

BD ACCURI C6 PLUS TUTORIAL

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BD Accuri C6 Plus Software controls the BD Accuri C6 Plus flow cytometer system, in order to acquire data, generate statistics, and analyze results.

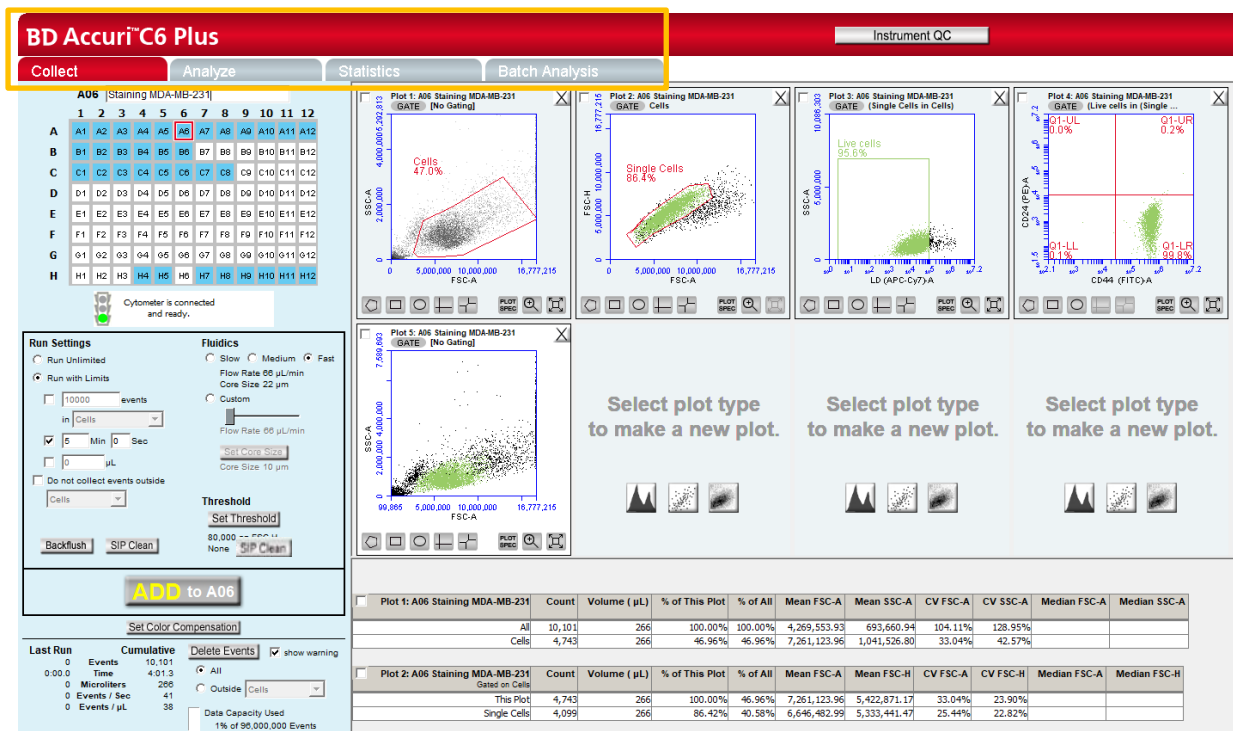
BD Accuri C6 Plus Software provides the following features:

- Tabbed views for collection, analysis, and statistics.
- Digital signal processing and color compensation at any time.
- Drag and drop plots.
- Export of files in FCS 3.0 format.
- Seamless data file importation into FCS Express.
- Enhanced Analysis upgrade adds.
- Drag and drop of publication-quality images, event coloring, live gating.
- Batch analysis of sample data.

To open BD Accuri C6 Plus Software, double-click on its icon on the computer desktop. BD Accuri C6 Plus Software opens a new blank workspace.

The workspace contains controls and displays that provide access to all functions required for data acquisition and analysis. The workspace is organized on four separate tabs:

- **Collect** – Contains controls for setting up data collection and acquiring data.
- **Analyze** – Allows analysis of multiple samples simultaneously.
- **Statistics** – Displays statistical information.
- **Batch Analysis** – Contains controls for analyzing batches of sample data and is available with the Enhanced Analysis upgrade.



The screenshot displays the BD Accuri C6 Plus software interface. The top navigation bar includes 'Collect', 'Analyze', 'Statistics', and 'Batch Analysis' tabs. The 'Collect' tab is active, showing 'Run Settings', 'Fluidics', and 'Threshold' sections. Below these are several flow cytometry plots (Plot 1 to Plot 5) and a statistics table.

Run Settings:

- Run Unlimited / Run with Limits (10000 events)
- Flow Rate: 65 µL/min
- Core Size: 22 µm
- Flow Rate: 65 µL/min
- Core Size: 10 µm
- Threshold: 80,000

Fluidics:

- Slow / Medium / Fast
- Flow Rate: 65 µL/min
- Core Size: 22 µm
- Flow Rate: 65 µL/min
- Core Size: 10 µm

Threshold:

- Set Threshold
- 80,000
- None

Plots:

- Plot 1: A06 Staining MDA-MB-231 (GATE) [No Gating] - Cells 47.0%
- Plot 2: A06 Staining MDA-MB-231 (GATE) Cells - Single Cells 88.4%
- Plot 3: A06 Staining MDA-MB-231 (GATE) (Single Cells in Cells) - Live cells 35.6%
- Plot 4: A06 Staining MDA-MB-231 (GATE) (Live cells in Single Cells) - Q1-LL 0.0%, Q1-UL 0.2%, Q1-LR 88.8%
- Plot 5: A06 Staining MDA-MB-231 (GATE) [No Gating]

