Flowlogic™

Flow Cytometry Analysis Software

Developed by scientists, for scientists

User Manual

Version 7.2.1

GateLogic:

Advanced Functions Drawer

PlateLogic, Set and View Statistics, Parameters, CompLogic, Metadata, Compensation

inivai® TECHNOLOGIES

TABLE OF CONTENTS

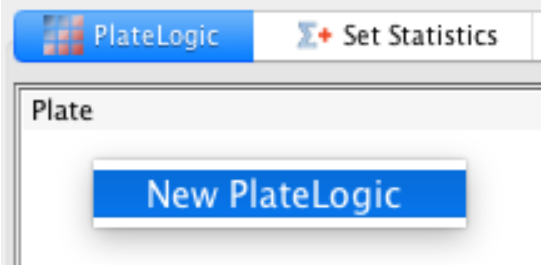
Advanced Functions Pull Up Drawer

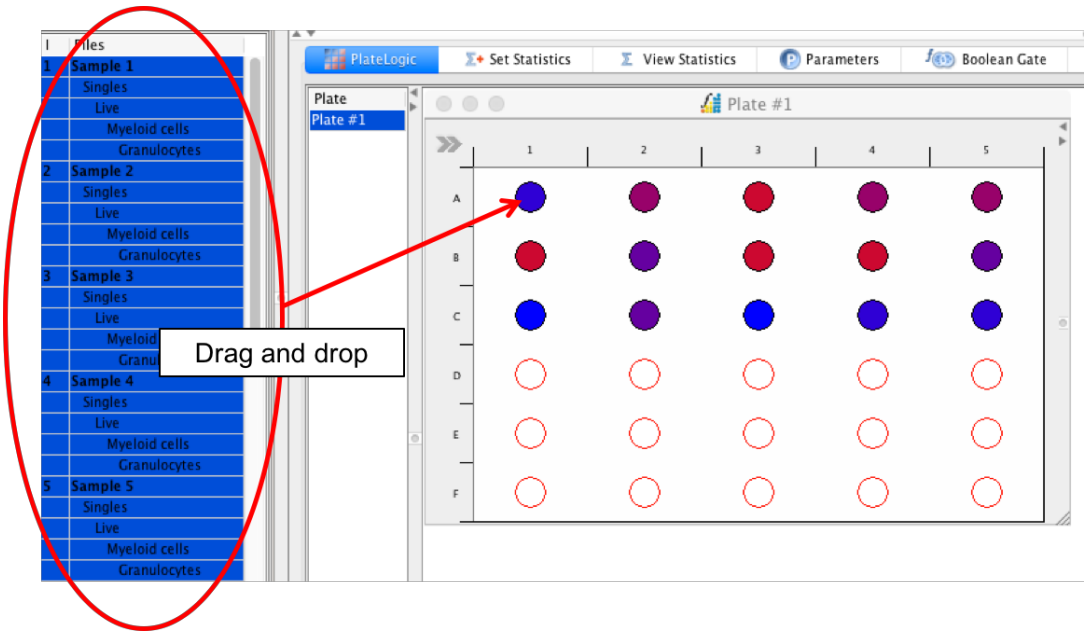
The Advanced Functions pull up drawer contains additional analysis features to improve efficiency and increase the data mining capabilities. To access the Advanced Functions drawer, click on the top of the drawer (located at the bottom of the Workspace) and drag it up. It can be pulled up to entirely cover the Workspace, if desired. The Advanced Functions drawer consists of a number of tabs. These are PlateLogic, Set Statistics, View Statistics, Parameters, Geometric Gates, Boolean Gates, CompLogic, MetaData, Compensation, Cell Cycle, Proliferation, Curve Fit and Kinetics.

PlateLogic – data array analysis

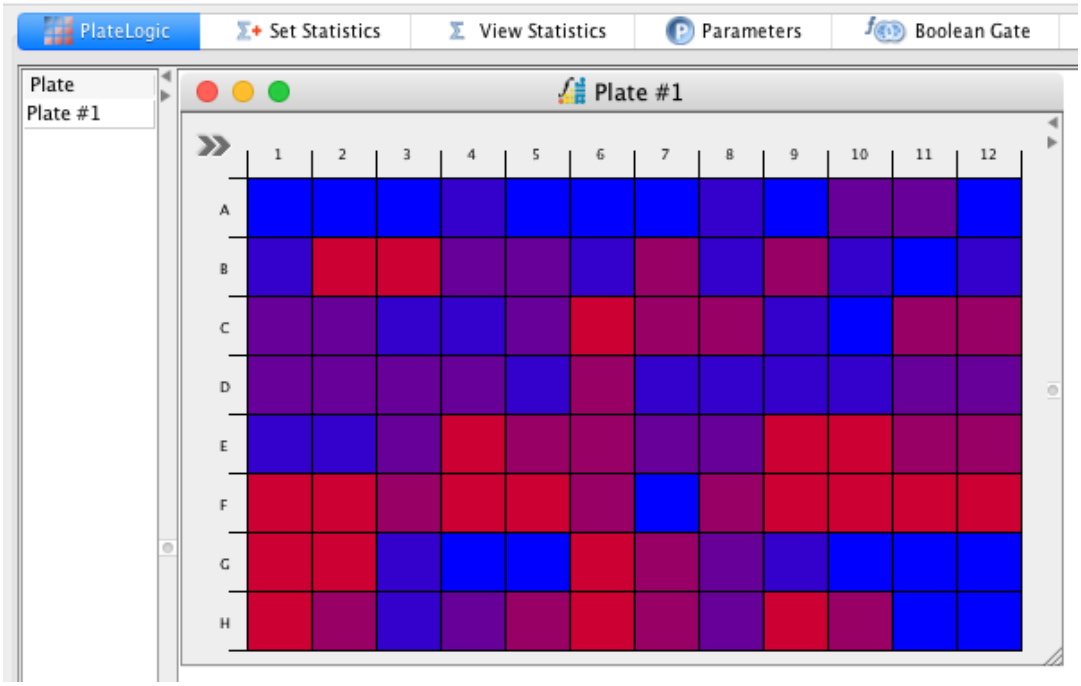
PlateLogic has been designed to assess large amounts of data, such as plate data, utilizing a plate layout. Highlighted FCS files can be dragged from the File Inspector and added to a plate, where a heatmap is automatically generated. Various statistics can be calculated, and tags can be applied to the analyzed files, allowing for the easy identification of samples with a specific characteristic. In addition, FCS data can be passed directly to GraphLogic in the format that they are arranged in a plate. This process does not rely on the selection of a gate or statistic in PlateLogic as the whole process is performed in GraphLogic.

To create a plate, click in the plate window and select New PlateLogic. To fill the plate, highlight a group of files in the File Inspector and drag them into the desired well in the plate. Continue filling the plate with all groups of files. It does not matter which gate or level of the gating hierarchy is selected and dragged into the plate as only the sample is recorded. Once in the plate, the specific subpopulation (gates) can be set and analyzed. It is also possible to add the same sample to multiple wells. If the same sample is added multiple times, each copy can be assigned to a different data set, with each dataset set to display a different level of the gating hierarchy.





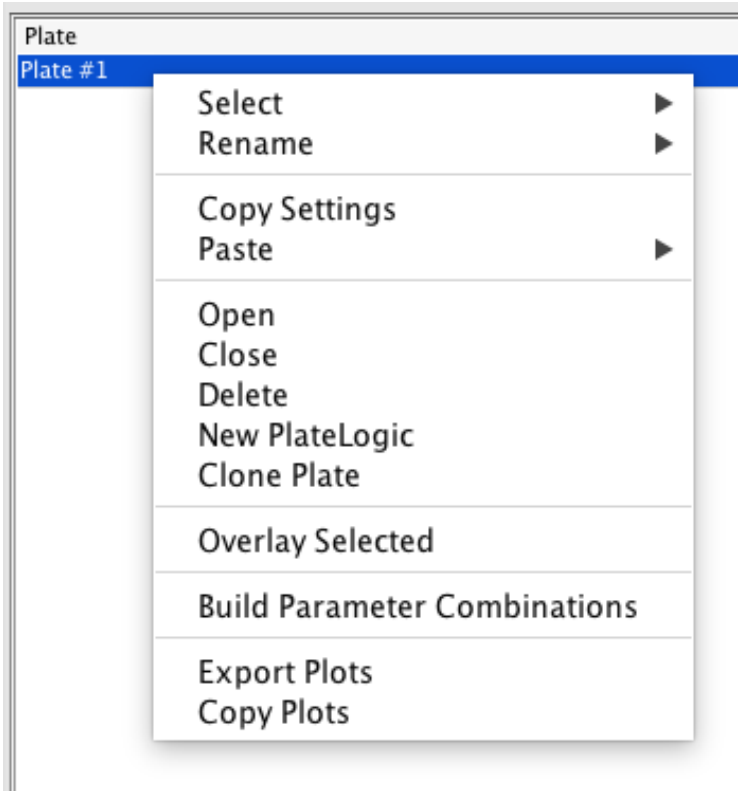
Once all files have been added to the plate, use the functions in the Plate Side Drawer to help analyze the samples.



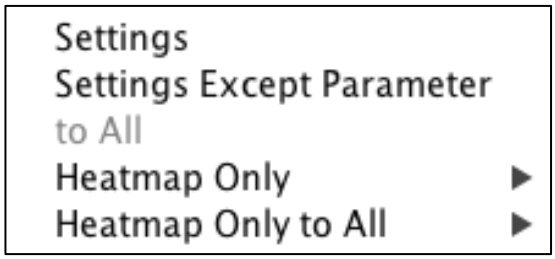
Here is an explanation of the features and elements located in the Data Array tab:

Plate list window – the Plate List window, located to the left of the plate workspace, displays all plates that have been created. To create a new plate, right click within the window (but not on an existing plate) and choose New PlateLogic.

- Right clicking on an existing plate name opens a different menu, with features to help manage the plates.



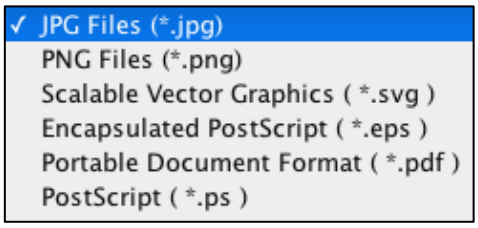
- Select – the select option is used to highlight specific plates in the Plate List window. The two selection options are All and Inverse. Clicking Select → All will highlight all the plates in the window. Select → Inverse will select all plates that were not highlighted, while deselecting all that were.
- Rename – select 'Rename' to access a field where a new plate name can be entered.
- Copy Settings – once a plate has been setup to suit the specific analysis, the settings from that plate can be copied and pasted onto another plate. Simply highlight the plate name, right click and choose Copy Settings. This copies the settings to the clipboard.
- Paste – once a plate's settings have been copied, right click the new plate and click Paste, followed by one of the following option:



    - Settings – will paste the plate settings to the selected plate.
    - Settings Except Parameter – will paste the plate settings to another plate except for changes made in the Parameter field of the Heatmap Settings tab.
    - To All – will paste the settings copied from one plate to all listed plates
    - Heatmap Only – will only paste settings associated with the heatmap.
    - Heatmap Only to All – will paste the settings associated with the heatmap to all other plates.

- Open – highlighting a plate or plates in the Plate List window, right clicking and selecting Open will open the plates in the plate workspace.
- Close – highlighting a plate or plates in the Plate List window, right clicking and selecting Close will close all plates in the plate workspace.
- Delete – highlighting a plate or plates, right clicking and selecting Delete will open a window asking if you wish to delete the specified plate. If confirmed, the plate will be deleted.

