# Getting started with FlowJo™ Software v10

An overview of analysis basics and how to get started in FlowJo™ 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, cell cycle analysis, proliferation, kinetics studies, quantitative population comparison, high-dimensional analysis and more. This document will go over how to begin a basic analysis. When you're finished, move forward with our Advanced FlowJo™ Software Tutorial.

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Loading samples

Drag and drop files into the Samples Pane or click **Add Samples**. FlowJo™ Software performs best if data are accessed from a local hard drive to avoid latency and disconnection issues. Alternatively, load files stored on BD® Research Cloud (BD RC) by selecting this option from the **Add Samples** dropdown in the workspace ribbon. BD RC intelligently handles your data to ensure that they are on the local hard drive while you're working on your analysis.
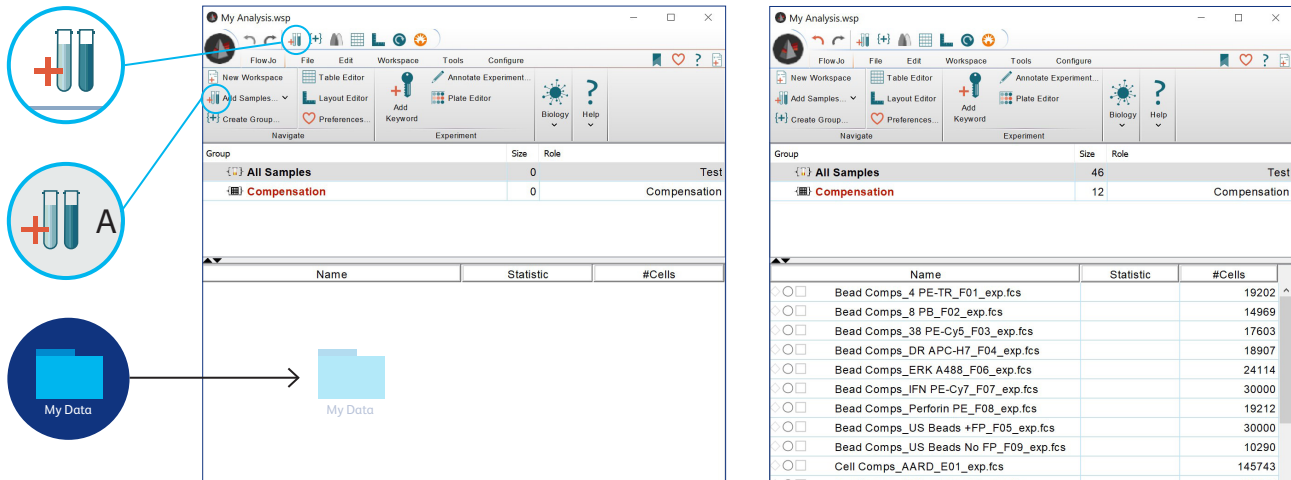

