Getting started with FlowJo™ v11 Software

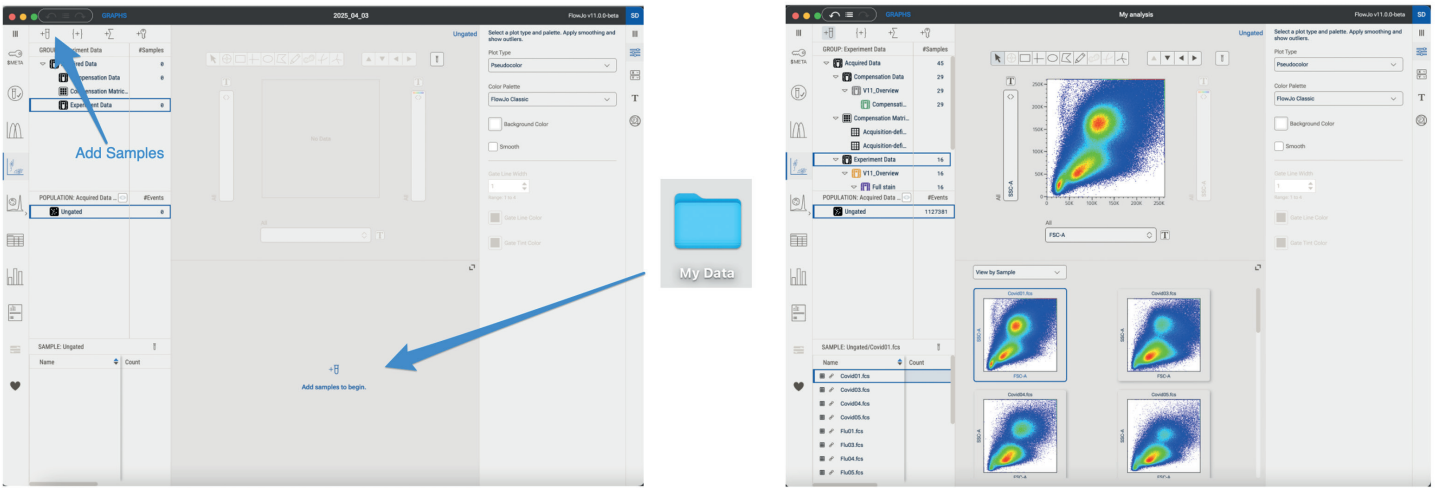
An overview of analysis basics and how to get started in FlowJo™ v11 Software

FlowJo™ Software is the leading platform for single-cell flow cytometry analysis that helps you interpret your data quickly and effectively with accessible features for immunophenotyping, quantitative population comparison, high-dimensional analysis and more. This document will go over how to begin a basic analysis.

BD

Loading samples

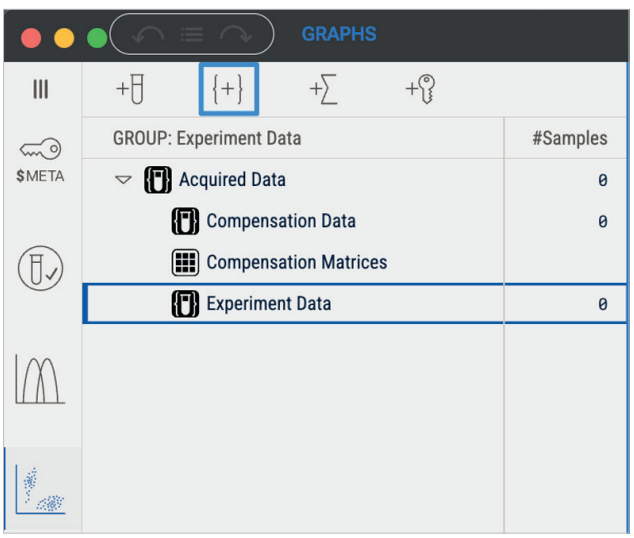
Drag and drop files into the sample pane or click Add Samples. FlowJo™ Software performs best if data is accessed from a local hard drive to avoid latency and disconnection issues.

