
VENTURIONE 

**Getting Started
Guide**
Version 7.5

AppliedCytometry
Pioneering Software Simplicity



VenturiOne Getting Started Guide

1	Welcome to VenturiOne® Software	2
2	Contact Details	3
3	License Mechanism	3
4	Open VenturiOne Software	10
5	Opening and Saving FCS Files	19
6	Using the Playlist	21
7	Playlist Groups.....	23
8	Creation of Plots Using the Home Tab	24
9	Preview Plots	26
10	Selection and Analysis of Plots in the Plots Area.....	28
11	Creating Gated plots Using Preview Plots Area.....	31
12	Plot View Options – Hierarchy View	33
13	Plot Format Options.....	34
14	Results.....	35
15	Reports	36
16	Create a Report with the Report Wizard	37
17	Modify a Report	42
18	Print a Report.....	42
19	Creating a Custom Field	42
20	Compensation.....	43
21	Calculate Compensation Values with the Compensation Wizard	44
22	Manually Adjust Compensation Using the Preview Area	49
23	HyperLog™ Scaling and HyperLog™ Sliders	50
24	Manually Adjust Compensation on an Individual Plot	51
25	Getting Started with Discovery	53

1 Welcome to VenturiOne® Software

VenturiOne software is a powerful flow cytometry analysis package. This guide provides a brief overview of the steps involved in analyzing your saved FCS data files using the VenturiOne software.

Further details of all the elements of the VenturiOne Software are provided in the **VenturiOne Operation Manual**.

FCS File Format

VenturiOne supports all the common FCS files and the following file formats:

- FCS 2.0
- FCS 3.0
- FCS 3.1

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VenturiOne Software is for research use only

2 Contact Details

To speak to one of our team email customersupport@appliedcytometry.com or telephone +44 (0)1909 547210

Or contact us by post at the following address:

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Matrix House,
Nobel Way,
Dinnington,
South Yorkshire S25 3QB
UK

Visit us online at www.appliedcytometry.com

3 License Mechanism

A license control mechanism, in the form of a DESkey device, an ACULA cloud license or a valid software license are used for the operation of the VenturiOne software.

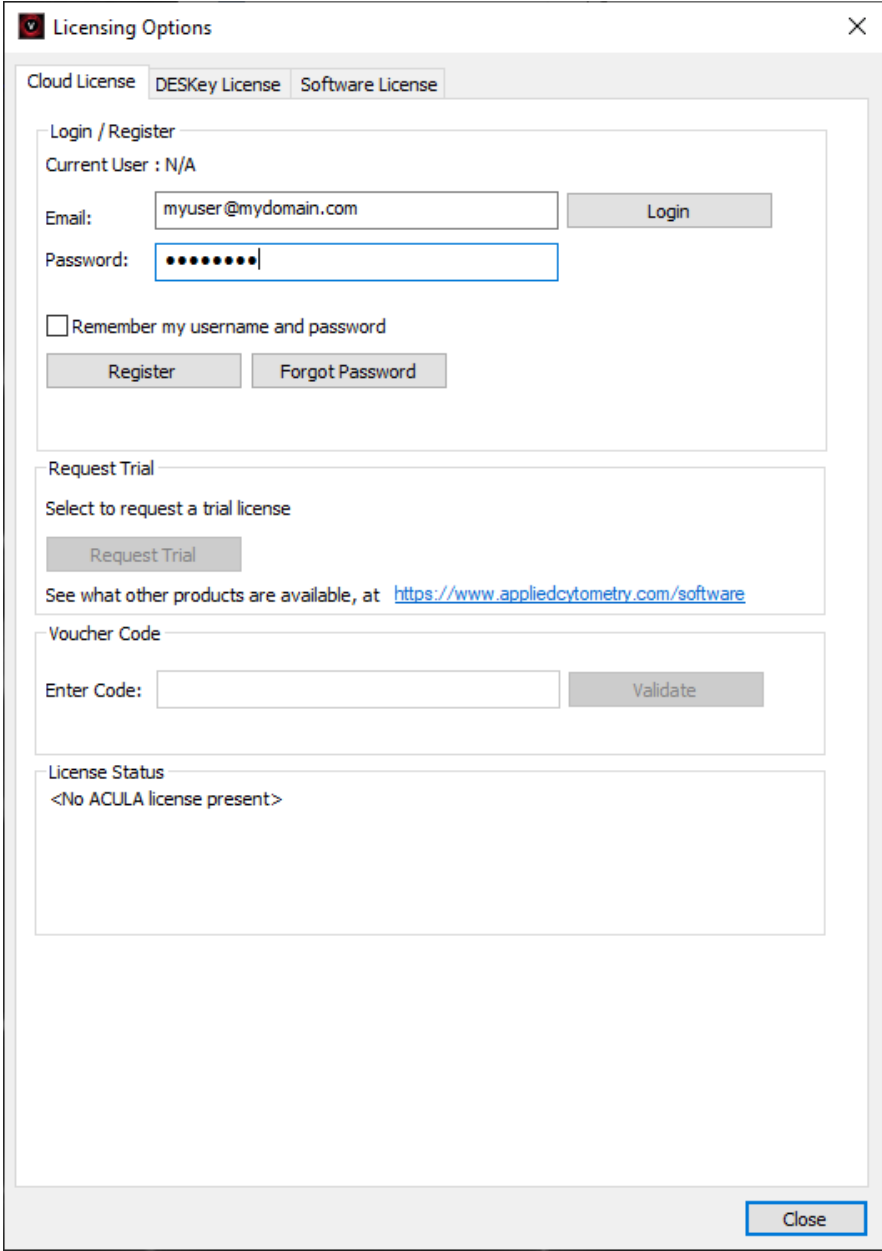
If a valid license mechanism is not detected when VenturiOne is started, a dialog is launched. This allows you to log in to the ACULA licensing system, enter a valid software license or attach a valid DESkey device.

Please see the VenturiOne Installation Guide for more information on installing the VenturiOne software.

3.1 ACULA cloud licensing page

The ACULA cloud licensing page allows you to register for a free trial, use a supplied license voucher code or use the free tier limited use version of the VenturiOne software.

The use of a cloud license requires an internet connection to validate upon logging in and periodically during software use.



The screenshot shows a window titled "Licensing Options" with a close button (X) in the top right corner. The window has three tabs: "Cloud License" (selected), "DESKey License", and "Software License".

Under the "Cloud License" tab, there are four sections:

- Login / Register:** Shows "Current User : N/A". It has an "Email:" field with "myuser@mydomain.com" and a "Login" button. Below it is a "Password:" field with masked characters "••••••••". There is an unchecked checkbox for "Remember my username and password", a "Register" button, and a "Forgot Password" button.
- Request Trial:** Says "Select to request a trial license" and has a "Request Trial" button. Below it, it says "See what other products are available, at" followed by a blue hyperlink: <https://www.appliedcytometry.com/software>.
- Voucher Code:** Has an "Enter Code:" field and a "Validate" button.
- License Status:** Displays "<No ACULA license present>".

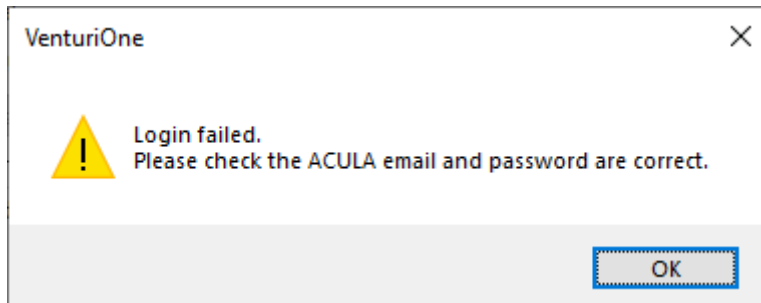
A "Close" button is located at the bottom right of the dialog box.

If you have not yet registered with the ACULA Cloud Licensing system, select the **Register** button to register your email address and password.

3.1.1 Login

If you have already registered with the ACULA Licensing system, enter the registered email and password, then select the **Login** button to log in to the software.

If you enter the incorrect details a warning message will be displayed, and you will not be logged in.



