

How to Look at CyTOF Data in FlowJo.

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Because of the inherent lack of compensation and a large number of zeroes in the CyTOF data, a lot of CyTOF data points will wind up on one of the axes. This makes accurate gating difficult.

The main thing you want to do is change some of your Preference settings. I've only tested this up through Mac v9.4.9, rather than v9.4.11, but I'm pretty sure it'll remain the same.

I have attached a screenshot of what you want your settings to look like.

1. Open FlowJo.
2. Close the new workspace that automatically appears.
3. Under "FlowJo", select "Preferences"
4. Make your Preferences window look like the left side of the attached picture. Especially note the lower right corner: select "CyTOF", and set log parameter range to "20000".
5. In the lower right corner, click the "Define" button. This opens the other window (right side of the attached picture).
6. Make this new window look like the right side of the attached picture. Especially note the upper right corner, where Time and Cell Length are *linear*, rather than log.
7. Hit "OK" in the second window.
8. In the first window, there's a drop-down menu in the bottom center. Select "Save this Set of Preferences", name it ("CyTOF preferences", or something), and hit "OK".
9. Hit "Save" in the first window. Close this first window.
10. Under "FlowJo", select "Switch to Preference Set", and select your new "CyTOF preferences" set.
11. Under "File", select "New Workspace". This new workspace should now have the new preferences set, and can drag in your FCS files.

* I do the biexponential transformation too, but I'm not sure whether you can do that with the workspace open already, or whether you can just do that once you have your FCS files in.

Note: if you're switching back and forth between flow preferences and CyTOF preferences, you *have* to close all workspaces, switch preferences, then open a new workspace.

I've been back and forth with FlowJo/Treestar back in August 2011 or so. Supposedly, you shouldn't have to close the new workspace and switch preferences before dragging in FCS files. In fact, supposedly you can have an open file and just switch preference sets.

However, I've *never* been able to get that to work. Just do it my way.

One thing you *could* do: after you create a workspace with the CyTOF preferences, just

save it as a template (*.jot) file you can just reopen as necessary. Any files added to this template automatically have the CyTOF preferences, b/c the template does. Same idea with the flow preferences... just have a CyTOF template and a Flow template, and let that work for you.

The image shows a screenshot of the 'Options for Reading 32 bit Data Files' dialog box in a software application. The dialog is divided into several sections with various settings and checkboxes.

Options for Reading 32 bit Data Files

Define the default values used when converting 32-bit parameter data. Changes only apply to data files subsequently added to workspaces—you cannot change the settings of files already in workspaces.
 NOTE: these values do not affect data files unless they contain 32-bit parameter data.

Select which parameters to display with logarithmic scaling

Forward Scatter All non-scatter parameters
 Side Scatter All fluorescence parameters

Time will always be shown with linear scaling
 Time:
 Cell_length:
 Add to list:

Ignore scaling suggested by the data file
 Some acquisition programs save the display settings used at acquisition. If not checked, these settings will override all settings in this box.

Standard Log Conversion Settings

Choose to define either the lowest displayed value or the highest displayed value:
 Lowest: Enter the linear channel value corresponding to left edge of log display (at least 1)
 Highest: Set the top end of scale as this many decades below the parameter's maximum value:

Then choose the number of decades to display
 Pulse Area Parameters:
 Pulse Height Parameters:

CXP Scale Conversion

Rescale CXP FCS3.0 data by a factor of:

Custom Visualization For Uncompensated Area Parameters (floating point only)

Enable Transformation
 If this is not checked, then parameters will be displayed using Standard Settings above
 Number of decades:
 Additional negative display size:
 Width basis (<0):

Transform Height Parameters too

Note: Caution is advised in changing these settings!

Preferences

These settings will apply to all workspaces that do not have custom preferences defined.

Workspaces | Graphs | Gates | Platforms | Tables/Layouts | Output | Text | General

Appearance

Show elapsed time since last "Save"
 Draw row borders Draw column borders
 Show subset frequency in workspace
 Gate statistic is Frequency of Parent
 Show all keywords in displays
 Create new Workspace when launched

Compensation

Allow custom visualization
 Checks to enable customized scale displays (Compensation->Define Transformation...)
 Additional negative display size:
 Positive decades of "log" display:
 Apply visualization to all compensations

How should data files be named:

Default (Sample Name Keyword)
 Use data file name
 Specify keywords:

Default Sort Order:

Conversions for 32 bit data files
 CXP files: Read only FCS2.0 portion
 CYTOF: Set log parameter ranges to:

The default column size and order can be copied from an open workspace, but currently none is open.

Use As Default

Preference Sets:

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