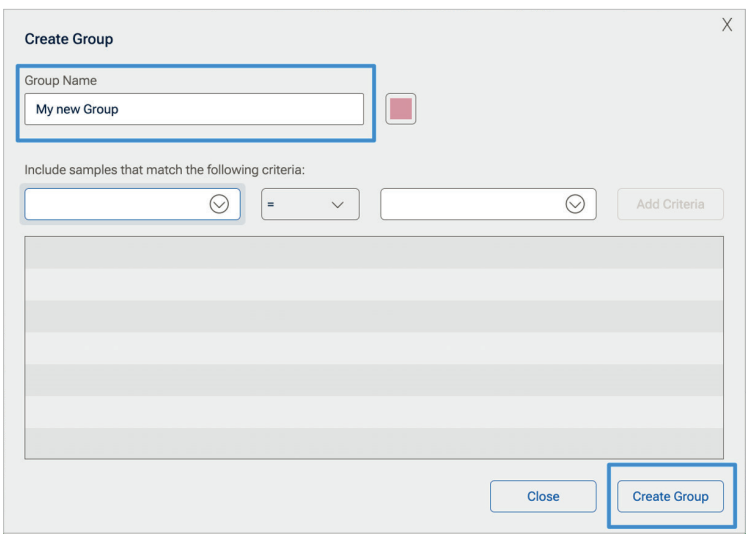


Creating and editing groups

Groups act as folders to collect similar samples, which organize the workspace, simplify application of gates and enable batch operations. Groups are hierarchical, meaning each group serves as a sample filter to pass samples from an upstream group to a downstream group. All gates pass downstream to all sub-groups. This allows you to apply analysis to a group that contains all appropriate samples but examine and create reports on any subset so that you can easily apply the broadest gates to all your samples. You can then add more specific gates in child groups to reduce the number of times you are redrawing the top-level gates.

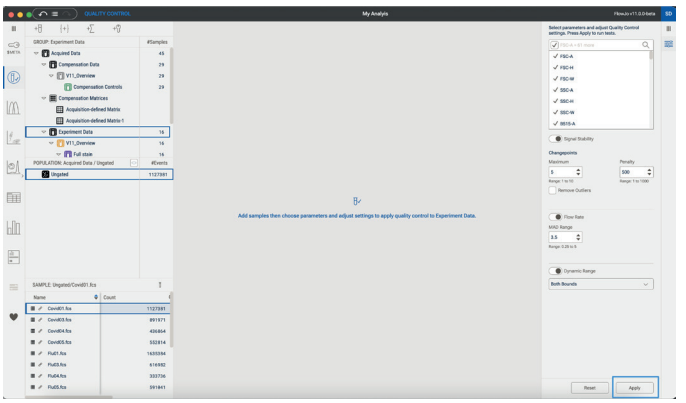
To create a group, click the **Create Group** icon  or right-click on a group at any level of the group hierarchy and select **Create Group**. Type a name for the group and click **Create Group**. Drag and drop samples from the samples pane to a group listed in the Group Pane. Double click on an existing group to edit its properties, including font appearance and sample inclusion criteria. Adding sample inclusion criteria allows FlowJo™ Software to automatically sort samples into a group and is the most efficient way to populate groups.

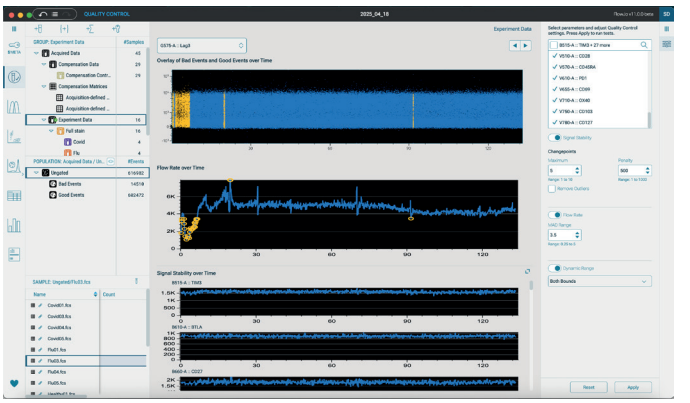




Quality control

Click the **Quality Control** icon to QC all the data added to the Experimental Data group. The platform can check for a consistent flow rate, stable signals and can remove events out of the dynamic range. Select Settings on the right in the Properties Panel to adjust the sensitivity. Click Apply to check the data. On completion, QC graphs will appear for the selected sample.

