



CFlow[®] SAMPLER

User Guide

Science is hard. Flow cytometry should be easy.[®]



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1 INTRODUCTION TO CSAMPLER

The Accuri CSampler is an optional accessory for the C6 Flow Cytometer® that allows for the aspiration of samples prepared in 48- and 96-well plates and standard 12 x 75 mm tubes from a 24-tube rack.

The 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: Do not use 24-tube racks other than the one supplied with the CSampler.

1.1 Installing the CSampler

Install and validate the C6 Cytometer with 6- and 8-peak beads before you install the CSampler. Inspect the CSampler Accessory Kit contents to ensure delivery of all components.

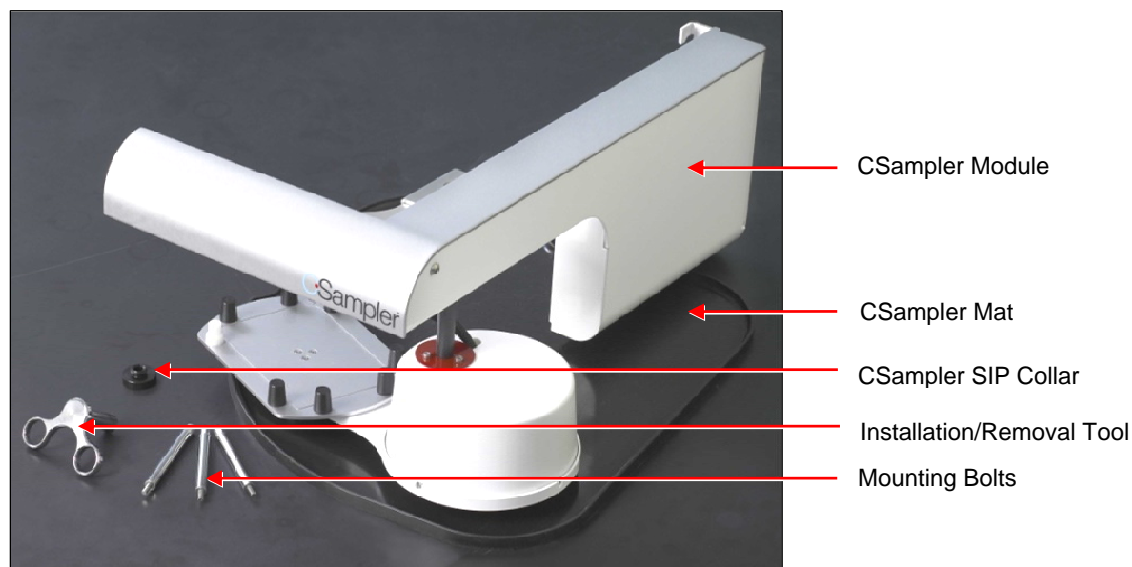


Figure 1-1. CSampler Accessory Kit

Table 1-1. CSampler Shipping Contents

Qty	Item
1	CSampler Module
1	CSampler Mat
3	Mounting Bolts
1	Installation and Removal Tool
1	CSampler Collar
1	CFlow® Sampler Software

To install the CSampler:

1. Shut down and power off the C6.

CAUTION: Failure to shut down the C6 during CSampler installation could result in damage to both C6 and CSampler electronic modules.

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

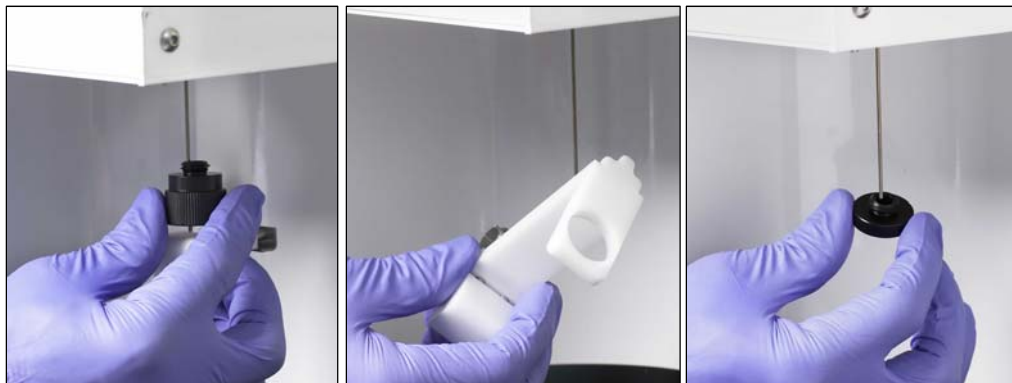


Figure 1-2. Installing the CSampler SIP Collar

3. Open the lid of the C6 and locate the bolt holes for mounting the CSampler.

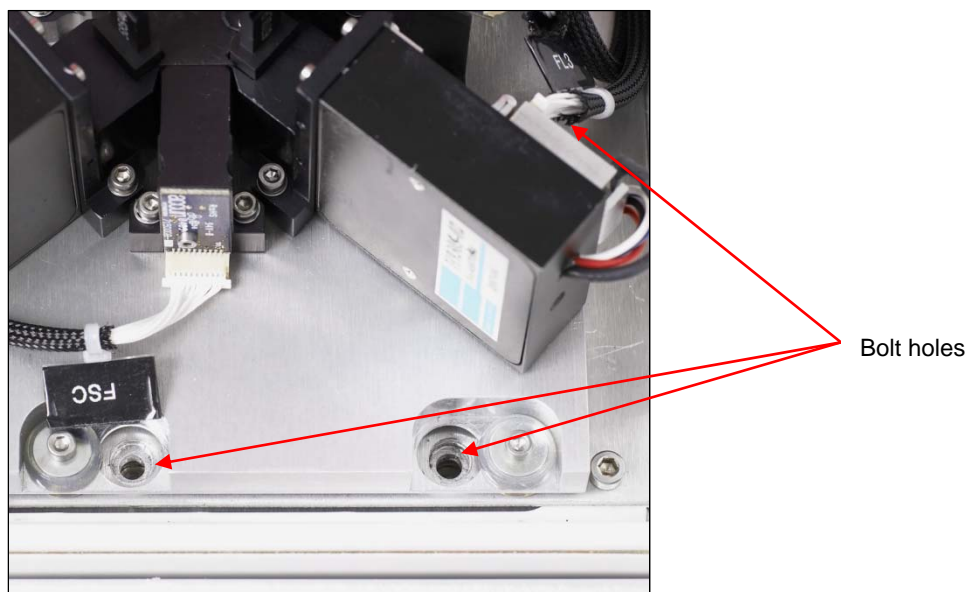


Figure 1-3. Location of Mounting Bolt Holes

4. Hold the CSampler chassis with both hands and insert the tab on the CSampler in the slot on the back of the C6.

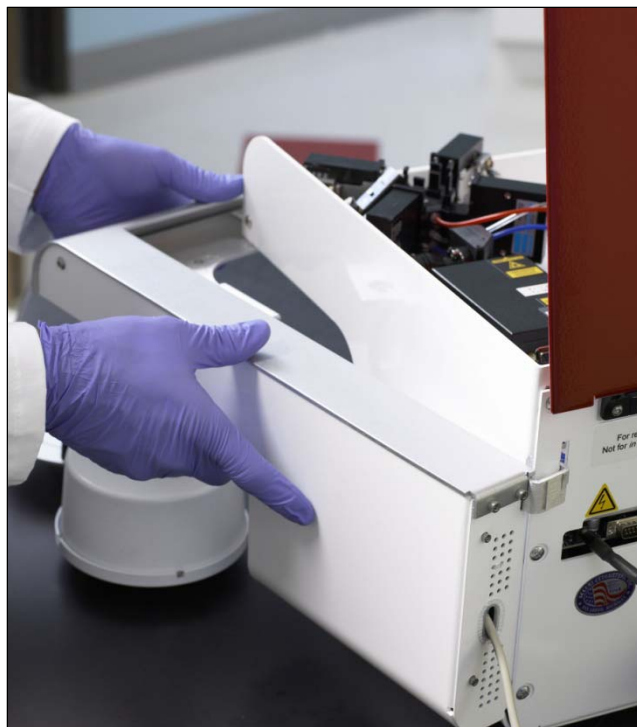


Figure 1-4. Insert CSampler Tab into Cytometer

5. Align the front of the CSampler so that the holes on the module are lined up with the three threaded holes in the C6.

6. Hold the module with one hand and partially screw in the right mounting bolt to secure the CSampler to the C6. Tighten with the installation and removal tool.



Figure 1-5. Secure the CSampler to the 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.

7. Partially screw in the remaining two mounting bolts.

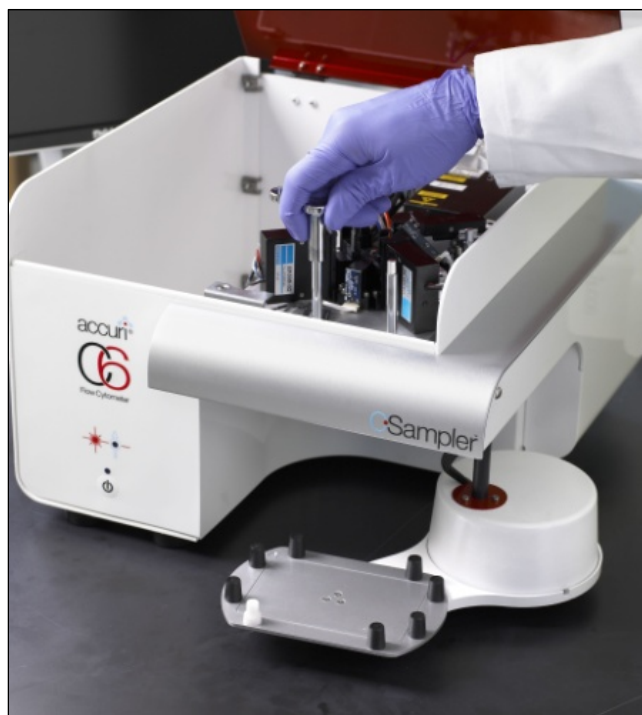


Figure 1-6. Secure the CSampler to the Cytometer

8. Confirm that the module is lined up properly with the C6, then completely tighten all three bolts.
9. Connect the serial cable to the socket at the back of the C6 then switch on the cytometer. The CSampler automatically aligns itself and rests in the home position.



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

10. Place the mat on the bench, under the arm of the CSampler.



Figure 1-8. CSampler in Home Position with Mat Installed

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

11. Install CFlow Sampler software from the installation CD (see section 2.1).
12. Run validation beads to ensure correct operation (see chapter 3, “Validating the Performance of the C6”).

1.2 Aligning the CSampler After a Collision

The CSampler performs an alignment to verify that the plate is aligned to the SIP every time you power up the cytometer or if the CSampler arm collides into an object. You can also perform an alignment in CFlow Sampler at any time.

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

To perform an alignment:

1. Remove any objects from the CSampler mat.

2. 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 CSampler*.

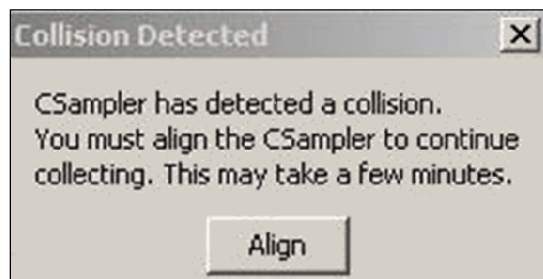


Figure 1-9. Collision Detected Window

If a second collision occurs, CFlow Sampler automatically performs a second alignment. If the second alignment fails, contact Accuri Technical Support at techsupport@accuricytometers.com (USA) or eurotech@accuricytometers.com (Europe). Samples left on the sample stage can be recovered by gently pushing down on the white cylindrical motor housing.

2 CFLOW SAMPLER OVERVIEW

The Accuri CFlow Sampler software (CFlow) is an easy to learn application program that allows you to control the C6 Flow Cytometer system to acquire data, generate statistics, and analyze results. CFlow contains controls that are both powerful and easy to use.

CFlow software provides the following features:

- Tabbed views for collection, analysis, and statistics
- Plots that display more than six decades of dynamic range
- Digital signal processing and color compensation at any time
- Drag and drop plots
- Export files in FCS 3.0 format
- Seamless data file importation into FCS Express

2.1 Installing CFlow Sampler

To install CFlow Sampler:

1. Insert the installation disk in your computer.
2. Navigate to the CD drive and double-click on the CFlow Sampler Installer.

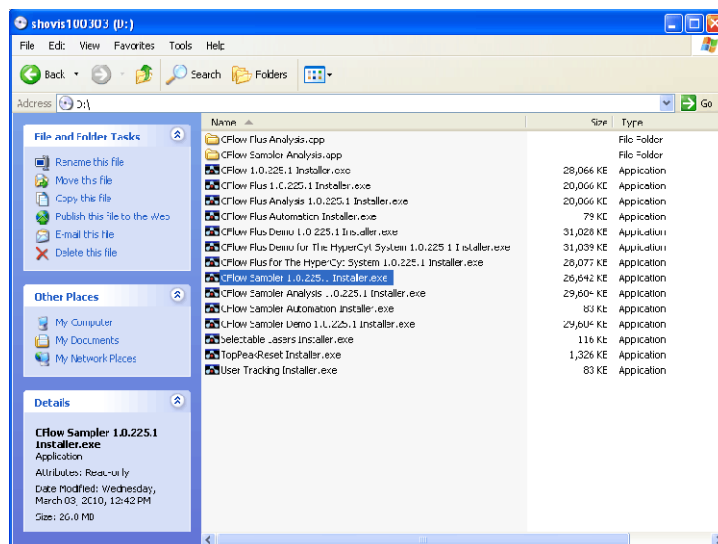


Figure 2-1. Install CFlow Sampler: Installation CD Directory

3. When prompted, click on the / Agree button to accept the Java license agreement.
4. Click on the / Agree button to accept the Accuri Cytometers license agreement.

5. Do one of the following:
 - Click on the *Install* button to install CFlow Sampler in the default location.
 - Use the *Browse* button or type a file path to assign a different location for CFlow Sampler. Click on the *Install* button.

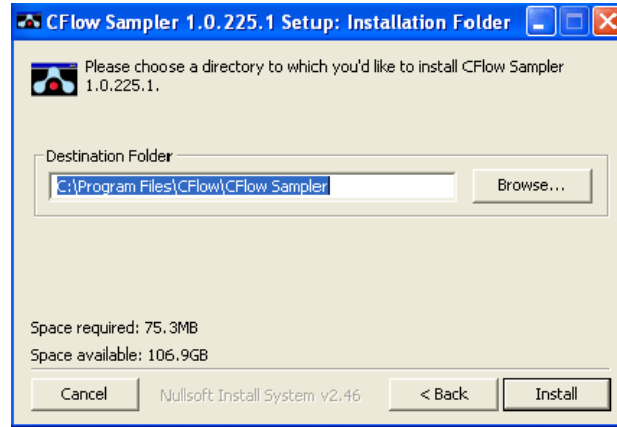


Figure 2-2. Install CFlow Sampler: Destination Folder

6. When CFlow Sampler is finished installing, click on the *OK* button.
7. Click on the *Close* button to close the installation wizard.

2.2 Starting CFlow Sampler

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

To open CFlow:

1. Double-click on the *CFlow Sampler* icon on the computer desktop. CFlow opens a new, blank workspace.

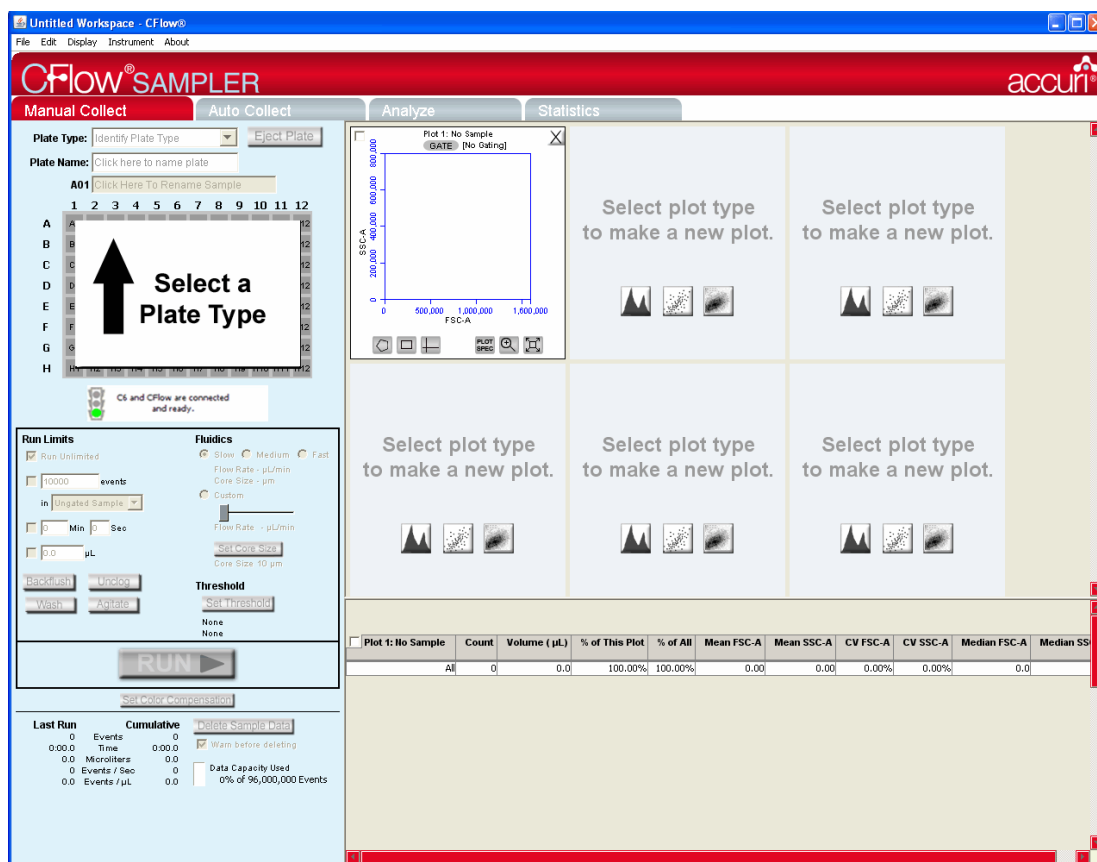


Figure 2-3. New CFlow Sampler Workspace

NOTE: If CFlow displays the message *Extra startup time needed due to cleaning or improper shutdown*, the C6 will take several more minutes than usual to recover and return to the green-light ready state. This may occur on initial C6 startup when you receive the unit. It will also occur after an interruption of power to the unit.

2.3 CFlow Workspace

The main CFlow window is called the CFlow workspace. The workspace contains controls and displays that provide access to all the functions for acquiring and analyzing data. The CFlow workspace is organized on four separate tabs:

- **Manual Collect**—Contains controls for setting up data collection and acquiring data in any order (see chapter 4, “Manual Data Acquisition,” for more information).
- **Auto Collect**—Contains controls for automatically collecting data from several wells in order (horizontally or vertically), starting at well A1 (see chapter 5, “Automatic Data Acquisition,” for more information).
- **Analyze**—Contains controls for analyzing data (see chapter 6, “Analyzing Sample Data,” for more information).
- **Statistics**—Displays statistical information (see chapter 7, “Viewing Statistics,” for details).

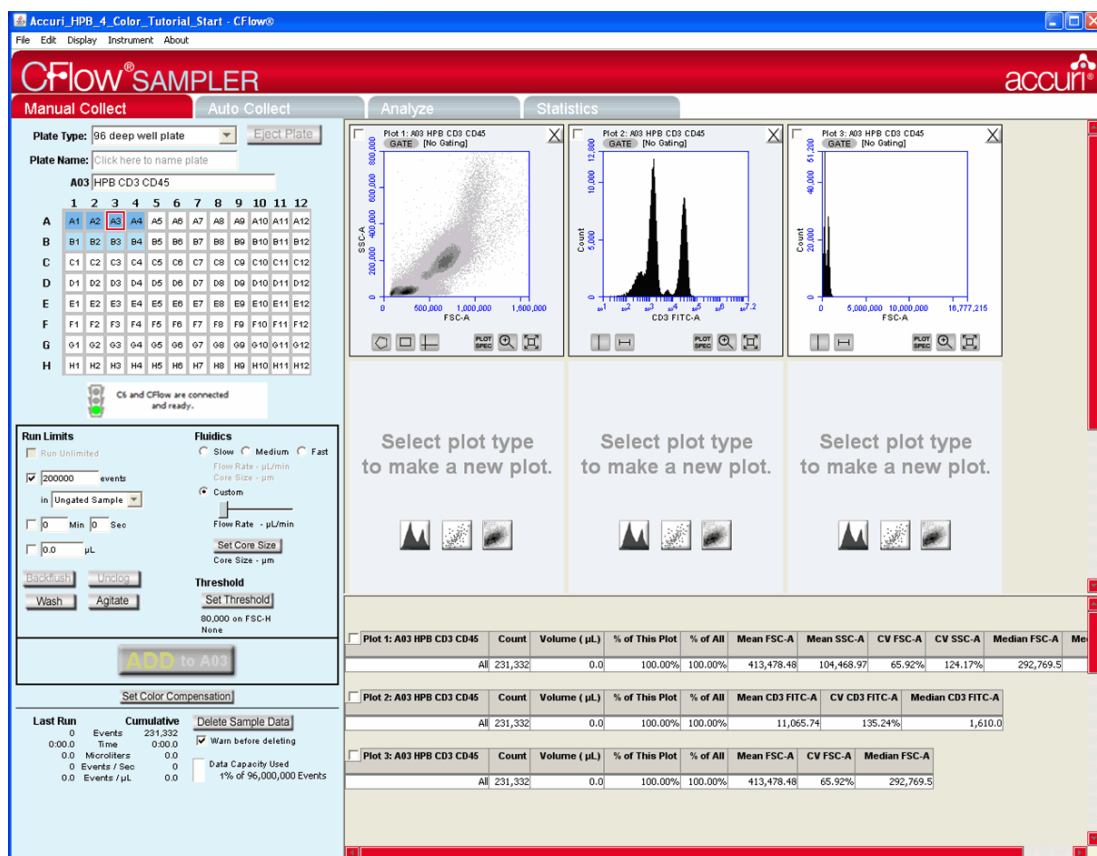


Figure 2-4. CFlow Sampler Window

2.4 Opening a New CFlow Workspace

When CFlow opens, it displays a blank workspace with no custom settings. You can use a new CFlow workspace for creating a new analysis template.

To open a new workspace:

- Do one of the following:
 - If CFlow is not already open, double-click on the CFlow Sampler icon on the computer desktop.
 - If CFlow is already open, select *File > New CFlow File*. CFlow prompts you to save any unsaved changes to the previous workspace.

2.5 Loading and Ejecting a Plate

CFlow Sampler can move the CSampler arm and plate platform into or out of the C6 so that you can easily place a plate or tube rack on the plate platform or remove a plate after sample acquisition.

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).

To move the CSampler arm to the load or eject position:

1. Click on the *Load Plate/Eject Plate* toggle button.

2.6 Exiting CFlow

To exit CFlow:

1. Select *File > Quit*.
2. If prompted to save changes to the CFlow workspace, do one of the following:
 - Click on the *Yes* button to save changes.
 - Click on the *No* button to close CFlow without saving changes.
 - Click on the *Cancel* button to cancel the exit and keep CFlow open.

2.7 Using the Example CFlow File

An example CFlow file of a four-color analysis of human peripheral blood (HPB 4 Color Tutorial.c6) can be downloaded from the Accuri website (www.accuricytometers.com/technical_information/tutorials/). You can use the data in this file to explore various tools in CFlow without the worry of corrupting or losing your own 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:

- Sample 1—Background control
- Sample 2—White blood cell gating control
- Sample 3—Control required for determining the percent of CD4⁺ and CD8⁺ cells within the CD45⁺CD3⁺ subset
- Sample 4—Actual test 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 single-stained fluorescence controls. The only channels where fluorescence spillover causes ambiguity for setting gates are FITC spillover into PE and PE-Cy7 spillover into the FITC and PE channels. For details on correcting fluorescence spillover, see section 4.12.

3 VALIDATING THE PERFORMANCE OF THE C6

Perform a validation of the fluidics at least once each day that you use the C6. This ensures that the C6 is working properly before running experimental samples.

Use the same CFlow file each day to collect validation bead data so that you can compare trends over time. When you run a validation, advance 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* when you name the sample for each day of validation. Start a new validation bead file when you fill up the wells.

Reagents required:

- SpheroTech 8-Peak Validation Beads (Accuri Part# QA-100, supplied with the initial C6 shipment)
- SpheroTech 6-Peak Validation Beads (Accuri Part# QA-110, supplied with the initial C6 shipment)
- Sheath fluid: Deionized, filtered water (0.2 μ m filter) plus Bacteriostatic Concentration Solution (Accuri Part# KR-220)

3.1 Running Validation Beads

To run the validation beads:

Setup:

1. If this is the first time using the C6 Cytometer, open a CFlow file and pull filtered de-ionized water from a 12x75 mm sample tube for at least 15 minutes.
2. Verify that the validation bead template file for CFlow Sampler (*not* for CFlow Plus) has been copied to the CFlow computer from the installation CD or the Accuri website (www.accuricytometers.com/technical_information/templates/).

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

3. In the *Manual Collect* tab, select *File > Open CFlow File or Template*.
4. In the Open dialog box, browse to the location of the template file and open the file.

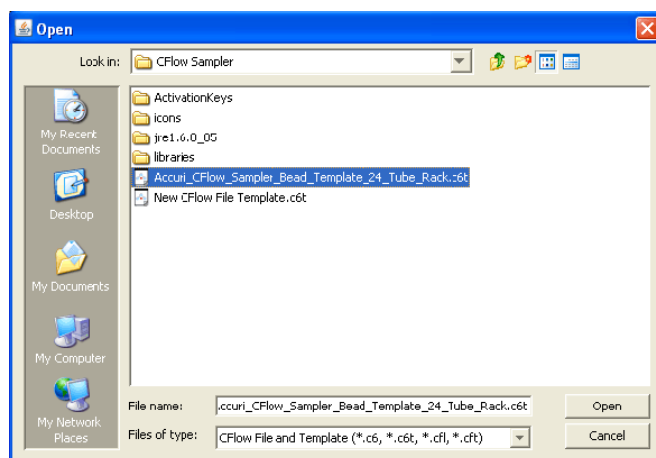


Figure 3-1. Open Validation Template

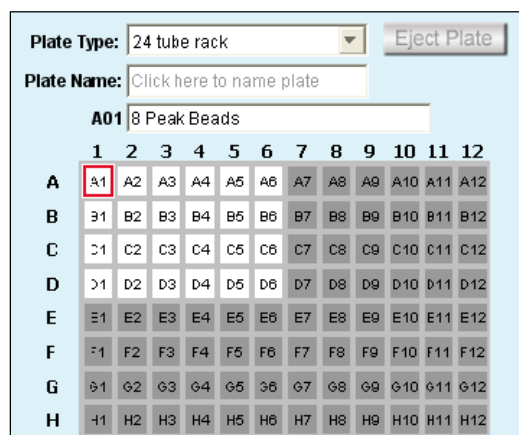
- Place the following tubes on the 24-tube rack:

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

Type of Sample	Location in CFlow Sample Grid/Tube Rack
2 mL of filtered, de-ionized water	A1
8-peak validation beads, prepared according to package instructions	First empty data well in rows A-B
6-peak validation beads, prepared according to package instructions	First empty data well in rows C-D

Clean the SIP:

- Perform a backflush (see section 8.1).
- Click on the *Load* button.
- Select well A1 in CFlow.

