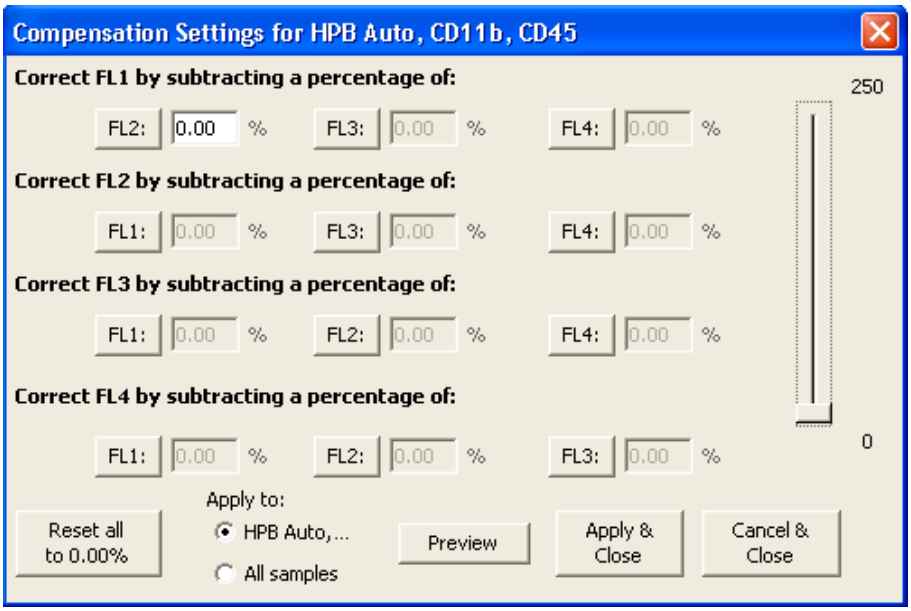


Figure 4-48. Compensation Settings Dialog Box

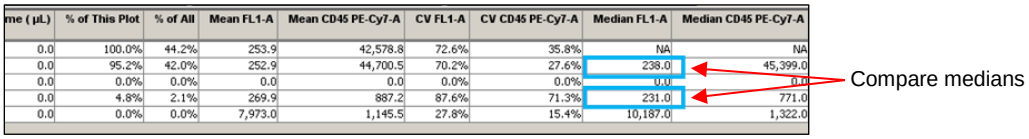
- In the row associated with the channel to correct, click on the *FL* button of the fluorescence channel that is spilling over.
- If needed, click on the *Reset all to 0.00%* button to clear all compensation values.
- Do one of the following:
  - In the text box next to the *FL* button, type an arbitrary percentage of the signal to subtract.
  - Use the C Comp Calculator Excel spreadsheet (available on the BD CSampler Software CD (or flash drive) or at http://www.bdbiosciences.com/support/resources/accuri/index.jsp) to calculate the subtraction values.

    See the appropriate tab of the C Comp Calculator for your instrument serial number for suggested fluorochrome-specific spillover values (Table 3-3 is an example).

Table 4-4. Fluorochrome Spillover per Channel

		Spillover Fluorochrome					
		FITC	**PE**	**PerCP**	**PerCPCy5.5**	**PE-Cy7**	**APC**
Channel to Correct	**FL1** (530BP)	N/A	3.2	0.00	0.0	0.5	N/A
	FL2 (585 BP)	6.5	N/A	0.00	0.00	1.5	0.0
	FL3 (670 LP)	1.0	19.5	N/A	N/A	N/A	0.8
	FL4 (675 BP)	0.0	0.0	3.00	12.00	0.00	N/A

- Click on the *Preview* button to update the plots and Statistics Table.
- In the Statistics Table, observe the values in the *Median* column of the FL channel of interest.
- Repeat steps 5-9 until the median value for the UL or LR quadrant is equal (or nearly equal) to the median value of the negative population (LL). This value is called the *compensation value*. The figure below shows the median values highlighted in blue.



me (µL)	% of This Plot	% of All	Mean FL1-A	Mean CD45 PE-Cy7-A	CV FL1-A	CV CD45 PE-Cy7-A	Median FL1-A	Median CD45 PE-Cy7-A
0.0	100.0%	44.2%	253.9	42,578.8	72.6%	35.8%	NA	NA
0.0	95.2%	42.0%	252.9	44,700.5	70.2%	27.6%	238.0	45,399.0
0.0	0.0%	0.0%	0.0	0.0	0.0%	0.0%	0.0	0.0
0.0	4.8%	2.1%	269.9	887.2	87.6%	71.3%	231.0	771.0
0.0	0.0%	0.0%	7,973.0	1,145.5	27.8%	15.4%	10,187.0	1,322.0

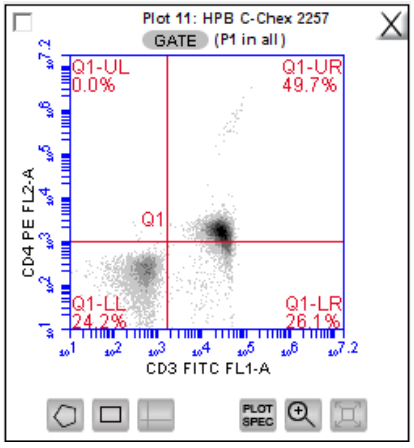
Figure 4-49. Results of Subtracting Spillover

- To apply the fluorescence subtraction to all samples, select the *Apply to All samples* radio button in the Compensation Settings dialog box.
- Click on the *Save & Close* button to apply the color compensation settings.

4.14.3 Troubleshooting Color Compensation

Occasionally, the plot might appear to have a smaller percentage of events in a quadrant than BD CSampler Software reports. For example, Figure 4-50 appears to have less than 26.1% of the population in the lower right quadrant, even though the statistics report 26.1%. This occurs when a decade containing data is hidden.

In the following figure, a number of events have been driven into channel 1 because of overcompensation. These events are not displayed in the plot if the first decade is hidden, but they are included in statistics calculations.



Plot 11: HPB C-Chex 2257 Gated on (P1 in all)	Count	Volume (µL)	% of This Plot
This Plot	21,479	73.3	100.0%
Q1-UL	7	73.3	0.0%
Q1-UR	10,669	73.3	49.7%
Q1-LL	5,198	73.3	24.2%
Q1-LR	5,605	73.3	26.1%

Figure 4-50. Plot Displaying Overcompensation

To fix overcompensation:

- Click on the Plot Spec Tool .

- In the Set Plot Specs dialog box, disable the *Hide 1st decade* check boxes for both X- and Y-axes.

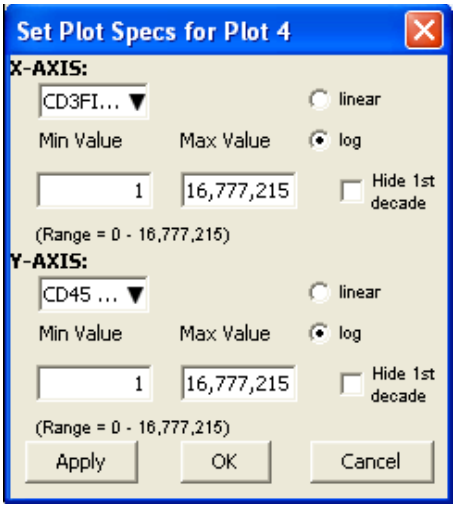
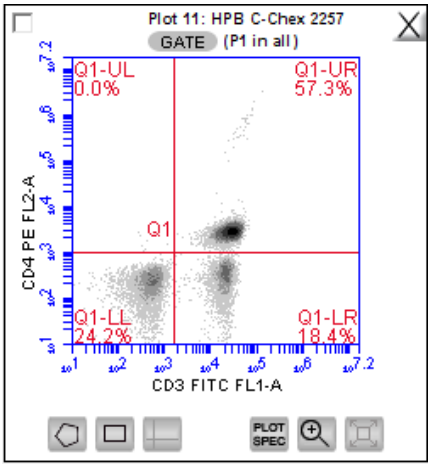


Figure 4-51. Set Plot Specs Dialog Box with *Hide 1st decade* Disabled

- Click on the *OK* button to apply the settings and close the dialog box.
- Open the Compensation Settings dialog box and reset the appropriate compensation values to zero. In this example, that would be “Correct FL2 by subtracting a percentage of FL1”.
- Perform the fluorescence compensation procedure described in section 4.14.2. The number of events displayed in Q1-LR agrees with the percent value of 18.4%, as shown on the plots and Statistics Table in Figure 4-52.



Plot 11: HPB C-Chex 2257 Gated on (P1 in all)	Count	Volume (µL)	% of This Plot
This Plot	21,479	73.3	100.0%
Q1-UL	8	73.3	0.0%
Q1-UR	12,315	73.3	57.3%
Q1-LL	5,197	73.3	24.2%
Q1-LR	3,959	73.3	18.4%

Figure 4-52. Properly Compensated Data After First Decade Is Rehidden

4.15 Changing Parameters

By default, BD CSampler Software displays the area parameter (signified with a suffix of *-A*), but parameters can also be displayed in height, width (of primary threshold channel only), or time.

To change a parameter:

- Click on the x- or y-axis label and select an option from the drop-down menu.

NOTE: The time parameter starts counting when the *Run* button is clicked and continues counting for 19 days (16 million tenths of a second), even if data is added to the sample at a later time. The time parameter cannot be reset to zero, even by deleting data.

4.16 Agitating Samples

The agitate feature is designed to maintain a sample in suspension, not to resuspend a completely settled sample.

CAUTION: Sample volume should not exceed 50% well capacity to effectively maintain suspension while avoiding spillover during agitation.

To perform an agitate cycle in the *Manual Collect* tab:

- Click on the *Agitate* button.

4.17 Copying and Pasting Plots

To copy and paste plots from the *Collect* or *Analyze* tab to a Microsoft® Office compatible application:

- Click anywhere on a plot and drag it to an open Microsoft application.

  NOTE: **Ctrl+C** and **Ctrl+V** cannot be used to copy and paste plots from BD CSampler Software into other applications.

If the Enhanced Analysis activation key is installed, one of two file formats can be selected for plots during drag and drop actions.

To select the file format to use:

- Select *File > Set Plot Drag and Drop Format.*
- In the dialog box, choose one of the following formats:
  - .png when lower resolution is sufficient. Drag and drop to Excel requires.png format.
  - .eps when higher resolution images are required for publication or posters.

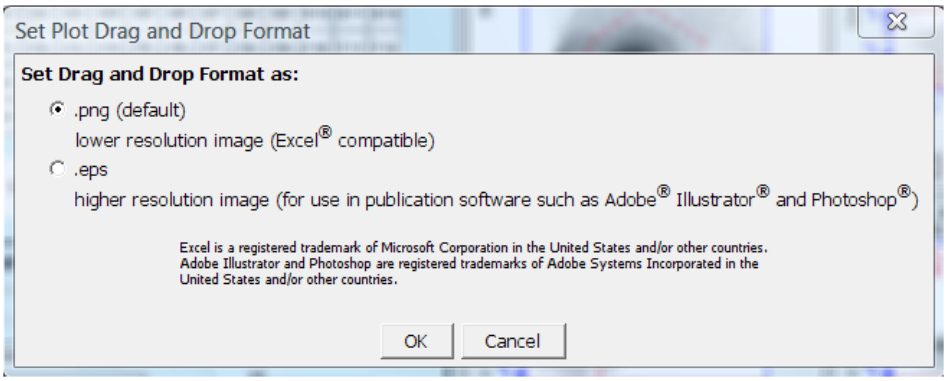


Figure 4-53. Set Plot Drag and Drop Format Dialog Box

NOTE: The Drag and Drop Format selection is BD CSampler Software file-specific, and will always revert to the default of .png when a new BD CSampler Software file is created or if the current BD CSampler Software file is not saved with the .eps option selected.

- Click on the *OK* button to save the settings and return to the BD CSampler Workspace.

4.18 Printing Data

To print selected plots and associated statistics from the *Manual Collect* or *Analyze* tab:

- Enable the check box in the upper left corner of one or more plots.

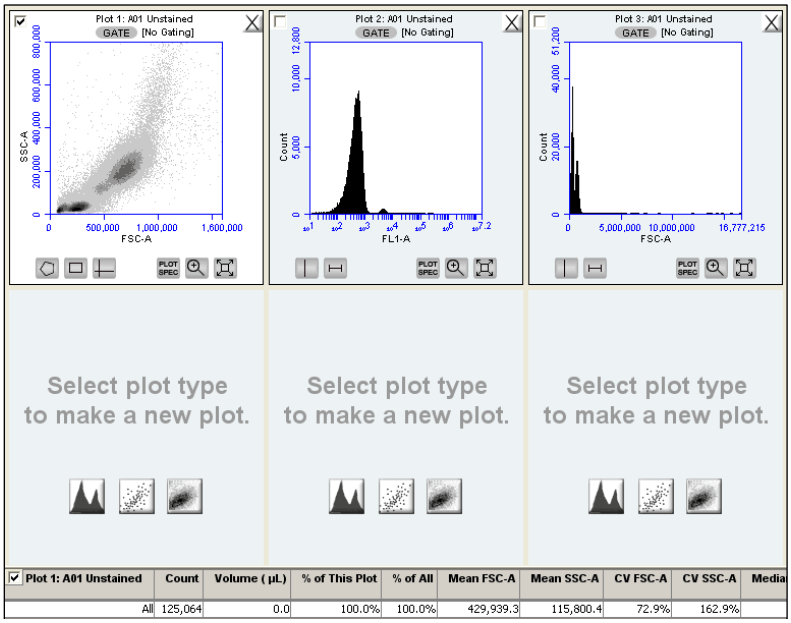


Figure 4-54. Plots Selected for Printing

The associated statistics are automatically selected for printing. This box can be de-selected if desired.

- Select *File* > *Print Selected Items*.

NOTE: Printing directly from BD CSampler Software will result in low resolution images even if the .eps option is selected in the Set Plot Drag and Drop Format dialog box.

4.19 Exporting and Importing Files

Data can be exported from sample wells from the *Manual Collect* or *Analyze* tab as individual FCS 3.0 files at any time.

NOTE: See Appendix F.4 for information about saving plots as publication-quality images.

To export data:

- Do one of the following:
  - Select *File* > *Export FCS File* to export and save the currently selected data well as an FCS 3.0 file.
  - Select *File* > *Export ALL Samples as FCS* to export and save all data wells as individual FCS 3.0 files.
  - Select *File* > *Export ALL Samples to Third Party* to export and save all data wells as individual FCS 3.0 files that enable autoscaling in third party applications (such as FlowJo).
  - Select *File* > *Export Plot Data as CSV* to save an individual file in .csv format (see APPENDIX H for an example .csv file).
- If prompted to confirm the export, click on the *OK* button.

To import an FCS data file into BD CSampler Software:

- Select an empty data well in a BD CSampler Software file or template. If the currently selected data well contains data, BD CSampler Software will start FCS file import in the first empty data well.
- Select *File* > *Import FCS File*.
- Navigate to the location of the file.

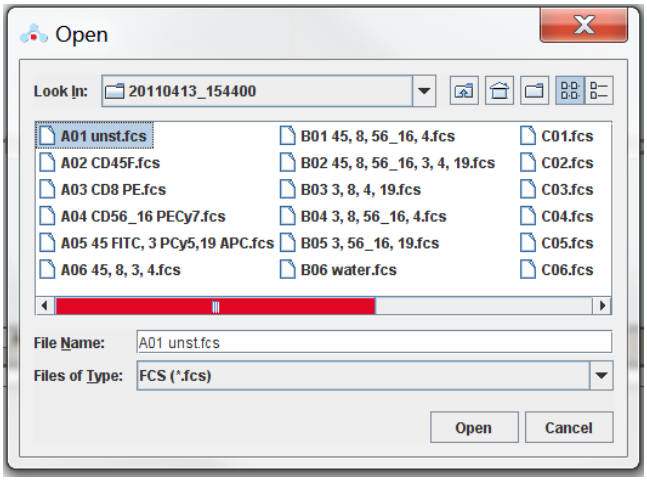


Figure 4-55. Open an FCS File

- Select the file and click on the *Open* button.
- Multiple FCS files from within a folder can be selected and imported as a group using **shift** + click. Files begin importing into the selected data well and proceed in succession, horizontally, from left to right, wrapping to the following row once the current row is filled.

  NOTE: Only FCS files created by BD CSampler Software can be imported into a BD CSampler Software file or template.

5 AUTOMATIC DATA ACQUISITION

The *Auto Collect* tab allows automated sampling from a well plate or sample tube rack. The tab contains buttons and controls for performing the following functions:

Loading and ejecting a plate

Creating sets of samples with the same acquisition settings

Acquiring data

Setting stop criteria and thresholds

Controlling the fluidics

Optionally washing the SIP between samples

Optionally agitating (mixing) samples

Viewing sample information

5.1 Viewing the *Auto Collect* Tab

The *Auto Collect* tab is organized into two major sections:

Instrument Control Panel—Panel on the left side of the window that contains controls for collecting data.

Sample Annotation Table—Large table on the right side of the window used to name samples, rename parameters, and add a notation to each sample.

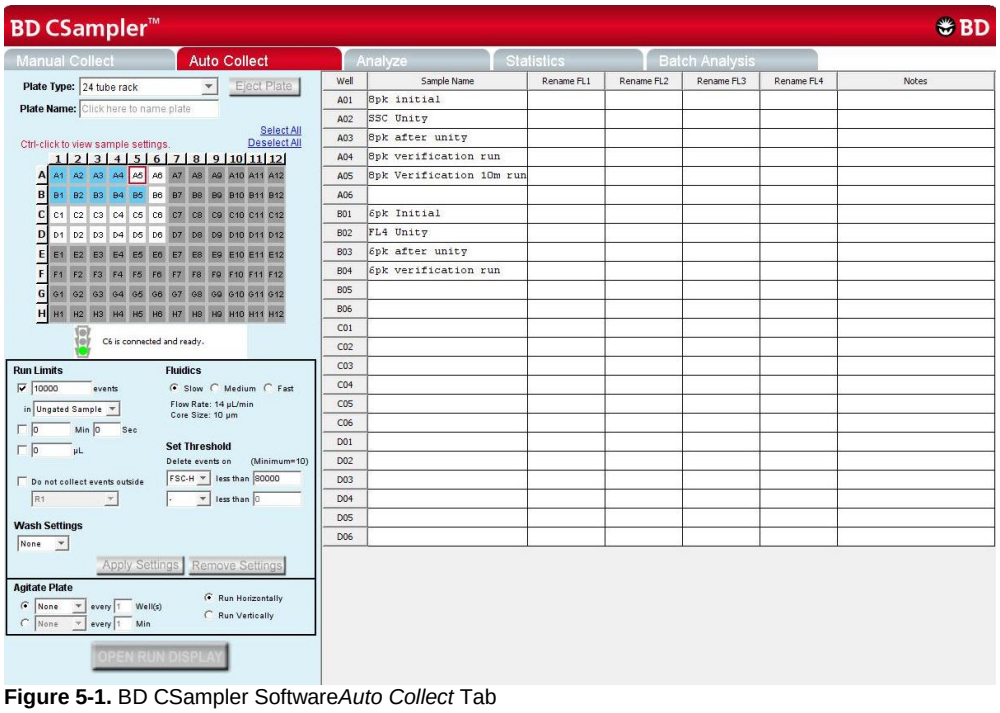


Figure 5-1. BD CSampler Software*Auto Collect* Tab

The following table describes each of the controls and indicators in the *Auto Collect* tab:

Table 5-1. *Auto Collect* Tab Controls

Control	*Description*
Plate Type	Drop-down list for setting the plate type.
Load Plate/Eject Plate	Moves the sample plate into position to be loaded or ejected from the flow cytometer.
Plate Name	Text box for naming the plate.
Sample Grid	Matrix laid out in the configuration of a multi-well plate to help organize experiments and collect data from sample tubes or wells. BD CSampler Software acquires each sample into its own well in the Sample Grid. The wells are color-coded: White—Does not contain data. Colored fill—acquisition settings are applied. **NOTE:** each unique acquisition setting is indicated by a color change. Small blue box—Contains data. Red outline—Currently selected for viewing or collecting data.
Traffic Light and Message	Indicator that displays BD CSampler Software's readiness and system messages. Before data collection can begin, BD CSampler Software must display a green Traffic Light  with the message *C6 is connected and ready.* The Traffic Light status is color-coded: Green—BD CSampler Software is ready to collect data or is collecting data. Yellow—The flow cytometer is preparing to perform an action, performing an action other than data collection (such as wash or agitate), or a non-critical error has occurred. Red—A critical error has occurred.
Run Limits	Contains a set of controls for defining criteria for automatically stopping the data collection. See section 5.6.3 for details.
Fluidics	Contains a set of controls for defining flow rate and core size. See section 5.6.1 for details.
Wash Settings	Performs a wash cycle to keep the SIP clean between samples. See section 5.6.5 for details.
Set Threshold	Sets the event threshold to gate out debris and noise from samples. The default value is 80,000 on FSC-H. See section 5.6.2 for details on setting threshold values.
Apply Settings/Remove Settings	To apply or remove sample settings such as run limits to a sample set. See sections 5.5.1 through 5.5.5 for details.
Agitate Plate	Performs an agitate cycle to keep particles in suspension. See section 5.6.4 for details.
Open Run Display	Opens the run display for starting and stopping a run, viewing data acquisition counters, and viewing two plots of data. See section 5.8 for more information.
Sample Annotation Table	Contains fields for naming samples, renaming parameters, and adding a notation to each sample. Information can be entered manually for each sample or copied and pasted from a spreadsheet program.

