

# Flowlogic<sup>TM</sup>

Flow Cytometry Analysis Software

Developed by scientists, for scientists

# User Manual

Version 7.2.1

## GraphLogic:

## Graphing and Statistical Analysis



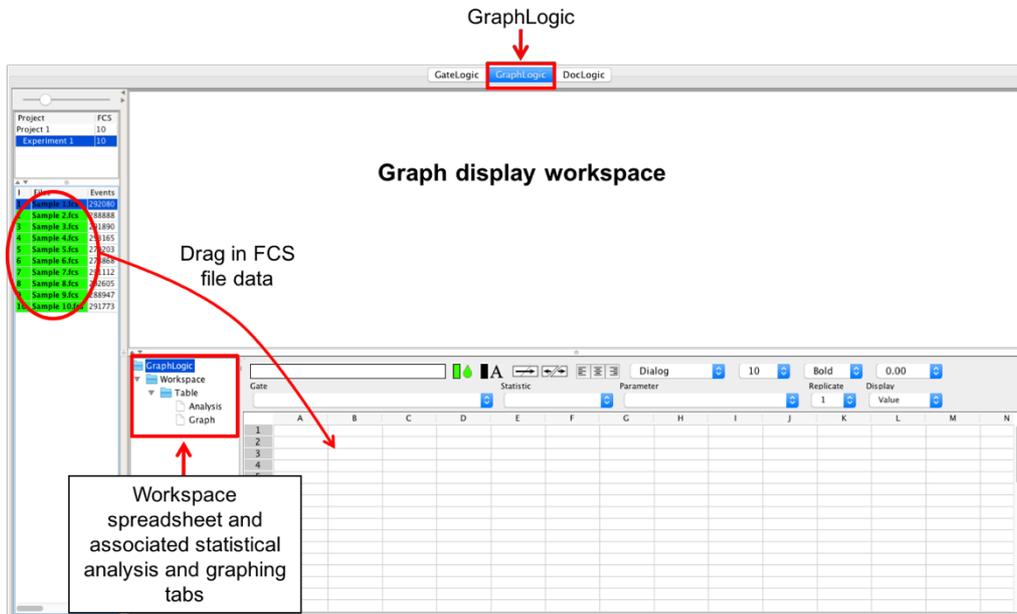
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# GraphLogic – Graphing and Statistical Analysis

## Overview

GraphLogic provides the ability to store, arrange and manipulate data derived from the FCS files and imported from other sources. This is achieved using the Workspace and Table subfolders, which themselves contain Analysis and Graph sheets.



## The GraphLogic File Inspector

The File Inspector within GraphLogic appears similarly to that in GateLogic but with a limited number of options available in the right click menu. The primary purpose of the GraphLogic File Inspector is to display the Files rather than the gating hierarchy (subpopulations can be selected within the workspace. Files are generally dragged and dropped into the Workspace in experimental groups (see [page 7](#)).

Right clicking within the File Inspector produces a menu that provides options for tagging and sorting, selecting particular files, revealing and hiding the gating hierarchy and renaming files or gates. The menu appears as shown:

1	Files	Events
1	Sample 1.fcs	
2	Sample 2.fcs	
3	Sample 3.fcs	
4	Sample 4.fcs	
5	Sample 5.fcs	
6	Sample 6.fcs	
7	Sample 7.fcs	
8	Sample 8.fcs	292605
9	Sample 9.fcs	288947
10	Sample 10.fcs	291773

- Tag
- Advanced Sort
- Show/Hide Gates
- Select
- Rename

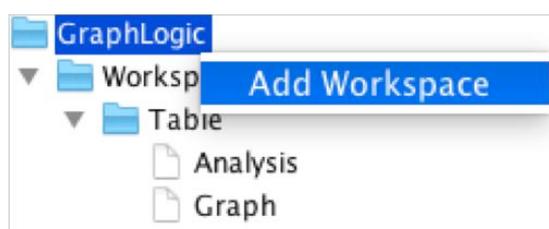
Tag – choose to add tags (user defined or a range of auto-tags) to highlighted samples. Once samples have been tagged, use the filter option in the tag menu to display a select number of samples based on their tags.

Show/Hide Gates – hiding gates results in only the file name being displayed. Showing gates reveals all levels of the gating hierarchy. In GraphLogic, it is generally only necessary to view the files themselves, with the ability to show gates only necessary to be reminded of the gating hierarchy without having to click back to GraphLogic.

Select → All/Inverse – select all highlights every row. The same result can be achieved with the keyboard shortcut 'Ctrl/⌘A'. Select Inverse highlights all but the files that are selected before clicking 'Inverse'.

Rename – rename individual files or gates by typing in a new name or choose from the original FCS file name or sample name created during acquisition.

## Workspace Folders

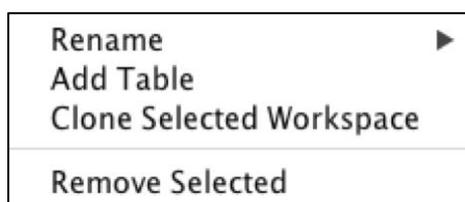


Data existing in the Workspace arranged within graph templates (see [page 7](#)) can be dragged and dropped into Table folders from which graphs are automatically generated and displayed in the Graph Display Window. Statistical tests can also be performed on data in the graph table folder in a few easy steps. As the FCS derived data in the tables remains linked to GraphLogic, all graphs and statistical analyses are updated automatically. Data can also be imported from CSV files or typed directly into the spreadsheet. Data contained in the Workspace can also be exported as a CSV file or as a MS Excel (.xlsx) file, with the latter exporting with all color and formatting.

### Right click menu for the GraphLogic folder:

The GraphLogic folder contains all subfolders. By default, one Workspace folder containing a Table folder with the associated Analysis and Graph sheets are created. Additional Workspace folders (with a table, analysis and graph) can be created by right clicking on the GraphLogic folder, followed by 'Add Workspace'.

### Right click menu for the Workspace folder:

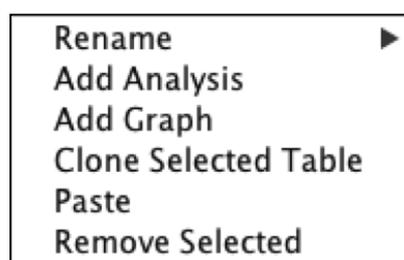


Workspace folders can be renamed using the first option in the right click menu. Additionally, new Table folders can be added to a single Workspace folder. In this way, multiple graphs can be generated and statistics test performed from all of the data in one Workspace spreadsheet.

There is also an option to clone a given Workspace and all of its subfolders. This can be very useful if you have a lot of formatting and calculations in one Workspace that you wish to apply to another. Once a Workspace has been cloned, the data can be deleted and new files or information can be imported.

Finally, a Workspace can be deleted or removed using the last option, 'Remove Selected', in the right click menu.

### Right click menu for the Table folder:



When FCS files are dragged into the Workspace, the naming template is automatically created around the data. When this same data is dragged into a Table, the Table folder name will take the name in the 'Table' cell from the Workspace. However, if you are importing non-FCS data, you have removed the 'Table' name from the Workspace or you wish to change it, this can be done from the 'Rename' option in the right click menu.

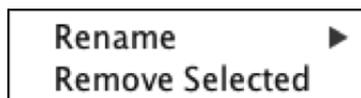
Other options include adding additional analyses and graphs. Whilst only one data set can be added to each Table folder, allowing multiple analysis and graph icons means that the same data can be displayed two or more different ways at the same time and different statistical tests can be performed without having overwrite that which has already been done.

Tables can also be cloned. This is useful if you have formatted graphs and performed particular statistical tests. The easiest way to replicate this is to clone a Table and then drag the new data into the clone. This will then update all current graphs and statistics.

