Beadlogic™

Multiplex Analysis Software

Developed by scientists, for scientists

User Manual

inivai™ TECHNOLOGIES

Table of Contents

Introduction

File Formats

Bead**Logic** is a multiplex data analysis program. A range of third-party data files, in addition to BeadLogic Bead Export data, can be imported directly into Bead**Logic**. These include:

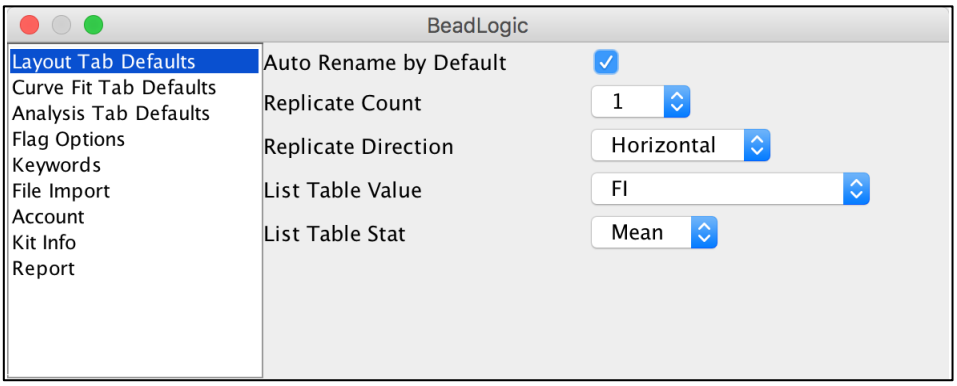
- Bioplex BPD
- Bioplex XML
- CSV (Luminex, etc.)
- BeadLogic Bead Export

Preferences

Before beginning an analysis, customize the program by setting the program preferences. From the menu bar, select 'BeadLogic' (Mac OSX, ⌘) or 'Edit' (Windows) and 'Preferences'. Select various Preference menu options from the left hand side of the Preferences window and optimize the settings using the options in the window on the right.

Explanations of the different Preference options are as follow:

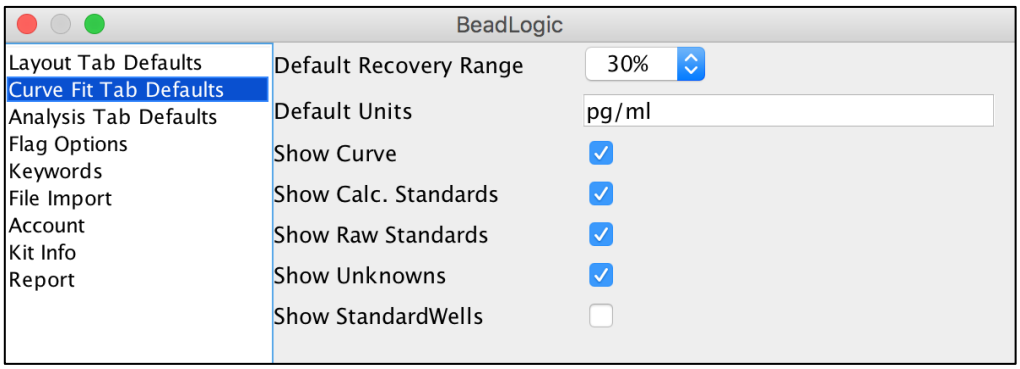
Layout Tab Defaults



- Auto Rename by Default – the auto-rename option forms part of the Sample Configuration panel, which is used to define the different types of samples in the plate (Standard, Blank, Unknown, Unlabeled and Control). When samples are defined as being a Standard, Blank, Unknown, Unlabeled or Control, a matching color is assigned and displayed in the Plate Layout. If the auto-rename option is selected, the corresponding name, e.g. "Standard" will also be added to the well. If the Auto-Number option in the Replicates panel is also selected, each sample name will also be numbered, e.g. "Standard1". This Auto-Rename option can be turned on by default by selecting the options in the Preferences.
- Replicate Count – set a default number of replicate wells used for each analyte (1-12).
- Replicate Direction – set the default direction that replicate wells are displayed in the plate.

    - Horizontal: replicates are placed to the right (or on the next row if at the end of a row).
    - Vertical: replicates are placed beneath (or in the next column if at the end of a column).
    - Natural: wells fill left to right until the defined number of replicates is reached. The next series of replicates begins in the row below, even if the entire row has not been filled. After filling all of the columns, the next sample will be added to the top row in the first available well and continue to fill left to right.
- List (Data) Table Value – set the default statistic to be displayed for each analyte in the table located under the List (Data) tab of the Layout section. The options are:
    - FI: Median fluorescent intensity
    - Observed: the concentration of an analyte derived from the interpolation of the regression curve
    - Observed (Limit): the concentration of an analyte derived from the interpolation of the regression curve but with concentrations existing above or below the top and bottom standards given the same value as that standard (bottom standard for low values and top standard for high values).
    - Observed (Removed): the concentration of an analyte derived from the interpolation of the regression curve but with concentrations existing above or below the top and bottom standards replaced with “OOR>” or “OOR<” (outside of range).
    - Bead Count – the bead count is the event count for the particular gated population
- List Table Stat – set default statistics for when replicates exist. A choice between a number of statistics derived from the replicate values can be displayed. These are:
    - Mean – the mean of the replicate values
    - CV% – the coefficient of variation (displayed as a percentage)
    - SD – the standard deviation
    - Min – the smallest value of all replicates
    - Max – the largest value of all replicates
    - Sum – the sum of all replicate values

Curve Fit Tab Defaults

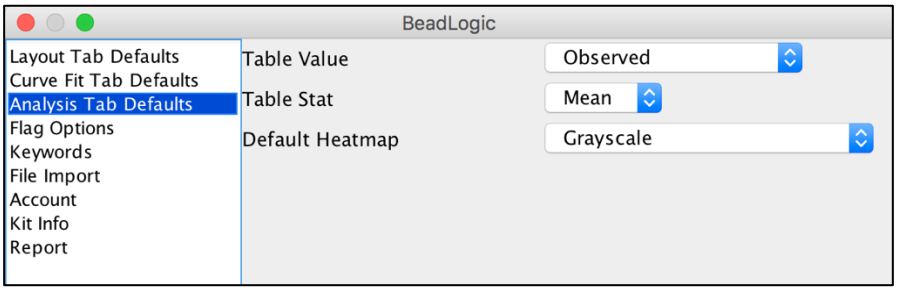


- Default Recovery Range: (10%, 20%, 30%, 40%, 50%, 60% or 70%) – when fitting the curve to the standards, the default acceptable recovery range, which is the error or the difference between the Observed and the Expected values as a percentage of the Expected value, can be set. Standards that are within the acceptable recovery range will be colored green on the regression tornado graph Standards that are outside of the acceptable recovery range will be flagged as a red bar on the same graph.
- Default Units: e.g. pg/ml – the default units for the concentration, which are displayed on the regression curve, can be defined for all new data sets. This can be changed for each analyte in the Regression Table window.

Other regression curve display options can be set by default and shown/hidden by the right-click menu in the regression curve window. These include:

- Show Curve – the curve itself
- Show Calc. Standards – the observed concentration values
- Show Raw Standards – the expected concentration values
- Show Unknowns – the unknown or test samples
- Show Standard Wells – the expected concentration values for all individual replicates

Analysis Tab Defaults

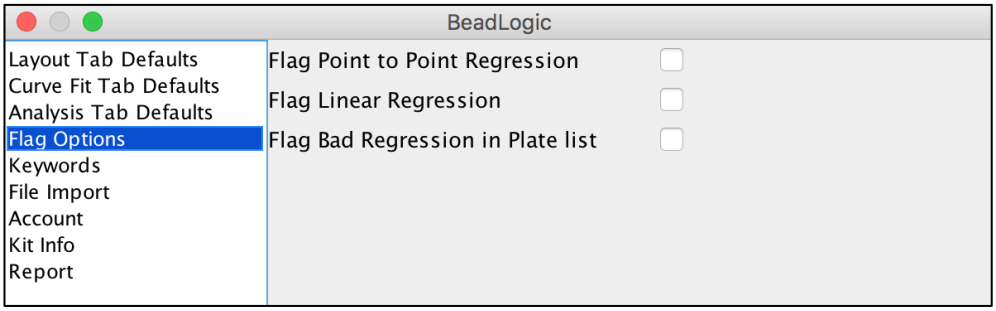


- Table Value – set the default statistic to be displayed for each analyte in the Data Table and defined in all three of the Basic, Intermediate and Advanced Table Setup tabs in the Analysis section. The options are:
    - FI: Fluorescent intensity
    - Observed: the concentration of an analyte derived from the interpolation of the regression curve
    - Observed (Limit): the concentration of an analyte derived from the interpolation of the regression curve but with concentrations existing above or below the top and bottom standards given the same value as that standard (bottom standard for low values and top standard for high values).
    - Observed (Removed): the concentration of an analyte derived from the interpolation of the regression curve but with concentrations existing above or below the top and bottom standards replaced with “OOR>” or “OOR<” (outside of range).
    - Bead Count – the bead count is the event count for the particular gated population

- Table Stat – set default statistics for when replicates exist. A choice between a number of statistics derived from the replicate values can be displayed. These are:
    - Mean – the mean of the replicate values
    - CV% – the coefficient of variation (displayed as a percentage)
    - SD – the standard deviation
    - Min – the smallest value of all replicates
    - Max – the largest value of all replicates
    - Sum – the sum of all replicate values

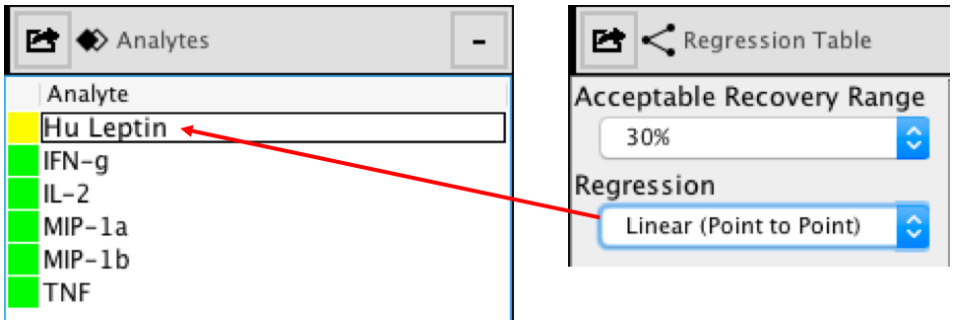
- Default Heatmap: set the default heatmap color scheme. Color schemes are displayed as the color corresponding to the low end of value range through to the color representing the high end of the value range (in some cases via black or white). There is also an option to choose a log color range. This option can allow for greater visualization of small differences at the bottom end of the data range. The options are:

Flag Options



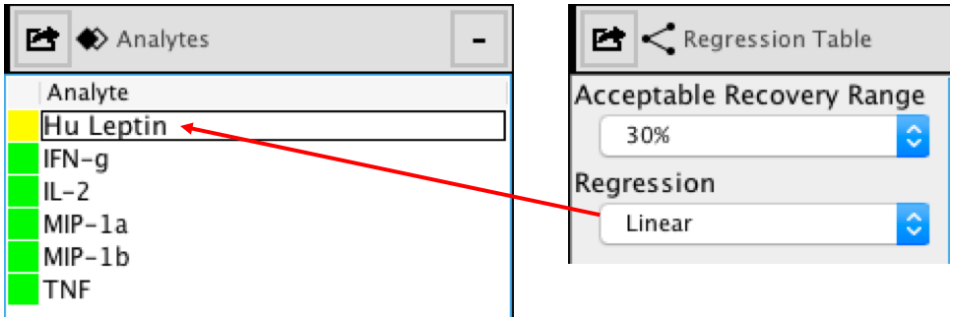
Flag Point to Point Regression

- With this option selected, if the regression type is set to Linear (Point to Point) in the Regression Table, then the analyte will be marked with a yellow square in the Analytes tab in the Layout and Curve Fit sections.
-



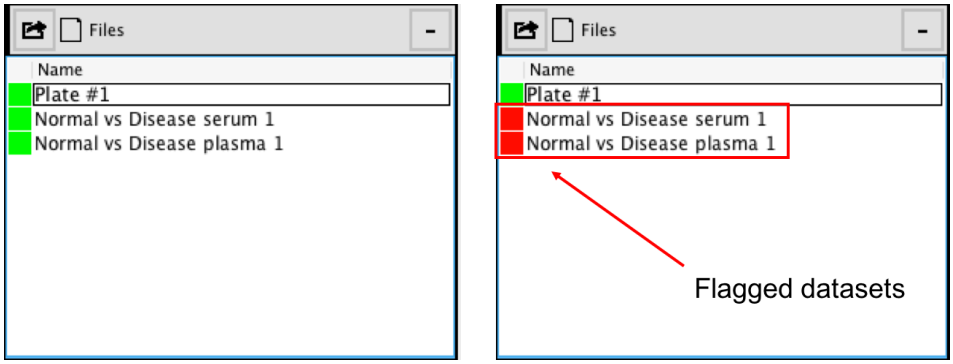
Flag Linear regression

- With this option selected, if the regression type is set to Linear in the Regression Table, then the analyte will be marked with a yellow square in the Analytes tab in the Layout and Curve Fit sections.
-

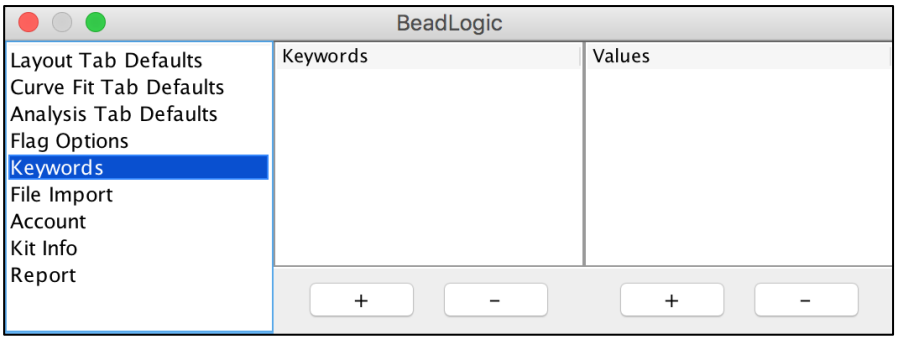


Flag bad regression in Plate list

- If any standard within a dataset in the Files list (in either the Layout or Curve Fit tabs) is outside of the Acceptable Recovery Range in the Regression Table/Regression Tornado in the Curve Fit section, then the dataset file name will be flagged with a red square. If all standards fit in the Acceptable Recovery Range, then the dataset will be marked with a green square.

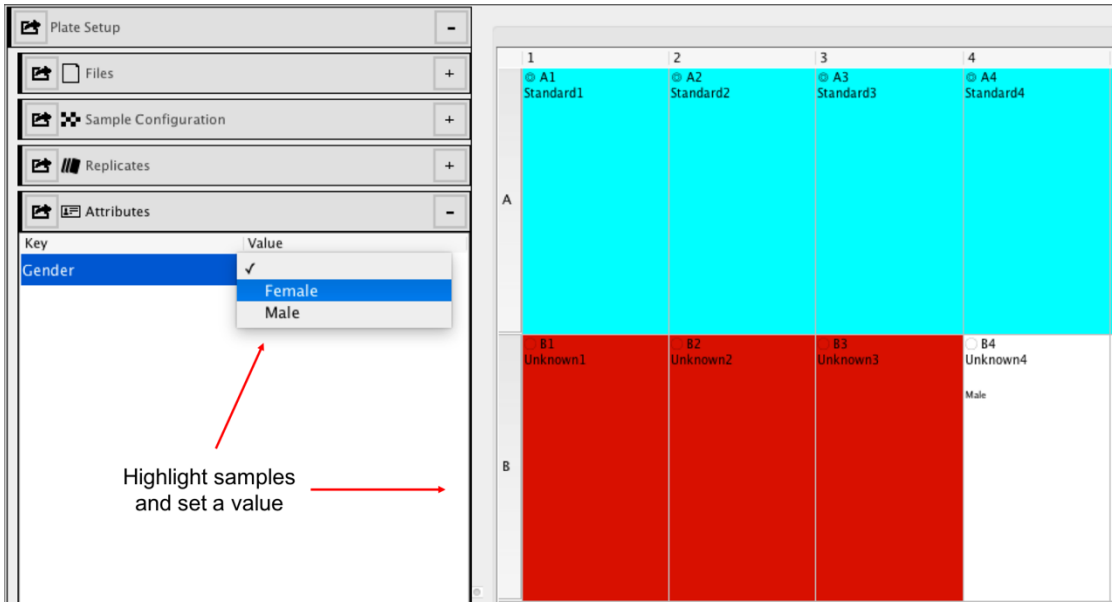


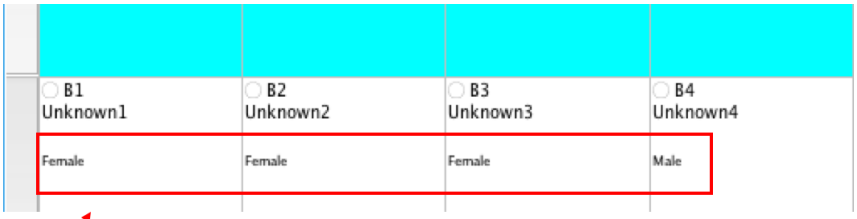
Keywords



Keywords and associated values can be added and displayed in the Attributes panel in the Layout and Curve Fit sections. These can then be assigned and displayed on selected wells in the plate plan. To add a keyword, click the '+' button at the bottom of the keywords window. By default, the new keyword will be 'Untitled Keyword'. Double click this text to rename it. To add a value to a keyword, highlight the keyword and then click the '+' button below the Values window. By default, the new value will be 'Untitled Value. Double click this text to rename it. To remove a Keyword or Value, highlight the text and click the '-' button below the relevant window.