Creating and editing groups

Groups act as folders to collect similar samples, which organizes the workspace, simplifies application of gates and enables batch operations. To create a group, click the **Create Group** icon **{+}** , type a Name and click **Create Group**. Drag and drop samples to a group listed in the Group Pane to add them to that group. A sample may belong to more than one group. Double click on an existing group to edit its properties, including font appearance, role and sample inclusion criteria. Adding sample inclusion criteria allows FlowJo™ Software to automatically sort samples into a group and is the most effceint way to populate groups.
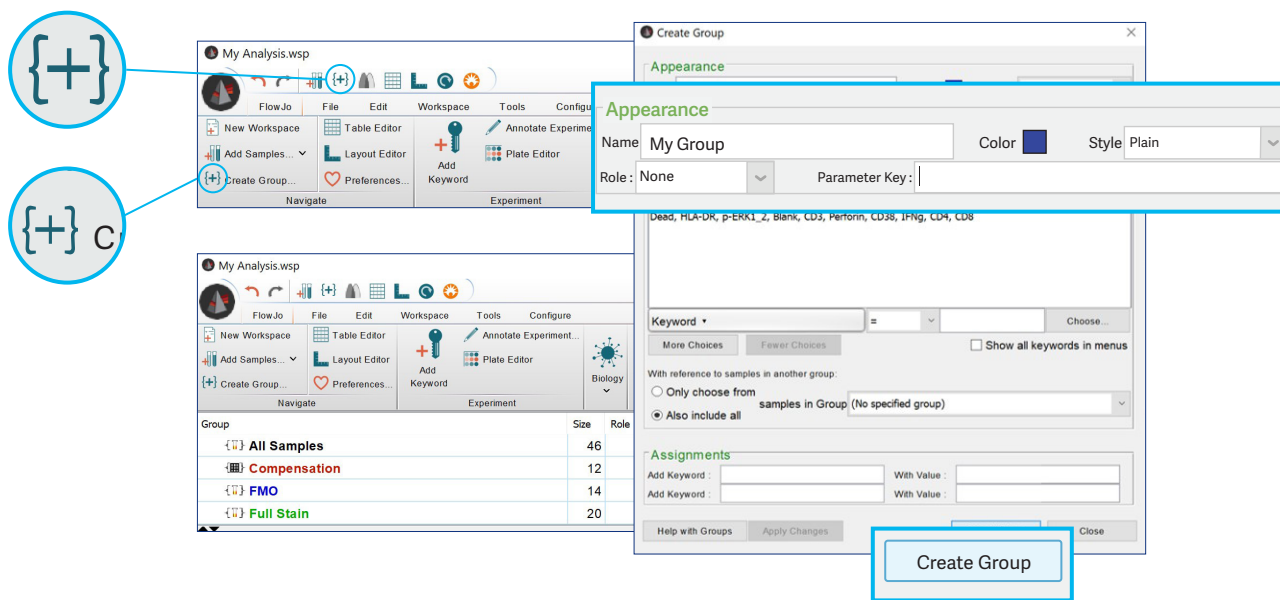
Displaying data

The Graph Window provides an interface to visualize and analyze your data, including functions to navigate between samples, add statistics and change plot views. Launch an interactive graph window by double-clicking a sample listed in the Sample Pane. Select the parameters to display using the dropdown menu along the x- and y-axes. In the Options dropdown, users can choose from one of several graph types, including histogram, dot, pseudo color and contour plots. Selecting **Heatmap Statistic** displays a statistical value (e.g., MFI) for a third parameter on cells in the current biaxial view, which can be selected from the dropdown below the Color Axis.
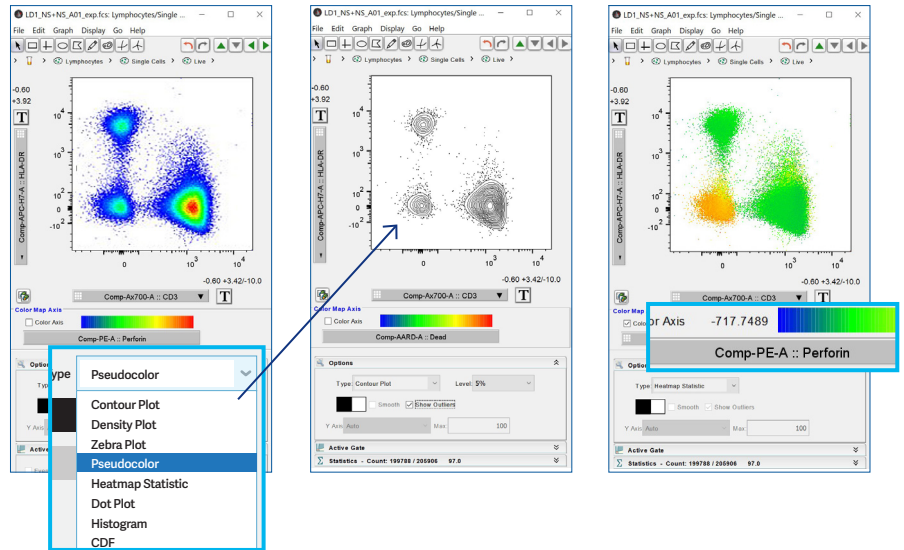

