

# WinList<sup>TM</sup>

## User Guide

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This guide is intended for use with WinList 7.0 and minor version updates.

Phone: (207) 729 6767  
Fax: (207) 729 5443  
Email: [verity@vsh.com](mailto:verity@vsh.com)  
Web: [www.vsh.com](http://www.vsh.com)

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## **WinList 7.0 User Guide**

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### **Verity Software House**

Phone: (207) 729 6767

Fax: (207) 729 5443

Email: [verity@vsh.com](mailto:verity@vsh.com)

Web: [www.vsh.com](http://www.vsh.com)



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## **Latest Versions**

To find the latest versions for this software and documentation, visit the Verity Software House web site at [www.vsh.com](http://www.vsh.com). This site provides a complete version of the latest documentation, service packs, and technical support information for the program.

You can also email us at [verity@vsh.com](mailto:verity@vsh.com) to determine whether you have the latest version. Please include the version that appears on the start-up dialog box and the program file date for the program.

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?

### 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™ 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 reports 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 bivariate histograms for parameters enabled for compensation. When the button is clicked, the system scans to see if any bivariate histograms 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 iCyt Mission Technology. 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 an 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 of DakoCytomation Summit data files.
- Results windows can now show statistics 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 data source.
- 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 accommodate 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 that is 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 an 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 HyperLogtm 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 toolbox 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 differentiate 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. (V11054)
- 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 accessed 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 XP SP3, Vista, Windows 7 or greater, 2GHz CPU or better, 2GB RAM or greater, 50MB free disk space for program files.

Faster CPU speeds 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.
- 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

If you purchased the Network version of the program, see Using the Network Version for details on installing, registering, and using 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 **Setup** or **Setup.exe**. Follow the directions on the screen to complete the installation.
- See the section on registering your software 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.

**Register Software**

Please enter the Serial # and CD Key for the application.

If connected to the internet, click the Web Registration button to get your Unlock Code. If not connected, take this information to a computer with Internet access and visit [www.vsh.com](http://www.vsh.com) to register, or contact Verity.

When you register, you will receive an Unlock code that allows the program to run normally.

Program: WinList 3D

Version: 7.0

Computer ID: CFB077DDF4FB

Serial #: ABCDEFGHIJ

CD Key: 12RT9F08IJS LZ

Unlock Code: ALICEINWONDERLAND

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. 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](mailto: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 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. 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](http://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.





## Installing a Software Battery

*You must have administrative rights to the computer during installation and when running the program for the first time. After the first time, the program can be run by users with restricted access to the computer.*

### What is a Software Battery?

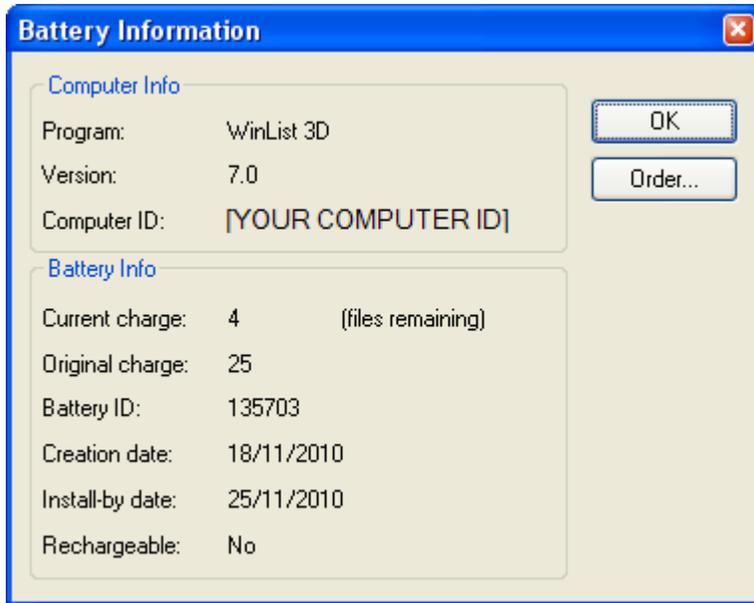
If you have a demo version of WinList, you can purchase “software batteries” from Verity Software House that enable the program to become fully functional. The software battery you purchase has a certain “charge” that allows the program to analyze a certain number of data files. A charge of 500, for example, allows the program to analyze 500 data files.

### How to Order

To use the software battery system, the first step is to install the software. After installation, run the software and click on the Battery button shown in the opening dialog.



The Battery Information dialog is displayed. You will need the information at the top of the dialog to order a battery: the program name, program version, and computer ID.



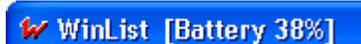
To make ordering as easy as possible, an Order button appears on the dialog. Click this button to connect to our web site and place an order for a new software battery. If you do not have internet access, you can call or mail the information to us and find out the current battery sizes and prices.

### Installing a Battery

The software battery is typically emailed to you as an attachment. In order to install the battery, follow this simple procedure.

- Close WinList, if it is running.
- Copy or save the battery file into the **My Documents\WinList 3D** folder. The full path depends on the operating system. A battery can only be installed for one user account.
- Restart WinList.

When the battery is recognized by the software, a confirmation dialog appears showing the amount of battery charge. As you work with the program, the main title bar displays the amount of charge remaining.



## Using the Network Version

The Network Version of WinList is designed to be installed and managed on a single “server” 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.

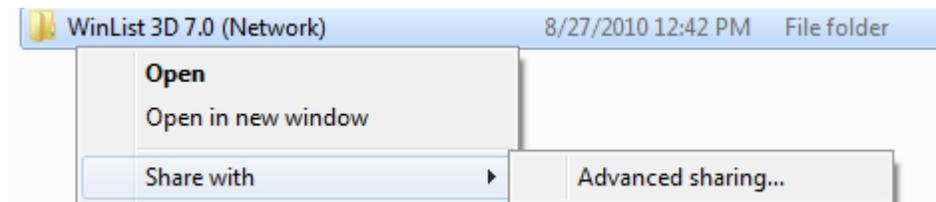
The “server” software can be set up on a machine running Windows XP SP3, Windows 7, or greater; or it can be installed on one of the Microsoft Server versions from 2003 or greater. One component of the WinList network version will be set up to run all the time on the server computer.

## Installation of the Network Version

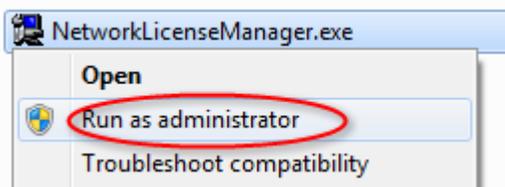
- Log on to the “server” computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Run the program named **Setup** or **Setup.exe**. Follow the directions on the screen to complete the installation. This will install WinList 3D as well as an application called the **Network License Manager** on the computer.

## Configuration of the Network Version

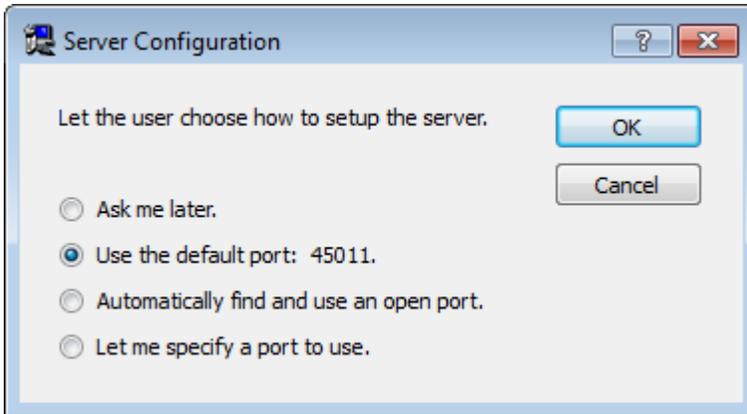
- Log on to the “server” computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- The WinList 3D 7.0 Network folder needs to be shared to allow network users to access and run WinList. Use the utilities on your computer to share the folder and provide Read-access rights to the users who need to run WinList.



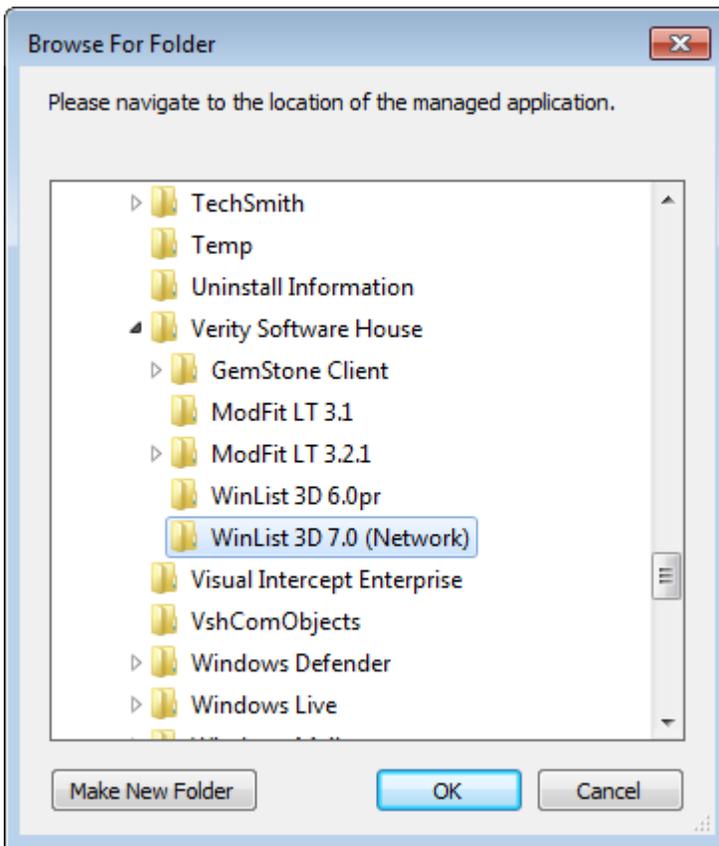
- Run the **Network License Manager** (NLM) application as administrator. To do this, login and as an administrator and right click on the application and choose “Run as administrator”.



- 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.



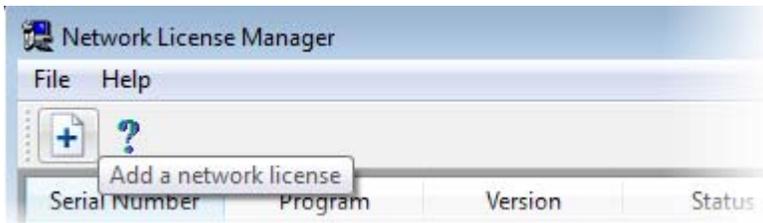
Next, you will be prompted to select the location of the WinList 3D 7.0 installation. The step is important because it will allow the NLM to write a special file (NLMServerInfo.txt) into the WinList directory that will allow WinList to connect to the NLM on your server.



### 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](http://www.vsh.com).



### Testing Your Installation

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

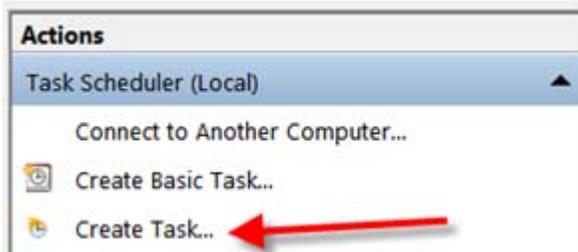
First, run WinList on the server itself. Launch WinList3D.exe. If the system is properly configured, the program should start and operate correctly. If the WinList cannot communicate with the Network License Manager, an error message will be displayed.

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.

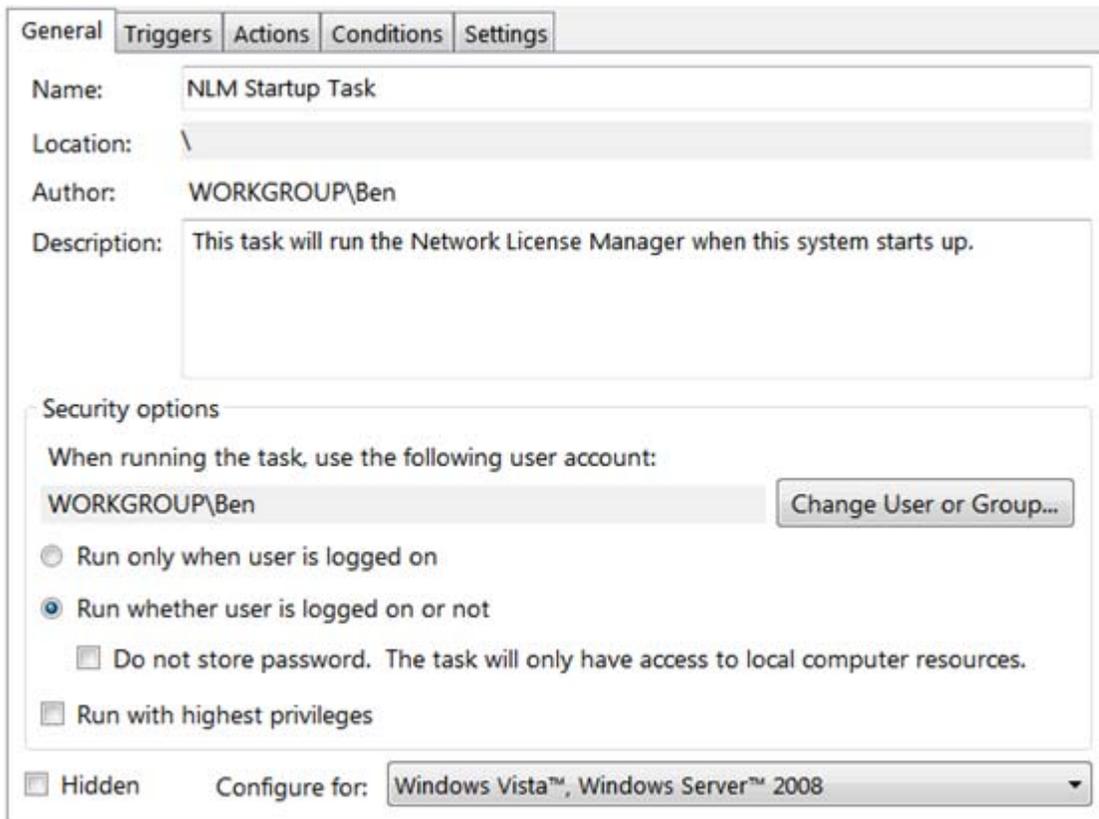
### Setup a Scheduled Task to Run NLM on Windows 7

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.

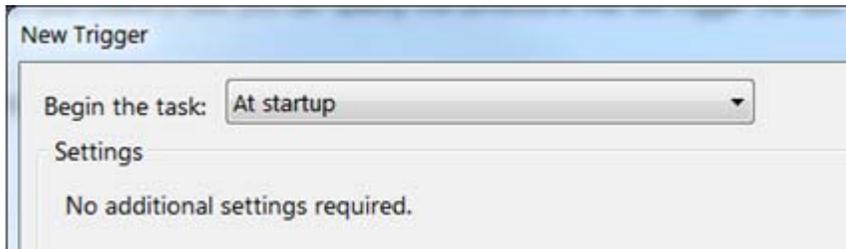
- From the System and Security section of the Control Panel choose **Schedule tasks** under Administrative Tools. Choose Create Task from the Actions pane.



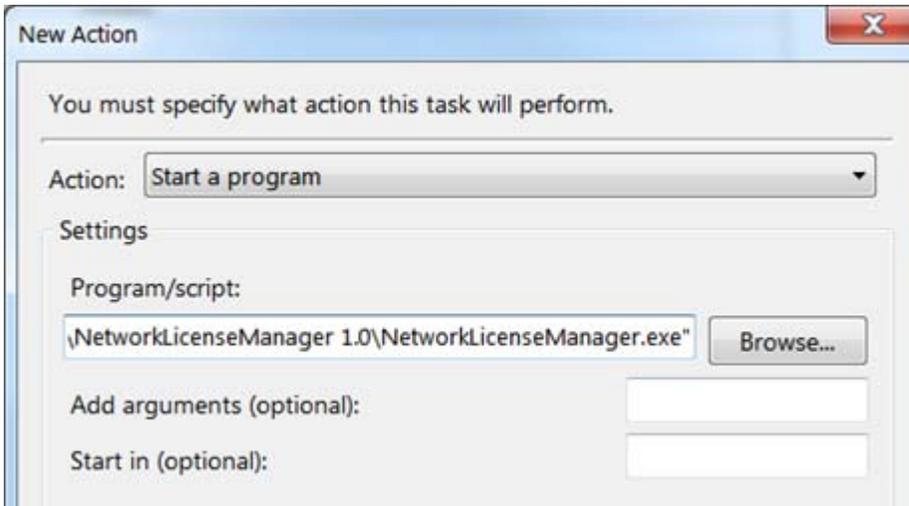
- On the general tab, give the task a name and description and select the Windows OS user that was used when configuring the NLM. Choose for the task to run even if the user is not logged in



- 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 Licence Manager, select **NetworkLicenseManager.exe** and 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.

General Triggers Actions Conditions Settings

Specify additional settings that affect the behavior of the task.

Allow task to be run on demand

Run task as soon as possible after a scheduled start is missed

If the task fails, restart every: 1 minute

Attempt to restart up to: 3 times

Stop the task if it runs longer than: 3 days

If the running task does not end when requested, force it to stop

If the task is not scheduled to run again, delete it after: 30 days

If the task is already running, then the following rule applies:

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.

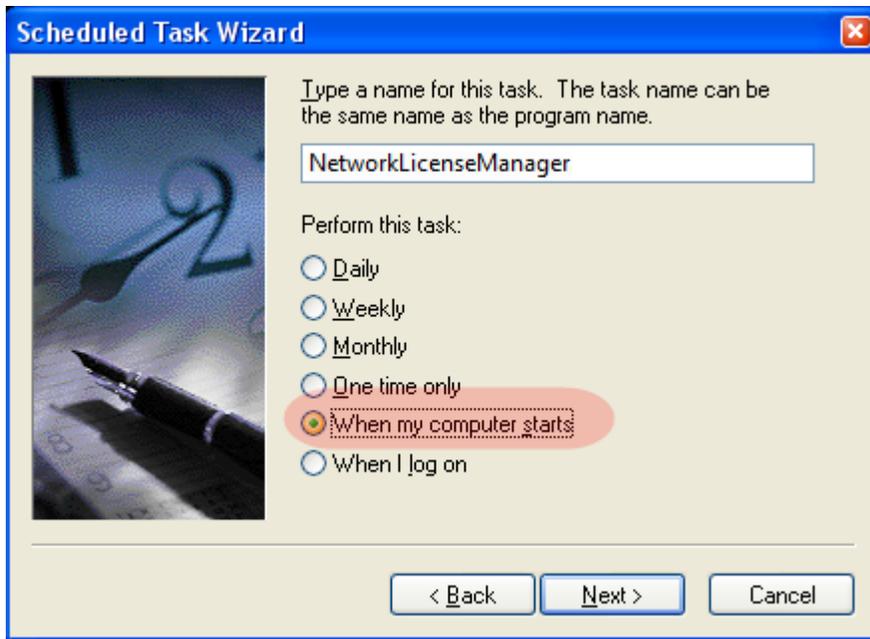
#### Setup a Scheduled Task to Run NLM on Windows XP

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.

- From the Control Panel, choose **Scheduled Tasks**, then **Add Scheduled Task**.



- Browse to the location of the Network License Manager executable file and select it.
- Set the task to run with the computer starts up.

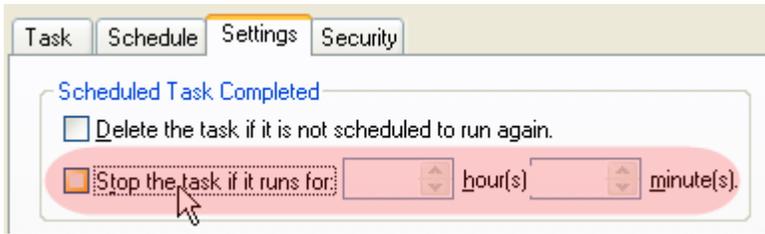


• Use the Windows OS user and password that was used to configure the NLM. This is the user name and password that you used to login to your computer.

• Choose to open advanced properties for the task.



• We do NOT want this task to be stopped. Uncheck the "Stop the task" checkbox.



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

General Email:	<a href="mailto:verity@vsh.com">verity@vsh.com</a>
Tech Email:	<a href="mailto:tech@vsh.com">tech@vsh.com</a>
Sales Email:	<a href="mailto:sales@vsh.com">sales@vsh.com</a>
Developer Email:	<a href="mailto:devteam@vsh.com">devteam@vsh.com</a>
Web:	<a href="http://www.vsh.com">www.vsh.com</a>
Toll-free Phone (US):	(877) 729 6767
Phone:	(207) 729 6767
Fax:	(207) 729 5443



## Tutorials

### Tutorials

#### Fundamentals

WinList Basics

Cascade Gating and Boolean Gates

Using Interactive N-Color Compensation™

Creating Reports

#### Example Applications

Using FCOM to Enumerate Phenotypes

Kinetic Analysis with Excel

Enhanced Proliferation Analysis

#### Extended Features

Remote connection to ModFit LT

Using MultiGraphs

[Databasing with WinList](#)

#### 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.

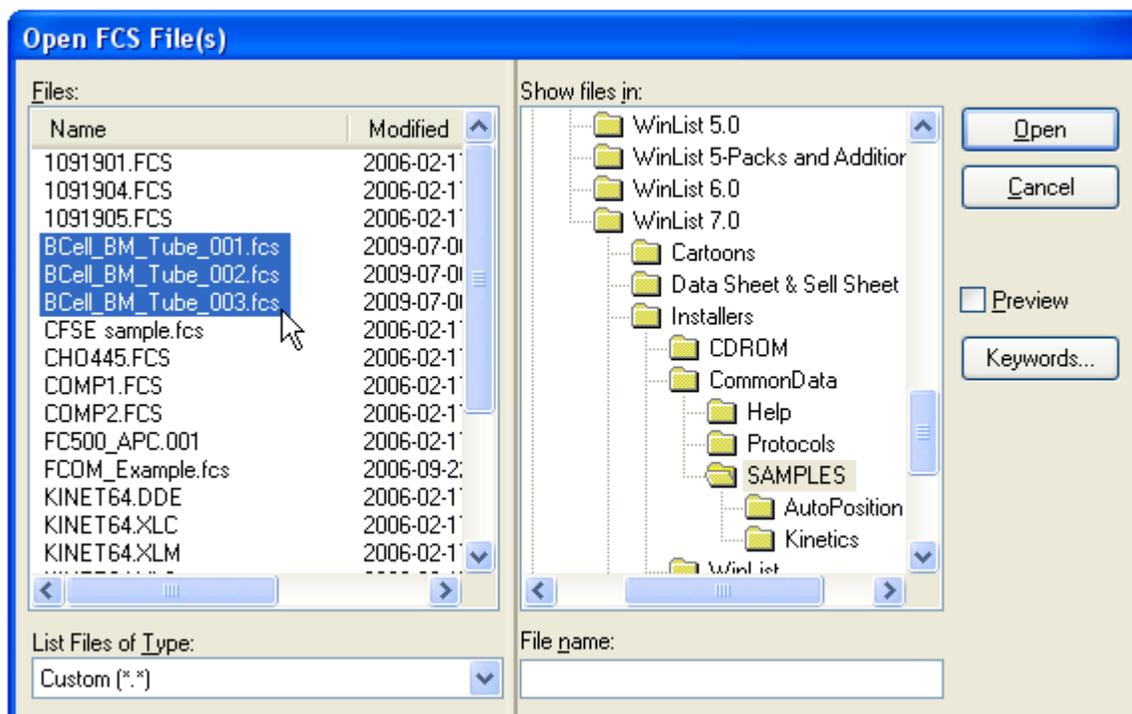
This introduction focuses on the fundamentals of working with WinList. In the course of the tutorial, you will see how quickly and easily you can create an analysis setup with WinList. Opening files, creating histograms, drawing regions and gates, and developing reports are all covered in this exercise.

#### Open files

1. Click the **Open FCS File** button on the main toolbar.



2. In the File Open dialog, navigate to the Samples folder 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**.



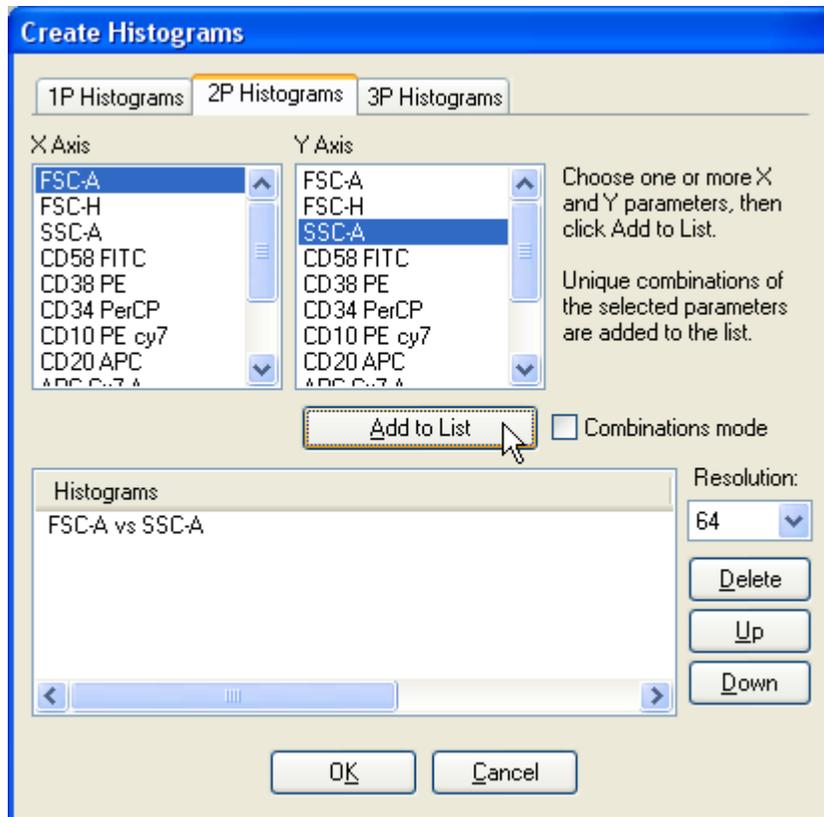
*On the PC, you can also drag and drop files from Windows Explorer or My Computer onto WinList. See [Drag and Drop](#) for details.*

The Create Histograms dialog will appear.

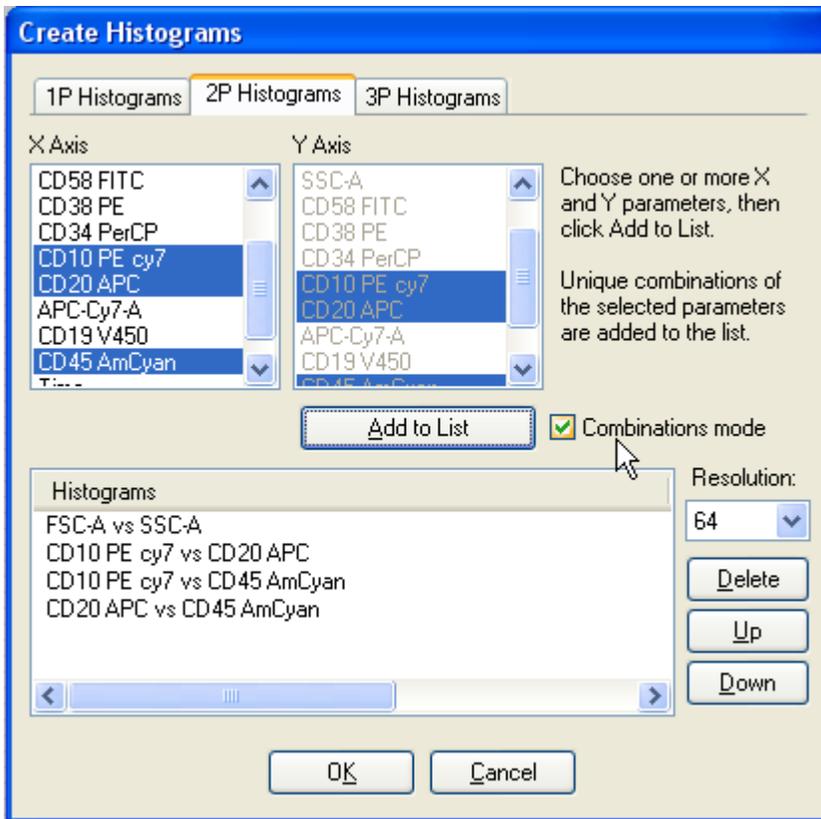
### Create histograms

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

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

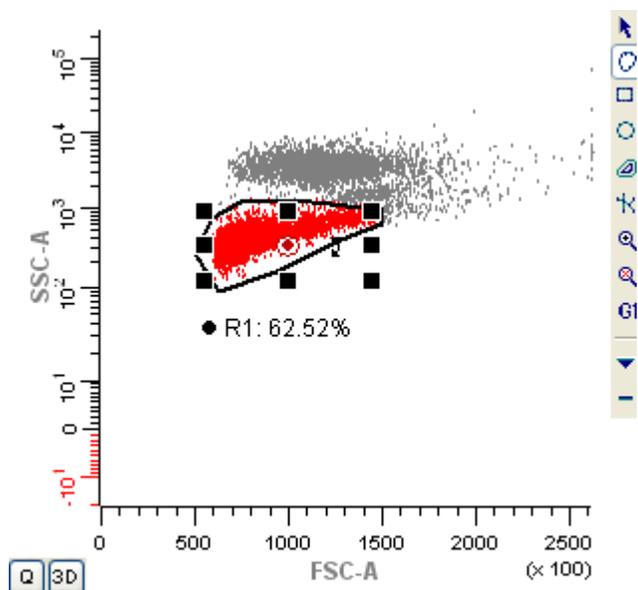
Next, we'll draw a light-scatter gate on the FSC-A vs. SSC-A histogram.

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.*

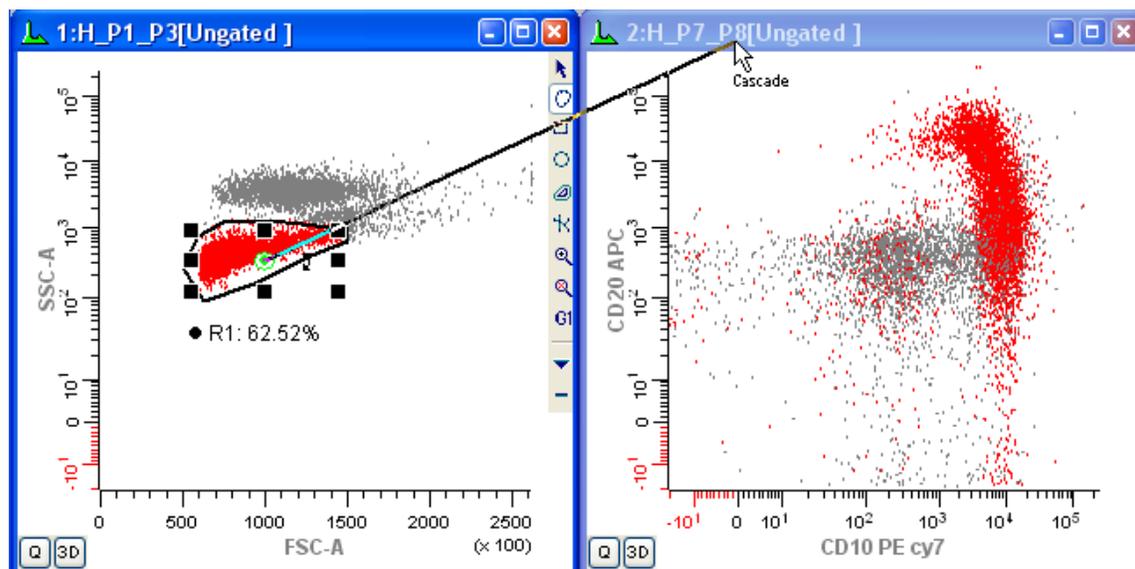


#### Gate the histograms

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.

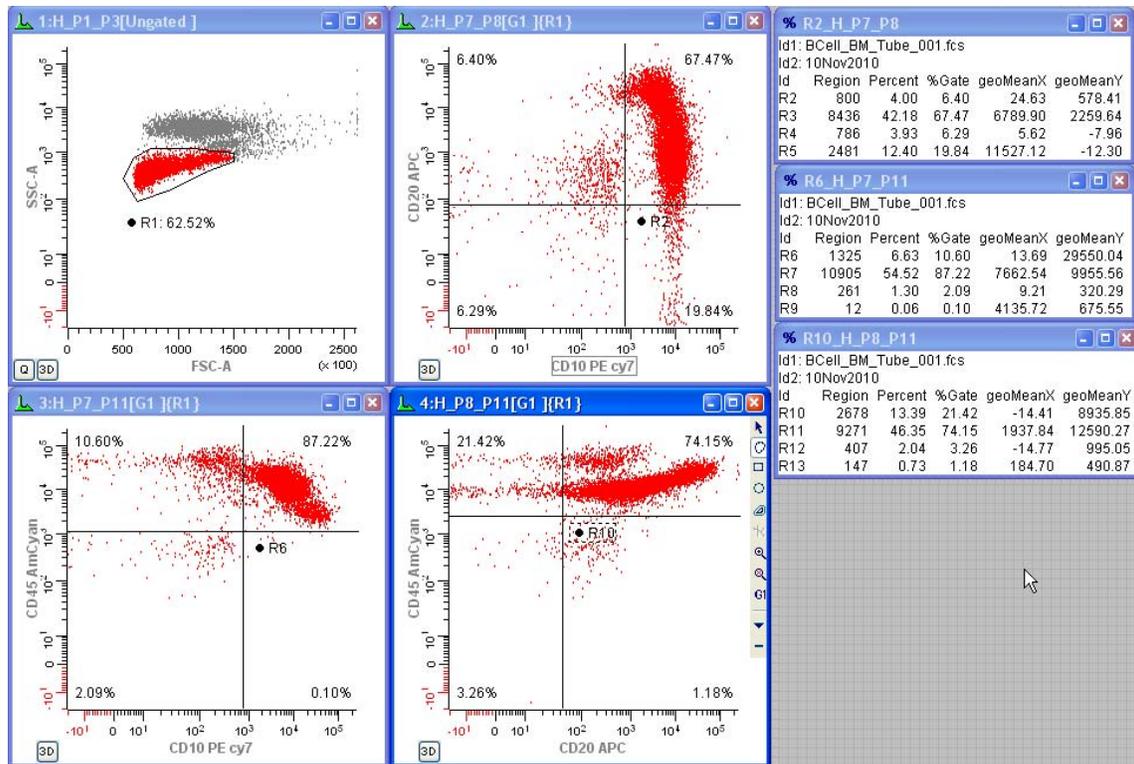
**Create QuadStats for statistics**

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. From the Report menu, choose **New Report**. 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 Properties for File Processor 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 Protocol 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 Protocol Bundle command creates a single file that also contains the listmode data file(s) that you opened.

The protocol bundle is also an excellent way to archive the analyses you perform. Simply use the Save Protocol Bundle command in the File menu 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 protocol bundle automatically.

### Summary

This quick tutorial has demonstrated how quickly and efficiently WinList helps you to create 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 simplicity 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 main toolbar.



2. In the File Open dialog, navigate to the Samples folder in the folder where WinList is installed. Select **BCell\_BM\_Tube\_001.fcs** and click **Open**.

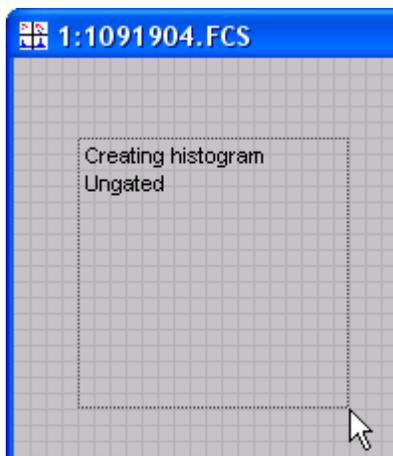
*On the PC, you can also drag and drop files from Windows Explorer or My Computer onto WinList. See Drag and Drop for details.*

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.

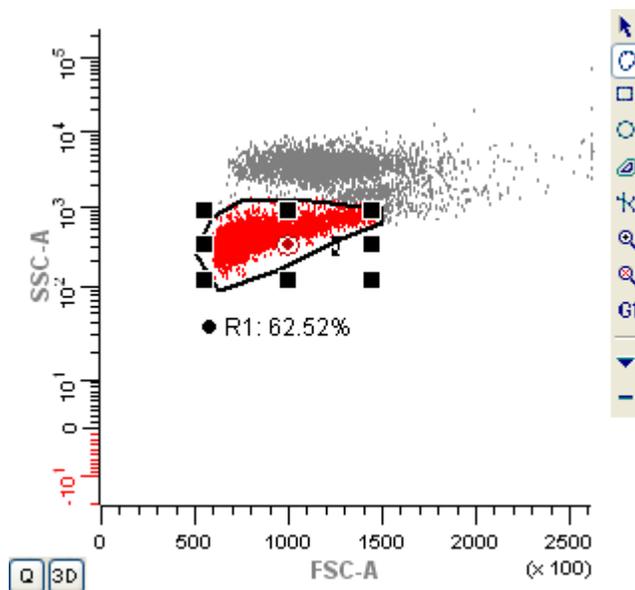
4. Click and hold the mouse button in the data source window. With the mouse button down, move the mouse to define the size and location for a new histogram window. Release the mouse to finish the histogram drawing operation.



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.*

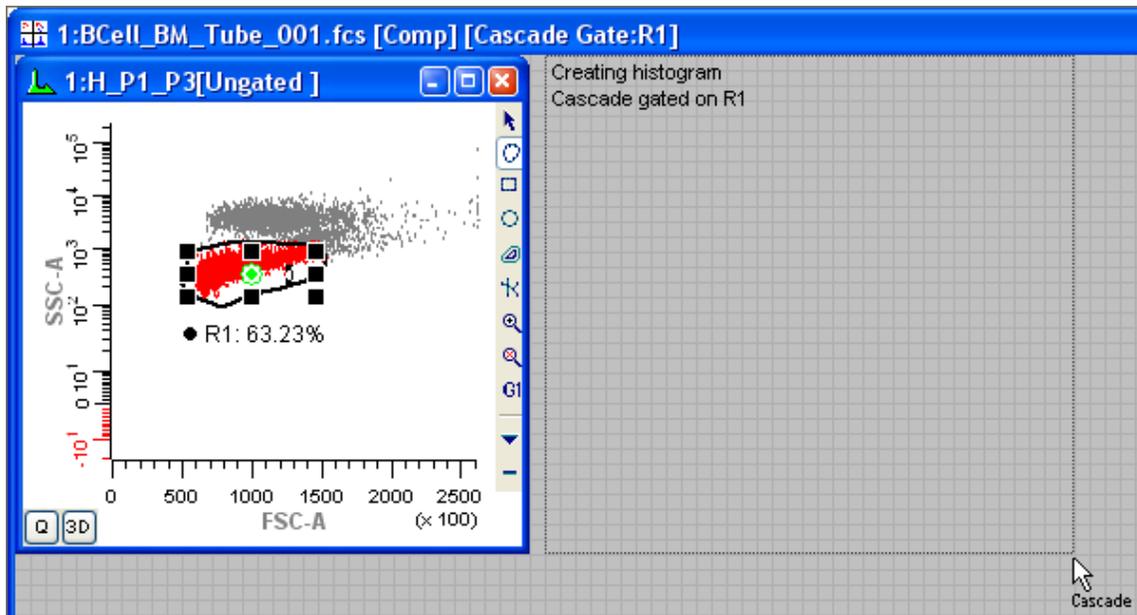
6. Click the **Freehand** region tool in the histogram toolbar, and draw a region around the population as shown. To draw a freehand region, click next to the population of interest and hold the mouse button down. Drag the mouse around the population shown. To finish drawing the region, click on the 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.



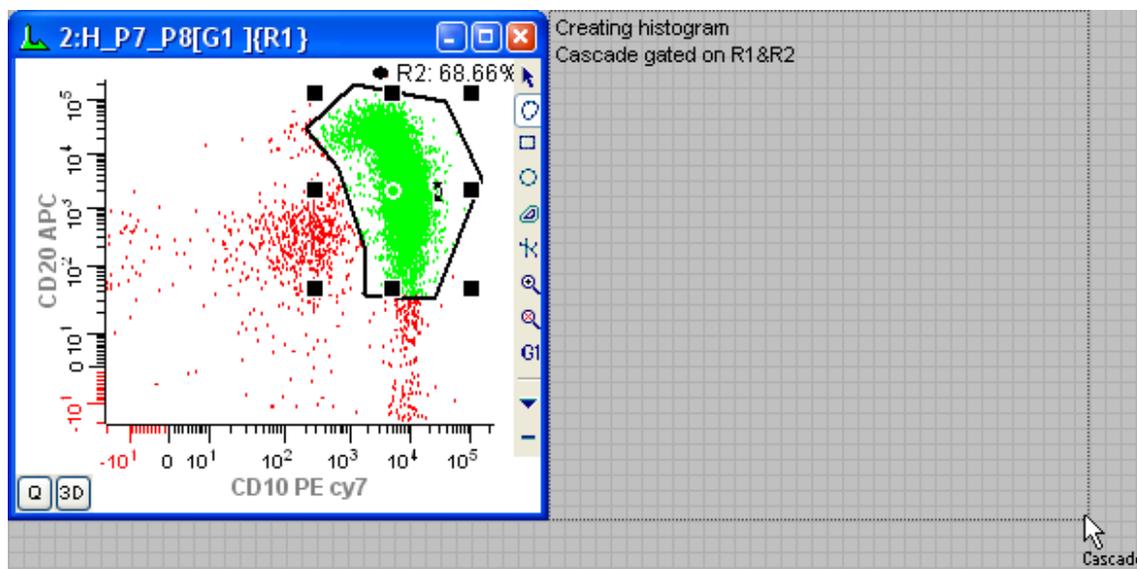
8. Release the mouse button. In the Edit Histogram dialog, select **CD10 PE cy7** for the X Axis and **CD20 APC** for the Y Axis. Click **OK**. This new histogram will be gated on R1.

#### 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 edit 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 main toolbar to display the **Edit Gates** dialog.

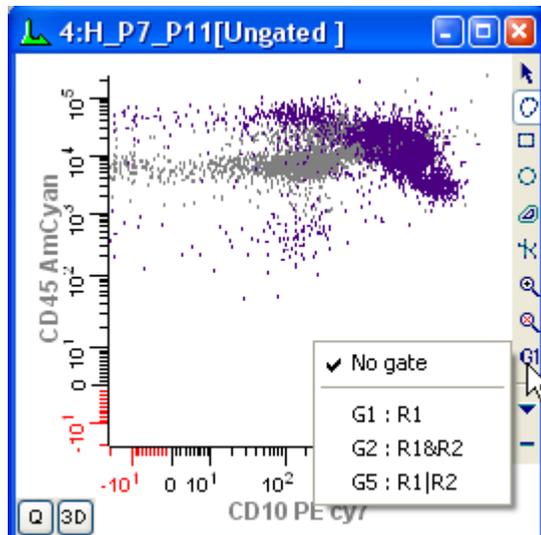
G1=  
G2=

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 click 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.

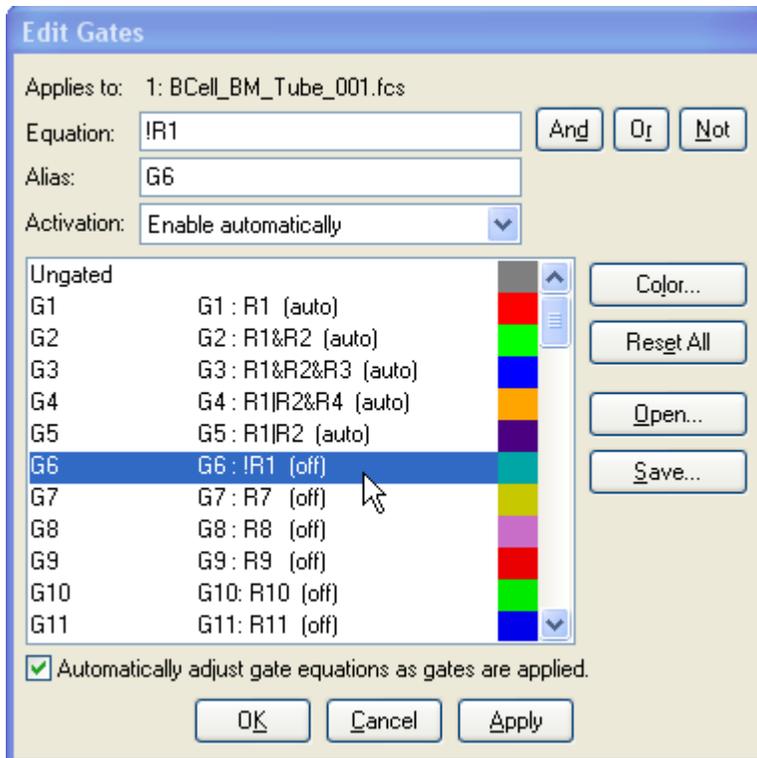
### The Boolean “NOT” operator

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.

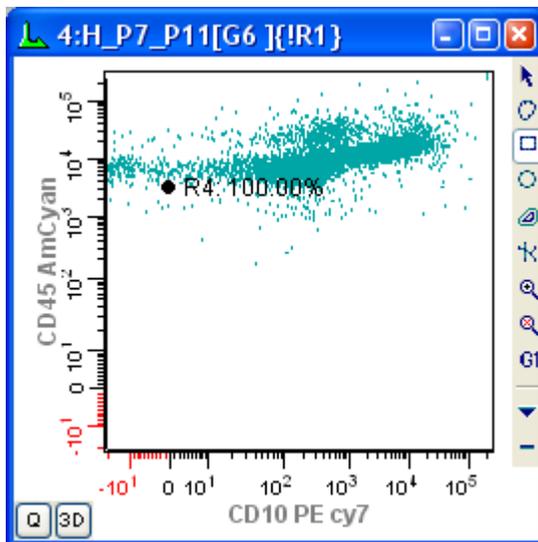
Let's define a gate that includes all events that are *not* in R1.

17. Click the Edit Gates button on the main toolbar 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 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

### 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

### 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.

Here 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, George Washington University. 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 main toolbar.



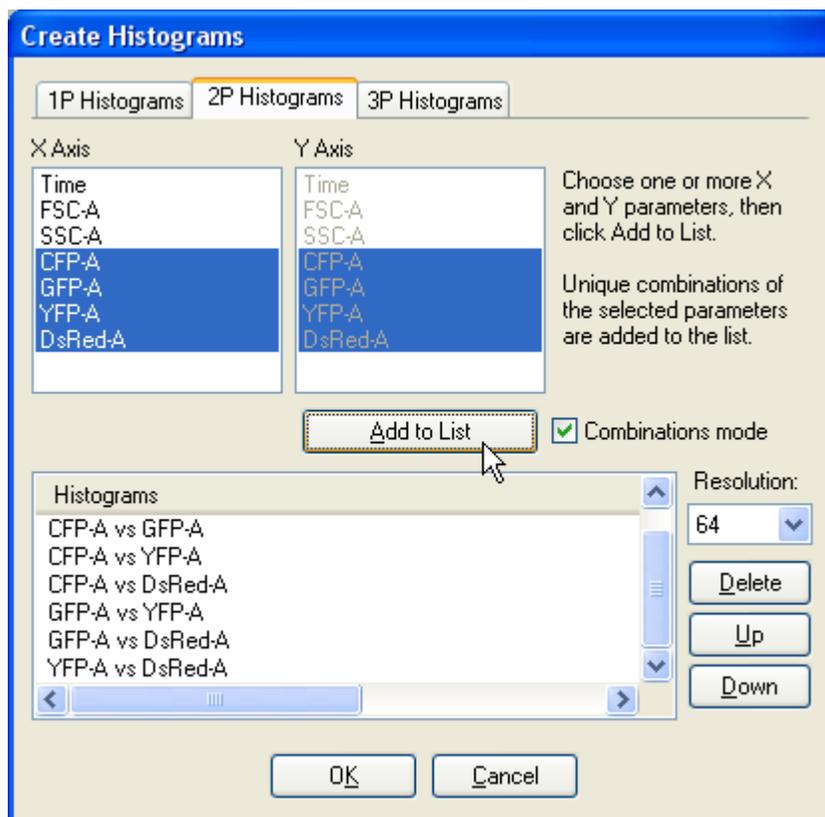
3. In the File Open dialog, navigate to the Samples folder in the folder where WinList is installed. Select four files: **SP2+CFP.fcs**, **SP2+DsRed.fcs**, **SP2+GFP.fcs** and **SP2+YFP.fcs**. You can press the Ctrl key on the keyboard to select more than one file. Click **Open**.

The first files selected are single-color control files for CFP, DsRes, 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.*

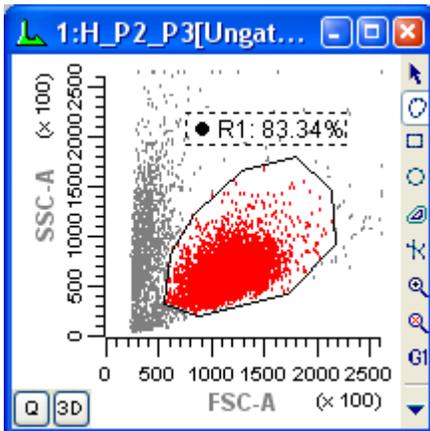
4. In the **Create Histograms** dialog, select **FSC-A** in the X Axis list, **SSC-A** in the Y Axis list, and click **Add to List**.

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 7 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.

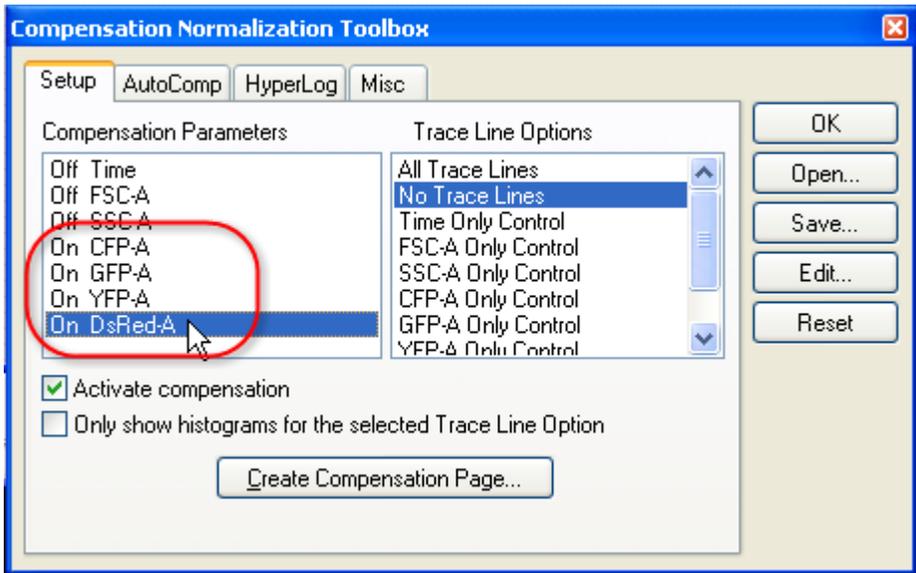


7. Apply this gate to each of the remaining dot plots.

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



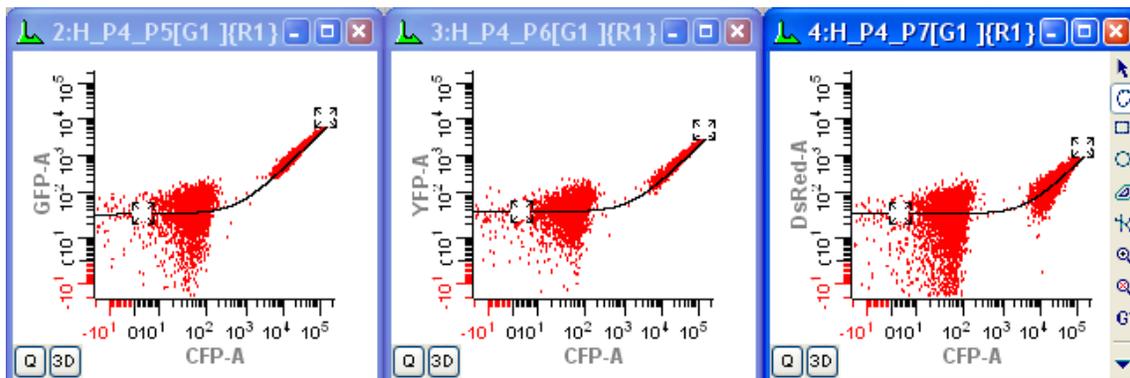
9. In the **Compensation Parameters** listbox, click **CFP-A**, **GFP-A**, **YFP-A**, and **DsRed-A** parameters to enable them for compensation. Notice the label changes from “Off” to “On” in the listbox.



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.*

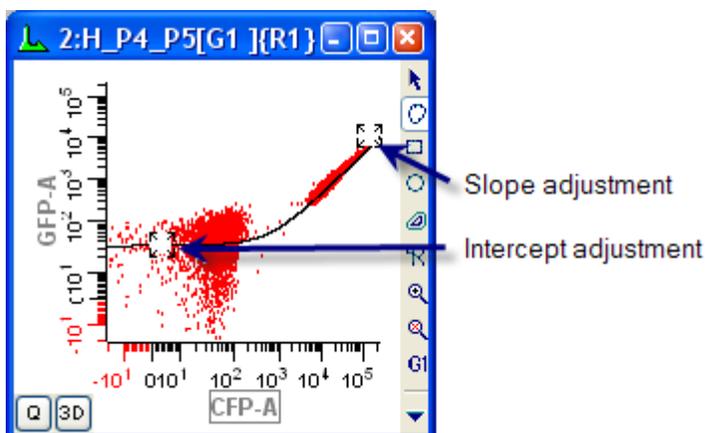
10. This first file is the CFP-only control file. Click *CFP-A Only Control* in the **Trace Line Options** listbox. Trace lines now appear only in the histograms containing CFP-A.



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, ignoring events in the first decade.

#### 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 linearized 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 Control” 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 Control” 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 Control” 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. From the File menu, choose **Next FCS File**. 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 scatter plot.

*You may have noticed that the log axes on the histograms show values less than zero. This is a feature in WinList called HyperLog™ that allows you to visualize compensated data. The HyperLog™ axis is like a log axis without the limitations of the log domain, 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 HyperLog tab of the Compensation Toolbox. This tab also provides a control to **Optimize HyperLog**.

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

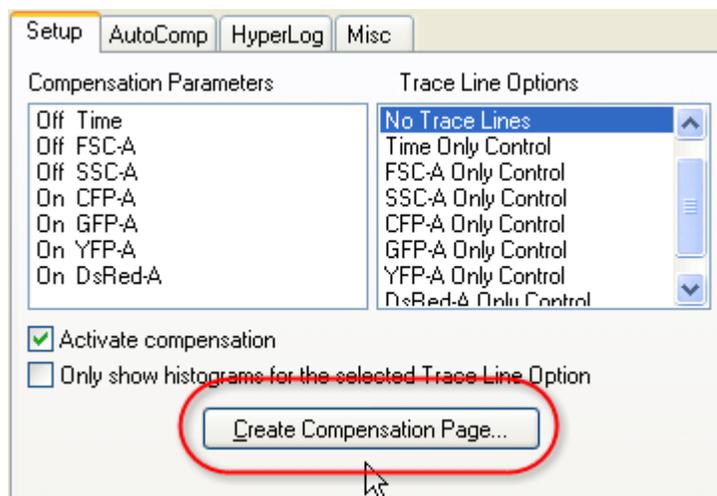
WinList uses a sophisticated data processing method to determine the optimal B-coefficient (the linear portion of the axis) and the lowest negative value for each parameter. The routine ensures that most negative values are visible and that populations near zero are not split into bimodal distributions.

## 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.

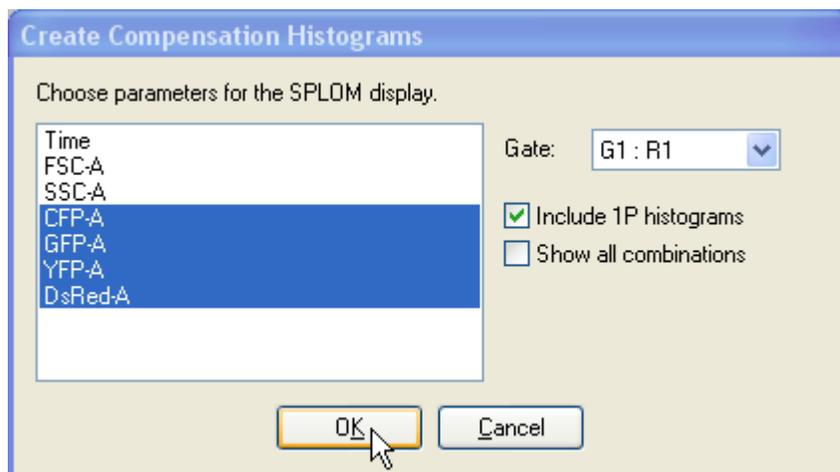
Let's see how the SPLOM works for this 4-color set up.

18. Click the **Create Compensation Page** button on the Setup tab of the Compensation Toolbox.



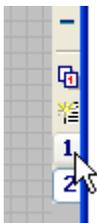
The Create Compensation Histograms dialog is displayed and the compensation parameters are already selected.

19. In the dialog, choose the 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.



### Reset Auto-Compensation

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

20. 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 option from the File menu 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 **Only show histograms for the selected Trace Line Option** on the Setup tab of the Compensation toolbox. When enabled, you can view a subset of histogram simply by selected 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 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

### 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.

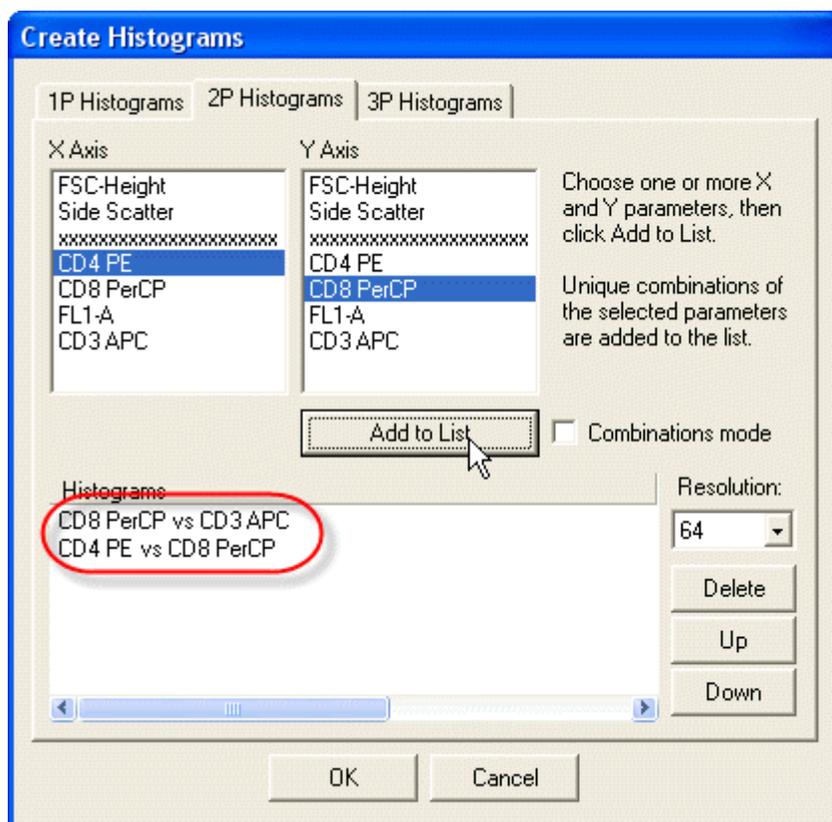
### Set up

We'll start by opening a listmode file and creating some histograms and results.

1. Click the **Open FCS File** button on the main toolbar and navigate to the Samples folder 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 for details.

2. When the **Create Histograms** dialog appears, select **CD8 PerCP** in the X Axis list, **CD3 APC** in the Y Axis list, and click **Add to List**. Select **CD4 PE** in the X Axis list, **CD8 PerCP** in the Y Axis list, and click **Add to List**. Click **OK**.



Select CD8 vs CD3 and click **Add to List**.

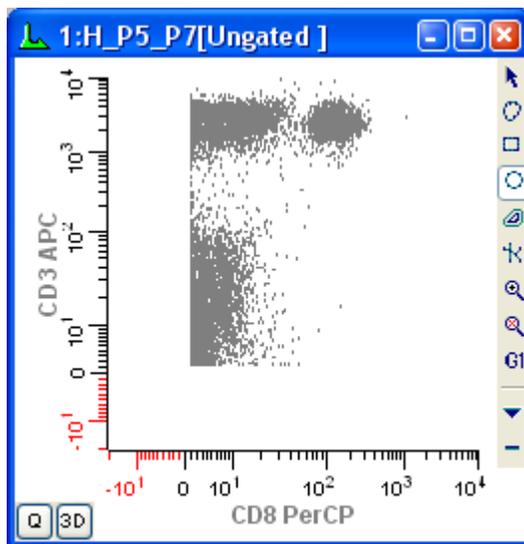
Select CD4 vs CD8 and click **Add to List**.

Click **OK**.

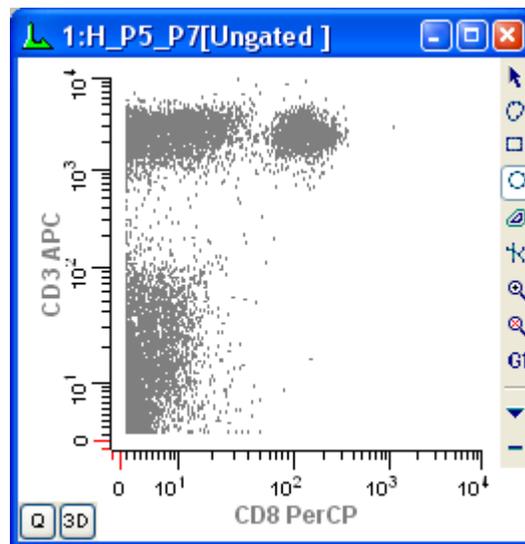
Now, if your preferences are set to use HyperLog by default, you'll notice that this data looks a bit odd. The file does not contain any negative values, and so there are no events plotted below 0. Let's use the Optimize HyperLog command to fix this situation.

3. Click the Compensation Toolbox button on the main toolbar to display the Compensation Normalization Toolbox. Select the **HyperLog** tab, and click the **Optimize HyperLog** button. Select all of the parameters and click **OK**. The negative portion of the HyperLog axis will be eliminated. You can close the Compensation Toolbox at this point.