To add the newly created keywords to samples, right click in the Attributes window → Add/Remove Keywords and select the keyword of interest. Then, highlight the relevant samples in the plate plan and choose a value associated with a particular keyword. The value will then be displayed in the well on the plate plan. The follow example uses ‘Gender’ as the keyword with ‘Female’ and ‘Male’ as the values:

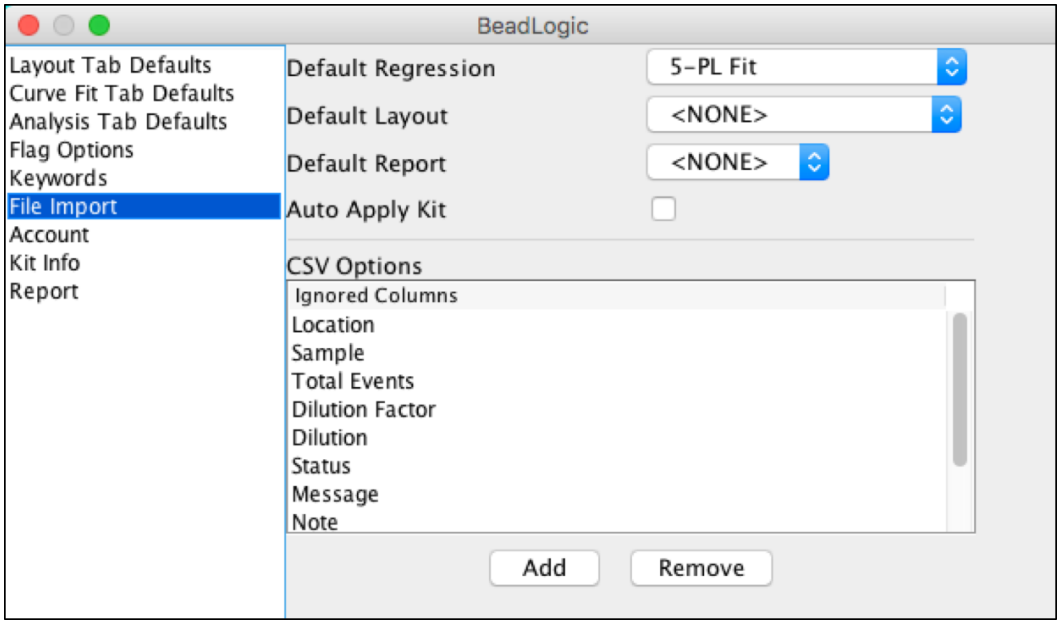




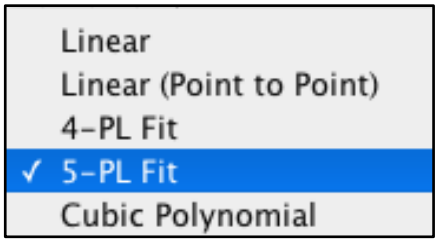
Value is displayed on
the selected samples

Keywords and values can also be edited by choosing Edit Keywords form the right click menu in the Attributes window.

File Import



Default automatic analysis settings can be defined and applied to data upon importation. These include:
Default Regression – the regression model used to fit the curve. This can be one of the following:



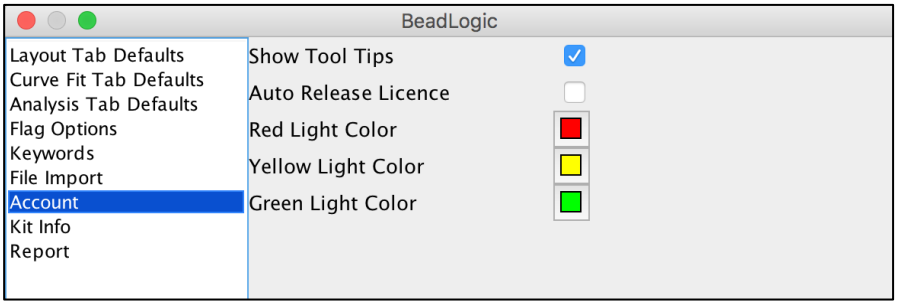
Default Layout – A plate layout can be saved and chosen to be loaded by default for new analyses.

Default Report – A report layout can be saved and loaded by default for new analyses.

Auto Apply Kit – A kit saved to the library can be chosen to be applied automatically upon importing data.

CSV Options – CSV files saved in the appropriate format can be imported into BeadLogic. Defined columns can be ignored for the process of importing. Add or remove the particular columns by way of the column titles.

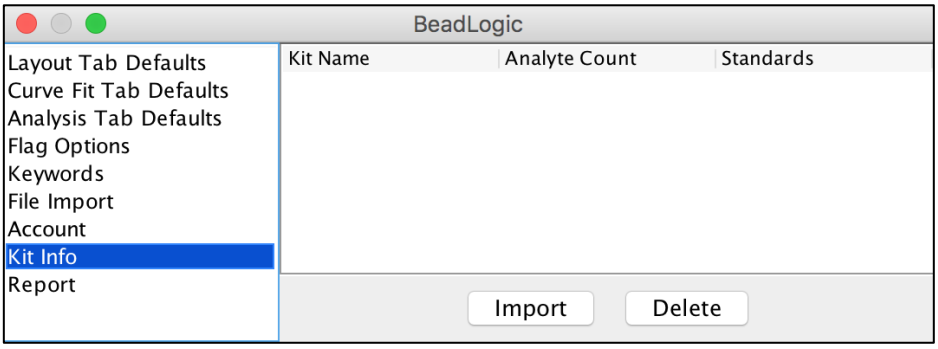
Account



Show Tool Tip – information that appears by hovering over certain objects, such as the samples in the heatmap or data table in the Analysis section, can be turned off.

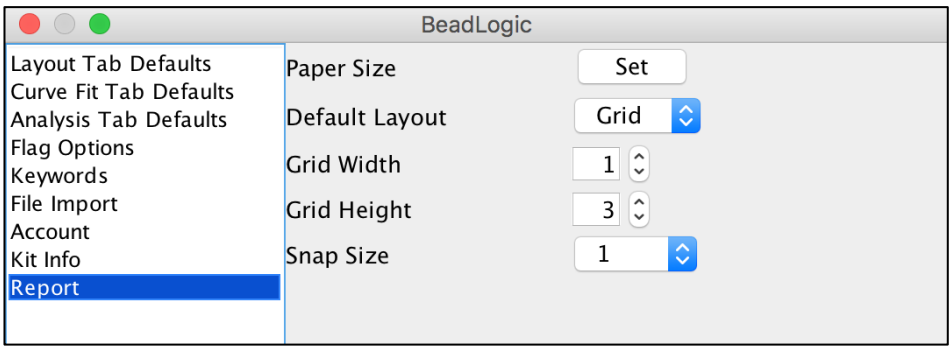
Auto Release Licence – the licence used to run BeadLogic can be released prior to quitting the program for use on another computer. This releasing of the licence can be done automatically every time the program is quit.

Kit Info



Import (or delete) specific kit details. Manufacturer kit information includes the list of analytes to be examined and the list of standards used to create the curves.

Report



- Paper Size: set the default paper size for when reports are created. To select a specific size, click **Set** from the Preferences window and then define the Page Setup.
- Default Layout: set elements to be added to the report in a defined grid layout or in a free layout. Free layout allows for elements to be placed anywhere on the page, even overlapping other elements.
- Grid Width: input a number to define the grid width
- Grid height: input a number to define the grid height
- Snap Size: click on the drop down menu to choose the snap size (1, 2, 4, 8, 16, 32, 64 or 128).

Main Menus

The options in the main menu contain general program operational functions. Many of these can be implemented using keyboard shortcuts, which are listed in the main menus next to the menu items. The four main menu options are:

BeadLogic (Mac OSX)

- About: this option opens a window displaying the program version along with the username, license type and the expiration date.
- Preferences… : Opens the Preferences window (see page 3 for a full description of the program preferences).
- Quit (⌘Q): select to exit or quite BeadLogic. A prompt window will ask if you would like to save the analysis prior to exiting. The options include **No**, **Yes** or to **Cancel** the action.

File

- New (Ctrl+N / ⌘N): creates a new analysis.
- Open (Ctrl+O / ⌘O): brings up a navigator window allowing the user to search for previously saved analyses. After locating and highlighting the saved analysis, select **Open** in the navigator window to load it.
- Save (Ctrl+S / ⌘S): saves the current analysis.
- Save As (Ctrl+Shift+S / ⇧⌘S): opens a window allowing the analysis to be named and saved in a desired location as a Beadlogic File.
- Recent: displays a list of recently opened Beadlogic files (.blf). Clicking on the name will load that analysis.
- Import (Ctrl+I / ⌘I): opens a window allowing data files to be imported. Only supported file formats can be selected. Select the data file and click **Open**.
- Recently Imported → : choose from a list of recently imported data files, such as .bpd or .flb (FlowLogic/BeadLogic bridge file). These are different to the .bfl Beadlogic files, which are saved analyses from within BeadLogic.
- Exit (Ctrl+Q / ⌘Q): select to exit or quite BeadLogic. A prompt window will ask if you would like to save the analysis prior to exiting. The options include **No**, **Yes** or to **Cancel** the action. In Mac OSX, the quit option is located under the **BeadLogic** menu option.

Edit

- Undo: undo the last action
- Redo: redo the last action if it has been undone
- Undo List: displays a chronological list of actions. Select a specific action to undo it.
- Preferences (Windows only): Opens the Preferences window. In Mac OSX, the Preferences are located under the BeadLogic menu (⌘,).

Window

- Show Window – brings the selected program window to the font of the display
- Library (Ctrl+L / ⌘L) – opens the BeadLogic plate layout library, where layouts can be saved, exported and imported.
- Close All Windows – closes all open windows

License

- Release**:** the current license, so that it can be used on another computer
- Renew: this option allows a new license code to be entered. Choosing to renew a license will open a window requesting the user name and the License Code. When these have been entered, choose **Renew**

Help

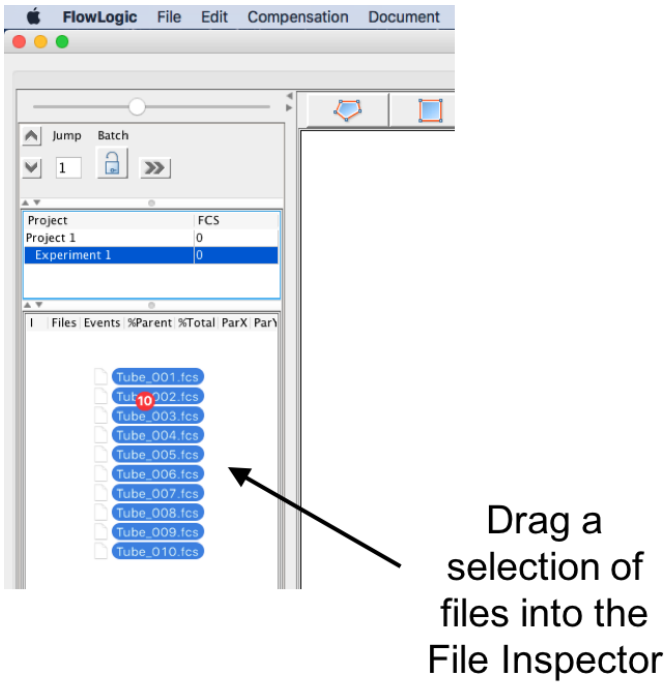
- Help F1: selecting help will launch a web browser and open the BeadLogic support page containing the BeadLogic manual and supporting videos.
- About (Windows): this option opens a window displaying the program version along with the username, license type and the expiration date. In Mac OSX, the **About** option is located under the **BeadLogic** menu.

FCS/MQD analysis and plate setup in FlowLogic

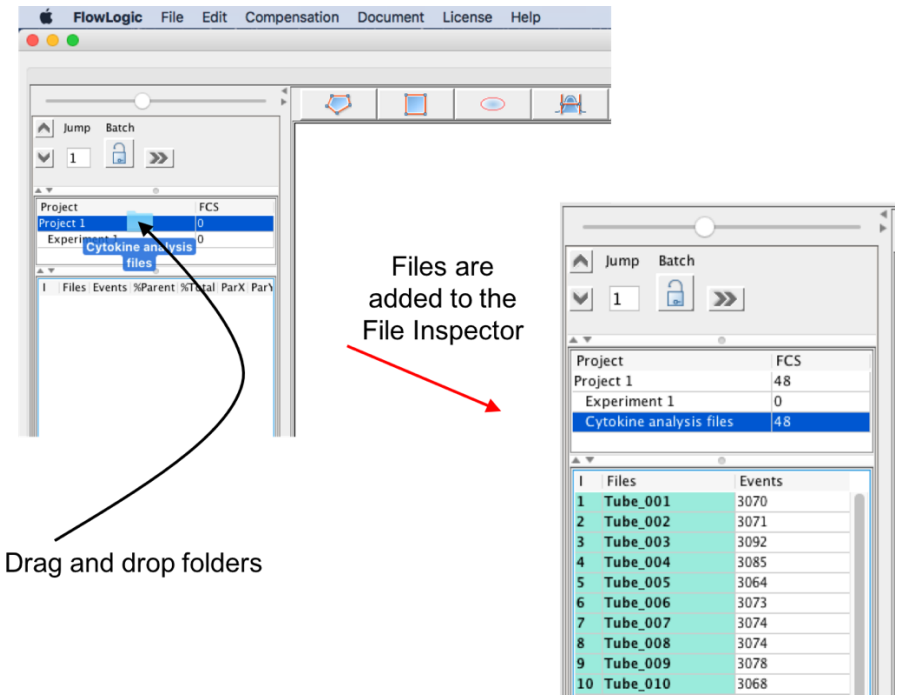
Data acquired on a flow cytometer can be analysed in FlowLogic flow cytometry analysis software and exported to BeadLogic with all plate setup parameters defined.
Here is a step-by-step description on how to perform this initial part of the sample analysis in FlowLogic. For a detailed explanation of the features FlowLogic, please consult the FlowLogic manual and video guides available online.

Importing FCS/MQD files

Files can be imported into FlowLogic by dragging a selection of FCS or MQD files directly into the Files Inspector. A folder containing these files can also be dragged into the File Navigator. Alternatively, from the main menu choose File→Import FCS-MQD-LMD-LXB… or Import Folders and select the files by their location.



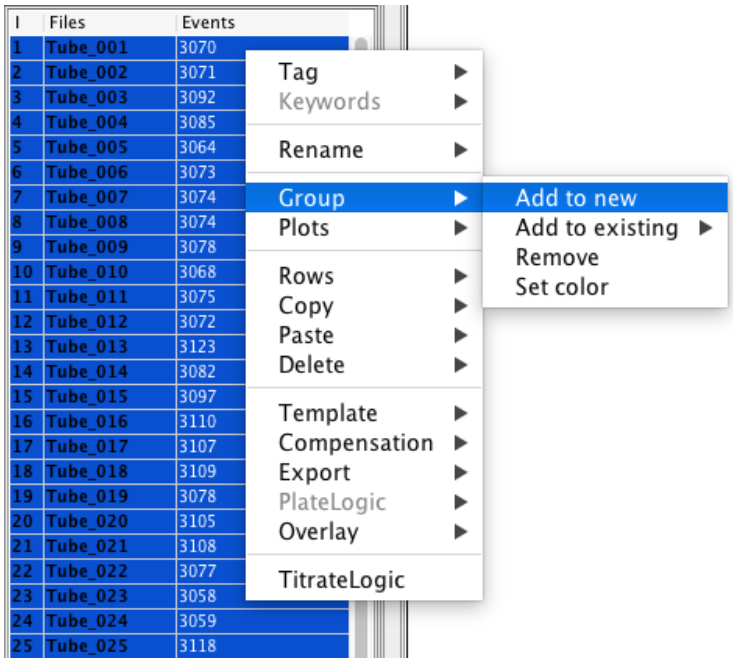
OR



Grouping files and gating on populations

Creating groups can make analysis of multiple samples very quick and easy. If a gate is applied to one file in a group, it is automatically applied to the same parameters on all other files in the group.

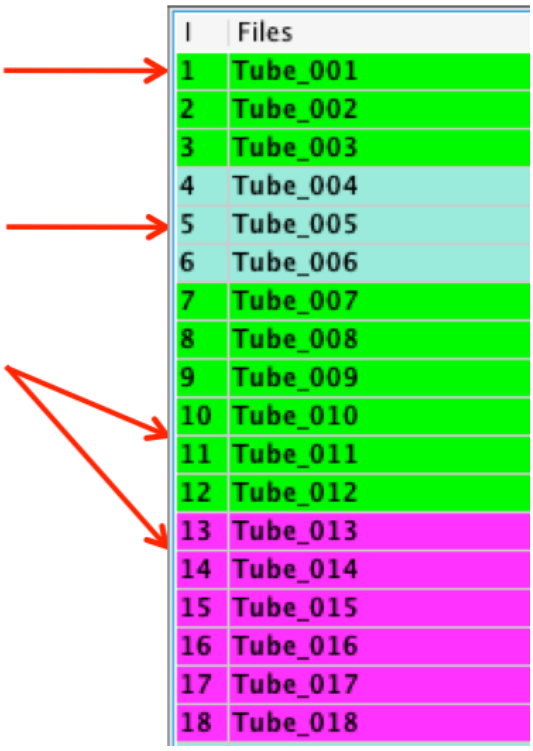
Select all files to be grouped, right click and select Group → Add to new.



Groups are assigned a color.

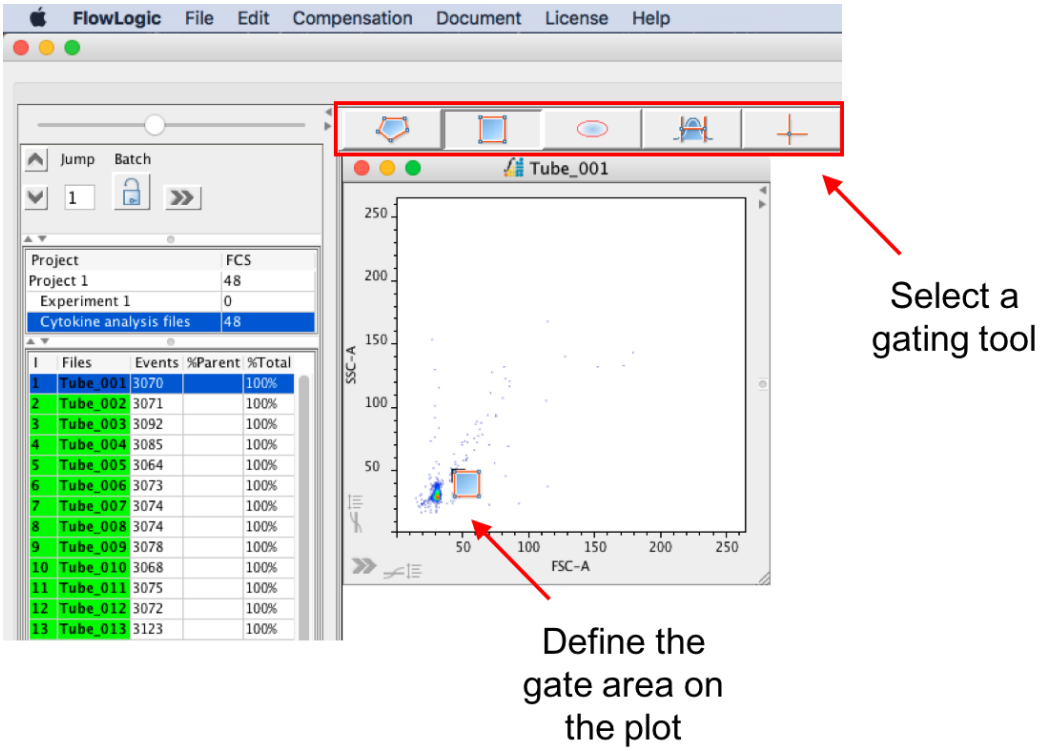
Individual files or even individual gates can be removed from a group.