Visually expand or compress data points on the plot by clicking the **Transformation [T]** button, then **Customize Axis**. In the Transform Window, users can select from one of various data scaling options, such as linear, log or bi-exponential. Use the "+" and "-" buttons or the Min and Max field boxes to adjust the range of the selected scale. For non-log and non-linear scales, there are also toggles to control the amount of visual space allotted to various regions of the data.

Access Transform Window
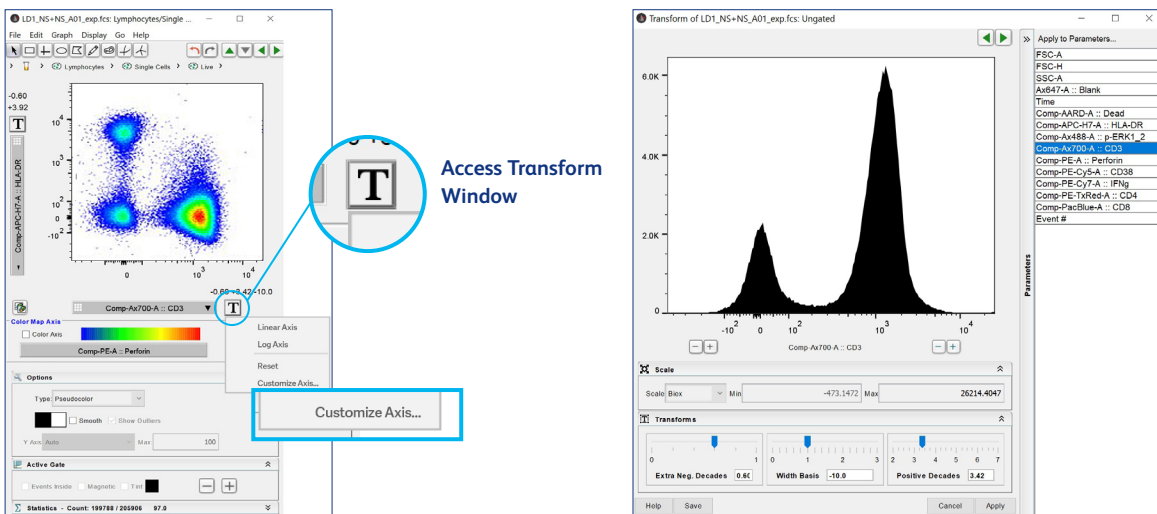
Customize Axis...

