WinList User Guide

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Updated 4 August 2021

This guide is intended for WinList 10.0 and minor version updates.

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WinList[™] 10 User Guide



Updated 3 August 2021 This guide is intended for WinList 10.0 and minor version updates.

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Network versions of software require one serial number for each simultaneous use. Network versions are intended for installation and use on a local area network; other uses are not supported.

Backups of the software must be used only for archival purposes.

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Latest Versions

To find the latest versions for this software and documentation, visit the Verity Software House web site at <u>www.vsh.com</u>. 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 <u>verity@vsh.com</u> 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 <u>Check for Updates</u> command in the Help menu. Use this command periodically to see if there are updates for your software.

What's new?

WinList 10.0

Implemented Cen-se' dimensionality-reduction algorithm to produce structured nearestneighbor embedding with fantastic speed and resolution, and process all events in the FCS file

Major performance improvements for t-SNE implementation, with the ability to process all events in the FCS file

Reports update much faster and without interruption to the user interface

Improve the drawing speed for 2P overlays using Dots.

Revise the Compensation Page to provide a more efficient interface for working with highdimensional data files

Improved transform defaults for high-resolution data from Helios, Aurora, and others

Provide a means to apply a transform defined on one measurement to other measurements

Provide more robust memory handling for large data files and low memory conditions

Scrollable data source windows allow for better visualization of histograms

Support for editing properties of individual overlay elements

Display histograms on more than one page in a data source to facilitate gating

Support import of tab-delimited text files containing listmode data

Support for FCS 3.2 files

Remove limitation on number of lines in a macro

Add support for "contains" operator in text-based Alert conditions

Improved support for high DPI monitors

Improved network license manager

Simplified registration for multiple users on the same computer

All reported bugs for previous versions fixed

System Requirements

Minimum System Configurations

PC: Windows 10 or greater, 3GHz CPU or better, 8GB RAM or greater, 8 or more logical processors, 100MB free disk space for program files.

Faster CPU with more cores, high-speed SSD drives, and greater RAM will improve performance, especially with large FCS files.

Installing and Running WinList

IMPORTANT NOTES:

- Stand-alone versions of the program are designed to be *installed and registered on a single computer*.
- 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.
- 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.
- If your program is an update version, you will need the serial number for the previous version in order to register and unlock the software.
- If your program is an additional computer license, you will need the serial number for the primary license in order to register and unlock the software.

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 downloaded the software, extract the installation files from the download into a temporary folder. If you have an installation CD, insert the program CD into the CD-ROM drive.
- Run the program named **WinListSetup.exe**. Follow the directions on the screen to complete the installation.
- See the section on <u>registering your software</u> for information on how to register your software.

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 navigate to the Verity folder. Choose **WinList 3D 10.0**.

Network Version

- Log on to the "server" computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- If you downloaded the software, extract the installation files from the download into a temporary folder. If you have an installation CD, insert the program CD into the CD-ROM drive.
- Double-click the **InstallAll.vbs** script to install all of the software components.
- This will install WinList 3D as well as an application called the Network License Manager on the computer. Alternatively, you can double-click each msi file that appears on the cd to install the Network License Manager and WinList.
- See Using the Network Version for details on configuring and using the network version of the software.

Registering Your Software

Why You Should Register

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, which requires you to register your software in order to receive an **Unlock Code** to make the program fully functional. An unregistered installation will be fully functional for 30-days, and then become a "trial version" with limited functionality until it is registered.

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 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:	1.0.9			
Computer ID:		Help		
Serial #:				
CD Key:	E-A7TBOR			
Unlock Code:	WOPYWHULK33PH/QLAZMPHOLIA	ОК		
Web Registration Deregister		Cancel		

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 program box or in email if the program was provided to you by download. Enter these exactly as they appear; they are case-sensitive. If you have lost or misplaced the Serial number or CD Key, contact Verity technical support at tech@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 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. The web site will generate an email to you with an *Unlock Code*.

When you receive your Unlock Code email, 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 <u>www.vsh.com</u>.

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. The web site will generate an email to you with an *Unlock Code*.

When you receive your Unlock Code email, 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 you have any questions, please contact us. See <u>Contacting Verity Software House</u> for phone numbers and addresses.

Contacting Verity Software House



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Phone:	(207) 729 6767	

Tutorials

WinList Basics

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

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

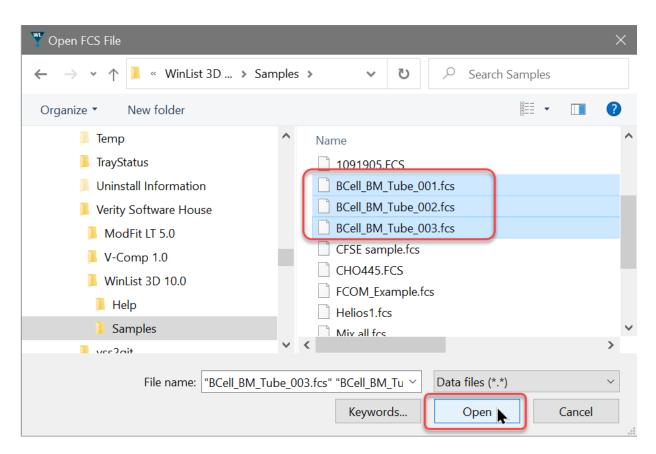
Open files

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



2. In the File Open dialog, navigate to the Samples folder located in the folder where WinList is installed. Select the three files **BCell_BM_Tube_001.fcs**, **BCell_BM_Tube_002.fcs**, and **BCell_BM_Tube_003.fcs** and click **Open**.

WinList Basics



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

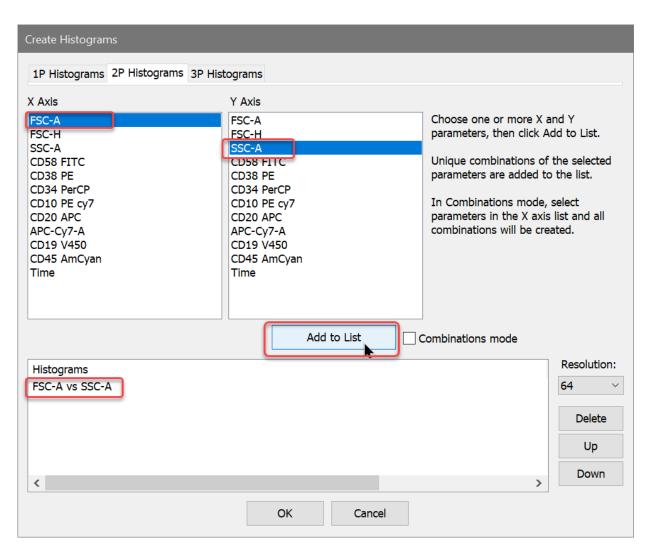
The Create Histograms dialog will appear.

Create histograms

This dialog allows you to create any number of histograms very quickly. We will create several 2P histograms. The dialog also allows you to create 1P and 3P histograms.

3. Select FSC-A in the X Axis list, and SSC-A in the Y Axis list. Then click the **Add to List** button.

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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.

WinList Basics

Create Histograms				
1P Histograms 2P Histograms 3P Histograms				
X Axis	Y Axis			
FSC-A FSC-H SSC-A CD58 FITC	FSC-A FSC-H SSC-A	Choose one or more X and Y parameters, then click Add to List. Unique combinations of the selected		
CD38 PE CD34 PerCP	CD58 FITC CD38 PE CD34 PerCP	parameters are added to the list.		
CD10 PE cy7 CD20 APC APC-Cy7-A	CD10 PE cy7 CD20 APC APC-Cy7-A	In Combinations mode, select parameters in the X axis list and all combinations will be created.		
CD19 V450 CD45 AmCyan	CD19 V450 CD45 AmCyan			
Time	Time			
Add to List				
Histograms Resolution FSC-A vs SSC-A 64 CD10 PE cy7 vs CD20 APC FSC-A				
CD10 PE cy7 vs CD45 AmCyan CD20 APC vs CD45 AmCyan		Delete		
		Up		
<		> Down		
OK Cancel				

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

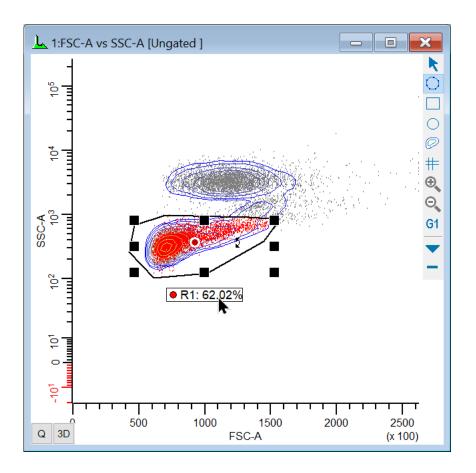
Next, we'll draw a light-scatter gate on the FSC-A vs. SSC-A histogram.

6. Click the title bar of the FSC-A vs. SSC-A histogram and select the Freehand region tool from the toolbar on the right side of the window.

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, click on the region's label and press the Delete key on the keyboard.

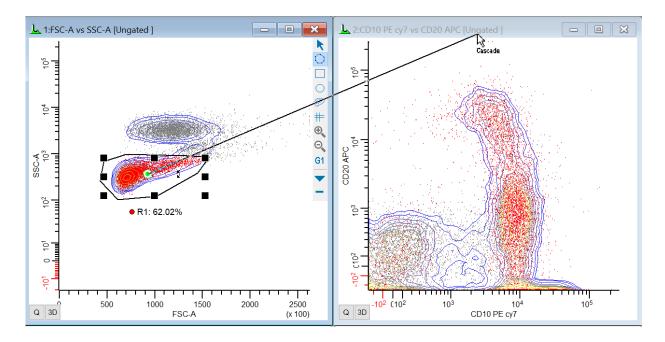


Gate the histograms

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 exit cascade gating mode.

Create QuadStats for statistics

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

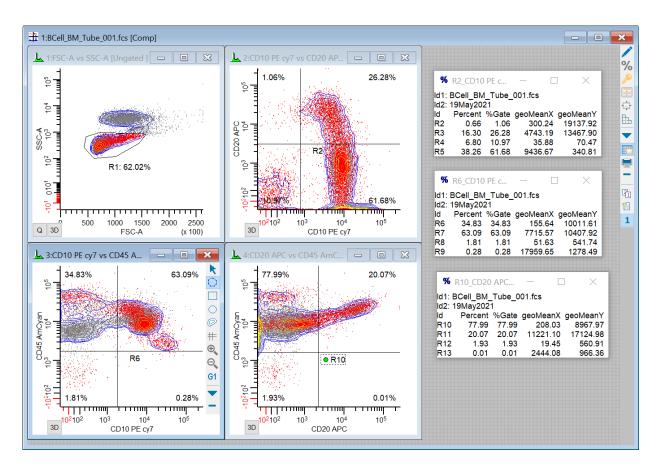
11. Click the **Q** button in the lower-left corner of each of the gated histograms. A QuadStat region will appear on each histogram, and a Results window for each QuadStat will be displayed.

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

You can select the statistics that are shown in a Results window by double-clicking the title bar and selecting the stats. This can be different for each Results window. You can also save your preference for which statistics to show.

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.

WinList User Guide



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 region.

Create a report

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

14. Click the **Report** button on the ribbon bar. Move and size the new report window so that it is not covering histograms and result windows.



15. Move the mouse over the icon on the left side of the first histogram's title bar. Drag and drop that icon onto the report.

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

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



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

Process the files

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

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



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

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

0

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.

When you reach the last file in the batch, the **Continue** button is enabled.



This allows you to make adjustments to the last file before the analysis is completed. After making adjustments, click the Continue button to finalize the analysis, save and print reports, and perform other batch operations on the last batch item.

Save the protocol

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

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

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

Summary

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

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

See also:

Tutorials Cascade Gating and Boolean Gates WinList User Guide

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

Open a file

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



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

You can also drag and drop files from Windows Explorer onto WinList. See <u>Drag and</u> <u>Drop</u> for details.

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

Draw 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.

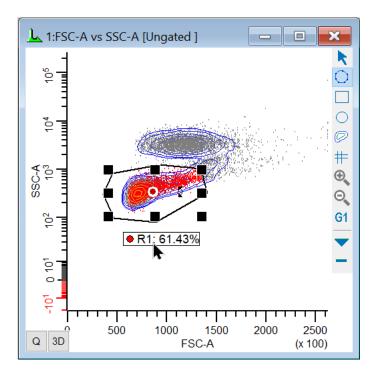
4. Click and hold the mouse button in the data source window. With the mouse button down, move the mouse to define the size and location for a new histogram window. Release the mouse to finish the histogram drawing operation.

해 1:BCell_BM_Tube_001.fcs			
Creating histogram Ungated			
	5		

5. The Edit Histogram dialog is displayed. Choose **FSC-A** for the X Axis and **SSC-A** for the Y Axis, and then click **OK**.

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

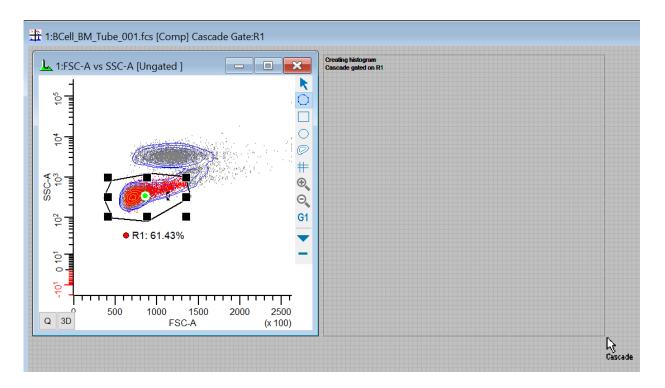
6. Click the **Freehand** region tool in the histogram toolbar, and draw a region around the population as shown. To draw a freehand region, click next to the population of interest and hold the mouse button down. Drag the mouse around the population as shown. To finish drawing the region, click on the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



Create a cascade-gated histogram

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

7. Click the cascade gate hotspot in R1; the red hotspot will change to green. Move the mouse to an empty portion of the data source window. Click, hold, and drag to define a new histogram window.

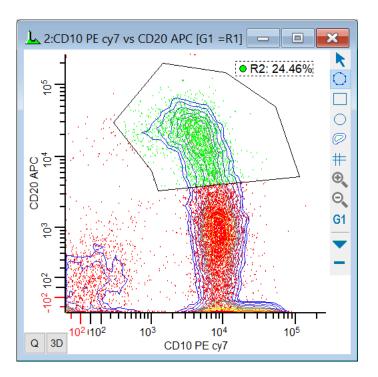


8. Release the mouse button. In the Edit Histogram dialog, select **CD10 PE Cy7** for the X Axis and **CD20 APC** for the Y Axis. Click **OK**. This new histogram will be gated on R1.

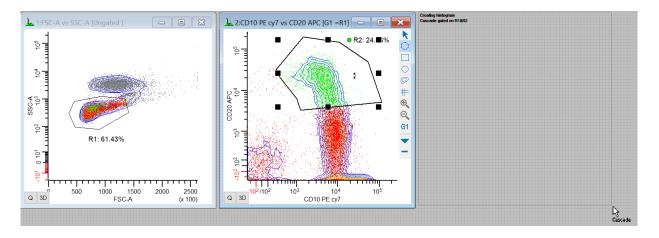
Additional cascade-gated histogram

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

9. Draw a freehand region in the CD10 PE Cy7 vs CD20 APC histogram to identify the CD20+ 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 write our own gate expression. Let's create a new histogram and then use the Edit Gates dialog to define an inclusive gate expression.

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

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

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

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



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

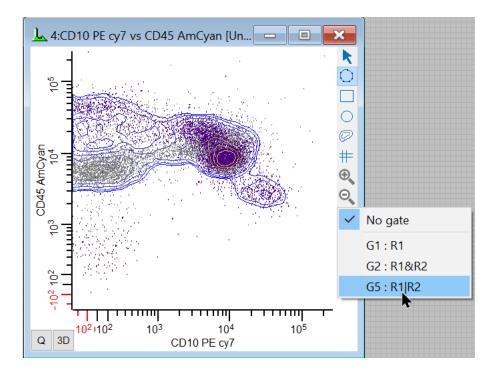
WinList User Guide

Edit Gates						
Applies to: 1: BCell_BM_Tube_001.fcs						
Equation:	R1 R2				And	Or Not
Alias:	G5					
Activation:	Enable auto	matically		~		
A Ungated	lias	Equ	ation	State auto	^	Color
G1		R1		auto		Reset All
G2		R1&R2		auto		
G3		R3		off		Open
G4		R4		off		
G5		R1 R2		off		Save
G6		R6		off		
G7		R7		off		
G8		R8		off		
G9		R9		off	~	
Enable cascade gating to create and adjust gates.						
	0	K	Cancel	Apply		

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

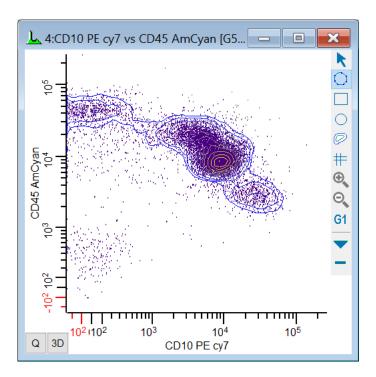
At this point, we have edited the gate expression for gate G5 so that it will include any events that are in *either R1 or R2*. Now we need to apply this gate to our new histogram.

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



There are other ways to apply a gate to a histogram. When the Edit Gate dialog is displayed, select the gate that you want to apply and then click the title bars of the histograms that you want to gate. This is a fast way to apply a gate to many histograms at once.

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



The Boolean "NOT" operator

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

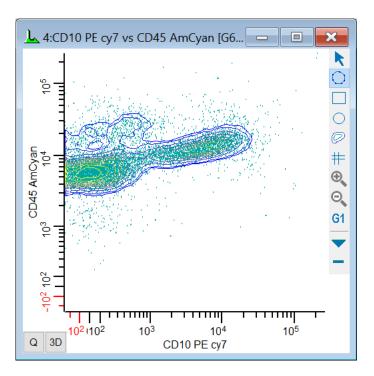
Let's define a gate that includes all events that are *not* in R1.

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

18. Select the "G6" gate in the list. In the **Equation** edit box, delete the current equation. Click the **Not** button to insert the "NOT" operator. Type **R1**. *Leave the dialog open.*

Edit Gates				
Applies to: 1: BCell_BM_Tube_001.fcs				
Equation:	!R1		And	i Or Not
Alias:	G6			
Activation:	Enable auto	omatically	~	
Ungated	Alias	Equation	State 🔨	Color
G1		R1	auto	Reset All
G2		R1&R2	auto	
G3		R3	off	Open
G4		R4	off	•
G5		R1 R2	auto	Save
G6		!R1	off	
G/		к/ 투	off	
G8		R8	off	
G9		R9	off	
Enable cascade gating to create and adjust gates.				
	O	K Cancel	Apply	

19. With G6 selected in the Edit Gate dialog, click on the title bar of the **last** histogram. The histogram's title bar will change to show the new gate name. Click **OK** to close the Edit Gate dialog.



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

More complex Boolean expressions

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

Not R1 And (R2 Or R3)

In WinList's syntax, this would be written:

! R1 & (R2 | R3)

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

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

(R2 | R3) & !R1

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

(R2 | R3) & !R1

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

G2 = **G1** & R4 & R5

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

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

Summary

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

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

See also

WinList Basics Edit Gates

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 <u>basics of working with WinList</u> 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. Probably the best way to explain how trace lines work is with an illustration.

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

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

Turn Off Auto-Compensation

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

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

Automatic compensation

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



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

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

You can also drag and drop files from Windows Explorer or My Computer onto WinList. See <u>Drag and Drop</u> 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**.

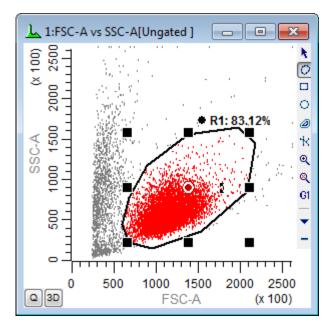
5. In the X Axis list, select **CFP-A**, **GFP-A**, **YFP-A**, **DsRed-A**. Enable the **Combinations mode** checkbox, and click **Add to List**. All combinations of the four fluorochromes will appear in the histogram list. Click the **OK** button.

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Create Histograms		
1P Histograms 2P Histograms 3P His	tograms	
X Axis	Y Axis	
Time FSC-A SSC-A CFP-A GFP-A YFP-A DsRed-A	Time FSC-A SSC-A GFP-A YFP-A DsRed-A	Choose one or more X and Y parameters, then click Add to List. Unique combinations of the selected parameters are added to the list. In Combinations mode, select parameters in the X axis list and all combinations will be created.
	Add to List	Combinations mode
Histograms CFP-A vs YFP-A CFP-A vs DsRed-A GFP-A vs YFP-A GFP-A vs DsRed-A YFP-A vs DsRed-A		Resolution: 64 Delete Up Down
	OK Cancel	

The program will open the first file and display seven scatter plots. The batch toolbar will appear at the bottom of the application window. We're ready to let WinList determine the correct compensation settings by reading each of the control files.

6. Draw a region around the cells of interest in the FSC-A vs SSC-A plot as shown.



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

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



9. In the **Compensate** listbox, click **CFP-A**, **GFP-A**, **YFP-A**, and **DsRed-A** measurements to enable them for compensation. Notice the label shows "On" in the listbox, and an entry appears in the **Show plots for** list for each enabled measurement.

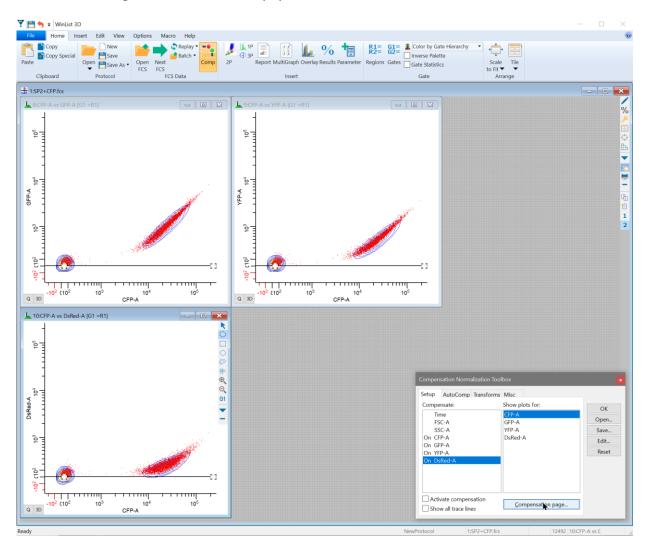
Compen	sation Norma	alization Tool	box		x
Setup	AutoComp	Transforms	Misc		
Compe			Show pl	ots for: OK	
	ne C-A		CFP-A GFP-A	Open	
SS	C-A		YFP-A	Save	
On CF			DsRed-	A Edit	
On GF On YF				Reset	
On Ds	Red-A				
	vate compens w all trace line		Со	npensation page	

Only enable the parameters that you want to compensate in the **Compensate** list.

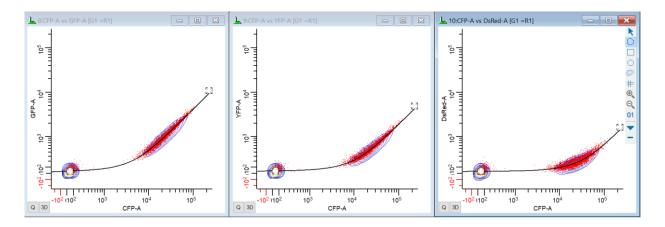
10. Next, click the **Compensation page...** button to create a page specifically designed for setting up compensation. Use this button any time you want to assign a gate to plots on the Compensation page. A dialog is displayed to select a gate for this page. Choose **G1** and click **OK**.

Create Compensation Page	
No gate G1 : R1	ОК
	Cancel
Select a gate for the compensation plots.	

The Compensation page (page 2) is created with three plots showing CFP-A on the X-axis. The Y-axis of each plot shows one of the other compensation measurements. The straight, horizontal lines on the plots are the trace lines. In the next step, WinList will position the trace lines through the centers of the populations.

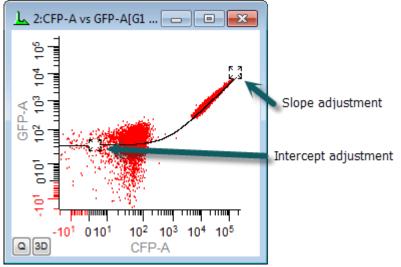


11. Click *CFP-A* in the **Show plots for** listbox, and WinList performs a regression through the events. It positions the trace lines through the median of the populations. This is WinList's automatic positioning routine at work.



Manual compensation adjustments

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



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

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

Processing the remaining control files

With the first single-color control all set, let's process the remaining single-color control files.

12. 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**. You will see the title in the data source window change to show the new file name.

C

13. Click the "DsRed-A" in the **Show plots for** listbox. WinList shows the correct plots for DsRed compensation and automatically positions the trace lines.

14. Click the **Next Batch Item** button to read in the next file, **SP2+GFP.fcs**. Choose the "GFP-A" from the **Show plots for** listbox. Once again, WinList automatically positions the Trace lines.

15. And once more, click the **Next Batch Item** button to read in the next file, **SP2+YFP.fcs**. Choose the "YFP-A" from the **Show plots for** listbox to tell WinList to position the trace lines.

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 spill coefficients have been determined for each fluorochrome, we are ready to apply the compensation.

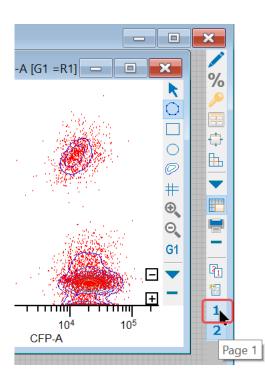
16. Click the **Activate compensation** checkbox.

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, enable the **Show all trace lines** checkbox.

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

17. On the data source toolbar, click the "1" button to return to page 1.

WinList User Guide



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



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

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

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

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

19. Let's save a protocol for this setup so that it can be used in the next tutorial. Click the **Save** button and name the protocol **"CompensationTutorial"**.

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

If you want to apply the same compensation to a series of files, you can use the Next File button on the ribbon bar to open each file into the compensated data source window.

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.

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, select a measurement in the **Show plots for** list, and make the adjustments. When you are finished, turn on the compensation system by checking the **Activate compensation** checkbox.

Summary

WinList's N-Color Compensation system is very quick and easy to use. You can use it to compensate any number of fluorescent measurements for signal crossover. This is best accomplished through the use of single-color control files, which are read one at a time to determine the best compensation settings.

Trace lines are graphical elements displayed in the histograms that you are compensating. WinList can automatically position trace lines using a robust fitting algorithm, and it uses the slopes of trace lines to set up a compensation matrix.

See Also:

<u>Compensation Toolbox</u> <u>Compensation Trace Lines</u> WinList User Guide

Compensation Review Mode

Cytometry assays continue to use more colors, providing a richer platform for understanding cells and biology. At the same time, as the number of colors increases, so does the complexity of properly compensating for fluorescence crossover. Let's explore a method for evaluating just how good your compensation setup is, confirming the strengths and revealing the weaknesses in a way the makes sense.

Compensation Review Mode in WinList is a visual tool that makes this possible. The process is simple: review each single-color control file, one by one, and examine how well the crossover values match the data. Compensation Review Mode performs some optimizations to make this process possible.

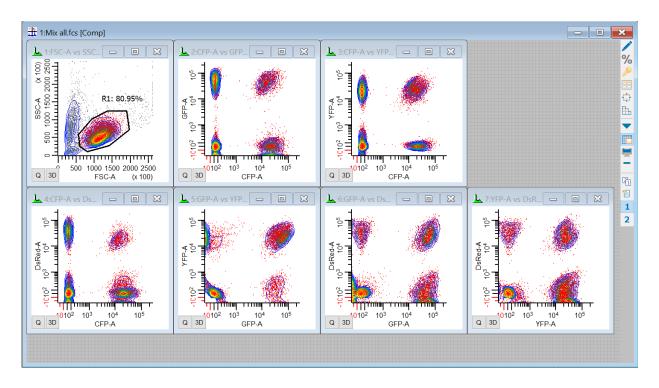
To use this mode you need a set of single-color control files that were used to set up the compensation. You may also want to review one or more all-color files.

Here's how it works. We'll use the compensation that we set up in <u>Using Interactive N-Color</u> <u>Compensation</u> as a test case, so make sure you do that tutorial first.

Open an all-color file

We start by opening an all-color file and applying the compensation that we want to review. That compensation might be loaded automatically by WinList by reading keywords from the file, or it may be compensation that you have set up in WinList.

1. Click the **Open Protocol** button and select "**CompensationTutorial**" - the protocol that was saved in the <u>Using Interactive N-Color Compensation</u> tutorial. There should be a FSC vs SSC plot, and 6 other plots for the fluorochromes.

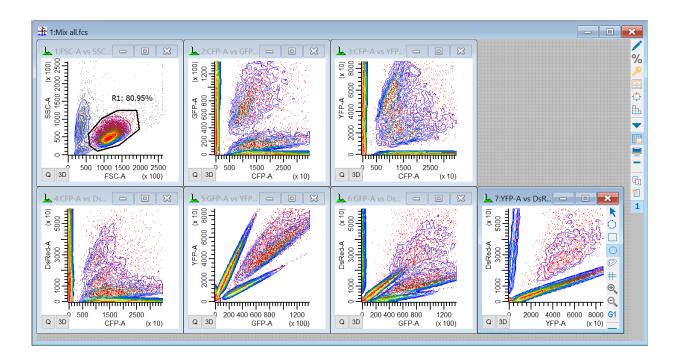


Turn on Compensation Review Mode

2. In the **Compensation Toolbox**, click the **Misc** tab and enabled **Compensation review**.

Compensation Normalization Toolbox	×
Setup AutoComp Transforms Misc	
Normalization Show normalization targets	ОК
	Open
Compensation Information	Save
Compensation review (linear, zoomed plots)	Edit
	Reset
View cross-over matrix Advice	

The display will change dramatically.



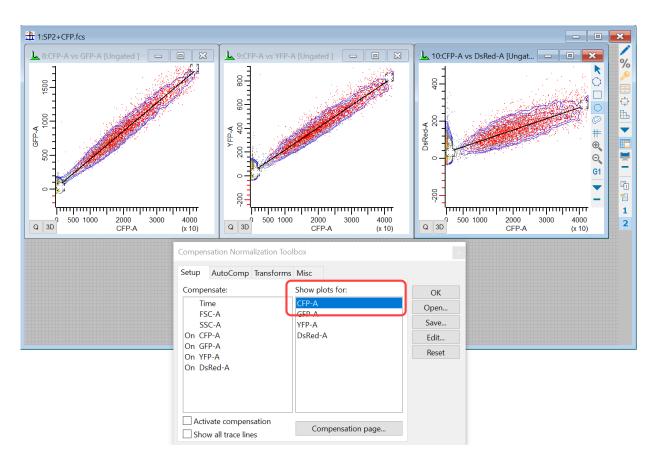
All of the plots that are normally showing a log-like axis will switch to a **linear** axis. Compensation has been temporarily disabled. In addition, the plots will "zoom" to the 95th-percentile. How is this going to help us review compensation? Let's keep going and find out.

Review the first control file

3. Click the **Next Batch** button on the batch toolbar to read in the first single-color control file that we used to set up the compensation.



4. On the **Setup** tab in the **Show plots for** list, select **CFP-A**, since this is the CFP-only control file. This will select the Compensation page, and you should see something like this:



Only 3 plots are shown: the ones that involve CFP-A. The X-axis for each plot is CFP-A, and the Y-axis shows one of the other fluorochromes. The view is zoomed in on the 95th-percentile, and we're looking at linear plots so there are no warping effects from log transformations.

Most importantly, note the position of the trace lines in the plots. If the compensation is set up properly, the trace lines will pass right through the center line of the data. That appears to be the case here, so it looks like the CFP-A compensation is set correctly for the CFP control.

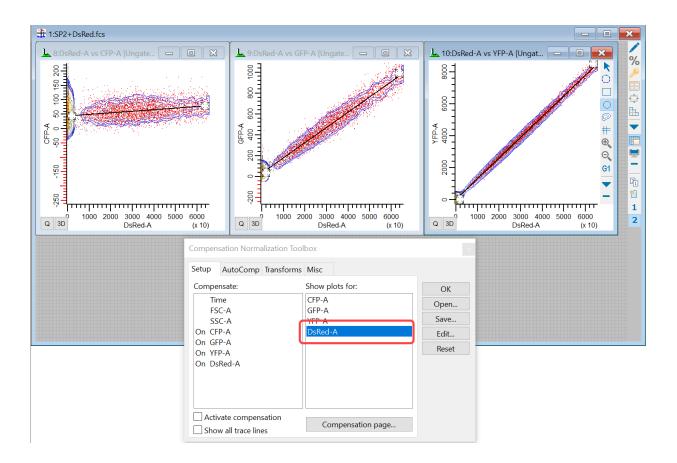
Let's check the other 3 controls in the same manner.

Review the other controls

5. Click the **Next Batch** button on the batch toolbar to read in the next single-color control file.



6. In the **Show plots for** list, select **DsRed-A**, since this is the DsRes-only control file.



Now we see DsRed-A on the X-axis plotted against each of the other markers. The trace lines appear to pass through the center of the linear-displayed clusters, so the DsRed looks correct.

7. Click the **Next Batch** button on the batch toolbar to read in the next single-color control file.



8. In the **Show options for** list, select **GFP-A** for the GFP-only control file.

1:SP2+GFP.fcs		
B:GFP-A vs CFP-A [Ungated]	3 9:GFP-A vs YFP-A [Ungated] (0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,000 0,00	10.GFP-A vs DsRed-A [Unget □ > 10.GFP-A vs DsRed-A [Unget □ > 000+ 000+ ○ ○ 0000+ 0000 ○ ○ ○ 0000+ 0000 ○ ○ ○ □ 0000+ 0000 ○ ○ ○ □ □ 0000- 0000- 0000- ○ ○ □ □ □ 0000- 0000- 0000- 0000- 0000- 0000- 0000- □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ □ <td< td=""></td<>
Setup Comp T P S On C On C On Y On L	FP-A	OK Open Save Edit Reset

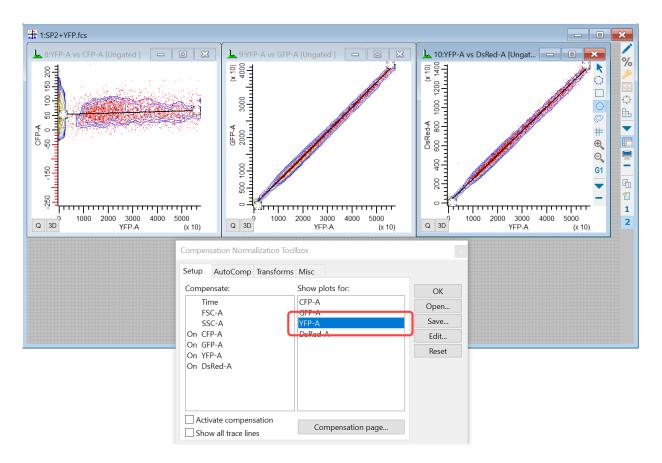
GFP also looks correct. We've got one more to review.

9. Click the **Next Batch** button on the batch toolbar to read in the last single-color control file.



10. In the **Show plots for** list, select **YFP-A** for the YFP-only control file.

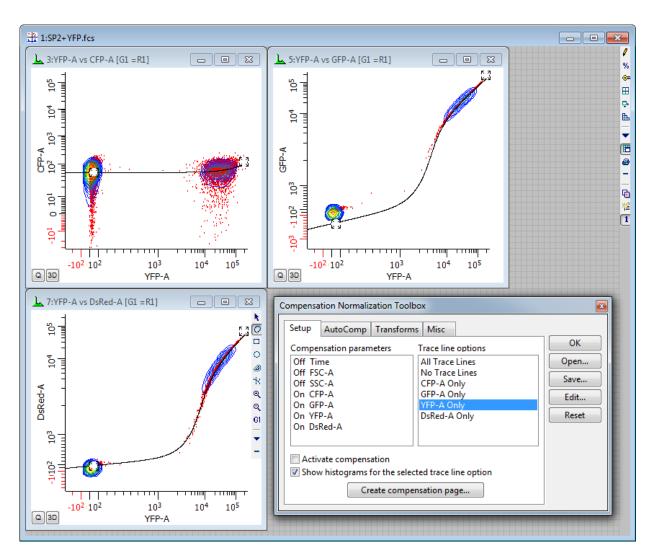
WinList User Guide



Once again, the trace line passes smack through the center of the distributions.

Interpretation

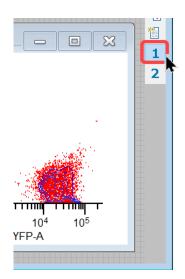
In Compensation Review mode, you are looking to see that the trace lines for each singlecolor control pass through the center (median) of the zoomed, linear distribution. That's one of the things Compensation Review mode does automatically for us. It's easier to see this when looking at linear axes with any high-end outliers clipped out. To demonstrate that point, here's the YPF control unzoomed and with Hyberboline Sine axes:



With this display, it is difficult to see what was quite simple and obvious in the zoomed, linear display.

You can also review the all-color file in Compensation Review mode. To do so, you open the all-color file and then use **Show plots for** to show each of the markers in turn.

11. When you finish with compensation review, uncheck the **Compensation review** option on the **Misc** tab of the **Compensation Toolbox**. Then switch back to page one of the data source to see all plots once again.



Summary

Compensation Review mode is a tool that simplifies the process of assessing fluorescence compensation. By switching transformations to linear and zooming in on the data, it becomes a simple task to determine whether a trace line passes through the center of a distribution. It is best used with single-color controls, but can also be used to review compensation on an all-color file.

Creating Reports

When you need to generate reports that contain graphics, text, and results, WinList's reporting system provides the tools to create multi-page documents with a professional, polished look. Reports are page layout windows that can contain any number of elements. These elements can update any time the data you are analyzing changes. Reports can be exported as PDF files so that you can easily share your analysis results with others who do not have access to WinList.

This tutorial takes an in-depth look at the reporting features of WinList. In the process, you will become familiar with the simplicity and flexibility of reports, and learn how to use reports in your own analysis protocols.

If you are unfamiliar with opening files and creating histograms with WinList, you might want to do the <u>WinList Basics</u> tutorial before continuing with this one.

Setup

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

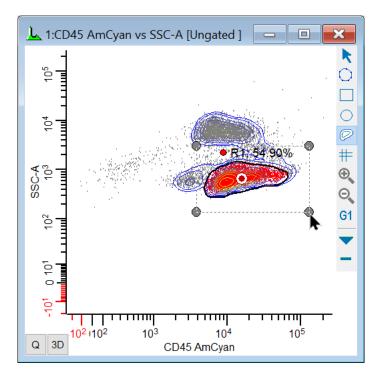
1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **BCell_BM_Tube_003.fcs** from the list and click **Open**.

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

2. When the **Create Histograms** dialog appears, select **CD45 AmCyan** in the X Axis list, **SSC-A** in the Y Axis list, and click **Add to List**. Select **CD10 PE** in the X Axis list, **CD20 APC** in the Y Axis list, and click **Add to List**. Click **OK**.

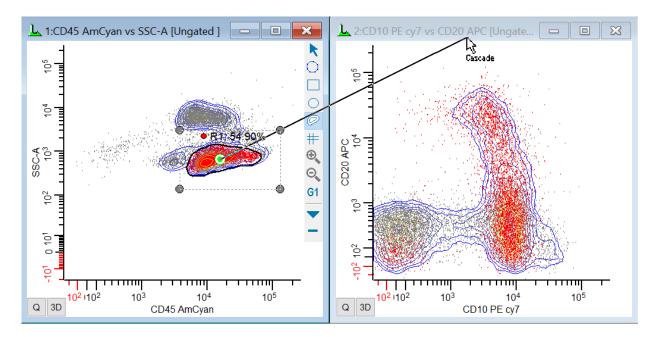
Create Histograms		
1P Histograms 2P Histograms 3P Hist	tograms	
X Axis	Y Axis	
FSC-A FSC-H SSC-A CD22 FITC CD9 PE CD34 PerCP CD10 PE cy7 CD20 APC APC-Cy7-A CD19 V450 CD45 AmCyan Time	FSC-A FSC-H SSC-A CD22 FITC CD9 PE CD34 PerCP CD10 PE cy7 CD20 APC APC-Cy7-A CD19 V450 CD45 AmCyan Time	Choose one or more X and Y parameters, then click Add to List. Unique combinations of the selected parameters are added to the list. In Combinations mode, select parameters in the X axis list and all combinations will be created.
	Add to List	ombinations mode
Histograms CD45 AmCyan vs SSC-A CD10 PE cy7 vs CD20 APC	<u>, r</u>	Resolution: 64 ~ Delete Up Down
	OK Cancel	

3. Use the Contour region tool to create a box around the lymphocytes in the CD45 vs SSC histogram.

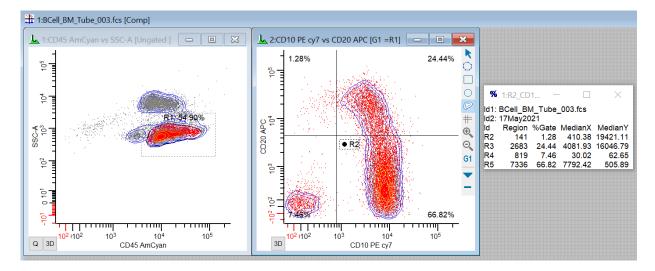


Contour regions are defined with a bounding rectangle. The contour region recomputes when data in the histogram changes.

4. Use the cascade gate hotspot in the center of R1 to apply the R1 gate to the CD10 PE vs CD20 APC histogram.



5. Click the \mathbf{Q} button in the lower-left corner of the CD10 PE vs CD20 APC histogram to create a QuadStat region. Adjust the QuadStat and move the associated Results window to a convenient location on the screen.



Create the report

Now that we have a simple analysis setup, let's create a report and add some elements to it.

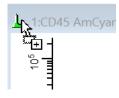
6. Click the **New Report** button on the ribbon bar to create a new, empty report window. Move and resize the new window so that you can see the histograms and results windows.



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 context menu for the report.

Adding histograms to the report

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



For a detailed explanation of drag-and-drop support, see the <u>Drag and Drop</u> section.

7. 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.

8. Double-click on the newly added histogram object on the report. The **Edit Histogram Link** dialog is displayed.

Fully 1 lists and as 1 in h	
Edit Histogram Link	
Choose a Data Source and Histogram:	
1:BCell_BM_Tube_003.fcs ~	ОК
1:CD45 AmCyan vs SSC-A	Connact
2:CD10 PE cy7 vs CD20 APC	Cancel
Caption:	
Show name and gate $\qquad \qquad \lor$	
Graphic quality:	
Normal quality \sim	
Update options:	
Update when histogram changes $~~$	
✓ Transparent background	

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.

9. Click the **Cancel** button to close the dialog without making changes.

10. Use the same drag-and-drop technique to add the second histogram to the report, placing the new object below the first one.

You can always adjust the size and position of objects after you've added them to a report. Click and drag and object to move it. When it is selected, use the sizing handles to re-size the object.

Adding results to the report

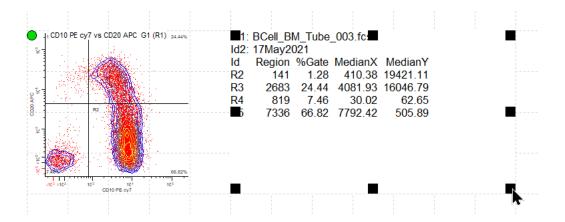
We could drag-and-drop the results window onto the report in the same manner, but let's use the report toolbar instead.

11. Click the Results button on the Report toolbar.

%

The **Edit Results Link** dialog is displayed. This is very similar to the Edit Histogram Link dialog, except that it allows us to select a Results window. There is also a **Properties** button that edits the font used by this object.

12. Click the **OK** button to accept the selection and create the object. Drag the new results object next to the second histogram object. Re-size the results object if it is not large enough to see all of the results.



Adding FCS keywords to the report

It is often useful to include on the report information contained in the FCS keywords from the listmode files that you analyze with WinList. The Keywords object makes it very simple to do this.

13. Right-click to the right of the first histogram on the report. This will display the report context menu.

14. Select **Insert Keywords** from the menu. The **Edit Keyword Object** is displayed. In the **Keyword Groups** tree on the left, under File Keywords choose **DataSource 1**. Notice that the **Available Keywords** list on the right fills with keywords contained in the file.

15. 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.

Edit Keyword Object			
Keyword Groups	Available Keywords		
Results File kowwords DataSource 1 G1 Ungated Histogram keywords DS1.H1 DS1.H2 Page Keywords Region keywords CR2 R3 R4 R5 System keywords	Keyword • ColorMode • DS1:\$BEGINANALYSIS • DS1:\$BEGINDATA • DS1:\$BEGINSTEXT • DS1:\$BTIM • DS1:\$BTTEORD • DS1:\$CYT • DS1:\$DATATYPE • DS1:\$DATE • DS1:\$DATE • Selected Keywords DS1:\$CYT: [Root Object/Results/	Value Color by 0 3172 0 15:15:2 4,3,2,1 FACSCa F 03-JUN- > File keyword	OK Cancel Add Properties • Edit • Preview
	Update when data changes	~	

16. Use this same technique to add **DS1:\$DATE** and **DS1:TUBE NAME**. You many need to scroll down in the Available Keywords list to locate these keywords. 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.

17. 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.

18. Click the Text tool on the report toolbar to create a new text object.



In the Text field, type **CD10/CD20 Report**. Click the **Font** button, select a typeface that you like, and change the font to size 36. Change the **Alignment** to **Center**. Click **OK**.

Edit Properties for Text				
-⊞ Text T Fi B T	Fext Font Background Fransparent	Current Value CD10/CD20 Repo Font Color Color	Description Enter text. Choose a font. Choose a background color Enable for a transparent object. Determines how the text is aligned.	OK Cancel Help

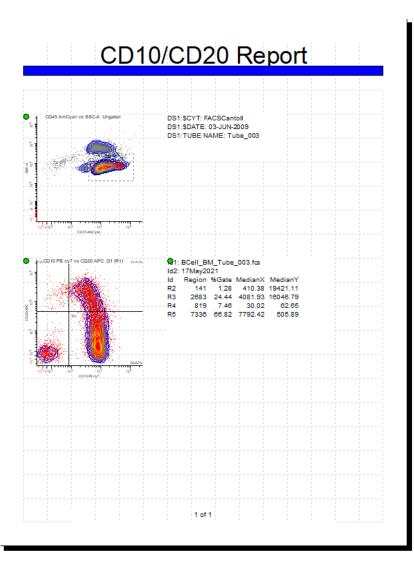
19. Move the new text object to the top of the report page and stretch it across the width of the page.

You can change the scaling of the report page by using the Zoom dropdown on the report toolbar.

20. 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,.

21. 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**.

22. Now, click the **Page 1** tab at the bottom of the report window to see the items from the master page and page 1. The report should look similar to the one shown below at this point.



Adding your own graphics

You can add your own graphics to your reports easily. Open the graphic you want to add in a graphics program and copy it to the clipboard. Then, activate the report window and paste the graphic by pressing Ctrl-V or using the right-click context menu.

Adding a gate legend and impressions

The report can include a legend that explains the colors that are shown in the histograms. Let's add a gate legend object, and then another text object that will allow us to enter comments about the analysis.

23. 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.

24. 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. An **Edit** dialog is displayed that allows you to type and view multiple lines of text. Press **Enter** again, and type "This analysis shows CD10 and CD20 expression for the selected lymphocytes.""

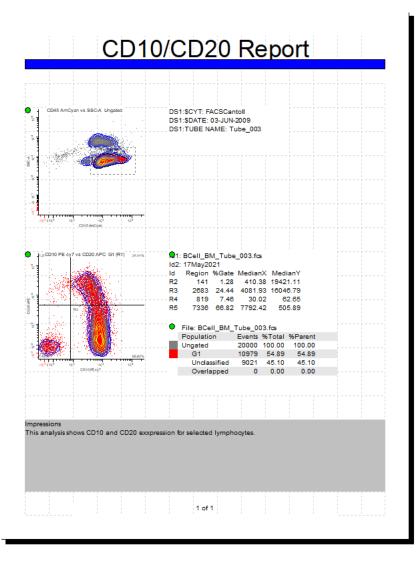
dit		
Impressions This analysis shows CD10 and CD20 e	xxpression for selected h	ymphocytes.
< Fype the text, then click OK.		>

25. 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.

26. Click the **Background** button, choose a light gray color from the color dialog, and click **OK**. Next, uncheck **Transparent** to disable transparency.

27. 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.

28. Move the R1 region on the first histogram window. Notice that after the histogram windows and results update, the objects on the report also update. Similarly, if you move the QuadStat region, the report will update to reflect the changes.

Histogram and result objects on the report display a symbol in the upper left corner to indicate whether the object is actively linked or not. The symbol for a linked object is a green-filled circle. When the object is not linked, the symbol is disappears.

Suppose that you want to make changes in the histograms and you do *not* want the report to change. This is easily accomplished by clicking the **Active Links** button on the report toolbar.





This is a toggle button. In the depressed position, the report objects update automatically, and when it is not depressed, the objects do not update.

Additional pages

Reports can include any number of pages. The **Pages** button on the report toolbar displays a menu of page commands for adding, removing, and navigating pages in the report.

29. 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.

30. 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

When you save a protocol or bundle, the report is automatically saved for you. The report will be reopened when the protocol or bundle is opened. You can also manually save a report file to use as a report template for other protocols.

31. To save the report for use in another protocol, 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.

32. To export the report as a PDF, 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:

<u>Report Windows</u> <u>Report Toolbar</u> <u>Report Context Menu</u> Report Page Menu

Using Overlays

Overlay windows are a great tool for graphically comparing a set of 1P or 2P plots. Offering different views and a wide variety of graphical options, overlays organize plots - typically from different files - so that you can easily see the similarities and differences among them. This tutorial explores how to create overlays and some of the properties you may find most useful.

If you are unfamiliar with opening files and creating histograms with WinList, you might want to do the <u>WinList Basics</u> tutorial before continuing with this one.

Getting Started

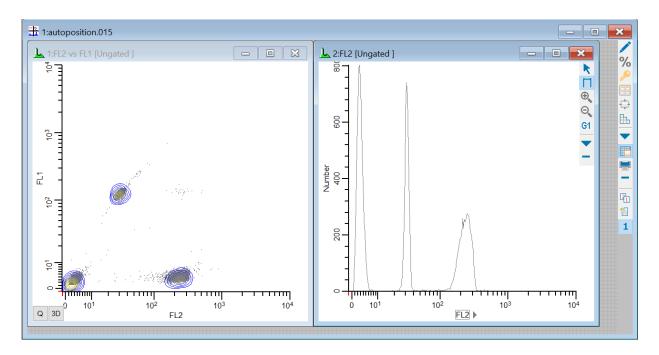
We'll start by opening a listmode file and creating some histograms.

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples\AutoPosition folder located in the folder where WinList is installed. Select **autoposition.015** from the list and click **Open**.

2. In the **Create Histograms** dialog, select **FL2** in the X Axis list and **FL1** in the Y Axis list. Click the **Add to List** button.

3. Click the **1P Histograms** tab in the dialog. Choose **FL2** in the X Axis list and click the **Add to List** button.

4. Click **OK** to close the dialog and display the histograms.



We're going to look at a series of samples in which the intensity of the marker in FL2 diminishes, and we will overlay the graphics to compare the changes. Let's create two overlay windows, one for the 1P graphics and the other for 2P graphics.

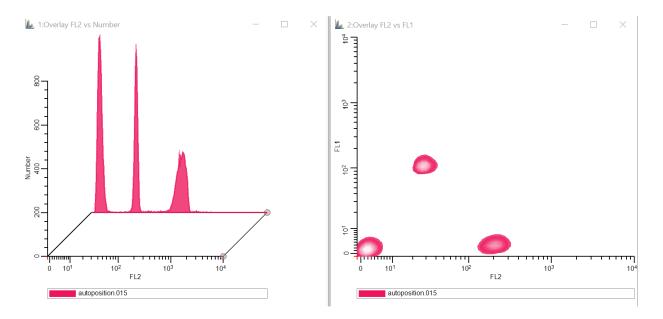
5. Click the **Overlay** button on the ribbon bar **two times** to create two overlay windows. Move the windows to a convenient location on your screen.



To add a histogram to an overlay, you simply drag-and-drop the histogram by the icon in the top left corner of its title bar.



6. Drag-and-drop the 1P histogram onto the first overlay window, and then drag-and-drop the 2P histogram onto the second overlay.



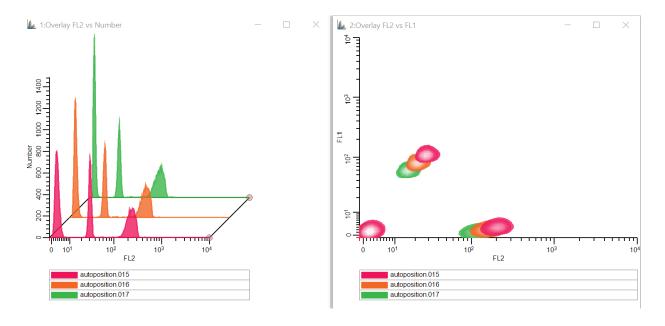
The first element added to an overlay window determines several key properties for the overlay. The overlay will become a 1P or 2P overlay depending on the type of histogram that is added. The axis displayed for the overlay is based on the first element, too. Let's add a couple more elements to each overlay.

7. Click the **Next FCS File** button on the ribbon bar and select the next file in the folder: **autoPosition.016**. Click Open to read the file into WinList.

8. Drag-and-drop each histogram onto the associated overlay window to add new elements for this new data file.

9. Click the **Next FCS File** button and select **autoPosition.017**. Click Open to read the file.

10. Drag-and-drop each histogram onto the associated overlay window. You should now have 3 elements in each overlay window.



Editing Overlays

Notice that the 1P overlay has a left-to-right arrangement, while the 2P overlay is a straight overlaid arrangement. These are the default settings for each overlay type, but they can be changed. We can also change other properties of the overlay and its elements. Let's explore.

11. Right-click inside the 1P overlay window and choose **Edit** from the context menu. The **Edit Properties** dialog for the overlay is displayed.

In this first panel of the dialog, we can change settings that affect the overall appearance of the overlay and some things common to all elements. For example, we could change the transparency or the line weight of the histograms, the fonts used, or whether to show grids. This is also where we can select the view of the overlay items.

Let's look at the properties that can be changed on individual elements of the overlay.

12. In the tree on the left side of the Edit Properties dialog, click the first element, **autoposition.015**. The properties for this element are displayed.

13. Edit the **Label** so that it reads **Time=Oh**. This label will now be used in the legend for the first item.

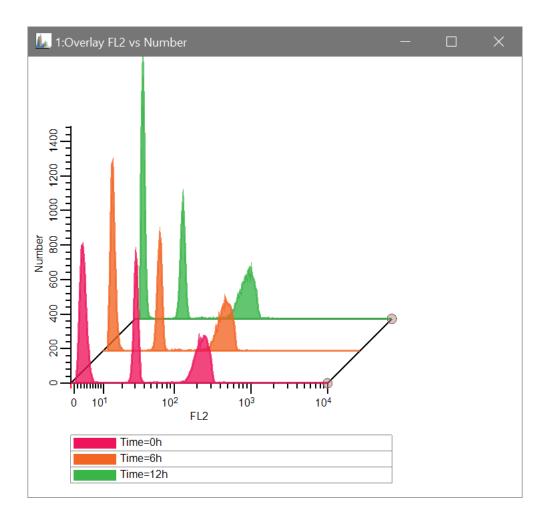
<u>Overlav EL2 vs Number</u>	Name	Current Value	Description	OK
autoposition.015	autoposition.015, FL2			
autoposition.016 autoposition.017	Label	Time=0h	Edit the label for this i	Cano
	Color	Color	Choose a color for the	Арр
	Line style	Solid	 Choose a line style. 	Help
	Filled histograms	✓ Enable	Fills the histograms wi	
	Transparency	200	0=transparent255=0	
	Line weight	3 ~	Choose a line weight.	
	Source: histogram 2 in data source	e 1		
	Update when histogram changes	Enable	When checked, this ite	

14. Select **autoposition.016** in the tree on the left, and edit its label to **Time=6h**.

15. Select **autoposition.017** in the tree on the left, and edit its label to **Time=12h**.

You can also change the color and line style with this editor. Of special interest is the **Update when histogram changes** option. With this option enabled, the element would update in the overlay any time that the histogram it came from changes - a very useful option for some or all elements in an overlay. In our example here, however, we will leave the option unchecked because we want the elements to remain unchanged.

16. Click **OK** to close the dialog and see the changes.



Did you notice the small handles on the right side of the axis in the 1P overlay? They can be dragged to change the left-to-right perspective. The lower handle moves left and right to adjust the width of the X-axis. The back handle adjusts up and down to control the tilt of the arrangement. These handles only appear in Left-to-Right view for an overlay.

Let's take a look at the 2P overlay. Suppose we want to change the order of the elements that we overlaid so that the first element was in the back of the stack and the last element was in the front. We do that by dragging elements in the legend at the bottom of the window.

17. Drag the **autoposition.017** element in the legend to the top of the legend and drop it. It becomes the front element in the stack.

18. Drag the **autoposition.015** element to the bottom of the legend and drop it. It becomes the last in the overlay stack.

Overlays are part of a WinList protocol file, so when you save your protocol, you save the overlays, too. This means that you can set up overlays as part of your experiments, adding to them as you work on new samples. Overlays can be copied in high-resolution to the

clipboard and pasted into other apps. They can also be saved to disk in many popular graphics formats for use with publications.

See also:

Overlay Windows Overlay Context Menu

Using FCOM to enumerate phenotypes

In this tutorial, we'll learn how to use the *FCOM* parameter to enumerate phenotypes. FCOM is defined as a calculated parameter function in WinList that analyzes any number of phenotypes defined by any number of gates. The best way of explaining this function is to use it. We'll start with a simple example and then discuss how you can extend it in more powerful ways.

Here is the general concept. We are going to open a listmode file and display a few histograms. We will set up some gates to identify the positive events for CD4 PE, CD8 PerCP, and CD3 APC. Then we'll create the FCOM parameter to see the complete phenotype.

Before you begin, make sure you understand the basics of working with WinList. The tutorial <u>WinList Basics</u> is a great place to start.

Open a data file

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **FCOM_Example.fcs** from the list and click **Open**.

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

2. When the **Create Histograms** dialog appears, click **Cancel**. We will create the histograms one at a time by drawing them on the data source.

This file does not contain any events with values less than 0, so in this case there is no utility to using the HyperLog transformation for it. Let's switch over to use the Log transform for all log-like parameters.

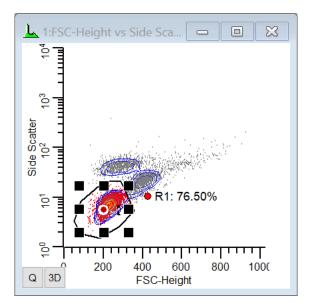
3. Click the **Comp** button on the ribbon bar to display the Compensation Normalization Toolbox. Select the **Transforms** tab, and click the **--> Log** button. You can close the Compensation Toolbox at this point.

Creating histograms and gates

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

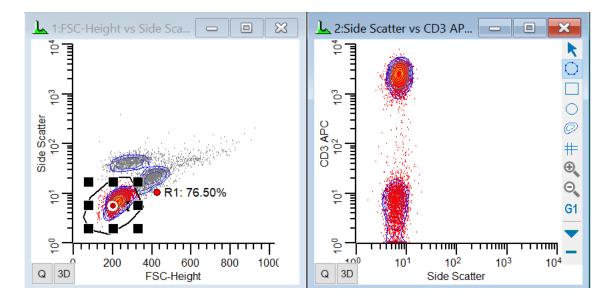
4. Click, drag, and release the mouse on the data source window to define an area where we want to display a histogram. In the **Edit Histogram** dialog, choose FSC-Height and Side Scatter for the X- and Y-Axis respectively, then click **OK**.

5. In this new histogram, draw a region around the cells of interest as shown below. You can use any region type to do this.



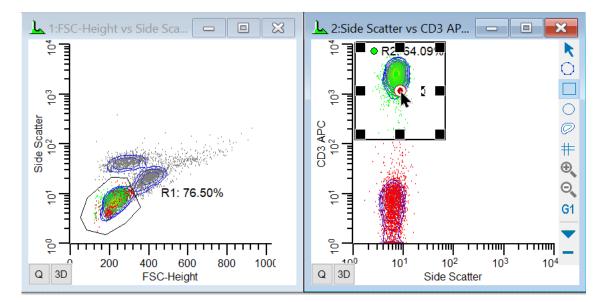
6. Click R1's label to display the cascade hotspot (the red bull's eye in the center of the region), move the mouse to an empty portion of the data source window, and draw a second histogram. In the **Edit Histogram** dialog, select Side Scatter for the X Axis and CD3 APC for the Y axis, and then click **OK**.

A new histogram gated on R1 is displayed.



Did you know that you can apply a cascade gate to any histogram by clicking on the region's hotspot and then clicking on the title bar of the histogram you want to gate? You can also hold down the Shift key if you want to apply a cascade gate to several histograms at once.

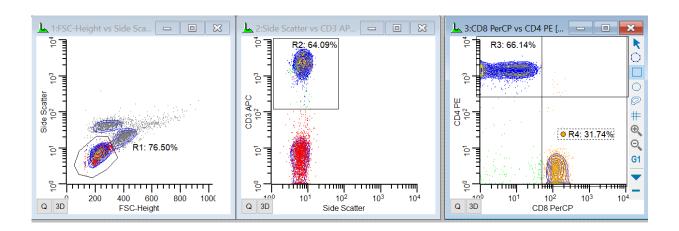
7. Draw region R2 around the CD3-positive population in the new histogram. Click on the cascade hotspot for R2, move the mouse onto the data source window, and draw a third histogram.



In the **Edit Histogram** dialog, select CD8 PerCP for the X Axis and CD4 PE for the Y axis, and then click **OK**.

8. Finally, draw two more regions: R3 to define the CD4 positives and R4 to define the CD8 positives, as shown in the following picture. Note that we intentionally capture some events as CD4+CD8+ in this example.

You can choose a different region drawing tool from the histogram toolbar to draw regions of different shapes.



Creating the FCOM parameter

OK, now for the fun part. Let's create the **FCOM** parameter.

9. Click the **Add Parameter** button on the main ribbon bar. Alternatively, you can right-click in the data source and choose Add Parameter from the context menu.



10. Click the **Add** button in the Edit Calculated Parameters dialog to add a new parameter.

Let's choose an example equation from the Functions list box.