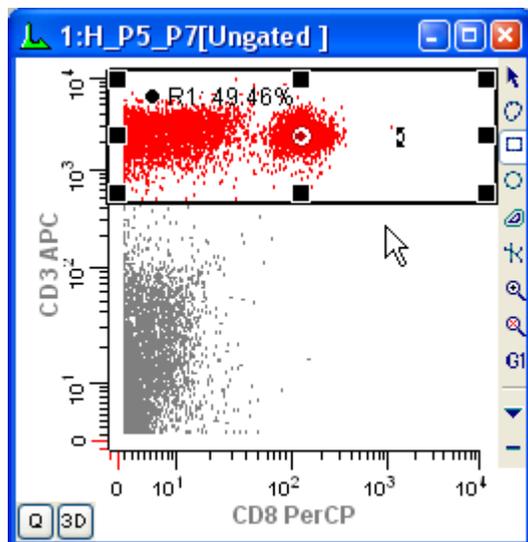
Before adjustment



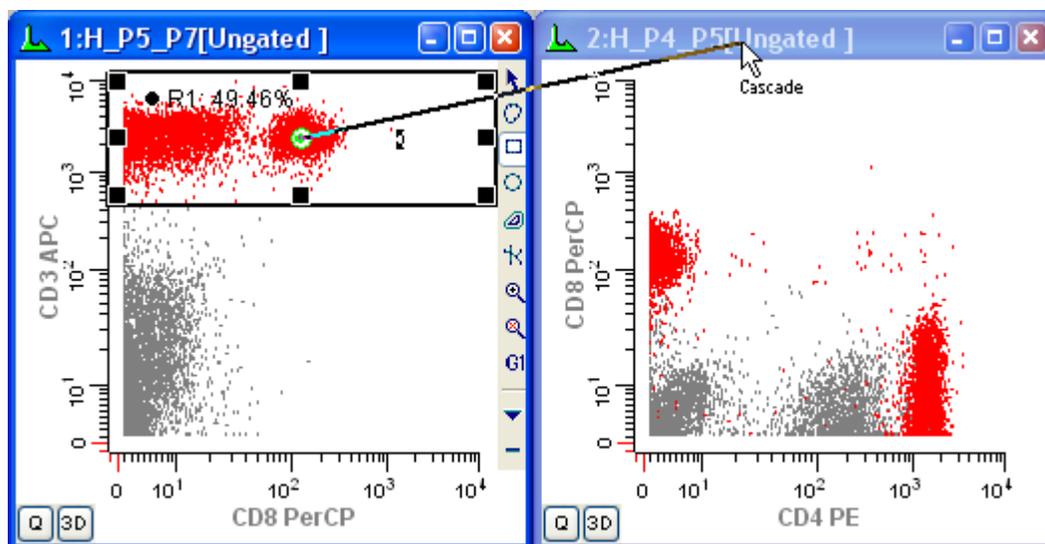
After adjustment



4. Use the Rectangle region tool to create a region around the CD3 positive events in the CD8 PerCP vs CD3 APC histogram.



5. Use the cascade gate hotspot in the center of R1 to apply the R1 gate to the CD4 PE vs CD8 PerCP histogram.



6. Click the **Q** button in the lower-left corner of the CD4 PE vs CD8 PerCP 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 main toolbar to create a new, empty report window. Move and resize the new window so that you can see the histograms and results windows.



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

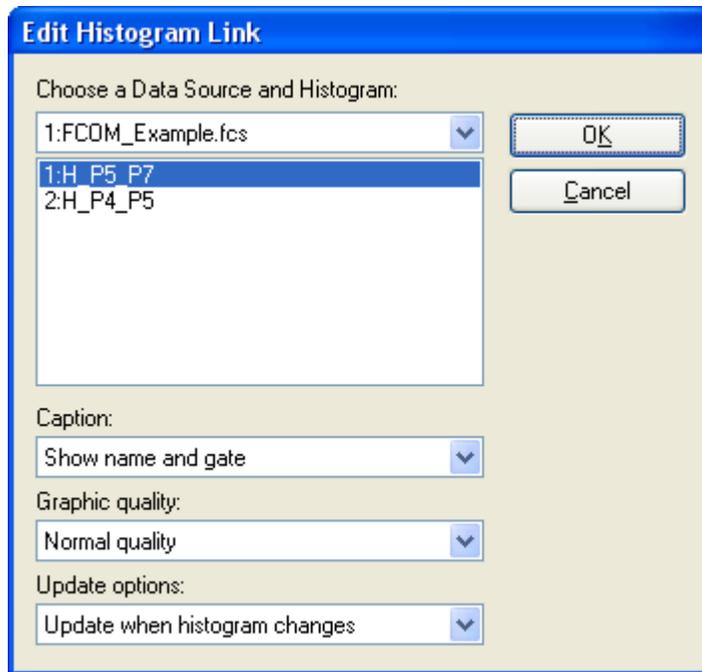
The most intuitive way to add objects to the report is to drag-and-drop them on the report. Each of WinList's windows has a drag-and-drop hotspot on the title bar. This is the small icon that appears on the left of the title bar.

*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.



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 resize the object.*

### Adding results to the report

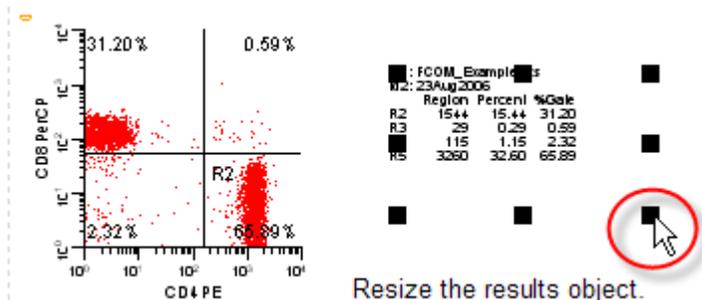
We could drag-and-drop the results window onto the report in the same manner, but let's use the report toolbar instead.

12. Click the Results tool 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. Resize the results object if it is not large enough to see all of the results.



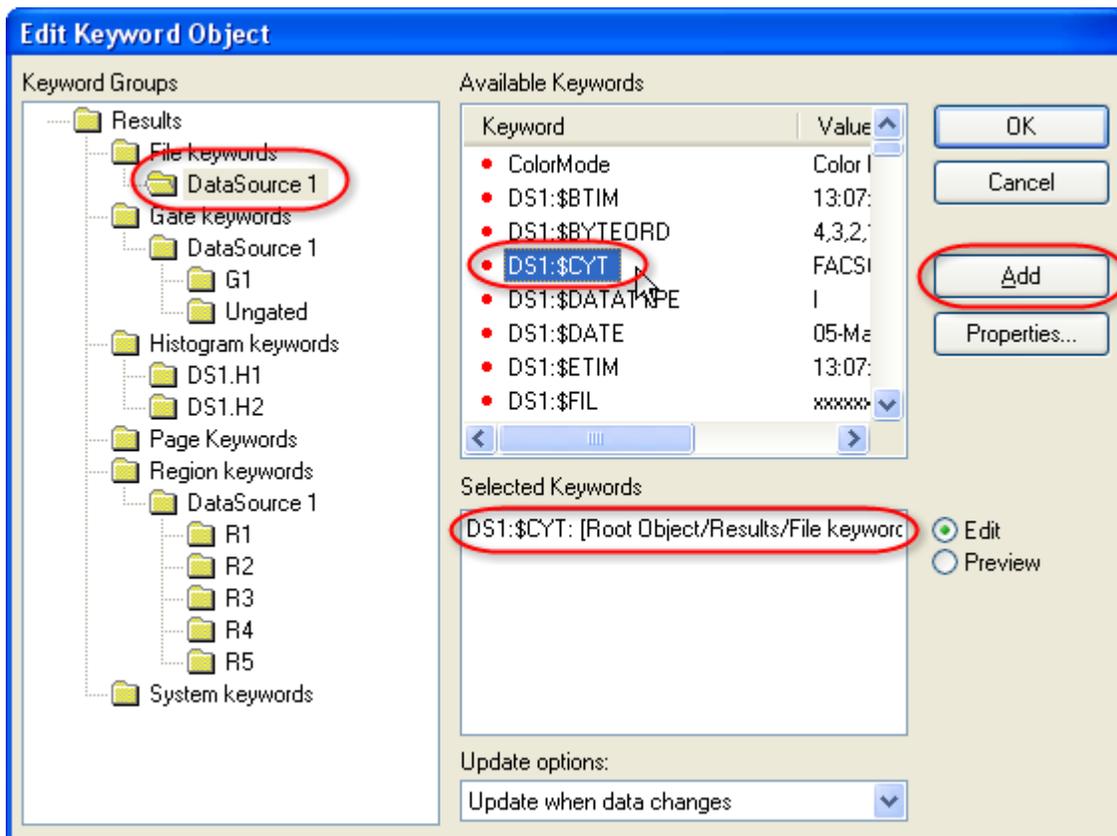
**Adding FCS keywords to the report**

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.

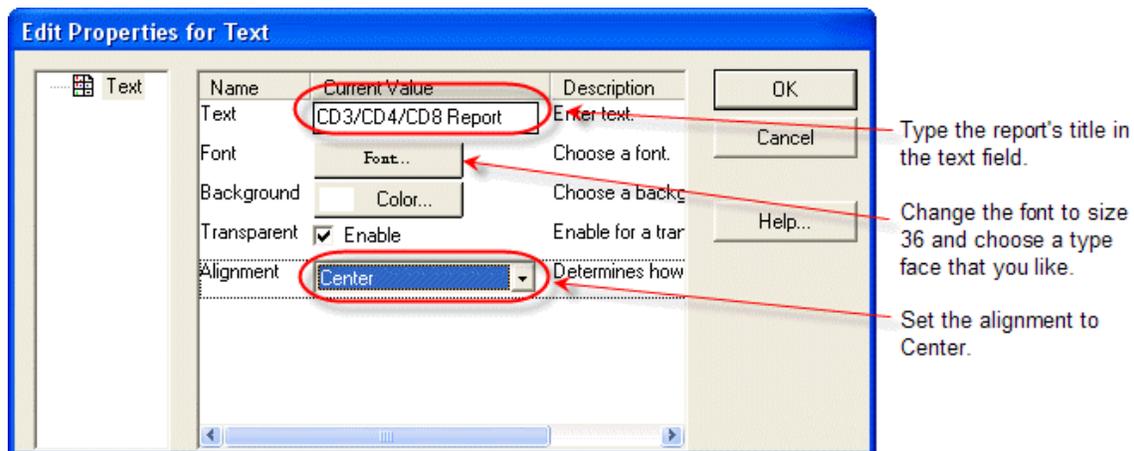
**Adding a title area to the Master Page**

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 **CD3/CD4/CD8 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**.



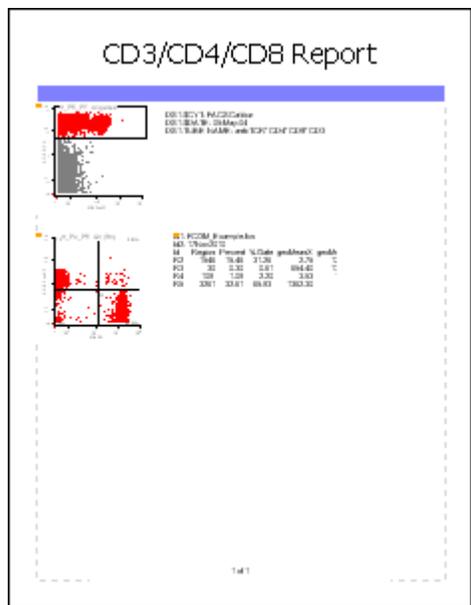
20. Move the new text object to the top of the report page and stretch it across the width of the page.

*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.



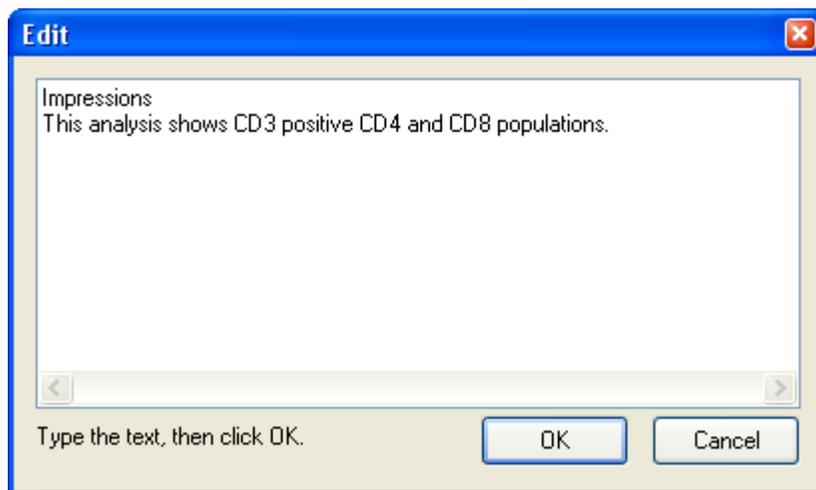
#### Adding a gate legend and impressions

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 CD3 positive CD4 and CD8 populations."



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 gold chain link. When the object is not linked, the symbol is a broken chain link.*

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 FCOM to enumerate phenotypes

In this tutorial, we'll learn how to use the *FCOM* parameter to enumerate phenotypes. The *FCOM* parameter is one of the most powerful calculated parameter functions 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 positives 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 is a great place to start.*

### Open a data file

1. Click the **Open FCS File** button on the main toolbar and navigate to the Samples folder 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 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 there is really no utility to using the HyperLog transformation for it. Let's switch over to use the Log transform for all log-like parameters.

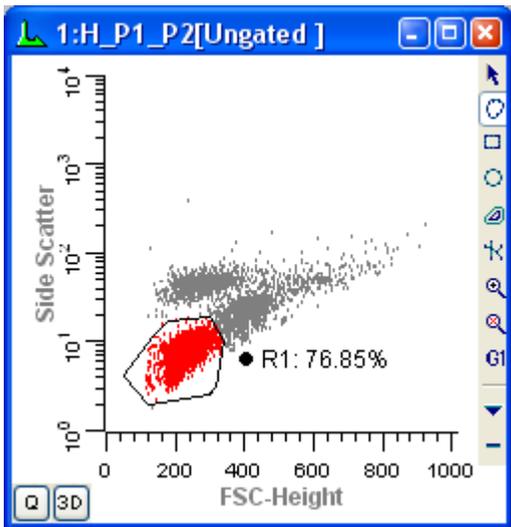
3. Click the Compensation Toolbox button on the main toolbar to display the Compensation Normalization Toolbox. Select the **HyperLog** tab, and click the **HyperLog > Log** button. You can close the Compensation Toolbox at this point.

### Creating histograms and gates

Next, we'll draw 3 histograms on the empty data source window and gate them appropriately.

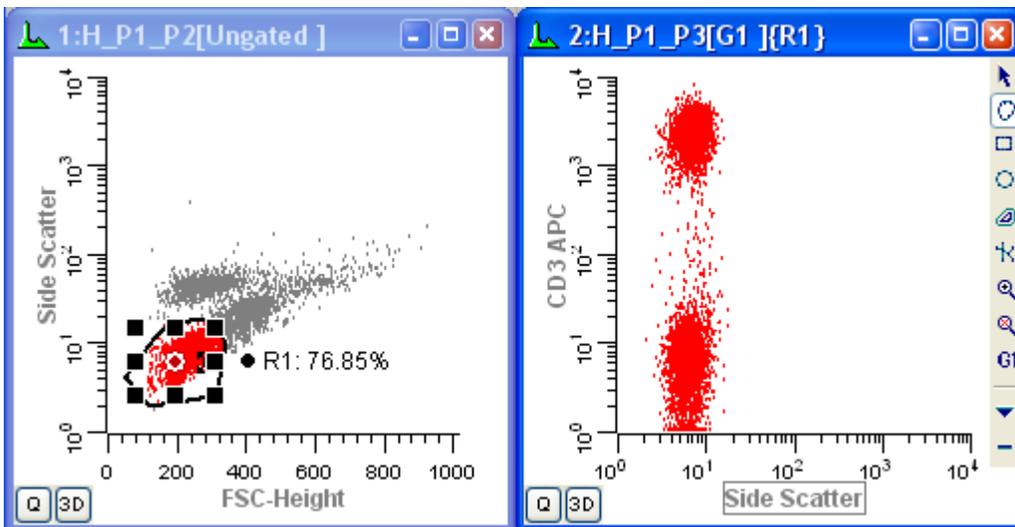
4. Click, drag, and release the mouse on the data source window to define an area where we want to display a histogram. In the **Edit Histogram** dialog, choose FSC-Height and Side Scatter for the X- and Y-Axis respectively, then click **OK**.

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 on R1's cascade hotspot (the red dot 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**.

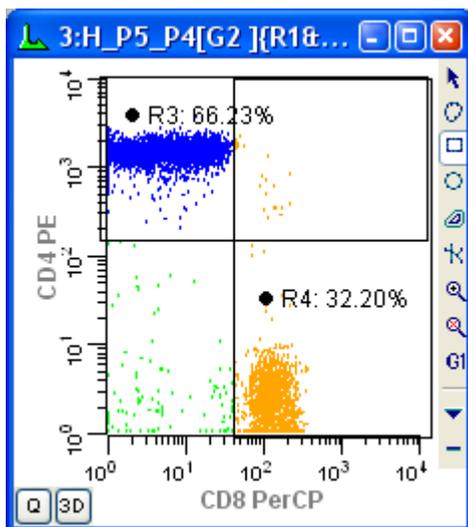
A new histogram gated on R1 is displayed.



*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+ for 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.

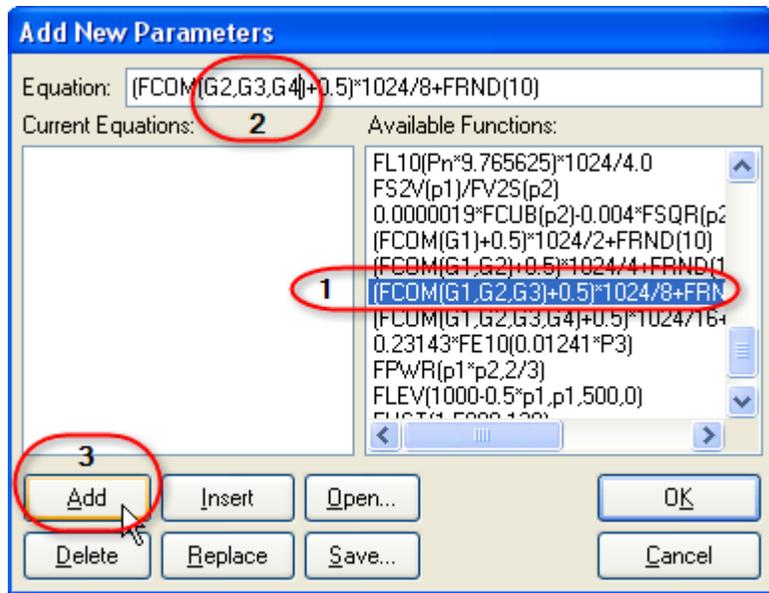
9. Choose **Edit->Data Source**. In the **Edit Data Source** dialog box, click **Add Parameters**.

Instead of typing the full FCOM equation, let's choose an example equation from the Available Functions list box.

10. Scroll the list box of Available Functions and select **(FCOM(G1,G2,G3)+0.5)\*1024/8+FRND(10)** from the list.

We need to edit this example equation to use gates **G2**, **G3**, and **G4**.

11. Edit the equation so that it reads **(FCOM(G2,G3,G4)+0.5)\*1024/8+FRND(10)**. Click **Add**.



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. With the 3 gates "G2, G3, G4" as its argument, 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 value for the G2, G3, and G4 gates.

G2	G3	G4	FCOM Value
-	-	-	0
+	-	-	1
-	+	-	2
+	+	-	3
-	-	+	4
+	-	+	5
-	+	+	6
+	+	+	7

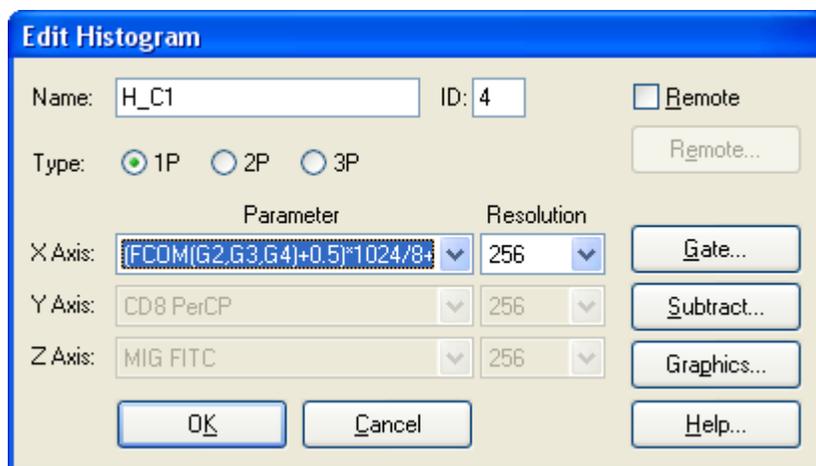
In essence, FCOM converts gate combinations to a single number identifying the phenotype. The more gates in the expression, the more combinations there are. The rest of the equation spreads the populations so that we can visualize them more easily on a histogram display.

12. Click **OK** to close the **Add Parameter** list box. Click **OK** again to close the **Edit Data Source**.

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.*

13. In an empty portion of the data source window, draw a new histogram with the mouse. In the Edit Histogram dialog, choose **1P** for the histogram type, and choose the FCOM parameter for the X Axis. Click **OK**.

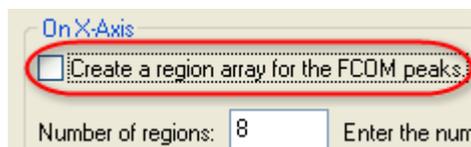


This new histogram has distinct peaks representing the eight possible states for events evaluated by gates G2, G3, and G4. The leftmost peak represents events outside of all three gates, the triple-negative events. The next peak contains events that are in G2 but not in G3 or G4. The last peak represents triple-positive events in all gates.

### Statistics

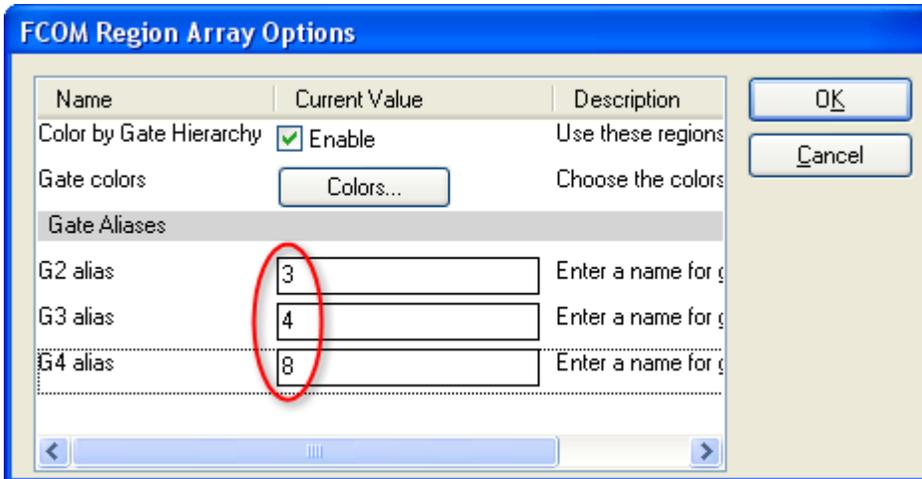
WinList makes it easy to get statistics for each phenotype and to see each distribution in the context of other histograms. To do this, we'll use the Create Region Array feature to create a set of regions and results.

- From the Create menu, choose **Region Array**. Click the checkbox **Create a region array for the FCOM peaks**. This is a special option that only appears when the active histogram contains an FCOM parameter.



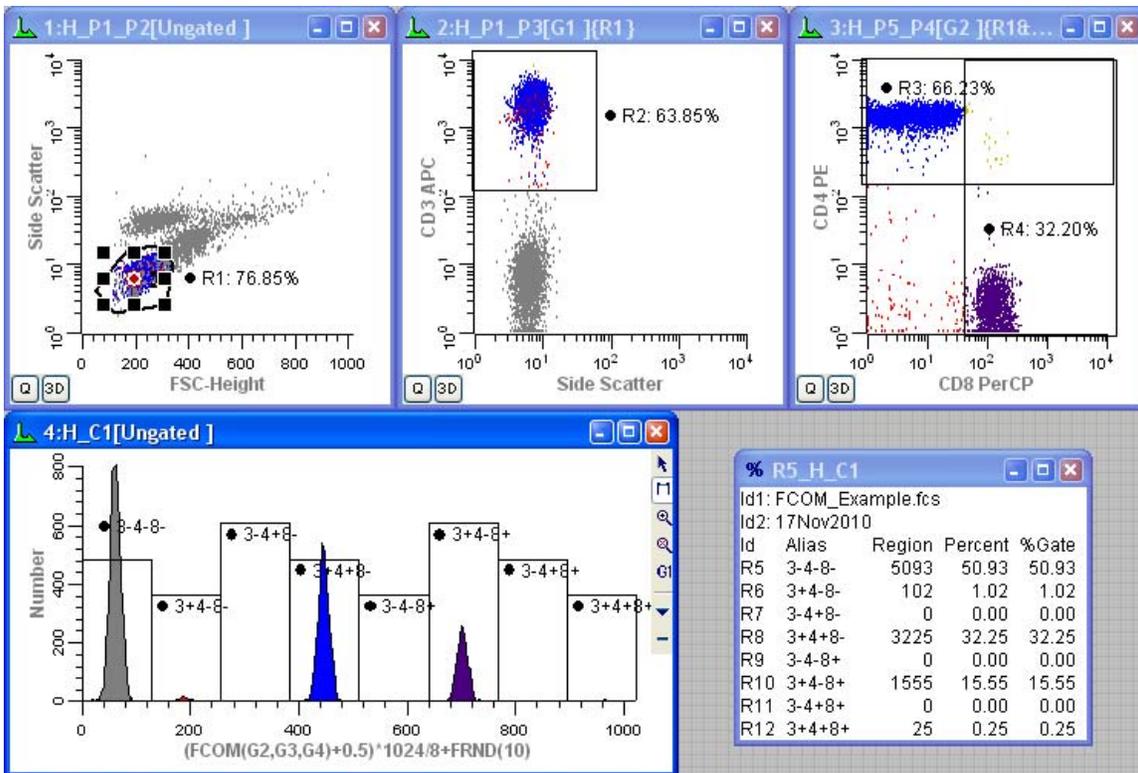
The **FCOM Region Array Options** dialog appears. This dialog allows you to turn on color gating based on the FCOM peaks, and to choose the colors used for those gates. It also allows us to name the gates to make it easier to identify phenotypes.

- Type 3 in the **G2 alias** edit box, 4 in the **G3 alias** edit box, and 8 in the **G3 alias** edit box.



16. 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

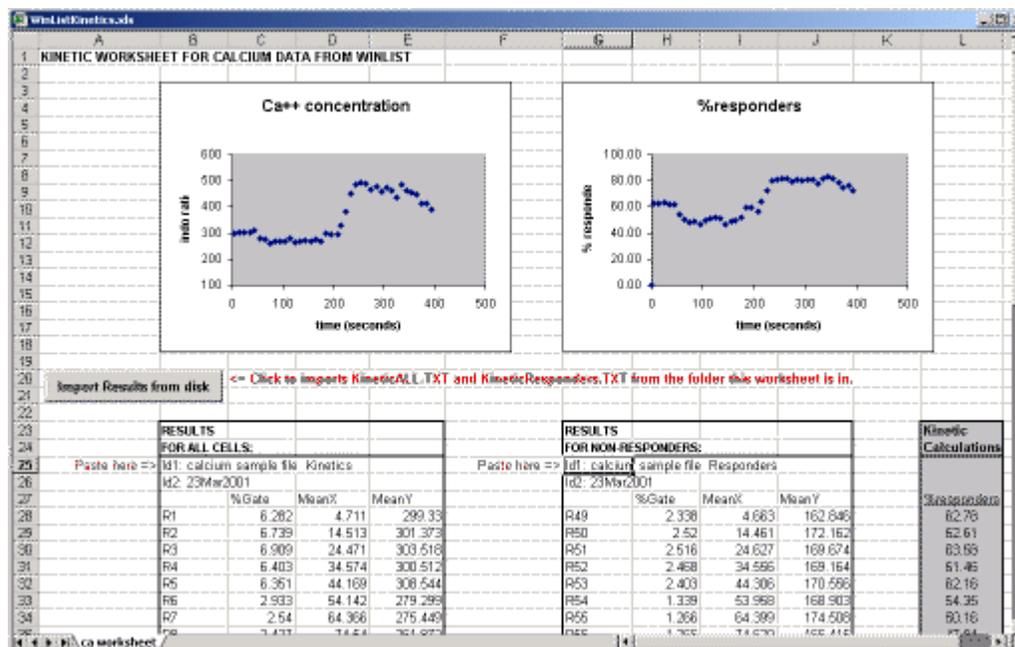
#### Kinetic Analysis with Excel

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**

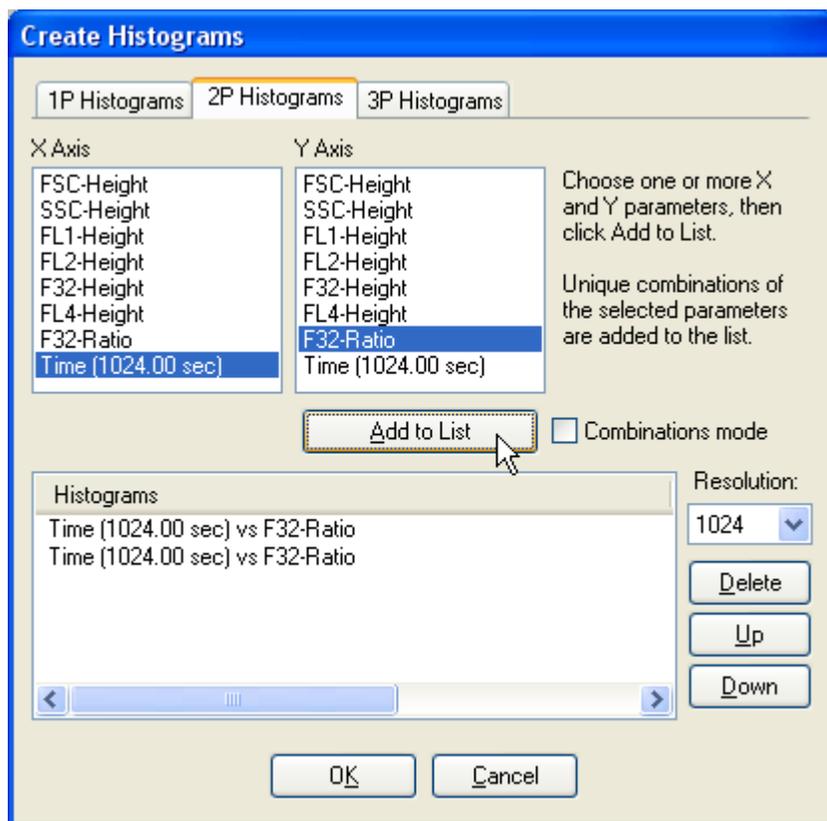
4. Start WinList, and from the File menu, choose **Open FCS File**. The File Open dialog is displayed.

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. Click the right-mouse button 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 Create menu, choose **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**.

The screenshot shows the 'Create Region Array' dialog box with the following settings:

- Options:**
  - Create a results window
  - Total regions to create: 48
- On X-Axis:**
  - Number of regions: 48
  - Starting at: 0
  - Ending at: 480
  - Space between: 0
- On Y-Axis:**
  - Number of regions: 1
  - Starting at: 0
  - Ending at: 1024
  - Space between: 0

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. Choose **Edit Results** from the Edit 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 *Create* menu, choose **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**.

**Create Region Array**

**Options**

Create a results window

Total regions to create: 48

**On X-Axis**

Number of regions: 48 Enter the number of regions in the X direction.

Starting at: 0 Enter the left edge of the first region.

Ending at: 480 Enter the right edge of the last region.

Space between: 0 Spacing (% of X scale).

**On Y-Axis**

Number of regions: 1 Enter the number of regions in the Y direction.

Starting at: 0 Enter the bottom edge of the first region.

Ending at: 250 Enter the top edge of the last region.

Space between: 0 Spacing (% of Y scale).

OK

Cancel

A second Results window will appear. Once again, we need to choose the statistics to display.

15. Choose **Edit Results** from the *Edit* 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.

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. From the **Replay** menu, choose **Active Data Source**.

17. Right click inside the **R1\_P8\_P7** 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.

The screenshot shows an Excel spreadsheet titled 'WinListKinetics.xls'. The columns are labeled A, B, and C. The rows are numbered 22, 23, 24, 25, and 26. Cell B25 is selected and contains the text 'Paste here => Id1: calcium sample'. Cell B26 contains the text 'Id2: 23Mar2001'. Above these cells, the text 'RESULTS FOR ALL CELLS:' is displayed in a bold font.

	A	B	C
22			
23		<b>RESULTS</b>	
24		<b>FOR ALL CELLS:</b>	
25	Paste here =>	Id1: calcium sample	
26		Id2: 23Mar2001	

Now, we repeat the process for the second set of results.

19. Activate WinList. Right click inside the **R49\_P8\_P7** 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 screenshot shows an Excel spreadsheet titled 'WinListKinetics.xls'. The columns are labeled F, G, and H. The rows are numbered 22, 23, 24, 25, and 26. Cell G25 is selected and contains the text 'Paste here => Id1: calcium sample'. Cell G26 contains the text 'Id2: 22Mar2001'. Above these cells, the text 'RESULTS FOR NON-RESPONDI' is displayed in a bold font.

	F	G	H
22			
23		<b>RESULTS</b>	
24		<b>FOR NON-RESPONDI</b>	
25	Paste here =>	Id1: calcium sample	
26		Id2: 22Mar2001	

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, let's put them to use here to streamline the process of getting results into Excel. We will create a macro in WinList that saves the content of the results windows to disk files in the same folder as the Excel worksheet. Then, we will use a pushbutton in the Excel worksheet to open these files and update the worksheet.

21. Activate WinList. Choose **Clear** from the Macro menu. This will clean out any macro in memory.

22. Choose **Record** from the Macro menu. This will turn on the macro recorder.

23. Click once on the title bar of the first results window, R1\_H\_P8\_P7. From the File menu, choose Save->Results. The name of the file in the **Save as** edit box should be *AllCells.TXT*. Navigate to the folder where the WinList Kinetics worksheet is located and click **Save**.

24. Next, click once on the title bar of the second results window, R50\_H\_P8\_P7. From the File menu, choose Save->Results. The name of the file in the **Save as** edit box should be *NonResponders.TXT*. Navigate to the folder where the WinList Kinetics worksheet is located and click **Save**.

25. Turn off the macro recorder by again choosing **Record** from the Macro menu.

We have recorded a macro that we want to use anytime, so we need to save the macro to disk.

26. From the Macro menu, choose **Save**. Navigate to the folder where the WinList Kinetics worksheet is located. Enter SaveKineticResults in the Save As edit box. Click **Save** to store the new macro on disk.

Now, let's run the macro to make sure it works.

27. From the Macro menu, choose **Run**.

The macro should replay, saving new copies of the two results windows to disk. Let's import the results into the Excel spreadsheet using an Excel macro that is already written for us.

28. Activate Excel. Click the **Import Results from disk** button.

Excel will read the two files we just saved, and paste the content into the correct locations on the worksheet. You may not notice any changes, since the numbers should be the same as the ones we copied and pasted manually.

### 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.

Using a WinList macro to save the results to files on disk, we imported them into Excel using a button that appears on the worksheet. The process could be further automated by attaching the macro that saves results to a button on the Macro Toolbox. Use this tutorial as a guideline to kinetic analysis of your own listmode files. If you collect files with the same parameters as the one we used in this example, you can use the same WinList protocol and you do not need to make any changes in the Excel worksheet. If your parameters are somewhat different, you may have a few edits to make in the Excel worksheet and the macro that saves WinList results.

### Enhanced Proliferation Analysis

Features in ModFit LT and WinList provide unprecedented capabilities to analyze cell proliferation.

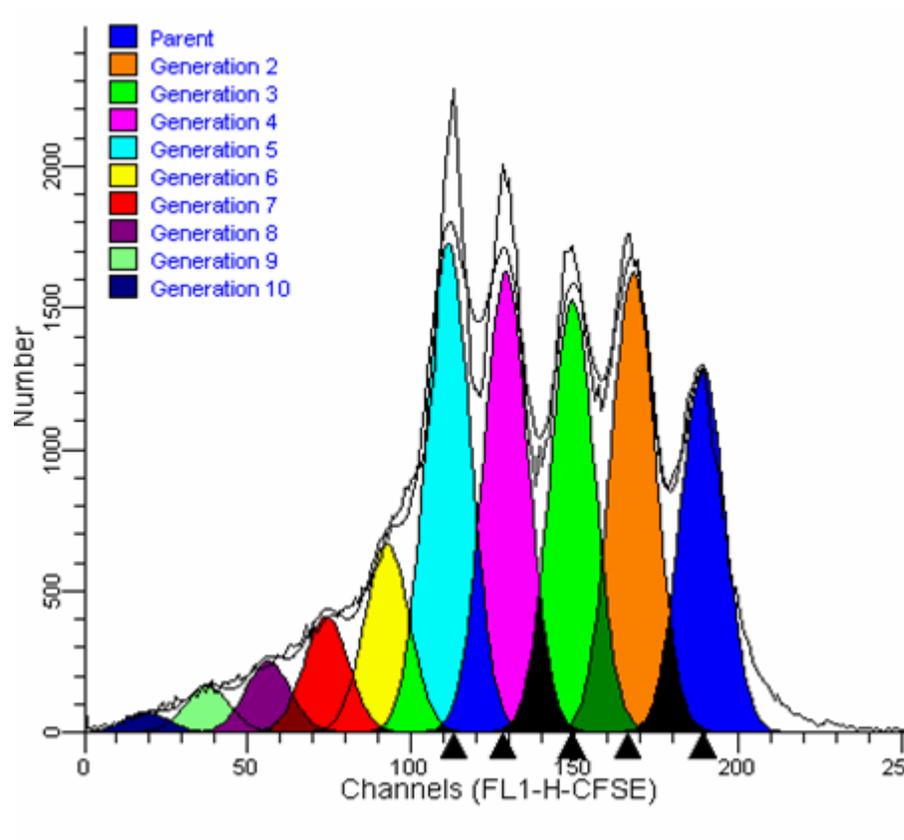
ModFit LT performs sophisticated non-linear least squares analysis in just a few clicks of a mouse and reveals the secrets of the proliferation data: proliferation index, non-proliferative fraction, and precursor frequency, and fractions for each proliferating generation. The proliferation results can 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.

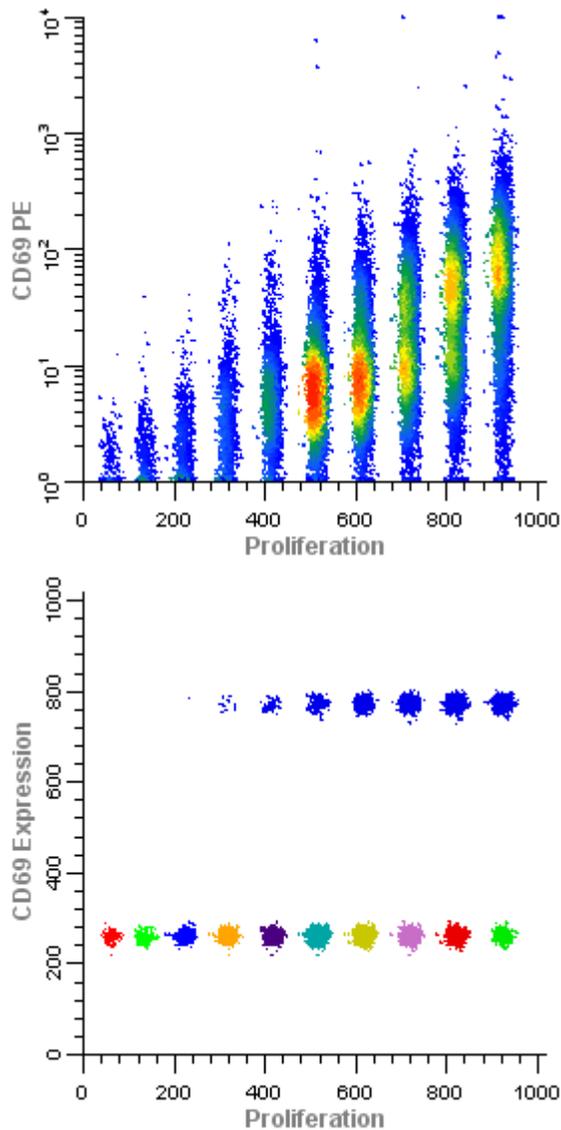
This tutorial will explore these new features to examine a CFSE-stained sample containing several other markers.

### The big picture

From the Proliferation Wizard in ModFit LT...



Into WinList for exciting, new, explorations of proliferation.

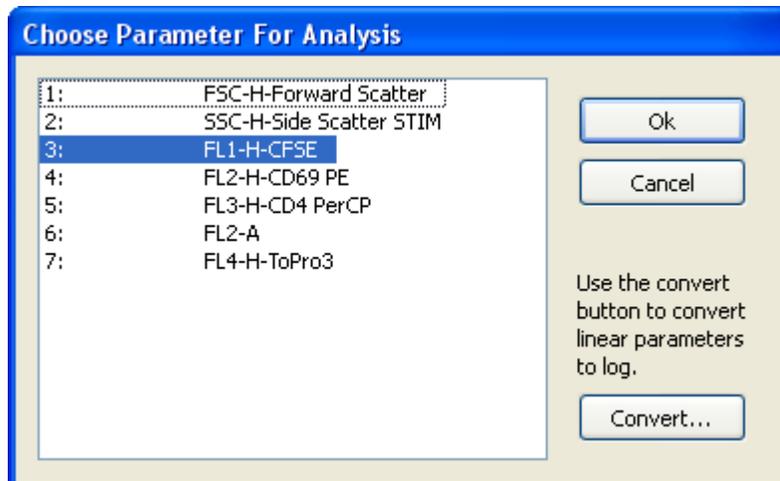


### Getting started

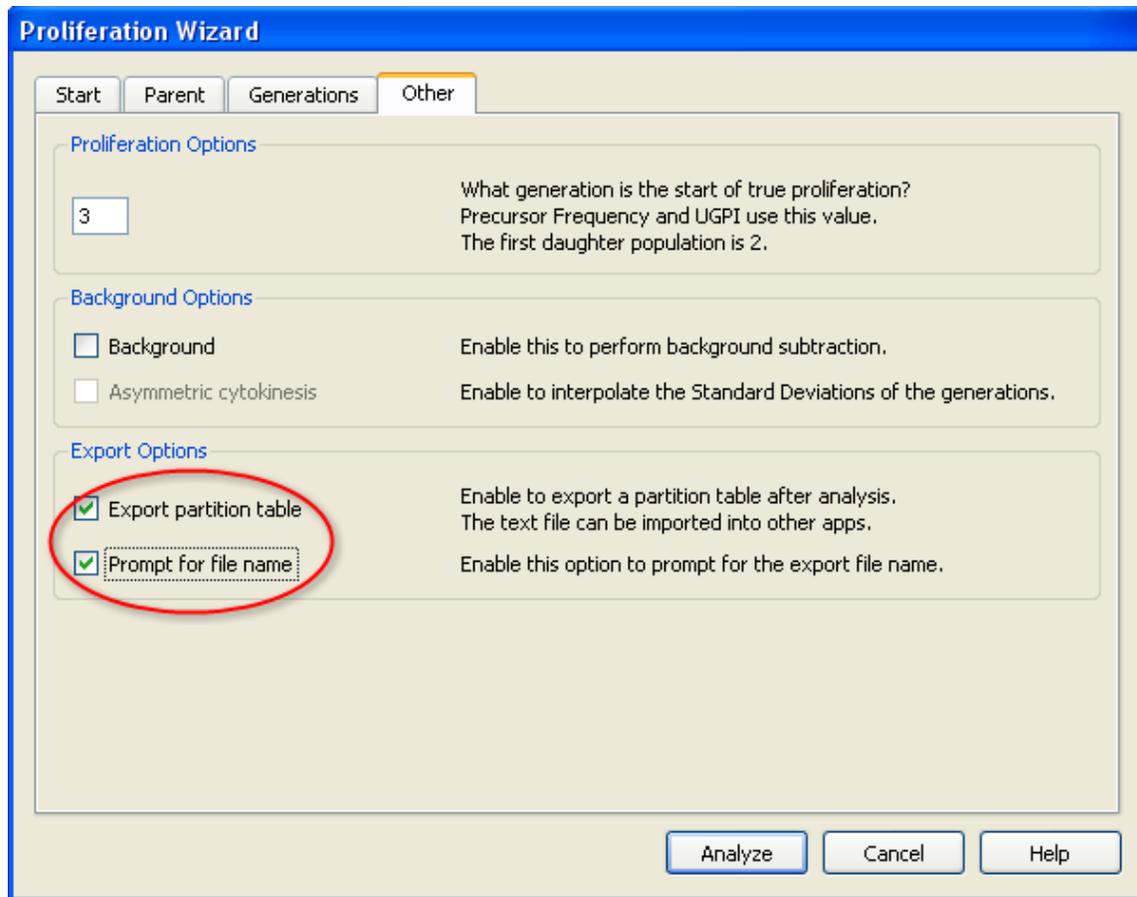
To start, we will use the Proliferation Wizard in ModFit LT and export its results. Then, you will set up WinList to import those results into an NStat region.

*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 in the WinList application folder. Select "CFSE sample.fcs" and click **Open**.



4. Choose "P3: FL1-H-CFSE" from the parameter list, and click **OK**.
5. In the next dialog, leave the Gate checkboxes unchecked and click **OK**.
6. From the Analysis menu, choose **Proliferation 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 Save dialog will be displayed. You can save the file to any location. For this tutorial, save the export file in the same folder as the data file.

*When you choose the Export option for the Proliferation 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.*

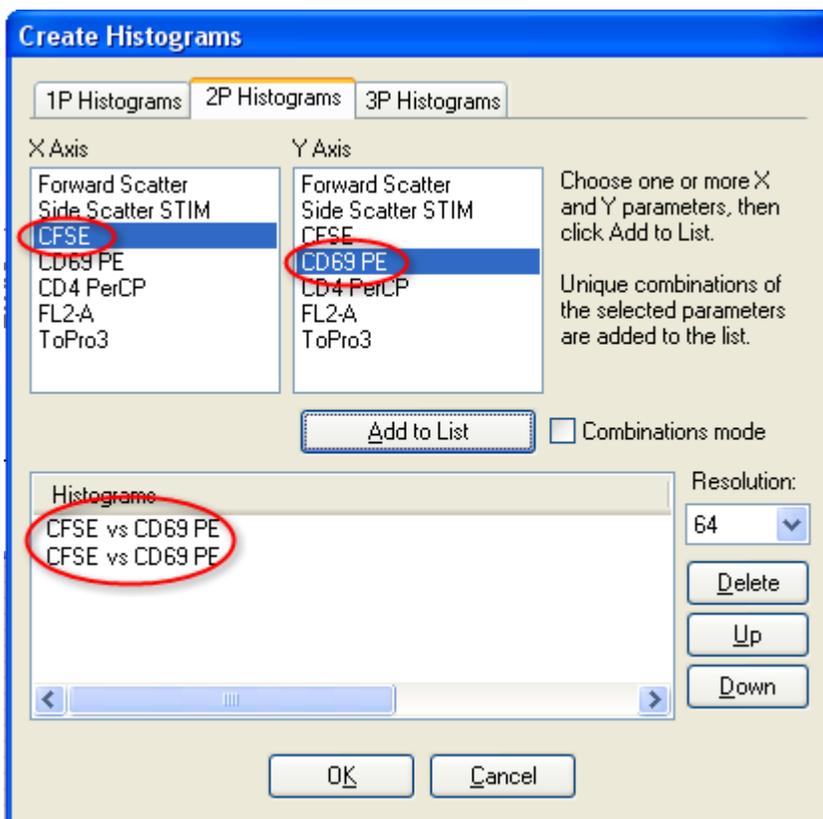
```

Prolif Wizard Partitions
File      CFSE sample.fcs
Regions  10
Scale    0      256
Gen10    0.00   26.20
Gen9     26.20  45.80
Gen8     45.80  63.82
Gen7     63.82  82.06
Gen6     82.06  99.51
Gen5     99.51  120.15
Gen4    120.15  139.11
Gen3    139.11  158.28
Gen2    158.28  178.87
Parent  178.87  256.00

```

#### Setting up WinList

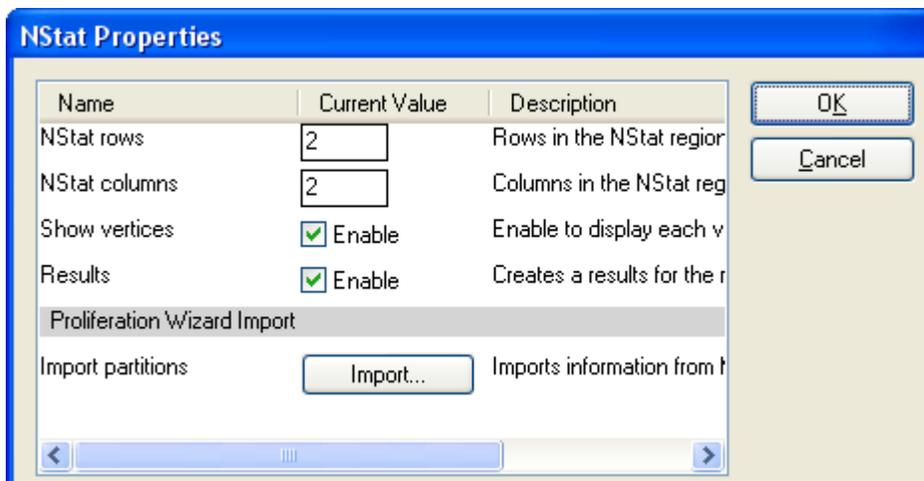
9. Activate WinList and click the **Open FCS File** button on the main toolbar.
10. Navigate to the Samples folder in the WinList application folder. Select the "CFSE sample.fcs" file and click **Open**.
11. In the **Create Histograms** dialog, select **CFSE** in the X Axis list and **CD69 PE** in the 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. Click the **Compensation Toolbox** button on the main toolbar. In the Compensation Toolbox, select the **HyperLog** tab. Click the **HyperLog > Log** button.
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 folder in which the Proliferation export file was saved. Select ProlifWizardPartitions.txt and click **Open**.

16. Notice now that the number of columns for the NStat Properties dialog is changed to 10. Edit the NStat rows to 1 and click **OK**.

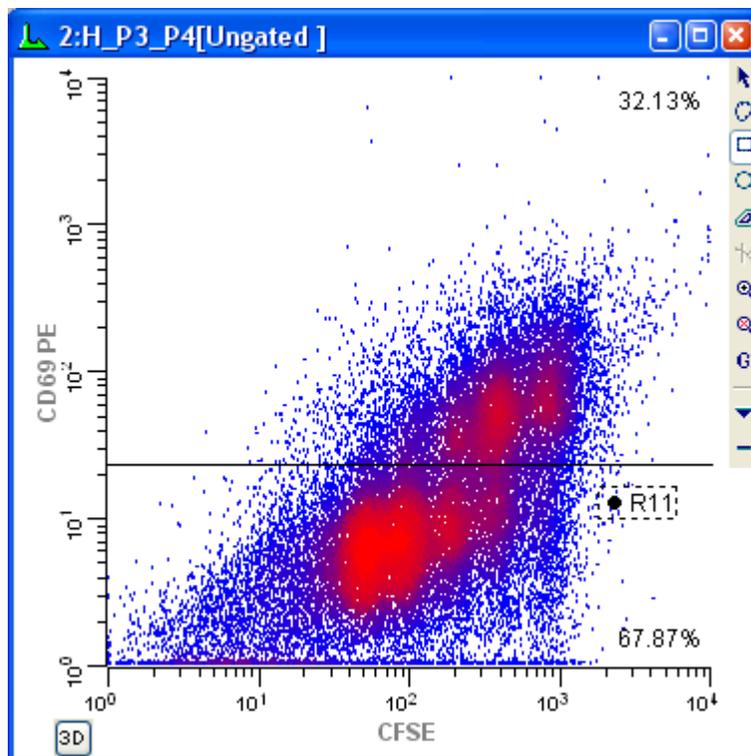
### Isolating the generations

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, NStat columns to 1, 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. Adjust the second NStat so that it approximately divides the two major populations in the second histogram, as shown.



To better analyze and visualize the generations, 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, so we start by enabling gates for the NStat regions we just created.

19. Display the **Edit Gates** dialog by clicking the Gate button on the toolbar.

20. Double-click the “G1” entry in the list to turn the gate on. In the same manner, double-click gates G2 through G11. Click **OK** to close the Edit Gate dialog.

Let's create a few FCOM calculated parameters to help isolate the generations.

21. Choose **Edit Data Source** from the Edit menu. Click **Add Parameters**.

22. Type or copy this expression into the Equation edit box, and then click **Add**.

$$\text{FLOG}(\text{FCOM}(\text{G1}, \text{G2}, \text{G3}, \text{G4}, \text{G5}, \text{G6}, \text{G7}, \text{G8}, \text{G9}, \text{G10}) + 0.5) * 1024 / \text{FLOG}(1024) + \text{FRND}(10)$$

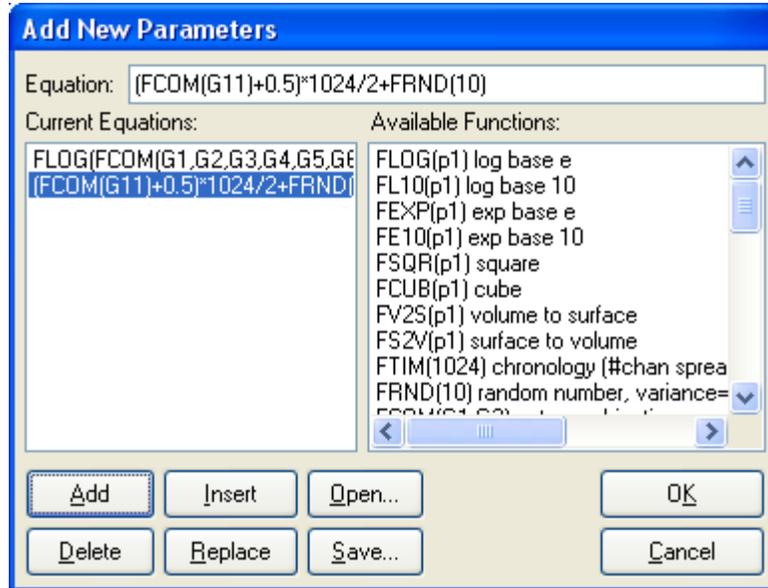
This expression deserves some explanation. Let's break it apart to understand it, starting with the FCOM portion. FCOM is a “gate combination” function. It takes any number of gates as arguments; the result of the equation depends on how many gates are in the expression. With a single gate, there are 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 1024 channels. The 10 population values after this rescaling are approximately 60, 135, 222, 309, 413, 516, 619, 723, 826 and 921. The final portion of the expression, “+ FRND(10)”, changes the spikes of events into narrow, 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.

23. Type this expression, and click Add.  
**(FCOM(G11)+0.5)\*1024/2+FRND(10)**



24. Click **OK**.

Let's assign more explanatory names to the two new parameters we just created.

25. Click **Names and Resolutions**. Click **P8** in the listbox. In the **Name** edit box, type *Proliferation*.

26. Click **P9** in the listbox, and in the **Name** edit box, type *CD69 Expression*. Click **OK**.

27. Click **OK** again to close the Edit Data Source dialog.

### 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.

28. From the Create menu, choose **Create Histogram**. In the X Axis dropdown list, choose *Proliferation*. In the Y Axis drop down, choose *CD69 PE*.

29. Click the Graphics button. For this display, dots will work better than contours. Choose the **Color Density** branch in the tree control, enable **Show all dots** and **Color density**. Disable **Contours**. Click **OK**. Click **OK** again to close the Edit Histogram dialog.

A new histogram is displayed. Let's create another histogram to isolate the CD69 positives and negatives.

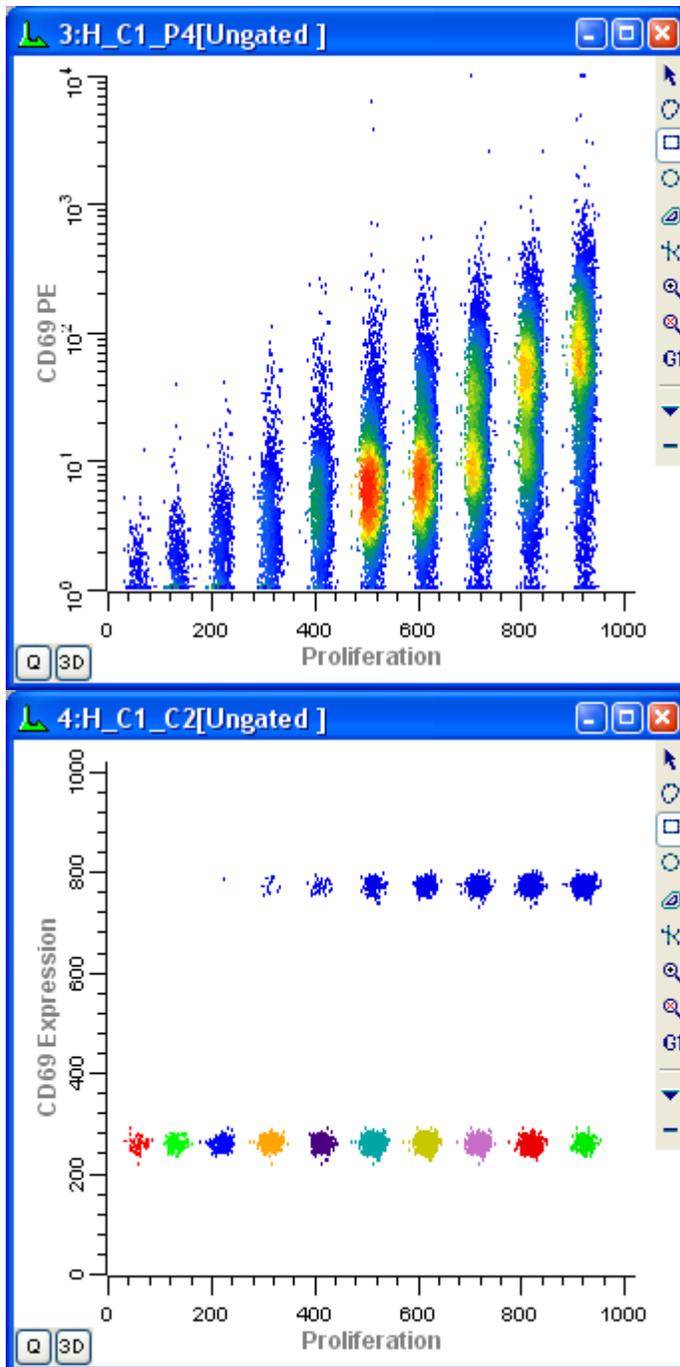
You can also display the Edit Graphics dialog by right-clicking on the histogram and choosing Edit Graphics from the context menu that appears.

30. From the Create menu, choose **Create Histogram**. In the X Axis dropdown list, choose *Proliferation*. In the Y Axis drop down, choose *CD69 Expression*. Click **OK** to close the Edit Histogram dialog.

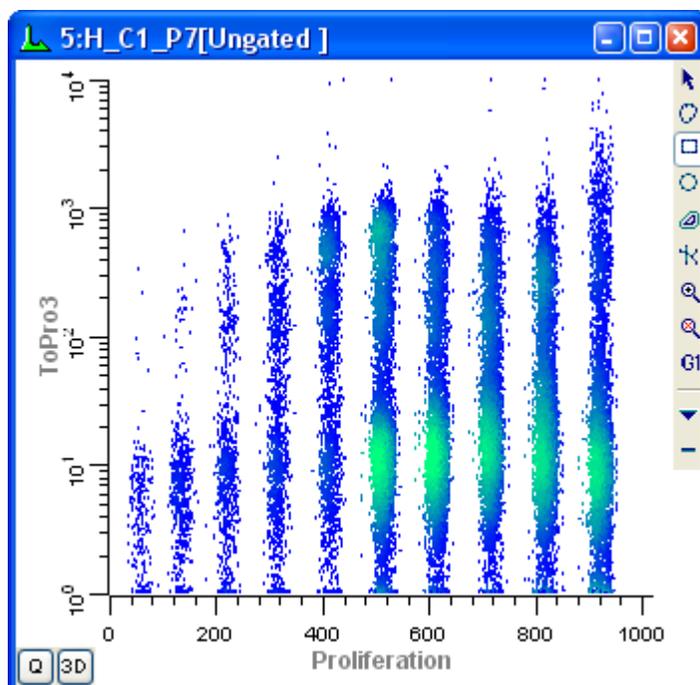
Another new histogram is displayed.