Preferences

Each user can specify their own personalized default functionality and appearance
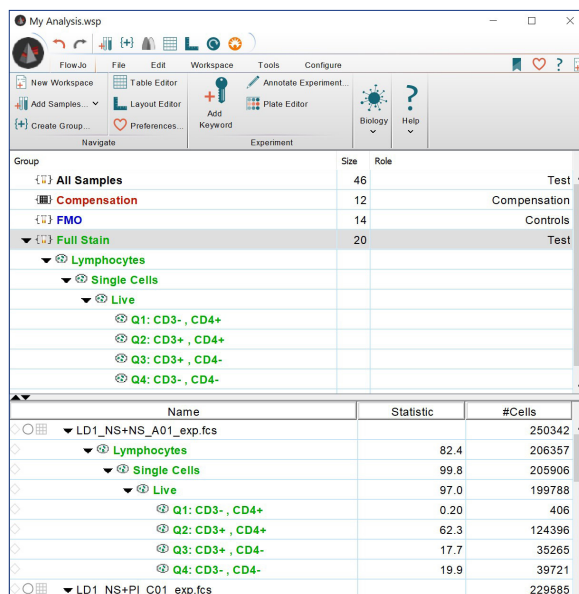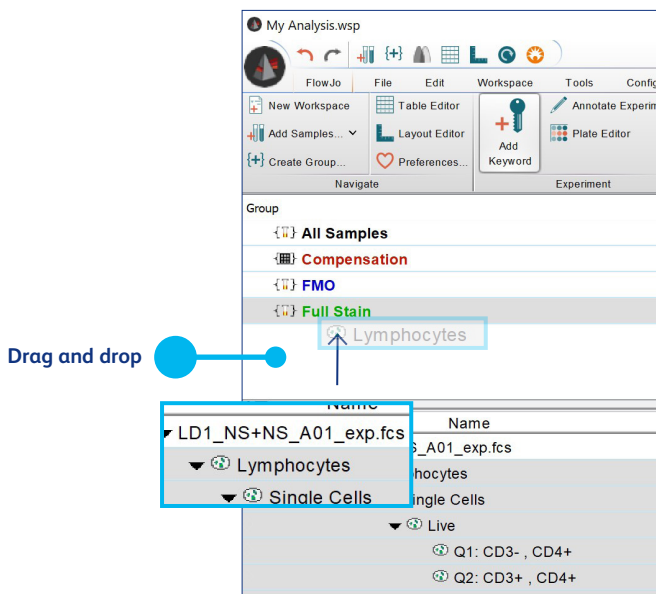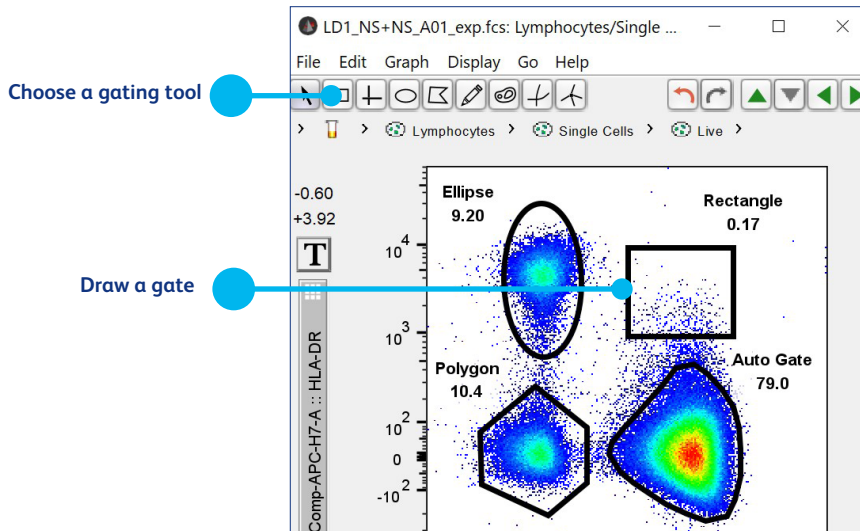
Nearly everything in FlowJo™ Software can be customized. The most common preference used is file naming. Under the Workspace tab in Preferences, the user can specify what aspect of the file information should be used as the name displayed throughout the program.
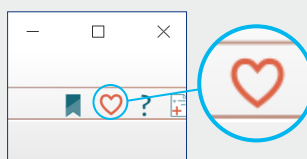
Click the heart icon at the top right corner of a window to access Preferences

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 hierarchy beneath the active sample. Double-click a gate in the graph window or a population node in the workspace to focus on that population in a new graph window. You can drag and drop single gates or entire gating hierarchies to other gates, samples or groups.