5.2 Running a Sample Plate in *Auto Collect*

The following procedure describes a typical setup and data collection workflow using BD CSampler Software.

- Perform pre-acquisition functions in the *Manual Collect* tab, such as creating plot displays and gates.
- Click on the *Auto Collect* tab.
- Create sample sets with specified acquisition, agitation, and wash settings.

  NOTE: Agitate settings are applied to the entire plate, while acquisition parameters, wash settings and run limits can be applied to individual sample sets.
- Define the file name for auto-save.
- Create the Sample Annotation Table.
- Select the direction to run the plate (horizontal or vertical).
- Open the Run Display.
- Select the plots to view during acquisition. Plots created using the manual collect tab are available for viewing.
- Click on the *AUTORUN* button.
- When the plate is complete, close the Run Display.

5.3 Assigning a Plate Type

When setting up an experiment, select the appropriate plate type.

NOTE: Changing to a new plate type results in BD CSampler Software opening a new blank workspace.

To assign a plate type:

- Select one of the following options from the *Plate Type* drop-down list:
  - 96-well plate: U-bottom
  - 96-well plate: flat bottom
  - 96-well plate: V-bottom
  - 96 deep well plate
  - 48-well plate
  - 24-tube rack

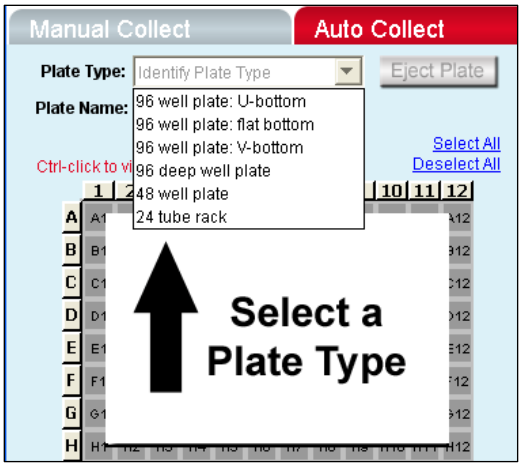


Figure 5-2. Plate Type Drop-Down Menu

The sample grid displays the available wells for data collection, based on the plate assignment:

96-well plate assignment—Displays all 96 wells

48-well plate assignment—Displays rows A-F, columns 1-8

24-tube rack assignment—Displays rows A-D, columns 1-6

5.4 Naming the Plate

The sample plate can be named to distinguish it from other sample plates in similar experiments (optional).

To name the plate:

- Name the plate by typing a label in the *Plate Name* field.

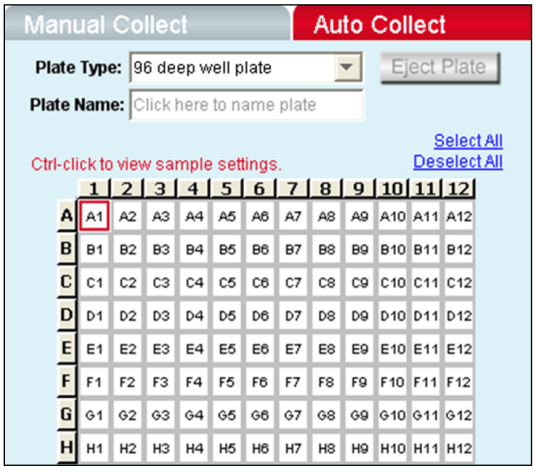


Figure 5-3. Plate Name Field

5.5 Using Sample Sets

A sample set is a group of samples that use the same data acquisition criteria (such as number of events or threshold setting). Acquisition criteria include:

- Run limits (number of total or gated events, sample volume, or time)
- Fluidics settings
- Wash settings
- Threshold(s) (default is FSC-H 80,000)

Each sample set is highlighted in a different color. Twelve colors are available, but up to 96 sets can be created (colors are reused).

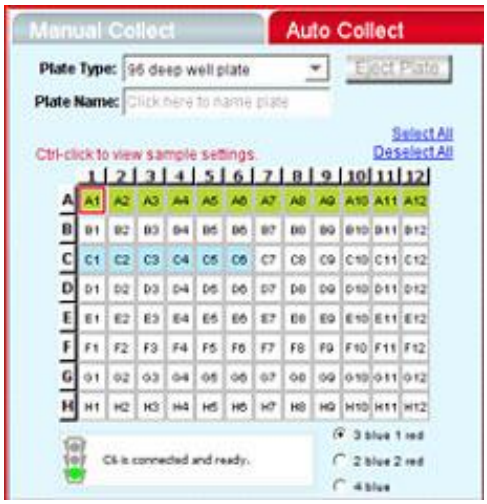


Figure 5-4. The *Auto Collect* Tab Displaying Two Data Sets

CAUTION: When a plate run is started, data will be collected in every well with settings applied, regardless of whether the well contains previously acquired data.

5.5.1 Creating Sample Sets

- Select one or more wells to include in the set by doing one of the following:
  - Click on individual wells.
  - Click on the column (1-12) or row header labels (A-H) to select the entire column or row. Multiple rows and columns can be selected simultaneously.
  - Click on *Select All* above the Sample Grid to select all the wells.

  Selected wells contain a black check mark.

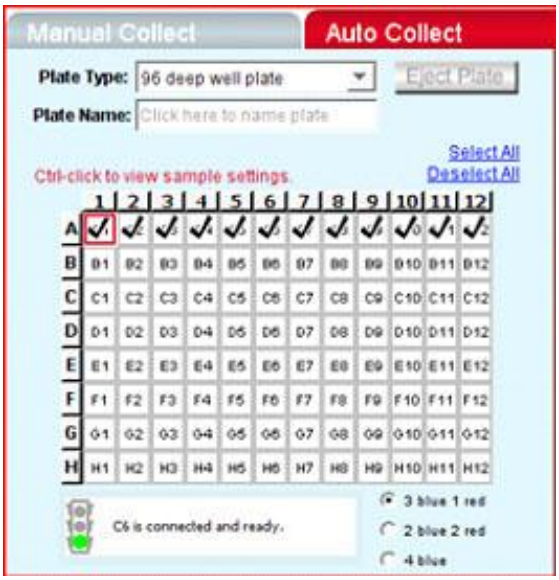


Figure 5-5. Selected Wells in the *Auto Collect* Tab

- Enter settings for the following:
  - Fluidics (see section 5.6.1)
  - Threshold (see section 5.6.2)—default is FSC-H 80,000
  - Run limits (see section 5.6.3)
  - Agitate cycles (see section 5.6.4) (optional)
  - Wash cycles (see section 5.6.5) (optional)

  NOTE: If run limit or fluidics settings were modified in the *Manual Collect* tab, those settings do not carry over in the *Auto Collect* tab. *Threshold settings and color compensation settings do carry over.*

- Click on the *Apply Settings* button. The selected samples are highlighted in the same color.

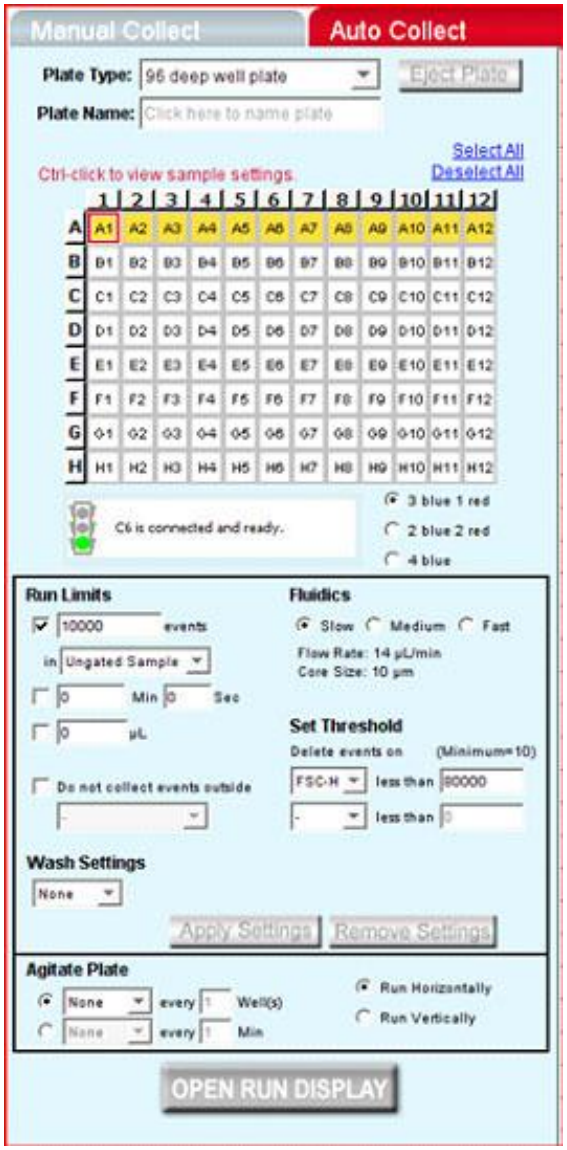


Figure 5-6. Samples in a Set Are the Same Color

5.5.2 Viewing Sample Settings

To view data acquisition settings for a particular well:

- Press **Ctrl** and click on the well of interest.

5.5.3 Modifying Sample Settings

To modify a set after it is created:

- Do one of the following:
  - Double-click on a well in the set to select the entire set.
  - Click on one or more wells within the set.
- Adjust the data acquisition settings.

- Click on the *Apply Settings* button. The selected wells change to a new color.

5.5.4 Saving a Sample Set

BD CSampler Software saves all sample settings each time the *Apply Settings* button is clicked. Data is automatically saved during a run, after each sample is complete.

It is also possible to manually save data at any time. To manually save sample settings:

- Click on the *Apply Settings* button. The first time settings are applied, BD CSampler Software prompts the user to name the file and designate a file location.

5.5.5 Removing Settings

To remove settings from a sample set:

- Do one of the following:
  - Select one or more wells in the set.
  - Double-click on a well in the set to select the entire set.
- Click on the *Remove Settings* button. Each selected sample well reverts to a white box and is removed from the set.

  NOTE: Acquisition settings displayed in the Control Panel do not change.

5.6 Defining Data Acquisition Settings

Data acquisition settings must be defined to create a sample set for an auto-run. This section gives details on assigning the following settings:

- Fluidics rate
- Threshold
- Run limits
- Agitate cycles (optional)
- Wash cycles (optional)
- Run direction

5.6.1 Setting the Fluidics Rate

The flow cytometer can accommodate an upper limit of 10,000 events per second, but it is recommended to acquire samples at a rate of 2,500 events per second or less to ensure the best data resolution.

NOTE: If fluidics settings were modified in the *Manual Collect* tab, they do not carry over in the *Auto Collect* tab.

To set the fluidics rate:

- Click on the *Slow, Medium,* or *Fast* radio button in the *Fluidics* section of the *Auto Collect* tab.

5.6.2 Setting the Threshold

Use thresholds to gate out light scatter and/or fluorescence signals caused by debris in cell samples and electronic noise inherent in the system. When set correctly for any given sample set, resolution of particle or cell light scatter and fluorescence signals is greatly improved and data set size often can be reduced. By default, BD CSampler Software is set to a primary threshold of channel 80,000 on FSC-H.

For more information on setting thresholds, see section 4.2.2.

5.6.3 Setting a Run Limit

The run limit defines when data acquisition will stop. The following parameters can be used individually or in combination to set a run limit.

- Time
- Volume
- Number of events (in a specified gate)

If multiple run limits are set, data collection stops on whichever limit is reached first.

NOTE: If run limit settings were modified in the *Manual Collect* tab, they do not carry over in the *Auto Collect* tab.

For more information on setting run limits, see section 4.2.6.

5.6.4 Agitating Samples

The agitate feature is designed to keep samples in uniform suspension during data collection. Agitate gently shakes the sample plate/tube rack in 15 second cycles.

BD CSampler Software can automatically perform one, two or three agitation cycles from the *Auto Collect* tab during the plate run. The user can specify whether to agitate after a given number of samples or in one-minute intervals for up to 30 minutes. If the interval is reached during acquire, the BD CSampler will complete data collection prior to performing the agitation.

Agitation is only performed when the SIP is clear of any well or tube and does not interrupt data collection.

CAUTION: Sample volume should not exceed 50% well capacity to effectively maintain suspension and avoid spillover with agitate. For best results, use U-bottom 96-well plates or standard 12x75 mm tubes.

NOTE: Agitate settings are applied to the entire plate or tube rack and cannot be applied to individual wells.

To set an agitate cycle:

- Select one of the following options:
  - To agitate before a given number of wells, click on the top radio button.
  - To agitate after a given number of minutes, click on the bottom radio button.

- Select the number of agitate cycles.

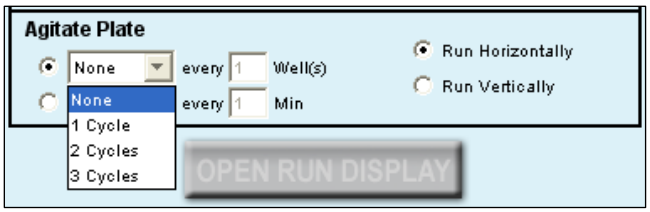


Figure 5-7. Agitate Plate Controls

- Do one of the following:
  - Type in the number of wells to increment between agitate cycles.
  - Type in the number of minutes to increment between agitate cycles.

5.6.5 Washing the SIP Between Samples

Multiple wash cycles of the SIP can be specified in the *Auto Collect* tab. Up to three wash cycles can be specified at a time. One wash cycle typically reduces carryover to < 1.0%. Two wash cycles typically reduce carryover to < 0.1%. The specified wash cycles are performed after each sample acquisition.

To wash the SIP:

- Select the number of wash cycles from the drop-down *Wash Settings* list.

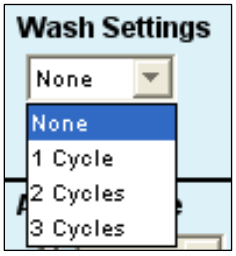


Figure 5-8. Wash Settings Controls

- Click on the *Apply Settings* button.

  NOTE: The *Apply Settings* button also applies run limits, thresholds, and fluidics.

Wash settings can be applied to individual wells.

5.6.6 Changing Run Direction

Data can be collected from plates either horizontally (A1, A2, A3, etc.) or vertically (A1, B1, C1, etc.).

To set the run direction:

- Do one of the following:

- Click on the *Run Horizontally* radio button in the Control Panel to collect the data horizontally.
- Click on the *Run Vertically* radio button to collect the data vertically.



Figure 5-9. Run Direction Controls

5.7 Creating the Sample Annotation Table

The Sample Annotation Table allows users to name samples, rename parameters, and add a notation to each sample. Information can be entered manually for each sample or copied and pasted from a spreadsheet program.

Well	Sample Name	Rename FL1	Rename FL2	Rename FL3	Rename FL4	Notes
A01	Unstained					
A02	HPB CD45-PE-Cy-7			CD45 PE-Cy7		
A03	HPB CD3 CD45	CD3 FITC		CD45 PE Cy7		
A04	HPB CD3, CD4, CD45, CD8	CD3 FITC	CD4 PE	CD45 PE-CY7	CD8 APC	
A05						
A06						
A07						
A08						
A09						
A10						
A11						
A12						
B01						
B02						
B03						
B04						

Figure 5-10. Sample Annotation Table

To manually create the Sample Annotation Table:

- For each sample well, type in the following information: Click on each cell and type appropriate information. Renaming parameters in the Sample Annotation Table will be also applied to data plots.

To copy and paste information from a spreadsheet:

- Highlight fields in the spreadsheet.
- Press **Ctrl + C**.
- In the Sample Annotation Table, click on *Sample Name* of well *A1* (or other appropriate cell).
- Press **Ctrl + V** to populate the Sample Annotation Table with the highlighted data from the spreadsheet.

5.8 Using the Run Display

The Run Display is used to begin, view, interrupt and abort data collections in *Auto Collect*.

The Run Display contains the following elements:

- Status banner
- Two large plots
- Data acquisition counters
- Two pause options

To open the Run Display:

- Apply settings to one or more wells (see section 5.5.1).
- Click on the *OPEN RUN DISPLAY* button. The Run Display opens in the main panel of the *Auto Collect* tab.

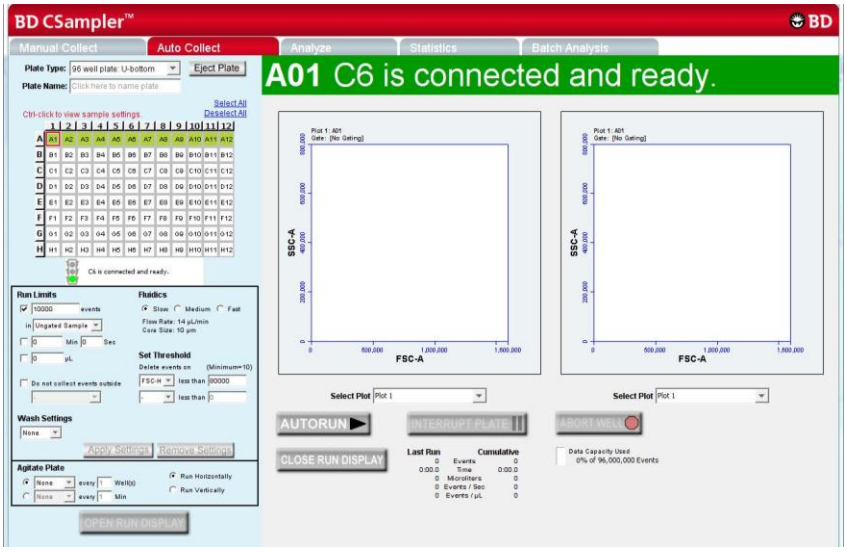


Figure 5-11. BD CSampler Run Display

To close the Run Display and return to the Control Panel:

- Click on the *CLOSE RUN DISPLAY* button.

5.8.1 Acquiring Data in the *Auto Collect* Tab

BD CSampler begins data collection in the first well that has settings applied (most commonly A1) and progresses horizontally or vertically as defined in the settings. As data is acquired and BD CSampler advances through the plate or tube rack, BD CSampler Software displays a small, blue square in the upper left corner of each completed well. A red border around a well indicates the sample that is currently being acquired. The current well also flashes blue during data acquisition.

CAUTION: Data is collected into all wells with settings applied, even if they already contain data.

To begin data acquisition:

- Click on the *AUTORUN* button. The status banner displays the current well and the message *Events are being recorded.* The Run Display also updates the acquisition counters and the sample data in the plots.

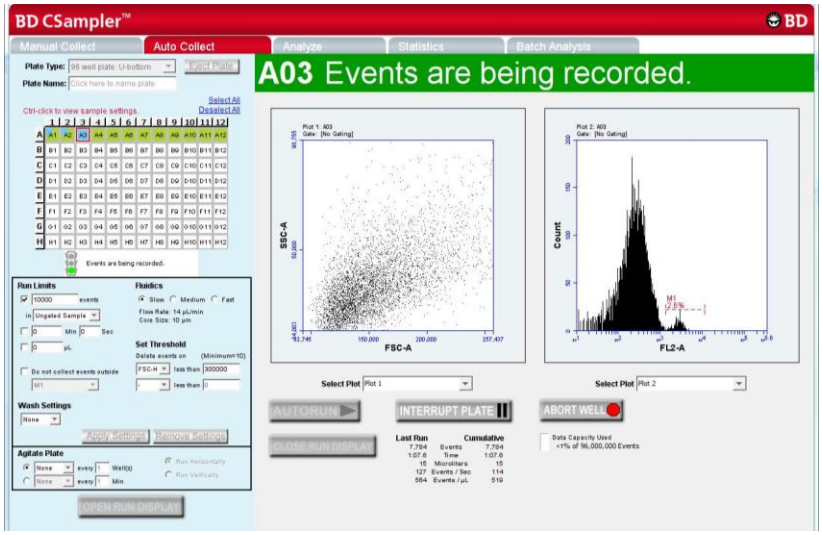


Figure 5-12. The Run Display While Collecting Data

When data collection is complete, BD CSampler Software displays ***DONE!*** prominently across the *Run* Display.

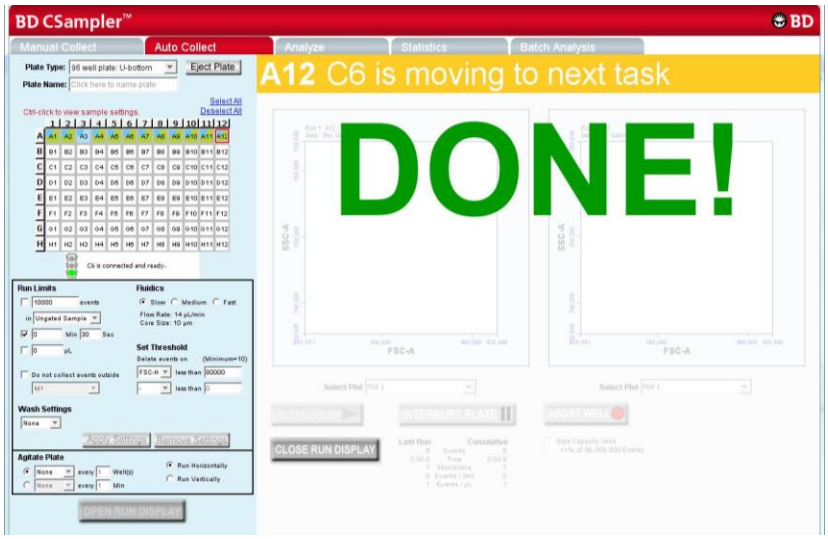


Figure 5-13. The BD CSampler Software Display After a Sample Run

When a plate is complete, click on the *CLOSE RUN DISPLAY* button to close the Run Display and continue using BD CSampler Software.

