Instructions for Use

Kaluza Analysis

Flow Cytometry Software

A75667AD
January 2014

Manufactured by
Beckman Coulter, Inc.
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Brea, CA 92821 U.S.A.

BECKMAN COULTER

Kaluza Analysis Software Instructions for Use
PN A75667AD (January 2014)

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Revision History

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released to the Beckman Coulter website. For labeling updates, go to www.beckmancoulter.com and download the most recent manual or system help for your instrument.

Initial Issue A, 09/2009
Software Version 1.0

Issue AB, 06/2010
Software Version 1.1

- Updated the following sections in the Introduction to Kaluza Analysis Software chapter:
  Application Menu Options
  Basic Editing for Plots, and Sheet Items
  Color Precedence Pane
  Components of the Main Workspace
  File/Protocol File Compatibility
  System Requirements
- Updated the following sections in the Data Analysis chapter:
  Accounting for Autofluorescence
  Applying a Protocol to a Raw Data Set
  Choosing Scale Type
  Establishing Color Precedence of Gates
  Gate Statistics Table
  Generating Compensation and Autofluorescence Vector Values
  Importing a Compensation File
  Kaluza File Type Summary
  Merge Data Sets
  Overlay Plots
  Setting Up Statistics
  Saving a Compensation File
  Setting Up Radar Plots
  Setting Up Tree Plots
  Updating Content within the Parameters Pane
  Using Kaluza with Other Applications
  Using the Coloring Menu
  Viewing Gate Logic
- Added Appendix A, Statistics.
- Added Appendix B, Quick Reference Sheet.
- Added the Index.

Issue AC, 09/2011
Software Version 1.2
Complete revision; therefore, change bars are not applicable.

Issue AD, 01/2014
Software Version 1.3
Change bars indicate updated or new content. Sections containing change bars are as follows:

- Chapter 1, *Introduction to Kaluza Analysis Software*:
  - *Overview*
  - *Getting Started*
  - *Main Workspace*
  - *Selecting Sheet Items*
- Chapter 2, *Data Analysis*
  - *Kaluza Analysis Files*
  - *Protocols*
  - *Composite Protocols*
  - *Plots & Tables*
  - *Gates & Tools*
  - *Parameters*
  - *Compensation*
  - *Using Kaluza Analysis With Other Applications*
- Chapter 3, *Sheet Setup*
  - *Using Sheets*
  - *Using the Sheet Tab Bar*
  - *Report Sheet*
- APPENDIX A, *Statistics*
- APPENDIX B, *Quick Reference Sheet*
- APPENDIX C, *Frequently Asked Questions*
- Added APPENDIX D, *Troubleshooting*

Safety Notice

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CHAPTER 1

Introduction to Kaluza Analysis

Overview

Kaluza Analysis is a stand-alone flow cytometry analysis software package, designed to work with *.fcs and *.lmd files from flow cytometers. This software includes remarkably advanced features, including processing multi-color files of up to 10 million events in real time and offering analytical speed that is dramatically faster than the previous version and several hundred times faster than other commercially-available software.

Researchers in the areas of regulatory T cells, NK cell subpopulations, and mesenchymal stem cells are focused on the analysis of the differentiation stage and on the function of very small subpopulations of cells. Data files of several million events or more are required for statistically significant results. Kaluza Analysis enables researchers in these areas to run their high-content flow cytometry files in real time, while maintaining an intuitive, user-friendly interface.

Product Description

Several of the innovative features that Kaluza Analysis offers are:

- very fast analysis speed
- compatible with a broad range of listmode files
- support for foreign language
- multi-parametric experimental design and data analysis

Distinguishing Features

Radial Menus

Radial Menus (Figure 1.1) provide quick access to the tools necessary for making changes on the plot sheet or report sheet. Radial Menus appear by right-clicking directly on a plot, gate, or on the whitespace of the sheet. As you hover over an icon, a menu appears for that icon, allowing you to make changes instantly.

Figure 1.1 Radial Menu

Tree Plot

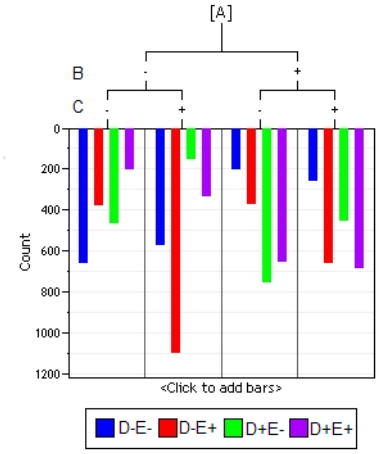
The tree plot (Figure 1.2) enables a unique and comprehensive approach to comparing the physical characteristics of the events included in your analysis. Tree plots provide a useful data comparison tool, as one tree plot can condense data from up to 28 bivariate plots.

The tree plot includes:

- **Branches**, which are used to categorize cell populations based on whether they have a negative or positive result for a specified phenotypic data type. Branches are located at the top of the plot.
- **Bars**, which are the event populations used to characterize every possible negative/positive branch combination. Bars are the central focus of the tree plot, as they are the pictorial representation of this phenotypic classification system. Bars can be viewed as either Count or % Gated.

Both bars and branches are based on gated data that has already been established within the Protocol.

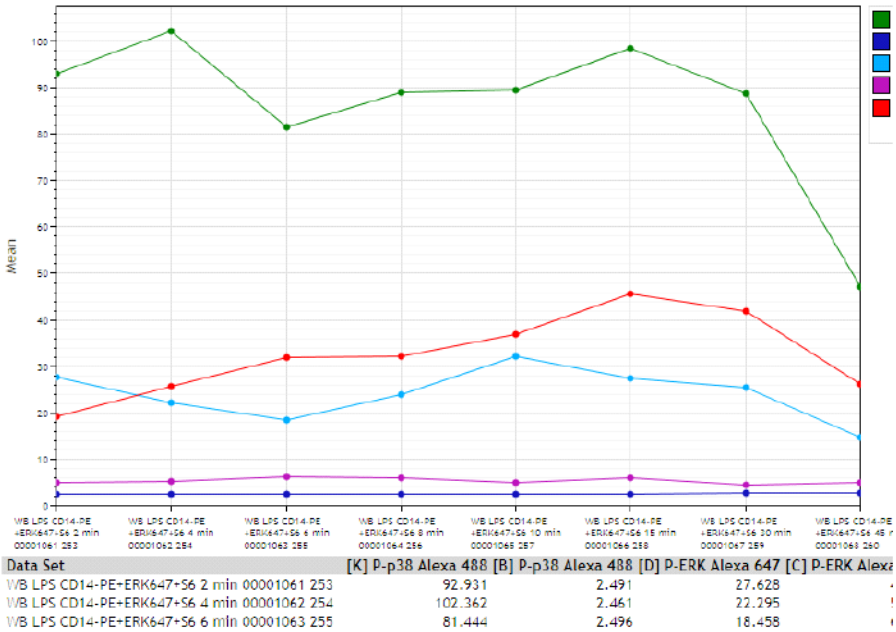
Figure 1.2 Tree Plot



Comparison Plot

Comparison plots (Figure 1.3) are used to compare statistics across data sets. When created within a protocol, the plot is displayed as a bar graph, by default, as this is only data set. When created in a composite or compensation composite, the plot is displayed as a line graph. See CHAPTER 2, *Comparison Plots*.

Figure 1.3 Comparison Plot



Radar Plots

The Radar Plot (Figure 1.4 and Figure 1.5) maps multi-dimensional data onto a two-dimensional surface; events are displayed by adding axes. As axes are moved, relationships become apparent; axes can be moved manually, or you may choose to animate one of the axes, which prompts automatic movement in the defined direction and rate of speed.

The appearance of the events on the plot can vary widely, depending on the input gate you choose, the number of axes you use to characterize your data, the resolution selected, the length of the axes, the placement of each axis, and the background color (white or black). Any parameter within the Data Set is available to use as an axis.

The radar plot is a very useful data comparison tool, combining data from many bivariate plots into one highly manipulatable plot.

Figure 1.4 Radar Plot with White Background

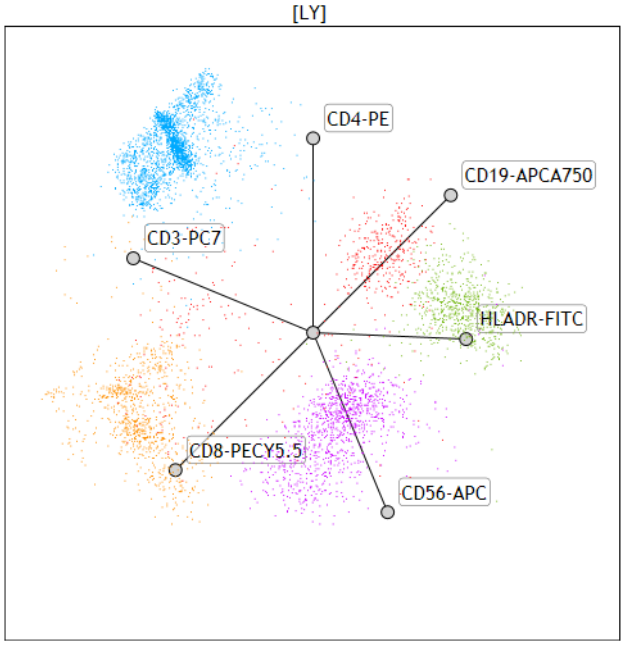
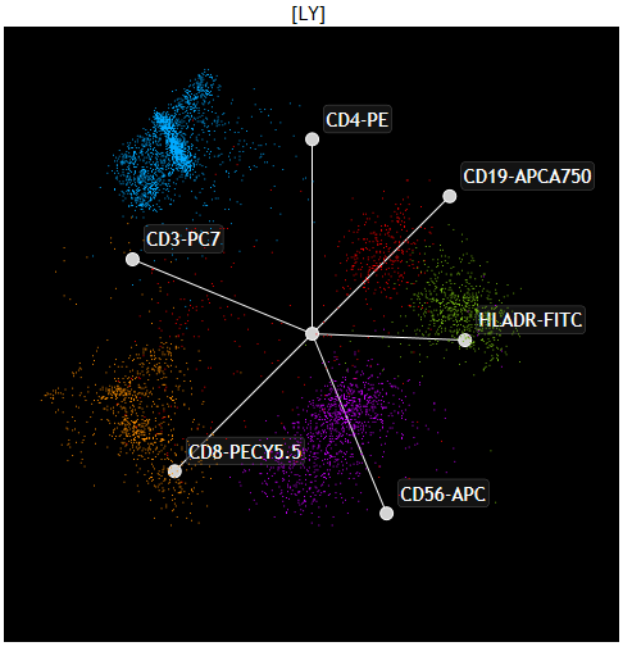


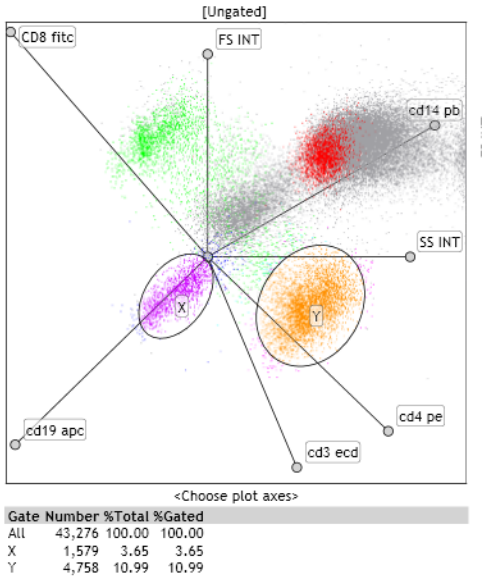
Figure 1.5 Radar Plot with Black Background



Radar Plot with Gates

You can create gates on a Radar Plot (Figure 1.6.), allowing you to use it much like a bivariate plot but with much more flexibility; thus providing added utility.

Figure 1.6 Radar Plot with Gates



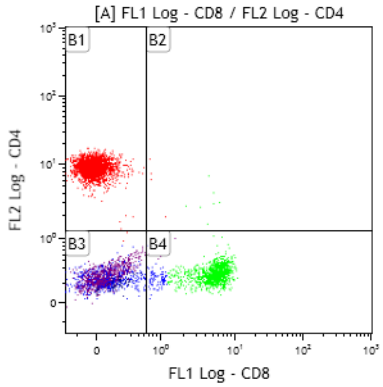
Gate	Number	%Total	%Gated
All	43,276	100.00	100.00
X	1,579	3.65	3.65
Y	4,758	10.99	10.99

Logicle Scale

Kaluza Analysis includes the Logicle Scale (Figure 1.7), which provides a means to correctly display compensated data. Changing an axis from log to logicle scale splits the axis into two different regions, where the positive values remain in log scale and negative values are transformed into linear scale. The two different scales are divided by a slider, which provides the ability to interactively control the width of each region. Using the logicle scale, negative values display correctly, preserving the desired symmetrical appearance of correctly compensated data.

When you are using the Log Scale, correctly compensated data may appear to be incorrectly over-compensated because events with negative values tend to pile along the axes; this distortion occurs because negative values do not exist on a Log Scale.

Figure 1.7 Logicle Scale



Interactive Report Sheet

You can customize the report sheet to suit your needs. It has the same functionality as the plot sheet, allowing you to add plots and edit the size, shape, and location of each plot. Additional options for the report sheet include changing the page size and adding the date, page numbers, text, and images. You can also choose to link plots on the report sheet to the plot sheet for simultaneous updating.

Getting Started

This section contains instructions and important information for improving your experience with Kaluza Analysis.

NOTE See Glossary for definitions of unfamiliar terms.

File/ Protocol File Compatibility

Kaluza Analysis is a stand-alone analysis software, meaning that you do not need to be connected to a flow cytometer to analyze listmode data. In fact, you can import and set up analyses using data files (*.lmd or *.fcs) from instruments of any manufacturer.

Kaluza Analysis can load any listmode file that is compliant with the FCS standard up through version 3.1.

IMPORTANT When working with data files containing embedded protocols, please consider the following:

- Kaluza Analysis imports gates, regions, plots, and color precedence information. **If, however, a gate and a region from the embedded source protocol have the same name, only the region is imported. The gate with the same name is not imported. Adjust gating as necessary.**
- Reports and statistics are not imported.

System Requirements

For Kaluza Analysis to install properly, your system must satisfy the following:

- Operating system:
  - Microsoft® Windows XP® 32-bit Operating System with Service Pack 3, or
  - Windows Vista® 32-bit Operating System with Service Pack 2, or
  - Microsoft® Windows® 7 32-bit or Windows 7 64-bit Operating System with Service Pack 1.

  NOTE If an nVidia Tesla® K20 card is present in your system, and you are running the Windows 7 64-bit Operating System, Kaluza Analysis uses the 2496 processors on the card.

  If an nVidia Tesla® C2075 card is present in your system, and you are running the Windows 7 64-bit Operating System, Kaluza Analysis uses the 448 processors on the card.

- Processor:
  - The processor must support the SSE2 Instruction Set.

- Minimum Resolution:

  The layout of the main workspace is optimized for high-resolution wide screen monitors; however, the software can function with a resolution as low as 1440 x 900.

- Language Packs:

  If installing the Japanese or Chinese language pack, ensure your Windows Operating System has the correct settings. This setting enables the programs that do not support Unicode to display menus and dialog boxes in their native language by installing the necessary code pages and fonts.

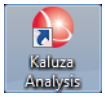
  To verify the Windows setting:

  1. Select **Start > Control Panel > Regional and Language Options**.
  2. Select the **Advanced** tab.
  3. Select language version of the non-Unicode program to be used (ex.: Japanese and/or Simplified Chinese).
  4. Select **OK**.

Launching Kaluza Analysis Software

The shortcut for Kaluza Analysis (Figure 1.8) was created on your desktop during the installation process (as described in the software CD package). To launch the software, double-click the Kaluza Analysis icon.

Figure 1.8 Kaluza Analysis Icon



Kaluza Analysis can also be started by selecting **Start > All Programs > Beckman Coulter > Kaluza Analysis**.

Command-line Switches to Kaluza Analysis

Command-line switches are very important for proper system functionality. The following switches instruct Kaluza Analysis to:

Table 1.1 Command-Line Switches

Command-Line Switch	Function
/HOST	Never use the Tesla board, even if it is normally installed and available to Kaluza Analysis.
/RESET	Resets: • Window sizes and position to the default location. • Kaluza Options to the default values. • **Add All Plots Options** to the default values.

Using the License Key

A license key enables the use of Kaluza Analysis after the trial period has ended. License keys are USB devices that are available in single-user or network (5-user or 10-user) license keys.

Setting-Up a Computer Using a Single License Key

To set up a single license key:

1. Install Kaluza Analysis on your computer using the instructions included in the software CD package.
2. Plug the USB key into host computer USB port. This allows full access to Kaluza Analysis.

NOTE For additional information regarding the license key, refer to the instructions on the website at **http://localhost:1947** (the computer do not need to be connected the network to access this site).

Setting-Up Computers Using a Network License Key

Prior to setting up user computers on a network license, you must set up the host computer. Follow the instructions in *Setting-Up a Computer Using a Single License Key*, to complete host computer setup.

To connect network computers to the host computer:

1. Install Kaluza Analysis on all computers that need to run Kaluza Analysis, as well as the computer that will host the license key.
2. Open a web browser program.
3. Enter the following address into the address bar: **http://localhost:1947**

   You are now connected to the HASP® License Manager Admin Control Center.
4. From the Administration Options section, select **Configuration**.
5. Select the **Access to Remote License Managers** tab.
6. Select the **Allow Access to Remote Licenses** check box.
7. Type the computer name of the host machine into the **Specify Search Parameters** field.

8 Select **Submit**, which connects the computer to the network license key and grants full access to Kaluza Analysis.

NOTE To verify that a computer is connected to the network license key, select the (**Information**) icon after launching Kaluza Analysis; this initiates the About screen. In the **License Type** section of the screen, a **Network** license type is indicated when the network license key is recognized by the computer.

NOTE For additional information regarding the HASP key, refer to the instructions on the website at **http://localhost:1947** (the computer do not need to be connected the network to access this site).

License Key Troubleshooting

If you currently have a HASP license key but are unable to access Kaluza Analysis due to a license expiration error similar to the one shown in Figure 1.9, your computer's virus scanner may be preventing access to the HASP license service. To enable access, contact your local Technical Support personnel to request to permission for **hasplms** (HASP License Manager) service on your computer. Refer to APPENDIX D, *Troubleshooting* for additional Kaluza Analysis troubleshooting topics.

Figure 1.9 Kaluza Analysis License Error Message

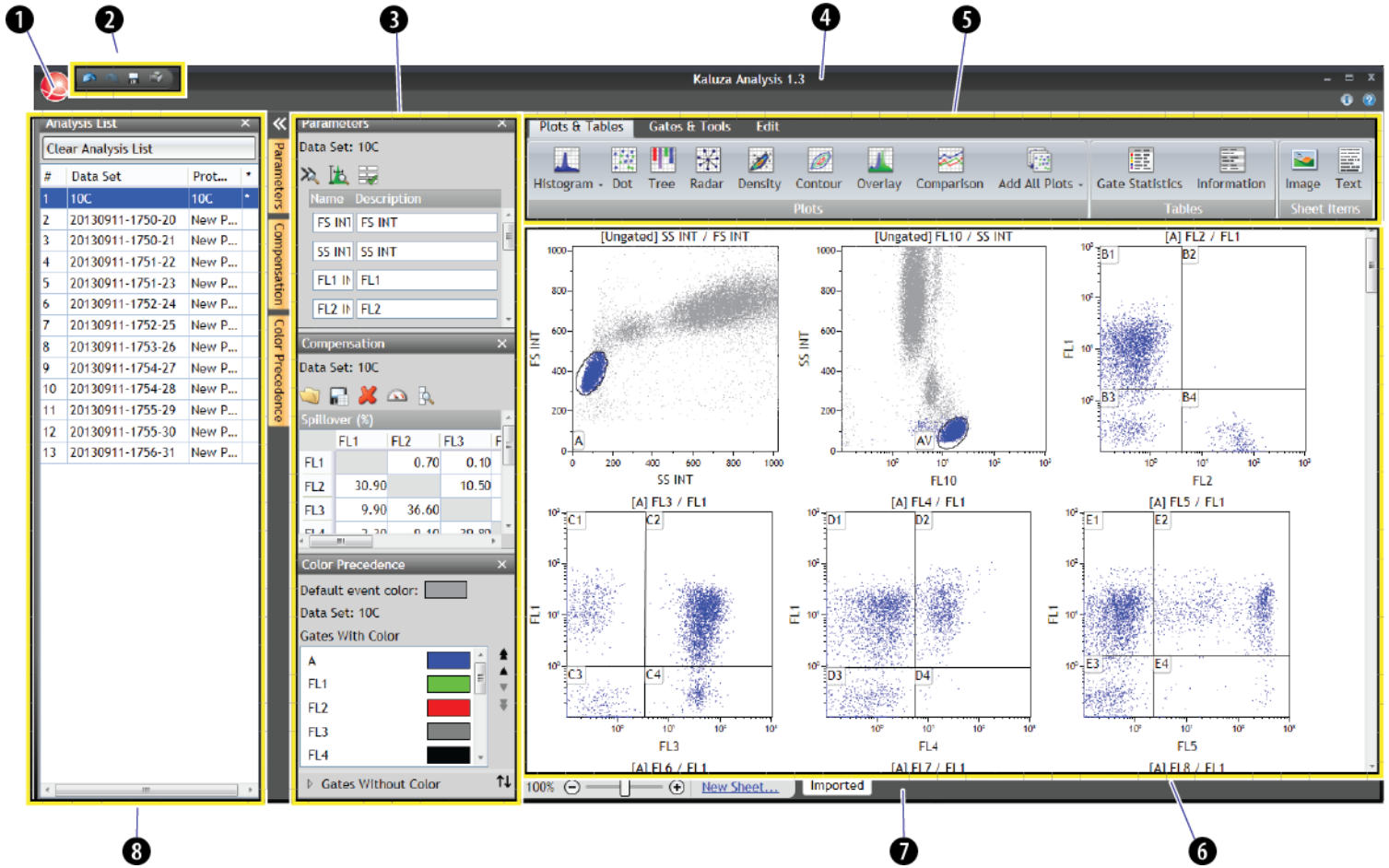
User Preferences and Security

On a shared system, you may wish to secure your Kaluza Analysis files and define options specifically for your needs. Kaluza Analysis works in conjunction with your operating system to provide you with the additional control you require. This is achieved through creating separate user accounts on your operating system. For instructions on creating multiple user accounts, consult **Windows Explorer > Help > View help**, and then enter "**User Accounts**" in the ***Search Help*** field and follow the instructions provided.

Components of the Main Workspace

The components of the Kaluza Analysis main workspace are shown in Figure 1.10, and descriptions of each component are found in Table 1.2.

Figure 1.10 Kaluza Analysis Main Workspace



1. Application Button
2. Quick Access Toolbar
3. Attributes Pane
4. Application Title Bar
5. Ribbon
6. Plot Sheet or Report Sheet
7. Sheet Tab Bar
8. Analysis List Pane

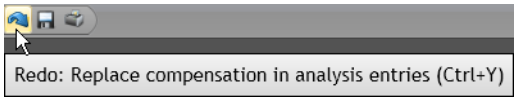
Table 1.2 Components of the Main Workspace

Component	Function
1. Application Button	Opens the Application menu.
2. Quick Access Toolbar	Allows access to Kaluza Analysis functions, including undo, redo, save, and print.
3. Attributes Pane	Allows viewing the Parameters, Compensation and Color Precedence panes.
4. Application Title Bar	Allows viewing the program name and version being used.
5. Ribbon	Allows performing a number of tasks from within the Plots & Tables, Gates & Tools, Edit, or Page Layout tab above the sheet area of the application screen.
6. Plot Sheet or Report Sheet	Allows viewing a graphical representation of the raw data collected from the flow cytometry sample. Plots are customized based on the parameters chosen to represent the data.
7. Sheet Tab Bar	Modify the zoom value, add a new sheet or switch between sheets.
8. Analysis List Pane	Allows viewing a list of open files within the application. The list may be comprised of up to 400 rows, including raw Data Sets, Analysis files, Protocols, Composites, and Compensation Composite files.

Tooltips

See Figure 1.11, which is an example of a tooltip that appears when the mouse cursor hovers over the Redo icon. Hover your mouse cursor over hotspot areas of the screen to display information related to your current location. This information, known as tooltips, provides clear instructions, saving you time and eliminating guesswork.

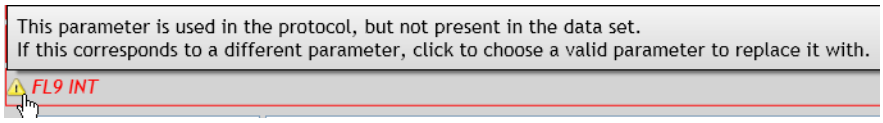
Figure 1.11 Tooltip Example



Warning and Information Messages

See Figure 1.12, which is a warning that appeared because the imported Protocol parameter names did not match those in the Data Set. When hovering over the warning, a tooltip appears, providing instructions for resolving the error. Warning and information messages display at the location of the issue and often provide instructions for a resolution.

Figure 1.12 Warning Message/Tooltip



Right-Click Options

NOTE Normally, right-click options provide alternatives to standard procedures and are not included in the instructions in this manual unless they are the only way to use a particular option.

When you click the right mouse button, menu options that apply to a particular region of the screen appear. Specifically, right-click menus are available in the Analysis List pane, the Attributes pane, and the Sheet Tab bar.

A unique type of menu, the Radial Menu, is available with a right-click in the plot or report sheet. See *Using Radial Menus*, for an overview on Radial Menu functionality.

Using Radial Menus

Radial Menus are incredibly useful tools, as they enable convenient access to the menu items that are applicable to your current location on the plot sheet or report sheet. Radial Menus appear by right-clicking on one of three areas: plots, gates, and sheet whitespace.

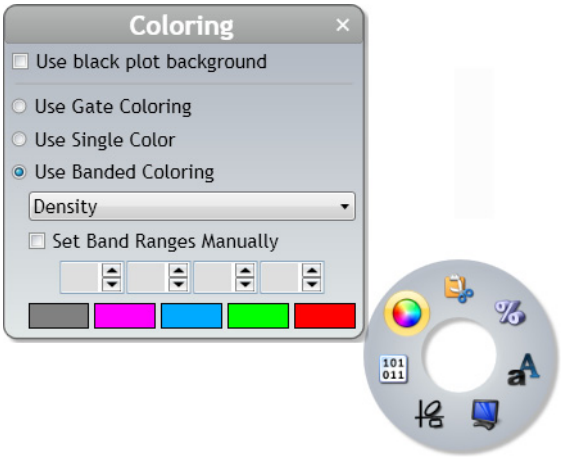
You can move a Radial Menu to any location of the screen. To move a Radial Menu, left-click on any blank part of the menu and drag it to the preferred location. See CHAPTER 2, *Plot Set-Up*, CHAPTER 2, *Setting Up Gates*, or CHAPTER 3, *Sheet Radial Menus Options*, for details.

To use a Radial Menu:

1. Right-click on the location that you wish to update. A Radial Menu appears.

2. Move your mouse over the menu. As you hover over the icons located on the Radial Menu, the menu for that icon appears. For example, hovering over the Coloring icon brings the Coloring menu, as shown in Figure 1.13.

Figure 1.13 Coloring Menu



3 Make the necessary changes within the appropriate menu. When you are satisfied with your changes, close the menu by selecting ✕ or by clicking on some other part of the software.

Drag and Drop

NOTE Kaluza Analysis includes multiple methods for achieving a particular outcome. When the "drag and drop" method is available for a task, it is the option that is noted in the instructions.

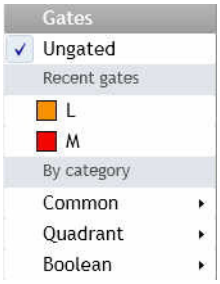
Many functions in Kaluza Analysis employ the drag and drop method. Examples include:

- Creating plots by dragging/dropping an icon from the Ribbon onto the sheet.
- Opening files by dragging/dropping into the Analysis List.
- Within the Analysis List, importing/replacing a Protocol associated with a Data Set.
- Within the Analysis List, importing/replacing a Data Set associated with a Protocol.
- Changing the order of entries on the Analysis List.
- Changing the order of data sets in the Batch Input list.
- Moving a sheet item to a different location.

Pop-up Menu Set-Up

Pop-up menus, which appear after selecting hyperlinks located on plots, may include headings and subheadings within the menu, both of which are not selectable; however, they do include information that is available for selecting under applicable headings. See Figure 1.14 to view an example of the Gates heading. Headings appear in white font/gray highlight. The subheadings (**Recent gates** and **By category** in Figure 1.14) use a dark gray font and are highlighted in light gray. An arrow located next to a menu item indicates that additional sub-menu options are available, as demonstrated by **Common**, **Quadrant**, and **Boolean**. Sub-menus pop-up when you hover your mouse cursor over a row that includes an arrow.

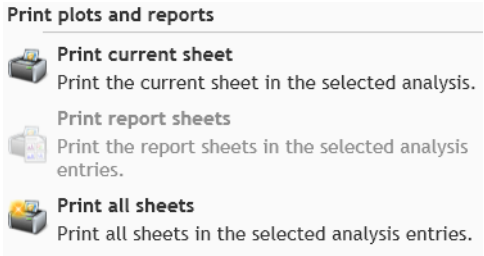
Figure 1.14 Pop-Up Menu Set-Up



Indication of Option Availability

See Figure 1.15, which shows that **Print report sheets** is not an available option because the selected analysis file does not contain a report sheet. The availability of options depends on the items that you have set up in your data analysis. When options are not available, they appear transparent compared to the options that are available.

Figure 1.15 Unavailable Options



System Performance

To optimize the performance of the application, consider closing all non-essential applications.

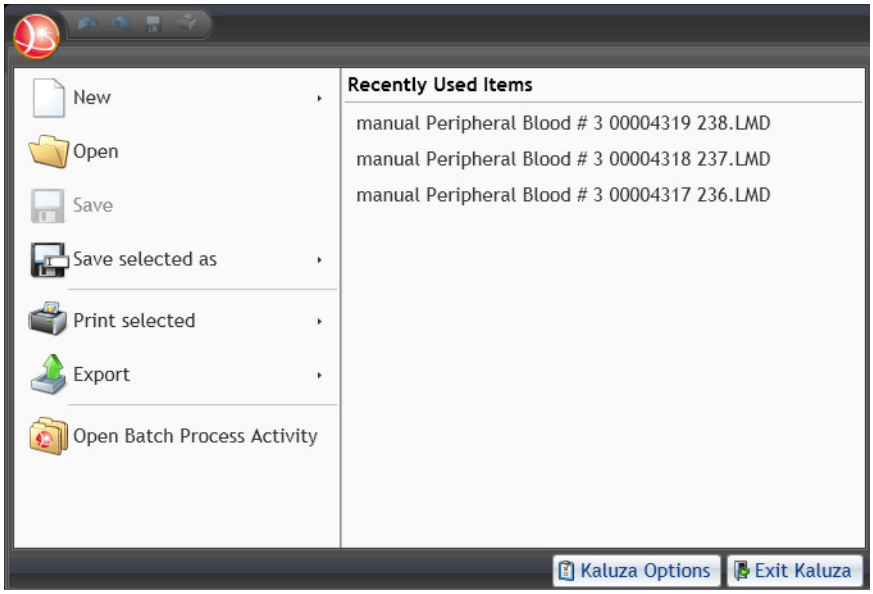
Main Workspace

See Figure 1.10, to view the location of each component in the Kaluza Analysis workspace. The components that make up the Kaluza Analysis workspace are described in detail in the following sections.

Application Menu

The Kaluza Analysis software Application button is located in the upper left-hand corner of the application workspace. Select this button to open the Application menu (Figure 1.16).

Figure 1.16 Application Menu



Application Menu Options

From the Application menu, select one of the following options:

- **Recently Used Items**: Provides access to the 13 most recently used files. The files are listed in chronological order, with the most recently used file at the top of the list. To open a file on the list, click on the file name.
- **New**: Creates a new entry in the Analysis List. Options include:
  - **Analysis List:** Creates a new Analysis List.
  - **Protocol**: Creates a new Protocol entry in the Analysis List.

    NOTE Ctrl + N also creates a new Protocol entry in the Analysis List.

  - **Composite**: Creates a new Composite entry in the Analysis List.
  - **Compensation**: Creates a new Compensation entry in the Analysis List.
- **Open**: Opens a file into the Kaluza Analysis application from the location you choose from the Open dialog box.

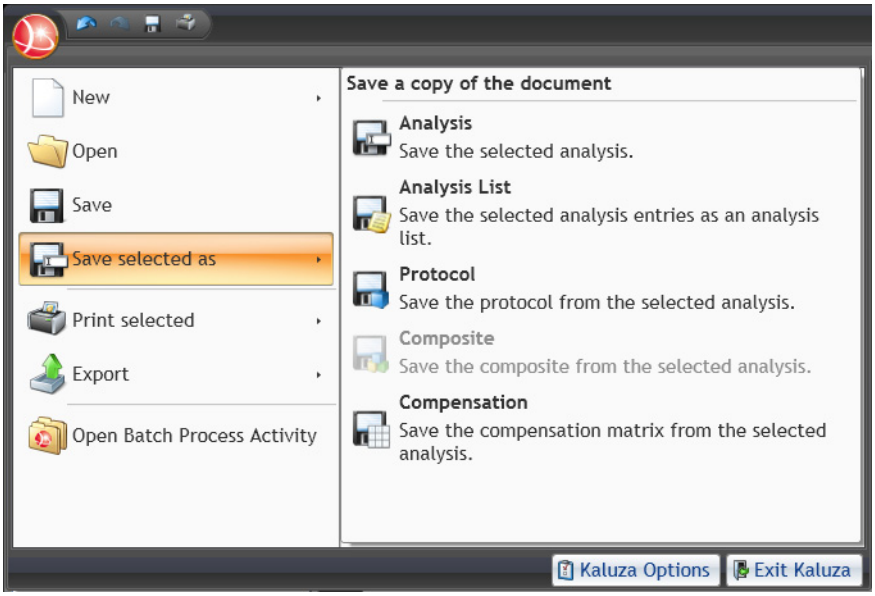
  NOTE Other options for opening files in Kaluza Analysis include:

  - Selecting Open files... from the main screen after opening the application.
  - Using the shortcut feature by pressing Ctrl + O keys on your keyboard to display the Open Dialog window.
- **Save**: Saves all the entries in the Analysis List as an *.analysis file, to the location of your choice.

  NOTE Ctrl + S also saves the entire Analysis List as an analysis.

- **Save selected as** (Figure 1.17): Provides a list of file types to which you can save the selected entry. Depending on the type of Analysis List entry you are saving, the options vary, and may include one or more of the following:
  - **Analysis:** Saves the selected analysis (*.analysis).
  - **Analysis List:** Saves the selected analysis entries as an Analysis List (*.analysis).
  - **Protocol:** Saves the Protocol from the selected analysis (*.protocol).
  - **Composite:** Saves the selected analysis files as a Composite (*.composite).
  - **Compensation:** Saves the Spillover Matrix and Autofluorescence Vector from the selected analysis (*.compensation).

Figure 1.17 File Types to Save As



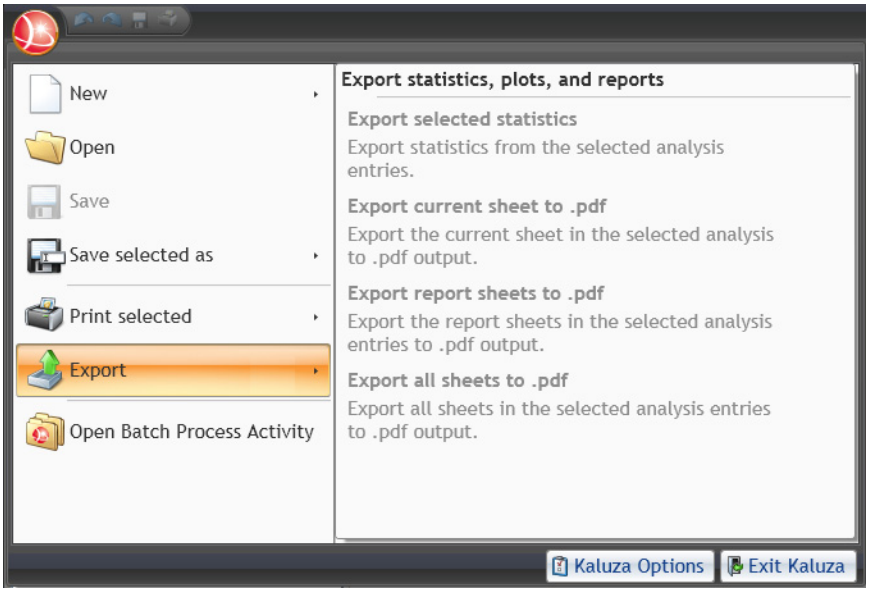
NOTE The Oki® B6300 printer has been tested in Kaluza Analysis and will produce expected results. Other printers have not been tested; therefore, quality of print has not been determined.

NOTE If multiple entries are selected on the Analysis List, only Analysis List is an option to Save As.

- **Print selected**: Provides options for printing plots and reports. Options include:
  - **Print current sheet**: Prints the current sheet in the selected analysis.
  - **Print report sheets**: Prints the report sheets in the selected analysis.
  - **Print all sheets**: Prints all sheets in the selected analysis.
  - Ctrl + P prints the current sheet in the selected analysis.

- **Export selected** (Figure 1.18): Provides options for exporting statistics or PDF files from selected entries/analyses within the Analysis List.
  - **Export selected statistics:** Export statistics as a .csv file from the selected analysis entries.
  - **Export current sheet to .pdf**: Export the current sheet in the selected analysis to .pdf output.
  - **Export report sheets to .pdf**: Export the report sheets in the selected analysis entries to .pdf output.
  - **Export all sheets to .pdf**: Export all sheets in the selected analysis entries to .pdf output.

Figure 1.18 Export



- **Open Batch Process Activity:** Allows you to run a batch of listmode files through the selected protocol. See CHAPTER 2, *Batch Processing*, for details.
- **Kaluza Options**: Allows you to adjust settings for the Kaluza Analysis application. See *Kaluza Options Menu*, for details.
- **Exit Kaluza**: Closes the application. Select Exit Kaluza to complete this operation.

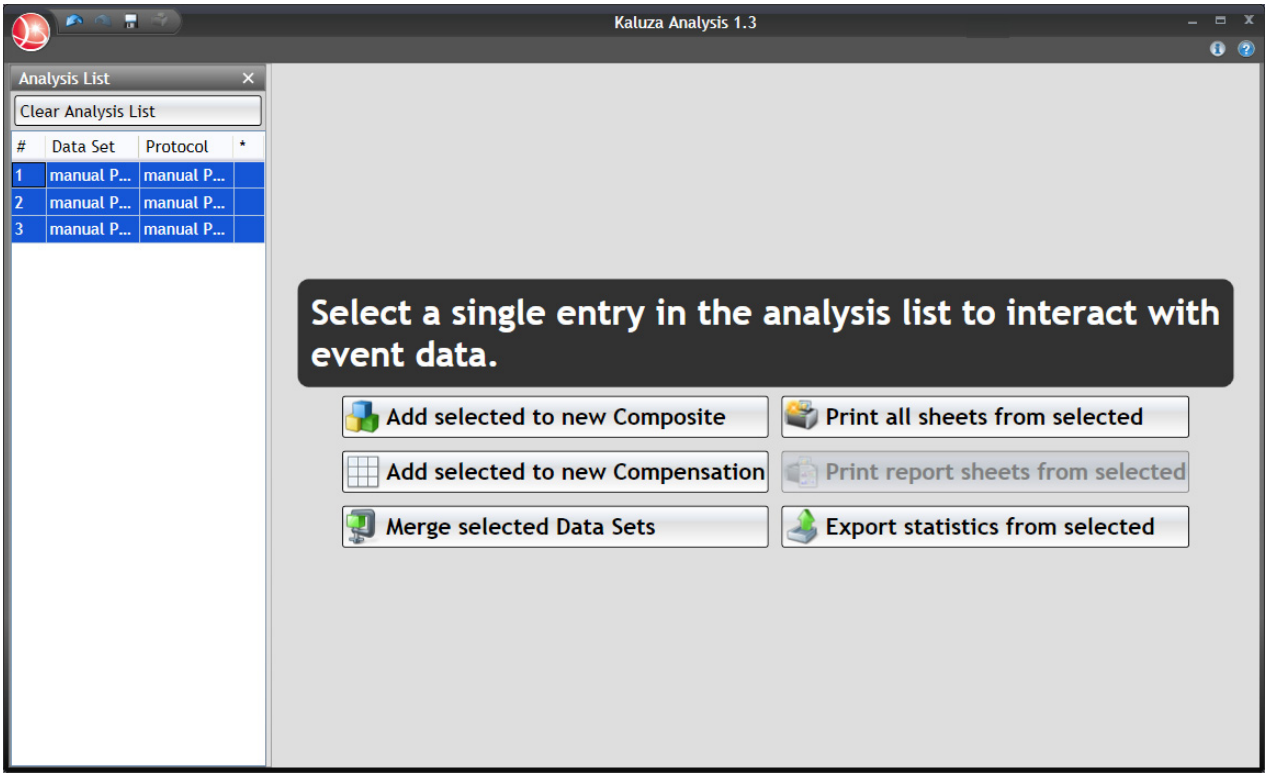
Analysis Options Screen

Figure 1.19 is an example of the Analysis Options screen, which appears when multiple entries are selected within the Analysis List. For more details, see:

- CHAPTER 2, *Composite Protocols*
- CHAPTER 2, *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature*
- CHAPTER 2, *Merged Data Histogram*
- CHAPTER 2, *Merged Data Sets*
- CHAPTER 2, *Exporting Statistics*

- *Application Menu* for:
  - Export Statistics
  - Print All Sheets from Selected
  - Print Report Sheets from Selected

Figure 1.19 Analysis Options Screen



Kaluza Options Menu

The Kaluza Options menu allows you to adjust settings for using the Kaluza Analysis application. Table 1.3 describes the settings that can be adjusted using this menu.

NOTE Kaluza Options are specific to individual User Accounts. For information on creating User Accounts, refer to *User Preferences and Security*.