Choose a gating tool

Draw a gate

Drag and drop

Resources

- **Searchable documentation**
  https://docs.flowjo.com/flowjo

- **Upcoming live webinars**
  https://www.flowjo.com/learn/webinars

- **Recorded webinars**
  https://www.flowjo.com/learn/webinars/recorded

- **FlowJo University**
  https://www.flowjo.com/learn/flowjo-university/flowjo

- **FlowJo newsletter**
  https://www.flowjo.com/newsletter

- **Download at**
  https://www.flowjo.com/solutions/flowjo/downloads

- **Start a free trial**
  https://www.flowjo.com/solutions/flowjo/free-trial

- **Explore BD® Research Cloud (BD RC) capabilities**
  https://www.flowjo.com/solutions/bd-research-cloud

- **Office team for purchasing and licensing**
  flowjooffice@bd.com

- **Technical support**
  flowjo@bd.com

**Scan the QR code for additional resources**

Creating graphical reports

The Layout Editor offers a canvas where users can generate visuals to display plots, create overlays, inspect gating strategies and add objects. The Layout Editor is opened by clicking on the ⌊ icon in the workspace ribbon. 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, double-click the plot to launch a window with customization options (plot view, font, legends, etc.). Create a single layout or apply a layout to all samples within a group by using one of the options in the Batch band. Reports can be exported from FlowJo™ Software as various image and file formats.

Zoom options

Click and drag intersection to set page breaks
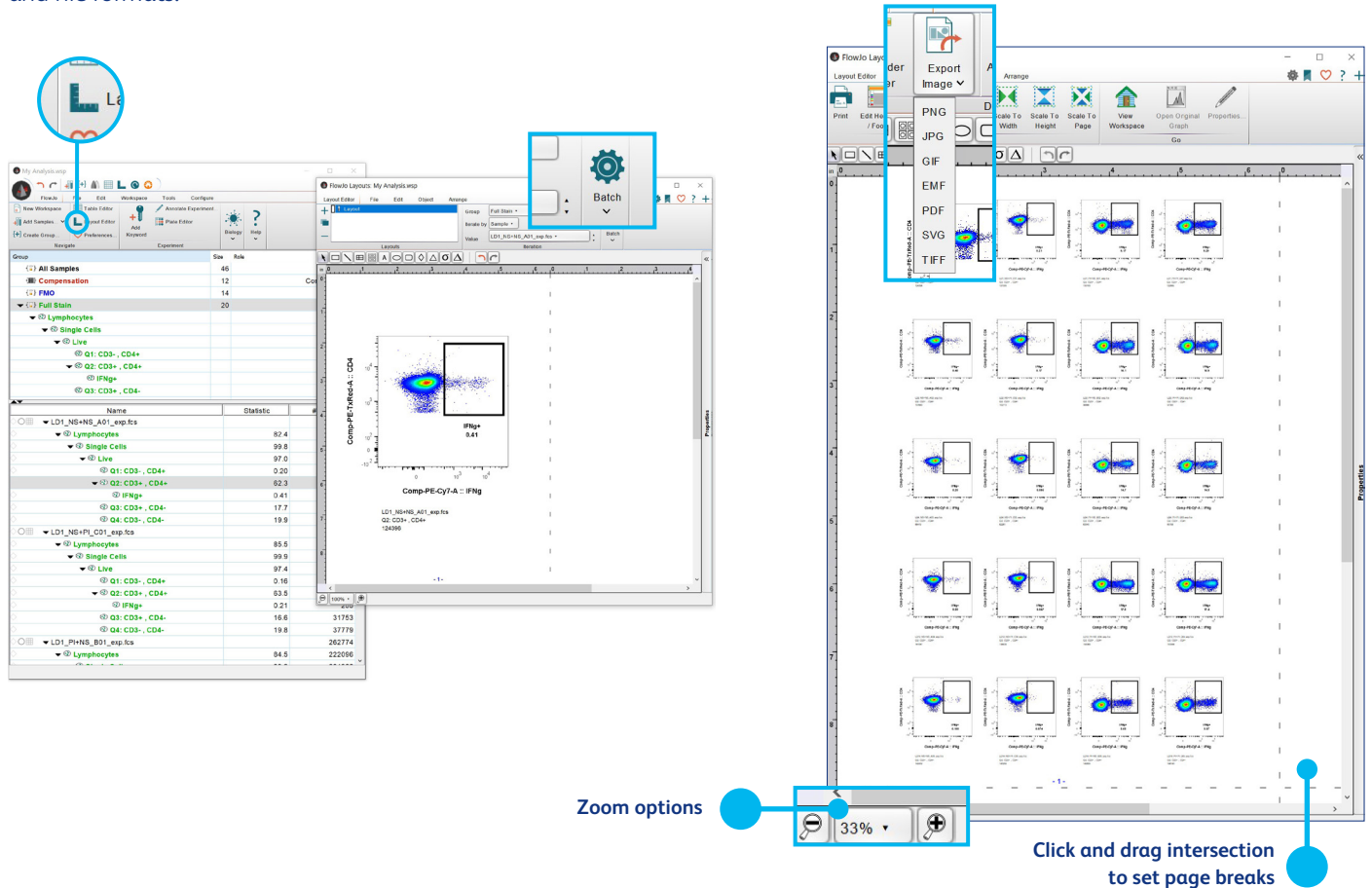
Generating statistics

