

Display of BD Accuri™ C6 Software-Generated FCS 3.0 Files Using FlowJo™ for PC and Mac® v7.6

Technical Note

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Introduction

Scaling of axes in earlier versions of FlowJo software is preset to display a maximum of 1,024 channels of data. FCS files from flow cytometers with 18-bit or higher digital signal processing, such as the BD Accuri™ C6 flow cytometer system, often require adjustment of FlowJo's scaling factors to properly visualize the data.

In BD Accuri™ C6 software, the range of axes viewed is set using the Zoom Tool or the Plot Spec box. This technical note describes how to access and change FlowJo axes settings to recreate plots zoomed in BD Accuri C6 software.

The FlowJo Preferences and settings outlined here can be saved for application to future analyses.



Before You Begin

Decide which parameters to display in linear and log scale. If there are no previously set Preferences, FlowJo will, by default, display FSC and SSC data in linear scale and all fluorescence parameters in log scale.

There are two ways to set up axes scaling in FlowJo. The first is to change settings under FlowJo's Preferences (Section A). We recommend this method for log-scaled parameters. The second method is to use the Transform button to change the displayed parameter range (Section B). This method works best for setting the axes scaling for linear parameters such as light scatter.

To recreate BD Accuri C6 software plots in FlowJo with the same axes scaling as the original BD Accuri C6 software file, note the channel range used to view each parameter (Figure 1).

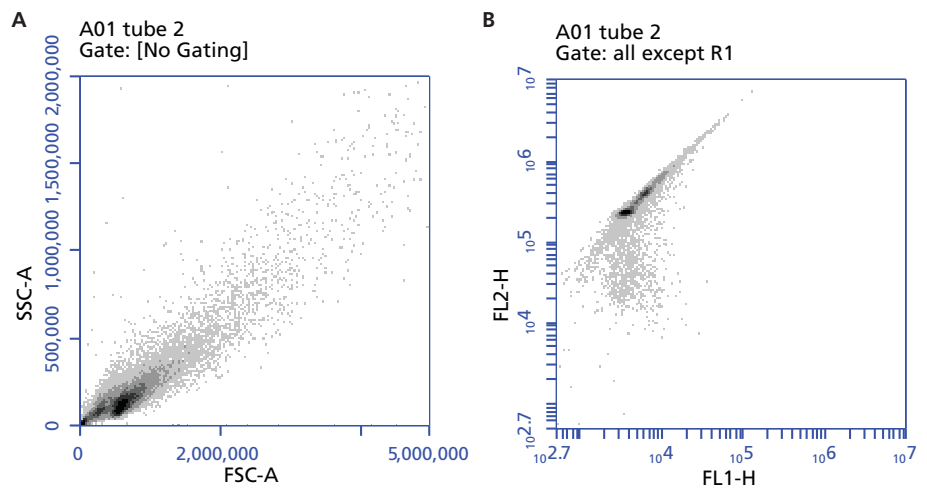


Figure 1. Examples of axes ranges used in a BD Accuri C6 software file.

- A. *Lin FSC-H, Channels 0 to 5×10^7*
Lin SSC-H, Channels 0 to 2×10^7
- B. *Log FL1-H, Channels 500 to 1×10^7*
Log FL2-H, Channels 500 to 1×10^7

A. Using the Global FlowJo Preferences to Set Parameter Axes Ranges

(recommended for log-scaled parameters)