Table 1.3 Kaluza Options Menu

Tab	Component	Description
Options (Figure 1.20)	**Display**	• Display the text in either standard or large font. **NOTE** This option does not affect the font size on the plots, since the plot fonts are configured on the **Defaults** tab. • **If the language pack is installed,** you can display the text and IFU based on the language selection (English is the default language in the settings).
	Statistics	• Include a thousands separator for both whole and fractional numbers. • Display between 0 and 4 decimal places for both fractional numbers and percents. **NOTE** If the **Exclude Empty Columns** option is checked, any blank columns in the statistics export function will not be written. However, if a fixed format is desired, for automation purposes, DO NOT check this option.
	Compensation	• Display between 0 and 4 decimal places in the Spillover Matrix, including the Autofluorescence Vector column.
	Printing	• Print plots with white background. **NOTE** If you had configured plots to display with a black background, Kaluza Analysis offers this option when printing, to help conserve toner in your printer.

Table 1.3 Kaluza Options Menu

Tab	Component	Description
Defaults (Figure 1.21)	**NOTE** When default settings are applied, this will affect only the newly created plots or newly loaded data files. **NOTE** Plots created by loading protocols embedded in *.lmd files will be affected by **Coloring** and **Font** defaults; however, plots in analysis files will not.	
	Coloring	• Allows configuring the default background color for plots.
	Font	• Allows configuring the default font style for plot titles, axes, and content.
	Parameter	• Allows configuration of the priority of signal measurement (area, height, width). The first signal measurement listed should be the one with the highest resolution on the hardware you acquire with most frequently. When there are multiple measurements captured for a given PMT, Kaluza will use this order to determine which parameters to use for compensation and for add all plots.
	Data Set Identifier	• Allows configuration of a default formula to be used to identify data sets when loading new data files (*.lmd, *.fcs). The Data Set identifier is used in the Data Set column in the Analysis List, as well as anywhere the data set name is shown throughout the software. See CHAPTER 2, *Using Formulas in Kaluza Analysis*. • The formula may include keywords from the FCS header information, or other custom content.
Available on both tabs	**Restore initial settings**	• Reinstates all settings listed in the **Kaluza Options** menu to the defaults. The **Restore initial settings** functionality applies to any settings made on both the **Options** and **Defaults** tabs. **NOTE** These defaults will apply to *.lmd and *.fcs files, but not *.analysis files. To configure priority on an existing analysis, see CHAPTER 2, *Parameters*.

Figure 1.20 Kaluza Options Menu > Application Options (With Language Pack Installed)

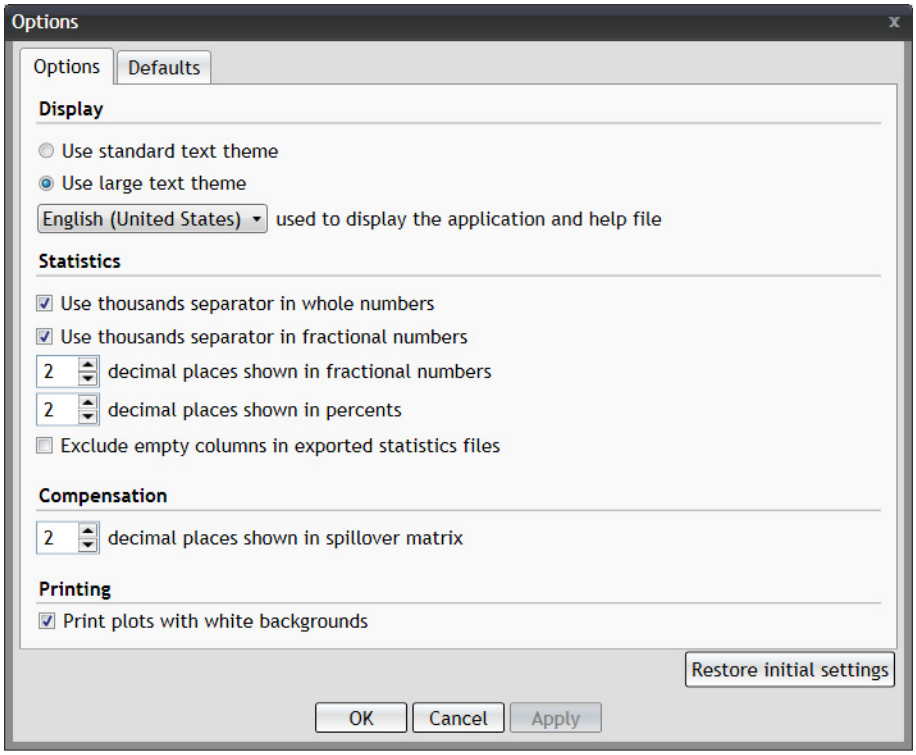
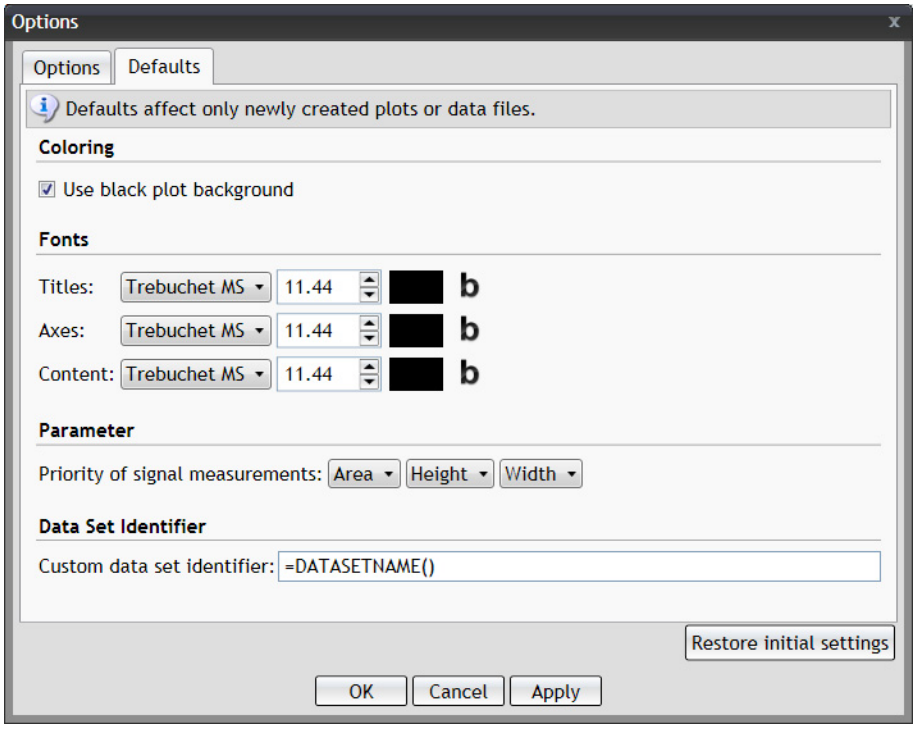


Figure 1.21 Kaluza Options Menu > Defaults



To make changes to the **Kaluza Options** menu:

1. Select > Kaluza Options. The **Kaluza Options** menu appears.

2. Make your changes using the radio buttons, check boxes, and up/down arrows, or use the Restore all defaults button to reset all values.

   If you wish to view the changes you made prior to closing the Kaluza Options menu, select **Apply**.

3. Click **OK** to implement changes and close the menu.

Quick-Access Toolbar

See Figure 1.22. The Quick-Access toolbar provides convenient access to Kaluza Analysis functions, including undo, redo, save, and print.

When you use the Quick-Access toolbar, the save and print functions are limited. Additional options for printing and saving are available through the Application menu. See *Application Menu*. The functions available on the Quick-Access toolbar are described in Table 1.4.

Figure 1.22 Quick-Access Toolbar

Table 1.4 Quick-Access Toolbar Functions

Icon	Description	Function
 	**Undo** **Redo**	• **Undo**: Steps the software back one action per click of this icon. **NOTE** Ctrl + Z is an additional method for undoing previous actions. • **Redo**: Steps the software forward one action per click of this icon (only available after using the undo function). **NOTE** Ctrl + Y is an additional method for redoing actions. Unlimited undo and redo is available within a session and is limited only by available memory and disk resources. **IMPORTANT** Undo/redo is not available on functions that do not impact program data. These functions include zoom, scrolling a window, selecting a different tab from the Sheet Tab Bar, etc.

Table 1.4 Quick-Access Toolbar Functions

Icon	Description	Function
	Save	Saves the entire Analysis List as a *.analysis file to a location of your choice.
	Print	Prints current sheet of the selected analysis entry.

Application Title Bar

The Application Title Bar (Figure 1.23) displays the software name and version, and contains the application button, the quick access toolbar, and the components described in Table 1.5.

Figure 1.23 Application Title Bar

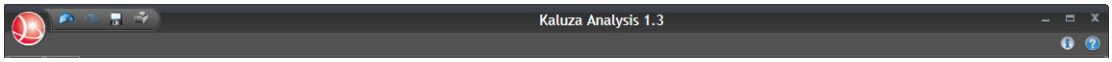


Table 1.5 Application Title Bar Functions

Icon	Description	Function
	Minimize	Minimizes the Kaluza Analysis screen.
	Maximize	Maximizes the Kaluza Analysis screen to fit the full dimensions of the monitor.
	Restore	Restores the Kaluza Analysis screen to the previous size.
	Close	Closes the application.
	Information	Provides information about Kaluza Analysis including the version, compute engine type, serial number, license type, copyright information, and provides access to the Log Directory. **NOTE** If an nVidia Tesla® K20 card is present in your system, and you are running the Windows 7, 64-bit Operating System, Kaluza Analysis uses the 2496 processors on the card. If an nVidia Tesla® C2075 card is present in your system, and you are running the Windows 7, 64-bit Operating System, Kaluza Analysis uses the 448 processors on the card.
	Help	Provides the complete Instructions for Use in a PDF file format.

Analysis List

The Analysis List, which occupies the left-hand side of the screen, is a list of Data Sets and Protocols currently open within the application. The Analysis List:

- contains a maximum of 400 entries or rows
- can be comprised of one or more protocol analyses, as well as Composite and Compensation Composite files.

NOTE Deleting any entry from the Analysis List does not delete the source file.

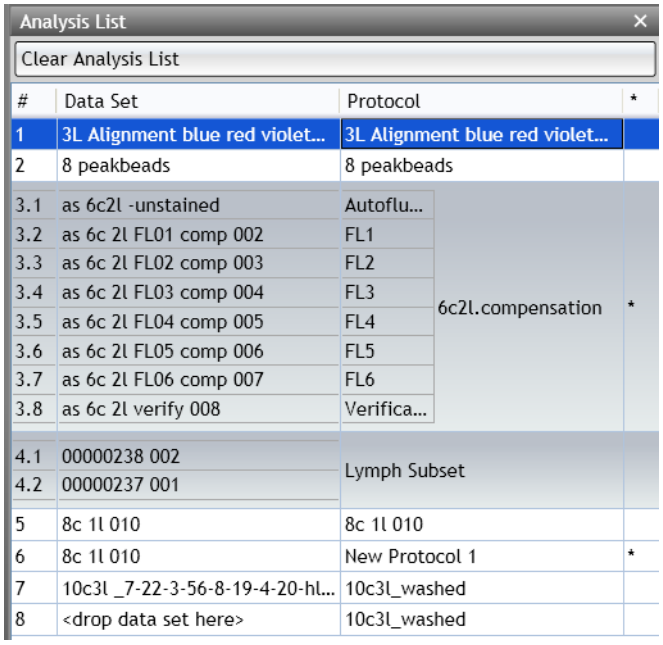
Analysis List Set-Up

There are four columns in the Analysis List, as shown in Figure 1.24:

- The **#** column shows the row number of each Analysis List entry.
- The **Data Set** column displays the identifier of the Data Set used in an Analysis List row.
- The **Protocol** column displays the file name of the Protocol used in an Analysis List row.
- The * column indicates that the Analysis List row has been modified and there are unsaved changes.

See *Display Options for the Analysis List and Attributes Pane*, for details on the Analysis List display.

Figure 1.24 Analysis List Example



Using the Analysis List

You can populate each row in the Analysis List with the Data Set and Protocol independently, allowing you to mix and match Data Sets and Protocols from different files. You can also replace Protocols or Data Sets currently within an analysis row by importing (dragging and dropping) a new file into the column you wish to update.

The Analysis List is the hub for setting up your data analyses, and Kaluza Analysis is designed to allow you to easily customize each analysis. Listed in Table 1.6 are the tasks you are able to complete from the Analysis List, as well as the methods that enable you to complete each function.

IMPORTANT Function availability depends on the type of Analysis List row(s) currently selected.

Table 1.6 Analysis List Function Availability

Function	Dragging and Dropping	Analysis List Menu (accessible by right-clicking)	Keyboard Shortcut
Select All	–	✓	Ctrl + A
Cut	–	✓	Ctrl + X
Copy	–	✓[a]	Ctrl + C
Paste	–	✓	Ctrl + V
Paste Special	–	✓[b]	–
Replace Data Set in an Analysis File or Composite	✓	–	–
Edit Data Set Label **NOTE** Left click on the cell will also gain access to editing the data set label.	–	✓	–
Clear Data Set	–	✓	–
Clear Protocol	–	✓	–
Import Protocol	✓	–	–
Delete Analysis **NOTE** When a row in a Composite is selected, it deletes the row from the Composite. Deleting an entry from the Analysis List does not delete the source file.	–	✓	Delete
Merge Data Sets	–	✓	–
Add to New Composite	✓	✓	–
Add to New Compensation	✓	✓	–
Import Compensation	✓	✓	–
Export: • Selected statistics • Current sheet to PDF • Report sheets to PDF • All sheets to PDF	–	✓	–

Table 1.6 Analysis List Function Availability

Function	Dragging and Dropping	Analysis List Menu (accessible by right-clicking)	Keyboard Shortcut
Save Analysis List	–	–	Ctrl + S
Save Selected As... • Analysis • Analysis List • Protocol • Composite • Compensation	–	✓	–
Print Selected... • Print Current Sheet • Print Report Sheets • Print All Sheets	–	✓	Ctrl + P (applies to Print Current Sheet only)

a. Kaluza Analysis provides ability to copy and paste special parameter information to other entries in the Analysis List. Deleting an entry from the Analysis List does not delete the source file.

b. Kaluza Analysis provides ability to paste special parameter information to other entries in the Analysis List.

Multi-Selecting Files

By selecting multiple rows on the Analysis List, additional options become available for the entries. See Figure 1.19, to view these options which appear immediately after a second row is selected. The following sections describe the methods for multi-selecting files on the Analysis List.

Multi-Selecting a Consecutive Group of Entries on the Analysis List

To multi-select a consecutive group of entries:

1. Select the entry located at the top of the group.
2. Press and hold the Shift key and select the entry located at the bottom of the group.
3. When you are finished, release the Shift key. The entries are now ready to act as a group.

Multi-Selecting Random Entries on the Analysis List

To multi-select random entries:

1. Press and hold the Ctrl key while selecting the entries you wish to include in your selection.

2 Release the Ctrl key when you have finished making your selections. The entries are now ready to act as a group.

Importing Files by Dragging into Analysis List

To import a file into the Analysis List by dragging and dropping from your computer:

1 Locate the file(s) you wish to include on the Analysis List.

2 Select the file(s), drag into the list, and release the mouse button. As an example, in Figure 1.25, four files are being dragged into the Analysis List. Figure 1.26 shows the Analysis List once the mouse button is released.

Figure 1.25 Dragging Files Into the Analysis List

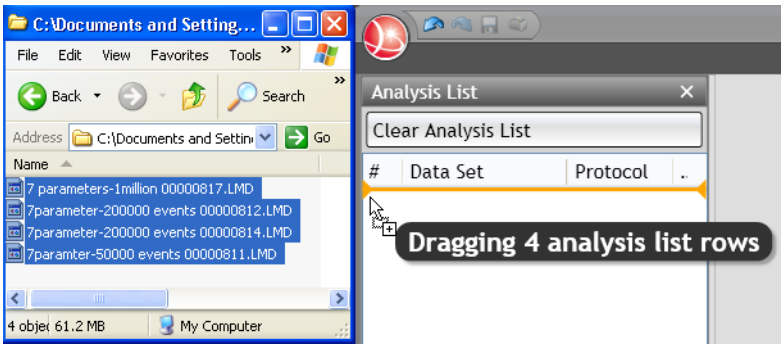
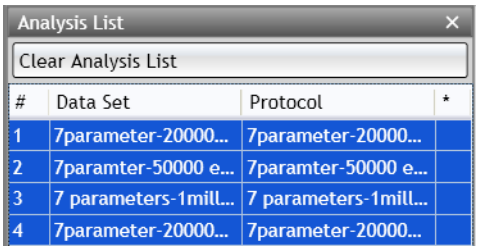


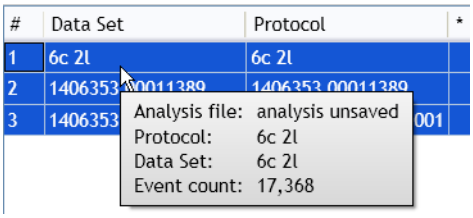
Figure 1.26 Analysis List Files



See Figure 1.21 to view the original file names that appear in the Data Set column, unless a custom Data Set identifier has been configured in the Options dialog.

Additionally, when you hover your mouse cursor over an Analysis List row, the tooltip shows pertinent details regarding the file, as shown in Figure 1.27.

Figure 1.27 File Details



Your file(s) are now imported into Kaluza Analysis and ready for you to begin your analysis. See CHAPTER 2, *Data Analysis*, for details on data analysis.

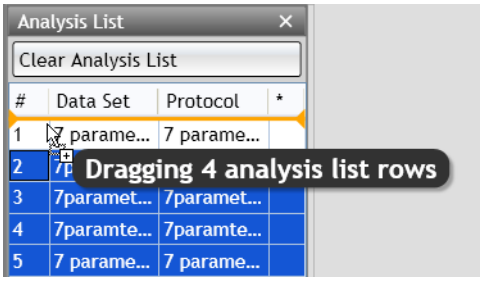
Changing the Sequence of Analysis List Rows

To move an Analysis List row to another location within the list:

1 Select the Analysis List row(s) you wish to move.

2 Click on the selected row(s) and, without releasing the mouse button, drag to the new location. When an orange line (as shown in Figure 1.28) is in the new location for the row(s), release the mouse button.

Figure 1.28 Relocating Analysis List Rows



NOTE If you moved multiple rows simultaneously, they appear in the new location in the same hierarchal order in which they were originally on the list.

Cutting and pasting Analysis List rows moves them to the bottom of the list. Dragging and dropping is the only way to change the order of files on the list.

Replacing or Importing a Data Set or Protocol into an Analysis List Row

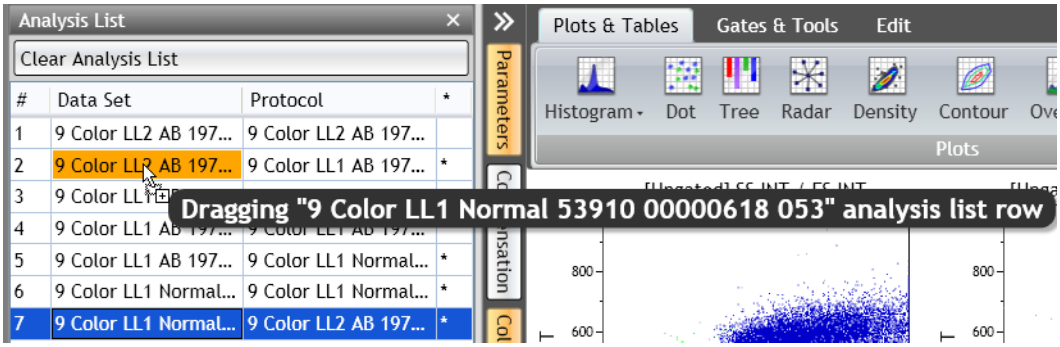
To assign a Data Set or a Protocol to a new entry or a saved file within the Analysis List:

1 Locate the file you wish to assign to the Analysis List row.

NOTE You may choose a file from your computer or from within the current Analysis List.

2 Select and drag the file into the column of the Analysis List row that you wish to update. For example, in Figure 1.29, the Data Set from the seventh row on the Analysis List is being moved to replace the Data Set on the second row *(this changes the second row to use the same data source as the seventh row, while retaining the protocol that defines the plots and gates used to visualize the data).*

Figure 1.29 Updating a Data Set



3 Once the appropriate Analysis List cell turns orange, release the mouse button.

Applying Data Sets to a Composite or Compensation Composite File

To apply a Data Set to a Composite or Compensation Composite entry on the Analysis List:

1 Depending on your starting point, the procedure will vary slightly:

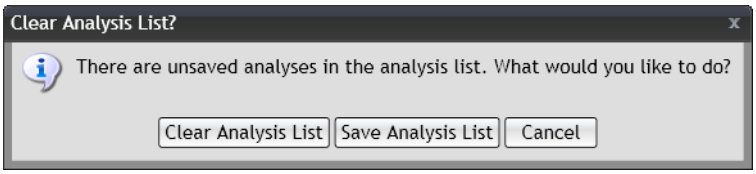
If...	Then...
Importing Data Sets into a new Composite or Compensation Composite entry,	Locate the files you wish to assign to the Composite or Compensation Composite entry. You may choose files from your computer or from within the current Analysis List. **1.** Multi-select the files and drag them into any cell within the Data Set column of the Composite/Compensation Composite file **or** drag them one-by-one into separate rows of the Data Set column. **NOTE** Even though new Composite and Compensation Composite entries have a preset number of rows, you can add rows by continuing to drag files into the Analysis List row entry. You can also delete rows. **2.** When the appropriate Analysis List cell (or row) is orange, release the mouse button.
Creating a Composite/Compensation Composite file using files that are currently on the Analysis List,	Multi-select the files on the Analysis List that you wish to include in the Composite or Compensation Composite file. The Analysis Options screen displays. Select the appropriate button from the Analysis Options screen.

2 Continue building your Composite or Compensation Composite file. See CHAPTER 2, *Composite Protocols* or CHAPTER 2, *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature*.

Clear Analysis List

To clear the current Analysis List, click the Clear Analysis List button, which is located below the Analysis List header. If there are no unsaved changes within the Analysis List, the Data Sets clear. If, however, there are unsaved changes within the Analysis List, the following message appears:

Figure 1.30 Clear Analysis List



Select the appropriate option based on the outcome you need:

- **Clear Analysis List**: Clears the list and does not save any changes made within the Data Sets.
- **Save Analysis List**: Saves all entries as single *.analysis file. See *Save Analysis List*.
- **Cancel**: Returns you to the current list without making any changes.

Save Analysis List

You can save multiple Analysis List entries as a single Analysis List file, allowing for easy retrieval. When saving, changes are applied to the *.Analysis file only. The original data file remains unchanged.

To save **selected entries** as an Analysis List:

1 Determine which files you wish to include in the Analysis List file.

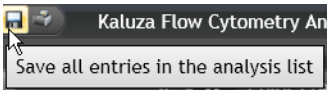
- To save **all** of the open files as an Analysis List, click once within the Analysis List pane and press Ctrl + A on your keyboard to select all the files.
- To save **a selected group** of open files as an Analysis List, Ctrl + click each file you wish to be included in the Analysis List file.

NOTE The options available when multiple files are selected are displayed on the Analysis Options screen.

2 Select **Save Selected as Analysis List** from the Analysis Options screen.

NOTE Additional options for saving an Analysis List include using the shortcut Ctrl + S on your keyboard or pressing the Save button on the Quick Access toolbar (Figure 1.31). Additionally you can either right-click on one of the selected files and click **Save selected as** > **Analysis List** or select the Application button and choose **Save selected as** > **Analysis List**.

Figure 1.31 Save Icon—Quick Access Toolbar



3 When the Save Analysis dialog box appears, select the destination for the file by navigating to the location using icons in the dialog box or the drop-down in the **Save in:** field.

4 Enter a file name in the **File name:** field.

5 Select **Save**.

To save a **subset** of the Analysis List:

1 Select the entries you wish to save.

2 Perform one of the following operations:

- Select > **Save Selected As** > **Analysis List**.

  OR
- Right click in the Analysis List > **Save Selected As** > **Analysis List.**

To save the **entire** Analysis List, select one of these options:

- Use the Quick Access toolbar Save option.

  OR
- From the Application Menu, click **Save** or use the shortcut Ctrl + S .

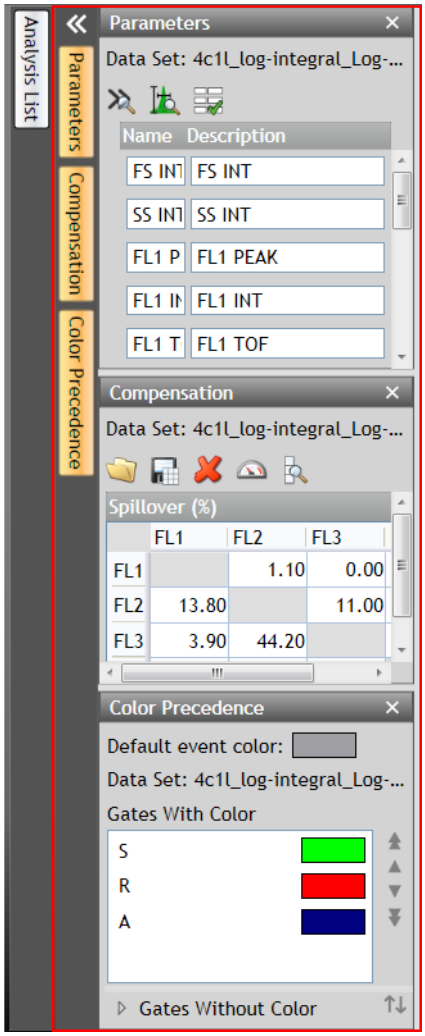
Attributes Panes

The Attributes Pane (Figure 1.32) is located next to the Analysis List and is comprised of three component panes: the Parameters, Compensation, and Color Precedence panes.

Attributes Pane

See *Display Options for the Analysis List and Attributes Pane*, for details on the Attributes Pane display.

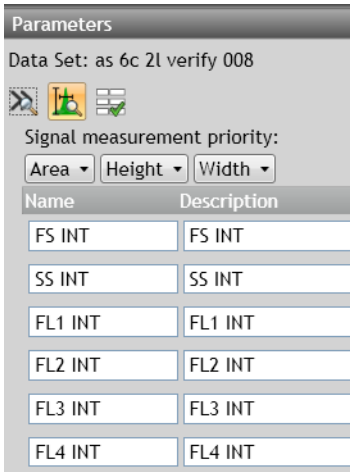
Figure 1.32 Attributes Pane



Parameters Pane

The parameters pane (Figure 1.33) is a list of the parameters collected in the original Data Set file. This pane enables you to alter parameter names, descriptions, types, detectors, and measurement type. See CHAPTER 2, *Parameters*, for complete instructions on updating parameters from within the Parameters pane.

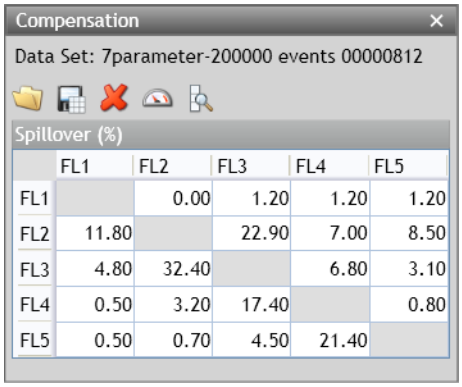
Figure 1.33 Parameters Pane



Compensation Pane

The Compensation pane (Figure 1.34) contains tools for adjusting the compensation Spillover and Autofluorescence Vector values related to a particular Data Set. See CHAPTER 2, *Adjusting Compensation*, for in-depth instructions on how to use the Compensation pane.

Figure 1.34 Compensation Pane

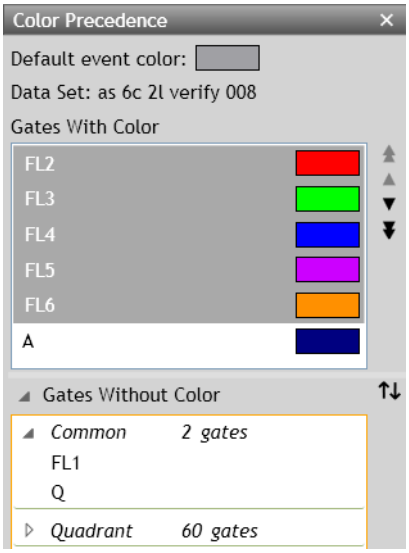


	FL1	FL2	FL3	FL4	FL5
FL1		0.00	1.20	1.20	1.20
FL2	11.80		22.90	7.00	8.50
FL3	4.80	32.40		6.80	3.10
FL4	0.50	3.20	17.40		0.80
FL5	0.50	0.70	4.50	21.40	

Color Precedence Pane

The Color Precedence pane (Figure 1.35) displays event coloring and precedence of coloring for gates in the current Protocol. See CHAPTER 2, *Establishing Color Precedence of Gates*, for in-depth instructions on how to use the Color Precedence pane.

Figure 1.35 Color Precedence Pane



Display Options for the Analysis List and Attributes Pane

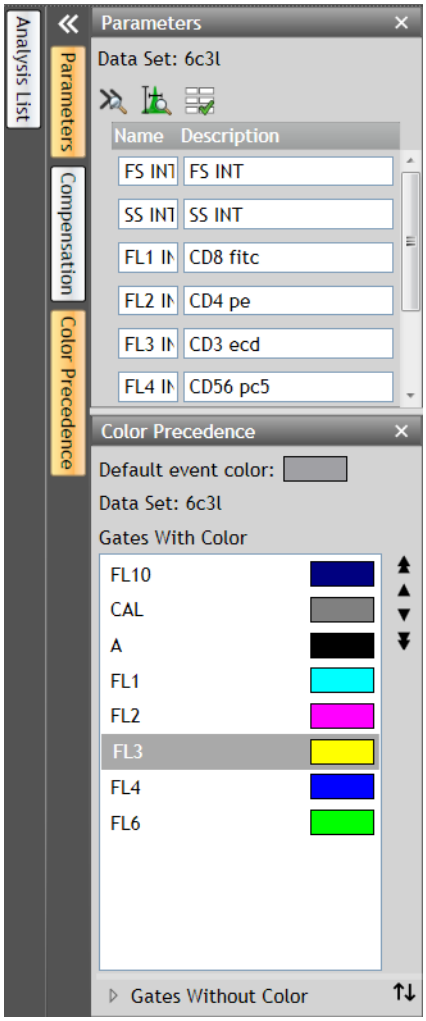
As a default, Kaluza Analysis displays the Analysis List and the three component panes of the Attributes pane. To optimize your workspace, you may wish to change the size or hide a component of a pane, or even the entire pane.

Hiding a Component Pane

To hide a component pane, select the button in the component pane you wish to close.

The three Attributes component panes each have vertically-docked buttons, where the color indicates the status of the pane. The white button indicates the pane is closed, and a gold button indicates that the pane is open. When the Analysis List has been closed, it is shown as a vertically-docked white button. For example, in Figure 1.36, the Analysis List and the Compensation panes had been closed.

Figure 1.36 Component Pane



NOTE An additional way to close an Attributes component pane is to select the gold button corresponding to the pane you wish to hide.

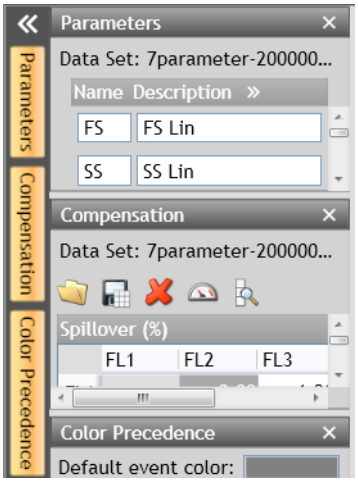
Displaying a Component Pane

To re-open a pane:

1. Select the white button corresponding to the pane you wish to open.

2. As a result, the button turns gold, and the pane appears. See Figure 1.37, which shows all three components of the Attributes pane open/active.

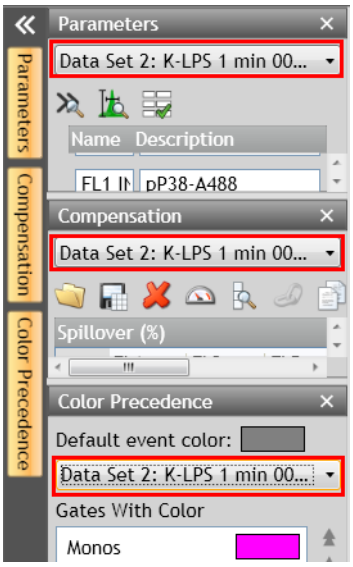
Figure 1.37 Attributes Pane - Components



Selecting the Data Set in a Composite

When working with a Composite, a Data Set selected in any of the three Attributes Pane components (Figure 1.38) will be correspondingly selected for all of the other Attributes Pane components: Parameters, Compensation and Color Precedence.

Figure 1.38 Attributes with Composite



Hiding the Attributes Pane

To hide the entire Attributes pane, select «, located at the top of the component pane buttons.

Displaying the Attributes Pane

Restore the hidden Attributes pane by selecting », which is located at the top of the component buttons.

NOTE When the Attributes pane is restored, it is restored with the same active component panes as when it was hidden from view.

Resizing the Analysis List or Attributes Panes

To resize the Analysis List or the Attributes pane:

1. Hover your mouse over the right edge of the pane until the cursor changes to a double-sided arrow (↔).
2. Click and drag the edge of the pane to the right or left, depending on whether you need to make it smaller or larger.
3. When you are satisfied with the size, release the mouse button.

Resizing Attribute Component Panes

To resize (lengthen or shorten) the Parameters, Compensation, or Color Precedence panes:

1. Hover your mouse over the bottom edge of the pane until the cursor changes to a double-sided arrow (↕).
2. Click and drag the edge of the pane up or down, depending on whether you need to make it smaller or larger.
3. When you are satisfied with the size, release the mouse button.

Ribbon

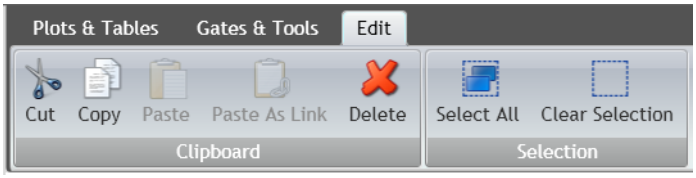
The Ribbon, which is located directly above the sheet workspace, contains tabs for convenient access to the most-used items within the application. The tabs that display can change, given the current task you are completing. Refer to the following sections for details on each tab type:

- *Plots & Tables Tab*
- *Gates & Tools Tab*
- *Edit Tab*
- *Page Layout Tab*

Switching Active Ribbon Tabs

See Figure 1.39. To switch between active tabs, select the title of a different tab on the Ribbon Toolbar.

Figure 1.39 Ribbon Toolbar



Hiding the Ribbon Toolbar

To maximize the sheet area, you can hide the contents of the Ribbon so that just the header is in view. To hide the Ribbon toolbar, double click on any of the Ribbon tabs.

Restoring the Ribbon Toolbar

There are two options for restoring a previously hidden toolbar.

- **Temporary restoration**: To temporarily restore the Ribbon toolbar, click once on the Ribbon tab you wish to view. The Ribbon toolbar appears until you click your mouse in another area of the application.
- **Complete restoration**: To completely restore the Ribbon toolbar, double-click on any Ribbon tab.

Using the Ribbon Toolbars

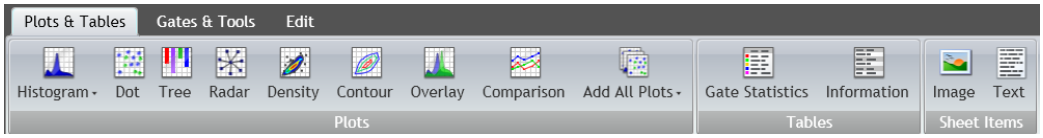
To make changes or add items to a sheet, use one or both methods described below:

- **Selecting the icon located on the tab**: Select the icon for the specific item you need; this either changes your cursor or adds the new item you selected below any items already on the sheet.
- **Dragging and dropping**: Select the item that you wish to add to the sheet, and then drag and drop it in the location of your choice.

Plots & Tables Tab

See Figure 1.40. The Plots & Tables Ribbon tab is divided into three sections: Plots, Tables and Sheet Items.

Figure 1.40 Plots & Tables Tab



Plots

The Plots section of the Plots & Tables tab displays all plots that are available. Refer to the following sections for details:

- CHAPTER 2, *Histograms*
- CHAPTER 2, *Dot, Density, and Contour Plots*
- CHAPTER 2, *Tree Plots*
- CHAPTER 2, *Radar Plots*
- CHAPTER 2, *Overlay Plots*
- CHAPTER 2, *Comparison Plots*
- CHAPTER 2, *Add All Plots*
- CHAPTER 2, *Merged Data Sets*

Tables

From the Tables section of the Plots & Tables tab, you can choose to add a Gate Statistics Table, which displays gate color, logic, and statistics, or an Information Table; which is a table showing the raw data keywords and calculated parameter results that you choose to display and output. See CHAPTER 2, *Information Table*, or CHAPTER 2, *Gate Statistics Table*.

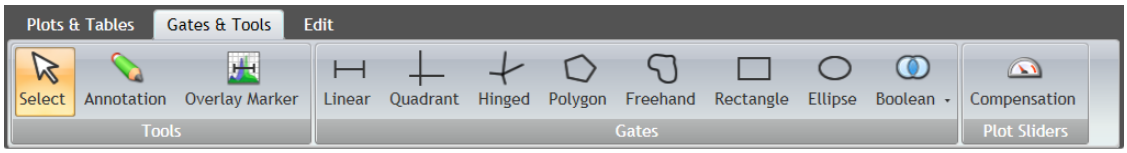
Sheet Items

The Sheet Items section of the Plots & Tables tab is used for adding an image or text to your sheet. See CHAPTER 3, *Adding an Image to a Sheet*, or CHAPTER 3, *Adding Text*.

Gates & Tools Tab

See Figure 1.41, for an example of the Gates & Tools Tab Ribbon.

Figure 1.41 Ribbon - Gates & Tools Tab



Tools

Change your cursor to a different mode by selecting one of the tools described in the following sections:

- CHAPTER 2, *Selection Tool*
- CHAPTER 2, *Annotation Tool*
- CHAPTER 2, *Overlay Marker*

Gates

The Gates section of this tab displays all options available for gating data. Refer to the following sections for details:

- CHAPTER 2, *Linear Gates*
- CHAPTER 2, *Quadrant Gates*
- CHAPTER 2, *Hinged Quadrant Gates*
- CHAPTER 2, *Polygon Gates*
- CHAPTER 2, *Freehand Gates*
- CHAPTER 2, *Rectangle Gates*
- CHAPTER 2, *Ellipse Gates*
- CHAPTER 2, *Boolean Gates*

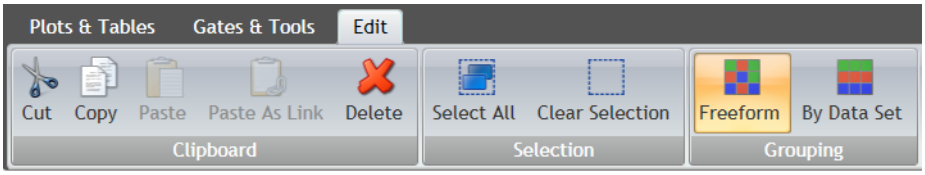
Plot Sliders

Choosing the Compensation icon from the Plot Sliders section of the Gates & Tools tab displays the Compensation Sliders on all applicable plots on the plot sheet. See CHAPTER 2, *Adjusting Spillover Using the Spillover Sliders Directly on the Plot(s)*, for details.

Edit Tab

The Edit tab contains the functions necessary for making edits to the Plot Sheet or Report Sheet. See Figure 1.42 for an example of the Edit tab while working on a Composite Analysis.

Figure 1.42 Ribbon - Edit Tab



Clipboard

The Clipboard section of this tab displays all of the editing options available for sheets. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*. These options include:

- Cut
- Copy
- Paste
- Paste as Link
- Delete

Selection

The Selection section of the Edit tab enables you to select/deselect items on your sheet. See *Selecting Sheet Items*.

Grouping

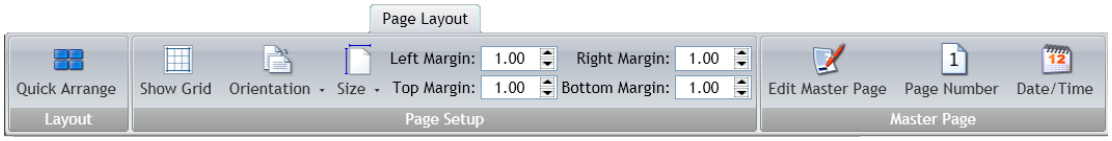
The Grouping section of the Edit tab includes two options for displaying plots in a Composite or Compensation Composite. Refer to the following sections for details:

- CHAPTER 2, *Freeform Arrangement*
- CHAPTER 2, *By Data Set Arrangement*

Page Layout Tab

See Figure 1.43, for an example of the Page Layout Ribbon tab.

Figure 1.43 Ribbon - Page Layout Tab



The Page Layout Ribbon tab is available only when using a report sheet. The options available from the Page Layout Ribbon tab include:

Layout

The Layout section of the Page Layout tab includes the Quick Arrange icon. See CHAPTER 3, *Layout*.

Page Setup

The Page Setup section of the Page Layout tab provides options to customize your report pages. See CHAPTER 3, *Page Setup*. These options include:

- Show Grid
- Orientation
- Size
- Margin

Master Page

The Master Page portion of the Page Layout tab gives options for creating or making changes to a master page. See CHAPTER 3, *Master Page*. These options include:

- Edit Master Page
- Page Number
- Date/Time

Sheet Tab Bar

The Sheet Tab Bar (Figure 1.44) is located at the bottom of the sheet area. The Sheet Tab Bar provides three main functions:

- Change zoom
- Add new sheet
- Switch between sheets using the sheet tabs

See CHAPTER 3, *Using the Sheet Tab Bar*, for additional information.

Figure 1.44 Sheet Tab Bar

Basic Editing for Plots, Gates, and Sheet Items

You can use the Edit Ribbon tab, Edit Radial Menu (available through the icon), or keyboard shortcuts to perform basic editing functions, including cut, copy, paste, paste as link, delete, and save as image. Table 1.7 provides details regarding the availability of these functions and any specific details regarding use.