5.8.2 Stopping Data Collection

Data acquisition can be stopped by interrupting the plate or aborting the well.

- *Interrupt Plate*—Completes data collection from the current well and performs wash and agitate cycles (if applied) before stopping the plate run. The user can eject the plate when the run is stopped. The settings in *Auto Collect* cannot be changed.
- *Abort Well*—Immediately stops data acquisition, stops the fluidics, and ejects the plate. Settings cannot be changed from the *Auto Collect* tab in the aborted state.

To stop acquiring data after it has started:

- Click on one of the following buttons:
  - *INTERRUPT PLATE*
  - *ABORT WELL*

To restart a run during an Interrupt state:

- Click on the *AUTORUN* button. BD CSampler Software continues acquiring data where it left off.

To restart a run during an Abort state:

- Do one of the following:
  - To begin collecting samples at the next well, click on the *Autorun* button.
  - To collect from the same well, close the run display on the *Auto Collect* tab, click on the *Manual Collect* tab and run the sample manually.

5.8.3 Viewing Sample Plots

All plots created in the *Manual Collect* tab are listed in the *Select Plot* drop-down list beneath the plot window. They can be selected at any time during data collection. Two plots can be viewed at a time.

NOTE: Plots can only be added, modified, or deleted from the *Manual Collect* tab.

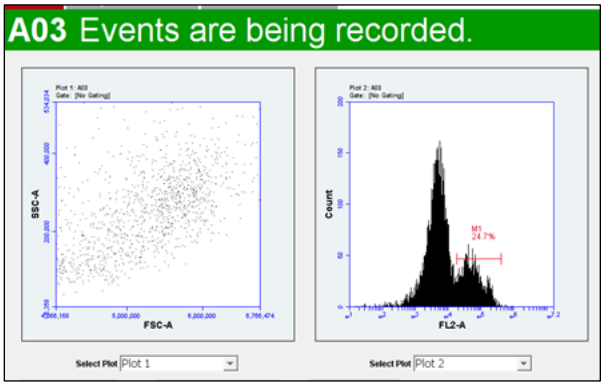


Figure 5-14. Run Display: Viewing Sample Plots

To view plots:

- Select plots to view from the *Select Plot* drop-down list under each plot corral.

5.9 Ending a Data Collection Session

Upon completion of each run, it is recommended to clean the SIP and fluidics lines using the following protocol:

- Load the tube holder with the following tubes:

Table 5-2. Plate Setup for Ending a Data Collection Session

Type of Sample	*Location in BD CSampler Software Sample Grid*
2 mL of filtered, deionized water	Empty data well (for example, H10)
2 mL of decontamination solution (PN 653154)	Empty data well (for example, H11)
2 mL of filtered, deionized water	Empty data well (for example, H12)

- In BD CSampler Software, select the well corresponding to the first tube of water. Make sure the well contains no data.
- Set the time limit for two minutes and the fluidics speed to fast.
- Run the sample.
- In BD CSampler Software, select the well corresponding to the decontamination solution. Make sure the well contains no data.
- Set the time limit for two minutes and the fluidics speed to fast.
- Run the sample.
- In BD CSampler Software, select the well corresponding to the second tube of water. Make sure the well contains no data.
- Set the time limit for two minutes and the fluidics speed to fast.
- Run the sample.
- When the run is finished, eject the plate/tube rack.

5.10 Saving a BD CSampler Software File

Always save BD Accuri C6 data as a BD CSampler Software file (.c6). A BD CSampler Software file is a comprehensive (and often large) data file that contains instrument settings, FCS files, and plot layouts. For details on saving a BD CSampler Software file, see section 4.12.

5.11 Creating a BD CSampler Software Template

A BD CSampler Software template contains a predefined BD CSampler Software workspace for quick and easy setup and analysis. All markers, regions, gates, parameter names, and sample names are saved without any data points. Accuri provides several templates (see the BD CSampler Software Installation CD, thumb drive or the BD website at http://www.bdbiosciences.com/support/resources/accuri/index.jsp). For details on creating custom templates, see section 4.13.

5.12 Exporting and Importing Files

Data from individual sample wells can be exported from any tab as FCS 3.0 files. For details on exporting and importing files, see section 4.19.

5.13 Exporting Sample Settings

Sample settings can be exported as a .csv file for viewing data in a spreadsheet. Sample settings include acquisition criteria, sample names, parameter names, and compensation values.

To export sample settings:

- Select *File* > *Export Sample Settings*.

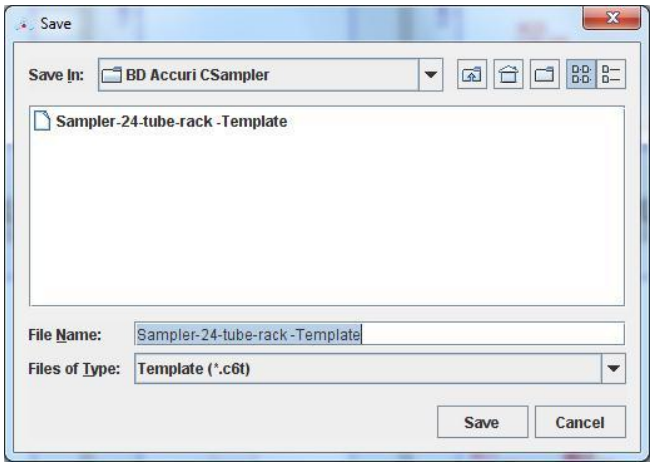


Figure 5-15. Export Sample Settings

- Navigate to the location to save the file.
- Type the name of the file in the *File Name* field and click on the *Save* button.

6 ANALYZING SAMPLE DATA

The *Analyze* tab allows data from multiple samples to be viewed simultaneously using the same plots and gating.

Use the tab to do the following:

- View several plots and samples in any combination for easy analysis.
- Compare specific samples from the 96-well grid.
- Create new plots, hide plots, or copy and reuse plots from the *Collect* tab.
- View different samples with the same plots.
- Create color overlay histograms.
- Print multiple plots.
- Adjust peak position.

6.1 Viewing the *Analyze* Tab

The *Analyze* tab is organized into two major sections:

- Setup panel—Panel on the left side of the window that contains controls for selecting samples and plots.
- Data display—Large area on the right side of the window that shows the sample data in plots and in a Statistics Table.

When the *Analyze* tab is opened for the first time, the workspace is empty. Plots can be copied from the *Manual Collect* tab or created from scratch. Gating strategies that were set up in the *Manual Collect* tab can be applied in *Analyze*, or new gates can be drawn and new gating strategies set up in *Analyze*.

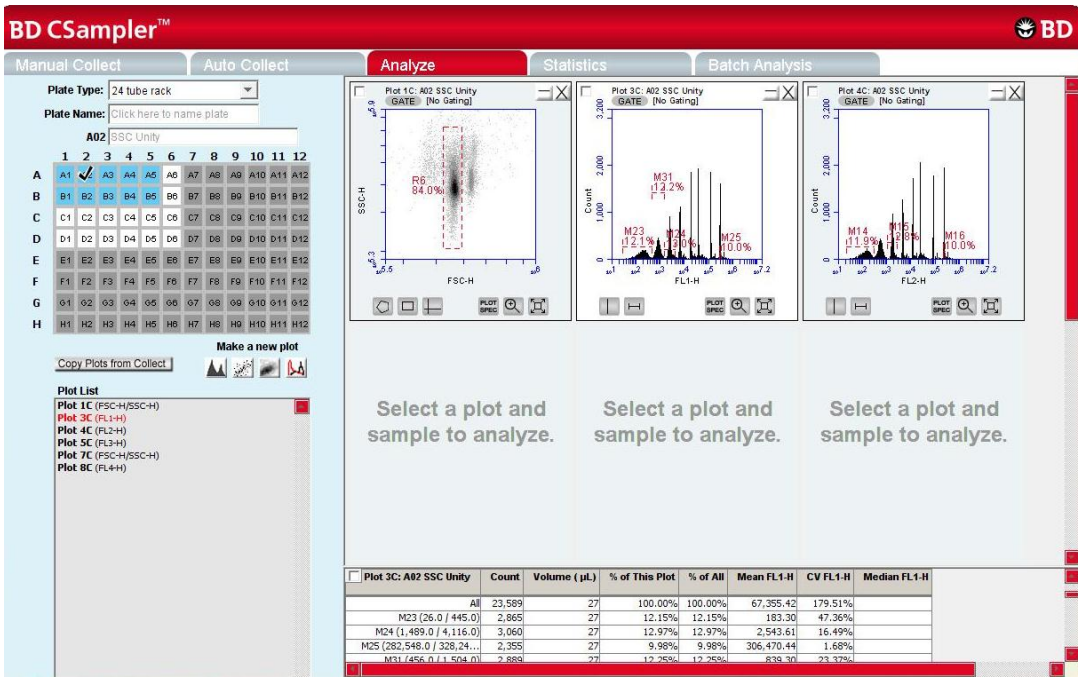


Figure 6-1. *Analyze* Tab Workspace

The following table describes each of the controls and indicators in the *Analyze* tab:

Table 6-1. *Analyze* Tab Controls

Control	*Description*
Plate Type	Disabled in the *Analyze* tab.
Plate Name	Text box for naming the plate.
Sample Naming Field	Text box for naming the current sample.
Sample Grid	Matrix laid out in the configuration of a multi-well plate to organize sample data. Each sample has its own well in the Sample Grid. The wells are color-coded: White—Does not contain data. Blue—Contains data. Black check mark—Currently selected for viewing data.
Copy Plots from Collect	Copies specified plots from the *Manual Collect* tab. See section 6.2.1 for details.
Plot Controls	Set of buttons for creating new plots or overlaying histograms. See section 6.2.3 for details. All plots created in the *Analyze* and *Manual Collect* tabs, including overlays, can be dragged and dropped into most Microsoft Office compatible programs.
Plot List	Lists the plots that are available in the *Analyze* tab. Available plots include plots copied from the *Manual Collect* tab or created in the *Analyze* tab.
Set Color Compensation	Opens the Compensation Settings dialog box for correcting fluorescence spillover. See section 4.14 for details.

Control	*Description*
Plot Corrals	Area displaying two rows of plot corrals. Scroll up or down to view more plots. For information on creating plots, see section 6.2.2.
Statistics Table	Table below the plots that displays statistical information on individual plots. Statistics Tables can be copied into most Microsoft Office compatible programs.

6.2 Setting up Plots

In the *Analyze* workspace, plots can be created or copied from the *Manual Collect* tab. Plots that are copied from the *Manual Collect* tab are appended with a "C" (for example, *Plot 1C*).

NOTE: See APPENDIX F.4 for details on saving plots as publication quality images.

6.2.1 Copying Plots from the *Manual Collect* Tab

- In the *Analyze* tab, click on the *Copy Plots from Collect* button.
- In the *Copy Plots from Collect* dialog box, do one of the following:
  - Select plots to copy by checking the box(es).
  - Enable the *All Plots* check box to copy all plots from the *Manual Collect* tab.

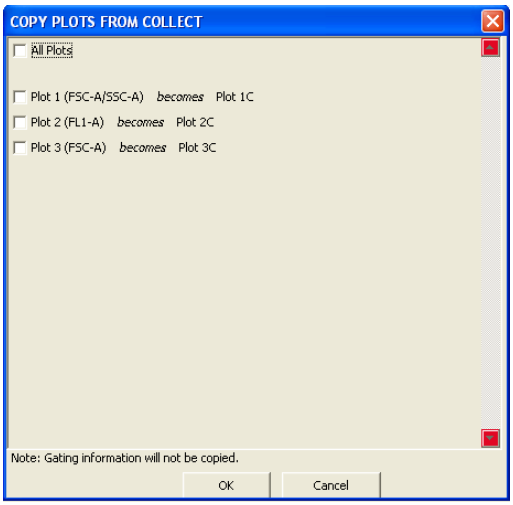


Figure 6-2. Selecting Plots to Copy from the *Collect* Tab

- Click on the *OK* button to close the dialog box. BD CSampler Software adds the selected plots to the Plot List in the *Analyze* tab.

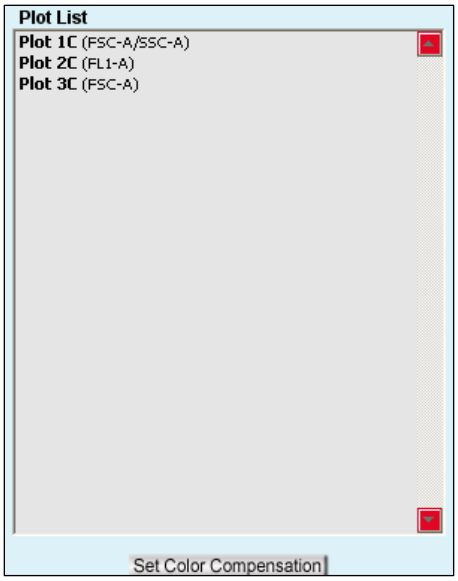


Figure 6-3. Plot List Containing Copied Plots

6.2.2 Creating Plots

- Click on an empty plot corral.
- Click on one of the following icons under the Sample Grid:
  - *Histogram*
  - *Dot*
  - *Density*
  - *Overlay Histogram* (see section 6.2.3 for details)
- Click on the sample well to view data.

6.2.3 Creating an Overlay Histogram

Creating an overlay histogram allows comparison of multiple distributions from up to 96 different samples at the same time.

To create an overlay histogram:

- Click on an empty plot corral.
- Click on the Overlay Histogram Tool to open a blank single-parameter FSC-A plot.

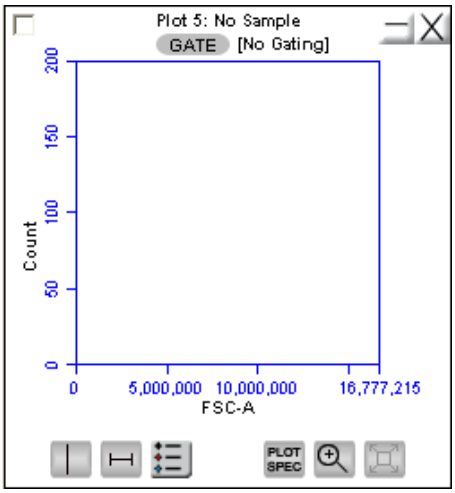


Figure 6-4. Blank Overlay Histogram Plot

- Click on the x-axis label *(FSC-A)* and select a different parameter in the drop-down list, if desired.
- Click on the *GATE* button and apply a gate, if desired (see section 4.7.2 for details).
- Select the samples to be overlaid from the 96-well grid.

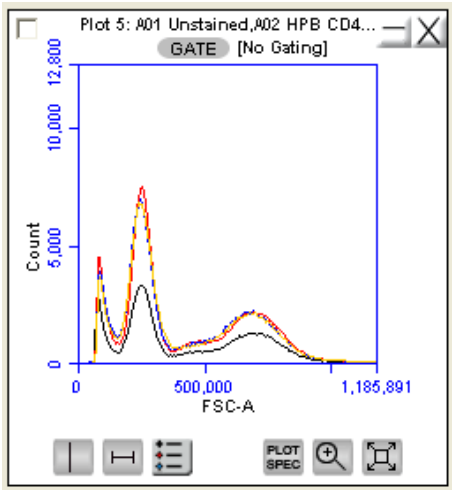


Figure 6-5. Overlay Histogram Plot with Data

- Click on the Overlay Histogram Legend Tool to view a legend for the overlay histogram.

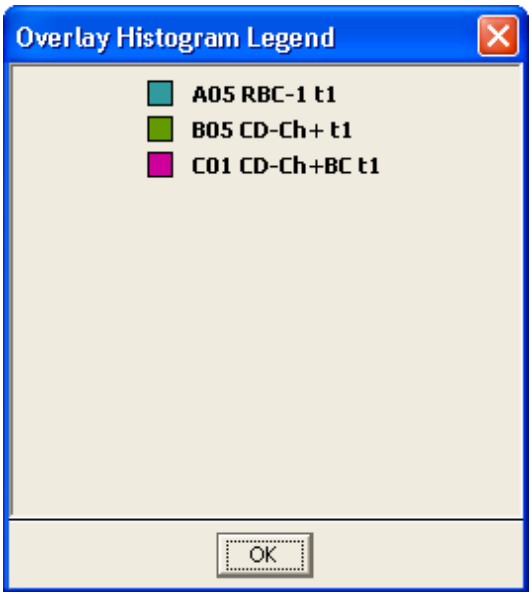


Figure 6-6. Overlay Histogram Legend

To change the color of one or more overlay plots, click on the square in the Overlay Histogram Legend and select the desired color from the pop-up color palette.

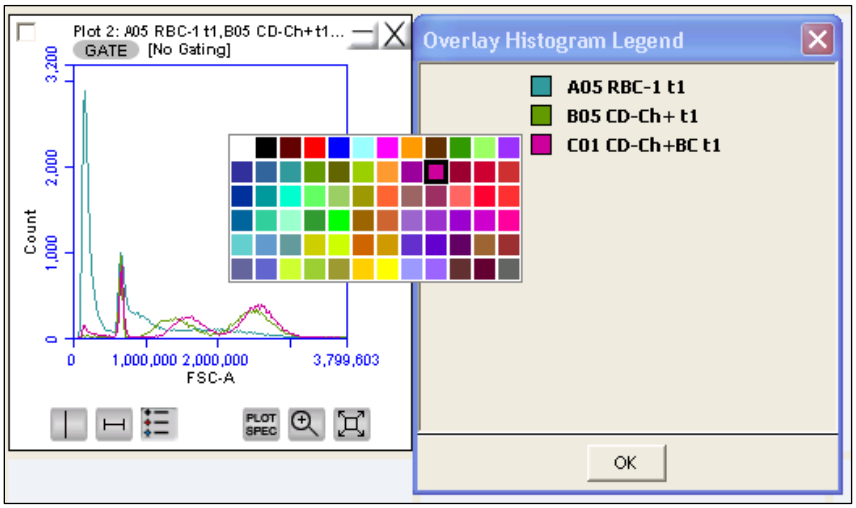


Figure 6-7. Overlay Histogram Legend with Color Palette

6.3 Viewing Plots

To view a plot in the Plot List:

- Click on an empty plot corral in the *Analyze* tab.
- Select a plot in the Plot List (Figure 6-8). BD CSampler Software displays the plot without any sample data.

NOTE: Any gating tools (markers, regions, etc.) carried over from the *Collect* tab are renamed (for example, P1 in *Collect* is P2 in *Analyze*). When these ”new” markers or regions are adjusted in the *Analyze* tab the original gate position in *Manual Collect* does not change.

- Click on a sample well to view data.
- Apply a gating strategy, if desired (see section 4.7.2 for details).

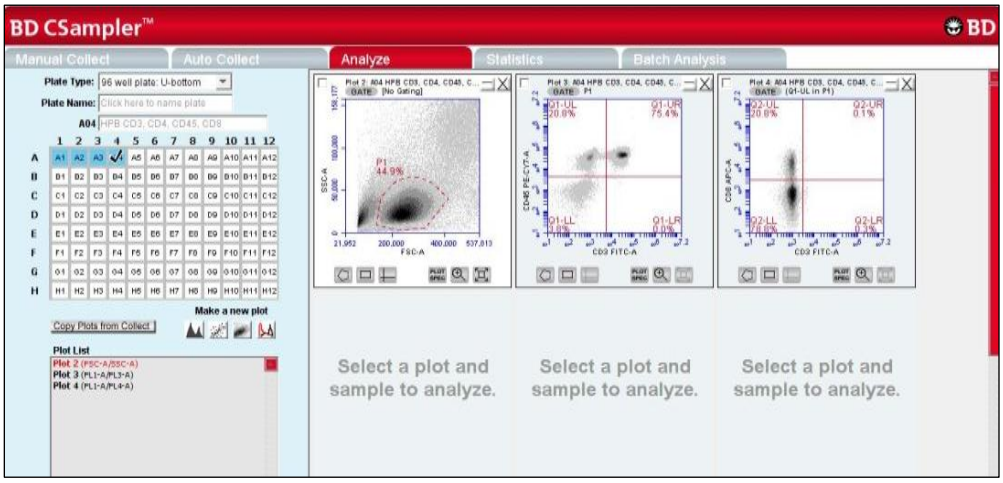


Figure 6-8. *Analyze* Tab: Plots with Gating Applied

To view data from another sample, open one or more plots from the Plot List (it is recommended to do this in another row of plot corrals) and choose the sample to be displayed in each plot. Gating strategies applied above are automatically applied to the corresponding plots in the new row.

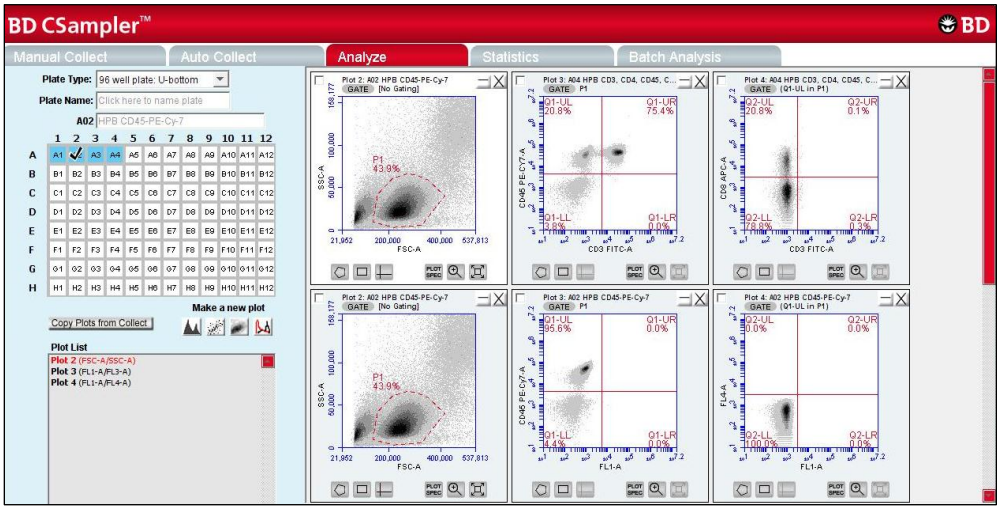


Figure 6-9. *Analyze* Tab: Two Samples with the Same Plots

Compare data and statistics between samples.