11. Select (FCOM(G1,G2,G3)+0.5)*1024/8+FRND(10) from the list of example equations.

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

12. Edit the equation so that it reads (FCOM(**G2,G3,G4**)+0.5)*1024/8+FRND(10). Type **FCOM** in the Name edit box.

Edit Calculate	ed Parameter	
Measuremen Second Meas	nt Name: FCOM surement Name:	OK Cancel
Equation:	Type an equation or select an example from the list. (FCOM G2,G3,G4)+0.5)*1024/8+FRND(10)	•
Functions:	All functions and examples	
Transform: Source:	Linear ~ Resolution: 1024 Compensated data ~	Help

This equation looks more complicated than it really is. The FCOM function can have any number of gates as arguments. The way it works is described in the reference section of the manual. Here is how it works with our example. Using the 3 gates "G2, G3, G4" as the arguments for our FCOM, there are 8 possible classifications for each event in the listmode file. An event can be negative for all gates, positive for all gates, or some other combination. The table below shows the possible FCOM values for the G2, G3, and G4 gates.

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

+	-	÷	5
-	+	+	6
+	+	+	7

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

13. Click **OK** to close the **Edit Calculated Parameter** dialog, and click **OK** again to close the **Edit Calculated Parameters** dialog.

WinList creates the new, calculated, listmode parameter that can be used like any other parameter in the listmode file. Let's create a histogram to see what it looks like graphically.

You can use FCOM parameters in 1P, 2P, and 3P histograms. You can plot it against another parameter in the listmode file to see the phenotype as a function of that parameter.

14. In an empty portion of the data source window, draw a new histogram with the mouse. We want to make this window wide, so hold down the **Alt** key as you draw the histogram. When you release the mouse, the Edit Histogram dialog is displayed. Choose **1P** for the histogram type, and choose the last parameter in the list, **FCOM**, for the X Axis. Click **OK**.

Edit Hist	ogram			
Name:	FCOM	ID:	4	<u>R</u> emote
Type:	● 1P ○ 2P ○	3P		Remote
	Paramete	er	Resolution	
X Axis:		~	256 🖂	<u>G</u> ate
Y Axis:	CD3 APC	\sim	256 🛛 🖂	<u>S</u> ubtract
Z Axis:	MIG FITC	\sim	256 ~	Graphics
	0 <u>K</u>	<u>C</u> ancel		<u>H</u> elp

This new histogram has distinct peaks representing the eight possible states for events evaluated by gates G2, G3, and G4. The left-most peak represents events outside of all three

gates, the triple-negative events. The next peak contains events that are in G2 but not in G3 or G4. The last peak represents triple-positive events in all gates.

Statistics

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

16. From the **Insert** tab of the ribbon bar, click the **Region Array** button. Click the checkbox labeled **Create a region array for the FCOM peaks**. This is a special option that only appears when the active histogram contains an FCOM parameter.

On X-Axis		
Create a region a	array for the	FCOM peaks.
Number of regions:	8	Enter the numb

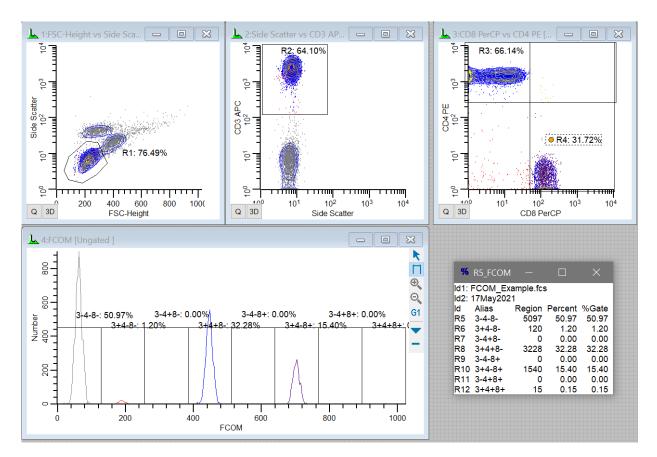
The **FCOM Region Array Options** dialog appears. This dialog allows you to turn on color gating based on the FCOM peaks, and to choose the colors used for those gates. It also allows us to name the gates to make it easier to identify phenotypes.

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

Name	Current Value	Description	ОК
Color by Gate Hierarchy	🗹 Enable	Use these regions for coloring.	Cancel
Gate colors	Colors	Choose the colors to use for gating.	Cancer
Gate Aliases			
G2 alias	3	Enter a name for gate G2.	
G3 alias	4	Enter a name for gate G3.	
G4 alias	8	Enter a name for gate G4.	

18. Click **OK** to close the dialog. Click **OK** again to close the Create Region Array dialog.

WinList creates 8 regions and positions them on the FCOM peaks. The regions are labeled to show the phenotypes. A result window shows the statistics for each peak, along with the phenotype labels. Each population is color-coded in the histogram to make it easy to identify the phenotype.



Extending this system

Since the FCOM parameter can take any number of gates, you could look at all the combinations of four gates (16 combinations) or more. You can create more than one FCOM parameter, each with the phenotype of a different set of gates, and then display a 2P histogram of the two FCOM parameters. You can even save the FCOM parameter into a new FCS file using the Save Data Source option in the File menu.

See Also:

Enhanced Proliferation Analysis FCOM Background and Usage

Population Identification with Cen-se' and t-SNE

A challenging aspect of modern cytometry is in how we deal with ever-increasing numbers of measurements and markers. New instruments provide us with more colors so that we can learn more about the cells we analyze. Traditional analysis strategies with 2P plots and gates become tedious, if not impossible - there are just too many combinations of markers to consider. This is not a problem unique to cytometry data, and several methods have been proposed which do a pretty good job of addressing the challenges of high-dimensional data.

In 2008, Laurens van der Maaten and G.E. Hinton proposed a method for visualizing highdimensional data called **t-SNE**. The name means "t-Distributed Stochastic Neighbor Embedding", and the method does a great job of reducing dimensionality to 2 or 3 dimensions. At the same time, the commonality of similar data points is preserved, making it a great tool for cytometry data. You can read more about this method on van der Maaten's web site: <u>Ivdmaaten.github.io/tsne</u>.

Verity Software House introduced a significant new dimensionality reduction algorithm derived from t-SNE called **Cen-se'** in 2019 (Bagwell, et al, <u>Improving the t-SNE Algorithms</u> <u>for Cytometry and Other Technologies: Cen-Se' Mapping</u>)</u>. Cen-se' (Cauchy Enhanced Nearest-neighbor Stochastic Embedding) corrects several issues with the t-SNE routine and provides better resolution of populations, along with greatly enhanced performance. WinList has integrated t-SNE and Cen-se' algorithms as calculated parameters, making it easy to generate these plots and explore your data. Let's take a look at how to do this with an example file acquired on a Fluidigm Helios instrument with 48 measurements.

Before you begin, make sure you understand the basics of working with WinList. The tutorial <u>WinList Basics</u> is a great place to start.

Open the FCS file

1. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **Helios1.fcs** from the list and click **Open**.

2. In the **Create Histograms** dialog, switch to the **1P Histograms** tab and select the CD4, CD8, and CD3 measurements. The full names for these are 143Nd_**CD4**, 144Nd_**CD8**, and 150Nd_**CD3**. With these selected, click **OK**.

Create Histograms	
1P Histograms 2P Histograms 3P Histograms	
X Axis	
115In_Dead A 191Ir_DNA1 A 193Ir_DNA2 A 175Lu_HLADR A 142Nd_CD19 A 143Nd_CD4 A 144Nd_CD8 A 146Nd_IgD A 148Nd_CD11c A 150Nd_CD3 Offset 208Pb Residual	
147Sm_CD85j 149Sm CD16	
Add to List	
Histograms	Resolution:
	Delete
	Up
<	Down
OK Cancel	

Create the Cen-se' and t-SNE parameters

Next, we want to create the Cen-se' and t-SNE calculated parameters. To do this, we use the Add Parameter feature of WinList.

3. Click the **Add Parameter** button on the main ribbon bar. Alternatively, you can right-click in the data source and choose Add Parameter from the context menu.



4. Click the **Add** button in the Edit Calculated Parameters dialog to add a new parameter.

Let's choose an example equation from the Functions list box. The function we're looking for is actually **FSNE** as opposed to t-SNE. All WinList function names start with the letter **F** and have a total of 4 letters, so *t-SNE* becomes *FSNE*.

5. Select **FSNE(P1,P2,P3,P4,P5,P6,G0,50,2)** from the list of example equations. This will get us started.

Examples	^
(FCOM(G1,G2)+0.5)*12000/4+FRND(10)	
(FCOM(G1,G2,G3)+0.5)*12000/8+FRND(10)	
(FCOM(G1,G2,G3,G4)+0.5)*12000/16+FRND(10)	
p1/p2	
FTIM(12000)	
FSNE(P1,P2,P3,P4,P5,P6,G0,50,2)	
FCEN(P1,P2,P3,P4,P5,P6,G0,200,2)	~

The function has 3 important parts to it. The first part is the list of parameters that we want t-SNE to evaluate. This can be **any number of parameters**, starting with P1, separated by commas:

FSNE(**P1,P2,P3,P4,P5,P6**,G0,50,2)

After the parameters, there is a **gate number** in the expression. This gate is used to filter the events that t-SNE evaluates. For example, you might have a gate the selects lymphocytes that you want to filter the events on. For no gate, we enter G0.

FSNE(P1,P2,P3,P4,P5,P6,**<u>G0</u>**,50,2)

You probably will not edit the last two arguments to the FSNE function. They relate to "perplexity" and the number of dimensions to return. Perplexity sets the number of effective nearest neighbors, which has a default value of 50. The number of return dimensions should be 2.

FSNE(P1,P2,P3,P4,P5,P6,G0,<u>50,2</u>)

Let's edit the example equation so that it suits our data file. This is easy to do with the **Choose** button that appears next to the Equation edit box.

6. Click the **Choose** button.

Edit Calculated	d Parameter			
Measurement	t Name:	t-SNE_1		ОК
Second Meas	surement Name:	t-SNE_2		Cancel
	Type an equation	or select an example from the list.		
Equation:	FSNE(P1,P2,P3,P	•		Choose
Functions:	All functions and	examples	~	

The Select t-SNE Parameters dialog is displayed, showing two lists of parameters. The Available list on the left contains parameters we have not included, and the Selected list on the right contains those that we have added. To move an item from one list to the other, you simply click the item.

7. Remove **P1**, **P2**, **P3**, **and P4** from the Selected list by clicking each one.

8. In the Available list, click **p7**, **p8**, **p9**, **p10**, **p11**, **p18**, **p21**, **p25**, **p26**, **p27**, **p28**, **p31**, **p36**, **p37**, **p38**, **p46**, **and p47** to add them to the Selected list. The dialog will look something like this when you are finished.

Availab	le		Selecte	ed	
P30	148Nd_CD11c	~	P5	162Dy_CD45RA	~
P32	Offset		P6	164Dy_CD20	
P33	208Pb		P7	166Er_CD33	
P34	Residual		P8	167Er_CD28	
P35	147Sm_CD85j		P9	168Er_CD24	
P39	120Sn		P10	170Er_CD161	
P40	159Tb_CXCR3		P11	151Eu_CD38	
P41	169Tm_ICOS		P18	160Gd_CCR7	
P42	131Xe		P21	113In_CD57	
P43	171Yb_TCRgd		P25	175Lu_HLADR	
P44	172Yb_PD-1		P26	142Nd_CD19	
P45	173Yb_CD123		P27	143Nd_CD4	
P48	Time		P28	144Nd_CD8	
P1	190BCKG		P31	150Nd_CD3	
P2	138Ba		P36	149Sm_CD16	
P3	140Ce_Bead		P37	152Sm_CD27	
P4	Center	\checkmark	P38	154Sm_CD14	~

9. Click OK to close the dialog.

The **Equation** field in Edit Calculated Parameter will now contain this expression:

FSNE(p5,p6,p7,p8,p9,p10,p11,p18,p21,p25,p26,p27,p28,p31,p36,p37,p38,p46,p47,G0,50,2)

Edit Calculate	d Parameter		
Measuremen	nt Name: t-SNE_1		ОК
Second Meas	surement Name:	t-SNE_2	Cancel
	Type an equation	or select an example from the list.	
Equation:	FSNE(P5,P6,P7,P	8,P9,P10,P11,P18,P21,P25,P26,P27,P28,P31,P36,P37,F	Choose
Functions:	All functions and		
	(FCOM(G1,G2)+0 (FCOM(G1,G2,G3	amples 0.5)*12000/4+FRND(10))+0.5)*12000/8+FRND(10) ,G4)+0.5)*12000/16+FRND(10)	
	FSNE(P1,P2,P3,P4 FCEN(P1,P2,P3,P4	4,P5,P6,G0,50,2) 4,P5,P6,G0,200,2)	
		tributed Stochastic Neighbor Embedding ne gate, perplexity(50), and return dimensions.	
Transform:	Linear	Resolution: 64	
Source:	Compensated dat	a v	Help

We are passing 19 ungated measurements to t-SNE and getting 2 dimensions in return. The measurements are CD45RA, CD20, CD33, CD28, CD24, CD161, CD38, CCR7, CD57, HLADR, CD19, CD4, CD8, CD3, CD16, CD27, CD14, CD56, and CD25. We will leave the gate at G0, and perplexity and dimensions at 50 and 2, respectively.

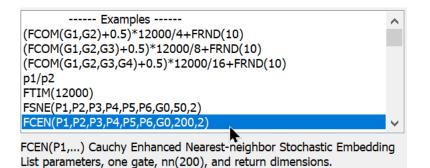
10. Click **OK** to closed the Edit Calculated Parameter dialog.

Next, we'll use the same process to add Cen-se'.

11. In Edit Calculated Parameters dialog, click the **Add** button again to add another parameter.

This time the function we're looking for is **FCEN** for the Cen-se' algorithm.

12. Select FCEN(P1,P2,P3,P4,P5,P6,G0,200,2) from the list of example equations.



The function is almost identical to the FSNE function. It starts with a list of parameters and then a gate. Next is the number of nearest neighbors, which is 200 by default. The last argument is always set to 2.

13. Click the **Choose** button. We'll select the same measurements that we used for the FSNE function.

14. Remove **P1**, **P2**, **P3**, **and P4** from the Selected list by clicking each one.

15. In the Available list, click **p7**, **p8**, **p9**, **p10**, **p11**, **p18**, **p21**, **p25**, **p26**, **p27**, **p28**, **p31**, **p36**, **p37**, **p38**, **p46**, **and p47** to add them to the Selected list.

Availab	le		Selecte	ed	
P23	191Ir_DNA1	~	P5	162Dy_CD45RA	^
P24	193Ir_DNA2		P6	164Dy_CD20	
P29	146Nd_IgD		P7	166Er_CD33	
P30	148Nd_CD11c		P8	167Er CD28	
P32	Offset		P9	168Er_CD24	
P33	208Pb		P10	170Er_CD161	
P34	Residual		P11	151Eu_CD38	
P35	147Sm_CD85j		P18	160Gd_CCR7	
P39	120Sn		P21	113In_CD57	
P40	159Tb_CXCR3		P25	175Lu_HLADR	
P41	169Tm_ICOS		P26	142Nd_CD19	
P42	131Xe		P27	143Nd_CD4	
P43	171Yb_TCRgd		P28	144Nd_CD8	
P44	172Yb_PD-1		P31	150Nd_CD3	
P45	173Yb_CD123		P36	149Sm CD16	
P48	Time		P37	152Sm_CD27	
P1	190BCKG	~	P38	154Sm_CD14	~

16. Click OK to close the dialog.

The **Equation** field in Edit Calculated Parameter will now contain this expression:

FCEN(p5,p6,p7,p8,p9,p10,p11,p18,p21,p25,p26,p27,p28,p31,p36,p37,p38,p46,p47,G0,200,2)

Edit Calculated Parameter						
Measurement Name:		Cen-se_1]	ОК
Second Meas	surement Name:	Cen-se_2]	Cancel
	Type an equation	or select a	n example fror	n the list.		
Equation:	FCEN(P5,P6,P7,P	8,P9,P10,P	11,P18,P21,P2	5,P26,P27,P28,	P31,P36,P37,F	Choose
Functions:	All functions and	examples			~	
	Examples (FCOM(G1,G2)+0.5)*12000/4+FRND(10) (FCOM(G1,G2,G3)+0.5)*12000/8+FRND(10) (FCOM(G1,G2,G3,G4)+0.5)*12000/16+FRND(10) p1/p2 FTIM(12000) FSNE(P1,P2,P3,P4,P5,P6,G0,50,2) FCEN(P1,P2,P3,P4,P5,P6,G0,200,2) V					
	List parameters, o	ne gate, nr	1(200), and ret	-	•	
Transform:	Linear		Resolution:	64	~	
Source:	Compensated dat	a ~				Help

17. Click **OK** in the Edit Calculated Parameter dialog. We now have two new calculated parameters.

• Edit Calco	ulated Parameters		×
Name t-SNE_1	Equation FSNE(P5,P6,P7,	P8,P9,P10,	ОК
Cen-se_1			Open
			Save
			Help
	_		
<		>	
Add	Delete	Edit	

18. Click **OK** in the Edit Calculated Parameters dialog.

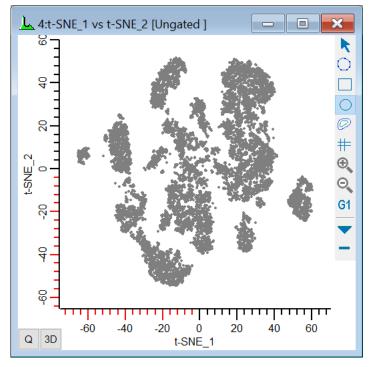
The program will begin to calculate the new parameters. Depending on your computer, this might take a few moments to a few minutes.

Computing Cen-se_1 13% Click to stop
Computing t-SNE_1 10% Click to stop
10000 3:150Nd_CD3

Let's create 2P plots that will display the Cen-se' and t-SNE parameters.

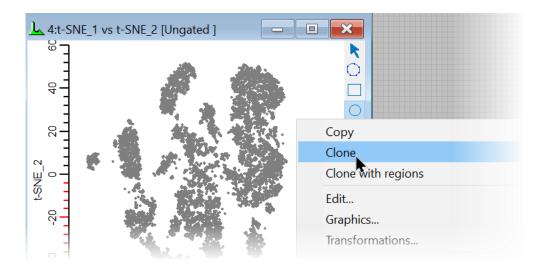
19. On the **Insert** tab of the ribbon bar, click the **Dots** button to add a dot plot to our layout. Move it and size it to your liking. You may want to increase the **Dot size** to 5 and change **Dot order** to **Low frequency on top** in the Edit Graphics dialog. These settings are helpful for t-SNE plots.

20. Change the X-axis to display **t-SNE_1** and the Y-axis to show **t-SNE_2**.



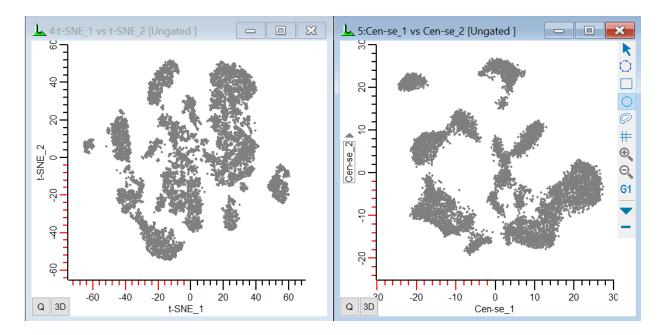
When the t-SNE function finishes computing, you should see a plot similar to this:

21. Right-click in the new t-SNE plot and choose **Clone** from the context menu.



This creates a copy of the t-SNE plot, which we can modify to show the Cen-se' measurements.

22. Change the X-axis to display **Cen-se_1** and the Y-axis to show **Cen-se_2**.

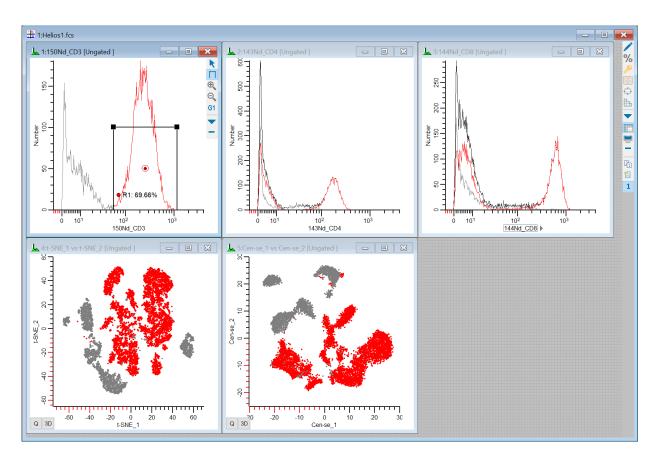


In both plots, we can see some distinct groups of events and some continuous distributions, but we have no idea what they mean. How does this help us understand our data? Well, it's always useful to start with something we know. Let's see where the CD3, CD4, and CD8 cells are in this plot.

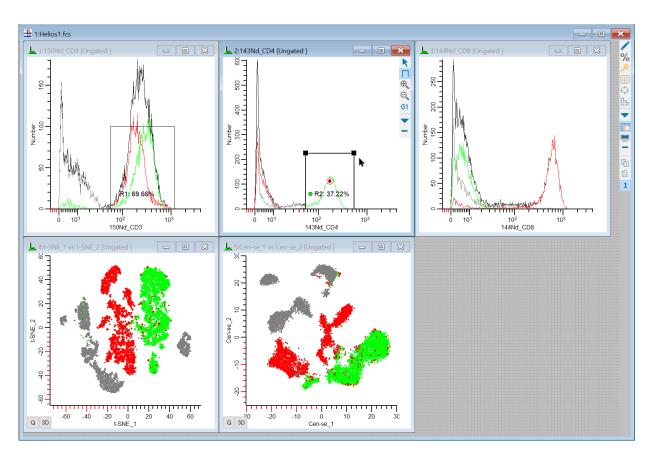
Using other plots to understand Cen-se' and t-SNE

Our workspace has 5 plots on it: 1P plots for CD3, CD4, and CD8, and 2P plots for t-SNE and Cen-se'. Let's put some regions on the 1P plots to see where the positives are the 2P plots.

23. Draw a region on the CD3 positive events in the 1P plot, and observe where they fall in the t-SNE and Cen-se' plots.

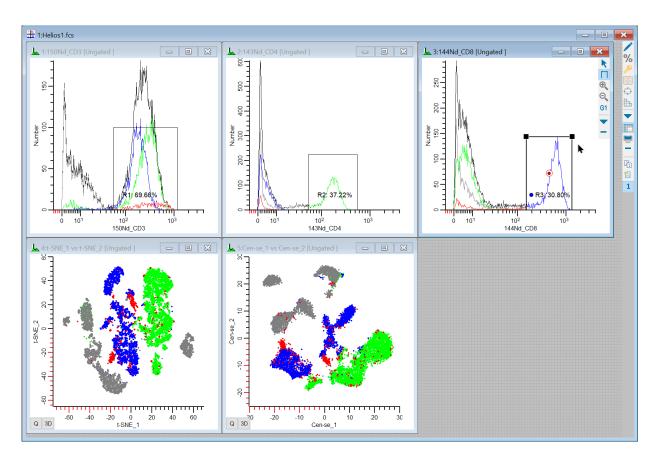


24. Draw a region on the CD4 positive events in the 1P plot and notice where they are in the t-SNE and Cen-se'.



Population Identification with Cen-se' and t-SNE

25. Draw region on the CD8 positive events. This should account for most of the remaining portion of the CD3 events in t-SNE and Cen-se' plots.



So we can see event coloring and gating in t-SNE and Cen-se' and figure out what the different populations relate to. Since these plots can change shape and location from file to file, this is always a good way to get started. It provides a backbone of information to help relate the plots to what we know about our data.

Using Cen-se' and t-SNE to understand other plots

The most exciting part of t-SNE and Cen-se' is that they reveal populations detected in multidimensional space. What can we learn if we draw a region around one of those populations? Let's find out.

26. On the **Insert** tab of the ribbon, click the **Multiple** button to display the **Create Histograms** dialog. Add 2P histograms for these markers:

164Dy_CD20 vs 142Nd_CD19

162Dy_CD45RA vs 175Lu_HLADR

154Sm_CD14 vs 166Er_CD33

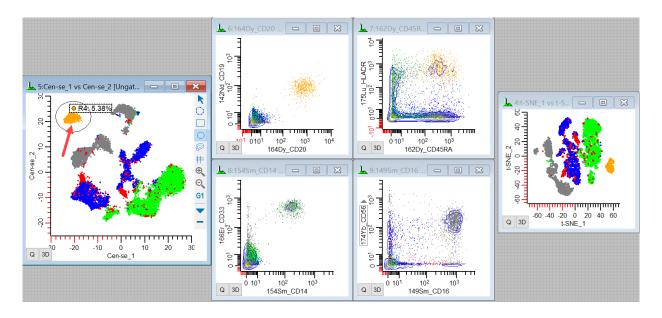
149Sm_CD16 vs 174Yb_CD56

1P Histograms 2P Histog		ograms			
(Axis		Y Axis			
Residual 1475m_CD85j 1495m_CD16 1525m_CD27 1545m_CD14 1205n 159Tb_CXCR3 169Tm_ICOS 131Xe 171Yb_TCRgd 172Yb_PD-1 173Yb_CD123 174Yb_CD56 176Yb_CD25 Time	~	120Sn 159Tb_CXCR3 169Tm_ICOS 131Xe 171Yb_TCRgd 172Yb_PD-1 173Yb_CD123 174Yb_CD56 176Yb_CD25 Time t-SNE_1 t-SNE_1 t-SNE_2 Cen-se_1 Cen-se_2	~	Choose one or more X and parameters, then click Add to Unique combinations of the parameters are added to the In Combinations mode, sele parameters in the X axis list combinations will be created	selected e list. ect and all
		Add to List		ombinations mode	
Histograms				R	esolution
164Dy_CD20 vs 142Nd_0	CD19)		64	4
162Dy_CD45RA vs 175Lu	_				
154Sm_CD14 vs 166Er_(Delete
149Sm_CD16 vs 174Yb_	CD56)			Up
				> [Down

27. Click OK to create the histograms. Arrange the histograms to your liking.

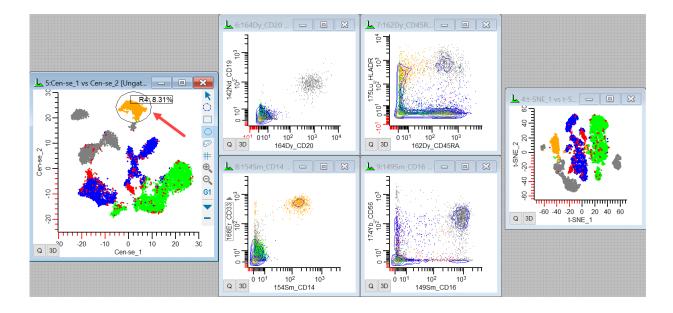
We'll use the Cen-se' plot to explore the additional populations.

28. Draw a region on the Cen-se' plot around the population in the left top of the plot.



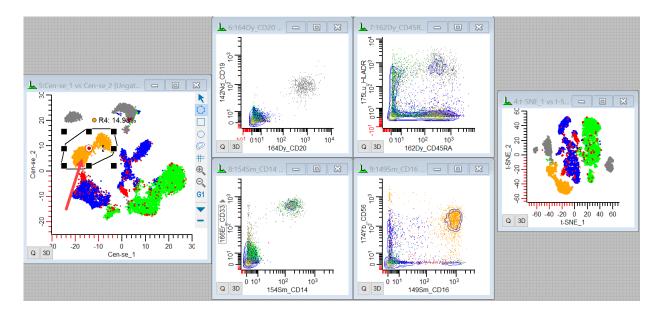
The events that light up are CD20+ CD19+ CD45RA+ and HLADR+. They are probably mature B-cells. We can also see where they fall in the t-SNE plot.

29. Move or redraw the region over the population in the top center.



We see the CD14+ CD33+ HLADR+ events light up, making these likely to be monocytes.

30. Finally, redraw the region around the remaining large distribution on the plot.



These are probably NK cells, as they are CD16+ CD56+ CD45RA+.

If you want to see the region events more dramatically in the other plots, you can right-click the region label and choose **Highlight events in R4**.

We're only displaying a few histograms in this tutorial to make the point, but you can see the power of the t-SNE and Cen-se' functions. They are good at finding related events and allowing us to see what they have in common by looking at the markers in our sample. As you probe the islands of events in Cen-se and t-SNE plots and see where they fall in other plots, you'll find some surprises and confirm some things that you already know.

Summary

WinList includes powerful Cen-se and t-SNE functions that take high-dimensional data and flattens it into 2-dimensional space so that it can be visualized in conventional 2P plots. Cense' is computed using the FCEN calculated parameter, and FSNE is WinList's implementation of t-SNE. You can display the measurements created by the functions as 2P plots that work just like any other 2P plots.

Sticky Regions and Magic Contours

You can probably think of cases where you wanted to have a set of regions sharing common vertices so that they fit perfectly together. It is difficult to create regions that do that, especially if you also want to be able to make adjustments. WinList simplifies this task with its ability to create "sticky regions" quickly and easily. It allows you to stick regions together, and just as easily to unstick them.

Similarly, there are times when you want a region to adjust its size and shape based on the data beneath it. For example, as you read a new file into an analysis setup, it would be really helpful if the lymphocyte gate would auto-adjust to the new distribution. The "magic contour" region in WinList does exactly this.

This short tutorial will introduce these new region capabilities so that you can add them into your own analysis protocols.

If you are unfamiliar with opening files and creating histograms with WinList, you might want to do the <u>WinList Basics</u> tutorial before continuing with this one.

Open the FCS file

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

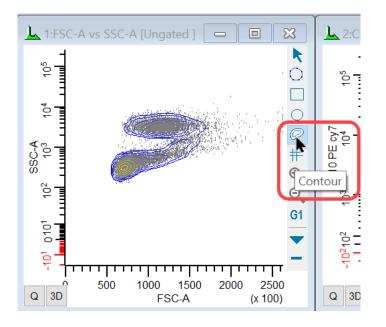


2. In the File Open dialog, navigate to the Samples folder located in the folder where WinList is installed. Select the **BCell_BM_Tube_001.fcs** and click **Open**.

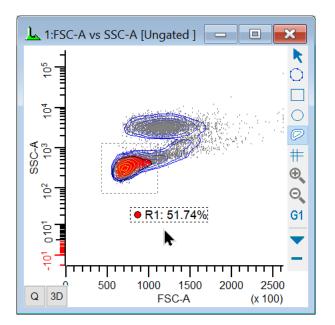
3. In the **Create Histograms** dialog, add **FSC-A vs SSC-A** and **CD38 vs CD10** histograms and click **OK**.

Create a contour region

4. Click the title bar of the FSC vs SSC plot to activate it, the click the **Contour region** tool in the histogram toolbar.



5. Click and drag to draw a box around the low SSC-A events. When you release the mouse, a contour region will be created from the data within the box you drew.



If you move the region (by dragging the label), the contour region will recompute on the data in the new location. Sometimes the resulting contour does not change because the bounding box contains the same probability distribution. For example, in the illustration above, moving one of the left handles to the left would not change the contour.

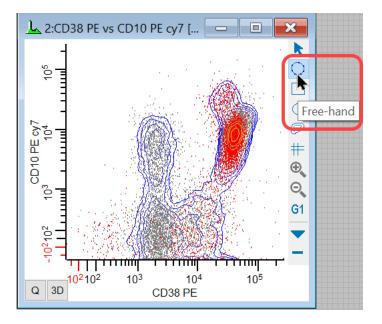
6. Right-click the region label and choose **Edit Properties**. The properties that relate to the contour region are grouped together. Edit the **Contour Probability** to be **0.95** and click OK.

Name	Current Value	Description
Show Name	✓ Enable	Show the region name in the label.
Show Alias	Enable	Show the region alias in the label.
Alias	R1	Edit the alias for the region.
Statistic	Percent gated ~	Choose a statistic to display in the label.
Line color	Color	Click to change color
Line weight	0 ~	Line weight for regions.
Allow delete		Allow or prevent the region from being deleted.
Contour Region		
Contour Probability	0.95	The probability level of the contour region. (0.01 to 0.99)
Smoothing	3 ~	Smoothing for the contour.
Show resizing handles	Enable	Display resizing handles when region is active.
Auto-positioning		
Auto-position	Enable	Automatically move region with data.
Auto-position method	Center on region median 🛛 🗠	Choose to method to auto-position.
X Percent	10.00	Enter the X percent to include. (1.00 to 99.00)
Y Percent	10.00	Enter the Y percent to include. (1.00 to 99.00)

The contour on the histogram will get a little bigger as a result of the edit, encompassing 95% of the events inside the contour region's bounding box.

Create sticky regions

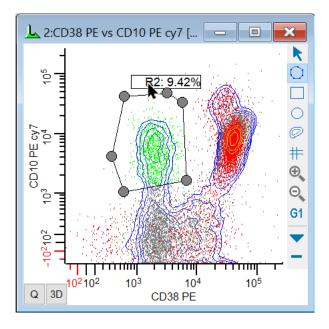
4. Click the title bar of the CD38 vs CD10 plot to activate it, the click the **Freehand region** tool in the histogram toolbar.



Let's create 3 adjacent regions in this plot using the point and click technique.

5. Click and hold the mouse for a second next to the CD10+ CD38- population, then release the mouse button. Move and click the mouse to define 6 or 7 points to surround the population, clicking back on the first point to close region R2.

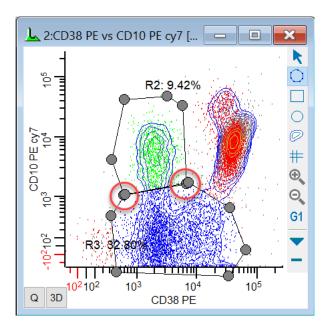
6. Right click the region label and choose **Show Vertices**.



7. Click and hold the mouse for a second next to the CD10- CD38 dim population, then release the mouse button. Move and click the mouse to define points in the region, and

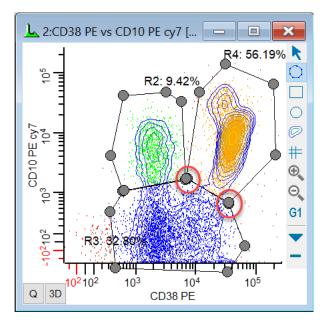
make sure to click on top of the vertices at the bottom of R2. Click on the first point of R3 to finish the region.

8. Right click the region label and choose **Show Vertices**.



9. Click and hold the mouse for a second next to the CD101 CD38+ population, then release the mouse button. Move and click the mouse to define points in the region, and *make sure to click on top of the vertices at the top of R3*. Click on the first point of R4 to finish the region.

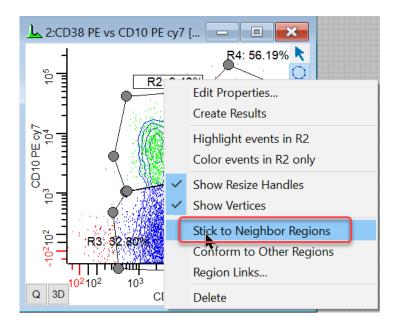
10. Right click the region label and choose **Show Vertices**.



So at this point, we have a set of adjacent regions that are completely independent of one another. If you move a region or common vertex, the other regions will be unaffected. We're about to change that.

Make it "sticky"

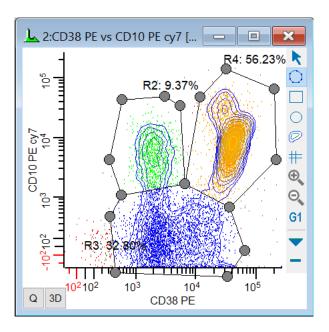
11. Right click the **R2** region label and choose **Stick to Neighbor Regions**.



A toast message will display indicating that 3 connections were made.

12. Repeat this for regions **R3** and **R4**.

Now your regions are dependent on one another. The common vertices are truly common: when you move a common vertex, all regions that share the vertex will be affected. Try it out.



If you move one of these regions (by the region label), the regions sharing vertices will be pulled to stay connected. You can also move individual vertices, and the connected vertices will move with them. This is a great tool for making sure that you single-count events and maintain adjacency even as you make adjustments to the regions.

Summary

This tutorial introduced the contour region and sticky regions. Contour regions are dynamic: they recompute the region whenever the data beneath them changes. They are a perfect tool for parts of your analysis where you want to reduce subjectivity in region placement and size, because they are computed based on probability.

Sticky regions make it possible to create a set of adjacent regions that maintain adjacency even if you move one region, or a vertex of a region.

Kinetic Analysis with Excel

This tutorial will introduce kinetic analysis of calcium flux experiments using WinList and Microsoft Excel. The first section of the tutorial shows how this can be done manually by copying results from WinList into a special Excel worksheet designed for kinetics. At the end of the tutorial, we will show how the process can be automated with a WinList macro and an Excel Visual Basic script.

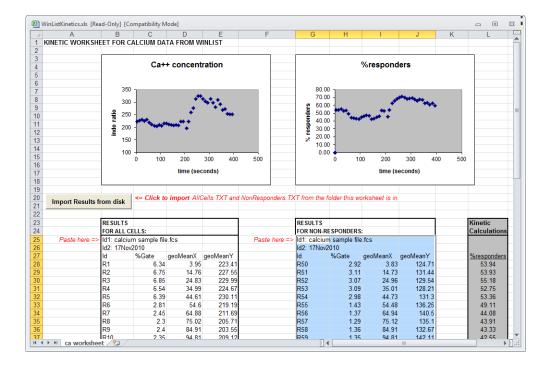
In order to perform this tutorial, you need WinList and Microsoft Excel installed on your computer. You will also need sample files that can be found in WinList's Samples\Kinetics folder.

Set up Excel

1. Start Microsoft Excel.

2. From Excel's file menu, choose **Open**. Navigate to the folder in which WinList is installed, and into the Samples\Kinetics folder. Select the file **WinListKinetics.xls**.

3. Click the **Open** button.



You may need to adjust the macro security settings in Excel to enable macros in this worksheet. Talk with your IT specialist to determine if this is appropriate for your institution.

This worksheet contains two charts, one showing calcium concentration for all cells, and a second showing the calcium response of responder cells. The charts plot data from two sets of results pasted from WinList. You will be pasting new results into this worksheet to update the charts with your analysis of a sample file.

If Excel displays a security warning, you will need to choose to Enable Content in order for the sheet to work properly.

Set up WinList

4. Start WinList, and click **Open FCS** on the ribbon bar. The Open FCS File dialog is displayed.

5. Navigate to the folder in which WinList is installed, and into the Samples\Kinetics folder. Select the file **calcium sample file.fcs**, and click the **Open** button.

The **Create Histograms** dialog is displayed. We want to create *two* copies of **Time** vs. **F32-Ratio** at a resolution of 1024 channels.

6. Set the Resolution dropdown list to **1024**.

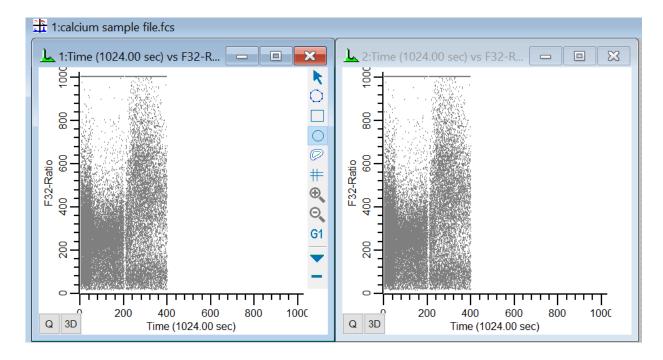
7. Select **Time** in the X Axis list, **F32-Ratio** in the Y Axis list, and then click **Add to List** *two times*. You should see two entries in the **Added Histograms** list. Click **OK** to close the dialog and display the histograms.

Create Histograms		
1P Histograms 2P Histograms 3P His	tograms	
X Axis	Y Axis	
FSC-Height SSC-Height FL1-Height	FSC-Height SSC-Height FL1-Height	Choose one or more X and Y parameters, then click Add to List.
FL2-Height F32-Height	FL2-Height F32-Height	Unique combinations of the selected parameters are added to the list.
FL4-Height F32-Ratie Time (1024.00 sec)	FL1 Height F32-Ratio Time (1024.00 sec)	In Combinations mode, select parameters in the X axis list and all
		combinations will be created.
	Add to List	Combinations mode
Histograms Time (1024.00 sec) vs F32-Ratio		Resolution:
Time (1024.00 sec) vs F32-Ratio		Delete
		Up
<		> Down
	OK Cancel	

We need to display all dots, and turn off contours if they are enabled.

8. Right-click the mouse on one of the histograms.

9. From the popup menu, choose **Graphics**. Select **Dots** in the tree on the left and make sure Dots are enabled. Check the **Show all dots** option. Select **Contours** in the tree and make sure that the Contours property is disabled. Scroll to the bottom of the property list and check **Apply to All**. Click **OK** to apply the changes to both histograms.



Create regions and results

Next, we need to create a set of regions on each histogram to provide statistics on the kinetics. In the histogram on the left, we will gather statistics about all cells, and in the right histogram, we will look at statistics for a subset of cells. We will use the Create Region Array option to simplify the process of creating 48 adjacent regions on each histogram.

10. Click the title bar of the histogram on the left to activate it. From the **Insert** tab on the ribbon bar, click **Region Array**.

We want to create 48 regions on the X-axis, each one being 10 channels wide.

11. In the **On X-Axis** group, enter 48 for the **Number of regions**. Enter 0 for **Starting at**, and 480 in the **Ending at** edit box. Click **OK**.

Create Region Array					
Options Create a results Total regions to creater 			OK Cancel		
On X-Axis					
Number of Starting at: Ending at: Space between:	48 0 480 0	Enter the number of regions in the X Enter the left edge of the first region Enter the right edge of the last regio Spacing (% of X scale).	ı.		
On Y-Axis					
Number of	1	Enter the number of regions in the Y	direction.		
Starting at:	Starting at: -1.02 Enter the bottom edge of the first region.				
Ending at:	1024 Enter the top edge of the last region.				
Space between:	0	Spacing (% of Y scale).			

After a moment or two, a results window containing results for the 48 regions will appear. We need to choose the statistics to display.

12. Right-click on the results window and choose **Edit** from the context menu. Enable the following options, and *disable all others*:

ID Field1 ID Field2 Percent Gated MeanX MeanY Use Geo Mean Use Robust SD Fields in Columns Click **OK** to close the dialog.

You can set the preferences for the results that you want in a new results window by clicking the **Save As Preference** button in the Edit Results dialog.

Next, we will repeat the process for the second histogram. This time, however, we will create slightly different regions.

13. Click the title bar of the histogram on the *right* to activate it. From the Insert tab on the ribbon bar, click **Region Array**.

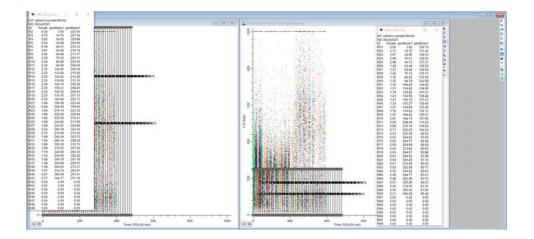
14. In the **On X-Axis** group, enter 48 for the **Number of regions**. Enter 0 for **Starting at** and 480 in the **Ending at** edit box. In the **On Y-Axis** group, enter 250 for the **Ending at** edit box. These regions will capture the non-responders. Click **OK**.

Create Region Array						
Options Create a results of the creater of the cr			OK Cancel			
On X-Axis						
Number of Starting at: Ending at: Space between:	48 0 480 0	Enter the number of regions in the X Enter the left edge of the first region Enter the right edge of the last region Spacing (% of X scale).	n.			
On Y-Axis						
Number of	1	Enter the number of regions in the	r direction.			
Starting at:	Starting at: -1.024 Enter the bottom edge of the first region.					
Ending at:						
Space between:	0	Spacing (% of Y scale).				

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

15. Right-click on the new Results window and choose **Edit**. Enable the following options again, and disable all others:

ID Field1 ID Field2 Percent Gated MeanX MeanY Use Geo Mean Use Robust SD 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 on the Home tab of the ribbon.

Copying results into Excel

We need to update the statistics for the entire file, and then copy the two sets of results into the Excel worksheet.

- 16. Click the **Replay** button on the Home tab of the ribbon bar.
- 17. Right click inside the first Results window. Choose **Copy**.

The first set of results is now in the clipboard, available to Excel. We need to paste these results into the correct cells in the Excel worksheet.

18. Activate Microsoft Excel. Select cell **B25** and then **Paste** the results.

🕺 WinListKinetics.xls					
	A	B	С		
22					
23		RESULTS			
24		FOR ALL CE	LLS:		
25	Paste here =>				
26	Paste here =>	Id2: 23Mar2	001		

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

19. Activate WinList. Right click inside the second Results window. Choose Copy.

The *second* set of results is now in the clipboard, ready to paste into Excel.

20. Activate Microsoft Excel. Select cell **G25** and **Paste** the results.

2	F	G	н
22		0	11
23		RESULTS	
24		FOR NON-RES	SPOND
25	Paste here =	> Id1 alcium	
26		Id2: 22Mar20	101

The Excel worksheet now displays two charts of the results you have just pasted into it. The chart on the left shows the Calcium concentration for all cells, and the chart on the right is for the "responder" cells. Notice that there is also a column of numbers that displays the percentage of responders for each time slice.

Automating the process

Since WinList and Excel both have automation capabilities, you can streamline the process of getting results into Excel. The best way to do this is with a Remote connection between each Result window and the Excel worksheet.

21. Activate WinList, right-click the first Results window, and choose Edit.

22. In the Edit Results dialog, enable the **Remote** checkbox and then click **Edit Remote**.

Edit Results			
Name: 1 me (1024.00 sec) v Show regions in data source: 1:calcium sample file.fcs I:calcium sample file.fcs R28: R28 R29: R29 R30: R30 R31: R31 R32: R32 R33: R33 R34: R34 R35: R35 R36: R36 R37: R37 R38: R38 R39: R39 R40: R40 R41: R41 R42: R42 R43: R43 R44: R44 R45: R45 R46: R46 R47: R47 R48: R48 R49: R49 ✓ Fields in Columns ✓ Auto-size window ✓ Save as preference	rs F32-Ratio		Gated Events Gated Events Career Gated Career GateGateGateGateGateGateGateGateGateGate
	ОК	Cancel	

23. The Edit Remote Link dialog is displayed. Select "Excel" in the **Link To** list, and then type "ca worksheet" for the **Topic**.

Edit Remote Link						
Link To: Excel		~	Update When data changes			
Topic: ca worksheet			On replay On command			
	Commands: [ACTIVATE("SHEET1")]					
POKE(R1C1	("SHEET1")) 1:R51C4,TEX R1C1:R51C4					
Add	Insert	Replace	Open	ОК		
Delete	Clear		Save	Cancel		

24. Next, select the *first* command in the Commands list, edit "SHEET1" to "ca worksheet", and then click the **Replace** button.

25. Select the *second* command in the Commands list, edit the text to "POKE(R25C2:R76C5,TEXT)", and then click the **Replace** button. This tells Excel where to put the results data that WinList will send to it.

26. Select the last command and click the **Delete** button.

Edit Remote Link				
Link To: Excel		~	Update When data	changes
Topic: ca worksheet			On replayOn command	
Commands:				
[ACTIVATE("ca worksheet")] POKE(R25C2:R76C5,TEXT)				
Add	Insert	Replace	Open	ОК
Delete	Clear		Save	Cancel

Click **OK** to close the dialog.

We have edited the script so that any time our data changes, WinList will send the new results to the correct location on the Excel spreadsheet. Let's make a similar connection for the second Results window.

27. Right-click the *second* Results window and choose Edit. Enable the **Remote** checkbox, and click **Edit Remote**.

28. Edit the Topic to "ca worksheet" and revise the script commands as shown. Note the text of the POKE command is different than the previous script: "POKE(R25C7:R76C10,TEXT)".

Edit Remote Link			
Link To: Excel ~	Update When data changes		
Topic: ca worksheet	 On replay On command 		
Commands: POKE(R25C7:R76C10,TEXT)			
[ACTIVATE("ca worksheet")] POKE(R25C7:R76C10,TEXT)			
Add Insert Replace	Open OK		
Delete Clear	Save Cancel		

29. Click OK to close the dialog, and click OK again to close the Edit Results dialog.

You now have a fully automated connection between Excel and WinList for both of the Result windows. Any time you load a new file or change a gate, WinList will send the new Results to Excel using the scripts we just created. If you save your protocol, the remote scripts will be stored and connections will be made automatically when the protocol is re-opened.

Summary and discussion

This tutorial introduced kinetic analysis using WinList and Microsoft Excel. We constructed a protocol in WinList that extracted statistics from 48 time slice regions in each of two histograms. We opened an Excel spreadsheet that is supplied with WinList, and then pasted two sets of results into the worksheet.

We streamlined the process of copying and pasting results by creating Remote connections between the Result windows and Excel.

Enhanced Proliferation Analysis

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

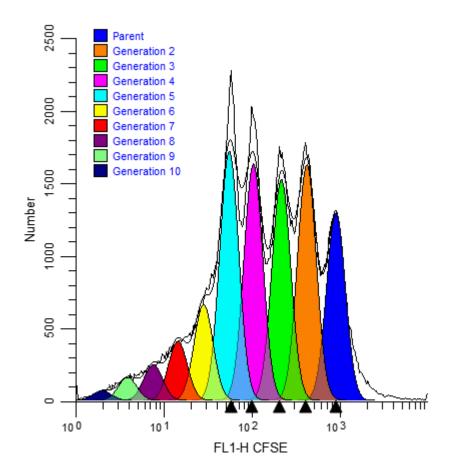
ModFit LT performs sophisticated least squares analysis in just a few clicks of a mouse and reveals the secrets of the proliferation data: proliferation index, non-proliferative fraction, and precursor frequency, and fractions for each proliferating generation. The proliferation results can be exported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

Enter WinList. NStat regions can import the proliferation results from ModFit LT, and then partition the generations according to the proliferation analysis. This enhancement sets the stage for exciting new analysis capabilities.

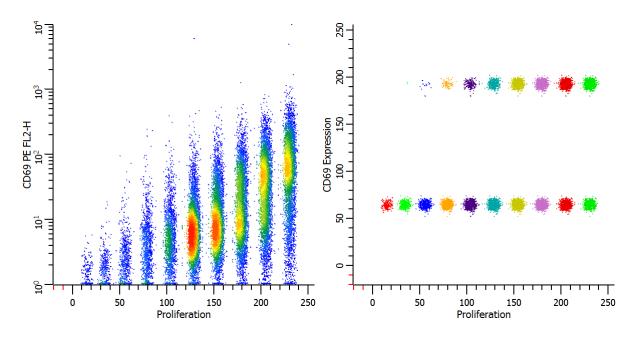
This tutorial will explore these new features to examine a CFSE-stained sample containing several other markers.

The big picture

From the Proliferation Wizard in ModFit LT...



... into WinList for exciting, new explorations of proliferation.



Getting started

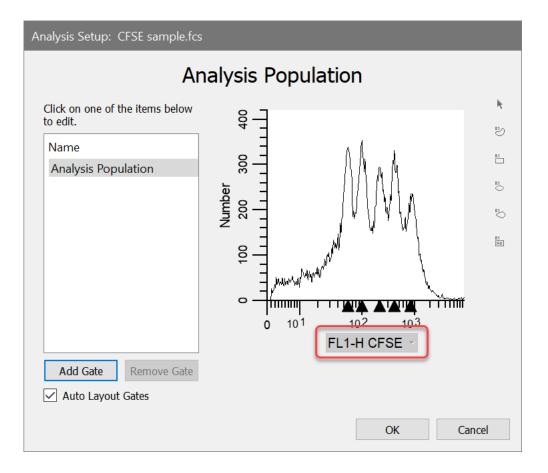
To start, we will use the Proliferation Wizard in ModFit LT and export its results. Then, you will set up WinList to import those results into an NStat region.

You will need both ModFit LT and WinList for this tutorial. ModFit LT must be at least version 3.2.1.

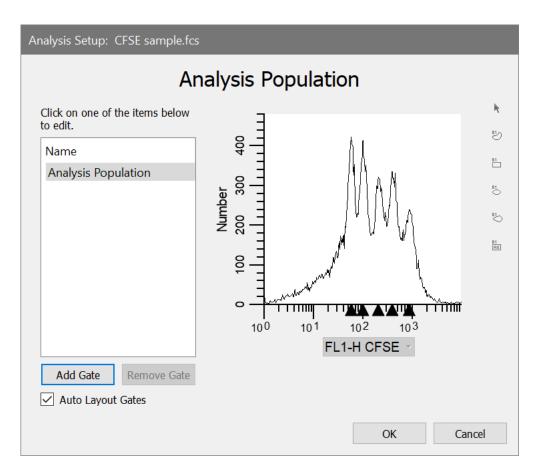
1. Launch WinList and pass through the opening dialogs.

2. Launch ModFit LT and pass through the opening dialogs.

3. Using ModFit LT's **Open FCS** command, navigate to the Samples folder located in the *WinList* application folder. Select "CFSE sample.fcs" and click **Open**. Note, the dialog may look different than the one shown below, depending on the version of ModFit LT you are using. If you have ModFit set up to show histogram channels, the X-axis will not match what is shown below, but the connection with WinList will still work fine.



4. Choose "FL1-H-CFSE" as the analysis parameter. Next, right-click on the histogram and choose **Edit X Parameter**. Choose **Log** for the **Transform** and click **OK**.



- 5. Click **OK** to close the Analysis Setup dialog.
- 6. On ModFit's home tab choose **Cell Track Wizard**.

7. In the Cell Tracking 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.

Cell Tracking Wizard			
Start Parent Generat	tions Other		
Cell Tracking Options			
Generation 3	What generation is the start of true proliferation? Precursor Frequency and UGPI use this value.		
Background Options			
Background	Enable this to perform background subtraction.		
Asymmetric cytokinesis	Enable to interpolate the Standard Deviations of the generations.		
Export Options			
Export partition table	Enable to export a partition table after analysis. The text file can be imported into other apps.		
Prompt for file name	Enable this option to prompt for the export file name.		
	Analyze Cancel Help		

8. When the analysis is complete, the Choose Location to Save Partitions dialog is displayed. You can save the file to any location. For this tutorial, save the export file to the desktop so that it's easy to find in later steps.

When you choose the Export option for the Cell Tracking Wizard, the program determines the boundaries between adjacent generations and writes that information to the export file. The boundaries are the points of intersection between the Gaussian components in the model.

Cell Tra	acking W	izard Partitions
File	CFSE sar	mple.fcs
Regions	10	
Scale	0	256
Gen10	0.00	24.83
Gen9	24.83	44.56
Gen8	44.56	62.77
Gen7	62.77	81.32
Gen6	81.32	98.89
Gen5	98.89	119.50
Gen4	119.50	138.40
Gen3	138.40	157.46
Gen2	157.46	177.94
Parent	177.94	256.00

Setting up WinList

9. Activate WinList and click the **Open FCS** button on the ribbon bar.

10. Navigate to the Samples folder located in the WinList application folder. Select the "CFSE sample.fcs" file and click **Open**.

11. In the **Create Histograms** dialog, select **CFSE** in the X Axis list and **CD69 PE** in the Y Axis list. Click **Add to List** *two times*. This will create two copies of CFSE vs. CD69 PE. Click **OK** to close the dialog.

Create Histograms					
1P Histograms 2P Histograms 3P Histograms					
X Axis Y Axis					
Forward Scatter	Forward Scatter Side Scatter STIM	Choose one or more X and Y parameters, then click Add to List.			
CFSE CD69 PE CD4 PerCP FL2-A ToPro3	CFSE CD69 PE CD4 PerCP FL2-A ToPro3	Unique combinations of the selected parameters are added to the list. In Combinations mode, select parameters in the X axis list and all combinations will be created.			
	Add to List	Combinations mode			
Histograms		Resolution:			
CFSE vs CD69 PE CFSE vs CD69 PE		64 ~ Delete Up			
<		> Down			
	OK Cancel				

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 because the exported information from ModFit LT is in Log units.

12. Move the mouse over the **CFSE** label on the X-axis and click the small triangle that appears. Select **Log** from the list of transformations. Repeat this for the **CD69** Y-axis.

	<u> </u>
10 ¹	10 ²
	CFSE FL1-H

13. Click on the title bar of the first dot plot to activate it. Click the \pm NStat region tool in the histogram toolbar.

14. In the NStat Properties dialog, click the **Import** button.

NStat Properties			
Name NStat rows NStat columns Show vertices Results	Current Value 2 2 Current Value Enable Enable	Description Rows in the NStat. Columns in the NStat. Enable to display each vertex. Creates a results for the region.	OK Cancel
Enable gates Cell Tracking Wizard Imp	Enable	Enables gates based on the subregions.	
Import partitions	Import	Imports information from ModFit LT Cell Tracking Wizard.	

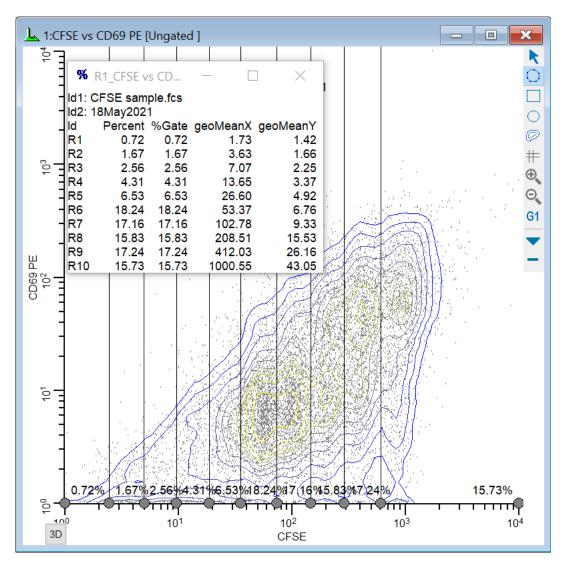
15. Navigate to the Desktop folder and select CellTrackingWizardPartitions.txt and click **Open**.

16. Edit the NStat rows to **1** and check the **Show Vertices**, **Results**, and **Enable gates** checkboxes. The number of columns should already be 10 from the Import. Click **OK**.

WinList User Guide

NStat Properties			
Name NStat rows NStat columns Show vertices Results Enable gates	Current Value 1 10 Current Value Labele Current Value Curr	Description Rows in the NStat. Columns in the NStat. Enable to display each vertex. Creates a results for the region. Enables gates based on the subregions.	OK Cancel
Cell Tracking Wizard Impo Import partitions	Import	Imports information from ModFit LT Cell Tracking Wizard.	

WinList creates the NStat partitions using the imported Cell Tracking information and displays a results window with statistics.



Isolating the generations

At this point, you have completed the basic import process. Let's extend the WinList analysis to explore other markers as a function of the proliferation analysis.

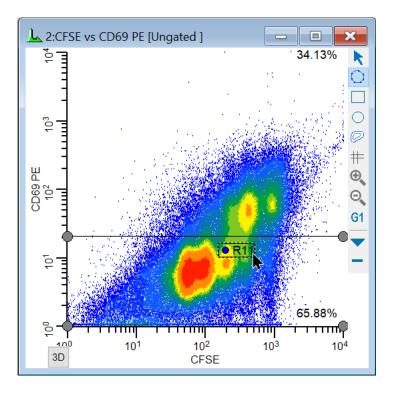
17. Click the title bar of the second histogram, and click the NStat region tool button to create another NStat. For this one, set NStat rows to **2** and NStat columns to **1**. Check the **Enable gates** option and uncheck other options. Click **OK**.

NStat Properties			
Name NStat rows NStat columns	Current Value	Description Rows in the NStat. Columns in the NStat.	OK Cancel
Show vertices Results	Enable Enable	Enable to display each vertex. Creates a results for the region.	
Enable gates Cell Tracking Wizard I	Enable	Enables gates based on the subregions.	
Import partitions	Import	Imports information from ModFit LT Cell Tracking Wizard.	

Now, one of the histograms contains an NStat that shows the proliferation partitions, and the other histogram has an NStat that will allow us to define the CD69 negatives and positives.

18. Drag the region by the label to adjust the second NStat so that it approximately divides the two major populations in the second histogram, as shown.

You can change the graphics for any histogram by choosing **Graphics** from the right-click context menu for a histogram. Try using Color Density with Rainbow colors for this one.



To analyze and visualize the generations better, we will create a calculated parameter using the *FCOM* function. This function creates a new parameter that will allow us to look at each population independently. It uses gates as input, and we'll use the gates from the NStat regions we just created.

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

19. Click the **Add Parameter** button on the main ribbon bar. Alternatively, you can rightclick in the data source and choose Add Parameter from the context menu.



20. Click the Add button in the Edit Calculated Parameters dialog.

21. Type **Proliferation** in the Name edit box. Type or copy this expression into the Equation edit box, and then click **OK**.

FLOG(FCOM(G1,G2,G3,G4,G5,G6,G7,G8,G9,G10)+0.5)*256/FLOG(1024)+FRN D(3)

This expression deserves some explanation. Let's break it apart, starting with the FCOM portion. FCOM is a "gate combination" function. It takes any number of gates as arguments;

the result of the equation depends on how many gates are in the expression. With a single gate, there are 2 possible event classifications: an event is "in" or "not in" the gate. With two gates, there are 4 possible event classifications. With the ten gates in our expression, FCOM can produce 1024 different event states.

However, since these gates do not overlap at all, most of the 1024 possible states have no events in them. In fact, the only states that contain events are the 10 single-positive event states, resulting in values of 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512. If we plotted the result of the FCOM function as a histogram, we would see 10 spikes of events, and the spikes would be exponentially spaced.

Our goal is to see these 10 spikes *evenly* spaced across the histogram. This is where the FLOG function comes in. By taking the log of the FCOM output and scaling it by 1024, we redistribute the 10 FCOM values evenly across 256 channels. The final portion of the expression, "+FRND(3)", changes the spikes of events into Gaussian distributions so that they can be better visualized.

Take a look at the reference section on <u>Add Parameters</u> and <u>FCOM</u> for details on the complete set of WinList functions.