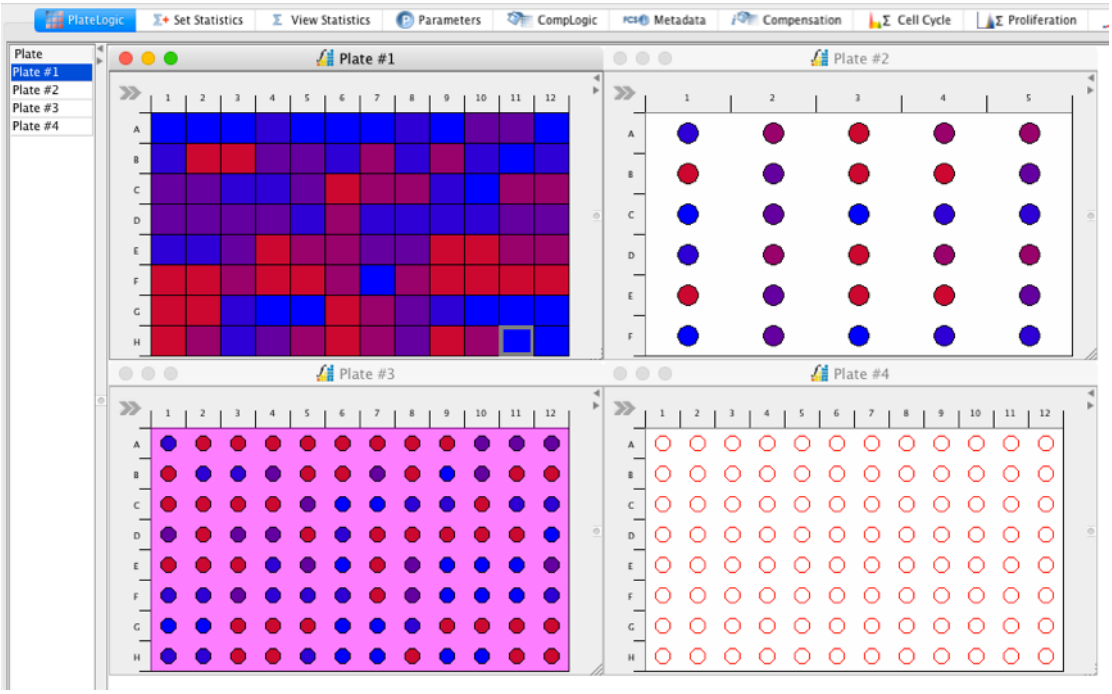
- New PlateLogic – this will create a new plate in the same way as selecting New PlateLogic after right clicking in the Plate List window.
- Clone Plate – Highlighting a plate, right clicking and selecting Clone Plate will create a copy of the selected plate and assign it the next available name.
- Overlay Selected – if two or more plates exist with FCS files that match (parameters and gates), then overlay plots can be created for all corresponding wells. So, the samples existing in well A1 will all be overlaid on one plot, while the next will be an overlay of all samples in well A2, etc.
- Build Parameter Combinations – highlighting a plate and selecting Build Parameter Combinations will result in a plate being created for every parameter contained within the FCS file. The parameter will then be set in the heatmap settings tab. If a parameter-dependent statistic is selected, then each plate will have a different result displayed in the statistics tab in the plate side drawer.
- Export Plots – highlighting a plate in the Plate List tab and selecting Export Plots will open a window allowing the heatmap (plate view) to be saved as one of six different file types, as shown:



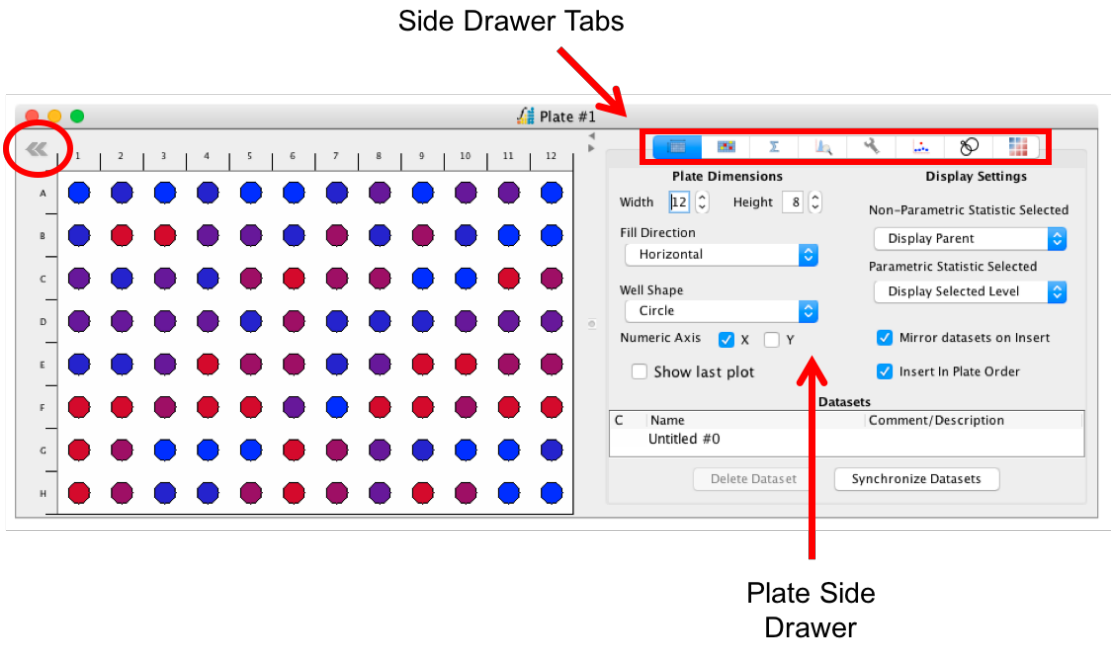
If multiple plates are highlighted when Export Plots is clicked, then each heatmap will be saved once after another.

- Copy Plots – in computers running Microsoft Windows, the plate image will be copied to the clipboard.
- Re-sizer – the re-sizer tool, located below the Plate List window, can be used to increase and decrease size of all plots open in the Data Array Workspace together. If one or more plates have been re-seized manually by clicking and dragging the bottom right hand corner of the plate, the re-sizer tool will return all plates back to the same size.

- Plate display window – plates are displayed in the Plate display window in a similar fashion to plots in the Workspace. The arrangement of plates in the display window can be optimized by clicking Edit → Plate Arrangement (see the topic about the Edit menu for more details).



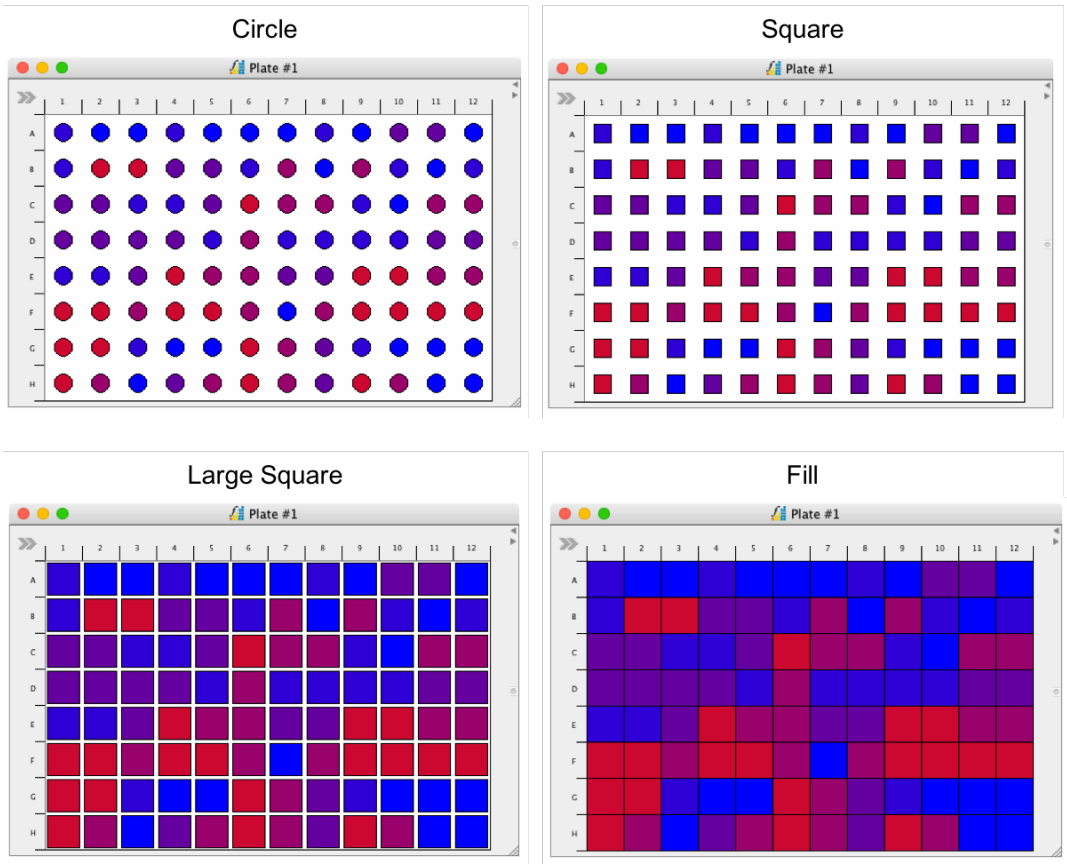
- Plate side drawer – each plate has a plate side drawer, which can be open by clicking the button in the top left-hand corner of the plate or by selecting the plate and pressing the space bar. Tabs are located at the top of the side drawer, containing a range of different display and analysis functions.



The plate side drawer tabs are as follow:

Plate Setup – set up the plate to best represent the design of your experiment. By default, the setup represents a 96 well plate.

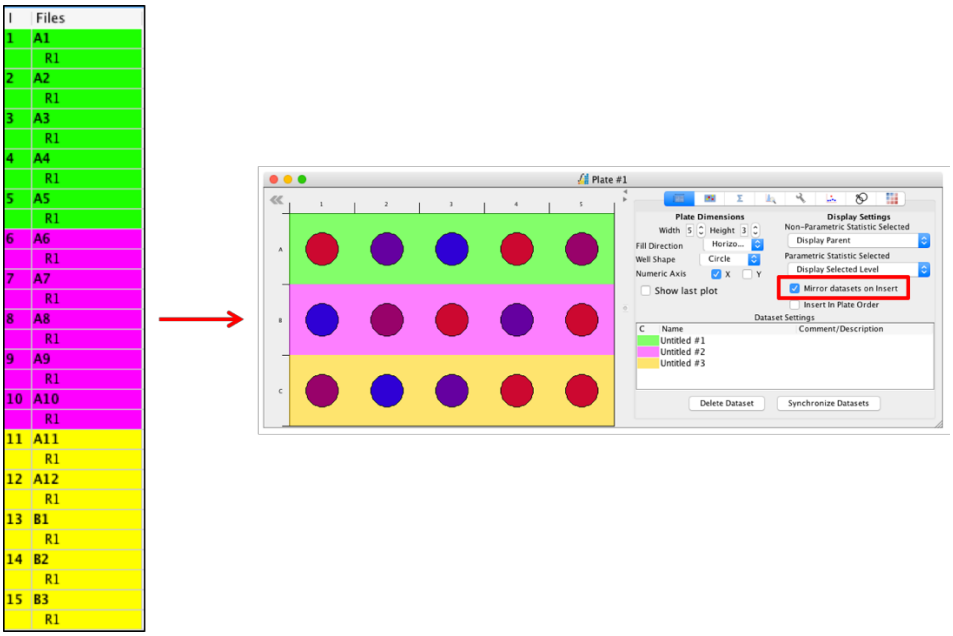
- **Plate Dimensions** – set the width and height of the plate (number of wells) by typing in a number into the field or by using the up and down arrows. The plate will update in real time.
  - Width – the width determines the number of columns.
  - Height – the height determines the number of rows.
  - Fill Direction – the fill direction determines where samples will be automatically placed when either imported one after the other or when a selection is imported at once.
  - Horizontal – horizontal fill direction means that samples will continue to fill up an entire row before moving to the second. E.g. sample one will be placed in well A1 and sample two in A2, etc.
  - Vertical – vertical fill direction means that samples will continue to fill up an entire column before moving to the second. E.g. sample one will be placed in well A1 and sample two in B1, etc.
  - Well Shape – choose to display the wells as circles, squares, large squares or fill.



