# WinList User Guide

This guide is intended for WinList 9.0 and minor version updates.

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**WinList™ 9.0 User Guide**

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**Verity Software House**

Phone:      (207) 729 6767

Fax:          (207) 729 5443

Email:       verity@vsh.com

Web:         www.vsh.com

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WinList also has a Check for Updates command in the Help menu. Use this command periodically to see if there are updates for your software. The command can download and install updates that are available, if you have administrative rights to your computer.

**What's new?**

<div style="background:#cccccc">

**WinList 9.0**

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**Regions, gates, and statistics**

Contour regions -
automatically update as the data beneath them changes. These regions feature a user-defined probability level and can also automatically move with data.

Sticky regions -
is a feature that allows vertices of adjacent regions to stick to one another, making a seamless boundary between the regions. As a common vertex is moved, it affects all of the regions that share it. A great tool for fitting regions together tightly.

Parameter statistics -
can be computed on gates using the Gate Statistics dialog. Select the parameters and statistics that you want to compute and the program will add them to the Gate Stats display. Values can be added to database and reports, too.

Highlight-this-region -
is a new coloring mode that allows you to see where the events for a given region are in all other dot plots. The events can be enhanced with increased size and blinking, making it easy to find those small populations even in noisy plots.

Improvements have been made to auto gating capabilities of regions. Regions can now position themselves based on the mean, median, or mode of the data beneath them, or of the data in the histogram.

Performance has been greatly improved for large, complex protocols with many regions and gates.

Alerts -
can now be defined to compare any two values that WinList stores as keywords. For example, you can now compare the number of events in two regions as the alert condition.

Robust SD (rSD) and CV (rCV) statistics have been added, using the interquartile range method for computation.

The database wizard has been enhanced to work with results windows and the gate statistics window. It provides a quick and easy way to add all of the results as fields to a database.

## Calculated Parameters

●New t-SNE parameter -
t-SNE is a method for clustering and dimension embedding. We've added a new calculated parameter that implements Laurens van der Maaten's clustering algorithm known as t-SNE: t-distributed Stochastic Neighbor Embedding. This is a great tool for finding related events in multidimensional space and displaying them in 2D space. See https://lvdmaaten.github.io/ for more info on the background of this routine and have a look at this youtube video of a google tech talk: https://www.youtube.com/watch?v=RJVL80Gg3lA.

●The dialogs for creating and editing calculated parameters have been greatly simplified.

●The resolution for calculated parameters can now be set independently for each parameter. There is no longer a common resolution of 1024 forced for each calculated parameter, so the natural range of the parameter expression can be directly controlled by the user.


## Compliance Mode

●Compliance Mode -
Support has been added for using WinList in "Compliance mode", which is designed to assist labs with regulatory requirements. In this mode, Windows user groups are set up to specify the permissions each authorized WinList user has. The program performs additional logging of user actions, and requires the user to confirm the Windows login when the program starts up.

●Logging has been enhanced to provide better support information for 21 CFR Part 11 compliance.

●WinList now computes a cyclical redundancy check (CRC) value on the FCS files it reads. Some FCS files contain a CRC value that allows WinList to verify that it has read the file correctly. This CRC value can be reported and databased to help support regulatory documentation requirements.


## Compensation

●The V-Comp app -
can now be launched directly from the Compensation Toolbox in WinList. V-Comp setups can be opened directly into WinList, and the V-Comp app can be easily launched to create additional setups.

●WinList now supports " label-specific " compensation setups created by V-Comp. These setups allow the program to use the best compensation match for any antibody-fluorochrome combination.

Compensation Review mode -
This option changes all transforms to Linear and zooms histograms to the 95th-percentile of the data. This is a great tool for evaluating compensation in single-color controls - you will *really* see how well your compensation is working.

## Transformations

New log-like transforms - Hyperboline Sine and V-Log - have been added. These provide several advantages over the HyperLog transform, maintaining evenly-spaced decades.

Linear to log is now quick as pushing a button. For FCS files that do not provide hints about log transformations, you can now switch linear parameters to your favorite log-like transform with one click.

Log-like transforms now support decades between 0.0 and 1.0

Linear transforms now support displaying negative values.

## More

File Export improvements -
WinList now supports sampled output, as well as file merging of compatible FCS files.

The Save button on the Home tab (or toolbar) can now be set up to function in 3 separate modes: as a Save (no dialog) button, as a Save As (with dialog) button, or as a Save Bundle (with dialog) button. The behavior is controlled by the "Save Button" setting in Options->Preferences->Miscellaneous. When the button is configured to Save Bundle, the Open button will also default to opening a bundle.

Histogram subtraction enhanced -
Subtraction settings are now saved in protocols and restored when a protocol is re-opened. We've also added the Mann-Whitney U Test and Area-Under-Curve (AUC).

## Bug fixes

In some cases it was not possible to create a new Compensation Page from the Compensation Toolbox. This could happen if a data source had 2 or more pages plus the Compensation page, and one of the other pages was then deleted. Corrected. (mz, 8Oct2015)

When reading integer FCS data files, WinList could incorrectly interpret data with resolutions that were not powers-of-two. As an example, the Time parameter in Stratedigm's SE520EXi files has a resolution that relates to the duration of the acquisition, and WinList did

not correctly read or display the Time parameter. This has been corrected. (vsh, wl-75, 6Aug2015)

On some computers, WL could crash when dropping an Overlay window onto a report. This has been corrected. (ks-rpci, 21Aug2015, wl-68)

The program did not correctly display parameters that were stored as log-encoded data values in the FCS file and then viewed as linear. This has been corrected. (dh, 3Jun2015)

1P regions that were turned off for coloring did not redraw correctly when the size was adjusted. This has been corrected. (bh, 24Jun2015)

When histogram or result window "Remote" scripts to other apps took a long time to complete, the user interface gave no indication that it was waiting for the script to finish. The user was able to manipulate virtually anything, include opening a new file, while waiting for the script to complete. This could cause the program to crash or display data that was out-of-sync. The interface is now disabled during Remote script processing and an hourglass cursor is displayed. (ab, 17mar2015, wl-25..29)

Index Analysis plots would cause the program to crash if the selected parameter had a lower range ($PnR) than the number of (rows x columns) in the plot. This has been corrected. (mcgill, 25mar2015, wl-30)

For some older file types, WinList used to detect log-encoded parameters based on the name of the parameter including "LOG". This logic interferes with newer file types that are actually stored as linear but have "LOG" in the parameter name. Logic has been revised to address this to work correctly for both old and new file types. (cb, 26mar2015, wl-31)

When reading a file from network or removable media, WinList could crash if the file became inaccessible during the read operation. The program now gracefully stops reading and alerts the user to the problem. (cp, 6apr2015, wl-36)

If a non-WinList compensation file was selected in the Open Compensation dialog, the program would crash. This has been corrected so that the user is alerted that the file is not the expected type. (dd, 7apr2015, wl-39)

The Export Histogram Data could fail if the histogram being saved had events that fell on the upper axis. This has been corrected. (jh, 13apr2015, wl-43,44)

If a histogram was "temporarily inactive" because of missing parameters, and it was edited, the program could crash if the user then chose to undo that edit. (jh, 13apr2015, wl-42)

In some cases where the number of files in a batch "group" was greater than 1, the program could create a non-fatal error that would render batch processing inoperable until the program was restarted. This has been corrected. (wl-11, am, 28Jan2015)

Export of listmode files did not remap all parameter keywords from the source file if only some of the source file parameters were selected for export. This created mismatches in voltage, gain, and several other keywords. Corrected. (wl-19, bh, 13Feb2015)

If parameters are enabled for compensation and Auto-Comp Each File is set, parameters could remain enabled for comp in a new file that does not specify compensation for them. Revised code now explicitly turns off any parameters that are not specified in the spill keyword. (wl-18, cb/bh, 23Feb2015)

Macro Toolbox limits file names and paths to 79 characters. Logic has been revised to handle full file path and name lengths for the toolbox. (wl-22, dh, 2Mar2015)

The program could crash during replay after acquisition if one or more histogram displayed dots and contours, and the packet size was smaller than the file size. Corrected. (jh, 3Jul2014)

Using the Create Region Array on a 1P histogram to create a single region caused the program to crash. This has been corrected. (mp, 24jul2014)

If a macro tool button is attached to a macro file and that file no longer exists, the program would crash when the macro button was clicked. This has been corrected. (ap, 21Aug2014)

Cascade gates did not work correctly when gating with a region whose name was contained by another region in the target histogram. For example, if gating from R11 onto a histogram that contained R1, the system would fail to build the new cascade gate expression. This has been corrected. (dh, 21nov2014)

The program could crash if Spots are defined on a file, and then a new file is opened which has fewer parameters than the original. Corrected. (ml, 1Dec2014)

## WinList 8.0
### Compensation

Introducing fully-automated compensation set up with V-Comp!

We've developed a new way to set up compensation that only requires the user to select the single-color controls and an unstained control.  No trace-line adjustments, no gates to position - it's hands-free comp set up that is fast and very accurate.  The system can also optimize HyperLog transforms and even adjust data based on the unstained control.  That means that "zero" on the axis can really mean "zero detectable fluorescence".

We call it Verity Comp, or V-Comp for short.  Click the "V-Comp Wizard" button on the AutoComp tab of the compensation toolbox to try it out.

If the compensation dialog is set for "Auto-comp each file", it no longer prompts for confirmation when you open a file with a different number of parameters.  It will automatically re-initialize the compensation based on the new file.  (mm/rs, 20Dec2013)

If AutoComp is enabled and the user adjusts a trace line, a notification is shown to the user. The notification also allows the user to turn off AutoComp.  (dh/bh, 16Sep2013)

The AutoComp tab of the Compensation Toolbox has been reorganized.  It now includes a field that shows how the current compensation was set up: "No compensation", "AutoComp from keywords", "V-Comp compensation", or "User-defined compensation".  (bh, 10Sep2013)

The Trace Lines list on the Setup tab of the Compensation Toolbox now only shows entries for parameters that have been enabled for compensation.  (la, 11Sep2013)

Several unused or unneeded options have been removed from the AutoComp tab of the Compensation Toolbox.  These include the Compensation Database options and the Auto-activate checkbox.  (bh, 28Aug2013)

N-Color Compensation has been enhanced to detect and ignore invalid compensation matrices. (cmb/bh, 18Dec2012)

**Overlay**

WinList now has a new window type specifically for overlays.  You can create overlays of 1p or 2p histograms, control the order and graphics, and select different viewing modes.  The new window has more options than were offered by MultiGraph overlays.

To create the new window type, choose "Overlay" in the Create menu.  Drag and drop a histogram onto the overlay to add an element.  All elements in the overlay must be compatible with the first element added.  For example, if the first item added is a 1p histogram showing a HyperLog axis with a low value of -200 and a high of 262144, all other elements in the overlay must also be 1p with a matching axis.

Elements in the overlay can be re-ordered by dragging rows in the overlay's legend.  A right-click context menu provides access to most of the actions that can be performed on the overlay.  Overlays can be added to reports, printed, saved as graphics, and copied to the clipboard.  (vsh, 10Dec2012)

**Reports and MultiGraphs**

●Several performance enhancements have been made for drawing large MultiGraphs.  (pw/bh, 5Dec2013)

●MultiGraph context menus now include "Save as Graphic" and "Export to PDF" commands. (bh, 3Dec2013)

●MultiGraph drawing on screen is now faster than previous versions, especially for MultiGraphs with elements that have lots of dots.  (pw, 25nov2013)

●Show All Results and Show All MultiGraphs now restore result and MultiGraph windows to the size and locations they used before they were hidden.  (bh, 19Jun2013)

●The Save MultiGraph Graphic option in the File menu now offers additional file formats for the graphic, including jpeg, png, tiff, and gif.  (rb, 22Jan2013)

●The Save MultiGraph Graphic dialog now points to the folder for MultiGraphs by default. The folder for MultiGraphs is now editable in the Wildcards and Pathways dialog. The name of the file to be saved is now based on the name of the MultiGraph. (ml, 23Oct2012)

●Conditional area objects on Reports can now be used to tile objects in a number of ways. The context menu for a conditional area has 3 new entries: "Tile objects RxC", "Tile objects to fit", and "Scale objects to fit".  The first command tiles the objects that overlap the container using the number of rows and columns defined in the properties of the container.  Tile object to fit will find the best arrangement of rows and columns to fit the objects into the conditional area object. Scale to fit will maintain the same relationship of objects that are contained by the conditional area, scaling them to fit into its confines.  (iCyt 1.5.5, 19Jun2012)

●Tiling commands and properties have been removed from the Report object.  They are now available in the Conditional Area object of the report. (bh, 20Jun2012)

●Reports now support multiple-selection of objects for moving and deleting.  (bh, 24Feb2012)

**Regions and Gates**

●The region context menu now has a "Color this region only" option.  When enabled, the coloring for the selected region is the only color used (except for "ungated" color).  This allows you to quickly check back-gating, identifying where the region's events appear in all other plots.

Create (Insert) Region Array now works much faster when creating large region arrays. This should eliminate "not responding" messages from Windows. (wm, 18Nov2013)

The handle for moving a region label is now drawn with the color of the gate that it relates to best. (bh, 9Oct2013)

The X and Y linear values for region vertices can now be edited by double-clicking the vertex. Note that linear values may be rounded when exiting the dialog. (jj, 11Jul2013)

The Region context menu now includes a "Links..." command that displays the Edit Region Links dialog box with the region selected in the list. (bh, 3May2013)

The Gate Statistics window now uses the "Alias" in the Edit Data Source dialog instead of displaying the file name. This allows users to customize the text of the Gate Statistics window (and gate legend on reports). The default alias for data sources is "[FILE]", which is editable in Preferences->Data Sources->Alias. The alias has also been added as a field that can be databased or displayed on reports. (be/la, 1May2013)

When a region was deleted by dragging its label off the left or bottom of the histogram, recreating the region could put the region's label in an invalid location. New logic now puts the region label in its default location when recreating a deleted region. Similarly, region labels are on-scale when undoing a region deletion that was done by dragging it off the left or bottom.

Logic has been added to update the gating hierarchy after edits are made in the Edit Gate dialog, as well as when regions are modified. This corrects situations where manually editing gates caused the hierarchy to be represented incorrectly in the Gate Statistics window. (dh/bh, 12Dec2012)

Region labels now only display the "move label" handle when the region or the label is active. The move label can still be selected, even when not visible. (vsh, 9Oct2012)

Auto-adjusting 1p and 2p regions can now adjust to capture the lower or upper percent of the histogram. Two new choices appear for the Auto Position Method: Include lower percent, Include upper percent. (bh, 7Sep2012)

The statistics shown in region labels are now independent of the alias for the region. Preferences and region dialogs have been modified to allow independent display of region name, alias, and a selectable statistic. WinList interprets and upgrades protocols created with previous builds to use the new region label properties. (iCyt 1.7.10, 5Jul2012)

Gates can now be set on multiple histograms at once.  Multiple-select the histograms by Ctrl-clicking the title bars.  Then use either the cascade gate tool or the gate tool on the histogram toolbar to choose a gate for the selected histograms.  (iCyt 1.6.2, 19Jun2012)

Freehand drawing of 2p regions now results in smoother regions.  (bh, 16Apr2012)

The region context menu (right-click on region label) now contains a command to initiate cascade gating.  This allows the region to be used for cascade gating even when the histogram is too small to show the region's cascade hot spot.  (la, 26Mar2012, iCyt 1.7.5)

Region vertices are now moved when the user changes the transform (linear, log, HyperLog) to maintain approximately the same position on the data. (bh, 22Mar2012, iCyt 1.7.7)

The Edit Regions dialog has been revised to use a table format instead of a simple list. (la, 14Mar2012, iCyt 1.7.11)

The mouse scroll wheel can now be used to inflate and deflate regions. Move the mouse inside a region and scroll up to inflate, or down to deflate the region. (vsh, 13Mar2012)

**Graphics**

Logic for coloring of 1P histograms in gate hierarchy mode has been improved to handle histograms with "No color subsets" selected in the graphic properties.  (dh/bh, 10Dec2013)

Index Analysis histogram and regions:

A new type of histogram window allows low-resolution classification parameters to be viewed in a 2 dimensional grid of wells.  The intended use for this Index Analysis histogram is for well index parameters from plate sorting experiments, but we expect there are other uses as well.

The Index Analysis histogram displays a filled circle for each index location that contains one or more events.  If more than one event falls in the same well location, a pie chart is used to show the colors of those events.

The Index Analysis histogram can be gated with any gate.

Regions can be created on the Index Analysis histogram.  These regions, called "groups", define a set of well locations.  The elements do not have to be adjacent wells.

Gates can be applied to Index Analysis windows, and gates defined in the Index Analysis can be applied to traditional histograms.

Histograms now have "Make bigger" and "Make smaller" entries in the context menu.  These commands map to the "+" and "-" key when the histogram is selected.  The commands increase or decrease the size of the histogram window by 50% for quick resizing up and down. (iCyt 1.7.2, 20Jun2012)

When a histogram is reduced in size so that the axis would take more than 25% of the space, the axis and fonts scale down automatically.  This only affects the display of a histogram window on the screen; it does not affect reports, MultiGraphs, or printing.  (la, 20Apr2012, iCyt 1.7.3)

Histograms on Reports now honor the "Keep square" histogram preference when resizing.  This restriction can be temporarily turned off by holding the Alt key down during the move/size. (la, 19Apr2012, iCyt 1.5.3)

**Performance and Usability**

Automatic recovery and crash reporting

We've designed a new automatic backup/recovery system that includes built-in crash reporting.  The system will save a copy of your protocol into "My Documents/WinList 3D/AutoRecover" every 2 minutes by default.  A Miscellaneous Preference "Auto-Backup Protocols" determines how often the backup is created.  This system is currently disabled in acquisition mode.  If the program closes normally, the auto-recovery files are deleted.  In the event of a crash or power failure, WinList will check the AutoRecover folder on next start-up and allow the user to recover from the last auto-save.

Integrated with this system is a new crash reporting system that creates a ZIP file with information that Verity can use to pinpoint the crash location in the software.  The Crash Reporter will offer to send a crash report to Verity, as well as to restart WinList if it crashes.  Users can choose whether to include the FCS file and WLX protocol with the crash report.  Crash reports are saved into "My Documents/WinList 3D/CrashReports".  (bh, 18Apr2013)

When WinList finds WLX files in the AutoRecover folder and the user chooses not to review those files, the program will now ask if the user wants to delete the files in the auto-recover folder.  If the user chooses "Yes" to this second question, all WLX files in the AutoRecover folder are deleted.  If the user chooses "No", the files are left in the folder.  (dtm, 12Jun2013)

Protocol bundles now open and save faster. (bh, 24Jl2013)

Protocol bundles can now store FCS files larger than 4GB.  (bh, 24Jul2013)

WinList now gracefully closes out when the user logs off the computer or Windows is shut down.  This is required for Windows 8 compatibility.  (bh, 12Jul2013)

⬤WinList displays the 10 most-recent protocols/bundles in the Ribbon Bar's "Recent Protocols" list.  This list is shown in the File menu (with the WinList icon on the left).  (bh, 8Jul2013)

⬤This build of WinList will use the Ribbon Bar interface by default (and it will announce that). Use Options->Preferences->Miscellaneous to change between ribbon bar and toolbar interface.  A restart of WinList is required after switching modes.  (bh, 5Jul2013)

⬤The program now displays a confirmation dialog when the user selects "Delete" from the Histogram context menu, allowing the user to cancel the deletion.  (bd, 5Jul2013)

⬤The batch toolbar is now displayed for batches of 1 or more files.  Previously it was displayed when there were 2 or more files.  (bg, 9Jul2013)

⬤There are several changes in the Edit Data Source dialog:

We removed the "New File" button. This functionality is handled by Get Next File or batch processing.

If a change is made in the Add Parameters or the Dataset dialogs, the Edit Data Source dialog is automatically closed.  This change was made because these two actions typically require the system to update everything.  Editing other aspects of the data source is not safe until after the update.  These changes were made to address several instabilities that arose from allowing edits to the different systems found in the Edit Data Source dialog.  (bh/rf, 18Dec2013)

⬤There are changes in the Names and Resolutions dialog (found in Edit Data Source).

The ability to edit the edit and lock the log bias for each parameter has been removed. If log bias is enabled (in the compensation toolbox), all parameters involved with compensation have the same log bias applied.  Once again, we highly recommend that users move away from using log bias at all.  (bh, 17Dec2013)

⬤The Batch button on the Home ribbon tab is now a splitter button.  By default, it executes the Create/Edit Batch command.  There are also commands for Next Batch and Previous Batch to allow navigation using the button.  (bg, 11Jul2013)

⬤We have added logic to handle Guava FCS files with invalid keyword/value formatting.  In addition, we no longer try to read keywords from the ANALYSIS segment of FCS files.  (bh, 12Jun2013)

⬤You can now protect histograms from being accidentally deleted.  Use the Edit Data Source dialog to enable the "Protect histograms" property.  With the property enabled, all

histograms on the data source are protected against user deletion.  The setting is also saved in a protocol or protocol bundle. (ms, vi 1352, 30jan2013)

Logging has been revised so that it can create larger (even unlimited) logs. A property has been added to determine the size limit, and the program checks this limit when it starts up and when it leaves the Edit Properties for Logging dialog.  When the log file reaches the size limit, it is renamed based on its modification date and time to preserve the log.

The log file now stores the newest entries at the end of the file to improve performance when logging is enabled.

There are now 2 logging modes: Basic and Debugging.  More detailed information is stored in Debugging mode.

Improved performance of FCOM calculated parameters and regions placed on FCOM histograms. (wm/bh, 22Mar2013)

Macro recording now supports creating histograms by clicking and dragging. (rf, 29jan2013)

Macro support has been added to allow recording and playback of selecting gates via the histogram toolbar.  (jb, 25jan2013)

Logic that fixes up FCS keywords in Beckman Coulter FC-500 files has been enhanced to work with more FC500 files.  This allows auto-compensation to work and for parameter names to be pulled from dataset 1 in more cases.  (bh/cmb, 20Dec2012)

A new option has been added to Miscellaneous preferences for loading preferences from bundles.  The new option is "Use bundle preferences".  This works like Prompt for preferences, except that no prompting is done.  With either of these settings, keep in mind that the preferences that are being loaded can change the setting of the setting.  In order for these settings to work optimally, all protocol bundles you save should have the preference set the way you want it.   (wr, 5sep2012, vi 1331)

Pressing and holding the Esc key will now cancel a macro without the Macro Status dialog showing.  (dh, 12Jul2012)

There is a new "Quick Next File" dialog box that can be used for the Next FCS File command.

The new dialog has the advantage that it selects the next (or previous) file in the list, making it quicker to process a sequential list of files.  To use the Quick Next File dialog, display the Preferences dialog and select "Data Sources".  The "Next File dialog" property allows you to choose the "Windows file dialog" or the "Quick file dialog".  (ml, 10Jul2012)

⬤When the region font preference is edited, existing regions now update their fonts to match the new preference.  (bh, 5Jul2012)

⬤When using "Open FCS File" or "Next FCS File", you can now select a WinList bundle. WinList will extract FCS file(s) from the bundle and allow it to be read into the current analysis setup.  (la, 18Apr2012, iCyt 1.4.6)

⬤Histograms in the Data Source window can be multiple-selected for moving, sizing, editing graphics, and adding to reports. To multiple-select, hold down the Ctrl or Shift key while clicking histogram title bars. To unselect, click on empty space in the data source window. To move the group, drag the hatched selection area. To size the group, drag one of the outside handles of the selection area. To edit the graphics, right click on a selected histogram and choose "Graphics".  To add the group to a report, right click and choose Add to Report.  To move or copy to another page, right click and choose Move or Copy to Page. To delete the group, right click and choose Delete.  (bh, 19Mar2012)

⬤XML Import:

You can now provide supplemental keyword information to WinList for the FCS files you analyze.  In Preferences->Data Sources, you will find an "Import Keywords" option.  Select one of the import options, and WinList will look for an XML file associated with each FCS file it reads. It can look for the XML in the same folder as the FCS file, or in some other folder you specify. The XML file must be named the same as the FCS file, with ".xml" appended to it.  WinList looks for the file when it reads an FCS file, and it parses the information in the file.  Keywords are added to the Keyword tree so that you can display the information on reports and in databases. (hv, 8Mar2012)

**Sony Plug-in**

⬤SY3200: Several protections have been added to region computation logic to prevent issues with regions created or modified during sort operations.  (ru, 31Jul2013)

⬤Sony: The file counter is now updated properly when edited in the Edit Acquisition dialog. (bh/jh, 15Nov2013)

⬤SY3200: Additional logic has been added to determine whether valid device data has been received from Haps Control prior to writing protocol files. (bh/jh, 18Jul2013)

⬤SY3200: Color density plots now reset correctly during acquisition. (bh, 24Jul2013)

🔵SY3200: After acquisition of very large files, WinList became unresponsive and the user interface did not update.  This has been corrected.  (bh/jh, 24Jul2013)

🔵SY3200: WinList now transmits sort information anytime it transitions from "W" mode into monitor or acquire via the toolbar buttons.  (ja/bh, 26Jun2013)

🔵SY3200: Several error checking routines have been added to ensure protocols are written and read properly. (bh, 15jan2013)

🔵SY3200: WinList now prevents the user from closing it directly when running in acquisition mode. The program is closed automatically by HAPS Control when that app shuts down.  (la, 11Jan2013)

🔵SY3200: Sort stream assignments are now stored in protocols.  When protocols are opened, the sort stream assignments are automatically sent to HAPS control.  (la, 15Oct2012)

🔵SY3200 preferences has a new property, "Monitor Mode Events", that determines the number of dots for 2p/3p plots and the area for 1p plots when monitoring.  Individual plots can still have customized settings for number of dots to display.  When the preference is changed, all plots update to the new preference value. (iCyt 1.7.9, 17Jul2012)

🔵SY3200: The Edit Sort dialog has been revised to be sort-stream-centric instead of gate-centric. Each sort stream is listed once.  A gate can be assigned to each stream.  (bh/la/jh, 12Jul2012)

🔵SY3200: Additional performance improvements for monitoring and acquisition. (1.2.1, bh, 14jun2012)

🔵SY3200: A quiet alert appears if transformations are edited during monitor/acquisition to allow the user to edit the sort gates. (dm, 12Jun2012)

🔵SY3200: A quiet alert appears if logging is enabled when the system starts monitoring, giving the user an opportunity to turn logging off to improve performance. (bh, 14jun2012)

🔵SY3200: Significant performance enhancements have been made in acquisition and real-time dot drawing.  (bh/cmb, 31May2012)

🔵SY3200: The Connection Manager has been removed.  All feature selection decisions are now made in Haps Control. (la, 18May2012)

**Bug fixes**

🌐Sony Bug fix: Under some restricted circumstances, it was possible to click the "X" to close a  histogram window as the Edit Histogram dialog was getting ready to display, causing the program to crash.  Protections have been added to prevent this problem.  (bm, 20Dec2013)

🌐Bug fix: The program crashed when drawing a Contour region on a 2P plot that had very few events.  Corrected.  (bg, 18Dec2013)

🌐Bug fix: Several fixes were made to support reading macro-based protocols created by WinList version 5 and 6.  (dh/bh, 12Dec2013)

🌐Bug fix: An FCS file with an invalid creation or modification date would cause the program to crash in the course of opening it.  Code has been added to detect and protect against this problem.  (pw, 13Dec2013)

🌐Bug fix: In the Edit Region Links dialog, if the mouse was clicked in the region listbox but not on an item in the list, the program crashed.  Corrected.  (bhd, 10Dec2013)

🌐Bug fix: Protocols that were based on 10-bit data files showing log could incorrectly show the parameters zoomed in histograms.  This occurred when the histogram contained regions and an active link for the histogram was part of a report.  Corrected.  (dh/bh, 10Dec2013)

🌐Bug fix: If a histogram name contained a forward slash "/", macro playback of region creation and region moves failed. This has been corrected.  (pw, 2Dec2013)

🌐Bug fix: If "Enable cascade gating to create and adjust gates" was enabled in Edit Gates, and a region file was opened from disk in the Edit Regions dialog, the gates were redefined as the region file was read.  This created problems for any custom gates that were edited by hand.  The corrected logic does not modify gate expressions when region files are read from disk.  (pw, 11Nov2013)

🌐Bug fix: Macro playback of region moving and sizing did not put the region label in the correct location. This has been corrected.  (pw, 18Nov2013)

🌐Bug fix: The 3D isometric contour display did not show populations correctly in color-by-hierarchy mode if several subsets had the same gate color.  This has been corrected.  (dh, 22Nov2013)

Bug fix: Color of some color subsets in 1P histograms was unexpected in some uses of Color-by-gate-hierarchy, especially when some colors were set to "no color". This has been corrected. (bd, 27Sep2013)

Bug fix: In the Edit Color By Hierarchy dialog, the Ungated gate was allowed to have "no color" assigned to it. This is no longer permitted; every event must have a color associated with it. (bh, 27Sep2013)

Bug fix: The program did not handle memory allocation failures for 2p dots properly. This has been enhanced to provide a more robust response during low memory conditions. (dh, 4Oct2013)

Bug fix: Arrow heads are now drawn correctly when larger line weights are selected. (dh, 17Oct2013)

Bug fix: Corrected a crash that could occur if the user clicked the close button ("X") on the main window while the program was reading a large protocol file. (ap, 2Aug2013)

Bug fix: Buttons on the macro toolbox are now blocked if a modal dialog is open or the program is in the process of opening or writing a protocol. (bg, 6Aug2013)

Bug fix: If preferences were set to "Autocomp each file" and the user adjusted the compensation manually after reading a file, a saved protocol would not display the data with the adjusted comp settings. This has been corrected so that when the protocol is loaded, WL uses the compensation that was in place when it was saved. (bh, 23Aug2013)

Bug fix: WinList did not correctly interpret the $SPILLOVER keyword for FCS3.1 files. Corrected. (bh/cb, 26Jul2013)

Bug fix: The program could crash when reading some older protocols with 1P histograms containing regions. Corrected. (jw, 12Jun2013)

SY3200 Bug fix: WinList would go into an infinite loop and eventually crash if a transformation was set to HyperLog and Auto-adjust was enabled when connected to Haps Control. The program now ignores Auto-adjust when connected to a Sony/iCyt instrument. (rc, 13Jun2013)

SY3200 Bug fix: If an acquisition is terminated by clicking the "W" button (entering Winlist mode), the program could crash when closing out or when opening a new protocol. It could also leave several temporary files on the disk. This has been corrected. (bh, 26Jun2013)

⬤SY3200 bug fix: If the user clicked a button on the SY3200 toolbar while WinList was writing out the final acquisition file, the program could crash.  This has been corrected.  (ru, 14May2013)

⬤SY3200 bug fix: If the program was set to continue monitoring after an acquisition, it could crash.  This has been corrected.  (bh, 14May2013)

⬤SY3200 bug fix: The program could crash if the user stopped an acquisition before it completed.   This has been corrected.  (bh, 15May2013)

⬤Bug fix: In some cases when opening protocols, region label statistics did not update if the region was over no events.  The label would update as soon as the region contained events.  This has been corrected.  (jh, 30Apr2013)

⬤Bug fix: When a compensation page was added and deleted, the program did not display the correct number of pages, and recreating the compensation page did not work correctly.  Fixed. (be, 6May2013)

⬤Bug fix: The page buttons on the data source toolbar did not update correctly when a page was deleted.  Corrected.  (be, 6May2013)

⬤SY3200 bug fix: A crash could occur when displaying 2 or more 2P histograms containing a "classification" feature during monitor or acquisition.  This has been corrected.  (jh, 3Apr2013)

⬤Bug fix: If a calculated parameter (Add Parameter dialog) referenced another calculated parameter in its expression, the program crashed due to an overrun.  Corrected. (bh/dh, 5Apr2013)

⬤Bug fix: When displaying low-resolution parameters such as "Event Classification", the program did not correctly dither the highest values.  Instead, the values were clipped to resolution-1.  This has been corrected.  (jh, 19Apr2013)

⬤Bug fix: When batch processing with one data source window, the program did not correctly pause after each batch file if multiple outline levels were created in the batch.  This has been corrected. (dh, 23Sep2013)

⬤Bug fix: After making edits in the Quantitation dialog, the program could appear to hang if there were a large number of regions.  This has been corrected.  (bh, 28Mar2013)

Bug fix: WL crashed when creating a region on a "Moflo Astrios" file with parameter resolutions of 4,294,967,296.  Corrected.  (dh, 25Mar2013)

Bug fix: The macro status dialog will be moved onto the visible portion of the screen if all or part of it is out of view when the macro starts running.  (vi 1353, 30jan2013)

Bug fix: Macro playback of the Save Data Source command and dialog did not work correctly. This has been corrected.  (bh, 5Feb2013)

Bug fix: In certain cases, if an NStat region label was moved and then clicked, the region would be repositioned incorrectly.  This has been corrected.  (dm, 8Feb2013)

Bug fix: For protocols with very large numbers of regions, changing the transformation for a parameter caused the program to appear to hang while processing the change.  The failing example contained 1041 regions.  This has been corrected.  (wm, 8Mar2013)

Bug fix: Some cascade gating scenarios (especially back-gates) caused the gate hierarchy to be incorrect.  Logic has been enhanced to allow back-gating, while maintaining the hierarchy. (bh, 21Mar2013)

SY3200 Bug fix: In some circumstances after a WL crash, ISB files would not open correctly.  A warning was displayed indicating that the protocol was designed for a different number of parameters. This has been fixed.  (jh/la, 16jan2013)

Bug fix: When running a macro to create a histogram, WinList created an invalid "undo" action.  If the user selected "Undo" after running the macro, the program would crash.  Corrected. (dr, 21Jan2013)

Bug fix: In some cases when an edge vertex for an NStat was moved, WL did not correctly compute the number of events in related sub regions.  This has been corrected.  (ap, 29Oct2012)

Bug fix: A crash occurred if a gate file was opened from disk and it contained more gates than the number currently defined for the data source.  (bh, 31Oct2012)

Bug fix: The file open/save dialog did not handle files with long names properly. This has been corrected. (bh/dh, 06Dec2012)

Bug fix: When gate aliases were edited, an extra set of keywords remained in the keyword list until a new protocol was loaded.  Corrected.  (dh, 13Dec2012)

Bug fix: Strings containing "&" did not display correctly in some report objects. (dh, 14Dec2012)

Bug fix: If batch toolbar was docked to top of app and a protocol was opened with minimized data source windows, the data source windows would be moved off screen. Corrected. (dm, 6sep2012)

Bug fix: Scale To Fit in the Window menu now ignores data source windows that are minimized. (dm, 6Sep2012)

Bug fix: Corrected a graphics issue where events on a HyperLog axis were not properly displayed if they fell on the lowest HyperLog channel.  (bh, 6Sep2012)

Bug fix: Double-click on an NStat region label did not create results when preference was set to do so.  This has been corrected.  (ml, 28Sep2012)

Bug fix: The program did not always update the gate statistics and event coloring when a region referenced by the gate was deleted.  If the gate is set to be "On" (instead of "auto-activate"), this problem would occur.  Corrected.  (dtm, 24Aug2012)

SY3200 Bug fix: files were being written with a value of "0.0" for $PnE.  It should be "0.0,0". This has been corrected.  (jh, 24Aug2012)

SY3200 Bug fix: Logic has been added to ensure that keywords generated during acquisition are not duplicated. In previous builds, two versions of the same keyword could be added, differing only in the case of letters.  (jh, 24Aug2012)

SY3200 Bug fix: Region flicker during monitor/acquire has been eliminated. (jh, 23Jul2012)

SY3200 Bug fix: When switching from Acquire to Monitor mode, an error message was displayed and the program switched to WinList mode.  This has been corrected.  (bh/cb, 20Jul2012)

SY3200 Bug fix: When drawing freehand regions during monitor/acquire, sometimes region segments disappeared until the region was completed.  Corrected.  (jh, 20Jul2012)

Bug fix: When loading a protocol with fewer regions defined than the current protocol, the database did not remove the region fields for the missing regions.  This bug was introduced in 7.1.1, and it has now been fixed.  (dy, 31Jul2012)

Bug fix: The ApplyRegion macro command would fail if the new region already existed in another histogram. This has been corrected. (rf, 21Aug2012)

SY3200 Bug fix: Regions would flicker in monitor and acquisition mode. Corrected. (la, 16Jul2012)

Bug fix: A text object on a histogram could cause a crash if the histogram was too small to display and it had a linked object on a report. Corrected. (cmb, 16Jul2012)

Bug fix: Corrected logic that finds the next unused gate in the process of cascade gating. The previous logic inappropriately selected an active gate in some cases. (bg, 17Jul2012)

Bug fix: In macro playback with "echo off", the Edit Regions and Edit Gates dialogs could cause other windows to be raised in the z-order, on top of WinList. Corrected. (pw, 27jun2012)

Bug fix: The Edit Gate dialog did not properly playback a macro to apply a gate to a histogram if the histogram name contained a comma. (pw, 24May2012)

Bug fix: Create Region Array took a very long time to complete for large arrays. This has been corrected, and a status window now shows the progress. (wm, 1Jun2012)

Bug fix: When processing a multi-level batch, the Pause command did not properly enable the "Continue" button on the batch toolbar. Corrected. (bh, 2Apr2012)

Bug fix: Quantitative results were incorrect with 7.1.1 for FCS2 data files with log-encoded data. Median, mode, and low/high statistics did not reflect quantitative units. (al/bh, 5Apr2012)

Bug fix: When 2p region vertices were shown and a user chose to display fewer than "all" vertices, the region resize handles were not always accessible and resizing was unpredictable. Corrected. (pw, 13Apr2012)

Bug fix: When using a preference file that was saved with "Inverse Palette", drawing new regions failed to use the correct color for region lines and text. Corrected. (pw, 16Apr2012)

Bug fix: The first file acquired via the SY3200 plug-in was missing $BEGINSTEXT and $ENDSTEXT keywords. Corrected. (jh/dm/bh, 2May2012)

Bug fix: WLB Bundle files were corrupted if they contained FCS files greater than about 2GB in size. This has been corrected.  (dm, 14May2012)

Bug fix: The "Automatically adjust gate equations..." checkbox in the Edit Gates dialog was not properly restored when reading a saved protocol or protocol bundle.  This has been corrected.  (bh, 23Feb2012)

Bug fix: Although there was no limit to the length of a gate expression, the gate compiler was using a limited buffer and imposing an invisible limit of 80.  This limit has been eliminated.  (bh, 23Feb2012)

Bug fix: When a region was made very narrow or very short, attempting to resize using a middle handle did not work.  In most cases, a new region was created.  This has been corrected.  (dm, 29Feb2012)

## WinList 7.1

### Performance and Usability

WinList now uses additional threads to improve processing performance.

A Miscellaneous preference has been added to enable/disable the use of threading. By default the "Threading" preference is enabled to improve performance. On computers with limited resources, you may need to disable threading.

The "Undo" command has been added to the Edit menu, with keyboard shortcut Ctrl+Z. Undoable actions: region deletion, moving and sizing editing histograms, changing parameters, and editing graphics.

Time parameter improvements: WinList now detects the "Time" parameter and sets it for auto-zooming. It also converts the axis into seconds if the data file contains the $TIMESTEP keyword. The Time parameter is also set to "Auto-zoom" by default.

WinList is now "large address aware", supporting greater than 2GB of memory space.

### MultiGraph

MultiGraph printing now takes advantage of some of the printing options for Reports. If "Fast Printing" is enabled for Reporting preferences, MultiGraphs will use the "Print Quality" report preference and printing can be significantly faster on some printers.

🔵MultiGraphs can now be saved as PDF files. Choosing File->Save->Save MultiGraph PDF will display the Export to PDF dialog, allowing you to navigate to a location and enter a file name.

**Regions and Gates**

🔵Improved region deletion behavior: When a region is deleted, WinList now keeps track of all its properties and vertices, as well as the histogram it was originally created in. The region can only be redrawn in the same histogram, unless the Edit Regions dialog is used to "Clear" the deleted region. The Edit Regions dialog allows the region to be restored after deletion. When the region is redrawn after deletion, its original properties are maintained. These changes provide more consistency in gate expressions that reference the region, as well as providing a means for undoing a region deletion.

🔵Holding the Shift key down when deleting a region will disassociate the region from the histogram so that it can be redrawn in another histogram. If the Shift key is not down, the deleted region remains associated with the histogram and will only be redraw in that histogram.

🔵A region preference has been added to control whether WinList associates regions with the histograms in which they are originally created. The default value for the "Associate with Histograms" is enabled, which means that a region will only be redrawn in the histogram where it was originally drawn. Disable the property to allow a deleted region to be redrawn in any histogram. You can also press the Shift key when deleting a region to disassociate it from the histogram, even if the property is enabled.

🔵NStat regions no longer limit rows and columns as a function of histogram resolution.

🔵A Region preference has been added for the display option for NStat region labels so that it can be set separately from other region default label options.

🔵A gate equation can now refer to other gates. For example, if G2=R1&R2 and G3=R3&R4, we could define G4=G2|G3. This would be equivalent to G4=(R1&R2)|(R3&R4). A gate can only reference gates that are defined ahead of it in the gate list.

A notification window now appears when a sort region is moved, sized, or deleted. The notification window can be clicked to edit the sort. The user can continue to work when the window appears; the window will close automatically.

🔵When the Edit Gates dialog Reset All button is clicked, the program now resets the histogram gates to "Ungated" in addition to resetting the gate expressions.

The Edit Program Controls dialog will now increase or decrease the number of gates and regions for each data source if these values are edited. Previous versions only increased the number.

The Context menu for 2P regions now includes a "Conform to Other Regions" command. This command adjusts the current region so that any of its vertices inside of other regions are removed, and any vertices of other regions inside of it are added.

**Graphics**

Edit Graphics dialogs now have an Apply button.

Histogram titles now update when a new file is loaded and when different parameters are selected for the histogram.

When the mouse hovers over a histogram title bar, a tooltip is now displayed to show the title bar text.

Default 2p graphic preferences have been modified to show Dots with Contour lines. This change will have no effect if you have already set 2p graphic preferences.

The Save Graphic command for histograms now supports JPEG, PNG, TIFF, and GIF formats, along with bitmap and metafile formats.

Contour drawing has been revised to provide improved graphics even with low event counts.

The 3D viewer has been enhanced. It now allows up to 1,000,000 spheres/dots. The user can choose the location for the axes, and there is an option to display the exponent only on the axis tics.

**General**

Histogram names now use the actual parameter names by default, instead of P1, P2, etc. Pre-existing protocols will use the same names they were saved with.

WinList now handles situations where the User Documents folder has been redirected to a network location. Registration information is now stored in a machine-specific section of the preferences file (WinList3D.INI). The program will automatically move existing information to the new section.

WinList now disables compensation if a new file is opened and its parameter resolutions differ from those that were used to set up the compensation.

WinList now quietly alerts users if Log Bias is turned on in a protocol. The alert offers to turn off log bias for the user.

Logic has been added to read FlowJo data files that do not conform to FCS standards for specifying data offsets.

The Transform dialog now supports Auto-zoom using several methods. With "Auto-Zoom to highest used channel" the program will automatically zoom to exclude empty channels at the upper end of the selected parameter. With "Auto-Zoom to Fixed Upper Boundary", you can specify a specific channel that will be used as the upper boundary for the parameter. And with "Auto-Zoom to Percentile", you can enter a percentile at which to set the upper boundary.

Quantitation has been revised and improved. WinList now supports quantitation on linear parameters, quantitation with HyperLog axis, and using slopes & intercepts derived from linear data. The Quantitation dialog (Edit Data Source) has been revised to improve efficiency.

WinList now ensures that its packet size is at least 200K events when it starts up with iCyt support. When starting up without iCyt support, the default packet size is 50K events.

Mousewheel scrolling in dialogs has been improved.

File Open and Save dialogs now use the native OS dialogs. The one exception is the File dialog displayed when adding files to the batch list, which cannot use the OS dialogs because it must be displayed at the same time as the Batch dialog.

When WinList is set to "autocompensation" files from keywords, it turns off compensation when it sees an identity crossover matrix (i.e. a matrix that specifies no compensation). Starting with this build, it also clears the compensation matrix in this condition.

When running on WinXP, the older XP-style choose folder dialog is used to pick a folder location.

The Preferences dialog now has a "Save preferences" checkbox on the page that allows opening of preferences from disk. Enable this option to make the newly opened preferences permanent.

A change has been made to the Save Data Source command. If the name of a parameter has been edited in the Names and Resolutions dialog, WinList will use that name as the $PnS

keyword value if the file is resaved using the Save Data Source command. The original $PnS value is stored as PnAlias.

⬤When the Open Macro command is used, WL now asks if you would like to run the macro you have just opened.

⬤A "DDE Timeout" setting has been added to Miscellaneous preferences to control the amount of time (milliseconds) that WinList will wait for a remote script command to complete. The default setting is 10000.


**iCyt Features**

⬤WinList now creates a $TIMESTEP keyword that allows conversion of the Time parameter to seconds

⬤If a HAPS had two features with the same name and they were both selected in WinList, no events would display. WinList now requests the features from HAPS based on the feature ID instead of feature name.

⬤Removed the "do you want to connect" prompt on app startup. If there's a server name saved in iCyt preferences, WL will attempt to make the connection.

⬤Output resolutions up to 16777216 can now be selected from the Connection Manager.


**Bug Fixes**

⬤Corrected a crash that could occur when Log Bias was enabled.

⬤Under some circumstances, region line segments would draw incorrectly when the region's label was moved.

⬤Code has been added to allow the user interface to update during lengthy operations.

⬤Several commands in submenus of the Window menu did not execute the correct command.

⬤When duplicating a data source, the state of the gate list "automatically adjust gates" checkbox is now preserved.

⬤If a report object had no width or height, the PDF creation routine would crash.

⬤Double-clicking a quadstat/nstat label did not perform the "Double-click action" that was set in region preferences. This has been corrected.

Enhanced region creation logic to ensure that region labels are on-scale after new regions are created.

Inactive gates were being recomputed when they did not need to be.

Color by Parameter Value did not assign the last color to as many events as it should have.

When exporting an FCS file using Save Data Source, the parameter resolutions were incorrect if the source file had log-encoded parameters.

When a new file is opened into an existing setup, histogram zoom will reset for any parameters that have a different resolution in the new file compared with the previous file.

When a connection file is not currently loaded and a protocol bundle is saved, WL now stores information about the last connection file in the protocol. It also saves the last connection file in the bundle. This allows the program to enable the "Open Last Connection File" command when the protocol bundle is stored.

Zoom is now reset if a new parameter is selected for a histogram and it has different transformation characteristics than the previous selection.

The auto-adjust routine for HyperLog did not properly handle data when there were no events in the negative portion of the data space. This has been corrected.

The Keywords button on the Open FCS Files dialog did not work correctly in some cases.

A misinterpretation of a Millipore InCyte FCS file's $SPILLOVER compensation information has been corrected.

Info windows now update correctly when added to reports.

Macro playback of assigning a gate to a histogram did not work correctly.

The Keywords button on the Open FCS dialog did not work properly.

If a macro-based protocol from an earlier version was opened and modified, WinList could overwrite the macro with a new xml-format protocol when quitting. Logic has been revised so that WinList does not attempt to save changes to a macro-based protocol.

When WL was on a second monitor, the Edit Batch dialog did not position the File dialog next to it correctly.

WL could hang if it moved auto-position regions to the edge of a histogram. On the edge, the logic always considered the region to have moved and tried to process it again.

Result window ID fields can now display keywords from the full results tree.

**WinList 7.0**

WinList 7.0 is a major version update with many new features and enhancements.

**New Features**

Network-based, shared "seats" allow multiple users to share WinList as needed, up to the available number of seats.

Enhanced OpenGL 3D graphics are now included in all versions for unique spatial views of 2P and 3P data with eye-catching display settings.

User defined keywords and equations create additional analysis results based on keywords and other stats. Create ratios and other mathematical expressions to extend your analysis.

Histograms now support data zooming to focus on an area of interest.

User-selectable transforms for each parameter: HyperLog, Log, and Linear.

Automatic adjustment of HyperLog display settings.

XML-based protocols store analysis setups and load significantly faster than macro-based protocols.

Create tube-specific compensation using the Compensation Database. Compensation of complex panels has never been so easy and efficient.

Enrich Data feature allows you to do a "virtual sort" to enhance a population of interest during analysis.

Downloadable WinList "Reader" provides an easy and free tool for sharing WinList reports and analysis protocols.

"Conditional update" areas on reports only update when user-defined conditions are met.

Run a macro script within batch file processing for highly customized batch analysis options.

●Report system now includes "smart" positioning of elements as they are added to the report.

●Duplicate Data Source now replicates all regions, gates, and colors – even keeping the same region IDs.

●Gate Statistics window provides a legend stats for all gates, as well as "Unclassified" and "Overclassified" events at each level of the gate hierarchy.

●Read and write FCS files in FCS 3.1 format.

●Create N-Stats that label the full phenotype of the FCOM vs FCOM.

●The Open FCS file dialog now includes a "Preview" checkbox to preview a set of FCS keywords, making it easy to identify files of interest.

●"Show Histograms based on Trace Line Option" arranges histograms based on the selection in the Trace line options listbox to simplify compensation setup.

●WinList's main toolbar now contains a drop-down list of coloring modes.
Color by Histogram Gate uses the color of the gate that is applied to the histogram.
Color by Gate Combinations is the new name for Color Event mode.
Color by Parameter Value colors events based on the intensity of a selected parameter.
Color by Gate Hierarchy uses the color of the last gate in the gate hierarchy that an event belongs to.

●Reports now have a "master page" containing items you want on all pages of the report.

●Keyboard shortcuts are now provided for adding objects to the report.


**More New Features**

A menu command has been added to Check for Updates online.

●Gate statistics are now available as keywords in reports and for databasing.

●The Edit Gate dialog now allows the user to choose how gates are activated, on a gate-by-gate basis.

●The Region preference for NStat sub region labels now includes the option to display name and percent of gate.

The Options menu has a new entry for the Database Update Wizard. This wizard is designed to fix-up region results in existing databases to work with the new structure of WinList. The wizard will check databases, and walk you through an update process for each one.

The macro commands for Save Protocol and Save Protocol Bundle can be edited to replace the file name portion with a keyword. This allows these macro commands to create file names that relate to the FCS file being analyzed.

The macro system can now record and play back the Open Protocol, Save Protocol, and Save Protocol Bundle commands.

1P histogram graphics have several new graphic options and preferences. Choices allow you to overlay color subsets, stack color subsets, or not display subsets.

The Window->Data Sources menu has two new commands to make it easy to activate data source windows. "Next Window" and "Previous Window" activate the next or previous data source windows, respectively.

The Save Data Source dialog has been redesigned to support revisions in FCS standards.

Report windows now have a tabbed control at the bottom which allows page selection and creation.

An option has been added to the Edit Gates dialog that controls whether "cascade gate" mode is on or off.

**Enhancements**

Reports now support region creation and modification directly.

Significant improvements have been made to 2P graphics.

Each data source now maintains its own regions and gates, rather than drawing from a common set of regions and gates.

Enhanced support for reading very large data files.

WinList interacts with ModFit LT 3.2.1 via DDE for integrated DNA analysis.

User preferences are now stored in protocol bundle files. This makes the bundle file a more complete snapshot of the analysis protocol.

●WinList is now compatible with Windows$^{TM}$ Vista and Windows 7.

●The palette and color management system has been redesigned to handle more colors.

●The Batch system has been revised to provide a more control over what actions are performed and when they take place. In "Basic" mode, simple options are selected via checkboxes and dropdown lists in the Edit Batch Options dialog. When you need more control, you can put the system into "Custom Scripts" mode. Batch processing levels allow custom actions to be assigned to specific files for detailed control of batch file output.

●Histograms can now be "cloned" to create copies with the same graphics and parameters.

●A new printing preference has been added to control the size of the output for Print Histogram.

●Printing of dots has been improved on high-resolution printer.

●A "Palette mode" preference has been added to Histogram preferences to support the new inverse palette option.

●WinList now activates any gate that contains at least one valid region. Prior versions required that all regions referenced in the gate expression exist in order for the gate to be valid.

●The program now allows the user to locate missing files when opening protocols.

●Preferences have been added to set the default transform and its properties.

●A WLX protocol file can now be dragged and dropped onto WinList to open the protocol.

●WinList now stores the locations of its toolbars, and restores those locations when it starts up.

●2P regions now support finer resolution for drawing and statistics.

●The Link Regions dialog has been redesigned. It provides support for bi-directional links, and simplifies selection by only showing "compatible regions" that can be linked.

●WinList now uses the PnDISPLAY keyword to set default transforms for new parameters.

●The cascade gating system has been enhanced so that it can update the chain of dependent cascade gates when an upstream gate is modified.

The Scale-to-Fit data source command now pays attention to the "Keep histograms square" graphics preference.

The quality of the PDF output for reports has been improved.

A new preference has been added to the Reporting preferences to control the "Printed quality" of histogram report graphics.

Gallios and Navios cytometer data files are now supported.

**Enhancements to iCyt Acquisition**

iCyt: WinList can now acquire very large data files, i.e., 20 features and 28-million events.

iCyt: The transformation settings in the Reflection Connection Manager now come from the Transformation preferences. Transformation changes made in the Connection Manager will automatically update preferences.

iCyt: The Connection Wizard now allows easier selection of features. Checkboxes have been added to the feature list to simplify multiple-selection without having to use the Ctrl or Shift keys.

iCyt: WinList now asks the user at start-up whether or not to connect to an iROC server.

iCyt: The Restart button on the iCyt toolbar now works for Monitor mode as well as acquisition mode. When clicked in Monitor mode, all histograms clear and rebuild with new data.

iCyt: The Feature Selection page of the Connection Wizard has been enhanced to provide a dynamic list of feature groups for filtering the feature selection list.

iCyt: 1p histograms now remove stale events if voltage is adjusted on the instrument while WL was monitoring.

iCyt: Sorting regions now work correctly if they are defined in 4-decade mode in WinList. Even with the correction, it is easier to make comparisons between WinList and HAPS Control if WinList is using "computed decades" mode.

iCyt: Text of some iCyt menus and dialogs has been modified so that it makes sense for both Reflection and Synergy instruments.

iCyt: The Restart button on the iCyt toolbar now works for Monitor mode as well as acquisition mode.

iCyt: Acquired files are now stored in floating-point FCS file format.

iCyt: Removed the popup status message that was displayed when data was not received within a timely period.

iCyt: Improved graphics when monitoring. The real-time dots shown while monitoring now correctly accumulate to the desired number of dots before dots are removed. Previous logic removed a certain fraction of dots and rarely displayed the desired number of dots. The new logic eliminates the need for the "Dot Persistence" preference, and provides more saturation in the dot displays.

iCyt: The behavior of color-density, contour plots, and 1P histogram has also been improved when monitoring. These plots will now accumulate events until the desired dot count is reached, and then reset and start accumulating again. For 1P plots, the reset occurs when a peak reaches 1000 events.

iCyt: Behavior change of Dots during acquisition. In previous versions, 2p dot plots would display up to a defined number of dots during acquisition, controlled by the "Max Acquisition Dots" property in iCyt preferences. Once that threshold was reached, no additional dots were displayed. Starting in this version, when that threshold is reached, the histograms showing dots will reset their dots and start accumulating them again. This change allows the operator to see changes in the display throughout the acquisition process.

iCyt: Behavior change when HAPS is pinched or connection is lost . The program now provides more consistent and obvious feedback when it is not receiving data. After 3 seconds of receiving 0-event buffers or no buffers, a small status window is displayed indicating that no data is being received. 1p and 2p plots will reset to show empty plots when monitoring.

iCyt: Logic has been improved (in some cases corrected) when the user start WinList acquisition mode with the HAPS Control in a "pinched" state. Previously, histograms and results might not reset properly in this circumstance. Now, all histograms and results are cleared and WinList enters a "waiting to acquire" state.

iCyt: WinList now provides support for storing instrument settings in its protocol files.

iCyt Bug fix: Spurious events in an acquired FCS file that had a 0 intensity when the file was reloaded after acquisition have been eliminated.

iCyt Bug fix: When the Finish Acquisition dialog was displayed and left on the screen, memory was being consumed by the acquisition system. Corrected.

iCyt Bug fix: Changing 2p graphics options while monitoring in iCyt mode could cause the program to crash. Corrected.

iCyt Bug fix: Corrected display of dots for low res parameters (event status, droplet status).

iCyt Bug fix: After opening protocol bundles, it was possible that the Edit Connection File command would show incorrect selections. This has been corrected.

iCyt Bug fix: If the Save Protocol Bundle option was set and an acquisition was started and then cancelled, acquisition data was deleted in some circumstances and an empty acquisition file was stored in the bundle. Corrected.

Numerous other bugs were corrected related to the iCyt acquisition plug-in.


**Earlier Versions**

**WinList 6.0**

Drag-and-drop support has been added throughout the program. Files can be dropped onto the application to automatically open them for analysis. Histograms and results can be dragged and dropped onto report windows to quickly add them to report layouts. See Drag and Drop for additional details.

Intuitive cascade gating of histograms makes it incredibly quick to create Boolean gates without having to type gate expressions. See Cascade Gating and Boolean Gates for details.

Convenient toolbars for histograms and data sources provide quick access to the most common tools and commands. Select region types, assign gates, add and manage analysis pages, and much more. See Histogram Toolbar for details.

A full, page-layout reporting system has been added to the program. Multi-page report s can be created with live links to analysis elements such as histograms and result window. It is quick and easy to add keywords, gate legends, bitmaps, logos, and of course free-form text. Report windows can be exported to PDF format for convenient archiving and information sharing. See Report Windows and Creating Reports for more.

WinList now provides an Alert system that allows users to define alert conditions based on region statistics. Each alert provides a user-defined name, an alert condition, and a keyword that stores the alert. Alert conditions are based on a user-selected region statistic, a mathematical test (=, <>, <, <=, >, >=), and a target value for the alert. Alert objects can be added to reports, displaying one color when the alert triggers, and a different color when it does not. See Edit Alerts for details.

●Improvements have been made in how histograms are created and edited. The Create Histograms dialog replaces the old Multiple Histogram Selection dialog to provide a highly efficient means for creating many histograms at once. In a similar way, the Edit Histogram dialog has been redesigned with a clean new look. Changing the parameters displayed in a histogram is a simple matter of clicking on the parameter label in the histogram and selecting a new parameter to view.

●With the histogram toolbar, you can draw any type of region by selecting the tool, clicking and dragging on the histogram. Contour regions can now be previewed by holding the mouse button down and moving the mouse, and you can use arrow keys on the keyboard to move the active region. Regions now have a new option for auto-positioning based on a user-defined percentile X and Y. The NStat region can now auto-position based on this method.

●Data source windows provide much more functionality than in previous versions. Histograms can be "drawn" directly onto the data source, and a convenient grid makes them easy to move, size, and align. Any number of pages can be added to a data source to manage the number of histograms viewed at once, and moving histograms from one page to another is a simple context menu action. See Data Source Windows for details.

●A new option, Parameter Names, has been added to Data Source preferences. This option allows you to choose the way that the program determines the names of parameters in your listmode files. The default is "Automatic", which uses the cytometer information to determine the appropriate keywords to use. You can also choose to name parameters based $PnS, $PnN, or a combination of these FCS keywords.

●Protocols can now be saved in a Zip-compatible file, the Protocol Bundle format. This provides a simple and effective means for sharing protocols with others. The Protocol Bundle contains all of the files that WinList normally generates using the Save Protocol command, along with the FCS files used by the protocol.

●WinList now supports automatic compensation of BD Biosciences Diva files and Beckman-Coulter FC500 files containing two datasets. Several user-preferences determine how and when this feature is applied. See Data Source Preferences for details.

●The Compensation Toolbox has a new button, "Create Missing Histograms", which can be used to create biviariate histograms for parameters enabled for compensation. When the button is clicked, the system scans to see if any bivariates involving compensation parameters are not currently shown. A dialog box with a list of the missing histogram is displayed, allowing the user to choose which histograms to create. This feature makes it very easy to create all of the 2P histograms that involved compensation parameters. The data source toolbar also provides an option to create Scatter Plot Matrix, "SPLOM ", of histograms that display all the combinations of parameters involved in compensation.

We've improved the compensation system's auto-positioning routines that automatically determine the best positions for the trace lines, resulting in more accurate compensation settings.

WinList is now capable of doing real-time acquisition and sorting with Reflection ™ cell sorting instruments from Sony Biotechnology. These instruments are designed for highly-automated parallel sorting and are capable of very high throughput. Since the connection between WinList and the Reflection is over TCP/IP, you can monitor experiments and acquire data on any computer that has a internet connection.

The histogram context menu now includes a Copy Data command. This command copies the histogram's data in text format to the clipboard, where it can be easily pasted into other applications.

The Copy Special dialog has been enhanced to allow selection of a target size and DPI for histogram graphics. This makes it much easier to generate high-quality histogram graphics for publications.

Corrected reading of some versions DakoCytomation Summit data files.

Results windows can now show statistic for "% Selected Gate". Choose a gate from a list of the active gates, and each region in the results window will show its events as a percentage of that gate. This option is useful to determine a region's percentage of an ancestor gate, or of any gate in the datasource.

A new wizard has been added to make it easy to add statistics in a Results window to a database of results. The Results Database Wizard can be accessed by right-clicking on a results window, or from the Options menu.

The Window menu has several changes and enhancements. The Arrange All submenu has been renamed to Data Sources to better reflect the windows that are affected. Added to this submenu are the names of all Data Source windows, making it easy to activate a data source when more than one is open at a time. A new Report submenu has been added to the Windows menu. This submenu has options to Show All or Hide All reports. In addition, it shows a list of all open reports so that a report can be easily activated.

Protocols now load faster because results window statistics are not updated until the end of the protocol.

The Create Splom dialog now allows the selection of a gate to apply to the new histograms.

Macros can now be up to 7000 lines long. Memory requirements on the Macintosh version have been adjusted to accomodate this change.

Batch processing now provides options to save and print reports.

Two new region preferences have been added: "Show resizing handles" and "Double-click action".

New statistics are available to display and database median values in ADC units.

A new region keyword has been added that contains the name of the gate applied to the histogram that contains the region. To extract the gate name, use the [R(id,stat)] keyword, and use GATENAME or GTN for the stat.

The program has a patch for Beckman Coulter FC 500 files to copy some of the keywords missing in dataset 2 from dataset 1.

Bug fix: The CC result could produce a error value for regions containing a single event.

Bug fix: Corrected problem with using Create Region Array to create FCOM regions for an FCOM that uses more than 4 gates.

Bug fix: In Service Pack 4, macros that used the [FINISHBATCH] keyword to loop through batch files could loop endlessly in some circumstances. This has been corrected.

Bug fix: corrected decoding of FILEROOT keyword during batch processing.

Bug fix: Revise batch processing logic so that the first batch file(s) is correctly opened when the Next Batch Item menu command is selected, or when a button on the batch toolbar is pressed.

Bug fix: Corrected problem with compensation routines for linear vs. log parameter compensation. In some cases, especially with high-resolution data, WinList's tracelines computed an incorrect slope when compensating linear vs. log parameters.

Bug fix: When reading some FCS3 listmode files, WinList could fail to find and display the data portion.

Bug fix: When reading floating point listmode files, events with the highest resolution value were sometimes incorrectly set to 0 during conversion to log.

Bug fix: The HyperLog transformation did not correctly handle Beckman-Coulter 32-bit data when displayed at "computed decades" (~6.01).

Bug fix: If a protocol was saved containing datasource(s) with IDs not starting with 1, the protocol did not replay properly. New protocols correctly handle this problem. To edit existing protocols that show this problem, contact technical support.

Bug fix: If saving a data source produced in a file that was larger than 99,999,999 bytes, the resulting output file was invalid due to restrictions in the FCS 2.0 spec. The program now saves datasources in FCS3 format which supports very large file sizes.

Bug fix: The program can now support reading FCS keywords of any length. This corrects a problem in the ability to interpret the SPILL compensation keyword for some Diva files. Some restrictions still apply to the length of keywords for display purposes.

Bug fix: Contours of low-frequency populations in histograms that also have high-frequency populations did not always display properly. Also, in some cases the contour display showed unexpected line segments crossing contours. Both of these issues have been corrected.

Bug fix: On fast PCs, remote scripts could sometimes appear to hang the program. This can be corrected by specifying a timeout value for DDE communications. Contact tech support for details.

Bug fix: When batch processing and printing files on the Mac, the Print Options dialog was inadvertently displayed. This dialog is now suppressed during batch processing and macro playback.

Bug fix: Print Screen on the Mac could fail in with some monitor configurations. This has been corrected.

Bug fix: If no histograms were displayed and the Edit button on the Compensation Toolbox was clicked, the program could crash trying to compute digital error values.

●2P region resizing handles now encompass only the region points; they do not include the region's label.

●1P range, 2P rectangle, and 2P ellipse regions now begin drawing as soon as the mouse button is pressed. This change is a result of the use of the new region toolbar, making the drawing operation more efficient.

●In Color Event mode, 1P histograms now show a polygon for the total histogram data in addition to the cluster polygons.

●The SED histogram subtraction algorithm contained a bug that could affect the reported % positives.

●The Close All Windows command now reinitializes the gates and regions to their default states.

●The Gate dialog and Region List dialog are now closed automatically by the Close All Windows command. This corrects a crash that occurred if either dialog was open and a protocol was loaded.

●Corrected parameter range checking logic for data stored floating-point format. Previous versions could crash if a floating-point parameter value exceeded the resolution specified in $PnR.


**WinList 5.0 Service Pack 4**

This version provides new features and fixes.

●A new HyperLog<sup>tm</sup> transform for displaying negative and positive compensated log data has been added. The option can be enabled in the Advanced section of the Compensation toolbox. With the option enabled, events that become zero or negative as a result of compensation can be visualized and analyzed. (12Aug2003)


●The Compensation toolbox now has a simple and advanced view. The advanced view is displayed when the "More >>" button on the toobox is clicked. This view contains most of the checkbox options and the Advice button. Click the "Less <<" button to restore the simple view. (19Aug2003)


●The Create Region Array command in the Create menu has been enhanced. When this command is selected and a 1P histogram containing an FCOM parameter is active, a new checkbox will appear in the Create Region Array dialog. Enabling this checkbox will allow you to automatically generate an array of regions with aliases that describe the FCOM peaks. You can also enable these regions for Color Event gating.

●The program now provides better accuracy for all routines that use the log function. (28Aug2003)


●Histograms now feature an improved axis display, including axis and grid line weight preference as well as quantitation axes. (27Aug2003)

●A "software battery" usage system that allows the program to run in a metered use mode has been added. The system is designed for users who have a limited project or want to try the program for a limited time. (26Sep2003)

●The PC version now supports drag-and-drop of data files onto a datasource window. When you drop listmode files on a datasource, WinList will automatically create a batch with those files. If files are already in the batch, the program will ask whether you want to append the files to the batch or make a new batch. (10Sep2003)

●Protocols now save a list of files in your batch, if you have created a batch. When the protocol is opened, the original batch will be reconstructed. (12Aug2003)

●A new batch toolbar with VCR-like controls is now displayed at the bottom of the application window when you have selected more than one file or created a batch of files. The toolbar provides easy navigation through the batch, as well as automatic advancing. You can select a macro to run after each batch item is read, as well as choose various print options. (12Aug2003)

●Additional keyboard shortcuts have been added for batch processing. The Right arrow and Up arrow will advance to the next batch item. The Left arrow and Down arrow will move to the previous batch item. (12Aug2003)

●The Hi-Res Options dialog in Edit Datasource and in Preferences has been renamed and made more general. It is now named Linear-to-Log Options. It can be used to convert *any* linear parameters in listmode files to log for display and analysis. The Linear-to-Log preference dialog now allows you to specify by name the parameters to convert to log. Previously, parameters were specified by parameter number. (8Aug2003)

●Color density on 2p histograms has been revised to provide better gradations in color. The maximum number of density levels has been increased to 40. An additional property allows you to control density enhancement, which can emphasize small or large populations. (15Apr2003)

●The NStat region now supports an Import function that allows its X-axis partitions to be defined by a text file import. This feature was designed to allow partition information from ModFit LT's Proliferation Wizard to be imported, and allow gating regions to be defined based on the Prolif Wizard's analysis. To use the feature, first create an NStat with the same number of columns as the number of generations in the Prolif Wizard. Then, right-click on the NStat label and choose Edit Properties. In the Edit Properties dialog, choose Import and select the file created by ModFit LT's Prolif Wizard. ModFit LT must have a build date of 4-April-2003 or later to support this feature. (4Apr2003)

Added trig functions for calculated expressions. The new functions are FSIN (sine), FCOS (cosine), FTAN (tangent), FCTN (cotangent), FASN (arcsine), FACS (arccosine), FATN (arctangent), and FACN (arccotangent). See Add Parameters for details on these new functions. (30Oct2003)

If compensation is active and a data file is opened that contains a different number of parameters than the current file, the program displays an alert dialog and disables compensation. (3Nov2003)

The 3D version of WinList on the PC now features 2 additional display options for 3-parameter data. In addition to the cloud-cluster display, the viewer can display data as 3D dots or 3D spheres. Additional properties have been added to control the size and number of dots or spheres. (7Nov2003)

Added 2 properties to Miscellaneous Preferences which determine what keywords will be evaluated for the "Choose Dataset" dialog box that appears for FCS files with multiple datasets. This allows the user to customize the keywords that are used to describe each dataset when making a selection. (30Sep2003)

The registration dialog now displays a confirmation dialog when a valid unlock code is entered. (26Mar2003)

Bug fix: Corrected function of Get Previous Batch command when processing multiple datasource batches. (2Jul2003, VI 1099)

Bug fix: Under some circumstances, regions were not reassigned to the correct histogram during protocol playback or loading a region file. (9May2003, ML)

Bug fix: Corrected problem in PC version with macros that set up Remote links. In some cases, the macro would stop processing and/or not apply all macro commands correctly to the Edit Remote dialog. (NP, 25Apr2003)

Bug fix: Corrected problem saving gated datasource in tab-delimited format. The selected gate was not applied, resulting in an ungated output file. (22Apr2003, VI 1089)

Bug fix: Corrected problem when color density, contours, and outlier dots were enabled at the same time. With these options selected, the program now turns off the outlier dot options, and draws contours with dot density. (9Apr2003, VI 1087)

Bug fix: The Save option in the Edit Spots dialog now works correctly.

Bug fix: Macros can again record movement and creation of regions. (17Sep2003)

Bug fix: Added protection on Mac for displaying database viewer with more than 50 records. The application displays a message recommending that the viewer be closed and offers to close the viewer for the user. (30Oct2003)

Bug fix: Corrected a problem with 3D version on Windows platform in which the program could stop responding when the 3D scene is spinning and a mouse action is performed. (5Nov2003)

Bug fix: When saving a data source as a new file (File->Save->Data Source), the program did not correctly export in text format in some circumstances. The column header was not written correctly. This has been corrected.

Bug fix: The median and mode of a region containing no events is now set to zero. Previous versions reported an interpolated value based on the region's boundaries. (25Nov2003)


**WinList 5.0 Service Pack 3**

This version provides new features and fixes.

The program now supports up to 32-bit resolution data. Hi-resolution data files from Becton Dickinson Bioscience LSR and FACSDiva instruments and from Beckman Coulter FC500 have been tested successfully. See the topic Understanding Hi-res Data Files for further details.

WinList now reads floating point listmode data. This data type can be created by some Becton Dickinson Bioscience and Guava Technologies products. WinList rounds the floating point values to 16-bit integer values for display and analysis.

2P graphics now provide an option to show "outlier dots " with contours. The program draws any dots that fall outside the boundaries of the first contour with this option enabled.

2P contour graphics now allow you to select a "base contour" level. This defines the area of the lowest probability contour, from 90-99%.

Improved 2P dot plot display to provide higher resolution placement and dithering of dots, regardless of histogram resolution.

The N-Color compensation system can now compute and display the effects of digital error on data files. The display appears on 2P histograms when enabled in the Compensation Toolbox.

New editing features allow visualization and fine-tuning of compensation settings.

WinList now allows normalization of listmode data, with special options in the Compensation Toolbox.

A new command, Change Macro Paths in the Macro menu, simplifies moving macros and protocols to a new pathway or computer.

A new Results preferences, Use ADC Resolution, allows WinList to display and analyze all linear parameters at the native, ADC resolution, even if the histogram resolution is less. This

allows histograms to use reasonable internal resolutions of 64-256 channels while providing full resolution for display and analysis.

The Edit Properties for Region dialog now contains an option that controls whether a region can be deleted or not. The option, Allow delete, is normally enabled, but can be disabled for regions you want to protect from accidental deletion.

1P regions now resize more easily, regardless of the position of the region label.

Graphics copied and pasted into other applications no longer draw the border.

A new region preference allows you to choose the default alias for regions. This feature makes it easy to display percentages of QuadStats and NStats directly on the histograms. Choose either "Region name" or "Percent of gate" from a list in the Regions branch of the Preferences dialog. New regions will use this alias. The Reset Alias button in the Edit Region List dialog will use the preference setting to initialize existing region aliases. Opening region files and protocols will apply stored region aliases to the regions.

Create Region Array now allows decimal values to be entered for locations and sizes of regions.

Several new options have been added to the Window Arrange All menu to align windows in a single column (vertically) or a single row (horizontally). The logic for tiling of histogram windows has been revised to keep histograms more square.

Linear axis labels will now automatically switch to number tic labels that are X 100 or X 1000 when labels exceed 10000 or 100000 respectively. This is indicated with text that appears next to the axis.

The Edit Trace dialog now allows 4 decimal places in each item.

The histogram name is now added as a keyword for remote DDE connection on the PC platform. This allows the remote app to diffentiate DDE data from different histograms in the same file. (10Dec2002)


Added support for FCS files with multiple datasets, e.g. Beckman Coulter's FC500, Guava Technologies. When WinList detects a multiple dataset file, it displays a dialog to allow the user to select the dataset of interest for analysis.

FC500 files now display the $PnS keyword for parameter names. (4Dec2002)


Added keyword [DEFAULTPATH], which returns the protocol pathway.

Added keyboard shortcut to close all windows. On PC, Ctrl-W. On Mac, Apple-W.

Revisions have been made to the compensation system to improve the reproducibility of statistical results.

Revisions have been made in calculated parameter expressions to scale all parameters to a common resolution. Whenever a parameter is referenced in a calculated expression, it is transformed to a floating point scale ranging from 0.0 to 1023.0. This change may require edits to previously written calculated parameter expressions. The benefits of the change are that calculated expressions can be applied to data files with different parameter resolutions, and all parameters can be displayed on scale.

⬤Save Datasource and Save Histogram no longer overwrite $SYS keyword from original file. Instead a new keyword, RESAVED_BY, is added with the Winlist signature.

⬤The MultiGraph selection listbox now shows the datasource and histogram IDs, as well as the descriptive names.

⬤Bug fix: Corrected byteswap problem in reading files created by Summit Software. Previously, files would read but not display or analyze correctly.

⬤Bug fix: On macro playback on the PC, the file selection dialog did not correctly select a network path if it was in UNC format.

⬤Bug fix: Long datasource filenames could create unexpected behavior with MultiGraph elements. In some cases, histogram axes would not display in the MultiGraph.

⬤Bug fix: When the program automatically allocated memory for additional regions beyond the initial protocol settings, it was possible to create a region with a duplicate name and ID. In most cases, two regions would appear to be R32. Corrected.

⬤Bug fix: Regions can be reassigned when protocol plays back, e.g. R4 assigned to R5. The problem could occur if regions were deleted and re-created before the protocol was saved. This has been corrected.

⬤Bug fix: Protocols with calculated parameters could appear to hang if WinList's logging option was enabled. This problem has been corrected.

⬤Bug fix: Macro playback could produce inconsistent layouts, with some windows positioned incorrectly and some gates not assigned. This problem relates to some internal timing that allowed the macro to process the next step before the current step completed. It has been corrected.

⬤Bug fix: Remote connections on the PC displayed pasted pictures even after the connection was terminated. Now, any pasted picture is deleted before the remote connection is updated.

⬤Bug fix: The PC version now correctly handles remote POKE command with keywords for the optional 3rd argument. (VI1054)

⬤Bug fix: 2P regions did not correctly show resize handles on protocol playback.

⬤Bug fix: In previous versions, if data source windows were positioned off the left or top of the WinList workspace, or if histogram windows were positioned off the left or top of the data source, protocols would not correctly encode the position of the windows. On playback, the affected windows would not appear on the screen. The save protocol logic has been corrected to ensure that windows are not stored in an illegal location.

⬤Bug fix: Macro playback of SetPath command now changes the file filter correctly.

⬤Bug fix: The program now chooses $PnS keywords for parameter names for Diva files.

⬤Bug fix: Corrected problem with reading compensation files cross-platform. Resave existing compensation files with the new version to upgrade them to the new cross platform format.

⬤Bug fix: Protocols with 1P regions could position the regions slightly differently on different computers. This has been corrected.

⬤Protocol macros now pause if a data file used by the macro cannot be found.

**WinList 5.0 Service Pack 2**

This version adds some significant enhancements to regions, making them easier to use.

MAJOR CHANGE: A new Create menu option, Enable Region Creation, has been added. There is an associated button on the toolbar. In order to create regions, you must enable this menu option (or toolbar button). When the menu is checked, new regions can be created. When unchecked, regions cannot be created. In either mode, existing regions can be moved, sized, and edited.

2P regions have an additional property that controls whether the resizing handles will appear. By default, the handles will NOT be displayed. You can change the property using the Edit Properties dialog for the region, or by choosing "Show resize handles" from the right-button popup menu. On the Mac, the right-button menu is accesses by pressing the Control key when clicking on the region label.

The Region preference "Shift key menu" has been replaced with "Show popup menu" to clarify its purpose. With this option enabled, WinList will display the region popup menu when you click on a histogram. When disabled, no menu is displayed and a new region is automatically created when you click. With the option disabled, you can display the popup menu by holding down the Shift key prior to clicking the mouse.

2P regions no longer show center and rotation handles when printing and when creating pictures for metafiles and clipboard.

A shortcut key combination has been added for Print Screen. Use Ctrl-S on the PC, or Command-S on the Mac.

The program now provides a "simple batch" feature that creates a batch automatically when you select more than one file in the Open FCS File dialog. You can move through the files you select using the Next Batch/Previous Batch commands in the file menu. You can edit the batch using the Open Batch files dialog (file menu).

Gate expressions can now be 64 characters long. Previously, the limit was 32 characters.

Save Data Source has an option allowing the user to specify the number of events to save, as well as allowing the data to be saved as tab delimited text.

Bug fix: Corrected problem with 1P regions in which a region would not move when the label was dragged. The problem related to invisible resizing handles on the border of the region.

Bug fix: A datasource created using the Duplicate Data Source didn't show "Q" buttons in some histograms.

**WinList 5.0 Service Pack 1**

This version corrects several issues found with version 5.0.

Speed of the file open/save dialog boxes has been improved for folders containing many files.

Bug fix: Corrected problem saving MultiGraph as graphic on Windows 98/ME.

Bug fix: Corrected bug with copying a MultiGraph with 4 or more columns or rows. This bug also occurs with saving a MultiGraph as a graphic. It is a Windows-only bug, and it does NOT affect Macintosh builds.

Bug fix: Corrected bug with Open Batch dialog that would crash the program if pathway or file names were too long.

## WinList 5.0

This version offers many new features, including a new region system, database support, and many new graphics options.

### Regions

WinList now features a brand new region system, making it easier and far more powerful than ever. The properties for each region can be customized.

New region types include Rectangle, Ellipse, and Contour regions, each created in just a few mouse clicks.

The new NStat region type adds great analytical power to 2-parameter histograms. NStat is a collection of regions, much like a QuadStat. With it, you can partition a histogram into any number of rows and columns. As with the new quadstat, you can move individual vertices of the NStat.

1P and 2P regions now have an Auto-position property, accessible from the Edit Properties for Region dialog. When enabled, the region positions its center on the median of the histogram data. You can move an Auto-position region to offset its position relative to the data median. This feature allows regions to adjust automatically as new data files are read into a protocol.

Region aliases now support the use of keywords. As a result, file keywords as well as statistics for the region can be displayed directly on the histogram.

2P Regions now support rotation. To rotate, activate the region by clicking on the region's label. A small handle will appear to the right of the center. Click on and drag the handle to rotate the region. Release the mouse to finish.

Region files have a new format that provides much smoother regions than previous versions. Old format region files can be read and translated by the new version of WinList.

Create Region Array now allows you to specify negative starting values of up to one-half the region size. The dialog now hides the edit controls that are not active for the type of array you are creating.

Individual line weight, font, alias, and other properties for regions can be set using the Edit Properties for Region dialog. This dialog is displayed when you double click on the region label.

### Database

⬤Database support has been added. Any number of tab-delimited ASCII databases can be created to store file keyword information, analysis results, and internal keywords as well. Database options are found in the Edit menu.

⬤The database system features a viewer window so that you can review records as they are added.

⬤A new record is triggered by pressing the Acquire button on the toolbar. New records can also be added with a button on the viewer window.

Graphics

⬤2P histograms now support probability contours.

⬤2P histograms support showing dots and contours simultaneously.

⬤Histograms now support the addition of text and other report objects, which can be placed anywhere in the histogram space. Objects added to the histogram will be copied into MultiGraphs and to the clipboard, and will be printed as well.

⬤2P and 3P histograms now provide finer control over dot size, and dot size is no longer based on channels. You can choose values from 0-10, with each increment equaling one half of a point. (A point is 0.35 mm. or 1/72 in.)

⬤WinList now provides control over 2P dot drawing order in color event mode. This makes it much easier to visualize low frequency and rare events in dot plots. Choices are Listmode order, Low frequency on top, or High frequency on top.

⬤The Apply to All option in the Edit Graphics dialog now spans data sources.

⬤Edit Paste now pastes text, bitmaps, and pictures into the active histogram as objects that can be dragged and sized. Text objects can also be edited.

⬤The File Save menu has a new entry, Save Histogram Graphic. This allows you to save the current histogram as a graphic to disk. It replaces the functionality of the Copy Special dialog "Save" option, which has been removed. Saving is now supported on the Mac as a PICT file.

⬤The display of log scale on Y-axis for Win95/Win98 has been corrected.

⬤Printing of Y-axis text on some Mac printers has been corrected.

⬤Dots of size 0 now draw much faster on Mac.

⬤Color density mode now supports gradient coloring, as well as the rainbow palette.

⬤Logic for determining major/minor axis tics has been revised to provide a more consistent interval. Properties for number of major and minor tics have been added to histogram graphics dialogs.

**N-Color Compensation™**

⬤Compensation trace lines can now be moved with greater freedom, allowing you to "compensate" populations that do not necessarily have signal crossover. If you need to move a trace line beyond the restricted space, hold down the Shift key on the keyboard before you start the drag operation. The low end of the trace line (intercept control) will move along the left and bottom edges, and the high end (slope control) will move along the top and right edges.

🔵Compensation Trace lines now display hollow handles when not selected. These handles are the only "hotspots" on the trace lines, so you have to click on one of these hotspots to pick a trace line. This allows trace lines to work along with regions and other objects on the histogram window.

🔵Regions can now be created and moved on histograms with N-Color Compensation™ activated and trace lines displayed.

### Histogram

🔵The histogram right button popup menu now includes "Annotate," which adds a text object to the histogram. This is the equivalent to choosing "Text" from the Report menu.

🔵Histogram subtraction now shows a linearized median for positives as well as a channel-based value. The subtraction routine has been further improved and is now called Super-enhanced DMax subtraction (SED). Subtraction results can be databased.

🔵An Auto-update menu option has been added to the Acquire menu. This option replaces the auto-update checkbox formerly on the Edit Region dialog. When unchecked, you can move regions without triggering the reprocessing of events. Reprocessing will occur when you re-acquire, or when you re-enable the option.

🔵Calculated parameters are now selectable as 1P and 2P histograms in the Multiple Histogram Selection dialog. Histograms for calculated parameters are listed after native parameters in the list boxes.

### Results

🔵WinList now supports Y median and Y mode results for 2P regions.

🔵The median computation has been revised to be more accurate.

🔵Additional statistics for low and high X and Y values are now available in Results windows and the database.

🔵Result windows now support a right button mouse click to display a popup menu. Options in the menu include edit, copy, and copy with no header. To access this on the Mac, press the Control key on the keyboard while clicking the mouse.

🔵When Results windows in auto size mode exceed the height of the application window, a vertical scroll bar is now added to the window. The height of the window is set to the application window height.

🔵Results windows now right-justify numbers.

🔵Results windows on Mac now auto size to show full content.

### Preferences

🔵Preference dialogs have been replaced with a new, tree-format preference dialog.

🔵New preferences have been added to support the numerous new graphics options.

🔵A graphics preference for grid color has been added.

🔵Preferences can now be saved to and opened from disk.

🔵Significant changes have been made to logic that creates pictures for MultiGraphs and for copy to the clipboard. Graphics Preferences contains a property that controls the target size

for a picture copied to the clipboard, defaulting to 10 cm. or 4.0 in. MultiGraph Preferences contains a similar property that controls the size used when creating a picture for a MultiGraph.

A new preference for MultiGraphs allows you to choose a separate dot size for dot plots when they are added to MultiGraphs. This allows you to work with dot plots showing dots of one size, and store them in MultiGraphs with a different dot size. Use this option if you are printing MultiGraphs with dot plots and dots are not showing up on the printout.

A graphics preference to allow control over the amount of border space around histograms has been added.

A MultiGraph preference to force elements to be square in tiled view has been added.

A MultiGraph preference for spacing between elements has been added.

A new preference section for results has been added, and a new preference for number of decimal places in floating-point results is available.

There is a new section for region preferences.

**Other**

The program uses a new file open/save dialog, which is identical on Mac and PC versions. This new dialog correctly displays long file names on all tested platforms. In addition, file date and size are displayed in the dialog to assist selection.

The file dialogs are now resizable, and the size is saved automatically. The program will use the new size when displaying the dialogs.

The file menu now has a "Get Previous Data Source" option. The shortcut for the option is Ctrl-P on the PC, Apple key-P on the Mac.

File names can include 0-9, A-Z, a-z, spaces, periods, hyphens, and underscores.

WinList now incorporates the same event logging system used in ModFit LT and QuantiCALC. Logging can assist tech support if you have a problem with the program.

Protocol macros now save edits in Names and Resolutions for each data source if edits have been made. Edited names, log decades, and log bias values are saved in the file, and reapplied when the protocol is opened.

The toolbar on the PC version now has the new "flat" look.

A button to create MultiGraphs has been added to the toolbar.

On PC version, a new command in the File menu allows you to save MultiGraph as a graphic file.

The Edit Macro Toolbox dialog now has a delete button to clear an item from the toolbox.

The SetPath macro command now supports evaluation of keywords in the second portion of the string.

Macro pause command has been revised. If the optional parameter is empty, the pause command will pause the macro without displaying a message.

The macro system now correctly handles a Pause command as the last command in the macro.

The program no longer supports a background metafile in the application client area.

●The Edit Region popup dialog box has been removed.

●On-line help is now in HTML format and can be viewed in most common browsers.

●A PAUSE command has been added to DDE scripting. It displays a dialog with an optional message and waits to proceed until the dialog is closed.

●ModFit LT 3.0 and WinList 5.0 on PC platform can now exchange file keywords in remote connection transactions.

●The Keyword dialog is resizable.

●Several datasource keywords have been enhanced. [FILE], [FILEROOT], and [FILEPATH] keywords now allow you to specify an optional datasource ID, using the form [FILE,id], [FILEROOT,id], and [FILEPATH,id]. When there is no ID specified, the active datasource is used.

●Two new stats for the [R(n,stat)] keyword are now available. The keyword returns the name of the datasource that contains the region when stat is *DS*. When stat is *HST*, the result is the combined file and histogram name.

●When the program closes, it now saves its size and position. When you start the program again, it will restore itself to that size and position (unless you change your screen resolution to prevent that size/position).


**3D version (Available as an option on the PC only)**


●The PC version now features newer 3D support with OpenInventor 2.5.

●3D graphics now provides control over cloud probability using the Display Options dialog in 3D viewer window.

●Axis labels for 3D display have been corrected.

Bug fixes

●When saving MultiGraphs to disk, memory resources were not properly released after the save operation. This could cause substantial memory demands when batch processing and saving MultiGraphs.

●When saving MultiGraphs on the Mac, some 2P dot plots could cause the program to write an invalid file to disk that subsequently couldn't be read. This has been corrected.

●If a compensation file cannot be opened by a macro, the macro will now pause and display an error message.

●Histogram subtraction did not work if the data file name had a space in it.

●If a remote DDE connection is broken during the processing of a remote script, WinList will now stop processing the script.

●Saving certain file types when running a macro could hang the system if the files already existed on disk. File saving by macros will now always overwrite target files.

●Cluster coloring now applies to 1P histograms for primary gate populations.

●Info windows now have appropriate limits on scrolling.

Auto-size of results window now takes into account the ID fields.

When a protocol is saved after deleting a histogram, the protocol will now playback correctly. This corrects several problems with protocols, region assignment, and histogram activation.

Adding a minimized histogram to a MultiGraph will now use correct dot size.

Adding a histogram to a MultiGraph now produces same result regardless of the size of the histogram window.

A problem with macros containing a Pause command has been corrected. The problem prevented the user from moving regions.

DDE scripts now open and save to selected path.

Saving a MultiGraph now prompts if the file already exists.

Open Batch and Open Macro dialogs did not show long file names on Win95/Win98. This has been corrected.

Print Report will no longer replace "&" symbol with "_" in gate expressions.

On Mac version, MultiGraph popup menu now works correctly.

If print screen fails because the printed image is too big, the program will scale down the image and try again.

Compensation of linear parameters has been corrected to prevent values from going out of bounds. Values after compensation are now forced to be greater than or equal to zero, and less than ADC resolution.

Temporary files on the Mac version are now cleaned up correctly.

Known problems printing regions on certain printers (HP and others) have been corrected.

When saving a histogram to an FCS file, the "SAMPLE ID" and "$SAMPLE" keywords now store the correct information. Previous versions stored time information.

## System Requirements

**Minimum System Configurations**

PC: Windows 7, 10, or greater, 2GHz dual core CPU or better, 2GB RAM or greater, 50MB free disk space for program files.

Faster CPU speeds with more CPU cores and greater RAM will improve performance, especially with large FCS files.

**Installing and Running WinList**

You must have administrative rights to the computer during installation and registration of the software. After registration, the program can be run by users with restricted access to the computer.

If your program is an update version, you will need the serial number for the previous version in order to register and unlock the software.

If your program is an additional computer license, you will need the serial number for the primary license in order to register and unlock the software.

You will need to register the software with Verity Software House in order to unlock the program on the computer. This can be done using the online registration process or by contacting technical support.

**Network Version**

Log on to the "server" computer with an account that has Administrator privileges. You *must* have administrative rights during installation.

If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.

If a start-up screen is displayed for the CD, follow the directions on the screen to complete the installation. If no start-up screen is displayed, navigate to and double-click the **InstallAll.vbs** script to install all of the software components.

This will install WinList 3D as well as an application called the **Network License Manager** on the computer.  Alternatively, you can double-click each **msi** file that appears on the cd to install the Network License Manager and WinList.

See Using the Network Version for details on configuring and using the network version of the software.

**Stand-Alone Version**

Stand-alone versions of the program are designed to be installed and registered on a single computer.

**Installation**

Log on to your computer with an account that has Administrator privileges. You *must* have administrative rights during installation.

If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.

Run the program named **WinList.msi**. Follow the directions on the screen to complete the installation.

See the section on <u>registering your software</u> for information on how to register your software, or…

If your version is a Software Battery version, see the section on Installing a Software Battery.

**Using the software**

Log on to your computer with your own user account. This should be the account that you normally use on the computer.

Open the Start menu, and choose All Programs. Then navigate to the Verity folder, and choose WinList 3D.

**Registering Your Software**

**Why You Should Register**

With this version of WinList, registration is a key step in setting up the software to run on your computer. In fact, *your software may not be fully functional until you register*. Here's why.

This version of the program uses software security that allows it to be used without a hardware lock. Software security requires you to register your software before you can receive an Unlock Code to make the program fully functional. You can run the program up to 10 times prior to registering the software.

Of course, registration is useful for other reasons, too. We can inform you of bug fixes and updates, and provide you with better technical support.

**How to Register**

When you start the program for the first time, it will automatically display the Registration dialog box shown below. You can access this dialog at any time by pressing the *Registration* button on the dialog box that is displayed when the program starts up.



This dialog has three fields that you need to fill in: *Serial #*, *CD Key*, and *Unlock Code*.

The *Serial number* and *CD Key* can be found on the outside of the box the program came in, on the CD sleeve, or they may be provided to you electronically by email or our website. Enter these exactly as they appear, as they are case-sensitive. If you have lost or misplaced the Serial number or CD Key, contact Verity technical support at verity@vsh.com or (207) 729 6767.

## If the installation computer has Internet access

Start by filling in the Serial Number and CD Key fields in the Register Software dialog. Use the Serial Number and CD Key that were provided with the software.

Next, to receive an Unlock Code using the Internet, click the *Web Registration* button on the dialog box. This button will launch your Internet browser software and connect you to the registration web site.

Follow instructions on the web site to register the product. If you are registering an *update version*, you will need the serial number of the previous version to complete the registration. If you are registering an *additional user license*, you will need the serial number of another copy of the program that is registered to your institution. If you have a network version – stop – you are in the wrong place. For network versions, see Using the Network Version .

*Getting an Unlock Code is a two-step process*. First, you fill in and submit the registration form on the web site. This sends a registration request is sent to Verity. The information is reviewed, and an Unlock Code is created for your installation. Your Unlock Code will be emailed to you, so make sure you provide a valid email address.

When you receive your Unlock Code, copy it from the email message into the Unlock Code field in the Registration dialog box. When possible, use Copy and Paste to do this, to ensure that the characters are correct. Then click the OK button.

Your program will become fully functional once the Unlock Code is entered correctly.

## If another computer has Internet access

If the computer you installed the software on does not have Internet access but another computer *does* have access, you can follow these steps to register.

*Write down the Program Name, Program Version, Computer ID, Serial number, and CD Key displayed in the Register Software dialog*.

Go to the computer that has Internet access, launch the browser, and navigate to www.vsh.com.

Follow the links to Register Software.

Follow instructions on the web site to register the product. If you are registering an *update version*, you will need the serial number of the previous version to complete the registration. If you are registering an *additional user license*, you will need the serial number of another copy of the program that is registered to your institution.

*Getting an Unlock Code is a two-step process*. First, you fill in and submit the registration form on the web site. This sends a registration request is sent to Verity. The information is reviewed, and an Unlock Code is created for your installation. Your Unlock Code is then emailed to you, so make sure you provide a valid email address.

When you receive your Unlock Code, copy it from the email message into the Unlock Code field in the Registration dialog box on the computer where the program is installed. When possible, use Copy and Paste to do this, to ensure that the characters are correct. Then click the OK button.

Your program will become fully functional once the Unlock Code is entered correctly.

**If you do not have Internet access**

If you do not have access to the Internet, you can send the registration information below to Verity Software House by fax, email, mail, or even telephone. Our technical support staff can register the software for you with the information you provide, and supply you with the Unlock Code to enter into the Registration dialog.

Here is the information we need. You can print this page and fill it in.

*These fields are found in the Registration dialog box.*

Program Name:
Program Version:
Computer ID:
Serial number:
CD Key:

*User Information*

Name:
Institution:
Address:
City:
State/Province:
Zip/Postal Code:
Country:
Phone:
Fax:
Email:

*Our technicians will use the information below to create a web user account for you on our web site.*

Name to use on VSH web:
Password to use on VSH web:
A question we can ask to verify who you are:
The answer to the question to verify who you are:

*If you are updating from a previous version, you will need to supply the serial number of the previous version.*

Serial Number of previous version:


If you have any questions, please contact us. See Contacting Verity Software House for phone numbers and addresses.

**Using the Network Version**

The Network Version of WinList is designed to be installed and managed on a single "server" computer by a user with administrative rights to the computer. The computer needs to be visible on the network to the users who need to run WinList. The server computer should be running 24/7 to provide access whenever those users need access; users will not be able to run the software if the "server" computer is shut down or off the network.

The "server" software can be set up on a machine running Windows 7, or greater; or it can be installed on one of the Microsoft Server versions from 2008 or greater. One component of the WinList network version will be set up to run all the time on the server computer.

**Configuration of the Network Version**

Log on to the "server" computer with the administrative user account that will be used to run the Network License Manager. This user *must* have administrative rights to the computer.

The WinList 3D Network folder needs to be shared to allow network users to access and run WinList. Use the utilities on your computer to share the folder and provide Read-access rights to the users who need to run WinList.
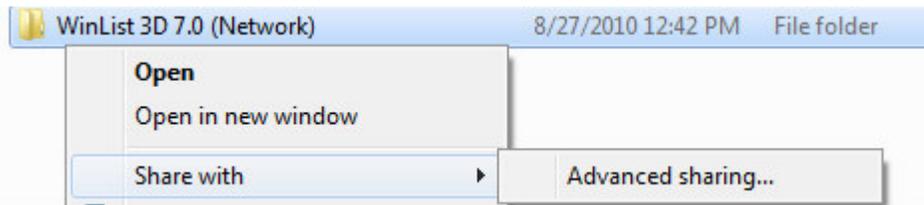


Run the **Network License Manager** (NLM) application. On Vista and newer operating systems, right click on the application and choose "Run as administrator".
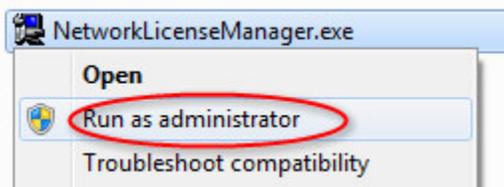


When NLM starts it will prompt you to choose a port to use. This is a TCP port that the software will monitor to communicate with network users who are running WinList. In most cases the default port is fine. You may be prompted by User Access Control on your computer to allow the software to use this port; allow the software to use the port if prompted.
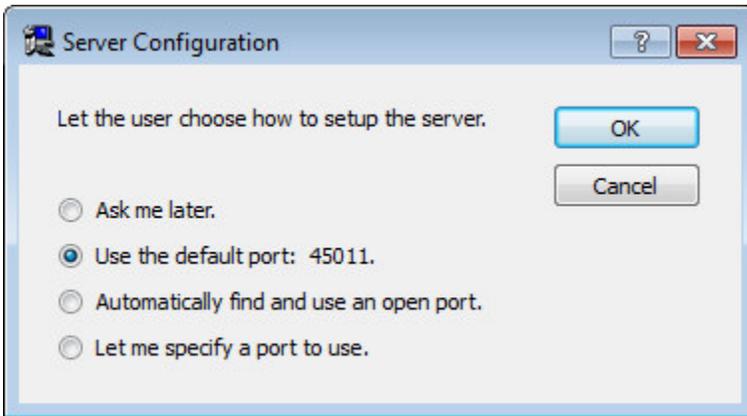
●Next, you will be prompted to select the location of the WinList 3D installation. The step is important because it will allow the NLM to write a special file (NLMServerInfo.txt) into the WinList directory that will allow WinList to connect to the NLM on your server.
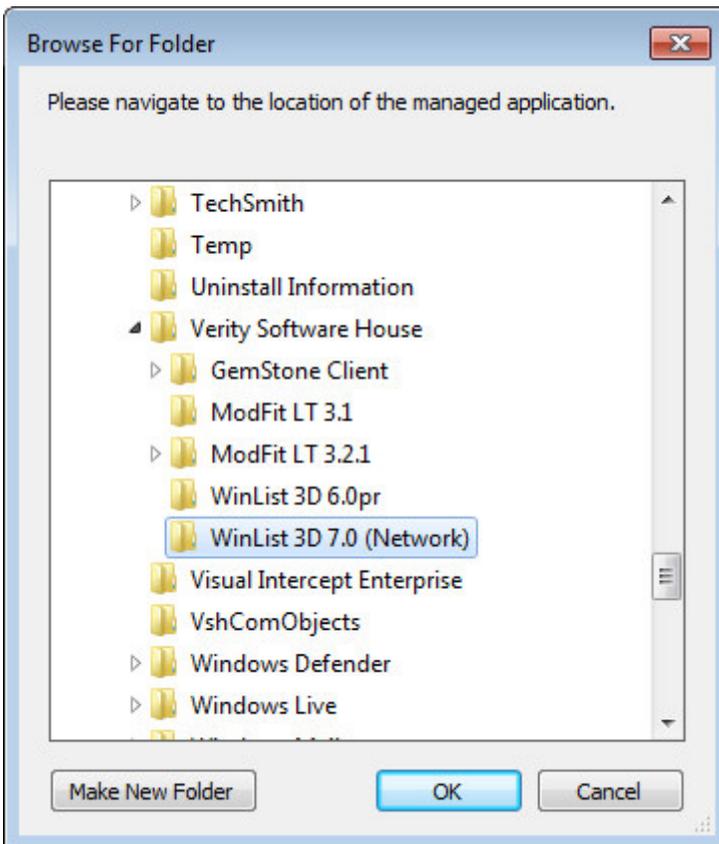


### Adding Licenses to the Network Version
Once the **Network License Manager** is installed and configured, you are ready to add licenses for WinList. Each license you add provides one use of WinList at a time on the

network. For example, if you add 5 licenses, NLM will support 5 simultaneous users on the network.

● Click the **Add a network license** button on the Network License Manager toolbar.
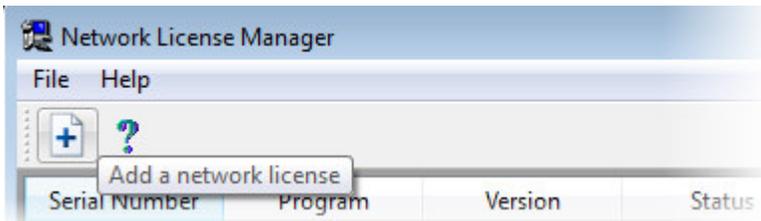


● The Registration Wizard will be displayed. For each network license, enter the Serial Number, CD Key and user account information to register the license. This process needs internet access to the Verity Software House web site, www.vsh.com.



**Testing Your Installation**

After installation and adding licenses to the **Network License Manager**, you are ready to test the setup.

First, run WinList on the server itself. Launch WinList3D.exe. If the system is properly configured, the program should start and operate correctly. If the WinList cannot communicate with the Network License Manager, an error message will be displayed.

After you have verified that WinList will run on the server itself, you are ready to test it from another network computer. From a network computer, navigate to the WinList share that you created on the server computer. The first time that you run WinList from a network computer, you should run the script "**WinList3D_NetworkSetup.vbs"** located in the WinList installation folder. This script will ensure that required DLLs are installed on the network computer. This script only needs to be run once; after that, the program can be run via a desktop shortcut on the network computer.

## Setup a Scheduled Task to Run NLM on Windows 7

The **Network License Manager** needs to run all of the time on the server computer, so it is useful to set up a task that will automatically launch NLM each time the server computer starts up. The specific instructions shown here for setting up a scheduled task will vary a bit from one OS to another.

Note: the scheduled task should be run with the same user account that was used to configure the Network License Manager.

From the System and Security section of the Control Panel choose **Schedule tasks** under Administrative Tools. Choose Create Task from the Actions pane.
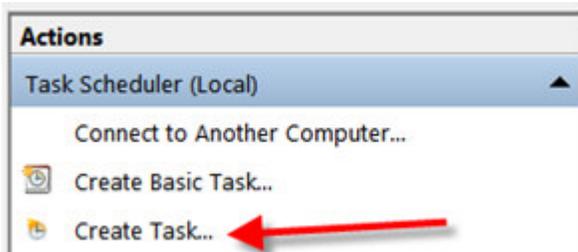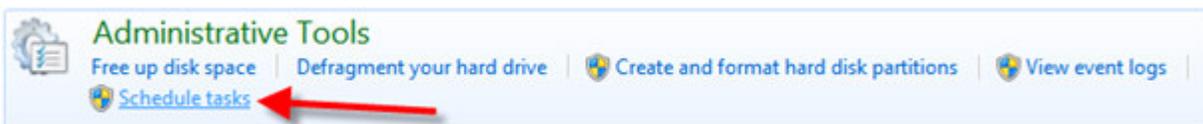




On the general tab, give the task a name and description and select the Windows OS user that was used when configuring the NLM. Choose for the task to run even if the user is not logged in

On the Triggers tab, click **New**. In the New Trigger dialog, choose "At startup" for the **Begin the task** dropdown and click OK.



On the Actions tab click **New**. Click **Browse**, navigate to the folder containing the Network License Manager, select **NetworkLicenseManager.exe**, click **Open**, and then click OK.
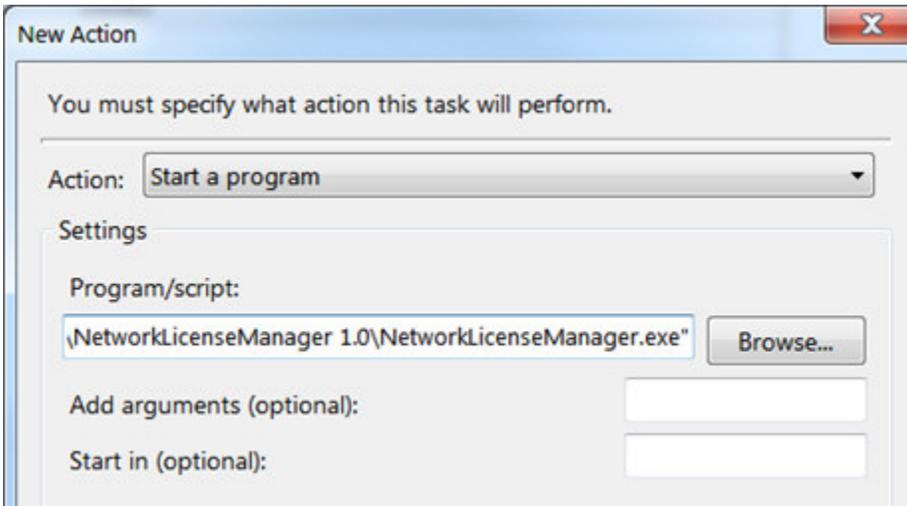
●On the Settings tab, **uncheck** the box next to "Stop the task if it runs longer than". We do NOT want this task to be stopped. Click **OK** to complete the task creation process.
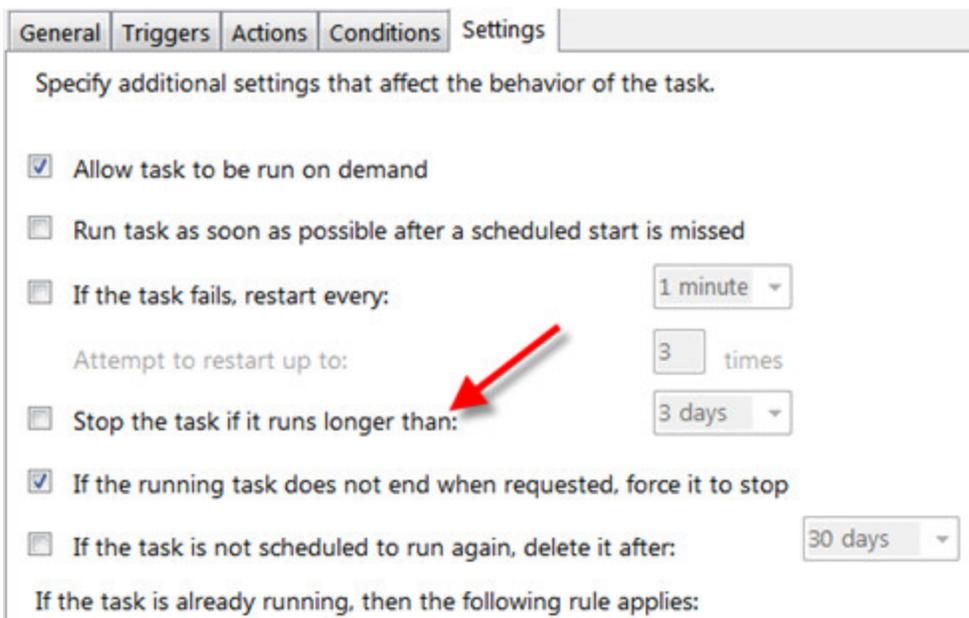


The next time that the server computer is restarted, the Network License Manager should automatically start up. You may want to restart the computer to verify this.

## Contacting Verity Software House

### Mailing Address:

Verity Software House
PO Box 247
Topsham, ME 04086
USA

### Shipping Address:

Verity Software House
45A Augusta Road
Topsham, ME 04086
USA

| | |
|---|---|
| Web: | www.vsh.com |
| General Email: | verity@vsh.com |
| Tech Email: | tech@vsh.com |
| Sales Email: | sales@vsh.com |
| Developer Email: | devteam@vsh.com |
| Toll-free Phone (US): | (877) 729 6767 |
| Phone: | (207) 729 6767 |
| Fax: | (207) 729 5443 |

**Tutorials**

**WinList Basics**

A key component in the design of WinList is fast, efficient performance. This applies not only to the inner workings of the program, but also to the user-interface. Everything you do with the program is designed to be easy to understand and highly efficient.

This introduction focuses on the fundamentals of working with WinList. In the course of the tutorial, you will see how quickly and easily you can create an analysis setup with WinList. Opening files, creating histograms, drawing regions and gates, and developing reports are all covered in this exercise.

**Open files**

1. Click the **Open FCS File** button on the ribbon bar.
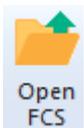


2. In the File Open dialog, navigate to the Samples folder located in the folder where WinList is installed. Select the three files **BCell_BM_Tube_001.fcs**, **BCell_BM_Tube_002.fcs**, and **BCell_BM_Tube_003.fcs** and click **Open**.
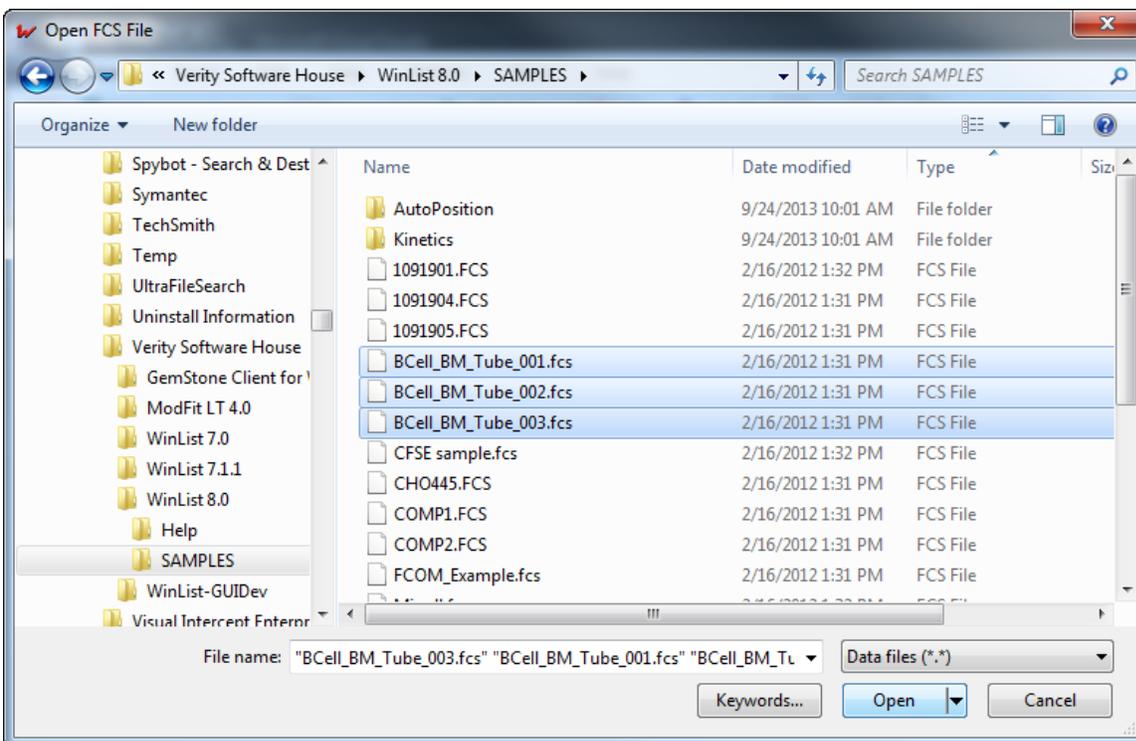
On the PC, you can also drag and drop files from Windows Explorer or My Computer onto WinList. See Drag and Drop for details.

The Create Histograms dialog will appear.

## Create histograms

This dialog allows you to create any number of histograms very quickly. In this tutorial, we will create several 2P histograms. The dialog also allows you to create 1P and 3P histograms.

3. Select FSC-A in the X Axis list, and SSC-A in the Y Axis list. Then click the **Add to List** button.
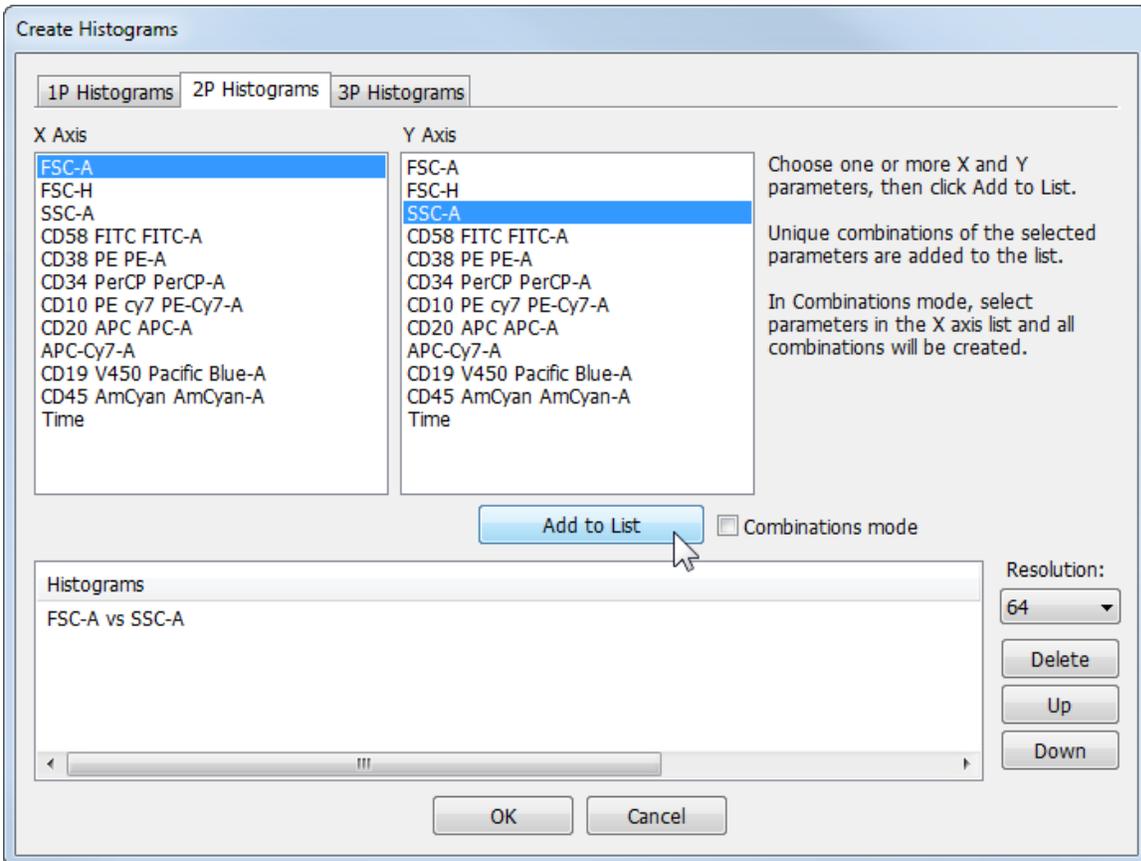
4. In the X Axis list, select CD10 PE Cy7, CD20 APC, and CD45 AmCyan. Enable the **Combinations mode** checkbox. Click the **Add to List** button. In this mode, histograms are created for all combinations of the selected parameters.
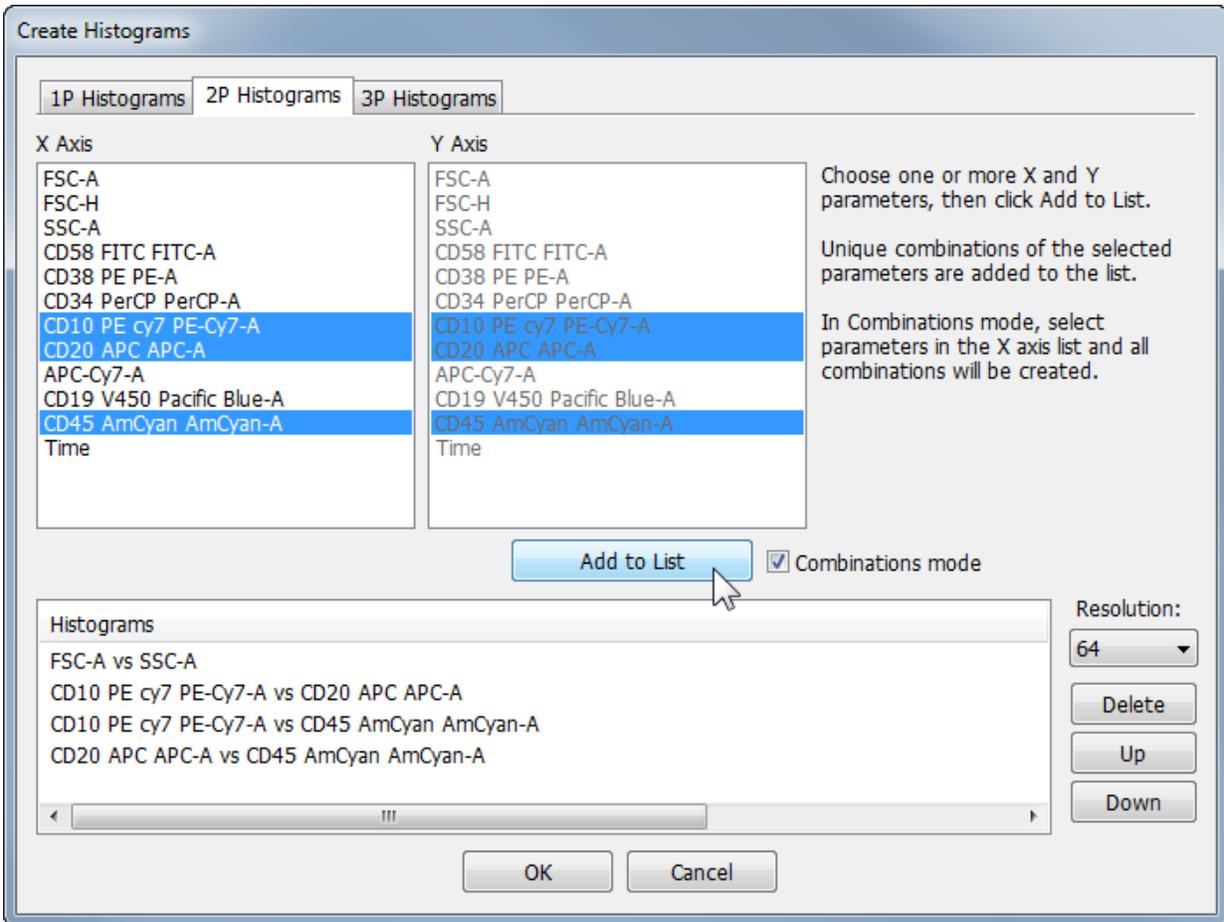
5. Click the **OK** button.

*You can also create one histogram at a time by clicking and dragging on a data source to define the area where you want the new histogram. The next tutorial illustrates this technique.*

**Create a light scatter region**

Next, we'll draw a light-scatter gate on the FSC-A vs. SSC-A histogram.

6. Click the title bar of the FSC-A vs. SSC-A histogram and select the Freehand region tool from the toolbar that appears.

*The histogram toolbar has tools for creating other types of regions.*

7. Draw a region around the population shown. To finish drawing the region, click on the point that appeared when the drawing began.

*If you want to delete a region, simply click on the region's label and press the Delete key on the keyboard.*
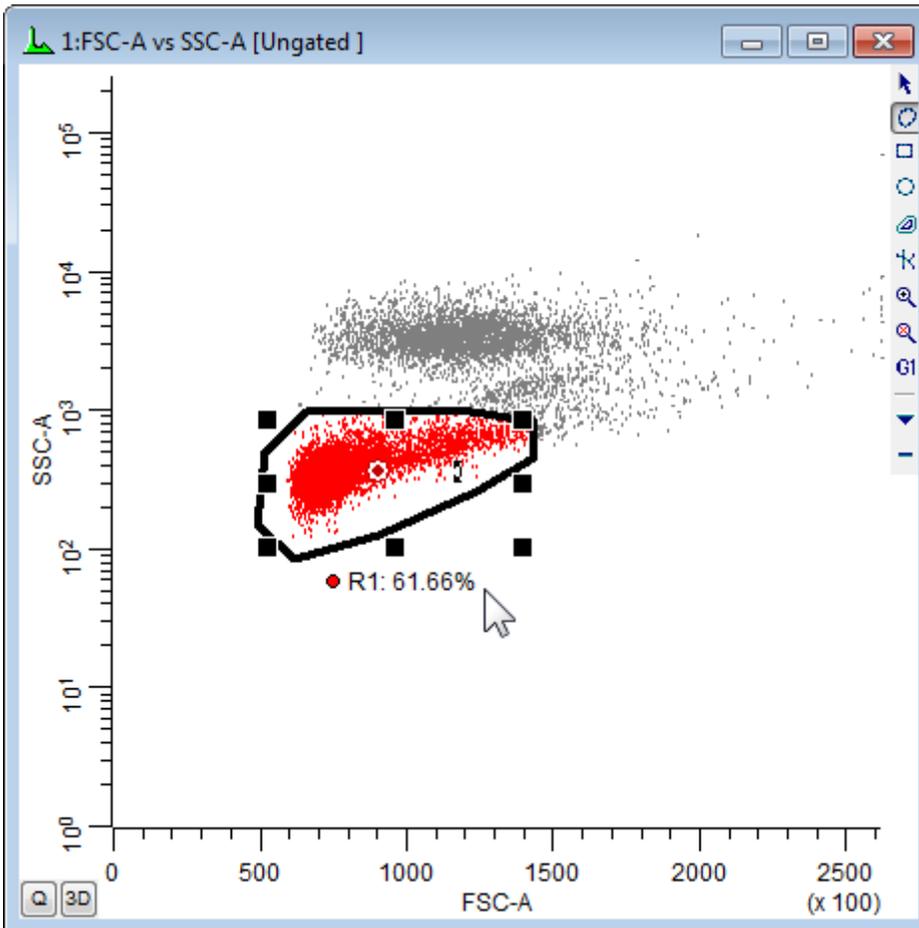


## Gate the histograms

With the R1 region drawn, we can now gate the other histograms on R1.

8. Click the mouse on the red bulls-eye in the middle of the R1 region. This is called the "cascade hotspot" and is used to apply the R1 gate to other histograms.

9. Press the Shift key on the keyboard and *click the title bar* of each of the remaining histograms. As you click, the gate is applied to the histograms.
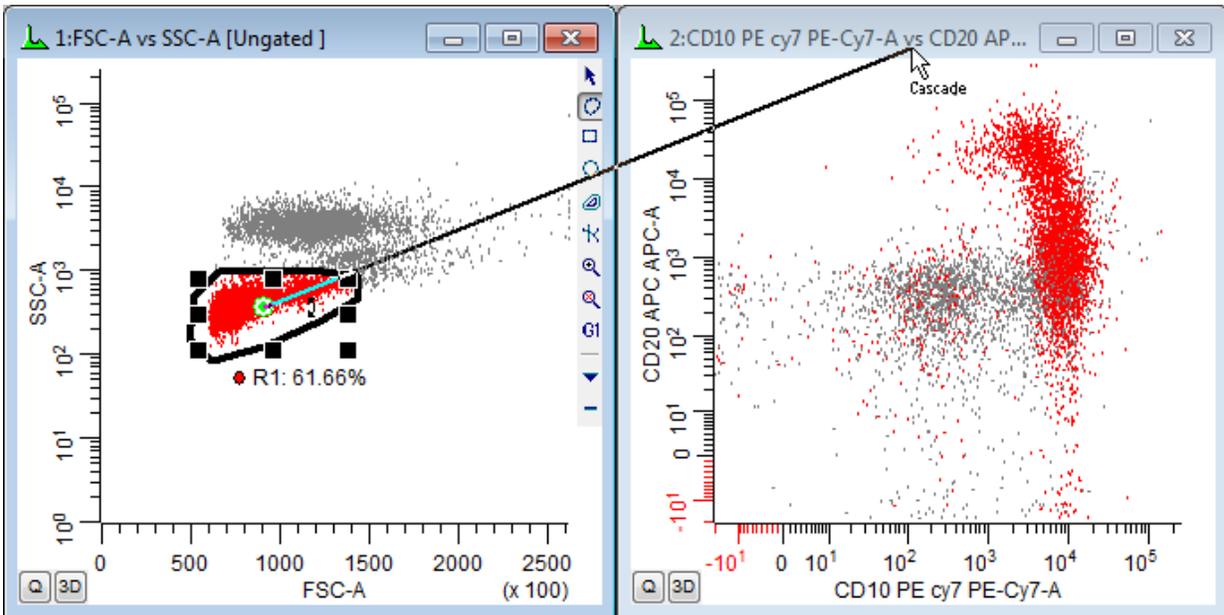
10. Press the Esc key on the keyboard to cancel cascade gating mode.

## Create QuadStats for Statistics

Let's create QuadStats for each of the gated histograms.

11. Click the **Q** button in the lower-left corner of each of the gated histograms. A QuadStat region will appear on each histogram, and a Results window for each QuadStat will be displayed.

12. Arrange the Results windows so that they are visible and not covering the QuadStats.

13. Adjust each QuadStat. To move the QuadStat, click and hold on the region label in the center of the QuadStat, drag the label to a new position, and release the mouse.
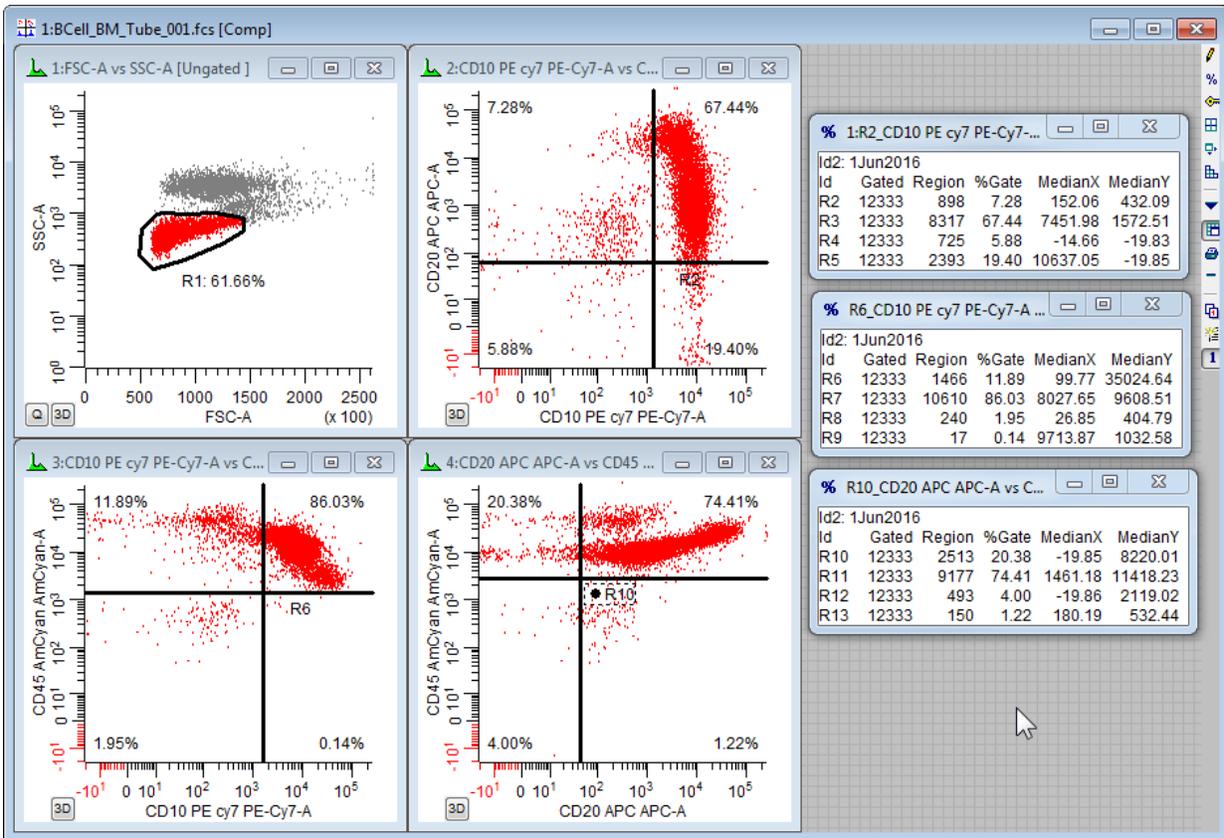
NStat regions are like QuadStats, but are much more flexible and powerful. With NStat regions, you can have any number of rows and columns, and the vertices do not have to remain orthogonal. Use the NStat tool on the histogram toolbar to create an NStat.

## Create a report

At this point, we have a basic analysis setup in place. Let's create a report that can include these graphics and other elements in a page layout format.

14. Click the **Report** button on the ribbon bar. Move and size the new report window so that it is not covering histograms and result windows.
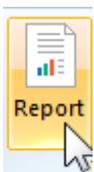
15. Move the mouse over the icon on the left side of the first histogram's title bar. Drag and drop that icon onto the report.
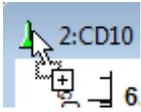


16. Add additional histograms in the same way, dragging and dropping each one onto the report.

17. Drag and drop the results windows onto the report in the same manner.
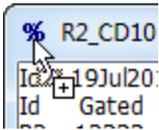


*Reports can also contain keywords, text, alerts, and other graphics. They can be saved for future use, as well as exported to PDF format. See Creating Reports for more information.*

**Process the files**

Since we selected several files for analysis, WinList has created a batch of files to analyze. The batch toolbar appears at the bottom of the WinList window whenever there is a batch of files selected.

18. On the batch toolbar, click the **Edit** button to display the Edit Batch Options dialog.



The options that appear in this dialog determine what happens when each new file is opened into the setup we have created. Review the options in this dialog. If connected to a printer, enable the **Print Report** option. Click **OK** to close the dialog.

19. Click the **Next batch item** button on the batch toolbar to advance to the next listmode file.



Elements on the report will update automatically as the new data is read in.

20. Adjust the gate in the FCS vs SSC histogram.

21. Click the **Next batch item** button again to process the last file in the batch.

*Other buttons on the batch toolbar allow you to move forward and backward in the batch, as well as to auto-advance through the entire batch list.*

### Save the protocol

Anytime that you have an analysis setup that you want to be able to re-open, use WinList's **Save Protocol** or **Save Bundle** command.

The Save Protocol command creates an XML file that WinList uses to re-create the setup. If you want to be able to use this setup on other computers, the Save Bundle command creates a single file that also contains the listmode data file that is currently opened.

The bundle is also an excellent way to archive the analyses you perform. Simply use the **Save As** button and choose Save Bundle to archive each data file that you open and analyze. Even better, use the batch toolbar to process your files, and set its options up to save a bundle automatically.

### Summary

This brief tutorial has demonstrated how quickly and efficiently WinList helps you to build an analysis setup. In just a few minutes, we were able to create histograms, draw regions, apply gates, and generate QuadStat statistics. We created a new report and dragged the elements we wanted onto the report. And finally, we processed a batch of files through the analysis setup.

Try out some of the additional tutorials to get more details on the powerful features of WinList.

**See also:**

Tutorials

Cascade Gating and Boolean Gates

**Cascade Gating and Boolean Gates**

Gating is a process of identifying subsets of populations in histograms. Regions are defined and used to "gate" the events that are displayed. Using Boolean expressions, gates can be created that are inclusive rather than restrictive in nature.

WinList is designed to make the common task of creating restrictive gates very fast and very simple, while at the same time offering open-ended Boolean gating expressions that are extremely flexible and powerful. This tutorial will explore the ease of creating restrictive, "cascade" gates, and look at how to use Boolean gate expressions to identify other populations.

## Open a file

1. Click the **Open FCS File** button on the ribbon bar.
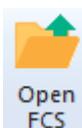


2. In the File Open dialog, navigate to the Samples folder located in the folder where WinList is installed. Select **BCell_BM_Tube_001.fcs** and click **Open**.

*On the PC, you can also drag and drop files from Windows Explorer or My Computer onto WinList. See [Drag and Drop](#) for details.*

3. The Create Histograms dialog will appear. Click the **Cancel** button to open the file without creating any histograms.

## Create a histogram and draw a region

There are several ways that histograms can be created in WinList. We'll draw a histogram on this empty data source window to get started.

4. Click and hold the mouse button in the data source window. With the mouse button down, move the mouse to define the size and location for a new histogram window. Release the mouse to finish the histogram drawing operation.
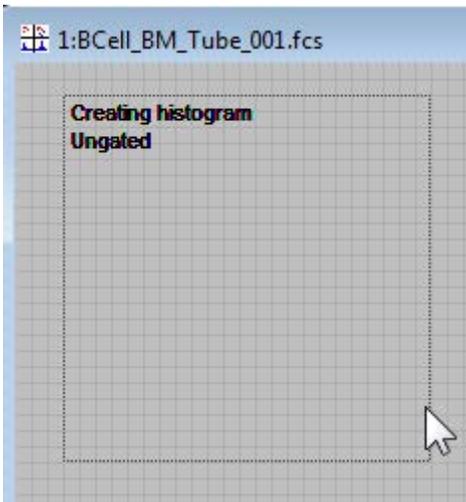
5. The Edit Histogram dialog is displayed. Choose **FSC-A** for the X Axis and **SSC-A** for the Y Axis, and then click **OK**.

> *On any histogram window, you can click on the axis label and select a new parameter. You can also change the parameters that are displayed using the Edit Histogram dialog.*

6. Click the **Freehand** region tool in the histogram toolbar, and draw a region around the population as shown. To draw a freehand region, click next to the population of interest and hold the mouse button down. Drag the mouse around the population as shown. To finish drawing the region, click on the point that appeared when the drawing began.
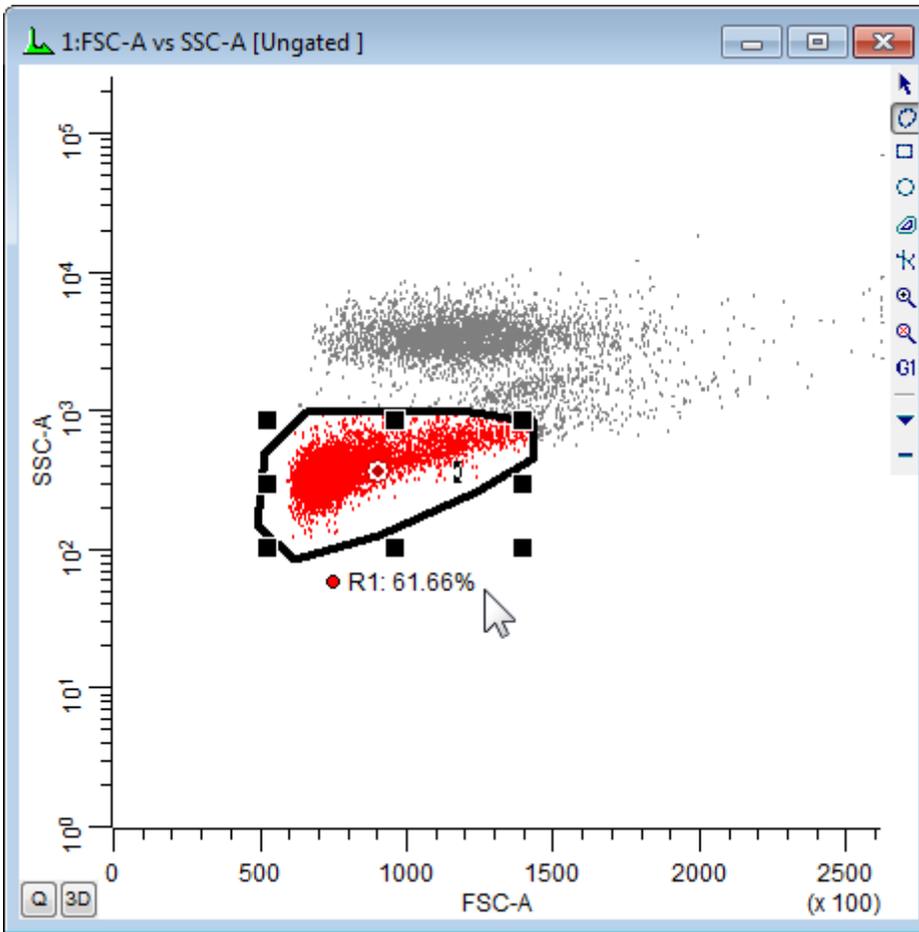
## Create a cascade-gated histogram

Notice the red bull's eye in the middle of region R1. This is the "cascade gate" hotspot, used to gate another histogram on this region.

7. Click the cascade gate hotspot in R1; the red hotspot will change to green. Move the mouse to an empty portion of the data source window. Click, hold, and drag to define a new histogram window.
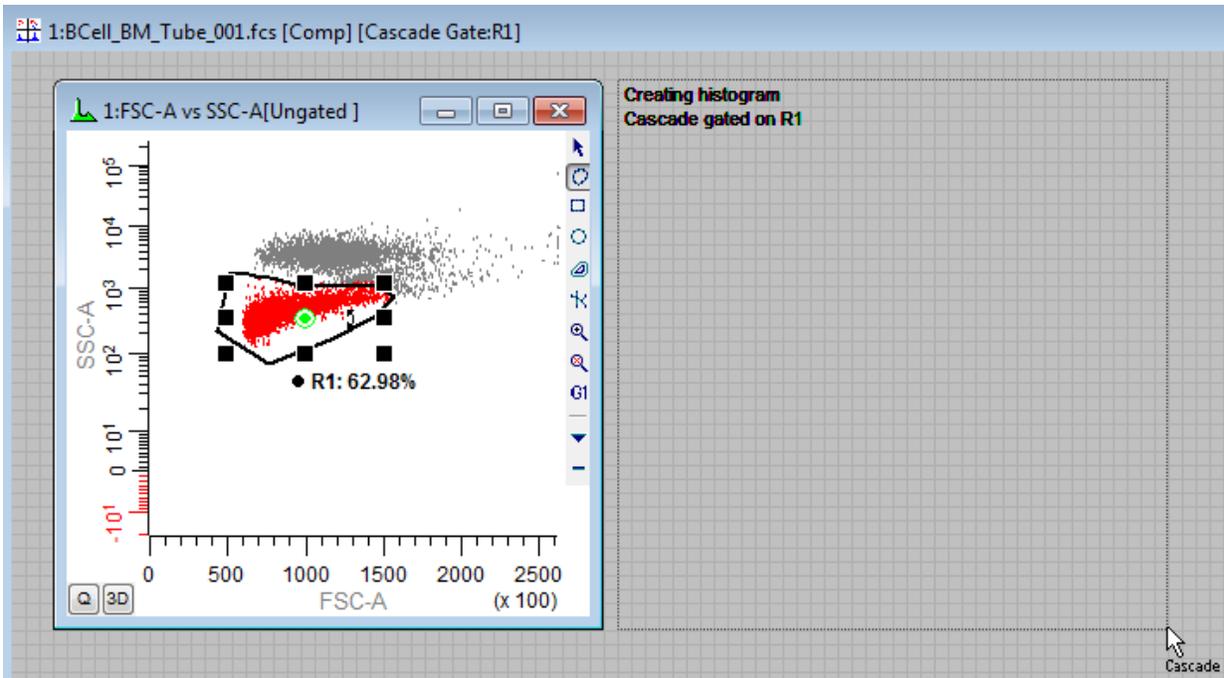
8. Release the mouse button. In the Edit Histogram dialog, select **CD10 PE Cy7** for the X Axis and **CD20 APC** for the Y Axis. Click **OK**. This new histogram will be gated on R1.