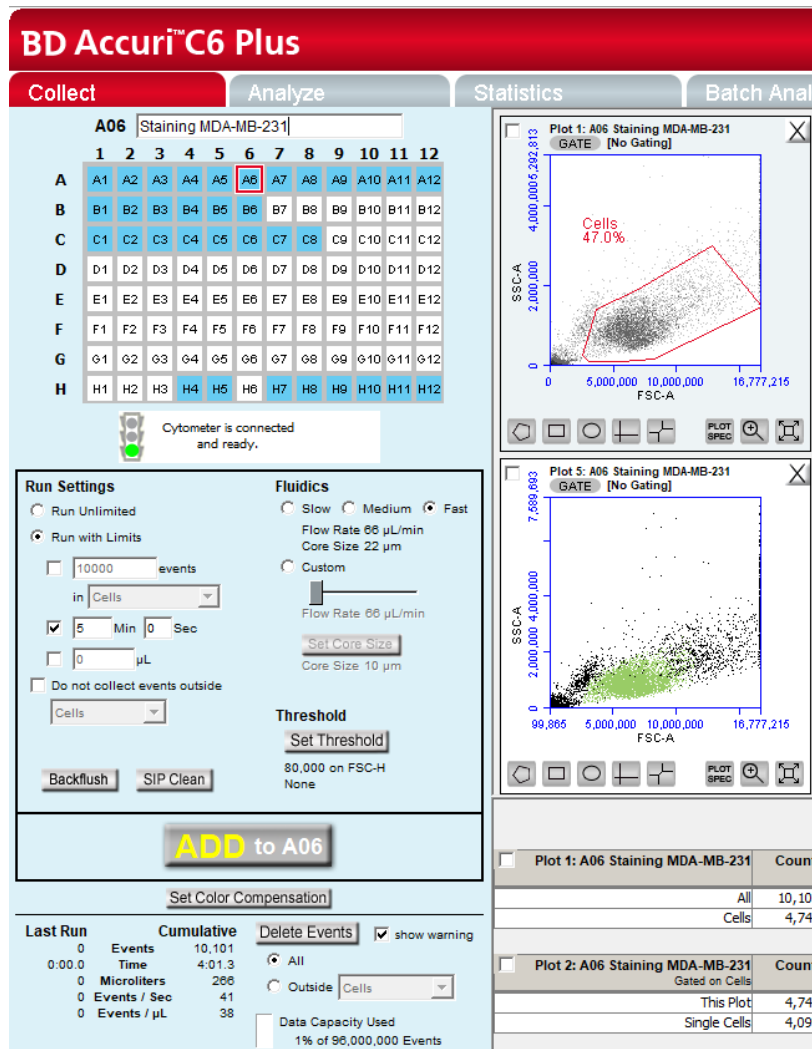
Statistics Table:

Plot	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
Plot 1: A06 Staining MDA-MB-231 (GATE) [No Gating]	All: 10,101	266	100.00%	100.00%	4,269,553.93	693,660.94	104.11%	128.95%		
	Cells: 4,743	266	46.95%	46.95%	7,261,123.96	1,041,526.80	33.04%	42.57%		
Plot 2: A06 Staining MDA-MB-231 (GATE) Cells	This Plot: 4,743	266	100.00%	46.96%	7,261,123.96	5,422,871.17	33.04%	23.90%		
	Single Cells: 4,099	266	86.42%	40.58%	6,646,482.99	5,333,441.47	25.44%	22.82%		

Collect

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

- Acquiring data.
- Creating plots (histogram, dot plot, or density) for viewing data.
- Setting stop criteria and thresholds.
- Controlling the fluidics.
- Using regions and markers to create gates and obtain statistics.
- Setting up gating strategies.
- Performing fluorescence compensation.



BD Accuri™ C6 Plus

Collect Analyze Statistics Batch Analy

A06 Staining MDA-MB-231

	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

Cytometer is connected and ready.

Run Settings

Run Unlimited
 Run with Limits
 10000 events in Cells
 5 Min 0 Sec
 0 µL
 Do not collect events outside Cells

Fluidics

Slow Medium Fast
 Flow Rate 66 µL/min
 Core Size 22 µm
 Custom
 Flow Rate 66 µL/min
 Core Size 10 µm
 Set Core Size

Threshold

Set Threshold
 80,000 on FSC-H
 None

Backflush SIP Clean

ADD to A06

Set Color Compensation

Plot 1: A06 Staining MDA-MB-231
GATE [No Gating]
Cells: 47.0%

Plot 5: A06 Staining MDA-MB-231
GATE [No Gating]

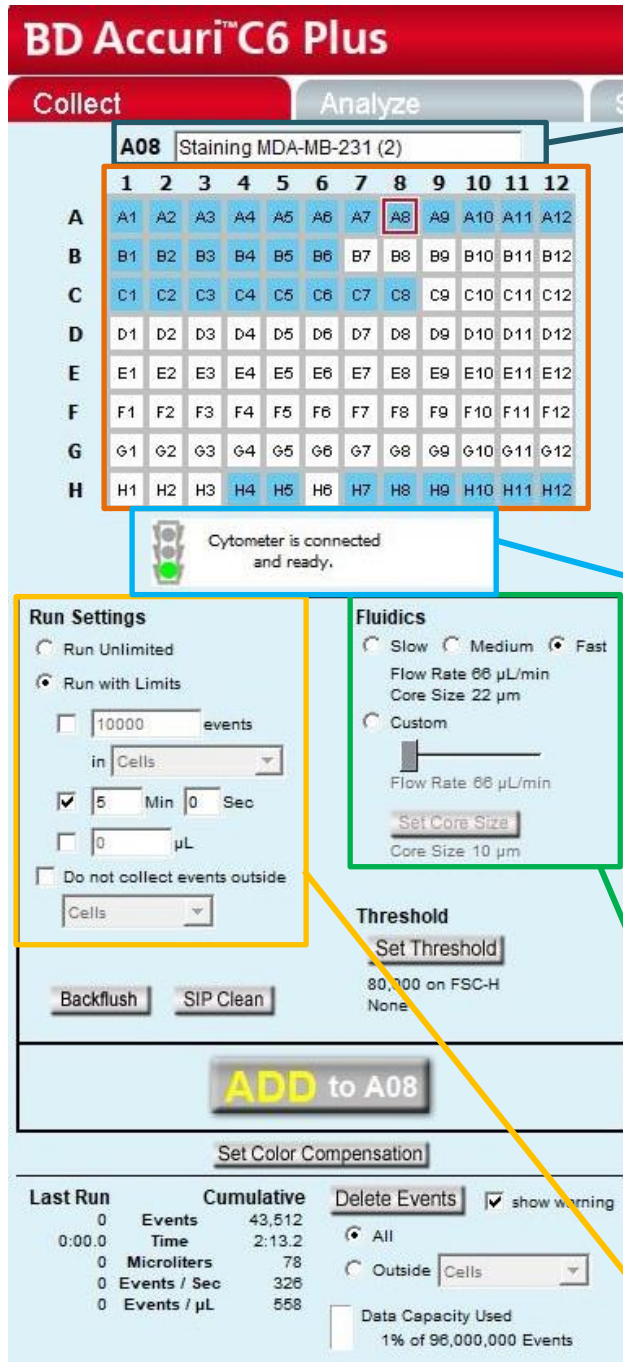
Plot 1: A06 Staining MDA-MB-231	Count
All	10,101
Cells	4,743

