BD Accuri®

Display of BD CFlow® Software-Generated FCS 3.0 Files Using FlowJo™ Version 8.6, 8.7, or 9.3.3 for the Mac®

Instruction Manual

BD

Introduction

This document describes how to adjust axes scaling factors in versions 8.6, 8.7, or 9.3.3 of FlowJo™ software to optimally visualize FCS 3.0 data files generated using the BD Accuri® C6 flow cytometer with BD CFlow® software.

In FlowJo software (Mac® versions 8.6, 8.7, and 9.3.3), axes scaling is preset to a maximum of 1024 data channels. The default settings display FSC and SSC data in linear scale and all fluorescence parameters in log scale. To set up axes scaling for log-scaled parameters, we recommend using the Preferences dialog. For linear parameters such as light scatter, we recommend using the Derive Parameters dialog. Make any changes to FlowJo settings before adding FCS files to the workspace.

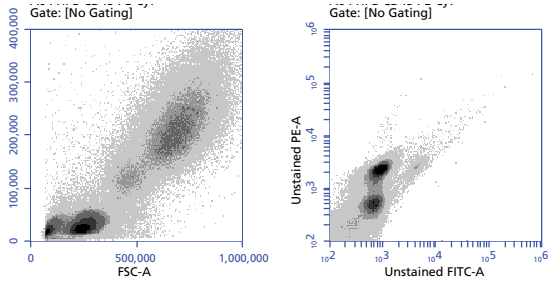

Figure 1. FlowJo examples of axes ranges used in a BD CFlow file.
Lin FSC-A, Channels 0 to 1 x 10^6 Log FL1-A, Channels 100 to 1 x 10^6
Lin SSC-A, Channels 0 to 4 x 10^5 Log FL2-A, Channels 50 to 1 x 10^6

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

Using FlowJo preferences to set parameter axes ranges (recommended for log-scaled parameters)

1. From the FlowJo **Home** screen, select **Preferences** from menu, then click **Define** in the **Reading Diva and other 32-bit data files** section.

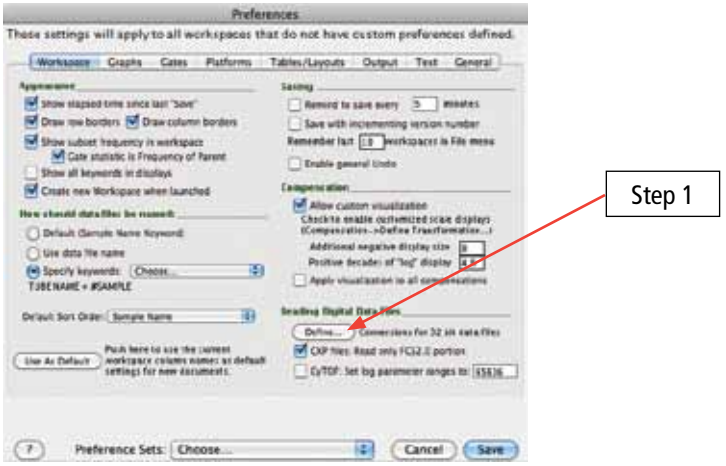

Figure 2. FlowJo Preferences dialog.

The **Options for Reading 32 bit Data Files** section expands.

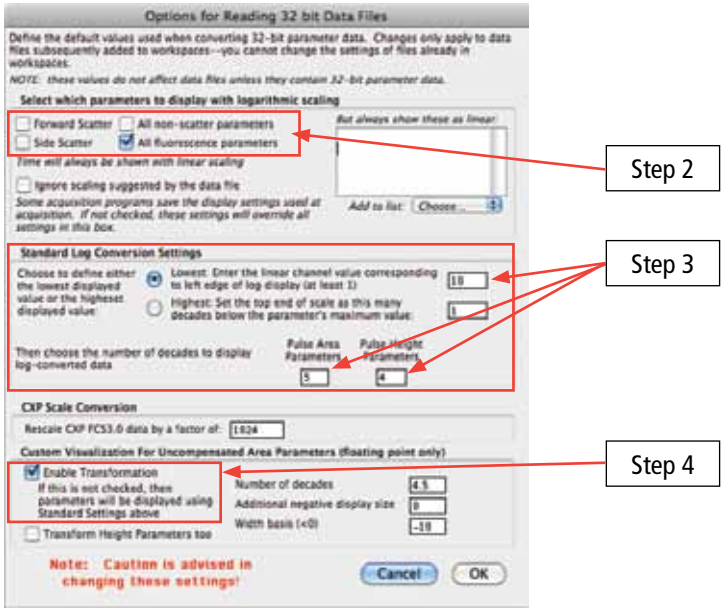

Figure 3. Setting standard log conversion settings.

2. From the **Select which parameters to display with logarithmic scaling** section, select **All Fluorescence Parameters** and clear the other options.
3. From the **Standard Log Conversion** Settings section, set the range of channels to be viewed on logarithmically displayed parameters.

If you know the range of channels spanned by the data in the BD CFlow file, use the information in Table 1 as a guide for entering values. See the next section of this document, Using the Derived Parameters, for information on how to adjust the channel range viewed on linear forward scatter and side scatter.