## Additional cascade-gated histogram

Now, let's draw a region in this new histogram and use it to create another gated histogram. The new histogram will be gated on R1 *and* R2.

9. Draw a freehand region in the CD10 PE Cy7 vs CD20 APC histogram to identify a subset of the events in that histogram.

10. Click the cascade gate hotspot in *R2*. Move the mouse to an empty portion of the data source window. Click, hold, and drag to define a new histogram window.
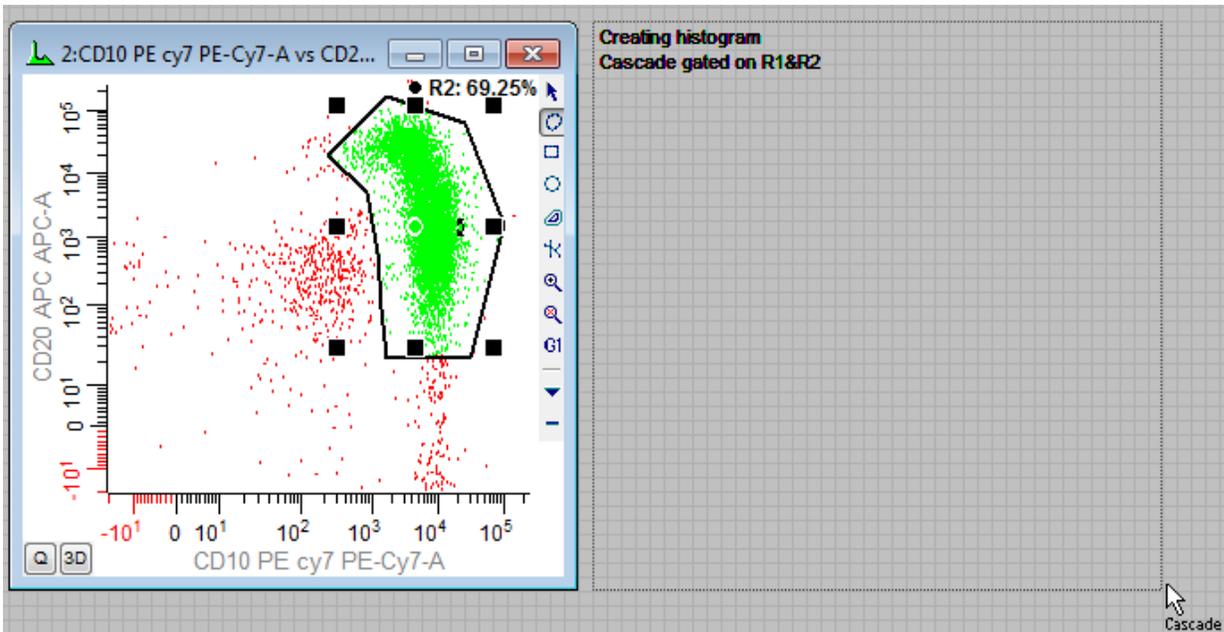
11. Release the mouse button. In the Edit Histogram dialog, select **CD10 PE Cy7** for the X Axis and **CD45 AmCyan** for the Y Axis. Click **OK**.

This new histogram is gated on R1 *and* R2, meaning that only the events that are in both R1 and R2 will be displayed. If we continued creating regions and cascade gating in this manner, each new histogram would contain a smaller and smaller set of events. This is an example of restrictive gating.

## A Boolean "OR" gate

What events would we see in the **CD10 PE Cy7** vs **CD45 AmCyan** histogram if we looked at events that are in R1 *OR* R2? The "OR" gate is sometimes called an "inclusive" gate, because events are accepted if they are in either part of the expression. It is often used to define "positives" for a fluorochrome, especially if the regions in the gate expression are parts of a QuadStat.

To create this type of gating condition, we'll need to write our own gate expression. Let's create a new histogram and then use the Edit Gates dialog to define an inclusive gate expression.

12. Click, hold, and drag in an empty portion of the data source window to define a new histogram window.

13. Release the mouse button. In the Edit Histogram dialog, once again select **CD10 PE Cy7** for the X Axis and **CD45 AmCyan** for the Y Axis. Click **OK**.

The new histogram is ungated at this point. We will use the Edit Gate dialog to create a gate expression for R1 OR R2.

14. Click the Edit Gates button on the ribbon bar to display the **Edit Gates** dialog.



15. Select the "G5" gate in the list. In the **Equation** edit box, type **R1**. Then click the **Or** button and type **R2**. Click the **OK** button to close the Edit Gate dialog.

> *You can also type the "|" character instead of clicking the "Or" button. Similarly, you can type the "&" character instead of clicking the "And" button, and type the "!" character instead of the "Not" button.*

At this point, we have edited the gate expression for gate G5 so that it will include any events that are in *either R1 or R2*. Now we need to apply this gate to our new histogram.

16. Click the Gate tool button on the histogram toolbar and select the G5 gate from the list to apply the new gate.
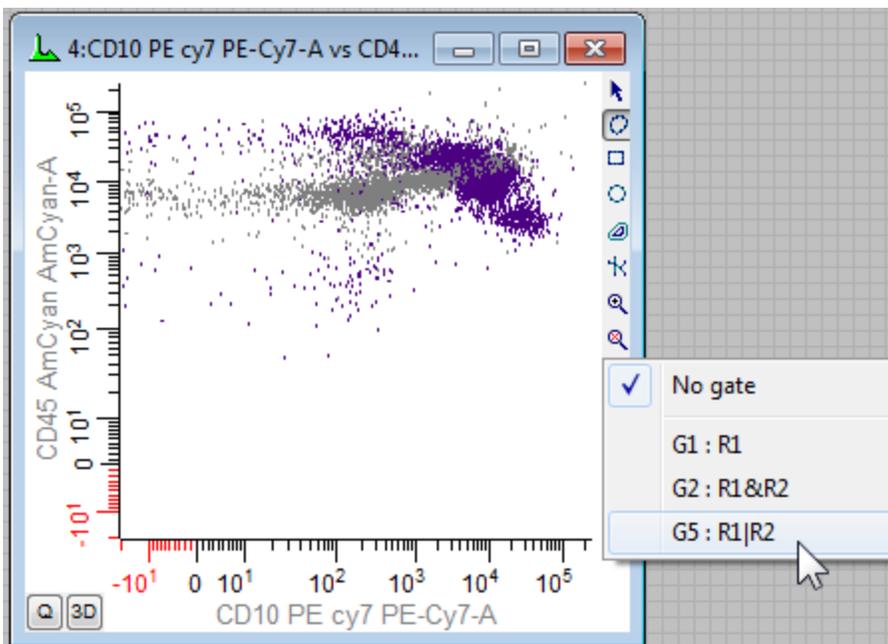


> *There are other ways to apply a gate to a histogram. When the Edit Gate dialog is displayed, select the gate that you want to apply and then click the title bars of the*

*histograms that you want to gate. This is a fast way to apply a gate to many histograms at once.*

As you might guess, the histogram gated on the "OR" gate contains more events because of the inclusive nature of this gating expression.

**The Boolean "NOT" operator**

There are certain cases where it is easier to define what you want by defining what you *don't* want. For example, if you have drawn a region that identifies fragments of cells or debris, you might want to see all events that are *not* debris. This is where the Boolean "NOT" operator is used.

Let's define a gate that includes all events that are *not* in R1.

17. Click the Edit Gates button on the ribbon bar to display the **Edit Gates** dialog.

18. Select the "G6" gate in the list. In the **Equation** edit box, delete the current equation. Click the **Not** button to insert the "NOT" operator. Type **R1**. *Leave the dialog open.*
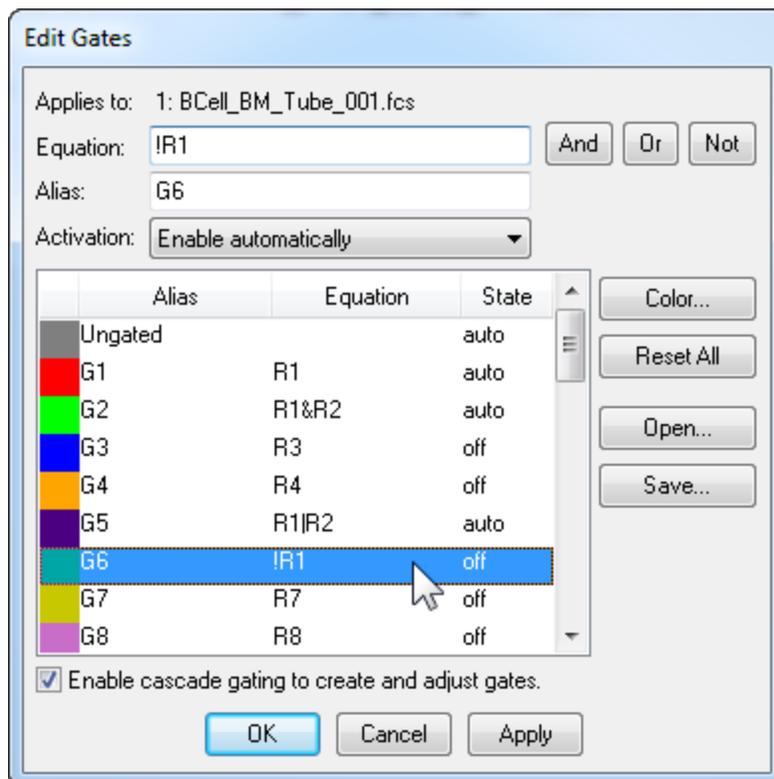
19. With G6 selected in the Edit Gate dialog, click on the title bar of the **last** histogram. The histogram's title bar will change to show the new gate name. Click **OK** to close the Edit Gate dialog.
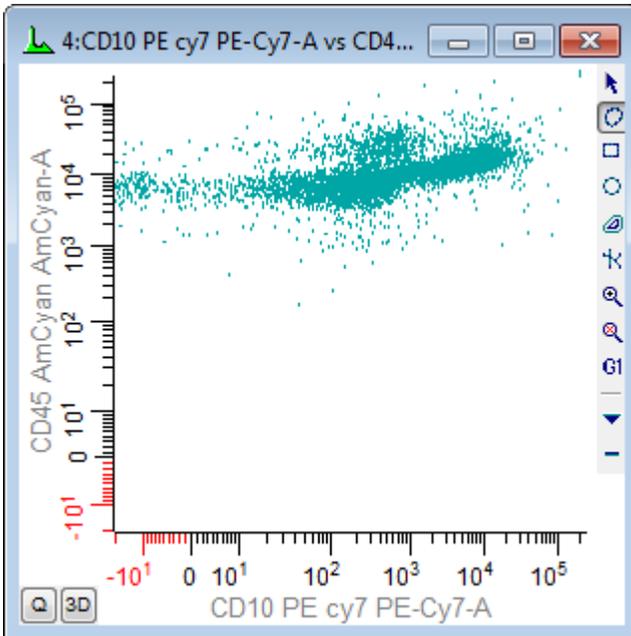


The events now shown in the histogram are all those that are *not* in region R1.

## More complex Boolean expressions

We have barely scratched the surface of what you can do with Boolean gate expressions. For example, let's suppose that you want to exclude debris, defined by R1, and include events that are in either of 2 other regions, R2 and R3. The gating expression would be:

  Not R1 And (R2 Or R3)

In WinList's syntax, this would be written:

  ! R1 & (R2 | R3)

WinList allows you to use parentheses to define the order of operations.

Be aware that if you use "NOT" later in the gate expression, it is must be preceded by "OR" or "AND". For example, if we rearrange the expression above so that "!R1" is at the end of the expression, it would read this way:

  (R2 | R3) & !R1

You can also use other gates in a gate expression, which is very handy when your gate expressions are lengthy. For example, if you have gate G1 defined as:

(R2 | R3) & !R1

You can reference G1 in other gate expressions, such as this one for G2:

G2 = **G1** & R4 & R5

The gate expression is expanded and surrounded with parentheses when it is evaluated. Here's the expanded form of G2 in this example:

((R2 | R3) & !R1) & R4 & R5

## Summary

In this section, we've taken a look at a number of ways that you can create and apply gates with WinList. Cascade gates are created and applied automatically when you click on a region's cascade hotspot and draw a new histogram (or click on an existing histogram). Cascade gates use the Boolean "And" to create restrictive gates.

We also examined how you can define gate expressions that use the Boolean "Or" and "Not" operators, and how parentheses can be used to create sophisticated gating expressions.

**See also**

[WinList Basics](#)
[Edit Gates](#)

**Compensation the Easy Way: V-Comp**

One of the things most feared in flow cytometry is setting up fluorescence compensation.  Sure, it's easy enough to set up for a 3 or 4 fluorescent markers, but as the number of fluors increases, compensation becomes a bigger and more challenging issue.  Historically, users have had to wade through numerous control files, carefully setting gates or trace lines, and making subjective decisions about populations that ultimately affect how well the compensation works.

There must be a better way.  Enter V-Comp™ - a stand-alone app that makes it easy to set up compensation for each of your panels, and do it automatically and objectively.

With a set of single-color controls and an unstained control, V-Comp™ can generate a crossover matrix without any user intervention.  Select the parameters to compensate, assign the controls to the parameters, and V-Comp creates the perfect compensation for your data.

WinList is tightly integrated with the V-Comp app. It knows how to launch the app so that you can create a new compensation setup.  It knows where to find your V-Comp setups.  And it knows how to read those compensation setups so that it can directly apply V-Comp compensation from within WinList.

Let's walk through how this system works.

> *You'll need V-Comp 1.0 installed on your computer to perform these steps.*

**Launching the V-Comp App**

Suppose that we have an FCS file open in WinList and we want to setup a V-Comp compensation for it.  We simply display the Compensation Toolbox, click the AutoComp tab, and click the **Launch V-Comp** button.
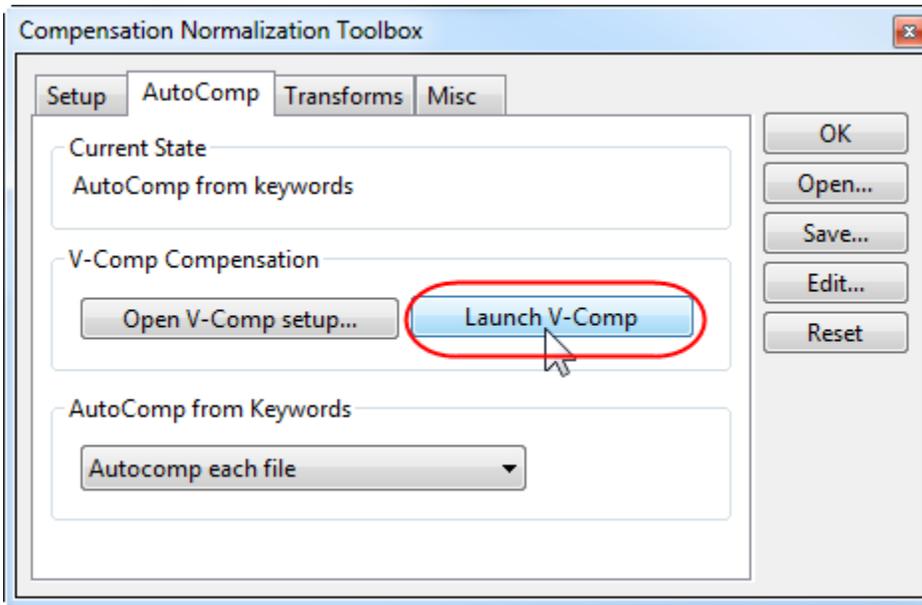
WinList will launch the V-Comp app.

## Working with the V-Comp app

The V-Comp all allows you to create new compensation setups as well as edit existing ones.  You can review the full user guide for V-Comp to see all of its options.  Here, we'll just give a quick overview of how easy it is to create a setup.

You create a new setup by clicking the **New** button on the ribbon bar, and working through the **Setup Wizard**.
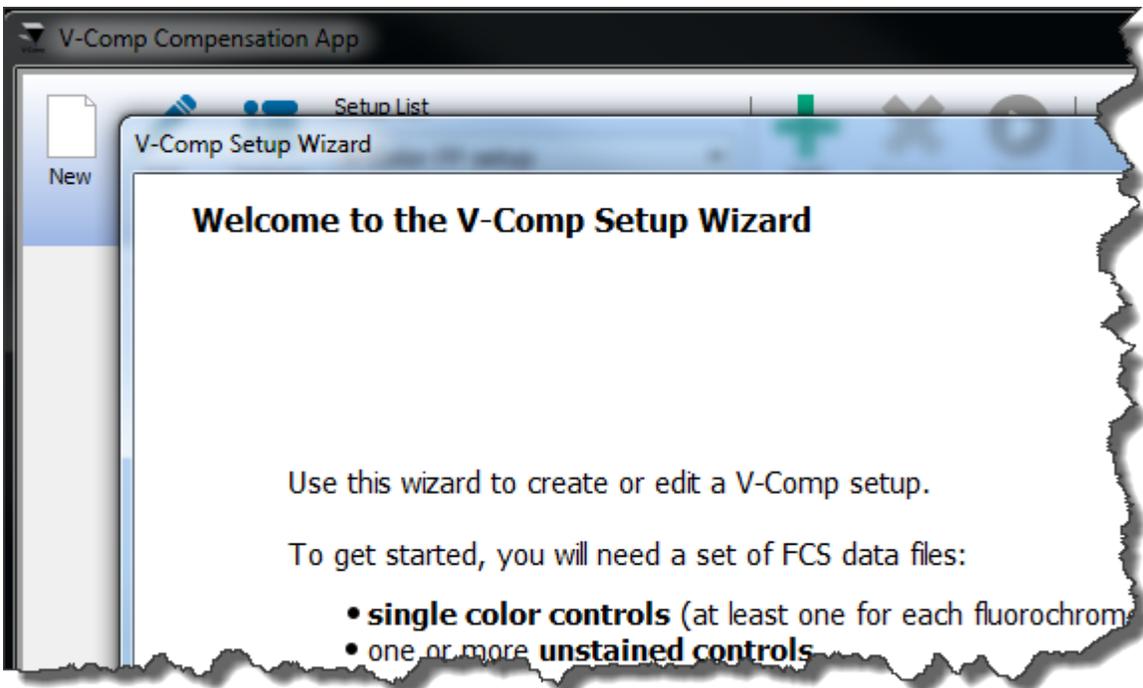
The wizard prompts you for **single-color control** files, as well as an **unstained control** file.
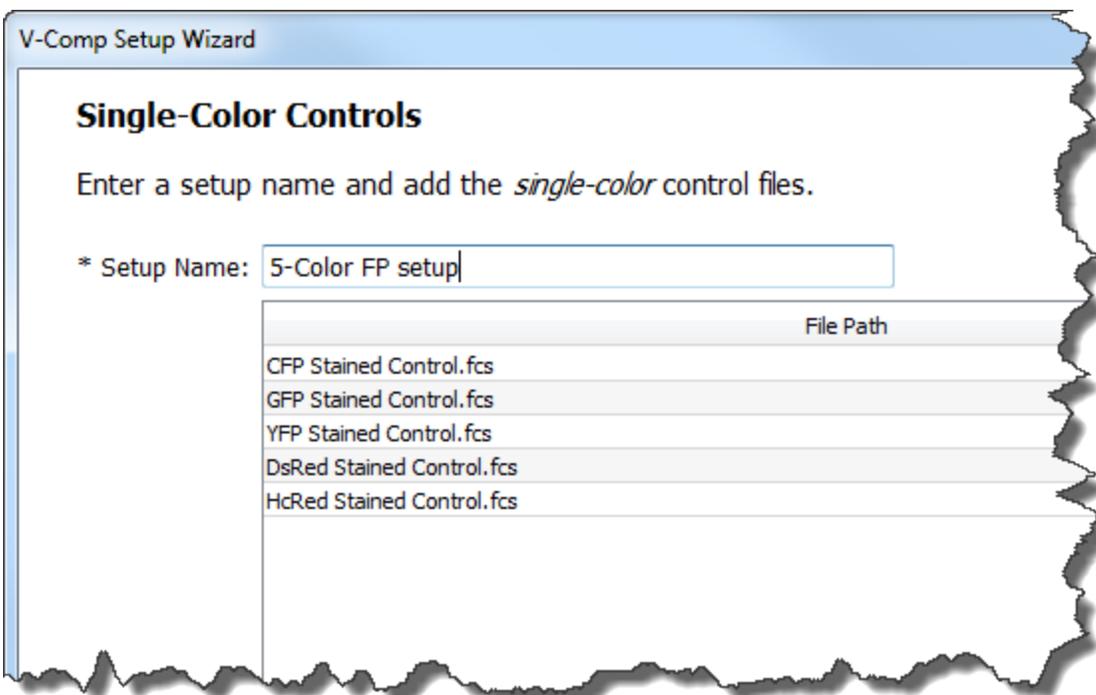
You identify the measurements that you want to compensate and match them up with the single-color controls.
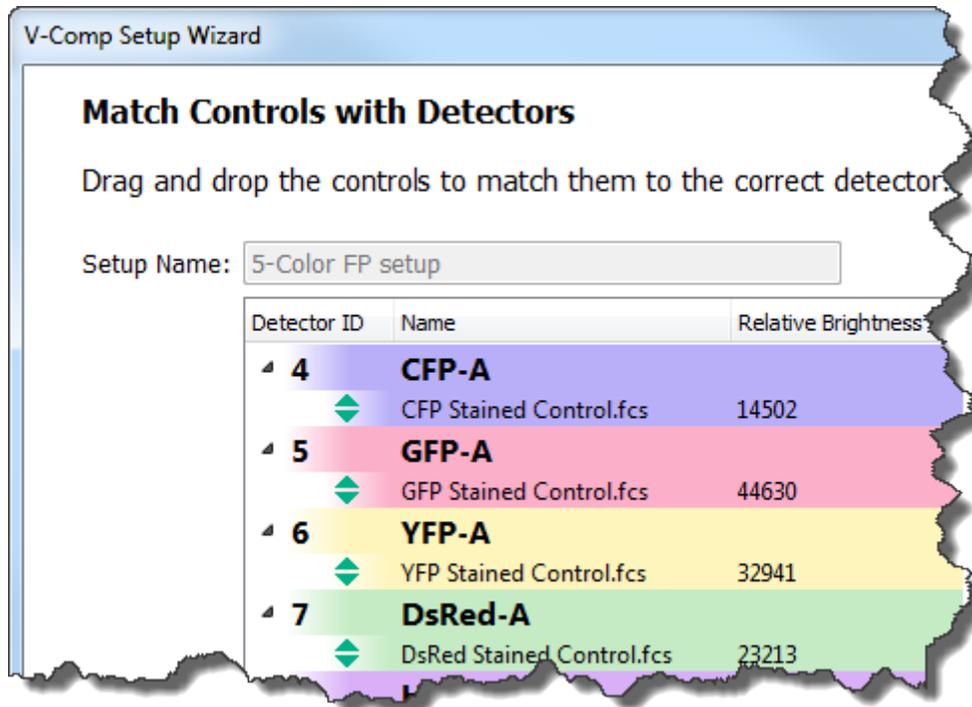


That's about it!  V-Comp will take the setup info and crunch the numbers to figure out an optimal compensation setup for the controls.  You can go ahead and directly apply that setup to any files that are created with the same panel.  You can also tell WinList to use the setup file for that panel.

## Using a V-Comp setup in WinList

So, back in WinList, we have a file opened and we want to apply a V-Comp setup to it.  How do we do that?  Easy.

On the **AutoComp** tab of the **Compensation Toolbox**, we click the **Open V-Comp setup** button.
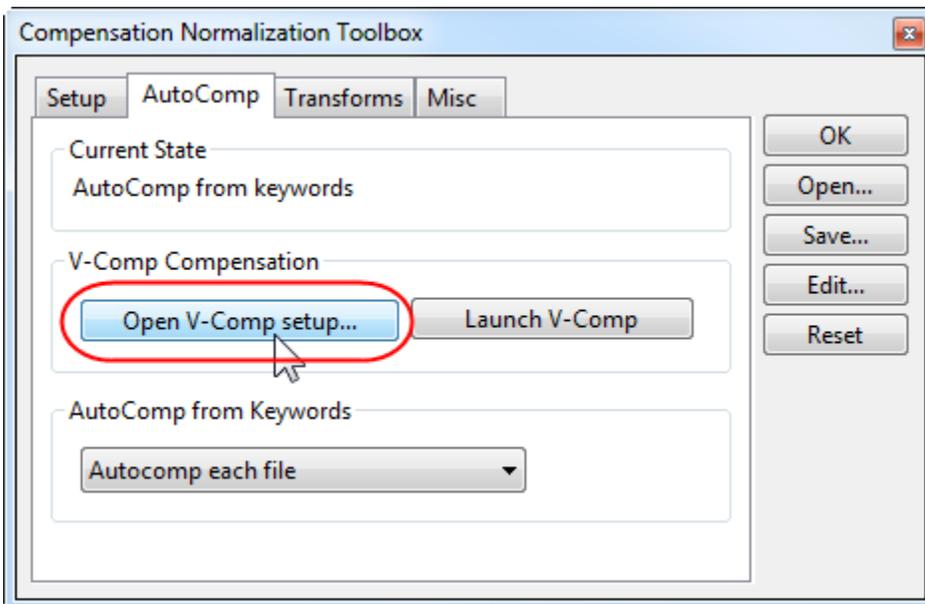
WinList will display the **Open Compensation File** dialog show V-Comp setups. Find the one that relates to the FCS file you have loaded, and open it.

The V-Comp setup will be read by WinList and used to set up the crossover values for compensation. WinList will enable the compensation for the correct parameters and turn compensation on.

**All Done!**

And that's it! If you are setting up an analysis protocol in WinList, the V-Comp compensation will be saved in the protocol file. You can read other FCS files into the protocol to apply the V-Comp compensation to them.

*Note that you will probably want to turn off "AutoComp from Keywords" when you use a V-Comp setup in WinList. Otherwise, it is possible that the compensation in WinList will be changed by keyword compensation from an FCS file that you read in.*

**See Also:**

Compensation Toolbox

Using Interactive N-Color Compensation

**Using Interactive N-Color Compensation ™**

WinList's interactive N-Color Compensation ™ system performs color compensation of any number of parameters through a simple-to-use Compensation Toolbox. With both automatic and manual control, you can use the system to perform post-acquisition compensation and even adjust previously compensated samples.

> *You should be familiar with the basics of working with WinList before performing this tutorial. You need to know how to open data files, draw regions, and apply gates to complete this tutorial.*

### Overview of WinList's interactive N-Color Compensation ™

Unlike most compensation systems, WinList provides you with a graphical interface using compensation *trace lines*. Trace lines make it very easy to adjust compensation using multiple intensity controls such as beads or with the more traditional single color and unstained controls. Probably the best way to explain how they work is with an illustration.

The following is an example of how to use the interactive compensation system with a four-color experiment. Data files for this tutorial were kindly provided by Teresa Hawley. The fluorochromes in this example are fluorescent proteins: CFP (Cyan), GFP (Green), YFP (Yellow), and dsRed (Red). We will open a single-color control file for each fluorochrome to set up the compensation. Then we'll open files with all four colors and apply the compensation to them.

The strategy used in this example can be easily extended to any number of fluorochromes.

### Turn Off Auto-Compensation

Before we get started, we need to make sure that WinList doesn't try to use compensation settings from keywords in these data files. We will need to turn off a preference; we can turn it back on at the end of the tutorial.

1. Choose Options->Preferences, and select the Data Source branch of the Preferences tree. Look for an option named **Auto-Compensation**, make a note of the current settings, and then set it to **Disable**.

### Automatic compensation

2. Click the **Open FCS File** button on the ribbon bar.
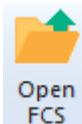
3. In the File Open dialog, navigate to the Samples folder located in the folder where WinList is installed. Select four files: **SP2+CFP.fcs, SP2+DsRed.fcs, SP2+GFP.fcs** and **SP2+YFP.fcs**. You can press the Ctrl key on the keyboard to select more than one file. Click **Open**.

The first files selected are single-color control files for CFP, DsRed, GFP, and YFP, respectively.

*You can also drag and drop files from Windows Explorer or My Computer onto WinList. See Drag and Drop for details.*

4. In the **Create Histograms** dialog, select **FSC-A** in the X Axis list, **SSC-A** in the Y Axis list, and click **Add to List**.

5. In the X Axis list, select **CFP-A**, **GFP-A**, **YFP-A**, **DsRed-A**. Enable the **Combinations mode** checkbox, and click **Add to List**. All combinations of the four fluorochromes will appear in the histogram list. Click the **OK** button.
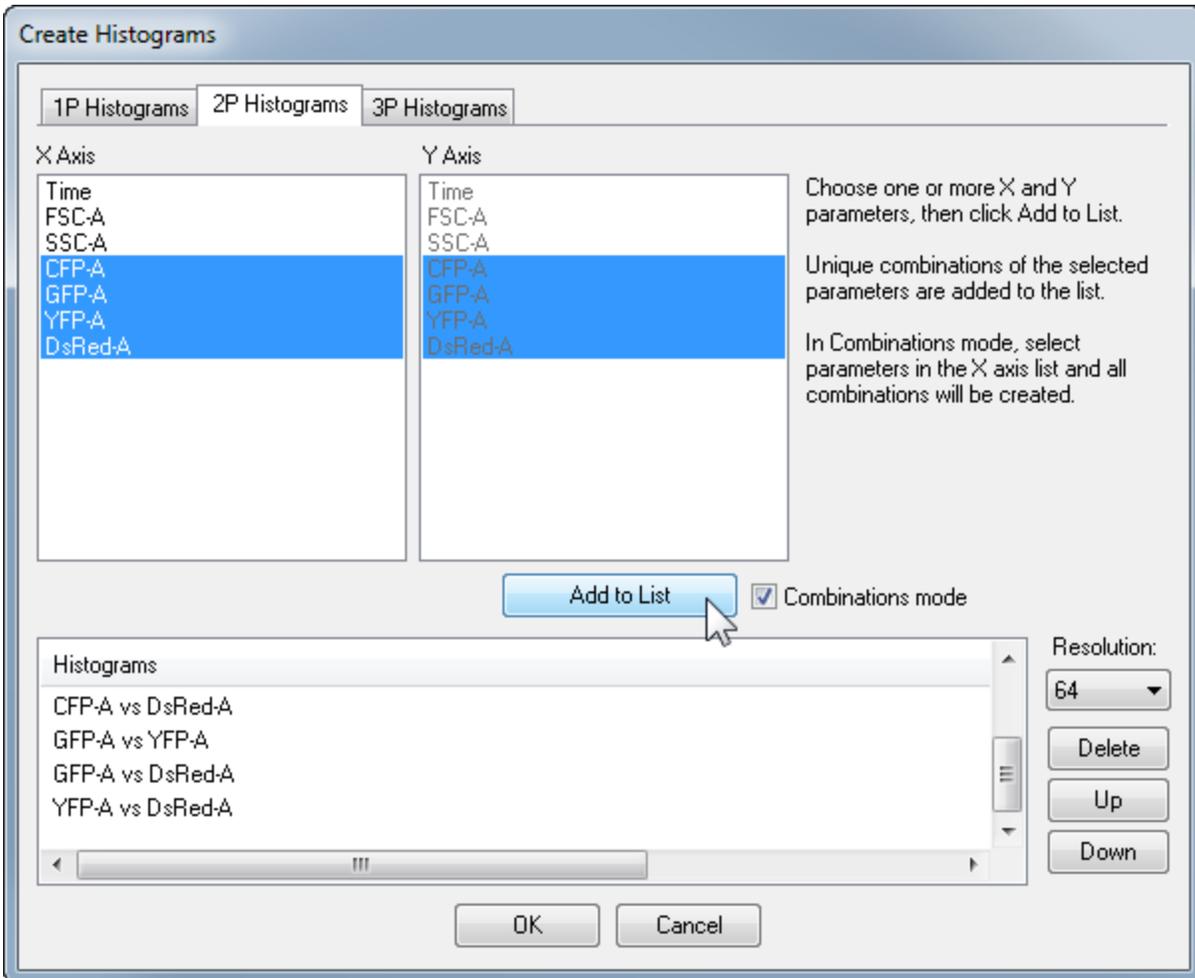
The program will open the first file and display seven scatter plots. The batch toolbar will appear at the bottom of the application window. We're ready to let WinList determine the correct compensation settings by reading each of the control files.

6. Draw a region around the cells of interest in the FSC-A vs SSC-A plot as shown.
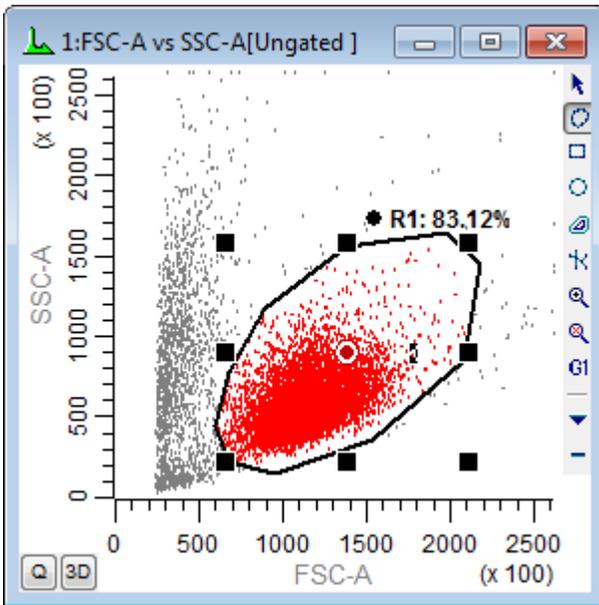
7. Apply this gate to each of the remaining dot plots.

8. Click the **Compensation Toolbox** button on the ribbon bar. The Compensation Normalization Toolbox will be displayed.



9. In the **Compensation Parameters** listbox, click **CFP-A**, **GFP-A**, **YFP-A**, and **DsRed-A** parameters to enable them for compensation. Notice the label changes from "Off" to "On" in the listbox, and an entry appears in the Trace Line Options list for each enabled parameter.
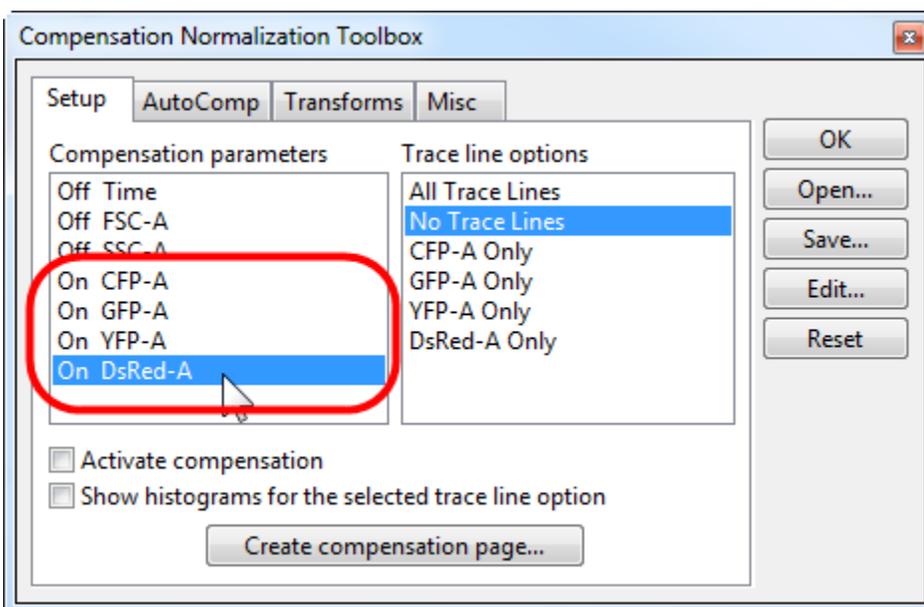
As you enable the parameters, you will notice lines appear on the histograms. These *trace lines* are used to adjust the compensation settings. In the next step, WinList will automatically position the trace lines so that they pass through the medians of the populations.

> *Only enable the parameters that you want to compensate in the* **Compensation Parameters** *list.*

10. This first file is the CFP-only control file. Click *CFP-A Only* in the **Trace Line Options** listbox. Trace lines now appear only in the histograms containing CFP-A.
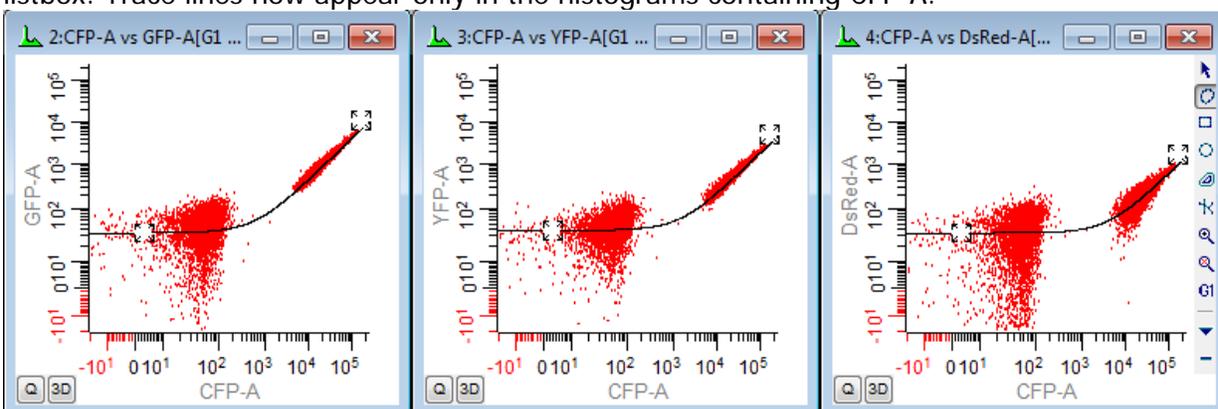


Notice that the Trace lines are positioned through the approximate center of the populations in the histograms. This is WinList's automatic positioning routine at work. The program performs a regression through the events.

## Manual compensation adjustments

With single-color controls, the program almost always does a better job of determining the correct slope than we can by eye. If, however, you need to make an adjustment to the trace lines, this is a simple drag operation. The square handles that appear at each end of the Trace line are used for this purpose.
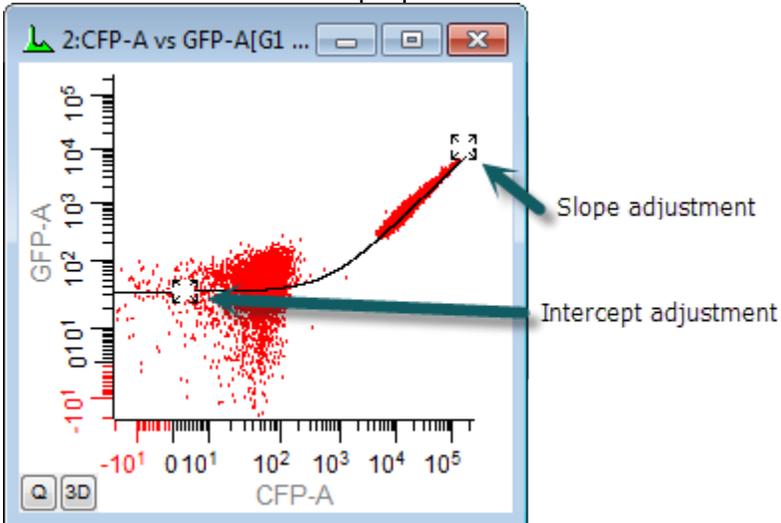


The position of a Trace line determines the slope and intercept of a line through the center of the data. Adjusting the handle in the low-intensity area sets the intercept, and the slope is determined by the position of the handle in the high-intensity area. As you move the slope handle toward the diagonal, you increase the amount of compensation that will be applied to the data. The intercept handle actually has very little impact on the compensation.

*In rare cases, you may need to apply more compensation than the trace lines normally permit. You can give greater freedom of movement to the trace lines by holding down the Shift key on the keyboard when moving its handles. This allows each trace line handle to move along two sides of the histogram, with slopes of greater than 1.0.*

## Processing the remaining control files

Let's continue with the next single color control file.

11. On the batch toolbar at the bottom of the application window, click the **Next Batch Item** button to read in the next file, SP2+DsRed.fcs.

12. Click the "DsRed-A Only" in the **Trace Line Options** listbox. WinList automatically positions the Trace lines.

13. Click the **Next Batch Item** button to read in the next file, SP2+GFP.fcs. Choose the "GFP-A Only" from the **Trace Line Options** listbox. Once again, WinList automatically positions the Trace lines.

14. And once more, click the **Next Batch Item** button to read in the next file, SP2+YFP.fcs. Choose the "YFP-A Only" from the **Trace Line Options** listbox to allow WinList to position the trace.

**Applying the compensation**

Believe or not, we just finished setting up the compensation! WinList did most of the work for us as each single color control was loaded and we selected the appropriate Trace line option. Now that the crossover coefficients have been determined for each fluorochrome, we are ready to apply the compensation.

15. Click the **Activate compensation** checkbox.

Two things will happen. First, the histograms will redraw to display the compensated data. Secondly, the Trace lines will disappear, replaced with small **+** and **-** buttons on the histograms. These buttons allow you to adjust the compensation settings with the compensation system activated. To see all of the adjustment buttons, choose *All Trace Lines* in the Trace Line Options list. To hide all of them, choose *No Trace Lines*.

Let's open a 4-color file and test the compensation settings.

16. Click the **Next FCS File** button on the ribbon bar. Choose **Mix all.fcs** and click **Open**.



The newly-defined compensation is applied to the data in the new file as it is read, and we can see well-compensated populations in each plot.

*You may have noticed that the log axes on the histograms show values less than zero. WinList supports several log-like transformations that allow negative values to be displayed: HyperLog, Hyperbolic Sine, and VLog. These transforms are log-like, but do not have the limitations of the log transform, in which zero and negative values are undefined.*

When compensation is applied to events, it is possible for the value of a parameter to become zero or negative. Color compensation is a subtractive process, and when significant compensation levels are applied to low intensity events, the result is often a negative number - sometimes a large, negative number.

You can toggle between Log and HyperLog transformations quickly using buttons that appear on the **Transforms** tab of the Compensation Toolbox. This tab also provides a control to **Optimize transforms**.

17. Click **Optimize transforms** on the Transforms tab. Click OK to allow it to optimize all parameters.

WinList uses a sophisticated data processing method to determine the optimal B-coefficient (the linear portion of the axis) and the lowest negative value for each parameter. The routine ensures that most negative values are visible and that populations near zero are not split into bimodal distributions.

## SPLOM

As the number of parameters needing compensation increases, it becomes more and more difficult to evaluate the compensation set up. It can be challenging to determine which bivariate plots to display. The solution to this is to create additional pages in the data source, and to use a special display called the *SPLOM*. A SPLOM is a scatter plot matrix that organizes histograms needed for compensation.

Let's see how the SPLOM works for this 4-color set up.

18. Click the **Create Compensation Page** button on the Setup tab of the Compensation Toolbox.
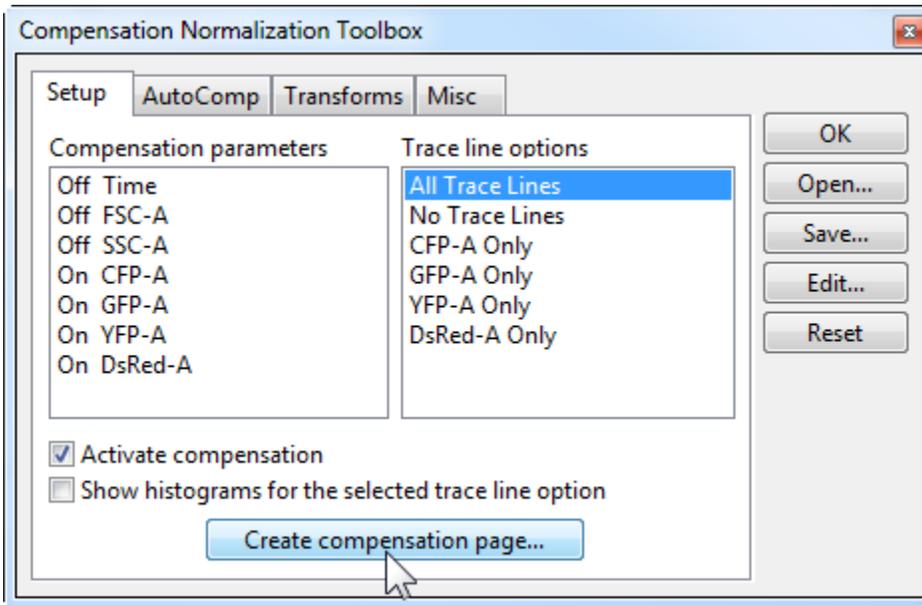
The Create Compensation Histograms dialog is displayed and the compensation parameters are already selected.

19. In the dialog, choose the **G1** gate. Click **OK**.
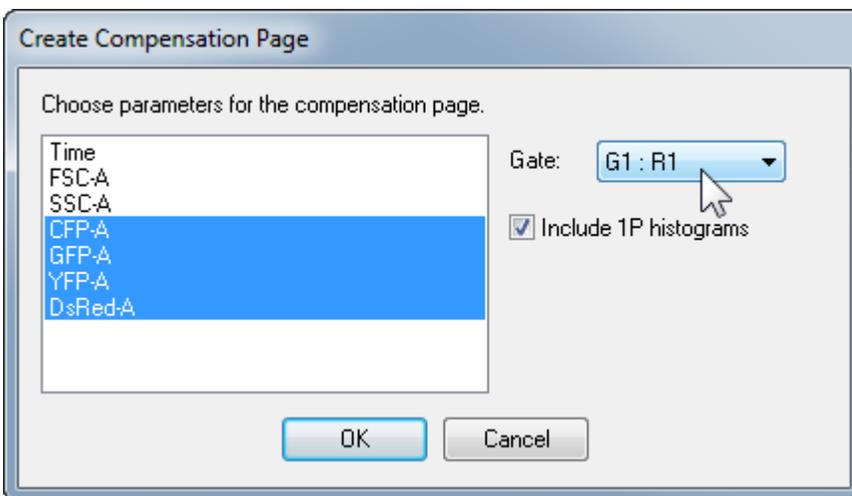


Each parameter appears in a row and a column of the SPLOM arrangement. This makes it easy to review the compensation settings, parameter by parameter, and to ensure that the compensation has been correctly applied.

You can return to Page 1 of the data source by clicking the appropriate page button on the data source toolbar.

20. Let's go ahead and save a protocol for this setup we've created.  Click the **Save** button and name the protocol **"CompensationTutorial"**.

## Reset Auto-Compensation

We need to restore your Auto-Compensation preference to its original setting.

21. Choose Options->Preferences, and select the Data Source branch of the Preferences tree. Look for an option named **Auto-Compensation** and set it to your original setting.

## A few more notes

Compensation is only active on the data source window that was used to set up the Trace lines. Different data source windows can have different compensation settings. If you want to apply the same compensation to a series of files, you can use the Next File button on the ribbon bar to open each file into the compensated data source window.

You can also use the compensation system to make adjustments to a previously compensated file. To do this, enable the parameters you want to adjust, turn on the Trace lines and make the adjustments, and then turn on the compensation system by checking the **Activate compensation** checkbox.

WinList needs a bivariate plot of each combination of compensation parameters, and this can present some spatial problems when compensating data with many fluorescent parameters. The solution to this is to enable **Show histograms for the selected Trace Line Option** on the Setup tab of the Compensation toolbox. When enabled, you can view a subset of histograms simply by selecting a different Trace Line option.

## Summary

WinList's N-Color Compensation system is very quick and easy to use. You can use it to compensate any number of fluorescent parameters for signal crossover. This is best accomplished through the use of single-color control files, which are read one at a time to determine the best compensation settings.

Trace lines are graphical elements displayed in the histograms that you are compensating. WinList can automatically position Trace lines using a robust fitting algorithm, and it uses the

slopes of Trace lines to set up a compensation matrix. Because of the simplicity of this system, it can be easily extended to any number of fluorescent parameters.

**See Also:**

Compensation Toolbox

Compensation Trace Lines

**Compensation Review Mode**

Cytometry assays continue to use more colors each year, providing a richer platform for understanding cells and biology. At the same time, as the number of colors increases, so does the complexity of properly compensating for fluorescence crossover. Let's explore a method for evaluating just how good your compensation setup is, confirming the strengths and revealing the weaknesses in a way the makes sense.

Compensation Review Mode in WinList is a visual tool that makes this possible. The process is simple: review each single-color control file, one by one, and examine how well the crossover values match the data. Compensation Review Mode performs some optimizations to make this process possible.

To use this mode you need a set of single-color control files that were used to set up the compensation. You may also want to review one or more all-color files.

Here's how it works. We'll use the compensation that we set up in Using Interactive N-Color Compensation as a test case, so make sure you do that tutorial first.

**Open an all-color file**

We start by opening an all-color file and applying the compensation that we want to review. That compensation might be loaded automatically by WinList by reading keywords from the file, or it may be compensation that you have set up in WinList.

1. Click the **Open Protocol** button and select "**CompensationTutorial**" - the protocol that was saved in the Using Interactive N-Color Compensation tutorial. There should be a FSC vs SSC plot, and 6 other plots for the fluorochromes.
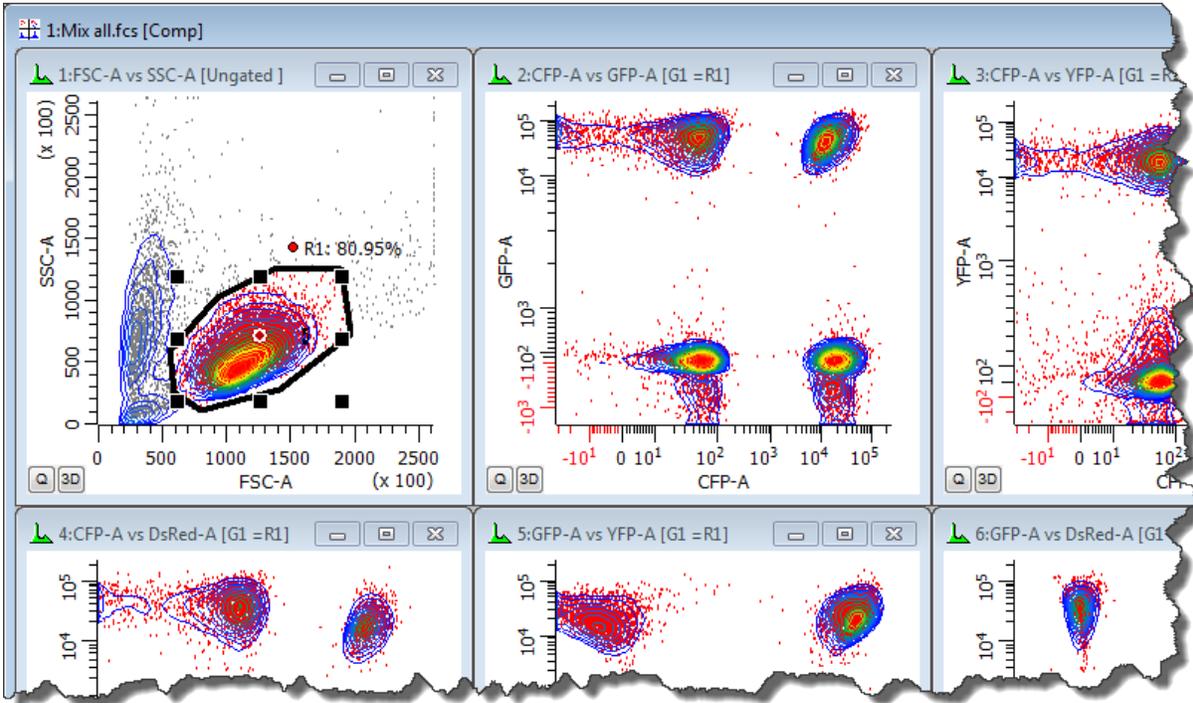
## Turn on Compensation Review Mode

2. In the **Compensation Toolbox**, click the **Misc** tab and enabled **Compensation review**.
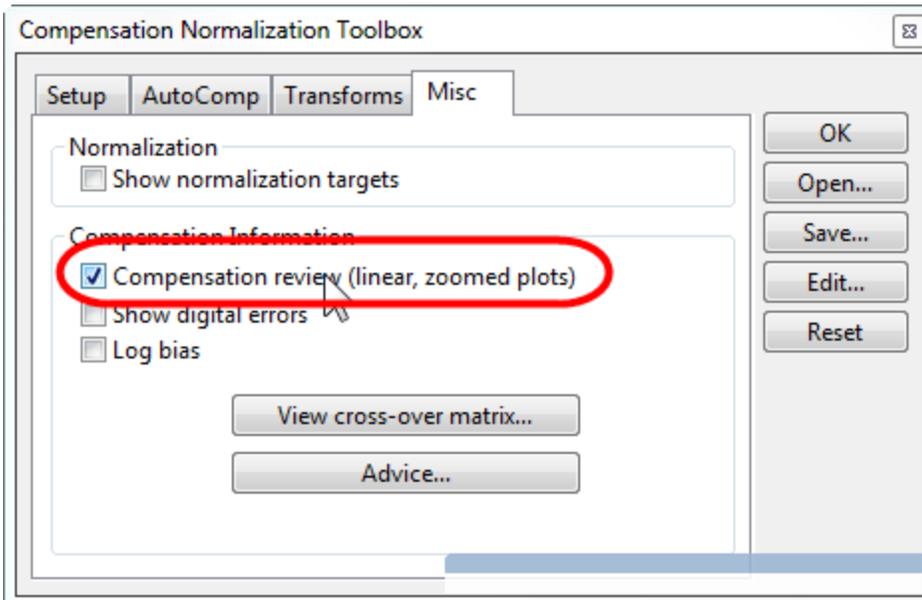
The display will change dramatically.  All of the plots that are normally showing a log-like axis will switch to a **linear** axis.  In addition, the plots will "zoom" to the 95th-percentile.  How is this going to help us review compensation?  Let's keep going and find out.

3. Click the **Setup** tab of the **Compensation Toolbox**. Select **All Trace Lines** in the Trace line options list, and enable the **Show histograms for the selected trace line option**.

We're ready to go.

## Review the first control file

4. Click the **Next Batch** button on the batch toolbar to read in the first single-color control file that we used to set up the compensation.

5. In the Trace line options list, select **CFP-A Only**, since this is the CFP-only control file.  You should see something like this:
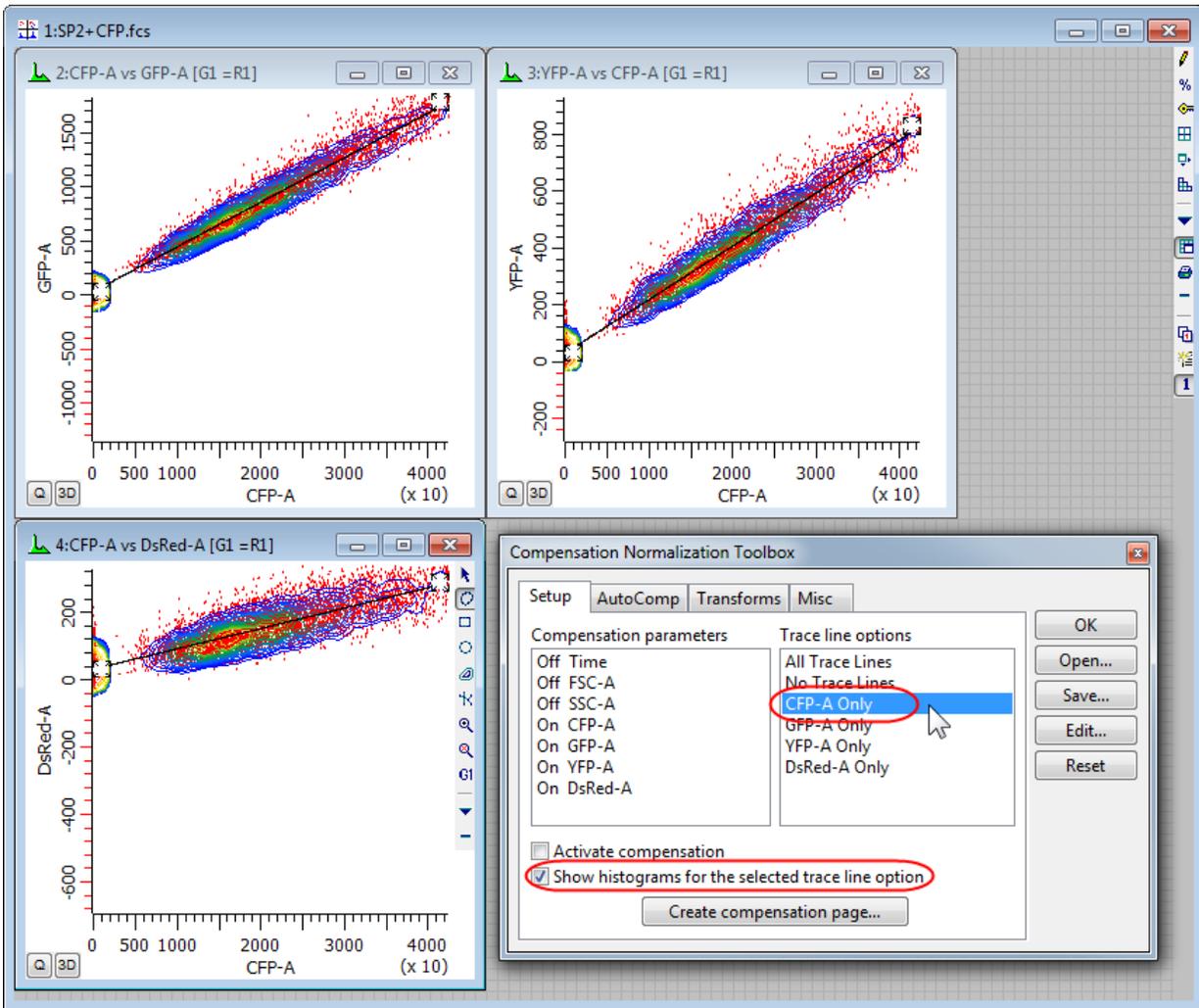
Only 3 plots are shown: the ones that involve CFP-A.  The X-axis for each plot is CFP-A, and the Y-axis show each of the other fluorochromes.  The view is zoomed in on the 95th-percentile, and we're looking at linear plots so there are no warping effects from log transformations.

Most importantly, note the trace lines in each of the plots.  If the compensation is set up properly, the trace lines will pass right through the center line of the data.  That appears to be the case here, so it looks like the CFP-A compensation is set correctly for the CFP control.

Let's check the other 3 controls in the same manner.

## Review the other controls

6. Click the **Next Batch** button on the batch toolbar to read in the next single-color control file.

7. In the Trace line options list, select **DsRed-A Only**, since this is the DsRes-only control file.
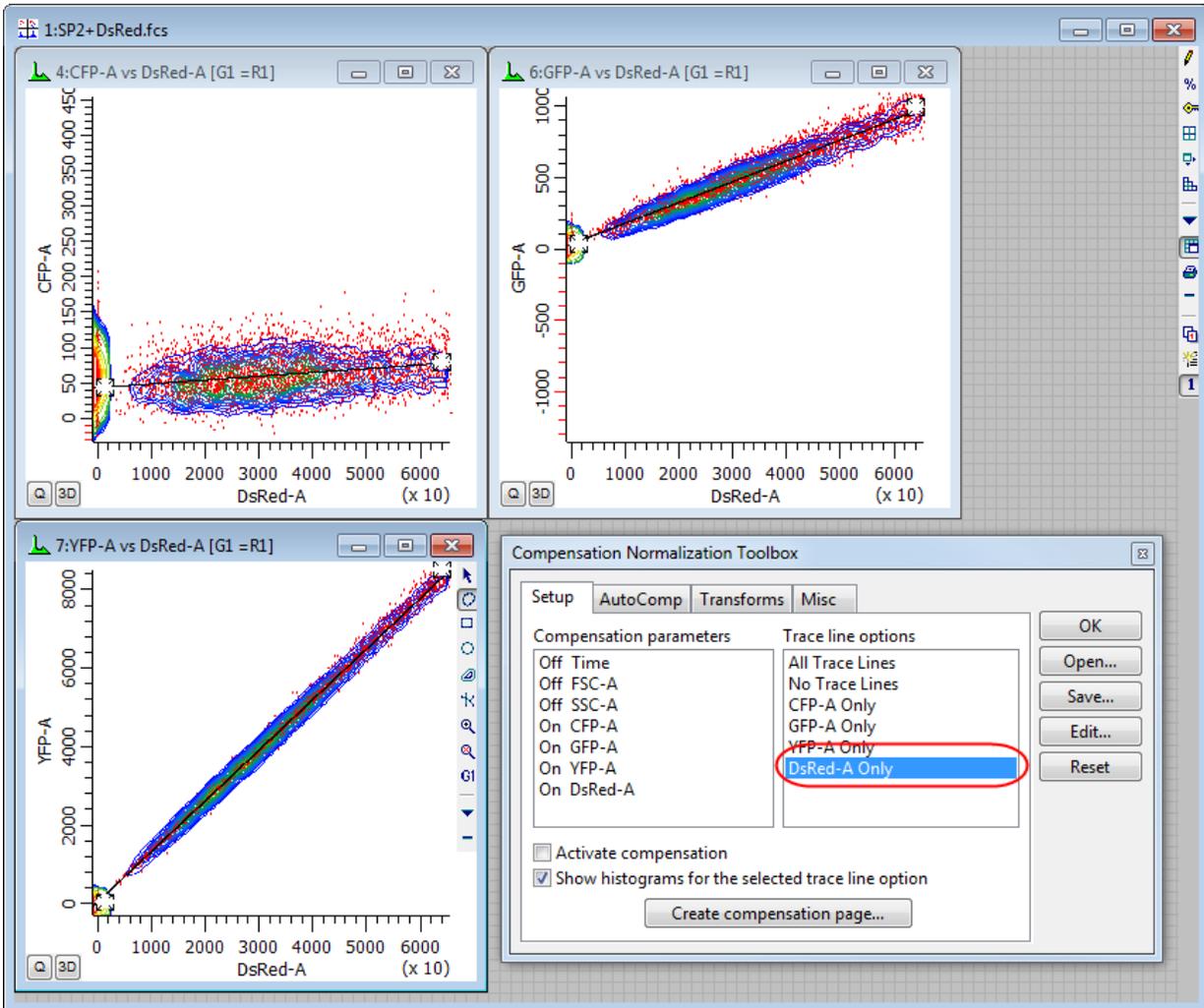


Now we see DsRed-A on the X-axis plotted against each of the other markers.  The trace lines appear to pass through the center of the linear-displayed clusters, so the DsRed looks correct.

8. Click the **Next Batch** button on the batch toolbar to read in the next single-color control file.

9. In the Trace line options list, select **GFP-A Only**, since this is the GFP-only control file.
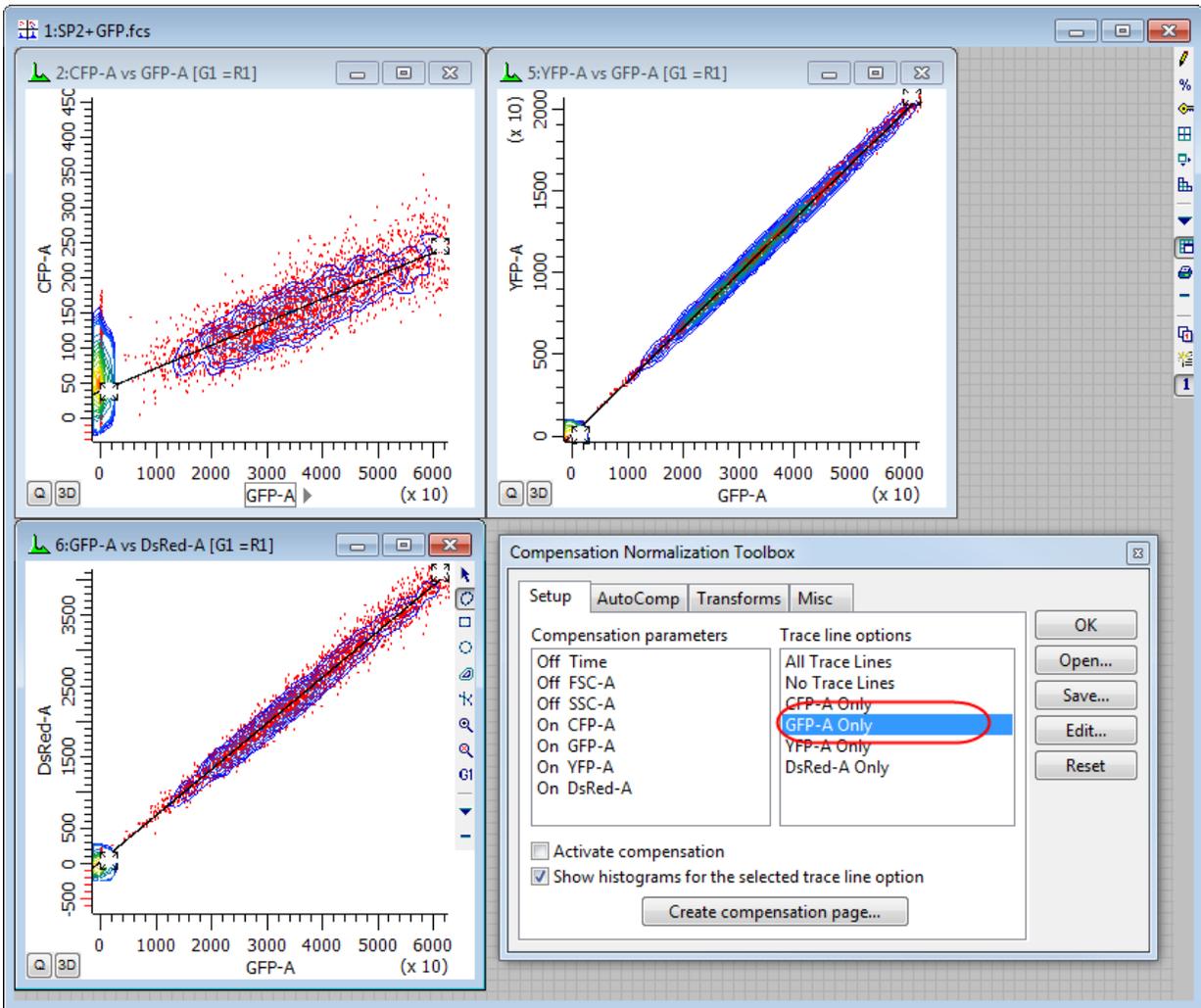
GFP also looks correct.  We've got one more to review.

10. Click the **Next Batch** button on the batch toolbar to read in the next single-color control file.

11. In the Trace line options list, select **YFP-A Only**, since this is the YFP-only control file.
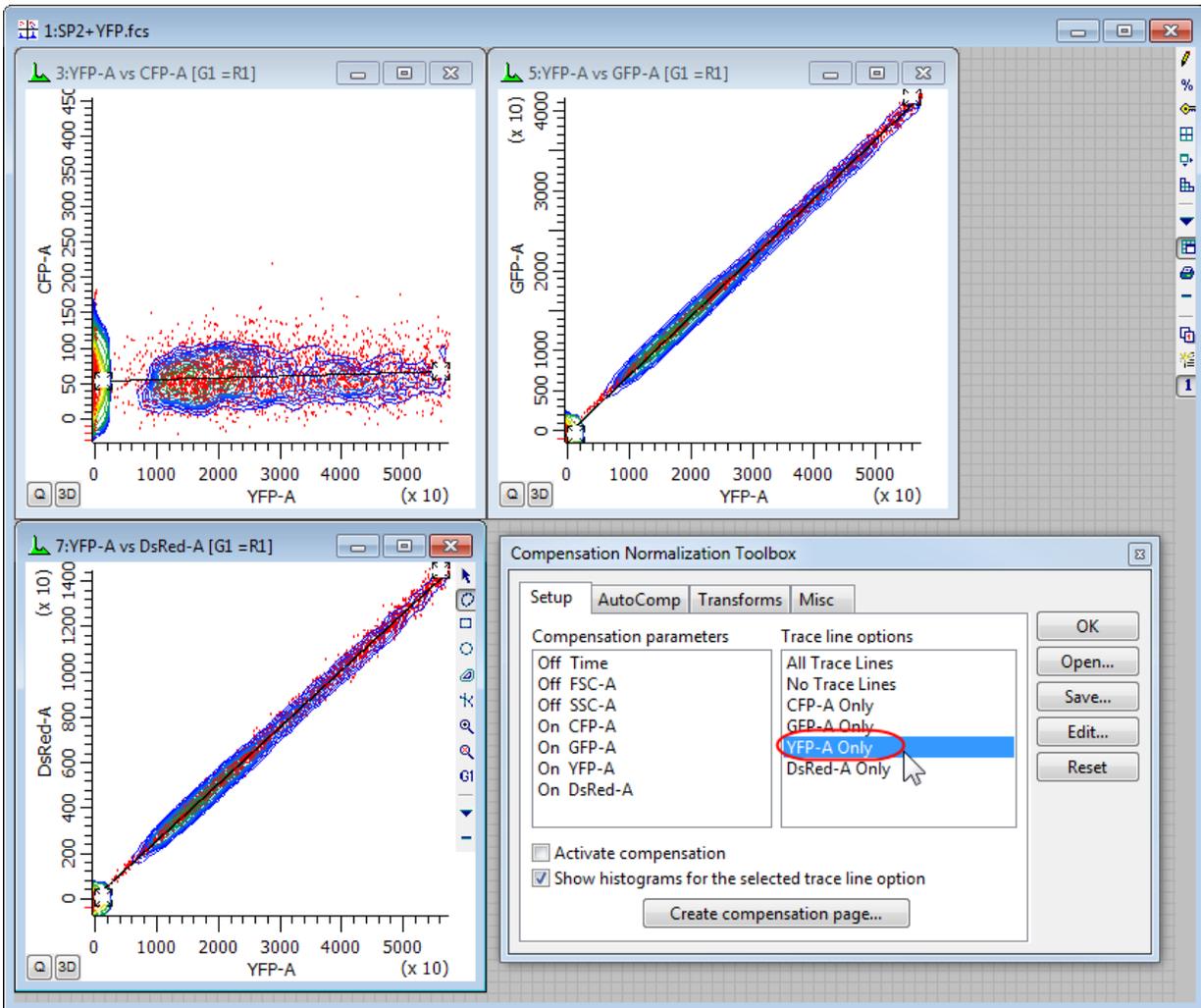
Once again, the trace line passes smack through the center of the clusters.

## Interpretation

In Compensation Review mode, you are looking to see that the trace lines for each single-color control pass through the center (median) of the zoomed, linear distribution. That's one of the things Compensation Review mode does automatically for us. It's a lot easier to see this when looking at linear axes, and more so when you clip out any high-end outliers. To demonstrate that point, here's the YPF control unzoomed and with Hyberboline Sine axes:
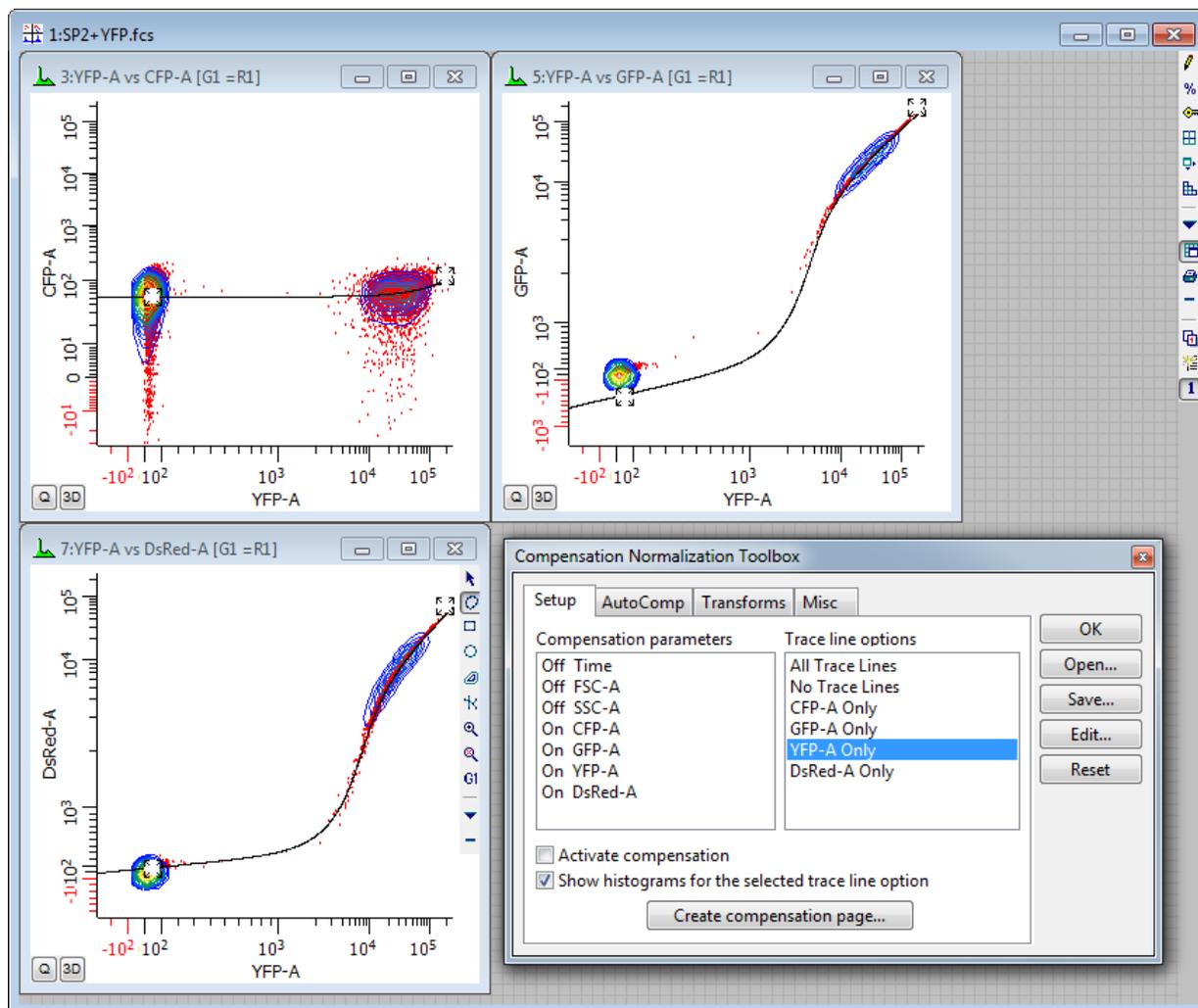
It is very difficult to see what was quite simple and obvious in the zoomed, linear display.

You can also review the all-color file in Compensation Review mode. To do so, you open the all-color file and then use the **Trace line options** to show each of the markers in turn.

12. When you finish with compensation review, uncheck the **Compensation review** option on the **Misc** tab of the **Compensation Toolbox.**

## Summary

Compensation Review mode is a tool that simplifies the process of assessing fluorescence compensation.  By switching transformations to linear and zooming in on the data, it becomes a simple task to determine whether a trace line passes through the center of a distribution.  It is best used with single-color controls, but can also be used to review compensation on an all-color file.

## Creating Reports

When you need to generate reports that contain graphics, text, and results, WinList's reporting system provides the tools to create multi-page documents with a professional, polished look. Reports are page layout windows that can contain any number of elements. These elements can update any time the data you are analyzing changes. Reports can be exported as PDF files so that you can easily share your analysis results with others who do not have access to WinList.

This tutorial takes an in-depth look at the reporting features of WinList. In the process, you will become familiar with the simplicity and flexibility of reports, and learn how to use reports in your own analysis protocols.

*If you are unfamiliar with opening files and creating histograms with WinList, you might want to do the WinList Basics tutorial before continuing with this one.*

### Setup

We'll start by opening a listmode file and creating some histograms and results.

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **BCell_BM_Tube_003.fcs** from the list and click **Open**.

*On the PC, you can also drag and drop files from Windows Explorer or My Computer onto WinList. See Drag and Drop for details.*

2. When the **Create Histograms** dialog appears, select **CD45 AmCyan** in the X Axis list, **SSC-A** in the Y Axis list, and click **Add to List**. Select **CD10 PE** in the X Axis list, **CD20 APC** in the Y Axis list, and click **Add to List**. Click **OK**.
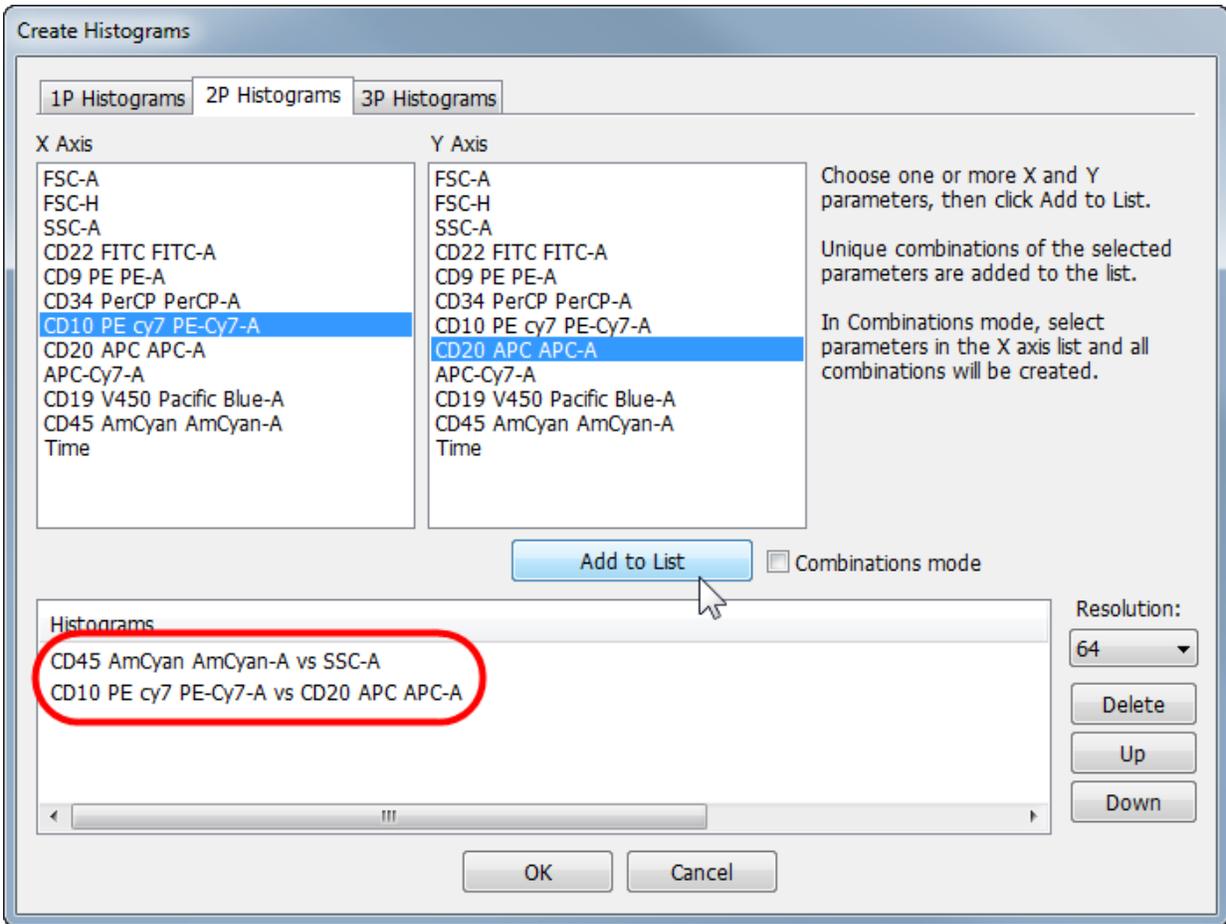
Now, if your preferences are set to use one of the log-like transforms, you'll notice that the CD10 vs CD20 plot has a lot of events piled on the axes. Let's use the Optimize Transforms command to fix this situation.

3. Click the Compensation Toolbox button on the ribbon bar to display the Compensation Normalization Toolbox. Select the **Transform** tab, and click the **Optimize transforms** button. Select all of the parameters and click **OK**. The program will adjust the transforms to provide an optimal distribution of the data. You can close the Compensation Toolbox at this point.

 Before adjustment                                    After adjustment
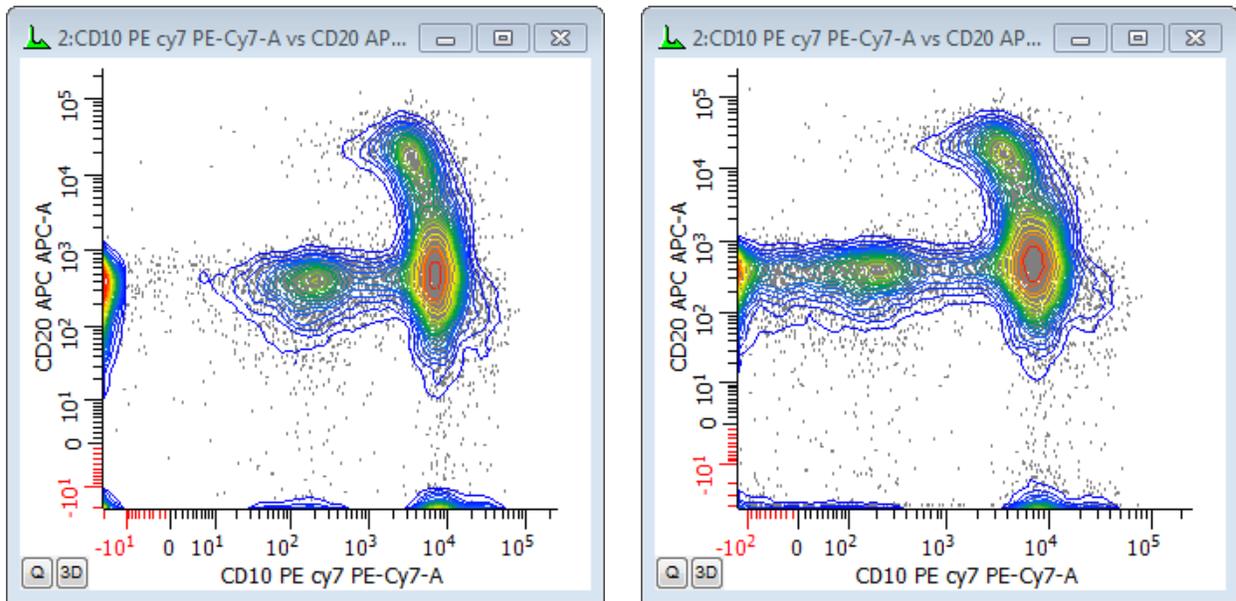
4. Use the Contour region tool to create a box around the lymphocytes in the CD45 vs SSC histogram.
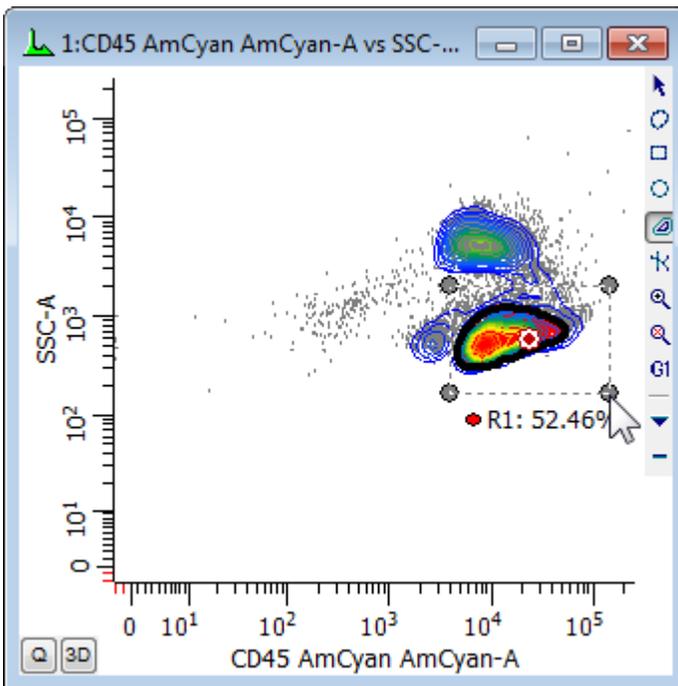


*Contour regions are defined with a bounding rectangle. The contour region recomputes when data in the histogram changes.*

5. Use the cascade gate hotspot in the center of R1 to apply the R1 gate to the CD10 PE vs CD20 APC histogram.
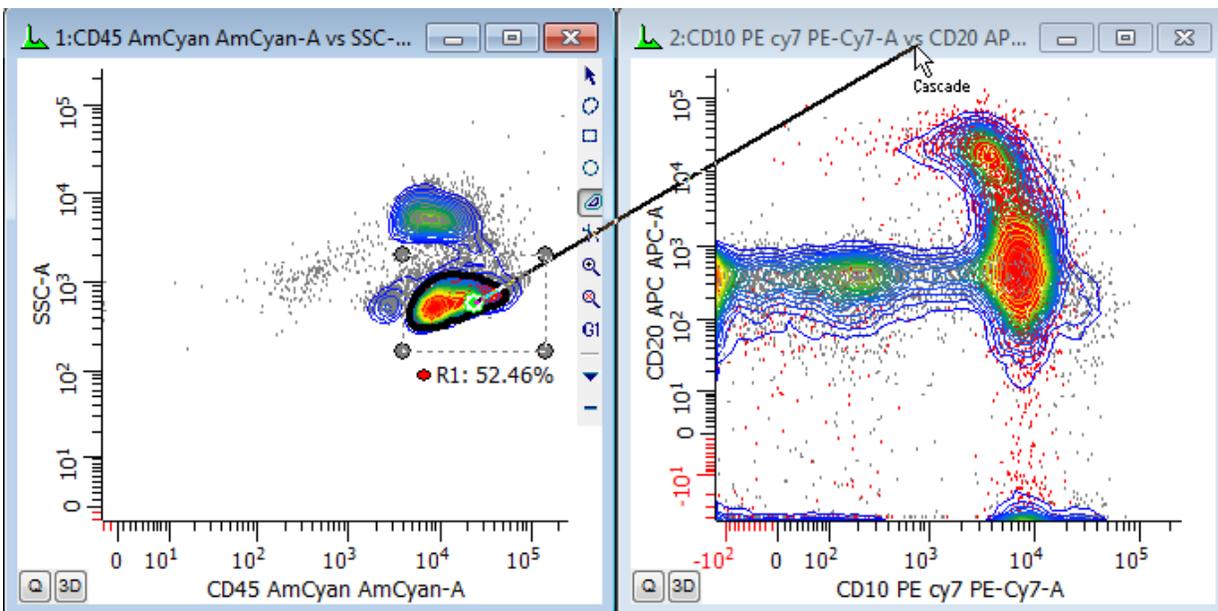


6. Click the **Q** button in the lower-left corner of the CD10 PE vs CD20 APC histogram to create a QuadStat region. Adjust the QuadStat and move the associated Results window to a convenient location on the screen.
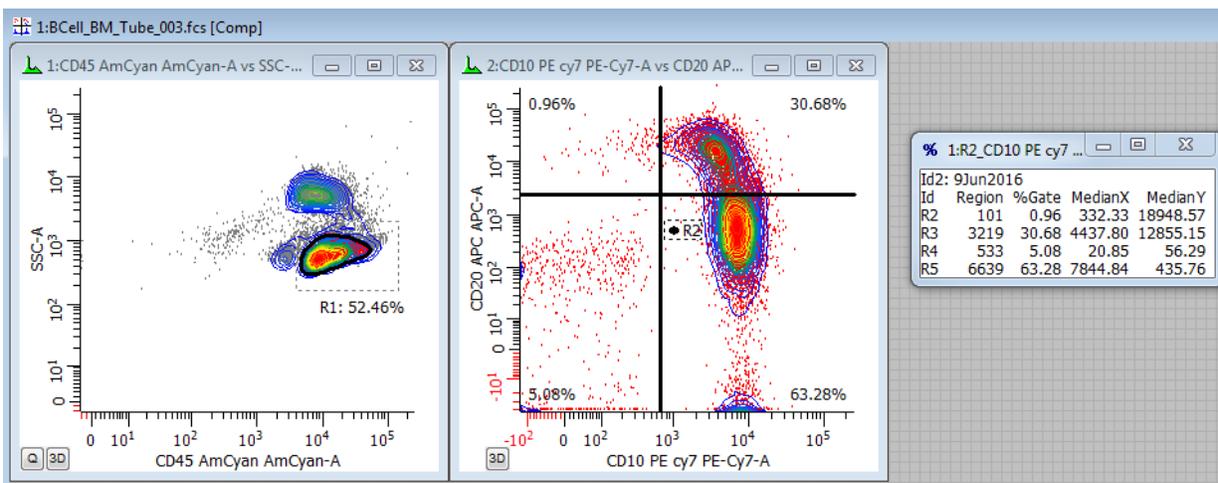


## Create the report

Now that we have a simple analysis setup, let's create a report and add some elements to it.

7. Click the **New Report** button on the ribbon bar to create a new, empty report window. Move and resize the new window so that you can see the histograms and results windows.
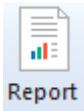
Report

Along the top of the report window is a toolbar containing commands to manage report files and to add objects to the report. Most of these commands also appear in the Report menu and in the menu that appears if you right-click on the report.

### Adding histograms to the report

The most intuitive way to add objects to the report is to drag-and-drop them on the report. Each of WinList's windows has a drag-and-drop hotspot on the title bar. This is the small icon that appears on the top left of the title bar.

*For a detailed explanation of drag-and-drop support, see the Drag and Drop section.*

8. Click and hold the mouse on the first histogram's drag-and-drop hotspot, drag it to the left side of the report page about an inch from the top, and release the mouse button. A copy of the histogram will appear on the report.

Let's examine the properties of this new object.

9. Double-click on the newly added histogram object on the report. The **Edit Histogram Link** dialog is displayed.

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**Edit Histogram Link**

Choose a Data Source and Histogram:

1:BCell_BM_Tube_003.fcs ▾

1:CD45 AmCyan AmCyan-A vs SSC-A
2:CD10 PE cy7 PE-Cy7-A vs CD20 APC APC-A

OK

Cancel

Caption:
Show name and gate ▾

Graphic quality:
Normal quality ▾

Update options:
Update when histogram changes ▾
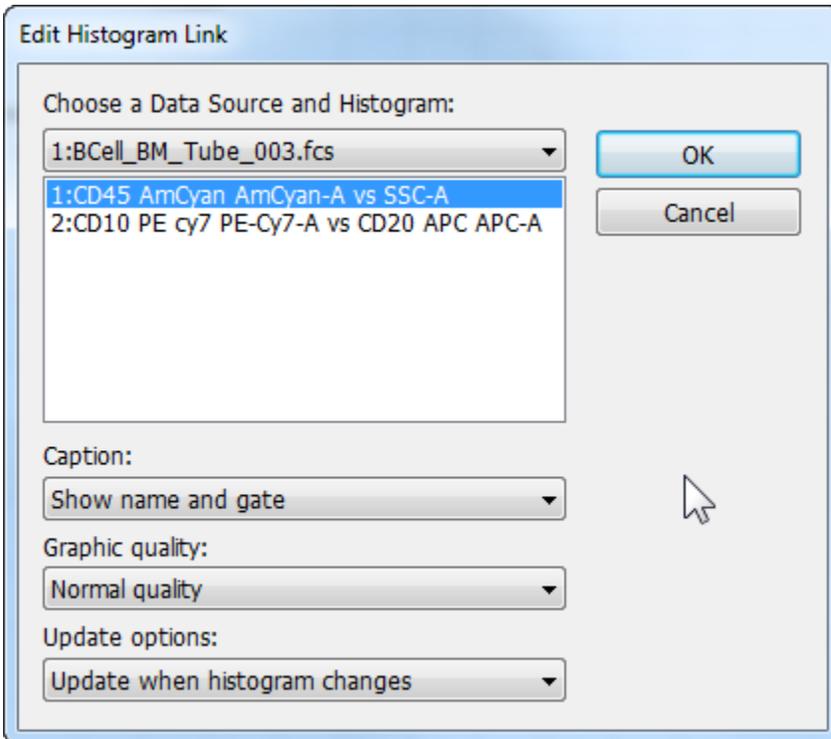
This dialog allows you to choose the histogram that this report object is linked to. It also has combo boxes that control the caption style, graphic quality, and how it will update when the linked histogram changes. You can edit these properties at any time. For now, we will leave the settings just as they are.

10. Click the **Cancel** button to close the dialog without making changes.

11. Use the same drag-and-drop technique to add the second histogram to the report, placing the new object below the first one.

*You can always adjust the size and position of objects after you've added them to a report. Click and drag and object to move it. When it is selected, use the sizing handles to re-size the object.*

**Adding results to the report**

We could drag-and-drop the results window onto the report in the same manner, but let's use the report toolbar instead.

12. Click the Results button on the Report toolbar.

The **Edit Results Link** dialog is displayed. This is very similar to the Edit Histogram Link dialog, except that it allows us to select a results window. There is also a **Properties** button that edits the font used by this object.

13. Click the **OK** button to accept the selection and create the object. Drag the new results object next to the second histogram object. Re-size the results object if it is not large enough to see all of the results.
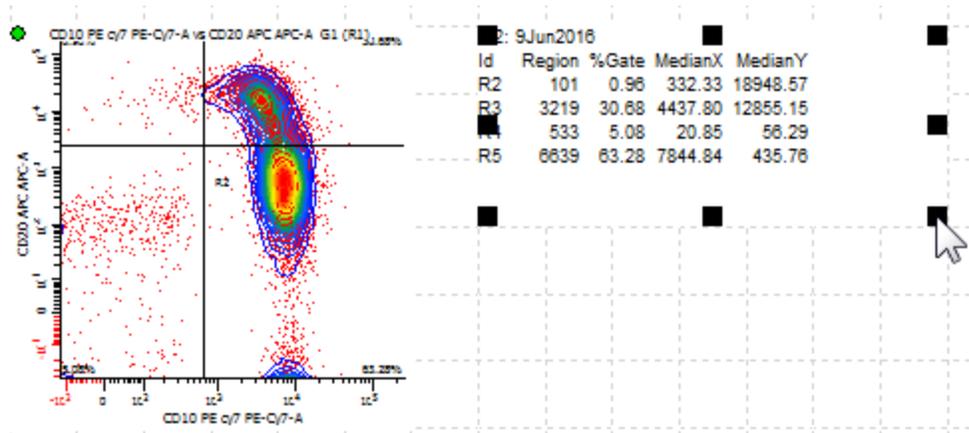


### Adding FCS keywords to the report

It is often useful to include on the report information contained in the FCS keywords from the listmode files that you analyze with WinList. The Keywords object makes it very simple to do this.

14. Right-click to the right of the first histogram on the report. This will display the report context menu.

15. Select **Insert Keywords** from the menu. The **Edit Keyword Object** is displayed. In the **Keyword Groups** tree on the left, choose **DataSource 1**. Notice that the **Available Keywords** list on the right fills with keywords contained in the file.

16. Choose **DS1:$CYT** in the list and click **Add**. A new line is added to the **Selected Keywords** window at the bottom of the dialog.
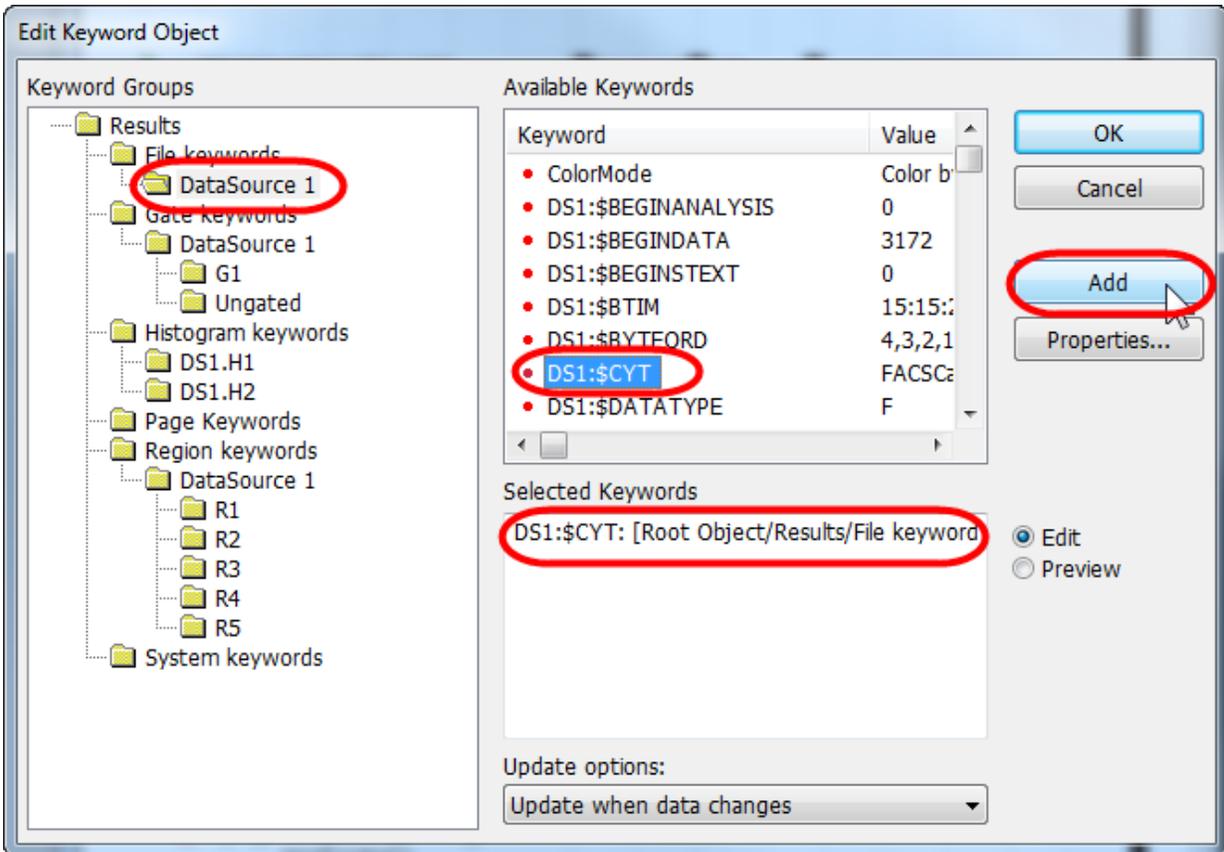
17. Use this same technique to add **DS1:$DATE** and **DS1:TUBE NAME**. Click **OK** to add the Keyword object to the report. Resize and adjust the position to show the content, if necessary.

## Adding a title area to the Master Page

Let's add some text and graphics at the top of the report to make it look more professional. We'll put these on the "Master Page" of the report so that they appear on all other pages.

> *Use the Master Page of the report to create header and footer items that should appear on all other pages of the report.*

18. At the bottom of the report window, click the **Master Page** tab. There is only one item on this page to start with, and that is the page numbering object at the bottom of the page.

19. Click the Text tool on the report toolbar to create a new text object. In the Text field, type **CD10/CD20 Report**. Click the **Font** button, select a typeface that you like, and change the font to size 36. Change the **Alignment** to **Center**. Click **OK**.
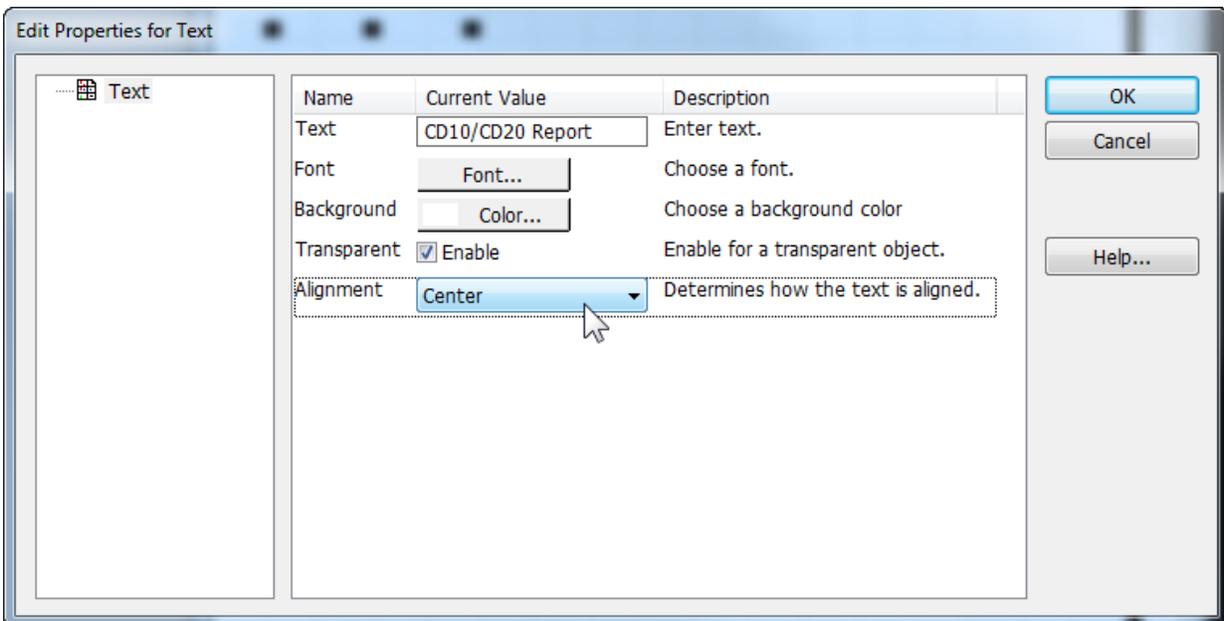
20. Move the new text object to the top of the report page and stretch it across the width of the page.

*You can change the scaling of the report page by using the Zoom dropdown on the report toolbar.*

21. Click the Rectangle tool on the report toolbar. Position the new rectangle under the title text, and make it a narrow band that stretches across the report page,.

22. Double-click the new rectangle object to display the **Edit Properties for Rectangle** dialog. Choose a **Fill Color** and **Border Color** that you like and click **OK**.

23. Now, click the **Page 1** tab at the bottom of the report window to see the items from the master page and page 1. The report should look similar to the one shown below at this point.
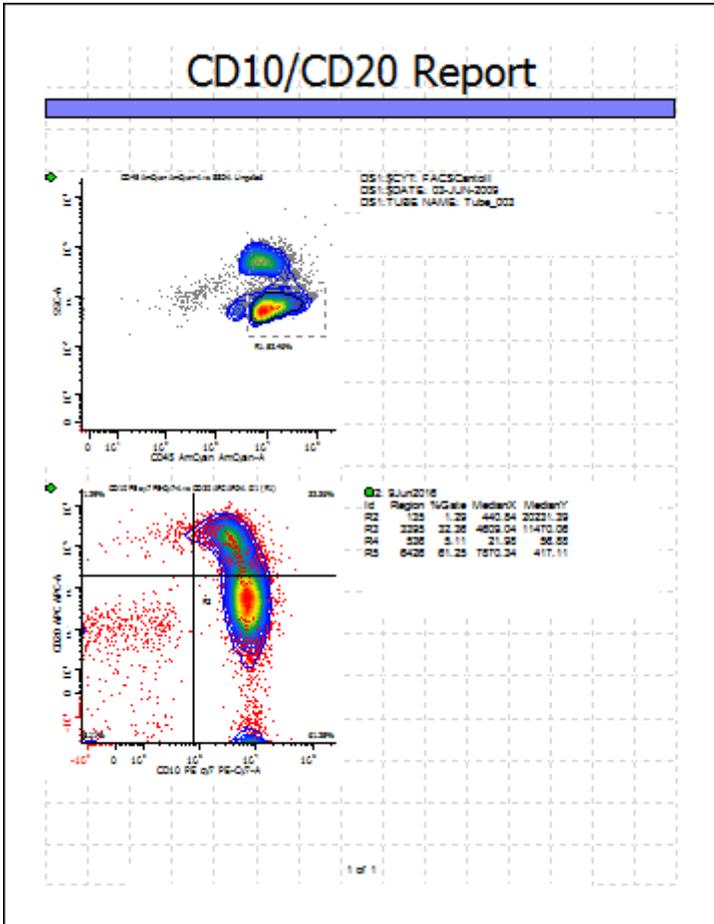
**Adding your own graphics**
You can add your own graphics to your reports easily. Open the graphic you want to add in a graphics program and copy it to the clipboard. Then, activate the report window and paste the graphic by pressing Ctrl-V or using the right-click context menu.

## Adding a gate legend and impressions

The report can include a legend that explains the colors that are shown in the histograms. Let's add a gate legend object, and then another text object that will allow us to enter comments about the analysis.

24. Right-click on the report page and choose **Insert Gate Legend** from the menu. Click **OK** in the Edit dialog. Move and size the new object on the report so that it doesn't obscure other objects.

*The Gate Legend object provides a key to the dot colors that appear in the histograms as well as statistics for each gate population.*

25. Right-click in the empty area near the bottom of the page and choose **Insert Text** from the menu. Type the word, "Impressions", and press the Enter key on the keyboard. When you press Enter, an **Edit** dialog is displayed that allows you to type and view multiple lines of text. Press **Enter** again, and type "This analysis shows CD10 and CD20 expression for the selected lymphocytes.""
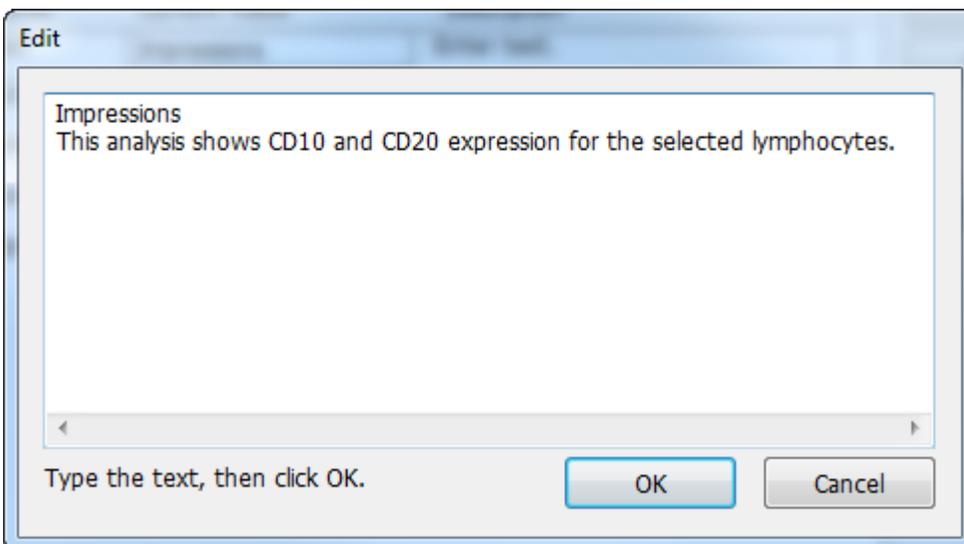


26. Click the **OK** button in the Edit dialog.

Now, suppose that we want this text object to have a light gray background. There are two edits to the object's properties we need to make.

27. Click the **Background** button, choose a light gray color from the color dialog, and click **OK**. Next, uncheck **Transparent** to disable transparency.

28. Click **OK** again to close the Edit Properties for Text dialog. Stretch the new text object across the width of the report page.
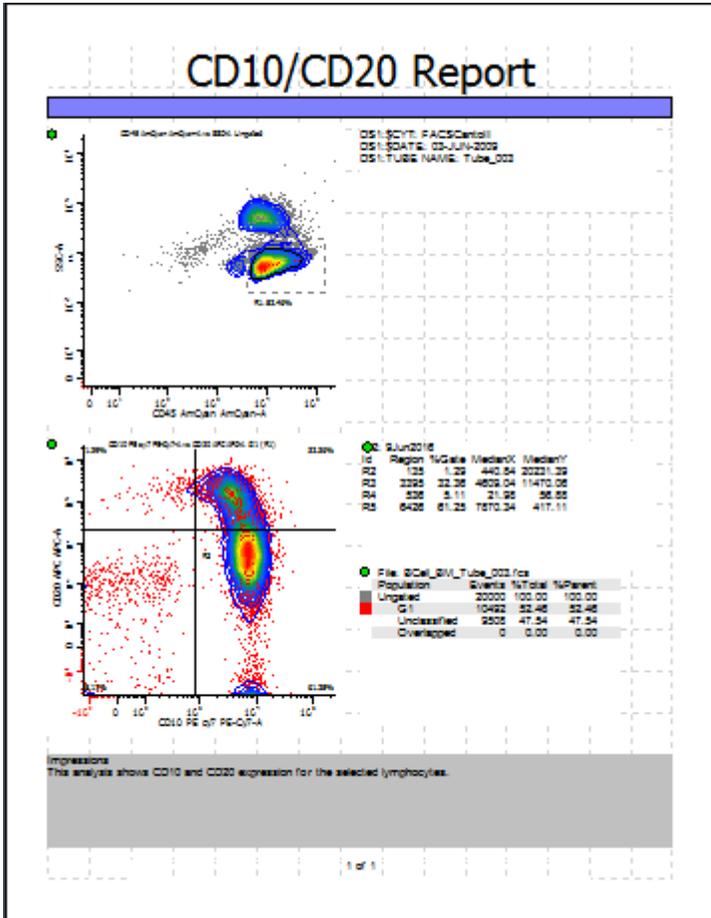
## Updating report objects

Now that the report is complete, let's see how it updates when we change something in the source histograms. The histogram and results objects we added to the report are all set to update when the data changes.

29. Move the R1 region on the first histogram window. Notice that after the histogram windows and results update, the objects on the report also update. Similarly, if you move the QuadStat region, the report will update to reflect the changes.

*Histogram and result objects on the report display a symbol in the upper left corner to indicate whether the object is actively linked or not. The symbol for a linked object is a green-filled circle. When the object is not linked, the symbol is disappears.*

Suppose that you want to make changes in the histograms and you do *not* want the report to change. This is easily accomplished by clicking the **Active Links** button on the report toolbar. This is a toggle button. In the depressed position, the report objects update automatically, and when it is not depressed, the objects do not update.

## Additional pages

Reports can include any number of pages. The **Pages** button on the report toolbar displays a menu of page commands for adding, removing, and navigating pages in the report.

30. At the bottom of the report window, click the **<Add>** tab. Click **OK** in the Add Page dialog to accept the default name for the new page. A new, empty page is displayed on the report. Notice that the items from the Master Page appear automatically.

Let's use this new page to show a quick way of adding many histograms at once. Suppose that you want to add all of the histograms from a data source to a report. Here's the quick way to do it.

31. Move the mouse over the drag-and-drop icon for the *data source* window. Drag the icon onto the report and release the mouse button. Both histograms from the data source are added at once. Move them so that they do not obscure the title.

You can navigate to the other report page by pressing the Page Up and Page Down keys on the keyboard, by clicking the Page tab at the bottom of the window, or by using commands in the **Pages** menu of the report toolbar.

## Saving and exporting the report

Saving a report to disk allows you to store it for later use or to serve as a reporting template. In addition to saving it for future use in WinList, it's often useful to export the report to PDF format so that you can share it with others.

32. Click the **Save** button on the report toolbar. Navigate to a location where you want to store the report. Type a name for the report and click **Save**.

Notice that the title bar of the report window changes to show the new name you typed in. Exporting the report to PDF is just as simple.

33. Click the **PDF** button on the report toolbar. Navigate to a location where you want to store the PDF version of the report. Type a name for the PDF file and click **Save**.

## Summary

Creating a report is a simple process of dragging elements onto a report page or adding elements using menus. Histograms, results, and keyword report objects automatically update when the source data changes, making the report a great tool for batch processing files.

Reports can contain any number of objects and pages, so that even very large experiments are easily supported.

**See Also:**

Report Windows

Report Toolbar

Report Context Menu

Report Page Menu

## Using Overlays

Overlay windows are a great tool for graphically comparing a set of 1P or 2P plots.  Offering different views and a wide variety of graphical options, overlays organize plots - typically from different files - so that you can easily see the similarities and differences among them.  This tutorial explores how to create overlays and some of the properties you may find most useful.

> *If you are unfamiliar with opening files and creating histograms with WinList, you might want to do the WinList Basics tutorial before continuing with this one.*

### Getting Started

We'll start by opening a listmode file and creating some histograms.

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples\AutoPosition folder located in the folder where WinList is installed. Select **autoposition.015** from the list and click **Open**.

2. In the **Create Histograms** dialog, select **FL2-H** in the X Axis list and **FL1-H** in the Y Axis list.  Click the **Add to List** button.

3. Click the **1P Histograms** tab in the dialog.  Choose **FL2-H** in the X Axis list and click the **Add to List** button.

4. Click **OK** to close the dialog and display the histograms.
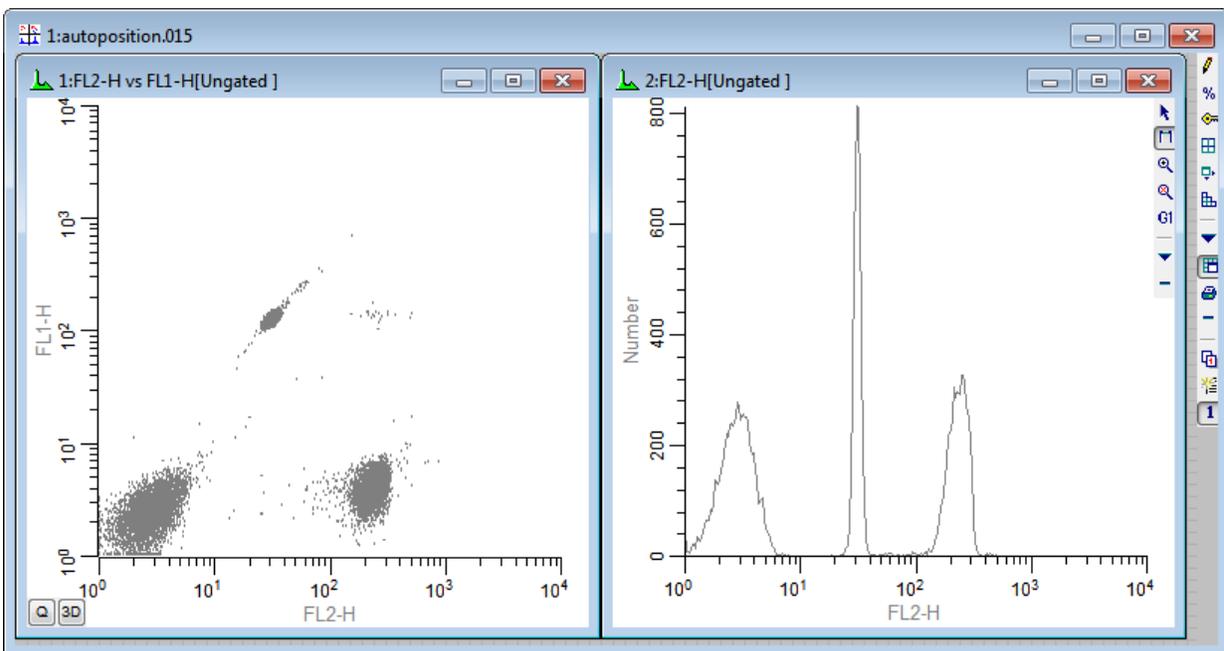
We're going to look at a series of samples in which the intensity of the marker in FL2-H diminishes, and we will overlay the graphics to compare the changes.  Let's create two overlay windows, one for the 1P graphics and the other for 2P graphics.

5. Click the **Overlay** button on the ribbon bar **two times** to create two overlay windows.  Move the windows to a convenient location on your screen.

To add a histogram to an overlay, you simply drag-and-drop the histogram by the icon in the top left corner of its title bar.
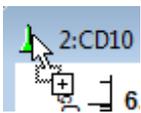


6. Drag-and-drop the 1P histogram onto the first overlay window, and then drag-and-drop the 2P histogram onto the second overlay.
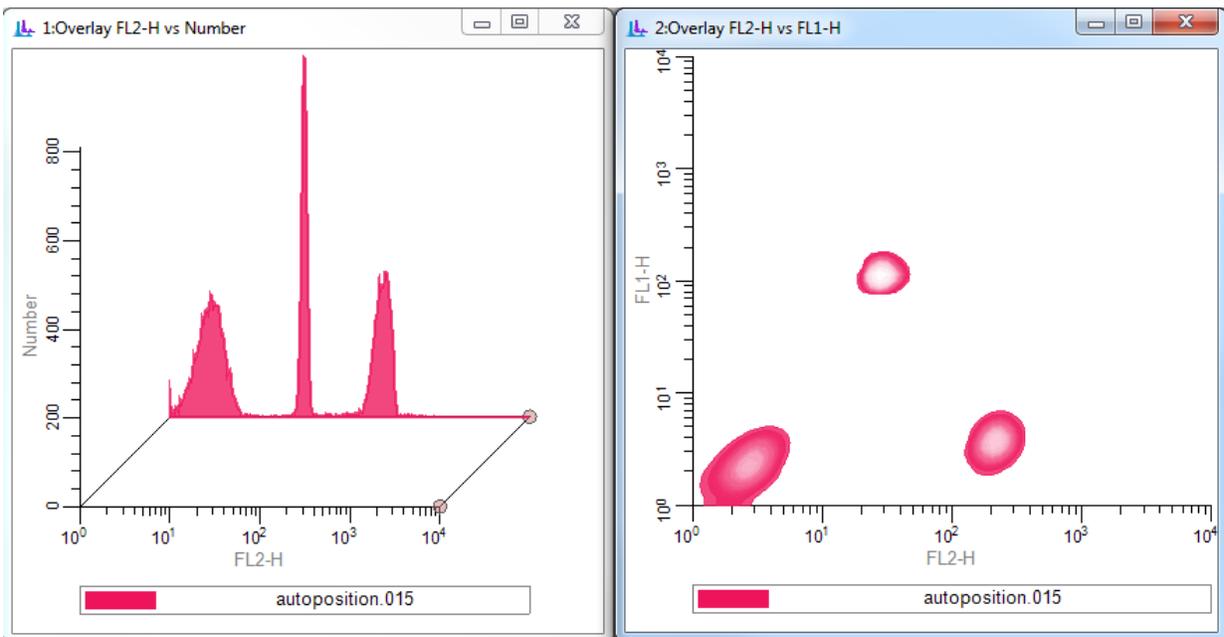


The first element added to an overlay window determines several key properties for the overlay.  The overlay will become a 1P or 2P overlay depending on the type of histogram that is added.  The axis displayed for the overlay is based on the first element, too.  Let's add a couple more elements to each overlay.

7. Click the **Next FCS File** button on the ribbon bar and select the next file in the folder: **autoPosition.016**.  Click Open to read the file into WinList.

8. Drag-and-drop each histogram onto the associated overlay window to add new elements for this new data file.

9. Click the **Next FCS File** button and select **autoPosition.017**.  Click Open to read the file.

10. Drag-and-drop each histogram onto the associated overlay window.  You should now have 3 elements in each overlay window.
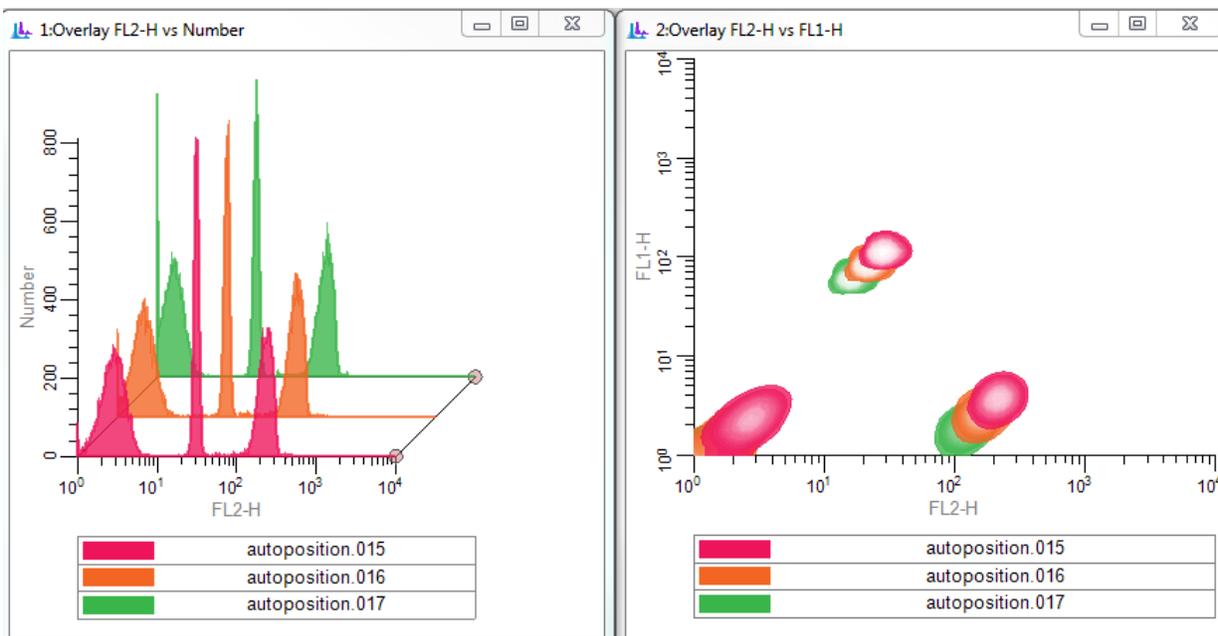


### Editing Overlays

Notice that the 1P overlay has a left-to-right arrangement, while the 2P overlay is a straight overlaid arrangement.  These are the default settings for each overlay type, but they can be changed.  We can also change other properties of the overlay and its elements.  Let's explore.

11. Right-click inside the 1P overlay window and choose **Edit** from the context menu.  The **Edit Properties** dialog for the overlay is displayed.

In this first panel of the dialog, we can change settings that affect the overall appearance of the overlay and some things common to all elements.  For example, we could change the transparency or the line weight of the histograms, the fonts used, or whether to show grids.  This is also where we can select the view of the overlay items.

Let's look at the properties that can be changed on individual elements of the overlay.

12. In the tree on the left side of the Edit Properties dialog, click the first element, **autoposition.015**.  The properties for this element are displayed.

13. Edit the **Label** so that it reads **Time=0h**.  This label will now be used in the legend for the first item.
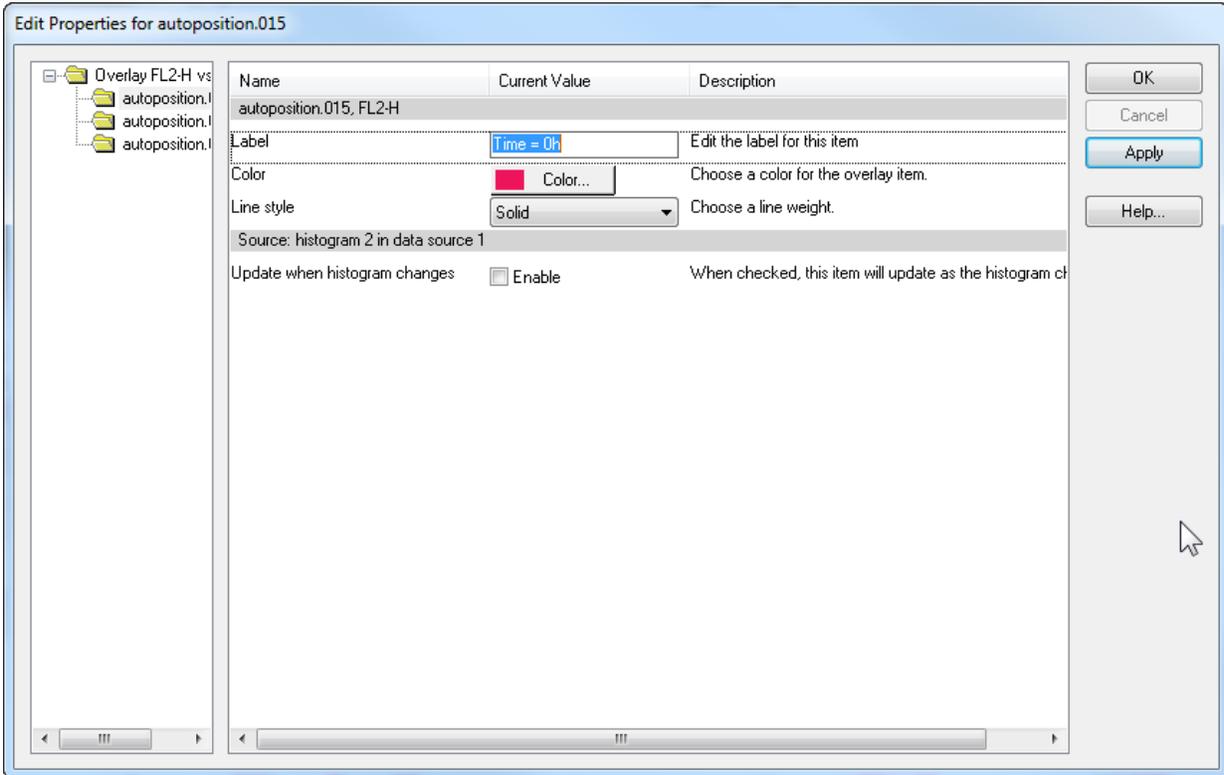


14. Select **autoposition.016** in the tree on the left, and edit its label to **Time=6h**.

15. Select **autoposition.017** in the tree on the left, and edit its label to **Time=12h**.

You can also change the color and line style with this editor.  Of special interest is the **Update when histogram changes** option.  With this option enabled, the element would update in the overlay any time that the histogram it came from changes - a very useful option for some or all elements in an overlay.  In our example here, however, we will leave the option unchecked because we want the elements to remain unchanged.

16. Click **OK** to close the dialog and see the changes.
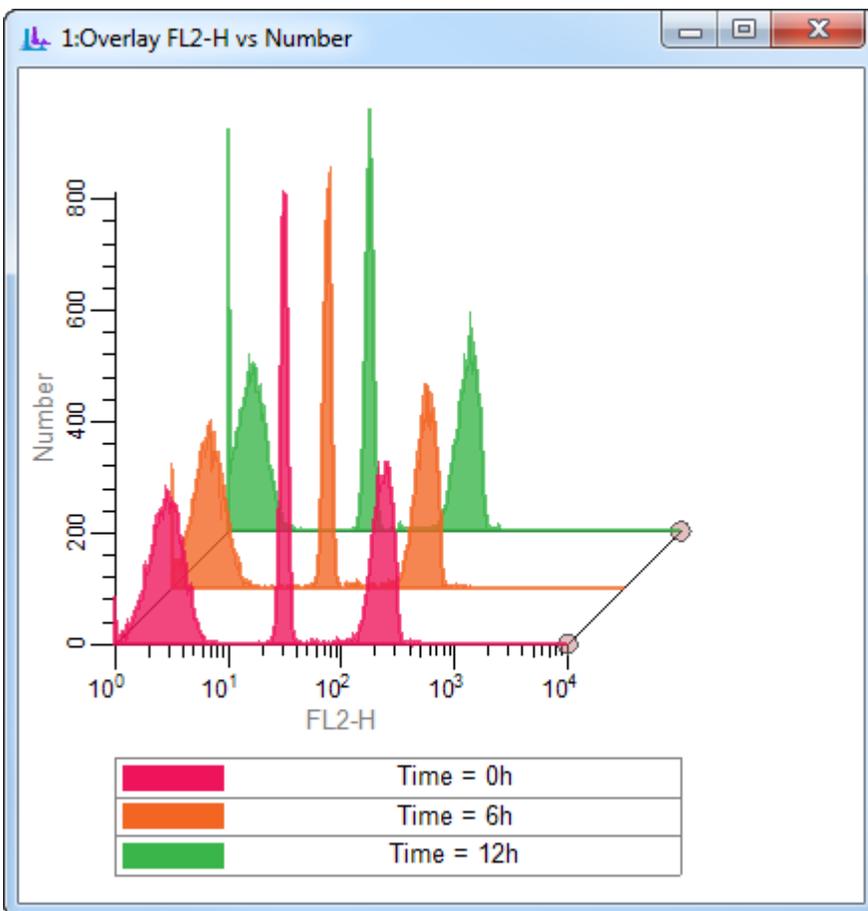
Did you notice the small handles on the right side of the axis in the 1P overlay? They can be dragged to change the left-to-right perspective. The lower handle moves left and right to adjust the width of the X-axis. The back handle adjusts up and down to control the tilt of the arrangement. These handles only appear in Left-to-Right view for an overlay.

Let's take a look at the 2P overlay. Suppose we want to change the order of the elements that we overlaid so that the first element was in the back of the stack and the last element was in the front. We do that by dragging elements in the legend at the bottom of the window.

17. Drag the **autoposition.017** element in the legend to the top of the legend and drop it. It becomes the front element in the stack.

18. Drag the **autoposition.015** element to the bottom of the legend and drop it. It becomes the last in the overlay stack.

Overlays are part of a WinList protocol file, so when you save your protocol, you save the overlays, too. This means that you can set up overlays as part of your experiments, adding

to them as you work on new samples.  Overlays can be copied in high-resolution to the clipboard and pasted into other apps.  They can also be saved to disk in many popular graphics formats for use with publications.

**See also:**

Overlay Windows

Overlay Context Menu

**Using FCOM to enumerate phenotypes**

In this tutorial, we'll learn how to use the *FCOM* parameter to enumerate phenotypes. FCOM is defined as a calculated parameter function in WinList that analyzes any number of phenotypes defined by any number of gates. The best way of explaining this function is to use it. We'll start with a simple example and then discuss how you can extend it in more powerful ways.

Here is the general concept. We are going to open a listmode file and display a few histograms. We will set up some gates to identify the positive events for CD4 PE, CD8 PerCP, and CD3 APC. Then we'll create the FCOM parameter to see the complete phenotype.

*Before you begin, make sure you understand the basics of working with WinList. The tutorial [WinList Basics](WinList Basics) is a great place to start.*

**Open a data file**

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **FCOM_Example.fcs** from the list and click **Open**.

*On the PC, you can also drag and drop files from Windows Explorer or My Computer onto WinList. See [Drag and Drop](Drag and Drop) for details.*

2. When the **Create Histograms** dialog appears, click **Cancel**. We will create the histograms one at a time by drawing them on the data source.

This file does not contain any events with values less than 0, so in this case there is no utility to using the HyperLog transformation for it. Let's switch over to use the Log transform for all log-like parameters.

3. Click the **Comp** button on the ribbon bar to display the Compensation Normalization Toolbox. Select the **Transforms** tab, and click the **--> Log** button. You can close the Compensation Toolbox at this point.

**Creating histograms and gates**

Next, we'll draw 3 histograms on the empty data source window and gate them appropriately.

4. Click, drag, and release the mouse on the data source window to define an area where we want to display a histogram. In the **Edit Histogram** dialog, choose FSC-Height and Side Scatter for the X- and Y-Axis respectively, then click **OK.**

5. In this new histogram, draw a region around the cells of interest as shown below. You can use any region type to do this.
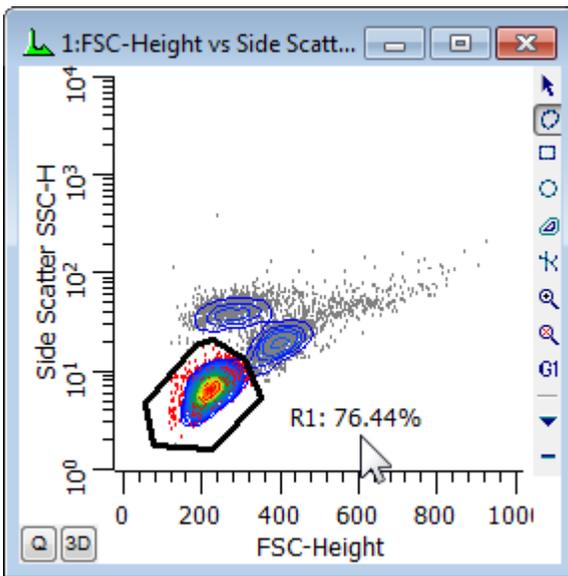


6. Click R1's label to display the cascade hotspot (the red bull's eye in the center of the region), move the mouse to an empty portion of the data source window, and draw a second histogram. In the **Edit Histogram** dialog, select Side Scatter for the X Axis and CD3 APC for the Y axis, and then click **OK**.

A new histogram gated on R1 is displayed.
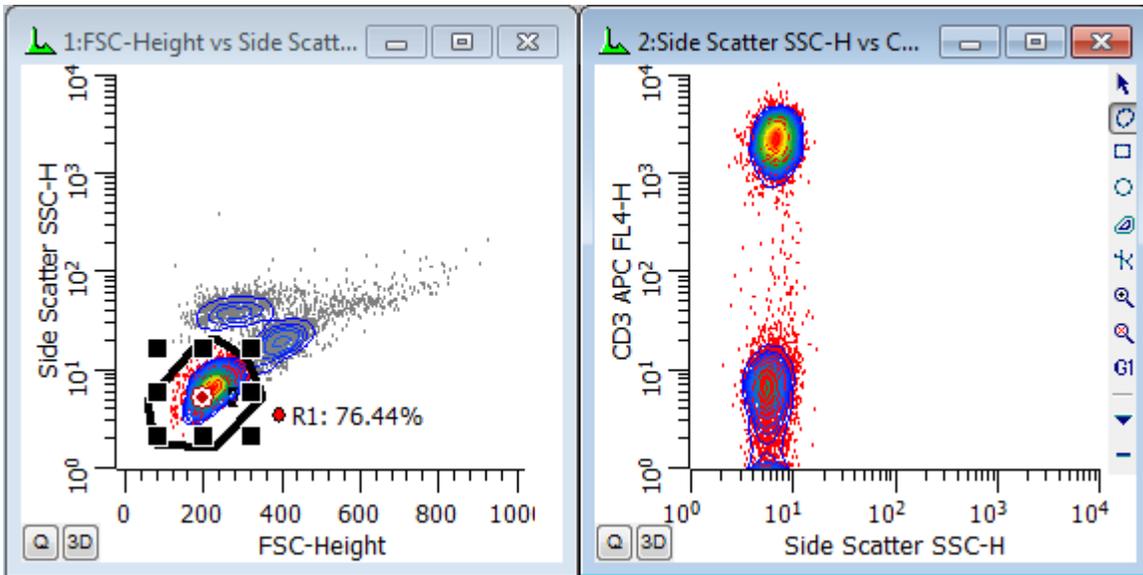
*Did you know that you can apply a cascade gate to any histogram by clicking on the region's hotspot and then clicking on the title bar of the histogram you want to gate? You can also hold down the Shift key if you want to apply a cascade gate to several histograms at once.*

7. Draw region R2 around the CD3-positive population in the new histogram. Click on the cascade hotspot for R2, move the mouse onto the data source window, and draw a third histogram. In the **Edit Histogram** dialog, select CD8 PerCP for the X Axis and CD4 PE for the Y axis, and then click **OK**.

8. Finally, draw two more regions: R3 to define the CD4 positives and R4 to define the CD8 positives, as shown in the following picture. Note that we intentionally capture some events as CD4+CD8+ in this example.

*You can choose a different region drawing tool from the histogram toolbar to draw regions of different shapes.*
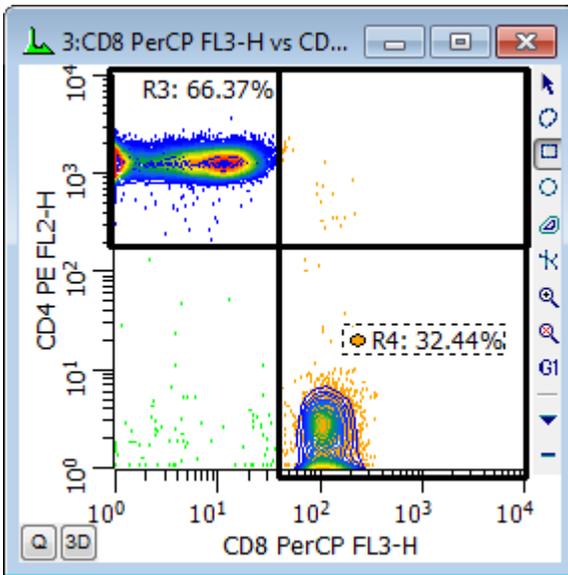
## Creating the FCOM parameter

OK, now for the fun part. Let's create the **FCOM** parameter.

9. Click the **Add Parameter** button on the main ribbon bar.  Alternatively, you can right-click in the data source and choose Add Parameter from the context menu.



10. Click the **Add** button in the Edit Calculated Parameters dialog to add a new parameter.

Let's choose an example equation from the Functions list box.

11. Select **(FCOM(G1,G2,G3)+0.5)*1024/8+FRND(10)** from the list of example equations.

We need to edit this example equation to use gates **G2**, **G3**, and **G4**.

12. Edit the equation so that it reads (FCOM(**G2,G3,G4**)+0.5)*1024/8+FRND(10). Type **FCOM** in the Name edit box.

This equation looks more complicated than it really is. The FCOM function can have any number of gates as arguments. The way it works is described in the reference section of the manual. Here is how it works with our example. Using the 3 gates "G2, G3, G4" as the arguments for our FCOM, there are 8 possible classifications for each event in the listmode file. An event can be negative for all gates, positive for all gates, or some other combination. The table below shows the possible FCOM values for the G2, G3, and G4 gates.

| G2 | G3 | G4 | FCOM Value |
|----|----|----|------------|
| -  | -  | -  | 0          |
| +  | -  | -  | 1          |
| -  | +  | -  | 2          |
| +  | +  | -  | 3          |
| -  | -  | +  | 4          |
| +  | -  | +  | 5          |
| -  | +  | +  | 6          |

| + | + | + | **7** |
|---|---|---|---|

In essence, FCOM converts gate combinations to a single number identifying the phenotype. The more gates in the expression, the more combinations there are. The rest of the equation spreads the populations so that we can visualize them more easily on a histogram display.

13. Click **OK** to close the **Edit Calculated Parameter** dialog, and click **OK** again to close the **Edit Calculated Parameters** dialog.

WinList creates the new, calculated, listmode parameter that can be used like any other parameter in the listmode file. Let's create a histogram to see what it looks like graphically.

*You can use FCOM parameters in 1P, 2P, and 3P histograms. You can plot it against another parameter in the listmode file to see the phenotype as a function of that parameter.*

14. In an empty portion of the data source window, draw a new histogram with the mouse. We want to make this window wide, so hold down the **Alt** key as you draw the histogram.  When you release the mouse, the Edit Histogram dialog is displayed. Choose **1P** for the histogram type, and choose the last parameter in the list, **FCOM**, for the X Axis. Click **OK**.
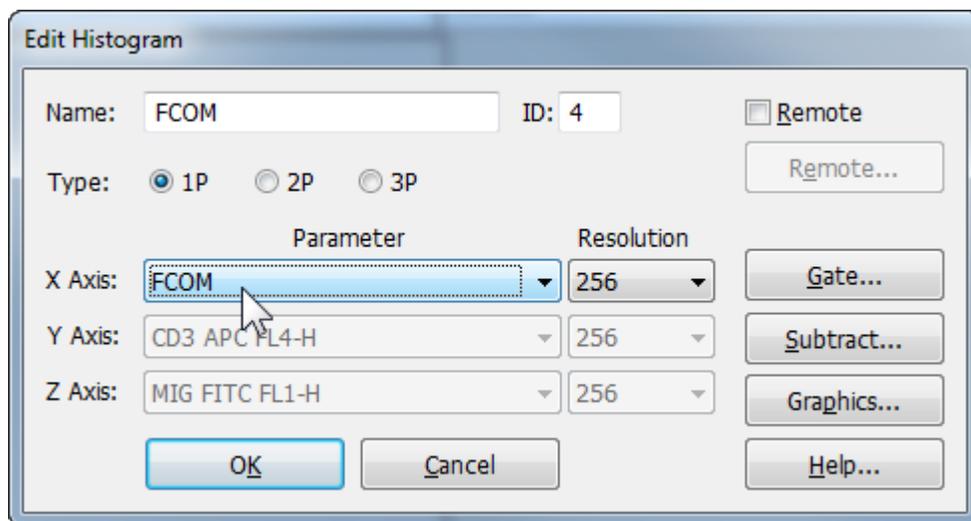


This new histogram has distinct peaks representing the eight possible states for events evaluated by gates G2, G3, and G4. The left-most peak represents events outside of all three gates, the triple-negative events. The next peak contains events that are in G2 but not in G3 or G4. The last peak represents triple-positive events in all gates.