22. Once again, click the **Add** button in the **Edit Calculated Parameters** dialog.

23. Type **CD69 Expression** in the Name edit box. Type or copy this expression, and click **OK**.

• Edit Calculated F	Parameters		×
Name Proliferation CD69 Expression	Equation FLOG(FCOM(G1,G2,G3,G4,G5,G6,G7,G8,G9,G10)+0.5)*256/FLOG(1024)+FRND(3) (FCOM(G11)+0.5)*256/2+FRND(3)	Resolution 256.000000 256.000000	OK Open Save Help
Add De	elete Edit		

(FCOM(G11)+0.5)*256/2+FRND(3)

24. Click **OK**.

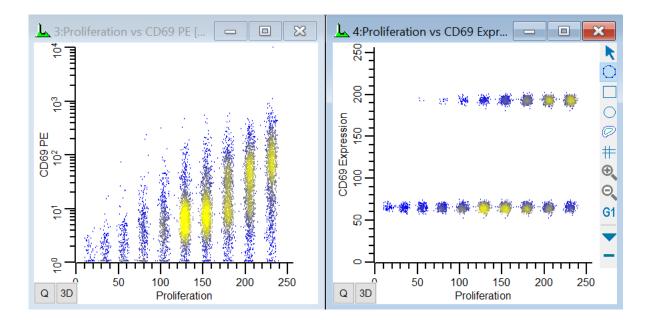
CD69 expression as a function of proliferation

We're now ready to explore this data file in really interesting ways. Wouldn't it be nice to see CD69 as it relates to proliferation, viewing each generation as a discrete cluster? Could we quantify the CD69 expression of CD69-positive cells for each generation? Yes and yes.

25. From the **Insert** tab on the ribbon bar, click the **Density** plot. This creates a Density plot in a default location on the data source. Click the X Axis label and choose *Proliferation*. Click the Y Axis label and choose *CD69 PE*.

Let's create another histogram to isolate the CD69 positives and negatives.

26. Right-click on the histogram that we just created and choose **Clone** from the context menu. A duplicate copy of the histogram is created.



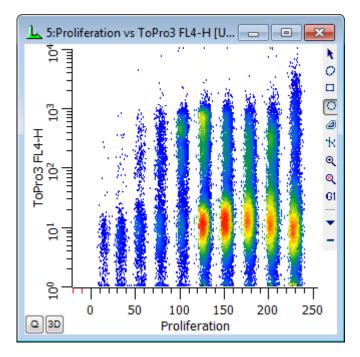
27. Change the Y Axis to show *CD69 Expression*.

In the graphics we can see the generations separated into discrete populations along the Xaxis. That's the first FCOM function we created. The Parent generation has the highest intensity.

The Y-axis shows the continuous CD69 parameter in the histogram on the left. We can see that the parent generation had the greatest expression of CD69, with diminishing expression for each subsequent daughter generation. In the second plot. the CD69 positives and negatives are distinctly separated using the CD69 Expression FCOM parameter that we

created. This makes it very easy to get statistics on the "CD69-activated" cells for each generation by simply creating regions on 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 WinList User Guide

Remote connection to ModFit LT

This feature is only available on PC platforms. ModFit version 3.2.1 or greater is required.

In this tutorial we will connect WinList to ModFit LT for enhanced DNA analysis. You will see how easily ModFit LT can be integrated into WinList. You will be using file **cho445.fcs** from the WinList sample files.

Before beginning, ModFit LT 3.2.1 or greater must be installed on your computer.

Set up

1. Start ModFit LT and pass through its opening dialogs. Minimize the program to clean up the desktop.

2. Start WinList.

3. Click the **Open FCS** button on the ribbon bar and navigate to the Samples folder located in the folder where WinList is installed. Select **cho445.fcs** from the list and click **Open**.

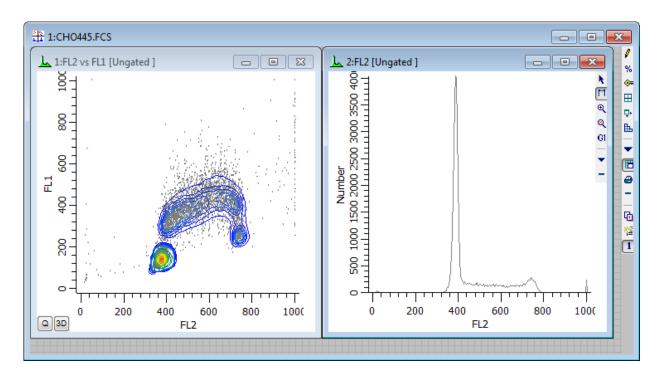
You can also drag and drop files from Windows Explorer or My Computer onto WinList. See <u>Drag and Drop</u> for details.

2. In the **Create Histograms** dialog, select FL2 vs FL1 and click **Add to List**.

3. Click the *1P Histograms* tab. Select FL2 in the list, and click **Add to List**.

4. Click **OK** to open the data source and create the histograms. You should have two histograms displayed.

Remote connection to ModFit LT



Making the remote connection

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

5. Double-click the title bar of the FL2 histogram. The **Edit Histogram** dialog box will appear.

6. Enable the **Remote** check box, and then click the **Remote** button.

Edit Hist	ogram		
Name:	FL2	ID: 2	Remote
Type:	● 1P ○ 2P ○ 3P		Remote
	Parameter	Resolution	
X Axis:	FL2	× 256 ×	Gate
Y Axis:	FS	× 256 ×	Subtract
Z Axis:	FS	× 256 ×	Graphics
	OK Cance	əl	Help

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.

Edit Remote	Link					
Link To: ModFitLT A	ModFitLT Auto Analysis When data changes					
Topic:			On replay			
EQUATION	l		On comma	ind		
Commands:						
POKE(DAT	A,VSH)					
	VORDS,VSH y(Decades=			▲✓		
Add	Insert	Replace	Open	ОК		
Delete	Clear		Save	Cancel		

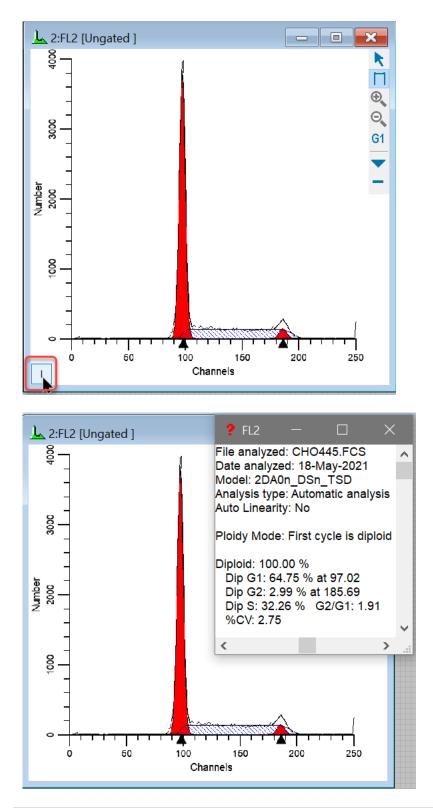
7. Click **OK** in the **Edit Remote Link** dialog box. WinList will make connection with ModFit LT and return us to the **Edit Histogram** dialog box.

If WinList cannot find ModFit LT, you receive an error message at this point. Make sure that ModFit LT is running, and repeat the last two steps. Remember that you need ModFit 3.2.1 or newer.

8. Click **OK** in the **Edit Histogram** dialog box.

Here's what happens. WinList begins to execute each command in the remote script for the histogram. That script tells WinList to send histogram data to ModFit LT and tells ModFit LT to Auto-Analyze the data. Next, the script tells WinList to copy ModFit's graphical and textual results and display them in the histogram window. WinList will update the analysis anytime it detects that the data for this histogram has changed. You could apply a gate to the DNA histogram, and any time a gate region is adjusted, the remote script would fire off, updating the ModFit analysis. You can modify a remote script using the **Edit Remote Link** dialog box.

9. To view the ModFit LT analysis, click the "**I**" button in the lower left corner of FL2 histogram. A window will appear, displaying the contents of the analysis results.



Modifying the update frequency

Depending on how WinList is set up, the analysis we just performed might not have sent *all* of the events for the FL2 histogram to ModFit LT. WinList only sends the number of events in

one of WinList's *packets.* Packet size is defined in Edit Program Controls, and determines the number of events WinList will keep *in memory* for quick analysis and gating.

Let's suppose that you want ModFit LT to see *all* of the events for FL2. The way to do this is to change the update frequency for the remote link. Here's how.

10. Double click the title bar of the FL2 histogram to show the **Edit Histogram** dialog. Click the **Remote** push button to display the **Edit Remote Link** dialog box.

11. Notice the **Update** group box, which contains several radio buttons. Click **On replay**. This tells WinList that the only time this link will update is when this file is replayed from disk. This ensures that *all* the data will be shipped to ModFit.

12. Click **OK** in the **Edit Remote Link** dialog box to accept the change, and click **OK** in the **Edit Histogram** dialog box.

13. Click the **Replay** button on the ribbon bar.

WinList will update the remote link with data that represents the entire listmode file. This technique has two distinct advantages: it allows ModFit LT to work with the full data set and it updates less frequently.

Summary

In this tutorial, we demonstrated how to use WinList's remote link capabilities to connect with ModFit LT. ModFit LT performed an Auto-Analysis of the data WinList sent to it, and WinList displayed ModFit's results.

To establish remote links, select **Edit Histogram**, and check the **Remote** check box. Then, click the **Remote** push button. The update frequency of the link, as well as the commands which are executed, can be edited in the **Edit Remote Link** dialog box.

Using MultiGraphs

MultiGraph windows can contain graphic and text elements from histogram and results windows, and allow you to display and print the collection in various views. They have a more restricted format than reports and do not update elements when data changes. However, they are a useful tool for creating summary information about experiments in a graphical record. In this tutorial, you will review how to open a listmode data file, create histograms, and learn how to select histograms and results for inclusion in a MultiGraph window.

Before you begin, make sure you understand the basics of working with WinList. The tutorial <u>WinList Basics</u> is a great place to start.

Setting up

1. Click the **Open FCS** button on the ribbon bar. Navigate to the Samples folder, select **1091901.fcs** in the list, and then click **Open**.

2. Use the Create Histograms dialog to create several 1P and 2P histograms. Click OK.

3. Click the **Q** button in the lower left corner of each of the 2P histograms. This will create a QuadStat region, and automatically display a results window for it. Adjust the QuadStats.

Creating the MultiGraph

At this point, we have created what we want to store in the MultiGraph. Next, we will create the MultiGraph and add this information to it.

4. Click the **MultiGraph** button on the ribbon bar. The **Edit MultiGraph** dialog box will appear. The dialog box displays all of the items that you can add into the MultiGraph in the **Available Items** list. As we add items, they will appear in the **MultiGraph Items** list.

5. In the **Available Items** list box, select all entries, except for *BLANK*. Click the **Add** button.

"BLANK" is always the first entry in the **Available Items** list box. It allows you to put a placeholder, of sorts, into the MultiGraph. It is useful when attempting to keep elements in proper columns and rows. 6. In the **Viewing Options** section, click **Tiled**, and enter 3 and 4 in the **Columns** and **Rows** edit boxes, respectively. This means that we will display the elements in a 3 x 4 array. If we were to add more than 12 elements, additional pages would be created automatically.

Edit MultiGraph	
Name: MultiGraph Id: 1 Available Items BLANK 1:1 1091901.FCS - MIG-FITC vs MIG-PE 1:2 1091901.FCS - MIG-FITC vs CD45-PERCP 1:3 1091901.FCS - MIG-PE vs CD45-PERCP	Add A-R 1:1 1091901.FCS MIG-FITC vs MIG-PE Add A-R 1:2 1091901.FCS MIG-FITC vs CD45-PERCP A-R 1:3 1091901.FCS MIG-FITC vs CD45-PERCP Remove A-R 1:4 1091901.FCS MIG-FITC
1:4 1091901.FCS - MIG-FITC 1:5 1091901.FCS - MIG-PE 1:6 1091901.FCS - CD45-PERCP R1_MIG-FITC vs MIG-PE R5_MIG-FITC vs CD45-PERCP R9_MIG-PE vs CD45-PERCP	A-R 1:5 1091901.FCS - MIG-PE Insert >> A-R 1:6 1091901.FCS - CD45-PERCP A-R R1_MIG-FITC vs MIG-PE A-R R5_MIG-FITC vs CD45-PERCP A-R R5_MIG-FITC vs CD45-PERCP A-R R9_MIG-PE vs CD45-PERCP
Viewing Options Left to Right Grids: Right to Left Overlay Tiled Cols: Rows: 4 	Item Label [\$SAMPLE] X pos. %: 60 Y pos. %: 95 Width %: 40
Save Open	OK Cancel

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

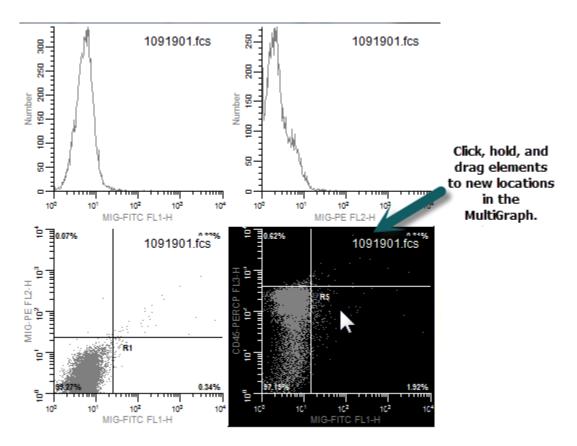
The MultiGraph will show the histograms and results that we added. Elements in the MultiGraph will not change, even if the histograms from which they were generated do. This means that you can use the MultiGraph to archive graphical and statistical information. You can also add elements from numerous data files to a single MultiGraph.

Note, the item label that is shown on each element of the MultiGraph is set by a setting in <u>MultiGraph Preferences</u>. By default it is set to the \$SAMPLE keyword, which the FCS file we have opened does not contain, so the label shows \$SAMPLE in this case. Set the preference to a keyword that provides useful information for your FCS files.

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 can also be exported to PDF format.

The **Edit MultiGraph** dialog box allows selection of new elements, editing of labels, and a choice of viewing options. MultiGraphs can also be saved to disk and opened from disk in this dialog box.

As with other types of windows in WinList, double-clicking on the title bar of the MultiGraph window is a shortcut to the **Edit MultiGraph** dialog box. There are also shortcuts for adding histograms and results windows to MultiGraphs.

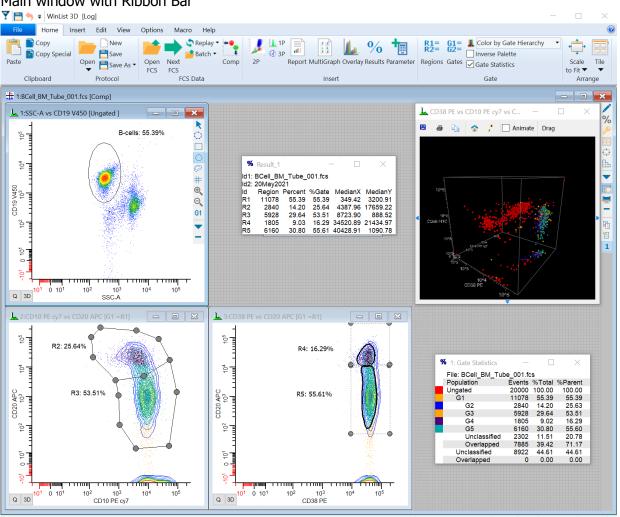
MultiGraphs have a popup menu that allows you to perform common MultiGraph tasks quickly. In addition, tiled MultiGraphs allow you to move elements by a click-hold-and-drag technique.

Reference

Main Window

WinList offers two interfaces for the main window. The Ribbon Bar provides easy access to the most common commands without having to drill down in menus. The Toolbar with Menu interface is a good option for users familiar with legacy versions of WinList.

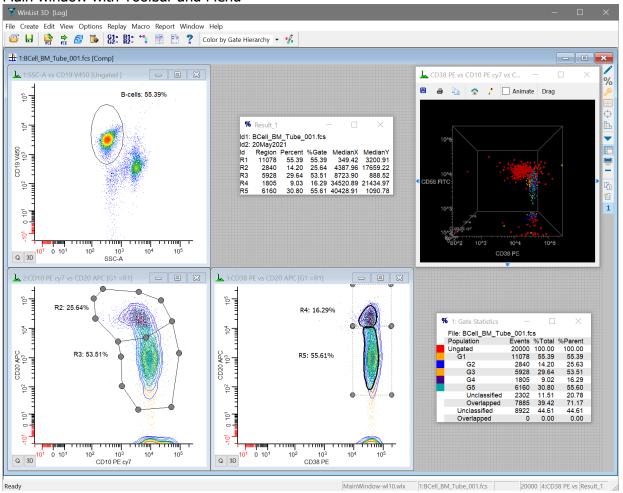
The **User Interface** property in Miscellaneous Preferences controls which interface the program will use. It can also be changed on the View tab of the ribbon or the View menu of the Toolbar. The program must be restarted when changing from one to the other.



Main window with Ribbon Bar

Click and drag onto a report.

MainWindow Ribbon-wl10 1:BCell BM Tube 001.fcs 20000 1:SSC-A vs Cl Result 1



Main window with Toolbar and Menu

See Also:

<u>Ribbon Bar</u> <u>Toolbar</u> <u>Status Bar</u>

Ribbon Bar

The Ribbon Bar provides easy access to the most common commands without having to drill down in menus. It is divided up into tabs that group commands based on function.

File (Application) Menu

This menu is accessed by clicking the WinList icon at the left side of the ribbon bar. It contains commands to open and save protocols, to open report and MultiGraph files, to export files, and to print.

🍸 💾 🦘 🗧 WinList	3D	[Log]					
File Home	Inse	rt Edit	View	Options	Macro	Help	
New Protocol		Recent Pr	otocols				
		<u>1</u> MainWi	ndow Rib	bon-wl10.w	lx		
<u>Open Protocol</u>	۲	<u>2</u> MainWi	ndow-wl	10.wlx			
Save Protocol		<u>3</u> MainWi	ndow Rib	bon.wlx			
<u>save Protocor</u>		4 cascadeGating-wl10.wlx					
Save <u>A</u> s	۲	<u>5</u> basics-wl10.wlx					
		<u>6</u> mf_remote-wl10.wlx					
Open <u>R</u> eport	Open <u>R</u> eport		<u>7</u> EnhancedProlifSetup WL10.wlx				
Open <u>M</u> ultiGrap	h	<u>8</u> Kinetic setup-wl10.wlx					
		<u>9</u> Cense-t	sne-wl10	.wlx			
Export	۲	1 <u>0</u> StickRe	gions-wl	10.wlx			
Print	٠						
Preferences							
						E <u>x</u> it	

Command	Description
New Protocol	Creates a new protocol, closing all open windows.
Open Protocol	Opens a protocol file.
<u>Open Bundle</u>	Opens a protocol bundle file.
Save Protocol	Saves a protocol.
Save Protocol As	Saves a protocol with a new name.

Save Bundle

<u>Open Report</u> <u>Open MultiGraph</u>

Export Listmode

Export Merge Files

Export Histogram

Export Histogram Graphic Save MultiGraph Graphic Export Report Graphic Export MultiGraph PDF Export Report PDF Export Results

Print Screen Print Report Print MultiGraph Print Overlay Print Histogram Print Data Source Print Setup

Preferences

Recent Files

Saves a protocol bundle file.

Opens a report file. Opens a MultiGraph file.

Saves a new listmode file in FCS or Text format based on the active data source. Merge FCS files into one output FCS file. Saves a new histogram file in FCS format based on the active histogram. Saves the active histogram as a graphic file format. Saves the active MultiGraph as a graphic file format. Saves the active Report as a graphic file format. Saves the active MultiGraph in PDF format. Saves the active report in PDF format. Saves the active results window in text format.

Prints the screen. Prints the active report. Prints the active MultiGraph. Prints the active overlay. Prints the active histogram. Prints the active data source window. Displays the Printer Setup dialog.

Displays the Preferences dialog.

Displays a list of recently opened protocols. Click an entry to open.

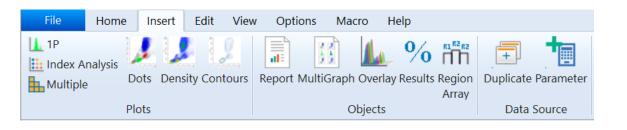
WinList User Guide

Home Tab

File Home Insert	Edit View Option	ns Macro Help			0
	New Save	Replay -		R1= G1= Color by Gate Hierarchy	
•	Save As Oper FCS	Next Comp FCS	2P Report MultiGraph Overlay Results Parameter	Regions Gates Gate Statistics	Scale Tile to Fit ▼ ▼
Clipboard	Protocol	FCS Data	Insert	Gate	Arrange

Clipboard panel	Protocol panel	FCS Data	Insert	Gate	Arrange
<u>Paste</u>	<u>Open</u>	<u>Open FCS</u>	<u>2P</u>	Regions	Scale to Fit
<u>Copy</u>	<u>Open Bundle</u>	<u>Next</u>	<u>1P</u>	<u>Gates</u>	<u>Tile</u>
Copy Special	New	<u>Replay</u>	<u>3P</u>	Color Mode	
	<u>Save</u>	<u>Batch</u>	<u>Report</u>	Inverse Palette	
	Save As	<u>Comp</u>	<u>MultiGraph</u>	Gate Statistics	
	Save Bundle A	<u>s</u>	<u>Overlay</u>		
			<u>Results</u>		
			Parameter		

Insert Tab



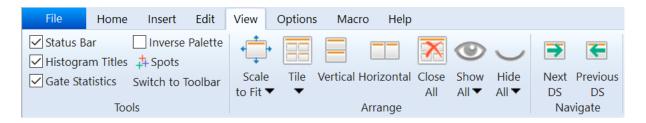
Plots	Objects	Data Source
<u>1P</u>	<u>Report</u>	Duplicate Data Source
Index Analysis	<u>MultiGraph</u>	Add Parameter
<u>Multiple</u>	<u>Overlay</u>	
<u>Dots</u>	<u>Results</u>	
<u>Density</u>	Region Array	
Contours		

Edit Tab

File	Home	Insert	Edit	Viev	w Op	otions	Macro	Help	
👆 Undo		🔑 User	Keywor	ds	R1= R2=	G1=	.		📮 Add Record
Q Logging	l .	A>B User	Equatio	ons			-		Database Wizard
📌 Progran	n Controls	Alerts	5	F	Regions	Gates	Palette	Setup	Auto-open
	Inform	ation				Gating			Databases

Information	Gating	Databases
Undo	Regions	<u>Setup</u>
Logging	<u>Gates</u>	Add Record
Program Controls	Palette	Database Wizard
User Keywords		Auto-open
User Equations		
<u>Alerts</u>		

View Tab



Tools	Arrange	Navigate	
Status Bar	Scale to Fit	Next DS	
Histogram Titles	<u>Tile</u>	Previous DS	
Gate Statistics	<u>Vertical</u>		
Inverse Palette	<u>Horizontal</u>		
Spots	Close All		
Switch to Toolbar	Show All		
	Hide All		

Options Tab Options File Home Edit View Macro Help Insert Auto-update Add to Report 🝷 Add to MultiGraph 🝷 Update Now Preferences Palette Wildcards Replay Enrich Update Remote Links and Paths Settings Replay and Update Utility Actions

Settings	Replay and Update	Utility Actions
Preferences	<u>Replay</u>	Add to Report
Palette	Enrich	Add to MultiGraph
Wildcards and Paths	Auto-update	
	Update Now	
	Update Remote Links	

Macro Tab

File	Home	Insert	Edit	View	Options	Macro	Help
Show To			-		Clear	5	Record
Edit Too		pen S	ave E		<mark>Batch</mark> Change Path	Run	Pause
Macro Too	olbox		Ma	cro File		Reco	rd and Run

Macro Toolbox	Macro File	Record and Run
Show Toolbox	<u>Open</u>	Run
Edit Toolbox	<u>Save</u>	Record
	<u>Edit</u>	Pause
	<u>Clear</u>	
	Batch	
	Change paths	



Quick Access Toolbar



The Quick Access Toolbar appears at the top left of the main window. Customize the commands that appear in this tool to include those that you need constant access to. To customize the commands, click the down-arrow button on the right side of the toolbar.

Toolbar

The toolbar is displayed across the top of the application window, below the menu bar. The toolbar provides quick mouse access to common WinList functions.

To hide or display the Toolbar, choose Toolbar from the View menu.

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

the functions they perform	
🖆 🔒 🙀 😤 🛃	🖌 🚱 🚼 👯 🗮 🖹 ? Color by Gate Hierarchy 💉 📢
Button	Function
B	Opens a protocol. This is equivalent to choosing <u>Open</u> <u>Protocol</u> from the File menu.
H	Saves a protocol. This is equivalent to choosing <u>Save</u> <u>Protocol</u> from the File menu.
FCS	Opens a listmode file. This is equivalent to choosing <u>Open FCS</u> <u>File</u> from the File menu.
rcs FCS	Opens the next file. This is equivalent to choosing <u>Next FCS</u> <u>File</u> from the File menu.
	To open the next file without displaying the File Open dialog, hold down the Shift key while clicking the button.
2	Replays the active data source. This is equivalent to choosing <u>Replay Active Data Source</u> from the Replay menu.
	To replay all data sources, hold down the Shift key while clicking the button.
6	Enrich the sampled data based on a selected gate.
G1= G2=	Displays the Edit Gates dialog. This is equivalent to choosing <u>Gate List</u> from the Options menu.

Toolbar

R1= R2=	Displays the region list dialog. This is equivalent to choosing <u>Region</u> <u>List</u> from the Options menu.
**	Displays the N-Color Compensation [™] Toolbox. This is equivalent to choosing <u>Compensation</u> 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 <u>New</u> <u>Report</u> from the Report menu.
?	Displays the on-line help system. This is equivalent to choosing <u>Index</u> from the Help menu.
Color by Gate Hierarchy	Selects the coloring mode used by WinList.
·l.	Edits the current coloring mode.

WinList User Guide

Status Bar

The status bar is displayed across the bottom of the main window. It displays prompts on the leftmost pane, and information about WinList objects in other panes.

	overlayTutorial finished.wlx	1:autoposition.017	15000	1:FL2-H vs FL1-H	R1_FL2-H vs FL
Dama	Mooning				
Pane	Meaning				
Left-most	Information about the curre	nt menu selection or	operation.		
1	Name of the current protoco	bl			
2	File name and ID for the active data source				
3	File index and size of the batch, if batch processing				
4	Number of events in the current file for the active data source				
5	Name and ID of the active h	istogram			
6	Name of the active results w	vindow			

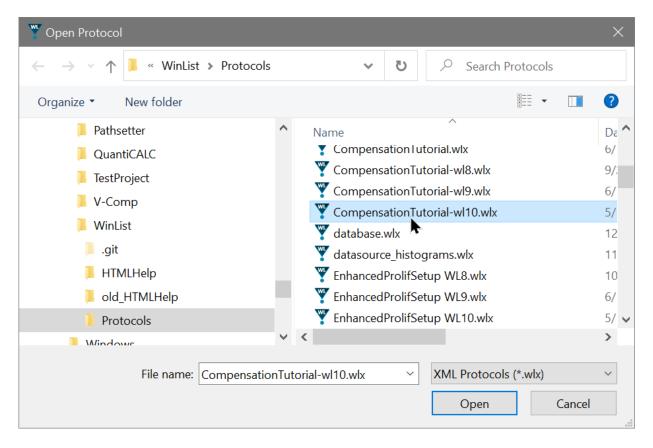
Open Protocol



This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display. It is used to open protocol files you have saved to set up an analysis in WinList. Protocols are created using the <u>Save Protocol</u> option.

You can also open Protocol Bundle files with this dialog by changing the filter drop-down to show Protocol Bundles.

The **Open Protocol** command displays the file open dialog box, allowing you to select a protocol (wlx or mac file) or a protocol bundle (wlb file). Select a protocol from the list and then click the Open button. Alternately, you can double-click the file in the list. The protocol will set up the screen, gates, regions and other components of the display.



Drag and Drop

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

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Open Bundle



This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display. It displays the <u>Open Protocol</u> dialog with the file type selection set for protocol bundle files. See <u>Save Bundle</u> for a description of the protocol bundle file.

Save Protocol

💾 Save

This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display. It is saves the current WinList analysis setup so that it can be reopened at a later time.

If the protocol has already been saved, this command updates the file on disk with the new setup. If the protocol has not yet been saved, it displays the <u>Save Protocol As...</u> dialog.

The function of this button is also controlled by several preferences. See <u>Miscellaneous</u> <u>Preferences</u> for additional information.

Save Protocol As...

💾 Save As 🝷

This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

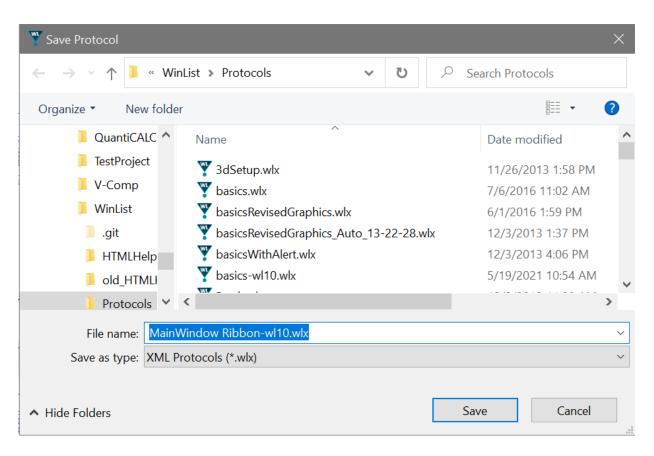
Sometimes the analysis you perform with WinList is something you use over and over again. In cases like this, it would be nice to save an analysis setup so that you can quickly reconstruct it and load new data files into it for analysis. This is where WinList protocols are used.

There are two kinds of protocols that WinList creates. The first is an XML-formatted protocol file that contains a complete description of the analysis you have setup. This kind of protocol requires that the FCS data files used in the setup are in the exact locations as they were when the protocol was saved.

A second type of protocol is called a protocol bundle. The protocol bundle stores an XML protocol file described above in a Zip-compatible file. In addition, the protocol bundle includes copies of the FCS files displayed in the protocol. Protocol bundles are convenient when you need to move protocols from one computer to another or to create an archive of your analysis. See <u>Save Bundle</u> for additional details.

This command displays the Save dialog box. Enter a name for the protocol, navigate to the folder where you want to save the protocol, and click the Save button.

Save Protocol As...



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 WinList User Guide

Save Bundle

💾 Save As 🝷

This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

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 <u>Save Protocol</u> command, but has several key differences that make it more powerful for some situations.

The significant differences between Save Protocol and Save Bundle are that Save Bundle creates a single file that contains the XML protocol *and* the FCS listmode files that the protocol uses. This makes the protocol bundle a better choice when you need to move a protocol from one place to another or to create an archive of your analysis.

To open a protocol bundle file, use the **Open Protocol** dialog and change the **Files of Type** selection to show Protocol Bundles.

Drag and Drop

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

See Also:

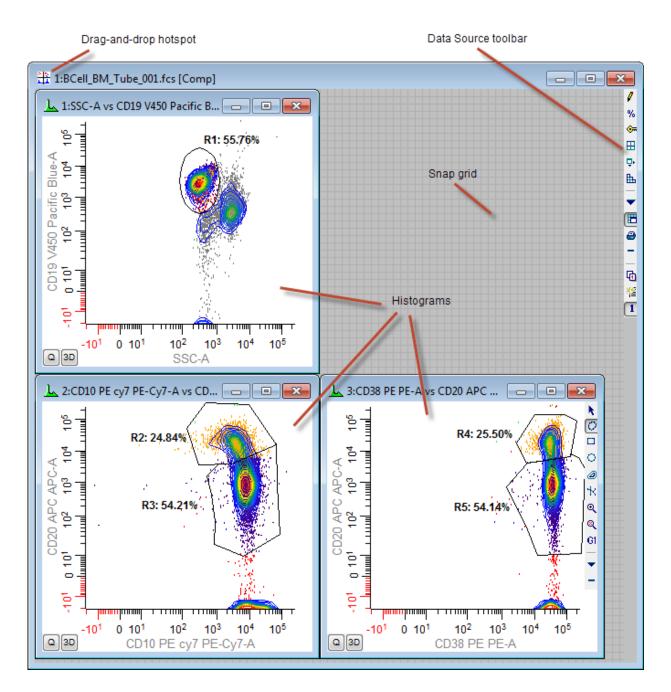
Save Protocol Open Protocol

Data Source Windows

WinList creates a data source window for each open listmode file. These windows are moveable and sizable within the confines of WinList's main window. Data source windows are automatically closed when WinList closes. They can contain any number of histograms and multiple pages of histograms.

To create a new data source window, click the Open FCS button on the ribbon or toolbar.

To read a new data file into an existing data source window, click the <u>Next FCS</u> button on the ribbon or toolbar.



A toolbar is positioned on the right edge of the data source window to provide quick access to many common commands. For details, see <u>Data Source Toolbar</u>.

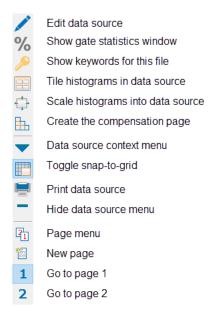
A data source window can be dragged and dropped onto a Report window to add all of its histograms to the report. The drag-and-drop hotspot is the upper-left portion of the title bar.

To edit a data source window, select **Edit Data Source** from the **Edit** menu. Alternately, you can double-click its title bar or click the Edit button on the data source toolbar.

Right-click a data source window to display its context menu.

Data Source Toolbar

This toolbar appears inside the right edge of the active data source window. Each icon represents a tool, described below.



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

Compensation Page

This button creates a special page in the data source for compensation. The compensation page shows histograms for one measurement at a time and is used when setting up N-Color compensation.

When you click the Compensation Page button on the toolbar, you can choose a gate to apply to the histograms on the compensation page.

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.

The page menu also allows you to select a page to view. This can also be done by clicking on the title bar of the data source and using the PgUp and PgDn keys on the keyboard.

Context menu

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

For more information, see Data Source Context Menu .

Snap

This button enables or disables snap-to-grid moving and sizing of histograms within a data source. With the option enabled, histogram windows move and size based on the background grid displayed on the data source. This makes it easy to create histograms that are the same size and to align them neatly. With snapping disabled, histograms can be moved and resized without constraint.

Data Source Context Menu

Edit	
Keywords	
Transformations	
Add parameter	
Print	
Add histograms to report	
Show gate statistics	
Add page	
Rename page	
Delete page	
Next page	PgDn
Previous page	PgUp
Move histograms to page	
Copy histograms to page	
Copy histograms with regions	
Delete histograms	

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

Edit

This command displays the Edit Data Source dialog.

Keywords

This command displays a list of all FCS keywords found in the current data file.

Transformations

This command displays the <u>Edit Transformations</u> dialog.

Add Parameter

Displays the Add Parameters dialog to create calculated parameters.

Print

This command prints the histograms on the current page of the data source.

Add Histograms to Report

This command adds the histograms on the current page of the data source to the active report. The histograms on the report are arranged in the same way they are on the data source window.

Show Gate Statistics

This command displays the <u>Gate Statistics</u> 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.

Copy Histograms with region

This command copies the histograms as well as the regions they contain to another page in the data source.

Delete Histograms

This command deletes the histograms on the current page.

Open FCS File



This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

Use this option to select an FCS listmode or delimited text file you want to open into WinList for display and analysis. When selected, the <u>Open</u> 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 <u>Next Batch</u> <u>File</u> and <u>Previous Batch File</u> commands, or edit the batch with the <u>Open Batch</u> command.

If the listmode file contains more than one *dataset*, the <u>Choose Dataset</u> 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.

If the selected file is a text file (comma- or tab-delimited), WinList will attempt to translate the file into an FCS file. For information about supported formats, see <u>Reading Delimited</u> <u>Text Files</u>,

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

You can select **Open FCS File** more than once to display more than one file at a time, or multiple copies of the same file. WinList is designed to handle any number of data sources. Your computer's memory, disk resources, and the demands of other applications that may be running limit the actual number you can display.

Open Dialog Box

This dialog box is common to most file opening operations in WinList. The tree on the left allows you to select the folder where files of interest are located. A list on the right displays files for the selected folder. A drop down list below the file list displays the types of files (file filters) that you see in the list. Buttons on the bottom allow you to open the file, view keywords stored in the file, or cancel the operation.

When opening listmode data files, this dialog allows you to select more than one file at a time. If you select more than one file, a batch is automatically created for you, and a convenient toolbar appears at the bottom of the main window to allow easy navigation through the batch. See <u>Batch toolbar</u> for details.

🍟 Open FCS File		\times
\leftarrow \rightarrow \checkmark \uparrow \blacksquare « WinList 3D » Sam	ples > v 🖸 🔎 Search Samples	
Organize 🔹 New folder		?
 Temp TrayStatus Uninstall Information Verity Software House ModFit LT 5.0 V-Comp 1.0 	 Name 1091904.FCS 1091905.FCS BCell_BM_Tube_001.fcs BCell_BM_Tube_002.fcs BCell_BM_Tube_003.fcs CFSE sample.fcs 	^
WinList 3D 10.0 Help Samples	CHO445.FCS FCOM_Example.fcs Helios1.fcs	~
File name: BCell_BM_Tube	• <	> ~

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 <u>Miscellaneous Preferences</u> dialog.

Choose Dataset	
This file contains more than one dataset. Which do you want to use?	OK Cancel
© 1. FCS2.0 212121 1024	
	2

To select a dataset, click the radio button next to it in the dialog and click OK.

When you need WinList to display several datasets from the same file at the same time, open the file with Open FCS File and select a different dataset each time.

Next FCS File



This command is found on the **Home** tab of the ribbon bar, or in the File menu when using the toolbar and menu display.

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

The Open dialog box is displayed so that you can select a new file. To open the new file, select it in the list box and click **Open**.

This dialog allows you to select more than one file at a time. If you select more than one file, a batch is automatically created for you, and a convenient toolbar appears at the bottom of the main window to allow easy navigation through the batch. See <u>Batch toolbar</u> 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 <u>Next FCS File</u>, except that it selects the previous file from the list.

Duplicate Data Source



This command is found on the **Insert** tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.

This command creates a copy of the data source window, duplicating the properties of the active data source and its histograms, regions, and gates. This provides a convenient means of setting up for multiple file analysis and keeping the setup of each data source consistent with the one from which it was created. One common use for this is in histogram subtraction, where one file contains control data and another file contains test data.

The layout of histograms, regions, and gates are replicated in the new data source window.

Edit Data Source

This dialog is displayed by choosing **Edit** in the data source context menu or by doubleclicking on the Data source title bar.

Edit Data	Source		
ID: 1	BCell_BM_Tu	be_001.fcs	
Alias:	[FILE]		
Events:	20000	Parameters: 12	
	FSC-A FSC-H		Add Parameter
P3 9 P4 0	SSC-A CD58 FITC CD38 PE		Transformations
P6 (CD34 PerCP		Names & Resolutions
P8 (P9 /	CD10 PE cy7 CD20 APC APC-Cy7-A		Quantitation
P11 (CD19 V450 CD45 AmCyan		Dataset
P12 -	Time		Protect histograms
			OK Cancel

Name and ID

The field displays the name and ID of the file data source. The ID is initially assigned by WinList, but it can be edited by the user. Each data source must have a unique ID.

Alias

Each data source has an alias that is displayed on its titlebar. By default, the alias is [FILE], which is a keyword that returns the name of the data file currently opened. You can enter another keyword or text in this field.

Events and Parameters

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

Parameter listbox

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

Add Parameters

This option allows you to add calculated parameters to the data source. See <u>Add New</u> <u>Parameter</u> for more.

When a parameter is added or deleted, the Edit Data Source dialog is automatically closed.

Transformations

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

Names and Resolutions

This button displays parameter names and resolutions for the data file. This also allows the user to adjust the log decades, if any, in the event that WinList was not able to automatically detect them correctly. See <u>Names and Resolutions</u> for more.

Quantitation

Selecting this option allows you to convert results values into quantitative units. See <u>Quantitation</u> for more.

Dataset

The option is enabled if the FCS file contains more than one dataset. It allows you to select which dataset to read and analyze with WinList.

When a new dataset is selected, the Edit Data Source dialog is automatically closed.

Protect histograms

When this option is enabled, histograms in the data source are protected from being deleted by the user.

ОК

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

Cancel

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

Add Parameters

This option allows you to create additional "calculated" parameters based on the native parameters of your listmode files. Calculated parameters can be displayed and manipulated just like native parameters. In addition to parameter ratios, WinList provides mathematical functions which can be applied to parameters.

All calculated parameters in WinList are computed in floating-point. They have a user-defined resolution, and can be computed using compensated or uncompensated data.



Display this dialog by clicking the **Add Parameter** button on the main ribbon bar or choosing the **Add Parameter** from the data source context menu. There is also a button in the **Edit Data Source** dialog box that shows this dialog.

• Edit (Calculated Parameters		×
Name Calc1	Equation (FCOM(G1,G2,G3)+0.5)*262144/8+FRND(10)	Resolution 262144.000000	ОК
Curci			Open
			Save
			Help
Add	Delete Edit		

Add

Adds a new calculated parameter to the data source. The <u>Edit Calculated Parameter</u> dialog will be displayed.

Delete

Deletes the currently selected calculated parameter in the list.

Edit

Displays the selected calculated parameter in the <u>Edit Calculated Parameter</u> dialog for editing.

Open

Opens a previously saved set of equations. The files have the default suffix of equ.

Save

Saves the current set of equations to disk.

ОК

Closes the dialog and accepts the changes. This causes all calculated parameters to be reevaluated and the workspace will be updated.

See also: Edit Calculated Parameter

Edit Calculated Parameter

This dialog is used to create and edit calculated parameters. It is displayed from the <u>Add</u> <u>Parameters</u> dialog.

One of the best ways to get started with a calculated parameter is to review the list of examples in the Functions listbox and select one that is similar to what you want to create. With an example selected, it is a simple matter to edit the specifics of the equation for your data.

The **Measurement Name** field can be any text you want. This is the name that will be displayed for this new calculated parameter on histograms and in parameter lists Some functions (FSNE and FCEN) create two measurements, and a second name field appears for those functions.

Edit Calculate	d Parameter		
Measuremen	t Name:	Cen-se_1	ОК
Second Meas	surement Name:	Cen-se_2	Cancel
	Type an equation	or select an example from the list.	
Equation:	FCEN(P1,P2,P3,P	4,P5,P6,G0,200,2)	Choose
Functions:	All functions and	examples ~	
	(FCOM(G1,G2)+0 (FCOM(G1,G2,G3		
		hy Enhanced Nearest-neighbor Stochastic Embedding ne gate, nn(200), and return dimensions.	
Transform:	Linear	Resolution: 64	
Source:	Compensated dat	a v	Help

Equation

Type an equation or select one from the example list to set this field. The equation can include parentheses for grouping, and supports +, -, *, and / operators. The parameters and their names are shown in a separate list box. The parameter and function letters are not case sensitive. For example, p1*2.5 is the same as P1*2.5.

When the Equation field contains **FCEN** or **FSNE** functions, the **Choose...** button is displayed next to the edit box. This button allows the user to select parameters for the FCEN or FSNE function quickly and efficiently.

Choose...

When the Equation field contains FCEN or FSNE functions, the Choose button is displayed next to the edit box. It allows you to select measurements to be used by the functions.

Parameters dialog box

A separate dialog box will be displayed showing the available measurements. The dialog will automatically be put away when you select either **Cancel** or **OK**.

Functions

All functions and examples are shown in this list box. A drop-down list allows you to show functions, examples, or both in the function list. Notice that all the functions start with the letter F.

FCOM(g1, g2,gn)	Gate combination function. The argument is a list of any number of gates separated by commas. See <u>FCOM</u> <u>Background</u> for further explanation.
FTIM(adcResolution)	Creates a chronological time variable over the number of channels, adcResolution , specified in the argument. This parameter allows you to gate out bad runs with DNA or to even perform kinetics calculations (assuming you are not interested in absolute time).
FSNE(p1,p2,,g0,50,2)	This function creates "t-SNE" (t-Distributed Stochastic Neighbor Embedding) parameters. The t-SNE function does a great job of reducing dimensionality to 2 dimensions.
	One FSNE equation will create 2 t-SNE parameters in the output.
	The first "p" arguments are parameters for the t-SNE function. Next is the "g" argument, which specifies a gate. Perplexity (50) sets the number of effective nearest neighbors. The last argument is 2, specifying the number of dimensions to return.

	See <u>Population Identification with Cen-se' and t-SNE</u> for further explanation.
FCEN(p1,p2,,g0,200,2)	This function creates "Cen-se" (Cauchy Enhanced Nearest-neighbor Stochastic Embedding) parameters. The function is a significant enhancement of the t-SNE algorithm, producing better resolution of clusters with more separation.
	One FCEN equation will create 2 Cen-se parameters in the output.
	The first "p" arguments are parameters for the FCEN function. Next is the "g" argument, which specifies a gate. The number of nearest neighbors to consider (200) is the next argument. The last argument is 2, specifying the number of dimensions to return.
	See <u>Population Identification with Cen-se' and t-SNE</u> for further explanation.
FLOG(param)	Returns the natural log of param . Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
FL10(param)	Returns the log to the base 10 of param . Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
FEXP(param)	Raises natural log to the param power.
FE10(param)	Raises 10 to the param power.
FSQR(param)	Returns the square of param .
FCUB(param)	Returns the cube of param .
FPWR(base, power)	Returns the value of base to the power .
FRND(variance)	Returns a random number with variance variability. If zero is entered as the argument, then it returns a uniform random number between 0 and 1.
FSIN(x)	returns the sine of x, where x is in radians.
FCOS(x)	returns the cosine of x, where x is in radians.
FTAN(x)	returns the tangent of x, where x is in radians.
FCTN(x)	returns the cotangent of x, where x is in radians.
FASN(x)	returns the arcsine of x, where x ranges from -1.0 to 1.0. Values for x outside of this range are forced to the nearest limit.
FACS(x)	returns the arccosine of x, where x ranges from -1.0 to 1.0. Values for x outside of this range are forced to the nearest limit.

FATN(x) FACN(x)	returns the arctangent of x. returns the arccotangent of x, equivalent to arctangent of $1/x$.
FV2S(param)	Returns param to the 2/3's power. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.
FS2V(param)	Returns param to the 3/2's power. Negative numbers and zero are evaluated to 0 since they are illegal values for log functions.

Transform

Sets the transform to be used for displaying the calculated parameter.

Source

In most cases, the calculated parameter can be computed on compensated or uncompensated data. A few functions (FCOM, FSNE, FTIM) ignore of this setting.

Resolution

Choose a resolution to use for the new calculated parameter. The list of resolutions is limited to the resolutions of parameters in the data file.

ОК

Accepts the changes to the equation and other settings and closes the dialog.

Cancel

Cancels the changes to the question. If the equation is being created, clicking the Cancel button will flag the equation to be deleted.

See also: <u>FCOM Background and Usage</u> <u>Population Identification with Cen-se' and t-SNE</u>

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 <u>Add New Parameters</u> dialog box, accessed from the Edit Listmode File Data Source dialog box.

To understand how this powerful function works, you must first understand something about binary numbers. A binary number is represented with only two digits: 0 and 1. A binary number is formatted similarly to a decimal number using powers of 2 instead of powers of 10. An example of a binary number is 1110. The decimal equivalent to this number is 0 + 2 + 4 + 8 = 14

The lowest four digit binary number is 0000 (0 decimal) and the highest four digit binary number is 1111 (15 decimal). Thus, a four digit binary number can encode 16 different numbers (0,1,...15). The number of encoded different numbers (n) is related to the number of binary digits (d) by the function n=2 to the d power. Thus, for this example, d=4 and n=16.

What does this all have to do with the FCOM parameter? Plenty! Since an event is either inside or outside a gate, its state can be represented by a single digit, 0=outside and 1=inside. Suppose we have two gates, G1 and G2. We can represent all possible combinations of gate states by simply forming a binary number where the first digit represents the state of G1 and the second digit represents the state of G2. Thus if we have two gates, then the binary number 01 represents an event outside G1 and inside G2. Similarly, 11 represents an event inside G1 and inside G2. The FCOM function assigns each event a decimal number reflecting the gate combination for that event. Thus, in the case of 2 gates, G1 and G2, FCOM will return a value of 0, 1, 2, or 3 depending on the particular gate combination satisfied.

If we multiply this FCOM number by a scaling value and we display the result as a single parameter histogram in WinList, we will see a number of spikes with the FCOM parameter on the X-axis. The height of each spike tells us how many events satisfy that particular gate state. Typically, we add a small random number to each FCOM number (e.g. FRND(10)), so that instead of spikes we see Gaussian distributions which are more familiar to us.

How do you know what combination corresponds to each peak? Easy! You just count in reverse binary, which is to say evaluate from left to right." Let's do a three gate example (e.g. FCOM(G1,G2,G3)).

G1	G2	G3	FCOM Derivation	Calculated FCOM value
0	0	0	0 + 0 + 0 =	0
1	0	0	1 + 0 + 0 =	1

0	1	0	0 + 2 + 0 =	2
1	1	0	1 + 2 + 0 =	3
0	0	1	0 + 0 + 4 =	4
1	0	1	1 + 0 + 4 =	5
0	1	1	0 + 2 + 4 =	6
1	1	1	1 + 2 + 4 =	7

OK, that's not so easy after all. It would be better if the program figured out which population was which and labeled them for us. This is something that the Create Region Array command does nicely. There's an example of that in the tutorial <u>Using FCOM to</u> enumerate phenotyptes.

You can create additional FCOM calculated parameters with different gate combinations to display two parameter dot plots. With some creativity on your part, you can readily visualize and analyze complex phenotypes with relatively little effort.

Edit Transformations

This dialog allows you to select linear parameters to convert from linear to log and log-like transforms.

Edit Transformations				
Name Scaling	Current Value	Description	^	ОК
Number of Decades	Computed ×	This applies to all log-like p		Cancel
FSC-A				
Transform for FSC-A	Linear ~			Help
Low Value for FSC-A	0.00	Negative values are ignored		neip
B-Coefficient for FSC-A	10 ~	Number of linear channels		
Auto-Adjust Transform for FSC-A	Enable	Optimize log-like transform		
Auto-Zoom for FSC-A	Off ×	Zoom-in on the parameter		
Zoom Fixed Boundary for FSC-A	0.00	Set the upper boundary to		
Zoom Percentile for FSC-A	99.00	Set the upper boundary ba		
FSC-H				
Transform for FSC-H	HyperLog ~			
Low Value for FSC-H	-20.00	Negative values are ignored		
B-Coefficient for FSC-H	10 ~	Number of linear channels	~	
<		>		_

Most flow cytometers store data in linear format, even if you view the data on log-like scale during acquisition and analysis. This dialog allows you to convert linear parameters to log-like for display and analysis in WinList.

Scaling

Number of Decades

This option determines how many log decades WinList will transform the linear data values into.

Choose *Computed* to allow the program to determine automatically the number of decades. With this option selected, the program computes the number of decades by taking the log of the ADC resolution of the parameter. For example, if the ADC resolution is 262144, the log10(262144) is approximately 5.42 decades.

Choose *4-decades* to force the program to map the linear data into 4-decades. This option allows comparisons with other software that only permits 4-decade displays. In the example above, compressing 262144 channels into 4-decades of log space compresses the data by 1.42 decades.

Measurements

For each measurement in the file, you can set several properties. Transform settings apply to all histograms showing the selected measurement.

Transform

The transform can be set to Linear, Log, HyperLog, Hyperbolic sine, or VLog.

Low Value

This is the lowest value shown on the axis.

B-Coefficient

This value controls the linear portion of a HyperLog axis. The selected value is the number of channels on both sides of 0 that will be displayed as linear. The remaining portions of the HyperLog axis are display log-like.

Auto-Adjust

Enable this option if you want WinList to automatically adjust the B-Coefficient and Max Negative value for this parameter. Each time a new file is opened, WinList will readjust B and Max Negative settings.

Auto-Zoom

Auto-zoom allows you to eliminate unnecessary channels at the upper end of a parameter's dynamic range automatically. It is very useful for parameters like "Time" which only use a portion of the range, and for some instruments which store data in the lower portion of the range. Auto-zoom is set up independently for each parameter.

Off: Turns off auto-zoom for the parameter.

To highest used channel: Choose this option if you want WinList to eliminate empty channels on the upper end of the parameter. It is useful, for example, for a TIME parameter that typically only uses a small portion of the dynamic range of the parameter.

To fixed upper boundary: This option allows you to zoom to a specific channel for the upper boundary of the data. Use the associated **Zoom Fixed Boundary** edit box to set the channel for zooming.

To percentile: This option allows you to zoom in on a specified percentile. If, for example, 99% of your data is in the lower half of the dynamic range, it may be useful to ignore the remaining 1% of the data so that you can better visualize and analyze the 99%. Use the associated **Zoom Percentile** edit box to set the percentile for zooming.

Save preferences

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

See also:

<u>Transformation Preferences</u> Understanding Hi-res Data Files

Names and Resolutions

This dialog box can be accessed from the **Edit Data Source** dialog box by clicking the **Names and Resolutions** push button. It allows the user to change the names of parameters, as well as set the number of log decades for any parameters.

Parar	neter Names and Resolution	ns			
	Name FSC-A			Res	Log 0.00
P1 P2	FSC-A FSC-H			262144 262144	0.00 5.42
P3	SSC-A			262144	5.42
P4 P5	CD58 FITC CD38 PE			262144 262144	5.42 5.42
P6	CD34 PerCP			262144	5.42
P7 P8	CD10 PE cy7 CD20 APC			262144 262144	5.42 5.42
P9	APC-Cy7-A			262144	5.42
	CD19 V450 CD45 AmCyan			262144 262144	
	Time			262144	0.00
		ОК	Cancel		

Parameter names are used by histograms on the axis labels.

To edit a parameter name, select the parameter in the list box and enter the new name for the parameter in the **Name** edit box. You can enter a keyword in square brackets, e.g. [\$P4S], to display information derived from the FCS file for the parameter name.

The number of log decades for parameters is displayed for informational purposes. The values can not be edited.

Whenever a name is edited by the user, WinList "locks" the name so that it does not change until the user "unlocks" the name, or the number of parameters changes. Locked names are designated with an asterisk (*) next to the name in the list box.

To manually lock or unlock a parameter name, select the parameter in the list box and click the **Lock** check box next to the name. If the check box is checked, the name is locked.

If you edit parameter names and you do not lock the edited entries, WinList will discard your edits the next time it updates the data source by replaying, opening a new file, or saying **OK** to the Edit Data Source dialog box.

Quantitation

WinList provides complete support for converting results values from channel-based units to units representing a quantitative value. Some of the units commonly used for quantitation are Antibody Binding Capacity (ABC), Mean Equivalent Soluble Fluorescein (MESF), and Molecules Per Cell. The great advantage to converting results into quantitative values is that they become meaningful when compared with data from other instruments and other users. Standardization among users and labs becomes a much simpler task.

If you have access to the quantitation calibration information, you can enter it manually into this dialog box.

If your data files contain special keywords, WinList can automatically apply calibration information to the relevant parameters and convert results into quantitative units. The keywords WinList looks for are PnQS, PnQI. The first defines the slope for parameter n; the second defines the intercept for parameter n. Some instrument manufacturers, BD Biosciences for example, store these keywords when acquiring data if relevant controls have been run.

Qu	antitation					
ID	Name	Mode	State	Slope	Intercept	Slope Type
1	FSC-A	Detect		0.00000000	0.00000000	Linear-based
2	FSC-H	Detect		0.00000000	0.00000000	Linear-based
3	SSC-A	Detect		0.00000000	0.00000000	Linear-based
4	CD58 FITC	Detect		0.00000000	0.00000000	Linear-based
5	CD38 PE	Detect		0.00000000	0.00000000	Linear-based
6	CD34 PerCP	Detect		0.00000000	0.00000000	Linear-based
7	CD10 PE cy7	Detect		0.00000000	0.00000000	Linear-based
8	CD20 APC	Detect		0.00000000	0.00000000	Linear-based
9	APC-Cy7-A	Detect		0.00000000	0.00000000	Linear-based
10	CD19 V450	Detect		0.00000000	0.00000000	Linear-based
11	CD45 AmCyan	Detect		0.00000000	0.00000000	Linear-based
12	Time	Detect	On	0.01000000	0.00000000	Linear-based
Edit	: Time	🗹 Detect	<mark>∕ O</mark> n	0.010000	0.000000	Linear-based ~
	Apply quantitation to all data sources				ОК	Cancel

The program can also automatically turn "TIME" into a quantitative parameter if there is a \$TIMESTEP keyword in the data file.

Detect

This option is normally checked, which tells WinList to automatically detect quantitation for the parameter. In this mode, WinList will look for quantitation keywords in the file and will apply the quantitation automatically. Most parameters are safely left with Detect enabled.

Uncheck this option if you want to enter you own slope and intercept values, or you do not want to detect quantitation keywords automatically.

On

If this option is checked, the parameter is enabled for quantitation. The slope and intercept values will be used to convert the parameter transformation into quantitative units.

Slope and Intercept

The slope and intercept provide information on how to convert an ADC channel number to a quantitative value. The values are typically provided by the instrument or reagent manufacturer to allow you to convert from "channels" into some other quantitative unit.

To compute your own slope and intercept values, you need to perform a linear regression of log (molecules per cell) versus channel numbers. WinList includes an Excel spreadsheet in the Samples folder that can be used to assist in this. See <u>Using the Quantitation Spreadsheet</u> for details.

Slope Type

The quantitative slope and intercept are typically defined using linear units. The \$TIME parameter, for example, is a linear parameter, its slope is determined by the \$TIMESTEP keyword, and the Slope Type should be "Linear".

However, there are cases where the slope and intercept relate to log-transformed units. For example, with older FCS files that were encoded in log, the slopes and intercepts provided by manufacturers were typically log-based. This means that they are applied to the log-transformed data rather than the linear form of the data.

Select the appropriate slope type for the quantitation for your files. If the quantitative information is supplied by a manufacturer, contact the manufacturer to determine whether the slope applies to linear units or log units.

ОК

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.

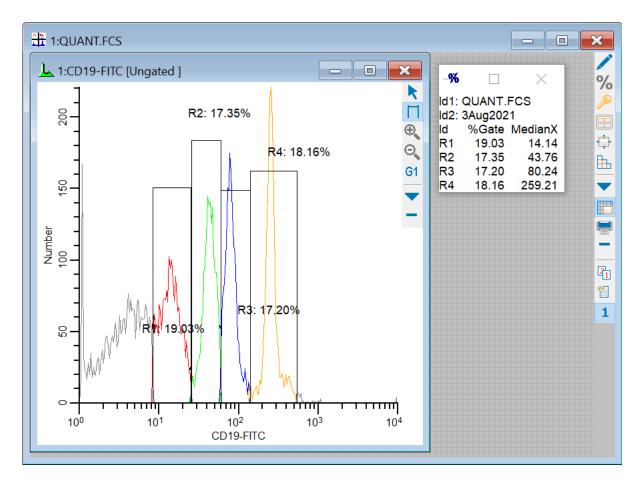
Using the Quantitation Spreadsheet

WinList includes an Excel spreadsheet that can assist in using its quantitative features. Use this sheet to compute slopes and intercepts that will convert the axes in WinList to quantitative units, such as MESF.

Typically, quantitation is set up with beads that have been assigned specific values by the bead manufacturer. When the bead sample is run on your instrument, there will typically be 4 to 6 peaks for the marker being quantified. Those peaks are analyzed in WinList, and median values are then entered into the quantitation spreadsheet to compute quantitation slopes and intercepts. Finally, the slope and intercept values are then entered in WinList to convert the axes to quantitative units.

Step-by-step instructions:

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 below using **QUANT.FCS** in the Samples folder.



2. In Excel, open the **WinlistQuantitationBead.xls** spreadsheet found in the Samples folder. Select the appropriate sheet for the number of bead peaks in your sample. In the example file, there are 4 beads so we select the **4-Bead** sheet.



3. Enter the **ADC range** (also called resolution) for the data file that you are analyzing. In the example file, this is 10,000. The sheet will compute the number of Decades represented by this value.

	Α	В	С
1	WinList S	lope Intercept (Calculation Sheet
2			
3	4-Bead pl	us Blank Conve	ersion
4			* Enter *
5		ADC range:	10000
6		Decades:	4.00

4. Next, enter the median values for each bead peak from WinList. Then enter the MESF (or ABC) values provided by your quantitative bead manufacturer.

4-Bead	plus Blank Conv	ersion			
		* Enter *			
	ADC range:	10000			
	Decades:	4.00			
		* Enter *	* Enter *		
		Linear Median	Linear MESF	Log Median	Log MESF
	Blank	0	0	n/a	n/a
	Bead 1	14.14	85	2876.1235	1.9294
	Decide 0	40.70		4400 0000	2 5070
	Bead 2	43.76	322	4102.6933	2.5079
	Bead 2 Bead 3	43.76 80.24	322 721	4102.6933 4760.9773	2.5079
	Bead 3	80.24	721	4760.9773	2.8579

The program will compute the slope and intercept using linear values and again using log values. Typically, the linear slope and intercept are a little more accurate. WinList can use either type.

5. Type a value into the **Testing** section to see how the value converts to MESF units. It's useful to test the bead linear median values and compare them with the MESF values for each peak. They should be similar results.

plus Blank Conv	ersion								
plus blank conv	* Enter *				3000 -				
ADC range:	10000								
Decades:	4.00				2500 -				*
Dooddoor	* Enter *	* Enter *			2000 -				
	Linear Median	Linear MESF	Log Median	Log MESF					
Blank	0	0	n/a	n/a	2000 -				
Bead 1	14.14	85	2876.1235	1.9294					
Bead 2	43.76	322	4102.6933	2.5079	Ц Ш Ш Ш				
Bead 3	80.24	721	4760.9773	2.8579	Σ				
Bead 4	256.21	2581	6021.4902	3.4118	1000 -				
					1000 -				
							×		
WinList Quan					500 -				
		Intercept				×			
Log-based	0.0004737593	0.573106035			0.				
						0	100	200	300
Linear-based	10.4409379	-102.0959655					Line	ar Units	
Testing	* Enter *	Log-based	Linear-based						
reating	Linear Value	MESF Result	MESF Result						

Now you are ready to enter the slope and intercept values into WinList.

6. Display the Edit Data Source dialog and choose **Quantitation**.

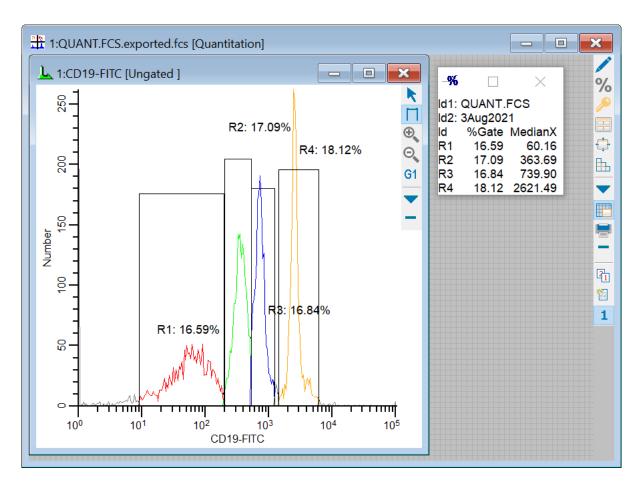
Edit Data Source	
ID: 1 QUANT.FCS Alias: [FILE]	
Events: 10000 Parameters: 5	I
P1 FSC-Height P2 SSC-Height	Add Parameter
P3 CD19-FITC P4 FL2-Height	Transformations
P5 FL3-Height	Names & Resolutions
	Quantitation
	Dataset
	Protect histograms
	OK Cancel

7. In the Quantitation dialog, select the measurement that has the quantitation information. Uncheck **Detect**, check **On**, copy and paste the linear slope and intercept

values from Excel, and select **Linear-based** for the type. In the example, the slope of 10.44 and intercept of -102.09 have been copied from Excel for the example file.