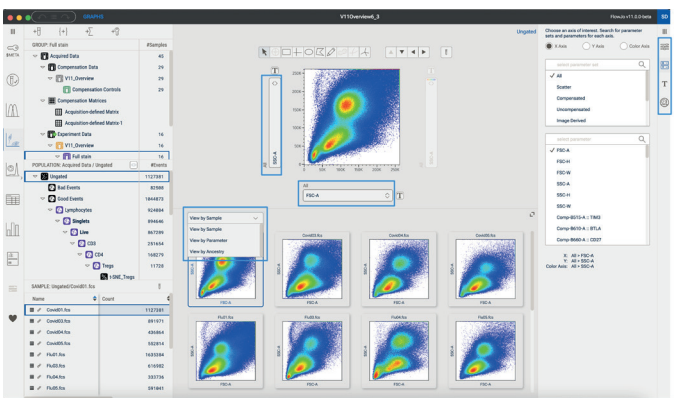




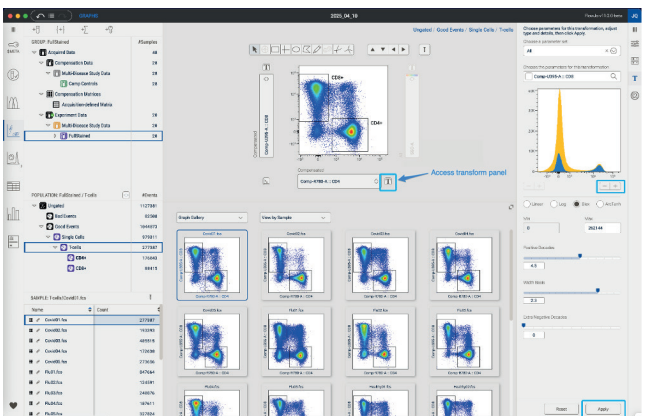
Other samples can be viewed by selecting them from the hierarchy. Good Events and Bad Events gates will be automatically created on the Experiment Data group. Click on the Good Events gate to proceed with your analysis using only the cleaned-up data.

Displaying data

The **Graphs** context provides an interface to visualize and analyze your data, including functions to navigate between samples, add statistics and change plot views. The Graph context includes a large primary plot that can be gated, along with a gallery of graphs below. These graphs can be configured to display either all other samples in a group, all parameter sets for the sample shown in the primary plot, or the ancestry of the displayed population.



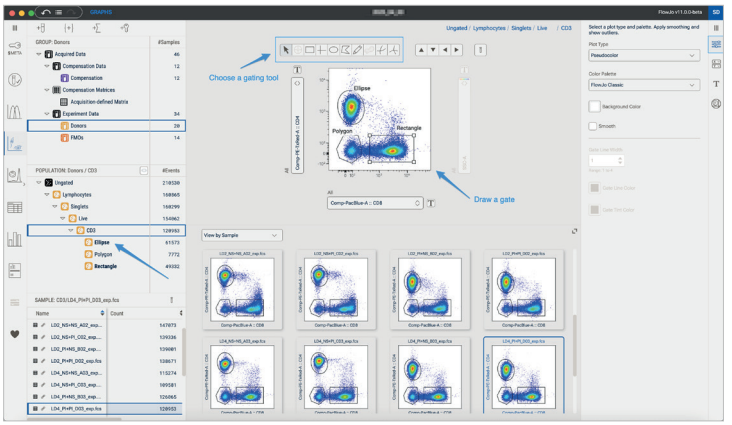
Fine level controls are available in the **Properties** panels on the far-right side of the screen. From top to bottom, the interface includes controls for **graph** settings, **parameter** selection, **transforms** and the **image wall** for data from the BD FACSDiscover™ Platform. You can select parameters from the drop-down menus below the primary plot, filtered by specific sets and choose them in the parameter selection **Properties** panel.