Table 1.7 Editing Plots, Gates, and Sheet Items

Description	Icon/Shortcut			Use on...		
	Edit Radial Menu	Edit Ribbon Tab	Keyboard Shortcut	Plots	Gates	Sheet Whitespace
Cut is used to remove an item from the sheet or plot. The removed item is available for pasting to any valid location.		Cut	Ctrl + X	✓	✓	-
Copy is used to duplicate selected items. The selected item is available for pasting to any valid location.		Copy	Ctrl + C	✓	✓	-
Paste inserts data made available by **Cut** or **Copy** to the location of your choice. **NOTE** Exception: Report Sheet Date/Time and Page Number cannot be pasted into Plot Sheets.		Paste	Ctrl + V	✓	✓	✓

Table 1.7 Editing Plots, Gates, and Sheet Items

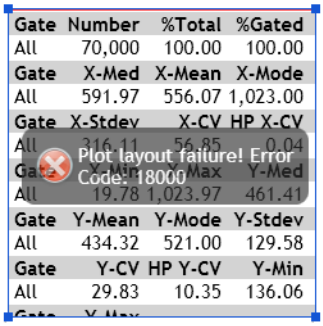
Description	Icon/Shortcut			Use on...		
	Edit Radial Menu	Edit Ribbon Tab	Keyboard Shortcut	Plots	Gates	Sheet Whitespace
Paste as Link allows you to paste a copied plot as a linked item, which means that the pasted plot, as well as any gates or other data located on the plot, change when the original changes and vice versa. Once a plot is linked to another, the symbol displays in the upper-left-hand corner of the both the original and the linked item. **NOTE** If you receive an error similar to the one shown in Figure 1.45 when pasting a plot as a link on a report sheet, resizing the plot so that all of the content is showing eliminates the error. **Figure 1.45** Paste as Link Error 	Paste As Link	-	✓	-	-
Delete eliminates a selected item. **NOTE** The only way to retrieve an item that has been deleted is by selecting the undo icon ( ).		Delete	Delete	✓	✓	-
Save as Image saves sheet items as a 600 dpi *.png or *.tif image file. **NOTE** 1. Only one item may be saved as an image at a time. 2. When you use another application to display your sheet item or export event data, icons and links either do not appear, or they appear as images only, not hyperlinks.		-	-	✓	-	-

Table 1.7 Editing Plots, Gates, and Sheet Items

Description	Icon/Shortcut			Use on...		
	Edit Radial Menu	Edit Ribbon Tab	Keyboard Shortcut	Plots	Gates	Sheet Whitespace
Export Compensated Event Data is an advanced feature that can be used to enable analysis in statistics packages or to validate the Kaluza Analysis statistics. The full-range, compensated event data for all enabled parameters in the selected plot or gate is written to a *.csv file. This feature allows: • Exporting only one item at a time. • Exporting data only from histograms, bivariate plots, radar plots, tree plots, and gates.		-	-	✓	✓	-

NOTE

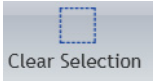
1. Plots, gates, and other sheet items must be selected prior to performing editing tasks. Most functions are available for multi-selection.

2. If the number of events is >102400 when exporting compensated event data, a progress bar will display allowing the export to be canceled, if desired.

Selecting Sheet Items

Select the icons from the Edit Ribbon tab to perform the functions described below:

Table 1.8 Edit Ribbon—Selection Descriptions

Icon	Function
	**Select All** selects all items located on the active sheet. **NOTE** Ctrl + A also selects all items located on the active sheet.
	**Clear Selection** removes any items currently selected on the active sheet from Selection mode. **NOTE** Clicking your mouse on the sheet whitespace also clears any selected items from Selection mode.

CHAPTER 2

Data Analysis

Kaluza Analysis Files

Table 2.1 lists the types of files that you can create using Kaluza Analysis, as well as important details about the content included in each file type. Review the table to determine the file type you need to create, and then refer to the appropriate section within this chapter for details on creating each file type.

NOTE For systems with multiple users, file security is achieved by setting up individual User Accounts on your operating system. For additional information, see CHAPTER 1, *User Preferences and Security*.

Table 2.1 Kaluza Analysis File Types

File Type	Extension	Saving Mechanism	What is Saved	What is NOT Saved
Analysis	*.analysis	Save As	• Data Set • Plots (including all customizations) • Tables • Gates (including gate coloring definitions) • Parameter definitions • Compensation Spillover Matrix and Autofluorescence Vector values • Annotations • All sheets included with the analysis	-
Analysis List	*.analysis	Save	All entries in the Analysis List (even if a Data Set is not present) are saved as a bundle.	-

Table 2.1 Kaluza Analysis File Types

File Type	Extension	Saving Mechanism	What is Saved	What is NOT Saved
Protocol	*.protocol	Save selected as[a]	• Plots (including all customizations) • Tables • Gates (including gate coloring definitions) • Annotations • All sheets included with the analysis • Compensation Spillover Matrix and Autofluorescence Vector values[b]	Data Sets are not saved with a ***.protocol** file.
Composite	*.composite	Save selected as[a]	• Plots (including all customizations) • Tables • Gates (including gate coloring definitions) • Annotations • All sheets included with the analysis • The number of available Data Set entries within the Analysis List • Compensation Spillover Matrix and Autofluorescence Vector values[b]	Data Sets are not saved with a ***.composite** file.
Compensation	*.compensation -or- *.txt	Save selected as[a]	Compensation Spillover Matrix and Autofluorescence Vector values only.	Protocols, Data Sets, sheets, etc.

a. **Save selected as** must be selected ***each*** time you wish to save an entry as any file type other than a ***.analysis** file.

b. Compensation and autofluorescence is imported by **Paste Special** when updating an entry in the Analysis List. Also available when importing the Protocol into Kaluza for Gallios.

Protocols

Rather than repeatedly setting up Analysis files for each raw Data Set, you can set up and save Protocols, allowing you to develop standards, save time, and provide consistent results for easier data comparison.

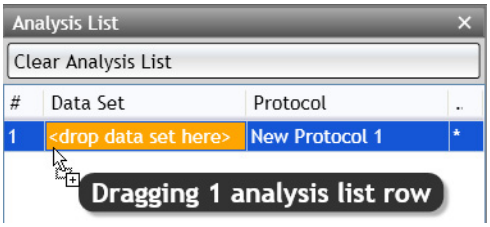
Creating a New Protocol

To create a new Protocol:

1 Select > **New** > **Protocol**. This will create a new entry in the Analysis List.

2 Locate the raw Data Set file from which you would like to create your Protocol. Drag and drop the file into the Data Set column. The cell contains the instructions "**<drop data set here>**," as shown in Figure 2.1. The raw Data Set file is now imported into Kaluza Analysis and is ready for you to begin your Analysis. See *Plots & Tables*, *Gates & Tools*, and *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature*, for complete details on creating the Analysis.

Figure 2.1 Importing a Data Set



NOTE Depending on the source of the raw data file, e.g., Kaluza for Gallios, the runtime protocol might automatically appear when the data is loaded into Kaluza Analysis.

3 When you are satisfied with your Protocol, you may save the Protocol alone, or you may save the Analysis.

- To save the Protocol only, see *Saving a Protocol*.
- To save the Analysis, see *Saving an Analysis*.

Saving a Protocol

To save the Protocol from an Analysis:

NOTE When saving a file as a Protocol, only the Protocol-related information will be saved; i.e., the plot types including the specific parameters associated with each plot and the gates. Saved Protocol files are used for the Analysis of raw Data Sets.

1 Select the Analysis List row of the protocol you wish to save.

2 Select > **Save selected as** > **Protocol**.

3 In the Save Protocol dialog box, select the destination for the file by navigating to the location using icons in the dialog box or the drop-down list in the **Save in** field.

4 Enter a file name in the **File name** field.

5 Select **Save**. The file is saved with the ***.protocol** extension and is now ready to apply to raw Data Sets.

Saving an Analysis

To save an Analysis:

1 Select the Analysis List row of the analysis you wish to save.

2 Select > **Save selected as** > **Analysis**.

3 In the Save Analysis dialog box, select the destination for the file by navigating to the location using icons in the dialog box or the drop-down list in the **Save in** field.

4 Enter a file name in the **File name** field.

5 Select **Save**. The file is saved with the ***.analysis** extension.

Applying a Protocol to a Raw Data Set

A protocol can be imported from several different file types, including *.protocol files, analysis files, *.lmd files, and *.fcs files produced by Kaluza for Gallios. See CHAPTER 1, *Replacing or Importing a Data Set or Protocol into an Analysis List Row* for additional information on applying a Protocol to a Data Set.

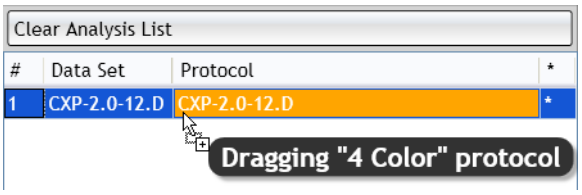
IMPORTANT Check the Parameters pane for data mismatch. See *Resolving Parameter Mismatch*.

When working with *.lmd files containing embedded protocols, consider the following:

1. Kaluza Analysis imports gates, regions, plots, and color precedence information. **If, however, a gate and a region from the embedded source protocol have the same name, only the region is imported. The gate with the same name is not imported. Adjust gating as necessary.**
2. If a *.lmd file is opened with an embedded protocol that contains an orphan gate, the orphaned gate will be present in color precedence, but there is no geometric entity (no gate viewed in a plot) for the user to manipulate. A plot displaying the orphan gate can be created, or the orphan gate can be deleted, from the Kaluza Analysis color precedence pane. See *Establishing Color Precedence of Gates*.
3. Statistics are not imported from *.lmd files.

To apply a protocol to a Raw Data Set, use one of the three methods described in Table 2.2:

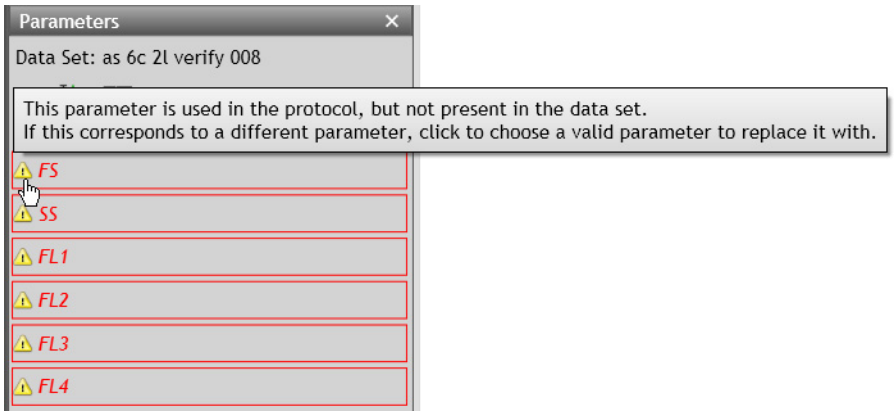
Table 2.2 Applying a Protocol to a Raw Data Set

Method	Procedure
Copying from Another Data Set	**1.** Select the Analysis List row containing the Protocol you wish to apply to another Data Set. **2.** Right click on the row, and select **Copy**. **3.** Select the Analysis List row(s) to which you wish to apply the copied Protocol. See CHAPTER 1, *Multi-Selecting Files* for additional information on selecting multiple Analysis List rows. **4.** Select **Paste Special** > **Protocol**.
Dragging from Another Data Set	**1.** From the Analysis List, select the Protocol you wish to use for another Raw Data Set. **2.** With the Protocol still selected, drag it to the cell in the Protocol column corresponding to the Data Set to which you wish to apply the Protocol, and then and release the mouse button when the cell becomes highlighted in orange.
Dragging from a File	**1.** Drag the Protocol file into the Protocol column of that Analysis List row (refer to Figure 2.2). **Figure 2.2** Applying a Protocol to a Raw Data Set 

Resolving Parameter Mismatch

If the parameters derived from the raw data file do not match with those that have been set up in the Protocol, you'll receive a warning message. Figure 2.3 is an example of a warning message showing for each mismatched parameter.

Figure 2.3 Parameter Mismatch



To resolve this parameter mismatch condition:

1. Click within the red rectangle; this prompts the appearance of a drop-down list, which provides options for replacing the mismatched reference with the correct parameter.

2. Choose the appropriate parameter from the list. This removes the warning and updates the parameter name/description.

NOTE References to the parameter on plots also appear in red, indicating a mismatch error. When the error is corrected, the parameter name/description is updated to match the one in the Protocol.

Applying a Different Protocol to an Analysis Entry

To change the Protocol used for Data Set analysis:

1. Open the Analysis file by dragging and dropping into the Analysis List.

2. Locate the file that contains the Protocol you wish to apply to the Data Set and drag the file into the Protocol column of that Analysis List row, replacing the current Protocol (make sure the designated cell is highlighted in orange prior to releasing the mouse button). The Data Set for this file now has the new Protocol associated with it.

   IMPORTANT This action completely replaces the Protocol previously associated with the Data Set.

Composite Protocols

Composite Protocols allow you to create one Protocol that links multiple Data Sets. When you save a Composite Protocol, it retains all of the plot types, the specific parameters associated with the plots, and the gates, allowing you to import raw Data Sets and conduct the same Analysis.

Kaluza Analysis also offers the ability to assign colors to gates on an individual data set basis. In a composite, each data set has its own set of gates. These gates are not shared across data sets. To position gates from different data sets in the same location, use the Link Gates feature (see *Link to Gates*).

Creating a New Composite Protocol

To create a new Composite:

1. Load the files into the Analysis List that you wish to include in your Composite.

2 Multi-select the Analysis List entries (CHAPTER 1, *Multi-Selecting Files*) that you wish to add to your Composite.

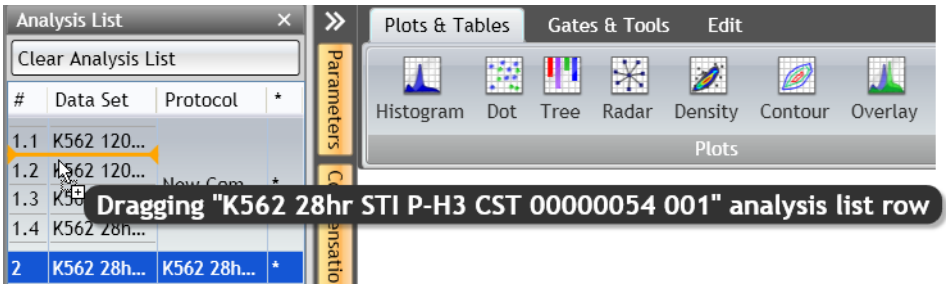
NOTE You can import up to 32 Data Sets/analyses into your Composite.

3 From the Analysis Options screen, select **Add selected to new Composite**.

4 If you wish to change the sequence of the entries in the Composite, follow the instructions provided in CHAPTER 1, *Changing the Sequence of Analysis List Rows*.

5 If you wish to add a data set to the Composite at any time, select, drag, and drop the file into the Composite entry on the Analysis List. Be sure to drop the Data Set between two rows if you do not wish to replace the current contents of the cell. As shown in Figure 2.4, the orange line indicates that an additional Data Set is being added to the Composite.

Figure 2.4 Adding a Data Set to a Composite Entry



The Composite Analysis is ready to be customized. See *Setting Up a Composite Protocol*, for instructions.

To save the Composite as an Analysis file so that you can return to it at a later time to complete the Composite Protocol, see *Saving a Composite Analysis*.

NOTE See CHAPTER 1, *Applying Data Sets to a Composite or Compensation Composite File*, for an alternative method for creating a Composite Protocol. When creating a new composite the plots, gates and sheets are automatically imported from the added dataset files.

Saving a Composite Analysis

To save the Composite Analysis, which includes all Data Sets, as well as the Composite Protocol:

1 On the Analysis List, select the Composite entry that you wish to save as a Composite Analysis.

2 Select > **Save selected analysis.**

3 In the Save Analysis dialog box, select the destination for the file by navigating to the location using icons in the dialog box or the drop-down list in the **Save in** field.

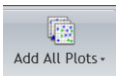
4 Enter a file name of your preference in the **File name** field.

5 Select **Save**. The file is saved with the ***.analysis** extension.

Setting Up a Composite Protocol

The following sections describe the options available for setting up a Composite Protocol. To create a Composite Protocol, see *Creating a New Composite Protocol*.

Add All Plots

Select the 

icon to add plots for each Data Set to the plot sheet. For each Data Set included in the Protocol, these plots include:

- A gating plot
- Fluorescence parameters compared to each other using dot plots
- Each parameter compared to count using a histogram

IMPORTANT

- The Add All Plots Option allows you to configure the inclusion of the gating plot and fluorescence plots and gates. See *Adding Plots to the Plot Sheet*.

- If the Add All Plots options are configured such that the number of plots or gates created will exceed the limit allowed by the software, an error message will be displayed.

Overlay Plots in a Composite Protocol

Histograms from any Data Set within the Composite may be added to an overlay plot. For additional details, see *Setting Up Overlay Plots*.

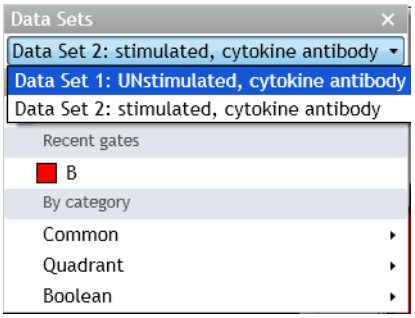
Changing the Data Set Associated with a Plot in a Composite Protocol

The Data Set associated with a plot may be selected or changed by completing the following steps:

1 Click on the plot header. A pop-up list appears.

2 Click where the current data set is shown to display a list of the Data Sets that you may choose from to apply to the plot, as shown in Figure 2.5.

Figure 2.5 Selecting a New Data Set



3 Select the appropriate Data Set.

Gating in Composite Protocol

In a Composite Protocol, each Data Set has its own set of gates. A gate created on one Data Set can only be used on that Data Set. To view the gates for a given Data Set, select the desired Data Set in the Color Precedence Pane. Gates from different data sets can have their coordinates linked together to keep them synchronized. See *Link to Gates*.

Linking Compensation Between All Data Sets

As a default, each Data Set within a Composite Analysis contains unique Spillover and Autofluorescence Vector values. Use the following steps to link Spillover and Autofluorescence Vector values so that all Data Sets contain the same values.

IMPORTANT For this option to be available, the fluorescence parameters that you wish to link must match between all data sets. Once the Spillover and Autofluorescence Vector values for each Data Set are linked, the values are retained. If the link is disabled, values do not return to those set prior to creating the link.

1 Update, if necessary, the Spillover and Autofluorescence Vector values that you would like to use as the default for all Data Sets within the Composite. Refer to *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature*, for details on various methods for adjusting Spillover values.

2 To update ALL Data Sets within the Composite to the Spillover and Autofluorescence Vector values currently displayed in the Spillover Matrix, select the **(Link compensation for all Data Sets)** icon, which is located in the Compensation pane. Once the Spillover and Autofluorescence Vector values are linked, the drop-down list located at the top of the Compensation pane is disabled.

NOTE As long as Spillover and Autofluorescence Vector values are linked, any changes made to the Spillover and Autofluorescence Vector values will update compensation for the corresponding parameter on all Data Sets.

To disable the compensation link between Data Sets, select the icon.

Copying Compensation to Other Data Sets

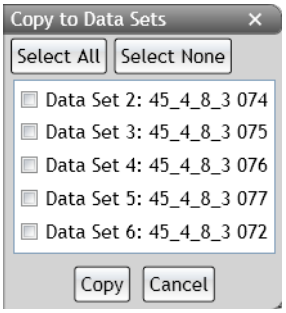
To copy Spillover and Autofluorescence Vector values to other Data Sets, use the following steps.

NOTE You cannot copy Spillover and Autofluorescence Vector values to a larger matrix. You can, however, copy Spillover and Autofluorescence Vector values to a matrix containing fewer fluorescence parameters.

1 From the drop-down list located in the Compensation pane header, select the Data Set that you wish to copy to other Data Sets.

2 Select the icon in the Compensation pane; this opens the **Copy to Data Sets** pop-up menu, as shown in Figure 2.6.

Figure 2.6 Copy to Data Sets Menu



3 Depending on the preferred outcome, do one of the following:

- **To copy to specific Data Sets**: Select the Data Set(s) you wish to copy Spillover and Autofluorescence Vector values to by clicking within the check box next to the Data Set name(s).
- **To copy to all Data Sets**: Click the Select All button.

You may remove any selections that have been made by clicking the Select None button or by deselecting the check box.

4 When you are satisfied with your selection(s), select Copy. The Spillover and Autofluorescence Vector values for all applicable Data Sets change.

IMPORTANT A notification of parameter name mismatch does not appear until after Copy is selected.

Saving a Composite Protocol

To save the Composite Protocol from an Analysis file:

NOTE When saving a file as a Composite, only the related information from a Protocol will be saved; i.e., the plot types, the specific parameters associated with the plots, and the gates. Raw Data Sets can be imported into saved Composite Protocol files.

1 On the Analysis List, select the Composite entry that you wish to save as a Composite Protocol.

2 Select > **Save selected as** > **Composite**.

3 In the Save Composite dialog box, select the destination for the file by navigating to the location using icons in the dialog box or the drop-down list in the **Save in** field.

4 Enter the file name in the **File name** field.

5 Select **Save**. The file has been saved with the ***.composite** extension and is now ready for importing raw Data Sets.

Arranging Data Sets

The Grouping section of the Edit Ribbon tab appears while working on Composite and Compensation Composite files. The contents of the Grouping section allow you to arrange data sets on the Plot Sheet. These options are described below.

Freeform Arrangement

The application defaults to the freeform arrangement. If the **By Data Set** arrangement had previously been selected, choose the 

icon to allow for selecting and moving plots to any location on the Plot sheet.

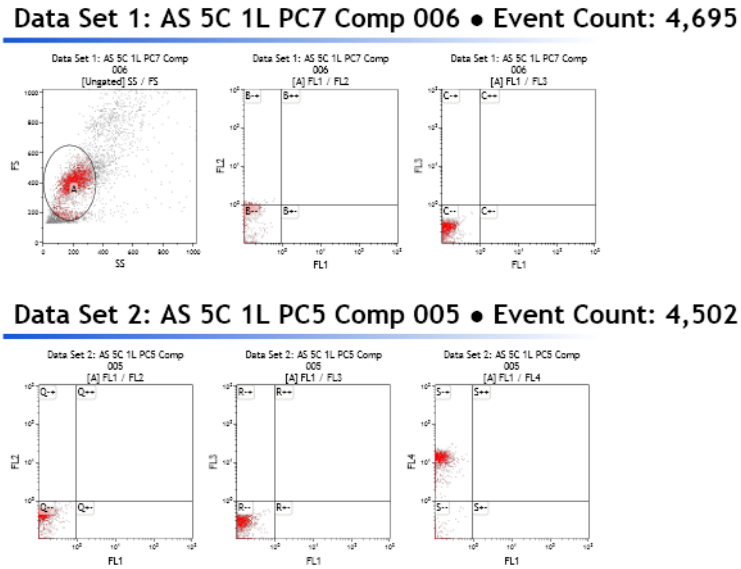
By Data Set Arrangement

Select the 

icon to arrange plots with an orderly separation by Data Set; the Plot Sheet displays as shown in Figure 2.7. Note that the separator displays not only the Data Set number, but the Data Set file name and the event count. Overlay plots are located in an **Other Items** category. Plots can only be moved within their own Data Set.

NOTE If you add new plots to the plot sheet from the Plots & Tables tab, plots default to Data Set 1.

Figure 2.7 By Data Set Arrangement



Plots & Tables

Kaluza Analysis supports histogram linear channel resolution up to 1024 channels, and offers eight different plot types and two tables, each of which can be customized to meet your needs.

The following sections describe the options available for setting up plots or tables.

Histograms

A Histogram represents a frequency distribution, where heights depict corresponding frequencies. The following parameter options are available for each axis:

Y-Axis:

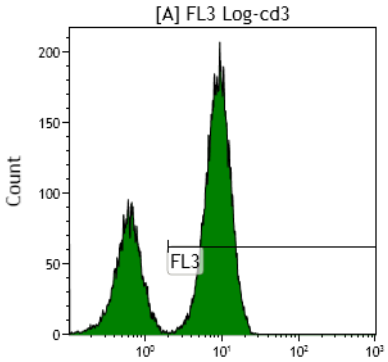
- Count
- % Gated

NOTE Selecting a parameter other than **Count** or **% Gated** for the Y-Axis parameter changes the plot to a dot plot. Any gates created for the histogram are removed when the plot type is changed.

X-Axis:

- Any parameter within the Data Set in linear, log, or logicle scale, or in integral seconds.

Figure 2.8 Histogram

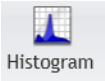


Setting Up Histograms

IMPORTANT The content within the Parameters pane directly affects how parameters are displayed on plots. See CHAPTER 2, *Copying and Pasting Parameters Between Data Sets* for complete instructions on updating parameter names, descriptions, types, detectors, and measurement types.

To set up a histogram:

---

1 From the Plots & Tables Ribbon tab, select the 

icon, and drag it to the preferred location on your sheet.

2 Hover your mouse over the parameter hyperlink at the bottom of the histogram.

3 Select the hyperlink. The list of parameters appears.

4 Select the new parameter.

If...	Then...
Using the default scale type for the parameter,	Select the parameter from the pop-up. You do not have to select the scale type. Default scale types are as follows: • **FS**/**SS**: Linear scale • **Fluorescence**: Log scale • **Time**: Seconds (integral)
Using a scale type other than the default,	1. Hover your mouse over the parameter. A pop-up appears, allowing you to choose the scale type for your parameter. 2. Select the scale that best suits your data. For additional details on available scale types, see *Choosing Scale Type*. **NOTE** The **Time** parameter only scales in seconds.

5 Select the hyperlink located on the Y-axis of the plot if you need to change the measurement type.

6 Choose the appropriate measurement type from the pop-up list.

NOTE Events in histograms default to being scaled on **Count**.

7 Choose a gate for your plot, if needed, using the hyperlink located at the top of the plot.

8 Continue customizing your plot using the Radial Menus. Table 2.3 provides specific information, as well as links to general options, for setting up a histogram.

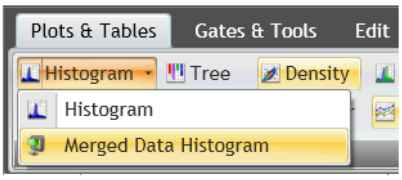
Table 2.3 Histogram Set-Up Options

Radial Menu	Plot Set-Up Details
Data	The **Histogram Options** section within the Data Radial Menu is for customizing your histogram for optimal data presentation. Specifically, these options are available: • Smoothing your data for a more pleasant appearance. • Clipping the first and last channels (for scaling purposes only). • Adjusting the Y-axis scale to better fit your data. See *Setting Up Plot Data*, for general information on making changes to plot data, including parameter axis data, and input gates.
Edit	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, paste as link, delete, export compensated event data, and save as image. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*.
Statistics	Use the Statistics Radial Menu to choose statistics to display at the bottom of the plot. See *Setting Up Statistics*.
Fonts	Use the Fonts Radial Menu to choose the font style and size.
Display	Use the Display Radial Menu to alter the size of a plot or to change the information that displays on a plot. See *Setting Up Plot Display*.
Gates & Tools	Use the Gates & Tools Radial Menu to add a gate, or annotation to a plot. See *Using the Gates & Tools Plot Radial Menu*. See *Linear Gates* for details on gating a histogram.
Coloring	Use the Coloring Radial Menu to change the background to black and to update the coloring associated with a plot. See *Using the Coloring Menu*.

Merged Data Histogram

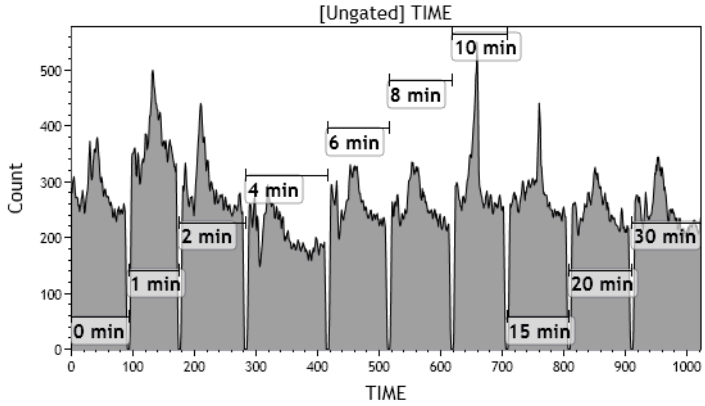
When a merged data set that includes the Time parameter is active, a drop-down option to create a Merged Data Histogram is available from the Plots & Tables Ribbon tab (Figure 2.9) or the Plots & Tables Radial Menu, which is accessed from the Plot or Report Sheet. Merged Data Histograms are automatically given the **Time** parameter on the X-Axis, and the **Count** parameter on the Y-Axis. For information on merging data sets, see *Merged Data Sets*.

Figure 2.9 Plots and Tables Menu



On Merged Data Histograms that use the Time parameter, Kaluza Analysis automatically creates gates on each Data Set, as well as a buffer between them. See Figure 2.10:

Figure 2.10 Merged Data Set Display—Using the "Time" Parameter

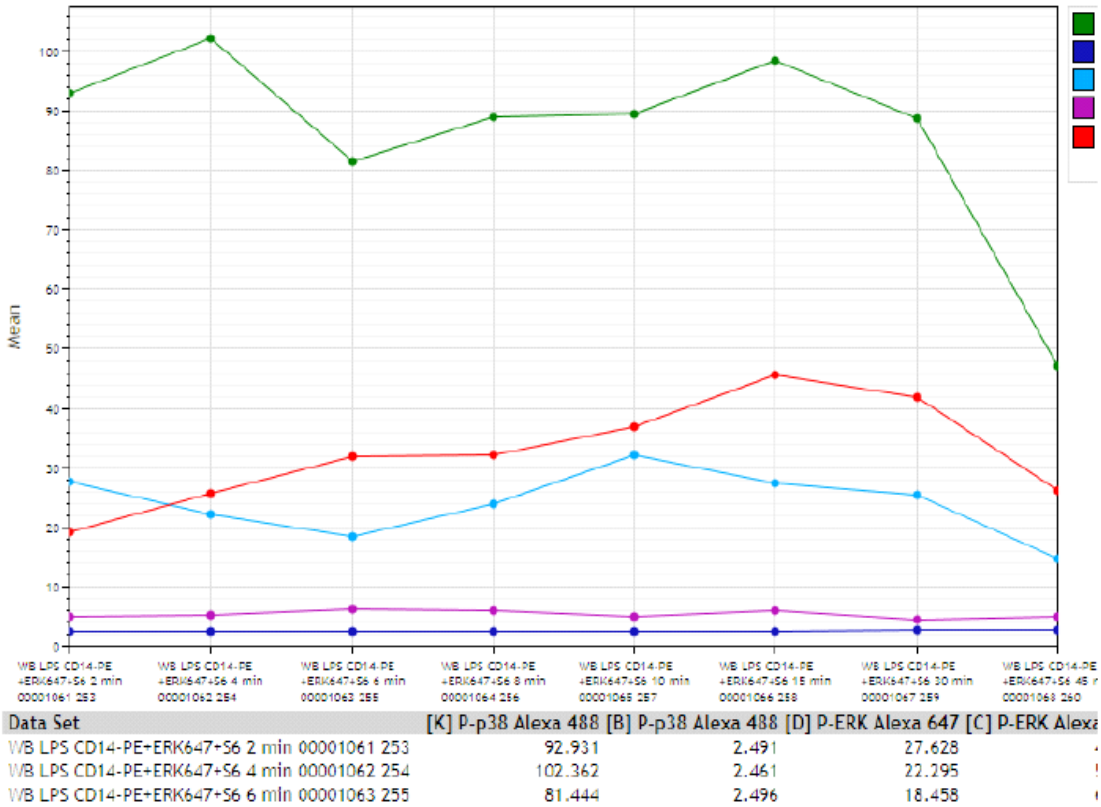


Comparison Plots

The Comparison Plot is a new plot type that allows:

- a given statistic to be compared across data sets and populations
- up to 12 series to be added to one Comparison Plot
- normalization based on an individual data set or a series selected control
- gate and color assignments for each series, as used to configure the plot
- statistics to be an optional item
- the data set name to display on the x-axis, while the y-axis offers several options of parameter statistics
- the plot to be used to track parameter statistics of multiple data sets associated with a kinetic studies. Figure 2.11, represents a kinetic assay for cell signaling.
- the Comparison Plot to default to a line-chart in Composite Protocols and a bar-chart in Single Data File Protocols.

Figure 2.11 Comparison Plot



Setting Up Comparison Plots

1 Select  from the Plots & Table Ribbon tab. This adds a new Comparison Plot to the sheet. By default, the x-axis will display the data set(s) identifier(s).

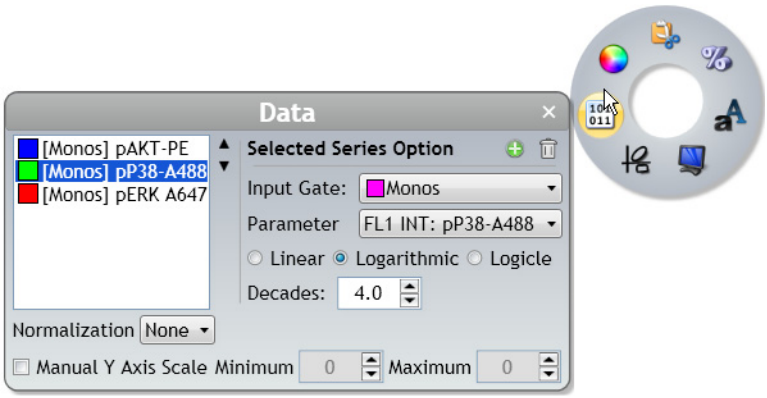
2 Select the corresponding Y-axis statistic on which you will gauge your histograms. Arithmetic Mean is the default statistic.

NOTE Select the Statistics (Arithmetic Mean) to change the statistics type from the pop-up list. Alternatively, you can also use the Statistics Radial Menu to select the desired statistic.

3 Select **Add series** on the upper right side of the plot.

4 Select the Series Color, Input Gate, and Parameter on the **Series Configuration** screen (Figure 2.12), for up to 12 series.

Figure 2.12 Series Configuration Screen



NOTE To have a complete graph display, the input gate must exist on all of the data sets in the composite.

5 Continue customizing the plot using the Radial Menus. Table 2.4 provides specific information, as well as links to general options for setting up a comparison plot.

NOTE Use the Radial Menu to change or delete a series, or click on the series name in the plot legend.

Table 2.4 Comparison Plot Set-Up Options

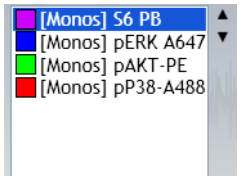
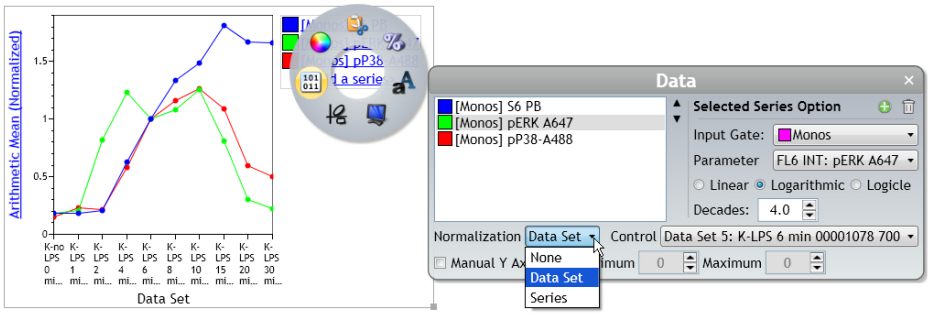
Radial Menu	Plot Set-Up Details
 **Data**	The **Series Selection** field is where a specific series can be selected for interacting within the **Selected Series Option** section of the Data Radial Menu. In addition, this field is where the order of the selected series can be changed by using the up or down arrows (located to the right of the field), as shown in Figure 2.13. **Figure 2.13** Up and Down Arrows  Specifically, these options are available: • Delete the selected histogram by clicking on the (**Delete**) icon. • Add a series by selecting the (**Add**) icon. • Input gate (alphabetical list of gates with color assignments indicated) • X-axis parameter/linear/log/logicle/decades • Normalization. Normalization of the data may be based on an individual data set or all data sets selected through the drop-down options (Figure 2.14). **Figure 2.14** Normalization Options  Normalization may be selected as None, Data Set or Series. If Normalization is selected to be based on Data Set or Series, a drop-down option display allowing you to select the Data Set or Series to use as the Control. The Y-axis label will indicate when data has been normalized. The **Y Parameter** section allows the manual adjustment of the Y-axis scale to better fit your data. You can enter scale limits manually or use the arrows to nudge them in either direction. Refer to *Setting Up Plot Data*, for general information on making changes to plot data, including parameter axis data and input gates.
 **Edit**	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, paste as link, delete, and save as image. Refer to CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for details.

Table 2.4 Comparison Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Statistics	Use the **Statistics** Radial Menu to choose statistics to display as the Y-axis of the Comparison Plot (the radio button allows for one statistic to be selected). Select the **Statistics Visible** checkbox to view the statistics at the bottom of the plot.
Fonts	Use the Fonts Radial Menu to choose the font style and size.
Display	Use the Display Radial Menu to alter the size of a plot or to change the information that displays on a plot. Use the **Graph Style** field to select between a Line Graph of Bar Graph display. When a comparison plot is created in a composite or compensation composite, by default it is displayed as a line graph. When a comparison plot is created in a single protocol, by default it is displayed as a bar graph. The **Axes** section offers options for the display of axis labels, tick marks, grid lines and legend. Additionally, the X-axis label may be customized by selecting “Use Custom Axes” and entering the desired X-axis label and/or X-axis tick mark labels. The Series section offers options to show/not show the input gate, name, description and scale. Additionally, the series names may be customized by selecting “Use Custom” and entering the desired names.
Gates & Tools	Use the Gates & Tools Radial Menu to add annotations. See *Annotation Tool*, for details.
Coloring	Use the Coloring Radial Menu to change the background to black and the colors of the series on the plot. See CHAPTER 2, *Using the Coloring Menu*, for details.

Dot, Density, and Contour Plots

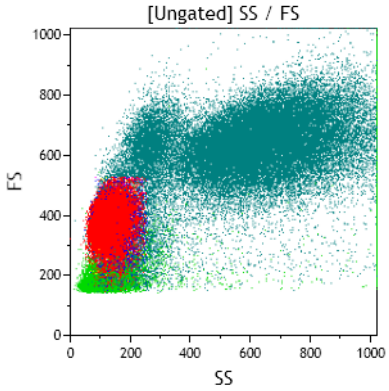
Dot, density, and contour plots compare two parameters to determine their relationship. You can choose any parameter within the Data Set for an axis. Density and contour plots are specific types of dot plots with different starting options for the coloring algorithm.

NOTE Selecting **Count** or **% Gated** as a Y-Axis parameter changes a dot, density, or contour plot to a histogram.

Dot Plot

A **dot plot** (Figure 2.15) compares two parameters to determine their relationship. Each event that contains markers for the two sets of data being compared appears as a dot.

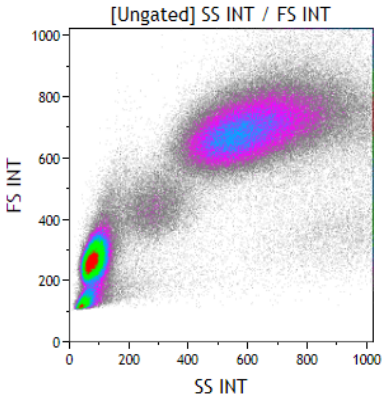
Figure 2.15 Dot Plot



Density Plot

A **density plot** (Figure 2.16) is a five-color representation of the distribution of events that occur in comparing X-axis and Y-axis parameters. Increases or decreases in event distribution are represented by different colors.

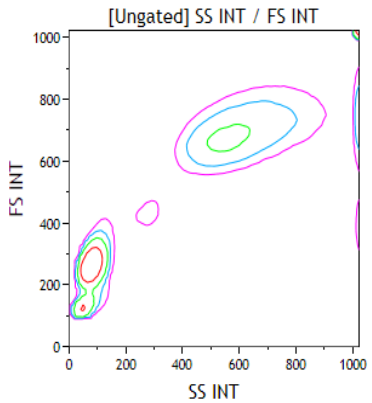
Figure 2.16 Density Plot



Contour Plot

A **contour plot** (Figure 2.17) is a colored outlined representation of the distribution of events that occur when comparing X-axis and Y-axis parameters. Increases or decreases in event distribution are represented by different colors. The number of colors in the display can vary (up to five), depending on the selected contour type, such as: plain contour (default), contour with density or contour with outliers.

Figure 2.17 Contour Plot



Setting Up Dot, Density, and Contour Plots

To set up a dot, density, or contour plot:

---

1 From the Plots & Tables Ribbon tab, select the 

, 

, or 

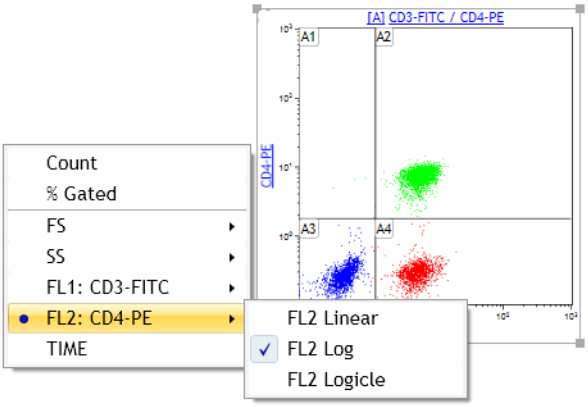
icon, and drag it to the preferred location on your sheet.

---

2 Select the **<Choose a parameter>** hyperlink. A pop-up appears with a list of parameters.

NOTE If you are updating a parameter, the currently selected parameter contains a dot next to the parameter name/description (**FL2** in Figure 2.18), and the scale used for the parameter contains a check mark next to the scale type (**FL2 Log** in Figure 2.18).

Figure 2.18 Selected Parameter and Parameter Scale



3 Make your selection by clicking on the appropriate parameter from the pop-up menu.

If...	Then...
Using the default scale type for the parameter,	Select the parameter from the pop-up. You do not have to select the scale type. Default scale types are as follows: • **FS**/**SS**: Linear scale • **Fluorescence**: Log scale • **Time**: Seconds (integral)
Using a scale type other than the default,	**1.** Hover your mouse over the parameter. A pop-up appears, allowing you to choose the scale type for your parameter. **2.** Select the scale that best suits your data. For additional details on available scale types, see *Choosing Scale Type*. **NOTE** The **Time** parameter only scales in seconds.