Plot 2: A06 Staining MDA-MB-231	Count
Gated on Cells	
This Plot	4,743
Single Cells	4,099

Last Run Cumulative Delete Events show warning

Last Run	Cumulative	Delete Events
0 Events	10,101	<input checked="" type="radio"/> All
0:00.0 Time	4:01.3	<input type="radio"/> Outside Cells
0 Microliters	266	
0 Events / Sec	41	
0 Events / µL	38	

Data Capacity Used
1% of 98,000,000 Events



The screenshot displays the BD Accuri C6 Plus software interface. At the top, there is a 'Collect' tab and an 'Analyze' tab. Below the tabs, a sample naming field contains 'A08 Staining MDA-MB-231 (2)'. A 96-well grid matrix is shown below, with columns 1-12 and rows A-H. The A8 well is highlighted with a red outline. Below the grid, a traffic light indicator shows a green light with the message 'Cytometer is connected and ready.'. The interface is divided into several sections: 'Run Settings' (with options for 'Run Unlimited' or 'Run with Limits' and various event and volume limits), 'Fluidics' (with options for 'Slow', 'Medium', or 'Fast' flow rates and 'Core Size' settings), and a 'Threshold' section. At the bottom, there is a 'Last Run' summary table and a 'Data Capacity Used' indicator.

	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

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 experiments and collect data from sample tubes. Each sample is acquired into its own well in the Sample Grid. The wells can be filled with data in any order. The wells are color-coded:

- White – Does not contain data.
- Blue – Contains data.
- Red outline – Currently selected for viewing or collecting data.

Traffic Light and Message: Indicator that displays BD Accuri C6 Plus Software's readiness and system messages. Before data collection can begin, the software must display a green Traffic Light with the message "Cytometer is connected and ready". The Traffic Light status is color-coded:

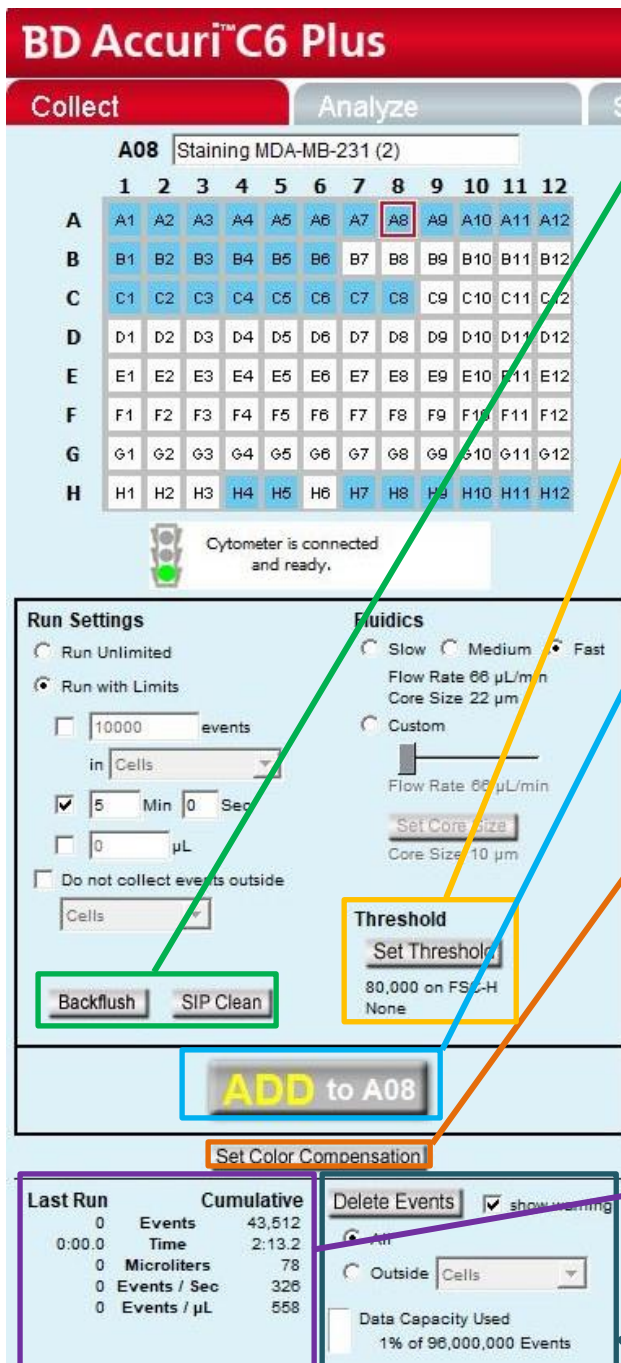
- Green – BD Accuri C6 Plus Software is ready to collect data or is collecting data.
- Yellow – The cytometer is preparing to perform an action, or a non-critical error has occurred.
- Red – A critical error has occurred.

Fluidics: Contains a set of controls for defining flow rate and core size. The system can accommodate an upper limit of 10,000 events per second, but it is recommended to acquire samples at a rate of 2,500 events per second or less to ensure the best data resolution.

Run Settings: Contains a set of controls that allow criteria definition for automatically stopping data collection. The following parameters can be used individually or in combination to set a run limit:

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

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



BD Accuri™ C6 Plus

Collect Analyze

A08 Staining MDA-MB-231 (2)

	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

Cytometer is connected and ready.

Run Settings

Run Unlimited
 Run with Limits
 10000 events
 in Cells
 5 Min 0 Sec
 0 µL
 Do not collect events outside
 Cells

Fluidics

Slow Medium Fast
 Flow Rate 66 µL/min
 Core Size 22 µm
 Custom
 Flow Rate 66 µL/min

 Core Size 10 µm

Threshold

80,000 on FSC-H
 None

ADD to A08

Last Run		Cumulative	
0	Events	43,512	
0:00.0	Time	2:13.2	
0	Microliters	78	
0	Events / Sec	326	
0	Events / µL	558	

show warning
 All
 Outside Cells
 Data Capacity Used
 1% of 96,000,000 Events

Backflush: Performs a backflush to clear the SIP.
SIP Clean: Performs small cleaning of the inside of the SIP.
 Both cleaning protocols provide instructions on the software.