- Numeric Axis – choose to have either of the axes labeled numerically. If the numerical option is not selected, the wells will be labeled alphabetically. In most cases, it is best to have one axis labeled numerically and the other alphabetically.

- **Display Settings** – the display setting options allow the viewing of different levels of the hierarchy, depending on the type of statistic that is being viewed.
  - Non-Parametric Statistic Selected – these are the statistics that are not related to the parameters where the gates have been created, such as event count and % parent. In many instances, it may be desired to view the statistics in relation to the parent plot. That is, if a gate is drawn, viewing the gate on the parent plot rather than the resulting daughter plot provides more information. The three different options are:
    - Display Selected Level
    - Display Parent
    - Display File
  - Parametric Statistic Selected – these are the statistics that are related to the parameters where the gates have been created, such as the mean fluorescence. In many instances, it may be desired to view the statistics of the populations resulting from a gate. The three different options are:
    - Display Selected Level
    - Display Parent
    - Display File

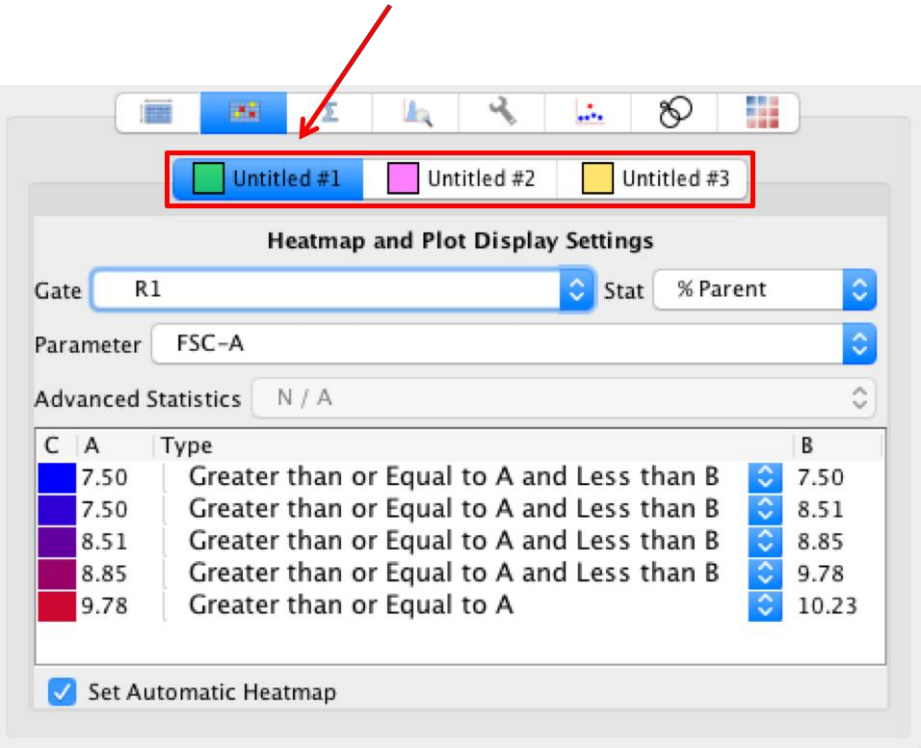
- Mirror datasets on Insert – this feature, when enabled, creates datasets in the plate that match groups from the File Inspector. The group color is applied to the matching dataset. Datasets are groups of wells/samples. Individual datasets can be modified without impacting other wells in the plate. For example, the same 5 files can be added to a plate three times, each in its own dataset. If there are a few gates associated with the files, then each dataset can be set to a different level of the hierarchy and/or statistic.



- Insert In Plate Order – samples are added to the plate in the order of A1, A2, A3, etc.
- Dataset Settings – the datasets can each be modified in terms of representative color and name. Comments or descriptions can also be added for each dataset.
  - C (color) – to change the dataset color, click on the color in the 'C' column and choose a new one from the options provided.
  - Name – double click on the dataset name to type in a new one.
  - Comment/Description – double click in the comment/description field to add a note relating to the dataset.
- Delete Dataset – to delete a dataset, highlight the dataset by clicking on its name and click Delete Dataset. This will delete the dataset but not the files in the wells.
- Synchronize Datasets – if FCS files are placed in groups in the File Inspector after they have been added to the plate, synchronizing the samples will create datasets based on the groups in the File Inspector. The result is the same as with mirrored datasets.

- **Heatmap Settings** – the heatmaps generated in the plate can be set up in the heatmap settings tab. Heatmaps are modified for each dataset individually.

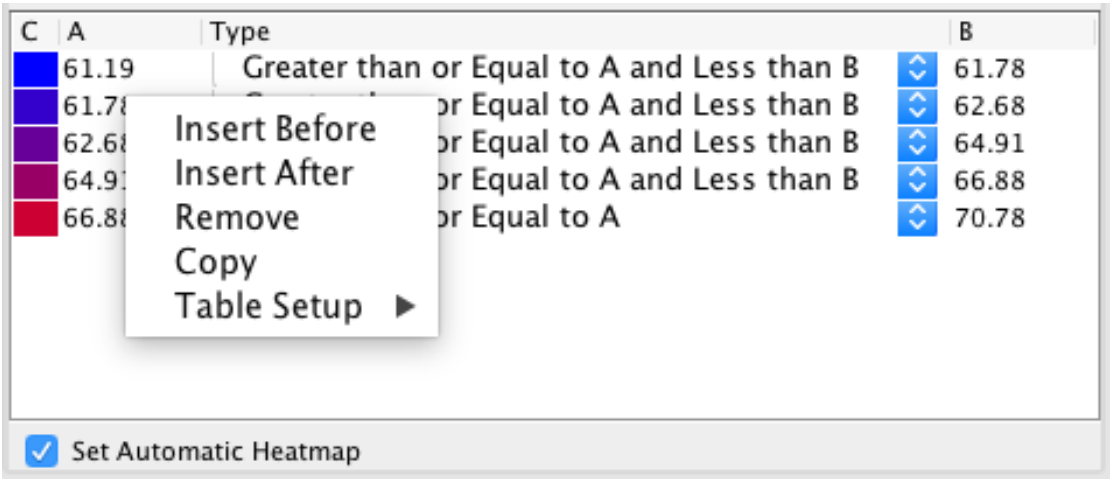
- Dataset name – defined datasets are listed at the top of the Heatmap Settings tab in the side drawer. To edit the heatmap settings for a dataset, click on the dataset name prior to changing the settings.



- Heatmap and Plot Display Settings
  - Gate – choose the population that you wish the data array feature to take the settings from to create the heatmap. Click on the Gate drop down menu to select the population by gate name.
  - Stat – click the statistic drop down menu to choose the statistic that you wish the heatmap to be generated for. The statistics available are: Mean, GeoMean, StdDev, CoefVar, RCoefVar, %Total, %Parent, Event Count, %Selected, RStdDev, Cells/ml, Cells/µ.
  - Parameter – for statistics that refer to a particular parameter, such as the mean, choose the parameter from the parameter drop down menu.
  - Advanced Statistics
    - C – the color for each range in the heatmap can be changed by clicking on the color in the in the column titled ‘C’.
    - A (bottom of range) – this column shows the value defining the bottom end of the range and can be changed by double clicking within the filed in the column labeled ‘A’ and typing a new one.
    - Type – this is the rule defining each particular range in the heatmap, in relation to the values in columns ‘A’ and ‘B’. The type options are:

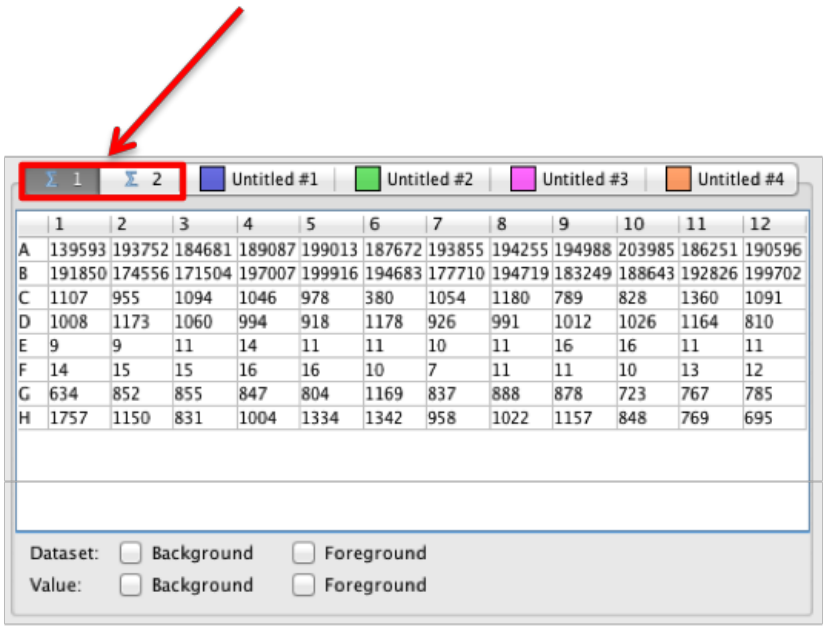
- Less than A
- Less than or Equal to A
- Greater than or Equal to A
- Equal to A
- Greater than or Equal to A and Less than B
- Not Greater than or Equal to A and Less than B
- B (top of range) – this column shows the value defining the top end of the range. The value can be changed by double clicking within the filed in the column labeled ‘B’ and typing a new one.

- Right click on a heatmap range to open a window with options to add a new range by inserting it before the selected range, after a selected range, delete a range or copy a range. Selecting copy range will add a copy of the selected range immediately after the copied range. If Set Automatic Heatmap is enabled, the range associated with the new copy will be recalculated. If Set Automatic Heatmap is not enabled, the range associated with the new copy will be the same as the range that it was copied from. To apply changes made to the heatmap settings for one dataset to another, click Apply to Dataset and select the dataset from the options. Click Table Setup to change the font and number of decimal places displayed in the heatmap setting table.
-



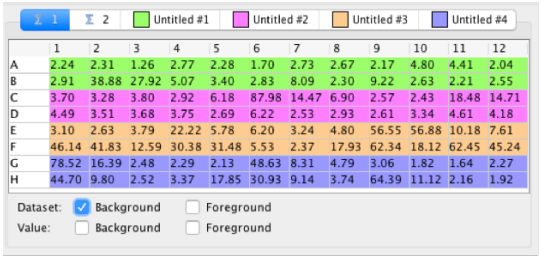
- Set Automatic Heatmap – when this option is selected, a heatmap will automatically be generate when samples are imported into a plate. The values for columns A and B cannot be edited if Set Automatic Heatmap is enabled.