Multiple groups can be created in each Experiment folder.

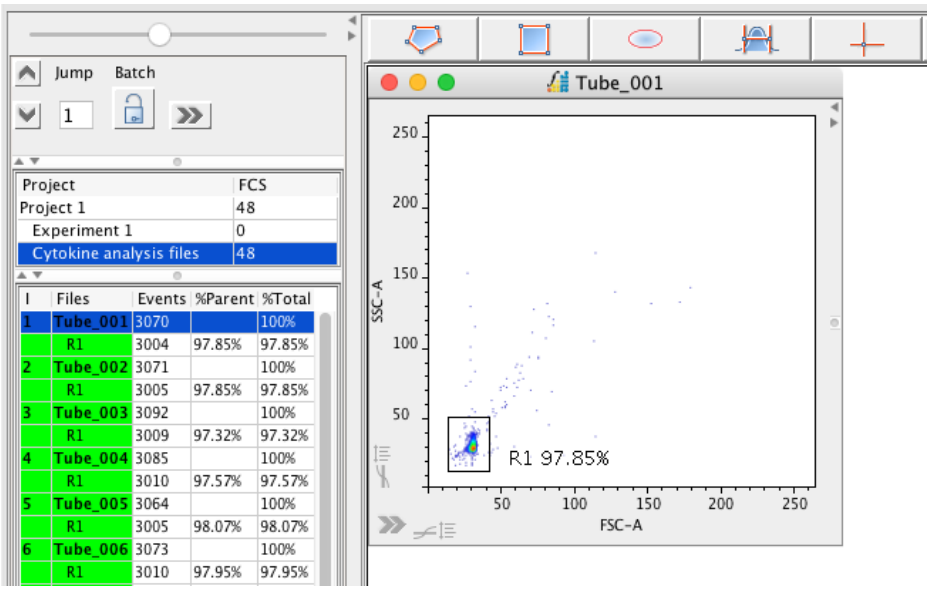


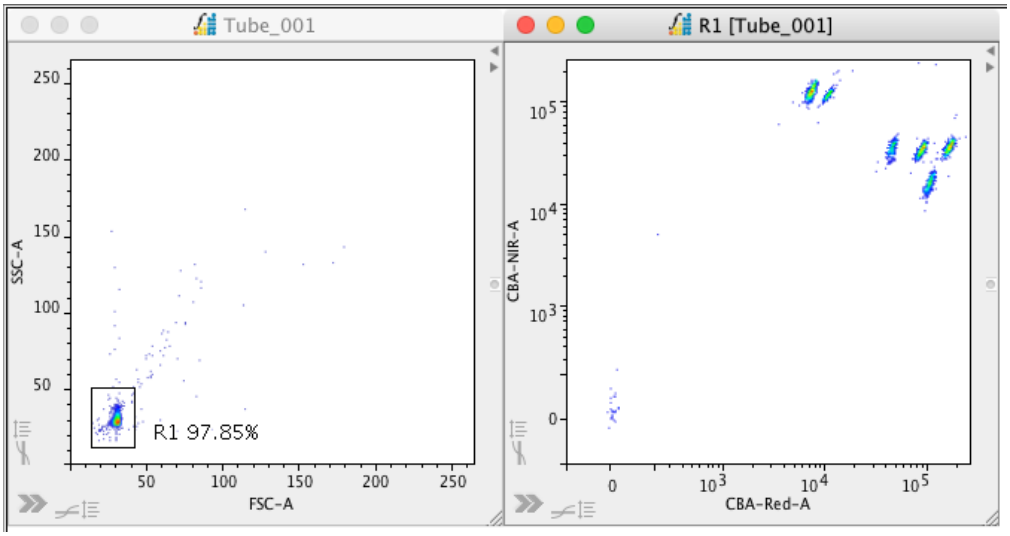
I	Files
1	Tube_001
2	Tube_002
3	Tube_003
4	Tube_004
5	Tube_005
6	Tube_006
7	Tube_007
8	Tube_008
9	Tube_009
10	Tube_010
11	Tube_011
12	Tube_012
13	Tube_013
14	Tube_014
15	Tube_015
16	Tube_016
17	Tube_017
18	Tube_018

Double click on a file in the File Inspector to open the plot in the Workspace. Click on the parameter labels on the plot to change them. Gate on populations by selecting the plot in the Workspace, clicking a gating tool from the Toolbar and then use the cursor in the plot to define the gate.



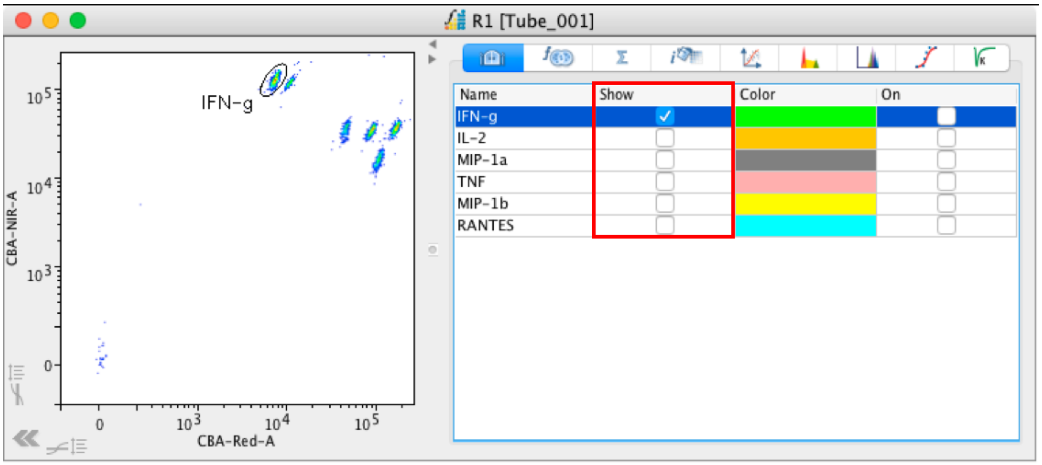
After a gate has been created, it will be listed beneath the sample in the File Inspector (in this case, R1). The gate name can be changed by right clicking on the gate in the File Inspector. To open the subpopulation, double click on the population in the File Inspector or double click within the gate on the plot.





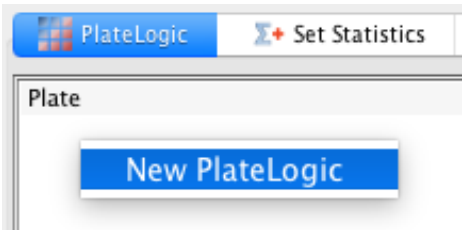
If the files have been grouped, as in the above Adjustments to individual gates are automatically updated to all within the group. If not, highlight the gates in the File Inspector, right click and choose Copy→Copy Gates. Then, highlight the files to which the gates need to be pasted to, right click and choose Paste→Gates.

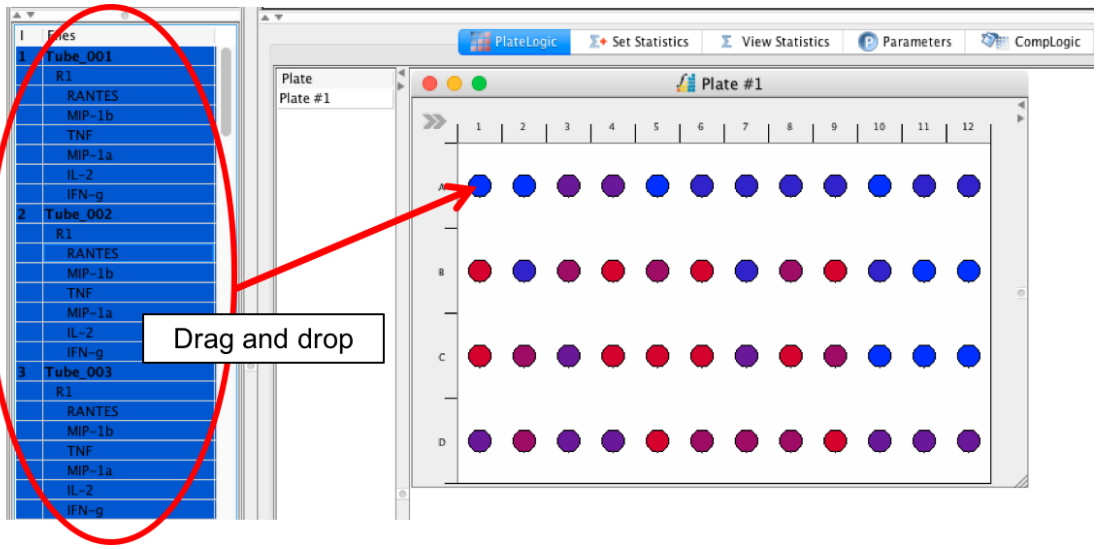
Continue gating on the specific bead populations that correspond to the different analytes. Naming the populations with the analyte name as the gates are created makes assessing the results in BeadLogic much easier. As the different populations can be relatively close together on the plot, a useful trick is to turn a gate off after it has been drawn. This is done by opening the plot side drawer by clicking on the button in the lower left of the plot window (or double clicking within the plot but not within a gate) and selecting the first tab called the Gate List tab. Untick the box in the column titled 'Show' to turn the gate off. This does not delete the gate, it just hides it from view and makes drawing new gates easier. Once all gates have been created, tick all of the boxes in the 'Show' column to make them visible again.



Adding samples to a plate in PlateLogic

After gating and naming the bead populations, add all of the samples to a plate in PlateLogic. PlateLogic is located in the Advanced Functions drawer located below the Workspace.
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. 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 (gate) can be set and analyzed.



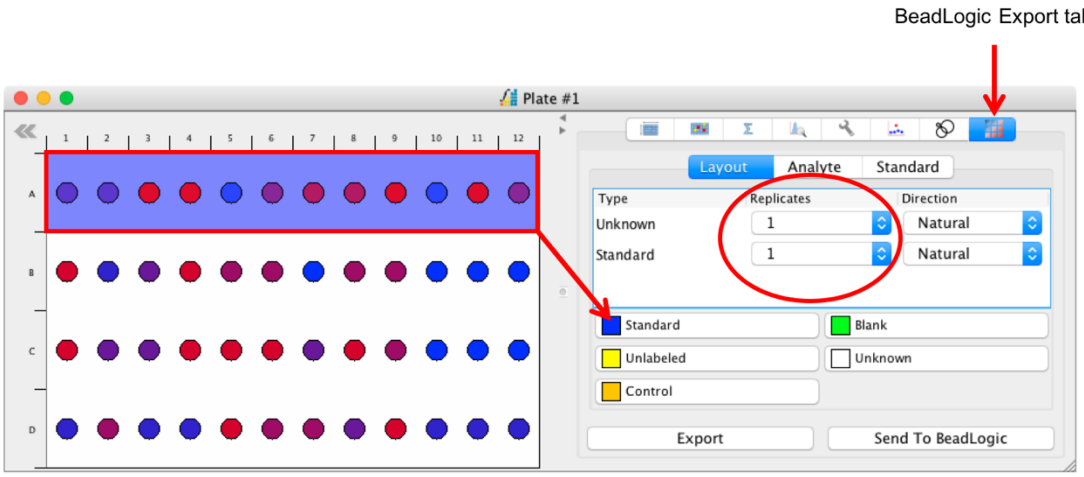


Once all files have been added to the plate, use the functions in the Plate Side Drawer to help analyze the samples. Specifically, the BeadLogic Export tab.

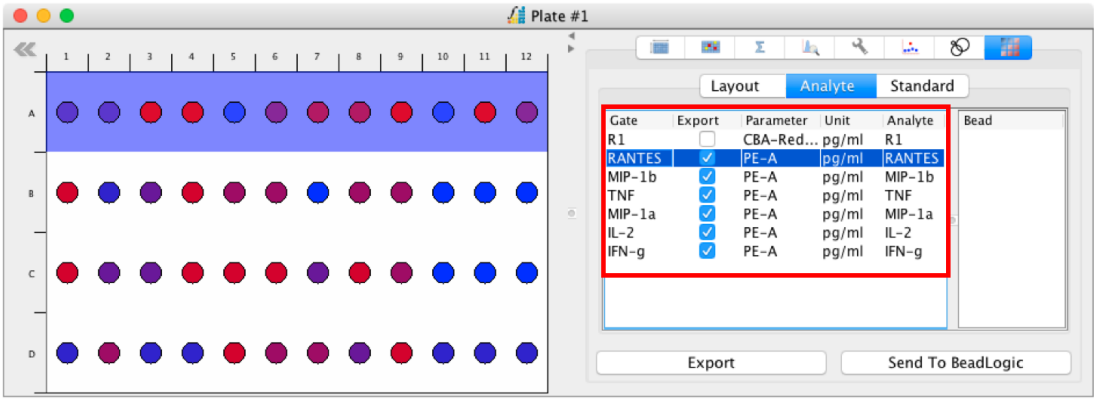
Plate setup

In the ‘Layout tab’, define each of the samples by highlighting them in the wells and clicking on one of the sample type buttons. This will color the background of the wells to reflect the different sample types, being Standards, Blanks, Unlabeled, Unknown or Controls.

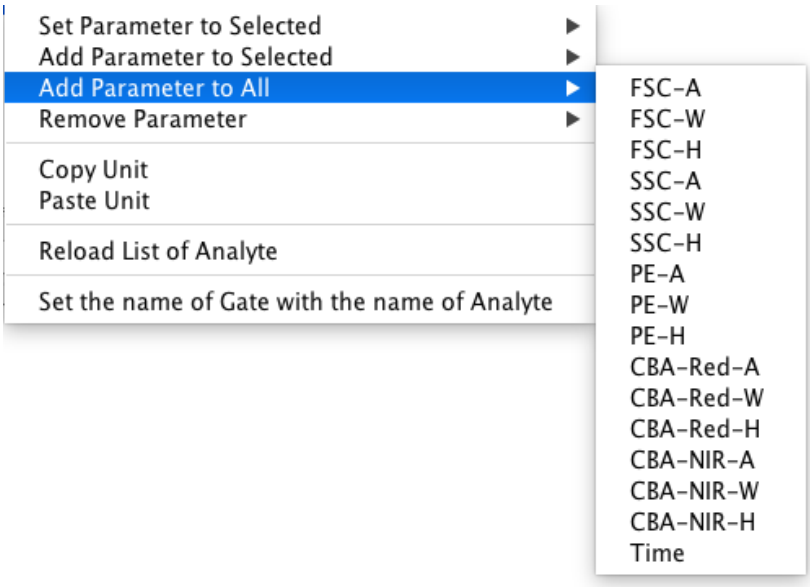
Also, define if technical replicates were used. To do this, choose a replicate number for the corresponding sample type. This will result in an average value for the replicates being used in BeadLogic.



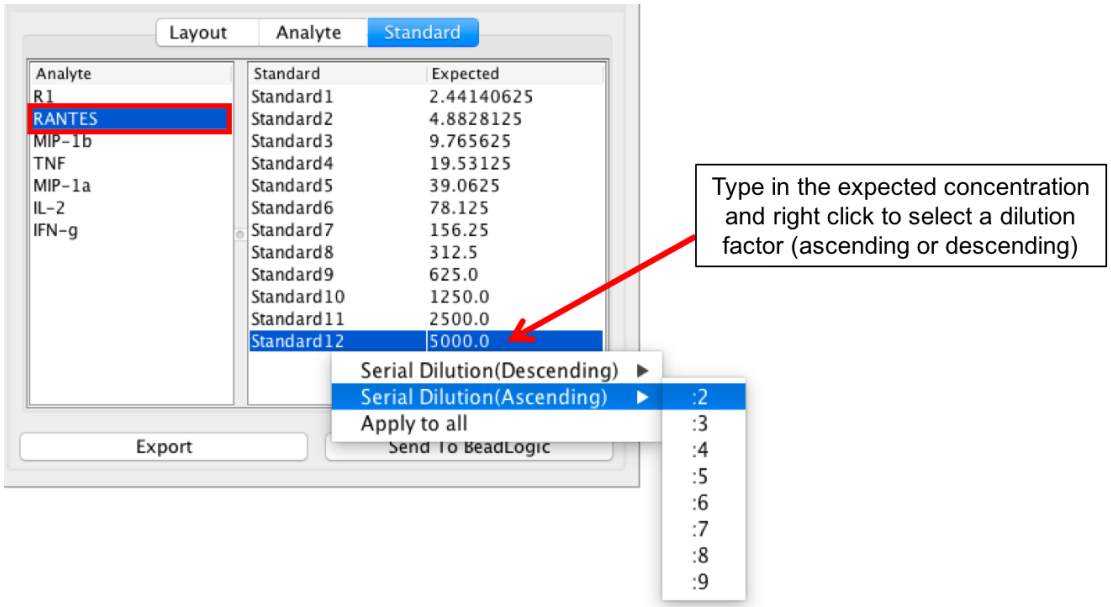
In the ‘Analyte tab’, choose the analytes to export, set the reporting parameter, define the units and name the analyte. These functions can be aided using the right click menu.