Visually expand or compress data points on the plot by clicking the **Transformation [T]** button, or by using the Transforms properties panel. You can select linear, log, bi-exponential, or ArcTanh scales. Use the "+" and "-" buttons or the Min and Max field boxes to adjust the range of the selected scale. For non-log and non-linear scales, there are sliders and data entry boxes to control the amount of visual space allotted to various regions of the data.

Drawing gates

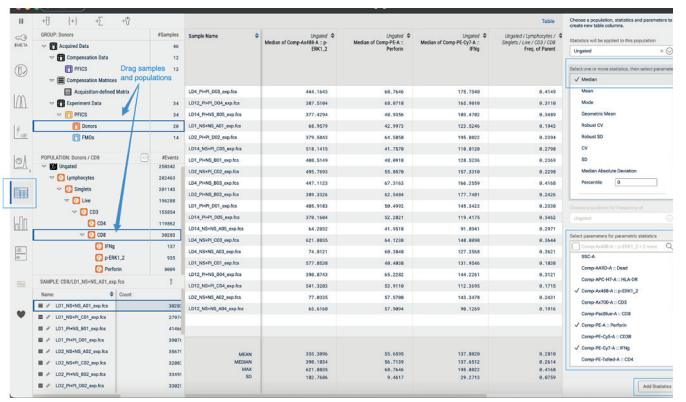
Gates subset events into populations based on marker expression. To begin gating, choose from one of several gating tools at the top of the graph window, such as rectangle, polygon or ellipse. Then click and drag the mouse over the plot to form the gate. Once the gate is set, a prompt to name the population will appear and it will be added to the Population hierarchy. All samples in the selected group have all gates displayed in the population pane. Any gate that has been edited on at least one sample will be formatted **bold**.



Right-clicking on a population gives you the option to copy the gate for pasting, copy and apply it to the group, or copy it and apply + resync so that the gate replaces any modified gates in that group. Double-click a gate in the graph window or on a population node in the hierarchy to focus the primary graph window on that population. You can drag and drop single gates or entire gating hierarchies to other gates, samples or groups.

Generating tables

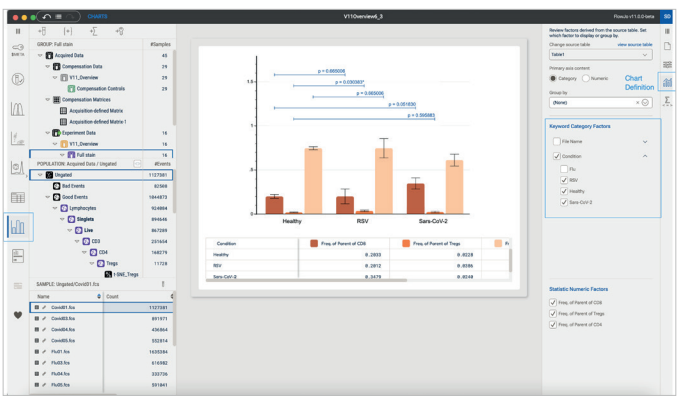
Open the **Table** context and drag samples or groups and one or more populations to add statistics to your analysis. Dragging in samples or groups adds rows to the table, dragging in a population adds columns. The Tables properties panel allows you to define what statistics or keywords will be added when dragging in samples.