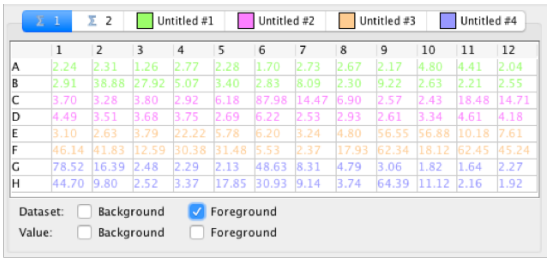
- **Statistics** – the statistics tab displays statistics that have been created in the Heatmap Settings tab. There are two table formats:
  - Statistic Table 1: table matches that in the plate setup tab.

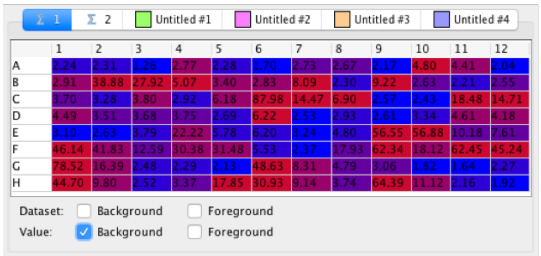


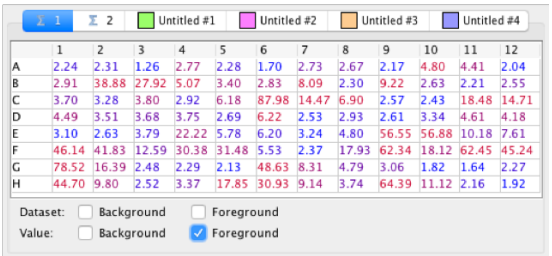
	1	2	3	4	5	6	7	8	9	10	11	12
A	139593	193752	184681	189087	199013	187672	193855	194255	194988	203985	186251	190596
B	191850	174556	171504	197007	199916	194683	177710	194719	183249	188643	192826	199702
C	1107	955	1094	1046	978	380	1054	1180	789	828	1360	1091
D	1008	1173	1060	994	918	1178	926	991	1012	1026	1164	810
E	9	9	11	14	11	11	10	11	16	16	11	11
F	14	15	15	16	16	10	7	11	11	10	13	12
G	634	852	855	847	804	1169	837	888	878	723	767	785
H	1757	1150	831	1004	1334	1342	958	1022	1157	848	769	695

  - Options at the bottom of the window allow the statistics to be colored based on the dataset or on their value where the colors match those from the heatmap. Both color options can be applied to the background (coloring the cell, not the text), the foreground (coloring the text, not the cell) or the background for one and the foreground for the other.



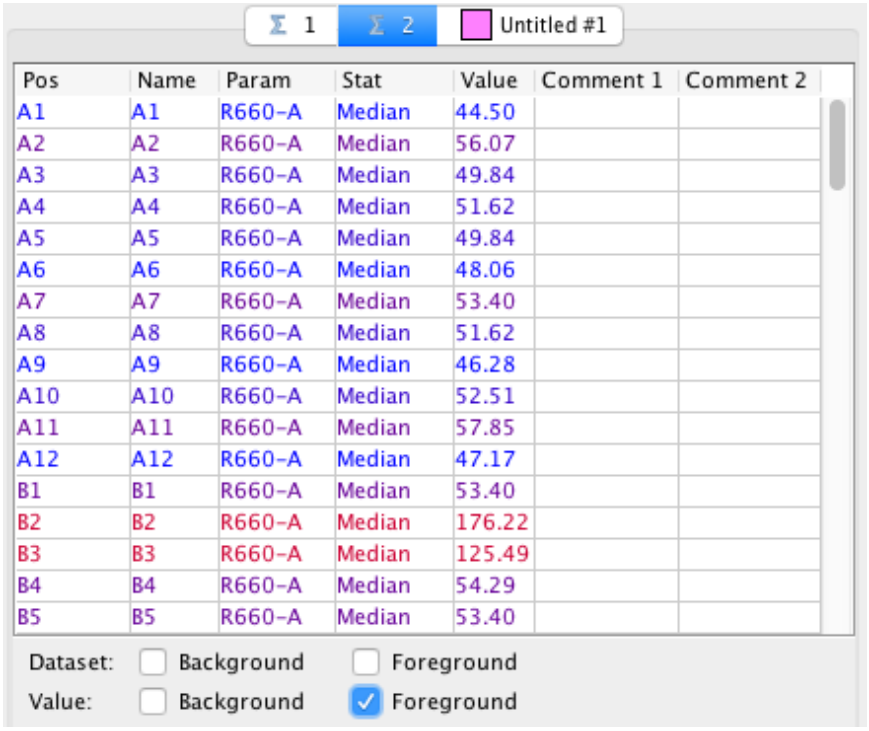




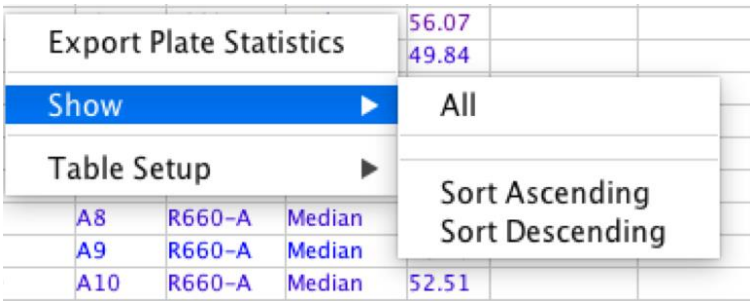


  - Right click on the table opens a window allowing the statistics to be saved as a CSV file.
  - Selecting Table Setup allows for the font and number of decimal places to be changed.

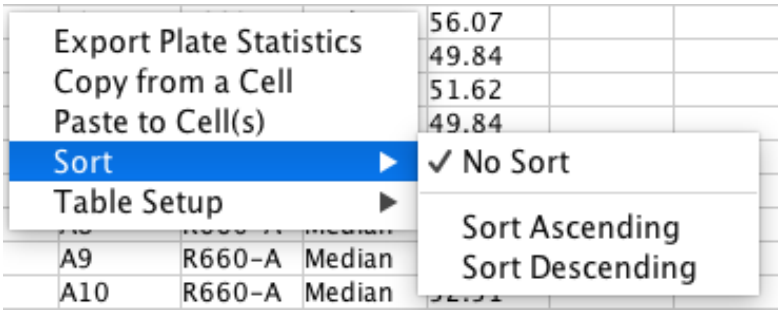
- Statistic Table 2: the data is set out in columns. The columns in Statistic Table 2 are Pos (plate position), Name, Param (parameter), Stat (statistic), Value, Comment 1 and Comment 2. Double click in the Comments columns to make notes related to specific samples.
- The same dataset and coloring options are available for Statistic Table 2 as described for Statistic Table 1.



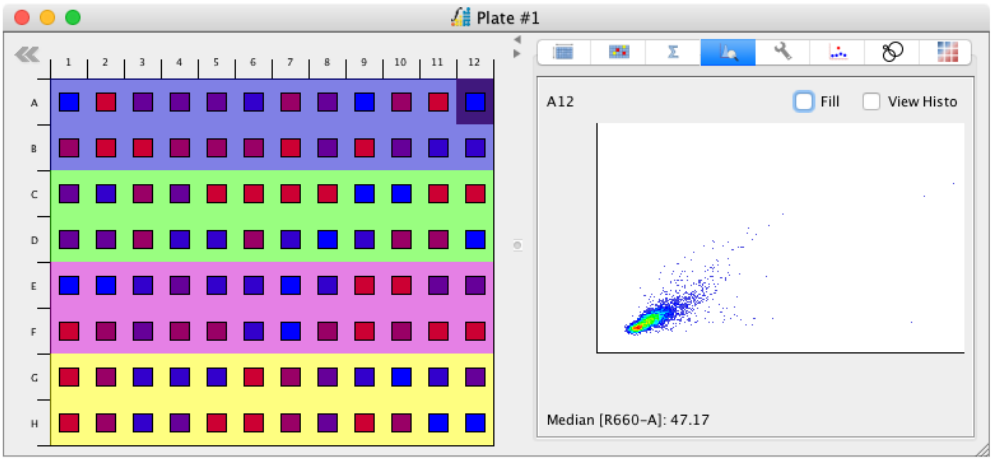
- Clicking on the dataset names above the table will display the results for the individual datasets in the same format as Statistic Table 2.
- The results in Statistic Table 2 can also be set to show an individual dataset by right clicking on the table, selecting Show and selecting the desired dataset. Right clicking, selecting Show and All will display all of the datasets.
- The results in Statistics Table 2 can also be sorted into ascending or descending numerical order by right clicking in the table, selecting Show and Sort Ascending or Sort Descending. Selecting No Sort (when a table has been sorted) will return the results back the sample order.

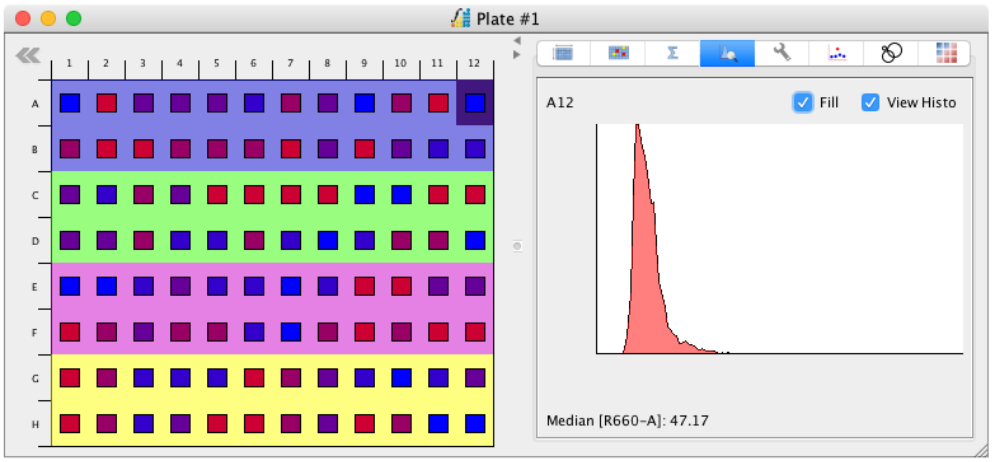


- Right click in the dataset table to:
  - Export Plate Statistics - opens a window allowing the statistics to be saved as a .csv file.
  - Copy from a Cell
  - Paste to Cell(s)
  - Sort: No Sort, Sort Ascending, Sort Descending
  - Table Setup: Change Font, choose number of decimal places



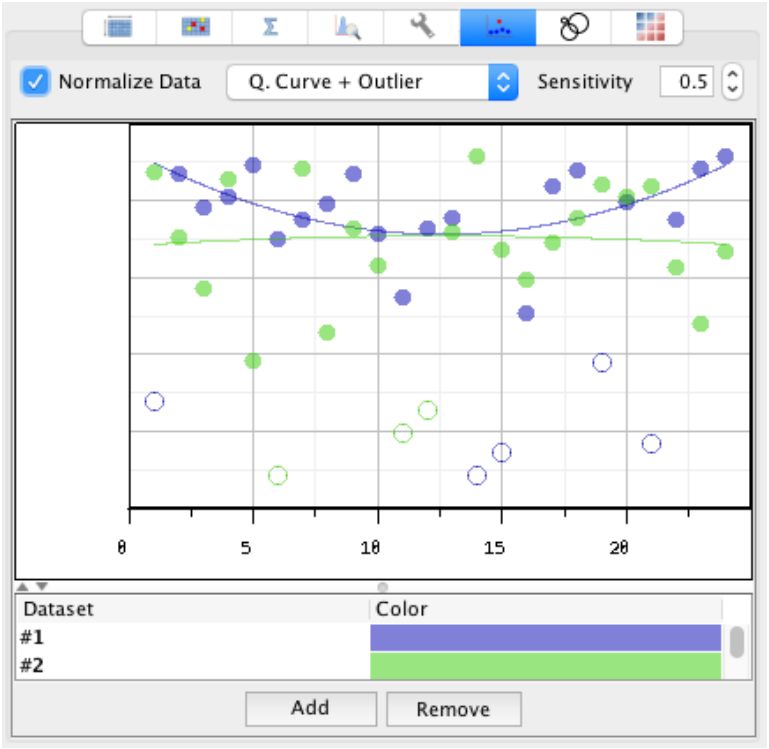
- **Plot Viewer** – the Plot Viewer tab allows the histogram or dot plot for each individual well to be inspected. Simply hover over the well in the plate to view the dot plot in the side drawer. The population displayed is determined by the setting in the Plate Setup tab under the Display Settings. The parameter to be displayed is determined by the settings in the Heatmap Settings tab.
  - Right Click: Remove Control – samples can be set as controls for data arrays by right clicking on the samples in the File Inspector and choosing Set as control. This will overlay the control in the plate when viewing the plots in the Plot Viewer tab in the plate side drawer. Right click → Remove Control removes control from the Plot Viewer tab when inspecting the wells.
  - View Histo – with this option selected, hovering over the wells will display a histogram.
  - Fill – with this option selected, a displayed histogram will be filled.
  - The dot plot display option will mirror what is set when the dot plot is open in the Workspace. To change this, double click on the well to open the plot in the Workspace. Then, choose from a new plot display option from the Toolbar. The dot plot in the plate side drawer will be updated to reflect the selection.

