

FlowJo 101

*Have questions? Contact Us:
flowjo@bd.com*

for research use only. not for diagnostic purposes.



Background

- Commercially available since 1996, FlowJo is part of BD since October 2017



- In the recent version 10.6. we see the results of this acquisition:
- Compatibility with Diva acquisition software, Plugins from BD:
X-Shift, Hyperfinder and Cluster Explorer

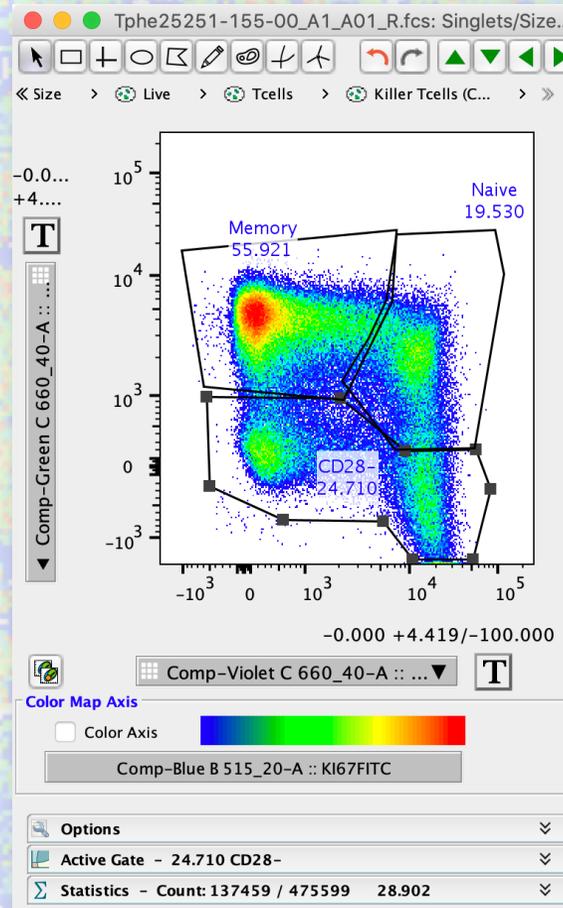
Turning Data into Results

Load data

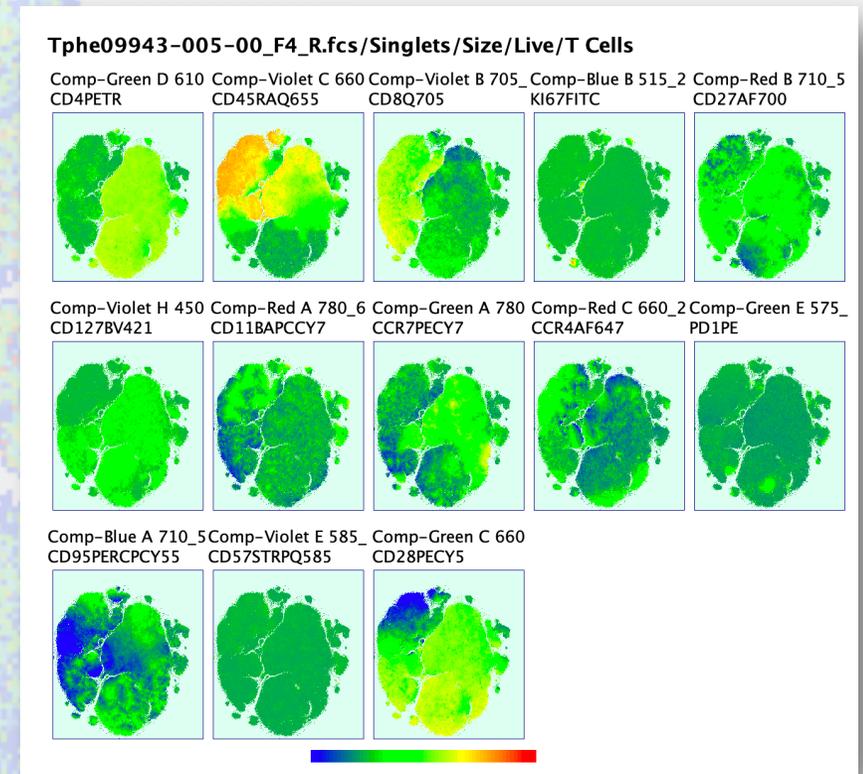
Group	Size	Role
{ } All Samples	68	Test
{ } Compensation	0	Compensation
{ } Day 0	68	Test
{ } Day 0 Old	34	Test
{ } Day 0 Young	34	Test