Select the **Remember my username and password** option to have the software automatically fill in your details next time you open the software. Uncheck this option to make the software forget your details.

3.1.2 Register

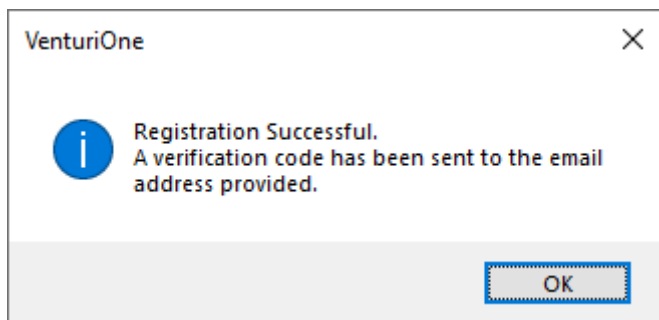
If you have not yet registered with the ACULA Cloud License system, select the Register button to register your email and password.

A screenshot of a registration dialog box titled "ACULA Registration" with a close button (X) in the top right corner. The form contains the following fields and elements: "Preferred Username:" with a text box containing "My Name"; "Email:" with a text box containing "myuser@mydomain.com"; "Password:" with a text box containing eight dots; "Confirm New Password:" with a text box containing eight dots; a checkbox labeled "Show Password" which is currently unchecked; a list of password requirements: "Password must conform to all of the following: - 8 or more characters long - Contain a lowercase letter - Contain an uppercase letter - Contain a number"; a privacy policy statement: "We will only use your personal information as set out in our Privacy Policy available on our website, at" followed by a blue hyperlink "https://www.appliedcytometry.com/privacy/"; a "Register" button; a "Verification Code:" label with an empty text box; a "Verify" button; and a "Close" button at the bottom.

Enter your preferred username, email address and password, then confirm your password. Select the **Show Password** option to display the current password text.

Select Register to register your email address

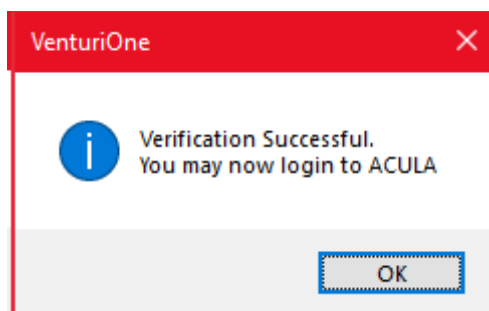
When you register with the ACULA licensing system you will be sent a verification code from [no-reply@acula.appliedcytometry.com](mailto:reply@acula.appliedcytometry.com)



if you do not receive a verification code, please check your spam folders.

To complete registration, enter the verification code sent to you and select **Verify**

You should then receive the verification successful message

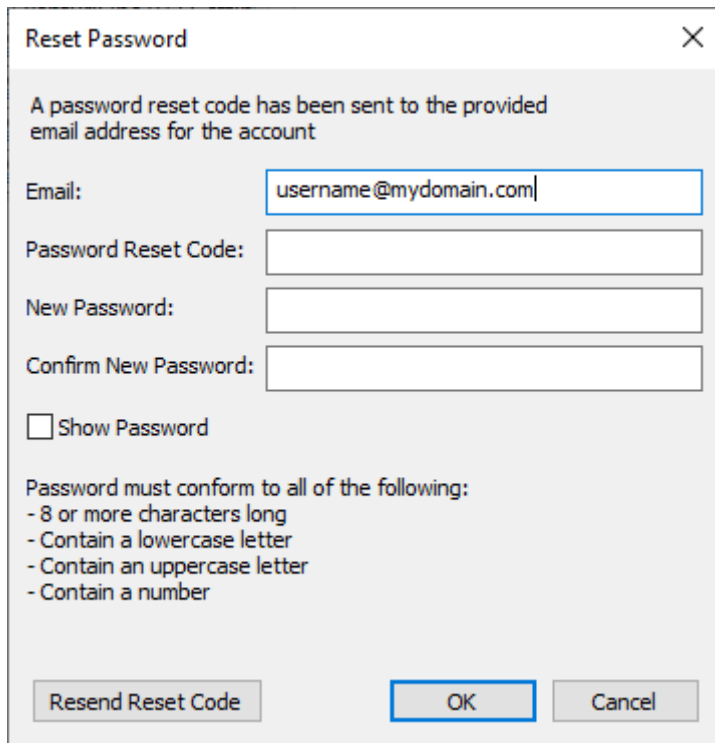


3.1.3 Forgot Password

If you have forgotten your password, select the **Forgot Password** button to initiate the process of entering a new password.

A verification code will be sent to the stated email address, enter the verification code here and the new password.

If you did not receive an email press the **Resend Reset Code** to resend the verification code, check your spam folders if the email does not arrive.



A dialog box titled "Reset Password" with a close button (X) in the top right corner. The message inside says: "A password reset code has been sent to the provided email address for the account". Below this, there are four input fields: "Email:" (containing "username@mydomain.com"), "Password Reset Code:", "New Password:", and "Confirm New Password:". Below the input fields is a checkbox labeled "Show Password". At the bottom, there is a list of password requirements: "Password must conform to all of the following:" followed by four bullet points: "- 8 or more characters long", "- Contain a lowercase letter", "- Contain an uppercase letter", and "- Contain a number". At the very bottom, there are three buttons: "Resend Reset Code", "OK", and "Cancel".

3.1.4 Request Trial

Once you have logged in you can use the **Request Trial** button to claim your free trial for this PC. You can only claim one free trial for each computer.

3.1.5 Voucher Code

If you have been given a voucher code, first register & and log in to ACULA then enter your voucher code into the voucher code box and press **Validate**.

3.1.6 License Status

The status of your ACULA Cloud License will be displayed in the License Status area.

If you are logged in to ACULA licensing system and do not have any other valid license, the software will operate in the Free Tier mode. In this mode you are allowed to analyze a limited number of files each month. The License Status area will display information relating to the number of files analyzed, the file limit and the date at which your allowance will be reset.

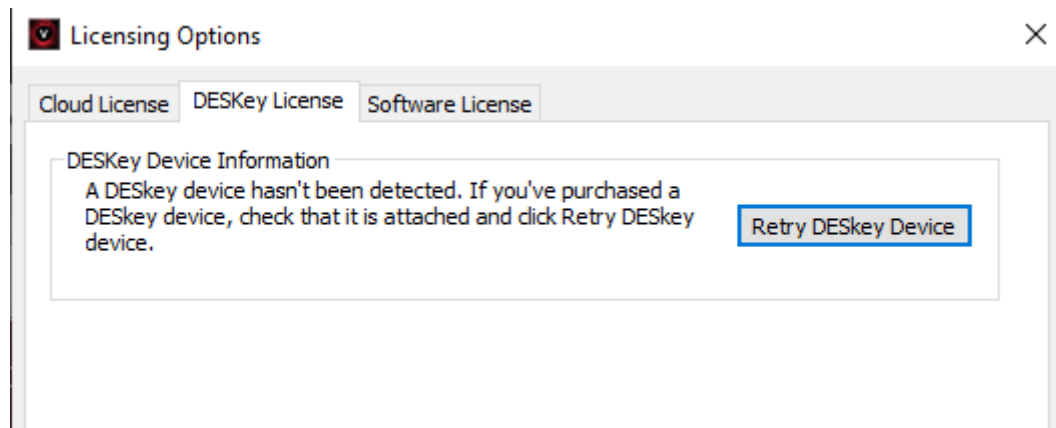
License Status

Trial License accepted. License expires at 22/03/2022

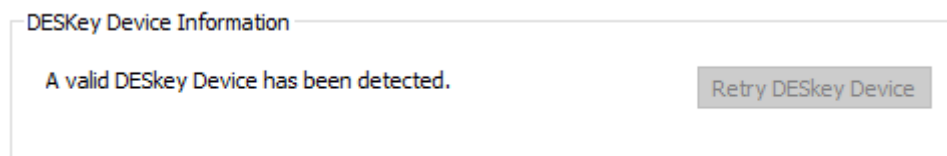
3.2 Deskey license page

If you have a valid Deskey USB license key, you can use this page to manually detect the key if it is not automatically detected.