Complete your analysis by enumerating properties of gated populations. Add a statistic to a population in the workspace by clicking **Add Statistic** and selecting one of the options in the left pane of the window; examples include standard deviation, median and frequency.


Select population(s) ①

② Click Add Statistic

③ Choose statistic

④ Choose parameter(s)

⑤ Click Add
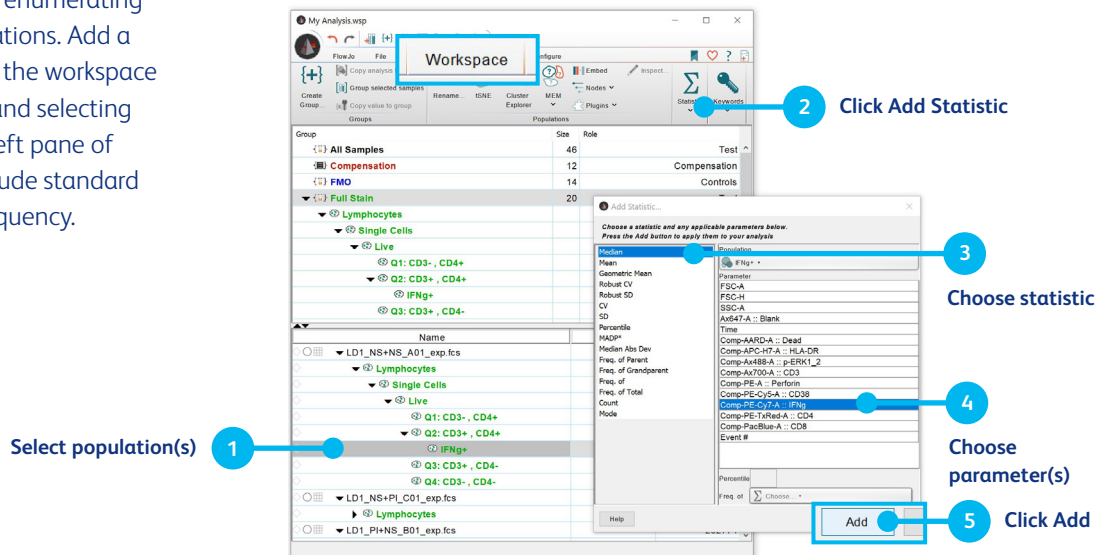
The Table Editor is a platform for gathering a collection of statistics. Access the Table Editor by clicking the ▦ icon at the top of the activity ribbon. Add a statistic from a single sample to the Table Editor by dragging in a population or statistical node from the workspace. Frequency of Parent is the default statistic used when dragging in a population node. Statistics, as well as keywords and formulas, can also be added to the Table Editor by navigating to the Edit tab and clicking **Add Column**. Various formatting, iteration and display options are available. Clicking **Create Table** will automatically batch all samples in the group and display the statistical values for each. Tables can be displayed in FlowJo™ Software, copied to the Layout Editor or exported as various text and spreadsheet files.
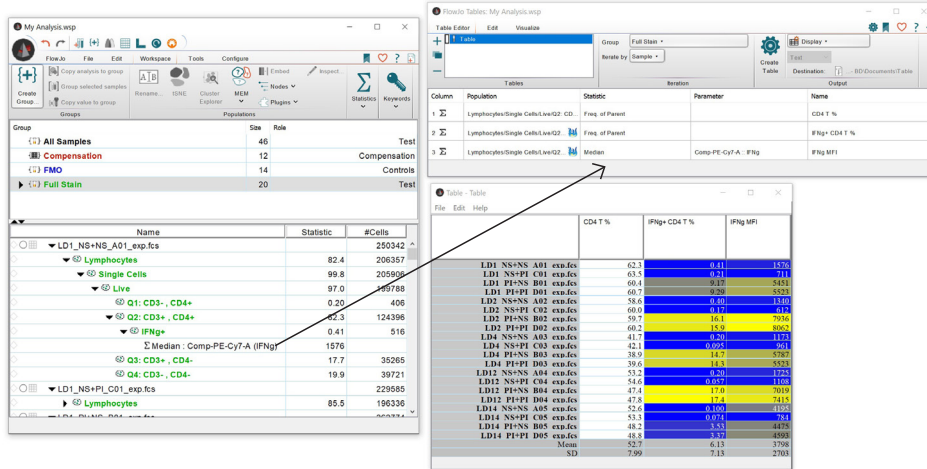
Saving your analysis