### Statistics

WinList makes it easy to get statistics for each phenotype and to see each distribution in the context of other histograms. To do this, we'll use the Create Region Array feature to create a set of regions and results.

16. From the **Insert** tab of the ribbon bar, click the **Region Array** button. Click the checkbox labeled **Create a region array for the FCOM peaks**. This is a special option that only appears when the active histogram contains an FCOM parameter.
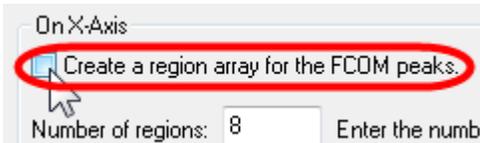


The **FCOM Region Array Options** dialog appears. This dialog allows you to turn on color gating based on the FCOM peaks, and to choose the colors used for those gates. It also allows us to name the gates to make it easier to identify phenotypes.

17. Type *3* in the **G2 alias** edit box, *4* in the **G3 alias** edit box, and *8* in the **G3 alias** edit box.
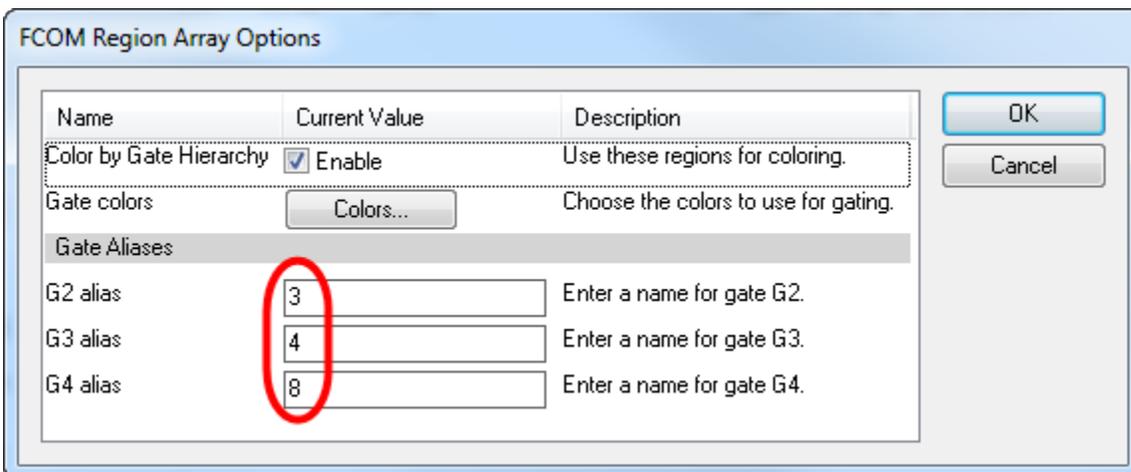


18. Click **OK** to close the dialog. Click **OK** again to close the Create Region Array dialog.

WinList creates 8 regions and positions them on the FCOM peaks. The regions are labeled to show the phenotypes. A result window shows the statistics for each peak, along with the phenotype labels. Each population is color-coded in the histogram to make it easy to identify the phenotype.
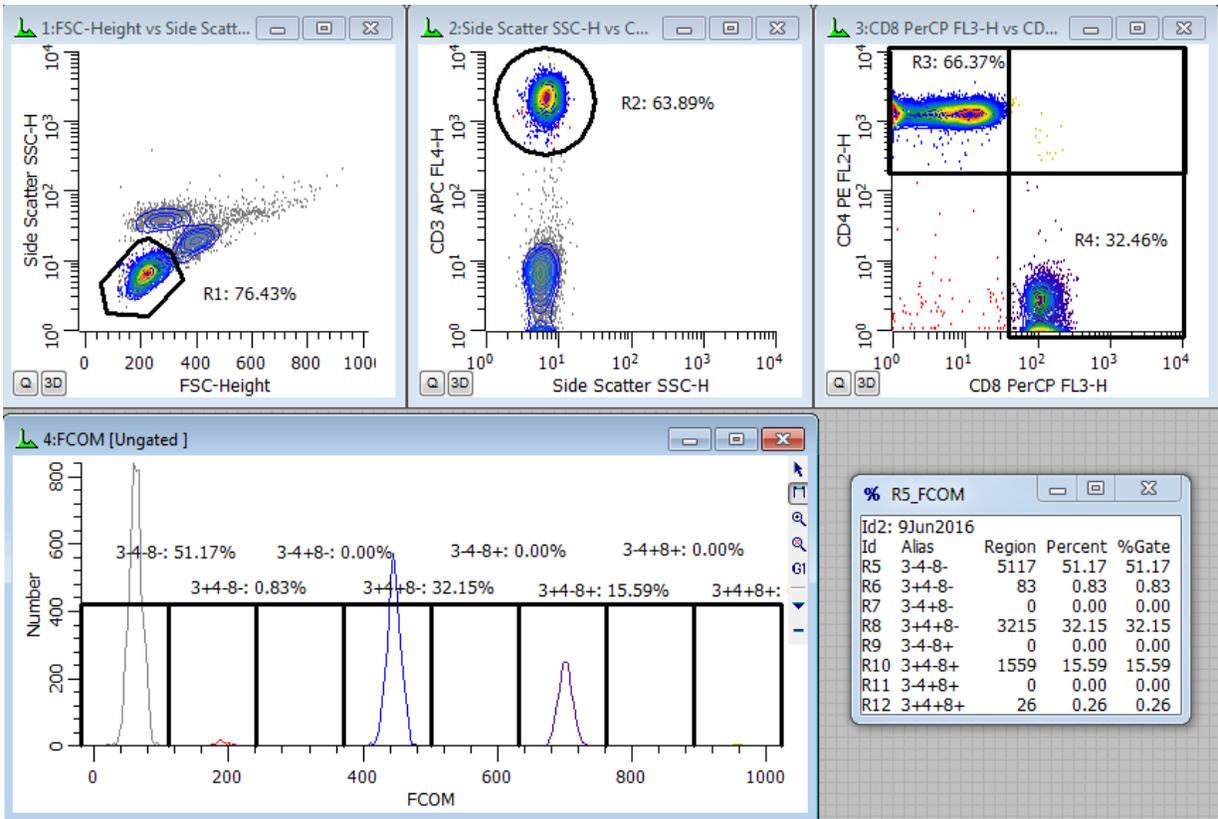
## Extending this system

Since the FCOM parameter can take any number of gates, you could look at all the combinations of four gates (16 combinations) or more. You can create more than one FCOM parameter, each with the phenotype of a different set of gates, and then display a 2P histogram of the two FCOM parameters. You can even save the FCOM parameter into a new FCS file using the Save Data Source option in the File menu.

**See Also:**

Enhanced Proliferation Analysis

FCOM Background and Usage

## Cluster Identification with t-SNE

A challenging aspect of modern cytometry is in how we deal with ever-increasing numbers of measurements and markers.  New instruments are introduced every year that provide us with more colors so that we can learn more about the cells we analyze.  Traditional analysis strategies with 2P plots and gates become tedious, if not impossible - there are just too many combinations of markers to consider.  This is not a problem unique to cytometry data, and several methods have been proposed which do a pretty good job of addressing the challenges of high-dimensional data.

In 2008, Laurens van der Maaten and G.E. Hinton proposed a method for visualizing high-dimensional data called **t-SNE** . The name means "t-Distributed Stochastic Neighbor Embedding", and the method does a great job of reducing dimensionality to 2 or 3 dimensions.  At the same time, the commonality of similar data points is preserved, making it a great tool for cytometry data.  You can read more about this method on van der Maaten's web site: lvdmaaten.github.io/tsne.

WinList has integrated t-SNE through its calculated parameter functions, making it easy to generate t-SNE plots and to explore your data with this tool.  Let's take a look at how to do this with an example file acquired on a Fluidigm Helios instrument with 48 measurements.

*Before you begin, make sure you understand the basics of working with WinList. The tutorial WinList Basics is a great place to start.*

### Open the FCS file

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **Helios1.fcs** from the list and click **Open**.

2. In the **Create Histograms** dialog, switch to the **1P Histograms** tab and select the CD4, CD8, and CD3 measurements.  The full names for these are 143Nd**_CD4** Nd143Di, 144Nd**_CD8** Nd144Di, and 150Nd**_CD3** Nd150Di.  With these selected, click **OK**.
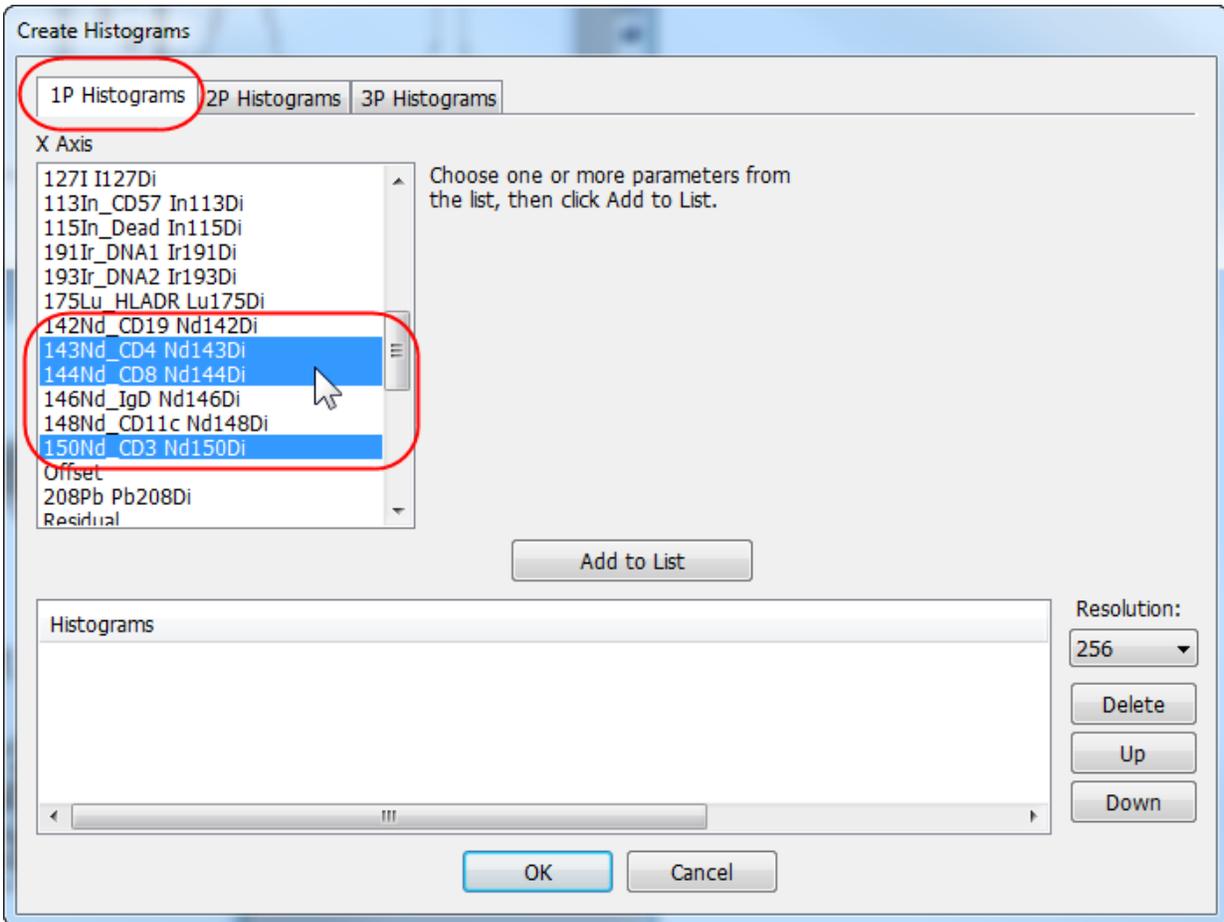
## Create the t-SNE parameter

Next, we want to create the t-SNE function.  To do this, we use the Add Parameter feature of WinList.

3. Click the **Add Parameter** button on the main ribbon bar.  Alternatively, you can right-click in the data source and choose Add Parameter from the context menu.



4. Click the **Add** button in the Edit Calculated Parameters dialog to add a new parameter.

Let's choose an example equation from the Functions list box.  The function we're looking for is actually **FSNE** as opposed to t-SNE.  All WinList function names start with the letter **F** and have a total of 4 letters, so *t-SNE* becomes *FSNE*.

5. Select **FSNE(P1,P2,P3,P4,P5,P6,G0,50,2)** from the list of example equations.  This will get us started.
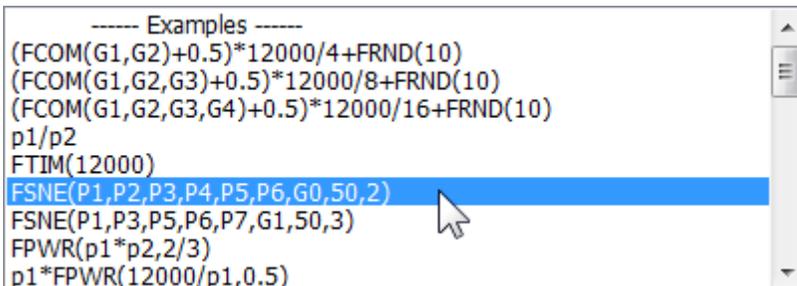


The function has 3 important parts to it.  The first part is the list of parameters that we want t-SNE to evaluate.  This can be **any number of parameters**, starting with P1, separated by commas:

FSNE(**P1,P2,P3,P4,P5,P6**,G0,50,2)


After the parameters, there is a **gate number** in the expression. This gate is used to filter the events that t-SNE evaluates.  For example, you might have a gate the selects lymphocytes that you want to filter the events on.   For no gate, we enter G0.

FSNE(P1,P2,P3,P4,P5,P6,**G0**,50,2)


You probably will not edit the last two arguments to the FSNE function.  They relate to "perplexity" and the number of dimensions to return.  Perplexity sets the number of effective nearest neighbors, which has a default value of 50.  The number of return dimensions is typically 2, but can be 3.

FSNE(P1,P2,P3,P4,P5,P6,G0,**50,2**)


Let's edit the example equation so that it suits our data file. This is easy to do with the **Choose** button that appears next to the Equation edit box.


6. Click the **Choose** button.
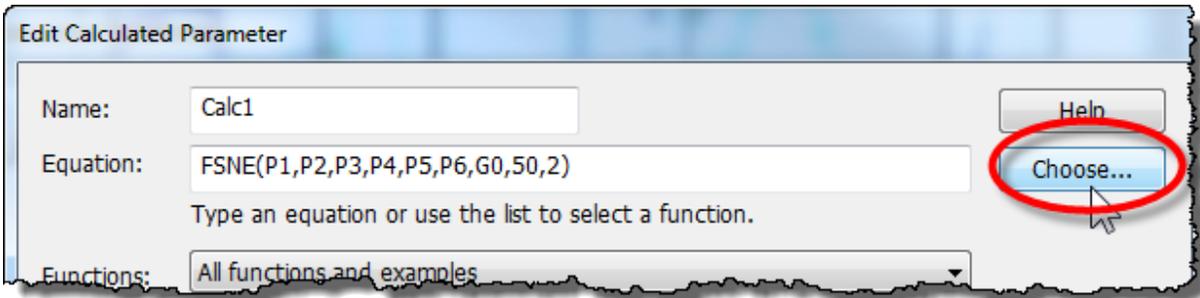
The Select t-SNE Parameters dialog is displayed, showing two lists of parameters. The Available list on the left contains parameters we have not included, and the Selected list on the right contains those that we have added. To move an item from one list to the other, you simply click the item.

7. Remove **P1, P2, P3, and P4** from the Selected list by clicking each one.

8. In the Available list, click **p7, p8, p9, p10, p11, p18, p21, p25, p26, p27, p28, p31, p36, p37, p38, p46, and p47** to add them to the Selected list. The dialog will look something like this when you are finished.
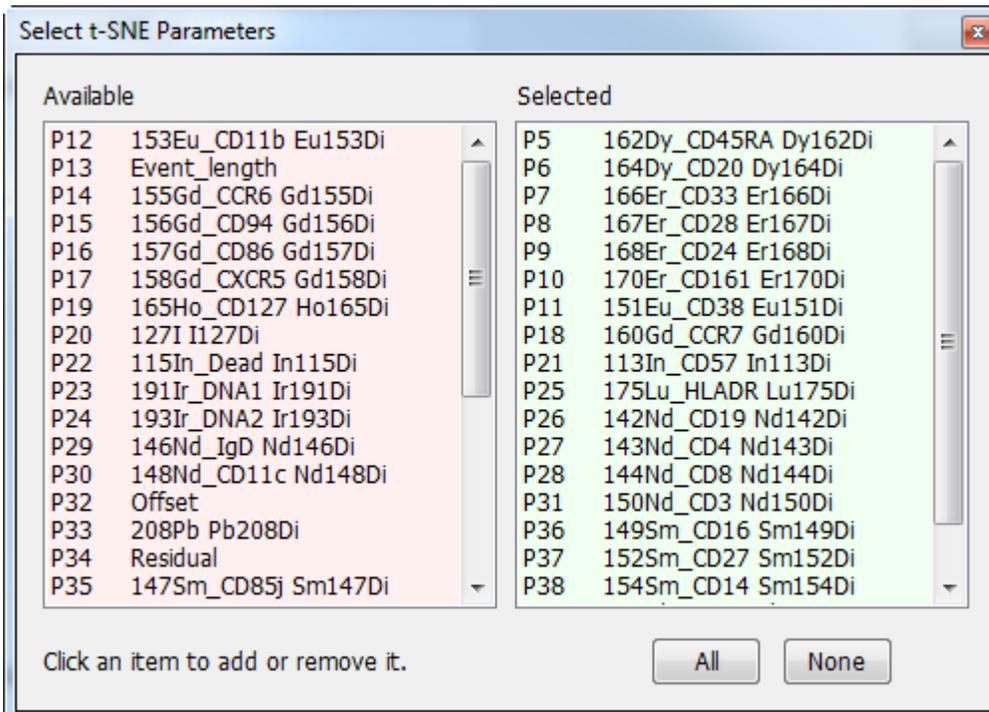


9. Click the X at the top of the dialog to close it. The **Equation** field in Edit Calculated Parameter will now contain this expression:

**FSNE(p5,p6,p7,p8,p9,p10,p11,p18,p21,p25,p26,p27,p28,p31,p36,p37,p38,p46,p47,G0,50,2)**
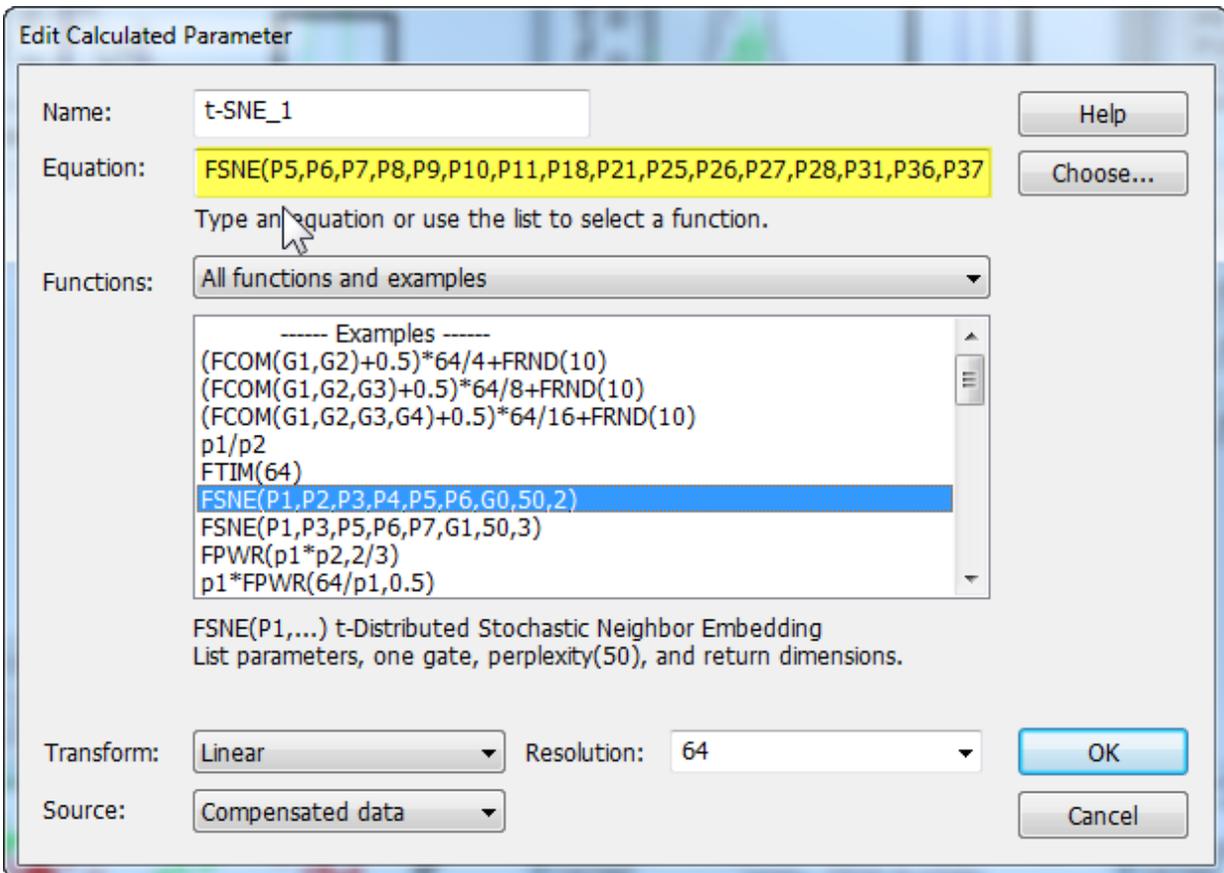


We are passing 19 ungated measurements to t-SNE and getting 2 dimensions in return. The measurements are CD45RA, CD20, CD33, CD28, CD24, CD161, CD38, CCR7, CD57, HLADR, CD19, CD4, CD8, CD3, CD16, CD27, CD14, CD56, and CD25. We will leave the gate at G0, and perplexity and dimensions at 50 and 2, respectively.

10. Click **OK** in the Edit Calculated Parameter dialog. You will notice that the program automatically named the calculated equation "t-SNE_1". Click **OK** in the Edit Calculated Parameters dialog. You may see a dialog with a sample size recommendation.  Click "Yes" if this dialog appears.
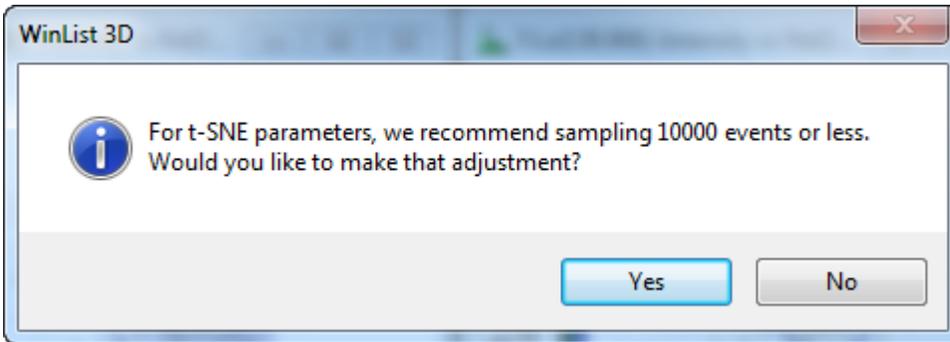
The program will begin to calculate the new parameters.
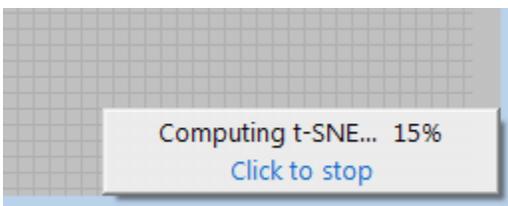


At this point, we can go ahead and work with the program. The t-SNE routine is working in background to figure things out. When it finishes, WinList will display a notification. Let's go ahead and create a 2P plot that will display the t-SNE parameters when it finishes.

11. On the **Insert** tab of the ribbon bar, click the **Dots** button to add a dot plot to our layout. Move it and size it to your liking. You may want to increase the **Dot size** to 5 and change **Dot order** to **Low frequency on top** in the Edit Graphics dialog. These settings are helpful for t-SNE plots.

12. Change the X-axis to display **t-SNE_1** and the Y-axis to show **t-SNE_2**.

When the t-SNE function finishes computing, you should see a plot similar to this:
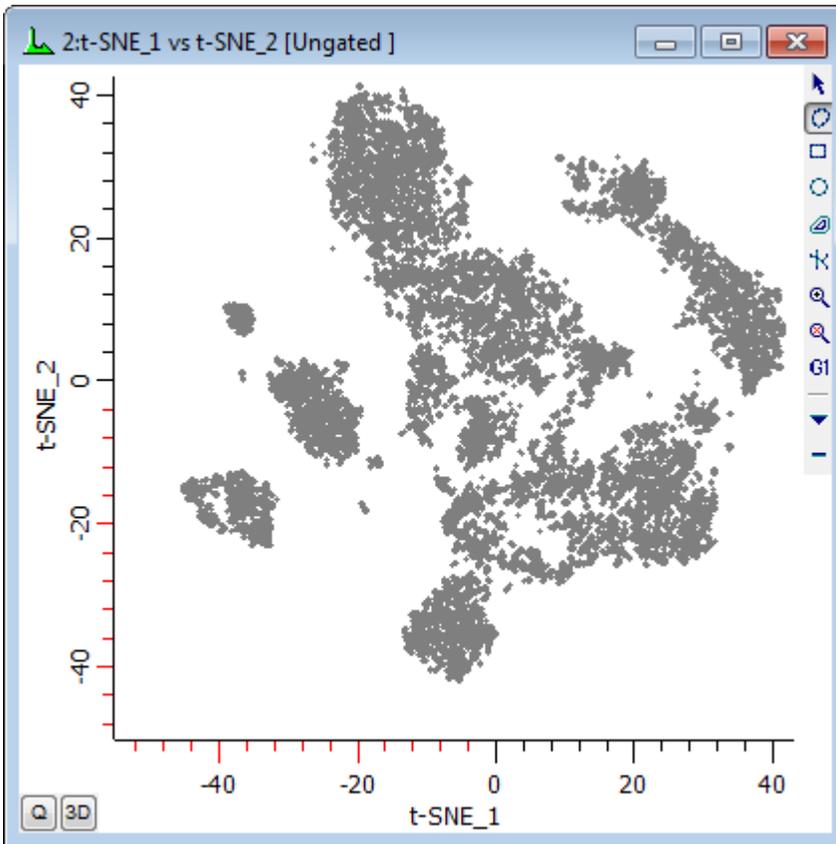
So we can see some distinct groups of events and some continuous distributions, but we have no idea what they mean. How does this help us understand our data? Well, it's always useful to start with something we know. Let's see where the CD3, CD4, and CD8 cells are in this plot.

## Using other plots to understand t-SNE

Our workspace has 4 plots on it: 1P plots for CD3, CD4, and CD8, and a 2P plot for t-SNE. Let's put some regions on the 1P plots to see where the positives are in t-SNE.

13. Draw a region on the CD3 positive events in the 1P plot, and observe where they fall in the t-SNE plot.
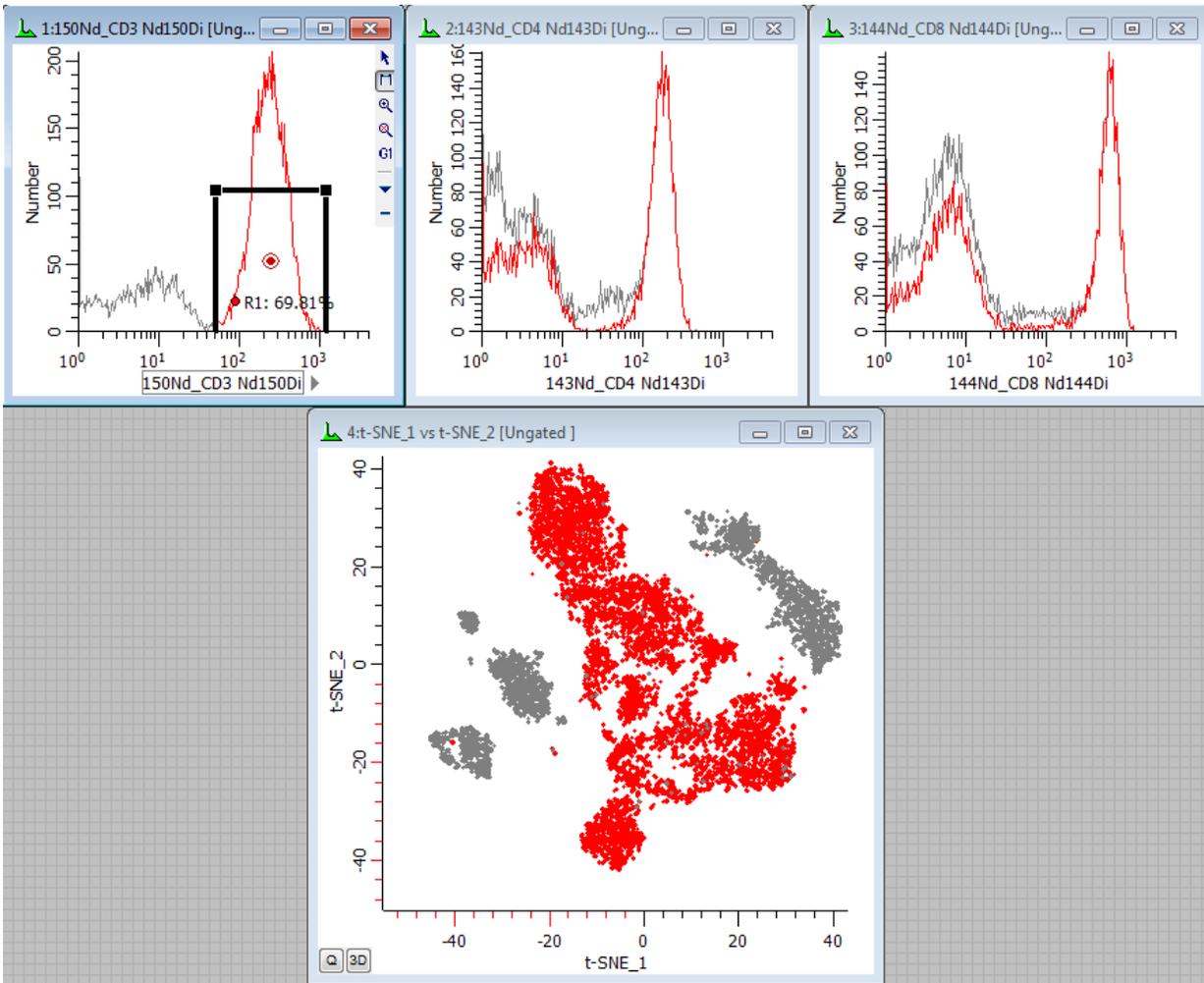
14. Draw a region on the CD4 positive events in the 1P plot and notice where they are in the t-SNE plot.
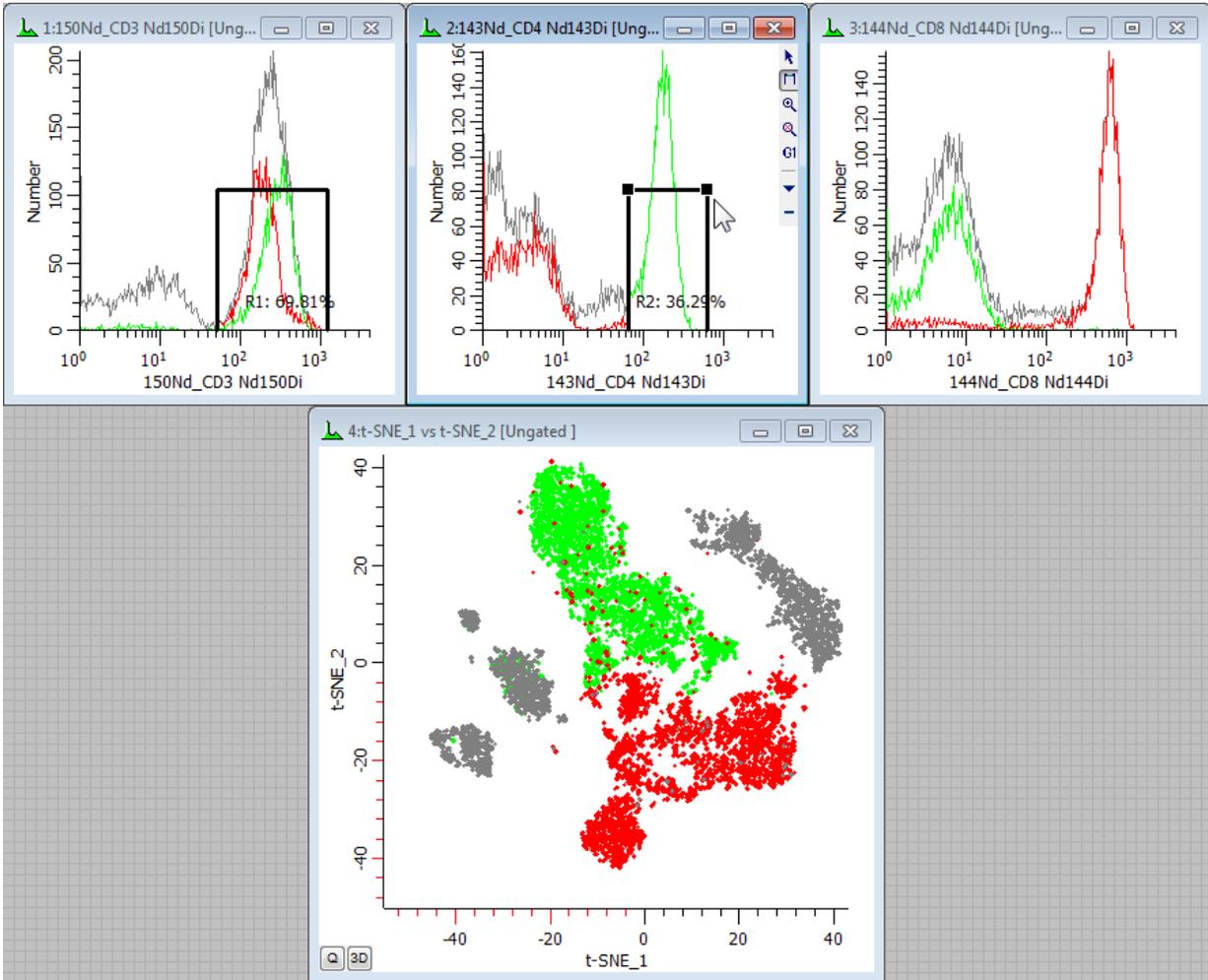
15. Draw region on the CD8 positive events. We expect then to take up most of the remaining portion of the CD3 events in the t-SNE plot.
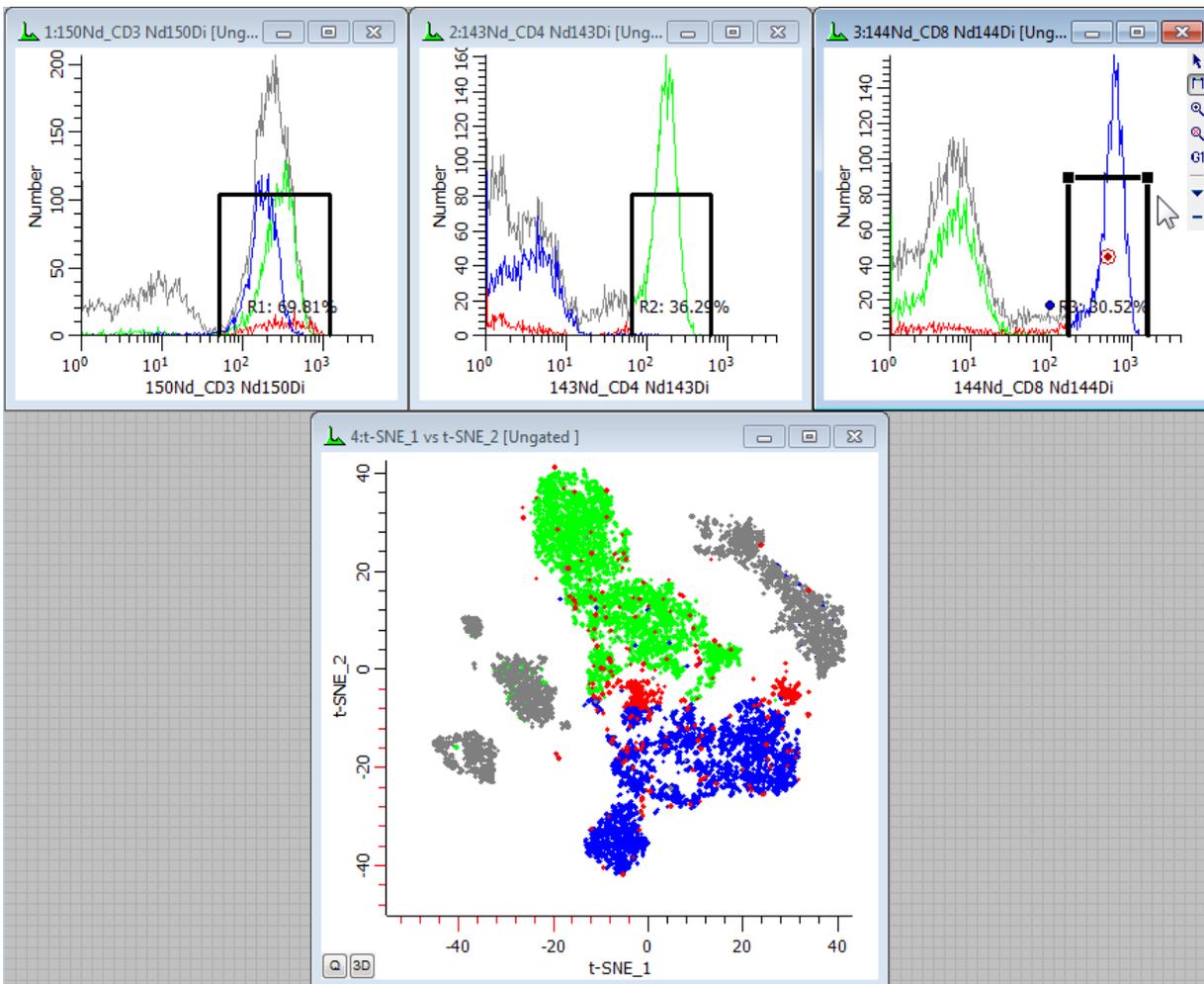
So we can see event coloring (and gating) in the t-SNE plot and figure out what the different clusters of events relate to. Since the t-SNE plot can change shape and location from file to file, this is always a good way to get started. It provides a backbone of information to help relate the t-SNE plot to what we know about our data.

## Using t-SNE to understand other plots

The most exciting part of the t-SNE plot is that it shows us clusters it has detected in multi-dimensional space. Clusters that have related phenotypes are generally near one another in the t-SNE plot. As we saw, the CD4+ and CD8+ events where next to one another at opposite polls of the CD3+ events. There are some CD3+ events that are CD4- and CD8- and they fall between the distributions on the t-SNE plot.

What can we learn from the t-SNE plot if we encircle one of the clusters with a region? Let's find out.

16. On the **Insert** tab of the ribbon, click the **Multiple** button to display the **Create Histograms** dialog. Add 2P histograms for these markers:

> 164Dy_**CD20** vs 142Nd_**CD19**
>
> 162Dy_**CD45RA** vs 175Lu_**HLADR**
>
> 154Sm_**CD14** vs 166Er_**CD33**
>
> 149Sm_**CD16** vs 174Yb_**CD56**
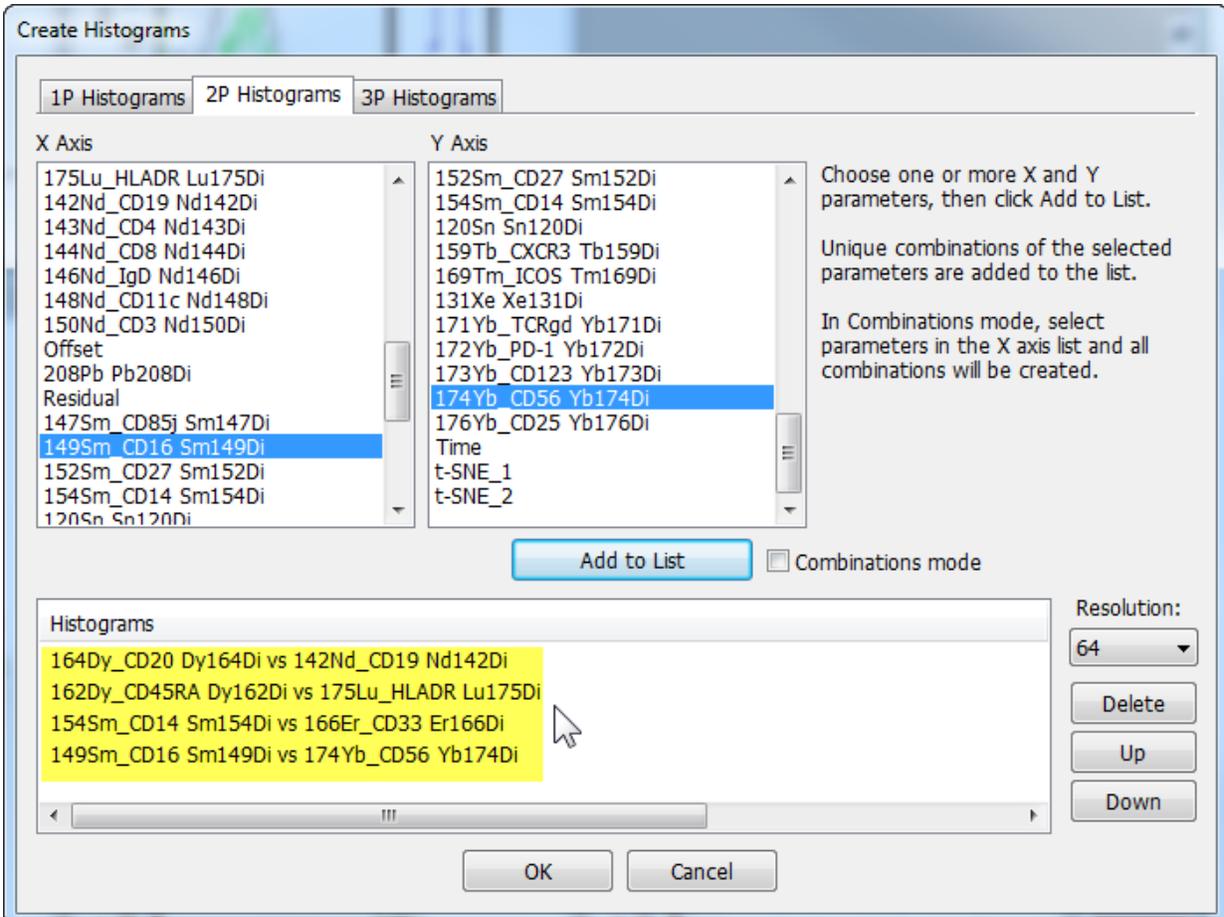


17. Click OK to create the histograms. Arrange the histograms to your liking.

18. Draw a region on the t-SNE plot around the population in the lower left of the plot.
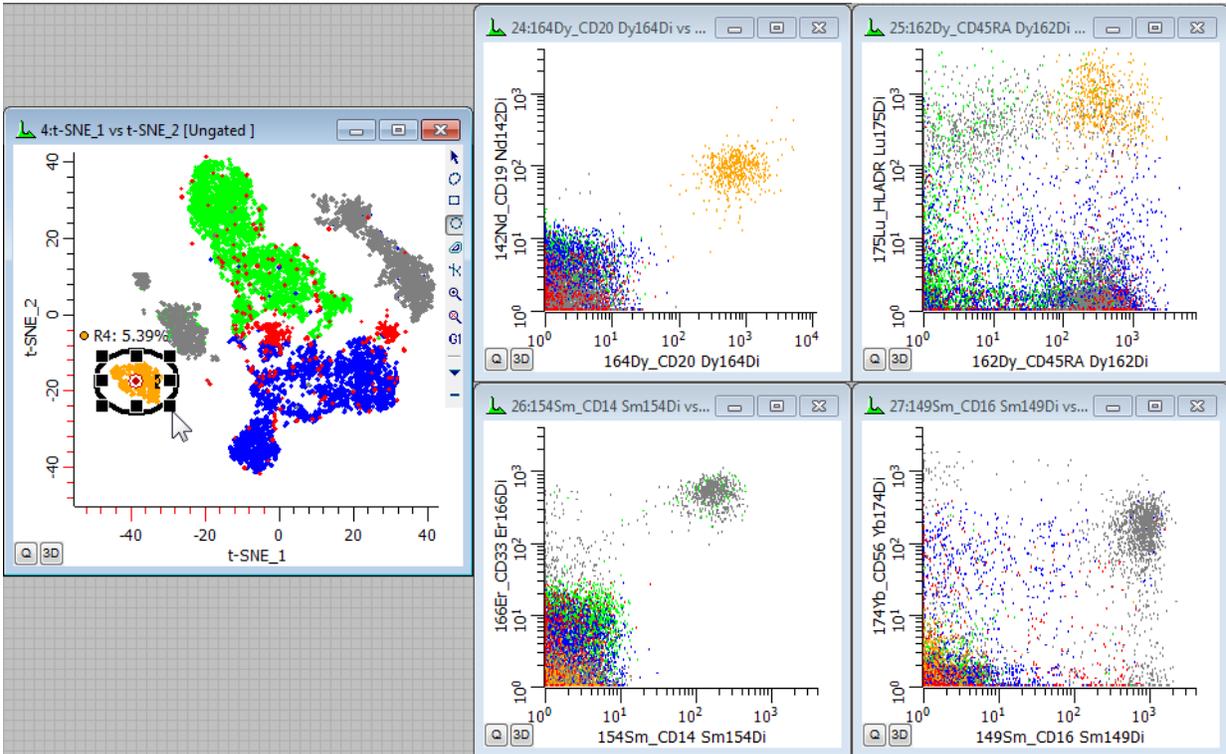
These events light up as CD20+ CD19+ CD45RA+ and HLADR+. They are probably mature B-cells with that phenotype.

19. Move or redraw the region to the population just above this one.
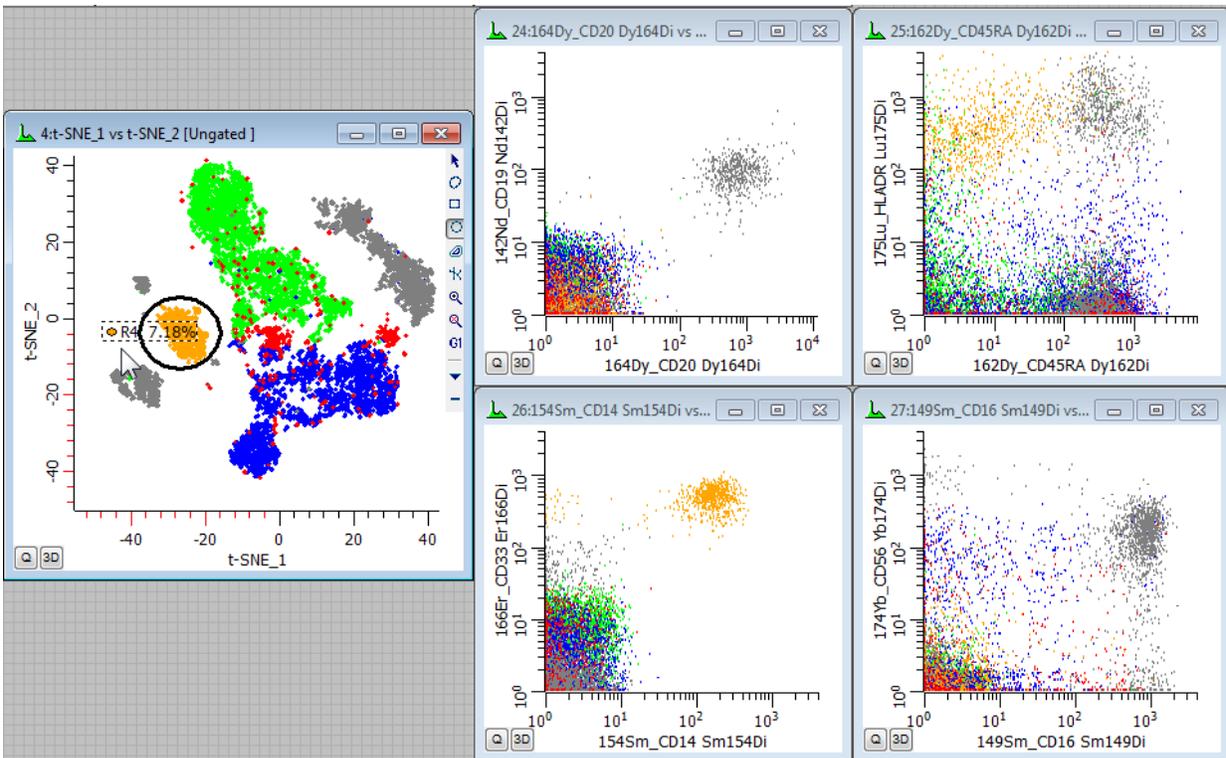
We see the CD14+ CD33+ HLADR+ events light up - making these likely to be monocytes.

20.  Finally, redraw the region around the distribution in the upper right portion of the t-SNE plot.
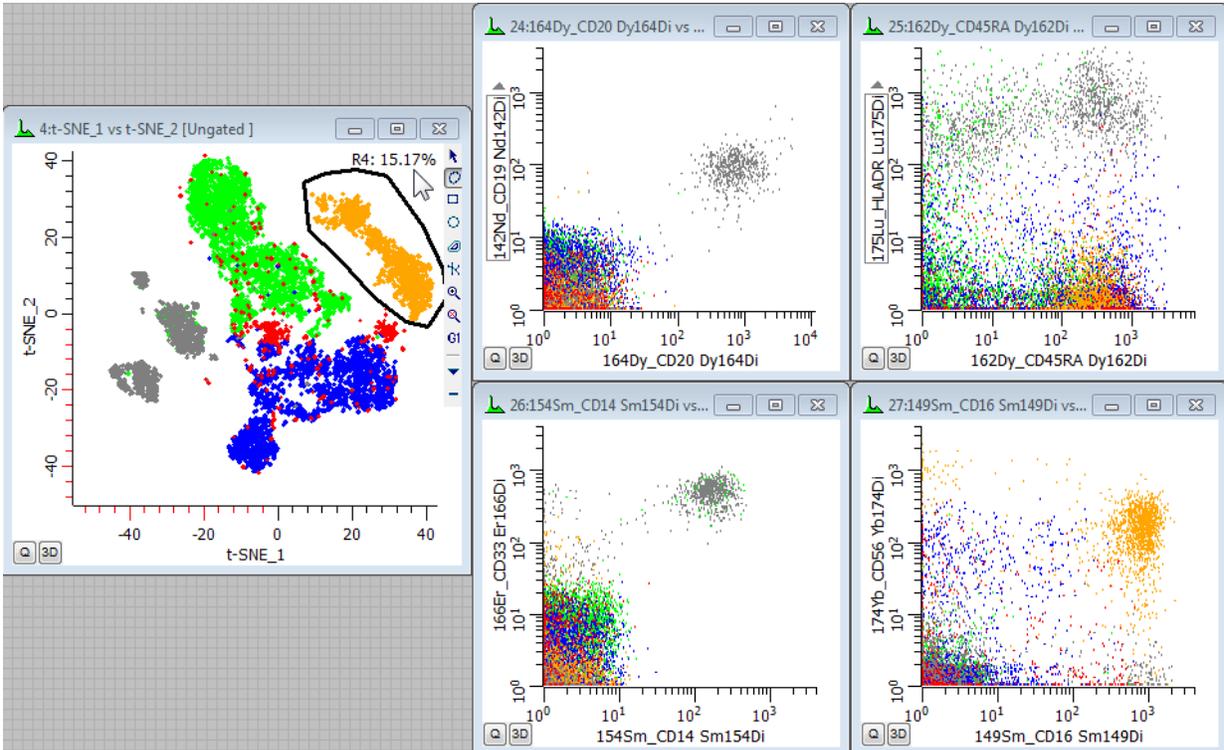
These are probably NK cells, as they are CD16+ CD56+ CD45RA+.

> *If you want to see the region events more dramatically in the other plots, you can right-click the region label and choose* **Highlight events in R4**.

We're only displaying a few histograms in this tutorial to make the point, but you can see the power of the t-SNE function. It is very good at finding related events, clustering them together, and allowing us to see what they have in common by looking at the markers in our sample. As you probe the islands of events in the t-SNE plot and see where they fall in other plots, you'll find some surprises and confirm some things that you already know.

## Summary

WinList includes a powerful function called t-SNE that takes high-dimensional data and flattens it into 2- or 3-dimension space so that it can be visualized in conventional 2P plots. The FSNE calculated parameter is WinList's implementation of t-SNE, creating these additional dimensions. Once the t-SNE function has completed, you can display it as a 2P plot, apply gates to it, and create regions on it to explore your data.

**Sticky Regions and Magic Contours**

You can probably think of cases where you wanted to have a set of regions sharing common vertices so that they fit perfectly together.  It is difficult to create regions that do that, especially if you also want to be able to make adjustments.  WinList simplifies this task with its ability to create "sticky regions" quickly and easily.  It allows you to stick regions together, and just as easily to unstick them.

Similarly, there are times when you want a region to adjust its size and shape based on the data beneath it.  For example, as you read a new file into an analysis setup, it would be really helpful if the lymphocyte gate would auto-adjust to the new distribution.  The "magic contour" region in WinList does exactly this.

This short tutorial will introduce these new region capabilities so that you can add them into your own analysis protocols.

*If you are unfamiliar with opening files and creating histograms with WinList, you might want to do the WinList Basics tutorial before continuing with this one.*

**Open the FCS file**

1. Click the **Open FCS File** button on the ribbon bar.
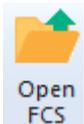


2. In the File Open dialog, navigate to the Samples folder located in the folder where WinList is installed. Select the **BCell_BM_Tube_001.fcs** and click **Open**.

3. In the **Create Histograms** dialog, add **FSC-A vs SSC-A** and **CD38 vs CD10** histograms and click **OK**.

**Create a contour region**

4. Click the title bar of the FSC vs SSC plot to activate it, the click the **Contour region** tool in the histogram toolbar.
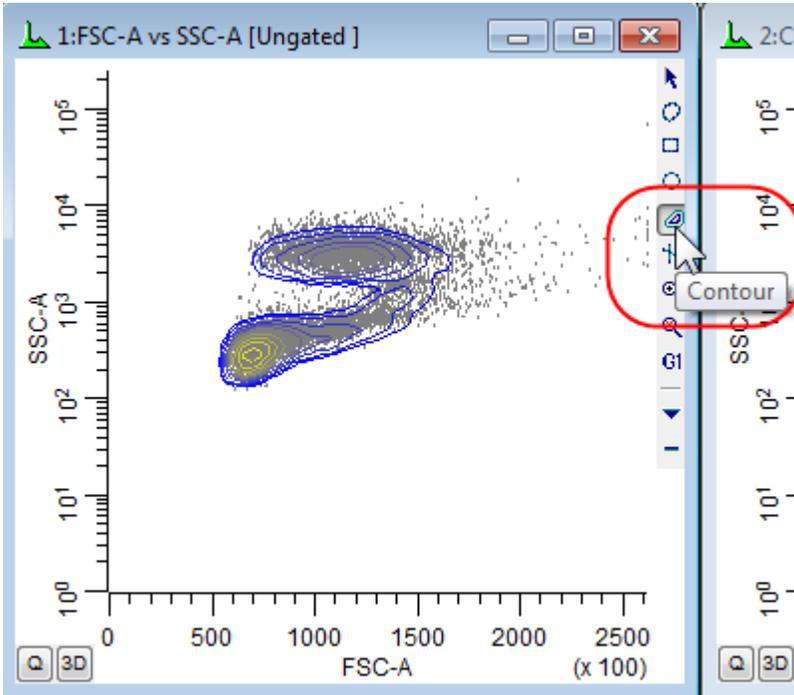
5. Click and drag to draw a box around the low SSC-A events.  When you release the mouse, a contour region will be created from the data within the box you drew.
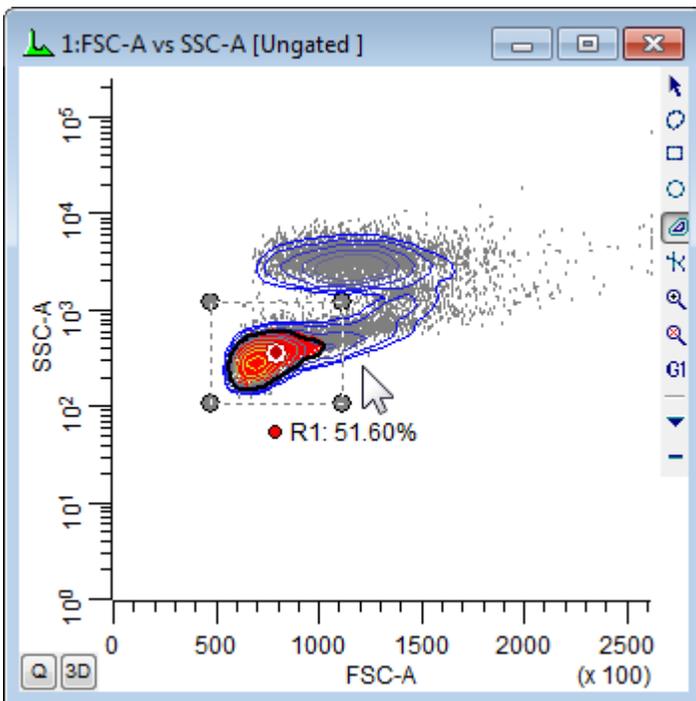
If you move the region (by dragging the label), the contour region will recompute on the data in the new location.  Sometimes the resulting contour does not change because the bounding box contains the same probability distribution.  For example, in the illustration above, moving one of the left handles to the left would not change the contour.

6. Right-click the region label and choose **Edit Properties**. The properties that relate to the contour region are grouped together.  Edit the **Contour Probability** to be **0.95** and click OK.

| Name | Current Value | Description |
|------|--------------|-------------|
| Show Name | ☑ Enable | Show the region name in the label. |
| Show Alias | ☐ Enable | Show the region alias in the label. |
| Alias | R1 | Edit the alias for the region. |
| Show statistic | Percent gated ▾ | Choose a statistic to display in the label. |
| Line color | ▓ Color... | Click to change color |
| Line weight | 0 ▾ | Line weight for regions. |
| Allow delete | ☑ Enable | Allow or prevent the region from being deleted. |
| **Contour Region** | | |
| Contour Probability | 0.95 | The probability level of the contour region. (0.01 to 0.9 |
| Smoothing | 3 ▾ | Smoothing for the contour. |
| Show resizing handles | ☑ Enable | Display resizing handles when region is active. |
| **Auto-positioning** | | |
| Auto-position | ☐ Enable | Automatically move region with data. |
| Auto-position method | Center on region median ▾ | Choose to method to auto-position. |
| X Percent | 10.00 | Enter the X percent to include. (1.00 to 99.00) |
| Y Percent | 10.00 | Enter the Y percent to include. (1.00 to 99.00) |

The contour on the histogram will get a little bigger as a result of the edit, encompassing 95% of the events inside the contour region's bounding box.

## Create sticky regions

4. Click the title bar of the CD38 vs CD10 plot to activate it, the click the **Freehand region** tool in the histogram toolbar.
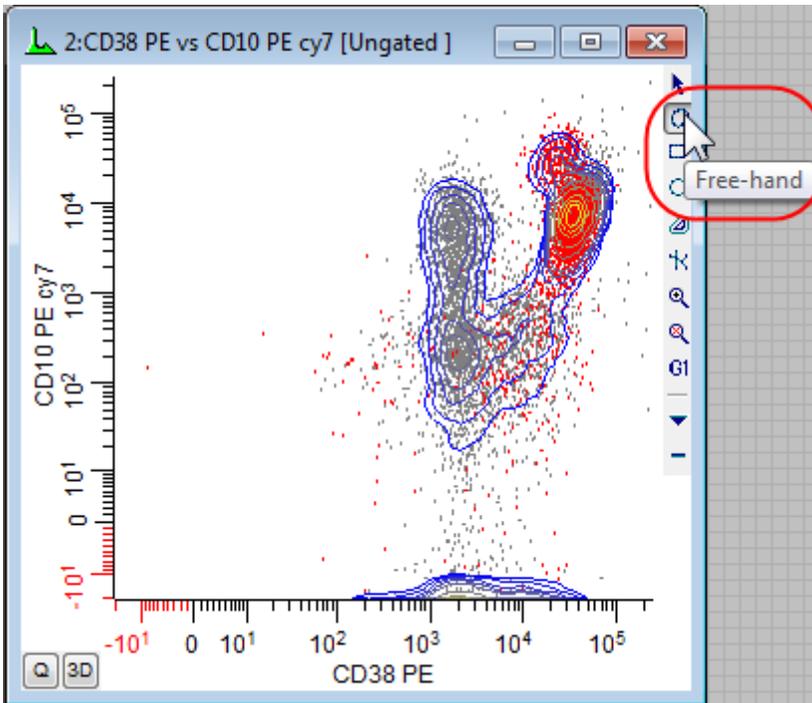
Let's create 3 adjacent regions in this plot using the point and click technique.

5. Click and hold the mouse for a second next to the CD10+ CD38- population, then release the mouse button.  Move and click the mouse to define 6 or 7 points to surround the population, clicking back on the first point to close region R2.

6. Right click the region label and choose **Show Vertices**.
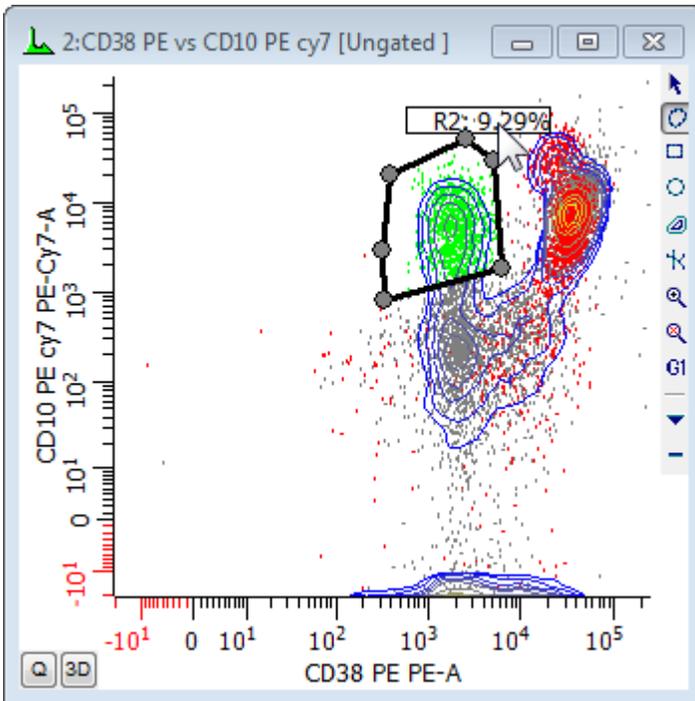
7. Click and hold the mouse for a second next to the CD10- CD38 dim population, then release the mouse button.  Move and click the mouse to define points in the region, and make sure to click on top of the vertices at the bottom of R2.  Click on the first point of R3 to finish the region.


8. Right click the region label and choose **Show Vertices**.
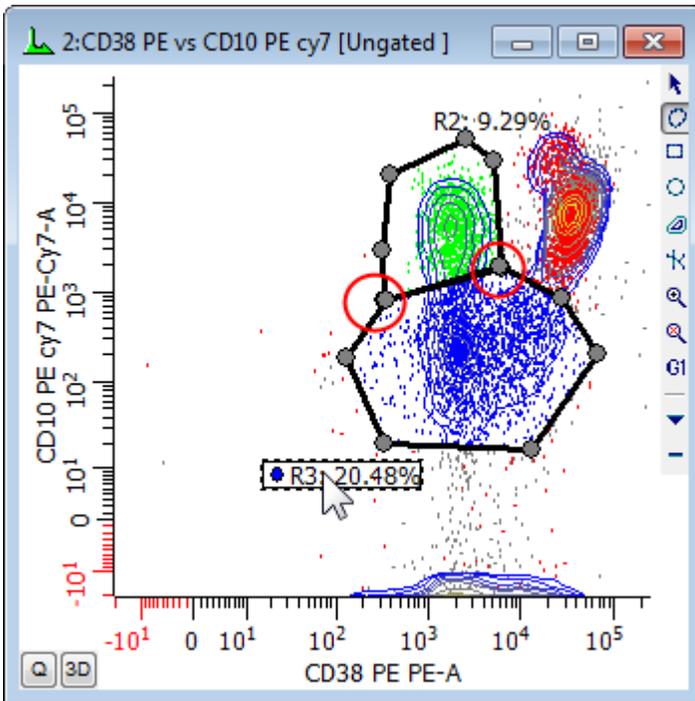
9. Click and hold the mouse for a second next to the CD101 CD38+ population, then release the mouse button.  Move and click the mouse to define points in the region, and make sure to click on top of the vertices at the top of R3.  Click on the first point of R4 to finish the region.

10. Right click the region label and choose **Show Vertices**.
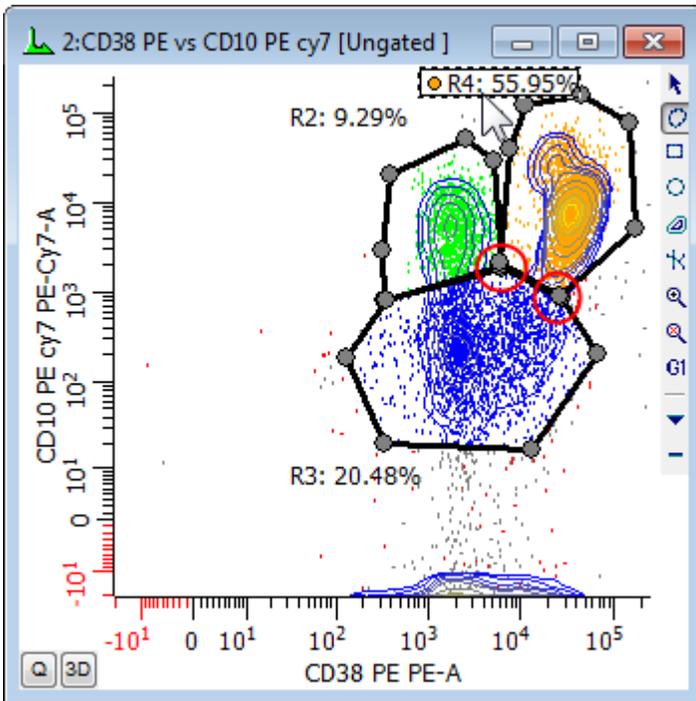
So at this point, we have a set of adjacent regions that are completely independent of one another. If you move a region or common vertex, the other regions will be unaffected. We're about to change that.

## Make it "sticky"

11. Right click the **R2** region label and choose **Stick to Neighbor Regions**.
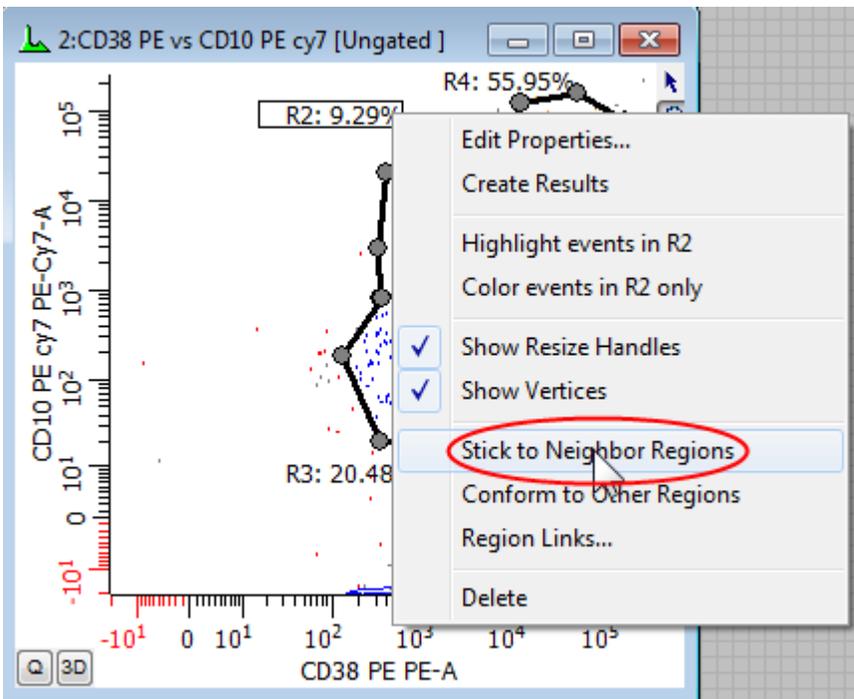
12. Repeat this for regions **R3** and **R4**.

Now your regions are dependent on one another.  The common vertices are truly common: when you move a common vertex, all regions that share the vertex will be affected.  Try it out.

176
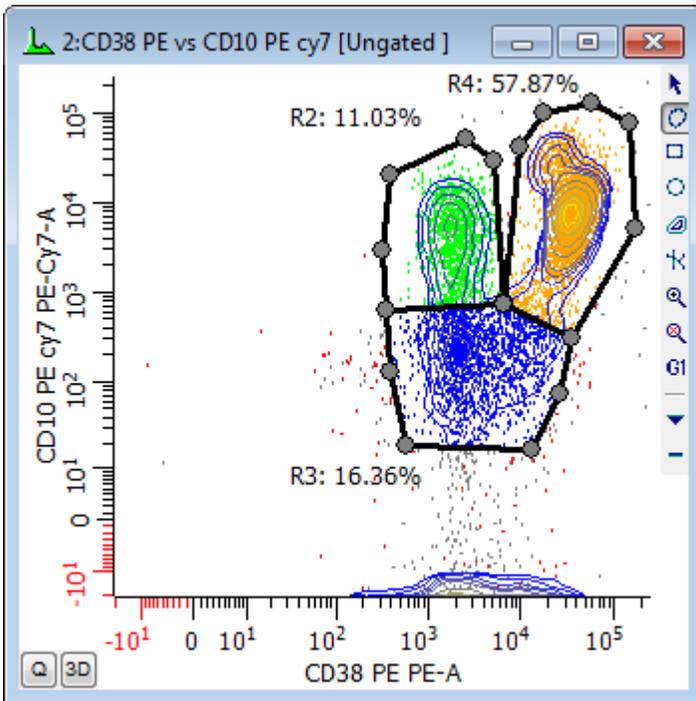
In fact, if you move one of these regions (by the region label), the regions sharing vertices will be pulled to stay connected. This is a great tool for making sure that you single-count events and maintain adjacency even as you make adjustments to the regions.

## Summary

This tutorial introduced the contour region and sticky regions.  Contour regions are dynamic: they recompute the region whenever the data beneath them changes.  They are a perfect tool for parts of your analysis where you want to reduce subjectivity in region placement and size, because they are computed based on probability.

Sticky regions make it possible to create a set of adjacent regions that maintain adjacency even if you move one region, or a vertex of a region.

**Kinetic Analysis with Excel**

This tutorial will introduce kinetic analysis of calcium flux experiments, using WinList and Microsoft Excel. The first section of the tutorial shows how this can be done manually by copying results from WinList into a special Excel worksheet designed for kinetics. At the end of the tutorial, we will show how the process can be automated with a WinList macro and an Excel Visual Basic script.

In order to perform this tutorial, you need WinList and Microsoft Excel installed on your computer. You will also need sample files that can be found in WinList's Samples\Kinetics folder.

## Set up Excel

1. Start Microsoft Excel.

2. From Excel's file menu, choose **Open**. Navigate to the folder in which WinList is installed, and into the Samples\Kinetics folder. Select the file **WinListKinetics.xls**.

3. Click the **Open** button.
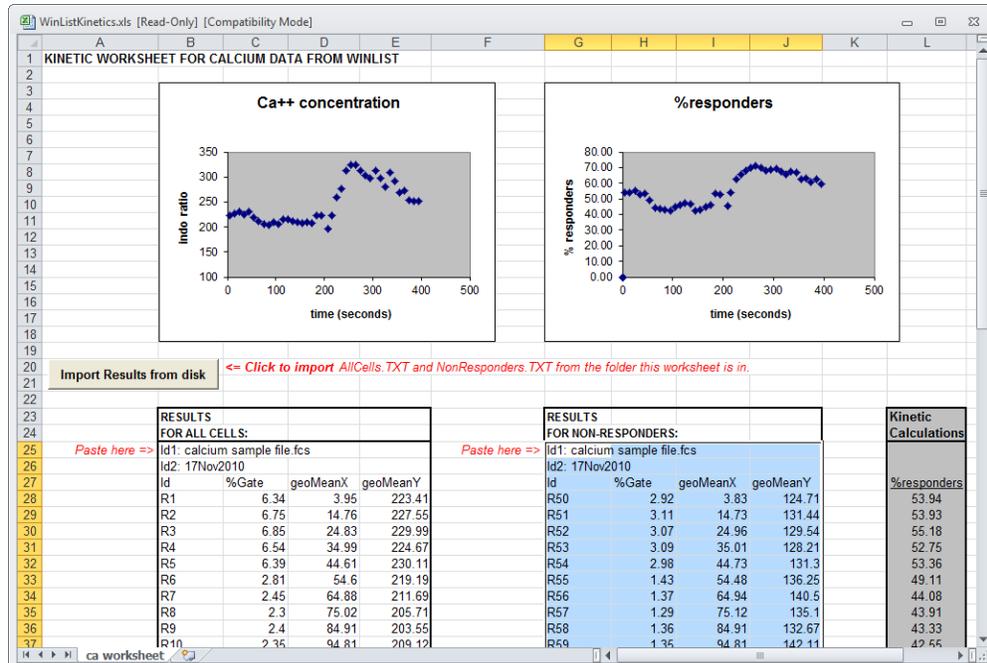


*You may need to adjust the macro security settings in Excel to enable macros in this worksheet. Talk with your IT specialist to determine if this is appropriate for your institution.*

This worksheet contains two charts, one showing calcium concentration for all cells, and a second showing the calcium response of responder cells. The charts plot data from two sets of results pasted from WinList. You will be pasting new results into this worksheet to update the charts with your analysis of a sample file.

## Set up WinList

4. Start WinList, and click **Open FCS** on the ribbon bar. The Open FCS File dialog is displayed.

5. Navigate to the folder in which WinList is installed, and into the Samples\Kinetics folder. Select the file **calcium sample file.fcs**, and click the **Open** button.

The **Create Histograms** dialog is displayed. We want to create *two* copies of **Time** vs. **F32-Ratio** at a resolution of 1024 channels.

6. Set the Resolution dropdown list to **1024**.

7. Select **Time** in the X Axis list, **F32-Ratio** in the Y Axis list, and then click **Add to List** *two times*. You should see two entries in the **Added Histograms** list. Click **OK** to close the dialog and display the histograms.
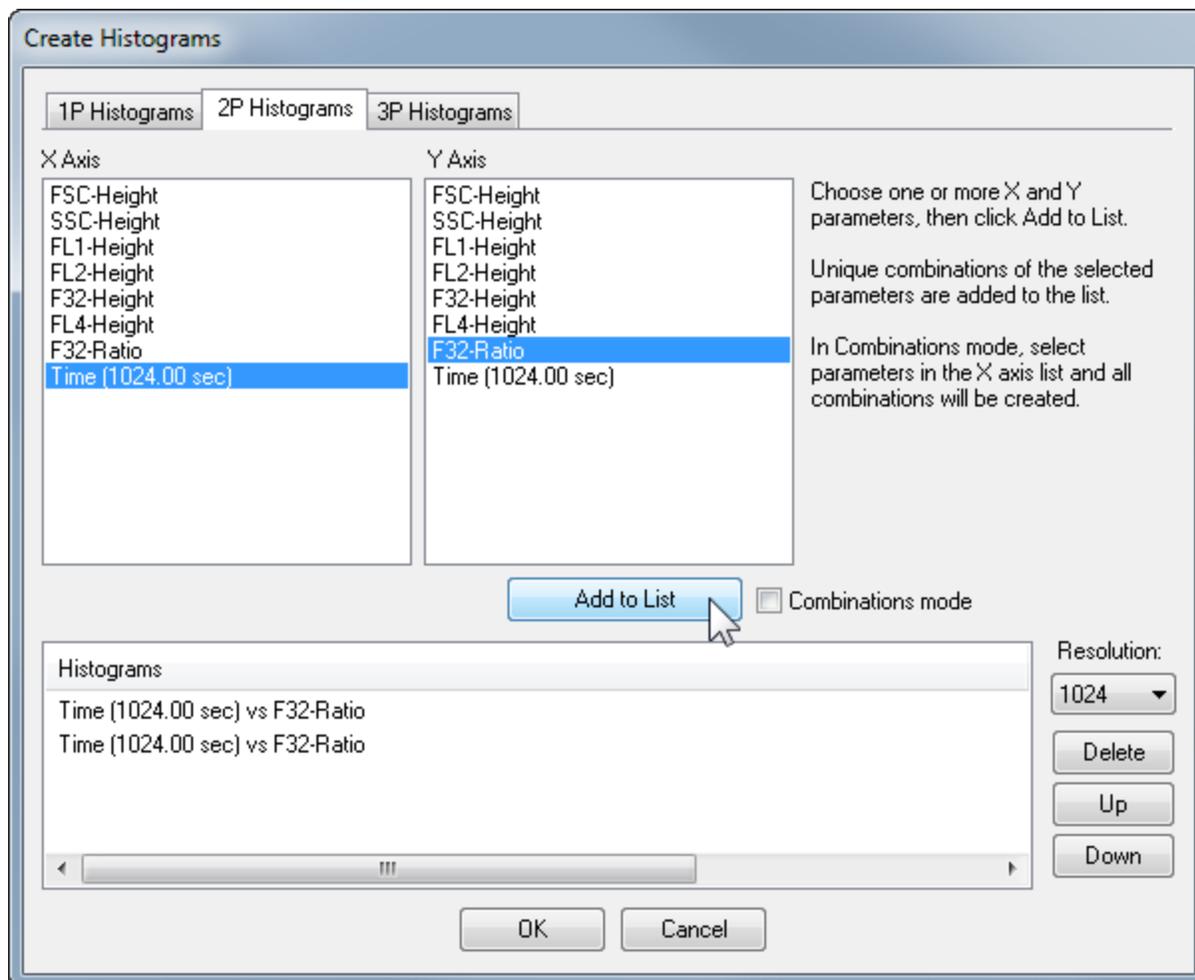
We need to display all dots, and turn off contours if they are enabled.

8. Right-click the mouse on one of the histograms.

9. From the popup menu, choose **Graphics**. Select **Dots** in the tree on the left and make sure Dots are enabled. Check the **Show all dots** option. Select **Contours** in the tree and make sure that the Contours property is disabled. Scroll to the bottom of the property list and check **Apply to All**. Click **OK** to apply the changes to both histograms.

## Create regions and results

Next, we need to create a set of regions on each histogram to provide statistics on the kinetics. In the histogram on the left, we will gather statistics about all cells, and in the right histogram, we will look at statistics for a subset of cells. We will use the Create Region Array option to simplify the process of creating 48 adjacent regions on each histogram.

10. Click the title bar of the histogram on the left to activate it. From the **Insert** tab on the ribbon bar, click **Region Array**.

We want to create 48 regions on the X-axis, each one being 10 channels wide.

11. In the **On X-Axis** group, enter 48 for the **Number of regions**. Enter 480 in the **Ending at** edit box. Click **OK**.



After a moment or two, a results window containing results for the 48 regions will appear. We need to choose the statistics to display.

12. Right-click on the results window and choose **Edit** from the context menu. Enable the following options, and *disable all others*:

    *ID Field1*

    *ID Field2*

    *Percent Gated*

    *MeanX*

    *MeanY*

    *Fields in Columns*

Click **OK** to close the dialog.

> *You can set the preferences for the results that you want in a new results window by clicking the Save As Preference button in the Edit Results dialog.*

Next, we will repeat the process for the second histogram. This time, however, we will create slightly different regions.

13. Click the title bar of the histogram on the *right* to activate it. From the Insert tab on the ribbon bar, click **Region Array**.

14. In the **On X-Axis** group, enter 48 for the **Number of regions**. Enter 480 in the **Ending at** edit box. In the **On Y-Axis** group, enter 250 for the **Ending at** edit box. These regions will capture the non-responders. Click **OK**.



A second Results window will appear. Once again, we need to choose the statistics to display.

15. Right-click on the new Results window and choose **Edit**. Enable the following options again, and disable all others:

*ID Field1*

*ID Field2*

*Percent Gated*

*MeanX*

*MeanY*

*Fields in Columns*

Click **OK** to close the dialog.

WinList is all set and ready to go. If you want to keep this setup as a protocol, use the Save Protocol option from the File menu to do so.

### Copying results into Excel

We need to update the statistics for the entire file, and then copy the two sets of results into the Excel worksheet.

16. Click the **Replay** button on the Home tab of the ribbon bar.

17. Right click inside the first Results window. Choose **Copy**.

The first set of results is now in the clipboard, available to Excel. We need to paste these results into the correct cells in the Excel worksheet.

18. Activate Microsoft Excel. Select cell **B25**. Choose **Paste** from Excel's Edit menu.



Now, we repeat the process for the second set of results.

19. Activate WinList. Right click inside the second Results window. Choose **Copy**.

The *second* set of results is now in the clipboard, ready to paste into Excel.

20. Activate Microsoft Excel. Select cell **G25**. Choose **Paste** from Excel's Edit menu.
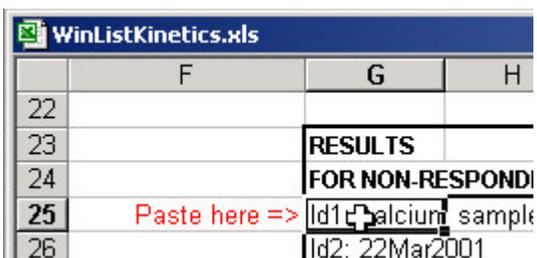
The Excel worksheet now displays two charts of the results you have just pasted into it. The chart on the left shows the Calcium concentration for all cells, and the chart on the right is for the "responder" cells. Notice that there is also a column of numbers that displays the percentage of responders for each time slice.

## Automating the process

Since WinList and Excel both have automation capabilities, you can streamline the process of getting results into Excel. The best way to do this is with a Remote connection between each Result window and the Excel worksheet.

21. Activate WinList, right-click the first Results window, and choose **Edit**.

22. In the Edit Results dialog, enable the **Remote** checkbox and then click **Edit Remote**.
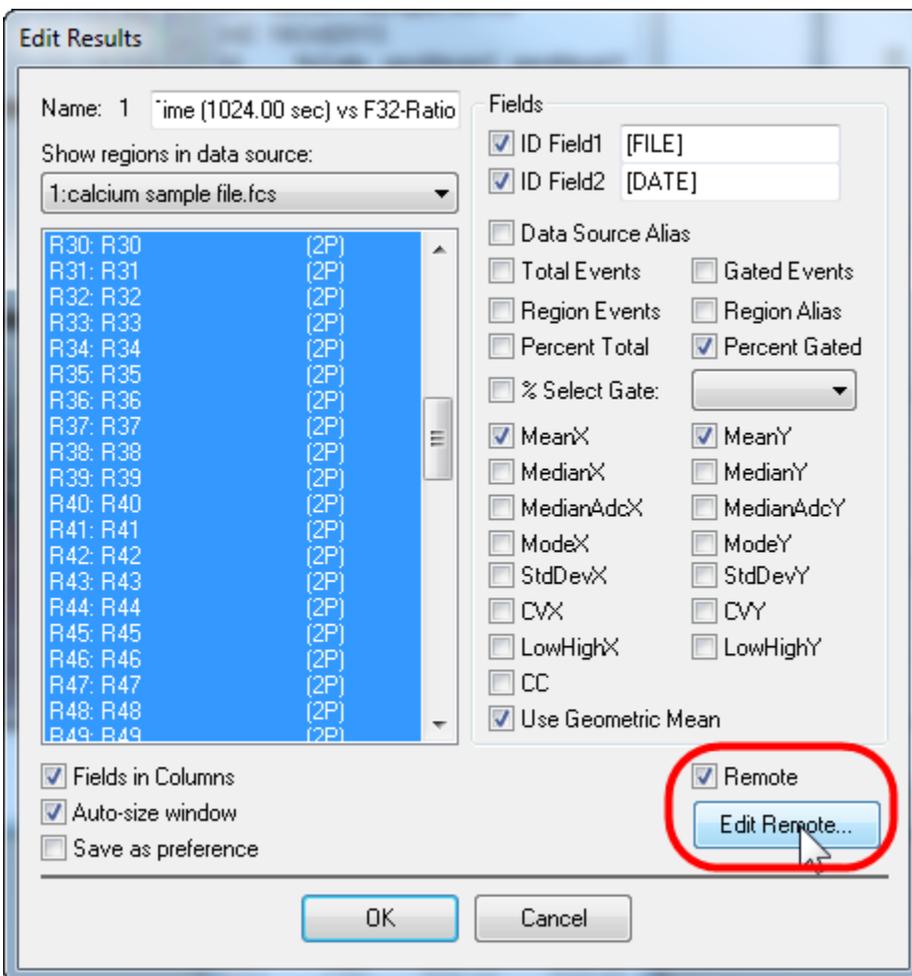
23. The Edit Remote Link dialog is displayed.  Select "Excel" in the **Link To** list, and then type "ca worksheet" for the **Topic**.
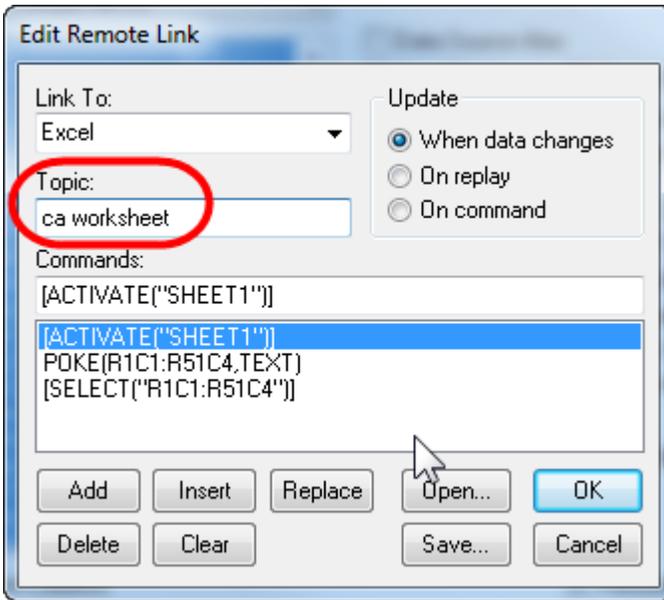
24. Next, select the *first* command in the Commands list, edit "SHEET1" to "ca worksheet", and then click the Replace button.

25. Select the *second* command in the Commands list, edit the text to "POKE(R25C2:R76C5,TEXT)", and then click the Replace button.

26. Select the last command and click the Delete button.
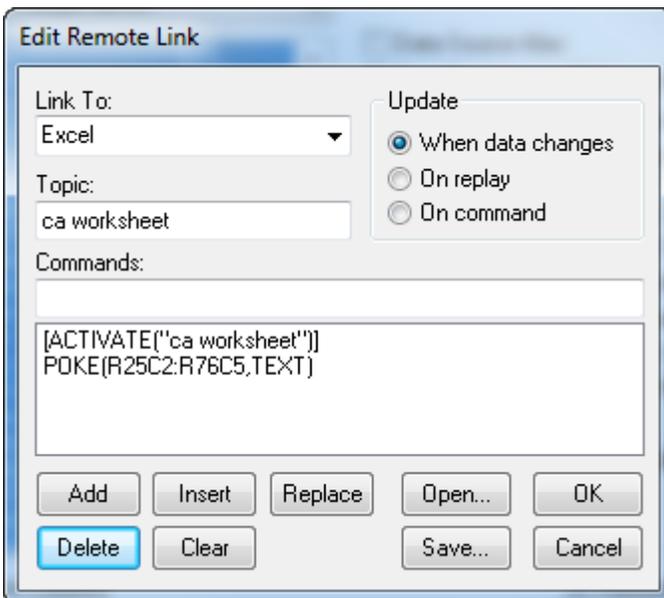
We have edited the script so that any time our data changes, WinList will send the new results to the correct location on the Excel spreadsheet.  Let's make a similar connection for the second Results window.

27. Right-click the *second* Results window and choose Edit.  Enable the **Remote** checkbox, and click **Edit Remote**.

28. Edit the Topic to "ca worksheet" and revise the script commands as shown.  Note the text of the POKE command is different than the previous script: "POKE(R25C7:R76C10,TEXT)".
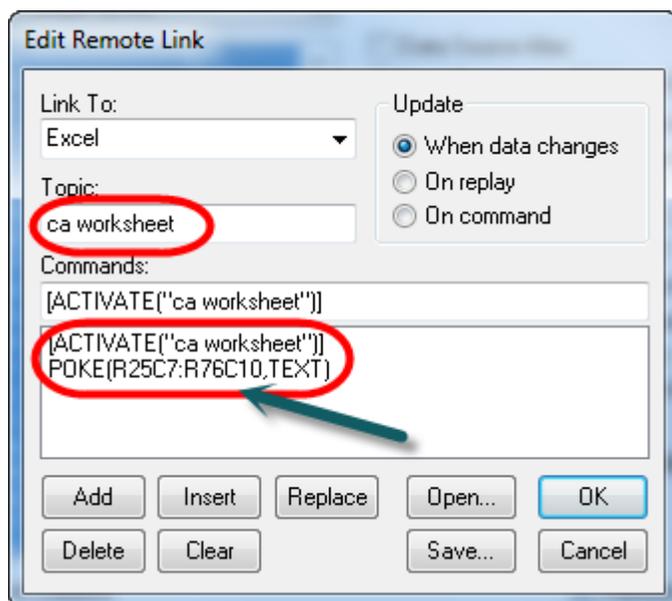


29. Click OK to close the dialog, and click OK again to close the Edit Results dialog.

You now have a fully automated connection between Excel and WinList for both of the Result windows.  Any time you load a new file or change a gate, WinList will send the new Results to Excel using the scripts we just created.  If you save your protocol, the remote scripts will be stored and connections will be made automatically when the protocol is re-opened.

## Summary and discussion

This tutorial introduced kinetic analysis using WinList and Microsoft Excel. We constructed a protocol in WinList that extracted statistics from 48 time slice regions in each of two histograms. We opened an Excel spreadsheet that is supplied with WinList, and then pasted two sets of results into the worksheet.

We streamlined the process of copying and pasting results by creating Remote connections between the Result windows and Excel.

**Enhanced Proliferation Analysis**

Features in ModFit LT and WinList provide unparalleled capabilities to analyze cell proliferation.

ModFit LT performs sophisticated least squares analysis in just a few clicks of a mouse and reveals the secrets of the proliferation data: proliferation index, non-proliferative fraction, and precursor frequency, and fractions for each proliferating generation. The proliferation results can be exported so that other applications can extend the analysis.

Enter WinList. NStat regions can import the proliferation results from ModFit LT, and then partition the generations according to the proliferation analysis. This enhancement sets the stage for exciting new analysis capabilities.

This tutorial will explore these new features to examine a CFSE-stained sample containing several other markers.

## The big picture

From the Proliferation Wizard in ModFit LT…
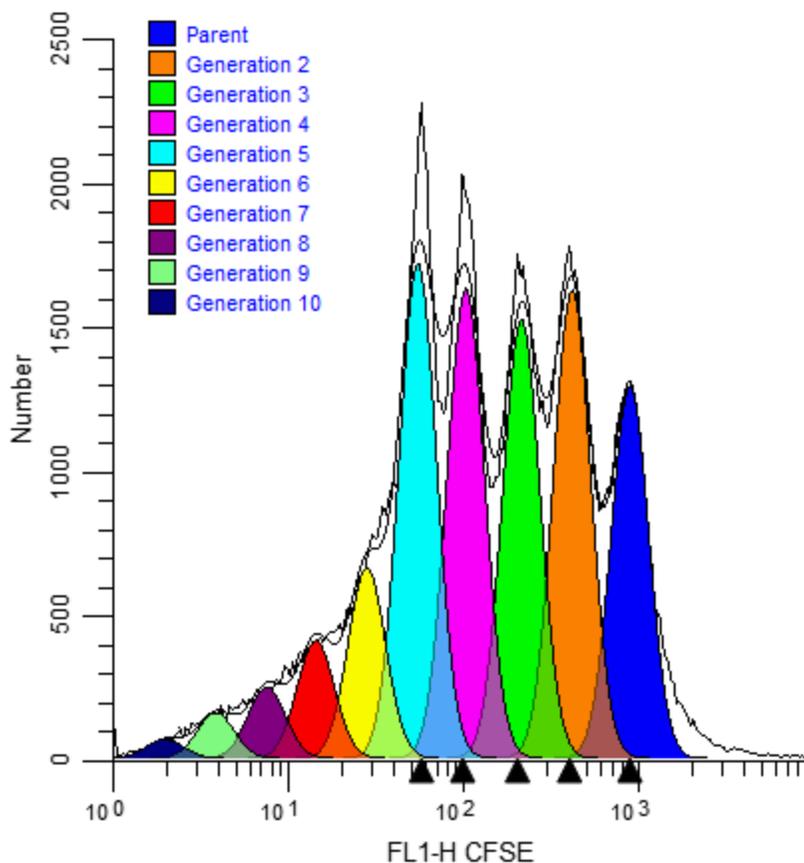
... into WinList for exciting, new explorations of proliferation.
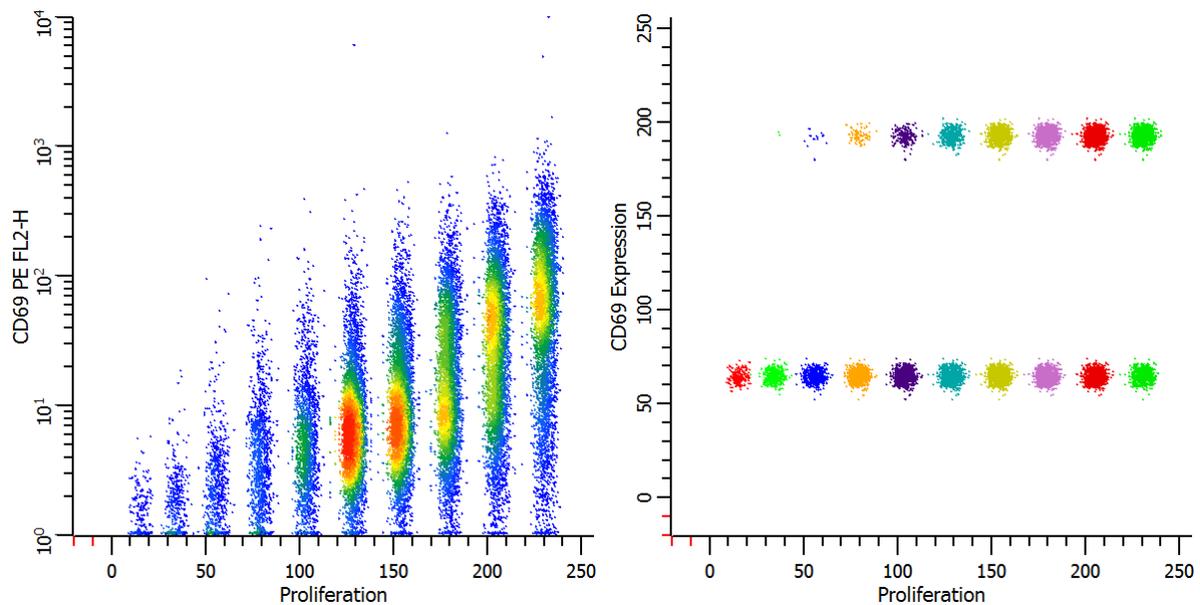


## Getting started

To start, we will use the Proliferation Wizard in ModFit LT and export its results. Then, you will set up WinList to import those results into an NStat region.

*You will need both ModFit LT and WinList for this tutorial. ModFit LT must be at least version 3.2.1.*

1. Launch WinList and pass through the opening dialogs.

2. Launch ModFit LT and pass through the opening dialogs.

3. Using ModFit LT's **Open Data** command, navigate to the Samples folder located in the *WinList* application folder. Select "CFSE sample.fcs" and click **Open**. Note, the dialog may look different than the one shown below, depending on the version of ModFit LT you are using.
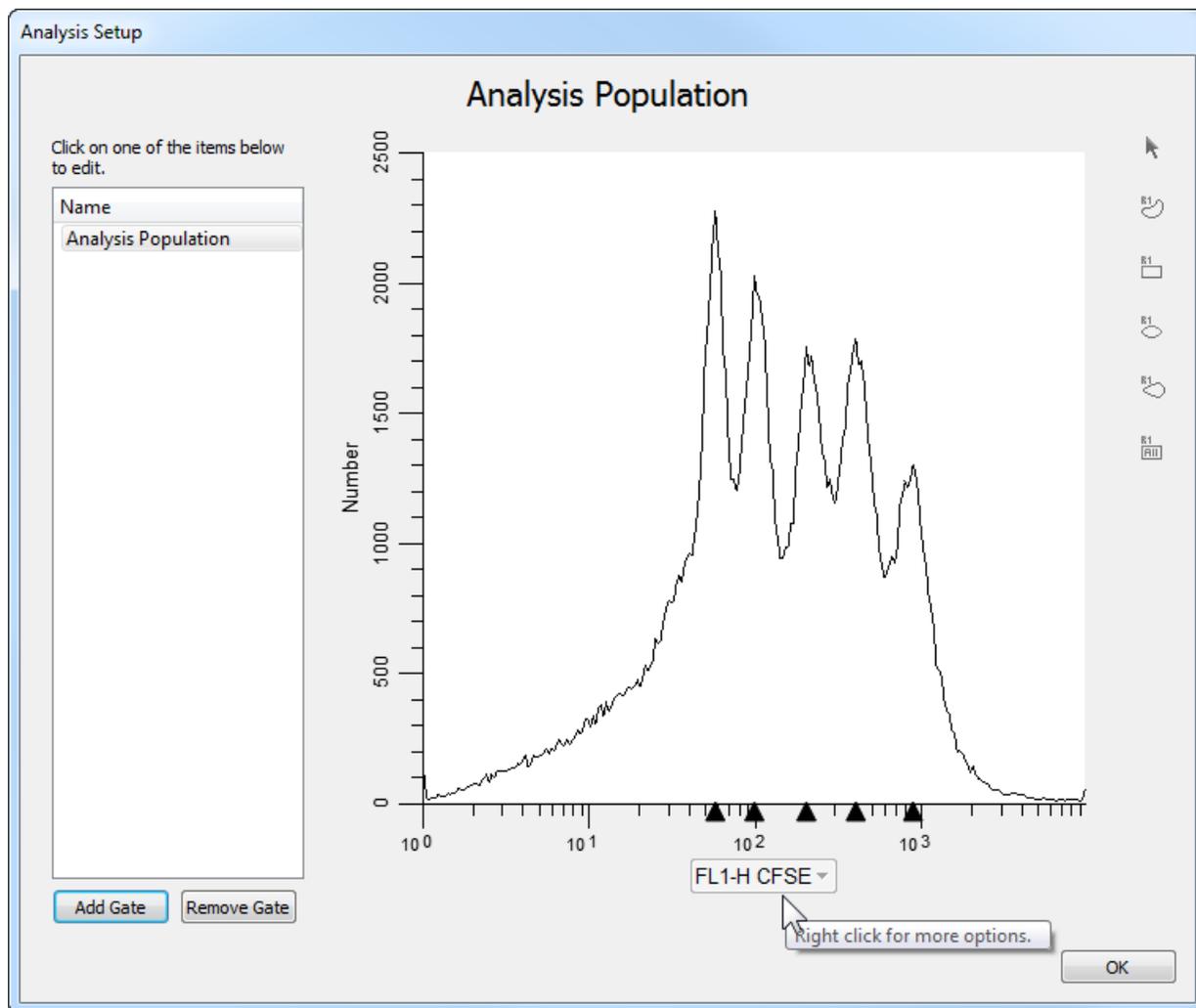
4. Choose "FL1-H-CFSE" as the analysis parameter, and click **OK**.

5. Do not create any gates for this file; we'll do that later in WinList.

6. From the Analysis menu, choose **Cell Tracking Wizard**, and then **Create or edit model**.

7. In the Proliferation Wizard dialog, click the **Generations** tab and choose the "Floating" model option from the drop-down list. Click the **Other** tab, and enable **Export partition table** and **Prompt for file name** checkboxes. These options will cause the program to create the export file after analysis and allow you to enter a name for the export file. Finally, click the **Analyze** button to perform the analysis.
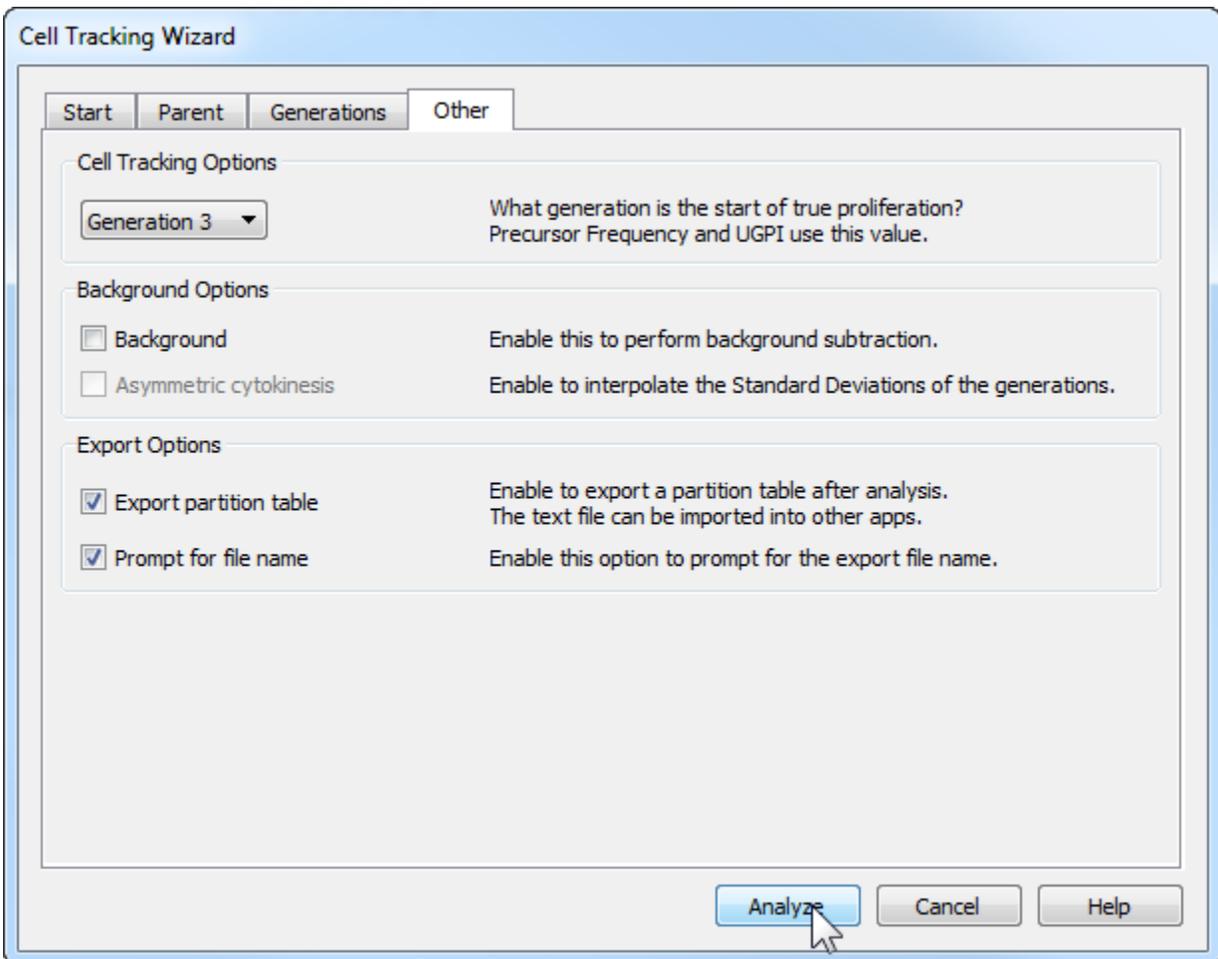
8. When the analysis is complete, the Choose Location to Save Partitions dialog will be displayed. You can save the file to any location. For this tutorial, save the export file to the desktop so that it's easy to find later.

*When you choose the Export option for the Cell Tracking Wizard, the program determines the boundaries between adjacent generations and writes that information to the export file. The boundaries are the points of intersection between the Gaussian components in the model.*

---

```
Cell Tracking Wizard Partitions
File    CFSE sample.fcs
Regions 10
Scale   0          256
Gen10   0.00       25.34
Gen9    25.34      45.03
Gen8    45.03      63.13
Gen7    63.13      81.51
Gen6    81.51      98.97
Gen5    98.97      119.54
Gen4    119.54     138.43
Gen3    138.43     157.50
Gen2    157.50     177.95
Parent  177.95     256.00
```

## Setting up WinList

9. Activate WinList and click the **Open FCS** button on the ribbon bar.

10. Navigate to the Samples folder located in the WinList application folder. Select the "CFSE sample.fcs" file and click **Open**.

11. In the **Create Histograms** dialog, select **CFSE** in the X Axis list and **CD69 PE** in the Y Axis list. Click **Add to List** *two times*. This will create two copies of CFSE vs. CD69 PE. Click **OK** to close the dialog.
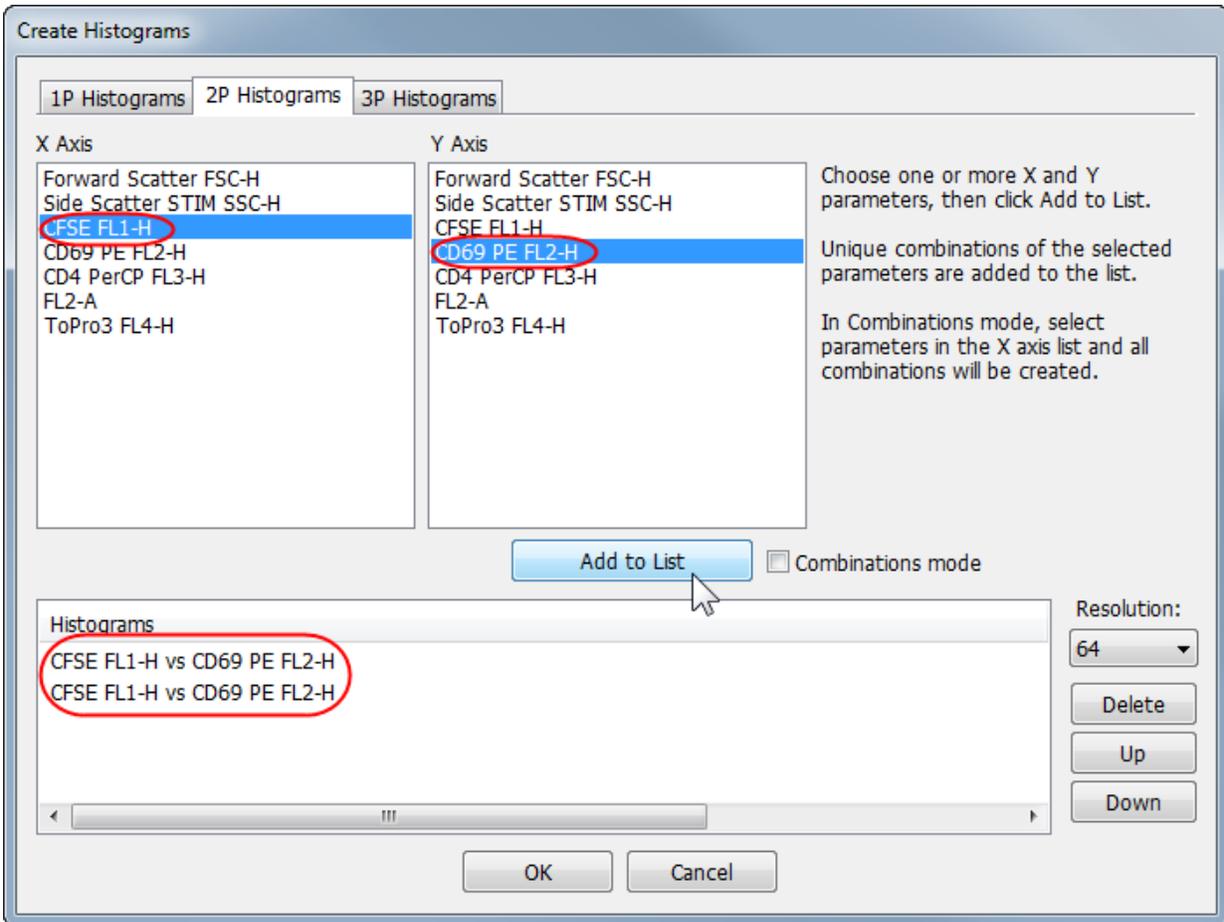
*You can change the graphical display for the histograms you've just created. Right click on the histogram and choose Graphics. Try out the different display options to see what you like best..*

For this analysis we need to use a standard **Log** transform for the X and Y axis, not HyperLog. The exported information from ModFit LT is in Log units.

12. Move the mouse over the **CFSE** label on the X-axis and click the small triangle that appears.  Select **Log** from the list of transformations.  Repeat this for the **CD69** Y-axis.
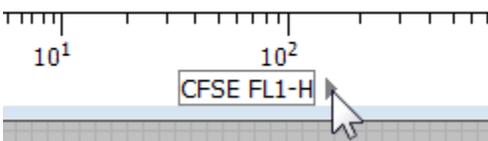
13. Click on the title bar of the first dot plot to activate it. Click the ⚹ NStat region tool in the histogram toolbar.

14. In the NStat Properties dialog, click the **Import** button.
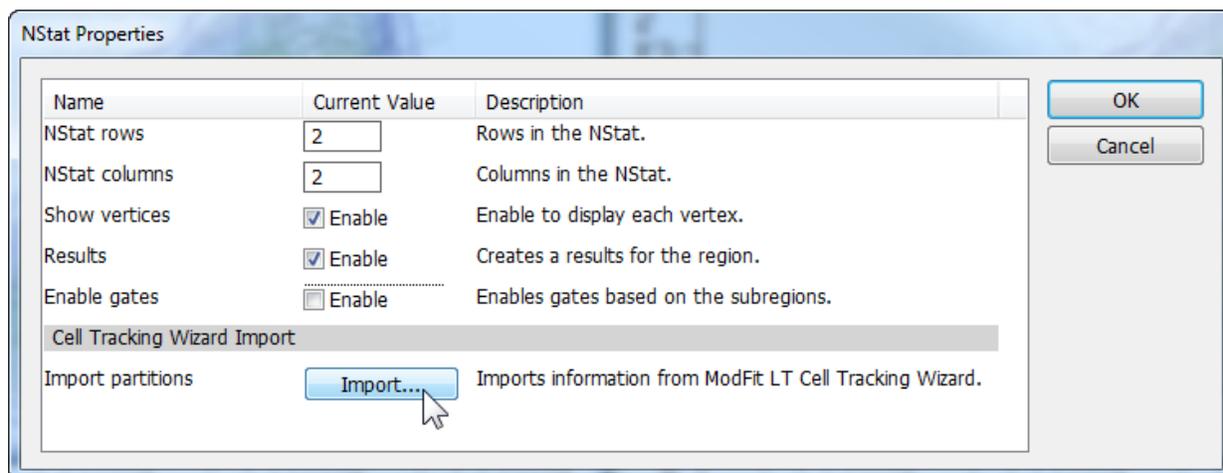


15. Navigate to the Desktop folder and select CellTrackingWizardPartitions.txt and click **Open**.

16. Edit the NStat rows to **1** and check the **Show Vertices**, **Results**, and **Enable gates** checkboxes. The number of columns should already be 10 from the Import.  Click **OK**.
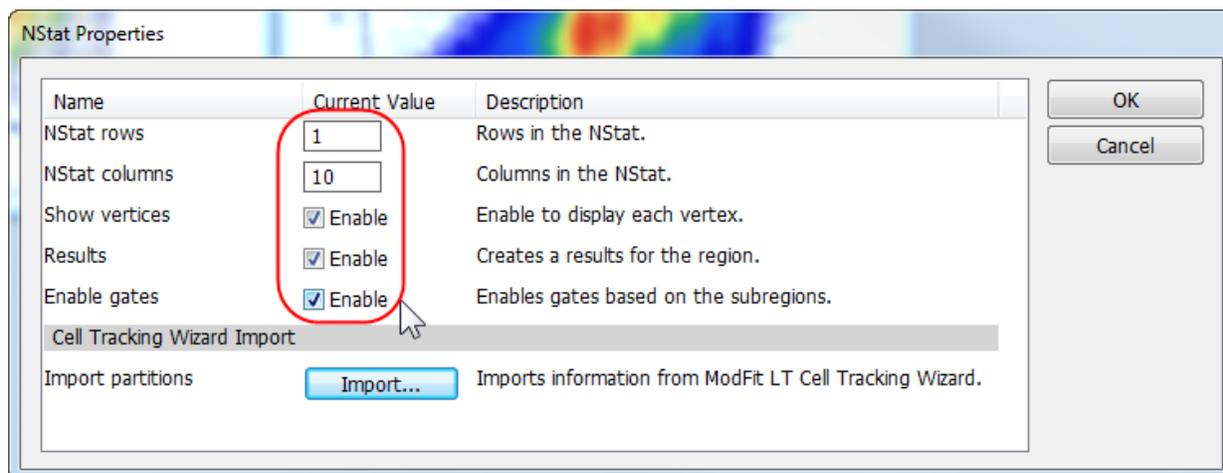


### Isolating the generations

At this point, you have completed the basic import process. Let's extend the WinList analysis to explore other markers as a function of the proliferation analysis.

17. Click the title bar of the second histogram, and click the NStat region tool button to create another NStat. For this one, set NStat rows to **2** and NStat columns to **1**. Check the **Enable gates** option and uncheck other options. Click **OK**.
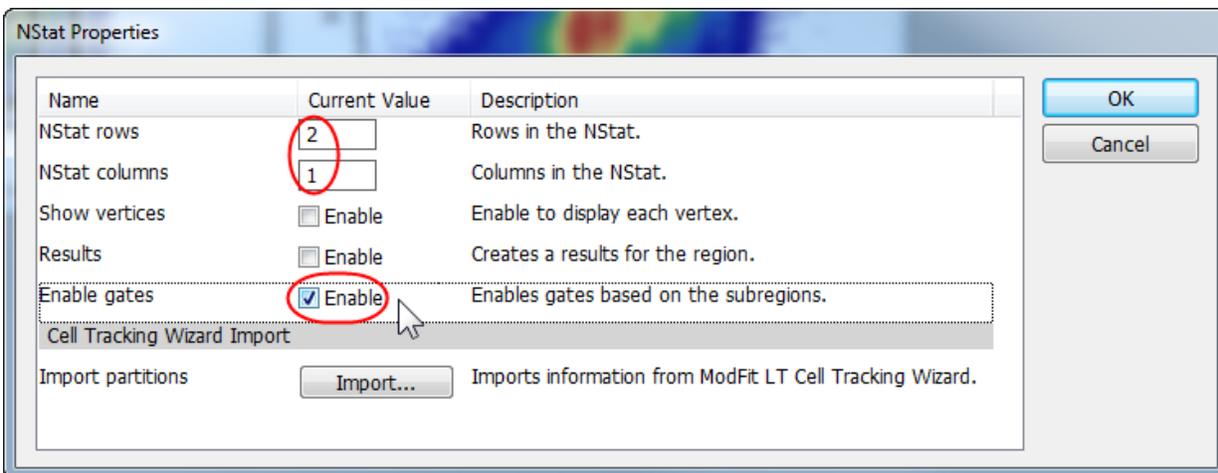


Now, one of the histograms contains an NStat that shows the proliferation partitions, and the other histogram has an NStat that will allow us to define the CD69 negatives and positives.

18. Drag the region by the label to adjust the second NStat so that it approximately divides the two major populations in the second histogram, as shown.

*You can change the graphics for any histogram by choosing* **Graphics** *from the right-click context menu for a histogram.*
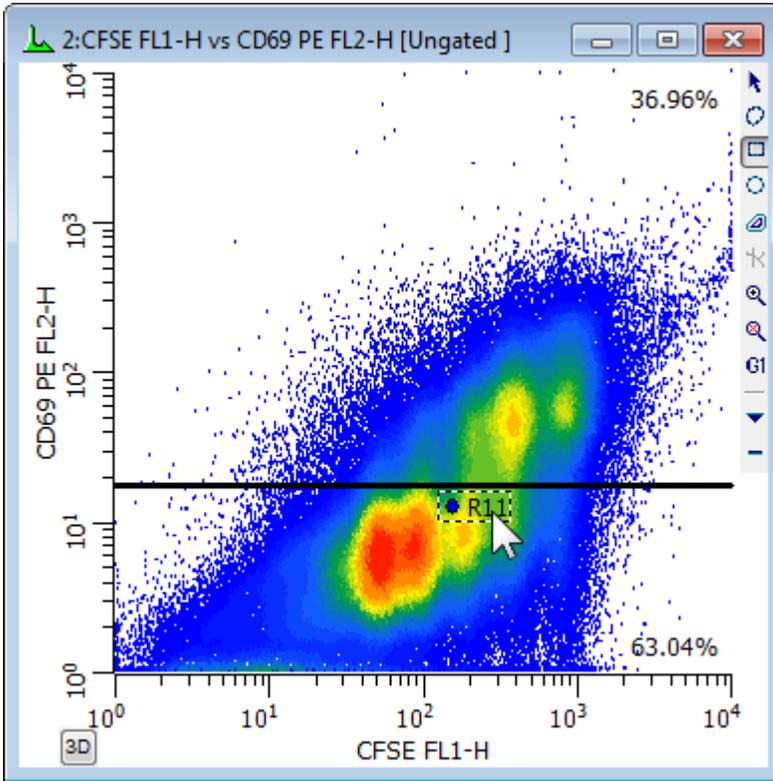
To analyze and visualize the generations better, we will create a calculated parameter using the *FCOM* function. This function creates a new parameter that will allow us to look at each population independently. It uses gates as input, and we'll use the gates from the NStat regions we just created.

Let's create a few FCOM calculated parameters to help isolate the generations.

19. Click the **Add Parameter** button on the main ribbon bar.  Alternatively, you can right-click in the data source and choose Add Parameter from the context menu.



20. Click the **Add** button in the **Edit Calculated Parameters** dialog.

21. Type **Proliferation** in the Name edit box.  Type or copy this expression into the Equation edit box, and then click **OK**.

> **FLOG(FCOM(G1,G2,G3,G4,G5,G6,G7,G8,G9,G10)+0.5)\*256/FLOG(1024)+FRND(3)**

This expression deserves some explanation. Let's break it apart, starting with the FCOM portion. FCOM is a "gate combination" function. It takes any number of gates as arguments; the result of the equation depends on how many gates are in the expression. With a single gate, there are 2 possible event classifications: an event is "in" or "not in" the gate. With two gates, there are 4 possible event classifications. With the ten gates in our expression, FCOM can produce 1024 different event states.

However, since these gates do not overlap at all, most of the 1024 possible states have no events in them. In fact, the only states that contain events are the 10 single-positive event states, resulting in values of 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512. If we plotted the result of the FCOM function as a histogram, we would see 10 spikes of events, and the spikes would be exponentially spaced.

Our goal is to see these 10 spikes *evenly* spaced across the histogram. This is where the FLOG function comes in. By taking the log of the FCOM output and scaling it by 1024, we redistribute the 10 FCOM values evenly across 256 channels. The final portion of the expression, "+FRND(3)", changes the spikes of events into Gaussian distributions so that they can be better visualized.

*Take a look at the reference section on Add Parameters and FCOM for details on the complete set of WinList functions.*

22. Once again, click the **Add** button in the **Edit Calculated Parameters** dialog.

23. Type **CD69 Expression** in the Name edit box.  Type or copy this expression, and click **OK**.

> **(FCOM(G11)+0.5)*256/2+FRND(3)**
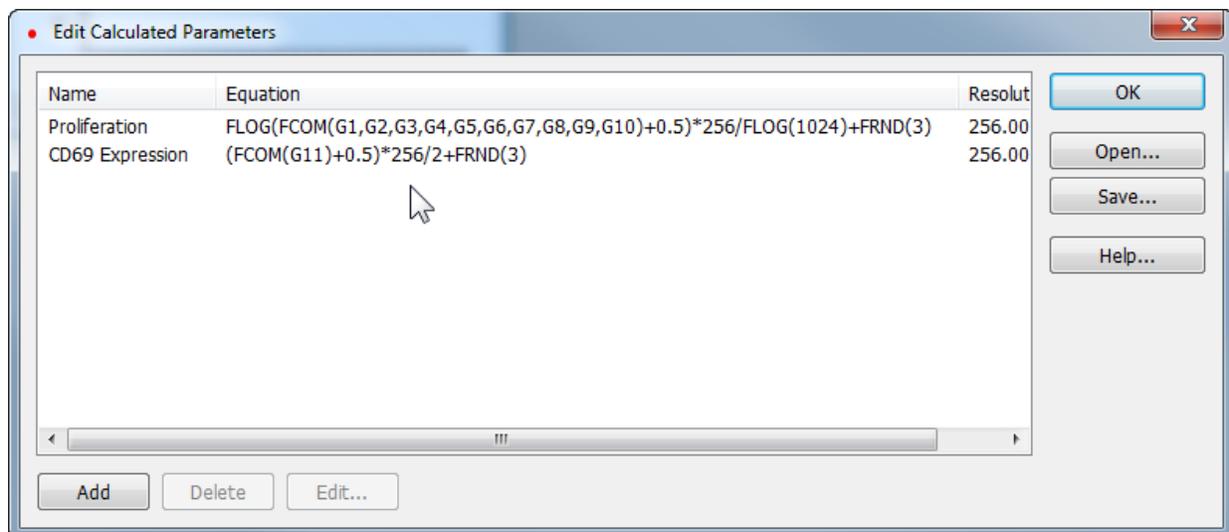


24. Click **OK**.

## CD69 expression as a function of proliferation

We're now ready to explore this data file in really interesting ways. Wouldn't it be nice to see CD69 as it relates to proliferation, viewing each generation as a discrete cluster? Could we quantify the CD69 expression of CD69-positive cells for each generation? Yes and yes.

25. From the **Insert** tab on the ribbon bar, click the **Density** plot. This creates a Density plot in a default location on the data source.  Click the X Axis label and choose *Proliferation*. Click the Y Axis label and choose *CD69 PE*.

Let's create another histogram to isolate the CD69 positives and negatives.

26. Right-click on the histogram that we just created and choose **Clone** from the context menu.  A duplicate copy of the histogram is created.

27. Change the Y Axis to show *CD69 Expression*.

In the graphics we can see the generations separated into discrete populations along the X-axis. That's the first FCOM function we created. The Y-axis shows the continuous CD69 parameter in one histogram and discrete subsets of CD69 positives and negatives in the other histogram. Regions can be created to quantify the subsets.
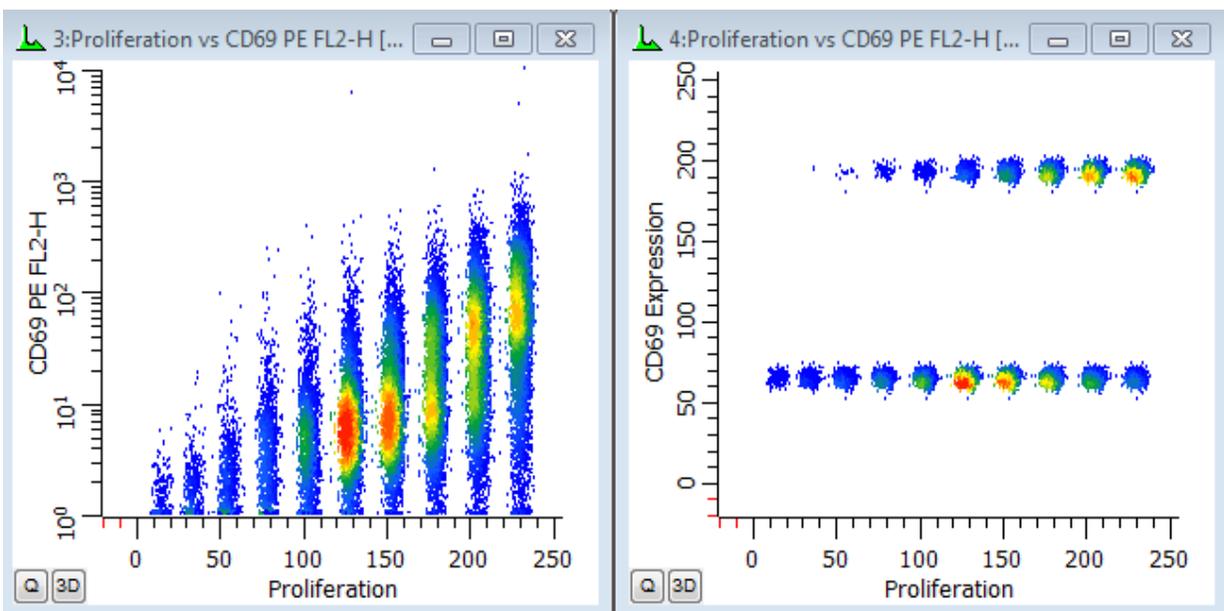


You can, of course, explore how other parameters relate to cell proliferation. Simply create additional histogram to display the parameter combinations you are interested in.
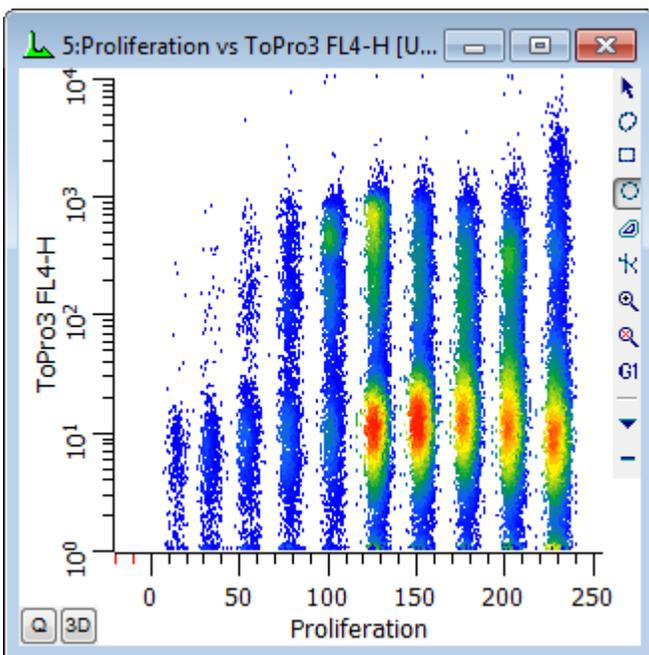
## Making life easier

You have done the real work in setting things up with this tutorial. If you save this setup as a WinList protocol or protocol bundle, the calculated FCOM parameters, NStat regions, and selected histogram can be recalled very easily from disk.

When you analyze new data files with ModFit LT's Proliferation Wizard, you can export new partition files. Then, open the same data file into your WinList protocol and import the partitions into the NStat region as we have demonstrated in this tutorial. All of the calculated parameters will update based on the new data and partitions.

**See also:**

How to use FCOM parameter to enumerate phenotypes

FCOM Background and Usage

Edit Properties for NStat Region

**Remote connection to ModFit LT**

*This feature is only available on PC platforms. ModFit version 3.2.1 or greater is required.*

In this tutorial we will connect WinList to ModFit LT for enhanced DNA analysis. You will see how easily ModFit LT can be integrated into WinList. You will be using file **cho445.fcs** from the WinList sample files.

*Before beginning, ModFit LT 3.2.1 or greater must be installed on your computer.*

**Set up**

1. Start ModFit LT and pass through its opening dialogs. Minimize the program to clean up the desktop.

2. Start WinList.

3. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **cho445.fcs** from the list and click **Open**.

*You can also drag and drop files from Windows Explorer or My Computer onto WinList. See Drag and Drop for details.*

2. In the **Create Histograms** dialog, select FL2 vs FL1 and click **Add to List**.

3. Click the *1P Histograms* tab. Select FL2 in the list, and click **Add to List**.

4. Click **OK** to open the data source and create the histograms. You should have two histograms displayed.
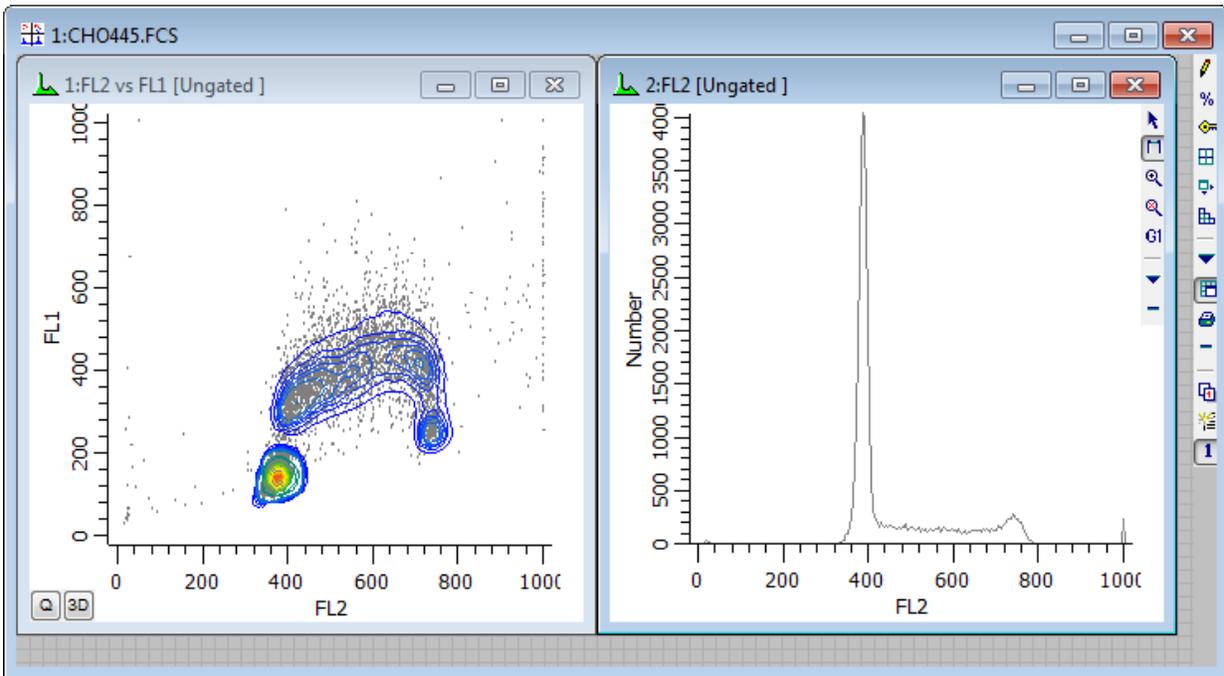
## Making the remote connection

Next, let's link the 1P FL2 histogram to ModFit. Here's how.

5. Double-click the title bar of the FL2 histogram. The **Edit Histogram** dialog box will appear.

6. Enable the **Remote** check box, and then click the **Remote** button.

The **Edit Remote Link** dialog box will appear. WinList assumes that you want to link to ModFit LT, and we do not need to make any changes to this dialog for this example.

7. Click **OK** in the **Edit Remote Link** dialog box. WinList will make connection with ModFit LT and return us to the **Edit Histogram** dialog box.

> *If WinList cannot find ModFit LT, you receive an error message at this point. Make sure that ModFit LT is running, and repeat the last two steps. Remember that you need ModFit 3.2.1 or newer.*

8. Click **OK** in the **Edit Histogram** dialog box.

Here's what happens. WinList begins to execute each command in the remote script for the histogram. That script tells WinList to send histogram data to ModFit LT and tells ModFit LT to Auto-Analyze the data. Next, the script tells WinList to copy ModFit's graphical and textual

results and display them in the histogram window. WinList will update the analysis anytime it detects that the data for this histogram has changed. You could apply a gate to the DNA histogram, and any time a gate region is adjusted, the remote script would fire off, updating the ModFit analysis. You can modify a remote script using the **Edit Remote Link** dialog box.

9. To view the ModFit LT analysis, click the "**I**" button in the lower left corner of FL2 histogram. A window will appear, displaying the contents of the analysis results.

### Modifying the update frequency

Depending on how WinList is set up, the analysis we just performed might not have sent *all* of the events for the FL2 histogram to ModFit LT. WinList only sends the number of events in one of WinList's *packets.* Packet size is defined in Edit Program Controls, and determines the number of events WinList will keep *in memory* for quick analysis and gating.

Let's suppose that you want ModFit LT to see *all* of the events for FL2. The way to do this is to change the update frequency for the remote link. Here's how.

10. Double click the title bar of the FL2 histogram to show the **Edit Histogram** dialog. Click the **Remote** push button to display the **Edit Remote Link** dialog box.

11. Notice the **Update** group box, which contains several radio buttons. Click **On replay**. This tells WinList that the only time this link will update is when this file is replayed from disk. This ensures that *all* the data will be shipped to ModFit.

12. Click **OK** in the **Edit Remote Link** dialog box to accept the change, and click **OK** in the **Edit Histogram** dialog box.

13. Click the **Replay** button on the ribbon bar.

WinList will update the remote link with data that represents the entire listmode file. This technique has two distinct advantages: it allows ModFit LT to work with the full data set and it updates less frequently.

### Summary

In this tutorial, we demonstrated how to use WinList's remote link capabilities to connect with ModFit LT. ModFit LT performed an Auto-Analysis of the data WinList sent to it, and WinList displayed ModFit's results.

To establish remote links, select **Edit Histogram**, and check the **Remote** check box. Then, click the **Remote** push button. The update frequency of the link, as well as the commands which are executed, can be edited in the **Edit Remote Link** dialog box.

## Using MultiGraphs

MultiGraph windows can contain graphic and text elements from histogram and results windows, and allow you to display and print the collection in various views. They have a more restricted format than reports and do not update elements when data changes. However, they are a useful tool for creating summary information about experiments in a graphical record. In this tutorial, you will review how to open a listmode data file, create histograms, and learn how to select histograms and results for inclusion in a MultiGraph window.

*Before you begin, make sure you understand the basics of working with WinList. The tutorial [WinList Basics](#) is a great place to start.*

### Setting up

1. Click the **Open FCS** button on the ribbon bar. Navigate to the Samples folder, select **1091901.fcs** in the list, and then click **Open**.

2. Use the **Create Histograms** dialog to create several 1P and 2P histograms. Click **OK**.

3. Click the **Q** button in the lower left corner of each of the 2P histograms. This will create a QuadStat region, and automatically display a results window for it. Adjust the QuadStats.

### Creating the MultiGraph

At this point, we have created what we want to store in the MultiGraph. Next, we will create the MultiGraph and add this information to it.

4. Click the **MultiGraph** button on the ribbon bar. The **Edit MultiGraph** dialog box will appear. The dialog box displays all of the items that you can add into the MultiGraph in the **Available Items** list. As we add items, they will appear in the **MultiGraph Items** list.

5. In the **Available Items** list box, select all entries, except for *BLANK*. Click the **Add** button.

*"BLANK" is always the first entry in the **Available Items** list box. It allows you to put a placeholder, of sorts, into the MultiGraph. It is useful when attempting to keep elements in proper columns and rows.*

6. In the **Viewing Options** section, click **Tiled**, and enter 3 and 4 in the **Columns** and **Rows** edit boxes, respectively. This means that we will display the elements in a 3 x 4 array. If we were to add more than 12 elements, additional pages would be created automatically.
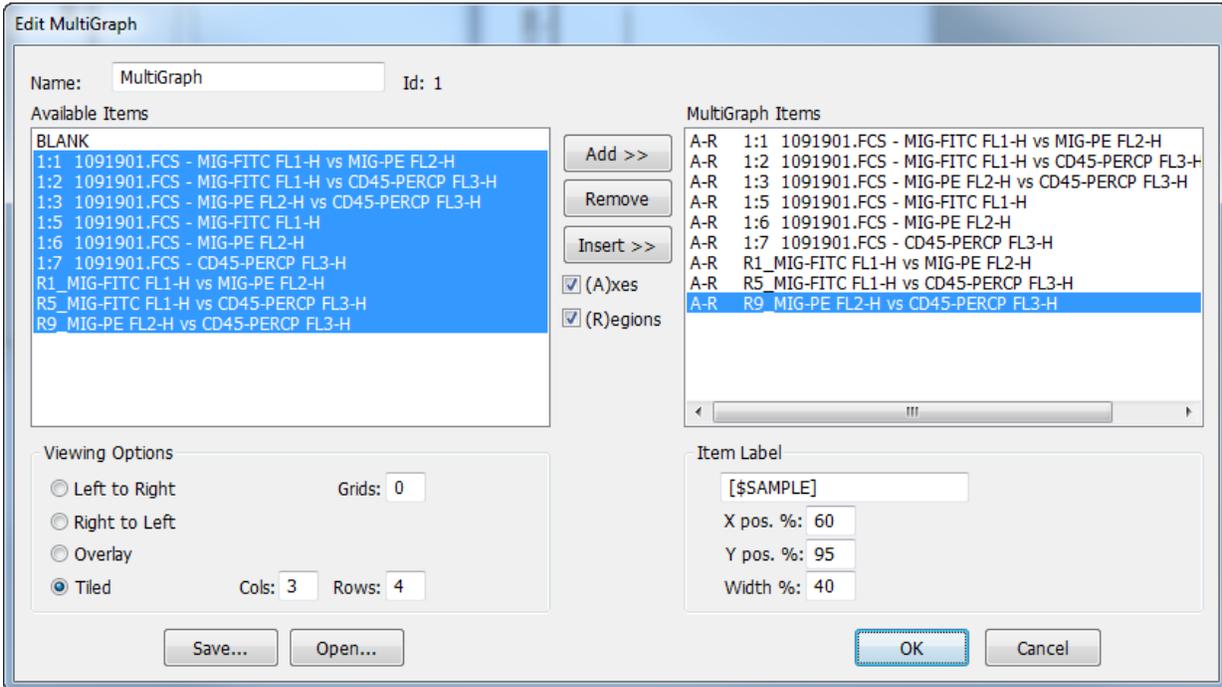
7. Click **OK** to close the dialog box and create the MultiGraph.

The MultiGraph will show the histograms and results that we added. Elements in the MultiGraph will not change, even if the histograms from which they were generated do. This means that you can use the MultiGraph to archive graphical and statistical information. You can also add elements from numerous data files to a single MultiGraph.

## Changing the arrangement

You can move elements around on a MultiGraph page by using the mouse.