7 CREATING A STATISTICS TABLE

The *Statistics* tab provides a way of tabulating data from multiple samples in one master table. It also allows users to do the following:

- View statistics for some or all samples.
- Display statistics of collected or imported samples.
- List all plots created on the *Manual Collect* and *Analyze* tabs.
- Display all plot name*s, gates, and* asso*ciated* statistics.
- Copy and paste data into a spreadsheet.

7.1 Viewing the *Statistics* Tab

The *Statistics* tab is organized into two major sections:

- Setup controls for the Master Statistics Table
- Master Statistics Table

When the *Statistics* tab is first opened, the workspace is empty. To create a Master Statistics Table select data from the setup controls.

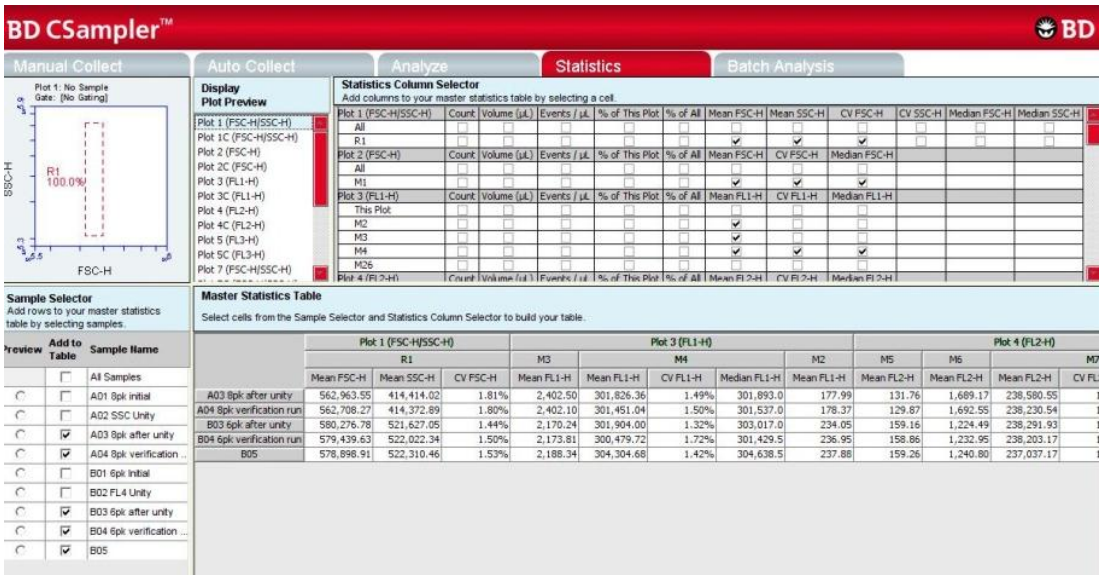


Figure 7-1. *Statistics* Tab Workspace

The following table describes each of the controls and indicators in the *Statistics* tab:

Table 7-1. *Statistics* Tab Controls

Control	*Description*
Plot	Previews the selected plot. See section 7.3 for details.
Display Plot Preview	List of plots imported from the *Manual Collect* and *Analyze* tabs that are available to preview.
Statistics Column Selector	Selects the data to view in the Master Statistics Table for each sample. See section 7.2 for details.
Sample Selector	Allows sample selection to view in the Master Statistics Table. See section 7.2 for details.
Master Statistics Table	Configurable table that displays data of selected samples. See section 7.2 for details.

7.2 Creating the Master Statistics Table

The Master Statistics Table enables creation of a customized data table for multiple samples within a given BD CSampler Software file.

To create the Master Statistics Table:

- In the Statistics Column Selector, enable the check boxes under the data items to view per plot. BD CSampler Software automatically adds columns to the Master Statistics Table.

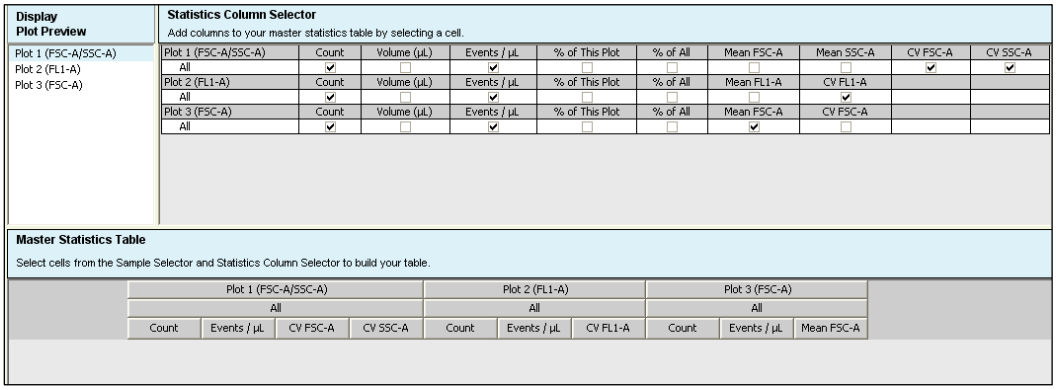


Figure 7-2. Creating Master Statistics Table: Adding Plots

- In the Sample Selector list, select samples by enabling check boxes. BD CSampler Software automatically adds the rows of samples to the Master Statistics Table and displays the sample data.

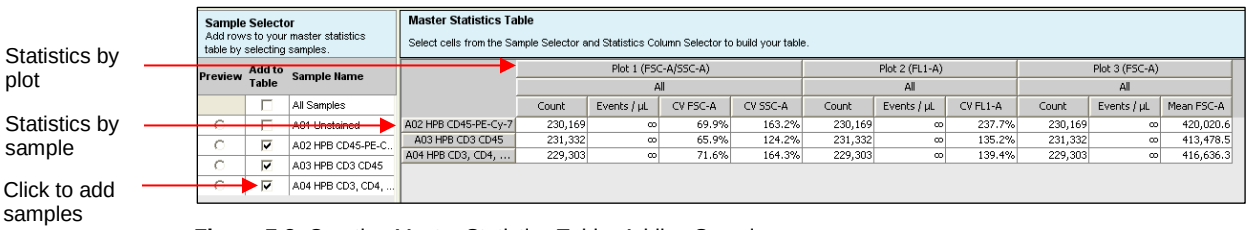


Figure 7-3. Creating Master Statistics Table: Adding Samples

7.3 Previewing a Plot in the *Statistics* Tab

To preview a plot in the *Statistics* tab:

- In the Display Plot Preview list, click on the appropriate plot to preview.

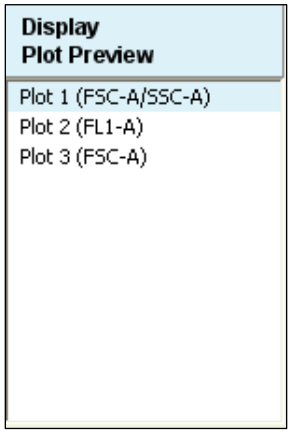


Figure 7-4. Display Plot Preview List

- In the Sample Selector list, select the radio button of a sample. BD CSampler Software displays the sample data in the plot.

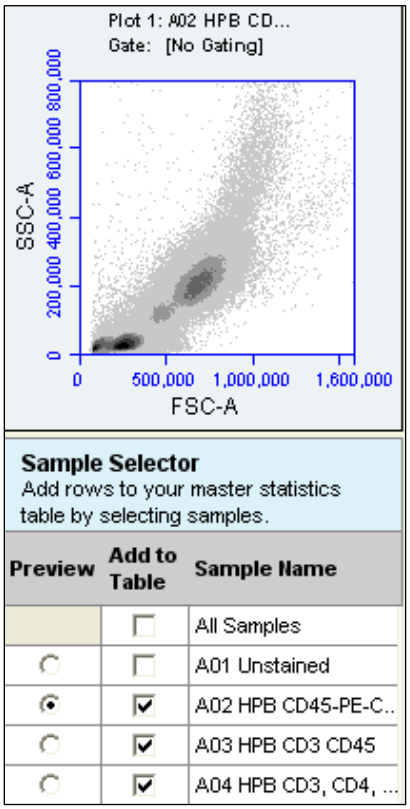


Figure 7-5. Plot Preview

The plot preview is available for viewing only. The zoom level and other plot settings must be modified in either the *Analyze* or *Manual Collect* tab.

7.4 Copying Data into Other Applications

Data can be copied and pasted from the Master Statistics Table into most Microsoft Office compatible applications.

To copy data:

- Use the mouse to highlight the fields. The column and row headers corresponding to the selected data fields are automatically copied.
- Press **Ctrl+C** to copy the data.
- In the Microsoft application, press **Ctrl+V** to paste the data.

NOTE: See Appendix F.4 for instructions on Batch Analysis.

8 MAINTAINING THE BD ACCURI C6 FLOW CYTOMETER

To maintain optimal performance, follow the routines in this chapter on a regular basis. See the *BD Accuri C6 Flow Cytometer Instrument Manual* for mechanical maintenance procedures (such as replacing tubing).

8.1 Cleaning the SIP

Run the Backflush Cycle to clean the SIP and remove clogs at the base of the SIP.

To perform a backflush:

- Do one of the following:
  - Click on the *Backflush* button in the *Manual Collect* tab.
  - Select *Instrument* > *Run Backflush Cycle.*
- Wait for the BD CSampler to move to the Eject position and place a container under the SIP.
- Click on the *Backflush* button in the Run Backflush Cycle dialog box.



Figure 8-1. The Run Backflush Cycle Window

8.2 Cleaning the Flow Cell

Clean the flow cell as a part of regular maintenance or to correct performance issues of the cytometer.

8.2.1 Running the Unclog Cycle

The Unclog Cycle purges the flow cell of debris.

To purge the flow cell:

- Do one of the following:
  - Click on the *Unclog* button in the *Manual Collect* tab.
  - Select *Instrument* > *Run Unclog Cycle.*
- Wait for the BD CSampler to move to the Eject position and place a container under the SIP.
- Click on the *Unclog* button in the Run Unclog Cycle dialog box.

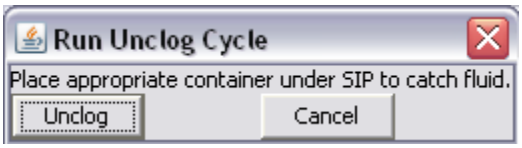


Figure 8-2. The Run Unclog Cycle Window

8.2.2 Running an Extended Clean of the Flow Cell

During the Extended Flow Cell Cleaning Cycle, the flow cell fills completely with the extended flow cell cleaning solution from a sample tube on the SIP. This cycle automatically shuts down the flow cytometer with the solution in the flow cell, allowing the flow cell to soak. It is recommended to perform this cleaning cycle monthly, or when a partial blockage of the SIP or flow cell is suspected.

.To run the Extended Clean of the Flow Cell Cycle:

- Place a tube with at least 500 µL of Extended Cell Flow Clean cleaning solution (PN 653159 —NOT cleaning solution, PN 653157) on the SIP.

  CAUTION: Never run the Extended Clean of the Flow Cell Cycle without a tube containing at least 500 µL of fluid.

- Select *Instrument* > *Extended clean of flow cell*.
- After the flow cytometer is shut down, leave the flow cytometer off for at least 30 minutes (up to overnight, for a more thorough cleaning).
- Restart the flow cytometer. The flow cytometer performs a longer fluidics startup cycle and BD CSampler Software displays the message *Extra startup time needed due to cleaning or improper shutdown*. This longer cycle purges cleaning solution from the flow cell and takes about 15 minutes to complete.
- Replace the tube containing cleaning solution with one containing 0.2 µm filtered deionized water, and run on Fast fluidics speed for at least 5 minutes to clear residual cleaner solution from the SIP and flow cell.
- Operate the flow cytometer as usual.

8.3 Cleaning the Fluidics Lines

The Cleaning Fluid Cycle pulls cleaner fluid from the cleaner tank and runs it through the fluidic lines. After filling the system with cleaner fluid, the cleaning fluid cycle purges the cytometer with fresh sheath fluid and performs a backflush. This cycle takes about five minutes.

To run a cleaning fluid cycle:

- Load a tube rack containing a tube of cleaning solution (PN 653157).
- Select *Instrument* > *Run cleaning fluid cycle*.

8.4 Decontaminating the Fluidics

BD CSampler Software automatically decontaminates the flow cytometer fluidics system at shut-down. The Decontamination Fluid Cycle can also be run manually at any time. During this process, the flow cytometer pulls decontamination fluid from the decontamination bottle then pulls sheath fluid from the sheath fluid bottle. Decontamination takes about 13 minutes.

To manually decontaminate the fluidics:

- Load a tube rack containing a tube of water.
- Select *Instrument* > *Run decontamination fluid cycle*.

8.5 Using the BD Accuri C6 Flow Cytometer for Precise Volume Measurements

Each BD Accuri C6 flow cytometer is calibrated at the factory for accurate volume measurement before shipping. Follow the guidelines below when accurate volume measurement is required during sample acquisition. For more detailed information on this subject, refer to the Technical Note, “A Guide to Absolute Counting” available at http://www.bdbiosciences.com/support/resources/accuri/index.jsp.

- Ensure that the peristaltic pump tubing and in-line sheath filter have been replaced within 60 days.
- Use 12x75 mm tubes (any type of plastic). Other tubes are not supported.
- Use sample volumes between 300 µL and 2 mL. Never acquire more than 750 µL from a single tube on *Medium* fluidics setting or 1500 µL on *Fast* fluidics setting.
- Adjust sample concentrations to fall approximately within the range of $1x10^3$ and 5×10^6 cells or particles per mL.
- Acquire data on *Medium* or *Fast* fluidics settings only. Custom fluidics settings above 15 µL and 16 µm may be used, but must be validated by an independent control/count bead.
- Only acquire once from any sample tube. Sample height within the tube is critical. Replicate measurements must be obtained from separate sample tubes made by aliquoting sample into the appropriate number of equal volumes.
- Always compare the same stop limit types. Do not compare concentrations collected with volume stop counts to event stop counts.
- Always validate accurate counting by using a reference count bead in the experimental buffer, using the same sample volume and tube as in the experiment. If bead counts are within 20% of the expected value (based on information provided by the bead manufacturer), proceed with sample collection. If bead counts are not within 20% of expected values, proceed with fluidics calibration as described below.
- Perform the following, in order:
  1. Ensure that the fluid levels in the Sheath, Cleaner and Decontamination bottles are sufficient to cover the inlet tubing and that there are no “kinked” fluidic lines.

2. Run a decontamination cycle from the Instrument Menu or by restarting the cytometer.
3. Within 5 minutes of completion of the decontamination cycle, place a 12x75 mm tube containing 750 µL of 70% ethanol on the SIP. Acquire 400 µL on *Fast* fluidics setting.
4. Within 5 minutes of completion of the ethanol run, place a 12x75 mm tube containing 1500 µL of filtered, deionized water on the SIP. Acquire 400 µL on *Fast* fluidics setting.
5. Within 5 minutes of completion of the water run, place a calibration sample on the SIP. Select Instrument > *Calibrate Fluidics*.
   - Calibration should be performed in the same tube as the experimental sample.
   - Calibration should be performed using a sample of the same or similar viscosity as the samples to be analyzed. For example, if lysed human peripheral blood samples are to be acquired, lysed human peripheral blood should be used during calibration.
   - The volume in the control sample tube should be 110 µL more than the volume used with subsequent test samples. For example, if using 1000 µL samples, perform calibration with 1110 µL in the tube. The calibration procedure consumes approximately 220 µL. The values determined by the BD Accuri C6 are based on the average sample height in the tube during the calibration.
   - If sample volumes >50 µL are to be acquired from the sample tube, the calibration volume should take this into account and the average volume in the sample tube during the acquisition should be used. For example, if 100 µL are to be acquired from a 1000 µL sample, the calibration volume would be 950 µL. The average volume [(Starting Volume + Ending Volume)/2] during the acquisition is [(1000+900)/2].
6. The cytometer will perform a calibration cycle, taking approximately 13 minutes. Once completed, the traffic light will revert to green with the *C6 is connected and ready* status message. Repeat fluidics validation using a reference count bead as described above.

   If calibration fails, BD CSampler Software will display a message that indicates that instrument calibration has failed.

   Troubleshooting Instrument Calibration:

   - Make sure that the calibration tube did not run dry during calibration.
   - Repeat calibration preparation routine, including running the decontamination cycle, 70% ethanol and water.
   - Repeat the calibration with a new calibration sample.
   - If calibration fails a second time, replace the peristaltic pump tubing in the cytometer and repeat the preparation/calibration routine.
   - If calibration fails a third time, contact Accuri Technical Support.
7. The cytometer will still operate normally after a failed calibration. However, the volume measurements for the desired samples may be incorrect because the cytometer will revert to the factory-set, default fluidics calibration settings. All other aspects of the data will be normal.

8.6 Technical Support

For Technical Support, contact:

- **In the USA:**
  Accuri Cytometers, Inc.
  173 Parkland Plaza
  Ann Arbor, MI 48103 USA
  Phone: +1.734.994.8000
  Fax: +1.734.994.8002
  Customer Support: CustomerService@AccuriCytometers.com
  Technical Support: TechSupport@AccuriCytometers.com

- **Europe, Middle East and Africa**
  BD Biosciences
  Erembodegem-Dorp 86
  9320 Erembodegem
  Belgium
  Phone: +32 53 720 882
  Customer Support: bd_accuri@europe.bd.com
  Technical Support: techsupport@europe.bd.com

- **Canada**
  BD Biosciences
  2100 Derry Rd West, Suite 100
  Mississauga, Ontario
  Canada L5N 0B3
  Email: canada@bd.com

  Direct Phone Numbers

  Austria: 0810 101 807
  Belgium: 078 16 60 50
  Canada: 1.800.268.5430
  Denmark: 8025 0622
  Finland: 0800 915582
  France: 0811 290 069
  Germany: 0180 100 1732
  Ireland: 1850 930 396
  Italy: 840 999 926
  Netherlands: 0900 0400 142
  Norway: 800 17 382
  Poland: 00800 121 4744
  Portugal: 800 855 717
  Spain: 901 988 155
  Sweden: 0200 883 736
  Switzerland: 0842 110 022
  UK: 0845 080 4769
  Other: +32 53 720 882

9 ADJUSTING PEAK POSITION WITH VIRTUALGAIN™

In certain instances, a particular peak should have the same position across different samples or be located at a specific channel number, regardless of the staining. Instruments that have voltage and amp gain controls allow peak position adjustments from sample to sample. BD CSampler Software uses VirtualGain instead of these controls.

VirtualGain is a software module that mimics voltage and amp gain adjustments to reposition data on the axis after the data has been collected. VirtualGain makes gross adjustments (approximate visual shifts of the data) of histogram plots. It is strictly an analysis tool and should not be used while collecting data.

For example, in Figure 9-1 the negative peaks in the control sample and in sample 1 fall in similar channels (mean value = 28.2 and 29.7, respectively). However, the negative population in sample 2 is farther to the right (mean value = 73.4). VirtualGain can be used to align the negative peak of sample 2 with the control sample.