Save As options are located in the File tab of the workspace. The various formats offer flexibility for storage or application. Saving as a workspace (WSP) preserves activity in the workspace (e.g., gates, layouts, tables) but does not save your FCS files as part of the workspace. Instead, FlowJo™ Software saves a reference to the FCS files within the WSP. Alternatively, an Archive (ACS) will save your FCS files and workspace into a single file, like a compressed .zip folder. Templates (WSPT) capture your analysis in the workspace without the corresponding FCS files, making templates very useful for repeated analyses. A Microsoft™ Excel™ (XLS) export recreates your workspace structure (gating and statistics) for use in a spreadsheet program. Saving your WSP to BD® Research Cloud uploads your workspace and corresponding FCS files to the site allowing your analysis to be directly opened within FlowJo™ Software or to be accessible from the site on another device.
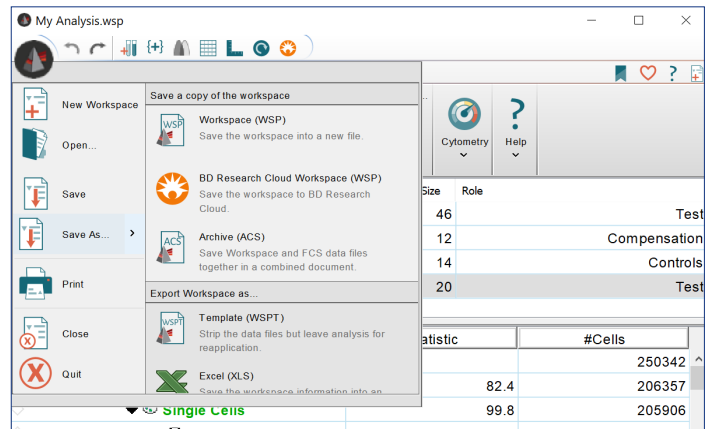
What's next? | Take your research to the next step with our Advanced FlowJo™ Software Guide! https://docs.flowjo.com/flowjo

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