Introduction

Axes scaling in earlier versions of FlowJo are preset to see a maximum of 1024 channels of data. FCS files from flow cytometers with 18-bit or higher digital signal processing, such as the Accuri C6 Flow Cytometer® System, often require adjustment of FlowJo's scaling factors in order to properly visualize the data.

In CFlow, the axes range 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 CFlow.

The FlowJo Preferences and/or Derive Parameters 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 **Derive Parameters** function (Section B). This method works best to set the axes scaling for linear parameters, such as light scatter.

To recreate CFlow plots in FlowJo with the same axes scaling as the original CFlow file, note the channel range used to view each parameter (Figure 1).
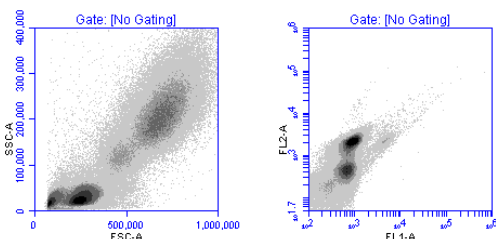
Figure 1. Examples of axes ranges used in a CFlow file.

Lin FSC-A, Channels 0 to 1×10^6
Lin SSC-A, Channels 0 to 4×10^5
Log FL1-A, Channels 100 to 1×10^6
Log FL2-A, Channels 50 to 1×10^6

The following directions will only take effect with newly created workspaces. It is recommended that the changes described below are made prior to adding FCS files to the workspace.

A. **Using the global FlowJo Preferences to set parameter axes ranges.** (Recommended for log-scaled parameters.)

1. From the FlowJo drop-down menu choose **Preferences,** then click on the **Define** box under the note **Reading Diva and other 32-bit data files** (Figure 2).
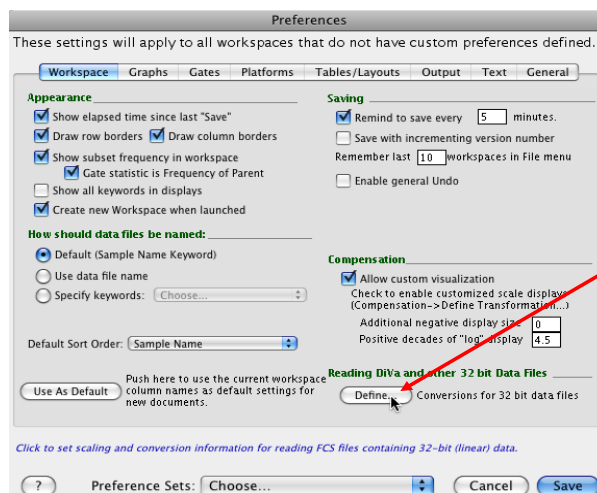
Figure 2. FlowJo **Preferences** dialog box

phone
USA +1.734.994.8000
UK +44 (0)1480 308380

support
TechSupport@AccuriCytometers.com
EuroTech@AccuriCytometers.com

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2. The Box **Options for Reading 32 bit Data Files** appears (Figure 3).

Figure 3. Setting "Standard Log Conversion Settings"

3. To view Forward and Side Scatter in linear scale, uncheck the boxes next to Forward Scatter and Side Scatter under **Select which parameters to display with logarithmic scaling** (Figure 3, Step 3). Refer to **Using the Derived Parameters Function** to adjust the channel range viewed on linear FSC and SSC.

4. Under **Standard Log Conversion Settings**, set the range of channels that will be viewed on all logarithmically displayed parameters (Figure 3, Step 4). If the range of channels spanned by the data in the CFlow file is known, Table 1 can be used as a guide in choosing values to enter. For most analyses, the data occurs over a range of 4 to 5 decades.

Channel Range to View (Log Scale)	Left Edge Channel #	# of Decades (Area & Height)
All (1 to 16.7 million)	1	7
10 to 16.7 million	10	6
10 to 10 million	10	5
10 to 1 million	10	4
1000 to 16.7 million	100	5
100 to 10 million	100	4

Table 1. Standard Log Conversion Settings: Some typical channel ranges.