Threshold: Sets the event threshold to gate out debris and noise from samples. The default value is 80,000 on FSC-H. When set correctly for any given sample set, resolution of particle or cell light scatter and fluorescence signals is greatly improved and data set size can often be reduced.

RUN/PAUSE/ADD TO: Toggle button that performs the following functions:

- RUN** – Starts the sample acquisition.
- PAUSE** – Pauses the acquisition. Click on **ADD TO** to resume data collection.
- ADD TO** – Allows additional sample collection into a well that already contains data.

Set Color Compensation: Opens the Color Compensation dialog box for correcting fluorescence spillover. It also allows changing fluorochrome labels.

Acquisition counters: Displays the following information about the most recent acquisition for the selected well (Last Run) and all acquisitions for the selected well (Cumulative) in real-time:

- Events** – Number of events sampled.
- Time** – Elapsed acquisition time.
- Microliters** – Volume of acquired sample.
- Events/sec** – Events acquired per second. When the run is completed, this is the average value.
- Events/µL** – Events acquired per microliter. When the run is completed, this is the average value.

Delete Events: Permanently deletes all events from the current sample. Enable the Show warning check box to display a warning message before deleting sample data. Also contains a *Data Capacity Used* meter that displays the amount of data storage capacity currently used in BD Accuri C6 Plus Software (max. of 1.000.000 events per well; 96.000.000 per workspace).

Setting the laser configuration

The detectors and lasers of the BD Accuri C6 Plus flow cytometer operate in a predefined configuration: detectors FL1, FL2, and FL3 read blue laser-excited fluorescence emissions, and detector FL4 reads red laser-excited emissions.

Note that, in order to adapt to your fluorochrome panel, the standard optical filters may need to be replaced with one or more of the filters that are supplied with the equipment. Ask us if you need to swap optical filters.

See on the equipment's "Laser and Filter Configuration" sheet (FORM.UCF.084) the default configuration and optical filter options.

Setting the fluidics rate

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

It is recommended to start data collection on *Slow* and observe the data rate. The setting can then be adjusted to *Medium* or *Fast*, if necessary.

There is also a *Custom* option, where you can use the toggle to customize the flow rate. Each flow rate is associated to a default core size. The core size can also be manipulated, to allow detection of bigger cells that tend to be out of scale. If you need to customize the core size, contact the UCF.

Setting a Threshold

Use thresholds to gate out light scatter and/or fluorescence signals caused by debris in cell samples and electronic noise inherent in the system. When set correctly for any given sample set, resolution of particle or cell light scatter and fluorescence signals is greatly improved and data set size is often reduced. By default, BD Accuri C6 Plus Software is set to a primary threshold of 80,000 on FSC-H.

Also, when analyzing small particles (smaller than 3.0 μm) such as bacteria, setting a lower threshold must be considered to ensure proper data acquisition.

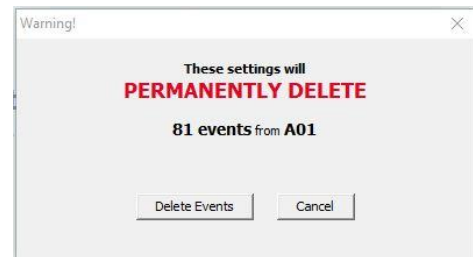
Notes on setting thresholds:

- Threshold settings can be changed before, during, or after data acquisition, but the most consistent, predictable results will be obtained if threshold settings are chosen before final data collection for any given experiment.
- All thresholds are set on the Height signal for any given parameter. For best results when setting or changing thresholds, create a plot that displays the Height signal for the threshold channel and observe the effect on data as the threshold is raised or lowered.

Suggested Starting FSC-H Threshold Settings for Various Cell Types

Cell Type	Start with FSC-H Threshold of	Notes
Cell Lines: Large (>20 µm)	500,000 to 1,000,000	Displaying FSC and SSC signals on Log scale is helpful
Cell Lines: Small (>20 µm)	200,000 to 500,000	Displaying FSC and SSC signals on Log scale is helpful
Unfixed, freshly isolated cells: white blood cells, spleen, thymus	200,000 to 500,000	Linear or Log scale display of FSC, SSC
Fixed cell suspensions: white blood cells, spleen, thymus	200,000 to 500,000	Linear or Log scale display of FSC, SSC
Platelets	10,000 to 30,000	Linear or Log scale display of FSC, SSC

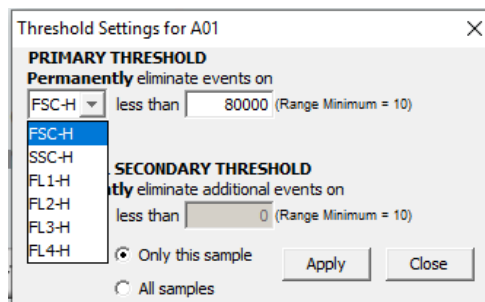
CAUTION: Take care when setting thresholds before or during data collection. Any event not meeting the threshold criteria will not be acquired or saved. When changes are made to the threshold values after data collection, BD Accuri C6 Plus Software displays a warning message if the new threshold value will result in permanent data loss.



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.



Primary Threshold Drop-Down List

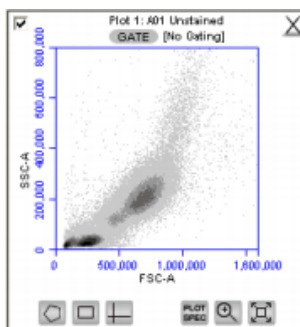
- Type a desired value in the *less than* edit box to set the threshold. A lower or higher than 80.000 FSC-H threshold may be required when working with small cells (such as platelets or bacteria) or large cells (such as cell lines), respectively.

Running the sample

- Gently resuspend the cells in the sample tube and place the tube on the SIP.
- Select an empty sample well in the BD Accuri C6 Plus Software Collect tab.
- Click on the RUN button to start the sample collection.
- BD Accuri C6 Plus Software begins fluidics initialization. During this time, the Traffic Light turns yellow and the software displays the message “*Preparing to analyze sample*”. Once initialization is complete, the Traffic Light turns green and the software displays the message “*Cytometer is running*”.
- The current well flashes blue during data collection. After the run limit is reached, the well stops flashing and remains blue, indicating that the well contains data.
- Additional data can be collected in a well that contains data by clicking on the ADD TO button. Note that run limits may need to be adjusted when adding data to a well.