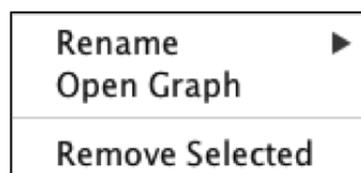
As an alternative to dragging selected data into a Table, data can be copied from the Workspace, (right click→Copy) and pasted into the Table (Right Click on Table folder→Paste). This is useful if there is a lot of data in the Workspace and dragging becomes impractical.

As with Workspaces, individual Table folders can be deleted by right clicking on the desired folder and choosing 'Remove Selected' from the menu.

#### **Right click menu for the Analysis icon:**



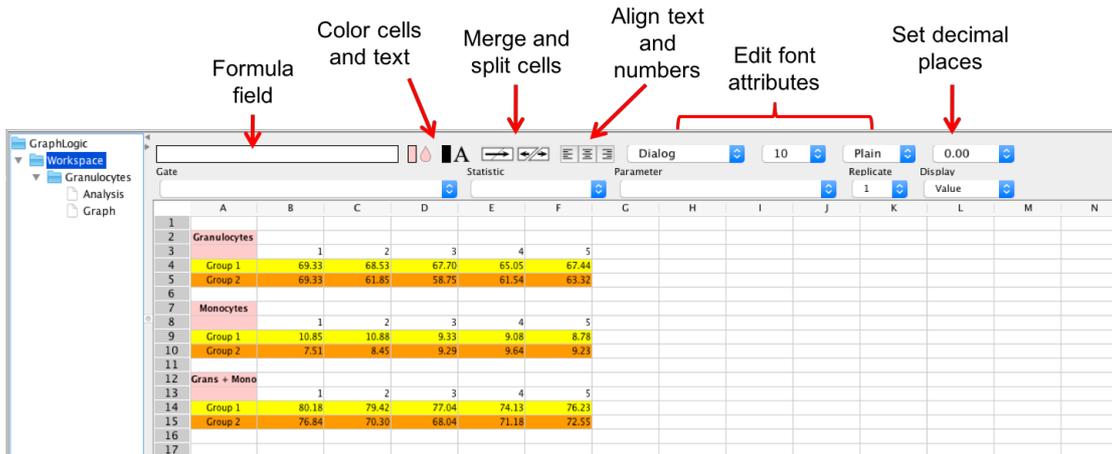
#### **Right click menu for the Graph icon:**



### **Data Workspace**

The Workspace is a spreadsheet that can accept FCS data, which remains linked to the original gates so that any adjustments in GateLogic are automatically updated in the GraphLogic Workspace. In addition, non-FCS data can be imported from CSV files and numbers and text can be typed directly into the cells.

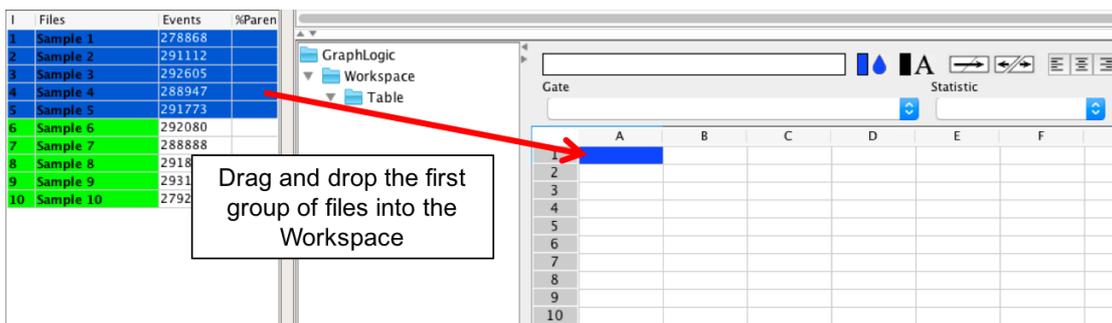
Common editing features exist, allowing cells and text to be colored, the merging and splitting of cells, changing the font type, style and size, changing text and number alignment within cells and selecting the number of decimal places to be displayed. A detailed description of each of these features can be found from [page 18](#).



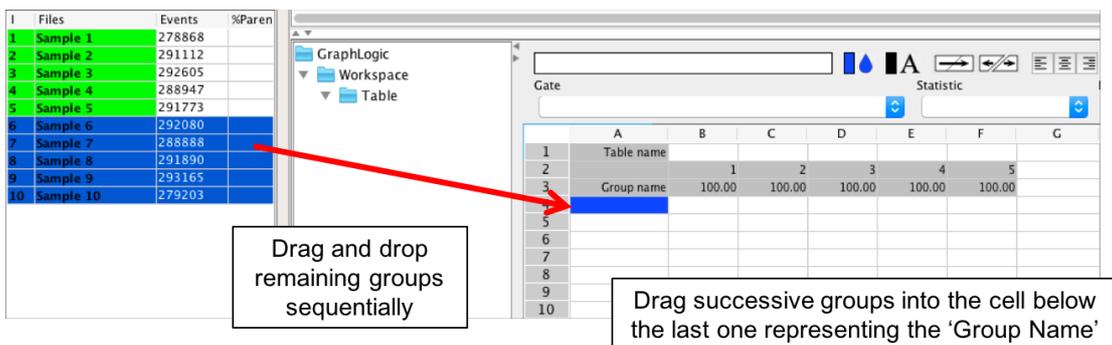
Functions can be applied to any data in the Workspace, such as adding, subtracting, multiplying or dividing one cell by another. The equation defining the calculation is created in the formula field at the top of the Workspace.

### Adding FCS-linked Data

The feature that makes the GraphLogic Workspace so useful is the ability to drag in FCS files, or import files contained in a plate, and then display any gate or parameter statistic using the drop-down menus at the top of the Workspace. Data can then be dragged into a Workspace Table, which automatically generates a graph. Statistical analyses can also be performed with a few clicks. In addition, FCS-linked data in the Workspace can be duplicated and set to a different gate or parameter statistic whilst maintaining all other generated graphs and tables.



Drag and drop the first group of files into the Workspace



Drag and drop remaining groups sequentially

Drag successive groups into the cell below the last one representing the 'Group Name'

When FCS files are dragged into the Workspace, a template is automatically created with titles for the Table (dataset) name, the experimental group names and the sample count.

- Table name: the table name refers to the table containing the dataset that the associated graph is derived from. For example, this could be Lymphocytes %Parent.
- Group names: the group name relate to the experimental groups, which are reflected as separate bars in a bar graph, for example. This is unrelated to the groups created for group analysis. In the same example, there might be two groups: treatment and control.
- Sample number: this refers to individual samples within each experimental group, or the *n* value.

	A	B	C	D	E	F
1	Table name					
2		1	2	3	4	5
3	Group name	100.00	100.00	100.00	100.00	100.00
4	Group name	100.00	100.00	100.00	100.00	100.00
5						

**Table name** (points to cell A1)

**Sample numbers** (points to cells B2-C2)

**Experimental group name** (points to cell A3)

**Gate or parameter statistics** (points to the red-bordered data area)

Graphs can be created by highlighting the gate/parameter statistics (within the red border above) or the entire data table (within the green border) and dragging them directly to a Table folder in the window. If the data exists within the template, as shown above, the Table name and group names will automatically be applied to the Table folder and graph, respectively. Updating these names in the Workspace after the data has been added to the table and a graph has been created will update the naming of these elements.

### Selecting gate and parameter statistics

When FCS files are dragged into the Workspace, by default the value for each sample will be 100.00, relating to the %Parent, All Events.