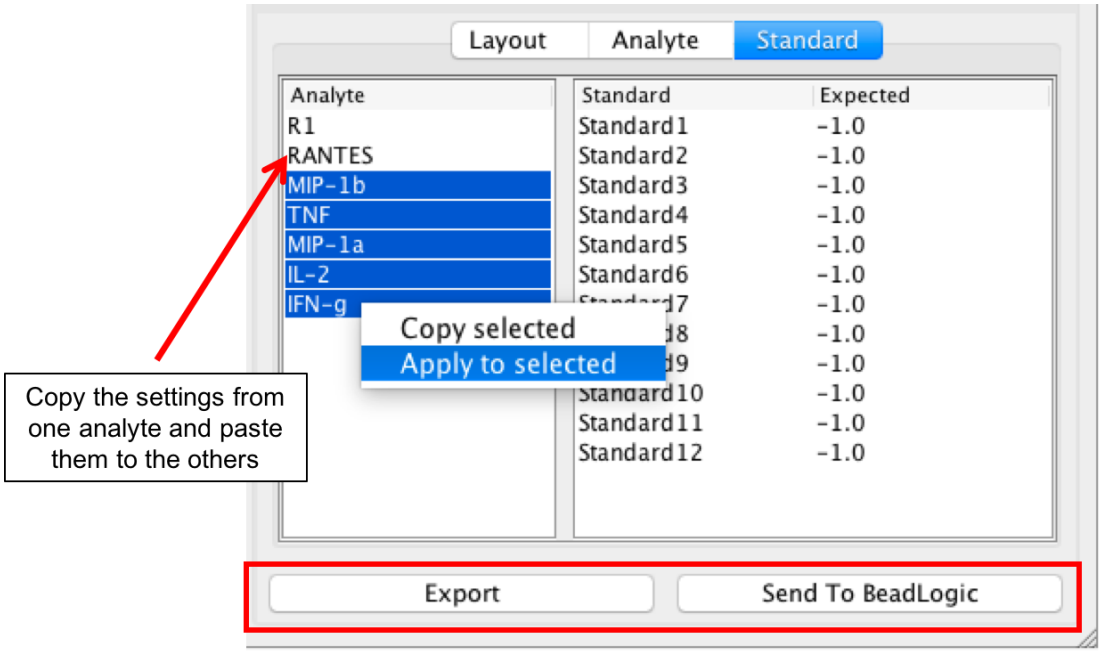
Right click within the ‘Analyte’ window for the following menu options:



In the ‘Standard’ window, define the expected concentrations for the standards. Highlight one standard and type a value in the ‘Expected’ column and right click to choose a serial dilution factor in an ascending or descending order, depending on which standard you begin with.



Once the expected values have been defined for one analyte, right click and choose ‘Copy selected’ to copy the settings. Then, highlight other analytes, right click and choose ‘Apply to selected’ to paste the same values to the highlighted analytes.



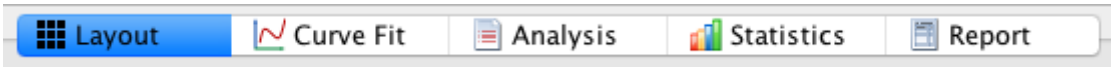
Export a FlowLogic/BeadLogic Bridge file to import into BeadLogic or send directly to BeadLogic (BeadLogic must be running)

Once all settings have been made, click either ‘Export’ to save a FlowLogic/BeadLogic Bridge file (.flb file) or send the analysis directly to BeadLogic by clicking ‘Send To BeadLogic’.

The FlowLogic/BeadLogic Bridge file can be imported into BeadLogic at any time. In order to send the analysis to BeadLogic, make sure to launch BeadLogic before clicking the ‘Send To BeadLogic’ button.

BeadLogic - Layout

After importing new files or loading a saved experiment, BeadLogic will load with the following toolbar displayed at the top of the screen:



To follow a basic workflow, use the functions located under each of the tabs, working from left to right, starting with **Layout**.

When **Layout** is selected, a range of Plate Setup features is located on the left hand side of the screen. Click on each of these to access options to select between plates, define the sample configuration (plate layout), define replicates, assign attributes to samples, define dilution factors, display the set of analytes tested and to view sample metadata.

In most cases, selecting one or more samples from the diagram to the right of the Plate Setup section will enable the various plate setup options to be accessed and changed.

To view the Plate Layout, select **Setup** from the sub toolbar under the **Layout** tab.

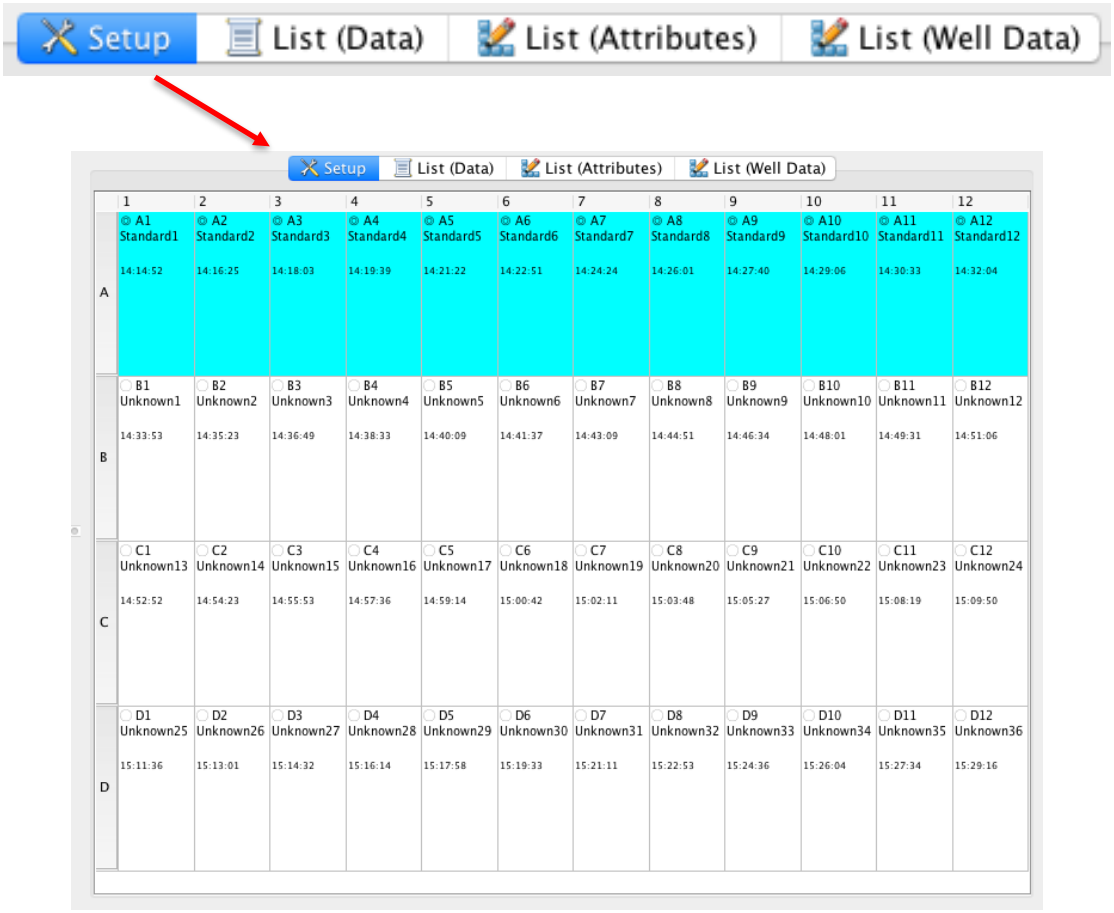
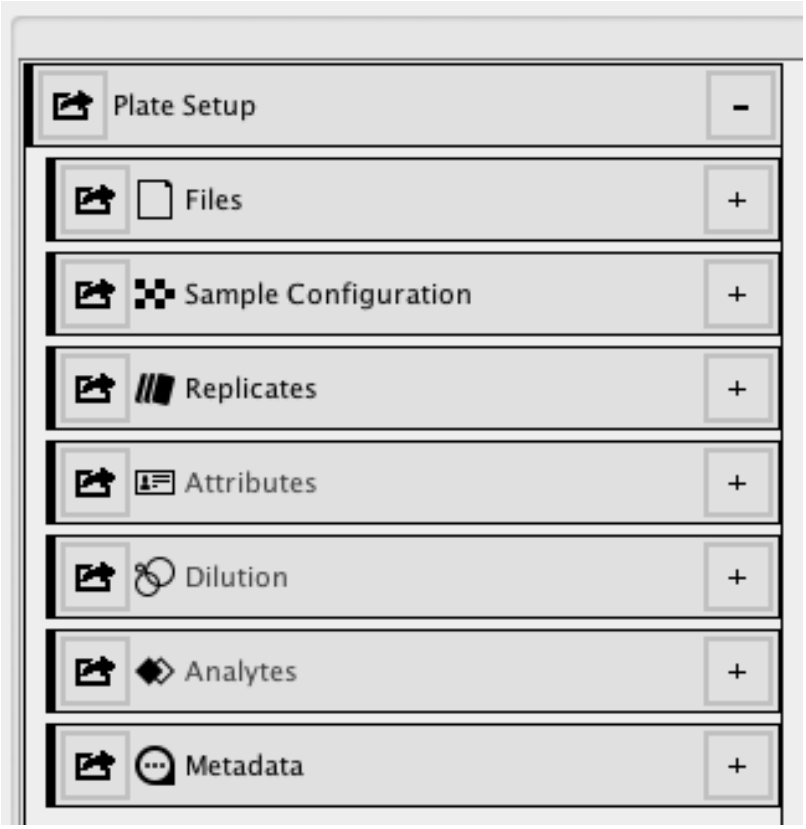
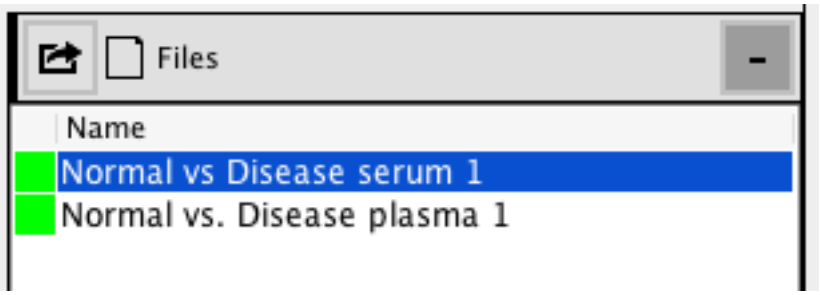


Plate Setup Features



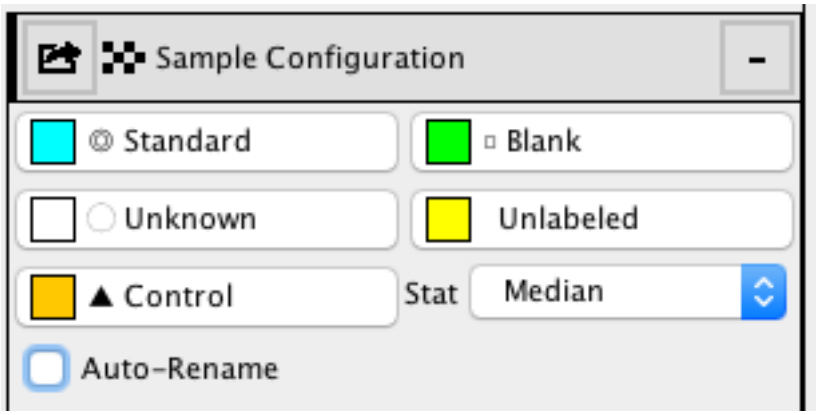
Files



Listed in the **Files** window is a list of all 'plate' files, which have been imported into BeadLogic. A green square beside the plate name indicates that appropriate standards have been defined in the plate layout. A red square indicates that appropriate standards have not been defined in the plate layout.

When a plate is highlighted in this panel, it is considered to be 'in focus' and the rest of the system will display its related properties and settings.

Sample Configuration



This panel allows for wells to be defined as a standard, blank, unknown, unlabeled or a control. One or more wells have to be highlighted in the plate layout for the options in this panel to use.

Standard: a well that is used for the calculation of the standard curve.
Unknown: a well with an 'Unknown' value (an experimental sample being analyzed).

Control: a 'Control' well, such as a high control or low control. These have a separate display on standard curves and output tables.

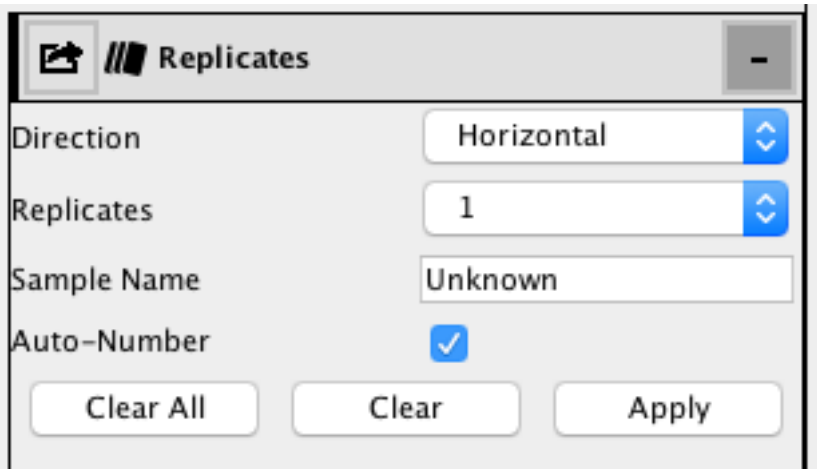
Blank: a well that is used to record background or non-specific readings. The values from 'blank' wells are subtracted from all fluorescence intensity (FI) readings.

Unlabeled: a well that is ignored from all output and reporting.

Stat: select the desired statistic for use in the selected plate. Some file formats do not provide any options while others may give a large number of possible statistics to choose from.

Auto-Rename: this will allow you to automatically apply replicate settings when assigning well types.

Replicates



Direction: allows for the 'direction' of replicates to be set. The options are:

- Horizontal - the next replicate in the series will be to the right of the previous one. Once the series of replicates is filled, the next series will start to the right of the previous series. When the end of the selection is reached horizontally, the allocation will continue from the next row.
- Vertical - the next replicate in the series will be below the previous. Once the series of replicates is filled, the next series will start below the previous series. Once the end of selection is reached vertically, the allocation will continue from the next column.
- Natural - the next replicate in the series will be to the right of the previous one (horizontal allocation). Once the series of replicates is filled, the next series of will start below the previous one until the end of the selection is reached vertically. The setting will then continue from the next column and allocate the wells horizontally.

Replicates: this drop-down menu allows you to set the number of replicates in a given series.

Sample Name: this text field allows you to set the 'sample name prefix' for wells prior to replication. A number is appended to the name to signal, which wells belong to which series.

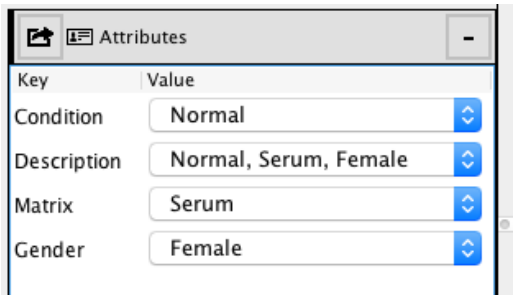
Auto-Number – if the name and replicate number is defined for a selection of wells, the auto-number feature will append each name with a number in ascending order.

Clear All: resets all wells in the current plate, renaming them with their well ID.

Clear: resets all selected wells, renaming them with their well ID.

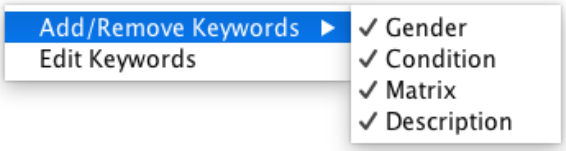
Apply: applies the settings to the selected wells.

Attributes

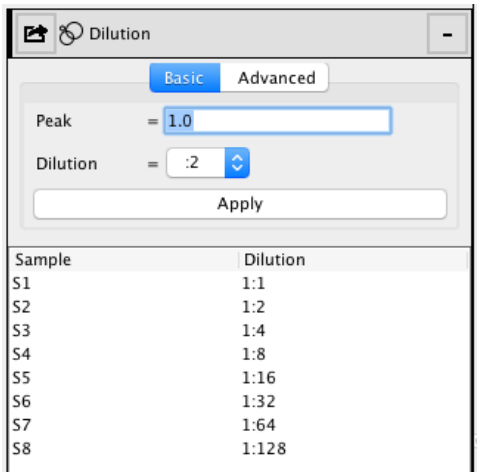


This window provides a list of ‘attributes’ or keywords for highlighted wells. A drop-down box populated with ‘*’ indicates that the selected wells have different values for the given attributes.

Right click within this window to access options to add or remove keywords from the current plate as well as edit the global attributes list.



Dilution



This panel can be used to apply dilution factors to wells. If dilution factors have been applied, BeadLogic can signal if there is a difference in dilution between different wells. Dilution factors are also used in conjunction with the Curve Fit section (accessed in the main toolbar) to define serial dilutions of standards without having to define expected values manually.

Dilutions can be defined using the features under either the **Basic** or **Advanced** tabs.

Basic:

- Peak: the ‘Initial’ or ‘Neat’ concentration.
- Dilution: the serial dilution ratio of subsequent wells.

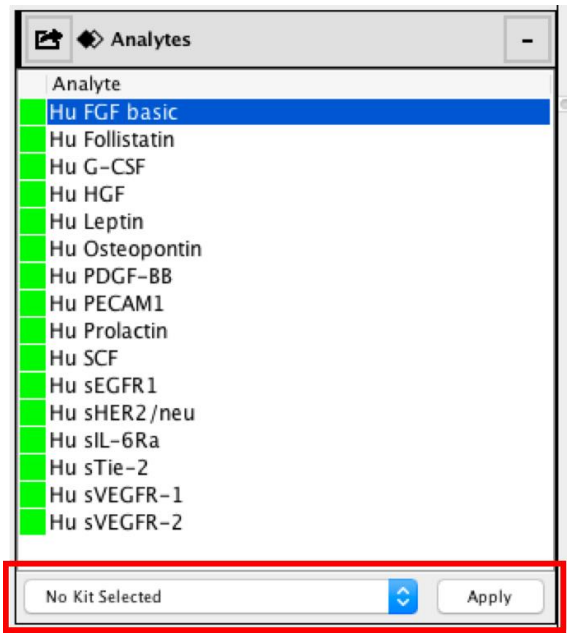
Advanced:

- y[0]: the ‘Initial’ or ‘Neat’ concentration.
- y[n+1]: an area where an equation can be entered for the calculation of the next value in the dilution series.