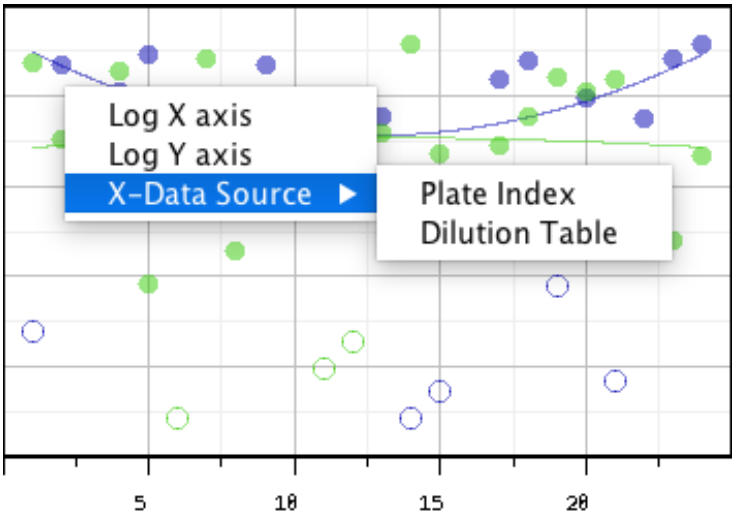




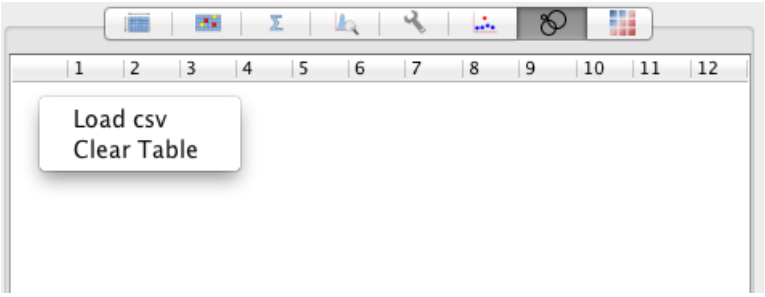
- **Advanced Functions** – the Advanced Functions tab allows for comparisons between different parameters, gates and datasets. Choose an option to compare, select the two statistics and choose the action to be performed. The row should read like an equation, e.g. *Gate 1 – Gate 2*. When the equation is set, select the input dataset from the Ratio Input window and click Apply. This will create a new dataset with the result. The new data set will also be displayed in the plate itself.

- **Plate Outlier Detection**
  - Data can be viewed in relation to a quadratic curve (Q. Curve), linear line or an asymmetrical 5PL curve, with outliers represented by a hollow circle. Double click on a circle to open the dot plot in the Workspace.
  - If multiple datasets are displayed on the same graph, normalize the data to so that they can all be viewed.
  - Pull up the drawer at the bottom of the graph for options to Add or Remove datasets.

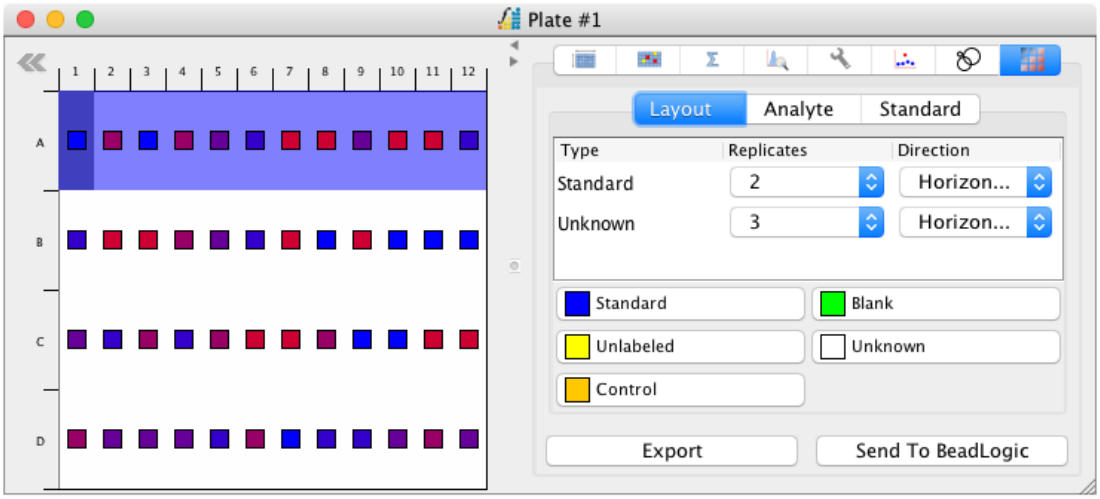




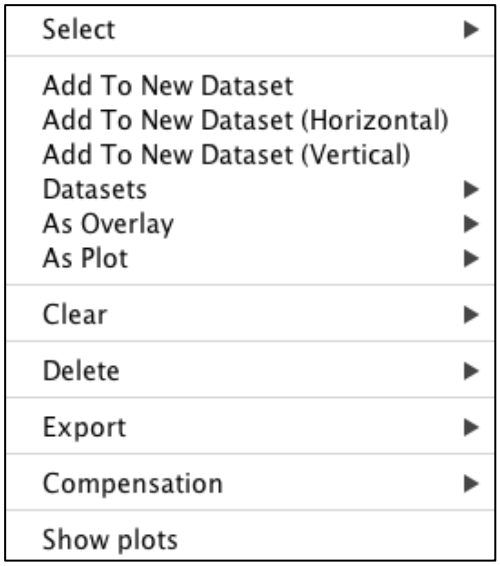
- **Dilution Table** – a dilution table saved as a .csv can be imported by right clicking in the side drawer under the dilution table tab and selecting 'Load csv'.



- **BeadLogic Export** – wells can be defined as Standards, Blanks, Unlabeled, Unknowns and Controls before exporting to Beadlogic for multiplex analysis.



- **Plate options** – right click on a well



- **Select** → allows the quick selection of groups of wells in a plate:
    - All – right click anywhere in a plate and choose 'Select' → 'All' to highlight all wells.
    - Extend Horizontally – highlight a well from one or more rows, right click and choose 'Select' → 'Extend Horizontally' to select all wells in the highlighted rows.
    - Extend Vertically – highlight a well from one or more columns, right click and choose 'Select' → 'Extend Vertically' to select all wells in the highlighted columns.
    - Dataset → to select all samples from a particular dataset, right click anywhere in the plate and choose 'Select' → 'Dataset' → and choose the dataset by name.
    - Inverse – an inverse selection is useful if you wish to exclude a relatively small number of wells. To make an inverse selection, highlight the wells to be excluded (using the options described above or by selecting them with the mouse), then choose 'Select' → 'Inverse'.
    - None – to clear all selections, right click anywhere in the plate and choose 'Select' → 'None'.

- **Datasets** – individual datasets can be manipulated separately, allowing the same group of samples to be imported multiple times into a single plate but analysed independently (e.g. different gates or stats of the same files in the one plate):
    - Add to new dataset – adds the selections to a new dataset.
    - Add to new dataset (horizontally) – creates a new dataset for each row in the plate.

    - Add to new dataset (vertically) – creates a new dataset for each column in the plate.
    - Dataset (set/delete) – deletes the dataset but samples remain in the plate.

- **As Overlay** will create overlays based on the specific selection of wells:
    - Selection – creates a dot plot overlay of the samples highlighted in the plate.
    - Selection (Horizontal) –creates an overlay containing all samples that exist in the same row as the selected well.
    - Selection (Vertical) – creates an overlay containing all samples that exist in the same column as the selected well.
    - Dataset → creates an overlay containing all samples that exist in the same dataset as the selected well.

- **As Plot** opens a dataset or selection of files as dot plots in the Workspace, either from the selection of individual wells or datasets.

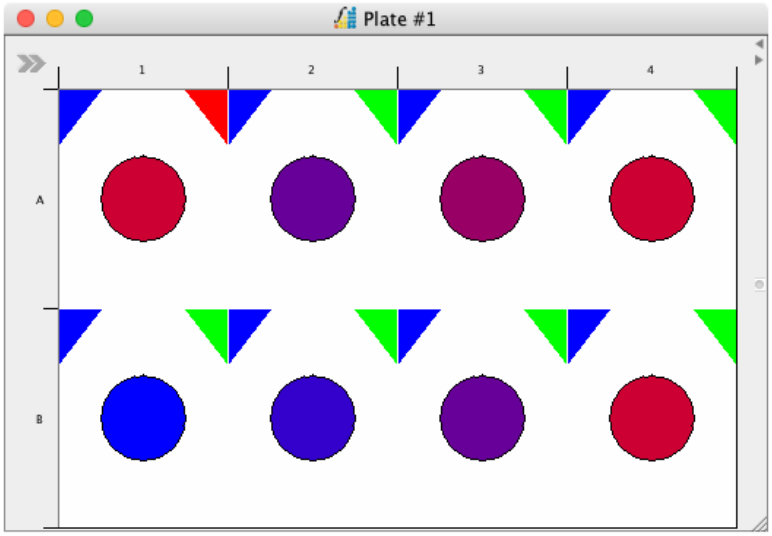
- **Clear** will clear all files from within selected wells. This does not delete the dataset:
    - All – deletes all samples from the plate
    - Selection – deletes the selected samples from the plate
    - Dataset → deletes the samples contained within a dataset.

- **Delete** will delete an entire row or column from the plate.
    - Selected Row(s) – deletes the selected row or rows and all data contained within them.
    - Selected Column(s) – deletes the selected column or columns and all data contained within them.

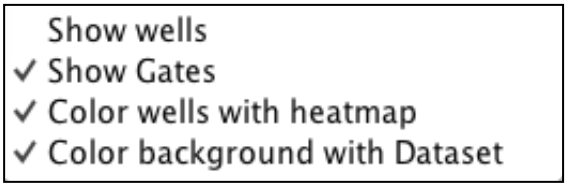
- **Export** a plate by either saving it as a file or copying it to the computer's clipboard.
    - To File – opens a window allowing the plate to be saved as one of a number of image file types.
    - To Clipboard – copies the image of the plate to the computer's clipboard to be pasted elsewhere.