Use the drop-down windows above the table to select the population (gate) and the type of statistic (event count, %parent, %total, mean, etc.) to graph. Finally, you can specify the number of replicates performed.

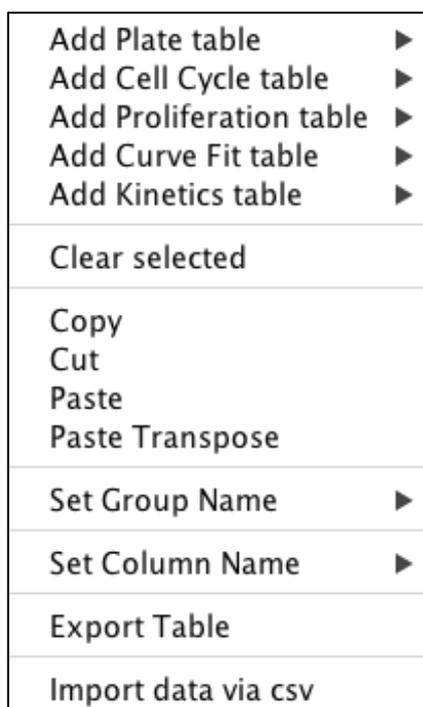
Gate	Statistic	Parameter
5 Monocytes	Median	Ly-6C FITC-A

## Importing PlateLogic and Advanced Analysis Datasets

An alternative method for adding FCS-derived data into a GraphLogic Workspace is to import files that are already arranged in plates within PlateLogic. This is achieved via the right click menu in the GraphLogic Workspace, as described below. The same process can be used to import data generated after performing cell cycle, proliferation, curve fitting and kinetics analyses.

Once any of these datasets have been imported into a GraphLogic Workspace, the data can be rearranged for graphing and statistical analysis. Data imported via PlateLogic remains linked to the gates and so any adjustment to gates in GateLogic will update the data in GraphLogic. If an adjustment is made to the gates used to derive the cycle, proliferation, curve fitting and kinetics analyses after importing into GraphLogic, re-import the data to display the updated values.

## The Workspace Right-Click Menu



## Add Plate Data

Files that have been imported into a plate in PlateLogic can be added directly to the Worksheet in GraphLogic right clicking, choosing 'Add Plate table' and selecting the desired plate by name. The data will be imported into the Worksheet in the same arrangement as the files exist in the plate. Files can be added to a plate before gating or at any time after gates have been created.

There is no need to define the statistics in PlateLogic as the selection can be made in GraphLogic. The data remains linked to the gates in GraphLogic and so will update automatically if a gate is adjusted. The same 'plate' can be imported multiple times or an imported dataset can be copied and pasted elsewhere in the Workspace. The data from either form of duplication can be changed using the drop-down menus to define a different population or statistic. When importing plate data, the table structure with a Table name, Group names and Sample counts will automatically be created in preparation for generating a graph.

	A	B	C	D	E	F	G	H	I
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									



Highlight samples and choose gate and parameter statistics



Gate	Statistic		Parameter		Replicate	Display								
1 RS	% Parent				1	Value								
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Plate data													
2		1	2	3	4	5	6	7	8	9	10	11	12	
3	A	2.83	3.15	1.78	3.64	2.94	2.32	3.57	3.41	2.82	5.84	5.61	2.84	
4	B	3.82	41.02	31.26	6.17	4.56	3.62	9.74	3.13	10.38	3.44	2.96	3.30	
5	C	4.79	4.25	5.25	3.84	7.76	88.50	15.65	8.37	3.25	3.19	21.45	16.57	
6	D	5.52	4.59	4.82	4.61	3.48	7.50	3.38	3.81	3.66	4.25	5.97	4.90	
7	E	4.06	3.40	4.93	24.84	6.91	7.53	4.10	5.67	58.62	58.66	11.48	9.34	
8	F	47.88	44.75	15.28	33.81	34.17	6.52	3.08	20.97	64.49	20.17	64.09	48.57	
9	G	80.22	17.28	3.27	3.20	2.81	51.17	9.21	6.00	3.89	2.45	2.30	2.92	
10	H	47.13	11.27	3.40	4.34	20.11	33.34	10.30	4.61	66.16	11.96	2.76	2.63	
11														
12														
13														
14														

### **Add Cell Cycle, Proliferation, Curve Fit and Kinetics table data**

Data derived from Cell Cycle, Proliferation, Curve Fit and Kinetics analyses can be added to the GraphLogic Workspace by right clicking, choosing the analysis type and selecting the dataset based on the Experiment Folder name where the files are located.

### **Clear Selected**

Selecting 'Clear Selected' cells from the right click menu after highlighting in the Workspace will clear all data, formulae, font formatting, cell alignment and color settings.

### **Copy**

Cells can be copied, by highlighting and selecting 'Copy' from the right click menu, and be pasted elsewhere in the same Workspace, in a different Workspace or into a Table folder. This process copies all data, formulae, font formatting, cell alignment and color settings. The original, copied data will remain after replicated data is pasted to another position in the Workspace.

### **Cut**

The Cut function removes the data in the selected cells, along with all formulae, font formatting, cell alignment and color settings and pastes it to the specified destination. When pasted, the Cut data will overwrite all data and formatting in the location where they are pasted.

### **Paste**

The Paste function adds the Copied or Cut data to a specified location in the same orientation.

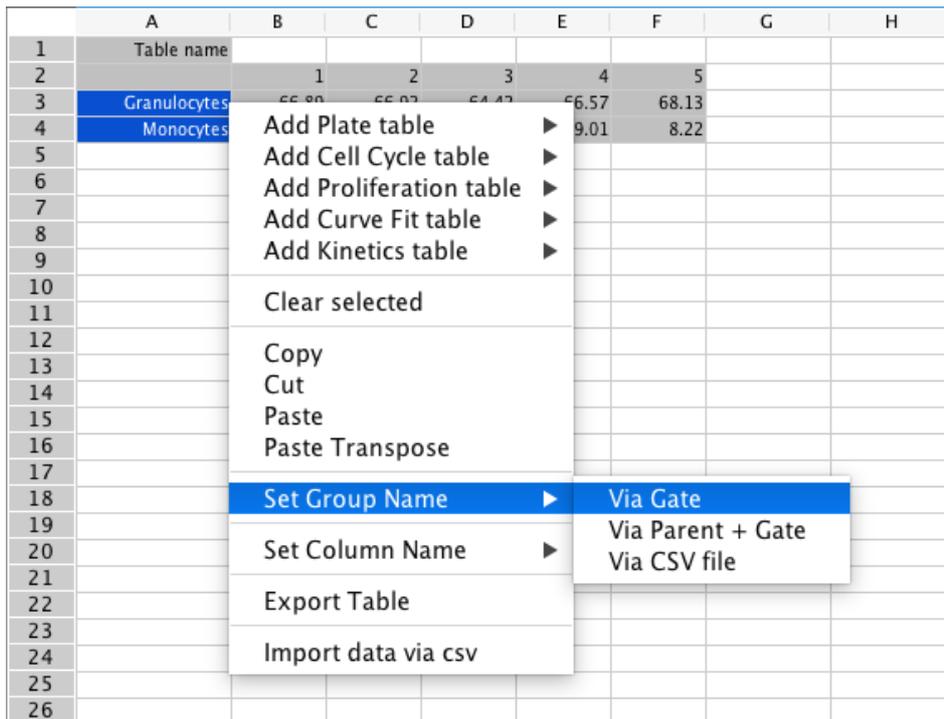
### **Paste Transpose**

The Paste Transpose function flips the orientation so that Row 1 becomes Column 1, Row 2 becomes Column 2, etc. Data can only be transposed within the Workspace and not into a Table folder.