31. On the Data Source toolbar, click the **Tile** button to arrange the histograms.

In the graphics we can see the generations separated into discrete populations along the X-axis. The Y-axis shows the continuous CD69 parameter in the lower-left histogram and discrete subsets of CD69 positives and negatives in the lower-right 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 a real-time DNA analysis. You will see how simply 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.*

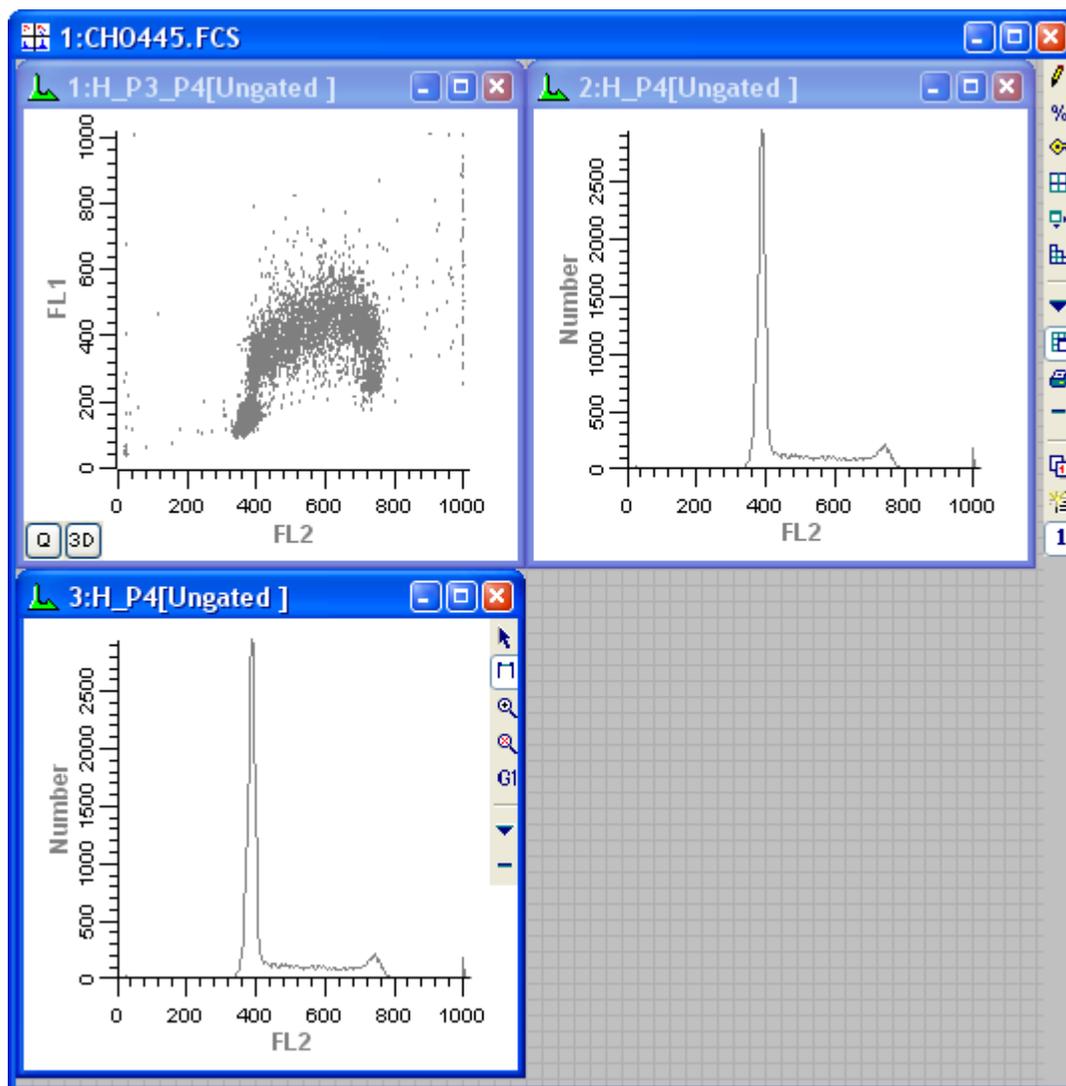
### Set up

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 File** button on the main toolbar and navigate to the Samples folder in the folder where WinList is installed. Select **cho445.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. 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** *twice* to add two copies of this histogram.
4. Click **OK** to open the data source and create the histograms. You should have three histograms displayed.



#### **Making the remote connection**

Next, let's link the first copy of the 1P FL2 histogram to ModFit. Here's how.

5. Double-click the title bar of the first H\_P4 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 you receive an error message at this point, WinList cannot find ModFit LT. 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 H\_P4. A window will appear, displaying the contents of the analysis results.

### Modifying the update frequency

The analysis we just performed did not allow ModFit LT to see *all* of the events for the FL2 histogram. WinList only sent it 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. From the **Edit** menu, choose **Edit Histogram**. 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. From the **Replay** menu, choose **Active Histogram**.

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 up to 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.

**See also:**

Edit Remote

### 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 – 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. Open the **File** menu and select **Open FCS File**. 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 regions.

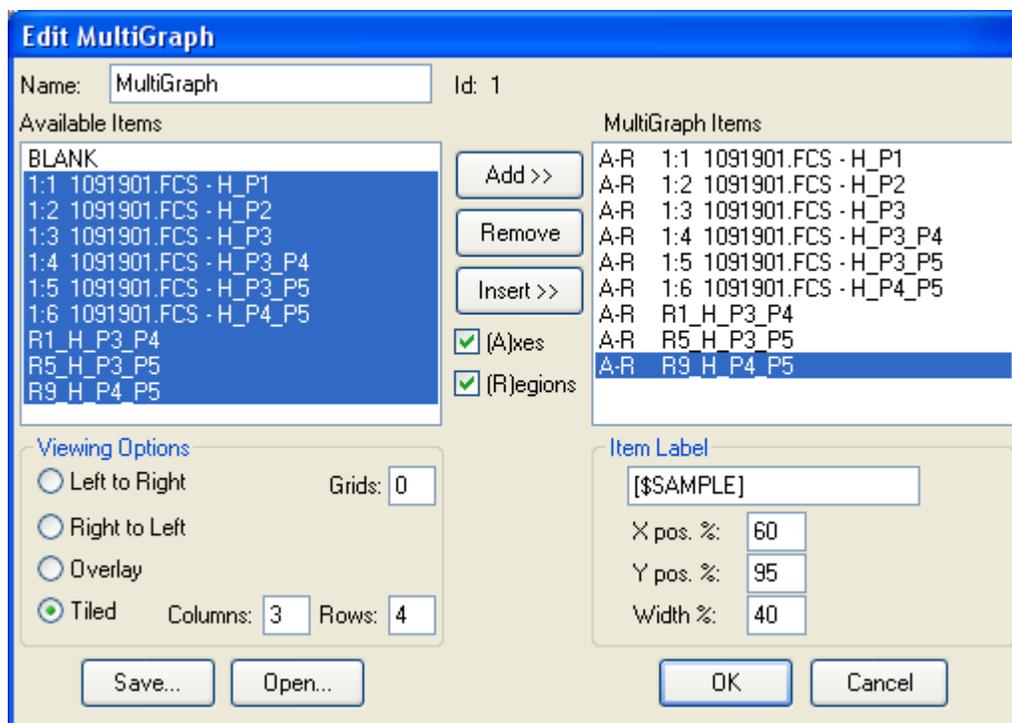
### Creating the MultiGraph

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. From the **Create** menu, select **MultiGraph**. 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.



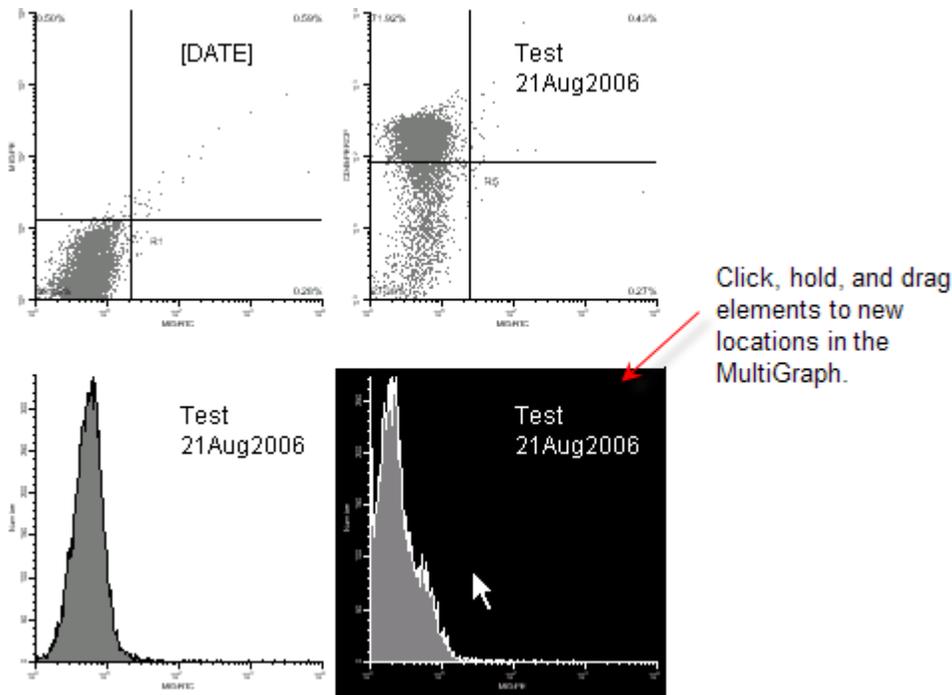
7. Click **OK** to close the dialog box and create the MultiGraph.

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.



### 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 **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.

### Databasing with WinList

This section introduces some of 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.

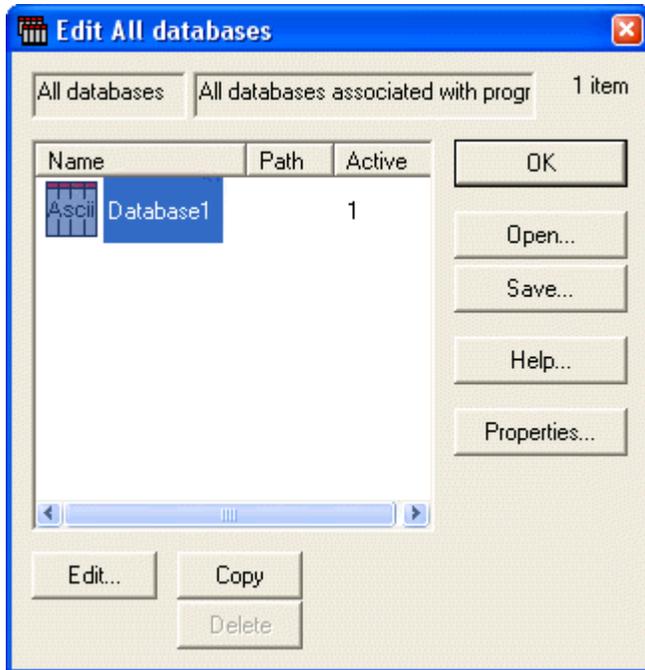
WinList has a simple, yet powerful database engine that allows you to store large amounts of data easily in one or more database files. You can view databases from within the program, manually add, delete, and replace records, and select the fields you want to store in each database. The databases are 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?

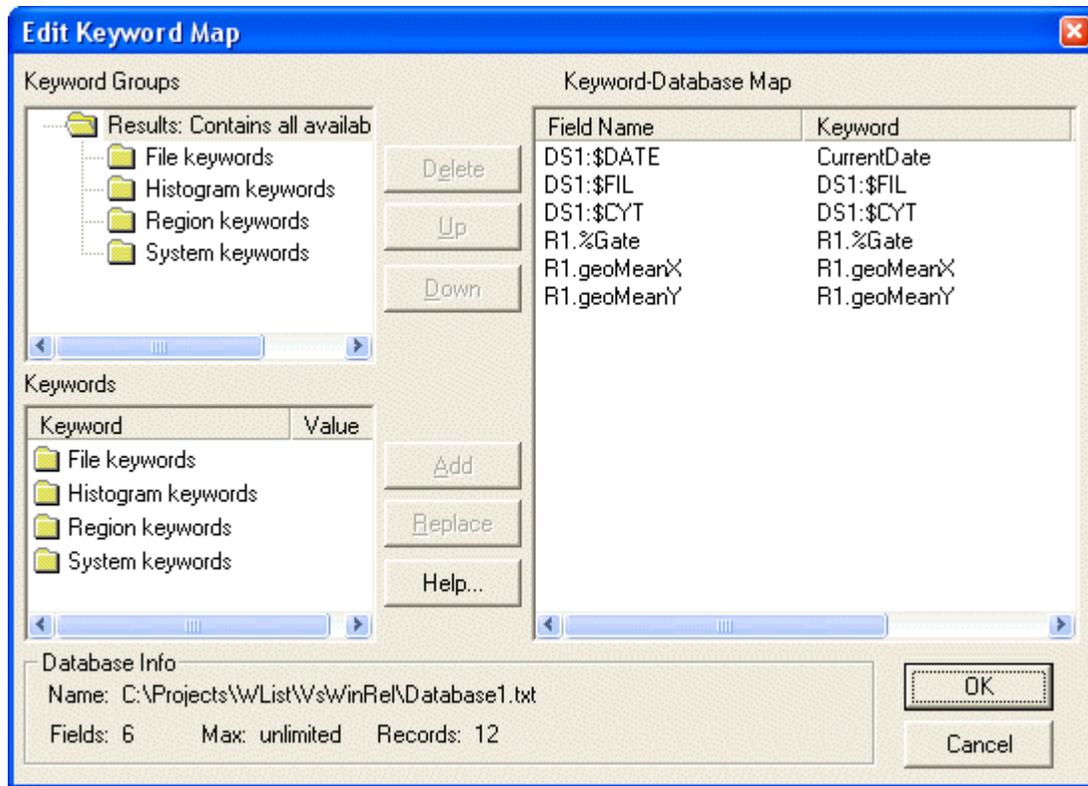
Set up WinList with a protocol before you create or edit a database. This includes opening one or more datasource files, selecting the histograms you want to work with, creating regions and gates, and displaying results. You can set

up a separate database for each protocol you use, one database for everything you do with the program, or anything in between.

Databases are accessible from the Edit menu. Select Edit Databases from the Edit menu, you will see 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 in the database. 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.

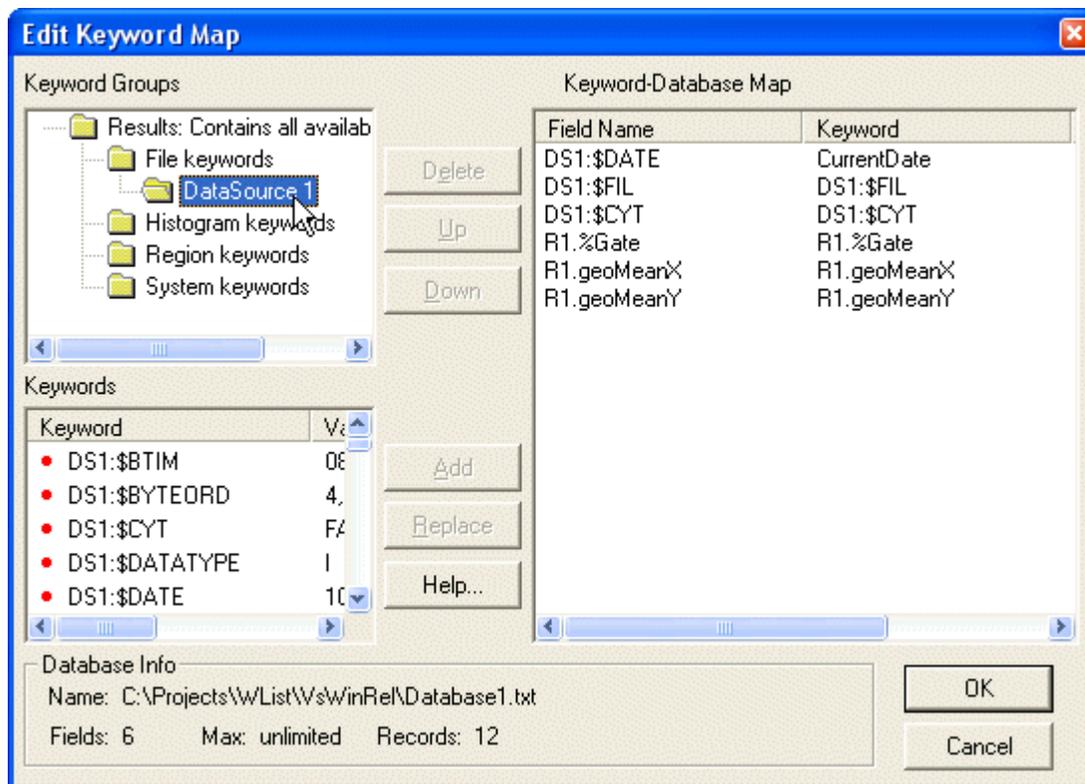


The Edit Keyword Map dialog is displayed. The left side of this dialog presents the information available to store, and the database fields are shown on the right. The two controls on the left are the Keyword Groups tree and the Keywords list. The right-hand list is the Keyword-Database Map, showing 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.

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 datasource; 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 datasource 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.

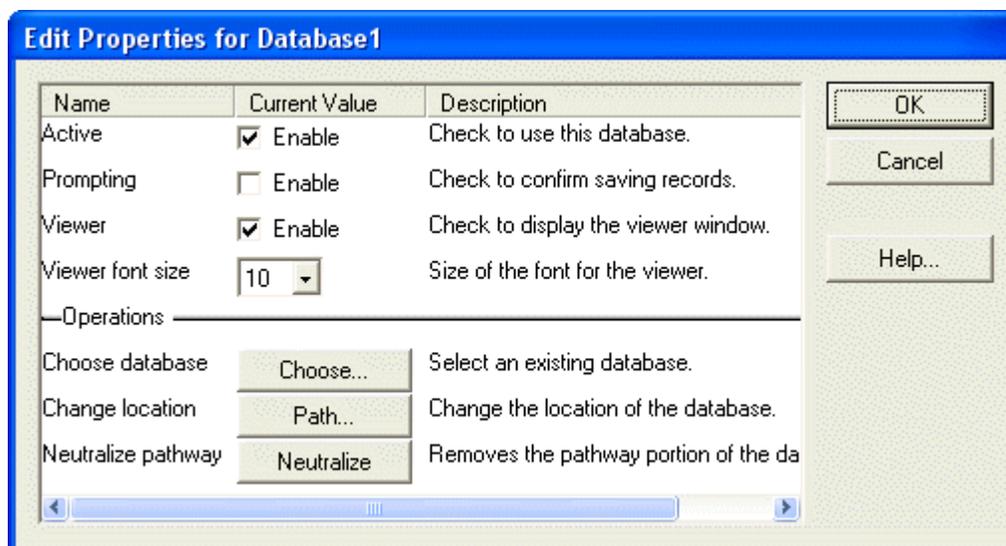
*If the database already exists on disk, WinList will automatically create a backup of the database before overwriting the existing one. The backup file will have the same name as the database, with " backup" appended to it.*

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 choose one of the Replay options in the Replay menu, or when you click the Replay button on the main toolbar. 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. 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

When you click OK in the Edit All Databases dialog, WinList will ask you if you would like to open the database setup when the program starts. If you choose "Yes", your database configuration will be restored and ready to go each time WinList starts.

#### Links:

Edit Databases

WinList User Guide

Edit Keyword Map

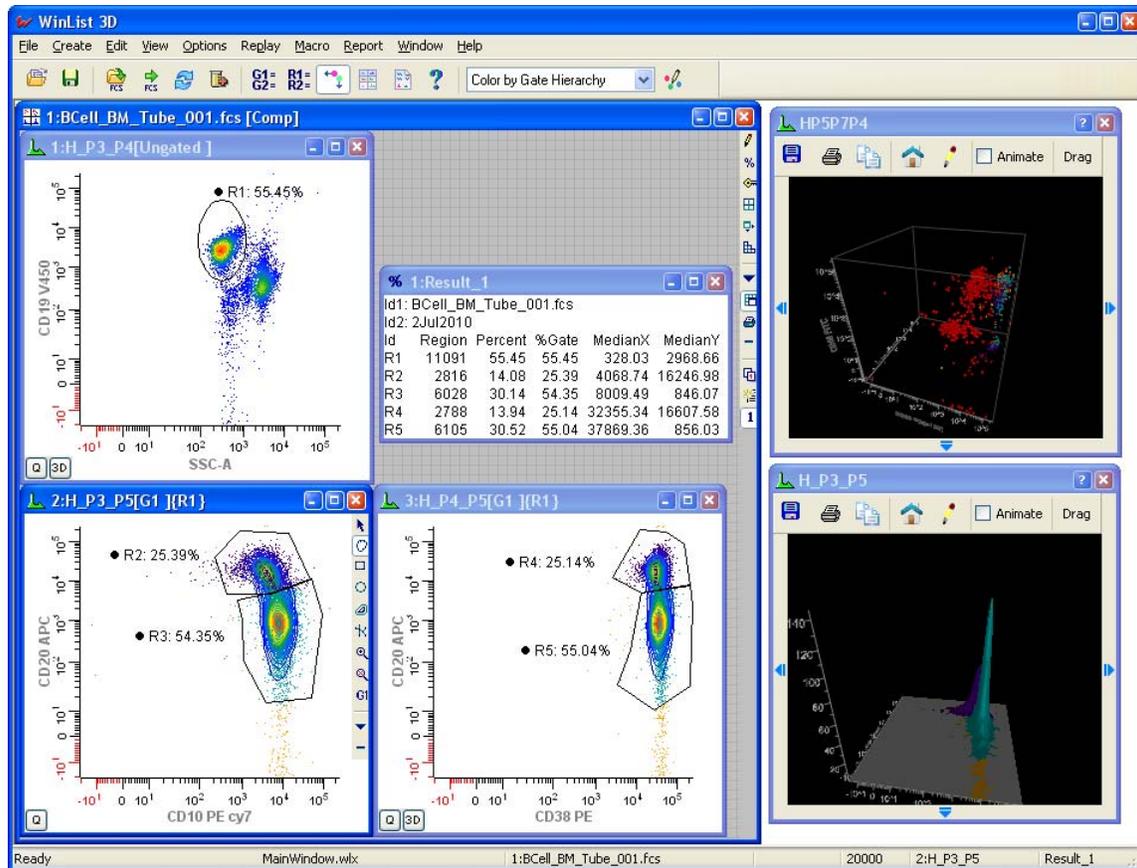
Edit Properties for All Databases

Edit Properties for Databasen

## Reference

### Window types and toolbars

#### Main Window

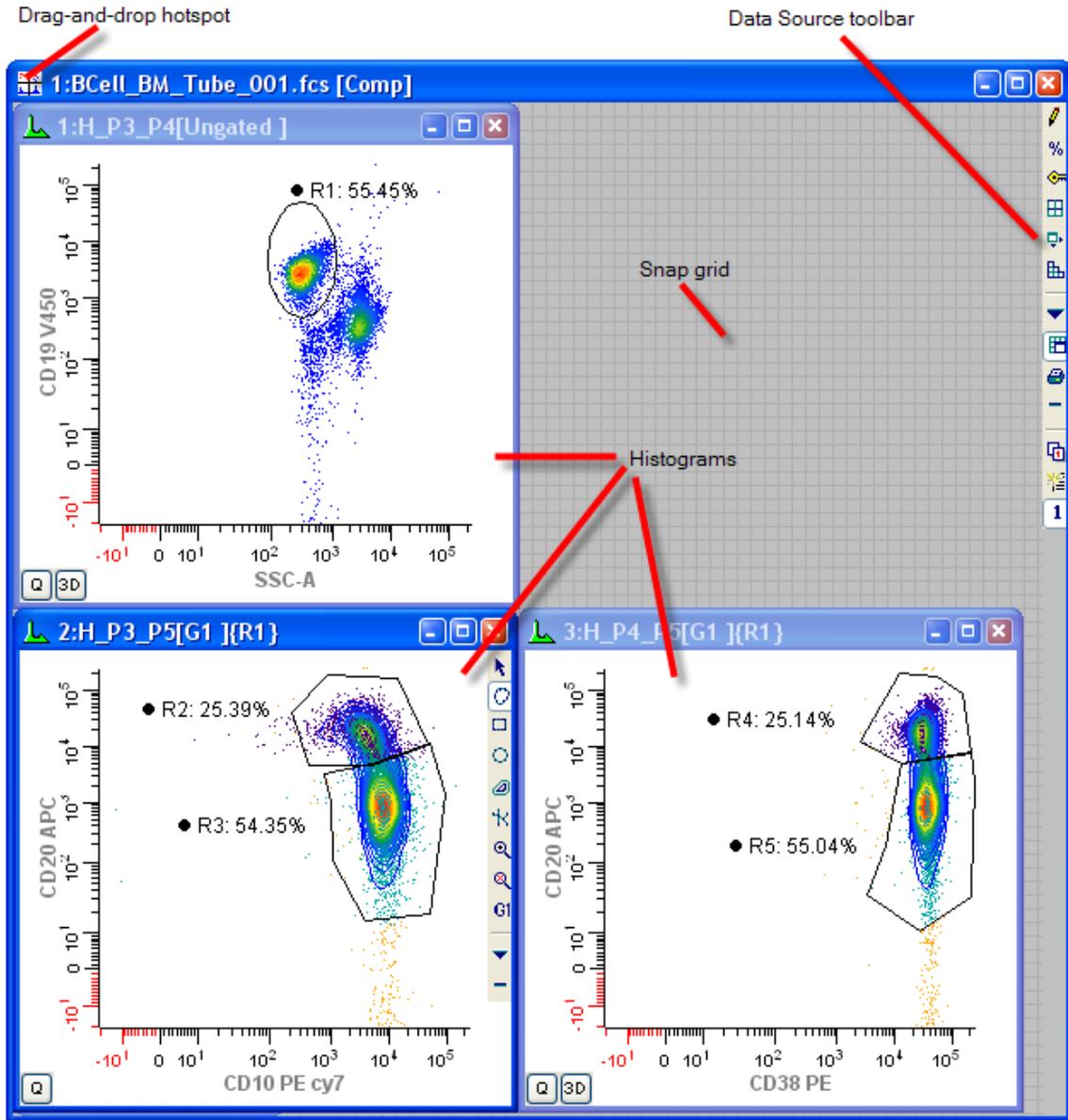


This is the main window of the WinList program. It has a **Title Bar**, **Toolbar** and **Menu**. It can be re-sized and moved to occupy whatever part of the display you choose. The **Status Bar** at the bottom of the window displays information about the selected menu options, and shows which objects are *active*.

See the **Toolbar** section for details on the commands in the main toolbar.

#### 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.



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.

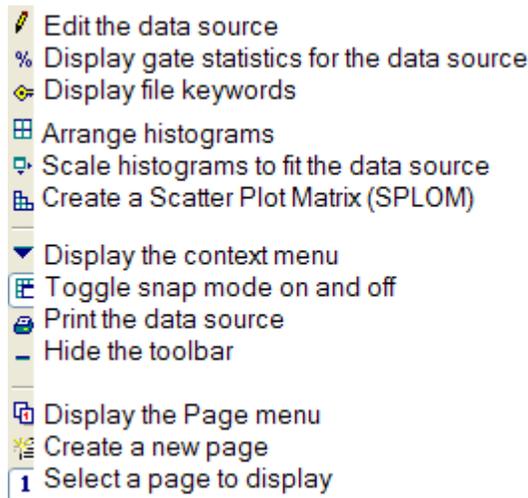
A data source window can be dragged and dropped onto a Report window to add all of its histograms to the report. The drag-and-drop hotspot is the upper-left portion of the titlebar.

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.



Many of these commands are explained elsewhere, but a few are only available through the toolbar. These are explained in more detail below.

### SPLOM

A Scatter Plot Matrix, or SPLOM, 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 Splom 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.

It is best to create a new page in the data source prior to pressing the Splom button, since the Splom command will remove any histograms on the current page (after prompting you).

### 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 titlebar of the data source and using the PgUp and PgDn keys on the keyboard.

### Context menu

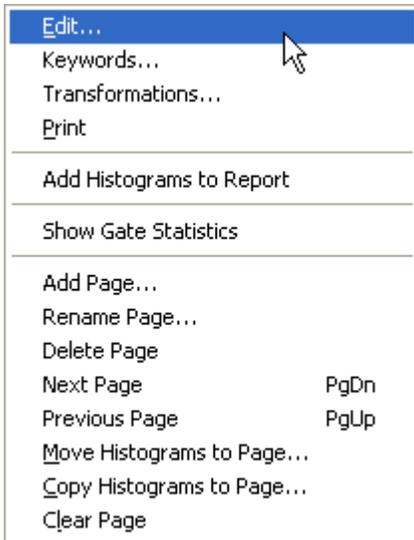
The context menu for the data source window contains many common commands. It can be displayed by clicking the Context menu button on the data source toolbar, or by right-clicking inside the data source window.

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



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.

**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...**

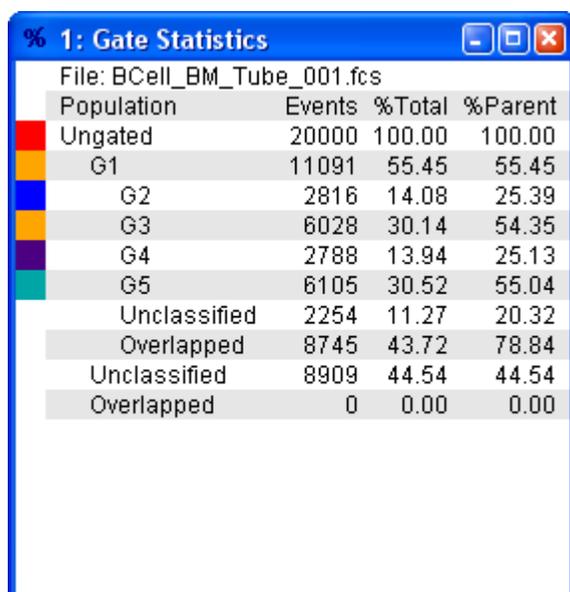
This command copies the histograms on the current page to another page in the data source.

**Clear Page**

This command deletes the histograms on the current page.

**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.



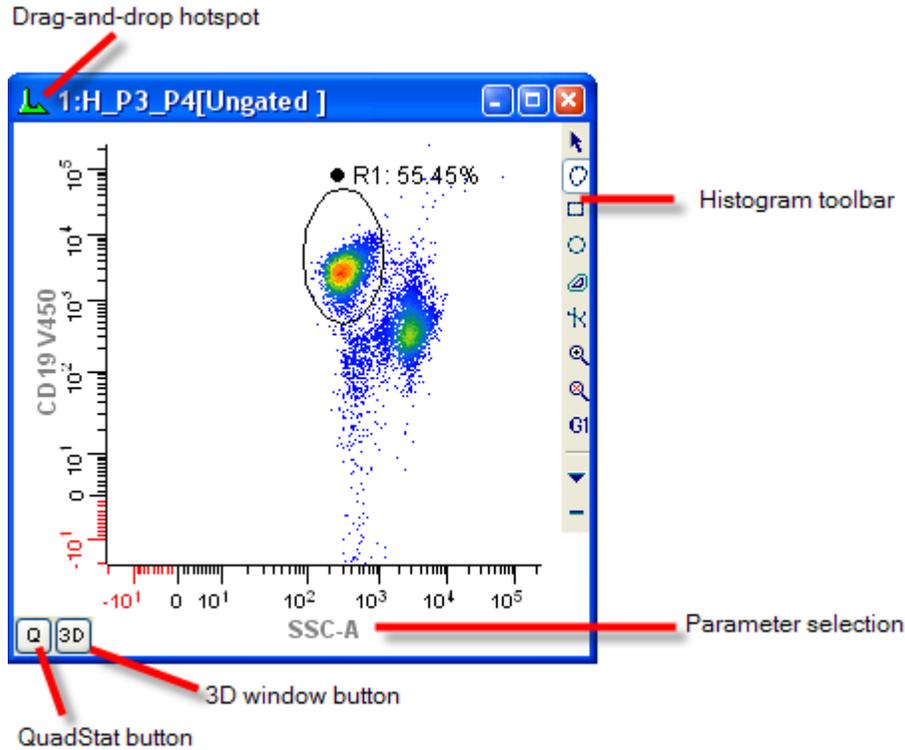
The screenshot shows a window titled "% 1: Gate Statistics" with a blue border and standard window controls. The window displays a table of statistics for a file named "BCell\_BM\_Tube\_001.fcs". The table has four columns: Population, Events, %Total, and %Parent. The rows represent different gates and their sub-categories, each with a small colored square to its left. The gates are Ungated, G1, G2, G3, G4, G5, Unclassified, Overlapped, Unclassified, and Overlapped.

Population	Events	%Total	%Parent
Ungated	20000	100.00	100.00
G1	11091	55.45	55.45
G2	2816	14.08	25.39
G3	6028	30.14	54.35
G4	2788	13.94	25.13
G5	6105	30.52	55.04
Unclassified	2254	11.27	20.32
Overlapped	8745	43.72	78.84
Unclassified	8909	44.54	44.54
Overlapped	0	0.00	0.00

The window can be displayed by right-clicking the data source and selecting Gate Statistics from the Data Source Context menu .

**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.



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.

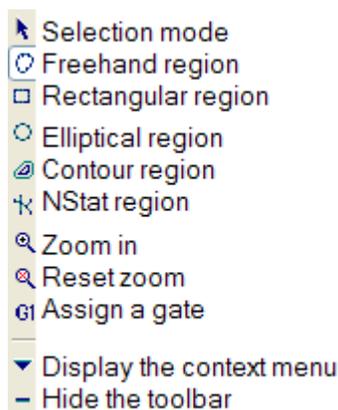
The button is also displayed for subtracted histograms. If you press the **I** button, a pop-up information window will appear that will allow you to scroll through the results.

### 3D Button

On the 3D version 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.



### 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 histogram, 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

Click and hold on the edge of a population in a 2P histogram. A preview of the contour region is displayed. Move the mouse to adjust the contour, and release the mouse button to finish the region.

### 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.

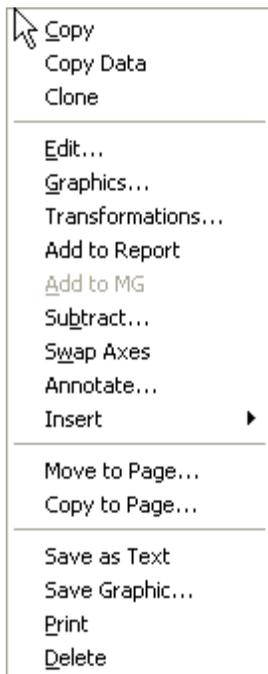
### Context Menu

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



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.

**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.

**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.

**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.

**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.

**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 spreadsheets.

**Save as Graphic**

This option saves the histogram graphic in either bitmap or metafile format.

**Print**

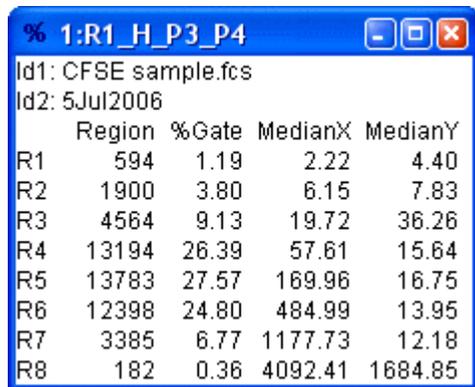
Print the histogram.

**Delete**

Delete the histogram window.

### Results Windows

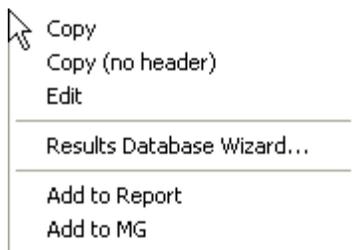
Results windows display statistics for regions and are displayed on top of histogram and data source windows. They can be positioned in any convenient location, including outside of the confines of the main window.



	Region	%Gate	MedianX	MedianY
R1	594	1.19	2.22	4.40
R2	1900	3.80	6.15	7.83
R3	4564	9.13	19.72	36.26
R4	13194	26.39	57.61	15.64
R5	13783	27.57	169.96	16.75
R6	12398	24.80	484.99	13.95
R7	3385	6.77	1177.73	12.18
R8	182	0.36	4092.41	1684.85

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 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.

#### Results Database Wizard

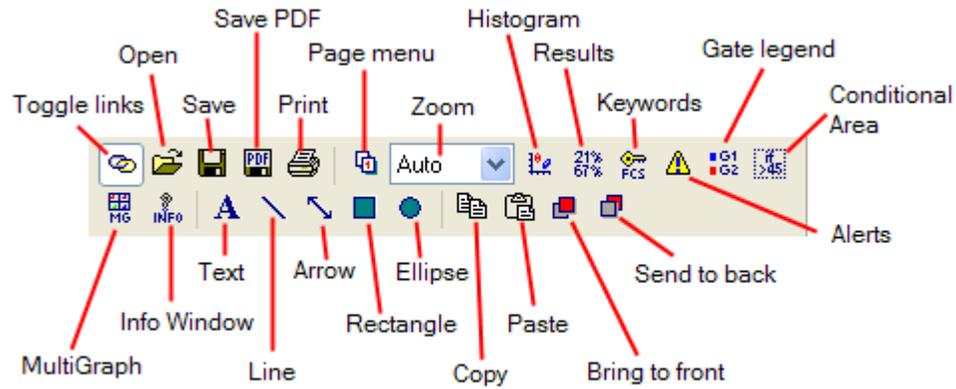
This command initiates a wizard that simplifies the task of adding results to a database. See Results Database Wizard for details.

#### Add to Report

This command adds a linked Results object to the active report. The report object updates anytime that the content of the results window changes.



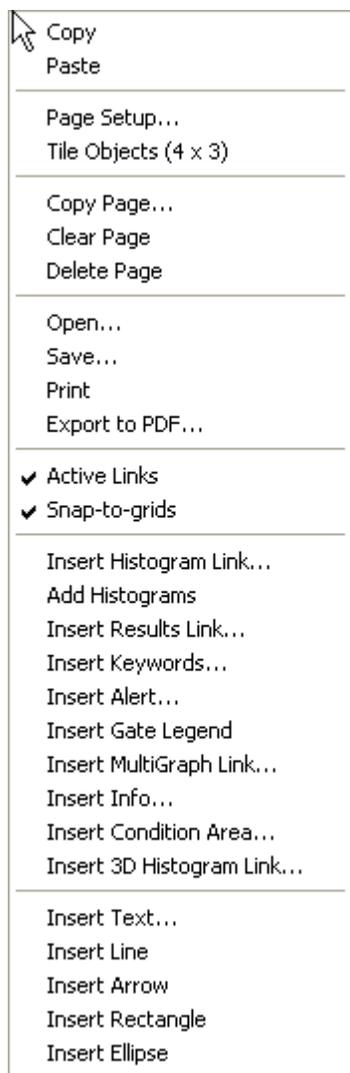
This toolbar is attached to the inside-top of each report window. Its icons initiate commands commonly used on reports.



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.



See individual report menu commands for details.

### MultiGraph Windows

These windows allow you to display multiple histograms and results in a single window. 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.

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.

WinList displays a popup menu 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, and print the MultiGraph.

To activate a MultiGraph window, click the mouse on its title bar. The title bar will change color to show it is active, and the MultiGraph will come to the top of other MultiGraph windows.

### 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.

### Drag and Drop

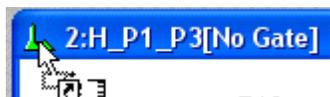
WinList supports a number of Drag and Drop operations that simplify common functions.

#### 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 titlebar, 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 titlebar of a window. When you position the mouse over this spot, the cursor will change to indicate the Drag and Drop functionality.



#### Drag and Drop Files onto WinList

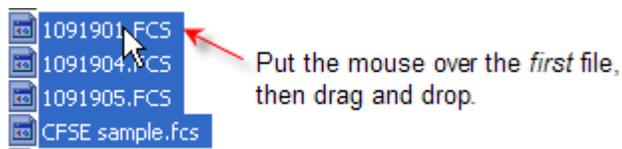
When you drag files from the operating system onto WinList's main window, WinList attempts to determine what kind of file you have dropped and open it appropriately.

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 (.mac or .wlb) are opened and run. Report files (.wlr) are opened in a new report window.

You can drop a compensation file (.cmp) onto a datasource 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 datasource 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.



## File menu

### Open FCS File

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.

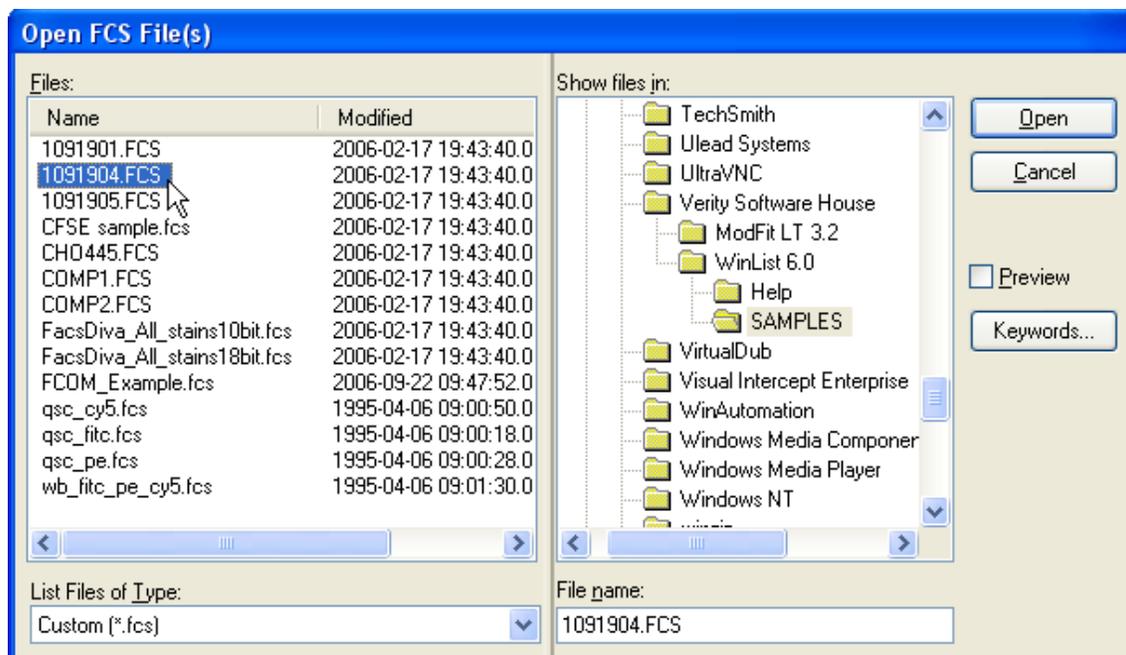
After selecting a file to open, the Create Multiple Histograms dialog box is displayed to provide a quick and efficient means for selecting any number and types of histograms for display. This dialog can simply be canceled if you prefer to create histograms one at a time.

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. A listbox on the left 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. The tree on the right allows you to select the folder where files of interest are located. Buttons on the right 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.



**Files**

Displays a list of files in the selected folder. Files must match the file filter displayed in the List Files of Type drop down list box. Select one or more files from this list to open (see note above). To display all files, choose "All files" from the List Files of Type drop down list box.

This list can be sorted based on file name or modification date. To sort by file name, click the Name column heading in the list. To sort on file modification date, click the Modified column heading.

**List Files of Type**

Displays available file filters to apply to the list of files. A custom filter can be set using the Wildcards and Pathways dialog.

**Show files in**

This tree displays all drives and network devices attached to your computer. Navigate down the tree by double-clicking on folders. As folders are opened, sub folders are displayed. Select the folder where your listmode files are stored.

**File name**

This edit box will display the selected file name. You can also type a file name and path directly into the edit box to select a specific file.

**Preview**

When this checkbox is enabled, the Files listbox will display some of the FCS keywords found in the files. You can control which keywords are displayed in the Data Source Preferences dialog.

**Keywords**

Click this button to display all keywords for the currently highlighted file. Keywords are displayed in a scrollable list box that can be left on the screen for convenience.

**Open**

Click this button to open the currently selected file in the **Files** list box. Optionally, you can double-click the file name in the list box to open a file. The dialog box closes when this button is clicked.

**Cancel**

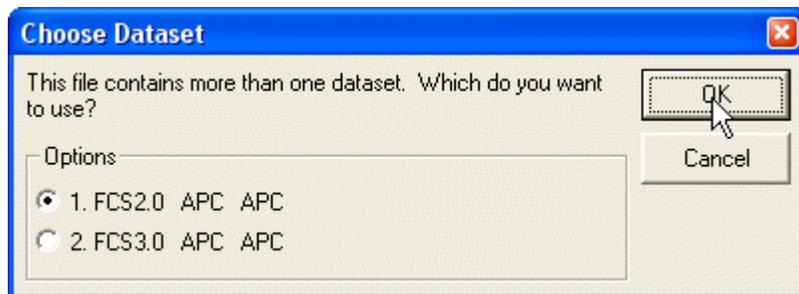
Click this button to cancel the file opening operation and close the dialog box.

**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 menu option is used to replace the data file for the current data source with another data file on disk.

Normally, the Open dialog box is displayed which allows the user to select a new file. The next file in the list box is automatically selected. To open the new file, select it in the list box and click **Open**.

Optionally, if you hold down the Shift key on the keyboard while choosing the Next FCS File option, WinList will attempt to open the Next FCS File *without* displaying the Open dialog.

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**

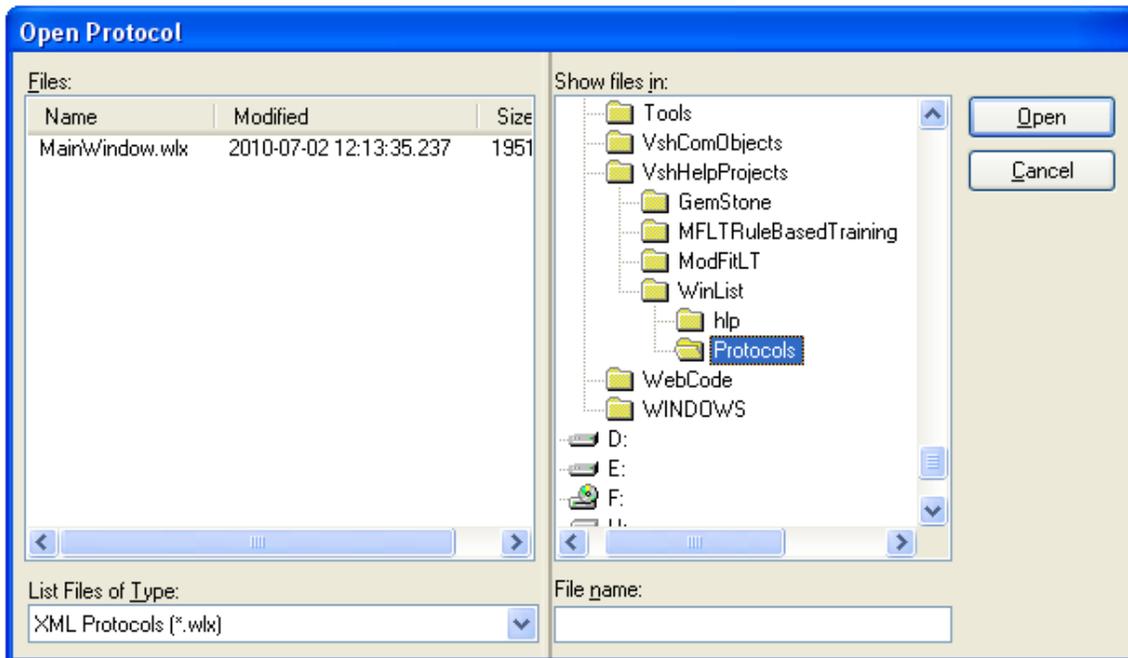
This menu option is used to replace the data file for the current data source with another data file on disk. It operates the same way as the Next FCS File, except that it selects the previous file from the list.

### **Open Protocol**

This command 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 Files of Type filter to show Protocol Bundles.

When **Open Protocol** is selected from the menu, a file open dialog box appears allowing you to select a protocol (w/x or mac file) or a protocol bundle (w/lb 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 be opened and run to set up the screen, gates, regions and palette.



### Drag and Drop

You can drag and drop a protocol file from the file system onto WinList to open a protocol.

### Save Protocol

This command 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 silently updates the file on disk with the new setup. If the protocol has not yet been saved, it displays the Save Protocol As... dialog.

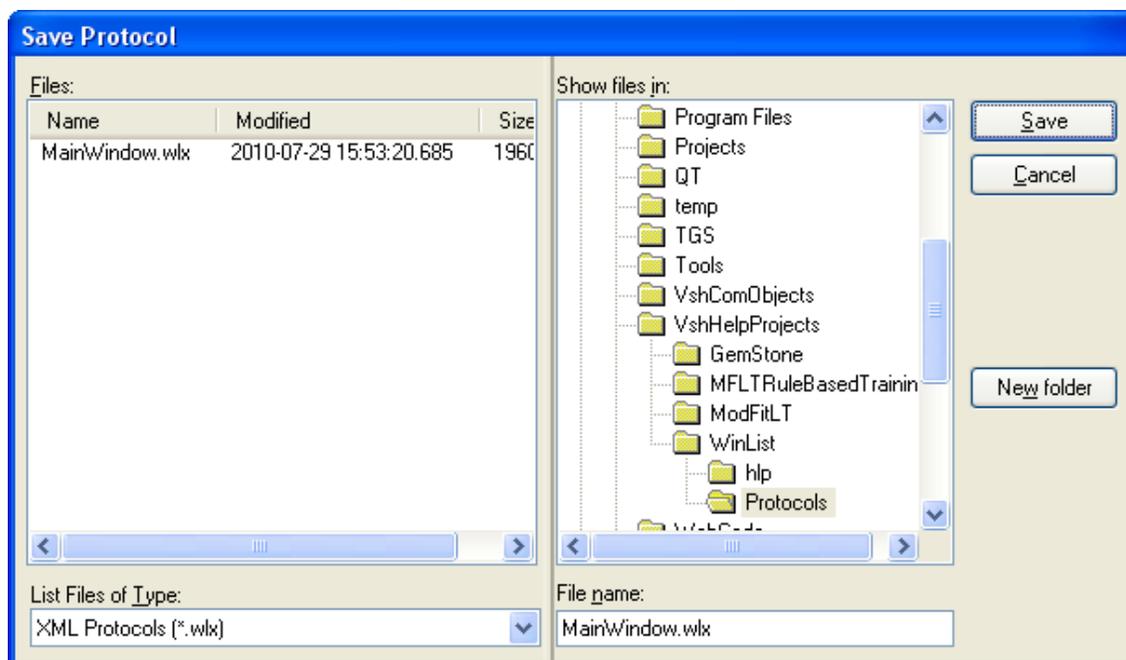
### Save Protocol As...

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 Protocol Bundle for additional details.

When you select this option from the menu, the Save dialog box will appear. 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

### Open Protocol Bundle

This command displays the Open Protocol dialog with the file type selection set for protocol bundle files. See Save Protocol Bundle for a description of the protocol bundle file.

### Save Protocol Bundle

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 Protocol Bundle are that Save Protocol 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

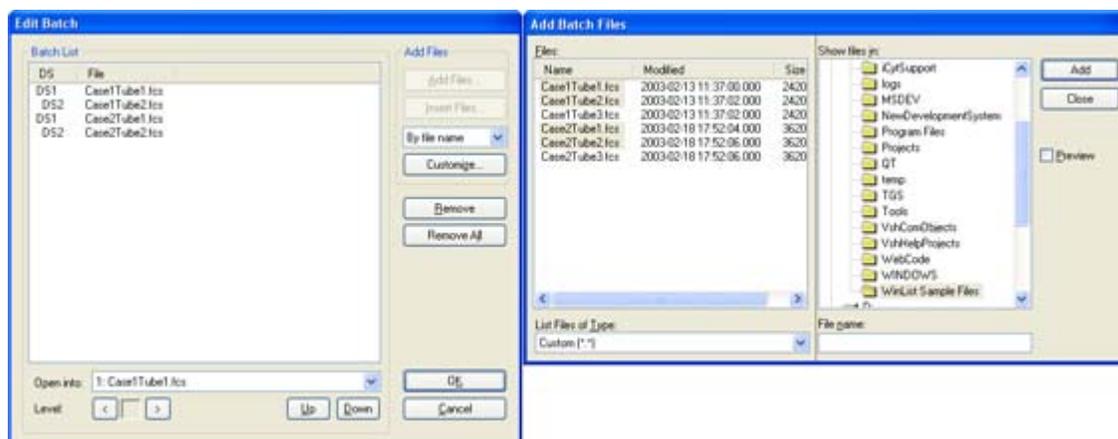
## Open Batch Files

**Note:** This function is disabled until there is an FCS file open in WinList.

A “batch” of files is a group of listmode files that will be processed through a WinList 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, the data source target ID's enable WinList to automatically associate a file with a particular data source. This system allows you to group the files to analyze 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 Open 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.

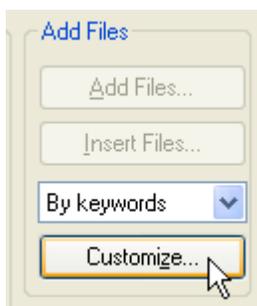
### Simple Batch

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.

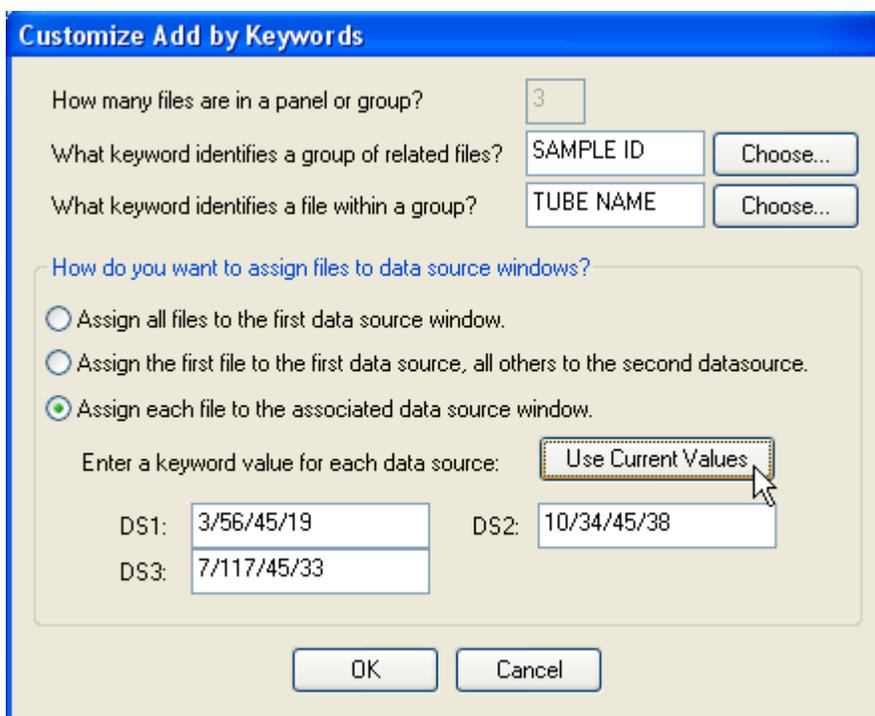
### More Sophisticated Batch

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.



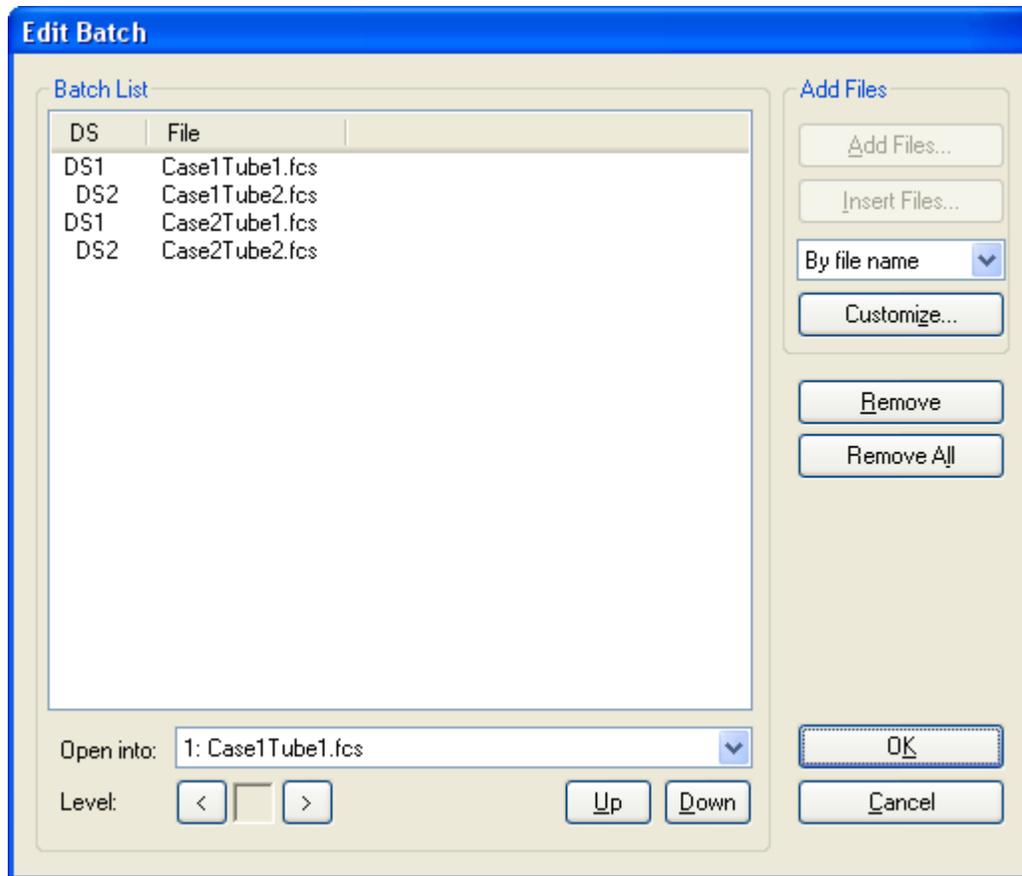
In the **Customize Add by Keywords** dialog, you would select "SAMPLE ID" for the keyword that identifies a group of files (a panel), and "TUBE NAME" as the keywords that identifies a file within the group. You would select "Assign each file to the associated data source window", and then choose or enter 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

The following is a listing of all the editable fields in this dialog box and their corresponding functions.



#### 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 dropdown 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.

**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 File(s)** 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 .

**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.



{bmct batch\_edit.bmp}

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.

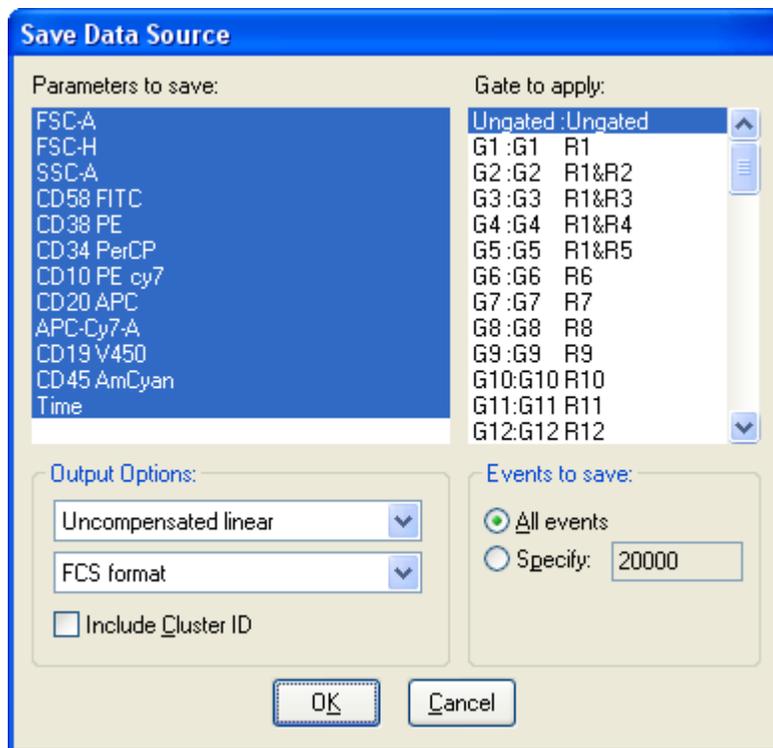
Stop the auto-advance process.

Pause/continue batch processing.

### Save Data Source

Use this command to create a new listmode file based on the file currently open. You can save original as well as calculated parameters, you can apply gates, and save compensated or uncompensated data. You can save a file in FCS or text file format.

When the **Save Data Source** command is selected, a dialog box entitled **Save Selected Listmode** appears on the desktop.



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 **Gate to apply** list box contains all gates defined for the data source. You can save ungated data or apply one of the gates in the list to the data as it is being saved.

The **Output Options** group contains several options for formatting the output. 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.

Enable the **Include Cluster ID** option to store color-event cluster identification as a parameter. The cluster ID becomes another parameter in the output file.

In the **Events to save** group, select **All** to store all of the events that pass the gate. If you only want a portion of the listmode file saved, select **Specify** and enter the number of events you want to save.

After the desired parameters are chosen and the active gate is selected, 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.

### Save Histogram

This command allows you to save the active histogram to disk in Flow Cytometry Standard (FCS) format. Choosing the option initiates a 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.

### Save Results

This command allows you to save the active results window to disk in tab-separated text format. Choosing the option initiates a 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.

### Save Histogram Graphic

This menu option allows you to save the active histogram graphic as a picture on disk. The graphic is saved as a Windows metafile picture (wmf) or as a bitmap (bmp), based on a setting in the Preferences dialog (Preferences->Histograms->Graphic format).

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.

### Save MultiGraph Graphics

This option appears on the PC version only. This menu options allows you to save the active MultiGraph graphic as a picture on disk. The graphic is saved as a Windows metafile picture (wmf).

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.

### **Print Report**

Select this option to print 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 Screen (Ctrl Shift P)**

Select this option to perform a high-speed bitmap screen dump to a graphics printer. The printout will display the visible contents of WinList's window. The resolution of the target printer may determine the usefulness of this option. Low-resolution printers may produce grainy graphics.

Use the Printer Setup option in the File menu to set print options.

### **Print Data Source**

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 Histogram**

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.

### **Print MultiGraph (Shift P)**

This option renders the current MultiGraph to the printer. The print margins set in Print Preferences are used and a border is placed around the MultiGraph.

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 than *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.

Because MultiGraphs can contain an enormous amount of graphical information, printing MultiGraphs can take some time. There are several ways you can reduce print time, however. If you reduce the number of dots displayed in dot plots prior to adding them to MultiGraphs, they will take less time to print. Make sure you are using the latest printer driver for your printer, and experiment with the resolution setting of the printer driver. Generally, print speed increases with lower resolutions, but you may find the graphics too grainy if the resolution is set too low. Other software and hardware options may be available to help improve print speed, so you may want to consult with your computer supplier.

Use the Printer Setup option in the File menu to set print options.

**Print Results**

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.

**Print Desktop**

Select this option to render WinList's printable area of the screen to the printer. Each histogram, MultiGraph, results window, and remote information window is drawn on the printer with the same relative positioning it has on the screen.

Use the Printer Setup option in the File menu to set print options.

Several options in the **Preferences** dialog affect the printed output. They appear in the Print Preferences dialog box and control margins, orientation, and more.

The sequence in which objects on the screen are drawn to the printer is important to understand in order to have reports appear on the printed page as they do on the screen. This only becomes an issue if objects overlap.

First, histograms are printed in the following order: first created histogram of the first data source, second created histogram of the first data source, and so on until all histograms of all data sources have been rendered.

After histograms, any information windows associated with histograms are printed.

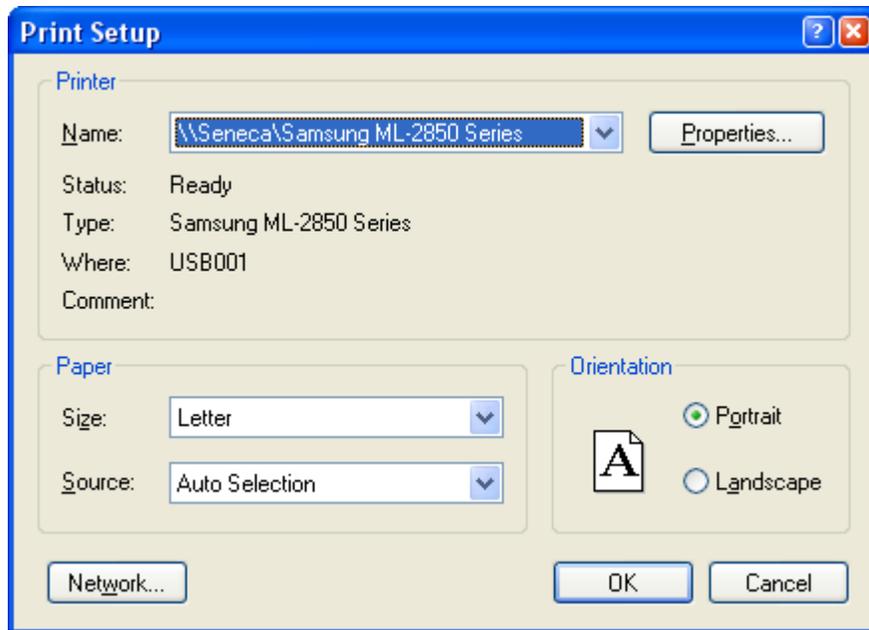
Next, MultiGraphs are printed, once again using the order in which they were created.

