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Ready, Set, Run!

The Collect tab is used to collect or import a sample, configure the run settings and set up plots and gates.

Collect a Sample

1. Start the C6 Flow Cytometer, open CFlow and wait for the green "traffic light" message.

2. Place sample tube on the sip.

3. Configure the run settings:
   • Set the run limits
   • Select fluidics settings
   • Set threshold (primary and optional secondary parameters)

4. Select a sample well in the grid and click **Run**.

Make a Plot

1. Click on the Histogram 📊 , Dot Plot 📈 or Density Plot 📉 button. The plot shows the selected sample.

2. Click the parameter name (e.g. FSC-A) to rename parameters from the drop down menu.

3. Click the Plot Spec PLOT SPEC button to change linear to log, set an axis range or change parameters.
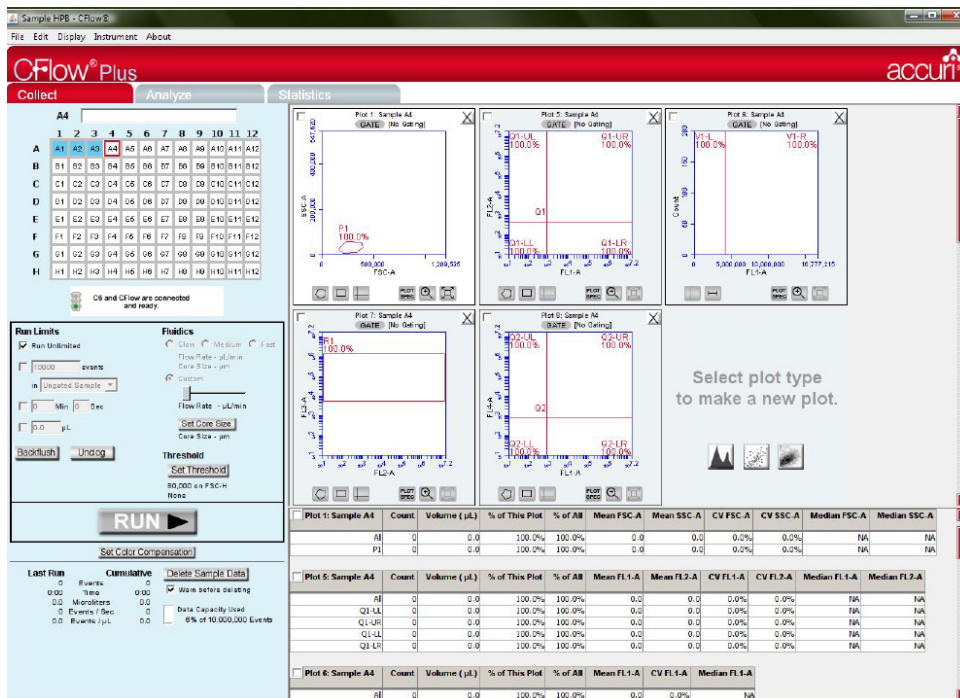
Note: Anything you do to your plot only changes the display of the data, not the data itself.

Tip! You can copy and paste the statistics tables into other applications and drag plots from CFlow to other applications.

Save a File

1. To save the samples and plots, select **Save CFlow File** from the **File** menu to be printed.

Tip! CFlow does not automatically save, so save often!

Make a Gate

1. Click on a **Marker** button 🔘 ⬜ ⊟ ⨆ ⊟ to draw a region to use as a gate.

2. Click the gate GATE button on the plot to which the gate should be applied.

3. In the Gating dialog, choose which regions to Include 🔲 , Exclude ⬜ or Intersect 🔳 and then click **Apply**.

Apply Color Compensation

1. Click the **Set Color Compensation** button.

2. In the Color Compensation dialog, select the parameters you want to compensate, and then enter the compensation percentages.

3. Apply settings to current or all samples.

4. Click **Save & Close**.

Zooming In or Out on a Plot

• To zoom in on a plot, click the **Zoom In** button 🔍 and encompass the region of interest using the cursor.

• Zoom can be used multiple times on the same plot.

• To zoom out on a plot, click the **Zoom Out** button ⛶ to return to the previous view.