### **Set Group Name →**

The group name for a data set can be set using several different references. This is achieved by selecting the cells containing the group titles to be renamed, right clicking and selecting 'Set Group Name' → and one of:

- *Via Gate*
- *Via Parent + Gate*
- *Via CSV file*



Via Gate – This function is for data derived from FCS files that are dragged into the Workspace from the File Inspector or imported via a plate. To rename the Group, highlight the Group Name cells, right click and select Set Group Name → Via Gate. The Group name will then be updated with the gate name, which is displayed in the gate drop down menu in the toolbar.

Via Parent + Gate – This function is also for data derived from FCS files that are dragged into the Workspace from the File Inspector or imported via a plate. It functions in the same way as the 'Via Gate' option but adds the parent gate name before the population gate name.

Via CSV file – This function allows groups to be named from a .csv file. Multiple Group Name cells can be named at once, as long as they are highlighted prior to naming. This feature can be very useful if the same experimental groups are used consistently over a number of analyses or experiments.

It is important, however, to set up the .csv file in the correct format. The names in the .csv file need to be listed horizontally, with one name per cell if created in a spreadsheet or having each name separated by a comma if created with a text editor. However, FlowLogic will apply these names vertically, assuming each experimental group is displayed one on top of the next. In addition, only the selected number of Group name cells will be named, even if the list in the .csv file is longer. For example, if the names in the .csv file are 'Group A', 'Group B', 'Group C', 'Group D' and 'Group E' but only two cells are highlighted in the Workspace, only the names 'Group A' and 'Group B' will be applied. Similarly, if more cells are highlighted than names exist in the CSV file, not all cells will be renamed.

It is also possible to set up a .csv file with spaces between the names. For example, here is a .csv file with a gap between 'Group 2' and 'Group 3':

Group 1	Group 2	Group 3	Group 4
---------	---------	---------	---------

```
Group 1,Group 2,,Group 3,Group 4
```

When cells A3 to A7 are highlighted and this .csv file is imported, the third name, corresponding to cell A5, is left blank.

	A	B	C	D	E	F
1	Table name					
2		1	2	3	4	5
3	Group name	8.23	4.95	4.04	6.23	5.61
4	Group name	27.46	38.18	36.43	28.15	35.51
5						
6	Group name	4.02	1.34	1.49	1.12	1.56
7	Group name	14.72	12.91	10.53	11.07	12.66

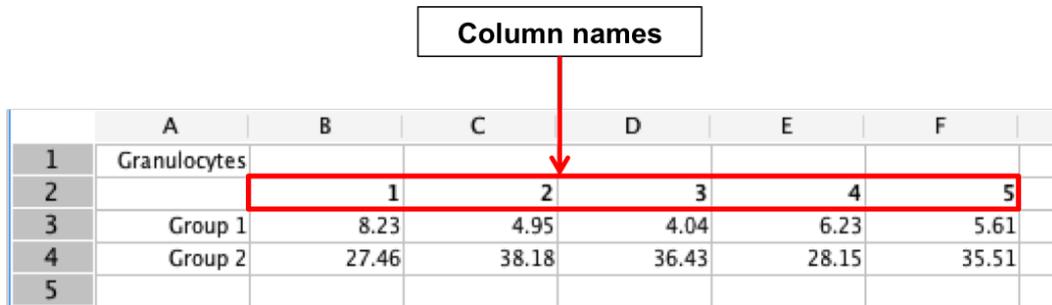
- Add Plate table
- Add Cell Cycle table
- Add Proliferation table
- Add Curve Fit table
- Add Kinetics table
- Clear selected
- Copy
- Cut
- Paste
- Paste Transpose
- Set Group Name**
  - Via Gate
  - Via Parent + Gate
  - Via CSV file**
- Set Column Name
- Export Table
- Import data via csv

	A	B	C
1	Table name		
2		1	2
3	Group 1	8.23	4.95
4	Group 2	27.46	38.18
5			
6	Group 3	4.02	1.34
7	Group 4	14.72	12.91

Gap in the naming

### Set Column Name →

The column name for a data set can be set using several different references. The column name refers to the title above each sample, which are numbered by default.



	A	B	C	D	E	F	
1	Granulocytes						
2		1	2	3	4	5	
3	Group 1	8.23	4.95	4.04	6.23	5.61	
4	Group 2	27.46	38.18	36.43	28.15	35.51	
5							

Select the cells containing the group titles to be renamed, right clicking and selecting 'Set Column Name' → and one of:

- *Via Count*
- *Via \$SRC*
- *Via \$CELLS*
- *Via TAG*
- *Via CSV file*

*Via \$SRC* – This function allows columns to be named from the \$SRC keyword contained in the FCS file. This keyword can differ from cytometer to cytometer and can be defined at the time of acquisition with certain acquisition programs.

*Via \$CELLS* – This is another keyword contained in the FCS file.

*Via TAG* – tags applied to a sample can be used to name the column titles. If multiple tags have been applied to a sample, these will all be listed in the column name.

*Via CSV file* – naming columns via a .csv file works in a similar way to naming the groups. Select the column titles to be named, right click and choose Set Column Name →Via CSV file. The names in the .csv file need to be listed horizontally and any spaces in the .csv file will result in a cell being missed in the Workspace.

### Exporting Table

The Workspace table can be exported as either a .csv file or Excel XML Workbooks (\*.xlsx) file. The .csv export option will record all text and numbers in the displayed table format without any formatting. Formulae will not be saved in the .csv file, only the resulting value displayed in the cell. The .xlsx file will save all the text and numbers along with text and cell formatting, such as coloring, alignment within cells, font style/type/size and formulae.

### Importing Data from CSV Files

Data saved in the .csv file format can be imported into the Workspace by right clicking in the destination cell and selecting 'Import data via CSV'.

## Manually Adding Data

Data can also be added to the Workspace by selecting a cell and typing text and numbers with the keyboard.

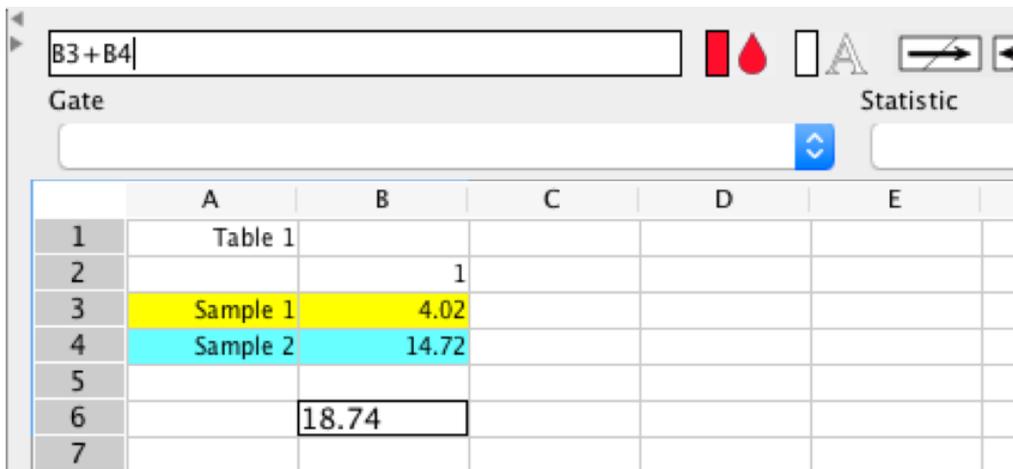
## Workspace Toolbar

### The Formula Field

Formulae can be created to apply mathematical functions to selected cells. Addition, subtraction, multiplication and division of cells, typed values or a combination of both can be performed by defining the equation in the formula field in the toolbar above the Workspace.