Creating plots

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






View of a Density Plot

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

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

To create a new plot:

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


BD Accuri C6 Plus Software displays an FSC-A vs. SSC-A density plot by default. Configure the plot specifications as needed.

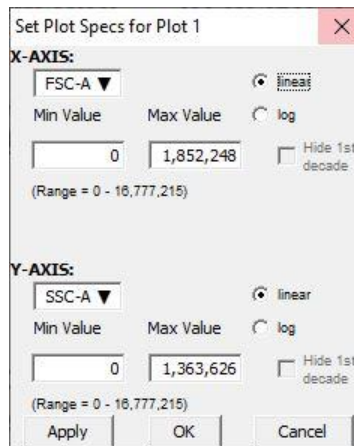
Changing plot specifications

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

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

To change the plot specifications:

- Click on the *Plot Spec* tool icon. 



Set Plot Specs Dialog Box

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

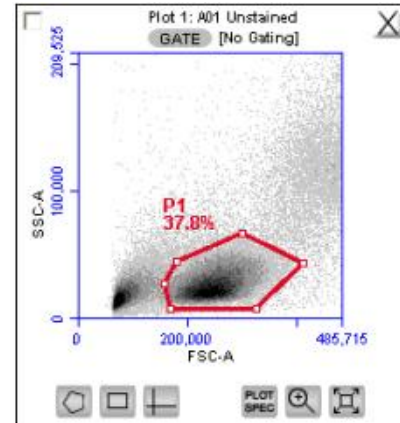
Creating a new gate

To create a gate in a density or dot plot:

- Click on one of the gating tools.
- Use the mouse to draw a region (labeled P1 for a polygonal gate, R1 for a rectilinear gate, or Q1 for a quadrant gate). To draw a polygon, click on the mouse to anchor each vertex and double-click to close the polygon.

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

BD Accuri C6 Plus Software automatically displays the percentage of cells within the region.

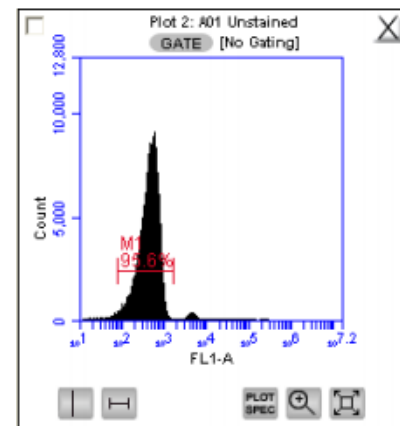


Example of a Polygonal Gate

To create an horizontal marker in a histogram plot:

- Click on the Horizontal Marker Tool.
- Click and drag the cursor horizontally across the area to apply the gate (labeled M1).

BD Accuri C6 Plus Software automatically displays the percentage of cells within the margins of the marker (labeled M1).



Example of a Horizontal Marker

Renaming plots/regions and coloring events

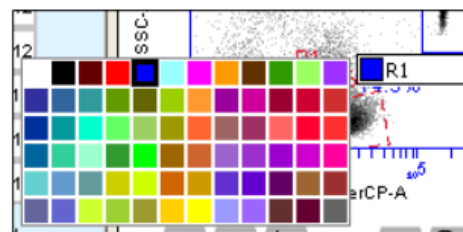
Plots, or regions in a plot, can be renamed. In each case the method is the same.

To rename a plot or region:

- Double-click on the plot or region name.
- In the text field, type in a new name.
- Press Enter.

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

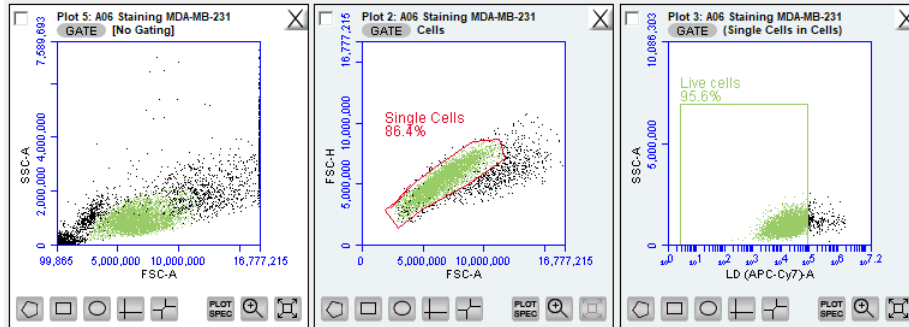
NOTE: Color will not appear in a density plot.



Event Coloring

To color events:

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



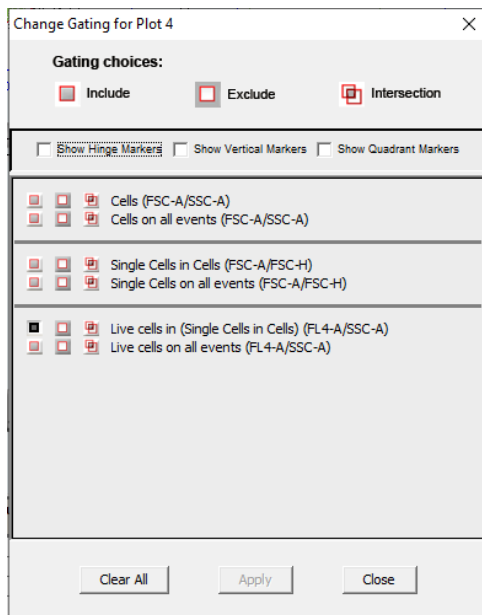
“Live cells” colored in green

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

Applying a gate to a plot

Click on the *GATE* button at the top of the plot. Only polygon (P), rectilinear (R), and marker (M) gating regions automatically appear in the *Gating* dialog box list of options.

To include hinge markers, vertical markers or quadrant markers in the list of gates, enable the associated check box(es) in the *Change Gating* dialog box.

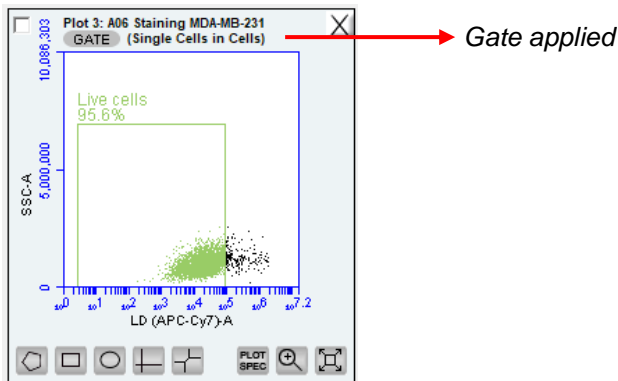


Gating dialog box

Select one of the following gating icons:

- Include – to analyze the events within the region. Multiple gates can be included in a single plot.
- Exclude icon – to analyze the events outside of the region.
- Intersection icon – to analyze the events within the intersection of two or more regions.