8. Click and hold the mouse on one of the elements in the MultiGraph. Move the mouse to a new location in the MultiGraph, and release the button. The element will move to the new position, and other elements will adjust to accommodate the change.

Click, hold, and drag elements to new locations in the MultiGraph.
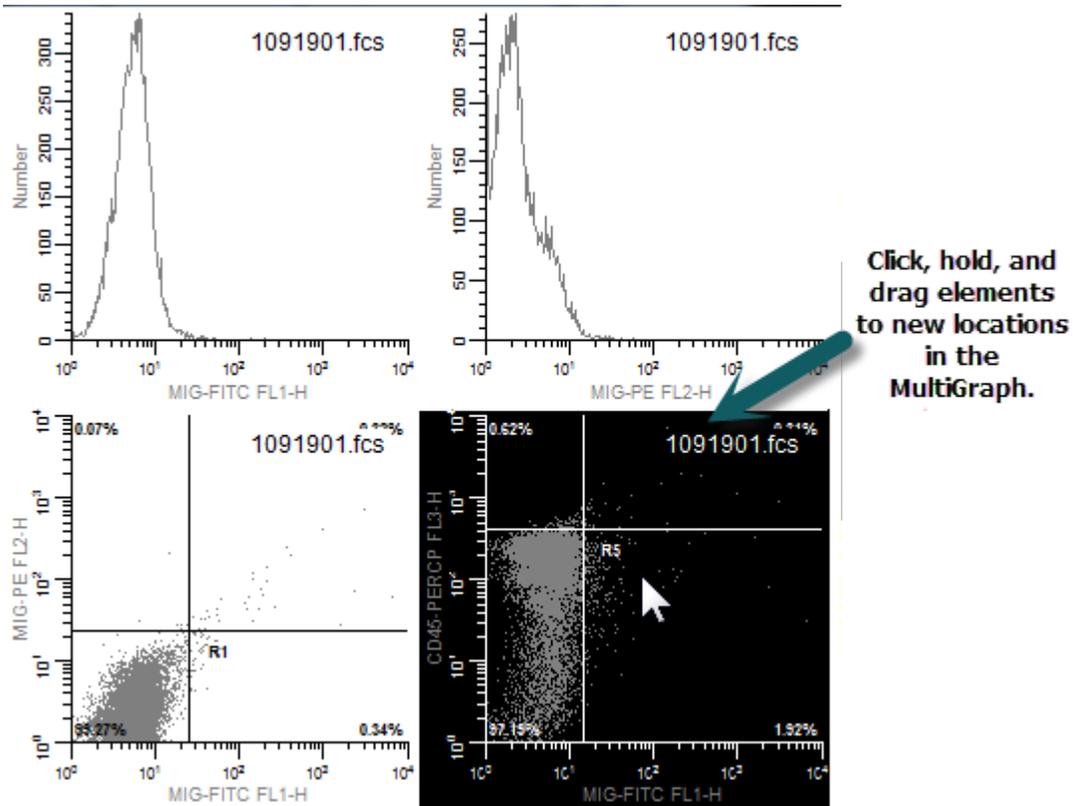
## Summary

In this tutorial, we introduced the **MultiGraph**, a type of window that can contain graphics and text from histogram and result windows. MultiGraphs can collect elements from numerous data files, display them in one window, and print them.  The can also be exported to PDF format.

The **Edit MultiGraph** dialog box allows selection of new elements, editing of labels, and a choice of viewing options. MultiGraphs can also be saved to disk and opened from disk in this dialog box.

As with other types of windows in WinList, double-clicking on the title bar of the MultiGraph window is a shortcut to the **Edit MultiGraph** dialog box. There are also shortcuts for adding histograms and results windows to MultiGraphs.

MultiGraphs have a popup menu that allows you to perform common MultiGraph tasks quickly. In addition, tiled MultiGraphs allow you to move elements by a click-hold-and-drag technique.

**Reference**

**Main Window**

WinList offers two interfaces for the main window.  The Ribbon Bar provides easy access to the most common commands without having to drill down in menus.  The Toolbar with Menu interface is a good option for users familiar with legacy versions of WinList.

The **User Interface** property in Miscellaneous Preferences controls which interface the program will use.  It can also be changed on the View tab of the ribbon or the View menu of the Toolbar.  The program must be restarted when changing from one to the other.

Main window with Ribbon Bar
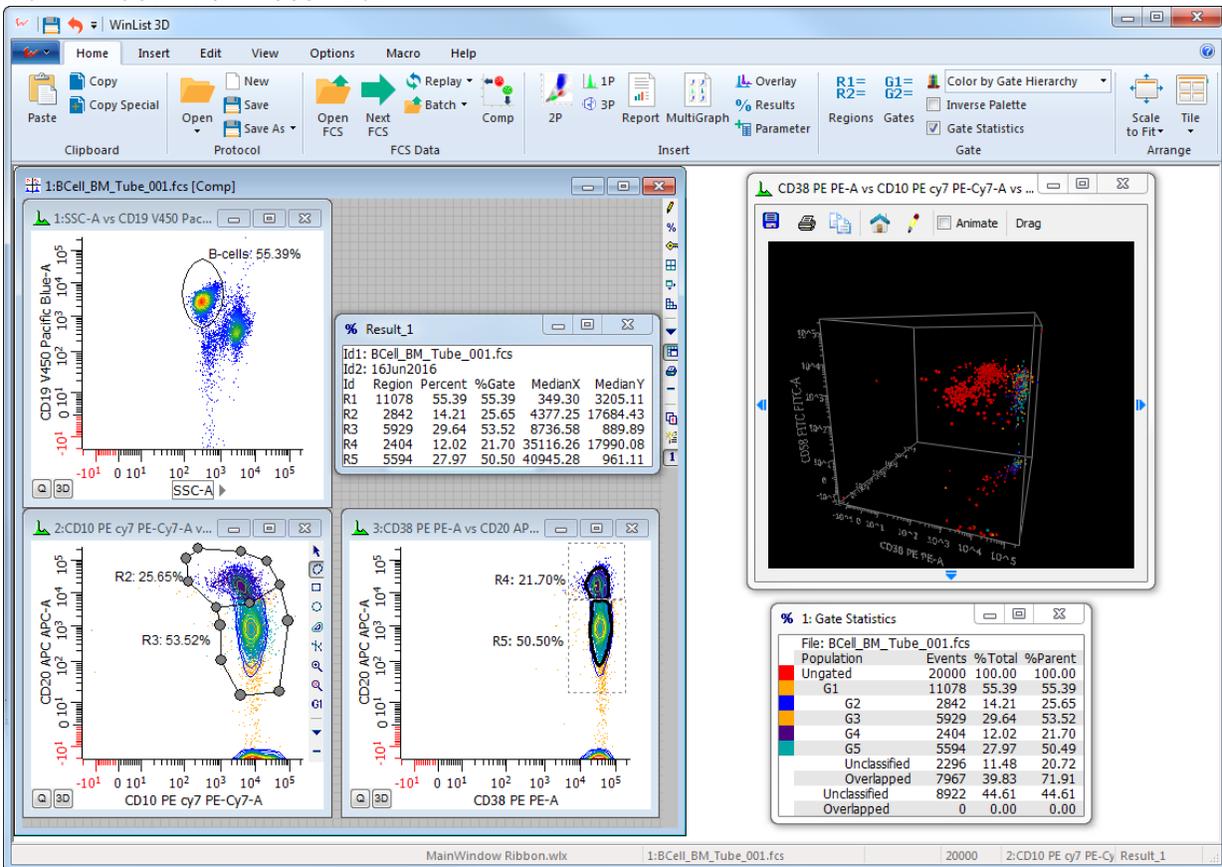
## Main window with Toolbar and Menu
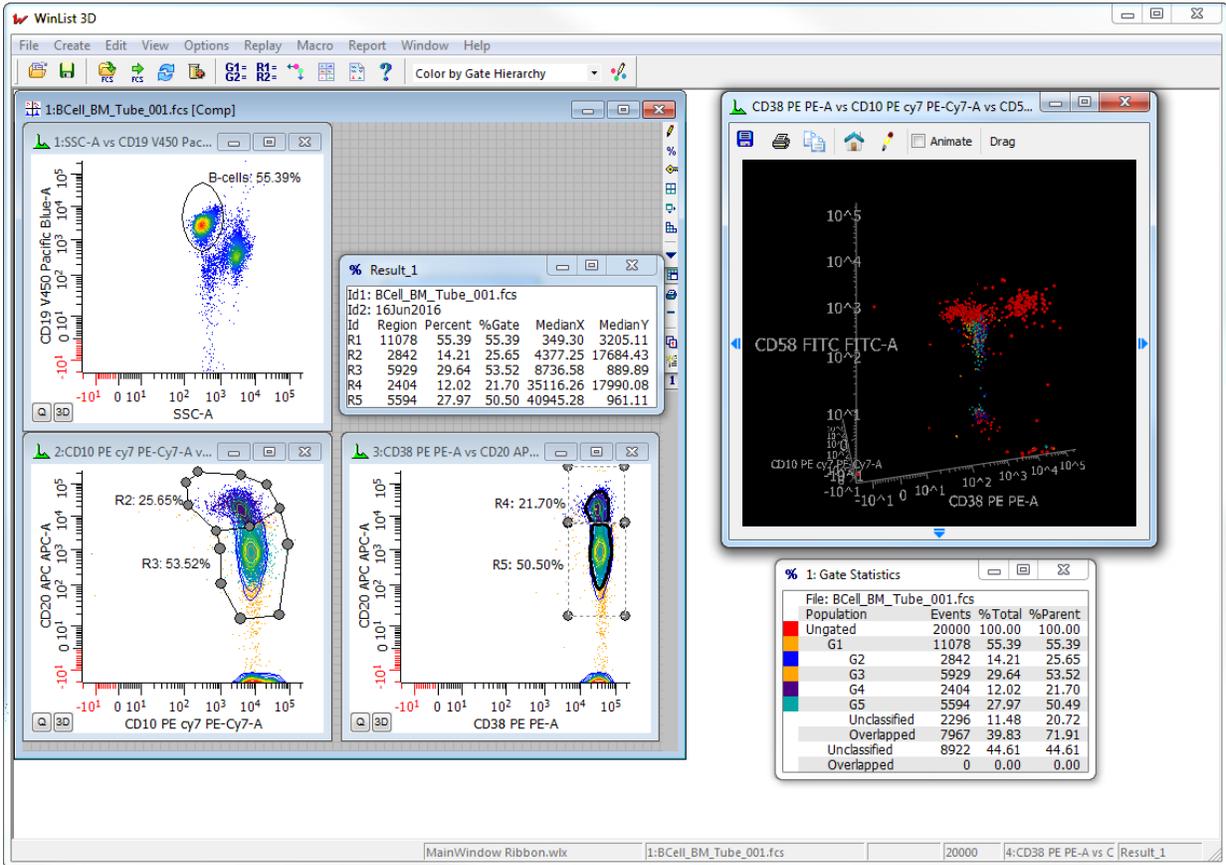


**See Also:**

Ribbon Bar
Toolbar
Status Bar

**Ribbon Bar**

The Ribbon Bar provides easy access to the most common commands without having to drill down in menus.  It is divided up into tabs that group commands based on function.

**File (Application) Menu**

This menu is accessed by clicking the WinList icon at the left side of the ribbon bar.  It contains commands to open and save protocols, to open report and MultiGraph files, to export files, and to print.
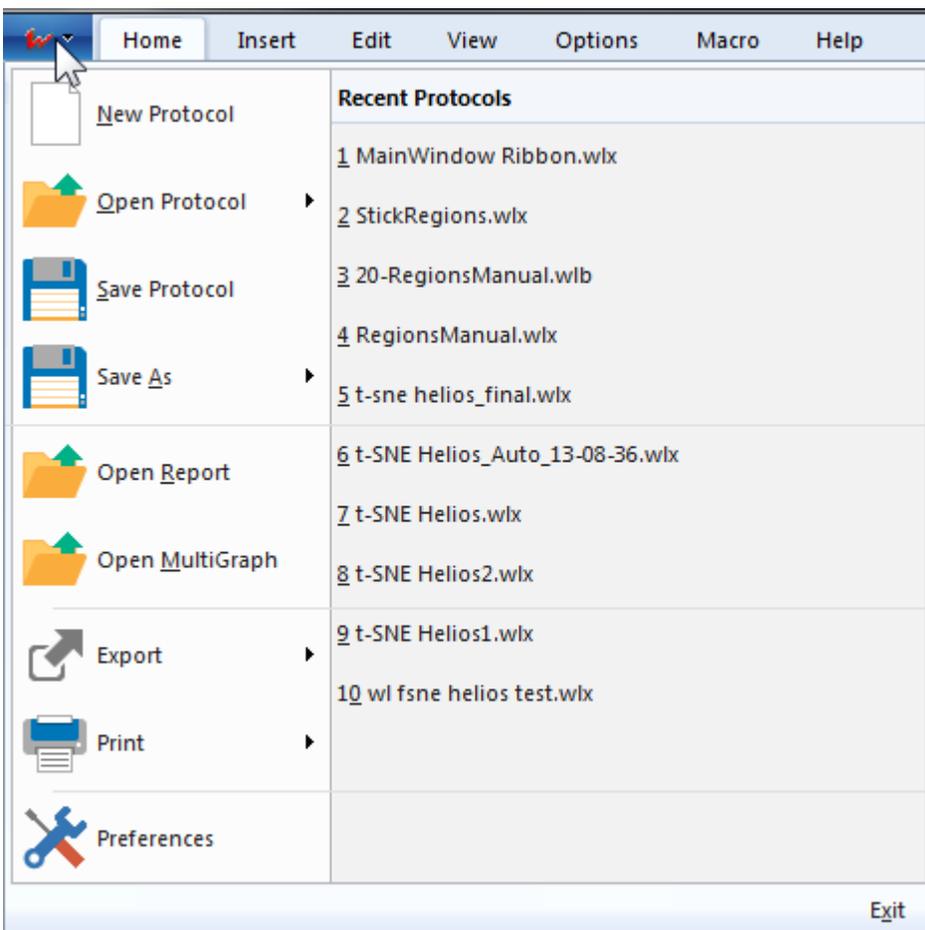


| Command | Description |
| --- | --- |
| New Protocol | Creates a new protocol, closing all open windows. |
| Open Protocol | Opens a protocol file. |
| Open Bundle | Opens a protocol bundle file. |

| | |
|---|---|
| Save Protocol | Saves a protocol. |
| Save Protocol As... | Saves a protocol with a new name. |
| Save Bundle | Saves a protocol bundle file. |
| | |
| Open Report | Opens a report file. |
| Open MultiGraph | Opens a MultiGraph file. |
| | |
| Export Listmode | Saves a new listmode file in FCS or Text format based on the active data source. |
| Export Merge Files | Merge FCS files into one output FCS file. |
| Export Histogram | Saves a new histogram file in FCS format based on the active histogram. |
| Export Histogram Graphic | Saves the active histogram as a graphic file format. |
| Save MultiGraph Graphic | Saves the active MultiGraph as a graphic file format. |
| Export Report Graphic | Saves the active Report as a graphic file format. |
| Export MultiGraph PDF | Saves the active MultiGraph in PDF format. |
| Export Report PDF | Saves the active report in PDF format. |
| Export Results | Saves the active results window in text format. |
| | |
| Print Screen | Prints the screen. |
| Print Report | Prints the active report. |
| Print MultiGraph | Prints the active MultiGraph. |
| Print Overlay | Prints the active overlay. |
| Print Histogram | Prints the active histogram. |
| Print Data Source | Prints the active data source window. |
| Print Setup | Displays the Printer Setup dialog. |
| | |
| Preferences | Displays the Preferences dialog. |
| | |
| Recent Files | Displays a list of recently opened protocols. Click an entry to open. |

## Home Tab
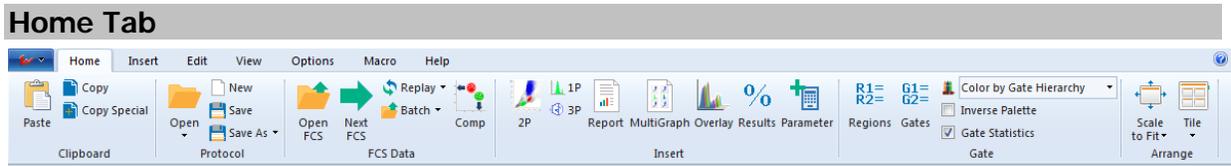


| Clipboard panel | Protocol panel | FCS Data | Insert | Gate | Arrange |
|---|---|---|---|---|---|
| Paste | Open | Open FCS | 2P | Regions | Scale to Fit |
| Copy | Open Bundle | Next | 1P | Gates | Tile |
| Copy Special | New | Replay | 3P | Color Mode | |
| | Save | Batch | Report | Inverse Palette | |
| | Save As | Comp | MultiGraph | Gate Statistics | |
| | Save Bundle As | | Overlay | | |
| | | | Results | | |
| | | | Parameter | | |

## Insert Tab
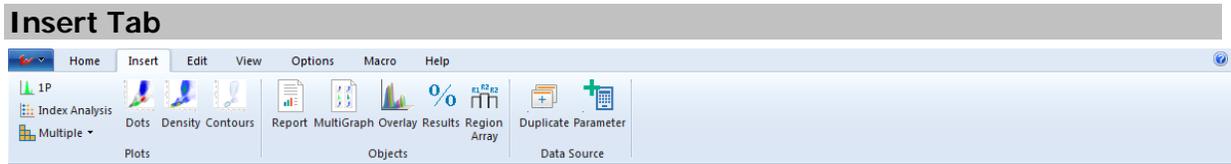


| Plots | Objects | Data Source |
|---|---|---|
| 1P | Report | Duplicate Data Source |
| Index Analysis | MultiGraph | Add Parameter |
| Multiple | Overlay | |
| Dots | Results | |
| Density | Region Array | |
| Contours | | |

## Edit Tab
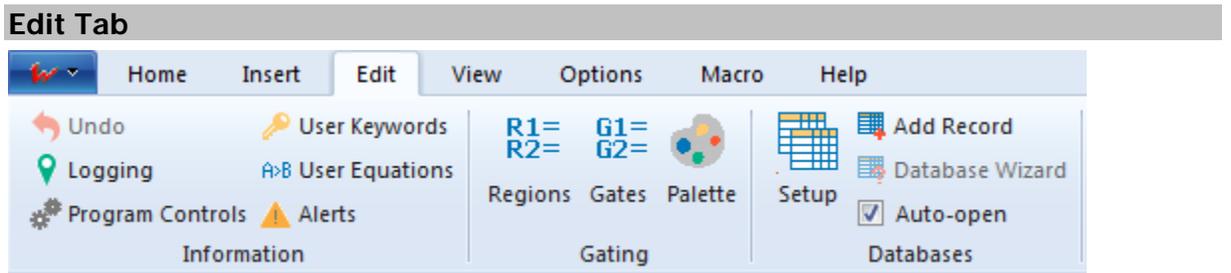
| Information | Gating | Databases |
|---|---|---|
| Undo | Regions | Setup |
| Logging | Gates | Add Record |
| Program Controls | Palette | Database Wizard |
| User Keywords | | Auto-open |
| User Equations | | |
| Alerts | | |

## View Tab
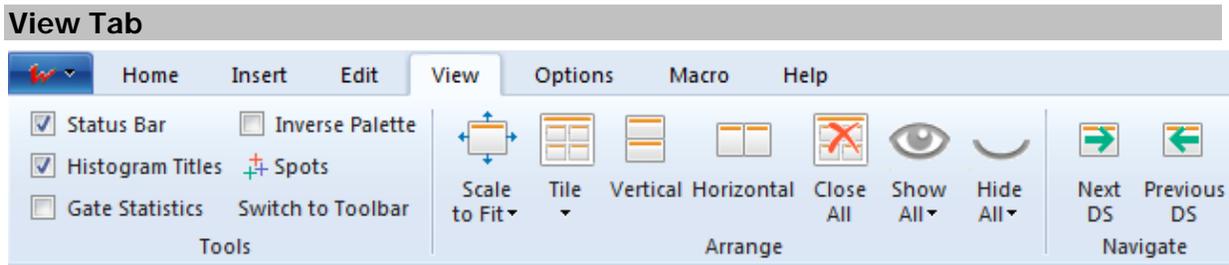


| Tools | Arrange | Navigate |
|---|---|---|
| Status Bar | Scale to Fit | Next DS |
| Histogram Titles | Tile | Previous DS |
| Gate Statistics | Vertical | |
| Inverse Palette | Horizontal | |
| Spots | Close All | |
| Switch to Toolbar | Show All | |
| | Hide All | |

**Options Tab**
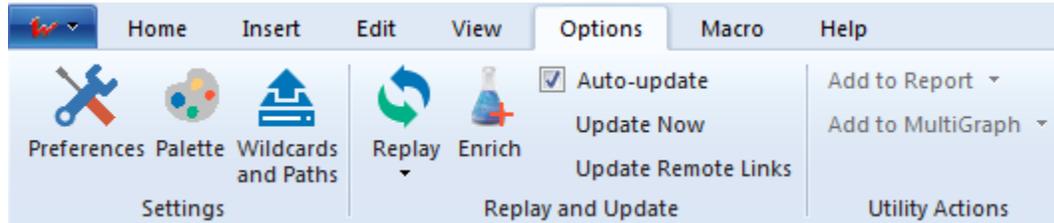


| Settings | Replay and Update | Utility Actions |
|---|---|---|
| Preferences | Replay | Add to Report |
| Palette | Enrich | Add to MultiGraph |
| Wildcards and Paths | Auto-update | |
| | Update Now | |
| | Update Remote Links | |

**Macro Tab**
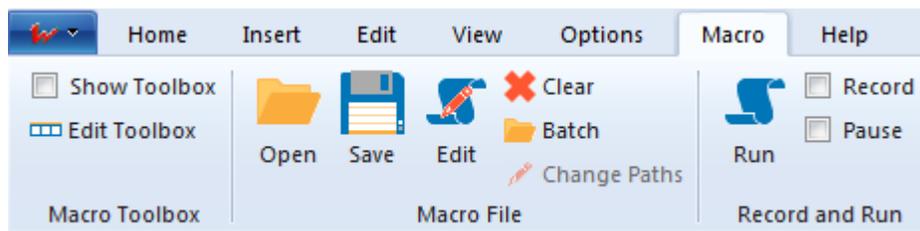


| Macro Toolbox | Macro File | Record and Run |
|---|---|---|
| Show Toolbox | Open | Run |
| Edit Toolbox | Save | Record |
| | Edit | Pause |
| | Clear | |
| | Batch | |
| | Change paths | |

## Help Tab
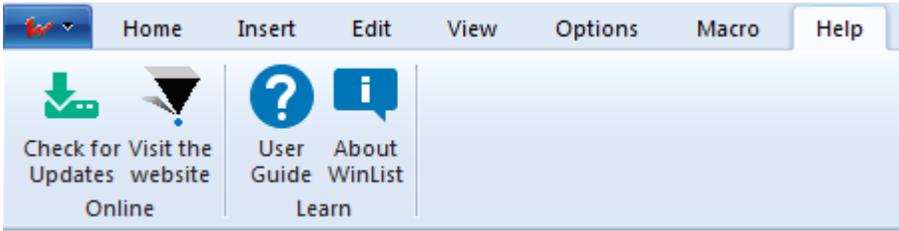


| Online | Learn |
|--------|-------|
| Check for Updates | User Guide |
| Visit the web site | About WinList |

## Quick Access Toolbar



The Quick Access Toolbar appears at the top left of the main window. Customize the commands that appear in this tool to include those that you need constant access to.

To customize the commands, click the down-arrow button on the right side of the toolbar.

**Toolbar**

The toolbar is displayed across the top of the application window, below the menu bar. The toolbar provides quick mouse access to common WinList functions.

To hide or display the Toolbar, choose Toolbar from the View menu.

The buttons appearing in the WinList Toolbar are shown below along with an explanation of the functions they perform.

| | |
| --- | --- |
| *Button* | *Function* |
| | Opens a protocol. This is equivalent to choosing Open Protocol from the File menu. |
| | Saves a protocol. This is equivalent to choosing Save Protocol from the File menu. |
| | Opens a listmode file. This is equivalent to choosing Open FCS File from the File menu. |
| | Opens the next file. This is equivalent to choosing Next FCS File from the File menu.<br><br>To open the next file without displaying the File Open dialog, hold down the Shift key while clicking the button. |
| | Replays the active data source. This is equivalent to choosing Replay Active Data Source from the Replay menu.<br><br>To replay all data sources, hold down the Shift key while clicking the button. |
| | Enrich the sampled data based on a selected gate. |
| | Displays the Edit Gates dialog. This is equivalent to choosing |

Gate List from the Options menu.

Displays the region list dialog. This is equivalent to choosing Region List from the Options menu.

Displays the N-Color Compensation™ Toolbox. This is equivalent to choosing Compensation from the Options menu.

Creates a new MultiGraph window. This is equivalent to choosing Create MultiGraph from the Create menu.

Creates a new Report window. This is equivalent to choosing New Report from the Report menu.

Displays the on-line help system. This is equivalent to choosing Index from the Help menu.

Selects the coloring mode used by WinList.

Edits the current coloring mode.

**Status Bar**

The status bar is displayed across the bottom of the main window. It displays prompts on the leftmost pane, and information about WinList objects in other panes.



| *Pane* | *Meaning* |
| --- | --- |
| Left-most | Information about the current menu selection or operation. |
| 1 | Name of the current protocol |
| 2 | File name and ID for the active data source |
| 3 | File index and size of the batch, if batch processing |
| 4 | Number of events in the current file for the active data source |
| 5 | Name and ID of the active histogram |
| 6 | Name of the active results window |

**Protocols**

**Open Protocol**
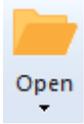


This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.  It is used to open protocol files you have saved to set up an analysis in WinList. Protocols are created using the Save Protocol option.

You can also open Protocol Bundle files with this dialog by changing the filter drop-down to show Protocol Bundles.

The **Open Protocol** command displays the file open dialog box, allowing you to select a protocol (wlx or mac file) or a protocol bundle (wlb file). Select a protocol from the list and then click the Open button. Alternately, you can double-click the file in the list. The protocol will set up the screen, gates, regions and other components of the display.
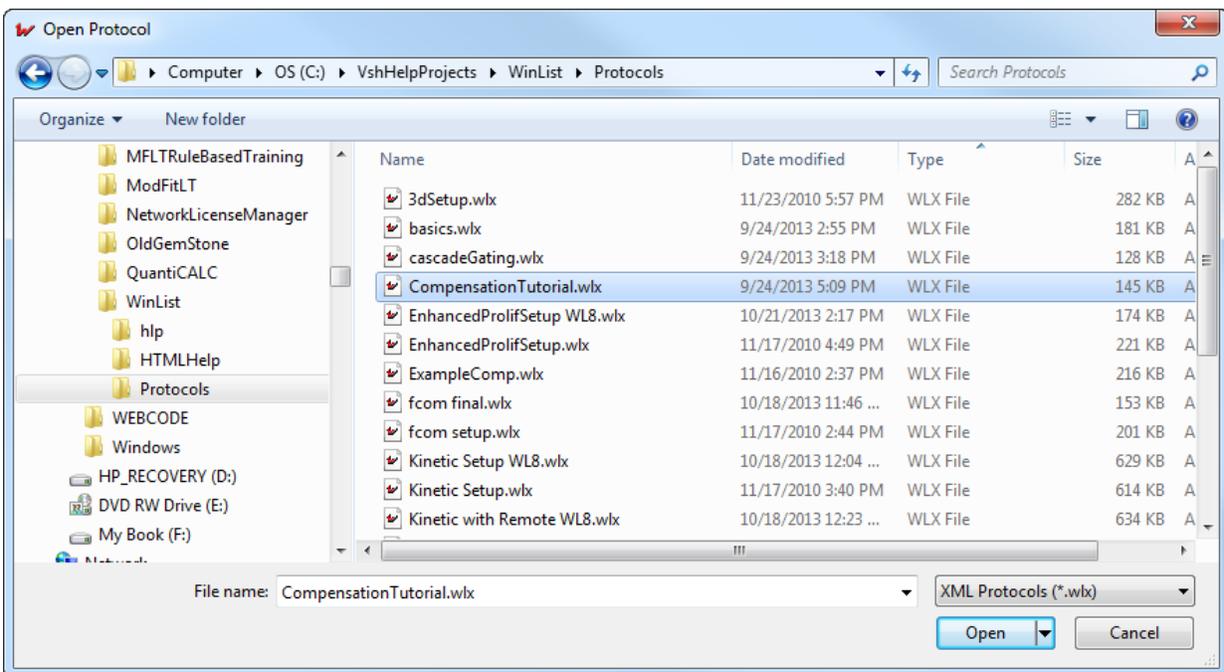


**Drag and Drop**
You can drag and drop a protocol file from the file system onto WinList to open a protocol.

**Open Bundle**
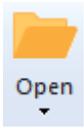


This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.  It displays the Open Protocol dialog with the file type selection set for protocol bundle files. See Save Bundle for a description of the protocol bundle file.
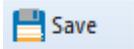
**Save Protocol**

Save

This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.  It is saves the current WinList analysis setup so that it can be reopened at a later time.

If the protocol has already been saved, this command updates the file on disk with the new setup. If the protocol has not yet been saved, it displays the Save Protocol As... dialog.

The function of this button is also controlled by several preferences.  See Miscellaneous Preferences for additional information.

**Save Protocol As...**
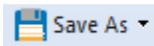
 Save As ▾

This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

Sometimes the analysis you perform with WinList is something you use over and over again. In cases like this, it would be nice to save an analysis setup so that you can quickly reconstruct it and load new data files into it for analysis. This is where WinList protocols are used.

There are two kinds of protocols that WinList creates. The first is an XML-formatted protocol file that contains a complete description of the analysis you have setup. This kind of protocol requires that the FCS data files used in the setup are in the exact locations as they were when the protocol was saved.

A second type of protocol is called a protocol bundle. The protocol bundle stores an XML protocol file described above in a Zip-compatible file. In addition, the protocol bundle includes copies of the FCS files displayed in the protocol. Protocol bundles are convenient when you need to move protocols from one computer to another or to create an archive of your analysis. See Save Bundle for additional details.

This command displays the Save dialog box. Enter a name for the protocol, navigate to the folder where you want to save the protocol, and click the Save button.
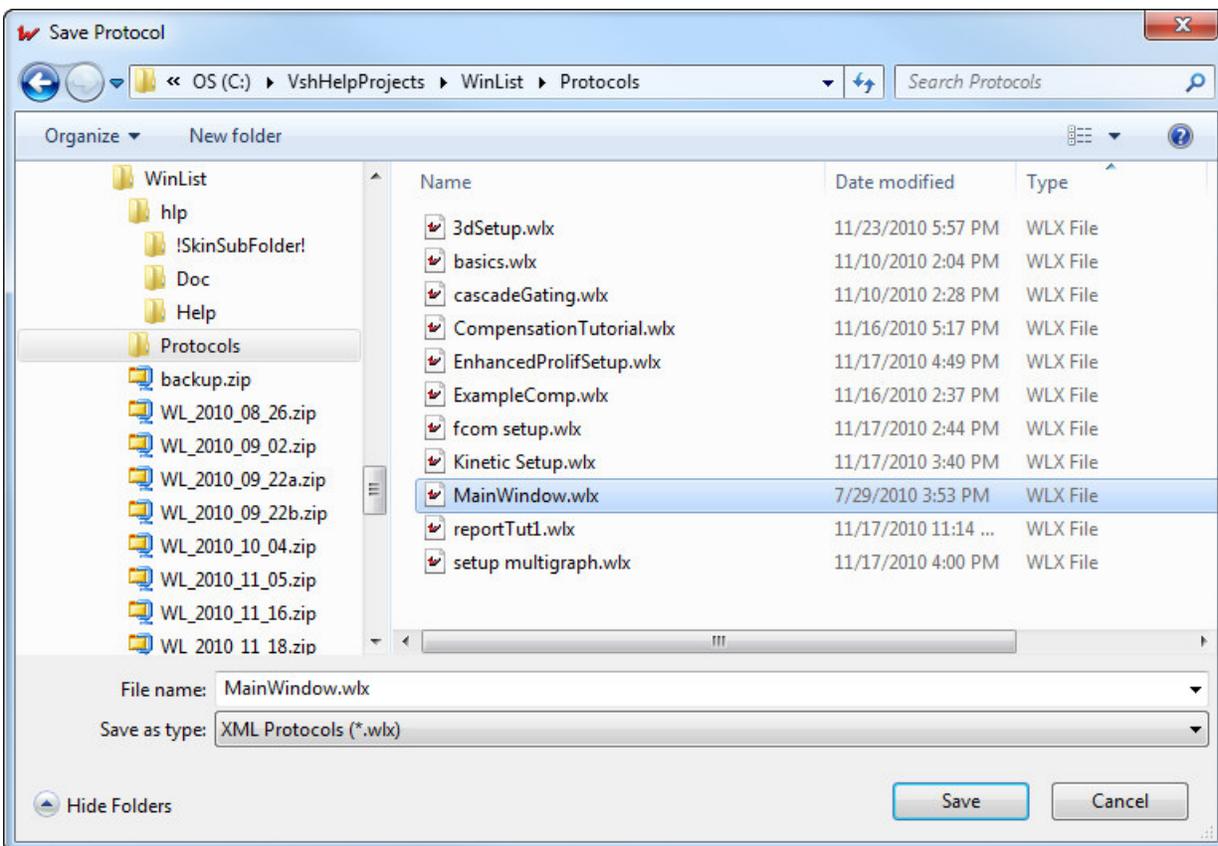
Several aspects of the WinList setup are *not* stored in protocols, including histogram subtraction and 3D viewer windows. These windows need to be re-created after you open your saved protocol.

**Drag and Drop**

You can drag and drop a protocol file from the file system onto WinList to open the protocol.

**See Also:**

Save Protocol Bundle

Open Protocol

**Save Bundle**
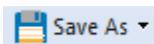


This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

This command creates a single, compressed file in Zip-compatible format that contains all information needed to reconstruct your analysis setup. It is similar to the Save Protocol command, but has several key differences that make it more powerful for some situations.

The significant differences between Save Protocol and Save Bundle are that Save Bundle creates a single file that contains the XML protocol *and* the FCS listmode files that the protocol uses. This makes the protocol bundle a better choice when you need to move a protocol from one place to another or to create an archive of your analysis.

To open a protocol bundle file, use the **Open Protocol** dialog and change the **Files of Type** selection to show Protocol Bundles.

**Drag and Drop**

You can drag and drop a protocol bundle file from the file system onto WinList to open a protocol.

**See Also:**

Save Protocol

Open Protocol

**Files and Data Sources**

**Data Source Windows**

WinList creates a data source window for each open listmode file. These windows are moveable and sizable within the confines of WinList's main window. Data source windows are automatically closed when WinList closes. They can contain any number of histograms and multiple pages of histograms.

To create a new data source window, click the Open FCS button on the ribbon or toolbar.

To read a new data file into an existing data source window, click the Next FCS button on the ribbon or toolbar.
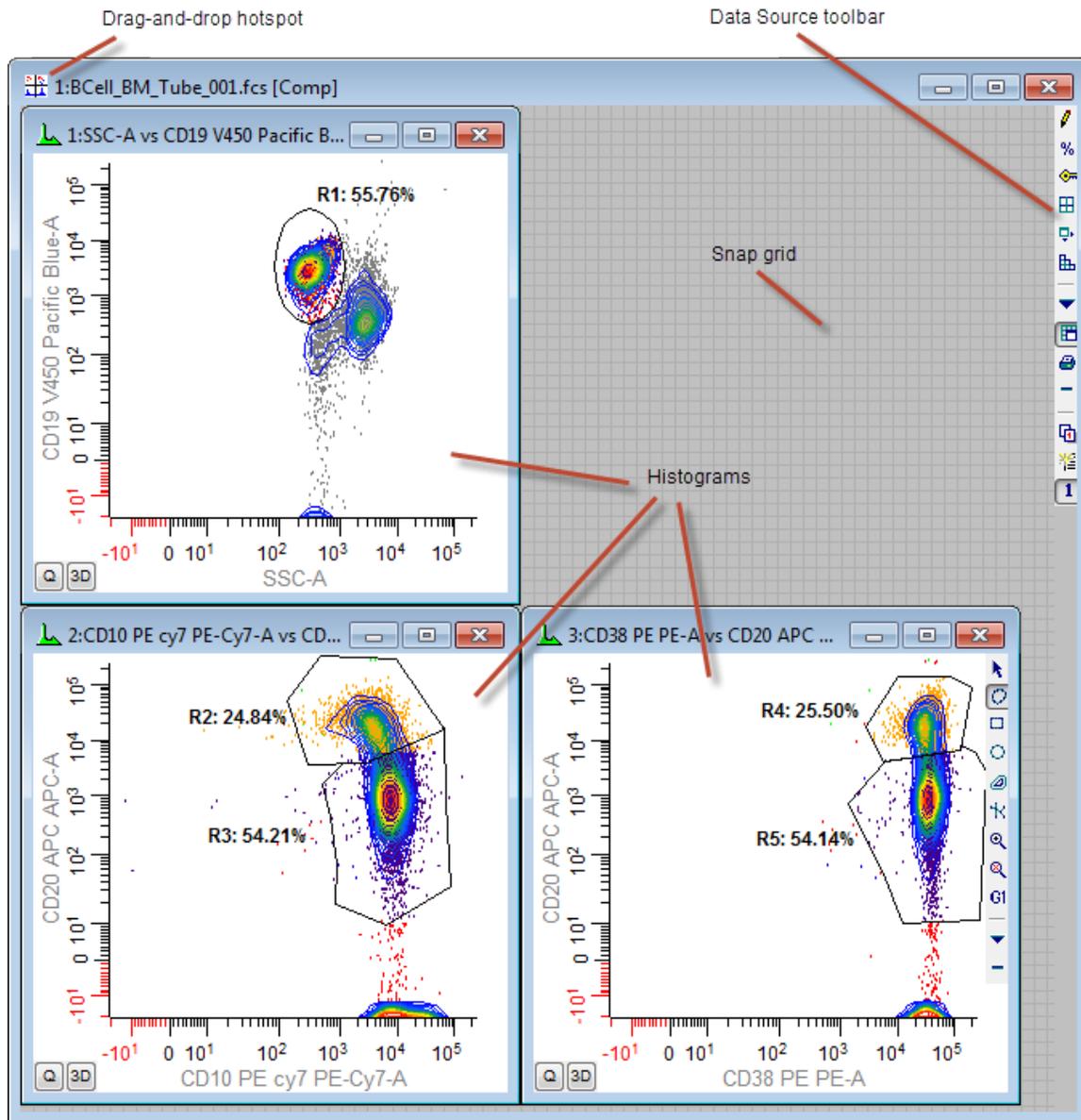
A toolbar is positioned on the right edge of the data source window to provide quick access to many common commands. For details, see [Data Source Toolbar](#).

A data source window can be dragged and dropped onto a Report window to add all of its histograms to the report. The drag-and-drop hotspot is the upper-left portion of the title bar.

To edit a data source window, select **Edit Data Source** from the **Edit** menu. Alternately, you can double-click its title bar or click the Edit button on the data source toolbar.

Right-click a data source window to display its context menu.

**Data Source Toolbar**

This toolbar appears inside the right edge of the active data source window. Each icon represents a tool, described below.

- ✐ Edit the data source
- % Display gate statistics for the data source
- ⊕ Display file keywords

- ⊞ Arrange histograms
- ⊡ Scale histograms to fit the data source
- ⊞ Create Compensation Page

- ▼ Display the context menu
- 臣 Toggle snap mode on and off
- 🖨 Print the data source
- – Hide the toolbar

- ⊡ Display the Page menu
- 🗋 Create a new page
- ⊡1 Select a page to display
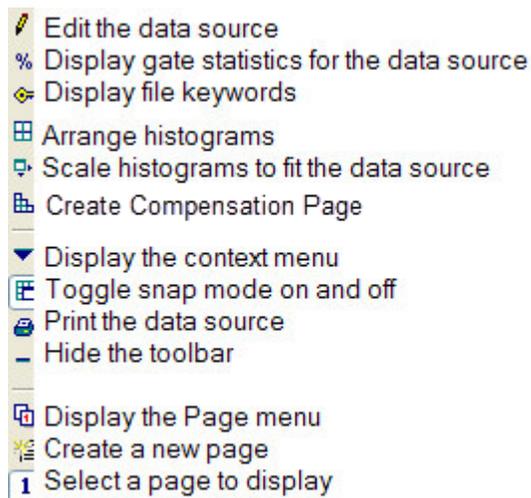
Many of these commands are explained elsewhere, but a few are only available through the toolbar. These are explained in more detail below.

**Compensation Page**

This button creates a special page in the data source for compensation, and sets up a SPLOM.  A SPLOM (Scatter Plot Matrix) is a collection of histograms representing all combinations of a selected set of parameters. It is a very useful arrangement to use when setting up N-Color compensation, because it presents all relevant histograms in a very logical layout.

When you click the Compensation Page button on the toolbar, you are presented with a dialog that allows you to choose the parameters you want to display and several other options. After making parameter selections and clicking OK, WinList creates all of the histograms and arranges them appropriately on the data source.

**Page menu**

The page menu allows you to add, rename, and delete pages in the data source. Use pages to group histograms when there are too many to display at once. For example, you may create a Splom arrangement of histograms on one page to review compensation, and use another page to set up gating and analysis.

The page menu also allows you to select a page to view. This can also be done by clicking on the title bar of the data source and using the PgUp and PgDn keys on the keyboard.

**Context menu**

The context menu for the data source window contains many common commands. It can be displayed by clicking the Context menu button on the data source toolbar, or by right-clicking inside the data source window.

For more information, see Data Source Context Menu .

**Snap**

This button enables or disables snap-to-grid moving and sizing of histograms within a data source. With the option enabled, histogram windows move and size based on the background grid displayed on the data source. This makes it easy to create histograms that are the same size and to align them neatly. With snapping disabled, histograms can be moved and resized without constraint.

**Data Source Context Menu**
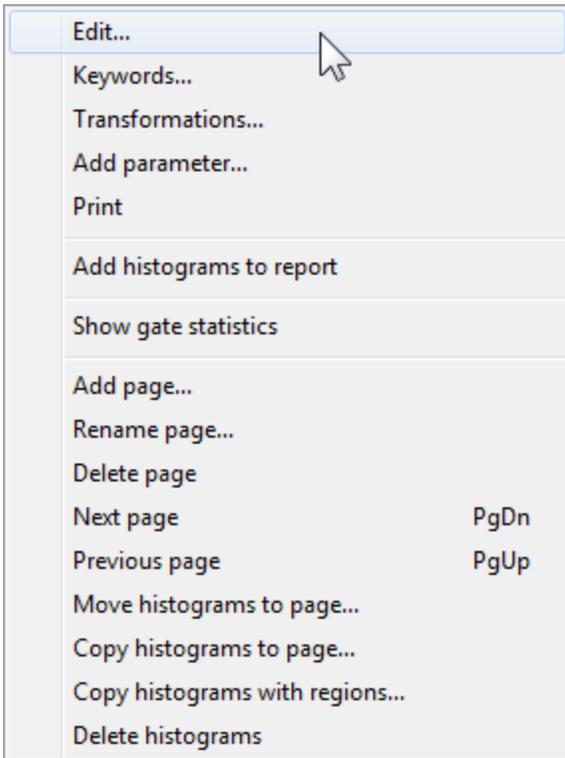
Edit...
Keywords...
Transformations...
Add parameter...
Print

Add histograms to report

Show gate statistics

Add page...
Rename page...
Delete page
Next page                    PgDn
Previous page                PgUp
Move histograms to page...
Copy histograms to page...
Copy histograms with regions...
Delete histograms

The context menu for the data source window contains many common commands. It can be displayed by clicking the Context menu button on the data source toolbar, or by right-clicking inside the data source window.

**Edit**

This command displays the Edit Data Source dialog.

**Keywords**

This command displays a list of all FCS keywords found in the current data file.

**Transformations**

This command displays the Edit Transformations dialog.

**Add Parameter**

Displays the Add Parameters dialog to create calculated parameters.

**Print**

This command prints the histograms on the current page of the data source.

## Add Histograms to Report

This command adds the histograms on the current page of the data source to the active report. The histograms on the report are arranged in the same way they are on the data source window.

## Show Gate Statistics

This command displays the Gate Statistics window.

## Add Page

This command adds a new, blank page to the data source.

## Rename Page

This command renames a page.

## Delete Page

This command deletes a page and the histograms it contains.

## Next Page

This command displays the next page in the data source.

## Previous Page

This command displays the previous page in the data source.

## Move Histograms to Page

This command moves the histograms on the current page to another page in the data source.

## Copy Histograms to Page

This command copies the histograms on the current page to another page in the data source.

## Delete Histograms

This command deletes the histograms on the current page.

**Open FCS File**
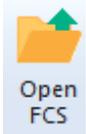


This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

Use this option to select a listmode file you want to open into WinList for display and analysis. When selected, the Open dialog box appears, allowing you to navigate to the correct folder to select files.

The dialog allows you to select one or more files from a folder. If you select more than one file, the program automatically creates a"batch" containing the selected files. When the Open button is clicked, the first file will be read. To process the additional files, use the Next Batch File and Previous Batch File commands, or edit the batch with the Open Batch command.

If the listmode file contains more than one *dataset*, the Choose Dataset dialog is displayed to select the dataset of interest. When there is only one dataset in the file, the Choose Dataset dialog does not appear.

After selecting a file to open, the Create Multiple Histograms dialog box is displayed to provide a quick and efficient means for selecting any number and types of histograms for display. This dialog can simply be canceled if you prefer to create histograms one at a time.

You can select **Open FCS File** more than once to display more than one file at a time, or multiple copies of the same file. WinList is designed to handle any number of data sources. Your computer's memory, disk resources, and the demands of other applications that may be running limit the actual number you can display.

## Open Dialog Box

This dialog box is common to most file opening operations in WinList. The tree on the left allows you to select the folder where files of interest are located. A list on the right displays files for the selected folder. A drop down list below the file list displays the types of files (file filters) that you see in the list. Buttons on the bottom allow you to open the file, view keywords stored in the file, or cancel the operation.

When opening *listmode data files*, this dialog allows you to select more than one file at a time. If you select more than one file, a batch is automatically created for you, and a convenient toolbar appears at the bottom of the main window to allow easy navigation through the batch. See Batch toolbar for details.
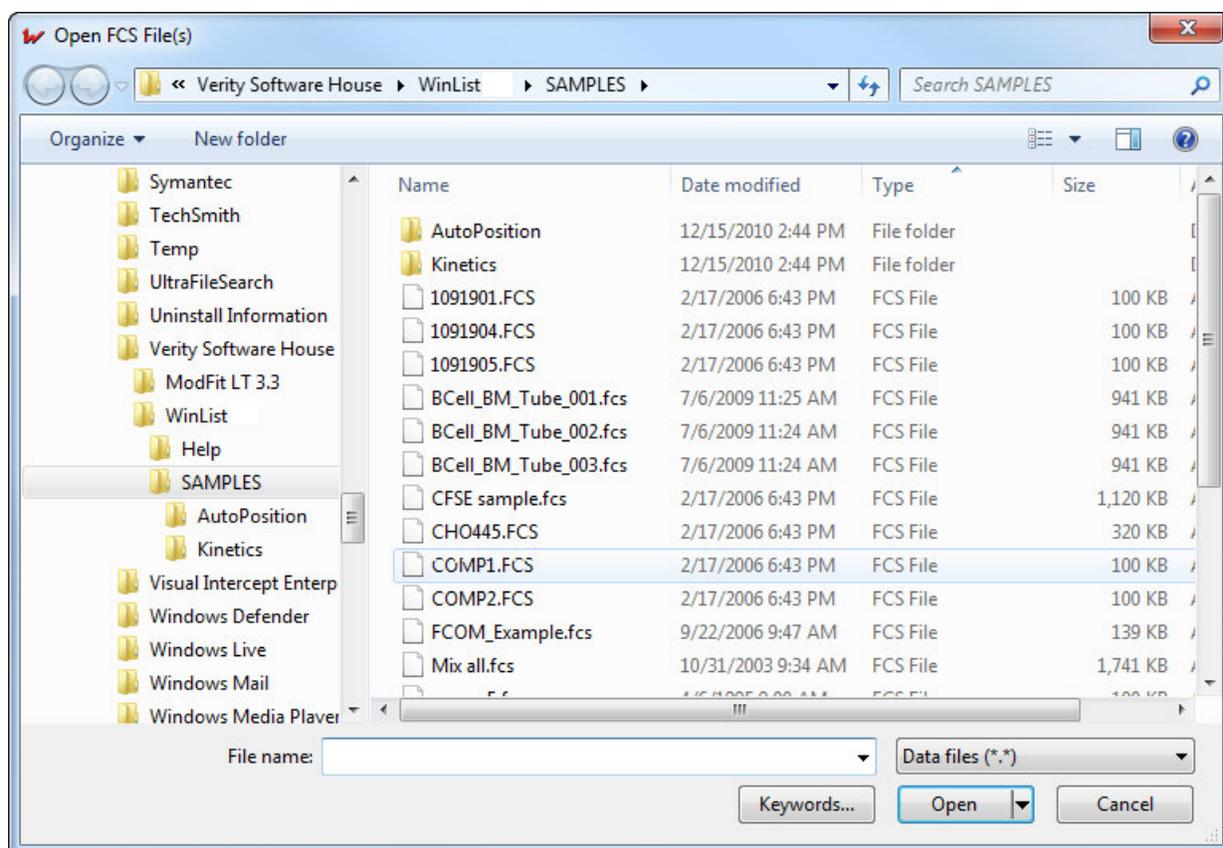


## Drag and Drop

You can drag and drop listmode files from the file system onto an existing data source window to add files to a batch.

**Choose Dataset**

This dialog appears when WinList detects more than one dataset in an FCS listmode file. It allows the user to select which dataset to read. The dialog displays a list of all datasets in the listmode file, along with the FCS version and two keywords. The keywords displayed are user-programmable in the Miscellaneous Preferences dialog.
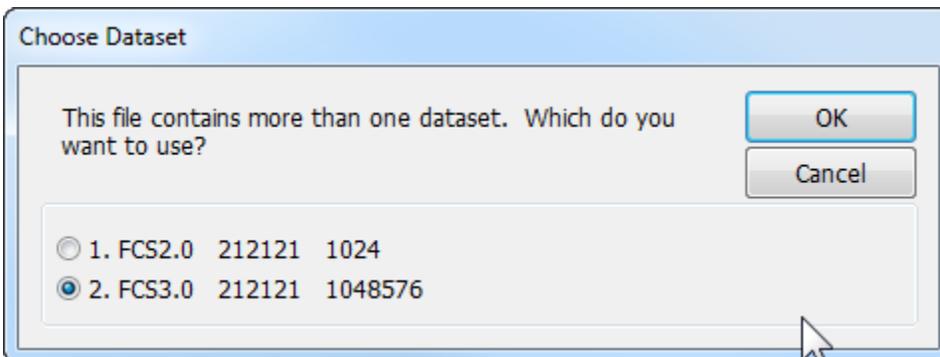


To select a dataset, click the radio button next to it in the dialog and click OK.

When you need WinList to display several datasets from the same file at the same time, open the file with Open FCS File and select a different dataset each time.

**Next FCS File**



This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

This menu option is used to replace the data file for the current data source with another data file on disk.

The Open dialog box is displayed so that you can select a new file. To open the new file, select it in the list box and click **Open**.

This dialog allows you to select more than one file at a time. If you select more than one file, a batch is automatically created for you, and a convenient toolbar appears at the bottom of the main window to allow easy navigation through the batch. See Batch toolbar for details.

**Previous FCS File**

This menu option is used to replace the data file for the current data source with another data file on disk. It operates the same way as the Next FCS File, except that it selects the previous file from the list.

**Duplicate Data Source**
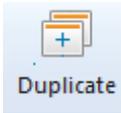


This command is found on the **Insert** tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.

This command creates a copy of the data source window, duplicating the properties of the active data source and its histograms, regions, and gates. This provides a convenient means of setting up for multiple file analysis and keeping the setup of each data source consistent with the one from which it was created. One common use for this is in histogram subtraction, where one file contains control data and another file contains test data.

The layout of histograms, regions, and gates are replicated in the new data source window.

### Edit Data Source

This dialog is displayed by choosing **Edit** in the data source context menu or by double-clicking on the Data source title bar.
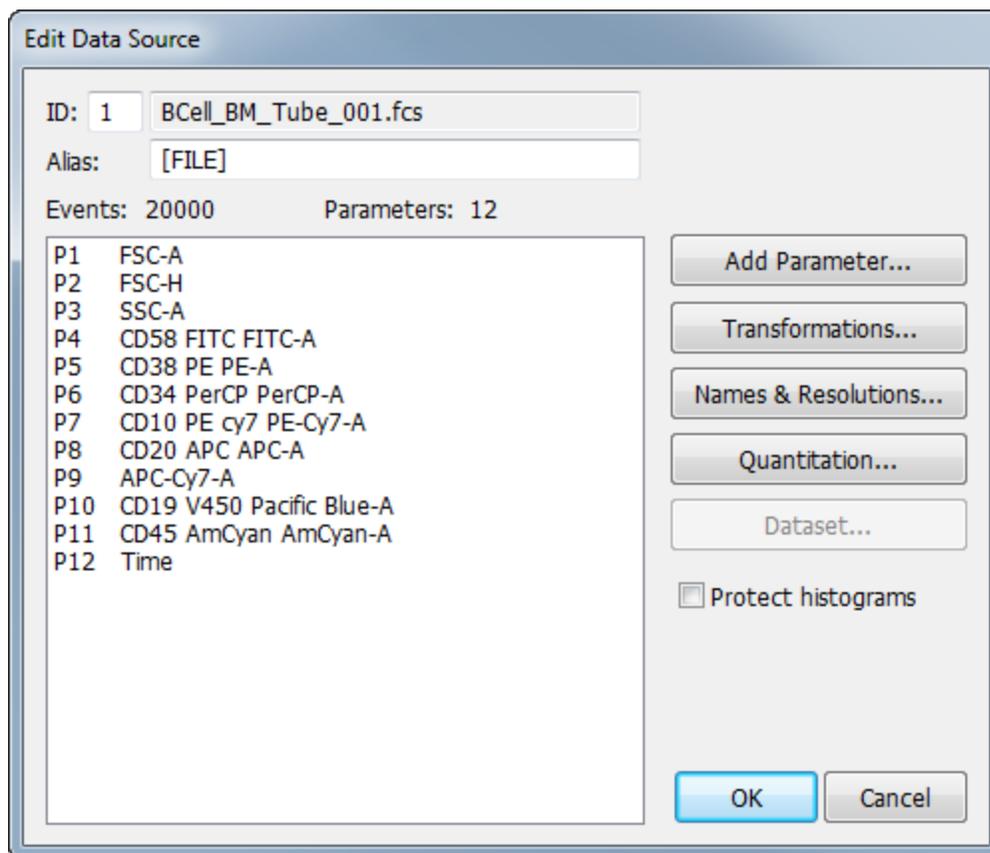


**Name** and **ID**

The field displays the name and ID of the file data source. The ID is initially assigned by WinList, but it can be edited by the user. Each data source must have a unique ID.

**Alias**

Each data source has an alias that is displayed on its titlebar. By default, the alias is [FILE], which is a keyword that returns the name of the data file currently opened. You can enter another keyword or text in this field.

**Events** and **Parameters**

This displays the total number of events and the number of parameters stored in the data source listmode file.

**Parameter listbox**

This list shows the parameter names stored in the data file.

**Add Parameters**

This option allows you to add calculated parameters to the data source. See Add New Parameter for more.

When a parameter is added or deleted, the Edit Data Source dialog is automatically closed.

**Transformations**

This option is allows you to select how each parameter is displayed in WinList. See Edit Transformations for more.

**Names and Resolutions**

This button displays parameter names and resolutions for the data file. This also allows the user to adjust the log decades, if any, in the event that WinList was not able to automatically detect them correctly. See Names and Resolutions for more.

**Quantitation**

Selecting this option allows you to convert results values into quantitative units. See Quantitation for more.

**Dataset**

The option is enabled if the FCS file contains more than one dataset. It allows you to select which dataset to read and analyze with WinList.

When a new dataset is selected, the Edit Data Source dialog is automatically closed.

**Protect histograms**

When this option is enabled, histograms in the data source are protected from being deleted by the user.

**OK**

Accepts changes to the data source and closes the dialog box.

**Cancel**

This option closes the dialog box with no changes to the data source.

**Add Parameters**

This option allows you to create additional "calculated" parameters based on the native parameters of your listmode files. Calculated parameters can be displayed and manipulated just like native parameters. In addition to parameter ratios, WinList provides mathematical functions which can be applied to parameters.

All calculated parameters in WinList are computed in floating-point. They have a user-defined resolution, and can be computed using compensated or uncompensated data.

Display this dialog by clicking the **Add Parameter** button on the main ribbon bar or choosing the **Add Parameter** from the data source context menu.  There is also a button in the **Edit Data Source** dialog box that shows this dialog.
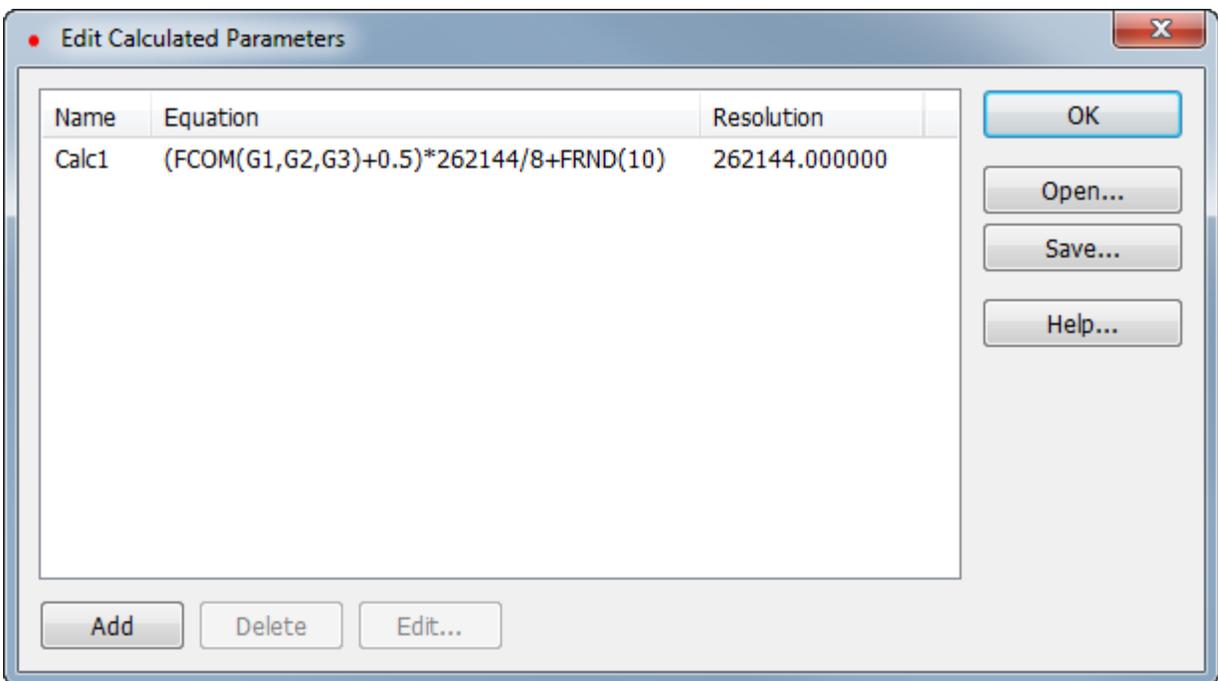
**Add**

Adds a new calculated parameter to the data source. The Edit Calculated Parameter dialog will be displayed.

**Delete**

Deletes the currently selected calculated parameter in the list.

**Edit**

Displays the selected calculated parameter in the Edit Calculated Parameter dialog for editing.

**Open**

Opens a previously saved set of equations. The files have the default suffix of equ.

**Save**

Saves the current set of equations to disk.

**OK**

Closes the dialog and accepts the changes.  This causes all calculated parameters to be re-evaluated and the workspace will be updated.


See also:

Edit Calculated Parameter

**Edit Calculated Parameter**

This dialog is used to create and edit calculated parameters.  It is displayed from the Add Parameters dialog.

One of the best ways to get started with a calculated parameter is to review the list of examples in the Functions listbox and select one that is similar to what you want to create.  With an example selected, it is a simple matter to edit the specifics of the equation for your data.

The **Name**  field can be any text you want.  This is the name that will be displayed for this new calculated parameter on histograms and in parameter lists.



**Equation**

Type an equation or select one from the example list to set this field. The equation can include parentheses for grouping, and supports +, -, *, and / operators. The parameters and

their names are shown in a separate list box. The parameter and function letters are not case sensitive. For example, p1*2.5 is the same as P1*2.5.

When the Equation field contains a **FSNE** function, the **Choose...** button is displayed next to the edit box. This button allows the user to select parameters for the FSNE function quickly and efficiently.

### Choose...

When the Equation field contains a FSNE function, the Choose button is displayed next to the edit box. This button allows the user to select parameters for the FSNE function quickly and efficiently.

### Parameters dialog box

All the available parameters and their names are displayed in a separate dialog box. The dialog will automatically be put away when you select either **Cancel** or **OK**.

### Functions

All functions and examples are shown in this list box. A drop-down list allows you to show functions, examples, or both in the function list. Notice that all the functions start with the letter F.

| | |
|---|---|
| **FCOM(g1, g2,...gn)** | Gate combination function. The argument is a list of any number of gates separated by commas. See FCOM Background for further explanation. |
| **FTIM(adcResolution)** | Creates a chronological time variable over the number of channels, **adcResolution**, specified in the argument. This parameter allows you to gate out bad runs with DNA or to even perform kinetics calculations (assuming you are not interested in absolute time). |
| **FSNE(p1,p2,...,g0,50,2)** | This function creates "t-SNE" (t-Distributed Stochastic Neighbor Embedding) parameters.  The t-SNE function does a great job of reducing dimensionality to 2 or 3 dimensions. |
| | One FSNE equation will create 2 or 3 t-SNE parameters in the output. |
| | The first "p" arguments are parameters for the t-SNE function.  Next is the "g" argument, which specifies a gate.  Perplexity (50) sets the number of effective nearest neighbors.  The last argument is 2 or 3, specifying the number of dimensions to return. |
| | See Cluster Identification with t-SNE for further explanation. |
| **FLOG(param)** | Returns the natural log of **param**. Negative numbers |

|  | and zero are evaluated to 0 since they are illegal values for log functions. |
|---|---|
| **FL10(param)** | Returns the log to the base 10 of **param**. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions. |
| **FEXP(param)** | Raises natural log to the **param** power. |
| **FE10(param)** | Raises 10 to the **param** power. |
| **FSQR(param)** | Returns the square of **param**. |
| **FCUB(param)** | Returns the cube of **param**. |
| **FPWR(base, power)** | Returns the value of **base** to the **power**. |
| **FRND(variance)** | Returns a random number with **variance** variability. If zero is entered as the argument, then it returns a uniform random number between 0 and 1. |
| **FSIN(x)** | returns the sine of x, where x is in radians. |
| **FCOS(x)** | returns the cosine of x, where x is in radians. |
| **FTAN(x)** | returns the tangent of x, where x is in radians. |
| **FCTN(x)** | returns the cotangent of x, where x is in radians. |
| **FASN(x)** | returns the arcsine of x, where x ranges from -1.0 to 1.0. Values for x outside of this range are forced to the nearest limit. |
| **FACS(x)** | returns the arccosine of x, where x ranges from -1.0 to 1.0. Values for x outside of this range are forced to the nearest limit. |
| **FATN(x)** | returns the arctangent of x. |
| **FACN(x)** | returns the arccotangent of x, equivalent to arctangent of 1/x. |
| **FV2S(param)** | Returns **param** to the 2/3's power. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions. |
| **FS2V(param)** | Returns **param** to the 3/2's power. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions. |

**Transform**

Sets the transform to be used for displaying the calculated parameter.

**Source**

---

In most cases, the calculated parameter can be computed on compensated or uncompensated data.  A few functions (FCOM, FSNE, FTIM) ignore of this setting.

**Resolution**

Choose a resolution to use for the new calculated parameter.  The list of resolutions is limited to the resolutions of parameters in the data file.

**OK**

Accepts the changes to the equation and other settings and closes the dialog.

**Cancel**

Cancels the changes to the question.  If the equation is being created, clicking the Cancel button will flag the equation to be deleted.

See also:

FCOM Background and Usage

**FCOM Background and Usage**

FCOM is a calculated parameter that can be used to classify events based on combinations of selected gates. It is commonly used in phenotypic analysis. You can create FCOM parameters using the Add New Parameters dialog box, accessed from the Edit Listmode File Data Source dialog box.

To understand how this powerful function works, you must first understand something about binary numbers. A binary number is represented with only two digits: 0 and 1. A binary number is formatted similarly to a decimal number using powers of 2 instead of powers of 10. An example of a binary number is 1110. The decimal equivalent to this number is $0 + 2 + 4 + 8 = 14$

The lowest four digit binary number is 0000 (0 decimal) and the highest four digit binary number is 1111 (15 decimal). Thus, a four digit binary number can encode 16 different numbers (0,1,...15). The number of encoded different numbers (n) is related to the number of binary digits (d) by the function $n = 2$ to the d power. Thus, for this example, d=4 and n=16.

What does this all have to do with the FCOM parameter? Plenty! Since an event is either inside or outside a gate, its state can be represented by a single digit, 0=outside and 1=inside. Suppose we have two gates, G1 and G2. We can represent all possible combinations of gate states by simply forming a binary number where the first digit represents the state of G1 and the second digit represents the state of G2. Thus if we have two gates, then the binary number 01 represents an event outside G1 and inside G2. Similarly, 11 represents an event inside G1 and inside G2. The FCOM function assigns each event a decimal number reflecting the gate combination for that event. Thus, in the case of 2 gates, G1 and G2, FCOM will return a value of 0, 1, 2, or 3 depending on the particular gate combination satisfied.

If we multiply this FCOM number by a scaling value and we display the result as a single parameter histogram in WinList, we will see a number of spikes with the FCOM parameter on the X-axis. The height of each spike tells us how many events satisfy that particular gate state. Typically, we add a small random number to each FCOM number (e.g. FRND(10)), so that instead of spikes we see Gaussian distributions which are more familiar to us.

How do you know what combination corresponds to each peak? Easy! You just count in reverse binary, which is to say evaluate from left to right." Let's do a three gate example (e.g. FCOM(G1,G2,G3)).

| G1 | G2 | G3 | FCOM Derivation | Calculated FCOM value |
|----|----|----|-----------------|-----------------------|
| 0 | 0 | 0 | 0 + 0 + 0 = | 0 |
| 1 | 0 | 0 | 1 + 0 + 0 = | 1 |
| 0 | 1 | 0 | 0 + 2 + 0 = | 2 |
| 1 | 1 | 0 | 1 + 2 + 0 = | 3 |
| 0 | 0 | 1 | 0 + 0 + 4 = | 4 |
| 1 | 0 | 1 | 1 + 0 + 4 = | 5 |
| 0 | 1 | 1 | 0 + 2 + 4 = | 6 |
| 1 | 1 | 1 | 1 + 2 + 4 = | 7 |

OK, that's not so easy after all.  It would be better if the program figured out which population was which and labeled them for us.  This is something that the Create Region Array command does nicely.  There's an example of that in the tutorial Using FCOM to enumerate phenotyptes.

You can create additional FCOM calculated parameters with different gate combinations to display two parameter dot plots. With some creativity on your part, you can readily visualize and analyze complex phenotypes with relatively little effort.

**Edit Transformations**

This dialog allows you to select linear parameters to convert from linear to log and log-like transforms.
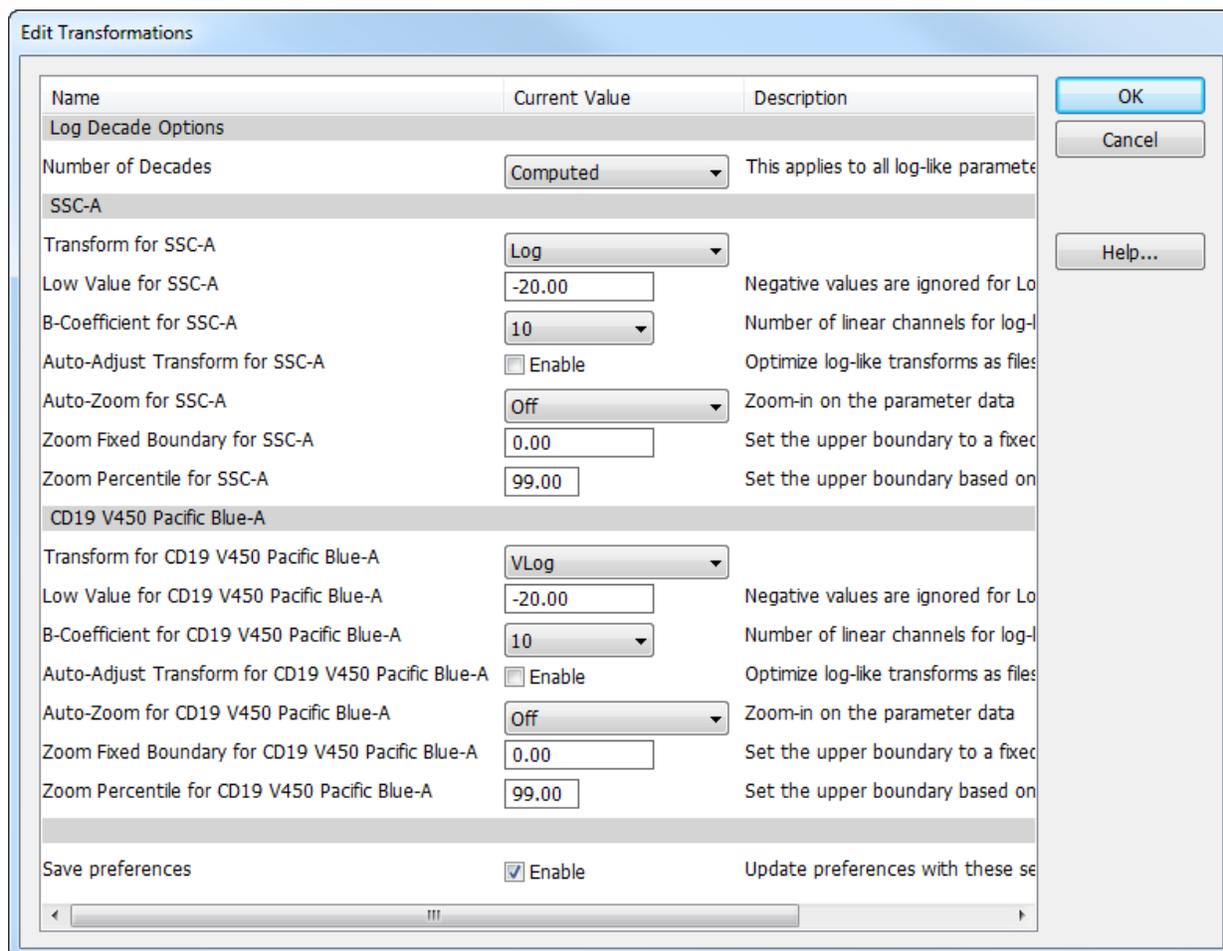


Most flow cytometers store data in linear format, even if you view the data on log-like scale during acquisition and analysis. This dialog allows you to convert linear parameters to log-like for display and analysis in WinList.

**Log Decade Options**
**Number of Decades**

This option determines how many log decades WinList will transform the linear data values into.

Choose *Computed* to allow the program to determine automatically the number of decades. With this option selected, the program computes the number of decades by taking the log of

the ADC resolution of the parameter. For example, if the ADC resolution is 262144, the log10(262144) is approximately 5.42 decades.

Choose *4-decades* to force the program to map the linear data into 4-decades. This option allows comparisons with other software that only permits 4-decade displays. In the example above, compressing 262144 channels into 4-decades of log space compresses the data by 1.42 decades.

**Parameters**

For each parameter in the file, you can set several properties. Transform settings apply to all histograms showing the selected parameter.

**Transform**

The transform can be set to Linear, Log, HyperLog, Hyperbolic sine, or VLog.

**Low Value**

This is the lowest value shown on the axis.

**B-Coefficient**

This value controls the linear portion of a HyperLog axis. The selected value is the number of channels on both sides of 0 that will be displayed as linear. The remaining portions of the HyperLog axis are display log-like.

**Auto-Adjust**

Enable this option if you want WinList to automatically adjust the B-Coefficient and Max Negative value for this parameter. Each time a new file is opened, WinList will readjust B and Max Negative settings.

**Auto-Zoom**

Auto-zoom allows you to eliminate unnecessary channels at the upper end of a parameter's dynamic range automatically. It is very useful for parameters like "Time" which only use a portion of the range, and for some instruments which store data in the lower portion of the range. Auto-zoom is set up independently for each parameter.

**Off**: Turns off auto-zoom for the parameter.

**To highest used channel**: Choose this option if you want WinList to eliminate empty channels on the upper end of the parameter. It is useful, for example, for a TIME parameter that typically only uses a small portion of the dynamic range of the parameter.

**To fixed upper boundary**: This option allows you to zoom to a specific channel for the upper boundary of the data. Use the associated **Zoom Fixed Boundary** edit box to set the channel for zooming.

**To percentile**: This option allows you to zoom in on a specified percentile. If, for example, 99% of your data is in the lower half of the dynamic range, it may be useful to ignore the remaining 1% of the data so that you can better visualize and analyze the 99%. Use the associated **Zoom Percentile** edit box to set the percentile for zooming.

**Save preferences**

When this option is enabled, the changes you make to the Transformations dialog are remembered.

**See also:**

Transformation Preferences

Understanding Hi-res Data Files

**Names and Resolutions**

This dialog box can be accessed from the **Edit Data Source** dialog box by clicking the
**Names and Resolutions** push button. It allows the user to change the names of
parameters, as well as set the number of log decades for any parameters.
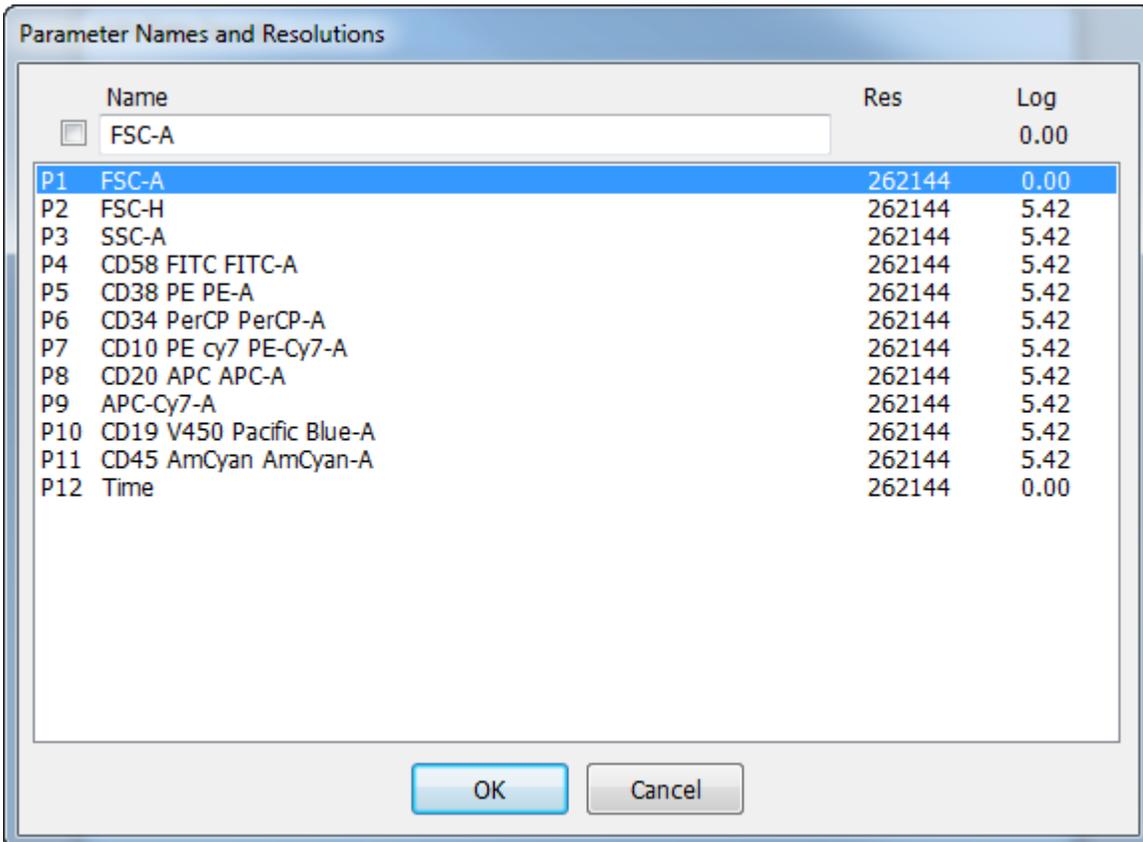


Parameter names are used by histograms on the axis labels.

To edit a parameter name, select the parameter in the list box and enter the new name for
the parameter in the **Name** edit box. You can enter a keyword in square brackets, e.g.
[$P4S], to display information derived from the FCS file for the parameter name.

The number of log decades for parameters is displayed for informational purposes.  The
values can not be edited.

Whenever a name is edited by the user, WinList "locks" the name so that it does not change
until the user "unlocks" the name, or the number of parameters changes. Locked names are
designated with an asterisk (*) next to the name in the list box.

To manually lock or unlock a parameter name, select the parameter in the list box and click
the **Lock** check box next to the name. If the check box is checked, the name is locked.

If you edit parameter names and you do not lock the edited entries, WinList will discard your edits the next time it updates the data source by replaying, opening a new file, or saying **OK** to the Edit Data Source dialog box.

**Quantitation**

WinList provides complete support for converting results values from channel-based units to units representing a quantitative value. Some of the units commonly used for quantitation are Antibody Binding Capacity (ABC), Mean Equivalent Soluble Fluorescein (MESF), and Molecules Per Cell. The great advantage to converting results into quantitative values is that they become meaningful when compared with data from other instruments and other users. Standardization among users and labs becomes a much simpler task.

If you have access to the quantitation calibration information, you can enter it manually into this dialog box.

If your data files contain special keywords, WinList can automatically apply calibration information to the relevant parameters and convert results into quantitative units. The keywords WinList looks for are PnQS, PnQI. The first defines the slope for parameter n; the second defines the intercept for parameter n. Some instrument manufacturers, BD Biosciences for example, store these keywords when acquiring data if relevant controls have been run.

The program can also automatically turn "TIME" into a quantitative parameter if there is a $TIMESTEP keyword in the data file.
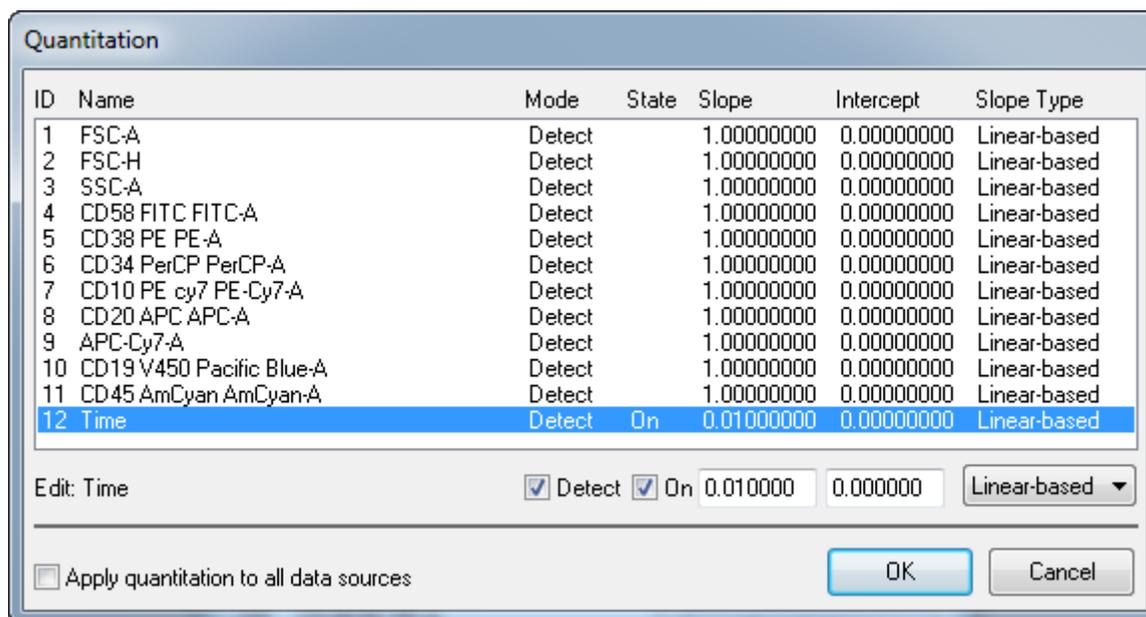


**Detect**

This option is normally checked, which tells WinList to automatically detect quantitation for the parameter. In this mode, WinList will look for quantitation keywords in the file and will apply the quantitation automatically.  Most parameters are safely left with Detect enabled.

Uncheck this option if you want to enter you own slope and intercept values, or you do not want to detect quantitation keywords automatically.

### On

If this option is checked, the parameter is enabled for quantitation.  The slope and intercept values will be used to convert the parameter transformation into quantitative units.

### Slope and Intercept

The slope and intercept provide information on how to convert an ADC channel number to a quantitative value. The values are typically provided by the instrument or reagent manufacturer to allow you to convert from "channels" into some other quantitative unit.

To compute your own slope and intercept values, you need to perform a linear regression of log (molecules per cell) versus channel numbers. WinList includes an Excel spreadsheet in the Samples folder that can be used to assist in this. See Using the Quantitation Spreadsheet for details.

### Slope Type

The quantitative slope and intercept are typically defined using linear units. The $TIME parameter, for example, is a linear parameter, its slope is determined by the $TIMESTEP keyword, and the Slope Type should be "Linear".

However, there are cases where the slope and intercept relate to log-transformed units. For example, with older FCS files that were encoded in log, the slopes and intercepts provided by manufacturers were typically log-based.  This means that they are applied to the log-transformed data rather than the linear form of the data.

Select the appropriate slope type for the quantitation for your files. If the quantitative information is supplied by a manufacturer, contact the manufacturer to determine whether the slope applies to linear units or log units.

### OK

Click this button to accept the changes you have made and close the dialog.

### Cancel

Click this button to cancel the changes you have made and close the dialog.

## Using the Quantitation Spreadsheet

WinList includes an Excel spreadsheet that can assist in using its quantitative features. The most versatile section of the spreadsheet is the 4-Bead Generic Conversion.

**4-Bead Generic Conversion**

| | | * Enter * | | * Enter * |
|---|---|---|---|---|
| | ADC range: | 1024 | | |
| | Decades: | 4.0000 | | |
| | | * Enter * | | * Enter * |
| | | Linear Median | | Linear MESF |
| | Blank | 0.00 | | 0 |
| | Bead 1 | 12.30 | | 2000 |
| | Bead 2 | 42.30 | | 8000 |
| | Bead 3 | 76.90 | | 15000 |
| | Bead 4 | 251.70 | | 55000 |
| | | | | |
| | | Slope | | Intercept |
| | WinList >> | 0.0042822228 | | 2.110870406 |
| | Quantitation | | | |

### To determine the Slope and Intercept for WinList:

1. In WinList, open the data file for the calibration beads. Using a 1P histogram, find the median values for each bead peak. An example is shown in the graphic below.
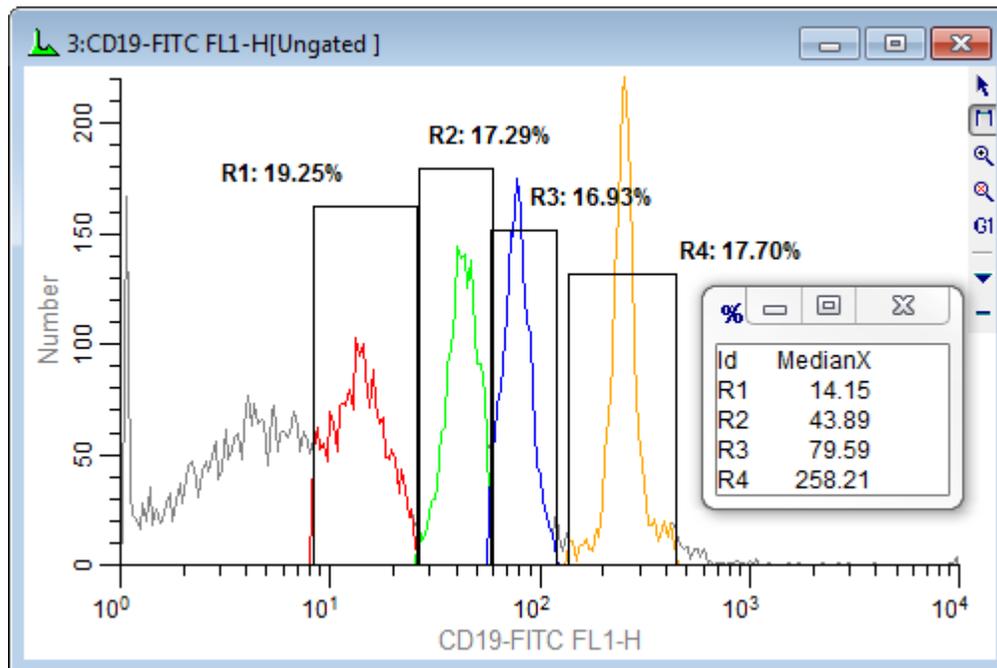
2. In Excel, open the WinlistQuantitation5Bead.xls spreadsheet found in the Samples folder. Enter the ADC range and the number of log decades for the data files that you are analyzing.

3. In the spreadsheet, enter the median values from WinList. Then enter the MESF (or ABC) values provided by your quantitative bead manufacturer.

4. Copy the Slope and Intercept from the spreadsheet into the Quantitation dialog in WinList.



WinList will now display quantitative results and axes for the parameter.
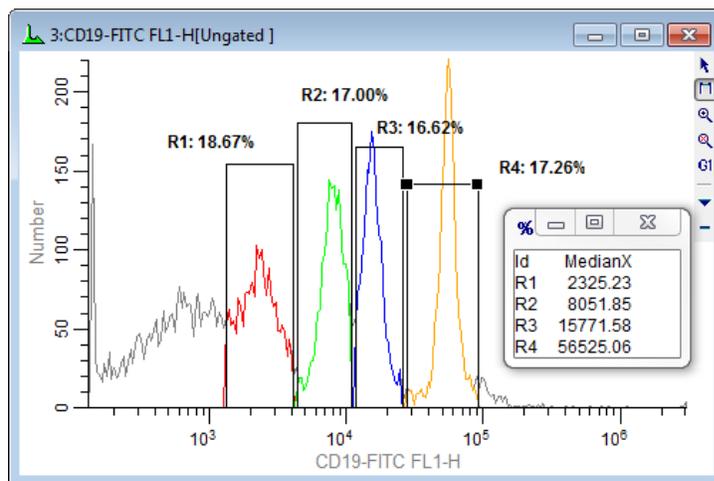
**Compensation**

**Compensation Toolbox**



This command is found on the **Home** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display. The Compensation Toolbox allows you to control WinList's interactive N-Color Compensation™ and normalization.

WinList's N-Color Compensation™ can handle any number of fluorochromes, and compensate them for signal crossover. In many cases, compensation can be set up automatically, but you can still manually override compensation settings. Compensation can be manually adjusted using 2-parameter dot plots.

If you have single-color controls as well as an unstained control file, you can use **V-Comp™** to set up your compensation automatically.  See Compensation the Easy Way: V-Comp for details on how to do this.

In order to set up compensation manually, you need a set of single-color control files, one for each fluorochrome you want to compensate. Using Interactive N-Color Compensation demonstrates this approach.

WinList also supports reading compensation information directly from FCS files created by many popular cytometers, as well as files containing the FCS 3.1-compliant $SPILLOVER keyword.

See also:

Compensation the Easy Way: V-Comp

Using Interactive N-Color Compensation™

Compensation Toolbox dialog

Compensation Trace Lines

**Compensation Toolbox dialog**

This dialog is displayed by clicking the **Comp** button on the ribbon bar or selecting **Compensation Toolbox** from the Options menu. It provides control over various components of the N-Color Compensation™ and the normalization system.
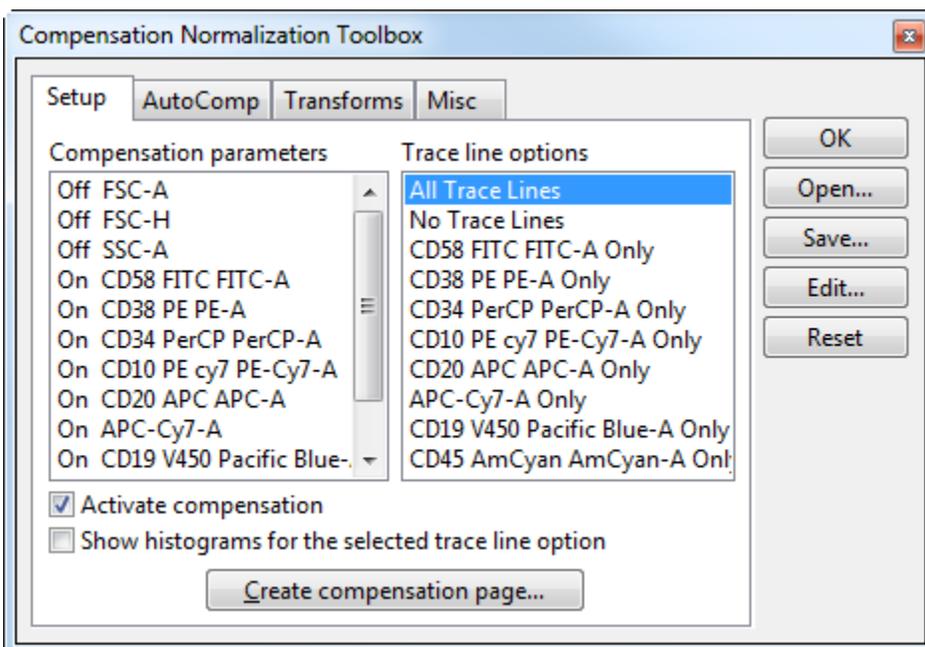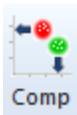




The dialog is separated into several tabs that group similar features together.

Compensation: Setup Tab

Compensation: AutoComp Tab

Compensation: Transforms Tab

Compensation: Miscellaneous Tab

**OK**

Click this button to close the dialog. This does *not* turn compensation off, it simply hides the dialog. To turn compensation off, uncheck the **Activate compensation** checkbox.

**Open**

Click this button to select a previously saved compensation file to apply to the current data source.

**Save**

Click this button to save compensation settings to disk for later use.

**Edit**

Displays the Edit Properties for Compensation System dialog. This dialog allows you to review and adjust settings in the compensation and normalization system.

**Reset**

Turns off compensation, clears the compensation matrix, and re-initializes all compensation and normalization properties.

**See also:**

Using Interactive N-Color Compensation™

Compensation Trace Lines

## Compensation: Setup Tab
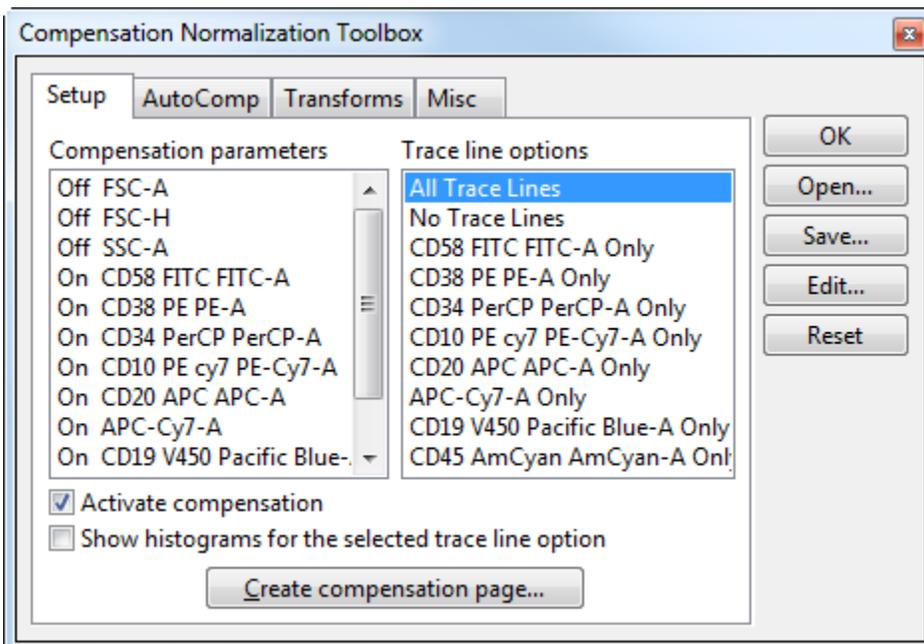


### Compensation Parameters

This list displays the parameters in the active data source. Click an item in the list to toggle it between **Off** and **On**.

### Trace Line Options

This list shows the options for displaying compensation trace lines. Trace lines set the slope and intercept used in the compensation system. They only appear when the **Activate** option is *not* checked. Display the trace lines for the parameter that you are currently compensating.

When you select one of the parameters in this list, WinList computes the slope and intercept so that the trace lines are properly positioned for the populations. If you have already positioned the trace lines, WinList will ask if you want to re-position them again. Use this feature with single-color controls for each parameter involved with compensation.

### Activate compensation

Enables or disables the N-Color Compensation and normalization systems. When unchecked, compensation and normalization is turned off. When checked, compensation and normalization are turned on.

Note: Trace lines disappear when compensation is activated. They are replaced by small **+** and **-** buttons. To hide these buttons, select the No Trace Lines option.

**Only show histograms for the selected Trace Line Option**

This option is useful when you have many histograms displayed and you are trying to set up compensation for your data. With the option checked, only the histograms that relate to the selected parameter in the Trace Line Options list will be shown.

**Create Compensation Page**

In order to effectively work with WinList's compensation system, you need to display a 2P histogram for each combination of parameters that are enabled for compensation. Click this button to automatically create a new page with all of the 2P histograms required to set up compensation.

**See also:**

Compensation Tool Box

Compensation: AutoComp Tab

Compensation: HyperLog Tab

Compensation: Miscellaneous Tab

### Compensation: AutoComp Tab

This tab in the Compensation Toolbox controls whether WinList will attempt to set up compensation automatically, either from keywords in a file or from the compensation database. The initial settings for the dialog are determined by preferences (Data Source Preferences) and it can be set up differently for each data source in a protocol.
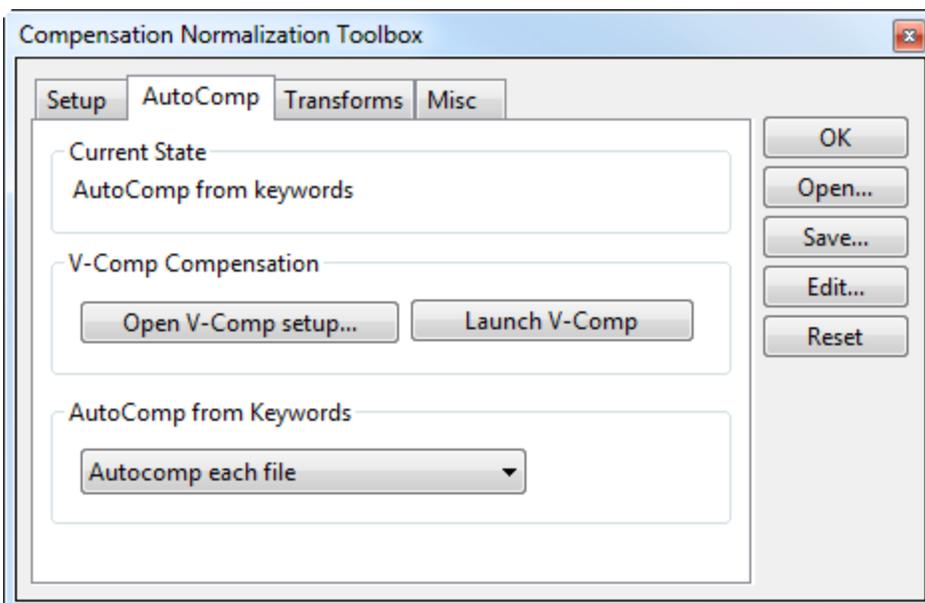


### Current State

This section displays the current state of compensation.

### V-Comp™ Compensation

This section provides quick access to the **V-Comp** stand-alone app, which can automatically compute compensation settings based on a set of single-color controls and an unstained control file.

### Open V-Comp setup

Click this button to select a V-Comp setup that you have already created.  A File Open dialog will be displayed showing the folder where V-Comp setups are typically stored.  Select the setup you want to use and click **Open**, and WinList will read the setup file and apply the compensation.

**Launch V-Comp**

If you have V-Comp installed on your computer, you can use this button to launch it directly from WinList.  This is useful when you want to create a new V-Comp setup or make edits to an existing setup.

**AutoComp from Keywords**

Many instrument vendors encode compensation information in the keywords of the FCS files they create. WinList can read and decode this information for many popular instruments.

| | |
|---|---|
| Disable | Do not use compensation from keywords. |
| Autocomp if not already set | Use keyword compensation values unless there is already compensation set up by a previous file or manually by the user. |
| Autocomp each file | Use keyword compensation values for each file. |

**See also:**

Compensation Tool Box

Compensation: Setup Tab

Compensation: Transforms Tab

Compensation: Miscellaneous Tab

## Compensation: Transforms Tab

This tab in the Compensation Toolbox offers a number of convenient commands to adjust transform settings and to toggle between different transformations for all parameters in the data source.
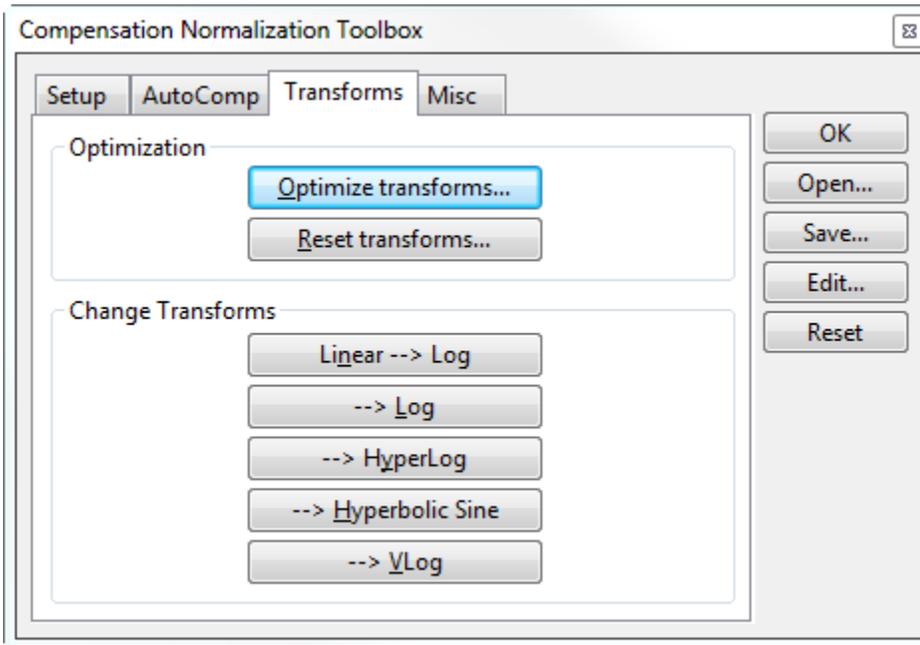


### Optimize transforms

This command triggers automatic adjustment of HyperLog, Hyperbolic Sine, and VLog transformations for selected parameters. A dialog is presented to allow you to choose which parameters to optimize. After selecting the parameters, click OK to begin the process.

WinList will determine the best B-Coefficient and the Low Value for each of the selected parameters.

### Reset transforms

This command resets the HyperLog™, Hyperbolic Sine, and VLog transformations for selected parameters. A dialog is presented to allow you to choose which parameters to reset. After selecting the parameters, click OK to begin the process.

WinList resets the B-Coefficient and the Low Value for each of the selected parameters, based on preference settings.

### Linear --> Log

This command switches linear parameters to the default log transformation. A dialog is displayed to allow you to choose the parameters to convert.

**- -> Log**

This command switches log-like parameters to use the Log transformation.

**--> HyperLog**

This command switches log-like parameters to HyperLog™ transformation.

**--> Hyperbolic Sine**

This command switches log-like parameters to Hyperbolic Sine transformation.

**--> VLog**

This command switches log-like parameters to VLog transformation.

Compensation Tool Box

Compensation: Setup Tab

Compensation: AutoComp Tab

Compensation: Miscellaneous Tab

### Compensation: Miscellaneous Tab
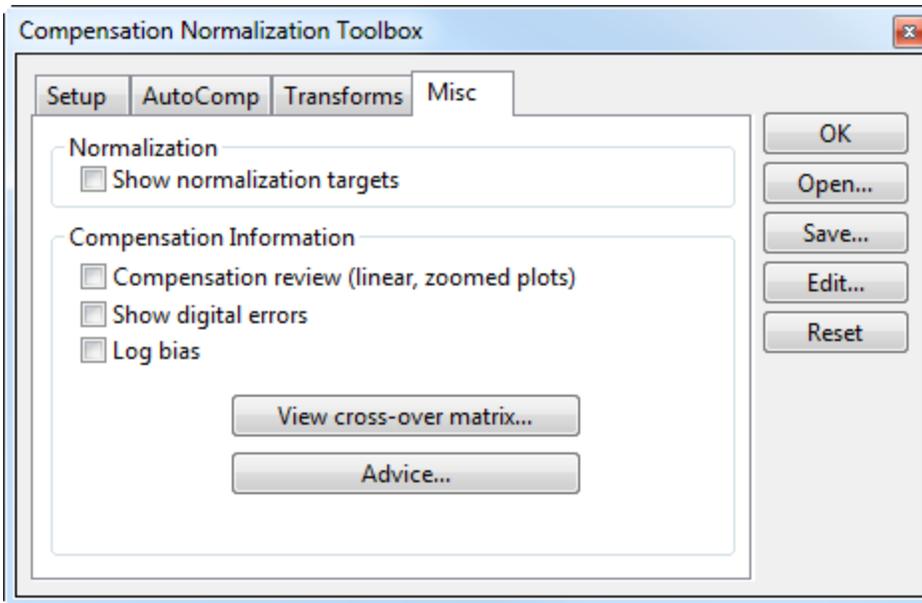


### Show normalization targets

Enable this option to display graphical elements that allow you to set normalization. Targets appear when the Activate option is enabled. See Normalization Targets for more information.

### Compensation review

This checkbox enables Compensation Review mode in WinList, a great tool for evaluating how well compensation matches the controls that were used to create it. See Compensation Review Mode for a walk-through.

### Show digital errors

When this option is enabled, the program draws dotted lines showing effects of digital error. The lines appear on 2P histograms that display parameters that are enabled for compensation in the Params to Compensate list. Use the Advice button to see information about the sources of digital error in the data.
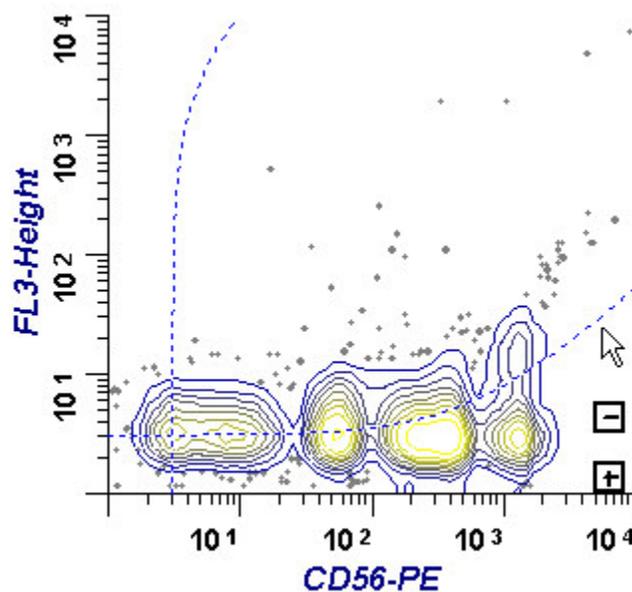
When analog data is converted to an integer, an error is introduced known as digital error. Normally this error is quite modest and presents little problem to cytometrists. However, when data is compensated, positive and negative errors are introduced that increase with parameter intensity. The digital error lines attempt to show the positive portion of the envelope of these error distributions for each parameter that is enabled for compensation. If intermediate intensity populations are present that tend to follow the digital error lines, it is possible that they are simply an artifact due to the effects of digital error.

**Log bias**

With the introduction of the HyperLog display, the Log Bias option is largely unnecessary. **It remains an option for backward compatibility but is not recommended.**

When checked, WinList applies a log bias value to events that would fall on the axis, simulating the bias in log amplifiers on older analog flow cytometers.

**Advice**

This command displays the Advice on Compensation dialog allowing you to view information about the compensation settings and sources of digital error in the data.

**View Cross-over Matrix**

This command displays the cross-over (also known as spillover) matrix for the current compensation settings. The matrix can be copied to the clipboard for use in other software.

**Compensation Trace Lines**

Trace Lines are graphical objects used to adjust N-Color Compensation in WinList. They appear on dual-parameter histograms when both parameters are enabled for compensation and the Trace Line Options are set to show trace lines for those parameters. The Compensation Toolbox controls these settings.
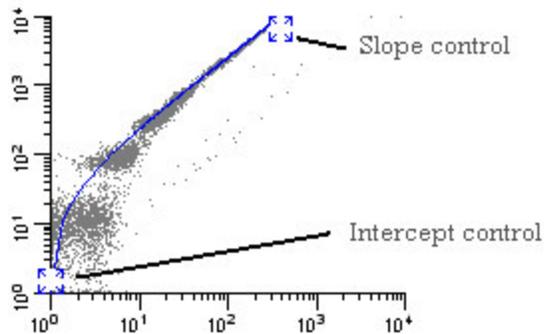


When displayed, a trace line can be moved by clicking on one of its handles. The high-intensity handle controls the slope of the trace line, and the low-intensity handle controls the intercept.

The Trace line is essentially setting the slope and intercept of a line through the center of the linearized data. Adjusting the handle in the low-intensity area sets the intercept. The slope is determined by the position of the handle in the high-intensity area relative to the intercept. As you increase the channel position of the slope handle, you increase the amount of compensation to be applied to the data, especially in the middle- to high-intensity range. Increasing the channel position of the intercept handle increases the compensation applied to the low intensity data.

In rare cases, you may need to apply more compensation than the trace lines normally permit. You can give greater freedom of movement to the trace lines by holding down the Shift key on the keyboard when moving its handles. This allows each trace line handle to move along two sides of the histogram, with slopes of greater than 1.0.
When the compensation is activated, trace lines are hidden and replaced by small **+** and **-** buttons. These buttons can increase and decrease the compensation settings with compensation activated.

See also:

Using Interactive N-Color Compensation™

Compensation Toolbox

Compensation Toolbox dialog

### Compensation Notice dialog

This dialog is displayed when a file with a different number of parameters is opened into a data source and some of the parameters in the data source are enabled for compensation.
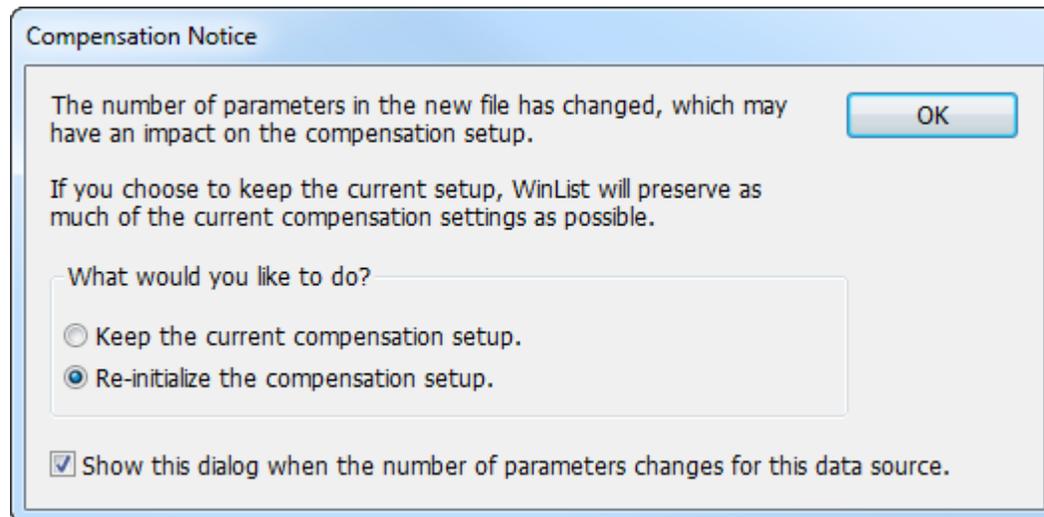


The first time WinList detects a change in number of parameters, it will display a dialog of choices for the user. The user can choose to maintain the existing compensation settings, to the extent that is possible, or to re-initialize the compensation system. The dialog has an option to not be displayed again, which applies to that data source.

**Keep the current compensation setup**

When this option is selected, the system makes temporary copies of all the compensation data structures. It then resizes the structures to suit the new number of parameters (larger or smaller), and copies what it can from the temporary structures. If the new file has more parameters, the new parameters will be turned off for compensation. If it has fewer parameters, the extra parameters are removed from the compensation setup. If compensation is turned on when this transition occurs, the compensation matrix will be reconstructed by inverting the crossover coefficients.

**Re-initialize the compensation setup**

When this option is selected, the system will turn off compensation, turn off the parameters selected for comp, and clear all of the crossover coefficients, normalization settings, and trace line settings.

**Show this dialog when the number of parameters changes in this data source.**

Enable this option to display this dialog anytime a file with a different number of parameters is opened, assuming that some of the parameters are enabled for compensation. Uncheck this option to suppress the dialog from appearing again.

**Edit Properties for Compensation**

This dialog is displayed when the **Edit** button in the Compensation Toolbox dialog is clicked. It allows you to edit many of the characteristics of the compensation and normalization system.
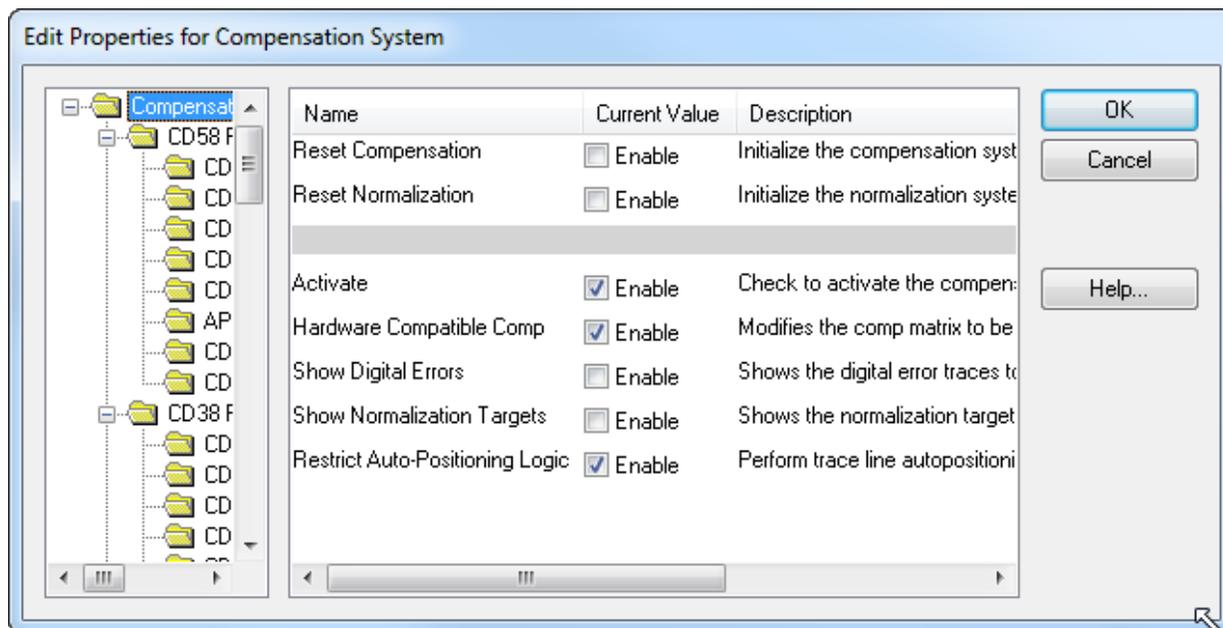


The left-hand pane of the dialog displays a tree of compensation objects. The right-hand pane displays properties for the compensation object selected in the tree. When the dialog is initially displayed, general properties of the compensation system are shown.

## Compensation System

This object shows general properties of the compensation and normalization system.

### Reset Compensation

Check this option to reset the compensation trace lines so that the data is uncompensated. The compensation matrix is initialized for all parameters in the active data file.

### Reset Normalization

Check this option to reset any normalization and restore the data to a non-normalized state. This affects normalization for all parameters in the active data file.

### Activate

Enables or disables the N-Color Compensation™ and normalization systems. When unchecked, compensation and normalization are turned off. When checked, compensation and normalization are turned on.

Note: Trace lines disappear when compensation is activated. They are replaced by small **+** and **-** buttons.

### Hardware Compatible Comp

When this option is enabled, the compensation matrix is constructed so that crossover is strictly subtractive. For example, these equations show hypothetical 3-color compensation with the Hardware option enabled:

```
Comp P3 = +1.000*P3 -0.010*P4 -0.000*P5
Comp P4 = -0.169*P3 +1.000*P4 -0.038*P5
Comp P5 = +0.008*P3 -0.283*P4 +1.000*P5
```

Notice that P3 is multiplied by 1.0 in the compensation of P3. This is also true for P4 in the P4 compensation, and P5 in the P5 compensation.

When the Hardware option is disabled, the compensation signal can be greater than 1.0 in the associated compensation expression:

```
Comp P3 = +1.002*P3 -0.011*P4 -0.000*P5
Comp P4 = -0.171*P3 +1.013*P4 -0.039*P5
Comp P5 = +0.008*P3 -0.286*P4 +1.011*P5
```

Notice that change in the expressions. In this setting, the loss of a signal due to signal crossover is adjusted for in the compensation matrix. This will result in slightly higher intensities than seen with the Hardware option enabled.

### Show Digital Errors

When this option is enabled, the program draws dotted lines showing effects of digital error. The lines appear on 2P histograms that display parameters that are enabled for compensation in the Params to Compensate list. Use the Advice button to see information about the sources of digital error in the data.

### Show Normalization Targets

Enable this option to display graphical elements that allow you to set normalization. Targets appear when the Activate option is enabled. See Normalization Targets for more information.

### Restrict Auto-Positioning Logic

When enabled, WinList uses a more restricted portion of histograms to determine how to automatically position compensation Trace Lines. For most cases, this option will have little effect on how the program positions the trace lines. In some cases where there is significant signal crossover, enabling this option can improve the auto-positioning.

## Signal Objects

Each parameter enabled for compensation has an associated *Signal* object in the tree control. When you select a Signal object, the following properties appear in the properties pane.

### Normalization Factor

This field shows the current normalization factor applied to the selected signal parameter. The default value is 1.0, which means that the parameter has no normalization applied. You can enter a specific factor to apply, or use the Normalization Targets to adjust normalization graphically.

### Max Negative Value

When the Use HyperLog option is selected above, this field determines the lowest value that the parameter will display in a histogram. Any events that compute to be lower than Max Negative Value will be set equal to it.

### B Linear Coefficient

This value specifies the number of linear channels displayed on the HyperLog axis, on both positive and negative sides of 0. It controls the visual compression of the populations that fall in that region. If populations near the origin split or spread too much, increasing the B Linear Coefficient will resolve the issue.

## Detector Objects

Each *Signal* object contains *Detector* objects, one for each of the other parameters that is enabled for compensation. When you select a Detector object, the following properties appear.

### Cross-over coefficient

This value represents the fraction of the *Signal* object parameter that is spilling over into this detector in the compensation system. A value of 1.00 (100%) is displayed for the detector associated with the signal parameter. A fractional value between 0.00 and 1.0 is displayed for the other detector objects. For example, a CD56-PE detector for a CD56-PE signal will display a value of 1.00. If 20% of a CD19-FITC signal is spilling into a PE detector, a value of 0.20 will be displayed for the PE detector of the CD19-FITC signal.

### Compensation coefficient

This read-only field shows the fraction of the signal to add or subtract from the detector. It is computed in the construction of the compensation matrix and is not editable.

### Digital Error Position

This read-only field displays the value between 0.0 and 1.0 that quantifies the digital error WinList computed for the parameter. Values of 0.85 and higher are relatively good, with little digital error. Values of 0.75 to 0.85 are fair. Values below 0.75 indicate that the parameter measurement contains a significant amount of digital error.

Use the Advice dialog to understand the sources of digital error.

**Advice on Compensation dialog**

This dialog presents information about compensation, normalization, and digital error for the active data file. Review the information in this dialog to determine the characteristics of compensation and the sources of digital error in your system.

**About Digital Error**

When analog data is converted to an integer, an error is introduced known as digital error. Normally this error is quite modest and presents little problem to cytometrists. However, when data is compensated, positive and negative errors are introduced that increase with parameter intensity. The digital error lines in WinList attempt to show the positive envelop of these error distributions for each parameter that is enabled for compensation. If intermediate intensity populations are present that tend to follow the digital error lines, it is possible that they are simply an artifact due to the effects of digital error.

## Normalization Targets

When compensation is **Active** and **Show Normalization Targets** is enabled in the Compensation Toolbox, WinList displays graphical objects to make it easy to set normalization for one or more parameters. The objects appear on 2P histograms.

Normalization allows you to change the location of populations in the data, usually to some normalized location. For example, suppose your lab routinely positions a bead population so that the median is at 10^2 for a log parameter. You open one file and find that the population is lower than usual because one of the gain settings was not quite right. You can use WinList's normalization to move that bead population to the correct location. Keep in mind, however, that WinList will reposition *all* of the data, not just the one population.

Use normalization with great care, and with a full understanding of what you are doing and why you are doing it. It cannot correct data that has been overcompensated or incorrectly stained. It should not be used to make a weak fluorochrome appear to be a bright fluorochrome.
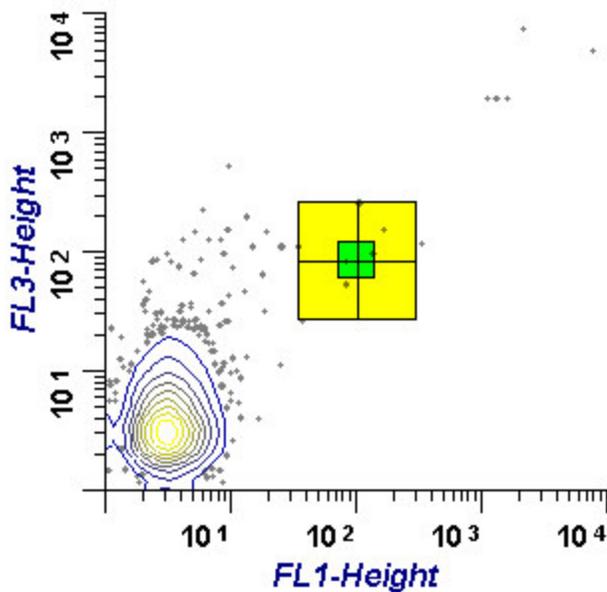


To set normalization, first drag the target object by grabbing the large, yellow outer portion with the mouse. Drag the entire object so that the center cross-hairs are located on the center of the population that you want to move.
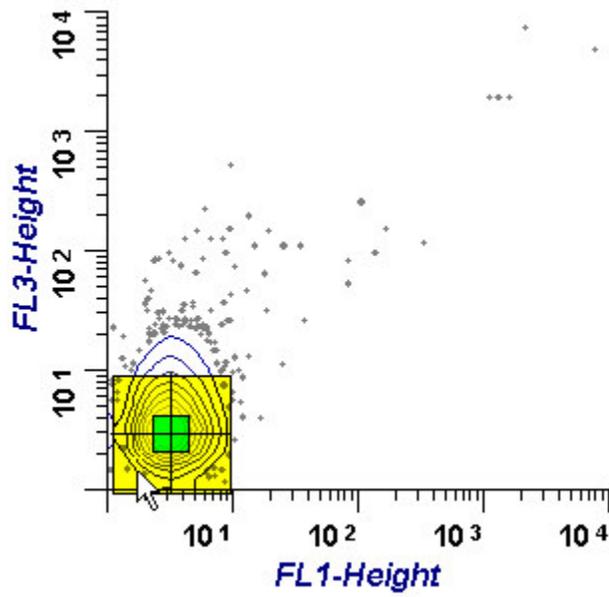
Next, drag the green center of the target to the place where you want to move the population.
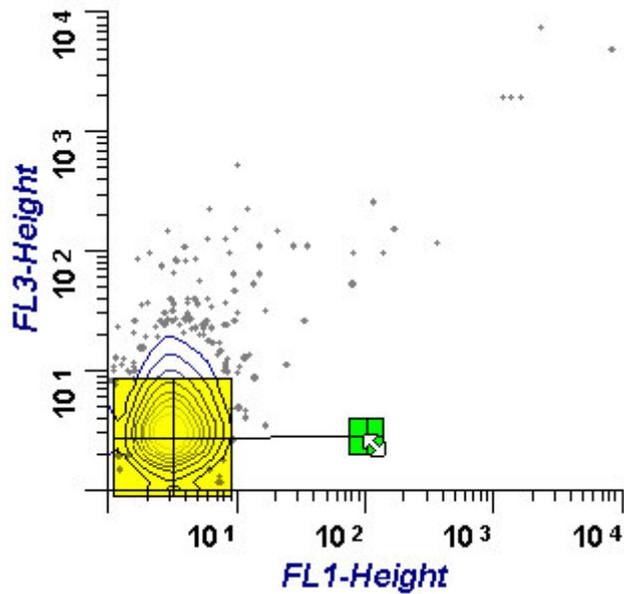


When you release the mouse, the normalization is applied to the data.
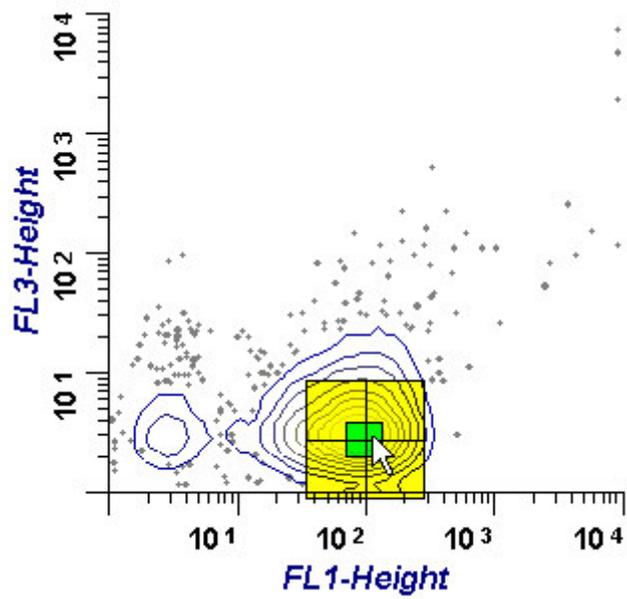
To reset the normalization, use the **Edit** button on the compensation toolbox, and choose the **Reset Normalization** option.

**Histograms**

**Histogram Windows**

Histogram windows belong to the data source that creates them. They are moveable and sizable within the data source. They can display 1P, 2P, and 3P data in a variety of graphical formats. Most data analysis begins with the creation of histograms and defining regions on the histograms.
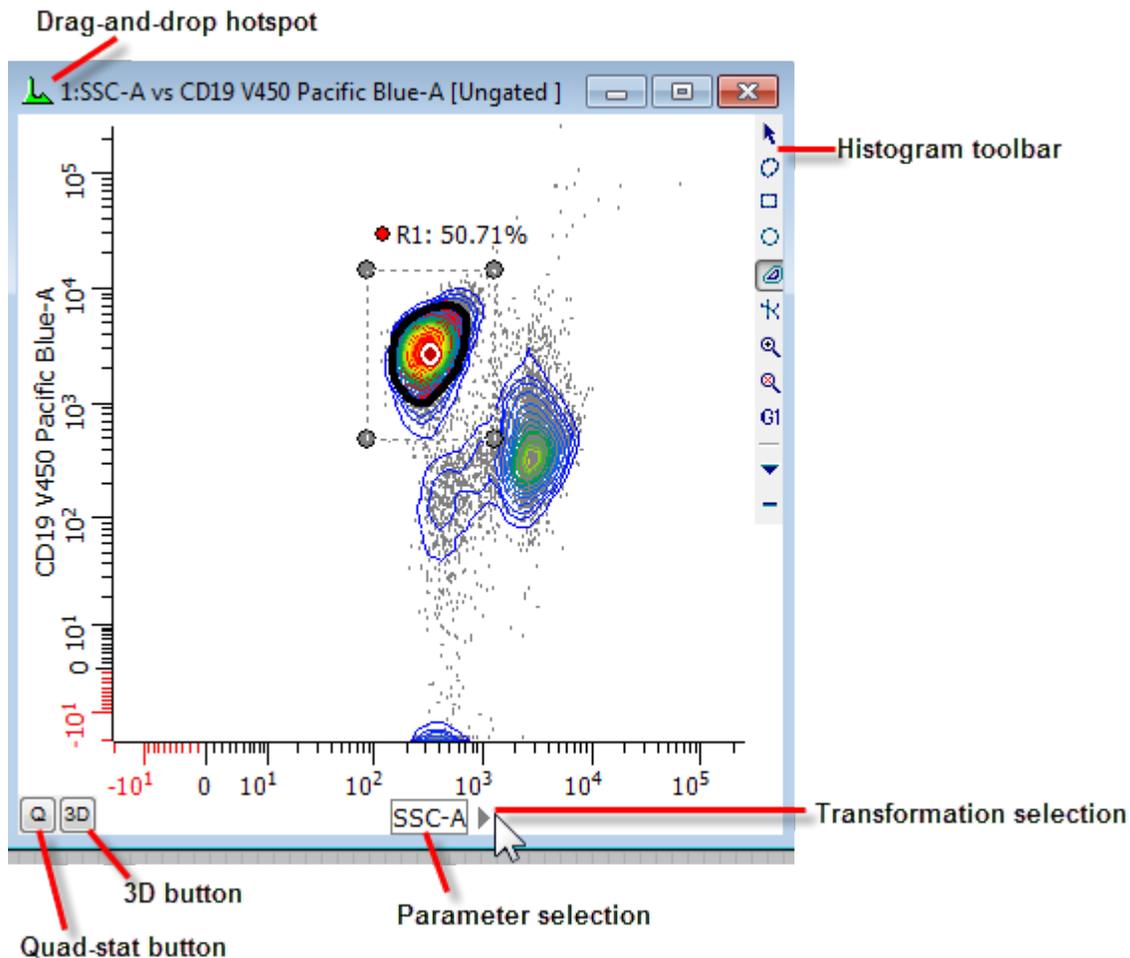


Click the mouse on an axis label to select a different parameter for that axis. When the mouse is over the axis label, click the small triangle next to the label to select a different transformation for the parameter.

A toolbar is positioned on the right edge of the histogram window to provide quick access to many common commands. For details, see Histogram Toolbar.

For quick access to commonly used histogram options, WinList displays the Histogram Context menu by clicking the right mouse button on a histogram. The histogram toolbar also provides access to this menu.

When you click the left mouse button within a histogram, several things may happen. If there is an object that can be moved or edited at that location, the object will be activated. Holding the mouse button down on an object will allow you to drag the object to a new location. If there is not an object under the mouse, the action is determined by the selected region creation mode. See the section on the Histogram Toolbar for more on region creation modes.

Close histogram windows by clicking the "X" button in the upper right corner. When a histogram window is closed, any regions it contains are also deleted.

Histogram windows can display 1, 2, or 3 parameter data from a listmode data source. They can also display information from other Windows programs if they are linked with a Dynamic Data Exchange (DDE) connection. See Edit Remote for more on DDE links.

Regions used for gating and statistics are drawn directly on histogram windows.

### Q or QuadStat Button

When a two-parameter histogram is created, a button appears in the lower left-hand corner labeled **Q**. Click this button to create a QuadStat and results window for it. Delete QuadStat regions by clicking on the region label, then pressing the Delete key on the keyboard. See Regions for more information on the QuadStat and other region types.

### I or Information Button

When you have put a one parameter or two parameter histogram in remote mode (DDE connection with another Windows application), under some circumstances you will see an **I** button in the lower left hand corner of the histogram window. If the remote application sent text back to WinList, you will see the **I** button.

The button is also displayed for subtracted histograms. If you press the **I** button, a pop-up information window will appear that will allow you to scroll through the results.

### 3D Button

On the 3D version of WinList, two- and three-parameter histogram windows also contain a **3D** button in the lower left-hand corner. When this button is clicked, WinList displays a **3D Viewer** window for the histogram. See WinList 3D Version Notes for more information.

**Histogram Toolbar**

This toolbar appears inside the right edge of the active histogram window. Each icon represents a tool, described below.

- Selection mode
- Freehand region
- Rectangular region
- Elliptical region
- Contour region
- NStat region
- Zoom in
- Reset zoom
- G1 Assign a gate
- Display the context menu
- Hide the toolbar
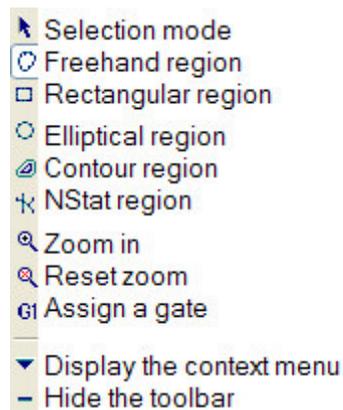
**Region Modes**

The first group of tool buttons is used to select a region drawing mode, with only one mode active at a time. Regions are drawn by pressing and holding the left mouse button when the cursor is inside the histogram, and moving the mouse to define the size and shape of the region. For 1P histograms, only the Select and Range modes are supported. 2P Histograms support Select, Freehand, Rectangle, Ellipse, and Contour modes. Currently, no region drawing is supported for 3P histograms.

**Select mode**

In this mode, regions can be selected and moved, but new regions are *not* created when the left mouse button is clicked on the histogram.

**Freehand mode**

Click, hold, and drag to create a freehand region in a 2P histogram. To complete the region, release the mouse button on the starting vertex of the region. Alternatively, click and hold for 1 second to define the first vertex, and then release the button. Define additional vertices by clicking the mouse.

**Rectangle mode**

Click, hold, and drag to define the boundaries of a rectangular region in a 2P histogram.

**Elliptical mode**

Click, hold, and drag to define the boundaries of an elliptical region in a 2P histogram.

## Contour mode

With a Contour region, a bounding rectangle is defined with the mouse, just as with Rectangle mode. Once that rectangle is defined, WinList computes a contour on the data within that rectangle. Each time the data in a histogram changes, the contour region will recompute based on the data in that rectangle.

## NStat region

This button initiates the creation of an NStat region in a 2P histogram. An NStat is similar to a QuadStat, except that it can have any number of rows and columns, and its vertices can be moved independently to change the shape of the array.

## Zoom in

Click the button to define a zoom area on the histogram. The histogram enters "zoom" mode. Click inside of the histogram and drag to define the zoom area. When the mouse is released, the histogram displays only the portion within the zoom area.

## Reset zoom

Use this button to reset the zoom on a histogram.

## Gate

This button displays a list of the gates that can be applied to the histogram. Select an item in the list to apply a gate to the histogram.

## Context Menu

This button displays the histogram Context menu containing additional commands relevant to the histogram.

## Hide

This button hides the histogram toolbar. The toolbar will reappear when the histogram is reactivated by clicking on its titlebar.

## Histogram Context Menu

Copy
Clone
Clone with regions

Edit...
Graphics...
Transformations...

Make bigger            +
Make smaller           -
Add to report
Add to MG
Swap axes
Annotate...
Insert                 ▶

Move to page...
Copy to page...

Copy data
Save as text...
Save graphic...
Print
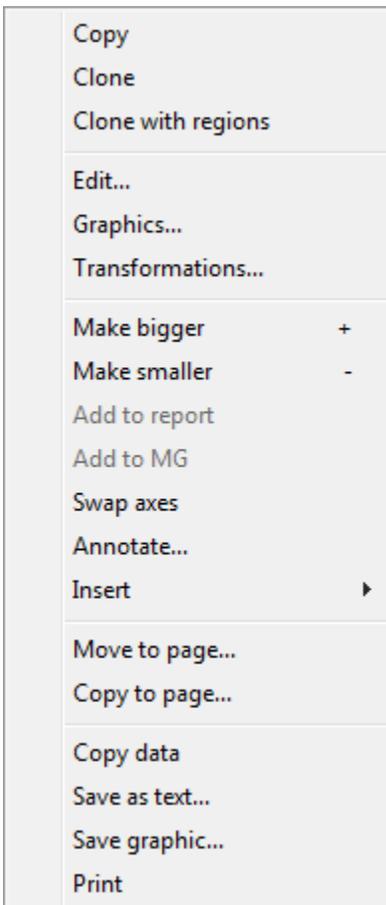
This menu is displayed when the right-mouse button is clicked on a histogram, or by choosing the Context menu button on the histogram toolbar. It displays a list of common commands related to histograms.

### Copy

Copy the histogram graphic to the clipboard. The graphic can then be pasted into a report or another application.

### Clone

This command creates an identical copy of the histogram, with the same parameters and graphic settings. Note, it *does not duplicate any regions* within the histogram.

### Clone with regions

Creates an identical copy of the histogram, like Clone, but also duplicates the regions.  The duplicated regions will have new region indexes.

**Edit**

Display the Edit Histogram dialog.

**Graphics**

Display the Edit 1P Graphics, Edit 2P Graphics, or Edit 3P Graphics dialog, depending on the histogram type.

**Transformations**

Display the Edit Transformations dialog to change the transformation(s) for the parameters displayed in the histogram.

**Make Bigger**

This command incrementally increases the size of the histogram by 50%.

**Make Smaller**

This command incrementally decreases the size of the histogram by 50%.

**Add to Report**

Add a linked copy of the histogram to the active report. The linked object on the report will update whenever the histogram's data changes.

**Add to MultiGraph**

Add a static copy of the histogram to the active MultiGraph.

**Subtract**

Currently only supported for 1P histograms, this option displays the Histogram Subtraction dialog to set up a Super-Enhanced D-Value subtraction.

**Swap Axes**

This option appears in the 2P histogram context menu. It swaps the parameters displayed on the X and Y axes.

**Annotate**

Insert a text annotation into the histogram. The text can be edited, moved and sized

**Insert**

Insert one of the graphic objects into the histogram. The object can be edited, moved and sized.

**Move to Page**

Move the histogram to another page of the data source. See Data Source Windows for more information about pages.

### Copy to Page

Copy the histogram to another page of the data source. See [Data Source Windows](#) for more information about pages.

### Copy Data

Copy the histogram data as a tab-delimited table of values to the clipboard. This is a convenient way to copy data into a spreadsheet or statistics application for further analysis.

### Save as Text

This option saves the histogram as a tab-delimited text file so that it can be imported into other applications, such as speadsheets.

### Save as Graphic

This option saves the histogram graphic in one of many common graphic formats.

### Print

Print the histogram.

**Create Histogram**

There are several ways to create a new histogram in WinList: from ribbon bar or menu, by drawing the histogram window, or by using the Create Multiple Histograms dialog.

The ribbon bar has several buttons on the Home tab that create 1P, 2P, and 3P histograms on the active data source.  These will show the last-used parameters, by default.  You'll find additional buttons on the Insert tab that allow you to create dot plots, density plots, and contour plots quickly. If you are using the toolbar and menu interface, you'll find the Create Histogram command in the Create menu.

Histograms can also be created by clicking and dragging the mouse in an empty portion of a data source window to define the size and location of a histogram. After defining the area, release the mouse button to display the Edit Histogram dialog and to choose the parameters to display.
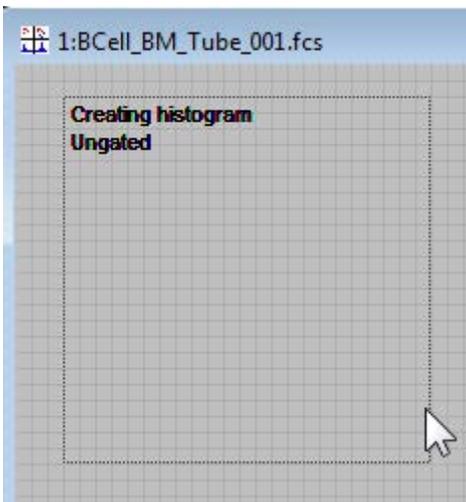


You can draw a gated histogram in a similar manner by first clicking on the Cascade Gate hotspot on an active region. The hotspot will change from red to green. As you draw the histogram, text will be displayed to show how the histogram will be gated.
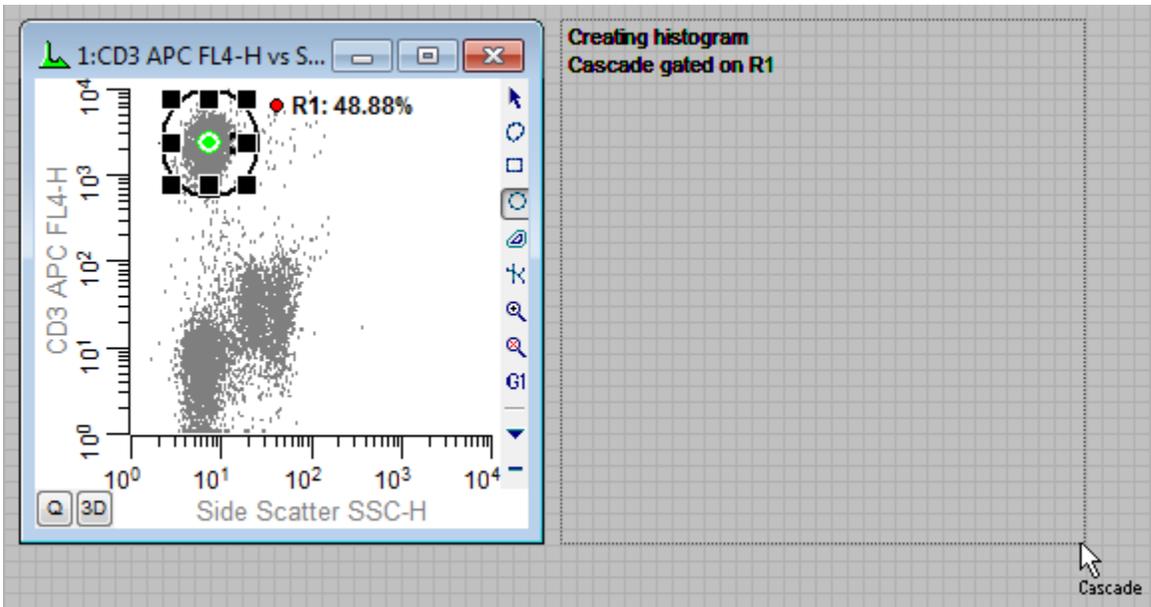
**See also:**

[Histogram Windows](#)

[Create Multiple Histograms](#)

## Create Multiple Histograms

This command displays the Create Histograms dialog box and provides a fast and efficient way to create one or more histograms at once.
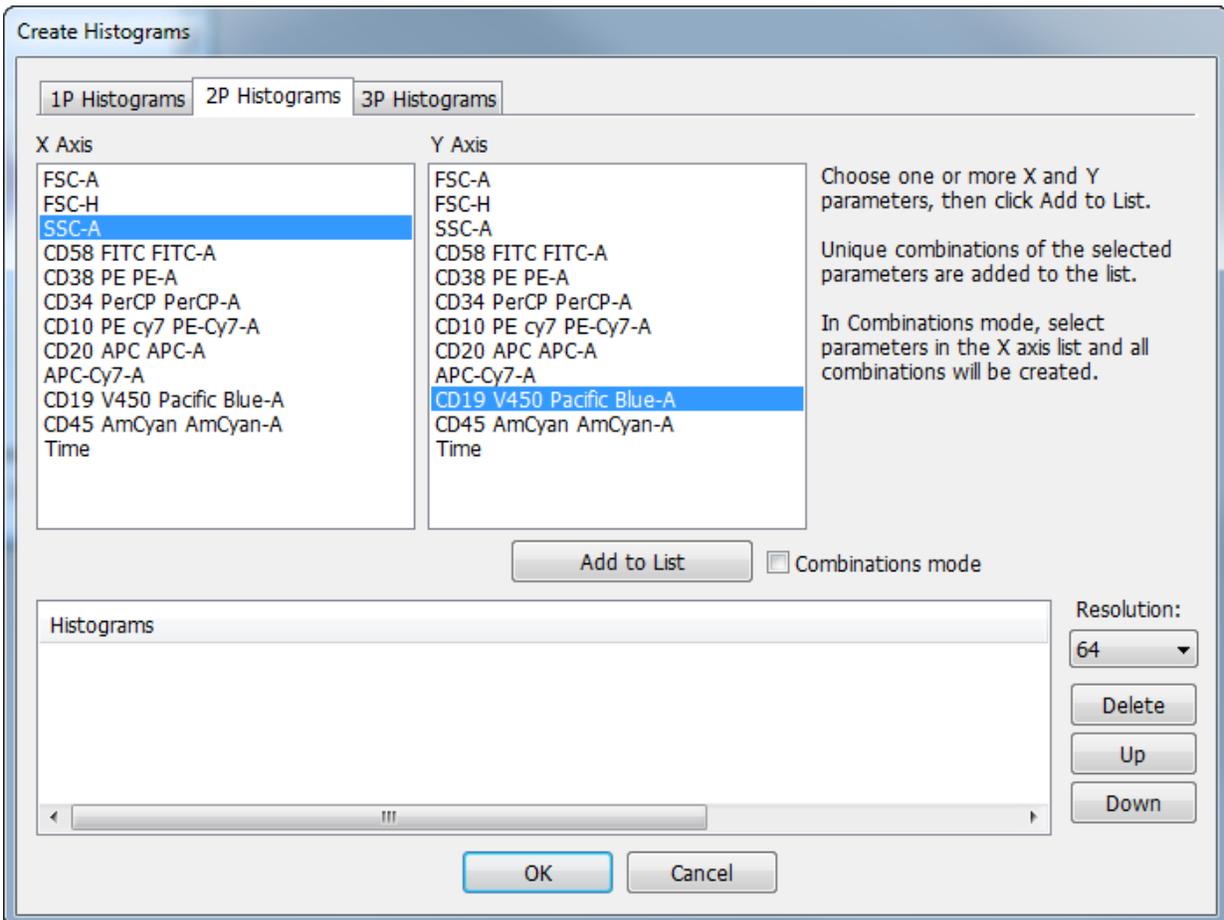


The dialog has a tab for 1P, 2P, and 3P histograms. The initial tab that is shown is the 2P Histograms tab.