Press the Retry Deskey Device to begin the detection process



The message will update if a valid Deskey device is detected.



3.3 Software license page

The software license page is used if you do not have an internet connection on the current computer so cannot use the ACULA cloud license system.

The screenshot shows a window titled "Licensing Options" with a close button (X) in the top right corner. It has three tabs: "Cloud License", "DESKey License", and "Software License", with the "Software License" tab selected. The window is divided into two main sections. The top section, "License Request", contains text explaining that a license key requires computer information and a contact email. It includes a text box for an email address and a "Save License Request File" button. The bottom section, "Licensing Information", contains text about entering a license key and a "Validate License" button. It also includes buttons for "Paste from Clipboard" and "From File...". A text box for the license key is present, and a "Not a valid license" message is displayed below it. A "Close" button is located at the bottom right of the window.

Licensing Options

Cloud License | DESKey License | **Software License**

License Request

To generate a license key, we need a few pieces of information about your computer. Click the button to save this to a file that can then be emailed to us at customersupport@appliedcytometry.com

We will only use your personal information as set out in our Privacy Policy available on our website, at <https://www.appliedcytometry.com/privacy/>

Email:

Save License Request File

Licensing Information

If you have a license key, enter it here and click Validate License.

Paste from Clipboard

From File...

Validate License

Not a valid license

Close

3.3.1 Requesting a software license

To request a key, enter your email address and voucher code (if you have one), then press the **Save License Request File** button.

A text file will be saved.

Send this text file to customersupport@appliedcytometry.com

We will then generate a license key for you and send that to the email entered above.

3.3.2 Entering the license key

Your license key will be sent to the email address you entered by one of the Applied Cytometry team. Your license key will be attached to this email.

Save the license file to a location on your Mac system

Start the VenturiOne software. When the **Licensing Options** dialog appears select the **From File...** button.

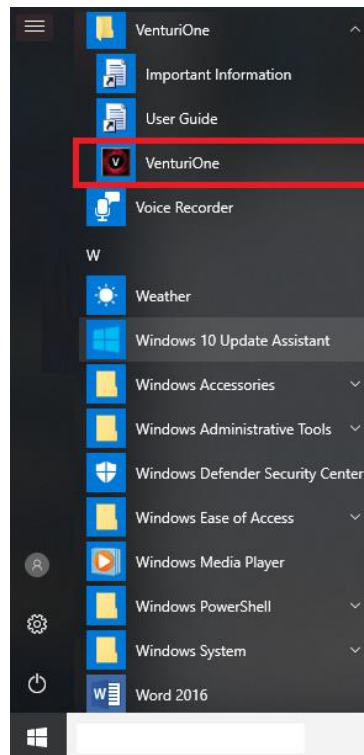
Select the license file and press ok.

You can also copy and paste the license key characters from the license file into the Licensing information box. Then press Validate license.

Note: The license file is only valid for the system on which the request file was generated, it is not transferable.

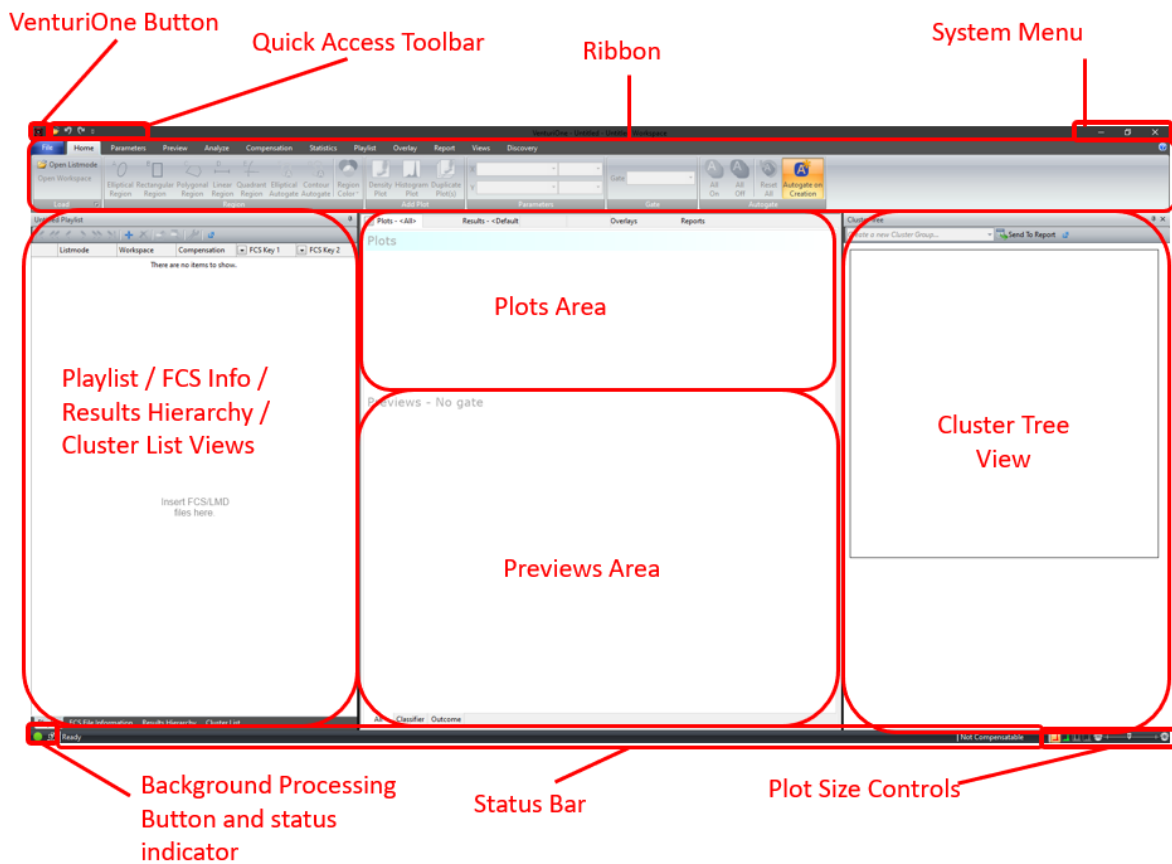
4 Open VenturiOne Software

To open the software, click the **VenturiOne** menu item from the **Start Menu**:

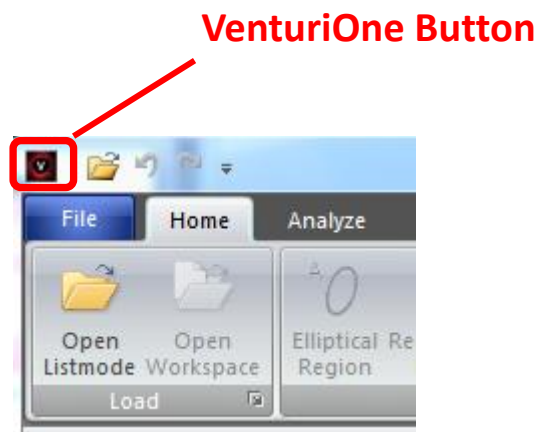


Double clicking a VenturiOne playlist file also launches the VenturiOne software.