1. From the **Edit** menu, select **Preferences**, then click the **Data Scaling** icon (Figure 2, Step 1).

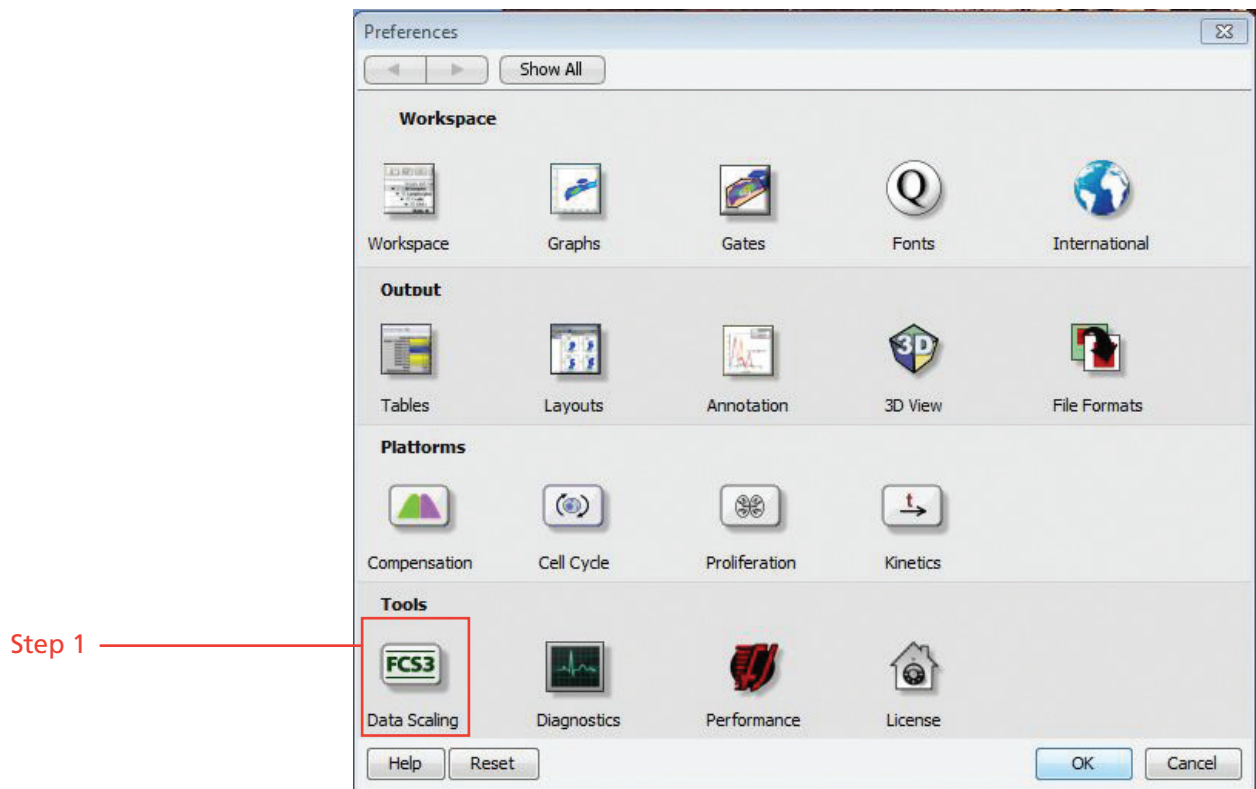


Figure 2. FlowJo Preferences dialog.