Quantitation						
ID Name	1	Mode	State	Slope	Intercept	Slope Type
1 FSC-Height		Detect		1.0000000	0.00000000	Linear-based
2 SSC-Height 3 CD19-FITC	¥	Detect Manual	On	1.00000000 10.44093790	0.00000000	Linear-based 50 Linear-based
4 FL2-Height 5 FL3-Height		Detect Detect		1.00000000 1.00000000	0.00000000 0.00000000	Linear-based Linear-based
				1	1	
Edit: CD19-FITC		Detect	⊡ Or	10.440937	-102.09596	Linear-based 🗸
Apply quantita	ation to all data sources				OK	Cancel

8. Click OK. WinList will now display quantitative results and axes for the parameter. Adjust the regions over the bead peaks, and the MedianX values should be close to the MESF values entered into the Excel sheet.



Compensation Toolbox



This command is found on the **Home** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display. The Compensation Toolbox allows you to control WinList's interactive N-Color Compensation[™] and normalization.

WinList's N-Color Compensation[™] can handle any number of fluorochromes and compensate them for signal crossover. In many cases, compensation can be set up automatically, but you can still manually override compensation settings. Compensation can be manually adjusted using 2-parameter dot plots.

If you have single-color controls as well as an unstained control file, you can use **V-Comp**[™] to set up your compensation automatically.

In order to set up compensation manually, you need a set of single-color control files, one for each fluorochrome you want to compensate. <u>Using Interactive N-Color Compensation</u> demonstrates this approach.

WinList also supports reading compensation information directly from FCS files created by many popular cytometers, as well as files containing the FCS 3.1-compliant \$SPILLOVER keyword.

See also: <u>Using Interactive N-Color Compensation</u>[™] <u>Compensation Toolbox dialog</u> Compensation Trace Lines

Compensation Toolbox dialog

This dialog is displayed by clicking the **Comp** button on the ribbon bar or selecting **Compensation Toolbox** from the Options menu. It provides control over various components of the N-Color Compensation[™] and the normalization system.



Setup AutoComp Trai	nsforms	Misc	
Compensate:		Show plots for:	ОК
FSC-A	~	CD58 FITC	
FSC-H		CD38 PE	Open
SSC-A		CD34 PerCP	Save
On CD58 FITC		CD10 PE cy7	Edit
On CD38 PE		CD20 APC	Luit
On CD34 PerCP		APC-Cy7-A	Reset
On CD10 PE cy7		CD19 V450	
On CD20 APC		CD45 AmCyan	
On APC-Cy7-A			
On CD19 V450			
On CD45 AmCvan	~		

The dialog is separated into several tabs that group similar features together.

Compensation: Setup Tab <u>Compensation: AutoComp Tab</u> <u>Compensation: Transforms Tab</u> <u>Compensation: Miscellaneous Tab</u>

ОК

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 <u>Edit Properties for Compensation System</u> 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 <u>Compensation Trace Lines</u>

Compensation: Setup Tab

Setup Au	toComp	Transforms	Misc	
Compensat	te:		Show plots for:	ОК
FSC-A		^	CD58 FITC	
FSC-H			CD38 PE	Open
SSC-A			CD34 PerCP	Save
On CD58	FITC		CD10 PE cy7	Edit
On CD38	PE		CD20 APC	Luit
On CD34	PerCP		APC-Cy7-A	Reset
On CD10	PE cy7		CD19 V450	
On CD20	APC		CD45 AmCyan	
On APC-C	у7-А			
On CD19	V450	~		
On CD45	AmCvan	*		

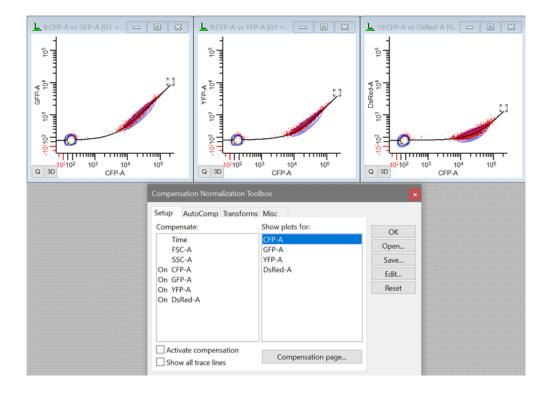
Compensate list

This list displays the parameters in the active data source. Items in the list will show **On** when they are enabled for compensation. Click an item in the list to toggle it between off and **On**.

Show plots for list

This list determines which plots are displayed on the compensation page. Select a measurement in the list to display 2P plots with that measurement on the X-axis and each of the other compensation measurements on the Y-axis. If compensation has not been set up for the measurement, the program will also compute the appropriate slopes to compensation the measurement. "Trace lines" are used to set and display the slope and intercept for compensation. They only appear when the **Activate** option is *not* checked.

Use this feature with single-color controls for each measurement 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.

Show all trace lines

With this option checked, trace lines are displayed on all plots showing compensation measurements. If the Activate compensation checkbox is also checked, small + and - buttons are shown instead of trace lines.

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 for the measurement selected in the **Show plots for** list. The program will display a dialog allow you to select a gate to apply to all of the plots, and then switch to the compensation page.

Use this button any time you want to assign a gate to plots on the Compensation page.

See also:

Compensation Tool Box

Compensation: AutoComp Tab

Compensation: HyperLog Tab

Compensation: Miscellaneous Tab

Compensation: AutoComp Tab

Settings on this tab in the Compensation Toolbox determine whether WinList will set up compensation automatically from keywords in the FCS file. The initial settings for the dialog are determined by <u>Data Source Preferences</u> and it can be set up differently for each data source in a protocol.

Compensation Normalization Toolbox				
Setup AutoComp Transforms Misc				
Current State	ОК			
User-defined compensation	Open			
V-Comp Compensation Save				
Open V-Comp setup Launch V-Comp				
AutoComp from Keywords				
Autocomp each file ~				

Current State

This section displays the current state of compensation.

V-Comp[™] Compensation

This section provides quick access to the **V-Comp** stand-alone app, which can automatically compute compensation settings based on a set of single-color controls and an unstained control file.

Open V-Comp setup

Click this button to select a V-Comp setup that you have already created. A File Open dialog will be displayed showing the folder where V-Comp setups are typically stored. Select the setup you want to use and click **Open**, and WinList will read the setup file and apply the compensation.

Launch V-Comp

If you have V-Comp installed on your computer, you can use this button to launch it directly from WinList. This is useful when you want to create a new V-Comp setup or make edits to an existing setup.

AutoComp from Keywords

Many instrument vendors encode compensation information in the keywords of the FCS files they create. WinList can read and decode this information for many popular instruments.

Disable	Do not use compensation from keywords.
Autocomp if not already set	Use keyword compensation values unless there is already compensation set up by a previous file or manually by the user.
Autocomp each file	Use keyword compensation values for each file.

See also:

Compensation Tool Box Compensation: Setup Tab Compensation: Transforms Tab Compensation: Miscellaneous Tab

Compensation: Transforms Tab

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

Compen	sation Norma	alization Tool	box				x
Setup	AutoComp	Transforms	Misc				
Optim	nization	Ontincing to	(OK	
	L	Optimize tr	ansionns.			Open	
	Reset transforms				Save		
Chang	je Transforms					Edit	
		Linear -	-> Log			Reset	
		>	Log				
		> Hy	perLog				
		> Hyper	bolic Sine	•			
		> \	/Log				

Optimize transforms

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

WinList will determine the best B-Coefficient and the Low Value for each of the selected parameters.

Reset transforms

This command resets the HyperLog[™], Hyperbolic Sine, and VLog transformations for selected parameters. A dialog is presented to allow you to choose which parameters to reset. After selecting the parameters, click OK to begin the process.

WinList resets the B-Coefficient and the Low Value for each of the selected parameters, based on preference settings.

Linear --> Log

This command switches linear parameters to the default log transformation. A dialog is displayed to allow you to choose the parameters to convert.

- -> Log

This command switches log-like parameters to use the Log transformation.

--> HyperLog

This command switches log-like parameters to HyperLog[™] transformation.

--> Hyperbolic Sine

This command switches log-like parameters to Hyperbolic Sine transformation.

--> VLog

This command switches log-like parameters to VLog transformation.

<u>Compensation Tool Box</u> Compensation: Setup Tab <u>Compensation: AutoComp Tab</u> <u>Compensation: Miscellaneous Tab</u>

Compensation: Miscellaneous Tab

Compensation Normalization Toolbox	×
Setup AutoComp Transforms Misc	
Normalization Show normalization targets Compensation Information Compensation review (linear, zoomed plots) Show digital errors Log bias	OK Open Save Edit Reset
<u>V</u> iew cross-over matrix <u>A</u> dvice	

Show normalization targets

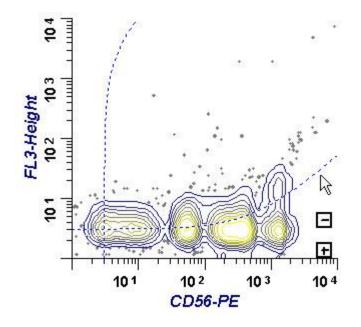
Enable this option to display graphical elements that allow you to set normalization. Targets appear when the Activate option is enabled. See Normalization Targets for more information.

Compensation review

This checkbox enables Compensation Review mode in WinList, a great tool for evaluating how well compensation matches the controls that were used to create it. See <u>Compensation Review</u> <u>Mode</u> for a walk-through.

Show digital errors

When this option is enabled, the program draws dotted lines showing effects of digital error. The lines appear on 2P histograms that display parameters that are enabled for compensation in the 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.

View Cross-over Matrix

This command displays the cross-over (also known as spillover) matrix for the current compensation settings. The matrix can be copied to the clipboard for use in other software.

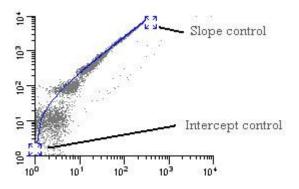
Advice

This command displays the <u>Advice on Compensation</u> dialog allowing you to view information about the compensation settings and sources of digital error in the data.

Compensation: Setup Tab Compensation: AutoComp Tab Compensation: Transforms 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. The <u>Compensation Toolbox</u> controls these settings.



When displayed, a trace line can be moved by clicking on one of its handles. The highintensity 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: <u>Using Interactive N-Color Compensation</u>[™] <u>Compensation Toolbox</u> <u>Compensation Toolbox dialog</u>

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.

Compensation Notice	
The number of parameters in the new file has changed, which may have an impact on the compensation setup.	ОК
If you choose to keep the current setup, WinList will preserve as much of the current compensation settings as possible.	
What would you like to do?	
Keep the current compensation setup.	
Re-initialize the compensation setup.	
Show this dialog when the number of parameters changes for this dat	ta source.

The first time WinList detects a change in number of parameters, it will display a dialog of choices for the user. The user can choose to maintain the existing compensation settings, to the extent that is possible, or to re-initialize the compensation system. The dialog has an option to not be displayed again, which applies to that data source.

Keep the current compensation setup

When this option is selected, the system makes temporary copies of all the compensation data structures. It then resizes the structures to suit the new number of parameters (larger or smaller), and copies what it can from the temporary structures. If the new file has more parameters, the new parameters will be turned off for compensation. If it has fewer parameters, the extra parameters are removed from the compensation setup. If compensation is turned on when this transition occurs, the compensation matrix will be reconstructed by inverting the crossover coefficients.

Re-initialize the compensation setup

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

Show this dialog when the number of parameters changes in this data source.

Enable this option to display this dialog anytime a file with a different number of parameters is opened, assuming that some of the parameters are enabled for compensation. Uncheck this option to suppress the dialog from appearing again.

Edit Properties for Compensation

This dialog is displayed when the **Edit** button in the <u>Compensation Toolbox</u> dialog is clicked. It allows you to edit many of the characteristics of the compensation and normalization system.

Edit Properties for Compensatic	on S <u>i</u>	ystem			
Compensation System	^	Name	Current Value	Description	ОК
🗆 🚍 CFP-A signal		Reset Compensation	Enable	Initialize the comp	Cancel
		Reset Normalization	Enable	Initialize the norma	
- YFP-A detector					
□ □ □ DsRed-A detector □ □ □ □ GFP-A signal		Activate	✓ Enable	Check to activate t	Help
CFP-A detector		Hardware Compatible Comp	✓ Enable	Modifies the comp	
- TFP-A detector		Show Digital Errors	✓ Enable	Shows the digital e	
□ □ DsRed-A detector □ □ □ YFP-A signal		Show Normalization Targets	Enable	Perform trace line	
		Restrict Auto-Positioning Logic	✓ Enable	Perform trace line a	
STP-A detector					
□ □ □ DsRed-A detector □ □ □ DsRed-A signal					
CFP-A detector	~				
		<		>	

The left-hand pane of the dialog displays a tree of compensation objects. The right-hand pane displays properties for the compensation object selected in the tree. When the dialog is initially displayed, general properties of the compensation system are shown.

Compensation System

This object shows general properties of the compensation and normalization system.

Reset Compensation

Check this option to reset the compensation trace lines so that the data is uncompensated. The compensation matrix is initialized for all parameters in the active data file.

Reset Normalization

Check this option to reset any normalization and restore the data to a non-normalized state. This affects normalization for all parameters in the active data file.

Activate

Enables or disables the N-Color Compensation[™] and normalization systems. When unchecked, compensation and normalization are turned off. When checked, compensation and normalization are turned on.

Note: Trace lines disappear when compensation is activated. They are replaced by small + and - buttons.

Hardware Compatible Comp

When this option is enabled, the compensation matrix is constructed so that crossover is strictly subtractive. For example, these equations show hypothetical 3-color compensation with the Hardware option enabled:

Comp P3 = +1.000*P3 -0.010*P4 -0.000*P5 Comp P4 = -0.169*P3 +1.000*P4 -0.038*P5 Comp P5 = +0.008*P3 -0.283*P4 +1.000*P5

Notice that P3 is multiplied by 1.0 in the compensation of P3. This is also true for P4 in the P4 compensation, and P5 in the P5 compensation.

When the Hardware option is disabled, the compensation signal can be greater than 1.0 in the associated compensation expression:

Comp P3 = +1.002*P3 -0.011*P4 -0.000*P5 Comp P4 = -0.171*P3 +1.013*P4 -0.039*P5 Comp P5 = +0.008*P3 -0.286*P4 +1.011*P5

Notice that change in the expressions. In this setting, the loss of a signal due to signal crossover is adjusted for in the compensation matrix. This will result in slightly higher intensities than seen with the Hardware option enabled.

Show Digital Errors

When this option is enabled, the program draws dotted lines showing effects of digital error. The lines appear on 2P histograms that display parameters that are enabled for compensation in the Params to Compensate list. Use the Advice button to see information about the sources of digital error in the data.

Show Normalization Targets

Enable this option to display graphical elements that allow you to set normalization. Targets appear when the Activate option is enabled. See Normalization Targets for more information.

Restrict Auto-Positioning Logic

When enabled, WinList uses a more restricted portion of histograms to determine how to automatically position compensation Trace Lines. For most cases, this option will have little effect on how the program positions the trace lines. In some cases where there is significant signal crossover, enabling this option can improve the auto-positioning.

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.

Detector Objects

Each *Signal* object contains *Detector* objects, one for each of the other parameters that is enabled for compensation. When you select a Detector object, the following properties appear.

Cross-over coefficient

This value represents the fraction of the *Signal* object parameter that is spilling over into this detector in the compensation system. A value of 1.00 (100%) is displayed for the detector associated with the signal parameter. A fractional value between 0.00 and 1.0 is displayed for the other detector objects. For example, a CD56-PE detector for a CD56-PE signal will display a value of 1.00. If 20% of a CD19-FITC signal is spilling into a PE detector, a value of 0.20 will be displayed for the PE detector of the CD19-FITC signal.

Compensation coefficient

This read-only field shows the fraction of the signal to add or subtract from the detector. It is computed in the construction of the compensation matrix and is not editable.

Digital Error Position

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

Use the <u>Advice</u> dialog to understand the sources of digital error.

Advice on Compensation dialog

This dialog presents information about compensation, normalization, and digital error for the active data file. Review the information in this dialog to determine the characteristics of compensation and the sources of digital error in your system.

About Digital Error

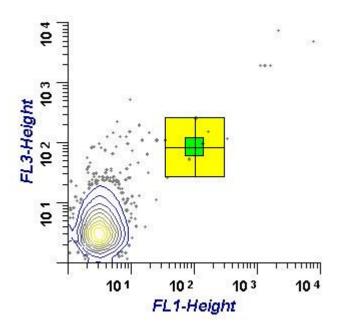
When analog data is converted to an integer, an error is introduced known as digital error. Normally this error is quite modest and presents little problem to cytometrists. However, when data is compensated, positive and negative errors are introduced that increase with parameter intensity. The digital error lines in WinList attempt to show the positive envelop of these error distributions for each parameter that is enabled for compensation. If intermediate intensity populations are present that tend to follow the digital error lines, it is possible that they are simply an artifact due to the effects of digital error.

Normalization Targets

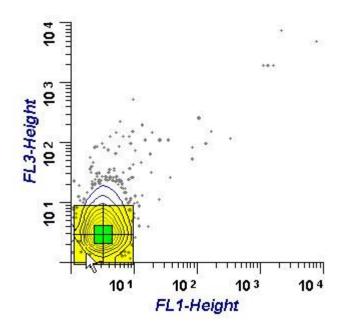
When compensation is **Active** and **Show Normalization Targets** is enabled in the Compensation Toolbox, WinList displays graphical objects to make it easy to set normalization for one or more parameters. The objects appear on 2P histograms.

Normalization allows you to change the location of populations in the data, usually to some normalized location. For example, suppose your lab routinely positions a bead population so that the median is at 10² for a log parameter. You open one file and find that the population is lower than usual because one of the gain settings was not quite right. You can use WinList's normalization to move that bead population to the correct location. Keep in mind, however, that WinList will reposition *all* of the data, not just the one population.

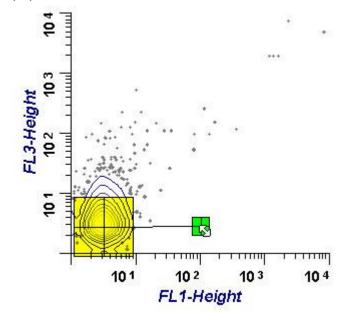
Use normalization with great care, and with a full understanding of what you are doing and why you are doing it. It cannot correct data that has been overcompensated or incorrectly stained. It should not be used to make a weak fluorochrome appear to be a bright fluorochrome.



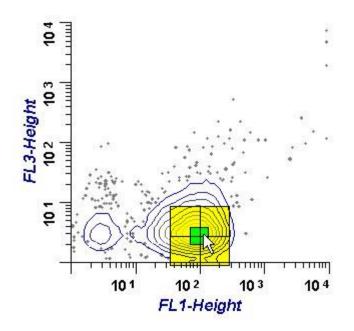
To set normalization, first drag the target object by grabbing the large, yellow outer portion with the mouse. Drag the entire object so that the center cross-hairs are located on the center of the population that you want to move.



Next, drag the green center of the target to the place where you want to move the population.



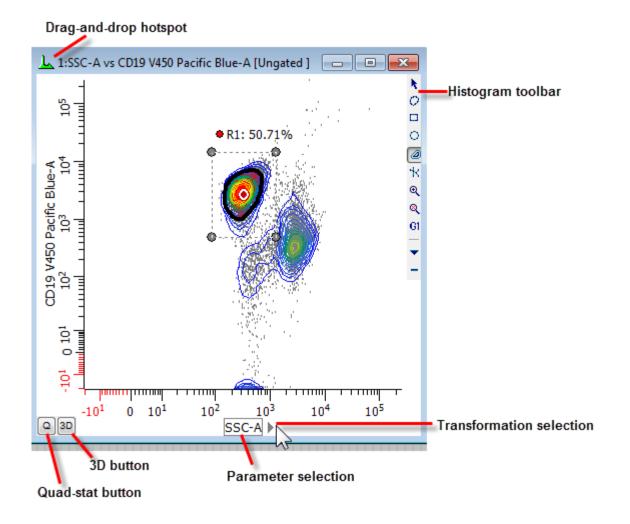
When you release the mouse, the normalization is applied to the data.



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

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 <u>regions</u> on the histograms.



Click the mouse on an axis label to select a different parameter for that axis. When the mouse is over the axis label, click the small triangle next to the label to select a different transformation for the parameter.

A toolbar is positioned on the right edge of the histogram window to provide quick access to many common commands. For details, see <u>Histogram Toolbar</u>.

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 <u>region</u> creation mode. See the section on the <u>Histogram Toolbar</u> 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 <u>Edit Remote</u> 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 \mathbf{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 <u>Regions</u> for more information on the QuadStat and other region types.

I or Information Button

When you have put a one parameter or two parameter histogram in remote mode (DDE connection with another Windows application), under some circumstances you will see an **I** button in the lower left hand corner of the histogram window. If the remote application sent text back to WinList, you will see the **I** button.

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

3D Button

On the 3D version of WinList, two- and three-parameter histogram windows also contain a **3D** button in the lower left-hand corner. When this button is clicked, WinList displays a **3D Viewer** window for the histogram. See <u>WinList 3D Version Notes</u> for more information.

Histogram Toolbar

This toolbar appears inside the right edge of the active histogram window. Each icon represents a tool, described below.

K	Selection mode			
\bigcirc	Freehand region			
	Rectangular region			
0	Elliptical region			
Ø	Contour region			
#	NStat region			
€	Zoom in			
Θ	Zoom out			
G1	Gate			
	Context menu			
_	Hide toolbar			

Region Modes

The first group of tool buttons is used to select a region drawing mode, with only one mode active at a time. Regions are drawn by pressing and holding the left mouse button when the cursor is inside the histogram, and moving the mouse to define the size and shape of the region. For 1P histograms, only the Select and Range modes are supported. 2P Histograms support Select, Freehand, Rectangle, Ellipse, and Contour modes. Currently, no region drawing is supported for 3P histograms.

Select mode

In this mode, regions can be selected and moved, but new regions are *not* created when the left mouse button is clicked on the histogram.

Range (not shown, 1P histograms)

The Range tool appears on 1P plots to draw a range region. Click, hold, and drag to create a range in a 1P histogram.

Freehand mode

Click, hold, and drag to create a freehand region in a 2P histogram. To complete the region, release the mouse button on the starting vertex of the region. Alternatively, click and hold for 1 second to define the first vertex, and then release the button. Define additional vertices by clicking the mouse.

Rectangle mode

Click, hold, and drag to define the boundaries of a rectangular region in a 2P histogram.

Elliptical mode

Click, hold, and drag to define the boundaries of an elliptical region in a 2P histogram.

Contour mode

With a Contour region, a bounding rectangle is defined with the mouse, just as with Rectangle mode. Once that rectangle is defined, WinList computes a contour on the data within that rectangle. Each time the data in a histogram changes, the contour region will recompute based on the data in that rectangle.

NStat region

This button initiates the creation of an NStat region in a 2P histogram. An NStat is similar to a QuadStat, except that it can have any number of rows and columns, and its vertices can be moved independently to change the shape of the array.

Zoom in

Click the button to define a zoom area on the histogram. The histogram enters "zoom" mode. Click inside of the histogram and drag to define the zoom area. When the mouse is released, the histogram displays only the portion within the zoom area.

Reset zoom

Use this button to reset the zoom on a histogram.

Gate

This button displays a list of the gates that can be applied to the histogram. Select an item in the list to apply a gate to the histogram.

Context Menu

This button displays the histogram Context menu containing additional commands relevant to the histogram.

Hide

This button hides the histogram toolbar. The toolbar will reappear when the histogram is reactivated by clicking on its titlebar.

Histogram Context Menu

Сору	
Clone	
Clone with regions	
Edit	
Graphics	
Transformations	
Make bigger	+
Make smaller	-
Add to report	
Add to MG	
Swap axes	
Annotate	
Insert	>
Move to page	
Copy to page	
Show on additional pages	
Copy data	
Save as text	
Save graphic	
Print	

This menu is displayed when the right-mouse button is clicked on a histogram, or by choosing the Context menu button on the histogram toolbar. It displays a list of common commands related to histograms.

Сору

Copy the histogram graphic to the clipboard. The graphic can then be pasted into a report or another application.

Clone

This command creates an identical copy of the histogram, with the same parameters and graphic settings. Note, it *does not duplicate any regions* within the histogram.

Clone with regions

Creates an identical copy of the histogram, like Clone, but also duplicates the regions. The duplicated regions will have new region indexes.

Edit

Display the Edit Histogram dialog.

Graphics

Display the <u>Edit 1P Graphics</u>, <u>Edit 2P Graphics</u>, or <u>Edit 3P Graphics</u> dialog, depending on the histogram type.

Transformations

Display the <u>Edit Transformations</u> dialog to change the transformation(s) for the parameters displayed in the histogram.

Make Bigger

This command incrementally increases the size of the histogram by 50%.

Make Smaller

This command incrementally decreases the size of the histogram by 50%.

Add to Report

Add a linked copy of the histogram to the active report. The linked object on the report will update whenever the histogram's data changes.

Add to MultiGraph

Add a static copy of the histogram to the active MultiGraph.

Subtract

Currently only supported for 1P histograms, this option displays the <u>Histogram Subtraction</u> 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 <u>Data Source Windows</u> for more information about pages.

Copy to Page

Copy the histogram to another page of the data source. See <u>Data Source Windows</u> for more information about pages.

Show on additional pages

Display the histogram on other pages of the data source. This makes it easier to see gating strategies across pages in the data source. See <u>Data Source Windows</u> for more information about pages.

Copy Data

Copy the histogram data as a tab-delimited table of values to the clipboard. This is a convenient way to copy data into a spreadsheet or statistics application for further analysis.

Save as Text

This option saves the histogram as a tab-delimited text file so that it can be imported into other applications, such as speadsheets.

Save as Graphic

This option saves the histogram graphic in one of many common graphic formats.

Print

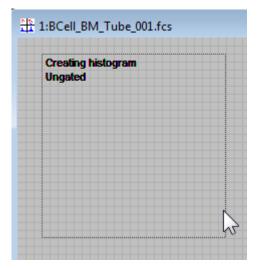
Print the histogram.

Create Histogram

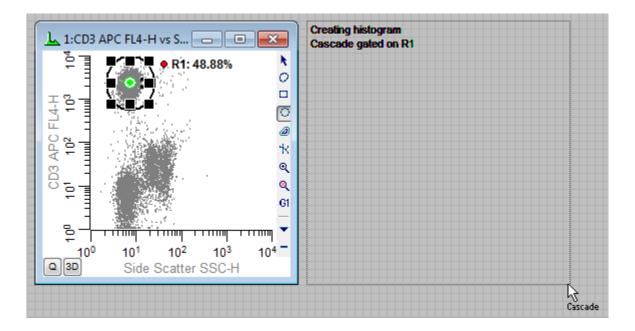
There are several ways to create a new histogram in WinList: from ribbon bar or menu, by drawing the histogram window, or by using the <u>Create Multiple Histograms</u> dialog.

The ribbon bar has several buttons on the <u>Home tab</u> that create 1P, 2P, and 3P histograms on the active data source. These will show the last-used parameters, by default. You'll find additional commands on the <u>Insert tab</u> that allow you to create dot plots, density plots, and contour plots quickly. If you are using the toolbar and menu interface, you'll find the Create Histogram command in the Create menu.

Histograms can also be created by clicking and dragging the mouse in an empty portion of a data source window to define the size and location of a histogram. After defining the area, release the mouse button to display the <u>Edit Histogram</u> dialog and to choose the parameters to display.



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



See also:

<u>Histogram Windows</u> <u>Create Multiple Histograms</u>

Create Multiple Histograms

This command displays the Create Histograms dialog box and provides a fast and efficient way to create a set of histograms.

Create Histograms		
1P Histograms 2P Histograms 3P His	tograms	
X Axis	Y Axis	
FSC-A FSC-H SSC-A CD58 FITC CD38 PE CD34 PerCP CD10 PE cy7 CD20 APC APC-Cy7-A CD19 V450 CD45 AmCyan Time	FSC-A FSC-H SSC-A CD58 FITC CD38 PE CD34 PerCP CD10 PE cy7 CD20 APC APC-Cy7-A CD19 V450 CD45 AmCyan Time	Choose one or more X and Y parameters, then click Add to List. Unique combinations of the selected parameters are added to the list. In Combinations mode, select parameters in the X axis list and all combinations will be created.
	Add to List	Combinations mode
Histograms		Resolution:
SSC-A vs CD19 V450		64 ~
		Delete
		Up
<		> Down
	OK Cancel	

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.

ОК

Click the button to close the dialog and create the selected items.

Cancel

Click the button to close the dialog and cancel the creation of multiple histograms.

Edit Histogram

Edit Hist	ogram	
Name:	SSC-A vs CD19 V450 ID: 1	Remote
Type:	○ 1P	Remote
	Parameter Resolution	
X Axis:	SSC-A ~ 64 ~	Gate
Y Axis:	CD19 V450 ~ 64 ~	Subtract
Z Axis:	FSC-A ~ 64 ~	Graphics
	OK Cancel	Help

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

Name

This field displays the name of the histogram, based on the selected parameters. You can also enter FCS keywords in this field surrounded by square brackets []. When the **OK** button is clicked, WinList will evaluate the name, looking for keywords and replacing them with keyword values. For example, if the listmode file opened has a keyword **\$PROJ** which has a value of **Unstimulated Control**, entering the name **[\$PROJ]** as the histogram name would display **Unstimulated Control** on the title bar of the histogram.

ID

This field displays the ID for the histogram. Each histogram must have a unique ID.

Туре

Select the radio button for the number of parameters you want the histogram to display. Based on this selection, other elements of the dialog are enabled or disabled.

Parameter drop-down lists

Select the parameters for each axis that you want to display. If you have created calculated parameters, they will appear at the end of the list of parameters.

Resolution drop-down lists

Select the resolution for each parameter in the histogram. Options will range from 2 up to the full resolution of the data at acquisition, to a maximum of 65536 channels. In 2 and 3 parameter histograms, you may vary the resolutions for each parameter independently. Higher resolutions generally require more of the computer's memory.

Remote check box

Check this box to enable the **Edit Remote** push button and establish a link with another Windows application using Dynamic Data Exchange (DDE). If a remote link is already established, you can use this check box to toggle between showing local data and remote data.

Remote button

If the **Remote** check box is checked, this button becomes available. Click the button to start or edit a connection with another application. For details, see <u>Edit Remote</u> dialog box.

Gate

This button displays a dialog box which allows the assignment, creation, and editing of gates. See <u>Edit Gates</u> for more information.

Subtract

This button displays a dialog box that allows a histogram to be subtracted from the active histogram. See <u>Subtract Histogram</u> for more information.

Graphics

This button allows editing of the histogram's graphical display. It leads to the <u>Edit 1P</u> <u>Graphics</u>, <u>Edit 2P Graphics</u>, or <u>Edit 3P Graphics</u> dialog box.

ОК

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

Cancel

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

Edit Remote

This dialog box allows the user to establish connections with other Windows applications using Dynamic Data Exchange, or DDE. Once a connection is made, WinList can interact with the other application whenever data changes. This can be useful for automatic databasing and reporting, for exporting WinList graphics, for allowing other applications to process WinList's results, or virtually any interchange with other programs.

Edit Remote Link				
Link To: Excel		~	Update When data On replay	changes
Topic: SHEET1				nd
ACTIVATE POKE(R1C1	E("SHEET1")] (<mark>"SHEET1")]</mark> L:R7C5,TEXT R1C1:R7C5")])		
Add	Insert	Replace	Open	ОК
Delete	Clear		Save	Cancel

The requirements for remote links are listed below:

- 1. The other application must support DDE.
- 2. You must know the DDE topics that the application supports.
- 3. The application must be in the computer's search path or it must already be running when WinList attempts to make a connection.
- 4. The application must provide DDE "Server" support. That is, it must be able to respond to requests and instructions from other programs, in this case WinList.

Examples of other programs with which WinList can communicate are ModFit LT, Microsoft Excel, and Microsoft Word. This is not a complete list, as many other applications also support DDE.

The dialog box has three critical areas: **Link To**, **Topic**, and **Commands**. These fields name the application to connect with, the topic used for the connection, and the internal and external commands to execute.

The **Update** group box determines when the link will be updated. All buttons relate to editing commands to send.

Link To

This drop-down list box contains a list of several programs that you may commonly want to connect to. If the program you want to connect with is listed, select it from the list. If not, select **Other** from the list and type in the name of the program.

Topic

A default topic is filled in for you if the **Link To** field contains a program known to WinList. If no topic is shown or you want to change the topic, you can edit this field. The topic you supply *must* be a topic the other application supports.

Commands

This section is comprised of an edit box and a list box. The commands which appear and those you enter must either be internal WinList commands, or external commands which the other application will execute.

Internal commands are listed in the <u>DDE Commands</u> section. They instruct WinList to perform some function. For example, *PASTE(metafile)* instructs WinList to paste a metafile (picture) from the clipboard and display it instead of the histogram graphics or results information.

External commands must be enclosed in square brackets []. Inside the square brackets enter the command that the other application will execute. For example, if a connection is established with Microsoft Excel, the command *[ACTIVATE("SHEET1")]* instructs Excel to make a spreadsheet called *Sheet1* the active spreadsheet. Notice that the command is surrounded by square brackets to let WinList know that the command is an *External* command.

As you enter commands, click the **Add** button to append the command to the list, or use the **Insert** or **Replace** buttons. *Commands are executed in the order in which they are listed*.

Add

Click this button to add the command in the Commands to Send edit box to the end of the list of commands.

Insert

Click this button to insert the command in the Commands to Send edit box in front of the currently selected command.

Delete

Click this button to remove the selected command from the list box.

Replace

Click this button to replace the selected command in the list box with the command in the Commands to Send edit box.

Open

This option allows you to retrieve a DDE command file previously saved to disk. The command file opened from disk will replace the one in memory.

Save

This option allows you to save a DDE command file to disk. You can reuse the command file by using the **Open** option. Several results or histogram windows may use a command file stored on disk at the same time.

ОК

Click this button to accept changes to the dialog box and make connection with the remote application. WinList will inform you if it cannot make connection.

Cancel

Click this button to discard changes made to the dialog box.

Update

Select an option to tell WinList how often to update the link with the other application. When it updates the link, WinList will run through all of the commands in the Commands to Send list, executing the *internal commands* and sending the *external commands* to the other (remote) application. Selecting **When data changes** will cause WinList to update the link whenever WinList changes the data related to the histogram or results window. Selecting **On Replay** tells WinList to wait until one of the options in the **Replay** menu has been selected. If you choose to update **On Command**, the link will be updated when you select **Update**

Remote Links from the macro menu, or when the equivalent command is encountered in running a WinList macro.

Edit 1P Graphics

Edit Properties for 1P Gra	aphics		
Name Styles	Current Value	Description	
Fill histogram Line style	Enable	Fill histogram with color Choose a line style. His	Cancel Apply
Coloring mode options Overlay style Scaling	Overlay color subset $ imes $	Mode for Color by Gate	Help
Scaling channel Y scale Auto scale	5 414 ✓ Enable	X Channel for scaling Number of events for Y Automatic Y scaling	
Zoom Zoom low X Zoom high X	1.00 262144.00	Sets the lowest linear X Sets the highest linear X	
General Major tics	5	Desired number of majo	
Minor tics Grids	5 Enable	Desired number of mino Display grids.	
Hide button Apply to all <	Enable	Hide buttons on histogra Apply these settings to :	

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.

ОК

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

Cancel

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

Apply

Click this button to apply the changes you have made and keep the dialog box open.

Edit 2P Graphics

This dialog allows you to change the graphical display of a 2P histogram. It is divided into 4 sections in the tree on the left of the dialog: 2P Graphics, Dots, Density, and Contours.

Edit Properties for 2P Graphics				
	Name Zoom	Current Value	Description	ОК
Color Density	Zoom low X	1.00	Sets the lowest X linear value fo	Cancel
- Ise Contours	Zoom high X	262144.00	Sets the highest X linear value f	Apply
	Zoom low Y	-262.14	Sets the lowest Y linear value fo	Liele
	Zoom high Y	262144.00	Sets the highest Y linear value f	Help
	Tics and Grids			
	Major tics	5	Desired number of major tics or	
	Minor tics	5	Desired number of minor tics. N	
	Grids	Enable	Display grids.	
	Hide buttons	Enable	Hide buttons on histogram.	
	Apply to all	Enable	Apply settings to all 2P histogra	
	<		>	

2P Graphics

Zoom

Zoom low X

This value defines the lowest X parameter value that is shown in the histogram. Values that are below this are ignored.

Zoom high X

This value defines the highest X value shown in the histogram. Values above this are ignored.

Zoom low Y

This value defines the lowest Y parameter value that is shown in the histogram. Values that are below this are ignored.

Zoom high Y

This value defines the highest Y value shown in the histogram. Values above this are ignored.

Tics and Grids

Major Tics

Enter an approximate number of major tics you want to appear on the axes. Major tics are always divisible by 10, so the actual number of major tics may not be equal to the value entered here.

Minor Tics

Enter the approximate number of minor tics you want to display. The actual number displayed will be 2, 5, 10 or a multiple of 10.

Grids

This check box allows you to turn on or off the display of grids that appear on the major tic intervals. If the box is checked, grids will be displayed.

Hide button

Check this option to hide the "I" or "Q" button. QuadStat buttons are typically displayed on 2P histograms. Info buttons appear in histograms that have remote links or histogram subtraction results.

Apply to all

When this option is enabled, the settings for the selected histogram will be applied to all other 2P histograms when the **OK** button is clicked.

Dots

Enable this option to display dot density display.

This option will uncheck automatically if Color Density is enabled on the next pane. Some Dot properties are shared with Color Density: Dot size, Dot order, Number, Show all dots, and Dot Filter. Changing them in one place will also change them in the other.

Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

Number

Enter the number of dots you want to display in the histogram window.

Note: The actual number of dots displayed may be less than the number you enter. If there are fewer events in the data than you specify, or if the *packet size* 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 <u>Edit Program Controls</u> 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.

Dot order

When using color event gating, this option controls the order in which dots are drawn. *Unsorted* draws dots in their natural event order based on the listmode acquisition. *Low frequency on top* draws the dots for the smallest populations last so that they can be seen on top of populations that have many events. It is useful for visualizing rare events. *High frequency on top* draws the dots for the largest populations last. It may be used to visually clean up low frequency events.

Color Density

Enable this option to display color-density dots.

This option will uncheck automatically if Dots are enabled on the previous pane. Some Dot properties are shared with Color Density: Dot size, Dot order, Number, Show all dots, and Dot Filter. Changing them in one place will also change them in the other.

Some Contour properties are shared with Color Density: Probability interval and Colors. Changing them in one place will also change them in the other.

Colors

Color mode

Choose either Color gradient or Rainbow mode. Color Gradient mode transitions from a starting color to an ending color as the number of events increases. Rainbow mode uses a set of colors which blend to define the color density levels.

Gradient start color

Choose a color to use as the starting color for color density dots and contours in Color Gradient mode. Click the color button to select a color

Gradient end color

Choose a color to use as the ending color for color density dots and contours in Color Gradient mode. Click the color button to select a color

Rainbow colors

Select the number of rainbow colors for Rainbow mode. By default this is 5.

Edit rainbow colors

Use this option to select colors used for Rainbow mode.

Contours

Enable this option to display probability contours.

Some Contour properties are shared with Color Density: Probability interval and Colors. Changing them in one place will also change them in the other.

Show outlier dots

To display dots that fall outside the first contour, check this option. The "Dots" section of this dialog controls the properties of the dots, such as size and number.

NOTE: If *Enable contours* and *Show outlier dots* are checked, the only dots drawn will be those that fall outside of the first contour level. To also draw dots that are inside the contours, uncheck the *Show outlier dots* option and check the *Enable dots* option above.

Probability interval

This value determines the probability used to compute contours. The default value draws 5% probability contours. With this setting, the largest contour level will contain 95% of the events in the histogram, the next level will contain 90%, and so on.

Base Contour

This dropdown list selects the level of the lowest, or base, contour. The level is selected as a percentage of events that it will include. The highest percentage, 99%, will include 99% of the events in the histogram. The lowest value for Base Contour is 90%.

Line weight

Choose a line weight for the contour border. The value is in points, a unit of 0.35 mm or 1/72 in.

Fill contours

When this option is checked, contours will be filled with colors defined in the Colors section. When unchecked, the contour is unfilled.

Smooth

Enable this option to smooth the histogram data used to create the contours. This creates contours that have fewer vertices and a rounder appearance.

Smoothing span

If the Smooth data option is enabled, this value determines how many channels are used in the smoothing algorithm. Using more channels creates rounder contours.

Colors

See the description of Color Density Colors for details.

ОК

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

Cancel

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

Apply

Click this button to apply the changes you have made and keep the dialog box open.

Edit 3P Graphics

Edit Properties for 3P Graphics			
Name Dots and plot	Current Value	Description	ОК
Draw dots	✓ Enable	Draw the dots in the 3P cloud.	Cancel Apply
Dot size Number	2 ~ 10000	0=Smallest Number of dots to display.	
Show all dots	Enable	Display all dots.	Help
Lines Background plots	Enable	Draw lines to base plane. Draw background dot plots.	
General	✓ Enable		
Major tics	5	Desired number of major tics on linear axes	
Minor tics Grids	5	Desired number of minor tics. Must be even divise Display grids.	
Zoom			
Zoom low X	-262.1	Sets the lowest X linear value for zooming. (-262.	
Zoom high X Zoom low Y	26214 ²	Sets the highest X linear value for zooming. (-262 Sets the lowest Y linear value for zooming. (-262.	
Zoom high Y	262144	Sets the highest Y linear value for zooming. (-262	
Zoom low Z	-20.00	Sets the lowest Z linear value for zooming. (-20.0	
Zoom high Z Apply to all	26214 ²	Sets the highest Z linear value for zooming. (-20. Apply these settings to similar histogram.	
<		>	

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

Dots and plot

Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

Number

Enter the number of dots you want to display in the histogram window.

Show all dots

By checking this option, all events will be displayed in the 3P histogram *after replaying*. See the note above for more on showing all dots.

Lines

When this option is enabled, line gray lines are drawn from each dot to the base plane. When displaying 1000 dots or fewer, this is useful to identify the position of dots.

Background plots

When this option is enabled, a bivariate dot plot is drawn for the 3 background planes.

General

Major Tics

Enter an approximate number of major tics you want to appear on the axes. Major tics are always divisible by 10, so the actual number of major tics may not be equal to the value entered here.

Minor Tics

Enter the approximate number of minor tics you want to display. The actual number displayed will be 2, 5, 10 or a multiple of 10.

Grids

This check box allows you to turn on or off the display of grids that appear on the major tic intervals. If the box is checked, grids will be displayed.

Zoom

Zoom low X

This value defines the lowest X parameter value that is shown in the histogram. Values that are below this are ignored.

Zoom high X

This value defines the highest X value shown in the histogram. Values above this are ignored.

Zoom low Y

This value defines the lowest Y parameter value that is shown in the histogram. Values that are below this are ignored.

Zoom high Y

This value defines the highest Y value shown in the histogram. Values above this are ignored.

Zoom low Z

This value defines the lowest Z parameter value that is shown in the histogram. Values that are below this are ignored.

Zoom high Z

This value defines the highest Z value shown in the histogram. Values above this are ignored.

Apply to all

When this option is enabled, the settings for the selected histogram will be applied to all other 3P histograms when the **OK** button is clicked.

ОК

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

Cancel

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

Apply

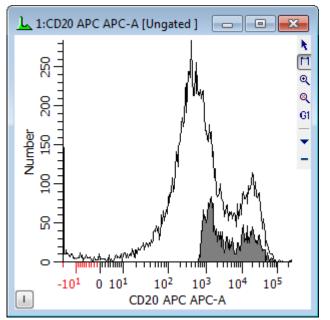
Click this button to apply the changes you have made and keep the dialog box open.

Subtract Histogram

This dialog box allows the user to perform histogram subtraction, where the control (negative) histogram is selected and subtracted from the current active (test) histogram. The dialog box can be accessed through the Edit Histogram dialog box, or from the histogram popup menu that appears when the right mouse button is clicked on a histogram. Only 1-parameter histograms are supported.

Often the two histograms are in separate listmode files; the control histogram is in one and the test histogram in another. In order to subtract one from the other, both files must be opened at the same time. One way to accomplish this is to set up a protocol with two data sources. Use the <u>Duplicate Data Source</u> 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.

Histogram Subtraction			
 ✓ Enable Histogram S Histogram ID: DataSource ID: Select the control hist 	1	btract from this histogra	OK Cancel
1:BCell_BM_Tube_00	1.fcs 1:CE	D58 FITC	

To subtract a histogram from the current histogram, select the negative control histogram that you want to subtract from the list box. All 1-parameter histograms in all open data sources will appear in the list box. Make sure that the **Enable Histogram Subtraction** option is checked and then click **OK**.

To disable histogram subtraction, clear the check from the **Histogram Subtraction On** check box.

There are times when you may receive a warning indicating that the histograms may be reversed." This message alerts you to a condition in the subtraction routine where the control histogram may have more "positives" than the positive histogram. Check to make sure you have not accidentally subtracted the positive histogram from the negative histogram or made a similar selection mistake. If you are certain that you are subtracting the correct histogram, the warning can be safely ignored.

Enable Histogram Subtraction

This option determines the state of histogram subtraction. When checked, subtraction is enabled. When unchecked, subtraction is disabled. *It is checked automatically each time you display the subtraction dialog.*

Histogram ID

This field displays the WinList ID of the histogram to subtract that is currently selected in the list box.

Data Source ID

This field displays the WinList ID of the data source owning the histogram that is currently selected in the list box.

Histogram list box

Displays a list of all 1-parameter histograms.

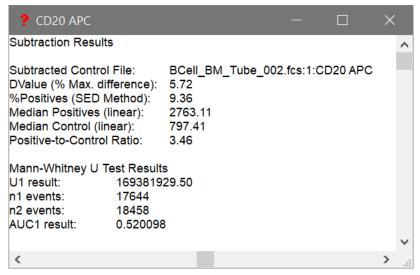
ОК

Accepts changes and closes the dialog box.

Cancel

Exits with no changes and closes the dialog box.

Statistical results of the subtraction can be viewed by clicking the **I** button in the lower left corner of the histogram window. When clicked, an information window will appear displaying several statistics as described below:



These results are also available for databasing.

Subtracted Control File

This field displays the name of the data source, the histogram ID, and the name of the subtracted control histogram.

DValue (% Max. difference)

In determining the difference between the test and control histograms, both are converted into cumulative histograms and normalized to a scale from 0 to 100. The point of maximum difference is found and the percent difference is calculated. Since the scale of the cumulative histograms is 0 to 100, the maximum difference is actually a percentage.

% Positives (SED Method)

This statistic uses the **Super-enhanced DMax (SED)** method to determine a more accurate percentage of the test histogram that is positive, or what portion remains after histogram subtraction. The method was presented at ISAC in 1996 as Enhanced Normalized Subtraction (ENS), and was further improved to become SED. Contact Customer Support for other references and a white paper on this method.

Median Positives (linear)

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

Median Control (linear)

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

Positive-Control Intensity Ratio

This field displays the ratio of the linearized positive median to the linearized control median. It provides an indication of the relative intensities of the positive and control distributions.

Mann-Whitney U Test Results

WinList computes the Mann-Whitney U Test for additional "Area-under-the-curve" (AUC) statistics for histogram subtraction. Detailed info on this test can be found <u>in this wiki article</u>. This provides another indication of the difference between two distributions - the test and control histograms.

The key statistic is the "Area-under-the-curve", or **AUC**. AUC is non-parametric and ranges from 0 to 1, such that if the histograms are the same AUC = 0.5. If test histogram shifts to the right, AUC will increase towards a value of 1.0, and if the test histogram shifts to the left, AUC decreases towards a value 0.0. The AUC as a metric gives both the proportion of

difference between histograms and direction of shift. AUC seems to be very robust and not affected by noise.

Information Windows

When a histogram window is <u>remotely linked</u> 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 <u>Histogram Subtraction</u> for details.

Index Analysis

The Index Analysis window is a special display for parameters that contain index-like data. Some plate-sorting cytometers create index parameters that indicate which well on the sort plate each event went into. The Index Analysis window is a great tool for viewing and investigating that information.



An Index Analysis window can be created for any index-like parameter in the FCS file. There are controls for setting the number of rows and columns to display, as well as how to label the rows and columns. In some cases, the FCS file may contain keywords to help the Index Analysis window figure out how to display the rows and columns. See <u>Edit Index Analysis</u> for details.

You can create "groups" of related wells on an Index Analysis window, much like regions on a histogram except that groups can contain locations that are not next to one another. In fact, each group you create has an associated region so that you can display statistics for it in a Results window as you would with other regions. See <u>Index Analysis Groups</u> for information on creating and editing groups.

Index Analysis plots can display the color-event information from regions in other histograms. You can also choose to color events based on the groups in the Index Analysis window.

Edit Index Analysis

This dialog is displayed when creating or editing an <u>Index Analysis</u> window.

Edit Index Analysis			
Parameter:	FTIM(96)	~	
Rows:	8	Columns: 12	
Row Labels:	• Letters		
Column Labels:		Numbers	
Grid Origin:	Upper Right	×.	
	ОК	Cancel	

Parameter

Choose the parameter in the FCS file that you want to display in the Index Analysis window. It is best if this parameter contains index-like information - whole numbers in the range defined by the number of rows and columns.

Rows and Columns

When you create a new Index Analysis window, you can edit the number of rows and columns. If the file you are analyzing has indexing keywords, this information will be filled in automatically for you. If it does not have keywords, create enough rows and columns to accommodate the range of values for the parameter. For example, if your parameter has 128 possible values, make sure that rows * columns = 128. In the illustration, the parameter has 96 possible values, so 8 rows by 12 columns will display all the possible values.

Row and Column Labels

You can label the rows and columns with letters or numbers to easily identify a particular well in the grid.

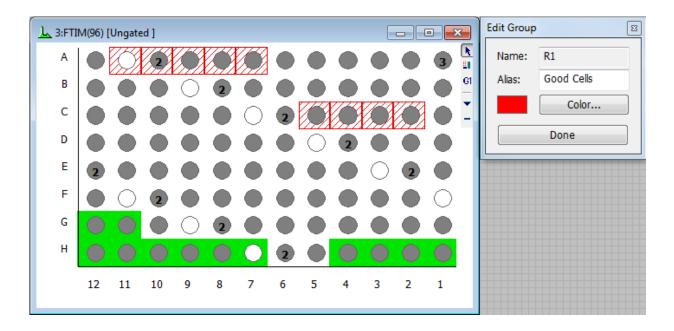
Grid Origin

The grid origin determines where the first index value is located on the window.

Index Analysis Groups

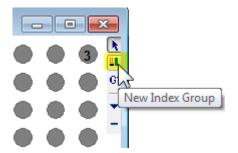
"Analysis Groups" in an Index Analysis window are the equivalent of "regions" in a histogram window. An analysis group identifies a set of wells in the Index Analysis window, and you can report statistics and perform event coloring with analysis groups just as you would with a region in a histogram.

In the image below, there are 2 groups defined. The red-colored boxes represent group R1, which is named "Good Cells". This group contains 5 wells on row A and 4 more wells on row C. The green-colored boxes represent group R2, named "Bad Cells".



Creating a Group

With an Index Analysis window created, use the Create Group tool in its toolbar to start a new group of wells.



This will display the Edit Group dialog, which identifies the results region associated with the group. You can type an alias and pick a color for the group you are creating in this dialog.

With the Edit Group dialog open, click and drag the mouse over the wells that you want to include in the group. If you want to add cells that are not next to one another, press and hold the **Ctrl** key on the keyboard before you click the mouse.

When you have finished with selection of wells, click the **Done** button to close the Edit Group dialog.

Editing a Group

Once a group has been created, you can edit the group by clicking on any one of its wells. This will display the Edit Group dialog. Just as with creating a group, you can select wells, change the alias and edit the color of the group. If you want to add cells that are not next to one another, press and hold the **Ctrl** key on the keyboard before you click the mouse.

When you have finished selecting wells, click the **Done** button to close the Edit Group dialog.

Deleting a Group

To delete a group, right-click the mouse on any well in the group and select **Delete** from the context menu.

Creating Results for a Group

To create results for a group, right-click the mouse on any well in the group and select **Create Results** in the context menu. The statistics that can be displayed are limited to frequency-based statistics; intensity and position statistics are not computed for Index Analysis groups.

Using a Group to Gate other Histograms

Sometimes it is useful to see where the wells in a group appear in other histograms. One method for doing this is to use the group to gate another histogram. Right-click on a group and select **Cascade Gate** from the context menu, and then click the title bar of the histogram you want to gate on the group. The histogram will show only those events that are in the wells selected in the group.

3D Histogram Viewing

WinList 3D can create high quality OpenGL 3D images from any 2P or 3P histogram. The 3D images are displayed in separate windows, and can be easily rotated and scaled. This feature generates wonderful publication graphics and greatly enhances the ability to visualize your data.

Each 2P and 3P histogram displays a small "3D" button in the lower left corner of the histogram window. To display the 3D viewer for a histogram, simply click the 3D button and a 3D Viewer window will appear.

You can set a preference for the type of 3D display that is created when you click the 3D button. See <u>2P Graphics Preferences</u> and <u>3P Graphics Preferences</u> for details.

2P histograms display an Iso3D image of the histogram data. For details on the display options for Iso3D displays, see the <u>2-Parameter Display Options</u>.

3P histograms display 3D clouds, dots, or spheres. For details on the 3-parameter display options, see <u>3-Parameter Display Options</u>.

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

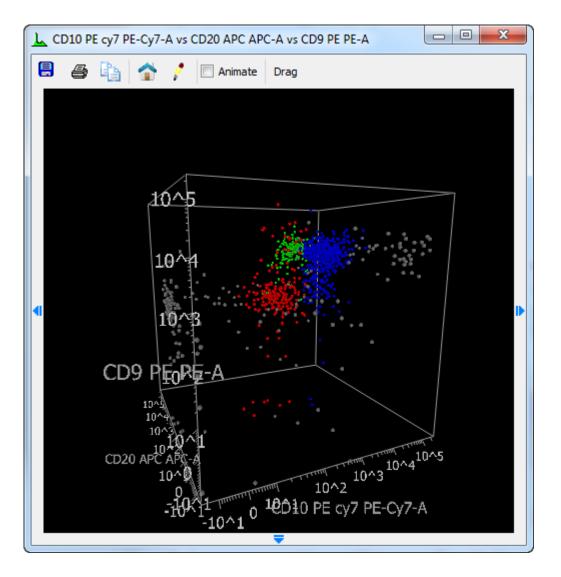
Using the 3D Viewer

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.

_ 0 23 L CD10 PE cy7 PE-Cy7-A vs CD20 APC APC-A 4 Animate Drag 160 140 120 1004 80 60 10^2 10^3 10^4 10^5 CD20 10010 PE cy7 PE-Cy7-A 0

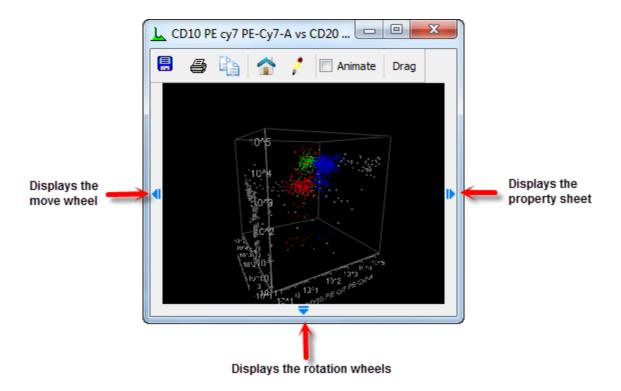
For details on the Iso3D display options for 2-parameter data, see <u>2-Parameter Display</u> <u>Options</u>.

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



Displaying Additional Controls and Options

The viewer has small arrows on its left, right, and bottom edges. Click or hover the mouse on one of the arrows to display the controls that it contains.



Editing Properties

Click or hover the mouse on the small arrow on the right edge of the viewer window to display the properties sheet. The property sheet contains a tree of objects and a property list for the selected object in the tree. You can also click the pencil icon on the viewer toolbar to display the properties sheet. See <u>2-Parameter Display Options</u> or <u>3-Parameter Display</u> <u>Options</u> 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: <u>3D Viewer: Additional Controls</u>

3D Viewer: Additional Controls

Mouse Button Controls

The mouse buttons can be used in the 3D Viewer as described in the table belowLeftSpin (rotate the virtual trackball)		
<shift> + Left</shift>	Pan (translate up/down/left/right)	
<ctrl> + <shift> + Left</shift></ctrl>	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.

4 🏕 Iso 3D Scene		
 Contour Base 		
A Contour Conta	iner	
All Contours		
🏕 G1		
🏕 G2		=
🛷 G3		
🛷 Ungate	b	
🔺 🕂 Axes		
		_
+ Y Axis		-
Name	Valu	e _
Name Contour Properties	Valu	e A
	Valu	e A
Contour Properties Minimum Count		
Contour Properties Minimum Count Auto Scale Frequency	0 ☑ On	
Contour Properties Minimum Count Auto Scale Frequency Maximum Frequency	0	
Contour Properties Minimum Count Auto Scale Frequency Maximum Frequency Background Color	0 On 173.00	
Contour Properties Minimum Count Auto Scale Frequency Maximum Frequency	0 ☑ On	

A pushpin at the top of the sheet allows you to pin the sheet open. The small "x" allows you to close the sheet.

The tree control at the top determines which set of properties are shown in the bottom section. Select an item in the tree to view and edit its properties.

Iso 3D Scene

Contour Properties

Minimum Count

Use this control to filter out low-level populations. Those that have fewer events than this value will not be drawn on the plot.

Auto Scale Frequency

When checked, the frequency axis is automatically scaled based on the data.

Maximum Frequency

When Auto Scale Frequency is not checked, the this field sets the height of the frequency axis.

Background Color

Select a background color for the viewer.

Show Rotation Point

Enable this option to see the point at the center of rotation.

Use Perspective

Enabled by default, this property determines whether perspective is applied to the 3D rendering.

Copy/Save/Print Options

Custom Size Width & Height

These values control the width and height for graphics when copied, saved, or printed.

Image Resolution

This field controls the resolutions for graphics when copied, saved, or printed.

Use White Background

Enable this option to use a white background when copying, saving, or printing.

Contour Base

Show Base

Enable this option to show the base plane of the contour plot.

Base Color

Choose a color for the base plane.

Percent Transparency

Choose a transparency for the base.

All Contours

These settings apply to ALL of the subset contours. Individual contours can be customized with different settings.

Contour Line Type

Select an option for displaying contour lines.

Minor Tic Contours

When contour lines are enabled, this option will draw contour lines for the minor tic levels.

Contour Line Color

Choose a color for the contour lines.

Percent Transparency

Choose a transparency for all contours.

Contours

Use these objects to customize individual clusters. To change the settings for *all* contours at once, select the All Contours object in the tree.

Contour Line Type

Select an option for displaying contour lines.

Minor Tic Contours

When contour lines are enabled, this option will draw contour lines for the minor tic levels.

Contour Line Color

Choose a color for the contour lines.

Percent Transparency

Choose a transparency for all contours.

Contour Color

The property displays the color used by the cluster. It is control by the gate dialogs in the main application.

Axes

Show Box

Enable this option to show the 3D box.

Box Line Weight

Choose a line weight for the 3D box.

Box and Axis Color

Choose a color for the 3D box and axis.

Y, Y, Z Axis

Show Choose an option for the axis display.

Axis Color Select a color for the axis.

Label Font Select a font for the axis label.

Number Font Select a font for the axis numbers.

Axis Line Weight

Select a line weight for the axis.

Axis Label Properties

Axis Label

Edit the text for the axis label.

Update Axis Label

Select an option for how the axis label is updated.

Axis Label Style

Choose a label style. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

Horizontal Alignment

Choose an alignment for the labels.

Axis Number Style

Choose a style for the axis numbers. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

Show Exponent Only

When enabled, only the exponent is shown on the axis for log-like transforms.

Axis position

Select a position for the axis.

3D Viewer: 3-Parameter Display Options

This property sheet allows you to change how the 3D-viewer window displays 3-parameter data.

			Ţ.
▲ 🗽 3D Clusters S	cene		
🔺 🌻 Cluster C	ontainer	<i>.</i>	
All Cluster	rs		
2.6	1		
Z G	2		
2.6	3		
🗷 U	ngated		
🔺 🕂 Axes			
👫 🛛 🕹 🕂 🕂 X Axi	;		
+⊁ YAxi	;		
+ Z Axi	;		
Name		Value	-
Background Cold	r		
Show Rotation Po	oint 📃 Off	F	Ξ
Use Perspective	On		
Copy/Save/Print O	ptions		
Custom Size Wid	h 4.00		
Custom Size Heig	ht 4.00		
		Resolution	
Image Resolution	Screen	Resolution	Ŧ

Show the scene using perspective or not.

A pushpin at the top of the sheet allows you to pin the sheet open. The small "x" allows you to close the sheet.

The tree control at the top determines which set of properties are shown in the bottom section. Select an item in the tree to view and edit its properties.

3D Clusters Scene

Background Color

Select a background color for the viewer.

Show Rotation Point

Enable this option to see the point at the center of rotation.

Use Perspective

Enabled by default, this property determines whether perspective is applied to the 3D rendering.

Copy/Save/Print Options

Custom Size Width & Height

These values control the width and height for graphics when copied, saved, or printed.

Image Resolution

This field controls the resolutions for graphics when copied, saved, or printed.

Use White Background

Enable this option to use a white background when copying, saving, or printing.

Dots/Spheres/Clouds

Display Mode

Select the type of 3D display to use: Dots, Spheres, or Clouds.

Dots/Spheres Properties

Enter the number of events to display for dots and spheres.

Cloud Properties

Probability

Select a probability threshold for the cloud display. This value is much like a contour in 2P space, in this case defining a 3P surface. Larger values include more events inside the cloud and result in a larger surface.

Necessary Events

This determines the minimum number of events that a cluster must contain in order for a cloud to be generated for it.

All Clusters

These settings are applied to ALL clusters. Individual clusters can be customized with different settings.

Dot/Sphere Size

Select a size for dots or spheres.

Percent Transparency

Choose a transparency for all clouds.