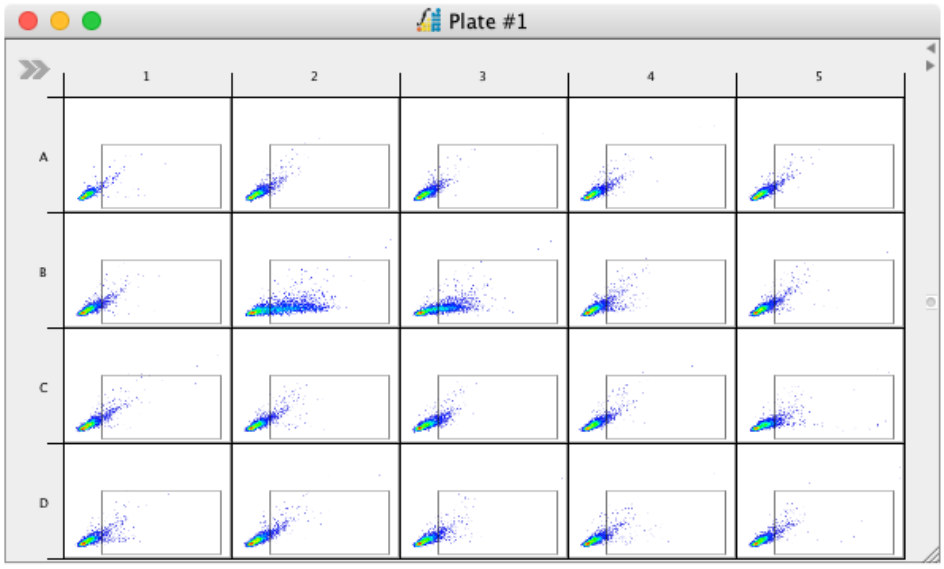
- **Compensation** displays differences in compensation matrices. The top corners of each well are colored. The left colored corner represents the original compensation matrix. If this is the same in multiple wells then they all have the same original matrix. The right corner represents a post-acquisition change in compensation. If the two corners of a well are different it indicates that the compensation has been changed. Compensation matrices can be copied from one file in the plate and pasted onto another.

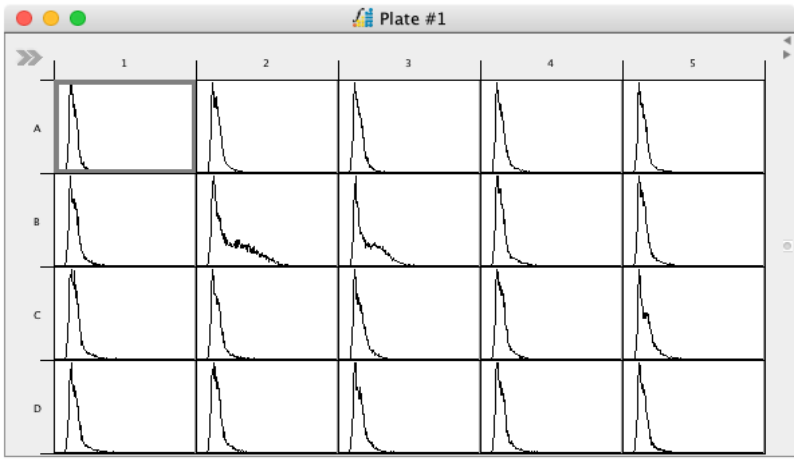
- Show Comp Matrix – colors the corners to depict the applied compensation
- Copy – copies the compensation matrix from a selected well/sample.
- Paste → All/Selection – applies the copied compensation matrix to a selection of samples or to all within a dataset.

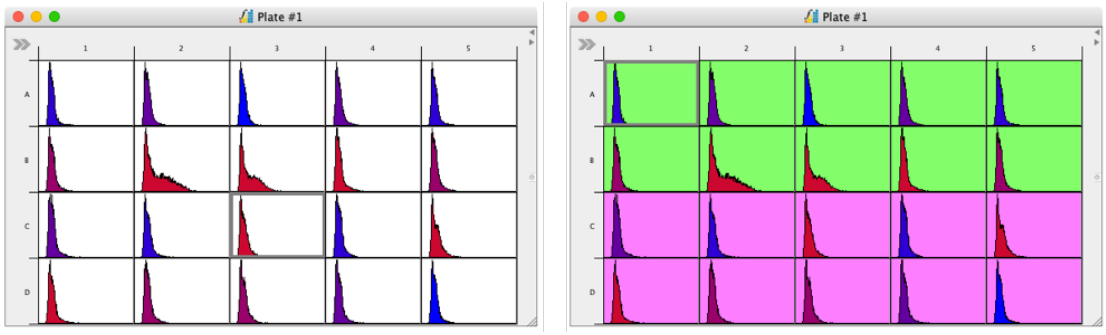


- **Show plots** displays each file as a dot plot. There are additional options to color the dots to match the heatmap colors, to color the background to reflect the different datasets and a combination of both.









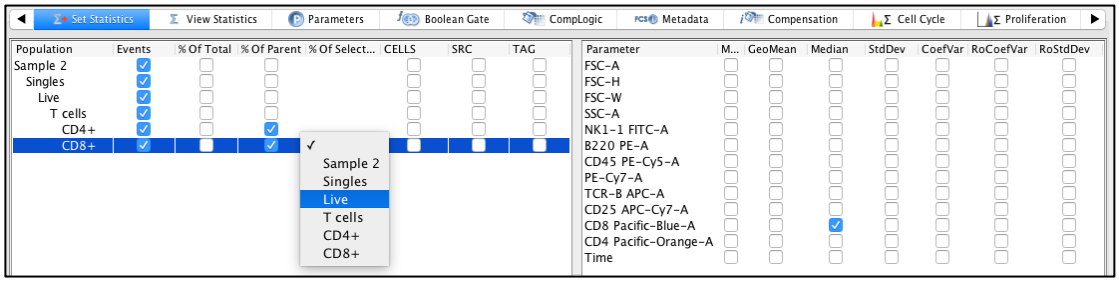
Building Plates from the File Navigator
Plates (data arrays) can be created directly from the File Navigator for all files contained in an experiment folder. This is particularly useful if you wish to display each gated population, as depicted in the population hierarchy) as a separate dataset. This enables all plots in the experiment to be displayed in the plate view.

Set Statistics

The set statistics tab is in the Advanced Functions is where the type of statistics to be calculated is set to display in the View Statistics tab.

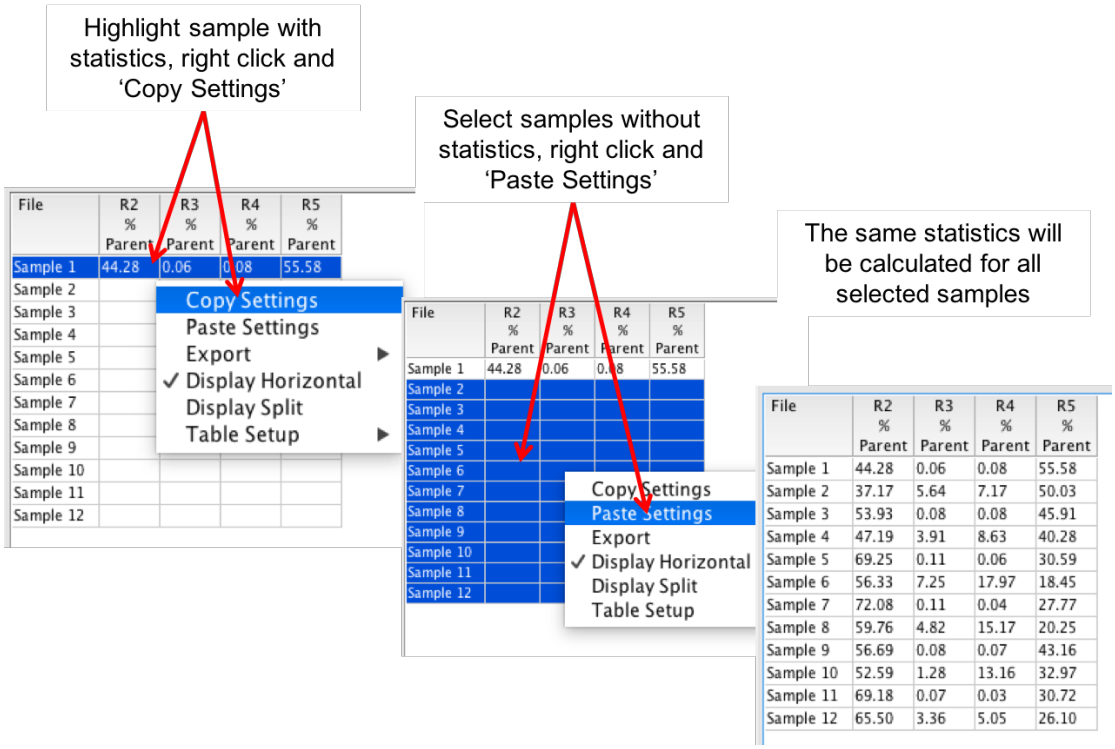
- Highlight the files in the File Inspector for which to calculate statistics. If files are contained in a group, selecting one of the grouped files will result in statistics being calculated for all files in the group.
- Open the Advanced Functions drawer and tick the boxes that correspond to the populations/gates. The statistics are separated into two windows. The left-hand window displays statistics that are linked to the selected files in the File Inspector. The right-hand window lists parameter-specific statistics that are linked to populations highlighted in the Populations column in the left-hand window. In the example that follows, the mean fluorescence will be calculated for the Pacific-Blue-A and Pacific-Orange-A parameters on the CD8+ population only. If multiple populations are highlighted in the Populations column prior to selecting a statistic in the right-hand window, then the parameter-specific statistic will be calculated for all selected populations.

- Population statistics include Number of Events, % Of Total, % Of Parent, % Of Selected, along with data contained in the keywords: CELLS, SRC and TAGs.
- % Of Selected means that a specific population can be shown as a percentage of any other population higher in the hierarchy, not just the parent (one level higher in the hierarchy) or total (the top level in the hierarchy). To define the selected population, click in the column in the row that corresponds with the gate of interest. Then, from the drop-down menu, choose the selected population.
- The parameter statistics include the mean fluorescence (Mean), geometric mean fluorescence (GeoMean), median fluorescence (Median), standard deviation (StdDev), coefficient of variation (CoefVar), robust coefficient of variation (RoCoefVar) and robust standard deviation (RoStdDev).
- Double clicking on the column title will tick all boxes in that column.
    - The columns in both windows can be reordered by dragging the header
- Double clicking on the parameter title in the right-hand window will tick all statistics for that parameter.
- To view the calculated statistics, click on the View Statistics tab in the Advanced Functions drawer.



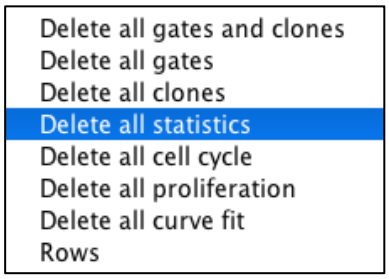
View Statistics

- Once statistics have been set in the Set Statistics tab, click on the View Statistics tab to display the statistics tables.
- Right click on the table to copy, export or change the table setup.
    - Within the View Statistics table, the settings for specific statistics can be copied and pasted from one file to another (or multiple). For example, if statistics for one file have been calculated, highlight that file in the table, right click → Copy Statistic Settings, highlight remaining files in the View Statistics table, right click → Paste Statistics Settings. All statistics calculated for the first file will then be calculated for all remaining files.