2. The **Data Scaling** window opens (Figure 3).

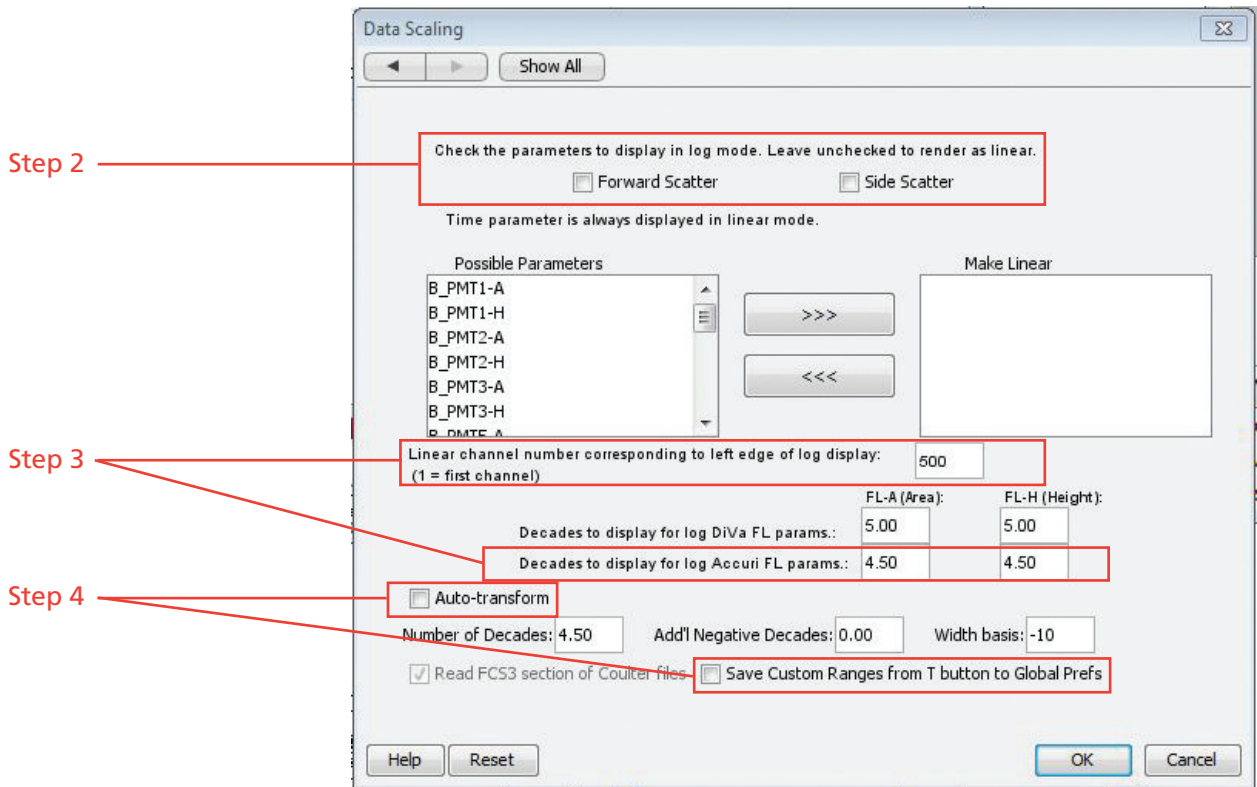


Figure 3. Setting logarithmic scaling.

- To view Forward and Side Scatter in linear scale, clear the checkboxes next to Forward Scatter and Side Scatter (Figure 3, Step 2) under **Check the parameters to display in log mode**. See Section B to adjust the channel range viewed on linear FSC and SSC.
- Next to **Decades to display for log Accuri FL params.**, set the range of channels that will be viewed on all logarithmically displayed parameters (Figure 3, Step 3). If the range of channels spanned by the data in the BD Accuri C6 software file is known, use Table 1 as a guide in choosing values to enter. For most analyses, the data occurs over a range of 4 to 5 decades.
- Set the **Linear channel number corresponding to left edge of log display** field (Figure 3, Step 3). See Table 1 as a guide.
- Make sure that the **Auto-transform** and the **Save Custom Ranges from T button to Global Prefs** checkboxes are left cleared (Figure 3, Step 4).
- Click **OK** to return to the **Workspace** screen.

Table 1. Standard log conversion settings: some typical channel ranges.