4 Repeat this process for the other parameter, if necessary.

5 If needed, choose a gate for your plot using the hyperlink located at the top of the plot.

6 Continue customizing your plot using the Radial Menus. Table 2.5 provides specific information, as well as links to general options, for setting up a dot, contour, or density plot.

Table 2.5 Dot, Contour, and Density Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Data	The **Bivariate Options** section of the data menu also provide the option to swap axes with the click of a button. This automatically reorients any gates on the plot. See *Setting Up Plot Data*, for general information on making changes to plot data including parameter axis data, and input gates.
Edit	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, paste as link, delete, export compensated data, and save as image. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*.
Statistics	Use the Statistics Radial Menu to choose statistics to display at the bottom of the plot. See *Setting Up Statistics*.
Fonts	Use the Fonts Radial Menu to choose the font style and size.
Display	Use the Display Radial Menu to: • Alter the size of a plot. • Change information that displays on a plot. • Change the bivariate resolution; options include: — 128 X 128 — 256 X 256 — 512 X 512 — 1024 X 1024 — 2048 x 2048 See *Setting Up Plot Display*.
Gates & Tools	Use the Gates & Tools Radial Menu to add a gate, annotation, or overlay marker to a plot. See *Using the Gates & Tools Plot Radial Menu*. See CHAPTER 2, *Gates & Tools* for details on gating the plot.
Coloring	**Banded coloring** uses five colors to show event density. When the **Use Banded Coloring** radio button is selected, select the drop-down arrow located directly under **Use Banded Coloring** and choose the option you prefer from the drop-down list. The available options include: • Density • Contour with outliers • Contour • Contour with density **Set Band Ranges Manually** allows you to specify the banded color ranges. Select the check box to enable manually setting band ranges. Select the up/down arrows or select the entry and type the new value into the field. You can also choose to use a black plot background. See *Using the Coloring Menu*, for additional details and options associated with using the Coloring Radial Menu.

Tree Plots

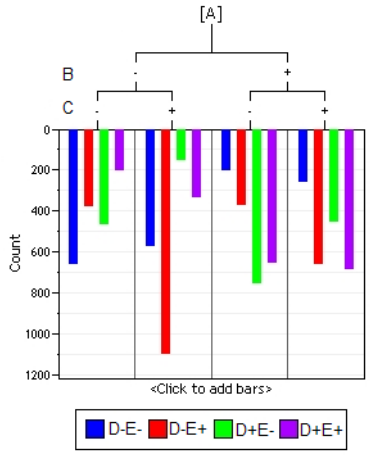
The tree plot (Figure 2.19) is a unique and comprehensive approach to comparing the physical characteristics of the events included in your Analysis. Tree plots provide a useful data comparison tool, as one tree plot can condense data from up to 28 bivariate plots.

The tree plot includes:

- **Branches**, which are used to categorize cell populations based on whether they have a negative or positive result for a specified phenotypic data type. Branches are located at the top of the plot.
- **Bars**, which are the event populations used to characterize every possible negative/positive branch combination. Bars are the central focus of the tree plot, as they are the pictorial representation of this phenotypic classification system. Bars can be viewed as either **Count** or **% Gated**.

Both bars and branches are based on gated data that has already been established within the Protocol.

Figure 2.19 Tree Plot

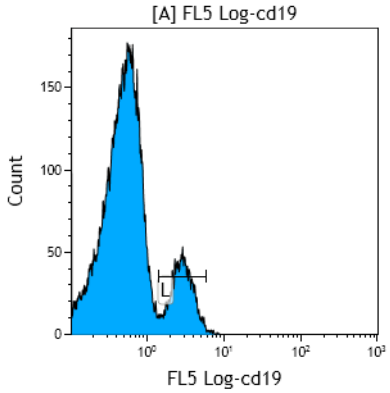


Setting Up Tree Plots

To set up a tree plot:

1. For each phenotype that you wish to include in your tree plot as branches or bars, create a plot for that phenotype. Then, on each plot, create a gate that includes the events that are positive for that phenotype. For example, in Figure 2.20, gate “L” includes the events that are positive for the CD-19 protein.

Figure 2.20 Gating Positive Events



2 Select  from the Plots & Tables Ribbon tab; this adds a new tree plot to the sheet.

3 Choose the input gate (if needed) to filter your data by selecting the **[Ungated]** hyperlink located at the top of the plot and choosing the gate from the pop-up menu.

4 Use the **<Choose Branches>** hyperlink to choose the branches of the tree; branches can be any gate within the Data Set. Each branch added to the tree further classifies gated events based on whether they are positive or negative for the phenotypic characteristic defined in the branch(es) of greater precedence.

NOTE The combined number of branches and bars may not total more than eight.

5 Use the **<Choose bars>** hyperlink to choose the bars that you wish to display on the plot. The plot displays all the positive and negative combinations for the bars that are selected. All possible positive/negative combinations are represented by a different color. These colored bars will display on the graph when they also meet the positive/negative criteria of the lowest branches. The length of the bars are directly correlated with the Y-axis data, which is either **Coun**t or **% Gated**.

NOTE

1. As you hover your mouse over a bar, the names of the gates, including the positive or negative classification, displays in the branches that are associated to that bar.

2. A legend for the bars, including the colors and the definition of the positive/negative phenotypic data classification specifically associated with each bar, is, by default, located at the bottom of the plot.

6 Select the appropriate Y-axis data type for viewing the bars. The default measurement type is **Count**. If you wish to change the measurement type to **% Gated**, select the **<Count>** hyperlink, and from the pop-up list, choose **% Gated**.

7 Continue customizing your plot using the Radial Menus. Table 2.7 provides specific information, as well as links to general options, for setting up a tree plot.

IMPORTANT You may use tree bars as an input gate for other plots, including other tree plots. To gate a plot using a tree bar, press the Alt key and select and drag the tree bar onto the appropriate plot and release your mouse button to complete the process.

Table 2.6 Tree Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Data	In addition to the Data Radial Menu options described in *Setting Up Plot Data*, the following options are available for tree plots. • **Input Gate**: Choose the input gate from which the branches and bars are gated. • **Branch Gates**: The Branch Gate section allows you to choose branches for the plot. To choose branches, select the **Choose branches** hyperlink, and then select gates from the popup menu/sub menus. This field lists the branches currently selected for that tree plot. To change the precedence of the branches, select a branch located in the field and then select the up or down arrow (located to the right of the field) to move in the preferred direction. • **Bar Gates:** The Bar Gates section allows you to choose bars for the plot. To choose bars, select the Choose bars hyperlink, and then select gates from the pop-up menu/sub menus. The field lists the branches currently selected for the tree plot. To change the precedence of the bars, select a bar from the field and select the up or down arrow (located to the right of the field) to move in the preferred direction. • **Y Parameter**: Select the radio button to choose the view type for your data as **Count** or **% Gated**. You can also create a manual Y-axis scale. Either enter scale limits manually or use the arrows to nudge them in either direction.
Edit	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, paste as link, delete, export compensated event data, and save as image. Refer to CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for details.
Statistics	Use the Statistics Radial Menu to choose statistics to display at the bottom of the plot, in addition to the population statistics described in *Setting Up Statistics*. An option to hide statistic rows containing no events for Tree Plot Statistics is also available.
Fonts	Use the Fonts Radial Menu to choose the font style and size.
Display	Use the Display Radial Menu to alter the size of a plot or to change the information that displays on a plot. See *Setting Up Plot Display*.

Table 2.6 Tree Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Gates & Tools	Use the Gates & Tools Radial Menu to add to a gate or annotation to a plot. See *Using the Gates & Tools Plot Radial Menu*. For details on gating your plot, see *Gates & Tools*.
Coloring	Use the Coloring Radial Menu to update the coloring associated with a plot and change the background color. See *Using the Coloring Menu*.

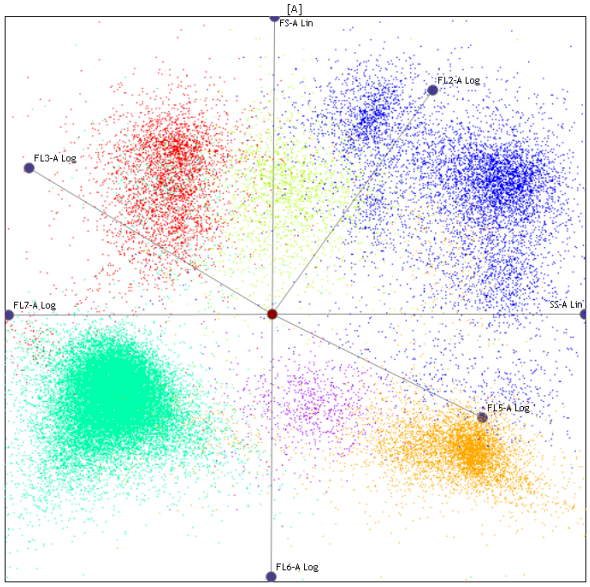
Radar Plots

The radar plot maps multi-dimensional data onto a two-dimensional surface; events are displayed by adding axes. When these axes are moved, relationships become apparent; axes can be moved manually or you may choose to animate of the axes, which prompts automatic movement in the defined direction and rate of speed.

The appearance of the events on the plot can vary widely, depending on the input gate you choose, the number of axes you use to characterize your data, the length of the axes, and the placement of each axis. Any parameter within the Data Set is available to use as an axis.

The radar plot is a very useful data comparison tool, combining data from many bivariate plots into one highly manipulatable plot.

Figure 2.21 Radar Plots



Gating on a Radar Plot and Population Statistics

Plot statistics may be added through the Radial Menu > Statistics. The available statistics are:

- Number
- % Total
- % Gated

Available Gate Types are:

- Polygon
- Ellipse
- Rectangular
- Freehand

Setting Up Radar Plots

To set up a radar plot:

1. Select 

from the Plots & Tables Ribbon tab. This adds a new radar plot to the sheet.

2. Choose an input gate, if necessary, using the hyperlink located at the top of the plot. See *Gates & Tools*, for details on gating your plot.

3. Choose the plot axes you wish to include in the plot by selecting **<Choose plot axes>** and choosing the parameters you want to display on your radar plot.

4. Arrange your plot by selecting axis handles and dragging to the preferred location, then release your mouse button. You can move axes in a 360° radius, and lengthen or shorten them.

5. Continue customizing your plot using the Radial Menus. Table 2.7 provides specific information, as well as links to general options, for setting up a radar plot.

Overlay Plots

An Overlay Plot is a combination of multiple histograms displayed in a single plot. Overlay plots can be used as a data comparison tool in individual data sets, merged data sets and composite data sets.

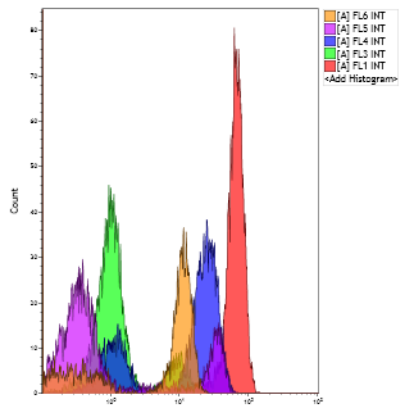
Table 2.7 Radar Plots Set-Up Options

Radial Menu	Plot Set-Up Details
Data	In addition to the Data Radial Menu options described in *Setting Up Plot Data*, the following options are available for radar plots. The **Axes** section is for choosing the axes to include in the plot. The options include: • **Choose plot axes**: Choose axes to include on the plot. To select parameters to use as plot axes, select the **Choose plot axes** hyperlink. From the pop-up menu, select the parameters you wish to include as plot axes, and use the additional pop-up menu to define the scale for each parameter. • **Add All**: Add all the parameters associated with the Data Set to the radar plot as axes. • **Remove All**: Remove all axes that had previously been added to the plot. • **Reset All**: Reset the length and placement of each axis. After resetting, axes are evenly spaced and appear in order by name in a counter-clockwise pattern. The **Axis Configuration** section is where the angle and length of a selected axis is defined. You may also animate an axis, which prompts automatic movement of the selected axis (in the rate and direction defined in the Radar Options section of this menu). The location of events displayed on the plot is updated in real-time as the animation is in process. To remove a selected axis, choose the (**Delete**) icon. In the **Radar Options** section, the following options are available: • **Origin**: Manually update X and Y coordinates of the location of the plot origin. Entering negative values moves the origin to the left or up, and entering positive values moves the origin down or to the right. • **Animation Rate**: Define the animation rate and direction of movement. Moving the animation slider to the left of center initiates clockwise movement; the farther to the left the slider is moved, the faster the animation. Moving the animation slider to the right initiates counter-clockwise movement; the farther to the right the slider is moved, the faster the animation. **NOTE** The radar plot animation is disabled when gates are present. • **Zoom**: Change the zoom. Moving the slider to the left to zooms out and to the right zooms in.
Edit	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, paste as link, delete, export compensated event data, and save as image. Refer to CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for details.
Statistics	Use the Statistics Radial Menu to choose statistics to display at the bottom of the plot. For details, refer to *Setting Up Statistics*.
Fonts	Use the Fonts Radial Menu to choose the font style and size.
Display	Use the Display Radial Menu to maximize/restore a plot, edit parameter labels, and set plot resolution (bivariate plots). See *Setting Up Plot Display*.

Table 2.7 Radar Plots Set-Up Options

Radial Menu	Plot Set-Up Details
Gates & Tools	Use the Gates & Tools Radial Menu to add to a gate, annotation, or overlay marker to a plot. Refer to *Using the Gates & Tools Plot Radial Menu*, for details. For details on gating your plot, see *Gates & Tools*.
Coloring	Use the Coloring Radial Menu to update the coloring associated with a plot and change the background color. See *Using the Coloring Menu*, for details.

Figure 2.22 Overlay Plot



Setting Up Overlay Plots

NOTE Up to 12 histograms may be added to an overlay plot. If all the histograms are on the same scale, the axis tick marks will display.

To set up an overlay plot:

---

1 Select  from the Plots & Tables Ribbon tab. This adds a new overlay plot to the sheet.

---

2 Hold the (ALT) key and drag an existing plot to add its parameters to the overlay.

OR

If using the Add Histogram hyperlink within the Overlay Plot, a Histogram Configuration screen displays allowing you to select the histogram color, input data set, input gate and parameters.

NOTE You may also multi-select plots to drag into the Overlay Plot, following the same method.

3 Continue customizing your plot using the Radial Menus. Table 2.8 provides specific information, as well as links to general options, for setting up an overlay plot.

Table 2.8 Overlay Plot Set-Up Options

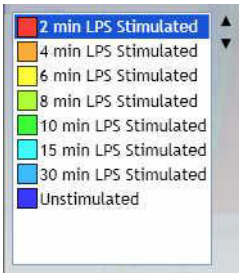
Radial Menu	Plot Set-Up Details
Data	The histogram selection field (see Figure 2.23) is where a specific histogram can be selected for interacting with in the **Selected Histogram Options** section of the Data Radial Menu. In addition, this field is where the precedence of the selected histogram can be changed using the up or down arrows (located to the right of the field). **Figure 2.23** Histogram Selection  The **Selected Histogram Options** section within the Data Radial Menu is for customizing overlay plots for optimal data presentation. Specifically, these options are available: • Delete the selected histogram by clicking on the (**Delete**) icon. • Add a histogram by selecting the (**Add**) icon. • Input gate (alphabetical list of gates with color assignments indicated) • X-axis parameter/scale/decades • Smooth your data for a more pleasant appearance. • Clip the first and last channels (for scaling purposes only). The **Y Parameter** section allows the manual adjustment of the Y-axis scale to better fit your data. You can enter scale limits manually or use the arrows to nudge them in either direction. See *Setting Up Plot Data*, for general information on making changes to plot data, including parameter axis data, input gates, and bivariate resolution.
Edit	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, paste as link, delete, and save as image. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*,
Statistics	Use the Statistics Radial Menu to choose statistics to display at the bottom of the plot. See *Setting Up Statistics*.
Fonts	Use the Fonts Radial Menu to choose the font style and size.

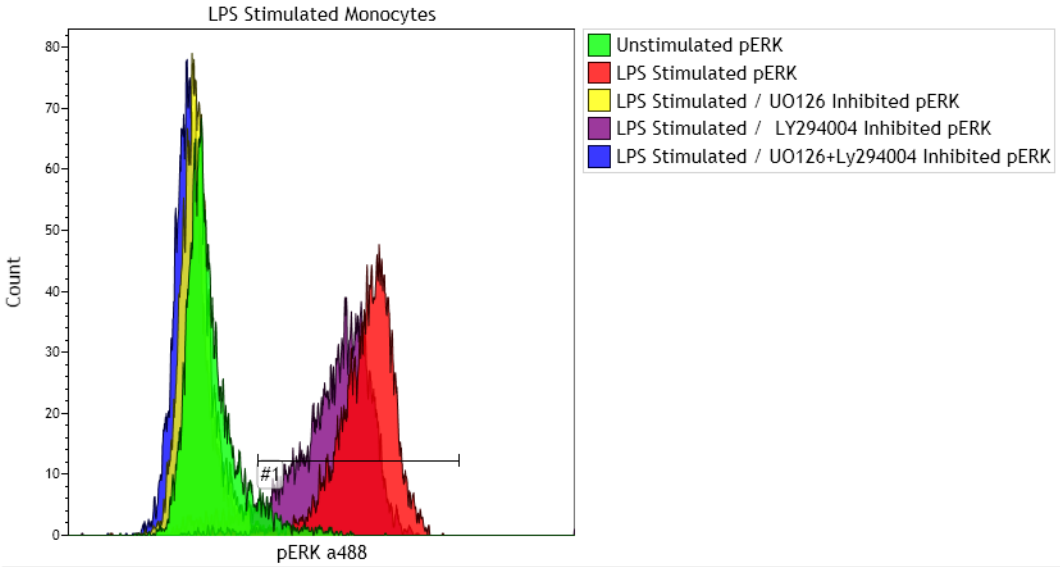
Table 2.8 Overlay Plot Set-Up Options

Radial Menu	Plot Set-Up Details
Display	Use the Display Radial Menu to alter the size of a plot or to change the information that displays on a plot. See *Setting Up Plot Display*. An additional section for titles is included in the Display Radial Menu for overlay plots, which is entitled **Histogram Titles**. In this section, you can customize the titles of all histograms included in the overlay plot. The Axis Section allows and offers options for the display of axis labels, scaling and legend. Additionally, the X-axis label may be customized by selecting “Use Custom Axes” and histograms can be customized by using the “Use Custom Titles.”
Gates & Tools	Use the Gates & Tools Radial Menu to add a gate, annotation, or overlay marker to a plot. See *Using the Gates & Tools Plot Radial Menu*. See *Overlay Marker*, for details on adding an Overlay Marker to your plot.
Coloring	Use the Coloring Radial Menu to update the coloring associated with a plot and change the background color. See *Using the Coloring Menu*.

Overlay Marker

The Overlay Marker tool is used for creating statistical markers within overlay plots. These statistical markers are applicable only to the overlay plot in which they reside and are used to produce statistics for histograms within that plot.

Figure 2.24 Overlay Plot Marker



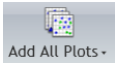
Marker	Number	X-Mean
All	4,971	1.59
All	4,967	25.40
All	5,133	1.14
All	5,385	14.90
All	5,336	1.03
#1	306	5.75
#1	4,896	25.74
#1	30	3.73
#1	4,914	15.95
#1	26	4.78

To create an Overlay Marker:

1. On the plot that you need to insert overlay markers, right-click to access the Plot Radial Menu.
2. Hover over the icon to access the Gates & Tools menu.
3. Select the Overlay Marker icon.
4. Click and drag across the histogram to create the marker and release the mouse button when completed. Repeat this procedure to create additional markers.

Add All Plots

The **Add All Plots Options** screen (Figure 2.25) allows you to configure the plot and gate types that are included when Add All Plots is selected. In addition, Verification Protocols in a Compensation Composite are created based on the Add All Plots configuration (see *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature* for details on Verification Protocols).

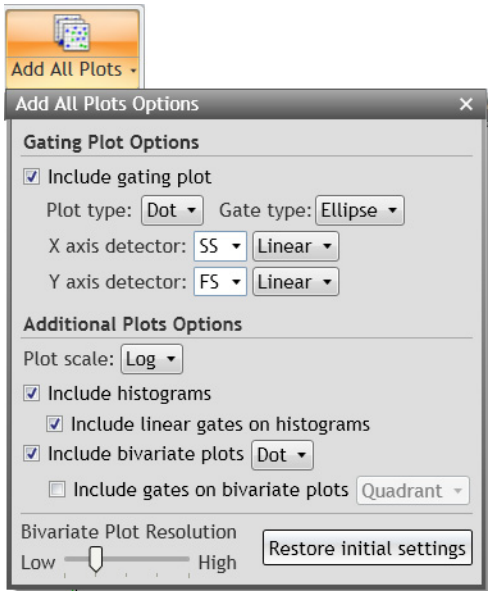
Use the drop-down arrow below the 

to customize which plots are to be created.

Gating Plot Options
Including Gate Plot: When this checkbox is selected, a gating plot is included.
Plot Type: Dot (default), Density or Contour.
Gate Type: Ellipse (default) or Rectangular
X and Y Axis Detector: Use to assign parameters to the X and Y axis of this gating plot.

Additional Plot Options
Plot Scale: Linear, Log (default), Logicle
Include Histograms: When this checkbox is selected, histograms of each fluorescence parameter are added.
Include linear gates on histograms: When this checkbox is selected, a linear gate is added to each histogram.
Include bivariate plots: When this checkbox is selected, bivariate plots comparing all fluorescence parameters to each other are added. Dot (default), Density, or Contour.
Include gates on bivariate plots: When this checkbox is selected, the selected gate type is added to the bivariate plots.
Gate Type Options: Quadrant (default), Hinged, Ellipse, Rectangle.
Bivariate Plot Resolution: Use the slider to control the plot resolution.
Restore initial settings : Use this button to restore the default selections.

Figure 2.25 Add All Plots



Gate Statistics Table

You can add a table showing gates, logic and population statistics to a sheet. Set up and customize the table using the Radial Menu. The gates that you choose to display in the table appear in the hierarchal order by default, as shown in Figure 2.27.

NOTE The percent (%) gated statistic for a Boolean Gate does not match the percent (%) gated statistic for the gate that defines the Boolean Gate.

Figure 2.26 Gates Table

Gate	Number	%Total	%Gated	Logic
All	17,368	100.00	100.00	Ungated
R	4,990	28.73	28.73	R
A	4,990	28.73	28.73	A

Figure 2.27 Gate Hierarchy

DonorA 041311 24Hr TBNK NoRead SCL 00000053 040

Gate	Number	%Total	%Gated
All	22,624	100.00	100.00
Lymph Capture	4,987	22.04	22.04
CD45+ Lymphs	4,913	21.72	98.52
CD14-	4,468	19.75	90.94
CD3-	746	3.30	16.70
CD16_56+	339	1.50	45.44
CD19-/CD16_56-	104	0.46	13.94
CD19+	296	1.31	39.68
CD19+/CD16_56+	7	0.03	0.94
CD3+	3,719	16.44	83.24
CD4-/CD8-	120	0.53	3.23
CD4+	2,708	11.97	72.82
CD4+/CD8+	5	0.02	0.13
CD8+	886	3.92	23.82
CAL	1,945	8.60	98.53

To create a Gate Statistics table:

1. From the Plots & Tables Ribbon tab, select the Gate Statistics icon, and drag it to the preferred location on your sheet.

2. Right-click within the empty table to access the Radial Menu.

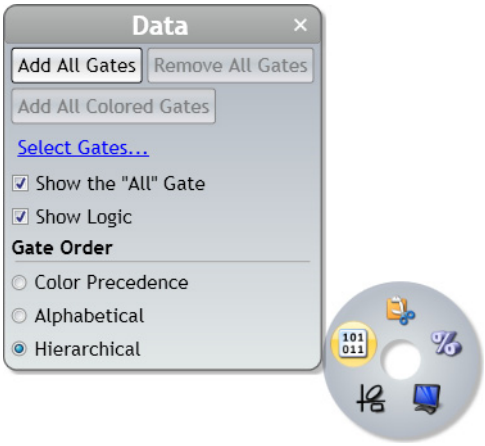
3. Hover over the (**Data**) icon to access the Data menu. The Data menu appears with the following options:
   - **Input Data Set**: In a composite, select the input data set.
   - **Add All Gates:** Adds all gates associated with the Data Set.
   - **Remove All Gates**: Removes all gates previously set up for the table.

- **Add All Colored Gates:** Adds gates that have been assigned a color.
- **Select Gates...:** Allows you to select the gates you wish to display on your Gate Statistics table. To make specific gate selections:
  - Select the **Select Gates...** hyperlink. A pop-up menu appears with gate categories.
  - Hover your mouse over the gate type you wish to add to the Gate Statistics table and, from the additional pop-up window, select the gate(s) you wish to add to the table. Continue this process for each type of gate you wish to add.
- **Show the "All" Gate:** Adds a row to the table for all the events associated with the Data Set. When you choose to show statistics for the table, this option serves as a gauge for the other statistics within the table.
- **Show Logic:** Shows the gate logic for Boolean gates.
- **Gate Order:** Choose to display the gates in color precedence, alphabetical, or hierarchical order.

NOTE Gates can also be added to the table, as follows:

- Select gates from within the Color Precedence pane, and then drag and drop them in the table.
- Press the Alt key, while selecting a gate from a plot, and then drag and drop them in the table.

Figure 2.28 Gate Statistics—Data Radial Menu

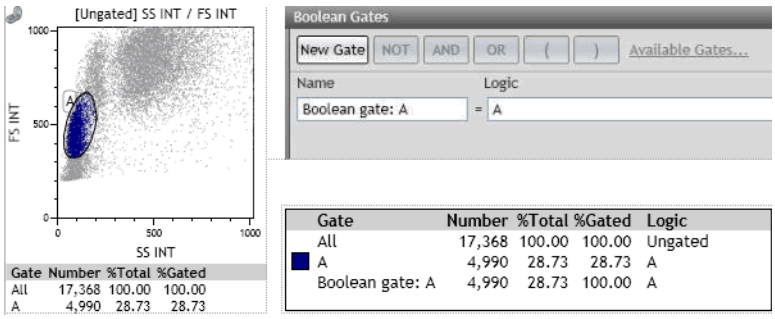


4 Make your selections. Select ✕ when changes are completed.

NOTE

1. To add statistics to the Gate Statistics table, access the Radial Menu by positioning your cursor over the table. Choose options from the (**Statistics**) menu to add features to the table.
2. The percent (%) gated statistic for a Boolean Gate does not match the percent (%) gated statistic for the gate that defines the Boolean Gate. Figure 2.29 demonstrates this behavior.

Figure 2.29 Percent Gated Statistic—Boolean Gate



Information Table

Use the Information Table (Figure 2.30) to:

- Add FCS data
- Add statistics (This can be a method of summarizing and exporting only statistics selected in the Information Plot, in lieu of displaying/ exporting statistics under or within a plot.)
- Create formulas to generate results, such as Absolute Counts.

See *Using Formulas in Kaluza Analysis*.

Figure 2.30 Information Summary Table

Immunophenotyping Summary

Lmd:	PAT B 00001431 069.LMD
Lmd:	PAT B 00001432 070.LMD
CD3% TUBE1	87.19
CD3% TUBE2	86.97
Avg CD3+ %	87.08
Avg CD3+ cells/uL	1,808.46
CD3+CD4+ cells/uL	1,063.67
CD3+CD8+ cells/ul	678.42
CD19+ cells/ul	135.35
CD3-CD56+	103.36
Lymphosum	98.32

To create an Information Table:

1. From the Plots & Tables Ribbon tab, select 

icon, and drag it to the preferred location on your sheet. An empty table appears in that location.

2. Click the <Add Keywords> hyperlink at the lower left of the table to select the keywords you wish to display in the table.

   NOTE Other than parameter names and descriptions (which always appear as defined in the Parameters pane) keywords cannot be changed; they appear exactly as derived from the source file.

3 Select whether to include the keyword description, name, or both. In a composite, select which data set to draw the keywords from, or select All Data Sets to include a copy of the keyword from each data set.

4 Select the keywords you wish to display in the Information table. When you are finished, select ✕.

5 Click the <Add Statistic> hyperlink and configure the data set, statistic, gate and parameter for each statistic you wish to display. When you are finished, select ✕.

6 Select the keywords you wish to display in the Information table. When you are finished, select ✕.

NOTE You can also click on the left or right side of the Information Plot to free-text in the Selected Value name or Value (formula).

7 See Table 2.9; then return to continue with step 8.

8 Select the input gate and statistic, and parameter, if required. Indicate whether to include the data set index, gate name, and parameter name, description and scale in the statistic description. In composites, select which data set to calculate the statistic on, or choose ALL Data Sets to include a copy of the statistic for each data set.

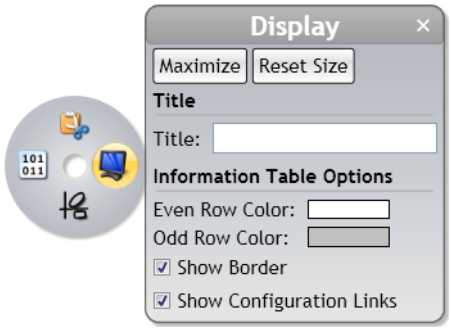
9 Apply formatting to your Information Table as follows:

a. Right-click in the table to access the Radial Menu.

b. Hover your mouse over the icon to access the Display menu. Here you can enter the title and select/configure Options such as: row colors and the inclusion of a border.

c. Use the **Information Table Options** (Figure 2.31) to format your table. This option includes changing the precedence of the data within your table. To move data up or down in your table, select the corresponding keyword, and then select the up or down arrow until it is located in the location you prefer.

Figure 2.31 Display Menu—Information Table Options



10 When you have completed your changes, select ✕.

Table 2.9 Information Plot Set Up Options

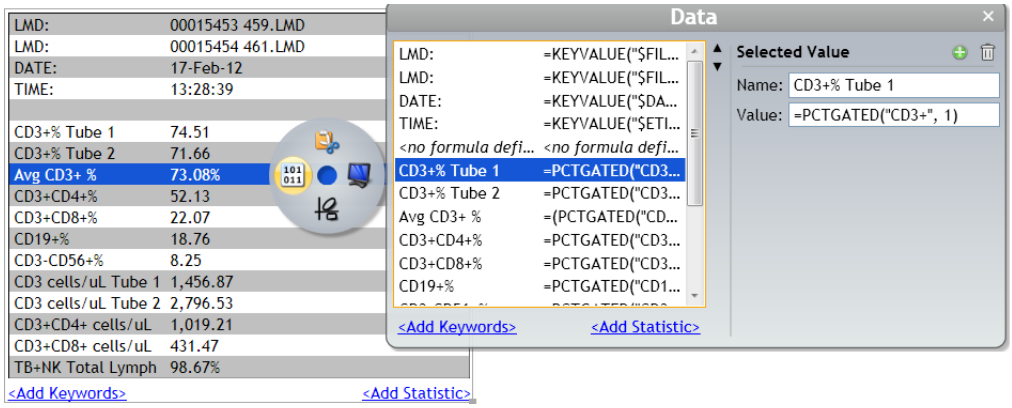
Radial Menu	Plot Set-Up Details
Data	The selection field allows the Keywords or Statistics to be reordered by using the up or down arrows (located to the right of the field). Additional Keywords or Statistics may be added using the hyperlinks at the bottom of this field. **Figure 2.32** Information Plot—Data Menu  The **Selected Value** section within the Data Radial Menu is for customizing the Information Table for optimal data presentation. The following specific options are available: • Remove the selected information table row by clicking the (**Delete**) icon • Add a row by selecting the (**Add**) icon. • Use the Name field to edit the name or description. • Use the Value field to enter a desired formula.
Edit	Use the Edit Radial Menu to perform basic plot editing functions, including cut, copy, paste, delete, and save as image. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*.

Table 2.9 Information Plot Set Up Options

Radial Menu	Plot Set-Up Details
Display	Use the Display Radial Menu to alter the size of a plot or to change the information that displays on a plot. An additional section for editing the plot **Title** is included in the Display Radial Menu for comparison plots. The Information Table Option provides these features: Even/Odd Row Color selection, Show, Show Border, and Show Configuration Links (hyperlinks).
Gates & Tools	Use the Gates & Tools Radial Menu to add to an annotation. See *Using the Gates & Tools Plot Radial Menu*.

Adding Plots to the Plot Sheet

To add a plot to the plot sheet, select the Plots & Tables Ribbon tab.

- **To add the new plot to the bottom of the plots already included on the sheet**: Select the icon corresponding to the type of plot you wish to include in your Analysis.
- **To add the new plot to a specific location on the plot sheet:** Click the icon and drag it to the location you prefer. Release the mouse button.

IMPORTANT If data seems to be missing from a plot, or if only a small number of events appear on the left or the bottom of the plot, the Data Set may have been acquired on an instrument with more than four decades of dynamic range. Adjust the number of displayed decades using the Data menu. See *Setting Up Plot Data*.

NOTE This option is not available for Add All Plots.

Plot Set-Up

Kaluza Analysis offers an array of options for customizing your plots. The following sections highlight these options and give instructions on how to employ the techniques for using these options. Because Radial Menus are the main source for making changes to your plots, the sections to follow will focus primarily on using the Radial Menus to make your changes.

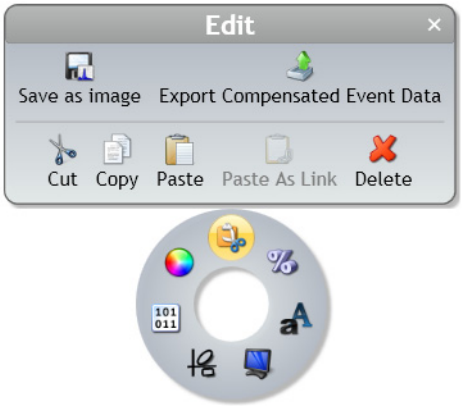
NOTE Radial Menu options will vary, depending on the plot type.

Editing Plots

To perform basic plot editing functions, including cut, copy, paste, paste as link, delete, and save as image, use the Edit Radial Menu (see Figure 2.33). See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for a description of the functions available on the Edit Radial Menu.

NOTE All Edit Radial Menu options are available for multi-selection, except for Save as Image and Export Compensated Event Data.

Figure 2.33 Edit Radial Menu



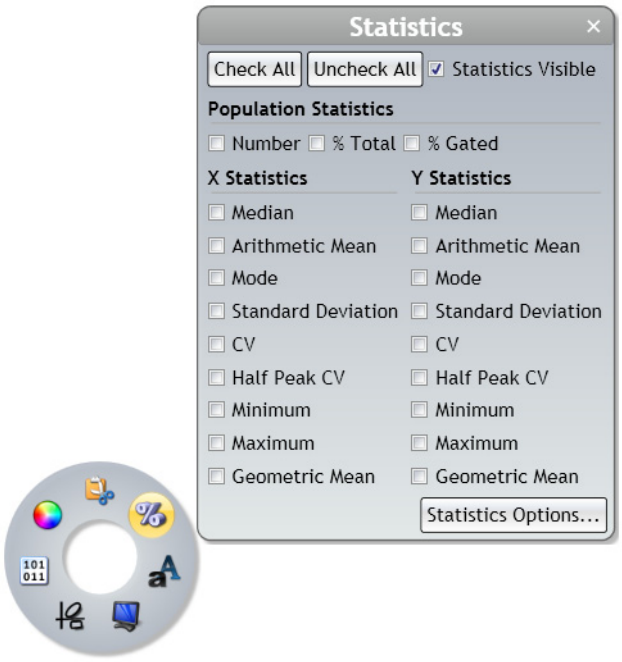
Setting Up Statistics

To choose statistics to display at the bottom of the plot, use the Statistics Radial Menu (see Figure 2.34), which provides access for selecting statistics to display on the plot.

- See *Using the Statistics Radial Menu*, for general instructions on using the Statistics Radial Menu.
- Figure 2.35 is an example of statistics displayed on a plot.

NOTE Available statistics are different, depending on the type of plot you are working with.

Figure 2.34 Statistics Radial Menu



The Statistics menu contains the following options:

- **Check All**: Selects all statistics available for a plot.
- **Uncheck All**: Removes all selections previously set for a plot.
- **Statistics Visible**: Displays chosen statistics on a plot. When deselected, any statistics you previously chose will be retained, but will not display on your plot. By default, **Statistics Visible** is selected.
- **Population Statistics**: Allows you to choose statistics related to all events within a plot, including **Number**, **% Total**, and **% Gated**.
- **X Statistics/Y Statistics**: Allows you to choose statistics related to the X and Y axes, including **Median**, **Arithmetic Mean**, **Mode**, **Standard Deviation, CV**, **Half Peak CV**, **Minimum**, **Maximum**, and **Geometric Mean**.

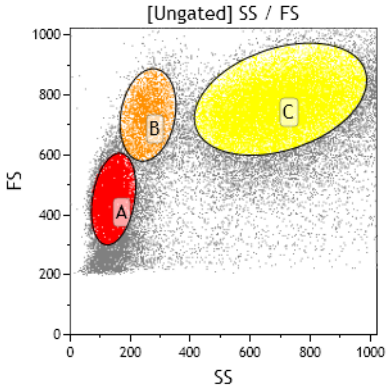
  NOTE

  1. This option is not available for the tree or radar plot. Histograms, including the overlay plot, only display X statistics.

  2. If selecting statistics by the Geometric Mean and exporting the statistics, DO NOT use scripts previously written against the fixed export format for exported statistics because said scripts could be operating against data that has moved in the output file.

- **Statistics Options...**: Allows you to make systemic changes to the appearance of statistics, including using thousands separators in whole numbers and/or fractions and choosing between 0 and 4 decimal places in fractional numbers and/or percents. See CHAPTER 1, *Kaluza Options Menu*.

Figure 2.35 Plot Statistics



Gate	Number	%Total	%Gated	X-Med
All	50,000	100.00	100.00	638.02
A	8,219	16.44	16.44	139.16
B	2,900	5.80	5.80	259.53
C	17,412	34.82	34.82	691.63

Gate	X-Mean	X-Mode	Y-Med	Y-Mean
All	592.44	1,023.00	698.97	651.13
A	140.39	132.00	445.28	446.73
B	259.43	261.00	725.19	724.84
C	696.93	691.00	764.67	766.44

Gate	Y-Mode
All	1,023.00
A	466.00
B	740.00
C	728.00

Using the Statistics Radial Menu

To use the Statistics menu:

1. Select the plot(s) for which you wish to display statistics.
2. Access the Statistic Radial Menu by right-clicking on the selected plot.
3. Hover your mouse over the (**Statistics**) icon to access the Statistics menu.
4. Make your selection(s) using the buttons and/or check boxes.
5. Select to complete the process.

Setting Up Plot Display

Use the Data Radial Menu to change data associated with a plot, including the parameter axis data, and input gates. (See Figure 2.36).

See *Using the Display Radial Menu*, for general instructions on using the Display Radial Menu.

NOTE Display menu options are different, depending on the type of plot you are working with.

Figure 2.36 Display Radial Menu

The Display menu contains the following options:

- **Maximize**: Increases the size of the plot to fit within the sheet portion of the application. The Maximize option is only available for items on the plot sheet, not the report sheet.

  NOTE Double-clicking on a plot also maximizes a plot, and double-clicking on a maximized plot returns the plot to the previous size.

  After you have maximized a plot, three buttons appear below the plot:

  — **Previous**: Shows the previous plot located on the sheet in the maximized view.

  — **Restore**: Returns the plot to its previous size.

  — **Next**: Shows the next plot located on the sheet in maximized view.

- **Reset Size**: Returns a plot that has been resized back to the default size and shape.
- **Plot Resolution**: Adjust the resolution of bivariate and radar plots using the slider. Options include 128 x 128, 256 x 256, 512 x 512, 1024 x 1024, and 2048 x 2048. Higher resolutions should be used on data sets with large numbers of events to assist in viewing populations.

- **Title**: Allows you to customize the title that appears at the top of the bivariate plots. You may select **Use Custom Title**, which prompts a **Title** field, allowing you to create your own title. Additional options include showing the following information in the plot title:
  - Input Gate
  - Data Set Name
  - Name
  - Description
  - Scale (linear, log, or logicle)
- **Axes**: Allows you to customize the axes included in your plot. Choose **Use Custom Axes** to prompt a field to display for each axis in the plot, which allows you to create your own title. If **Use Custom Axes** is not selected, you may include:
  - Name
  - Description
  - Scale

  Additional options include the following:
  - Axis Tick Marks
  - Axis Grid Lines

Using the Display Radial Menu

To use the Display menu:

1. Select the plot for which you wish to change the display.
2. Access the Display Radial Menu by right-clicking on the selected plot.
3. Hover your mouse over the (**Display**) icon to access the Display menu.
4. Make your updates in the Display menu.
5. Select to complete the process.

Using the Gates & Tools Plot Radial Menu

To add a gate, annotation, or overlay marker to a plot, the Gates & Tools menu (Figure 2.37) provides immediate access for choosing these options.

NOTE The Gates & Tools menu includes the same options no matter where you are currently located on the sheet. If a gate selected from the menu is not appropriate for the current sheet location, the cursor appears as a ⊘ symbol. When you are in an appropriate location for the gate type, the cursor changes to the symbol for the gate type you selected, indicating that you may begin drawing.

See *Gates & Tools*, for an in-depth description of gating.

Figure 2.37 Gates & Tools Radial Menu

Gates

Gates that are accessible through the Gates & Tools menu include the following:

- Linear
- Quadrant
- Hinged
- Polygon
- Freehand
- Rectangle
- Ellipse

To enable Gate-Drawing mode:

1. Access the Gates & Tools Radial Menu by right-clicking on a plot.

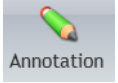
2. Hover your mouse over the (**Gates & Tools**) icon to access the Gates & Tools menu.

3. Select the gate type you need from the menu.

   NOTE As you hover your mouse over each icon, the tooltip shows the name of the gate that corresponds with the icon.

4 Your cursor changes to resemble the type of gate you wish to draw. See *Gates & Tools*, for details on drawing specific gate types.

Annotation Tool

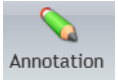


The **Annotation** tool allows you to add text to a plot.

To enable Annotation mode:

1 Access the Radial Menu by right-clicking on a plot.

2 Hover your mouse over the (**Gates & Tools**) icon to access the Gates & Tools menu.

3 Select the 

icon from the menu.

4 Click and drag your mouse over the plot to create a text box. Release your mouse button when the box is the size you prefer.

NOTE Text boxes can be resized at any time by selecting the box and then clicking a handle and dragging to the size you prefer.

5 Right-click on the text box. The Radial Menu appears.

6 Hover over the (**Data**) icon, which initiates a data field.

7 Click within the data field and type your annotation.

8 Select when finished.

NOTE See CHAPTER 3, *Formatting a Text Box*, to customize the annotation.