File	R2 % Parent	R3 % Parent	R4 % Parent	R5 % Parent
Sample 1	44.28	0.06	0.08	55.58
Sample 2	37.17	5.64	7.17	50.03
Sample 3	53.93	0.08	0.08	45.91
Sample 4	47.19	3.91	8.63	40.28
Sample 5	69.25	0.11	0.06	30.59
Sample 6	56.33	7.25	17.97	18.45
Sample 7	72.08	0.11	0.04	27.77
Sample 8	59.76	4.82	15.17	20.25
Sample 9	56.69	0.08	0.07	43.16
Sample 10	52.59	1.28	13.16	32.97
Sample 11	69.18	0.07	0.03	30.72
Sample 12	65.50	3.36	5.05	26.10

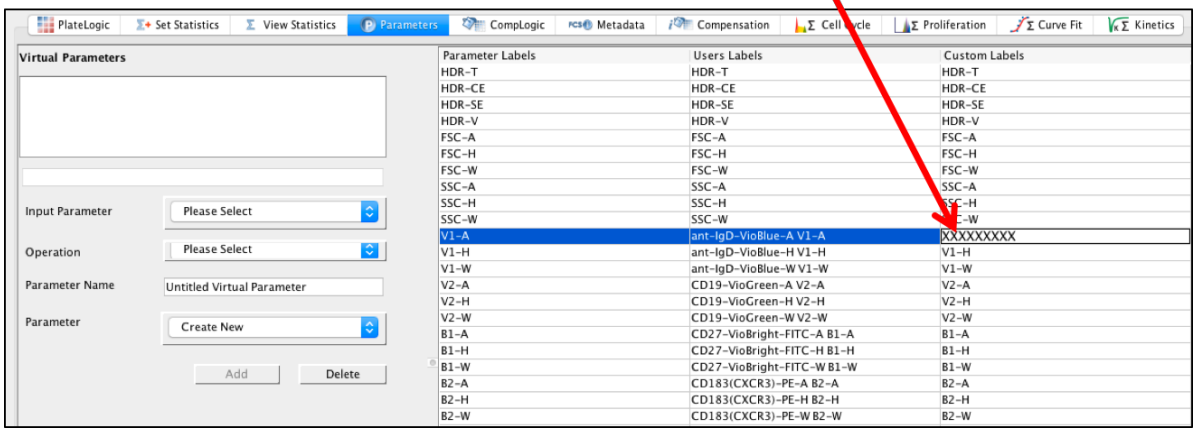
- To clear the statistics, right click in the File Inspector and choose Delete → Delete all statistics.



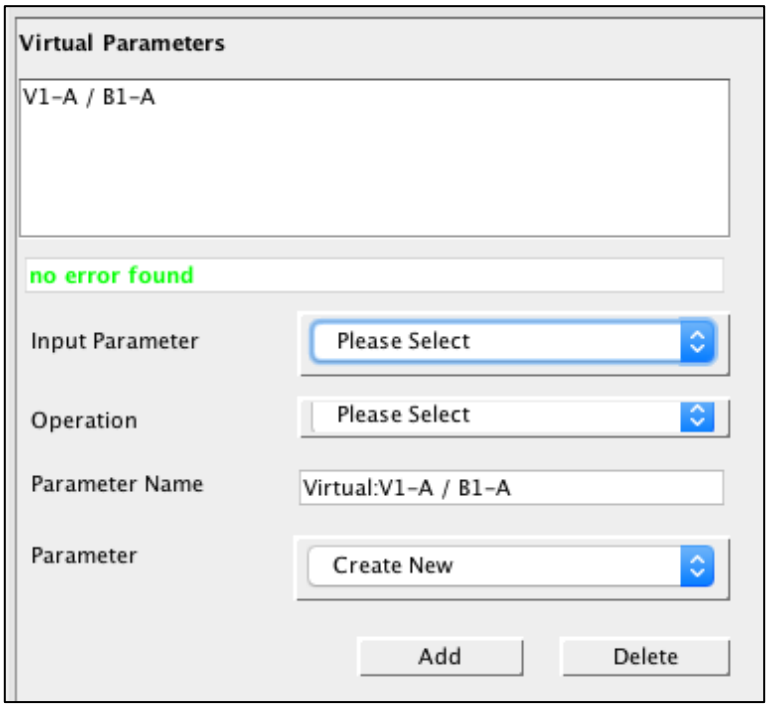
Parameters

- All parameters are listed in the Parameters tab.
- Custom Labels can be changed by double clicking in the field and typing the new name. Display the custom labels on plots by selecting to display Custom Labels in the program preferences.

Double click to rename and choose to display Custom Labels in the program Preferences



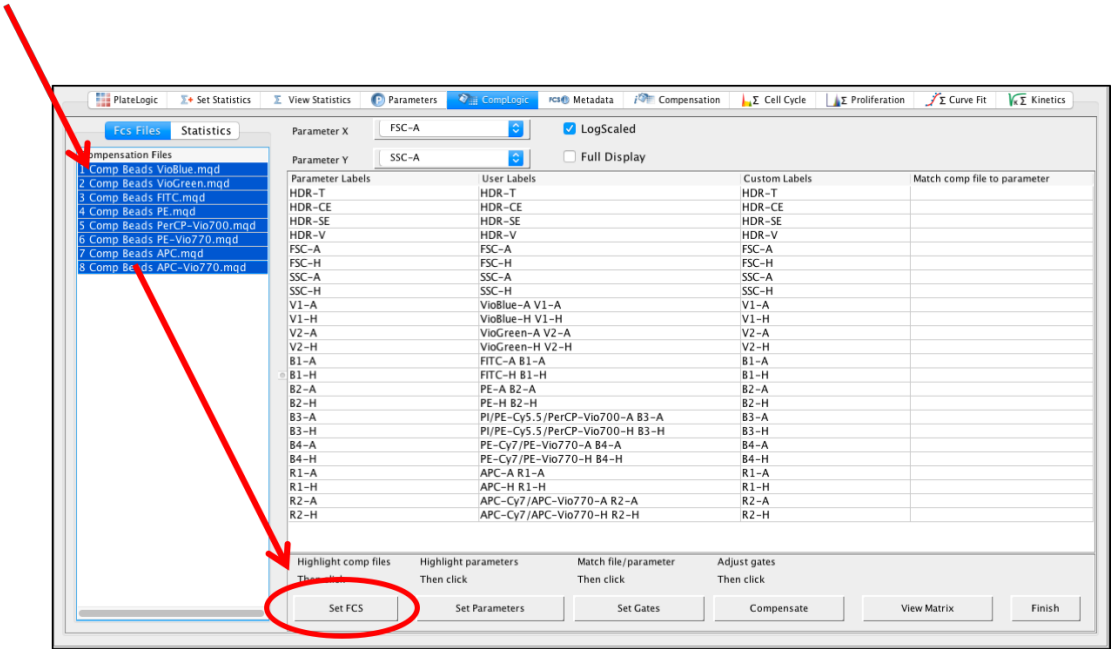
Virtual parameters can be created by selecting an input parameter followed by an operation and another input parameter or value. Click ‘Add’ to create the new parameter. To delete a virtual parameter, select the parameter in the ‘Parameter’ drop-down list and click ‘Delete’.



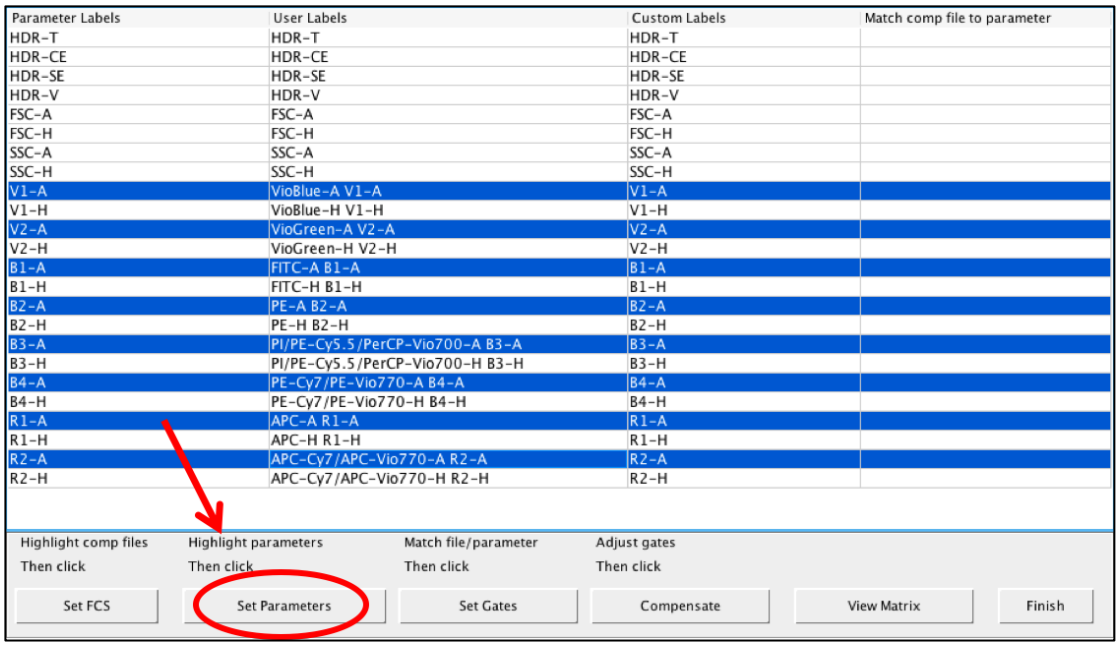
CompLogic – Auto-compensation

FlowLogic can perform auto-compensation given appropriate single-color controls.

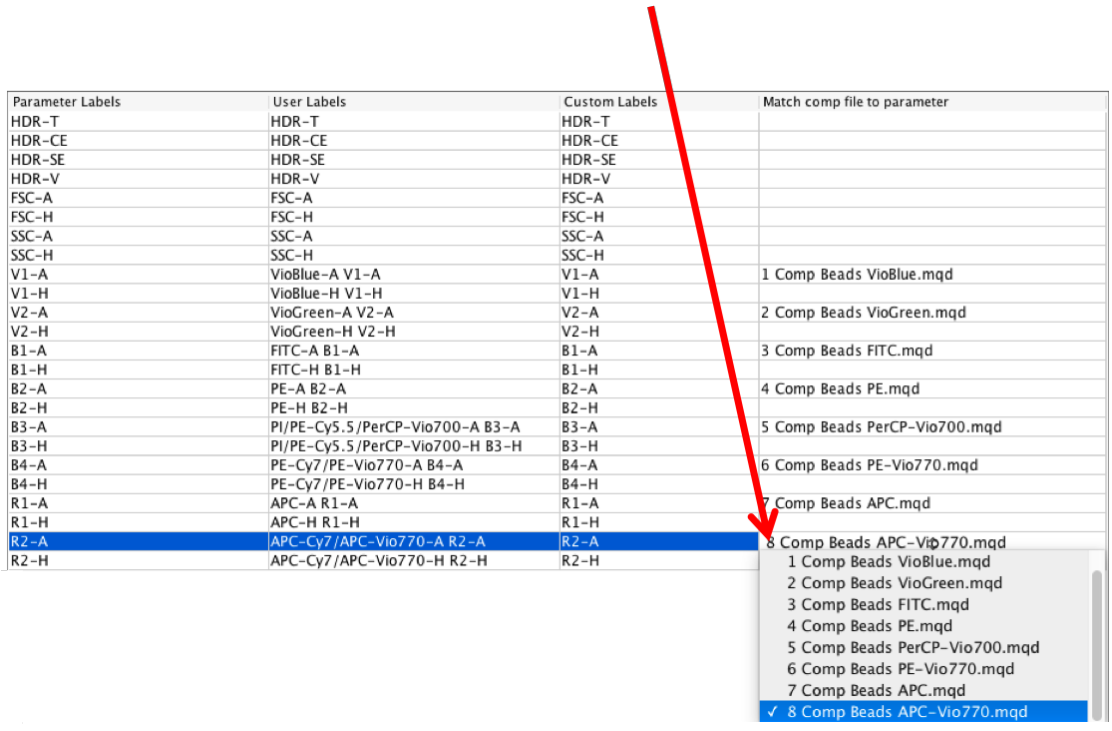
- From the Advanced Functions drawer, select the CompLogic tab. From the samples list on the left of the drawer, select your single-color controls then click ‘Set FCS’.
- In the parameters box highlight the parameters that you want to compensate and click ‘Set Parameters’. The boxes in the ‘Auto’ column will be ticked.