Name	Statistic	#Cells
Tphe09943-028-00_C10_R.fcs		1554993
Tphe25251-164-00_A6_A06_R.fcs		816961
Tphe09943-032-00_D10_R.fcs		1014669
Tphe25251-167-00_C2_C02_R.fcs		1290506
Tphe25251-173-00_G1_G01_R.fcs		1745525
Tphe09943-017-00_A4_R.fcs		1440992
Tphe09943-018-00_A7_R.fcs		1221374
Tphe09943-031-00_D7_R.fcs		1100435
Tphe09943-015-00_H10_R.fcs		614214
Tphe25251-162-00_F3_F03_R.fcs		1143921
Tphe25251-165-00_B4_B04_R.fcs		1369745
Tphe09943-011-00_G10_R.fcs		1546299
Tphe09943-033-00_E1_R.fcs		974351
Tphe25251-160-00_D6_D06_R.fcs		1555864
Tphe09943-013-00_H4_R.fcs		770923
Tphe25251-176-00_D1_D01_R.fcs		870846
Tphe25251-178-00_G10_G10_R.fcs		690919
Tphe09943-008-00_G1_R.fcs		563332
Tphe09943-026-00_C4_R.fcs		1956416
Tphe25251-181-00_D11_D11_R.fcs		1728710

Analyze



Export Results



The FlowJo Workspace

The screenshot shows the FlowJo workspace interface. At the top is a menu bar with 'FlowJo', 'File', 'Edit', 'Workspace', 'Tools', and 'Configure'. Below the menu bar is a ribbon with various tool icons. The main workspace is divided into several sections: a 'Group' list on the left, a table with columns 'Name', 'Statistic', and '#Cells' in the middle, and a large empty area at the bottom with the text 'Drag Samples Here'. A large, semi-transparent flow cytometry plot is visible in the background on the right side of the workspace.