### Selection Methods

Selection on the 1P Histograms tab is fairly obvious: highlight one or more parameters in the X Axis listbox and click **Add to List**. If you click Add to List more than once, you can create more than one copy of the histogram to allow different gating strategies.

For 2P Histograms, there are a variety of ways to select parameters. The X Axis and Y Axis listboxes allow multiple selections, and when the Add to List button is clicked, the program generates a list entry for all combinations of the parameters selected in the X and Y lists. For example, if you want Side Scatter vs Forward Scatter and Side Scatter vs CD45, you can

select Side Scatter in the X Axis list, and both Forward Scatter and CD45 in the Y Axis list and click the Add to List button.

If there is a set of parameters for which you want to see all possible plots, you can select the parameters in the X Axis list, and then check the **Combinations mode** checkbox. In this mode, the Y Axis list is grayed out. All combinations of the selected parameters are added to the list when you click Add to List.

The 3P Histograms list works in a similar manner, but does not include the Combinations mode option.

### Add to List

This button generates one or more entries in the Histograms list, based on the selected items and the selection mode. Click the button more than once to create additional copies of the selected items.

**Note**: If all of the items that you want to create are of the same type (e.g. 2P histograms) and can be selected at the same time, it is not necessary to click the Add to List button. When the OK button is clicked, the selected items will be used to generate the histograms.

### Resolution

This drop-down list allows you to select the resolution for the histograms. Select a resolution before clicking the Add to List button.

### Delete

This button deletes the selected items in the Histograms list.

### Up/Down

These buttons allow you to change the order of items that have been added to the list. Select one or more items in the list and click Up to move them up in the list, or Down to move them lower in the list.

### OK

Click the button to close the dialog and create the selected items.

### Cancel

Click the button to close the dialog and cancel the creation of multiple histograms.

**Edit Histogram**

This dialog box is displayed by choosing **Edit Histogram** in the histogram's context menu or by double-clicking on the histogram window's title bar.



### Name

This field displays the name of the histogram, based on the selected parameters. You can also enter FCS keywords in this field surrounded by square brackets [ ]. When the **OK** button is clicked, WinList will evaluate the name, looking for keywords and replacing them with keyword values. For example, if the listmode file opened has a keyword **$PROJ** which has a value of **Unstimulated Control**, entering the name **[$PROJ]** as the histogram name would display **Unstimulated Control** on the title bar of the histogram.

### ID

This field displays the ID for the histogram. Each histogram must have a unique ID.

### Type

Select the radio button for the number of parameters you want the histogram to display. Based on this selection, other elements of the dialog are enabled or disabled.

### Parameter drop-down lists

Select the parameters for each axis that you want to display. If you have created calculated parameters, they will appear at the end of the list of parameters.

### Resolution drop-down lists

Select the resolution for each parameter in the histogram. Options will range from 2 up to the full resolution of the data at acquisition, to a maximum of 65536 channels. In 2 and 3

parameter histograms, you may vary the resolutions for each parameter independently. Higher resolutions generally require more of the computer's memory.

### Remote check box

Check this box to enable the **Edit Remote** push button and establish a link with another Windows application using Dynamic Data Exchange (DDE). If a remote link is already established, you can use this check box to toggle between showing local data and remote data.

### Remote button

If the **Remote** check box is checked, this button becomes available. Click the button to start or edit a connection with another application. For details, see Edit Remote dialog box.

### Gate

This button displays a dialog box which allows the assignment, creation, and editing of gates. See Edit Gates for more information.

### Subtract

This button displays a dialog box that allows a histogram to be subtracted from the active histogram. See Subtract Histogram for more information.

### Graphics

This button allows editing of the histogram's graphical display. It leads to the Edit 1P Graphics, Edit 2P Graphics , or Edit 3P Graphics dialog box.

### OK

Click this button to confirm the changes to the histogram and close the dialog box.

### Cancel

Click this button to abandon changes to the histogram, and close the dialog box.

**Edit Remote**

This dialog box allows the user to establish connections with other Windows applications using Dynamic Data Exchange, or DDE. Once a connection is made, WinList can interact with the other application whenever data changes. This can be useful for automatic databasing and reporting, for exporting WinList graphics, for allowing other applications to process WinList's results, or virtually any interchange with other programs.
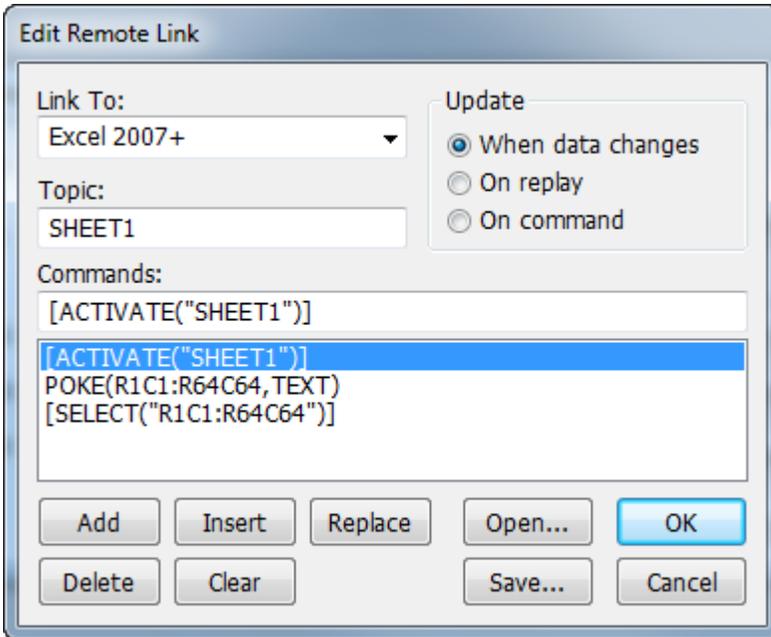


The requirements for remote links are listed below:

1. The other application must support DDE.

2. You must know the DDE topics that the application supports.

3. The application must be in the computer's search path or it must already be running when WinList attempts to make a connection.

4. The application must provide DDE "Server" support. That is, it must be able to respond to requests and instructions from other programs, in this case WinList.

Examples of other programs with which WinList can communicate are ModFit LT, Microsoft Excel, and Microsoft Word. This is not a complete list, as many other applications also support DDE.

The dialog box has three critical areas: **Link To**, **Topic**, and **Commands to Send**. These fields name the application to connect with, the topic used for the connection, and the internal and external commands to execute.

The **Update** group box determines when the link will be updated. All buttons relate to editing commands to send.

### Link To

This drop-down list box contains a list of several programs that you may commonly want to connect to. If the program you want to connect with is listed, select it from the list. If not, select **Other** from the list and type in the name of the program.

### Topic

A default topic is filled in for you if the **Link To** field contains a program known to WinList. If no topic is shown or you want to change the topic, you can edit this field. The topic you supply *must* be a topic the other application supports.

### Commands

This section is comprised of an edit box and a list box. The commands which appear and those you enter must either be internal WinList commands, or external commands which the other application will execute.

*Internal commands* are listed in the DDE Commands section. They instruct WinList to perform some function. For example, *PASTE(metafile)* instructs WinList to paste a metafile (picture) from the clipboard and display it instead of the histogram graphics or results information.

*External commands must be enclosed in square brackets [ ]*. Inside the square brackets enter the command that the other application will execute. For example, if a connection is established with Microsoft Excel, the command *[ACTIVATE("SHEET1")]* instructs Excel to make a spreadsheet called *Sheet1* the active spreadsheet. Notice that the command is surrounded by square brackets to let WinList know that the command is an *External* command.

As you enter commands, click the **Add** button to append the command to the list, or use the **Insert** or **Replace** buttons. *Commands are executed in the order in which they are listed*.

### Add

Click this button to add the command in the Commands to Send edit box to the end of the list of commands.

### Insert

Click this button to insert the command in the Commands to Send edit box in front of the currently selected command.

### Delete

Click this button to remove the selected command from the list box.

**Replace**

Click this button to replace the selected command in the list box with the command in the Commands to Send edit box.

**Open**

This option allows you to retrieve a DDE command file previously saved to disk. The command file opened from disk will replace the one in memory.

**Save**

This option allows you to save a DDE command file to disk. You can reuse the command file by using the **Open** option. Several results or histogram windows may use a command file stored on disk at the same time.

**OK**

Click this button to accept changes to the dialog box and make connection with the remote application. WinList will inform you if it cannot make connection.

**Cancel**

Click this button to discard changes made to the dialog box.

**Update**

Select an option to tell WinList how often to update the link with the other application. When it updates the link, WinList will run through all of the commands in the Commands to Send list, executing the *internal commands* and sending the *external commands* to the other (remote) application. Selecting **When data changes** will cause WinList to update the link whenever WinList changes the data related to the histogram or results window. Selecting **On Replay** tells WinList to wait until one of the options in the **Replay** menu has been selected. If you choose to update **On Command**, the link will be updated when you select **Update Remote Links** from the macro menu, or when the equivalent command is encountered in running a WinList macro.

**Edit 1P Graphics**

This dialog allows you to change the graphical display of a 1P histogram.
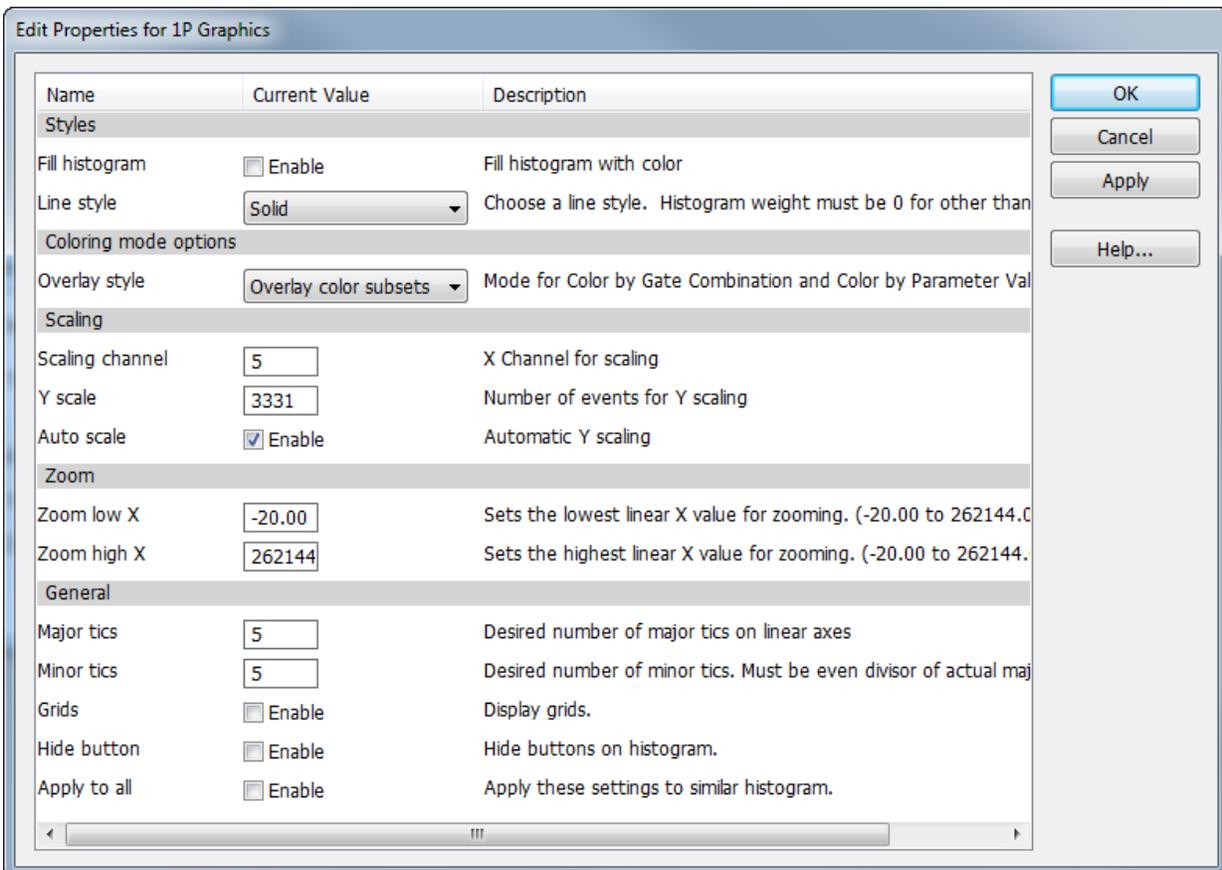


**Styles**

**Fill histogram**

This check box determines whether this histogram is filled with color or drawn as an outline. The default setting for this comes from Preferences - Graphics.

**Line Style**

This option allows you to assign a line type to the border of the 1P histogram. Six line types are supported. Click the scroll bars to select a new style. As you click, the new style is displayed for previewing.

**Coloring mode options**

**Overlay style**

When a color gating mode is used (i.e. Color by Gate Hierarchy or Color by Gate Combinations), a 1P histogram can display population subsets. The subsets can be stacks or overlays. Use this option to select how to display subsets for the 1P histogram.

**Scaling**

**Scaling channel**

Enter a channel number to define where the program will start looking for the peak channel in the histogram. This is useful if your data has an accumulation of events in low channels that should not be considered when looking for the peak. WinList uses the peak height to scale the histogram when the Auto Scale option is enabled.

**Y Scale**

If the **Auto Scale** is enabled, this edit box displays the highest peak value for the histogram. To set a value manually, uncheck the **Auto Scale** option, and then enter a number to set the highest value on the Y-axis of the histogram.

**Auto Scale**

If checked, WinList sets the vertical, y-axis scale of the histogram to the highest peak value. It will look for peaks starting at the channel set in **Scaling channel**. If Auto Scale is unchecked, the number in the **Y Scale** edit box determines the scale of the y-axis of the histogram.

**Zoom**

**Zoom low X**

This value defines the lowest parameter value that is shown in the histogram. Values that are below this are ignored.

**Zoom high X**

This value defines the highest value shown in the histogram. Values above this are ignored.

**General**

**Major Tics**

Enter an approximate number of major tics you want to appear on the axes. Major tics are always divisible by 10, so the actual number of major tics may not be equal to the value entered here.

**Minor Tics**

Enter the approximate number of minor tics you want to display. The actual number of displayed will be 2, 5, 10 or a multiple of 10.

**Grids**

This check box allows you to turn on or off the display of grids that appear on the major tic intervals. If the box is checked, grids will be displayed.

**Hide button**

Check this option to hide the "Info" button. Info buttons appear in histograms that have remote links or histogram subtraction results.

**Apply to all**

When this option is enabled, the settings for the selected histogram will be applied to all other 1P histograms when the **OK** button is clicked.

**OK**

Click this button to accept the changes you have made and close the dialog box.

**Cancel**

Click this button to exit with no changes made and close the dialog box.

**Apply**

Click this button to apply the changes you have made and keep the dialog box open.

**Edit 2P Graphics**

This dialog allows you to change the graphical display of a 2P histogram. It is divided into 4 sections in the tree on the left of the dialog: 2P Graphics, Dots, Density, and Contours.
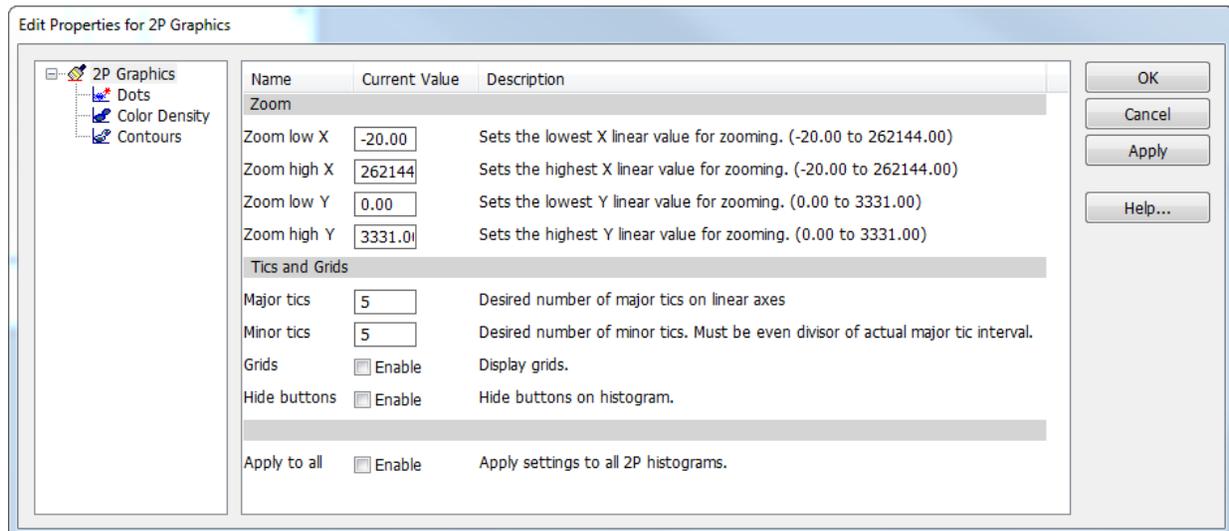


## 2P Graphics
### Zoom
### Zoom low X

This value defines the lowest X parameter value that is shown in the histogram. Values that are below this are ignored.

### Zoom high X

This value defines the highest X value shown in the histogram. Values above this are ignored.

### Zoom low Y

This value defines the lowest Y parameter value that is shown in the histogram. Values that are below this are ignored.

### Zoom high Y

This value defines the highest Y value shown in the histogram. Values above this are ignored.

## Tics and Grids
### Major Tics

Enter an approximate number of major tics you want to appear on the axes. Major tics are always divisible by 10, so the actual number of major tics may not be equal to the value entered here.

### Minor Tics

Enter the approximate number of minor tics you want to display. The actual number displayed will be 2, 5, 10 or a multiple of 10.

### Grids

This check box allows you to turn on or off the display of grids that appear on the major tic intervals. If the box is checked, grids will be displayed.

### Hide button

Check this option to hide the "I" or "Q" button. QuadStat buttons are typically displayed on 2P histograms. Info buttons appear in histograms that have remote links or histogram subtraction results.

### Apply to all

When this option is enabled, the settings for the selected histogram will be applied to all other 2P histograms when the **OK** button is clicked.


## Dots

Enable this option to display dot density display.

This option will uncheck automatically if Color Density is enabled on the next pane. Some Dot properties are shared with Color Density: Dot size, Dot order, Number, Show all dots, and Dot Filter. Changing them in one place will also change them in the other.

### Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

### Number

Enter the number of dots you want to display in the histogram window.

Note: The actual number of dots displayed may be less than the number you enter. If there are fewer events in the data than you specify, or if the *packet size* you specify is less than the number of dots, fewer dots will appear.

In the second case where the packet size is smaller than the number of dots, you can display the full number of dots by replaying data for that histogram, or you can adjust the packet size to store the entire listmode file. See Edit Program Controls for further discussion of *packet size.*

### Show all dots

By checking this option, all events will be displayed in the 2P histogram *after replaying*. See the note above for more on showing all dots.

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**Dot Filter**

This option provides a means for visually eliminating dots in low-frequency channels of the plot. WinList will hide dots in any channel with fewer than the selected number of events.

**Dot order**

When using color event gating, this option controls the order in which dots are drawn. *Unsorted* draws dots in their natural event order based on the listmode acquisition. *Low frequency on top* draws the dots for the smallest populations last so that they can be seen on top of populations that have many events. It is useful for visualizing rare events. *High frequency on top* draws the dots for the largest populations last. It may be used to visually clean up low frequency events.

## Color Density

Enable this option to display color-density dots.

This option will uncheck automatically if Dots are enabled on the previous pane. Some Dot properties are shared with Color Density: Dot size, Dot order, Number, Show all dots, and Dot Filter. Changing them in one place will also change them in the other.

Some Contour properties are shared with Color Density: Probability interval and Colors. Changing them in one place will also change them in the other.

**Colors**

**Color mode**

Choose either Color gradient or Rainbow mode. Color Gradient mode transitions from a starting color to an ending color as the number of events increases. Rainbow mode uses a set of colors which blend to define the color density levels.

**Gradient start color**

Choose a color to use as the starting color for color density dots and contours in Color Gradient mode. Click the color button to select a color

**Gradient end color**

Choose a color to use as the ending color for color density dots and contours in Color Gradient mode. Click the color button to select a color

**Rainbow colors**

Select the number of rainbow colors for Rainbow mode. By default this is 5.

**Edit rainbow colors**

Use this option to select colors used for Rainbow mode.

## Contours

Enable this option to display probability contours.

Some Contour properties are shared with Color Density: Probability interval and Colors. Changing them in one place will also change them in the other.

**Show outlier dots**

To display dots that fall outside the first contour, check this option. The "Dots" section of this dialog controls the properties of the dots, such as size and number.

NOTE: If *Enable contours* and *Show outlier dots* are checked, the only dots drawn will be those that fall outside of the first contour level. To also draw dots that are inside the contours, uncheck the *Show outlier dots* option and check the *Enable dots* option above.

**Probability interval**

This value determines the probability used to compute contours. The default value draws 5% probability contours. With this setting, the largest contour level will contain 95% of the events in the histogram, the next level will contain 90%, and so on.

**Base Contour**

This dropdown list selects the level of the lowest, or base, contour. The level is selected as a percentage of events that it will include. The highest percentage, 99%, will include 99% of the events in the histogram. The lowest value for Base Contour is 90%.

**Line weight**

Choose a line weight for the contour border. The value is in points, a unit of 0.35 mm or 1/72 in.

**Fill contours**

When this option is checked, contours will be filled with colors defined in the Colors section. When unchecked, the contour is unfilled.

**Smooth**

Enable this option to smooth the histogram data used to create the contours. This creates contours that have fewer vertices and a rounder appearance.

**Smoothing span**

If the Smooth data option is enabled, this value determines how many channels are used in the smoothing algorithm. Using more channels creates rounder contours.

**Colors**

See the description of Color Density Colors for details.

**OK**

Click this button to accept the changes you have made and close the dialog box.

**Cancel**

Click this button to exit with no changes made and close the dialog box.

**Apply**

Click this button to apply the changes you have made and keep the dialog box open.

**Edit 3P Graphics**

This dialog allows you to change the graphical display of a 3P histogram.
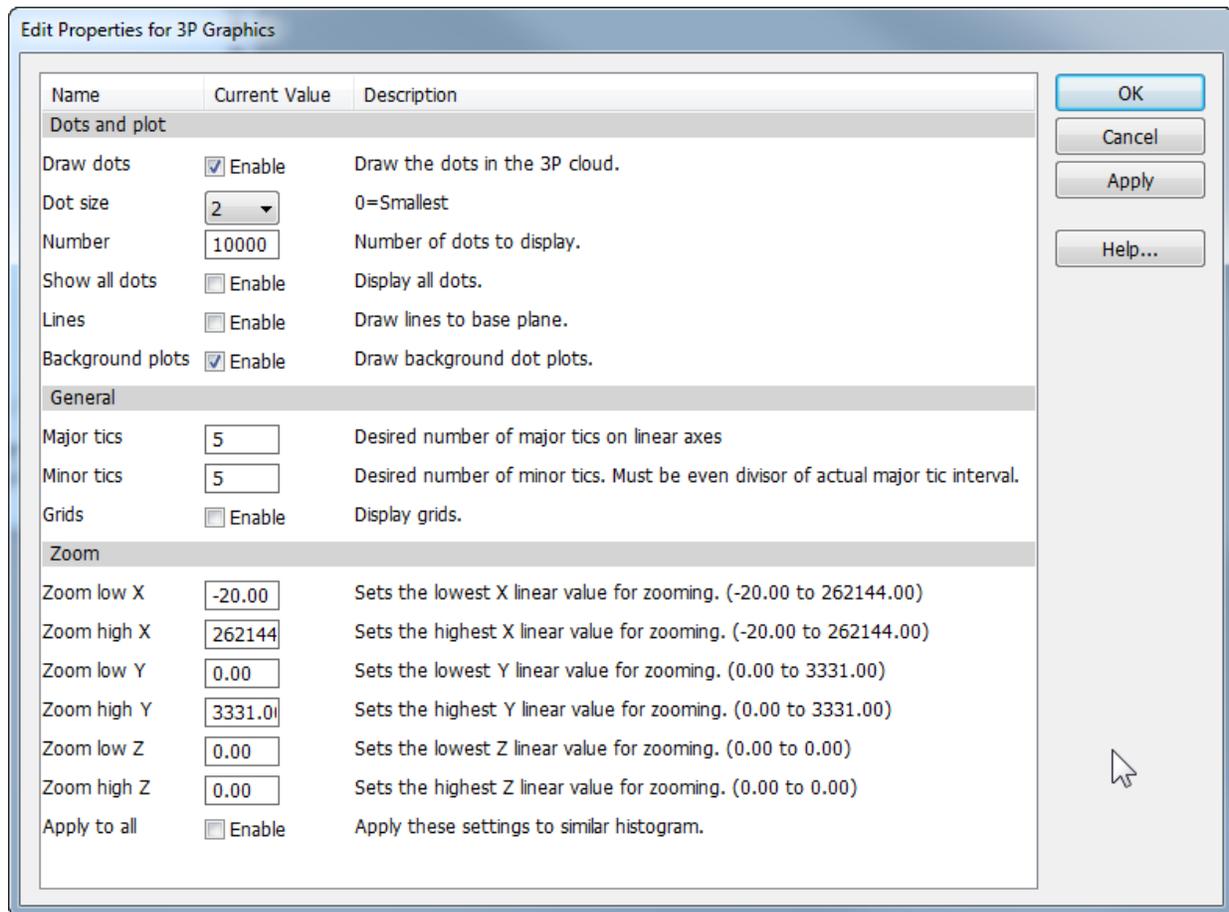


**Dots and plot**

**Dot size**

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

**Number**

Enter the number of dots you want to display in the histogram window.

**Show all dots**

By checking this option, all events will be displayed in the 3P histogram *after replaying*. See the note above for more on showing all dots.

**Lines**

When this option is enabled, line gray lines are drawn from each dot to the base plane. When displaying 1000 dots or fewer, this is useful to identify the position of dots.

**Background plots**

When this option is enabled, a bivariate dot plot is drawn for the 3 background planes.

## General

**Major Tics**

Enter an approximate number of major tics you want to appear on the axes. Major tics are always divisible by 10, so the actual number of major tics may not be equal to the value entered here.

**Minor Tics**

Enter the approximate number of minor tics you want to display. The actual number displayed will be 2, 5, 10 or a multiple of 10.

**Grids**

This check box allows you to turn on or off the display of grids that appear on the major tic intervals. If the box is checked, grids will be displayed.

## Zoom

**Zoom low X**

This value defines the lowest X parameter value that is shown in the histogram. Values that are below this are ignored.

**Zoom high X**

This value defines the highest X value shown in the histogram. Values above this are ignored.

**Zoom low Y**

This value defines the lowest Y parameter value that is shown in the histogram. Values that are below this are ignored.

**Zoom high Y**

This value defines the highest Y value shown in the histogram. Values above this are ignored.

**Zoom low Z**

This value defines the lowest Z parameter value that is shown in the histogram. Values that are below this are ignored.

**Zoom high Z**

This value defines the highest Z value shown in the histogram. Values above this are ignored.

**Apply to all**

When this option is enabled, the settings for the selected histogram will be applied to all other 3P histograms when the **OK** button is clicked.

**OK**

Click this button to accept the changes you have made and close the dialog box.

**Cancel**

Click this button to exit with no changes made and close the dialog box.

**Apply**

Click this button to apply the changes you have made and keep the dialog box open.

**Subtract Histogram**


This dialog box allows the user to perform histogram subtraction, where the control (negative) histogram is selected and subtracted from the current active (test) histogram. The dialog box can be accessed through the Edit Histogram dialog box, or from the histogram popup menu that appears when the right mouse button is clicked on a histogram. Only 1-parameter histograms are supported.


Often the two histograms are in separate listmode files; the control histogram is in one and the test histogram in another. In order to subtract one from the other, both files must be opened at the same time. One way to accomplish this is to set up a protocol with two data sources. Use the Duplicate Data Source option in the Create menu to easily set up a second data source.


When activated, WinList will change the graphical display of the target histogram so that the outline of the original histogram appears, and the histogram resulting from the subtraction is displayed in the foreground. The title bar of the histogram will indicate that subtraction is on. A sample display is shown below.
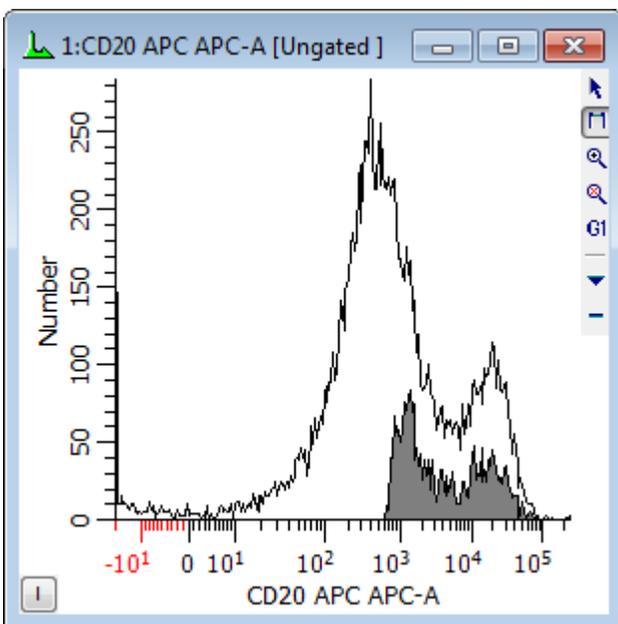


When the subtract histogram command is selected, the following dialog box appears.
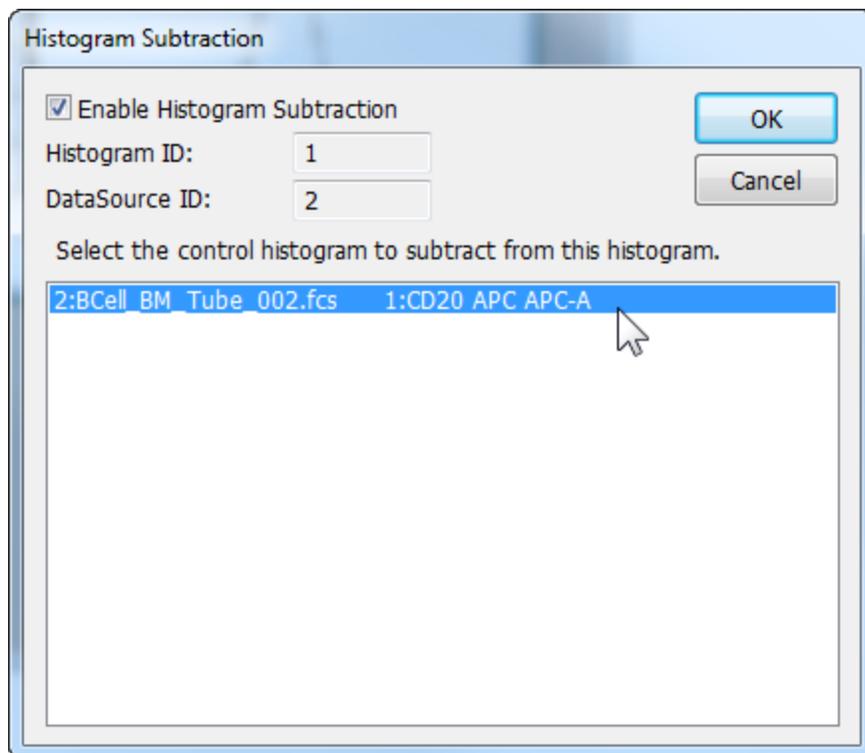
To subtract a histogram from the current histogram, select the negative control histogram that you want to subtract from the list box. All 1-parameter histograms in all open data sources will appear in the list box. Make sure that the **Enable Histogram Subtraction** option is checked and then click **OK**.

To disable histogram subtraction, clear the check from the **Histogram Subtraction On** check box.

There are times when you may receive a warning indicating that the histograms may be reversed." This message alerts you to a condition in the subtraction routine where the control histogram may have more "positives" than the positive histogram. Check to make sure you have not accidentally subtracted the positive histogram from the negative histogram or made a similar selection mistake. If you are certain that you are subtracting the correct histogram, the warning can be safely ignored.

### Enable Histogram Subtraction

This option determines the state of histogram subtraction. When checked, subtraction is enabled. When unchecked, subtraction is disabled. *It is checked automatically each time you display the subtraction dialog.*

### Histogram ID

This field displays the WinList ID of the histogram to subtract that is currently selected in the list box.

**Data Source ID**

This field displays the WinList ID of the data source owning the histogram that is currently selected in the list box.

**Histogram list box**

Displays a list of all 1-parameter histograms.

**OK**

Accepts changes and closes the dialog box.

**Cancel**

Exits with no changes and closes the dialog box.

Statistical results of the subtraction can be viewed by clicking the **I** button in the lower left corner of the histogram window. When clicked, an information window will appear displaying several statistics as described below:
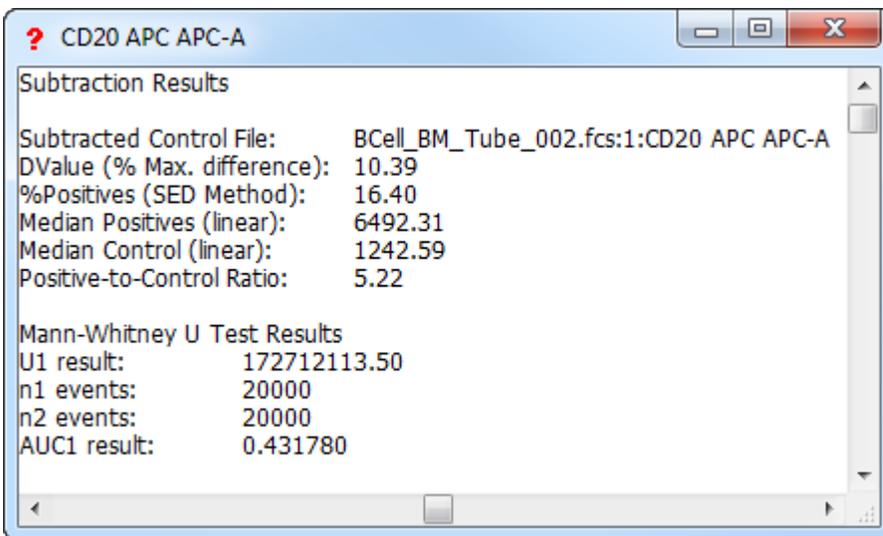


These results are also available for databasing.

**Subtracted Control File**

This field displays the name of the data source, the histogram ID, and the name of the subtracted control histogram.

**DValue (% Max. difference)**

In determining the difference between the test and control histograms, both are converted into cumulative histograms and normalized to a scale from 0 to 100. The point of maximum difference is found and the percent difference is calculated. Since the scale of the cumulative histograms is 0 to 100, the maximum difference is actually a percentage.

### % Positives (SED Method)

This statistic uses the **Super-enhanced DMax (SED)** method to determine a more accurate percentage of the test histogram that is positive, or what portion remains after histogram subtraction. The method was presented at ISAC in 1996 as Enhanced Normalized Subtraction (ENS), and was further improved to become SED. Contact Customer Support for other references and a white paper on this method.

### Median Positives (linear)

This field displays the statistical median for the positive distribution computed using the SED method. It is in linear ADC units.

### Median Control (linear)

This field displays the statistical median for the control histogram. It is in linear ADC units.

### Positive-Control Intensity Ratio

This field displays the ratio of the linearized positive median to the linearized control median. It provides an indication of the relative intensities of the positive and control distributions.

### Mann-Whitney U Test Results

WinList computes the Mann-Whitney U Test for additional "Area-under-the-curve" (AUC) statistics for histogram subtraction. Detailed info on this test can be found in this wiki article. This provides another indication of the difference between two distributions - the test and control histograms.

The key statistic is the "Area-under-the-curve", or **AUC**. AUC is non-parametric and ranges from 0 to 1, such that if the histograms are the same AUC = 0.5.  If test histogram shifts to the right, AUC will increase towards a value of 1.0, and if the test histogram shifts to the left, AUC decreases towards a value 0.0. The AUC as a metric gives both the proportion of difference between histograms and direction of shift. AUC seems to be very robust and not affected by noise.

**Information Windows**

When a histogram window is *remotely linked* to another program and it has received textual information, an Information window can display the textual or graphical information. For example, if a DNA histogram in WinList is linked to ModFit for analysis, ModFit will send back a graphic as well as its analysis results.

WinList indicates that remote information is available for a window by displaying a button labeled with the letter **I** in the lower left hand corner of the histogram window.

The Information window is also available when a histogram has subtraction results to display. See Histogram Subtraction for details.

### Index Analysis

The Index Analysis window is a special display for parameters that contain index-like data.  Some plate-sorting cytometers, like Sony's SY3200, create index parameters that indicate which well on the sort plate each event went into.  The Index Analysis window is a great tool for viewing and investigating that information.
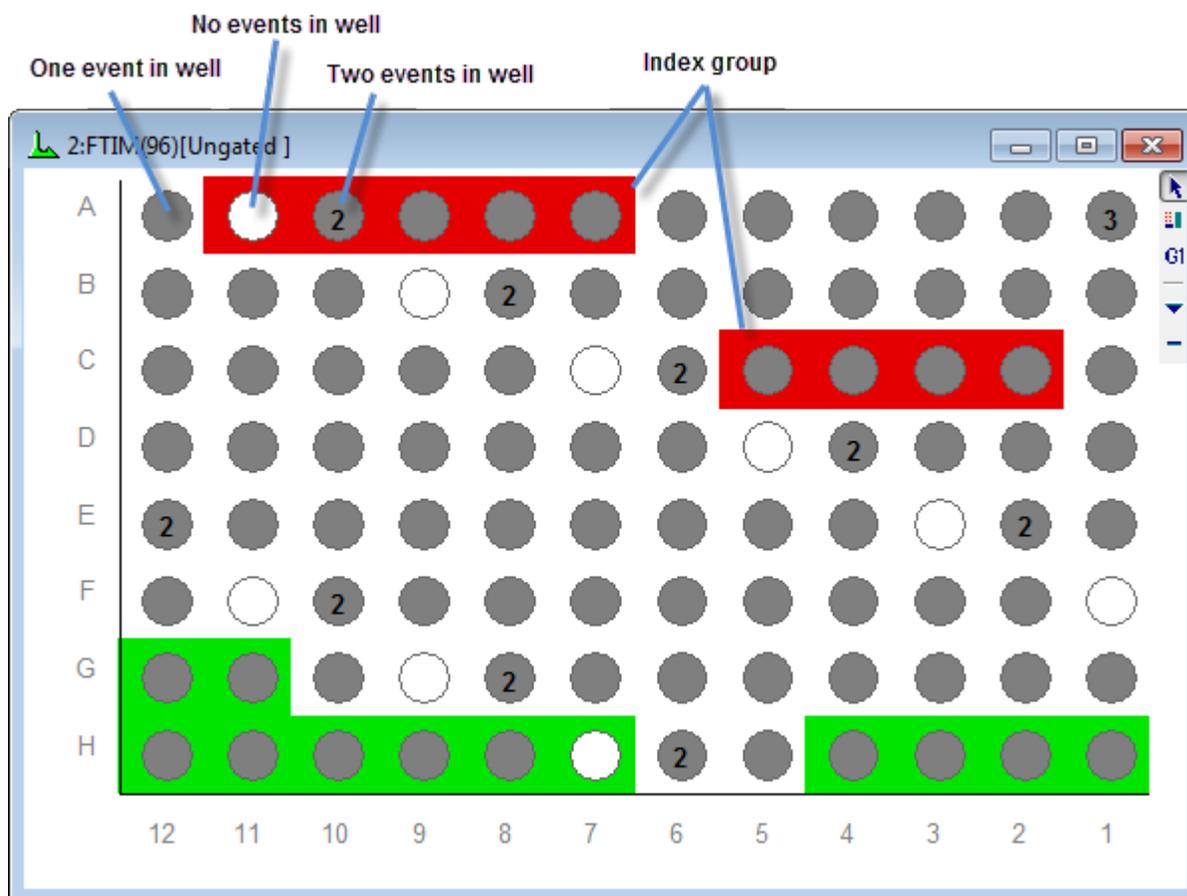


An Index Analysis window can be created for any index-like parameter in the FCS file.  There are controls for setting the number of rows and columns to display, as well as how to label the rows and columns.  In some cases, the FCS file may contain keywords to help the Index Analysis window figure out how to display the rows and columns.  See Edit Index Analysis for details.

You can create "groups" of related wells on an Index Analysis window, much like regions on a histogram except that groups can contain locations that are not next to one another.  In fact, each group you create has an associated region so that you can display statistics for it

in a Results window as you would with other regions.  See [Index Analysis Groups](#) for information on creating and editing groups.

Index Analysis plots can display the color-event information from regions in other histograms.  You can also choose to color events based on the groups in the Index Analysis window.

**Edit Index Analysis**

This dialog is displayed when creating or editing an Index Analysis window.
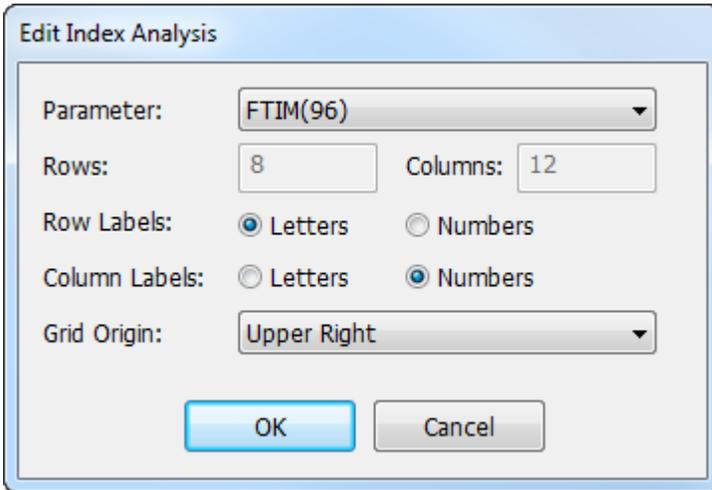
**Parameter**

Choose the parameter in the FCS file that you want to display in the Index Analysis window.  It is best if this parameter contains index-like information - whole numbers in the range defined by the number of rows and columns.

**Rows and Columns**

When you create a new Index Analysis window, you can edit the number of rows and columns.  If the file you are analyzing has indexing keywords, this information will be filled in automatically for you.  If it does not have keywords, create enough rows and columns to accommodate the range of values for the parameter.  For example, if your parameter has 128 possible values, make sure that rows * columns = 128.  In the illustration, the parameter has 96 possible values, so 8 rows by 12 columns will display all the possible values.

**Row and Column Labels**

You can label the rows and columns with letters or numbers to easily identify a particular well in the grid.

**Grid Origin**

The grid origin determines where the first index value is located on the window.

**Index Analysis Groups**

"Analysis Groups" in an Index Analysis window are the equivalent of "regions" in a histogram window.  An analysis group identifies a set of wells in the Index Analysis window, and you can report statistics and perform event coloring with analysis groups just as you would with a region in a histogram.

In the image below, there are 2 groups defined.  The red-colored boxes represent group R1, which is named "Good Cells".  This group contains 5 wells on row A and 4 more wells on row C.  The green-colored boxes represent group R2, named "Bad Cells".
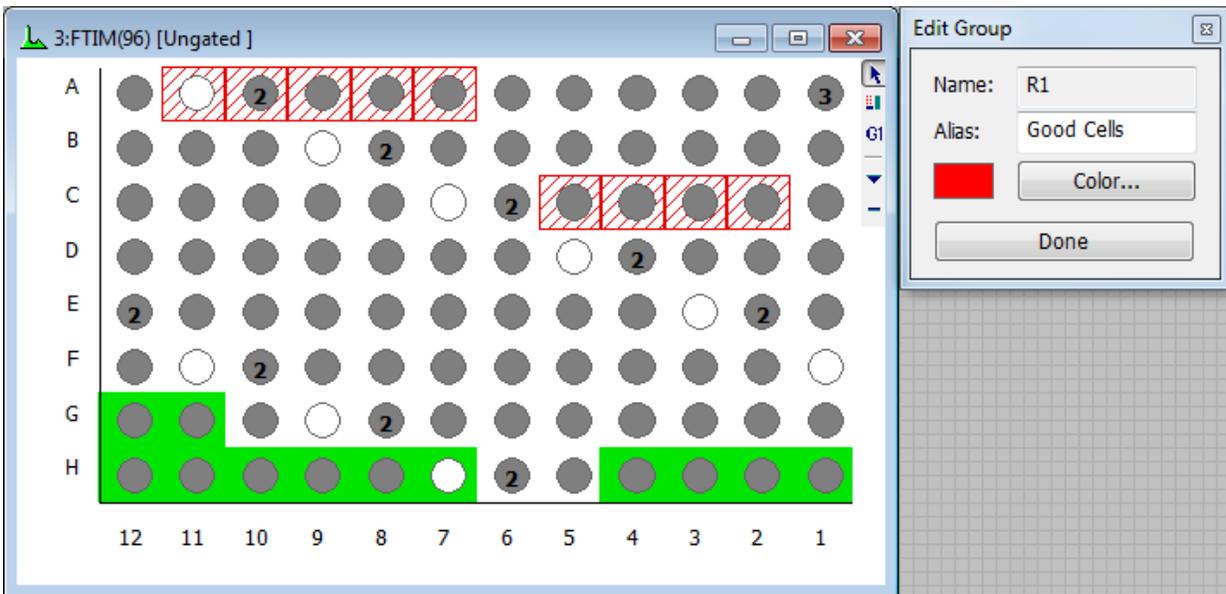


**Creating a Group**

With an Index Analysis window created, use the Create Group tool in its toolbar to start a new group of wells.
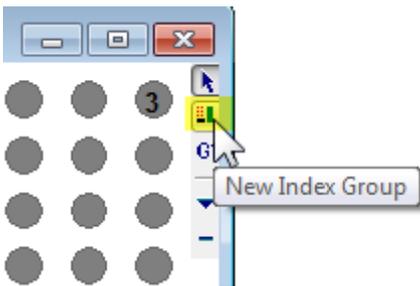
This will display the Edit Group dialog, which identifies the results region associated with the group.  You can type an alias and pick a color for the group you are creating in this dialog.

With the Edit Group dialog open, click and drag the mouse over the wells that you want to include in the group.  If you want to add cells that are not next to one another, press and hold the **Ctrl** key on the keyboard before you click the mouse.

When you have finished with selection of wells, click the **Done** button to close the Edit Group dialog.

## Editing a Group

Once a group has been created, you can edit the group by clicking on any one of its wells.  This will display the Edit Group dialog.  Just as with creating a group, you can select wells, change the alias and edit the color of the group.  If you want to add cells that are not next to one another, press and hold the **Ctrl** key on the keyboard before you click the mouse.

When you have finished selecting wells, click the **Done** button to close the Edit Group dialog.

## Deleting a Group

To delete a group, right-click the mouse on any well in the group and select **Delete** from the context menu.

## Creating Results for a Group

To create results for a group, right-click the mouse on any well in the group and select **Create Results** in the context menu.  The statistics that can be displayed are limited to frequency-based statistics; intensity and position statistics are not computed for Index Analysis groups.

## Using a Group to Gate other Histograms

Sometimes it is useful to see where the wells in a group appear in other histograms.  One method for doing this is to use the group to gate another histogram.  Right-click on a group and select **Cascade Gate** from the context menu, and then click the title bar of the histogram you want to gate on the group.  The histogram will show only those events that are in the wells selected in the group.

**3D Views**

**3D Histogram Viewing**

WinList 3D can create high quality OpenGL 3D images from any 2P or 3P histogram. The 3D images are displayed in separate windows, and can be easily rotated and scaled. This feature generates wonderful publication graphics and greatly enhances the ability to visualize your data.

Each 2P and 3P histogram displays a small "3D" button in the lower left corner of the histogram window. To display the 3D viewer for a histogram, simply click the 3D button and a 3D Viewer window will appear.

You can set a preference for the type of 3D display that is created when you click the 3D button. See 2P Graphics Preferences and 3P Graphics Preferences for details.

2P histograms display an Iso3D image of the histogram data. For details on the display options for Iso3D displays, see the 2-Parameter Display Options.

3P histograms display 3D clouds, dots, or spheres. For details on the 3-parameter display options, see 3-Parameter Display Options.

Zoom settings that you make on the histograms will be used by the 3D viewer window.

For more on the 3D Viewer, see:
Using the 3D Viewer

**Using the 3D Viewer**

The 3D viewer can display 2- or 3-parameter data in a number of different formats. The colors that are applied to the 3D data are same as those shown in the standard display. Zoom settings that you make on the histograms will be used by the 3D viewer window. The 3D viewer provides additional flexibility, allowing rotation and zooming on the data display.

For details on the Iso3D display options for 2-parameter data, see 2-Parameter Display Options.
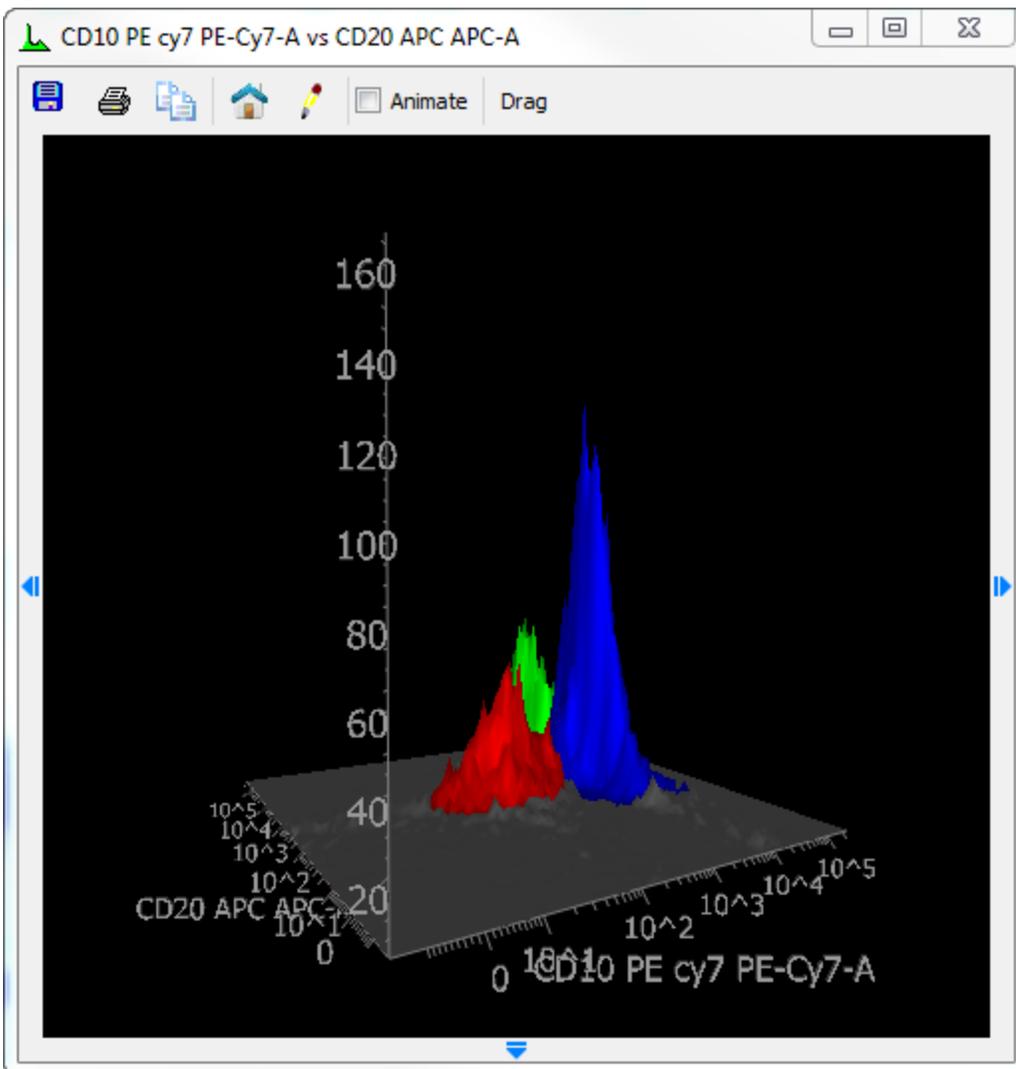


For details on the 3D display options for 3-parameter data, see 3-Parameter Display Options.
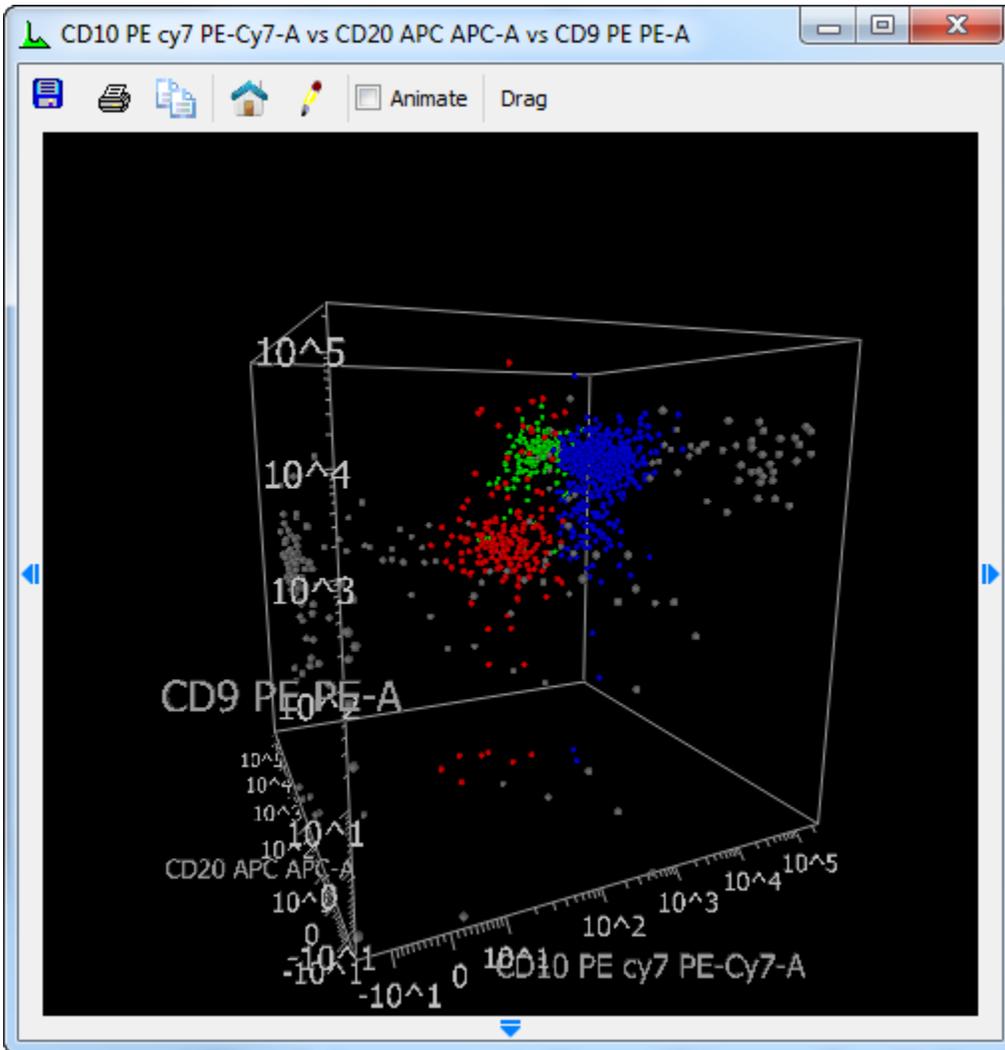
## Displaying Additional Controls and Options

The viewer has small arrows on its left, right, and bottom edges. Click or hover the mouse on one of the arrows to display the controls that it contains.
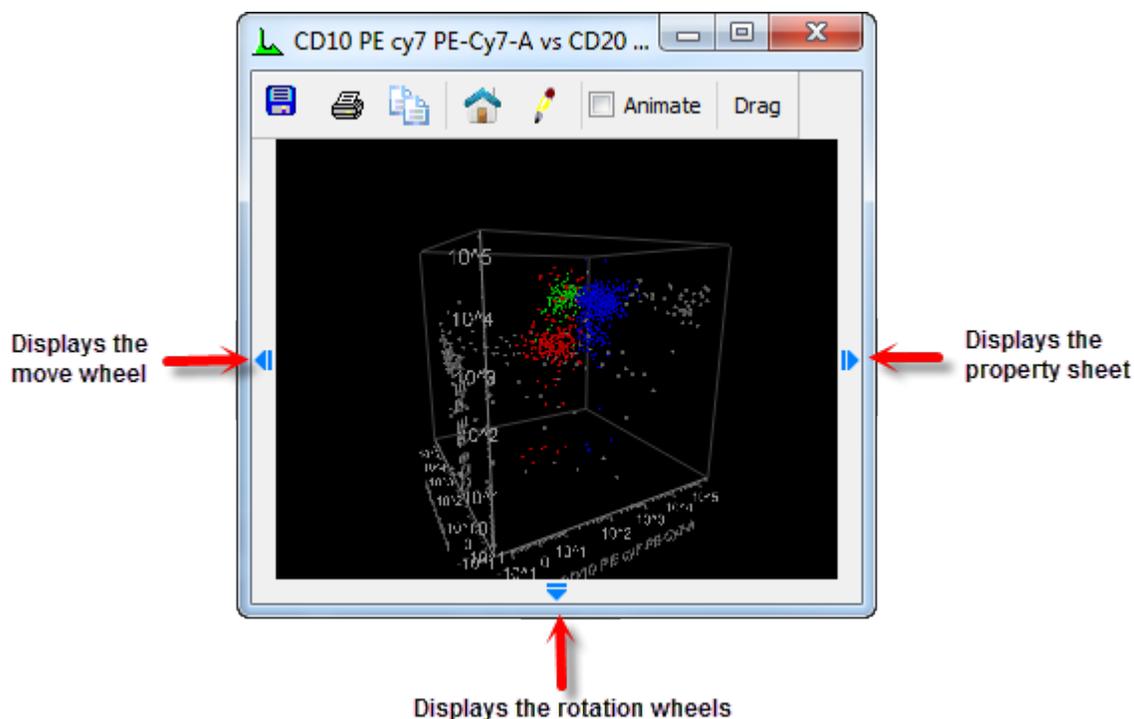
### Editing Properties

Click or hover the mouse on the small arrow on the right edge of the viewer window to display the properties sheet. The property sheet contains a tree of objects and a property list for the selected object in the tree. You can also click the pencil icon on the viewer toolbar to display the properties sheet. See 2-Parameter Display Options or 3-Parameter Display Options for details on properties.

### Rotate the image

Click, hold, and drag on the viewer window. The image in the window will rotate as you drag the mouse. Release the mouse button to complete the rotation. You can also use the rotation wheels contained in the property sheet at the bottom of the viewer window.



### Animate the image

When you enable the **Animate** checkbox at the top of the viewer, the image will rotate. You can control the direction and speed of rotation by using the mouse. Click, drag, and release to set the speed and direction.

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**Save the image**

Click the Save icon on the viewer toolbar to save the 3D image as a graphic file on disk. A dialog is displayed to set the size and resolution of the image. Adjust the properties as needed and then click OK. The Save Image dialog is displayed to select a name and format for the image.

**Print the image**

Click the Print icon on the viewer toolbar to print the image.

**Copy the image**

Click the Copy icon on the viewer toolbar to copy the image to the clipboard.

**The Home position**

Click the Home icon to return the image to its default rotation and position.

**Drag and Drop**

Use the Drag button on the toolbar to begin a drag-and-drop operation. Click the button, drag it to the destination (e.g. a Report window), then release the button to finish the action.

See also:

3D Viewer: Additional Controls

**3D Viewer: Additional Controls**

**Mouse Button Controls**

The mouse buttons can be used in the 3D Viewer as described in the table below.

| Left | Spin (rotate the virtual trackball) |
|---|---|
| <SHIFT> + Left | Pan (translate up/down/left/right) |
| <CTRL> + <SHIFT> + Left | Zoom in and out |
| Right Mouse | Display the Viewer Popup Menu |

**3D Viewer: 2-Parameter Display Options**

This property sheet allows you to change how the 3D-viewer window displays 2-parameter data.
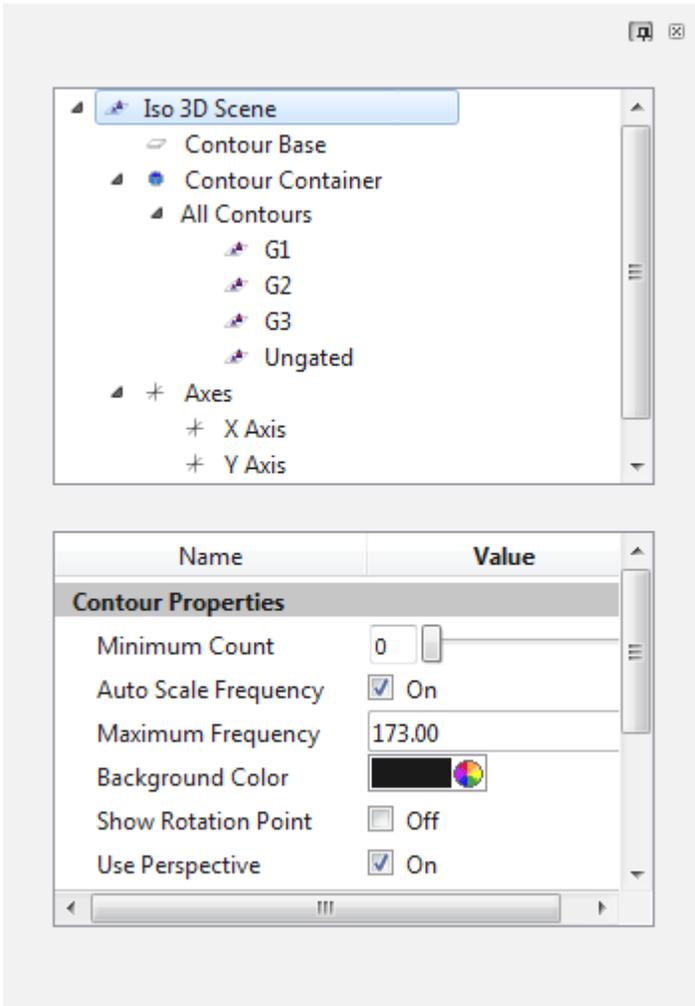


A pushpin at the top of the sheet allows you to pin the sheet open. The small "x" allows you to close the sheet.

The tree control at the top determines which set of properties are shown in the bottom section. Select an item in the tree to view and edit its properties.

## Iso 3D Scene

**Contour Properties**

**Minimum Count**

Use this control to filter out low-level populations. Those that have fewer events than this value will not be drawn on the plot.

**Auto Scale Frequency**

When checked, the frequency axis is automatically scaled based on the data.

**Maximum Frequency**

When Auto Scale Frequency is not checked, the this field sets the height of the frequency axis.

**Background Color**

Select a background color for the viewer.

**Show Rotation Point**

Enable this option to see the point at the center of rotation.

**Use Perspective**

Enabled by default, this property determines whether perspective is applied to the 3D rendering.

**Copy/Save/Print Options**
**Custom Size Width & Height**

These values control the width and height for graphics when copied, saved, or printed.

**Image Resolution**

This field controls the resolutions for graphics when copied, saved, or printed.

**Use White Background**

Enable this option to use a white background when copying, saving, or printing.

## Contour Base
**Show Base**

Enable this option to show the base plane of the contour plot.

**Base Color**

Choose a color for the base plane.

**Percent Transparency**

Choose a transparency for the base.

## All Contours

These settings apply to ALL of the subset contours. Individual contours can be customized with different settings.

### Contour Line Type

Select an option for displaying contour lines.

### Minor Tic Contours

When contour lines are enabled, this option will draw contour lines for the minor tic levels.

### Contour Line Color

Choose a color for the contour lines.

### Percent Transparency

Choose a transparency for all contours.

## Contours

Use these objects to customize individual clusters. To change the settings for *all* contours at once, select the All Contours object in the tree.

### Contour Line Type

Select an option for displaying contour lines.

### Minor Tic Contours

When contour lines are enabled, this option will draw contour lines for the minor tic levels.

### Contour Line Color

Choose a color for the contour lines.

### Percent Transparency

Choose a transparency for all contours.

### Contour Color

The property displays the color used by the cluster.  It is control by the gate dialogs in the main application.

## Axes

### Show Box

Enable this option to show the 3D box.

**Box Line Weight**

Choose a line weight for the 3D box.

**Box and Axis Color**

Choose a color for the 3D box and axis.

## Y, Y, Z Axis

**Show**

Choose an option for the axis display.

**Axis Color**

Select a color for the axis.

**Label Font**

Select a font for the axis label.

**Number Font**

Select a font for the axis numbers.

**Axis Line Weight**

Select a line weight for the axis.

**Axis Label Properties**
**Axis Label**

Edit the text for the axis label.

**Update Axis Label**

Select an option for how the axis label is updated.

**Axis Label Style**

Choose a label style. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

**Horizontal Alignment**

Choose an alignment for the labels.

**Axis Number Style**

Choose a style for the axis numbers. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

**Show Exponent Only**

When enabled, only the exponent is shown on the axis for log-like transforms.

**Axis position**

Select a position for the axis.

**3D Viewer: 3-Parameter Display Options**

This property sheet allows you to change how the 3D-viewer window displays 3-parameter data.
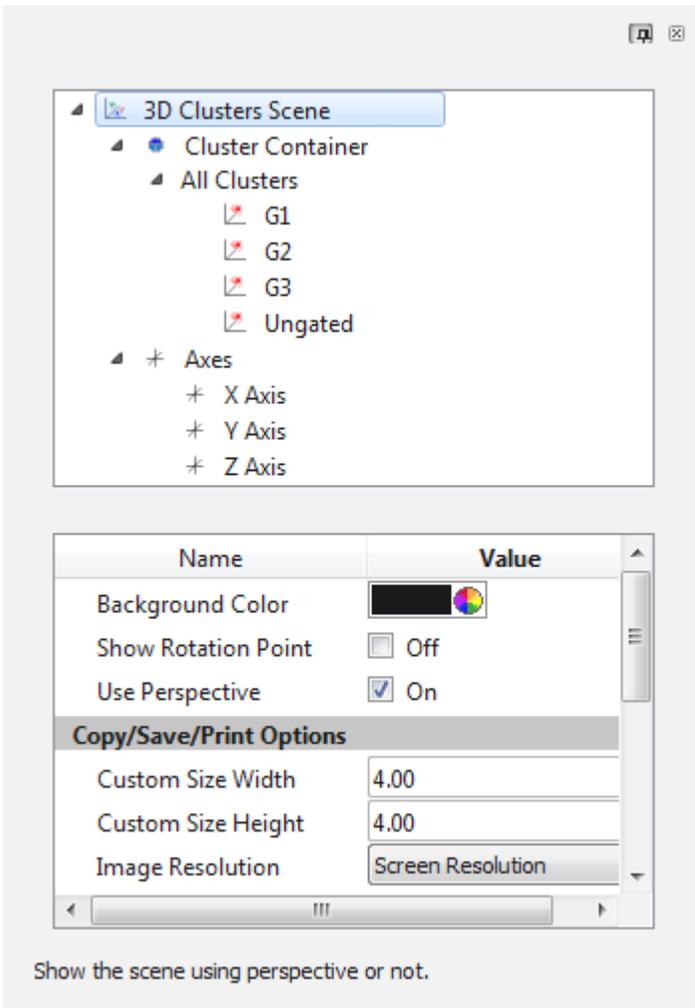


A pushpin at the top of the sheet allows you to pin the sheet open. The small "x" allows you to close the sheet.

The tree control at the top determines which set of properties are shown in the bottom section. Select an item in the tree to view and edit its properties.

**3D Clusters Scene**
**Background Color**

Select a background color for the viewer.

**Show Rotation Point**

Enable this option to see the point at the center of rotation.

**Use Perspective**

Enabled by default, this property determines whether perspective is applied to the 3D rendering.

**Copy/Save/Print Options**
**Custom Size Width & Height**

These values control the width and height for graphics when copied, saved, or printed.

**Image Resolution**

This field controls the resolutions for graphics when copied, saved, or printed.

**Use White Background**

Enable this option to use a white background when copying, saving, or printing.

**Dots/Spheres/Clouds**
**Display Mode**

Select the type of 3D display to use: Dots, Spheres, or Clouds.

**Dots/Spheres Properties**

Enter the number of events to display for dots and spheres.

**Cloud Properties**
**Probability**

Select a probability threshold for the cloud display. This value is much like a contour in 2P space, in this case defining a 3P surface. Larger values include more events inside the cloud and result in a larger surface.

**Necessary Events**

This determines the minimum number of events that a cluster must contain in order for a cloud to be generated for it.

## All Clusters

These settings are applied to ALL clusters. Individual clusters can be customized with different settings.

**Dot/Sphere Size**

Select a size for dots or spheres.

**Percent Transparency**

Choose a transparency for all clouds.

**Clusters**

**Dot/Sphere Size**

Select a size for dots or spheres.

**Percent Transparency**

Choose a transparency.

**Cluster Color**

Choose a color for the cluster.

**Axes**

**Show Box**

Enable this option to show the 3D box.

**Box Line Weight**

Choose a line weight for the 3D box.

**Box and Axis Color**

Choose a color for the 3D box and axis.

**Y, Y, Z Axis**

**Show**

Choose an option for the axis display.

**Axis Color**

Select a color for the axis.

**Label Font**

Select a font for the axis label.

**Number Font**

Select a font for the axis numbers.

**Axis Line Weight**

Select a line weight for the axis.

**Axis Label Properties**
**Axis Label**

Edit the text for the axis label.

**Update Axis Label**

Select an option for how the axis label is updated.

**Axis Label Style**

Choose a label style. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

**Horizontal Alignment**

Choose an alignment for the labels.

**Axis Number Style**

Choose a style for the axis numbers. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

**Show Exponent Only**

When enabled, only the exponent is shown on the axis for log-like transforms.

**Axis position**

Select a position for the axis.

**3D Output Options Dialog**

This dialog controls the size and resolution of the image generated when printing or saving to disk.
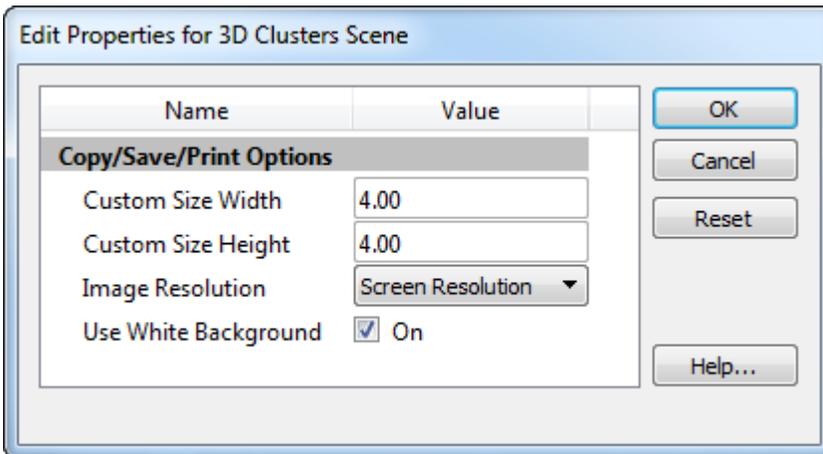


**Custom Size Width and Height**

Enter values for Width and Height, in inches.

**Image Resolution**

Select a resolution for the output. The resolution is the number of dots per inch (DPI). Keep in mind that as you increase the resolution, the size of the image increases, and the memory demands on your computer can become very great. You may want to try a low resolution first to see how long rendering takes and to assess the quality of the output. Increase the resolution gradually until you reach the quality you desire.

**Use white for background**

Check this option to render the image with a white background. Clear the check if you want to use a black background as seen in the viewer.

**OK**

Click this button to accept the settings in the dialog.

**Cancel**

Click this button to cancel any changes.

**Spots**

**Edit Spot**

This dialog box can be brought to the screen by choosing **Spots** from the **View** tab of the ribbon bar, or **Edit Spots** in the **Edit** menu.
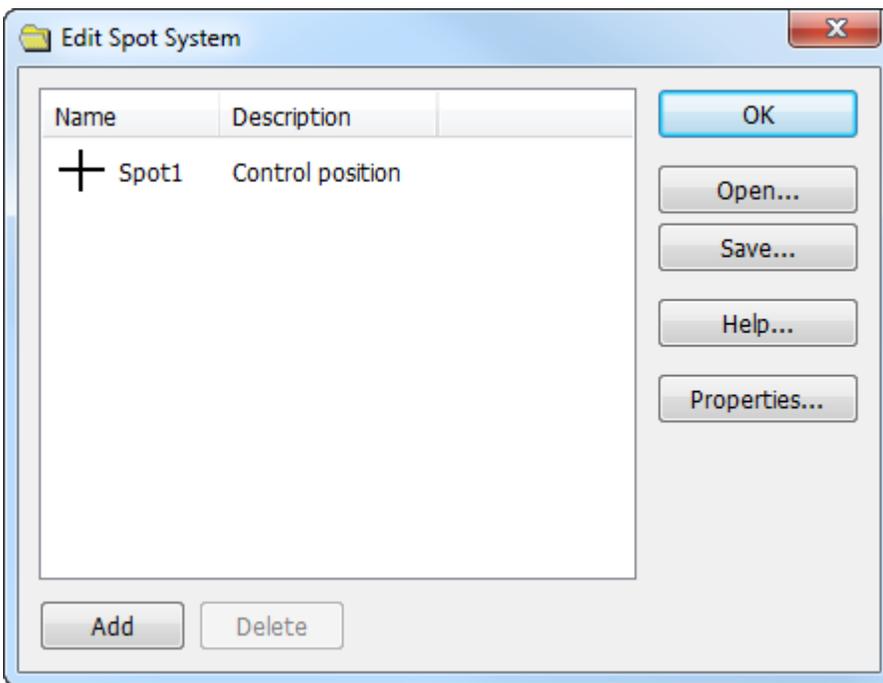


**What is a spot?**

Spots are graphical elements that you can use to mark locations in histograms. Each Spot stores its location for each parameter in the listmode file, so the spot becomes an n-dimensional placeholder. You can use spots to identify peak positions or cluster locations in one file, and then read a test file into the program and see how the positions have changed. They are useful when working with software compensation to see how clusters move when compensation is applied.

**Positioning the Spot**

After a spot is created and the dialog closed, the spot can be positioned with the mouse by clicking and dragging the center of the spot.
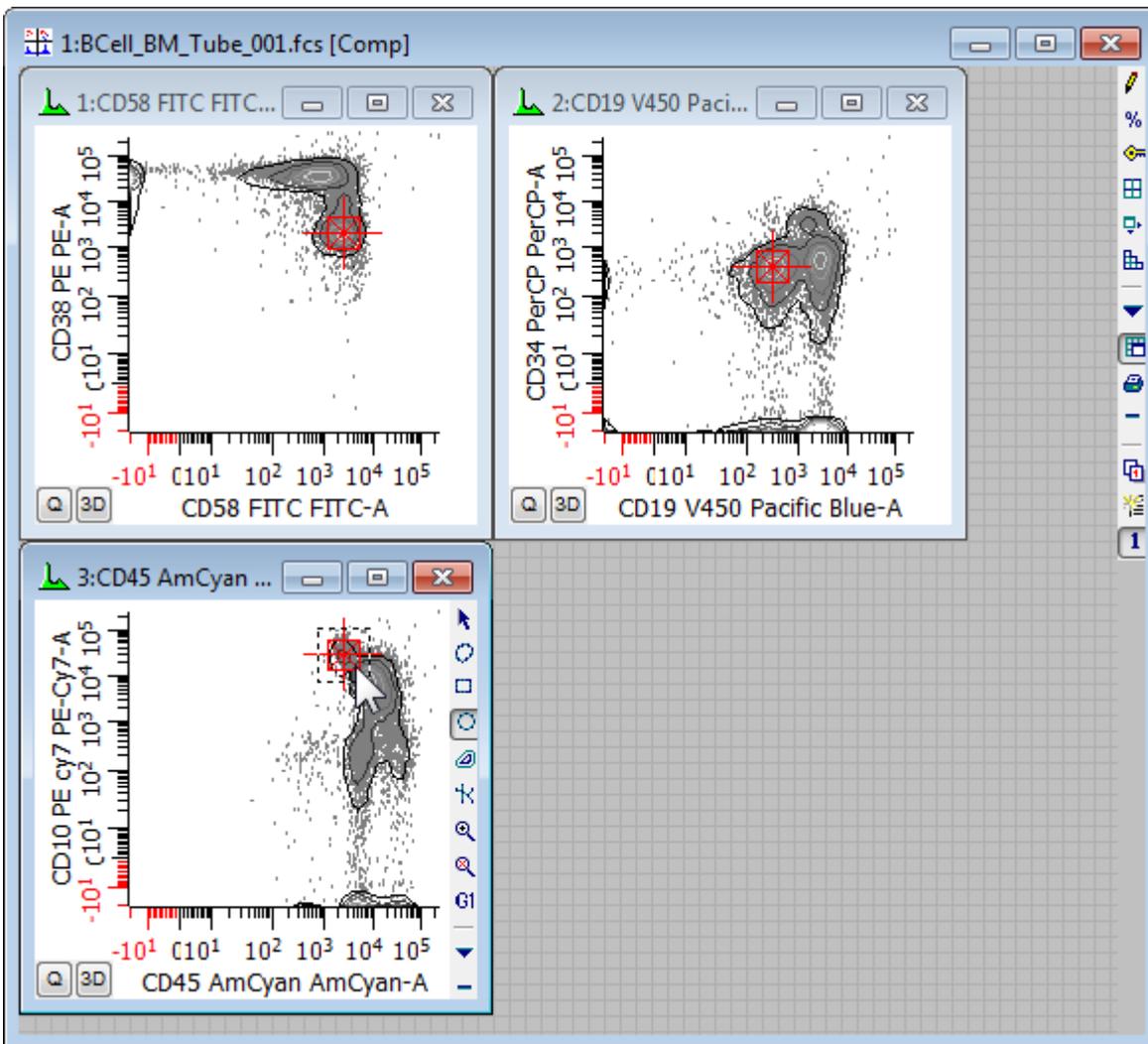
## Add

To create a new spot, click the Add button. Any number of spots can be created. When a new spot is created, the Edit Properties for Spot dialog is displayed to allow the spot to be named and a color selected.

## Delete

To delete a spot, select it in the list and click the Delete button.

## OK

Click this button to close the dialog.

## Open/Save

You can save a set of spots to a file on disk by clicking the Save button. Use the Open button to reopen a saved spot file.

**Properties**

If a spot is selected in the list, the Properties button will display the Edit Properties for Spot dialog to allow editing of the spot's description and color.

If nothing is selected in the list, the properties dialog for all spots is displayed. This dialog allows you to show or hide all spots.

**Edit Properties for Spot**

This dialog is displayed by double-clicking a spot, or from the Edit Spots dialog.
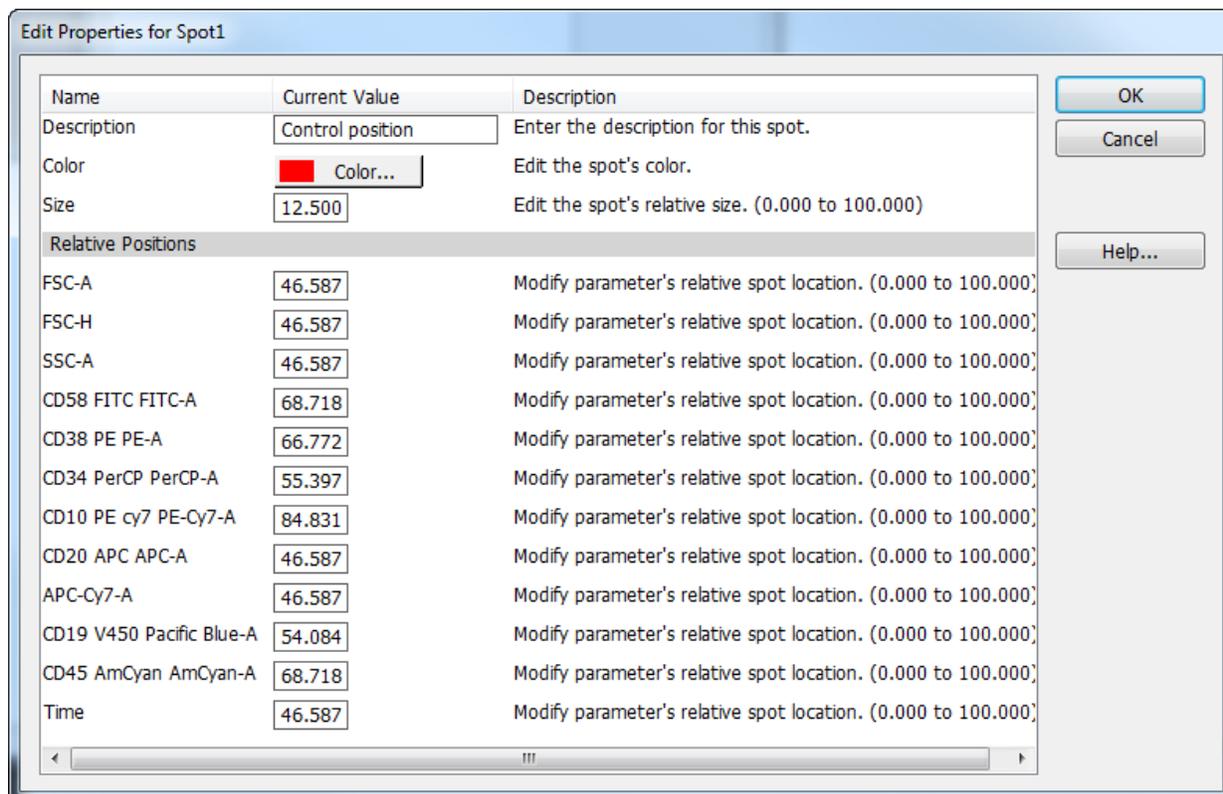


**Description**

Type a description, if desired. The description is not displayed except in this dialog.

**Color**

Click this button to choose a color for the spot. The standard color selection dialog is displayed.

**Size**

Spots can be of different sizes. The size field is a percentage of the histogram area. So, a value of 10 causes the spot to be 10% of the width and height of the histogram.

**Relative positions**

The spot keeps track of its position in each parameter of the listmode file. The positions are in percentage units, so a value of 50.0 positions the spot at the halfway point in the parameter. These values can be edited directly, or you can use the mouse to move the spot graphically.

**Regions and Gates**

**Regions**

WinList can create a number of different types of regions.  Each is created in a different way as described below.

The way regions are moved, resized, and deleted is common to all region types. Move a region by clicking on the region's label, and then dragging the label to a new location. To move the label *without* moving the region, click on the left portion of the region label, called the hotspot, and drag the label.
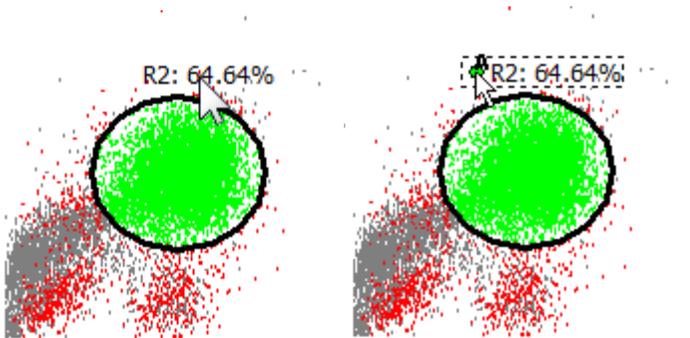


Resize a 1P or 2P region by clicking on the region's label to activate the region. Sizing handles are displayed. Click on one of the handles and drag it with the mouse to adjust the size of the region.
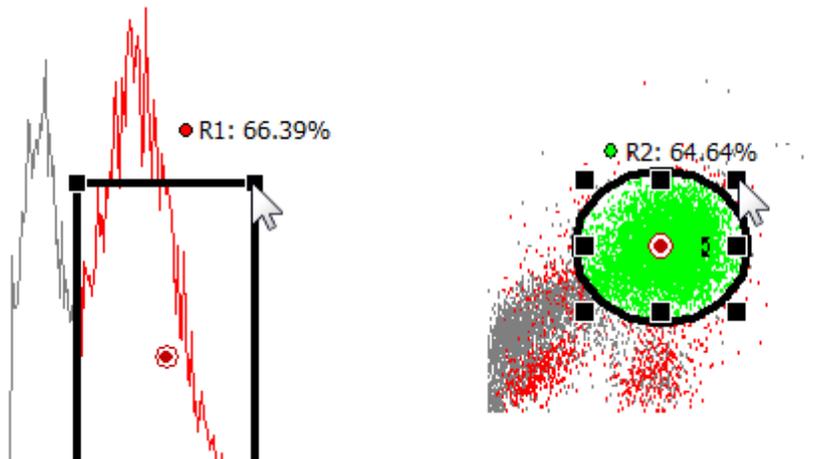


Delete a region by clicking on the region label, and then pressing the Delete key on the keyboard. You can also delete regions (except for NStat regions) by dragging the region label off the left edge of the histogram and releasing the mouse.

## 1P Region Creation

To define a new region in 1P histograms, click on the histogram title bar to activate it. This will also display the histogram toolbar. Make sure that the Range tool is selected in the histogram toolbar. Click and draw on the histogram to define the left and right boundaries of the range, and release the mouse button when finished.

1P regions can automatically position themselves relative to the median of the histogram that contains them. The attribute can be enabled in the Edit Properties for 1P Region dialog box.

## 2P Region Creation

In 2P histograms, you can define many types of regions, including free-hand, contour, rectangular, elliptical, and NStat. To create a new region, first click on the histogram's title bar to activate it and show the histogram toolbar. Choose the type of region you want from the toolbar that appears on the right side of the histogram.

To draw a *free-hand region*, select the **Free-hand** tool, then click, hold, and drag the mouse to create additional points. Finish the region by releasing the mouse on the first point that was created. Free hand regions can also be created using the *point-and-click* technique to define each point in the region. Start this method in the same way described above, and then click the mouse button for each point you want to define. Finish the region by clicking on the first point that was created.

To create a *rectangular region*, select the **Rectangle** tool. Click and hold the mouse button to create the first corner of the rectangle. Move the mouse and release the button to define the opposite corner of the rectangle.

To create an *elliptical region*, select the **Ellipse** tool. The ellipse is created in the same way as the rectangle region described above.

To create a *contour region*, select the **Contour** tool. With a Contour region, a bounding rectangle is defined with the mouse, just as with the Rectangle tool.  Once that rectangle is defined, WinList computes a contour on the data within that rectangle.  Each time the data in a histogram changes, the contour region will recompute based on the data in that rectangle.

To size a 2P region, first click on the region label to activate the region. Sizing handles will appear around the perimeter of the region. Drag a handle to resize the region.

To rotate a 2P region, first click on the region label to activate the region. Along with sizing handles, a small double-headed arrow will appear to the right of the region's center. Drag this handle to rotate the region.

The Edit Properties for 2P Region dialog is displayed when you double-click a 2P region label.

A 2P region can also display all or some of its vertices, allowing you to adjust one or more points in the region. To display the vertices, display the Edit Properties for 2P Region dialog and enable the Show Vertices option. Each vertex point can be moved with the mouse.

2P regions can automatically position themselves relative to the median of the histogram that contains them. This attribute can be enabled in the Edit Properties for 2P Region dialog box.

**NStat and QuadStat Region Creation**

An NStat region is an array of adjacent regions with a defined number of rows and columns on a 2P histogram. A QuadStat region is a particular type of NStat region with 2 rows and 2 columns. NStats are very useful when you need a set of adjacent regions that must move together.

To create an *NStat region*, click the **NStat** tool in the histogram toolbar. The NStat Properties dialog is displayed, allowing you to select the number of rows and columns for the NStat. Select the options you want for the NStat and click OK to finish the creation process.
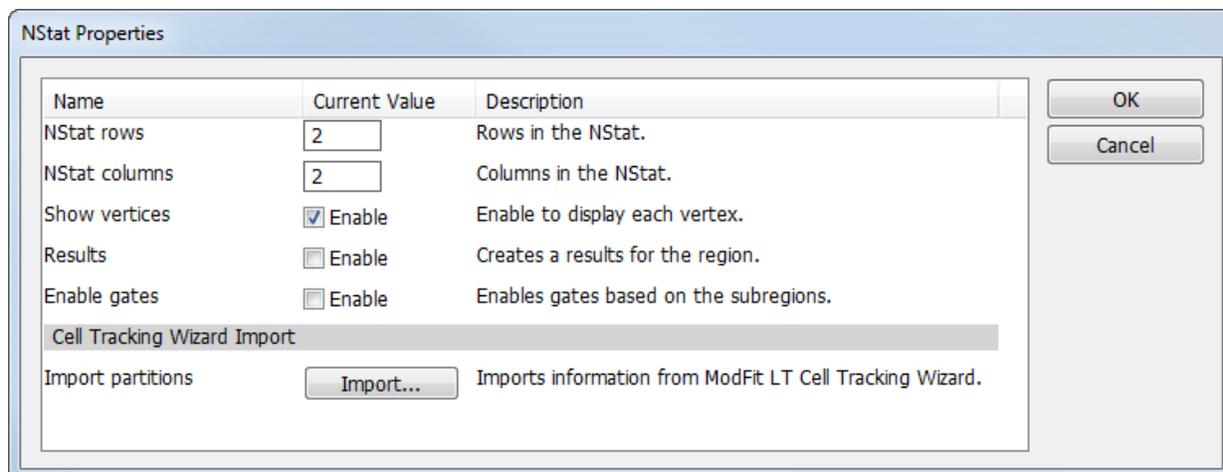


The Edit Properties for NStat Region dialog is displayed when you double-click an NStat region label. Click on the region label and press the Delete key to delete these regions.

An NStat region can display its vertices, allowing you to adjust individual points in the region. To display the vertices, display the Edit Properties for NStat Region dialog and enable the Show Vertices option. Each vertex point can be moved with the mouse.

**Region Array Creation**

To create an entire set of individual, independent regions at once on a histogram, use the Create Region Array option in the **Create** menu. This command can create sets of 1P or 2P regions, depending on what histogram is active at the time.

**Edit Regions**



This command is found on the **Home** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display.

The **Edit Regions** dialog box displays a list of the regions that have been created in the active data source. It can be used to delete regions, link regions together, and to save and open regions from disk.
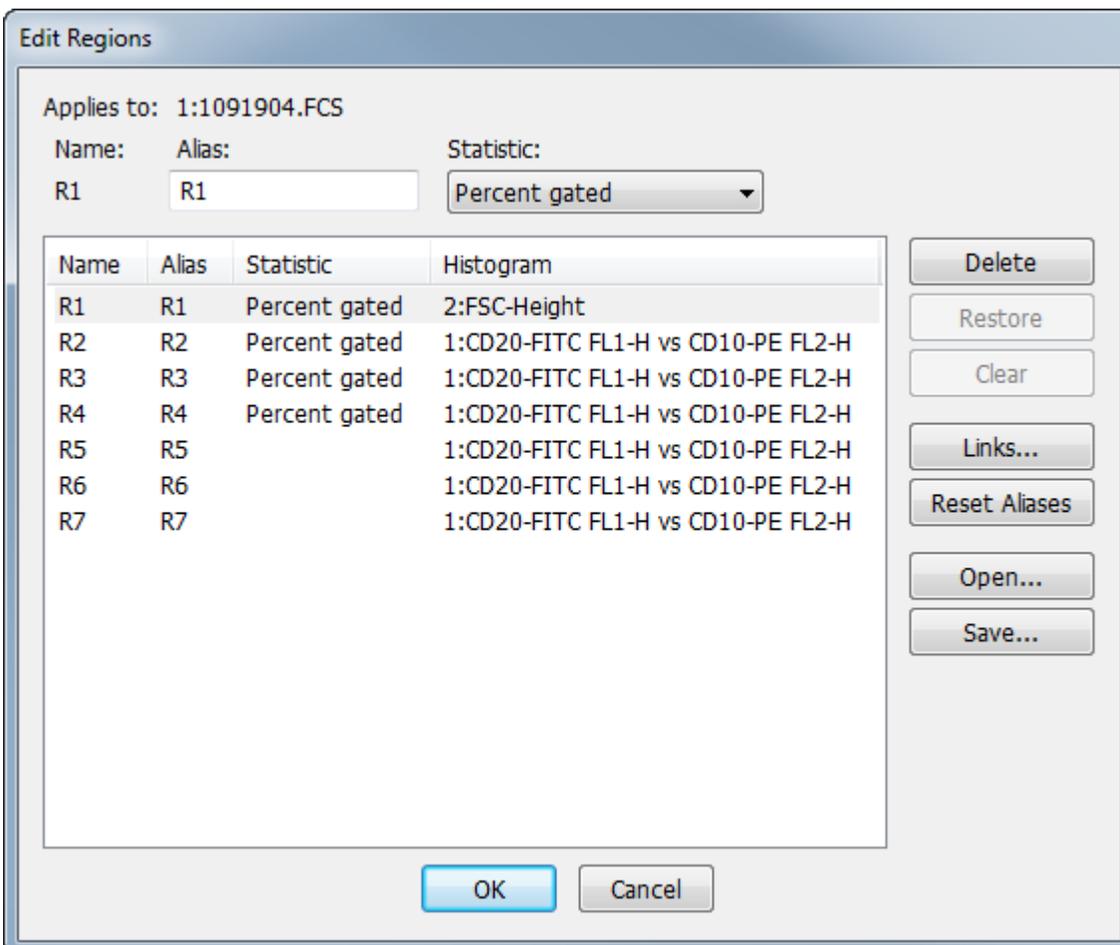


The region list box is a multiple select list box, which means that any number of regions can be selected at once. To select regions that are not adjacent in the list, hold the **Ctrl** key down on the keyboard while clicking on the Region name.

**Applies to**

The data source that this region dialog relates to is shown here.

**Name**

This field shows the name of the selected region.

**Alias**

This edit box allows you to enter an alias name for the selected region. The name can be displayed in results windows to make region identification easier. You can also display the region name or alias when you draw regions on histograms, so you can edit the Alias to more meaningful names such as Lymphs or Monos.

In addition, region aliases can be FCS or internal keywords. Any part of an alias that is surrounded with square brackets [ ] will be evaluated as a keyword. See Internal Keywords for more on keywords.

**Statistic**

This field shows the statistic that the selected region will display in its label. *No statistic*, *Percent Gated*, *Percent Total*, and *Region Events* are the statistics that can be selected.

**Region list box**

This list box shows all regions that have been created. This is a multiple selection list box, so more than one item may be selected at a time. Each time a selection is made, the alias for the first selected item is displayed in the Alias edit box.

**Delete**

This command deletes the selected regions.

**Restore**

This command restores a deleted region from the Undo buffer.

**Clear**

This command clears a deleted region from the Undo buffer so that it is no longer associated with the histogram.

**Links**

Click this button to link one or more regions together, or to view existing region links. The Edit Region Links dialog is displayed.

**Reset Aliases**

This command changes all the current aliases to their default values.

**Open/Save**

These buttons allow you to save and retrieve regions. When regions are opened from disk, they replace the ones currently in memory. WinList will attempt to re-establish histogram, results windows, and linking associations.

**OK**

Click this button to accept the changes made and close the dialog box.

**Edit Properties for 1P Region**

This dialog can be displayed by double-clicking the mouse on the label of a 1P region, or by right-button clicking on the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the Preferences dialog, found in the Options menu.



## Elements in the tree
**Region**

Select the region in the tree on the left to edit the properties of the region.

**Label**

Choose Label in the tree on the left to edit the font, background color, and transparency for the region label.

## Properties
**Show Name**

Enable this checkbox to display the name of the region.

**Show Alias**

Enable this checkbox to display the alias of the region.

**Alias**

This edit box allows you to edit the alias of the region.

**Show Statistic**

Select a statistic to display in the region label. *No statistic*, *Percent Gated*, *Percent Total*, and *Region Events* are the statistics that can be selected.

**Line color**

Click this button to choose a different color for the region.

**Line weight**

Select a line weight from the list of options. Larger values are heavier line weights.

**Allow delete**

With this option enabled, the region can be deleted by the user. To prevent a region from being deleted, uncheck this option.

**Region style**

Select the style for the 1P region: Bar with legs, Bar with tics.

**Auto-positioning**
**Auto-position**

Enable this option when you want the region to automatically adjust its position relative to the data in the histogram. The region will center itself on the X median or mode value, depending on the selection in the Auto-position method option below. When you move a region with the auto-position property enabled, you offset its auto-position location relative to the data. When the data changes in the histogram containing the region, the region will adjust its position relative to the new data.

The auto-position property will be disabled in a few circumstances where it is invalid. When a region is used in a self-gate on the histogram in which it resides, the property is disabled. Similarly, if a region is a dependent linked region, the property is disabled.

**Auto-position method**

This option determines what statistic is used when the auto-positioning is enabled for a region. The center of the region is positioned on the specified value. Choose *Center on histogram median* if you want to track the central tendency of the data or *Center on histogram mode* to track the location of highest frequency events. Choose *Include lower percent* to position the region to include events on the low intensity end of the histogram, or *Include upper percent* to include the high intensity events. Choose *Center on region median* or *Center on region mode* to track the central tendency of the data within the region, not the entire histogram.

**X Percent**

If Auto-Position is enabled and the method is *Include lower percent,* enter the percentile value where the right leg of the region should be positioned. The left leg will be set at the origin.

If Auto-Position is enabled and the method is *Include upper percent,* enter the percentile value where the left leg of the region should be positioned. The right leg will be set at the upper boundary.

**OK**

Click this button to accept changes and close the dialog box.

**Cancel**

Click this button to close the dialog box and cancel changes.

**Help**

Click this button to display on-line help for this dialog.

### 1P Region Context Menu

This menu is displayed by right-clicking a 1P region label. It provides commands that relate to the region.

### Edit Properties

Choose this command to display the Edit Properties dialog for the 1P region.

### Create Results

This command creates a Results window to display statistics for the region.

### Highlight events in Rn

This is a toggle option that can be enabled for one region at a time. When enabled, the events that are in the region will be highlighted in all histograms.  Highlighting, by default, displays these events larger and blinking. See Region preferences for highlight properties.

### Color events in Rn only

This is a toggle command that enables or disables coloring using this region only.  It is a convenient way to explore where the events that are in the region appear in all other histograms.  Selecting the option once will enable coloring for the region.  Selecting it again (or choosing it for another region) will turn off the function.

### Stick to Neighbor Regions

This command scans the vertices of the region to see if any are overlapping vertices from neighboring regions.  For any vertex that overlaps another region's vertex, a linkage is made so that if either vertex is moved, both are moved. Sticky regions make it possible to create a set of adjacent regions that maintain adjacency even if you move one region, or a vertex of a region. See Sticky Regions and Magic Contours for more info.

### Region Links…

This command displays the Edit Region Links for the region to allow this region to control the size and shape of other regions, and vice versa.

### Delete

This command deletes the region, if it is not locked.

**Edit Properties for 2P Region**

This dialog can be displayed by double-clicking the mouse on the label of a 2P region, or by right-clicking the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the Preferences dialog, found in the Options menu.



## Elements in the tree
**Region**

Select the region in the tree on the left to edit the properties of the region.

**Label**

Choose Label in the tree on the left to edit the font, background color, and transparency for the region label.

## Properties
**Show Name**

Enable this checkbox to display the name of the region.

**Show Alias**

Enable this checkbox to display the alias of the region.

**Alias**

This edit box allows you to edit the alias of the region.

**Show Statistic**

Select a statistic to display in the region label. *No statistic*, *Percent Gated*, *Percent Total*, and *Region Events* are the statistics that can be selected.

**Line color**

Click this button to choose a different color for the region.

**Line weight**

Select a line weight from the list of options. Larger values are heavier line weights.

**Allow delete**

This option determines whether a region can be deleted by the user. When enabled, regions can be deleted by clicking on the region label and pressing the Delete key on the keyboard. Some regions can also be deleted by dragging them off the left or bottom edge of the histogram. Uncheck this option to protect a region from being deleted accidentally. Any region can be deleted using the Region List dialog box, even if the Allow delete option is disabled.

**Vertices**

**Show resizing handles**

Enable this option to display resize handles on the region when it is active. Resize handles form a bounding rectangle around a region and can be used to adjust size or shape of a region. The handles are hidden when the region is not selected.

**Show vertices**

Enable this option to display the individual points that comprise the region. When displayed, the vertex points can be moved individually.

**Number of vertices**

This option allows you to choose how many of the region vertices to display when the Show Vertices option is enabled. Choosing 0 displays all vertices. Other choices include 5, 10, and 20 vertices. By displaying a subset of the vertices, it is possible to adjust regions with many vertices using just a few control points.

**Vertex size**

This option determines the size of the region's vertices, if they are displayed.

**Change shape**

This option allows you to change the shape of an existing region to either an ellipse or a rectangle.

**Auto-positioning**

**Auto-position**

Enable this option when you want the region to automatically adjust its position relative to data in the histogram. The region will center itself on the X and Y medians or modes, depending on the selection in the Auto-position method option below. When you move a region with the auto-position property enabled, you offset its auto-position location relative to the data. When the data changes in the histogram containing the region, the region will adjust its position relative to the new data.

The auto-position property will be disabled in a few circumstances where it is invalid. When a region is used in a self-gate on the histogram in which it resides, the property is disabled. Similarly, if a region is a dependent linked region, the property is disabled.

**Auto-position method**

This option determines what statistic is used when the auto-positioning is enabled for a region. The center of the region is positioned on the specified value. Choose *Center on histogram median* if you want to track the central tendency of the data or *Center on histogram mode* to track the location of highest frequency events.  Choose *Include lower percent* to position the region to include events on the low intensity end of the histogram, or *Include upper percent* to include the high intensity events.  Choose *Center on region median* or *Center on region mode* to track the central tendency of the data within the region, not the entire histogram.

**X Percent, Y Percent**

If Auto-Position is enabled and the method is *Include lower percent,* enter the percentile value where the top and right sides of the region should be positioned. The left and bottom edges will be set at the origin.
If Auto-Position is enabled and the method is *Include upper percent,* enter the percentile value where the left and bottom edges of the region should be positioned. The top and right edges will be set at the upper boundary.

**OK**

Click this button to accept changes and close the dialog box.

**Cancel**

Click this button to close the dialog box and cancel changes.

**Help**

Click this button to display on-line help for this dialog.

## 2P Region Context Menu

This menu is displayed by right-clicking a 2P region's label. It provides commands that relate to the region.

### Edit Properties

Choose this command to display the Edit Properties dialog for the 2P region.

### Create Results

This command creates a Results window to display statistics for the region.

### Highlight events in Rn

This is a toggle option that can be enabled for one region at a time. When enabled, the events that are in the region will be highlighted in all histograms.  Highlighting, by default, displays these events larger and blinking. See Region preferences for highlight properties.

### Color R*n* Only

This is a toggle command that enables or disables coloring using this region only.  It is a convenient way to explore where the events that are in the region appear in all other histograms.  Selecting the option once will enable coloring for the region.  Selecting it again (or choosing it for another region) will turn off the function.

### Show Resize Handles

Enable this option to show the resizing handles for the 2p region.

### Show Vertices

Enable this option to show the vertices of the 2p region.

### Stick to Neighbor Regions

This command scans the vertices of the region to see if any are overlapping vertices from neighboring regions.  For any vertex that overlaps another region's vertex, a linkage is made so that if either vertex is moved, both are moved. Sticky regions make it possible to create a set of adjacent regions that maintain adjacency even if you move one region, or a vertex of a region. See Sticky Regions and Magic Contours for more info.

### Conform to Other Regions

This command adjusts the vertices of the region so that they do not overlap other 2P regions in the histogram. Vertices of this region that are inside of other regions are eliminated, and vertices of other regions that are inside of this region are added.

**Region Links...**

This command displays the Edit Region Links for the region to allow this region to control the size and shape of other regions, and vice versa.

**Delete**

This command deletes the region, if it is not locked.

**Edit Properties for Contour Region**

This dialog can be displayed by double-clicking the mouse on the label of a Contour region, or by right-clicking the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the Preferences dialog, found in the Options menu.



## Elements in the tree
### Region
Select the region in the tree on the left to edit the properties of the region.

### Label
Choose Label in the tree on the left to edit the font, background color, and transparency for the region label.

## Properties
### Show Name
Enable this checkbox to display the name of the region.

### Show Alias
Enable this checkbox to display the alias of the region.

**Alias**

This edit box allows you to edit the alias of the region.

**Show Statistic**

Select a statistic to display in the region label. *No statistic*, *Percent Gated*, *Percent Total*, and *Region Events* are the statistics that can be selected.

**Line color**

Click this button to choose a different color for the region.

**Line weight**

Select a line weight from the list of options. Larger values are heavier line weights.

**Allow delete**

This option determines whether a region can be deleted by the user. When enabled, regions can be deleted by clicking on the region label and pressing the Delete key on the keyboard. Some regions can also be deleted by dragging them off the left or bottom edge of the histogram. Uncheck this option to protect a region from being deleted accidentally. Any region can be deleted using the Region List dialog box, even if the Allow delete option is disabled.

**Contour Region**

**Contour Probability**

This field determines the size of the contour region, with a probability value ranging from 0.01 (smallest) to 0.99 (largest).  With a larger probability value, more events are included.

**Smoothing**

This field controls the smoothness of the contour region, with a larger value creating a smoother region.

**Show resizing handles**

Enable this option to display resize handles on the region when it is active. Resize handles form a bounding rectangle around a region and can be used to adjust size or shape of a region. The handles are hidden when the region is not selected.

**Auto-positioning**

**Auto-position**

Enable this option when you want the region to automatically adjust its position relative to data in the histogram. The region will center itself on the X and Y medians or modes, depending on the selection in the Auto-position method option below. When you move a region with the auto-position property enabled, you offset its auto-position location relative to the data. When the data changes in the histogram containing the region, the region will adjust its position relative to the new data.

The auto-position property will be disabled in a few circumstances where it is invalid. When a region is used in a self-gate on the histogram in which it resides, the property is disabled. Similarly, if a region is a dependent linked region, the property is disabled.

### Auto-position method

This option determines what statistic is used when the auto-positioning is enabled for a region. The center of the region is positioned on the specified value. Choose *Center on histogram median* if you want to track the central tendency of the data or *Center on histogram mode* to track the location of highest frequency events.  Choose *Include lower percent* to position the region to include events on the low intensity end of the histogram, or *Include upper percent* to include the high intensity events.  Choose *Center on region median* or *Center on region mode* to track the central tendency of the data within the region, not the entire histogram.

### X Percent, Y Percent

If Auto-Position is enabled and the method is *Include lower percent,* enter the percentile value where the top and right sides of the region should be positioned. The left and bottom edges will be set at the origin.
If Auto-Position is enabled and the method is *Include upper percent,* enter the percentile value where the left and bottom edges of the region should be positioned. The top and right edges will be set at the upper boundary.

### OK

Click this button to accept changes and close the dialog box.

### Cancel

Click this button to close the dialog box and cancel changes.

### Help

Click this button to display on-line help for this dialog.

## Edit Properties for NStat Region

This dialog can be displayed by double-clicking the mouse on the label of an NStat region, or by right-button clicking on the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the Preferences dialog, found in the Options menu.
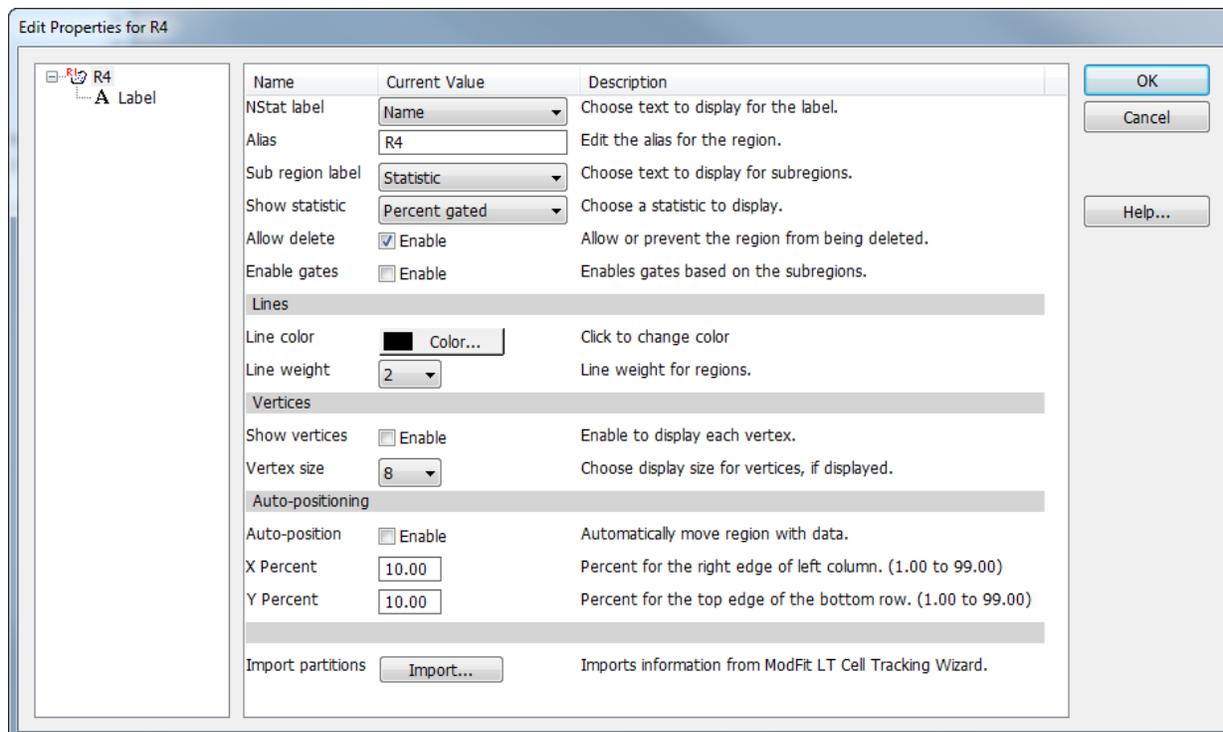


## Elements in the tree
### Region

Select the region in the tree on the left to edit the properties of the region.

### Label

Choose Label in the tree on the left to edit the font, background color, and transparency for the region label.

## Properties
### NStat label

This list allows you to choose what to display for the movable region label. Choose from Name, Alias, or both Name and alias.

**Alias**

This edit box allows you to edit the alias of the region.

**Label**

Choose this item in the tree on the left of the dialog to edit the font, background color, and transparency for the region label.

**Sub region label**

This list allows you to choose what to display for the sub region labels. Choose from Name, Alias, or both Name and alias. The sub region label appears in the corner of the subregion and is not movable.

**Show Statistic**

Select a statistic to display in the region label. *No statistic*, *Percent Gated*, *Percent Total*, and *Region Events* are the statistics that can be selected.

**Allow delete**

This option determines whether a region can be deleted by the user. When enabled, regions can be deleted by clicking on the region label and pressing the Delete key on the keyboard. Some regions can also be deleted by dragging them off the left or bottom edge of the histogram. Uncheck this option to protect a region from being deleted accidentally. Any region can be deleted using the Region List dialog box, even if the Allow delete option is disabled.

**Enable gates**

Enable this option to turn on gates that are associated with each sub region of the NStat. Normally, WinList does not turn off N-Stat subregions for gating. If you want to use an N-Stat in a gate expression, this option needs to be enabled.

**Lines**

**Line color**

Click this button to choose a different color for the region.

**Line weight**

Select a line weight from the list of options. Larger values are heavier line weights.

**Vertices**

**Show vertices**

Enable this option to display the individual points that comprise the region. When displayed, the vertex points can be moved individually.

**Vertex size**

This option determines the size of the region vertices, if they are displayed.

**Auto-positioning**

**Auto-position**

Enable this option when you want the region to automatically adjust its position relative to data in the histogram. For NStat regions, the auto-positioning is always performed based on an X and Y percentile value that you enter.

**X Percent, Y Percent**

If Auto-Position is enabled, enter the X and Y percentile values for positioning the NStat. The left-most column of the NStat is positioned at the X Percentile, and the bottommost row is positioned at the Y Percentile value.

**Import partitions**

This option allows region partitions to be imported from file on disk. It is designed to work with ModFit LT's capability to export partitions from the Proliferation Wizard. The format of the file is tab-delimited ASCII. An example is shown below:

```
Prolif Wizard Partitions
File    ab#28 CSFE Norm 72h.011
Regions 10
Scale   0       256
Gen10   0.00    23.23
Gen9    23.23   43.78
Gen8    43.78   62.89
Gen7    62.89   82.19
Gen6    82.19   99.98
Gen5    99.98   122.44
Gen4    122.44  140.95
Gen3    140.95  160.32
Gen2    160.32  179.90
Parent  179.90  256.00
```

When the Import option is used to read a partition file, the NStat region boundaries are adjusted to match those defined in the partition file. This allows WinList to take advantage of a proliferation analysis in ModFit LT, to perform additional analysis on the daughter populations and their relationship to other markers.

**OK**

Click this button to accept changes and close the dialog box.

**Cancel**

Click this button to close the dialog box and cancel changes.

**Help**

Click this button to display on-line help for this dialog.

**NStat Context Menu**

This menu is displayed by right-clicking an NStat region label. It provides commands that relate to the region.

**Edit Properties**

Choose this command to display the Edit Properties dialog for the NStat region.

**Create Results**

This command creates a Results window to display statistics for the region.

**Show Vertices**

Enable this option to show the vertices of the NStat region.

**Region Links…**

This command displays the Edit Region Links for the region to allow this region to control the size and shape of other regions, and vice versa.

**Delete**

This command deletes the region, if it is not locked.

**Edit Region Links**

The **Edit Regions Links** dialog box is used to create links between regions. Linked regions take on the same shape and position. The link can be bidirectional, where a change in either region affects the other. They can also be one-way links. One region can be linked to many others.

A region in one data source can be linked to a region in another, allowing you to set up identical gating conditions in both.
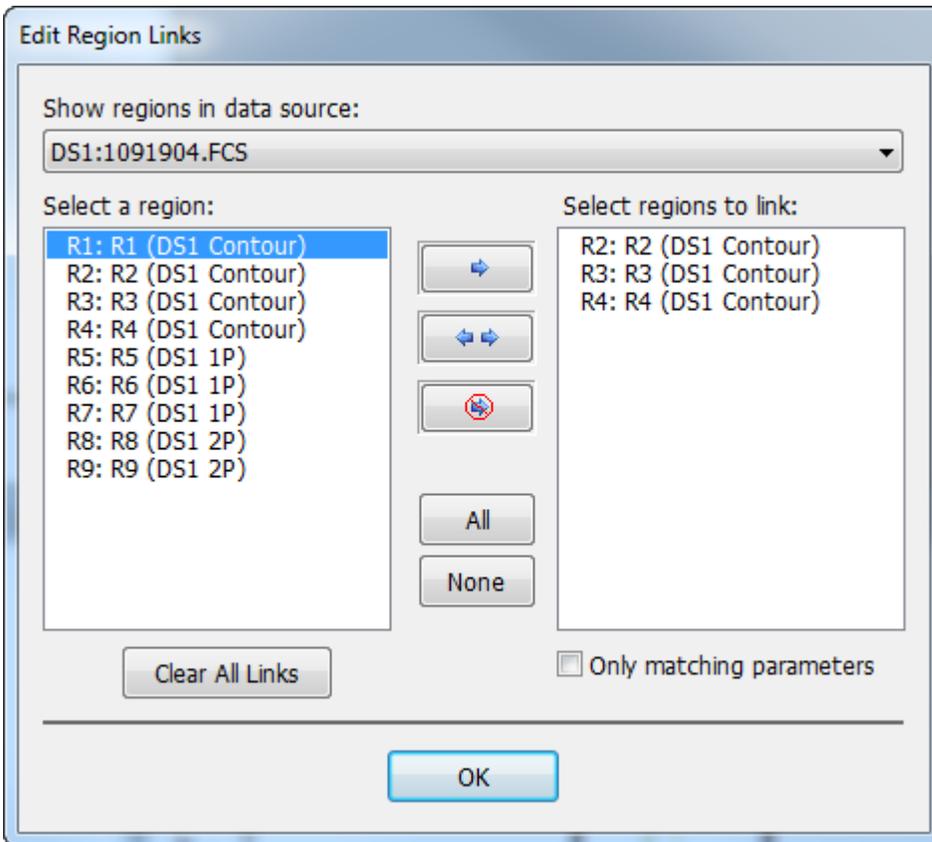


**Show regions in data source:**

Select a data source from this dropdown to view/edit the links for its regions.

**Select a region:**

In the list on the left, select one region. Once selected, the list on the right fills with compatible regions that are or can be linked to the selected region.

**Select regions to link:**

When a region is selected from the list on the left, this list fills with compatible regions that are or can be linked to the selected region. Click a region to select it. Press the **Ctrl** key and click to select more than one region.

Regions with an asterisk (*) are linked to the selected region. Regions are not linked until one of the link buttons below is clicked.

**Only matching parameters:**

With this option enabled, only regions in histograms with identical parameters to the source region will be displayed in the list.

**One-way link**
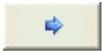


Click this button to link the region on the left to the regions selected on the right. The link is a one-way link, meaning that when the left-hand region is moved or resized, the other regions will move and resize accordingly.

**Two-way link**



Click this button to link the region on the left **bidirectionally** to the regions selected on the right. The links are two-way links, meaning that when either region is moved or resized, the other will move and resize accordingly.

**Unlink**



This push button will unlink the selected regions.

**Clear All Links**

This push button will unlink the all regions.

**OK**

To put away the Link Regions dialog box, select this push button.

### Create Region Array

This menu option displays the Create Region Array dialog box, which is used to create one or more rectilinear regions. Use this dialog when you want to create a set of regions spanning a given range of channels.

The main difference between a region array and an NStat region is that the region array creates a set of separate regions. Each region in the region array can be moved independently. The NStat is a collection of regions that are always adjacent and can be moved as a collection. See Regions and Edit Properties for NStat Region for more about NStat regions.

The dialog provides control over the number of regions, starting and ending channels, and the spacing between the regions. A result window can be created automatically for the regions.



### Create a results window

Enable this option if you want WinList to create a results window with the statistics for the regions created. Clear the check if you do not want a results window.

**Number of regions**

Enter the number of regions you want to create in a given direction. For single-parameter histograms, enter the number to create on the X-axis. For two-parameter histograms enter both X-axis and Y-axis values.

**Starting at**

Enter the value where the first region will begin. Values are in the same units shown on the axis.

**Ending at**

Enter the value where the last region will end. Values are in the same units shown on the axis.

**Space between**

If you want the regions to be separated, enter a value in this edit box. The value is in units representing a percentage of the axis, and the same value is used between all regions.

**OK**

Choose this option to create the region or regions defined by the dialog box.

**Cancel**

Choose this option to cancel the dialog box without creating any regions or results.

Some of the content of the dialog depends on the properties of the active histogram. If a 2-parameter histogram is active, options are displayed for X-Axis and Y-Axis as shown above. If a 1-parameter histogram is active, only the X-axis options are displayed. In addition, if the 1-parameter histogram contains an FCOM calculated parameter, a special option is added to the dialog.



**Create a region array for the FCOM peaks**

This option only appears if the active histogram is 1-parameter containing an FCOM calculated parameter. It creates a region array for the FCOM histogram, with annotated region labels and color event gating options. See FCOM Region Array options for details.

**FCOM Region Array Options**

This dialog is displayed by the Create Region Array dialog to create an array of regions specially designed for FCOM calculated parameters. It is only available when the active histogram is a 1P-histogram containing an FCOM parameter.
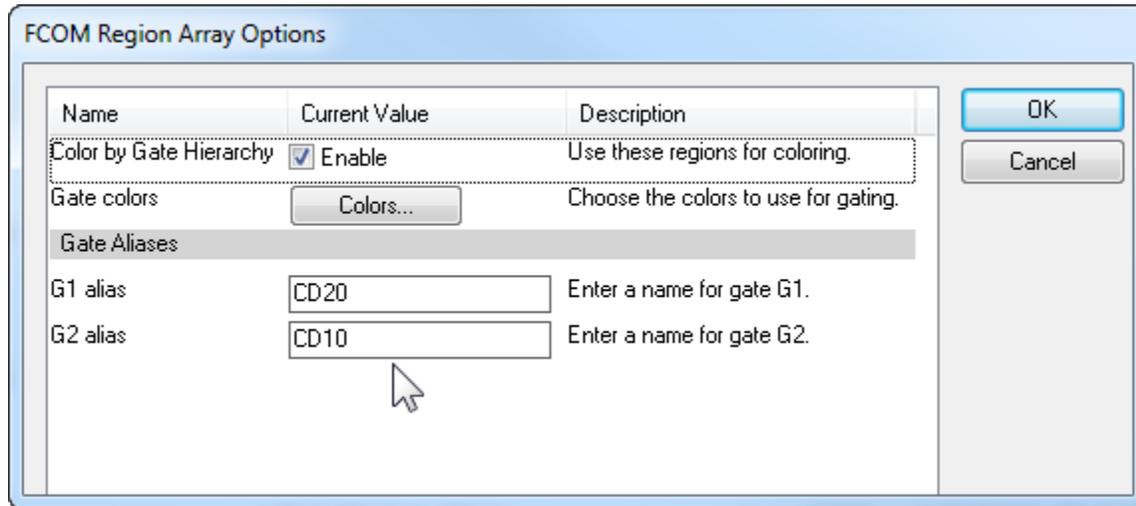


**Color by Gate Hierarchy:**

Enable this option if you want to use the FCOM peaks to color the dots in histogram. Since FCOM peaks are discrete phenotypes, it is often very useful to use them for color gating so that the populations can be seen in the context of all other histograms.

**Gate Colors:**

If you enable color-event gating with the option above, the colors used for each gate can be selected with this option.

**Gate Aliases**

The FCOM parameter uses gates to create phenotypes. The Gate Aliases edit boxes allow you to give the gates more descriptive names. For example, if G1 identifies CD20 positive cells and G2 is CD10 positive cells, you might type "CD20" and "CD10" respectively as the gate aliases. It is best not to include the "positive" or "negative" in the alias, and short aliases are more practical than long ones.

The program will create region aliases from the gate aliases you enter. Each peak in the FCOM histogram will have one region associated with it, and the region's alias will identify the phenotype. The region aliases created for the example given above would be:

CD20- CD10-

CD20+ CD10-

CD20- CD10+

CD20+ CD10+
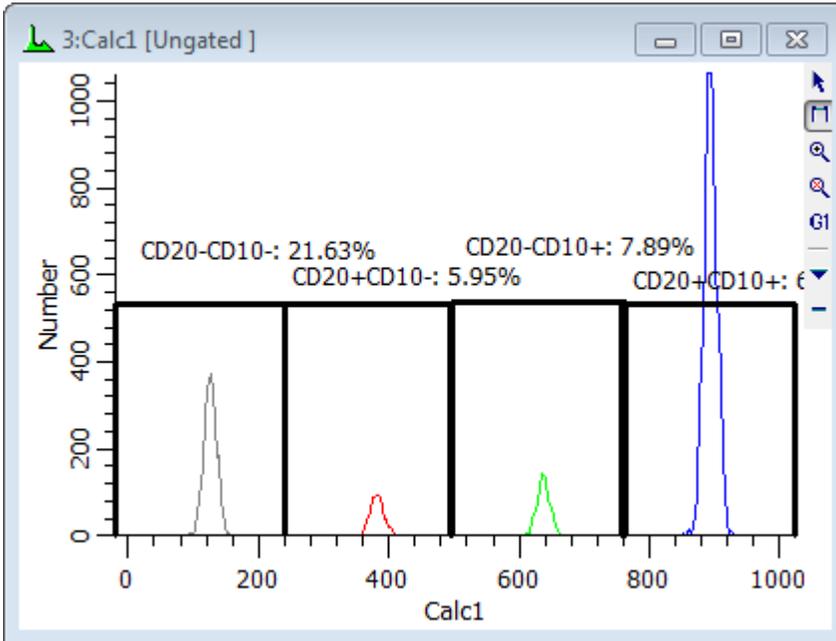
Obviously, if the FCOM parameter involves more gates, the need for short aliases becomes even greater.

**Edit Gates**
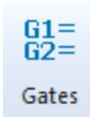


This command is found on the **Home** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display.

This dialog manages gates for one data source. You can redefine gates, change colors, assign them to histograms, save and open them from disk.
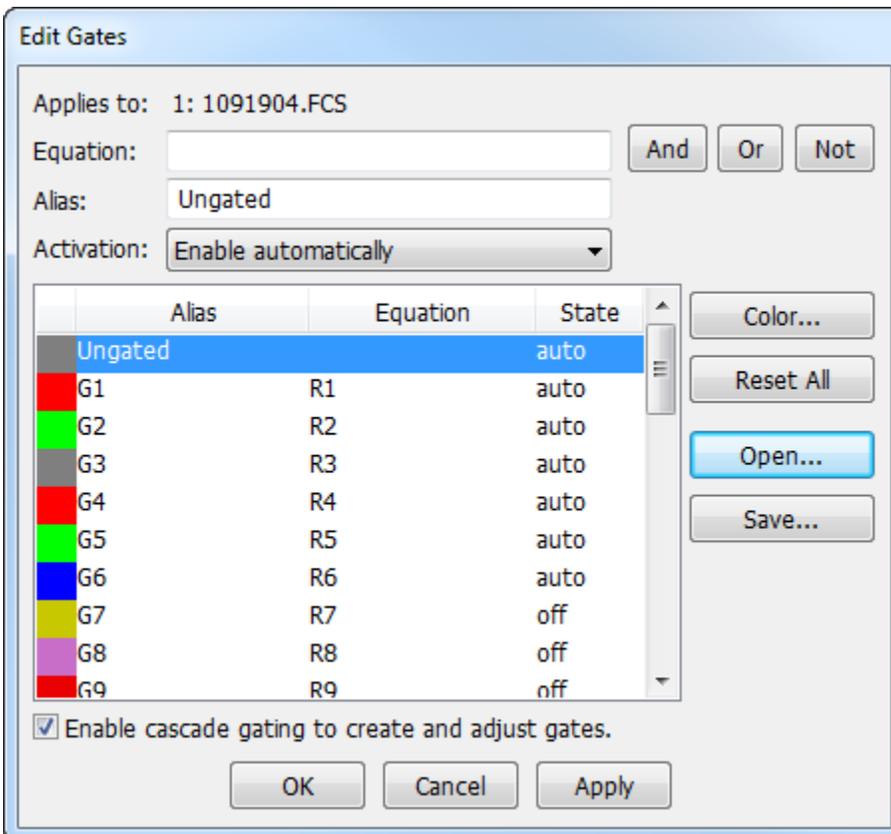


**Applies to**

The data source that this gate dialog relates to is shown here.

**Equation**

This field contains the gating expression. See **Writing a gate containing a Boolean region expression** below for a detailed explanation.

### And, Or, and Not Buttons

These buttons aid in the writing of Boolean equations. If you push the **And** button, a **&** will be inserted at the current location in the **Equation** edit box. The **Or** button inserts the **|** character and the **Not** button inserts the **!** character.

### Alias

Each gate can have an alias. The alias is the name that appears when the gate is shown in other locations in the program.

### Activation

This field controls how WinList activates the gate.

"Enable automatically" will turn the gate on when all of the regions referenced by the Equation have been created. The gate will be disabled if any of the regions are missing.

"Always enabled" causes the gate to remain enabled even if some of the regions in its Equation are missing.

### Gate List box

The gate name, equation, activation, and color assignment are shown in this list box. The number of gates is set in the Edit Program Controls dialog box.

Click an entry in the list to select the gate for editing.

Click the color swatch to select a new color for the gate. Double-clicking the gate will toggle it between "Enable automatically" and "Always enabled".

To assign a gate to one or more histograms, select the gate in the listbox and click the histogram title bars.

### Enable cascade gating to create and adjust gates

This check box turns the Cascade Gate system on and off. When Cascade Gating is on, gate expressions are automatically adjusted based on the gates that are applied to different histograms and the regions in those histograms.

### Color

Selects a color for the selected gate.

### Reset All

This option puts the system in its default condition where all gates are off and are associated with R1, R2, ... Rn respectively.

### Open

This option retrieves the contents of the gate list from disk.

**Save**

The contents of the gate list box can be saved to disk when this option is selected. This option may be useful if you have created a number of complex gates and you don't want to waste time re-creating them each time. It is important to realize that this option does not save the regions themselves (see Region List) and it does not save the actual absolute colors (see Show/Edit Palette).

**Cancel**

The **Cancel** button allows you to exit with no changes.

**OK**

Click **OK** to accept the changes you have made.

**Apply**

This button acts like the **OK** button, except that the dialog remains open.

**Writing a gate containing a Boolean region expression**

Normally when working with the gates, each gate is assigned a single region. For example, gate 1 or G1 is assigned to region 1 or R1. This initial setup can be changed extensively to better serve your gating needs. When a histogram is assigned a gate, say G1, and that gate is associated with R1 and the gate is active, the program will only allow those events that are inside R1 to be displayed in the gated histograms.

While this system allows some powerful gating, you can perform much more complex analytical gating. For example, if you wanted a histogram to be gated on two regions, say R1 and R2, and you wanted the histogram associated with this gate to have access to events that satisfy **both** regions, you could create a gate associated with the expression **R1 & R2**. The **&** symbol tells WinList to look only at events that satisfy both R1 AND R2. Click the **And** button to insert the **&** symbol in an expression.

The Boolean expression can be quite complex and may involve several regions. If you wanted to look at events that satisfy either R1 or R2, you would click the **Or** button. The expression would be written as **R1 | R2**.

Occasionally it is important to look at events that are **NOT** within a particular region. To do this we use the **!** operator. For example, to look at events that are *not* in R1, one would write **!R1**.

The gate expression can be made much more complex if needed by nested parentheses. For example, the gate expression

!(R1&R2) | R3

is true for events that do not satisfy both R1 and R2 or that satisfy R3. As you can see, these Boolean expressions can be quite powerful. A typical use of complex gates is to find rare events that satisfy a number of gating criteria. You can create a gate such as R1&R2&R3&R4 and turn on event coloring just for this gate. The events that satisfy all these regions will be highlighted by the gate color.

You can also use other gates in your gate expressions (as long as they do not create circular references). This is a convenient way to make your gate expressions more readable. For example, suppose that gate G1 is defined as (R1&R2)&!(R3&R4). We can use G1 as a shorthand for the expression in other gates:

> G2 = G1 & R6

which expands to

> ((R1&R2)&!(R3&R4)) & R6

when it is evaluated.

In summary, WinList's Boolean operators include **&** (AND), **|** (OR), and **!** (NOT). The operands are the regions defined as **R1**, **R2**, ... **Rn**. The steps to create a more complex gate expression are to first choose the gate that needs to be redefined, then to write the Boolean expression in the edit box, and finally to press the Replace button. To assign that gate to a particular histogram, click the histogram's title bar with the gate you want selected in the **Edit Gates** dialog box. When the **OK** button is selected, the new gate will be applied to all the gated histogram. You can save the gates at any time for future use by using the Save option.

**Gate Statistics**

The Gate Statistics window can be display for each data source. It contains a list of the active gates arranged by hierarchy, along with statistics for each gate.  The gate Alias is used to identify the gates.
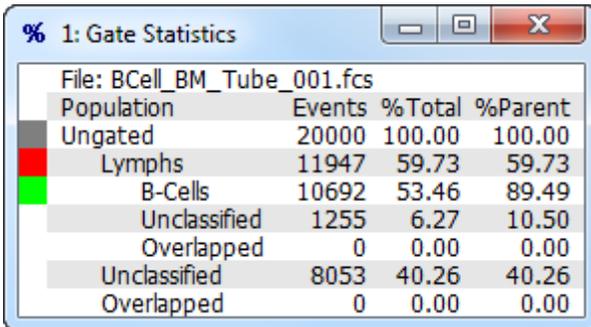
| Population | Events | %Total | %Parent |
|---|---|---|---|
| Ungated | 20000 | 100.00 | 100.00 |
| Lymphs | 11947 | 59.73 | 59.73 |
| B-Cells | 10692 | 53.46 | 89.49 |
| Unclassified | 1255 | 6.27 | 10.50 |
| Overlapped | 0 | 0.00 | 0.00 |
| Unclassified | 8053 | 40.26 | 40.26 |
| Overlapped | 0 | 0.00 | 0.00 |

File: BCell_BM_Tube_001.fcs

The window can be displayed by right-clicking the data source and selecting Gate Statistics from the Data Source Context menu.

The content of the Gate Statistics window can be edited with the Edit Properties for Gate Statistics dialog.

**Edit Properties for Gate Statistics**

This dialog controls the content of the Gate Statistics window.
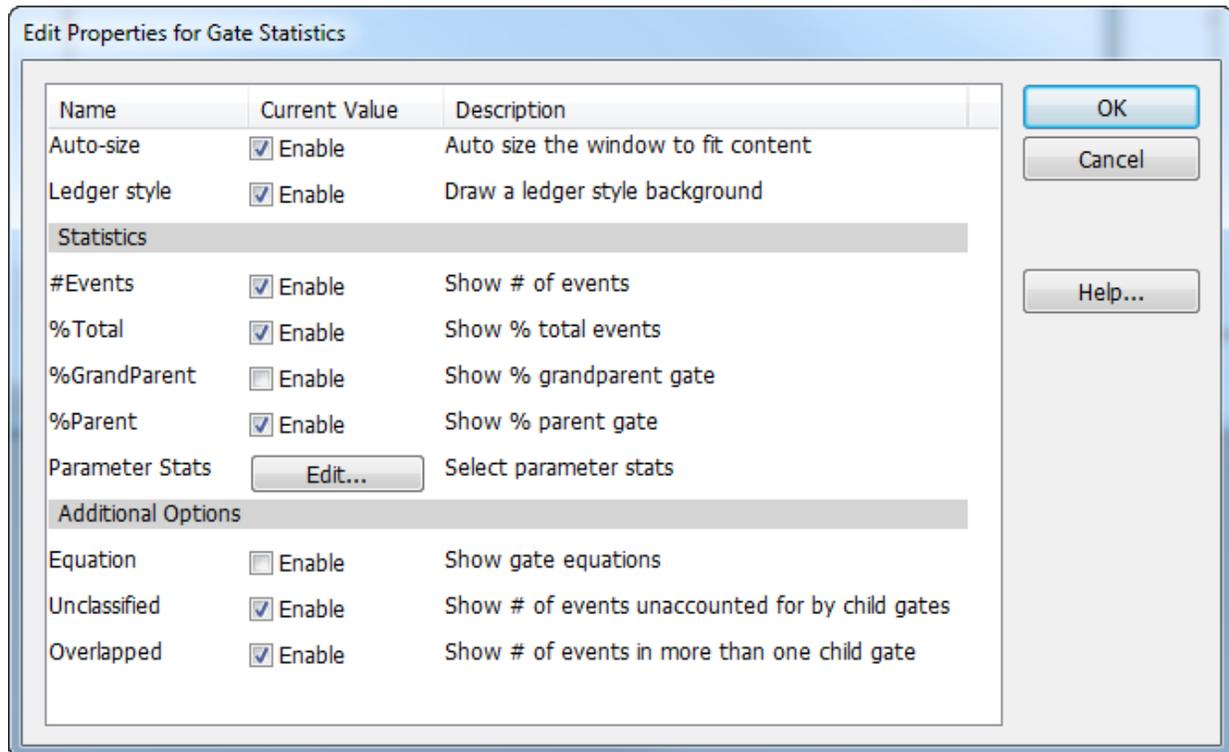


**Auto-size**

Enable this option to auto-size the Gate Statistics window to fit the content.

**Ledger style**

When this option is enabled, the Gate Statistics window uses alternating white and gray background for each line.

**Statistics**
**#Events**

Displays the number of events in the gates.

**%Total**

Displays number of events as a percentage of total events in the gate.

## %GrandParent

Displays number of events as a percentage of the grandparent gate.

## %Parent

Displays number of events as a percentage of the parent gate.

## Parameter Stats

This button displays the Edit Parameter Stats dialog, which allows selection of parameters statistics to be computed for each gate.

## Additional Options
### Equation

Displays the gate equation in the Gate Statistics window.