Channel Range to View (Log Scale)	Left Edge Channel No.	No. of Decades (Area & Height)
All	1	7.2
10 to 16.7 million	10	6.2
10 to 10 million	10	6
10 to 1 million	10	5
1,000 to 16.7 million	1,000	4.2
100 to 10 million	100	5

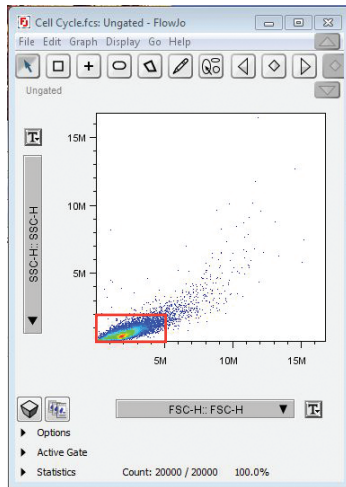


Figure 4. FCS file exported from BD Accuri C6 software and viewed in FlowJo before changing the displayed parameter range to zoom in on the red-boxed area.

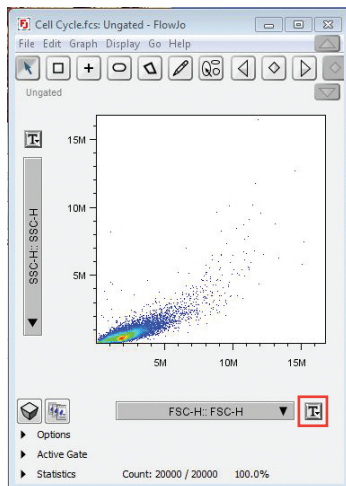


Figure 5. Transform (T) button.

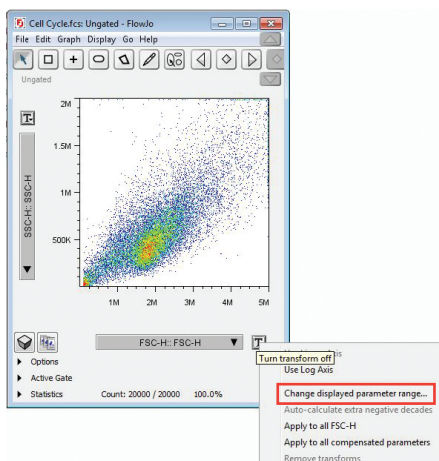


Figure 6. Change displayed parameter range... (red box).

B. Using the Transform Button (T Button) to Choose a Channel Range for Data Display (recommended for use with linear parameters)

The FlowJo **Data Scaling** option may not allow enough flexibility in choosing the channel range. To “zoom in” on a specific data range, use the T button and then change the displayed parameter range.

An example is the FSC-H vs SSC-H plot in Figure 4. When the data was collected in BD Accuri C6 software, the user had zoomed in on the area shown in the red box, so that the maximal channels were 5×10^6 on FSC-H and 2×10^6 on SSC-H. Figure 6 shows how the FCS file data will look when initially opened in FlowJo. The following directions illustrate how to zoom in on this area.

1. In the FlowJo workspace view, double-click on a sample to open the dot plot view, and then click the T button (Figure 5, red box).
2. Select **Change displayed parameter range** (Figure 6).
3. Place the appropriate values in **Min:** and **Max:** for both **FSC-H** and **SSC-H** (Figure 7). In this case, the values used are 1 and 5,000,000 for FSC-H and 1 and 2,000,000 for SSC-H. (See the placement of the red box in Figure 4.)
4. Click **Apply** to preview the changes and **OK** to save settings.

The dialog box 'Set Display Range for FSC-H, SSC-H' has a section 'Enter ranges manually' with a table for FSC-H and SSC-H. Below the table are buttons for 'Auto', 'Reset All', and 'Show All'. At the bottom are 'Apply', 'Reset', 'OK', and 'Cancel' buttons.

	FSC-H	SSC-H
Min:	1	1
Max:	5000000	2000000

Figure 7. Setting the display range.

C. Saving Settings

The scaling changes made in Preferences and Displayed Parameter ranges will be saved with the Workspace. In addition, you can also save your settings as a template.

1. To save a Workspace, click **File**, then **Save** or **Save As**.
2. To save a Template, click **File**, then **Save as Template**.