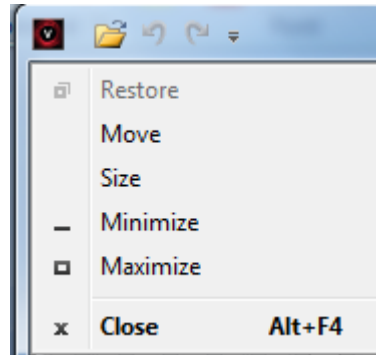
The **Application Window** is displayed, as illustrated below:



At the top left corner of the application is the **VenturiOne** button:

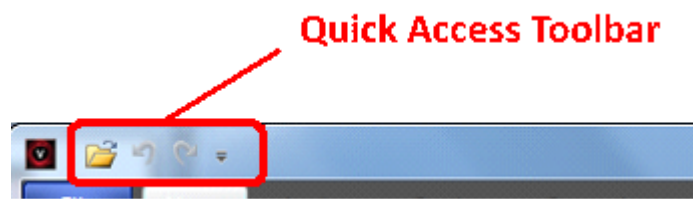


Click the VenturiOne button to display the **VenturiOne Menu** pictured below:



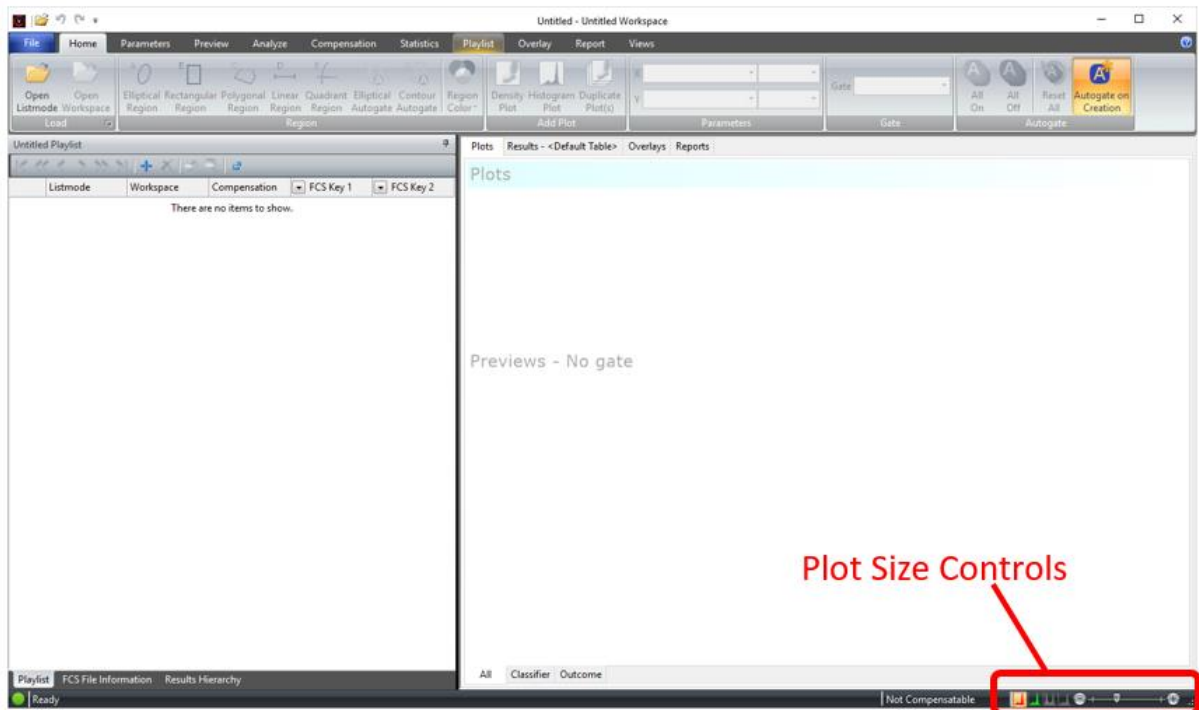
These options allow you to Minimize, Maximize, Move, or Resize the application window. The application can also be closed from here.

Next to the **VenturiOne** button is the **Quick Access** toolbar:



The **Quick Access** toolbar provides quick access to some of the most commonly used options in VenturiOne. The displayed options are user adjustable.

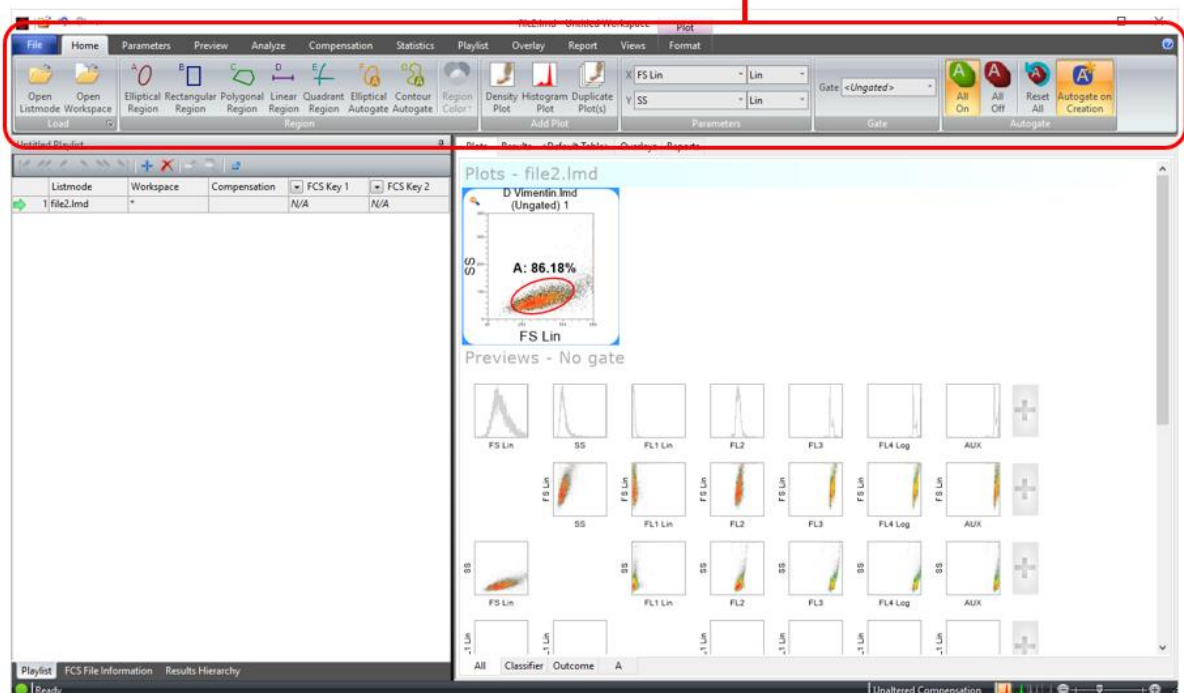
Plots sizing controls are located on the lower right-hand side of the application window.



The plot sizing controls allow the resizing of plots in the Plots area, Previews area as well as the Overlay and Gallery areas.

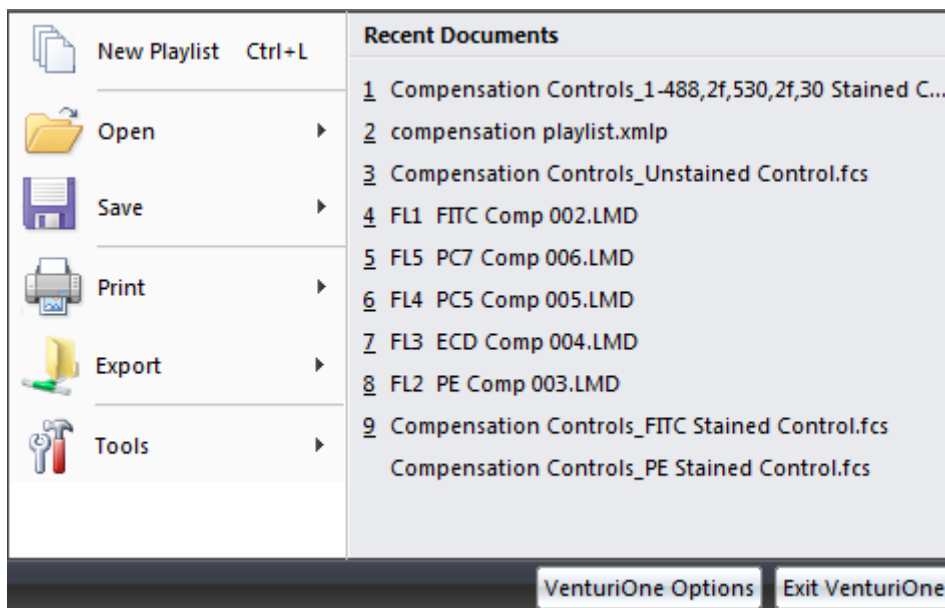
The **Ribbon** is the area at the top of the VenturiOne **Application Window** that presents an easy-to-browse main set of commands for the VenturiOne software. Commands are organized in logical groups that are collected together under tabs. To reduce clutter, some tabs are shown only when needed.