Group	Size	Role
{ } All Samples	0	Test
{ } Compensation	0	Compensation

The Ribbon

The Groups

The Columns

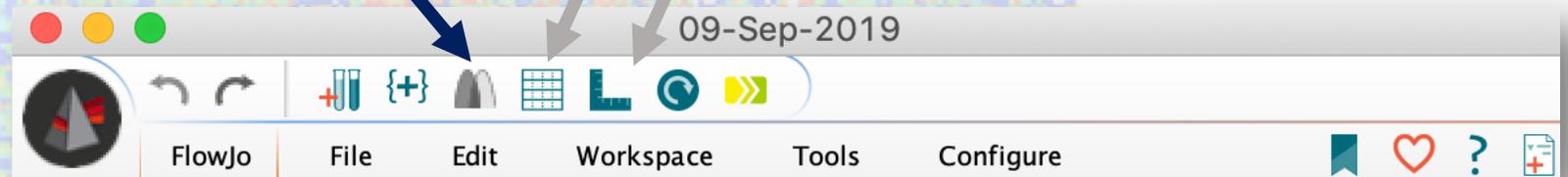
The Sample Area

Drag Samples Here

Shortcuts

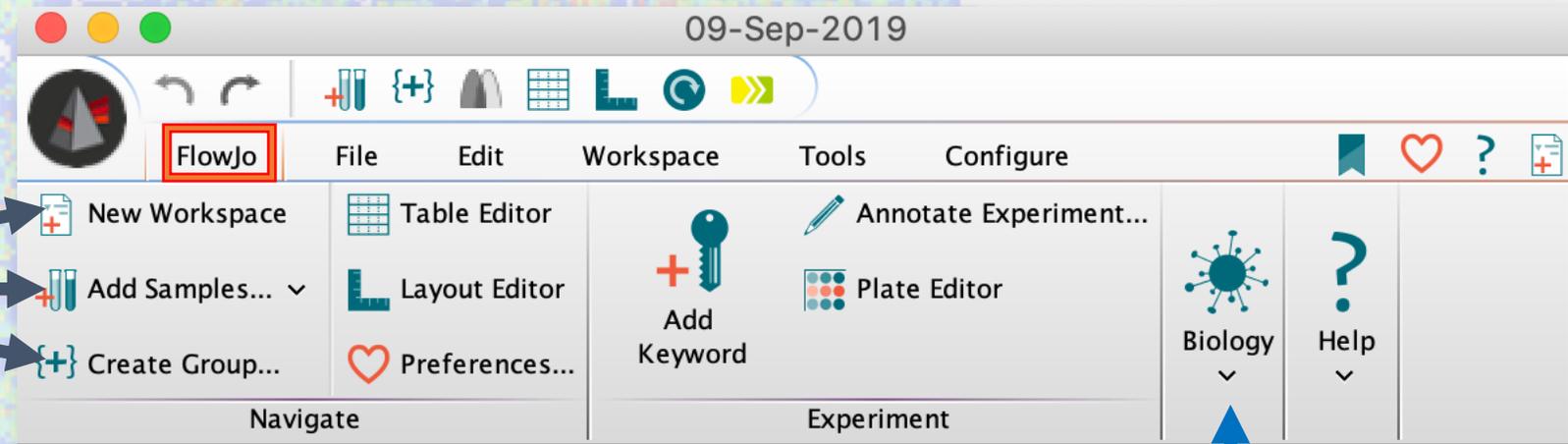
Compensation wizard

Table and layout editors.



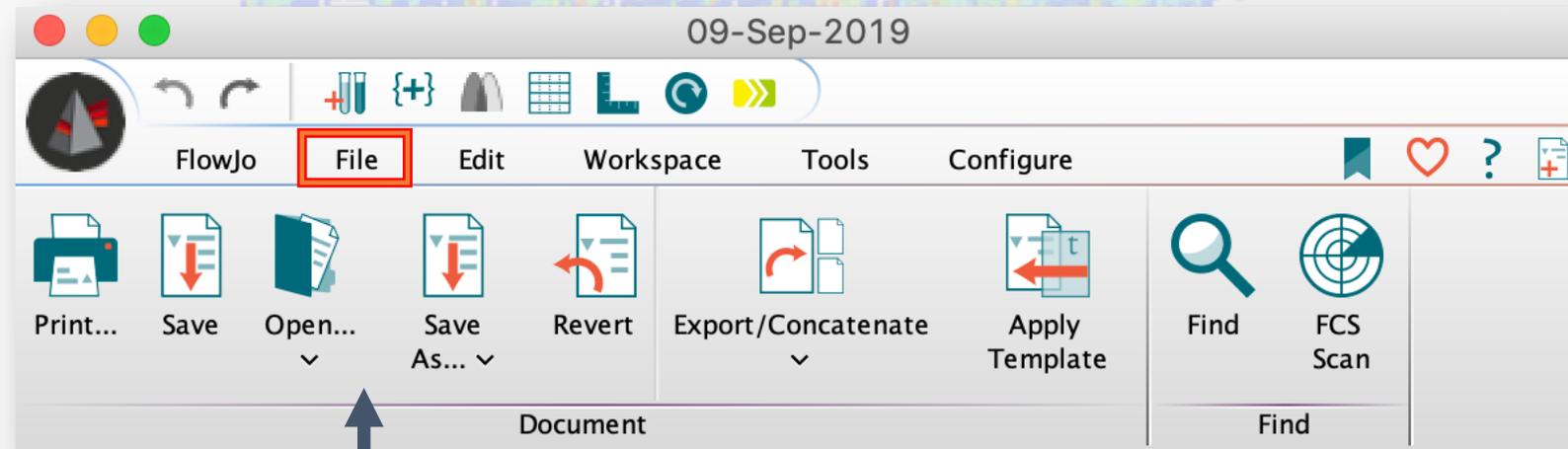
The FlowJo Tab

These buttons can be used to add samples/groups.



The biology button holds Platforms

The File Tab



Options for saving/opening workspaces.

The FlowJo Workspace

The screenshot displays the FlowJo workspace interface. At the top is a menu bar with options: FlowJo, File, Edit, Workspace, Tools, and Configure. Below the menu is a ribbon with various tool icons such as 'Compensation', 'Check Sample Quality', 'Derive Parameters...', 'Script Editor', 'Biology', and 'Boolean'. On the left side, there is a 'Groups' panel showing a hierarchical tree structure of samples and gates. The main area of the workspace is a table with three columns: 'Name', 'Statistic', and '#Cells'. The table contains data for various cell populations and gates, including 'Singlets', 'Size', 'Live', 'Monocytes', 'NK Cells', 'Tcells', and 'Killer Tcells'. A large, semi-transparent flow cytometry plot is visible in the background on the right side of the interface.