Collecting Using the Manual Collect Tab

The Manual Collect Tab is used to acquire a sample from one well (or tube) at a time with CSampler.
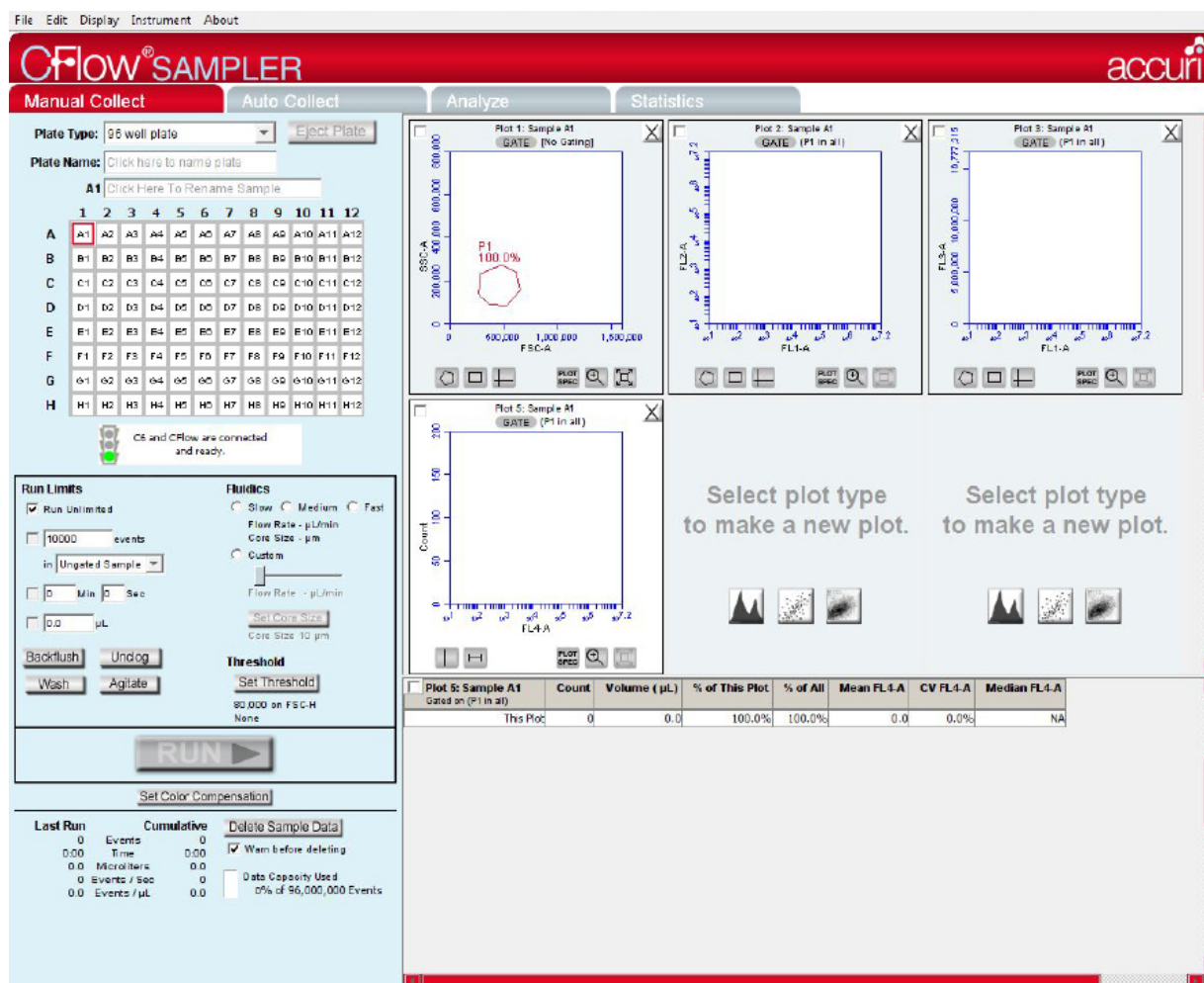
To Start Collecting

1. Identify your Plate Type.

2. Place your plate or tube rack on the CSampler and press **Load Plate**.

3. Select a sample well in the well grid.

4. Configure the run settings:
   • Run Limits
   • Fluidics
   • Threshold

5. Press **Run**. CSampler will move to the selected well and begin to collect.

Manual Controls

• **Backflush** and **Unclog**: CSampler will eject the arm to allow placement of an appropriate container under the SIP.

• **Wash**: CSampler will move the wash station to the SIP and perform one wash cycle.

• **Agitate**: CSampler will perform one agitation cycle.

• **Calibrate Fluidics**: CSampler will calibrate the fluidics system. Calibration occurs in the currently selected well.

Other Features

• AutoSave: Before collection can begin, CFlow Sampler requires a file name in order to save your data. Sample data is saved incrementally during collection.

• Run Limits: CFlow Sampler supports multiple run limits. Collection will stop when the first of the specified limits is reached.

Ready, Set, Go!

Use Manual Collect to set gates, setup plots for viewing in Auto Collect. Also use Manual Collect to determine appropriate thresholds, best flow rates and event limit that are then entered in the Auto Collect tab.

To Set Your Plate

1. Identify the location of your samples by:
   - Clicking on a well
   - Clicking on the row or column header buttons
   - Clicking on **Select All**

2. Configure your run settings for the checked wells. These include:
   - Run Limits
   - Wash settings
   - Fluidics
   - Threshold

3. Press the **Apply Settings** button.
   - CFlow Sampler requires a file name in order to save your sample automatically during collection.
   - The selected samples are now a uniform color to indicate they have the same run settings.