**Figure 3-2.** Select Well A1

- Disable the *Run Unlimited* check box in the Instrument Control Panel.

**Figure 3-3.** Run Limits Controls

- Enable the *Time* check box next to the *Min* and *Sec* fields in the Instrument Control Panel and type in a run time of two minutes.

11. Select the *Fast* radio button in the Fluidics section of the Control Panel.

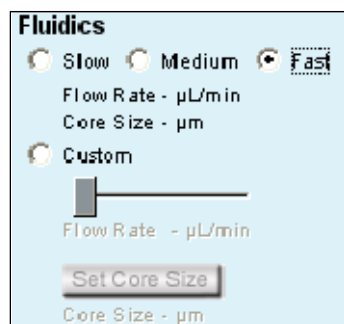


Figure 3-4. Fluidics Settings Controls

12. Click on the *RUN* button to rinse out the SIP.
13. Once the run is finished, click on the *Delete Sample Data* button to delete data collected during the rinse.

Run 8-Peak Validation Beads:

14. Select the *Slow* radio button in the Fluidics section of the Control Panel.
15. Disable the *Time* check box next to the *Min* and *Sec* fields and enable the *Events* check box to stop the run when the specified number of events has been acquired.



Figure 3-5. Run Limits for 8-Peak Beads

16. Type *50000* in the *events* edit box and select *Ungated Sample* from the associated drop-down list.
17. Click on the *Agitate* button to resuspend the beads in the 24-tube rack.
18. In CFlow, select the first empty data well in rows A-B. This should correspond to the tube containing 8-peak beads.
19. Click on the *RUN* button to start acquisition. Acquisition automatically stops after 50,000 total events are acquired.

CAUTION: Make sure the well in CFlow 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.

20. Type a name of the sample 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: You can also name samples before, during, or after collection.

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

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

Run 6-Peak Validation Beads:

22. Click on the *Agitate* button to resuspend the beads in the 24-tube rack.
23. Click on the first empty well in rows C-D to advance to the well. This should correspond to the tube containing 6-peak beads.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Figure 3-7. Select Well for 6-Peak Beads

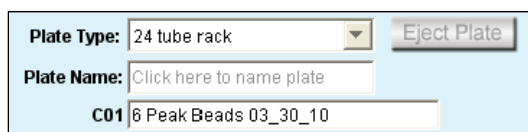
24. Verify that *Events* is still enabled and set at 50,000 in *Ungated Sample*.

Figure 3-8. Run Limits for 6-Peak Beads

25. 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.

26. Name the sample (include the date processed).



The screenshot shows a software interface for naming a sample plate. It includes a 'Plate Type' dropdown menu set to '24 tube rack', an 'Eject Plate' button, and a 'Plate Name' text input field. Below the 'Plate Name' field, there is a label 'C01' followed by the text '6 Peak Beads 03_30_10'.

Figure 3-9. Sample Name for 6-Peak Beads

27. When the collection is finished, eject the plate and click on the *Wash* button to minimize sample carryover.

End the Procedure:

28. Place a tube with 2 mL of filtered, de-ionized water in the A1 position of the 24-tube rack and advance to the data well in CFlow Sampler.

29. Select the *Time* check box (*Min Sec*) in the Instrument Control Panel and set it for two minutes.

30. Click on the *RUN* button.

31. Click on the *Delete Sample Data* button when the run is complete.

32. When the run is finished, eject the tube rack and load an empty tray.

3.2 Saving Validation Bead Data

By default, CFlow automatically saves validation bead data at the end of each sample. You can also save data manually at any time. For information on saving data, see section 4.10.

3.3 Analyzing and Recording Validation Bead Data

After you collect the bead data, analyze the data using the *Manual Collect* tab of CFlow to ensure that the C6 is functioning properly.

To analyze the bead data:

1. Click on the well that contains the most recent 8-peak bead data (in rows A-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 75-85% of all 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.

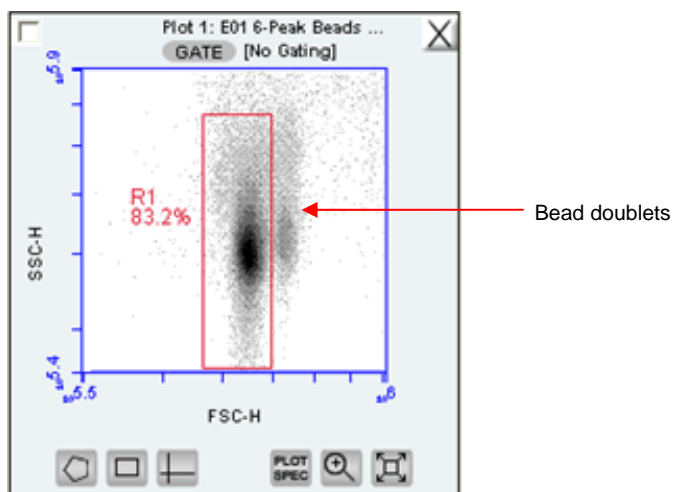


Figure 3-10. Plot with Bead Doublets

- 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).

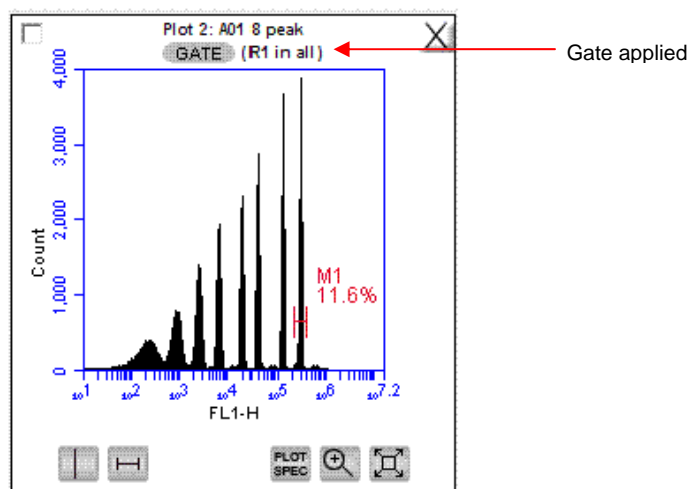




Figure 3-11. Gate Applied to 8-Peak Bead Plot

4. Measure the CV of the top (brightest, far right) peak on each of the three fluorescence plots. Do the following to place the predrawn horizontal marker tightly around the peaks:
 - Use the Zoom Tool  in the plot to zoom in on the top peak (see section 4.9).
 - 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.

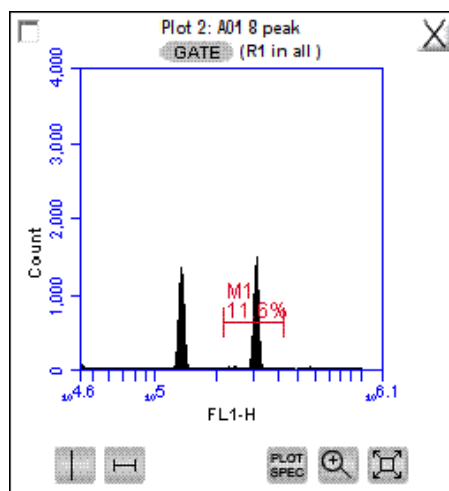


Figure 3-12. Zoomed View of Plot

5. Compare the bead run to the manufacturing results sent with the C6. If the C6 is performing properly, the data plots should look similar to the 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-H

NOTE: FL4-H performance is checked with the 6-peak beads in steps 6-9.

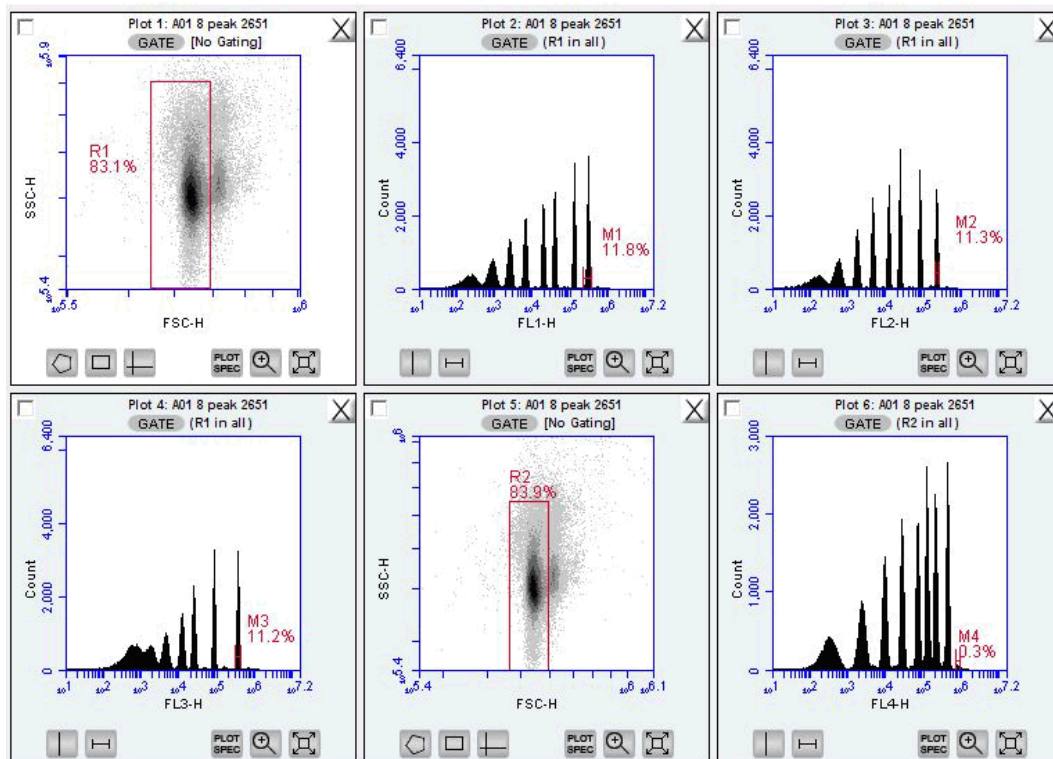


Figure 3-13. 8-Peak Validation Bead Data

6. Select the well containing the most recent 6-peak bead data (in rows C-D).

7. 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).

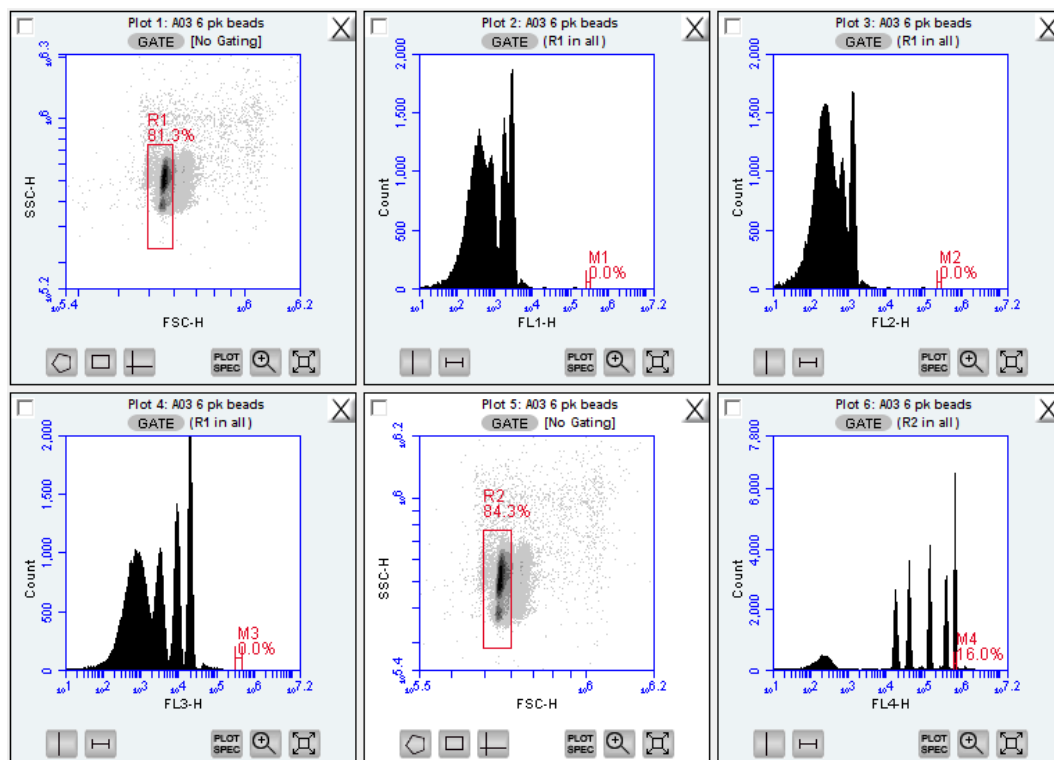


Figure 3-14. 6-Peak Validation Bead Data

8. 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 (for information about gates, see section 4.7).
9. 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 good 6-peak bead data. Look for the following:
 - One main population of beads on FSC-H vs. SSC-H
 - Six peaks on FL4-H
10. If you want, you can record the following information for each parameter in the Accuri C6 Cytometer Log (available on the CFlow CD):
 - Number of peaks
 - Mean channel numbers for the top peaks and forward scatter
 - CVs for the top peaks and forward scatter
11. If this is your first validation run, send a copy of the file to Accuri Technical Support: techsupport@accuricytometers.com (USA) or eurotech@accuricytometers.com (Europe) for analysis.

3.4 Monitoring Validation Bead Data

You can easily monitor validation bead data (and thus the C6 performance) over time in one CFlow file to determine if the C6 performance is stable.

To monitor the bead data:

1. Save the 8- and 6-peak validation bead data from each day in separate wells.
2. Create a table in the *Statistics* tab that contains the mean channel numbers and CVs for the top peaks and forward scatters (see chapter 7 for details on creating tables in the *Statistics* tab). The C6 reports arithmetic means.

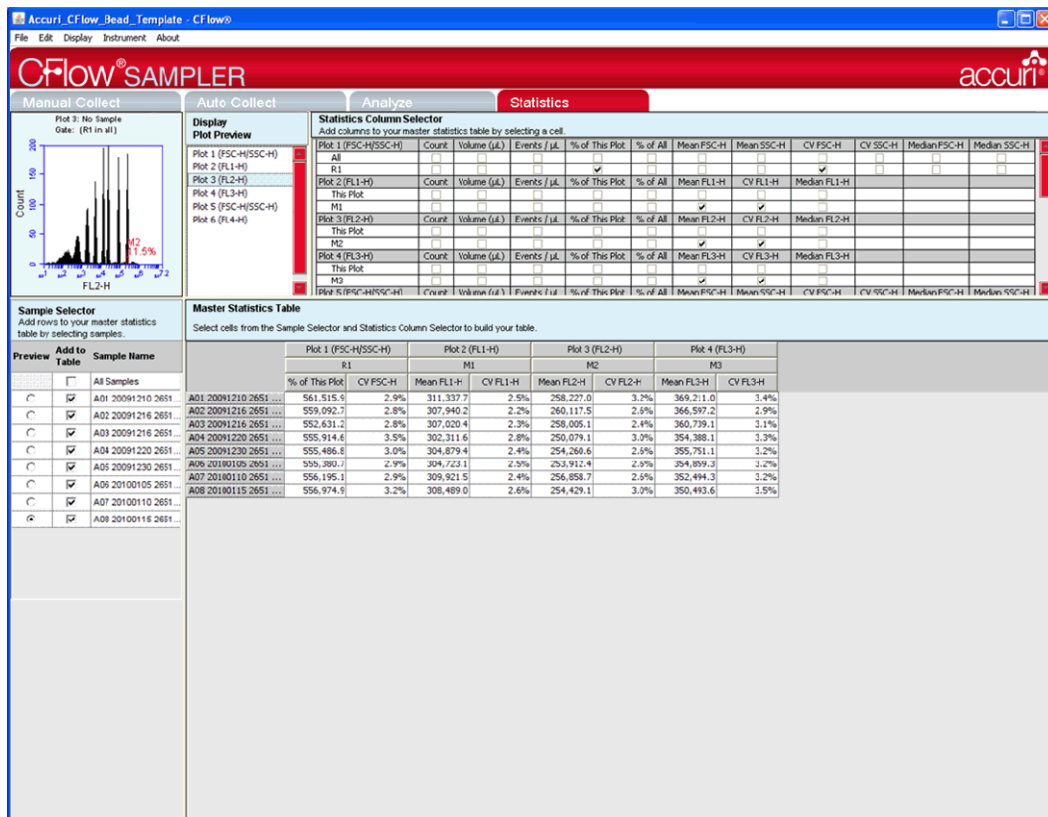


Figure 3-15. Statistics Tab: 8-Peak Bead Data from Successive Days

3. Compare statistics over time for trends or sudden changes in mean values to assess the cytometer's performance.

3.5 Troubleshooting Validation

The following conditions may indicate a problem with the C6 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.
- Fewer than six peaks for FL4.
- The brightest peak CVs are > 5.0%.

To troubleshoot validation:

1. If bead data were not acquired using the slow rate, select *Slow*, resuspend the beads, and recollect the data.
2. 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 degraded. Make new bead suspensions and run the bead sample again.
3. There may be a bubble or clog in the flow cell. Do one or more of the following:
 - Run the bead sample again.
 - Replace the current tube with an empty one, reload the rack, and click on the *Unclog* button. When the unclog cycle is finished (CFlow displays a green Traffic Light), run the bead sample again.
 - Replace the current tube with an empty one, reload the rack, and click on the *Backflush* button. When the cycle is finished (CFlow displays a green Traffic Light), run the bead sample again.

4 MANUAL DATA ACQUISITION

The *Manual Collect* tab allows you to set the data collection criteria, start and stop data acquisition, and view data on collected samples. The *Manual Collect* tab allows you to perform the following functions:

- Run individual samples from a plate or tube rack. Settings such as run limits, 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. You can do the following in the *Manual Collect* tab before switching to the *Auto Collect* tab:
 - Create plots
 - Set up gating strategies
 - 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.

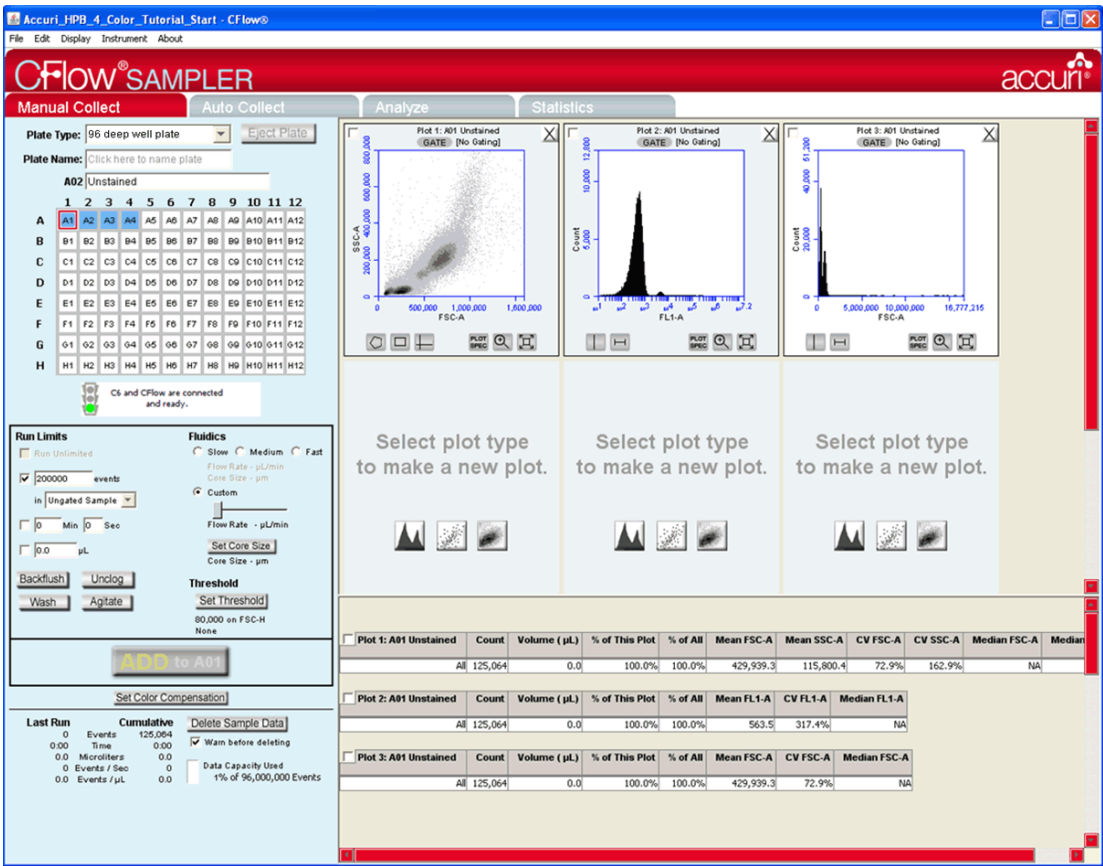



Figure 4-1. CFlow Sampler *Manual Collect* Tab

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 the type of plate you are using.
Load Plate/Eject Plate	Moves the sample plate into position to be loaded on to or ejected from the C6.
Plate Name	Text box for naming the plate you are using.
Sample Naming Field	Text box for naming the sample that you are collecting.
Sample Grid	<p>Matrix laid out in the configuration of a 96-well plate to help organize experiments and collect data from sample tubes. CFlow acquires each sample into its own well in the Sample Grid. The wells can be filled with data in any order.</p> <p>The wells are color-coded:</p> <ul style="list-style-type: none">• White—Does not contain data.• Blue—Contains data.• Red outline—Currently selected for viewing or collecting data.

Control	Description
<i>Traffic Light and Message</i>	<p>Indicator that displays CFlow's readiness and system messages. Before data collection can begin, CFlow must display a green Traffic Light  with the message <i>C6 and CFlow are connected and ready</i>.</p> <p>The Traffic Light status is color-coded:</p> <ul style="list-style-type: none"> Green—CFlow is ready to collect data or is collecting data. Yellow—The C6 is preparing to perform an action or a non-critical error has occurred. Red—A critical error has occurred.
<i>Run Limits</i>	Contains a set of controls that allow you to define criteria for automatically stopping the data collection. See section 4.2.6 for details.
<i>Fluidics</i>	Contains a set of controls for defining flow rate and core size. See section 4.2.1 for details.
<i>Backflush</i>	Performs a backflush to eject sample from the SIP. See section 8.1 for details.
<i>Unclog</i>	Performs an unclog cycle to purge the flow cell of debris. See section 8.2.1 for details.
<i>Wash</i>	Performs a wash cycle to keep the SIP clean between samples. See section 4.2.8 for details.
<i>Agitate</i>	Performs an agitate cycle to keep particles in suspension. See section 4.14 for details.
<i>Set Threshold</i>	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.
<i>RUN/PAUSE/ADD TO</i>	<p>Toggle button that performs the following functions:</p> <ul style="list-style-type: none"> <i>RUN</i>—Starts the sample acquisition. <i>PAUSE</i>—Pauses the acquisition. Click on <i>ADD TO</i> to resume data collection. <i>ADD TO</i>—Allows you to run another sample in a well that already contains data.
<i>Set Color Compensation</i>	Opens the Color Compensation dialog box for correcting fluorescence spillover. See section 4.12 for details.
<i>Acquisition Counters</i>	<p>Displays the following information about the most recent acquisition for the selected well (<i>Last Run</i>) and all acquisitions for the selected well (<i>Cumulative</i>) in real-time:</p> <ul style="list-style-type: none"> <i>Events</i>—Number of events sampled. <i>Time</i>—Elapsed acquisition time. <i>Microliters</i>—Volume of acquired sample. <i>Events/sec</i>—Events acquired per second. When the run is completed, this is the average value. <i>Events/μL</i>—Events acquired per microliter. When the run is completed, this is the average value.
<i>Delete Sample Data</i>	Permanently deletes all events from the current sample. Enable the <i>Warn before deleting</i> check box to display a warning message before deleting sample data. Also contains a <i>Data Capacity Used</i> meter that displays the amount of data storage capacity currently used in CFlow.

Control	Description
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. You can create multiple plots for each sample. Each plot corral contains buttons for creating histogram, scatter, 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

Each data well holds a maximum of 1 million events. You can add more events, up to a total of 1 million, to a well at any time, even if the well already contains data. When a data well already contains data, the *RUN* button displays *ADD TO*.

CFlow must display a green Traffic Light and the message *C6 and CFlow are connected and ready* to collect data.

The figure below 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.

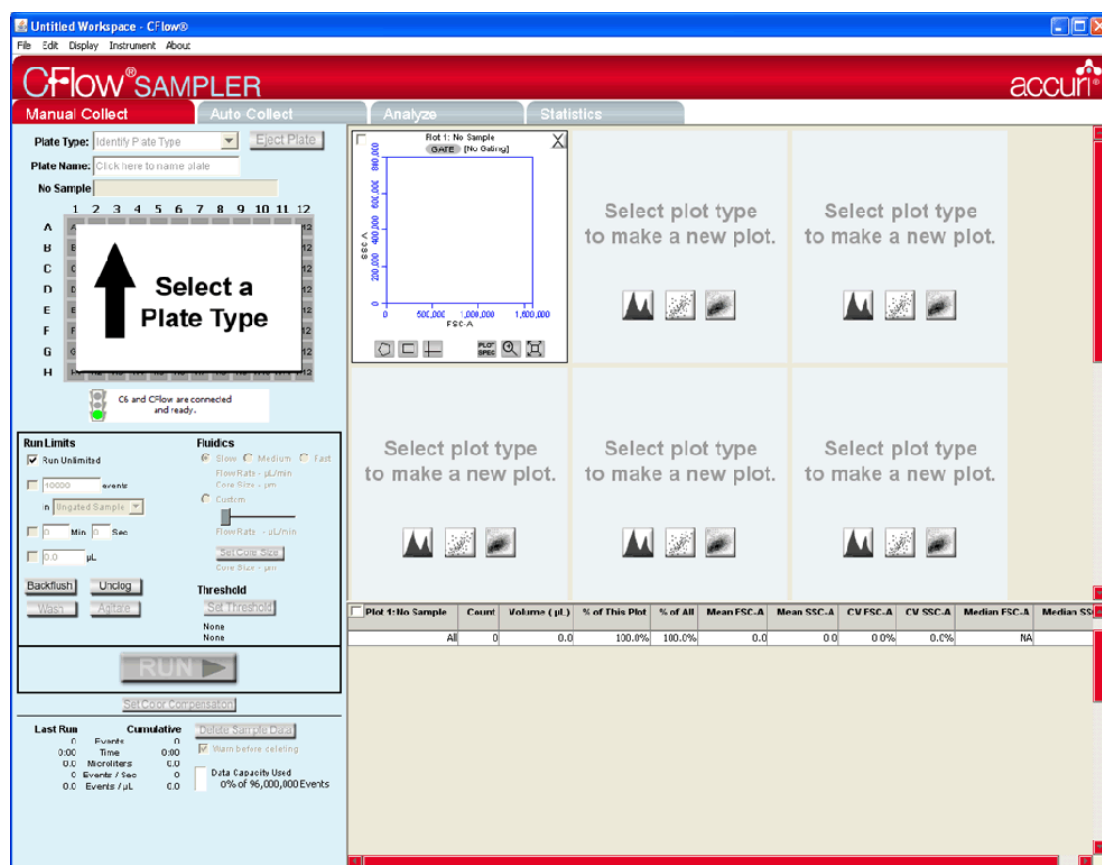


Figure 4-2. New CFlow Workspace

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

To collect sample data:

1. In the *Manual Collect* tab, select a plate type and name the plate.
2. Create the plots for running the plate, or load a template.
3. Define acquisition settings.
4. If necessary, acquire some events to define regions, gating, and other settings.
5. Click on the *Auto Collect* tab.
6. Define acquisition settings, create the Sample Annotation Table, and create sample sets.
7. Start data acquisition.
8. 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 2500 events per second or less to ensure the best data resolution.