Finally, results windows are printed. These windows may change in size on the printer since the size of the text is kept consistent with the size of the text on the screen.

**Printer Setup**

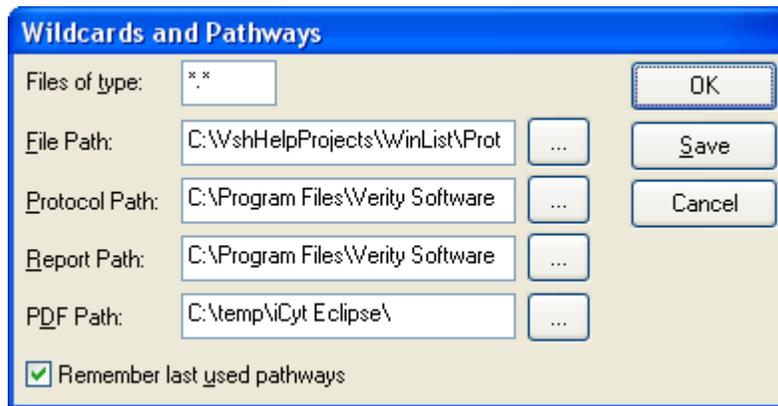
Select this option to display the printer setup dialog box. In the dialog box you may select a printer that you want WinList to use, as well as the orientation you want to use. The specific dialog box will vary somewhat depending on the operating system your computer is running.

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.



**Wildcards and Pathways**

This menu option initiates a dialog box that allows the user to set search criteria and pathways for various file types.



**Files of type**

Enter the 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**

Enter the pathway to WinList. 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 in which to store 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 in which to store PDF report 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.

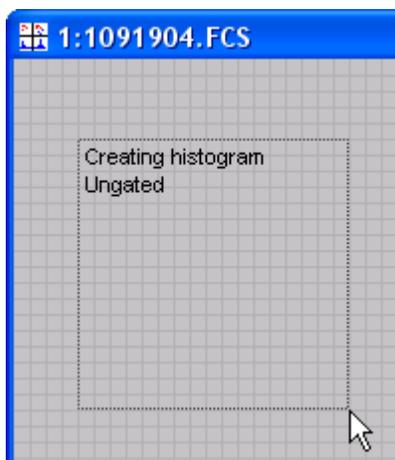
**Exit**

Select this option to end your WinList session.

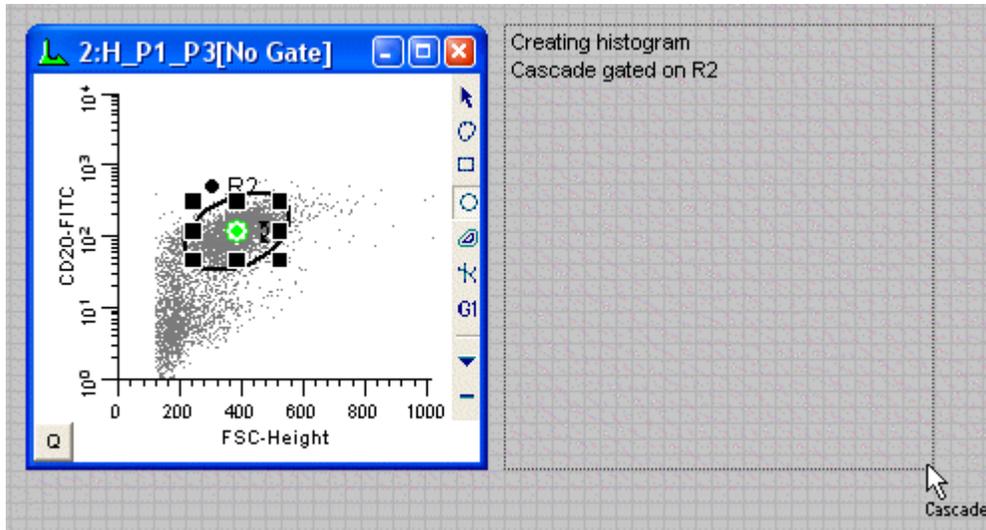
**Create menu****Create Histogram**

This command creates a histogram in the active data source. The Edit Histogram dialog is displayed.

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.

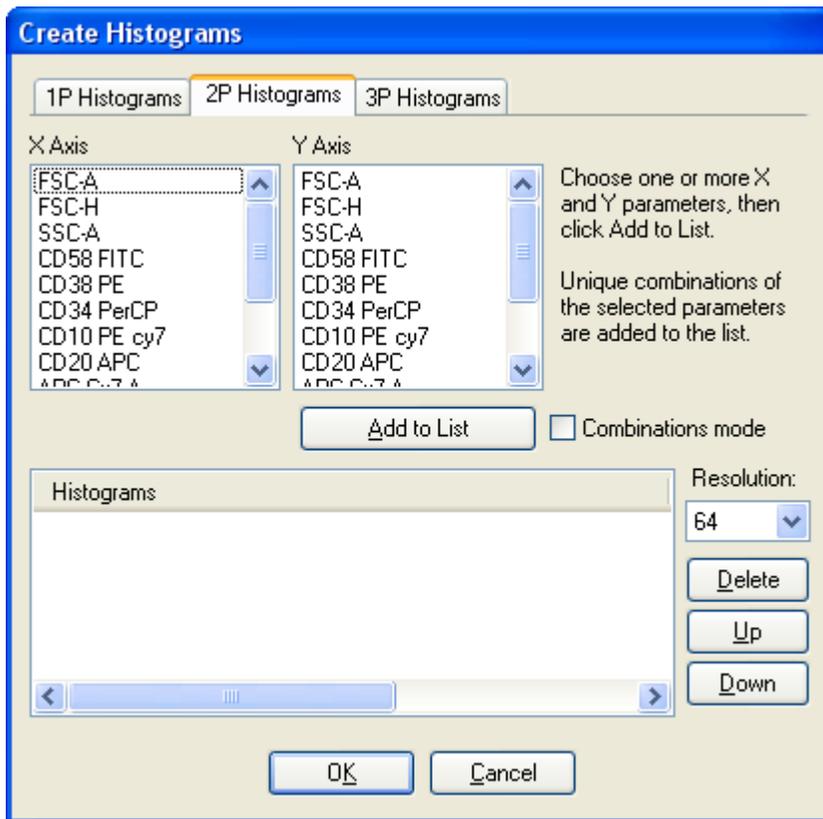


You can draw a gated histogram in a similar manner by first clicking on the Cascade Gate hotspot on an active region. The hotspot will change from red to green. As you draw the histogram, text will be displayed to show how the histogram will be gated.



### 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.

### Create Results

Results windows are used to display statistics for one or more regions. In 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.

### Create 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 and opened from disk. 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.

Some characteristics of MultiGraphs can be edited by selecting **Edit MultiGraph** from the Edit menu. These include the **Viewing Options**, as well as **Item Labels** and positions. You can also add or remove histograms at any time. Other characteristics do not change: these include the state of axes and regions for histograms already generated in the MultiGraph. To change these display options, the histograms must be deleted from the MultiGraph and recreated from the original data source. Item labels may contain keywords or text.

The **Create MultiGraph** menu option leads to the Edit MultiGraph dialog box. It allows the creation of a new MultiGraph, or opening of a MultiGraph from disk. Once the MultiGraph has been created or opened, it is displayed on the WinList window. Double-clicking on the title bar allows you to edit and add histograms to the MultiGraph, as does selecting **Edit MultiGraph** from the Edit menu.

To configure the default characteristics of MultiGraphs, use the Preferences command in the Options menu.

### 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 Region Array**

**Options**

Create a results window

Total regions to create: 1

**On X-Axis**

Number of regions:	<input type="text" value="1"/>	Enter the number of regions in the X direction.
Starting at:	<input type="text" value="-20"/>	Enter the left edge of the first region.
Ending at:	<input type="text" value="262144"/>	Enter the right edge of the last region.
Space between:	<input type="text" value="0"/>	Spacing (% of X scale).

**On Y-Axis**

Number of regions:	<input type="text" value="1"/>	Enter the number of regions in the Y direction.
Starting at:	<input type="text" value="-20"/>	Enter the bottom edge of the first region.
Ending at:	<input type="text" value="262144"/>	Enter the top edge of the last region.
Space between:	<input type="text" value="0"/>	Spacing (% of Y scale).

OK

Cancel

**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.

On X-Axis

Create a region array for the FCOM peaks.

Number of regions:  Enter the number of regions in the X direction.

Starting channel:  Enter the starting channel of the first region.

Region size:  Enter the width in channels. Regions are same size.

Ending channel:  Enter the ending channel of the last region.

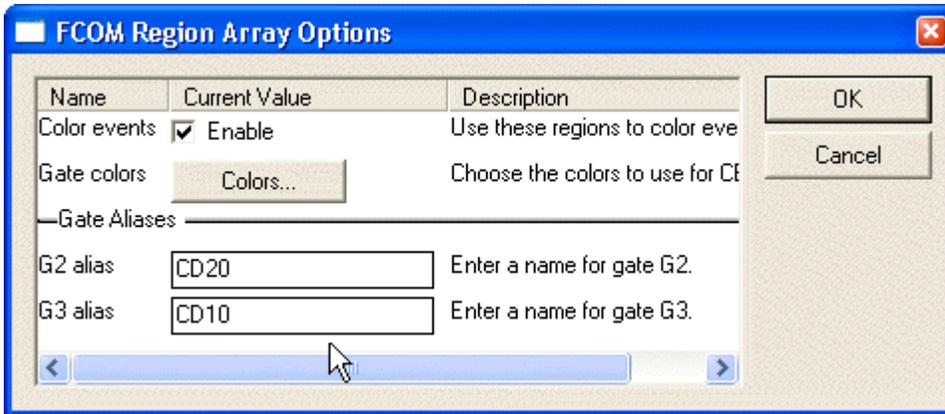
Space between:  Enter the number of channels between regions.

**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:**

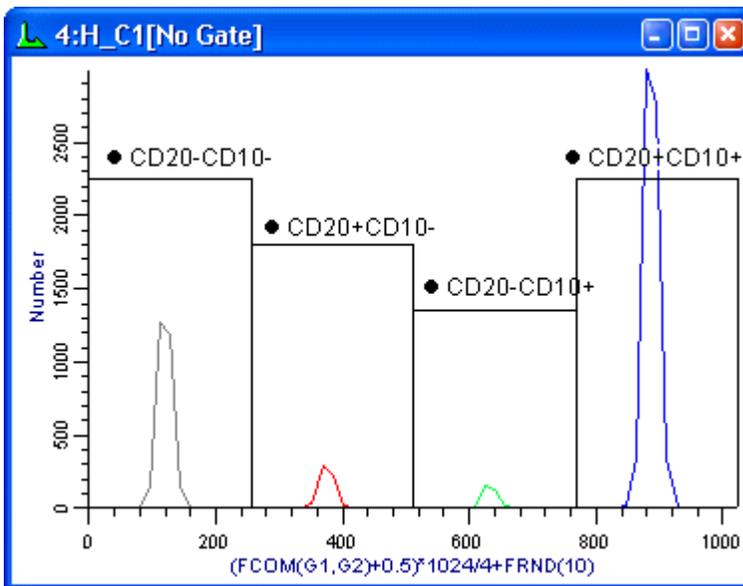
If you enable color-event gating with the option above, the colors used for each gate can be selected with this option.

**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.

### Duplicate Data Source

When selected, this option will create another data source window that will duplicate the active data source and its histograms. 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 menu

#### Copy (Ctrl C)

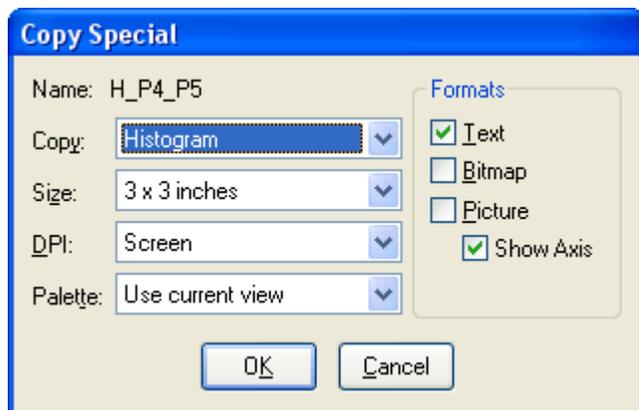
Select this option to copy the active histogram, results, or report window to the clipboard. A histogram will be copied as a bitmap and a metafile by default, two formats that many other programs can paste as pictures. The format(s) used can be changed using Copy Special.

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

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)

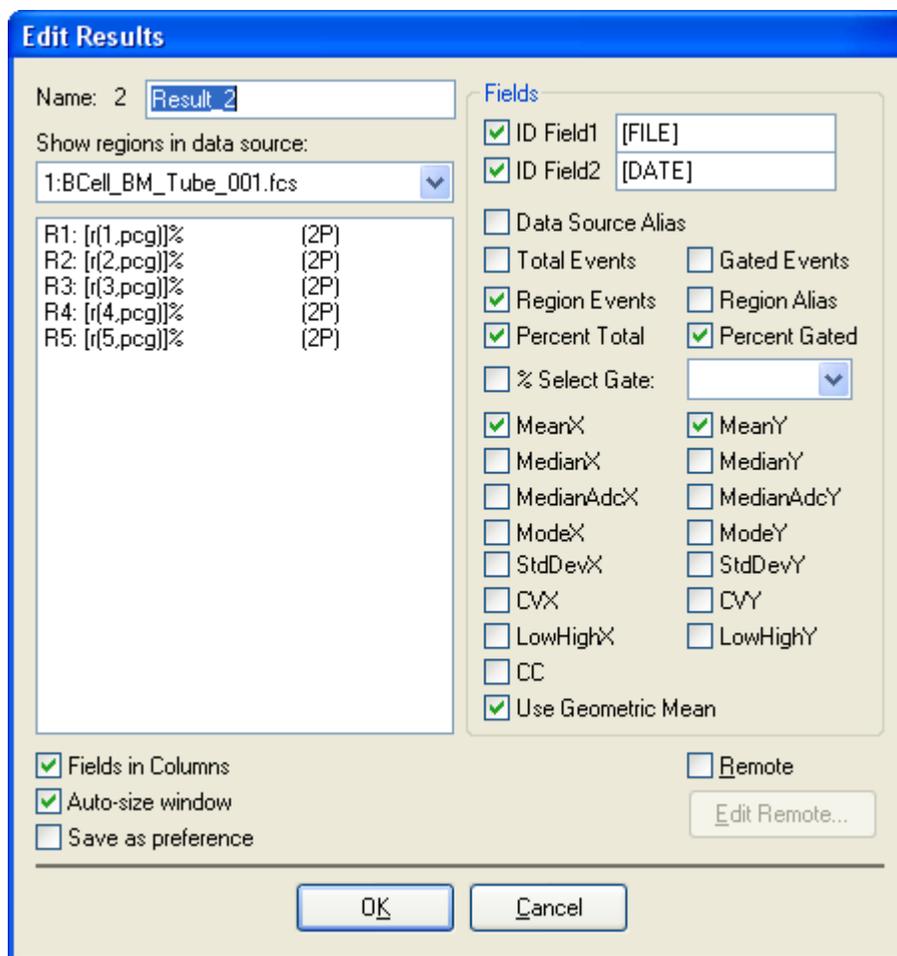
This command allows you to paste a picture or text object from the clipboard into the active histogram or report. The option is available if the clipboard contains bitmap, metafile, or text formats. The pasted object can be moved and sized on the histogram or report window.

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.

## 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. **[SSAMPLE]**), WinList will search the currently opened file's keywords for a match. If it finds a matching keyword, the keyword's value 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, if any, which the histogram containing the region is assigned. 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.

The numerator of this statistic is the number of gated events reported for the region. To compute the denominator, the program evaluates each event for several conditions. First, an event must pass the gate that is applied to the histogram that contains the region whose results are being computed. Then, the event must pass the gate specified by the **% Selected Gate**.

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.

**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 pushbutton**

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.

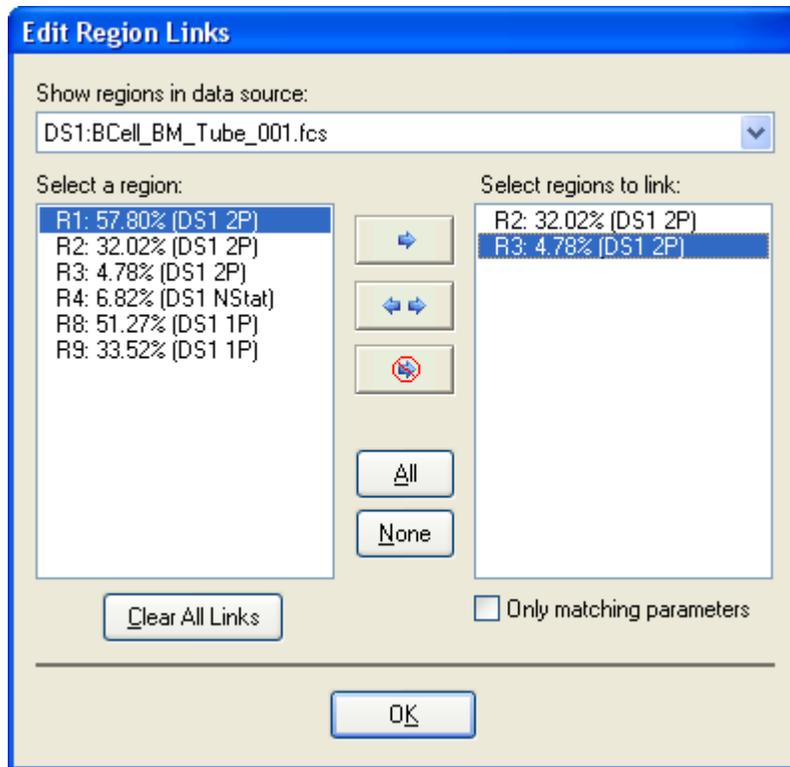
**Cancel**

Click this button to exit the dialog box with no changes.

**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.



### Controls

#### 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 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



When this push button is pressed, the region on the left is linked to the region(s) on the right. The link is a one-way link, meaning that when the lefthand region is moved or resized, the other regions will move and resize accordingly.

#### Two-way link



When this push button is pressed, the region on the left is **bidirectionally** linked to the region(s) 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 allregions.

**OK**

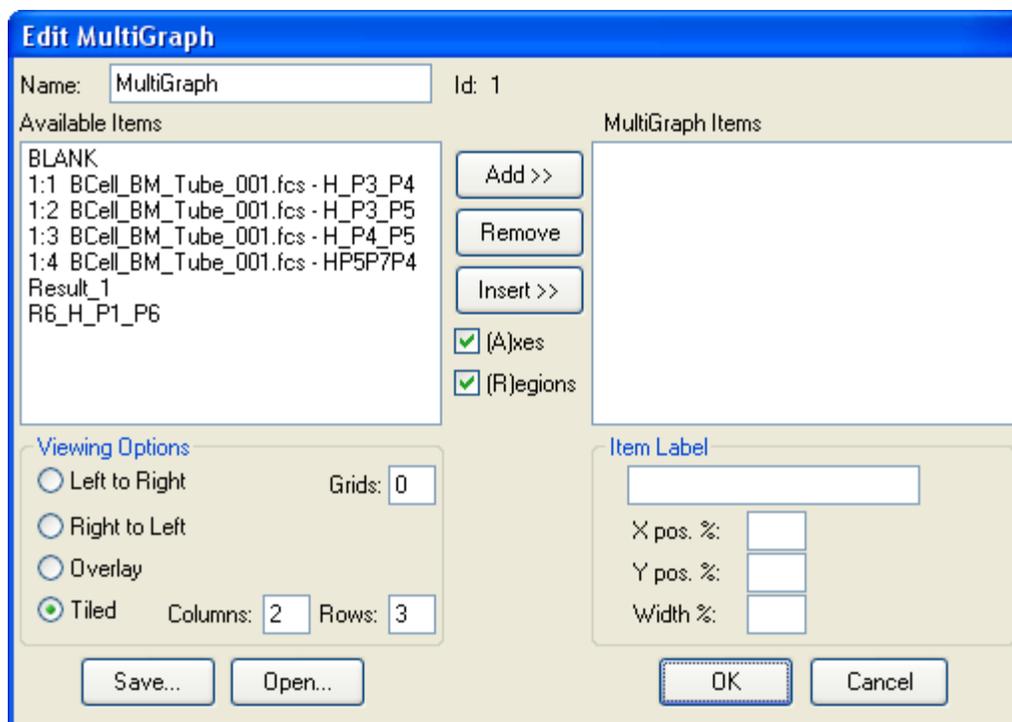
To put away the Link Regions dialog box, select this push button.

**Cancel**

Click this button to close the dialog without making changes. This push button grays out if any regions are linked or unlinked.

**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.



Some characteristics of MultiGraphs can be edited by selecting **Edit MultiGraph** from the Edit menu. These include the **Viewing Options**, as well as **Labels** and positions. You can also add or remove histograms at any time. Other characteristics do not change. These include the state of axes and regions for histograms already generated in the MultiGraph. To change these display options, the histograms must be deleted from the MultiGraph and recreated from the original data source.

The **Edit MultiGraph** dialog box has the following controls.

### Name

Allows the user to type in 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

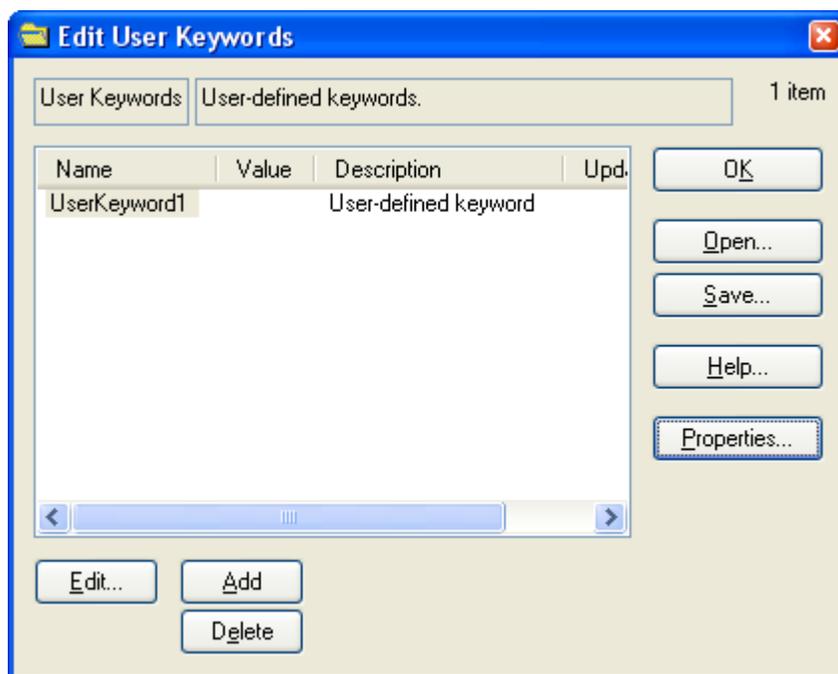
Click **OK** when you've made your changes and selections and the new MultiGraph elements will be generated. The amount of time to generate a MultiGraph depends on how many elements you have selected and how complicated each one is.

### 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.

### Edit User Keywords

This dialog 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.

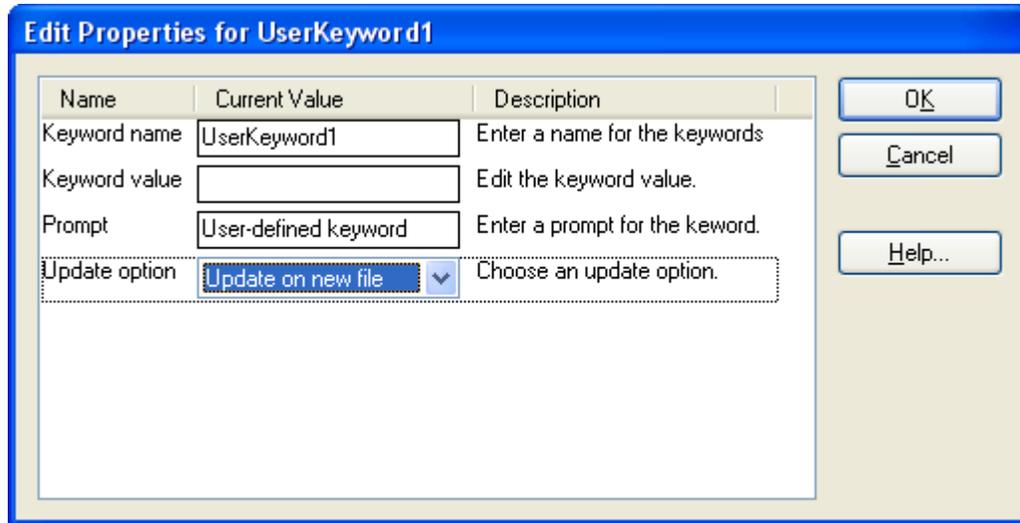
Click the **Add** button to create a new user keyword. The Edit Properties for User Keyword will be displayed allowing you to create a name, default value, and prompt for the keyword. You can also set when WinList should update the keyword.

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.

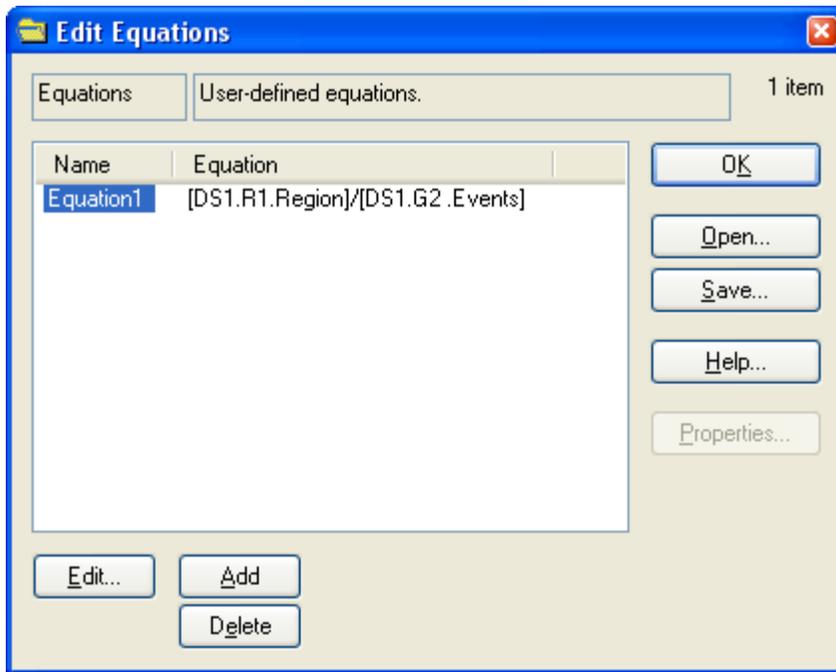
Update on replay: this option causes WinList to prompt for a new value when one of the Replay options is used.

See also:

Edit User Keywords

### Edit Equations

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.

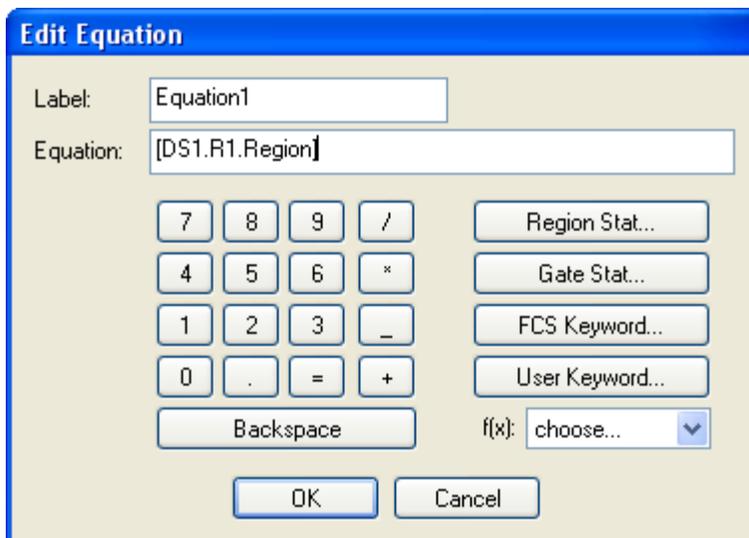


Create a new equation by click **Add**, or edit an existing equation using the **Edit** button. The Edit Equation dialog is displayed to define the equation.

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

This dialog is designed to make it easy to create your own equations. It is layed out 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**

This dialog allows you to define and edit alert conditions that WinList will monitor. Alerts are defined by selecting a region statistic from a list, choosing a logical operator, and entering a test value. 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.

**Edit Alerts**

Alert Name:

Applies to:

Condition:  <

Alert Keyword:  Alert Text:

Show alert prompt.

Alert	Applies to	Condition	Logic	Value	Prompt
Alert1	DS 1	DS1.R2...	<	20.00	Yes

Buttons: OK, Cancel, New, Delete, Open..., Save...

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 region statistic, a logical operator, and a threshold value. Select the region statistic that you want to monitor in the first drop-down list, then choose a logical operator and enter a 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**

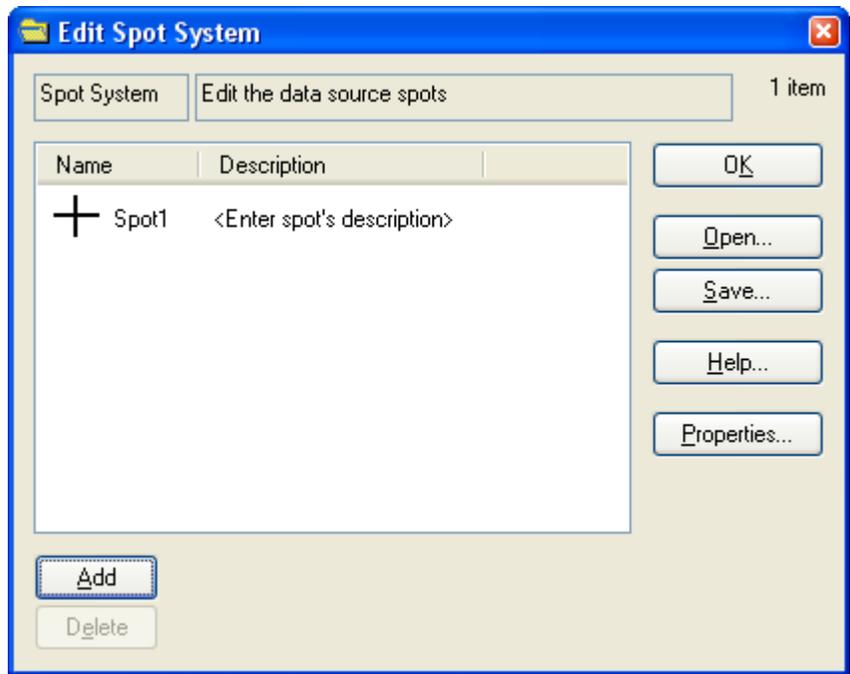
Close the dialog and accept the changes.

**Cancel**

Close the dialog and discard the changes.

**Edit Spot**

This dialog box can be brought to the screen by choosing **Edit Spot** in the **Edit** menu.



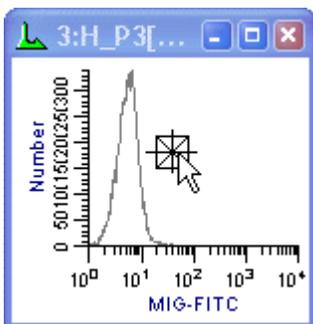
**What is a spot?**

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.

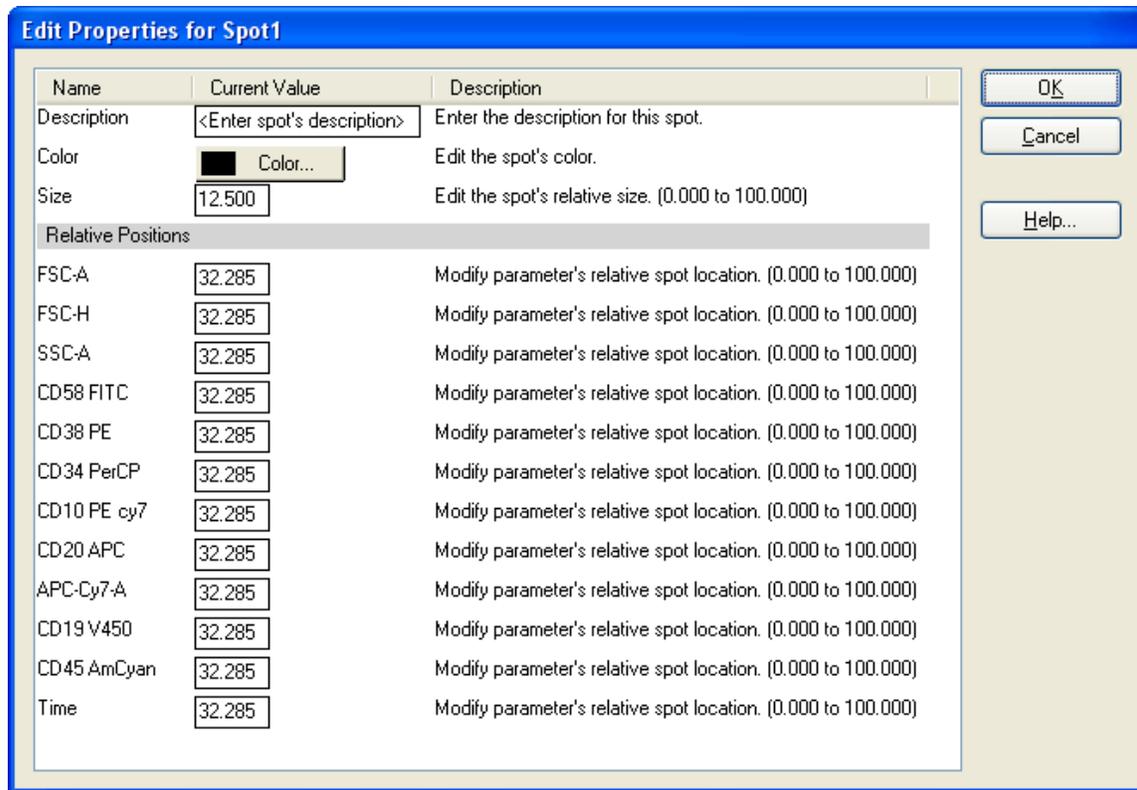
### Properties

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.

**Edit Log**

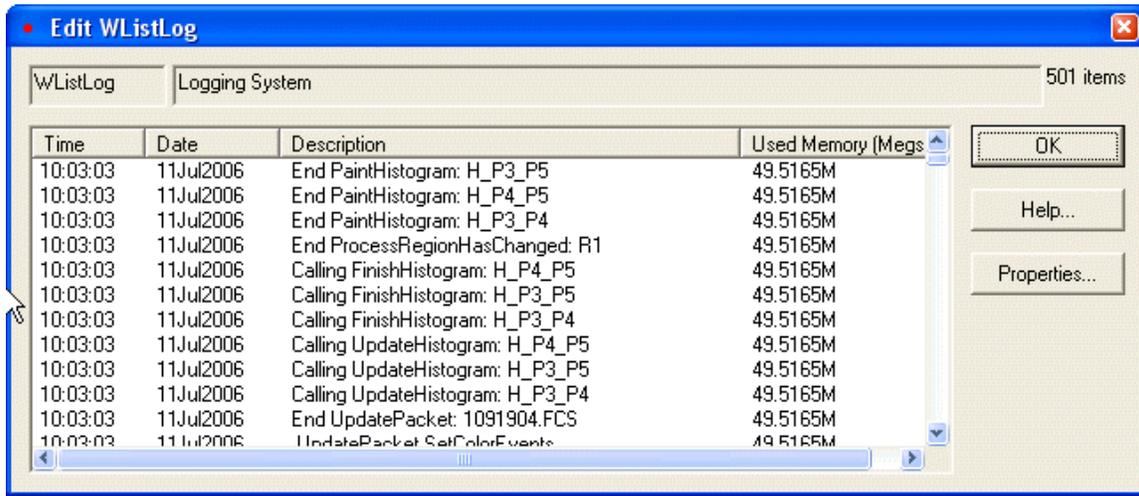
This option displays the Edit WListLog dialog box, which allows you to view and set properties for session logging. Logging can be used to help Technical Support resolve problems with your software.

You can turn logging on and off by pressing the F2 key on the keyboard. The application titlebar will indicate when logging is enabled.

See Edit WListLog and Edit Properties for WListLog for more information.

**Edit WListLog**

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.

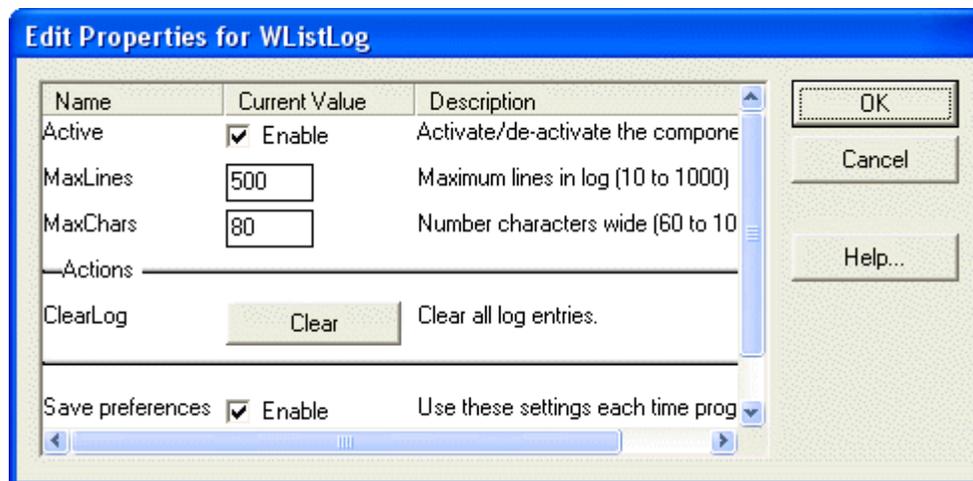
#### Properties

Click this button to display the Edit Properties dialog for the logging object.

See also Edit Properties for WListLog and Edit Log.

#### Edit Properties for WListLog

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 same folder as the application. The file will store operations you perform as you work with the program.

**MaxLines**

This option defines how large the log file can be, in number of lines. When the limit is reached, the oldest line is discarded and a new line is added. You can increase this value to retain more entries in the log.

**MaxChars**

This option defines the length of a line in the log file. Normally, you do not need to adjust this value.

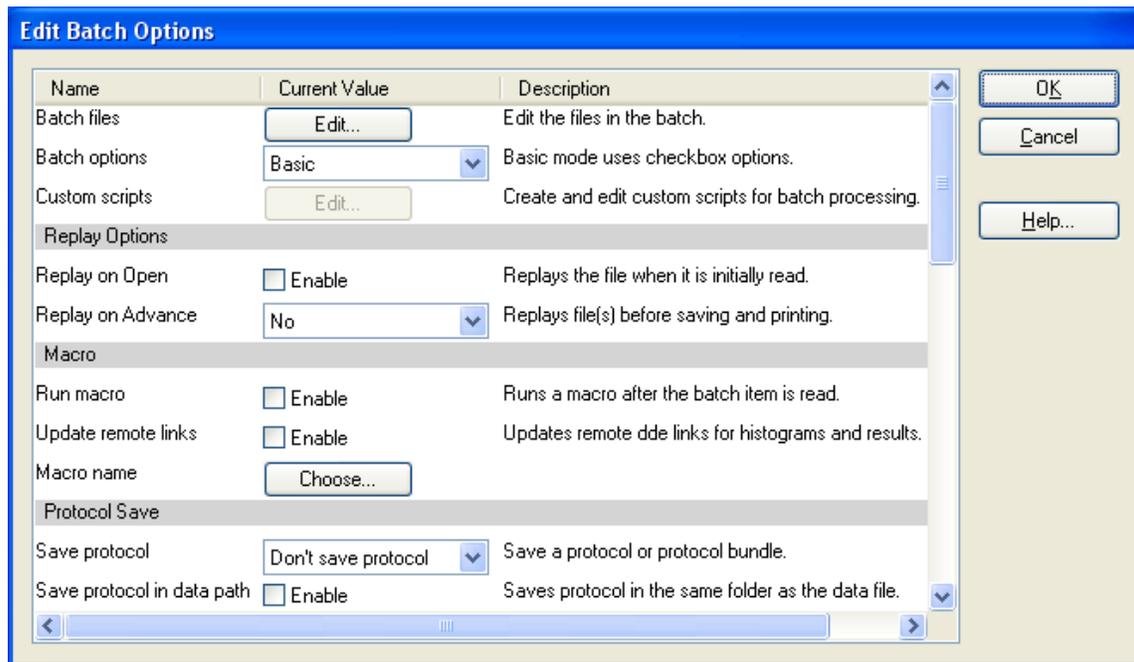
**ClearLog**

Click this button to clean out the log file.

See also Edit Log and Edit WListLog.

**Edit Batch Options**

This dialog can be accessed from the batch toolbar by clicking the leftmost button. 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 protocols 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**

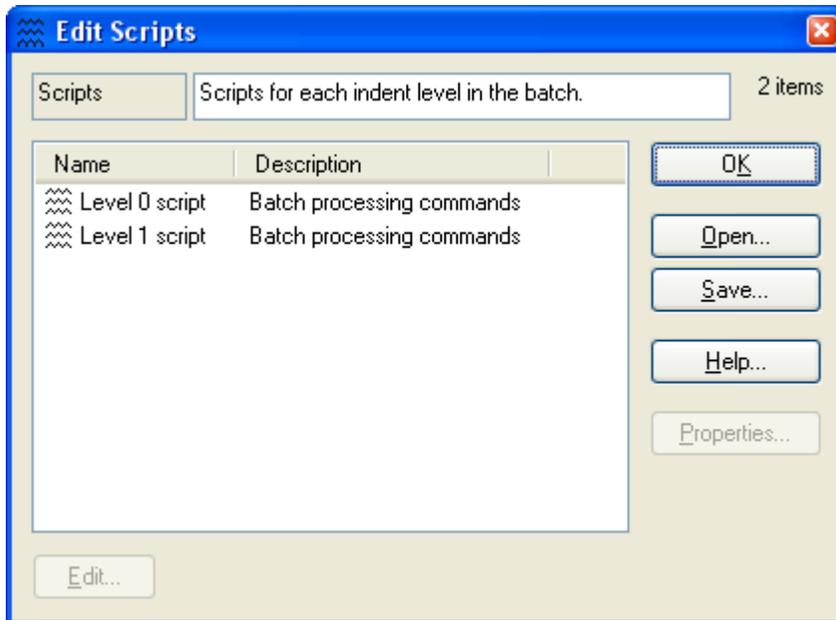
Normally, WinList hides the batch toolbar when the batch list is empty. Enable this option to always show the batch toolbar.

### **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**.

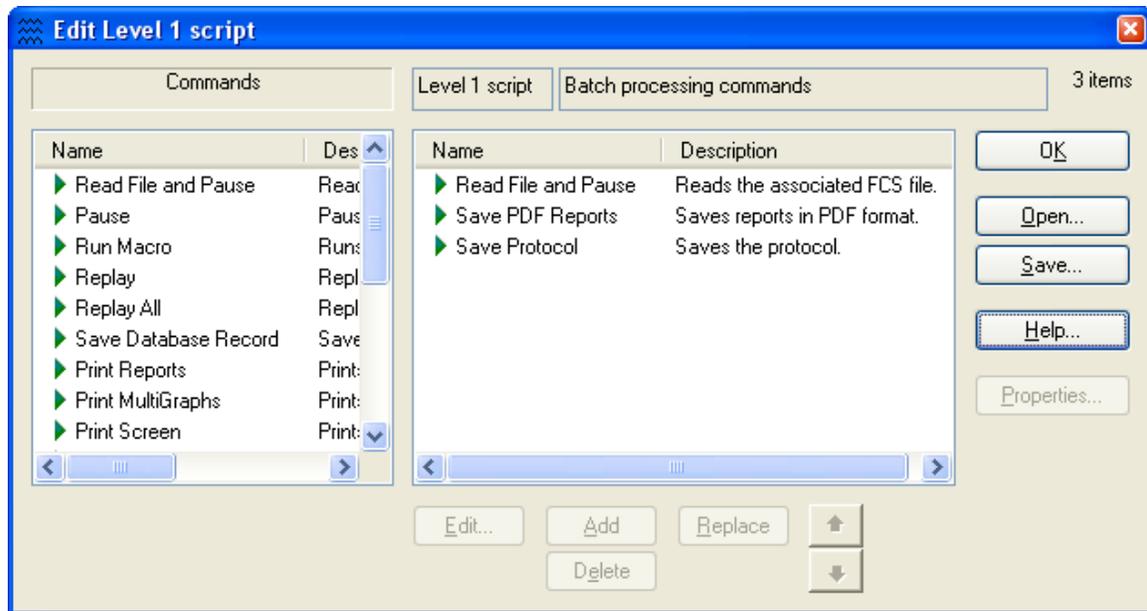
Use the **Open** and **Save** buttons to save collections of customized scripts so that you can reuse them in other protocols. Scripts are also saved as part of the protocol and protocol bundle file formats.

### 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 lefthand 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:

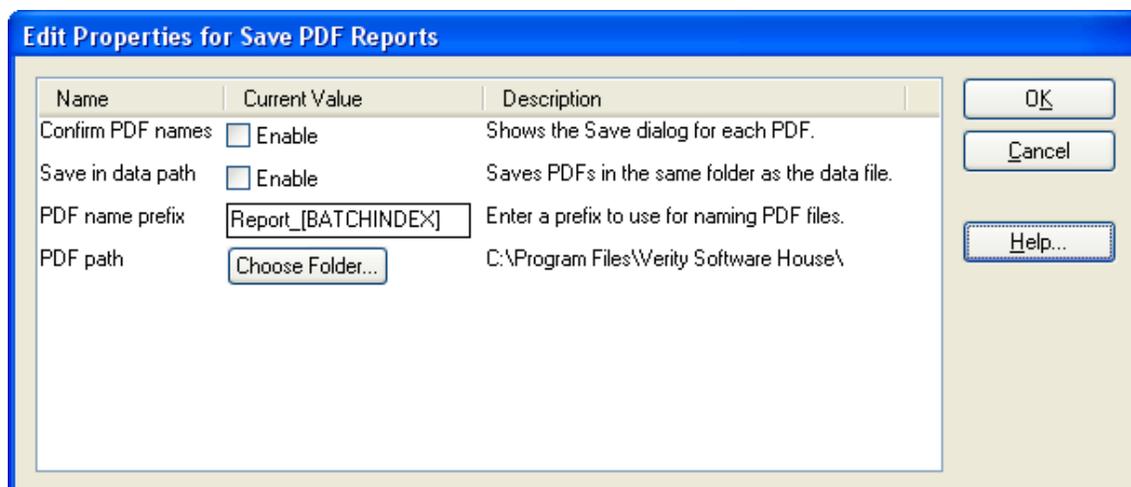
Edit Batch Command

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. Alternatively, you can select the command in the list and click the **Properties** button.

#### See also:

Edit Script

Edit Scripts

Edit Batch Options

#### Edit Compensation Database

The Compensation Database provides a time-saving means for setting up and applying fluorescence compensation. Using the Compensation Database explains how to create and use it.

The main advantage to using the compensation database is that it breaks apart the compensation matrix into interactions between 2 fluors at a time. This allows you to compensate new combinations of fluors by updating the database with only the part of the compensation that needs to be changed.

#### See also:

Using the Compensation Database

Setup Compensation Database

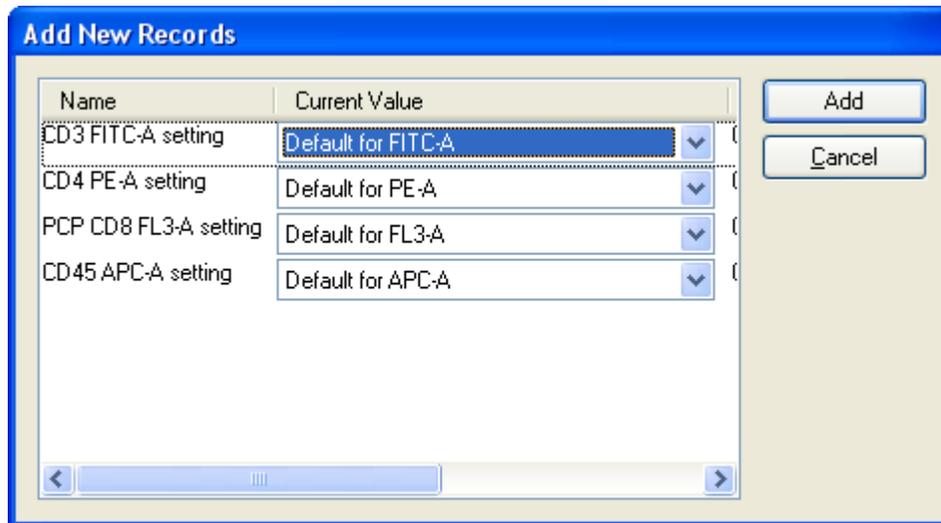
#### Using the Compensation Database

The Compensation Database provides a time-saving means for setting up and applying fluorescence compensation. It allows you to streamline the process of setting up compensation for large panels and to create single-tube compensation adjustments.

There are two parts to working with the Compensation Database: teaching the system how and what to compensate, and using the system to apply compensation.

#### Teaching the system

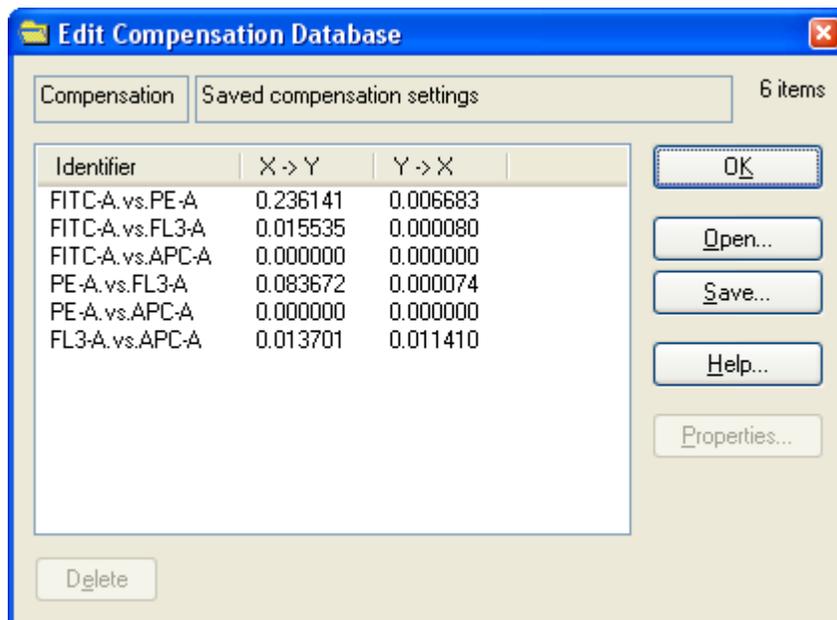
First, you set up some compensation, either from single color controls or from auto-compensation from the FCS file's keywords. When a compensation setup is ready to be "learned" by the system, you click **Learn Compensation Settings** on the **AutoComp** tab of the **Compensation Toolbox**. A dialog is displayed.



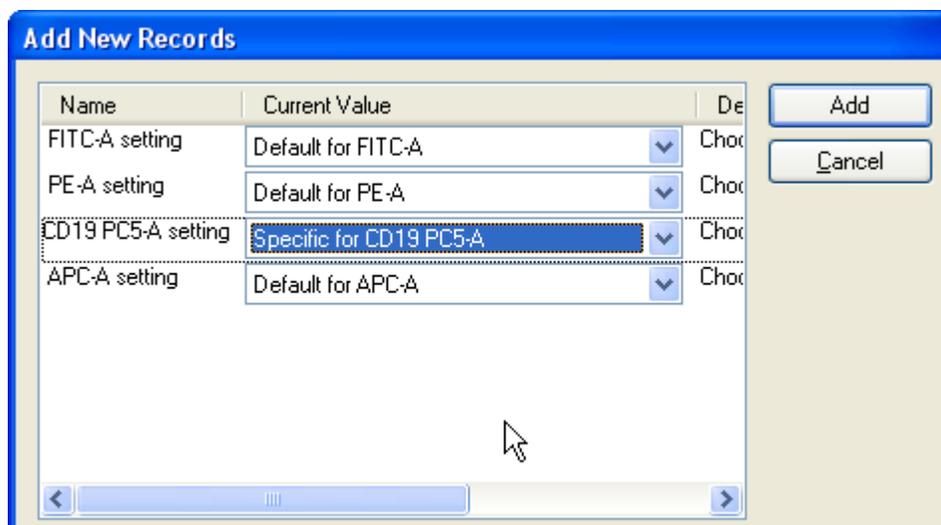
An entry is displayed for each parameter in the compensation. The names of the entries are based on keywords found in the files. The keywords used by default are \$PnN and \$PnS, but these can be selected in the Setup Compensation Database dialog. Each entry can either be set as “**Default for fluor**” or “**Specific for antibody & fluor**”.

The best way to start is to set up compensation for the default case first and choose “Default for fluor” for each of the parameters. Then click the **Add** button to store the settings. At this point, the system knows how to set up compensation whenever it sees these fluors in combination.

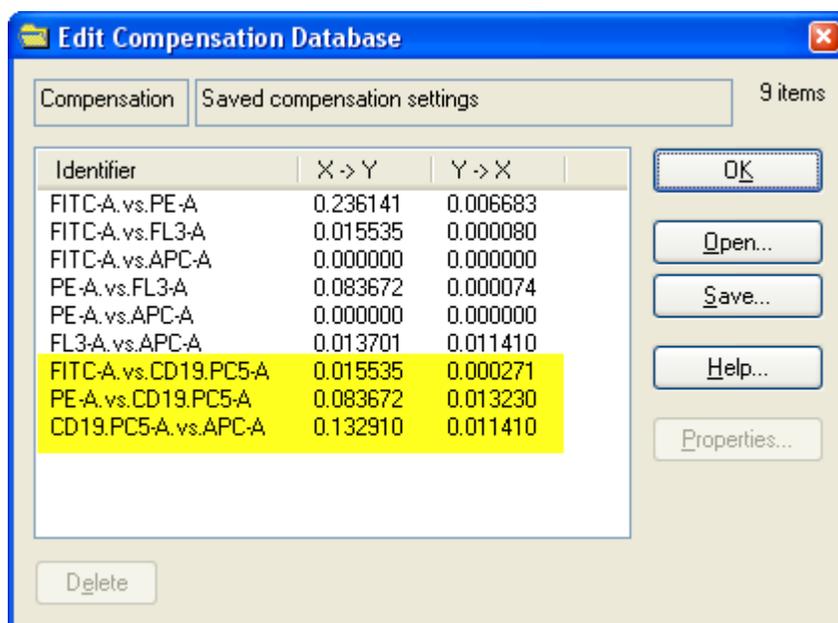
When you click Add, a record is added to the database for each combination of fluors that are involved with compensation.



Now suppose that you have the same panel except that there is one different fluor. You make the appropriate adjustments to the compensation, based on a new single color control. Then again click **Learn Compensation Settings** on the **AutoComp** tab of the **Compensation Toolbox**.



This time you would select **"Specific for fluor & antibody"** for the one fluor that is different, and click **Add**. The database would now have some records with more specific names.

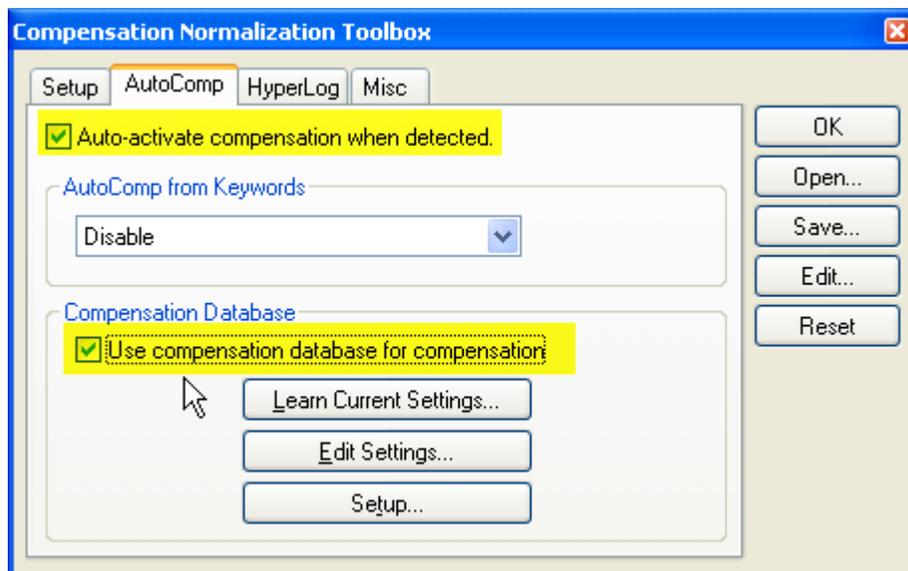


The Compensation Database now knows how to compensate a more specific combination of fluors and antibodies. It will always use the most specific compensation that it has for each pair of markers in a file.

Once you have the default compensation set up for a combination of fluors, you can create exceptions for specific combinations of fluors and antibodies. Each time you set up a new variation, click the **Learn Compensation Settings** button and select **"Specific for fluor & antibody"**.

### Using the system

Once you set up the compensation database, you are ready to start using it to apply compensation to your data. There are two options that need to be turned on, both found on the **AutoComp** tab of the **Compensation Toolbox**.



First, enable the **Auto-activate compensation** checkbox so that compensation is automatically applied. Next, enable the **Use compensation database for compensation**.

You can use the Compensation Database in conjunction with AutoComp from Keywords. When both options are enabled, the program sets up compensation based on keyword information first, and then searches the compensation database for matching fluors and antibodies.

As each file is read, the compensation database is scanned to see if there are any entries that match the parameters in the FCS file you opened. When matches are found, the crossover matrix is updated with values from the database.

### Setup Compensation Database

This dialog allows you to customize several aspects of the Compensation Database.

**Setup Compensation Database**

**Fluorochrome Keyword**  
 Keyword that identifies the fluorochrome: \$PnN  
 Current value for P1: FSC-A

**Antibody Keyword**  
 Keyword that identifies the antibody: \$PnS  
 Current value for P1:

**Voltage Alert**  
 Alert when voltage of a parameter differs by: 50

Be sure to use a consistent naming strategy for antibodies and fluorochromes during acquisition.

OK Cancel

**Fluorochrome Keyword:**

Select the keyword that identifies the fluorochrome. Typically, this is \$PnN.

**Antibody Keyword:**

Select the keyword that identifies the fluorochrome. Typically, this is \$PnS.

**Voltage Alert:**

Enter the amount of voltage difference between the compensation database and an FCS file that will trigger a user alert. Compensation settings are affected by the voltage settings on the instrument, and the Compensation Database stores the voltage that was set when the crossover matrix was created. This setting allows the program to notify you if a file is loaded with a voltage difference that could cause the compensation to be incorrect.

**Edit Program Controls**

This dialog box can be brought to the screen by choosing **Edit Program Controls** 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**. You must be careful when resetting this value since there may be memory-related consequences in making some of the entries too large.

**Edit Program Controls**

Initial regions: 64

Initial gates: 64

Palette size: 32

Data Packet Size: 30000

OK Save Cancel

**Initial regions**

This field sets the initial number of regions allocated by WinList. The program will automatically allocate for more regions as needed.

**Initial 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 increase 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.

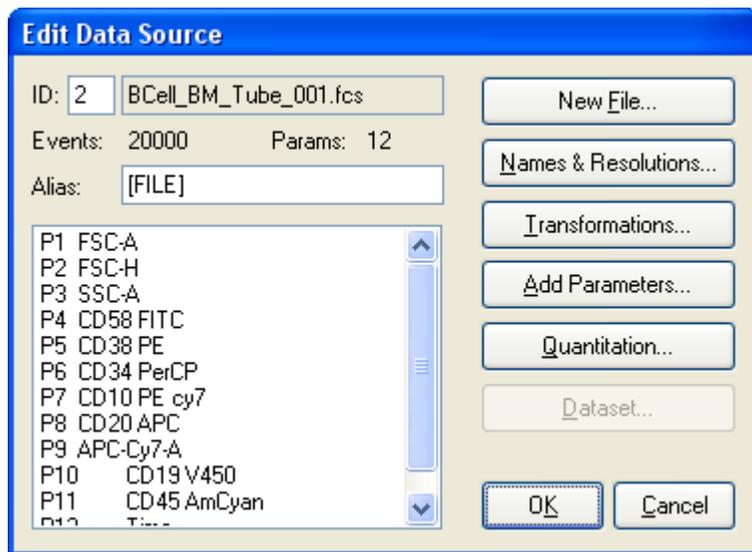
**Cancel**

Click this button to exit with no changes.

**Edit Data Source**

**Edit Data Source**

This dialog box can be brought to the screen by either choosing **Edit Data Source** in the **Edit** menu or by double-clicking on the Data source window's 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.

**Events and Params**

This displays the total number of events and the number of parameters stored in the data source listmode file.

**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.

**Parameter listbox**

This list shows the parameter names stored in the data file.

**New File**

Use this button to select a different data file. This option leads to the Open Listmode File dialog box.

**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.

**Transformations**

This option is allows you to select how each parameter is displayed in WinList. See Edit Transformations for more.

**Add Parameters**

This option allows you to add calculated parameters to the data source. See Add New Parameter 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.

**OK**

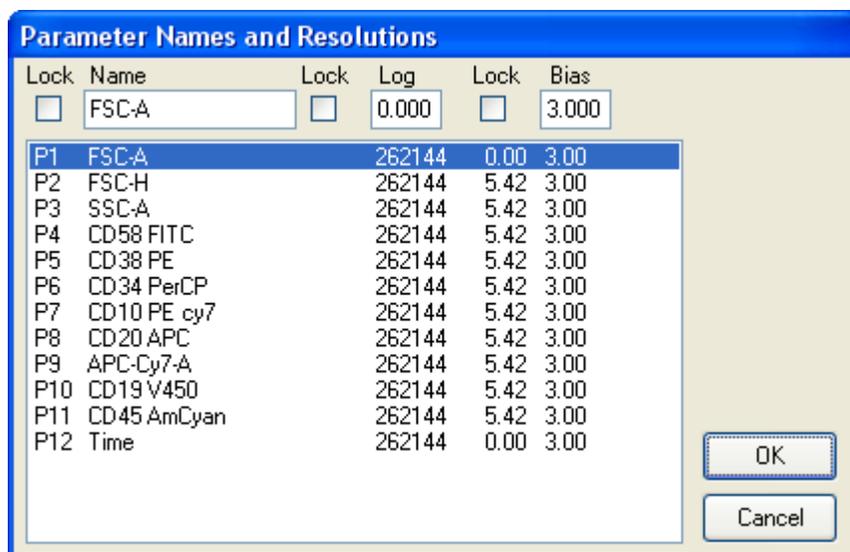
Accepts changes to the data source and closes the dialog box.