- In the parameters box highlight the parameters that you want to compensate and click ‘Set Parameters’. The boxes in the Auto column will be ticked.

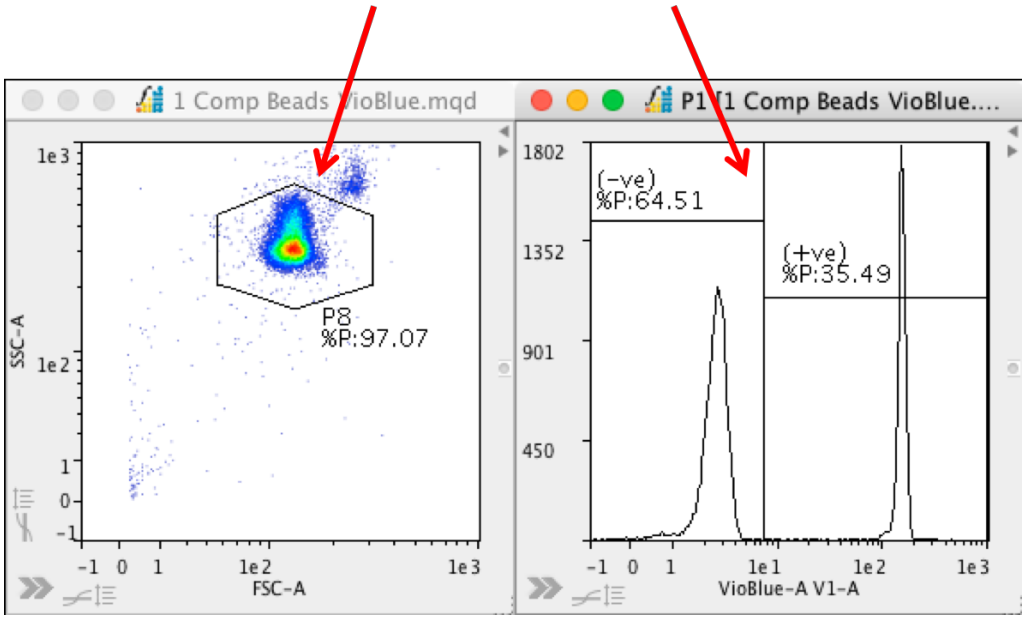


- Verify that the suggested file listed in the FCS Files column matches the parameter as shown in the Parameters column. If not, click on the FCS file title and choose the matching FCS file from the available options.



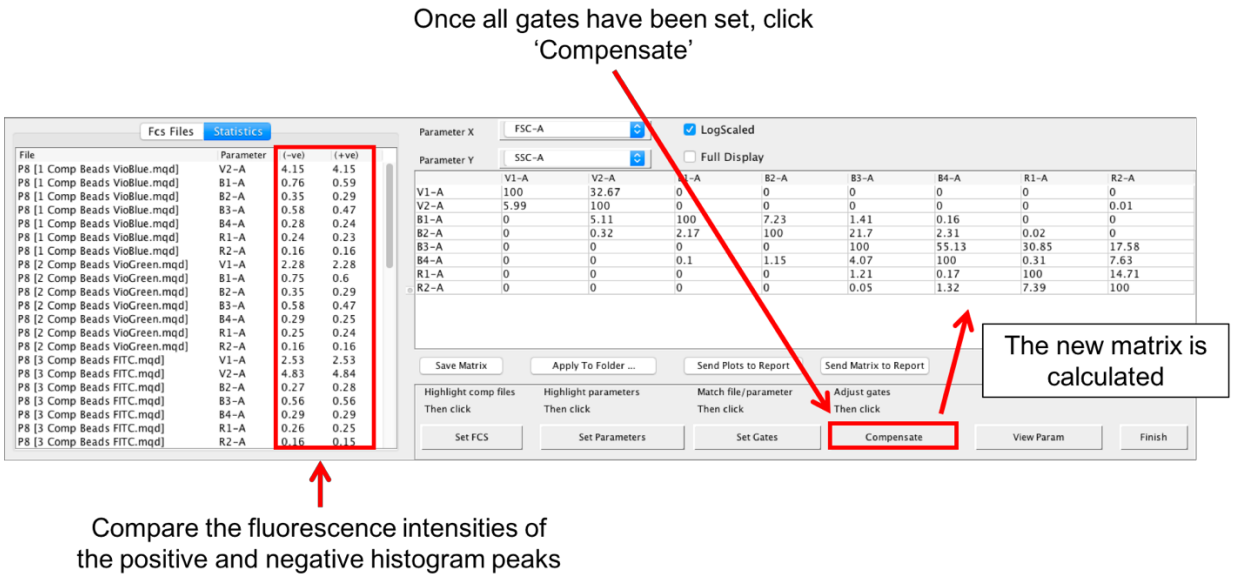
- When you have matched the FCS files and parameters click ‘Set Gates’. FlowLogic will create a FSC-A vs. SSC-A plot with a polygon gate and the daughter population histogram for each parameter. The polygon gate itself, will automatically detect the population of greatest density. Adjust the polygon and histogram markers to select the negative and positive populations.

- To make viewing easier, change the number of windows displayed per row to two. Click Edit → Plot Arrangement → Number of Windows → 2.



- When all gates have been corrected, click Compensate. FlowLogic will then calculate the compensation matrix.

- The newly calculated compensation matrix will be displayed. You can now save the compensation matrix or apply it to all samples within a folder with one click.

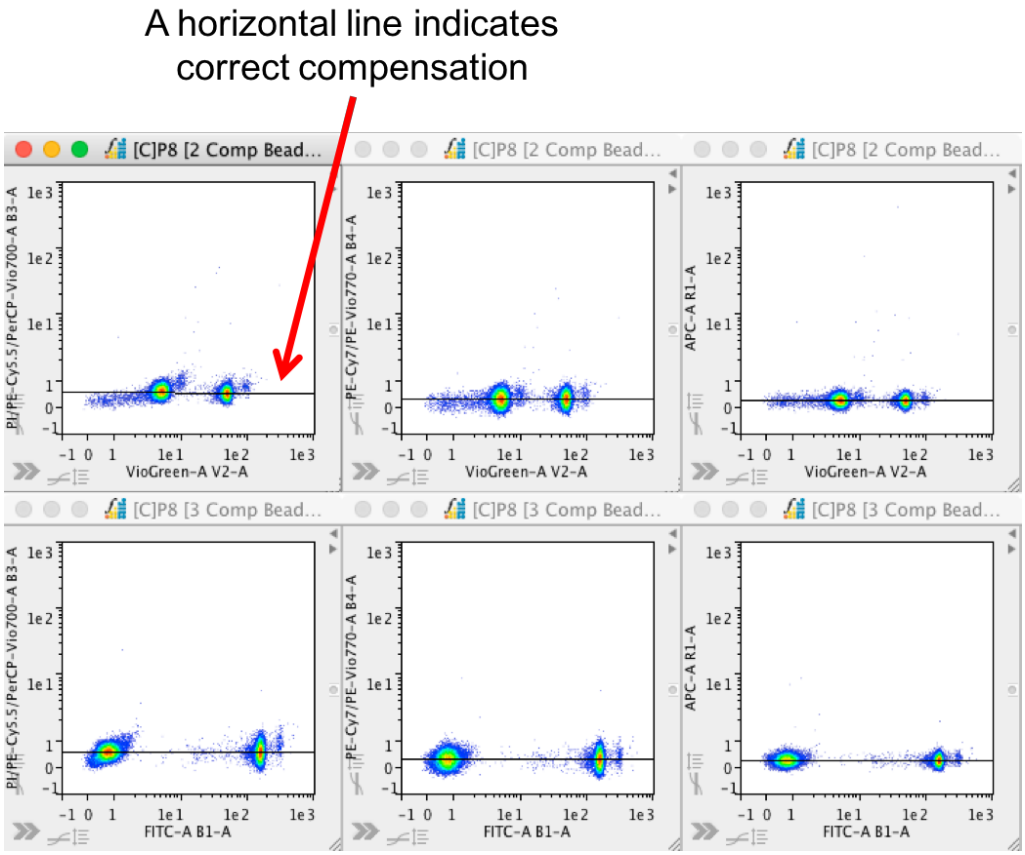


- The MFI is calculated for the positive and negative populations in all spillover channels. A line is subsequently drawn connecting these two values. A horizontal line indicates correct compensation.

- The original compensation created at acquisition remains saved with the file and can be re-applied at any time.

- Auto-compensation can be performed as many times as you wish.

- The compensation for each individual file can be checked by opening the appropriate dot plots. Fine adjustments can be made in the plot side drawer and the new matrix saved and applied to all other files.

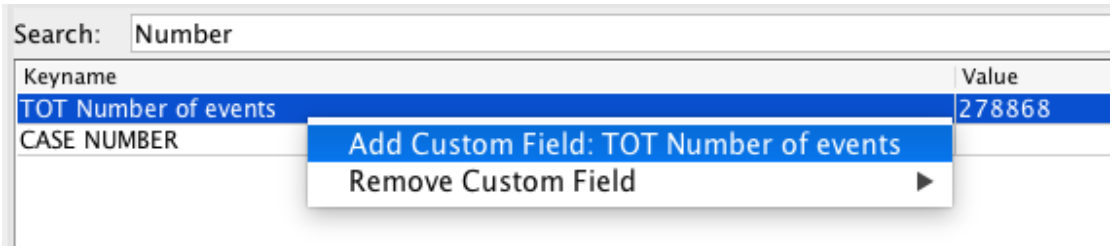


In the CompLogic window, tick ‘Full Display’ to view all events on each plot. Unticking ‘Logscaled’ will revert the axis to a logarithmic scale for all plots.

Metadata

Each FCS file has associated metadata that can be viewed in this tab.

- A specific metadata keyword and its associated value can be added to the File Inspector table for each sample. To add the keyword to the File Inspector, right click on the specific row and choose 'Add Custom Field'.
- To remove a keyword from the File Inspector, right-click in the Metadata table and select 'Remove Custom Field →'.
- Use the Search field at the top of the pull up drawer to search for specific items within the metadata.



Compensation

The compensation matrix for each FCS/MQD file can be viewed in this tab.

- To view a compensation matrix, highlight the FCS/MQD file in the File Inspector. The matrix will automatically be displayed.
- Right clicking on the matrix will provide an option to export the matrix as a TXT file.

Cell Cycle, Proliferation, Curve Fit and Kinetics Tabs

These tabs provide statistical summary tables from the Cell Cycle, Proliferation, Curve Fit and Kinetics features. Statistics can be exported from the tables by right clicking within the table and choosing the export option. This data can also be imported directly into GraphLogic by right clicking in the Workspace and choosing to add the table related to the specific analysis type.