NOTE: If using FlowJo version 8.8, changes in the left edge channel number may not be reflected in the data plot. Accuri suggests using either FlowJo MAC versions 8.6 or 8.7, or FlowJo for PC/MAC 7.6.
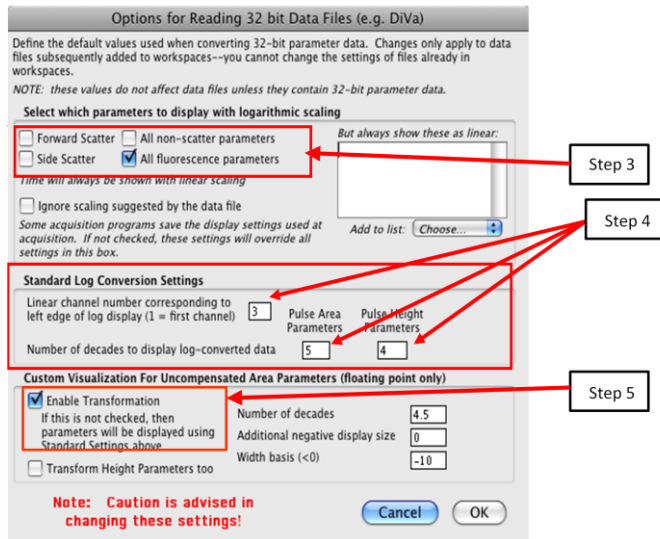
5. In many cases, the removal of the bi-exponential display function will be desired. In FlowJo, it is automatically enabled unless the Enable Transformation box is unchecked (Figure 3, Step 5).

6. Click "OK", to return to the **Preferences** screen.

7. Click on the **Save** button to save these settings as the new global FlowJo Preferences or click **Choose** (next to Preference Sets) and assign a new name before saving.

8. Please note that while being intended to be global, FlowJo Preferences are not applied to a workspace that has been customized. With Accuri data, most workspaces will be customized. Therefore, the settings applied in FlowJo Preferences should also be applied to Workspace Preferences:

   a. Select **Workspace Preferences** under the Workspace drop down menu

   b. Click on the Define box under the heading **Reading Diva and other 32-bit data files**

   c. Enter the same settings as entered in FlowJo Preferences in steps 3 to 5.

   d. Click **OK** and then **Save** on the next screen that appears.

9. To apply these Preference Settings to the current workspace:
   a. Save and close the Workspace file.
   b. Re-open the Workspace file.

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USA +1.734.994.8000
UK   +44 (0)1480 308380

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B. Using the Derived Parameters function to choose a channel range for data display. (Recommended for use with linear parameters.)

The FlowJo **Preferences Options for Reading 32-bit Data Files** does not allow much flexibility in choosing the channel range. To "zoom in" on a specific data range, use the Derived Parameters Function.

An example is the FSC-A vs. SSC-A plot in Figure 4. When the data was collected in CFlow, the user had zoomed in on the area shown in the red box, so that the maximal channels were $5x10^6$ on FSC-A and $2x10^6$ on SSC-A. 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.
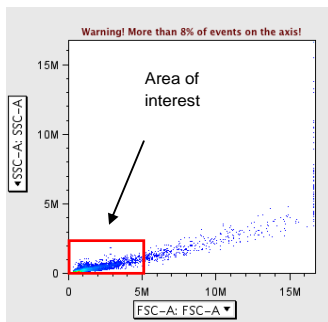
Figure 4. FCS file exported from CFlow and viewed in FlowJo before Deriving Parameters to zoom in on red-boxed area.

Deriving new linear parameters and re-setting the axis scale (i.e. zooming)

1. In FlowJo click **Platform,** then select **Derive Parameters** from the drop down menu, followed by **Define new or change.**

2. Under **Custom Derived Parameters**, click on **Choose** and select FSC-A : FSC-A. (Figure 5).

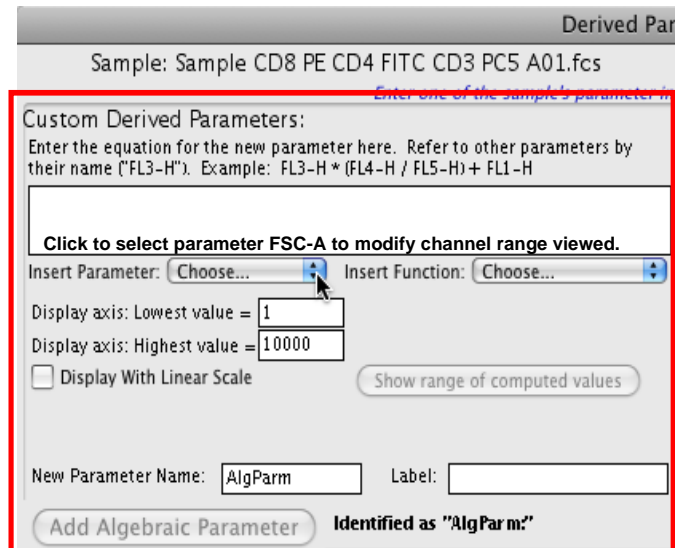3. Click on the **Display with Linear Scale** box (Figure 6, Step 3).