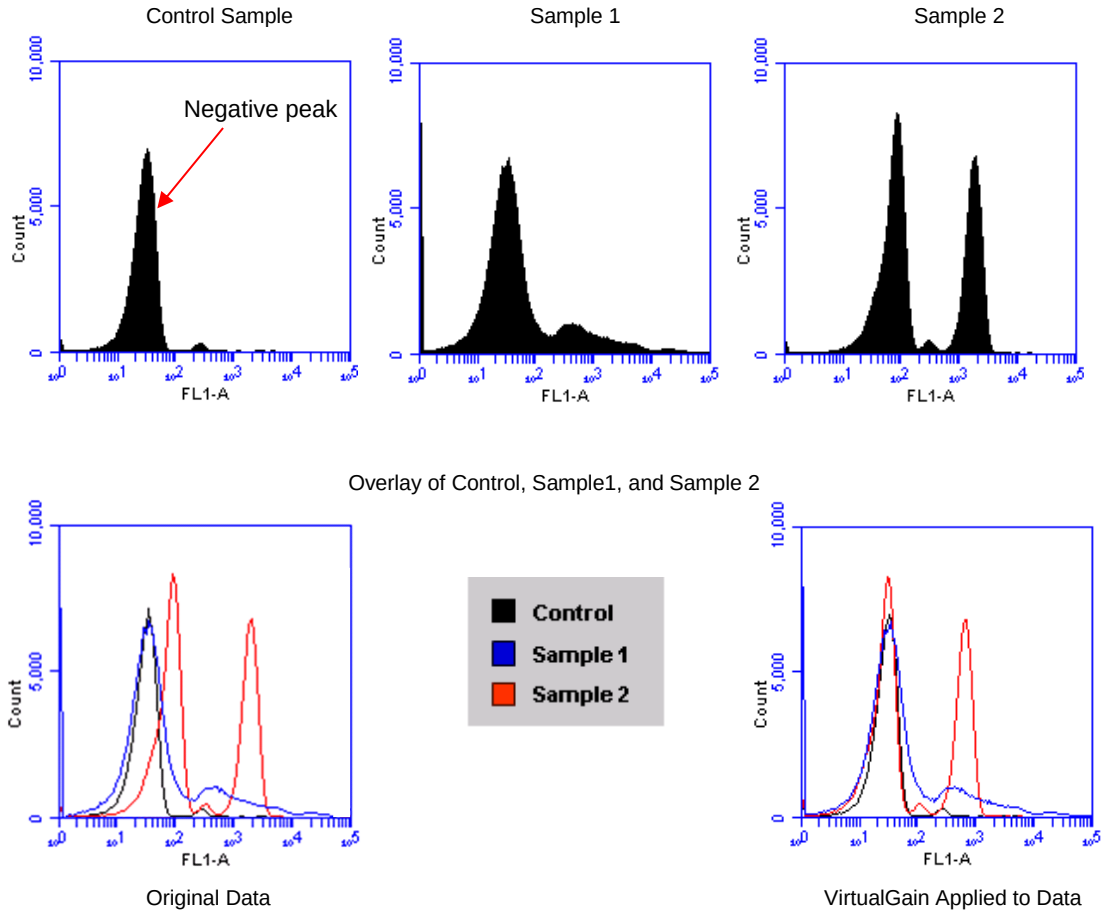


Figure 9-1. Before and After Applying VirtualGain

9.1 Applying VirtualGain

Only apply VirtualGain on a histogram plot and only on one parameter at a time. After VirtualGain is applied, data can be viewed in any type of plot and VirtualGain can be toggled on and off.

VirtualGain is only applied to the displayed data and does not alter FCS data. The adjustment is recorded only in the BD CSampler Software file.

To apply VirtualGain:

- In the *Analyze* tab, do one of the following:
  - Recreate the histogram of interest (see section 6.2 for details).
  - Copy plots from the *Manual Collect* tab (see section 6.2 for details).

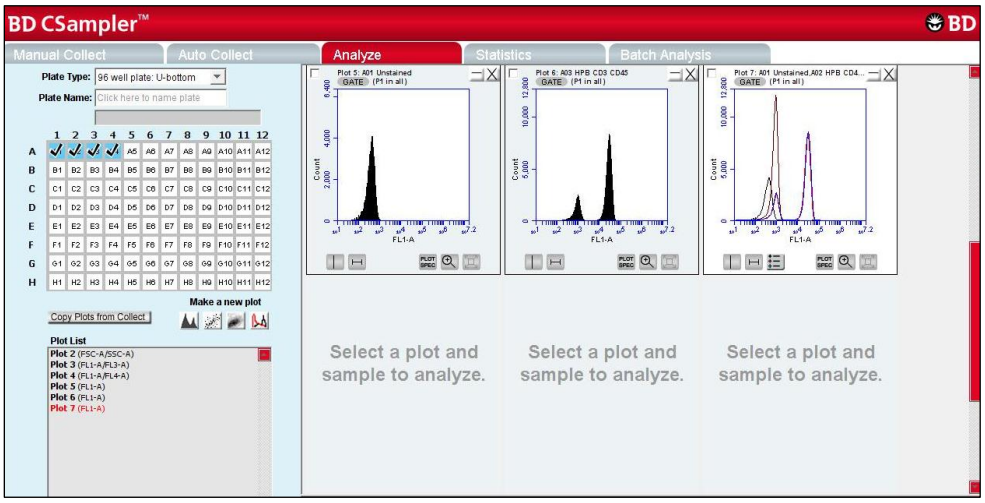


Figure 9-2. Setting Up Histograms for VirtualGain

- Apply the appropriate gating to the plots in the *Analyze* tab.
- Do one of the following:
  - Select a histogram plot from the sample to which the other samples will be aligned. This sample is the *standard sample.*
  - Select an empty well to align data to a *specific channel* instead of a collected sample.
- Click on the x-axis label on the standard sample and select *VirtualGain* from the pop-up Parameter List.
- In the VirtualGain dialog box, do one of the following:
  - Move the peak definition marker (vertical line) in the Standard Sample plot to the center of the peak that will be the reference point. Other samples will be aligned to this position.
  - If an empty well was selected in step 3, move the peak definition marker (vertical line) to the channel to assign as the reference point.

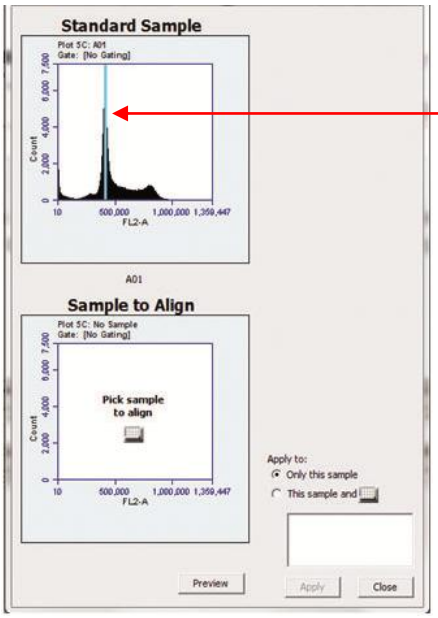


Figure 9-3. Aligning Plots

- If needed, use the Zoom Tools in the *Analyze* tab to change the zoom level in the VirtualGain dialog box.
- Click on the small sample grid icon in the center of the Sample to Align plot.
- Open the sample to be aligned by clicking on the corresponding blue well in the pop-up sample grid (the gray well indicates the standard sample currently selected). Ensure that this plot has been zoomed to the required level before setting VirtualGain.

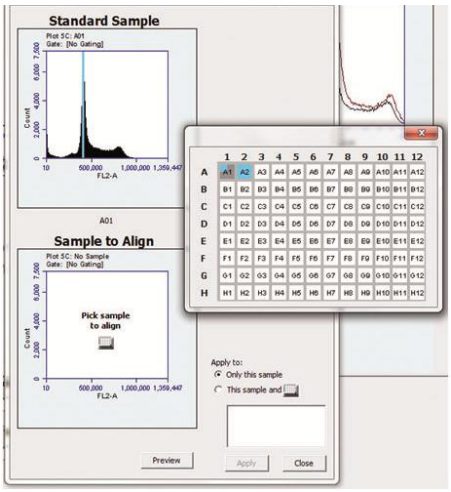


Figure 9-4. Pick the Sample to Align

- Move the peak definition marker in the Sample to Align plot to the center of the peak to align.

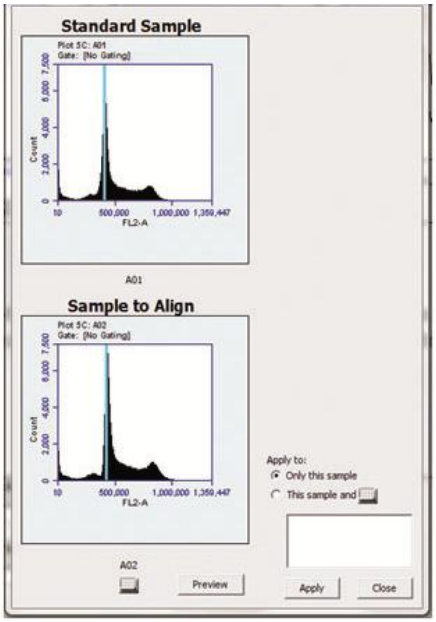


Figure 9-5. Move the Peak Definition Marker

- Click on the *Preview* button to view the aligned sample with VirtualGain applied. BD CSampler Software aligns the peak of interest in both plots.
- Repeat steps 9-10 to make additional adjustments, if needed.
- To align additional samples exactly as the first aligned sample, select the *This sample and* radio button and click on the well(s) in the pop-up sample grid to be included. If the other samples need a different amount of VirtualGain, set VirtualGain separately for each sample.
- Click on the *Apply* button to apply VirtualGain to the data. BD CSampler Software displays a black asterisk under the Sample to Align plot to indicate that VirtualGain has been applied to the specified parameter for that sample.

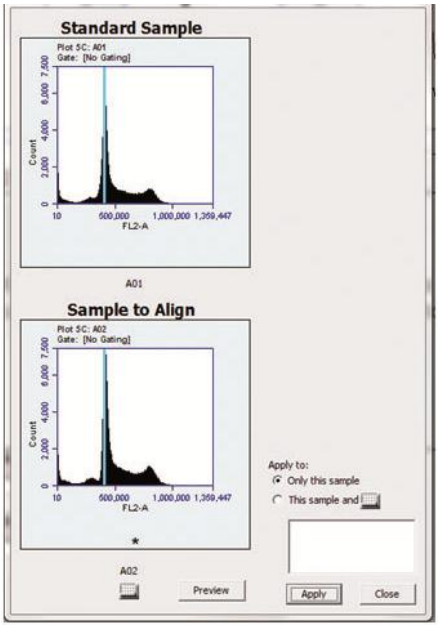


Figure 9-6. Black Asterisk Identifier

- Click on the *Close* button to close the VirtualGain dialog box.

9.2 Viewing VirtualGain

When VirtualGain is applied to a sample, BD CSampler Software displays a black asterisk under the parameter label in the associated plot.

The asterisk is color-coded:

- Black—VirtualGain has been applied.
- Gray—BD CSampler Software is currently displaying the original data.

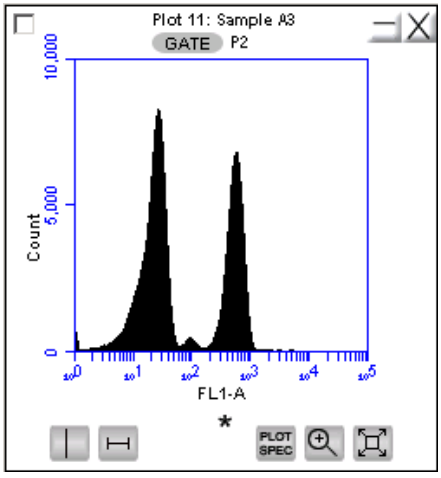


Figure 9-7. Plot with Black Asterisk

Overlays automatically display VirtualGain when it is applied (Figure 9-8). The asterisk is not displayed in overlays when VirtualGain is applied to some or all of the samples in the overlay.

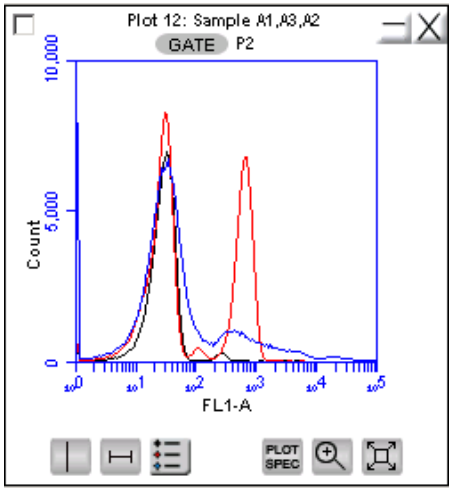


Figure 9-8. VirtualGain Applied in an Overlay Histogram

To toggle between views with VirtualGain applied and not applied:

- Click on the asterisk in the plot.

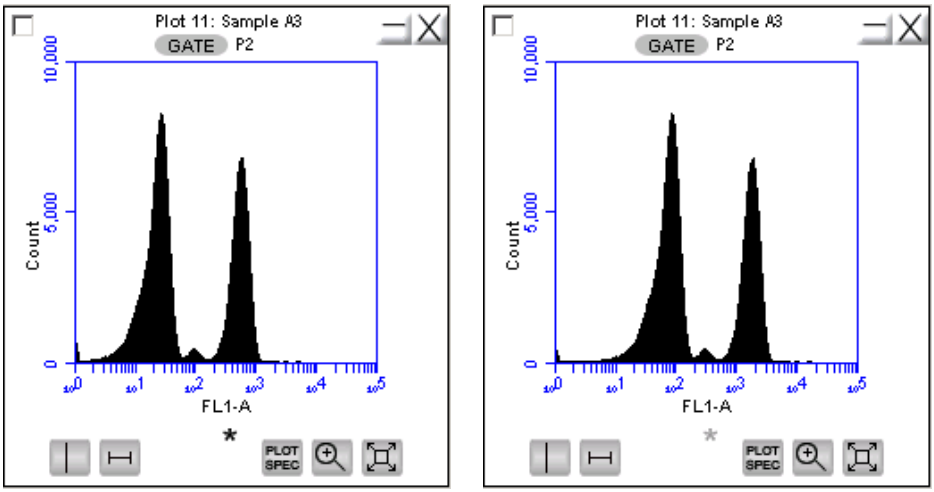


Figure 9-9. Toggle between VirtualGain Applied (Left) and Not Applied (Right)

9.3 Removing VirtualGain

To permanently remove VirtualGain from every parameter in every sample in the BD CSampler Software file:

- Select *Display* > *Remove All VirtualGain*.

CAUTION: This is a permanent action: Undo cannot be applied.

APPENDIX A BD CSAMPLER SOFTWARE MENU QUICK REFERENCE

The following table provides a description of all menu options in BD CSampler Software**Table A-1.** BD CSampler Software Menu Options

Menu Item	*Menu pick*	*Description*
File	Open BD CSampler Software File or Template	Opens a previously saved BD CSampler Software file or BD CSampler Software template. Only one BD CSampler Software workspace can be open at a time.
	New BD CSampler Software File	Opens a new, blank BD CSampler Software workspace. Replaces any previously open workspace.
	Save	Saves the open BD CSampler Software workspace under the current name. If the file has not already been named, BD CSampler Software prompts the user to name the file.
	Save BD CSampler Software File as	Saves the open BD CSampler Software workspace under a new name.
	Save BD CSampler Software Template as	Creates a template from the currently open BD CSampler Software workspace. All markers, regions, gates, parameter names, and sample names are saved without any data points.
	Auto-save Settings	Allows auto-save feature to be enabled or disabled.
	Import FCS File	Imports an FCS file previously exported from another BD CSampler Software file to the currently open workspace. Only FCS files created on a BD Accuri C6 flow cytometer can be imported into BD CSampler Software.
	Export FCS File	Exports and saves the currently selected data well as an FCS 3.0 file to a specified folder. Exported files are compatible with off-line analysis programs such as FCS Express, FlowJo, and WinList.
	Export ALL Samples as FCS	Saves all of the data wells as individual FCS 3.0 files in the folder *FCS Exports* on the computer desktop. Exported files are compatible with off-line analysis programs such as FCS Express, FlowJo, and WinList.
	Export ALL Samples to Third Party	Exports and saves all data wells as individual FCS 3.0 files that enable autoscaling in third party applications (such as FlowJo).
	Export Plot Data as CSV	Saves an individual file in .csv format for further analysis in spreadsheet programs. All data for every event in the selected plot is exported. See APPENDIX H for an example .csv file.

Menu Item	*Menu pick*	*Description*
File (cont.)	Export Sample Settings	Exports sample settings as a .csv file for viewing data in a spreadsheet. Sample settings include acquisition criteria, sample names, parameter names, and compensation values.
	Set Plot Drag and Drop Format	Available with Enhanced Analysis Features. Allows plot format to be toggled between .png (low resolution) or .eps (high resolution).
	Print Selected Items	Prints the selected plots and associated statistics.
	Quit	Quits BD CSampler Software and closes the application.
Edit	Undo	Undoes the last action that was performed in BD CSampler Software. Not all actions are undoable.
	Redo	Reverses an *Undo* action.
	Copy	Copies a selected marker or region from a plot, or selected statistics from the tables in BD CSampler Software.
	Paste	Pastes copied markers and regions into new plots.
	Rename Parameters	Allows individual parameters in either the current sample or all samples to be renamed.
Display	Events Display Settings	Opens a dialog to change the number of events displayed in all plots.
	Auto-Select next well	Opens a dialog to configure whether BD CSampler Software automatically selects wells vertically, horizontally, or not at all at the completion of each sample collection.
	Remove All VirtualGain	Removes all VirtualGain settings from the entire BD CSampler workspace.
	Hide/Show Median Statistics	Hides/shows the median statistics in the Statistics Table.
Instrument	Set threshold	Opens the Threshold dialog box for setting the primary threshold value, and setting an optional secondary threshold.
	Set Compensation	Opens the Compensation Settings dialog box for correcting fluorescence spillover.
	Run Cleaning Fluid Cycle	Runs a cleaning fluid cycle.
	Run Decontamination Fluid Cycle	Runs decontamination fluid cycle.
	Run Unclog Cycle	Runs the unclog cycle to clean the flow cell.
	Run Backflush Cycle	Runs the backflush cycle to clean the SIP and remove clogs at the base of the SIP.
	Extended Clean of Flow Cell	Cleans the flow cell for an extended time.
	Align CSampler	Aligns the BD CSampler arm to the SIP.
	Calibrate Fluidics	Initiates fluidics calibration to ensure that the BD Accuri C6 provides accurate volume measurement
	Update Firmware	Updates the flow cytometer firmware. Use only when directed by BD Accuri to upgrade the firmware with an official firmware release.

Menu Item	*Menu pick*	*Description*
Instrument (cont.)	Remote Control	Optional feature that allows control of the flow cytometer from a remote location.
About	About BD CSampler Software	Opens a dialog box that displays the version of BD CSampler Software and Accuri Technical Support contact information.
	Technical Support Information	Opens a dialog box that displays information about BD CSampler Software and the BD Accuri C6 flow cytometer. Each time an activation key is used to install a new BD CSampler Software component, the dialog box is updated to reflect the change.
	Users	Allows addition, deletion or modification of user accounts with the optional user tracking feature.

APPENDIX B ADVANCED FLUIDICS SETTINGS

In the Manual mode, advanced users can customize the fluidics rate and core size for collecting samples.

To customize the fluidics rate:

- Select the *Custom* radio button in the Fluidics section of the *Manual Collect* tab.

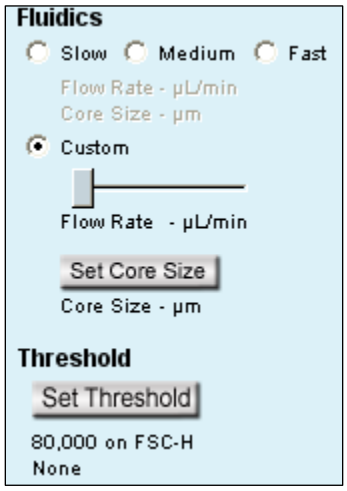


Figure B-1. Custom Radio Button

- Move the Custom slider to adjust the flow rate.

To customize the sample core size:

- Click on the *Set Core Size* button in the Fluidics section of the *Manual Collect* tab.
- Move the slider to adjust the core size.

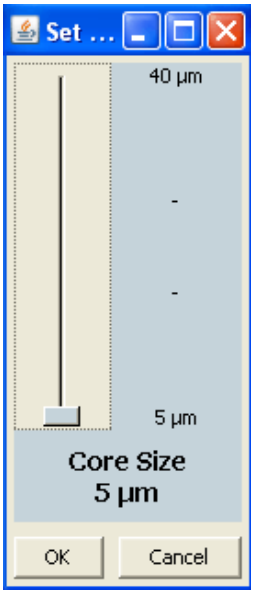


Figure B-2. Set Core Size

NOTE: Certain core sizes are not compatible with certain flow rates. BD CSampler Software does not allow these combinations to be set. Use the following table to determine allowable combinations.

Table B-1. Core Size and Flow Rate Guide

Core Size	*Min Flow Rate*	*Max Flow Rate*
5	10	11
6	10	16
7	10	22
8	10	29
9	10	36
10	10	45
11	10	54
12	10	65
13	10	76
14	12	88
15	14	100
16	15	100
17	17	100
18	19	100
19	22	100
20	24	100
21	26	100
22	29	100
23	32	100
24	35	100
25	38	100
26	41	100
27	44	100
28	47	100
29	50	100
30	54	100
31	58	100
32	61	100
33	65	100
34	69	100
35	74	100
36	78	100
37	82	100
38	87	100
39	91	100
40	96	100

- Click on the *OK* button to set the core size and close the slider.

APPENDIX C TRACKING USER ACTIVITY

User tracking allows laboratory administrators to track the activities of flow cytometer operators by assigning a user name and password to each individual. Passwords are created and used in BD CSampler Software and are unrelated to any Windows passwords used on the host computer or network.

User Tracking is an optional upgrade for BD CSampler Software and requires the use of BD CSampler Software version 227 or above.

C.1 Installing the User Tracking Module

- Ensure BD CSampler Software version 227 or above is loaded on the computer.
- Copy the User Tracking Activation Key from the installation CD to the computer desktop.
- Double-click on the User Tracking Installer icon on the desktop.



Figure C-1. User Tracking Installer Icon

- In the installation wizard, choose the directory in which to install the Activation Key. The location depends on where BD CSampler Software has been installed. In most cases, the location is in C:\Program Files\BD Accuri\BD CSampler\ActivationKeys

  If necessary, use the browse button ... to navigate to the correct folder.