To set the fluidics rate:

1. 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. You can then adjust the setting to medium or fast, if necessary.

You can customize your fluidics rate settings and adjust the sample core size for very large or very small cells. For information on customizing these settings, see APPENDIX B.

4.2.2 Setting the Threshold

Thresholds gate out debris and noise signals from cell samples so that sample data are not compromised. By default, CFlow filters out events lower than channel 80,000.

You can change the threshold settings at any time before, during, or after data acquisition. The primary threshold is the parameter that triggers data collection. You can optionally set a secondary threshold to filter out additional data. FSC-H is the default primary threshold.

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

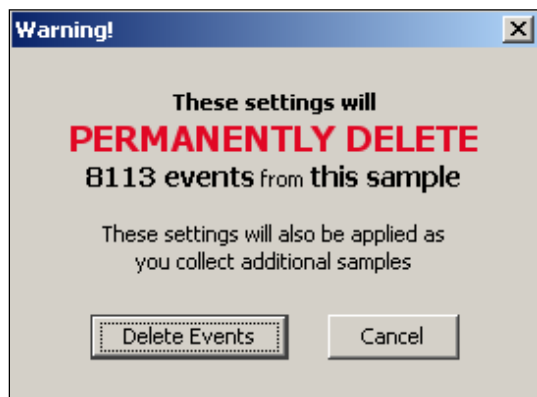


Figure 4-3. Threshold Settings Warning Message

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.

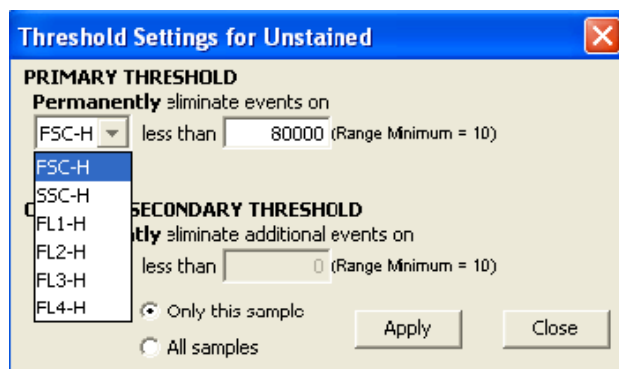


Figure 4-4. Primary Threshold Drop-Down List

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

NOTE: You may need to set a lower or higher FSC-H threshold when working with small cells (such as platelets or bacteria) or large cells (such as cell lines).

- If you want to apply a secondary threshold for filtering out more data, do the following:
 - 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.

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

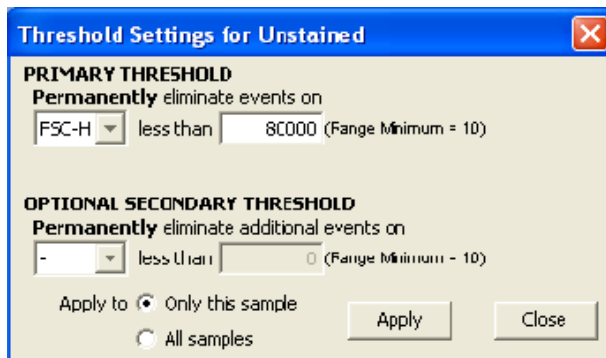


Figure 4-5. Threshold Settings Dialog Box

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

4.2.3 Assigning a Plate Type

When setting up an experiment, select the plate type that you are using.

NOTE: If you change to a new plate type, CFlow opens a new blank workspace.

To assign a plate type:

1. 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

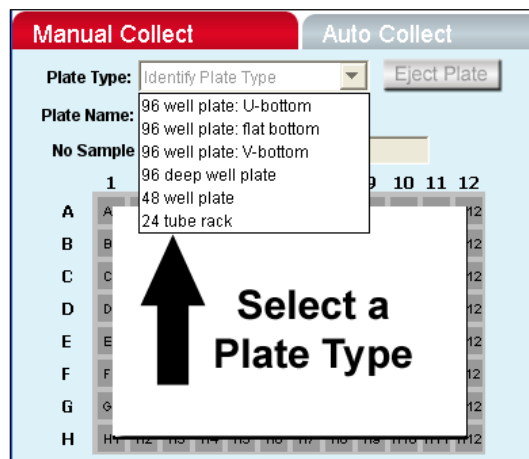


Figure 4-6. Plate Type Drop-Down Menu

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

- 96-well plate assignment—Displays all 96 wells
- 48-well plate assignment—Displays wells A1-F8
- 24-tube rack assignment—Displays wells A1-D6

4.2.4 Naming the Plate

You can optionally name the sample plate to distinguish it from other sample plates in similar experiments.

To name the sample plate:

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

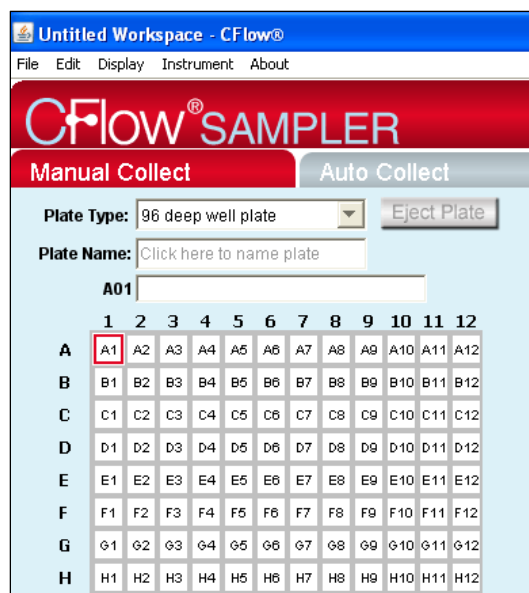


Figure 4-7. Plate Name Field

4.2.5 Naming the Sample

You can name samples at any time. If you do not type in a name, CFlow names the sample according to the well location (for example, *Sample A01*).

To name the sample:

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

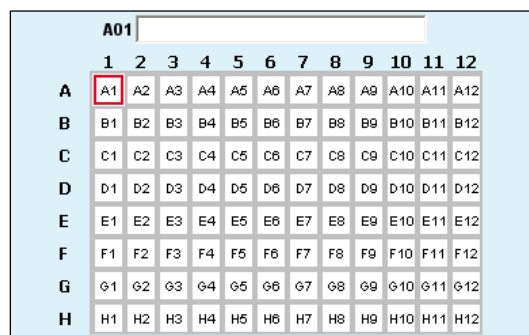


Figure 4-8. Sample Name Field

4.2.6 Setting a Run Limit

Set a run limit to indicate to CFlow when to stop collecting data. You can set a run limit based on any of the following criteria:

- After a specified time
- After a specified volume
- After a specified number of events
- For an unlimited time (until you manually stop the run)

You can also choose multiple run limits (the C6 stops on the first limit reached).

For the first sample of your experiment, it is recommended to collect a total of 100,000 to 200,000 ungated events. You can change the run limit once a gate has been set on the population of interest.

Figure 4-9. Run Limits Controls

To collect samples without setting a run limit:

1. Disable all check boxes in the Run Limits section of the *Collect* tab.
2. Enable the *Run Unlimited* check box.

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

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

To stop the run after a time has expired:

1. Enable the check box next to the *min* and *sec* fields.
2. 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:

1. Enable the check box next to the μL field.
2. Type the volume in microliters (μL) at which to stop the run.

4.2.7 Running the Sample

To run the sample:

1. Gently resuspend the cells in the sample tube, place the tube in the tube rack, and load the rack.
2. Select a well in the sample grid that corresponds to the sample on the plate.
3. Click on the **RUN** button to start the sample collection.

CFlow begins fluidics initialization. During this time the Traffic Light turns yellow and CFlow displays the message *Preparing to analyze sample*. Once initialization is complete, the Traffic Light turns green and CFlow displays the message *C6 is collecting data*. Well A1 flashes blue during data collection. After the run limit is reached, well A1 stops flashing and remains blue, indicating that the well contains data.

4. If desired, you can collect more data into a well that contains data at any time by clicking on the **ADD TO** button.

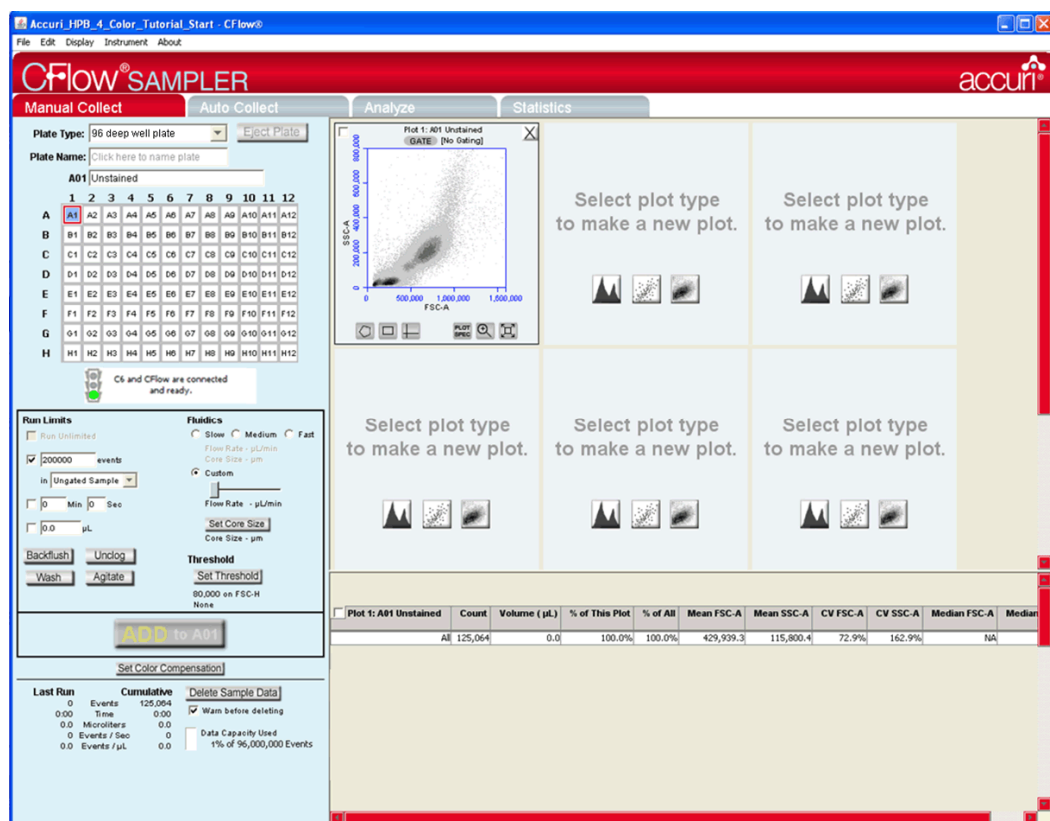


Figure 4-10. CFlow Workspace after Collecting a Sample

4.2.8 Washing the SIP Between Samples

You can use CFlow Sampler to perform a single wash cycle to thoroughly clean the SIP of a previous sample. The C6 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:

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

4.2.9 Adding New Sample Data to a CFlow File

You can collect new samples and add the data to a CFlow file that already contains sample data. You can add data to an empty sample well or to a well that already contains data.

To add data to a CFlow file:

1. Gently resuspend a new sample (if necessary) and load the plate. You can perform a wash if you are concerned about carryover between samples.
2. Click on a data well in the 96-well sample grid. If you select an empty well, any plots and gates you created earlier are still displayed, but they do not contain any data, as shown in the following figure.

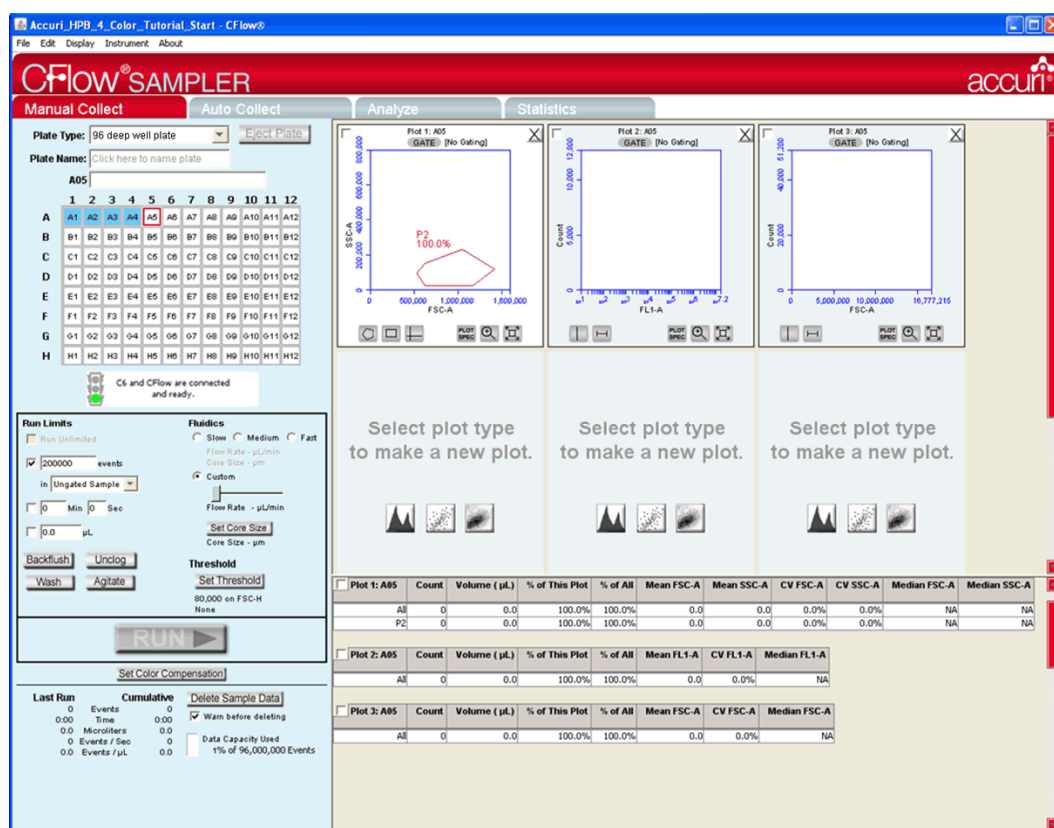


Figure 4-11. CFlow Workspace with Empty Sample Well and Empty Plots

- Click on the *RUN* (or *ADD TO*) button to start a sample collection. CFlow displays and updates data in real time during the collection. The C6 stops sampling from the tube when the run limit is reached.

CAUTION: If you click on the *ADD TO* button, CFlow will collect data into a well that already contains data.

4.3 Ending a Data Collection Session

Whenever you are finished collecting samples, rinse out the SIP to ensure cells or other particles are not left in the SIP.

To end a data collection session:

NOTE: If you plan to collect more samples later in the day, you can skip steps 2-7.

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

Table 4-2. Plate Setup for Ending a Data Collection Session

Type of Sample	Location in CFlow Sample Grid
2 mL of filtered, de-ionized water	Empty data well (for example, D4)
2 mL of decontamination solution (Accuri Part #KR-200)	Empty data well (for example, D5)
2 mL of filtered, de-ionized water	Empty data well (for example, D6)

- In CFlow, select the well corresponding to the first tube of water. Make sure the well contains no data.
- Set the time limit for two minutes.
- Click on the *RUN* button.
- In CFlow, 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.
- Click on the *RUN* button.
- In CFlow, select the well corresponding to the second tube of water. Make sure the well contains no data.
- Set the time limit for two minutes.
- Click on the *RUN* button.
- When the run is finished, eject the plate/tube rack and load an empty tray.

4.4 Creating Plots

Plots allow you to view sample data in histogram, density, and dot graphs. You can view multiple plots for each sample you collect.

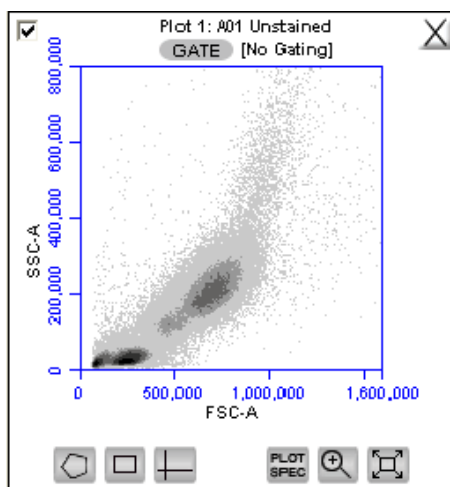


Figure 4-12. View of a Plot

Each plot contains a set of gating and marking tools and a set of viewing tools:

- Gating and marking tools:
 - Gate button —Opens the Change Gating dialog box for applying gates to a plot.
 - Polygonal Gating Tool —Used for drawing irregularly shaped gates around a population of events.
 - Rectilinear Gating Tool —Used for drawing a rectilinear gate around a population of events.
 - Quadrant Gating Tool —Used for gating the plot in quadrants.
 - Vertical Marker Tool —Used for gating histograms to the right or left of a vertical marker.
 - Horizontal Marker Tool —Used 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 —Allows you to draw a rectilinear range in the plot to zoom in on.
 - Expand Tool —Zooms out one level.

To create a new plot:

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

CFlow displays an FSC-A vs. SSC-A plot by default.

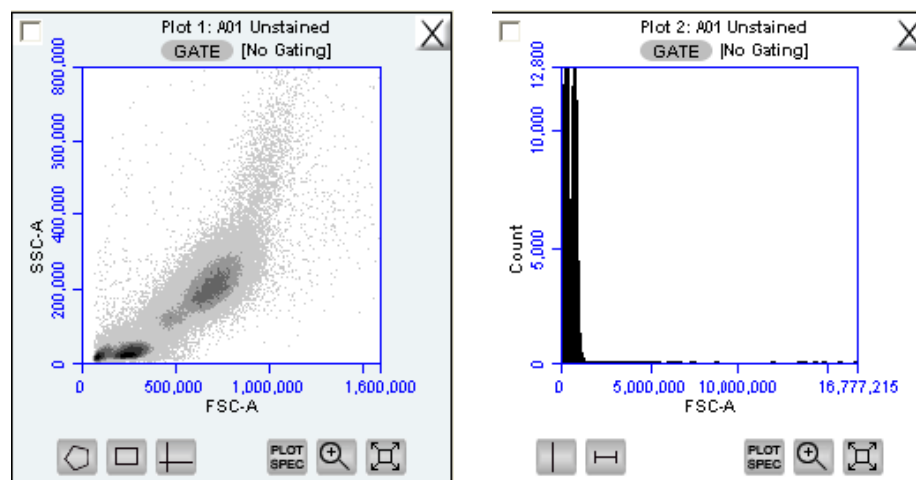


Figure 4-13. New Density and Histogram Plots

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

4.5 Changing Plot Specifications

The Plot Spec Tool allows you to change the way data are displayed in a plot. You can change axis parameters, specify channel ranges, and toggle between linear and logarithmic scales. The Plot Spec Tool is available in the *Collect* and *Analyze* tabs.

You can set up or modify plot specifications at any time before or after collecting data.

To change the plot specifications:

1. Click on the *Plot Spec Tool*  icon.



Figure 4-14. Set Plot Specs Dialog Box

2. In the Set Plot Specs dialog box, do the following for each axis:
 - Select the parameter you want to view in the parameter drop-down list.
 - Select the *linear* or *log* radio button to specify how data is displayed.
 - Type in the minimum and maximum channels to set the channel range to view.
 - Enable or disable the *Hide 1st decade* check box to indicate whether you want CFlow to display the first decade of channels in the plot.
3. 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 your changes.

4.6 Changing the Number of Events in a Plot

You can change the number of events displayed in all plots across all samples to make it easier to view data. This option allows you to visually remove a number of events from the plot without deleting data.

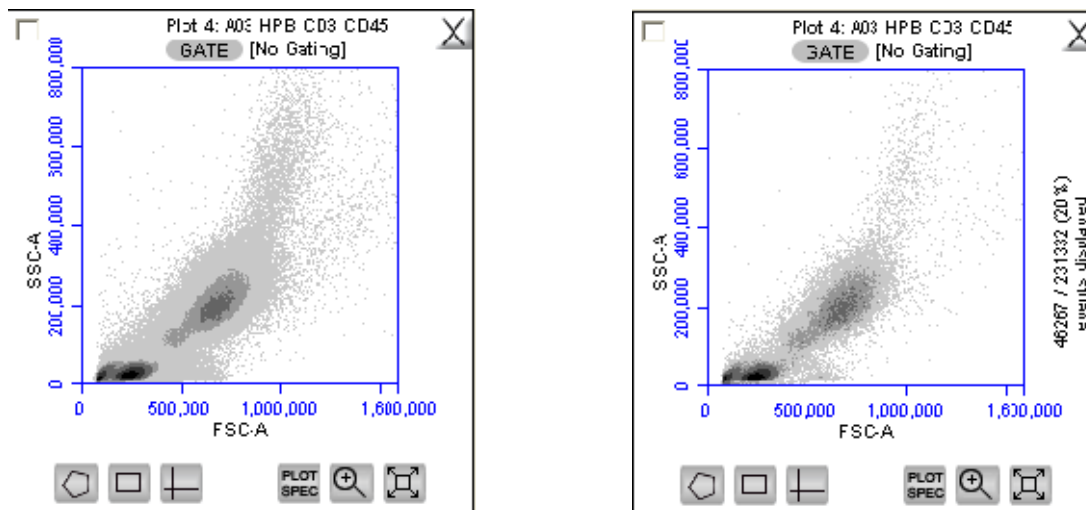


Figure 4-15. Before and After Changing Events Displayed

To change the events displayed in a plot:

1. Select *Display > Events Display Settings*.

2. 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).

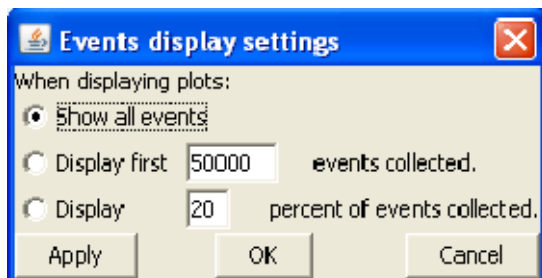


Figure 4-16. Events Display Settings Dialog Box

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

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

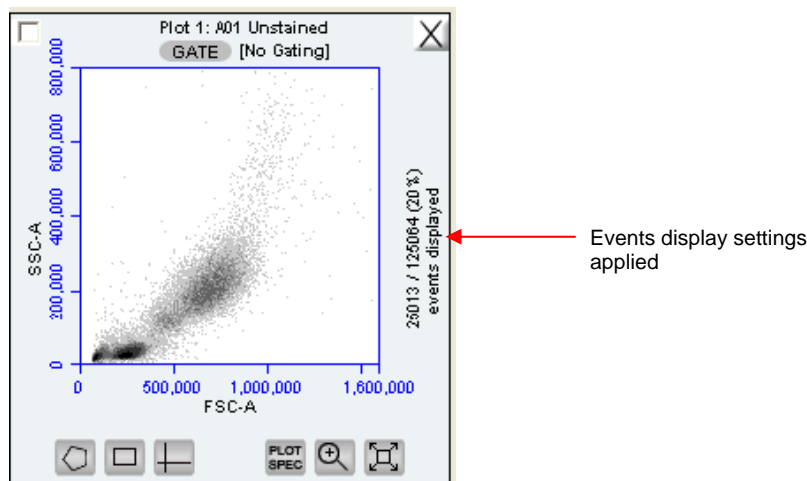


Figure 4-17. Plot with Events Display Settings Applied

4.7 Using Gates and Markers




A gate is a specified area within a plot that is used to designate a set of events to analyze. You can create 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 a horizontal marker.

When you draw a gating region in a plot, you must then apply it to another plot for the gate to take effect.

4.7.1 Creating a New Gate

To create a gate in a density or dot plot:

1. 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 fluorescence compensation (see section 4.12 for details).
2. Use the mouse to draw a region (labeled *P1* for a polygonal gate, *R1* for a rectilinear gate, or *Q1* for a quadrant gate). If you are drawing a polygon, click on the mouse to anchor each vertex and double-click to close the polygon.

CFlow automatically displays the percentage of cells within the region.

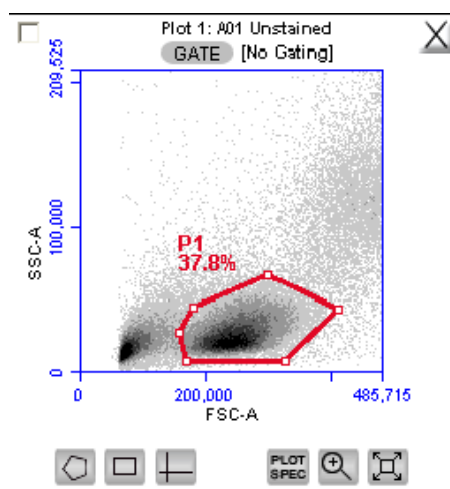


Figure 4-18. Using Polygonal Gating Tool

To create a vertical marker in a histogram plot:

1. Click on the *Vertical Marker Tool* .

- Click the cursor at the point along the x-axis where you want to place the marker. CFlow automatically displays the percentage of cells to the left (V1-L) and right (V1-R) of the marker.

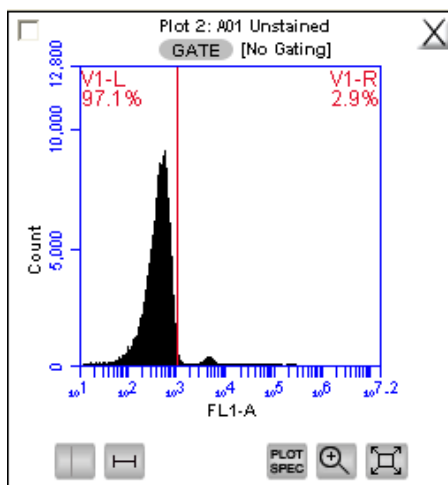



Figure 4-19. Using the Vertical Marker

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 you want to gate. CFlow automatically displays the percentage of cells within the margins of the marker (labeled M1).