Overlay Marker

An **Overlay Marker** is for creating statistical markers within overlay plots. These are applicable only to the overlay plot in which they reside and are used to produce statistics for each of the histograms within the plot. See *Overlay Plots*.

Setting Up Plot Data

To change data associated with a plot, including the parameter axis data, input gates, and scale use the Data Radial Menu (see Figure 2.38).

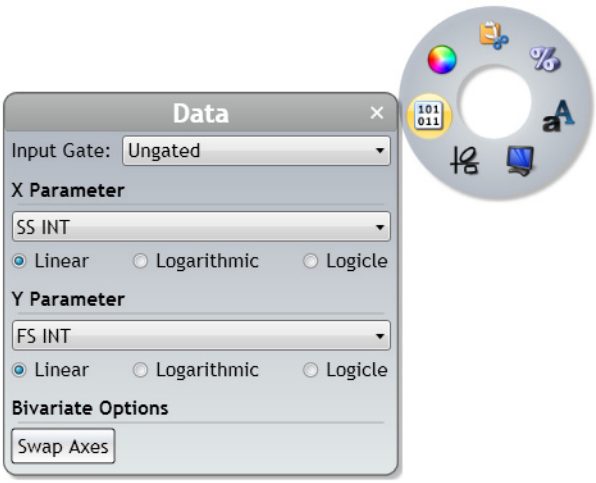
For general instructions on using the Data Radial Menu, see *Using the Data Radial Menu*.

Data Menu Options—All Plots

The Data menu for all plots contain the following options:

- **Input Gate**: Allows you to change the input gate from which a plot is gated.
- **X Parameter/Y Parameter**: Allows you to make axis-related data changes, including the following:
  - Change the X or Y parameter.
  - Select a different scale; options include linear, log, or logicle.
  - Select the number of decades displayed (log or logicle).
  - Change the negative percentage (logicle only).
  - Swap axes.

Figure 2.38 Data Radial Menu



Using the Data Radial Menu

To use the Data menu:

1 Select the plot for which you wish to change the data.

2 Access the Data Radial Menu by right-clicking on the selected plot.

3 Hover your mouse over the (**Data**) icon to access the Data menu. The Data menu for the specific plot type appears.

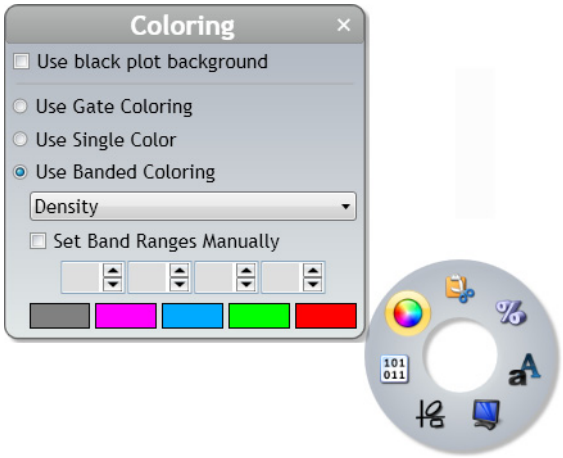
4 Enter your updates.

5 Select to complete the process.

Using the Coloring Menu

To update the coloring associated with a plot, use the Coloring Radial Menu (Figure 2.39 is an example of the Coloring menu when on a dot plot), which provides access to making multiple types of coloring updates to plots.

Figure 2.39 Coloring Radial Menu



Updating Colors

Color blocks are designed to allow you to change the color of bands in contour and density plots, the bars in tree plots, and the histograms in overlay plots.

NOTE Other than the ability to turn on/off gate coloring, changes to gate coloring are not made through the Plot Radial Menu. Use the Color Precedence pane or the Gating Radial Menu to change the color associated with events in a gate (see *Color Events*, for details).

To make changes to coloring:

1 Access the Updating Colors Radial Menu by right-clicking on a plot.

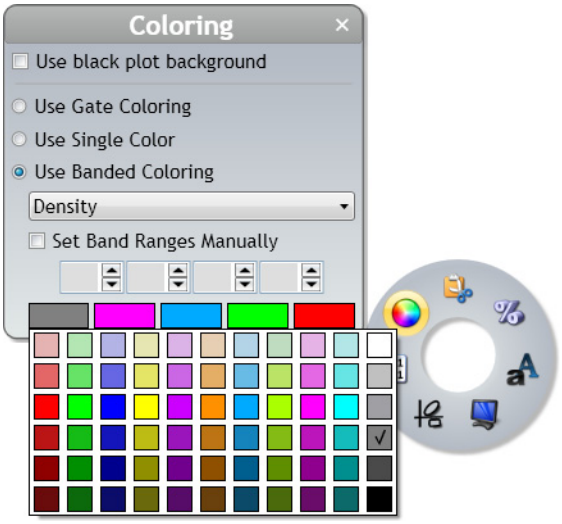
2 Hover your mouse over the (**Coloring**) icon to access the Coloring menu.

3 Click **Use Banded Coloring**.

4 Select the color block you wish to change. The color palette appears.

5 Select any color from the palette that does not contain a check mark (these colors are already used). Refer to the details in Figure 2.40.

Figure 2.40 Color Palette



NOTE The current color contains a check mark.

6 Select to finish the process.

Gate Coloring

You may choose between displaying the gate coloring set up in your Protocol or using single color for the plot.

- **Use Single Color**: Changes all events on the plot to the default event color (set up in the Color Precedence pane).
- **Use Gate Coloring**: Displays gate coloring used in the Protocol. Use the Color Precedence pane to change gate coloring.

NOTE The **Use Banded Coloring** is a third option for Dot, Contour, and Density plots, as described in Table 2.5.

To turn on/off gate coloring on a plot:

1. Access the Gate Coloring Radial Menu by right-clicking on the plot.
2. Hover your mouse over the (**Coloring**) icon to access the Coloring menu.
3. Choose the radio button for the option you prefer.
4. Select to finish the process.

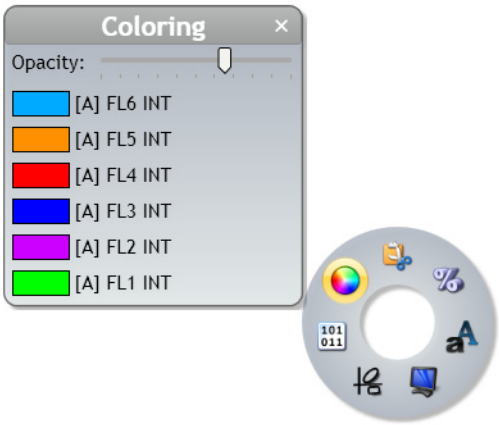
Opacity

Opacity allows you to change the opacity associated with the histograms on an Overlay Plot.

To change the opacity:

1. Access the Coloring Radial Menu by right-clicking on a plot.
2. Hover your mouse over the (**Coloring**) icon to access the Coloring menu.
3. Select the slider and move to the left or right depending on the opacity level you prefer. Refer to Figure 2.41.

Figure 2.41 Opacity Slider



4 Select ✕ to finish the process.

Gates & Tools

Kaluza Analysis offers eight different gate types, allowing you to precisely define your data. These gate types are described in detail in the following sections.

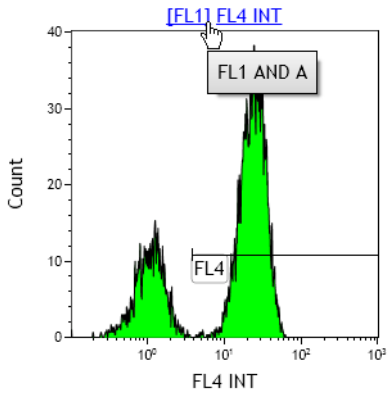
IMPORTANT If you wish to make multiple gates of the same type, hold down the Shift key to remain in drawing mode. Release the Shift key to escape that mode.

Each Data Set being analyzed may contain a maximum of 318 gates, which can be spread across multiple sheets. Up to 31 gates can be assigned a color for each data set.

Viewing Gate Logic

Hovering over the gate assignment at the top of a plot, as well as hovering over the gate on the Color Precedence List, allows viewing the gate logic for any gate.

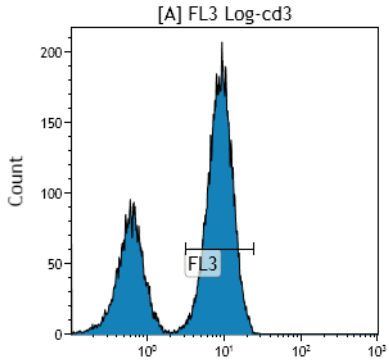
Figure 2.42 Gate Logic



Linear Gates

Linear gates are used for histograms. A linear gate (Figure 2.43) encompasses events that fall within the linear range that you define and includes all events within the vertical/horizontal frame.

Figure 2.43 Linear Gate



To create a linear gate:

---

1 From the Gates & Tools Ribbon tab, select the 

icon.

---

2 On the histogram, click and drag your mouse over the area that you wish to be included in the gate.

3 Release your mouse button when you are satisfied.

4 Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

5 Customize your gate by following instructions in *Setting Up Gates*.

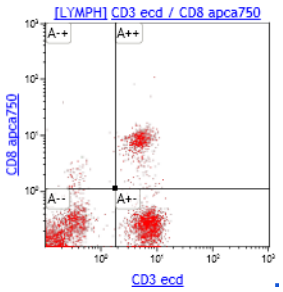
Quadrant Gates

The quadrant gate (Figure 2.44) is available for use on dot, density, and contour plots. When you choose this option, each plot is divided into four gated sections by perpendicular lines. Quadrant gates can be moved at your discretion. Each quadrant of the gate is assigned positive/negative values, depending on the position:

- -+ (upper left quadrant)
- ++ (upper right quadrant)
- -- (lower left quadrant)
- +- (lower right quadrant)

NOTE Multiple quadrants can be placed on a single plot.

Figure 2.44 Quadrant Gate



To create a quadrant gate:

1 From the Gates & Tools Ribbon tab, select the 

icon.

2 Click your mouse anywhere within the dot, contour, or density plot to create the new gate; this adds a quadrant gate to the plot, with the center-point in the location where you clicked your mouse.

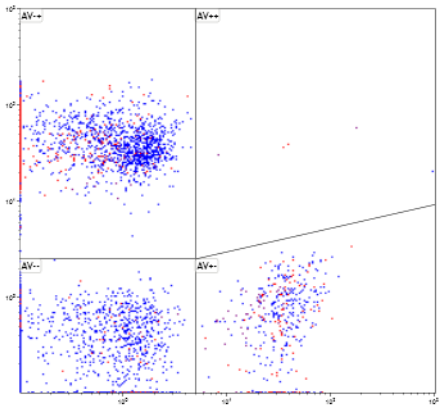
3 Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

4 Customize your gate by following instructions in *Setting Up Gates*.

Hinged Quadrant Gates

Similar to the quadrant gate, the hinged quadrant gate (Figure 2.45) divides plots into four sections, each containing positive/negative values depending on the location of the quadrant. However, unlike the quadrant gate, the hinged quadrant allows you the flexibility to move each quadrant borderline to an angle of your choosing. The movement of each quadrant borderline is limited to its current plot axis.

Figure 2.45 Hinged Quadrant Gate



To create a hinged quadrant gate:

1 From the Gates & Tools Ribbon tab, select the 

icon.

2 Click your mouse where you would like the center-point of the hinged quadrant in the dot, contour, or density plot.

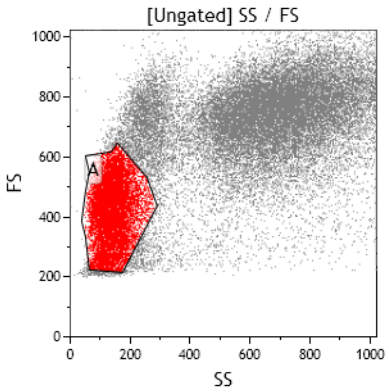
3 Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.

4 Customize your gate by following instructions in *Setting Up Gates*.

Polygon Gates

The polygon gate (Figure 2.46) allows you to create a gate with up to 128 points; this allows you set up a very specific zone of events to include in your gate. The polygon gate is available on dot, density, contour and radar plots.

Figure 2.46 Polygon Gate



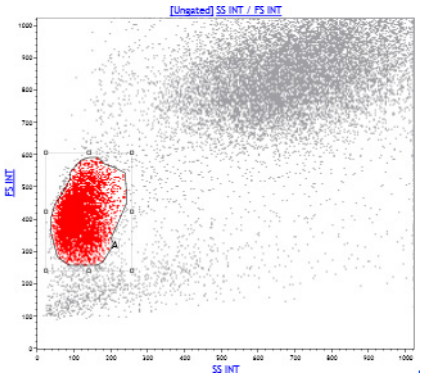
To create a polygon gate:

1. From the Gates & Tools Ribbon tab, select the Polygon icon.
2. Click your mouse where you wish to begin creating your gate.
3. Determine the path you need for your gate and continue clicking your mouse at the location of each direction change. As you draw the gate, a new line will be added each time you click your mouse, and the default gate color will display, working as a guide to show your progress.
4. Double-click or select the initial point when the gate is completed.
5. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.
6. Customize your gate by following instructions in *Setting Up Gates*.

Freehand Gates

The freehand gate (Figure 2.47) is a very flexible option that gives you complete control over the size and shape of your gate. The freehand gate is available on dot, density, contour and radar plots.

Figure 2.47 Freehand Gates



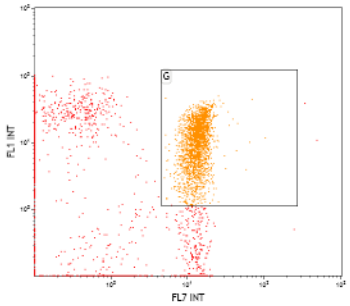
To create a freehand gate:

1. From the Gates & Tools Ribbon tab, select the  icon.
2. Determine the path you need for your gate.
3. Click your mouse where you wish to begin creating your gate, and, without releasing your mouse button, draw your gate to the size and shape you need. As you draw the gate, the default gate color will display, working as a guide to show your progress.
4. Release your mouse when you are finished.
5. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.
6. Customize your gate by following instructions in *Setting Up Gates*.

Rectangle Gates

The rectangle gate (Figure 2.48) is available on dot, density, contour and radar plots.

Figure 2.48 Rectangle Gates



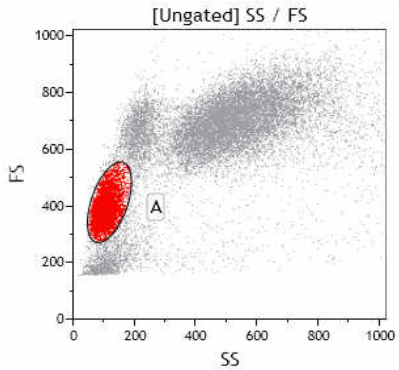
To create a rectangular gate:

1. From the Gates & Tools Ribbon tab, select the Rectangle icon.
2. Determine the size you need for your rectangular gate.
3. Click your mouse where you would like to begin your rectangle and drag to the size you need. Release your mouse when finished.
4. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.
5. Customize your gate by following instructions in *Setting Up Gates*.

Ellipse Gates

The Ellipse gate (Figure 2.49) consists of curved lines. These gates can be sized and shaped using the eight default handles and rotated using the handle. The ellipse gate is available on the dot, density, contour and radar plots.

Figure 2.49 Ellipse Gates



To create an ellipse gate:

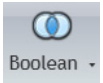
1. From the Gates & Tools Ribbon tab, select the  icon.
2. Determine the path you need for your gate.
3. Click your mouse where you would like to begin your ellipse and drag to the size you need. Release your mouse when finished.
4. Move or resize your gate by following instructions in *Resizing, Reshaping, and Moving Gates*.
5. Customize your gate by following instructions in *Setting Up Gates*.

Boolean Gates

The boolean gate allows you to set up a new gate by selecting current gates and defining whether to include and/or exclude the events that are located within those gates. Once defined, the boolean gate can be used for gating other plots.

To create a Boolean gate:

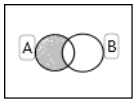
---

1 From the Gates & Tools Ribbon tab, select the 

icon. The Boolean Gates menu appears, which is where boolean gates are defined. The menu contains the following:

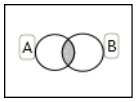
- **New Gate**: Adds a new entry; this is where the gate is defined.
- **Available gates...**: Choose from current gates to create the boolean gate.
- **NOT**: Excluded events are defined by **NOT**. For example, **A AND (NOT B)** includes all events from gate A, but all events that are included in gate B or gates A and B are excluded (see Figure 2.50).

Figure 2.50 Boolean Gating—Example Using "NOT"



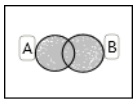
- **AND**: When using **AND**, only the events located within the overlap of all gates defined by **AND** are included. For example, **A AND B** only includes the events that fall within the overlap of both gates (see Figure 2.51).

Figure 2.51 Boolean Gating—Example Using "AND"



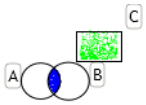
- **OR**: Events in all of the gates defined with an **OR** will be included in the boolean gate. For example, **A OR B** includes all events in both gates (see Figure 2.52).

Figure 2.52 Boolean Gating—Example Using "OR"



- Parentheses enclose terms to be combined with an operator when more than one type of Boolean operator appears in the same statement. For example, **C OR (A AND B)** contains all events in gate C and those that overlap between A and B (see Figure 2.53).

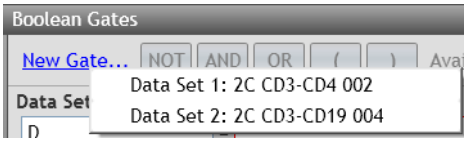
Figure 2.53 Boolean Gating—Example Using Parenthesis



-  : When selected, deletes the entire gate.

2 Select **New Gate** (Figure 2.54).

Figure 2.54 New Gates Link



NOTE If working with a composite when a new gate is selected, Kaluza Analysis lists the data sets included in the composite. You need to select a data set from which to create the Boolean Gate.

3 Use the buttons and the **Available gates...** hyperlink to create your logic expression in the **Logic** field. Rename the boolean gate by updating the text located in the **Name** field.

The expression is in red with a red outline until the expression is determined to be logical.

NOTE You can also type the logic expression in the Logic field but must use quotation marks when the name of the gate contains spaces; for example, **NOT "CD45 Dim" AND "CD34 POS" AND Leuks**.

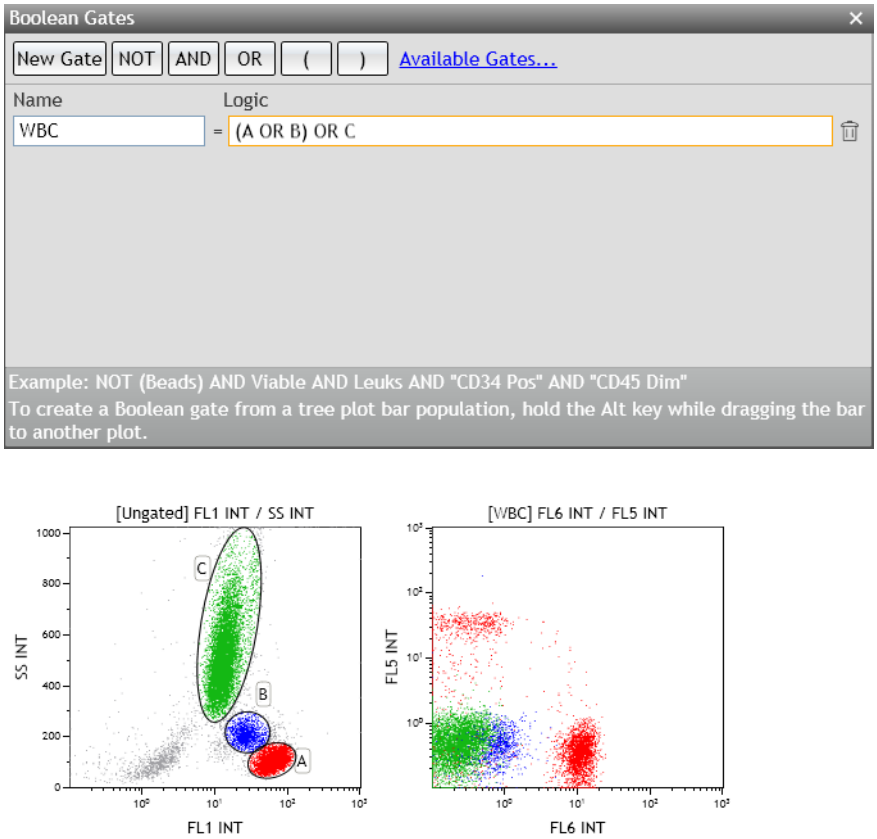
There is a limit to the number of terms you can include in a logic expression; this limit is based on the number **and** complexity of terms included in a logic expression. An error message displays when this limit is reached.

IMPORTANT Complex logic expressions slow the application response time.

4 Select ✕ when finished. The newly created boolean expression is now available for gating plots.

Figure 2.55 is an example of a boolean gate, defined as WBC, created to encompass all events within gates A, B, and C. The plot in the lower right corner of Figure 2.55 is gated on WBC.

Figure 2.55 Boolean Gate Example



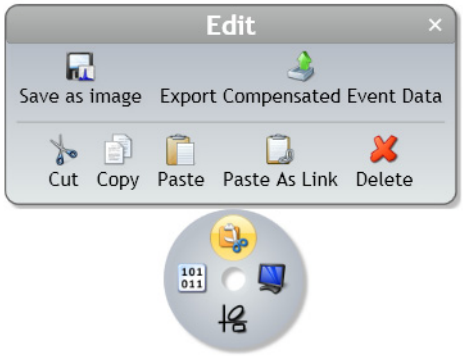
Setting Up Gates

Kaluza Analysis offers an array of options for customizing your gates. The following sections highlight these options and give instructions on how to employ the techniques for using these options.

Editing Gates

Use the Edit Radial Menu (see Figure 2.56) to perform basic editing functions for a selected gate, including cut, copy, paste, delete, and Export Compensated Event Data. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for a description of the functions.

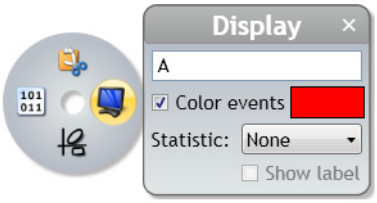
Figure 2.56 Edit Radial Menu



Setting Up Gate Display

Use the Display Radial Menu (see Figure 2.57) to alter the name of a gate, change the color of events that fall within the gate, and choose a statistic to appear next to the gate.

Figure 2.57 Display Radial Menu



Gate Name

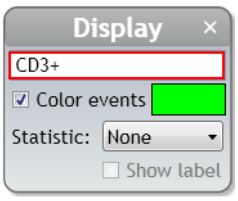
The **Gate Name** field allows you to enter a custom name for your gate. To update a gate name:

1. With your mouse positioned over the gate, right-click to access the Radial Menu.

2. Hover over the (**Display**) icon to access the Display menu. The Display menu appears.

3. Delete the current gate name and enter the revised name into the field (field location is outlined in red in Figure 2.58).

   Figure 2.58 Updating the Gate Name



4 Select ✕ to complete the process.

Color Events

Use the **Color events** section of the Display menu to define a new color for your gate or remove gate coloring. The two procedures below describe the process for completing these tasks.

To update the color of the events that fall within a gate:

1. With your mouse positioned over the gate, right-click to access the Radial Menu.
2. Hover over the (**Display**) icon to access the Display menu. The Display menu appears.
3. Select the color block. The color palette appears.
4. Choose the new color for the gated events.
5. Select ✕ to complete the process.

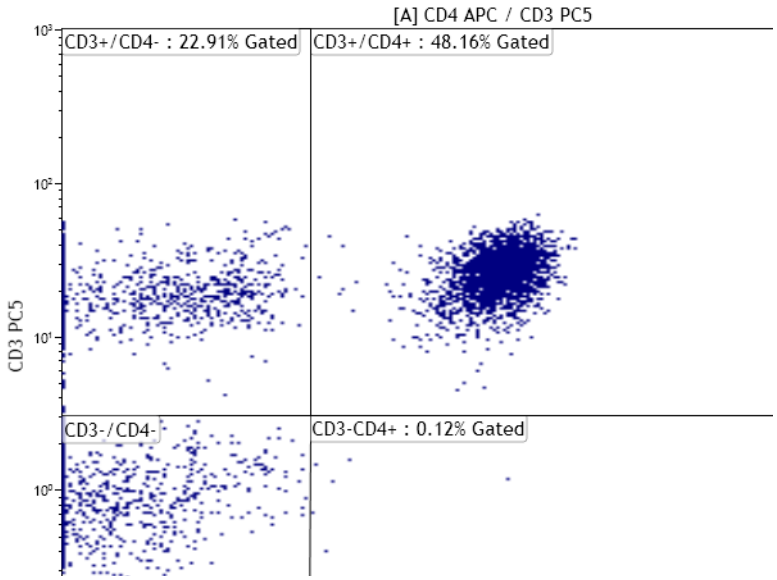
To add/remove gate coloring:

1. With your mouse positioned over the gate, right-click to access the Radial Menu.
2. Hover over the (**Display**) icon to access the Display menu. The Display menu appears.
3. Select/deselect the **Color events** check box to change your event coloring preference.
4. Select ✕ to complete the process.

Gate Statistics

The **Statistic:** drop-down is where you can choose to display a statistic directly on the plot. Statistic options include **Number**, **% Total**, and **% Gated**. You can also choose to include a statistic label. Figure 2.59 shows an example of a gate displaying the **% Gated** statistic, including the statistic label.

Figure 2.59 % Gated Statistic and Statistic Label



To display a statistic directly on the plot:

1. With your mouse positioned over the gate, right-click to access the Radial Menu.
2. Hover over the (**Display**) icon to access the Display menu. The Display menu appears.
3. Select the **Statistic:** drop-down list and choose the preferred statistic.
4. If you wish to show a label, select the checkbox located next to **Show Label.**
5. Select to complete the process.

Adding a New Gate

Use the Gates & Tools Radial Menu (see Figure 2.60) to add a gate, annotation, or overlay marker to a plot.

NOTE The Gates & Tools menu includes the same options no matter where you are currently located on the sheet. If a gate selected from the menu is not appropriate for the current sheet location, the cursor will display as a ⃠ symbol. When you are in an appropriate location for the gate type, the cursor changes to the symbol for the gate type you selected, indicating that you may begin drawing.

See *Using the Gates & Tools Plot Radial Menu*, for details on using this menu.

See *Gates & Tools*, for an in-depth description of gating.

Figure 2.60 Gates & Tools Radial Menu

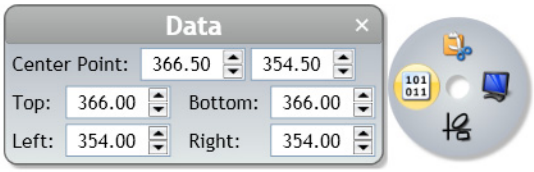
Data Menu

Use the Data Radial Menu (see Figure 2.61) for one or more of the following activities:

- Viewing coordinates
- Changing location
- Changing the size or angle
- Linking to other gates

For additional methods for changing data, see *Resizing, Reshaping, and Moving Gates*.

Figure 2.61 Data Radial Menu



Center Point

Center Point allows you to change the location of the center point of the gate. (Applies to elliptical, quadrant, and hinged quadrant gates.) To change the center point location using the Radial Menu:

---

1 With your mouse positioned over the gate, right-click to access the Data Radial Menu.

2 Hover over the (**Data**) icon to access the Data menu. The Data menu appears.

3 The Center Point field for the X-axis is on the left, and the Y-axis field is on the right. Update the coordinates either by deleting the number currently located within the fields and entering the new value, or by using the up/down arrows to nudge in either direction.

4 Select to complete the process.

Top/Bottom/Left/Right

The **Top**, **Bottom**, **Left**, and **Right** fields allow you to enter coordinates of the intersection points for each side of hinged quadrant gates. To update coordinates using the Radial Menu:

1 With your mouse positioned over the gate, right-click to access the Radial Menu.

2 Hover over the (**Data**) icon to access the Data menu. The Data menu appears.

3 Update the **Top**, **Bottom**, **Left**, and/or **Right** fields either by deleting the number currently located within the fields and entering the new value or by using the up/down arrows to nudge in either direction.

4 Select to complete the process.

X Radius/Y Radius/Angle

The **X Radius**, **Y Radius**, and **Angle** fields allow you to enter the size of the radius in relation to the X/Y axes and the angle of an elliptical gate. To update coordinates using the Radial Menu:

1 With your mouse positioned over the gate, right-click to access the Radial Menu.

2 Hover over the (**Data**) icon to access the Data menu. The Data menu appears.

3 Update the **X Radius**, **Y Radius**, and **Angle** fields either by deleting the number currently located within the fields and entering the new value or by using the up/down arrows to nudge in either direction.

4 Select ✕ to complete the process.

X0/X1/Y0/Y1

The **X0**, **X1**, **Y0**, and **Y1** fields allow you to change the size of a rectangle gate by changing the coordinates of each side of the rectangle. To update coordinates using the Radial Menu:

1 With your mouse positioned over the gate, right-click to access the Radial Menu.

2 Hover over the (**Data**) icon to access the Data menu. The Data menu appears.

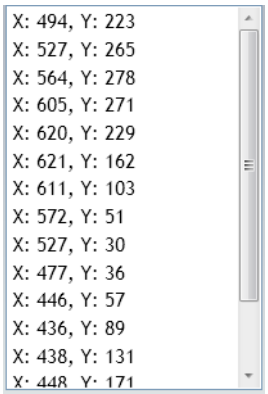
3 Update the **X0**, **X1**, **Y0**, and **Y1** fields either by deleting the number currently located within the fields and entering the new value or by using the up/down arrows to nudge in either direction.

4 Select ✕ to complete the process.

X/Y Coordinates

In polygon and freehand gates, the Data menu displays the X and Y coordinates of each handle (change of direction), as well as the angle and length located on the gate. Figure 2.62 shows the X/Y coordinates list for a freehand gate.

Figure 2.62 X/Y Coordinates List



NOTE To make changes to the shape of polygon or freehand gates, follow the methods described in *Resizing, Reshaping, and Moving Gates*.

Link to Gates

Link to Gates... allows you to change other freehand, polygon, linear, rectangle, and ellipse gates within your Protocol to the same size, shape and X/Y coordinate locations as the current gate. Gates cannot be linked to gates of other types, except for polygon and freehand, which can be linked

together. When gates are linked, changes made to one gate automatically apply to all gates that are linked.

NOTE You may only link gates of the same type. Changes to the axes of a plot on which one linked gate is defined will not affect the other linked gates.

To link gates:

1. With your mouse positioned over the gate, right-click to access the Radial Menu.
2. Hover over the (**Data**) icon to access the Data menu. The Data menu appears.
3. Select the **Link to Gates...** hyperlink. A pop-up window appears with a list of gates applicable for linking.
4. Select the gate(s) that you wish to link to the current gate.
5. Select to complete the process.

X0/X1/Height

The **X0**, **X1**, and **Height** fields allow you to change the length and height of a linear gate or overlay marker by changing the coordinates of each side of the line or the height coordinate on the Y-axis. To update coordinates using the Radial Menu:

1. With your mouse positioned over the linear gate or overlay marker, right-click to access the Radial Menu.
2. Hover over the (**Data**) icon to access the Data menu. The Data menu appears.
3. Update the **X0**, **X1**, and **Height** fields either by deleting the number currently located within the fields and entering a new value or by using the up/down arrows to nudge in either direction.
4. Select to complete the process.

Resizing, Reshaping, and Moving Gates

See Table 2.10 for complete instructions on updating the physical characteristics of a gate.

Table 2.10 Resizing, Reshaping, and Moving Gates

Item	Details
	Resize and Reshape Gates: Handles allow for resizing and/or reshaping a portion of, or an entire, gate.
	Move Gates: When your mouse enters the confines of a gate, the cursor changes to a , indicating that the movement of an entire gate is enabled. Move a gate by selecting and dragging when you see this cursor. • To move the gate a fixed amount, select the gate, and then press the appropriate arrow key(s) on your keyboard until you are satisfied with the position. • Smaller adjustments can be made by pressing the Ctrl key while using the arrow keys.
	Lengthen or Shorten, Reposition, Change Angle, and Stretch or Compress Gates: The cursor changes to a double-sided arrow when you move your mouse near a handle. The type of arrow indicates the direction of movement. Select and drag a handle to: • Lengthen or shorten **linear** gates or **overlay markers**. • Reposition quadrants in the **quadrant** and **hinged** gates. • Change angles of the quadrants in the **hinged** gates. • Horizontally, vertically, or diagonally stretch or compress entire gates, including **polygon**, **freehand**, **rectangular**, and **elliptical** gates.
	Reshape Polygon Gates: The cursor changes to a + when you hover your mouse over a polygon handle. This cursor indicates that you can reshape the polygon. Select and drag a handle to reshape.
	Rotate Elliptical Gates: By moving your mouse over the circular handle, the indicates it is ready for you to rotate an elliptical gate on the center of axis.
	Move a Gate Name: When you move your mouse over a gate name, the indicates that a gate name is ready for movement. Select and drag the name to move to a new location.

Methods for Applying Gates to Plots

In addition to using the Data menu (see *Setting Up Plot Data*), there are three other methods for assigning a gate to a plot.

Gating Plots Using the Plot Hyperlink

To gate a plot using the hyperlink:

1 Select the hyperlink located at the top of a plot. A pop-up menu appears, which contains a list of gates, including recently created gates and gates by category (common, quadrant, and boolean).

2 Select the gate for your plot from the pop-up (s). The events within your plot are now filtered, based on the events in the gate you selected.

Gating Plots Using the Color Precedence Pane

To gate a plot using the Color Precedence pane:

1 From within the Color Precedence pane, select the row of the gate that you wish to apply to the plot.

2 Drag the gate onto the plot and release your mouse button to complete the process.

Gating Plots by Dragging/Dropping

To gate a plot using the drag/drop method:

NOTE Not only will the following procedure work for all gate types, you can also gate plots on tree bars from a tree plot.

1 Press the (Alt) key, and, while pressed, select the gate or tree bar that you wish to use as the input gate for a plot.

2 Drag the gate or tree bar onto the plot and release your mouse button/ (Alt) key to complete the process.

Establishing Color Precedence of Gates

The Color Precedence pane displays the event coloring and precedence of coloring for gates in the current Protocol. The pane contains three main sections, including Default Event Color, Gates With Color, and Gates Without Color. These sections, along with the procedures for making changes within the Color Precedence pane, are described in the following section:

IMPORTANT

1. If a *.lmd file is opened with an embedded protocol that contains an orphan gate, the orphaned gate will be present in color precedence, but there is no geometric entity (no gate viewed in a plot) for the user to manipulate. A plot can be created for the gate using Show Gate option, or the gate can be deleted from the Color Precedence pane.

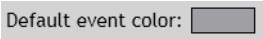
2. Changing the Data Set here also affects the Compensation and Parameters pane. See *Selecting the Data Set in a Composite* for selection of individual Data Sets when working with color precedence on composites.

NOTE An orphan gate in the embedded runtime protocol is a gate that is included in a protocol, but not present on any plot.

Default Event Color

Default Event Color (Figure 2.63) displays the color of events that have not been assigned to a gate. This color is also the default color of events on a plot when **Use Single Color** is selected from the Coloring Radial Menu (see *Using the Coloring Menu* for details).

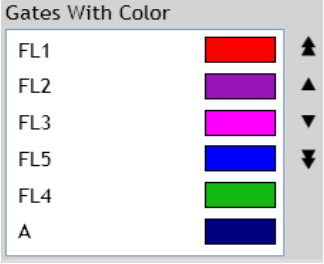
Figure 2.63 Default Event Color



Gates With Color

Gates With Color (Figure 2.64) displays gates in order of precedence. The gate located at the top of the list has the highest precedence, and the gate at the bottom has the lowest precedence. When an event belongs to more than one gate, it appears on the plot sheet in the color with the highest applicable precedence. All enabled gates in the current Protocol are displayed in the Gates With Color section of the Color Data Set Precedence pane.

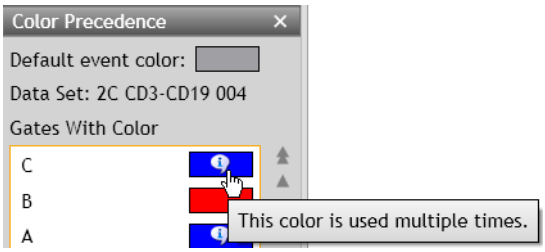
Figure 2.64 Gates with Color



NOTE

1. You can assign color to up to 31 gates per Data Set. Colored gates can be located on multiple sheets within the same Protocol/Analysis.
2. If the same color is selected for more than one gate, an Information icon displays indicating "This color is used multiple times." See Figure 2.65.

Figure 2.65 Duplicate Color Icon



NOTE Multi-selection is available for the following procedures.

Updating Color Precedence

To change the precedence of a gate:

---

1 From the Color Precedence pane, select anywhere within the row of the gate you need to move.

2 At this point, there are two ways to change the gate precedence hierarchy:

a. **Drag and Drop Method**: With the row still selected, drag it to the new location and release the mouse button.

OR

b. **Using Buttons (located on the right-hand side of the pane)**:

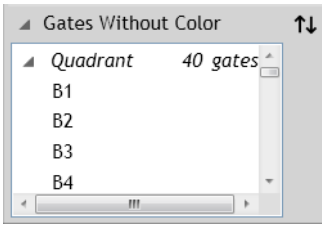
- **To move the gate up or down one space**: select the ▲ or ▼ buttons until the gate is in the appropriate location.
- **To move the gate to the top or bottom of the list:** select the ⏶ or ⏷ button.

Gates Without Color

Gates Without Color (Figure 2.66) displays a list of gates that are not color enabled. These gates are separated into Common, Quadrant, and Boolean gates, but only applicable categories are visible. The Gates Without Color section also displays gates that had been previously assigned a color, but are now disabled.

As a default, the Color Precedence pane does not display the list of gates without color. To enable this listing, see *Displaying the Gates Without Color Listing*.

Figure 2.66 Gates Without Color



Displaying the Gates Without Color Listing

To display/hide the Gates Without Color section of the Color Precedence pane:

1 Click the arrow (outlined in red in the figure below) located at the bottom of the Color Precedence pane, to display gates without color. The Gates Without Color section uses categories (Common, Quadrant, and Boolean).

To hide this portion of the Color Precedence screen, click on the ◢ icon located next to Gates Without Color.

2 The software defaults to a collapsed view of the Common/Quadrant/Boolean gate categories.

To display the contents a category, click on the icon located next to the section you wish to expand.

To collapse the list, click on the icon located next to the section you wish to collapse.

Assigning Color to a Gate Without Color

To assign color to a gate without color:

1 Select anywhere within the row of the gate you need to color.

2 There are three ways to enable gate coloring:

- **Click and Drag:** With the row still selected, drag the row to the Gates With Color section of the pane and release the mouse button.
- **Up/Down Icon:** Select the button to move the gate to the Gates With Color section.

  NOTE If gate coloring had been disabled, the gate will retain the former level of precedence when re-enabled.

- **Right-Click:** Right-click on the selected gate(s) and select **Enable coloring**.

Changing the Name of a Gate

Gate names are editable and can be changed by:

NOTE Changing the gate name using the Color Precedence pane changes the gate name in all locations within the application.

1 Position your mouse over the gate name that you wish to change and click twice. An editable field is indicated by the text appearing highlighted.

NOTE Copy, cut, and paste is available by right-clicking on the text field or using keyboard shortcuts once the field is editable (highlighted).

2 Enter the new name in the field.

IMPORTANT When changing gate names, keep the following in mind:

- Gate names cannot be left blank.
- The maximum length is 255 characters.
- Gate names cannot be repeated.

3 To save your changes, press (Enter) on your keyboard or click on another gate.

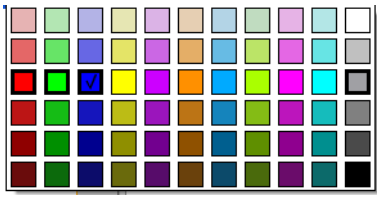
Changing the Color of a Gate

New gates are automatically assigned a unique color. Gates that have been moved from the Gates Without Color section, however, might not be assigned a unique color. When this occurs, you will see an Information icon. To correct the error, update one of the colors by following the procedure below.

To change the color of a gate:

1 Click on the color swatch of the gate color you wish to change. The color palette appears (Figure 2.67). The colors that have a bold, black outline are the colors that are already in use. The color that contains a check mark is the current color assigned to that gate. Refer to the figure below for an example.

Figure 2.67 Color Palette



2 Choose a new color from the palette. This action changes the color of all events shown on the plot sheet that fall within that gate and have the higher precedence.

Deleting a Gate From the Color Precedence Pane

To delete a gate:

1 From the Color Precedence pane, select within the row of the gate you wish to delete.

2 There are two ways to delete a gate:

a. **Right-Click:** Right-click on the selected gate and select **Delete** from the menu.

b. **Keyboard:** Press Delete on your keyboard.

NOTE You may also delete a gate directly from the plot by right-clicking on the gate and selecting from the Edit Radial Menu.

NOTE If you have imported a Protocol from a *.lmd file, it may include gates that are not visible on any plots. To delete these gates, right-click on the gate from within the Color Precedence pane and select **Delete** from the menu.

Disabling Color From the Color Precedence Pane

To disable a gate:

1 From the Color Precedence pane, select within the row of the gate you wish to disable coloring on.

2 Right-click and select **Disable Coloring.** This places the gate in the **Gates without Color** section of the pane.

Show Gate From the Color Precedence Pane

To locate a gate:

1 From the Color Precedence pane, select within the row of the gate you wish to locate.

2 Right-click and select **Show Gate.** This places into view, on the screen, the plot where a specific gate is located, or creates a plot if one does not exist.

NOTE This option is not available for multi-selection.

3 Locate a gate in the sheet area or create a plot for an orphan gate.

Tools

The tools described in the following sections allow you to customize your plots/sheet.

Annotation Tool

The annotation tool allows you to add a text box to a plot by clicking and dragging within the plot.

To annotate a plot:

1. Right-click on the plot you wish to annotate.
2. Hover over the (**Gates & Tools**) icon to access the Gates & Tools menu.
3. Select Annotation.
4. Click and drag your mouse over the plot to create a text box. Release the mouse button when the box is the size you prefer.