Clusters

Dot/Sphere Size

Select a size for dots or spheres.

Percent Transparency

Choose a transparency.

Cluster Color

Choose a color for the cluster.

Axes

Show Box

Enable this option to show the 3D box.

Box Line Weight

Choose a line weight for the 3D box.

Box and Axis Color

Choose a color for the 3D box and axis.

Y, Y, Z Axis

Show Choose an option for the axis display.

Axis Color Select a color for the axis.

Label Font Select a font for the axis label.

Number Font Select a font for the axis numbers.

Axis Line Weight Select a line weight for the axis.

Axis Label Properties

Axis Label

Edit the text for the axis label.

Update Axis Label

Select an option for how the axis label is updated.

Axis Label Style

Choose a label style. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

Horizontal Alignment

Choose an alignment for the labels.

Axis Number Style

Choose a style for the axis numbers. **3D Fixed** maintains the same label orientation as the plot is rotated. **3D Facing Viewer** rotates the labels to face the viewer and remain readable as the plot is rotated.

Show Exponent Only

When enabled, only the exponent is shown on the axis for log-like transforms.

Axis position

Select a position for the axis.

3D Output Options Dialog

This dialog controls the size and resolution of the image generated when printing or saving to disk.

Name	Value	OK
Copy/Save/Print Options		Cancel
Custom Size Width	4.00	
Custom Size Height	4.00	Reset
Image Resolution	Screen Resolution -	
Use White Background	🗹 On	Help

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.

ОК

Click this button to accept the settings in the dialog.

Cancel

Click this button to cancel any changes.

Edit Spot

This dialog box can be brought to the screen by choosing **Spots** from the **View** tab of the ribbon bar or **Edit Spots** in the **Edit** menu.

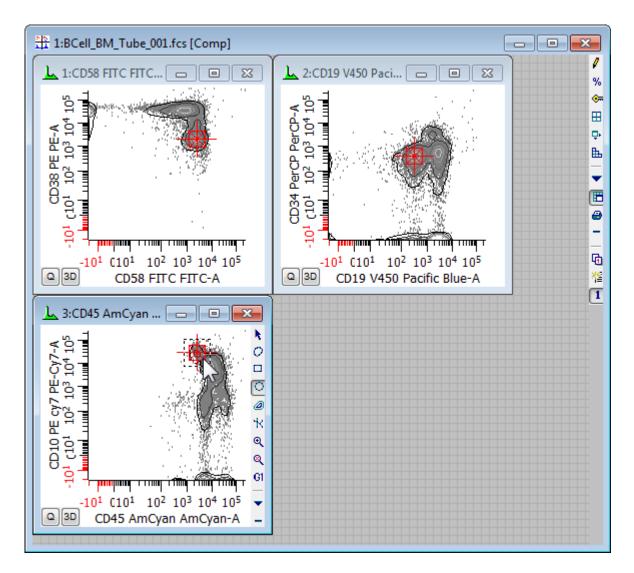
🔁 Edit Spot	System	×
Name + Spot1	Description control position	ОК
1 oper		Open
		Save
		Help
		Properties
Add	Delete	

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 <u>Edit Properties for Spot</u> 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.

ОК

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 <u>Edit Spots</u> dialog.

Name	Current Value	Description	ОК
Description	control position	Enter the description for this spot.	
Color	Color	Edit the spot's color.	Cancel
Size	12.500	Edit the spot's relative size. (0.000 to 100.000)	
Relative Position	ons		Help
Time	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	neip
FSC-A	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	
SSC-A	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	
CFP-A	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	
GFP-A	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	
YFP-A	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	
DsRed-A	45.360	Modify parameter's relative spot location. (0.000 to 100.000)	

Description

Type a description, if desired. The description is not displayed except in this dialog.

Color

Click this button to choose a color for the spot. The standard color selection dialog is displayed.

Size

Spots can be of different sizes. The size field is a percentage of the histogram area. So, a value of 10 causes the spot to be 10% of the width and height of the histogram.

Relative positions

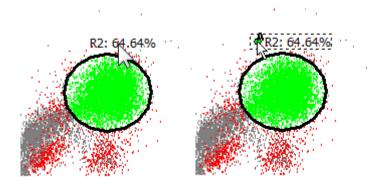
The spot keeps track of its position in each parameter of the listmode file. The positions are in percentage units, so a value of 50.0 positions the spot at the halfway point in the

parameter. These values can be edited directly, or you can use the mouse to move the spot graphically.

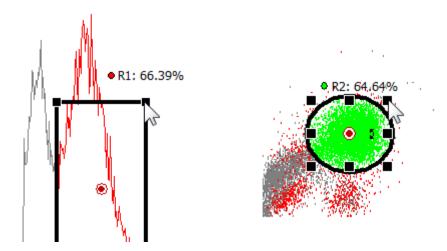
Regions

WinList can create a number of different types of regions. Each is created in a different way as described below.

The way regions are moved, resized, and deleted is common to all region types. Move a region by clicking on the region's label, and then dragging the label to a new location. To move the label *without* moving the region, click on the left portion of the region label, called the hotspot, and drag the label.



Resize a 1P or 2P region by clicking on the region's label to activate the region. Sizing handles are displayed. Click on one of the handles and drag it with the mouse to adjust the size of the region.



Delete a region by clicking on the region label, and then pressing the Delete key on the keyboard. You can also delete regions (except for NStat regions) by dragging the region label off the left edge of the histogram and releasing the mouse.

1P Region Creation

To define a new region in 1P histograms, click on the histogram title bar to activate it. This will also display the histogram toolbar. Make sure that the Range tool is selected in the histogram toolbar. Click and draw on the histogram to define the left and right boundaries of the range, and release the mouse button when finished.

1P regions can automatically position themselves relative to the median of the histogram that contains them. The attribute can be enabled in the <u>Edit Properties for 1P Region</u> dialog box.

2P Region Creation

In 2P histograms, you can define many types of regions, including free-hand, contour, rectangular, elliptical, and NStat. To create a new region, first click on the histogram's title bar to activate it and show the histogram toolbar. Choose the type of region you want from the toolbar that appears on the right side of the histogram.

To draw a *free-hand region*, select the **Free-hand** tool, then click, hold, and drag the mouse to create additional points. Finish the region by releasing the mouse on the first point that was created. Free hand regions can also be created using the *point-and-click* technique to define each point in the region. Start this method in the same way described above, and then click the mouse button for each point you want to define. Finish the region by clicking on the first point that was created.

To create a *rectangular region*, select the **Rectangle** tool. Click and hold the mouse button to create the first corner of the rectangle. Move the mouse and release the button to define the opposite corner of the rectangle.

To create an *elliptical region*, select the **Ellipse** tool. The ellipse is created in the same way as the rectangle region described above.

To create a *contour region*, select the **Contour** tool. With a Contour region, a bounding rectangle is defined with the mouse, just as with the Rectangle tool. Once that rectangle is defined, WinList computes a contour on the data within that rectangle. Each time the data in a histogram changes, the contour region will recompute based on the data in that rectangle.

To size a 2P region, first click on the region label to activate the region. Sizing handles will appear around the perimeter of the region. Drag a handle to resize the region.

To rotate a 2P region, first click on the region label to activate the region. Along with sizing handles, a small double-headed arrow will appear to the right of the region's center. Drag this handle to rotate the region.

The Edit Properties for 2P Region dialog is displayed when you double-click a 2P region label.

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

2P regions can automatically position themselves relative to the median of the histogram that contains them. This attribute can be enabled in the <u>Edit Properties for 2P Region</u> 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.

NStat Properties			
Name NStat rows NStat columns Show vertices Results Enable gates	Current Value 2 2 Current Value 2 Current Valu	Description Rows in the NStat. Columns in the NStat. Enable to display each vertex. Creates a results for the region. Enables gates based on the subregions.	OK Cancel
Cell Tracking Wizard In			
Import partitions	Import	Imports information from ModFit LT Cell Tracking Wizard.	

The <u>Edit Properties for NStat Region</u> 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 <u>Create Region Array</u> option in the **Create** menu. This command can create sets of 1P or 2P regions, depending on what histogram is active at the time.

Edit Regions



This command is found on the **Home** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display.

The **Edit Regions** dialog box displays a list of the regions that have been created in the active data source. It can be used to delete regions, link regions together, and to save and open regions from disk.

Name:	Alias	Cell_BM_Tube_00 s:	Statistic:	
R1	R1		Percent gated ~	
Name	Alias	Statistic	Histogram	Delete
R1 R2 R3 R4	R1 R2 R3 R4	Percent gated Percent gated	1:FSC-A vs SSC-A 2:CD10 PE cy7 vs CD20 APC 2:CD10 PE cy7 vs CD20 APC 2:CD10 PE cy7 vs CD20 APC	Restore Clear
R5 R6 R7 R8	R5 R6 R7 R8	Percent gated	2:CD10 PE cy7 vs CD20 APC 3:CD10 PE cy7 vs CD45 AmCyan 3:CD10 PE cy7 vs CD45 AmCyan 3:CD10 PE cy7 vs CD45 AmCyan	Links Reset Aliases
R9 R10 R11 R12	R9 R10 R11 R12	Percent gated	3:CD10 PE cy7 vs CD45 AmCyan 4:CD20 APC vs CD45 AmCyan 4:CD20 APC vs CD45 AmCyan	Open Save
R12 R13	R12 R13		4:CD20 APC vs CD45 AmCyan 4:CD20 APC vs CD45 AmCyan	

The region list box is a multiple select list box, which means that any number of regions can be selected at once. To select regions that are not adjacent in the list, hold the **Ctrl** key down on the keyboard while clicking on the Region name.

Applies to

The data source that this region dialog relates to is shown here.

Name

This field shows the name of the selected region.

Alias

This edit box allows you to enter an alias name for the selected region. The name can be displayed in results windows to make region identification easier. You can also display the region name or alias when you draw regions on histograms, so you can edit the Alias to more meaningful names such as Lymphs or Monos.

In addition, region aliases can be FCS or internal keywords. Any part of an alias that is surrounded with square brackets [] will be evaluated as a keyword. See <u>Internal Keywords</u> for more on keywords.

Statistic

This field shows the statistic that the selected region will display in its label. *No statistic, Percent Gated, Percent Total,* and *Region Events* are the statistics that can be selected.

Region list box

This list box shows all regions that have been created. This is a multiple selection list box, so more than one item may be selected at a time. Each time a selection is made, the alias for the first selected item is displayed in the Alias edit box.

Delete

This command deletes the selected regions.

Restore

This command restores a deleted region from the Undo buffer.

Clear

This command clears a deleted region from the Undo buffer so that it is no longer associated with the histogram.

Links

Click this button to link one or more regions together, or to view existing region links. The <u>Edit Region Links</u> dialog is displayed.

Reset Aliases

This command changes all the current aliases to their default values.

Open/Save

These buttons allow you to save and retrieve regions. When regions are opened from disk, they replace the ones currently in memory. WinList will attempt to re-establish histogram, results windows, and linking associations.

ОК

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

Edit Properties for 1P Region

This dialog can be displayed by double-clicking the mouse on the label of a 1P region, or by right-button clicking on the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the <u>Preferences</u> dialog, found in the Options menu.

Edit Properties for R14				
Bta LA Label	Name Show Name Show Alias Alias Statistic Line color Line weight Allow delete Region style	Current Value Enable Enable R14 Percent gated Color Color Enable Bar with legs	Description Show the region name Show the region alias ir Edit the alias for the reg Choose a statistic to dis Click to change color Line weight for regions. Allow or prevent the reg Choose the style.	OK Cancel Help
	Auto-positioning Auto-position Auto-position method Percent <	Enable Center on region median 10.00	Automatically move reg Choose to method to a Enter the percent to inc	

Elements in the tree

Region

Select the region in the tree on the left to edit the properties of the region.

Label

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

Properties

Show Name

Enable this checkbox to display the name of the region.

Show Alias

Enable this checkbox to display the alias of the region.

Alias

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

Show Statistic

Select a statistic to display in the region label. *No statistic, Percent Gated, Percent Total,* and *Region Events* are the statistics that can be selected.

Line color

Click this button to choose a different color for the region.

Line weight

Select a line weight from the list of options. Larger values are heavier line weights.

Allow delete

With this option enabled, the region can be deleted by the user. To prevent a region from being deleted, uncheck this option.

Region style

Select the style for the 1P region: Bar with legs, Bar with tics.

Auto-positioning

Auto-position

Enable this option when you want the region to automatically adjust its position relative to the data in the histogram. The region will center itself on the X median or mode value, depending on the selection in the Auto-position method option below. When you move a region with the auto-position property enabled, you offset its auto-position location relative to the data. When the data changes in the histogram containing the region, the region will adjust its position relative to the new data.

The auto-position property will be disabled in a few circumstances where it is invalid. When a region is used in a self-gate on the histogram in which it resides, the property is disabled. Similarly, if a region is a dependent linked region, the property is disabled.

Auto-position method

This option determines what statistic is used when the auto-positioning is enabled for a region. The center of the region is positioned on the specified value. Choose *Center on histogram median* if you want to track the central tendency of the data or *Center on histogram mode* to track the location of highest frequency events. Choose *Include lower percent* to position the region to include events on the low intensity end of the histogram, or *Include upper percent* to include the high intensity events. Choose *Center on region median* or *Center on region mode* to track the central tendency of the data within the region, not the entire histogram.

X Percent

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

If Auto-Position is enabled and the method is *Include upper percent,* enter the percentile value where the left leg of the region should be positioned. The right leg will be set at the upper boundary.

ОК

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

Cancel

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

Help

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

1P Region Context Menu

This menu is displayed by right-clicking a 1P region label. It provides commands that relate to the region.

Edit Properties

Choose this command to display the <u>Edit Properties</u> dialog for the 1P region.

Create Results

This command creates a <u>Results window</u> to display statistics for the region.

Highlight events in Rn

This is a toggle option that can be enabled for one region at a time. When enabled, the events that are in the region will be highlighted in all histograms. Highlighting, by default, displays these events larger and blinking. See <u>Region preferences</u> for highlight properties.

Color events in Rn only

This is a toggle command that enables or disables coloring using this region only. It is a convenient way to explore where the events that are in the region appear in all other histograms. Selecting the option once will enable coloring for the region. Selecting it again (or choosing it for another region) will turn off the function.

Stick to Neighbor Regions

This command scans the vertices of the region to see if any are overlapping vertices from neighboring regions. For any vertex that overlaps another region's vertex, a linkage is made so that if either vertex is moved, both are moved. Sticky regions make it possible to create a set of adjacent regions that maintain adjacency even if you move one region, or a vertex of a region. See <u>Sticky Regions and Magic Contours</u> for more info.

Region Links...

This command displays the <u>Edit Region Links</u> for the region to allow this region to control the size and shape of other regions, and vice versa.

Delete

This command deletes the region, if it is not locked.

Edit Properties for 2P Region

This dialog can be displayed by double-clicking the mouse on the label of a 2P region, or by right-clicking the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the <u>Preferences</u> dialog, found in the Options menu.

Edit Properties for R1				
R1 └─A Label	Name Show Name	Current Value	Description Show the region name in the lal	OK Cancel
	Show Alias	Enable	Show the region alias in the lab	Cancel
	Alias	R1	Edit the alias for the region.	
	Statistic	Percent gated \sim	Choose a statistic to display in t	Help
	Line color	Color	Click to change color	i icipiii
	Line weight	0 ~	Line weight for regions.	
	Allow delete	✓ Enable	Allow or prevent the region from	
	Vertices			
	Show resizing handles	✓ Enable	Display resizing handles when re	
	Show vertices	Enable	Enable to display each vertex.	
	Number of vertices	0 ~	Limits number of displayed vert	
	Vertex size	8 ~	Choose display size for vertices,	
	Change shape	Custom ~	Choose to change the shape	
	Auto-positioning			
	Auto-position	Enable	Automatically move region with	
	Auto-position method	Center on region median \sim	Choose to method to auto-posit	
	X Percent	10.00	Enter the X percent to include. (
	Y Percent	10.00	Enter the Y percent to include. (
	<		>	

Elements in the tree

Region

Select the region in the tree on the left to edit the properties of the region.

Label

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

Properties

Show Name

Enable this checkbox to display the name of the region.

Show Alias

Enable this checkbox to display the alias of the region.

Alias

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

Show Statistic

Select a statistic to display in the region label. *No statistic, Percent Gated, Percent Total,* and *Region Events* are the statistics that can be selected.

Line color

Click this button to choose a different color for the region.

Line weight

Select a line weight from the list of options. Larger values are heavier line weights.

Allow delete

This option determines whether a region can be deleted by the user. When enabled, regions can be deleted by clicking on the region label and pressing the Delete key on the keyboard. Some regions can also be deleted by dragging them off the left or bottom edge of the histogram. Uncheck this option to protect a region from being deleted accidentally. Any region can be deleted using the Region List dialog box, even if the Allow delete option is disabled.

Vertices

Show resizing handles

Enable this option to display resize handles on the region when it is active. Resize handles form a bounding rectangle around a region and can be used to adjust size or shape of a region. The handles are hidden when the region is not selected.

Show vertices

Enable this option to display the individual points that comprise the region. When displayed, the vertex points can be moved individually.

Number of vertices

This option allows you to choose how many of the region vertices to display when the Show Vertices option is enabled. Choosing 0 displays all vertices. Other choices include 5, 10, and 20 vertices. By displaying a subset of the vertices, it is possible to adjust regions with many vertices using just a few control points.

Vertex size

This option determines the size of the region's vertices, if they are displayed.

Change shape

This option allows you to change the shape of an existing region to either an ellipse or a rectangle.

Auto-positioning

Auto-position

Enable this option when you want the region to automatically adjust its position relative to data in the histogram. The region will center itself on the X and Y medians or modes, depending on the selection in the Auto-position method option below. When you move a region with the auto-position property enabled, you offset its auto-position location relative to the data. When the data changes in the histogram containing the region, the region will adjust its position relative to the new data.

The auto-position property will be disabled in a few circumstances where it is invalid. When a region is used in a self-gate on the histogram in which it resides, the property is disabled. Similarly, if a region is a dependent linked region, the property is disabled.

Auto-position method

This option determines what statistic is used when the auto-positioning is enabled for a region. The center of the region is positioned on the specified value. Choose *Center on histogram median* if you want to track the central tendency of the data or *Center on histogram mode* to track the location of highest frequency events. Choose *Include lower percent* to position the region to include events on the low intensity end of the histogram, or *Include upper percent* to include the high intensity events. Choose *Center on region median* or *Center on region mode* to track the central tendency of the data within the region, not the entire histogram.

X Percent, Y Percent

If Auto-Position is enabled and the method is *Include lower percent,* enter the percentile value where the top and right sides of the region should be positioned. The left and bottom edges will be set at the origin.

If Auto-Position is enabled and the method is *Include upper percent,* enter the percentile value where the left and bottom edges of the region should be positioned. The top and right edges will be set at the upper boundary.

ОК

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

Cancel

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

Help

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

2P Region Context Menu

This menu is displayed by right-clicking a 2P region's label. It provides commands that relate to the region.

Edit Properties

Choose this command to display the <u>Edit Properties</u> dialog for the 2P region.

Create Results

This command creates a <u>Results window</u> to display statistics for the region.

Highlight events in Rn

This is a toggle option that can be enabled for one region at a time. When enabled, the events that are in the region will be highlighted in all histograms. Highlighting, by default, displays these events larger and blinking. See <u>Region preferences</u> for highlight properties.

Color Rn Only

This is a toggle command that enables or disables coloring using this region only. It is a convenient way to explore where the events that are in the region appear in all other histograms. Selecting the option once will enable coloring for the region. Selecting it again (or choosing it for another region) will turn off the function.

Show Resize Handles

Enable this option to show the resizing handles for the 2p region.

Show Vertices

Enable this option to show the vertices of the 2p region.

Stick to Neighbor Regions

This command scans the vertices of the region to see if any are overlapping vertices from neighboring regions. For any vertex that overlaps another region's vertex, a linkage is made

so that if either vertex is moved, both are moved. Sticky regions make it possible to create a set of adjacent regions that maintain adjacency even if you move one region, or a vertex of a region. See <u>Sticky Regions and Magic Contours</u> for more info.

Conform to Other Regions

This command adjusts the vertices of the region so that they do not overlap other 2P regions in the histogram. Vertices of this region that are inside of other regions are eliminated, and vertices of other regions that are inside of this region are added.

Region Links...

This command displays the <u>Edit Region Links</u> for the region to allow this region to control the size and shape of other regions, and vice versa.

Delete

This command deletes the region, if it is not locked.

Edit Properties for Contour Region

This dialog can be displayed by double-clicking the mouse on the label of a Contour region, or by right-clicking the label and choosing Edit Properties.

Default properties for regions are set in the dialog found in the <u>Preferences</u> dialog, found in the Options menu.

Edit Properties for R15				
লি% R15 A Label	Name Show Name	Current Value	Description Show the region na	ОК
	Show Alias	 ✓ Enable ☐ Enable 	Show the region ali	Cancel
	Alias	R15	Edit the alias for the	
	Statistic	Percent gated ~	Choose a statistic to	Help
	Line color	Color	Click to change colc	пер
	Line weight	0 ~	Line weight for regi	
	Allow delete	🗸 Enable	Allow or prevent the	
	Contour Region			
	Contour Probability	0.90	The probability leve	
	Smoothing	3 ~	Smoothing for the (
	Show resizing handles	^S Enable	Display resizing han	
	Auto-positioning			
	Auto-position	Enable	Automatically move	
	Auto-position method	Center on region median	Choose to method 1	
	X Percent	10.00	Enter the X percent	
	Y Percent	10.00	Enter the Y percent	
	<		>	

Elements in the tree

Region

Select the region in the tree on the left to edit the properties of the region.

Label

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

Properties

Show Name

Enable this checkbox to display the name of the region.

Show Alias

Enable this checkbox to display the alias of the region.

Alias

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

Show Statistic

Select a statistic to display in the region label. *No statistic, Percent Gated, Percent Total,* and *Region Events* are the statistics that can be selected.

Line color

Click this button to choose a different color for the region.

Line weight

Select a line weight from the list of options. Larger values are heavier line weights.

Allow delete

This option determines whether a region can be deleted by the user. When enabled, regions can be deleted by clicking on the region label and pressing the Delete key on the keyboard. Some regions can also be deleted by dragging them off the left or bottom edge of the histogram. Uncheck this option to protect a region from being deleted accidentally. Any region can be deleted using the Region List dialog box, even if the Allow delete option is disabled.

Contour Region

Contour Probability

This field determines the size of the contour region, with a probability value ranging from 0.01 (smallest) to 0.99 (largest). With a larger probability value, more events are included.

Smoothing

This field controls the smoothness of the contour region, with a larger value creating a smoother region.

Show resizing handles

Enable this option to display resize handles on the region when it is active. Resize handles form a bounding rectangle around a region and can be used to adjust size or shape of a region. The handles are hidden when the region is not selected.

Auto-positioning

Auto-position

Enable this option when you want the region to automatically adjust its position relative to data in the histogram. The region will center itself on the X and Y medians or modes, depending on the selection in the Auto-position method option below. When you move a region with the auto-position property enabled, you offset its auto-position location relative to the data. When the data changes in the histogram containing the region, the region will adjust its position relative to the new data.

The auto-position property will be disabled in a few circumstances where it is invalid. When a region is used in a self-gate on the histogram in which it resides, the property is disabled. Similarly, if a region is a dependent linked region, the property is disabled.

Auto-position method

This option determines what statistic is used when the auto-positioning is enabled for a region. The center of the region is positioned on the specified value. Choose *Center on histogram median* if you want to track the central tendency of the data or *Center on histogram mode* to track the location of highest frequency events. Choose *Include lower percent* to position the region to include events on the low intensity end of the histogram, or *Include upper percent* to include the high intensity events. Choose *Center on region median* or *Center on region mode* to track the central tendency of the data within the region, not the entire histogram.

X Percent, Y Percent

If Auto-Position is enabled and the method is *Include lower percent,* enter the percentile value where the top and right sides of the region should be positioned. The left and bottom edges will be set at the origin.

If Auto-Position is enabled and the method is *Include upper percent,* enter the percentile value where the left and bottom edges of the region should be positioned. The top and right edges will be set at the upper boundary.

ОК

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 <u>Preferences</u> dialog, found in the Options menu.

Edit Properties for R2				
R2 □ A Label	Name NStat label	Current Value	Description	ОК
		Name ~	Choose text to display for the la	Cancel
	Alias	R2	Edit the alias for the region.	
	Sub region label	Statistic ~	Choose text to display for subre	
	Statistic	Percent gated \sim	Choose a statistic to display.	Help
	Allow delete	🗹 Enable	Allow or prevent the region fron	
	Enable gates	Enable	Enables gates based on the sub	
	Lines			
	Line color	Color	Click to change color	
	Line weight	0 ~	Line weight for regions.	
	Vertices			
	Show vertices	Enable	Enable to display each vertex.	
	Vertex size	8 ~	Choose display size for vertices,	
	Auto-positioning			
	Auto-position	Enable	Automatically move region with	
	X Percent	10.00	Percent for the right edge of lef	
	Y Percent	10.00	Percent for the top edge of the	
	Import partitions	Import	Imports information from ModF	
	<		>	

Elements in the tree

Region

Select the region in the tree on the left to edit the properties of the region.

Label

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

Properties

NStat label

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

Alias

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

Label

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

Sub region label

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

Show Statistic

Select a statistic to display in the region label. *No statistic, Percent Gated, Percent Total,* and *Region Events* are the statistics that can be selected.

Allow delete

This option determines whether a region can be deleted by the user. When enabled, regions can be deleted by clicking on the region label and pressing the Delete key on the keyboard. Some regions can also be deleted by dragging them off the left or bottom edge of the histogram. Uncheck this option to protect a region from being deleted accidentally. Any region can be deleted using the Region List dialog box, even if the Allow delete option is disabled.

Enable gates

Enable this option to turn on gates that are associated with each sub region of the NStat. Normally, WinList does not turn off N-Stat subregions for gating. If you want to use an N-Stat in a gate expression, this option needs to be enabled.

Lines

Line color

Click this button to choose a different color for the region.

Line weight

Select a line weight from the list of options. Larger values are heavier line weights.

Vertices

Show vertices

Enable this option to display the individual points that comprise the region. When displayed, the vertex points can be moved individually.

Vertex size

This option determines the size of the region vertices, if they are displayed.

Auto-positioning

Auto-position

Enable this option when you want the region to automatically adjust its position relative to data in the histogram. For NStat regions, the auto-positioning is always performed based on an X and Y percentile value that you enter.

X Percent, Y Percent

If Auto-Position is enabled, enter the X and Y percentile values for positioning the NStat. The left-most column of the NStat is positioned at the X Percentile, and the bottommost row is positioned at the Y Percentile value.

Import partitions

This option allows region partitions to be imported from file on disk. It is designed to work with ModFit LT's capability to export partitions from the Proliferation Wizard. The format of the file is tab-delimited ASCII. An example is shown below:

Prolif	Wizard	Partitions
File	ab#28	CSFE Norm 72h.011
Regions	10	
Scale	0	256
Gen10	0.00	23.23
Gen9	23.23	43.78
Gen8	43.78	62.89
Gen7	62.89	82.19
Gen6	82.19	99.98
Gen5	99.98	122.44
Gen4	122.44	140.95
Gen3	140.95	160.32
Gen2	160.32	179.90
Parent	179.90	256.00

When the Import option is used to read a partition file, the NStat region boundaries are adjusted to match those defined in the partition file. This allows WinList to take advantage of a proliferation analysis in ModFit LT, to perform additional analysis on the daughter populations and their relationship to other markers.

ОК

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

Cancel

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

Help

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

NStat Context Menu

This menu is displayed by right-clicking an NStat region label. It provides commands that relate to the region.

Edit Properties

Choose this command to display the Edit Properties dialog for the NStat region.

Create Results

This command creates a <u>Results window</u> to display statistics for the region.

Show Vertices

Enable this option to show the vertices of the NStat region.

Region Links...

This command displays the <u>Edit Region Links</u> for the region to allow this region to control the size and shape of other regions, and vice versa.

Delete

This command deletes the region, if it is not locked.

Edit Region Links

The **Edit Regions Links** dialog box is used to create links between regions. Linked regions take on the same shape and position. The link can be bidirectional, where a change in either region affects the other. They can also be one-way links. One region can be linked to many others.

A region in one data source can be linked to a region in another, allowing you to set up identical gating conditions in both.

Edit Region Links			
Show regions in data source:			
DS1:BCell_BM_Tube_001.fcs			\sim
Select a region:		Select regions to link:	
R1: R1 (DS1 2P) R2: R2 (DS1 NStat) R6: R6 (DS1 NStat) R10: R10 (DS1 NStat) R14: R14 (DS1 1P) R15: R15 (DS1 Contour)	Let	R15: R15 (DS2 Contour)	
Clear All Links		Only matching parameter	S
	ОК]	

Show regions in data source:

Select a data source from this dropdown to view/edit the links for its regions.

Select a region:

In the list on the left, select one region. Once selected, the list on the right fills with compatible regions that are or can be linked to the selected region.

Select regions to link:

When a region is selected from the list on the left, this list fills with compatible regions that are or can be linked to the selected region. Click a region to select it. Press the **Ctrl** key and click to select more than one region.

Regions with an asterisk (*) are linked to the selected region. Regions are not linked until one of the link buttons below is clicked.

Only matching parameters:

With this option enabled, only regions in histograms with identical parameters to the source region will be displayed in the list.

One-way link

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Click this button to link the region on the left to the regions selected on the right. The link is a one-way link, meaning that when the left-hand region is moved or resized, the other regions will move and resize accordingly.

Two-way link



Click this button to link the region on the left **bidirectionally** to the regions selected on the right. The links are two-way links, meaning that when either region is moved or resized, the other will move and resize accordingly.

Unlink



This push button will unlink the selected regions.

Clear All Links

This push button will unlink the all regions.

ОК

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

Create Region Array

This menu option displays the Create Region Array dialog box, which is used to create one or more rectilinear regions. Use this dialog when you want to create a set of regions spanning a given range of channels.

The main difference between a region array and an NStat region is that the region array creates a set of separate regions. Each region in the region array can be moved independently. The NStat is a collection of regions that are always adjacent and can be moved as a collection. See <u>Regions</u> and <u>Edit Properties for NStat Region</u> 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 Total regions to crea			OK Cancel
On X-Axis			
Number of Starting at: Ending at: Space between:	1 -20 262144 0	Enter the number of regions in the X Enter the left edge of the first region Enter the right edge of the last region Spacing (% of X scale).	1.
On Y-Axis	10]	
Number of Starting at:	-20	Enter the number of regions in the Y Enter the bottom edge of the first re	
Ending at:	262144	Enter the top edge of the last region	
Space between:	0	Spacing (% of Y scale).	

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.

ОК

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 2parameter 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 1parameter 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:	1	Enter the number of regions in the $ imes$ direction.			
Starting at:	0	Enter the left edge of the first region.			
Ending at:	1024	Enter the right edge of the last region.			
Space between:	0	Spacing (% of X scale).			

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 <u>FCOM Region Array options</u> 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.

COM Region Array Optic	ons	
Name	Current Value	Description
Color by Gate Hierarchy	🗸 Enable	Use these regions for coloring.
Gate colors	Colors	Choose the colors to use for gating.
Gate Aliases		
G2 alias	3	Enter a name for gate G2.
G3 alias	4	Enter a name for gate G3.
G4 alias	8	Enter a name for gate G4.

Color by Gate Hierarchy:

Enable this option if you want to use the FCOM peaks to color the dots in histogram. Since FCOM peaks are discrete phenotypes, it is often very useful to use them for color gating so that the populations can be seen in the context of all other histograms.

Gate Colors:

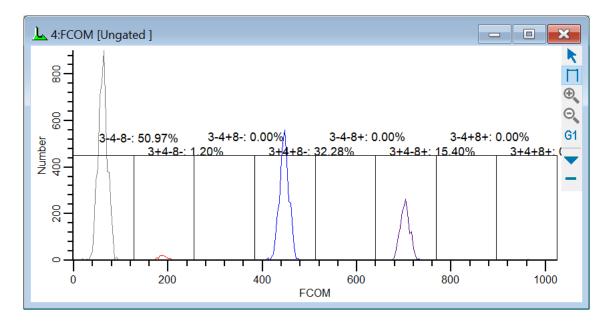
If you enable color-event gating with the option above, the colors used for each gate can be selected with this option.

Gate Aliases

The FCOM parameter uses gates to create phenotypes. The Gate Aliases edit boxes allow you to give the gates more descriptive names. For example, if G1 identifies CD20 positive cells and G2 is CD10 positive cells, you might type "CD20" and "CD10" respectively as the gate aliases. It is best not to include the "positive" or "negative" in the alias, and short aliases are more practical than long ones.

The program will create region aliases from the gate aliases you enter. Each peak in the FCOM histogram will have one region associated with it, and the region's alias will identify the phenotype. The region aliases created for the example given above would be:

CD20- CD10-CD20+ CD10-CD20- CD10+ CD20+ CD10+



Obviously, if the FCOM parameter involves more gates, the need for short aliases becomes even greater.

Edit Gates

G1= G2=	
Gates	

This command is found on the **Home** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display.

This dialog manages gates for one data source. You can redefine gates, change colors, assign them to histograms, save and open them from disk.

Edit Gates						
Applies to:	1: FCOM_E	Example.	fcs	_		
Equation:					And	Or Not
Alias:	Ungated					
Activation:	Enable aut	omaticall	ly	.~		
Δ	lias	E	quation	State	^	Color
Ungated				auto		0001
G1		R1		auto		Reset All
G2		R1&R2		auto		
G3		R1&R2&	R3	auto		Open
G4		R1&R2&	R4	auto		
G5		R5		auto		Save
G6		R6		auto		
G7		R7		auto		
G8		R8		auto		
G9		R9		auto	\checkmark	
Enable ca	ascade gatir	ng to cre	ate and adjus	t gates.	Ŧ	
	(ОК	Cancel	Apply		

Applies to

The data source that this gate dialog relates to is shown here.

Equation

This field contains the gating expression. See **Writing a gate containing a Boolean region expression** below for a detailed explanation.

And, Or, and Not Buttons

These buttons aid in the writing of Boolean equations. If you push the **And** button, a **&** will be inserted at the current location in the **Equation** edit box. The **Or** button inserts the **|** character and the **Not** button inserts the **!** character.

Alias

Each gate can have an alias. The alias is the name that appears when the gate is shown in other locations in the program.

Activation

This field controls how WinList activates the gate.

"Enable automatically" will turn the gate on when all of the regions referenced by the Equation have been created. The gate will be disabled if any of the regions are missing.

"Always enabled" causes the gate to remain enabled even if some of the regions in its Equation are missing.

Gate List box

The gate name, equation, activation, and color assignment are shown in this list box. The number of gates is set in the <u>Edit Program Controls</u> dialog box.

Click an entry in the list to select the gate for editing.

Click the color swatch to select a new color for the gate. Double-clicking the gate will toggle it between "Enable automatically" and "Always enabled".

To assign a gate to one or more histograms, select the gate in the listbox and click the histogram title bars.

Enable cascade gating to create and adjust gates

This check box turns the Cascade Gate system on and off. When Cascade Gating is on, gate expressions are automatically adjusted based on the gates that are applied to different histograms and the regions in those histograms.

Color

Selects a color for the selected gate.

Reset All

This option puts the system in its default condition where all gates are off and are associated with R1, R2, ... Rn respectively.

Open

This option retrieves the contents of the gate list from disk.

Save

The contents of the gate list box can be saved to disk when this option is selected. This option may be useful if you have created a number of complex gates and you don't want to waste time re-creating them each time. It is important to realize that this option does not save the regions themselves (see Region List) and it does not save the actual absolute colors (see Show/Edit Palette).

Cancel

The **Cancel** button allows you to exit with no changes.

ОК

Click **OK** to accept the changes you have made.

Apply

This button acts like the **OK** button, except that the dialog remains open.

Writing a gate containing a Boolean region expression

Normally when working with the gates, each gate is assigned a single region. For example, gate 1 or G1 is assigned to region 1 or R1. This initial setup can be changed extensively to better serve your gating needs. When a histogram is assigned a gate, say G1, and that gate is associated with R1 and the gate is active, the program will only allow those events that are inside R1 to be displayed in the gated histograms.

While this system allows some powerful gating, you can perform much more complex analytical gating. For example, if you wanted a histogram to be gated on two regions, say R1 and R2, and you wanted the histogram associated with this gate to have access to events that satisfy **both** regions, you could create a gate associated with the expression **R1 & R2**. The **&** symbol tells WinList to look only at events that satisfy both R1 AND R2. Click the **And** button to insert the **&** symbol in an expression.

The Boolean expression can be quite complex and may involve several regions. If you wanted to look at events that satisfy either R1 or R2, you would click the **Or** button. The expression would be written as **R1 | R2**.

Occasionally it is important to look at events that are **NOT** within a particular region. To do this we use the **!** operator. For example, to look at events that are *not* in R1, one would write **!R1**.

The gate expression can be made much more complex if needed by nested parentheses. For example, the gate expression

!(R1&R2) | R3

is true for events that do not satisfy both R1 and R2 or that satisfy R3. As you can see, these Boolean expressions can be quite powerful. A typical use of complex gates is to find rare events that satisfy a number of gating criteria. You can create a gate such as R1&R2&R3&R4 and turn on event coloring just for this gate. The events that satisfy all these regions will be highlighted by the gate color.

You can also use other gates in your gate expressions (as long as they do not create circular references). This is a convenient way to make your gate expressions more readable. For example, suppose that gate G1 is defined as (R1&R2)&!(R3&R4). We can use G1 as a shorthand for the expression in other gates:

G2 = G1 & R6

which expands to

((R1&R2)&!(R3&R4)) & R6

when it is evaluated.

In summary, WinList's Boolean operators include **&** (AND), | (OR), and ! (NOT). The operands are the regions defined as **R1**, **R2**, ... **Rn**. The steps to create a more complex gate expression are to first choose the gate that needs to be redefined, then to write the Boolean expression in the edit box, and finally to press the Replace button. To assign that

gate to a particular histogram, click the histogram's title bar with the gate you want selected in the **Edit Gates** dialog box. When the **OK** button is selected, the new gate will be applied to all the gated histogram. You can save the gates at any time for future use by using the Save option.

Gate Statistics

The Gate Statistics window can be display for each data source. It contains a list of the active gates arranged by hierarchy, along with statistics for each gate. The gate Alias is used to identify the gates.

% 1: Gate Statistics	—		×
File: FCOM_Example.fo	cs		
Population	Events	%Total	%Parent
V Ungated	10000	100.00	100.00
Lymphs	7649	76.49	76.49
CD3+	4903	49.03	64.10
CD4 TCells	3243	32.43	66.14
CD8 TCells	1555	15.55	31.71
Unclassified	120	1.20	2.44
Overlapped	15	0.15	0.30
Unclassified	2746	27.46	35.90
Overlapped	0	0.00	0.00
G5	5097	50.97	50.97
G6	120	1.20	1.20
G7	0	0.00	0.00
G8	3228	32.28	32.28
G9	0	0.00	0.00
G10	1540	15.40	15.40
G11	0	0.00	0.00
G12	15	0.15	0.15
Unclassified	0	0.00	0.00
Overlapped	7649	76.49	76.49

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

The content of the Gate Statistics window can be edited with the <u>Edit Properties for Gate</u> <u>Statistics</u> dialog.

Edit Properties for Gate Statistics

Edit Properties for	Gate Statistics		
Name Auto-size Ledger style	Current Value Current Value Enable Current Value	Description Auto size the window to fit content Draw a ledger style background	OK Cancel
Statistics #Events %Total %GrandParent %Parent Parameter Stats	 Enable Enable Enable Enable Enable 	Show # of events Show % total events Show % grandparent gate Show % parent gate Select parameter stats	Help
Additional Option Equation Unclassified Overlapped		Show gate equations Show # of events unaccounted for by child gates Show # of events in more than one child gate	

This dialog controls the content of the Gate Statistics window.

Auto-size

Enable this option to auto-size the Gate Statistics window to fit the content.

Ledger style

When this option is enabled, the Gate Statistics window uses alternating white and gray background for each line.

Statistics

#Events

Displays the number of events in the gates.

%Total

Displays number of events as a percentage of total events in the gate.

%GrandParent

Displays number of events as a percentage of the grandparent gate.

%Parent

Displays number of events as a percentage of the parent gate.

Parameter Stats

This button displays the <u>Edit Parameter Stats dialog</u>, which allows selection of parameters statistics to be computed for each gate.

Additional Options

Equation

Displays the gate equation in the Gate Statistics window.

Unclassified

Displays "Unclassified" statistics for each level of the gate hierarchy. Unclassified events are those that do not belong in any gate at that level.

Overlapped

Displays "Overlapped" statistics for each level of the gate hierarchy. Overlapped events are those that belong to more than one gate at that level.

Edit Parameter Stats

Edit Parameter Stats		
Parameters FSC-Height Side Scatter MIG FITC CD4 PE CD8 PerCP FL1-A CD3 APC FCOM	Statistics Median ✓ Add to List -> Remove from List	Parameter Stats List Median CD4 PE Median CD8 PerCP
	OK Cancel	

This dialog is displayed from the <u>Edit Properties for Gate Statistics</u> dialog.

This dialog is used to select parameter statistics to be computed for each gate. It provides an easy method to collect parameter intensity statistics for gates without having to create histograms.

Any number of parameter statistics can be selected. The statistics are computed for every active gate and displayed in the Gate Statistics window. Stats are also available to be databased and added to reports.

Parameters

Lists the parameter for the current data file. Select one or more parameters for which you want to compute a gate statistic.

Statistics

Select the statistic that you want to compute for the selected items in the Parameters list. Available statistics are mean, median, SD, and CV%.

Parameter Stats List

This list shows the parameter stats that have been added.

Add to List ->

When this button is clicked, the current Statistics selection is added for each of the parameters selected in the Parameters list to the Parameter Stats List.

Remove from List

Use this button to remove the selected items in the Parameter Stats List.

ОК

Closes the dialog and stores the changes.

Cancel

Closes the dialog and discards any changes.

Palette



This command is found on the **Options** tab of the ribbon bar, or in the Options menu when using the toolbar and menu display.

The **Edit Palette** dialog box is used to display and edit the colors used by gates, background, and axes.

	1	
Name View inverse palette	Current Value	Description OK Enable to display histograms in inverted
	Enable	Cancel
Brightness	Moderate	 Controls the brightness of the colors.
Reset colors	Reset	Regenerates the palette colors.
		Help
Background color	Color	Background color for window
Axis color	Color	Histogram axis color
Gate colors		
Color 1	Color	Gate color
Color 2	Color	Gate color
Color 3	Color	Gate color
Color 4	Color	Gate color
Color 5	Color	Gate color
Color 6	Color	Gate color
Color 7	Color	Gate color
Color 8	Color	Gate color
Color 9	Color	Gate color
Color 10	Color	Gate color
Color 11	Color	Gate color

View inverse palette

Enable <u>inverse palette</u> mode.

Brightness

Select the brightness level to use for generating palette colors. After selecting a level, use the **Reset colors** button to regenerate the palette.

Reset colors

Regenerate the colors in the palette.

Background color

The color used for background of histograms, results, etc. Click the button to select a different color.

Axis color

The color used for drawing the axis and tics. Click the button to select a different color.

Gate colors

A set of colors used for gates. Click the button to select a different color.

Open

Open a palette file from disk.

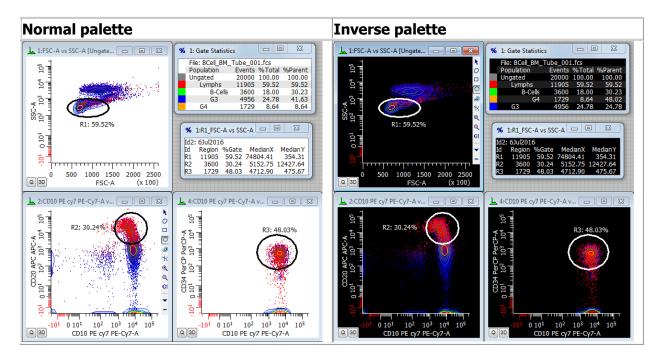
Save

Save a palette file to disk.

Inverse Palette

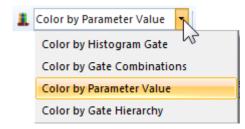
This option is found in the View tab of the ribbon bar, or the View menu when using toolbar and menu.

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



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

Color Modes



The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. Color modes mostly affect 2P histograms that are display Dots, but also have an impact on 1P and 3P histograms.

Four color modes are available:

Color by Gate Hierarchy

This is the default coloring mode. In this mode, dots are assigned the color of the last gate in the gate hierarchy that they belong to. Typically, this means that the dot color is the color of the most refined subset. See <u>Edit Color by Gate Hierarchy</u> for additional details.

Color by Histogram Gate

In this mode, dots are assigned the color of the gate that is applied to the histogram. This mode makes it easy to see how a histogram has been gated.

Color by Gate Combinations

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

Ŧ

🧸 Color by Gate Hierarchy

The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. In this coloring mode, dots are assigned the color of the last gate in the gate hierarchy that they belong to. Typically, this means that the dot color is the color of the most refined subset.

Jngated		In this mode, an even	nt is colored by the
Lymphs (G1 :R1)	\square	last gate it satisfies.	
CD3+ (G2 :R1&R2)	\square		
CD4 TCells (G3 :R1&R2&R3)	\square	Click a color swatch to change a color. Gates with X in the color box do not co the events.	
CD8 TCells (G4 :R1&R2&R4)	\square		
G5 (G5 :R5)		Edit aliance by coloctin	a aster and tuning
G6 (G6 :R6)		Edit aliases by selectir The Tab key moves t	
G7 (G7 :R7)		item.	
G8 (G8 :R8)			
G9 (G9 :R9)			
G10 (G10:R10)			
G11 (G11:R11)		Open	Save

The gate hierarchy is displayed in a list, with a color for each gate shown.

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

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

Edit Color by Gate Combinations

🧸 Color by Gate Combinatior 🝷

The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. In this mode, an event is colored based on *all* of the gates that it belongs to. So if an event belongs to more than one gate, a new color is created for the gate combination. This mode works well when only a few gates are used and there is not much overlap between them

Edit Color by Gate Combinations		
Available Gates	Selected Gates	
G5 :G5 G6 :G6 G7 :G7 G8 :G8 G9 :G9 G10:G10 G11:G11 G12:G12	Ungated G1 :Lymphs G2 :CD3+ G3 :CD4 TCells G4 :CD8 TCells	In this mode, events are assigned additive colors of each of the gates they satisfy. Customize Color Combinations
Add Drag gates into the Selected	Remove Gates list to include them, the	n select a color for each.
	OK Cancel	Apply

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 <u>Customize Color Combinations</u> button allows further control over the color used for overlapping gates.

Customize Color Combinations

This dialog box provides full control of the coloring of dots in 2P and 3P histograms when using Color by Gate Combinations. You can choose a color for each cluster created by the color gates.

For example, with gates G1 and G2 enabled for Gate Combinations mode, there are 4 clusters created: G1- G2-, G1- G2+, G1+ G2-, and G1+ G2+. Each cluster represents a unique set of events. This dialog provides a way of viewing and editing the color of each of these clusters.

Customize Gate Combination Colors	
Search:	Applies to: 1:FCOM_Example.fcs
Lymphs+CD3++CD4 TCells-CD8 TCells+ Lymphs+CD3+-CD4 TCells+CD8 TCells- Lymphs+CD3+-CD4 TCells+CD8 TCells+ Lymphs+CD3+-CD4 TCells-CD8 TCells- Lymphs-CD3++CD4 TCells-CD8 TCells+ Lymphs-CD3++CD4 TCells+CD8 TCells- Lymphs-CD3++CD4 TCells+CD8 TCells+ Lymphs-CD3++CD4 TCells-CD8 TCells+ Lymphs-CD3++CD4 TCells-CD8 TCells+ Lymphs-CD3++CD4 TCells+CD8 TCells+ Lymphs-CD3++CD4 TCells+CD8 TCells+	This list contains all combinations for the Color by Gate Combination Colors dialog. Type a gate subset or scroll the list to review combinations Click the color box to select a new color for the subset.
OK Cancel Reset	All

Search

This control allows you to search for the phenotype of the gate combination. As you type the combination (e.g. G1+G2-), the matching entry is selected in the list

Combination List

This list displays all gate combinations for the selected gates. Click the color swatch next to a list item to change its color.

ОК

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

Cancel

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

Reset All

Click this button to restore cluster colors to the default settings and close the dialog.

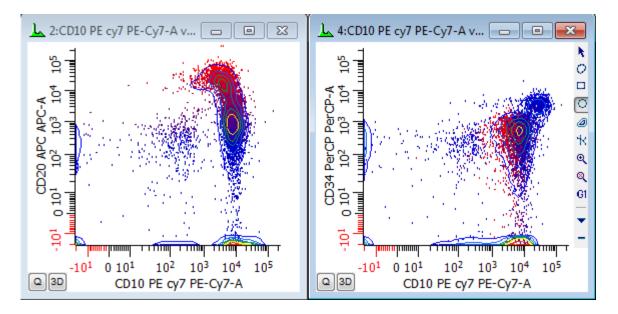
Edit Color by Parameter Value

🧸 Color by Parameter Value 🔹

The Home tab of the ribbon bar includes a drop-down control that allows you to select different coloring modes for WinList. In this mode, one parameter is selected to be used to color dots. The intensity of that parameter for each event determines the color of dots representing that event. It effectively adds information from a third parameter to every 2P histogram.

Edit Color by Parameter Value		
Parameters FSC-Height Side Scatter MIG FITC CD4 PE CD8 PerCP FL1-A CD3 APC FCOM	Color by: CD3 APC Colors: 8 ~ Starting color Ending color	In this mode, an event color is based on its intensity for the selected parameter. Gradient Colors Palette Colors Starting at: 1 Ending at: 1024
	OK Cancel	Apply

In the example below, CD20 was selected as the parameter, with dark blue representing low CD20 transitioning to red for high CD20. We have set the starting point for the transition at channel 100, so anything below 100 will be dark blue. The ending point for the transition to red is at 100,000, so anything above that will be red. Note that the coloring appears in the CD10 vs CD34 plot to help identify the low and high intensity CD20 events.



Select a parameter from the **Parameters** list. The intensity of the selected parameter will be used to color the dot for each event.

Choose the number of color levels with the **Colors** control.

Colors can be changed by clicking the color swatch for an entry in the Colors list.

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

Edit the **Starting at** value to set the channel where the transition begins. Any events below this value will be colored with the first color.

Edit the **Ending at** value to set the change where the transition ends. Any events above this value will be colored with the last color.

Results Windows



To create a results window, click the Results button on the Home tab of the ribbon bar. If WinList is set up to use a menu and toolbar, choose Results from the Create menu.

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

A new, results window will appear and the <u>Edit Results</u> dialog will be displayed to allow selection of regions and statistics.

% 2:R4_CD10 PE cy7 PE-Cy7-A 📼 💷 🕺						
Id1: BCell_BM_Tube_001.fcs						
Id2:	26Nov20	013				
ld	Gated	Region	%Gate	MedianX	MedianY	
R1	20000	12597	62.98	75398.78	361.11	
R2	12597	8723	69.25	7391.24	1602.82	
R3	20000	7251	36.26	121549.56	3064.13	
R4	8723	1697	19.45	3462.72	20790.28	
R5	8723	718	8.23	7746.37	18696.28	
R6	8723	1242	14.24	4659.79	11200.90	
R7	8723	5066	58.08	9263.23	8986.75	

A single results window can display information for any number of regions simultaneously. Users can choose which statistics appear and whether they are column- or row-oriented using the <u>Edit Results</u> 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.

Сору
Copy (no header)
Edit
Save
Print
Database wizard

Сору

Copy the results in tab-delimited text format to the clipboard. The text can then be pasted into a report or another application.

Copy (no header)

This command is similar to Copy, except that the column names for the results are omitted.

Edit

Display the Edit Results dialog.

Save

Use this command to save the results as a text file on disk. The standard <u>Save</u> dialog is displayed..

Print

Use this command to print the results window to the current printer.

Database Wizard

This command initiates a wizard that simplifies the task of adding statistics from a Results window or the Gate Statistics window to a database. See <u>Database Wizard</u> for details.

Create Results

Results windows are used to display statistics for one or more regions. It most cases, the results relate to a single data source and file. However, you can add results from any data source to a Results window.

To create a results window, choose the **Create Results** menu option. The **Edit Results** dialog box appears, enabling you to calculate and display one or more region's results in a window. The function of each item in this dialog box is described in the <u>Edit Results</u> dialog box.

Edit Results

This dialog box appears when creating or editing a results window. It allows the user to choose what statistics will be displayed and for which regions. You can also establish remote links with other programs through this dialog box.

One of the important concepts relating to WinList's results has to do with the sampling of events involved with the calculations. Normally, WinList uses a sample of the data, called a *packet*, for performing calculations and displaying statistical and graphical information. The number of events in the sample packet is set in <u>Edit Program Controls</u>. As the packet size increases, so does the number of events evaluated by WinList.

Edit Results Name: 2 R2_Side Scatter vs CD3 APC Show regions in data source: 1:FCOM_Example.fcs 1:FCOM_Example.fcs (2P) R2: R2 (2P) R3: R3 (2P) R4: R4 (2P) R5: 3-4-8- (1P) R6: 3+4-8- (1P) R7: 3-4+8- (1P) R9: 3-4-8+ (1P) R10: 3+4-8+ (1P) R11: 3-4+8+ (1P) R12: 3+4+8+ (1P)	ID Field3 Data Source Alias Total Events Gated Events Region Events Region Alias ✓ Percent Total ✓ Percent Gated % Select Gate: ✓ ✓ MeanX ✓ MeanY MedianAdcX MedianAdcY ModeX ModeY StdDevX StdDevY CVX CVY
	StdDevX StdDevY
 ✓ Fields in Columns ✓ Auto-size window Save as preference 	Edit Remote
ОК	Cancel

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 <u>Appendix B: Internal Keywords</u> for more information.

Show regions in data source:

This dropdown contains a list of all data source windows. Select a data source from the list to see the regions that have been created in that data source. Then select the regions you want to appear in this Results window.

You can select regions from any number of data sources to be included in the results window.

Region list

This list displays the regions that are created in the selected data source. Select the region you want to include in this results window.

Fields

ID Field 1 and 2

If ID Field1 or ID Field2 check boxes are checked, WinList displays the contents of the edit boxes next to these check boxes at the top of the results window. If the content of the edit box is braced by square brackets (e.g. **[\$SAMPLE]**), WinList will search the keywords of the current file for a match. If it finds a matching keyword, the value of the keyword will be displayed within the results window. This option is handy to identify file-specific information about the current data source.

Data Source Alias

When this field is checked, the name of the data source window will appear to the left of each region in the result window. This is useful when one results window is used to display results from several data sources.

Total Events

This option displays the total number of events currently used by WinList. This number is normally *Packet Size* number of events or the number of events in the data file. In order for WinList to display and analyze the total number of events in the data file, replay the data using one of the options in the Replay menu whenever you want to update the statistics. For more information on *Packet Size*, see Edit Program Controls.

Gated Events

This option displays the number of events that passed through the gate assigned to the histogram containing the region. If there is no gate, this number will equal **Total Events**. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

Region Events

This option displays the number of events that fall within the region. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

Region Alias

This option displays the region's alias name for each region in the results window. Region alias names can be edited in the Region List dialog box.

Percent Total

This option displays the number of events within the region as a percentage of **Total Events**. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

Percent Gated

This option displays the number of events within the region as a percentage of **Gated Events**. See the discussion of Total Events above for the effect of *Packet Size* on the number of events considered.

% Select Gate

This option has two elements: a checkbox to enable or disable it, and a drop-down list of all active gates. Similar in concept to Percent Gated, this option allows you to choose *any* gate on which to compute percentages for the regions in the results window. Since this statistic can be selected for regions that do not have an obvious relationship to the selected gate, it is important to understand how this computation is performed.

If the region represents a child gate of the selected Gate in the gating hierarchy, the statistic is very simple. It is the number of events in the region, multiplied by 100 and divided by the number of events in the selected Gate. This is the typical case.

Id	Gated	Region	%Gate	%G8
R1	20000	12595	62.98	14151.69
R7	12595	904	7.18	1015.73
R8	904	89	9.85	100
R8 is a				

However, if the region is *not* a child gate of the selected gate, the statistic is a little more complicated. The numerator of this statistic is the number of events in the region. The denominator is the number of events in the selected Gate, which may not be the same as the number of events in the region associated with the selected Gate.

ю	Gated	Region	%Gate	%G8
KAS	20000	12595	62.98	3279.95
R7	12595	904	7.18	235.42
R8	904	89	9.85	23.18
R8 is I				
384				

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.

Median X and Median Y

This option displays the X and Y median for the selected regions. The units reported are linearized units.

Median ADC X and Median ADC Y

This option displays the X and Y median in ADC units.

Mode X and Mode Y

This option displays the X and Y mode for the selected regions. The units reported are linearized units.

StdDevX and StdDevY

This option displays the X and Y standard deviation for the selected regions. The units reported are linearized units.

CVX and **CVY**

This option displays the X and Y percent coefficient of variation (CV) for the selected regions.

LowHighX and LowHighY

This option displays the low and high X and Y values for the selected regions. The units reported are linearized units.

CC

This option displays the correlation coefficient for the selected regions.

Use Geometric Mean

If a parameter is displayed on a log scale, this option controls whether the mean value is an arithmetic or geometric mean. A geometric mean calculates the arithmetic mean of the log data, and then linearizes the result. With the option unchecked, the mean is computed on linearized data.

Use Robust SD

The Robust SD is a variation on the SD calculation that is less influenced by outlier events. When enabled, the SD and CV computations use the <u>Interquartile Range method</u> for the computation. This is equivalent to BD Biosciences' rSD and %rCV computation.

Other options

Fields in Columns

When checked, the results window will display statistics, or fields, in columns. Each row will represent a different region. If unchecked, regions are in columns, and a different statistic appears in each row.

Auto Sizing

If this option is checked, WinList will automatically calculate the size that the results window needs to be in order to display the selected statistics and re-size the window as necessary. If the option is not checked, WinList will not adjust the results window's size.

Save Preference

Click this button to keep the current settings as default settings for all results windows.

Remote checkbox

This check box, in conjunction with the **Edit Remote** button, allows WinList to establish a Dynamic Data Exchange (DDE) link with another Windows application. If checked, the Edit Remote button is enabled.

Edit Remote

Click this button to establish a remote link with another application, or to edit the script used for that connection. See <u>Edit Remote</u> for more details on Remote links and DDE.

ОК

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

Cancel

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

Database Wizard

This command simplifies the task of adding the statistics from a <u>Results window</u> or the <u>Gate</u> <u>Statistics window</u> to a <u>database</u>.

To use the command, first activate a Results window or Gate Statistics window by clicking its title bar. Then, right-click the window to display its context menu and choose Database Wizard. A wizard dialog appears.

Database Wizard	\times		
Welcome to the database wizard.			
This tool makes it easy to add fields to a database from a Results window or the Gate Statistics window.			
All of the fields from the window will be added when you click Finish to complete the wizard. Source window: R2_Side Scatter vs CD3 APC			
< Back Next > Cancel Help			

Click Next to display the next pane in the wizard.

Database	×
Available Databases	
Database1	Show database viewer
Database2	✓ Activate the database
Choose the database you want to use	e, the click Next to continue.
< Back Nex	t > Cancel Help

In this pane, select a database to which you want to add statistics. Additional options allow you to display and activate the database when the wizard finishes.

Click Next to display the next pane in the wizard.

Options	×
Field Options ● Add fields to existing database. ○ Replace existing fields. Record Options	
 Keep existing records in the database. Remove existing records. 	
< Back Finish Cancel Help	

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: <u>Databasing with WinList</u> <u>Edit Properties for Database</u>

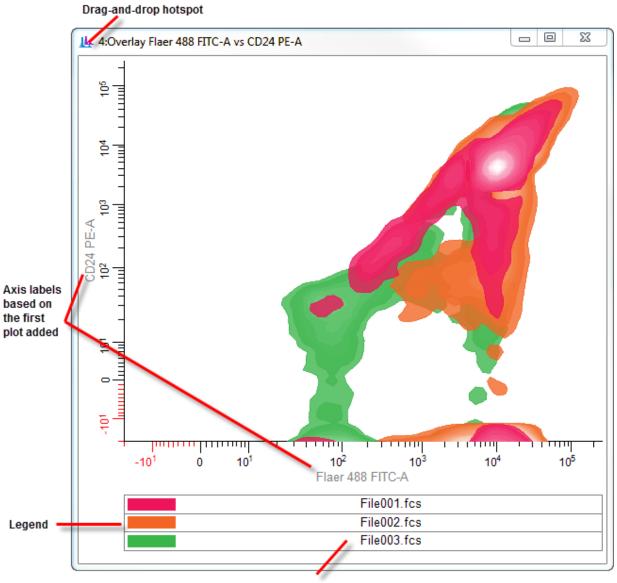
Overlay Windows



To create a new overlay window, click the Overlay button on the Home tab of the ribbon bar. If you are set up to use a menu and toolbar instead, choose Overlay from the Create menu.

Overlay windows are a great tool for graphically comparing a set of 1P or 2P plots. You can set up different views and select a wide variety of graphical options. Overlays organize plots - typically from different files - so that you can easily see the similarities and differences among them.

A new, empty overlay window will appear.



Plots added to the overlay

To add a plot to an overlay, drag a histogram by the icon on the left side of its toolbar and drop it onto the overlay. The first plot that is added to an overlay determines whether it is a 1P or 2P overlay window. It also determines the axes that will be displayed. If you subsequently add a plot with different parameters than the first plot, the overlay will ask if you really intend to do so.

To remove a plot from an overlay, click the item *in the legend* to select it. Then, right-click and choose **Delete Item** from the context menu.

To change the order of items in the overlay, click the item that you want to move in the legend, and then drag the item to the desired order in the legend. When the mouse is released, the overlay will redraw with items in the new order.

To edit the properties of the overlay or overlay items, right-click the overlay and choose **Edit** from the <u>context menu</u>. The <u>Edit Overlay</u> dialog will be displayed. This is where you can choose the kind of overlay you want and set the graphical properties.

Overlay Context Menu

The overlay context menu is displayed when the right mouse button is clicked on an overlay window.

Сору
Edit
Add to Report
Save
Print

If an item is selected in the legend and that item is right-clicked, an additional entry appears in the menu allowing the selected item to be deleted.

All.fcs CFP Stained Control.fcs	
DsRed Stained Control.fcs	Сору
	Edit
	Add to Report
	Save
	Print
	Delete Item

Сору

This command copies an image of the overlay to the clipboard in bitmap and picture (metafile) formats. The image can be pasted into other applications.

Edit

This command displays the <u>Edit Overlay</u> dialog to set the display properties for the overlay.