4. Repeat this process in order to collect other samples using different run settings.

Additional Controls

1. Agitate Plate: Specify the agitation frequency. Agitation will not interrupt sample collection. It will only occur between wells.

2. Run orientation:

   Run Horizontally: CSampler begins at A1 and collects in a horizontal pattern through H12.

   Run Vertically: CSampler begins at A1 and collects in a vertical pattern through H12.

Using the Sample Table

- Data can be pasted into the sample spreadsheet. You can populate a spreadsheet with appropriate sample names, FL names and sample notes. These cells can be copied and pasted into the sample table. You can also enter them manually.

- All data entered into this table is saved in the CFlow Sampler file.

A01 C6 and CFlow are connected and ready.
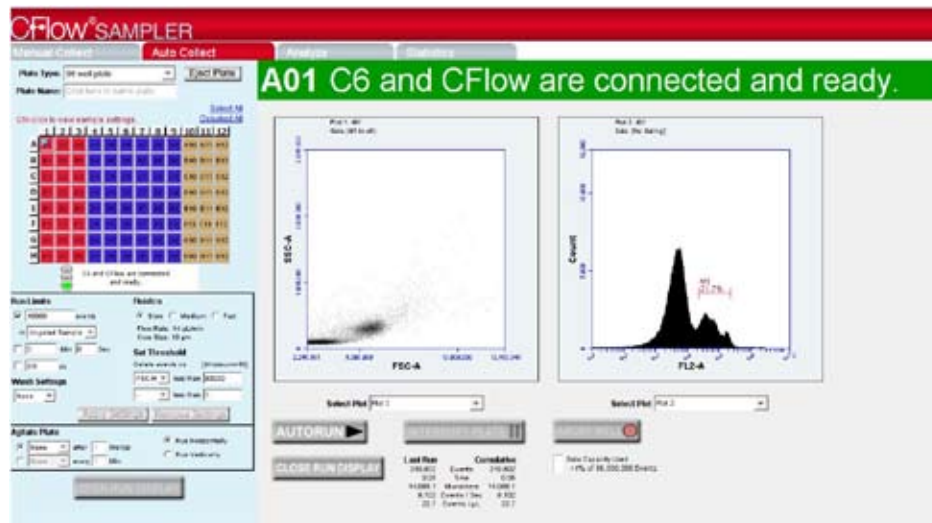
Begin Your Automated Collection

Click on the **Open Run Display** button.

1. **Autorun**: Begins the automated collection cycle.

2. **Interrupt Plate**: Stops collection after completing the current well. Collection can be restarted with the next well by pressing the **AUTORUN** button.

3. **Abort Well**: Stops collection immediately. Collection can be restarted with the next well by pressing the **AUTORUN** button.

4. **Close Run Display**: Closes the run display window.

5. Once **Auto Run** is pressed no changes can be made in setup or run sequence. The only change that can be made is the plot display.

Tips!

- Color compensation is a global setting. Threshold and color compensation settings carry over between the **Manual Collect** and **Auto Collect** tabs.

- After beginning an automated collection, sample settings can be changed by interrupting the plate, closing the run display and then making the desired change. The run display can then be re-opened and the automated collection resumed.

- During automated collection, only one of the plots created in **Manual Collect** may be viewed at a time. This plot cannot be manipulated during collection but the view plot can be changed during **Auto Collect**.

- At any point (except during collection) you can export your current sample settings in a .csv file format. From the **File** menu, select the **Export Sample Settings** menu item.

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Time to Analyze!

The Analyze tab is used to display multiple plots and samples in any combination, supporting easy analysis.

Make a New Plot

1. Click on an open plot grid, and then click on the Historgram 📊, Dot Plot 📊, Density Plot 📊, or Overlay Historgram 📊 button. The new plot displays in the Plot List.

2. Select a plot and click on a sample in the 96 well grid to view data.

Tip! The Analyze tab allows simultaneous display of multiple samples.

Hide or Delete a Plot

1. To hide a plot from view, click on the Minimize — button. To show it again, click on the plot name in the plot list.

2. To delete a plot from the plot list, click on the Delete ✕ button.

Make an Overlay Histogram

1. Click on an open plot grid and then click on the Overlay Historgram 📊 button.

2. Select up to six samples in the well grid.

3. Click on the Legend 📋 button to view the sample color assignments.

Tip! You can drag plots from CFlow to other applications.