Examples of each of these situations are as follow:

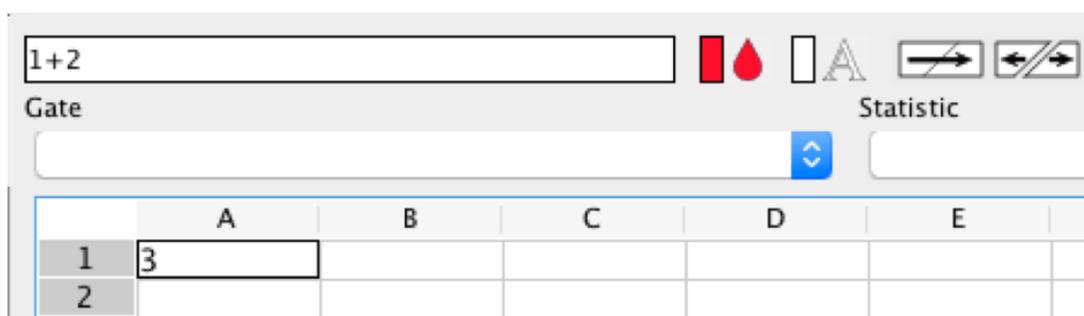
- To add the values in cells B3 and B4, select a destination cell (B6 in this example), then click in the formula field, click cell B3 followed by B4, click inside the formula field again and finish by pressing enter on the keyboard.



The screenshot shows the workspace toolbar with the formula field containing "B3+B4". Below the toolbar is a table with the following data:

	A	B	C	D	E
1	Table 1				
2		1			
3	Sample 1	4.02			
4	Sample 2	14.72			
5					
6		18.74			
7					

- To add a series of numbers and display the result in one cell, select a destination cell, then click in the formula field and type the equation, e.g. 1+2. Then press enter on the keyboard and the result (3) will be displayed in the destination cell.



The screenshot shows the workspace toolbar with the formula field containing "1+2". Below the toolbar is a table with the following data:

	A	B	C	D	E
1	3				
2					

- To multiply a cell by a number (e.g. B3\*100), select a destination cell (B5 in this example), then click in the formula field, click cell B3 and in the formula field type “\*100”. Finish by pressing enter on the keyboard. The final value will be calculated and displayed in the destination cell.

The screenshot shows a spreadsheet interface. At the top, a formula field contains the text "B3\*100". Below it, a "Gate" field is empty and a "Statistic" field is empty. The main spreadsheet area has columns labeled A, B, C, D, and E, and rows labeled 1 through 6. Cell A1 contains "Table 1". Cell B2 contains "1". Cell B3 is highlighted in yellow and contains "4.02". Cell B5 contains "402.18".

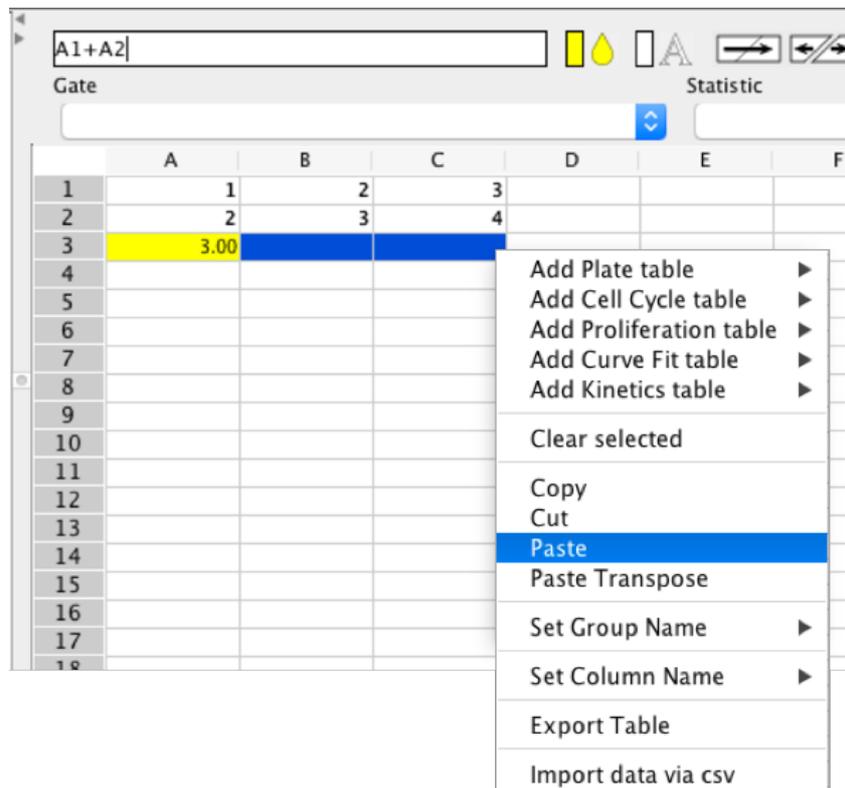
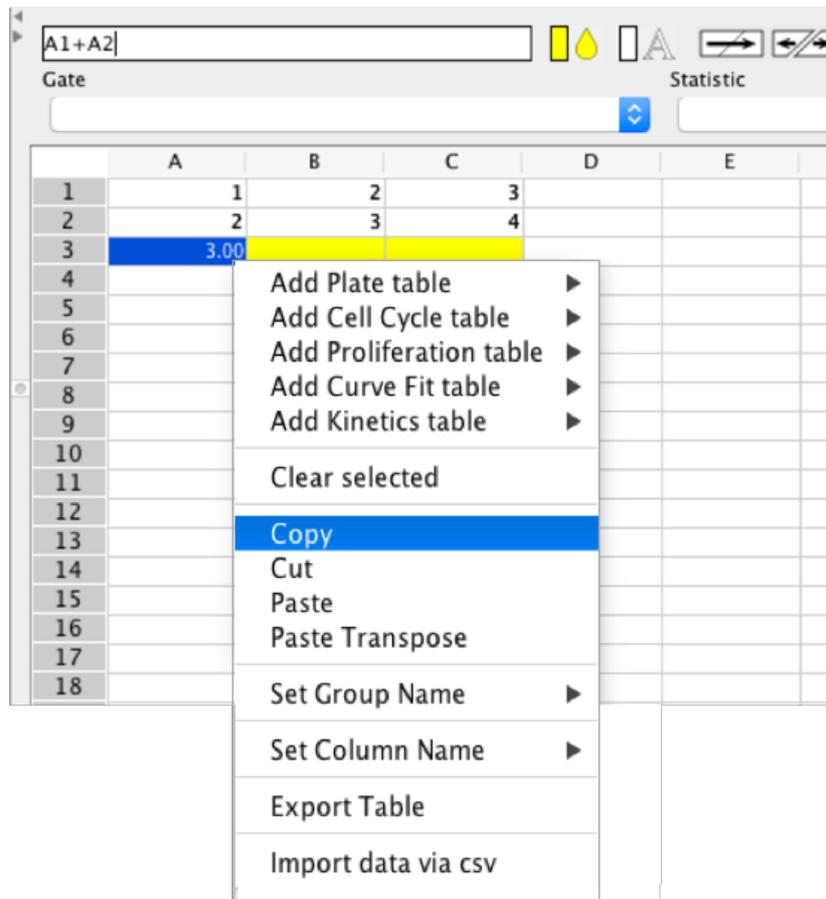
	A	B	C	D	E
1	Table 1				
2		1			
3	Group 1	4.02			
4					
5		402.18			
6					

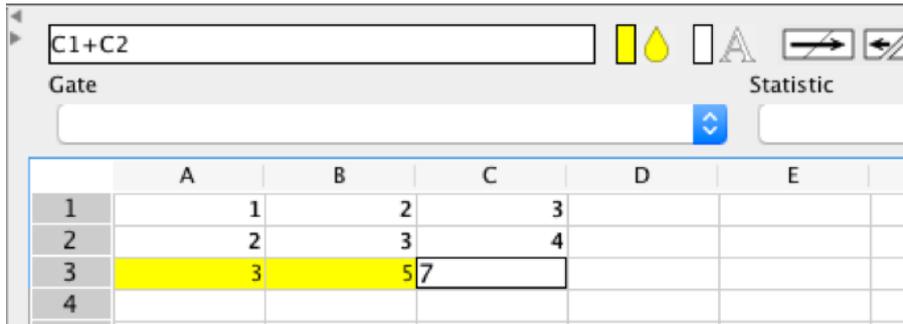
Much more complex formulae can be created using all combinations of mathematical functions (+, -, \*, /).