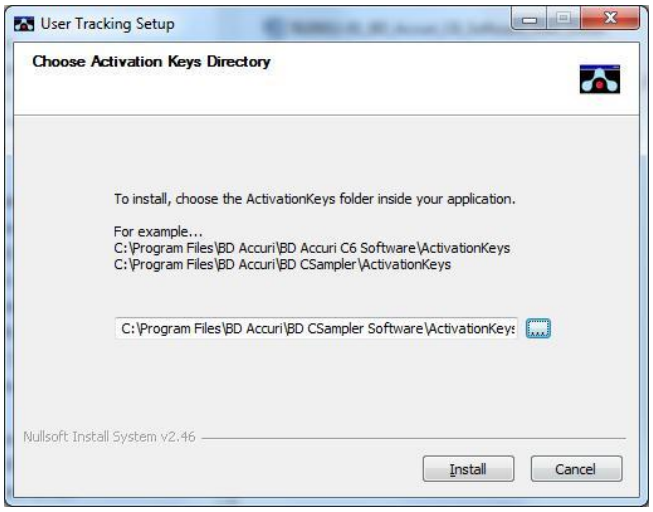


Figure C-2. Installation Dialog Box

- Click on the *Install* button. BD CSampler Software displays a confirmation message after successful installation.

C.2 Using the Tracking Feature for the First Time

To use the tracking feature for the first time:

- Open BD CSampler Software.
- When prompted, type *admin* in the *Username* text box.

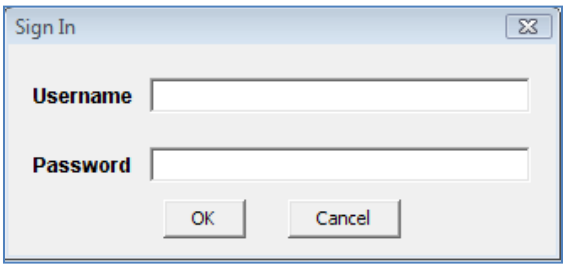


Figure C-3. Username and Password Dialog Box

- Type *Admin* in the *Password* text box (case sensitive). The Administrator password can be changed later.
- Click on the *OK* button
- Use BD CSampler Software as usual.

C.3 Adding, Deleting, and Modifying User Accounts

The administrator can add new users, delete existing users, and change passwords.

C.3.1 Adding User Accounts

- Sign in as the administrator.
- Select *About* > *Users* in BD CSampler Software.

  NOTE: The *Users* menu option is only visible to the administrator.
- In the Users dialog box, click on *Add New User.*

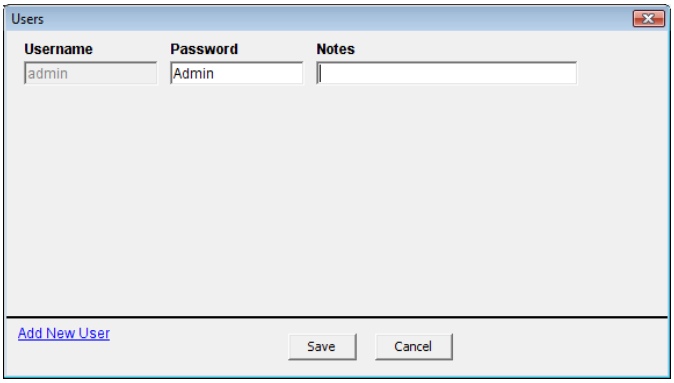


Figure C-4. The Users Information Box

- Type the *Username*, *Password* and *Notes* (optional) in the blank text boxes. Notes are only visible in the Users dialog box.

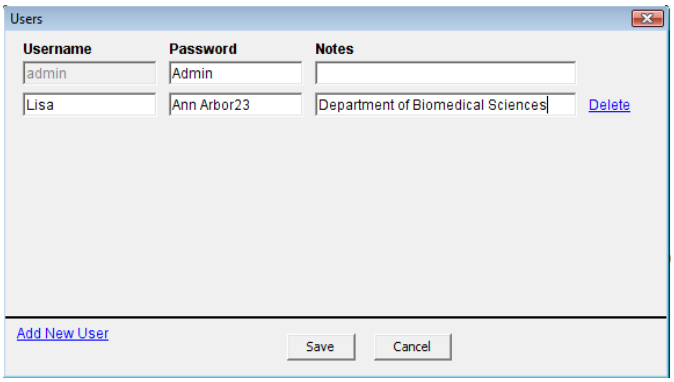


Figure C-5. Adding a New User

- Click on the *Save* button to save changes.

C.3.2 Deleting User Accounts

- Select *About* > *Users* in BD CSampler Software.
- Click on *Delete* next to the user account to be removed.

  NOTE: The administrator's account cannot be deleted.

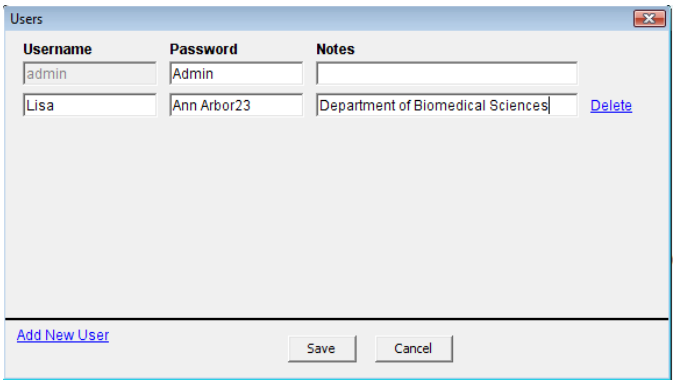


Figure C-6. Deleting a User

- Click on the *Save* button to save changes.

C.3.3 Changing a Password

The administrator can change a user's password at any time.

To change a user password:

- Select *About* > *Users* in BD CSampler Software.
- Delete the text in the *Password* field and type a new password.
- Click on the *Save* button.

C.4 Signing In and Signing Out

Once user tracking is set up, users must sign in for each session.

To sign in to BD CSampler Software:

- Open BD CSampler Software.
- When prompted, enter *Username* in the text box.

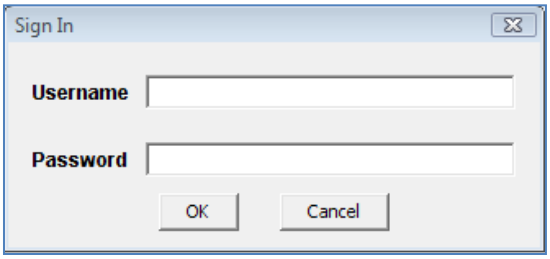


Figure C-7. Username and Password Dialog Box

- Enter *Password* in the text box.
- Click on the *OK* button
- Use BD CSampler Software as usual.

BD CSampler Software will continue to log time until the user signs out.

To sign out of BD CSampler Software:

- Select *File* > *Quit.*

C.5 Restoring a Forgotten Administrator Password

The password file is encrypted and contains all username and password information created in the User Logging feature. If the Administrator password is forgotten, the password file can be deleted and the Administrator account recreated.

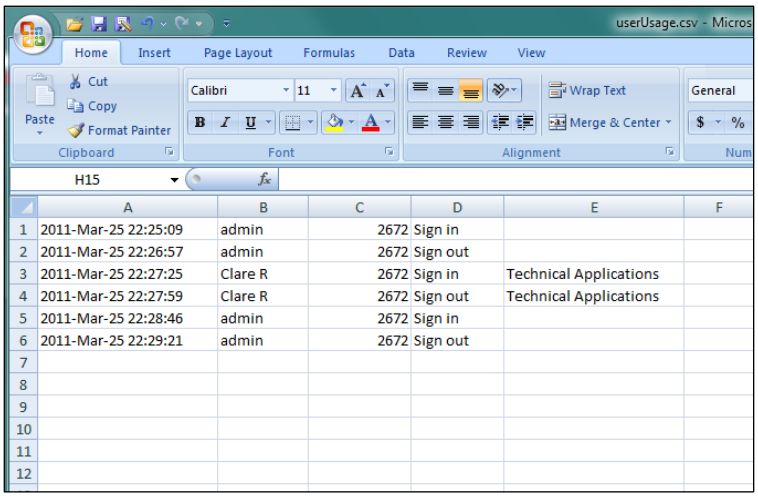
CAUTION: This procedure deletes *all* user names and passwords; each account must be manually recreated.

To restore the administrator password:

- If BD CSampler Software is running, shut down BD CSampler Software.
- Navigate to the CytometerSupportFiles folder (typically in the root directory of the BD CSampler Software computer).
- Locate the Password file and delete the file.
- Follow the procedures in sections C.2 and C.3.1.

C.6 Monitoring User Activity

Each time a user signs into or signs out of BD CSampler Software, an entry is made in the userUsage Log. The userUsage log is a .csv file.



	A	B	C	D	E	F
1	2011-Mar-25 22:25:09	admin	2672	Sign in		
2	2011-Mar-25 22:26:57	admin	2672	Sign out		
3	2011-Mar-25 22:27:25	Clare R	2672	Sign in	Technical Applications	
4	2011-Mar-25 22:27:59	Clare R	2672	Sign out	Technical Applications	
5	2011-Mar-25 22:28:46	admin	2672	Sign in		
6	2011-Mar-25 22:29:21	admin	2672	Sign out		
7						
8						
9						
10						
11						
12						

Figure C-8. userUsage Log

The userUsage Log contains the following information:

- Date of a sign in/sign out
- Time of a sign in/sign out
- Username of the operator
- Serial number of the BD Accuri C6
- Type of activity (sign in or sign out)

To view the userUsage log:

- Navigate to the CytometerSupportFiles folder (typically in the root directory of the BD CSampler Software computer) and open the folder.

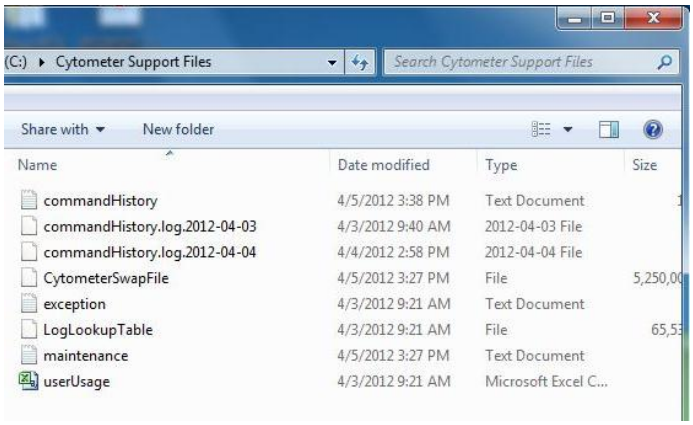


Figure C-9. CytometerSupportFiles Folder

- Open the userUsage.log file.

APPENDIX D BD ACCURI C6 ANALYSIS SOFTWARE

The optional BD Accuri C6 Analysis Software allows analysis of BD Accuri C6 Software files on a computer that does not have a connection to the BD Accuri C6 flow cytometer. BD Accuri C6 Analysis Software is available in two forms:

- PN 653122—BD Accuri C6 Analysis Software for PC or Mac
- PN 653123—BD CSampler Analysis Software for PC or Mac

BD CSampler Analysis Software has all the functionality of BD CSampler Software, with the exception of instrument control functions. Thresholds and compensation can still be set. BD C6Sampler Analysis Software is not intended for operating the flow cytometer.

MINIMUM SYSTEM REQUIREMENTS

- PC version:
  Intel Core Duo processor, 2.8GHz
  2GB RAM
  CD-ROM Drive
  Windows® XP (Service Pack 2), Windows Vista, or Windows 7 (recommended)
  Language selection: US or UK English
  5GB hard disk space
  Display resolution 1280 x 1024 or higher
- Mac version:
  Intel or PowerPC processor, 1.66 GHz (Intel processor recommended)
  1GB RAM
  CD-ROM Drive
  Mac OS X software
  Language selection: US or UK English
  5GB hard disk space
  Display resolution 1152 x 864 or higher (1280 x 1024 recommended)

SOFTWARE OPERATION

See the relevant chapters in this manual for operational information.

APPENDIX E SELECTABLE LASERS

The detectors and lasers of the BD Accuri C6 flow cytometer operate in a predefined configuration: detectors FL1, FL2, and FL3 read blue laser-excited fluorescence emissions and detector FL4 reads red laser-excited emissions. This configuration is referred to as *3 blue 1 red*.

The Selectable Lasers Module (PN 653126) allows operation of the flow cytometer in two alternate configurations which significantly expands the fluorochrome combinations that can be analyzed (see section E.4 for examples):

- *2 blue 2 red:*

  FL1 and FL2 read blue laser-excited emissions

  FL3 and FL4 read red laser-excited emissions
- *4 blue:* All 4 detectors read blue laser-excited emissions

Components Supplied:

- Selectable Lasers Activation Key (software)
- Three optical filters:
  - 780/60 BP (PN 653187)
  - 610/20 BP (PN 653186)
  - 630/30 BP (only available with Selectable Lasers Module)

NOTE: The Selectable Lasers upgrade requires prior installation of BD Accuri C6 Software or BD CSampler Software.

E.1 Installing the Selectable Lasers Module

- Verify that BD Accuri C6 Software or BD CSampler Software version 227.4 or above is loaded on the computer. The Selectable Lasers Module can also be installed on any computer where BD Accuri C6 Analysis Software (version 227.4 or higher) is installed.
- Copy the Selectable Lasers Activation Key from the installation CD (or flash drive) to the desktop.
- Double-click on Selectable Lasers Activation Key icon.



Figure E-1. Selectable Lasers Installer

- In the installation wizard, choose the directory in which to install the Activation Key. The location depends on where BD Accuri C6 Software or BD CSampler Software has been installed. In most cases, the location will be in one of the following places:
  - C:\Program Files\BD Accuri\BD Accuri C6Software\ActivationKeys
  - C:\Program Files\BD \CSampler\ActivationKeys

  NOTE: If BD Accuri C6 Software and BD CSampler Software are installed on the same computer, install the Selectable Lasers Activation Key twice: once in the BD Accuri C6 Software directory, and again in the BD CSampler Software directory.
- Click on the browse button [...] to navigate to the correct location.

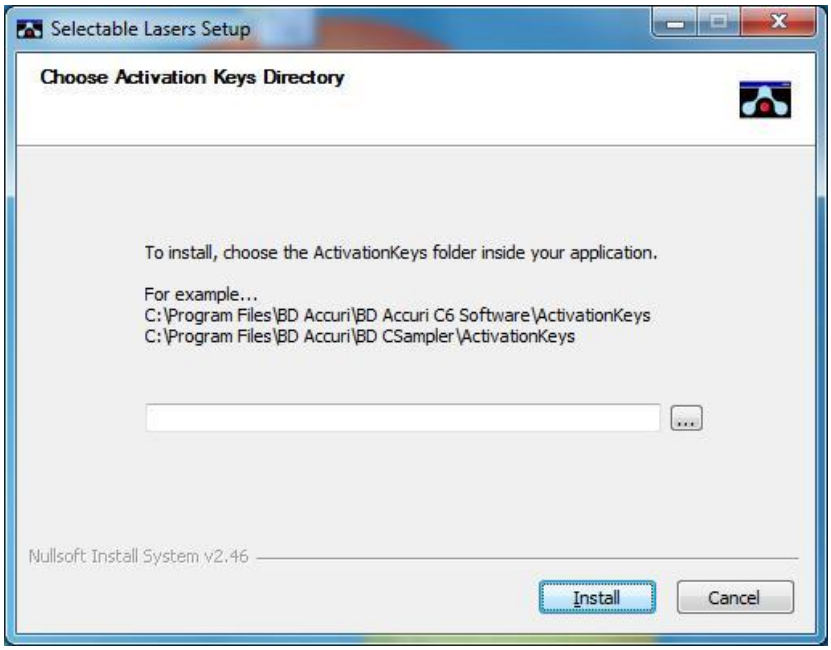


Figure E-2. Install Wizard: Choose Activation Keys Directory

- Click on the *Install* button.

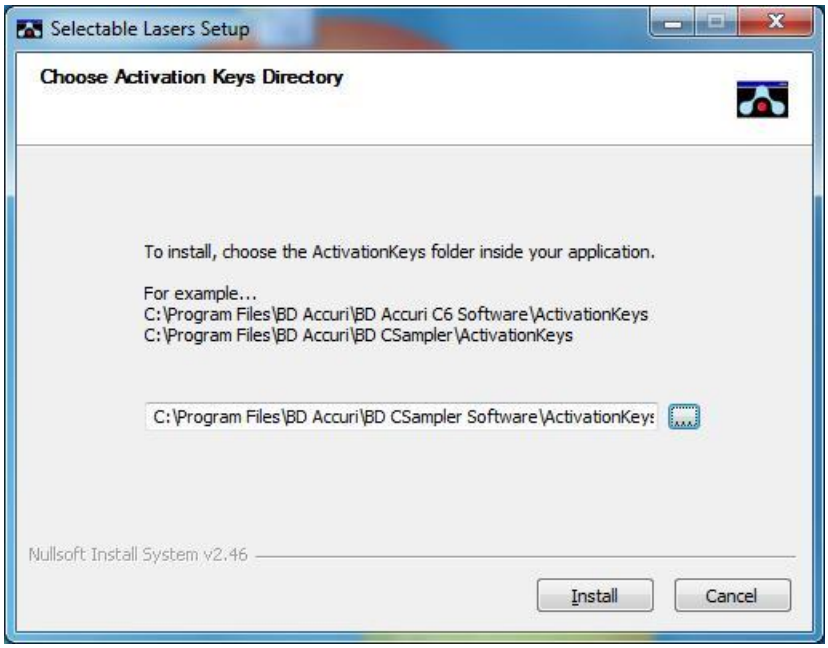


Figure E-3. Install Wizard: Install the Module

- After installation, click on the *Close* button.

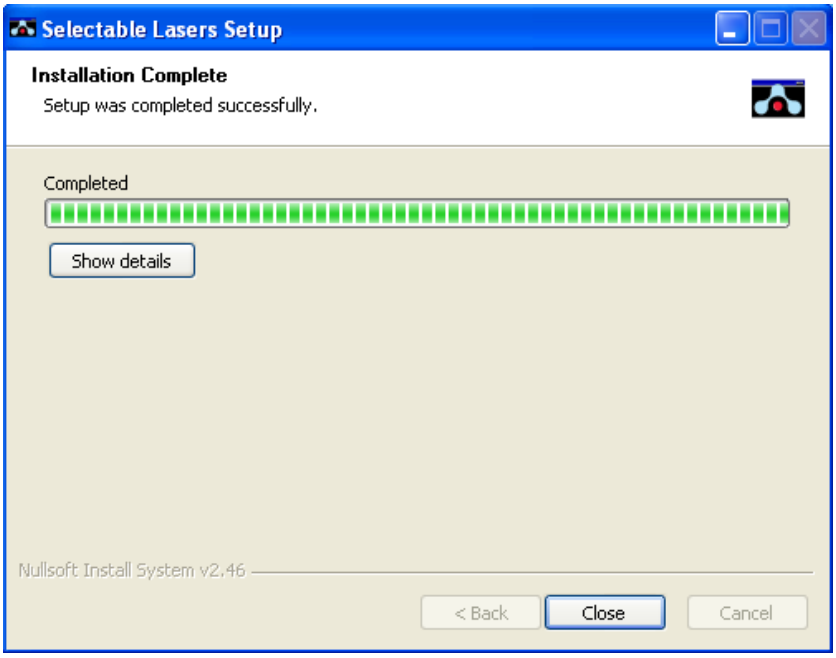


Figure E-4. Install Wizard: Setup Completed Successfully

- Open BD CSampler Software. The Selectable Lasers controls are displayed next to the BD CSampler Software traffic light message.

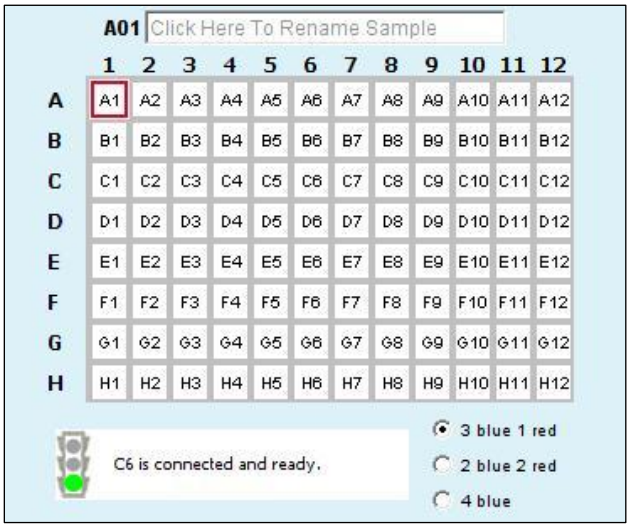


Figure E-5. Selectable Laser Controls in the BD CSampler Workspace

- Depending on the laser configuration to be used, the standard optical filters may need to be replaced with one or more of the filters that are supplied with the Selectable Lasers software. See section E.4 for details.

E.2 Validating Proper Function After Installation

After installing the Selectable Lasers Module, verify that the module is operating properly. To verify the Selectable Lasers function:

- Leave the standard optical filters in place.
- With BD CSampler Software open and the flow cytometer powered on, select the *3 blue 1 red* laser configuration.

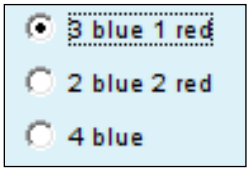


Figure E-6. Selectable Laser Options: *3 Blue 1 Red*

- Set the run limit to 30,000 events in all and set the fluidics rate to slow.
- Collect data files for the 8- and 6-Peak Validation Beads (PN 653144 and PN 653145).
- Confirm that the flow cytometer is operating within specification (see Chapter 3).

- Select the *2 blue 2 red* option and collect data using the 6-Peak Validation Beads (PN 653145) into a new data well.