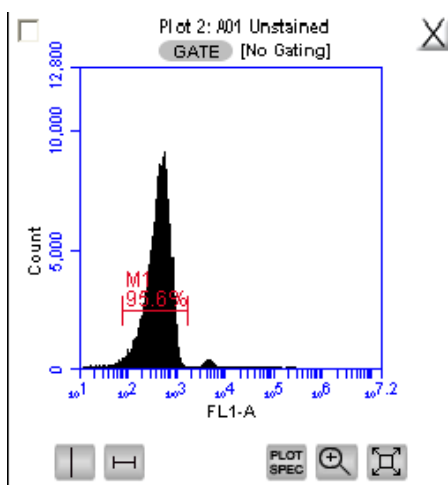


Figure 4-20. Using the Horizontal Marker

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

4.7.2 Applying a Gate to a Plot

To apply a gate to a plot:

1. Click on the *GATE* button at the top of the plot to which you want the gate applied.

NOTE: You cannot apply a gate to the same plot in which you drew the region.

2. Only polygon (P), rectilinear (R), and marker (M) gating regions automatically appear in the Gating dialog box list of options. If you want to view a list of vertical markers or quadrant markers, enable the associated check box(es) in the Change Gating dialog box.

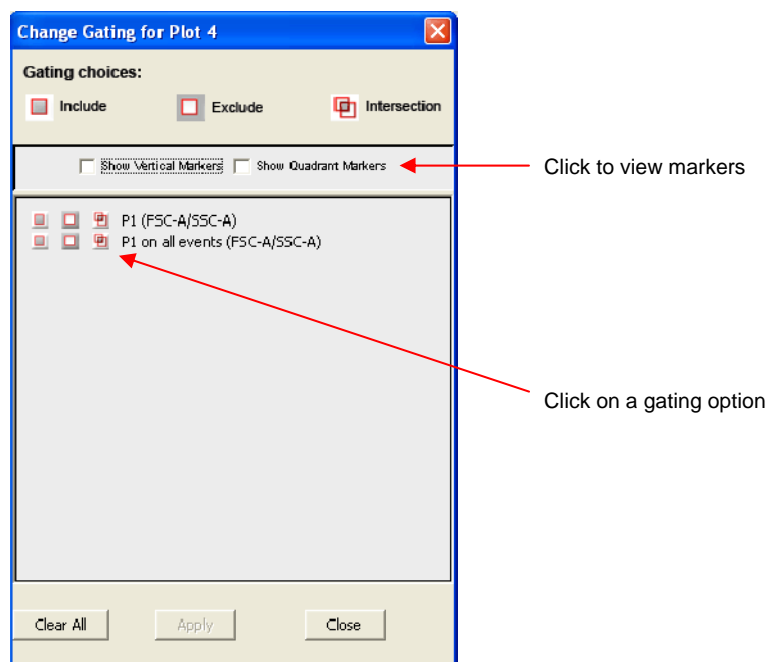





Figure 4-21. Selecting a Gating Option

3. Select one of the following gating icons associated with the gate you want to use:
 - *Include* icon —to analyze the events within the region.
 - *Exclude* icon —to analyze the events outside of the region.
 - *Intersection* icon —to analyze the events within the intersection of two regions (select this icon for each region you want to intersect).

- Click on the *Apply* button. CFlow displays the gate that is applied next to the *GATE* button in the plot.

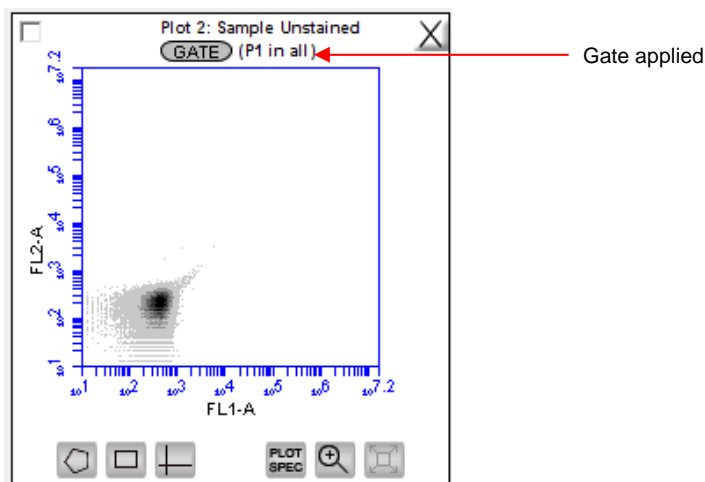


Figure 4-22. Plot Gated to Include P1

4.7.3 Creating and Applying Nested Gates

You can create a series of nested gates in which each gate is a subset of the previous one. This allows you to fine tune your gating strategy for viewing and analyzing specific subsets of data.

To create nested gates:

- Draw any region or marker around a population of events.

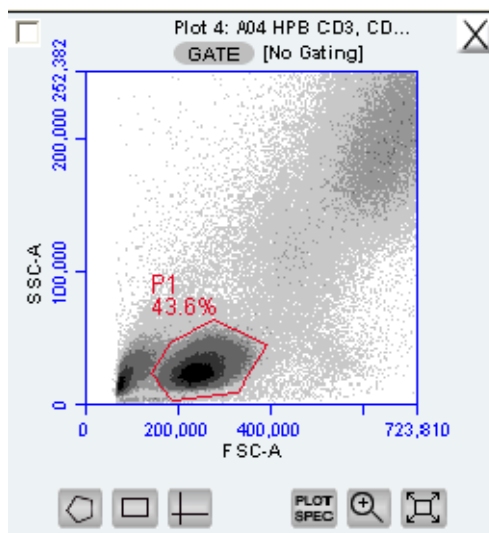


Figure 4-23. First Gate for Creating Nested Gates, Drawn in Plot 4

- Click on the **GATE** button in a second plot and apply the gate. This is the *parent gate*.

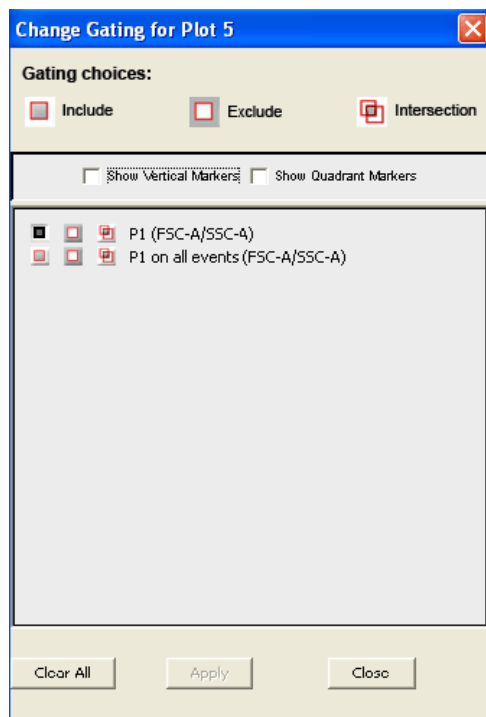


Figure 4-24. Applying the Parent Gate to Plot 5

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

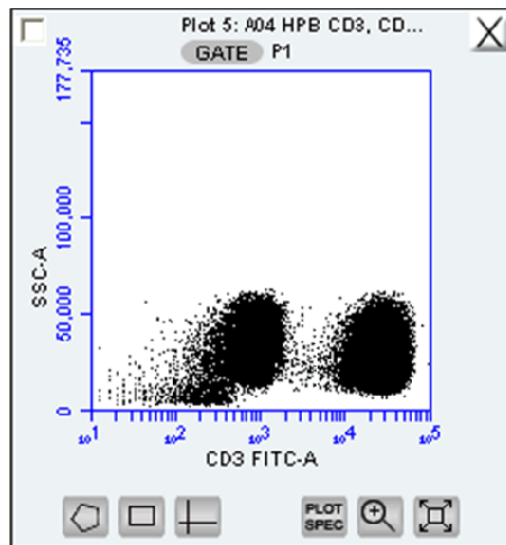


Figure 4-25. Parent Gate Applied to Dot Plot

4. In the plot gated on the parent gate (Plot 5 in this example), draw a second region or marker around a subset of the populations displayed in the plot.

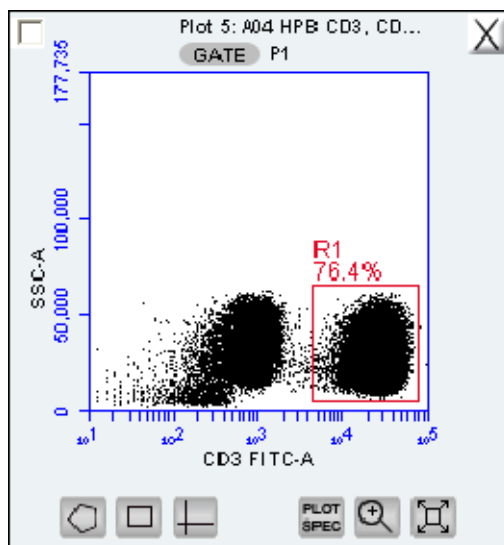


Figure 4-26. Second Gate for Creating Nested Gates

5. Open a third plot and click on the *GATE* button.
6. In the Change Gating dialog box, select the option in which the second gate is “in” the parent gate (see the following figure). Do not select the *on all events* option.

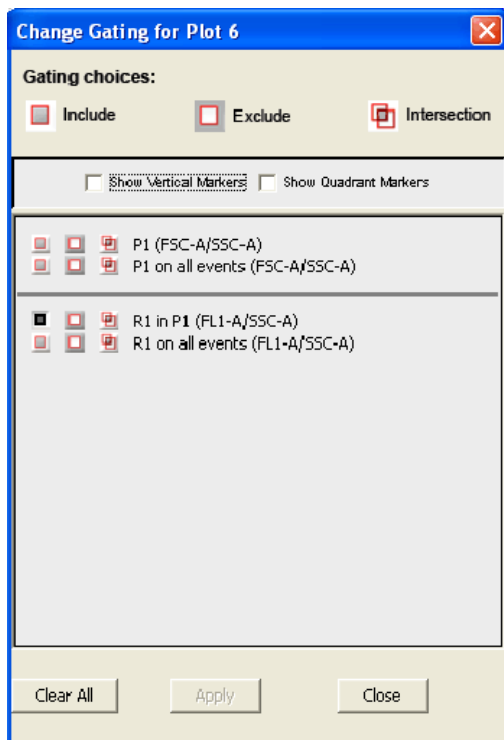


Figure 4-27. Applying the Child Gate

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

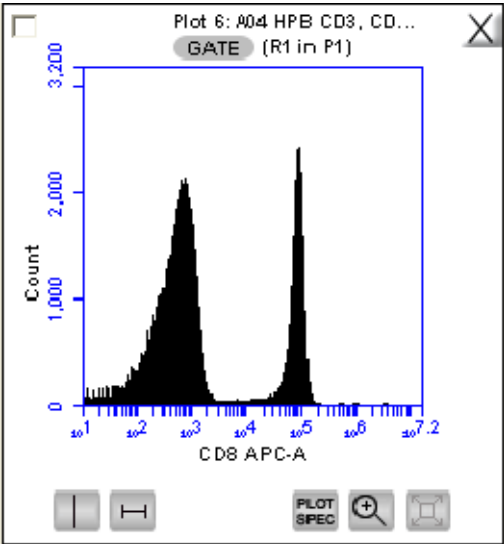


Figure 4-28. Third Plot with Nested Gate Applied (R1 in P1)

8. View the statistics in the Statistics Table.

Plot 6: A04 HPB CD3, CD4, CD45, CD8 Gated on (R1 in P1)	Count	Volume (μ L)	% 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

9. Close the dialog box.

4.8 Naming Plot Axes

You can rename the axis labels in a plot from the *Manual Collect* or *Analyze* tab to identify the antibody staining or fluorochrome used in the sample.

To name a plot axis:

1. Click on an axis label and select *Rename Parameters* from the pop-up menu.

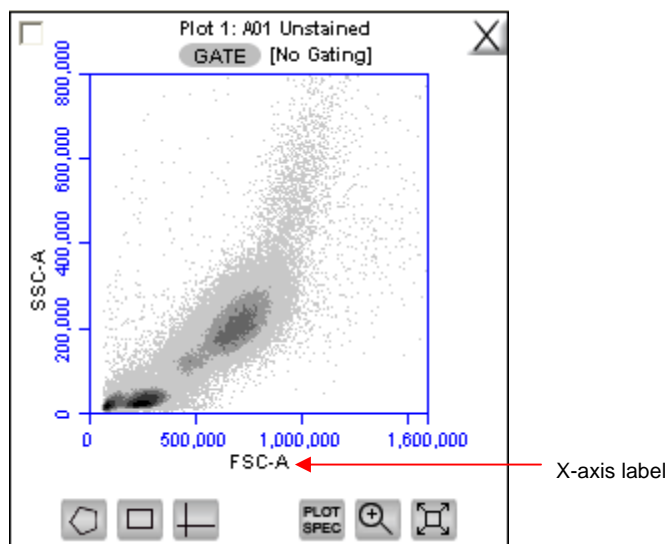


Figure 4-30. X-Axis Label

2. In the Rename Parameters dialog box, type the new label in the edit box of the parameter you want to rename.

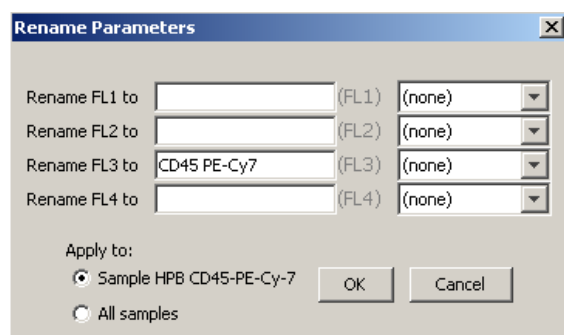


Figure 4-31. Rename Parameters Dialog Box

3. 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.

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

1. Select another sample from the 96-well grid.
2. Click on an axis label in a plot and select *Rename Parameters* from the pop-up menu.

3. Select the name from the drop-down list associated with the parameter.

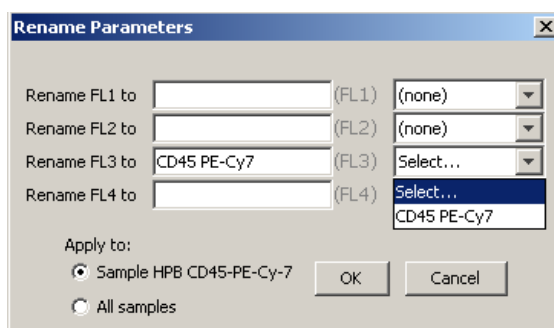



Figure 4-32. Rename Parameters Dialog Box with Axis Label Drop-Down List

4.9 Zooming on a Plot

CFlow automatically zooms the initial display of any parameter on a logarithmic scale from channel 10 to 16.7×10^6 . 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 gating regions, especially when setting fluorescence compensation (see section 4.12).

4.9.1 Basic Zoom

To zoom on a population:

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

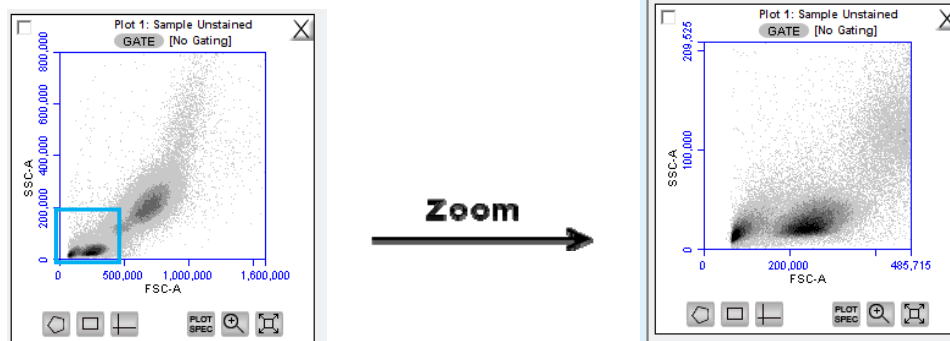



Figure 4-33. Before and After Using Zoom Tool

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

To zoom out:

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

4.9.2 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:

1. Click on the Plot Spec Tool  in the plot you want to zoom.

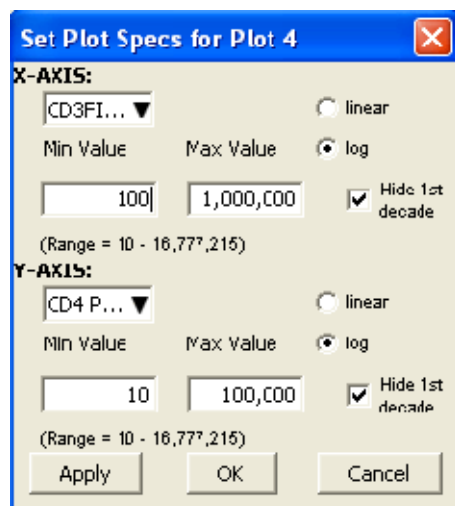


Figure 4-34. Plot Spec Dialog Box: Set Min and Max Channel Values for the X- and Y-Axes

2. 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.
3. Specify the y-axis channel range by typing a minimum and maximum value under Y-Axis.
4. Click on the *Apply* button to apply the changes and click on the *OK* button to close the Plot Spec dialog box.

4.10 Saving a CFlow File

Always save C6 data as a CFlow file (.c6). A CFlow file is a comprehensive (and often large) data file that contains instrument settings, FCS files, and plot layouts.

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

- Sample data
- Plot layouts
- Gating
- Color compensation
- Threshold settings
- *Collect* tab settings
- Changes made in the *Analysis* or *Statistics* tabs

By default, CFlow automatically saves CFlow data at the end of each sample run. You can also manually save data at any time. To save the entire CFlow file, save the file manually (see section 4.10.2).

When a CFlow file is saved, CFlow displays the file name in the upper left corner of the workspace (Figure 4-35).



Figure 4-35. Title Bar with File Name

4.10.1 Auto-Saving Files

By default, CFlow automatically saves the event data any time the C6 reaches a run limit or if you click on the *Pause* button during a run. Auto-save does not save acquisition settings, plots, or gating strategies.

CAUTION: If you make changes after a run or after pausing a run, CFlow *does not* automatically save the file. Save these changes manually (see section 4.10.2).

To enable or disable auto-save:

1. Select *File > Auto-save Settings*.
2. 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.

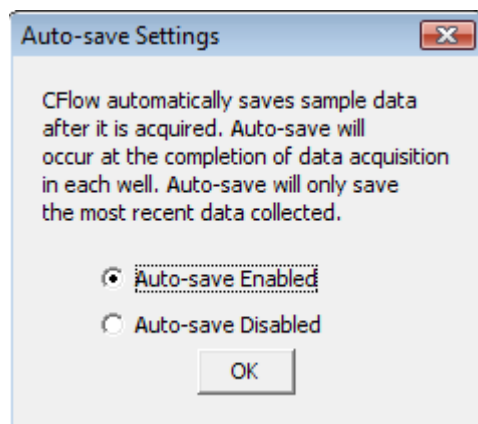


Figure 4-36. Auto-Save Settings Dialog Box

3. Click on the *OK* button to accept the change and close the dialog box.
4. 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.

4.10.2 Manually Saving Files

You can manually save a CFlow file at any time.

To manually save a CFlow file:

1. Select *File > Save*.

To manually save a CFlow file with a new name:

1. Select *File > Save CFlow File As*.

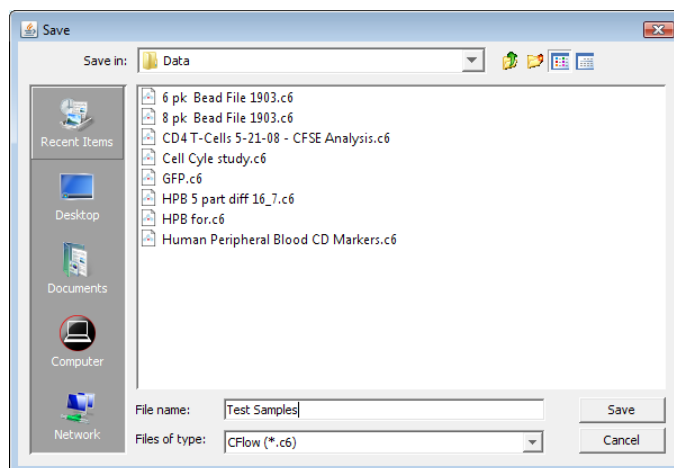


Figure 4-37. Save CFlow File

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

4.11 Creating a CFlow Template

A CFlow template contains a predefined CFlow 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 CFlow Installation CD or the Accuri website at

www.accuricytometers.com/technical_information/templates/) or you can create your own customized templates.

To create a template:

1. Define plot, gating, and acquisition settings in a blank workspace, or use the current .c6 file.

2. Select *File > Save CFlow template as*.

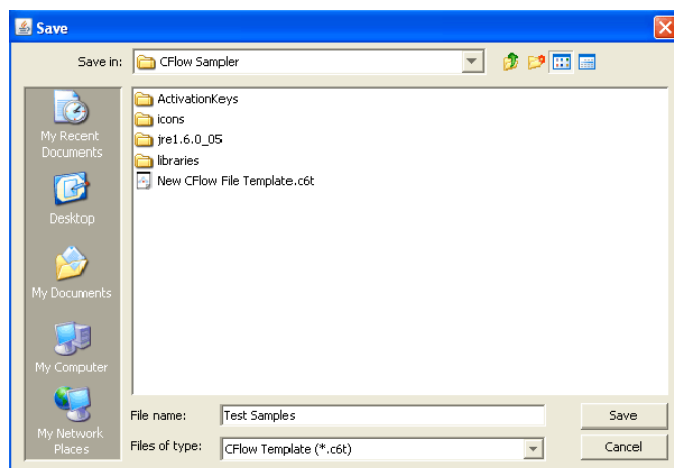


Figure 4-38. Save CFlow Template

3. If necessary, navigate to the location to save the file.
4. In the Save dialog box, enter the file name and click on the **Save** button. CFlow saves the file with the extension **.c6t**.

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

4.12 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 of a cytometer 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.

4.12.1 Recognizing Fluorescence Spillover

Whenever performing a multi-color experiment, prepare a set of control samples, each stained with one individual fluorochrome. These single-stained controls will allow you to determine 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 was detected in the FL3 (670 LP), as expected. However, there is also PE-Cy7 signal detected in FL1 (530 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.

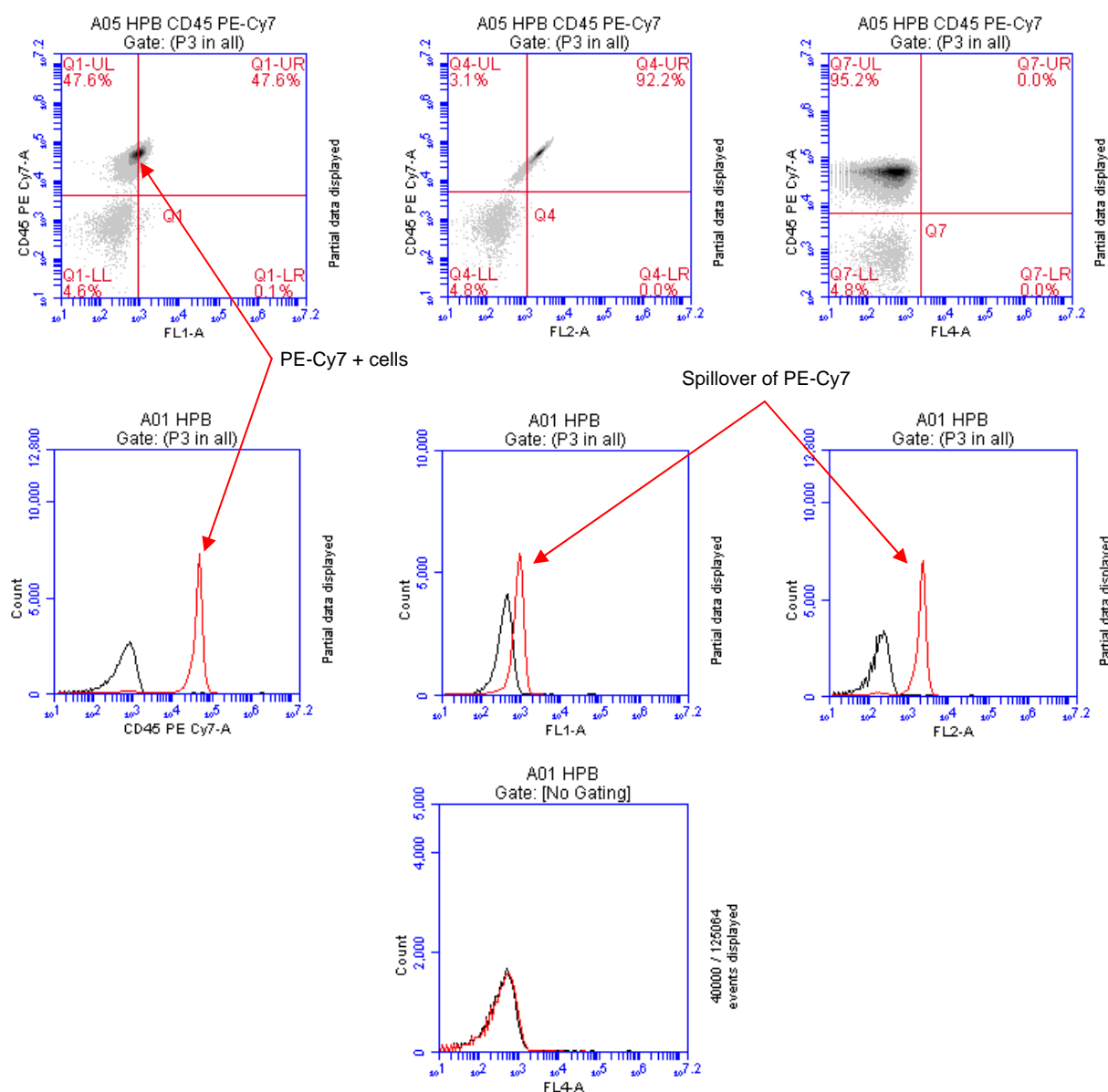


Figure 4-39. Fluorescence Spillover in Different Plots

4.12.2 Correcting Fluorescence Spillover

You can remove fluorescence spillover 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 C6 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 of 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.