Creating charts

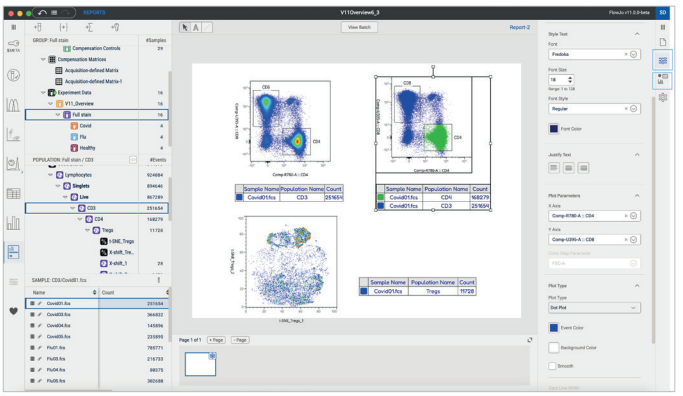
Once you have created a table, open the **Charts** context to use the data for creating bar or pie charts to compare data across categories. You can import categories into charts by including a keyword in the input table that specifies the category for each sample.

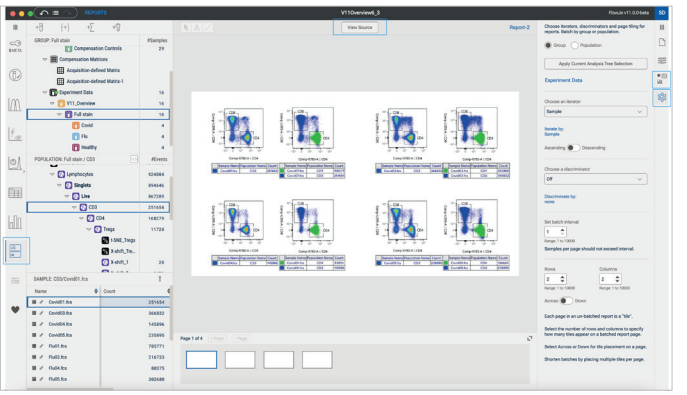
The **Charts** properties panel includes three controls: the settings, definitions, and comparison. Click **Definition** and select a table that you created in the table context to use as the data source for a chart. Choose categorical and statistical factors to populate the chart by checking on/off the top-level factors, or specific sub-factors. Click **Settings** to format the appearance of the chart. Click **Comparison** to add a statistical test to your chart.



Creating graphical reports

Open the **Reports**  context to reveal a canvas where you can display plots, create overlays, inspect gating strategies and add charts and tables. Add a plot to the blank canvas by dragging in a population from a sample's hierarchy. To make any changes to the graph's appearance open the **Settings** properties panel and use the customization options (plot view, font, legends, etc.). Create an overlay by dropping one population onto the plot of another.

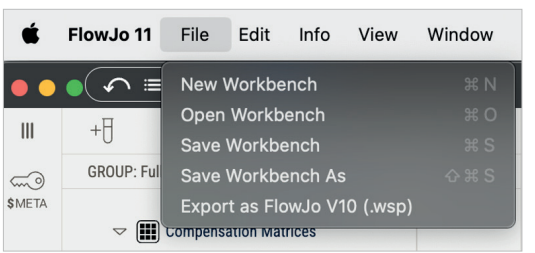




Use the **Assets** properties panel to add a chart or a table to a report. To apply the format of that report page you have made to other samples, open the **Batch Options** properties panel and select the group, cadence and inclusion criteria to apply the design of your report to, then click the **View batch** button. **Batch view** and **Source view** can be toggled. Reports are live and will update with any change to a gate or population selection. A batched view can be saved as a new report to preserve it from updating when viewing other populations.

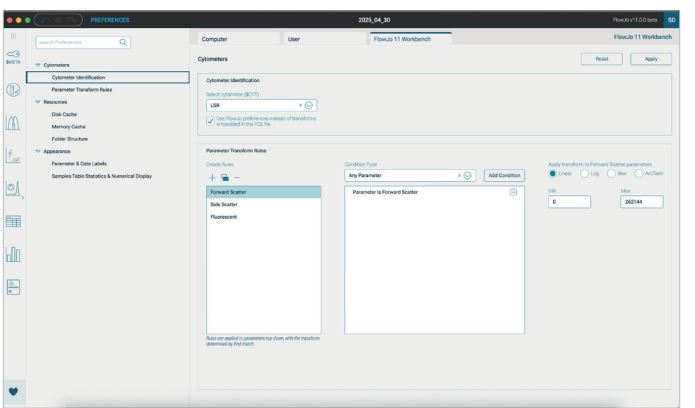
Saving your analysis

Save options are located in the **File** tab of the workspace. You may either Save, Save As, or Export as a FlowJo™ v10 Software workspace. Exported FlowJo™ v10 Software workspaces include groups and gates but not tables, charts, or reports.