## Unclassified

Displays "Unclassified" statistics for each level of the gate hierarchy. Unclassified events are those that do not belong in any gate at that level.

## Overlapped

Displays "Overlapped" statistics for each level of the gate hierarchy. Overlapped events are those that belong to more than one gate at that level.

**Edit Parameter Stats**

This dialog is displayed from the Edit Properties for Gate Statistics dialog.
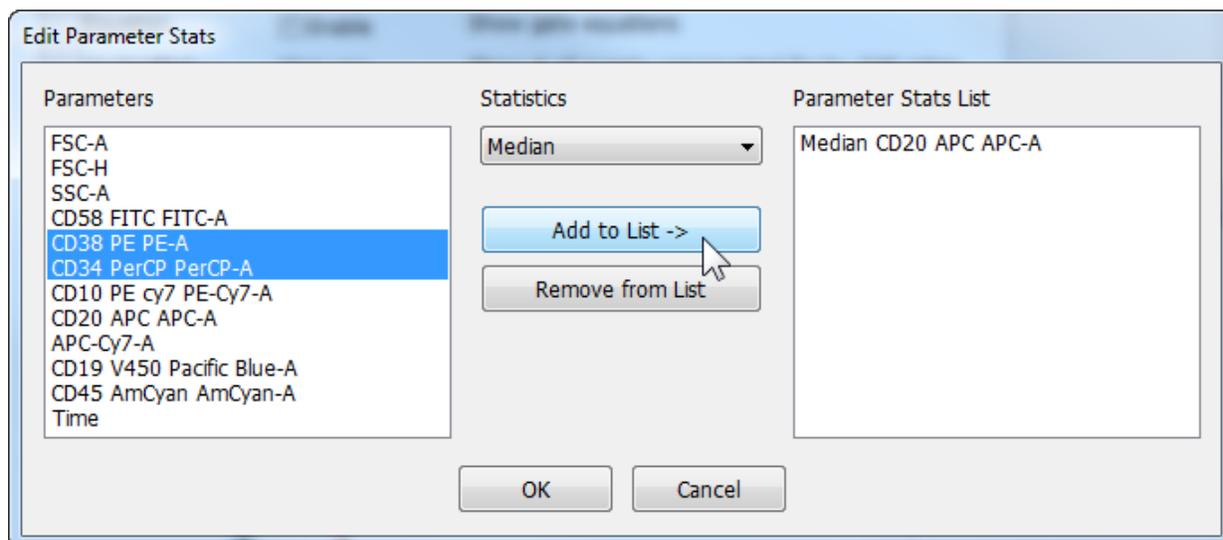


This dialog is used to select parameter statistics to be computed for each gate. It provides an easy method to collect parameter intensity statistics for gates without having to create histograms.

Any number of parameter statistics can be selected. The statistics are computed for every active gate and displayed in the Gate Statistics window. Stats are also available to be databased and added to reports.

**Parameters**
Lists the parameter for the current data file. Select one or more parameters for which you want to compute a gate statistic.

**Statistics**
Select the statistic that you want to compute for the selected items in the Parameters list. Available statistics are mean, median, SD, and CV%.

**Parameter Stats List**
This list shows the parameter stats that have been added.

**Add to List ->**

When this button is clicked, the current Statistics selection is added for each of the parameters selected in the Parameters list to the Parameter Stats List.

**Remove from List**

Use this button to remove the selected items in the Parameter Stats List.

**OK**

Closes the dialog and stores the changes.

**Cancel**

Closes the dialog and discards any changes.

**Coloring**

**Palette**



This command is found on the **Options** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display.

The **Edit Palette** dialog box is used to display and edit the colors used by gates, background, and axes.
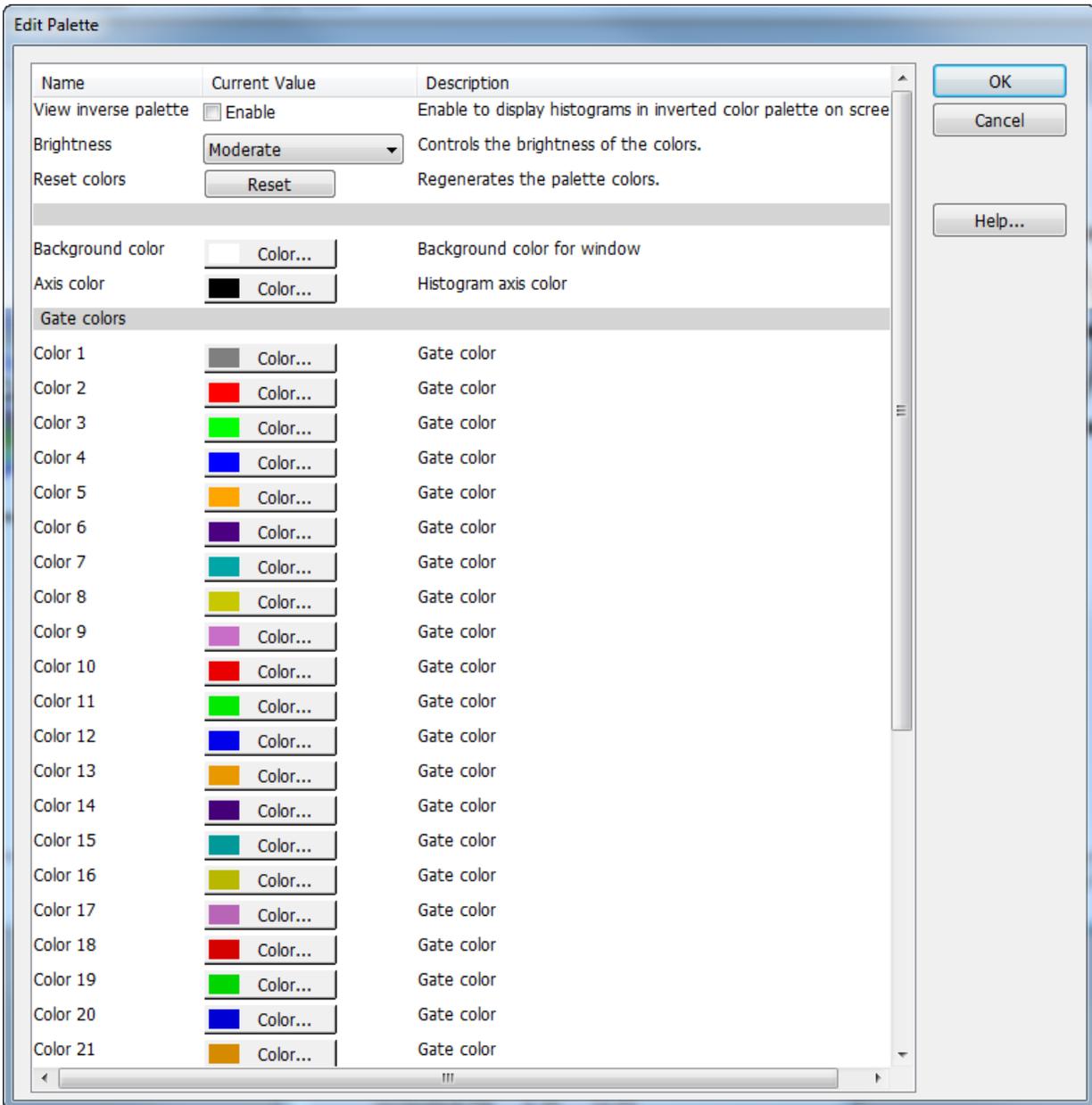
**View inverse palette**

Enable inverse palette mode.

**Brightness**

Select the brightness level to use for generating palette colors. After selecting a level, use the **Reset colors** button to regenerate the palette.

**Reset colors**

Regenerate the colors in the palette.

**Background color**

The color used for background of histograms, results, etc. Click the button to select a different color.

**Axis color**

The color used for drawing the axis and tics. Click the button to select a different color.

**Gate colors**

A set of colors used for gates. Click the button to select a different color.

**Open**

Open a palette file from disk.

**Save**

Save a palette file to disk.

## Inverse Palette

This option is found in the View tab of the ribbon bar, or the View menu when using toolbar and menu.

Inverse Palette is a great visualization aid. When you select this command, histograms and result windows switch the foreground and background colors - effectively inverting the color palette. You can quickly switch between white and black background, which is often used to enhance the small, outlier populations.
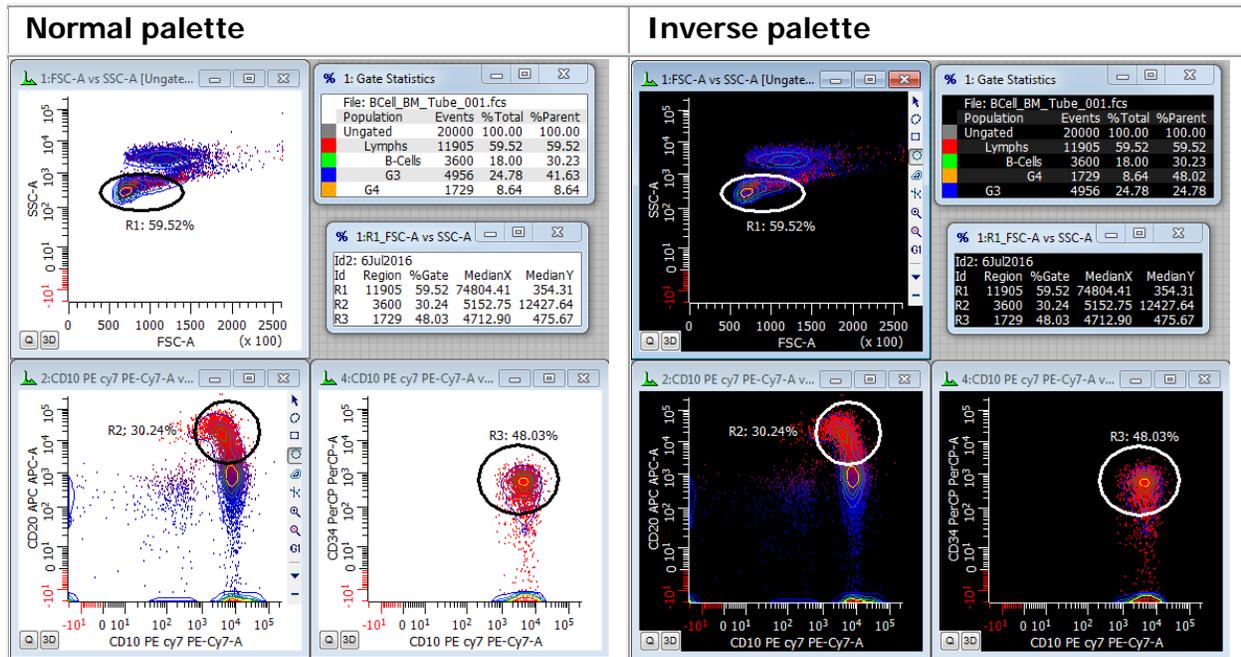


Preferences determine whether the inverse palette mode is used by reports, for printing, and when copying to the clipboard.

## Color Modes
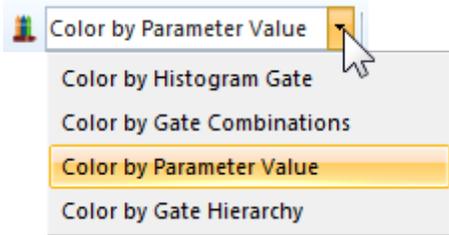


The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. Color modes mostly affect 2P histograms that are display Dots, but also have an impact on 1P and 3P histograms.

Four color modes are available:

### Color by Gate Hierarchy

This is the default coloring mode. In this mode, dots are assigned the color of the last gate in the gate hierarchy that they belong to. Typically, this means that the dot color is the color of the most refined subset. See Edit Color by Gate Hierarchy for additional details.

### Color by Histogram Gate

In this mode, dots are assigned the color of the gate that is applied to the histogram. This mode makes it easy to see how a histogram has been gated.

### Color by Gate Combinations

Similar to Color by Gate Hierarchy, this mode *adds* colors of the gates that classify the dots. It creates new colors for events that fall into more than one gate. This mode works well when only a few gates are used and there is not much overlap between them. See Edit Color by Gate Combinations for details.

### Color by Parameter Value

In this mode, one parameter is selected to be used to color dots. The intensity of that parameter for each event determines the color of dots representing that event. It effectively adds information from a third parameter to every 2P histogram. See Edit Color by Parameter Value for details.

**Edit Color by Gate Hierarchy**



The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. In this coloring mode, dots are assigned the color of the last gate in the gate hierarchy that they belong to. Typically, this means that the dot color is the color of the most refined subset.
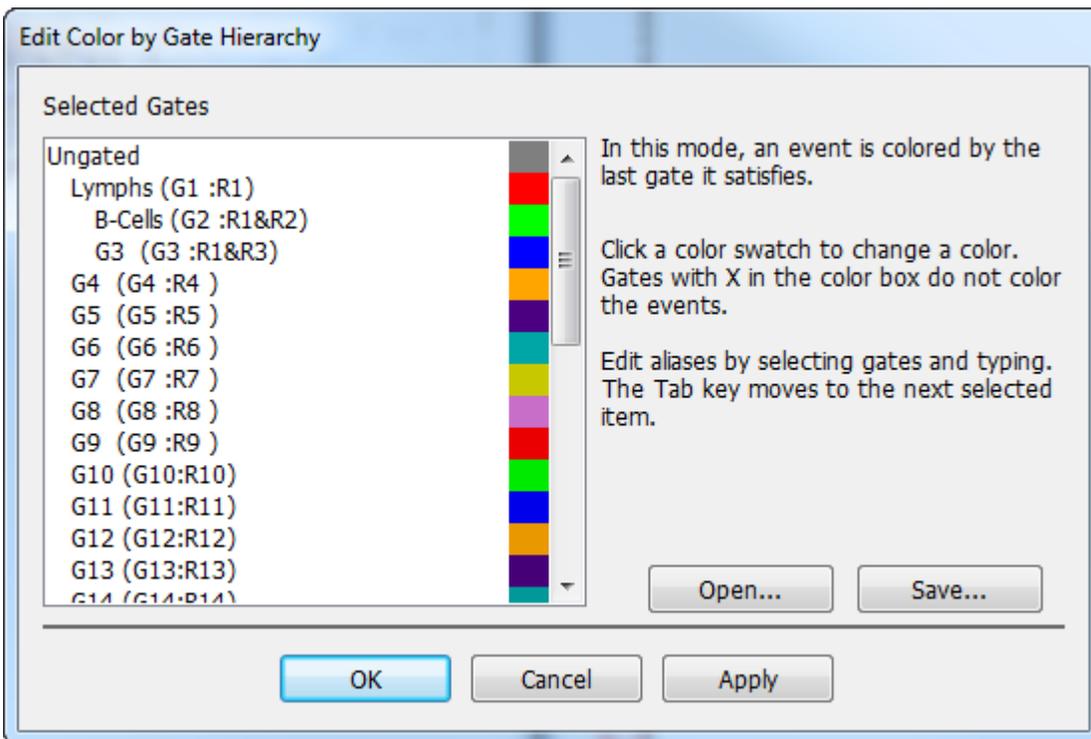


The gate hierarchy is displayed in a list, with a color for each gate shown.

To change the color of a gate, click the color swatch next to it. The palette of colors is displayed, including an "X". Selecting "X" will disable coloring for that gate.

Use the Save and Open buttons to store and recall customized color sets.
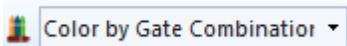
375

**Edit Color by Gate Combinations**



The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. In this mode, an event is colored based on *all* of the gates that it belongs to. So if an event belongs to more than one gate, a new color is created for the gate combination. This mode works well when only a few gates are used and there is not much overlap between them
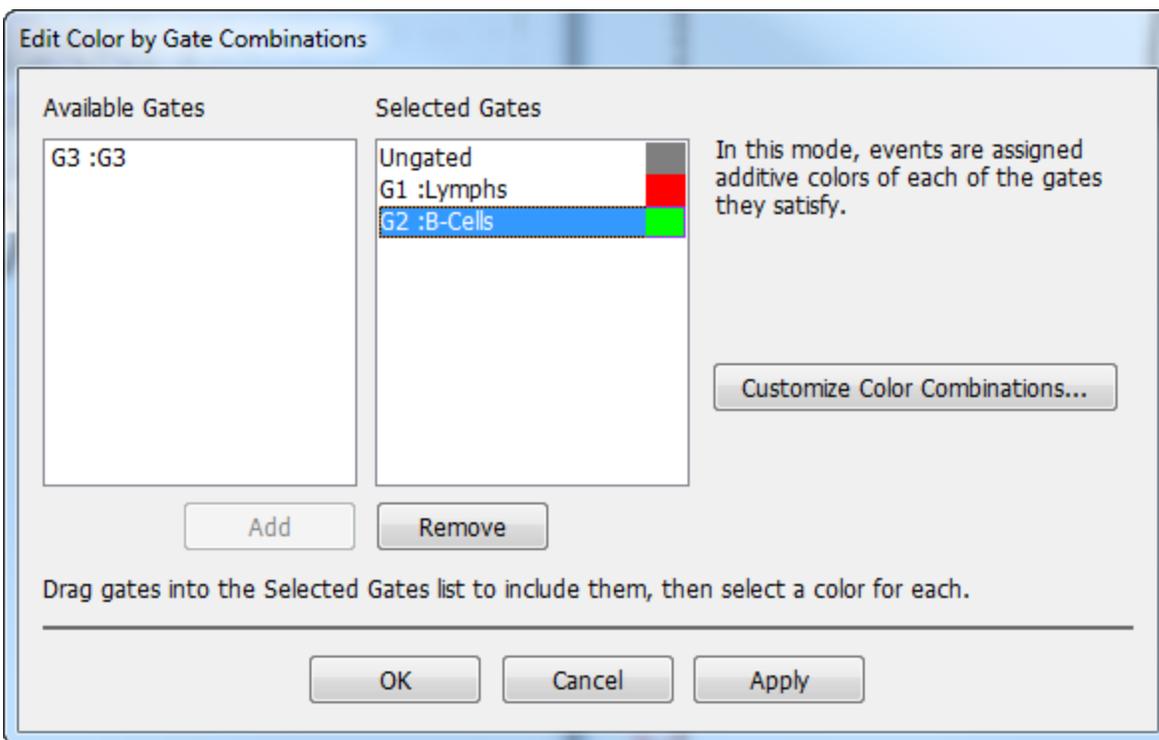


Active gates appear in the Available Gate list. They can be included for coloring by using the Add button. Once a gate is added, click the color swatch next to the gate to choose color for it.

The Customize Color Combinations button allows further control over the color used for overlapping gates.

**Customize Color Combinations**


This dialog box provides full control of the coloring of dots in 2P and 3P histograms when using Color by Gate Combinations. You can choose a color for each cluster created by the color gates.


For example, with gates G1 and G2 enabled for Gate Combinations mode, there are 4 clusters created: G1- G2-, G1- G2+, G1+ G2-, and G1+ G2+. Each cluster represents a unique set of events. This dialog provides a way of viewing and editing the color of each of these clusters.
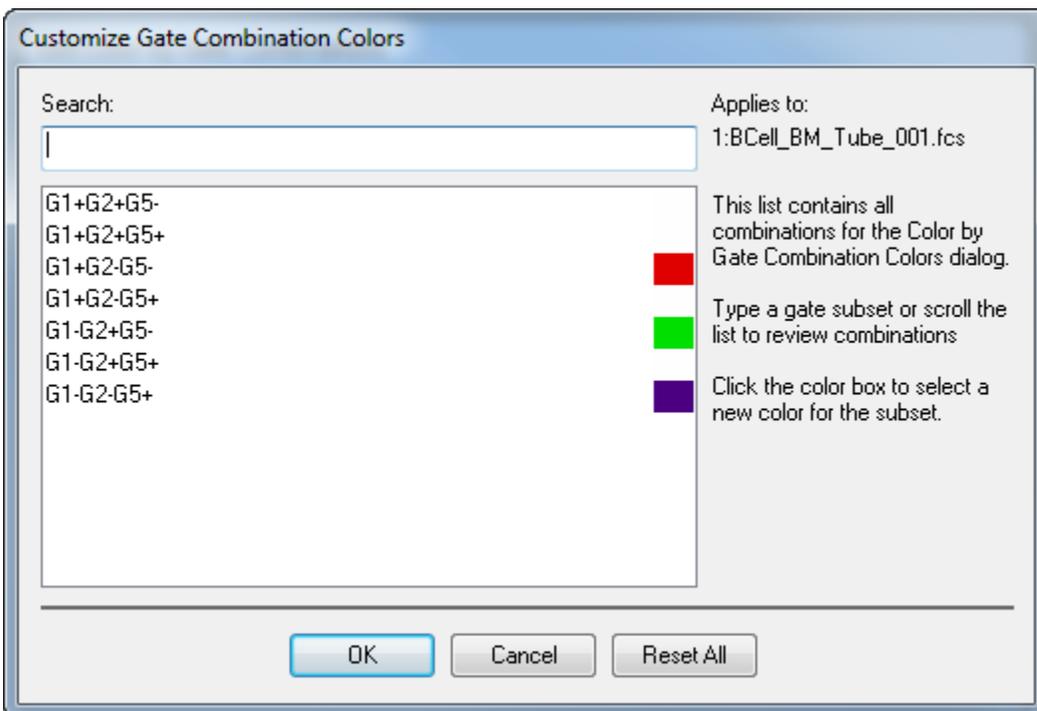


**Search**

This control allows you to search for the phenotype of the gate combination. As you type the combination (e.g. G1+G2-), the matching entry is selected in the list

**Combination List**

This list displays all gate combinations for the selected gates. Click the color swatch next to a list item to change its color.

**OK**

Click this button to accept changes and close the dialog box.

**Cancel**

Click this button to cancel the changes and close the dialog box.

**Reset All**

Click this button to restore cluster colors to the default settings and close the dialog.

**Edit Color by Parameter Value**



The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList.  In this mode, one parameter is selected to be used to color dots. The intensity of that parameter for each event determines the color of dots representing that event. It effectively adds information from a third parameter to every 2P histogram.
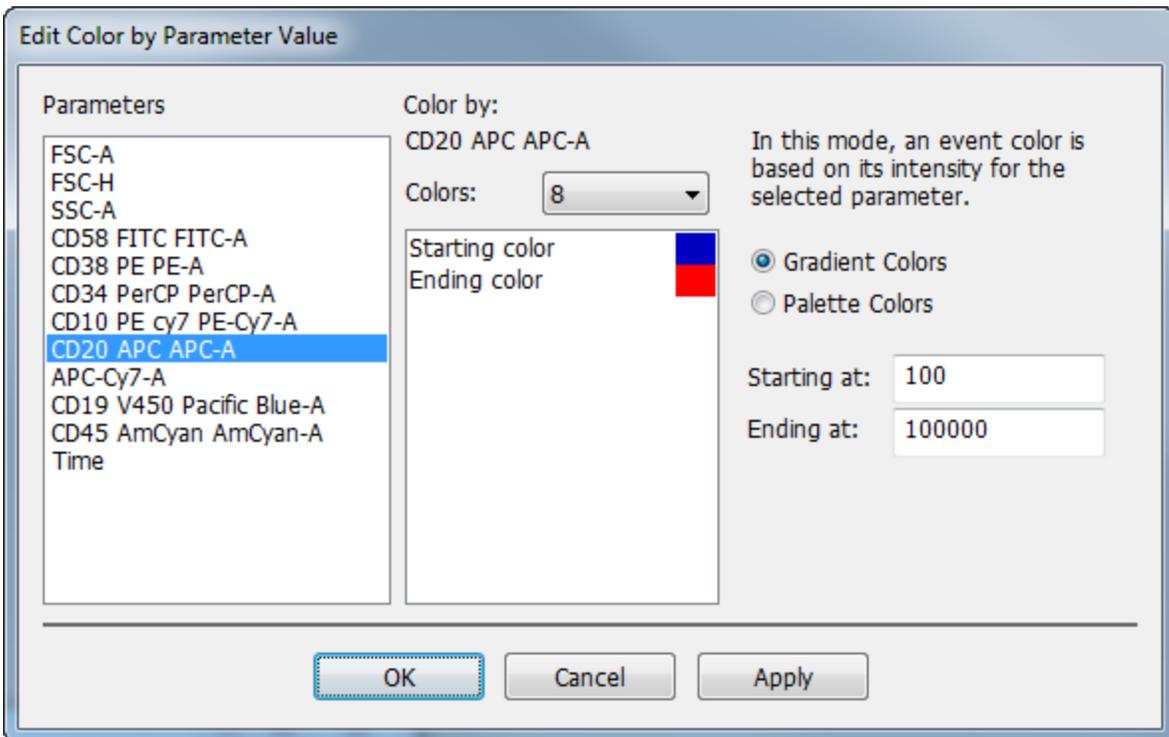


In the example below, CD20 was selected as the parameter, with dark blue representing low CD20 transitioning to red for high CD20.  We have set the starting point for the transition at channel 100, so anything below 100 will be dark blue. The ending point for the transition to red is at 100,000, so anything above that will be red. Note that the coloring appears in the CD10 vs CD34 plot to help identify the low and high intensity CD20 events.
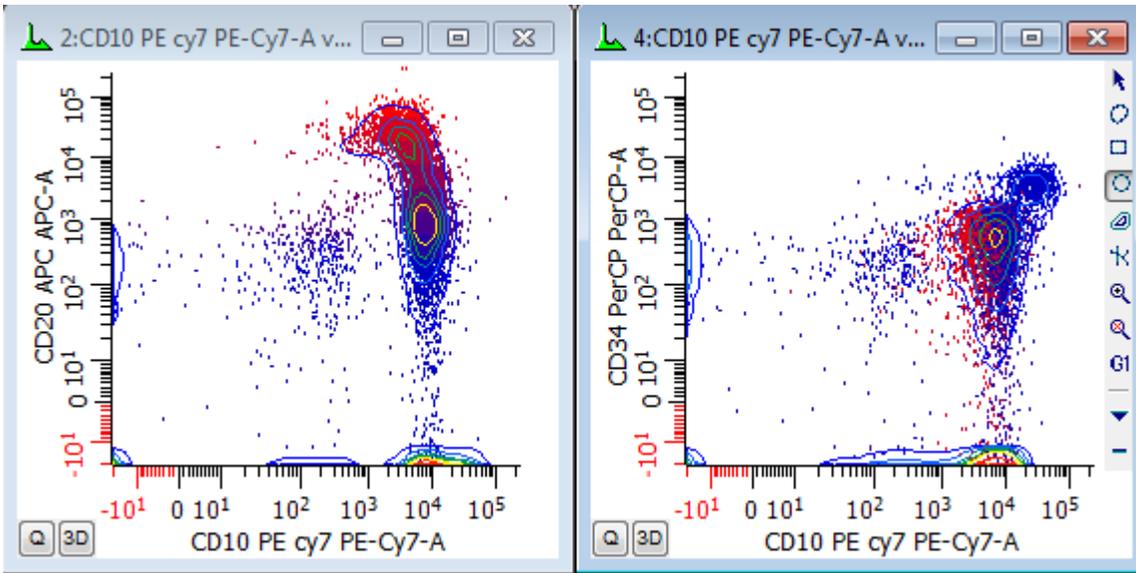
Select a parameter from the **Parameters** list. The intensity of the selected parameter will be used to color the dot for each event.

Choose the number of color levels with the **Colors** control.

Colors can be changed by clicking the color swatch for an entry in the Colors list.

Select **Gradient Colors** to use a 2-color gradient, or **Palette Colors** to select a specific set of colors to use.

Edit the **Starting at** value to set the channel where the transition begins. Any events below this value will be colored with the first color.

Edit the **Ending at** value to set the change where the transition ends.  Any events above this value will be colored with the last color.

**Results**

**Results Windows**



To create a results window, click the Results button on the Home tab of the ribbon bar.  If WinList is set up to use a menu and toolbar, choose Results from the Create menu.

Results windows display statistics for regions and are displayed on top of histogram and data source windows. They can be positioned in any convenient location, including outside of the confines of the main window.

 A new, results window will appear and the Edit Results dialog will be displayed to allow selection of regions and statistics.
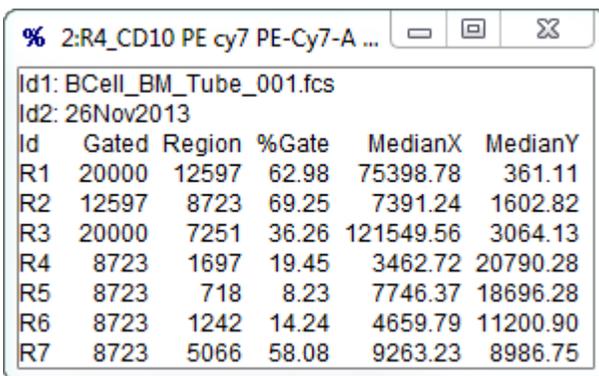


| Id | Gated | Region | %Gate | MedianX | MedianY |
|----|-------|--------|-------|---------|---------|
| R1 | 20000 | 12597 | 62.98 | 75398.78 | 361.11 |
| R2 | 12597 | 8723 | 69.25 | 7391.24 | 1602.82 |
| R3 | 20000 | 7251 | 36.26 | 121549.56 | 3064.13 |
| R4 | 8723 | 1697 | 19.45 | 3462.72 | 20790.28 |
| R5 | 8723 | 718 | 8.23 | 7746.37 | 18696.28 |
| R6 | 8723 | 1242 | 14.24 | 4659.79 | 11200.90 |
| R7 | 8723 | 5066 | 58.08 | 9263.23 | 8986.75 |

A single results window can display information for any number of regions simultaneously. Users can choose which statistics appear and whether they are column- or row-oriented using the Edit Results dialog box.

### Results Context Menu

This menu is displayed when the right-mouse button is clicked on a results window. It displays a list of common commands related to results windows.

```
Copy
Copy (no header)

Edit
Save
Print

Database wizard...
```
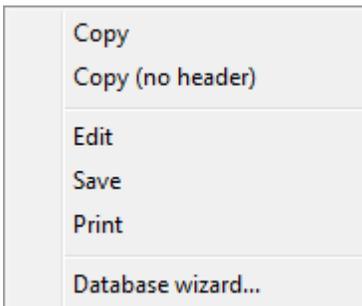
### Copy

Copy the results in tab-delimited text format to the clipboard. The text can then be pasted into a report or another application.

### Copy (no header)

This command is similar to Copy, except that the column names for the results are omitted.

### Edit

Display the Edit Results dialog.

### Save

Use this command to save the results as a text file on disk.  The standard Save dialog is displayed..

### Print

Use this command to print the results window to the current printer.

### Database Wizard

This command initiates a wizard that simplifies the task of adding statistics from a Results window or the Gate Statistics window to a database. See Database Wizard for details.

## Create Results

Results windows are used to display statistics for one or more regions. It most cases, the results relate to a single data source and file. However, you can add results from any data source to a Results window.

To create a results window, choose the **Create Results** menu option. The **Edit Results** dialog box appears, enabling you to calculate and display one or more region's results in a window. The function of each item in this dialog box is described in the Edit Results dialog box.

**Edit Results**

This dialog box appears when creating or editing a results window. It allows the user to choose what statistics will be displayed and for which regions. You can also establish remote links with other programs through this dialog box.

One of the important concepts relating to WinList's results has to do with the sampling of events involved with the calculations. Normally, WinList uses a sample of the data, called a *packet*, for performing calculations and displaying statistical and graphical information. The number of events in the sample packet is set in Edit Program Controls. As the packet size increases, so does the number of events evaluated by WinList.
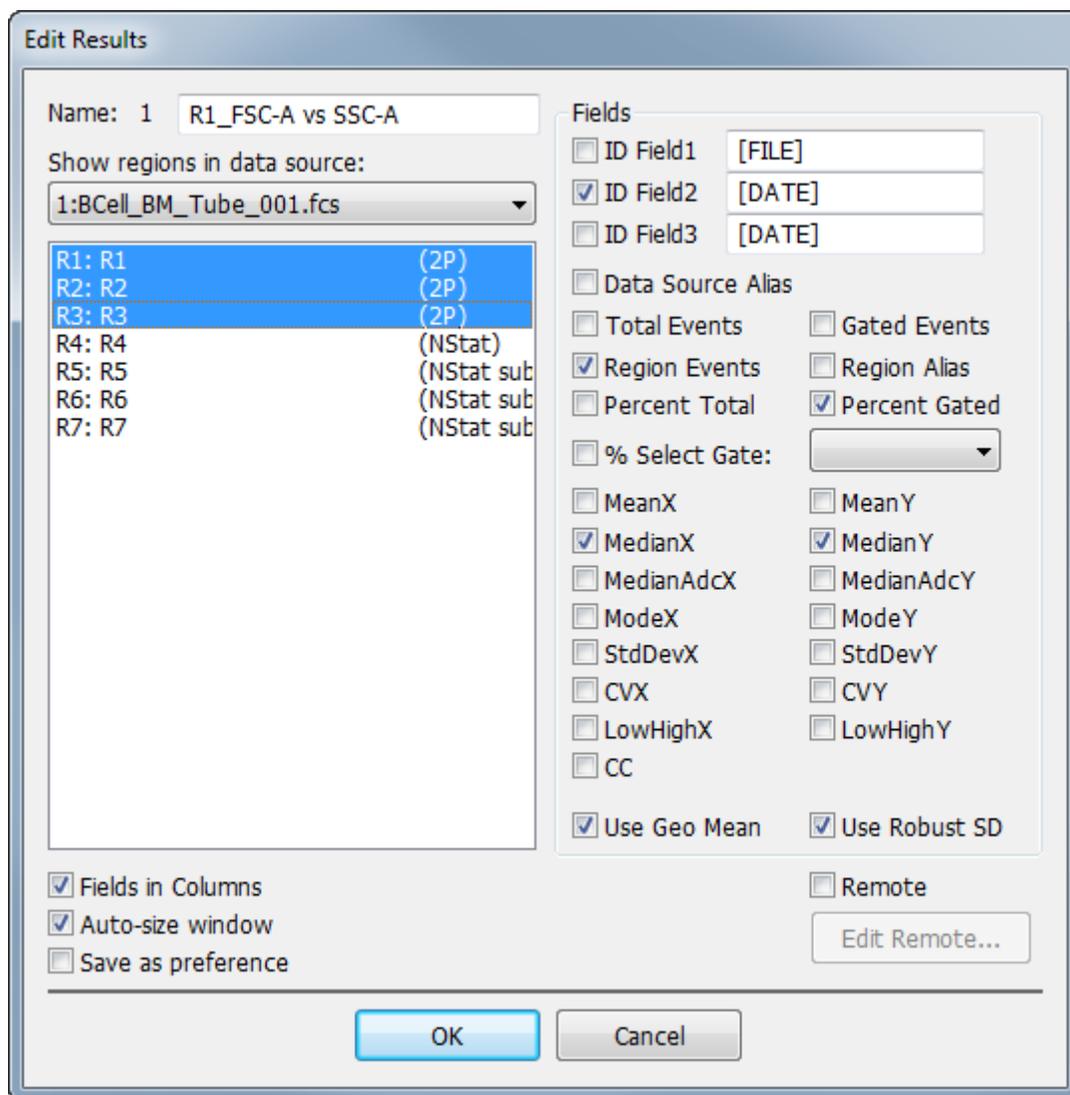
If you want to see statistics for the *entire* data file, you need to use one of the options in the **Replay** menu to re-read the data, or click the **Replay Data Source** button on the **Toolbar**.

### Name

The ID and name of the Results window are shown. An edit box displays an editable name up to 250 characters long. The name can include *FCS Keywords*, in which case WinList will extract the keyword values from the data file for display as the results window's name. See Appendix B: Internal Keywords for more information.

### Show regions in data source:

This dropdown contains a list of all data source windows. Select a data source from the list to see the regions that have been created in that data source. Then select the regions you want to appear in this Results window.

You can select regions from any number of data sources to be included in the results window.

### Region list

This list displays the regions that are created in the selected data source. Select the region you want to include in this results window.

### Fields

#### ID Field 1 and 2

If ID Field1 or ID Field2 check boxes are checked, WinList displays the contents of the edit boxes next to these check boxes at the top of the results window. If the content of the edit box is braced by square brackets (e.g. **[$SAMPLE]**), WinList will search the keywords of the current file for a match. If it finds a matching keyword, the value of the keyword will be displayed within the results window. This option is handy to identify file-specific information about the current data source.

#### Data Source Alias

When this field is checked, the name of the data source window will appear to the left of each region in the result window. This is useful when one results window is used to display results from several data sources.

#### Total Events

This option displays the total number of events currently used by WinList. This number is normally *Packet Size* number of events. In order for WinList to display and analyze the total number of events in the data file, replay the data using one of the options in the Replay menu whenever you want to update the statistics. For more information on *Packet Size*, see Edit Program Controls.

#### Gated Events

This option displays the number of events that passed through the gate assigned to the histogram containing the region. If there is no gate, this number will equal **Total Events**.

See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

### Region Events

This option displays the number of events that fall within the region. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

### Region Alias

This option displays the region's alias name for each region in the results window. Region alias names can be edited in the Region List dialog box.

### Percent Total

This option displays the number of events within the region as a percentage of **Total Events**. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

### Percent Gated

This option displays the number of events within the region as a percentage of **Gated Events**. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

### % Select Gate

This option has two elements: a checkbox to enable or disable it, and a drop-down list of all active gates. Similar in concept to Percent Gated, this option allows you to choose *any* gate on which to compute percentages for the regions in the results window. Since this statistic can be selected for regions that do not have an obvious relationship to the selected gate, it is important to understand how this computation is performed.

If the region represents a child gate of the selected Gate in the gating hierarchy, the statistic is very simple. It is the number of events in the region, multiplied by 100 and divided by the number of events in the selected Gate. This is the typical case.

| Id | Gated | Region | %Gate | %G8 |
|----|-------|--------|-------|-----|
| R1 | 20000 | 12595 | 62.98 | 14151.69 |
| R7 | 12595 | 904 | 7.18 | 1015.73 |
| R8 | 904 | 89 | 9.85 | 100 |

R8 is a child gate of R1 and R7

However, if the region is *not* a child gate of the selected gate, the statistic is a little more complicated. The numerator of this statistic is the number of events in the region. The denominator is the number of events in the selected Gate, which may not be the same as the number of events in the region associated with the selected Gate.

| Id | Gated | Region | %Gate | %G8 |
|----|-------|--------|-------|-----|
| R1 | 20000 | 12595 | 62.98 | 3279.95 |
| R7 | 12595 | 904 | 7.18 | 235.42 |
| R8 | 904 | 89 | 9.85 | 23.18 |
| R8 is NOT a child of R1 and R7 | | | | |
| 384 events in G8 (ungated R8) | | | | |

If the region whose results are being computed is contained in a data source that does *not* own the gate in **% Select Gate**, the statistic will show 0.00 regardless of the number of events in the region or in the gate.

### Mean X and Mean Y

This option displays the X and Y arithmetic mean for the selected regions. The units reported are linearized units. The computation will be slightly different for log parameters when the **Use Geometric Mean** option is toggled on or off. See also Understanding Mean values in WinList for more details.

### Median X and Median Y

This option displays the X and Y median for the selected regions. The units reported are linearized units.

### Median ADC X and Median ADC Y

This option displays the X and Y median in ADC units.

### Mode X and Mode Y

This option displays the X and Y mode for the selected regions. The units reported are linearized units.

### StdDevX and StdDevY

This option displays the X and Y standard deviation for the selected regions. The units reported are linearized units.

### CVX and CVY

This option displays the X and Y percent coefficient of variation (CV) for the selected regions.

### LowHighX and LowHighY

This option displays the low and high X and Y values for the selected regions. The units reported are linearized units.

### CC

This option displays the correlation coefficient for the selected regions.

## Use Geometric Mean

If a parameter is displayed on a log scale, this option controls whether the mean value is an arithmetic or geometric mean. A geometric mean calculates the arithmetic mean of the log data, and then linearizes the result. With the option unchecked, the mean is computed on linearized data.

## Use Robust SD

The Robust SD is a variation on the SD calculation that is less influenced by outlier events. When enabled, the SD and CV computations use the [Interquartile Range method](#) for the computation. This is equivalent to BD Biosciences' rSD and %rCV computation.

## Other options

### Fields in Columns

When checked, the results window will display statistics, or fields, in columns. Each row will represent a different region. If unchecked, regions are in columns, and a different statistic appears in each row.

### Auto Sizing

If this option is checked, WinList will automatically calculate the size that the results window needs to be in order to display the selected statistics and re-size the window as necessary. If the option is not checked, WinList will not adjust the results window's size.

### Save Preference

Click this button to keep the current settings as default settings for all results windows.

### Remote checkbox

This check box, in conjunction with the **Edit Remote** button, allows WinList to establish a Dynamic Data Exchange (DDE) link with another Windows application. If checked, the Edit Remote button is enabled.

### Edit Remote

Click this button to establish a remote link with another application, or to edit the script used for that connection. See [Edit Remote](#) for more details on Remote links and DDE.

### OK

Click this button to accept all changes and close the dialog box.

### Cancel

Click this button to exit the dialog box with no changes.

**Database Wizard**

This command simplifies the task of adding the statistics from a Results window or the Gate Statistics window to a database.

To use the command, first activate a Results window or Gate Statistics window by clicking its title bar. Then, right-click the window to display its context menu and choose Database Wizard. A wizard dialog appears.

Database Wizard

Welcome to the database wizard.

This tool makes it easy to add fields to a database from a Results window or the Gate Statistics window.

All of the fields from the window will be added when you click Finish to complete the wizard.

Source window: Gate Statistics

< Back    Next >    Cancel    Help

Click Next to display the next pane in the wizard.

Database

Available Databases

Database1
03-FCSReadTestDB

☑ Show database viewer
☑ Activate the database

Choose the database you want to use, the click Next to continue.

< Back    Next >    Cancel    Help

In this pane, select a database to which you want to add statistics. Additional options allow you to display and activate the database when the wizard finishes.

Click Next to display the next pane in the wizard.
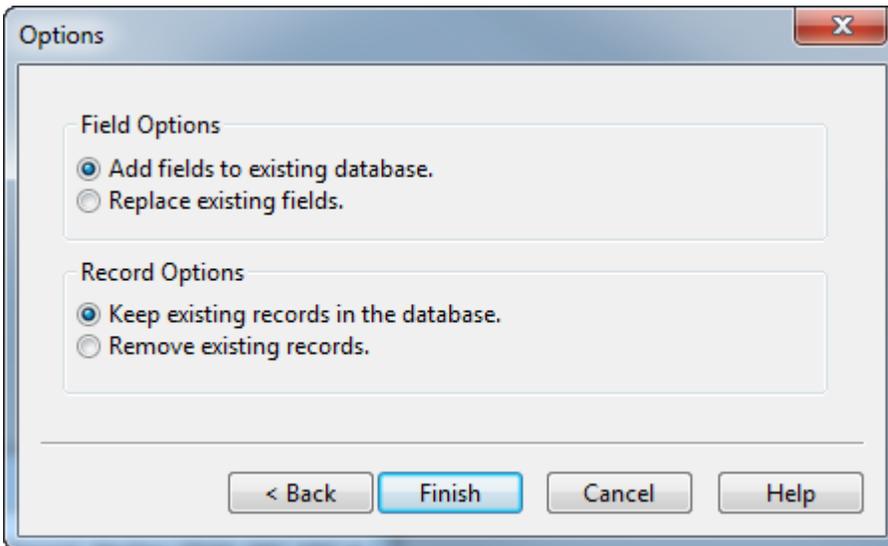


This pane provides options related to how the new fields are added to the database. Options allow you to replace any fields that are already there, or to append new fields. In addition, you can choose whether to remove any records that are already in the database or keep them.

Click Finish to complete the wizard.

See also:

Databasing with WinList
Edit Properties for Database

**Overlays**

**Overlay Windows**



To create a new overlay window, click the Overlay button on the Home tab of the ribbon bar.  If you are set up to use a menu and toolbar instead, choose Overlay from the Create menu.

Overlay windows are a great tool for graphically comparing a set of 1P or 2P plots.  You can set up different views and select a wide variety of graphical options.  Overlays organize plots - typically from different files - so that you can easily see the similarities and differences among them.

A new, empty overlay window will appear.
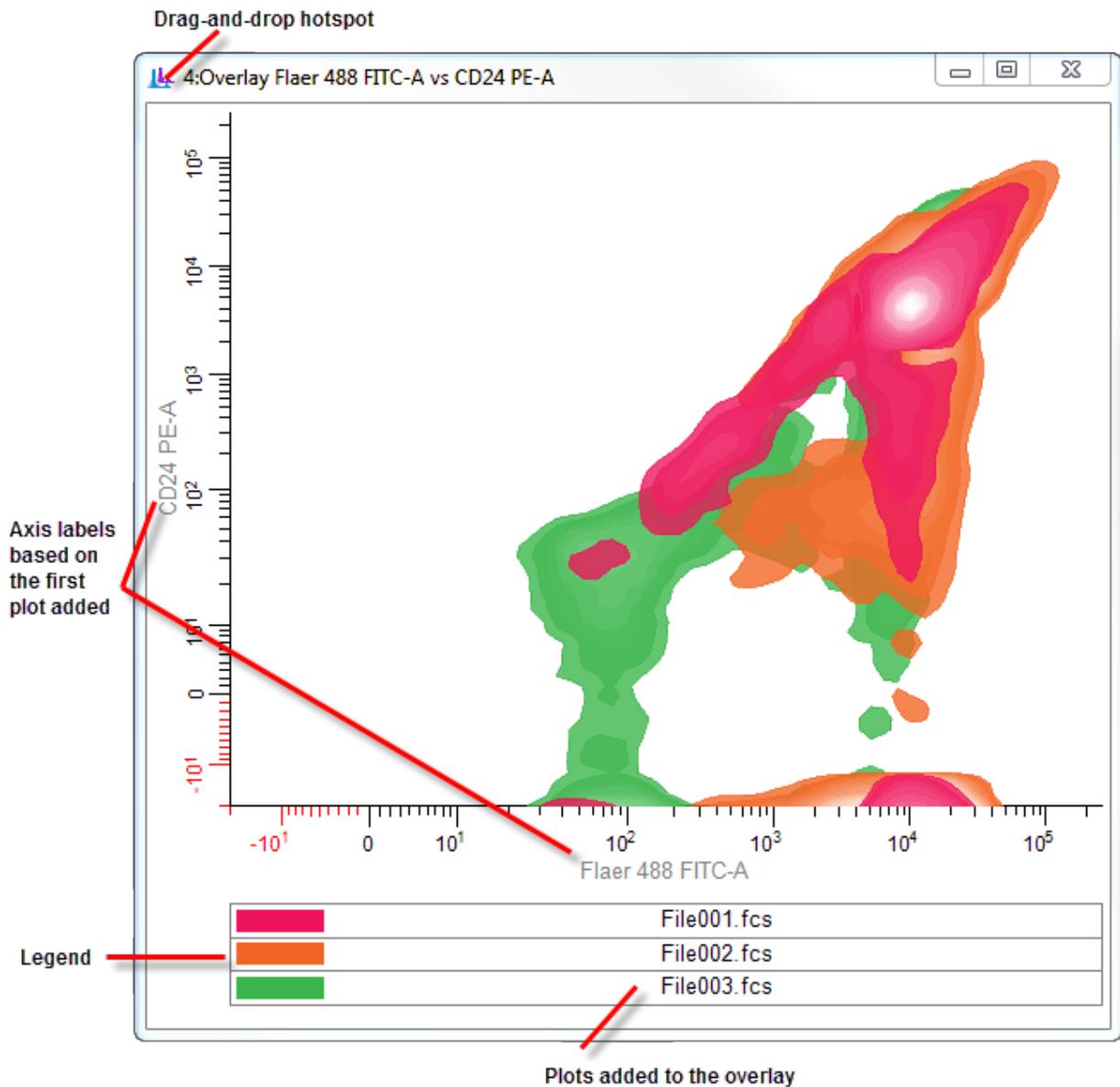
To add a plot to an overlay, drag a histogram by the icon on the left side of its toolbar and drop it onto the overlay. The first plot that is added to an overlay determines whether it is a 1P or 2P overlay window. It also determines the axes that will be displayed. If you subsequently add a plot with different parameters than the first plot, the overlay will ask if you really intend to do so.

To remove a plot from an overlay, click the item *in the legend* to select it. Then, right-click and choose **Delete Item** from the context menu.

To change the order of items in the overlay, click the item that you want to move in the legend, and then drag the item to the desired order in the legend.  When the mouse is released, the overlay will redraw with items in the new order.


To edit the properties of the overlay or overlay items, right-click the overlay and choose **Edit** from the context menu.  The Edit Overlay dialog will be displayed.  This is where you can choose the kind of overlay you want and set the graphical properties.

### Overlay Context Menu

The overlay context menu is displayed when the right mouse button is clicked on an overlay window.
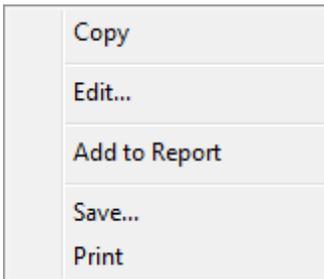


If an item is selected in the legend and that item is right-clicked, an additional entry appears in the menu allowing the selected item to be deleted.
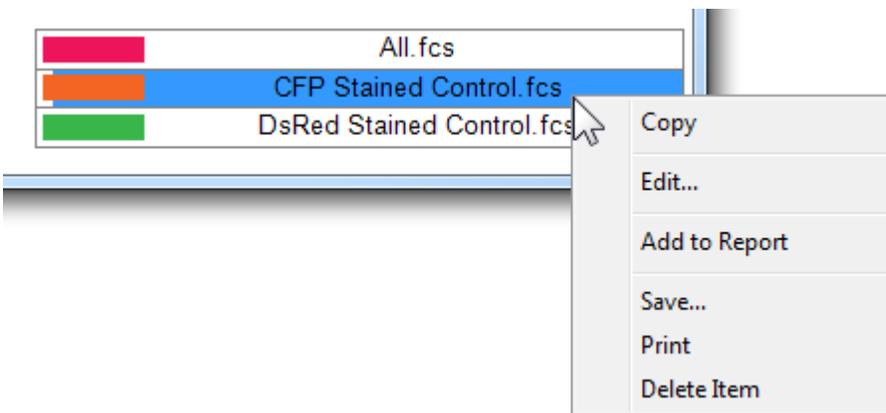


### Copy

This command copies an image of the overlay to the clipboard in bitmap and picture (metafile) formats.  The image can be pasted into other applications.

### Edit

This command displays the <u>Edit Overlay</u> dialog to set the display properties for the overlay.

### Add to report

This command adds the overlay to a report.  The report item is actively linked to the overlay so that as changes are made to the overlay, they automatically appear in the report.

---

**Save**

This command allows the overlay to be saved to disk in a number of popular graphics file formats.

**Print**

This command prints the overlay to the active printer.

**Delete Item**

This command deletes a selected item from the overlay.

**Edit Overlay**

This dialog determines the display properties for an overlay window.  It is accessed by double-clicking an overlay title bar, or by right-clicking the overlay and choosing **Edit** from the context menu.

The options shown in the dialog differ, depending on whether the overlay is for 1P or 2P plots.  The image below shows the 2P overlay dialog.
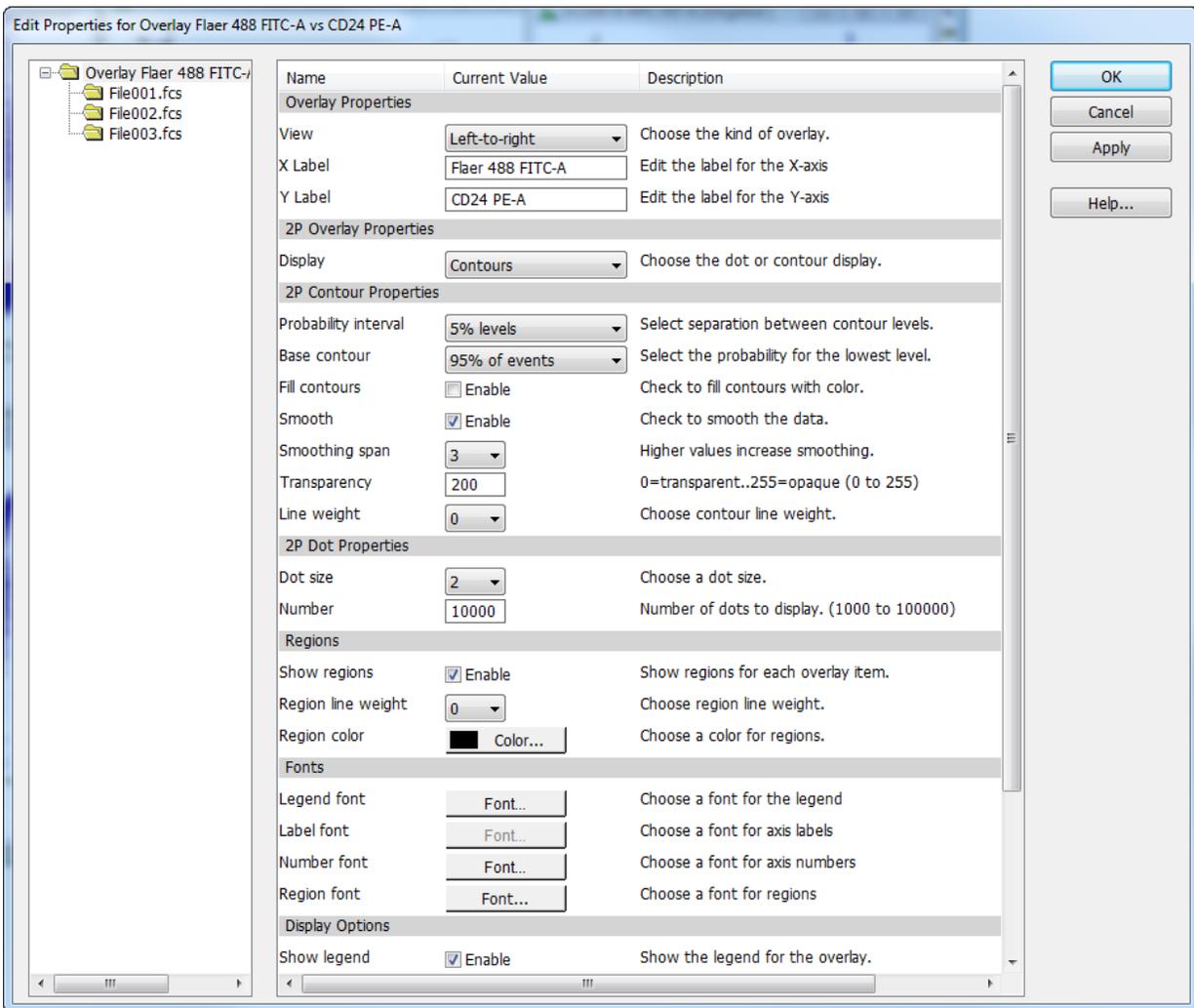


## Overlay Properties
**View**

There are 4 viewing modes for overlay windows.
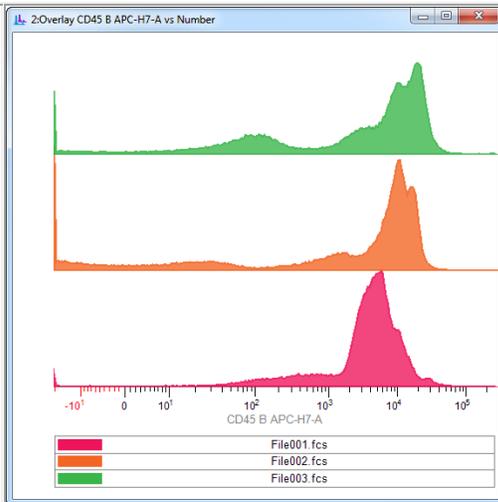
| | |
|---|---|
| **Offset** |  |
| **Offset overlapped** |  |
| **Left-to-right** |  |
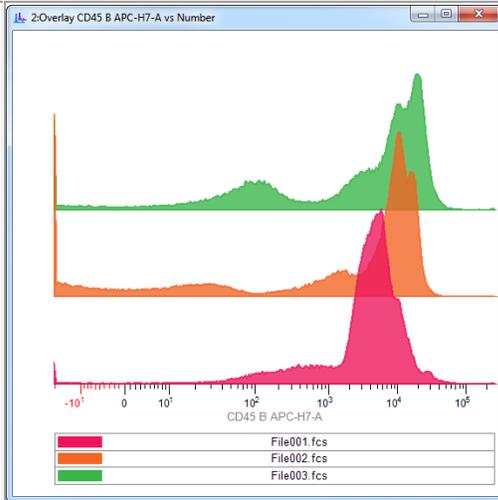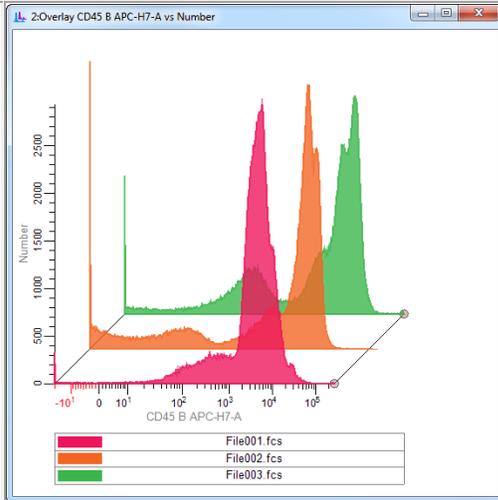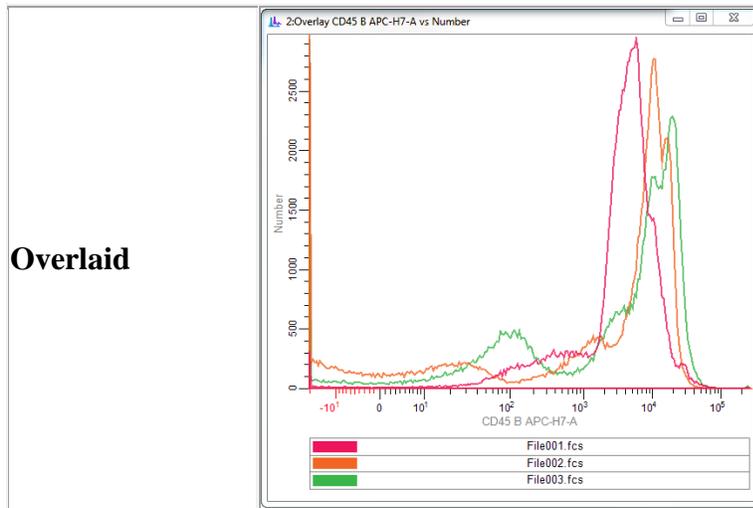
**Overlaid**



## X Label

This is the label that appears on the X axis of the overlay.


## Y Label

This is the label that appears on the Y axis of the overlay.


## 1P Overlay Properties

### Filled Histograms

Enable this option to draw 1P histograms filled with the selected color.  Disable the option to draw 1P histograms with no fill.


### Y Scale

Choose **Absolute** or **Normalized**.  In Absolute scaling mode, each 1P histogram in the overlay is drawn to its actual height, and the Y axis scale of the overlay is based on the tallest histogram in the overlay.  In Normalized scaling, each 1P histogram is drawn to 100% of its own scale - they will all appear to have the same height - and the Y axis switches to show a 0 to 100 scale.  Normalized scaling is useful when you are more interested in the intensity shifts of the overlay items rather than the actual areas.

| Absolute | Normalized |
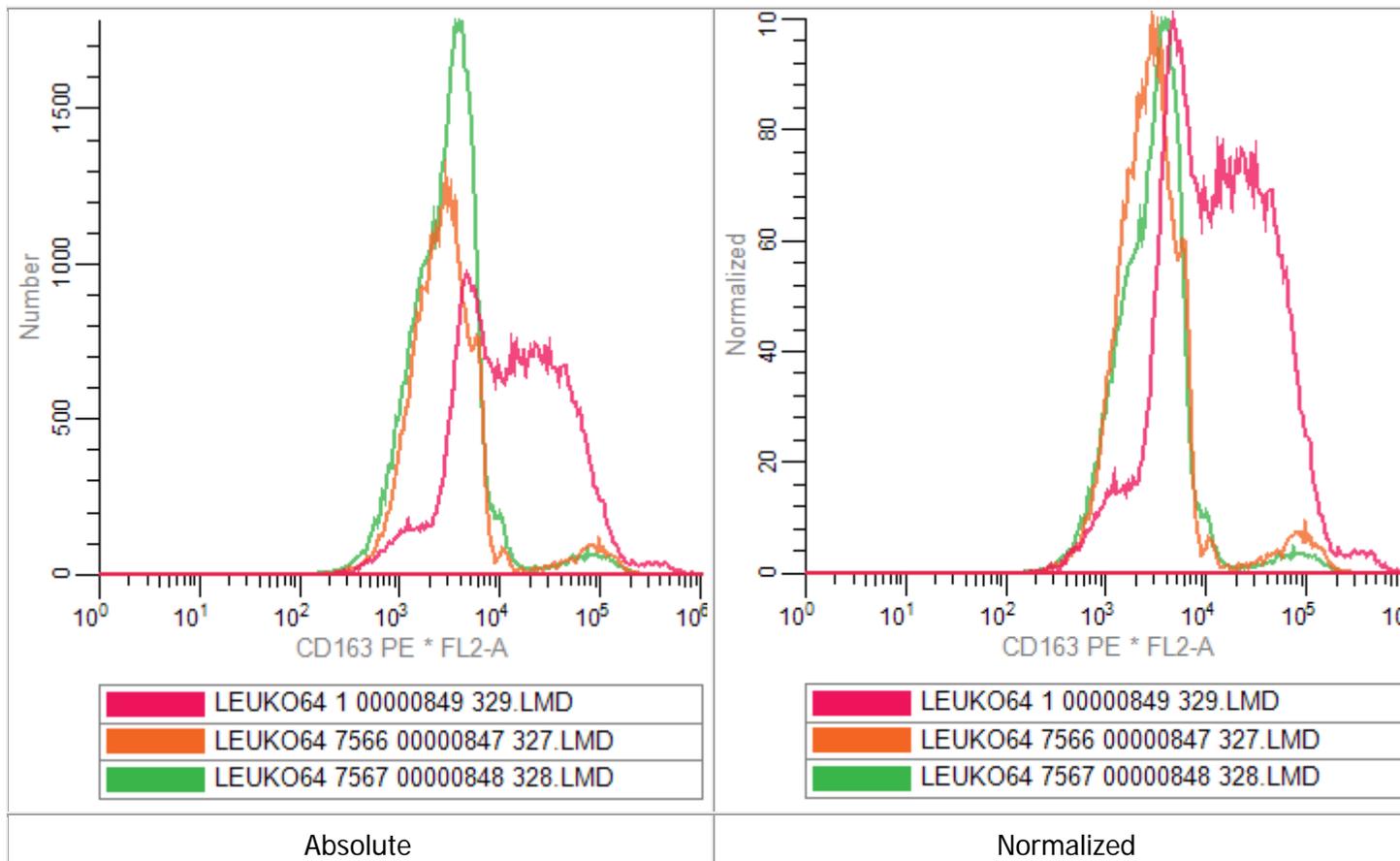
**Transparency**

This value determines the transparency of the plots.  A value of 0 makes the plots completely transparent; a value of 255 makes the plots opaque.

**Line Weight**

Select a line weight for the outline of the 1P plot.

**2P Overlay Properties**

**Display**

The 2P overlay can display **Dots** or **Contours** for the plots.  Contours will draw faster and are somewhat easier to interpret.

**2P Contour Properties**

These properties are used when **Contours** is the selected Display option.  The color assigned to each item in the overlay is used as the base color for the contours.  Colors transition to white for the highest contour.

**Probability interval**

This option determines the number of contours that will be drawn, based on the separation between them.

**Base contour**

This option determines the probability for the lowest contour.

**Fill contours**

Enable this option to fill the contours with color.  Disable the option to draw unfilled contours.

**Smooth**

Enable this option to smooth the contours.

**Smoothing span**

With Smooth enabled, this setting controls how much smoothing is applied.  Higher values create smoother contours, but show less detail.

**Transparency**

This value determines the transparency of the contours.  A value of 0 makes the plots completely transparent; a value of 255 makes the plots opaque.

**Line Weight**

Select a line weight for the outline of the contours.

## 2P Dot Properties

These properties are used when **Dots** is the selected Display option.  The color assigned to each item in the overlay is used for the dots.

**Dot size**

Select a size for the dots.

**Number**

Select the number of dots to display for each overlay item.

## Fonts
**Legend font**

Select a font to use for the legend.

**Label font**

Select a font to use for the axis labels.

**Number font**

Select a font to use for the numbers on the axes.

**Region font**

Select a font to use for the region labels in the plots.

## Display Options

**Show legend**

Enable this option to display the legend with colors and item names on the overlay.  The legend appears at the bottom of the overlay window.  It is also used to re-order items in the overlay.

**Show X axis**

Enable this option to display the X axis.

**Show Y axis**

Enable this option to display the Y axis.

**Show grid**

Enable this option to show a grid based on the major tics of the axes.

**X-axis size, Y-axis size**

These options are only used for **Left-to-right** viewing mode.  They control how much of the width or height of the plot area is used by the axis.  By default, these are 80% (0.80).  You can manually edit these values to change the perspective in Left-to-right viewing mode.  You can also edit these directly on the Left-to-right overlay window by moving the small handles at the right side of the plot.
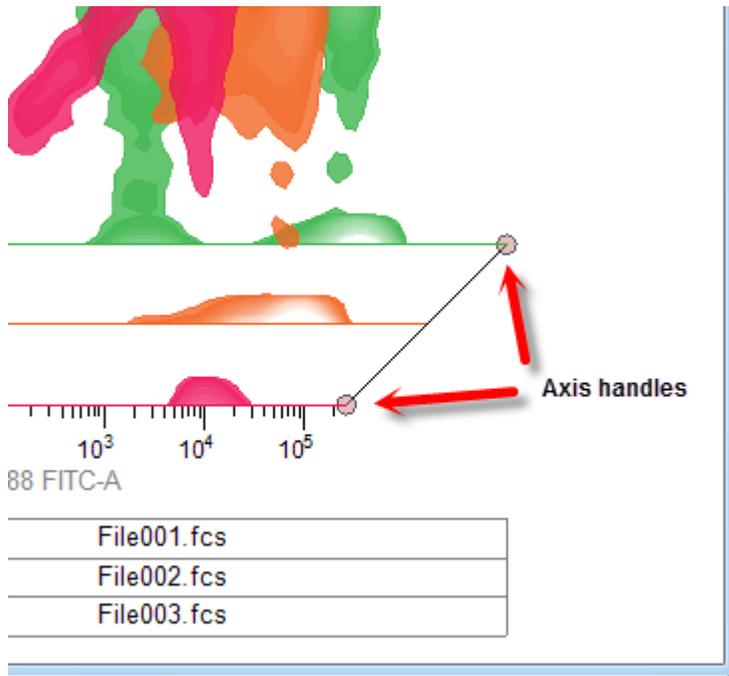
**Edit Properties for Overlay Item**

This dialog determines the display properties for an item in an overlay window.  It is accessed by double-clicking an overlay title bar, or by right-clicking the overlay and choosing **Edit** from the context menu.  Select the item you want to edit from the tree on the left of the dialog.
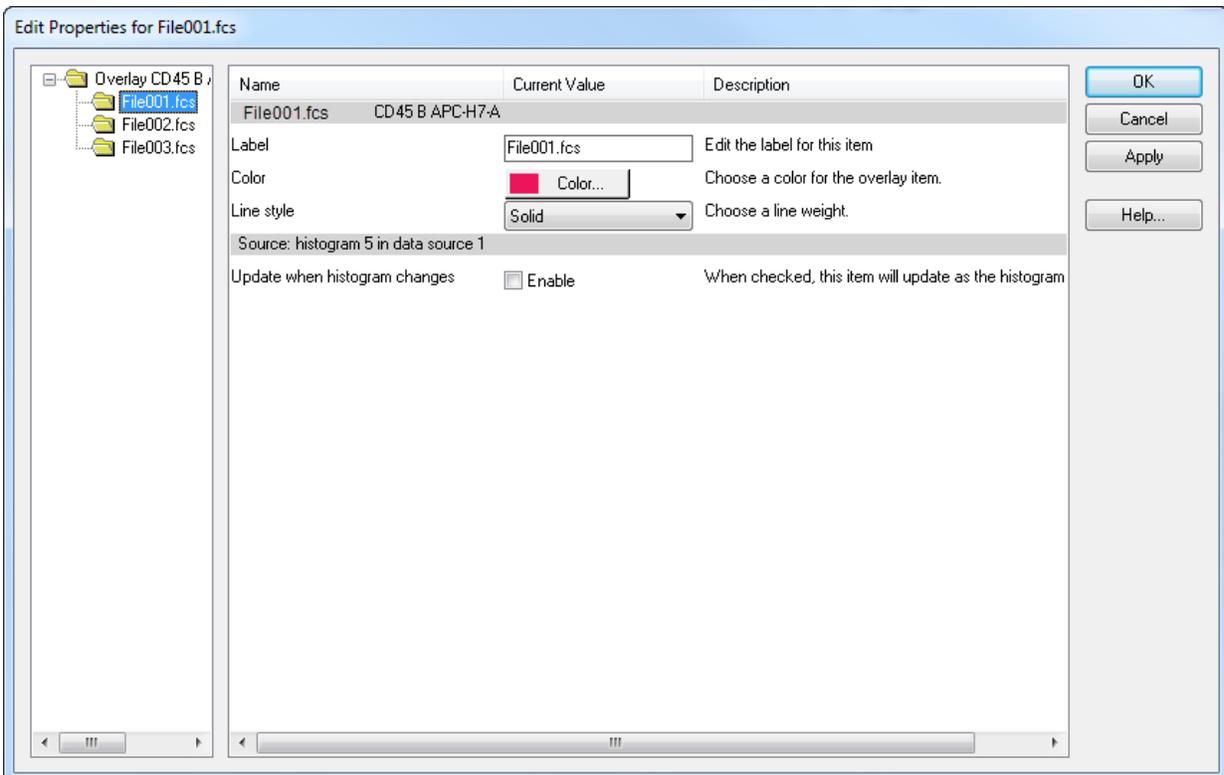


The first entry in the property list shows the source FCS file and the parameter(s) that the plot is displaying.  These are not editable; they are determined at the time the item is added to the overlay.

**Label**

This field shows the label that appears on the legend for the item.  It can be edited.

**Color**

Select the color to associate with the item.  This color appears on the legend, and is used to draw the item.  If the item is a 1P plot, the selected color determines the line and fill color.  For 2P plots showing contours, the color is used as the base contour color, transitioning to white at the highest contour.  The color is used to draw dots in dot plots.

**Line style**

1P overlays only: this determines the style of the line used to outline 1P plots.

**Source**

This entry is not editable.  It displays the histogram and data source IDs that this item is associated with.

**Update when histogram changes**

Enable this option if you want the overlay item to update in the overlay when the source histogram changes.

**MultiGraphs**

**MultiGraph Windows**
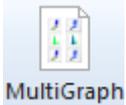


To create a MultiGraph window, click the MultiGraph button on the Home tab of the ribbon bar.  If you are set up to use a menu and toolbar instead, choose MultiGraph from the Create menu.  The Edit MultiGraph dialog will be displayed.

These windows allow you to display multiple histograms and results.  The items in a MultiGraph are static - they do not change when a new file is opened or a gate is changed. MultiGraphs can display a mix of single, dual, and three parameter histograms and results in several different views. Additionally, MultiGraphs can be saved to disk, opened from disk, and added to at any time. They can also be exported in PDF format.
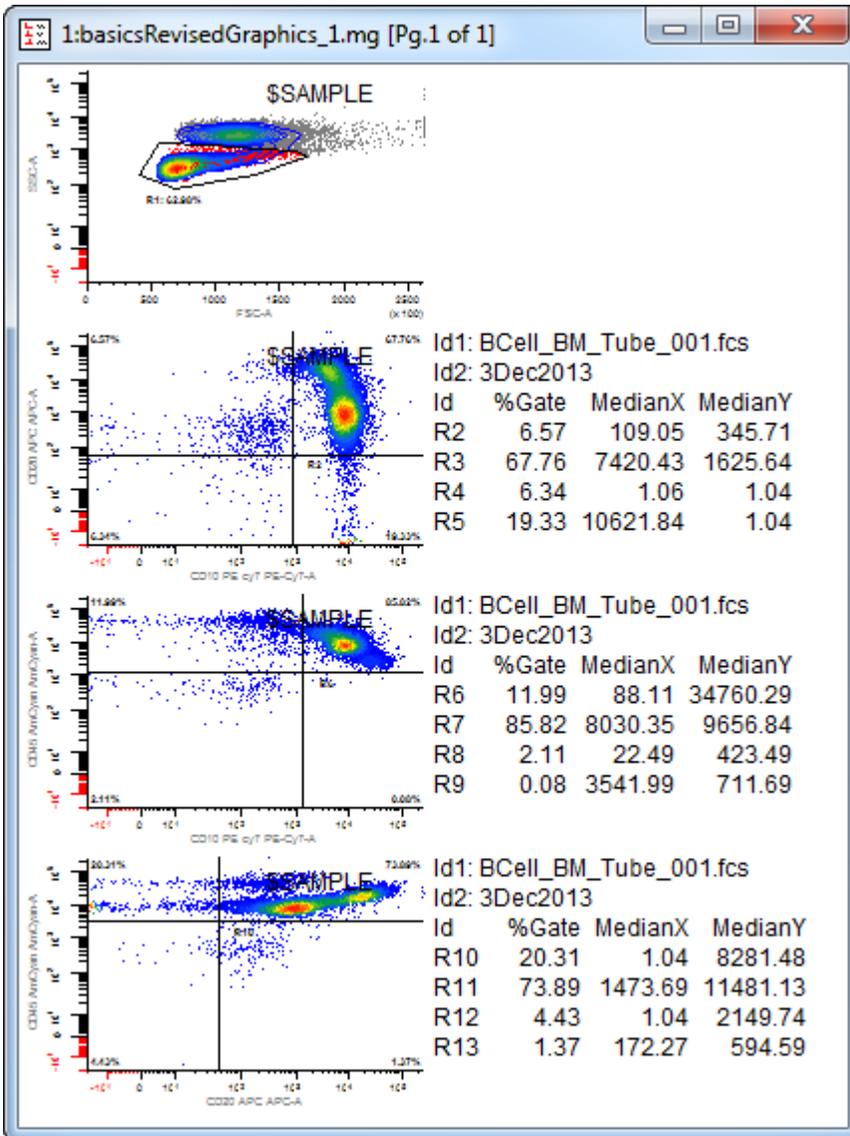
MultiGraph graphic elements are stored in Windows "metafile" format, also called "picture" format. Results window information is stored as text. Each element is like a picture snap-shot: once added, an element in a MultiGraph does not change even if the histogram it represents changes.

If a MultiGraph is in Tiled mode, you can move its elements by clicking the left mouse button on the item to move, holding the button down, and dragging the element to a new location in the MultiGraph. When you release the button, the MultiGraph will be re-drawn with the elements in their new locations.

The MultiGraph context menu is displayed when you click the right mouse button on a MultiGraph. Options appearing in the menu allow you to copy or delete a single element or the entire MultiGraph, edit the MultiGraph, print the MultiGraph, and more.

To edit a MultiGraph, double-click the title bar.

To configure the default characteristics of MultiGraphs, use the Preferences command in the Options menu.

**MultiGraph Context Menu**

This context menu is displayed when the right mouse button is clicked on a MultiGraph window.

```
Copy Element
Delete Element
Copy

Edit
Save Graphic...
Export to PDF...
Print

Clear
```
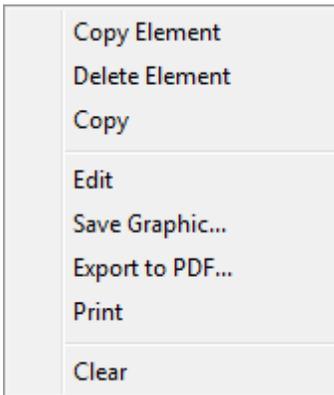
**Copy Element**

This command appears in Tiled view.  It copies the selected MultiGraph item to the clipboard as a graphic or text object.

**Delete Element**

This command appears in Tiled view.  It removes the selected item from the MultiGraph.

**Copy**

This command copies the MultiGraph to the clipboard in graphic format.

**Edit**

This command displays the Edit MultiGraph dialog to edit the elements and the style of the MultiGraph.

**Save Graphic**

This command displays the Save As dialog to save the MultiGraph to disk in one of several popular graphic formats.

**Export to PDF**

This command displays the Save As dialog to export the MultiGraph to disk in PDF format.

**Print**

This command prints the MultiGraph to the current printer.

**Clear**

This command clears all elements from the MultiGraph, leaving an empty MultiGraph window.

**Edit MultiGraph**

A **MultiGraph** is a window that can display multiple histograms and results windows in various arrangements. The histograms can be from different data sources, and they can be a mix of 1, 2, and 3 parameter. MultiGraphs can be saved to disk, reopened and new histograms and results windows can be added to them, making them useful for archival storage. Each histogram in the MultiGraph may have a label assigned to it which can be positioned anywhere in the MultiGraph window. You can choose from several different views of the histograms, and change the views easily. See **Create MultiGraph** for more information.

To display this dialog, choose **Edit** from the MultiGraph context menu or double-click the MultiGraph's title bar.
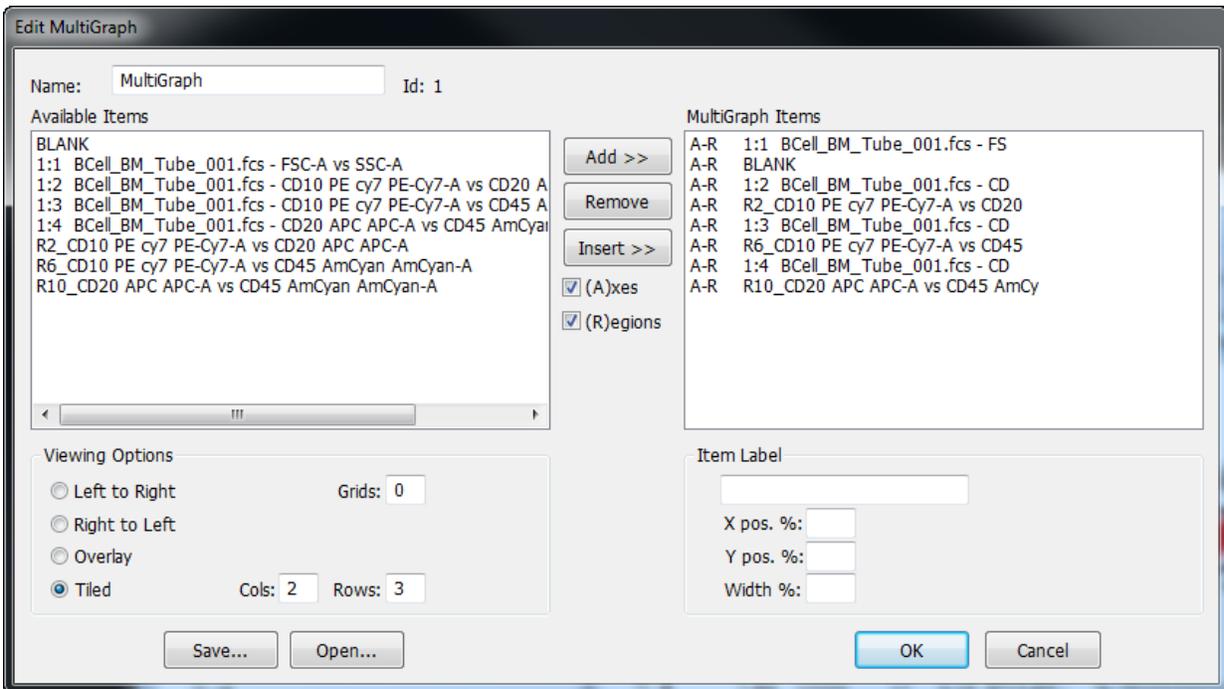


**Name**

Enter a name for the MultiGraph window. You can enter text, or type a keyword enclosed in square brackets.

**Available Items**

Displays a list of all histograms in all data sources currently opened in WinList, as well as the results windows. This is a multiple selection list box, so you can select the name of the histograms and results windows you want, drag the mouse in the list box to select a range, then click the **Add** button to add those histograms to the **Selected Items** list. An additional

entry, **BLANK**, allows you to insert a placeholder with no graphic. This can be useful if you have a wide results window and want to allow it more room on the MultiGraph in a tiled view.

### MultiGraph Items

Displays a list of histograms existing in the MultiGraph and those you want to add to it. It is a multiple select list box. You can click the left mouse button and multiple select the histograms you want to remove or edit. The first three characters in each entry of this list indicate the states of the **Axes** and **Regions** check boxes. The MultiGraph entries will include or exclude the X and Y-axes, and any regions that are within the histograms based on these characters. For example, if an entry in Selected Histograms starts with **A-R**, axes and regions will be included in the MultiGraph.

### Add

This button copies the histograms currently selected in the **Available Items** list box into the **MultiGraph Items** list box. As histograms are added, the state of the check boxes **Axes** and **Regions** is also copied for each item added.

### Remove

This button deletes the highlighted items from the **MultiGraph Items** list box and subsequently from the MultiGraph.

### Insert

Use this button to add items from the **Available Items** list box in front of the currently selected item in the MultiGraph **Items** list box.

### Axes, Regions

These check boxes determine whether x and y axes, and regions will be displayed for items as they are added to the **MultiGraph Items** list box. If **Axes** is checked, the letter **A** will appear as the first character in the **MultiGraph Items** entry. If it is not checked, a hyphen (**-**) will appear. Similarly, **R** will appear for regions. Once the **OK** button is clicked, these options become permanent for the selected histograms.

### Viewing Options

Radio buttons allow you to select from several viewing arrangements: **Left to Right**, which stacks the histograms from the upper left to the lower right, **Right to Left**, which stacks from upper right to lower left, **Overlay**, which allows histograms to be superimposed on one another, and **Tiled**, which provides a row-column arrangement. If you select **Tiled**, the **Col** and **Rows** edit boxes allow you to control the row-column arrangement. Setting **Rows** to 0 will fit all elements of the MultiGraph onto one page. Setting **Rows** to a positive number will display a maximum of that many rows per page, in some cases creating a multiple page printout. For **Left to Right** and **Right to Left**, the edit box **Grids** allows you to lay down evenly spaced grid lines connecting the bases of the histograms.

**Label**

This group of controls allows you to enter a label for each item and to determine its size and position on the MultiGraph. The label may include up to 80 characters of text.

Labels can include any FCS keywords embedded in the data file and internal WinList keywords. Keywords must be surrounded by square brackets []. Keywords are only evaluated at the time the elements are created.

See Internal Keywords for more on keywords.

Examples of labels:

*[HISTOGRAM] [GATE] [DATE]*

 displays name of the histogram, the gating information, and today's date

*[FILE] contains [R('3,PERCENT')] percent positives*

 displays the name of the file and the percent of region R3.

*[$SAMPLE] [$PROJ]*

 displays the FCS keyword values for $SAMPLE and $PROJ.

**Label position and width**

The X and Y positions and the width can be entered in these edit boxes. The numbers are in *percentage of the windows* where 0 percent x and y is the lower left corner of the window, and 100 percent x and y is the upper right. X and Y positions determine the origin of the label. The width, again in percent, determines how wide the label can extend. Usually, the width and the x position values should not add up to more than 100 or the label will spill out of that histogram's portion of the MultiGraph.

**Save and Open**

These push buttons allow you to save MultiGraphs to disk, or reopen existing MultiGraphs. Opening a MultiGraph from disk will replace any histograms in the current MultiGraph.

**OK**

Click **OK** when you've made your changes and selections and the new MultiGraph elements will be generated. The amount of time to generate a MultiGraph depends on how many elements you have selected and how complicated each one is.

**Cancel**

The **Cancel** button allows you to undo the changes you have made. In some cases it is grayed out, indicated that you cannot cancel the operation. This occurs if you are editing an existing MultiGraph and you *remove* one or more of the histograms from the **Selected Items** list box. Removing existing items is not a recoverable operation.

**Open MultiGraph**

This command displays the File Open dialog to select a MultiGraph from disk.  It is found in
the **File (Application)** menu of the Ribbon bar.

**Export MultiGraph Graphics**

**MultiGraph Graphic**
Export a MultiGraph in a graphic format.

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This menu options allows you to save the active MultiGraph as a graphic file on disk. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, or GIF.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box and choose the graphic format by using the Save as Type drop down menu. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

**Export MultiGraph PDF**

MultiGraph PDF
Export a MultiGraph as a PDF

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This option saves the active MultiGraph window as a PDF file.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

**Add Histogram to MultiGraph (Ctrl A)**

This is a shortcut used to add the active histogram to the currently selected MultiGraph. It allows the user to bypass the **Edit MultiGraph** dialog box. If there is no histogram to add or no MultiGraph present, the option does nothing.

The histogram is added using the current settings of the **Edit MultiGraph** dialog box for Axes and Regions, as well as labels and label positions.

See also:

Edit MultiGraph

**Add Results to MultiGraph (Ctrl R)**

This is a shortcut used to add the active results window to the currently selected MultiGraph. It allows the user to bypass the **Edit MultiGraph** dialog box. If there are no results to add or no MultiGraph present, the option does nothing.

See also:

Edit MultiGraph

**Add Blank to MultiGraph (Ctrl K)**

This is a shortcut used to add a " blank" place holder to the currently selected MultiGraph. It allows the user to bypass the **Edit MultiGraph** dialog box. A blank is an element for which there is no graphic or text, but which occupies space like any other element in the MultiGraph.

See also:

Edit MultiGraph

**Add Info to MultiGraph (Ctrl I)**

This option allows you to add the text contained in the active histogram's Info window, if there is one, to the currently selected MultiGraph. Info windows appear when doing histogram subtraction, or if the histogram has a remote DDE connection with another application that has returned text to the histogram. When a button with the letter **I** appears in the lower left of a histogram window, you can click the button to display the histogram's Info window.

Use this option to add histogram subtraction results and remote DDE results to a MultiGraph.

See also:

Edit MultiGraph

**Reports**

**Report Windows**
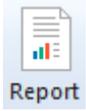


To create a report window, click the Report button on the Home tab of the ribbon bar.  If you are set up to use a menu and toolbar instead, choose New from the Report menu.

Reports are used to create multi-page documents that can contain histograms, results, alerts, and many other kinds of information that WinList generates. The elements added to a report can come from different data sources, and can automatically update when the source objects change.
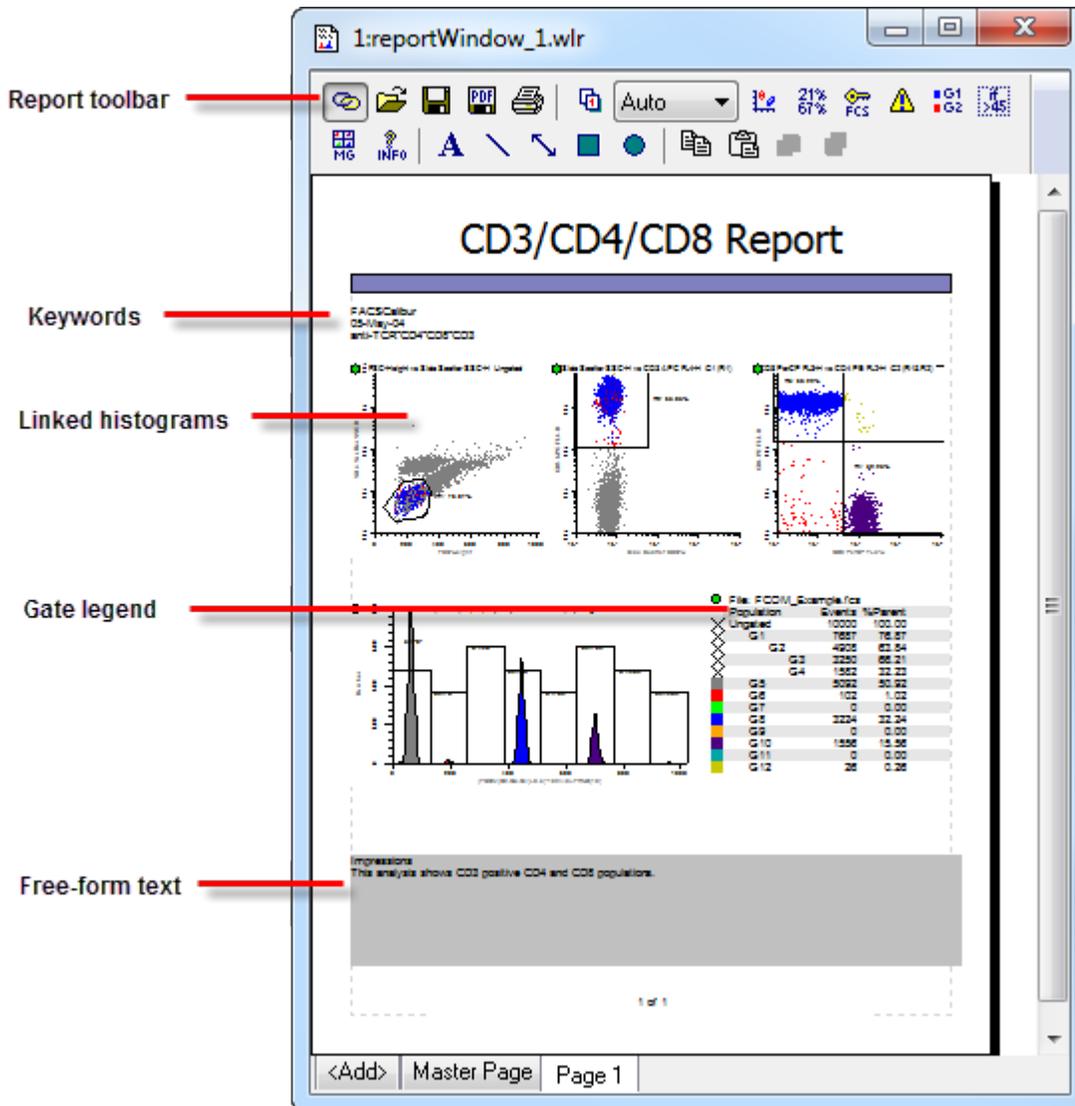
Elements can be added to reports by dragging-and-dropping them onto the report, or by using a command from the toolbar or menu. Once added, objects can be moved and resized using the mouse. See Drag and Drop for details.

Right-click the mouse on an element of the report to see additional commands that can be performed on the selected element. The commands shown will differ depending on the type of object selected.

Reports can be saved to and opened from disk files. They can also be exported in PDF format, a convenient means for sharing reports with others who may not have access to WinList. You can create and open any number of reports, providing a simple mechanism for producing internal and external versions of reports.

**Report Toolbar**

This toolbar is attached to the inside-top of each report window. Its icons initiate commands commonly used on reports.
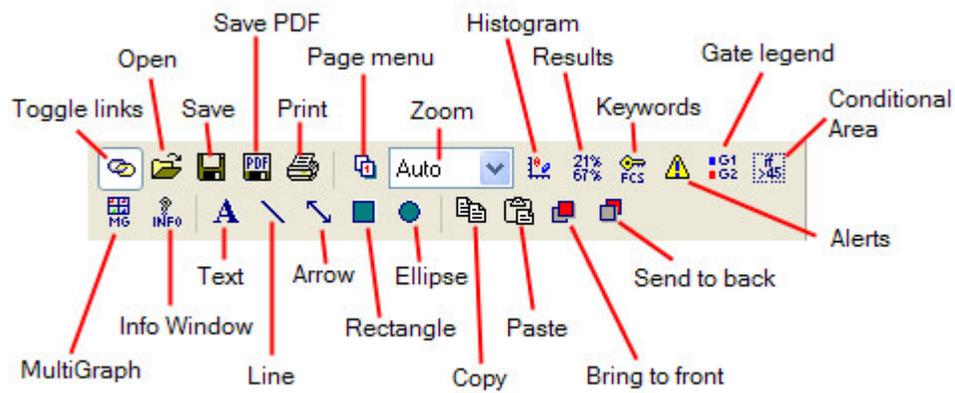
See individual report menu commands for details.

**Report Context Menu**

This context menu is displayed when the right-mouse button is clicked on a report.  The menu also lists commands commonly used on reports, many of which are also accessed from the report toolbar.

| | |
|---|---|
| | Copy |
| | Paste |
| | Page Setup... |
| | Copy Page... |
| | Clear Page |
| | Delete Page |
| | Open... |
| | Save... |
| | Print |
| | Export to PDF... |
| ✓ | Active Links |
| ✓ | Snap-to-grid |
| | Display grid |
| | Insert Histogram Link... |
| | Add Histograms |
| | Insert Results Link... |
| | Insert Keywords... |
| | Insert Alert... |
| | Insert Gate Legend |
| | Insert MultiGraph Link... |
| | Insert Overlay Link... |
| | Insert Info... |
| | Insert Condition Area... |
| | Insert 3D Histogram Link... |
| | Insert Text... |
| | Insert Line |
| | Insert Arrow |
| | Insert Rectangle |
| | Insert Ellipse |

See individual report menu commands for details.

**New Report**

This command creates a new, empty report window.

To create a report window, click the Report button on the Home tab of the ribbon bar.  If you are set up to use a menu and toolbar instead, choose New from the Report menu.
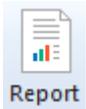


Report

**Open Report**

This command displays the Open Report dialog to select a report to open from disk.

**Save Report**



This command displays the Save Report dialog to save the active report to disk.

**Print Report**



This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.  It is also found on the toolbar for the active Report window.

Use the Printer Setup option in the File menu to set print options.

Several options in the **Preferences** dialog affect the print report option. They appear in the Print Preferences dialog box and control margins, orientation, and more.

**Export Report PDF**

**Report PDF**
Export a report in PDF format

This command is found in the File (Application) menu of the <u>ribbon bar</u>.  When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command displays the Export PDF dialog to save the active report as a PDF file. PDF stands for Portable Document Format, and is a standard format for exchanging information containing graphics and text. It is very useful for creating archival reports or for sharing analysis results with others.

**Export Report Graphic**
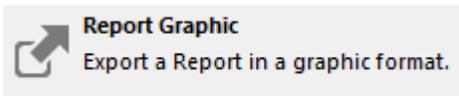
Report Graphic
Export a Report in a graphic format.

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This command displays the Save Report Graphic dialog to save the active report as a graphic file. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, and GIF.

**Report Page Menu**

This command displays the page menu. The command is available in the report toolbar at the top of each report window.

| | |
|---|---|
| ⬉ | Add page... |
| | Rename page... |
| | Copy page... |
| | Clear page |
| | Delete page |
| | Page Setup... |
| | Next page |
| | Previous page |
| | Master Page |
| ✓ | Page 1 |
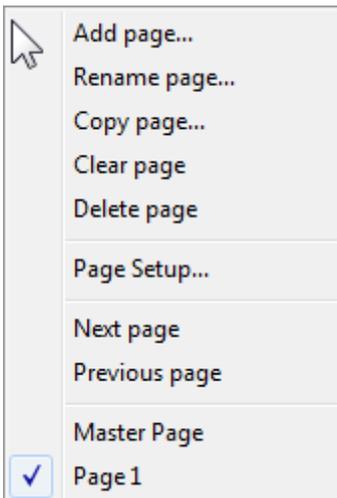
This menu allows you to add, rename, and delete pages in the report. It also provides commands to navigate to pages. Note: you can also navigate to pages by clicking on the title bar of the report, and then using the PgUp and PgDn keys on the keyboard.

**Add Page**

This command creates a new, blank page in the report and displays a dialog that allows you to name the page.

**Rename Page**

This command displays a dialog that allows you to rename a page.

**Copy Page**

This command copies all of the objects on the current page to another page in the report. The Choose Page dialog is displayed to allow you to select the page to copy to.
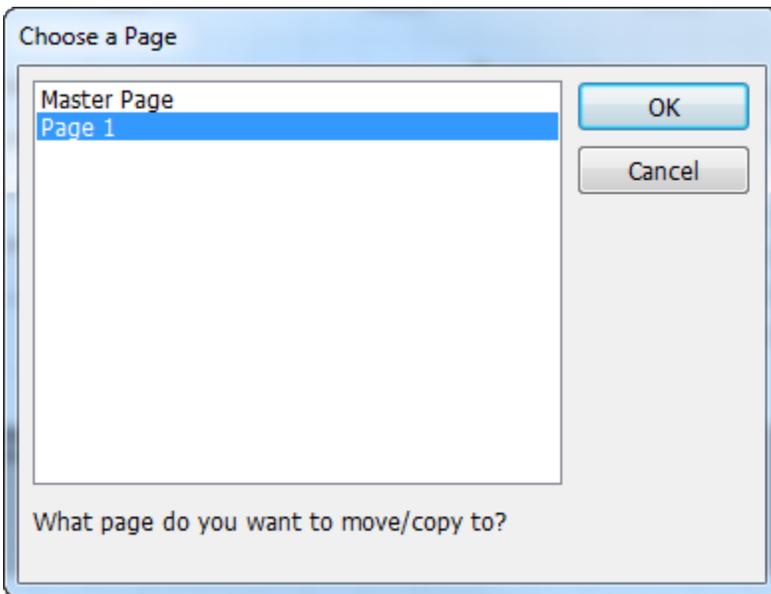
## Clear Page

This command removes all of the objects on the page, leaving a blank page.

## Delete Page

This command deletes the current page of the report and all of the objects on the page.

## Page Setup

This command displays the Page Setup dialog, which determines page size and margins.

## Next Page

This command navigates to the next page in the report. The PgDn key is a shortcut for this command.

## Previous Page

This command navigates to the previous page in the report. The PgUp key is a shortcut for this command.

**Report Zoom**



This drop-down provides options for zooming in or out on the active report. The default setting, **Auto**, scales the report height to fit the report window. Other settings select specific magnifications for the report page.

**Active Links**



This command toggles the "active link" state for all linked objects on the report. When the button is in a pressed state, the linked histograms and results objects on the report will update whenever the windows to which they are linked change. When the button is up, linked objects do not update automatically and will display information from the last time they updated.

If you want to save a report for a specific experiment and you do not want it to update when you re-open it, toggle the button to the "up" state before saving it.

If you are about to perform a number of actions on a large data file and you do not want objects to update during that process, toggle the active links button to the "up" state before performing the actions. Once you've finished, you can re-enable active links by pressing the button again.

This command is also available in the report context menu.

**Snap to Grid**

This command is found in the Report Context menu.  It controls whether objects on a report are aligned to a user-defined grid when moving or sizing. The grid size is set in the Reporting Preferences pane of the Preferences dialog.

Enable this item to force objects to align to the snapping grid.

Uncheck the command to allow objects to move freely on the report page.

### Insert Histogram Link

This command is found in the Report Context menu.  It adds a histogram link object to the current page of a report. Histogram link objects display a picture of a histogram. Because the object is linked to a histogram, it updates whenever the histogram changes.

A dialog is displayed that allows you to select the specific histogram that you want to link the object to.

```
Edit Histogram Link

Choose a Data Source and Histogram:

1:FCOM_Example.fcs           ▼      [   OK   ]

1:FSC-Height vs Side Scatter SSC-H  [ Cancel ]
2:Side Scatter SSC-H vs CD3 APC FL4-H
3:CD8 PerCP FL3-H vs CD4 PE FL2-H
4:(FCOM(G2




Caption:
Show name and gate              ▼
Graphic quality:
Normal quality                  ▼
Update options:
Update when histogram changes   ▼
```
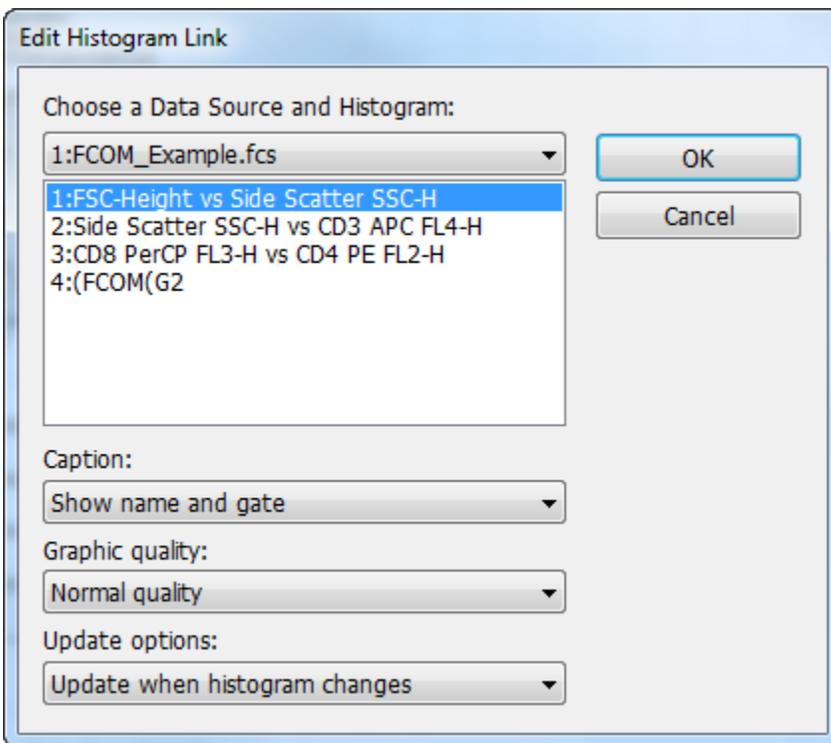
Select the data source from the drop-down list, and then select the histogram from the listbox.

**Caption**

This option control determines whether the histogram object shows a caption. The caption can contain the histogram name, gate, the name and gate, or nothing at all.

**Graphic quality**

This option controls the quality of the histogram graphics generated when the object updates on the report. Lower quality settings will produce graphics that require less memory, draw faster, and print faster. Perfect quality requires the most memory and draws the slowest, but

produces the best output possible from the program. Experiment with this setting to find a suitable quality for your purposes.

**Update options**

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to update when a specific condition is met, or not to update at all. Updating can also be controlled by placing the object in a Condition Area object on the report.

There are two other methods you can use to add histogram links to a report.

1. You can drag and drop a histogram onto the report. See Report Windows for details.

2. You can use the histogram context menu **Add to Report** command.

**Insert Results Link**

21%
67%

This command is found in the Report Context menu.  It adds an object linked to a results window to the current page of a report. Results link objects display the textual information shown in the Results window. Because the object is linked to a results window, it updates whenever the results window changes.

A dialog is displayed that allows you to select the specific results window that you want to link the object to.
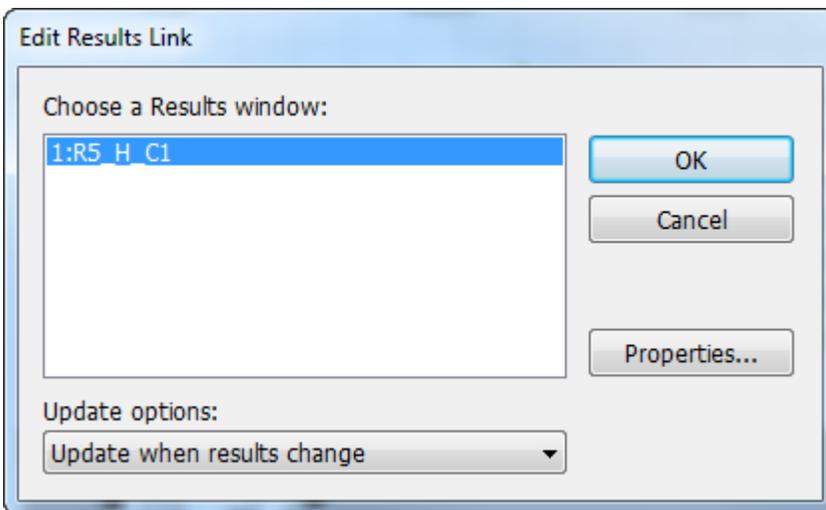
Select the results window you want to link to from the listbox.

**Update options**

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to update when a specific condition is met, or not to update at all. Updating can also be controlled by placing the object in a Condition Area object on the report.

There are two other methods you can use to add results window links to a report.

1. You can drag and drop a results window onto the report. See Report Windows for details.

2. You can use the results context menu **Add to Report** command.

### Insert Keywords



This command is found in the Report Context menu.  It adds a keyword object to the current page of a report. Keyword objects display textual information derived from analysis results and FCS keywords in the listmode data files. Keyword objects update when the values for keywords they contain change.

A dialog is displayed that allows you to select the keywords that you want to display.
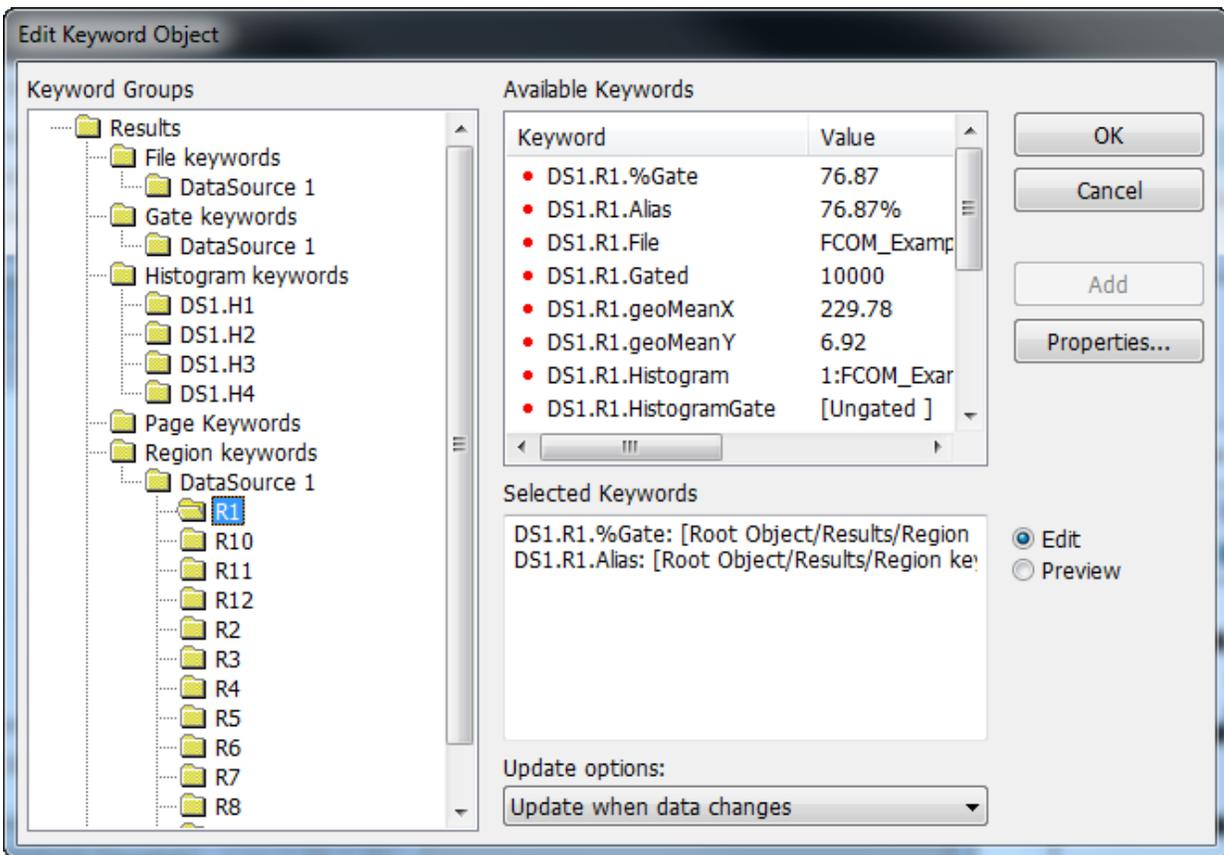


**Keyword Group**

The Keyword Group tree displays groups of keywords. When you select a node in the tree, the keywords for that group are displayed in the Available Keywords list.

**Available Keywords**

This list shows the keywords that are available for the item selected in the Keyword Group tree. The list is a multiple-selection list; any number of keywords can be selected and added to the report object.

To add keywords from this list to the keywords that you want to display, use one of these methods:

1. Drag and drop keywords from this list into the Edit and Format edit box.

2. Select the keyword you want to add, and then click the Add button.

**Selected Keywords**

This edit control displays the keywords that have been selected for display on the report. When the Edit radio button is selected, you can directly edit keyword labels. For example, suppose that you have added the keyword "R1.%Gate" and you want the label to read "T Cells (% of Live Cells)". You would select the text "R1.%Gate" and type "T Cells (% of Live Cells)".

**Update options**

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to update when a specific condition is met, or not to update at all. Updating can also be controlled by placing the object in a Condition Area object on the report.

**Add**

Click this button to add keywords selected in the Available Keywords list to the Edit and Format control. Keywords that are added will be displayed on the report.

**Properties**

This button displays the Edit Properties for Text dialog, to allow you to choose a font and other characteristics of the object.

**Edit/Preview**

These radio buttons determine whether the Edit and Format control shows editable keywords or a preview of what the report object will display.

**OK**

Click OK to accept the changes to the object and close the dialog.

**Cancel**

Click Cancel to discard the changes to the object and close the dialog.

**Insert Alert**

⚠️

This command is found in the Report Context menu.  It adds an alert object to the current page of a report. An alert object is linked to an alert that is defined in Edit Alerts, and shows the current state of the alert.

A dialog is displayed that allows you to select an alert that you want to link the object to.
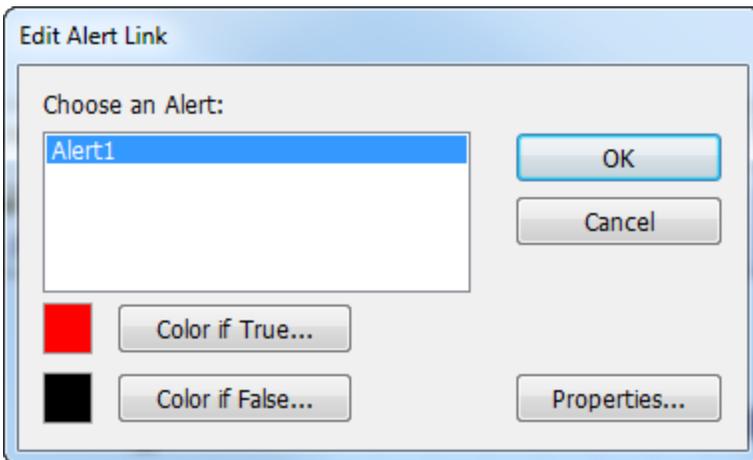


This dialog also allows you to select two colors for the text of the alert: one color for when the alert is True, and another for when it is False (i.e. the alert condition has not been met). You can use the Properties button to choose a font for the alert object.

**Insert Gate Legend**



This command is found in the Report Context menu.  It adds a gate legend object to the current page of the report.  This object relates to the Gate Statistics window for a data source.

| | Population | Events | %Parent |
|---|---|---|---|
| File: FCOM_Example.fcs | | | |
| | Ungated | 10000 | 100.00 |
| | G1 | 7687 | 76.87 |
| | G2 | 4908 | 63.84 |
| | G3 | 3250 | 66.21 |
| | G4 | 1582 | 32.23 |
| | G5 | 5092 | 50.92 |
| | G6 | 102 | 1.02 |
| | G7 | 0 | 0.00 |
| | G8 | 3224 | 32.24 |
| | G9 | 0 | 0.00 |
| | G10 | 1556 | 15.56 |
| | G11 | 0 | 0.00 |
| | G12 | 26 | 0.26 |

The gate legend displays a list of all active gates. For each gate, the gate color, gate alias, and statistics are displayed.

Use the Edit Gates dialog to edit gate colors, aliases, and expressions.

**Insert Condition Area**



This command is found in the Report Context menu.  It adds a rectangular "conditional update" object to the report. Anything that falls within the conditional update object will only update if the condition specified is satisfied.
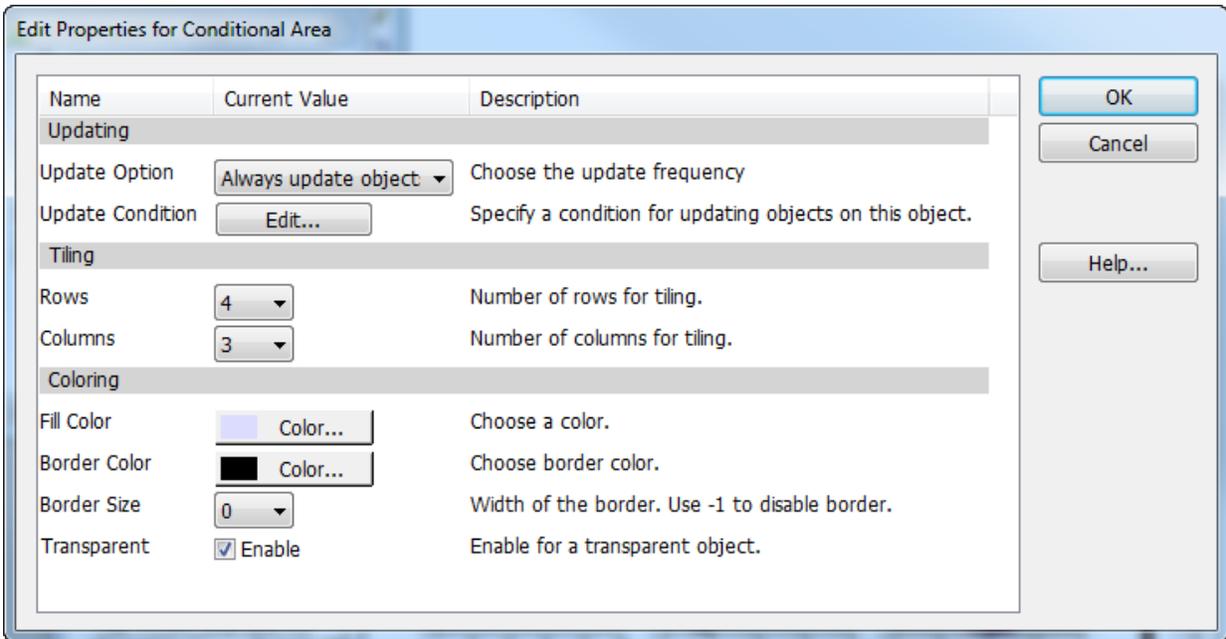


**Updating**
**Update Option**

Select how you want objects placed in this condition area to update. By default, objects update automatically whenever the associated data changes. You can also set the object to update when a specific condition is met, or not to update at all.

**Update Condition**

Edit the condition for this area to update.

**Tiling**

A condition area object can automatically tile elements that fall within its boundaries.  Tiling is performed on-demand by right-clicking on the condition area object and choosing a tiling option from the context menu.

**Rows**

Enter the number of rows in the tiling arrangement that you want this area to have.

**Columns**

Enter the number of columns in the tiling arrangement that you want this area to have.

**Coloring**

**Fill Color**

If the object is not set to Transparent, this is the color that is used to fill the area.

**Border Color**

Select a color for the border of the area.

**Border Size**

Select a size for the border. Selecting -1 will disable the border.

**Transparent**

When this option is enabled, the fill color is ignored.

**Edit Condition**

This dialog is used to define an update condition for some kinds of report objects.
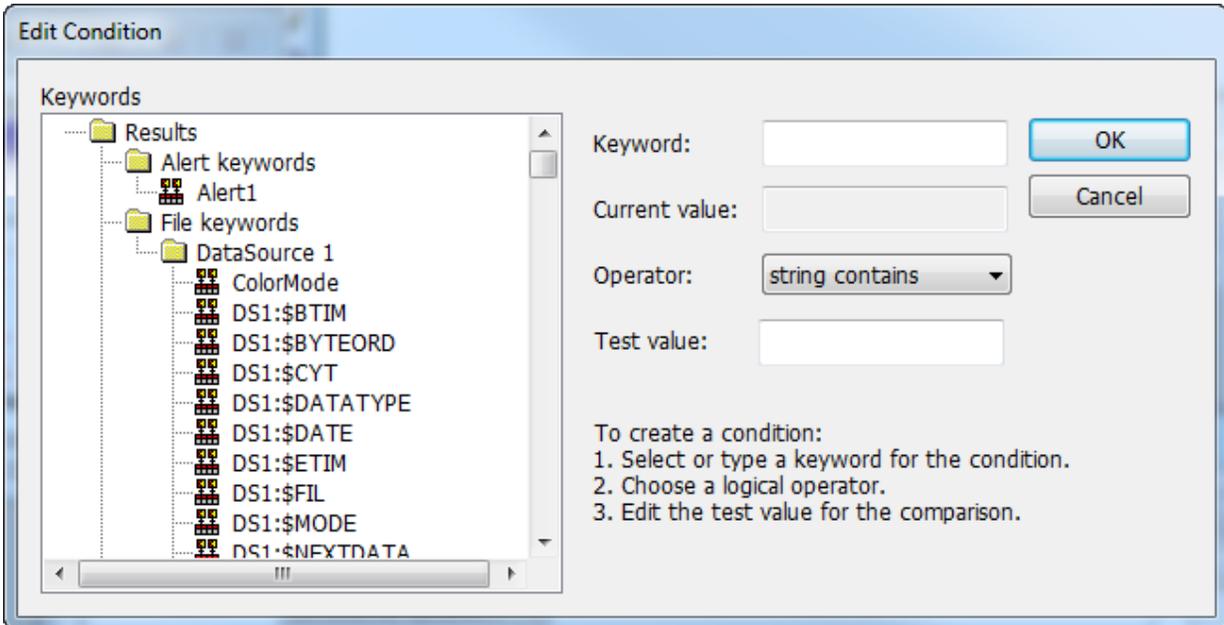


Conditions are based on keyword values. Keyword values can be extracted from the FCS files being analyzed, from regions, from user equations, or from user keywords.

**To create a condition**

1. Select a keyword from the **Keywords tree**. The selected keyword will appear in the **Keyword** edit control and its current value is also displayed.
2. Select how the keyword will be evaluated by choosing an **Operator**. Common mathematical operators, as well as string operators, are available in the list.
3. Enter or edit the **Test value**.

The test value is compared with the current value using the selected operator. If the condition represented by the expression is TRUE, the condition is met and the update will proceed. If the condition evaluates as FALSE, the update will be suppressed.

## Insert MultiGraph Link



This command is found in the Report Context menu.  It adds an object linked to a MultiGraph window to the current page of a report. A MultiGraph link object displays the content of a MultiGraph window. Because the object is linked, it updates whenever the MultiGraph window changes.

A dialog is displayed that allows you to select the specific MultiGraph window that you want to link the object to.
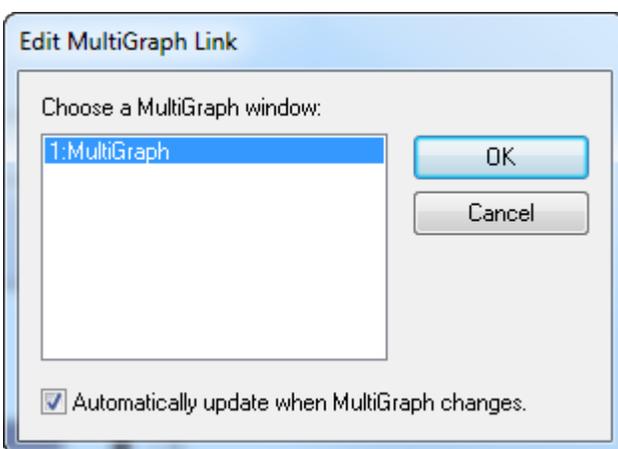


Select the MultiGraph window you want to link to from the listbox. A checkbox is provided that determines controls whether the new object will update automatically as the MultiGraph window changes.

You can also drag and drop a MultiGraph window onto the report to create a MultiGraph link object. See Report Windows for details.

**Insert Info Window**



This command is found in the [Report Context menu](#).  It adds an object linked to a histogram's Info window to the current page of a report. An Info window link object displays the content of an Info window. Because the object is linked, it updates whenever the Info window changes.

A dialog is displayed that allows you to select the histogram that owns the Info window.

Select the data source from the drop-down list, and then select the histogram from the listbox. A checkbox is provided that determines controls whether the new object will update automatically as the Info window changes.

## Insert Text

**A**

This command is found in the Report Context menu.  It adds a text object to the report. The object can be moved, sized, and edited.

See Edit Properties for Text for details.

**Edit Properties for Text**

This dialog allows you to edit the text, font, background, and other properties of a text report object.
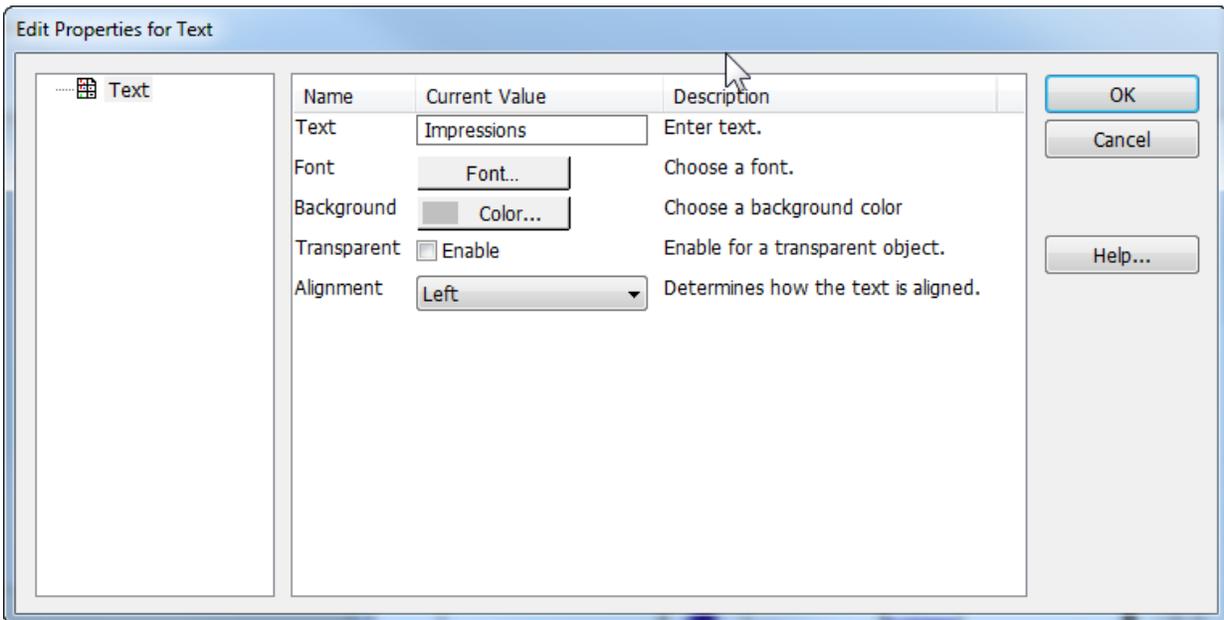


**Text**

Edit the text to display in the object. If the text has more than one forced line, a text edit dialog can be displayed by clicking in the edit box and pressing the Enter key.

**Font**

Use this button to select the font to use for the text object.

**Background**

Select a background color for the text with this object. If the **Transparent** option is enabled below, this color is ignored.

**Transparent**

When enabled, the graphics under the text object will visible. Disable this option to display text on a solid background.

**Alignment**

Choose the alignment option for the text object.

**OK**

Choose this button to close the dialog and accept the changes.

**Cancel**

Choose this button to close the dialog and discard any changes you may have made.

**Help**

Choose this button to display online help for the dialog.

**Insert Line**

This command is found in the Report Context menu.  It adds a line object to the report. The object can be moved, sized, and edited.

See Edit Properties for Line for details.

**Edit Properties for Line**

This dialog allows you to edit the properties of lines and arrows.
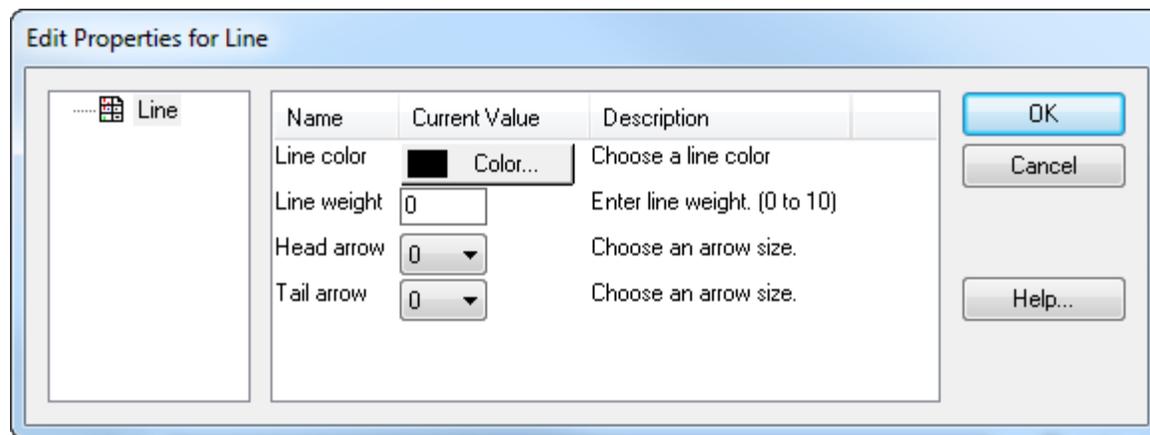


**Line color**
Select the color to use for the object.

**Line weight**
Enter a line weight to use for the object. This value is in points, a unit of 0.35 mm or 1/72 in.

**Head arrow**
Choose the size for the arrow at the head of the line. Size of 0 disables the head arrow.

**Tail arrow**
Choose the size for the arrow at the tail of the line. Size of 0 disables the tail arrow.

**OK**
Choose this button to close the dialog and accept the changes.

**Cancel**
Choose this button to close the dialog and discard any changes you may have made.

**Help**
Choose this button to display online help for the dialog.

**Insert Arrow**

This command is found in the Report Context menu.  It adds an arrow object to the report. The object can be moved, sized, and edited.

See Edit Properties for Arrow for details.

**Edit Properties for Arrow**

These options are the same as those for line objects. See [Edit Properties for Line](#) for details.

## Insert Ellipse

This command is found in the [Report Context menu](#).  It adds an ellipse object to the report. The object can be moved, sized, and edited.

**Edit Properties for Ellipse**

This dialog allows you to change the properties of an ellipse report object.
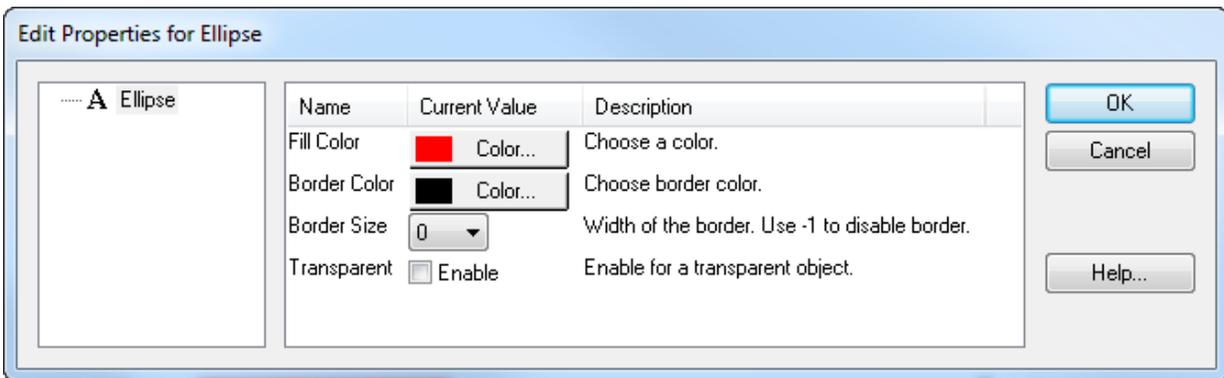


**Fill Color**
Choose a fill color for the object.

**Border color**
Choose a color for the edge of the object.

**Border size**
Choose a thickness for the border of the ellipse. A size of -1 turns off the border.

**Transparent**
Check this option to display the object without any fill color.

**OK**
Choose this button to close the dialog and accept the changes.

**Cancel**
Choose this button to close the dialog and discard any changes you may have made.

**Help**
Choose this button to display online help for the dialog.

## Insert Rectangle

This command is found in the [Report Context menu](#).  It adds a rectangle object to the report. The object can be moved, sized, and edited.

**Edit Properties for Rectangle**
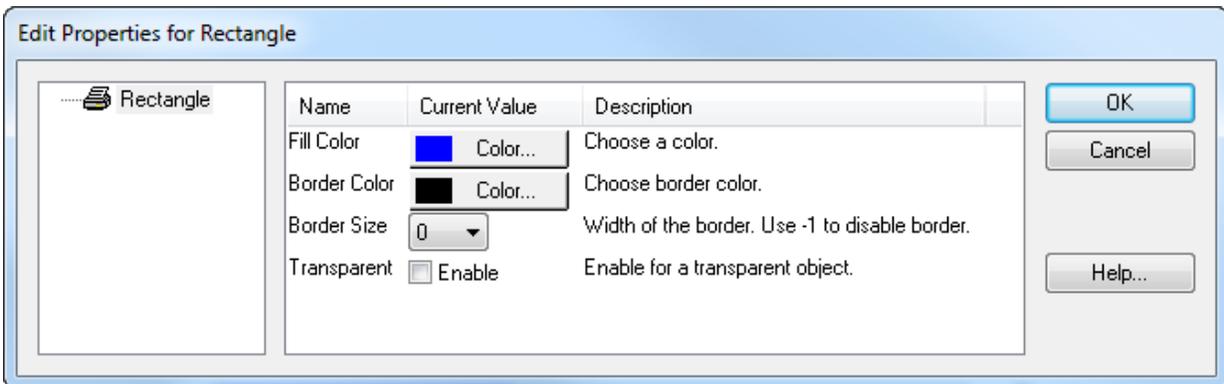


**Color**

Choose a fill color for the object.

**Border color**

Choose a color for the edge of the object.

**Border size**

Choose a thickness for the border of the object. A size of -1 turns off the border.

**Transparent**

Check this option to display the object without any fill color.

**OK**

Choose this button to close the dialog and accept the changes.

**Cancel**

Choose this button to close the dialog and discard any changes you may have made.

**Help**

Choose this button to display online help for the dialog.

**Insert 3D Histogram Link**

This command is found in the Report Context menu.  It adds a link to a 3D window to the current page of a report. 3D Histogram link objects display a picture of a 3D window. Because the object is linked, it updates whenever the 3D window changes.

A dialog is displayed that allows you to select the specific histogram that you want to link the object to. Choose the histogram that owns the 3D window you want to add.
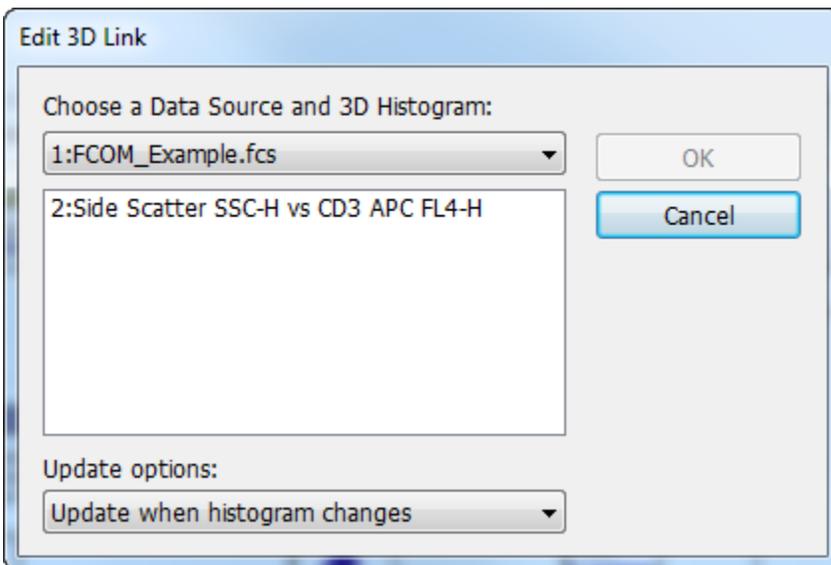


Select the data source from the drop-down list, and then select the histogram from the listbox. A checkbox is provided that determines controls whether the new object will update automatically as the histogram changes.

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to update when a specific condition is met, or not to update at all. Updating can also be controlled by placing the object in a Condition Area object on the report.

You can also drag and drop a 3D window onto the report to create a 3D link object in the report. See Report Windows for details.

## Copy (Report Toolbar)

This command is found in the Report Context menu.  It copies either the whole report or the selected object to the clipboard.

If an object is selected, it is copied to the clipboard in its native format. Text objects are copied as text. Object containing graphics or a mix of graphics and text are copied as bitmaps.

If no object is selected on the report, the entire report is copied in bitmap format.

**Paste (Report Toolbar)**

This command is found in the Report Context menu.  It will attempt to paste the data into the report.

If the clipboard contains text formatted data, a new text object is inserted and the data is pasted into it.

If the clipboard contains a compatible graphic format, a new graphic object is inserted and the date is pasted into it.

If more than one compatible format is available in the clipboard, the program displays a dialog to allow you to select the format you want to paste.

**Bring to Front**

This command is found in the [Report Context menu](#).  It is enabled when an object is selected in the report. It will move the selected object in front of (i.e. on top of) all other objects in the report.

The command is disabled if no object is selected.

## Send to Back

This command is found in the Report Context menu.  It is enabled when an object is selected in the report. It will move the selected object behind (i.e. in back of) all other objects in the report.

The command is disabled if no object is selected.

**Add Histogram to Report**

This command is found in the Report Context menu.  It adds the active histogram window to the active report. The histogram object on the report is located in a default position and size.

**Add Results to Report**

This command is found in the Report Context menu.  It adds the active results window to the active report. The results object on the report is located in a default position and size.

**Equations, Keywords, and Alerts**

**Edit User Keywords**

🔑 User Keywords

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.  It allows the user to create and modify user-defined keywords.
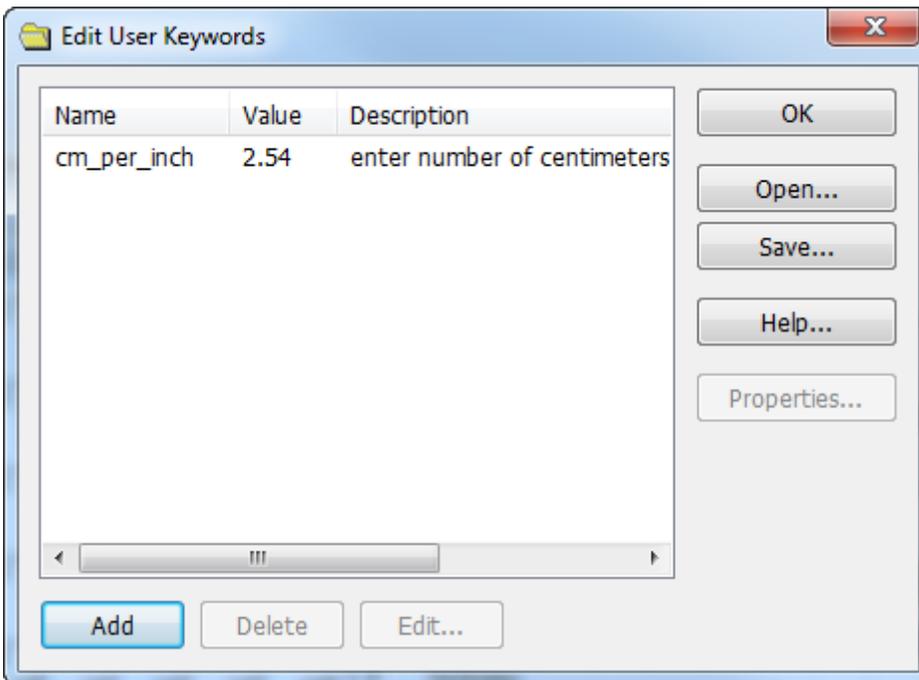


User-defined keywords allow you to enter information that is not otherwise available to WinList, and to use the information for display and computation of other expressions. WinList can prompt for a new value when a new file is opened, when a file is replayed, or never.

User-defined keywords can be databased and added to reports. They can also be used in User Equations, as part of arithmetic expressions. They are stored with protocols and protocol bundle files.

Click the **Add** button to create a new user keyword. The Edit Properties for User Keyword will be displayed allowing you to create a name, default value, and prompt for the keyword. You can also set when WinList should update the keyword.

See also:

Edit Properties for User Keyword

**Edit Properties for User Keyword**

This dialog edits the properties for a user-defined keyword.



**Keyword name**

Type a name for the keyword. This name is used to identify the keyword when it is used in the database, reports, or user equations.

**Keyword value**

Enter the value for this keyword. This can be a text or a number. If the keyword is intended to be used in a user equation, the value should be a number.

**Prompt**

This is the prompt that is displayed when WinList needs to update this keyword. It can be any helpful message.

**Update option**

Never update: this option suppresses updates for the keyword. The value will remain unchanged.

Update on new file: this option causes WinList to prompt for a new keyword value whenever a new file is opened.

Update on replay: this option causes WinList to prompt for a new value when one of the Replay options is used.

See also:

Edit User Keywords

**Edit Equations**

A>B User Equations

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.  User equations are used to compute new statistics that WinList does not otherwise compute. Any numeric information that WinList stores can be used in mathematical expression, and user-defined information can be included too.
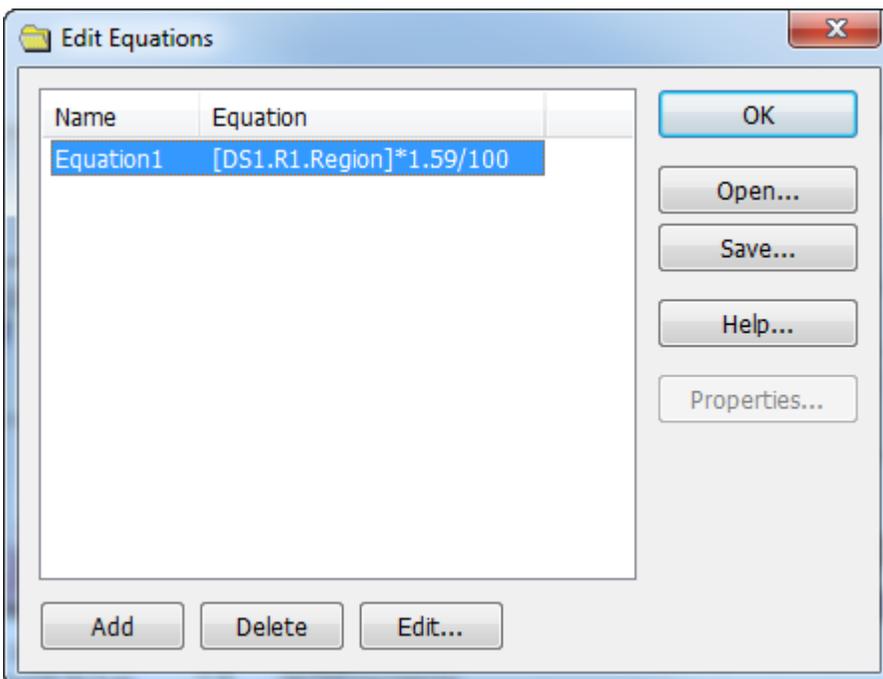


Create a new equation by clicking **Add**, or edit an existing equation using the **Edit** button. The Edit Equation dialog is displayed to define the equation.

Equations can be saved to disk and opened in other protocols, so that complex expression do not have to be re-entered manually.

**Edit Equation**

WinList equations can be used to compute new values based on other information in the analysis setup.  Regions, gates, keywords, and built-in functions are available to the equation editor.  Parentheses allow grouping of terms in the expression.