Ribbon



The individual tabs are described below:

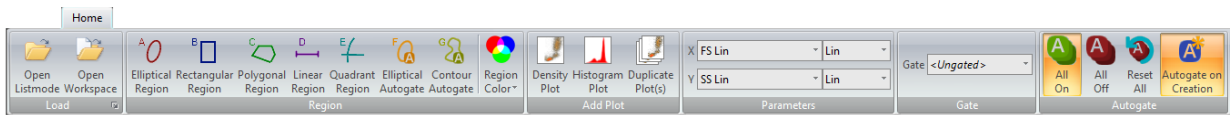
File Button - provides File and Print options:



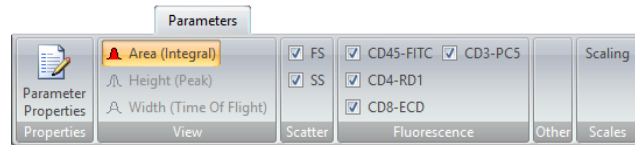
The **File** menu provides you with options to:

- Open a new or saved playlist file
- Open a saved workspace file
- Open an FCS file or compensation file
- Save FCS files, a playlist, workspace, compensation or Plot image file
- Print
- View the Print Preview and Print Setup
- Export VenturiOne results, reports and playlists
- View and/or open **Recent Documents**
- View FCS file keywords
- Access the **VenturiOne Options** dialog to set system default options.
- Exit the VenturiOne software

Home tab – provides all the essential controls to create and analyze plots in one place:



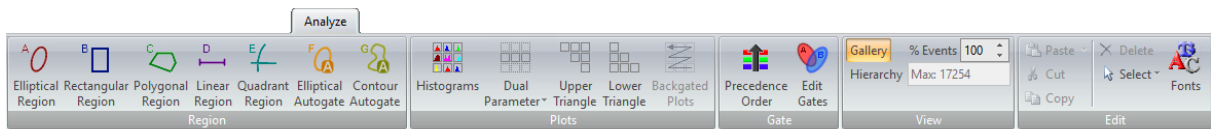
Parameters tab - provides controls to select the parameters for display:



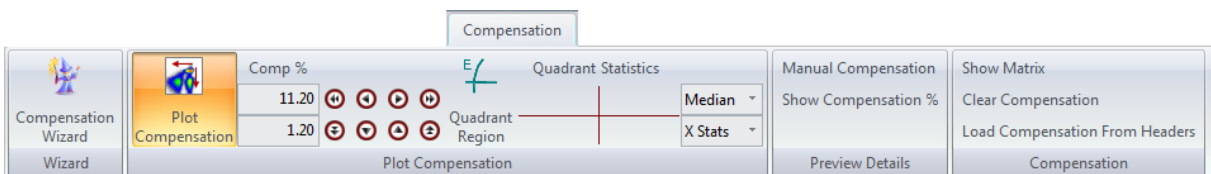
Preview tab - provides controls to modify the plots in the **Previews** area:



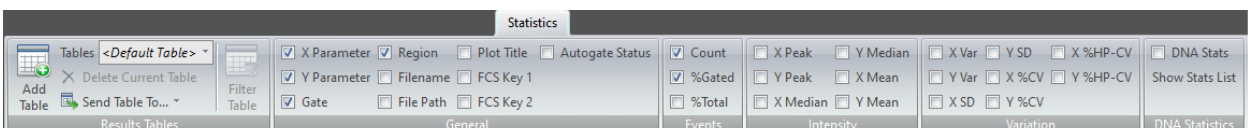
Analyze tab – provides additional controls to analyze and modify the plots in the **Plots** area:



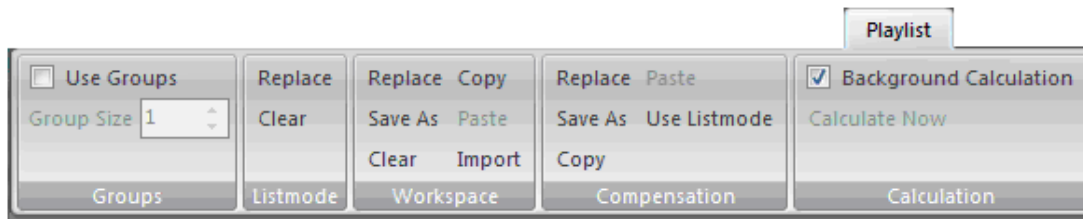
Compensation tab - provides controls to calculate fluorescence compensation:



Statistics tab - provides controls to select a statistic to display on plots or select statistics for display in the results table. Additional tables can be created which allow display of filtered results:



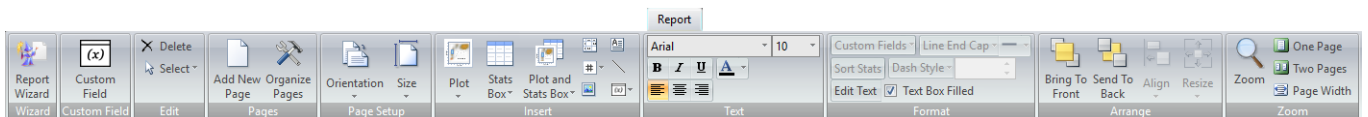
Playlist tab - provides controls to modify a **Playlist** including playlist items, FCS files, workspace files and compensation files:



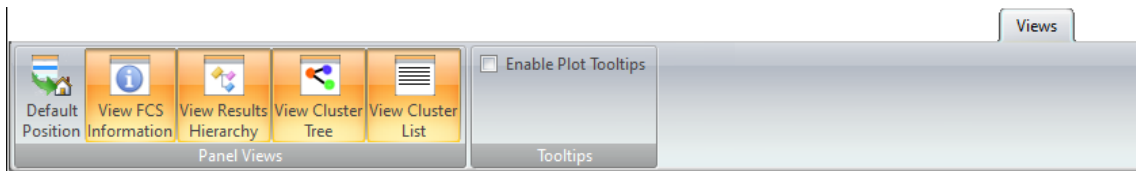
Overlay Tab – provides the controls for adding plots and regions to Overlay plots.



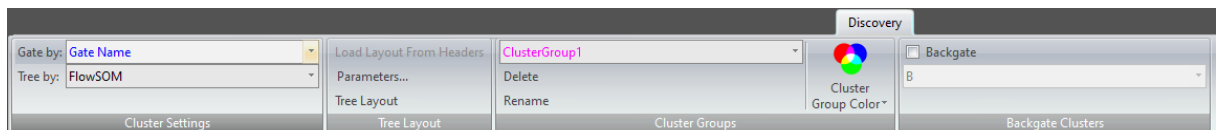
Report tab – provides controls to create page-based reports from a single playlist item or a group of playlist items:



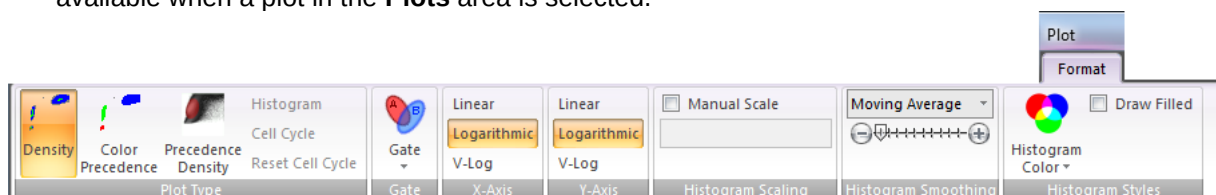
Views tab – provides controls to display and reset the position of the Worklist pane, FCS Information pane, the Results Hierarchy pane, the cluster tree and the cluster list:



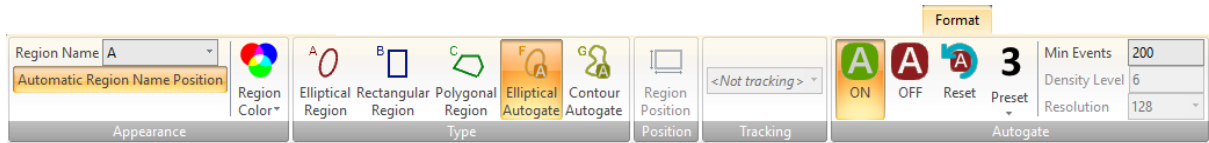
Discovery tab – provides controls to enable the display of cluster parameters in the **Cluster Tree plot** and allow the creation and modification of **Cluster Groups** on the Cluster tree.