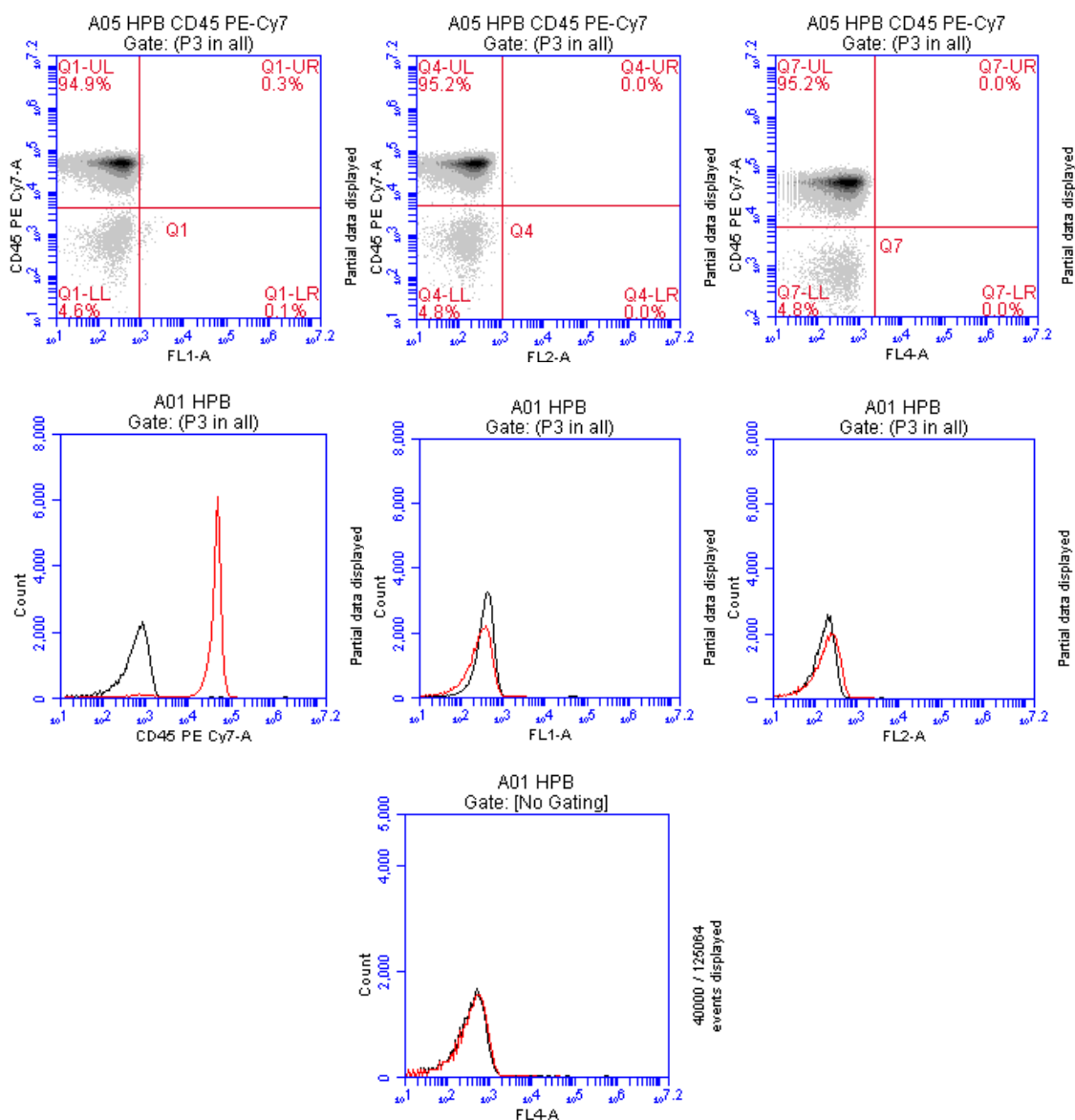



Figure 4-40. Corrected Fluorescence Spillover

To correct fluorescence spillover:

1. Click on the Quadrant Tool  of a plot you want to correct and click inside the plot.

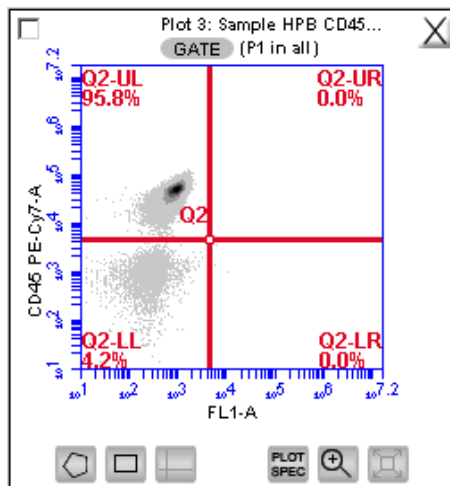


Figure 4-41. Placing a Quadrant Tool

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

CFlow displays the median fluorescence channel value for the events in each quadrant in the Statistics Table (shown below). No median is calculated for the line named *All* or *This Plot* because this line contains summary data for the entire plot.

<input type="checkbox"/> 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 4-42. Statistics Table Displaying Median Values

3. 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), you will need to perform fluorescence compensation.

- Click on the *Set Color Compensation* button in the *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.

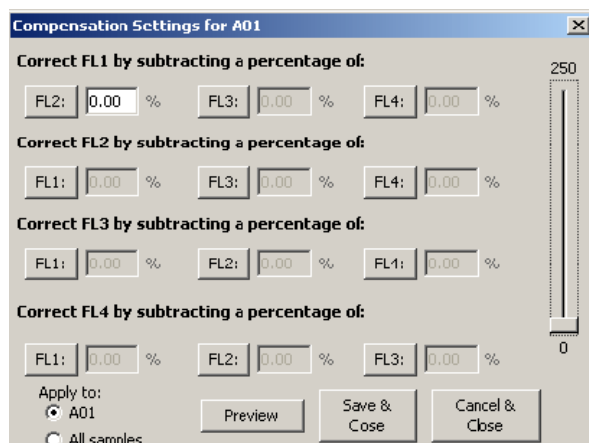


Figure 4-43. Compensation Settings Dialog Box

- In the row associated with the channel to correct, click on the *FL* button of the fluorescence channel that is spilling over.
- 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 provided by Accuri (available on the CFlow installation CD or at www accuricytometers.com/technical_information/Brochures/manuals/) to calculate the subtraction values. The spreadsheet is more accurate and saves time.
 - Use the following table as a guide:

Table 4-3. Fluorochrome Spillover per Channel

		Spillover Fluorochrome					
		FITC	PE	PerCP	PerCP Cy5.5	PE-Cy7	APC
Channel to Correct	FL1 (530BP)	N/A	3.5	0.00	0.0	1.00	N/A
	FL2 (585 BP)	7.0	N/A	0.00	0.00	3.50	0.0
	FL3 (670 LP)	1.0	14.5	N/A	N/A	N/A	1.2
	FL4 (675 BP)	0.0	0.0	3.00	12.00	0.00	N/A

- Click on the *Preview* button to update the Statistics Table.
- In the Statistics Table, observe the values in the *Median* column of the FL channel you are correcting.

9. Repeat steps 5-8 until the 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.

Vol (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

Compare medians

Figure 4-44. Results of Subtracting Spillover

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

4.12.3 Troubleshooting Color Compensation

Occasionally, the plot might appear to have a smaller percentage of events in a quadrant than CFlow reports as present. For example, Figure 4-45 appears to have less than 26.1% of the population in the lower right quadrant. 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.

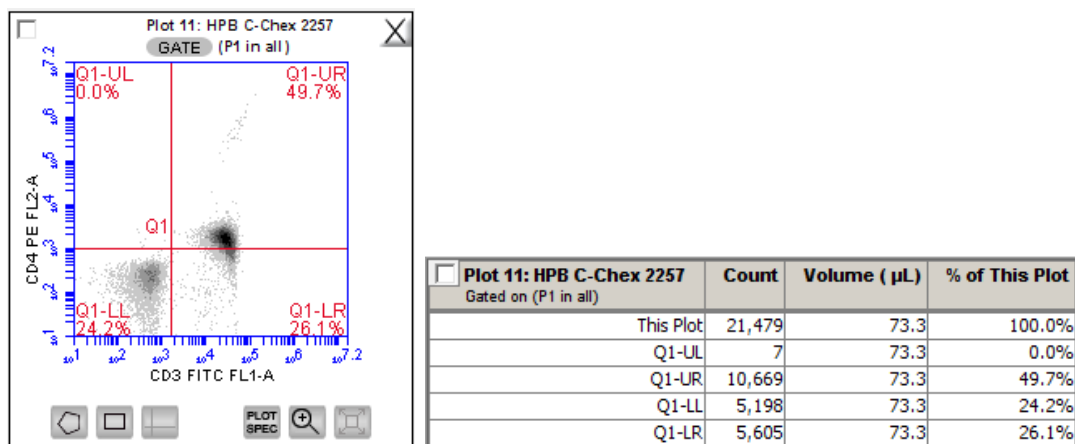


Figure 4-45. Plot Displaying Overcompensation

To fix overcompensation:

1. 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.

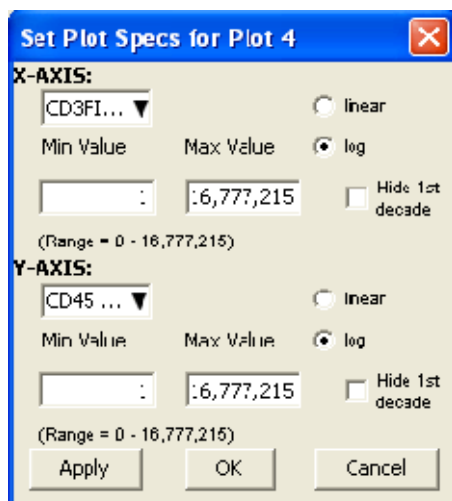
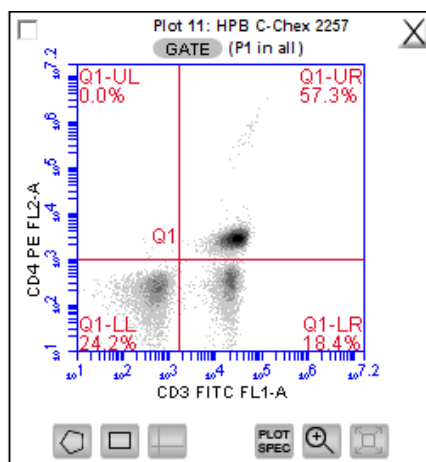


Figure 4-46. Set Plot Specs Dialog Box with *Hide 1st decade* Disabled

- Click on the **OK** button to apply the settings and close the dialog box.
- Perform the fluorescence compensation procedure described in section 4.12.2. 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 4-47.



<input type="checkbox"/> Plot 11: HPB C-Chex 2257 Gated on (P1 in all)	Count	Volume (μ L)	% 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 4-47. Properly Compensated Data After First Decade Is Rehidden

4.13 Changing Parameters

You can change the parameters that a plot displays along the x- and y-axes. By default, CFlow displays the area parameter (signified with a suffix of -A), but you can also choose a height, width, or time parameter.

To change a parameter:

1. Click on the x- or y-axis label and select the option you want from the pop-up menu.

NOTE: The time parameter starts counting when you click on the *Run* button and does not stop until the run is completed, even if you pause the run. You cannot reset the parameter to zero, even by deleting data.

4.14 Agitating Samples

The agitate feature allows you to keep a sample in suspension. It is not designed to resuspend a completely settled sample. You can perform a single agitation cycle in the *Manual Collect* tab.

CAUTION: Accuri recommends that sample volume not exceed 50% well capacity to effectively maintain suspension with agitate, and that sample volume not exceed 70% well capacity to avoid spillover during agitation.

To perform an agitate cycle:

1. Click on the *Agitate* button.

4.15 Copying and Pasting Plots

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

1. Click anywhere on a plot and drag it to an open Microsoft application. You cannot use **Ctrl+C** and **Ctrl+V** to copy and paste plots from CFlow into other applications.

4.16 Printing Data

You can print selected plots and their associated statistics from the *Manual Collect* or *Analyze* tab.

To print plot data:

1. Enable the check box in the upper left corner of each plot that you want to print.

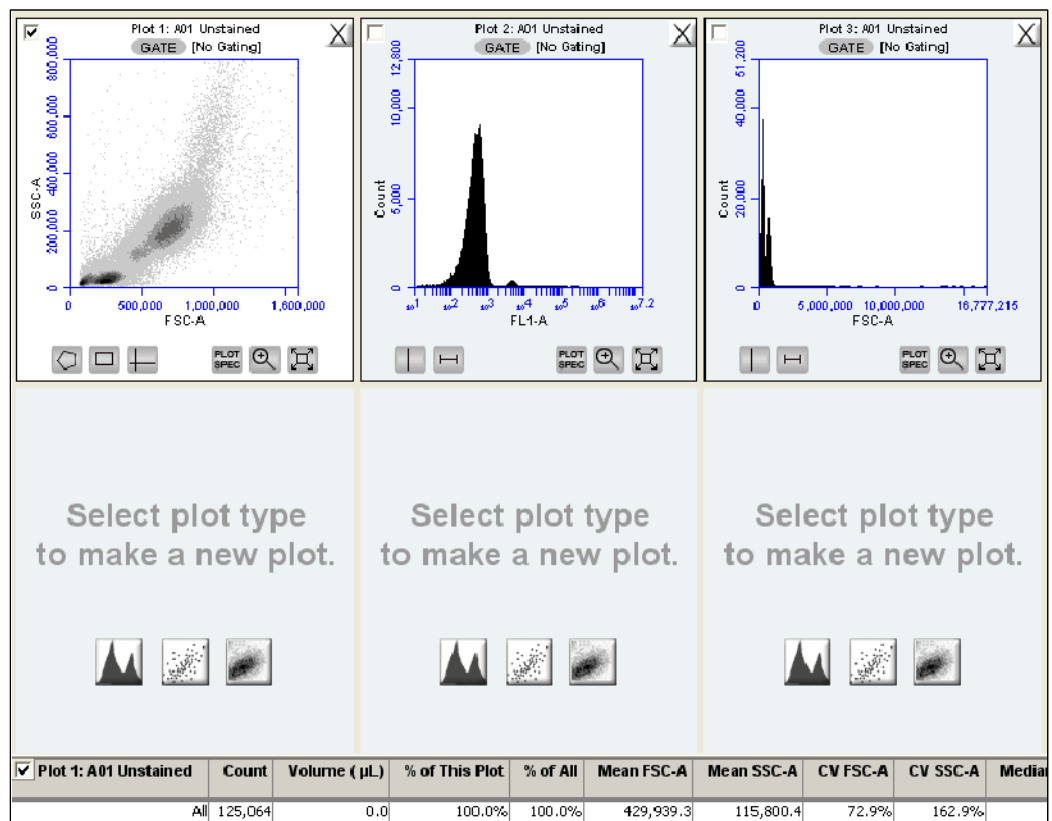


Figure 4-48. Plots Selected for Printing

2. If you do not want to print the associated Statistics Tables, disable the check box in the upper left corner of each table.
3. Select *File > Print Selected Items*.

4.17 Exporting and Importing Files

You can export data from individual sample wells from any tab as FCS 3.0 files at any time.

To export data:

1. 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 F for an example .csv file).
2. If prompted to confirm the export, click on the *OK* button.

To import an FCS data file into CFlow:

1. Select an empty data well in a CFlow file or template.
2. Select *File > Import FCS File*.
3. Navigate to the location of the file.

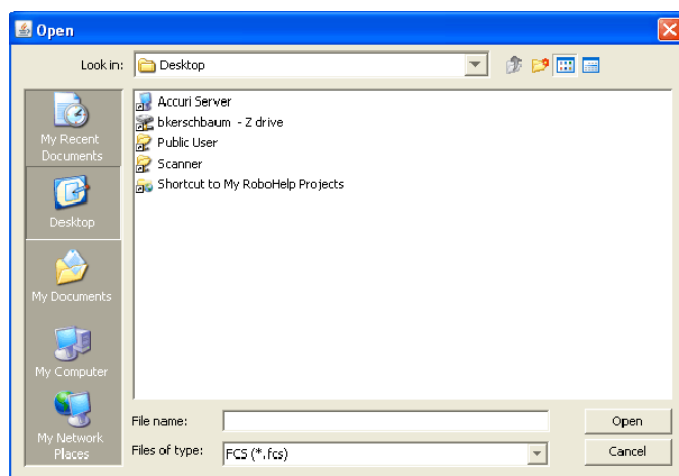


Figure 4-49. Open an FCS file

4. Select the file and click on the *Open* button.

5 AUTOMATIC DATA ACQUISITION

The *Auto Collect* tab allows you to automatically run multiple samples from a well plate or the sample tube rack. You can also easily label a large number of samples and create sets of samples. 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
- Importing and exporting data

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 that allows you to name samples, rename parameters, and add a notation to each sample.

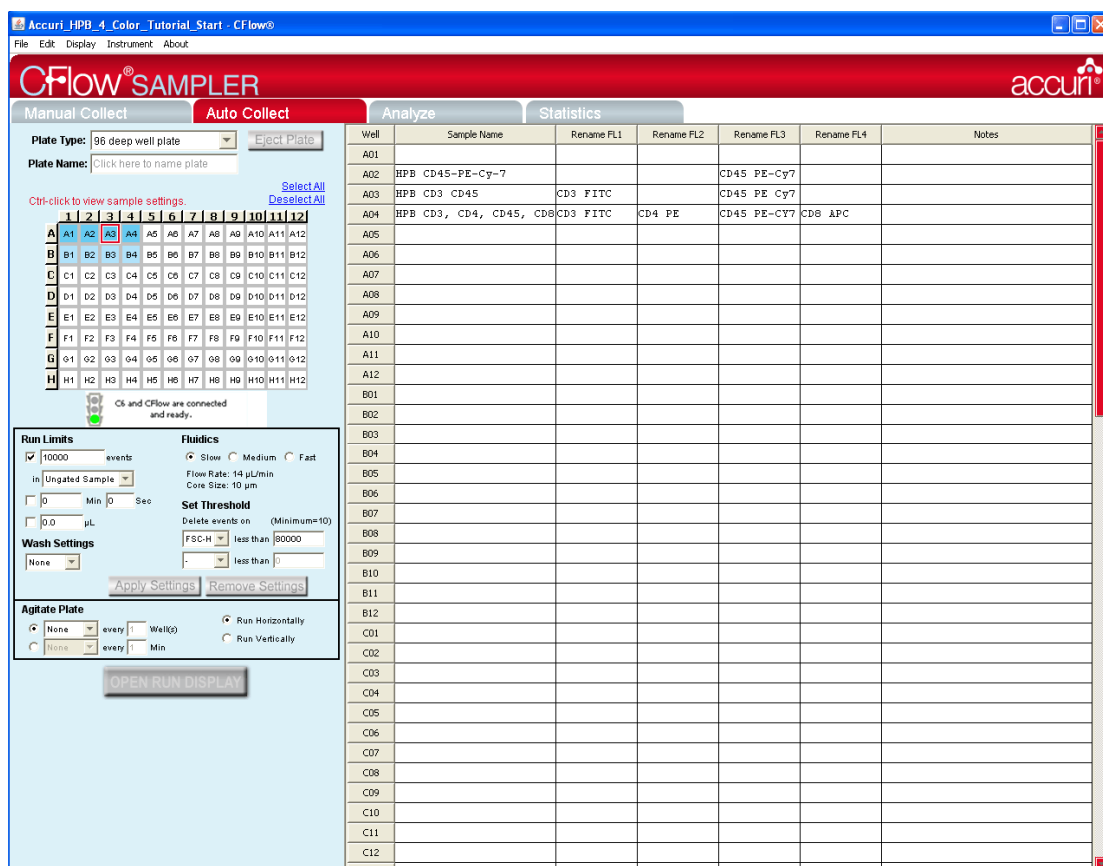



Figure 5-1. CFlow Sampler Auto Collect Tab

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

Table 5-1. Auto Collect Tab Controls

Control	Description
<i>Plate Type</i>	Drop-down list for setting the type of plate you are using.
<i>Load Plate/Eject Plate</i>	Moves the sample plate into position to be loaded on or ejected from the C6.
<i>Plate Name</i>	Text box for naming the plate you are using.
<i>Sample Grid</i>	<p>Matrix laid out in the configuration of a 96-well plate to help organize experiments and collect data from sample tubes. CFlow acquires each sample into its own well in the Sample Grid. The wells can be filled with data in any order.</p> <p>The wells are color-coded:</p> <ul style="list-style-type: none"> • White—Does not contain data. • Blue—Contains data. • Red outline—Currently selected for viewing or collecting data.

<i>Control</i>	<i>Description</i>
<i>Traffic Light and Message</i>	<p>Indicator that displays CFlow's readiness and system messages. Before data collection can begin, CFlow must display a green Traffic Light  with the message <i>C6 and CFlow are connected and ready</i>.</p> <p>The Traffic Light status is color-coded:</p> <ul style="list-style-type: none"> Green—CFlow is ready to collect data or is collecting data. Yellow—The C6 is preparing to perform an action or a non-critical error has occurred. Red—A critical error has occurred.
<i>Run Limits</i>	Contains a set of controls that allow you to define criteria for automatically stopping the data collection. See section 5.6.3 for details.
<i>Fluidics</i>	Contains a set of controls for defining flow rate and core size. See section 5.6.1 for details.
<i>Wash Settings</i>	Performs a wash cycle to keep the SIP clean between samples. See section 5.6.5 for details.
<i>Set Threshold</i>	Sets the event threshold to gate out debris and noise from cell samples. The default value is 80,000 on FSC-H. See section 5.6.2 for details on setting threshold values.
<i>Apply Settings/Remove Settings</i>	Allows you to apply or remove sample settings to a sample set. See sections 5.5.1 through 5.5.5 for details.
<i>Agitate Plate</i>	Performs an agitate cycle to keep particles in suspension. See section 5.6.4 for details.
<i>OPEN RUN DISPLAY</i>	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.
<i>Sample Annotation Table</i>	Allows you to name samples, rename parameters, and add a notation to each sample. You can manually enter information for each sample or copy and paste it 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 CFlow Sampler.

1. Perform preacquisition functions in the *Manual Collect* tab.
2. Click on the *Auto Collect* tab.
3. Create sample sets with specified acquisition, agitation, and wash settings.
4. Define the file name for auto-save.
5. Create the Sample Annotation Table.
6. Select the direction to run the plate.
7. Open the Run Display.
8. Select the plots to view during acquisition.
9. Click on the *AUTORUN* button.

10. When the plate is complete, close the Run Display.

5.3 Assigning a Plate Type

When setting up an experiment, select the plate type that you are using.

NOTE: If you change to a new plate type, CFlow opens a new blank workspace.

To assign a plate type:

1. 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

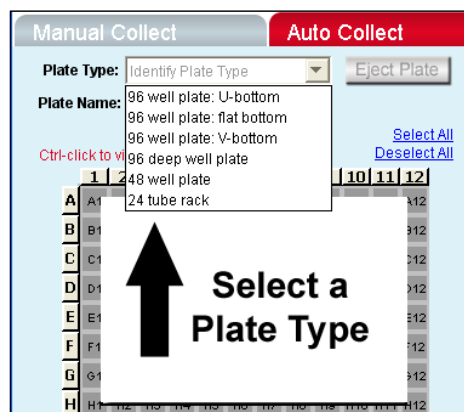


Figure 5-2. Plate Type Drop-Down Menu

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

- 96-well plate assignment—Displays all 96 wells
- 48-well plate assignment—Displays wells A1-F8
- 24-tube rack assignment—Displays wells A1-D6

5.4 Naming the Plate

You can optionally name the sample plate you are using, to distinguish it from other sample plates in similar experiments.

To name the plate:

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

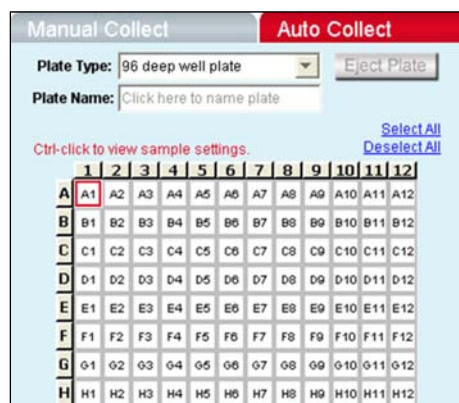


Figure 5-3. Plate Name Field

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). You can set up one or more of the following criteria to determine how the samples are to be collected:

- Run limits
- Fluidics settings
- Threshold(s)
- Wash cycles

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

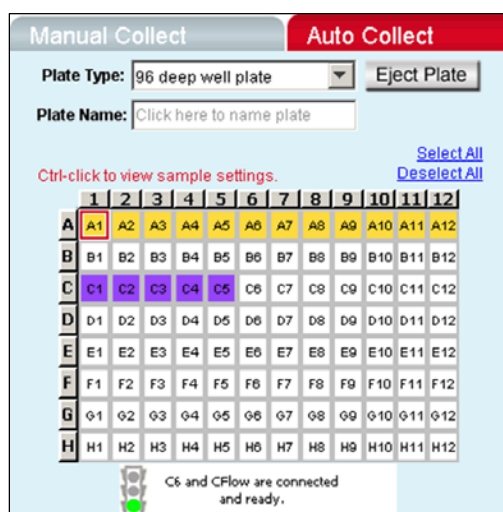


Figure 5-4. The *Auto Collect* Tab displaying two data sets

CAUTION: Once you apply settings and start a plate run, the C6 runs every well in the set, even if the well already contains data.

5.5.1 Creating Sample Sets

To create a sample set:

1. 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.

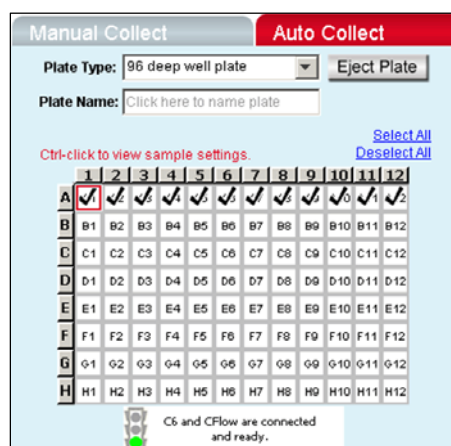


Figure 5-5. Selected Wells in the *Auto Collect* Tab

2. Enter settings for one or more of the following:
 - Fluidics (see section 5.6.1)
 - Threshold (see section 5.6.2)
 - Run limits (see section 5.6.3)
 - Agitate cycles (see section 5.6.4)
 - Wash cycles (see section 5.6.5)

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.

Manual Collect Auto Collect

Plate Type: 96 deep well plate Eject Plate

Plate Name: Click here to name plate

Select All
Deselect All

Ctrl-click to view sample settings.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

C6 and CFlow are connected and ready.

Run Limits
☐ Run Unlimited
☒ 10000 events
 in Ungated Sample
☐ 0 Min 0 Sec
☐ 0.0 µL

Fluidics
☒ Slow ☐ Medium ☐ Fast
 Flow Rate: 14 µL/min
 Core Size: 10 µm

Set Threshold
 Delete events on (Minimum=10)
 FSC-H less than 80000
 less than 0

Wash Settings
 None
 1 cycle takes about 1 min.

Apply Settings Remove Settings

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

5.5.2 Viewing Sample Settings

To view a well's data acquisition settings:

- Press **Ctrl** and click on the well you want to view.

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.
- Change the data acquisition settings that you want to modify.
- Click on the *Apply Settings* button. The selected wells change to a new color.

5.5.4 Saving a Sample Set

CFlow Sampler saves all sample settings every time you click on the *Apply Settings* button. Data is automatically saved during a run, after each sample is complete.

To manually save sample settings:

1. Click on the *Apply Settings* button. The first time you apply the settings, CSampler prompts you to name the file and designate a file location.

5.5.5 Removing Settings

To remove settings from a set:

1. 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.
2. 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

You must define data acquisition settings when you create a sample set for an auto-run. This section gives details on assigning the following settings:

- Fluidics rate
- Threshold
- Run limits
- Agitate cycles
- Wash cycles
- Run direction

5.6.1 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 2500 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:

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

NOTE: It is recommended to start data collection at slow and observe the data rate. You can then adjust the setting to medium or fast, if necessary.

5.6.2 Setting the Threshold

Thresholds gate out debris and noise from cell samples so that sample data are not compromised. By default, CFlow filters out events lower than channel 80,000.

You can change the threshold settings at any time before, during, or after data acquisition. The primary threshold is the parameter that triggers data collection. You can optionally set a secondary threshold to filter out additional data. FSC-H is the default primary threshold.