This dialog is designed to make it easy to create your own equations. The layout is like a calculator, with the addition of a number of special function buttons on the right.
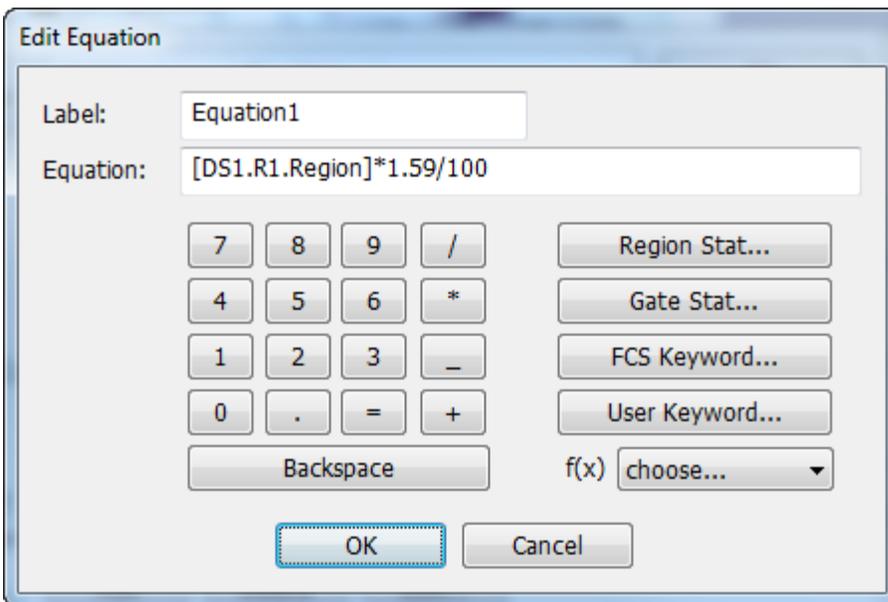


**Fields**

**Label**

The label is used as a name for the equations. This is how the equation is identified in other parts of WinList, like reports, alerts, and keywords.

**Equation**

The equation can be directly edited in this edit control. Values that are surrounded by square brackets [ ] contain keywords. Math symbols, numbers, parentheses can also be used in this control.

**Calculator buttons**

As you click one of the calculator buttons, its value is inserted into the Equation edit box at the location of the cursor.

**Region Stat**

To include a region statistic in the equation, use the Region Stat button. A tree with all region keywords is displayed. Select the statistic you want and click OK. The region statistic will be inserted into the equation.

**Gate Stat**

To include a gate statistic in the equation, use the Gate Stat button. A tree with all gate keywords is displayed. Select the statistic you want and click OK. The gate statistic will be inserted into the equation.

**FCS Keyword**

To include an FCS keyword in the equation, use the FCS Keyword button. A tree with all FCS keywords is displayed. Select the keyword you want and click OK. The keyword will be inserted into the equation.

Note: you should only use keywords that have number values. The program will attempt to convert text values to numbers, but the result of that conversion is not predictable.

**User Keyword**

If you have created user-defined keywords, they can be added to the equation using this button.

**F(x)**

The F(X) button displays a list of math functions that can be used in the equation. The selected function is inserted at the current location in the equation. Its arguments need to be filled in with appropriate values.

**OK**

Click OK to save the changes to the equation.
**Cancel**

Click Cancel to discard changes.

**Edit Alerts**

⚠ Alerts

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.  It allows you to define and edit alert conditions that WinList will monitor. Alerts are defined by selecting a region statistic or other keyword from a list, choosing a logical operator, and entering a test value or another keyword. An Alert can compare keywords, statistics, and constant values.  For example, an alert can test to see if the number of events in region R1 is less than events in R2.  Instead of comparing with R2, it could compare with a constant value, like 250.

WinList can database alert conditions when it batch processes files, so that you can keep a record of any results that trigger the alert. In addition, you can choose to have WinList pause and display an alert dialog box when an alert triggers.
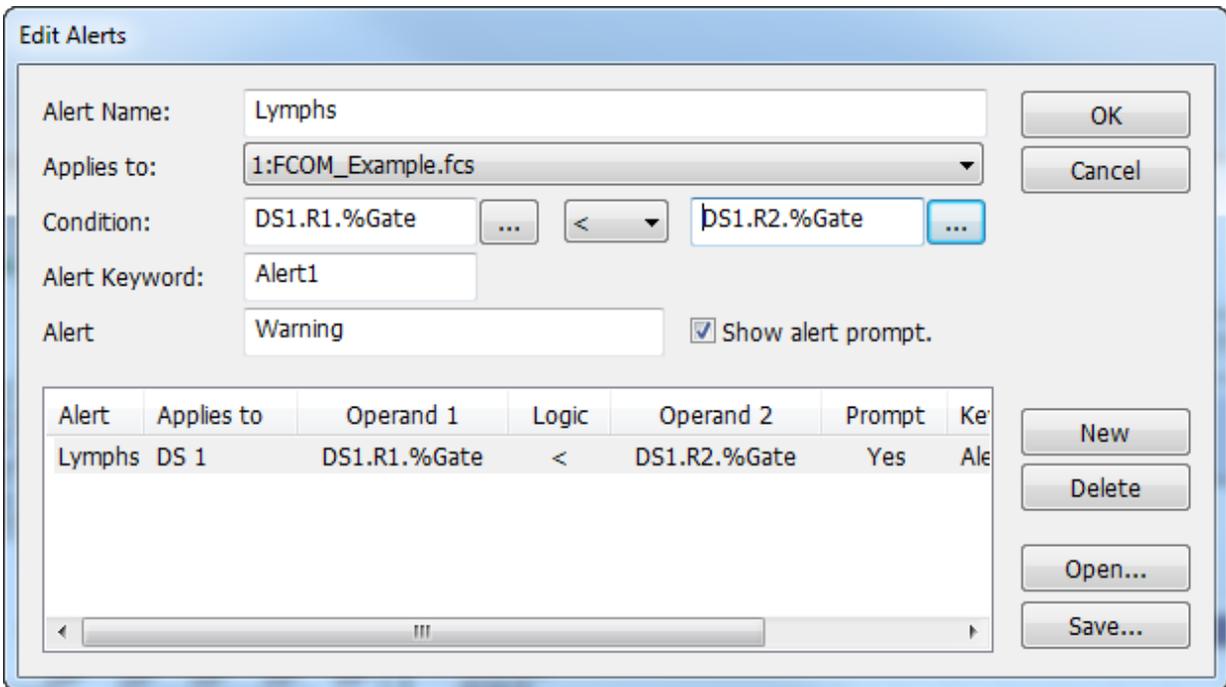


To create a new alert, click the **New** button and fill in the fields described below.

To edit an alert, select the alert in the listbox and edit the fields.

**Alert Name**

Enter a name for the alert. This name is displayed when the alert is triggered, so it is useful to enter a name that relates to the alert condition.

**Applies to**

Select the data source that the alert applies to. Each alert relates to only one data source.

**Condition**

The Condition contains three parts: a left-hand value, a logical operator, and a right-hand value. The left- and right-hand values can be region statistics, keywords, or constant values that you enter.  Click the **...** button next to a condition field to select a keyword from a list.  Choose a logical operator with the dropdown list in the middle of the condition.  And again, click the **...** button to select a keyword for the right-hand value. If the condition described by the expression is true during analysis, WinList will trigger the alert.

**Alert Keyword**

This is a user-defined keyword that WinList will use to store the alert in a database.

**Alert Text**

This text will be used as the value for the alert keyword if the alert is triggered.

**Show alert prompt**

Enable this field if you want WinList to pause and display a warning dialog whenever the alert condition is triggered.
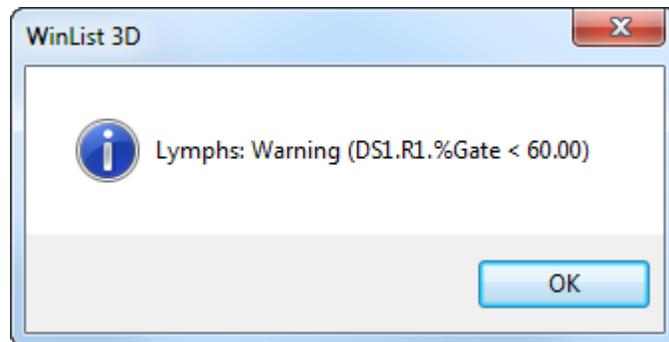


**Alert Listbox**

This list contains all alerts currently defined. To edit an alert, select it in the list and then edit its fields.

**New**

Click this button to create a new alert condition. This adds an entry to the alert listbox and selects it for editing.

**Delete**

This command deletes the currently selected alert in the alert listbox.

**Open**

This command displays a File Open dialog to select a previously saved set of alerts. When an alert file is selected and opened, the alerts in the file replace any that were previously displayed in the alert listbox.

**Save**

This command displays the File Save dialog, allow you to save the currently defined alerts to a file on disk.

**OK**

Close the dialog and accept the changes.

**Cancel**

Close the dialog and discard the changes.

**Updating and Replay**

**Replay**



The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.  It provides several ways to re-read data into data source windows. Use these options to do the following:

- Trigger the saving of a database record. See Databasing with WinList for more.

- Display all data events in single parameter histograms.

- Display all data events in dual and three parameter histograms after enabling the **Show all dots** option in the dual or three parameter **Graphics** dialog boxes. See also Edit 2P Graphics and Edit 3P Graphics .

- Update display and statistics for gating if **Auto Update** in the **Replay menu** is turned off. See also Auto-update.

- Trigger a *remote link* that is set to update on replay. See also Edit Remote.

WinList is normally working with a *packet* of events from the data source, not the entire data source. This allows the instantaneous update of statistics and graphics as you manipulate regions and gates. The packet is only a sample of events from the entire data source, though, and there are times when you want to see all of the events, or statistics relevant to all events. This is when you will use **Replay** options.

See also:

Auto-update

Replay Active Histogram

Replay Active Data Source

Replay All Data Sources

## Replay All Data Sources



This command replays data for all histograms in all data sources.

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

When this option is selected, any active databases will attempt to save a record. See Databasing with WinList for more.

**Replay Active Data Source**



This command replays data for all histograms in the active data source.

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

When this option is selected, any active databases will attempt to save a record. See Databasing with WinList for more.

## Replay Active Histogram



This command replays data for the active histogram in the active data source.

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

When this option is selected, any active databases will attempt to save a record. See Databasing with WinList for more.

**Enrich Data Source**



This command is used to process a data source through a user-selected gate, selecting only the events that are in the gate into the sample packet that WinList uses for analysis. It is most useful for analyzing low-frequency populations (e.g. B-cells).

The Enrich command can be found on the Options tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

When you select the command, a list of the active gates is displayed. Select the gate you want to enrich on. The data file will be re-read, this time selecting on the events "In" the selected gate.

To cancel the enriched analysis, select the option again and choose Ungated from the list.

**Auto-update**
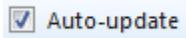


When this option is checked, Auto-update is enabled. Each time you move a region, the program will update results for the region and associated gates.  This is the normal operating mode for WinList.

When this option is *not* checked, you can move regions without triggering the update of results, gates, and histograms. It can be useful when you need to adjust more than one region in a protocol that takes a long time to update.  When the option is enabled (checked) again, the program will update any results, gates, and histograms.

**Update Now**

This command appears in the Replay menu in Toolbar mode.  It is displayed on the Options tab of the Ribbon bar.  It can be triggered by the shortcut Ctrl-J.

When selected this command tells WinList to reanalyze the current data in the packet and update results and graphics.  It can be used after a Replay command to revert WinList to packet-mode operation.

**Update Remote Links**

This command is found on the Options tab of the ribbon bar, or the Options menu when not using the ribbon bar.

The command updates "remote links" for histograms and results windows if they are set to update **On Command** (see Edit Remote dialog box). It has no effect on other remote links nor on windows which have no remote connections.

This option is useful for remote connections to database programs. It gives the greatest control over when information will be exchanged between a WinList window and some other application. For example, suppose a results window has a remote DDE link with Microsoft Excel to database results. Setting the link to update On Command will allow you to adjust regions, replay the data, even open a new file, without causing the link to update. Once you have the data on the screen the way you want to database it, selecting Update Remote Links will initiate the update process.

**Arranging the View**

**Scale to Fit**
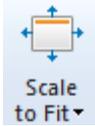


This command is found on the Home tab as well as the View tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the Window menu.

**Scale to Fit** adjusts all data source windows proportionally to fit into the main application window. The command automatically scales the histograms of each data source to fit the new space of the data source, too.

You can click the down-arrow on the button to select Scale to Fit for histogram windows in the current data source.

**Tile**
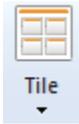


This command is found on the Home tab as well as the View tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the Window menu.

Select **Tile** to fit all data sources in a grid format. Histograms contained in the data sources are automatically rearranged in a tiled format.

You can click the down-arrow on the button to select Scale to Fit for histogram windows in the current data source.

## Vertical
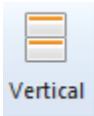


This command is found on the View tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the Window menu.

Select **Vertical** to arrange data source windows vertically, in a single column. Histograms contained in the data sources are automatically rearranged in a tiled format.

**Horizontal**
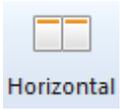


This command is found on the View tab of the ribbon bar.  When WinList is configured to use the [classic toolbar and menu interface](#), the command is found in the Window menu.

Select **Horizontal** to arrange data source windows horizontally, in a single row. Histograms contained in the data sources are automatically rearranged in a tiled format.

**Close All**



This command is found on the View tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the Window menu.

This option performs the same function as the New Protocol command. WinList will close all data source, histogram, MultiGraph, results, and report windows. It also resets other settings to defaults.

**Show All**



This command is found on the View tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the Window menu.

Select **Show All** to display any hidden windows of the selected type and ensure that they are within the viewable area of the screen.

**Hide All**



This command is found on the View tab of the ribbon bar.  When WinList is configured to use the [classic toolbar and menu interface](), the command is found in the Window menu.

Select **Hide All** to hide all windows of the selected type from view. The windows are not destroyed by this command; they are only hidden.

## Next - Previous Data Source
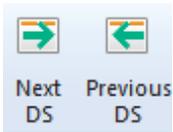
This command is found on the View tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the Window menu.

These commands are useful when you have more than one data source window open.  It allows you to navigate the data source windows easily.

**Printing**

**Print Screen (Ctrl Shift P)**



This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

Print Screen perform a high-speed bitmap screen dump to a graphics printer. The printout will display the visible contents of WinList's window.

Use the Printer Setup option in the File menu to set print options.

**Print Report**



This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.  It is also found on the toolbar for the active Report window.

Use the Printer Setup option in the File menu to set print options.

Several options in the **Preferences** dialog affect the print report option. They appear in the Print Preferences dialog box and control margins, orientation, and more.

**Print MultiGraph (Shift P)**



This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This command draws the current MultiGraph to the printer. The print margins set in Print Preferences are used and a border is placed around the MultiGraph.  The Fast Printing preference controls how MultiGraphs are printed.

If the MultiGraph is in **Tiled** mode and you have specified a fixed number of rows, the page is divided up into columns and rows and each cell will have a fixed size. If there are fewer than *rows X columns* MultiGraph elements, there will be empty space left on the page. If there are greater that *rows X columns* elements, multiple pages will be printed. If Rows is set to zero (0), all elements of the tiled MultiGraph will be scaled to fit on a single page.

All other views of MultiGraphs produce single pages when printed.

Use the Printer Setup option in the File menu to set print options.

**Print Overlay**

Print

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

Choose this command to print the active overlay window to the current printer. The print margins set in Print Preferences are used and a border is placed around the overlay.

Use the Printer Setup option in the File menu to set print options.

**Print Histogram**



This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

Select this option to print the current histogram to the printer. The print margins set in Print Preferences are used and a border is placed around the histogram.

Use the Printer Setup option in the File menu to set print options.

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**Print Data Source**



This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This command prints all of the histograms contained on the current page of the active data source window. The arrangement of histograms is scaled to fit the printed page.

## Print Results



This command is found in the File (Application) menu of the [ribbon bar](#).  When WinList is configured to use the [classic toolbar and menu interface](#), the command is found in the File menu.

Select this option to print the text of the currently selected results window to the printer.

Use the [Printer Setup](#) option in the File menu to set print options.

**Printer Setup**

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

Select this option to display the printer setup dialog box. In the dialog box you may select a printer that you want WinList to use, as well as the orientation you want to use. The specific dialog box will vary somewhat depending on the operating system your computer is running.

WinList will use the selected printer as its default printer until you change this setting, even if you change your default printer in the Windows Control Panel.

## Batch Processing

## Create Batch



This dialog is displayed by choosing the **Batch** button on the Home tab of the ribbon bar.  If you are showing toolbar and menu, it is found in the File menu.  This function is disabled until there is an FCS file open in WinList.

A batch is a group of listmode files that will be processed through a WinList analysis protocol. The Open Batch Files dialog allows you to select the files you want to analyze, and to assign them to different data source windows. After you select files with this dialog, you can navigate through them with Get Next Batch and Get Previous Batch commands, or use the batch toolbar that appears at the bottom of the main window.

If your protocol has more than one data source window, WinList can automatically associate a file with a particular data source. You can group the files into convenient analysis groups, based on the outline level shown in the Batch list. Use **Level >** and **Level <** to change the indentation level of items in the batch list.

The Create Batch command displays 2 dialogs side-by-side: the Edit Batch dialog and the Add Batch Files dialog.
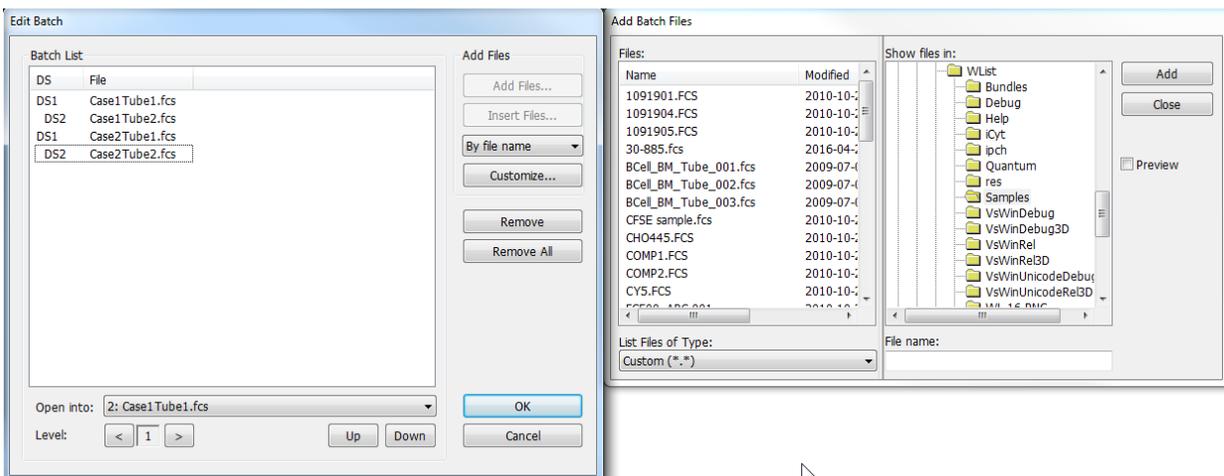


Edit Batch contains the list of all files already added to the batch, and the Add Batch Files dialog is used to add files to the batch. Settings in the Edit Batch dialog determine *how* files will be added. As files are added, they are automatically assigned to a data source based on rules that you set up in the Edit Batch dialog.

**Simple Batch**

In the simplest case, when you add files to the batch they are sorted based on **File Name** or **File Date**. If your analysis setup has one data source window, all of the selected files will be read into that data source. With more than one data source window, files are assigned sequentially to the data source windows in the analysis setup.

**More Sophisticated Batch**

You can also select and assign files based on *keywords* that are contained in them. FCS files use keywords to contain information about the sample, the case, the tube in the panel, and many other useful properties. Any keyword in your files can be used to select files and assign them to the appropriate data source window.

For example, suppose you have a 3-tube panel, and that your files include keywords "SAMPLE ID" to identify the case and "TUBE NAME" to identify which tube in the panel the file represents. You set up three data source windows in WinList, one for each tube in the panel. Then you can tell the Edit Batch dialog that you want to select files **By Keywords**, and click the **Customize** button to select the keywords to use.
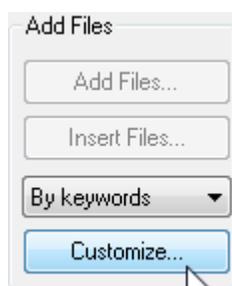


In the **Customize Add by Keywords** dialog, you would select "SAMPLE ID" for the keyword that identifies a group of files (a panel), and "TUBE NAME" as the keywords that identifies a file within the group. You would select "Assign each file to the associated data source window", and then choose or enter the keyword values for "TUBE_NAME" that relate to each data source. Here's an example of that set up:

By taking the time initially to set up file selection **By Keywords**, you can save time and reduce selection errors during your day-to-day analysis.

## Controls in the dialog
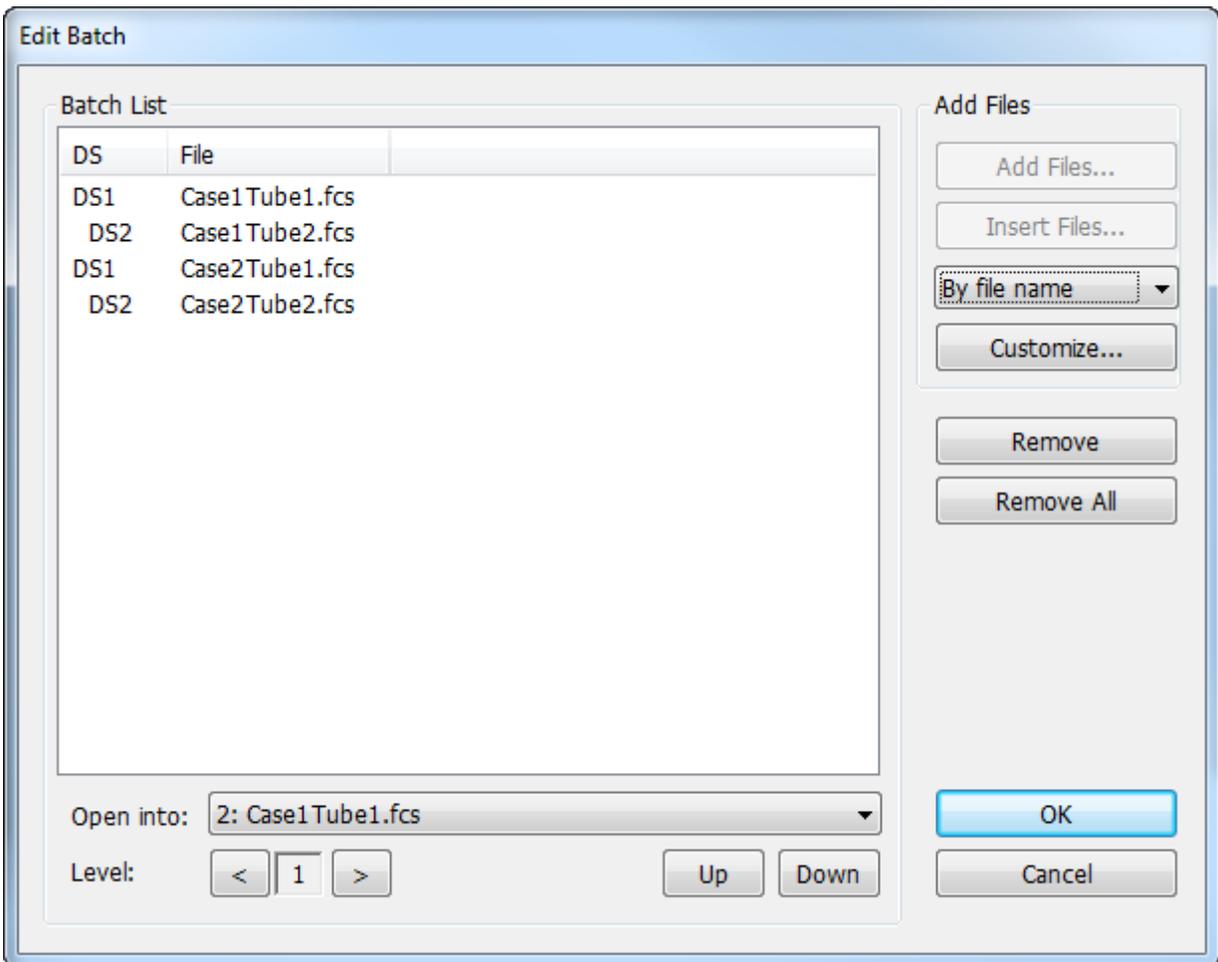


### Batch List

This shows the current list of files and data source to which each will be assigned.

To change the target data source, select one or more items in the list and change the selection in the **Open into** dropdown control.

To change the indentation level, select one or more items and use the **<** and **>** buttons.

To remove items from the list, highlight the items and click **Remove**.

### Open into

This drop-down control lists the current data source names. Changing the selection will change the data source for the items selected in the Batch List.

### Level >

This option increases the level of all selected batch files by one.

**Level <**

This option decreases the level of all selected batch files by one.

**Up & Down**

Select one or more items and click **Up** or **Down** to change the order of items in the Batch List.

**Add Files**
**Insert Files**

These buttons are disabled when the **Add Batch Files** dialog is displayed. If you close the Add Batch Files dialog, use **Add Files** or **Insert Files** buttons to display the dialog and select additional files.

**OK**

Click this button to save the **Batch List** entries close the dialog.

**Cancel**

Click this button to cancel changes in the dialog.

**Batch toolbar**

This toolbar appears when more than one listmode file is selected in the Open dialog. It provides a simple tool for navigating through the batch of files.



As you navigate through the batch, the actions and options in the [Edit Batch Options dialog](#) are performed on each file that is opened. This allows you to perform a series of automatic operations just by moving to another batch item, such as acquiring all events, running a macro, or printing. The function of each button is described below.

|  |  |
|---|---|
| ✏ | Display the [Edit Batch Options](#) dialog. |
| 📂 | Add files to the batch. |
| ◁◁ | Go to the first file in the batch. |
| ◁ | Go to the previous file in the batch. |
| ↻ | Redo the current file in the batch. |
| ▷ | Go to the next file in the batch. |
| ▷▷ | Go to the last file in the batch. |
| ◁▷ | Select a specific batch item to open. |
| ≫ | Auto-advance through the batch. |
| ■ | Stop the auto-advance process. |
| ❚❚ | Pause/continue batch processing. |

**Edit Batch Options**

This dialog can be accessed from the batch toolbar by clicking the left-most button. It is also found by clicking the down-arrow on the Batch button on the Home tab of the ribbon bar.  It controls the actions that are performed as each file in the batch is read.



**Batch files**

This option displays the Open Batch Files dialog to select or modify files for the batch.

**Batch options**

This option selects either **Basic** or **Custom Scripts** mode for batch processing.

In Basic mode, processing options are selected using the checkboxes and other controls shown in this dialog.

In Custom Scripts mode, you can create custom scripts of actions for each "level" in the batch list outline shown in the Open Batch Files dialog. Custom scripts can perform all of the same operations as Basic mode processing, as well as additional commands. You can also set the order of operation and include things like user prompts with Custom Scripts. See Edit Scripts for details.

**Custom scripts**

In Custom Script mode, use the **Edit** button to view and edit the custom scripts you have created for your batch.

**Replay Options**

**Replay on Open**

When enabled, the FCS file will be replayed as soon as it is opened. Replaying a file allows the statistics and graphics to represent the entire data file.

**Replay on Advance**

This dropdown contains several choices related to replaying the batch processor is about to move to the next file in the batch.

**No**: No replay action is taken when a new batch file is opened.

**Replay active data source**: WinList replays the active data source and computes statistics on the full listmode file. This option also triggers any active databases to store a new record.

**Replay all data sources**: WinList replays all data sources and computes statistics on the full listmode file. This option also triggers any active databases to store a new record.

**Macro**

**Run macro**

Enable this option if you want to run a WinList macro after each batch file is opened. If you want to perform detailed tasks that are not directly supported with options in this dialog, use the Run macro and Macro name fields to perform a custom task.

**Update remote links**

When this option is enabled, WinList will update all Remote connections for Result and Histogram windows.

**Macro name**

When the Run macro option is enabled, this option allows you to select the macro that you want to run. Click the button, navigate to the folder containing the macro, and select the macro.

**Protocol Save**

**Save protocol**

There are several options for saving protocols: **Don't save protocol**, **Save XML protocol**, and **Save protocol bundle**. When one of the saving options is selected, the other properties in the Protocol Save section also need to be set.

**Save protocol in data path**

Enable this option to store the protocol in the same path as the data file that is being analyzed. When not enabled, the protocol is saved in the path specified by Protocol Path.

**Confirm protocol names**

Enable this option to be prompted to confirm the protocol name before it is saved by the batch processor.

**Protocol name prefix**

This field contains text that is used for naming protocols. It can contain a mix of text and keywords; keywords are enclosed in square brackets. The default name prefix is "Protocol_[BATCHINDEX]", which will name a protocol based on the index of the file in the batch list.

**Protocol path**

Select a path for storing protocols. This path is ignored if **Save protocol in data path** is enabled.

**Reports**

**Save reports**

Enable this option to save reports after each batch file is loaded. Reports will be automatically named based on the Report name prefix below.

**Save reports in data path**

Enable this option to store the reports in the same path as the data file that is being analyzed. When not enabled, the report is saved in the path specified by Report Path.

**Confirm report names**

Enable this option to confirm and/or edit report names prior to saving.

### Report name prefix

Enter text to use as the first part of each report name. This can contain internal and file keywords by enclosing the keyword in square brackets. For example, to name the reports based on FCS file name for the active data source, you might use Report_[FILE], excluding the quotation marks. See Internal Keywords for other keywords that can be used for this purpose.

### Report path

Choose the location in which to save reports. This path is ignored if **Save reports in data path** is enabled.

### PDF Export

### Export PDFs

Enable this option to save reports in PDF format after each batch file is loaded. Reports will be automatically named based on the PDF name prefix below.

### Save PDF in data path

Enable this option to store the PDF reports in the same path as the data file that is being analyzed. When not enabled, the PDF is saved in the path specified by PDF Path.

### Confirm PDF names

Enable this option to confirm and/or edit PDF file names prior to saving.

### PDF name prefix

Enter text to use as the first part of each PDF report name. This can contain internal and file keywords by enclosing the keyword in square brackets. The default name prefix is Report_[BATCHINDEX]″, which will name PDF based on the index of the file in the batch list.

### PDF path

Choose the location in which to save PDF reports. This path is ignored if **Save PDF in data path** is enabled.

### Printing

### Print report

### Print screen

### Print MultiGraphs

When selected, WinList performs the print operation after each file is opened.

### Always show toolbar

Normally, WinList hides the batch toolbar when the batch list is empty. Enable this option to always show the batch toolbar.

**Save Preferences**

If this option is enabled, the choices made in the dialog are stored as preferences.

**Edit Scripts**

This dialog is accessed from the Edit Batch Options dialog when you choose Custom Scripts. It allows the batch processing scripts to be customized.
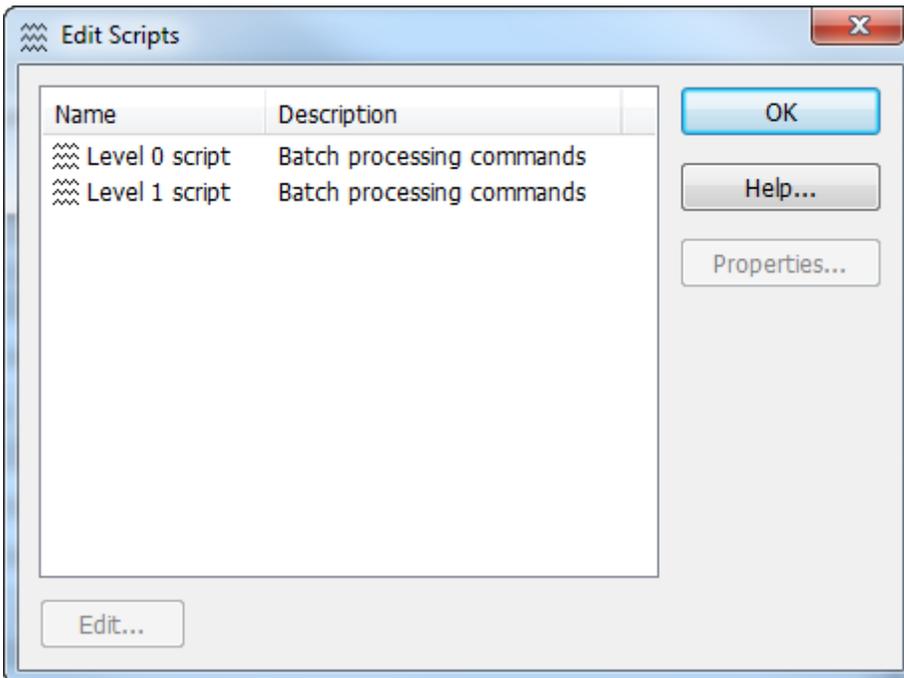


WinList automatically creates a batch processing script for each level in the outline of files in the batch file list. Levels are used to differentiate how you want to process files. For example, you can have files on Level 1 simply read in and update the results, while files on Level 2 will cause reports to be saved and printed.

To edit a script for a level, select it in the list and click **Edit**.

Use the **Open** and **Save** buttons to save collections of customized scripts so that you can reuse them in other protocols. Scripts are also saved as part of the protocol and protocol bundle file formats.

**See also:**

Edit Script

Edit Batch Options

**Edit Script**

This dialog allows you to edit the commands for a batch processing script. There is one batch processing script for each level in the outline of files in the batch file list.
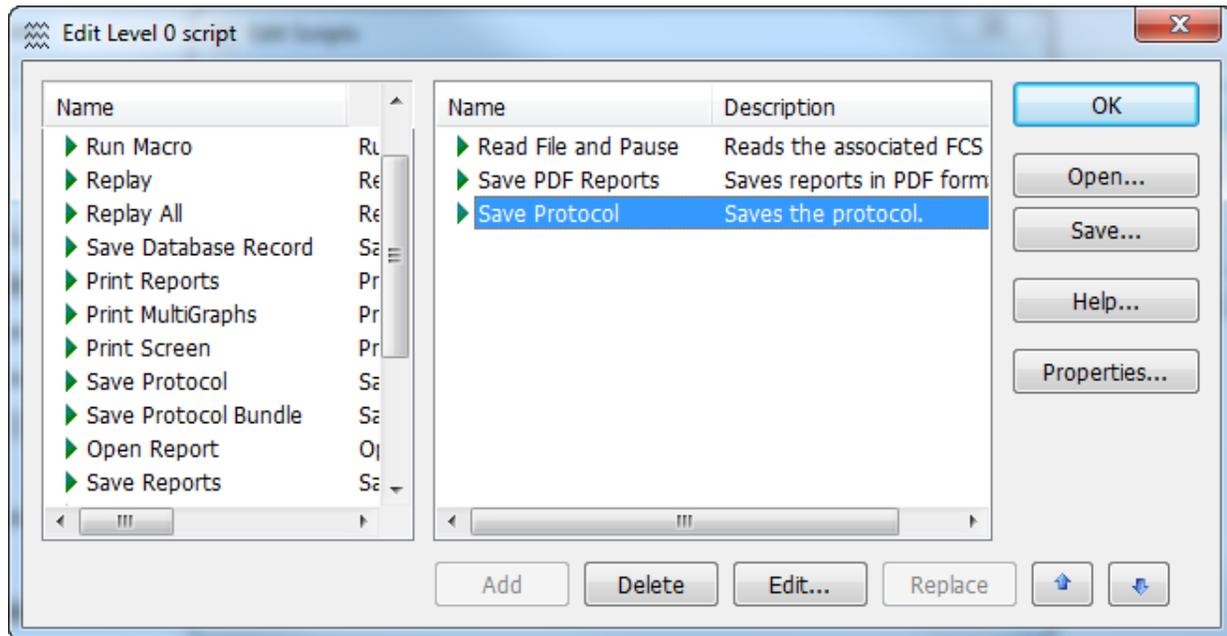


The left listbox contains a list of all commands that are available. The list on the right side contains the commands that the script will execute.

To add a command to a script, select the command in the list on the left and click **Add**.

To replace a command, select the new command in the list on the left and the command to replace in the list on the right, then click **Replace**.

To change the order of a command in the script, select the command and use the ⬆ and ⬇ buttons.

To edit the properties for a command in the script, select the command and click **Edit** or **Properties**. The Edit Batch Command dialog will be displayed.

**See also:**

Edit Batch Command

Edit Scripts

Edit Batch Options

**Edit Batch Command**

This dialog allows you to edit the properties for a batch command.
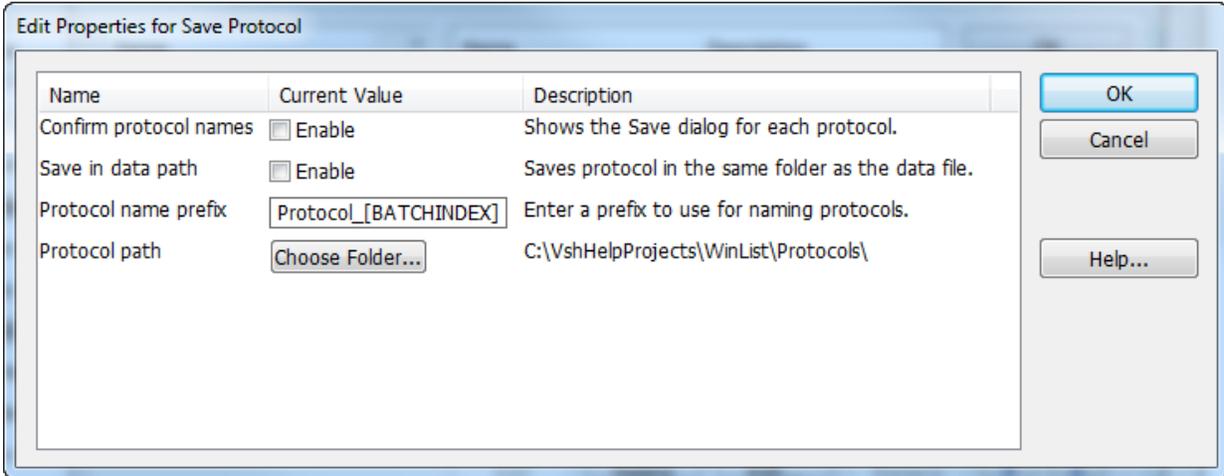


Each batch command has its own unique properties. For example, commands that relate to saving files typically have properties that allow you to set the path and file name, and commands that pause have properties that allow you to specify a prompt to display to the user.

To edit the properties for a batch command, double-click the command in the Edit Script dialog, or select the item and click the **Properties** button.

**See also:**

Edit Script

Edit Scripts

Edit Batch Options

**Next Batch Files (Ctrl B)**

**Note:** This function is disabled until there is a data source open in WinList.

**Note:** The batch toolbar provides another method for moving through batch files. It automatically appears at the bottom of the main window when more than one file is in the batch.

The **Next Batch Files** menu option will open one group of FCS files from the batch list into the data sources. A group of files is defined by the outline **Level** in the **Edit Batch** dialog. Typically, a group represents a panel of files for an analysis setup.

The option **Previous Batch Files** backs up one group of files in the batch list.

**Previous Batch Files (Shift B)**

**Note:** This function is disabled until there is a data source open in WinList.

**Note:** The batch toolbar provides another method for moving through batch files. It automatically appears at the bottom of the main window when more than one file is in the batch.

This option backs up one group of files in the batch list. It is the converse of Next Batch Files.

**Databases**

**Databasing with WinList**

This section introduces the concepts and functions needed to work with WinList's database features. It is more of a discussion than a tutorial, however there are examples to clarify some aspects of the system.

WinList has a simple, yet powerful database engine that allows you to store large amounts of data easily in one or more database files. You can view databases from within the program, manually add, delete, and replace records, and select the fields you want to store in each database. The databases are created in a simple, text format known as tab-delimited ASCII text, which can be read by common database and spreadsheet programs. These features give you a great deal of flexibility in choosing what information to store, where to store it, and how to integrate the information into other database systems.

**How do you create a database?**

Set up WinList with a protocol before you create or edit a database. This includes opening one or more data source files, selecting the histograms you want to work with, creating regions and gates, and displaying results. You can set up a separate database for each protocol you use, one database for everything you do with the program, or anything in between.

*It's important to have an FCS file open and regions defined before you create a database. The information available to the database depends on what you have set up on the screen.*



Databases are accessible from the **Edit** tab on the ribbon bar. Click the **Setup** button in the Databases group to display the Edit All Databases dialog.
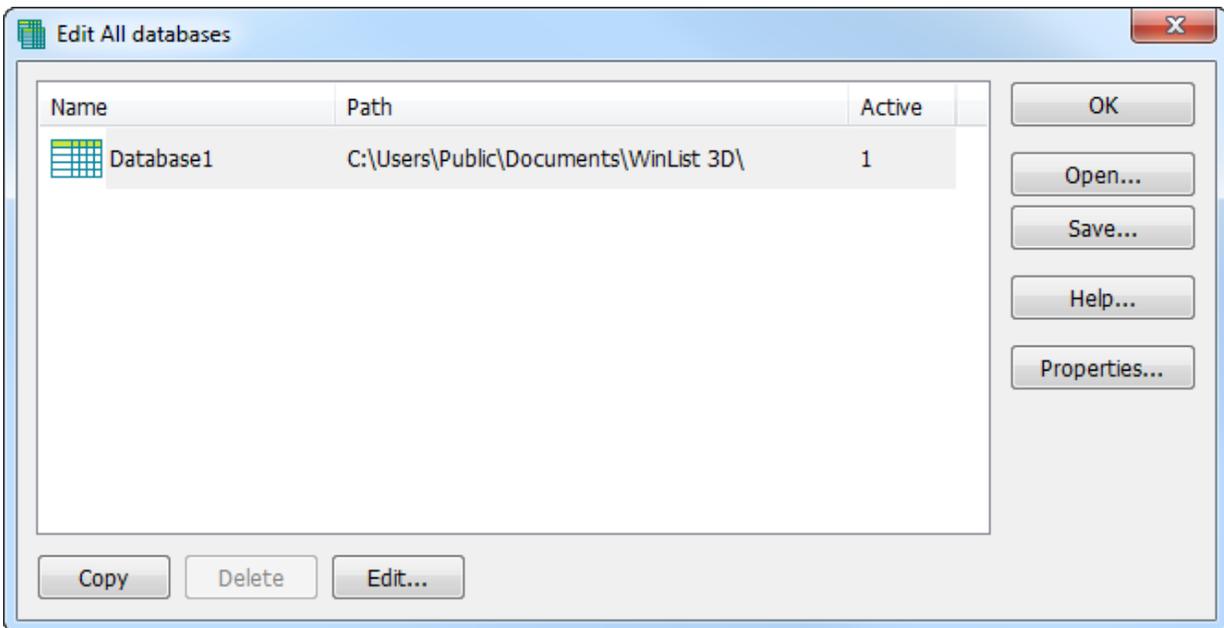
There is one database, "Database1", created by the program automatically. This database will not store anything until you tell the program what information you want to save. To do so, you need to select the database in the list and click the Edit button. You can also double-click on the item in the list to do this.
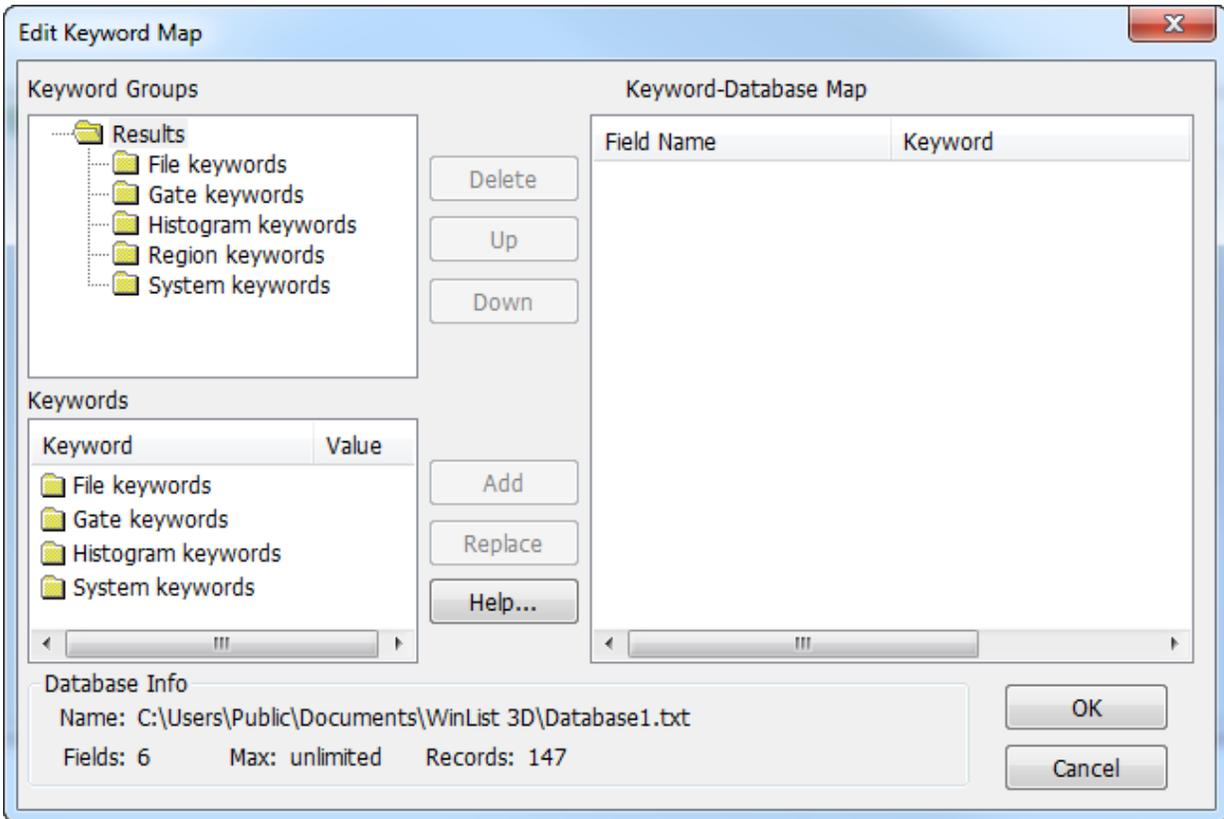
The Edit Keyword Map dialog is displayed. The left side of this dialog presents the information available to store, and the database fields already added are shown on the right. The two controls on the left are the Keyword Groups tree and the Keywords list. The right-hand list is the Keyword-Database Map, showing the fields you will store in the database. Initially, the right-hand list is empty.

## What can you database?

WinList allows you to database virtually any statistic it generates, as well as keyword information from the files that you analyze. The tree in the top left of the dialog can be expanded to display branches of keywords. To open a branch of the tree, double-click on it with the mouse.

> *Sometimes it's easier to create a database directly from Results windows that you have already created.  The Database Wizard makes that an easy task by automatically adding fields for the selected Results window.*

As you select a branch in the tree, the list below the tree will fill with the objects beneath the branch. For example, when you double-click to open the Region keywords branch, the Keywords list below the tree will fill with a folder for each region that has been created.

These also appear in the tree because they are branches that can be expanded further. File keywords come from the FCS keywords for each data source; System keywords are items like current date, and current time.
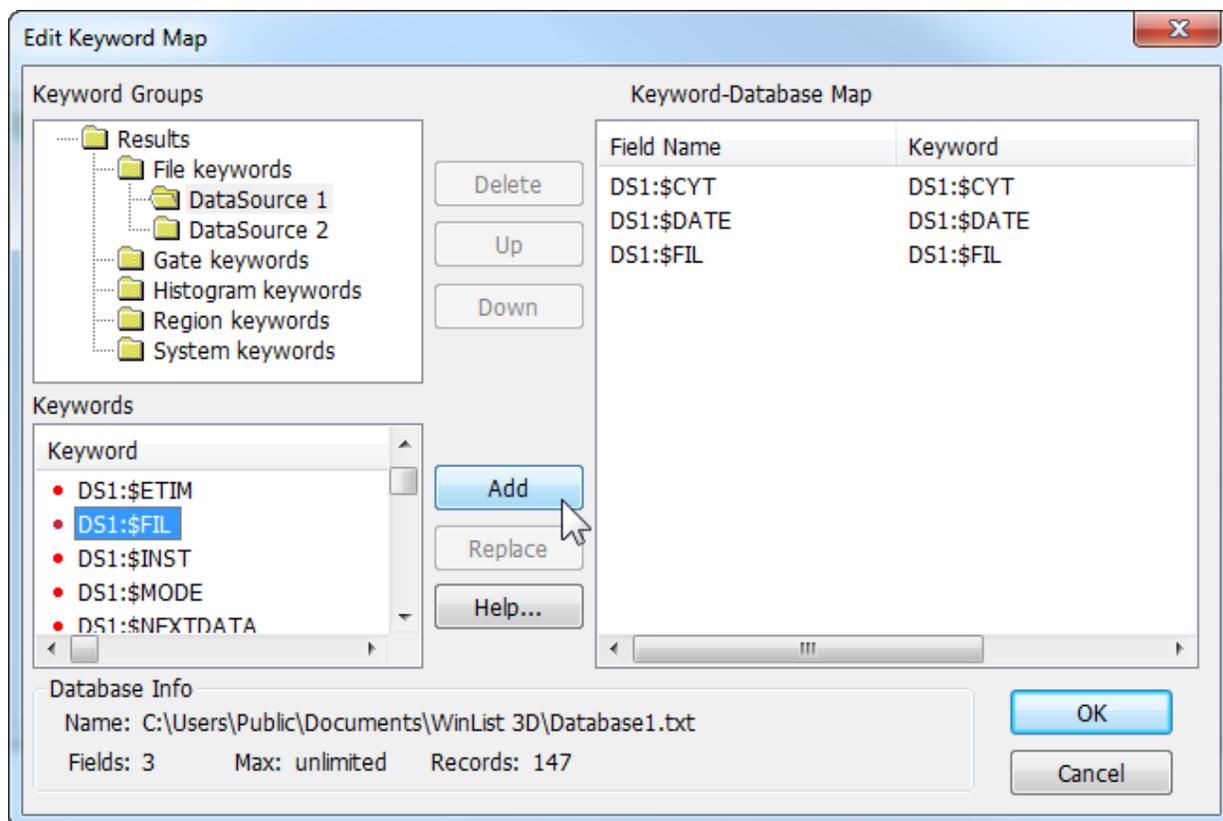


When you see an item in the Keywords list that you want to database, select it and click the Add button. One or more fields will be created, depending on what you have selected to add. Here is the important thing to understand about adding items to the database: *everything contained in the selected item will be added to the database.* This means that if you select the File keywords, the Region keywords, and the System keywords list items and click Add, *everything* that WinList can store for the current Protocol will be added to the database.

> *There is a downside to this simplicity. It is very easy to create a database with much more information than you want or need. In fact, you can create a database that other programs will not read because it has too many fields.*

In most cases, you probably want to store most of the Region keywords, but *rarely* would you want to store all of the File keywords. File keywords come from the files you are analyzing, and many of them are meaningless to anything except the program that created the files. For example, storing how many bytes each parameter for each event uses in the listmode file is not useful information, in most cases. On the other hand, keywords that identify the sample, experiment, and file name will be useful to database.

Another reason you would not want to store all the File keywords is the limitation that most database and spreadsheet programs impose. Other programs usually will not read a database with more than 255 fields of data. If, for example, your files contained 100 keywords and you set up a protocol with 10 data sources open at once, there would be 1000 keywords available to you in the File keywords branch. Reading such a database is well beyond the capabilities of most other programs. Since it is likely that you *will* want to open WinList databases with other software, avoid creating databases with more than 255 fields.

A better solution, if you need to store more information, is to break the storage into several smaller databases. This is possible because WinList can work with any number of database files simultaneously.

The field names initially assigned when you click the Add button are designed to help you know what keyword has been added. For example, all of the keywords relating to Region R1 are prefixed with "R1:", so you can differentiate "MeanX" for regions R1 and R2 easily.

File keywords are prefixed with the data source id.

### Editing fields

You can edit the names for the fields after they are added to the Keyword-Database Map list, if you wish. Click once on the field name to select it. After a brief pause, click slowly again. An edit control will appear around the field name, allowing you to type a new name for the field.

To remove one or more fields from the database, select the fields in the Keyword-Database Map and click the Delete button.

To replace an existing field with a new keyword, select the new field in the Keywords list and the one to replace in the Keyword-Database Map list. Then, click the Replace button. The name of the field will not change, only the keyword used to evaluate the field.

If you need assistance understanding the dialog, click the Help button to display the on-line help.

When you have finished selecting information to store in the database, click the OK button to close the Edit Keyword Map dialog.

### What files are created?

When you select keywords to store in a database, WinList creates three or four files on disk to manage the database. The files are created in the WinList application folder by default. However, you can choose another location by editing the database's properties, discussed below.

First, a file is created to store a "map" of keywords to database fields. The map file name uses the name of the database object and appends ".map" to it. For "Database1", the map file is named "Database1.map".

Next, the program creates or revises the actual database file. This is the tab-delimited, ASCII text file that you may open in other database or spreadsheet programs. The database file name uses the database object name and appends ".txt" to it. This file is readable in virtually

any program that can open unformatted text. The first line of the file contains the names of the fields separated by tabs; it is known as the database header or definition. Each subsequent line contains a record of information.

> *If the database already exists on disk, WinList will automatically create a backup of the database before overwriting the existing one. The backup file will have the same name as the database, with " backup" appended to it.*

Finally, the program creates a text file called a "key" file. This is a text file that explains the database and mapping files. You can open this file in a text editor to review the structure of the database you have created. Its name is based on the database object name, with "key.txt" appended to it.

### How do you activate the database?

Once a database has been defined, there are several additional properties you can specify. These properties determine whether the database is active, whether it will prompt you before saving a record, whether or not you want to see the records in a database viewer, and where you want to store the database.

To edit these properties for a database, select the database in the Edit All Databases dialog box and click the Properties button. The Edit Properties for Database dialog will be displayed.
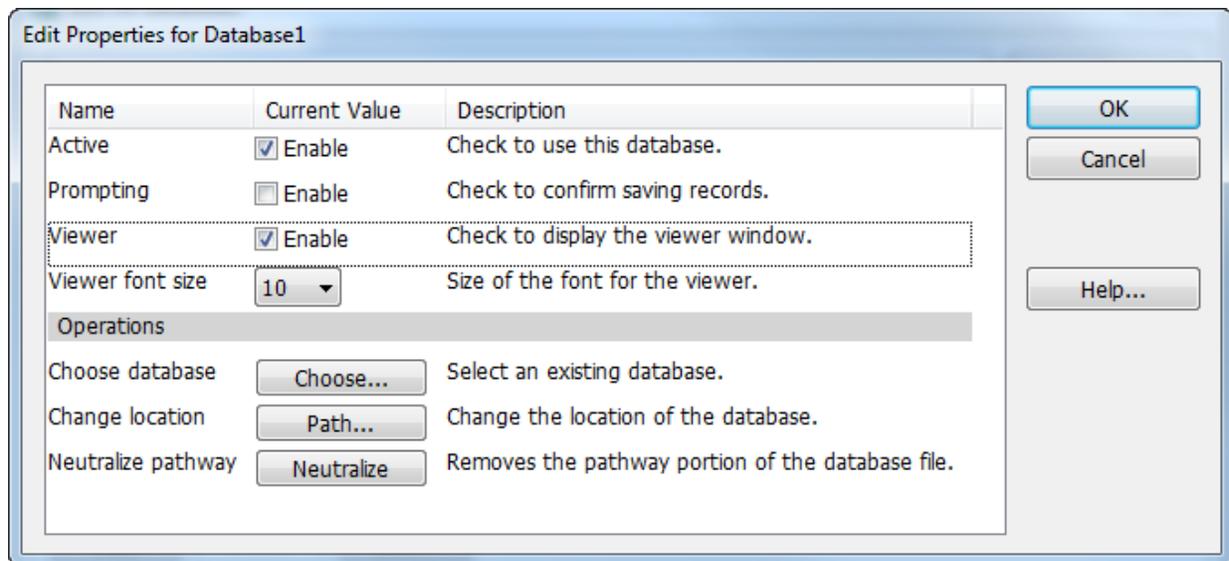


The properties are enabled or disabled with simple check boxes. Path selection is initiated by clicking the Database Path Folder button.

In order to store records of information in a database, it must be "active". To make a database active, check the Active checkbox in the Edit Properties dialog. Database records are saved when you click the Replay button on the ribbon bar, or when you click the Replay

options in the Replay menu. Additionally, you can save a record at any time by choosing Save Current Results from the Edit menu, or by clicking the "**+**" button on the database viewer.

If the Prompting checkbox is checked, WinList will display a message each time it is about to save a record to the database. You can choose at that time whether or not you want to save the record. If Prompting is disabled, the database will save a record without prompting.

To view the database while you work with WinList, check the Viewer checkbox in the Edit Properties for Database dialog. A database viewer window will be created and displayed. The database viewer can be moved, resized, and scrolled horizontally or vertically. It has a toolbar that provides some additional manual database functions. See the topic Database Viewer for more information.


**How do you rename or create additional databases?**

It is useful to rename a database so that its name relates to its purpose. For example, if you have an activation study for which you have created a WinList protocol called "Activation", it makes sense to name the database "Activation" as well.

To rename a database, display the Edit All Database dialog box by selecting Edit Databases from the Edit menu. Click once to select the database you want to rename. After a brief pause, click again on the object to display an edit box in the Name column. Type the new name for the database.

The name you type must contain only alphabetic, numeric, or space characters, and must be at least 4 characters shorter than the maximum file name length for the operating system you are using. If a file with the new name already exists, you will be given a chance to overwrite the existing file, use the existing file, or cancel the operation.

When you want to create an additional database, select the first database in the Edit All Databases dialog and click the Copy button. This will create an identical database to the one copied, but will give the database a new name.

Each database has its own map of keywords to fields, and its properties determine whether it is active, prompting, and showing a viewer.


**Making the databases open automatically when WinList starts**

If you would like to open the database setup each time the program starts, enable the Auto-open check box.

☑ Auto-open
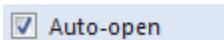

**Links:**

Edit Databases

Edit Keyword Map

Edit Properties for All Databases

Edit Properties for Database

### Edit Databases

This dialog allows you to add, delete or edit one or more databases.

The Edit Databases dialog can be displayed by choosing **Setup** on the **Edit** tab of the ribbon bar.  If you are using the toolbar interface, it is found in the Edit menu.
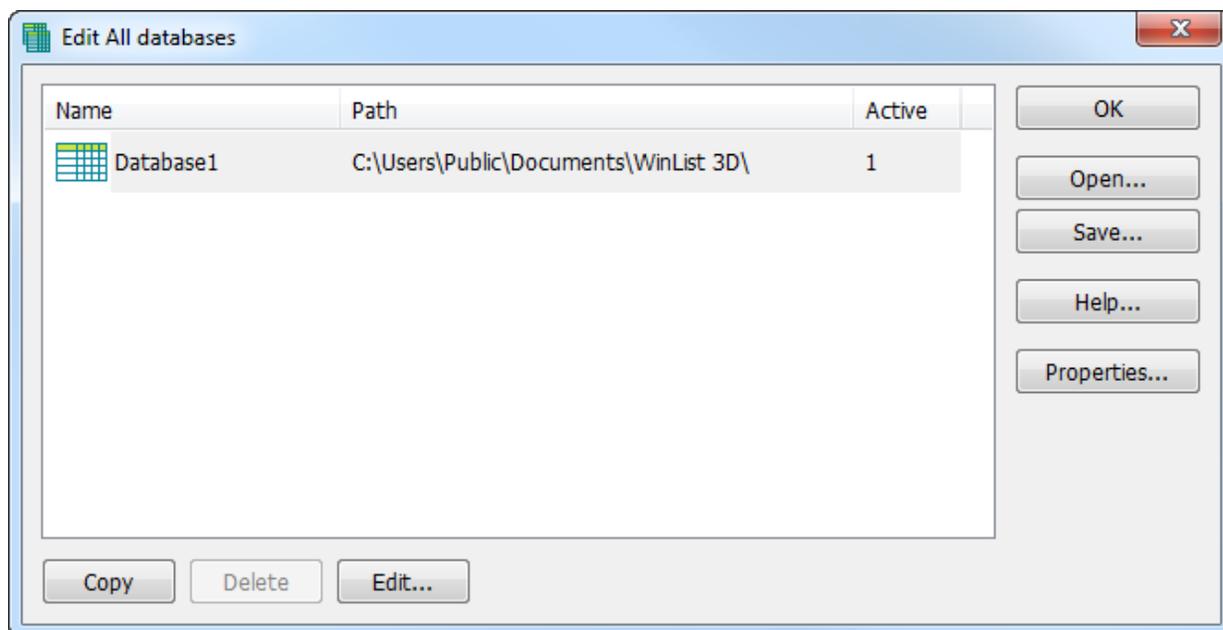


#### Overview

WinList can have one or more databases defined at any time. The database format is ASCII-formatted, tab-delimited database. Any FCS keyword or analysis result can be mapped to a database field. A complete description of the functionality of the database system can be found in the Databasing with WinList tutorial.

#### Edit

If a Database is selected, this option shows the Edit Keyword Map dialog box. You can also double-click on the Database item to activate this option.

#### Copy

If a Database is selected, this option creates a new Database. You may edit the name of the database by clicking on the name, and after a brief pause, clicking again. The name will then appear in an edit box.

#### Delete

If any Databases, other than the first one, are selected, this option will delete the Databases.

**Open**

Opens a file containing a set of database definitions previously saved.

**Save**

Save the database definitions to a disk file.

**Properties**

If no Database is selected, clicking on Properties will show the Edit Properties for All Databases dialog box. If a Database is selected, clicking on Properties will show the Edit Properties for Database dialog box.

**Help**

Show this help topic.

**OK**

Put away the Edit All Databases dialog box.

**Edit Properties for All Databases**

Choosing Edit Databases from the Edit menu, then clicking the Properties button can display the Edit Properties for All Databases dialog. Note: Make sure nothing is selected in the Edit All Databases list when clicking the Properties button.
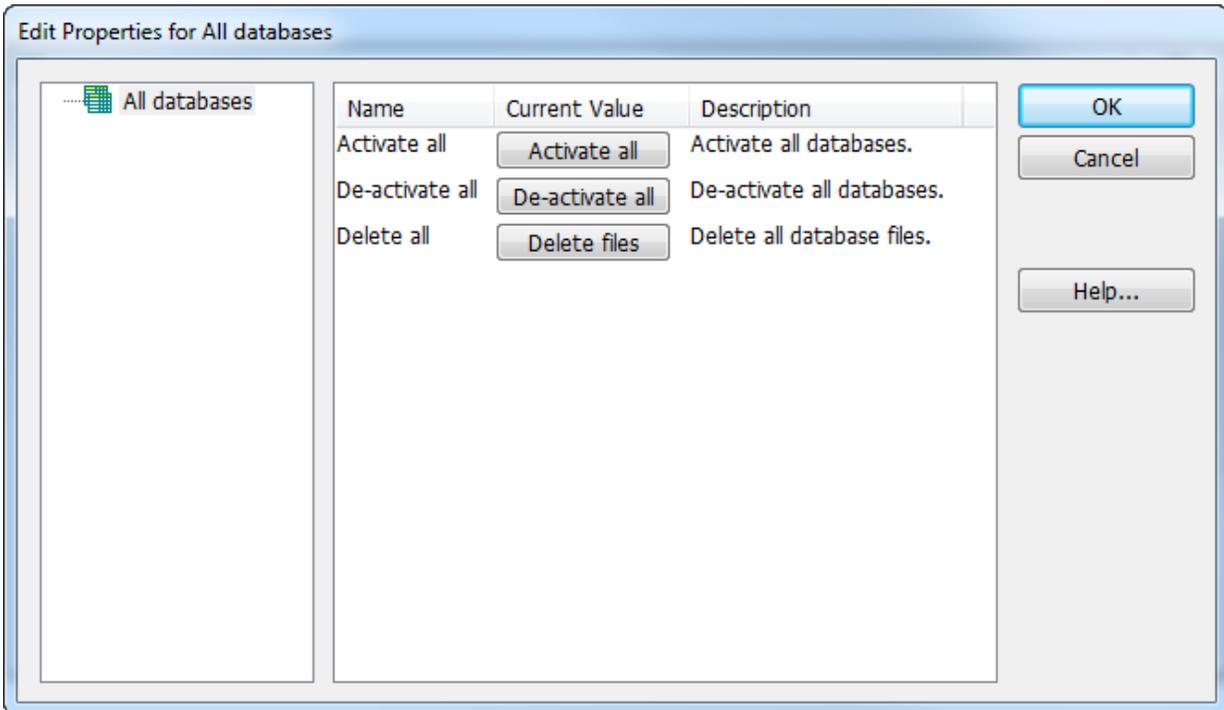


**Overview**

The dialog is displayed when the Properties button is clicked in the Edit All Databases dialog, *and* no databases are selected in the list. It allows you to perform action on all of the defined databases at once.

**Activate all**

Activates all databases. When a database is active, it can store records of information as you analyze files in WinList.

**De-activate all**

Disables all databases. No database will store information until it is reactivated.

**Delete files**

Removes all database files on disks for databases in the Edit All Databases dialog. This is a convenient file management button, but should be used with great caution. Make sure you have adequate backup copies of important databases before using this option.

**OK**

Close the dialog and accept changes.

**Cancel**

Since the options in this dialog box are immediate, Cancel is the same as clicking OK.

**Help**

Show this help topic.

### Edit Properties for Database

### Purpose

To activate, display, and set other attributes of a database.

The Edit Properties for Database dialog can be displayed by choosing **Edit Databases** from the **Edit** menu, selecting the database you want, then clicking the **Properties** button. If the Database Viewer is displayed, clicking a button on the viewer's toolbar can also access this dialog.
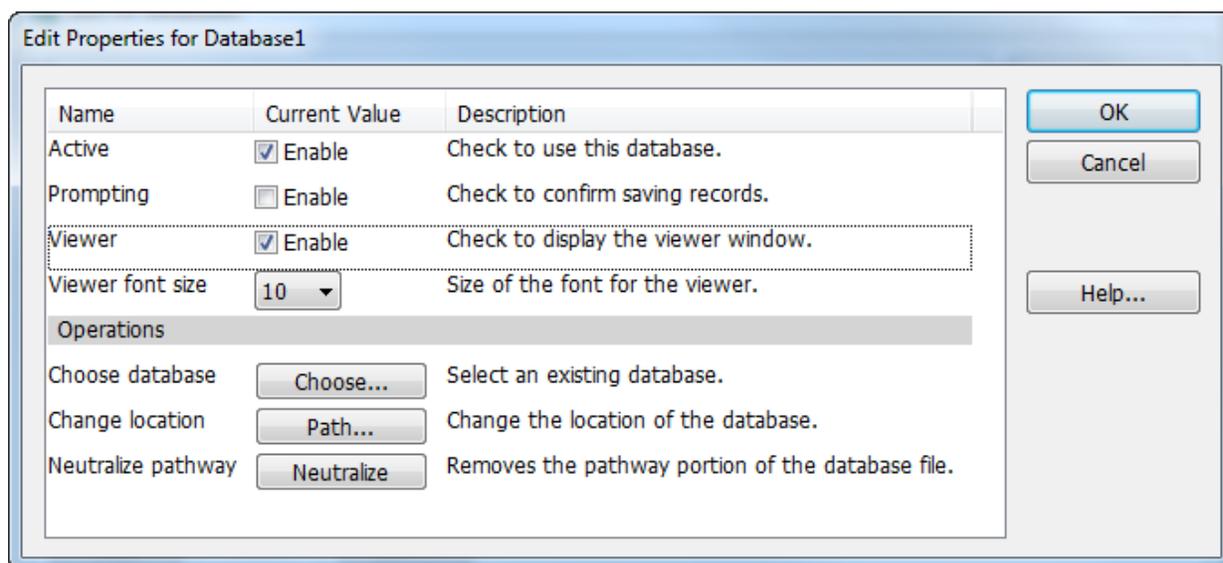


### Overview

Each database can be activated independently in WinList. Additionally, the database can be set for prompting or automatic mode, and a viewer window can be displayed. This dialog also allows you to set the path for the database.

### Active

Check this option to activate the database. When the database is active, it will attempt to store a record each time an analysis is performed. Uncheck this option to disable a database; records will not be saved in databases that are not active.

### Prompting

Check this option if you want the program to prompt you each time it is ready to save a record. The prompt will allow you to confirm or cancel the saving of the record into each active database. Uncheck this option to store the information without prompting.

### Viewer

Check this option to display a [Database Viewer](#) for the database. The viewer displays the fields in columns and each row represents a record in the database. Additional options are available on the viewer window.

### Viewer font size

Choose a font size to use in the database viewer. Choose a larger font to make the text easier to read, or a smaller font to view more information in the same space.

### Choose database

This option allows you to choose an existing database on disk. Click the "Link to" button. A file selection dialog box will appear, allowing you to navigate to the existing database and select it. Linking to an existing database will rename the database in memory to match the one selected on disk, and the associated database map on disk will be loaded as well.

### Change location

This option allows you to change the path for the defined database. Click the "Path" button to display a Choose database location dialog. Navigate to the location in which you want to store the database.

If a database of the same name already exists in that folder, you will be offered three choices. First, you can choose to overwrite the database with the one you have defined. Secondly, you can choose to use the database that exists in the new location, replacing the structure you have defined in memory. This is convenient when you have a database already defined that you want to use. Finally, you can choose to cancel the action.

### Neutralize pathway

This option is useful when you need to move a database to another installation of WinList. When the button is clicked, WinList copies the database to the application folder. It then empties the internal variable that stores the pathway to the database. This allows the program to open the database from the application folder on any installation.

### OK

Save property settings and put away the Edit Properties for Database dialog box.

### Cancel

Close the dialog and discard any changes you have made.

### Help

Show this help topic.

**Edit Keyword Map**

Purpose: Select the information you want to save in a database. See Databasing with WinList for more on databases.

The Edit Keyword Map dialog can be displayed by choosing **Setup** from the **Edit** tab on the ribbon bar, selecting the database you want to edit, then clicking the **Edit** button. If the Database Viewer is displayed, clicking a button on the viewer's toolbar can also access this dialog.
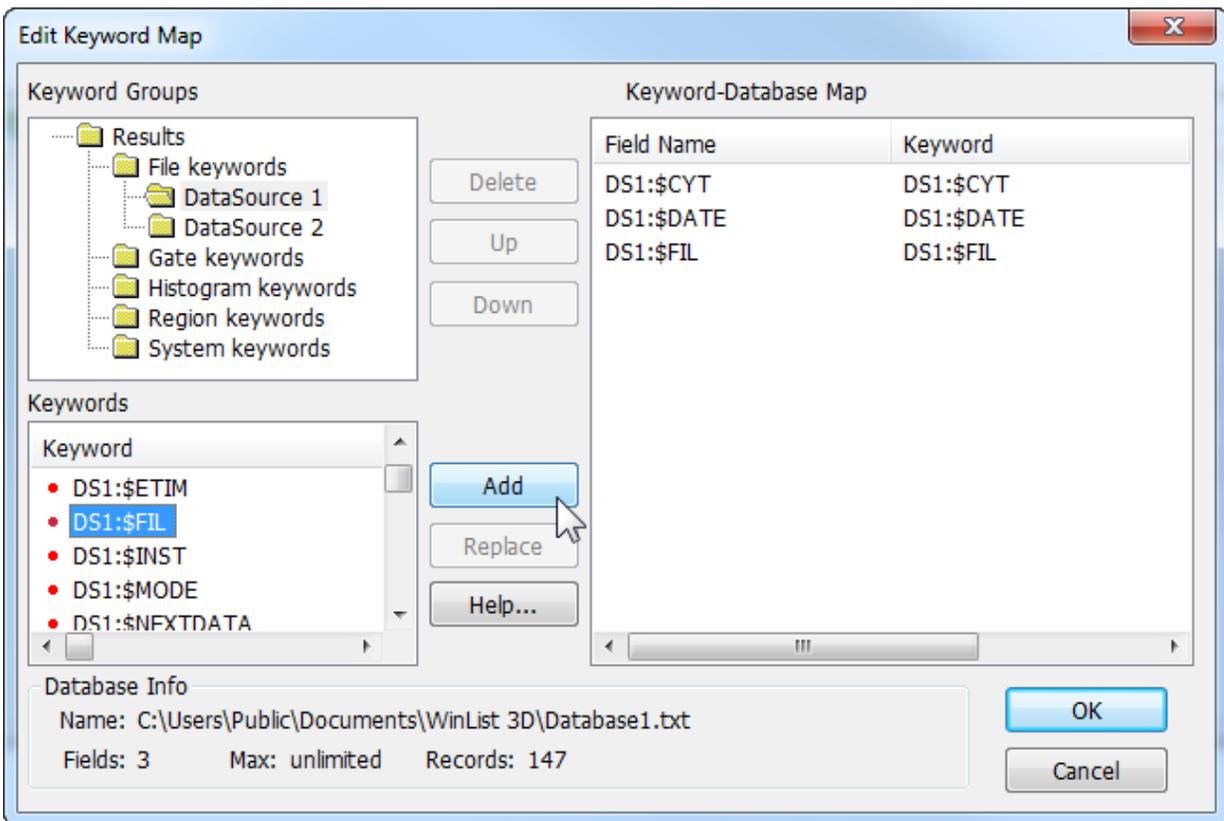


**Overview**

Each database in WinList has its own structure, made up of a set of keywords to database and a matching set of fields in a database. The Edit Keyword Map dialog controls how the keywords are "mapped" to fields in the database.

**Keyword Groups**

This tree expands to display all of the available groups of keywords. A branch of the tree can be expanded by double-clicking the mouse on it. Select a branch of the tree by clicking once on it. Elements contained in the selected branch are displayed in the Keywords list below the tree.

### Keywords

This list is used to select the keywords you want to store in the database. It shows elements contained in the selected branch of the Keywords Groups tree above. The list allows multiple selections. Two types of elements are displayed in the Keywords list: keywords and folders. When you select one or more keywords in the list and click Add, the selected keywords will be added to the database. When you select one or more folders in the list and click the Add button, *all of the branches and keywords beneath those folders in the tree are added to the database*. It is very important to understand the practical limitations of databasing before adding folders of keywords to the database. See the discussion in Databasing with WinList for more on proper selection of information to database.

### Keyword-Database Map

This list displays the fields, keywords, and keyword paths currently in the database. This list allows multiple selections. The first column showing the field name can be edited; other columns are for display only. To edit a field name, click once on the field to select it, then click again after a moment to enter edit mode.

### Database Info

This section displays the file name of the database, the number of fields defined, the limit for number of fields, and the number of records currently stored in the database.

### Delete

Click this button to delete the fields selected in the Keyword-Database Map list from the database. The button will be grayed if no fields are selected in the list.

### Up/Down

Use these buttons to change the order of fields in the Keyword-Database Map list. The Up button moves the selected items toward the beginning of the record definition, and the Down button moves them toward the end. The buttons will be grayed if no fields are selected in the list.

### Add

Click this button to add the keywords and folders selected in the Keywords list to the database definition shown in the Keyword-Database Map list. Fields are named automatically to show the source of the information. You can also double-click on a keyword to add it to the definition.

### Replace

The option is available when you select an item in the Keywords list and an item in the Keyword-Database Map list. It allows you to map a new keyword to an existing field in the database.

**OK**

Save changes to the database. If records already exist in the database, the structure of the records will be revised to match the new structure you have defined.

**Cancel**

Close the dialog and discard any changes you have made.

**Help**

Show this help topic.

**Database Viewer**

Purpose: Display the contents of a database.

The Database Viewer can be displayed by choosing to show the viewer in the database properties. To do so, choose **Edit Databases** from the **Edit** menu, select the database you want, and then click the **Properties** button. In the Edit Properties for Databasen dialog, enable the viewer and click OK.
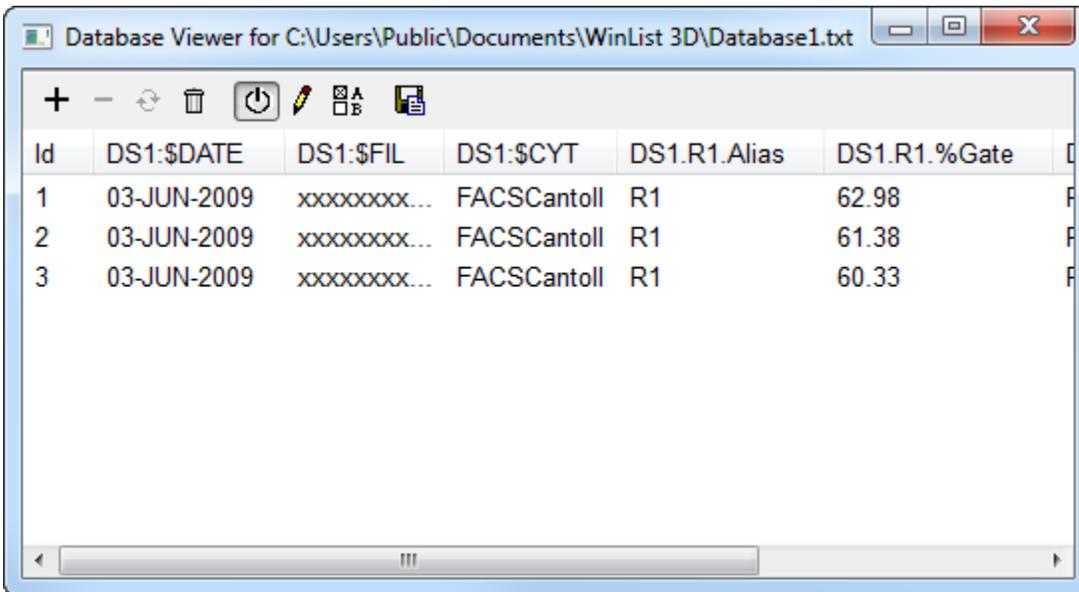


**Overview**

This window can be displayed for any database you have created. The viewer displays the fields defined in the database as column headings. Each record in the database is shown as a row of information. The window can be scrolled horizontally and vertically if there is information out of view. Each column can be resized by dragging the partition between the column headings with the mouse.



At the top, a toolbar provides buttons to manually add, delete, replace and delete all records.

Click the "+" button to add the current analysis information to the database.

Click the "-" button to delete the selected records from the database; if the button is grayed, there are no records selected.

Click the replace button to store the current analysis into an existing record in the database.

Click the trash can button to delete all records in the database. You will be prompted to confirm this action.

Additional buttons toggle the database on and off, display the Edit Keyword Map dialog, display the Edit Properties for Database dialog, and backup the database.

See Databasing with WinList for more on databases.

## Auto-open databases

☑ Auto-open

When this option is selected, WinList saves the database configuration when you exit the program, and automatically reopens the database when the program is started.

With this option checked, databases and database viewers are automatically opened and restored to the state they were in when the program was last used.

If you uncheck this option, you will need to use the Edit Databases command to open the databases that you want to use with the program.

**Add Record (Ctrl D)**

This command allows you to manually trigger any open, active databases to save the current results as a new record. Normally, database records are saved when you click the Replay button or use one of the options in the Replay menu.

The command is found on the **Edit** tab of the ribbon bar.  Pressing Ctrl D on the keyboard is a shortcut for this command.

**Clipboard**

**Copy (Ctrl C)**
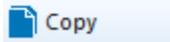
Copy

Select this option to copy the active histogram, results, or report window to the clipboard. A histogram will be copied as a bitmap and a metafile by default, two formats that many other programs can paste as pictures. The format(s) used can be changed using Copy Special.

Click the title bar of the window you want to copy before using the command.

Pressing Ctrl -C on the keyboard is a shortcut for the Copy command.

**Copy Special**

 Copy Special
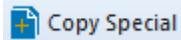
This option displays a dialog box, which allows full control over what is copied to the clipboard and in what format(s) it is copied.
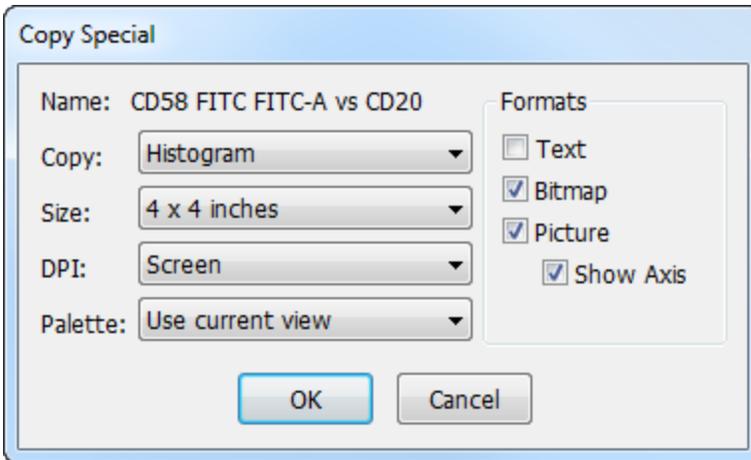


The name of the object that will be copied is displayed at the top of the dialog.

**Copy**

This drop down list allows you to choose what type of window to copy. The name of the active object appears just above the drop down list.

**Size**

This drop down list provides a list of target sizes for the Bitmap or Picture copy. For best results, choose the size that best matches the size of the image that you want to paste.

**DPI**

DPI, or Dots-Per-Inch, is a measure of graphic resolution. Higher DPI settings produce finer graphic output, and demand more memory of the computer as well. Choose a DPI setting that provides good quality for your use. For example, Screen resolution is usually plenty for in-house documents, but publications may want 1200 DPI for the highest quality printed output.

**Palette**

WinList supports normal and "inverse mode" palettes for viewing histograms and result windows. This option allows you to copy an object in the current mode or a specific palette mode.

**Formats**

**Text**

This format is available for copying Results windows to the clipboard.

**Bitmap**

Bitmap format is a pixel-by-pixel representation of the selected window, also referred to as a raster image. The dimensions and resolution of the bitmap depends on the settings for Size and DPI.

**Picture**

This format is only available for histogram windows. Picture format is a vector-based image format (Windows metafile) that can be resized without a loss of image quality. The optimal dimensions and resolution of the picture depends on the settings for Size and DPI.

**Show Axis**

With this option checked, the histogram axis is enabled in the copy operation. If unchecked, the data area of the histogram is copied, without displaying the axis tics and labels.

**Paste (Ctrl V)**
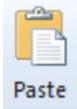


This command allows you to paste a picture or text object from the clipboard into the active histogram or report. The option is available if the clipboard contains bitmap, metafile, or text formats. The pasted object can be moved and sized on the histogram or report window.

Note: To paste an object into a Report window, use the Paste button on the report toolbar or press Ctrl-V after activating a report window.

### Drag and Drop

WinList supports a number of drag and drop operations that simplify common functions.

### Drag and Drop Object onto Reports

Many objects in WinList can be dragged onto report windows to simplify adding the object to the report.

To drag and drop a histogram, MultiGraph, or results window onto a report, position the mouse over the drag hotspot on the title bar, press and hold the mouse button, and drag the window onto the report. When you release the mouse button, a linked object will appear on the report.

The drag hotspot is the left corner of the title bar of a window. When you position the mouse over this spot, the cursor will change to indicate the Drag and Drop functionality.
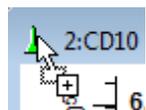


The drag hotspot on the upper left corner of the data source window will drag the entire contents of the window onto the report.
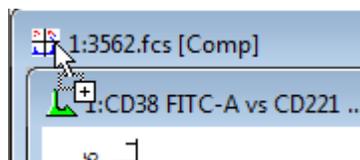


### Drag and Drop Files onto WinList

When you drag files from the operating system onto WinList's main window, WinList attempts to determine what kind of file you have dropped and open it appropriately.

You can locate files using Windows Explorer or My Computer on the PC, select them with the mouse, and drop them onto the application. Protocol and macro files (wlx, mac, and wlb) are opened and run. Report files (wlr) and MultiGraphs (mg) are opened in a new report window.

You can drop a compensation file (cmp) onto a data source to automatically apply the compensation settings to it.

Other file types are treated as FCS listmode files and the program attempts to open them. If you drop files onto an existing data source window, WinList will assume that you want to add these files to the batch list for that data source window. If you drop files onto the main

application window, WinList will attempt to open the first one as a new data source window, and then add the remaining files to the batch for this new data source.

Note: When selecting files for drag and drop, the file under the mouse cursor will be the first file added. In most cases, you should make the selection and then press and hold the mouse on the first file to begin the drag and drop operation.
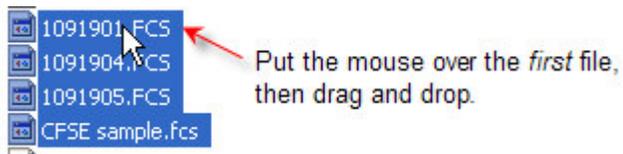
## Exporting

### Export Listmode



**Listmode File**
Export data in FCS or Text format

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

Use this command to create a new listmode file based on the current file.  A gate can be applied to restrict the events in the exported data file. You can save original parameters as well as calculated parameters, either compensated or uncompensated.  Output formats include FCS or text file format.

When the **Export Listmode** command is selected, a dialog box is displayed.
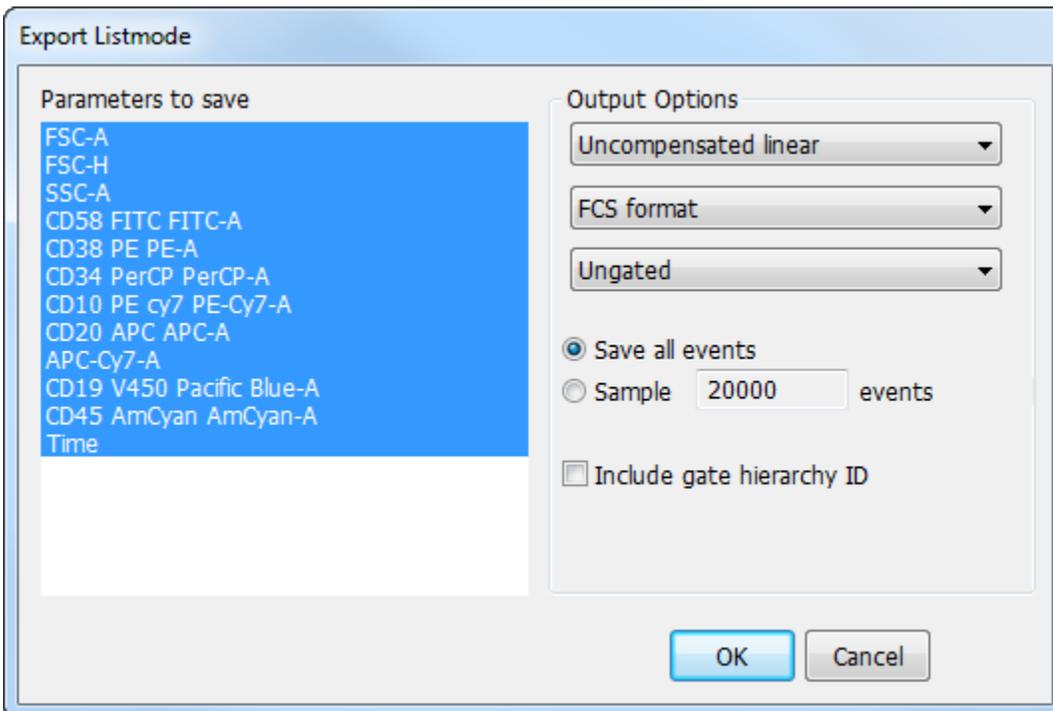


The **Parameters to save** list box shows the parameters available to save. Select parameters in this list that you want in the new listmode file.

The **Output Options** group includes several controls the determine how the data is written into the new file.

Three types of data can be saved: **Uncompensated linear**, **Compensated linear**, or **Compensated log**. In most cases, Uncompensated linear format is the best choice. With this option, compensation information is stored in keywords so that other software can read the new file and apply compensation dynamically. If you need to read the new file into software that does not perform compensation, use Compensated linear format. In a few rare cases, you might use Compensated log format to save the log-transformed compensated data.

The file can be saved as **FCS format** or **Text format**. FCS format is Flow Cytometry Standard format, compatible with most flow cytometry software programs. Text format is **tab-delimited text**, useful for exporting to database and spreadsheet programs.

You can save **ungated** data or apply one of the **active gate**s to the data as it is being saved.

The program can **save all events** from the source file, or **sample** a specified number of events. Sampling will divide the number of events in the source file by the number of events you want to sample, and use that value to select events to write into the new file.

Enable the **Include gate hierarchy ID** option to store gate identification as a parameter. The gate's ID becomes another parameter in the output file.

After making the selections for parameters and output options, press **OK**. When the file save dialog box appears, type the file name in the edit box and click **Save**. Use the **Cancel** button close the dialog without saving.

**Export Merge Files**
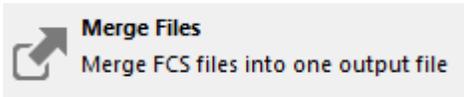

Merge Files
Merge FCS files into one output file

This command is found in the File (Application) menu of the ribbon bar. When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

Use this command to create a new listmode file that is a concatenation of a set of FCS files. A gate can be applied to restrict the events in the exported data file. You can save original parameters as well as calculated parameters, either compensated or uncompensated. Output formats include FCS or text file format.

This option does *not* create missing parameters or synthesize data to fill in missing parameters. It concatenates files into one, combined, new file. You can include all the events from all files, or sample a number of events from each.

When the **Export Merge Files** command is selected, a dialog box is displayed.
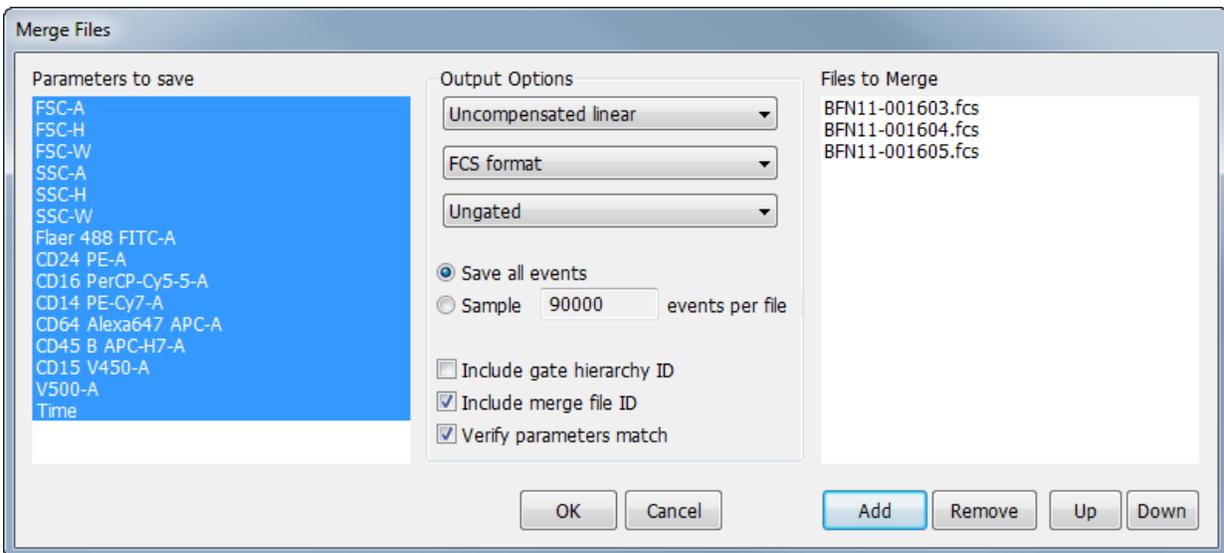


Many of these options are the same as those in the Export Listmode dialog box. See that topic for descriptions.

Additionally, the Merge Files dialog includes checkboxes to **include merge file ID** and to **verify parameters match** across the files in the merge.

The **Add** button displays a File Open dialog to select the files that you want to merge. The **Remove** button will remove selected files from the Files to Merge list.  The **Up** and **Down** buttons allow the items in the merge file list to be rearranged.

Once parameters and files are selected and output options are set, click the OK button to initiate the merge.

### Export Histogram File

**Histogram File**
Export a histogram in FCS format

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This command allows you to save the active histogram to disk in Flow Cytometry Standard (FCS) format. Choosing the option initiates the Save dialog box.

Navigate to the folder in which you want to save the histogram by double-clicking on items in the tree. Enter a name for the file in the File edit box. By default the name is the same as the name appearing on the histogram's title bar. Click **Save** to store the histogram on disk, or **Cancel** to exit the process.

Only one and two parameter histograms are supported by this option.

**See also:**
Export Listmode

**Export Histogram Graphic**
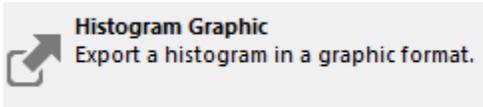
**Histogram Graphic**
Export a histogram in a graphic format.

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This menu option allows you to save the active histogram graphic as a file on disk. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, or GIF.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box and choose the graphic format by using the Save as Type drop down menu. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

**Export MultiGraph Graphics**

**MultiGraph Graphic**
Export a MultiGraph in a graphic format.

This command is found in the File (Application) menu of the <u>ribbon bar</u>.  When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This menu options allows you to save the active MultiGraph as a graphic file on disk. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, or GIF.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box and choose the graphic format by using the Save as Type drop down menu. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

**Export Report Graphic**

**Report Graphic**
Export a Report in a graphic format.

This command is found in the File (Application) menu of the [ribbon bar](#).  When WinList is configured to use the [classic toolbar and menu interface](#), the command is found in the File menu.

This command displays the Save Report Graphic dialog to save the active report as a graphic file. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, and GIF.

**Export MultiGraph PDF**

MultiGraph PDF
Export a MultiGraph as a PDF

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This option saves the active MultiGraph window as a PDF file.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

**Export Report PDF**

**Report PDF**
Export a report in PDF format

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This command displays the Export PDF dialog to save the active report as a PDF file. PDF stands for Portable Document Format, and is a standard format for exchanging information containing graphics and text. It is very useful for creating archival reports or for sharing analysis results with others.

**Export Results**

This command is found in the File (Application) menu of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the command is found in the File menu.

This command allows you to save the active results window to disk in tab-separated text format. Choosing the option initiates the Save dialog box.

Navigate to the folder in which you want to save the results by double-clicking on items in the tree. Enter a name for the file in the File edit box. By default the name is the name appearing on the results window's title bar. Click **Save** to store the results to disk, or **Cancel** to exit the process.

**Macros**

**Macros**
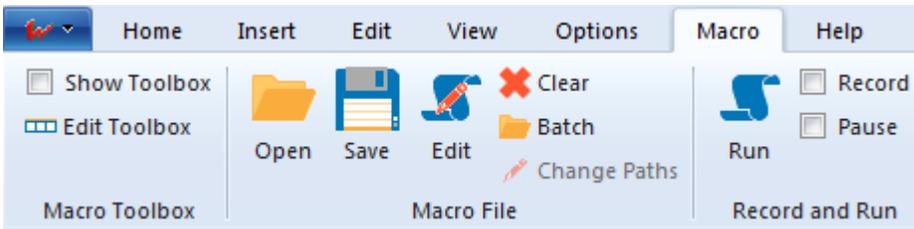


Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

Macros are command scripts that can be used to automate things that you do repeatedly in WinList.  They can be "recorded" as you perform a set of tasks, then saved to disk so that they can be "played" later.  You can also directly edit macros to create customized processes.

All macro-related commands are found on the **Macro** tab of the ribbon bar.

**Record**

Macro commands are found on the Macro tab of the [ribbon bar](#).  When WinList is configured to use the [classic toolbar and menu interface](#), the commands are found in the Macro menu.

To begin recording a macro, click the **Record** checkbox on the Macro tab. A check mark will appear indicating that WinList is recording. In Record mode, the WinList functions you perform are recorded. You can record an entire sequence of operations, then uncheck the Record checkbox to stop recording.

There are a few macro commands that cannot be recorded automatically in Record mode. Any of these commands may be added to a macro using the Edit macro command.

See also

[Edit Macro](#)

[Macro Commands](#)

**Run (Ctrl U)**

Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

When the Run option is selected, WinList begins executing commands from the macro stored in memory. The macro commands can be created with the Record, Open macro, or Edit macro options. When running macros, a *Macro Status* dialog box appears on the screen. The recorded macro commands are displayed, and as each command is executed, the list box highlights the current macro command. The Macro Status dialog box can be re-sized and moved to any convenient location on the screen. WinList will remember the size and location you select for the Macro Status dialog and use them the next time a macro runs.

The dialog box contains three buttons: **Pause**, **Continue**, and **Cancel**. Click the **Pause** button to pause the macro.  This is useful when you need to adjust something before letting the macro continue. Click **Continue** to resume running the macro.

To cancel the macro while it is running, select the Macro Status **Cancel** button.

**Edit Macro**

Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

This command displays the Edit Macro dialog box used to edit the currently stored set of macros.
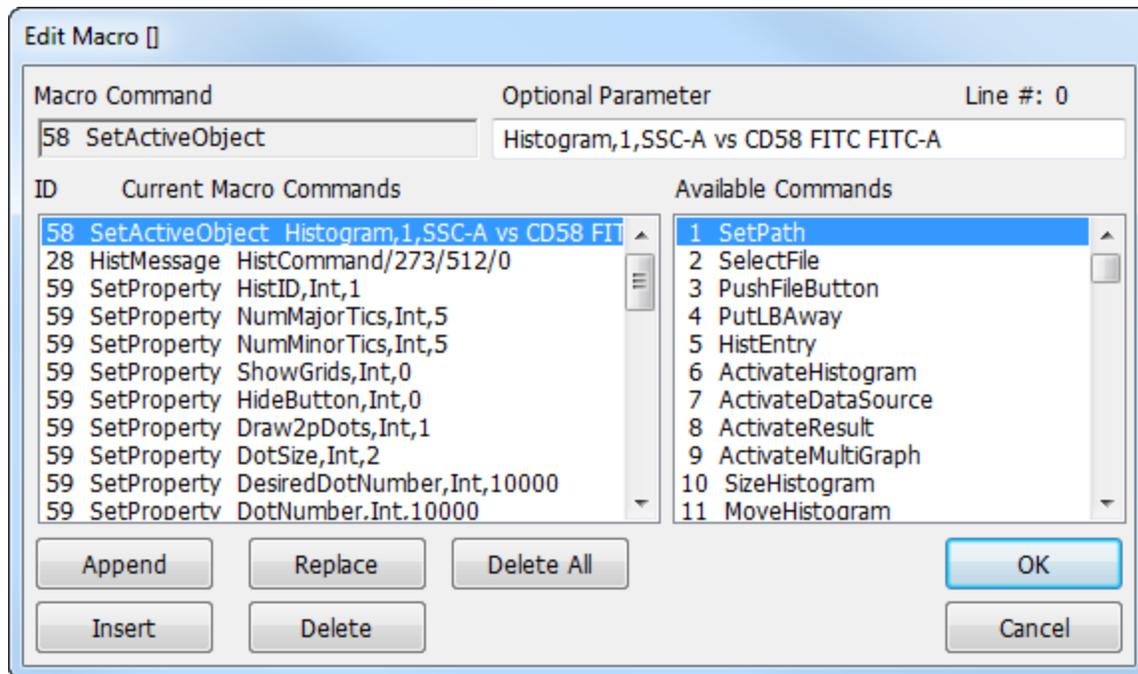


**Macro Command**

This field displays the currently select entry from either the Current Macro Command list box on the left or from the Available Commands list box on the right. WinList does not register any changes made to the command until you press Append, Insert, or Replace. Simply pressing the ENTER key will *not* store the edited command.

**Optional Parameter**

This field is used for parameters specific to particular macro commands. For details on valid parameters for each command, see Macro Commands. WinList does not register any changes made to the optional parameter until you press Append, Insert, or Replace. Simply pressing the ENTER key will *not* store the edited parameter.

**Current Macro Commands**

This list box displays commands contained in the current macro. Use the vertical scroll bars immediately to the right of the list box to see commands not currently in view. To select a command for editing, select it in the list. Once selected, the command portion will appear in

the Macro Command field and the parameter portion, if any, will appear in the Optional Parameter edit box.

**Available Commands**

This list box contains all of WinList's macro commands. When a desired command is selected, it is displayed in the Macro Command field. If a desired command is not in view, scroll in the list box.

**Append**

The Append push button appends the contents of the Macro edit boxes to the end of the list of commands.

**Insert**

The Insert push button inserts the contents of the Macro edit boxes above the current selected command list box entry.

**Replace**

The Replace push button replaces the contents of the Macro edit boxes with the current selected command list box entry.

**Delete**

The Delete push button deletes the current selected command list box entry.

**Delete All**

The Delete All push button deletes all the command list box entries.

**OK**

The OK push button puts away the Edit Macro dialog box and saves any changes made. To permanently store the changes to disk, use the macro Save As menu selection.

**Cancel**

The Cancel push button puts away the Edit Macro dialog box without saving any changes that might have been made.

See also:

Macro Commands

**Macro Commands**

Most macro commands are equivalent to mouse operations and menu selections you make. They are stored during macro recording. However, there are a few commands that are only available to you from within the Edit Macro dialog box, e.g. ResetMacroLine, Step, and NoStep.

Note: Currently a maximum of 8000 macro lines is supported.

If you record a complex macro and view it with the Edit Macro dialog box, you will be presented with a list of command lines that may at first be unfamiliar. It is the purpose of this section of the manual to explain what these commands do and what their arguments mean and perhaps give you some tips on when to change them. Some commands such as AppMessage and HistMessage have a format that is really only relevant to Windows and are best created by recording. The numbers associated with the commands shown below are the order in which they were implemented and the order they appear in the Macro Command list box. For the purpose of explaining their functionality, we will arrange them into more meaningful groups. The following are the currently supported macro commands for WinList:

**1      SetPath                [*.*][D:\winlist\]**

**2      SelectFile           bag101.fcs**

The **SetPath** command sets the search wild card for current **File IO** dialog box (e.g. **\*.\***) and sets the pathway to the files of interest (e.g. **D:\winlist\**). The square brackets are mandatory because they allow WinList to parse the two sections of the command correctly. Also the terminating "**\**" in the path is required. The **SelectFile** command generally follows the **SetPath** command if you are recording a **File IO** selection. Its argument is simply the file selected. If the file has no suffix a period must be placed after the name (e.g. **100222.**).

It's important to realize that WinList uses the same **File IO** dialog box for several functions (e.g. **File Data Source selection**, **Gate selection**, **Palette selection**, **Region selections**, etc.); therefore, these commands must be presented to WinList when the **File IO** box is on the screen. The best way of doing this is to use the record macro option. If you do, you will always be assured that these commands are presented to WinList at the proper time. A useful technique in modifying these macro commands is to insert a **Pause** command right after the **SelectFile** command and then delete the **SetPath** and **SelectFile** commands. WinList will stop the macro when it reaches the **Pause** command and then you can manually select the file of interest and then continue on with the macro by pressing the **Continue** button on the **Macro Status** dialog box. It's an easy way of making a macro that is file-specific to be more general. There are, however, more powerful ways of doing this with WinList's Batch features.

**3      PushFileButton**

No longer implemented.

**4        PutLBAway**

If you put away the keyword or parameter list box, this command will be recorded. It is usually not necessary because WinList will put it away for you automatically.

**5        HistEntry              1 1 2 0 64 H_P1_P2**

This command is important and may be one that you want to later modify so let's go through it in some detail. This command is recorded when you are selecting which histograms to automatically create in a Data Source. The first number indicates the type of histogram (**0=single parameter, 1=dual parameter, and 2=three parameter**). The next three numbers (e.g. **1 2 0**) indicate which parameters are selected. In the example, we have a dual parameter histogram and the X-axis will be parameter **1** and the Y-axis will be parameter **2**. The third parameter is not used for dual parameter but is used for three parameter histograms. The next number (e.g. **64**) indicates what resolution to use for the histogram. Finally, the ending text is the name of the histogram (e.g. **H_P1_P2**). After a macro is written, you might want to change the resolutions of some of the histograms. All you need do is to find the **HistEntry** command that corresponds to the histogram to change and then edit the resolution portion of the command. Another typical change might be to add another histogram. Just insert another **HistEntry** command and you're done.

The following group of commands activates the designated window in WinList. WinList uses a double naming technique for finding a particular window. Every window has a numeric id as well as a name. We use both methods for finding a particular Window so that your macro has the best chance of finding the appropriate window even if the name of the Window has changed. The id follows the name in the command arguments. Therefore, in all the Activate commands both the name and id are specified. If WinList can't find the id, it then tries to find the window with the designated name.

**6        ActivateHistogram    [bag101.fcs][1][H_P1_P2][1]**

Activates histogram window id **1** with name **H_P1_P2** in data source id **1** named **bag101.fcs**.

**7        ActivateDataSource    [bag101.fcs][1]**

Activates the data source window that has an id of **1** or a name of **bag101.fcs**. The square brackets are required.

**8        ActivateResult        [R1 H_P1_P2][1]**

Activates the results window that has an id of **1** or a name of **R1 H_P1_P2**. The square brackets are required.

**9        ActivateMultiGraph    [MGraph-1][1]**

Activates the MultiGraph window that has an id of **1** or a name of **MGraph-1**. The square brackets are required.

Whenever a window is sized or moved and macro record is on, one or more of the following macro commands is generated. The format of all the arguments is the same. The first number is the id of the window and the second two numbers are either the position (**Move**) or size (**Size**) of the x and y coordinates of the window. The units are in client units which

are pixels relative to the upper left-hand corner of the parent window. The **Size** commands have an additional argument which is **0** if **normal** sizing is taking place, **1** if the window is becoming an **icon**, or **2** if the window is **maximizing**.

| 10 | **SizeHistogram** | **1 367 386 0** |
|----|----|----|
| 11 | **MoveHistogram** | **1 346 4** |
| 12 | **SizeDataSource** | **1 788 444 0** |
| 13 | **MoveDataSource** | **1 0 0** |
| 14 | **SizeResults** | **1 112 111 0** |
| 15 | **MoveResults** | **1 4 42** |
| 16 | **SizeMultiGraph** | **1 459 245 0** |
| 17 | **MoveMultiGraph** | **1 295 230** |

| 18 | **DialogBox** | **Button {FileIO_OK}1 0 0** |
|----|----|----|

This command is used many times in macros. It enables the macro to set all the types of objects found in dialog boxes. For example, the **Button** part of the argument tells WinList to press a button. The **FileIO_OK** is simply descriptive text for you and is not interpreted by WinList. You do however need to have some description and it must be braced with the curly brackets. The next number tells WinList what button to press. The next two fields are not used for buttons but have meanings for other dialog box objects. The different types of objects supported by this command are **INTEGER, RADIO BUTTON, CHECK BOX, BUTTON, LIST BOX, MLIST BOX, CLIST BOX, and STRING**. It's really not important for you to know all the details for each of these command variants. If you do ever want to modify one, just record a small macro that sets the dialog box the way you want and then examine it.

| 19 | **AppMessage** | **CreateFile WM_COMMAND 60 0** |
|----|----|----|

This is a very common macro command that issues a Windows message to the WinList application. Any selection of a menu item creates this command. The first part of the argument list is a description of the command invoked (e.g. **CreateFile**). The second argument is a Windows-related parameter that indicates the type of message being sent to Windows and its arguments. The 60, for example, tells Windows that this is menu item 60. We recommend not changing this parameter without first contacting Technical Support.

| 20 | **SetPaletteID** | **4** |
|----|----|----|

This command is relevant only when the **Edit Palette** dialog box is on the screen. It sets the palette id that can be edited. Currently WinList supports only 16 colors in its default palette.

The next set of macro commands is only relevant to macro processing. Except for the **Pause** command, all of these macro commands need to be inserted into the set of macro commands either with the **Edit Macro** dialog box or by a text editor.

**21     Pause                       Choose the appropriate file to open**

When executed, the **Pause** command temporarily suspends further macro processing and then displays the text for **5** seconds. The macro system will hold in this state until you press the **Continue** button in the **Macro Status** dialog box. Typical uses of **Pause** are to add manual selection capability to a macro or to simply communicate with the user. You can insert **Pauses** into the macro by either selecting the **Pause** menu option in the **Macro** menu or by inserting the **Pause** command from the **Edit Macro** dialog box.

**22     Step                        Turns step debugging on**

**37     NoStep                      Turns step debugging off**

The **Step** and **NoStep** commands can help you work through complex macros. If you insert a **Step** command in a macro, the macro will stop after each macro command is executed and will wait for you to press the **Continue** button in **Macro Status**. The **NoStep** command simply turns this stepping mode off. A very powerful feature of the **Macro Status** dialog box is that in a paused state, you can highlight another macro command; when **Continue** is pressed the macro will begin running from the selected command. Thus, when you are in **Step** mode you can pick and choose which commands to execute.

**27     Remark**

The function of this macro command is simply to document the various sections of a macro. The macro processor simply skips this command when it is executing.

**29     ResetMacroLine      0**

The **ResetMacroLine** command is a powerful macro processing command that should be used very carefully. When this command is executed by the macro processor, the argument is used to reset the macro counter. Thus, if a **0** is in the argument, the macro counter is reset to **0** and the macro processor begins executing the macros starting at the beginning. This cycle will repeat **indefinitely** until you **Pause** or **Abort** using the **Macro Status** dialog box. There is another way for the macro processor to break the infinite cycle set up by ResetMacroLine. If you have recorded the command

**19 AppMessage GetNextBatch WM_COMMAND 15 0**

by executing the **GetNextBatch** menu option under **File**, WinList will set an internal flag if there are no more batch files that need processing. If the macro processor executes the **ResetMacroLine** and that batch file flag is set, it will continue with the **next** macro command. This construction enables you to create a very powerful macro system. For example,

19 AppMessage GetNextBatch WM_COMMAND 15 0

… other processing here, analysis, printing, etc.

29 ResetMacroLine 0

... further processing perhaps.

The above lines enable you to open up a series of batch files from the **Open Batch Files** dialog box and then to run this one macro. The system will analyze all the batch files automatically until there are no more batch files in the queue. The system will then continue through the **ResetMacroLine** command and do some further processing. This is the method we recommend for automating your listmode processing.

The following group of macro commands controls the creation, movement, sizing, and deletion of all regions in WinList. Because of the complexity of the arguments for these commands, we highly suggest you create them by recording. Only under very special circumstances should you attempt to edit them.

### 23      StartRegion          1/R2/CD4/1/19

When a region is being manipulated, this command is issued first. The first argument (e.g. **1**) is the type of region being created (**0=1P range, 1=2P region, 3=First Quad stat, 4=2nd,3rd, and 4th Quad stat**). The next argument is the name of the region (e.g. **R2**). The next argument is the region's alias (see Region List, e.g. **CD4**). The next argument is the region's numeric index (e.g. **1**) and the last argument is the number of points that will be in the region (e.g. **19**). If there is already a region defined (e.g. **R2**), it will be erased from the screen and deleted from memory.

### 24      AddToRegion          0/24/51

This macro command is issued for each point in the region defined in the **StartRegion** macro command. The first argument (e.g. **0**) is the 0th or first point, the next argument is the x value in the parent histogram channel units (e.g. **24**) and the last argument is the y value (51).

### 25      ApplyRegion          1/bag103.fcs/1/H_P1_P2/1

This macro command creates and displays the region. The arguments are the region index (e.g. **1**), the data source name (e.g. **bag103.fcs**), the data source id (e.g. **1**), the histogram name (e.g. **H_P1_P2**), and finally the histogram id (e.g. **1**).

### 26      RegionNameSize       1/24/55/30/58

This command sets the position and size of the region's name. The arguments are the region index (e.g. **1**), the left corner (e.g. **24**), the bottom left corner (e.g. **55**), the right corner (e.g. **30**), and the upper right corner (e.g. **58**).

The following commands are Windows oriented messages and have rather obscure arguments. It is best that you simply record these and leave it at that. The only argument that may be of importance to you is the descriptive argument. These commands are typically generated when you delete the window using mouse actions.

| 28 | HistMessage | HistCommand/273/1/8624 |
|---|---|---|
| 30 | ResultsMessage | DeleteResults/16/0/0 |
| 31 | DataSourceMessage | DeleteDataSource/16/0/0 |
| 32 | MultiGraphMessage | DeleteMultiGraph/16/0/0 |
| 33 | InfoMessage | DeleteInfo/16/0/0 |

### 34    ScaleHistogram        1411

When a 1P histogram is re-scaled and we are in record mode, this command will be issued. The argument (e.g. **1411**) is the new maximum Y-scale for the histogram.

### 35    SetPrintArea        0/0/765/424

This command sets the print area for the report printing options of WinList. The arguments are the upper left-hand x-value (e.g. **0**) and y-value (e.g. **0**) and the lower right-hand X-value (e.g. **765**) and Y-value (e.g. **424**).

### 36    SetSpot        1/1/416/2/512

The **SetSpot** command sets the current spot to the positions specified in the argument list. The arguments are the histogram type (**0=1P, 1=2P; e.g. 1**), the parameter number (e.g. **1**), the value for this parameter in ADC resolution (e.g. **416**), the next parameter number (e.g. **2**), and its value (e.g. **512**).

### 38    ModifyUserCounter    1,1

This command changes the value of one of the user-programmable counters by the amount specified as the argument to the command. The first number in the argument specifies which of five counters to modify (1,2,3,4 or 5), and the second part of the argument specifies the amount to change the counter. The counter can be accessed as an internal keyword **[COUNTER*n*]**, where *n* is a number from 1 to 5 identifying which counter you want to access. The [COUNTERn] keyword can be used for naming results windows, MultiGraphs, and any other places where internal keywords are accessible. The counter will be increased by the amount specified if no sign is present; it will be decreased by the amount if the minus sign (-) is specified. For example, ModifiyUserCounter with 1,3 as an argument will increase Counter#1 by 3. Using 3,-2 as an argument will decrease the Counter#3 by 2.

### 39    ResetUserCounter    1

This command sets the value of one of five user-programmable counters to zero (0). The argument specifies which counter to reset. The counter can be accessed as an internal keyword **[COUNTERn]** for naming results windows, MultiGraphs, and any other places where internal keywords are accessible. See also command #38: **ModifyUserCounter**.

### 40    NextBatchItem

This command tells WinList to process the next available batch item in the list. This command is particularly valuable in creating complex looping commands that involve the RepeatUntil command, (see below).

### 41      RepeatUntil         LL <Keyword><Condition><Value>

The arguments to the RepeatUntil command are:

LL: is the absolute macro line number,

<Keyword>: is usually either [COUNTERn] (see above), [LEVEL] which returns the level of the current batch item, [NEXTLEVEL] which returns the level of the next batch item, [FINISHBATCH] which returns 1 if the last batch item has been processed, or [NEWLEVEL] which returns 1 if the next batch item level is different from the current level, otherwise it returns 0.

<Condition>: is one of the following conditions: "<","<=","==",">",">=".

<Value>: is some integer number.

There can be intervening blanks between these tokens. A simple example of the use of RepeatUntil is shown below:

    0 NextBatchItem

    1 RepeatUntil 0 [NEXTLEVEL]==1

    2 RepeatUntil 0 [FINISHBATCH]==1

Suppose you have created a batch list that looks like:
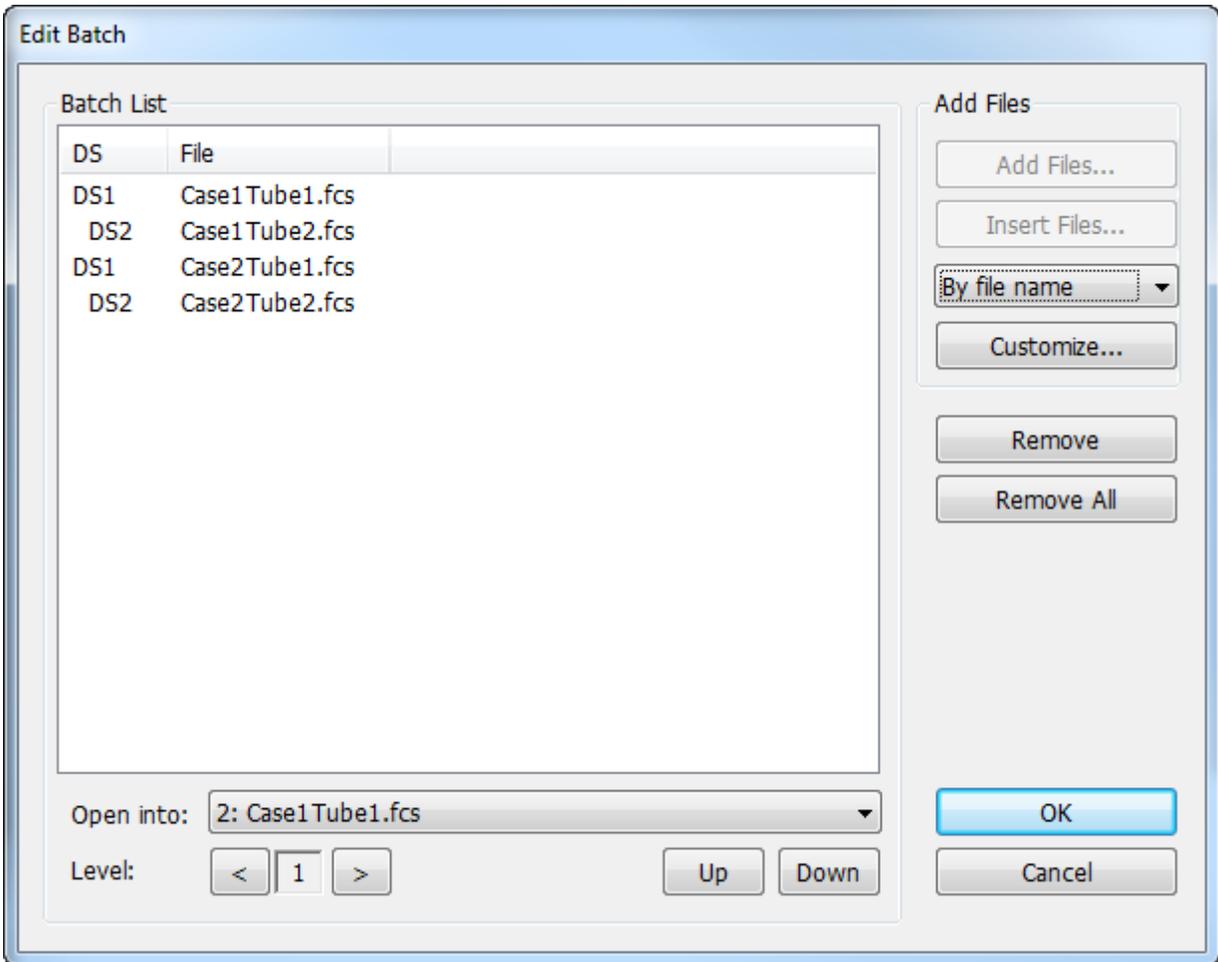
When you run the macro it will open the first file, 1543303 and assign it to datasource #1. Since this first line is at level 1 (no indentation), when we get to the next line, RepeatUntil 0 [NEXTLEVEL]==1, the [NEXTLEVEL] finds the level of the next entry in the batch list which is 2. Since [NEXTLEVEL]==1 is false under that condition, the RepeatUntil loops to the designated line number (#0). The macro executes another NextBatchIndex and hits the RepeatUntil again. This time, however, [NEXTLEVEL]==1 is true and the macro drops down to the next macro line (RepeatUntil 0 [FINISHBATCH]==1). This line checks to see if we are done with the batch. In this case, we are not done so it jumps to macro line #0 again.

The pattern repeats until we are done with the batch. Keep in mind that you can create any number of levels in the batch list box so you can do some pretty complicated stuff automatically. We've created some fairly powerful examples of this looping capability. If you let us know you are interested in obtaining copies, we'll send them to you.

### 42      GetResourceFiles    <Name>

When played, instructs WinList to open the stored palette, regions, and gates. <Name> is any legal DOS filename excluding the "." and suffix. This command is automatically generated by the Save Protocol command.

### 43      ClearProtocol

When played, instructs WinList to initialize the protocol. This is a dangerous command. It should only be used if you need to ensure that WinList is in a default starting condition. The Save Protocol command automatically generates this command.

### 44   SetProtocol    <Version><maxRegions><maxGates><maxSpots><maxColors> <maxParams><dataPacketSize>

When played, instructs WinList to set the protocol with the above specifications for the resources. This is a dangerous command. It should only be used if you need to ensure that WinList is in a default starting condition. The Save Protocol command automatically generates this command.

### 45      SetWindowName    <Type>,<Name>

When played, instructs WinList to set the **<Type>** window's name to **<Name>**. The current active window is the one edited. **<Type>** can be **PROTOCOL**, **DATASOURCE**, **HISTOGRAM**, **RESULTS**, or **MULTIGRAPH**. The **<Name>** field can be a name of the window or a keyword that when evaluated results in the name (e.g. **[SAMPLE]**).

### 46      ClearMultiGraph

When played, instructs WinList to clear the contents of the current MultiGraph.

### 47      Echo                        <Status>

When <Status> is set to OFF, histograms, results, and MultiGraphs will not be repainted on the screen. When <Status> is ON, the program automatically repaints all windows on the screen. This macro command is useful in speeding up macro playing especially if there are numerous events in each two parameter histogram. If you are inserting a Pause command within a dialog box, make sure the state of Echo is On; otherwise, you will not see the dialog box after the Pause executes.

### 48      GetNumberRegions    <counter id>

This command takes as its argument a number which identifies a counter id, and sets that counter to the number of regions in the active histogram. For example, if the active histogram has 4 regions in it, GetNumberRegions 5 will set user counter 5 equal to 4. It can be used for creating looping macros which loop depending on the number of regions in a histogram.

### 49      UpdateRemoteLinks

This command has no arguments. When executed, this command will initiate the DDE scripts for all histogram and results windows which have remote connections set to update *On Command*. Scripts are executed in order of lowest data source-histogram window id to highest data source-histogram window id, then results windows from lowest id to highest.

**50      ExecuteMacro          <macro name>**

This command opens a macro file, **<macro name>** and runs the macro. It allows you to chain macros together. A macro which is opened and run with the ExecuteMacro command does not return to the macro from which it was executed. To use the command, select it from the Available Commands list in the Edit Macro dialog box, enter the macro name as its optional parameter, and append it to your macro. If you insert ExecuteMacro in the body of a macro, any macro lines following it will not be executed.

**51    PositionRange    rangeNum/type/lowBoundary/highBoundary**

This command can be used to reposition a region on a single parameter histogram. It positions one leg of a single parameter region in the lowest channel between lowBoundary and highBoundary.

rangeNum: Identifies the region. It must be a 1 parameter region. R1 corresponds to a rangeNum of 1.

type: Identifies whether the left side (0) or the right side (1) is being repositioned.

lowBoundary: Starting point for the search. The value is in units of **percent** of X-axis resolution. This allows the same command arguments to be used with listmode files of different resolution parameters.

highBoundary: Ending point for the search. The value is in units of **percent** of X-axis resolution. This allows the same command arguments to be used with listmode files of different resolution parameters.

| | | |
|---|---|---|
| **52** | **Save Compensation File** | **<file path name>** |
| **53** | **Open Compensation File** | **<file path name>** |
| **54** | **Save Equation File** | **<file path name>** |
| **55** | **Open Equation File** | **<file path name>** |
| **56** | **Save Cluster File** | **<file path name>** |
| **57** | **Open Cluster File** | **<file path name>** |

Commands 52 through 57 allow compensation, calculated parameters, and cluster definitions to be saved to disk and opened from disk.

**58      SetActiveObject          type, id, name**

This command can be used to activate different objects. Presently, histogram and data source objects are the only supported values for *type*. The values for *id* and *name* represent the object's ID and name, shown in the titlebar. Once activated, you can send other commands and change object properties using the SetProperty and DoCommand commands below.

**59      SetProperty                property, type, value**

This command changes properties for the active object. Presently, histogram objects are the only supported objects. Determine the possible values for *property* by recording a macro that edits the properties of an object, and then looking at the SetProperty lines in the macro. *Type* and *value* should be determined in the same manner.

**60      DoCommand                command, option**

This command can instruct an object to do certain operations. Presently, histogram objects are the only supported objects. The object that receives the DoCommand should be activated with a SetActiveObject command prior to using DoCommand.

Histograms support two commands.

*UpdateObject* command has no option. It causes a histogram to update and redraw.

*EditObject* command can be used with no option to display the Edit Histogram dialog box, or it can be used in the form *EditObject,Graphics* to display the Edit Graphics dialog for the histogram. Note, there are no spaces in this form, just a comma to separate EditObject from Graphics.

**61      reserved**

**62      Open Names File        <file path name>**

Command 62 opens files for the Names and Resolutions dialog box. When saving protocols, a names and resolutions file is automatically saved if you have used the Names and Resolutions dialog to edit parameter names or log decades.

**63      Close Report**

This command closes the active report file. There are no arguments to the command.

**64      Open Report                <file path name>**

Command 64 opens the specified report file. When saving protocols, reports are automatically saved and a command is added to the protocol to open the report.

**65      reserved**

**66      Open Alerts                <file path name>**

Command 66 opens the specified alerts file. This command is automatically generated when you save a protocol if you have created alert conditions.

**67      reserved**

**68      Open Acquisition        <file path name>
         Settings**

Command 68 opens a file containing the settings in the Acquisition dialog for the SY3200-enabled version of WinList.

**69       Set Color Mode              <mode>**

This command selects a color display mode for the active data source. Currently support values for <mode> are listed below.

| Value | Meaning |
|-------|---------|
| 0 | Color by Gate Color |
| 1 | Color by Gate Combinations |
| 2 | Color by Parameter Value |
| 3 | Color by Gate Hierarchy |

**70       Open Protocol              <file path name>**

**71       Save Protocol              <file path name>**

Commands 70 through 71 allow protocols to be saved to disk and opened from disk.

**72       Save Protocol Bundle       <file path name>**

This command is used to save the current setup as a protocol bundle.

**73       reserved**

**74       Open Compensation          <file path name>
          Database**

Command 74 opens a compensation database file for use by WinList's compensation database system.

**75       Open Palette File          <file path name>**

This command is used to open a palette file to set the colors used by gates.

**Important Macro Tips**

When editing macros, save the revised macro frequently under a new name and test the changes you make incrementally. It is much easier to debug your changes if you have only made a few at a time.

Use the Close All Windows menu option at the beginning of a macro if you want to be certain that the macro starts with a clean slate. Even if no other windows are open when you start recording the macro, you can still record the command to close all windows. When the macro is run, the command will be ignored if no other windows are open.

We suggest that when you are creating a complex macro you should break it up into logical sub-units. For example, record a macro that simply arranges the screen to your design specifications. Record another macro that sets up all the regions. When macros are in smaller sub-units, they become more manageable and easier to edit. You can use a word processor to concatenate all the macros together or you can use the multiple macro capability of Open Macro to run all the pieces at one time. The latter is probably the best choice because it gives you the most flexibility.

See also:

Edit Macro Toolbox

**Open**

Macro commands are found on the Macro tab of the <u>ribbon bar</u>.  When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

WinList displays the standard file open dialog box, allowing you to navigate to a drive and directory and enter a name for the macro file.

Click the **Open** button to read the macro from disk.

Click **Cancel** to cancel the dialog.

**Save**

Macro commands are found on the Macro tab of the <u>ribbon bar</u>.  When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

WinList displays the file save dialog box, allowing you to navigate to a drive and directory and enter a name for the macro file.

Click the **Save** button to store the macro in the folder on disk.

Click **Cancel** to cancel the dialog.

**Clear**

Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

Selecting the Clear command will clear the current macro from memory. It has the same function as the **Delete All** button in the **Edit Macro** dialog box.

If the macro was saved to disk, the disk file is not affected by this action.

**Macro Batch**

Macro commands are found on the Macro tab of the [ribbon bar](#).  When WinList is configured to use the [classic toolbar and menu interface](#), the commands are found in the Macro menu.

This menu option is used to select a set of macros that you want WinList to run in sequence.

```
Load Macro

Path:    C:\VshHelpProjects\WinList\Protocols\              [ ... ]

Macro files              ▼      Batch List           Items:  000

ChangeGatesMacro.mac                  ┌─────────────────────┐
EditHistogramMacro.mac                │                     │
                                      │                     │
                                      │                     │
                                      │                     │
                                      │                     │
                                      │                     │
 ◄   III        ►                     └─────────────────────┘

                              ┌ Batch List Controls ──────────┐
   [    Open    ]             │  [   Add   ]    [ Run Batch ]  │
                              │                                │
   [   Cancel   ]            │  [  Delete  ]   [ Delete All ] │
                              └────────────────────────────────┘
```
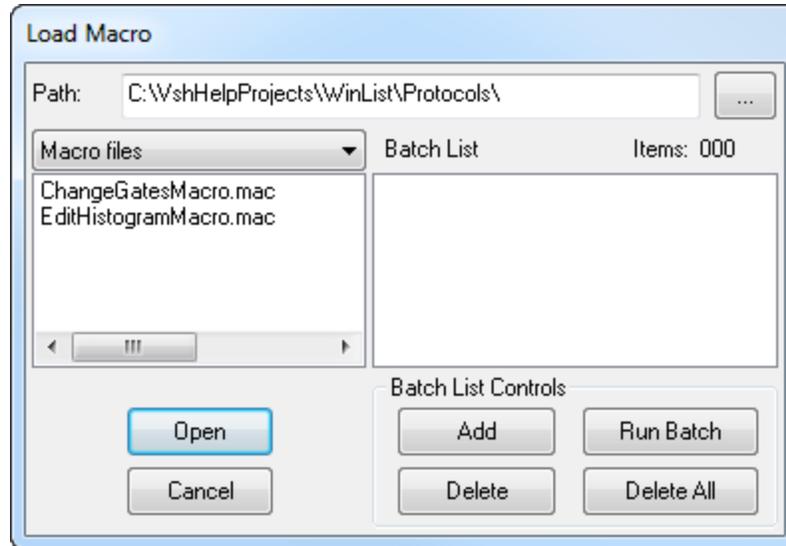
**Path**

This displays the complete pathway to the directory containing the macro files. To choose a new path, click the "**…**" button.

**Macro List**

The macro list box contains all the files in the selected pathway. Files are displayed alphabetically. On the right is a vertical scroll bar that can be used to find the files not displayed in the box. Clicking on the up or down arrow in the bar scrolls entries in that direction. To open a particular macro, click the left button of the mouse once on the file in the list box, and then click the Open button. The dialog box will disappear from the screen shortly after the Open button is pressed. Alternately, double-clicking on a macro name will open the macro, close the dialog box, and run the macro.

**Open**

Selecting this option opens the macro selected in the macro list box and closes the Open Macro dialog box. Alternately, double-clicking on a macro name in the **Macro List** will open the macro, close the dialog box, and run the macro.

**Cancel**

The Cancel button closes the Open Macro dialog box without opening a macro.

**Batch List**

This listbox displays the macros that you have added to the macro batch using the Add button. These macros will run in sequence when the Run Batch button is clicked.

**Add**

Clicking the Add button copies the highlighted files in the Macro list box into the Batch list box.

**Delete**

Select this option to remove all files highlighted from the Batch list box.

**Delete All**

Click this button to clear the macro batch list box.

**Run Batch**

Clicking the Run Batch button launches, in sequence, each macro listed in the Batch list box. The Open Macro dialog box will be hidden during the batch execution, but a Batch Status dialog box will display the number of macros left in the batch, and allow canceling of the batch.

**Change Macro Paths**

Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

This command provides a simple method for revising macros to run from a different folder or disk.

Macros and legacy protocol macros contain many references to specific files on disk. The listmode files that are used by protocols, the compensation files, and many others are identified by complete pathways. This can create problems when you need to run a macro or open a protocol from a different location on your computer. To resolve this problem, the Change Macro Paths command changes *all* pathways in the macro or protocol to a new path that you select.

To use this command, follow these steps.

1. Copy all of the files used by the protocol or macro into the new location. It is important to place *all* of the files, include the data files that are used directly by the protocol, in one folder in the new location.

2. Using the Open command in the Macro menu, read the macro *from the new location* into WinList. *Do not use the Open Protocol command for this purpose, because the macro will still refer to the old locations.*

3. Choose the Change Macro Paths command. The Change Macro Paths dialog will appear.
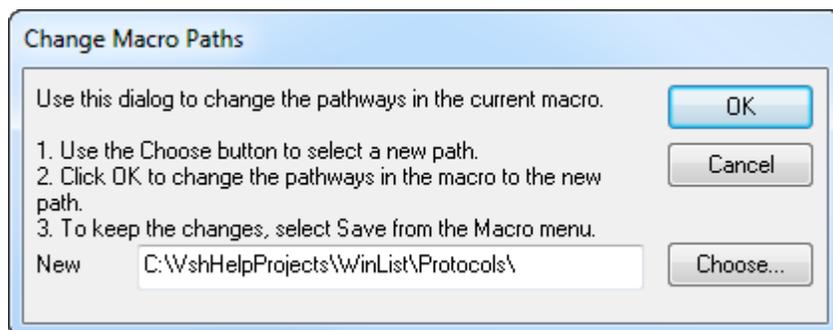


4. If the path shown in the New Path edit field is not the path you want to use, click the **Choose** button to select a new path.

5. Click the OK button to change the pathways in the macro to the new path. The program will change all pathways in the macro to the new path.

6. If you want to store these changes to disk, use the Save command in the Macro menu to re-save the file.

---

**Pause Macro**

Macro commands are found on the Macro tab of the [ribbon bar](#).  When WinList is configured to use the [classic toolbar and menu interface](#), the commands are found in the Macro menu.

This option can be selected while recording a macro to record a Pause command in the macro. After recording a Pause command, a custom message can be defined using the Edit Macro dialog box.

**Show Macro Toolbox**

Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

This command displays or hides the Macro Toolbox. The Macro Toolbox is a popup window with programmable buttons on it. The buttons can be hooked up to macros or protocols files on disk.  When a toolbox button is pushed, the associated macro or protocol file is opened, and if it's a macro file, the macro runs. It is especially useful for running macros that automate commonly performed operations.
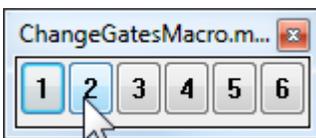


Once the Macro Toolbox is displayed, you can move it to any convenient location on the screen. Its position will be stored by WinList so that each time you run the program, the toolbox will appear where you left it.

You can control the number of buttons and the macros associated with them by using the Edit Macro Toolbox dialog box.

**Edit Macro Toolbox**

Macro commands are found on the Macro tab of the ribbon bar.  When WinList is configured to use the classic toolbar and menu interface, the commands are found in the Macro menu.

Select this command to program WinList's Macro Toolbox. The Macro Toolbox is a popup window that contains programmable buttons. The buttons can be "hooked up" to macros stored on disk so that when a button is pushed, the associated macro is executed. It is especially useful for running screen setup macros and macros that automate commonly performed operations.

The Show Macro Toolbox menu option allows you to display or hide the toolbox.

To program the toolbox, first adjust the number of rows and columns to suit your needs and click the **Set size** button. Next, select the first line in the list box below and click the **Select macro** button. From the file list, select a macro you want to assign to a button and click "Select." The macro you selected will be assigned. Continue assigning macros in this manner until all of the macros you want in the toolbox have been assigned to a button. Then click OK to display and use the toolbox. You can drag the toolbox to any location on the screen.
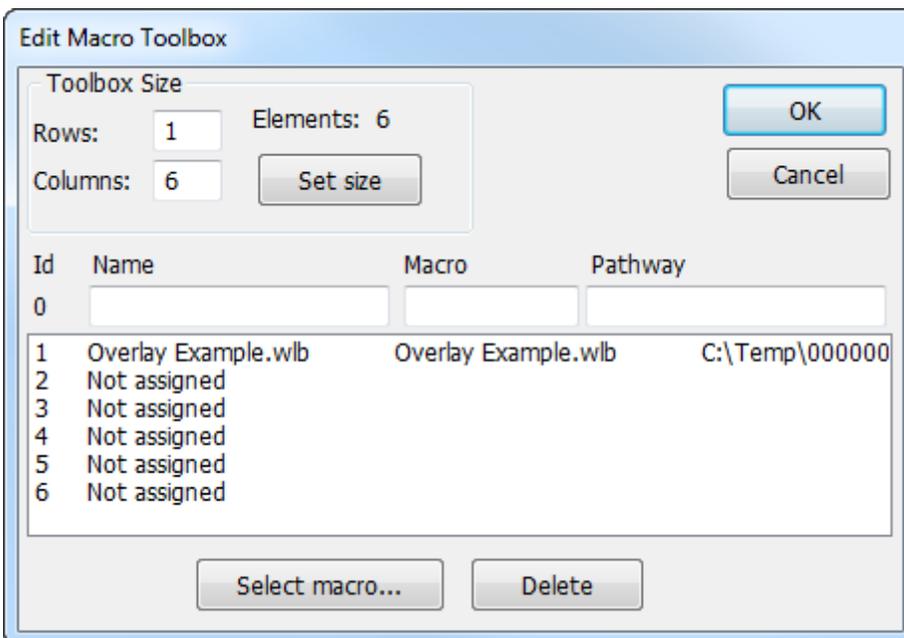


**Rows**

Enter a number, 1 or greater, to determine the number of rows of buttons you want to display in the Macro Toolbox.

**Columns**

Enter a number, 1 or greater, to determine the number of columns of buttons you want to display in the Macro Toolbox.

Note: The maximum number of buttons (Rows X Columns) is 100.

**Elements**

This field displays the number of buttons that will be displayed in the macro toolbox (rows X columns).

**Set size**

Click this button to change the toolbox rows and columns settings. The number of items in the list box will increase or decrease depending on whether the size is increasing or decreasing.

**Id**

The ID is the button associated with a macro.  Buttons on the toolbox are labeled with the ID.

**Name**

This edit box displays the name of a macro assigned to the button. You may edit this field, keeping the name's length under 30 characters. The name entered here is displayed in the Macro Toolbox title bar as you move your mouse over the buttons. By default, WinList will use the macro's name on disk as its toolbox name.

**Macro**

This edit box displays the name of the macro file on disk. It is filled in automatically if you select the macro using the Select Macro button described below.

**Pathway**

This edit box displays the pathway of the macro file on disk. It is filled in automatically if you select the macro using the Select Macro button described below.

**Select macro**

Clicking this button will display a file list dialog box. You can select a macro using this dialog box, and the selected macro will be assigned to the currently selected toolbox id in the list box.

**Delete**

Click this button to remove a macro assignment from the list.

**OK**

Click this button after you have finished making your edits to the Macro Toolbox. The changes you make will be accepted and the Macro Toolbox will be displayed after the Edit Macro Toolbox dialog is closed.

**Cancel**

Select this button to exit with no changes made to the Macro Toolbox.

**Logging**

**Edit Log**



This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.

The Edit Log dialog box allows you to view and set properties for session logging. Logging can be used to help Technical Support resolve problems with your software.

To display the Edit Log dialog with the ribbon bar interface, click **Logging** on the Edit tab.  With the menu & toolbar interface, choose **Log** from the Edit menu.

You can turn logging on and off by pressing the F2 key on the keyboard. The application title bar will indicate when logging is enabled.

See Edit Log and Edit Properties for Log for more information.

**Edit Log**

This dialog allows you to view the WinList log file, if logging is enabled.