Apply: when clicked, the Basic or Advanced settings will auto-populate the selected wells with the appropriate dilutions.

Dilution Table: a table where dilutions can be both viewed and manually entered on a sample-by-sample basis.

Analytes

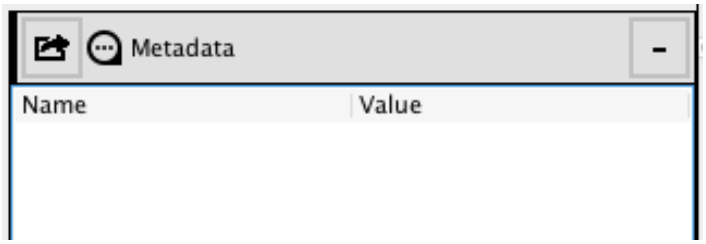


The analytes being analysed are displayed in this window. A green square next to the analytes indicates that all of the standards for that analytes fit within a defined acceptable error range. A red square indicates that at least one standard falls outside of the defined acceptable error range. To automatically fit the curves to the data, right click on an analyte and select Auto-Fit.
Kit Info files, containing all of the analyte information, can be imported into BeadLogic by clicking Edit-> Preferences -> Kit Info -> Import.

Kit Info files can be applied to a plate from the Analytes tab in the Plate Setup section.

If Automatic Kit Info is enabled in Preferences, the first available Kit Info will be preloaded.

Metadata

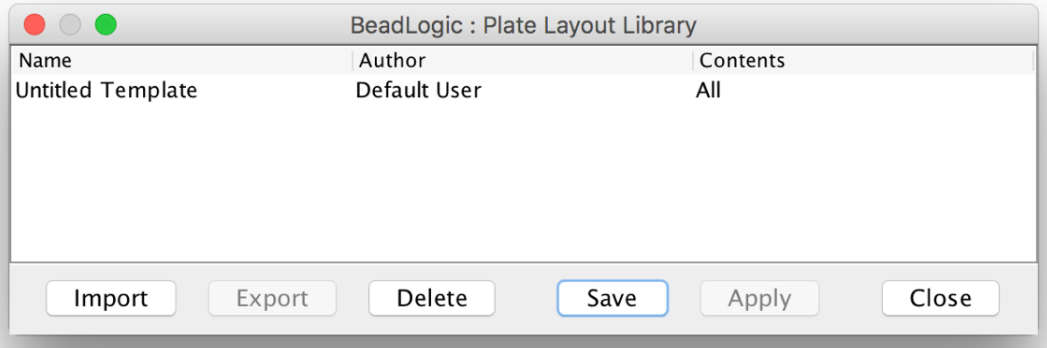


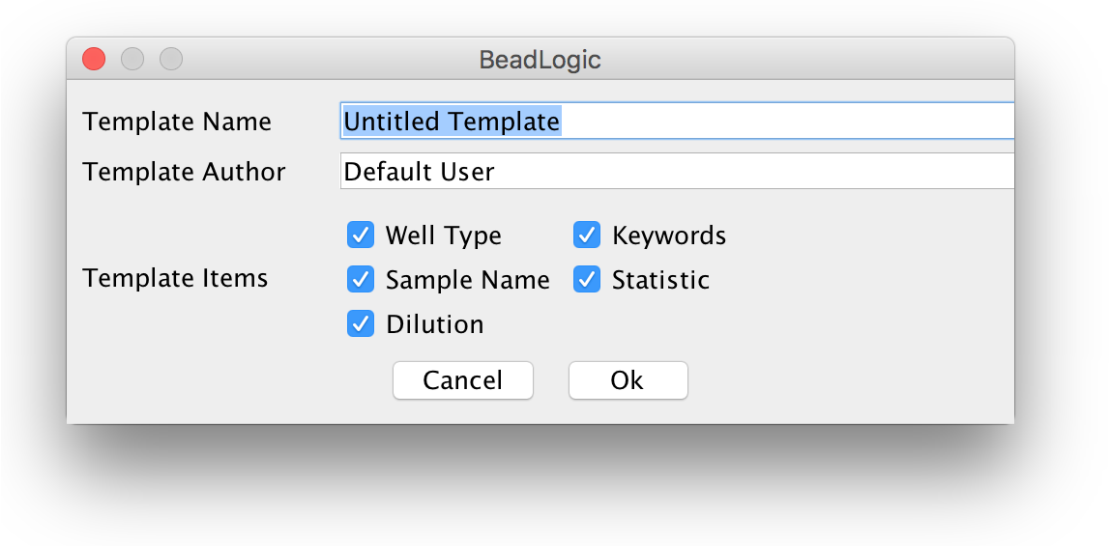
The metadata window displays all of metadata associated with an imported file. Metadata can be exported by right clicking in the metadata window and choosing **Export Table.** Choose to export the metadata as either a CSV or an Excel XML Workbooks file. Note: some file formats do not provide metadata.

Library

Click this button, located at the bottom of the Plate Setup window, to import and export all settings created in the Layout tab.

To save a template of the current template, select **Save** from the bottom of the window. This will open another window with options to name the template, name the author and define which template parameters to save with the file. Once the template has been created, click to highlight it and choose export to save it as a 'Bead Plate Setup Template' (.blpst file). This can be imported in subsequent analyses



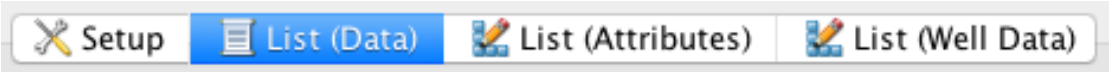


- Import: import a library item that has been saved externally.
- Export: export of a library item as an external file.
- Delete: deletes the currently selected library item from the library
- Save: saves the current plate layout into the library with the ability to select which items are saved in the template.
- Template Name: the human readable name for the template you wish to save
- Template Author: the author of the template
- Well Type: if enabled then the well types for the currently selected plate will be saved (e.g. Standard, Unknown, Control, Blank, Unlabeled)
- Sample Name: if enabled then the sample names (replicate definitions) are saved
- Dilution: if enabled then the dilution information will be saved.
- Keywords: if enabled then the Keywords and their values will be saved.
- Statistic: if enabled then the statistic definition for the plate will be saved.
- Ok: if clicked the Save process will be completed.
- Cancel: if clicked the save process will be aborted.
- Apply: will apply the currently selected library item to the current plate. A dialog box will allow unwanted items to be turned off/excluded from the template. (The definition and meanings of the items is the same as in the 'Save' dialog)
- Close: causes the Plate Layout Library to disappear.

From the plate **Setup** tab you can highlight wells and apply the settings from the menus from the left and side.

Right click in the plate layout and choose 'Discontinuous Selection' to make a selection of wells from across the plate (not just connected horizontally or vertically.

List (Data)



The **List (Data)** tab provides a table display of the current data. To export the data, right click within the table and select **Export Table**.

Above the table are a number of table format settings. These are:

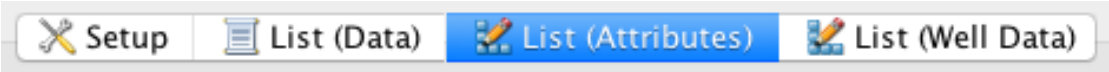
- Table Value: select the type of value to be displayed in the table for the current plate
    - FI (Fluorescence Intensity): when selected the statistic for the table is the raw fluorescence intensity.
    - Observed: this is the observed (regressed) value without any markings whether the value is OOR or not
    - Observed (limit): this is the observed value, although if a value is considered to be OOR, it will be limited to the relevant upper or lower limit of quantization.
    - Observed (removed): this is the observed value but when it is considered OOR, it will be marked OOR> or OOR< appropriately.
    - Bead count: this is the recorded bead count for the sample. Note: some file formats do not provide bead count data.

- Table Statistic: the aggregate statistic for the replicates used for the table.
    - Mean: the mean of all the selected 'Table Values' for the appropriate sample.
    - CV%: the Coefficient of Variation of the selected 'Table Values' for each sample.
    - SD: the Standard Deviation of the selected 'Table Values' for each sample.
    - Min: the minimum value of the selected 'Table Values' for each sample.
    - Max: the maximum value of the selected 'Table Values' for each sample.
    - Sum: the sum of the 'Table Values' for each selected sample.

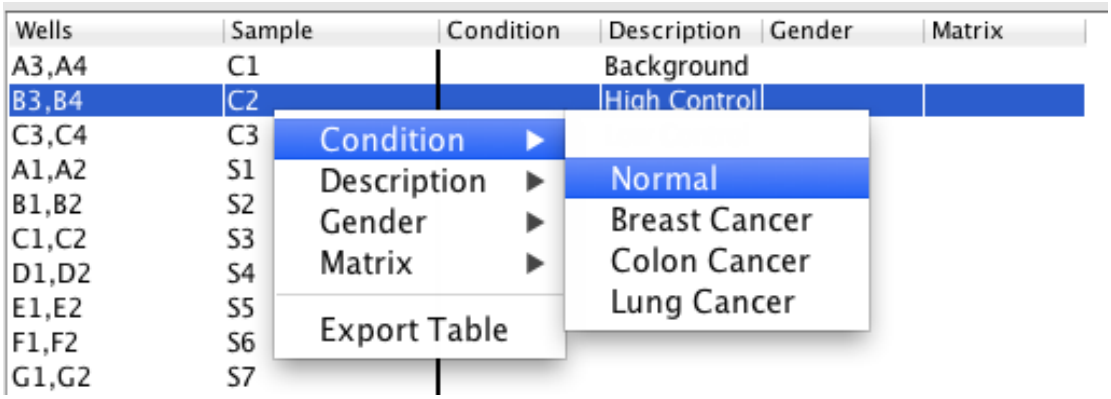
- Highlight OOR: if enabled then the 'Out of Range' samples will be highlighted in red.

- Background Color: provides the rules for the background color of the given wells.
    - No Background: If selected then there is no background color coloring
    - CV FI: if selected then the cell background will be colored yellow when the CV of the fluorescence intensity is outside the threshold
    - CV Obs: if selected then the cell background is colored yellow when the CV of the observed values of the given cells is outside of the threshold
- CV% Thresh: The threshold that is applied to the background color settings.

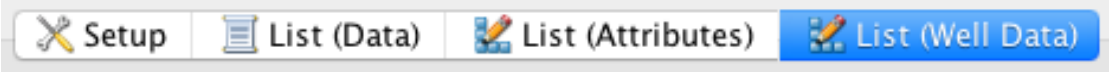
List (Attributes)



The **List (Attributes)** tab provides a list of all samples and the Attributes/Keywords that have been applied to them. Right click to export the entire table or to apply keywords to selected samples.

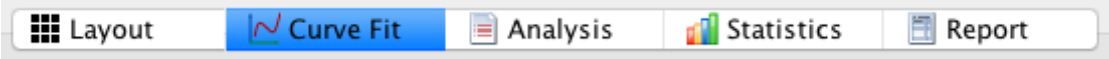


List (Well Data)



The **List (Well Data)** provides a quick view of all the raw data in the table not separated by sample.

Curve Fit

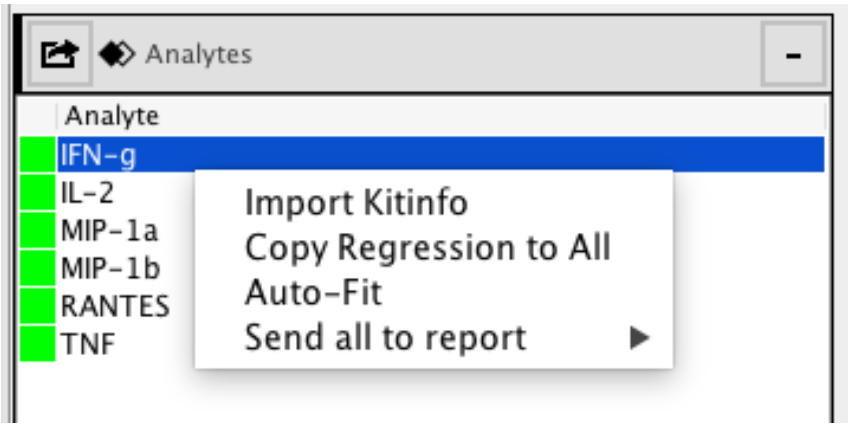


In the Curve Fit tab, curve-fitting functions can be performed manually or automatically. Manual adjustments (enabling and disabling standards) are performed individually, on a per analyte basis.

Within the **Curve Fit** tab there are five windows. On the left hand side are the Files and Analytes windows and in the workspace on the right hand side are the Regression Curve, Regression Tornado and Regression Table windows. Click on the window headings to expand and collapse them.

Files
See Layout → Files for a more detailed explanation.

Analytes



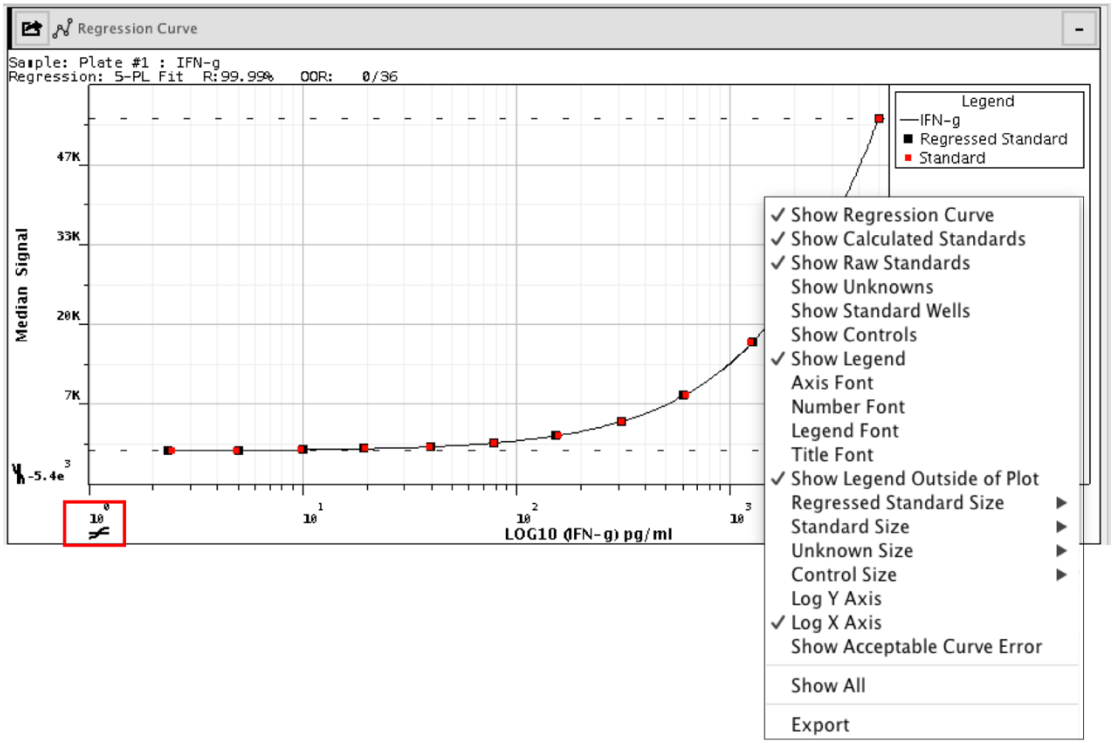
The analytes in the current plate are displayed in this window. When an analyte is selected within this window the output in the Regression Curve, Regression Tornado and Regression Table windows will update to display the result for the specific analyte.

A red square next to the analyte name signals that it has at least one standard with an error outside of the acceptable recovery range. If a given plate provides 'bead Id' information, this can be used to apply a compatible kit info to the plate, which will pre-fill expected concentrations of standards.

The right click menu is as follows:

- Import Kit Info: import a new kit info file into the preferences.
- Copy Regression to All: copy the Acceptable Recovery Range and regression settings and apply it to all analytes in the plate.
- Auto-Fit: runs the 'Auto-Fit' algorithm for all analytes in the plate. The results can then be checked visually. This algorithm progressively disables standards at the upper and lower limits of the range until all standards sit within the acceptable recovery range.
- Send all to report: sends all analytes to an 'analyte only report template'.