   NOTE You can resize text boxes at any time by selecting the box and clicking and dragging a handle.
5. Right-click on the text box. The Gating Radial Menu appears.
6. Hover over the (**Data**) icon, which initiates a Data field.
7. Click within the data field and type your annotation.
8. Select when finished.

   NOTE To customize the annotation, see CHAPTER 3, *Formatting a Text Box*.

Overlay Marker

Overlay Marker

An **Overlay Marker** is for creating statistical markers within overlay plots. These are applicable only to the overlay plot in which they reside and are used to produce statistics for each of the histograms within the plot. See *Overlay Plots*.

Selection Tool

Choose Select to change the cursor to Selection mode, which allows you to make changes to the plot sheet, as well as individual plots.

Parameters

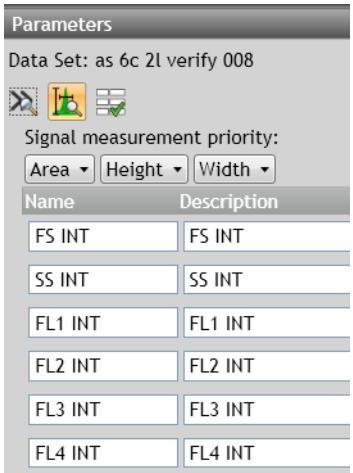
The Parameters pane displays a list of the parameters collected from the raw flow cytometry Data Set file. This pane enables you to edit parameter names, descriptions, types, detectors, and measurement type. The three icons on the Parameters pane (Figure 2.68) allow you to:

- Show more information
- Show signal measurement priority preferences for area, height and width
- Edit loaded parameters. You can change what parameters are shown in the list.

IMPORTANT If Kaluza Analysis does not initially recognize a parameter from an imported raw Data Set file, it will flag the parameter as a possible unknown in the Type field (parameter fields are described in detail throughout this section). If an incorrect assumption is made, the parameter may not appear in the Spillover Matrix (see *Adjusting Compensation*).

IMPORTANT Imported Data Sets that contain custom plot labels are configured as plot specific custom *axis* labels in Kaluza Analysis (see *Axes* for details). New plots using a custom label in another software platform will not use the custom label but will use the official parameter name or stain name assigned in the original software. For example, a plot from the imported Protocol used the custom parameter label "CD3+**Lymph**," which was created for the FL1 parameter. Switching the axis to the FL2 parameter does not change the axis label, and it will remain "CD3+**Lymph**," as custom axis labels in Kaluza Analysis apply specifically to an axis, not the parameter. The originating data set parameter name(s) and description(s) can be viewed in the Parameters pane **Name** and **Description**.

Figure 2.68 Parameters Pane

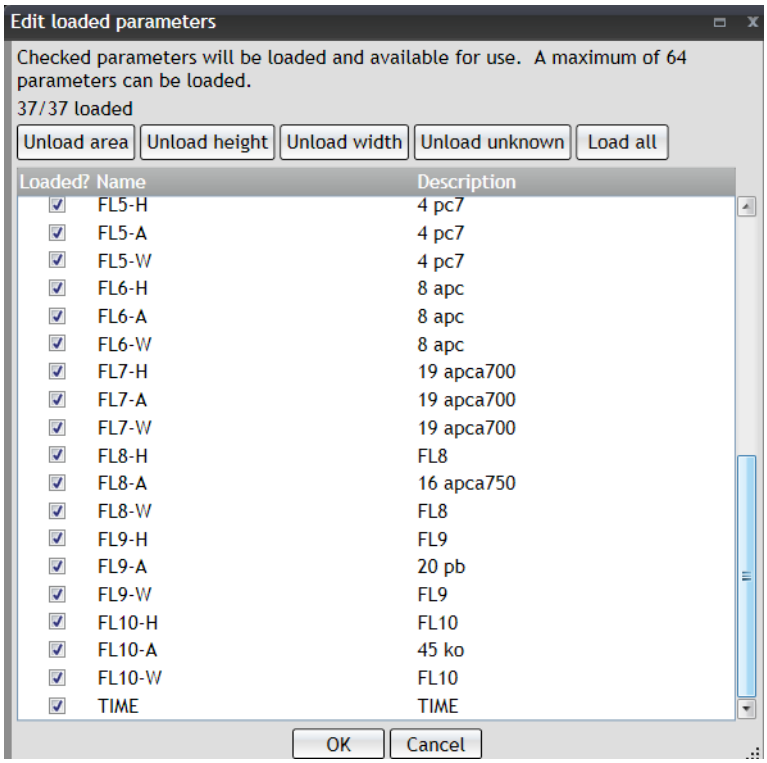


Changing the Parameters Pane Display

As a default, the Parameters pane displays Name (FCS $PnN) and Description (FCS $PnS) attributes only.

- Click on the **Show more information** icon . The Type, Detector, and Measurement attributes will also be visible. To collapse the additional attributes click the button again.
- Click on the **Show Signal Measurement Preference** icon located on the table header (see Figure 2.68) to show the preferences set up for the current data set. This priority is used to determine which measurement to use in compensation and for Add All Plots. The default for this setting can be set in the Kaluza Options; see CHAPTER 1, *Kaluza Options Menu*.
- Click on the **Edit Loaded Parameters** icon located on the table header to select which parameters are available for analysis. Files with more than 64 parameters may be loaded into Kaluza Analysis; however, only 64 parameters can be analyzed. All parameters beyond this limit will automatically be hidden. Use "Edit loaded parameters" to select which ones will be shown. When you have completed determining which parameters to load, select **OK**. The data set will be reloaded with the selected parameters.

Figure 2.69 Edit Loaded Parameters

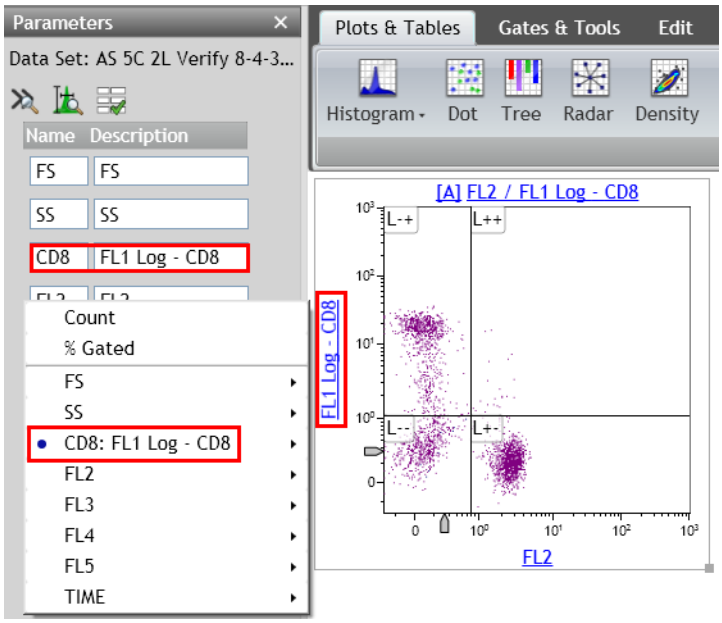


Updating Parameters

When parameter attributes are updated from the Parameters pane, all references to that parameter are updated. For instance, in Figure 2.70, the **Name** attribute changed from **FL1** to **CD8**, and the **Description** changed from **FL1** to **FL1 Log-CD8**. This action updated all instances of FL1 on the plot sheet, including the Y-axis parameter label, as well as the label on the drop-down list when the Y-axis parameter is selected.

You can copy and paste parameter configuration (via **Paste Special**) in the Analysis List as long as you use matching parameter names.

Figure 2.70 Updating Parameters



Complete instructions for updating content within the Parameters pane are included below.

Updating Content Within the Parameters Pane

IMPORTANT Kaluza Analysis is not guaranteed to make the correct assignment; always review the results and correct as deemed proper.

To update content within the **Name**, **Description**, or **Detector** field:

1. Highlight the text in the field that you wish to change.

2. Press the (Delete) key on your keyboard.

3 Type the new content into the field and press (Enter) .

NOTE There is a limit of:

- 5,000 characters for parameter name.
- 5,000 characters for parameter description.
- 5,000 characters for parameter detector.

IMPORTANT To ensure a parameter displays on the Spillover Matrix (see *Adjusting Spillover Values in the Compensation Pane*), the fluorescence detector **Type** must be set to "Fluorescence."

To update the **Type** or **Measurement** field:

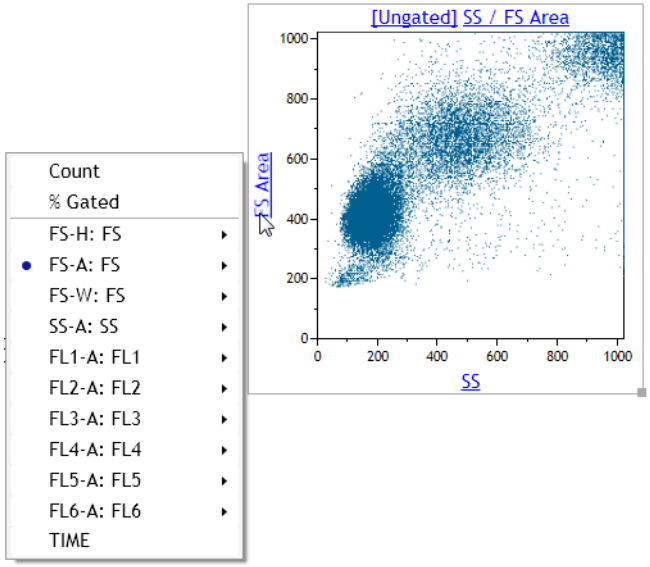
1 Click on the drop-down arrow on the right-hand side of the field you wish to change.

2 Select a new value.

NOTE When the **Type** field is set to "**Unknown**," the parameter does not appear on the sheet when **Add All Plots** is selected, and the parameter does not appear on the Spillover Matrix.

NOTE If multiple measurements are available for a parameter, the measurement type displays with the parameter name on the plot. For example, in Figure 2.71, FS was measured on Height, Area, and Width during acquisition; because the Area measurement was selected for FS, **FS Area** displays on the axis.

Figure 2.71 Parameters/Measurement Types



Copying and Pasting Parameters Between Data Sets

This function allows you to copy parameter details (e.g., Name or Description) to multiple data sets.

IMPORTANT The procedure below changes FCS Keywords $PnN (Name) and $PnS (Description) in files saved in Kaluza Analysis only. The raw data file keywords remain unchanged.

1. Right-click on the Analysis List and select **Copy**.

2. Select one or more data sets to **Paste Special** (by right-clicking on the Analysis List).

Additional Information for Composite Protocols

When a Composite Protocol is the active Analysis, the data within the Parameters pane can be updated for the individual Data Sets within the Composite. The pane's title block changes to a drop-down, allowing you to select an individual Data Set. To change to a different Data Set, simply select the drop-down arrow located on the right-hand side of the top of the pane and select the new Data Set.

NOTE When the Data Set in the Parameters pane is changed, the Compensation and Color Precedence panes simultaneously change to that Data Set.

Choosing Scale Type

Choosing the appropriate scale for your data is very important. The ability to produce meaningful plots is dependent upon selecting the appropriate scale. Scale type selections may also be controlled through the Data Radial Menu.

Logarithmic (Log) Scale

The log scale is useful when the data includes a large range of values, as the log scale changes the range by using ratios (for example, cell surface marker fluorescence parameters). Fluorescence parameters are best displayed in log scales because the scale is expanded to display weak signals and compressed to show strong signals. In Kaluza Analysis, decade width can be adjusted to fit the data.

Linear Scale

Linear scales contain divisions that are uniformly spaced. The linear scale is good for showing forward scatter and side scatter parameters, as well as width measurements.

Logicle Scale

When using the logarithmic (log) scale, correctly compensated data may appear to be incorrectly over-compensated because events with negative values tend to pile along the axes; this distortion occurs because negative values do not exist on a log scale.

Kaluza Analysis includes the logicle scale, which provides a means to correctly display compensated data. Changing an axis from log to logicle scale splits the axis into two different regions, where the positive values remain in log scale and negative values are transformed into linear scale. The two

different scales are divided by a slider, which provides the ability to interactively control the width of each region. When you use the logicle scale, negative values display correctly, preserving the desired symmetrical appearance of correctly compensated data.

Compensation

Introduction to Compensation

To understand the need for fluorescence compensation, you must start with basic flow cytometry concepts. These concepts are described in this section.

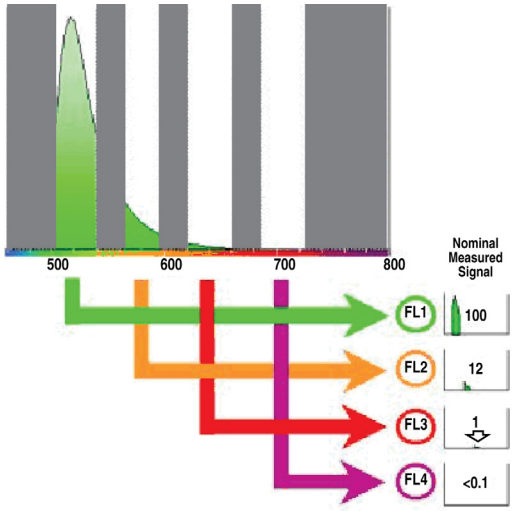
When particles are processed through a flow cytometer, they (or the attached fluorochromes) are excited by a laser. As these laser-excited events return to their former, unexcited state, they release energy. The intensity of the released energy depends on two factors, including:

- The type of fluorochrome(s) attached to, or intrinsic to (autofluorescence) the event.
- The amount of fluorescence molecules that are attached to each event.

The energy released from each event is detected by photomultiplier tubes (PMTs), via a series of dichroic mirrors and optical band-pass filters that allow only a specific region of the spectrum to reach each PMT. Each PMT located within a flow cytometer detects a different color range; however, because the emission spectra for different fluorescence stains overlap and signals cross over to PMTs other than the one specified for a particular fluorochrome, it is necessary to correct spillover.

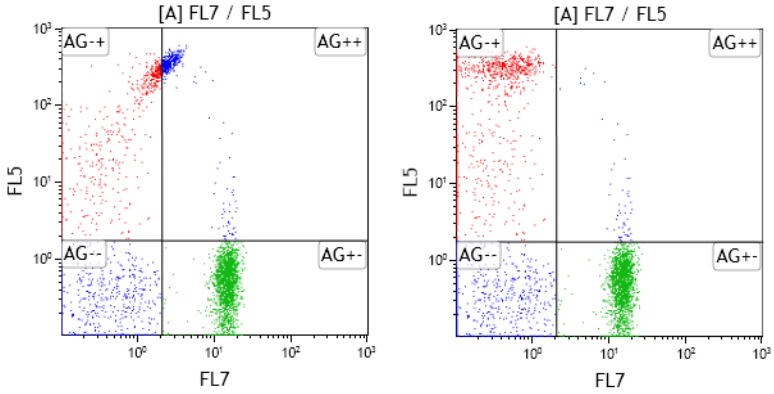
Figure 2.72 illustrates fluorescence spillover (FL1) from a particle labeled with FITC, a green dye fluorescing into PMT2 (FL2), PMT3 (FL3), and (PMT4) FL4. Some of the FITC-emitted light reaches every one of the four fluorescence detectors shown in this example. Because PMTs are very high-gain devices, even a very small amount of light can be measured, and it is quite possible for three, or even all four, detectors (as in this example), to generate a measurable signal. This phenomenon, where part of the signal from a fluorochrome spills over into a detector other than its primary or “intended” detector, is called “crosstalk” or "spillover." This may occur for all fluorochromes in use and must be dealt with. Color compensation electronically removes the crosstalk or spillover. The Spillover Matrix shows the combined spillover effects for all fluorochromes present.

Figure 2.72 Fluorescence Spillover—FITC



In the figure below, the plot on the left is not compensated, and the plot on the right shows correctly compensated fluorescence parameters. When fluorescence parameters are correctly compensated, they are aligned both horizontally and vertically.

Figure 2.73 Compensating Event Populations - Before and After Correctly Compensating



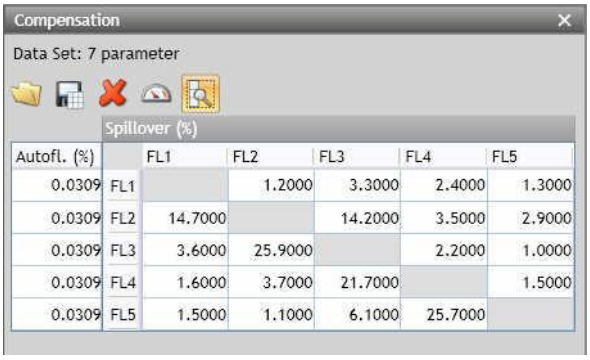
The following sections describe the methods for automatically generating Spillover and Autofluorescence Vector values (see *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature*), and making manual adjustments to compensation using Spillover Sliders (see *Adjusting Spillover Using the Spillover Sliders Directly on the Plot(s)*) or the Spillover Matrix (see *Adjusting Spillover Values in the Compensation Pane*).

Compensation Pane

The Compensation pane (see Figure 2.74) contains tools for adjusting Spillover and Autofluorescence Vector values related to a particular Data Set. The main component of the Compensation pane is the Spillover Matrix, which includes all fluorescence parameters associated with a Data Set and, if applicable, a column that displays the autofluorescence vector. Other tools, which are described in the following sections, are also available in the Compensation pane:

- *Saving a Compensation File*
- *Importing a Compensation File*
- *Resetting Spillover and Autofluorescence Vector Values*
- *Adjusting Spillover Using the Spillover Sliders Directly on the Plot(s)*
- *Displaying the Autofluorescence Vector*

Figure 2.74 Compensation Pane



Autofl. (%)		FL1	FL2	FL3	FL4	FL5
0.0309	FL1		1.2000	3.3000	2.4000	1.3000
0.0309	FL2	14.7000		14.2000	3.5000	2.9000
0.0309	FL3	3.6000	25.9000		2.2000	1.0000
0.0309	FL4	1.6000	3.7000	21.7000		1.5000
0.0309	FL5	1.5000	1.1000	6.1000	25.7000	

The Compensation matrix uses the following convention: FL# (vertical axis) - %FL# (horizontal axis). For example, in Figure 2.74, **FL2-%FL1 = 14.7**.

Adjusting Compensation

The following sections describe the methods for adjusting compensation.

Adjusting Spillover Values in the Compensation Pane

The Spillover Matrix allows you to manually enter Spillover percentages into the matrix and/or to view Spillover percentages that have already been adjusted using the Spillover Sliders on the plot sheet.

To manually update a value within a cell of the Spillover Matrix:

1 Click your mouse button within the cell you wish to update. As shown in Figure 2.75, the cell is highlighted in blue when it is ready to update.

Figure 2.75 Manually Updating a Cell

Spillover (%)

	FL1	FL2	FL3	FL4	FL5	FL6
FL1		1.20	0.30	0.20	0.60	0.00
FL2	15.10		11.50	2.10	8.20	0.00
FL3	4.30	43.10		1.90	3.20	0.00
FL4	0.50	7.10	24.50		0.10	0.60
FL5	0.00	0.00	0.80	4.50		0.00
FL6	0.00	0.00	0.00	37.80	0.00	

2 Type the new Spillover percentage into the cell and press (Enter) on your keyboard or click your mouse on another location of your screen.

NOTE Values between 0 to 100% are considered to be expected values and appear in black font in the Spillover Matrix. Values that are outside of the expected range are allowed, but appear in gold font in the Spillover Matrix (Figure 2.83), indicating that something unexpected has been entered. However, there is a limited range for unexpected values; spillover values lower than -500% or above 500% are not accepted and appear in red font (and will be cancelled if not corrected while editing).

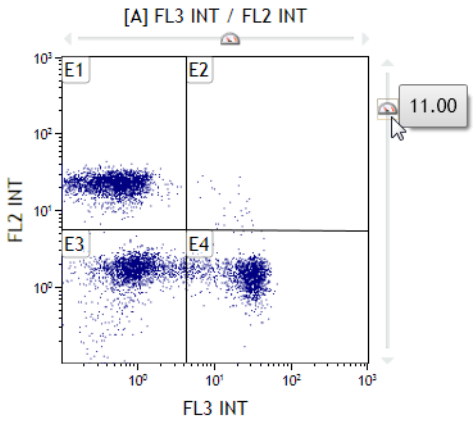
IMPORTANT If a fluorescence detector does not appear in the Spillover Matrix, make sure that the **Type** field within the Parameters pane is not set to "Unknown." See *Updating Parameters*, for instructions on changing the parameter type.

Adjusting Spillover Using the Spillover Sliders Directly on the Plot(s)

Spillover Sliders allow you to compensate for fluorescence Spillover by using real-time visual cues on plots. The sliders can be generated on all applicable plots.

- **To enable the Spillover Sliders on the plot sheet:** Select the icon from the Compensation pane or the Gates & Tools Ribbon tab.
- **To remove the Spillover Sliders from the plot sheet:** Select the icon from the Compensation pane or the Gates & Tools Ribbon tab.
- **To update Spillover using the Spillover Sliders on the plot sheet:** Select the slider and drag in the direction you wish to change. The Spillover value is displayed next to the slider, as shown in Figure 2.76.
- **To make incremental adjustments on the plot sheet:**
  - **To move in increments of .1%:** Select the appropriate arrow located on either end of the slider. Each time an arrow is selected, the slider handle moves 0.1%. This change can also be viewed in the Compensation pane.
  - **To move in increments of 1%:** Click on the slider bar; whichever side of the handle that you click on moves the slider 1% in that direction.

Figure 2.76 Spillover Sliders



Saving a Compensation File

The Spillover Matrix and Autofluorescence Vector can be saved into a ***.compensation** or ***.txt** file for use with other Data Sets or Protocols from Kaluza for Gallios. To save a ***.compensation** or ***.txt** file:

1. From the Compensation pane, select the (**Save Compensation As**) icon, which is located above the Spillover Matrix. The Save Compensation dialog box appears.
2. Select the destination for the file by navigating to the location using icons in the dialog box or the drop-down list in the **Save in** field.
3. Enter a file name into the **File name** field.
4. Select **Save**.

NOTE Additional options for saving a ***.compensation** or *.txt file are described in CHAPTER 1, *Using the Analysis List*.

Importing a Compensation File

The Spillover Matrix and Autofluorescence Vector from a previously saved file can be applied to a Data Set.

To import a ***.compensation** or ***.txt** file:

1. Select the (**Import compensation file**) icon above the Spillover Matrix. The Import Compensation dialog box appears.

2. Navigate to the ***.compensation** or ***.txt** file using the icons in the dialog box or the drop-down list in the **Look in** field. Once you find the file, select it, and verify that the file name appears in the **File Name** field.

3. Select **Open.** The new Spillover percentages appear in the Spillover Matrix.

NOTE Additional options for importing a ***.compensation** or *.txt file are described in CHAPTER 1, *Using the Analysis List*.

Resetting Spillover and Autofluorescence Vector Values

The (**Clear spillover and autofluorescence**) icon located above the Spillover Matrix resets Spillover and Autofluorescence Vector values associated with the Data Set to **0.00**.

Accounting for Autofluorescence

To increase the accuracy of fluorescence compensation, Autofluorescence Vector values need to be factored in prior to determining Spillover percentages. An Autofluorescence Vector is the value that is subtracted from the data prior to Spillover compensation and then added back afterwards.

Accounting for autofluorescence allows a greater degree of accuracy when interpreting data due to the increased ability to decipher which fluorochromes have attached to the surface of a cell. The true level of absorption of fluorochromes, or fluorescence intensity, is also more evident when factoring in autofluorescence.

When autofluorescence is considered, it enables a more “true” compensation because it changes the coordinates of each event to a state as if they contained no autofluorescence, compensates the events based on this assumption, and then adds the value back in to re-account for the intensity level.

Displaying the Autofluorescence Vector

To show the Autofluorescence Vector in the Spillover Matrix, select the (**Show autofluorescence vector**) icon; this adds a column to the left-hand side of the Spillover Matrix (highlighted in red in Figure 2.77).

Figure 2.77 Autofluorescence Vector Column (2 decimals)

	Spillover (%)					
Autofl.(%)		FL1	FL2	FL3	FL4	FL5
0.03	FL1		0.90	1.40	4.90	3.70
0.03	FL2	13.00		11.90	5.10	15.90
0.03	FL3	4.60	37.60		3.80	6.80
0.03	FL4	1.00	2.20	10.70		5.10
0.03	FL5	1.00	0.70	1.20	8.20	

Figure 2.78 Autofluorescence Vector Column (4 decimals)

	Spillover (%)					
Autofl.(%)		FL1	FL2	FL3	FL4	FL5
0.0309	FL1		0.9000	1.4000	4.9000	3.7000
0.0309	FL2	13.0000		11.9000	5.1000	15.9000
0.0309	FL3	4.6000	37.6000		3.8000	6.8000
0.0309	FL4	1.0000	2.2000	10.7000		5.1000
0.0309	FL5	1.0000	0.7000	1.2000	8.2000	

The Autofluorescence Vector is one of the following:

- **0.0309**, if using a *.lmd file derived from some Beckman Coulter instruments or *.fcs file from Kaluza for Gallios.
- the value established using the "Generate Compensation" feature (see *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature*), where the Spillover Matrix is generated with an Autofluorescence Data Set input.
- **0.000,** if the Spillover Matrix is generated without an autofluorescence Data Set input, or if the Data Set is derived from an non-Beckman Coulter instrument.

NOTE The Autofluorescence Vector value might be rounded, depending on the number of decimal places you elected to display in the Kaluza Options dialog box.

To hide the Autofluorescence Vector, select the  icon.

Determining Autofluorescence Vector Values

To manually determine an Autofluorescence Vector value for a fluorescence parameter:

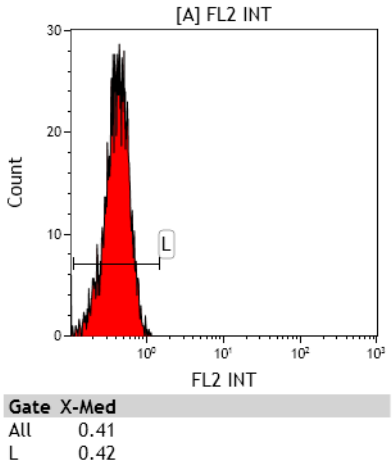
IMPORTANT Optimally, you should start by using a Data Set from an unstained sample. If this is not available, negative populations allow for Autofluorescence Vector calculations.

1 Create a histogram for the fluorescence parameter by setting the Y-axis to **Count** and the X-axis to the specific fluorescence parameter.

---

2 Create a linear gate on the histogram that includes events from the negative population (the events located in the first decade, as shown in Figure 2.79).

Figure 2.79 Gated Negative Population



3 Using the Statistics Radial Menu, choose to display the X-Median value.

4 Find the value corresponding to negative population for the gate that you created in step 2.

For example, in the figure in step 2, the "L" gate includes the negative population for FL2. Take the X-Med value shown, divide by 1024 and multiply by 100 to get a percentage.

5 Enter the result from step 4 for the negative population into the Autofluorescence Vector column in the Compensation pane.

Updating the Autofluorescence Vector

To update the Autofluorescence Vector:

1 Click within the cell you wish to update. The cell is highlighted in blue when it is ready to update.

2 Type the new autofluorescence value into the cell and press (Enter) .

NOTE Valid Autofluorescence Vector values range from **0** to **100, since it is a percentage value**.

Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature

To enable the most accurate data analyses, Kaluza Analysis has a feature that calculates Spillover and Autofluorescence Vector values. These values are viewed in the Compensation pane and can be saved and applied to Data Sets containing the same fluorescence parameters.

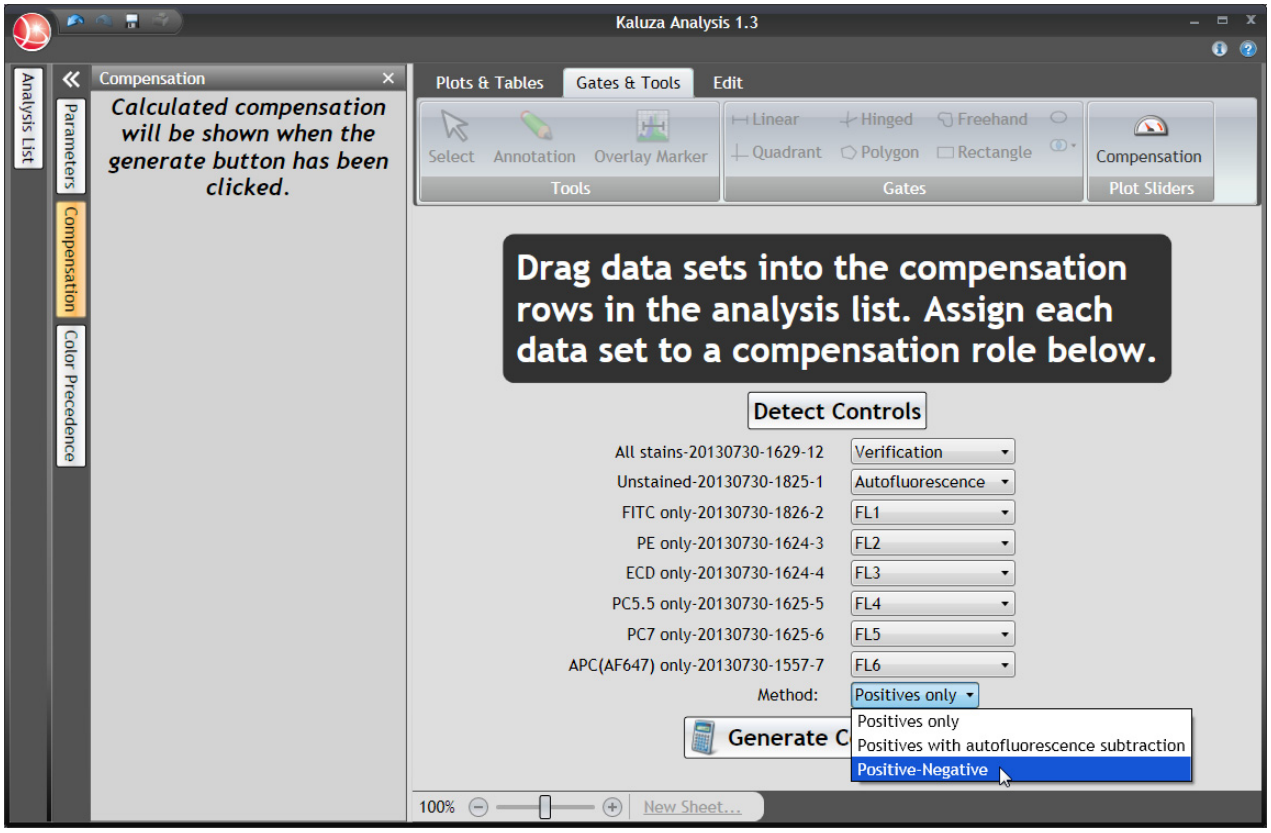
NOTE If you add or replace a row within the Compensation file that contains different parameters than the other files, errors will display in the Parameters pane, and the data will not display in the Verification Data Set.

Kaluza Analysis provides three methods for calculating spillover:

- Positives Only - This algorithm calculates the spillover matrix based on the ratio of the Arithmetic Mean on a detector without dye to the Arithmetic Mean of a positive population on a detector with dye. It assumes the negative events are centered at zero.
- Positives with Autofluorescence Subtraction - This algorithm is similar to the Positives Only algorithm, although it subtracts off autofluorescence values first. If an autofluorescence control is included, the values calculated from it will be in use; otherwise it is assumed that the negative events are in the middle of the first decade.
- Positive-Negative - This algorithm uses the slope of a line between the center of a bright gate and the center of a dim gate (as calculated by the median) for the spillover. The bright and dim gates are linked by default within each single-color control, but can be unlinked if you prefer. To use this method, your single-color control data must include some unstained cells.

  NOTE By default, the control population gates for the single-color controls are linked together.

Figure 2.80 Select Compensation Methods



Prior to generating Spillover and Autofluorescence Vector calculations, you need one Data Set file for each fluorescence parameter containing events stained using only one fluorescent dye. The following are optional:

- An unstained Data Set (to determine autofluorescence)

  NOTE If an unstained Data Set is not included in the calculation, the Autofluorescence Vector value is set to **0.00**.

- One Data Set that includes dyes from all fluorescence parameters (as verification)

Generating Spillover and Autofluorescence Vector Values

To create the Spillover Matrix and Autofluorescence Vector (if applicable):

1 Select > **New** > **Compensation**. This creates a new Compensation entry in the Analysis List.

2 Choose the files to use for the compensation calculation. You can either choose Analysis files and/or Data Set files that are already located within the Analysis List, or you can choose files that are stored on your computer.

NOTE Even though the default for a new Compensation Composite file contains six entries, you can import up to 32 Data Sets/analyses into your Spillover and Autofluorescence Vector calculation; the total number that you can assign roles to, however, depends on the number of fluorescence parameters in your calculation.

3 Multi-select the files and drag into the **<drop data set here>** cells within the Compensation Composite. When the cells become highlighted in orange, release your mouse button to complete the importing process.

NOTE

1. For details on multi-selecting, see *Multi-Selecting Files*.
2. If you wish to add a single row to the Compensation entry, select, drag, and drop the Data Set into the Compensation entry within the Analysis List. Be sure to drop the Data Set between two rows if you do not wish to replace the current contents of the cell. The indication that you are dropping data between rows appears as an orange line.

Use the Generate Compensation screen in the main portion of the application to assign files from which Spillover and Autofluorescence Vector values are calculated.

4 On the Generate Compensation screen:

- Click the drop-down arrow and choose the Compensation role corresponding to each Data Set included in the Compensation file.

  OR

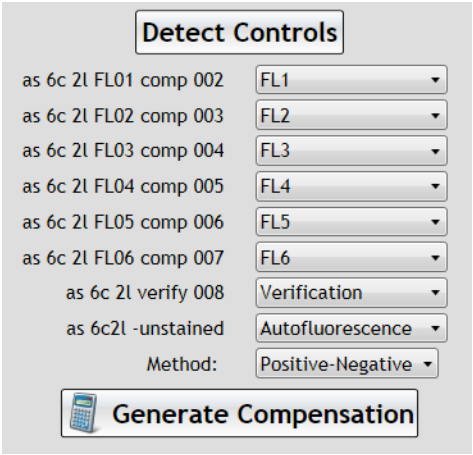
- Click the **Detect Controls** button so Kaluza Analysis can attempt to detect what the assignment should be.

IMPORTANT Keep in mind the following when using the Generate Compensation feature:

- Though Kaluza Analysis attempts to make logical assignments, always review the assignments as they may vary depending on your data.
- Each Compensation role can only be selected once.
- The application does not allow for a Compensation Composite file to be created from Data Sets containing mismatched fluorescence detectors. When this occurs, the icon appears on the Generate Compensation screen. You must replace the Data Set within the Analysis List to resolve this error.

Figure 2.81 is an example of the roles assigned to files for a 6-color Analysis.

Figure 2.81 Roles for a 6-Color Analysis



5 Choose which algorithm is to be used.

6 Select Generate Compensation. There are three routines available to choose from:

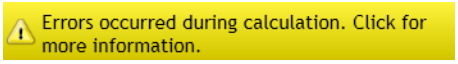
- Positives only
- Positives with Autofluorescence Subtraction
- Positives - Negative

This creates the Spillover Matrix and Autofluorescence Vector (if applicable) based on the data in the files used to make the calculations. See *Automatic Spillover and Autofluorescence Vector Generation: Using the Generate Compensation Feature* for a description of each compensation method.

NOTE Keep in mind the following when generating compensation:

- If an unstained Data Set is not included in the calculation, the Autofluorescence Vector value is set to **0.00**.
- Changes made to the Spillover Matrix and autofluorescence values update the Verification Data Set and may be further edited. If you elected not to include a Verification Data Set, the Spillover Matrix and Autofluorescence Vector values are not editable, but can be saved as a *.compensation file.
- If you receive an error message as shown in Figure 2.82, select it to view the complete list of errors. Errors can be resolved by interacting with the data sheets (see *Using Compensation Sheets*) or by checking your original file to ensure the Data Set is appropriate.

Figure 2.82 Errors During Calculation



- Values between 0 to 100% are considered to be expected values and appear in black font in the Spillover Matrix. Values that are outside of the expected range are allowed, but appear in gold font in the Spillover Matrix (Figure 2.83), indicating that something unexpected has been entered. However, there is a limited range for unexpected values; spillover values lower than -500% or above 500% are not accepted and appear in red font (and will be cancelled if not corrected while editing).

Figure 2.83 Unexpected Spillover Values

Compensation

Calculated Compensation: Verification Data Set

Spillover (%)

	FL1	FL2	FL3	FL4	FL5	FL6
FL1		0.96	0.27	0.19	0.64	0.00
FL2	15.40		10.97	2.63	5.99	-0.05
FL3	4.48	36.72		1.92	2.16	-0.04
FL4	0.55	5.39	23.32		0.67	0.42
FL5	0.00	0.19	1.19	5.95		0.00
FL6	0.00	0.00	0.02	45.28	0.00	

NOTE There are two ways to reenter the Generate Compensation screen:

- Select (**Undo**) until the screen reappears.
- Delete all sheets in the Protocol by one of the following methods:
  - Right-click on the Compensation entry in Analysis List row and select **Clear Protocol**.
  - Right-click on each sheet tab and select **Delete Sheet**. The sheet tabs are on the Sheet Tab Bar, which is located in the lower right portion of the application.

Using Compensation Sheets

When a Spillover and Autofluorescence Vector calculation is generated, a new sheet for each Compensation role is also added to the application. As a default, each Spillover sheet includes:

- An information block in the upper left corner of the sheet, describing how the calculation is derived for autofluorescence or spillover, and indicating which algorithm is used.
- One dot plot showing forward scatter vs. side scatter. This plot includes a default gate and statistics showing the % **Gated** and **Number** of events exhibiting fluorescence markers for that role, as well as the total number of events in that Data Set. The gate on this plot has its coordinates linked to the corresponding gate on the sheets for the other compensation roles by default. See *Link to Gates*.
- A set of additional plots and gates which are dependent on the compensation algorithm chosen. You can adjust any of the gates to improve the calculated values.

NOTE By default, the Verification sheet contains the plots and gates defined in **Add All Plots Options** (except the plots will use the logicle scale rather than the log scale). Make sure that the **Add All Plots Options** are set the way you want *prior to* generating the Verification Protocol. See *Add All Plots* for details.

To alternate between sheets, select the Compensation role on the Sheet Tab Bar (located at the bottom of the application) that you wish to view. The active role is highlighted in white, as with the FL1 sheet in the following figure:

Figure 2.84 Sheet Tab Bar

Adding Plots From the Original Analysis File

You can create additional plots, as deemed necessary.

Arranging Plots

For details on arranging plots on sheets, see *Moving Plots/Sheet Items*.

Saving Options

There are two options available for saving after using the Generate Compensation feature:

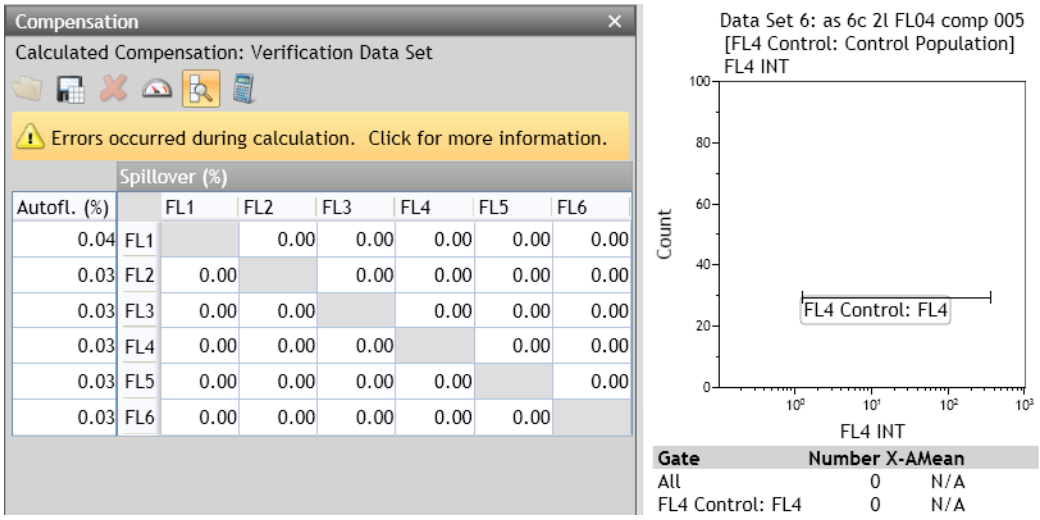
- Save the compensation values alone (see *Saving a Compensation File*).
- Save the entire composite analysis (see *Saving a Composite Analysis*). This file can be reused for other data sets.

Recalculating the Generated Compensation and Autofluorescence Vector Values by Editing Gate Position

To make updates to calculations, edit the gate positions located on the sheet belonging to the Compensation role that needs to be changed. The Spillover Matrix updates in real time when you move a gate.

NOTE A common error that you might receive occurs when the default gate encompasses no events. When that happens, the statistics in the histograms show as N/A, and the plots will be empty (as shown in the example figure below). If you move the gate in the forward scatter/side scatter plot to encompass events, the errors disappear.

Figure 2.85 Compensation Error



Manual Updates to the Generated Spillover Matrix

To fine-tune the generated Compensation calculations, manual adjustments can be made by following the procedure described in *Adjusting Spillover Values in the Compensation Pane*, or *Adjusting Spillover Using the Spillover Sliders Directly on the Plot(s)*.

IMPORTANT When making manual updates, keep in mind the following:

- After you manually adjust the Spillover Matrix, any adjustments made within the fluorescence sheets recalculate the entire matrix.
- If you wish to remove manual adjustments, select the  icon for a recalculation based on the fluorescence data inputs.

Saving, Importing, and Resetting the Generated Spillover Matrix

To save, import, or reset the generated Spillover Matrix, refer to the procedures in the following sections:

- *Saving a Compensation File*
- *Importing a Compensation File*
- *Resetting Spillover and Autofluorescence Vector Values*

Using the Logicle Scale

To display the logicle scale (see *Logicle Scale* for an overview) and use the sliders, follow the steps below.

---

1 Hover your mouse over the parameter you wish to change to logicle. The parameter changes to a hyperlink.

---

2 Select the hyperlink. The list of parameters appears.

NOTE Currently selected parameters contain a dot next to the parameter name/description, and the current scale used for the parameter (if applicable) contains a check mark next to the scale type.