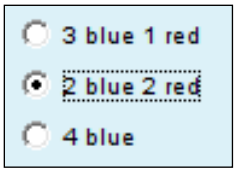


Figure E-7. Selectable Laser Options: *2 Blue 2 Red*

- Select the *4 blue* option and collect data using the 8-Peak Validation Beads (PN 653144) into a new data well.



Figure E-8. Selectable Laser Options: *4 Blue*

- Confirm proper operation of the *2 blue 2 red* configuration by comparing the 6-peak bead distributions for FL3 and FL4 to those obtained with the standard *3 blue 1 red* configuration. Results for the *2 blue 2 red* selection should show 6 peaks for both FL3 and FL4.

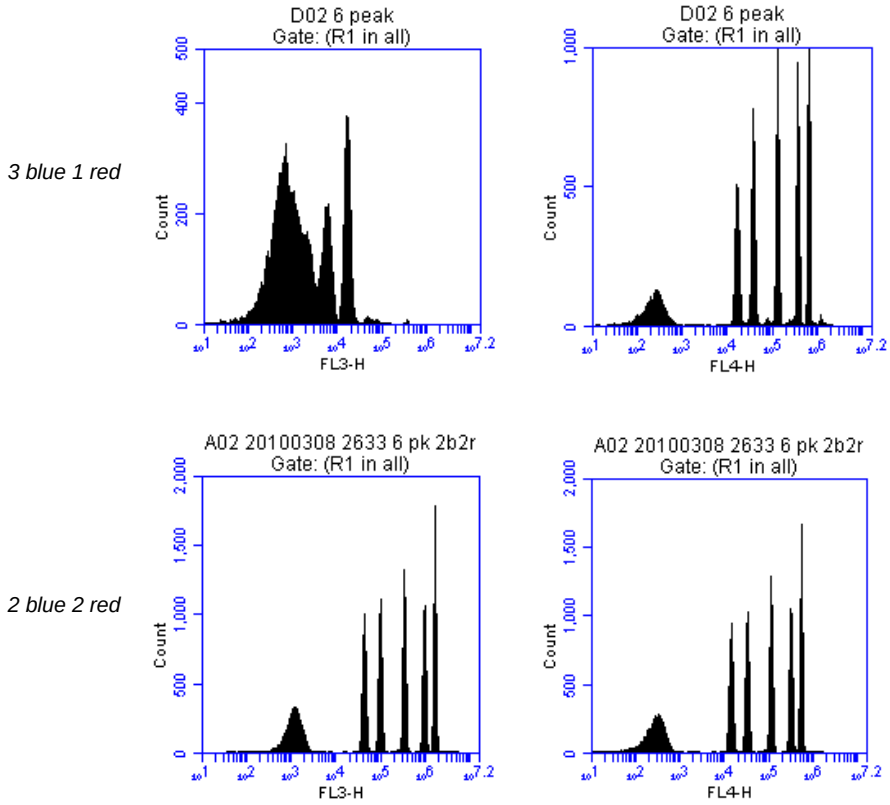


Figure E-9. Evaluation of 6-Peak Validation Beads for the *2 Blue 2 Red* Configuration

- Confirm proper operation of the *4 blue* configuration by comparing the 8-peak distributions for FL3 and FL4 to those obtained with the standard *3 blue 1 red* configuration. Results for the *4 blue* selection should show similar peak profiles for FL3 and FL4.

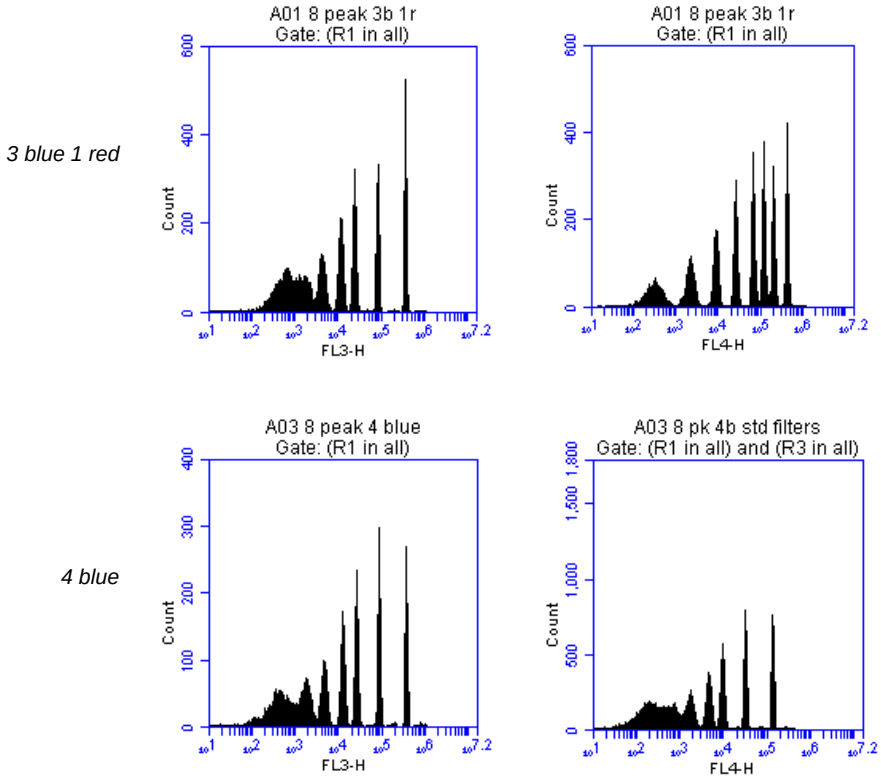


Figure E-10. Evaluation of 8-Peak Validation Beads for the *4 Blue* Configuration

E.3 Annotating Selected Laser Configuration

The selectable lasers radio buttons show the *most recently* selected configuration only. BD CSampler Software does not retain this setting for each well. Therefore, it is recommended to annotate the data wells in the naming field of BD CSampler Software to indicate the laser configuration used during data collection of each well, especially when using alternate configurations. For example, name a sample “HPB 4b” to indicate that the *4 blue* option was selected during data collection.

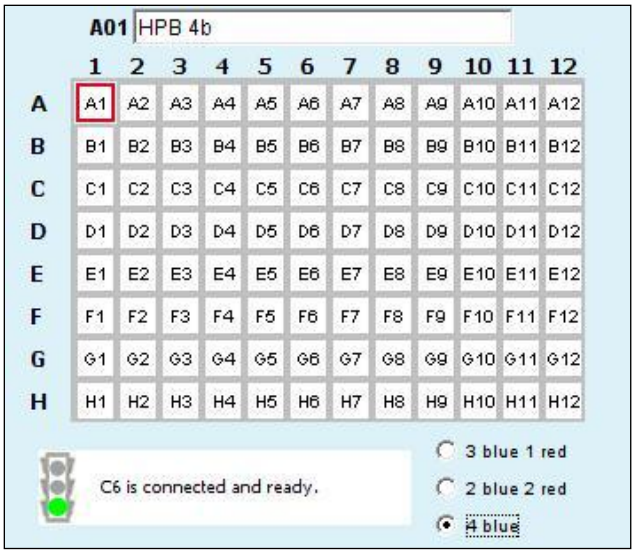


Figure E-11. Renamed Data Well to Indicate *4* Blue Configuration

Laser configurations are *not saved* in the BD CSampler Software data file or with a BD CSampler Software template. Any previously saved BD CSampler Software file will default back to the *3 blue 1 red* option when it is opened.

The last laser configuration used to collect a given well of data is written in the FCS file header using the custom keyword **#LASERCONFIGURATION.** FCS file headers can be viewed by opening an exported FCS file in a text editor (such as Microsoft® Notepad).

E.4 Optical Filter Placement

Due to the unique optical layout of the BD C6 flow cytometer, it is critical that any optional filters used with the Selectable Lasers Module are placed in the proper position for optimal performance.

WARNING: The 630/30 bandpass filter provided with the Selectable Lasers module should only be used when operating in the *4 blue* configuration. Using the 630/30 filter when operating in any other configuration may damage the corresponding detector due to unfiltered red laser signal.

Use the tables below as a guide to optical filter placement for various fluorochrome combinations:

Table E-1. *3 Blue 1 Red:* Configuration 1 (Standard Filters)

Detector	*Filter*	*Fluorochrome*
FL1	**530/30 or 533/30**	FITC, GFP, CFSE
FL2	**585/40**	PE, PI, PE-Texas Red®
FL3	**670 LP**	PerCP-Cy™5.5, PE-Cy5, PE-Cy7

FL4	**675/25**	APC, Alexa-647, PE-Cy5

Table E-2. *3 Blue 1 Red:* Configuration 2

Detector	*Filter*	*Fluorochrome*
FL1	**530/30 or 533/30**	FITC, GFP, CFSE
FL2	**585/40**	PE
FL3	**610/20**	PI, PE-Texas Red
FL4	**675/25**	APC, Alexa-647, PE-Cy5

NOTE: When operating in the *3 blue 1 red* configuration place either the 610/20 bandpass or the 670 LP filters in position FL3. For best results when analyzing PE and PE-Texas Red (PE-TR) simultaneously, select the filters with the following signal-intensity considerations in mind:

- PE-bright, PE-TR-moderate to bright: FL3 = 670 LP
- PE-dim to moderate, PE-TR any level: FL3 = 610/20
- PE-bright, PE-TR dim: may be difficult to separate; consider using the *4 blue* configuration with a 630/30 in FL4 to detect PE-TR.

Table E-3. *2 Blue 2 Red* Configuration

Detector	*Filter*	*Fluorochrome*
FL1	530/30 or 533/30	FITC, GFP, CFSE
FL2	585/40	PE, PI, PE-Texas Red
FL3	780/60	APC-Cy7 (and equivalents)
FL4	675/25	APC (and equivalents)

Table E-4. *4 Blue:* Configuration 1

Detector	*Filter*	*Fluorochrome*
FL1	530/30 or 533/30	FITC, GFP, CFSE
FL2	585/40	PE, PI, PE-Texas Red
FL3	780/60	PE-Cy7 (and equivalents)
FL4	675/25	PerCP-Cy5.5, PE-Cy5

Table E-5. *4 Blue:* Configuration 2

Detector	*Filter*	*Fluorochrome*
FL1	530/30 or 533/30	FITC, GFP, CFSE
FL2	585/40	PE
FL3	780/60	PE-Cy7 (and equivalents)
FL4	610/20	PI
	630/30	PE-Texas Red

Table E-6. *4 Blue:* Configuration 3

Detector	*Filter*	*Fluorochrome*
FL1	530/30 or 533/30	FITC, GFP, CFSE
FL2	585/40	PE
FL3	675/25	PerCP-Cy5.5, PE-Cy5
FL4	610/20	PI
	630/30	PE-Texas Red

E.5 Selectable Laser Application Examples

E.5.1 *2 Blue 2 Red* Configuration Examples

Table E-7. Detector and Filter Configuration for *2 Blue 2 Red*, Examples 1 and 2

Detector	*Filter*	*Fluorochrome*
FL1	530/30 or 533/30	FITC
FL2	585/40	PE
FL3	780/60	APC-Cy7
FL4	675/25	APC

Example 1: *2 Blue 2 Red* for Simultaneous FITC, PE, APC and APC-Cy7

The following images illustrate a 4-color analysis of $CD3^+CD4^+$ cells. Human peripheral blood (HPB) was stained with CD3-APC-Cy7, CD4-APC, CD45RA-FITC, and CD45RO-PE.

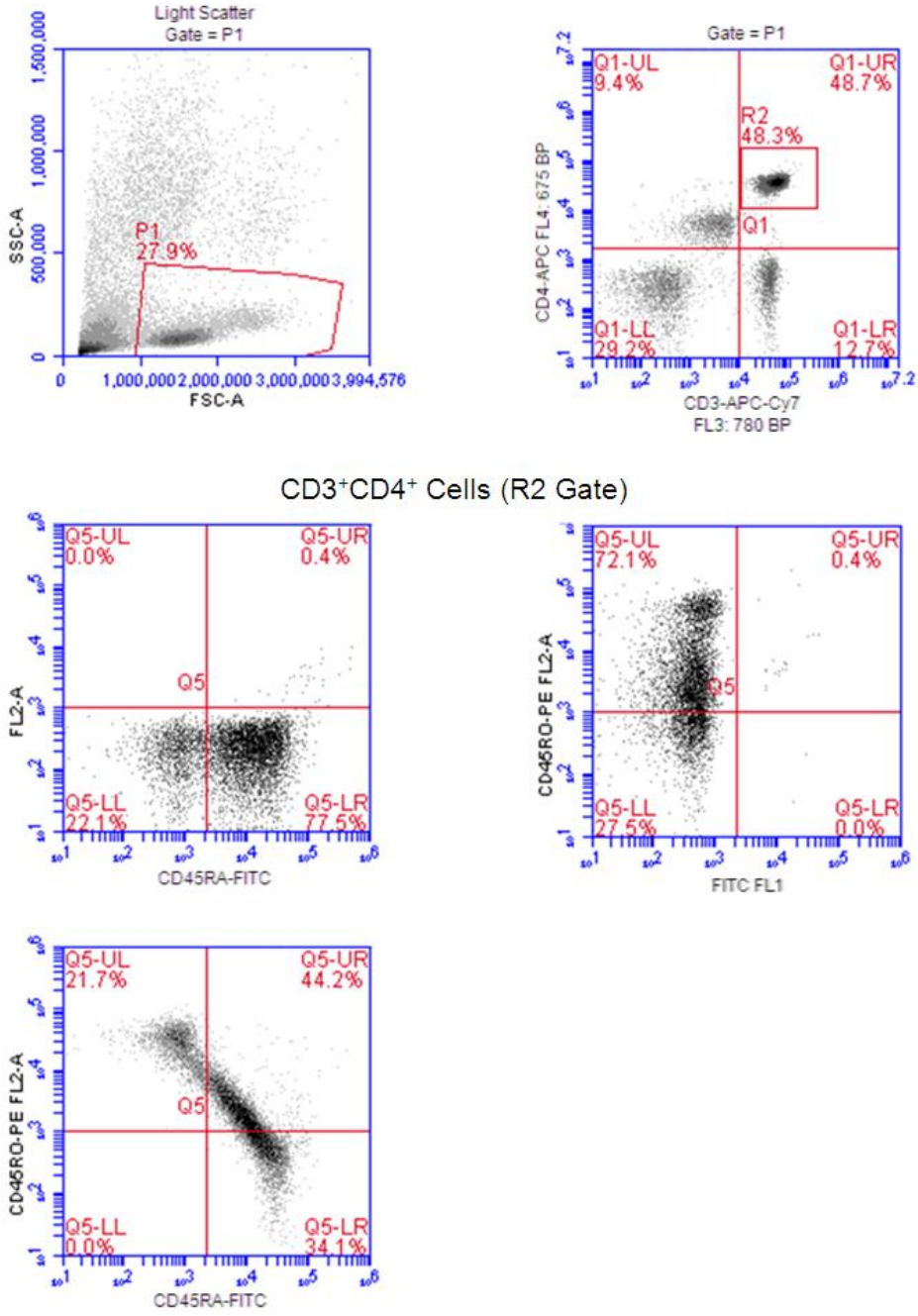
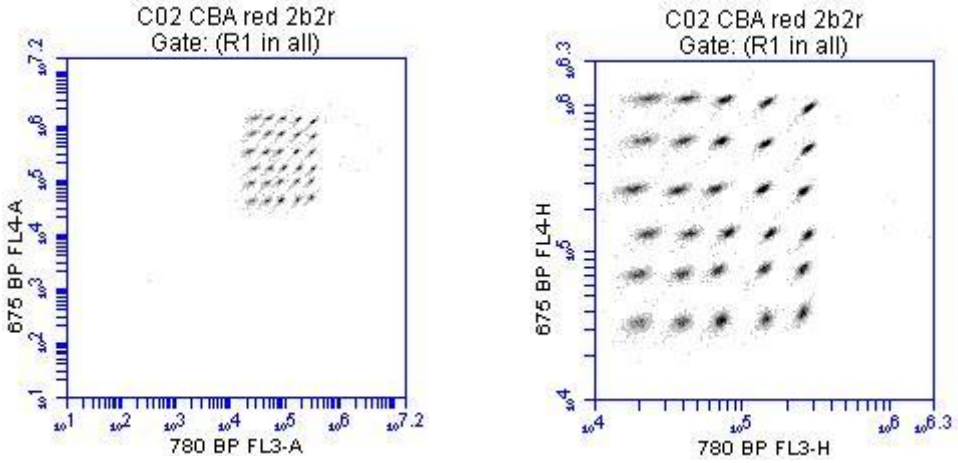


Figure E-12. Gating Example Using the *2 Blue 2 Red* Configuration

Example 2: *2 Blue 2 Red* with BD Cytometric Cytokine Bead Array (CBA) 30 Plex Bead Mixture (BD 51-9004679)

The following images show the results for bead separation using the entire 30 plex bead mixture



Unzoomed View, Area

Zoomed View, Height

Figure E-13. BD Cytometric CBA 30 Plex Bead Mixture collected on the BD Accuri C6 run in “2 Blue 2 Red” mode using the Selectable Lasers Module (PN 653126).

E.5.2 *4 Blue* Configuration Examples

Example 1: *4 Blue* FITC, PE, PE-Cy5, PE-Cy7: Human Peripheral Blood Subsets

Table E-8. Detector and Filter Configuration for Selectable Lasers *4 Blue* Example 1

Detector	*Filter*	*Fluorochrome*
FL1	530/30 or 533/30	FITC
FL2	585/40	PE
FL3	780/60	PE-Cy7
FL4	675/25	PE-Cy5

The following images show CD45-FITC vs. SSC gating of lymphocytes (zoomed in, left; zoomed out, right). HPB was stained with CD45-FITC, CD8-PE, CD3-PE-Cy5, and CD4-PE-Cy7.

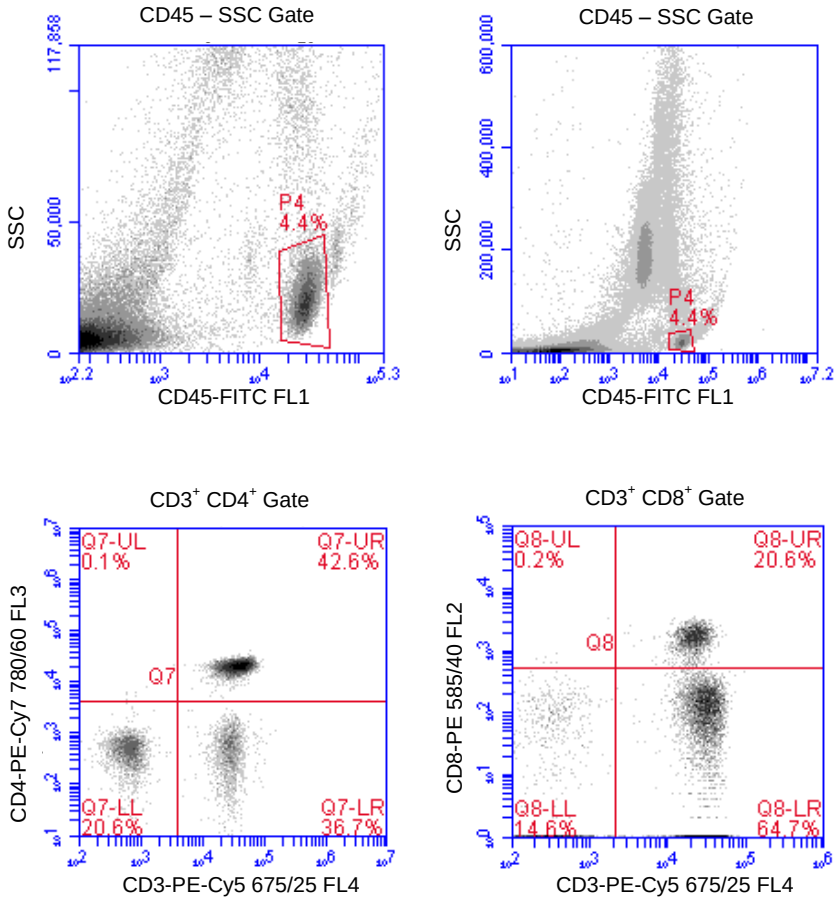


Figure E-14. *4 Blue* Configuration and the Optional 780/60 Optical Filter at Detector FL3

Example 2: *4 Blue* FITC, PE, PE-Cy5, PE-Texas Red: Human Peripheral Blood Subsets

Table E-9. Detector and Filter Configuration for Selectable Lasers *4 Blue,* Example 2

Detector	*Filter*	*Fluorochrome*
FL1	**530/30 or 533/30**	FITC
FL2	**585/40**	PE
FL3	**675/25**	PE-Cy5
FL4	**630/30**	PE-Texas Red

The following images show the results for example 2. The color compensation value to correct the spillover of PE (FL2) into the PE-Texas Red (FL4) detector may be in the range of 70% to 90%.