If a formula has been created based on the relationship between a specific selection of cells and numbers, it can be copied and pasted to a new selection of cells, with the formula in the new cells referring to cells with the same spatial relationship. To copy a cell, right click in the cell and select 'Copy'. Then, select the destination cells, right click and select 'Paste'.

For example, if the formula in A3 is A1+A2, then copying A3 to B3 and C3 will result in:

- A3=A1+A2
- B3=B1+B2
- C3=C1+C2



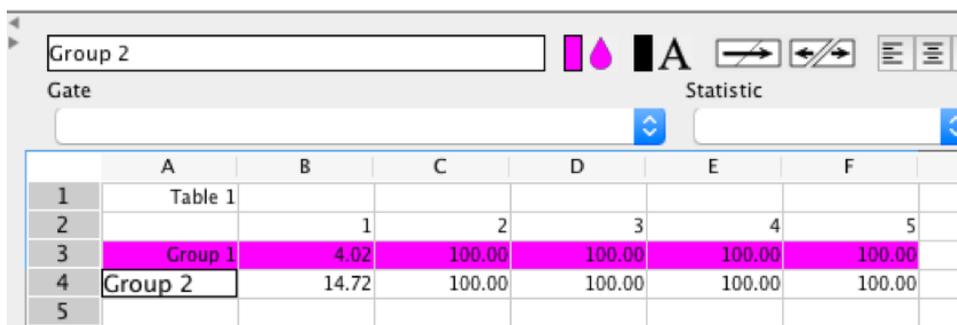
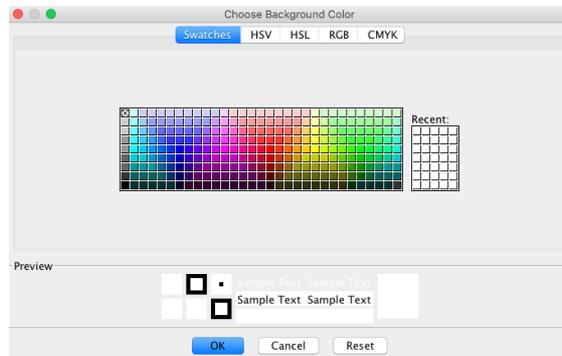


### Coloring Text and Cells

Color can be applied to cells and text in the Workspace spreadsheet. This can be achieved using the icons in the toolbar. Each function (background color and foreground/text color) has two associated icons: select color (the colored rectangle icon) and apply color (drop icon for cells and 'A' icon for text).



To color the cell (background), highlight the cells to be colored and click the background color selection icon. This will open a color palette. Click to choose the desired color and then click 'OK'. This will apply the chosen color to the highlighted cells. You will notice that the Select Color and Apply Color icons have taken the most recent color choice. To continue filling cells with the same color, simply highlight the cells and click the Drop icon.



The same process applies to coloring text within cells. Text is colored black by default but to change the text color, highlight the desired cells and click the select color icon next to the 'A' icon. Choose a color and click 'OK'. The 'A' icon is now colored with the most recent selection. To continue to color text with the selected color, highlight the desired cells and click the 'A' icon.

	A	B	C	D	E	F
1	Table 1					
2		1	2	3	4	5
3	Group 1	4.02	100.00	100.00	100.00	100.00
4	Group 2	14.72	100.00	100.00	100.00	100.00
5						
6						

### Split and Merge Cells

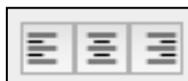
Multiple cells in the Workspace can be merged to form a single larger cell and merged cells can be split back into their original components. To merge or split cells, highlight them in the Workspace and then click the appropriate button in the toolbar.



Cells running horizontally, vertically or a block spanning a number of columns and rows can be merged (are subsequently split).

### Text Alignment

Text can be aligned to either the left, centre or right of a cell using the three icons in the toolbar. To change the alignment, select the cell(s) and click on the appropriate icon.



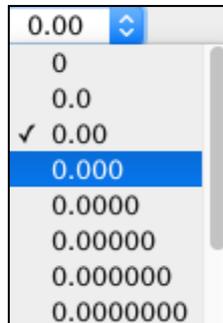
### Font Settings

Three font setting options can be accessed in the toolbar, being the font type, size and style. To change the font settings, highlight the cells in the Workspace and select from the different options in the font settings drop down menus.



## Decimal Places

The number of decimal places that are displayed in individual cells can be set by selecting the cell(s) and choosing an option ranging from no decimal places through to ten. Individual cells within the same Workspace can be set to display different numbers of decimal places.

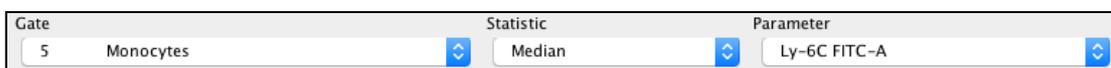


## Population Statistic Menus

The population statistics menu allows for the selection of specific gate and parameter statistics to be displayed for a single FCS sample, or a group of FCS samples, that have been dragged into the Workspace.

To define a statistic, highlight the cell or cells containing data derived from an imported FCS file and select the desired gate, statistic and parameter (if applicable). The value in the cell will update to reflect the defined statistic and can be changed to any other statistic without having to re-import the sample. The statistics will also update following any changes or adjustments to the defining gates in GateLogic.

The Population Statistic Menus can also be used to verify the statistic in a selected cell. Clicking on any cell containing FCS-derived data will prompt the three population statistic menus to display the gate, statistic and parameter for that cell. If multiple cells containing different statistics are selected, the display in the menus will reflect the first cell in the selection.



For more information regarding importing and displaying FCS-derived data, see [page 7](#).

## Defining Replicates

If values from all technical replicates have been imported, the number of replicates can be defined with the average of the technical replicates being displayed in merged cells.



## Gate/Value Display

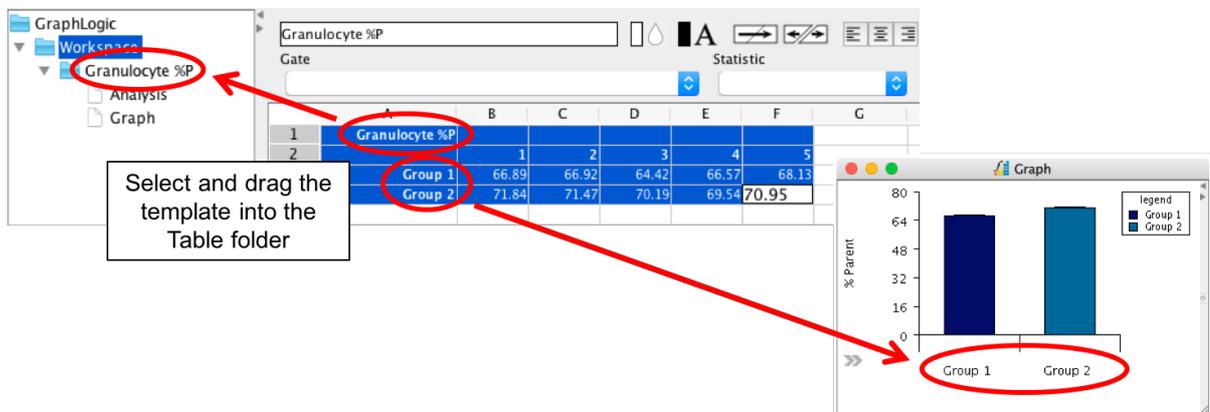
The Gate/Value Display function allows the for the gate name linked to the gate/parameter statistics to be displayed within the cells instead of the statistic itself. This can be useful if you wish to check the origin of a selection of cells without having to click on the cells one-by-one to view the gate in the Population Statistic 'Gate' menu.