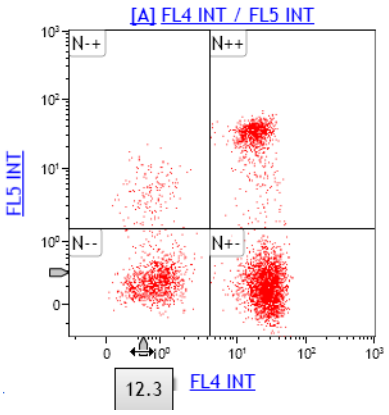
---

3 Hover your mouse over the parameter that you wish to display using the logicle scale, and from the pop-up menu, choose the **logicle** scale type for that parameter.

Repeat this step for the other plot parameter, if necessary.

Changing a parameter to the logicle scale adds a slider to the axis, as shown in Figure 2.86.

Figure 2.86 Logicle Sliders



4 Select and drag the slider to adjust the scale to display negative values. As demonstrated on the X-axis in the figure above, the numerical value appears and changes as you move the slider.

5 Release your mouse button once you are satisfied with the display of the compensated events.

Composite Analysis Compensation Options

When a Composite Protocol is the active Analysis, you can update the data within the Compensation pane for the individual Data Sets within the Composite. The title block for that pane changes to a drop-down, allowing you to select an individual Data Set. To change to a different Data Set, select the drop-down arrow located on the right-hand side of the top of the pane and select the new Data Set you wish to display.

NOTE When the Data Set in the Compensation pane changes, the Parameters pane and Color Precedence pane simultaneously change to that Data Set.

In addition to the options for changing compensation already mentioned in this section, the options listed in Table 2.11 are available for a Composite Analysis.

Table 2.11 Compensation Options

Icon	Description
	**Link compensation for all data sets:** Links Spillover and Autofluorescence Vector values to the other Data Sets in the active Composite Protocol.
	**Copy compensation to other data sets:** Copies Spillover and Autofluorescence Vector values to any or all other Data Sets within the Composite Protocol.

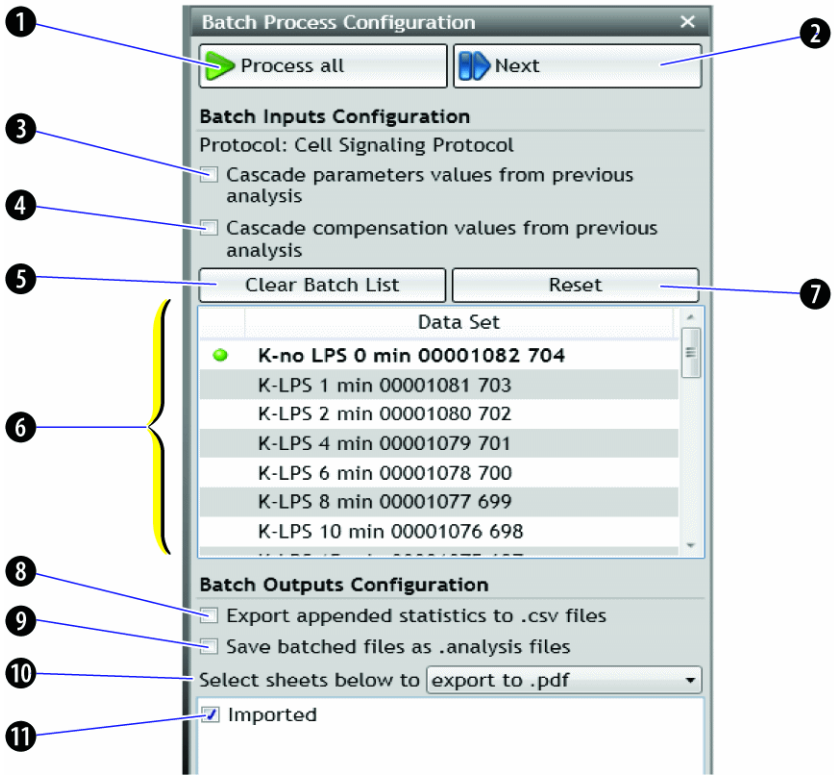
Batch Processing

The Batch Process Activity provides a means to generate output from a specified list ("batch") of data sets through a selected protocol or composite protocol. Each data set (when analyzing with a protocol) or group of data sets (when analyzing with a composite) can produce printed outputs, *.pdf outputs, *.analysis files, and/or statistics outputs.

The Batch Process Activity is laid out similarly to the Analysis Activity. The left side of the Batch Process Activity is the Batch Process Configuration pane and controls what is shown on the right hand side. The right hand side of the Batch Process Activity application (parameters/compensation/color precedence/sheets) behaves the same as the right hand side of the Analysis Activity.

NOTE Only one batch process activity can be performed at a time, and Batch Processing does not support the undo and redo functions.

Figure 2.87 Batch Process Activity



Component	Function
1. **Process all**	Allows the replay of the entire batch of data files.
2. **Next**	Allows manual sequencing through the batch list.
3. **Cascade parameter values from previous analysis**	Use to copy the parameter values in the currently edited entry and paste them into the next entry in the Batch List.
4. **Cascade compensation values from previous analysis**	Use to copy the compensation values in the currently edited entry and paste them into the next entry in the Batch List.

Component	Function
5. Clear Batch List	Clears all data files from the Batch List. A message is displayed requesting confirmation of this action or providing the option to cancel this action.
6. Batch List	Display of your current Batch List.
7. Reset	Use to reset the current file pointer to the first data set in the Batch List.
8. Export appended statistics to *.csv files	Enables automatic creation of a *.csv file for the entire data set batch.
9. Save batched files as *.analysis files	Enables automatic creation/saving of a *.analysis file for each data set file in the batch.
10. Select sheets below to export to *.pdf or print	Displays a checkbox next to a list of Plot Sheet(s) and Report Sheet(s) in the protocol. An individual *.pdf file or printout will be created for each sheet and for each data file in the batch.
11. Imported	This is the title of the plot sheet used for this example. If there were additional plot or report sheets, they would also be listed here.

Additional Batch Processing features:

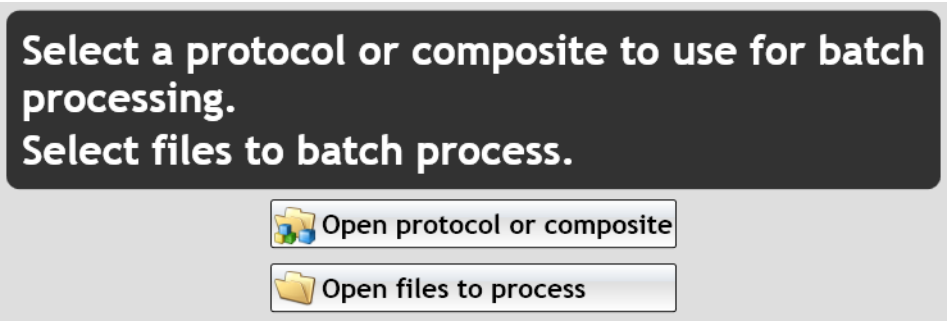
- Batch files can be reordered or removed.
- If any editing to the protocol takes place during a batch analysis, the remaining files will use these protocol changes.
- If a composite protocol is configured, the batch file distinguishes the groups of files that are processed together.
- An asterisk shown beside the protocol indicates changes to the protocol have not been saved.
- The **Batch Input Configuration** feature identifies the protocol selected for the batch analysis.

NOTE The **Cascade compensation values from previous analysis** option is not available if you are processing a group of files through a compensation composite.

Initiate Batch Processing

1 Select **Open Batch Process Activity** from the Application menu. The screen shown in Figure 2.88 appears.

Figure 2.88 Batch Process Activity Screen



NOTE The **Batch Process Activity** tab may remain open while you resume use of the Analysis Activity tab at the upper left of the Kaluza Analysis workspace.

2 Select the desired protocol or composite protocol to replay the data using the **Open Protocol** or Composite button. This button is available from the Batch Process Activity's application menu and from an assistive view.

3 Select data files to be processed using the **Open files to process** button. The selected data files will be listed in the Batch List in the Batch Process Configuration pane, and the fist data file will automatically be viewed in the sheet area.

Additional data sets may be added by using the Batch Process Activity's application menu or by using the Ctrl + O key option.

4 Optionally, arrange data sets in the desired order using drag and drop within the Batch List. This is especially important when batching data sets through a composite protocol, in which order of files is important.

5 Configure the desired outputs that should be generated by the Batch Processing Activity using the Batch Processing Configuration pane. This may include *.csv, *.analysis, *.pdf, and printed outputs.

6 Optionally, edit the protocol in the sheet area, as desired. This may include manipulating plots, gates, or importing compensation. Protocol changes made persist to all subsequent data sets within the Batch List.

The parameter and compensation values used during batch processing are provided by the Data Set, unless otherwise specified. Therefore, if changes are made to the parameters pane and the compensation pane, and you wish to persist the changes to subsequent data sets in the Batch List, the following options must be checked:

- Cascade parameters values from previous analysis
- Cascade compensation values from previous analysis

NOTE The currently processed data sets are denoted by a green icon in the Batch List.

7 Select **Process All** to start automatically processing the files. Alternatively, select **Next** to process the next Data Set in the Batch List.

NOTE Kaluza Analysis will prompt you by displaying a **Browse for Folder** window to specify a folder for file outputs. It will also display a **Print Configuration** window if printed outputs are requested.

8 The Batch Process is complete when all Data Sets within the Batch List have been processed. Once the Batch Process is complete, the user is presented with options.

a. **Process more files** - Allows the user to select more files to process with the already configured protocol.

   NOTE Cascaded parameter and compensation values, if configured, will be applied to the newly selected files.

b. **Setup next batch process** - Clears the protocol, Batch List, cascaded parameter, and cascaded compensation values, preparing Kaluza Analysis for another Batch Process.

c. **Close Batch Process Activity** - Closes the Batch Processing activity tab. This can also be achieved by using the Batch Processing Activity's application menu.

Errors During Batch Processing

If errors occur during processing, they will be displayed in the user interface. An error pane is displayed listing the errors, and the Data Sets with errors are indicated in the Batch List. When errors occur while processing a file, outputs for that file will not be generated.

Using Kaluza Analysis With Other Applications

Kaluza Analysis offers the ability to drag and drop, copy and paste, and save images into other applications. These features work with Microsoft Office products, as well as other applications.

IMPORTANT When using Kaluza Analysis with language packs such as Chinese or Japanese, dragging and dropping or copying and pasting statistics to Microsoft Excel may yield unexpected characters in the row and column headers. The "Export Selected Statistics" feature should be used to import statistics into Microsoft Excel when these language packs are active.

Copy/paste individual or multi-selected items to MSOffice 2010 (Excel, PowerPoint, and Word), has been tested in Kaluza Analysis and produces expected results. Other applications have not been tested; therefore, quality has not been determined.

The response of the application when dragging/dropping or copying/pasting items from Kaluza Analysis is dependent upon on the type of sheet item(s) you are working with. The tables below describe the response of the application for each type of sheet item.

Table 2.12 Using Kaluza Sheet Items in Spreadsheet Applications

When Copying/Pasting or Dragging/Dropping...	Spreadsheet Application Response
A Plot	If statistics are not selected in the plot, a 96 dpi image of the plot appears. If statistics are selected, then the selected statistics appear in column format in the spreadsheet application. One column for each statistic available within the Kaluza Analysis application appears. Blank columns may appear depending on the **Exclude empty columns exported statistics files** option in **Kaluza Options**.
A Gate Statistics Table	If statistics are not selected in the plot, a 96 dpi image of the plot appears. If statistics are selected, then the selected statistics appear in column format in the spreadsheet application. One column for each statistic available within the Kaluza Analysis application appears. Blank columns may appear depending on the **Exclude empty columns exported statistics files** option in **Kaluza Options**.
An Information Table	Descriptions appear in columns headers. A row is created per analysis list entry which contains the value.
A Text Box	All contents of the text box are located within one cell; text is editable.
Multiple Plots, FCS Information Tables, and/or Gate Statistics Tables	Gate statistics tables and plots contain the same set of statistics, and are in the format described above. Information Table data appears below the plot and Gate Statistics Table data. Comparison plot data appears below the Information Table data.

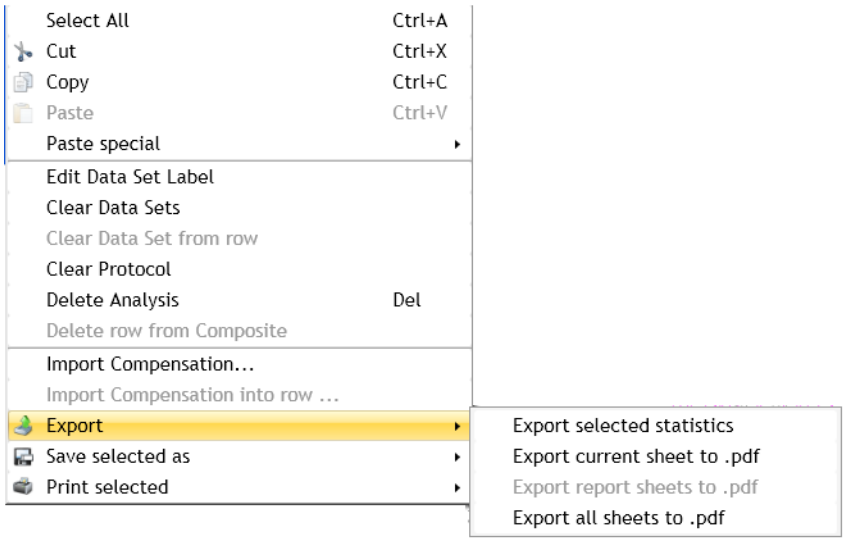
Table 2.13 Using Kaluza Sheet Items in Word-Processing Applications

When Copying/Pasting or Dragging/Dropping...	Word-Processing Application Response
A Plot	A 96 dpi image. **IMPORTANT** Using the **Save As Image** option saves the image with a 600 dpi resolution. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for details. **NOTE** When you use another application to display your sheet item, icons and links either do not appear, or they appear as images only, not hyperlinks.
A Gate Statistics Table	
An Information Table	
A Text Box	Editable text.
Multiple Plots, Information Tables, and/or Gate Statistics Tables	A 96 dpi image of all of the plots which can be ungrouped within Microsoft Word or Microsoft PowerPoint to allow customization.

Exporting Statistics

Statistics that have been selected for your plots from the Statistics Radial Menu can be exported into a *.csv spreadsheet, and sheets can be exported into a *.pdf file. For additional information on setting up statistics for your plots, see *Setting Up Statistics*.

Figure 2.89 Exporting Statistics



To export statistics currently on plots within the selected Protocol (statistics must be selected for plots prior to completing this procedure):

1 Select > **Export > Export Selected Statistics.** The Export dialog box displays.

2 Enter a file name in the **File name** field.

NOTE Kaluza Analysis can produce up to three *.csv files (statistics.csv, comparison.csv, and information.csv.) depending on what plots are in the analysis. If only one file is generated, you will be prompted for a filename; otherwise, you will be prompted for a directory where all the files will be saved.

3 Select **Save** to complete the process.

Additionally, you may review statistics in spreadsheet format for individual or multiple selected plots by following the instructions in *Using Kaluza Analysis With Other Applications*.

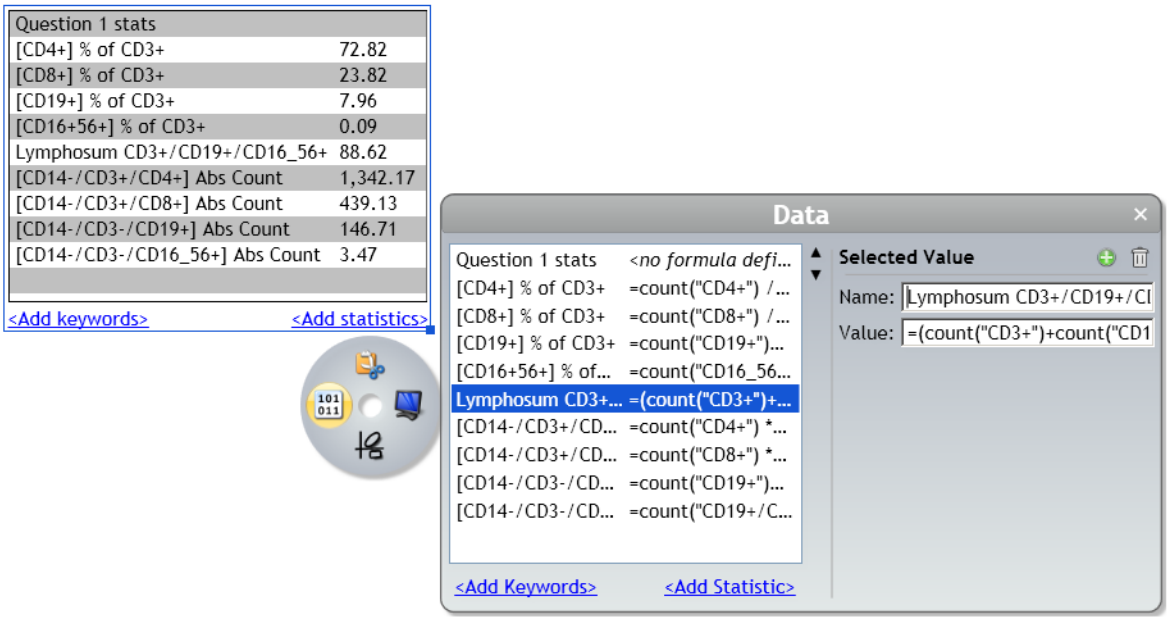
Using Formulas in Kaluza Analysis

Kaluza Analysis allows you to extract and display information from the analysis using formulas. You can specify formulas in similar fashion as that in Microsoft Excel and other common spreadsheet software.

Use the various operator functions supported by the software to create your customized formula(s) on the Information Table, and/or in the Analysis List, and/or in the Data Set Identifier option (in the **Kaluza Options** dialog). The Analysis List and Data Set Identifier options only support functions that extract information related to the keywords in the data file.

If a formula cannot be evaluated, the ### (in red) error message displays, indicating there is an error in the formula.

Figure 2.90 Information Table Formulas



Information Table

The Information Table evaluates formulas you specify, such as calculate absolute count, and displays the result in a table format. It can be used to display FCS keywords by placing a description of the keyword in the first column and using a formula to retrieve the value of a keyword in the second column.

Every cell in the Information Table can have its own formula (based on addition, subtraction, multiplication, division, and text concatenation), so you can highly customize the Information Table by using custom formulas to display statistics, calculations, or keywords.

Single data sets and composites have different requirements:

- For composites, the index of the data set in the composite must be specified, and the scale must be identified (e.g.., the data set index = 1, as in **=CV("A", "FL1", LOG, 1)**).
- For a single data set, the scale can be omitted, as the default scale is used (e.g., in **=ARITHMETICMEAN("A", "FL1")**, the log scale is used by default).

The formula that appears in the Information Table supports the functions described in Table 2.14.

NOTE In the examples below, you may substitute **LIN**, **LINEAR**, **LOGICLE**, or **LOGARITHMIC** for **LOG**.

Table 2.14 Using Formulas in Kaluza Analysis - Information Table

Function	Example	Description
ARITHMETICMEAN	=ARITHMETICMEAN("A", "FL1", LOG) =ARITHMETICMEAN("A", "FL1", LOG, 1)	Returns the arithmetic mean for the specified gate, parameter, scale, and data set.
COUNT	=COUNT("A") =COUNT("A",1)	Returns the number of events in the specified gate and data set.
CV	=CV("A", "FL1", LOG) =CV("A", "FL1", LOG, 1)	Returns the CV for the specified gate, parameter, scale, and data set.
DATASETNAME	=DATASETNAME() =DATASETNAME(1)	Returns the name of the specified data set.
FIND	=FIND("FC", KEYVALUE("$CYT"))	Returns the starting position of one text string within another text string. FIND is case-sensitive.
GATENAME	=GATENAME("A") =GATENAME("A",1)	Returns the name of the specified gate and data set.
GEOMETRICMEAN	=GEOMETRICMEAN("A", "FL1", LOG) =GEOMETRICMEAN("A", "FL1", LOG, 1)	Returns the geometric mean for the gate, parameter, scale, and data set.
HPCV	=HPCV("A", "FL1", LOG) =HPCV("A", "FL1", LOG, 1)	Returns the HPCV for the specified gate, parameter, scale, and data set.
KEYDESCRIPTION	=KEYDESCRIPTION("$CYT")	Returns the verbose, localized description of the specified keyword. If the verbose description is not available, the keyword name itself is returned.
KEYNAME	=KEYNAME("$CYT") =KEYNAME("$CYT",1)	Returns the name of the specified keyword in the specified data set.
KEYVALUE	=KEYVALUE("$CYT") =KEYVALUE("$CYT",1)	Returns the value of the specified keyword in the specified data set.
LEN	=LEN(KEYVALUE("$CYT"))	Returns the length of the specified text.
MAX	=MAX("A", "FL1", LOG) =MAX("A", "FL1", LOG, 1)	Returns the maximum value for the specified gate, parameter, scale, and data set.
MEDIAN	=MEDIAN("A", "FL1", LOG) =MEDIAN“A”, "FL1", LOG, 1)	Returns the median for the specified gate, parameter, scale, and data set.
MID	=MID(KEYVALUE("$CYT"), 6)	Returns the characters from the middle of a text string given a starting position and length.
MIN	=MIN("A", "FL1", LOG) =MIN("A", "FL1", LOG,1)	Returns the minimum value for the specified gate, parameter, scale, and data set.
MODE	=MODE("A", "FL1", LOG) =MODE("A", "FL1", LOG,1)	Returns the mode for the specified gate, parameter, scale, and data set.

Table 2.14 Using Formulas in Kaluza Analysis - Information Table

Function	Example	Description
PARAMETERDESCRIPTION	=PARAMETERDESCRIPTION("FL1") =PARAMETERDESCRIPTION("FL1", 1) *(do not specify scale)*	Returns the description of the specified parameter in the specified data set.
PARAMETERNAME	=PARAMETERNAME("FL1") =PARAMETERNAME("FL1", 1) *(do not specify scale)*	Returns the name of the specified parameter and data set.
PCT	=PCT(50) =PCT(50)	Returns the percent value after dividing the specific value by 100.
PCTGATED	=PCTGATED("A") =PCTGATED("A",1)	Returns the percent of gated events in the specified gate and data set.
PCTTOTAL	=PCTTOTAL("A") =PCTTOTAL("A",1)	Returns the percent of total events in the specified gate and data set.
STDEV	=STDEV("A", "FL1", LOG) =STDEV("A", "FL1", LOG,1)	Returns the standard deviation for the specified gate, parameter, scale, and data set.

***Common Examples*:**

Absolute Count: =COUNT("target gate") * KEYVALUE("@ABSCALFACTOR")/COUNT("CAL")

Perentage of Grandparent Gate - For a gating hierarchy A -> B ->C ->D, & grandparent for D:
=COUNT(D)/COUNT(B) * 100 & "%"

Analysis List and Options Dialog

Use the following formulas to extract the relevant information from the data files and identify the Analysis List entries.

Table 2.15 Using Formulas in Kaluza Analysis - Analysis List Options

Function	Example	Description
DATASETNAME	=DATASETNAME() =DATASETNAME(1)	Returns the name of the specified data set.
FIND	=FIND("FC", KEYVALUE("$CYT"))	Returns the starting position of one text string within another text string. FIND is case-sensitive.
KEYDESCRIPTION	=KEYDESCRIPTION("$CYT")	Returns the verbose, localized description of the specified keyword. If the verbose description is not available, the keyword name itself is returned.
KEYNAME	=KEYNAME("$CYT") =KEYNAME("$CYT",1)	Returns the name of the specified keyword in the specified data set.

Table 2.15 Using Formulas in Kaluza Analysis - Analysis List Options

Function	Example	Description
KEYVALUE	=KEYVALUE("$CYT") =KEYVALUE("$CYT",1)	Returns the value of the specified keyword in the specified data set.
LEN	=LEN(KEYVALUE("$CYT"))	Returns the length of the specified text.
MID	=MID(KEYVALUE("$CYT"), 10, 6)	Returns the characters from the middle of a text string given a starting position and length.

***Common Examples*:**

- **MID:** If the name on the cytometer on which the data was collected is specified as "Cytomics Gallios" and you are interested in only displaying "Gallios", use the following formula to extract the interested part of the name:

  Example: =MID(KEYVALUE("$CYT"), 10, 6)

- **FIND**: If you want to ignore all the irrelevant text without having to know what else is in the text:

  Example: =MID(KEYVALUE("$CYT"), FIND("FC",KEYVALUE("$CYT")), 6)

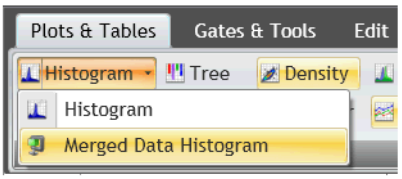
Merged Data Sets

You can merge up to 48 Data Sets into one file. Prior to merging Data Sets, ensure that each of the following are identical:

- Number of Parameters
- Parameter Names
- Range
- Scale
- Number of decades (when using log scale), and
- Spillover matrix values (FCS files).

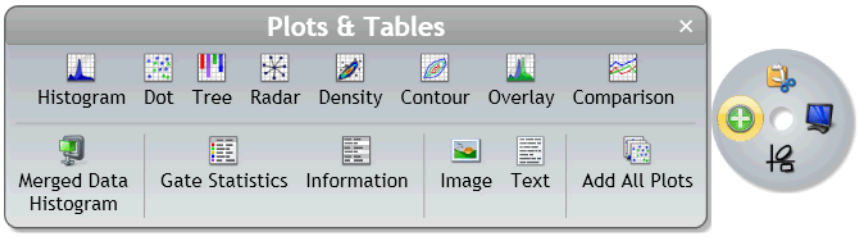
When a merged data set that includes the time parameter is active, a drop-down option to create a Merged Data Histogram is available from the Plots & Tables Ribbon tab.

Figure 2.91 Histogram Options—Plots & Tables Ribbon



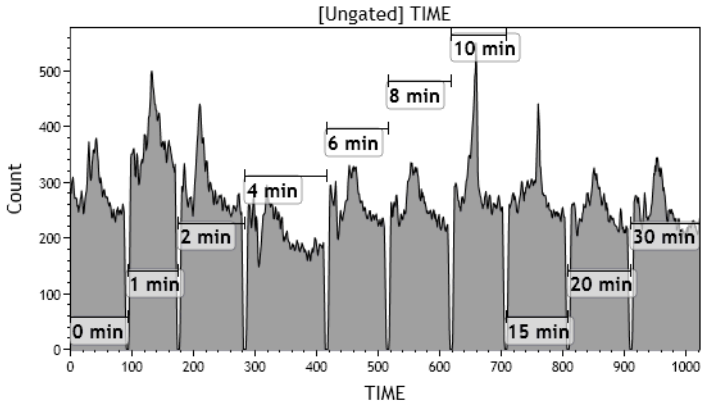
The Merged Data Histogram can also be accessed from the Plots & Tables Radial Menu, which is accessed from the Plot or Report Sheet.

Figure 2.92 Merged Data Histogram—Plots & Tables Radial Menu



Kaluza Analysis automatically creates gates on this merged time histogram, as shown in Figure 2.93.

Figure 2.93 Merged Data Set Display—Using the "Time" Parameter



To merge Data Sets:

1 Drag and drop the files you wish to merge into the Analysis List. Reorder the Data Sets as desired (see CHAPTER 1, *Changing the Sequence of Analysis List Rows*).

NOTE If you choose to merge Analysis files, only the Protocol from the file of the highest precedence on the Analysis List is transferred into the merged file.

2 Multi-select the files you wish to include in your merged file. The Analysis Options screen appears.

3 Select the 

button. The Data Sets are now merged into a single listmode file. Note that the merged file contains the name of the file of highest precedence on the Analysis List, and the file appears with the “merged” icon in the Data Set column, as shown in the following figure:

#	Data Set
1	K562 Contr P-H3 restain 00000053 001

NOTE Plots containing the Time parameter include a time buffer between each merged Data Set file, as shown in Figure 2.10.

CHAPTER 3

Sheet Set-Up

Using Sheets

The sheet, which makes up the main portion of the Kaluza Analysis workspace, is where you will spend most of your time, as this is where you set up Protocols and Composites, analyze your data, and create interactive reports. The following sections describe the options available to design your sheet for optimal data presentation and also include some time-saving techniques. Descriptions will focus primarily on the Radial Menus and Ribbon items since they contain the majority of tools to update your sheets.

IMPORTANT Each sheet can contain a maximum of 200 items.

Sheet Radial Menus Options

Sheet Radial Menu

The Sheet Radial Menu (Figure 3.1) provides access for changing or adding items to your sheet. The following sections provide an overview of the options available when you right-click on the sheet white space to access the Radial Menu.

Figure 3.1 Sheet Radial Menu

Display Menu

Use the (**Display**) menu to:

- Add compensation sliders to applicable plots (see CHAPTER 2, *Adjusting Spillover Using the Spillover Sliders Directly on the Plot(s)*).
- Switch between Freeform and By Data Set grouping when working on a Composite or Compensation Composite. For additional information, see:
  - CHAPTER 2, *Freeform Arrangement*
  - CHAPTER 2, *By Data Set Arrangement*
- Align data on the Report Sheet via the Quick Arrange or Show Grid. For additional information, see *Page Layout* for details.

Gates & Tools Menu

Use the (**Gates & Tools**) menu to draw a gate, change to Selection mode, make an annotation, or to add overlay markers to a overlay plot. See CHAPTER 2, *Gates & Tools*.

Plots & Tables Menu

Use the (**Plots & Tables**) menu to select plots, a Gate Statistics or Information table, or an image or text to add the sheet. See CHAPTER 2, *Plots & Tables*, *Adding an Image to a Sheet*, or *Adding Text*.

Edit Menu

Use the (**Edit**) menu to paste an item or paste an item as a link. See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*.

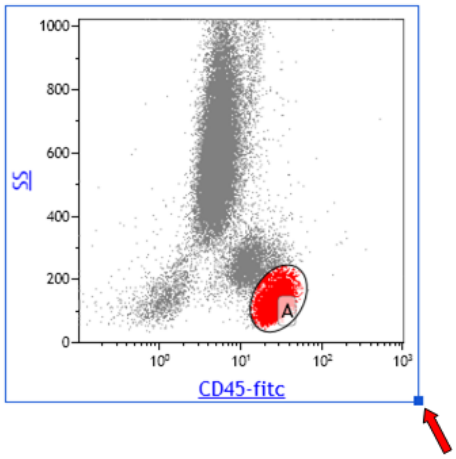
Adjusting the Size of a Sheet Item

To adjust the size of a single sheet item:

1 Select the item you wish to adjust.

2 Select the handle (indicated by the red arrow in Figure 3.2) and drag in the direction you wish to make your change.

Figure 3.2 Adjustment Handle



3 Release your mouse button when you are satisfied with the size.

TIP If you hold down the Shift key while you resize the item, the height and width adjust by the same amount.

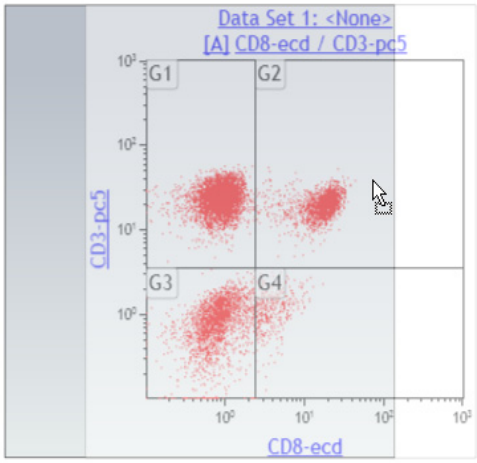
Moving Plots/Sheet Items

To move a sheet item to a new location on your sheet:

1 Select the plot you wish to move.

2 Drag the plot to the new location. As you drag you plot, a gray box will appear (as shown in Figure 3.3) indicating the locations you can drop the plot. When the gray box appears in the location of your preference, release your mouse button.

Figure 3.3 Moving a Plot



NOTE See CHAPTER 1, *Basic Editing for Plots, Gates, and Sheet Items*, for additional methods for moving plot/sheet items.

Adding an Image to a Sheet

To add an image to a sheet:

1 Right-click in the whitespace where you would like to add an image, and the Sheet Radial Menu displays.

2 Hover over the (**Add**) icon to view the Plots & Tables menu, and select the Image icon from the menu. An outline, indicating the location of the image you choose appears on the sheet, as shown in Figure 3.4.

Figure 3.4 New Item Selection

IMPORTANT The following image file types have been tested in Kaluza Analysis and will produce expected results. Other available file types have not been tested, and therefore, their quality is not guaranteed.

- *.bmp
- *.jpg

3 Click on the **Browse** button within the image outline. The Open Image dialog box appears.

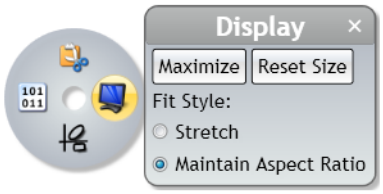
4 Navigate to your image file using the **Look in:** field or the icons/folders located in the dialog box.

5 Select the image file, and then select **Open**. The image appears on your sheet.

Formatting an Image

The Display Radial Menu allows you to choose options for resizing an image. The options included within the Display menu are described below. Changes made within the menu occur in real time, allowing you to refine the appearance until you are satisfied.

Figure 3.5 Formatting an Image



- **Maximize**: Select **Maximize** to increase the size of the image to fit within the sheet portion of the application. See CHAPTER 2, *Setting Up Plot Display* for additional information.
- **Reset Size**: Select **Reset Size** to return an item that has been resized back to the default size and shape.
- **Stretch**: Choose the **Stretch** radio button to allow the image to stretch/compress when resizing.
- **Maintain Aspect Ratio**: Choose the **Maintain Aspect Ratio** radio button to constrain the image to the original aspect ratio when resizing.

To change the display of an image:

1 Right-click within the confines of the image to access the Radial Menu.

2 Hover your mouse over the (**Display**) icon to access the Display menu.

3 Make changes within the menu. Refer to the descriptions above for details.

4 Select when you are finished making your selections.

NOTE You can replace the current image by right-clicking on an image and accessing the (**Data**) menu. Select **Browse** and locate/select the new image using the Open Image dialog box.

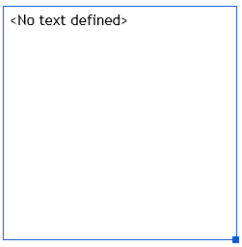
Adding Text

Use the Sheet Radial Menu to add text.

1 Right-click in the whitespace where you would like to add text. The Sheet Radial Menu appears.

2 Hover over the (**Add**) icon to view the Plots & Tables menu, and select the Text icon. An outline, indicating the location of the text, appears on the sheet as shown in Figure 3.6.

Figure 3.6 Text Box



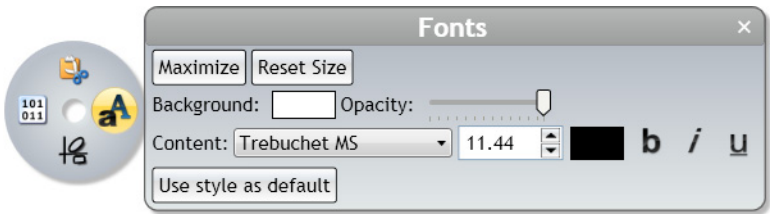
3 Click once within the text box, and type your text. When you are finished, click on the sheet whitespace or another plot to exit edit mode.

NOTE To format the text box, see *Formatting a Text Box*. For instructions on resizing the text box, see *Adjusting the Size of a Sheet Item*.

Formatting a Text Box

The Fonts Radial Menu allows you to customize your text box. The options included within the Fonts and Display menu are described below. Changes made within the menu occur in real time, allowing you to refine the appearance until you are satisfied.

Figure 3.7 Fonts Radial Menu



- **Maximize**: Select **Maximize** to increase the size of the item to fit within the sheet portion of the application. See CHAPTER 2, *Setting Up Plot Display* for additional information.
- **Reset Size**: Select **Reset Size** to return an item that has been resized back to the default size and shape.
- **Font Drop-down**: Use the font drop-down field to change the font used in the text box. To change the font, select the new font from the drop-down list.

  NOTE The following fonts have been tested in Kaluza Analysis and will produce expected results. The other available fonts have not been tested; therefore, their quality is not guaranteed.

  — Trebuchet MS (default font)

  — Arial

  — Times New Roman

- **Font Size Field**: Use the font size field to either manually enter a font size or to incrementally increase/decrease the current font size by one point each time the arrow is clicked.

  NOTE Font sizes 9.75 and 11.44 have been tested in Kaluza Analysis and will produce expected results. The other available font sizes have not been tested; therefore, their quality is not guaranteed.

- **Foreground Color**: Use the foreground color field to choose the color of the text by selecting the current color, and then selecting the new color from the palette.
- **Background Color**: Use the background color field to choose the color background for the entire text box. To change the color, select the current color; then select the new color from the palette.
- **Bold**: Select **b** to change the text to bold font.
- **Italics**: Select *i* to italicize the text.
- **Underline**: Select u to underline the text.
- **Opacity Slider**: Use the opacity slider to change the degree of opacity of the text box. Select and drag the slider to the preferred level of opacity and release the mouse button when you are satisfied.

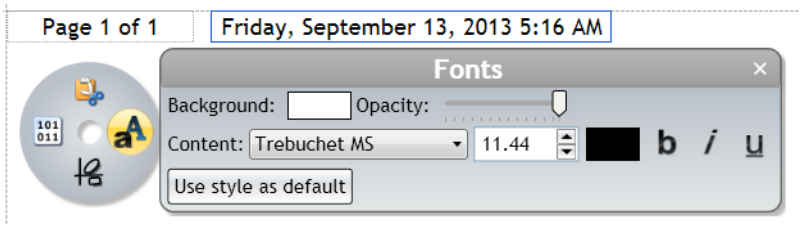
To customize a text box:

1 Right-click within the confines of the text box to access the Radial Menu.

2 Hover your mouse over the (**Fonts**) menu button.

3 Make changes within the menu (see Figure 3.8). Refer to the descriptions above for details.

Figure 3.8 Fonts Radial Menu—Text Box



4 Select when you are finished making your selections.

Using the Sheet Tab Bar

See Figure 1.44 to view the Sheet Tab bar, located at the bottom of the sheet area (see Figure 1.10). The Sheet Tab Bar provides the three main functions: Zoom, New Sheet, Sheet Tabs.

Table 3.1 Sheet Tab Bar Functions

Item	Function/Procedure
100%	**Zoom** uses a slider. Ranges are between 50% and 500%. To change the zoom, select and drag the slider, or select the - or + buttons.

Table 3.1 Sheet Tab Bar Functions

Item	Function/Procedure
New Sheet...	**New Sheet...** adds a new plot sheet or report sheet. To add a new sheet: **1.** Select the hyperlink. A drop-down appears that enables you to choose between adding a new plot sheet or a new report sheet. **2.** Select the type of sheet you need. A new sheet is added as the active sheet. **NOTE** You can add up to 1024 sheets per Protocol file.
Plot Sheet 1 Report Sheet 1	**Sheet Tabs** allow you to do the following: • **Move between open sheets**: The active sheet is highlighted in white (Plot Sheet 1 in the figure to the left is the active sheet). To display a different sheet, select the name of the sheet tab that you wish to display. • **Rename sheets**: To rename a sheet: **1.** Right click on the tab and select **Rename**. **2.** Enter the new sheet name into the highlighted field. **3.** Click your mouse away from the tab or press (Enter) to save the change. OR **1.** Double-click the sheet tab. **2.** Enter the new sheet name into the highlighted field. **3.** Click your mouse away from the tab or press (Enter) to save the change. • **Reorder sheets**: To reorder sheets, select the sheet tab that you wish to move and drag it to the preferred location. While moving the sheet an arrow displays, showing the location that the sheet can be dropped. When the arrow is in the appropriate location, release the mouse button. • **Print sheets**: Right click on the sheet tab, and select **Print**. • **Delete Sheets**: Right click on the sheet tab that you wish to delete, and select **Delete**.

Report Sheet

The following features are available either from the Ribbon or the Radial Menu. The Report Sheet is where you create reports from your data plots. You can perform the following functions while working in a report sheet, besides the options listed in *Using Sheets*. See *Using the Sheet Tab Bar*, for instructions on adding a report sheet.

- Arrange plots.
- Change page orientation, page size, and margin size.
- Add page numbers.
- Add a date and time stamp.
- Create and edit a master page, which can include plots, tables, graphics, text, page numbers, and/or a date and time stamp.

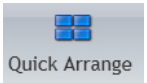
Page Layout

The Page Layout Ribbon tab is available only when you use a Report Sheet. The sections below detail the functions available within the Page Layout tab.

Layout

See Table 3.2 to perform a Quick Arrange on your report sheet.

Table 3.2 Page Layout Ribbon—Layout

Icon	Function
	**Quick Arrange** arranges the plots on the report sheet so that plots are not overlapping. Plots retain the same order they were in prior to selecting this option.

Page Setup

See Table 3.3 to view the options to customize your report pages.

Table 3.3 Page Layout Ribbon—Page Setup

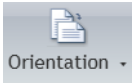
Icon	Function
	**Show Grid** toggles the grid on the background of the report sheet on/off.
	**Orientation** contains a drop-down list that allows you to choose between the portrait or landscape page orientation. The adjustment is made instantly when the new orientation is selected.

Table 3.3 Page Layout Ribbon—Page Setup

Icon	Function
	**Size** contains a drop-down list that allows you to change the page size to one that is appropriate for your needs. There are many sizes to choose from, including standard, A, and JIS sizes. Your selection applies to all pages within the active report sheet. **IMPORTANT** The following page sizes have been tested in Kaluza Analysis and produce the expected results. Other page sizes have not been tested, and therefore, quality is not guaranteed. • Letter (8.5 X 11”) • A4 (210 X 297 mm)
	**Margin** fields contain editable fields that you can customize for each margin. Use the up and down arrows located next to the appropriate margin field to incrementally increase or decrease the margin by 0.10. You can also manually type the preferred size into the field. Any changes you make within margin fields occur instantly on the report sheet.

Master Page

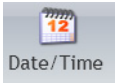
The Master Page portion of the Page Layout tab give options for creating or making changes to a master page for the current report sheet in the selected Protocol.

See Table 3.4. Anything that can be added to a Plot Sheet can also be added to the Master Page.

Table 3.4 Page Layout Ribbon—Master Page

Icon	Function
	**Edit Master Page** allows you to make changes to the master page. The changes you make to the master page are displayed on all pages within the active report sheet. You can add the following items to the master page: • Any sheet item • Page numbers • Date and time • Text • Graphics **IMPORTANT** Make sure **Edit Master Page** is selected prior to creating your master page. Otherwise, the items you choose will only appear on the current page of the report, and not the master page. To escape master page mode, select **Edit Master Page** once again.