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

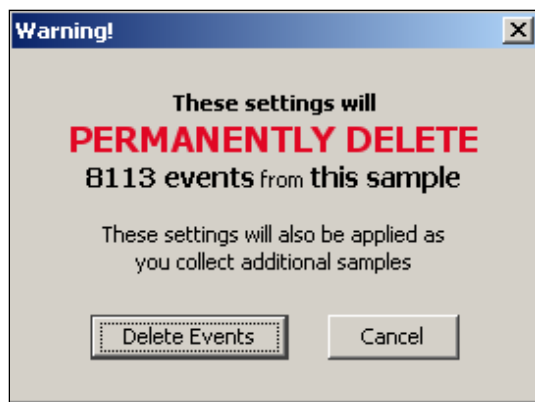


Figure 5-7. Threshold Settings Warning Message

To set the threshold:

- Under the Set Threshold controls, select the primary threshold parameter from the top drop-down list.

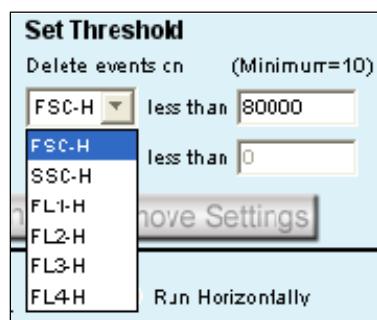


Figure 5-8. Threshold Settings Controls

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

NOTE: You may need to set a lower or higher FSC-H threshold when working with small cells (such as platelets or bacteria) or large cells (such as cell lines).

4. If you want to apply a secondary threshold for filtering out more data, do the following:
 - Select the threshold parameter from the bottom drop-down list.
 - Type a value in the *less than* edit box to set the threshold minimum.

5.6.3 Setting a Run Limit

Set a run limit to indicate to CFlow when to stop collecting data. You can set a run limit based on any of the following criteria::

- After a specified number of events
- After a specified time
- After a specified volume

You can also choose multiple run limits (the C6 stops on the first limit reached).

For the first sample of your experiment, it is recommended to collect a total of 100,000 to 200,000 ungated events. You can change the run limit once a gate has been set on the population of interest.

Figure 5-9. Run Limits Controls

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

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

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

To stop the run after a time has expired:

1. Enable the check box next to the *min* and *sec* fields.
2. 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:

1. Enable the check box next to the *µL* field.

2. Type the volume in microliters (μL) at which to stop the run.

5.6.4 Agitating Samples

You can keep your samples in suspension by using the Agitate feature. Agitate gently shakes the sample plate/tube rack in 15 second cycles.

CFlow Sampler can automatically perform up to three agitation cycles from the *Auto Collect* tab during the plate run. You can set agitation after a given number of samples or in one minute intervals for up to 30 minutes. If the interval is reached during acquire, CFlow Sampler waits for the collection to finish then performs the agitation.

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

CAUTION: Accuri recommends that sample volume not exceed 50% well capacity to effectively maintain suspension with agitate, and that sample volume not exceed 70% well capacity to avoid spillover during agitation.

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

To set an agitate cycle:

1. 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.
2. Select the number of agitate cycles you want to run at a time.

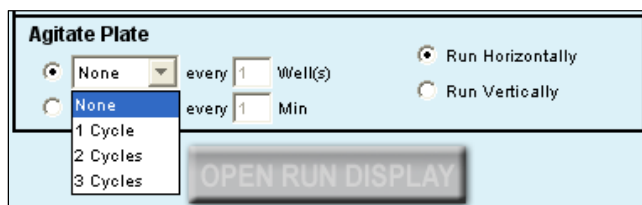


Figure 5-10. Agitate Plate Controls

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

5.6.5 Washing the SIP Between Samples

The *Auto Collect* tab allows you to automatically perform multiple wash cycles of the SIP after each well is complete. You can select up to three wash cycles per wash. One wash cycle typically reduces carryover to < 1.0%. Two wash cycles typically reduce carryover to < 0.1%.

To wash the SIP:

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

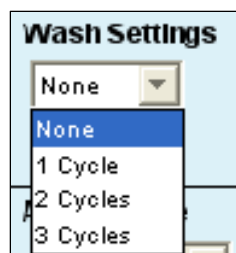


Figure 5-11. Wash Settings Controls

2. Click on the *Apply Settings* button.

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

5.6.6 Changing Run Direction

You can collect data from plates either horizontally (A1, A2, A3, etc.) or vertically (A1, B1, C1, etc.) in the *Auto Collect* tab.

To set the run direction:

1. 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 5-12. Run Direction Controls

5.7 Creating the Sample Annotation Table

The Sample Annotation Table allows you to name samples, rename parameters, and add a notation to each sample. You can manually enter information for each sample or copy and paste it 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 5-13. Sample Annotation Table

To manually create the Sample Annotation Table:

- For each sample well, type in the following information:
 - Sample Name*—Name of the sample
 - Rename FL1-Rename FL4*—Renamed labels for parameters FL1 through FL4
 - Notes*—Any annotations you want to note about the sample

To copy and paste information from a spreadsheet:

- In the spreadsheet, highlight the fields you want to copy to CFlow.
- Press **Ctrl + C**.
- In the Sample Annotation Table, click on the first cell you want to copy the information into (usually *Sample Name* of well A1).
- Press **Ctrl + V** to populate the Sample Annotation Table with all of the data from the spreadsheet.

5.8 Using the Run Display to Perform an Autorun

The Run Display allows you to run data collections, view acquisition data, and view sample data while it is being collected from *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:

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

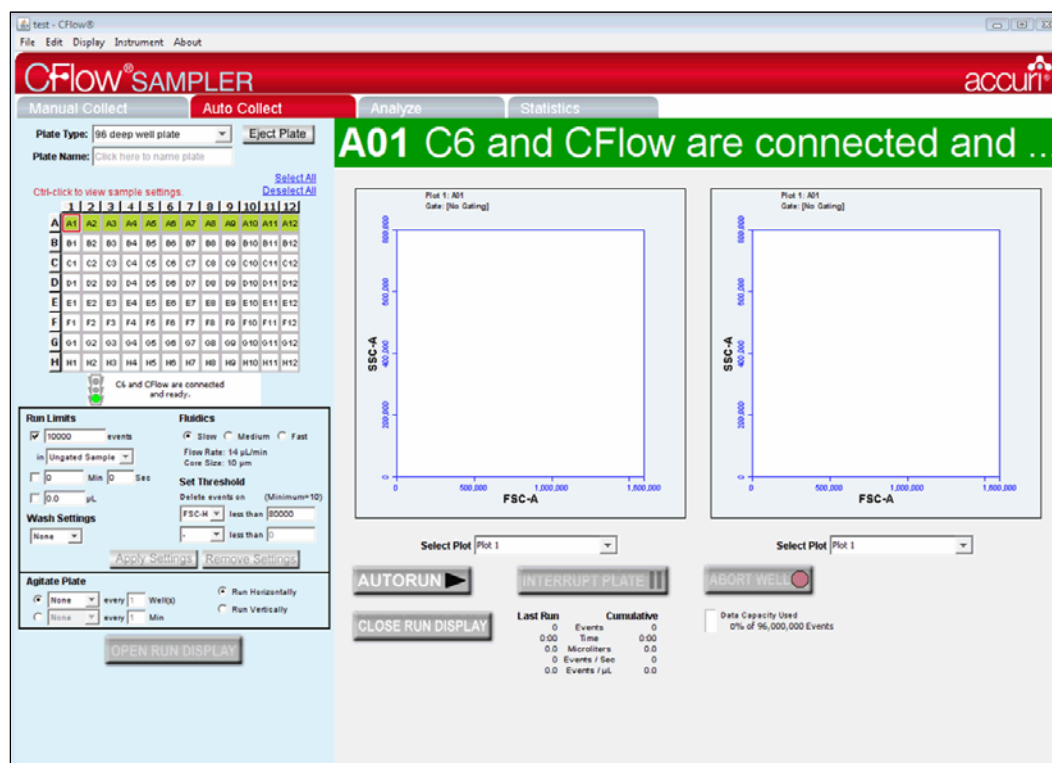


Figure 5-14. CFlow Sampler Run Display

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

1. Click on the *CLOSE RUN DISPLAY* button.

When data collection is complete, CFlow Sampler displays **DONE!** prominently across the Run Display.

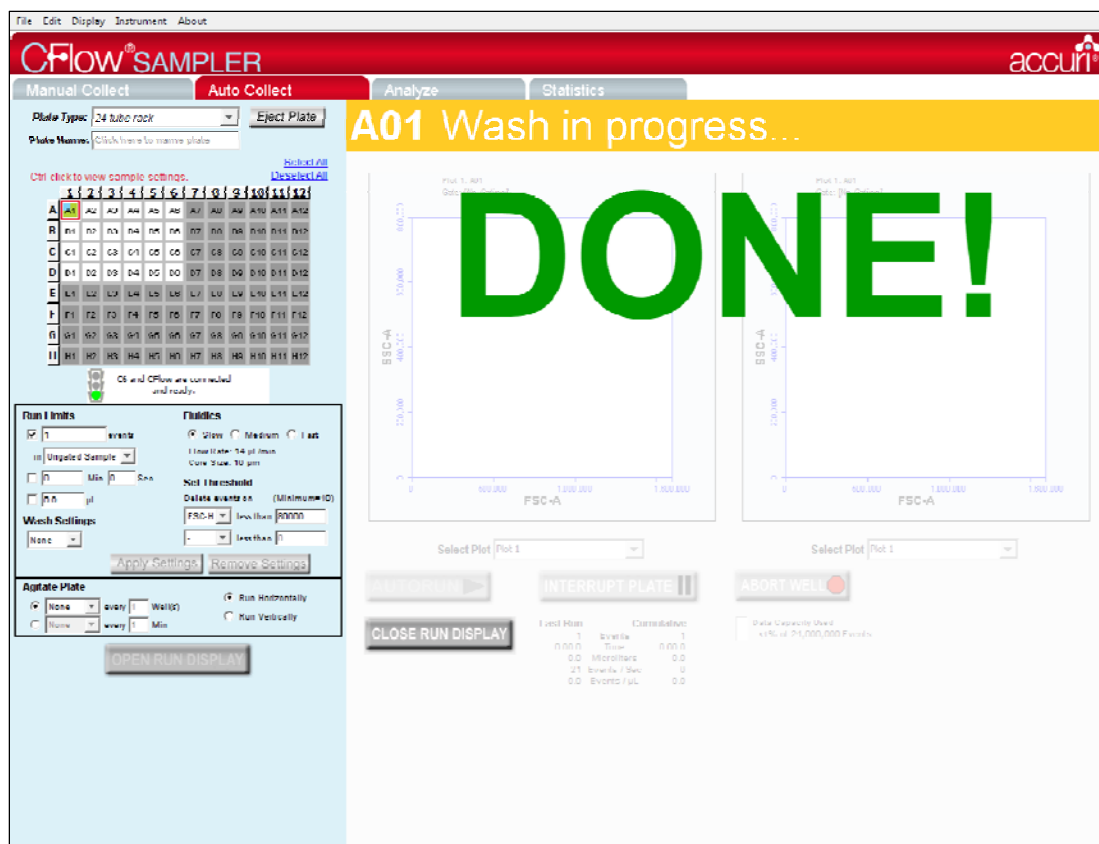


Figure 5-16. The CFlow Sampler Display After a Sample Run

5.8.2 Stopping Data Collection

You can stop acquiring data during a collection by interrupting the plate or aborting the well.

- **Interrupt Plate**—Completes data collection from the current well and performs wash and agitate cycles before stopping the plate run. You 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:

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

To restart a run during an Interrupt state:

1. Use the *Manual Collect* tab to ensure that the run settings of the interrupted plate are retained when the plate run is restarted.

- Click on the *RUN* button. CFlow Sampler begins acquiring data at the next sample well.

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, go to 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. You can view two plots at a time.

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

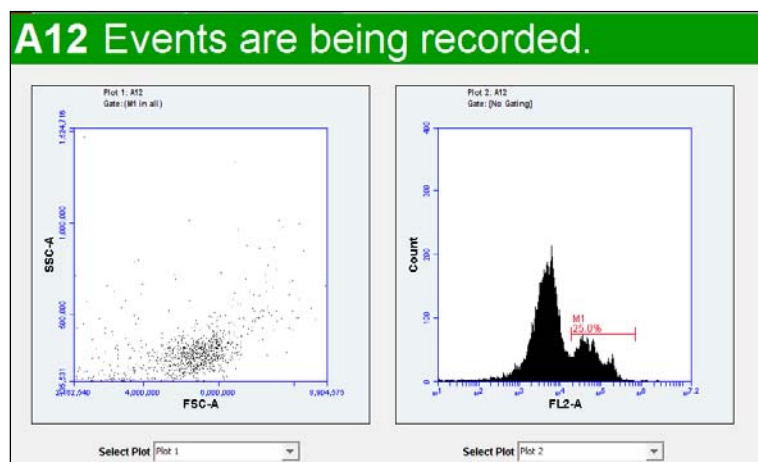


Figure 5-17. Run Display: Viewing Sample Plots

To view plots:

- Under each plot corral, select the plot you want to view from the *Select Plot* drop-down list.

5.9 Ending a Data Collection Session

When you are finished collecting samples, rinse out the SIP to ensure cells or other particles are not left in the SIP.

To end a data collection session:

NOTE: If you plan to collect more samples later in the day, you can skip steps 2-7.

1. Load the tube holder with the following tubes:

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

Type of Sample	Location in CFlow Sample Grid
2 mL of filtered, de-ionized water	Empty data well (for example, H10)
2 mL of decontamination solution (Accuri Part #KR-200)	Empty data well (for example, H11)
2 mL of filtered, de-ionized water	Empty data well (for example, H12)

2. In CFlow, select the well corresponding to the first tube of water. Make sure the well contains no data.
3. Set the time limit for two minutes.
4. Run the sample.
5. In CFlow, select the well corresponding to the decontamination solution. Make sure the well contains no data.
6. Set the time limit for two minutes and the fluidics speed to fast.
7. Run the sample.
8. In CFlow, select the well corresponding to the second tube of water. Make sure the well contains no data.
9. Set the time limit for two minutes.
10. Run the sample.
11. When the run is finished, eject the plate/tube rack and load an empty tray.

5.10 Saving a CFlow File

Always save C6 data as a CFlow file (.c6). A CFlow file is a comprehensive (and often large) data file that contains instrument settings, FCS files, and plot layouts. For details on saving a CFlow file, see section 4.10.

5.11 Creating a CFlow Template

A CFlow template contains a predefined CFlow 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 CFlow Installation CD or the Accuri website at www.accuricytometers.com/technical_information/templates/) or you can create your own customized templates. For details on creating a template, see section 4.11.

5.12 Exporting and Importing Files

You can export data from individual sample wells from any tab as FCS 3.0 files at any time. For details on exporting and importing files, see section 4.17.

5.13 Exporting Sample Settings

You can export sample settings 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:

1. Select *File > Export Sample Settings*.

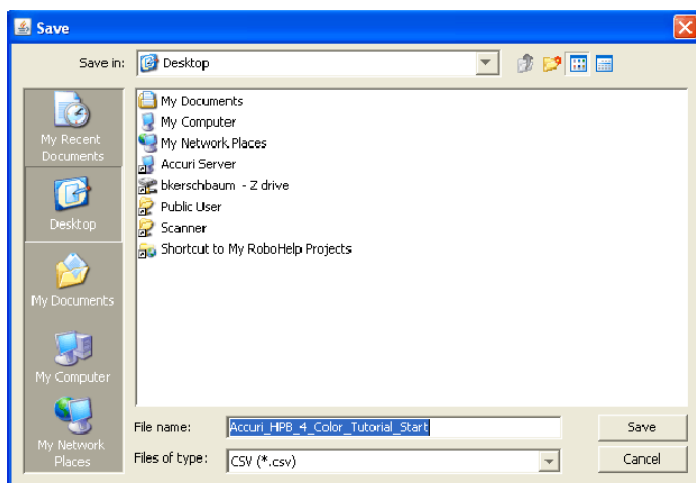


Figure 5-18. Export Sample Settings

2. Navigate to the location to save the file.
3. 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 you to simultaneously view data from multiple samples 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 or delete plots, or copy and reuse plots from the *Manual Collect* tab.
- View different samples with the same plots.
- Create color overlay histograms.
- Print multiple plots.
- Adjust peak position.
- Calculate median statistics.

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 you open the *Analyze* tab for the first time, the workspace is empty. Only the Sample Grid displays any information. To set up the *Analyze* tab, you can copy plots from the *Collect* tab, create new plots, and set up gating strategies.

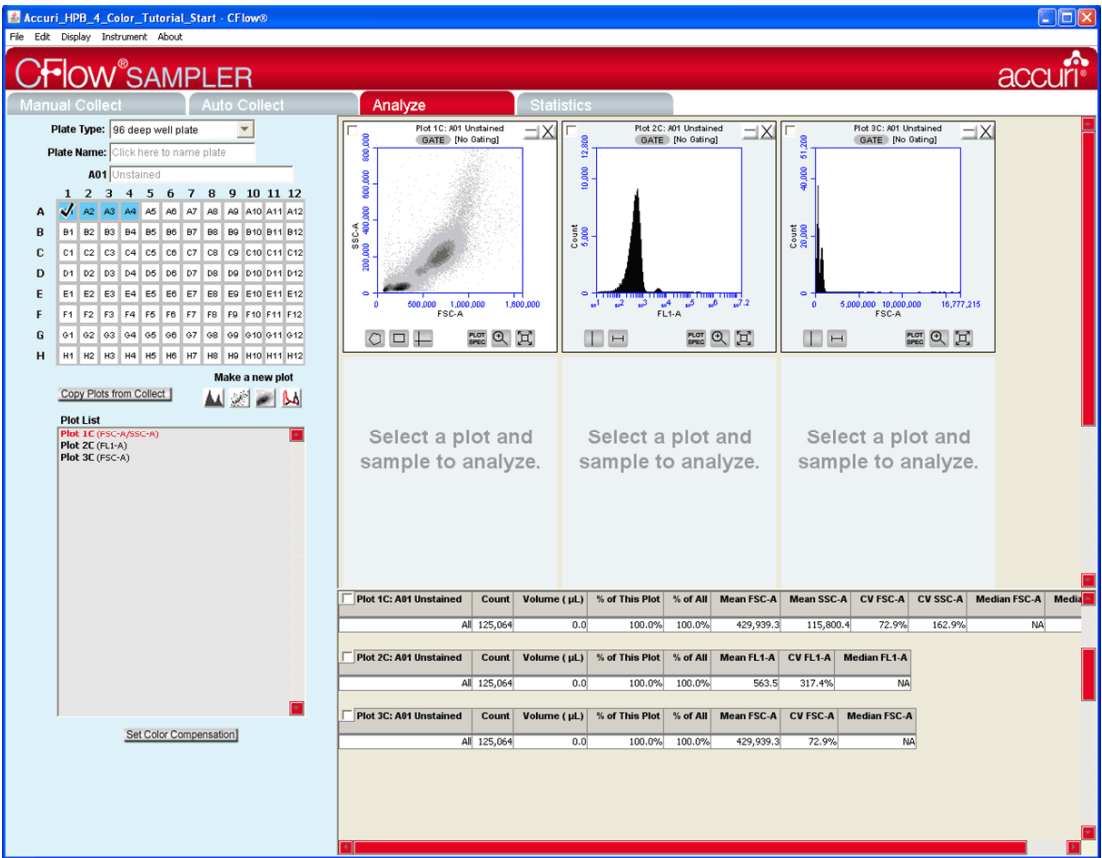


Figure 6-1. Analyze Tab Workspace

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

Table 6-1. Analyze Tab Controls

Control	Description
Plate Type	Drop-down list for setting the type of plate you are using.
Plate Name	Text box for naming the plate you are using.
Sample Naming Field	Text box for naming the current sample.
Sample Grid	Matrix laid out in the configuration of a 96-well plate to help organize sample data. Each sample has its own well in the Sample Grid. The wells are color-coded: <ul style="list-style-type: none">• White—Does not contain data.• Blue—Contains data.• Black check mark—Currently selected for viewing or collecting data.
Copy Plots from Collect	Allows you to copy specified plots from the <i>Collect</i> tab. See section 6.2.1 for details.

<i>Control</i>	<i>Description</i>
<i>Plot Controls</i>	Set of buttons for creating new plots or overlaying histograms. See section 6.2.3 for details. All plots created in the <i>Analyze</i> and <i>Collect</i> tabs, including overlays, can be dragged and dropped into most Microsoft Office compatible programs.
<i>Plot List</i>	Lists the plots that are available in the <i>Analyze</i> tab. Available plots include plots copied from the <i>Collect</i> tab or created in the <i>Analyze</i> tab.
<i>Set Color Compensation</i>	Opens the Compensation Settings dialog box for correcting fluorescence spillover. See section 4.12 for details.
<i>Plot Corrals</i>	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.
<i>Statistics Table</i>	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, you can copy plots from the *Manual Collect* tab or create new plots. Plots that are copied from the *Manual Collect* tab are appended with a “C” (for example, *Plot 1C*).

6.2.1 Copying Plots from the *Collect* Tab

To copy plots:

1. In the *Analyze* tab, click on the *Copy Plots from Collect* button.

2. In the Copy Plots from Collect dialog box, do one of the following:
 - Select the check boxes of the plots you want to copy.
 - Enable the *All Plots* check box to copy all of the plots from the *Collect* tab.

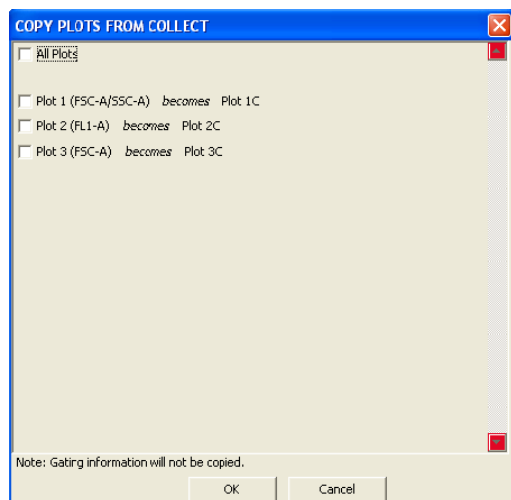


Figure 6-2. Selecting Plots to Copy from the *Collect* Tab

3. Click on the *OK* button to close the dialog box. CFlow adds the selected plots to the Plot List in the *Analyze* tab.

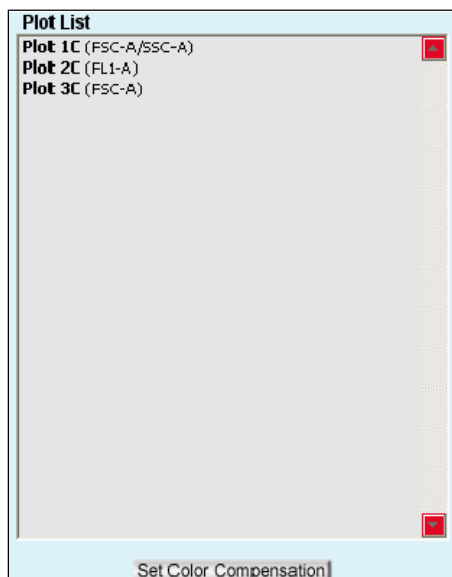






Figure 6-3. Plot List Containing Copied Plots

6.2.2 Creating Plots

To create a new plot:


1. 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 that contains the data you want to view.

6.2.3 Creating an Overlay Histogram

You can compare multiple distributions from different samples at the same time by creating an overlay histogram.

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.

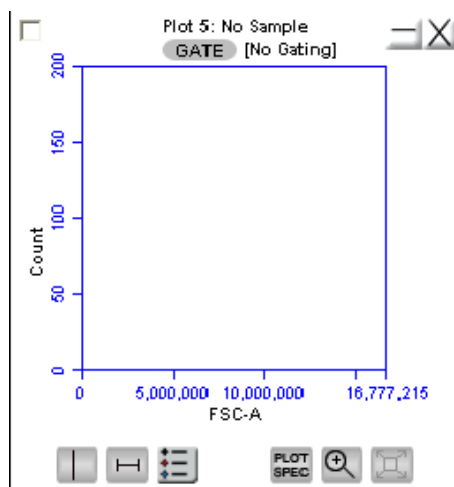


Figure 6-4. Blank Overlay Histogram Plot

- Click on the x-axis label (*FSC-A*) and assign a parameter in the pop-up list.
- Click on the *GATE* button and apply a gate (see section 4.7.2) as appropriate.

5. Select the samples to be overlaid from the 96-well grid.

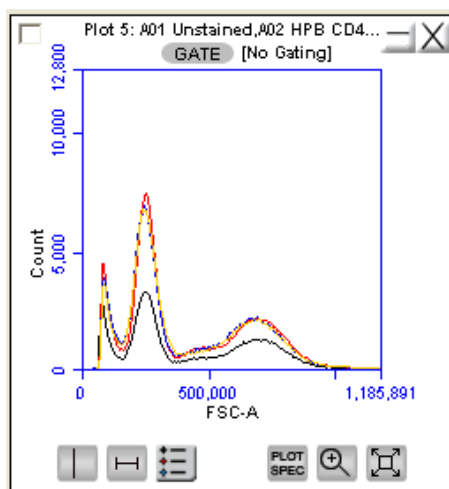



Figure 6-5. Overlay Histogram Plot with Data

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

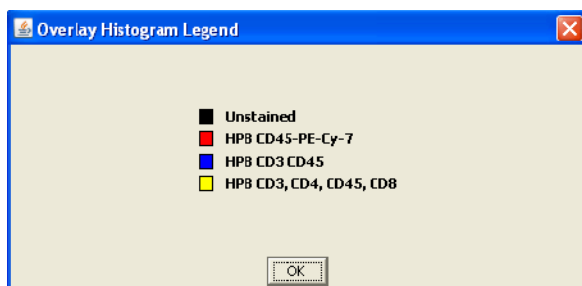


Figure 6-6. Overlay Histogram Legend

6.3 Viewing Plots

To view a plot in the Plot List:

1. Click on an empty plot corral in the *Analyze* tab.

- Click a plot in the Plot List (Figure 6-7) to open the plot. CFlow displays the plot without any sample data.

NOTE: Any gates copied from the *Collect* tab are renamed (for example, P1 in *Collect* is P2 in *Analyze*). These gates can be adjusted in the *Analyze* tab without changing the position of the gates in *Collect*.

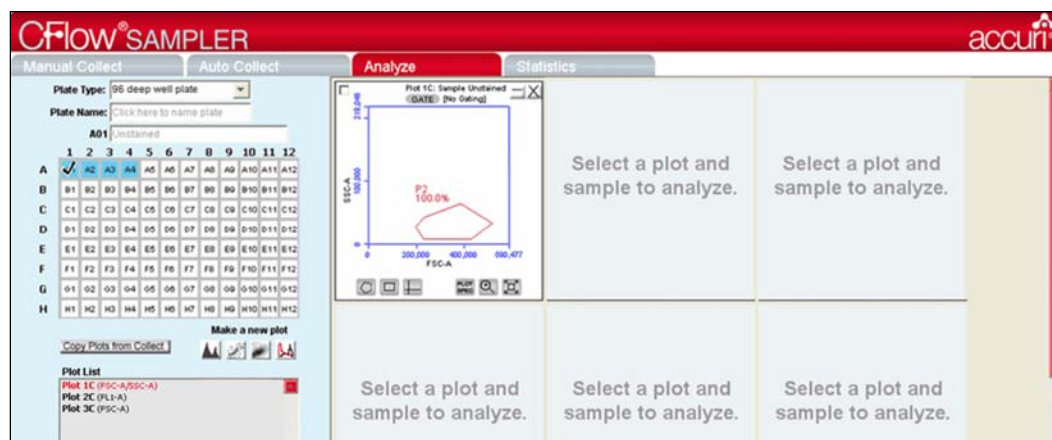


Figure 6-7. Analyze Tab with Opened Plot

- Click on the well of the sample you want to view.