**Cancel**

This option closes the dialog box with no changes to the data source.

## 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. Therefore, editing the names of data source parameters will affect all of the histograms it displays.

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 for the name of a parameter in order to over-ride our method of finding the parameter names.

The number of log decades for parameters should only be edited if WinList has not correctly determined the value for a parameter.

To edit a parameter log decade value, select the parameter in the list box and enter the new log value for the parameter in the **Log** edit box.

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.

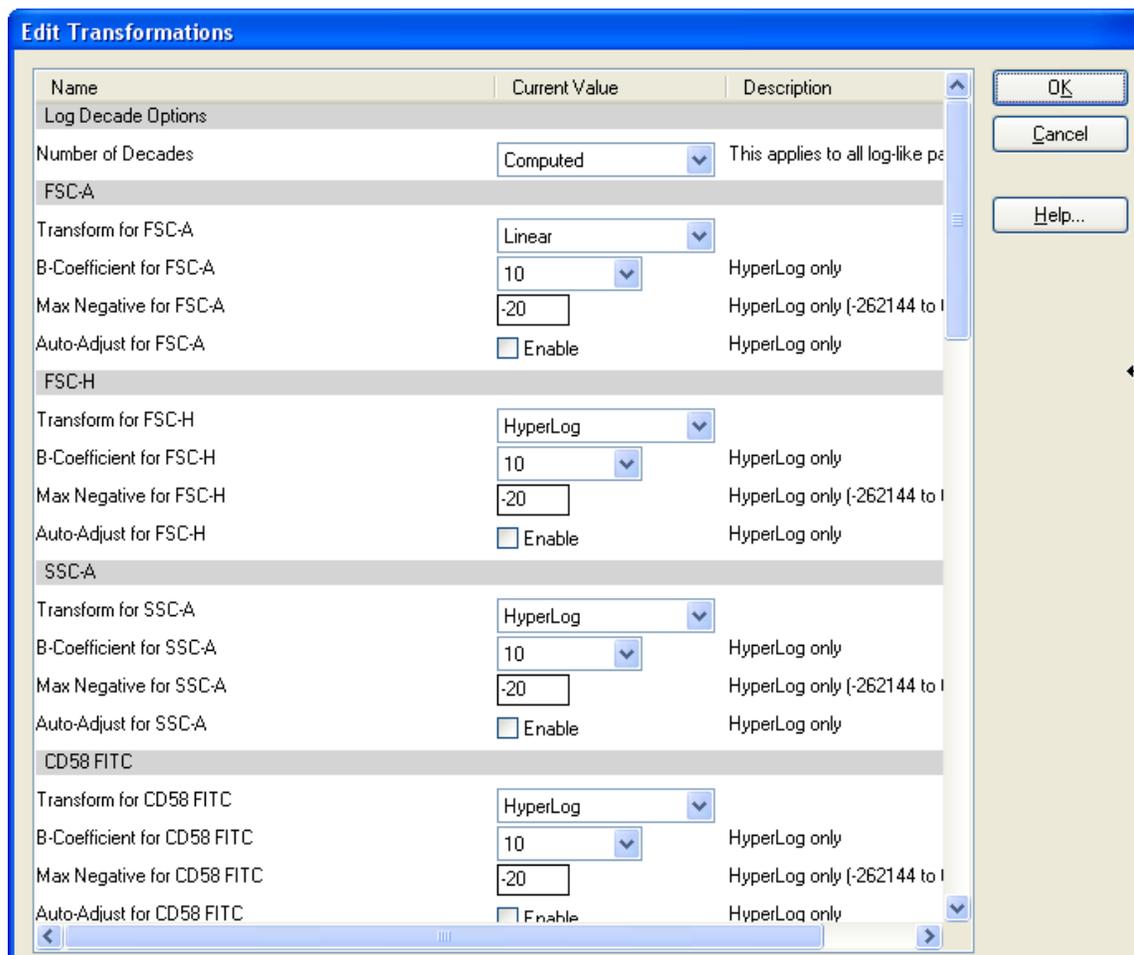
Similarly, WinList "locks" the number of log decades for any parameter that you edit. A **Lock** check box appears next to the **Log** edit box allowing you to toggle the lock on and off.

The **Bias** field for each entry is applied only when using WinList's N-Color Compensation™ system. *This option is not recommended for most cases; it should only be used for historical comparisons.* When compensation is applied to a parameter, WinList applies the Bias value to any events that fall on the axis. This simulates the behavior of log amplifiers on older flow cytometers. In applying the Bias, WinList will change a 0-value for a parameter to a value between 0 and the bias value. By default, the bias is 3, which means that compensated parameters will spread 0-value events across 3 linearized channels.

If you edit parameter names or log decade values 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.

## Edit Transformations

This dialog allows you to select linear parameters to convert from linear to log.



Most newer flow cytometers store all data in linear format, even if you view the data on log scale during acquisition and analysis. This dialog allows you to convert linear parameters to log 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  $\log_{10}(262144)$  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. However, since the linear data contains more than 4-decades (10,000 linear units) of information, there is a loss of information in this transformation. In the example above, compressing 262144 channels into 4-decades of log space causes the loss of 1.42 decades of information.

#### 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, or HyperLog.

**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.

**Max Negative value**

This is the lowest value shown on the HyperLog axis.

**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.

**Save preferences**

When this option is enabled, the changes you make to the Transformations dialog are remembered.

**See also:**

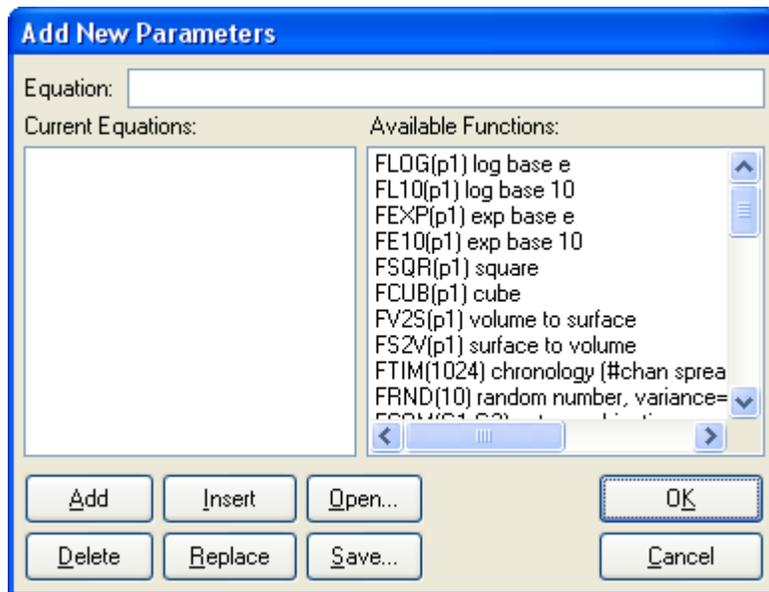
- Transformation Preferences
- Understanding Hi-res Data Files

## Add New Parameters

This option allows you to create calculated parameters based on the native parameters of your listmode files. Calculated parameters can be displayed and manipulated in the same ways as native parameters. In addition to parameter ratios, WinList provides numerous mathematical functions which can be applied to parameters.

All calculated parameters in WinList are computed in floating-point. When native parameters are referenced in a calculated parameter expression, they are transformed to a common, floating-point scale of 0.0 to 1023.0. The result of the calculated parameter expression is converted to an interger with a range of 0 to 1023.

You can display this dialog by choosing the **Add New Parameter** button in the **Edit Data Source** dialog box. The Add New Parameters dialog box appears.



**Equation**

Insert or type an equation to be created or edited in this edit box. The equation calculator accepts any algebraic function. The parameters and their meanings are shown in the parameter list box. The parameter and function letters are not case sensitive. For example, p1\*2.5 is the same as P1\*2.5. Equations can also use the functions displayed in the **Available Functions** list box. If you select a function or an example in the **Available Functions** list box, its value is automatically entered in the **Equation** edit box. Once you have created an equation for the calculated parameter, **Add** it, **Insert** it, or **Replace** the currently selected equation. In the case where operator precedence is ambiguous,

parentheses should be used. **Note:** In order to prevent binning effect in calculated parameters, WinList adds a random floating point number between 0 and 1 to each parameter prior to evaluating it in the algebraic equation.

**Parameters List box**

All the available parameters and their meanings are displayed in this list box. The list box will automatically be put away when you select either **Cancel** or **OK**.

**Available Functions**

All available functions and some examples are shown in this list box. To see the examples you will probably need to use the scroll down list box arrows. The arguments to these functions can be expressions. For example,  $FLOG(2*p1/p2)$  is a valid calculated parameter. Notice that all the functions start with the letter F. The functions are:

<b>FLOG(param)</b>	Returns the natural log of <b>param</b> . Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
<b>FL10(param)</b>	Returns the log to the base 10 of <b>param</b> . Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
<b>FEXP(param)</b>	Raises natural log to the <b>param</b> power.
<b>FE10(param)</b>	Raises 10 to the <b>param</b> power.
<b>FSQR(param)</b>	Returns the square of <b>param</b> .
<b>FCUB(param)</b>	Returns the cube of <b>param</b> .
<b>FPWR(base, power)</b>	Returns the value of <b>base</b> to the <b>power</b> .
<b>FLEV(exp, testExp, threshold, level)</b>	Returns <b>exp</b> unless <b>testExp</b> is greater or equal to <b>threshold</b> ; then it returns <b>level</b> .
<b>FV2S(param)</b>	Returns <b>param</b> to the 2/3's power. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
<b>FS2V(param)</b>	Returns <b>param</b> to the 3/2's power. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
<b>FTIM(adcResolution)</b>	Creates a chronological time variable over the number of channels, <b>adcResolution</b> , 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).
<b>FRND(variance)</b>	Returns a random number with <b>variance</b> variability. If zero is entered as the argument, then it returns a uniform random number between 0 and 1.
<b>FHST(param, area, resolution)</b>	Creates a frequency distribution for parameter <b>param</b> . Normally, after creating this parameter you would create a 2 parameter histogram displaying FHST on the Y-axis and <b>param</b> on the X-axis. You can create a 2-parameter region around a normal distribution using this technique.  You can normalize the area of the generated distribution by adjusting <b>area</b> . The default value is 5000. Additionally, the internal resolution used by WinList can be adjusted using <b>resolution</b> : default resolution is 128 channels.
<b>FCOM(g1, g2,...gn)</b>	Gate combination function. The argument is a list of any number of gates separated by commas. See FCOM Background and Usage for further explanation.
<b>FSIN(x)</b>	returns the sine of x, where x is in radians.
<b>FCOS(x)</b>	returns the cosine of x, where x is in radians.
<b>FTAN(x)</b>	returns the tangent of x, where x is in radians.
<b>FCTN(x)</b>	returns the cotangent of x, where x is in radians.
<b>FASN(x)</b>	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.
<b>FACS(x)</b>	returns the arccosine of x, where x ranges

**FATN(x)**

from -1.0 to 1.0. Values for x outside of this range are forced to the nearest limit.

**FACN(x)**

returns the arctangent of x.

returns the arccotangent of x, equivalent to arctangent of 1/x.

**Equation List box**

Any equations that are in the **Equation** list box will be calculated when WinList exits from the **Edit Data Source** dialog box. These parameters can be displayed or analyzed by creating a histogram that involves a calculated parameter.

**Add**

Adds the **Equation** contents to the end of the **Equation List box**. There are no limits (other than memory considerations) on the number of calculated parameters.

**Insert**

Inserts the **Equation** contents just above the currently selected line in the **Equation List box**.

**Delete**

Deletes the currently selected line in the **Equation List box**.

**Replace**

Replaces the currently selected line in the **Equation List box** with the contents of the **Equation** edit box.

**Open**

Opens a previously stored file of equations. The files have the default suffix of equ.

**Save**

Saves the current set of equations to disk.

**OK**

Adds the equations in the Equation List box as new parameters to the data source. The Cancel button in the Edit Data Source dialog box will be grayed out after clicking the OK button. The new calculated parameters can be saved as part of a data source listmode file by choosing the **Save Data Source** option from the File menu.

**Cancel**

Quit the process. No changes will be registered.

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

You may create regions around the corresponding FCOM histogram peaks for statistics, gates, or color eventing. You may also 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.

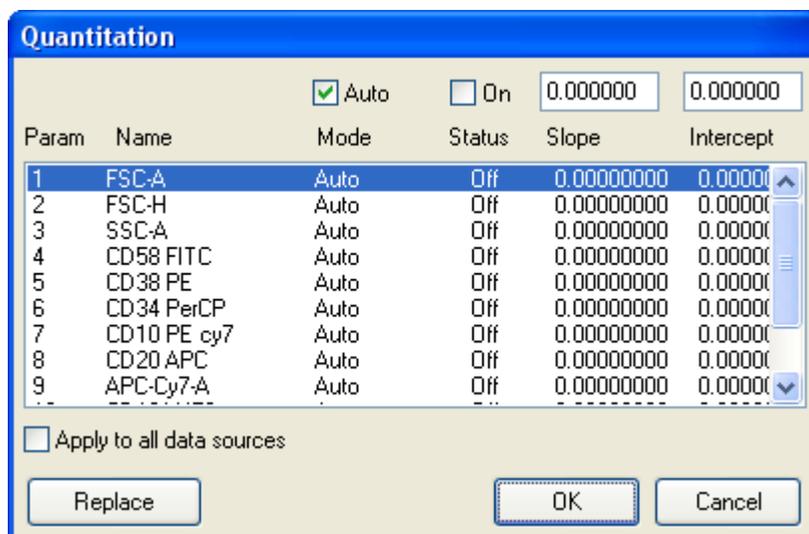
For more on using FCOM, review the Using FCOM parameter to enumerate phenotypes tutorial.

## 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, Becton Dickinson Immunocytometry Systems for example, store these keywords when acquiring data if relevant controls have been run.



### Auto

This option is normally checked, which sets WinList for “Auto” mode for quantitation. In Auto mode, WinList will look in the data file for quantitation keywords, and if found, will apply the quantitation and enable the relevant parameters automatically. Uncheck this option if you want to enter you own slope and intercept values, or you do not want to use quantitation.

### On

If this option is checked, the parameter is enabled for quantitation.

### Slope and Intercept

The slope and intercept provide information on how to convert an ADC channel number to a quantitative value. The formula used by WinList in “Auto” mode to convert keyword information is as follows:

$$\text{Molecules Per Cell} = 4.0 * \text{ADC channel} / (\text{ADC resolution} * \text{slope}) - \text{intercept} / \text{slope}$$

Note: Formula assumes the parameter is logarithmic.

To compute your own slope and intercept values, you need to perform a linear regression of log (molecules per cell) versus ADC 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.

### Replace

Click this button to update the options for the selected entry in the listbox.

### 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.

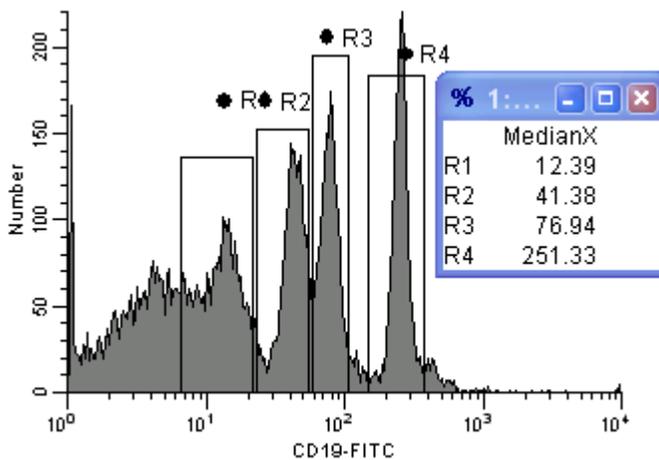
## Using the Quantitation Spreadsheet

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 *
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 >> Quantitation	0.0042822228	2.110870406

**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.

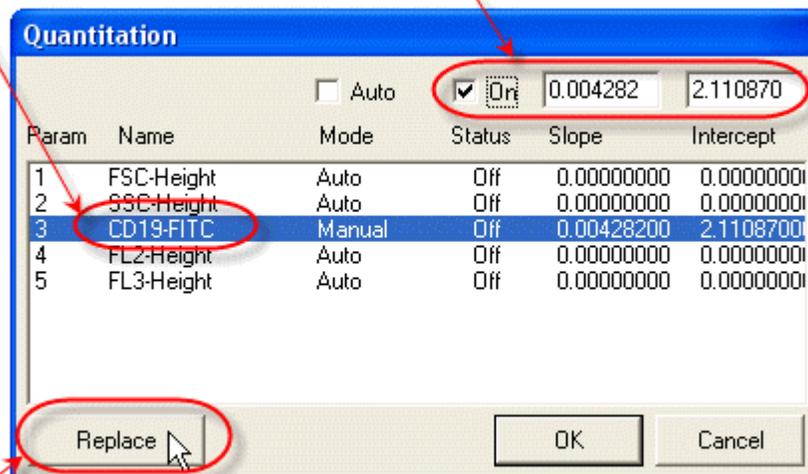


2. In Excel, open the WinlistQuantitation5Bead.xls spreadsheet found in the Samples folder. Enter the ADC range and the number of log decades for the data files that you are analyzing.

3. In the spreadsheet, enter the median values from WinList. Then enter the MESF (or ABC) values provided by your quantitative bead manufacturer.

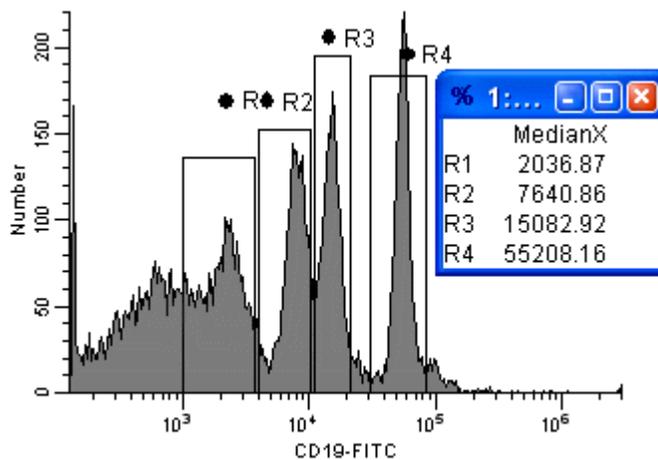
4. Copy the Slope and Intercept from the spreadsheet into the Quantitation dialog in WinList.

1. Choose the quantitation parameter.
2. Enter the slope and intercept and check "On".



3. Click the Replace button, then click OK.

WinList will now display quantitative results and axes for the parameter.



### Edit Histogram

#### Edit Histogram

This dialog box is displayed either by choosing **Edit Histogram** in the **Edit** menu or by double-clicking on the histogram window's title bar.

**Name**

This field displays the name of the histogram. 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. In some cases, the program may not be able to display a histogram because there may not be enough memory available.

**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 graphics dialog box.

**OK**

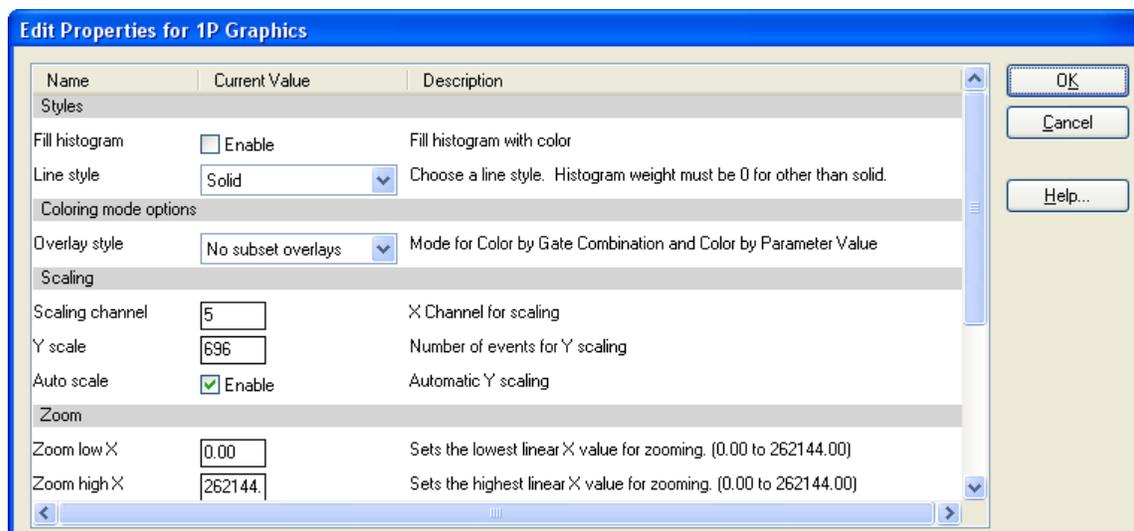
Click this button to confirm the changes to the histogram and close the dialog box.

**Cancel**

Click this button to abandon changes to the histogram, and close the dialog box.

## Edit 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**

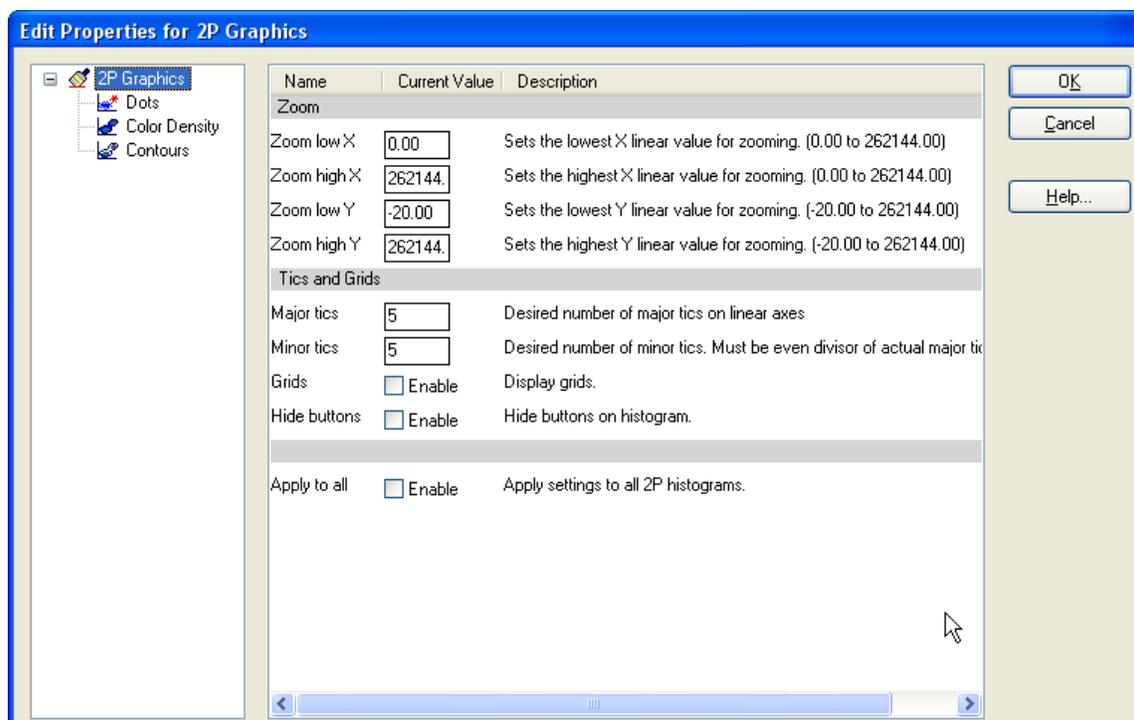
Click this button to accept the changes you have made and close the dialog box.

#### **Cancel**

Click this button to exit with no changes made and close the dialog box.

## **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.

## Ticks 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 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 "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.

### 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.

### 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.

### 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.

## 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 colorsr**

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.

## Edit 3P Graphics

This dialog allows you to change the graphical display of a 3P histogram.

Name	Current Value	Description
<b>Dots and plot</b>		
Dot size	2	0=Smallest
Number	10000	Number of dots to display.
Show all dots	<input type="checkbox"/> Enable	Display all dots.
Lines	<input type="checkbox"/> Enable	Draw lines to base plane.
Background plots	<input checked="" type="checkbox"/> Enable	Draw background dot plots.
<b>General</b>		
Major tics	5	Desired number of major tics on linear axes
Minor tics	5	Desired number of minor tics. Must be even divisor of actual major tic interval.
Grids	<input type="checkbox"/> Enable	Display grids.
<b>Zoom</b>		
Zoom low X	-20.00	Sets the lowest X linear value for zooming. (-20.00 to 10000.00)
Zoom high X	10000.0	Sets the highest X linear value for zooming. (-20.00 to 10000.00)
Zoom low Y	0.00	Sets the lowest Y linear value for zooming. (0.00 to 10000.00)
Zoom high Y	10000.0	Sets the highest Y linear value for zooming. (0.00 to 10000.00)
Zoom low Z	-20.00	Sets the lowest Z linear value for zooming. (-20.00 to 10000.00)
Zoom high Z	10000.0	Sets the highest Z linear value for zooming. (-20.00 to 10000.00)
Apply to all	<input type="checkbox"/> Enable	Apply these settings to similar 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 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.

**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**

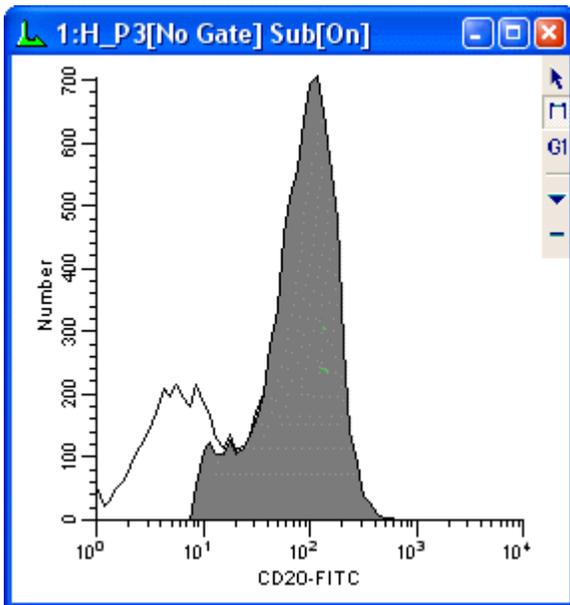
Click this button to exit with no changes made and close the dialog box.

## Subtract Histogram

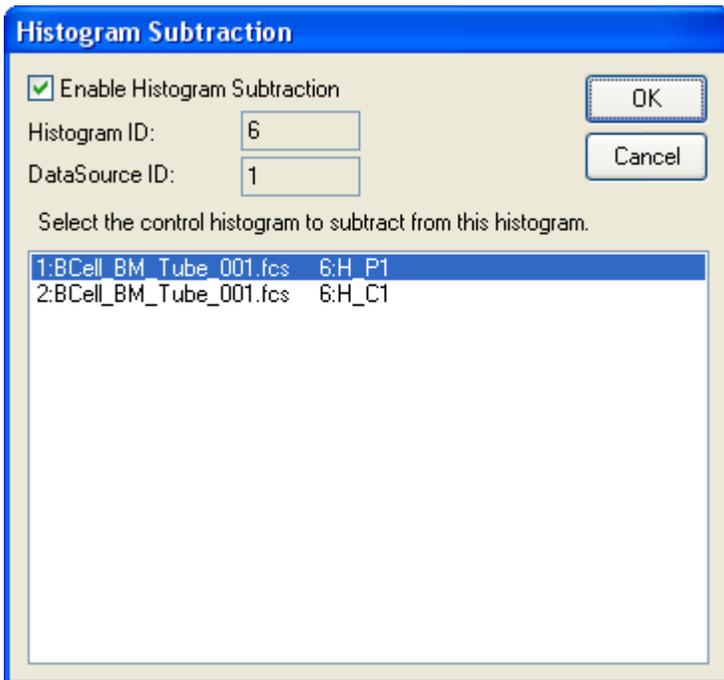
This dialog box allows the user to perform histogram subtraction, where the control (negative) histogram is selected and subtracted from the current active (test) histogram. The dialog box can be accessed through the Edit Histogram dialog box, or from the histogram popup menu that appears when the right 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.

#### DataSource 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.

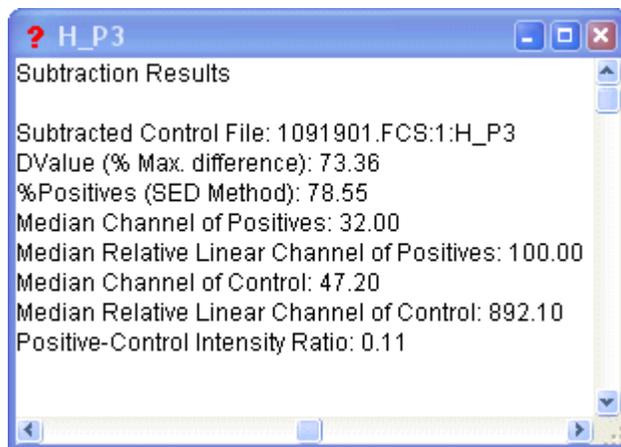
#### OK

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 Channel of Positives

This field displays the statistical median for the positive distribution computed using the SED method. It is in histogram channel units.

### Median Relative Linear Channel of Positives

This field displays the median for the positive distribution in linearized units.

### Median Channel of Control

This field displays the statistical median for the control histogram. It is in histogram channel units.

### Median Relative Linear Channel of Control

This field displays the median for the control histogram in linearized 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.

## Edit Regions

### Regions

WinList can create a number of different types of regions, each created in a different way 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's label, and then pressing the Delete key on the keyboard. You can also delete regions (except for NStat regions) by dragging the region's label off of 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 titlebar 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 titlebar 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 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 tool **Contour** tool. Click and hold the mouse on a point that you want the contour region to include. As you move the mouse, a preview of the probability contour region is shown. When the preview shows the contour that you want, release the mouse button to complete the region.

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.

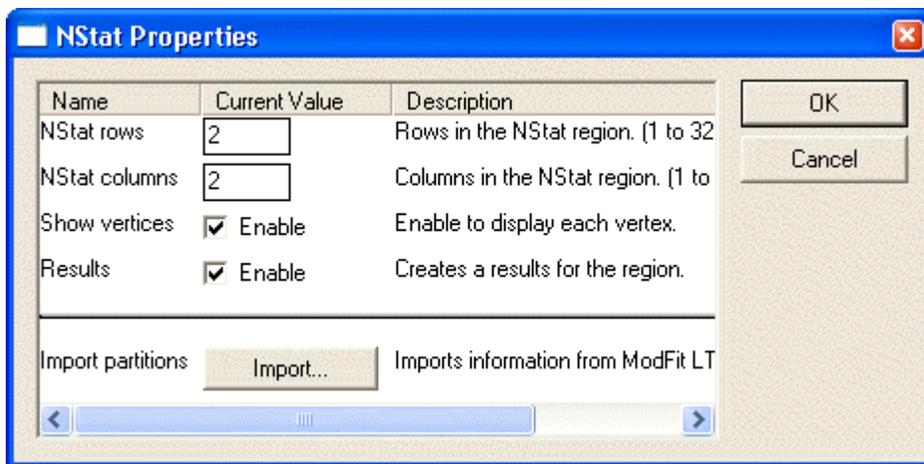
A 2P region can also display all or some of its vertices, allowing you to adjust one or more points in the region. To display the vertices, display the Edit Properties for 2P Region dialog and enable the Show Vertices option. Each vertex point can be moved with the mouse.

2P regions can automatically position themselves relative to the median of the histogram that contains them. The attribute can be enabled in the Edit Properties for 1P 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.

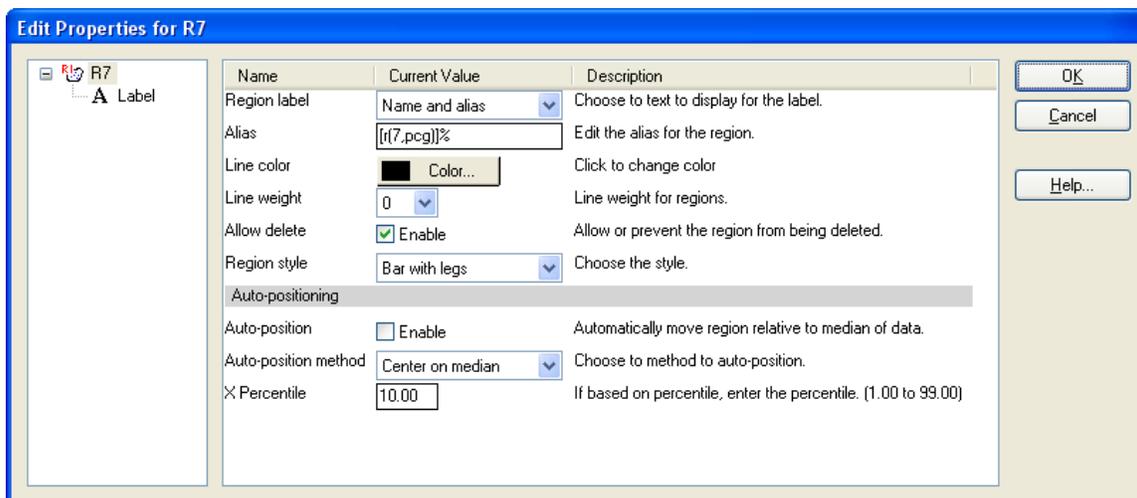
### Region Array Creation

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 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.



**Region label**

This list allows you to choose what to display for the region label. Choose from Name, Alias, or both Name and alias.

**Label**

Choose Label in the tree on the left of the dialog to edit the font, background color, and transparency for the region's label.

**Alias**

This edit box allows you to edit the alias of the region.

**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-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 median* if you want to track the central tendency of the data, *Center on mode* to track the location of highest frequency events, or *Adjust to Percentile* to position the region based on a user-defined frequency.

**X Percentile**

If Auto-Position is enabled and the method is *Adjust to Percentile*, enter the percentile value where the right leg of the region should be positioned. The left leg will be set at the origin.

**OK**

Click this button to accept changes and close the dialog box.

**Cancel**

Click this button to close the dialog box and cancel changes.

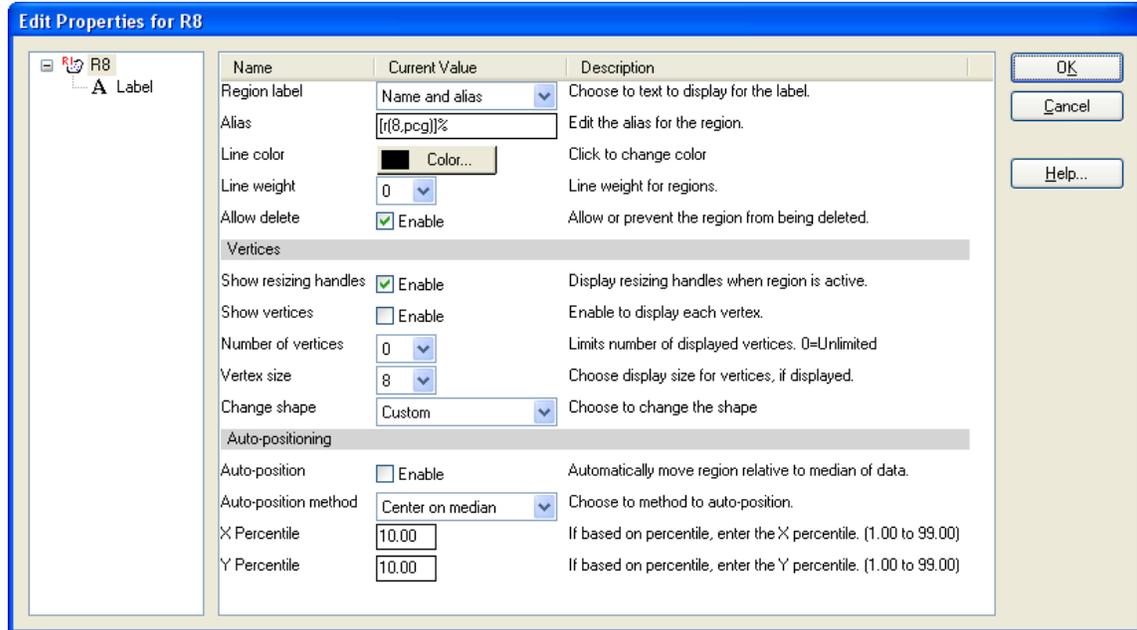
**Help**

Click this button to display on-line help for this dialog.

## Edit Properties for 2P Region

This dialog can be displayed by double-clicking the mouse on the label of a 2P 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.



### Region label

This list allows you to choose what to display for the region label. Choose from Name, Alias, or both Name and alias.

### Label

Choose this item in the tree on the left of the dialog to edit the font, background color, and transparency for the region's label.

### Alias

This edit box allows you to edit the alias of the region.

### 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 use 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's 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 median* if you want to track the central tendency of the data, *Center on mode* to track the location of highest frequency events, or *Adjust to Percentile* to position the region based on a user-defined frequency.

**X Percentile, Y Percentile**

If Auto-Position is enabled and the method is *Adjust to Percentile*, enter the Y percentile value for the top edge of the region, and the X percentile for the right edge. The left and bottom edges will be set at the origin.

**OK**

Click this button to accept changes and close the dialog box.

**Cancel**

Click this button to close the dialog box and cancel changes.

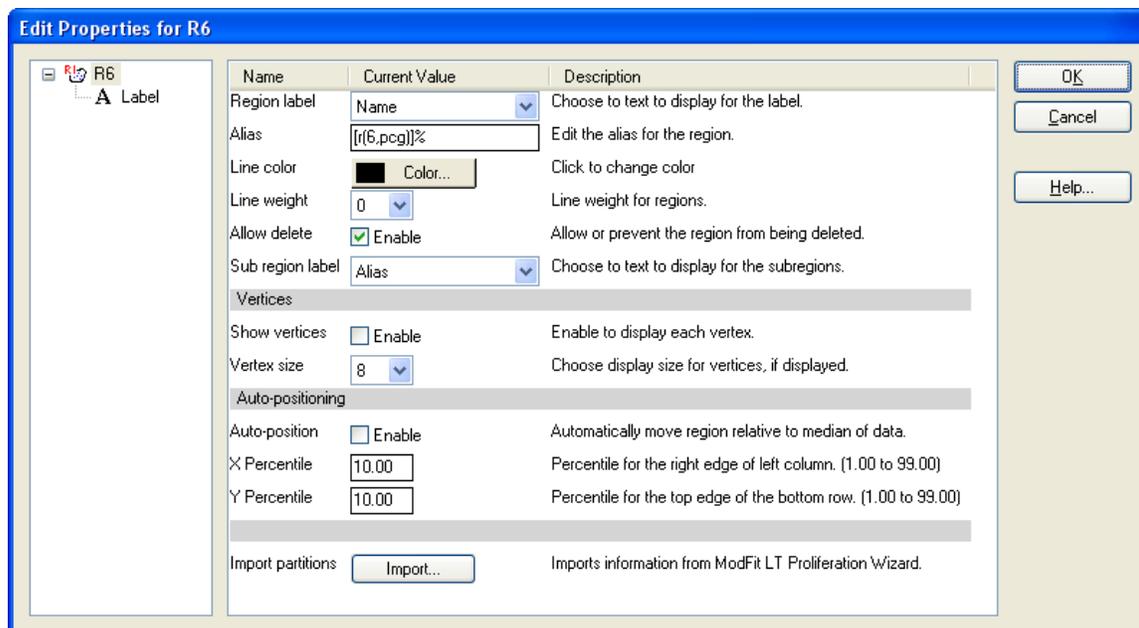
**Help**

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.



### Region 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's label.

### 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 use the Region List dialog box, even if the Allow delete option is disabled.

### 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.

### 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's 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 Percentile, Y Percentile**

If Auto-Position is enabled, enter the X and Y percentile values for positioning the NStat. The leftmost 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's 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.

**Edit Color Mode****Color Modes**

The main toolbar includes a dropdown 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

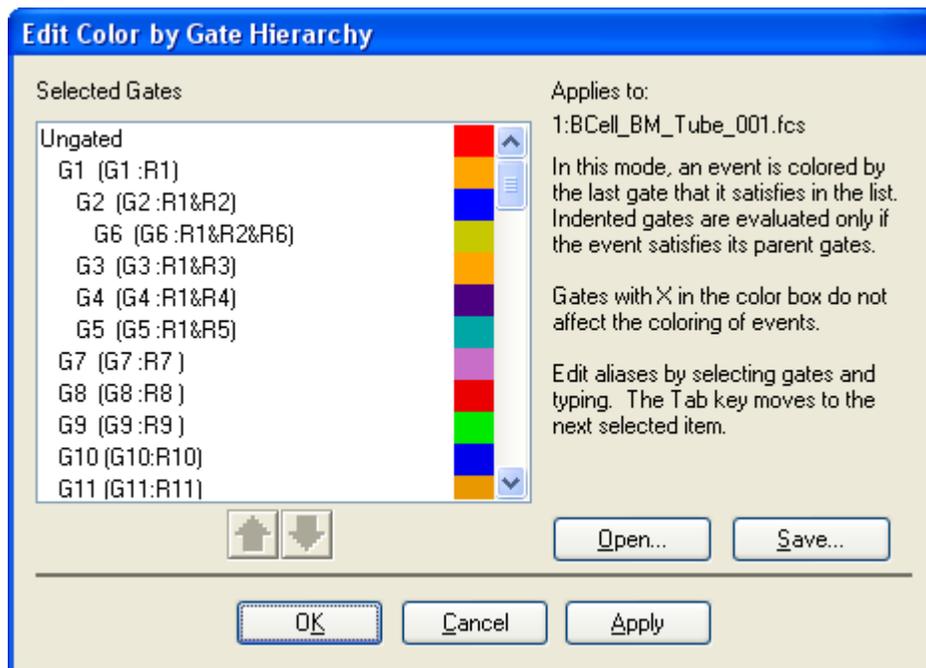
Similar to Color by Gate Hierarchy, this mode *add* colors of the gates that classify the dots. It creates new colors for events that fall into more than one gate. This mode works well when only a few gates are used and there is not much overlap between them. See Edit Color by Gate Combinations for details.

### 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

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.



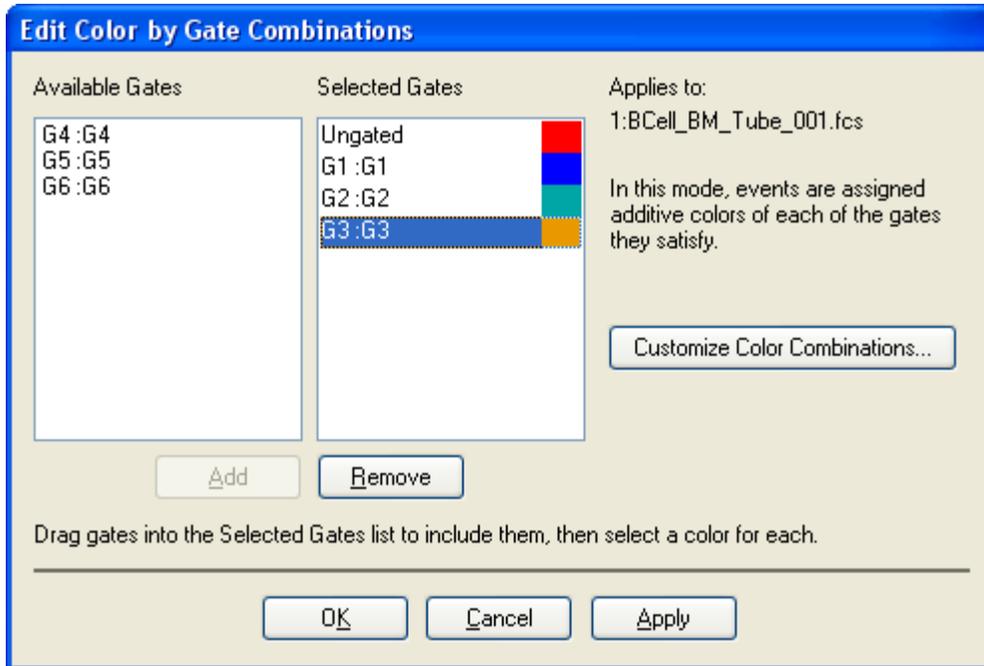
The gate hierarchy is displayed in a list, with a color for each gate shown. Gates can be moved up and down in the hierarchy, within their current level. When a parent gate is moved up or down, all of its descendant gates move with it.

To change the color of a gate, click the color swatch next to it. The palette of colors is displayed, including an "X". Selected "X" will disable coloring for that gate.

Use the Save and Open buttons to store and recall customized color sets.

## Edit Color by Gate Combinations

Similar to Color by Gate Hierarchy, this mode *add* 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

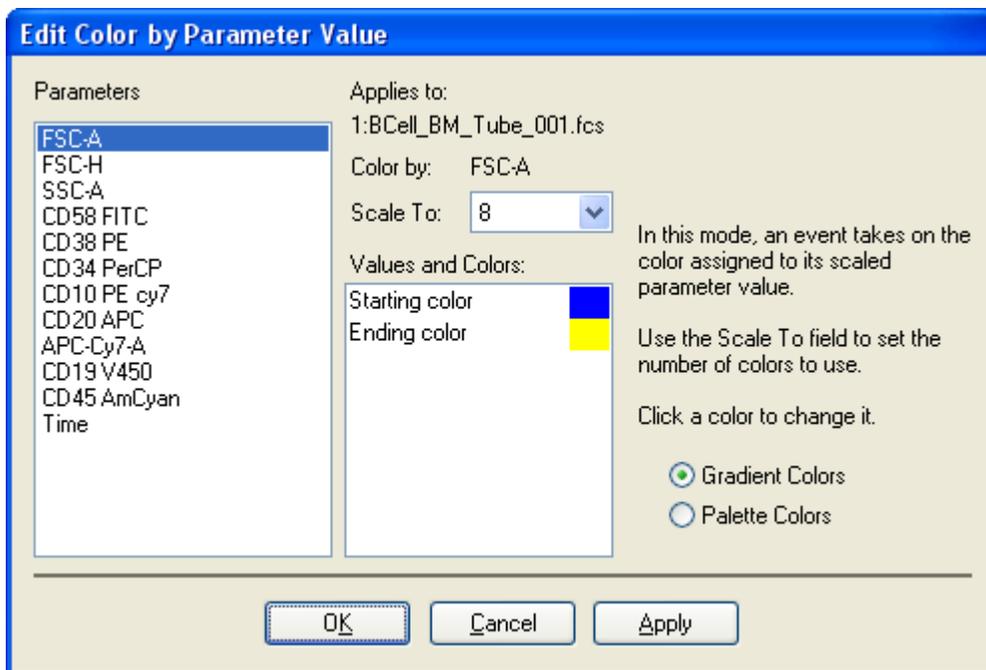


Active gates appear in the Available Gate list. They can be included for coloring by using the Add button. Once a gate is added, click the color swatch next to the gate to choose color for it.

The Customize Color Combinations button allows further control over the color used for overlapping gates.

## Edit 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



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 **Scale To** control.

Colors can be change by clicking the color swatch for an entry in the **Values and Colors** list.

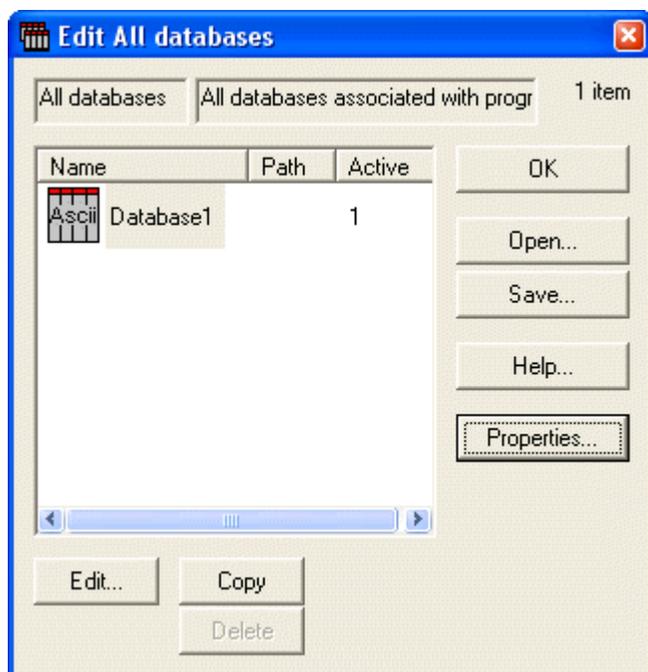
Select **Gradient Colors** to use a 2-color gradient, or **Palette Colors** to select a specific set of colors to use.

## Edit Databases

### Edit Databases

This dialog allows you to add, delete or edit one or more databases.

The Edit Databases dialog can be displayed by choosing **Edit Databases** from 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

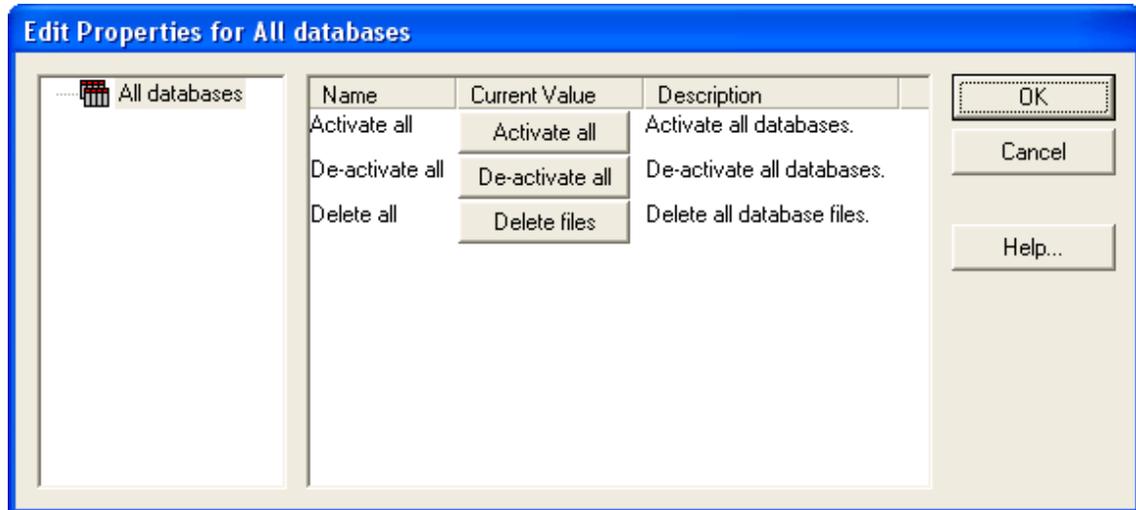
Show this help topic.

#### 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.

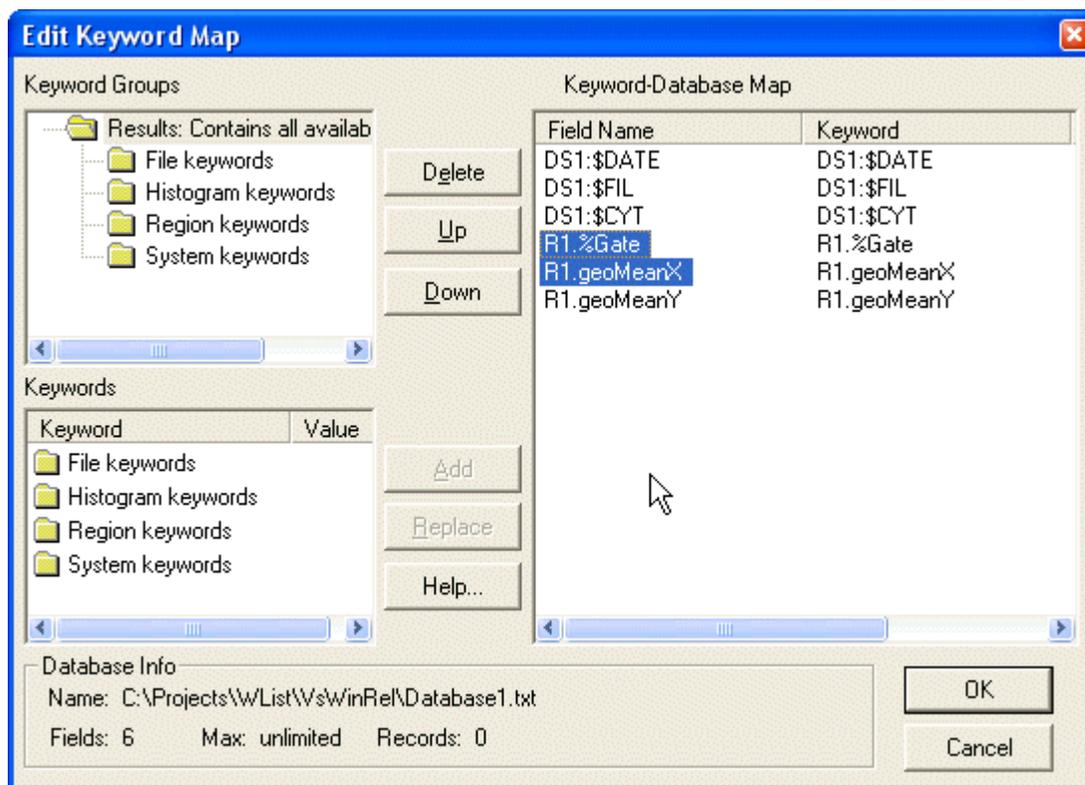
### Help

Show this help topic.

## Edit 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 **Edit Databases** from the **Edit** menu, 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.

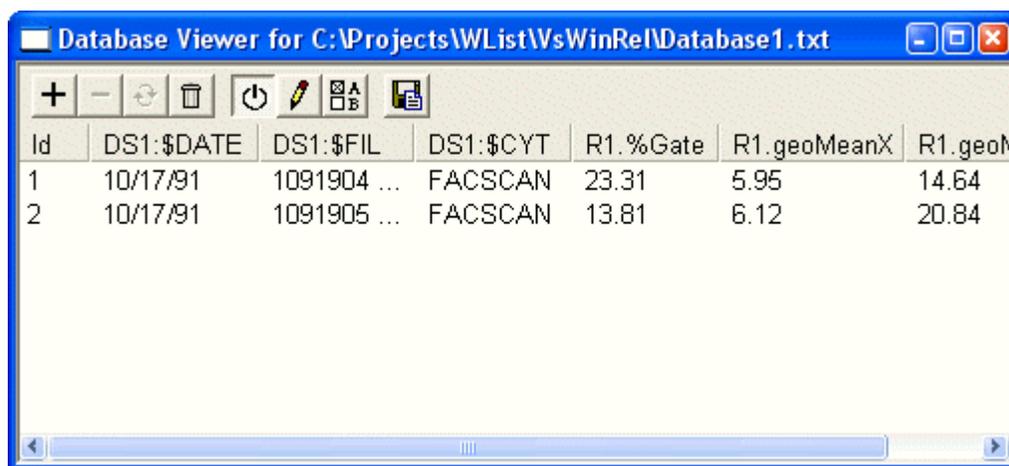
**Help**

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 Databases 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

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.

## Database current results (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.

Pressing Ctrl D on the keyboard is a shortcut for this command.

### View menu

#### View Toolbar

Use this command to display and hide the Toolbar, which includes buttons for some of the common commands in WinList. A check mark appears next to the menu item when the Toolbar is displayed.

Clicking and holding the left mouse button down on a blank portion of the toolbar, then dragging to a new location can move the toolbar.

See also:

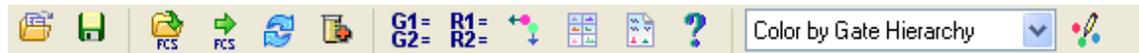
Toolbar

**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.

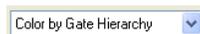
The buttons appearing in the WinList Toolbar are shown below along with an explanation of the functions they perform.



<b>Button</b>	<b>Function</b>
	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. 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. 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.

### View Status Bar

The status bar is displayed across the bottom of the application window. It displays prompts on the leftmost pane, and information about WinList objects in other panes. Below is a table describing each pane of the status bar.

<b>Pane</b>	<b>Meaning</b>
Leftmost	Information about the current menu selection or operation.
1	Name of the current protocol
2	Name and ID of the active data source
3	File index and size of the batch, if batch processing
4	Number of events in the active data source
5	Name and ID of the active histogram
6	Name of the active results window

To hide or display the Status Bar, choose Status Bar from the View menu.

### Histogram Titles

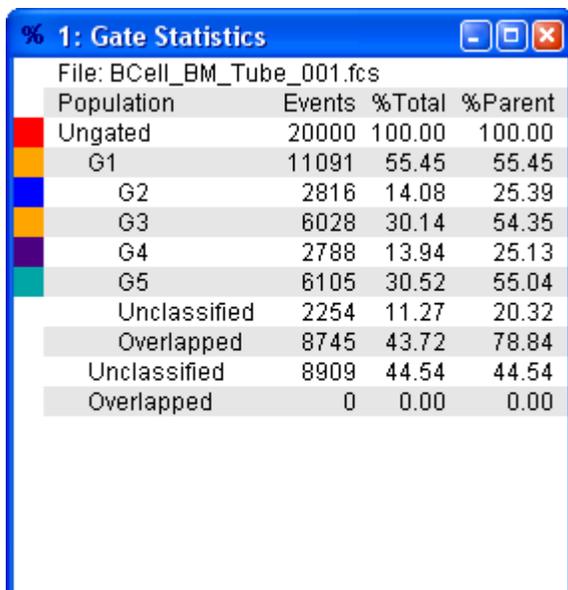
This option is not available on the Mac. Selecting this option toggles histogram title bars off and on. A check appears next to the option if title bars will be shown.

Disabling this option (turning off the display of titles) allows the histogram graphics to occupy more space on the screen. Moving and sizing the histograms becomes a two-step process, however.

**To move or size a histogram without a title bar**, click your left mouse button on one corner of the histogram window, hold the button down and drag that corner to its new location. Release the button. Click on the diagonally opposite corner, drag it and release to complete the moving-sizing operation.

### 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 screenshot shows a window titled "% 1: Gate Statistics" with a blue title bar. Below the title bar, the file path "File: BCell\_BM\_Tube\_001.fcs" is displayed. The main content is a table with four columns: Population, Events, %Total, and %Parent. The rows represent different gates and their sub-populations, each with a small colored square to its left.

Population	Events	%Total	%Parent
Ungated	20000	100.00	100.00
G1	11091	55.45	55.45
G2	2816	14.08	25.39
G3	6028	30.14	54.35
G4	2788	13.94	25.13
G5	6105	30.52	55.04
Unclassified	2254	11.27	20.32
Overlapped	8745	43.72	78.84
Unclassified	8909	44.54	44.54
Overlapped	0	0.00	0.00

The window can be displayed by right-clicking the data source and selecting Gate Statistics from the Data Source Context menu .

### Inverse Palette

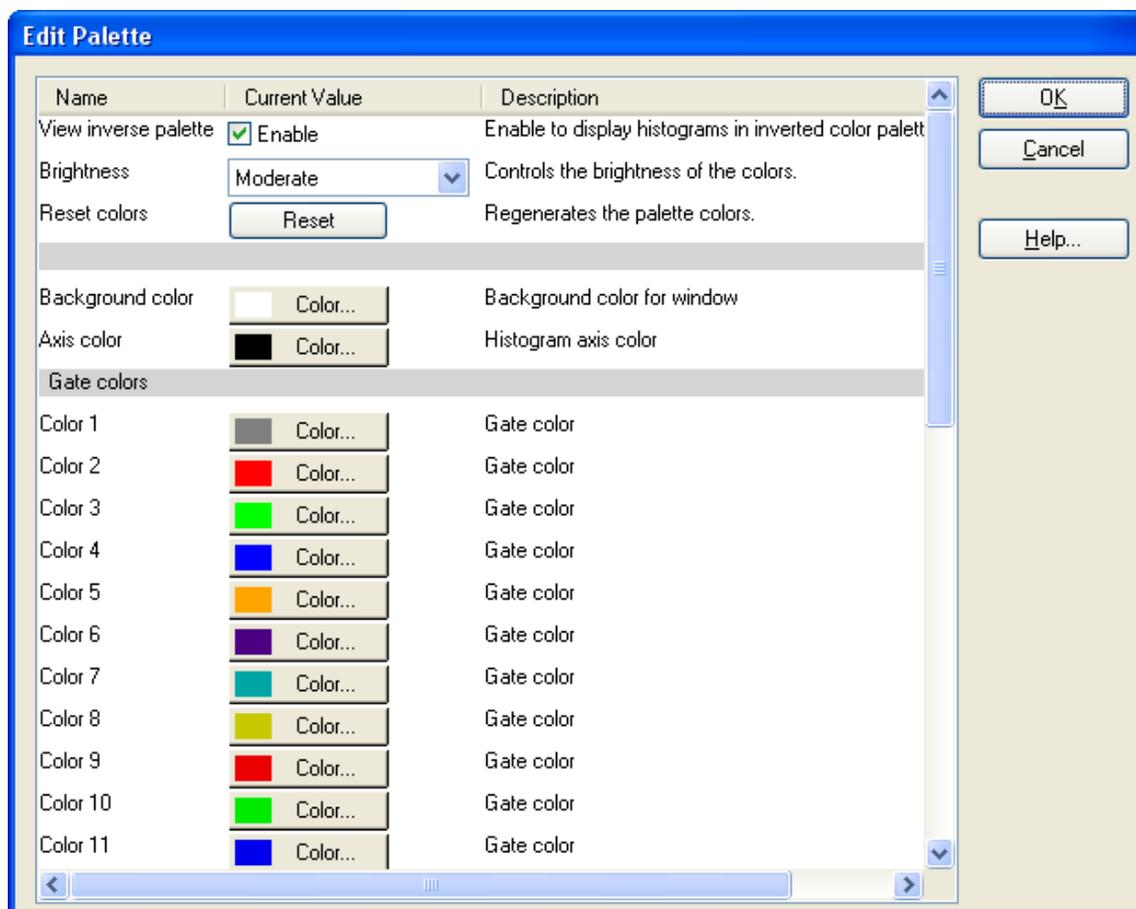
Inverse Palette is a great visualization aid. When you select this command, histograms and result windows switch the foreground and background colors – effectively inverting the color palette. You can quickly switch between white and black background, which is often used to enhance the small, outlier populations.

Preferences determine whether the inverse palette mode is used by reports, for printing, and when copying to the clipboard.

