FlowJo™ Software Cheat Sheet

Adding Data

The easiest way to add data to a workspace is to drag the entire folder of data and drop it into the workspace (see **Drag Samples Here**).

Groups

Groups are the basic organizational unit in FlowJo™. When you do something to a group (make a gate, add a stat, etc.) it will apply to all the files in the group.
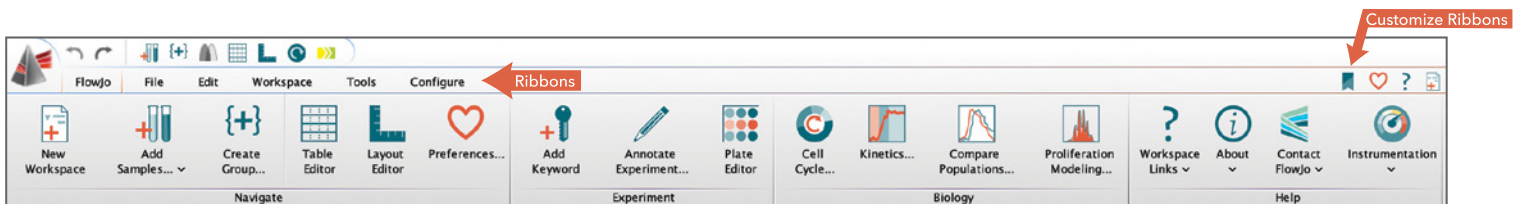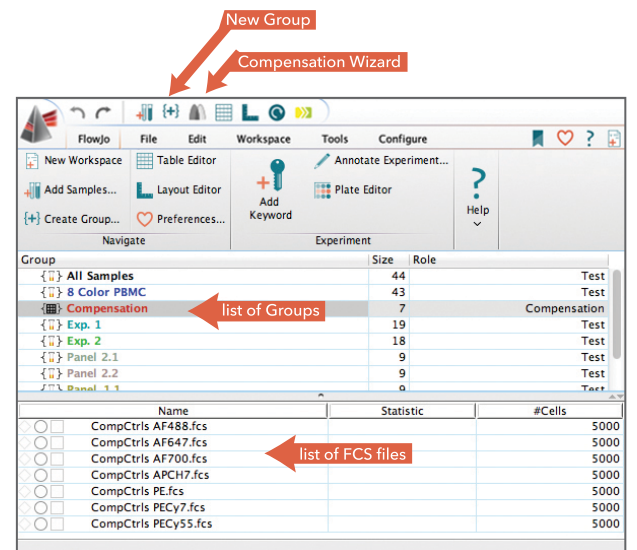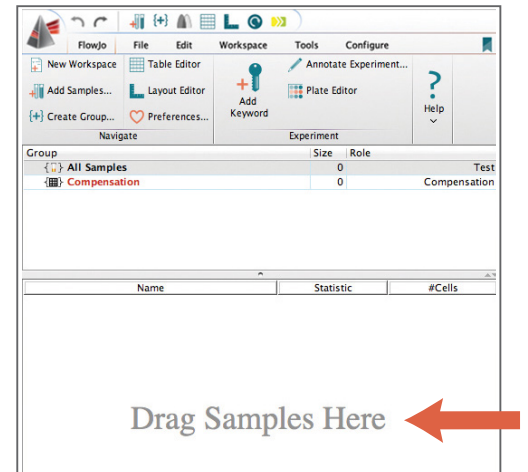
- When you drag a folder of FCS files into FlowJo, a group is automatically created with the same name as the folder.
- You can make a new group by clicking the New Group **{+}** button.
- Double-clicking on a group allows you to assign the group criteria that FlowJo will use to automatically assign samples to your group.

Compensation

FlowJo will automatically assign any file with the word 'comp' or 'unstained' in the file name to the **Compensation Group.** You can drag-and-drop any other sample that you would like to use as a comp control into the **Compensation Group**. Once populated, click on the **Compensation Group**, and then click the Compensation Wizard button. The **Wizard** will automate the process, but allows you, the expert, to make strategic edits when you see something to optimize.

Ribbons

Tools within the FlowJo v10 workspace are located in ribbons. There are 6 ribbons - **FlowJo, File, Edit, Workspace, Tools** and **Configure** - that contain one or more bands. Bands are collections of related tools. The ribbons can be customized by dragging bands off a ribbon, or by clicking the Customize Ribbons button, and dragging a band onto a ribbon.