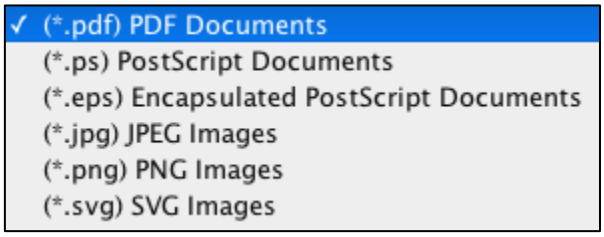
Regression Curve



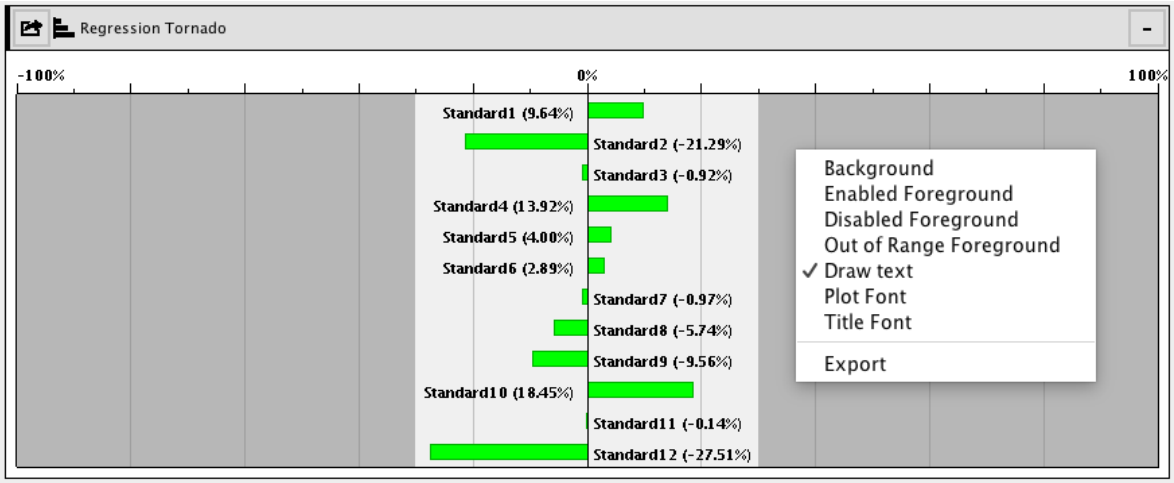
The Regression Curve window displays the standard regression curve. There is one 'Log → Lin' button on each axis to allow for the switching of axis transformations. This display is configurable from the right click menu:

- Show regression Curve: enables/disables the regression curve from the display.
- Show Calculated Standards: enables/disables the 'calculated' standard values.
- Show Raw Standards: enables/disables the 'Raw' standard values (non-regressed).
- Show Unknowns: enables/disables display of unknowns on the curve
- Show Standard Wells: enables/disables the actual 'wells' that make up each standard sample. All wells are included in the calculation for a quick visual display of CV within a standard sample.
- Show Controls: enables/disables the display of controls from the standard curve.
- Show Legend: enables/disables the display of the Legend on the regression curve display.
- Axis Font: allows the axis label font to be changed.
- Number Font: allows the numeric axis label font to be changed.
- Legend Font: allows legend font to be changed.
- Title Font: allows regression plot title font to be changed.
- Show Legend Outside of Plot: allows the location of the legend to be changed:
    - outer right hand side of the plot area
    - top left hand side inside the plot

- Regressed Standard Size: allows the size of the 'dots' for the regressed standards to be changed.
- Standard Size: allows the size of the 'dots' for the raw-standards to be changed.
- Unknown Size: allows the size of the 'dots' for the unknowns to be changed.
- Control Size: allows the size of the 'dots' for the controls to be changed.
- Show All: displays all plot items for the standard curve.
- Export: export the standard curve in one of a number of external file formats:



Regression Tornado

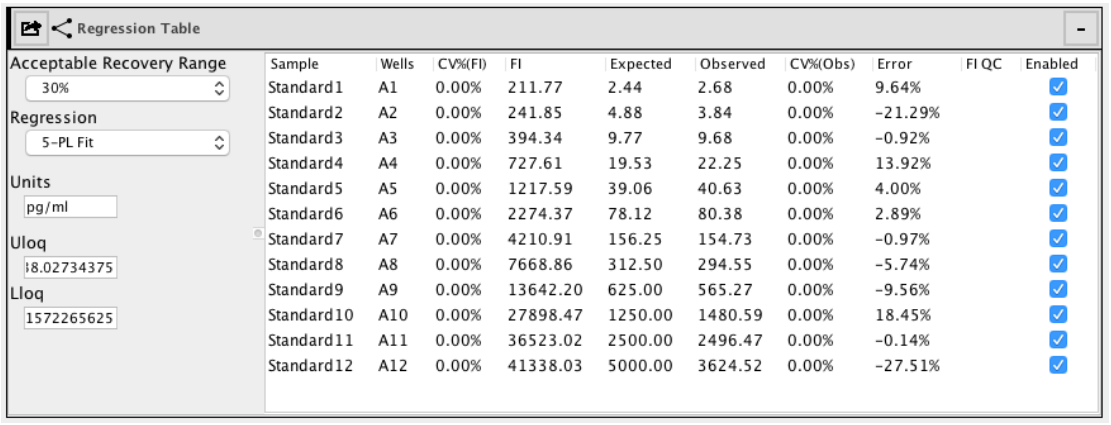


The Regression Tornado window provides a simple display to view the difference between expected and actual values for the standards.

The plot is configurable via the right click menu:

- Background: change the background color for the plot.
- Enabled Foreground: set the foreground color for a standard that is both enabled and within the acceptable recovery range.
- Disabled Foreground: change the foreground color for a standard that has been disabled.
- Out of Range foreground: set the foreground color for a standard that is both enabled and out of range.
- Draw Text: if enabled, text appears on the inside of the tornado plot.
- Plot Font: change the font inside the plot.
- Title Font: change the font for the title (axis).

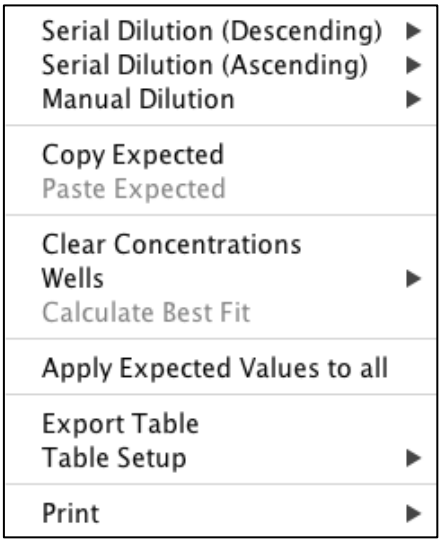
Regression Table



Sample	Wells	CV%(FI)	FI	Expected	Observed	CV%(Obs)	Error	FI QC	Enabled
Standard1	A1	0.00%	211.77	2.44	2.68	0.00%	9.64%		☑
Standard2	A2	0.00%	241.85	4.88	3.84	0.00%	-21.29%		☑
Standard3	A3	0.00%	394.34	9.77	9.68	0.00%	-0.92%		☑
Standard4	A4	0.00%	727.61	19.53	22.25	0.00%	13.92%		☑
Standard5	A5	0.00%	1217.59	39.06	40.63	0.00%	4.00%		☑
Standard6	A6	0.00%	2274.37	78.12	80.38	0.00%	2.89%		☑
Standard7	A7	0.00%	4210.91	156.25	154.73	0.00%	-0.97%		☑
Standard8	A8	0.00%	7668.86	312.50	294.55	0.00%	-5.74%		☑
Standard9	A9	0.00%	13642.20	625.00	565.27	0.00%	-9.56%		☑
Standard10	A10	0.00%	27898.47	1250.00	1480.59	0.00%	18.45%		☑
Standard11	A11	0.00%	36523.02	2500.00	2496.47	0.00%	-0.14%		☑
Standard12	A12	0.00%	41338.03	5000.00	3624.52	0.00%	-27.51%		☑

Within the Regression Table, standards can be enabled and disabled. The manual entry of expected concentrations can also be performed in this window.

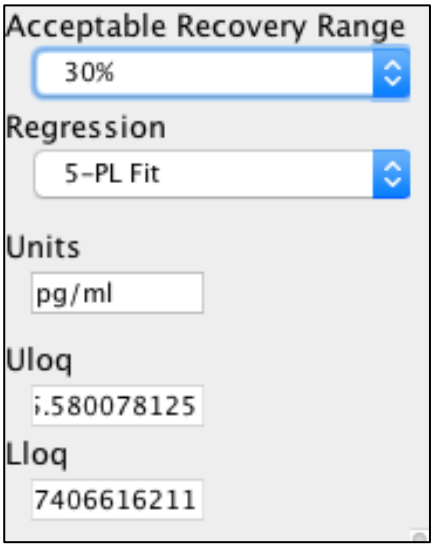
Right click to display options to configure the table:



- Serial Dilution (Descending and Ascending): allows for the setting of expected concentrations via a serial dilution (starting at the value of Standard 1).
- Manual Dilution: set the defined dilution factor using the format: *x (where x is the dilution factor)
- Copy Expected: once the dilution factor has been defined, the settings determining the Expected Values can be copied from one analyte and pasted into the Regression Table of other analytes.
- Paste Expected: this option becomes available after copying the dilution settings from one analyte. This option allows the settings to be pasted into the Regression Table of other analytes.
- Clear Concentrations: clears all concentration settings in the Expected column.

- Wells: displays the well ID and FI for an individual sample.
- Calculate best fit: performs the best fit algorithm for the standards.
- Apply Expected Values to All: this applies the Expected values from one analyte to all others in the analysis.
- Export Table: export the table as either a CSV file or an Excel XML Workbooks file.
- Table Setup: define the number of decimal places and the text font used in the table.
- Print: allows for the printing of the Regression Table.

Within the Regression Table panel are settings to define the Acceptable Recovery Range, Regression type and the concentration units. These are as follow:

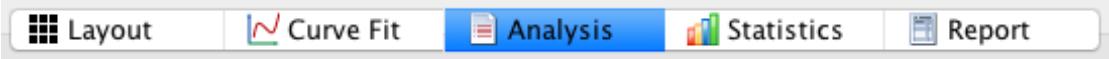


- Acceptable Recovery Range: the percentage for which a given standard's expected value can fall in based on the regressed value.
- Regression: use to set the current regression type. The options are:
    - Linear
    - Linear (Point to Point)
    - 4-PL Fit
    - 5-PL Fit
    - Cubic Polynomial
- Units: the units to be displayed in reports and tables for a given analyte
- Uloq and Lloq: the upper and lower limits of quantification. These values represent the highest and lowest fluorescence intensity values from the standards.

Library

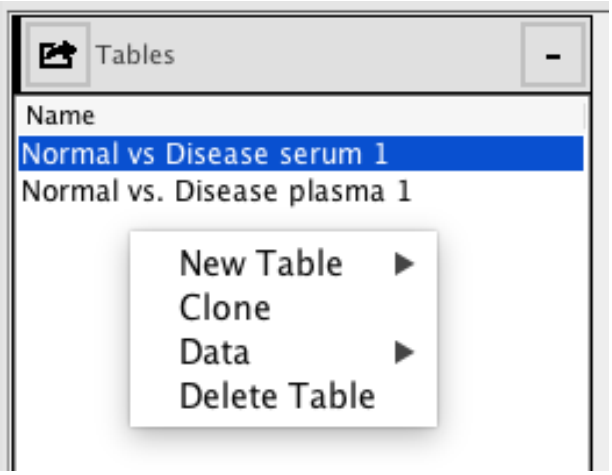
Click this button to import and export all settings created in the Layout tab. See page 29 for more details.

Analysis



The core of the analysis area is data mining and preparation for statistical analysis and reporting.

Tables



This is the primary list of tables, which are used for data mining and analysis. When a plate is imported a table is automatically created but a table should not be mistaken for a plate. Tables can contain the data from many plates. Rename a table by double clicking on its name and typing the new name.

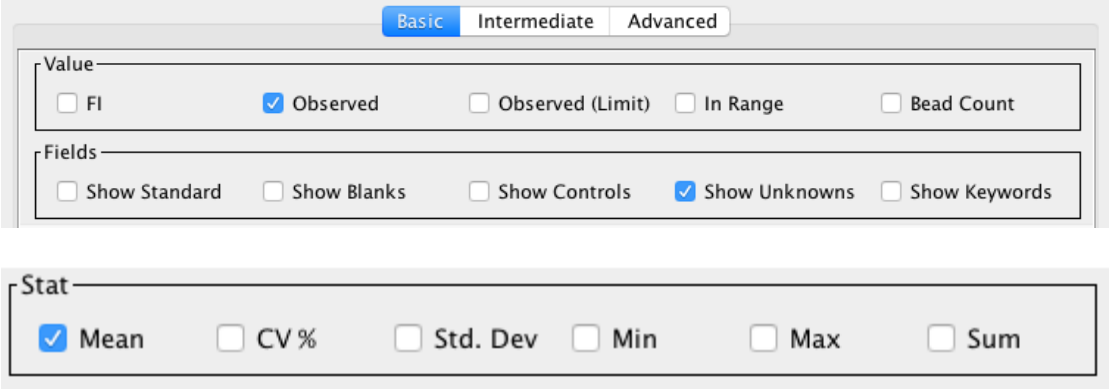
A right click within the table window brings up a menu with the following features:

- New Table: creates a new table from a plate containing data.
- Clone: creates an identical copy of the highlighted table.
- Data: allows data from individual plates to be enabled/disabled.
- Delete Table: deletes the highlighted table.

Table Setup

Three levels of control are available for data mining: Basic, Intermediate and Advanced.

Basic



This is the simplest form of table generation. It does not provide any mining or aggregation tools, although it does make it simple to quickly produce a table of results. There is a number of buttons that enable/disable columns and rows in the data table. These are located in different subsections.

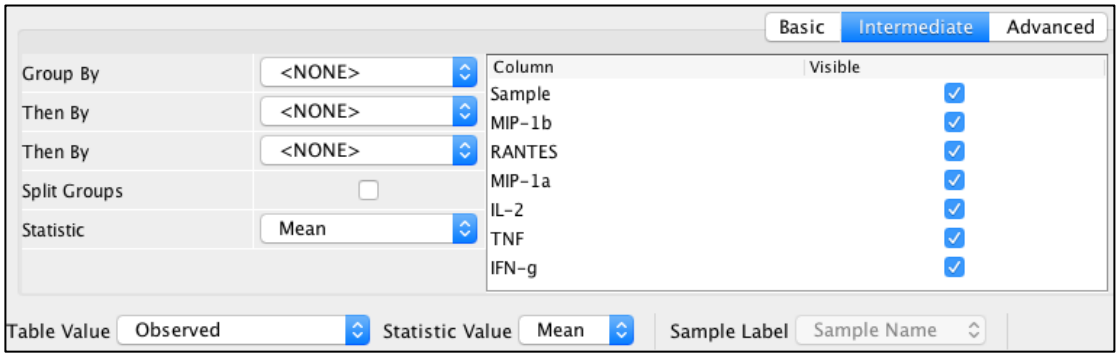
- Value
  - FL: displays the Fluorescence Intensity columns in the table for each analyte.
  - Observed: displays the Observed value columns for each analyte.
  - Observed (Limit): displays the Observed (Limit) columns for each analyte.
  - In Range: displays the In Range columns for each analyte.
  - Bead Count: displays the Bead Count columns for each analyte.

- Fields
  - Show Standard: displays all rows that where wells have been marked as standards.
  - Show Blanks: displays all rows where wells have been marked as blanks.
  - Show Controls: displays all the rows where samples have been marked as controls.
  - Show Unknowns: displays all rows where wells have been marked as unknowns.
  - Show Keywords: displays all the ‘Text’ columns for a given table.
- Stat
  - Mean: displays the mean of the replicates for each displayed column.
  - CV %: displays the coefficient of variation between replicates for each displayed column.
  - Std. Dev.: displays the standard deviation between replicates for each displayed column.
  - Min: displays the minimum value of the replicates for each sample for the displayed columns.
  - Max: displays the maximum value of the replicates for each sample for the displayed columns.

- Sum: displays the sum of replicate values for each given sample for the displayed columns.

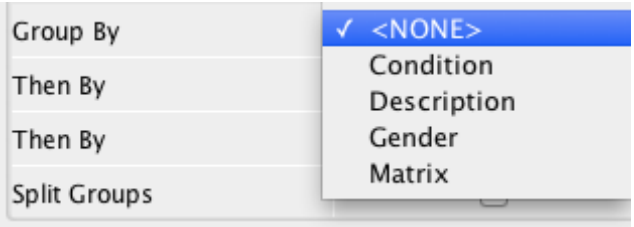
- Sample Label: choose between displaying the sample name or other sample descriptions as defined in the Attributes panel of the Layout tab, through the right-click menu and the Add/Remove Keywords option.

Intermediate



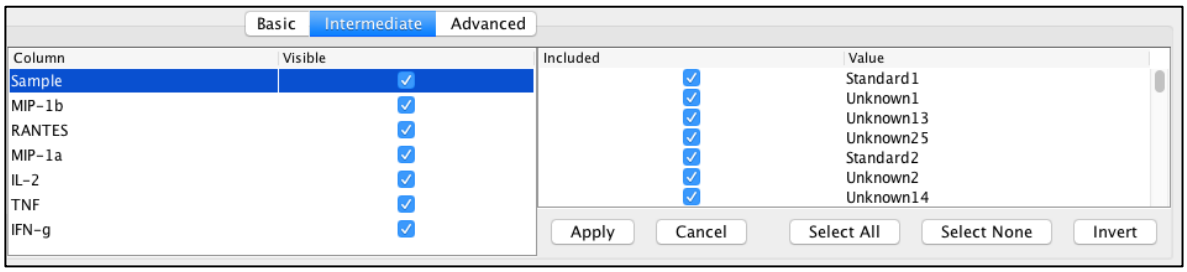
The intermediate table setup allows for both data aggregation as well as data filtering and grouping. The features contained within the Intermediate window include:

- Group By: creates an initial level of grouping. Once a column is selected as available for grouping, all of the rows will be 'merged' with the selected aggregation statistic.

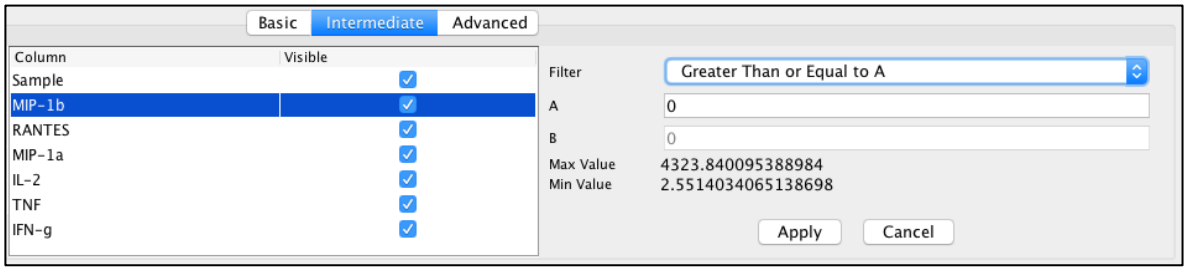


- Then By: provides the second and third levels of grouping.
- Split Groups: when enabled, the sorting is kept but the groups are split back into their samples. This can be useful for a quick view of the source data without having to modify grouping settings.
- Statistic: allows for the selection of the aggregation statistic for grouped data.
- Table Value: allows for the selection of the source statistic for each sample.
- Statistic Value: allows for the selection of the statistic calculation used to generate a single sample value.
- Sample Label: allows the Value in the 'sample' column to be changed.
- Visible (column): allows data columns to be made visible/invisible.
- Sample Filter: Click on 'Sample' under 'Column' in the table to view the entire sample list to the right. Make a selection using the check boxes in the 'Included' column, or by using the buttons below the panel, and click apply.

The resulting sample list and statistics will be displayed in the Data Table below the Table Setup window.