**OK**
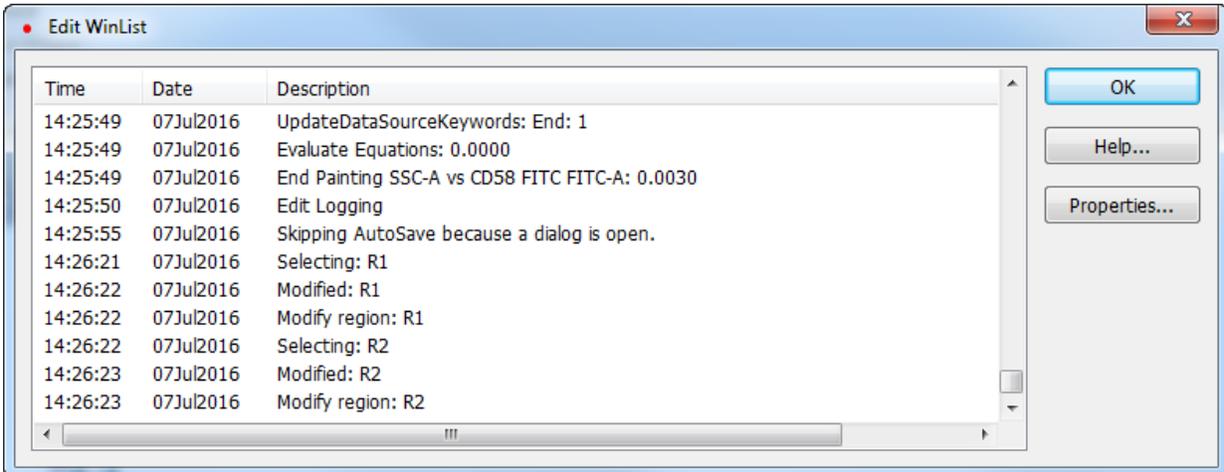
Click this button to close the dialog and accept any changes.

**Cancel**

Click this button to close the dialog and discard changes.

**Properties**

Click this button to display the Edit Properties dialog for the logging object.

See also Edit Properties for Log and Logging.

**Edit Properties for Log**

This dialog allows you to control the properties of the WinList logging function. Logging can be used to create a list of actions that you performed using the program, and can assist Technical Support in trouble-shooting problems. Normally, this option should be disabled.
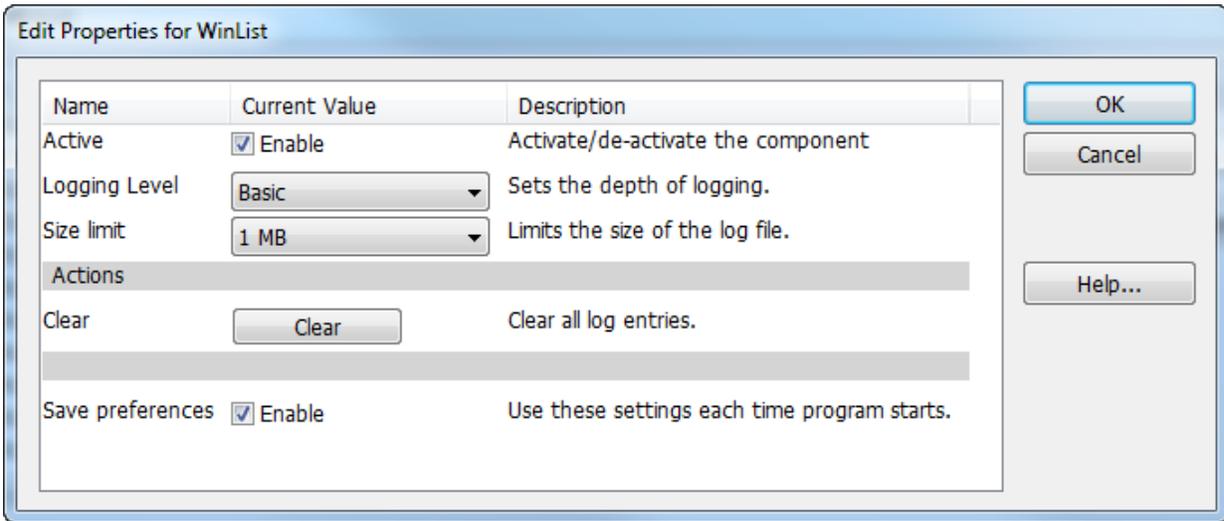


**Active**

Check this option to enable the logging function. WinList will create a file named WListLog.txt in the WinList 3D folder under the user's documents. The file will store operations you perform as you work with the program.

**Logging Level**

Choose between Basic and Debugging.  Debugging level includes all the Basic information plus additional logging to help trouble-shoot problems.

**Size limit**

Select the size limit for the logging file.

**Clear**

Click this button to clean out the log file.

See also Logging and Edit Log.

**Preferences**

**Preferences**



To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

The **Preferences** command displays a dialog box that is used to set up default values for many options in WinList.

The dialog displays a tree of categories on the left. Click a category item in the tree to view and edit its properties.
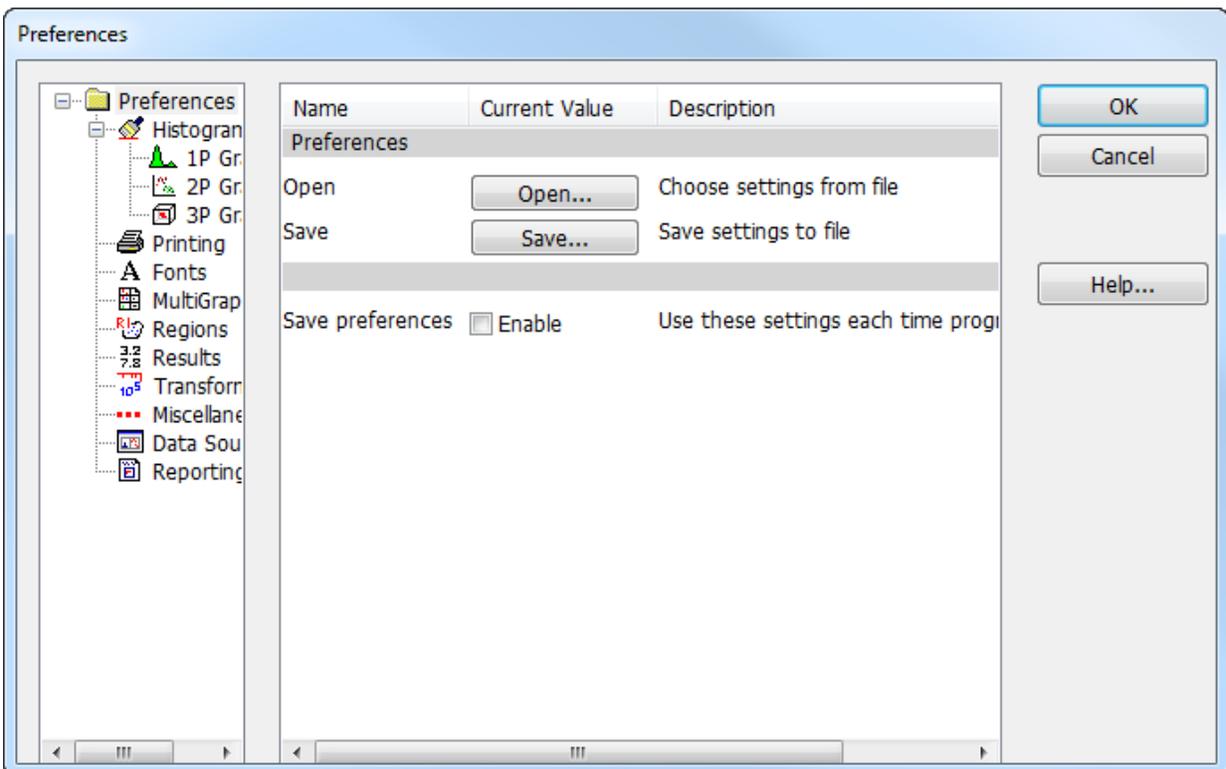


**Open**

Choose this command to open a WinList preferences file from disk. All of the preferences in the stored file will be read and used to set preferences for the program.

**Save**

Choose this command to save the WinList preferences as a file. This file can be used to configure WinList on different computers to use the same settings.

**Save preferences**

Enable this option if you have opened a preference file with the Open command above and you want to store the new preferences so that they will be used the next time you start WinList.

## Histogram Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to all histogram types.
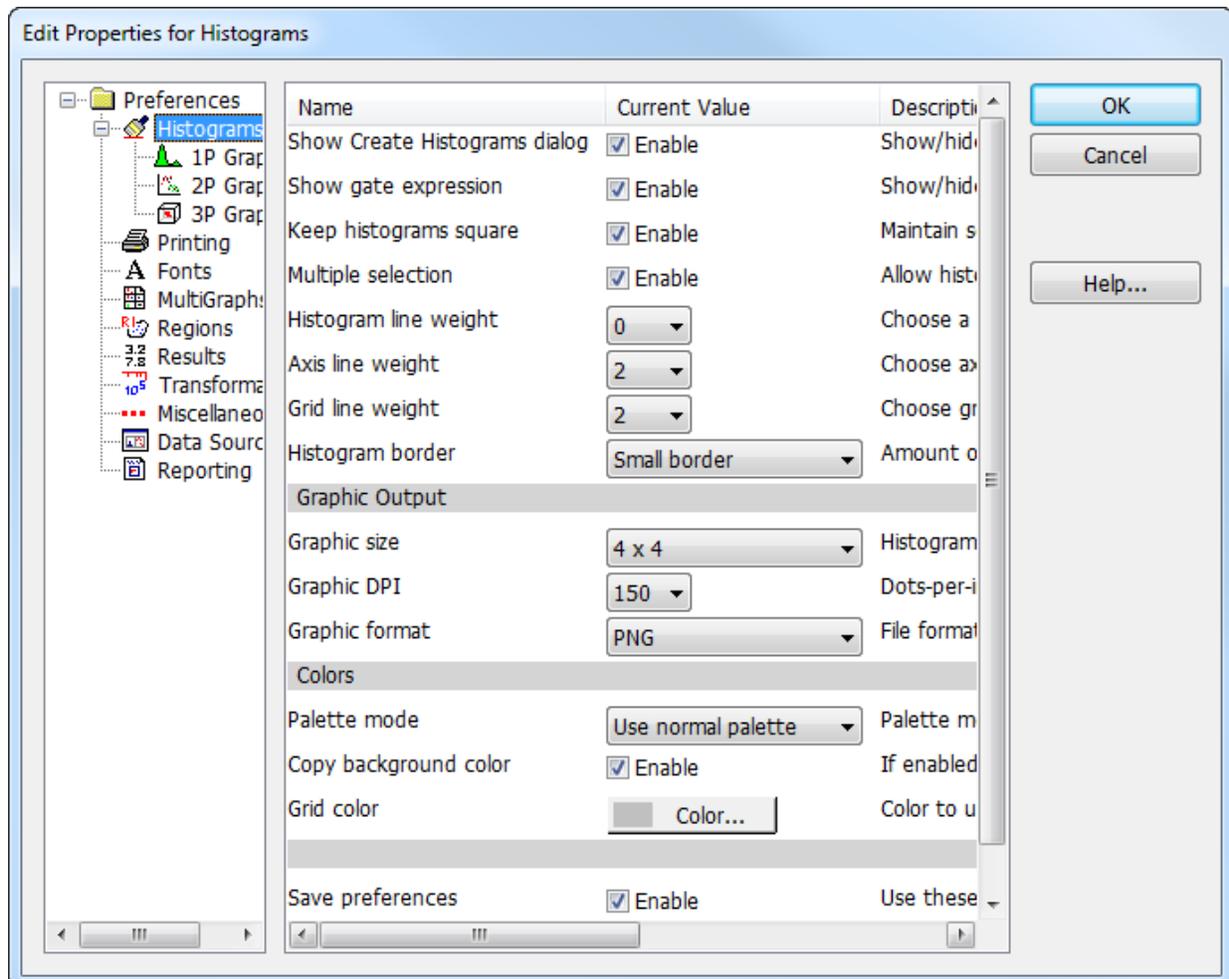


### Show Create Histograms dialog

When enabled, the Create Histograms dialog is displayed whenever a new data source is created.

### Show gate expression

When enabled, the gate expression appears in the title bar of each histogram

**Keep histograms square**

When enabled, the window arranging commands will always keep histograms windows square.

**Multiple selection**

When enabled, you can select more than one histogram window by pressing the **Ctrl** key on the keyboard.  This can be used for moving and sizing histograms, as well as for drag-and-drop and some graphics properties.

**Histogram line weight**

Choose a weight of the line used to draw 1P histograms. Sizes are in 1/2 points, (a point is 0.35 mm or 1/72 in).

**Axis line weight**

Choose a weight of the line used to draw axis borders and tics. Sizes are in 1/2 points, (a point is 0.35 mm or 1/72 in).

**Grid line weight**

Choose a weight of the line used to grids on histograms. Sizes are in 1/2 points, (a point is 0.35 mm or 1/72 in).

**Histogram border**

Choose how much space to allow between the graphics and the window border for all histograms.

**Graphic Output**

**Graphic size**

The value specifies the virtual size for a histogram window when creating a picture (metafile) of the histogram to copy to the clipboard. When you paste the picture into another application and display it at the specified size, it will look best at the target size you select here. Enter a larger value to reduce the size of fonts in the picture relative to other aspects of the histogram, or a smaller size to increase the fonts.

See also MultiGraph Preferences for setting the target size for images added to MultiGraphs.

**Graphic DPI**

This property specifies the dots-per-inch, or DPI, used when copying a histogram graphic to the clipboard or saving it to disk.  Higher values provide better quality output but require more memory.

**Graphic format**

This property determines the file format used when saving graphics to disk. Choose the format that you most commonly use.

**Colors**

**Palette mode**

This property determines what palette mode is used when histograms are copied, printed, or updated on a report.

**Use normal palette** uses the normal background and foreground colors, **Use inverse palette** uses the reverse background and foreground colors, and **Use current view** will use same palette mode that is currently displayed.

**Copy background color**

Enable this property if you want the background color of histograms included when the histogram is copied.

**Grid color**

Choose a color from the standard color dialog to use for grids on histograms.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**1P Graphics Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to 1P histograms. Individual histograms can override these settings.
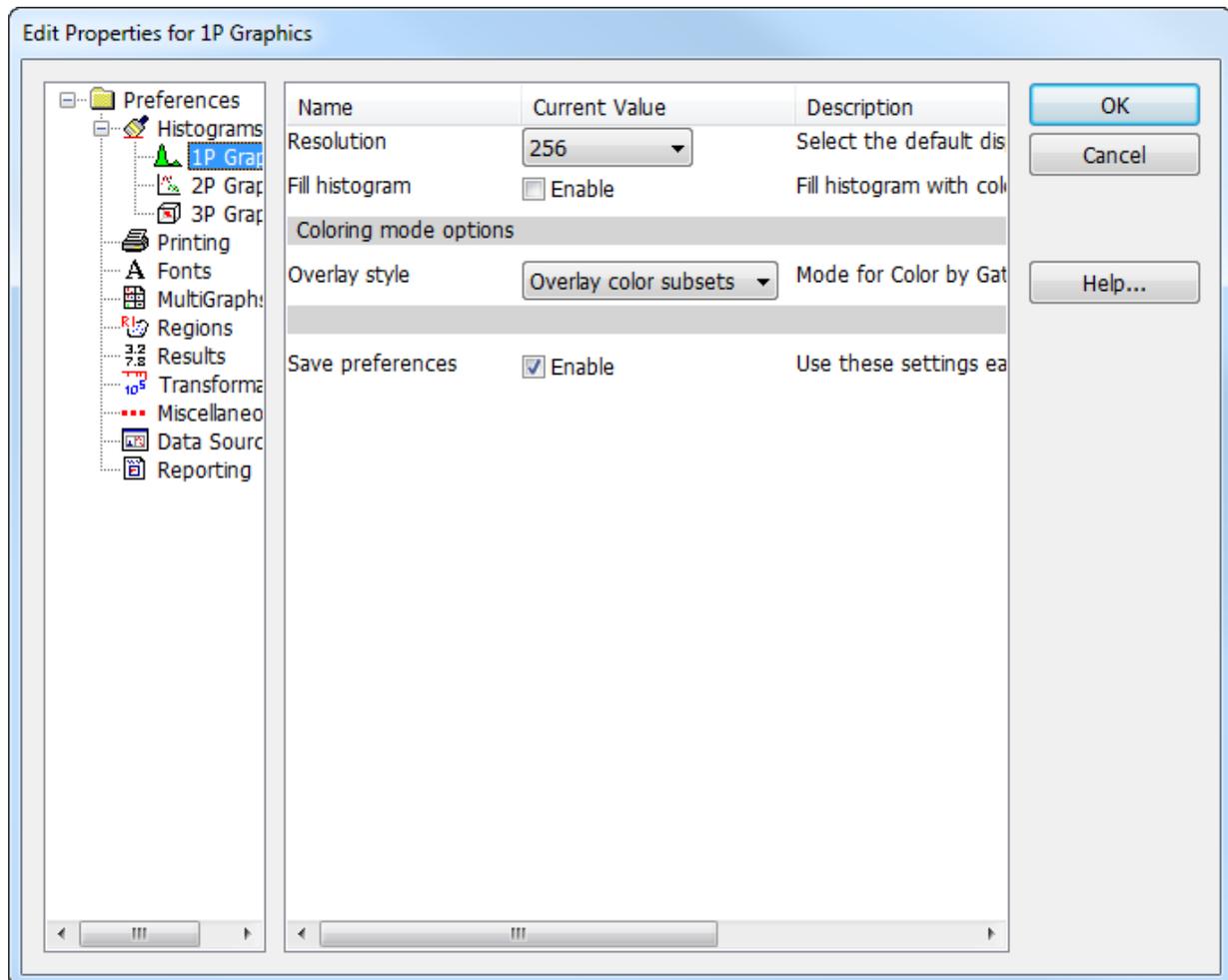


**Resolution**

Choose a desired histogram resolution for 1P histograms.

**Fill histogram**

When checked, histograms will be filled with color by default. Uncheck the option to display histograms without a fill.

**Coloring mode options**

**Overlay style**

This option determines how 1P histograms display in coloring modes **Color by Gate Combinations** and **Color by Gate Hierarchy**.

"No subset overlays" displays a single polygon for the 1P histogram.

"Overlay color subsets" draws additional polygons, one for each color. Each polygon is drawn from the baseline.

"Stacked color subsets" draws additional polygons, one for each color. The first polygon is drawn from the baseline, and the bottom of the next polygon is the top of the previous polygon.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**2P Graphics Preferences**


To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.


These preferences apply to 2P histograms. Individual histograms can override these settings.
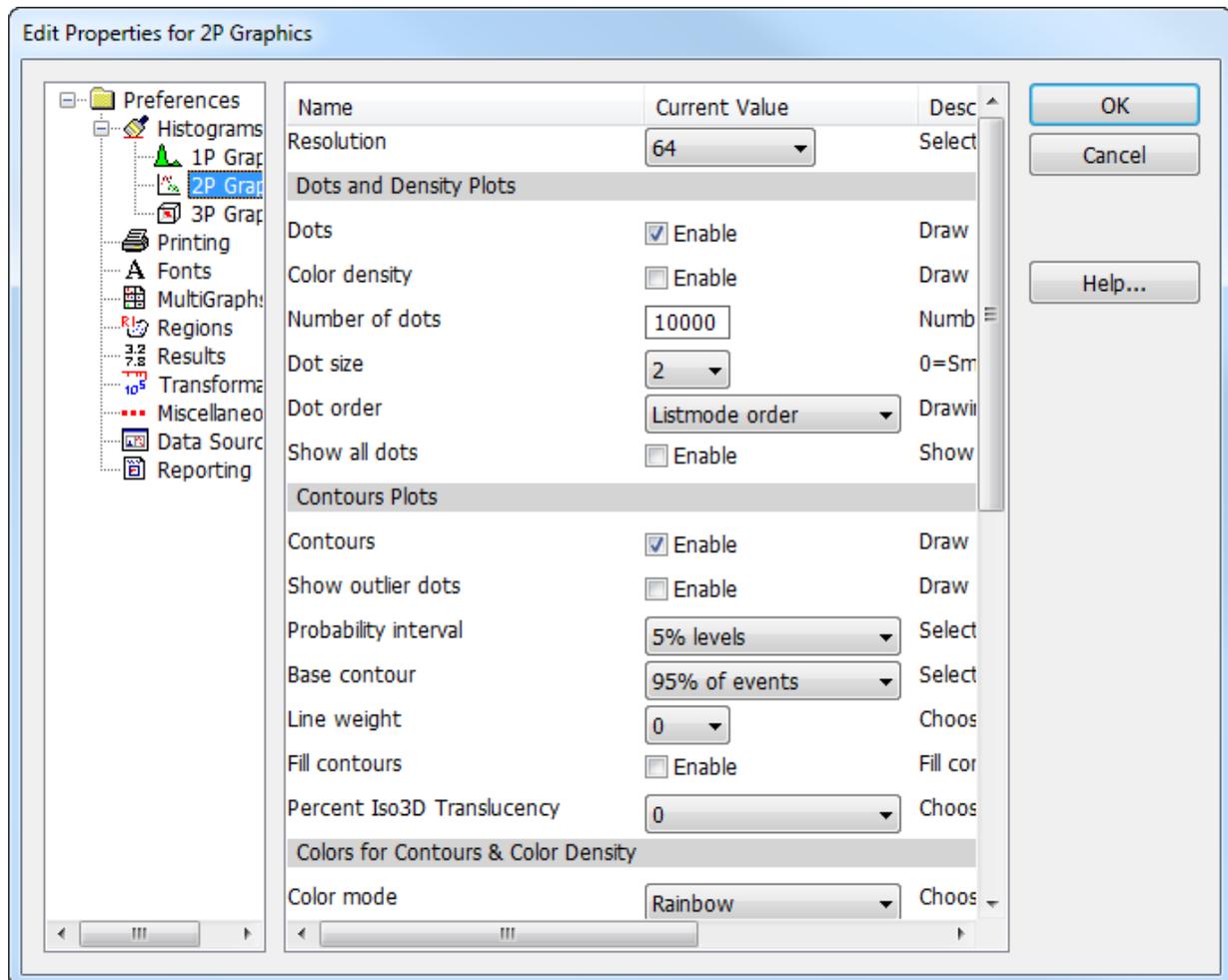


**Resolution**

Choose a desired histogram resolution for 2P histograms. If the chosen value is greater than the resolution of the listmode file, the maximum resolution of the listmode file will be used.

## Dots and Density Plots

### Dots

To display dots density by default, check this option. When the option is unchecked, dots will not be displayed on the histogram.

### Color density

To display color density by default, check this option. When the option is unchecked, color density dots will not be displayed on the histogram.

### Number of dots

Enter the number of dots you want to display in the histogram window.

Note: The actual number of dots displayed may be less that the number you enter. If there are fewer events in the data than you specify, or if the *packet size* you specify is less than the number of dots, fewer dots will appear.

In the second case where the packet size is smaller than the number of dots, you can display the full number of dots by replaying data for that histogram, or you can adjust the packet size to store the entire listmode file. See [Edit Program Controls](#) for further discussion of *packet size.*

### Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

### Dot order

When using color event gating, this option controls the order in which dots are drawn. *Listmode order* draws dots in their natural event order based on the listmode acquisition. *Low frequency on top* draws the dots for the smallest populations last so that they can be seen on top of populations that have many events. It is useful for visualizing rare events. *High frequency on top* draws the dots for the largest populations last. It may be used to visually clean up low frequency events.

### Show all dots

By checking this option, all events will be displayed in the 2P histogram *after replaying*. See the note for "Number of dots" above for more on showing all dots.

## Contour Plots

### Contours

To display contours, check this option. Uncheck the option if you do not want contours to display.

**Show outlier dots**

To display dots that fall outside the first contour, enable this option. NOTE: When this option is enabled, the *only* dots displayed are those that fall outside the lowest or base contour. It effectively disables the Enable Dots option in the Dots and Density graphics section.

**Probability interval**

This value determines the probability used to compute contours.

**Base Contour**

This dropdown list selects the level of the lowest, or base, contour. The level is selected as a percentage of events that it will include. The highest percentage, 99%, will include 99% of the events in the histogram.

**Line weight**

Choose a line weight for the contour border. The value is in points, a unit of 0.35 mm or 1/72 in.

**Fill contours**

When this option is checked, contours will be filled with colors defined in the Colors section. When unchecked, the contour is unfilled.

**Iso3D Translucency**

Select the default translucency to use for Iso3D displays. Higher values make the populations more translucent.

**Colors for Contours and Color Density**
**Color mode**

For color density and contours, this option determines how colors are assigned to different levels. *Color gradient* mode assigns **Gradient Start color** to the first level and gradually changes colors to **Gradient End color** for the last level. *Rainbow* mode uses colors from WinList's color palette, starting with color 1 and sequentially selecting colors from the palette for each level. If there are more levels than colors, colors are repeated.

**Gradient Start color**

Choose a color to use as the starting color for color density dots and contours in Color Gradient mode. When you click the color button, the standard color selection dialog box is displayed.

**Gradient End color**

Choose a color to use as the ending color for color density dots and contours in Color Gradient mode. When you click the color button, the standard color selection dialog box is displayed.

**Rainbow colors**

Select the number of rainbow colors to use in Rainbow color mode. The default, 5, serves most cases very well.

**Rainbow 1 - n**

Select a default color for each Rainbow mode level.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**3P Graphics Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to 3P histograms. Individual histograms can override these settings.
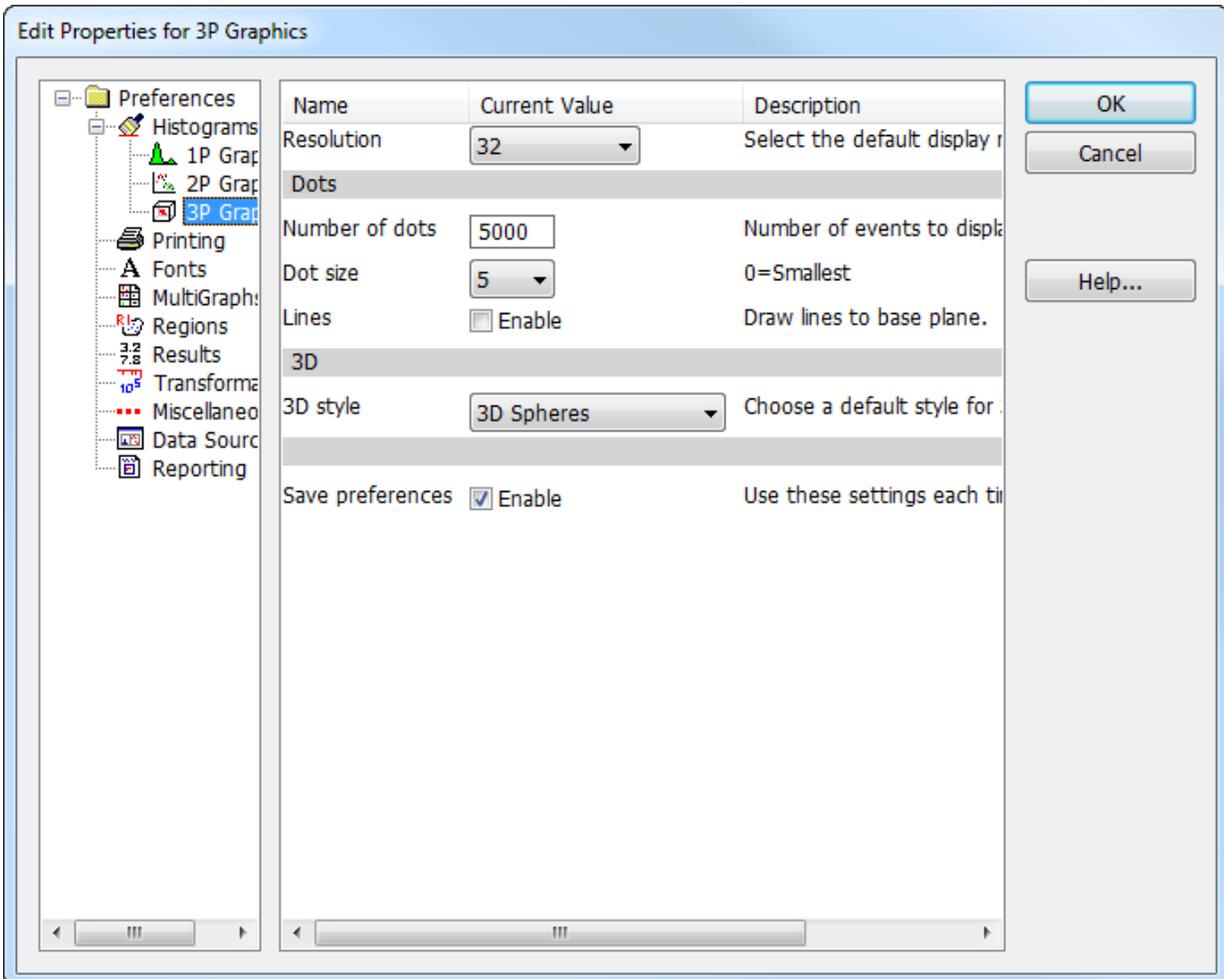


**Resolution**

Choose a desired histogram resolution for 3P histograms. If the chosen value is greater than the resolution of the listmode file, the maximum resolution of the listmode file will be used. On PC versions that support the 3D viewer, it is recommended that you choose a value of 32 channels.

**Dots**

### Number

Enter the number of dots you want to display in the histogram window.

### Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

### Lines

When this option is enabled, gray lines are drawn from each dot to the base plane. When displaying 1000 dots or fewer, this is useful to identify the position of dots.

**3D**

### 3D style

Select the default 3D display.

### Save preferences

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**Print Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences control various aspects of printing.
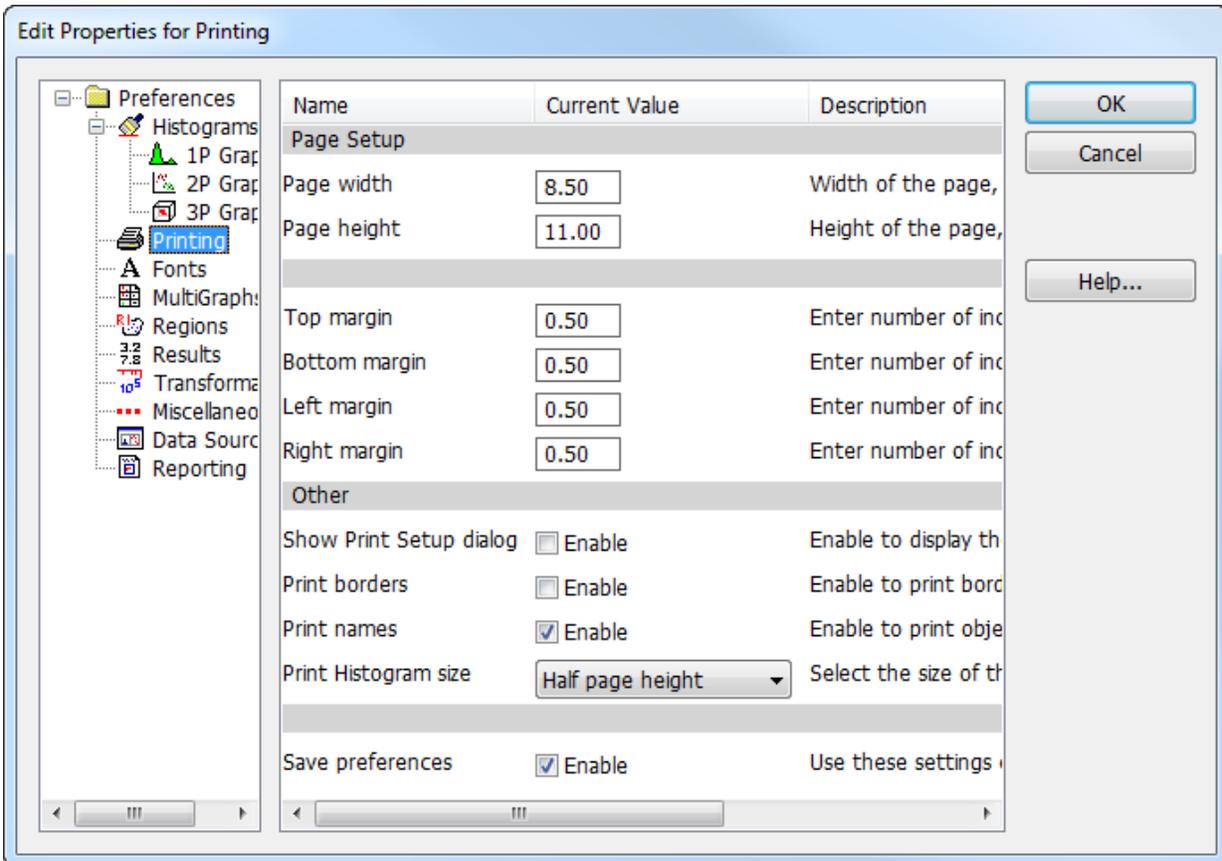


**Page Setup**

**Page width**

Enter the page width, in inches. This setting is used when printing reports, histograms, and MultiGraphs.

**Page height**

Enter the page height, in inches. This setting is used when printing reports, histograms, and MultiGraphs.

**Margins**

**Top, Bottom, Left, and Right margins**

Enter the number of inches for each margin. These margins are used when printing reports, histograms, and MultiGraphs.

**Other**

**Show Print Setup dialog**

Enable this option to display the Print Setup dialog prior to each print job.

**Print borders**

Enable this option if you want the program to print a line border around reports, histogram, and MultiGraphs. Clear the check to disable this option.

**Print names**

Enable this option to print the names of objects on reports, histograms, and MultiGraphs.

**Print Histogram size**

This option selects the page size to use for the Print Histogram command.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**Font Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences control the fonts that are used by WinList. Each font is selected by clicking the associated button to display the standard font dialog, choosing a font, and clicking OK.
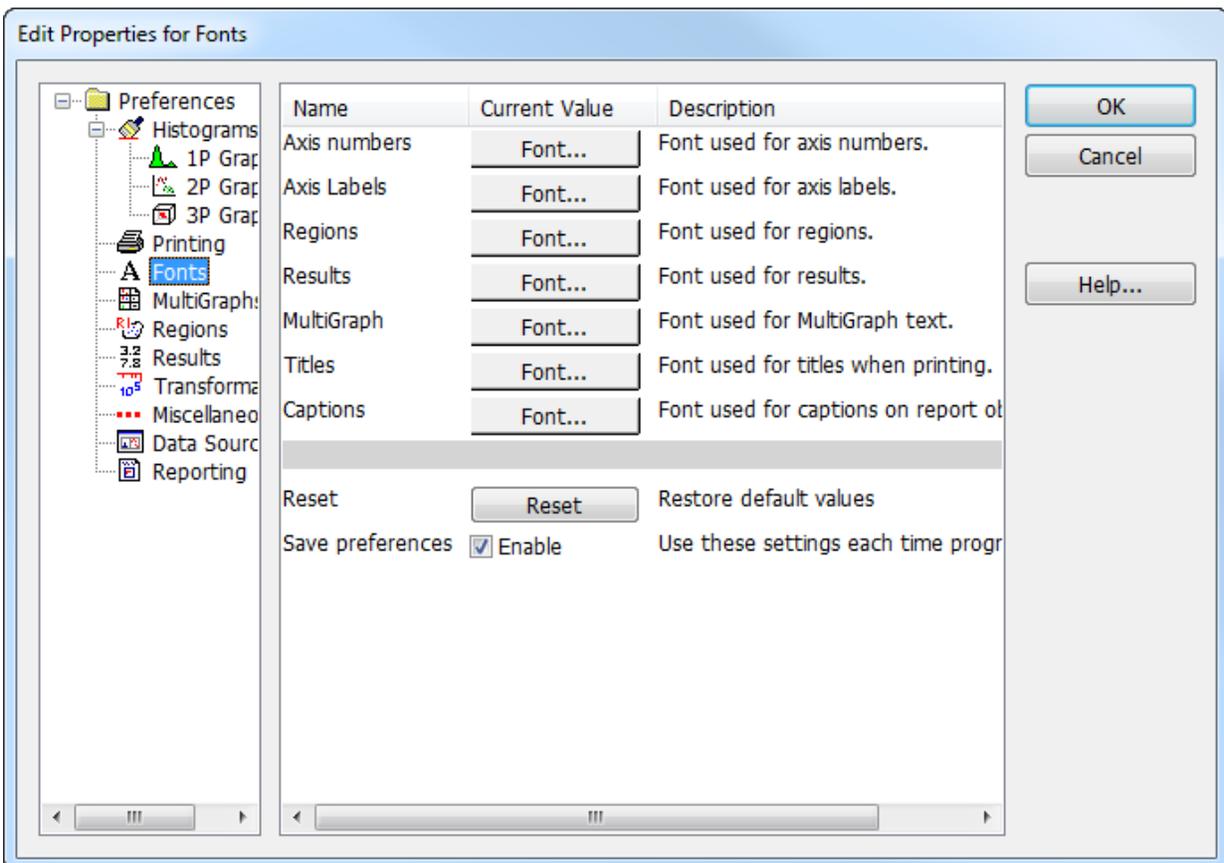


**Axis numbers**

Choose the font used for histogram axis numbers.

**Axis labels**

Choose the font used for histogram axis labels.

**Regions**

Choose the default font used for region labels. Individual regions can override this font by editing region properties.

**Results**

Choose the font used for result windows.

**MultiGraph**

Choose the font used for text in MultiGraph windows.

**Titles**

Choose the font used for titles when printing reports.

**Captions**

Choose the font used for captions of histogram report objects.

**Reset**

Click this button to reset all fonts to the default state for the program.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**MultiGraph Preferences**


To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.


These preferences are used by MultiGraph windows.
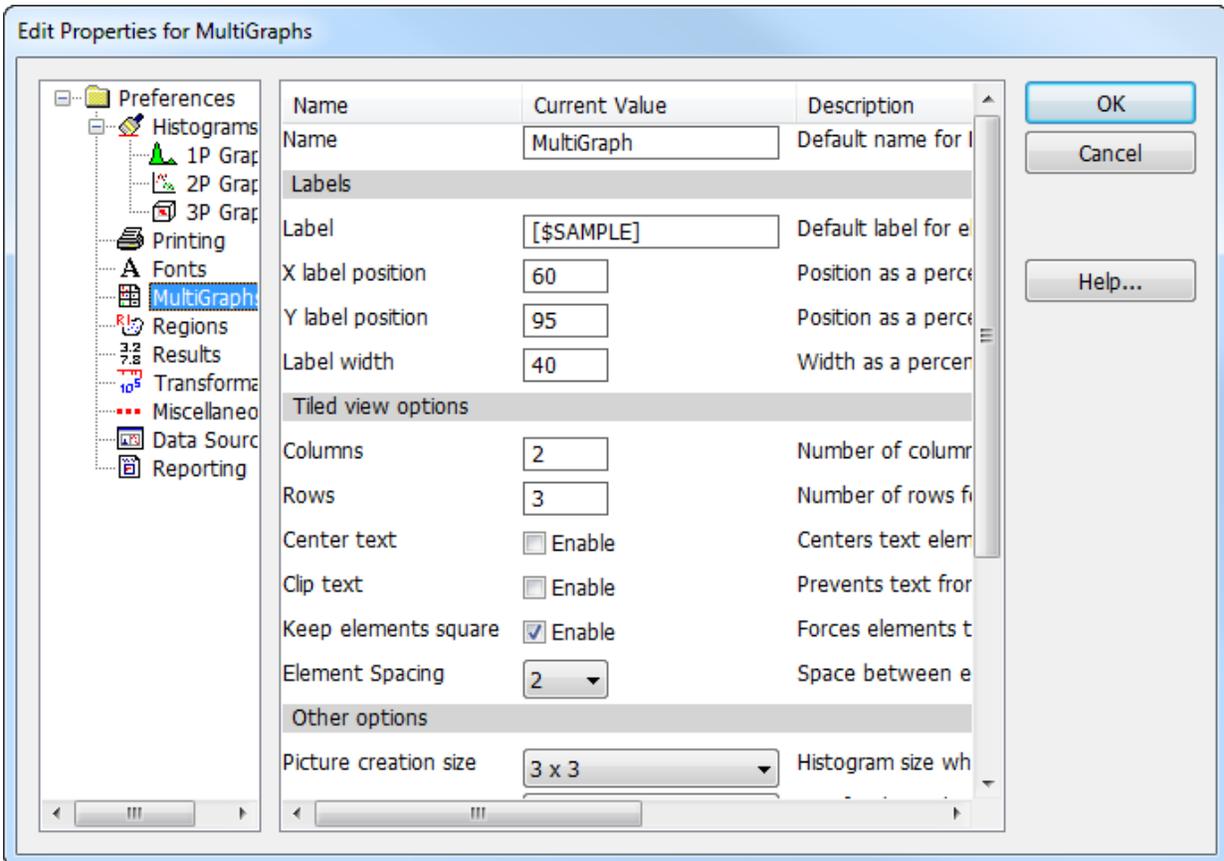




**Name**

Enter the default name for the MultiGraph window. You can enter text, or type a keyword enclosed in square brackets. For example, [FILE] will cause a new MultiGraph to be named with the listmode file name of the active data source.


**Labels**

**Label**

Enter the default label to use for graphical elements in a MultiGraph.

Labels can include any FCS keywords embedded in the data file and internal WinList keywords. Keywords must be surrounded by square brackets [ ]. See Internal Keywords for more on keywords.

### Label position and width

The X and Y positions and the width can be entered in these edit boxes. The numbers are in *percentage of the windows* where 0 percent x and y is the lower left corner of the window, and 100 percent x and y is the upper right. X and Y positions determine the origin of the label. The width, again in percent, determines how wide the label can extend. Usually, the width and the x position values should not add up to more than 100 or the label will spill out of that histogram's portion of the MultiGraph.

## Tiled view options

### Columns

Enter the default number of columns for tiled MultiGraphs.

### Rows

Enter the default number of rows for tiled MultiGraphs. If you want an unlimited number of rows on one MultiGraph page, enter 0 for rows.

### Center text

Check this item to center results text in the element space. If unchecked, results text is left aligned.

### Clip text

Check this item to keep results text from entering adjacent element space.

### Keep elements square

When checked, the histogram elements in the MultiGraph will always be square, preventing distortion of the histogram graphics. The overall size of the elements may shrink with this checked, since the program will use the lesser of the X and Y element size to determine the space in which to draw.

### Element spacing

Choose the amount of space, in pixels, to leave between elements in a MultiGraph.

## Other options

### Picture creation size

The value specifies the virtual size for a histogram window when creating a picture (metafile) of the histogram to add to the MultiGraph. Enter a larger value to reduce the size of fonts in the picture relative to other aspects of the histogram, or a smaller size to increase the fonts. Default value, 7.6 cm. or 3.0 in., works well in most cases.

### Dot size

This option allows you to select a dot size to use when adding dot plots to the MultiGraph. The dot size can be the same as the histogram dot size, or you can choose a different setting. This may be useful if your MultiGraphs are not printing dots correctly to high-resolution printers, in which case you would choose a larger dot size for the MultiGraph than what you have for the histograms normally.

### Histogram quality

This option controls the quality of the histogram graphics that are generated when histograms are added to a MultiGraph. Lower quality settings will produce graphics that require less memory, draw faster, and print faster. Perfect quality requires the most memory and draws the slowest, but produces the best output possible from the program. Experiment with this setting to find a suitable quality for your purposes.

### Copy background color

When enabled, the background color for histograms is copied with the histogram graphic. When this option is not enabled, the background color of the MultiGraph appears behind transparent histogram graphics.

### Text lines per element

When copying an entire MultiGraph to the clipboard, this setting determines how to scale the font used for results window elements. Enter a value that is at least as big as the maximum number of rows for the results elements contained in the MultiGraph.

### Grid offset

This option allows you to align grids for left-to-right and right-to-left view MultiGraphs. The value is the fraction of the MultiGraph window to offset the grids so that they align with the axes of the elements. Default value is 0.15.

### Save preferences

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

## Region Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to regions created by WinList. Individual regions can override these preferences using the Edit Properties for Region dialog.
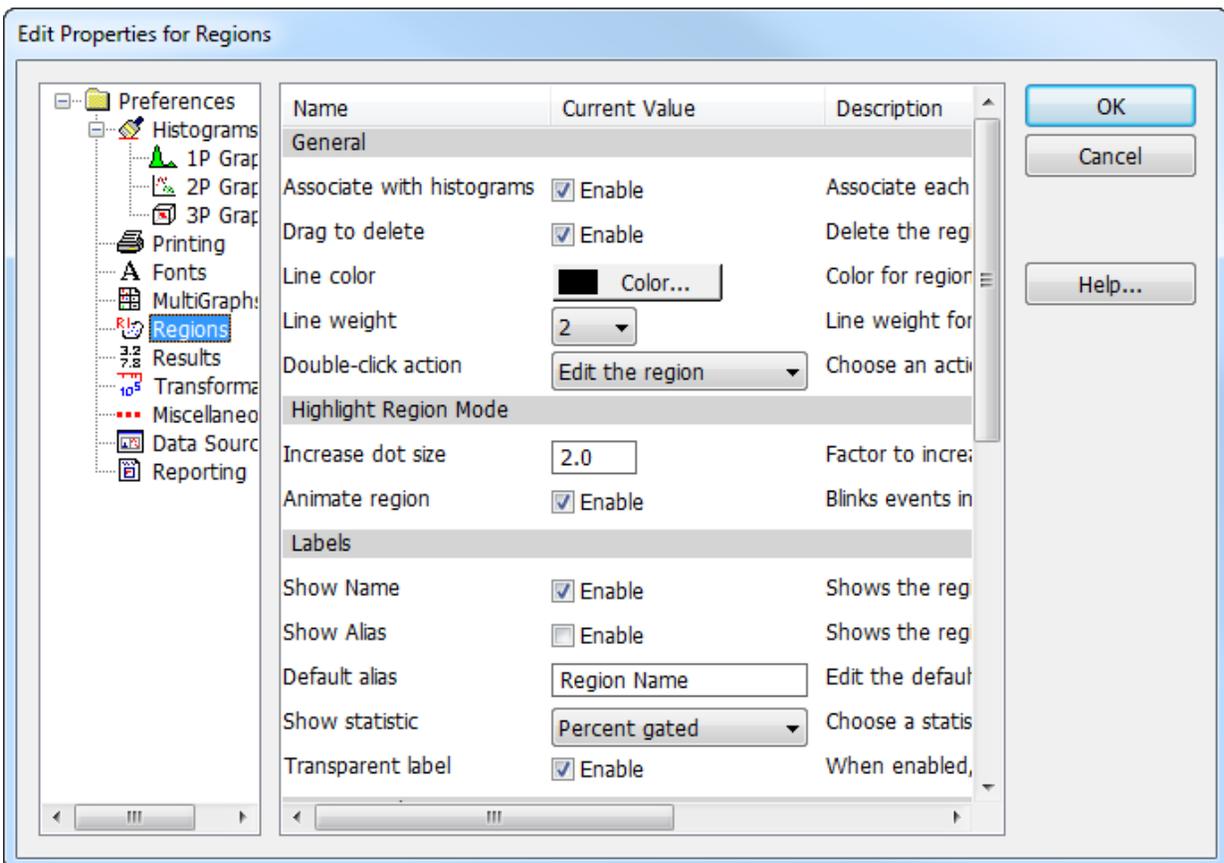


## General

### Associate with histograms

When this option is enabled, a region becomes associated with the histogram in which it was originally created. If you delete the region, the program remembers the association and will only recreate the region in the same histogram.

With the option disabled, you can delete a region from one histogram and recreate it in another histogram.

**Drag to delete**

When enabled, region labels can be dragged off the left or bottom of the histogram window to delete them.

**Line color**

Choose a color to use for region lines.

**Line weight**

Choose a line weight to use for region lines. The value is in points, a unit of 0.35 mm or 1/72 in.

**Double-click action**

This option allows you to choose what happens when you double click on a region's label. Options include editing the region or creating a results window.

**Highlight Region Mode**

Highlight-this-region mode is enabled for a region by using the region's context menu.  It will highlight the region's events in all of the dot plot histograms.

**Increase dot size**

This field is the factor to increase 2P dots by when a region is in "highlight this region" mode.

**Animate region**

Enable this preference to blink the dots when a region is in "highlight this region" mode.

**Labels**

**Show Name**

Enable this option to show the region's name (e.g. "R1") in the label for new regions.

**Show Alias**

Enable this option to show the region's alias (e.g. "Lymphocytes") in the label for new regions.

**Default alias**

This option allows you to choose the default alias for regions. The alias can be displayed instead of or in addition to the region's name.

**Show statistic**

This option allows you to show a statistic in region labels, by default.  Choices include **None**, **Percent Gated**, **Percent Total**, and **Region Events**.

**Transparent label**

Enable this option to allow graphics under the region label show through. With the option disabled (unchecked), the region label is filled with a solid background color.

## Region Tools

**Restore Selection tool**

When this option is enabled, the region selection tool is reselected after you finish working with one of the region creation tools. This behavior is similar to how PowerPoint tools work.

**1P region tool**

Select the region tool for 1P histograms that you want selected each time WinList starts-up. For 1P regions, the options include the Select tool and the Range tool. These tools are found in the histogram toolbar and determine what happens when you click the mouse in a 1P histogram.

**2P region tool**

Select the region tool for 2P histograms that you want selected each time WinList starts-up. For 2P regions, the options include the Select, Freehand, Rectangle, Ellipse, and Contour tool. These tools are found in the histogram toolbar and determine what happens when you click the mouse in a 2P histogram.

## 1P Regions

**1P region style**

Select a style to use when drawing 1P regions. Region options are available in the 1P Region Context Menu .

## 2P Regions

**Show resizing handles**

This option determines whether the resizing handles are automatically shown when a 2P region is activated. Note, you can always show resizing handles for individual regions by using the 2P region context menu.

**Show vertices**

This option determines whether vertices are shown when a 2P region is activated.

**Number of vertices**

This option controls the number of vertices that are *displayed* for 2P regions when you enable that option. Choose 0 to display all vertices for the region. Note, this does not affect the actual number of vertices for a region, only the number that are displayed.

## NStat Regions

### NStat label

This list allows you to choose what to display for the NStat region labels. Choose from Name, Alias, or both. The label appears on the movable label for the NStat. Region options are available in the [NStat Context Menu](#) .

### Sub region label

This list allows you to choose what to display for the sub region labels. Choose from Name, Percent gated, or both. The sub region label appears in the corner of the subregion and is not movable.

### Save preferences

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**Results Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to results windows.
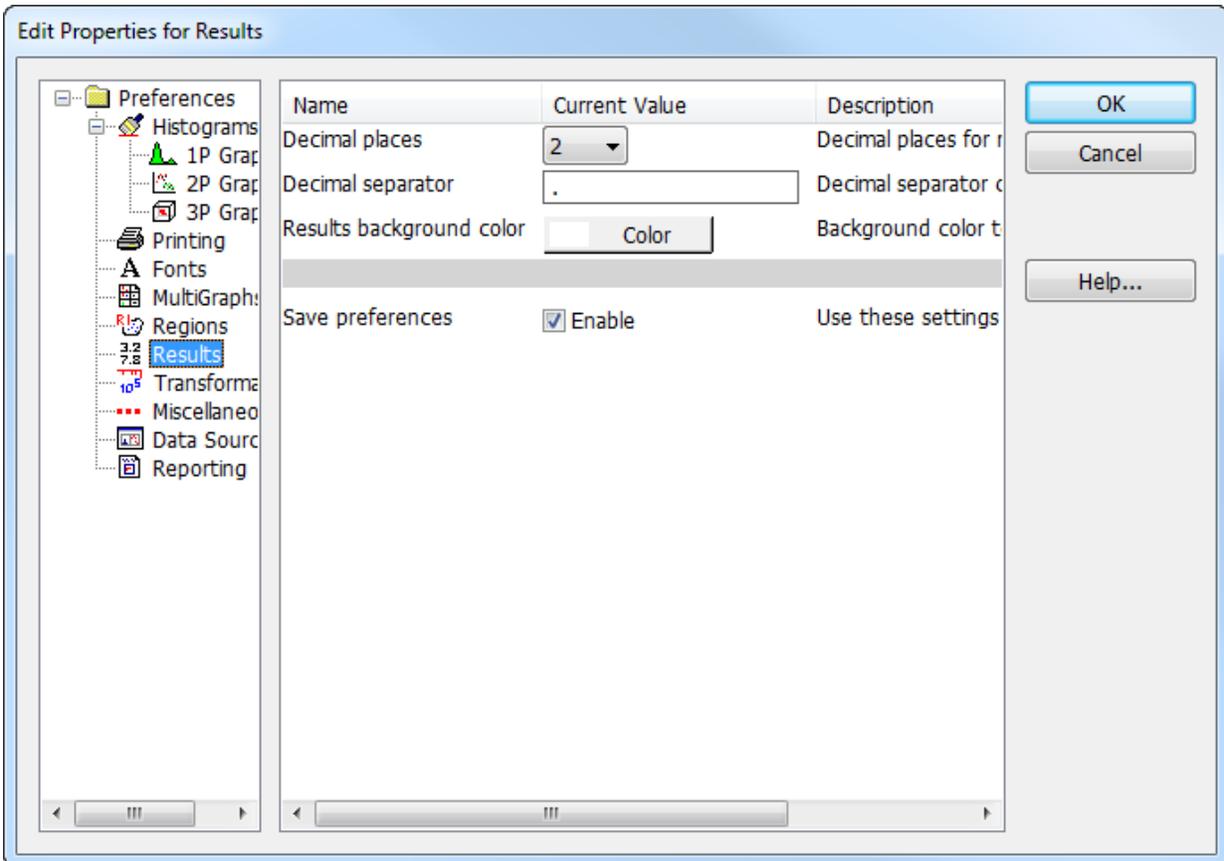


**Decimal places**

Choose the number of decimal places you want to display in results windows.

**Decimal separator**

Type the character that you want to use as the decimal separator. The default value is a period (.).

**Results background color**

Choose a color to use as the background for results windows.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**Transformation Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.
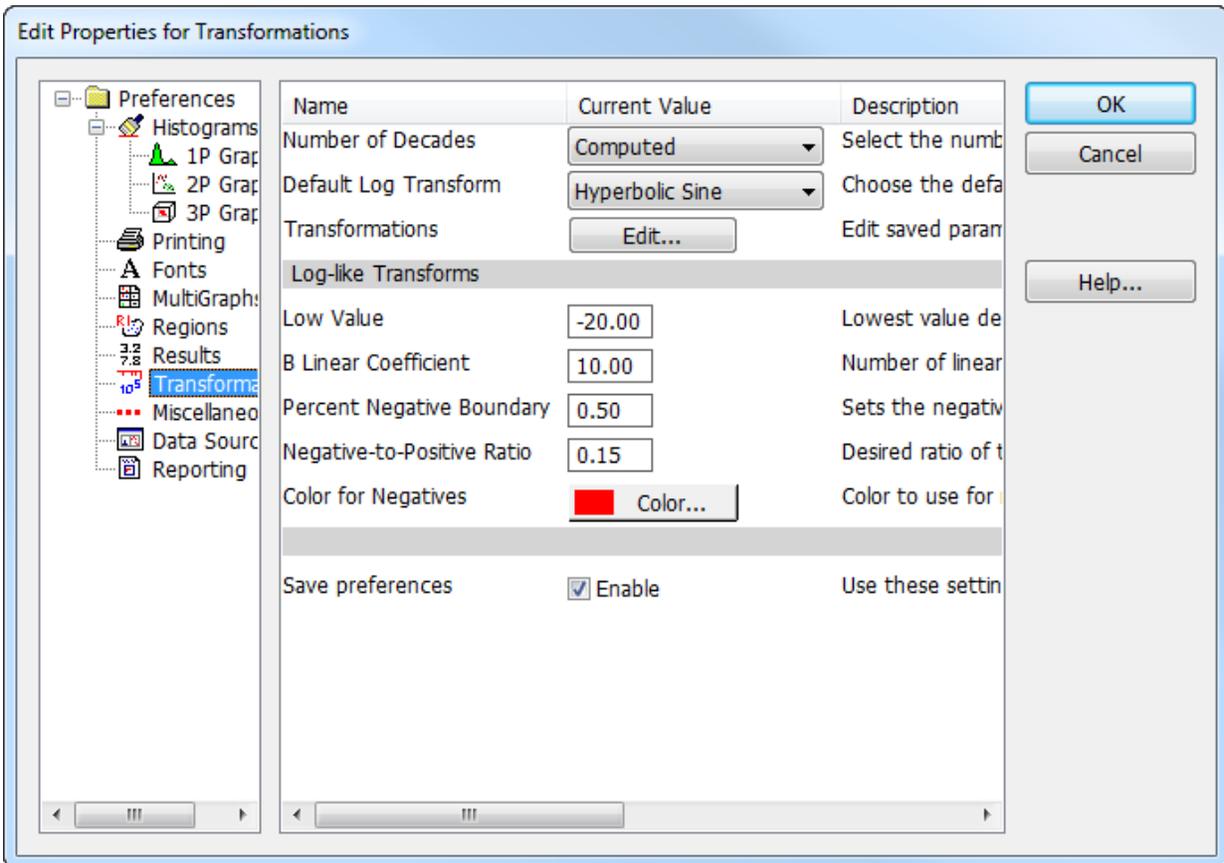


This preference section controls what parameters WinList will automatically convert from linear to log, and how that conversion will be performed. These preferences are most important for data files from newer, digital instruments, which store all parameters in linear form.

WinList approaches this problem by making internal calculations to convert parameters from linear to log, and allowing you to set preferences to control which parameters are converted to log. You can override the preferences for individual data files.

**Number of Decades**

This option determines how many log decades WinList will transform the linear data values into.

Choose *Computed* to allow the program to determine automatically the number of decades. With this option selected, the program computes the number of decades by taking the log of the ADC resolution of the parameter. For example, if the ADC resolution is 262144, the log10(262144) is approximately 5.42 decades.

Choose *4-decades* to force the program to map the linear data into 4-decades. This can change the shape of distributions because the scaling is performed in linear space to map to 10,000 linear units.

## Default Transform

This selects the default transform to use for log-like data: Log, HyperLog, Hyperbolic Sine, or VLog.

## Transformation

This command displays the [Edit Transformations](Edit Transformations) dialog to edit transformations for all saved parameters.

## Log-like Transforms

### Low Value

This sets the default lowest value shown on the axis.

### B Linear Coefficient

This sets the default B Coefficient value for HyperLog, Hyperbolic Sine, and VLog transformations.

### Percent Negative Boundary

This value is used for parameters that have Auto-Adjust enabled for HyperLog. The value represents the percentage of negative events that will be allowed to be off-scale in the HyperLog axis. For example, with the default value of 3.0 approximately 3% of negative events will pile up on the lowest displayed channel, and 97% of events will be visible after the Auto-Adjustment is complete.

### Negative-to-positive ratio

This value is used for parameters that have Auto-Adjust enabled for HyperLog. The value represents the relationship of negative and positive portions of the axis. With the default value of 0.2 and a parameter that has a maximum value of 262144, the negative portion of the axis will be approximately 0.2 * 262144, or 52428.8 channels.

### Color for Negatives

Select a color to use for the negative portion of a HyperLog axis.

### Save preferences

When this option is enabled, the changes you make to the Transformations dialog are remembered.

You can override these settings for individual data files.

See also:

Edit Transformations

Understanding Hi-res Data Files

## Miscellaneous Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to various WinList objects.
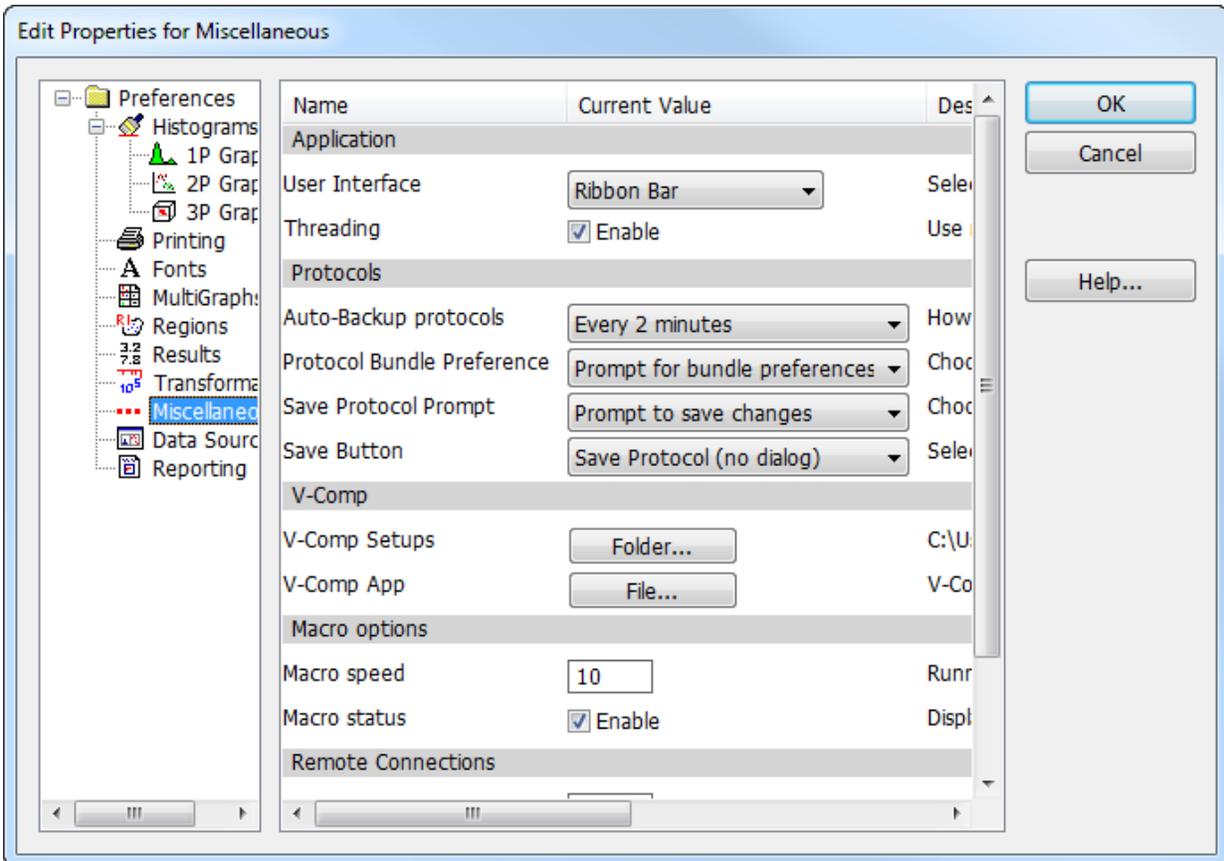


## Application
### User Interface

WinList provides two user-interface options: **Toolbar & menu** or **Ribbon Bar**.  The default interface is the Ribbon Bar.  For details on the differences between these interfaces, see Main Window, Toolbar, and Ribbon Bar.

### Threading

Enable this option to improve performance on computers with multi-core processors.  Most users can leave this option enabled, and disable the option only if advised by tech support.

**Protocols**

### Auto-Backup protocols

WinList can automatically save a backup of the work you are doing in the program. This option allows you to choose how often to save a backup. The backup files are automatically removed when the program closes normally. If the program does not close normally, the backup files will be detected by the program when it starts up again, and it will offer to recover your work from the backup.

### Protocol Bundle Preference

This preference determines what happens when WinList opens a protocol bundle that contains a preference file.

"Don't use bundle preferences" will ignore the preference file that is in the bundle.

"Prompt for bundle preferences" will ask the user whether the preferences from the bundle should be used.

"Use bundle preferences" will use the preference file that is in the bundle without asking.

### Save Protocol Prompt

When closing the program, WinList can detect whether there are unsaved changes in your protocol. This option controls whether or not the program alerts you that the protocol has changed.

### Save button

The Save Protocol button on the main toolbar can behave like the Save Protocol command, the Save Protocol As command, or the Save Bundle command. Select the behavior for the button with this preference. The program will need to be restarted for the change to take effect.

**V-Comp**

### V-Comp Setups

This property contains the path to V-Comp setup files. Typically, WinList can determine this path correctly without intervention. However, if you store V-Comp setup files in a common location with other users, you may need to set this path explicitly.

### V-Comp App

This property points to the V-Comp application, if you have it installed on your computer. It allows WinList to launch V-Comp directly from the Compensation toolbox.

**Macro options**

### Macro speed

This option controls how fast macros and protocols playback. It is the minimum amount of time, in milliseconds, between each line of a macro. The default value is 10 milliseconds.

**Macro status**

This option controls whether or not the macro status dialog is displayed as a macro is played back.

**Remote Connections**

**DDE Timeout**

This option determines how long WinList waits for a remote script command to complete. The default value is 10000 milliseconds.

On some computers, the operating system waits for the full timeout period before completing. In these cases, experiment with lower settings for the DDE Timeout. On newer hardware, a setting of 1000 typically works fine, and it can save 9 seconds of unnecessary waiting for the transaction to complete.

**Save preferences**

When checked, these preferences will be stored as new default preferences when you click OK to close the dialog. If unchecked, changes are only applied to this session of the program.

**Data Source Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to Data Source windows and listmode data files.
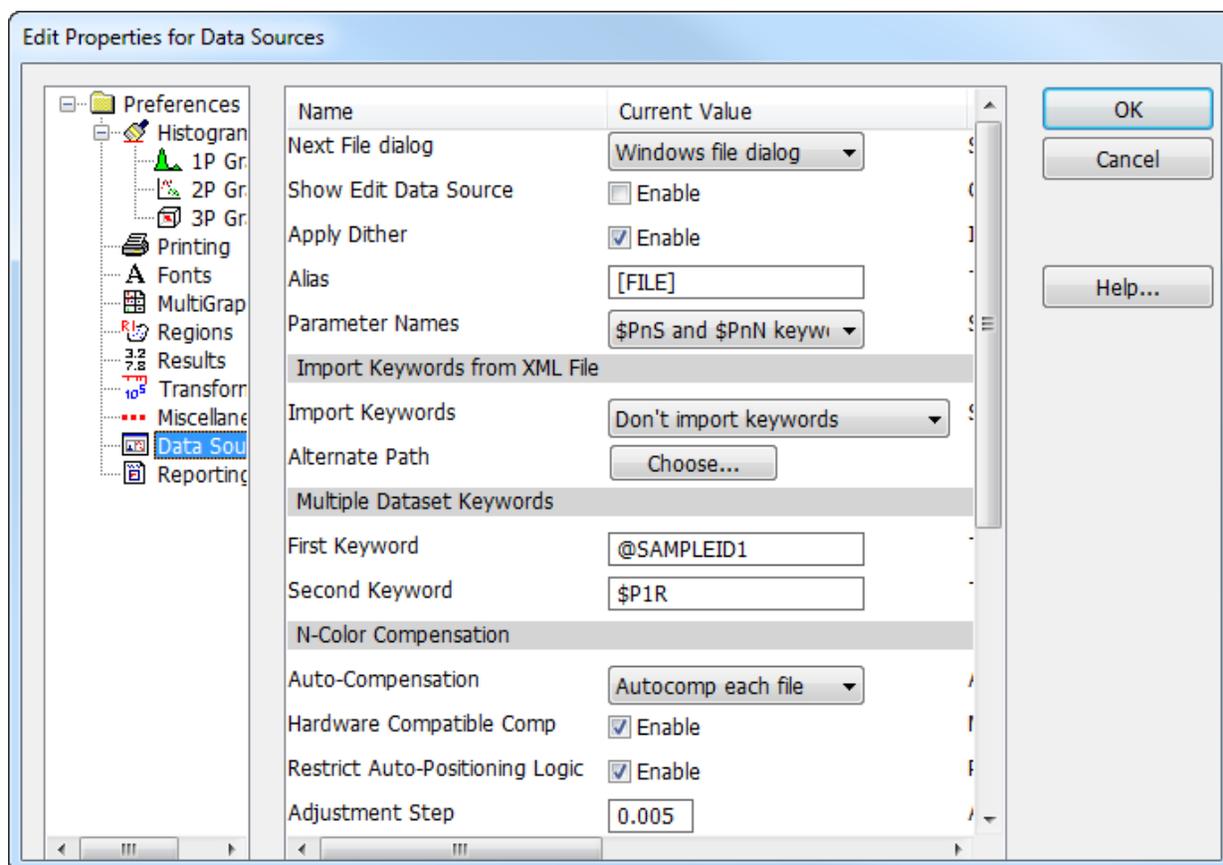


**Next File dialog**

This option determines what dialog you see when you use the Next File command to read a new FCS file into a data source.  The **Windows file dialog** is the standard file dialog you are familiar with, and this is the default option recommended for most users.  The **Quick file dialog** is a simplified dialog that displays faster and has limited choices.  It is most useful when files are in the same folder.

**Show Edit Data Source dialog**

Enable this option if you want the Edit Data Source dialog to be displayed when creating a new data source window with the Open Listmode Data Source command.

**Apply Dither**

This option determines whether or not WinList applies a small dithering value to each event as it is read from disk. Dithering is used to spread values across histograms for better visualization. In theory, dithering corrects for a partial truncation that occurs on many instruments during acquisition. Dithering has virtually no impact on statistics.

**Alias**

Enter the keyword to use to set the alias for data sources. By default, the name of the FCS file that is opened is used.

**Parameter Names**

This preference determines what strategy WinList will use to assign names to the parameters in the data files you open. Choose **Automatic** to allow WinList to try to determine the best strategy. Other options allow you to specify various combinations of the $PnS and $PnN keywords that are stored in the data file.

**Import Keywords from XML File**

**Import Keywords**

This option allows WinList to import additional keyword information from an XML file and add those keywords to the keywords from the current FCS file.  The XML file must be named the same as the FCS file, with "**.xml**" appended to it.

There are three options for this:

| Don't import keywords: | This option disables import of XML keywords. |
|---|---|
| Import XML from FCS file path: | With this option selected, WinList will look for an XML file in the same path as the FCS file.  For example, if the FCS file's name and path are "C:\Data\PatientFile123.fcs", the program would look for "C:\Data\PatientFile123.fcs.xml" for the keywords. |
| Import XML from Alternate Path: | With this option selected, WinList will look for an XML file in the path specified by the **Alternate path** preference below. For example, if the FCS file's name and path are "C:\Data\PatientFile123.fcs" and the Alternate Path is "D:\XML", the program would look for "D:\XML\PatientFile123.fcs.xml" for the keywords. |

**Alternate Path**

This option is only used if **Import XML from Alternate Path** is selected for the **Import Keywords** property above.  The property defines where the XML files will be located.  Click the Choose button to navigate to a folder.

## Multiple Dataset Keywords

### First Keyword

Enter the name of a keyword to display when WinList detects an FCS file with more than one dataset in it.

### Second Keyword

Enter the name of a keyword to display when WinList detects an FCS file with more than one dataset in it.

## N-Color Compensation

### Auto-Compensation

Some instrument manufacturers encode compensation settings directly into the FCS files that you read into WinList. If you want WinList to use the compensation settings that you set up on the instrument, you can enable one of the options provided in this list.

### Hardware compatible comp

Enable this option to use the force compensation settings to behave like hardware-based compensation. This makes all values subtractive.

### Restrict Auto-Positioning Logic

When enabled, WinList uses a more restricted portion of histograms to determine how to automatically position compensation Trace Lines. For most cases, this option will have little effect on how the program positions the trace lines. In some cases where there is significant signal cross-over, enabling this option can improve the auto-positioning.

### Adjustment Step

Set the amount of change associated with the "**+**" and "**-**" compensation controls.

## FCS

### FCS Version

Select the FCS version to use for FCS files that WinList creates.

## Snap Properties

### Snap-to-grid

With this option enabled, histograms are aligned to the data source grid when they are moved or sized.

### Grid spacing

This option specifies the size of the snapping grid, in pixels.

### Grid color

Choose a color for the snapping grid lines displayed in the data source.

**Background color**

Choose a color for the background of the data source.

**Reporting Preferences**

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to Report windows.
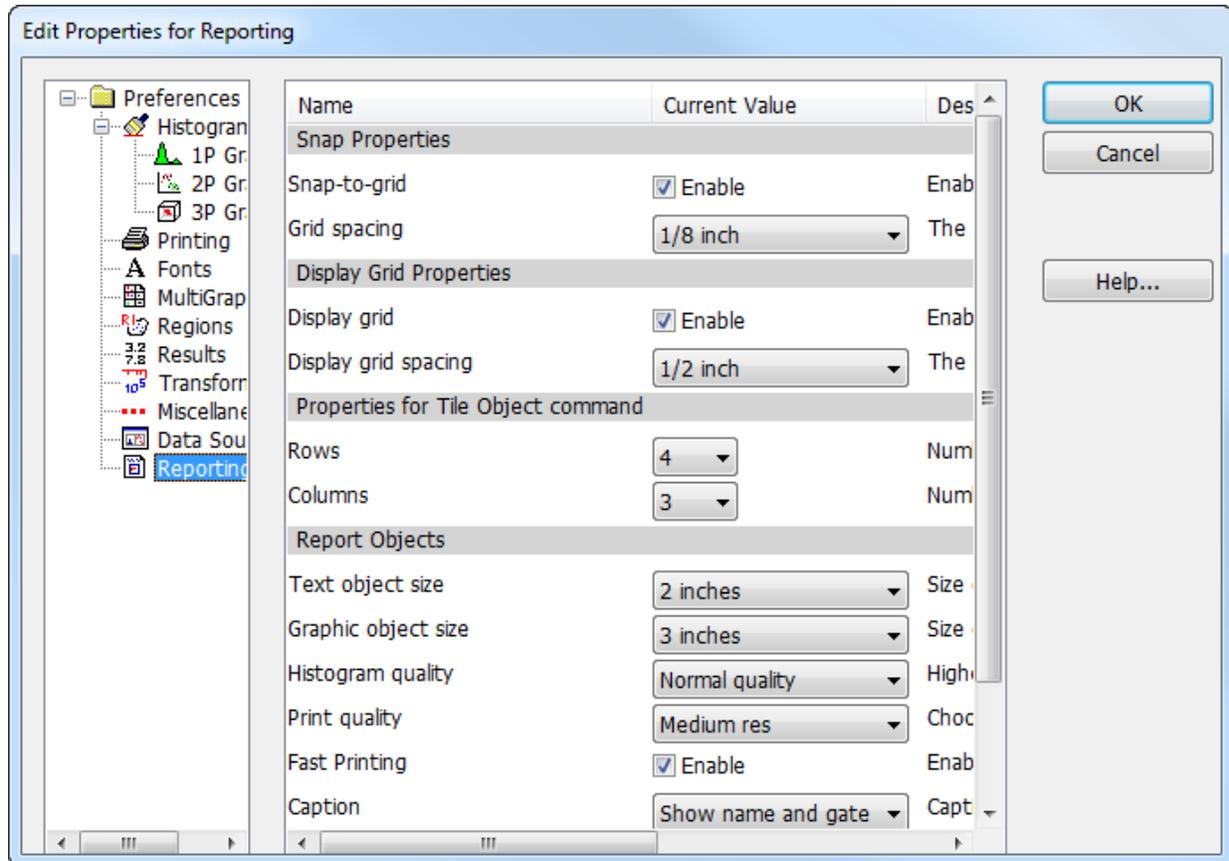


**Snap Properties**

**Snap-to-grid**

With this option enabled, objects are aligned to the report grid when they are moved or sized.

**Grid spacing**

This option specifies the size of the snapping grid, in inches.

## Display Grid Properties

**Display Grid**

Enable this option to show a grid on the report background. The display grid does not affect how objects are aligned automatically, but can be used for visual alignment of objects.

**Display grid spacing**

This option specifies the size of the display grid, in inches.

## Properties for Tile Object command

**Rows and Columns**

Enter the number of rows and columns that you want to create when the Tile command is selected for a report.

## Report Object

**Text object size**

This setting determines the size of text objects when they are added to a report. Note: Objects can be resized after they are initially created.

**Graphic object size**

This setting determines the size of graphic objects when they are added to a report. Note: Objects can be resized after they are initially created.

**Histogram quality**

This option controls the quality of the histogram graphics that are generated when linked histogram objects update on the report. Lower quality settings will produce graphics that require less memory, draw faster, and print faster. Perfect quality requires the most memory and draws the slowest, but produces the best output possible from the program. Experiment with this setting to find a suitable quality for your purposes.

This preference can be overridden for individual histogram objects on a report, using the Edit Histogram Link dialog.

**Print quality**

This option controls the quality of used when printing reports. Select the resolution that provides the best speed and quality for your use.

**Fast Printing**

With this option enabled, WinList renders graphics to bitmaps before sending them to the printer.  With most printers, this results in faster printing.  If your printer seems to print graphics from WinList slowly, compare printing speeds with the option on and off.

**Caption**

This option determines how captions will be displayed for histograms, by default. Individual histograms on reports can override this preference.

**Edit Program Controls**

This dialog box can be brought to the screen by choosing **Edit Program Controls** in the **Edit** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Edit** menu.

The settings within this dialog box control many of WinList's resources such as **regions**, **gates**, and the **data packet size**. The settings have an impact on how much memory the program uses.
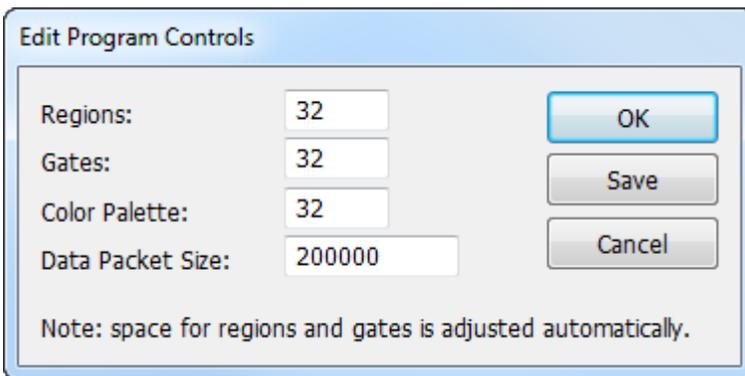


**Regions**

This field sets the initial number of regions allocated by WinList. The program will automatically allocate for more regions as needed.

**Gates**

This field sets the initial number of gates allocated by WinList. The program will automatically allocate for more gates as needed.

**Palette size**

This field sets the number of colors in WinList's color palette. The palette is used by gates and other graphical objects.

**Data Packet Size**

This field sets the packet size used for sampling your FCS files. This sample packet allows the program to update the histograms and results rapidly, without replaying the entire file for every change you make.

You can increase this value to work with more events from your file. Increasing the packet size also increases the amount of memory used by the program, so you may need to experiment to find a value that works well for the computer you are using.

The program can display results for the entire listmode file at any time by clicking the **Replay** button.

After you change the Data Packet Size, you need to re-read any FCS files that are currently open in order for the new packet size to take effect. Use the **Next FCS File** in the File menu to read the files again.

### Save

Click this button to cause WinList to use the displayed settings each time the program is started.

### OK

Click this button to close the dialog and apply any changes to open data sources.

### Cancel

Click this button to exit with no changes.

**Wildcards and Pathways**



This command is found on the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **File** menu.

This command initiates a dialog box that allows the user to set search criteria and pathways for various file types.
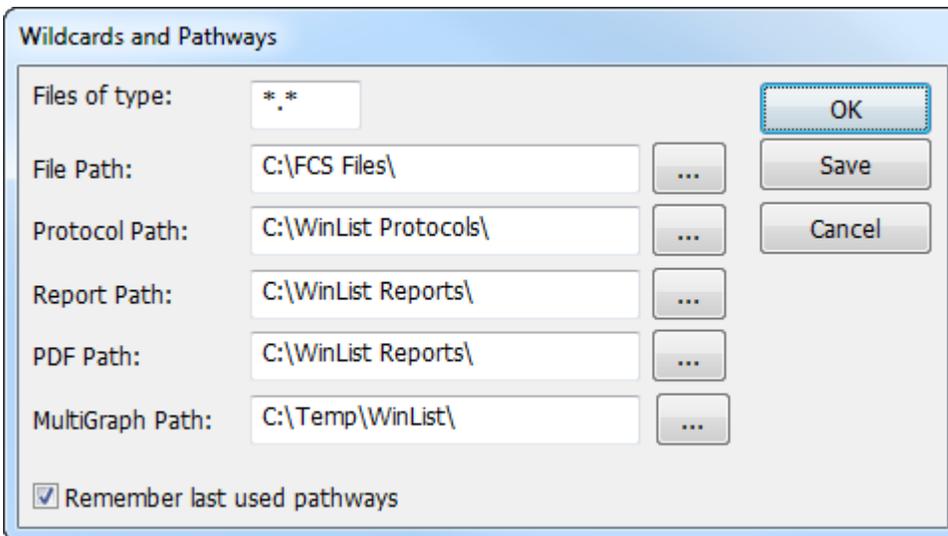


**Files of type**

Enter the FCS file specification that WinList will use to display listmode files.

**File Path**

This edit box displays the pathway where WinList will look for listmode data files. You may manually enter the pathway, or click the "**...**" button to display a dialog that allows you to pick a folder. The PC version uses a backslash (\) to separate parts of a path.

**Protocol Path**

This is the default location for WinList protocols, bundles, and many other files that WinList reads and writes. You may manually enter the pathway, or click the "**...**" button to display a dialog that allows you to pick a folder.

### Report Path

This is the default location for report files. You may manually enter the pathway, or click the "**...**" button to display a dialog that allows you to pick a folder.

### PDF Path

This is the default location for PDF report files. You may manually enter the pathway, or click the "**...**" button to display a dialog that allows you to pick a folder.

### MultiGraph Path

This is the default location for MultiGraph files. You may manually enter the pathway, or click the "**...**" button to display a dialog that allows you to pick a folder.

### Remember last used pathway

If this option is checked, the program will automatically save the last location where you opened or saved data files. Uncheck this option if you want WinList to use the pathways you enter in this dialog each time it starts up. Check this option if you want it to use the last pathway you were in each time it starts.

### OK

Click this button to accept changes to the dialog box. Changes will be used for the current session of WinList only.

### Save

Click this button to accept changes to the dialog box and store them as default settings for WinList.

### Cancel

Click this option to exit with no changes to the dialog box.

**Help**

**User Guide**

Help commands are found on the **Help** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Help** menu.

Selecting this menu option launches WinList's on-line help system and displays the index to the system. Most of WinList's documentation is available through this system.

**Check for Updates**

This command is found on the **Help** tab of the ribbon bar.  In the menu & toolbar interface, the command is in the **Help** menu.

Use this command to check the Verity Software House web site for program updates. This requires internet connectivity, and if updates are found you will also need administrative rights for the computer.

*On some operating systems, you may be presented with an option that offers to protect your computer from unauthorized program activity. This option interferes with the ability of the updater program, and must be **unchecked**.*



The command launches another application, VSH_Updater.exe, which interacts with Verity's web site and does the actual downloading and updating. The updater application must be in the same folder as the WinList application in order to operate correctly.

**About WinList**

This command is found on the **Help** tab of the ribbon bar.  In the menu and toolbar interface, the command is in the **Help** menu.

Selecting this option displays WinList's opening banner dialog. This dialog displays information about the version of the program, the registered user, system information, and contact information for Verity Software House.

**Research and Compliance Modes**

**Research and Compliance Modes**

WinList can be set up to run in either a Research-Use-Only mode, or in Compliance mode. There are some differences in how the program operates in each mode.

- Compliance mode depends on operating system user groups to control the level of access to program features. Research mode allows any user to perform any operation.
- Compliance mode displays a login dialog when the WinList is starting up to verify the user and determine the group to which the user belongs.
- Compliance mode enables logging so that there is a record of the activities of each user. Research mode can be run with logging enabled or disabled.
- Compliance mode is designed to support clinical labs and can be part of a compliance plan for the US-FDA's 21 CFR Part 11 regulations. Research mode does not provide compliance with 21 CFR Part 11.
- Both modes use the same intuitive interface and WinList engine.

## Setting Up Research Use Only Mode

After initial installation, WinList will automatically be in Research mode. There is no further configuration required.

To revert to Research mode after setting up Compliance mode, the user groups that were created for Compliance mode must be removed. Once the user groups are removed, the program will revert to Research mode.

## Setting Up Compliance Mode

*Setting up Compliance mode is something that should be performed by IT personnel.*

While it is not a complicated task, it requires administrative access rights and a clear understanding of user groups and permissions. It is therefore something that we strongly recommend be performed by the IT team at your institution. The task involves creating several *required* WinList user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode. Click here for details.

## 21 CFR Part 11 Compliance Matrix

This matrix provides a simple summary of how WinList in Compliance mode helps you with 21 CFR Part 11 compliance. Click here for details.

**Setting Up Compliance Mode**

**Setting up Compliance mode should be performed by your IT team. It requires administrative rights and a clear understanding of your network operating system.**

The task involves creating several *required* WinList user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode.

## WinList User Groups

There are three (3) user groups for Compliance mode: **WinListAdmins**, **WinListEditors**, and **WinListUsers**.  Optionally, you can define an additional user group, **WinListResearchers,** for research users within the Compliance setting who are not restricted by Compliance requirements.

The table below shows the features that group members can access.

| Feature | WinListAdmins | WinListEditors | WinListUser | WinListResearchers |
|---|---|---|---|---|
| Run a batch of files | Yes | Yes | Yes | Yes |
| Add & remove files from batch | Yes | Yes | Yes | Yes |
| Open protocols | Yes | Yes | Yes | Yes |
| Edit databases | Yes | Yes | | Yes |
| Create & edit protocols | Yes | Yes | | Yes |
| Edit logging options | Yes | | | Yes |
| Check for program updates | Yes | | | Yes |
| Edit preferences | Yes | | | Yes |

## Creating User Groups

The program requires at least one of the WinList user groups to be defined in order to run in Compliance mode.  WinList user groups can be defined for your computer or for your entire domain using tools provided by the operating system.  This is typically done by information technology personnel because it requires administrative rights.

The advantage of defining the user groups at the domain (network) level is that the groups are defined *for all computers* on the domain at the same time.  If you define the user groups on an individual computer, those groups are available *only on that computer*.

Below we describe how to create user groups on a Windows server or on a Windows compute as examples.  The specific methods you need to use may differ depending on the version of the operating system you are working with.

After you have defined the user groups and added users to them, WinList will detect the groups when it starts up and operate in Compliance mode automatically.

## Windows Server User Groups

Follow these steps to define user groups for an entire network or domain. **Note: if you use this technique, you do not need to define user groups on each individual computer.**

- On a domain server, launch the "Active Directory Users and Computers" administrative tool.
- Right click the "Users" folder in the left panel and choose New->Group.  The New Group dialog is displayed.
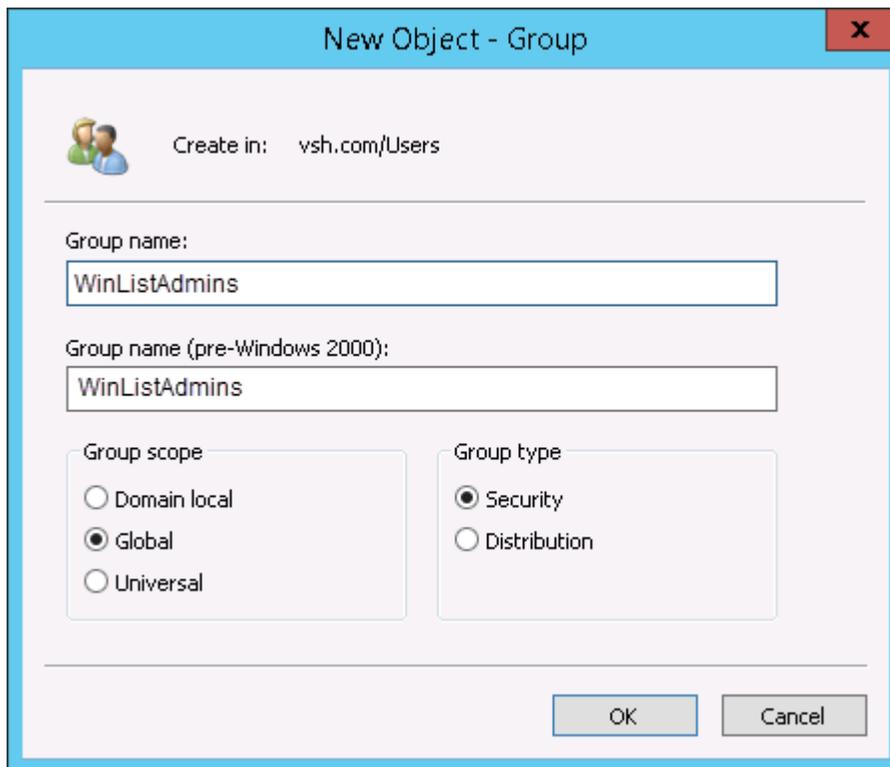
- Type **WinListAdmins** for the group name and click OK.
- Once the group is created, double-click the group in the list to display the Properties for the group.
- Click the **Members** tab and click **Add** to select members for the WinListAdmins group.  You should have at least one user in the WinListAdmins group. Members can be added by typing user names, or by using the dialog's search features.
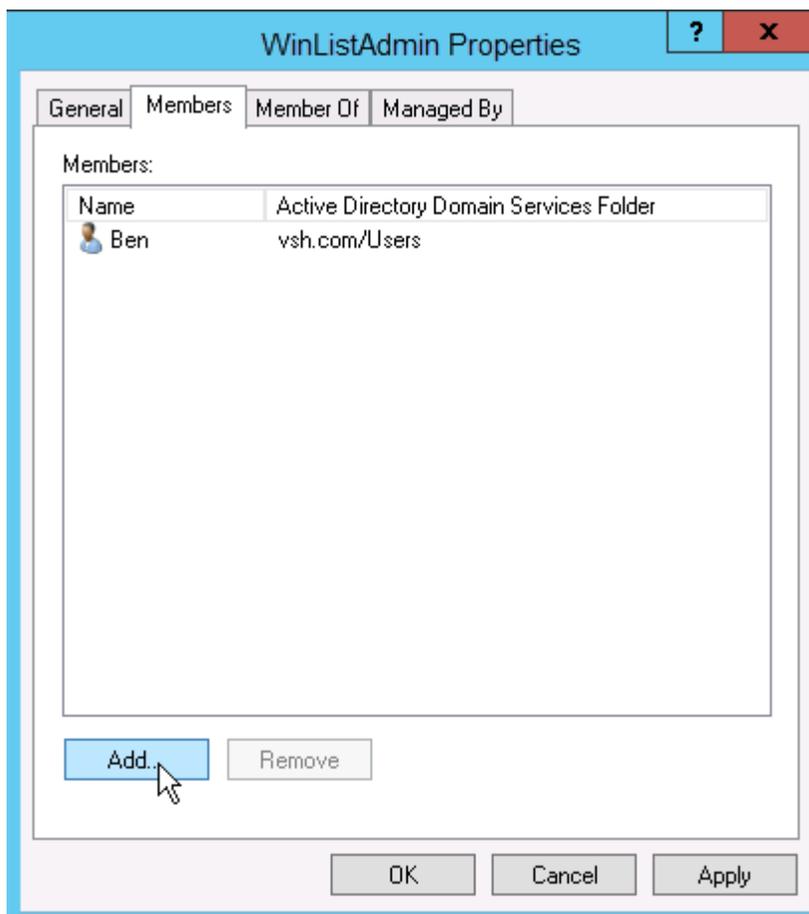
- Repeat this process for the **WinListEditors**, **WinListUsers**, and optionally **WinListResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up and is connected to the domain.

## Windows Computer User Groups

Follow these steps to define user groups for an individual Windows computer. **Note, if you already defined user groups for your domain, you do not need to define the groups again for each computer.**

- On a Windows 7 computer, right click Computer and choose "Manage". For Windows 8 and newer, right click the Windows button (lower left) and choose "Computer Management".  The Computer Management app will display.
- Expand "System Tools" and "Local Users and Groups" in the left panel.
- Right click the "Groups" folder and choose "New Group".  The New Group dialog is displayed.
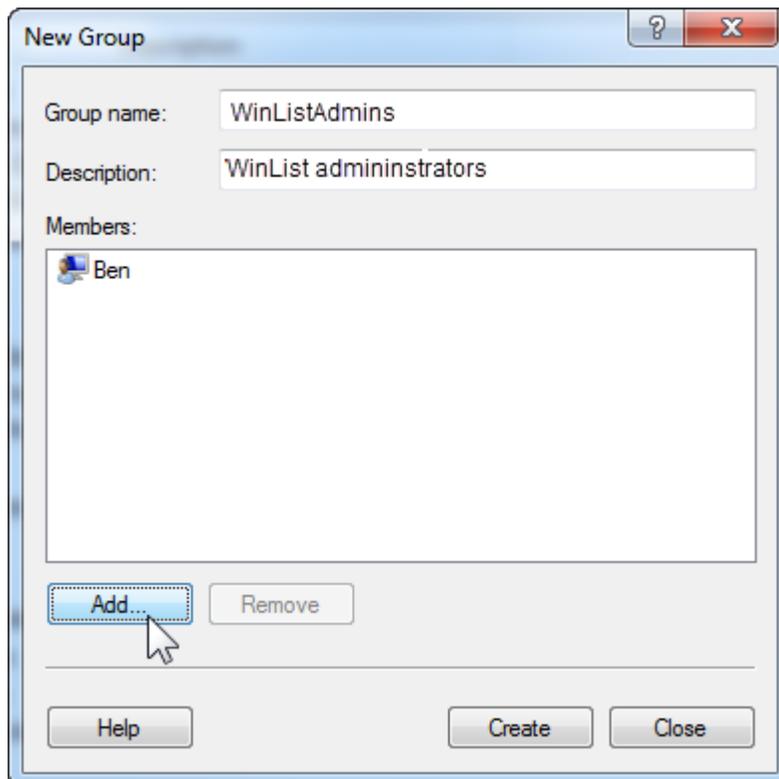
- Type **WinListAdmins** for the group name and enter a description for the group.
- Click **Add** to select members for the WinListAdmins group.  You should have at least one user in the WinListAdmins group.  Members can be added by typing user names, or by using the dialog's search features.
- Click **Create** to create the new local user group.
- Repeat this process for the **WinListEditors**, **WinListUsers**, and opytionally **WinListResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up on that computer.


See also:

[21 CFR Part 11 Compliance](#)

## 21 CFR Part 11 Compliance

WinList can be part of your lab's compliance strategy for 21 CFR Part 11 when it is setup and operated in Compliance mode.  It has been developed under a quality management system and validated with a suite of quality assurance tests. The table below summarizes how the key elements of Part 11 relate to WinList and its use in Compliance mode settings.

| Section | Summary of Rule | Compliance Strategy |
|---|---|---|
| 11.10 | Controls for closed systems | WinList can be accessed only through the user interface; it is a closed system. |
| 11.10 (a) | Validation and accuracy. | WinList uses externally-generated data to validate its results and employs proprietary binary format, static PDF, and/or CRC methods to ensure valid files. |
| 11.10 (b) | Record generation and readability | WinList generates Xml-format protocol files, CRC-checked log files, and static PDF that are human-readable and machine-readable. |
| 11.10 (c) | Record protection and retrieval | Record retention and retrieval plan is managed by the laboratory's file management practices. |
| 11.10 (d) | Authorized access | WinList uses the operating system's user login validation, and user group membership determines permissions within the software. |
| 11.10 (e) | Audit trails | WinList employs a CRC-checked logging system with time stamps, and log files are preserved. Log files cannot be deleted by ordinary means. |
| 11.10 (f) | Operational system checks | WinList enables and disables portions of the UI to enforce permitted sequencing of steps and events. |
| 11.10 (g) | Authority checks | WinList uses the operating system user groups and login validation to assure authorized access. |
| 11.10 (h) | Device checks and validity | WinList performs validation checks on the FCS data files. |
| 11.10 (i) | Education, training and experience | WinList provides a User Guide, technical support, and tutorial videos to assist with the proper, intended use of WinList. Specific on-site training may be arranged. |
| 11.10 (j) | Electronic signatures | This is an individual laboratory requirement beyond the scope of use of WinList. |
| 11.10 (k) | Systems documentation | WinList version releases are accompanied by an updated User Guide. Verity employs document control procedures. |
| 11.30 | Controls for open systems | WinList is designed as a closed system. |
| 11.50 | Signature manifestations | WinList does not require or offer electronic signatures. |
| 11.70 | Signature linking | WinList does not require or offer electronic signatures. |

| | | |
|---|---|---|
| 11.100 (a) | Unique electronic signatures | Your laboratory must ensure that electronic signatures are unique to each user on the network. |
| 11.100 (b) | Identity verification | Each laboratory must verify its own employment records and IDs. |
| 11.100 (c) | Signature verification | Each laboratory must verify electronic signature eligibility with the FDA. |
| 11.200 (a)(1) | Two-component signing | WinList does not require or offer electronic signatures. |
| 11.200 (a)(2,3) | Authenticity and falsification | WinList does not require or offer electronic signatures. |
| 11.200 (b) | Biometric signatures | WinList does not utilize biometric IDs. |
| 11.300 (a) | Controls for identification | WinList uses the operating system user groups and login validation to assure unique user identities. |
| 11.300 (b) | Identification aging | The individual laboratory IT structure must control password checks and aging requirements. |
| 11.300 (c) | External ID validation | Not applicable. WinList does not utilize external tokens, cards, RFID, or other physical identity-confirming devices. |
| 11.300 (d) | Unauthorized use | The individual laboratory IT structure must control login authenticity and manage safeguards to prevent unauthorized use. |
| 11.300 (e) | External device testing | Not applicable. WinList does not utilize external tokens, cards, RFID, or other physical identity-confirming devices. |

**SY3200 Acquisition and Sort Control**

**SY3200: Overview**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

When the SY3200 Acquisition plug-in for WinList is installed with the program, WinList adds features that allow you to monitor data, acquire and sort data on SY3200 and Reflection cell sorting instruments.



Using WinList's powerful gating capabilities, it's fast and easy to create regions and define gates for sort decisions. With a one click, WinList transmits sort gates and N-Color Compensation to the SY3200, enabling extensive sort classifications. Events can even be color-coded in WinList based on how they actually sorted on the device.

**Next:**

SY3200: Monitoring

**SY3200: Monitoring**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

WinList is automatically launched by the SY3200 software. It establishes a connection with the SY3200 and has access to the parameters that are currently configured. Communication flows in both directions, with the SY3200 software sending data and commands to WinList, and WinList supplying information about sort gates and compensation. As changes are made on one side, they are communicated to the other.

**Creating or Opening a Protocol**

When the SY3200 software starts up, it displays the Create/Open Protocol dialog. This dialog is used to create new protocols for WinList as well as to open protocols you saved previously.
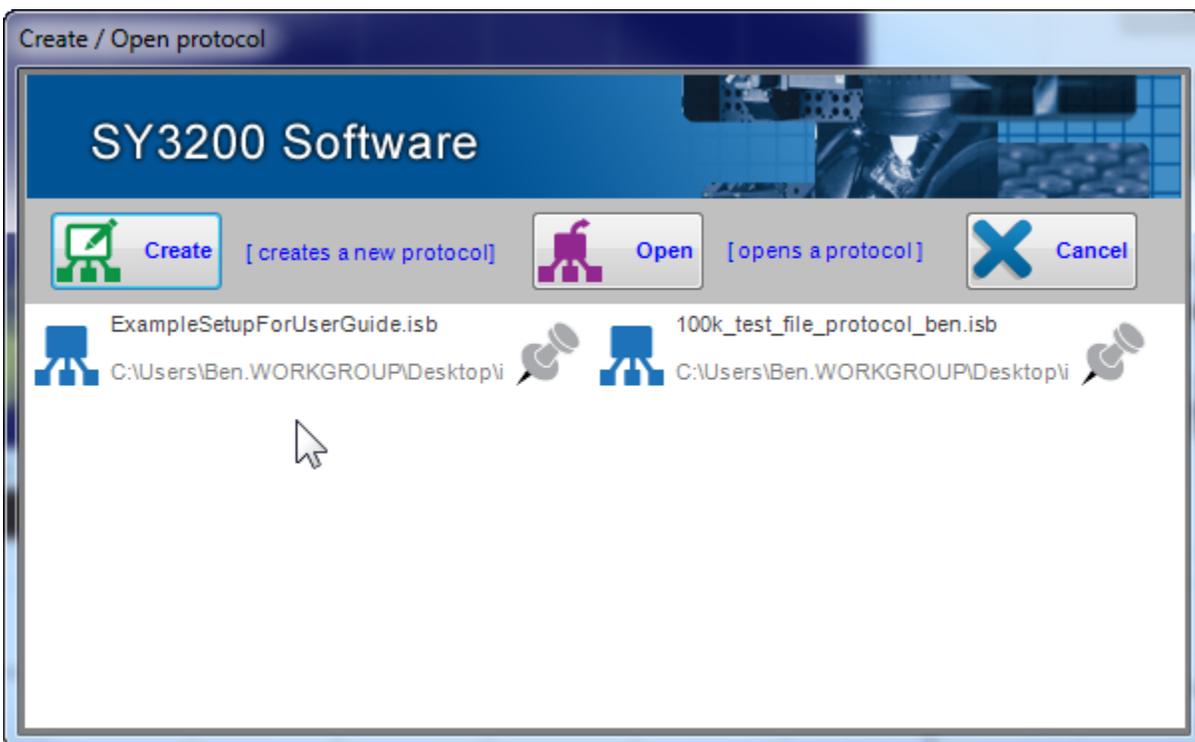


If you open an existing protocol, WinList will recreate the setup you had with histograms, regions, gates, and reports. Sort gates are remembered and transmitted to the SY3200 software. If you are creating a new protocol, define your histograms, regions, and gates as you normally would with WinList. See WinList Basics for an introduction to this.

WinList displays a special SY3200 toolbar at the bottom of the data source window, and it begins to monitor data transmitted from the instrument.
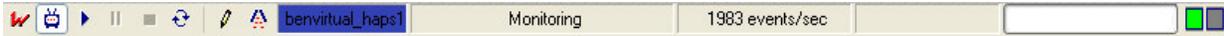


When monitoring, WinList continually updates the graphics and statistics with the latest data received from the SY3200. The toolbar and the data source title bar provide information about the sample and the number of events processed. WinList is not accumulating the data it receives in monitor mode; the most recent events are stored temporarily and they are replaced when new data is received.

Most of WinList's features are available during monitor mode. You can draw regions, define gates, view statistics, set up N-Color Compensation, and much more.

**Next:**

SY3200: Acquiring Data

**More Info:**

WinList Basics

Histogram Windows

Using Interactive N-Color Compensation

**SY3200: Acquiring Data**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

Once WinList is monitoring an SY3200 and you have a protocol defined, you can acquire data in just a few steps.

**To review and edit the properties of acquisition:**

1. Click the Acquisition Properties button on the SY3200 toolbar at the bottom of the data source window.



2. The Edit Acquisition dialog is displayed. Review and edit the properties for the acquisition, and then click OK.

**To acquire data:**

1. Click the Acquire button on the SY3200 toolbar.



2. Depending on the settings in the Edit Acquisition dialog, the Start Acquisition dialog may appear. If it does, you can edit the keyword values that will be stored in the file, and then click OK.

During acquisition, you can use the Pause, Stop, and Restart buttons on the toolbar. See the Toolbar section for details on these commands.

3. When the acquisition is complete, the Acquisition Complete dialog is displayed. In this dialog you can again edit the keywords that will be stored in the file, you can rename the output file, and you can review summary information about the acquisition.



4. Click OK to close the dialog and finish the acquisition.

**SY3200: Sorting**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

WinList provides very flexible and powerful sorting control. Sort decisions are made by creating regions and gates on histograms, setting up N-Color Compensation, and using the Edit Sort dialog to transmit the sort decisions to the SY3200.

Once the sort decisions have been sent to the module, they remain in effect until new sorting decisions are sent. If you modify or delete a region involved with sorting, you will be prompted to re-send the sort information to the SY3200.

**To create sort decisions:**

1. Create regions on histograms that identify the populations that you want to sort. See Regions for additional details.
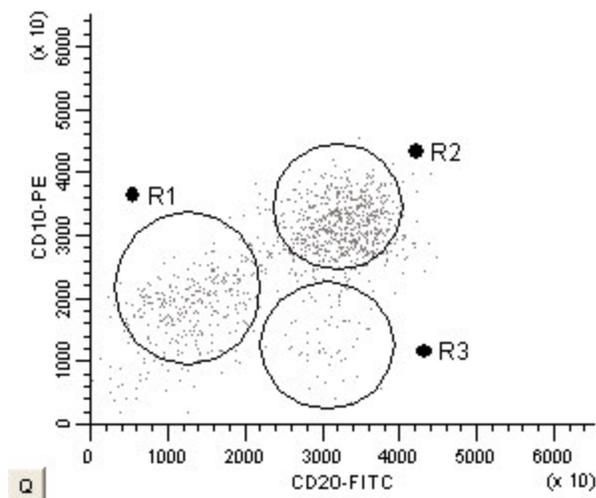


2. Click the Edit Sort button on the SY3200 toolbar.



3. In the Edit Sort dialog, associate the gates you created in step 1 with sort streams on the SY3200. Each gate should be assigned to only one sort stream.
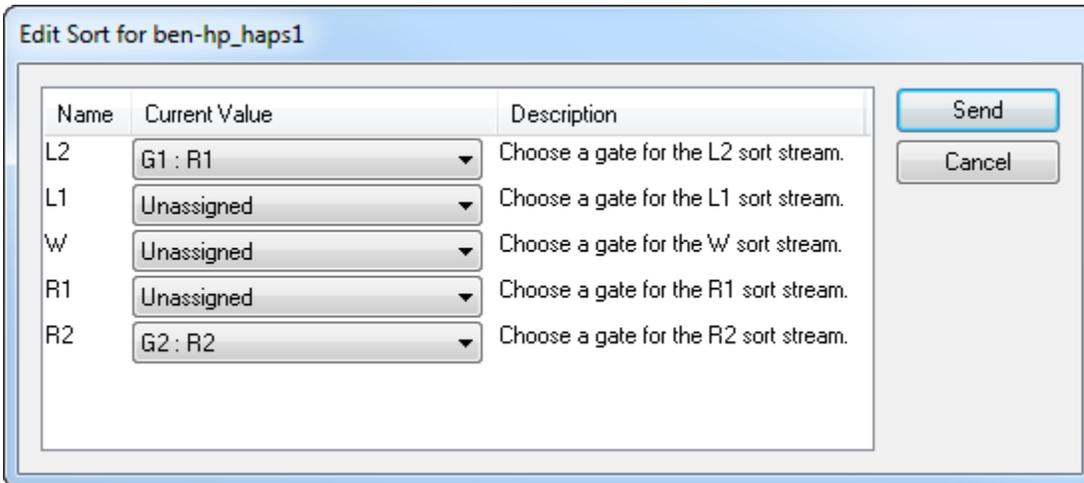
4. Click the Send button to transmit the sorting regions, gate expressions, and N-Color Compensation to the SY3200 and close the dialog.

**More Info:**

Edit Sort dialog

Using Interactive N-Color Compensation

Toolbar

**SY3200: Toolbar**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

At the bottom of the data source window, the SY3200 toolbar provides additional controls and status indicators related to the Sony acquisition system.



The left portion of the toolbar contains a set of buttons that control monitoring, acquisition, and sorting. These are described in the Toolbar Commands table below.

Status panes indicate the name and associated color of the SY3200 module, and information about monitoring, acquisition, and event rates. A progress meter appears during acquisition to indicate the status of the acquisition.

Two LED indicators appear on the right of the toolbar. The first one turns green when a packet of information is received from the SY3200. It turns yellow when a packet is requested and the server does not supply one. The second LED turns green when WinList is processing a packet of data received from the server.

**Toolbar Commands**

Button    Purpose

W         WinList mode
          Stop the connection with the SY3200 and enter
          WinList mode.

          Monitor
          Connect with the SY3200 and request data.
          Real-time events are displayed.
          Events are not stored.

▶         Acquire
          Accumulate data from the SY3200 and store it in an
          FCS file on disk.
          Real-time events are displayed.
          Events are stored.

II        Pause
          Pause or un-pause acquisition
          Real-time events are displayed.
          Events are not stored.

■         Stop acquisition

The Acquisition Complete dialog is displayed.
Events that have been accumulated are stored in an
FCS file on disk.

Restart acquisition
Events accumulated prior to restart are discarded and
acquisition continues.

Edit Acquisition
Edit the acquisition properties, output paths, stop
gate, etc.
See Edit Properties of Acquisition for details.

Edit Sort
Edit the sort gate assignments, color event options,
and send sort info.
See Edit Sort for details.

## SY3200: Edit Properties of Acquisition

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

This dialog allows you to configure the acquisition characteristics for the SY3200. You can choose preferences for most of these properties in the SY3200 Preferences dialog.



### Stop
### Stop Gate

Select a gate to use for calculating the stop count. If you select a gate, the acquisition finishes when the number of events that satisfy the stop gate reaches the stop count. If no

gate is selected, acquisition finishes when the number of events received reaches the stop count.

## Stop Count

Set the stop count for acquisition. See comments on Stop Gate above.

## Save
### FCS File Prefix

Enter an option prefix for the FCS files created during acquisition. This field can be left empty.

### FCS File Pathway

Choose the location where FCS files are stored during acquisition.

### File Counter

This is a simple counter that increments as files are acquired during a WinList session. You can edit the value to any number you want.

### Event sampling

This value determines the rate at which events are sampled into the acquisition output file from the buffers received. The default setting is 1, which means that every event received will be stored. With a setting of 5, WinList would store every 5th event that it receives from the instrument. A setting of 500 would store 1 out of 500 events. This can be useful when running long acquisitions where you do not need to store every event.

## Pre-Acquisition
### Prompt Before

When this option is enabled, WinList will display a dialog before acquisition. The dialog allows the user to enter keywords for the sample.

## Post-Acquisition
### After Acquisition

Choose what you want the program to do after you acquire a new file. Options include **Enter WinList mode & Replay** and **Continue Monitoring**.

### Prompt After

When this option is enabled, WinList will display a dialog after acquisition that allows the user to enter keywords for the sample.

### Update Report Links

When this option is enabled, WinList will automatically update all linked objects on reports when an acquisition finishes.

**Save Reports**

When this option is enabled, WinList will automatically save any open reports when an acquisition finishes.

**Save PDF Reports**

When this option is enabled, WinList will automatically save any open reports in PDF format when an acquisition finishes.

**Print Reports**

When this option is enabled, WinList will print open reports when acquisition finishes.

**Save Protocol Bundle**

When enabled, WinList will automatically save a protocol bundle for the newly acquired file after each acquisition.

**User-Defined Keywords**

**Keyword 1**

This field contains the name of the first user-defined keyword for the FCS file created during acquisition.

**Keyword 1 Description**

If you edit Keyword 1, it is a good idea to edit this field to describe the keyword that you have entered. This description is displayed to the operator when the system prompts for the keyword value.

**Keyword 1 Value**

Enter a default value for Keyword 1, or leave the field blank.

**Keyword 2**

This field contains the name of the second user-defined keyword for the FCS file created during acquisition.

**Keyword 2 Description**

If you edit Keyword 2, it is a good idea to edit this field to describe the keyword that you have entered. This description is displayed to the operator when the system prompts for the keyword value.

**Keyword 2 Value**

Enter a default value for Keyword 2, or leave the field blank.

**SY3200: Edit Sort**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

This dialog allows you to make sorting decisions for the SY3200. No changes are made until the **Send** button is clicked.



**Sort Streams**

The top section of the dialog shows each sort stream that is defined on the SY3200.

To use a gate for a sort decision, select a gate from the drop-down list for the sort location you want. This will associate the gate expression with the Sort Stream decision.

Sort streams that show "Unassigned" for the gate will not receive any events during the sort.

**Other Cells** and **Empty Drops**

The system allows you to capture all cells that are not identified by other gate assignments, as well as droplets that did not contain cells.  Any sort destination can be set to "Other Cells", "Empty Drops", or "Other Cells and Empty Drops".

**Send**

Click this button to transmit the sorting regions, sort gate expressions, and N-Color Compensation to the SY3200 module. The dialog will close, and a message will be displayed to indicate whether or not the operation was successful.

**Cancel**

Click this button to close the dialog without transmitting the sorting decisions.

**SY3200: Preferences**

*This option is only available in special versions of the WinList supplied through Sony Biotechnology.*

This dialog displays settings that control various aspects of the SY3200 acquisition system.
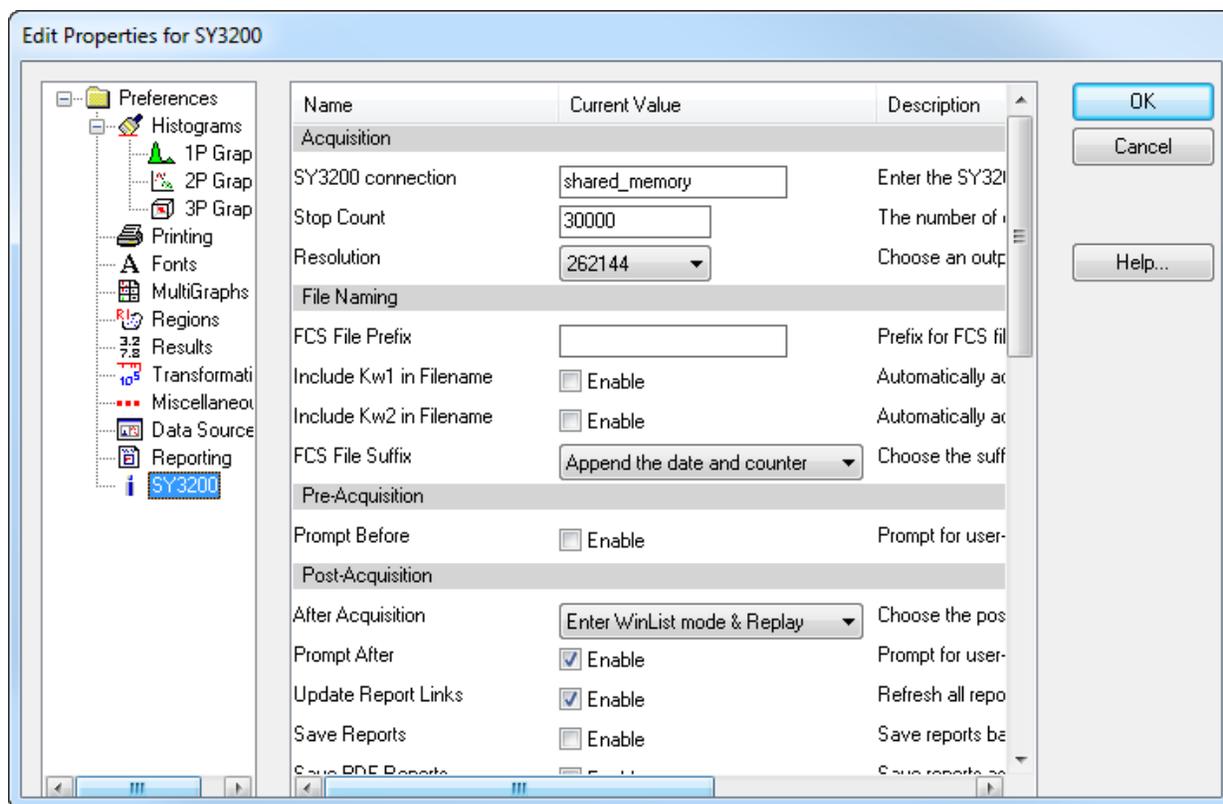


**Acquisition**

**SY3200 Connection**

This field defines how WinList connects to the SY3200.  It should only be changed if advised by your Sony technician.

**Stop Count**

Set the default stop count for acquisition.

**Resolution**

Select a resolution to use when writing acquired data to FCS files.

## File Naming

### FCS File Prefix

Enter an optional prefix for the FCS files created during acquisition. This field can be left empty.

### Include Kw1 in File name

Enable this option to include the Keyword 1 in the name of files you acquire.

### Include Kw2 in File name

Enable this option to include the Keyword 2 in the name of files you acquire.

### FCS File Suffix

Select an option for a file naming suffix.

## Pre-Acquisition

### Prompt Before

When this option is enabled, WinList will display a dialog before acquisition. The dialog allows the user to enter keywords for the sample.

## Post-Acquisition

### After Acquisition

This option determines what WinList will do following an acquisition.

Choose **Continue Monitoring** to automatically switch back into monitoring mode.

Choose **Enter WinList Mode and Replay** to work with the data that has just been acquired.

### Prompt After

When this option is enabled, WinList will display a dialog after acquisition. The dialog allows the user to enter keywords for the sample and shows summary information about the acquisition.

### Update Report Links

When this option is enabled, WinList will automatically update all linked objects on reports when an acquisition finishes.

### Save Reports

When this option is enabled, WinList will automatically save any open reports when an acquisition finishes.

### Save PDF Reports

When this option is enabled, WinList will automatically save any open reports in PDF format when an acquisition finishes.

**Print Reports**

When this option is enabled, WinList will print open reports when acquisition finishes.

**Save Protocol Bundle**

When this option is enabled, WinList will automatically save a protocol bundle after acquiring an FCS file.

**Include Acquired File in Bundle**

When this option is enabled, WinList will include the newly acquired FCS file in the protocol bundle.

**User-Defined Keywords**

**Auto-close Dialog**

If enabled, WinList will automatically close the Post Acquisition dialog after a user-defined time passes.

**Auto-close Time**

Select the amount of time to keep the Post Acquisition dialog open before automatically closing it.

**Keyword 1 and 2**

This field contains the name of the first user-defined keyword. The default keyword name is $SMNO, which is a standard FCS keyword used to describe the specimen.

**Keyword 1 and 2 Description**

If you edit Keyword 1, it is a good idea to edit this field to describe the keyword that you have entered. This description is displayed to the operator when the system prompts for the keyword value.

**Keyword 1 and 2 Value**

Enter a default value for Keyword 1, or leave the field blank.

**Keyword 1 and 2 Separator**

This separator is used when adding this keyword to the file name.

**Real-Time Display**

**Show LEDs**

Enable this option to display LEDs on the SY3200 toolbar.

**Monitor Mode Events**

This setting determines the number of events WinList will try to show in histograms during Monitor mode.

**Max Acquisition Dots**

During acquisition, it is possible for WinList to accumulate and display all of the events that are being acquired. In most cases, however, this is not helpful or practical because it requires more and more memory and new dots are drawn on top of existing dots.

This setting limits the number of dots that 2P histogram will try to display during acquisition.

**Throughput Control**

**Maximum Events Per Second**

Choose the maximum throughput allowed for the WinList/SY3200 connection. In most cases this setting will not need to be changed. However, on slow network connections or slow computers, you may choose to lower this setting to ensure that the computer remains responsive.

**Save preferences**

Enable this option to save the settings as preferences.

**Appendices**

**Appendix A: DDE Commands**

Dynamic Data Exchange (DDE) provides a way for Windows programs to share data and interact with one another. WinList supports DDE as a client of other applications. In other words, it can request information and instruct other applications to do things. It does not support DDE as a server of information.

When connecting WinList to other applications with DDE, there are several internal commands which WinList recognizes. These commands allow you to ship data to other programs and to retrieve data from them. Below is a list of the current internal DDE commands for WinList and an explanation of usage for each.

**POKE**
This command is used to send histogram or results data to another program in either TEXT format or VSH format. The syntax of the command is:

POKE(externalRef, format, {*optional internal reference}* )

where externalRef is a reference the other application uses to determine where to put or how to use the data, and format is either TEXT, TEXTNOHEADER, or VSH. TEXT format is supported by most applications that can paste text from the clipboard. TEXTNOHEADER format is identical to TEXT except that it sends a result window's text as a table of information, without column headings. It can only be used with results windows. VSH is a Verity Software House binary data format, which provides a more efficient use of memory. It pertains only to histogram connections and should be used when interacting with other VSH products or with products known to support the format.
The *optional internal reference* can be literal text or any keyword in the keyword tree. This allows you to send any kind of information that WinList knows about to the remote application. If a keyword is used for this option argument, it must be enclosed in curly braces { }. Literal text does not need any special formatting.

Examples:

POKE(DATA,VSH) - used to send data to ModFit or IsoContour.

POKE(R1C1:R64C64,TEXT) - used to send a 64 x 64 resolution histograms to Microsoft Excel in TEXT format. Note the reference tells Excel to put the data into the active spreadsheet at row-1 column-1 to row-64 column-64.

POKE(RC:R[+6]C[+6],TEXTNOHEADER) - used to send a results window to Microsoft Excel in TEXT format, excluding the column headings and id fields in the results window. The reference tells Excel to put the data into the active spreadsheet at a location starting at the current row and column, and extending through 6 rows and 6 columns. Relative references of this sort are very useful for adding results to a database without overwriting previous results.

POKE(KEYWORDS,VSH,KEYWORDS) - used in a histogram connection to send keywords from the listmode file to a Verity application like ModFit LT 3.0 or greater.

POKE(R1C1:R1C255,TEXT,KEYWORDS) - sends keywords from the listmode file to an application like Microsoft Excel. The format of the keywords is *keyword* followed by a tab character, then the *keywordValue* and another tab character.

POKE(R1C1,TEXT,R1 Events) - sends the label "R1 Events" as text to an application like Microsoft Excel to place in row 1 column 1.

POKE(R1C2,TEXT, {DS1:$DATE}) – evaluates the string "{DS1:$DATE}" as a keyword, and then sends the result as text to an application like Microsoft Excel to place in row 1 column 2. See Internal Keywords for a list of special internal keywords supported by WinList.

POKE(R2C1,TEXT,{DS1.R1.%Gate}) - sends the %Gate statistic for R1 in data source 1 to row 2 column 1 in Excel.

POKE(R3C1,TEXT,{DS2:SPILL}) - sends the SPILL keyword value from data source 2 to row 3 column 1 in Excel.

## PASTE
This command tells WinList to paste a copy of the contents of the Windows clipboard into the histogram or results window for display. The syntax is PASTE(format) where format is METAFILE or TEXT. METAFILE is a picture format supported by many Windows graphic programs and can be used by both Results and Histogram windows. TEXT format should only be used with Results windows. When WinList successfully completes the PASTE command, the pasted object will be displayed in the window.

Examples:

PASTE(METAFILE) is used to get a copy of a picture from the clipboard. It can be used both by Results and Histogram windows.

PASTE(TEXT) is used to get a copy of text from the clipboard. It can be used only by Results windows.

## COPY

This command tells WinList to copy the results or histogram window to the Windows Clipboard so that another application can paste it into its workspace. Its syntax is COPY(format) where format can be METAFILE, TEXT, or BITMAP.

Examples:

COPY(METAFILE) is used to copy a picture of a histogram to the clipboard. It can be used only by Histogram windows.

COPY(TEXT) is used to copy text to the clipboard. It can be used only by Results windows.

COPY(TEXTNOHEADER) is used to copy text to the clipboard. It can be used only by Results windows. The only difference between this format and TEXT format is that TEXTNOHEADER strips off all lines up to and including the column headings. It is useful for databasing of results in another program using a DDE connection.

**SETREGIONRESULT**

This command tells WinList to set the value of a specific region's result to a value extracted from a remote application. Its syntax is SETREGIONRESULT("*region reference*","*external request*") where *region reference* is in *R(n,stat)* format and *external request* is a string which is requested from another application. For more information on *R(n, stat)* format, see Internal Keywords. *External Request* is expected to be a value number of the same type as the field it is copied to.

Examples:

 If connected to ModFit, SETREGIONRESULT("R(1,REGIONEVENTS)","A[1,0]") tells WinList to ask ModFit for the answer to "A[1,0]", and to put the result into the Region Events field for Region 1.

**PAUSE**

This command is supported by histograms. It displays a dialog with optional message and waits to proceed until the dialog is closed. The dialog appends "Press OK to continue or Cancel to stop the script." Its syntax is PAUSE(*message*) where *message* is optional text for the message.

Example:

PAUSE(Please check results in ModFit LT.)

This command in a histogram script displays dialog "Please check results in ModFit LT. Press OK to continue or Cancel to stop the script."

**SLEEP**

This command is used to allow WinList to "sleep" for a specified period of time. It is useful in cases where the remote application needs to perform some time-consuming task, which could cause the script to timeout.

The command syntax is:

SLEEP(milliseconds)

For example, adding a script line

SLEEP(2000)

would cause WinList to sleep for 2 seconds.

### Appendix B: Internal Keywords

WinList allows users to access two types of keywords: FCS keywords stored in data files, and internal keywords that WinList calculates.

Users can access keywords for histogram, MultiGraph, and results window titles, as well as text labels in MultiGraphs. Keywords are identified by surrounding specific words with square brackets [ ]. When WinList sees a keyword, it replaces it with the keyword's value. For example, **[DATE]** is an internal keyword that would display the current date. **[$TOT]** is an FCS keyword which tells how many total events were stored in the listmode file. Generally, when the user can edit a text field, keywords are available for those fields.

WinList also supports mixed text and keywords. It is possible, therefore, to display a label for a MultiGraph that would display the date and time as follows:

     Date: [DATE] Time: [TIME]

In this example, if the current date is 01-Mar-2013 and the time is 09:20:45, WinList would display:

     Date: 01-Mar-2013 Time: 09:20:45

The internal keywords supported by WinList are listed below.

**[DATE]**: Displays the current system date reported by your computer.
**[TIME]**: Displays the current system time reported by your computer.
**[FILE]**: Displays the filename of the active data source.
**[FILE,*dsID*]**: Displays the filename of the data source with an ID of *dsID.*
**[FILEROOT]**: Displays the base of the active data source file name. For example, if the file name is 190594.FCS, the FILEROOT keyword returns 190594, leaving off the file extension.
**[FILEROOT,*dsID*]**: Displays the base of the filename for the data source with an ID of *dsID.*.
**[FILEPATH]**: Displays the pathway to the active data source file.
**[FILEPATH,*dsID*]**: Displays the pathway for the data source with an ID of *dsID.*

**[DEFAULTPATH]**: Displays the pathway to protocols and other WinList file types.

**[HISTOGRAM]**: Displays the name of the active histogram.

**[GATE]**: Displays gate information of the active histogram.

**[COUNTER*n*]**: Displays the current value of user-programmable counter *n*. This value can be changed with macro commands **ModifyUserCounter** and **ResetUserCounter**. See Edit Macro and Macro Commands for more on changing the counter's value.

**[LEVEL]:** Returns the level of the current batch item.
**[NEXTLEVEL]:** Returns the level of the next batch item.

**[FINISHBATCH]:** Returns 1 if the last batch item has been processed.

**[NEWLEVEL]:** Returns 1 if the next batch item level is different from the current level.

**[BATCHINDEX]:** Returns the index of the current file in the batch list.

There are two forms of the following keyword, used to display a region result. Note that *no spaces* are permitted in the keyword syntax below.

**[R(*n*,*stat*)]:** Displays a statistic *stat* for region *n*, where

 *n* is the number of the region, and

 *stat* is the textual name of the statistic as shown in the table below.

**[R(*n*,PCN,*g*)]:** Displays the percent-of-gate statistic for region *n* and gate *g*, where

 *n* is the number of the region, and

 **PCN** is a literal that specifies the Percent of Gate N statistic, and

 *g* is the gate ID of for the gate of interest.

| To display | stat equals | or |
|---|---|---|
| Total events | TOTALEVENTS | TOT |
| Gated events | GATEDEVENTS | GAT |
| Region events | REGIONEVENTS | REG |
| Percent Total | PERCENT | PCT |
| Percent Gated | PERCENTGATED | PCG |
| Name of the gate applied to the histogram containing region *n* | GATENAME | GTN |
| Mean X | MEANX | MNX |
| Mean Y | MEANY | MNY |
| Median X | MEDIANX | MDX |
| Median Y | MEDIANY | MDY |
| Median X in ADC units | MEDIANADCX | MDADCX |
| Median Y in ADC units | MEDIANADCY | MDADCY |
| Mode X | MODEX | MOX |
| Mode Y | MODEY | MOY |
| CV X | CVX | CVX |
| CV Y | CVY | CVY |

| Standard Deviation X | STDDEVX | SDX |
|---|---|---|
| Standard Deviation Y | STDDEVY | SDY |
| Low X | LOWX | LOX |
| Low Y | LOWY | LOY |
| High X | HIGHX | HIX |
| High Y | HIGHY | HIY |
| Correlation coefficient. | CC | CC |
| Data source filename containing the region | DS | DS |
| Name of the histogram containing the region | HST | HST |

For example, to display the percent of gate statistic for region R5, type:

[R(5,PCG)]

To display "percent of gate N" statistic for R6, where gate N is gate 2, type:

[R(6,PCN,2)]

Another group of keywords is used in DDE scripts and is specific to histogram windows. These keywords can be put into script statements and surrounded by curly brackets { }. WinList will replace them with histogram-specific information before sending the DDE command to the remote application.

For example, to pass the parameter names of a two-parameter dot plot to IsoContour, you can enter **[SetLabelText('3,{XNAME} -->')]**. The portion of the command in curly brackets, XNAME, will be replaced with the name of the parameter on the X-axis, then the command will be sent to IsoContour.

DDE keywords are listed below:

*For Histograms:*

**{XNAME}** displays the name of the X parameter (1st parameter) in a histogram

**{YNAME}** displays the name of the Y parameter (2nd parameter) in a histogram

**{ZNAME}** displays the name of the Z parameter (3rd parameter) in a histogram

**Appendix C: Understanding Mean values**

Earlier versions of WinList provided options to compute the mean for log parameters in terms of histogram channels or linearized units. This created some confusion for users that made the reported values unnecessarily difficult to understand. The program has been modified to always report statistics for log parameters in linearized units.

What is the difference between **Linear Mean** and **Geometric Mean** for data collected in log domain? Here is an explanation.

**Linear Mean**

Here is how the mean is computed when the Use Geometric Mean option is *not* enabled in the EDIT RESULTS dialog. The parameter values for each event are converted to the equivalent value on a linear scale and added to a running sum. The mean is computed by dividing this sum by the number of events analyzed. For a 4-decade log amplifier, the values would be from 1 to 10,000. This is method can be sensitive to a small number of events in the higher decades.
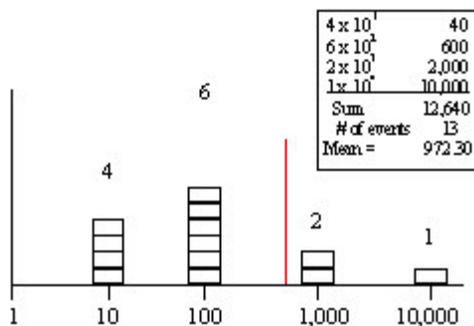


**Geometric Mean**

When the Use Geometric Mean option is checked, the mean is computed differently. The parameter values for each event are added to a running sum without being converted to the linear scale. The sum is divided by the number of events, and then converted to a linear value. Geometric mean calculations are generally less influenced by outlier events.

| | |
|---|---|
| 4 x 256 | 1024 |
| 6 x 512 | 3072 |
| 2 x 768 | 1536 |
| 1 x 1024 | 1024 |
| Sum | 6656 |
| # of events | 13 |
| Mean = | 512 |

Linearize mean value (512)

= (value / resolution) x (# log decades)
= (512 / 1024) x 4.0
= 0.500 x 4.0
= 2.00
10 raised to the 2.00 power = 100

**Geometric Mean = 100.00**
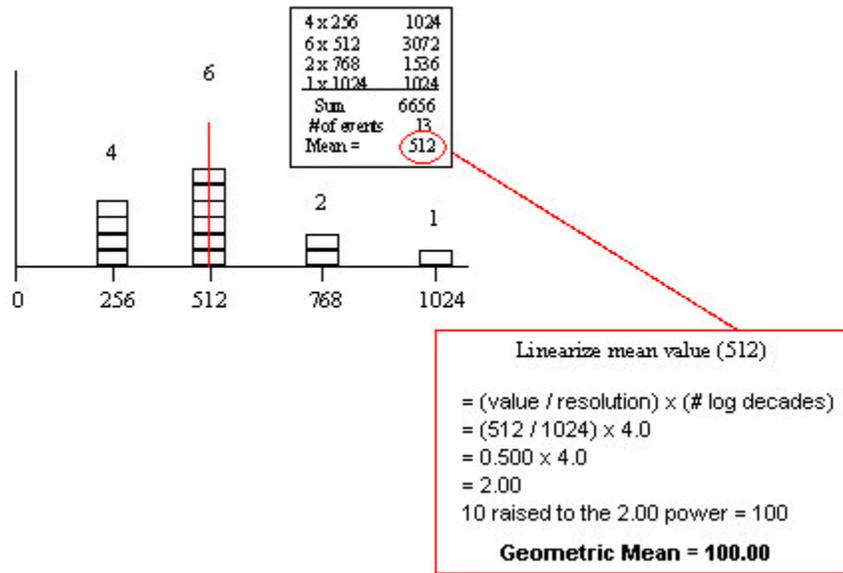
### Appendix D: Data Conversions

The following section explains how to convert listmode parameter data from log scale to linear scale, and vice versa. The expressions for scaling the new parameter for display on histograms are also shown.  Note, you can use the Edit Transformations dialog to convert parameters from linear to log or HyperLog without having to create additional calculated parameters.  What is shown below is useful for cases where a calculated parameter needs to be based on data in log data space instead of linear.

WinList allows you to create new parameters in a listmode file using the Add Parameters button in the Edit Data Source dialog box. Each parameter you create with this dialog has a floating-point resolution of 1024, regardless of the resolution of other parameters in the listmode file. For this reason, it is often necessary to scale calculated parameter expressions, including log to linear and linear to log conversions.

Calculated parameter expressions are stored as floating-point values ranging from 0.0 to 1023.0.

The formulas in the example use the expressions defined below:

| | |
|---|---|
| *parameter* | linear or log listmode parameter to convert |
| *adcRes* | ADC resolution of the parameter |
| *decades* | number of decades for the log amplifiers in the flow cytometer |
| *linearScale* | scale of linearized values |
| *linearScaleFactor* | value that will scale *parameter* from 1024 channels to *linearScale* |

### Equation for converting Log to Linear Data

To create an unscaled parameter converting log to linear:

FE10(*decades* * *parameter* / *adcRes*)

To scale the parameter for display, the expression would be:

FE10(*decades* * *parameter* / *adcRes*) * (1024 / FE10(*decades*))

For example, to linearize log parameter 3 in a listmode file acquired on 4-decade log amps at 1024 channel resolution, the WinList calculated parameter expression would be:

FE10(4 * p3 / 1024)

The result of this example produces values between 1 and 10,000. If you want to display this linearized expression in a histogram, you need to scale the data. The scaling can be calculated by dividing the 1024 by $10^{decades}$. We explicitly use 1024 here because that is the defined resolution for all calculated parameters in WinList.

In the example, that is:

1024 / $10^4$, or approximately 1/10.

The calculated parameter expression you would use if you wanted to properly scale the data for display would be:

FE10(4 * p3 / 1024)/10

**Equation for converting Linear to Log Data**

FL10(*parameter * linearScaleFactor*) * 1024 / FL10( *linearScale* )

When *parameter* appears in this equation, WinList automatically transforms the value of parameter to a floating point number between 0.0 and 1024.0. Because of this, it is necessary to determine the *linearScaleFactor* that will scale the value to *linearScale*.

For example, suppose that you want to transform a linear parameter P1 into a 5.42-decade scale. The linear value equivalent to 5.42 decades is 262144. In order to scale a value from 1024 to 262144, it must be multiplied by 256. So, the expression would be:

FL10(P1 * 256) * 1024 / FL10(262144), or

FL10(P1 * 256) * 1024 / 5.42

Similarly, suppose you want to scale a linear parameter, P2, to a 4-decade log scale. This represents 10000 linear units, so we determine the *linearScaleFactor* by dividing 10000 by 1024, giving a result of 9.765625. The expression for the transformation is:

FL10(P2 * 9.765625) * 1024 / 4.0

Note: Computing the log for a value of 0 is an error condition, so the program returns a value of 0 for FL10( 0 ).

**Appendix E: Understanding Hi-res Data Files**

WinList provides support for reading and analyzing high-resolution parameters created by digital flow cytometers. If you analyze these new files in WinList, there are some additional preferences and settings you should know how to find and use.

**What is a High-Resolution listmode file?**

Newer flow cytometers acquire listmode data at 16-bit and higher resolutions and store data in linear format. This means that the analysis software (e.g. WinList) needs to transform the data in order for you to view it in log or HyperLog format. Some of these data files contain keyword information that allows WinList to determine which parameters should be displayed linear and which should be displayed log or HyperLog.  Other data files do not include information to identify which parameters should be displayed in log, and the Transformation dialog can help resolve this issue.

You can use the Transformations dialog to select how each parameter in a data file will be displayed. There is a section in Preferences for transformations, as well as access through the Edit Data Source dialog.
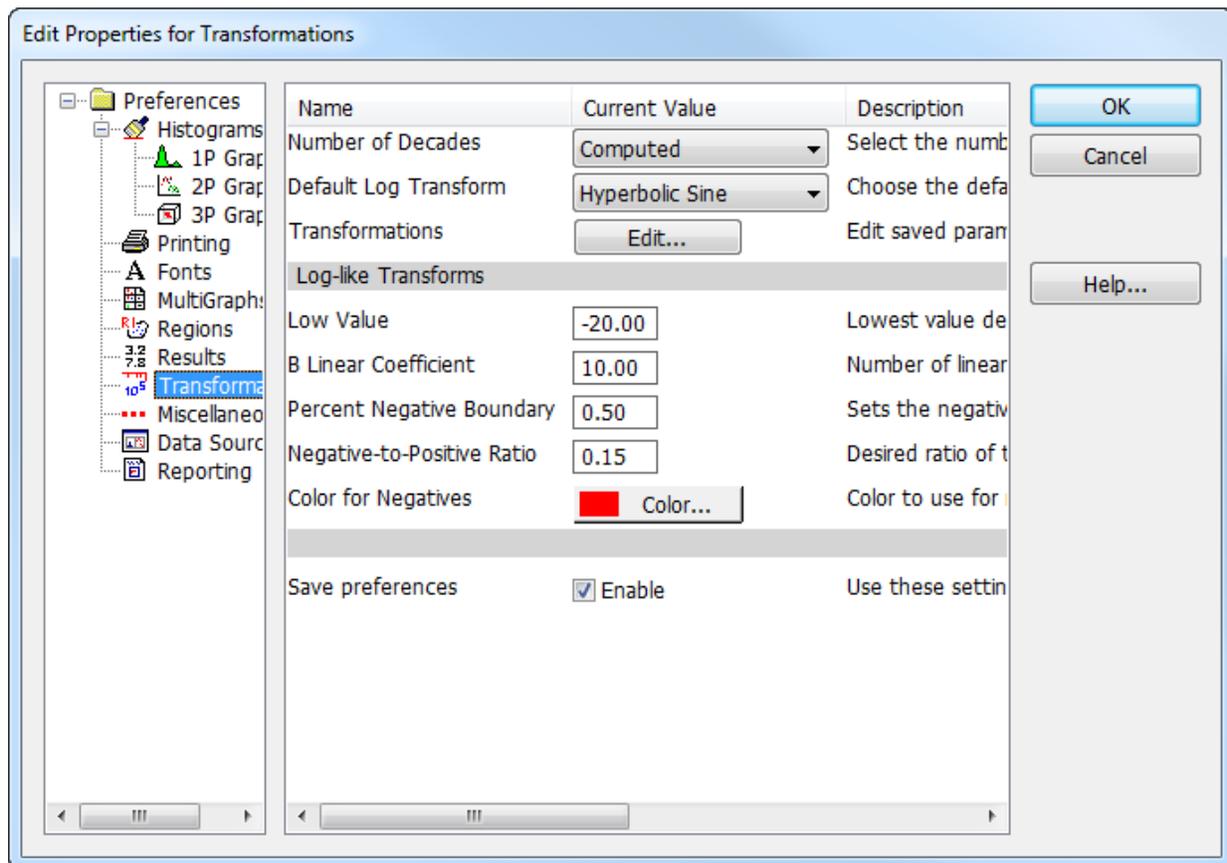
Use this preference section to specify whether to convert the data to log scale or leave the parameter linear for each parameter in the listmode file.

**Under the Hood**

When you enable a parameter for log conversion, WinList does some extra work when reading the parameter. First, it determines how many log decades can be extracted from the linear data by taking the log of the resolution. For example, if the parameter resolution is 262144, the number of log decades it contains is log(262144), or approximately 5.42. This is the number of decades the program converts to if you have the *Number of Decades* option set for *Computed*. If you have selected *4-decade* for the *Number of Decades*, the program will transform the linear values to a 4-decade scale, regardless of the computed number of decades.

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