Add to report

This command adds the overlay to a report. The report item is actively linked to the overlay so that as changes are made to the overlay, they automatically appear in the report.

Save

This command allows the overlay to be saved to disk in a number of popular graphics file formats.

Print

This command prints the overlay to the active printer.

Delete Item

This command deletes a selected item from the overlay.

Edit Overlay

This dialog determines the display properties for an overlay window. It is accessed by double-clicking an overlay title bar, or by right-clicking the overlay and choosing **Edit** from the <u>context menu</u>.

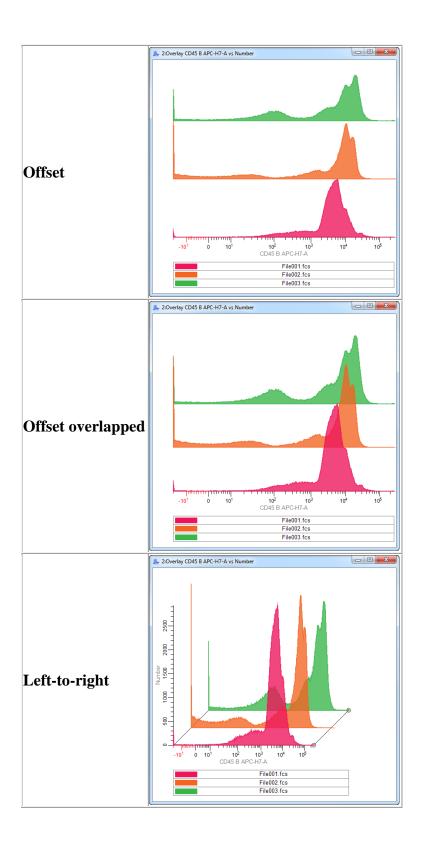
The options shown in the dialog differ, depending on whether the overlay is for 1P or 2P plots. The image below shows the 2P overlay dialog.

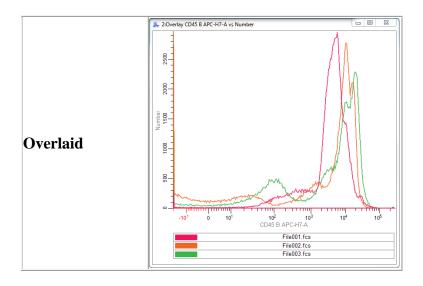
Edit Properties for Overlay FL2 vs Number					
Galactic Overlay FL2 vs Number	Name Overlay Properties	Current Value	Description	^	ОК
- Time=6h - Time=12h	View	Left-to-right ×	Choose the kind of overlay.		Cancel
	X Label	FL2	Edit the label for the X-axis		Apply
	Y Label	Number	Edit the label for the Y-axis		Help
	1P Overlay Propertie	95			•
	Filled histograms	✓ Enable	Fills the histograms with color by		
	Y Scale	Absolute ~	In Normalized, all items will have		
	Transparency	200	Default transparency, 0=transpare		
	Line weight	3 ~	Choose a default line weight.		
	Regions				
	Show regions	✓ Enable	Show regions for each overlay ite		
	Region line weight	0 ~	Choose region line weight.		
	Region color	Color	Choose a color for regions.		
	Fonts			\sim	
	<		>		

Overlay Properties

View

There are 4 viewing modes for overlay windows.





X Label

This is the label that appears on the X axis of the overlay.

Y Label

This is the label that appears on the Y axis of the overlay.

1P Overlay Properties

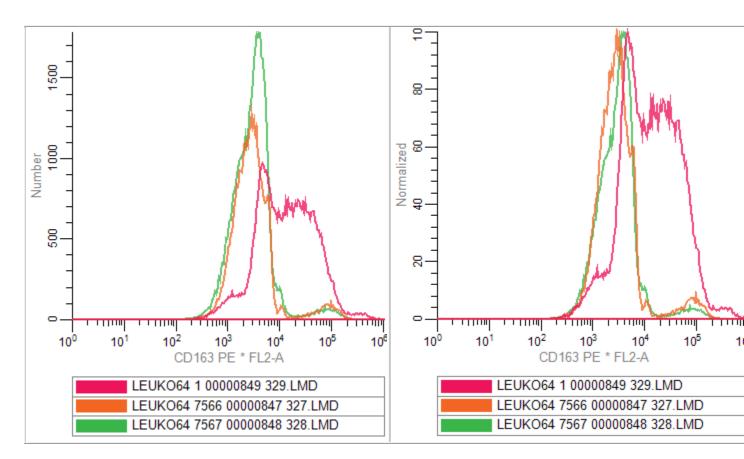
Filled Histograms

Enable this option to draw 1P histograms filled with the selected color. Disable the option to draw 1P histograms with no fill.

Y Scale

Choose **Absolute** or **Normalized**. In Absolute scaling mode, each 1P histogram in the overlay is drawn to its actual height, and the Y axis scale of the overlay is based on the tallest histogram in the overlay. In Normalized scaling, each 1P histogram is drawn to 100% of its own scale - they will all appear to have the same height - and the Y axis switches to show a 0 to 100 scale. Normalized scaling is useful when you are more interested in the intensity shifts of the overlay items rather than the actual areas.

Absolute	Normalized



Transparency

This value determines the transparency of the plots. A value of 0 makes the plots completely transparent; a value of 255 makes the plots opaque.

Line Weight

Select a line weight for the outline of the 1P plot.

2P Overlay Properties

Display

The 2P overlay can display **Dots** or **Contours** for the plots. Contours will draw faster and are somewhat easier to interpret.

2P Contour Properties

These properties are used when **Contours** is the selected Display option. The color assigned to each item in the overlay is used as the base color for the contours. Colors transition to white for the highest contour.

Probability interval

This option determines the number of contours that will be drawn, based on the separation between them.

Base contour

This option determines the probability for the lowest contour.

Fill contours

Enable this option to fill the contours with color. Disable the option to draw unfilled contours.

Smooth

Enable this option to smooth the contours.

Smoothing span

With Smooth enabled, this setting controls how much smoothing is applied. Higher values create smoother contours, but show less detail.

Transparency

This value determines the transparency of the contours. A value of 0 makes the plots completely transparent; a value of 255 makes the plots opaque.

Line Weight

Select a line weight for the outline of the contours.

2P Dot Properties

These properties are used when **Dots** is the selected Display option. The color assigned to each item in the overlay is used for the dots.

Dot size

Select a size for the dots.

Number

Select the number of dots to display for each overlay item.

Regions

These properties determine whether regions are included in the overlay.

Show regions

Enable this option to show the regions for the overlay items.

Region line weight

Select the line weight for regions.

Region color

Select the color to use for the region line.

Fonts

Legend font

Select a font to use for the legend.

Label font

Select a font to use for the axis labels.

Number font

Select a font to use for the numbers on the axes.

Region font

Select a font to use for the region labels in the plots.

Display Options

Show legend

Enable this option to display the legend with colors and item names on the overlay. The legend appears at the bottom of the overlay window. It is also used to re-order items in the overlay.

Show X axis

Enable this option to display the X axis.

Show Y axis

Enable this option to display the Y axis.

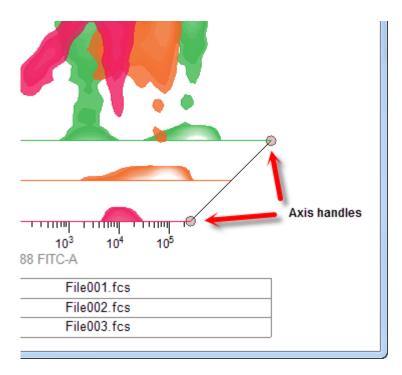
Show grid

Enable this option to show a grid based on the major tics of the axes.

X-axis size, Y-axis size

These options are only used for **Left-to-right** viewing mode. They control how much of the width or height of the plot area is used by the axis. By default, these are 80% (0.80). You can manually edit these values to change the perspective in Left-to-right viewing mode. You can also edit these directly on the Left-to-right overlay window by moving the small handles at the right side of the plot.

Edit Overlay



Edit Properties for Overlay Item

This dialog determines the display properties for an item in an overlay window. It is accessed by double-clicking an overlay title bar, or by right-clicking the overlay and choosing **Edit** from the <u>context menu</u>. Select the item you want to edit from the tree on the left of the dialog.

Edit Properties for autoposition.015					
Coverlay FL2 vs FL1	Name autoposition.015, FL2 vs FL1 Label	Current Value	Description Edit the label for this	OK Cancel	
autoposition.017	Color Fill contours	Color	Choose a color for th Check to fill contours	Apply Help	
	Transparency Line weight Dot size	200 0 ~ 2 ~	Default transparency, Choose default contc Choose a dot size.		
	Source: histogram 1 in data source 1 Update when histogram changes	Enable	When checked, this it		
	<		>		

The first entry in the property list shows the source FCS file and the parameter(s) that the plot is displaying. These are not editable; they are determined at the time the item is added to the overlay.

Label

This field shows the label that appears on the legend for the item. It can be edited.

Color

Select the color to associate with the item. This color appears on the legend, and is used to draw the item. If the item is a 1P plot, the selected color determines the line and fill color. For 2P plots showing contours, the color is used as the base contour color, transitioning to white at the highest contour. The color is used to draw dots in dot plots.

Fill contours

2P overlays only: this determines whether contours will be filled for this item.

Transparency

Sets the transparency level for the item, with 0=transparent and 255=opaque.

Line weight

Sets the weight of lines in this item.

Dot size

2P overlays only: this determines how large dots are for 2P items..

Line style

1P overlays only: this determines the style of the line used to outline 1P plots.

Source

This entry is not editable. It displays the histogram and data source IDs that this item is associated with.

Update when histogram changes

Enable this option if you want the overlay item to update in the overlay when the source histogram changes.

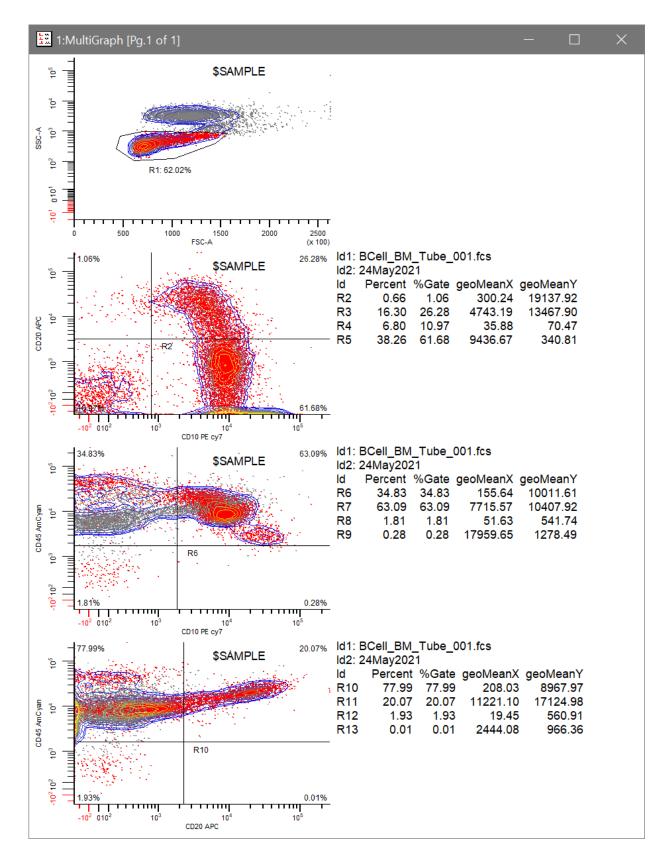
MultiGraph Windows



To create a MultiGraph window, click the MultiGraph button on the Home tab of the ribbon bar. If you are set up to use a menu and toolbar instead, choose MultiGraph from the Create menu. The <u>Edit MultiGraph</u> dialog will be displayed.

These windows allow you to display multiple histograms and results. The items in a MultiGraph are static - they do not change when a new file is opened or a gate is changed. MultiGraphs can display a mix of single, dual, and three parameter histograms and results in several different views. Additionally, MultiGraphs can be saved to disk, opened from disk, and added to at any time. They can also be exported in PDF format.

MultiGraph Windows



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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 snapshot: once added, an element in a MultiGraph does not change even if the histogram it represents changes.

If a MultiGraph is in Tiled mode, you can move its elements by clicking the left mouse button on the item to move, holding the button down, and dragging the element to a new location in the MultiGraph. When you release the button, the MultiGraph will be re-drawn with the elements in their new locations.

The <u>MultiGraph context menu</u> is displayed when you click the right mouse button on a MultiGraph. Options appearing in the menu allow you to copy or delete a single element or the entire MultiGraph, edit the MultiGraph, print the MultiGraph, and more.

To edit a MultiGraph, double-click the title bar.

To configure the default characteristics of MultiGraphs, use the <u>Preferences</u> command in the Options menu.

MultiGraph Context Menu

This context menu is displayed when the right mouse button is clicked on a MultiGraph window.

Copy Element
Delete Element
Сору
Edit
Save Graphic
Export to PDF
Print
Clear

Copy Element

This command appears in Tiled view. It copies the selected MultiGraph item to the clipboard as a graphic or text object.

Delete Element

This command appears in Tiled view. It removes the selected item from the MultiGraph.

Сору

This command copies the MultiGraph to the clipboard in graphic format.

Edit

This command displays the <u>Edit MultiGraph</u> dialog to edit the elements and the style of the MultiGraph.

Save Graphic

This command displays the Save As dialog to save the MultiGraph to disk in one of several popular graphic formats.

Export to PDF

This command displays the Save As dialog to export the MultiGraph to disk in PDF format.

Print

This command prints the MultiGraph to the current printer.

Clear

This command clears all elements from the MultiGraph, leaving an empty MultiGraph window.

Edit MultiGraph

A **MultiGraph** is a window that can display multiple histograms and results windows in various arrangements. The histograms can be from different data sources, and they can be a mix of 1, 2, and 3 parameter. MultiGraphs can be saved to disk, reopened and new histograms and results windows can be added to them, making them useful for archival storage. Each histogram in the MultiGraph may have a label assigned to it which can be positioned anywhere in the MultiGraph window. You can choose from several different views of the histograms, and change the views easily. See **Create MultiGraph** for more information.

To display this dialog, choose **Edit** from the MultiGraph context menu or double-click the MultiGraph's title bar.

Edit MultiGraph				
Name: MultiGraph Id: 1 Available Items BLANK	MultiGraph Items A-R 1:1 BCell_BM_Tube_001.fcs - FS			
1:1 BCell_BM_Tube_001.fcs - FSC-A vs SSC-A 1:2 BCell_BM_Tube_001.fcs - CD10 PE cy7 vs CD20 APC 1:3 BCell_BM_Tube_001.fcs - CD10 PE cy7 vs CD45 AmCyan 1:4 BCell_BM_Tube_001.fcs - CD20 APC vs CD45 AmCyan R2_CD10 PE cy7 vs CD20 APC R6_CD10 PE cy7 vs CD45 AmCyan R10_CD20 APC vs CD45 AmCyan	Add >> A-R BLANK Remove I:2 BCell_BM_Tube_001.fcs - CD Insert >> Ref_CD10 PE cy7 vs CD20 APC A-R 1:3 BCell_BM_Tube_001.fcs - CD Insert >> Ref_CD10 PE cy7 vs CD45 AmCyan A-R 1:4 BCell_BM_Tube_001.fcs - CD (A)xes R10_CD20 APC vs CD45 AmCyan			
Viewing Options Left to Right Right to Left Overlay Tiled Cols: 2 Rows: 4 	Item Label X pos. %: Y pos. %: Width %:			
Save Open	OK Cancel			

Name

Enter a name for the MultiGraph window. You can enter text, or type a keyword enclosed in square brackets.

Available Items

Displays a list of all histograms in all data sources currently opened in WinList, as well as the results windows. This is a multiple selection list box, so you can select the name of the histograms and results windows you want, drag the mouse in the list box to select a range, then click the **Add** button to add those histograms to the **Selected Items** list. An additional entry, **BLANK**, allows you to insert a placeholder with no graphic. This can be useful if you have a wide results window and want to allow it more room on the MultiGraph in a tiled view.

MultiGraph Items

Displays a list of histograms existing in the MultiGraph and those you want to add to it. It is a multiple select list box. You can click the left mouse button and multiple select the histograms you want to remove or edit. The first three characters in each entry of this list indicate the states of the **Axes** and **Regions** check boxes. The MultiGraph entries will include or exclude the X and Y-axes, and any regions that are within the histograms based on these characters. For example, if an entry in Selected Histograms starts with **A-R**, axes and regions will be included in the MultiGraph.

Add

This button copies the histograms currently selected in the **Available Items** list box into the **MultiGraph Items** list box. As histograms are added, the state of the check boxes **Axes** and **Regions** is also copied for each item added.

Remove

This button deletes the highlighted items from the **MultiGraph Items** list box and subsequently from the MultiGraph.

Insert

Use this button to add items from the **Available Items** list box in front of the currently selected item in the MultiGraph **Items** list box.

Axes, Regions

These check boxes determine whether x and y axes, and regions will be displayed for items as they are added to the **MultiGraph Items** list box. If **Axes** is checked, the letter **A** will appear as the first character in the **MultiGraph Items** entry. If it is not checked, a hyphen (-) will appear. Similarly, **R** will appear for regions. Once the **OK** button is clicked, these options become permanent for the selected histograms.

Viewing Options

Radio buttons allow you to select from several viewing arrangements: **Left to Right**, which stacks the histograms from the upper left to the lower right, **Right to Left**, which stacks from upper right to lower left, **Overlay**, which allows histograms to be superimposed on one another, and **Tiled**, which provides a row-column arrangement. If you select **Tiled**, the **Col** and **Rows** edit boxes allow you to control the row-column arrangement. Setting **Rows** to 0 will fit all elements of the MultiGraph onto one page. Setting **Rows** to a positive number will display a maximum of that many rows per page, in some cases creating a multiple page printout. For **Left to Right** and **Right to Left**, the edit box **Grids** allows you to lay down evenly spaced grid lines connecting the bases of the histograms.

Label

This group of controls allows you to enter a label for each item and to determine its size and position on the MultiGraph. The label may include up to 80 characters of text.

Labels can include any FCS keywords embedded in the data file and internal WinList keywords. Keywords must be surrounded by square brackets []. Keywords are only evaluated at the time the elements are created.

See Internal Keywords for more on keywords.

Examples of labels:

[HISTOGRAM] [GATE] [DATE]

displays name of the histogram, the gating information, and today's date

[FILE] contains [R('3, PERCENT')] percent positives

displays the name of the file and the percent of region R3.

[\$SAMPLE] [\$PROJ]

displays the FCS keyword values for \$SAMPLE and \$PROJ.

Label position and width

The X and Y positions and the width can be entered in these edit boxes. The numbers are in *percentage of the windows* where 0 percent x and y is the lower left corner of the window, and 100 percent x and y is the upper right. X and Y positions determine the origin of the label. The width, again in percent, determines how wide the label can extend. Usually, the width and the x position values should not add up to more than 100 or the label will spill out of that histogram's portion of the MultiGraph.

Save and Open

These push buttons allow you to save MultiGraphs to disk, or reopen existing MultiGraphs. Opening a MultiGraph from disk will replace any histograms in the current MultiGraph. Click **OK** when you've made your changes and selections and the new MultiGraph elements will be generated. The amount of time to generate a MultiGraph depends on how many elements you have selected and how complicated each one is.

Cancel

The **Cancel** button allows you to undo the changes you have made. In some cases it is grayed out, indicated that you cannot cancel the operation. This occurs if you are editing an existing MultiGraph and you *remove* one or more of the histograms from the **Selected Items** list box. Removing existing items is not a recoverable operation.

Open MultiGraph

This command displays the File Open dialog to select a MultiGraph from disk. It is found in the **File (Application)** menu of the Ribbon bar.

🐓 💾 🤚 🖘 🗐 WinList 3D						
🐓 🕶 Home Insert						
New Protocol						
Open Protocol						
save Protocol						
Save <u>A</u> s						
Open <u>R</u> eport						
Open <u>M</u> ultiGraph						
Export +						
Print •						
Preferences						

Export MultiGraph Graphics



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This menu options allows you to save the active MultiGraph as a graphic file on disk. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, or GIF.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box and choose the graphic format by using the Save as Type drop down menu. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

Export MultiGraph PDF



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This option saves the active MultiGraph window as a PDF file.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

Add Histogram to MultiGraph (Ctrl A)

This is a shortcut used to add the active histogram to the currently selected MultiGraph. It allows the user to bypass the **Edit MultiGraph** dialog box. If there is no histogram to add or no MultiGraph present, the option does nothing.

The histogram is added using the current settings of the **Edit MultiGraph** dialog box for Axes and Regions, as well as labels and label positions.

Add Results to MultiGraph (Ctrl R)

This is a shortcut used to add the active results window to the currently selected MultiGraph. It allows the user to bypass the **Edit MultiGraph** dialog box. If there are no results to add or no MultiGraph present, the option does nothing.

Add Blank to MultiGraph (Ctrl K)

This is a shortcut used to add a " blank" place holder to the currently selected MultiGraph. It allows the user to bypass the **Edit MultiGraph** dialog box. A blank is an element for which there is no graphic or text, but which occupies space like any other element in the MultiGraph.

Add Info to MultiGraph (Ctrl I)

This option allows you to add the text contained in the active histogram's Info window, if there is one, to the currently selected MultiGraph. Info windows appear when doing histogram subtraction, or if the histogram has a remote DDE connection with another application that has returned text to the histogram. When a button with the letter **I** appears in the lower left of a histogram window, you can click the button to display the histogram's Info window.

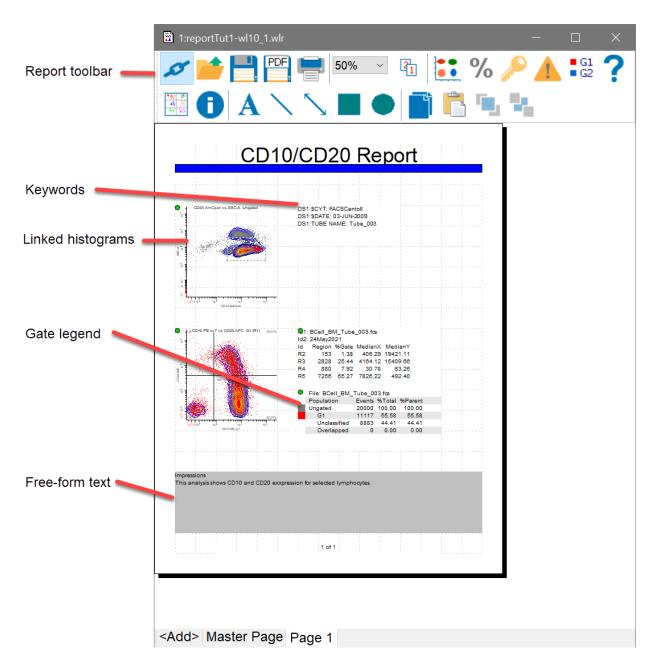
Use this option to add histogram subtraction results and remote DDE results to a MultiGraph.

Report Windows

	Ξ	1	
l	đ	-	
R	en	ort	

To create a report window, click the Report button on the Home tab of the ribbon bar. If you are set up to use a menu and toolbar instead, choose New from the Report menu.

Reports are used to create multi-page documents that can contain histograms, results, alerts, and many other kinds of information that WinList generates. The elements added to a report can come from different data sources, and can automatically update when the source objects change.



Elements can be added to reports by dragging-and-dropping them onto the report, or by using a command from the toolbar or menu. Once added, objects can be moved and resized using the mouse. See <u>Drag and Drop</u> for details.

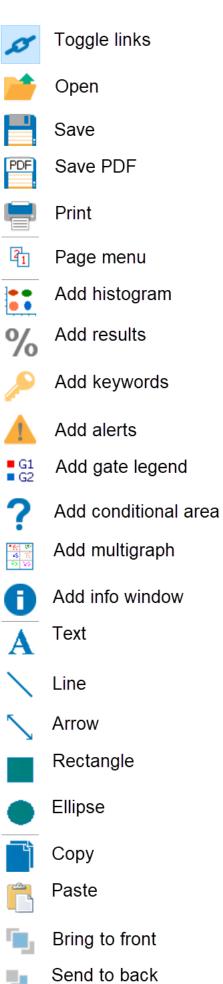
Right-click the mouse on an element of the report to see additional commands that can be performed on the selected element. The commands shown will differ depending on the type of object selected.

Reports can be saved to and opened from disk files. They can also be exported in PDF format, a convenient means for sharing reports with others who may not have access to

WinList. You can create and open any number of reports, providing a simple mechanism for producing internal and external versions of reports.

Report Toolbar

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



See individual report menu commands for details.

Report Context Menu

This context menu is displayed when the right-mouse button is clicked on a report. The menu also lists commands commonly used on reports, many of which are also accessed from the report toolbar.

	Сору
	Paste
	Page Setup
	Copy Page
	Clear Page
	Delete Page
	Open
	Save
	Print
	Export to PDF
✓ ✓	Active Links
✓	Snap-to-grid
	Display grid
	Insert Histogram Link
	Add Histograms
	Insert Results Link
	Insert Keywords
	Insert Alert
	Insert Gate Legend
	Insert MultiGraph Link
	Insert Overlay Link
	Insert Info
	Insert Condition Area
	Insert 3D Histogram Link
	Insert Text
	Insert Line
	Insert Arrow
	Insert Rectangle
	Insert Ellipse

See individual report menu commands for details.

New Report

	Ξ'n
	12
Rei	port

This command creates a new, empty report window.

To create a report window, click the Report button on the Home tab of the ribbon bar. If you are set up to use a menu and toolbar instead, choose New from the Report menu.

Open Report



This command displays the Open Report dialog to select a report to open from disk.

Save Report



This command displays the Save Report dialog to save the active report to disk.

Print Report



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu. It is also found on the toolbar for the active Report window.

Use the **<u>Printer Setup</u>** option in the File menu to set print options.

Several options in the **Preferences** dialog affect the print report option. They appear in the <u>Print Preferences</u> dialog box and control margins, orientation, and more.

Export Report PDF



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command displays the Export PDF dialog to save the active report as a PDF file. PDF stands for Portable Document Format, and is a standard format for exchanging information containing graphics and text. It is very useful for creating archival reports or for sharing analysis results with others.

Export Report Graphic

Report Graphic Export a Report in a graphic format.

This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command displays the Save Report Graphic dialog to save the active report as a graphic file. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, and GIF.

Report Page Menu

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

	Add page					
~	Rename page					
	Copy page					
	Clear page					
	Delete page					
	Page Setup					
	Next page					
	Previous page					
	Master Page					
\checkmark	Page 1					

This menu allows you to add, rename, and delete pages in the report. It also provides commands to navigate to pages. Note: you can also navigate to pages by clicking on the title bar of the report, and then using the PgUp and PgDn keys on the keyboard.

Add Page

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

Rename Page

This command displays a dialog that allows you to rename a page.

Copy Page

This command copies all of the objects on the current page to another page in the report. The Choose Page dialog is displayed to allow you to select the page to copy to.

Choose a Page	
Master Page Page 1	OK Cancel
What page do you want to move/copy to?	

Clear Page

This command removes all of the objects on the page, leaving a blank page.

Delete Page

This command deletes the current page of the report and all of the objects on the page.

Page Setup

This command displays the Page Setup dialog, which determines page size and margins.

Next Page

This command navigates to the next page in the report. The PgDn key is a shortcut for this command.

Previous Page

This command navigates to the previous page in the report. The PgUp key is a shortcut for this command.

Report Zoom

Auto	-
200%	
150%	
100%	
75%	
50%	
Auto	

This drop-down provides options for zooming in or out on the active report. The default setting, **Auto**, scales the report height to fit the report window. Other settings select specific magnifications for the report page.

Active Links



This command toggles the "active link" state for all linked objects on the report. When the button is in a pressed state, the linked histograms and results objects on the report will update whenever the windows to which they are linked change. When the button is up, linked objects do not update automatically and will display information from the last time they updated.

If you want to save a report for a specific experiment and you do not want it to update when you re-open it, toggle the button to the "up" state before saving it.

If you are about to perform a number of actions on a large data file and you do not want objects to update during that process, toggle the active links button to the "up" state before performing the actions. Once you've finished, you can re-enable active links by pressing the button again.

This command is also available in the report context menu.

Snap to Grid

This command is found in the <u>Report Context menu</u>. It controls whether objects on a report are aligned to a user-defined grid when moving or sizing. The grid size is set in the Reporting Preferences pane of the Preferences dialog.

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

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

Insert Histogram Link



This command is found in the <u>Report Context menu</u>. It adds a histogram link object to the current page of a report. Histogram link objects display a picture of a histogram. Because the object is linked to a histogram, it updates whenever the histogram changes.

A dialog is displayed that allows you to select the specific histogram that you want to link the object to.

Edit Histogram Link	
Choose a Data Source and Histogram:	
1:BCell_BM_Tube_003.fcs ~	ОК
1:CD45 AmCyan vs SSC-A	Cancel
2:CD10 PE cy7 vs CD20 APC	Curreer
Caption:	
Show name and gate $\qquad \qquad \qquad$	
Graphic quality:	
Normal quality $\qquad \qquad \qquad$	
Update options:	
Update when histogram changes \checkmark	
✓ Transparent background	

Select the data source from the drop-down list, and then select the histogram from the listbox.

Caption

This option control determines whether the histogram object shows a caption. The caption can contain the histogram name, gate, the name and gate, or nothing at all.

Graphic quality

This option controls the quality of the histogram graphics generated when the object updates on the report. Lower quality settings will produce graphics that require less memory, draw faster, and print faster. Perfect quality requires the most memory and draws the slowest, but produces the best output possible from the program. Experiment with this setting to find a suitable quality for your purposes.

Update options

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to <u>update when a specific</u> <u>condition is met</u>, or not to update at all. Updating can also be controlled by placing the object in a <u>Condition Area</u> 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 <u>Report Windows</u> for details.
- 2. You can use the histogram context menu **Add to Report** command.

Transparent background

Enable this option if you want the object to have a transparent background on the report. This is useful if the object will be on top of another object that you want to show through.

Insert Results Link



This command is found in the <u>Report Context menu</u>. It adds an object linked to a results window to the current page of a report. Results link objects display the textual information shown in the Results window. Because the object is linked to a results window, it updates whenever the results window changes.

A dialog is displayed that allows you to select the specific results window that you want to link the object to.

Edit Results Link				
Choose a Results window:				
1:R2_CD10 PE cy7 vs CD20 APC	ОК			
	Cancel			
	Properties			
Update options:				
Update when results change $\qquad \qquad \qquad$				

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 <u>update when a specific</u> <u>condition is met</u>, or not to update at all. Updating can also be controlled by placing the object in a <u>Condition Area</u> 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 <u>Report Windows</u> for details.
- 2. You can use the results context menu **Add to Report** command.

Insert Keywords



This command is found in the <u>Report Context menu</u>. It adds a keyword object to the current page of a report. Keyword objects display textual information derived from analysis results and FCS keywords in the listmode data files. Keyword objects update when the values for keywords they contain change.

A dialog is displayed that allows you to select the keywords that you want to display.

Edit Keyword Object		
Keyword Groups	Available Keywords	
Results Res	KeywordValueFile keywordsGate keywordsHistogram keywordsPage KeywordsRegion keywordsSystem keywords	OK Cancel Add Properties
DataSource 1 R1 R2 R3 R4 DataSource 2 System keywords	Selected Keywords DS1:\$CYT: [Root Object/Results/File keyword DS1:\$DATE: [Root Object/Results/File keyword DS1:TUBE NAME: [Root Object/Results/File k	 Edit Preview
	Update options:	
	Update when data changes \sim	

Keyword Group

The Keyword Group tree displays groups of keywords. When you select a node in the tree, the keywords for that group are displayed in the Available Keywords list.

Available Keywords

This list shows the keywords that are available for the item selected in the Keyword Group tree. The list is a multiple-selection list; any number of keywords can be selected and added to the report object.

To add keywords from this list to the keywords that you want to display, use one of these methods:

- 1. Drag and drop keywords from this list into the Edit and Format edit box.
- 2. Select the keyword you want to add, and then click the Add button.

Selected Keywords

This edit control displays the keywords that have been selected for display on the report. When the Edit radio button is selected, you can directly edit keyword labels. For example, suppose that you have added the keyword "R1.%Gate" and you want the label to read "T Cells (% of Live Cells)". You would select the text "R1.%Gate" and type "T Cells (% of Live Cells)".

Update options

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to <u>update when a specific</u> <u>condition is met</u>, or not to update at all. Updating can also be controlled by placing the object in a <u>Condition Area</u> 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 <u>Edit Properties for Text</u> 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.

ОК

Click OK to accept the changes to the object and close the dialog.

Cancel

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

Insert Alert



This command is found in the <u>Report Context menu</u>. It adds an alert object to the current page of a report. An alert object is linked to an alert that is defined in <u>Edit Alerts</u>, 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.

Edit Alert Link					
Choose an Alert:					
Alert1	ОК				
	Cancel				
Color if True					
Color if False	Properties				

This dialog also allows you to select two colors for the text of the alert: one color for when the alert is True, and another for when it is False (i.e. the alert condition has not been met). You can use the Properties button to choose a font for the alert object.

Insert Gate Legend



This command is found in the <u>Report Context menu</u>. It adds a gate legend object to the current page of the report. This object relates to the <u>Gate Statistics</u> window for a data source.

۲	File: FCOM_Example.fcs						
	Populatio	n	Events	%Parent			
\times	Ungated		10000	100.00			
\times	G1		7687	76.87			
\times	G2	2	4908	63.84			
\times		G3	3250	66.21			
\times		G4	1582	32.23			
	G5		5092	50.92			
	G6		102	1.02			
	G7		0	0.00			
	G8		3224	32.24			
	G9		0	0.00			
	G10		1556	15.56			
	G11		0	0.00			
	G12		26	0.26			

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

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

Insert Condition Area



This command is found in the <u>Report Context menu</u>. It adds a rectangular "conditional update" object to the report. Anything that falls within the conditional update object will only update if the condition specified is satisfied.

Name	Current Value	Description	ОК
Updating			ÖK
Update Option	Always update objec $ imes $	Choose the update frequency	Cancel
Update Condition	Edit	Specify a condition for updating objects on this object.	
Tiling			Liele
Rows	4 ~	Number of rows for tiling.	Help
Columns	3 ~	Number of columns for tiling.	
Coloring			
Fill Color	Color	Choose a color.	
Border Color	Color	Choose border color.	
Border Size	0 ~	Width of the border. Use -1 to disable border.	
Transparent	🗸 Enable	Enable for a transparent object.	

Updating

Update Option

Select how you want objects placed in this condition area to update. By default, objects update automatically whenever the associated data changes. You can also set the object to <u>update when a specific condition is met</u>, or not to update at all.

Update Condition

Edit the condition for this area to update.

Tiling

A condition area object can automatically tile elements that fall within its boundaries. Tiling is performed on-demand by right-clicking on the condition area object and choosing a tiling option from the context menu.

Rows

Enter the number of rows in the tiling arrangement that you want this area to have.

Columns

Enter the number of columns in the tiling arrangement that you want this area to have.

Coloring

Fill Color

If the object is not set to Transparent, this is the color that is used to fill the area.

Border Color

Select a color for the border of the area.

Border Size

Select a size for the border. Selecting -1 will disable the border.

Transparent

When this option is enabled, the fill color is ignored.

Edit Condition

Edit Condition				
Keywords				
-뾻 DS1:P9BS -뾻 DS1:P9DISPLAY	^	Keyword:	DS1:TUBE NAME	ОК
一器 DS1:P9MS 一器 DS1:SPILL		Current value:	Tube_003	Cancel
뾻 DS1:THRESHOLD		Operator:	string contains	
Gate keywords		Test value:	Tube_003	
G1 BS1.G1.Alias BS1.G1.Equation BS1.G1.Events BS1.G1.PcntParent BS1.G1.PcntTotal	~	2. Choose a log	e a keyword for the condi	
< >				

This dialog is used to define an update condition for some kinds of report objects.

Conditions are based on keyword values. Keyword values can be extracted from the FCS files being analyzed, from regions, from user equations, or from user keywords.

To create a condition

- 1. Select a keyword from the **Keywords tree**. The selected keyword will appear in the **Keyword** edit control and its current value is also displayed.
- 2. Select how the keyword will be evaluated by choosing an **Operator**. Common mathematical operators, as well as string operators, are available in the list.
- 3. Enter or edit the **Test value**.

The test value is compared with the current value using the selected operator. If the condition represented by the expression is TRUE, the condition is met and the update will proceed. If the condition evaluates as FALSE, the update will be suppressed.

Insert MultiGraph Link



This command is found in the <u>Report Context menu</u>. It adds an object linked to a MultiGraph window to the current page of a report. A MultiGraph link object displays the content of a MultiGraph window. Because the object is linked, it updates whenever the MultiGraph window changes.

A dialog is displayed that allows you to select the specific MultiGraph window that you want to link the object to.

Edit MultiGraph Link	
Choose a MultiGraph window:	
1:MultiGraph	ОК
	Cancel
✓ Automatically update when MultiGra	ph changes.

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

You can also drag and drop a MultiGraph window onto the report to create a MultiGraph link object. See Report Windows for details.

Insert Info Window



This command is found in the <u>Report Context menu</u>. It adds an object linked to a histogram's Info window to the current page of a report. An Info window link object displays the content of an Info window. Because the object is linked, it updates whenever the Info window changes.

A dialog is displayed that allows you to select the histogram that owns the Info window.

Select the data source from the drop-down list, and then select the histogram from the listbox. A checkbox is provided that determines controls whether the new object will update automatically as the Info window changes.

Insert Text



This command is found in the <u>Report Context menu</u>. It adds a text object to the report. The object can be moved, sized, and edited.

See Edit Properties for Text for details.

Edit Properties for Text

This dialog allows you to edit the text, font, background, and other properties of a text report object.

Edit Properties for Text				
-⊞ Text	Name Text Font Background Transparent Alignment	Current Value Impressions Font Color Enable Left ~	Description Enter text. Choose a font. Choose a background color Enable for a transparent object. Determines how the text is aligne	OK Cancel Help
	<		>	

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.

ОК

Choose this button to close the dialog and accept the changes.

Cancel

Choose this button to close the dialog and discard any changes you may have made.

Help

Choose this button to display online help for the dialog.

Insert Line



This command is found in the <u>Report Context menu</u>. It adds a line object to the report. The object can be moved, sized, and edited.

See Edit Properties for Line for details.

Edit Properties for Line

This dialog allows you to edit the properties of lines and arrows.

Edit Properties for Line			
Line	me Current Value color Color e weight 0 ad arrow 0 ~ arrow 0 ~	Description Choose a line color Enter line weight. (0 to 10) Choose an arrow size. Choose an arrow size.	OK Cancel Help

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.

ОК

Choose this button to close the dialog and accept the changes.

Cancel

Choose this button to close the dialog and discard any changes you may have made.

Help

Choose this button to display online help for the dialog.

Insert Arrow



This command is found in the <u>Report Context menu</u>. It adds an arrow object to the report. The object can be moved, sized, and edited.

See Edit Properties for Arrow for details.

Edit Properties for Arrow

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

Insert Ellipse



This command is found in the <u>Report Context menu</u>. It adds an ellipse object to the report. The object can be moved, sized, and edited.

Edit Properties for Ellipse

Edit Properties for Ellipse					
-A Ellipse	Name Fill Color Border Color Border Size Transparent	Current Value Color Color 0 ~ Enable	Description Choose a color. Choose border color. Width of the border. Use -: Enable for a transparent ob		OK Cancel Help
	<			>	

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.

ОК

Choose this button to close the dialog and accept the changes.

Cancel

Choose this button to close the dialog and discard any changes you may have made.

Help

Choose this button to display online help for the dialog.

Insert Rectangle



This command is found in the <u>Report Context menu</u>. It adds a rectangle object to the report. The object can be moved, sized, and edited.

Edit Properties for Rectangle

Edit Properties for Rectangle				
Rectangle	Name Fill Color Border Color Border Size Transparent	Current Value Color Color 0 ~ Enable	Description Choose a color. Choose border color. Width of the border. Use -1 to disable Enable for a transparent object.	OK Cancel Help
	<		>	

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.

ОК

Choose this button to close the dialog and accept the changes.

Cancel

Choose this button to close the dialog and discard any changes you may have made.

Help

Choose this button to display online help for the dialog.

Insert 3D Histogram Link

This command is found in the <u>Report Context menu</u>. It adds a link to a 3D window to the current page of a report. 3D Histogram link objects display a picture of a 3D window. Because the object is linked, it updates whenever the 3D window changes.

A dialog is displayed that allows you to select the specific histogram that you want to link the object to. Choose the histogram that owns the 3D window you want to add.

Edit 3D Link Choose a Data Source and 3D Histogram:	
-	
1:BCell_BM_Tube_003.fcs ~	OK
2:CD10 PE cy7 vs CD20 APC	Cancel
Update options:	
Update when histogram changes $\qquad \qquad \lor$	

Select the data source from the drop-down list, and then select the histogram from the listbox. A checkbox is provided that determines controls whether the new object will update automatically as the histogram changes.

Select how you want this object to update. By default, the object updates automatically whenever the associated data changes. You can also set the object to <u>update when a specific</u> <u>condition is met</u>, or not to update at all. Updating can also be controlled by placing the object in a <u>Condition Area</u> object on the report.

You can also drag and drop a 3D window onto the report to create a 3D link object in the report. See <u>Report Windows</u> for details.

Copy (Report Toolbar)



This command is found in the <u>Report Context menu</u>. It copies either the whole report or the selected object to the clipboard.

If an object is selected, it is copied to the clipboard in its native format. Text objects are copied as text. Object containing graphics or a mix of graphics and text are copied as bitmaps.

If no object is selected on the report, the entire report is copied in bitmap format.

Paste (Report Toolbar)



This command is found in the <u>Report Context menu</u>. It will attempt to paste the data into the report.

If the clipboard contains text formatted data, a new text object is inserted and the data is pasted into it.

If the clipboard contains a compatible graphic format, a new graphic object is inserted and the date is pasted into it.

If more than one compatible format is available in the clipboard, the program displays a dialog to allow you to select the format you want to paste.

Bring to Front



This command is found in the <u>Report Context menu</u>. It is enabled when an object is selected in the report. It will move the selected object in front of (i.e. on top of) all other objects in the report.

The command is disabled if no object is selected.

Send to Back



This command is found in the <u>Report Context menu</u>. It is enabled when an object is selected in the report. It will move the selected object behind (i.e. in back of) all other objects in the report.

The command is disabled if no object is selected.

Add Histogram to Report

This command is found in the <u>Report Context menu</u>. It adds the active histogram window to the active report. The histogram object on the report is located in a default position and size.

Add Results to Report

This command is found in the <u>Report Context menu</u>. It adds the active results window to the active report. The results object on the report is located in a default position and size.

Edit User Keywords

🔑 User Keywords

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display. It allows the user to create and modify user-defined keywords.

🔁 Edit User Ke	ywords		×
Name cm_per_inch	Value 2.54	Description enter number of centimeters	ОК
			Open
			Save
			Help
			Properties
<		>	
Add	Delete	Edit	

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 <u>Edit Properties for User Keyword</u> 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

Name	Current Value	Description	ОК
Keyword name	cm_per_inch	Enter a name for the keywords	Cancel
Keyword value	2.54	Edit the keyword value.	Cancer
Prompt	of centimeters per inch	Enter a prompt for the keyword.	
Jpdate option	Update on new file $\ \ \sim$	Choose an update option.	Help
ate option	Update on new file $\ \ \sim$	Choose an update option.	Help

This dialog edits the properties for a user-defined keyword.

Keyword name

Type a name for the keyword. This name is used to identify the keyword when it is used in the database, reports, or user equations.

Keyword value

Enter the value for this keyword. This can be a text or a number. If the keyword is intended to be used in a user equation, the value should be a number.

Prompt

This is the prompt that is displayed when WinList needs to update this keyword. It can be any helpful message.

Update option

Never update: this option suppresses updates for the keyword. The value will remain unchanged.

Update on new file: this option causes WinList to prompt for a new keyword value whenever a new file is opened.

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

See also:

Edit User Keywords

Edit Equations

A>B User Equations

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display. User equations are used to compute new statistics that WinList does not otherwise compute. Any numeric information that WinList stores can be used in mathematical expression, and user-defined information can be included too.

🔁 Edit Equat	tions	×
Name Equation1	Equation [DS1.R1.Region]*1.59	ОК
		Open
		Save
		Help
		Properties
<	>	
Add	Delete Edit	

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

Equations can be saved to disk and opened in other protocols, so that complex expression do not have to be re-entered manually.

Edit Equation

WinList equations can be used to compute new values based on other information in the analysis setup. Regions, gates, keywords, and built-in functions are available to the equation editor. Parentheses allow grouping of terms in the expression.

This dialog is designed to make it easy to create your own equations. The layout is like a calculator, with the addition of a number of special function buttons on the right.

Edit Equatior	۱				
Label:	Equati	on1			
Equation:	[DS1.F	R1.Regi	ion]*1.5	59/100	o
	7	8	9	1	Region Stat
	4	5	6	*	Gate Stat
	1	2	3	_	FCS Keyword
	0		=	+	User Keyword
		Back	space		f(x) choose ~
			ОК		Cancel

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.

ОК

Click OK to save the changes to the equation.

Cancel

Click Cancel to discard changes.

Edit Alerts

🔺 Alerts

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display. It allows you to define and edit alert conditions that WinList will monitor. Alerts are defined by selecting a region statistic or other keyword from a list, choosing a logical operator, and entering a test value or another keyword. An Alert can compare keywords, statistics, and constant values. For example, an alert can test to see if the number of events in region R1 is less than events in R2. Instead of comparing with R2, it could compare with a constant value, like 250.

WinList can database alert conditions when it batch processes files, so that you can keep a record of any results that trigger the alert. In addition, you can choose to have WinList pause and display an alert dialog box when an alert triggers.

Edit Alerts							
Alert Name:	Ly	mphs		ОК			
Applies to:	1:E	Cell_BM_Tube_003	.fcs			~	Cancel
Condition:	DS	1.R1.%Gate		· · · 60			
Alert Keyword:	Ale	ert1					
Alert Prompt:	Wa	arning		Show aler	t prompt.		
Alert Appli	es to	Operand 1	Logic	Operand 2	Prompt	Ke	
Lymp DS 1		DS1.R1.%Gate	<	60	Yes	Ale	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 left-hand value, a logical operator, and a right-hand value. The left- and right-hand values can be region statistics, keywords, or constant values that you enter. Click the ... button next to a condition field to select a keyword from a list. Choose a logical operator with the dropdown list in the middle of the condition. And again, click the ... button to select a keyword for the right-hand value. If the condition described by the expression is true during analysis, WinList will trigger the alert.

Alert Keyword

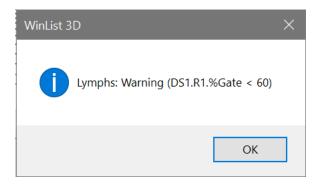
This is a user-defined keyword that WinList will use to store the alert in a database.

Alert Text

This text will be used as the value for the alert keyword if the alert is triggered.

Show alert prompt

Enable this field if you want WinList to pause and display a warning dialog whenever the alert condition is triggered.



Alert Listbox

This list contains all alerts currently defined. To edit an alert, select it in the list and then edit its fields.

New

Click this button to create a new alert condition. This adds an entry to the alert listbox and selects it for editing.

Delete

This command deletes the currently selected alert in the alert listbox.

Open

This command displays a File Open dialog to select a previously saved set of alerts. When an alert file is selected and opened, the alerts in the file replace any that were previously displayed in the alert listbox.

Save

This command displays the File Save dialog, allow you to save the currently defined alerts to a file on disk.

ОК

Close the dialog and accept the changes.

Cancel

Close the dialog and discard the changes.

Replay

🔷 Replay 🝷

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars. It provides several ways to re-read data into data source windows. Use these options to do the following:

- Trigger the saving of a database record. See <u>Databasing with WinList</u> 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 <u>Edit 2P</u> <u>Graphics</u> and <u>Edit 3P Graphics</u>.

- Update display and statistics for gating if **Auto Update** in the **Replay menu** is turned off. See also <u>Auto-update</u>.

- Trigger a *remote link* that is set to update on replay. See also <u>Edit Remote</u>.

WinList is normally working with a *packet* of events from the data source, not the entire data source. This allows the instantaneous update of statistics and graphics as you manipulate regions and gates. The packet is only a sample of events from the entire data source, though, and there are times when you want to see all of the events, or statistics relevant to all events. This is when you will use **Replay** options.

See also: <u>Auto-update</u> <u>Replay Active Histogram</u> <u>Replay Active Data Source</u> Replay All Data Sources

Replay All Data Sources

🔷 Replay 🝷

This command replays data for all histograms in all data sources.

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

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

Replay Active Data Source



This command replays data for all histograms in the active data source.

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

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

Replay Active Histogram

🔷 Replay 🝷

This command replays data for the active histogram in the active data source.

The Replay command can be found on the Home tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

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

Enrich Data Source



This command is used to process a data source through a user-selected gate, selecting only the events that are in the gate into the sample packet that WinList uses for analysis. It is most useful for analyzing low-frequency populations (e.g. B-cells).

The Enrich command can be found on the Options tab of the ribbon bar, or on the main toolbar when using menus and toolbars.

When you select the command, a list of the active gates is displayed. Select the gate you want to enrich on. The data file will be re-read, this time selecting on the events "In" the selected gate.

To cancel the enriched analysis, select the option again and choose Ungated from the list.

Auto-update

Auto-update

When this option is checked, Auto-update is enabled. Each time you move a region, the program will update results for the region and associated gates. This is the normal operating mode for WinList.

When this option is *not* checked, you can move regions without triggering the update of results, gates, and histograms. It can be useful when you need to adjust more than one region in a protocol that takes a long time to update. When the option is enabled (checked) again, the program will update any results, gates, and histograms.

Update Now

This command appears in the Replay menu in Toolbar mode. It is displayed on the Options tab of the Ribbon bar. It can be triggered by the shortcut Ctrl-J.

When selected this command tells WinList to reanalyze the current data in the packet and update results and graphics. It can be used after a Replay command to revert WinList to packet-mode operation.

Update Remote Links

This command is found on the Options tab of the ribbon bar, or the Options menu when not using the ribbon bar.

The command updates "remote links" for histograms and results windows if they are set to update **On Command** (see Edit Remote dialog box). It has no effect on other remote links nor on windows which have no remote connections.

This option is useful for remote connections to database programs. It gives the greatest control over when information will be exchanged between a WinList window and some other application. For example, suppose a results window has a remote DDE link with Microsoft Excel to database results. Setting the link to update On Command will allow you to adjust regions, replay the data, even open a new file, without causing the link to update. Once you have the data on the screen the way you want to database it, selecting Update Remote Links will initiate the update process.

Scale to Fit



This command is found on the Home tab as well as the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

Scale to Fit adjusts all data source windows proportionally to fit into the main application window. The command automatically scales the histograms of each data source to fit the new space of the data source, too.

You can click the down-arrow on the button to select Scale to Fit for histogram windows in the current data source.

Tile

Tile					

This command is found on the Home tab as well as the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

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

You can click the down-arrow on the button to select Scale to Fit for histogram windows in the current data source.

Vertical



This command is found on the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

Select **Vertical** to arrange data source windows vertically, in a single column. Histograms contained in the data sources are automatically rearranged in a tiled format.

Horizontal



This command is found on the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

Select **Horizontal** to arrange data source windows horizontally, in a single row. Histograms contained in the data sources are automatically rearranged in a tiled format.

Close All



This command is found on the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

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

Show All



This command is found on the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

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

Hide All



This command is found on the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

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

Next - Previous Data Source



This command is found on the View tab of the ribbon bar. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the Window menu.

These commands are useful when you have more than one data source window open. It allows you to navigate the data source windows easily.

Print Screen (Ctrl Shift P)



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

Print Screen perform a high-speed bitmap screen dump to a graphics printer. The printout will display the visible contents of WinList's window.

Print Report



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu. It is also found on the toolbar for the active Report window.

Use the **<u>Printer Setup</u>** option in the File menu to set print options.

Several options in the **Preferences** dialog affect the print report option. They appear in the <u>Print Preferences</u> dialog box and control margins, orientation, and more.

Print MultiGraph (Shift P)



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command draws the current MultiGraph to the printer. The print margins set in <u>Print</u> <u>Preferences</u> are used and a border is placed around the MultiGraph. The <u>Fast Printing</u> preference controls how MultiGraphs are printed.

If the MultiGraph is in **Tiled** mode and you have specified a fixed number of rows, the page is divided up into columns and rows and each cell will have a fixed size. If there are fewer than *rows X columns* MultiGraph elements, there will be empty space left on the page. If there are greater that *rows X columns* elements, multiple pages will be printed. If Rows is set to zero (0), all elements of the tiled MultiGraph will be scaled to fit on a single page.

All other views of MultiGraphs produce single pages when printed.

Print Overlay



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

Choose this command to print the active overlay window to the current printer. The print margins set in <u>Print Preferences</u> are used and a border is placed around the overlay.

Print Histogram



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

Select this option to print the current histogram to the printer. The print margins set in <u>Print</u> <u>Preferences</u> are used and a border is placed around the histogram.

Print Data Source



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command prints all of the histograms contained on the current page of the active data source window. The arrangement of histograms is scaled to fit the printed page.

Print Results



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

Select this option to print the text of the currently selected results window to the printer.

Printer Setup

This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

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.

Create Batch



This dialog is displayed by choosing the **Batch** button on the Home tab of the ribbon bar. If you are showing toolbar and menu, it is found in the File menu. This function is disabled until there is an FCS file open in WinList.

A batch is a group of listmode files that will be processed through a WinList analysis protocol. The Open Batch Files dialog allows you to select the files you want to analyze, and to assign them to different data source windows. After you select files with this dialog, you can navigate through them with <u>Get Next Batch</u> and <u>Get Previous Batch</u> commands, or use the <u>batch toolbar</u> that appears at the bottom of the main window.

If your protocol has more than one data source window, WinList can automatically associate a file with a particular data source. You can group the files into convenient analysis groups, based on the outline level shown in the Batch list. Use **Level** > and **Level** < to change the indentation level of items in the batch list.

The Create Batch command displays 2 dialogs side-by-side: the Edit Batch dialog and the Add Batch Files dialog.

Edit Batch		Add Batch Files			
Batch List DS File DS1 Case1Tube1.fcs DS2 Case1Tube2.fcs DS1 Case2Tube1.fcs DS2 Case2Tube2.fcs	Add Files Add Files By file name ~ Customize Remove Remove All	Files: Name 1091901.FCS 1091904.FCS 1091905.FCS BCell_BM_Tube_001.fcs BCell_BM_Tube_003.fcs CF5E sample.fcs CHO445.FCS FC500_APC.001 FC500_APC.001 FC0M_Example.fcs Helios1.fcs KINET64.DDE Mix all.fcs SP2+CFP.fcs List Files of Type: All Files (*.*)	Modified ∧ 2006-02-: 2006-02-: 2009-07-(2009-07-(2009-07-(2006-02-: 2006-02-: 2006-02-: 2016-06-(2016-06-(2016-06-(> >	Show files in: NVIDIA Corporation ProDAD Quicken Realtek Realtek Samsung Stegisoft Systhemals TechSmith TrayStatus VecComp 1.0 WinList 3D 10.0 Help Samples Ves2oit File name:	Add Close
Open into: 1: Case1Tube1.fcs Level: <	OK Cancel				

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:

Customize Add by Keywords					
How many files are in a panel or					
What keyword identifies a group of related	SAMPLE ID	Choose			
What keyword identifies a file within a	TUBE NAME	Choose			
How do you want to assign files to data source windows?					
Assign all files to the first data source window.					
O Assign the first file to the first data source, all others to the second datasource.					
• Assign each file to the associated data source window.					
Enter a keyword value for each data source: Use Current Values					
DS1: Tube_001 DS2: Tube_002					
OK Cancel					

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

Create Batch

Edit Batch					
Batch Li DS DS1 DS2 DS1					Add Files Add Files Insert Files By file name ~ Customize Remove Remove All
Open i Level:	nto: 1: Case1Tube1.	fcs	Up	~ Down	OK Cancel

Batch List

This shows the current list of files and data source to which each will be assigned.

To change the target data source, select one or more items in the list and change the selection in the **Open into** dropdown control.

To change the indentation level, select one or more items and use the < and > buttons. To remove items from the list, highlight the items and click **Remove**.

Open into

This drop-down control lists the current data source names. Changing the selection will change the data source for the items selected in the Batch List.

Level >

This option increases the level of all selected batch files by one.

Level <

This option decreases the level of all selected batch files by one.

Up & Down

Select one or more items and click **Up** or **Down** to change the order of items in the Batch List.

Add Files

Insert Files

These buttons are disabled when the **Add Batch Files** dialog is displayed. If you close the Add Batch Files dialog, use **Add Files** or **Insert Files** buttons to display the dialog and select additional files.

ОК

Click this button to save the **Batch List** entries close the dialog.

Cancel

Click this button to cancel changes in the dialog.

Batch toolbar

This toolbar appears when more than one listmode file is selected in the Open dialog. It provides a simple tool for navigating through the batch of files.



As you navigate through the batch, the actions and options in the <u>Edit Batch Options dialog</u> are performed on each file that is opened. This allows you to perform a series of automatic operations just by moving to another batch item, such as acquiring all events, running a macro, or printing. The function of each button is described below.

	Display the Edit Batch Options dialog.
*	Add files to the batch.
	Go to the first file in the batch.
0	Go to the previous file in the batch.
e	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.
\mathbf{O}	Auto-advance through the batch.
0	Stop the auto-advance process.
0	Pause/continue batch processing.

Edit Batch Options

This dialog can be accessed from the batch toolbar by clicking the left-most button. It is also found by clicking the down-arrow on the Batch button on the Home tab of the ribbon bar. It controls the actions that are performed as each file in the batch is read.



Batch files

This option displays the Open Batch Files dialog to select or modify files for the batch.

Batch options

This option selects either **Basic** or **Custom Scripts** mode for batch processing.

In Basic mode, processing options are selected using the checkboxes and other controls shown in this dialog.

In Custom Scripts mode, you can create custom scripts of actions for each "level" in the batch list outline shown in the <u>Open Batch Files</u> 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 <u>Edit</u> <u>Scripts</u> for details.

Custom scripts

In Custom Script mode, use the **Edit** button to view and edit the custom scripts you have created for your batch.

Replay Options

Replay on Open

When enabled, the FCS file will be replayed as soon as it is opened. Replaying a file allows the statistics and graphics to represent the entire data file.

Replay on Advance

This dropdown contains several choices related to replaying the batch processor is about to move to the next file in the batch.

No: No replay action is taken when a new batch file is opened.

Replay active data source: WinList replays the active data source and computes statistics on the full listmode file. This option also triggers any active databases to store a new record.

Replay all data sources: WinList replays all data sources and computes statistics on the full listmode file. This option also triggers any active databases to store a new record.

Macro

Run macro

Enable this option if you want to run a WinList macro after each batch file is opened. If you want to perform detailed tasks that are not directly supported with options in this dialog, use the Run macro and Macro name fields to perform a custom task.

Update remote links

When this option is enabled, WinList will update all Remote connections for Result and Histogram windows.

Macro name

When the Run macro option is enabled, this option allows you to select the macro that you want to run. Click the button, navigate to the folder containing the macro, and select the macro.

Protocol Save

Save protocol

There are several options for saving protocols: **Don't save protocol**, **Save XML protocol**, and **Save protocol bundle**. When one of the saving options is selected, the other properties in the Protocol Save section also need to be set.

Save protocol in data path

Enable this option to store the protocol in the same path as the data file that is being analyzed. When not enabled, the protocol is saved in the path specified by Protocol Path.

Confirm protocol names

Enable this option to be prompted to confirm the protocol name before it is saved by the batch processor.

Protocol name prefix

This field contains text that is used for naming protocols. It can contain a mix of text and keywords; keywords are enclosed in square brackets. The default name prefix is "Protocol_[BATCHINDEX]", which will name a protocol based on the index of the file in the batch list.

Protocol path

Select a path for storing protocols. This path is ignored if **Save protocol in data path** is enabled.

Reports

Save reports

Enable this option to save reports after each batch file is loaded. Reports will be automatically named based on the Report name prefix below.

Save reports in data path

Enable this option to store the reports in the same path as the data file that is being analyzed. When not enabled, the report is saved in the path specified by Report Path.

Confirm report names

Enable this option to confirm and/or edit report names prior to saving.

Report name prefix

Enter text to use as the first part of each report name. This can contain internal and file keywords by enclosing the keyword in square brackets. For example, to name the reports based on FCS file name for the active data source, you might use Report_[FILE], excluding the quotation marks. See <u>Internal Keywords</u> 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.

💥 Edit Scripts		×
Name	Description Batch processing commands	ОК
Eevel 0 script	Batch processing commands	Help
		Properties
Edit		

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.

Edit Level 1 script					×
Name	^	Name	Description	ОК	
 Read File and Paus Pause 		 Read File and Pause Save Reports 	Reads the as Saves all rep	Open	
 Run Macro Replay 		Save Protocol Bundle	Saves all file	Save	
 Replay All Save Database Rec 				Help	
Print Reports				· .	
 Print MultiGraphs Print Screen 				Properties	
 Save Protocol Save Protocol Bund 					
 Open Report Save Reports 	~				
< >		<	>		
		Add Delete	Edit	Replace 🍿	

The left listbox contains a list of all commands that are available. The list on the right side contains the commands that the script will execute.

To add a command to a script, select the command in the list on the left and click **Add**.

To replace a command, select the new command in the list on the left and the command to replace in the list on the right, then click **Replace**.

To change the order of a command in the script, select the command and use the and

buttons.

To edit the properties for a command in the script, select the command and click **Edit** or **Properties**. The <u>Edit Batch Command</u> dialog will be displayed.

See also:

Edit Batch Command Edit Scripts Edit Batch Options

Edit Batch Command

Name Current Value Description ОК Confirm protocol names Shows the Save dialog for each protocol. Enable Cancel Saves protocol in the same folder as the data file. Save in data path Enable Protocol_[BATCHINDE> Enter a prefix to use for naming protocols. Protocol name prefix Protocol path C:\6_color\Horacio\ Choose Folder... Help...

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 <u>Edit Script</u> <u>dialog</u>, or select the item and click the **Properties** button.

See also:

Edit Script Edit Scripts Edit Batch Options

Next Batch Files (Ctrl B)

Note: This function is disabled until there is a data source open in WinList.

Note: The <u>batch toolbar</u> provides another method for moving through batch files. It automatically appears at the bottom of the main window when more than one file is in the batch.

The **Next Batch Files** menu option will open one group of FCS files from the batch list into the data sources. A group of files is defined by the outline **Level** in the **Edit Batch** dialog. Typically, a group represents a panel of files for an analysis setup.

The option **Previous Batch Files** backs up one group of files in the batch list.