Click on *Apply*. BD Accuri C6 Plus Software displays the type of gate that is applied next to the *GATE* button in the plot.

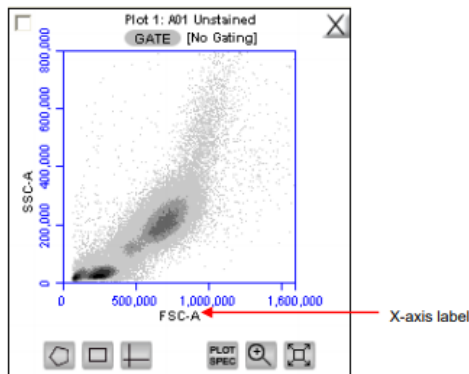


Naming plot axes

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

To name a plot axis:

- Click on an axis label and select *Rename Parameters/Set Color Compensation* from the drop-down menu. This option can also be accessed from the *Edit* drop-down menu.



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

Rename Parameters/Set Color Compensation dialog box

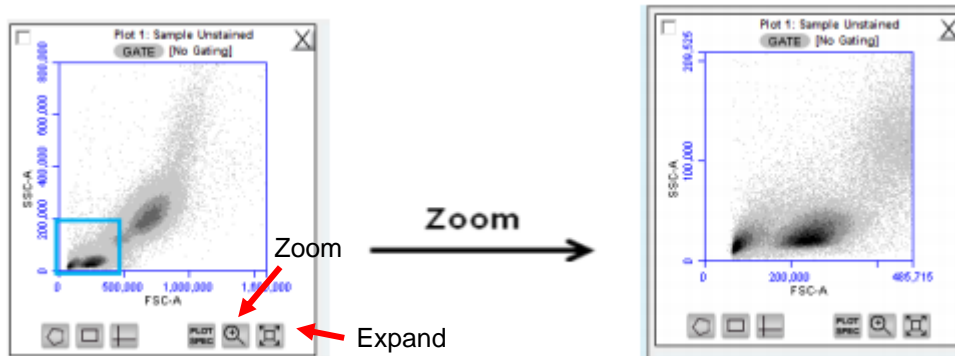
- Do one of the following
 - *Apply* – applies parameter names to the current sample
 - Check the box “*Apply to all samples*” if you want to apply changes to all samples
- Click *Close*.

Zooming on a plot

Click on the *Zoom* tool.

Click and drag the mouse in the plot to draw a zoom area.

Repeat these 2 steps as needed to zoom in.



Before and After Using Zoom Tool

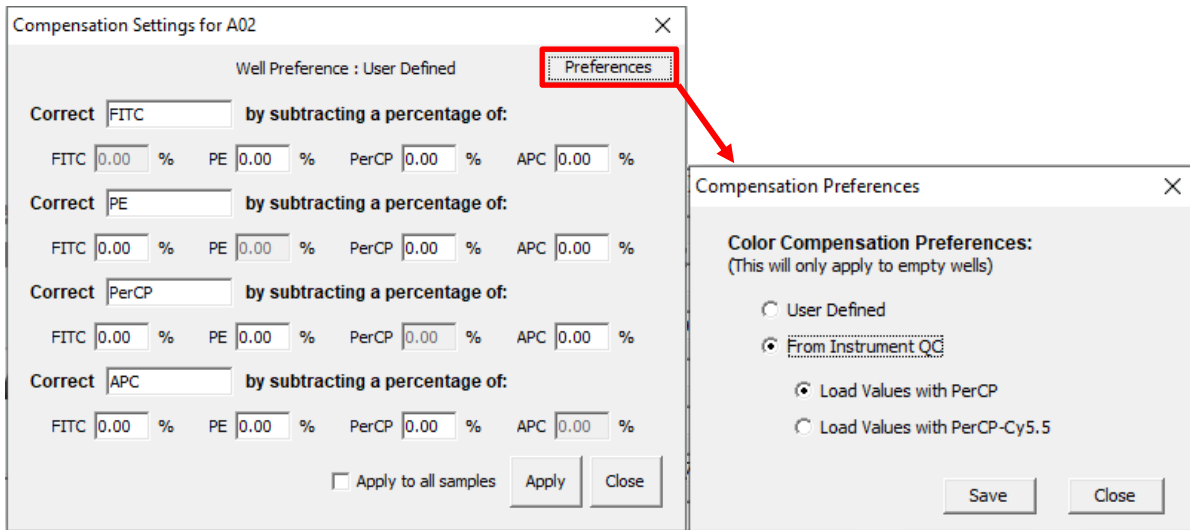
To zoom out:

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

Correcting Fluorescence Spillover

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

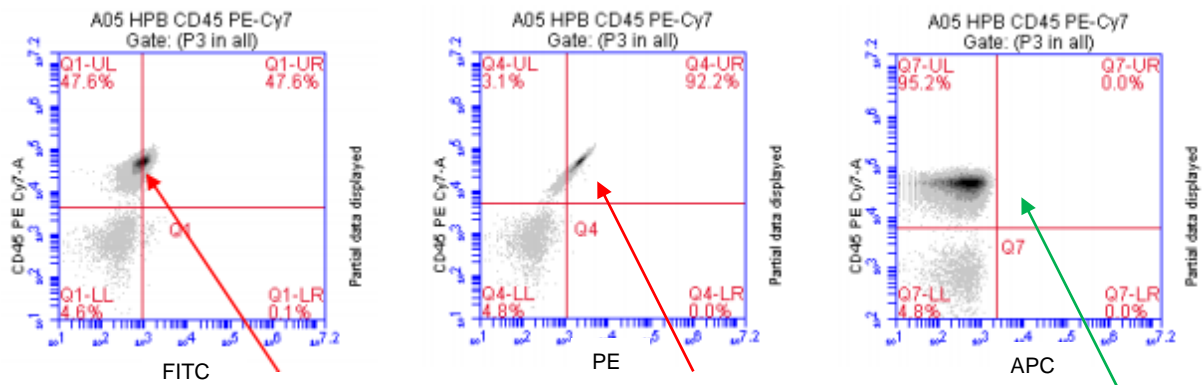
On *Set Color Compensation > Preferences*, you can choose between using your own custom compensation settings (User Defined), or using the compensation settings obtained from instrument quality control (QC), prepared for the standard fluorophores: FITC, PE, PerCPCy5.5 and APC. The second option cannot be used if you change the optical configuration of the equipment (optical filters).