### Options menu

#### Palette

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**

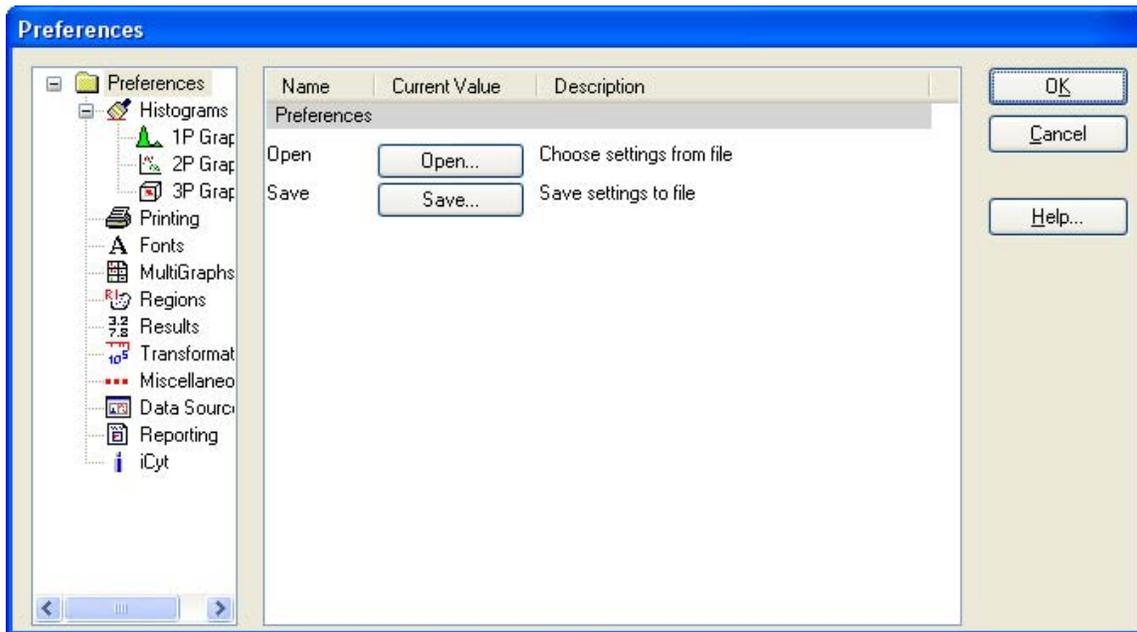
Open a palette file from disk.

**Save**

Save a palette file to disk.

## Preferences

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 to edit its properties. Double-click an item to expand its subcategories.



### Open

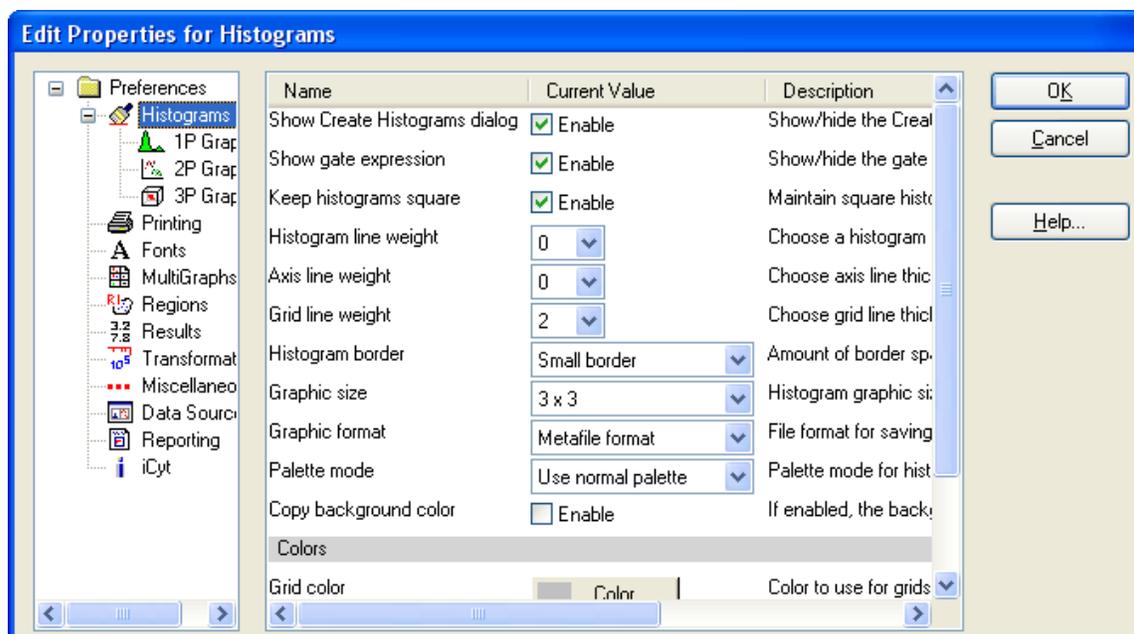
Choose this command to open a WinList preferences file from disk. All of the preferences in the stored file will be read and used to set preferences for the program.

### 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.

### Histogram Preferences

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.

#### 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 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 format

This property determines the file format used when saving graphics to disk. Choose the format that you most commonly use.

**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.

**Colors**

**Grid color**

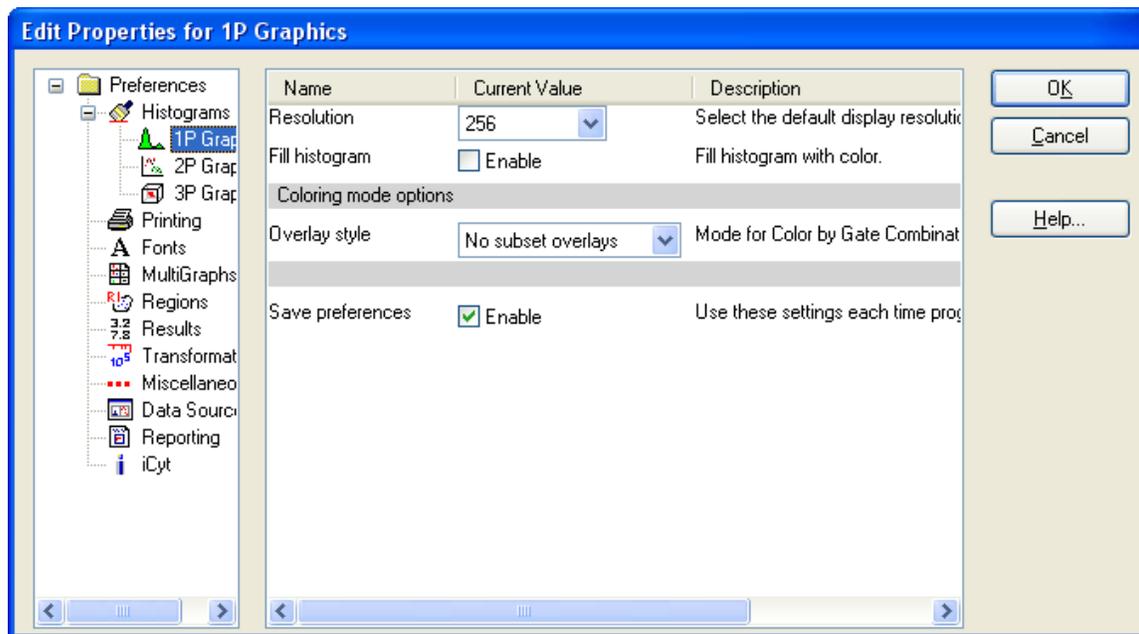
Choose a color from the standard color dialog to use for grids on histograms.

**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**

These preferences apply to 1P histograms. Individual histograms can override these settings.



**Resolution**

Choose a desired histogram resolution for 1P histograms. If the chosen value is greater than the resolution of the listmode file, the maximum resolution of the listmode file will be used.

**Fill histogram**

When checked, histograms will be filled with color by default. Uncheck the option to display histograms without a fill.

**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.

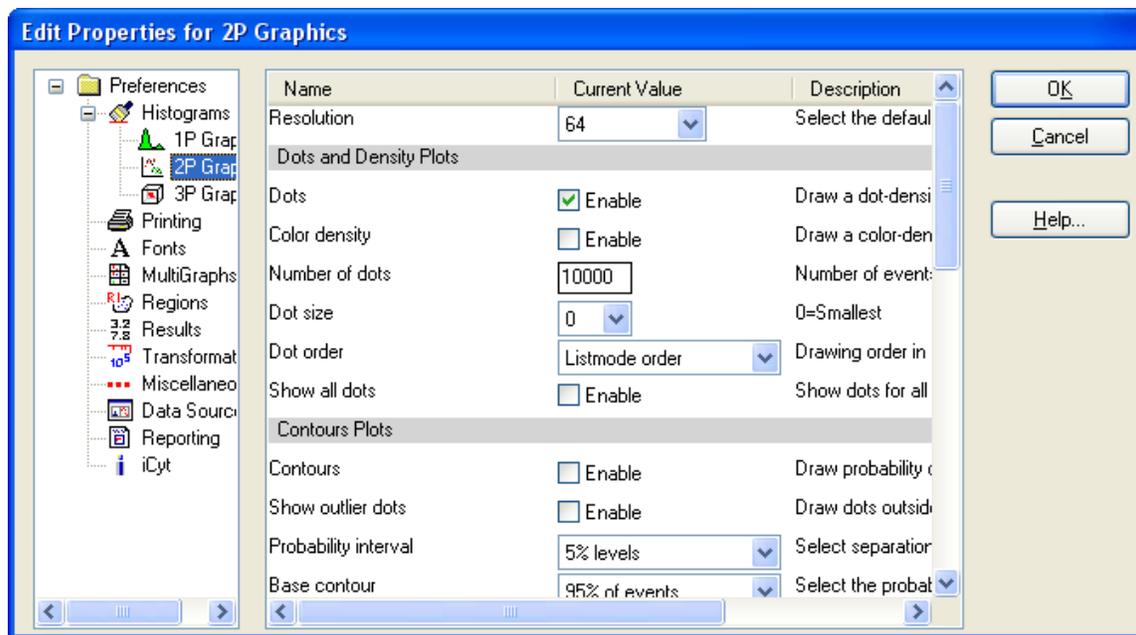
“Stacked color subsets” draws additional polygons, one for each color. The first polygon is drawn from the baseline, and the bottom of the next polygon is the top of the previous polygon.

### 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

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 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*.

#### 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' 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 **Start color** to the first level and gradually changes colors to **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.

#### 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.

#### 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 Rainbo color mode. The default, 5, serves most cases very well.

### Rainbow 1 - n

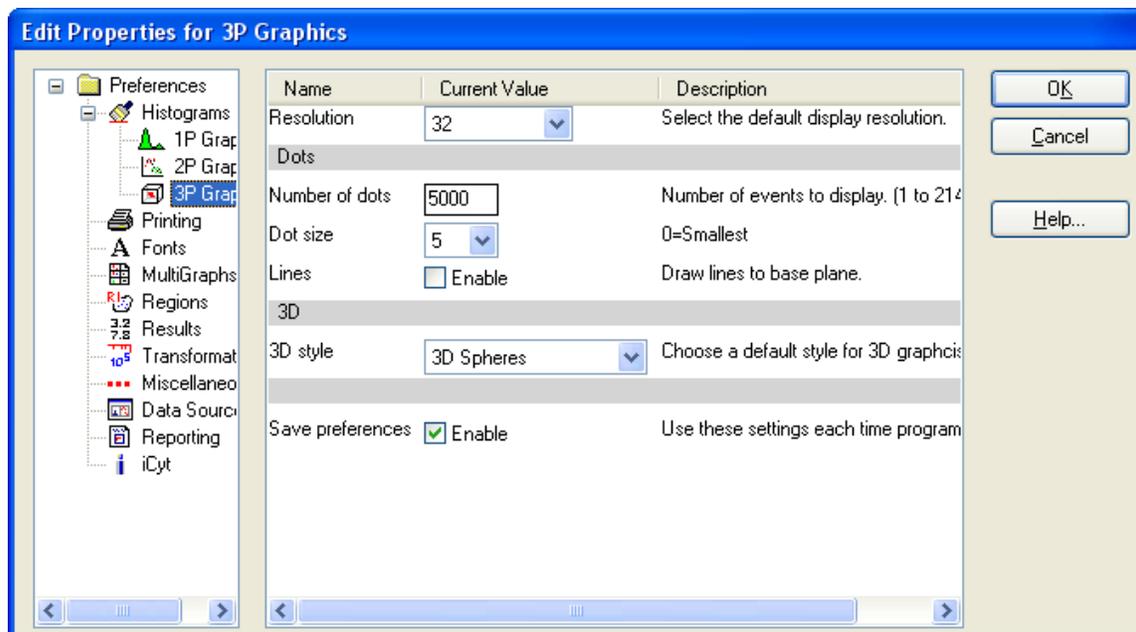
Select a default color for each Rainbow mode level.

### 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

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 style

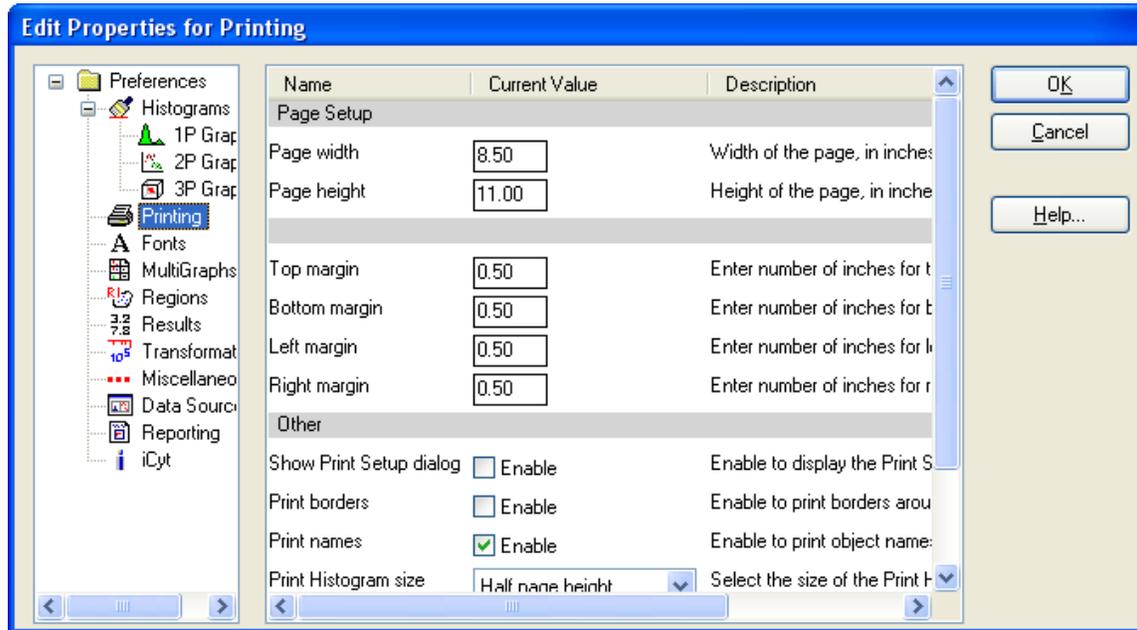
Select the default 3D display.

## 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

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

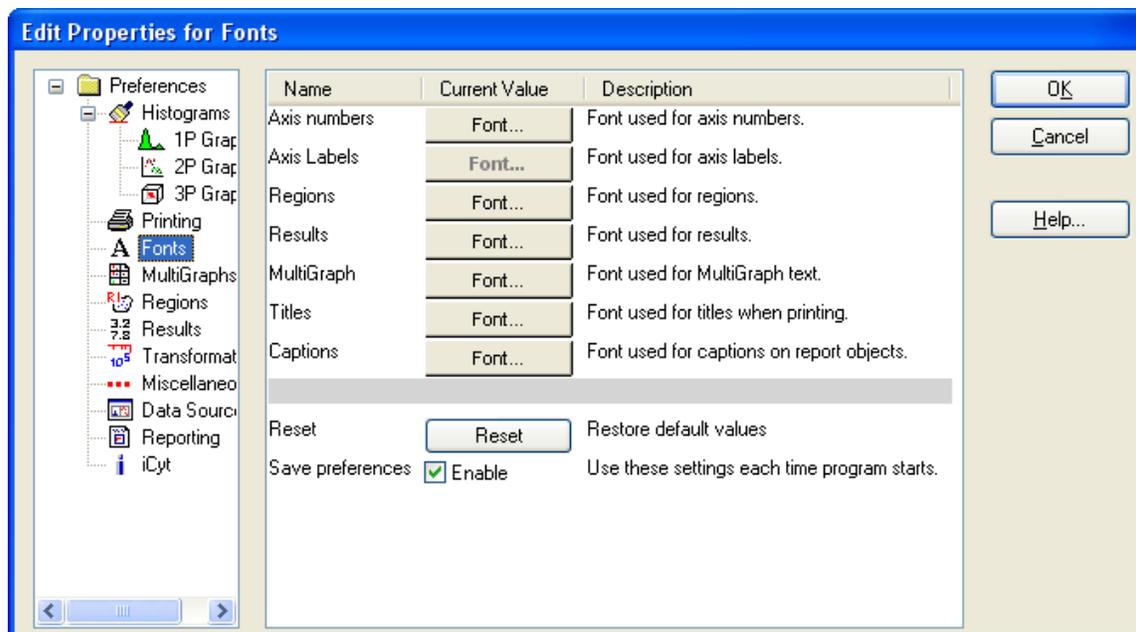
This option selects the page size to use for the Print Histogram command.

### 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

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

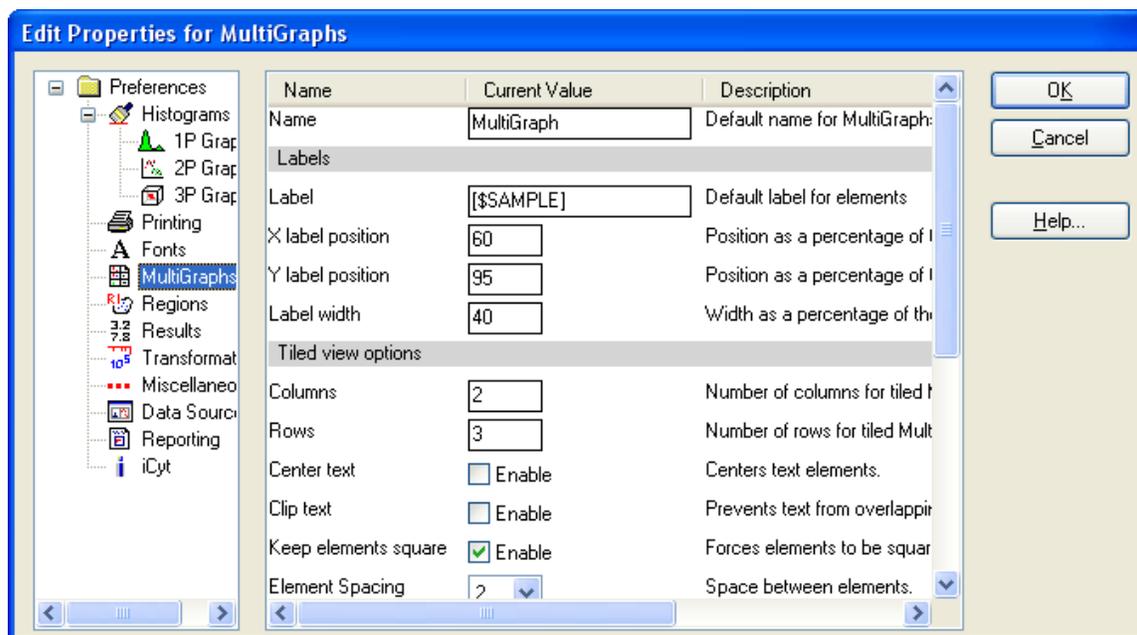
Click this button to reset all fonts to the default state for the program.

## 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

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**

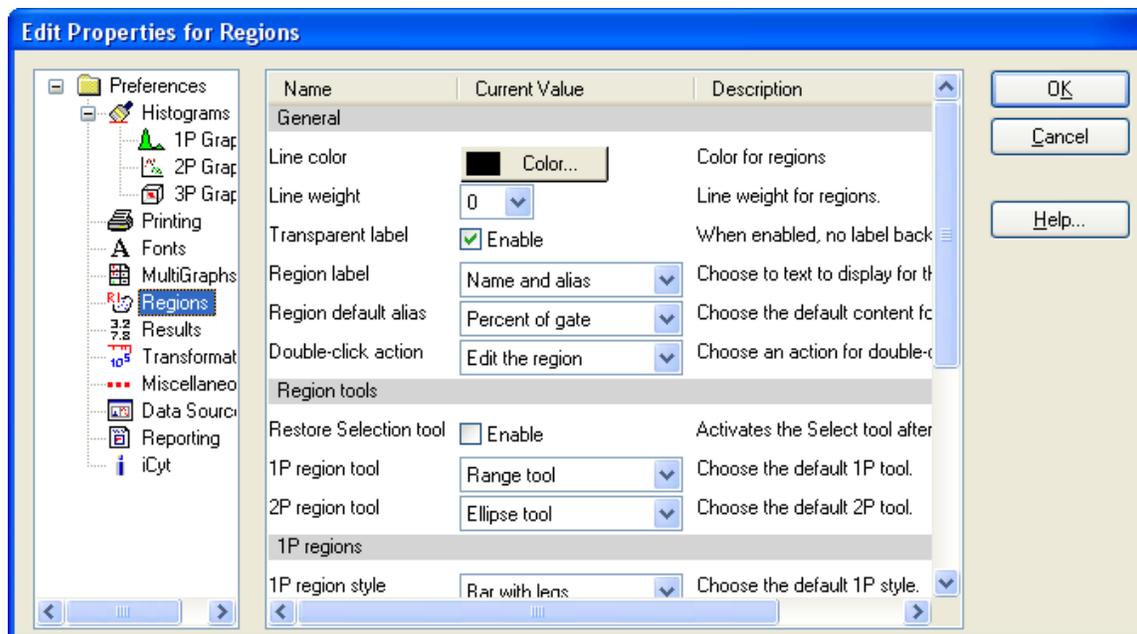
This option allows you to align grids for left-to-right and right-to-left view MultiGraphs. The value is the fraction of the MultiGraph window to offset the grids so that they align with the axes of the elements. Default value is 0.15.

**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**

These preferences apply to regions created by WinList. Individual regions can override these preferences using the Edit Properties for Region dialog.



## General

### 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.

### 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 label

This list allows you to choose what to display for the region label. Choose from Name, Alias, or both Name and alias.

### Region 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. Choose from the options in the list.

### 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.

## 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.

## 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 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.

## NStat Regions

### Sub region label

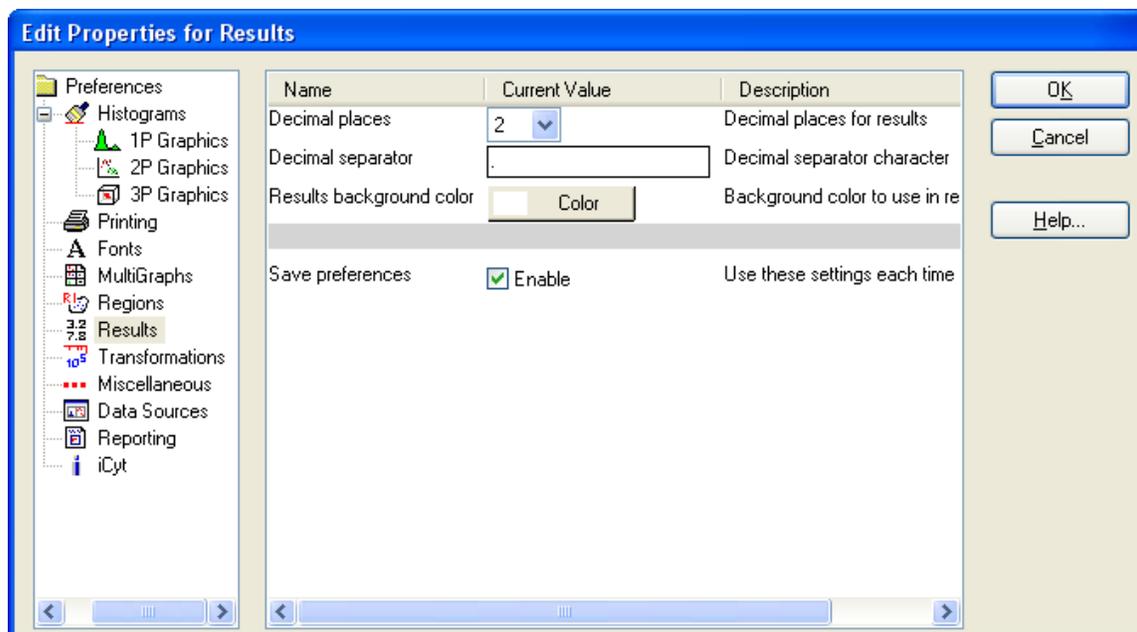
This list allows you to choose what to display for the sub region labels. Choose from Name, Alias, or both Name and alias. The sub region label appears in the corner of the subregion and is not movable.

### 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

These preferences apply to results windows.



**Decimal places**

Choose the number of decimal places you want to display in results windows.

**Decimal separator**

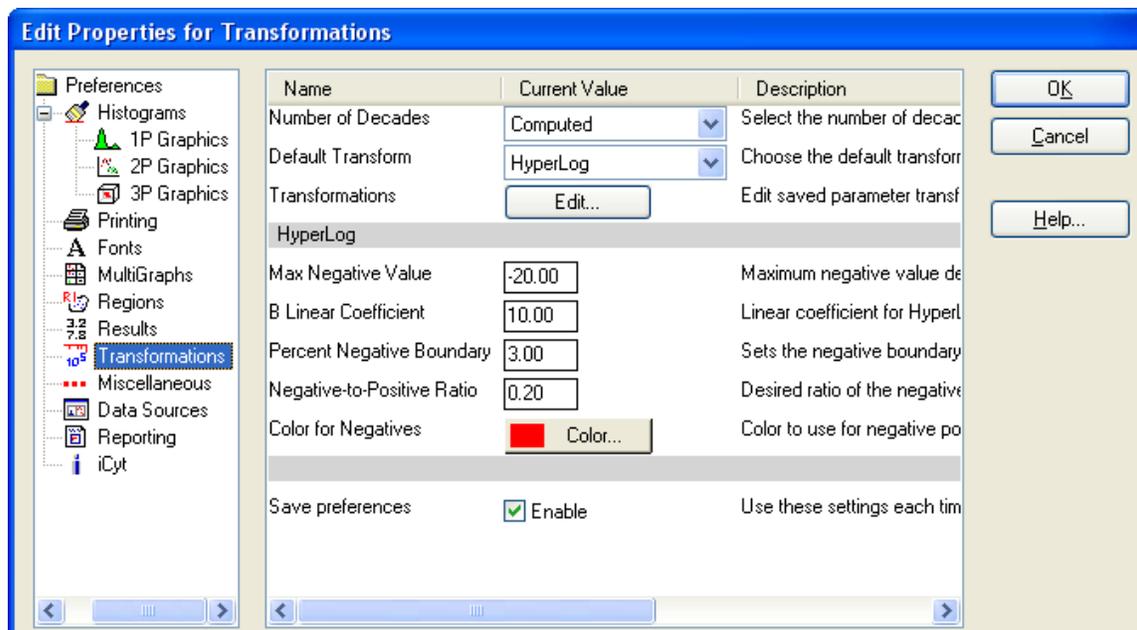
Type the character that you want to use as the decimal separator. The default value is a period (.).

**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**

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 useful for data files from some of the newer, digital instruments, which store all parameters in linear form. At present, there is no standard keyword information to convey which parameters are best viewed on log scales, nor is there information about the number of log decades over which to distribute the data.

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  $\log_{10}(262144)$  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. However, since the linear data contains more than 4-decades

(10,000 linear units) of information, there is a loss of information in this transformation. In the example above, compressing 262144 channels into 4-decades of log space causes the loss of 1.42 decades of information.

### **Default Transform**

This selects the default transform to use for log-like data: Log or HyperLog.

### **Transformation**

This command displays the Edit Transformations dialog to edit transformations for all saved parameters.

### **HyperLog**

#### **Max Negative Value**

This sets the default Max Negative value for HyperLog parameters.

#### **B Linear Coefficient**

This sets the default B Coefficient value for HyperLog parameters.

#### **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.

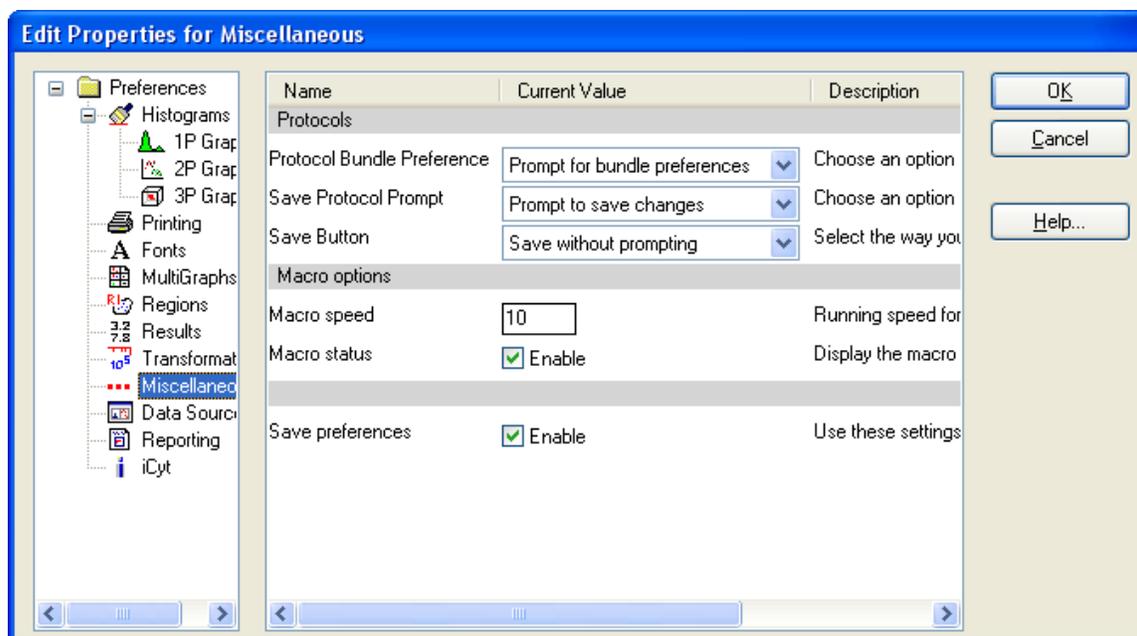
See also:

Linear-to-log

Understanding Hi-res Data Files

#### **Miscellaneous Preferences**

These preferences apply to various WinList objects.



## Protocols

### 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.

### 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 or the Save Protocol As command. Select the behavior for the button with this preference.

## 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

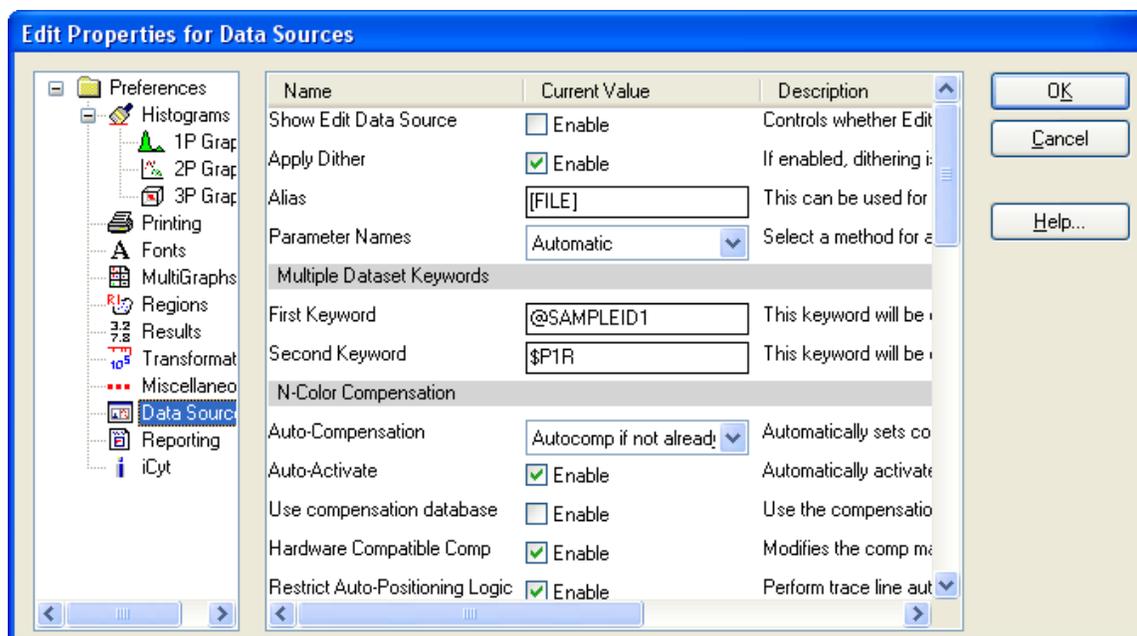
This option controls whether or not the macro status dialog is displayed as a macro is played back.

### 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

These preferences apply to Data Source windows and listmode data files.



#### 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.

#### Multiple Dataset Keywords

##### First Keyword, Second Keyword

These options allow you to choose what keywords you want WinList to evaluate and display in the Choose Dataset dialog box. When the program opens an FCS listmode file that contains more than one dataset, it evaluates these keywords for each dataset and presents the result in the Choose Dataset dialog. The keywords that you enter here should be selected to help you select the dataset that you are interested in opening.

#### 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.

##### Auto-Activate

If an auto-compensation option is enabled (above), you can enable this option to automatically turn on the compensation and apply it when the file is read. If this option is disabled, WinList will read the compensation information from the file, if available, but it will not turn apply the compensation; you will need to Activate the compensation manually.

### **Use compensation database**

Enable this option to use the Compensation Database by default. See Edit Compensation Database for additional details.

### **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.

### **Keyword 1 through 16**

These keywords are used when previewing FCS file keywords in the Open FCS File dialog. You can enter any valid keywords that you want to preview.

### **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**

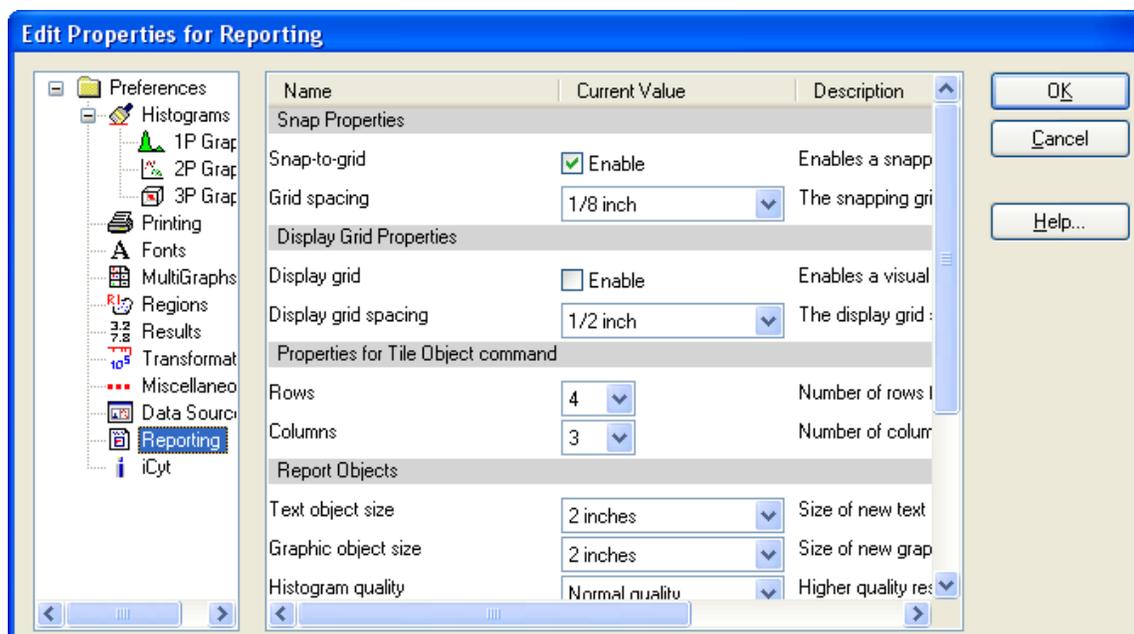
Choose a color for the snapping grid lines displayed in the data source.

#### **Background color**

Choose a color for the background of the data source.

### **Reporting Preferences**

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**

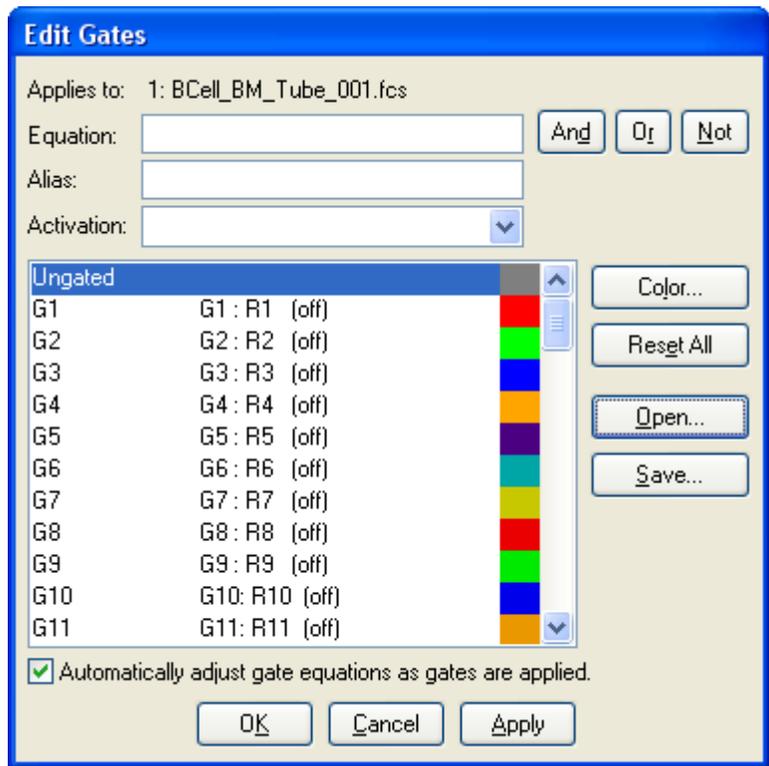
This option causes reports to print faster, at the sacrifice of some quality.

**Caption**

This option determines how captions will be displayed for histograms, by default. Individual histograms on reports can override this preference.

**Edit Gates**

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 listed in this list box. The number of gates is set in the Edit Program Controls dialog box. Click 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.

**Automatically adjust gate equations as gates are applied**

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 histogram window(s).

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 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 color eventing just for this gate. The events that satisfy all these regions will be highlighted by the gate color.

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.

### Compensation Toolbox

This option displays the Compensation Toolbox dialog, equivalent to clicking the Compensation Toolbox button on the WinList toolbar. The Compensation Toolbox allows you to control WinList's interactive N-Color Compensation<sup>™</sup> and normalization systems.

WinList's N-Color Compensation<sup>™</sup> 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 override compensation settings. Compensation is adjusted using 2-parameter dot plots.

In order to set up compensation, you need a set of single-color control files, one for each fluorochrome you want to compensate. Each of these single-color file is loaded into WinList, one at a time. You tell the program which fluorochrome you have loaded using **Trace line** options, and WinList computes the slope and intercept of a line that runs through the median of the data. Once all the single-color files have been processed, you can activate the compensation to apply the settings to multicolor data files.

WinList also supports reading compensation information from FCS files created by many popular cytometers:

Becton Dickinson files created by Diva software

Beckman Coulter files created by FC500, Gallios, and Navios

Attune

Accuri

FCS 3.1 compliant \$SPILLOVER files

See also:

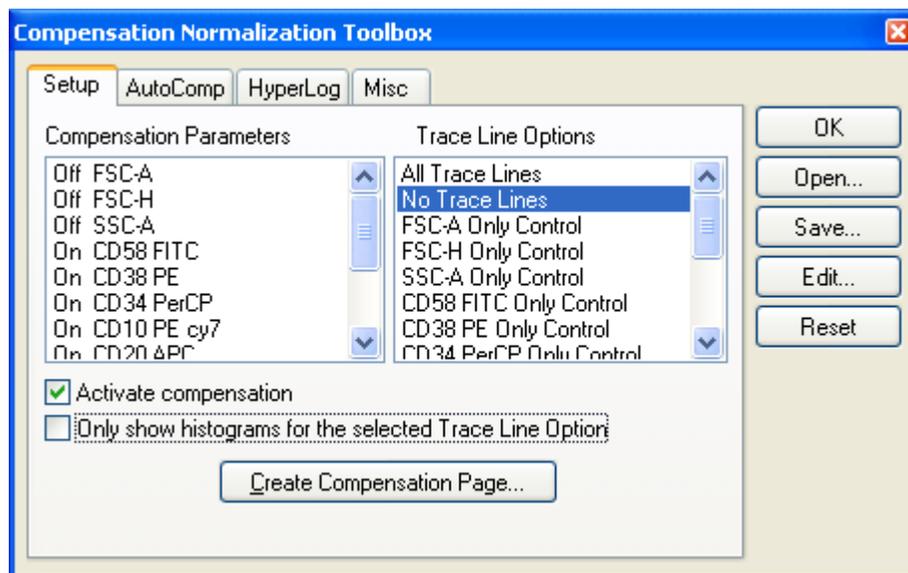
Using Interactive N-Color Compensation™

Compensation Toolbox dialog

[Compensation Trace Lines](#) Compensation\_Trace\_Lines

#### Compensation Toolbox dialog

This dialog is displayed by selecting **Compensation Toolbox** from the Options menu or clicking the  button on the main toolbar. 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: HyperLog 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.

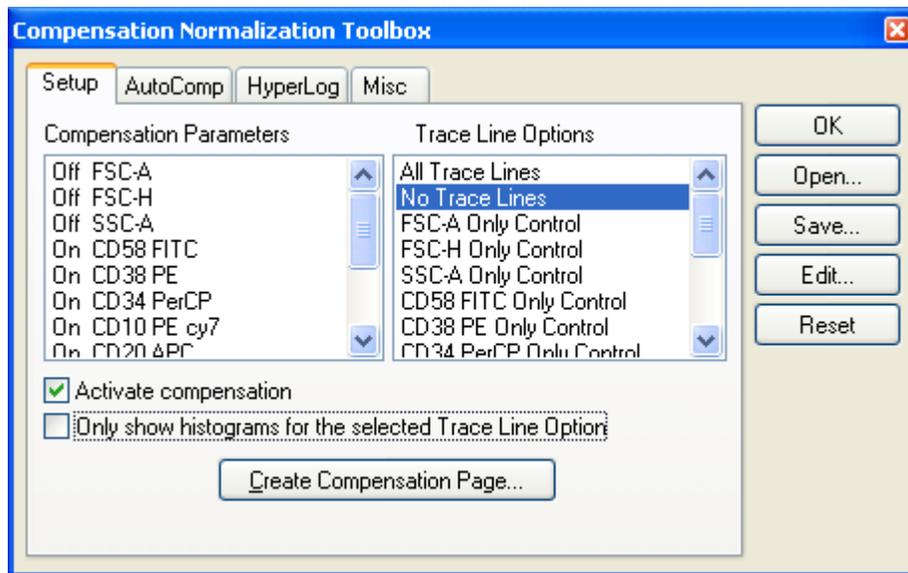
**See also:**

Using Interactive N-Color Compensation™

Compensation Trace Lines

Compensation Tool Box

**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

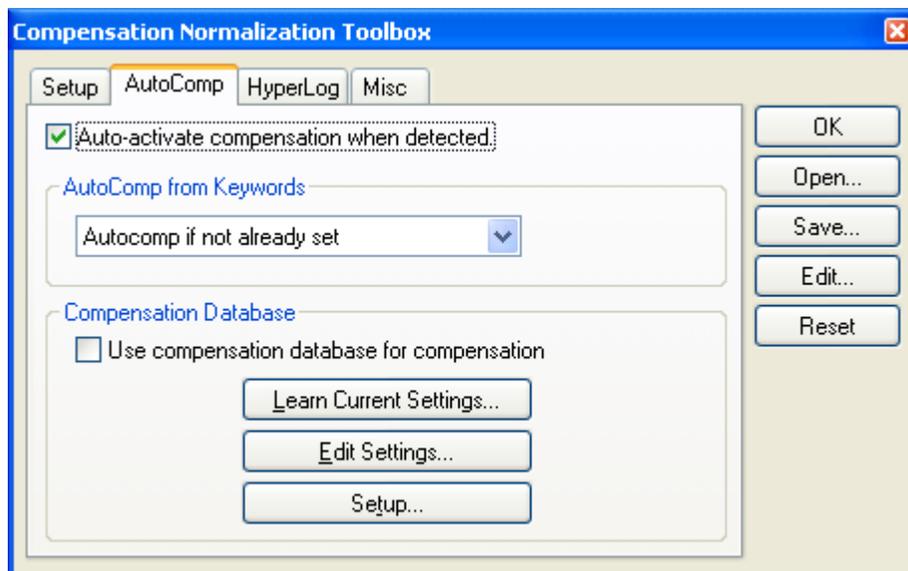
Compensation: AutoComp Tab

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.



#### Auto-activate compensation when detected

When this option is enabled, compensation will automatically be enabled if it is detected either from keywords or from the compensation database.

#### 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.

#### Compensation Database

WinList can create and use a database of compensation settings. This becomes a real efficiency when you have several panels that only differ by one marker. Rather than setting up the full compensation for each panel variant, you can set up compensation for the individual components (e.g. PE vs FITC) and re-use the components in the different panel setups.

#### Use compensation database for compensation

When this option is enabled, compensation settings are extracted from the compensation database.

#### Learn Current Settings

See Using the Compensation Database for details.

#### Edit Settings...

See Edit Compensation Database for details.

#### Setup...

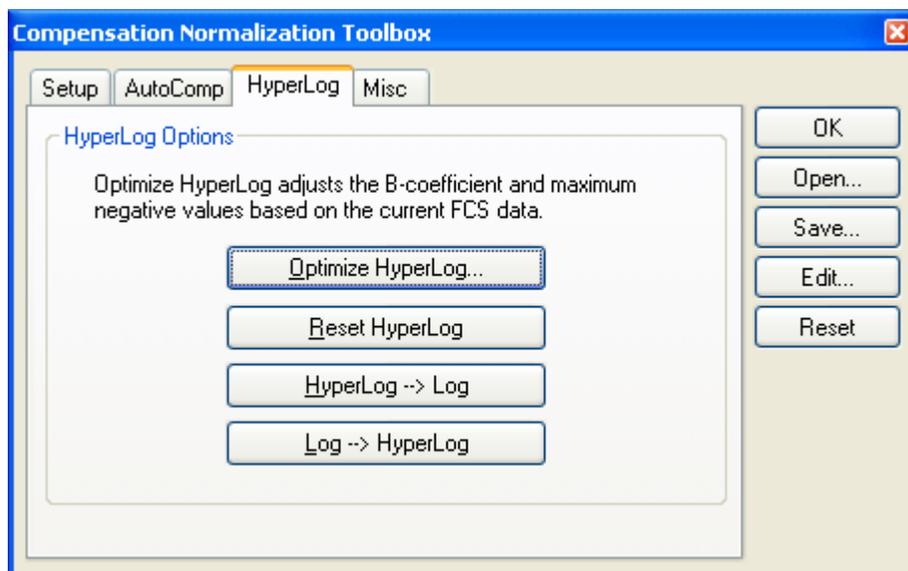
See Setup Compensation Database for details.

#### See also:

Compensation Tool Box  
 Compensation: Setup Tab  
 Compensation: HyperLog Tab  
 Compensation: Miscellaneous Tab

### Compensation: HyperLog Tab

This tab in the Compensation Toolbox offers a number of convenience commands to adjust HyperLog settings and to toggle between HyperLog and Log transformations for all parameters in the data source.



#### Optimize HyperLog

This command triggers automatic adjustment of the HyperLog™ transformations for selected parameters. A dialog is presented to allow you to choose which parameters to optimize. After selected the parameters, click OK to begin the process.

WinList will determine the best B Linear Coefficient and the Max Negative Value for each of the selected parameters.

#### Reset HyperLog

This command resets the HyperLog™ transformations for selected parameters. A dialog is presented to allow you to choose which parameters to optimize. After selected the parameters, click OK to begin the process.

WinList resets the B Linear Coefficient and the Max Negative Value for each of the selected parameters, based on preference settings.

#### HyperLog → Log

This command switches all parameters showing HyperLog™ transformation to log transformations.

#### Log → HyperLog

This command switches all parameters showing log transformations to HyperLog™ transformation.

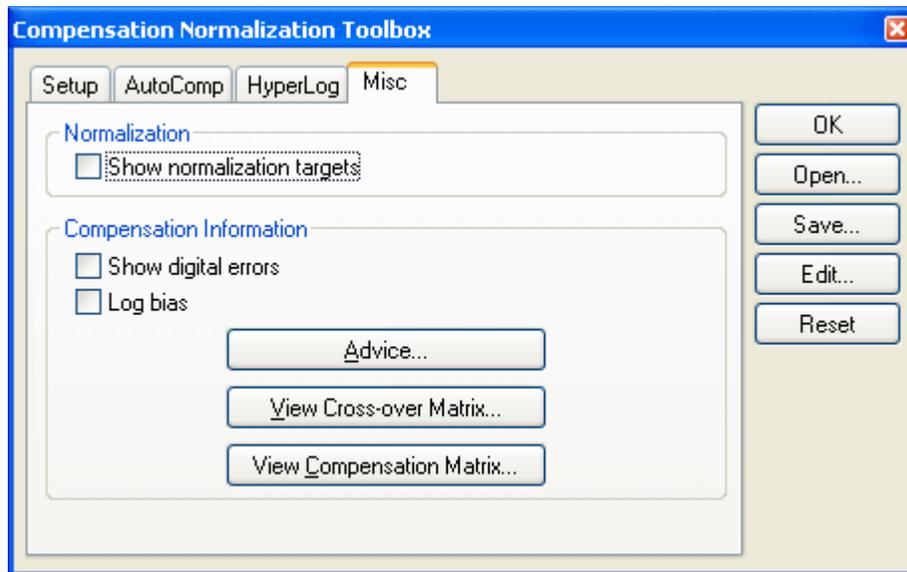
Compensation Tool Box

Compensation: Setup Tab

Compensation: AutoComp Tab

Compensation: Miscellaneous Tab

### Compensation: Miscellaneous Tab

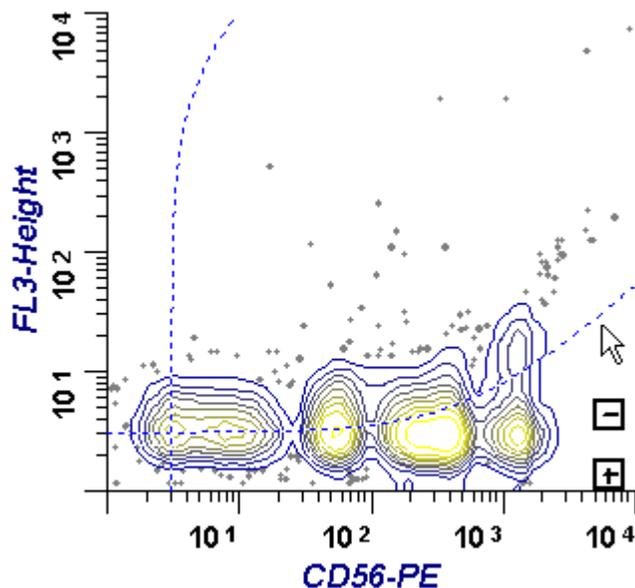


#### Show normalization targets

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.

#### 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. The values can be adjusted for each parameter in the Names and Resolution dialog box accessed through **Edit Data Source**. The default log bias setting is 3 linear channels.

### 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 (aka "spillover") matrix for the current compensation settings. The matrix can be copied to the clipboard for use in other software.

### View Compensation Matrix

This command displays the compensation matrix for the current compensation settings. The matrix can be copied to the clipboard for use in other software.

The compensation matrix is the inverted cross-over matrix. The coefficients in this matrix are used to apply compensation to the data.

Compensation Tool Box

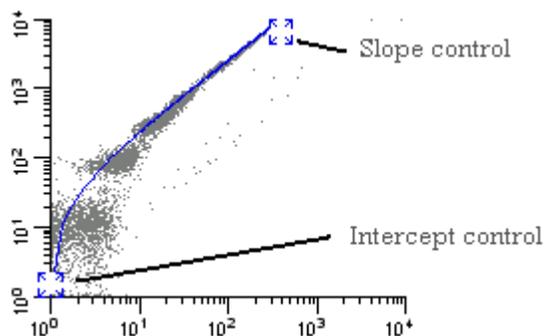
Compensation: Setup Tab

Compensation: AutoComp Tab

Compensation: HyperLog Tab

### 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:

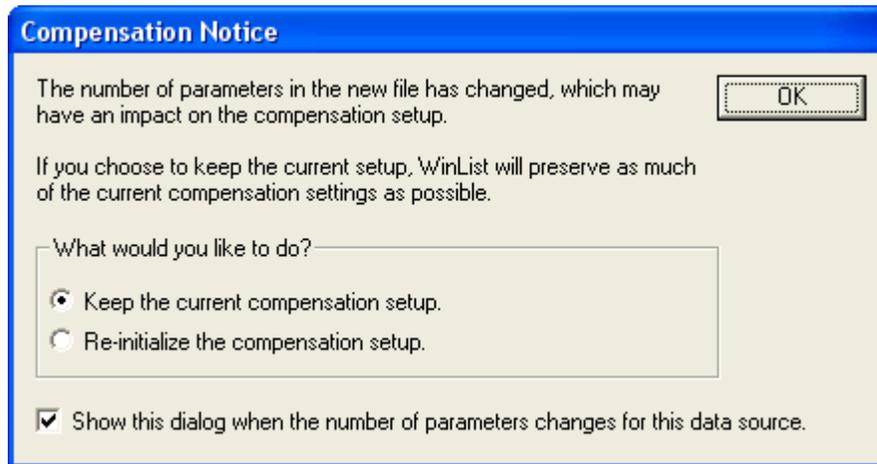
Using Interactive N-Color Compensation™

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

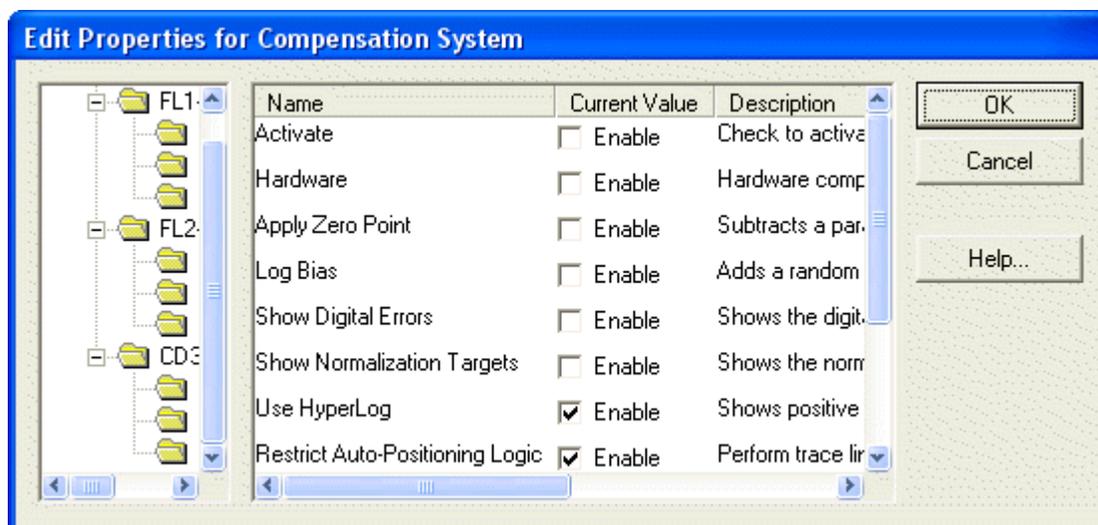
When this option is selected, the system will turn off compensation, turn off the parameters selected for comp, and clear all of the crossover coefficients, normalization settings, and trace line settings.

#### Show this dialog whenever 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 trachelines 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:

$$\begin{aligned} \text{Comp P3} &= +1.000*P3 - 0.010*P4 - 0.000*P5 \\ \text{Comp P4} &= -0.169*P3 + 1.000*P4 - 0.038*P5 \\ \text{Comp P5} &= +0.008*P3 - 0.283*P4 + 1.000*P5 \end{aligned}$$

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:

$$\begin{aligned} \text{Comp P3} &= +1.002*P3 - 0.011*P4 - 0.000*P5 \\ \text{Comp P4} &= -0.171*P3 + 1.013*P4 - 0.039*P5 \\ \text{Comp P5} &= +0.008*P3 - 0.286*P4 + 1.011*P5 \end{aligned}$$

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.

### Apply Zero Point

This option enables Zero Point adjustments for normalization and compensation. See Zero Point Adjustment for a full explanation.

### 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 cross-over, 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.

### Zero Point

When the Zero Point Adjustment option is enabled (see above), this field should be set to the linear channel median of the background fluorescence. See Zero Point Adjustment for a full explanation.

### 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 CD65-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

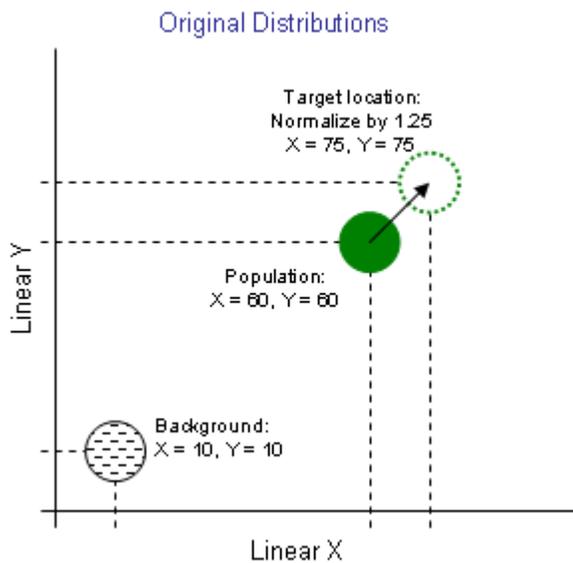
This read-only field displays the value between 0.0 and 1.0 that quantifies the digital error WinList computed for the parameter. Values of 0.85 and higher are relatively good, with little digital error. Values of 0.75 to 0.85 are fair. Values below 0.75 indicate that the parameter measurement contains a significant amount of digital error.

Use the Advice dialog to understand the sources of digital error.

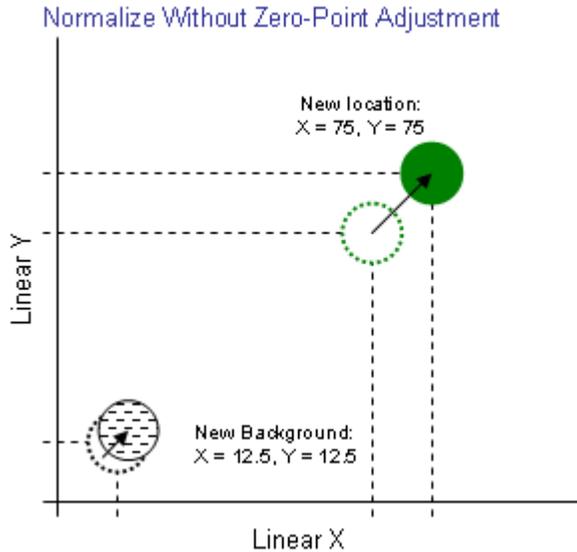
### Zero Point Adjustment

The best way to understand the Zero Point adjustment is to look at an example of its use.

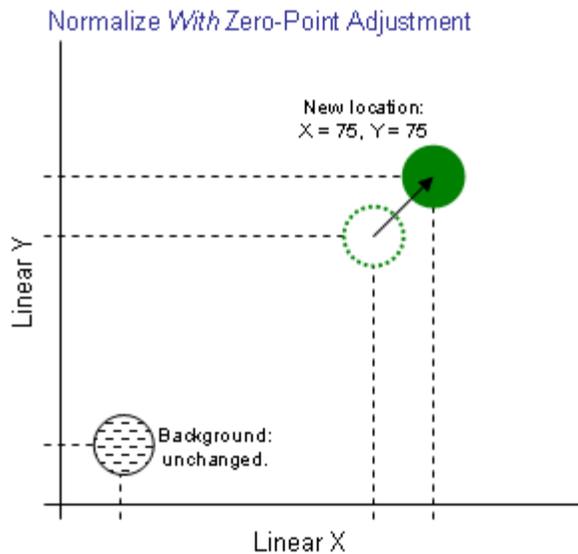
Suppose that we have two populations in linear space: a background population with linear median X and Y at channel 10, and a bright population with linear median X and Y at channel 60. We want to use the Normalization in WinList to center the bright population on  $X=75$ ,  $Y=75$ .



We set the normalization factor for both X and Y parameters to 1.25. This can be done using the Normalization Targets or by typing the values directly into the Edit Properties of Compensation dialog. *Without* the Zero-Point Adjustment, the program simply multiplies the intensity of each event by 1.25. Both the background and the bright population shift positions, as shown below.



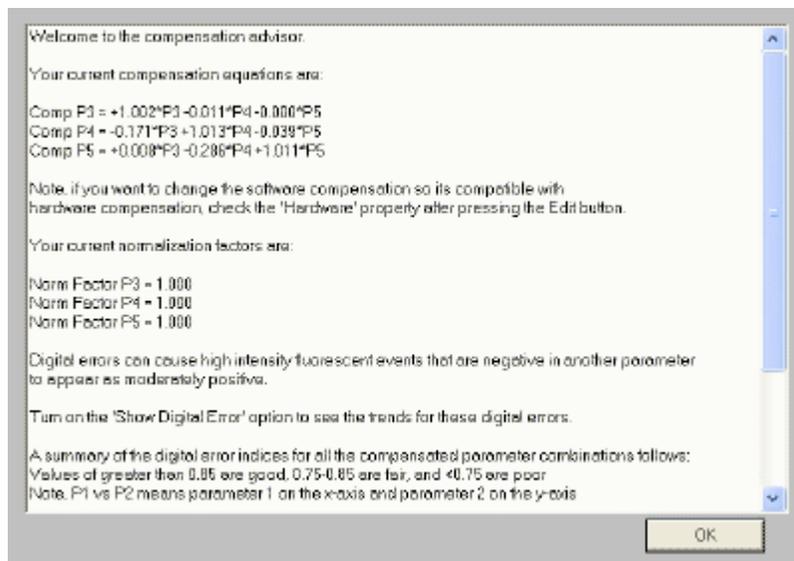
With the Zero-Point Adjustment enabled and set for 10 on both parameters, the background population is unchanged by the normalization process, shown below. Before applying the normalization value to each event, the program first subtracts the Zero Point value. This effectively moves events in the background population to a median position of 0, making them unaffected by the normalization factor. After the normalization factor is applied, the Zero Point value is added back to all events. This moves the background population back to its original position.