Name	Statistic	#Cells
Tphe09943-005-00_F4_R.fcs		578441
Derived		
Singlets	84.1	486264
Size	82.6	401509
Live	91.1	365862
Monocytes	16.2	59309
NK Cells	0.17	612
Tcells	52.4	191892
Helper Tcells (CD4+, C	75.0	143968
Median : Comp-Green	3942	
Inflammatory TCells	0.044	64
Memory TCells (CD	43.4	62447
Naive TCells (CD45	58.6	84371
Freq. of Live	23.1	
Regulatory TCells	26.8	38572
Killer Tcells (CD4-, C	19.3	37047
CD28-	42.0	15550
Cytokine Marker (C	7.65	2835
Cytokine Marker (P	3.14	1164
Immunosuppression	45.0	16688
Memory Immunosup	21.7	8048
Naive Immunosupp	23.5	8722

The Ribbon

The Groups

The Columns

Samples and Gates

The Groups

All the samples loaded in the workspace will be in the "All Sample" group always.

The screenshot shows the FlowJo software interface with a workspace titled '*unsaved* GatedTestSpace.wsp'. The menu bar includes FlowJo, File, Edit, Workspace, Tools, and Configure. The toolbar contains icons for Compensation, Check Sample Quality, Derive Parameters..., and Script Editor. The main panel displays a list of groups with columns for Group, Size, and Role. The groups are: All Samples (Size 68, Role Test), Compensation (Size 0, Role Compensation), Day 0 (Size 68, Role Test), and its sub-groups: Singlets, Size, Live, Monocytes, and NK Cells.

Group	Size	Role
{ } All Samples	68	Test
{ } Compensation	0	Compensation
{ } Day 0	68	Test
{ } Singlets		
{ } Size		
{ } Live		
{ } Monocytes		
{ } NK Cells		

A custom group.

Group owned gating.

Workspace Samples

The compensation matrix applied to the sample.

The gating tree. Subpopulations are under parent populations.

Statistic nodes

The sample

Name	Statistic	#Cells
Tphe09943-005-00_F4_R.fcs		578441
Derived		
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Monocytes	16.2	59309
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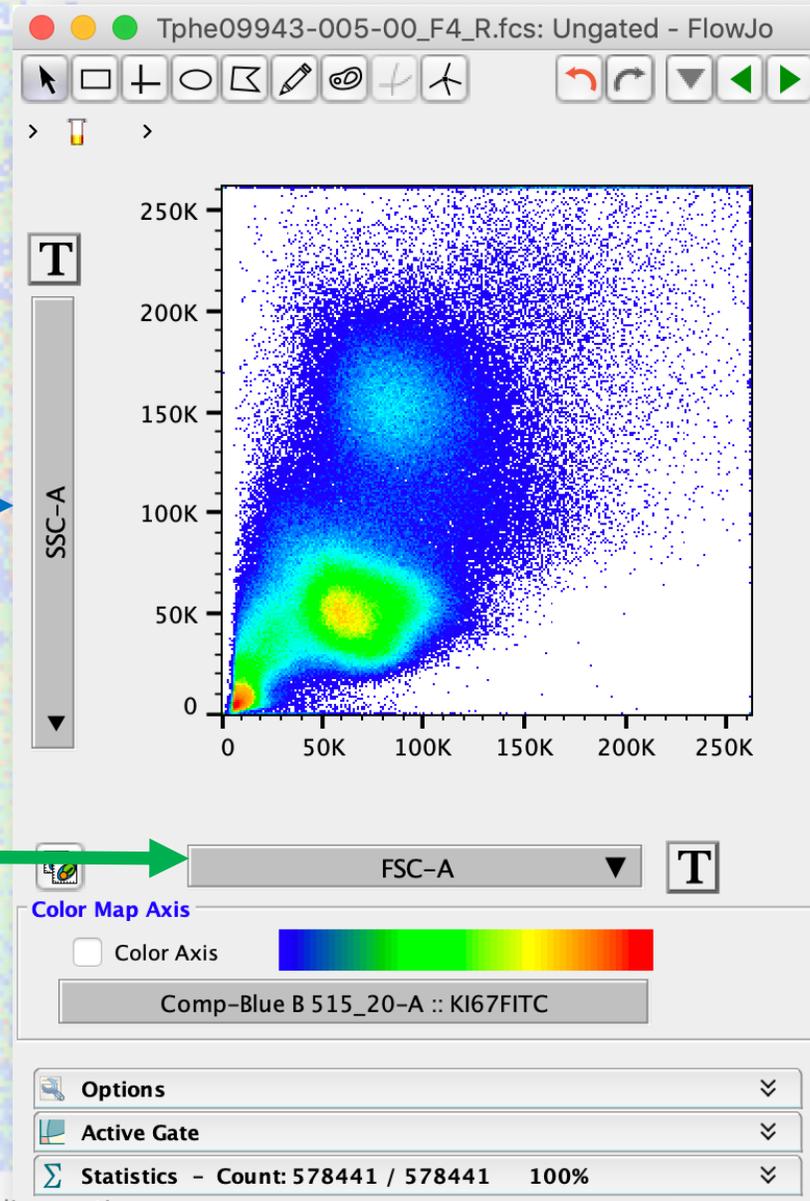
The Graph Window