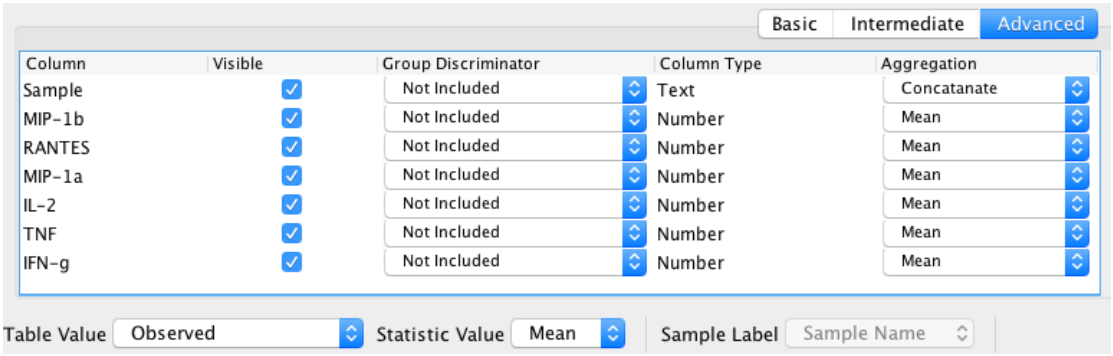


- Numeric Filter: use the numeric filter to remove rows that do not meet certain criteria. The numeric filter will appear when a row is selected in the table.
-



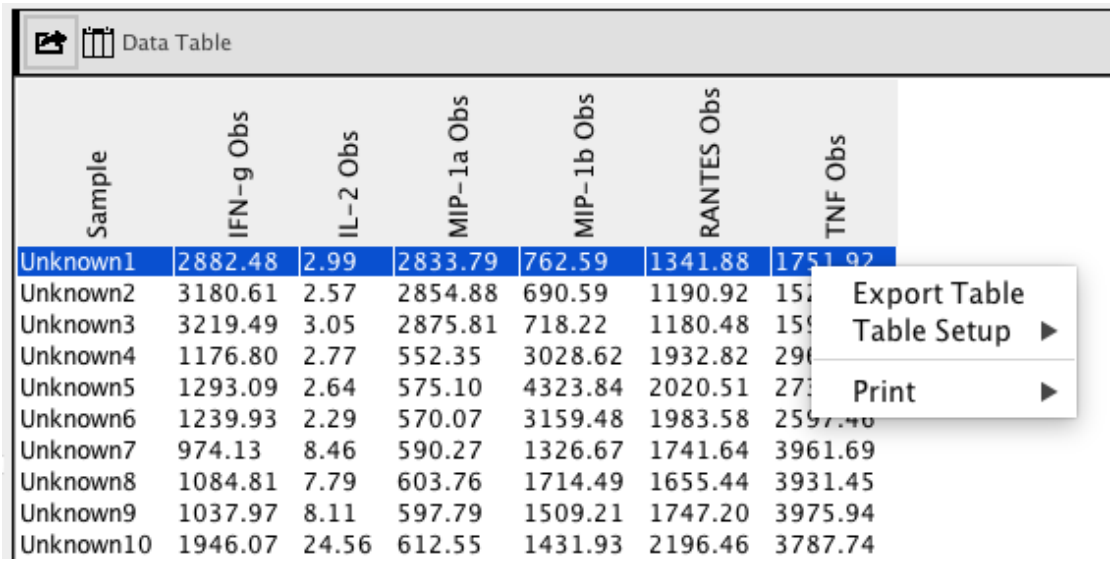
- List table: once the setup is complete, click the 'Apply' button to apply the filter. There are a number of filter options available.
    - None: no filtering is performed.
    - Equals A: when a value from the given column matches the value entered in the text box for 'A' it will be included in the output.
    - Not Equals A: when a value from the given column does not match the value entered in the text box for 'A' it will be included in the output.
    - Less than A: when the value for the given column is less than A it will be included in the output.
    - Greater than A: when the value for the given column is greater than A it will be included in the output.
    - Less than or Equal to A: when the value for the given column is less than or equal to A it will be included in the output.
    - Greater than or equal to A: when the value for the given column is greater than or equal to A it will be included in the output.

Advanced



The advanced table setup is similar to the intermediate table with the exception of having a full grouping window where the grouping level of any given analyte can be set from the main editor.

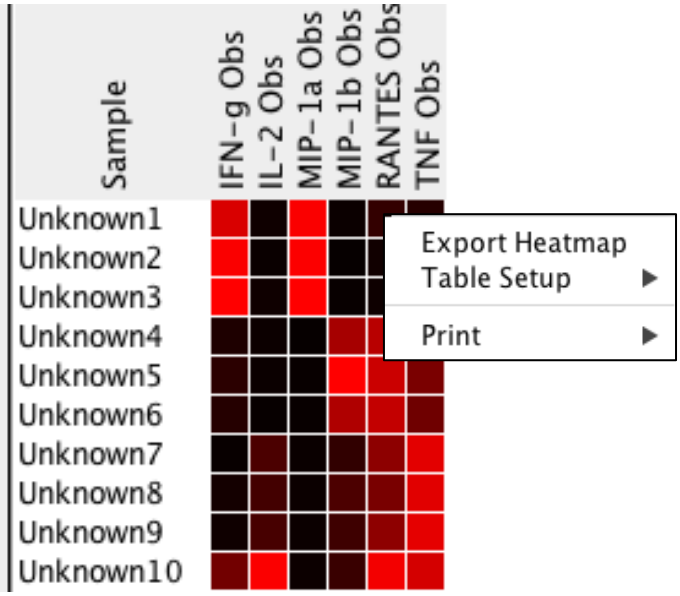
Data Table



Right click in the table and select from the options to Export the data, define the number of decimal places, change the font and print.

By clicking on the table column headers you can sort the rows by a given column's values in either ascending or descending order.

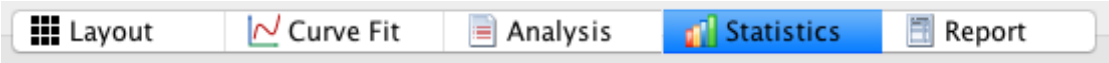
Quick Heatmap



The quick heatmap has all of the basic functionality of the 'Data Table'. Each column's values are separate in regards to the heatmap calculation and therefore the color, which is defined as the 'maximum' for that particular statistic. The heatmap is updated as the selections made in the Table Setup tab are changed.

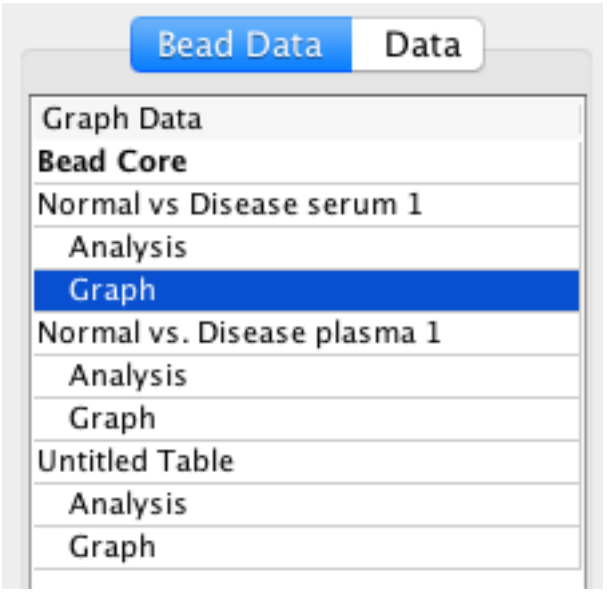
Color Scheme: this drop down box different color options for the heatmap. The different color options are:

Statistics

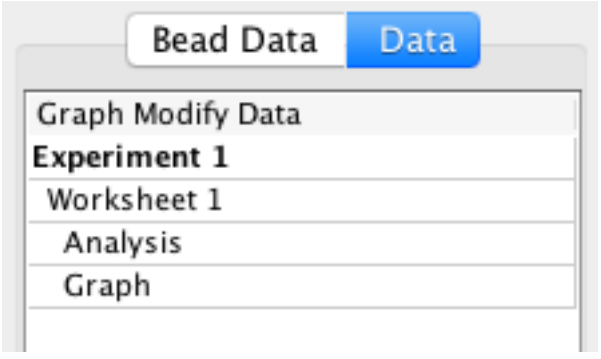


Within the Statistics tab, values displayed in the Data Table of the Analysis tab can be graphed and analyzed.

Bead Data: located to the left of the worksheets, the Bead Data window contains a list of all the 'tables' that have been built in the Analysis section. A default graph and analysis is created for each table. Double click the graph name to open it in the workspace above the worksheet. Right click on the graph name for options to rename it (along with adding a new experiment, opening a graph in the graph workspace and deleted the highlighted row.



Data: the data section allows for mathematical manipulation and concatenation of data tables.

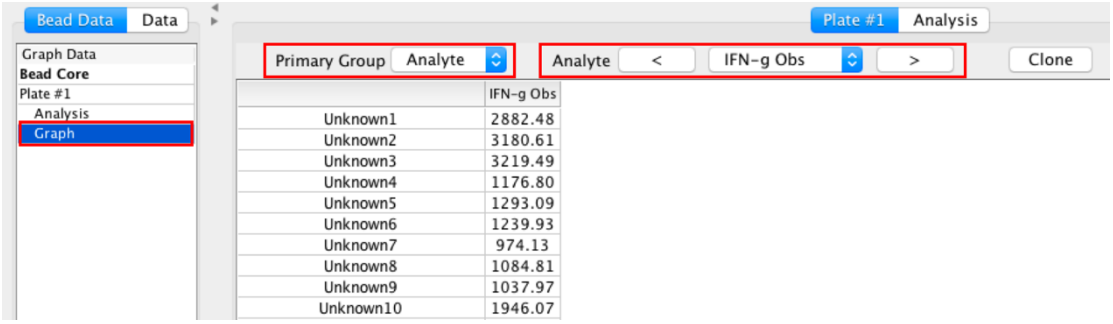


Graphing data

Display all of the relevant data in the Data Table of the Analysis tab and choose the specific samples that you wish to be included in a graph. Note that the different values and specific analytes can be chosen in the Statistics tab but the selections of samples cannot.

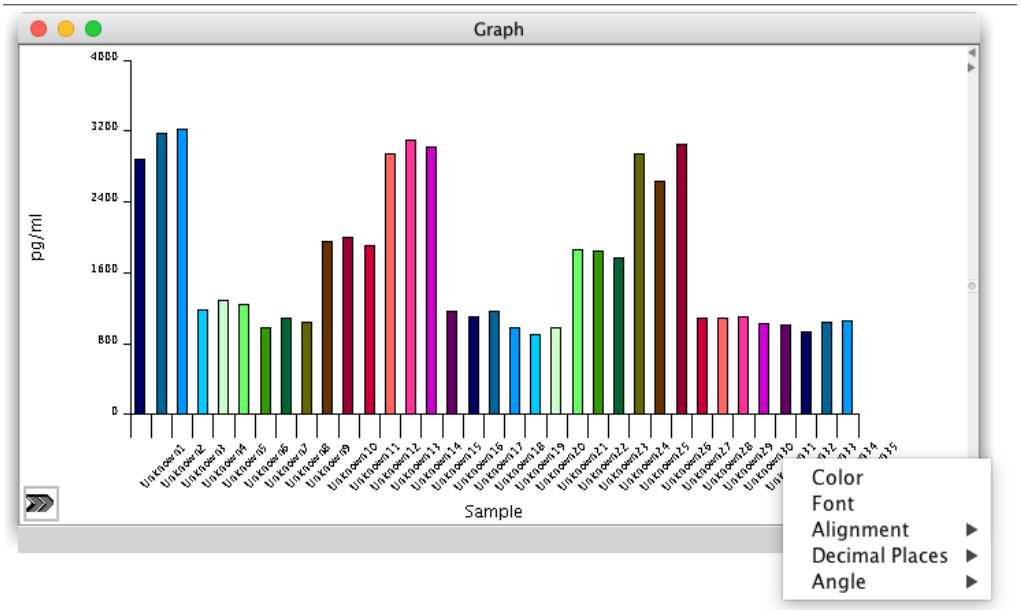
Once all desired data and the correct samples selection has been performed, return to the Statistics section. Choose the specific plate in the Bead Data window and the same data displayed in the Data Table will be displayed in the data window to the right.

Select populations from the drop down menus for the Primary Group and the Analyte. The table will automatically update to show the defined data. To open a graph for the defined data, double click or right click → Open Graph the 'Graph' tab in the Bead Data tab. The graph will be displayed in the graph workspace above the data tables.

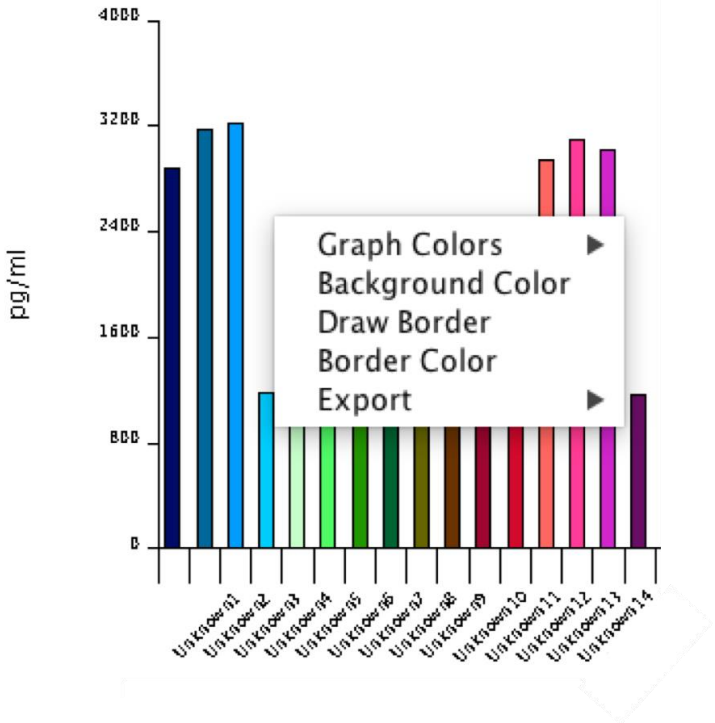


	IFN-g Obs
Unknown1	2882.48
Unknown2	3180.61
Unknown3	3219.49
Unknown4	1176.80
Unknown5	1293.09
Unknown6	1239.93
Unknown7	974.13
Unknown8	1084.81
Unknown9	1037.97
Unknown10	1946.07

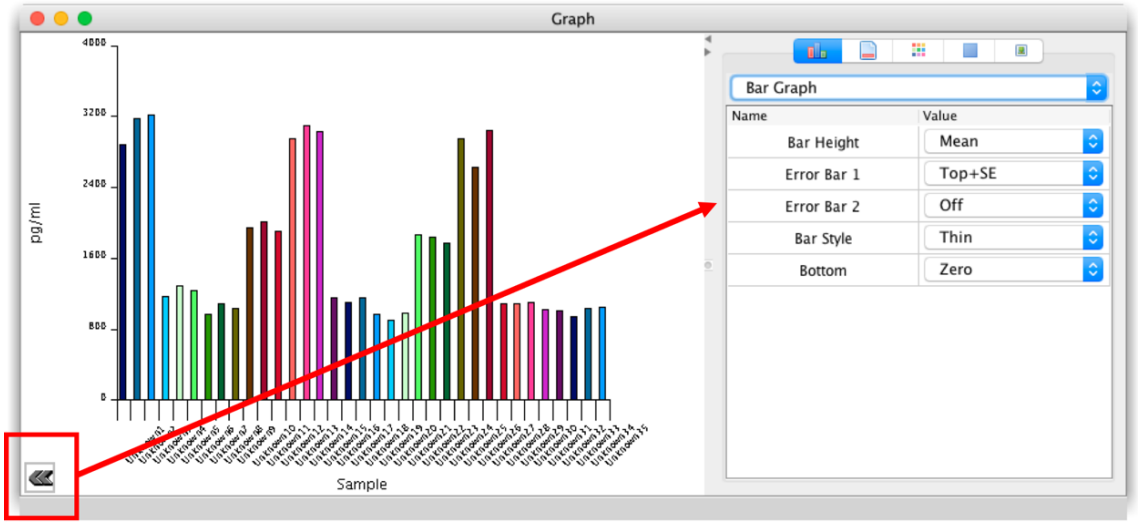
Right click on each axis of the resulting graph to display options to set the font type, color and alignment, define the number of decimal places and change the angle of the text.



Right click within the graph area to display a different menu with options to change the color of individual samples, the background color, turn the graph border on or off, set the border color and export the graph.

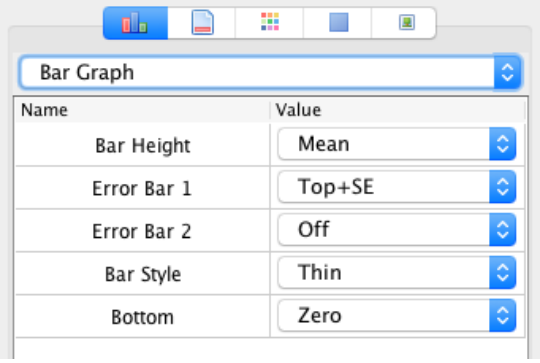


Each graph has a side drawer containing a range of formatting options. This can be opened by clicking on the double arrows at the bottom right of the graph.

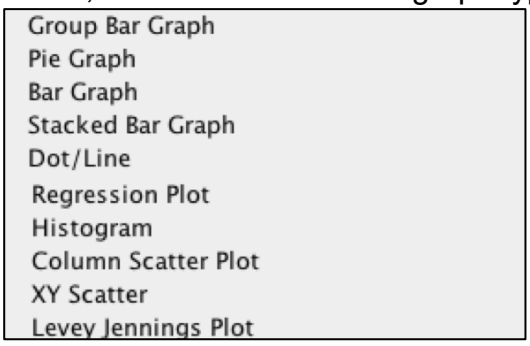


The side drawer is divided into five tabs: Graph Settings, Graph Axis Settings, Graph Color Settings, Graph Legend Settings and Graph Border and Background.