Previous Batch Files (Shift B)

Note: This function is disabled until there is a data source open in WinList.

Note: The <u>batch toolbar</u> 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 <u>Next Batch Files</u>.

Databasing with WinList

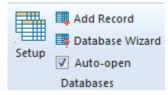
This section introduces the concepts and functions needed to work with WinList's database features. It is more of a discussion than a tutorial, however there are examples to clarify some aspects of the system.

WinList has a simple, yet powerful database engine that allows you to store large amounts of data easily in one or more database files. You can view databases from within the program, manually add, delete, and replace records, and select the fields you want to store in each database. The databases are created in a simple, text format known as tab-delimited ASCII text, which can be read by common database and spreadsheet programs. These features give you a great deal of flexibility in choosing what information to store, where to store it, and how to integrate the information into other database systems.

How do you create a database?

Set up WinList with a protocol before you create or edit a database. This includes opening one or more data source files, selecting the histograms you want to work with, creating regions and gates, and displaying results. You can set up a separate database for each protocol you use, one database for everything you do with the program, or anything in between.

It's important to have an FCS file open and regions defined before you create a database. The information available to the database depends on what you have set up on the screen.



Databases are accessible from the **Edit** tab on the ribbon bar. Click the **Setup** button in the Databases group to display the Edit All Databases dialog.

📑 Edit All databa	ses		×
Name Database1 Database2	Path C:\Users\Ben.WORKGROUP\Documents\WinList 3D\ C:\Users\Ben.WORKGROUP\Documents\WinList 3D\	Active 0 0	OK Open Save Help Properties
Сору	Delete Edit		

There is one database, "Database1", created by the program automatically. This database will not store anything until you tell the program what information you want to save. To do so, you need to select the database in the list and click the Edit button. You can also double-click on the item in the list to do this.

eyword Groups		Keyword-Database N	1ар	
		Field Name	Keyword	-
File keywords	Delete	DS1:\$FIL	DS1:\$FIL	
Gate keywords	Delete	DS1.R1.Alias	DS1.R1.Alias	
Histogram keywords	Up	DS1.R1.Region	DS1.R1.Region	
Region keywords		DS1.R1.MedianX	DS1.R1.MedianX	
System keywords	Down	DS1.R2.Alias	DS1.R2.Alias	
	Down	DS1.R2.Region	DS1.R2.Region	
		DS1.R2.MedianX	DS1.R2.MedianX	
eywords		DS1.R3.Alias	DS1.R3.Alias	
,		DS1.R3.Region	DS1.R3.Region	
Keyword Value		DS1.R3.MedianX	DS1.R3.MedianX	
File keywords	Add	DS1.R13.DsId	DS1.R13.DsId	
Gate keywords		DS1.R13.Alias	DS1.R13.Alias	
Histogram keywords	Replace	DS1.R13.Region	DS1.R13.Region	
Region keywords		DS1.R13.Percent	DS1.R13.Percent	
System keywords	Help	DS1.R13.%Gate	DS1.R13.%Gate	
		<		>
Database Info				
Name: C:\Users\Ben.WORKGRO	UP\Documents\V	VinList 3D\Database1 txt	OK	

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

What can you database?

WinList allows you to database virtually any statistic it generates, as well as keyword information from the files that you analyze. The tree in the top left of the dialog can be expanded to display branches of keywords. To open a branch of the tree, double-click on it with the mouse.

Sometimes it's easier to create a database directly from Results windows that you have already created. The <u>Database Wizard</u> makes that an easy task by automatically adding fields for the selected Results window.

As you select a branch in the tree, the list below the tree will fill with the objects beneath the branch. For example, when you double-click to open the Region keywords branch, the Keywords list below the tree will fill with a folder for each region that has been created. These also appear in the tree because they are branches that can be expanded further. File keywords come from the FCS keywords for each data source; System keywords are items like current date, and current time.

Edit Keyword Map					\times
Keyword Groups			Keyword-Database Ma	p	
🖻 R3	~		Field Name	Keyword	^
🖻 R4		Delete	DS1.R15.DsId	DS1.R15.DsId	
- 🖻 R5		Delete	DS1.R15.Alias	DS1.R15.Alias	
- 🖻 R6		Up	DS1.R15.Region	DS1.R15.Region	
R7		00	DS1.R15.Percent	DS1.R15.Percent	
🗀 R8 🔁 R9		Down	DS1.R15.%Gate	DS1.R15.%Gate	
DataSource 2		Down	DS1.R15.geoMeanX	DS1.R15.geoMeanX	
System keywords	~		DS1.R15.geoMeanY	DS1.R15.geoMeanY	
			DS1.R16.DsId	DS1.R16.DsId	
Keywords			DS1.R16.Alias	DS1.R16.Alias	
Keyword	Valu 🔨		DS1.R16.Region	DS1.R16.Region	
 DS1.R9.%Gate 	0.28	Add	DS1.R16.Percent	DS1.R16.Percent	
 DS1.R9.Alias 	R9	.	DS1.R16.%Gate	DS1.R16.%Gate	
DS1.R9.File	BCe	Replace	DS1.R16.geoMeanX	DS1.R16.geoMeanX	
DS1.R9.Gated	200		DS1.R16.geoMeanY	DS1.R16.geoMeanY	
 DS1.R9.geoMeanX 	179	Help	DS1.R9.Gated	DS1.R9.Gated	
DS1 R9 deoMeanY	127: *		<		>
-					/
Database Info				ОК	
Name: C:\Users\Ben.W	ORKGROUP	\Documents\W	/inList 3D\Database1.txt	OK	
Fields: 39 Max: u	Inlimited	Records: 63		Cancel	
				Curreer	

When you see an item in the Keywords list that you want to database, select it and click the Add button. One or more fields will be created, depending on what you have selected to add. Here is the important thing to understand about adding items to the database: *everything contained in the selected item will be added to the database.* This means that if you select the File keywords, the Region keywords, and the System keywords list items and click Add, *everything* that WinList can store for the current Protocol will be added to the database.

There is a downside to this simplicity. It is very easy to create a database with much more information than you want or need. In fact, you can create a database that other programs will not read because it has too many fields. In most cases, you probably want to store most of the Region keywords, but *rarely* would you want to store all of the File keywords. File keywords come from the files you are analyzing, and many of them are meaningless to anything except the program that created the files. For example, storing how many bytes each parameter for each event uses in the listmode file is not useful information, in most cases. On the other hand, keywords that identify the sample, experiment, and file name will be useful to database.

Another reason you would not want to store all the File keywords is the limitation that most database and spreadsheet programs impose. Other programs usually will not read a database with more than 255 fields of data. If, for example, your files contained 100 keywords and you set up a protocol with 10 data sources open at once, there would be 1000 keywords available to you in the File keywords branch. Reading such a database is well beyond the capabilities of most other programs. Since it is likely that you *will* want to open WinList databases with other software, avoid creating databases with more than 255 fields.

A better solution, if you need to store more information, is to break the storage into several smaller databases. This is possible because WinList can work with any number of database files simultaneously.

The field names initially assigned when you click the Add button are designed to help you know what keyword has been added. For example, all of the keywords relating to Region R1 are prefixed with "R1:", so you can differentiate "MeanX" for regions R1 and R2 easily.

File keywords are prefixed with the data source id.

Editing fields

You can edit the names for the fields after they are added to the Keyword-Database Map list, if you wish. Click once on the field name to select it. After a brief pause, click slowly again. An edit control will appear around the field name, allowing you to type a new name for the field.

To remove one or more fields from the database, select the fields in the Keyword-Database Map and click the Delete button.

To replace an existing field with a new keyword, select the new field in the Keywords list and the one to replace in the Keyword-Database Map list. Then, click the Replace button. The name of the field will not change, only the keyword used to evaluate the field.

If you need assistance understanding the dialog, click the Help button to display the on-line help.

When you have finished selecting information to store in the database, click the OK button to close the Edit Keyword Map dialog.

What files are created?

When you select keywords to store in a database, WinList creates three or four files on disk to manage the database. The files are created in the WinList application folder by default.

However, you can choose another location by editing the database's properties, discussed below.

First, a file is created to store a "map" of keywords to database fields. The map file name uses the name of the database object and appends ".map" to it. For "Database1", the map file is named "Database1.map".

Next, the program creates or revises the actual database file. This is the tab-delimited, ASCII text file that you may open in other database or spreadsheet programs. The database file name uses the database object name and appends ".txt" to it. This file is readable in virtually any program that can open unformatted text. The first line of the file contains the names of the fields separated by tabs; it is known as the database header or definition. Each subsequent line contains a record of information.

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

Finally, the program creates a text file called a "key" file. This is a text file that explains the database and mapping files. You can open this file in a text editor to review the structure of the database you have created. Its name is based on the database object name, with "key.txt" appended to it.

How do you activate the database?

Once a database has been defined, there are several additional properties you can specify. These properties determine whether the database is active, whether it will prompt you before saving a record, whether or not you want to see the records in a database viewer, and where you want to store the database.

To edit these properties for a database, select the database in the Edit All Databases dialog box and click the Properties button. The Edit Properties for Database dialog will be displayed.

Name	Current Value	Description	ОК
Active	🗸 Enable	Check to use this database.	
Prompting	Enable	Check to confirm saving records.	Cancel
Viewer	✓ Enable	Check to display the viewer window.	
Viewer font size	10 ~	Size of the font for the viewer.	Help
Operations			nop
Choose database	Choose	Select an existing database.	
Change location	Path	Change the location of the database.	
Neutralize pathway	Neutralize	Removes the pathway portion of the database file.	

The properties are enabled or disabled with simple check boxes. Path selection is initiated by clicking the Database Path Folder button.

In order to store records of information in a database, it must be "active". To make a database active, check the Active checkbox in the Edit Properties dialog. Database records are saved when you click the Replay button on the ribbon bar, or when you click the Replay options in the Replay menu. Additionally, you can save a record at any time by choosing Save Current Results from the Edit menu, or by clicking the "+" button on the database viewer.

If the Prompting checkbox is checked, WinList will display a message each time it is about to save a record to the database. You can choose at that time whether or not you want to save the record. If Prompting is disabled, the database will save a record without prompting.

To view the database while you work with WinList, check the Viewer checkbox in the <u>Edit</u> <u>Properties for Database</u> dialog. A database viewer window will be created and displayed. The database viewer can be moved, resized, and scrolled horizontally or vertically. It has a toolbar that provides some additional manual database functions. See the topic <u>Database</u> <u>Viewer</u> for more information.

How do you rename or create additional databases?

It is useful to rename a database so that its name relates to its purpose. For example, if you have an activation study for which you have created a WinList protocol called "Activation", it makes sense to name the database "Activation" as well.

To rename a database, display the Edit All Database dialog box by selecting Edit Databases from the Edit menu. Click once to select the database you want to rename. After a brief pause, click again on the object to display an edit box in the Name column. Type the new name for the database.

The name you type must contain only alphabetic, numeric, or space characters, and must be at least 4 characters shorter than the maximum file name length for the operating system you are using. If a file with the new name already exists, you will be given a chance to overwrite the existing file, use the existing file, or cancel the operation.

When you want to create an additional database, select the first database in the Edit All Databases dialog and click the Copy button. This will create an identical database to the one copied, but will give the database a new name.

Each database has its own map of keywords to fields, and its properties determine whether it is active, prompting, and showing a viewer.

Making the databases open automatically when WinList starts

If you would like to open the database setup each time the program starts, enable the Autoopen check box.

🔽 Auto-open

Links:

Edit Databases Edit Keyword Map Edit Properties for All Databases Edit Properties for Database

Edit Databases

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

The Edit Databases dialog can be displayed by choosing **Setup** on the **Edit** tab of the ribbon bar. If you are using the toolbar interface, it is found in the Edit menu.

📑 Edit All databa	ises		×
Name Database1 Database2	Path C:\Users\Ben.WORKGROUP\Documents\WinList 3D\ C:\Users\Ben.WORKGROUP\Documents\WinList 3D\	Active 0 0	OK Open Save Help Properties
Сору	Delete Edit		

Overview

WinList can have one or more databases defined at any time. The database format is ASCIIformatted, 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 <u>Databasing with WinList</u> tutorial.

Edit

If a Database is selected, this option shows the <u>Edit Keyword Map</u> dialog box. You can also double-click on the Database item to activate this option.

Сору

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 <u>Edit Properties for All</u> <u>Databases</u> dialog box. If a Database is selected, clicking on Properties will show the <u>Edit</u> <u>Properties for Database</u> dialog box.

Help

Show this help topic.

ОК

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.

Edit Properties for All databases	5			
All databases	Name Activate all De-activate all Delete all	Current Value Activate all De-activate all Delete files	Description Activate all databases. De-activate all databases. Delete all database files.	OK Cancel Help

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.

ОК

Close the dialog and accept changes.

Cancel

Since the options in this dialog box are immediate, Cancel is the same as clicking OK.

Help

Show this help topic.

Edit Properties for Database

Purpose

To activate, display, and set other attributes of a database.

The Edit Properties for Database dialog can be displayed by choosing **Edit Databases** from the **Edit** menu, selecting the database you want, then clicking the **Properties** button. If the <u>Database Viewer</u> is displayed, clicking a button on the viewer's toolbar can also access this dialog.

Name	Current Value	Description	ОК
Active	🗸 Enable	Check to use this database.	
Prompting	Enable	Check to confirm saving records.	Cancel
Viewer	✓ Enable	Check to display the viewer window.	
Viewer font size	10 ~	Size of the font for the viewer.	Help
Operations			neip
Choose database	Choose	Select an existing database.	
Change location	Path	Change the location of the database.	
Neutralize pathway	Neutralize	Removes the pathway portion of the database file.	

Overview

Each database can be activated independently in WinList. Additionally, the database can be set for prompting or automatic mode, and a viewer window can be displayed. This dialog also allows you to set the path for the database.

Active

Check this option to activate the database. When the database is active, it will attempt to store a record each time an analysis is performed. Uncheck this option to disable a database; records will not be saved in databases that are not active.

Prompting

Check this option if you want the program to prompt you each time it is ready to save a record. The prompt will allow you to confirm or cancel the saving of the record into each active database. Uncheck this option to store the information without prompting.

Viewer

Check this option to display a <u>Database Viewer</u> for the database. The viewer displays the fields in columns and each row represents a record in the database. Additional options are available on the viewer window.

Viewer font size

Choose a font size to use in the database viewer. Choose a larger font to make the text easier to read, or a smaller font to view more information in the same space.

Choose database

This option allows you to choose an existing database on disk. Click the "Link to" button. A file selection dialog box will appear, allowing you to navigate to the existing database and select it. Linking to an existing database will rename the database in memory to match the one selected on disk, and the associated database map on disk will be loaded as well.

Change location

This option allows you to change the path for the defined database. Click the "Path" button to display a Choose database location dialog. Navigate to the location in which you want to store the database.

If a database of the same name already exists in that folder, you will be offered three choices. First, you can choose to overwrite the database with the one you have defined. Secondly, you can choose to use the database that exists in the new location, replacing the structure you have defined in memory. This is convenient when you have a database already defined that you want to use. Finally, you can choose to cancel the action.

Neutralize pathway

This option is useful when you need to move a database to another installation of WinList. When the button is clicked, WinList copies the database to the application folder. It then empties the internal variable that stores the pathway to the database. This allows the program to open the database from the application folder on any installation.

Save property settings and put away the Edit Properties for Database dialog box.

Cancel

Close the dialog and discard any changes you have made.

Help

Show this help topic.

Edit Keyword Map

Purpose: Select the information you want to save in a database. See <u>Databasing with WinList</u> for more on databases.

The Edit Keyword Map dialog can be displayed by choosing **Setup** from the **Edit** tab on the ribbon bar, selecting the database you want to edit, then clicking the **Edit** button. If the <u>Database Viewer</u> is displayed, clicking a button on the viewer's toolbar can also access this dialog.

eyword Groups			Keyword-Database Ma	n	
-			•	•	
🗀 R3 🗀 R4	^		Field Name	Keyword	^
- R5		Delete	DS1.R15.DsId	DS1.R15.DsId	
- R6			DS1.R15.Alias	DS1.R15.Alias	
- R7		Up	DS1.R15.Region	DS1.R15.Region	
- R8			DS1.R15.Percent	DS1.R15.Percent	
		Down	DS1.R15.%Gate	DS1.R15.%Gate	
DataSource 2		20111	DS1.R15.geoMeanX	DS1.R15.geoMeanX	
System keywords	$\mathbf{\mathbf{v}}$		DS1.R15.geoMeanY	DS1.R15.geoMeanY	
			DS1.R16.DsId	DS1.R16.DsId	
(eywords			DS1.R16.Alias	DS1.R16.Alias	
Keyword	Valu 🔨		DS1.R16.Region	DS1.R16.Region	
 DS1.R9.%Gate 	0.28	Add	DS1.R16.Percent	DS1.R16.Percent	
 DS1.R9.Alias 	R9		DS1.R16.%Gate	DS1.R16.%Gate	
DS1.R9.File	BCe	Replace	DS1.R16.geoMeanX	DS1.R16.geoMeanX	
 DS1.R9.Gated 	200		DS1.R16.geoMeanY	DS1.R16.geoMeanY	
 DS1.R9.geoMeanX 	179	Help	DS1.R9.Gated	DS1.R9.Gated	
• DS1 R9 deoMeanY	127: 🗡	•			_ `
<	>		<		>
Database Info					
Name: C:\Users\Ben.W	ORKGROUP	\Documents\V	/inList 3D\Database1.txt	ОК	
Fields: 39 Max: (unlimited	Records: 63			

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 <u>Databasing with WinList</u> 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.

ОК

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 <u>Edit Properties for Databasen</u> dialog, enable the viewer and click OK.

🔳 D	atabase Viewer	for C:\Users\Ben.W	'ORK — 🗆	×
+ -	e 🗊 🕐 👫			
ld	DS1:\$FIL	DS1.R1.Alias	DS1.R1.Region	DS1.I 🔨
55	100Events			
56	20130308	R1	0	
57	20130308	R1	0	
58	20140328	R1	3771	
59	BL000050	Lymphs	53395	
60	BL000050	Lymphs	53395	
61	RI 000050	Lymnhs	53395	×
<				>:

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.

+ -	0	01		
		 	the second s	

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 <u>Databasing with WinList</u> for more on databases.

Auto-open databases

🗸 Auto-open

When this option is selected, WinList saves the database configuration when you exit the program, and automatically reopens the database when the program is started.

With this option checked, databases and database viewers are automatically opened and restored to the state they were in when the program was last used.

If you uncheck this option, you will need to use the <u>Edit Databases</u> command to open the databases that you want to use with the program.

Add Record (Ctrl D)

This command allows you to manually trigger any open, active databases to save the current results as a new record. Normally, database records are saved when you click the Replay button or use one of the options in the Replay menu.

The command is found on the **Edit** tab of the ribbon bar. Pressing Ctrl D on the keyboard is a shortcut for this command.

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 <u>Copy Special</u>.

Click the title bar of the window you want to copy before using the command.

Pressing Ctrl -C on the keyboard is a shortcut for the Copy command.

Copy Special

Copy Special

This option displays a dialog box, which allows full control over what is copied to the clipboard and in what format(s) it is copied.

Copy Special					
Name:	CD20 APC vs CD45 AmCyan	Formats			
Copy:	Histogram \vee	Text			
Size:	4 x 4 inches ~	✓ Bitmap ✓ Picture			
DPI:	Screen ~				
Palette:	Use current view $\qquad \qquad \qquad$				
	OK Canc	el			

The name of the object that will be copied is displayed at the top of the dialog.

Сору

This drop down list allows you to choose what type of window to copy. The name of the active object appears just above the drop down list.

Size

This drop down list provides a list of target sizes for the Bitmap or Picture copy. For best results, choose the size that best matches the size of the image that you want to paste.

DPI

DPI, or Dots-Per-Inch, is a measure of graphic resolution. Higher DPI settings produce finer graphic output, and demand more memory of the computer as well. Choose a DPI setting that provides good quality for your use. For example, Screen resolution is usually plenty for in-house documents, but publications may want 1200 DPI for the highest quality printed output.

Palette

WinList supports normal and "inverse mode" palettes for viewing histograms and result windows. This option allows you to copy an object in the current mode or a specific palette mode.

Formats

Text

This format is available for copying Results windows to the clipboard.

Bitmap

Bitmap format is a pixel-by-pixel representation of the selected window, also referred to as a raster image. The dimensions and resolution of the bitmap depends on the settings for Size and DPI.

Picture

This format is only available for histogram windows. Picture format is a vector-based image format (Windows metafile) that can be resized without a loss of image quality. The optimal dimensions and resolution of the picture depends on the settings for Size and DPI.

Show Axis

With this option checked, the histogram axis is enabled in the copy operation. If unchecked, the data area of the histogram is copied, without displaying the axis tics and labels.

Paste (Ctrl V)



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

Note: To paste an object into a Report window, use the Paste button on the report toolbar or press Ctrl-V after activating a report window.

Drag and Drop

WinList supports a number of drag and drop operations that simplify common functions.

Drag and Drop Object onto Reports

Many objects in WinList can be dragged onto report windows to simplify adding the object to the report.

To drag and drop a histogram, MultiGraph, or results window onto a report, position the mouse over the drag hotspot on the title bar, press and hold the mouse button, and drag the window onto the report. When you release the mouse button, a linked object will appear on the report.

The drag hotspot is the left corner of the title bar of a window. When you position the mouse over this spot, the cursor will change to indicate the Drag and Drop functionality.

The drag hotspot on the upper left corner of the data source window will drag the entire contents of the window onto the report.

Drag and Drop Files onto WinList

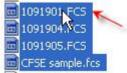
When you drag files from the operating system onto WinList's main window, WinList attempts to determine what kind of file you have dropped and open it appropriately.

You can locate files using Windows Explorer or My Computer on the PC, select them with the mouse, and drop them onto the application. Protocol and macro files (wlx, mac, and wlb) are opened and run. Report files (wlr) and MultiGraphs (mg) are opened in a new report window.

You can drop a compensation file (cmp) onto a data source to automatically apply the compensation settings to it.

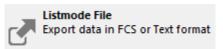
Other file types are treated as FCS listmode files and the program attempts to open them. If you drop files onto an existing data source window, WinList will assume that you want to add these files to the batch list for that data source window. If you drop files onto the main application window, WinList will attempt to open the first one as a new data source window, and then add the remaining files to the batch for this new data source.

Note: When selecting files for drag and drop, the file under the mouse cursor will be the first file added. In most cases, you should make the selection and then press and hold the mouse on the first file to begin the drag and drop operation.



Put the mouse over the *first* file, then drag and drop.

Export Listmode



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

Use this command to create a new listmode file based on the current file. A gate can be applied to restrict the events in the exported data file. You can save original parameters as well as calculated parameters, either compensated or uncompensated. Output formats include FCS or text file format.

When the **Export Listmode** command is selected, a dialog box is displayed.

Export Listmode	
Parameters to save	Output Options
FSC-A FSC-H	Uncompensated linear \sim
SSC-A CD58 FITC	FCS format
CD38 PE CD34 PerCP	Ungated ~
CD10 PE cy7 CD20 APC APC-Cy7-A CD19 V450 CD45 AmCyan Time	 Save all events Sample 20000 events
	Include gate hierarchy ID
	OK Cancel

The **Parameters to save** list box shows the parameters available to save. Select parameters in this list that you want in the new listmode file.

The **Output Options** group includes several controls the determine how the data is written into the new file.

Three types of data can be saved: **Uncompensated linear**, **Compensated linear**, or **Compensated log**. In most cases, Uncompensated linear format is the best choice. With this option, compensation information is stored in keywords so that other software can read the new file and apply compensation dynamically. If you need to read the new file into software that does not perform compensation, use Compensated linear format. In a few rare cases, you might use Compensated log format to save the log-transformed compensated data.

The file can be saved as **FCS format** or **Text format**. FCS format is Flow Cytometry Standard format, compatible with most flow cytometry software programs. Text format is **tab-delimited text**, useful for exporting to database and spreadsheet programs.

You can save **ungated** data or apply one of the **active gate**s to the data as it is being saved.

The program can **save all events** from the source file, or **sample** a specified number of events. Sampling will divide the number of events in the source file by the number of events you want to sample, and use that value to select events to write into the new file.

Enable the **Include gate hierarchy ID** option to store gate identification as a parameter. The gate's ID becomes another parameter in the output file.

After making the selections for parameters and output options, press **OK**. When the file save dialog box appears, type the file name in the edit box and click **Save**. Use the **Cancel** button close the dialog without saving.

Export Merge Files

Merge Files Merge FCS files into one output file

This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

Use this command to create a new listmode file that is a concatenation of a set of FCS files. A gate can be applied to restrict the events in the exported data file. You can save original parameters as well as calculated parameters, either compensated or uncompensated. Output formats include FCS or text file format.

This option does *not* create missing parameters or synthesize data to fill in missing parameters. It concatenates files into one, combined, new file. You can include all the events from all files, or sample a number of events from each.

Merge Files		
Parameters to save FSC-A FSC-H SSC-A CD58 FITC CD38 PE CD34 PerCP CD10 PE cy7 CD20 APC APC-Cy7-A CD19 V450 CD45 AmCyan Time	Output Options Uncompensated linear FCS format Ungated Save all events Sample 20000 events per file Include gate hierarchy ID Include merge file ID Verify parameters match	Files to Merge BCell_BM_Tube_001.fcs BCell_BM_Tube_002.fcs BCell_BM_Tube_003.fcs
	OK Cancel	Add Remove Up Down

When the **Export Merge Files** command is selected, a dialog box is displayed.

Many of these options are the same as those in the <u>Export Listmode</u> dialog box. See that topic for descriptions.

Additionally, the Merge Files dialog includes checkboxes to **include merge file ID** and to **verify parameters match** across the files in the merge.

The **Add** button displays a File Open dialog to select the files that you want to merge. The **Remove** button will remove selected files from the Files to Merge list. The **Up** and **Down** buttons allow the items in the merge file list to be rearranged.

Once parameters and files are selected and output options are set, click the OK button to initiate the merge.

Export Histogram File



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

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

Navigate to the folder in which you want to save the histogram by double-clicking on items in the tree. Enter a name for the file in the File edit box. By default the name is the same as the name appearing on the histogram's title bar. Click **Save** to store the histogram on disk, or **Cancel** to exit the process.

Only one and two parameter histograms are supported by this option.

See also:

Export Listmode

Export Histogram Graphic



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This menu option allows you to save the active histogram graphic as a file on disk. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, or GIF.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box and choose the graphic format by using the Save as Type drop down menu. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

Export MultiGraph Graphics



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This menu options allows you to save the active MultiGraph as a graphic file on disk. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, or GIF.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box and choose the graphic format by using the Save as Type drop down menu. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

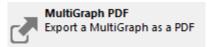
Export Report Graphic

Report Graphic Export a Report in a graphic format.

This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command displays the Save Report Graphic dialog to save the active report as a graphic file. The graphic can be saved in a number of popular graphics formats, including WMF, BMP, JPG, TIF, PNG, and GIF.

Export MultiGraph PDF



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This option saves the active MultiGraph window as a PDF file.

Navigate to the folder in which you want to save the graphic by double-clicking on items in the tree. Enter a name for the file in the File edit box. Click **Save** to save the graphic to disk, or **Cancel** to exit the process.

Export Report PDF



This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

This command displays the Export PDF dialog to save the active report as a PDF file. PDF stands for Portable Document Format, and is a standard format for exchanging information containing graphics and text. It is very useful for creating archival reports or for sharing analysis results with others.

Export Results

This command is found in the File (Application) menu of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the command is found in the File menu.

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

Navigate to the folder in which you want to save the results by double-clicking on items in the tree. Enter a name for the file in the File edit box. By default the name is the name appearing on the results window's title bar. Click **Save** to store the results to disk, or **Cancel** to exit the process.

Macros

File	Home	Insert	Ed	it ۱	/iew	Options	Macro	Help
Show To	olbox			ø	• 🗶 🕻	Clear		Record
🚥 Edit Too	lbox			2	E 📄	Batch		Pause
	(Open !	Save	Edit	/ (Change Path	s Run	
Macro Too	olbox			Macro	File		Recor	rd and Run

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

Macros are command scripts that can be used to automate things that you do repeatedly in WinList. They can be "recorded" as you perform a set of tasks, then saved to disk so that they can be "played" later. You can also directly edit macros to create customized processes.

All macro-related commands are found on the **Macro** tab of the ribbon bar.

Record

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

To begin recording a macro, click the **Record** checkbox on the Macro tab. A check mark will appear indicating that WinList is recording. In Record mode, the WinList functions you perform are recorded. You can record an entire sequence of operations, then uncheck the Record checkbox to stop recording.

There are a few macro commands that cannot be recorded automatically in Record mode. Any of these commands may be added to a macro using the Edit macro command.

See also Edit Macro Macro Commands

Run (Ctrl U)

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

When the Run option is selected, WinList begins executing commands from the macro stored in memory. The macro commands can be created with the Record, Open macro, or Edit macro options. When running macros, a *Macro Status* dialog box appears on the screen. The recorded macro commands are displayed, and as each command is executed, the list box highlights the current macro command. The Macro Status dialog box can be re-sized and moved to any convenient location on the screen. WinList will remember the size and location you select for the Macro Status dialog and use them the next time a macro runs.

The dialog box contains three buttons: **Pause**, **Continue**, and **Cancel**. Click the **Pause** button to pause the macro. This is useful when you need to adjust something before letting the macro continue. Click **Continue** to resume running the macro.

To cancel the macro while it is running, select the Macro Status **Cancel** button.

Edit Macro

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

This command displays the Edit Macro dialog box used to edit the currently stored set of macros.

Edit Macro []								
Mac	ro Command		Optional Pa	aram	nete	r	Line #: 0	
58	SetActiveObj	ject	Histogram,	1,FS	SC-A	vs SSC-A		
ID	Current N	lacro Commands			Av	ailable Commands		
58	SetActiveOb	ject Histogram,1,FSC-A	vs SSC-A	~	1	SetPath		^
28	HistMessage	HistCommand/273/51	5/0		2	SelectFile		
59	SetProperty	HistogramName,Str,FSC	C-A vs SSC-A		3	PushFileButton		
59	SetProperty	HistogramType,Int,1			4	PutLBAway		
59	SetProperty	XParam,Int,0			5	,		
59	SetProperty	YParam,Int,2			6	ActivateHistogram		
59	SetProperty	ZParam,Int,0			7	ActivateDataSource		
59	SetProperty	XRes,Int,64			8	ActivateResult		
59	SetProperty	YRes,Int,64			9	ActivateMultiGraph		
59	SetProperty	ZRes,Int,64			10	SizeHistogram		
59	SetProperty	HistogramType,Int,1		$\mathbf{\mathbf{v}}$	11	MoveHistogram		$\mathbf{\mathbf{v}}$
	Append	Replace	Delete All				ОК	
	Insert	Delete					Cancel	

Macro Command

This field displays the currently select entry from either the Current Macro Command list box on the left or from the Available Commands list box on the right. WinList does not register any changes made to the command until you press Append, Insert, or Replace. Simply pressing the ENTER key will *not* store the edited command.

Optional Parameter

This field is used for parameters specific to particular macro commands. For details on valid parameters for each command, see <u>Macro Commands</u>. 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.

ОК

The OK push button puts away the Edit Macro dialog box and saves any changes made. To permanently store the changes to disk, use the macro Save As menu selection.

Cancel

The Cancel push button puts away the Edit Macro dialog box without saving any changes that might have been made.

See also: Macro Commands

Macro Commands

Most macro commands are equivalent to mouse operations and menu selections you make. They are stored during macro recording. However, there are a few commands that are only available to you from within the Edit Macro dialog box, e.g. ResetMacroLine, Step, and NoStep.

Note: Currently a maximum of 8000 macro lines is supported.

If you record a complex macro and view it with the Edit Macro dialog box, you will be presented with a list of command lines that may at first be unfamiliar. It is the purpose of this section of the manual to explain what these commands do and what their arguments mean and perhaps give you some tips on when to change them. Some commands such as AppMessage and HistMessage have a format that is really only relevant to Windows and are best created by recording. The numbers associated with the commands shown below are the order in which they were implemented and the order they appear in the Macro Command list box. For the purpose of explaining their functionality, we will arrange them into more meaningful groups. The following are the currently supported macro commands for WinList:

1 SetPath [*.*][D:\winlist\]

2 SelectFile bag101.fcs

The **SetPath** command sets the search wild card for current **File IO** dialog box (e.g. *.*) and sets the pathway to the files of interest (e.g. **D:\winlist**). The square brackets are mandatory because they allow WinList to parse the two sections of the command correctly. Also the terminating "****" in the path is required. The **SelectFile** command generally follows the **SetPath** command if you are recording a **File IO** selection. Its argument is simply the file selected. If the file has no suffix a period must be placed after the name (e.g. **100222.**).

It's important to realize that WinList uses the same **File IO** dialog box for several functions (e.g. **File Data Source selection, Gate selection, Palette selection, Region selections**, etc.); therefore, these commands must be presented to WinList when the **File IO** box is on the screen. The best way of doing this is to use the record macro option. If you do, you will always be assured that these commands are presented to WinList at the proper time. A useful technique in modifying these macro commands is to insert a **Pause** command right after the **SelectFile** command and then delete the **SetPath** and **SelectFile** commands. WinList will stop the macro when it reaches the **Pause** command and then you can manually select the file of interest and then continue on with the macro by pressing the **Continue** button on the **Macro Status** dialog box. It's an easy way of making a macro that is file-specific to be more general. There are, however, more powerful ways of doing this with WinList's Batch features.

3 PushFileButton

No longer implemented.

4 PutLBAway

If you put away the keyword or parameter list box, this command will be recorded. It is usually not necessary because WinList will put it away for you automatically.

5 HistEntry 1 1 2 0 64 H_P1_P2

This command is important and may be one that you want to later modify so let's go through it in some detail. This command is recorded when you are selecting which histograms to automatically create in a Data Source. The first number indicates the type of histogram (**0=single parameter, 1=dual parameter, and 2=three parameter**). The next three numbers (e.g. **1 2 0**) indicate which parameters are selected. In the example, we have a dual parameter histogram and the X-axis will be parameter **1** and the Y-axis will be parameter **2**. The third parameter is not used for dual parameter but is used for three parameter histograms. The next number (e.g. **64**) indicates what resolution to use for the histogram. Finally, the ending text is the name of the histogram (e.g. **H_P1_P2**). After a macro is written, you might want to change the resolutions of some of the histograms. All you need do is to find the **HistEntry** command that corresponds to the histogram to change and then edit the resolution portion of the command. Another typical change might be to add another histogram. Just insert another **HistEntry** command and you're done.

The following group of commands activates the designated window in WinList. WinList uses a double naming technique for finding a particular window. Every window has a numeric id as well as a name. We use both methods for finding a particular Window so that your macro has the best chance of finding the appropriate window even if the name of the Window has changed. The id follows the name in the command arguments. Therefore, in all the Activate commands both the name and id are specified. If WinList can't find the id, it then tries to find the window with the designated name.

6 ActivateHistogram [bag101.fcs][1][H_P1_P2][1]

Activates histogram window id **1** with name **H_P1_P2** in data source id **1** named **bag101.fcs**.

7 ActivateDataSource [bag101.fcs][1]

Activates the data source window that has an id of **1** or a name of **bag101.fcs**. The square brackets are required.

8 ActivateResult [R1 H_P1_P2][1]

Activates the results window that has an id of **1** or a name of **R1 H_P1_P2**. The square brackets are required.

9 ActivateMultiGraph [MGraph-1][1]

Activates the MultiGraph window that has an id of **1** or a name of **MGraph-1**. The square brackets are required.

Whenever a window is sized or moved and macro record is on, one or more of the following macro commands is generated. The format of all the arguments is the same. The first number is the id of the window and the second two numbers are either the position (**Move**) or size (**Size**) of the x and y coordinates of the window. The units are in client units which are pixels relative to the upper left-hand corner of the parent window. The **Size** commands have an additional argument which is **0** if **normal** sizing is taking place, **1** if the window is becoming an **icon**, or **2** if the window is **maximizing**.

10	SizeHistogram	1 367 386 0
11	MoveHistogram	1 346 4
12	SizeDataSource	1 788 444 0
13	MoveDataSource	100
14	SizeResults	1 112 111 0
15	MoveResults	1 4 42
16	SizeMultiGraph	1 459 245 0
17	MoveMultiGraph	1 295 230
18	DialogBox	Button {FileIO_OK}1 0

0

This command is used many times in macros. It enables the macro to set all the types of objects found in dialog boxes. For example, the **Button** part of the argument tells WinList to press a button. The **FileIO_OK** is simply descriptive text for you and is not interpreted by WinList. You do however need to have some description and it must be braced with the curly brackets. The next number tells WinList what button to press. The next two fields are not used for buttons but have meanings for other dialog box objects. The different types of objects supported by this command are **INTEGER, RADIO BUTTON, CHECK BOX, BUTTON, LIST BOX, MLIST BOX, CLIST BOX, and STRING**. It's really not important for you to know all the details for each of these command variants. If you do ever want to modify one, just record a small macro that sets the dialog box the way you want and then examine it.

19 AppMessage CreateFile WM_COMMAND 60 0

This is a very common macro command that issues a Windows message to the WinList application. Any selection of a menu item creates this command. The first part of the argument list is a description of the command invoked (e.g. **CreateFile**). The second argument is a Windows-related parameter that indicates the type of message being sent to Windows and its arguments. The 60, for example, tells Windows that this is menu item 60. We recommend not changing this parameter without first contacting Technical Support.

20 SetPaletteID 4

This command is relevant only when the **Edit Palette** dialog box is on the screen. It sets the palette id that can be edited. Currently WinList supports only 16 colors in its default palette.

The next set of macro commands is only relevant to macro processing. Except for the **Pause** command, all of these macro commands need to be inserted into the set of macro commands either with the **Edit Macro** dialog box or by a text editor.

21 Pause Choose the appropriate file to open

When executed, the **Pause** command temporarily suspends further macro processing and then displays the text for **5** seconds. The macro system will hold in this state until you press the **Continue** button in the **Macro Status** dialog box. Typical uses of **Pause** are to add manual selection capability to a macro or to simply communicate with the user. You can insert **Pauses** into the macro by either selecting the **Pause** menu option in the **Macro** menu or by inserting the **Pause** command from the **Edit Macro** dialog box.

22 Step Turns step debugging on

37 NoStep Turns step debugging off

The **Step** and **NoStep** commands can help you work through complex macros. If you insert a **Step** command in a macro, the macro will stop after each macro command is executed and will wait for you to press the **Continue** button in **Macro Status**. The **NoStep** command simply turns this stepping mode off. A very powerful feature of the **Macro Status** dialog box is that in a paused state, you can highlight another macro command; when **Continue** is pressed the macro will begin running from the selected command. Thus, when you are in **Step** mode you can pick and choose which commands to execute.

27 Remark

The function of this macro command is simply to document the various sections of a macro. The macro processor simply skips this command when it is executing.

29 ResetMacroLine 0

The **ResetMacroLine** command is a powerful macro processing command that should be used very carefully. When this command is executed by the macro processor, the argument is used to reset the macro counter. Thus, if a **0** is in the argument, the macro counter is reset to **0** and the macro processor begins executing the macros starting at the beginning. This cycle will repeat **indefinitely** until you **Pause** or **Abort** using the **Macro Status** dialog box. There is another way for the macro processor to break the infinite cycle set up by ResetMacroLine. If you have recorded the command

19 AppMessage GetNextBatch WM_COMMAND 15 0

by executing the **GetNextBatch** menu option under **File**, WinList will set an internal flag if there are no more batch files that need processing. If the macro processor executes the **ResetMacroLine** and that batch file flag is set, it will continue with the **next** macro command. This construction enables you to create a very powerful macro system. For example,

- 19 AppMessage GetNextBatch WM_COMMAND 15 0
- ... other processing here, analysis, printing, etc.
- 29 ResetMacroLine 0
- ... further processing perhaps.

The above lines enable you to open up a series of batch files from the **Open Batch Files** dialog box and then to run this one macro. The system will analyze all the batch files automatically until there are no more batch files in the queue. The system will then continue through the **ResetMacroLine** command and do some further processing. This is the method we recommend for automating your listmode processing.

The following group of macro commands controls the creation, movement, sizing, and deletion of all regions in WinList. Because of the complexity of the arguments for these commands, we highly suggest you create them by recording. Only under very special circumstances should you attempt to edit them.

23 StartRegion 1/R2/CD4/1/19

When a region is being manipulated, this command is issued first. The first argument (e.g. 1) is the type of region being created (**0=1P range, 1=2P region, 3=First Quad stat, 4=2nd,3rd, and 4th Quad stat**). The next argument is the name of the region (e.g. **R2**). The next argument is the region's alias (see Region List, e.g. **CD4**). The next argument is the region's numeric index (e.g. **1**) and the last argument is the number of points that will be in the region (e.g. **19**). If there is already a region defined (e.g. **R2**), it will be erased from the screen and deleted from memory.

24 AddToRegion 0/24/51

This macro command is issued for each point in the region defined in the **StartRegion** macro command. The first argument (e.g. 0) is the 0th or first point, the next argument is the x value in the parent histogram channel units (e.g. 24) and the last argument is the y value (51).

25 ApplyRegion 1/bag103.fcs/1/H_P1_P2/1

This macro command creates and displays the region. The arguments are the region index (e.g. **1**), the data source name (e.g. **bag103.fcs**), the data source id (e.g. **1**), the histogram name (e.g. **H_P1_P2**), and finally the histogram id (e.g. **1**).

26 RegionNameSize 1/24/55/30/58

This command sets the position and size of the region's name. The arguments are the region index (e.g. **1**), the left corner (e.g. **24**), the bottom left corner (e.g. **55**), the right corner (e.g. **30**), and the upper right corner (e.g. **58**).

The following commands are Windows oriented messages and have rather obscure arguments. It is best that you simply record these and leave it at that. The only argument that may be of importance to you is the descriptive argument. These commands are typically generated when you delete the window using mouse actions.

28	HistMessage	HistCommand/273/1/8624
30	ResultsMessage	DeleteResults/16/0/0
31	DataSourceMessage	DeleteDataSource/16/0/0
32	MultiGraphMessage	DeleteMultiGraph/16/0/0
33	InfoMessage	DeleteInfo/16/0/0

34 ScaleHistogram 1411

When a 1P histogram is re-scaled and we are in record mode, this command will be issued. The argument (e.g. **1411**) is the new maximum Y-scale for the histogram.

35 SetPrintArea 0/0/765/424

This command sets the print area for the report printing options of WinList. The arguments are the upper left-hand x-value (e.g. **0**) and y-value (e.g. **0**) and the lower right-hand X-value (e.g. **765**) and Y-value (e.g. **424**).

36 SetSpot 1/1/416/2/512

The **SetSpot** command sets the current spot to the positions specified in the argument list. The arguments are the histogram type (**0=1P**, **1=2P**; **e.g. 1**), the parameter number (e.g. **1**), the value for this parameter in ADC resolution (e.g. **416**), the next parameter number (e.g. **2**), and its value (e.g. **512**).

38 ModifyUserCounter 1,1

This command changes the value of one of the user-programmable counters by the amount specified as the argument to the command. The first number in the argument specifies which of five counters to modify (1,2,3,4 or 5), and the second part of the argument specifies the amount to change the counter. The counter can be accessed as an internal keyword **[COUNTER***n*], where *n* is a number from 1 to 5 identifying which counter you want to access. The [COUNTERn] keyword can be used for naming results windows, MultiGraphs, and any other places where internal keywords are accessible. The counter will be increased by the amount if the minus sign (-) is specified if no sign is present; it will be decreased by the amount if the minus 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:

Edit Batch	
Batch List	Add Files
DS File DS1 Case1Tube1.fcs DS2 Case1Tube2.fcs DS1 Case2Tube1.fcs	Add Files Insert Files
DS2 Case2Tube2.fcs	By file name
	Customize
	Remove
	Remove All
Open into: 1: Case1Tube1.fcs ~	ОК
Level: < > Up Down	Cancel

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.

4 SetProtoco <Version><maxRegions><maxGates><maxSpots><maxColo 4 I rs>

<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 name="" path=""></file>
53	Open Compensation File	<file name="" path=""></file>
54	Save Equation File	<file name="" path=""></file>
55	Open Equation File	<file name="" path=""></file>
56	Save Cluster File	<file name="" path=""></file>
57	Open Cluster File	<file name="" path=""></file>

Commands 52 through 57 allow compensation, calculated parameters, and cluster definitions to be saved to disk and opened from disk.

58 SetActiveObject type, id, name

This command can be used to activate different objects. Presently, histogram and data source objects are the only supported values for *type*. The values for *id* and *name* represent the object's ID and name, shown in the titlebar. Once activated, you can send other commands and change object properties using the SetProperty and DoCommand commands below.

59 SetProperty property, type, value

This command changes properties for the active object. Presently, histogram objects are the only supported objects. Determine the possible values for *property* by recording a macro that edits the properties of an object, and then looking at the SetProperty lines in the macro. *Type* and *value* should be determined in the same manner.

60 DoCommand command, option

This command can instruct an object to do certain operations. Presently, histogram objects are the only supported objects. The object that receives the DoCommand should be activated with a SetActiveObject command prior to using DoCommand.

Histograms support two commands.

UpdateObject command has no option. It causes a histogram to update and redraw.

EditObject command can be used with no option to display the Edit Histogram dialog box, or it can be used in the form *EditObject, Graphics* to display the Edit Graphics dialog for the histogram. Note, there are no spaces in this form, just a comma to separate EditObject from Graphics.

61 reserved

62 Open Names File <file path name>

Command 62 opens files for the Names and Resolutions dialog box. When saving protocols, a names and resolutions file is automatically saved if you have used the Names and Resolutions dialog to edit parameter names or log decades.

63 Close Report

This command closes the active report file. There are no arguments to the command.

64 Open Report <file path name>

Command 64 opens the specified report file. When saving protocols, reports are automatically saved and a command is added to the protocol to open the report.

65 reserved

66 Open Alerts <file path name>

Command 66 opens the specified alerts file. This command is automatically generated when you save a protocol if you have created alert conditions.

67 reserved

68 Open Acquisition <file path name> Settings

Command 68 opens a file containing the settings in the Acquisition dialog for the Cytometer Plug-in-enabled version of WinList.

69 Set Color Mode <mode>

This command selects a color display mode for the active data source. Currently support values for <mode> are listed below.

Value Meaning

- 0 Color by Gate Color
- 1 Color by Gate Combinations
- 2 Color by Parameter Value
- 3 Color by Gate Hierarchy

70 Open Protocol <file path name>

71 Save Protocol <file path name>

Commands 70 through 71 allow protocols to be saved to disk and opened from disk.

72 Save Protocol Bundle <file path name>

This command is used to save the current setup as a protocol bundle.

73 reserved

74 Open Compensation <file path name> Database

Command 74 opens a compensation database file for use by WinList's compensation database system.

75 Open Palette File <file path name>

This command is used to open a palette file to set the colors used by gates.

Important Macro Tips

When editing macros, save the revised macro frequently under a new name and test the changes you make incrementally. It is much easier to debug your changes if you have only made a few at a time.

Use the Close All Windows menu option at the beginning of a macro if you want to be certain that the macro starts with a clean slate. Even if no other windows are open when you start recording the macro, you can still record the command to close all windows. When the macro is run, the command will be ignored if no other windows are open.

We suggest that when you are creating a complex macro you should break it up into logical sub-units. For example, record a macro that simply arranges the screen to your design specifications. Record another macro that sets up all the regions. When macros are in smaller sub-units, they become more manageable and easier to edit. You can use a word processor to concatenate all the macros together or you can use the multiple macro capability of Open Macro to run all the pieces at one time. The latter is probably the best choice because it gives you the most flexibility.

See also: Edit Macro Toolbox

Open

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

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

Click the **Open** button to read the macro from disk.

Click **Cancel** to cancel the dialog.

Save

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

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

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

Click **Cancel** to cancel the dialog.

Clear

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

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

If the macro was saved to disk, the disk file is not affected by this action.

Macro Batch

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

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

Load Macro							
Path:	Path: C:\VshHelpProjects\WinList\Protocols\						
Macro	o files (*.mac)	~	Batch List Items: 0				
	geGatesMacro.mac listogramMacro.mac		Batch List Controls				
	Open Cancel		Add Delete Delete All Run Batch	I			

Path

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

Macro List

The macro list box contains all the files in the selected pathway. Files are displayed alphabetically. On the right is a vertical scroll bar that can be used to find the files not displayed in the box. Clicking on the up or down arrow in the bar scrolls entries in that direction. To open a particular macro, click the left button of the mouse once on the file in the list box, and then click the Open button. The dialog box will disappear from the screen

shortly after the Open button is pressed. Alternately, double-clicking on a macro name will open the macro, close the dialog box, and run the macro.

Open

Selecting this option opens the macro selected in the macro list box and closes the Open Macro dialog box. Alternately, double-clicking on a macro name in the **Macro List** will open the macro, close the dialog box, and run the macro.

Cancel

The Cancel button closes the Open Macro dialog box without opening a macro.

Batch List

This listbox displays the macros that you have added to the macro batch using the Add button. These macros will run in sequence when the Run Batch button is clicked.

Add

Clicking the Add button copies the highlighted files in the Macro list box into the Batch list box.

Delete

Select this option to remove all files highlighted from the Batch list box.

Delete All

Click this button to clear the macro batch list box.

Run Batch

Clicking the Run Batch button launches, in sequence, each macro listed in the Batch list box. The Open Macro dialog box will be hidden during the batch execution, but a Batch Status dialog box will display the number of macros left in the batch, and allow canceling of the batch.

Change Macro Paths

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

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.

Change Macro Paths								
Use this dialog t	ОК							
1. Use the Choo 2. Click OK to ch	Cancel							
3. To keep the o								
New path:	C:\WinList\Macros	Choose						

4. If the path shown in the New Path edit field is not the path you want to use, click the **Choose** button to select a new path.

5. Click the OK button to change the pathways in the macro to the new path. The program will change all pathways in the macro to the new path.

6. If you want to store these changes to disk, use the Save command in the Macro menu to re-save the file.

Pause Macro

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

This option can be selected while recording a macro to record a Pause command in the macro. After recording a Pause command, a custom message can be defined using the Edit Macro dialog box.

Show Macro Toolbox

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

This command displays or hides the Macro Toolbox. The Macro Toolbox is a popup window with programmable buttons on it. The buttons can be hooked up to macros or protocols files on disk. When a toolbox button is pushed, the associated macro or protocol file is opened, and if it's a macro file, the macro runs. It is especially useful for running macros that automate commonly performed operations.



Once the Macro Toolbox is displayed, you can move it to any convenient location on the screen. Its position will be stored by WinList so that each time you run the program, the toolbox will appear where you left it.

You can control the number of buttons and the macros associated with them by using the <u>Edit Macro Toolbox</u> dialog box.

Edit Macro Toolbox

Macro commands are found on the Macro tab of the <u>ribbon bar</u>. When WinList is configured to use the <u>classic toolbar and menu interface</u>, the commands are found in the Macro menu.

Select this command to program WinList's Macro Toolbox. The Macro Toolbox is a popup window that contains programmable buttons. The buttons can be "hooked up" to macros stored on disk so that when a button is pushed, the associated macro is executed. It is especially useful for running screen setup macros and macros that automate commonly performed operations.

The <u>Show Macro Toolbox</u> 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.

	Macro Toolbo	X					
-Tool Rows Colui		Elements: 6 Set size					OK ancel
Id 1	Name EditHistogra	mMacro.mac	Macro EditH	listograml	Pathway C:\VshHelp	oProject	s\WinLis
1 2 3 4 5 6	ChangeGate EditHistogra Not assigned Not assigned Not assigned	mMacro.mac d d d			atesMacro.n gramMacro.		C:\VshI C:\VshI
		Select macro		Delete	e		

Rows

Enter a number, 1 or greater, to determine the number of rows of buttons you want to display in the Macro Toolbox.

Columns

Enter a number, 1 or greater, to determine the number of columns of buttons you want to display in the Macro Toolbox.

Note: The maximum number of buttons (Rows X Columns) is 100.

Elements

This field displays the number of buttons that will be displayed in the macro toolbox (rows X columns).

Set size

Click this button to change the toolbox rows and columns settings. The number of items in the list box will increase or decrease depending on whether the size is increasing or decreasing.

Id

The ID is the button associated with a macro. Buttons on the toolbox are labeled with the ID.

Name

This edit box displays the name of a macro assigned to the button. You may edit this field, keeping the name's length under 30 characters. The name entered here is displayed in the Macro Toolbox title bar as you move your mouse over the buttons. By default, WinList will use the macro's name on disk as its toolbox name.

Macro

This edit box displays the name of the macro file on disk. It is filled in automatically if you select the macro using the Select Macro button described below.

Pathway

This edit box displays the pathway of the macro file on disk. It is filled in automatically if you select the macro using the Select Macro button described below.

Select macro

Clicking this button will display a file list dialog box. You can select a macro using this dialog box, and the selected macro will be assigned to the currently selected toolbox id in the list box.

Delete

Click this button to remove a macro assignment from the list.

ОК

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.

Edit Log

💡 Logging

This command is found on the Edit tab of the ribbon bar, or in the Edit menu when using the toolbar and menu display.

The Edit Log dialog box allows you to view and set properties for session logging. Logging can be used to help Technical Support resolve problems with your software.

To display the Edit Log dialog with the ribbon bar interface, click **Logging** on the Edit tab. With the menu & toolbar interface, choose **Log** from the Edit menu.

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

See <u>Edit Log</u> and <u>Edit Properties for Log</u> for more information.

Edit Log

This dialog allows you to view the WinList log file, if logging is enabled.

• Edit V	VinList				×
Time	Date	Description	CRC	^	ОК
10:06	18May	<========== NEW LOG FILE CREATED ===	62751		
10:06	18May	<============ START LOGGING ====	63699		Help
10:06	18May	<= APPLICATION STARTING =>	28978		•
10:06	18May	Program: WinList 3D	16483		Properties
10:06	18May	User:	29596		•
10:06	18May	Computer:	34688		
10:06	18May	IP: 192 348 308 309	49404		
10:06	18May	OS: Windows 10 or greater	8566		
10:06	18May	AcqMode: 0	24422		
10:06	18May	AcqDllPath:	37572		
10:06	18May	ApplicationMode: Research-use-only mode	11841		
10:06	18May	Total memory: 4095.88MB	20889		
10:06	18May	Available memory: 3903.59MB	29997		
10:06	18Mav	GetBannerIniInfo: Start	8813	~	

ОК

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

Cancel

Click this button to close the dialog and discard changes.

Properties

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

See also Edit Properties for Log and Logging.

Edit Properties for Log

This dialog allows you to control the properties of the WinList logging function. Logging can be used to create a list of actions that you performed using the program, and can assist Technical Support in trouble-shooting problems. Normally, this option should be disabled.

Edit Properties for V	WinList		
Name Active Logging Level Size limit Full Error Report	Current Value Current Value Enable Basic S MB Enable Enable	Description Activate/de-activate the component Sets the depth of logging. Limits the size of the log file. Generate a full error report on a program crash.	OK Cancel
Path			Help
Use Custom Path Custom Log Path	Enable	Use the Custom Log Path	
Actions			
Clear	Clear	Clear all log entries.	
Save preferences	🗹 Enable	Use these settings each time program starts.	

Active

Check this option to enable the logging function. WinList will create a file named WListLog.txt in the WinList 3D folder under the user's documents. The file will store operations you perform as you work with the program.

Logging Level

Choose between Basic and Debugging. Debugging level includes all the Basic information plus additional logging to help trouble-shoot problems.

Size limit

Select the size limit for the logging file.

Full Error Report

Enable this option to create a more detailed error report for VSH technical support. The error report will also be larger on disk.

Use Custom Path

Enable this option to specify the location where you want log files to be stored.

Custom Log Path

When Use Custom Path is enabled, this property specifies the location of the log files.

Clear

Click this button to clean out the log file.

See also Logging and Edit Log.

Preferences



To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

The **Preferences** command displays a dialog box that is used to set up default values for many options in WinList.

The dialog displays a tree of categories on the left. Click a category item in the tree to view and edit its properties.

Preferences				
Preferences	Name Preferences Open Save Save	Current Value Open Save	Description Choose settings from file Save settings to file Use these settings each time progra	OK Cancel Help
⊢© Reporting	۲		>	

Open

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

Save

Choose this command to save the WinList preferences as a file. This file can be used to configure WinList on different computers to use the same settings.

Save preferences

Enable this option if you have opened a preference file with the Open command above and you want to store the new preferences so that they will be used the next time you start WinList.

Histogram Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to all histogram types.

Preferences	Name	Current Value	Description \land	ОК
Mathematical Histograms	Show Create Histograms dialog	Contraction Enable	Show/hide t	
▲ 1P Graphics 2P Graphics	Show gate expression	✓ Enable	Show/hide t	Cance
3P Graphics Printing	Keep histograms square	🗸 Enable	Maintain squ	
A Fonts	Multiple selection	✓ Enable	Allow histog	Lista
MultiGraphs Regions	Histogram line weight	0 ~	Choose a his	Help
- ⅔ Results	Axis line weight	2 ~	Choose axis	
Miscellaneous	Grid line weight	2 ~	Choose grid	
Data Sources	Histogram border	Small border	✓ Amount of t	
	Graphic Output			
	Graphic size	4 x 4	→ Histogram g	
	Graphic DPI	150 🗠	Dots-per-inc	

Show Create Histograms dialog

When enabled, the Create Histograms dialog is displayed whenever a new data source is created.

Show gate expression

When enabled, the gate expression appears in the title bar of each histogram

Keep histograms square

When enabled, the window arranging commands will always keep histograms windows square.

Multiple selection

When enabled, you can select more than one histogram window by pressing the **Ctrl** key on the keyboard. This can be used for moving and sizing histograms, as well as for drag-and-drop and some graphics properties.

Histogram line weight

Choose a weight of the line used to draw 1P histograms. Sizes are in 1/2 points, (a point is 0.35 mm or 1/72 in).

Axis line weight

Choose a weight of the line used to draw axis borders and tics. Sizes are in 1/2 points, (a point is 0.35 mm or 1/72 in).

Grid line weight

Choose a weight of the line used to grids on histograms. Sizes are in 1/2 points, (a point is 0.35 mm or 1/72 in).

Histogram border

Choose how much space to allow between the graphics and the window border for all histograms.

Graphic Output

Graphic size

The value specifies the virtual size for a histogram window when creating a picture (metafile) of the histogram to copy to the clipboard. When you paste the picture into another application and display it at the specified size, it will look best at the target size you select here. Enter a larger value to reduce the size of fonts in the picture relative to other aspects of the histogram, or a smaller size to increase the fonts.

See also MultiGraph Preferences for setting the target size for images added to MultiGraphs.

Graphic DPI

This property specifies the dots-per-inch, or DPI, used when copying a histogram graphic to the clipboard or saving it to disk. Higher values provide better quality output but require more memory.

Graphic format

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

Colors

Palette mode

This property determines what palette mode is used when histograms are copied, printed, or updated on a report.

Use normal palette uses the normal background and foreground colors, **Use inverse palette** uses the reverse background and foreground colors, and **Use current view** will use same palette mode that is currently displayed.

Copy background color

Enable this property if you want the background color of histograms included when the histogram is copied.

Grid color

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

Save preferences

1P Graphics Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

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

Edit Properties for 1P Graphics				
	Name Resolution Fill histogram Coloring mode options	Current Value 256 ~ Enable	Description Select the default displa Fill histogram with color	OK Cancel
-A Fonts -⊞ MultiGraphs -≌ Regions	Overlay style	Overlay color subset \vee	Mode for Color by Gate	Help
- ⅔ Results - ☞ Transformations Miscellaneous Data Sources 	Save preferences	✓ Enable	Use these settings each	
	<		>	

Resolution

Choose a desired histogram resolution for 1P histograms.

Fill histogram

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

Coloring mode options

Overlay style

This option determines how 1P histograms display in coloring modes **Color by Gate Combinations** and **Color by Gate Hierarchy**.

"No subset overlays" displays a single polygon for the 1P histogram.

"Overlay color subsets" draws additional polygons, one for each color. Each polygon is drawn from the baseline.

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

Save preferences

2P Graphics Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to 2P histograms. Individual histograms can override these settings.

Preferences	Name	Current Value	Descrip 🔨	ОК
Mistograms	Resolution	64 ~	Select t	Cance
- 12 Craphics	Dots and Density Plots			Cance
 ☑ 3P Graphics ☑ Printing 	Dots	🖌 Enable	Draw a	
A Fonts	Color density	Enable	Draw a	Liele
-⊞ MultiGraphs -≌ Regions	Number of dots	10000	Number	Help
Results	Dot size	2 ~	0=Smal	
Miscellaneous	Dot order	Listmode order	✓ Drawinc	
Data Sources B Reporting	Show all dots	Enable	Show d	
	Contours Plots			
	Contours	🗸 Enable	Draw pi	
	Show outlier dots	Enable	Draw de	

Resolution

Choose a desired histogram resolution for 2P histograms. If the chosen value is greater than the resolution of the listmode file, the maximum resolution of the listmode file will be used.

Dots and Density Plots

Dots

To display dots density by default, check this option. When the option is unchecked, dots will not be displayed on the histogram.

Color density

To display color density by default, check this option. When the option is unchecked, color density dots will not be displayed on the histogram.

Number of dots

Enter the number of dots you want to display in the histogram window.

Note: The actual number of dots displayed may be less that the number you enter. If there are fewer events in the data than you specify, or if the *packet size* you specify is less than the number of dots, fewer dots will appear.

In the second case where the packet size is smaller than the number of dots, you can display the full number of dots by replaying data for that histogram, or you can adjust the packet size to store the entire listmode file. See <u>Edit Program Controls</u> for further discussion of *packet size*.

Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

Dot order

When using color event gating, this option controls the order in which dots are drawn. *Listmode order* draws dots in their natural event order based on the listmode acquisition. *Low frequency on top* draws the dots for the smallest populations last so that they can be seen on top of populations that have many events. It is useful for visualizing rare events. *High frequency on top* draws the dots for the largest populations last. It may be used to visually clean up low frequency events.

Show all dots

By checking this option, all events will be displayed in the 2P histogram *after replaying*. See the note for "Number of dots" above for more on showing all dots.

Contour Plots

Contours

To display contours, check this option. Uncheck the option if you do not want contours to display.

Show outlier dots

To display dots that fall outside the first contour, enable this option. NOTE: When this option is enabled, the *only* dots displayed are those that fall outside the lowest or base contour. It effectively disables the Enable Dots option in the Dots and Density graphics section.

Probability interval

This value determines the probability used to compute contours.

Base Contour

This dropdown list selects the level of the lowest, or base, contour. The level is selected as a percentage of events that it will include. The highest percentage, 99%, will include 99% of the events in the histogram.

Line weight

Choose a line weight for the contour border. The value is in points, a unit of 0.35 mm or 1/72 in.