Preferences

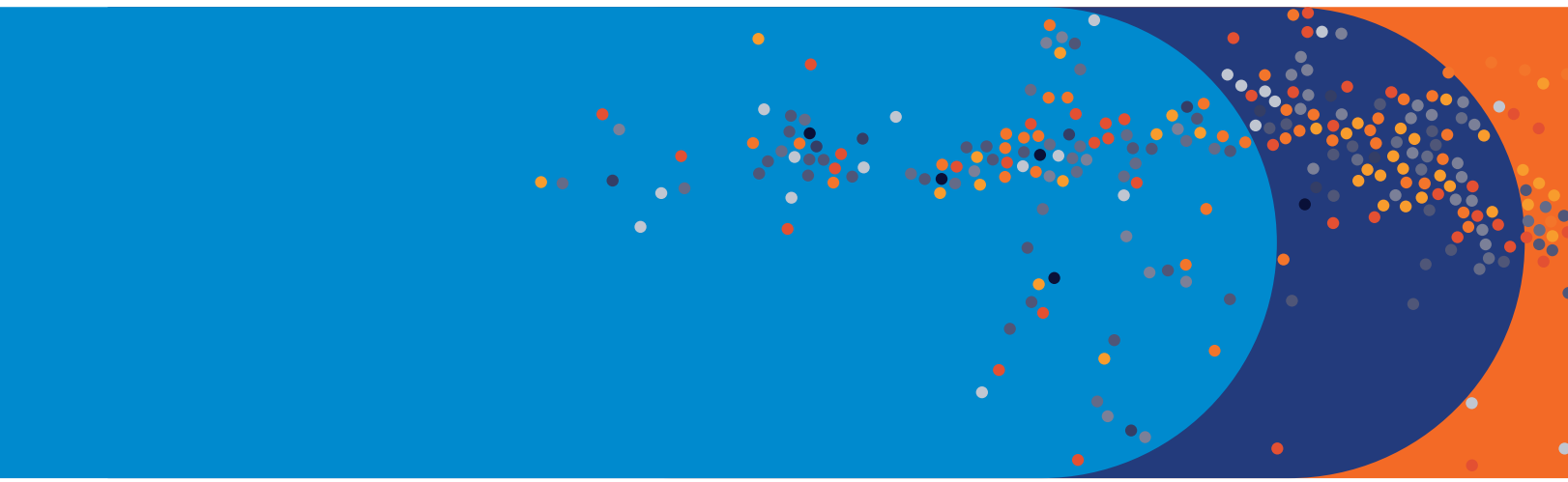
Access **Preferences** via the heart icon  in the bottom left corner of the workbench. One of the most important preferences in FlowJo™ Software is the ability to set default values for each cytometer. This ensures that most data displays correctly by default and is the ideal place for making comprehensive changes. For spot changes, the T-button is more appropriate.



Resources

- FlowJo™ v11 Software documentation: https://www.flowjo.com/docs/flowjo11
- Upcoming live and recorded webinars: https://www.flowjo.com/learn/webinars
- FlowJo University: https://www.flowjo.com/learn/flowjo-university/flowjo
- FlowJo newsletter: https://www.flowjo.com/newsletter
- Download at: https://www.flowjo.com/flowjo/download
- Start a free trial: https://www.flowjo.com/flowjo/free-trial
- Technical Support: flowjo@bd.com
- Office team for purchasing and licensing: flowjooffice@bd.com
- Explore BD® Research Cloud capabilities: https://www.flowjo.com/bd-research-cloud/overview

To learn more about FlowJo™ v11 Software, check out the resources at **flowjo.com**



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