Figure 5. Custom Derived Parameters section of Derived Parameters dialog box.

4. Place the appropriate values in **Display Axis: Lowest Value and Highest Value** (Figure 6, Steps 4 and 5). In this case, the values used are 1 and 5,000,000 for FSC-A. (Refer to the placement of the red box in Figure 4).
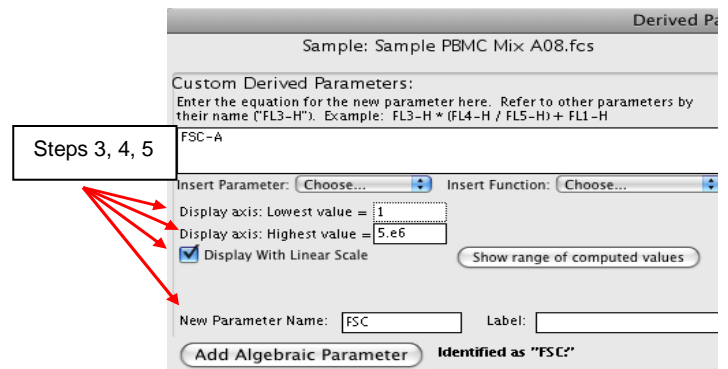
Figure 6. Custom Derived Parameters choices have been modified

5. Name and label this *new* parameter then click **Add Algebraic Parameter**. (In the example in Figure 7, the new parameter name is FSC). The name must be different than the original parameter name (FSC-A in this case). The new parameter will appear as a **Currently Defined Derived Parameter** (Figure 7).

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USA +1.734.994.8000
UK   +44 (0)1480 308380

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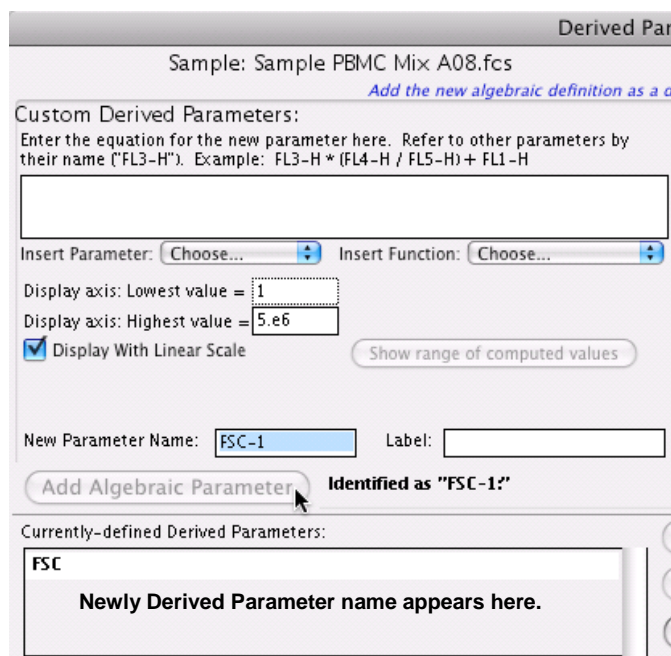
Figure 7. New parameter **FSC** has been assigned a channel range for viewing (1 to 5×10^6) and added to the **Currently-Defined Derived Parameters** list.

6. Repeat this process with SSC-A (or any other desired parameters). Then click **Done**. It is not necessary to restart FlowJo after **Deriving New Parameters** on a plot or histogram. If a FSC-A vs. SSC-A plot is already open in the workspace, click on the parameter name on the axis and scroll down to choose the new parameter. The plot will update.

Applying settings to all files

Once the new parameters are derived and **Done** is clicked, return to the pull-down menu under **Platform**, click **Derive Parameters**, and select **Copy to Group's Samples**. Click **Add** when the box opens asking to add new parameters to all the samples in the Workspace.

Additional Notes on using FlowJo

In order to apply the Workspace axes scaling changes to multiple groups of data files, save the Workspace with one renamed sample file that contains the desired scaling factors and **Derived Parameters**. Next, **Add** the new group of samples and follow **Applying Settings to all files** to apply the settings to all the samples added to the Workspace. This way multiple Workspace files, containing one sample, can be designed with settings usable for a variety of analysis types, e.g.:

- Mouse Spleen Immunofluorescence
- Jurkat Cell Apoptosis
- Cell Cycle/ BrdU
- GFP Transfectants

For more information on FlowJo please visit http://flowjo.com/home/macintosh.html