Graph Settings



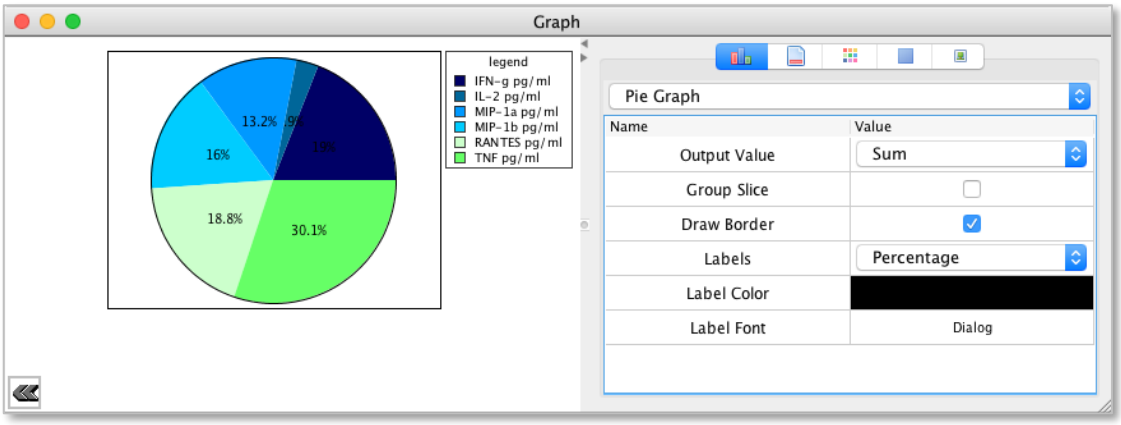
From the drop down menu, choose from different graph types:



Different graph formatting options will appear in the window depending the type of graph chosen.

Graph types:

Pie Graph



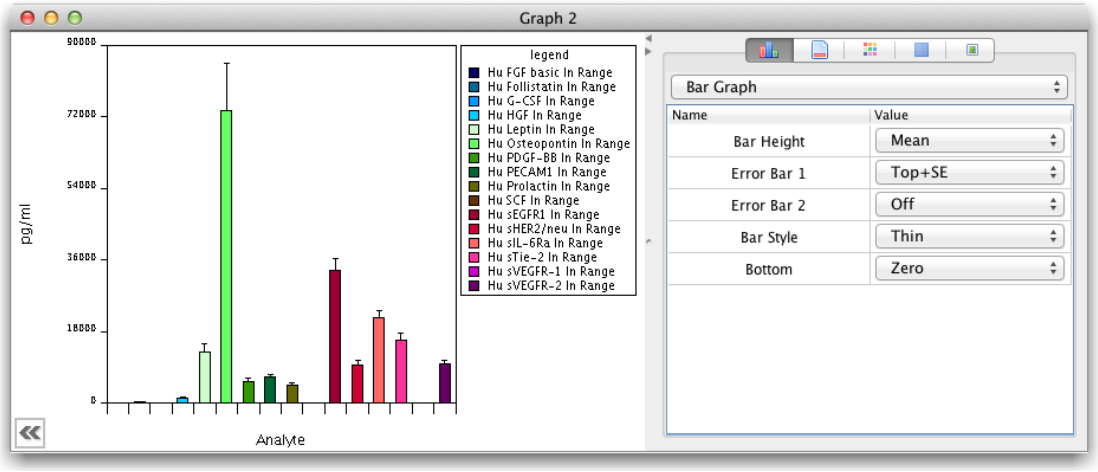
- Output Value: allows for the selection of the statistic, which determines the output value of each slice.
- Group Slice: turns the pie graph into a ringed pie graph.
- Draw Border: enables/disables the border of each ring.
- Labels: allows the Selection of ‘None’, ‘Percentage’, ‘Value’ or ‘Name’ as the labels for each slice.

- Label Color: allows the color of the labels to be changed
- Label font: allows the font of the labels to be changed.

Group Bar Graph

- A bar graph that shows individual values without statistical manipulation.
- Interleaved: if enabled, each group column is interleaved with the others.
- Draw Border: enables/disables the border around each column
- Border Color: allows the color of the column border to be changed.
- Gap: define the gap between columns.
- Group Gap: define the gap between groups.
- Gap Alignment: allows groups of columns to be justified in different parts on the x-axis.

Bar Graph



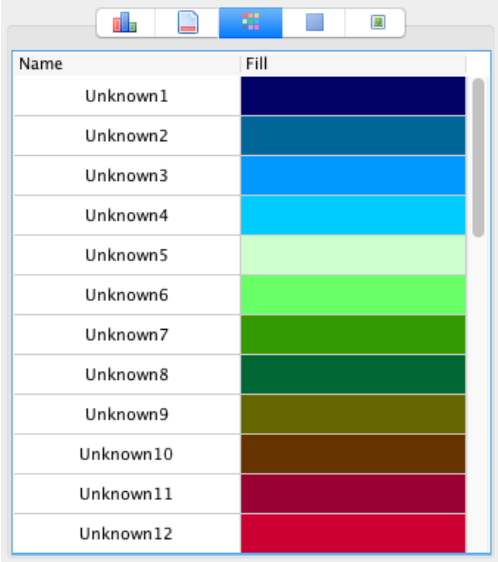
- Includes statistical manipulation on values within groups to provide each portion of the bar. This Graph type can also be used to create box and whisker plots.
- Bar Height: allows the height of each column to be defined based on a statistic for a given group
- Error Bar 1: allows the position of one of the error bars to be defined based on a statistic for a given group
- Error Bar 2: allows the position of the other error bars to be defined based on a different statistic for a given group
- Bar Style: allows for the manipulation of how the error bars are drawn
- Bottom: allows for the location of the 'bottom' of the bar to be set.
- Stacked Bar Graph: the stacked bar graph shows multiple bars either stacked or overlapped
- Superimposed: when enabled the bars are overlapped. Otherwise, they are stacked on top of each other.
- Gap: allows for the setting of a 'gap' between bars.
- Alignment: the alignment of the bars on the X axis.
- Dot/Line
- Draw Lines: if enabled, lines are drawn

- Draw Points: if enabled, points are drawn instead of lines.
- Regression Plot
- Histogram
- Column Scatter

Graph Axis Settings

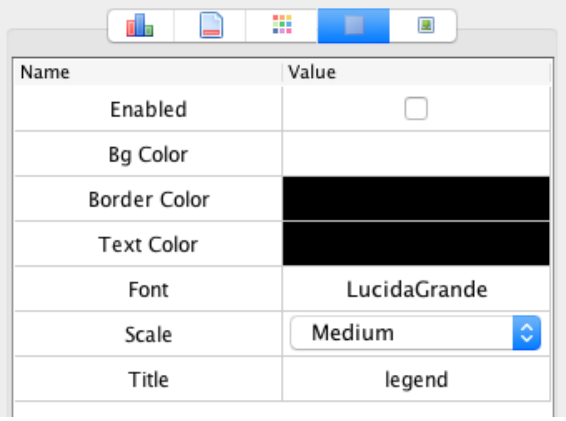
Various formatting options for the x and y axes are available in the Graph Axis Settings tab. These are divided into the categories of General, Labels, Scale, Major Tick Lines and Minor Tick Lines.

Graph Color Settings



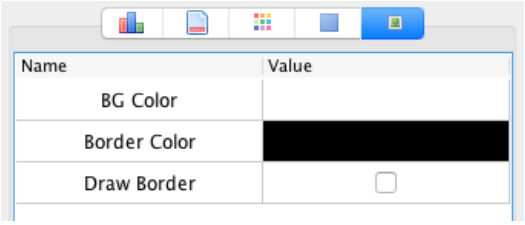
Click on the Fill color associated with a particular sample to choose a new color.

Graph Legend Settings



Format various elements related to the graph legend.

Graph Border and Background

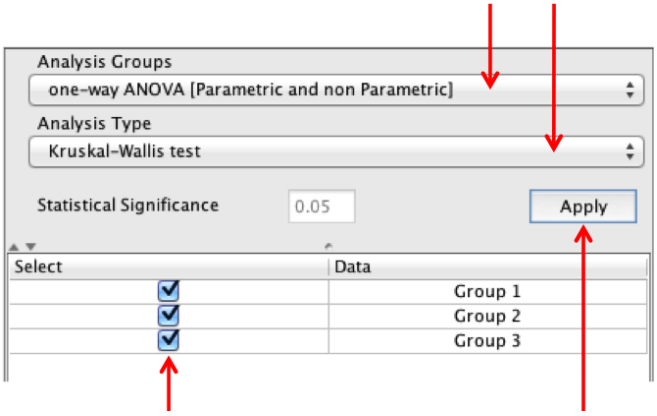


Choose a color for the border and graph background. Choose to display or hide the border.

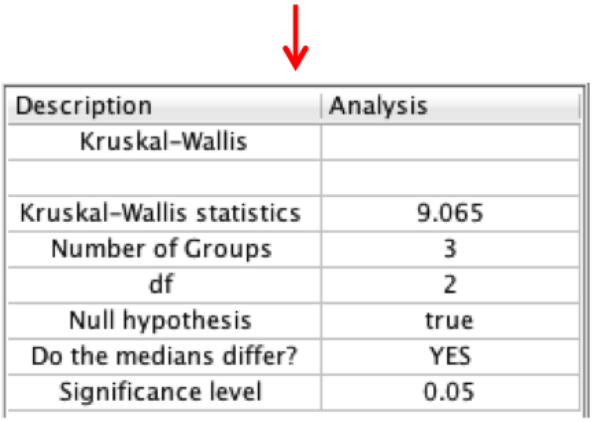
Statistical Analysis

To perform a statistical analysis on data contained in a graph, in the Statistics section click ‘Analysis’ associated with the particular graph in the Bead Data window. This displays the available statistical tests and the list of samples to compare in the ‘Analysis’ window.

To perform a statistical test, choose an Analysis Group along with an Analysis Type from the drop down menus.



Select the groups that you wish to compare and click ‘Apply’. The result will be displayed in the Description and Analysis columns adjacent.



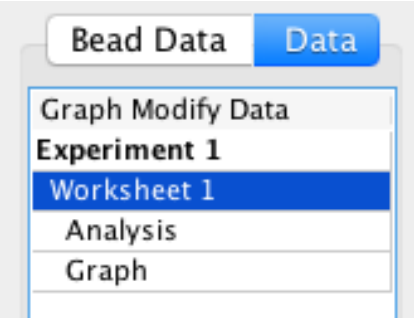
Description	Analysis
Kruskal-Wallis	
Kruskal-Wallis statistics	9.065
Number of Groups	3
df	2
Null hypothesis	true
Do the medians differ?	YES
Significance level	0.05

The options with the Analysis Group are:
- t tests (Parametric and non-Parametric)
- One-way ANOVA (Parametric and non-Parametric)

For t tests, the options with the Analysis Type are:
- Unpaired t tests with Welch-Satterthwaite approximation
- Unpaired t tests
- Unpaired t test Equal Variance
- Paired t tests
- Mann-Whitney

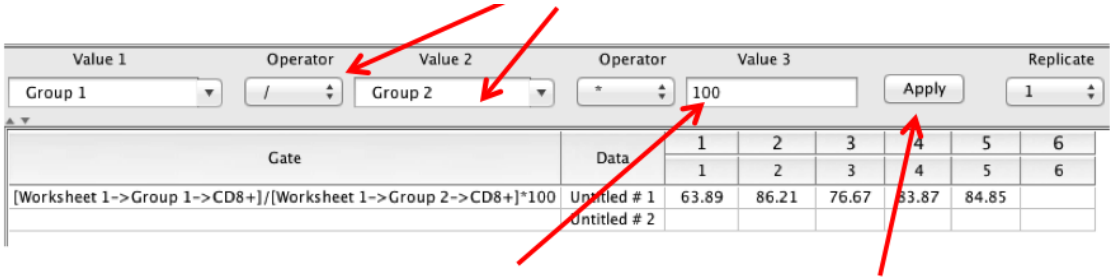
For **One-way ANOVA,** the options with the **Analysis Type** are:
• One-way analysis of Variance
• Kruskal-Wallis test

Another option for graphing data is in the Graph Modify Data Window. This can be accessed by selecting a Worksheet in the Data window (next to the tab for the Bead Data window.



This is where calculations involving group data (as displayed in the Graph Data Worksheets) can be performed. For example, proportions of one group (or individual samples) can be compared to another, with the result automatically converted into a new graph.

For example, the percentage that Group 1 is of Group 2 can be calculated as follows:

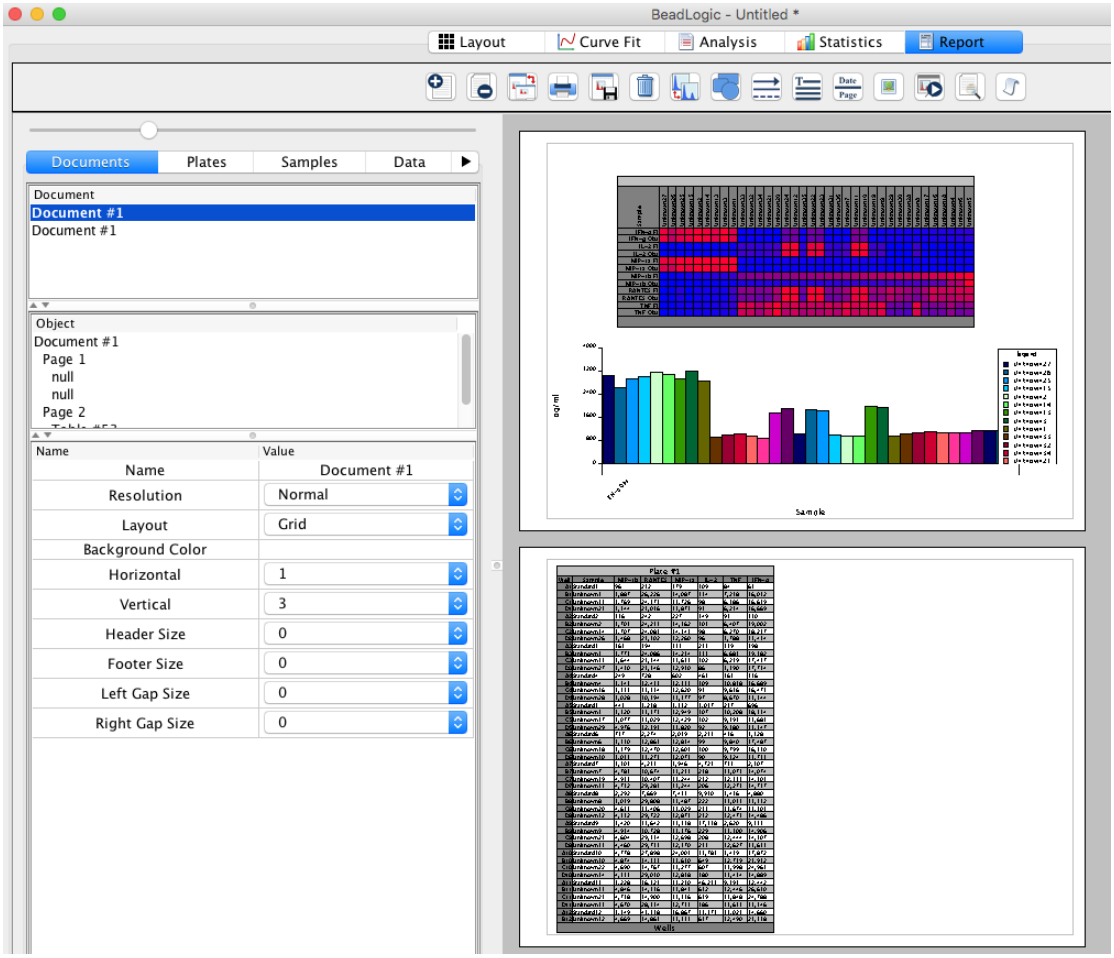


Enter a number in the Value 3 field and click ‘Apply’.

By default, the first graph in the Graph Data window is titled ‘Graph 1’ and the first Graph in the Graph Modify Data window is ‘Graph 2’. This is why when adding a new graph to the Graph Data window it appears as ‘Graph 3’. Names of all graphs, analyses, worksheets and experiments can be renamed by clicking on the title when it is selected.

Report

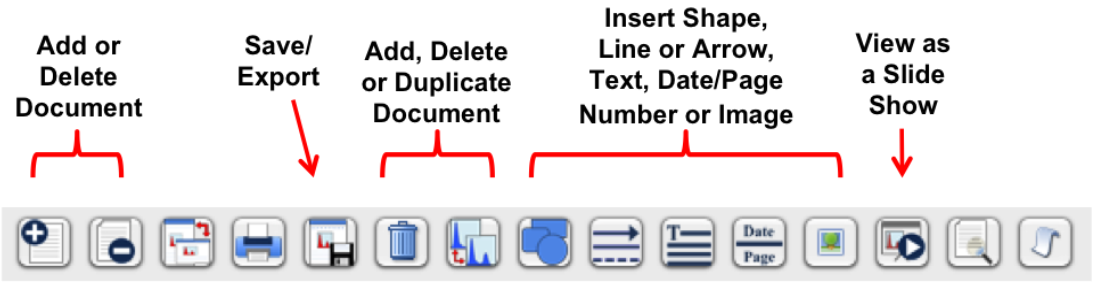
Reports can be generated, containing any of the elements created as part of the data analysis.



These elements along with various formatting options can be selected from the panels below the tabs to the left of the page layout. These tabs are:

To add an element, click and drag it from the panel directly onto the page.

Reports can be annotated and edited using the elements in the toolbar at the top of the screen. These include:



Layout and library templates: To create a layout template, click the template button in the toolbar and press ‘Save’. This allows the user to save a basic report template including headers and footers. Data is then added to the report manually. To save a Library Template, create a report including all data (graphs, tables, heatmaps, etc.). Then, click Library at the bottom of the screen and click ‘Save’. Enter the library name and description and click ‘Ok’. To load a Library Template, select ‘Library’, choose a library and click ‘Apply’. This will load the library template and repopulate all the data-associated elements with the results from the new analysis.

New or Delete a Document - Documents can be organized in the **List of Documents** tab above the file inspector.

Page Setup - Change the page layout between portrait (**Screen**) and landscape (**Paper**).

Print and **Save as PDF**.

Add a plot, delete item and duplicate plot - Highlight the appropriate population or document, either in the samples list or on the page layout, to add, delete or duplicate it.

Add stats table – Highlight the populations in the Navigator that have had statistics applied to them. Then, click the button and drag it onto the page.

Add shapes, arrows/lines and text – click on the appropriate button and drag it to the page layout. These can be resized once they are on the page.

Date and Page Numbers – click to add the date or page numbers to the document.

Add Image – insert an image from a file

Slide Show – Once in slide show mode, progress the slides forward by clicking the mouse. Right click to provide more navigation options.

Display Preview Page – Click on any page in the preview to jump straight to that page.

Click through the tabs above the File Inspector to customize and manage your document.

A default report showing all regression curves can be generated by right clicking on an analyte in the Analytes panel in the Curve Fit tab and selecting ‘Send all to report’ → ‘Default Analyte Report’.