Table 3.4 Page Layout Ribbon—Master Page

Icon	Function
	**Page Number** displays the page number on the report sheet. The page number only appears on each page if you place it on the master page. You can move the page number by selecting within the text box and dragging to the preferred location. See *Page Number Format*, and *Customizing the Appearance of the Date/Time and Page Number*, to make changes to the page number.
	**Date/Time** adds the date and time to the report sheet. The date/time only appears on each page if you place it on the master page. You can move the date/time by selecting within the text box and dragging to the preferred location. See *Date/Time Format*, and *Customizing the Appearance of the Date/Time and Page Number*, to make changes to the time/date. **NOTE** The date/time is updated when the report is printed.

Formatting the Date/Time and Page Number Display

The Data menu within the Radial Menu allows you to customize the appearance of page numbers and the date/time. The options included within the Data menu are described below. Changes made within the menu occur in real time, allowing you to refine the appearance until you are satisfied.

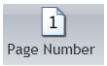
Page Number Format

See Table 3.5 for the options available to format page numbers.

Table 3.5 Page Number Options

Option	Example	Description
Current Page Only	Page 1	Displays the current page number.
Long	Page 1 of 3	Displays the current page number and the total number of pages within the report.
Short	1	Displays only the page number.

To update the page number format:

1. If the page number is not already located on the master page, from the Page Layout Ribbon tab, select the  icon. Then, select the  icon, which adds the page number using the default format to the master page.

2. Right-click within the confines of the page number text box.

3. Hover your mouse over the  (**Data**) icon to access the Data menu.

4 Select the drop-down arrow and, from the list, choose the page number format of your preference.

5 Select ✕ to exit the menu.

Date/Time Format

See Table 3.6 for the options available to format the date and time.

Table 3.6 Date/Time Options

Option	Example
Long Date	Sunday, June 28, 2009
Long Date and Time	Sunday, June 28, 2009 1:30 PM
Short Date	06/28/2009
Short Date and Time	06/28/2009 1:30 PM
Time	1:30 PM

To update the date/time format:

1 If the date and time is not already located on the master page, from the Page Layout Ribbon tab, select the Edit Master Page icon. Then, select the Date/Time icon, which adds the date and time using the default format to the master page.

2 Right-click within the confines of the date/time text box.

3 Hover your mouse over the (**Data**) icon to access the Data menu.

4 Select the drop-down arrow and, from the list, choose the date/time format of your preference.

5 Select ✕ to exit the menu.

Customizing the Appearance of the Date/Time and Page Number

The Fonts menu within the Radial Menu allows you to customize the appearance of page numbers and the date/time. The options included within the Fonts menu are described below. Changes made within the menu occur in real time, allowing you to refine the appearance until you are satisfied.

- **Background**: Use the background color field to choose the color background for the entire text box. To change the color, select the current color; then select the new color from the palette.
- **Opacity Slider**: Use the opacity slider to change the degree of opacity of the background in the text box. Select and drag the slider to the preferred level of opacity and release the mouse button when you are satisfied.
- **Content**: This feature encompasses the font style and size, as follows:
  - **Font Style drop-down**: Use the font drop-down field to change the font style used in the text box. To change the font, select the new font from the drop-down list.

    NOTE The following font styles have been tested in Kaluza Analysis and produce expected results. The other available font styles have not been tested; therefore, their quality is not guaranteed.
    - Trebuchet MS (default font)
    - Arial
    - Times New Roman
  - **Font Size Field**: Use the font size field to either manually enter a font size or to incrementally increase/decrease the current font size by one point each time the arrow is clicked.

    NOTE The following font sizes have been tested in Kaluza Analysis and produce expected results. The other available font sizes have not been tested; therefore, their quality is not guaranteed.
    - 9.75 (default size when application is in small font theme)
    - 11.44 (default size when application is in large font theme)
- **Bold**: Select b to change the text to bold font.
- **Italics**: Select i to italicize the text.
- **Underline**: Select u to underline the text.

To customize the page number or date/time:

1. If the page number is not already located on the master page, from the Page Layout Ribbon tab, select the Edit Master Page icon. Then, select the Page Number or the Date/Time icon, which adds the default page number or date/time to the report sheet.

2. Right-click within the confines of the text box to access the Radial Menu.

3. Hover your mouse over the (**Fonts**) icon to access the Fonts menu.

4 Make changes within the menu. Refer to the descriptions above for details.

5 Select ✕ when you are finished making your selections.

Printing Report Sheets

IMPORTANT The Oki B6300 printer has been tested in Kaluza Analysis and produces expected results. Other printers have not been tested; therefore, print quality has not been determined.

To print a report sheet:

1 Select the Analysis List row of the report you wish to print.

2 Select > **Print Selected** > **Print report sheets**.

3 In the Print dialog box, select the printer you wish to use and, if necessary, select printing preferences for your report.

4 Select **Print**.

APPENDIX A

Statistics

Overview

This chapter contains information about the statistics that Kaluza Analysis supports:

Component	Definition
1. Number	The number of events in the Input Gate.
2. % Total	Ratio of the number of events in the input gate to the total events in the protocol. $$\%Total = 100 * \frac{Count}{Total\ number\ of\ events\ in\ protocol}$$
3. % Gated	Ratio of the number of events in the input gate to the number of events in the parent gate. $$\%Gated = 100 * \frac{Count}{Parent\ Gate\ Count}$$

Component	Definition
4. Median	Median of the values of the events in the input gate. Kaluza Analysis computes the frequency histogram of the events to generate this statistic. The frequency histogram has 1024 bins. Using the frequency histogram avoids the time consuming sort operation that is typically used to find the median. $$b = i \left| \sum_{i=1}^{j} C_i > \frac{n}{2} \right.$$ $[j \leq n, C_i = Count\ in\ bin\ i, n = Total\ number\ of\ events\ in\ input\ gate]$ The median *m* can then be computed using the following steps. 1. $A = \frac{\sum_{i=1}^{b-1} x_i}{n} * 100$ 2. $B = 50 - A$ 3. $D = \frac{k_b}{n} * 100$ 4. $E = \frac{B}{D}$ 5. $m = b + E$ In step 3, k_b is the count in bin *b*. **NOTE** If the parameter that the statistics are computed on are displayed using the log or logicle transformation, the frequency histogram computed for the mode, HPCV and median statistics are computed in that transformation space. The following computations need to be performed for the result to be reported in linear transformation space. If the display parameter is log: $mode_{linear} = LinearTransform(LogarithmicTransform^{-1}(mode))$ $HPCV_{linear} = LinearTransform(LogarithmicTransform^{-1}(HPCV))$ $m_{linear} = LinearTransform(LogarithmicTransform^{-1}(m))$ If the display parameter is logicle: $mode_{linear} = LinearTransform(LogicleTransform^{-1}(mode))$ $HPCV_{linear} = LinearTransform(LogicleTransform^{-1}(HPCV))$ $m_{linear} = LinearTransform(LogicleTransform^{-1}(m))$

Component	Definition
	The various transformations used by Kaluza Analysis are based on the following definitions of the transformations: $LinearTransform(x) = a * x + b$ $LogarithmicTransform(x) = \log_{10}\left(\max(a * x, 1)\right) * b$ $LogicleTransform^{-1}(x) = BiExponentialTransform(x) = ae^{bx} - ce^{-dx} + f$ The coefficients ***a, b, c, d*** and ***f*** in these transformations are computed dynamically based on factors like the range of the data, number of decades, width of the negative zone (in case of the logicle) etc. Some of these coefficients change as the user interacts with Kaluza Analysis.
5. Arithmetic Mean	The Arithmetic Mean of the values of the events in the input gate. The arithmetic mean of a sequence of numbers {a1, a2, ...ai... an} is defined by: $\mu = \frac{1}{n}\sum_{i=1}^{n} a_i$
6. Mode	The channel number with largest population among the events in the input gate. For multimodal data, the mode with the smallest value is returned. Kaluza Analysis computes the frequency histogram of the events to generate this statistic. The frequency histogram has 1024 bins. If every value occurs only once, the Mode is set to NaN. E.g.: If there were only one (1) event or if there were two (2) events each in different channels.
7. Standard Deviation	The standard deviation of the values of the events in the input gate. The standard deviation of the sample with a sequence of numbers {a1, a2, ...ai... an} and arithmetic mean μ, is the square root of the sample variance and is defined by: $S = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(a_i - \mu)^2}$
8. CV (Coefficient of Variation)	The coefficient of variation of the values of the events in the input gate. $CV = \frac{S}{\mu}$

Component	Definition
9. HPCV (Half Peak of Coefficient of Variation)	Kaluza Analysis computes the frequency histogram of the events to generate this statistic. The frequency histogram has 1024 bins. Where FWHM = Full Width Half Max value of a Normal or Gaussian peak. $HPCV = \frac{1}{2.36} * \frac{FWHM}{Mode} *100$ Refer to *Practical Flow Cytometry*, by Howard M. Shapiro, Fourth Edition 2003, Wiley-Liss, Inc., page 235 for the definition.
10. Minimum	The minimum value among all events in the input gate. $x_{min} = \min_{1 \le i \le n} \{x_i\}$ $[n = Total\ number\ of\ events\ in\ input\ gate]$
11. Maximum	The maximum value among all events in the input gate. $x_{max} = \max_{1 \le i \le n} \{x_i\}$ $[n = Total\ number\ of\ events\ in\ input\ gate]$
12. Geometric Mean	The mean of the values of the events in the input gate. The geometric mean of a sequence of numbers {a1, a2, ...ai... an} is defined by: $G = \left(\prod_{i=1}^{n} a_i\right)^{1/n}$

NOTE

1. Kaluza Analysis statistics may not match exactly to the other software platform statistics. This is expected behavior due to use of different computation methods employed by different software platforms. For example: Kaluza Analysis computes several statistics from raw data, whereas many systems use binned data. Methods for computing events within a gate may differ, and smoothing algorithms may also differ.

2. Under certain conditions, “N/A” will display in a given statistics field. For example, 0% will display if there are events on your plot, but none are encompassed in the particular gate. However, if there are no events in the input gate to the plot, then any gates on that plot will display N/A. This is because in the first case the % gated is 0/N which equals 0; whereas in the second case it is 0/0 which is a divide by zero error and will display “N/A.”

APPENDIX B

Quick Reference Sheet

This chapter contains a quick reference list about limits on Kaluza Analysis features.

Kaluza Analysis Feature	Limit
Analysis List	400 entries or rows
Annotations per Plot	32
Batch Processing	2000 Data Sets
Comparison Plot	12 series
Data Sets per Composite Protocol	32
Gate Logic	16 gates/logical statement
Gates per Protocol (Data Set)	318 • "All" stat is considered a gate • Up to 31 gates can be assigned a color • 128 points per polygon gate
Histograms per Overlay	12
Merged Data Sets	48
Parameters	64 (total). *Kaluza can open files with more parameters, but additional parameters are disabled and cannot be used for analysis.* **NOTE** *Add All Plots* does not work when all options are enabled for loaded *Data Sets* with more than 18 fluorescence parameters due to the possibility of surpassing the limit of gates/plots allowed by the software.
Plots per Sheet	200 per plots/sheet
Sheets per Analysis/ Protocol	1024
Tree Plot	Total of eight (8) branches and bars (in any combination, as long as the total is eight).

APPENDIX C

Frequently Asked Questions

Overview

The purpose of this addendum is to provide our customers with a list of the frequently asked questions.

Frequently Asked Questions

1. What is Kaluza Analysis?

   Kaluza Analysis is a stand-alone software package for the analysis of flow cytometry listmode data stored as *.fcs or *.lmd files.

2. What operating systems does Kaluza Analysis run on? Does it run on the Mac?

   Kaluza Analysis v1.3 is compatible with:

   - Microsoft Windows XP (32-bit), SP3
   - Microsoft Windows Vista (32-bit), SP1
   - Microsoft Windows 7 (32-bit), SP1
   - Microsoft Windows 7 (64-bit), SP1

   Kaluza Analysis has been independently shown to operate on Intel-based Apple Mac hardware while running the above operating system configurations in a virtual environment. However, these configurations have not been validated and are chosen at the user's risk.

3. What software libraries does Kaluza Analysis need?

   The following libraries are required to install Kaluza Analysis and are included as part of the Kaluza Analysis installation program:

   a. Microsoft.NET Framework 3.5 SP1
   b. Microsoft Visual C++ 2008 SP1 Redistributable version 9.0
   c. Sentinel Runtime 6.56
   d. A PDF Reader is required to open the *Kaluza Analysis Software Instructions for Use*. It is included on the Kaluza Analysis installation CD, as an optional install.

4. What kind of computer hardware does Kaluza Analysis need?

   The minimum requirement for a computer to run Kaluza Analysis is one that meets the requirements for operating systems and libraries above. For optimal performance, please take the following into consideration:

   a. For optimal performance, greater system capabilities may be required in some situations. These include:
      - Larger files (greater number of events)
      - Higher number of parameters (more colors)
      - More complicated protocols (many levels of gating, larger numbers of plots, etc.)

      Also see Does Kaluza Analysis use any optional hardware?

   b. The performance of Kaluza Analysis benefits from larger amounts of RAM. For 32-bit operating systems, up to 4 GB is recommended. For 64-bit operating systems, more than 4 GB is recommended.

   c. The performance of Kaluza Analysis benefits from multi-core and multi-CPU systems.

   d. Kaluza Analysis works at screen resolutions as low as 1440 x 900, but larger resolutions and a wide-screen aspect ratio are recommended.

   e. Kaluza Analysis may require up to 200 MB of disk space to install, depending on what libraries are installed. Beyond that, the only requirement is for space to store listmode data. Listmode files can be quite large and faster disk subsystems are recommended in that case.

5. Does Kaluza Analysis use any optional hardware?

   Kaluza Analysis supports the nVidia Tesla C2075 board with 488 CPUs and 6 GB of RAM, and the K20 board with 2496 CPUs and 5 GB of RAM. If any of these boards is installed, Kaluza Analysis automatically will use it if the correct drivers are installed. (The correct level of drivers from nVidia are required and are included on the Kaluza Analysis installation disc, but must be installed by the user.) This greatly accelerates analyses.

   NOTE The nVidia Tesla C1060 and C2050 boards supported in earlier versions of the Kaluza Analysis have been shown to operate with Kaluza Analysis v 1.3; however, these configurations have not been validated.

6. Does Kaluza Analysis use any optional software?

   Language packs for Kaluza Analysis may be found at www.kaluzasoftware.com. Users may install these to view Kaluza Analysis in their local language (if available). Each user's language preference is stored separately.

7. Does Kaluza Analysis affect my existing files in any way?

   No. However, the Kaluza Analysis Software installer can optionally associate *.fcs and/or *.lmd files with Kaluza Analysis. If this is done, then any associated file will be displayed with the Kaluza Analysis icon and opening it from the operating system will cause it to be loaded into Kaluza Analysis, automatically starting Kaluza Analysis, if necessary. These file associations can be changed by accessing the Kaluza Analysis installer via the control panel (Add/Remove Programs, Change.)

Please note that associating listmode files with Kaluza Analysis utilizes a feature of Microsoft Windows to change the icon that they are displayed with. The listmode files are not modified in any way by Microsoft Windows or by Kaluza Analysis. The listmode files will continue to function as before with other flow cytometry analysis software, although the Kaluza Analysis icon may be used if these files are sent as an attachment via email. Files sent this way are still standard listmodes, despite the icon, and are not "Kaluza Analysis files." Kaluza Analysis will not be required by the recipient to open them.

8. Does Kaluza Analysis have any network requirements?

The Kaluza Analysis does not interact with any network, with the following exceptions:

- If a network license is used, it interacts with the network (see What kind of licensing technology does Kaluza Analysis use?)
- The About Box within the software contains hyperlinks to the Kaluza page on www.kaluzasoftware.com and to the Tesla page on www.nvidia.com. Clicking on these links opens the appropriate page in the default web browser. No information is sent when this is done.

9. What kind of licensing technology does Kaluza Analysis use?

Kaluza Analysis is licensed. This license is enforced by technology called "Sentinel HASP" by SafeNet Inc. (formerly Aladdin Systems). This technology is automatically installed by the Kaluza Analysis installation program and is required for use of the software. The SafeNet website is
http://www.safenet-inc.com/. Several configurations are available:

a. Trial License: The trial license is a software-based license. It is installed automatically when Kaluza Analysis is installed. It expires 30 days from the first time it is used. The trial license is intended to make it easy to evaluate the software.

NOTE The expiration date starts when the license is first used, not when Kaluza Analysis is installed.

b. Single User License: The single user license is provided on a USB device commonly referred to as a "key" or a "dongle". This key must be plugged into a free USB port on the computer that Kaluza Analysis is being used on, to enable Kaluza Analysis to function.

1) It does not appear in the operating system's file system.

2) The user cannot store data on it.

3) Kaluza Analysis can read from it, but not write to, using special software provided by the vendor.

NOTE

1. The single user license will not work via a Microsoft Windows remote desktop. A network license is recommended for this scenario.
2. This device is not a USB flash drive.

c. Timed Single User License: As single user license (as stated above), but limited to use for one year from the time the license is first accessed.

d. Network License: The network license allows multiple Kaluza Analysis licenses to be stored on a single computer which can then be accessed by other computers across an Ethernet network. The network license is provided by a USB device similar to the single user license. The computer which hosts the network license must be capable of installing Kaluza

Analysis and have a free USB port. There are no other requirements for this computer, other than (optionally) high uptime and physical security. Unique characteristics of the network license:

1) Network license follows a "concurrent use" model. Kaluza Analysis can be installed on an unlimited number of computers, but the number of users operating the software on these computers at once is limited to the number of available network licenses.

2) Each computer accessing a network license must be configured to do so. This is done by accessing http://localhost:1947 in a web browser, at each computer. Instructions for this procedure can be found in CHAPTER 1, *Using the License Key*.

10. How does Kaluza Analysis interact with the file system?

Kaluza Analysis reads user-provided listmode data from *.fcs and *.lmd files stored in the file system. These files must be in FCS1.0, FCS2.0, FCS3.0 or FCS3.1 format. Kaluza Analysis will never overwrite, change, or delete these files.

Kaluza Analysis can read images in most standard formats and can read CSV files, as instructed to by the user.

Kaluza Analysis can store the user's work in one of several formats, if instructed to do so, as follows:

a. An *.analysis file containing copies of one or more listmode files and the user's protocol(s).

b. A *.protocol file containing the user's protocol.

c. A *.compensation file containing the spillover matrix for a protocol.

d. A *.txt representation of the spillover matrix.

e. A *.csv with selected statistics from their analysis.

If Kaluza Analysis exits unexpectedly, it will write a log file to one of the following locations:

- Windows 7 and Vista:
  C:\Users\<user-name>\AppData\Local\Beckman Coulter\Kaluza Analysis\<version>
- On Microsoft Windows XP:
  C:\Documents and Settings\<user-name>\Local Settings\Application Data\Beckman Coulter\Kaluza Analysis\<version>

Kaluza Analysis uses an SQLite database to log additional performance information. The database files are maintained in the same directory as the log file.

Kaluza Analysis uses temporary files in the location specified by the operating system configuration.

Kaluza Analysis stores user options in a user configuration file which can be found at:

- Windows 7 and Vista:
  C:\Users\<user-name>\AppData\Local\Beckman_Coulter,_Inc\Kaluza.exe_Url_<random>\<version>\user.config
- On Microsoft Windows XP:
  C:\Documents and Settings\<user-name>\Local Settings\Application Data\Beckman_Coulter,_Inc\Kaluza.exe_Url_<random>\<version>\user.config

11. What are the database requirements for Kaluza Analysis?

Kaluza Analysis uses a SQLite database to maintain logging information. The SQLite software is included in the Kaluza Analysis software package. No additional software is required.

12. What kind of security requirements does Kaluza Analysis have?

Kaluza Analysis has the following security requirements for the account of the user operating it:

a. Elevated privileges (typically administrator) are required to install the software.

b. For operation, Kaluza Analysis requires privileges no greater than “restricted user”.

c. Kaluza Analysis must have read access to file system data that it is programmed to read.

d. Kaluza Analysis must have write access to file system folders where it is programmed to write.

Other than the above, Kaluza Analysis does not interact with the operating system’s security features.

13. What security features does Kaluza Analysis have? Does it support 21 CFR Part 11?

Refer to CHAPTER 1, *User Preferences and Security* for information on Kaluza Analysis user security.

APPENDIX D

Troubleshooting

Overview

The purpose of this appendix is to provide tips for troubleshooting Kaluza Analysis.

Log Files

Log files document exceptions within Kaluza Analysis and provide clues to understanding issues and viewing user set-up configurations. In *Program Failure Log Files* are instructions for providing Kaluza Analysis log files to a Beckman Coulter Representative for troubleshooting. *User Options File* provides the location of user configuration log files.

Program Failure Log Files

When contacting Beckman Coulter for troubleshooting information, you will need to provide the log files.

To access the log files and upload for troubleshooting:

1. Locate the Kaluza Analysis log directory:
   - **a.** Select the (**About**) icon in the upper right corner of the Kaluza Analysis screen.
   - **b.** In the **About Kaluza Analysis** screen, select the Open Log Directory... button, which takes you directly to the location of the files.

   OR

   Locate the files manually by navigating to the appropriate location listed below:

Operating System	Storage Location
Microsoft Windows 7 or **Microsoft Windows Vista**	C:\Users\<user-name>\AppData\Local\Beckman Coulter\Kaluza Analysis\<version>
Microsoft Windows XP	C:\Documents and Settings\<user-name>\Local Settings\Application Data\Beckman Coulter\Kaluza Analysis\<version>

2 Close Kaluza Analysis.

3 Zip the entire contents of the log directory, and place the zip file in the location of your choice.

4 Access the Kaluza troubleshooting page by entering *www.kaluzasoftware.com* into your internet browser, and then selecting the **FAQ** tab.

5 Select the **Troubleshooting File Upload** button.

6 Fill out the short form, describing the problem, and then select **Submit your information** to access the upload page.

7 Upload the zip file to complete the process. Once the file is uploaded, it will be listed on the page. You can upload additional files or close the browser when you are finished.

System NFO Files

When contacting Beckman Coulter for troubleshooting information, you might be requested to provide a *.nfo file. To access the *.nfo file and upload for troubleshooting:

1 From the Windows **Start** button, select **All Programs** > **Accessories** > **System Tools**.

2 Select **System Information**.

3 In the System Information window, select **File** > **Save**.

4 In the **File name** field, enter your name, navigate to the location where you wish to save the file, and then select **Save**. The default file type is ***.nfo**.

NOTE It could take between 2 and 4 minutes for the *.nfo file to be created.

5 Access the Kaluza troubleshooting page by entering *www.kaluzasoftware.com* into your internet browser, and then selecting the **FAQ** tab.

6 Select the **Troubleshooting File Upload** button.

7 Fill out the short form, describing the problem, and then select **Submit your information** to access the upload page.

8 Upload the *.nfo file to complete the process. Once the file is uploaded, it will be listed on the page. You can upload additional files or close the browser when you are finished.

User Options File

Kaluza Analysis stores user options in a user configuration file that can be found in the following locations:

- Windows 7 and Vista:
  C:\Users\<user-name>\AppData\Local\Beckman_Coulter,_Inc\Kaluza.exe_Url_<random>\<version>\user.config
- On Microsoft Windows XP:
  C:\Documents and Settings\<user-name>\Local Settings\Application Data\Beckman_Coulter,_Inc\Kaluza.exe_Url_<random>\<version>\user.config

Troubleshooting Kaluza Analysis Software

The table below lists tips to guide you if you encounter any problem during installation, as well as the respective recommended action. If you are unable to solve the problem, contact your Beckman Coulter Representative.

NOTE Some troubleshooting issues are due to the Sentinel HASP subsystem. Sentinel HASP is the technology used to license-protect Kaluza Analysis. The names “Sentinel” and “HASP” are used interchangeably in error text.

Issue: .NET Framework installation errors.	
Comment:	**Recommended Action:**
• Error messages display during .NET installation. • Kaluza Analysis crashes during launch.	• Contact your IT Department. • See *Log Files*.

Issue: Kaluza Analysis installation prerequisites dialog box persists that the .NET is not installed on a Windows 7 PC.	
Comment:	**Recommended Action:**
The Kaluza Analysis Setup.exe properties are set to Compatibility Mode. (This can be viewed in Windows Explorer by right-clicking the Kaluza Analysis Setup.exe file and selecting **Properties**.)	If installing on Windows 7 and the install is being run by Windows XP in Compatibility Mode, the .NET installation will always show a warning on the Prerequisite screen (even after successful installation). If this is the case, deactivate Compatibility Mode via the Install File and repeat the installation. If this does not work, try copyig the Kaluza Analysis Setup.exe file from the installation disc to a hard drive aand repeating the steps.

Issue: • **Unable to start Aksfridge Service, error occurs at the end of the installation.** • **Unable to start Aksfridge Service with parameters 1080 1058 1. Error code: 48 5 550 1058.**	
Comment:	**Recommended Action:**
This error occurs because the Sentinel HASP service cannot get started correctly.	Reboot the computer and attempt to run the application one more time.

Issue: Sentinel HASP error during installation.	
Comment:	**Recommended Action:**
This error occurs because the Sentinel HASP service is not installed correctly. Three possible causes are: • Other “Sentinel” services are running and are interfering with or have modified the Kaluza Analysis licensing service. • A firewall is blocking port 1947 for the Sentinel HASP. • Another program is holding on to port 1947 for Sentinel HASP.	• Reboot and repeat install. • If error persists, temporarily disable virus checker and repeat installation. • If error persists, uninstall any instances of Kaluza Analysis and then remove any Sentinel HASP Local License Manager license keys installed on the PC, reboot, and then re-install Kaluza Analysis. • If error still persists, browse to http://localhost:1947 in your web browser. Click the Sentinel HASP keys on the left side of the screen, take a screen shot and save this image file. Click on **Features** on the left side of the screen and take another screenshot, and save the file. Files (.JPG, .GIF, .PDF, .TXT) can be uploaded for troubleshooting. To access the troubleshooting page, go to *www.kaluzasoftware.com*, and select the **FAQ** tab. Fill out the short form, describing the problem. Select **Submit** to access the upload page.

Issue: Unable to access HASP SRM Run-Time Environment (H0033) displays on the HASP Protection System for the Kaluza Analysis window.	
Comment:	**Recommended Action:**
This occurs because the Sentinel HASP run-time has not been started.	**1.** Find Kaluza Analysis in **Add**/**Remove** programs. **2.** Select the **Repair** option to ensure all necessary files are in place. **3.** Reboot the computer. **4.** Attempt to launch Kaluza Analysis. If the error message still persists: **1.** Click **START** > **My Computer** > right-click **Manage**. **2.** Select **Services** from the left side of the screen. **3.** Locate **HASP License Manager for Kaluza.** **4.** Right-click on the **HASP service**. **5.** Select **Properties**. **6.** Ensure that the **Startup Type** is set to **Automatic**. **7.** Ensure that the message **Service is started** is displayed. **8.** Attempt to launch Kaluza Analysis. **9.** If the problem persists, contact your Beckman Coulter Representative.

Issue: Unable to start hardlock service displays on the HASP SRM Run-time installation window.	
Comment:	**Recommended Action:**
This is known to occur occasionally at the end of the installation. It occurs because the HASP service could not get started correctly.	Reboot the computer and attempt to run the application one more time.

Issue: Unable to start hasplms service displays on the HASP SRM Run-time installation window.	
Comment:	**Recommended Action:**
This is likely a bad interaction with McAfee antivirus software.	**1.** Reboot the computer. **2.** Rerun the Kaluza Analysis installer. If the error message still persists: **1.** Click **START** > **My Computer** > right-click **Manage**. **2.** Select **Services** from the left side of the screen. **3.** Locate any McAfee services. **4.** Attempt to right-click and halt the McAfee services. **5.** If problem persists, contact your local IT department to verify McAfee services are halted. **NOTE** HASP contains a known interaction McAfee Host Intrusion Protection Service.

Issue: Upon launching the software, Kaluza Analysis displays an H0007 error message.	
Comment:	**Recommended Action:**
Error displays: "Kaluza requires a hardware key. Please insert the USB hardware key. (H0007)" This occurs because Kaluza Analysis cannot locate a trial license or a physical hardware key to connect to. Kaluza Analysis should install a trial license by default.	**1.** Ensure that the computer was rebooted after Kaluza Analysis was installed. This will ensure that HASP services have been started. Instructions for starting HASP services can be found in steps 1-7 in "Issue: Unable to access HASP SRM Run-Time Environment (H0033) displays on the HASP Protection System for the Kaluza Analysis window." above. **2.** Locate Kaluza Analysis on your hard-drive. ***C:\Program Files\Beckman Coulter\Flow Cytometry\Kaluza (version number)\Kaluza.Trialinstall.exe.*** **3.** Run the Kaluza Analysis **Trialinstall.exe** file. **4.** Attempt to launch Kaluza Analysis. If the error message still persists: **1.** Find Kaluza Analysis in the **Add**/**Remove** programs. **2.** Select the **Repair** option to ensure all necessary files are in place. **3.** Reboot the computer. **4.** Attempt to launch Kaluza Analysis. If the error message still persists: **1.** Check the file path ***C:\Program Files\Common Files\Aladdin Shared\Hasp*** or ***C:\Program Files (x86)\Common Files\Aladdin Shared\HASP*** to ensure it does not contain any non-Latin characters. (This sometimes happens on non-English Windows installations.) **2.** If problem persists, contact your Beckman Coulter Technical Support Desk. **NOTE** Users with the Danish version of Windows XP installation may experience this difficulty. HASP is aware of this difficulty, and currently there is no workaround the situation. Users must purchase the license with a hardware key for the software to work fine. This issue does not occur on Danish Vista or Danish Windows 7 operating systems.

Issue: Using the Repair option from Add/Remove programs on a 64-bit version of Windows 7 leads to an uninstallable Sentinel HASP License Manager service.	
Comment:	**Recommended Action:**
If the user repairs the Kaluza Analysis installation on a 64-bit version of Windows 7 and later uninstalls Kaluza Analysis, Sentinal HASP License Manager service remains installed.	If software repair is needed, uninstall/install Kaluza Analysis by doing the following: **1.** Find Kaluza Analysis in the **Add**/**Remove** programs. **2.** Uninstall Kaluza Analysis. **3.** Reboot the computer. **4.** Reinstall Kaluza Analysis using the installation disk.

Issue: AVG flags HASP as a virus. Using the AVG antivirus software version 9.0.818 or prior version.	
Comment:	**Recommended Action:**
This is a known interaction between AVG and HASP. Currently .AVG flags the file **haspvlib_87749**.dll as a virus. This .dll file is specific the Kaluza Analysis. AVG has been contacted about this issue, and they have now updated their product so this file will no longer be flagged as a virus.	Upgrade the AVG software to version 9.0.819 or later.

Issue: You currently have a HASP license key but are unable to access Kaluza Analysis due to a license expiration error. Error code 11009.	
Comment:	**Recommended Action:**
Either you have a trial license that has expired or your computer is having trouble recognizing the hardware (USB) license key.	• If you have a **single user license key**, unplug and then plug the hardware key back in. Ensure that the red light on the hardware key is turned on. • If using a **network license key**, refer to the *Using the Network Key* section in the Instructions for Use manual.

Issue: Kaluza Analysis crashes when opening a large file while the Windows Picture and Fax Viewer application is open.	
Comment:	**Recommended Action:**
N/A	• Contact your IT Department and ensure that the latest video drivers are installed. • Avoid using Windows Picture and Fax Viewer when working with large listmode files in Kaluza Analysis. • See *Log Files*.

Issue: Report Sheet page size layout is ignored when printing ALL sheets.	
Comment:	**Recommended Action:**
The page size selected in the Windows Print dialog box persists when printing ALL sheets; regardless of the Report Sheet page size selection.	Select to print ALL Report Sheets when Report Sheets are setup with a custom page size selection.
Issue: Tree plot statistics is clipped on the printout.	
Comment:	**Recommended Action:**
This may occur when the size of the displayed plot causes a wrap-around of the statistics.	Increase the width of the plot enough so that the selected statistics do not wrap around.

Issue: EXPO32 *.lmd files does not open with their embedded runtime protocol conditions.	
Comment:	**Recommended Action:**
This is due to FCS keyword incompatibilities causing the embedded protocol information to not be read. Viewing the *.lmd file's FCS Info plot demonstrates some FCS keyword information is missing. For example, the "@" is present, but the corresponding keyword description or value in the keyword is missing.	Created plots manually for these EXPO32 *.lmd files.

Issue: System II data has an unexpected presentation in Kaluza Analysis with Logicle scale view.	
Comment:	**Recommended Action:**
The artifacts are a result of the way that System II stored the FCS data in the file. System II stored the data in FCS 2.0 format, meaning that the data is stored compensated, transformed to a log scale, and any negative values produced by compensation are clamped to the lowest axis value (i.e., 0.1024). The clamping in the source file is the reason the events are lined up at 10^-1 in the plot.	None.

Issue: Error on startup. The Kaluza Analysis application used to operate properly, but now will not load.	
Comment:	**Recommended Action:**
This issue could be due to the license manager service no longer running.	**1.** Verify that the license manager service that Kaluza Analysis uses is properly running. **a.** Navigate to http://localhost:1947. **b.** If the website displays with content, then the service is running. If the error still persists: **1.** Select **Run** from the Windows **Start** menu. **2.** Enter **services.msc** in the **Open** field. **3.** Locate **Sentinel HASP License Manager Service**. and right-click. **4.** Start or restart **Sentinel HASP**.

Issue: Out of memory.	
Comment:	**Recommended Action:**
Out of memory conditions may yield: • Statistics displaying as “O” or ####, even though there is data present in the gate; or • Statistics present even though no data is displayed on the plot.	**1.** Restart the Kaluza Analysis application. **2.** If problem persists, scale back the analysis sequence triggering the out of memory condition.

Issue: (Applies to v1.2 or higher.) “Kaluza encountered an error and needs to exit!” Error code: 99999 (while printing to .PDF).	
Comment:	**Recommended Action:**
Having Kaluza Analysis v1.0 or v1.1 .PDF drivers and attempting to print to a .PDF file will cause Kaluza Analysis to become non-responsive.	**1.** Use the built-in export to .PDF functionally in Kaluza v1.2 or higher rather than the Kaluza .PDF printer. **2.** Uninstall/reinstall the Kaluza Analysis .PDF printer. **3.** If problem persists, contact your Beckman Coulter Representative.

Issue: When loading compensation files generated from a non-BCI system (e.g.: BD FACSAria) into the auto-compensation module of Kaluza Analysis and *Generate Compensation* is selected, an error message is generated and Kaluza Analysis shuts down. **Kaluza Analysis, v1.1 - Error Code 9999.** **Kaluza Analysis, v1.2 and v1.3 - Error Code 19028.**	
Comment:	**Recommended Action:**
Kaluza Analysis has a limit of 31 characters for the detector name, and the auto-compensation is trying to create a sheet exceeding the character limitation for the **Name** field.	• **Kaluza Analysis, v1.1:** **1.** Click the **Show more information button**. **2.** Scroll to find the value in the Detector column for the parameter with the lengthy parameter detector name. This must be done for both the Area and Height of the parameters on each data file. **3.** Modify the values. **4.** Click **OK**. **5.** Resume with the Generate Compensation activities. • **Kaluza Analysis, v1.2 and v1.3**: Follow the instructions on the screen.

Issue: Mean/Mode Statistics are significantly different between Kaluza Analysis and the originating system the data was generated on.	
Comment:	**Recommended Action:**
In some Beckman Coulter systems there is an option to change the plot scaling. This is identified by the PnE FCS keyword. Kaluza Analysis always sets the highest decade to 10^3 and then goes down from there. Kaluza Analysis has no way to make the first decade label be 10^1. Statistics are always going to differ when customized offsets are used in the Acquisition Protocol.	**1.** See the *Statistics* section of the Instructions for Use manual for v1.2 and above. **NOTE** Kaluza Analysis statistics may not match exactly to the other software platform statistics. This is expected behavior due to the use of different computation methods employed by different software platforms. For example, Kaluza Analysis computes statistics from raw data, whereas Gallios uses binned data. Methods for computing events within a gate may differ, and smoothing algorithms may also differ. **2.** Verify the PnE FCS keyword value. If it does not have the default set to 0.1, then user-customized scaling has been used in acquisition.

Issue: • **Version 1.1 - On initial startup, Kaluza Analysis displays the following message: "Kaluza encountered an error and needs to exit!" Error code: 99999.** **NOTE** A 99999 error can occur at times other than initial startup, as this error indicates a catastrophic failure, causing the application to crash. The Recommended Action below only applies to a 99999 error at startup. **- OR -** • **Version 1.2 and 1.3 - "An error occurred while trying to load persisted settings for the Kaluza application. Kaluza settings, including any user configured options, will be restored to initial values." Error code: 99991.**	
Comment:	**Recommended Action:**
The user's configuration is saved in a file titled **user.config** when Kaluza Analysis closes. In this case, something was saved in the **user.config** file that Kaluza Analysis is unable to load.	Delete the **user.config** file from the location specified in *User Options File*. **1.** Close and re-launch the Kaluza Analysis. **2.** If the error persists after re-launching, zip the entire log directory as specified in *Log Files*. **3.** Report the issue to your local Beckman Coulter representative. Files (.JPG, .GIF, .PDF, .TXT) can be uploaded for troubleshooting. To access the troubleshooting page, go to *www.kaluzasoftware.com*, and select the **FAQ** tab. Fill out the short form, describing the problem. Select **Submit** to access the upload page. **NOTE** The **user.config** file stores the pane positions, collapsed pane states and the recently used list. Kaluza Analysis automatically recreates this file when the software is re-launched.

Issue: Images copied from Kaluza Analysis and pasted unto other Windows applications are not the best quality for printing.	
Comment:	**Recommended Action:**
Images saved from copy/paste functions are saved at 96 dpi; whereas images saved by the **Save as Image** tool from the Kaluza Analysis radial menu **Edit** function are saved at 600 dpi.	Use the **Save as Image** tool from the **Edit** function on the Kaluza Analysis radial menu.

Issue: A Summit file loaded into Kaluza Analysis displays scrambled plots.	
Comment:	**Recommended Action:**
This is related to the different byte orders that can be used for binary data saved from Summit. The bands go from thick near zero to thin as they get into the higher decades. Summit can save as five different file types that use one of four different byte orders.	**1.** Reload the files into Summit 4. **2.** Save them in a different byte order.

Glossary

This glossary is a collection of specialized terms and their meanings that are either used in this manual or related to the information in it. If a term has more than one meaning, all meanings relevant to this manual are included.

21 CFR 11
FDA Guideline that applies to records in electronic format that are created, modified, maintained, archived, retrieved or transmitted, where FDA will then consider these records equivalent to paper documents or written signatures.

Analysis/ Analysis File
A file that contains raw data as well as the protocol used to analyze the data.

Analysis List
A list of open files within the application. The list may be comprised of up to 400 rows, including raw Data Sets, Analysis files, Protocols, Composites, and Compensation Composite files.

Autofluorescence*
Fluorescence associated with a cell, usually caused by components and chemicals within the cell structure itself.

Autofluorescence Vector
The value that is subtracted from the data prior to spillover compensation and then added back afterwards. This increases the accuracy of fluorescence compensation.

Bivariate Plot
A plot containing two variables, one is displayed on the X-axis and the other on the Y-axis.

Boolean Gate*
Gates created by adding or subtracting populations, and utilizes the “and,” “not,” and “or” functions when defining the population of choice.

Color Precedence
The hierarchy of colors associated with gated events or when an event falls within several different gates, then the color precedence determines the display color.

Compensation
The mathematical process by which multi-parameter flow cytometric data is corrected for spectral overlap.

Composite
A file that contains multiple Data Sets analyzed by one protocol.

Data Set
Raw data derived from events within a sample, as captured by the flow cytometer.

Event
An individual particle, detected by a flow cytometer, from which raw data is derived.

FCS (Flow Cytometry Standard)*
(FCS 2.0 or 3.0) File format – format used to save flow cytometry data. Includes identifying information about the sample in the “header” and measurement information for each cell or event analyzed. The header is in text format while the measurement information is in binary format.

Fluorescence*
Excitation light energy is absorbed by fluorescent molecule, the molecule transitions to an excited state and as it returns to unexcited ground-state, a specific wavelength of light is emitted.

Fluorochrome*
Fluorescent substance used in biological staining to produce fluorescence in a specimen.

Gate
A subset of events, defined by a boundary, that allows for further examination.

Linear Scale
A scale that contains divisions that are uniformly spaced. The linear scale is good for showing forward scatter and side scatter parameters.

Logarithmic (Log) Scale
A scale that contains divisions based on exponential values. Log scales are useful when the data includes a large range of values. Fluorescence parameters are best displayed in the log scale because both weak and strong signals are appropriately accounted for.

Logicle Scale
A scale that allows for correctly displaying compensated data. When using the logicle scale, negative space can be displayed on one or both axes. This optimizes your ability to compensate fluorescence parameters, giving events a Gaussian appearance.

Opacity
The degree to which light can/cannot penetrate an object. The greater the opacity, the more defined the object appears.

Parameter
Types of data collected from the flow cytometer detectors, including such measurements as light scatter and fluorescence, area, height, and width.

Plot
Used as a data analysis tool, a plot is a graphical representation of the raw data collected from the flow cytometric sample. Plots are customized based on the parameters chosen to represent the data.

Protocol
The plots, parameters, and gates used for data analysis.

Ribbon
The section above the sheet area of the application screen for which you may perform a number of tasks from within the Plots & Tables, Gates & Tools, Edit, Page Layout or Galleries & Grouping tab.

Spillover
The amount of light emitted from a fluorochrome that is detected by unintended detectors.

Standardization
The use of a control material to establish the appropriate cytometer hardware settings to consistently run an application eliminating hardware variability; can be beads or unstained control sample.

Verification

The act of verifying that the statistics obtained after optimizing application cytometer settings match known values.

* Flow Cytometry Glossary http:/www.flocyte.com. October 14, 2013
http://www.flocyte.com/glossary

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Related Documents

Your Kaluza documentation can be found on our website at www.beckmancoulter.com.

Kaluza for Gallios Instructions for Use
P/N B25062

- Chapter 1, *Use and Function*
- Chapter 2, *Installation*
- Chapter 3, *Operation Principles*
- Chapter 4, *Specifications*
- Chapter 5, *System Overview*
- Chapter 6, *Introduction to Kaluza for Gallios*
- Chapter 7, *Protocols*
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- Appendix A, *Barcode Specifications*
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