Channel Range to View (Log Scale)	Left Edge Channel No.	No. of Decades (Area and Height)
All (1 to 16.7 million)	1	7
10 to 16.7million	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.

4. If you are not using bi-exponential display, from the **Custom Visualization for Uncompensated Area Parameters** section, select the **Enable Transformation** checkbox.
5. Click **OK** to enable these changes and return to the **Preferences** dialog.
6. [Optional] In the **Preference Sets** field, select **Choose** to assign a new name for the new global FlowJo preferences.
7. Click **Save** to save these settings as the new global FlowJo preferences.

Note that global FlowJo preferences are not applied to customized workspaces. Since most workspaces that use BD Accuri data are customized, apply the new FlowJo preferences settings to workspace preferences.

To apply new FlowJo preferences settings to workspace preferences.

1. From the **Workspace** menu, select **Workspace Preferences**.
2. From the **Reading Diva and other 32-bit Data Files** section, click **Define** to expand the section, and enter the settings from the FlowJo preferences.
3. From the 32-bit data scaling preference panel screen, click **OK** and then **Save**.

To apply these preference settings to the current workspace, save and close the workspace file and reopen it. The new preferences are automatically applied.

Using the Derived Parameters dialog to choose a channel range for data display (recommended for linear parameters)

The FlowJo preferences options for reading 32-bit data files do not allow much flexibility in choosing the channel range. To "zoom in" on a specific data range, use the **Derived Parameters** dialog.

For example, in the FSC-A vs SSC-A plot shown in the following figure, when the data was collected in BD CFlow software, the user had zoomed in on the area shown in the red box, so that the maximal channels were 5 x 10^6 on FSC-A and 2 x 10^6 on SSC-A. Figure 4 shows how the FCS file data will look when initially opened in FlowJo software. The following directions describe how to zoom in on this area.

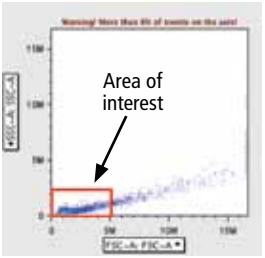

Figure 4. FCS file exported from BD CFlow software and viewed in FlowJo before deriving parameters to zoom in on red-boxed area.

Deriving new linear parameters and resetting the axis scale (zooming)

1. In FlowJo software, select **Platform.**
2. From the menu, select **Derive Parameters,** followed by **Define new or change.**
3. Under **Custom Derived Parameters,** select **Choose** from the **Insert Parameter** field and select **FSC-A: FSC-A.**

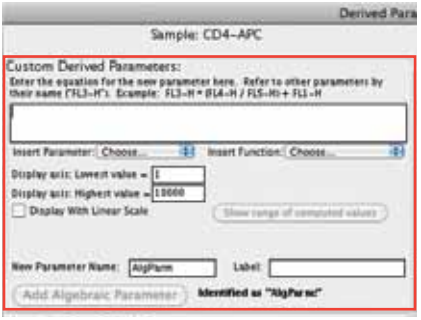

Figure 5. Custom Derived Parameters section or Derived Parameters dialog.

4. Select the **Display with Linear Scale** checkbox.
5. Enter the values you want in the **Display Axis: Lowest Value** and **Highest Value** fields.

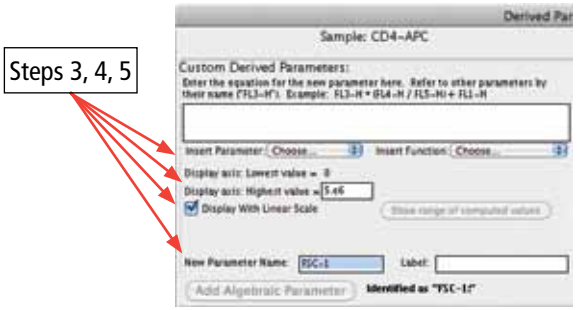

Figure 6. Custom Derived Parameters choices have been modified.

6. In this example dialog, the values used are 1 and 5,000,000 for FSC-A. They would be applied to the placement of the red box in Figure 4 in the example plot figure.
7. Enter a new name and label for the new parameter. The name must be different from the original name.
8. Select **Add Algebraic Parameter.** The new parameter is displayed in the **Currently Defined Derived Parameter** field.

Applying the new settings to all files

1. After the new parameters are derived, click **Done**.
2. From the menu under **Platform**, select **Derive Parameters**.
3. Select **Copy to Group's Samples**.

   A message is displayed to confirm adding new parameters to all the samples in the workspace.
4. Select **Add.**

Additional Notes on using FlowJo

To apply the workspace axes scaling changes to multiple groups of data files, save the workspace with one renamed sample file that contains the scaling factors you want and the derived parameters settings. Next, add the new group of samples and follow the preceding instructions on how to apply the new settings to all files, which applies 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, eg, mouse spleen immunofluorescence, Jurkat cell apoptosis, cell cycle/BrdU, and GFP transfectants.

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