Fill contours

When this option is checked, contours will be filled with colors defined in the Colors section. When unchecked, the contour is unfilled.

Percent Iso3D Translucency

Select the default translucency to use for Iso3D displays. Higher values make the populations more translucent.

Colors for Contours and Color Density

Color mode

For color density and contours, this option determines how colors are assigned to different levels. *Color gradient* mode assigns **Gradient Start color** to the first level and gradually changes colors to **Gradient End color** for the last level. *Rainbow* mode uses colors from WinList's color palette, starting with color 1 and sequentially selecting colors from the palette for each level. If there are more levels than colors, colors are repeated.

Gradient Start color

Choose a color to use as the starting color for color density dots and contours in Color Gradient mode. When you click the color button, the standard color selection dialog box is displayed.

Gradient End color

Choose a color to use as the ending color for color density dots and contours in Color Gradient mode. When you click the color button, the standard color selection dialog box is displayed.

Rainbow colors

Select the number of rainbow colors to use in Rainbow color mode. The default, 5, serves most cases very well.

Rainbow 1 - n

Select a default color for each Rainbow mode level.

Save preferences

3P Graphics Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to 3P histograms. Individual histograms can override these settings.

Preferences	Name	Current Value	Description	ОК
Mistograms	Resolution	32 🗠	Select the default display reso	Cance
- 2P Graphics	Dots			
□ 3P Graphics Printing	Number of dots	5000	Number of events to display.	
A Fonts	Dot size	5 ~	0=Smallest	Lista
聞 MultiGraphs 🕼 Regions	Lines	Enable	Draw lines to base plane.	Help
32 Results	3D			
- 📅 Transformations Miscellaneous Data Sources	3D style	3D Spheres V	Choose a default style for 3D	
Reporting	Save preferences	🗹 Enable	Use these settings each time I	

Resolution

Choose a desired histogram resolution for 3P histograms. If the chosen value is greater than the resolution of the listmode file, the maximum resolution of the listmode file will be used. On PC versions that support the 3D viewer, it is recommended that you choose a value of 32 channels.

Dots

Number

Enter the number of dots you want to display in the histogram window.

Dot size

This option controls how large each event (or dot) will be on the screen and printer. A size of 0 will display the smallest unit on the device. Other sizes are in points, a unit of 0.35 mm or 1/72 in.

Lines

When this option is enabled, gray lines are drawn from each dot to the base plane. When displaying 1000 dots or fewer, this is useful to identify the position of dots.

3D

3D style

Select the default 3D display.

Save preferences

Print Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences control various aspects of printing.

Preferences	Name	Current Value	Description 🔨	ОК
🖉 Histograms	Page Setup			
- 🚣 1P Graphics - 🖾 2P Graphics	Page width	8.50	Width of the page,	Cance
	Page height	11.00	Height of the page,	
A Fonts				Liele
MultiGraphs Regions	Top margin	0.50	Enter number of ind	Help
	Bottom margin	0.50	Enter number of inc	
··· Miscellaneous	Left margin	0.50	Enter number of inc	
Data Sources Reporting	Right margin	0.50	Enter number of inc	
	Other			
	Show Print Setup di	alog 🗌 Enable	Enable to display th	
	Print borders	Enable	Enable to print bord	

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

Font Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences control the fonts that are used by WinList. Each font is selected by clicking the associated button to display the standard font dialog, choosing a font, and clicking OK.

Edit Properties for Fonts				
Edit Properties for Fonts	Name Axis numbers Axis Labels Regions Results MultiGraph Titles Captions Reset Save preferences	Current Value Font Font Font Font Font Font Reset Seset	Description Font used for axis numbers. Font used for axis labels. Font used for regions. Font used for results. Font used for MultiGraph text. Font used for titles when printing. Font used for captions on report obj Restore default values Use these settings each time program	
	<		>	

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

MultiGraph Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences are used by MultiGraph windows.

Preferences	Name	Current Value	Description	ОК		
Market Histograms	Name	MultiGraph	Default name for Mu			
▲ 1P Graphics ▲ 2P Graphics	Labels			Cance		
SP Graphics	Label	[\$SAMPLE]	Default label for eler			
A Fonts	X label position	60	Position as a percent			
MultiGraphs Regions	Y label position	95	Position as a percent	Help		
Results	Label width	40	Width as a percentag			
Miscellaneous	Tiled view options	Tiled view options				
Data Sources	Columns	2	Number of columns			
1 5	Rows	3	Number of rows for			
	Center text	Enable	Centers text element			
	Clip text	Enable	Prevents text from c			
	Keep elements square		Forces elements to h			

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 <u>Internal Keywords</u> 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 highresolution 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

Region Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

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

Edit Properties for Regions				
Edit Properties for Regions	Name General Associate with histograms Line color Line weight Double-click action Highlight Region Mode Increase dot size	Current Value Current Value Color Color Current Color Current Color Current	Description Associate each re Color for regions Line weight for re Choose an action Factor to increase	OK Cancel Help
-⊡ Data Sources -⊡ Reporting	Animate region Labels Show Name Show Alias Default alias	Enable Enable Enable Region Name	Blinks events in t Shows the region Shows the region Use 'Region Name >	

General

Associate with histograms

When this option is enabled, a region becomes associated with the histogram in which it was originally created. If you delete the region, the program remembers the association and will only recreate the region in the same histogram.

With the option disabled, you can delete a region from one histogram and recreate it in another histogram.

Line color

Choose a color to use for region lines.

Line weight

Choose a line weight to use for region lines. The value is in points, a unit of 0.35 mm or 1/72 in.

Double-click action

This option allows you to choose what happens when you double click on a region's label. Options include editing the region or creating a results window.

Highlight Region Mode

Highlight-this-region mode is enabled for a region by using the region's context menu. It will highlight the region's events in all of the dot plot histograms.

Increase dot size

This field is the factor to increase 2P dots by when a region is in "highlight this region" mode.

Animate region

Enable this preference to blink the dots when a region is in "highlight this region" mode.

Labels

Show Name

Enable this option to show the region's name (e.g. "R1") in the label for new regions.

Show Alias

Enable this option to show the region's alias (e.g. "Lymphocytes") in the label for new regions.

Default alias

This option allows you to choose the default alias for regions. The alias can be displayed instead of or in addition to the region's name.

Show statistic

This option allows you to show a statistic in region labels, by default. Choices include **None**, **Percent Gated**, **Percent Total**, and **Region Events**.

Transparent label

Enable this option to allow graphics under the region label show through. With the option disabled (unchecked), the region label is filled with a solid background color.

Region Tools

Restore Selection tool

When this option is enabled, the region selection tool is reselected after you finish working with one of the region creation tools. This behavior is similar to how PowerPoint tools work.

1P region tool

Select the region tool for 1P histograms that you want selected each time WinList starts-up. For 1P regions, the options include the Select tool and the Range tool. These tools are found in the <u>histogram toolbar</u> 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 <u>histogram toolbar</u> and determine what happens when you click the mouse in a 2P histogram.

1P Regions

1P region style

Select a style to use when drawing 1P regions. Region options are available in the 1P Region Context Menu .

2P Regions

Show resizing handles

This option determines whether the resizing handles are automatically shown when a 2P region is activated. Note, you can always show resizing handles for individual regions by using the 2P region context menu.

Show vertices

This option determines whether vertices are shown when a 2P region is activated.

Number of vertices

This option controls the number of vertices that are *displayed* for 2P regions when you enable that option. Choose 0 to display all vertices for the region. Note, this does not affect the actual number of vertices for a region, only the number that are displayed.

NStat Regions

NStat label

This list allows you to choose what to display for the NStat region labels. Choose from Name, Alias, or both. The label appears on the movable label for the NStat. Region options are available in the <u>NStat Context Menu</u>.

Sub region label

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

Save preferences

Results Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to results windows.

Edit Properties for Results				
 Preferences Histograms IP Graphics 2P Graphics 3P Graphics 3P Graphics Printing A Fonts MultiGraphs Regions Results Transformations Miscellaneous Data Sources Reporting 	Name Decimal places Decimal separator Results background color Save preferences	Current Value 2 Color Color Enable	Description Decimal places for res Decimal separator cha Background color to u Use these settings eac	OK Cancel Help

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

Transformation Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

Edit Properties for Transformations				
Preferences Kite Alternative Alternati	Name Scaling	Current Value	Descripti 🔨	OK Cancel
→ IP Graphics → 2P Graphics → 3 P Graphics → Printing	Number of Decades Log-like Transforms	Computed ~	Select the	Curcer
—A Fonts —⊞ MultiGraphs	Transformations Default Log Transform	Edit	Edit save Choose th	Help
- 10 Regions - 32 Results - 33 Transformations	Min Linear Option	VLog ~ Percent of Max Linear ~	Choose h	
Miscellaneous 	Percent of Max Linear Percent of Area	0.10 3.00	Percent u Percent u	
	Negative-to-Positive Ratio	0.20	Desired ra	
	Color for Negatives	Color	Color to ı	
	Save preferences		Use these ×	

This preference section controls what parameters WinList will automatically convert from linear to log, and how that conversion will be performed. These preferences are most important for data files from newer, digital instruments, which store all parameters in linear form.

WinList approaches this problem by making internal calculations to convert parameters from linear to log, and allowing you to set preferences to control which parameters are converted to log. You can override the preferences for individual data files.

Number of Decades

This option determines how many log decades WinList will transform the linear data values into.

Choose *Computed* to allow the program to determine automatically the number of decades. With this option selected, the program computes the number of decades by taking the log of the ADC resolution of the parameter. For example, if the ADC resolution is 262144, the log10(262144) is approximately 5.42 decades.

Choose *4-decades* to force the program to map the linear data into 4-decades. This can change the shape of distributions because the scaling is performed in linear space to map to 10,000 linear units.

Log-like Transforms

Transformation

This command displays the <u>Edit Transformations</u> dialog to edit transformations for all saved parameters.

Default Transform

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

Min Linear Option

Determines how the low value for the axis will be determined: **Percent of Max Linear** or **Percent of Area**

Percent of Max Linear

When Min Linear Option is Percent of Max Linear, this field specifies the percentage to use. This max value is multiplied by this field, and the negative of this is used for the low value of the axis.

Percent of Area

When Min Linear Option is Percent of Area, this field specifies the percentage of the measurement's area to use. The negative of this is used for the low value.

Percent Negative Boundary

This value is used for parameters that have Auto-Adjust enabled for log-like transforms. The value represents the percentage of negative events that will be allowed to be off-scale in the log-like 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.

Color for Negatives

Select a color to use for the negative portion of a HyperLog axis.

Save preferences

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

You can override these settings for individual data files.

See also: Edit Transformations

Understanding Hi-res Data Files

Miscellaneous Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to various WinList objects.

Edit Properties for Miscellaneous				
 Preferences Histograms IP Graphics 2P Graphics 3P Graphics 3P Graphics Printing A Fonts MultiGraphs Regions Results Transformations Miscellaneous Data Sources Reporting 	Name Application User Interface Threading Protocols Auto-Backup protocols Protocol Bundle Preference Database Preference Save Protocol Prompt Save Button V-Comp V-Comp Setups	Current Value Ribbon Bar Ribbon Bar Enable Every 2 minutes Prompt for bundle preferenc Do nothing Prompt to save changes Save Protocol (no dialog) Folder	Descript Select th Use mult How ofte Choose i Select th Choose i Select th C:\Users V-Comp	OK Cancel Help
	-		-	

Application

User Interface

WinList provides two user-interface options: **Toolbar & menu** or **Ribbon Bar**. The default interface is the Ribbon Bar. For details on the differences between these interfaces, see <u>Main</u> <u>Window</u>, <u>Toolbar</u>, and <u>Ribbon Bar</u>.

Threading

Enable this option to improve performance on computers with multi-core processors. Most users can leave this option enabled, and disable the option only if advised by tech support.

Protocols

Auto-Backup protocols

WinList can automatically save a backup of the work you are doing in the program. This option allows you to choose how often to save a backup. The backup files are automatically removed when the program closes normally. If the program does not close normally, the backup files will be detected by the program when it starts up again, and it will offer to recover your work from the backup.

Protocol Bundle Preference

This preference determines what happens when WinList opens a protocol bundle that contains a preference file.

"Don't use bundle preferences" will ignore the preference file that is in the bundle.

"Prompt for bundle preferences" will ask the user whether the preferences from the bundle should be used.

"Use bundle preferences" will use the preference file that is in the bundle without asking.

Database Preference

This preference determines what protocols do to databases when protocols are opened.

"Do nothing" will ignore leave databases unchanged when a protocol is opened..

"Set database properties" will set the properties of databases when a protocol is opened. For example, if "Database1" was active and showing the database viewer when the protocol was saved, the protocol can make sure the database is activated and the viewer is showing.

Save Protocol Prompt

When closing the program, WinList can detect whether there are unsaved changes in your protocol. This option controls whether or not the program alerts you that the protocol has changed.

Save button

The Save Protocol button on the main toolbar can behave like the Save Protocol command, the Save Protocol As command, or the Save Bundle command. Select the behavior for the button with this preference. The program will need to be restarted for the change to take effect.

V-Comp Setups

This property contains the path to V-Comp setup files. Typically, WinList can determine this path correctly without intervention. However, if you store V-Comp setup files in a common location with other users, you may need to set this path explicitly.

V-Comp App

This property points to the V-Comp application, if you have it installed on your computer. It allows WinList to launch V-Comp directly from the <u>Compensation toolbox</u>.

Macro options

Macro speed

This option controls how fast macros and protocols playback. It is the minimum amount of time, in milliseconds, between each line of a macro. The default value is 10 milliseconds.

Macro status

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

Remote Connections

DDE Timeout

This option determines how long WinList waits for a remote script command to complete. The default value is 10000 milliseconds.

On some computers, the operating system waits for the full timeout period before completing. In these cases, experiment with lower settings for the DDE Timeout. On newer hardware, a setting of 1000 typically works fine, and it can save 9 seconds of unnecessary waiting for the transaction to complete.

Save preferences

Data Source Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

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

Preferences	Name	Current Value	De \land	ОК
d Histograms	Next File dialog	Windows file dialog $~\sim~$	Sele	
- 1P Graphics - 🗠 2P Graphics	Show Edit Data Source	Enable	Cor	Cance
□ 3P Graphics Printing	Apply Dither	└─ Enable	If e	
A Fonts	Alias	[FILE]	Thi	Liele
- 聞 MultiGraphs - 🕼 Regions	Parameter Names	Automatic ~	Sele	Help
33 Results	N-Color Compensation			
 Transformations Miscellaneous 	Auto-Compensation	Autocomp if not alre $$	Aut	
 □ Data Sources □ Reporting 	Hardware Compatible Comp	🗸 Enable	Moc	
,	Restrict Auto-Positioning Logic	🗸 Enable	Pert	
	Adjustment Step	0.005	Am	
	Add Crossover Keywords	Enable	Adc	

Next File dialog

This option determines what dialog you see when you use the Next File command to read a new FCS file into a data source. The **Windows file dialog** is the standard file dialog you are familiar with, and this is the default option recommended for most users. The **Quick file dialog** is a simplified dialog that displays faster and has limited choices. It is most useful when files are in the same folder.

Show Edit Data Source dialog

Enable this option if you want the Edit Data Source dialog to be displayed when creating a new data source window with the <u>Open Listmode Data Source</u> 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.

N-Color Compensation

Auto-Compensation

Some instrument manufacturers encode compensation settings directly into the FCS files that you read into WinList. If you want WinList to use the compensation settings that you set up on the instrument, you can enable one of the options provided in this list.

Hardware compatible comp

Enable this option to use the force compensation settings to behave like hardware-based compensation. This makes all values subtractive.

Restrict Auto-Positioning Logic

When enabled, WinList uses a more restricted portion of histograms to determine how to automatically position compensation Trace Lines. For most cases, this option will have little effect on how the program positions the trace lines. In some cases where there is significant signal cross-over, enabling this option can improve the auto-positioning.

Adjustment Step

Set the amount of change associated with the "+" and "-" compensation controls.

Add Crossover Keywords

When enabled, WinList will add keywords to describe the crossover for all measurements involved in compensation. Since this can create a lot of keywords, this option is disabled by default. Only enable it if you need to store the crossover information in a database or add it to reports.

FCS

FCS Version

Select the FCS version to use for FCS files that WinList creates.

Import Keywords from XML File

Import Keywords

This option allows WinList to import additional keyword information from an XML file and add those keywords to the keywords from the current FCS file. The XML file must be named the same as the FCS file, with "**.xml**" appended to it.

There are three options for this:

Don't import keywords:	This option disables import of XML keywords.
Import XML from FCS file path:	With this option selected, WinList will look for an XML file in the same path as the FCS file. For example, if the FCS file's name and path are "C:\Data\PatientFile123.fcs", the program would look for "C:\Data\PatientFile123.fcs.xml" for the keywords.
Import XML from Alternate Path:	With this option selected, WinList will look for an XML file in the path specified by the Alternate path preference below. For example, if the FCS file's name and path are "C:\Data\PatientFile123.fcs" and the Alternate Path is "D:\XML", the program would look for "D:\XML\PatientFile123.fcs.xml" for the keywords.

Alternate Path

This option is only used if **Import XML from Alternate Path** is selected for the **Import Keywords** property above. The property defines where the XML files will be located. Click the Choose button to navigate to a folder.

Multiple Dataset Keywords

First Keyword

Enter the name of a keyword to display when WinList detects an FCS file with more than one dataset in it.

Second Keyword

Enter the name of a keyword to display when WinList detects an FCS file with more than one dataset in it.

Snap Properties

Snap-to-grid

With this option enabled, histograms are aligned to the data source grid when they are moved or sized.

Grid spacing

This option specifies the size of the snapping grid, in pixels.

Grid color

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

Background color

Choose a color for the background of the data source.

Reporting Preferences

To display the dialog, choose **Preferences** from the **File (Application)** menu or the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Options** menu.

These preferences apply to Report windows.

Edit Properties for Reporting				
Preferences Preferences Histograms A. 1P Graphics S 2P Graphics Printing A Fonts MultiGraphs Regions Regions Results Transformations Miscellaneous	Name Snap Properties Snap-to-grid Grid spacing Display Grid Properties Display grid Display grid spacing Properties for Tile Object commar	Current Value Cu	Descrip ^ Enables The sna Enables The dis;	OK Cancel Help
■ Data Sources ■ Reporting	Rows Columns Report Objects Text object size Graphic object size	4 ~ 3 ~ 2 inches ~	Number Number Size of I	

Snap Properties

Snap-to-grid

With this option enabled, objects are aligned to the report grid when they are moved or sized.

Grid spacing

This option specifies the size of the snapping grid, in inches.

Display Grid Properties

Display Grid

Enable this option to show a grid on the report background. The display grid does not affect how objects are aligned automatically, but can be used for visual alignment of objects.

Display grid spacing

This option specifies the size of the display grid, in inches.

Properties for Tile Object command

Rows and Columns

Enter the number of rows and columns that you want to create when the Tile command is selected for a report.

Report Object

Text object size

This setting determines the size of text objects when they are added to a report. Note: Objects can be resized after they are initially created.

Graphic object size

This setting determines the size of graphic objects when they are added to a report. Note: Objects can be resized after they are initially created.

Histogram quality

This option controls the quality of the histogram graphics that are generated when linked histogram objects update on the report. Lower quality settings will produce graphics that require less memory, draw faster, and print faster. Perfect quality requires the most memory and draws the slowest, but produces the best output possible from the program. Experiment with this setting to find a suitable quality for your purposes.

This preference can be overridden for individual histogram objects on a report, using the Edit Histogram Link dialog.

Print quality

This option controls the quality of used when printing reports. Select the resolution that provides the best speed and quality for your use.

Fast Printing

With this option enabled, WinList renders graphics to bitmaps before sending them to the printer. With most printers, this results in faster printing. If your printer seems to print graphics from WinList slowly, compare printing speeds with the option on and off.

Caption

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

Edit Program Controls

This dialog box can be brought to the screen by choosing **Edit Program Controls** in the **Edit** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Edit** menu.

The settings within this dialog box control many of WinList's resources such as **regions**, **gates**, and the **packet size**. The settings have an impact on how much memory the program uses.

Edit Program Controls	5			
Regions:	32	ОК		
Gates:	32	Save		
Color Palette:	32			
Packet Size Limit:	200000	Cancel		
Note: space for regions and gates is adjusted automatically.				

Regions

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

Gates

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

Palette size

This field sets the number of colors in WinList's color palette. The palette is used by gates and other graphical objects.

Packet Size Limit

This field sets the limit on the packet size used for sampling your FCS files. When WinList creates a packet to store events from the FCS file, the packet will be at most Packet Size Limit. If the file contains fewer events than Packet Size Limit, or if memory is limited on the computer, the actual packet size will be less than Packet Size Limit.

You can increase this value to work with more events from your file. Increasing the packet size also increases the amount of memory that may be used by the program, so you may need to experiment to find a value that works well for the computer you are using. Ideally, this value should be set to the number of events in the largest files that you open with WinList. Practically, that may be greater than your computer can support.

If the entire file cannot fit into memory at once, the program can display graphics and results for the entire file at any time by clicking the **Replay** button.

After you change the Packet Size Packet, WinList automatically applies the change to data sources.

Save

Click this button to cause WinList to use the displayed settings each time the program is started.

OK

Click this button to close the dialog and apply any changes to open data sources.

Cancel

Click this button to exit with no changes.

Wildcards and Pathways



This command is found on the **Options** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **File** menu.

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

Wildcards and Pathways					
Files of type:	**		ОК		
File Path:	C:\Program Files (x86)\Verity Soft		Save		
Protocol Path:	C:\VshHelpProjects\WinList\Protoc		Cancel		
Report Path:	C:\temp\				
PDF Path:	C:\Temp\				
MultiGraph Path:	C:\Temp\				
Remember last used pathways					

Files of type

Enter the FCS file specification that WinList will use to display listmode files.

File Path

This edit box displays the pathway where WinList will look for listmode data files. You may manually enter the pathway, or click the "..." button to display a dialog that allows you to pick a folder. The PC version uses a backslash (\) to separate parts of a path.

Protocol Path

This is the default location for WinList protocols, bundles, and many other files that WinList reads and writes. You may manually enter the pathway, or click the "..." button to display a dialog that allows you to pick a folder.

Report Path

This is the default location for report files. You may manually enter the pathway, or click the "..." button to display a dialog that allows you to pick a folder.

PDF Path

This is the default location for PDF report files. You may manually enter the pathway, or click the "..." button to display a dialog that allows you to pick a folder.

MultiGraph Path

This is the default location for MultiGraph files. You may manually enter the pathway, or click the "..." button to display a dialog that allows you to pick a folder.

Remember last used pathway

If this option is checked, the program will automatically save the last location where you opened or saved data files. Uncheck this option if you want WinList to use the pathways you enter in this dialog each time it starts up. Check this option if you want it to use the last pathway you were in each time it starts.

ОК

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.

User Guide

Help commands are found on the **Help** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Help** menu.

Selecting this menu option launches WinList's on-line help system and displays the index to the system. Most of WinList's documentation is available through this system.

Check for Updates

This command is found on the **Help** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Help** menu.

Use this command to check the Verity Software House web site for program updates. It will launch your default browser and display a list of available updates for the version of WinList you are running.

About WinList

This command is found on the **Help** tab of the ribbon bar. In the menu and toolbar interface, the command is in the **Help** menu.

Selecting this option displays WinList's opening banner dialog. This dialog displays information about the version of the program, the registered user, system information, and contact information for Verity Software House.

Research and Compliance Modes

WinList can be set up to run in either a Research-Use-Only mode, or in Compliance mode. There are some differences in how the program operates in each mode.

- Compliance mode depends on operating system user groups to control the level of access to program features. Research mode allows any user to perform any operation.
- Compliance mode displays a login dialog when the WinList is starting up to verify the user and determine the group to which the user belongs.
- Compliance mode enables logging so that there is a record of the activities of each user. Research mode can be run with logging enabled or disabled.
- Compliance mode is designed to support clinical labs and can be part of a compliance plan for the US-FDA's 21 CFR Part 11 regulations. Research mode does not provide compliance with 21 CFR Part 11.
- Both modes use the same intuitive interface and WinList engine.

Setting Up Research Use Only Mode

After initial installation, WinList will automatically be in Research mode. There is no further configuration required.

To revert to Research mode after setting up Compliance mode, the user groups that were created for Compliance mode must be removed. Once the user groups are removed, the program will revert to Research mode.

Setting Up Compliance Mode

Setting up Compliance mode is something that should be performed by IT personnel.

While it is not a complicated task, it requires administrative access rights and a clear understanding of user groups and permissions. It is therefore something that we strongly recommend be performed by the IT team at your institution. The task involves creating several *required* WinList user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode. <u>Click here for details</u>.

21 CFR Part 11 Compliance Matrix

This matrix provides a simple summary of how WinList in Compliance mode helps you with 21 CFR Part 11 compliance. <u>Click here for details</u>.

Setting Up Compliance Mode

Setting up Compliance mode should be performed by your IT team. It requires administrative rights and a clear understanding of your network operating system.

The task involves creating several *required* WinList user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode.

WinList User Groups

There are three (3) user groups for Compliance mode: **WinListAdmins**, **WinListEditors**, and **WinListUsers**. Optionally, you can define an additional user group, **WinListResearchers**, for research users within the Compliance setting who are not restricted by Compliance requirements.

The table below shows the features that group members can access.

Feature	WinListAdmins	WinListEditors	WinListUser	WinListResearchers
Run a batch of files	Yes	Yes	Yes	Yes
Add & remove files from batch	Yes	Yes	Yes	Yes
Open protocols	Yes	Yes	Yes	Yes
Edit databases	Yes	Yes		Yes
Create & edit protocols	Yes	Yes		Yes
Edit logging options	Yes			Yes
Check for program updates	Yes			Yes
Edit preferences	Yes			Yes

Creating User Groups

The program requires at least one of the WinList user groups to be defined in order to run in Compliance mode. WinList user groups can be defined for your computer or for your entire domain using tools provided by the operating system. This is typically done by information technology personnel because it requires administrative rights.

The advantage of defining the user groups at the domain (network) level is that the groups are defined *for all computers* on the domain at the same time. If you define the user groups on an individual computer, those groups are available *only on that computer*.

Below we describe how to create user groups on a Windows server or on a Windows compute as examples. The specific methods you need to use may differ depending on the version of the operating system you are working with.

After you have defined the user groups and added users to them, WinList will detect the groups when it starts up and operate in Compliance mode automatically.

Windows Server User Groups

Follow these steps to define user groups for an entire network or domain. **Note: if you use this technique, you do not need to define user groups on each individual computer.**

- On a domain server, launch the "Active Directory Users and Computers" administrative tool.
- Right click the "Users" folder in the left panel and choose New->Group. The New Group dialog is displayed.

New O	bject - Group 🛛 🗙
Create in: vsh.com/Us	ers
Group name:	
WinListAdmins	
Group name (pre-Windows 2000):	
WinListAdmins	
Group scope	Group type
O Domain local	 Security
 Global 	
O Universal	
	OK Cancel
	OK Cancel

- Type **WinListAdmins** for the group name and click OK.
- Once the group is created, double-click the group in the list to display the Properties for the group.
- Click the **Members** tab and click **Add** to select members for the WinListAdmins group. You should have at least one user in the WinListAdmins group. Members can be added by typing user names, or by using the dialog's search features.

	WinListAdmin Properties ? ×
General Members	Member Of Managed By
Members:	
Name	Active Directory Domain Services Folder
🚴 Ben	vsh.com/Users
Add	Remove
	OK Cancel Apply

- Repeat this process for the **WinListEditors**, **WinListUsers**, and optionally **WinListResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up and is connected to the domain.

Windows Computer User Groups

Follow these steps to define user groups for an individual Windows computer. **Note, if you** already defined user groups for your domain, you do not need to define the groups again for each computer.

- On a Windows 7 computer, right click Computer and choose "Manage". For Windows 8 and newer, right click the Windows button (lower left) and choose "Computer Management". The Computer Management app will display.
- Expand "System Tools" and "Local Users and Groups" in the left panel.
- Right click the "Groups" folder and choose "New Group". The New Group dialog is displayed.

New Group		9	X	J
Group name:	WinListAdmins			
Description:	WinList admininstrators			
Members:				
Sen .				
Add	Remove			
Help	Create	Clo	se	

- Type **WinListAdmins** for the group name and enter a description for the group.
- Click **Add** to select members for the WinListAdmins group. You should have at least one user in the WinListAdmins group. Members can be added by typing user names, or by using the dialog's search features.
- Click **Create** to create the new local user group.
- Repeat this process for the **WinListEditors**, **WinListUsers**, and opytionally **WinListResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up on that computer.

See also: 21 CFR Part 11 Compliance

21 CFR Part 11 Compliance

WinList can be part of your lab's compliance strategy for 21 CFR Part 11 when it is setup and operated in <u>Compliance mode</u>. It has been developed under a quality management system and validated with a suite of quality assurance tests. The table below summarizes how the key elements of Part 11 relate to WinList and its use in Compliance mode settings.

Section	Summary of Rule	Compliance Strategy
11.10	Controls for closed	WinList can be accessed only through the user
11.10 (a)	systems Validation and accuracy.	interface; it is a closed system. WinList uses externally-generated data to validate its results and employs proprietary binary format, static PDF, and/or CRC methods to ensure valid files.
11.10 (b)	Record generation and readability	WinList generates Xml-format protocol files, CRC- checked log files, and static PDF that are human- readable and machine-readable.
11.10 (c)	Record protection and retrieval	Record retention and retrieval plan is managed by the laboratory's file management practices.
11.10 (d)	Authorized access	WinList uses the operating system's user login validation, and user group membership determines permissions within the software.
11.10 (e)	Audit trails	WinList employs a CRC-checked logging system with time stamps, and log files are preserved. Log files cannot be deleted by ordinary means.
11.10 (f)	Operational system checks	WinList enables and disables portions of the UI to enforce permitted sequencing of steps and events.
11.10 (g)	Authority checks	WinList uses the operating system user groups and login validation to assure authorized access.
11.10 (h)	Device checks and validity	WinList performs validation checks on the FCS data files.
11.10 (i)	Education, training and experience	WinList provides a User Guide, technical support, and tutorial videos to assist with the proper, intended use of WinList. Specific on-site training may be arranged.
11.10 (j)	Electronic signatures	This is an individual laboratory requirement beyond the scope of use of WinList.
11.10 (k)	Systems documentation	WinList version releases are accompanied by an updated User Guide. Verity employs document control procedures.
11.30	Controls for open systems	WinList is designed as a closed system.
11.50	Signature manifestations	WinList does not require or offer electronic signatures.
11.70	Signature linking	WinList does not require or offer electronic signatures.
11.100 (a)	Unique electronic signatures	Your laboratory must ensure that electronic signatures are unique to each user on the network.

11.100 (b)	Identity verification	Each laboratory must verify its own employment records and IDs.
11.100 (c)	Signature verification	Each laboratory must verify electronic signature eligibility with the FDA.
11.200 (a)(1)	Two-component signing	WinList does not require or offer electronic signatures.
11.200 (a)(2,3)	Authenticity and falsification	WinList does not require or offer electronic signatures.
11.200 (b)	Biometric signatures	WinList does not utilize biometric IDs.
11.300 (a)	Controls for identification	WinList uses the operating system user groups and login validation to assure unique user identities.
11.300 (b)	Identification aging	The individual laboratory IT structure must control password checks and aging requirements.
11.300 (c)	External ID validation	Not applicable. WinList does not utilize external tokens, cards, RFID, or other physical identity-confirming devices.
11.300 (d)	Unauthorized use	The individual laboratory IT structure must control login authenticity and manage safeguards to prevent unauthorized use.
11.300 (e)	External device testing	Not applicable. WinList does not utilize external tokens, cards, RFID, or other physical identity-confirming devices.

Cytometer Plug-in

Cytometer Plug-in: Overview

This option is only available if you have a Cytometer Plug-in supplied with WinList.

When the Cytometer Plug-in Acquisition plug-in for WinList is installed with the program, WinList adds features that allow you to monitor data, acquire and sort data on an instrument with a supplied Cytometer Plug-in.

Using WinList's powerful gating capabilities, it's fast and easy to create regions and define gates for sort decisions. With a one click, WinList transmits sort gates and N-Color Compensation to the Cytometer Plug-in, enabling extensive sort classifications. Events can even be color-coded in WinList based on how they actually sorted on the device.

Next:

Cytometer Plug-in: Monitoring

Cytometer Plug-in: Overview

This option is only available if you have a Cytometer Plug-in supplied with WinList.

When the Cytometer Plug-in Acquisition plug-in for WinList is installed with the program, WinList adds features that allow you to monitor data, acquire and sort data on an instrument with a supplied Cytometer Plug-in.

Using WinList's powerful gating capabilities, it's fast and easy to create regions and define gates for sort decisions. With a one click, WinList transmits sort gates and N-Color Compensation to the Cytometer Plug-in, enabling extensive sort classifications. Events can even be color-coded in WinList based on how they actually sorted on the device.

Next:

Cytometer Plug-in: Monitoring

Cytometer Plug-in: Monitoring

This option is only available if you have a Cytometer Plug-in supplied with WinList.

WinList is automatically launched by the Cytometer Plug-in software. It establishes a connection with the Cytometer Plug-in and has access to the parameters that are currently configured. Communication flows in both directions, with the Cytometer Plug-in software sending data and commands to WinList, and WinList supplying information about sort gates and compensation. As changes are made on one side, they are communicated to the other.

Creating or Opening a Protocol

When the Cytometer Plug-in software starts up, it displays the Create/Open Protocol dialog. This dialog is used to create new protocols for WinList as well as to open protocols you saved previously.

If you open an existing protocol, WinList will recreate the setup you had with histograms, regions, gates, and reports. Sort gates are remembered and transmitted to the Cytometer Plug-in software. If you are creating a new protocol, define your histograms, regions, and gates as you normally would with WinList. See <u>WinList Basics</u> for an introduction to this.

WinList displays a special Cytometer Plug-in toolbar at the bottom of the data source window, and it begins to monitor data transmitted from the instrument.

			Current rate: Last block:	38657 eps 3995 events
			Process time: Sleep time:	0.2s 198ms
Ÿ 🚾 O O O 🕐 🚠	Monitoring	32974 events/sec		

When monitoring, WinList continually updates the graphics and statistics with the latest data received from the Cytometer Plug-in. The toolbar and the data source title bar provide information about the sample and the number of events processed. WinList is not accumulating the data it receives in monitor mode; the most recent events are stored temporarily and they are replaced when new data is received.

Most of WinList's features are available during monitor mode. You can draw regions, define gates, view statistics, set up N-Color Compensation, and much more.

Next:

Cytometer Plug-in: Acquiring Data

Cytometer Plug-in: Acquiring Data

This option is only available if you have a Cytometer Plug-in supplied with WinList.

Once WinList is monitoring an Cytometer Plug-in and you have a protocol defined, you can acquire data in just a few steps.

To review and edit the properties of acquisition:

1. Click the Acquisition Properties button on the Cytometer Plug-in toolbar at the bottom of the data source window.



2. The <u>Edit Acquisition</u> dialog is displayed. Review and edit the properties for the acquisition, and then click OK.

Edit Acquisition for Cytor	nomeEvalToolDevice		
Name	Current Value	Description	ОК
Stop			Cancel
Stop Gate	Ungated :	Select a gate to use for the stop count.	
Stop Count	10000	Number of events to acquire.	
Save			Help
FCS File Prefix		Prefix for FCS files.	
FCS File Pathway	Choose	C:\Program Files (x86)\Verity Software House\WinList 3[
File Counter	1	Set the starting file counter, used for naming the FCS file	
Event sampling	1	Save every nth event into the FCS file. (1 to 214748364	
Pre-Acquisition			
Prompt Before	✓ Enable	Prompt for user-keywords before starting.	
Post-Acquisition			
After Acquisition	Enter WinList mode & Replar $ \sim $	Choose the post-acquisition mode.	
Prompt After	✓ Enable	Prompt for user-keywords after acquisition.	
Update Report Links	🗸 Enable	Refresh all report links after acquisition.	
Save Reports	Enable	Save reports based on FCS file name.	
Save PDF Reports	Enable	Save reports as PDFs based on FCS file name.	
Print Reports	Enable	Print reports after acquisition.	
Save Protocol Bundle	Enable	Save a protocol bundle based on FCS file name.	
User-Defined Keywords	5		
Keyword 1	\$SMNO	Name of the first user keyword.	
Keyword 1 Description	Enter the specimen labe	Description of the first user keyword.	
Keyword 1 Value	unknown	Default value of the first user keyword.	
Keyword 2	\$SRC	Name of the second user keyword.	
Keyword 2 Description	Enter the source of the	Description of the second user keyword.	
Keyword 2 Value	unknown	Default value of the second user keyword.	
<		>	

To acquire data:

1. Click the Acquire button on the Cytometer Plug-in toolbar.

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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.

Device: Starting Acquisition	
CytonomeEvalToolDevice	
Auto-close this dialog	
Run ID: DfltRun00001 Enter the specimen label (e.g. tube name, well number)	ОК
\$SMNO unknown	Cancel
Enter the source of the specimen (e.g. patient name, cell types)	
\$SRC unknown	

During acquisition, you can use the Pause, Stop, and Restart buttons on the toolbar. See the <u>Toolbar</u> 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.

Dev	vice: Acquisition Complete	
	CytonomeEvalToolDevice	
Auto-close this d	ialog	
Run ID: DfltRun0000	01	•
Enter the specimen	label (e.g. tube name, well number)	OK
\$SMNO	unknown	Cancel
Enter the source of \$SRC	the specimen (e.g. patient name, cell types)	
Acquisition Summary	/	
File name:	DfltRun00001.fcs	Browse
File path:	C:\Program Files (x86)\Verity Software	View Path
File counter:	1	
Stop gate events:	10000	
Total events:	10000	
		.

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

More Info:

Cytometer Plug-in: Sorting

Cytometer Plug-in: Sorting

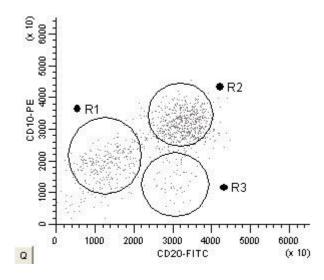
This option is only available if you have a Cytometer Plug-in supplied with WinList.

WinList provides very flexible and powerful sorting control. Sort decisions are made by creating regions and gates on histograms, setting up N-Color Compensation, and using the Edit Sort dialog to transmit the sort decisions to the Cytometer Plug-in.

Once the sort decisions have been sent to the module, they remain in effect until new sorting decisions are sent. If you modify or delete a region involved with sorting, you will be prompted to re-send the sort information to the Cytometer Plug-in.

To create sort decisions:

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



2. Click the Edit Sort button on the Cytometer Plug-in toolbar.

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3. In the <u>Edit Sort dialog</u>, associate the gates you created in step 1 with sort streams on the Cytometer Plug-in. Each gate should be assigned to only one sort stream.

Name	Current Value		Description	Send
L2	G1 : B1	-	Choose a gate for the L2 sort stream.	Cancel
L1	Unassigned	•	Choose a gate for the L1 sort stream.	
W	Unassigned	•	Choose a gate for the W sort stream.	
R1	Unassigned	-	Choose a gate for the R1 sort stream.	
R2	G2: R2	•	Choose a gate for the R2 sort stream.	

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

More Info:

Edit Sort dialog Using Interactive N-Color Compensation Toolbar

Cytometer Plug-in: Toolbar

This option is only available if you have a Cytometer Plug-in supplied with WinList.

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

			Current rate: Last block:	38657 eps 3995 events
			Process time: Sleep time:	0.2s 198ms
Ψ 🔼 🖸 Ο Ο Ο 🚺 🖍 📩	Monitoring	32974 events/sec		

The left portion of the toolbar contains a set of buttons that control monitoring, acquisition, and sorting. These are described in the Toolbar Commands table below.

Status panes indicate the name and associated color of the Cytometer Plug-in module, and information about monitoring, acquisition, and event rates. A progress meter appears during acquisition to indicate the status of the acquisition.

Two LED indicators appear on the right of the toolbar. The first one turns green when a packet of information is received from the Cytometer Plug-in. It turns yellow when a packet is requested and the server does not supply one. The second LED turns green when WinList is processing a packet of data received from the server.

Toolbar Commands

Button	Purpose
Y	WinList mode Stop the connection with the Cytometer Plug-in and enter WinList mode.
1-1	Monitor Connect with the Cytometer Plug-in and request data. Real-time events are displayed. Events are not stored.
0	Acquire Accumulate data from the Cytometer Plug-in and store it in an FCS file on disk. Real-time events are displayed. Events are stored.
0	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.

Cytometer Plug-in: Edit Properties of Acquisition

This option is only available if you have a Cytometer Plug-in supplied with WinList.

This dialog allows you to configure the acquisition characteristics for the Cytometer Plug-in. You can choose preferences for most of these properties in the <u>Cytometer Plug-in</u> <u>Preferences dialog</u>.

Edit Acquisition for Cytor	nomeEvalToolDevice		
Name	Current Value	Description	ОК
Stop			Cancel
Stop Gate	Ungated :	Select a gate to use for the stop count.	Cancer
Stop Count	10000	Number of events to acquire.	
Save			Lista
FCS File Prefix		Prefix for FCS files.	Help
FCS File Pathway	Choose	C:\Program Files (x86)\Verity Software House\WinList 3[
File Counter	1	Set the starting file counter, used for naming the FCS file	
Event sampling	1	Save every nth event into the FCS file. (1 to 214748364	
Pre-Acquisition			
Prompt Before	✓ Enable	Prompt for user-keywords before starting.	
Post-Acquisition			
After Acquisition	Enter WinList mode & Replar $ imes $	Choose the post-acquisition mode.	
Prompt After	✓ Enable	Prompt for user-keywords after acquisition.	
Update Report Links	✓ Enable	Refresh all report links after acquisition.	
Save Reports	Enable	Save reports based on FCS file name.	
Save PDF Reports	Enable	Save reports as PDFs based on FCS file name.	
Print Reports	Enable	Print reports after acquisition.	
Save Protocol Bundle	Enable	Save a protocol bundle based on FCS file name.	
User-Defined Keywords	•		
Keyword 1	\$SMNO	Name of the first user keyword.	
Keyword 1 Description	Enter the specimen labe	Description of the first user keyword.	
Keyword 1 Value	unknown	Default value of the first user keyword.	
Keyword 2	\$SRC	Name of the second user keyword.	
Keyword 2 Description	Enter the source of the	Description of the second user keyword.	
Keyword 2 Value	unknown	Default value of the second user keyword.	
<		>	

Stop

Stop Gate

Select a gate to use for calculating the stop count. If you select a gate, the acquisition finishes when the number of events that satisfy the stop gate reaches the stop count. If no gate is selected, acquisition finishes when the number of events received reaches the stop count.

Stop Count

Set the stop count for acquisition. See comments on Stop Gate above.

Save

FCS File Prefix

Enter an option prefix for the FCS files created during acquisition. This field can be left empty.

FCS File Pathway

Choose the location where FCS files are stored during acquisition.

File Counter

This is a simple counter that increments as files are acquired during a WinList session. You can edit the value to any number you want.

Event sampling

This value determines the rate at which events are sampled into the acquisition output file from the buffers received. The default setting is 1, which means that every event received will be stored. With a setting of 5, WinList would store every 5th event that it receives from the instrument. A setting of 500 would store 1 out of 500 events. This can be useful when running long acquisitions where you do not need to store every event.

Pre-Acquisition

Prompt Before

When this option is enabled, WinList will display a dialog before acquisition. The dialog allows the user to enter keywords for the sample.

Post-Acquisition

After Acquisition

Choose what you want the program to do after you acquire a new file. Options include **Enter WinList mode & Replay** and **Continue Monitoring**.

Prompt After

When this option is enabled, WinList will display a dialog after acquisition that allows the user to enter keywords for the sample.

Update Report Links

When this option is enabled, WinList will automatically update all linked objects on reports when an acquisition finishes.

Save Reports

When this option is enabled, WinList will automatically save any open reports when an acquisition finishes.

Save PDF Reports

When this option is enabled, WinList will automatically save any open reports in PDF format when an acquisition finishes.

Print Reports

When this option is enabled, WinList will print open reports when acquisition finishes.

Save Protocol Bundle

When enabled, WinList will automatically save a protocol bundle for the newly acquired file after each acquisition.

User-Defined Keywords

Keyword 1

This field contains the name of the first user-defined keyword for the FCS file created during acquisition.

Keyword 1 Description

If you edit Keyword 1, it is a good idea to edit this field to describe the keyword that you have entered. This description is displayed to the operator when the system prompts for the keyword value.

Keyword 1 Value

Enter a default value for Keyword 1, or leave the field blank.

Keyword 2

This field contains the name of the second user-defined keyword for the FCS file created during acquisition.

Keyword 2 Description

If you edit Keyword 2, it is a good idea to edit this field to describe the keyword that you have entered. This description is displayed to the operator when the system prompts for the keyword value.

Keyword 2 Value

Enter a default value for Keyword 2, or leave the field blank.

Cytometer Plug-in: Edit Sort

This option is only available if you have a Cytometer Plug-in supplied with WinList.

This dialog allows you to make sorting decisions for the Cytometer Plug-in. No changes are made until the **Send** button is clicked.

Name	or ben-hp_haps1 Current Value		Description	Send
L2	G1 : R1		Choose a gate for the L2 sort stream.	Cancel
L1	Unassigned	•	Choose a gate for the L1 sort stream.	
W	Unassigned		Choose a gate for the W sort stream.	
R1	Unassigned		Choose a gate for the R1 sort stream.	
R2	G2: R2	•	Choose a gate for the R2 sort stream.	

Sort Streams

The top section of the dialog shows each sort stream that is defined on the Cytometer Plugin.

To use a gate for a sort decision, select a gate from the drop-down list for the sort location you want. This will associate the gate expression with the Sort Stream decision.

Sort streams that show "Unassigned" for the gate will not receive any events during the sort.

Other Cells and Empty Drops

The system allows you to capture all cells that are not identified by other gate assignments, as well as droplets that did not contain cells. Any sort destination can be set to "Other Cells", "Empty Drops", or "Other Cells and Empty Drops".

Send

Click this button to transmit the sorting regions, sort gate expressions, and N-Color Compensation to the Cytometer Plug-in 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.

Cytometer Plug-in: Preferences

This option is only available if you have a Cytometer Plug-in supplied with WinList.

This dialog displays settings that control various aspects of the Cytometer Plug-in acquisition system.

Edit Properties for Acquisition Plu	gin				
	Name Acquisition	Current Value	Des ^	ОК	
	Acquisition connection Stop Count	shared_memory 10000	Ente The	Cancel	
- A Fonts	File Naming	File Naming			
⊞ MultiGraphs ™ Regions	FCS File Prefix		Prefi	Help	
	Include Kw1 in Filename	Enable	Autc		
Miscellaneous	Include Kw2 in Filename	Enable	Autc		
Reporting	FCS File Suffix	Append Run ID \sim	Chor		
Acquisition Plugin	Pre-Acquisition				
	Prompt Before	🗹 Enable	Pron		
	Post-Acquisition				
	After Acquisition	Enter WinList mode & Repla $^{\vee}$	Cho		
	Prompt After	🗹 Enable	Pron		
	Update Report Links	✓ Enable	Refr		
	Save Reports	Enable	Save		
	Save PDF Reports	Enable	Save		
	Print Reports	Enable	Print ~		
	<		>		

Acquisition

Cytometer Plug-in Connection

This field defines how WinList connects to the Cytometer Plug-in. This should be supplied by your Cytometer Plug-in manufacturer.

Stop Count

Set the default stop count for acquisition.

Resolution

Select a resolution to use when writing acquired data to FCS files.

File Naming

FCS File Prefix

Enter an optional prefix for the FCS files created during acquisition. This field can be left empty.

Include Kw1 in File name

Enable this option to include the Keyword 1 in the name of files you acquire.

Include Kw2 in File name

Enable this option to include the Keyword 2 in the name of files you acquire.

FCS File Suffix

Select an option for a file naming suffix.

Pre-Acquisition

Prompt Before

When this option is enabled, WinList will display a dialog before acquisition. The dialog allows the user to enter keywords for the sample.

Post-Acquisition

After Acquisition

This option determines what WinList will do following an acquisition.

Choose **Continue Monitoring** to automatically switch back into monitoring mode.

Choose **Enter WinList Mode and Replay** to work with the data that has just been acquired.

Prompt After

When this option is enabled, WinList will display a dialog after acquisition. The dialog allows the user to enter keywords for the sample and shows summary information about the acquisition.

Update Report Links

When this option is enabled, WinList will automatically update all linked objects on reports when an acquisition finishes.

Save Reports

When this option is enabled, WinList will automatically save any open reports when an acquisition finishes.

Save PDF Reports

When this option is enabled, WinList will automatically save any open reports in PDF format when an acquisition finishes.

Print Reports

When this option is enabled, WinList will print open reports when acquisition finishes.

Save Protocol Bundle

When this option is enabled, WinList will automatically save a protocol bundle after acquiring an FCS file.

Include Acquired File in Bundle

When this option is enabled, WinList will include the newly acquired FCS file in the protocol bundle.

User-Defined Keywords

Auto-close Dialog

If enabled, WinList will automatically close the Post Acquisition dialog after a user-defined time passes.

Auto-close Time

Select the amount of time to keep the Post Acquisition dialog open before automatically closing it.

Keyword 1 and 2

This field contains the name of the first user-defined keyword. The default keyword name is \$SMNO, which is a standard FCS keyword used to describe the specimen.

Keyword 1 and 2 Description

If you edit Keyword 1, it is a good idea to edit this field to describe the keyword that you have entered. This description is displayed to the operator when the system prompts for the keyword value.

Keyword 1 and 2 Value

Enter a default value for Keyword 1, or leave the field blank.

Keyword 1 and 2 Separator

This separator is used when adding this keyword to the file name.

Real-Time Display

Show LEDs

Enable this option to display LEDs on the Cytometer Plug-in toolbar.

Monitor Mode Events

This setting determines the number of events WinList will try to show in histograms during Monitor mode.

Max Acquisition Dots

During acquisition, it is possible for WinList to accumulate and display all of the events that are being acquired. In most cases, however, this is not helpful or practical because it requires more and more memory and new dots are drawn on top of existing dots.

This setting limits the number of dots that 2P histogram will try to display during acquisition.

Throughput Control

Maximum Events Per Second

Choose the maximum throughput allowed for the WinList/Cytometer Plug-in connection. In most cases this setting will not need to be changed. However, on slow network connections or slow computers, you may choose to lower this setting to ensure that the computer remains responsive.

Save preferences

Enable this option to save the settings as preferences.

Appendices

DDE Commands

Dynamic Data Exchange (DDE) provides a way for Windows programs to share data and interact with one another. WinList supports DDE as a client of other applications. In other words, it can request information and instruct other applications to do things. It does not support DDE as a server of information.

When connecting WinList to other applications with DDE, there are several internal commands which WinList recognizes. These commands allow you to ship data to other programs and to retrieve data from them. Below is a list of the current internal DDE commands for WinList and an explanation of usage for each.

POKE

This command is used to send histogram or results data to another program in either TEXT format or VSH format. The syntax of the command is:

POKE(externalRef, format, { optional internal reference})

where externalRef is a reference the other application uses to determine where to put or how to use the data, and format is either TEXT, TEXTNOHEADER, or VSH. TEXT format is supported by most applications that can paste text from the clipboard. TEXTNOHEADER format is identical to TEXT except that it sends a result window's text as a table of information, without column headings. It can only be used with results windows. VSH is a Verity Software House binary data format, which provides a more efficient use of memory. It pertains only to histogram connections and should be used when interacting with other VSH products or with products known to support the format.

The *optional internal reference* can be literal text or any keyword in the keyword tree. This allows you to send any kind of information that WinList knows about to the remote application. If a keyword is used for this option argument, it must be enclosed in curly braces { }. Literal text does not need any special formatting.

Examples:

POKE(DATA,VSH) - used to send data to ModFit or IsoContour.

POKE(R1C1:R64C64,TEXT) - used to send a 64 x 64 resolution histograms to Microsoft Excel in TEXT format. Note the reference tells Excel to put the data into the active spreadsheet at row-1 column-1 to row-64 column-64.

POKE(RC:R[+6]C[+6],TEXTNOHEADER) - used to send a results window to Microsoft Excel in TEXT format, excluding the column headings and id fields in the results window. The reference tells Excel to put the data into the active spreadsheet at a location starting at the current row and column, and extending through 6 rows and 6 columns.

Relative references of this sort are very useful for adding results to a database without overwriting previous results.

POKE(KEYWORDS,VSH,KEYWORDS) - used in a histogram connection to send keywords from the listmode file to a Verity application like ModFit LT 3.0 or greater.

POKE(R1C1:R1C255,TEXT,KEYWORDS) - sends keywords from the listmode file to an application like Microsoft Excel. The format of the keywords is *keyword* followed by a tab character, then the *keywordValue* and another tab character.

POKE(R1C1,TEXT,R1 Events) - sends the label "R1 Events" as text to an application like Microsoft Excel to place in row 1 column 1.

POKE(R1C2,TEXT, {DS1:\$DATE}) – evaluates the string "{DS1:\$DATE}" as a keyword, and then sends the result as text to an application like Microsoft Excel to place in row 1 column 2. See Internal Keywords for a list of special internal keywords supported by WinList.

POKE(R2C1,TEXT,{DS1.R1.%Gate}) - sends the %Gate statistic for R1 in data source 1 to row 2 column 1 in Excel.

POKE(R3C1,TEXT,{DS2:SPILL}) - sends the SPILL keyword value from data source 2 to row 3 column 1 in Excel.

PASTE

This command tells WinList to paste a copy of the contents of the Windows clipboard into the histogram or results window for display. The syntax is PASTE(format) where format is METAFILE or TEXT. METAFILE is a picture format supported by many Windows graphic programs and can be used by both Results and Histogram windows. TEXT format should only be used with Results windows. When WinList successfully completes the PASTE command, the pasted object will be displayed in the window.

Examples:

PASTE(METAFILE) is used to get a copy of a picture from the clipboard. It can be used both by Results and Histogram windows.

PASTE(TEXT) is used to get a copy of text from the clipboard. It can be used only by Results windows.

COPY

This command tells WinList to copy the results or histogram window to the Windows Clipboard so that another application can paste it into its workspace. Its syntax is COPY(format) where format can be METAFILE, TEXT, or BITMAP.

Examples:

COPY(METAFILE) is used to copy a picture of a histogram to the clipboard. It can be used only by Histogram windows.

COPY(TEXT) is used to copy text to the clipboard. It can be used only by Results windows.

COPY(TEXTNOHEADER) is used to copy text to the clipboard. It can be used only by Results windows. The only difference between this format and TEXT format is that TEXTNOHEADER strips off all lines up to and including the column headings. It is useful for databasing of results in another program using a DDE connection.

SETREGIONRESULT

This command tells WinList to set the value of a specific region's result to a value extracted from a remote application. Its syntax is SETREGIONRESULT("*region reference*", "*external request*") where *region reference* is in R(n, stat) format and *external request* is a string which is requested from another application. For more information on R(n, stat) format, see Internal Keywords. *External Request* is expected to be a value number of the same type as the field it is copied to.

Examples:

If connected to ModFit, SETREGIONRESULT("R(1,REGIONEVENTS)","A[1,0]") tells WinList to ask ModFit for the answer to "A[1,0]", and to put the result into the Region Events field for Region 1.

PAUSE

This command is supported by histograms. It displays a dialog with optional message and waits to proceed until the dialog is closed. The dialog appends "Press OK to continue or Cancel to stop the script." Its syntax is PAUSE(*message*) where *message* is optional text for the message.

Example:

PAUSE(Please check results in ModFit LT.)

This command in a histogram script displays dialog "Please check results in ModFit LT. Press OK to continue or Cancel to stop the script."

SLEEP

This command is used to allow WinList to "sleep" for a specified period of time. It is useful in cases where the remote application needs to perform some time-consuming task, which could cause the script to timeout.

The command syntax is:

SLEEP(milliseconds)

For example, adding a script line

SLEEP(2000)

would cause WinList to sleep for 2 seconds.

Internal Keywords

WinList allows users to access two types of keywords: FCS keywords stored in data files, and internal keywords that WinList calculates.

Users can access keywords for histogram, MultiGraph, and results window titles, as well as text labels in MultiGraphs. Keywords are identified by surrounding specific words with square brackets []. When WinList sees a keyword, it replaces it with the keyword's value. For example, **[DATE]** is an internal keyword that would display the current date. **[\$TOT]** is an FCS keyword which tells how many total events were stored in the listmode file. Generally, when the user can edit a text field, keywords are available for those fields.

WinList also supports mixed text and keywords. It is possible, therefore, to display a label for a MultiGraph that would display the date and time as follows:

Date: [DATE] Time: [TIME]

In this example, if the current date is 01-Mar-2013 and the time is 09:20:45, WinList would display:

Date: 01-Mar-2013 Time: 09:20:45

The internal keywords supported by WinList are listed below.

[DATE]: Displays the current system date reported by your computer.

[TIME]: Displays the current system time reported by your computer.

[FILE]: Displays the filename of the active data source.

[FILE, dsID]: Displays the filename of the data source with an ID of dsID.

[FILEROOT]: Displays the base of the active data source file name. For example, if the file name is 190594.FCS, the FILEROOT keyword returns 190594, leaving off the file extension. **[FILEROOT**, *dsID*]: Displays the base of the filename for the data source with an ID of *dsID*.

[FILEPATH]: Displays the pathway to the active data source file.

[FILEPATH, dsID]: Displays the pathway for the data source with an ID of dsID.

[DEFAULTPATH]: Displays the pathway to protocols and other WinList file types.

[HISTOGRAM]: Displays the name of the active histogram.

[GATE]: Displays gate information of the active histogram.

[COUNTER*n***]**: Displays the current value of user-programmable counter *n*. This value can be changed with macro commands **ModifyUserCounter** and **ResetUserCounter**. See Edit Macro and <u>Macro Commands</u> 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	тот
Gated events	GATEDEVENTS	GAT
Region events	REGIONEVENTS	REG
Percent Total	PERCENT	РСТ
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.	СС	СС
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

Reading Delimited Text Files

WinList can convert certain text files into FCS listmode files so that they can be read and analyzed. This provides a convenient way to read files from programs like Microsoft Excel and other applications that do not know about FCS file formatting.

When WinList initially opens a data files, it tries to open it as an FCS format file. If it determines the file is not FCS format and that it contains ASCII text characters, it will attempt to interpret the file as a comma-delimited or tab-delimited text file.

In the first pass, the file is scanned for the first occurrence of a comma or tab character. It will treat that first found tab or comma as the delimiter for the file.

Next, the lines are scanned for non-numeric information that can be interpreted as keywordvalue pairs. For example, "Experiment Name, Covid-19 Exp 21" would be interpreted as a keyword "Experiment Name" with a value of "Covid-19 Exp 21", and it would be added to the list of keywords for the file. These lines are optional.

Example of comma-delimited text file with keywords, header row, and event data:

Experiment Name,Covid-19 Exp21 Sample Type,Blood Date,20June2020 FSC,SSC,CD14 FITC,CD33 PE,CD45 PerCP,Unused,CD34 - APC,Time 500.32730102,23.97476196,2.48005509,12.11952114,76.91445159,0.08981653,15.66742992,0.68804007 350.73910522,23.81124496,3.64244961,1.00029993,133.74858093,0.27155384,1.00362289,0.85104483 604.67559814,233.84678649,7.89220094,104.74340057,129.41178894,16.72563743,7.80934953,0.46642997 404.19461059,198.41729736,11.91999912,56.67926025,169.21188354,7.57181167,1.28422546,0.66259807 436.83917236,23.94353294,5.2218418,61.92754745,129.70245361,8.84675407,1369.01867675,0.37820205 511.32632446,134.78446960,19.62970352,31.06235122,83.55042266,2.76216840,6.23621845,0.89229768 234.57890319,14.62403297,1.94246792,1.00125622,519.26617431,0.87409734,6.30185270,0.3307272 571.65905761,125.00919342,10.14484119,49.64657592,145.86990356,5.11629390,14.54989719,0.96987426 139.30685424,100.70596313,2.75440049,4.02659416,6.44094133,0.47582840,6.36307287,0.74844944 239.69461059,17.00057601,1.06224739,1.44625675,490.00079345,0.92500466,2.36994695,0.49123454

During this pass, the logic is also looking for a line that contains the names of the measurements - a header row. For example, "FSC,SSC,CD14 FITC,CD33 PE,CD45 PerCP,Unused,CD34 - APC,Time" on a line would be interpreted as defining the names for 8 measurements.

Example of tab-delimited text file with header row and no additional keyword information:

FSC	SSC	CD14 FITC	CD33 PE	CD45 PerCP	Unused	CD34 - APC	Time
500.327301	23.97476196	2.48005509	12.11952114	76.91445159	0.08981653	15.66742992	0.68804007
350.7391052	23.81124496	3.64244961	1.00029993	133.7485809	0.27155384	1.00362289	0.85104483
604.6755981	233.8467865	7.89220094	104.7434006	129.4117889	16.72563743	7.80934953	0.46642997
404.1946106	198.4172974	11.91999912	56.67926025	169.2118835	7.57181167	1.28422546	0.66259807
436.8391724	23.94353294	5.22218418	61.92754745	129.7024536	8.84675407	1369.018677	0.37820205
511.3263245	134.7844696	19.62970352	31.06235122	83.55042266	2.7621684	6.23621845	0.89229768
234.5789032	14.62403297	1.94246792	1.00125622	519.2661743	0.87409734	6.3018527	0.33072727
571.6590576	125.0091934	10.14484119	49.64657592	145.8699036	5.1162939	14.54989719	0.96987426
139.3068542	100.7059631	2.75440049	4.02659416	6.44094133	0.4758284	6.36307287	0.74844944
239.6946106	17.00057601	1.06224739	1.44625675	490.0007935	0.92500466	2.36994695	0.49123454
145.0820465	7.34243822	2.04113459	3.3355658	3.24733758	0.23381255	10.10052108	0.8525846
417.8861694	82.34845733	14.13291072	36.79376983	130.2686462	3.07494664	8.58093452	0.91702514
337.8833313	26.50701904	2.23525166	2.71089744	712.3223877	0.90674597	1.00242233	0.40314322
493.5660706	97.68910217	33.57312774	25.14200019	92.15415191	0.28692317	1.00533556	0.71002495
453.609436	112.3068466	13.69358062	24.19342613	183.3162994	0.05160796	6.41166877	0.22030788

When a line is read that contains only numerical values, it is interpreted as the beginning of event data. It should have the same number of fields as the header row. This line and all subsequent lines will be read as the event values for the file.

If no formatting errors are encountered, the program will create an FCS file with the same name as the original file and the ".fcs" extension. The FCS file will then be read into WinList as it would normally read an FCS file.

The FCS file is not removed by WinList when it is finished, so it may be read into other FCS file readers for further analysis.

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