New Group

Compensation Wizard

list of Groups

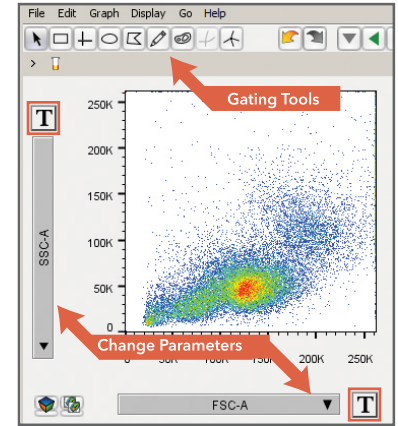list of FCS files

Customize Ribbons

Ribbons

Annotation

Right-clicking on the **column header** above the list of FCS files allows you to select which keywords (metadata about the experiment are displayed as columns in the workspace. Almost any keyword you display can be edited by double-clicking on the cell you want to change. You can add new keywords or a set of keywords through the tools in the Keywords band in the workspace ribbon.

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Gates

Double-click on any FCS file in the workspace to open its **Graph Window** (GW).
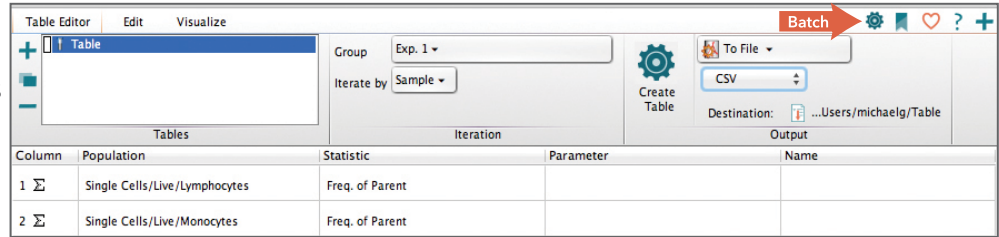
- Change parameters by clicking on the label on either axis.
- Make gates by selecting a gating tool and drawing the gate on the graph.
- Double-click inside the gate to open a new graph window displaying just the gated population.
- Apply a gate to all the samples in a group by dragging the gate and dropping it onto the group hierarchy where you want it.

Rescaling or Transforming Data

Rescaling data in FlowJo v10 is as simple as clicking a button. The **T** button next to the axis parameter selectors allows you to quickly change the current linear or log.
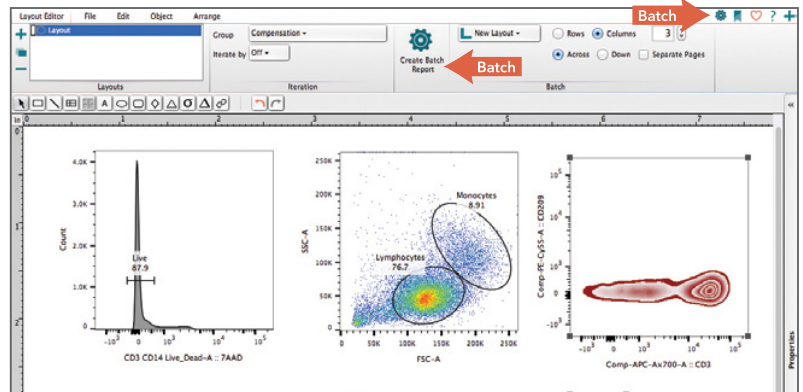
You can implement a more complex transform and specify a custom range by selecting the **Customize Axis** option. Make your adjustments in the **Transform** window and apply to multiple parameters or save to preferences if desired.

Tables

Once you have gated the data, build a table of statistics using the Table Editor ⊞ (TE).
Open the TE by clicking the button at the top of the workspace. A window will open that allows you to drag-and-drop the population for which you would like to see a statistic. Double-click on the statistic to change after dropping it. Once you have added all the statistics you need, click the Batch ⚙ button. This will generate a table of the selected statistics for all of the samples in the selected group.

Layouts

The Layout Editor ᴸ (LE) is where plots for all FCS files in a group can be created. Drag-and-drop any populations or statistics into the layout editor. Align them how you like, then click **Batch** ⚙ . FlowJo will generate that set of plots for all FCS files in the selected group.

Saving

Save the entire FlowJo workspace including the LE and TE by clicking on the FlowJo progam icon in the top left-hand corner of the workspace. There are options for saving as a workspace, a template, an archive (a zip file with all FCS files and the workspace), or an Excel file of the workspace.

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