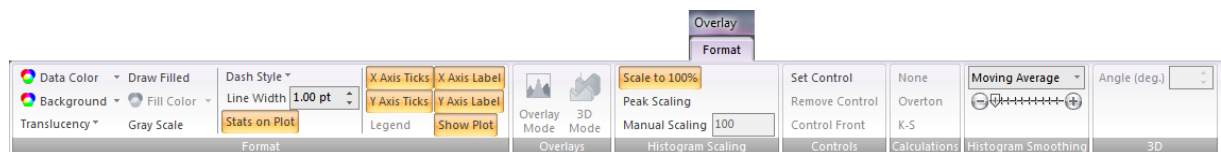
Plot Format tab – provides controls to adjust settings on a per plot basis. This tab is only available when a plot in the **Plots** area is selected.



Region Format tab - The options here modify the appearance and type of region displayed on the selected plot, reposition the region label and track one region to another. Autogate settings can be customized here. This tab is only available when a region is selected on a plot.

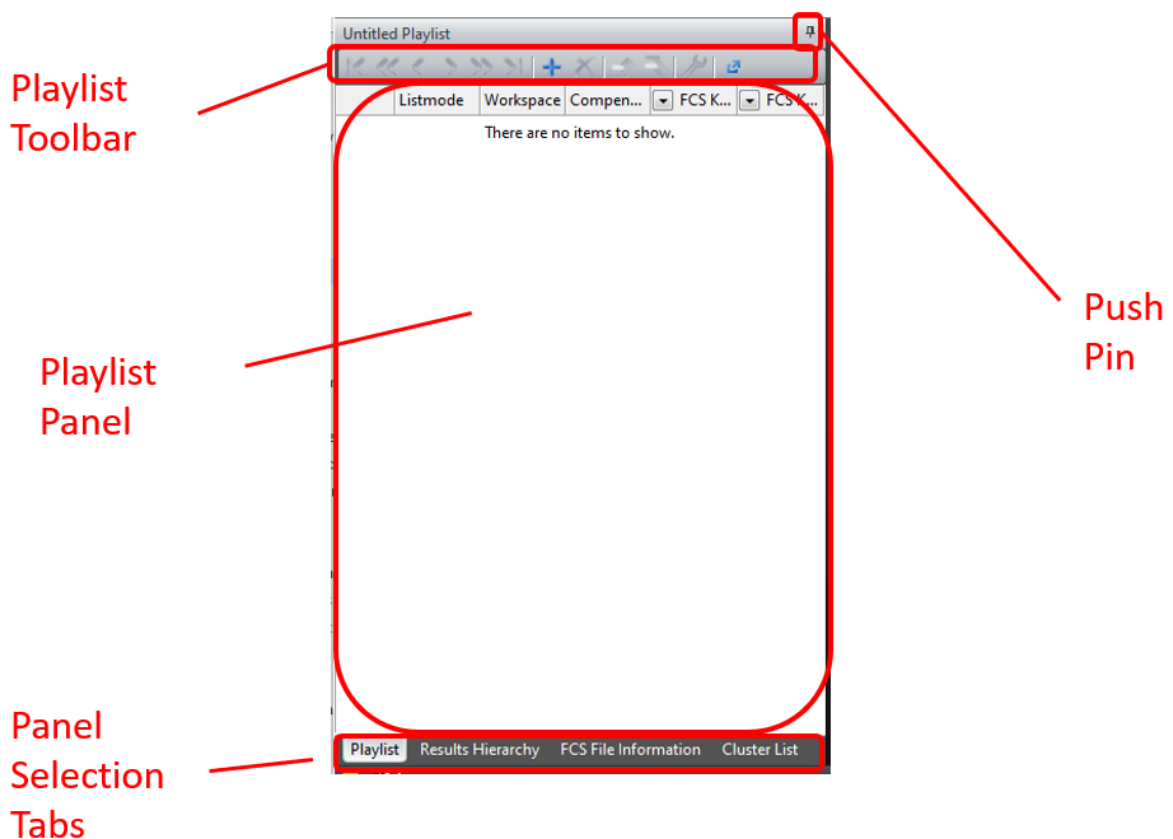


Overlay Format tab - The options here modify the appearance and type of region displayed on the selected plot, reposition the region label and track one region to another. This tab is only available when an Overlay or Gallery plot is selected in the Overlays tab.

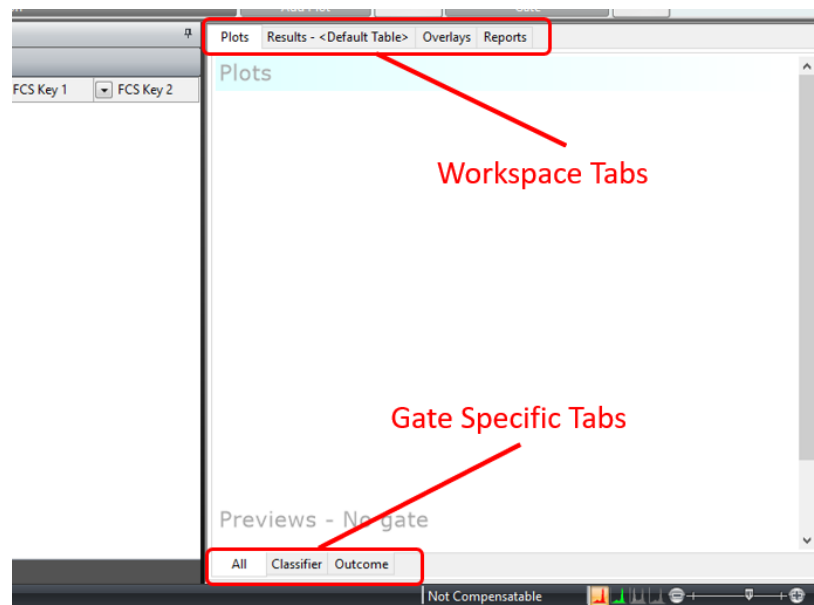


Under the **Ribbon** are the **Playlist** panel and the **Workspace**.

The **Playlist** allows selection of up to 400 FCS files for simultaneous analysis. Files can be reordered with workspace and compensation applied to each as desired.



The **Workspace** has four tabs:



Plots tab - When the VenturiOne software is launched the **Plots** tab is opened by default. The **Plots** tab contains two areas: **Plots** and **Previews**. The **Previews** area automatically displays preview plots once an FCS file is opened. Plots can be added to the **Plots** area in a number of ways. See Section 9 Preview Plots for further detail.

The Plots tab workspace contains gate tabs at the bottom of the page. The All, Classifier and Outcome tabs are always present.

- The All tab displays all plots currently available.
- The Classifier tab shows only those plots which contain regions which are parent gates.
- The Outcome tab shows all other daughter plots

Additional gate tabs are dynamically added or removed and only show those plots gated on the specific gate displayed.

Results tab - displays the statistics selected in the Statistics tab of the Ribbon.

Overlays tab – allows the creation and display of single and dual parameter overlay plots.

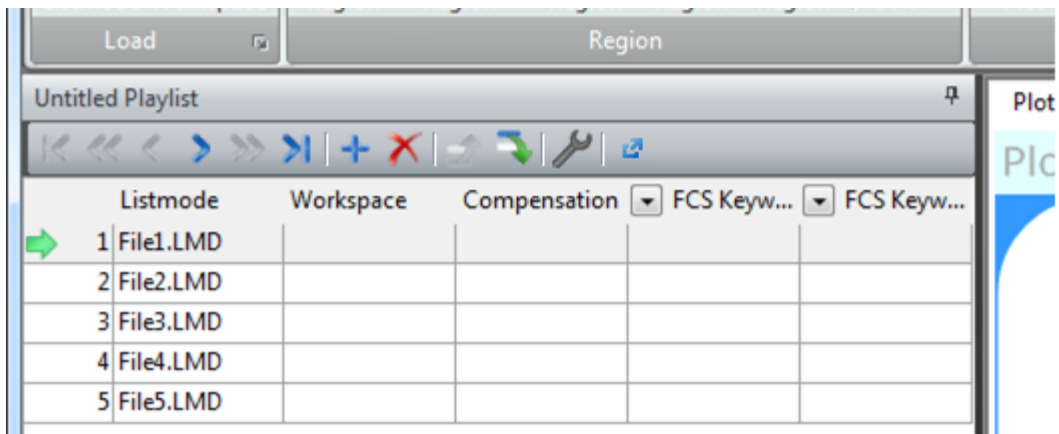
Reports tab - allows the creation and modification of page-based reports.