The idea is that the background level is constant; we do not want it to be affected by the normalization. To use this feature correctly, start by identifying the median intensities of the background populations using control files. Use those intensities to fill in the Zero Point values for each parameter you want to normalize. Then enable the Zero Point Adjustment settings. When you apply a normalization factor, the background population will be unchanged.

**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

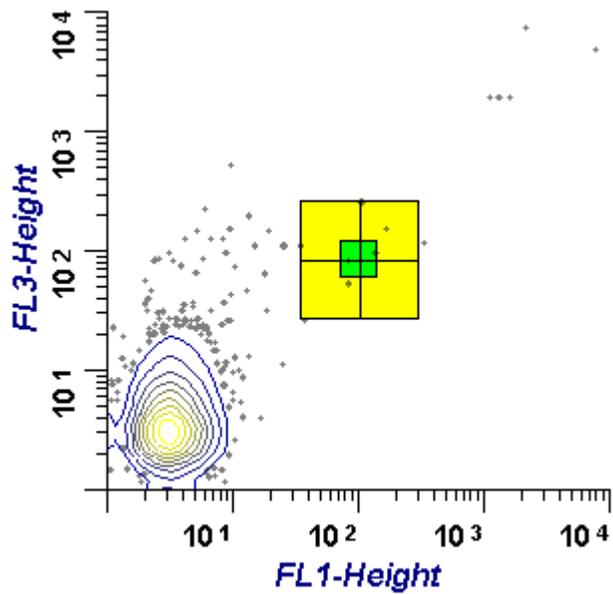
When analog data is converted to an integer, an error is introduced known as digital error. Normally this error is quite modest and presents little problem to cytometrists. However, when data is compensated, positive and negative errors are introduced that increase with parameter intensity. The digital 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

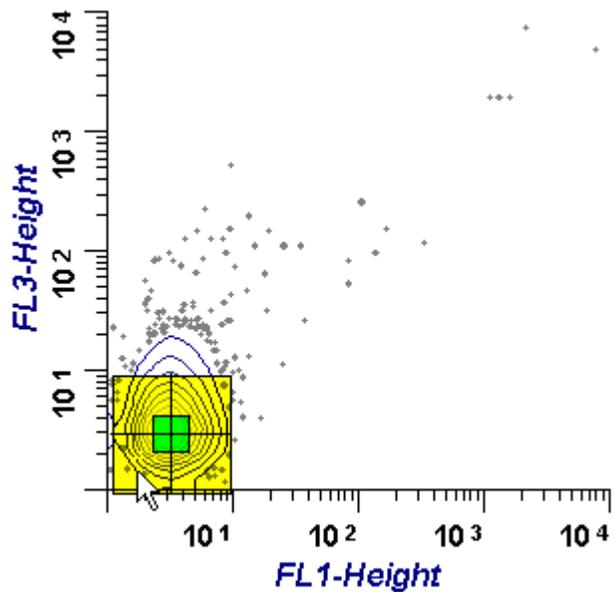
When compensation is **Active** and **Show Normalization Targets** is enabled in the Compensation Toolbox, WinList displays graphical objects to make it easy to set normalization for one or more parameters. The objects appear on 2P histograms.

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.

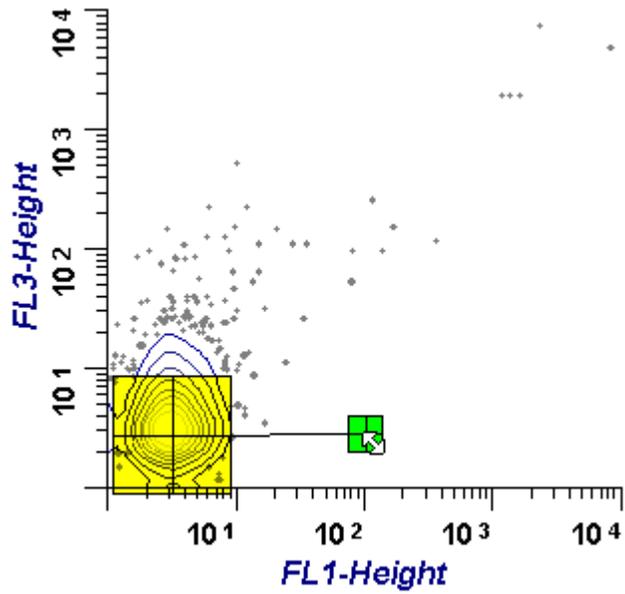
Use normalization with great care, and with a full understanding of what you are doing and why you are doing it. It cannot correct data that has been overcompensated or incorrectly stained. It should not be used to make a weak fluorochrome appear to be a bright fluorochrome.



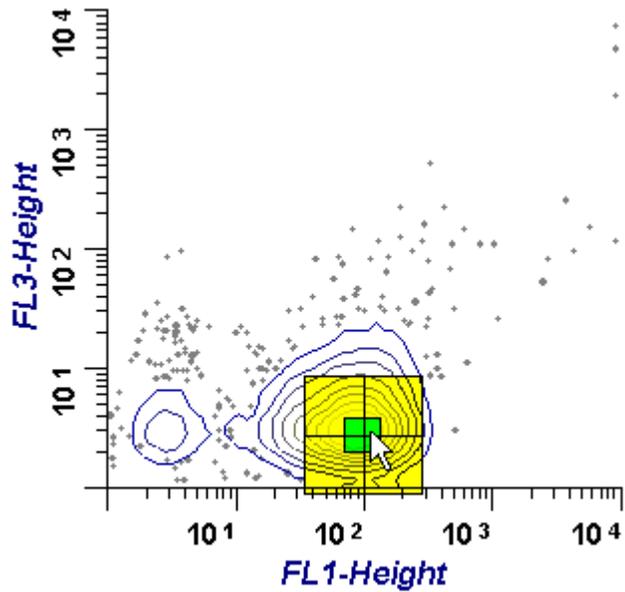
To set normalization, first drag the target object by grabbing the large, yellow outer portion with the mouse. Drag the entire object so that the center crosshairs are located on the center of the population that you want to move.



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.

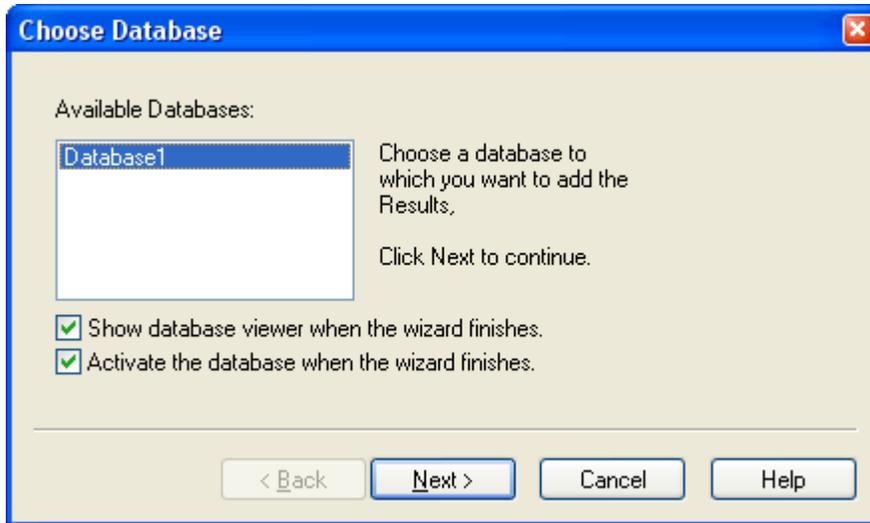


To reset the normalization, use the **Edit** button on the compensation toolbox, and choose the **Init Normalization** option.

### Results Database Wizard

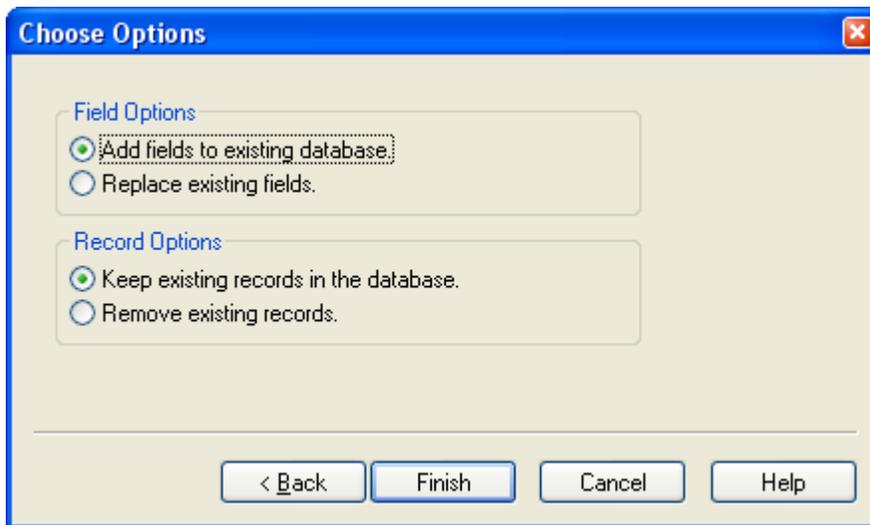
This command simplifies the task of adding the results from a Results window to a database.

To use the command, first activate a Results window by clicking its titlebar. Then, choose the command from the Options menu or the Results window context menu. A wizard dialog appears.



In the first pane in the wizard, select a database for the results. Additional options allow you to display and activate the database when the wizard finishes.

Click Next to display the second 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

### Database Update Wizard

Use this wizard to update a WinList database from an earlier version. Changes were made to the structure of the results that WinList uses to create databases in version 6.0, and this wizard can help fix databases that were created by earlier versions of WinList.

When the wizard it started, it identifies the database that it will attempt to update and suggests that you make backups of the database. Be sure to follow the advice and backup the database before the wizard makes changes, in case you need to go back to the original version.

Click Next to proceed with the wizard.

WinList will evaluate the selected database. When it finishes, a message will be displayed to indicate the result of the update.

### **Add Histogram to Report**

This command 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 adds the active results window to the active report. The results object on the report is located in a default position and size.

### **Add Histogram to MultiGraph (Ctrl A on PC)**

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 Z on PC)**

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 on PC)**

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 on PC)

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

### Replay menu

#### Replay Menu

The Replay menu provides several ways to re-read data into data source windows. You will use these options when you want to do one or more of 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 options from the **Replay** menu.

See also:

Auto-update

Replay Active Histogram

Replay Active Data Source

Replay All Data Sources

### 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 if the region is used in any gates, the gates and histograms using the gates are updated automatically.

When this option is not checked, Auto-update is disabled. You can move regions without triggering the update of results, gates, and histogram. It is useful to use this option when you need to adjust more than one region in a protocol that takes a long time to update.

When the option is changed from disabled (unchecked) to enabled (checked), the program will update any results, gates, and histograms that need updating.

### Replay Active Histogram

This menu option can be selected to replay data for the active histogram in the active data source. WinList displays the active histogram name in the Status bar at the bottom of the WinList window.

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 menu option can be selected to replay data for all histograms in the active data source. It is equivalent to clicking the **Replay** button on WinList's toolbar. WinList displays the active data source name in the Status bar at the bottom of the WinList window.

When this option is selected, any active databases will attempt to save a record. See *Databasing with WinList* for more.

### Replay All Data Sources

This menu option can be selected to replay data for all histograms in all data sources on the desktop. It is equivalent to holding down the Shift key and clicking the **Replay** button on WinList's toolbar.

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).

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" for the list.

## Macro menu

### Record

To begin recording a macro, select the Record menu entry. A check mark will appear next to the left of the menu entry indicating that WinList is recording. When you are in Record mode, the program will store into a macro file the WinList functions you perform. As each command is stored, the program will issue an audible "beep" to indicate it has stored the command.

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 option, or a text processor.

See also

Edit Macro

Macro Commands

### Run (Ctrl U on PC)

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 executing macros, a *Macro Status* dialog box appears in the lower right portion of the screen. A list of all the recorded macro commands is 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.

At the bottom of the dialog box are three push buttons: **Pause**, **Continue**, and **Cancel**. If the **Pause** button is selected, WinList will interrupt the macro as soon as possible and return control to the application window. At this

point any WinList command can be executed. You can scroll back and forth in the Macro Status list box and select a new starting point for the macro interpreter. When the **Continue** button is selected, the macro will begin running at the newly selected point.

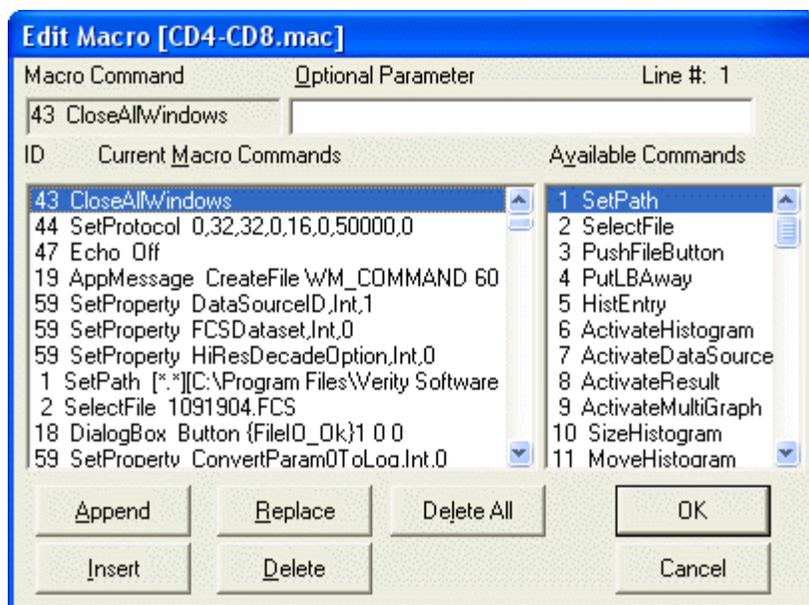
To cancel the macro while it is running, select the Macro Status **Cancel** button. After a few moments, the Macro Status dialog box will disappear and control will be passed to the application window.

### Clear

Selecting the Clear macro command will clear the current macro from memory. It has the same function as the **Delete All** button in the **Edit Macro** dialog box.

### Edit Macro

Choosing this option initiates a dialog box used to edit the currently stored set of macros.



### Macro Command

This field displays the currently selected 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 editable 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

**Open**

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**

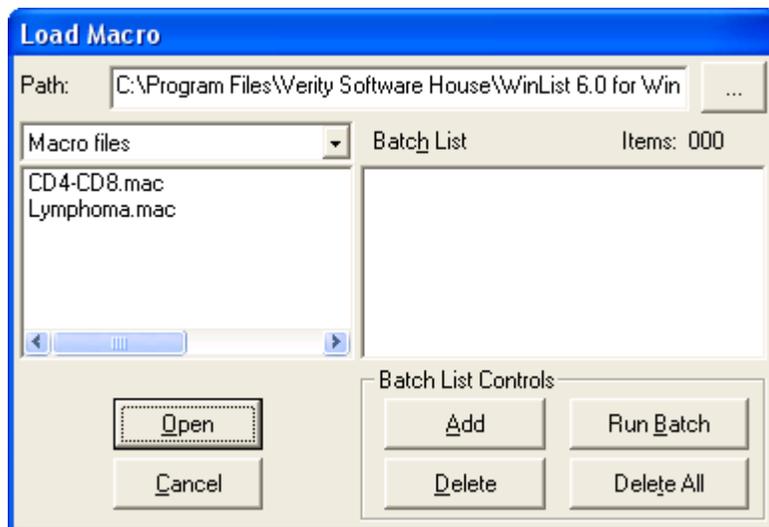
WinList displays the standard file save dialog box, allowing you to navigate to a drive and directory and enter a name for the macro file.

Click the **Save** button to store the macro in the folder on disk.

Click **Cancel** to cancel the dialog.

**Macro Batch**

This menu option is used to select a set of macros that you want WinList to run in sequence.

**Path**

This displays the complete pathway to the directory containing the macro files. To choose a new path, click the "..." button.

**File type drop down list**

This drop down allows you to display macro files, or all files in the chosen directory.

**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 macro 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.

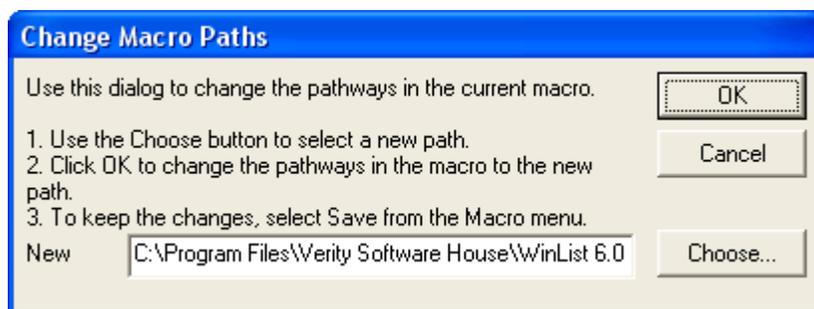
### Change Macro Paths

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.

### 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	<b>MoveHistogram</b>	<b>1 346 4</b>
12	<b>SizeDataSource</b>	<b>1 788 444 0</b>
13	<b>MoveDataSource</b>	<b>1 0 0</b>
14	<b>SizeResults</b>	<b>1 112 111 0</b>
15	<b>MoveResults</b>	<b>1 4 42</b>
16	<b>SizeMultiGraph</b>	<b>1 459 245 0</b>
17	<b>MoveMultiGraph</b>	<b>1 295 230</b>

**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      SetPalettID                      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 at best. 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 [**COUNTERn**], 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, ModifyUserCounter 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.

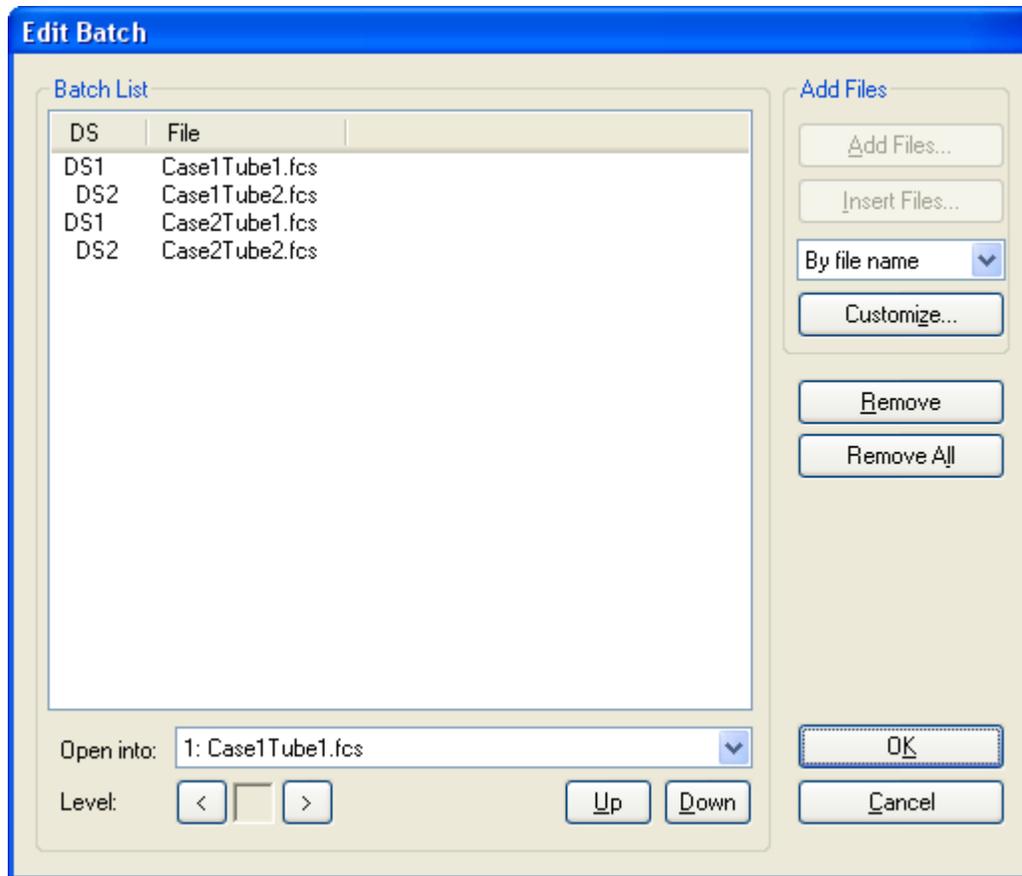
<Condition>: is one of the following conditions: "<","<=","=",">",">=".

<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.



**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 Database            <file path name>**

Command 74 opens a compensation database file for use by WinLists compensation database system.

**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

**Pause Macro**

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.

**Update Remote Links**

*This feature is available only on PC platforms. It is not supported in the Macintosh version.*

This menu option forces WinList to update the remote DDE 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 DDE 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.

**Show Macro Toolbox**

This menu option toggles the display of the Macro Toolbox. The Macro Toolbox is a popup window with programmable buttons on it. The buttons can be "hooked up" to macros stored on disk so that when a button is pushed, the associated macro is run. It is especially useful for running screen setup macros and 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.

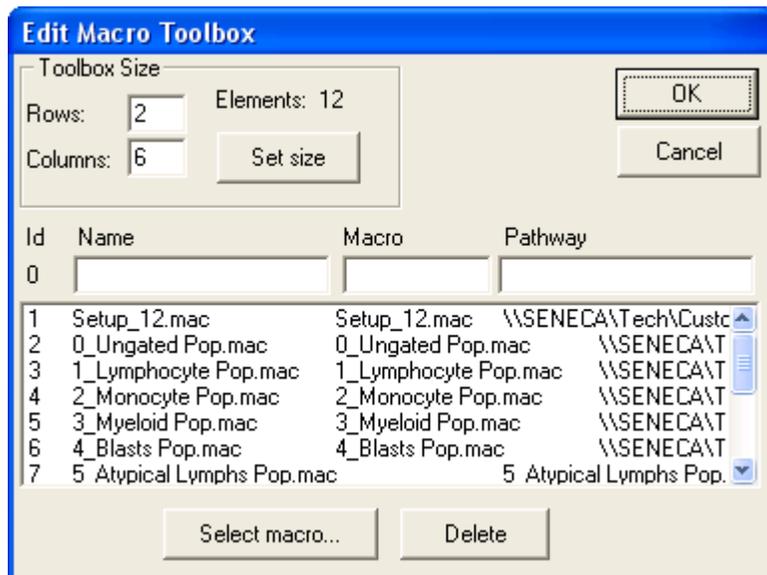
**Edit Macro Toolbox**

Select this menu option 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.

The dialog box and its options are discussed below.



**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**

This field displays the numeric id of the selected macro line. This id appears on the button face in the toolbox.

**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**

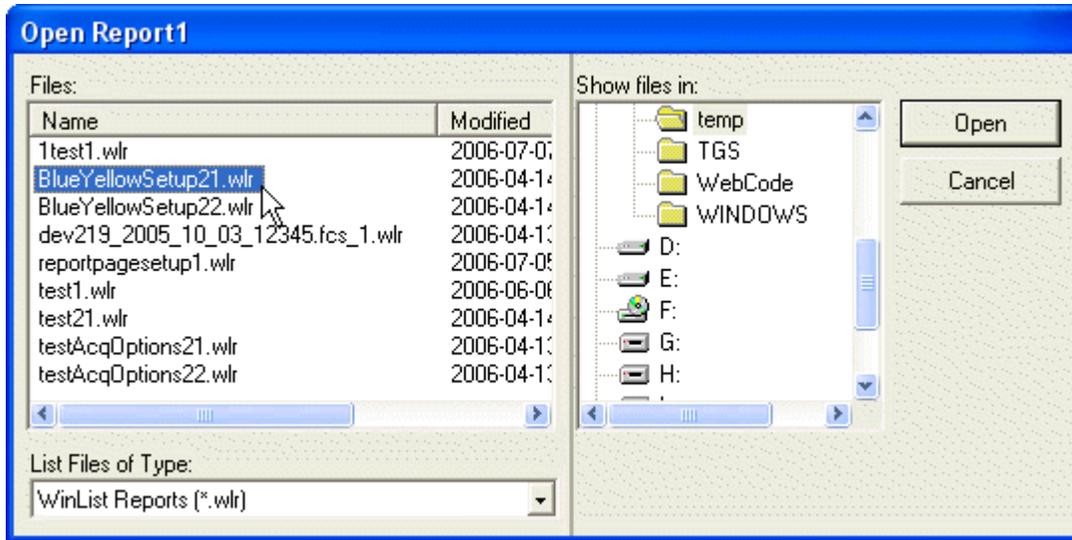
Select this button to exit with no changes made to the Macro Toolbox.

**Report****New Report**

This command creates a new, empty report window.

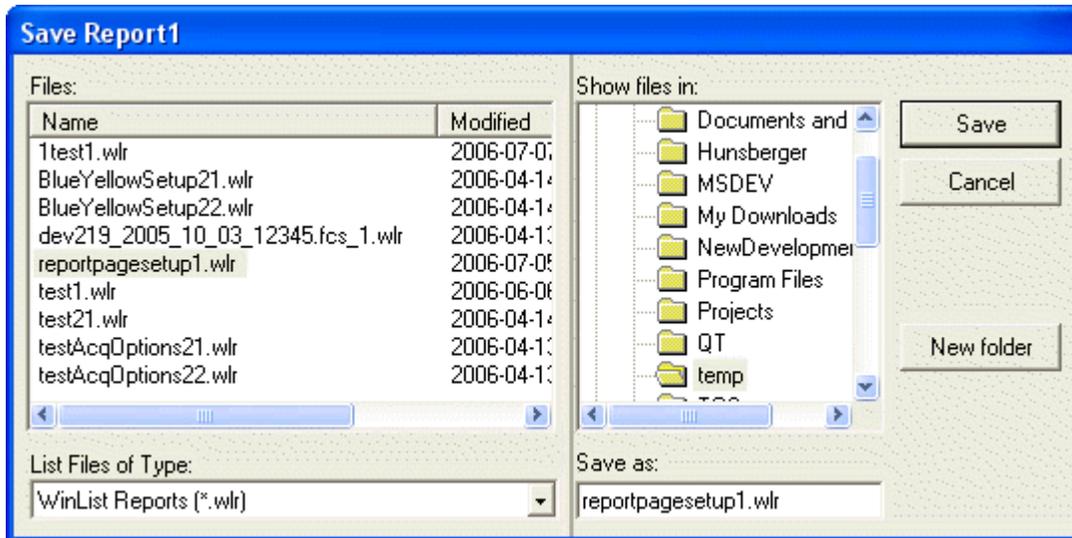
**Open Report**

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

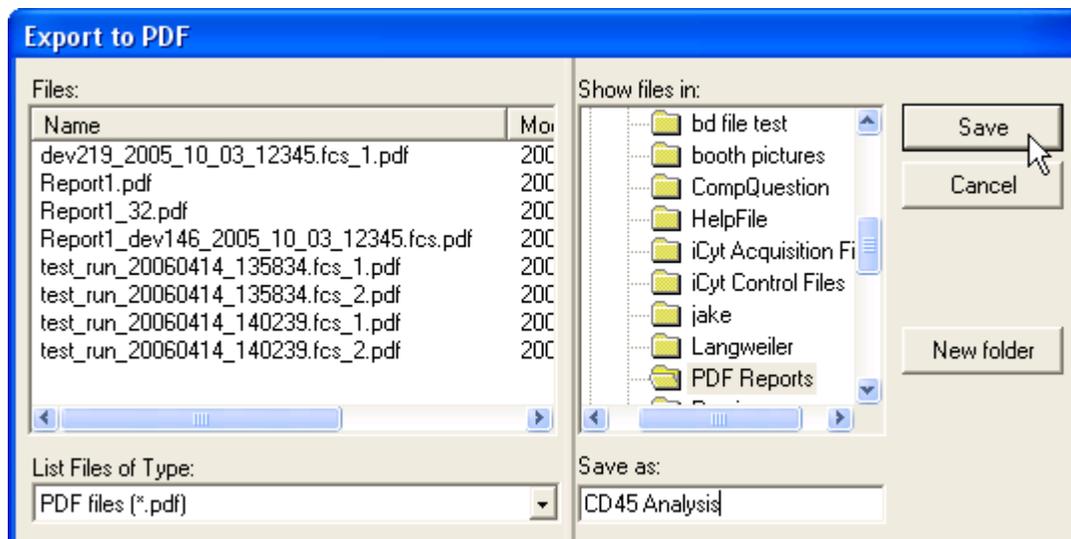
Select this option to print the active Report window.

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.

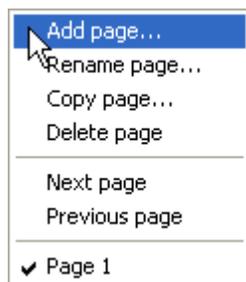
### Export to PDF

This command displays the Export to 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.



### Report Page Menu

This command displays the page menu. The command is available in the report toolbar at the top of each report window.



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 titlebar of the report, and then using the PgUp and PgDn keys on the keyboard.

### Add Page

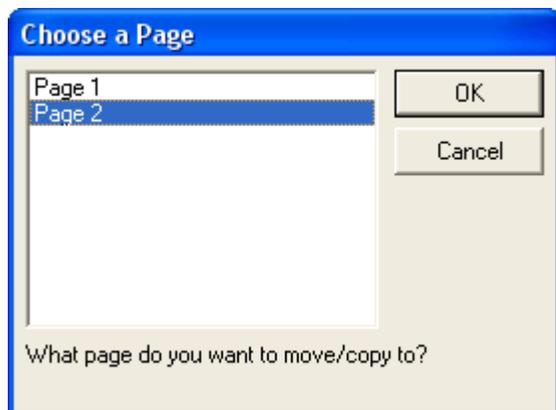
This command creates a new, blank page in the report and displays a dialog that allows you to name the page.

### 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.

#### **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 dropdown 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 don't 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.

#### **Snap to Grid**

This command 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.

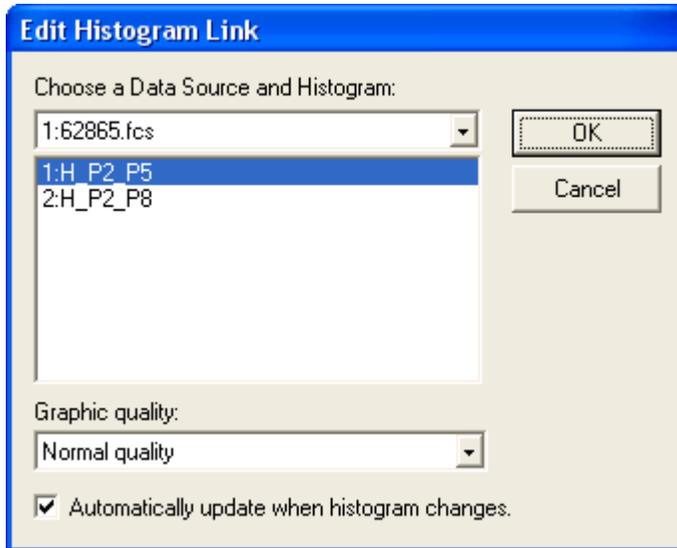
Enable this item to force objects to align to the snapping grid.

Uncheck the command to allow objects to move freely on the report page.

#### **Insert Histogram Link**

This command 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.



Select the data source from the dropdown 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.

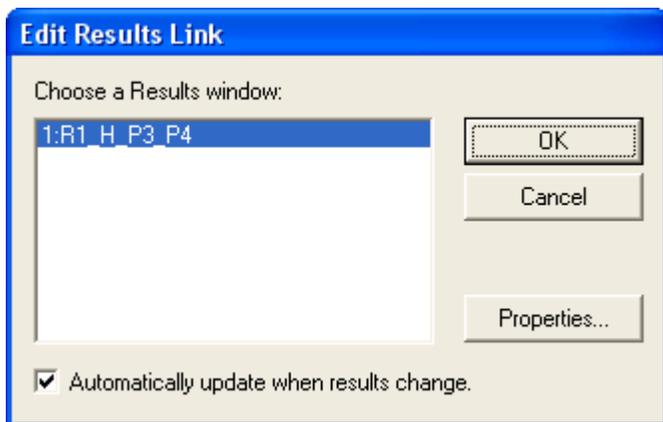
There are two other methods you can use to add histogram links to a report.

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

This command 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.

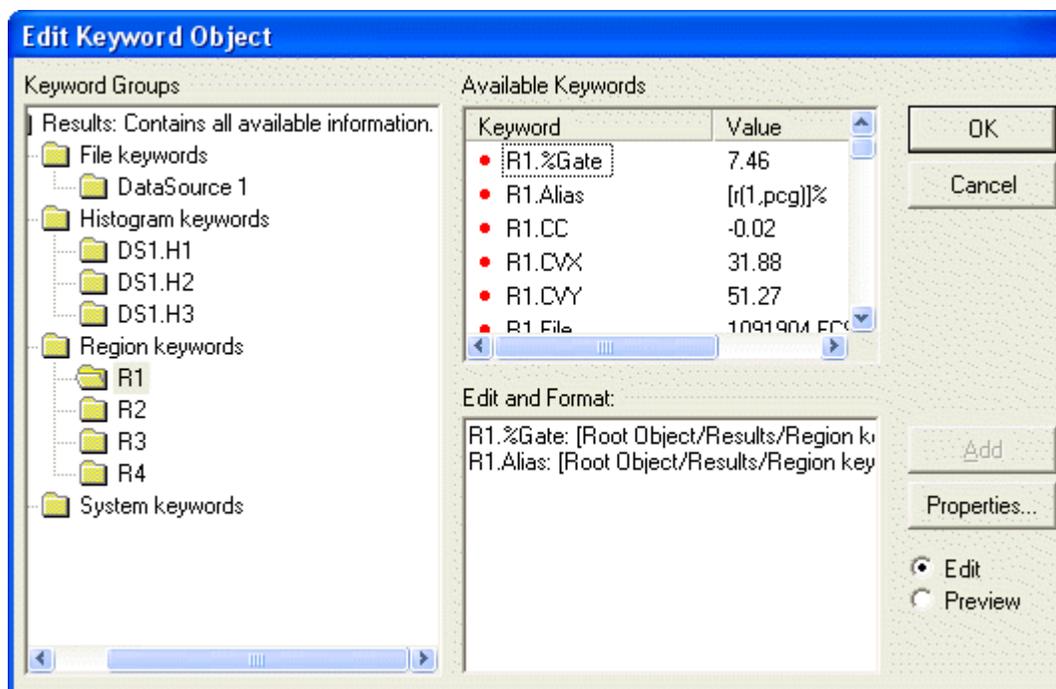
There are two other methods you can use to add results window links to a report.

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 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.

### Edit and Format

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.

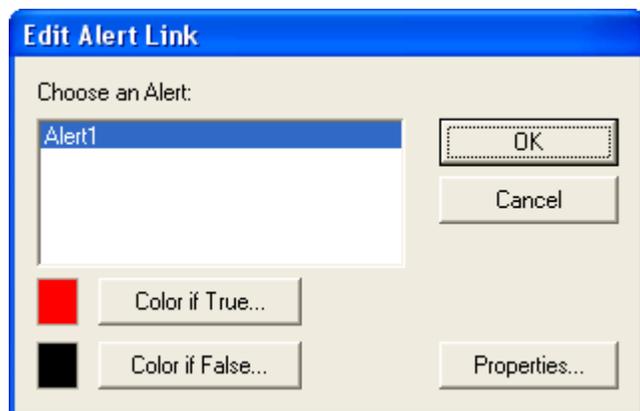
### Cancel

Click Cancel to discard the changes to the object and close the dialog.

### Insert Alert

This command 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 adds a gate legend object to the current page of the report.

- No gate
- Lymphs (R1)
- Live Cells (R2)
- Monos (R3)
- T cells (R4)

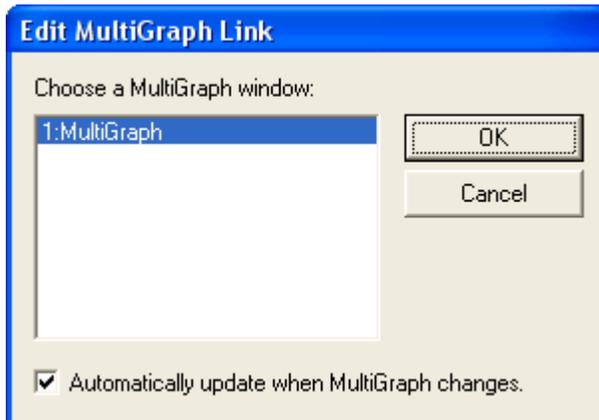
The gate legend displays a list of all active gates. For each gate, the gate color, gate alias, and gate expression are displayed.

Use the Edit Gates dialog to edit gate colors, aliases, and expressions.

### Insert MultiGraph Link

This command 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.



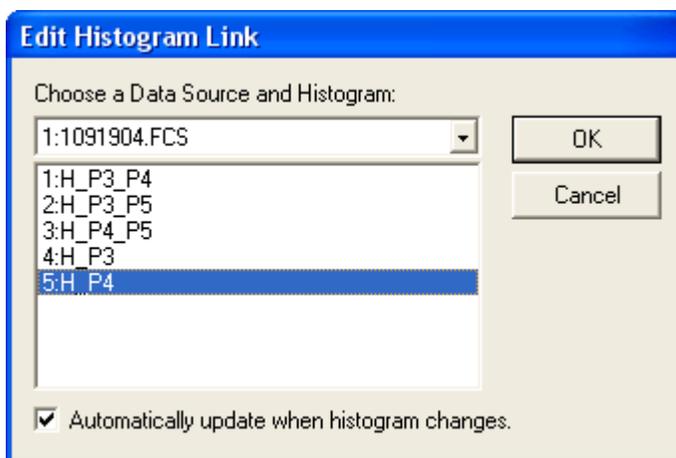
Select the MultiGraph window you want to link to from the listbox. A checkbox is provided that determines controls whether the new object will update automatically as the MultiGraph window changes.

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 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 specific histogram that owns the Info window that you want to link the object to.



Select the data source from the dropdown 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.

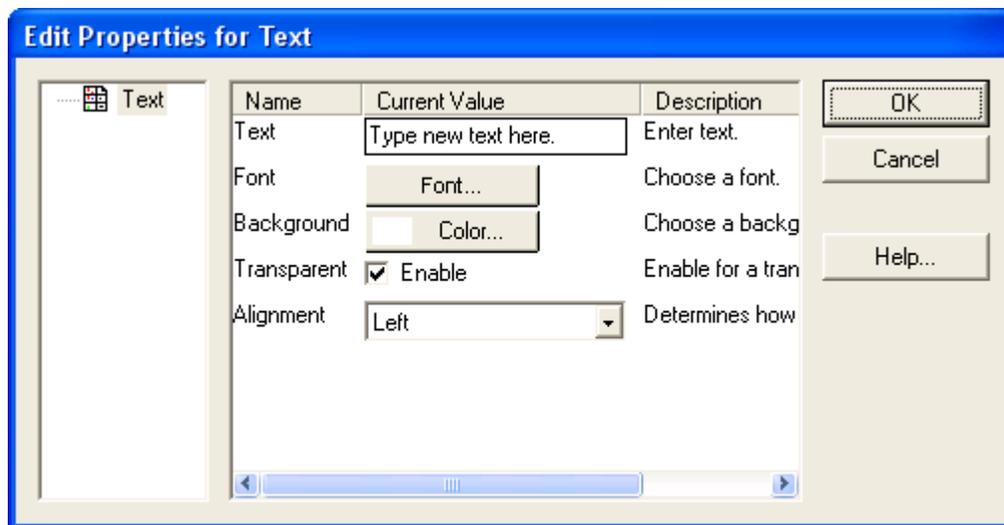
### Insert Text

This command 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.

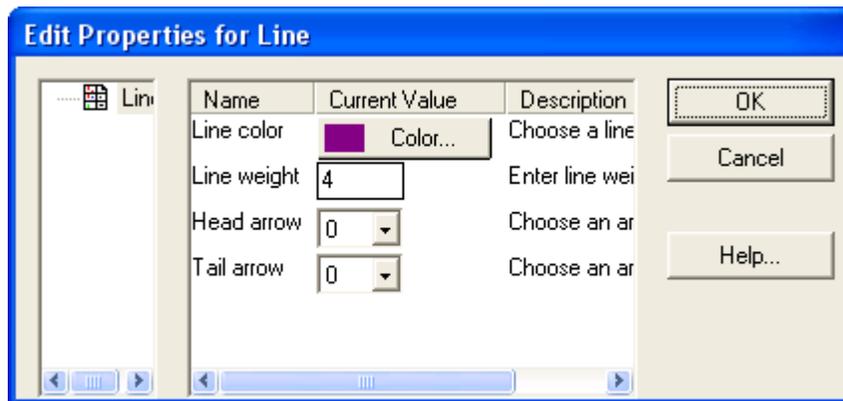
**Insert Line**

This command 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 adds an arrow object to the report. The object can be moved, sized, and edited.

See Edit Properties for Line for details.

### Edit Properties for Arrow

These options are the same as those for line objects. See Edit Properties for Line for details.

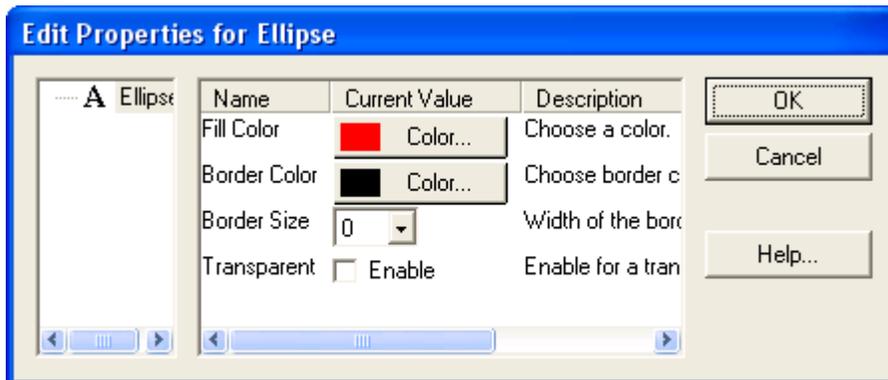
### Insert Ellipse

This command adds an ellipse object to the report. The object can be moved, sized, and edited.

See Edit Properties for Ellipse for details.

### 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.

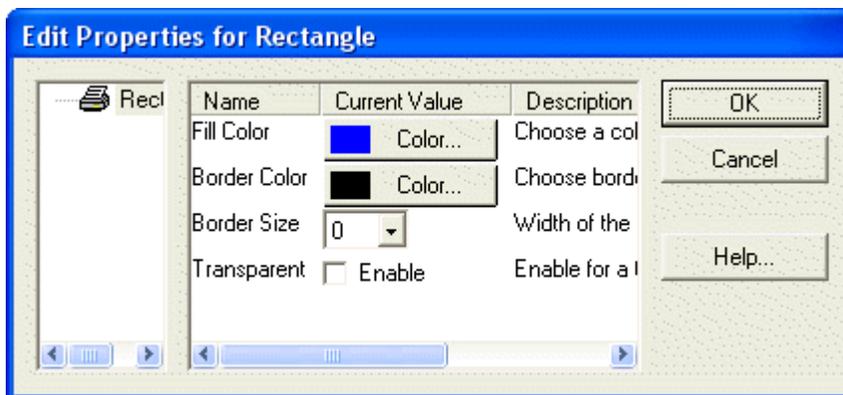
**Help**

Choose this button to display online help for the dialog.

**Insert Rectangle**

This command adds a rectangle object to the report. The object can be moved, sized, and edited.

See Edit Properties for Rectangle for details.

**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**

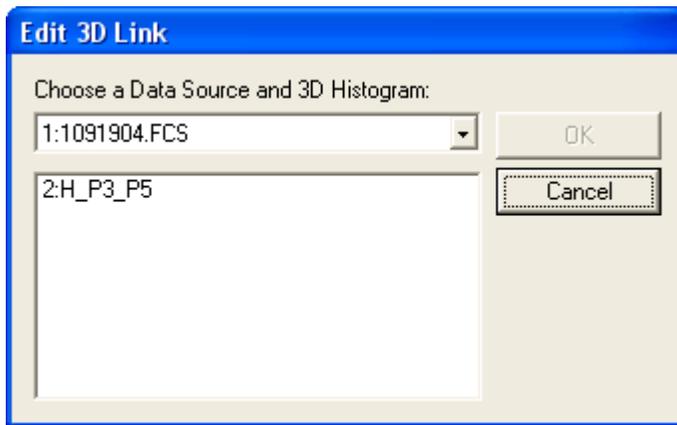
Choose this button to display online help for the dialog.

**Insert 3D Histogram Link**

Note: This command is only available in the 3D Version.

This command 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 dropdown 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.

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 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 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 object 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 object 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.

## **Window menu**

### **Window: Data Sources**

This menu contains commands that apply to data source windows.

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.

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.

Select **Scale to Fit** to adjust 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.

Select **Tile** to fit all data sources in a grid format. Histograms contained in the data sources are automatically rearranged in a tiled format.

Select **Cascade** to arrange data sources from upper right to lower left. Histograms contained in the data sources are automatically rearranged in a tiled format.

The **Minimize** and **Restore** commands appear only on the PC version. When selected, all data source windows are minimized or restored to normal size, respectively.

### **Window: Histograms**

**Arrange Histograms** contains several options: **Tile**, **Cascade**, **Scale to Fit**, **Minimize**, and **Restore**.

Selecting **Tile** will fit all histograms into the data source so that they are fully visible.

Selecting **Cascade** overlaps the histograms from upper right to lower left. Only histograms which are not minimized will be stacked or tiled.

Select **Scale to Fit** to adjust all histograms proportionally to fit into the data source window.

**Minimize** reduces all histograms in the current data source to icons.

**Restore** will return all minimized histograms in the current data source to their last normal positions.

### **Window: MultiGraphs**

This menu contains options for arranging MultiGraph windows.

Select **Show All** to display all hidden MultiGraph windows and ensure that they are within the viewable area of the screen.

Select **Hide All** to hide all MultiGraph windows from view. The MultiGraphs are not destroyed by this command; they are only hidden.

### **Window: Results**

This menu contains options for arranging Results windows.

Select **Show All** to display all hidden Results windows and ensure that they are within the viewable area of the screen.

Select **Hide All** to hide all Results windows from view. The Results are not destroyed by this command; they are only hidden.

### **Window: Reports**

This menu contains options for arranging Report windows.

Select **Show All Reports** to display all hidden Report windows and ensure that they are within the viewable area of the screen.

Select **Hide All Reports** to hide all Report windows from view. The Reports are not destroyed by this command; they are only hidden.

### **Window: Scale to Fit**

This command in the Window menu performs the same action as the Scale to Fit command in the Data Source submenu. It appears here for convenience.

### **Close All Windows**

This option performs the same function as the File->New Protocol command. WinList will close all data source, histogram, MultiGraph, results, and report windows. It also resets other settings to defaults.

### **Help menu**

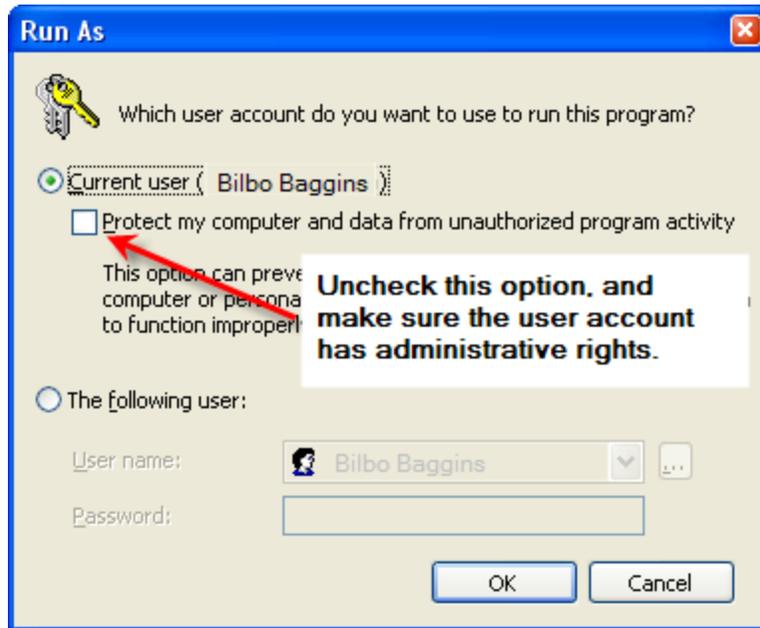
#### **Help Index**

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**

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

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.



## **WinList 3D Version Notes**

### **WinList 3D Version Notes**

WinList 3D can create high quality OpenGL 3D images from any 2P or 3P histograms. 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.

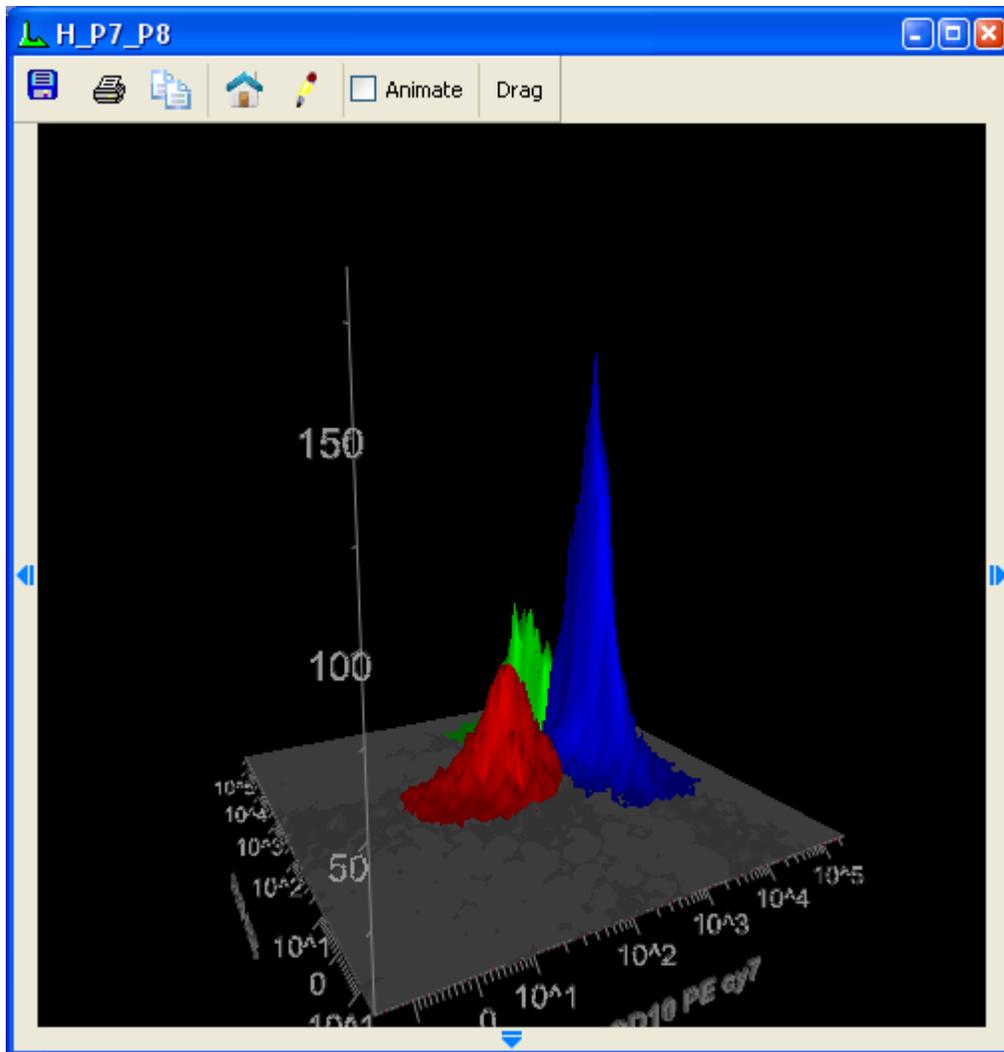
For more on the 3D Viewer, see:

Using the 3D Viewer

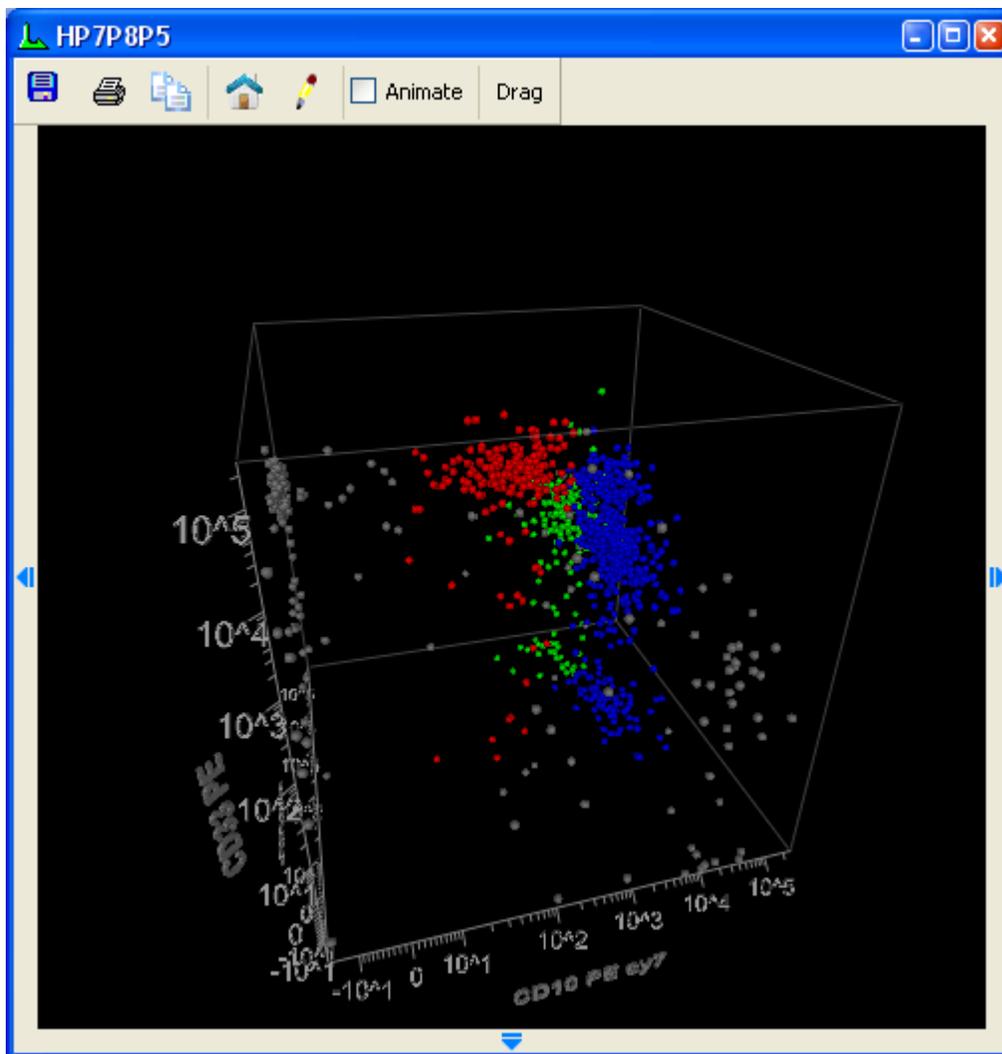
### **Using the 3D Viewer**

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.

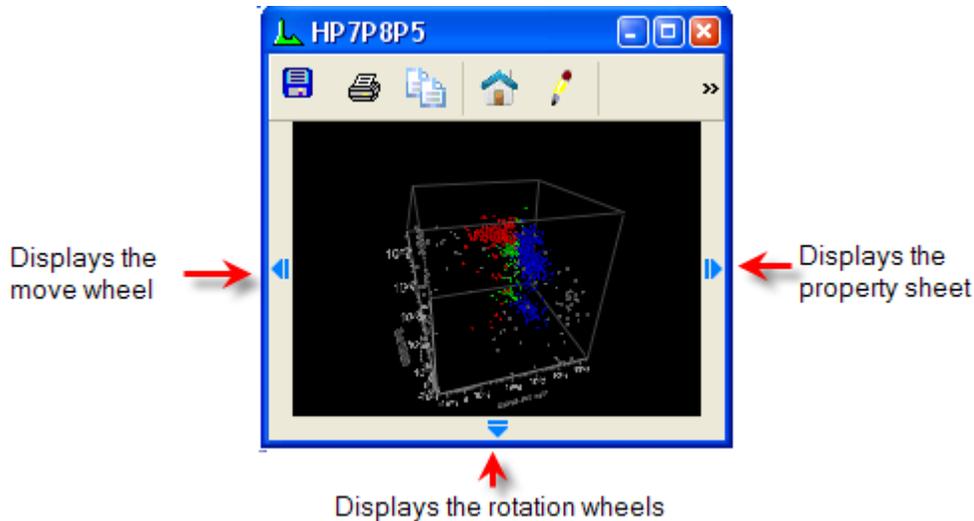


For details on the 3D display options for 3-parameter data, see 3-Parameter Display Options.



#### 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's 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.

**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

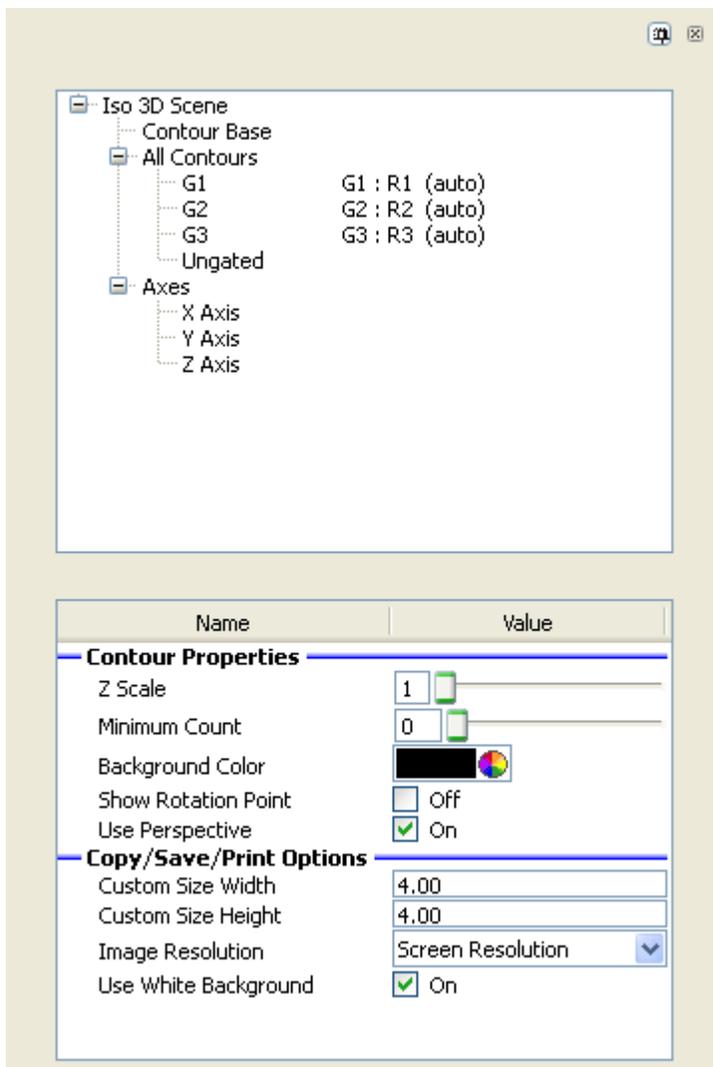
**3D Viewer: Additional Controls****Mouse Button Controls**

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

##### Z Scale

This controls the scaling factor for the Z dimension. Typically it is 1.

##### 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.

##### 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**

Choose a color for the contours.

## **Axes**

### **Show Box**

Enable this option to show the 3D box.

### **Box Line Weight**

Choose a line weight for the 3D box.

## **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**

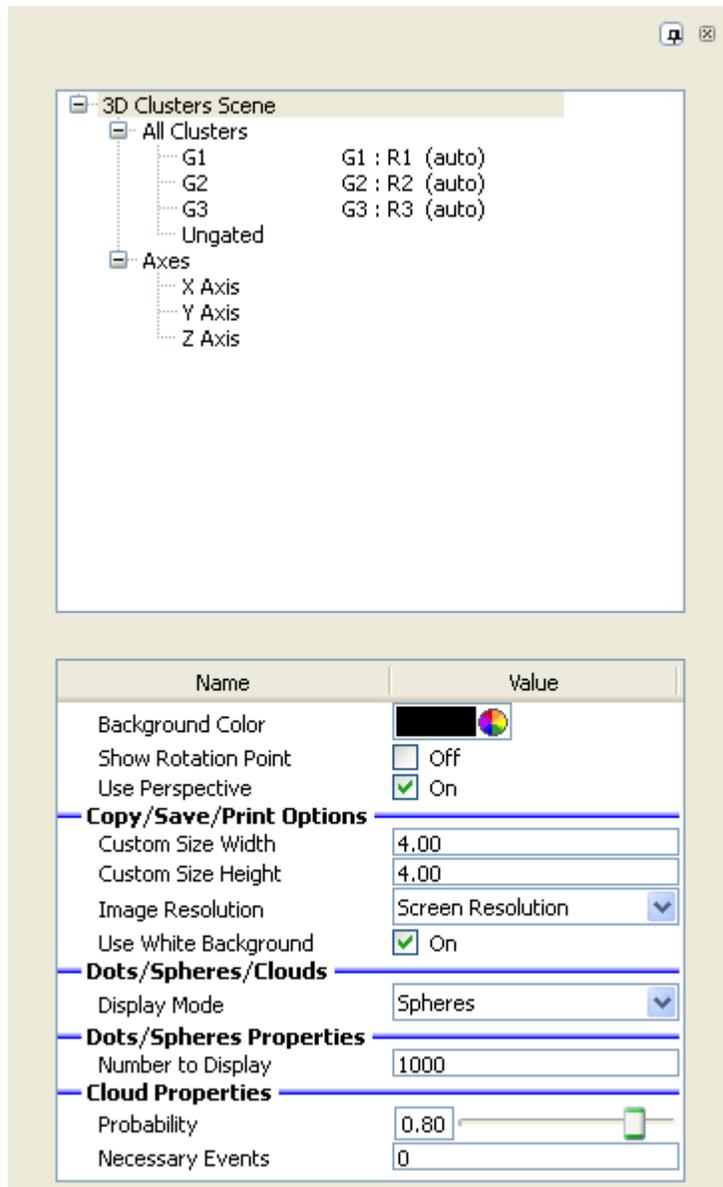
Choose an alignment for the labels.

#### **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.

### **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.

### **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**

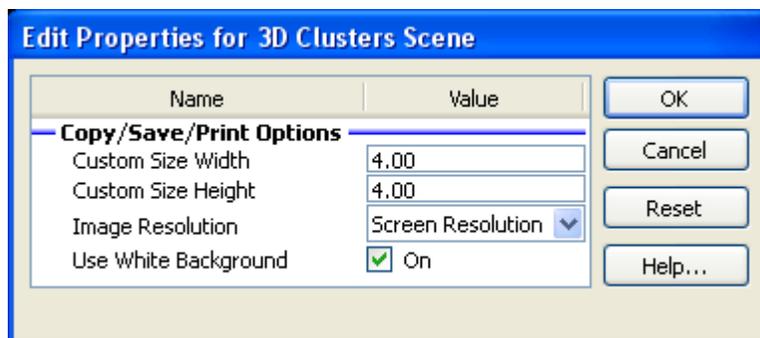
Choose an alignment for the labels.