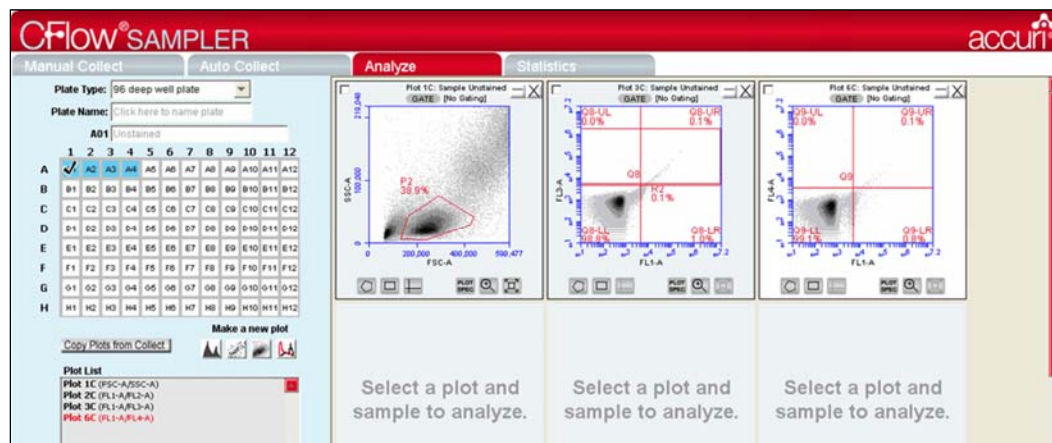


Figure 6-8. Analyze Tab with Populated Plots

4. Apply a gating strategy, if desired (see section 4.7.2 for details).

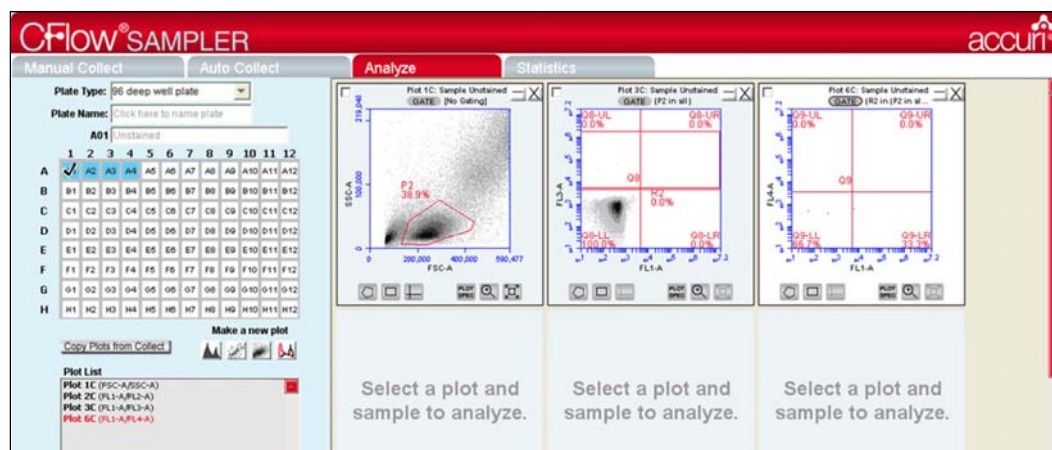


Figure 6-9. Analyze Tab: Plots with Gating Applied

5. 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 display in each plot. The gates that you applied above are automatically applied to the corresponding plots in the new row.

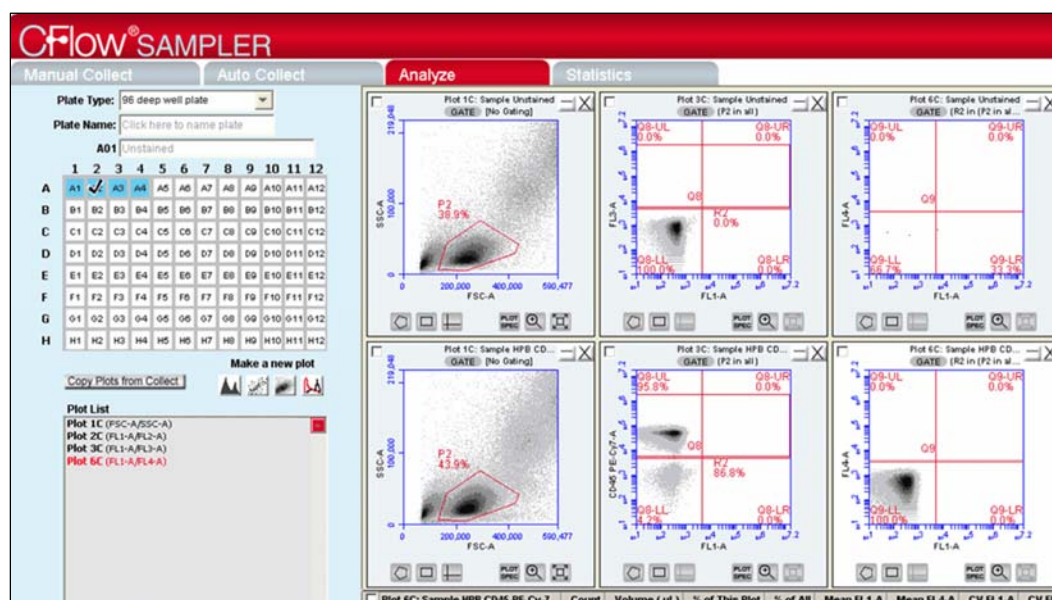


Figure 6-10. Analyze Tab: Two Samples with the Same Plots

6. Compare data and statistics between samples.

7 VIEWING STATISTICS

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

- View statistics for some or all of your collected samples.
- Display statistics of collected or imported samples.
- List all plots created on the *Collect* and *Analyze* tabs.
- Display all plot names, gates, and associated statistics.
- Cut 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 you open the *Statistics* tab for the first time, the workspace is empty. To set up the *Statistics* tab, you can select items from the setup controls for the Master Statistics Table.

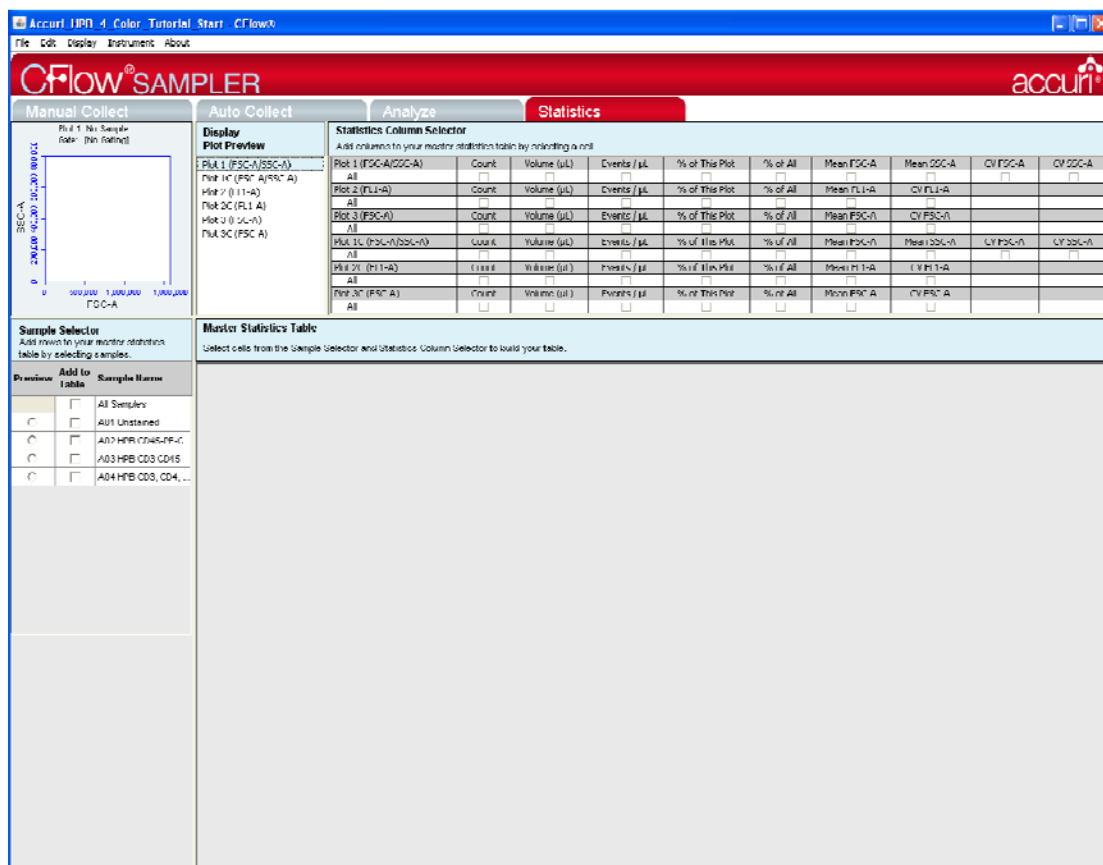


Figure 7-1. *Statistics* Tab Workspace

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

Table 7-1. Statistics Tab Controls

Control	Description
<i>Plot</i>	Previews the selected plot. See section 7.3 for details.
<i>Display Plot Preview</i>	List of plots imported from the <i>Collect</i> and <i>Analyze</i> tabs that allows you to select the plot to preview.
<i>Statistics Column Selector</i>	Allows you to select the data to view in the Master Statistics Table for each sample. See section 7.2 for details.
<i>Sample Selector</i>	Allows you to select the samples to view in the Master Statistics Table. See section 7.2 for details.
<i>Master Statistics Table</i>	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 allows you to view selected data across multiple samples at once. You can customize which data you want to view for each plot, and you can modify the table at any time.

To create the Master Statistics Table:

1. In the Statistics Column Selector, enable the check boxes of the data items you want to view per plot. CFlow automatically adds the columns to the Master Statistics Table.

Display Plot Preview		Statistics Column Selector									
Plot 1 (FSC-A/SSC-A)		Add columns to your master statistics table by selecting a cell.									
Plot 2 (FL1-A)		Count	Volume (μL)	Events / μL	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	
Plot 3 (FSC-A)		All	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	
		Count	Volume (μL)	Events / μL	% of This Plot	% of All	Mean FL1-A	CV FL1-A			
		All	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>			
		Count	Volume (μL)	Events / μL	% of This Plot	% of All	Mean FSC-A	CV FSC-A			
		All	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>			

Master Statistics Table										
Select cells from the Sample Selector and Statistics Column Selector to build your table.										
Plot 1 (FSC-A/SSC-A)				Plot 2 (FL1-A)			Plot 3 (FSC-A)			
All				All			All			
Count	Events / μL	CV FSC-A	CV SSC-A	Count	Events / μL	CV FL1-A	Count	Events / μL	Mean FSC-A	

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

2. In the Sample Selector list, enable the check box of each sample you want to view. CFlow automatically adds the rows of samples to the Master Statistics Table and displays the sample data.

Sample Selector		Master Statistics Table									
Add rows to your master statistics table by selecting samples.		Select cells from the Sample Selector and Statistics Column Selector to build your table.									
Preview	Add to Table	Plot 1 (FSC-A/SSC-A)				Plot 2 (FL1-A)			Plot 3 (FSC-A)		
	Sample Name	All				All			All		
	<input type="checkbox"/> All Samples	Count	Events / μL	CV FSC-A	CV SSC-A	Count	Events / μL	CV FL1-A	Count	Events / μL	Mean FSC-A
	<input checked="" type="checkbox"/> A01 Unstained	230,169	∞	69.9%	163.2%	230,169	∞	237.7%	230,169	∞	420,020.6
	<input checked="" type="checkbox"/> A02 HPB CD45-PE-C...	231,332	∞	65.9%	124.2%	231,332	∞	135.2%	231,332	∞	413,478.5
	<input checked="" type="checkbox"/> A03 HPB CD3 CD45	229,303	∞	71.6%	164.3%	229,303	∞	139.4%	229,303	∞	416,636.3
	<input checked="" type="checkbox"/> A04 HPB CD3, CD4, ...										

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:

1. In the Display Plot Preview list, click on the plot you want to preview.

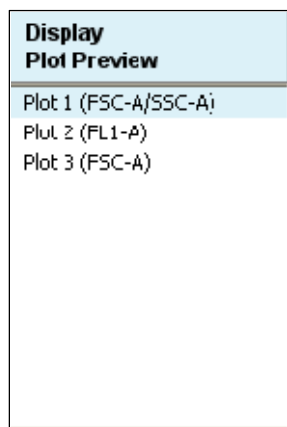


Figure 7-4. Display Plot Preview List

2. In the Sample Selector list, select the radio button of a sample. CFlow displays the sample's data in the plot.

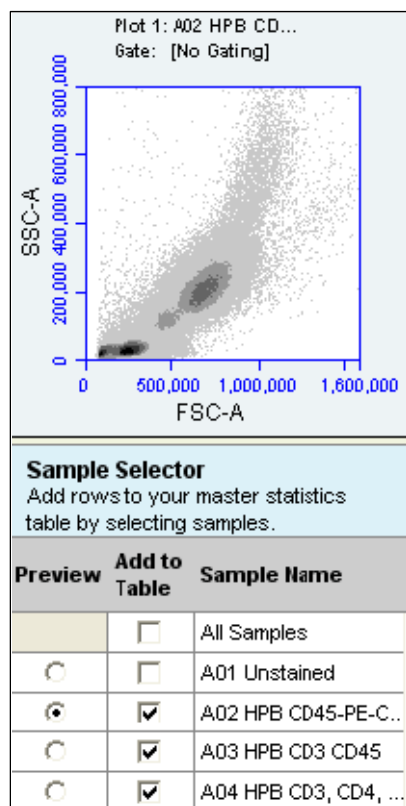


Figure 7-5. Plot Preview

The plot preview is available for viewing only. You can modify the zoom level and other plot settings in the *Analyze* or *Manual Collect* tab.

7.4 Copying Data into Other Applications

You can copy and paste data from the Master Statistics Table into most Microsoft Office compatible applications.

To copy data:

1. Use the mouse to highlight the fields you want to copy.
2. Press **Ctrl+C** to copy the data.
3. In the Microsoft application, press **Ctrl+V** to paste the data.

8 MAINTAINING THE C6

To ensure that your C6 Flow Cytometer continues optimal performance, follow the routines in this chapter on a regular basis. See the *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:

1. Click on the *Backflush* button.
2. Wait for the CSampler to move to the Eject position and place a container under the SIP.
3. Click on the *Backflush* button in the Run Backflush Cycle dialog box.

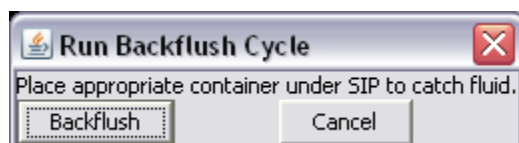


Figure 8-1. The Run Backflush Cycle Window

4. When the backflush is completed, eject the plate.

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 Unclogging the Flow Cell

The unclog cycle purges the flow cell of debris.

To purge the flow cell:

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

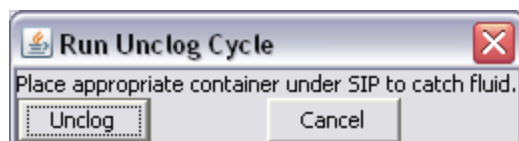


Figure 8-2. The Run Unclog Cycle Window

8.2.2 Running an Extended Clean of the Flow Cell

In cases when a more thorough cleaning is needed, you can clean the flow cell for an extended time. During extended flow cell cleaning, the flow cell fills completely with cleaning solution from the sample tube. This cycle automatically shuts down the C6 with cleaning reagent in the flow cell, allowing the flow cell to soak.

To run the extended clean of the flow cell:

1. Load the 24-tube rack with a tube containing at least 500 μ L of cleaning solution.
CAUTION: Never run the Extended Clean of the Flow Cell cycle without a tube containing at least 500 μ L of fluid.
2. Select *Instrument > Extended clean of flow cell*.
3. After the C6 is shut down, allow the cytometer to rest for at least 30 minutes (or longer, for a more thorough cleaning).
4. Restart the cytometer. The C6 performs a longer fluidics startup cycle and CFlow displays the message *Extra startup time needed due to cleaning or improper shutdown*. This longer cycle purges cleaning reagent from the flow cell.
5. Operate the C6 as usual when startup is complete.

8.3 Cleaning the Fluidics Lines

The cleaning fluid cycle pulls cleaner fluid from the cleaner tank and runs it through the fluidic lines of the fluidics system. 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:

1. Load a tube rack containing a tube of cleaning solution.
2. Select *Instrument > Run cleaning fluid cycle*.

8.4 Decontaminating the Fluidics

CFlow decontaminates the fluidics system of biohazardous materials every time you shut down the C6. You can also manually run the decontamination fluid cycle at any time. Decontamination takes about 13 minutes.

To manually decontaminate the fluidics:

1. Load a tube rack containing a tube of water.
2. Select *Instrument > Run decontamination fluid cycle*. The C6 pulls decontamination fluid from the decontamination bottle then pulls sheath fluid from the sheath fluid container.

8.5 Calibrating the Fluidics System for Precise Volume Measurements

If you validate performance with the Accuri volumetric validation beads (Accuri Part #QA-120) and validation fails, you can recalibrate the C6 Cytometer.

Do the following on a regular basis to ensure that the C6 continues to be correctly calibrated:

- Replace any peristaltic pump tubing that is at least two months old.
- Run samples at medium or fast speeds.
- Use the same sample volume/fluid height in the tube during calibration as you will use during your experiment.

To calibrate the C6 for precise volume measurements:

1. Place a 12x75 mm tube with 500 μ L of sheath fluid in well A1 of the sample plate.
2. Load the plate.
3. Select well A1 in the Sample Grid in CFlow Sampler.
4. Select *Instrument > Calibrate fluidics* to begin calibration. The calibration takes about five minutes.
5. When prompted, confirm that you want to calibrate fluidics for the type of plate indicated, using the well indicated.

NOTE: Do not use a 96-well plate for calibration.

If calibration fails, the C6 automatically reverts to the factory calibration settings and operates normally. However, the volumes recorded will not be optimized for the new sample volume. If more precise measurement is essential, try calibrating again.

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 you to adjust peak position from sample to sample. CFlow 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 data collection. 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 the figures below, 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). You can use VirtualGain to align the negative peak of sample 2 with the control sample.

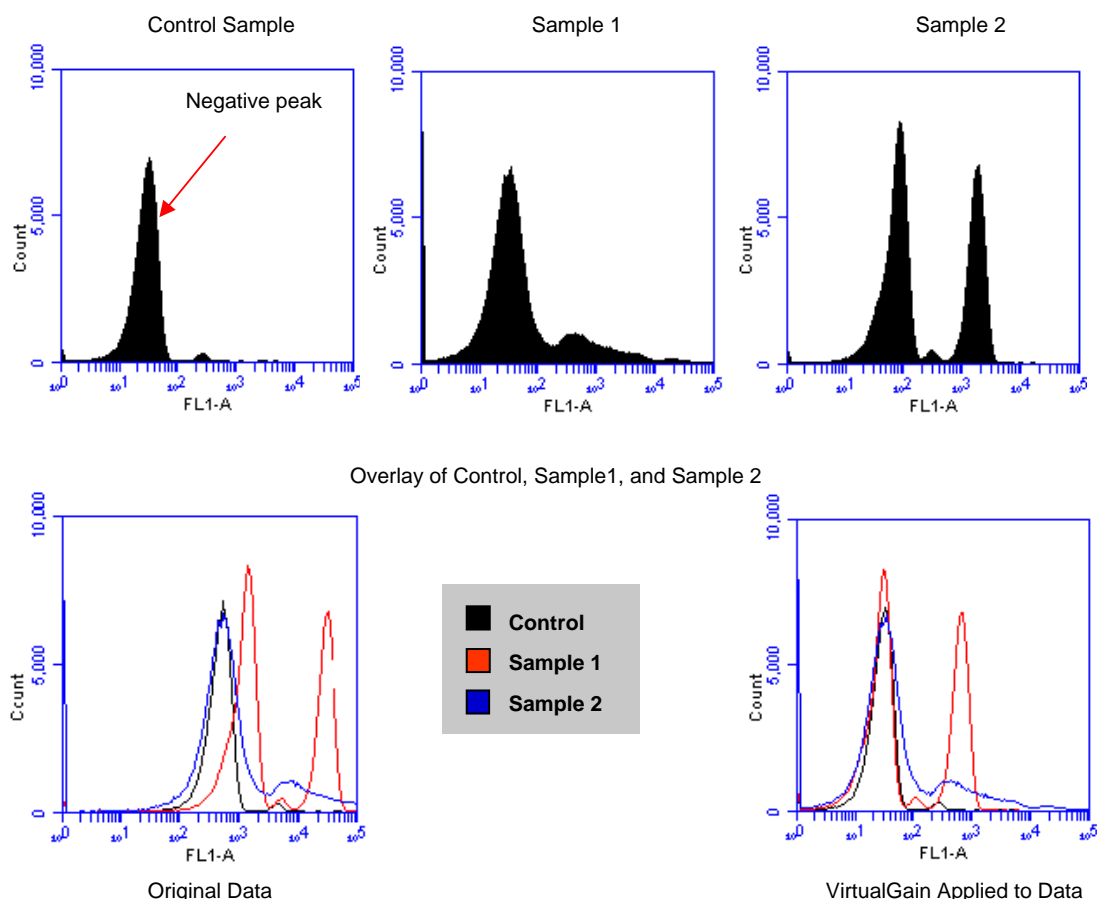


Figure 9-1. Before and After Applying VirtualGain

9.1 Applying VirtualGain

Apply VirtualGain only on a histogram plot, and only on one parameter at a time. After VirtualGain is applied, you can view data in any type of plot and toggle VirtualGain on and off.

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

To apply VirtualGain:

1. In the *Analyze* tab, do one of the following:
 - Recreate the histogram to which you want to apply VirtualGain (see section 6.2 for details).
 - Copy plots from the *Manual Collect* tab (see section 6.2 for details).

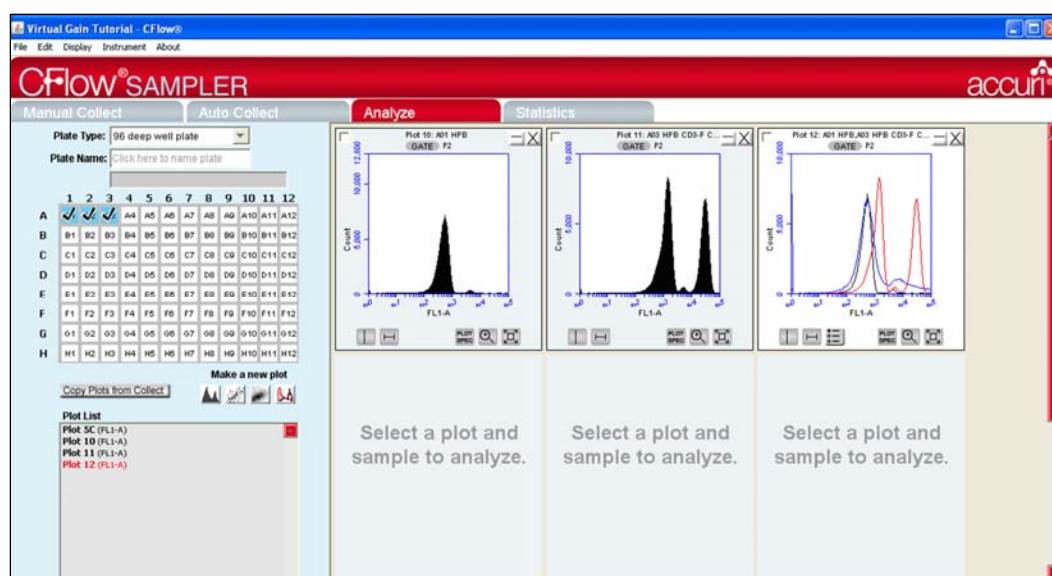


Figure 9-2. Setting Up Histograms for VirtualGain

2. Apply the appropriate gating to the plots in the *Analyze* tab.
3. 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 if you want to align data to a *specific channel* instead of a collected sample.
4. Click on the x-axis label on the standard sample and select *VirtualGain* from the pop-up Parameter List.

5. 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 you selected an empty well in step 3, move the peak definition marker (vertical line) to the channel that you want to assign as the reference point.

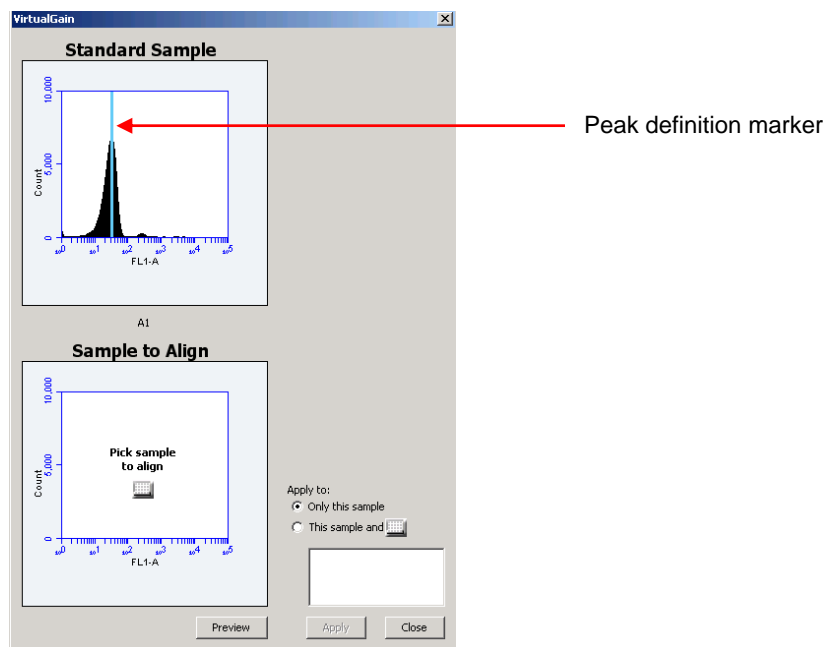


Figure 9-3. Aligning Plots

6. If needed, use the Zoom Tools in the *Analyze* tab to change the zoom level in the VirtualGain dialog box.
7. Click on the small sample grid icon in the center of the Sample to Align plot.

8. 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.