5 Opening and Saving FCS Files

To open an FCS file in VenturiOne click the  icon on the **Quick Access** toolbar or select **Open Listmode** from the **Home** tab or from the **File** options choose **Open** and then **Open Listmode**. A **File Open** dialog is displayed. Select the desired files.

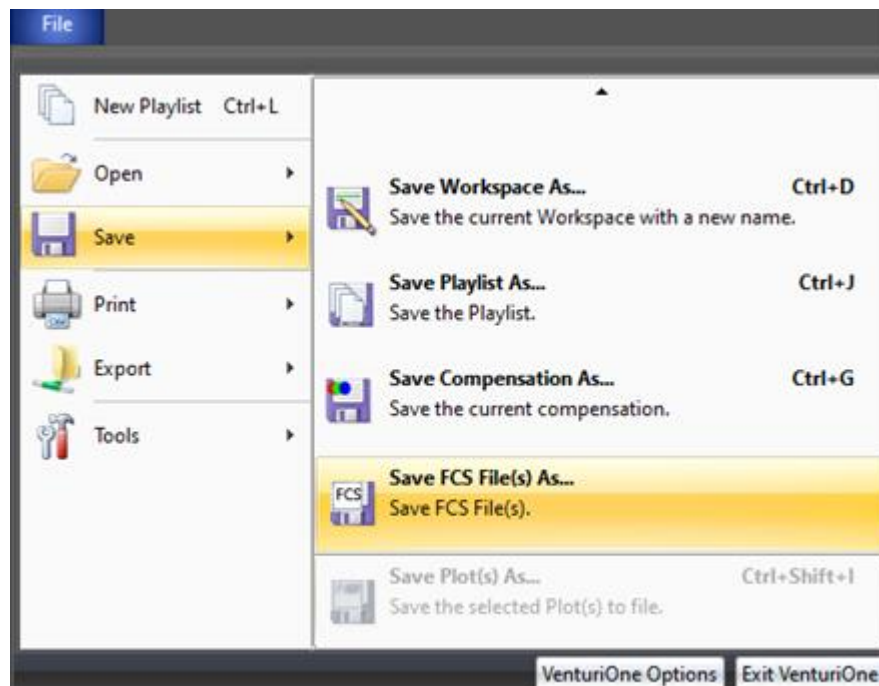
You can also select FCS files from a specific folder location in Windows Explorer and then drag and drop them onto the VenturiOne **Playlist**.

Files are added to the **Playlist** as shown below.

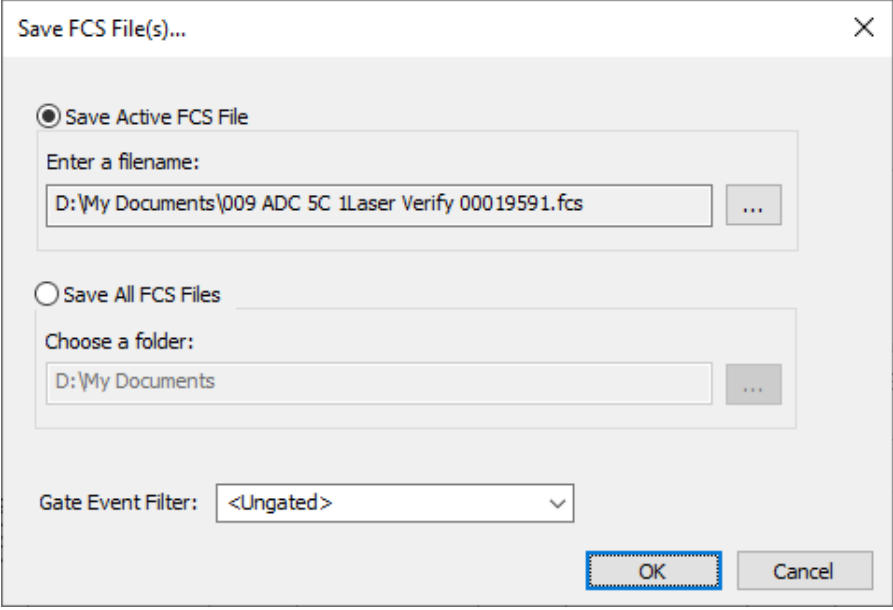


Files will be automatically added to the playlist in logical file name order, if you want files to be added using date and time order that option is selectable in the VenturiOne Options dialog / Playlist page.

To Save an FCS file, select the **File / Save FCS As..** option, you may need to scroll down on the **File** menu to see the option.



You can then choose to select either just the current file or select all files in the playlist. You can also select a gate from the currently active file to save only data from within that gate. When saving all files, the gate position from the current file will be used to filter the data for all files.



The image shows a 'Save FCS File(s)...' dialog box with a close button (X) in the top right corner. It contains two radio button options: 'Save Active FCS File' (selected) and 'Save All FCS Files'. The 'Save Active FCS File' section has a text field labeled 'Enter a filename:' containing 'D:\My Documents\009 ADC 5C 1Laser Verify 00019591.fcs' and a browse button (...). The 'Save All FCS Files' section has a text field labeled 'Choose a folder:' containing 'D:\My Documents' and a browse button (...). At the bottom, there is a 'Gate Event Filter:' label followed by a dropdown menu showing '<Ungated>' with a downward arrow. At the bottom right are 'OK' and 'Cancel' buttons.

Save FCS File(s)...

☒ Save Active FCS File

Enter a filename:

D:\My Documents\009 ADC 5C 1Laser Verify 00019591.fcs ...

☐ Save All FCS Files

Choose a folder:

D:\My Documents ...

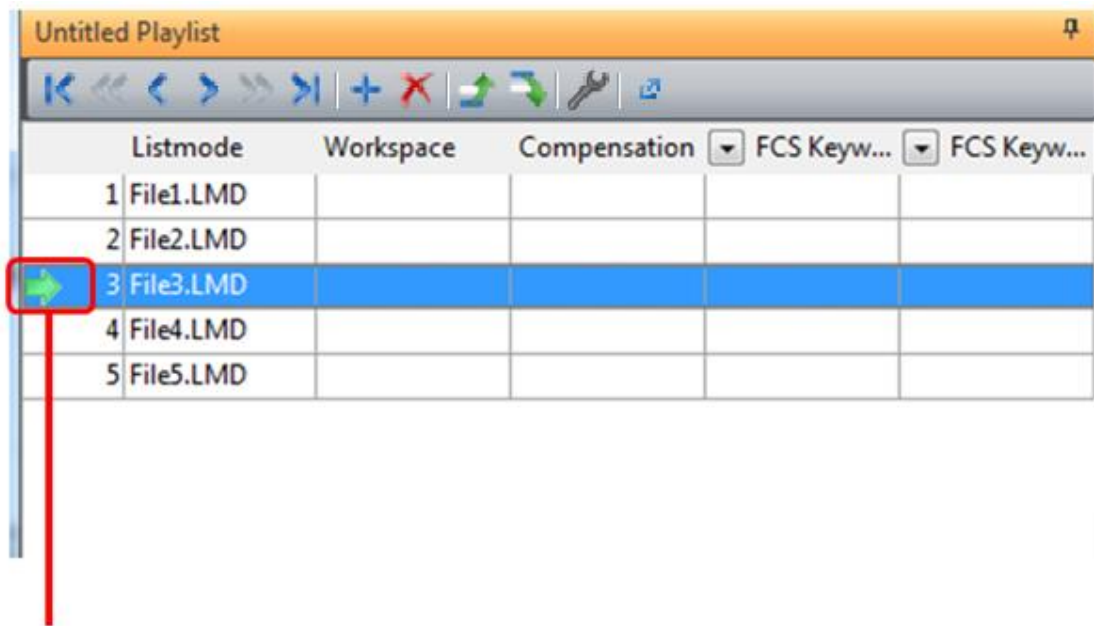
Gate Event Filter: <Ungated> ▼

OK Cancel

6 Using the Playlist

VenturiOne allows you to organize your files using the **Playlist**. A **Playlist** can contain a maximum of 400 files.