The gating tools

*The axis transform button.
Used to scale the axis.*

Y-axis Parameter

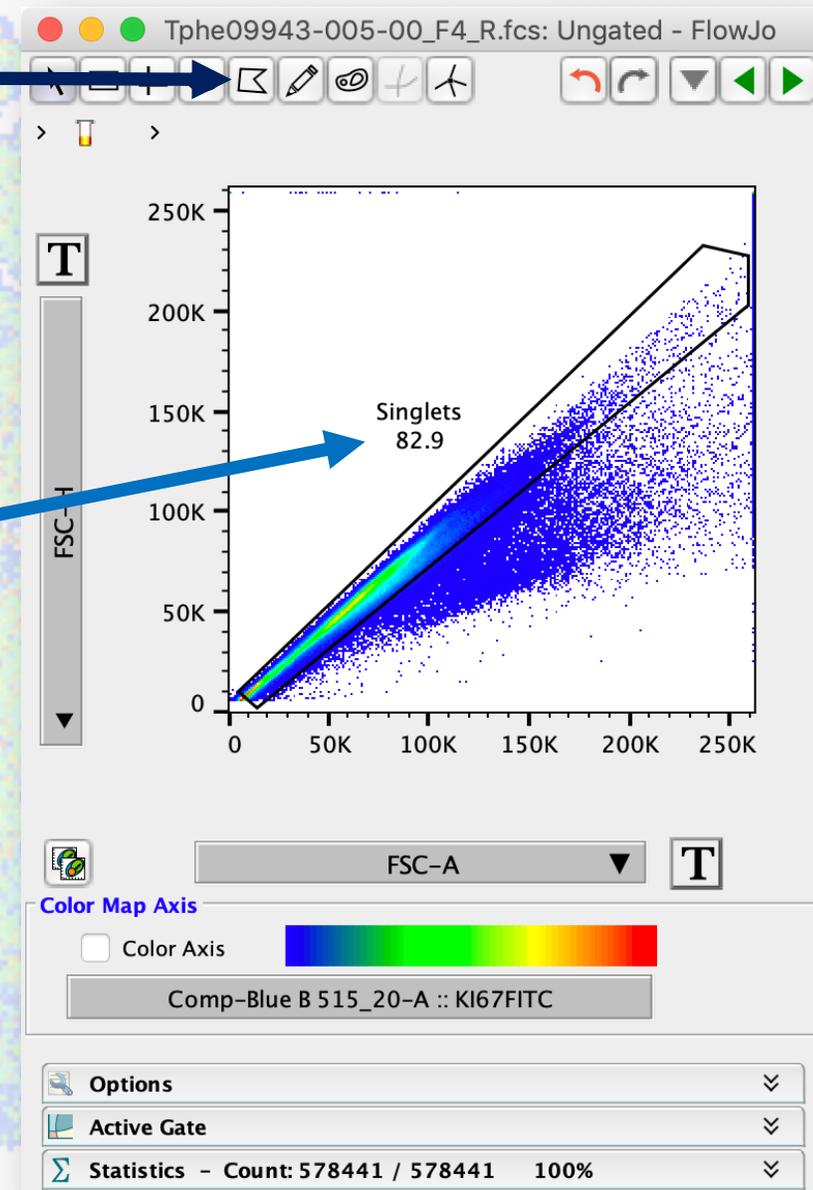
X-axis Parameter



Gating

This gate was drawn with the polygon shape

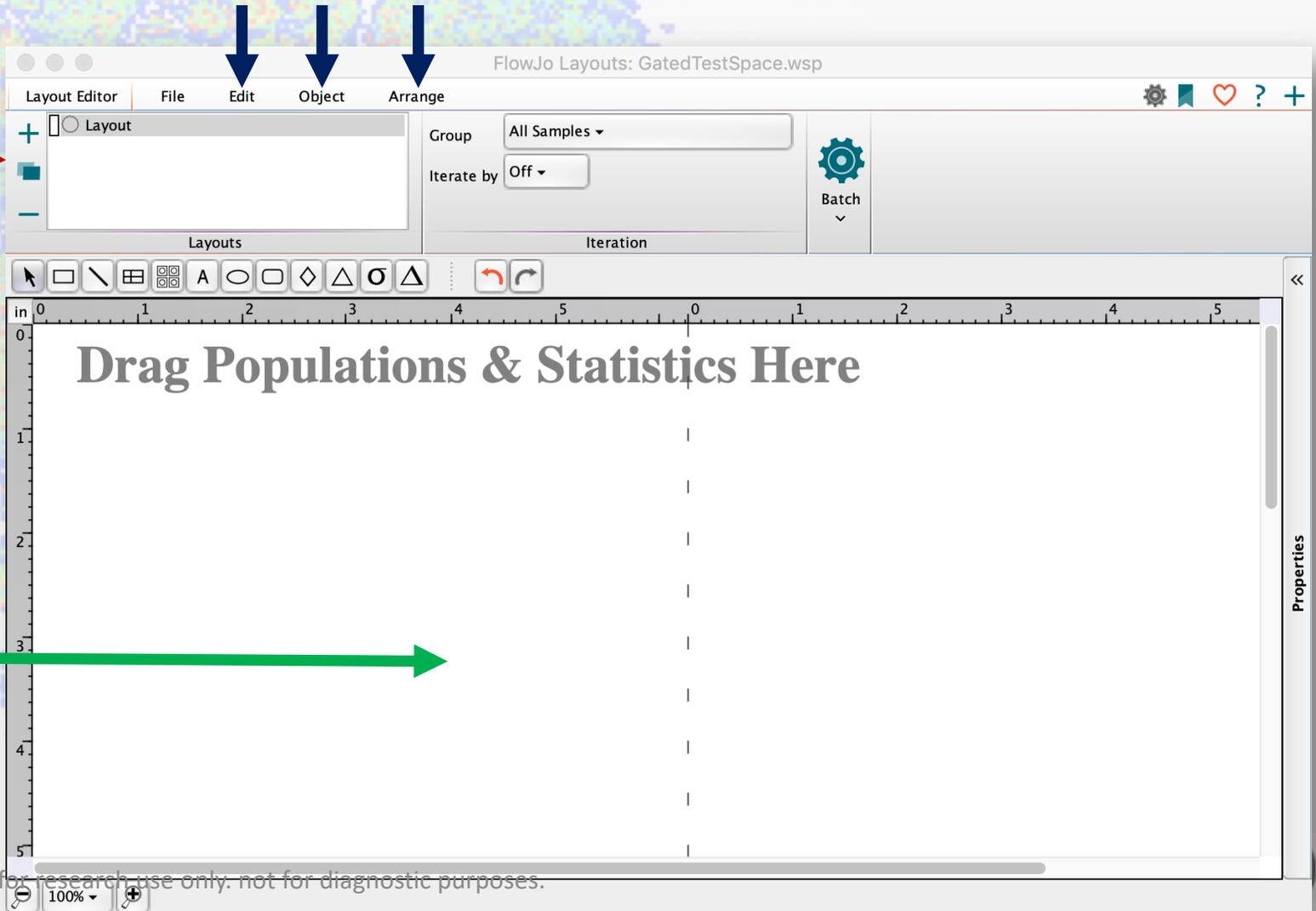
Displays the name given to the gated population, as well as its frequency (%) within the parent population.



Layout Editor

The Edit, Options and Arrange tab all hold formatting options.

Create new layouts



This space displays the plots for editing

Table Editor

These tools create new tables and switch between tables.

The iteration and create table tools quickly generate tables.

Change to export statistics.

The screenshot shows the FlowJo Table Editor window. A red arrow points to the 'Table' list on the left. A blue arrow points to the 'Iteration' icon. Another blue arrow points to the 'Create Table' button. A dark blue arrow points to the 'Display' dropdown menu. A green arrow points to the main table area.

Column	Population	Statistic	Parameter	Name
1 Σ	Singlets/Size	Median	Comp-Blue B 515_20-A :: KI67FITC	

This space displays the statistics for editing

More Information

flowjo.com

docs.flowjo.com/d2

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Up Next: Live Demo Analysis...