**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.

**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.

## iCyt Acquisition and Sort Control

### iCyt: Acquisition and Sort Control Overview

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

When the iCyt Acquisition plug-in for WinList is installed with the program, WinList adds a number of features that allow you to monitor data, acquire data, and sort data on Synergy™ and Reflection™ cell sorting instruments. WinList allows you to select a Highly Automated Parallel Sorting (HAPS) module on an iCyt Remote Operation Cytometer (iROC) server and then connects to it via TCP/IP, the standard protocol of the Internet. Choose the instruments and features of interest and monitor events in real-time.



The connection allows you to take advantage of the full power of WinList, monitoring or acquiring from any number of iCyt instruments on a given iROC server simultaneously. WinList connects to a user-defined iROC server, which in turn provides access to all of its attached HAPS modules.

Using WinList's powerful gating capabilities, it's fast and easy to create regions and define gates that can be used for sort decisions. With a simple click, WinList transmits sort gates and N-Color Compensation™ to the iCyt HAPS module via the iROC server, enabling extensive sort classifications. Events can even be color-coded in WinList based on how they actually sorted on the device.

#### What It Does and What It Doesn't Do

The WinList-iCyt connection is certainly very powerful. However, there are some aspects of interacting with an iCyt instrument that are handled outside of WinList. The table below outlines some of the functions that are performed from within WinList and others that are not.

Feature	WinList	Instrument Control Software
Monitoring events	Yes	Yes
Creating sort gates	Yes	No
Establishing sort decisions	Yes	No

Viewing any number of histograms, dot plots, and 3D plots	Yes	No
Acquiring data to FCS files	Yes	Yes
Defining N-Color Compensation™	Yes	No
Setting voltages	No	Yes
Starting and stopping the flow	No	Yes

### Getting Started

A good starting point for getting to know the WinList-iCyt system is to review the Terminology section, and then move on to the Walk-through. A detailed topic reference for all features is found in the table of contents.

Links:

- Terminology
- Walk-through
- Troubleshooting

### iCyt: Terminology

#### HAPS modules

The iCyt cytometer system uses one or more *Highly Automated Parallel Sort* modules, referred to as HAPS modules. Each HAPS module has the capabilities of a typical flow cytometer.

#### Features

Typical flow cytometers create listmode data files containing a number of *parameters* that were measured by the instrument. The measurements that are made by an iCyt HAPS module are referred to as *features*.

#### iROC server

The *iCyt Remote Operation Cytometer Server* is designed to manage one or more iCyt HAPS modules. iROC servers provide network connectivity between the HAPS modules and WinList on remote workstations.

Links:

- Walk-through

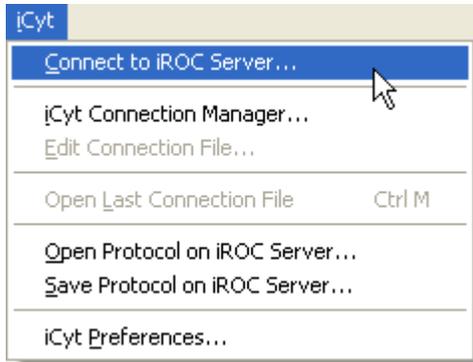
### iCyt: Connecting to an iROC Server

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

The first step in working with WinList's iCyt features is to establish a connection between WinList and an *iCyt Remote Operation Cytometer Server* (iROC) server. The iROC server provides access to one or more iCyt HAPS modules.

#### To create a connection:

1. From the iCyt menu, choose **Connect to iROC Server**.



2. In the dialog, enter the name or the IP address of the iROC server. IP addresses are used to identify computers on the internet.



3. Click OK to establish the connection.

WinList will inform you whether or not the connection has been made.

**To set a default connection:**

1. From the iCyt menu, choose **iCyt Preferences**.
2. In the **iROC Server** field, enter the name or IP address of the iROC server.
3. Click **OK**.

**Next:**

Selecting an iCyt HAPS Module

**More Info:**

Terminology  
 Troubleshooting

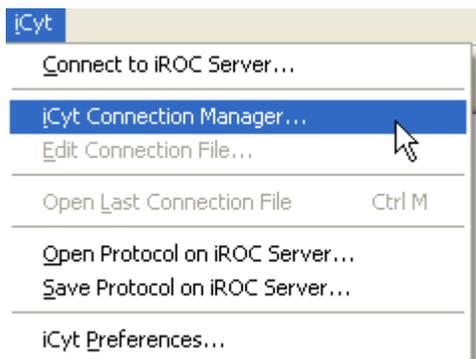
**iCyt: Selecting an iCyt HAPS Module**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

After making a connection to an iROC server, the next step is to choose an iCyt *HAPS Module* that you want to monitor or acquire data from. You can create connections to more than one HAPS module, but the connections are made one at a time.

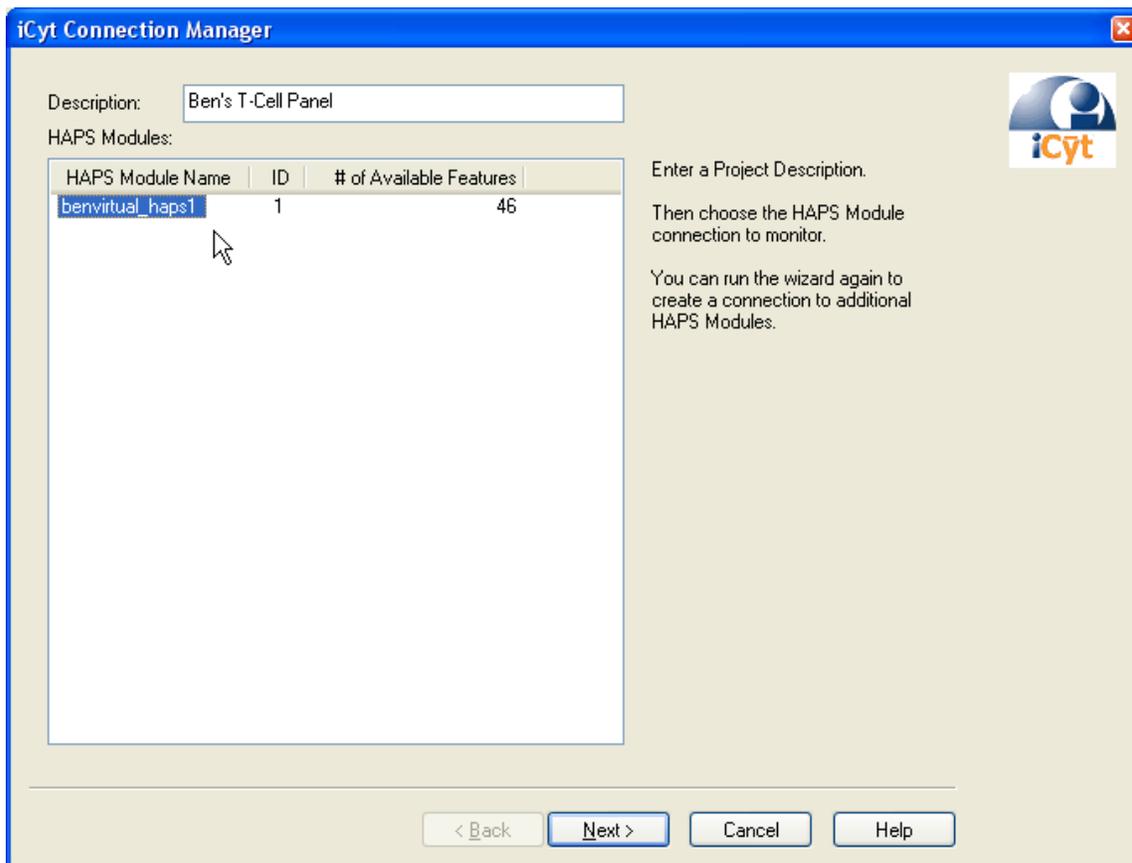
**To make a new connection to an iCyt HAPS Module:**

1. From the iCyt menu, choose **iCyt Connection Manager**.



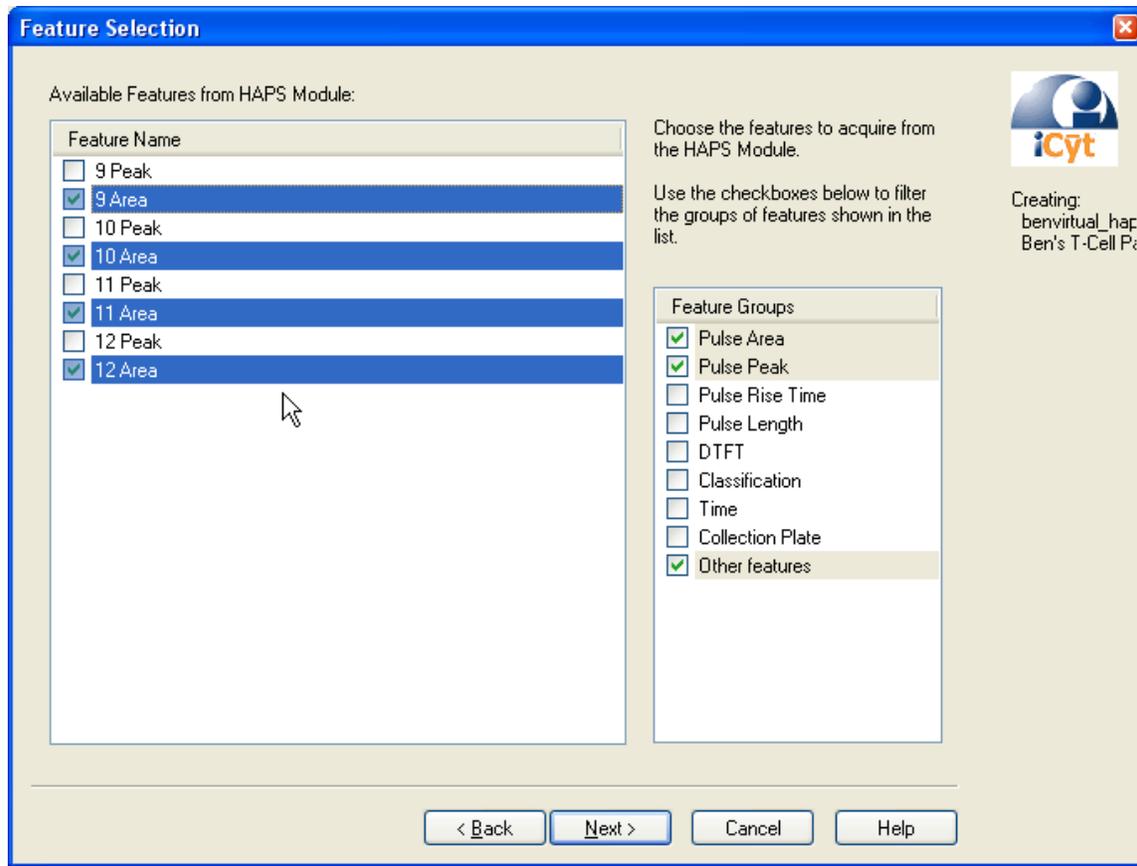
2. From the list of the iCyt HAPS modules, select the HAPS module you want to monitor and click **Next**.

Example



3. Select the features that you are interested in from the list and click **Next**. You can use the Feature Group filters to filter the list of features.

Example

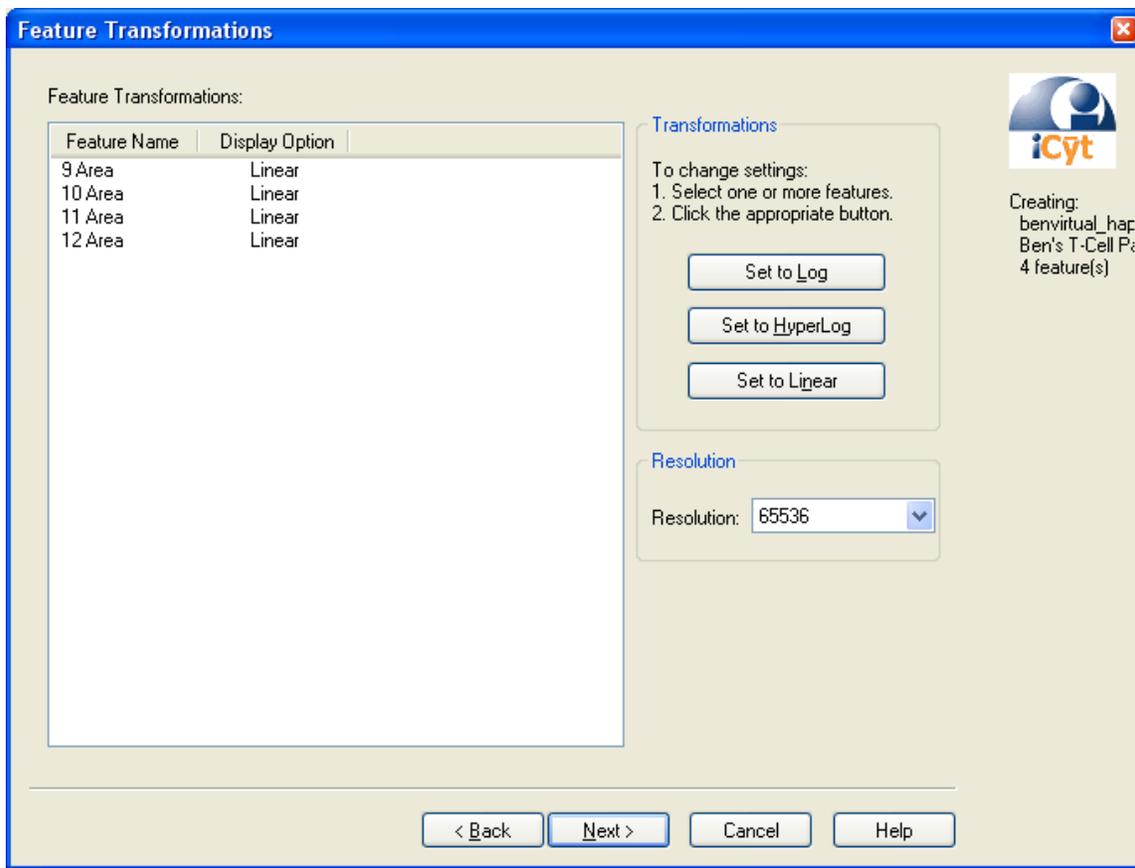


4. On the Feature Transformations pane, you can set resolution and transformations for any of the parameters.

To change the transformation settings:

- a. Select one or more features.
- b. Click **Set to Log**, **Set to HyperLog**, or **Set to Linear**.

Example

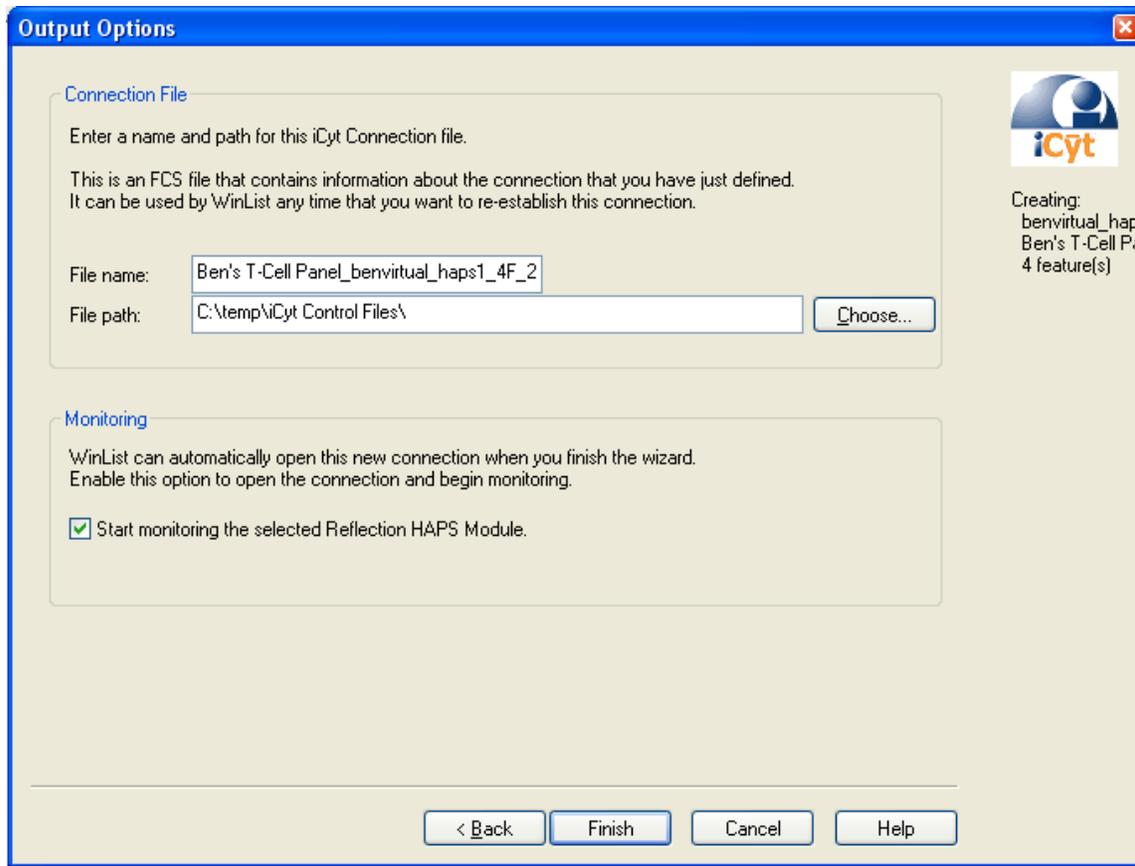


Click **Next** to proceed to the next pane in the wizard.

5. On the Output Options pane, you can enter a name for the iCyt Connection file and choose a location for it. Leave the **Start monitoring** option checked so that WinList will automatically open the connection file for you. The iCyt Connection file you are about to create is an FCS file that WinList uses to create a connection with the selected iCyt HAPS module. You can use this file over and over again once it is created.

Click the **Finish** button.

Example



After the iCyt Connection Wizard closes, WinList will open the iCyt Connection file you just created.

**To open an existing iCyt Connection file:**

1. From the File menu, choose **Open FCS File**. The Open dialog is displayed.
2. In the tree control in the dialog, navigate to the folder that contains the iCyt Connection file that you want to open.
3. Select the iCyt Connection file from the list of files, and click **Open**.

**Next:**

Monitoring a HAPS Module

**More Info:**

Terminology  
 Troubleshooting

**iCyt: Monitoring a HAPS Module**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

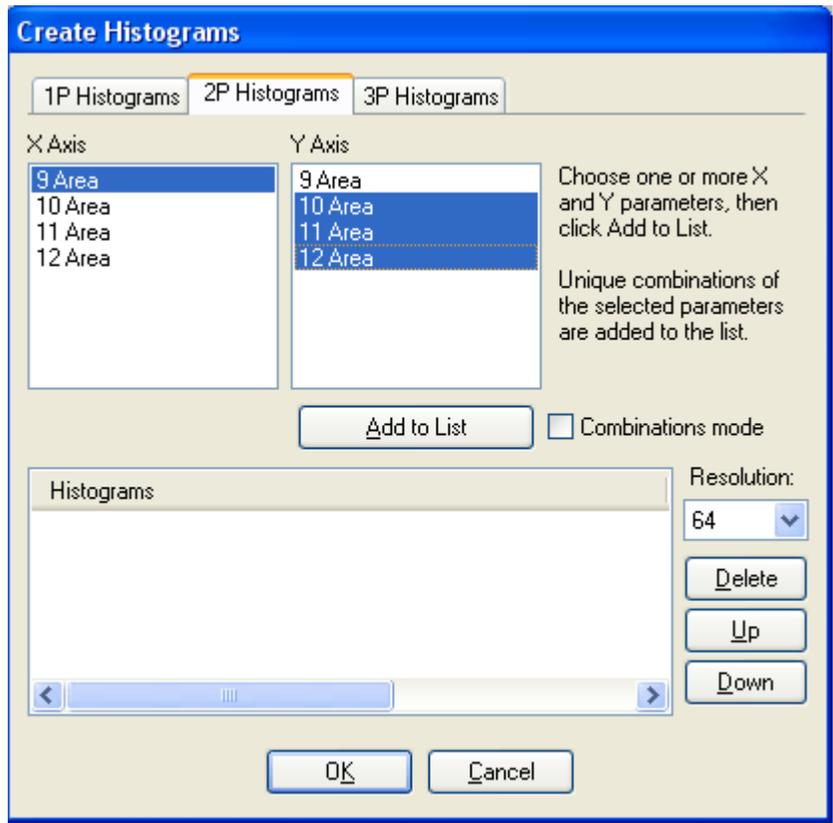
To monitor data for a HAPS module, use one of the methods below.

1. Create an iCyt Connection file with the iCyt Connection Manager, or
2. Open an iCyt Connection file that was previously created with the iCyt Connection Manager, or

3. Open a protocol or protocol bundle that was saved with a connection.

When WinList opens an iCyt Connection file, it establishes a connection with the iCyt HAPS Module that the file relates to. The iCyt Connection file contains information about the features that were selected for monitoring, and WinList uses this information to communicate with the HAPS module.

Initially, the Create Histograms dialog will be displayed. This dialog is displayed anytime WinList opens a new listmode data file (depending on a property in the Histogram Preferences dialog).



You can quickly create any number of 1P, 2P, and 3P histograms with this dialog (see Create Multiple Histograms for more details). After making selections, click the OK button to continue.

WinList will create a data source window containing any of the histograms you selected. It displays the iCyt toolbar at the bottom of the data source window, and it automatically begins to monitor the HAPS module.



When monitoring, WinList continually updates the graphics and statistics with the latest data received from the HAPS module. The iCyt toolbar and the data source title bar provide details about the Run ID of 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.

Virtually all of WinList's features are available during monitor mode. You can draw regions, define gates, view statistics, create calculated parameters, and even set up N-Color Compensation™.

**Next:**

Acquiring Data

**More Info:**

WinList Basics

Histogram Windows

Using Interactive N-Color Compensation

Troubleshooting

**iCyt: Acquiring Data**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

To acquire data from an iCyt HAPS module, you first need to establish a connection with an iROC server and have a working connection with a specific iCyt HAPS module. These actions are described in previous sections Connecting to an iROC Server, Selecting an iCyt HAPS Module, and Monitoring a HAPS Module. Once WinList is monitoring an iCyt HAPS module, 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 iCyt 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.

**Edit Acquisition for benvirtual\_haps1**

Name	Current Value	Description
<b>Stop</b>		
Stop Gate	Ungated :	Select a gate to use for the stop count.
Stop Count	10000	Number of events to acquire.
<b>Save</b>		
FCS File Prefix	Ben	Prefix for FCS files.
FCS File Pathway	Choose...	C:\VshHelpProjects\WinList\Protocols\
File Counter	1	Set the starting file counter, used for naming the FCS file. (0
Event sampling	1	Save every nth event into the FCS file. (1 to 2147483647)
<b>Pre-Acquisition</b>		
Auto Acquisition	<input type="checkbox"/> Enable	Automatically acquire data when run id changes.
Prompt Before	<input type="checkbox"/> Enable	Prompt for user-keywords before starting.
<b>Post-Acquisition</b>		
After Acquisition	Enter WinList mode & Replay	Choose the post-acquisition mode.
Prompt After	<input checked="" type="checkbox"/> Enable	Prompt for user-keywords after acquisition.
Update Report Links	<input checked="" type="checkbox"/> Enable	Refresh all report links after acquisition.
Save Reports	<input type="checkbox"/> Enable	Save reports based on FCS file name.
Save PDF Reports	<input type="checkbox"/> Enable	Save reports as PDFs based on FCS file name.
Print Reports	<input type="checkbox"/> Enable	Print reports after acquisition.
Save Protocol Bundle	<input type="checkbox"/> Enable	Save a protocol bundle based on FCS file name.
<b>User-Defined Keywords</b>		

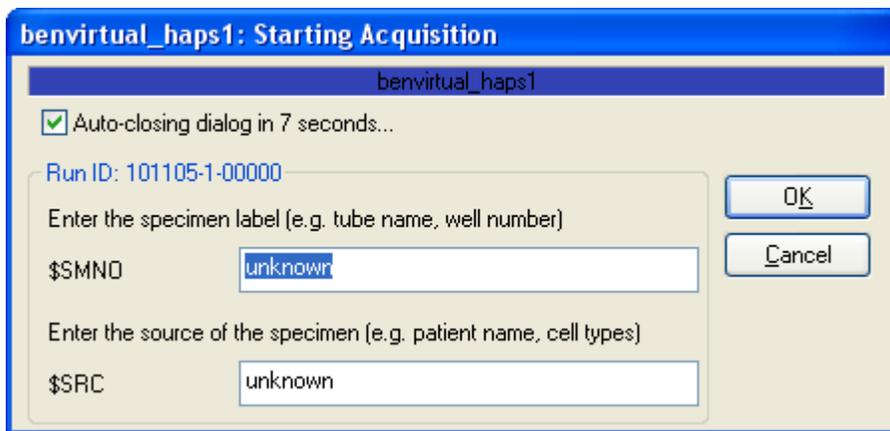
Buttons: OK, Cancel, Help...

**To acquire data:**

1. Click the Acquire button on the iCyt 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.

**benvirtual\_haps1: Acquisition Complete**

benvirtual\_haps1

Auto-close

Run ID: 101105-1-00000

Enter the specimen label (e.g. tube name, well number)

\$SMNO

Enter the source of the specimen (e.g. patient name, cell types)

\$SRC

OK

Cancel

**Acquisition Summary**

File name:  Browse...

File path:  View Path

File counter:

Stop gate events: 10000

Total events: 10000

4. Click OK to close the dialog and finish the acquisition.

**Next:**

Sorting

**More Info:**

Terminology

Troubleshooting

**iCyt: Sorting**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

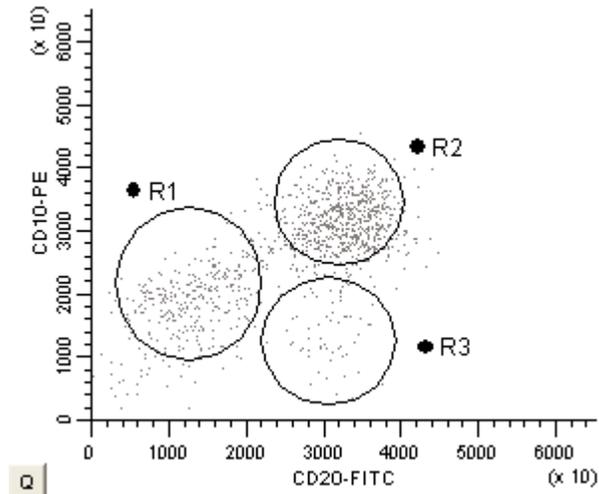
To sort data from an iCyt HAPS module, you first need to establish a connection with an iROC server and have a working connection with a specific iCyt HAPS module. These actions are described in previous sections Connecting to an iROC Server, Selecting an iCyt HAPS Module, and Monitoring a HAPS Module.

WinList provides very flexible and powerful sorting control for each iCyt HAPS module that it is connected to. 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 HAPS module.

Once the sort decisions have been sent to the module, they remain in effect until new sorting decisions are sent. Even if you close out of your WinList session with the iCyt HAPS module, the sorting decisions remain in effect.

**To create sort decisions:**

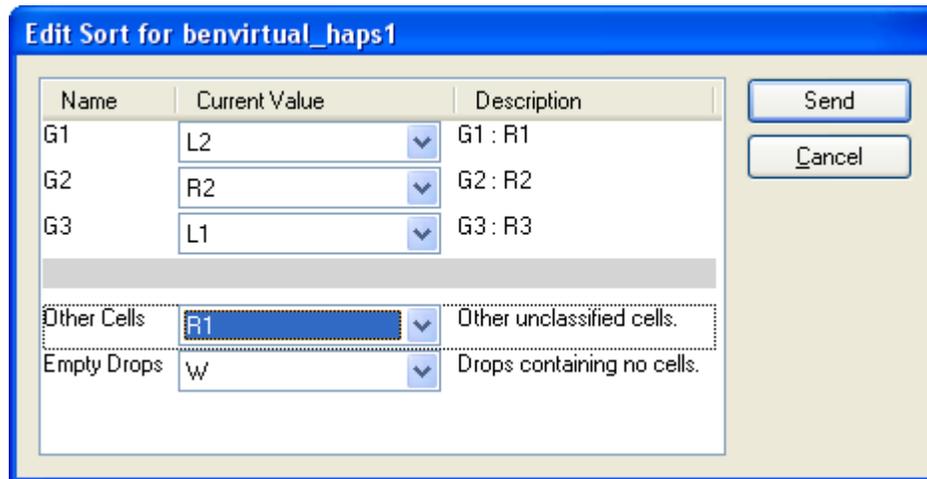
1. Create regions on histograms that identify the populations that you want to sort. See Regions for additional details.



2. Click the Edit Sort button on the iCyt toolbar.



3. In the Edit Sort dialog, associate the gates you created in step 1 with sort streams on the iCyt HAPS module. Each sort stream should be assigned to only one gate.



4. Click the Send button to transmit the sorting regions, gate expressions, and N-Color Compensation to the HAPS module and close the dialog.

**More Info:**

- Edit Sort dialog
- Using Interactive N-Color Compensation
- Toolbar
- Terminology
- Troubleshooting

**iCyt: Toolbar**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

At the bottom of the data source window, the iCyt toolbar provides additional controls and status indicators related to the iCyt acquisition system.



The left portion of the toolbar contains a set of command 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 iCyt HAPS module, and information about monitoring, acquisition, and events processed per second. A progress meter appears during acquisition to indicate how far along the acquisition is.

Two LED indicators appear on the right of the toolbar. The first one turns green when a packet of information is received from the iROC server. It turns yellow when a packet is requested and the server doesn't supply one. The second LED turns green when WinList is processing a packet of data received from the server.

### Toolbar Commands

Button	Purpose
	WinList mode Stop the connection with the HAPS module and enter WinList mode.
	Monitor Connect with the HAPS module and request data. Real-time events are displayed. Events are not stored.
	Acquire Accumulate data from the HAPS module and store it in an FCS file on disk. Real-time events are displayed. Events are stored.
	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.

### Links:

Terminology  
Walk-through  
Troubleshooting

### iCyt: Edit Properties of Acquisition

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

This dialog allows you to configure the acquisition characteristics for the HAPS module connection. You can choose preferences for most of these properties in the iCyt Preferences dialog.

Name	Current Value	Description
<b>Stop</b>		
Stop Gate	Ungated :	Select a gate to use for the stop count.
Stop Count	10000	Number of events to acquire.
<b>Save</b>		
FCS File Prefix	Ben	Prefix for FCS files.
FCS File Pathway	Choose...	C:\WshHelpProjects\WinList\Protocols\
File Counter	1	Set the starting file counter, used for naming the FCS file. (0
Event sampling	1	Save every nth event into the FCS file. (1 to 2147483647)
<b>Pre-Acquisition</b>		
Auto Acquisition	<input type="checkbox"/> Enable	Automatically acquire data when run id changes.
Prompt Before	<input type="checkbox"/> Enable	Prompt for user-keywords before starting.
<b>Post-Acquisition</b>		
After Acquisition	Enter WinList mode & Replay	Choose the post-acquisition mode.
Prompt After	<input checked="" type="checkbox"/> Enable	Prompt for user-keywords after acquisition.
Update Report Links	<input checked="" type="checkbox"/> Enable	Refresh all report links after acquisition.
Save Reports	<input type="checkbox"/> Enable	Save reports based on FCS file name.
Save PDF Reports	<input type="checkbox"/> Enable	Save reports as PDFs based on FCS file name.
Print Reports	<input type="checkbox"/> Enable	Print reports after acquisition.
Save Protocol Bundle	<input type="checkbox"/> Enable	Save a protocol bundle based on FCS file name.
<b>User-Defined Keywords</b>		

## 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 HAPS module. A setting of 500 would store 1 out of 500 events. This can be useful when running long acquisitions where you don't need to store every event.

**Pre-Acquisition****Auto Acquisition**

When this option is enabled, WinList will automatically begin to acquire from connected iCyt HAPS modules whenever the Run ID on the instrument changes. This allows the program to automatically store FCS files as tubes are being changed at the instrument.

**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.

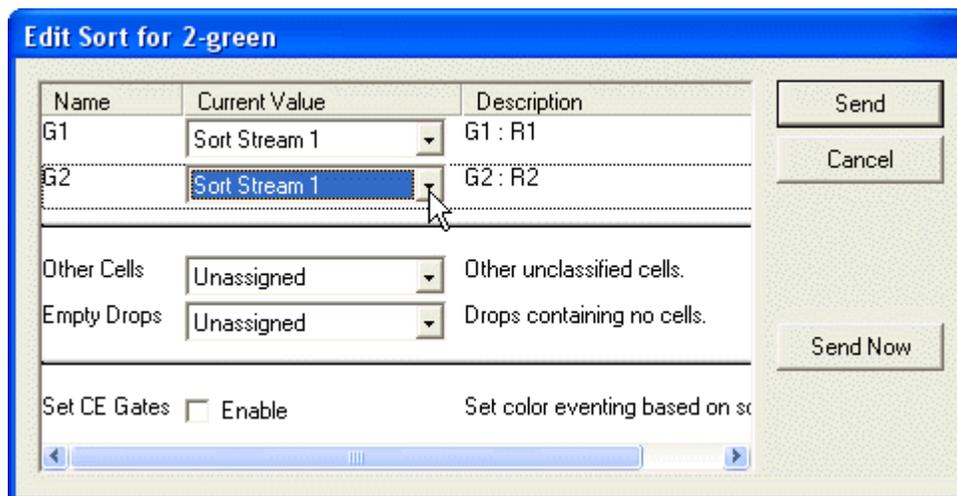
### Links:

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### iCyt: Edit Sort

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

This dialog allows you to make sorting decisions for the HAPS module. No changes are made at the HAPS module until the **Send** or **Send Now** button is clicked.



### Gates

The top section of the dialog shows each gate that is currently defined.

To use a gate for a sort decision, select one of the available **Sort Stream** entries in the second column. This will associate the gate expression with the Sort Stream decision.

To exclude a gate from sort decisions, set it to "Unassigned".

### Other Cells

The system allows you to sort all cells that are not identified by other gate assignments.

To sort all other unclassified cells into a specific sort stream, select the Sort Stream from the drop-down list in the second column.

To ignore other cells when sorting, set it to "Unassigned". In this case, these cells will be sorted to the middle stream. If there is an even number of streams, they will sort to the stream just to the left of middle.

### Empty Drops

This option allows you to sort droplets that contain no cells.

To sort empty droplets into a specific sort stream, select the Sort Stream from the drop-down list in the second column.

To ignore empty drops when sorting, set it to "Unassigned". In this case, these cells will be sort to the middle stream. If there is an even number of streams, they will sort to the stream just to the left of middle.

**Send**

Click this button to transmit the sorting regions, sort gate expressions, and N-Color Compensation™ to the iCyt HAPS 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 to the iCyt HAPS module.

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**iCyt: iCyt Connection Manager**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

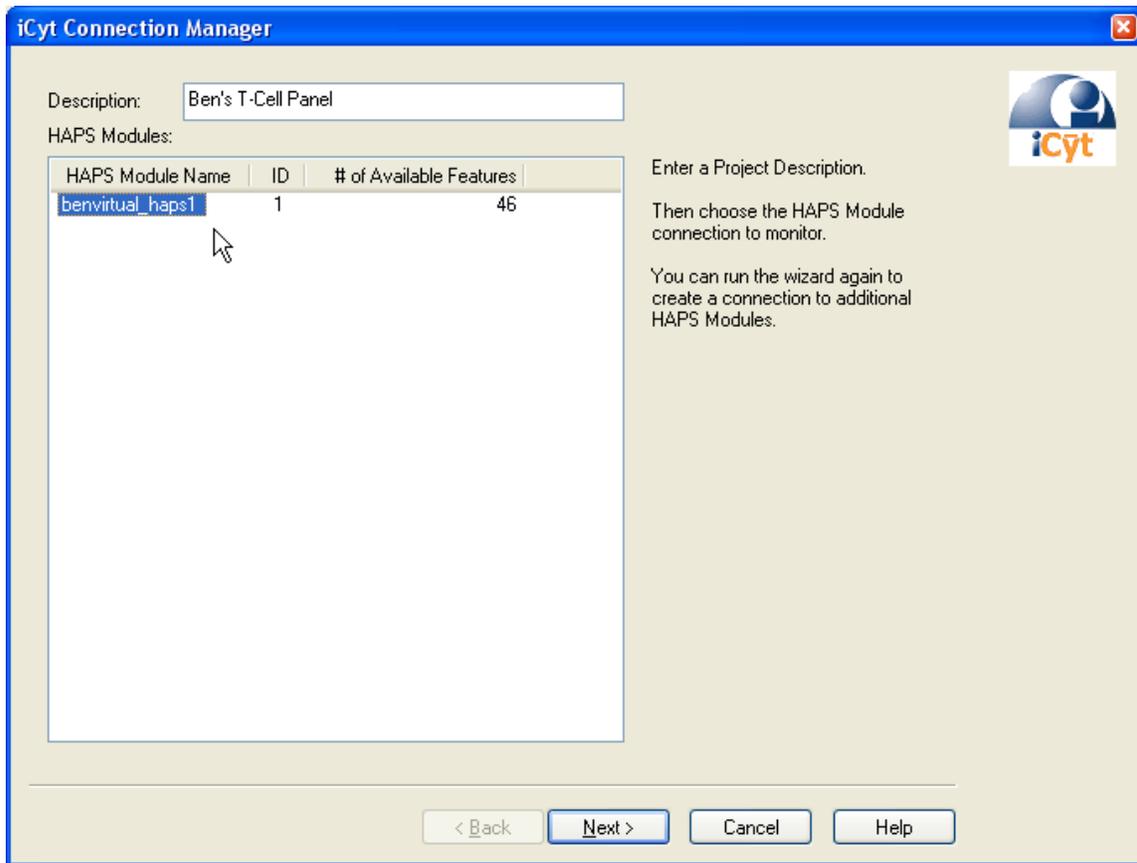
This wizard is used to create an iCyt Connection file that allows WinList to monitor and acquire data from an iCyt HAPS module on an iROC server. The wizard is only available when WinList is connected to an iROC server.

To display the iCyt Connection Manager, use one of the methods below:

1. From the iCyt menu, choose **iCyt Connection Manager**.
2. Click the iCyt button on WinList's main toolbar.

Each pane in the iCyt Connection Manager is described below.

### iCyt Connection Manager

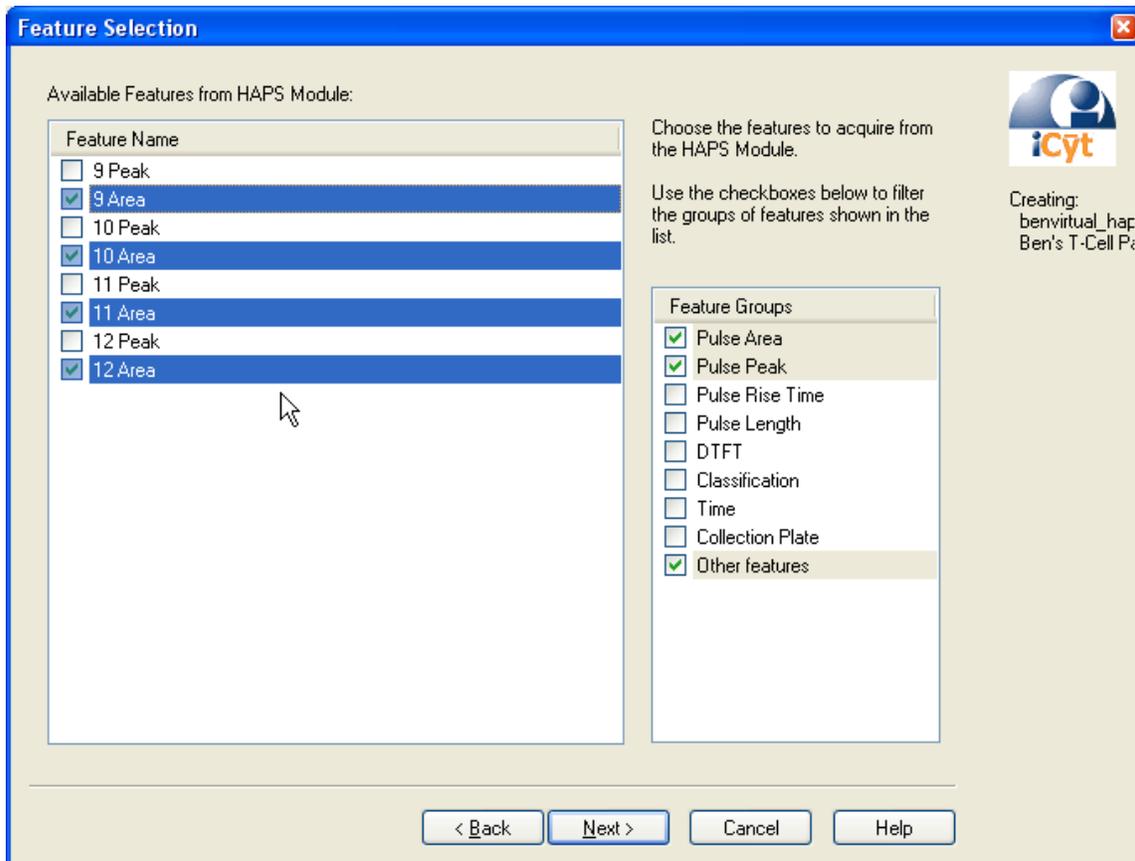


#### Available HAPS Modules

This list displays all of the iCyt HAPS Modules connected to the iROC server, and the number of features each has for selection.

Choose one entry in the list and click **Next** to continue to the next pane of the wizard.

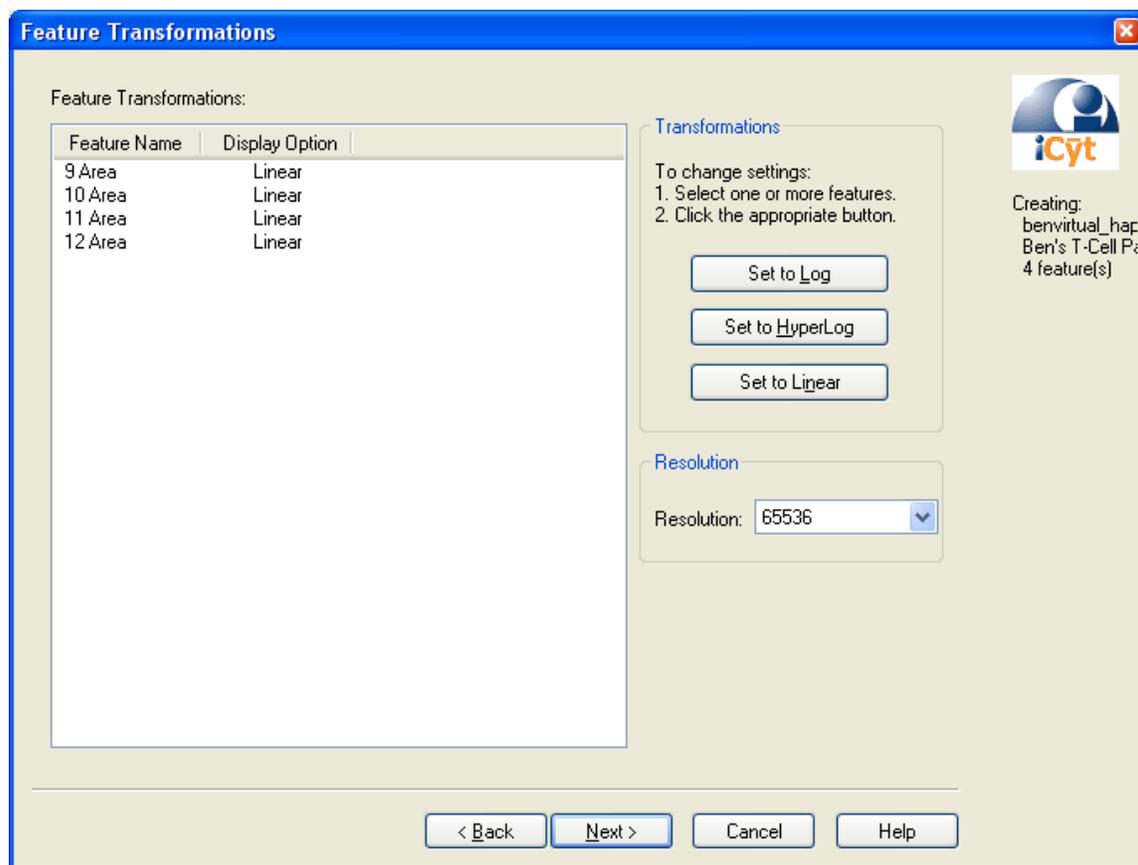
## iCyt Feature Selection

**Available Features from HAPS Module**

This list displays the features available on the iCyt HAPS Module that was selected on the previous pane in the wizard. Information about each feature is displayed in additional columns of the list control.

Choose the features in the list that you want to monitor or acquire. Click **Next** to continue to the next pane of the wizard.

## Feature Transformations



### Feature Transformations

This list displays the selected features and their transformations, along with the resolution for all features.

Choose a resolution from the Resolution dropdown list. The Resolution setting will be used as the number of channels for the feature when you view it in WinList. In most cases, it is not necessary to change this setting from the default value of 65536 channels.

To change the transformation settings:

- a. Select one or more features.
- b. Click **Set to Log**, **Set to HyperLog**, or **Set to Linear**.

After making adjustments to the resolutions and transformations, click **Next** to continue to the next pane of the wizard.

**Output Option**

**Output Options**

**Connection File**

Enter a name and path for this iCyt Connection file.

This is an FCS file that contains information about the connection that you have just defined. It can be used by WinList any time that you want to re-establish this connection.

File name: Ben's T-Cell Panel\_benvirtual\_haps1\_4F\_2

File path: C:\temp\iCyt Control Files\

**Monitoring**

WinList can automatically open this new connection when you finish the wizard. Enable this option to open the connection and begin monitoring.

Start monitoring the selected Reflection HAPS Module.

< Back   Finish   Cancel   Help

Creating: benvirtual\_hap  
Ben's T-Cell P:  
4 feature(s)

**File name**

This editable field displays the suggested name for the new iCyt Connection file, based on the name of the selected iCyt HAPS module.

**File path**

This field displays the path for the new iCyt Connection file. You can directly edit the path, or use the **Choose** button to select a path.

**Start monitoring the selected iCyt HAPS Module.**

When this option is checked, WinList will automatically open the newly created iCyt Connection file and establish a connection with the Reflection HAPS Module when the Finish button is clicked.

**Finish**

Click this button to complete the wizard. WinList will close the wizard and create an iCyt Connection file.

**Cancel**

Click this button to cancel the operation and close the dialog.

**Back**

Move back to the previous pane in the wizard.

**Next**

Move to the next pane in the wizard.

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**iCyt: Edit Connection File**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

The command allows you to edit a current connection. It displays the iCyt Connection Wizard with the settings for the connection. See iCyt: Reflection Connection Manager for details.

**iCyt: Open Last Connection File**

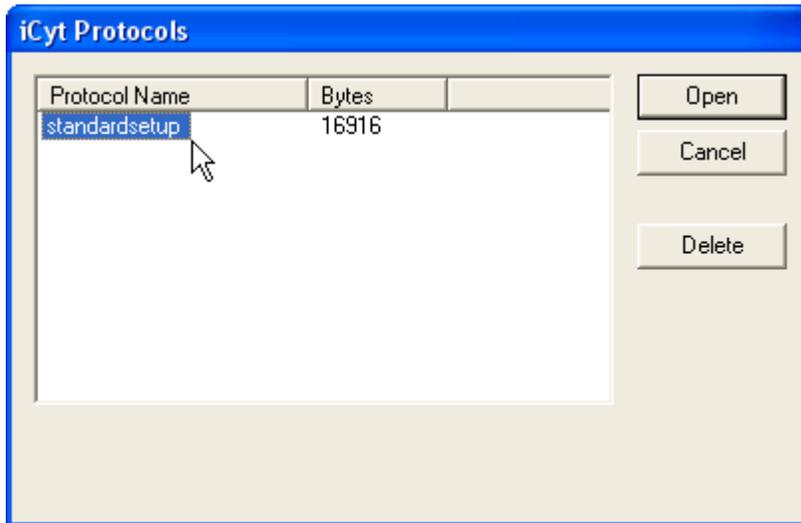
*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

Use this command to reopen a connection file that was previously opened in the current data source window.

**iCyt: Open Protocol on iROC Server**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

This dialog is used to open a WinList protocol that has been previously stored on an iROC Server. Saved protocols are available to all users connected to the iROC server, regardless of who saved the protocol.



The dialog displays a list of protocols that are available on the server. If the list is empty, no protocols have been saved.

**To open a protocol:**

Select a protocol in the list and click the Open button.

**To delete a protocol from the server:**

Select a protocol from the list and click the Delete button.

**Links:**

[Terminology](#)

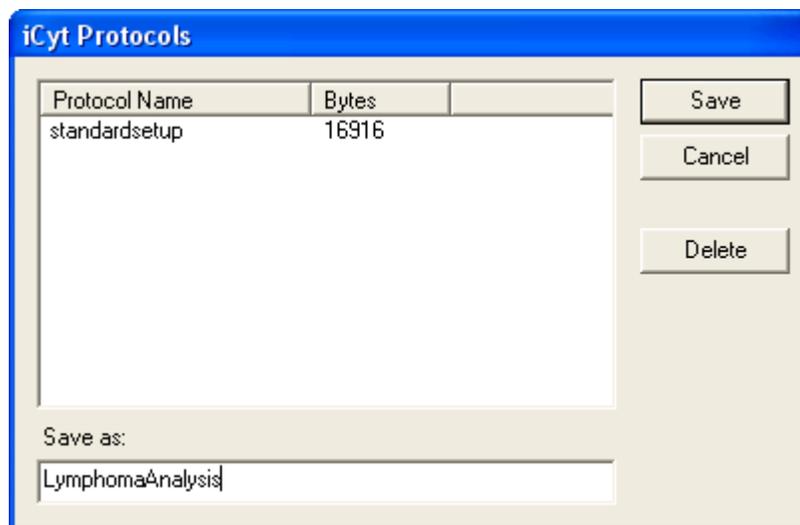
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**iCyt: Save Protocol on iROC Server**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

This dialog is used to save a WinList protocol to an iROC Server. Once saved, the protocol becomes available to all users connected to the iROC server.



The dialog displays a list of protocols that are available on the server. If the list is empty, no protocols have been saved.

**To save a protocol:**

Type the name for the new protocol in the Save As edit control, or select an existing protocol in the list.

Click the Save button.

**To delete a protocol from the server:**

Select a protocol from the list and click the Delete button.

**Links:**

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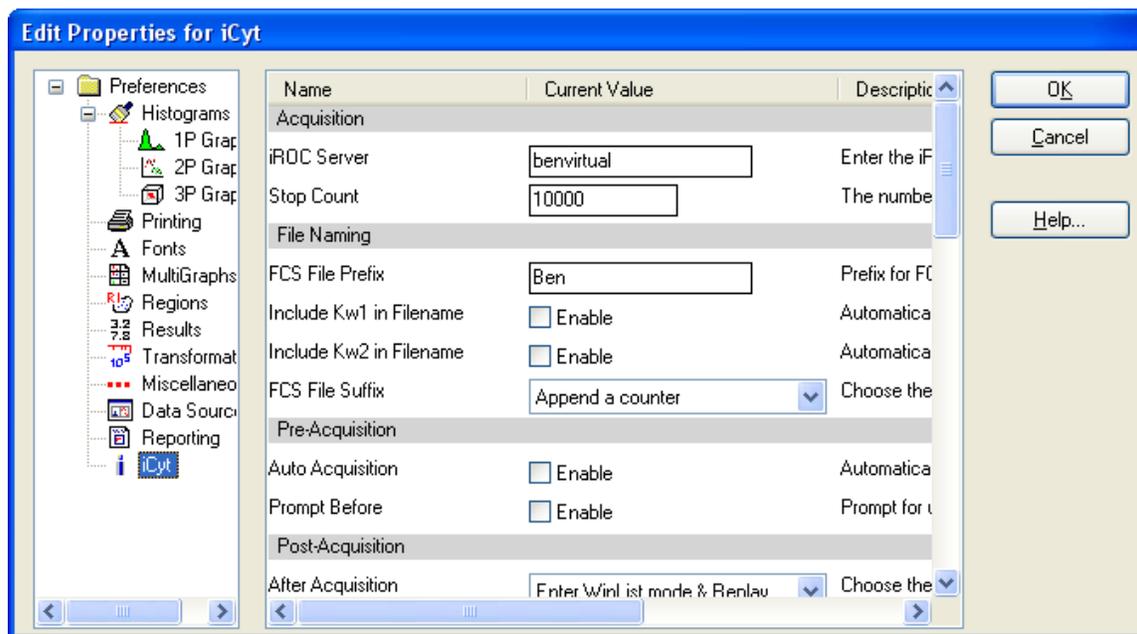
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**iCyt: Preferences**

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

This dialog displays settings that control various aspects of the iCyt acquisition system.



#### Acquisition

##### iROC Server

Enter the name or IP address of the iROC server that you want to connect to by default. You can use the Connect to iROC Server command to connect to a different server at any time.

If you leave this field empty, WinList will not connect to an iROC server when it starts up.

##### Stop Count

Set the default stop count for acquisition.

#### 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

##### Auto Acquisition

When this option is enabled, WinList will automatically begin to acquire from connected iCyt HAPS modules whenever the Run ID on the instrument changes. This allows the program to automatically store files as tubes are being changed at the instrument.

**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 iCyt toolbar.

### 1P Histogram Area

This setting determines how frequently WinList resets 1P histograms during Monitor mode. When the selected area is reached, the 1P histogram will clear and start accumulating again.

### 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/iCyt 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.

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### iCyt: Troubleshooting

*This option is only available in special versions of the WinList supplied through iCyt Mission Technology.*

### Connecting with an iROC Server

If there is a problem establishing a connection to an iROC server, there are a number of steps you can perform to troubleshoot the problem.

1. Ensure that you have network connectivity. Can your browser connect to web pages?
2. Check for firewalls that may be blocking connections. Firewalls are commonly used to protect networks from unauthorized access, and you may need to interact with your IT department to gain access to the iROC server.
3. Try using an IP address instead of a computer name for the iROC server. If there is no name resolution service available, your computer may not be able to connect to the iROC server by using its name.
4. Try to "ping" the iROC server, either by name or by IP address. Ping is a command-line utility that is used to detect connectivity to an IP address. An example is shown below.

```

C:\WINDOWS\system32\cmd.exe

C:\temp>ping Ben8200

Pinging ben8200.vsh.com [192.168.100.111] with 32 bytes of data:

Reply from 192.168.100.111: bytes=32 time<1ms TTL=128

Ping statistics for 192.168.100.111:
    Packets: Sent = 4, Received = 4, Lost = 0 (0% loss),
    Approximate round trip times in milli-seconds:
        Minimum = 0ms, Maximum = 0ms, Average = 0ms

C:\temp>

```

### Sluggish Performance

There are two primary factors to consider if the performance of the WinList-iCyt connection is slow: the capabilities of the computer running WinList, and the speed of the network connection.

If you notice that dot plots do not display a consistent number of dots while you are monitoring a HAPS module, this is often an indication of a problem with the connection speed over the network. Use the LEDs on the iCyt toolbar to determine whether WinList is waiting for data packets too often (see iCyt: Preferences). Your network administrator should be able to determine whether there are any bottlenecks in the connection between WinList and the iROC server. Keep in mind that the slowest part of the connection will determine the speed of the connection.

#### To adjust the throughput for a connection:

WinList has a preference setting that can be used to smooth out the performance over slow connections: *Maximum Events Per Second*. This is found in the iCyt: Preferences dialog Throughput Control section. Here's how to adjust the settings for a slower connection.

Note: This adjustment does not affect the speed at which the HAPS module is operating. WinList may drop some events if the connection speed is slower than the HAPS module speed.

1. Run WinList, connect to an iROC server, and establish a monitoring connection with an iCyt HAPS module.
2. Create a typical analysis setup with histograms, regions, and results.
3. Click the WinList mode button to stop monitoring.
4. In the iCyt Preferences dialog, set *Maximum Events Per Second* to the lowest possible setting and then click OK.
5. Click the Monitor button to begin monitoring again and re-establish a connection. This new connection will use the new setting.
6. Repeat steps 3 through 5, each time selecting the next higher setting for *Maximum Events Per Second*. When the performance becomes sluggish, you have reached the capacity of the connection. Choose a lower setting.

#### To determine the capabilities of the computer:

1. Create a connection to an iROC server and an iCyt HAPS module.
2. Create one dot plot, and then click the "Q" button on the plot to create a quadstat.
3. Add additional plots, one at a time, and gradually add complexity to the WinList setup.

As you add more and more plots and complexity, you will begin to see the system reach a point where it becomes less responsive to user actions.

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## Appendices

### Appendix A: DDE Commands

*This feature is available only on PC platforms.*

Dynamic Data Exchange (DDE) provides a way for Windows programs to share data and interact with one another. WinList currently 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 currently 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 datasource 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. [STOT] 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 were 01-Mar-96 and the time were 09:20:45, WinList would display:

Date: 01-Mar-96 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 <i>n</i>	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

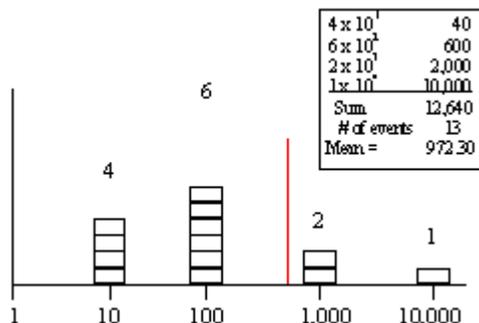
### Appendix C: Understanding Mean values

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's an explanation.

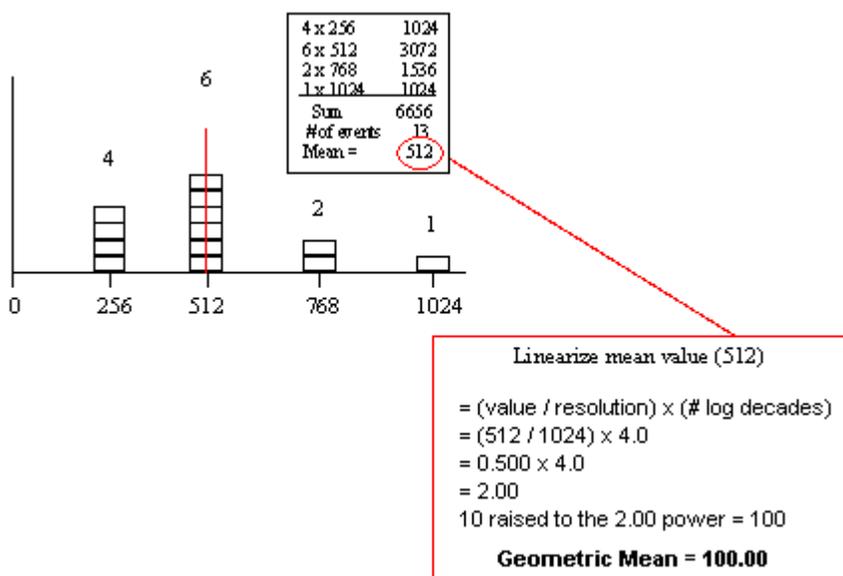
### Linear Mean

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.



### 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.

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 channel 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.

Even though the results of the calculated parameter expression are stored as integers ranging from 0 to 1023, all intermediate calculations are performed in floating point.

The formulas in the example use the expressions defined below:

<i>parameter</i>	linear or log listmode parameter to convert
<i>adcRes</i>	ADC resolution of the parameter
<i>decades</i>	number of decades for the log amplifiers in the flow cytometer
<i>linearScale</i>	scale of linearized values
<i>linearScaleFactor</i>	value that will scale <i>parameter</i> from 1024 channels to <i>linearScale</i>

#### 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  $FL10(0)$ .

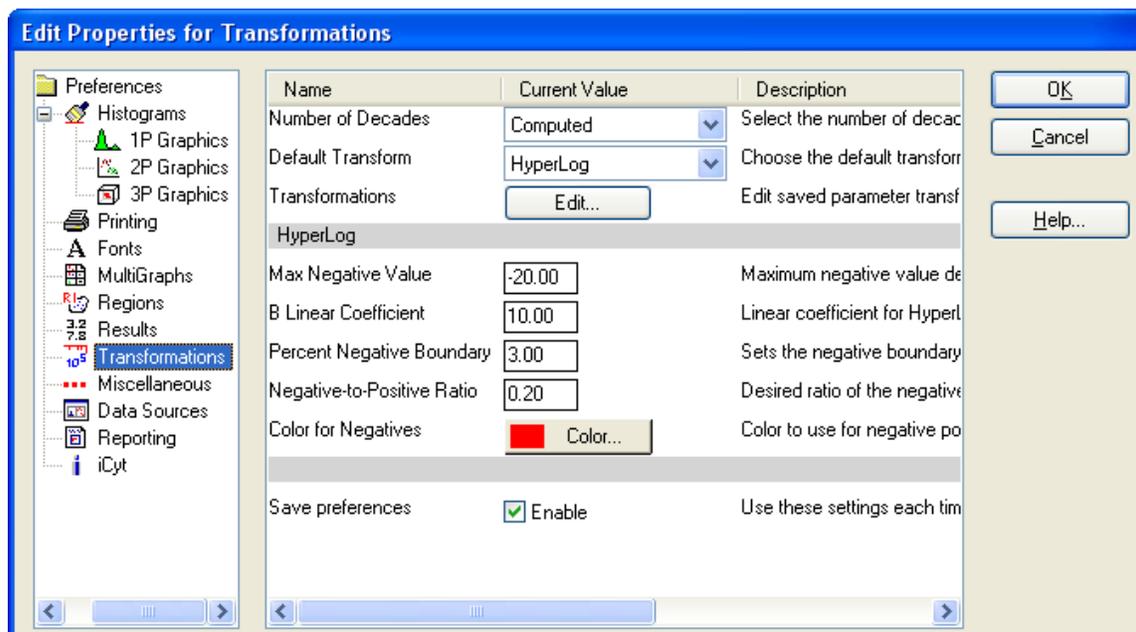
## Appendix E: Understanding Hi-res Data Files

WinList provides support for reading and analyzing high-resolution parameters created by the new 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?

The newer digital flow cytometers acquire listmode data at 16-bit and higher resolutions. Most of these instruments 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.

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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