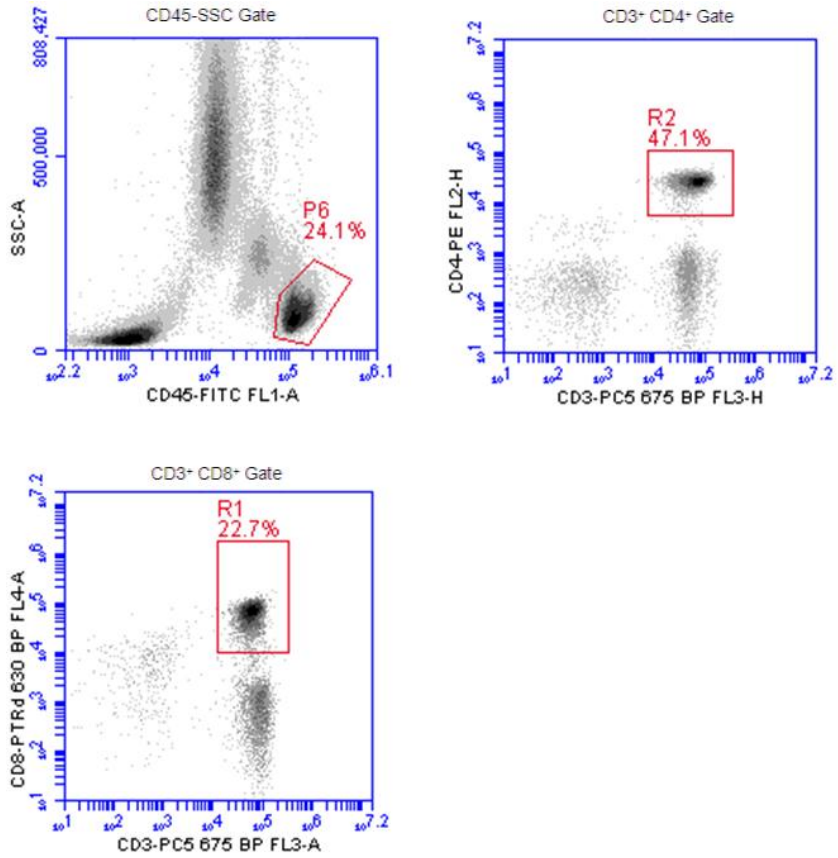


Figure E-15. HPB Stained with CD45-FITC, CD4-PE, CD8-PE-Texas Red and CD3-PE-Cy5

APPENDIX F ENHANCED ANALYSIS FEATURES

The Enhanced Analysis module includes several advanced functions in BD CSampler Software:

- Live (or “dump”) gating
- Plot and region renaming
- Event coloring in a region
- Vector scalable graphics
- Batch analysis

NOTE: Enhanced Analysis is compatible with BD Accuri C6 Software and BD CSampler Software version 264 and higher.

F.1 Creating a Live Gate

Live gating, also called dump gating, is used to exclude events outside a designated region from being displayed and stored as part of the c6 or fcs file. Any region may be used as a live gate. This selection can be remembered as part of the template if desired.

CAUTION: Once a live gate is executed, there is no way of recapturing any excluded data.

To create a live gate:

- Create the region to use in the live gate in the *Collect* tab.
- Click on the *Do not collect events outside* check box and select the region from the drop-down list, located under *Run Settings* in the *Collect* tab.

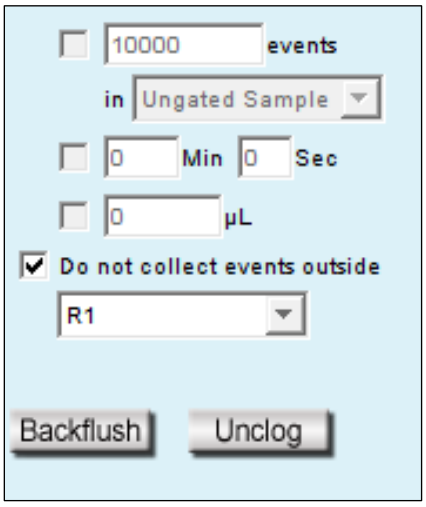


Figure F-4. Creating a Live Gate

F.2 Renaming Plots and Regions

Plots or regions in a plot can be renamed. In each case the method is the same.

To rename a plot or region:

- Double-click on the plot or region name.



Figure F-5. Enable Renaming of the Plot

- In the text field, type in a new name.

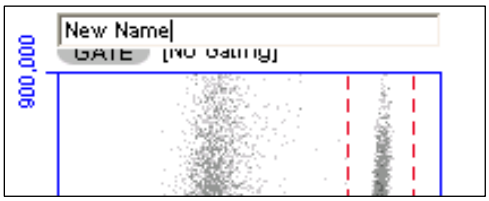


Figure F-6. Type a New Plot Name

- Press **Enter.**

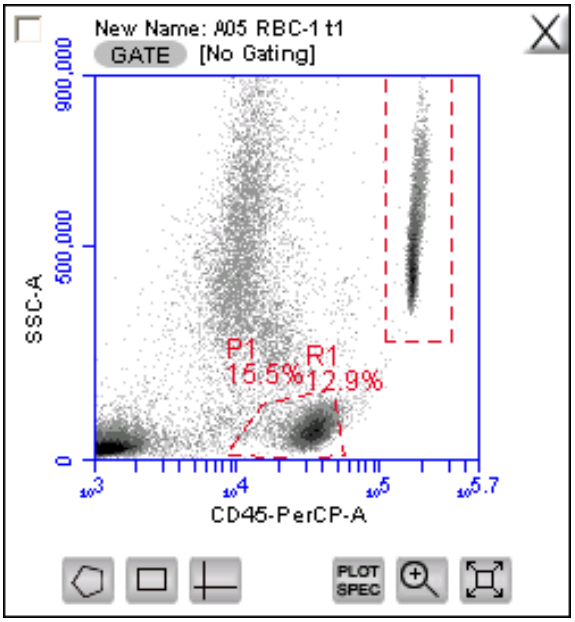


Figure F-7. New Plot Name

F.3 Coloring Events in a Region

All events within one or more regions can be designated to appear as a specified color within other histogram or dot plots. The event coloring will remain and automatically update during data acquisition.

NOTE: Color will not appear in a density plot.

To color events:

- Create a region in a histogram, dot or density plot.
- Double-click on the region name and click on the small white square to display a color palette. The most commonly used colors appear on the top row.

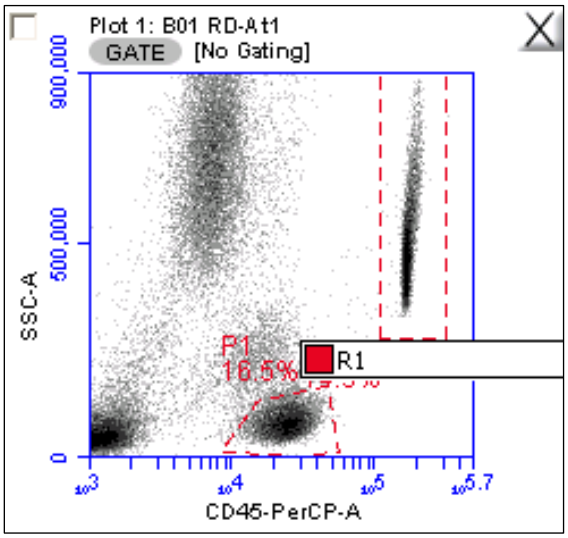


Figure F-8. Select a Region to Color

- Select a color.

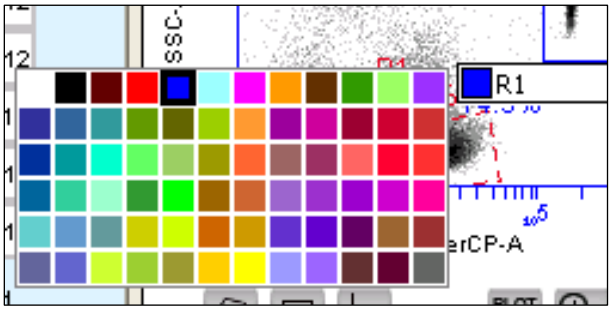


Figure F-9. Select a Color for a Region

- Click outside the region to make the change take effect. Events that fall within the region in other dot and histogram plots are now colored and displayed in the same color.

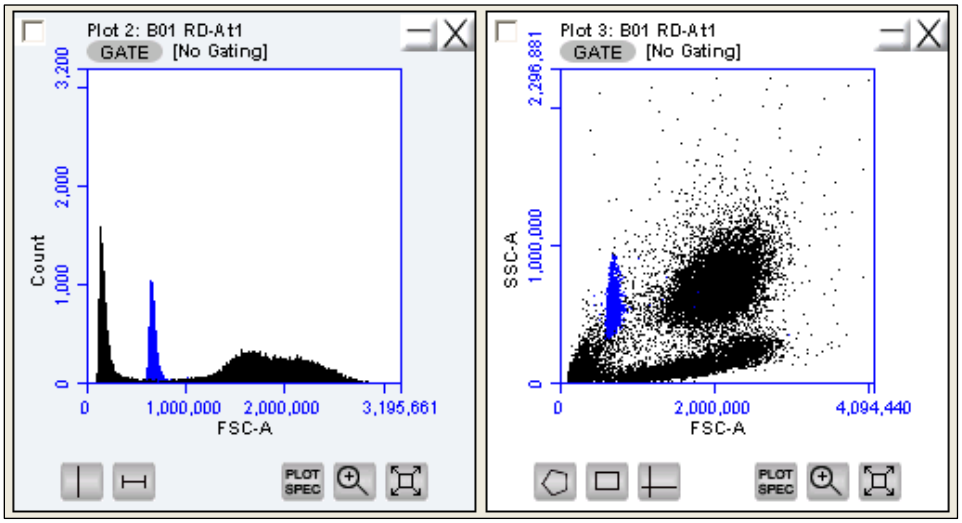


Figure F-10. Colored Events with a Region

F.4 Creating Publication-Quality Images of Plots

High resolution (publication quality) images are stored in eps format, which is vector scalable.

To create a high resolution image of a plot:

- Select *File* > *Set Plot Drag and Drop Format.*
- Select the .eps option in the dialog box.
- Click on the *OK* button.
- In the BD CSampler Workspace, click on a plot and drag it onto the desktop.
- Open an image editing application (e.g., Photoshop) and ensure the image resolution is set to 300 dpi, or higher if required, and import the eps image.
- Save the image.

F.5 Analyzing Batches of Samples

The *Batch Analysis* tab allows an automated analysis to be performed on multiple samples at the same time. The plot types created during acquisition will appear at the top of the screen. Statistics can be displayed for individual files or all files selected. After analysis, regions can be moved or resized on plots for individual samples without affecting the plots for other samples.

F.5.1 Viewing the *Batch Analysis* Tab

The *Batch Analysis* tab is organized into two major sections:

- Setup panel—Panel on the top half of the window that contains controls for selecting samples and plots for analysis.
- Data display—Large area on the bottom half of the window that shows sample data in plots and in a Statistics Table.

When the *Batch Analysis* tab is opened for the first time, the workspace is empty. To set up the *Batch Analysis* tab, plots can be created or copied from the *Manual Collect or Analyze* tabs.

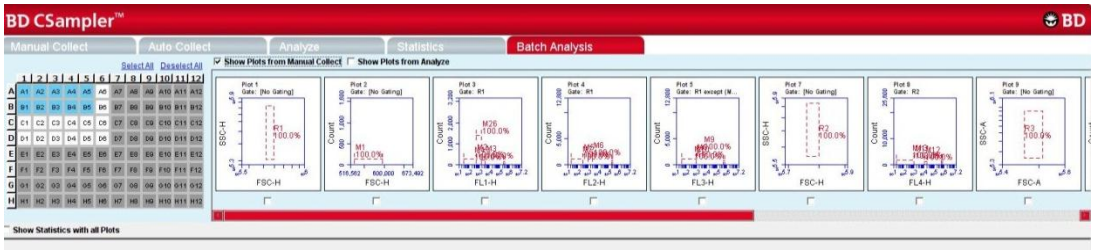


Figure F-11. *Batch* Analysis Tab Workspace

The following table describes each of the controls and indicators in the *Batch Analysis* tab:

Table F-1. *Batch Analysis* Tab Controls

Control	*Description*
Sample Grid	Matrix laid out in the configuration of a multi-well plate for selecting the samples to analyze. The wells are color-coded: White—Does not contain data. Blue—Contains data. Black check mark—Currently selected for batch analysis.
Available Plots	Displays plots (without data) that can be included in the batch analysis. Available plots include plots copied from the *Collect* tab or created in the *Analyze* tab. See section F.5.2 for details.
Analysis Pane	Rows of analysis data in table and plot format, based on the plots that were selected for use in analysis. Statistics Tables can be copied into most Microsoft Office compatible programs.

F.5.2 Running a Batch Analysis

- Run a number of samples.
- After running the samples, click on the *Batch Analysis* tab.
- Select the samples to analyze in the Sample Grid by clicking on the wells.
- Do one or both of the following:
  - Enable the *Show Plots from Collect* check box to make all of the plots from the *Collect* tab available for batch analysis.

  - Enable the *Show Plots from Analyze* check box to make all of the plots from the *Analyze* tab available for batch analysis.
- Select the specific plots to include in the batch analysis by enabling the check boxes under those plots.
- To show the statistics associated with samples, do one of the following:
  - Enable the *Show Statistics with All Plots* check box to show statistics in every row.
  - Enable the *Show Statistics* check box in individual rows to view statistics for specific samples. Regions can be moved and resized (see section □).

F.5.3 Exporting Data

Plots and statistics can be exported in two ways, either as a PowerPoint file or as an Excel spreadsheet. To export data:

- Do one of the following in the lower right-hand corner of the *Batch Analysis* tab:
  - Click on the *Export to PowerPoint* button.
  - Click on the *Export Stats to* Excel button.

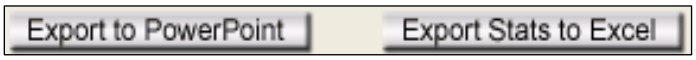


Figure F-12. Export Buttons in the *Batch Analysis* Tab

- In the Save dialog box, navigate to the destination folder and type a file name. BD CSampler Software exports the file to the specified location.

APPENDIX G FCS KEYWORDS

This appendix lists all of the keywords BD CSampler Software uses in FCS files.

STANDARD FCS TAGS IN FCS FILES EXPORTED BY BD CSAMPLER SOFTWARE

Table G-1. Standard FCS Tags

FCS Tag	*Description*	*BD CSampler Software*
$FIL	Filename including .fcs extension.	Save file dialog.
$SMNO	Sample name.	Sample name field above the Well Grid.
$DATATYPE	The data type of the actual values for each event. It is always “I” for unsigned binary integers.	Unchangeable.
$MODE	The mode of the data. It is always “L” for list mode where the data is in the order described by the $Pn keywords.	Unchangeable.
$BYTEORD	Order in which data bytes are written, least to most significant. It is always 4,3,2,1.	Unchangeable.
$NEXTDATA	The byte offset for an additional dataset in the file. BD CSampler Software files always specify 0 since the files only contain 1 dataset.	Unchangeable.
$PAR	Total number of parameters stored in the dataset. All datasets have 14 parameters.	Unchangeable.
$PnB	For parameter N, the number of bits for each binary value. The number is always 32 since integers are stored in 32 bits in Java.	Unchangeable.
$PnR	The range of parameter N. The range for all parameters is always 16777216.	Unchangeable.
$PnN	The name of parameter N. Parameters are the default values from BD CSampler Software.	Unchangeable.
$PnE	For parameter N, this denotes if linear or logarithmic amplifiers are used. It is always 0,0 because linear values for data are always saved. This is an optional tag.	Unchangeable.

FCS Tag	Description	BD CSampler Software
$PnS	The name of the fluorescence stain or probe used for parameter N. This tag is used for the custom parameter name.	Rename parameter dialog.
$TOT	Number of objects stored in the data list. The cumulative event total for the sample.	Determined by the amount of events acquired by user.
$DATE	The date the represented sample was last acquired into DD-MMM-YYYY.	Defined by the beginning time of the most recent acquisition. Set by the computer's clock.
$CYT	The name of the cytometer used for the measurement. It is always BD Accuri C6.	Unchangeable.
$SPILLOVER	The standard tag for color compensation.	Determined by values entered into Color Compensation dialog.
$TIMESTEP	Hard-coded value of 0.1, which is in seconds.	Unchangeable.
$PROJ	Represents the name of the workspace, which is also the name of the .c6 file (before the .c6 extension). If exporting an FCS file, this value is the name of the file before the .fcs extension.	Saving as a different file, importing an FCS file, or exporting an FCS file would change this value.
$BTIM	The beginning time of acquisition of the first event.	Unchangeable.
$ETIM	The end time of acquisition of the last event.	Unchangeable.
$VOL	Total volume in nanoliters.	Collecting more events would change the total volume.
$BEGINSTEXT	Default FCS 3.0 tag to mark the beginning of the text section.	Unchangeable.
$ENDSTEXT	Default FCS 3.0 tag to mark the end of the text section.	Unchangeable.
$BEGINANALYSIS	Default FCS 3.0 tag to mark the beginning of the analysis section.	Unchangeable.
$ENDANALYSIS	Default FCS 3.0 tag to mark the end of the analysis section.	Unchangeable.
$BEGINDATA	Default FCS 3.0 tag to mark the beginning of the data section.	Unchangeable.
$ENDDATA	Default FCS 3.0 tag to mark the end of the data section.	Unchangeable.

CUSTOM TAGS IN FCS FILES EXPORTED BY BD CSAMPLER SOFTWARE

Table G-2. Custom FCS Tags

Tag	*Description*	*BD CSampler Software*
#BDACCURIDECADESN	The number of decades for parameter N. It is always 7.224719870049579.	Unchangeable.
#BDACCURI4COLORCOMP	A list of the percent values as entered into the color compensation window.	Determined by values entered into Color Compensation dialog.
#BDACCURICAPTUREDDATE	The date of the last time the represented sample was acquired into expressed in milliseconds since Jan 1, 1970.	Defined by the beginning time of the most recent acquisition. Set by the computer's clock.
#PNVIRTUALGAIN	The virtual gain set for parameter N, where 1.0 means no virtual gain.	Set by user in the VirtualGain window.
#SAMPLE	Value is either the well code or the sample rename (if one exists).	Can be changed by renaming the sample in the application. If no rename exists, importing an FCS file into a different well than the one collected in will change this value.
#ATIM	Cumulative acquisition time.	Can be changed by acquiring more data.
#SPACERS	Used to pad the text, analysis, and data sections of the FCS file.	Unchangeable.

FCS 3.0 DEFINED TAGS NOT IN BD CSAMPLER SOFTWARE EXPORTED FCS FILES

Table G-3. FCS Tags not in BD CSampler Software Exported Files

Tag	*Description*
$COMP	Amount of fluorescence compensation employed during collection. This replaces the $DFCiTOj tag from FCS 2.0.
$TIMESTEP	Absolute measure of time used in kinetic analysis.
$UNICODE	Enables usage of certain keywords in non-English languages. This is optional.
$PnE	For parameter N, this denotes if linear or logarithmic amplifiers are used. BD CSampler Software always uses 0,0 because BD CSampler Software always saves linear values for the data. Mandatory in 3.0.
$PnN	When time is collected, the keyword value of the time-parameter name must now be the string "TIME".
CRC	An optional 16-bit Cyclic Redundancy Check has been added to the end of each dataset.

APPENDIX H EXAMPLE .CSV FILE

The following table is an exported bead log file in .csv format.

Each row represents the parameter data associated with a single event. The number of rows will equal the total number of events in the data file. A data well that contains 125,000 events also contains 125,000 rows of data in the spreadsheet display of the .csv data. The Time value is rounded to the nearest second during data export. The Width parameter is that for the primary threshold parameter.

Table H-1. Example .csv File

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	FSC-A	SSC-A	FL1-A	FL2-A	FL3-A	FL4-A	FSC-H	SSC-H	FL1-H	FL2-H	FL3-H	FL4-H	Width	Time
2	747359	206049	785	147	0	423	1883688	704036	486	60	161	382	82	137
3	308831	36583	390	221	456	0	787489	127133	274	114	438	383	75	137
4	656756	159245	685	179	870	286	1935215	555473	443	114	780	652	78	137
5	519654	138403	746	329	225	763	1175444	463213	501	152	118	506	86	137
6	818135	257287	767	72	1068	691	2171916	876805	591	0	586	629	80	137
7	257396	19821	203	254	75	76	688493	71597	110	96	182	298	73	137
8	268765	16276	168	5	0	26	742169	58897	46	55	275	362	71	137
9	741749	183316	329	145	517	526	2209348	664629	200	48	431	458	77	137
10	74788	19503	295	204	70	194	279103	76864	154	70	0	278	44	137
11	310347	28687	405	213	513	206	811460	101016	259	79	345	631	75	137
12	676065	201903	585	500	1374	775	1755862	709407	432	208	702	594	81	137
13	611517	252169	788	242	400	0	1461590	889724	427	177	274	229	82	137
14	321267	42064	149	194	244	660	823497	146225	319	46	314	732	76	137
15	301525	56315	314	133	442	118	734258	189713	150	56	414	442	78	137
16	787700	328238	926	92	1402	15	2011475	1183340	798	66	823	454	82	137
17	712542	188615	567	84	376	0	1939139	664650	415	48	343	234	79	137
18	940993	240525	871	224	122	0	2740219	850548	669	85	121	252	79	137
19	93345	18538	65	110	786	378	340737	73594	34	14	668	293	49	137
20	1120179	898131	6328	1672	1878	3020	3199136	3339490	5642	1524	1869	2551	80	137
21	735806	205623	342	158	776	565	2127351	755878	229	131	364	480	76	137
22	93556	28907	425	178	1106	217	312696	108595	204	66	464	318	51	138
23	539263	150426	657	220	626	435	1257974	519183	631	124	330	390	82	138
24	390039	401617	720	240	1270	542	1519975	1555340	604	108	747	613	57	138
25	656006	159032	570	198	433	0	1809412	567177	340	205	243	380	79	138
26	749099	186852	526	177	437	374	2095595	670857	287	81	187	407	79	138
27	249477	24622	598	267	757	0	640125	90865	424	97	486	378	74	138

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