To change the information displayed in the cells, select them in the Workspace and choose the desired option in the Display drop down menu.



## Adding Data to a Table Folder for Statistical Analysis and Graphing

When data exists in the Workspace within the data template as shown below, it is very easy to add it to a Table folder in order to perform statistical analyses and graph. Simply select the data (not the Group or column names) and drag it into a Table folder.



The screenshot shows a workspace with a table and a graph. The table has columns labeled A through G and rows labeled 1 and 2. The data in the table is as follows:

	A	B	C	D	E	F	G
1	Granulocyte %P	1	2	3	4	5	
2	Group 1	66.89	66.92	64.42	66.57	68.13	
	Group 2	71.84	71.47	70.19	69.54	70.95	

A callout box with the text "Select and drag the template into the Table folder" points to the 'Granulocyte %P' cell in the table. A red arrow points from the 'Group 1' and 'Group 2' rows of the table to a bar graph. The graph shows two bars: a dark blue bar for 'Group 1' and a lighter blue bar for 'Group 2'. The y-axis is labeled '% Percent' and ranges from 0 to 80. The legend indicates 'Group 1' and 'Group 2'.

The Table folder will be automatically renamed the dataset title (the yellow cell in the example above) and the groups in the graph and statistical analyses will be named automatically renamed from the group names in the Workspace.

## Performing Statistical Analyses

The screenshot shows the software interface. On the left, a tree view under 'Workspace' has 'Analysis' selected, indicated by a red arrow pointing to a box labeled 'Statistical analysis sheet'. The main 'Analysis Groups' window is open, showing 't tests (Parametric and non Parametric)' selected. The 'Analysis Type' is 'Unpaired t tests with Welch-Satterthwaite approximation'. The 'Post Analysis Type' is 'Post Analysis'. The 'Statistical Significance' is set to 0.05. Below this, a table shows 'Select' and 'Data' columns with 'Group 1' and 'Group 2' selected. To the right, a 'Dialog' window displays statistical results for an unpaired t test, including p-values and t-statistics. A box labeled 'Analysis window' points to the 'Analysis Groups' window.

- After data has been added to the data table (see [page 7](#)), select a statistical analysis sheet. This opens the Analysis window. The workspace from where the data originates can be viewed by selecting the Workspace in the Graph Data window or by clicking on the Table folder associated with the particular analysis sheet.
- To perform a statistical test, select the experimental groups to analyse and the specific statistical analysis test (and post-test if applicable) from the drop-down menus.

This close-up shows the 'Analysis Groups' dialog box. Three red arrows point to the 'Analysis Groups' dropdown (set to 'one-way ANOVA [Parametric and non Parametric]'), the 'Analysis Type' dropdown (set to 'One-way analysis of Variance'), and the 'Post Analysis Type' dropdown (set to 'Tukey'). Below these is the 'Statistical Significance' field (0.05) and an 'Apply' button. At the bottom, a table shows 'Select' and 'Data' columns with 'Group 1', 'Group 2', and 'Group 3' listed. A red arrow points to the 'Select' column, which has checkmarks for all three groups.

- The result will be displayed in the Description and Analysis columns adjacent.

Description	Analysis
One Way Anova	
Number of groups	3.00
F Value	13.42
P Value	0.00
Null hypothesis	false
Are means different?	YES
Significance level	0.05
Number	12.00
Mean	35.15
SD	11.78
Variance	138.86
Skewness	0.29
Kurtosis	-0.94
Min	18.45
Max	55.58
Tukey	
Groups Group 1 Vs Group 2	
Mean Diff.	23.69
Standesized Error	3.26
Critical Value	3.95
Lower	10.80
Upper	36.57
Sig Diff?	NO

## Analysis Groups

✓ t tests [Parametric and non Parametric]
one-way ANOVA [Parametric and non Parametric]
Simple Statistics
Regression Analysis

## Analysis Type

✓ Unpaired t tests with Welch-Satterthwaite approximation
Unpaired t tests
Unpaired t test Equal Variance
Paired t tests
Mann-Whitney

✓ One-way analysis of Variance
Kruskal-Wallis test

✓ Basic Statistics

✓ Linear Regression  
4 Parameter Log Regression  
5 Parameter Log Regression

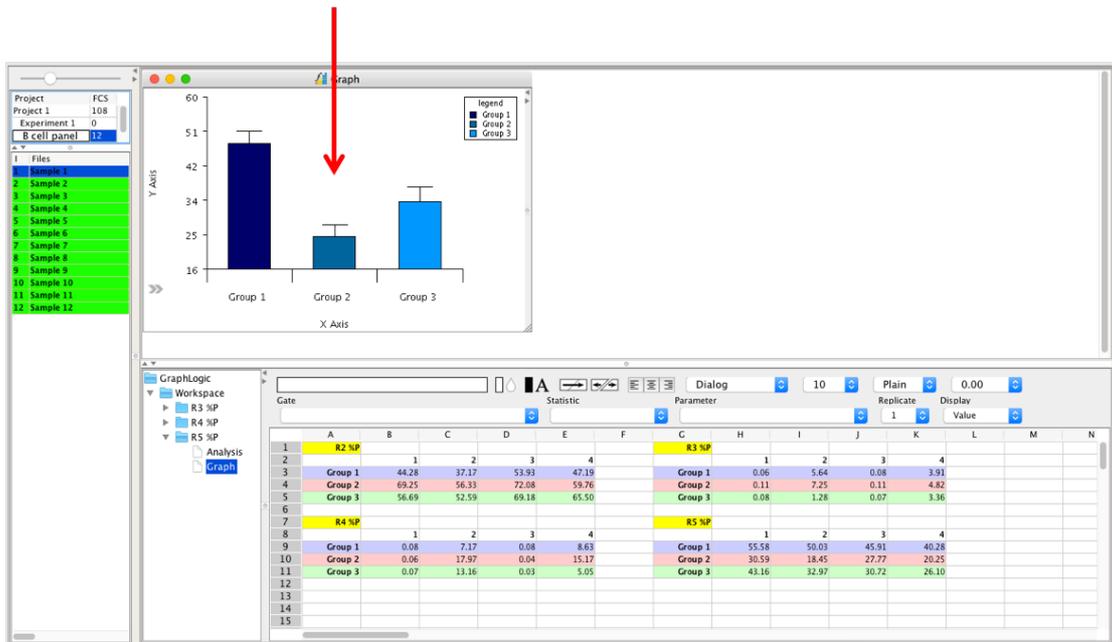
### Post Analysis Type

✓ Post Analysis  
Bonferroni  
Tukey

### Creating a Graph

To graph your data, select the Statistics window at the top of the program and select Workspace 1 in the Graph Data window. Then, highlight the data within a workspace template and drag it into a Table folder (see [page 21](#)).