Set Color Compensation and Compensation preferences dialog boxes

In the following example, we have data collected from a **PE-Cy7 single-stained control**.

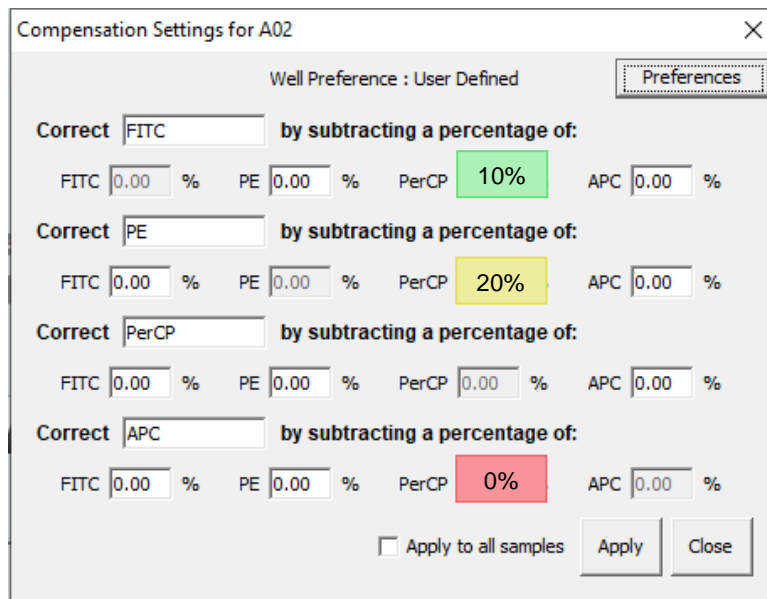
Most of the fluorescence signal from PE-Cy7 positive cells is detected on the PerCP channel (FL3; 670 LP), as expected. However, there is also PE-Cy7 signal detected on the FITC channel (FL1; 533 BP) and on the PE channel (FL2; 585 BP), so that plots of data for those detectors appear to have positively fluorescent cells. No signal from PE-Cy7 appears on the APC channel (FL4, 675 BP).



Fluorescence spillover of PE-Cy7 into the FITC, PE and APC channels

This means we need to correct the FITC and the PE channels, by subtracting a percentage of the PE-Cy7 fluorescence that is spilling over. Since the APC detector was not affected by PE-Cy7 fluorescence, there is no need for corrections on that channel.

To perform these changes, click on the *Set Color Compensation* icon in the Collect menu.



Compensation Settings for A02

Well Preference : User Defined

Correct FITC by subtracting a percentage of:

FITC 0.00 % PE 0.00 % PerCP 10% APC 0.00 %

Correct PE by subtracting a percentage of:

FITC 0.00 % PE 0.00 % PerCP 20% APC 0.00 %

Correct PerCP by subtracting a percentage of:

FITC 0.00 % PE 0.00 % PerCP 0.00 % APC 0.00 %

Correct APC by subtracting a percentage of:

FITC 0.00 % PE 0.00 % PerCP 0% APC 0.00 %

Apply to all samples

Apply Close

Set Color Compensation dialog box with compensation settings applied

To change the compensation matrix:

- In the text box next to the channel button, type an arbitrary percentage of the signal to subtract. According to the previous example, we applied a 10% compensation to the FITC channel, 20% to PE, and 0% to APC.
- Click on *Apply* to update the plots and statistics tables.
- Evaluate the data and adjust the values if needed.
- To apply the fluorescence subtraction to all samples, select *Apply to all samples*.
- Click on *Apply* and then *Close* to apply the color compensation settings.

Saving a BD Accuri C6 Plus Software file

Saving a workspace (.ciplus)

The BD Accuri C6 Plus Software workspace file includes the following elements:

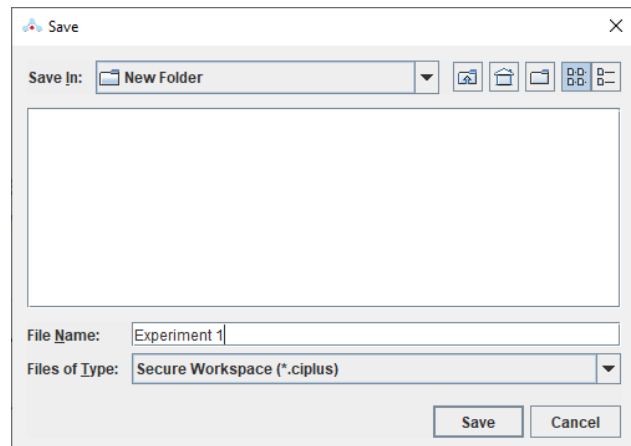
- Sample data.
- Plot layouts.
- Zoom levels.
- Gating.
- Color compensation.
- Threshold settings.
- Collect tab settings.
- Changes made in the Analysis or Statistics tabs.

To manually save a BD Accuri C6 Plus Software file

- Select *File > Save*.

To manually save a BD Accuri C6 Plus Software file with a new name:

- Select *File > Save Workspace As*.
- If necessary, navigate to the location to save the file.
- In the Save dialog box, enter the file name and click on the Save button. The file is saved with the extension .ciplus.



Save dialog box

Saving a template (.ciplust)

The template file includes everything except the sample data.

Used for quick and easy setup and analysis. All markers, regions, gates, parameter names, and sample names are saved without any data.

- Select *File > Save Template as*.
- If necessary, navigate to the location to save the template file.
- Save BD Accuri C6 Plus Software Template.

Exporting and Importing files

Data can be exported from sample wells from the Collect or Analyze tab as individual FCS 3.0 files at any time. Only FCS files created by BD Accuri C6 Plus Software can be imported into a BD Accuri C6 Plus Software file or template.

To export data:

- Do one of the following:
 - Select *File > Export FCS File* – to export and save the currently selected data well as an FCS 3.0 file.
 - Select *File > Export ALL Samples as FCS* – to export and save all data wells as individual FCS 3.0 files.
 - Select *File > Export Plot Data as CSV* – to save an individual file in .csv format.