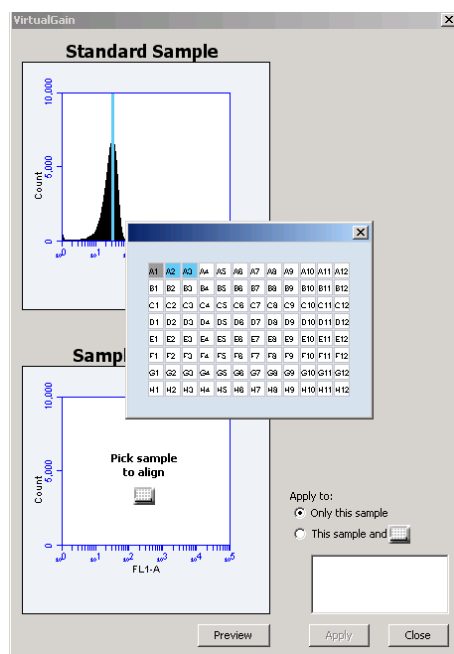


Figure 9-4. Pick the Sample to Align

9. Move the peak definition marker in the Sample to Align plot to the center of the peak that you want to align.

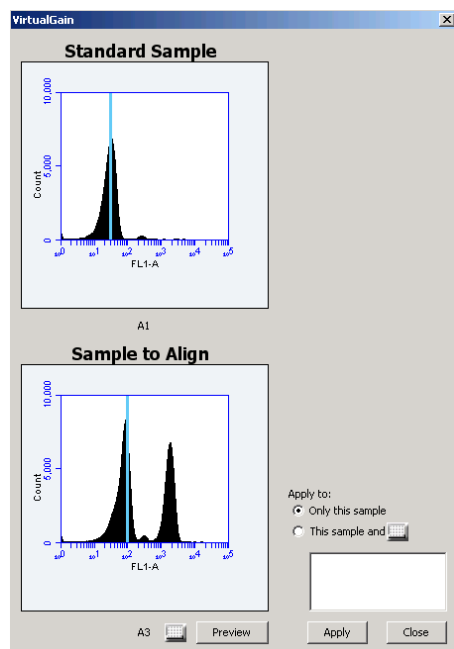


Figure 9-5. Move the Peak Definition Marker

10. Click on the *Preview* button to view the aligned sample with VirtualGain applied. CFlow aligns the peak of interest in both plots.
11. Repeat steps 9-10 to make additional adjustments, if needed.
12. 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 that you want to include. If the other samples need a different amount of VirtualGain, set VirtualGain separately for each one.

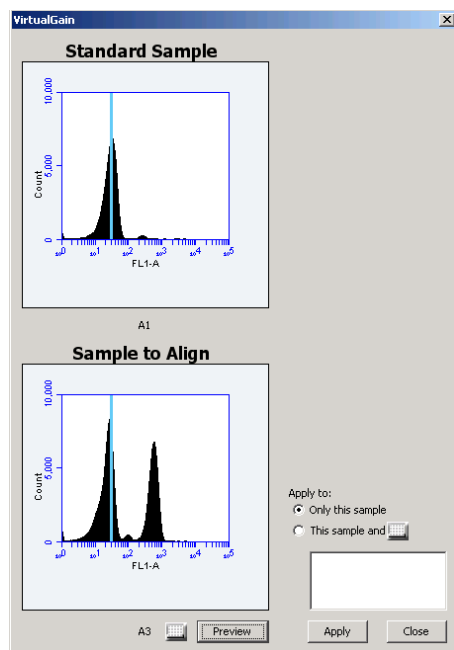


Figure 9-6. Align Additional Samples

13. Click on the *Apply* button to apply VirtualGain to the data. CFlow displays a black asterisk under the Sample to Align plot to indicate that VirtualGain has been applied to the specified parameter for that sample.

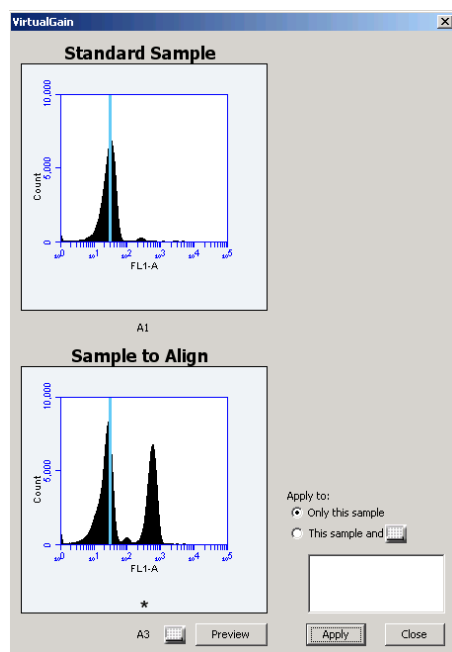


Figure 9-7. Black Asterisk Identifier

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

9.2 Viewing VirtualGain

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

The asterisk is color-coded:

- Black—VirtualGain has been applied.
- Gray—CFlow is currently displaying the original data.

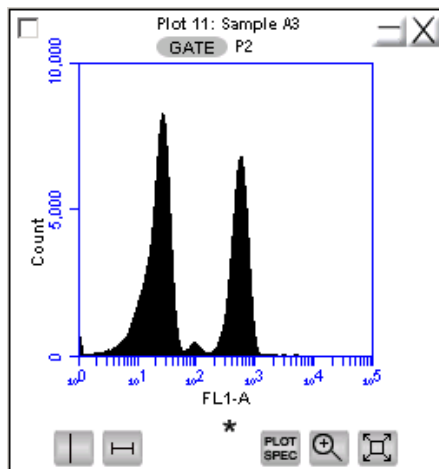


Figure 9-8. Plot with Black Asterisk

Overlays automatically display VirtualGain when it is applied (as shown in the following figure). The asterisk is not displayed in overlays when VirtualGain is applied to some or all of the samples in the overlay.

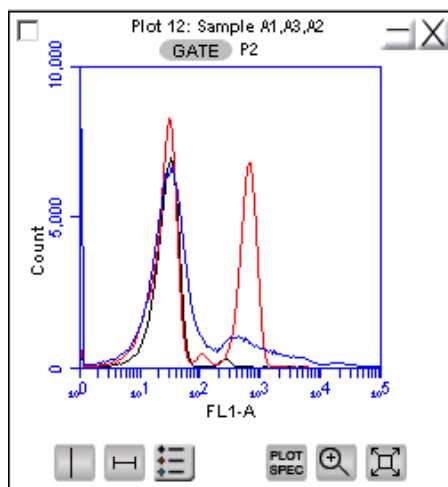


Figure 9-9. VirtualGain Applied in an Overlay Histogram

To toggle between views with VirtualGain applied and not applied:

1. Click on the asterisk in the plot.

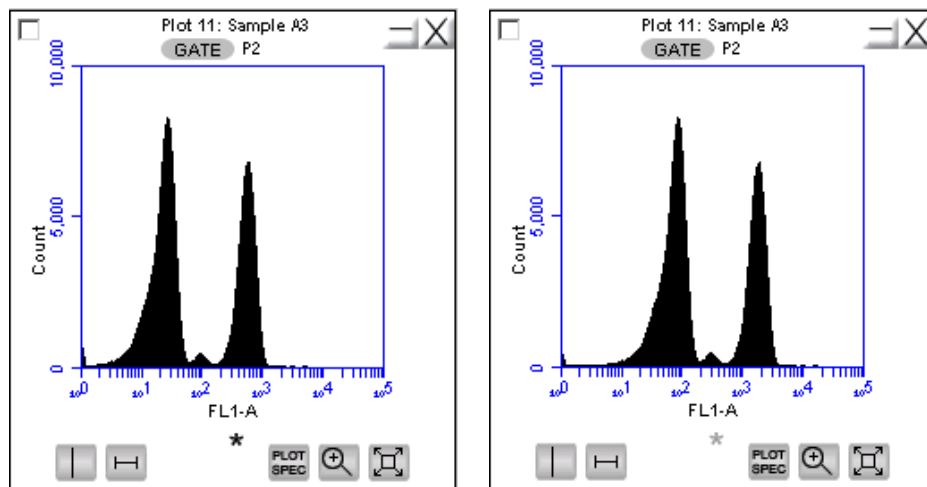


Figure 9-10. Toggle between VirtualGain Applied (Left) and Not Applied (Right)

9.3 Removing VirtualGain

To permanently remove VirtualGain from every parameter in every sample in the CFlow file:

1. Select *Display > Remove All VirtualGain*.
2. When prompted, confirm that you want to remove VirtualGain.

CAUTION: You cannot undo this action.

APPENDIX A CFLOW MENU QUICK REFERENCE

This appendix provides a description of all the menu options in CFlow.

Table A-1. CFlow Sampler Menu Options

<i>Menu Item</i>	<i>Menu pick</i>	<i>Description</i>
File	Open CFlow File or Template	Opens a previously saved CFlow file or CFlow template. Only one CFlow workspace can be open at a time.
	New CFlow File	Opens a new, blank CFlow workspace. Replaces any previously open workspace.
	Save	Saves the open CFlow workspace under the current name. If the file has not already been named, CFlow prompts you to name the file.
	Save CFlow File as	Saves the open CFlow workspace under a new name.
	Save CFlow Template as	Creates a template from the currently open CFlow workspace. All markers, regions, gates, parameter names, and sample names are saved without any data points.
	Auto-save Settings	Allows you to enable or disable the auto-save feature.
	Import FCS File	Imports an FCS file previously exported from another CFlow file to the currently open workspace. Only FCS files created on a C6 Cytometer can be imported into CFlow.
	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 <code>CFlow-FCS Exports</code> 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 F for an example .csv file.
	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.
	Print Selected Items	Prints the selected plots and associated statistics.
	Quit	Quits CFlow and closes the application.

<i>Menu Item</i>	<i>Menu pick</i>	<i>Description</i>
Edit	Undo	Undoes the last action that was performed in CFlow. Not all actions are undoable.
	Redo	Reverses an <i>Undo</i> action.
	Copy	Copies a marker or region from a plot, or statistics from the tables in CFlow.
	Paste	Pastes copied markers and regions into new plots.
	Rename Parameters	Allows you to rename individual parameters in either the current sample or all samples at the same time.
Display	Events Display Settings	Opens a dialog that allows you to change the number of events displayed in all plots.
	Remove All VirtualGain	Removes all VirtualGain settings from the entire CFlow workspace. VirtualGain settings are removed from all wells of data.
	Hide/Show Median Statistics	Hides/shows the median statistics in the Statistics Table.
Instrument	Set threshold	Opens the Threshold dialog box for selecting the trigger channel, setting the primary threshold value, and setting an optional secondary threshold.
	Set Compensation	Opens the Compensation Settings dialog box for subtracting fluorescence spillover.
	Run Cleaning Fluid Cycle	Runs a cleaning fluid cycle.
	Run Decontamination Fluid Cycle	Allows you to manually run the 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 CSampler arm to the SIP.
	Calibrate Fluidics	Initiates fluidics calibration to ensure that the C6 provides accurate measurement of the volume aspirated from samples.
	Update Firmware	Updates the C6 firmware. Use only when directed by Accuri Cytometers to upgrade the C6 firmware with an official firmware release.
	Remote Control	Optional feature that allows control of the C6 from a remote location.
About	About CFlow	Opens a dialog box that displays the version of CFlow and Accuri Technical Support contact information.
	Technical Support Information	Opens a dialog box that displays information about CFlow and the C6. Each time an activation key is used to install a new CFlow component, the dialog box is updated to reflect the change.
	Users	Optional feature that allows you to add, delete, or modify user accounts for usage tracking.

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:

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

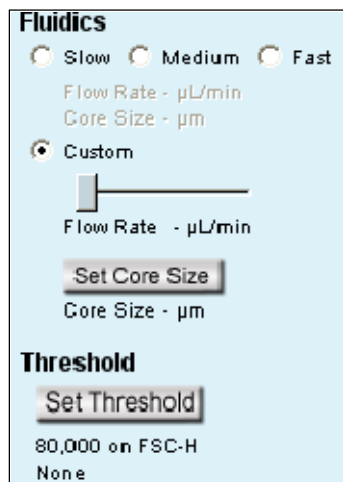


Figure B-1. Custom Fluidics Settings

2. Move the *Custom* slider to the rate you want to set.

To customize the sample core size:

1. Click on the *Set Core Size* button in the Fluidics section of the *Collect* tab.
2. Move the slider to the core size you want to set.

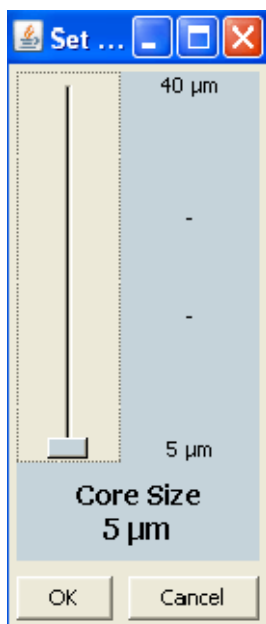


Figure B-2. Core Size Slider

NOTE: Certain core sizes are not possible at certain flow rates. CFlow does not allow you to set these combinations. You can 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 C6 operators by assigning a user name and password to each individual. Passwords are created and used in CFlow and are unrelated to any Windows passwords used on the host computer or network.

User Tracking is an optional upgrade for CFlow Sampler and requires the use of CFlow version 227 or above.

C.1 Installing the User Tracking Module

To install user tracking on your CFlow computer:

1. Ensure CFlow Sampler version 227 or above is loaded on the computer.
2. Copy the User Tracking Activation Key from the installation CD to the computer desktop.
3. Double-click on the User Tracking Installer icon on the desktop.



Figure C-1. User Tracking Installer Icon

4. In the installation wizard, choose the correct directory to install the Activation Key. The location depends on where CFlow Sampler has been installed. In most cases, the location is in C:\Program Files\CFlow\CFlow Sampler\ActivationKeys

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

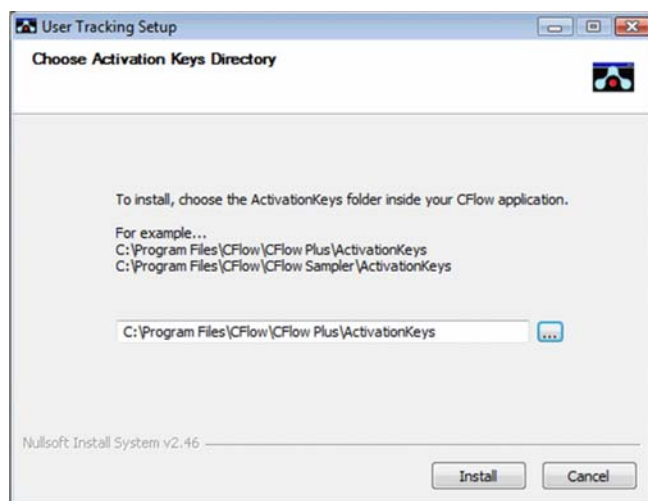


Figure C-2. Installation Dialog Box

5. Click on the *Install* button. CFlow 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:

1. Open CFlow.
2. When prompted, type *admin* in the *Username* text box.

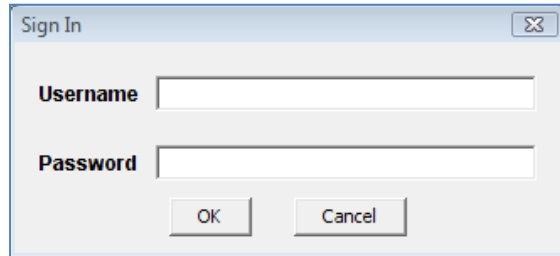


Figure C-3. Username and Password Dialog Box

3. Type *Admin* in the *Password* text box (case sensitive). You can change the Administrator password later.
4. Click on the *OK* button
5. Use CFlow as usual.

C.3 Adding, Deleting, and Modifying User Accounts

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

C.3.1 Adding User Accounts

To add new user accounts:

1. Sign in as the administrator.
2. Select *About > Users* in CFlow.
NOTE: The *Users* menu option is only visible to the administrator.
3. In the Users dialog box, click on *Add New User*.

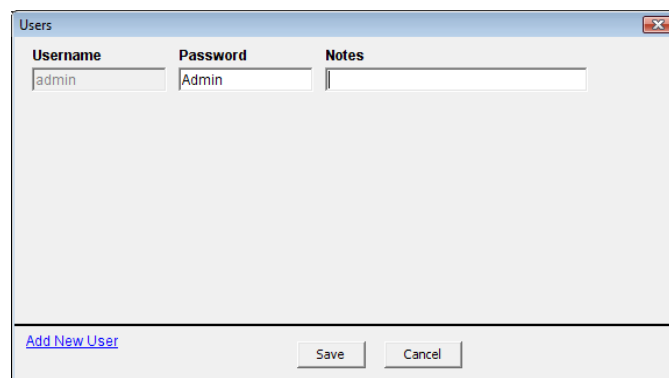
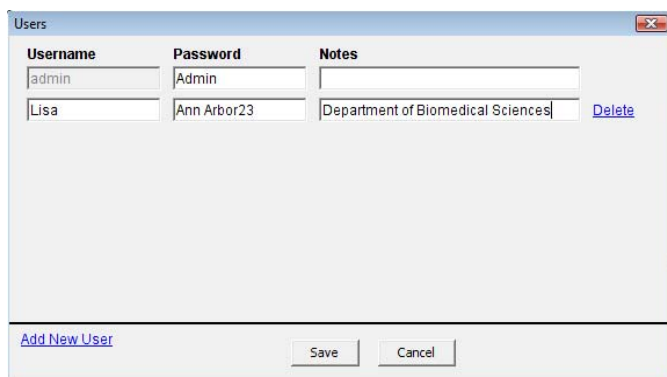


Figure C-4. The Users Information Box

4. Type the *Username* and *Password* for the user in the blank text boxes. Type any notes you want to add in the *Notes* text box (this information is only visible in the Users dialog box).



The 'Users' dialog box contains a table with the following data:

Username	Password	Notes
admin	Admin	
Lisa	Ann Arbor23	Department of Biomedical Sciences

Below the table is a link labeled 'Delete' next to the 'Department of Biomedical Sciences' note. At the bottom of the dialog box, there is a link labeled 'Add New User', and two buttons labeled 'Save' and 'Cancel'.

Figure C-5. Adding a New User

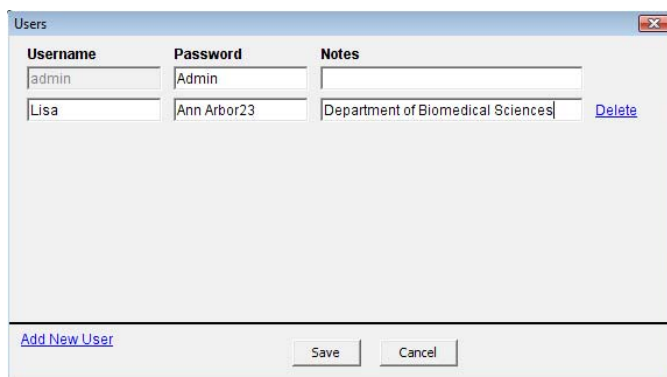
5. Click on the *Save* button to save your changes.

C.3.2 Deleting User Accounts

To delete a user account:

1. Select *About > Users* in CFlow.
2. Click on *Delete* next to the user account you want to remove.

NOTE: You cannot delete the administrator's account.



The 'Users' dialog box contains a table with the following data:

Username	Password	Notes
admin	Admin	
Lisa	Ann Arbor23	Department of Biomedical Sciences

Below the table is a link labeled 'Delete' next to the 'Department of Biomedical Sciences' note. At the bottom of the dialog box, there is a link labeled 'Add New User', and two buttons labeled 'Save' and 'Cancel'.

Figure C-6. Deleting a User

3. Click on the *Save* button to save your changes.

C.3.3 Changing a Password

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

To change a user password:

1. Select *About > Users* in CFlow.

2. Delete the text in the *Password* field and type a new password.
3. Click on the *Save* button.

C.4 Signing In and Signing Out

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

To sign in to CFlow:

1. Open CFlow.
2. When prompted, type your *Username* in the text box.

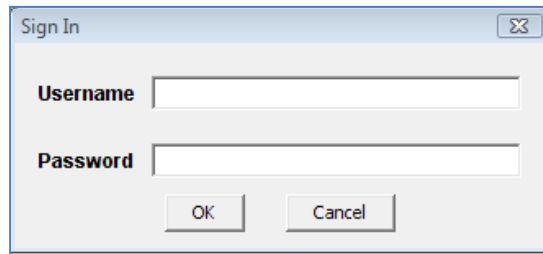


Figure C-7. Username and Password Dialog Box

3. Type your *Password* in the text box.
4. Click on the *OK* button
5. Use CFlow as usual.

Be sure you sign out of CFlow each time you end your session. If you do not sign out, CFlow will continue to log your time.

To sign out of CFlow:

1. In CFlow, 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 you forget your Administrator password, you can delete the password file and recreate your Administrator account.

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

To restore the administrator password:

1. If CFlow is running, shut down CFlow.
2. Navigate to the CFlowSupportFiles folder (typically in the root directory of the CFlow computer).
3. Locate the Password file and delete the file.
4. 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 CFlow, an entry is made in the userUsage Log. The userUsage log is a text file that you can view in a text editor (such as Notepad) or copy into Microsoft Excel as a comma delimited file.

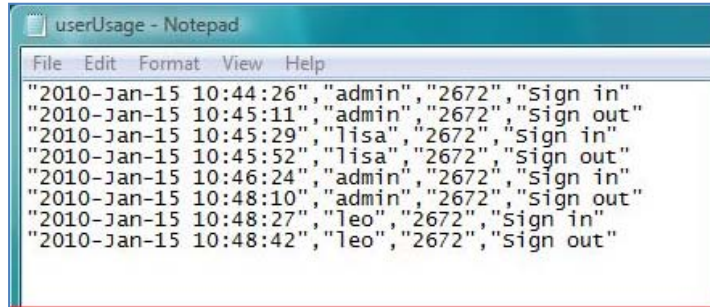


Figure C-8. userUsage Log

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 C6
- Type of activity (sign in or sign out)

To view the userUsage log:

1. Navigate to the CFlowSupportFiles folder (typically in the root directory of the CFlow computer) and open the folder.

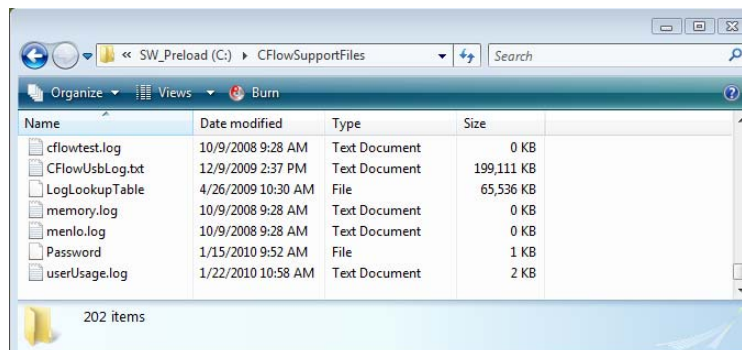


Figure C-9. CFlowSupportFiles Folder

2. Open the userUsage.log file.

APPENDIX D CFLOW ANALYSIS SOFTWARE

The optional CFlow Analysis software allows you to analyze CFlow files on a computer that does not have a connection to the C6 Cytometer. CFlow Analysis is available in two forms:

- CF-20—CFlow Plus Analysis Software for PC or Mac
- CF-30—CFlow Sampler Analysis Software for PC or Mac

CFlow Analysis has all the functionality of CFlow, but the instrument control functions in the *Instrument* menu are disabled (except *Set Threshold* and *Set Compensation*). CFlow Analysis software is not intended for controlling the C6 instrument for data acquisition.

MINIMUM SYSTEM REQUIREMENTS

- PC version:
 - Intel Core Duo processor, 2.8GHz
 - 2GB RAM
 - CD-ROM Drive
 - Windows® XP (Service Pack 2) or Windows Vista (recommended)
 - Language selection: US or UK English
 - 5GB hard disk space
 - Display resolution 1152 x 864 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

SOFTWARE OPERATION

See the relevant chapters in this manual for operational information.

APPENDIX E FCS KEYWORDS

This appendix lists all of the keywords CFlow uses in FCS files.

STANDARD FCS TAGS IN FCS FILES EXPORTED BY CFLOW SOFTWARE

Table E-1. Standard FCS Tags

<i>FCS Tag</i>	<i>Description</i>	<i>CFlow</i>
\$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. Accuri 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 CFlow.	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.
\$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.

<i>FCS Tag</i>	<i>Description</i>	<i>CFlow</i>
\$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 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.
\$BEGINTEXT	Default FCS 3.0 tag to mark the beginning of the text section.	Unchangeable.
\$ENDTEXT	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 CFLOW

Table E-2. Custom FCS Tags

<i>Tag</i>	<i>Description</i>	<i>CFlow</i>
#CFLOWDECADESN	The number of decades for parameter N. It is always 7.224719870049579.	Unchangeable.
#CFLOW4COLORCOMP	A list of the percent values as entered into the color compensation window.	Determined by values entered into Color Compensation dialog.
#CFLOWCAPTUREDDATE	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 CFLOW EXPORTED FCS FILES

Table E-3. FCS Tags not in CFlow Exported Files

<i>Tag</i>	<i>Description</i>
\$COMP	Amount of fluorescence compensation employed during collection. This replaces the \$DFC _i T _{0j} 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. CFlow always uses 0,0 because CFlow 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 F EXAMPLE .CSV FILE

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

Table F-1. Example .csv File.

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
118075	95988	456	294	272	851	607048	422139	1473	815	714	2875	43	121
117720	88712	576	268	283	881	601519	374399	1417	719	533	2521	43	121
110042	85241	2866	1872	2126	7954	583439	372881	7325	5042	5356	28644	41	121
114296	67129	9034	5419	6515	24375	583940	287922	23759	14273	18009	91638	42	121
118532	101077	56704	38096	37950	73004	591859	437256	147685	95718	89739	233762	43	121
114148	89976	1035	714	496	2868	586298	383138	2897	1998	2454	10256	42	121
116901	77515	136085	104961	155282	133610	581299	331039	344734	272212	394709	440201	42	121
111757	80760	16897	10281	11171	39681	580904	354623	45060	26759	29697	149886	43	122
113937	86275	1193	763	921	2540	580677	380168	2632	1748	2965	8335	42	122
112549	105640	125303	101393	154812	137072	563859	450332	313047	252427	393460	470855	43	122
112085	71263	1029	759	227	2902	567995	316559	2683	1915	1352	10755	41	122
118052	96038	95	72	0	129	592987	405663	384	121	599	799	43	122
110943	89761	133064	100253	161526	143719	576133	385371	355359	266257	414414	478612	42	122
115882	84264	1047	593	984	3045	573668	347709	3082	1507	2678	9212	42	122
109415	85656	1315	671	523	2890	583643	380640	3530	1980	2950	10823	41	122
112818	87317	0	0	0	0	583281	385421	317	146	397	463	42	122
116118	87517	8382	5371	5645	22063	575042	362503	20362	14123	11901	73736	43	122
122745	100658	8036	5433	5483	30389	612061	426570	20973	13186	14029	100238	45	122
117550	94310	422	107	140	695	594473	403387	1165	492	604	2492	42	122
107921	90101	1198	647	654	2808	572735	398355	3718	1765	1884	9203	41	122
110195	111820	21375	12491	12746	72825	546675	464189	55839	32764	32063	216207	43	122
115341	93977	16101	10748	11758	37634	588991	393280	41618	27052	29625	133265	44	122
110606	73288	8681	4925	5365	21686	572786	320110	24384	13815	12568	77871	42	122
112173	66739	129974	96461	151427	124808	574229	291566	354745	262399	416530	469631	42	122

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