Print Multiple Plots

1. Click on the checkbox in the upper left hand corner of the plot or the statistics table to be printed.

2. Select the **Print Selected Items** option in the File menu.
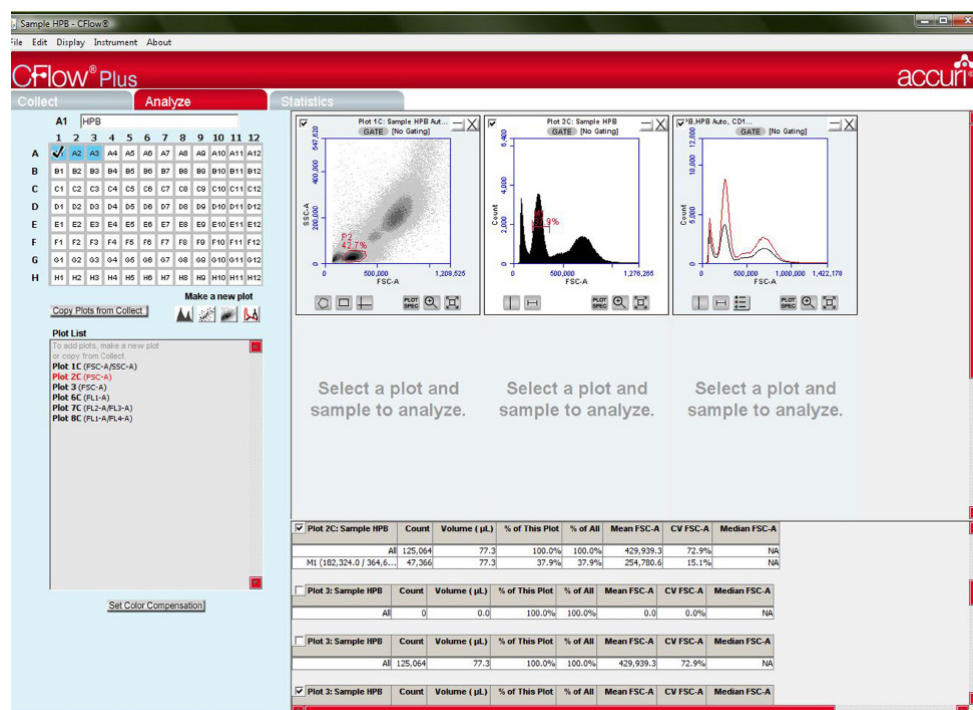
Copy a Plot from the Collect Tab

1. Click on the **Copy Plots** from Collect button.

2. In the **Copy Plots** dialog, select the plots that you want to display in the analyze tab. The plot names display in the **Plot List** followed by **-C** to indicate copies.

3. Click on an open plot grid, select the plot that you want to display, and then click on a sample in the well grid. Gating information from Collect does not copy to Analyze.

Calculate Median Statistics

1. Click on a Marker button 📊 📊 📊 📊 📊 to draw a region on the plot.

2. Click on a maker or region in a plot to select it.

3. Select **Calculate Median** from the **Display** menu. The median statistics calculate and display in the statistics table for the plot.

Set VirtualGain™

VirtualGain is used to standardize the position of a given peak from sample to sample.

1. On a histogram plot, click on the parameter name FSC-A and then click on the **VirtualGain** option from the drop down menu.

2. In the **VirtualGain** dialog, select the sample that you want to align, move the markers to define the peak, and then click on the **Preview** button. When **VirtualGain** is applied as desired, click on the **Apply** button. A black asterisk * indicates that Gained data.

Developing Your Statistics

The Statistics tab is used to display statistics for some or all of your collected samples. You will need at least one sample and a plot to view statistics.

A   **Preview** shows a preview image of the selected plot/sample combination.

B   **Display Plot Preview** lists all plots created on the Collect and Analyze tabs.

C   **Statics Column Selector** displays all available plot names, their gates, and associated statistics columns.

D   **Sample Selector** displays all samples collected or imported.

E   **Master Statistics Table** shows a custom table of statistics selected from Column and Sample Selectors.

Build a Statistics Table

1. Include a statistic in your table by clicking the corresponding check box in the **Statistics Column Selector** area.

2. Uncheck a column to remove it from the table.

Select Samples

1. Select the sample to be included by clicking on the check boxes from the 'Add to Table' column.

2. The sample names are displayed and the statistics are added to the **Master Statistics Table**.

3. Uncheck samples to remove them from the **Master Statistics Table**.

Preview a Plot or Sample

1. Use Display Plot Preview to select a plot to be previewed.

2. Use **Preview** column of the **Sample Selector** to select a sample to be previewed.

Tip! Previews are for viewing purposes only and cannot be edited from the Statistics tab.

Master Statistics Table

1. The Master Statistics Table is saved as part of the workspace by saving the CFlow file.

2. You can also copy and paste statistics into spreadsheet applicaitons for calculations or printing purposes.

Tip! This is a convenient way to record validation bead data into a spreadsheet for tracking instrument performance.