To import an FCS data file:

- Select an empty data well in a BD Accuri C6 Plus Software file or template. If the currently selected data well contains data, the software will start FCS file import in the first empty data well.
- Select *File > Import FCS File*.
 - Navigate to the location of the file.
 - Select the file and click on the *Open* button.
- Multiple FCS files from within a folder can be selected and imported as a group using Shift + Click. Files begin importing into the selected data well and proceed in succession, horizontally, from left to right, wrapping to the following row once the current row is filled.

Analysis and Statistics Menus

The Analysis and Statistics menus are not used in the system. The cytometer should only be used for acquisition. You should always export your data as FCS files, so you can analyze afterwards.

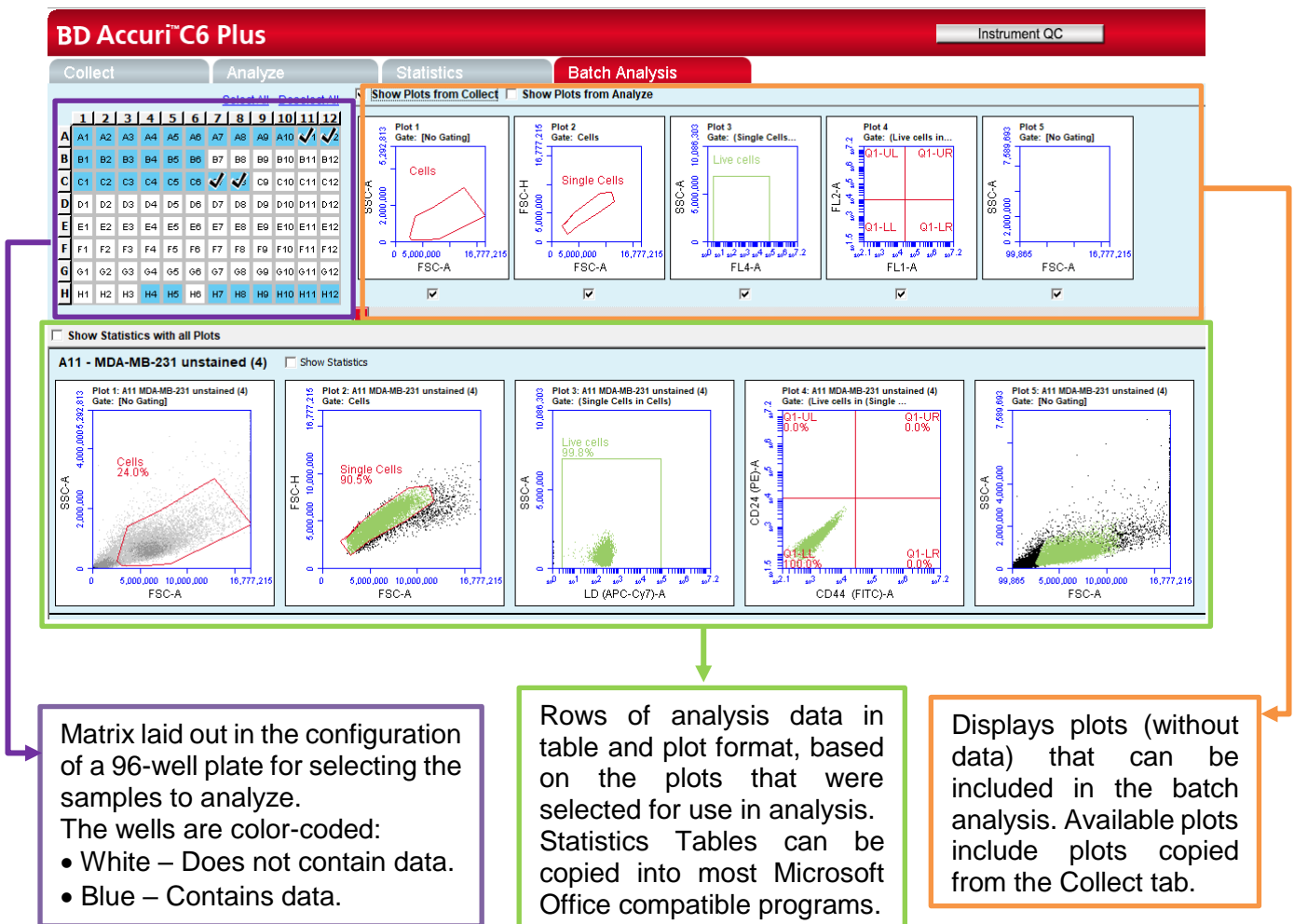
Batch Analysis Menu

The Batch Analysis tab allows an automated analysis to be performed on multiple samples at the same time. The plot types created during acquisition will appear at the top of the screen. Statistics can be displayed for all files selected. This way, you can export your plots quickly into a PowerPoint document and your statistic value table into the Excel (for instance if you want to take the volume data of each sample, for cell counting).

The Batch Analysis tab is organized into two major sections:

Setup panel – Panel on the top half of the window that contains controls for selecting samples and plots for analysis.

Data display – Large area on the bottom half of the window that shows sample data in plots and in a Statistics Table.



Matrix laid out in the configuration of a 96-well plate for selecting the samples to analyze. The wells are color-coded:

- White – Does not contain data.
- Blue – Contains data.

Rows of analysis data in table and plot format, based on the plots that were selected for use in analysis. Statistics Tables can be copied into most Microsoft Office compatible programs.

Displays plots (without data) that can be included in the batch analysis. Available plots include plots copied from the Collect tab.

To perform Batch Analysis:

- Run a number of samples.
- After running the samples, click on the Batch Analysis tab.
- Select the samples to analyze in the Sample Grid by clicking on the wells.
- Do one or both of the following:
 - Enable the *Show Plots from Collect* check box to make all of the plots from the Collect tab available for batch analysis.
 - Enable the *Show Plots from Analyze* check box to make all of the plots from the Analyze tab available for batch analysis.
- Select the specific plots to include in the batch analysis by enabling the check boxes under those plots.
- Enable the *Show Statistics with All Plots* check box to show statistics in every row.

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

To export data:

- Do one of the following in the lower right-hand corner of the Batch Analysis tab:
 - Click on the *Export to PowerPoint* button.
 - Click on the *Export Stats to Excel* button.
- In the Save dialog box, navigate to the destination folder and type a file name.
- BD Accuri C6 Plus Software exports the file to the specified location.