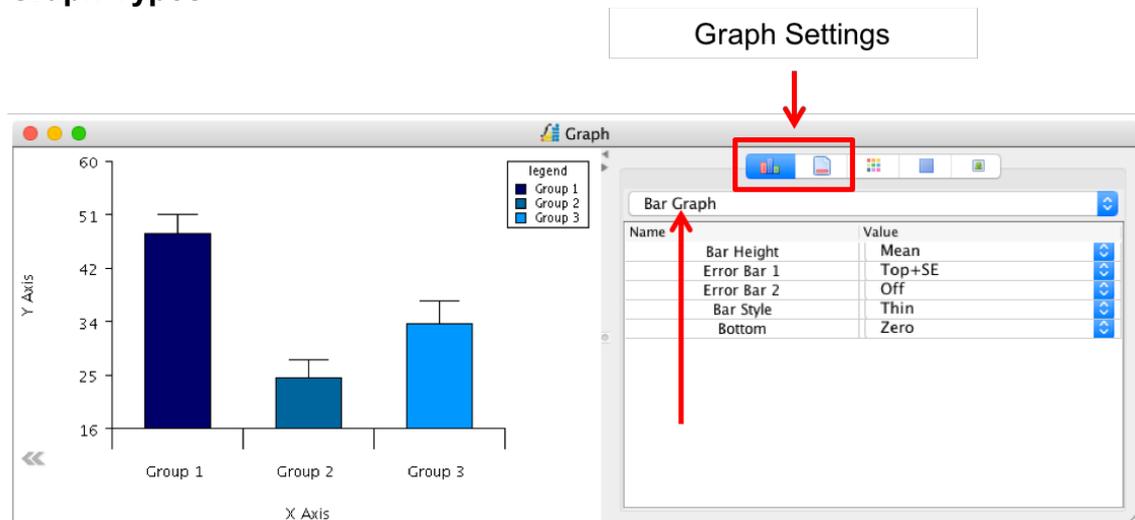
Double click Graph in the Data Folder window to create a bar graph of the imported data. The graph appears in the Graph Window.



- Open the graph side drawer and use the functions in the Graph Settings, Color Settings, Legend Settings and Border & Background Settings to customize your graph.

- Graphs are exported or displayed in reports as they appear here. Take a few moments to adjust the settings and appearance of the graphs. Of course, graphs in a Report will be automatically updated if settings are changed at a later time.

## Graph Types



From the drop-down menu, you can choose from a range of graph types, including Group Bar Graph, Pie Graph, Bar Graph, Stacked Bar Graph and Dot/Line Graph.

## Editing Graphs

Bars and error bars can be edited using the other options in the side drawer. These include the Bar Height (Min, Median, Mean or Max), Error Bars (Off, Min, Median, Mean, Max, Top+SD, Top-SD, Top+SE, Top-SE) and the Bar Style (Thin, Dash, Medium, Medium Dash, Thick, Thick Dash and None).

## Graph Settings

Turn on/off axes and define the spaces surrounding the graph.

Edit axis labels, including color, font, size, alignment, decimal places and angle to the axis.

Change the scale of the axes or select Auto Max and Auto Min.

Set and customize major and minor tick lines.

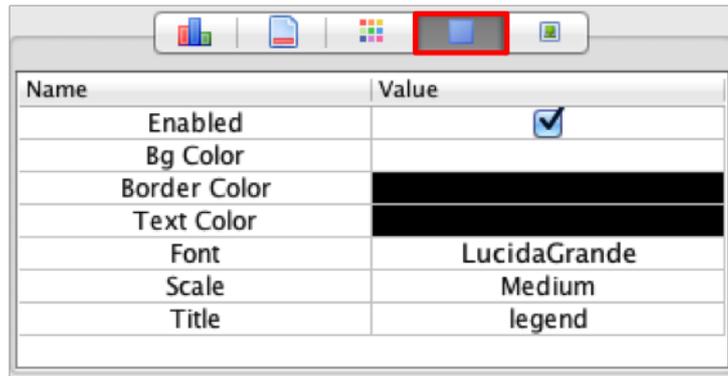
	X-Axis	Y-Axis
<b>General</b>		
On/Off	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Size	80	80
Space	10	100
Draw Frame	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<b>Labels</b>		
Label Color		
Scale Font	Dialog	Dialog
Label Font	Dialog	Dialog
Label	X Axis	Y Axis
Alignment	Center	Above
Units		
Decimal Places	0	0
Angle	0	0
<b>Scale</b>		
Max	6.0	40.0
Auto Max	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Min	0.0	0.0
Auto Min	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<b>Major Tick Lines</b>		
On/Off	<input type="checkbox"/>	<input type="checkbox"/>
Number of Lines		
Color		
Weight		
Tick Length		
Style		
<b>Minor Tick Lines</b>		
On/Off	<input type="checkbox"/>	<input type="checkbox"/>
Number of Lines		
Color		
Weight		
Tick Length		
Style		

## Graph color settings

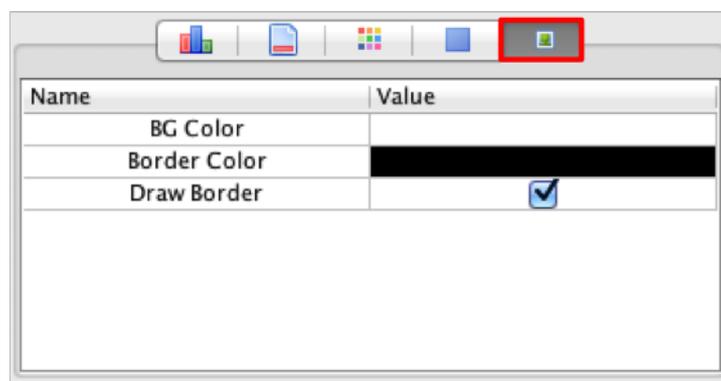
Name	Fill
Group 1	
Group 2	
Group 3	

Click on the colors and choose a new one from the color palette. Alternatively, right click on the graph and choose 'Graph Colors'.

Graph legend settings – edit the appearance of the legend, including its background color, border color, text color, font and size. Double click on 'legend' to rename its title.

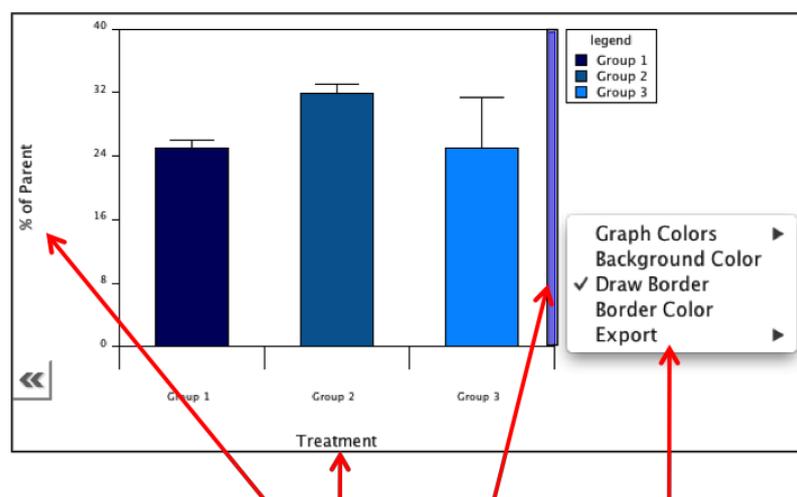


Graph border and background – edit the color of the graph background and border or remove the border completely.



Editing the graph space – the graph can be edited directly or through the right click menu. Click and drag the axes to adjust the size of the graph within the space.

Double click on the axis titles to edit the name.  
 Click and drag the legend to a new location.  
 Right click in the legend to bring up more editing options.



Creating additional graphs – graphs are located within Workspaces as displayed in the Graph Data window of the pull up drawer.