Depending on how you selected the FCS files, a file may already be open in the workspace, if not you can double click on an item in the **Playlist** to open a file. The **Playlist** displays a green arrow to show the currently open file.



Denotes Currently Open File

If you previously saved workspace and compensation files, you can associate those with an FCS file.

Select **Save Playlist As...** from the **VenturiOne Menu** to save a **Playlist** for future use.

To open an existing playlist file, you can use the **Open Playlist...** option, from the **VenturiOne Menu** **Open** option. You can also use Windows Explorer to drag a playlist file onto **VenturiOne**.

Asterisks are used when modifications have been made to workspace or compensation settings.

*Playlist items colored **red** are invalid and denote files which are not compatible with the current Workspace or compensation coefficients in the FCS file.*

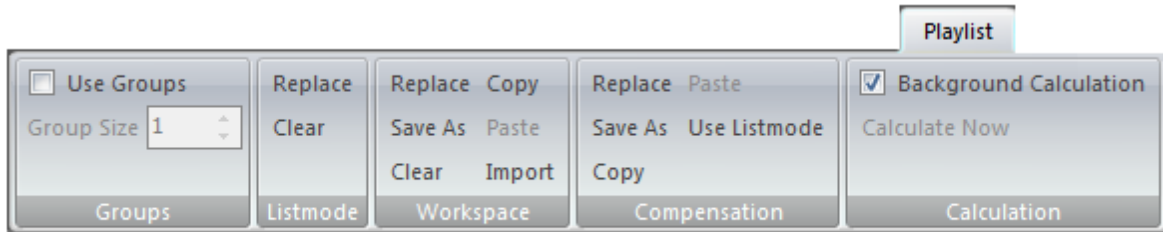
*Playlist items that have **orange** text have FCS files with different scaling information to their associated workspace file.*

Files will be automatically added to the playlist in logical file name order, if you want files to be added using date and time order that option is selectable in the VenturiOne Options dialog / Playlist page.

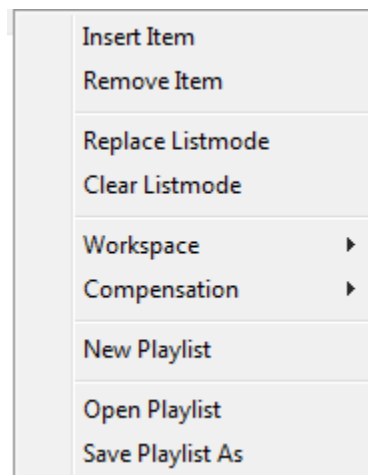
*Drag and drop items in the **Playlist** to reorder.*

- ✎ If you open an existing playlist, and the FCS files have been moved from their original location stored in the playlist, you can use the Repair Path option to point the playlist to the folder containing the files.

Other controls for the **Playlist** are available from the **Playlist** tab on the ribbon:



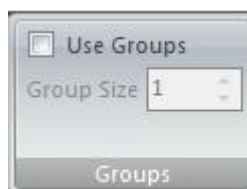
Right click on a playlist item to display a context menu from which most of these options are available.



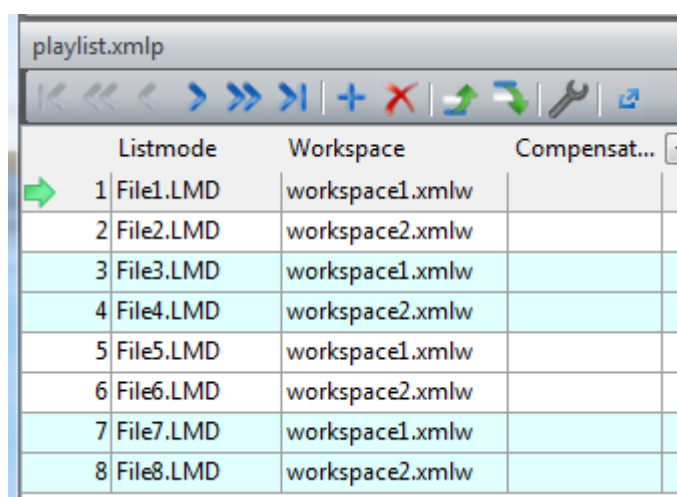
7 Playlist Groups

You can use groups in the **Playlist** to simplify the analysis and report of similar samples in your analysis.

To group samples, check the **Use Groups** checkbox on the **Playlist** tab and set the group size in the adjacent spin control:



Shading is used in the **Playlist** to show which files are grouped together as illustrated below:



	Listmode	Workspace	Compensat...
➡	1 File1.LMD	workspace1.xmlw	
	2 File2.LMD	workspace2.xmlw	
	3 File3.LMD	workspace1.xmlw	
	4 File4.LMD	workspace2.xmlw	
	5 File5.LMD	workspace1.xmlw	
	6 File6.LMD	workspace2.xmlw	
	7 File7.LMD	workspace1.xmlw	
	8 File8.LMD	workspace2.xmlw	

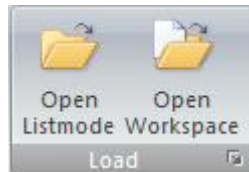
Apply the same workspace for corresponding files within a group to ensure that the plots required for the report are present for each sample.

8 Creation of Plots Using the Home Tab

The Home tab provides all the essential tools for creating a workspace to analyze data files.



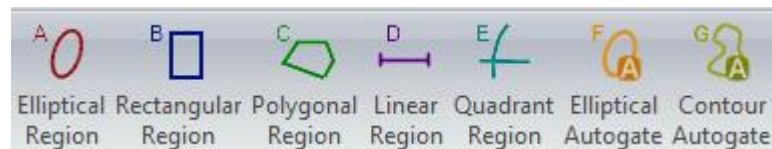
Use the Open Listmode button to select FCS files for analysis.



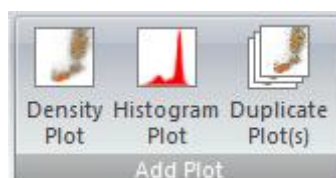
The Open Workspace button allows the selection of existing workspace files.

If Preview plots are not displayed a plot of the first two parameters will automatically be displayed in the **Plots** area.

Regions can be created and edited from the **Region** group on the **Home** or **Analyze** tabs. There are seven region types, as illustrated below:

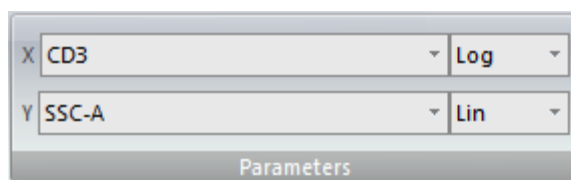


The **Add Plot** options are used to create additional density or histogram plots, the **Duplicate Plot** option allows you to duplicate an existing plot with any gating that has been applied to the plot.



Duplicating a plot with Cell Cycle analysis enabled will duplicate the plot only. Cell Cycle analysis will need to be enabled on the new plot if required.

Use the **Parameters** options to change the parameters of selected plots.



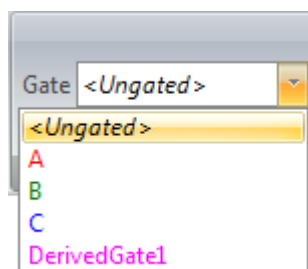
If multiple plots are selected all selected plots will be updated with the selected Parameter.

Individual plot parameters can be modified by right clicking on the parameter name.

The **Gate** options allow the selection of the gating criteria for the currently selected plots.



All existing gates and currently displayed regions will be available for selection from the drop-down list.



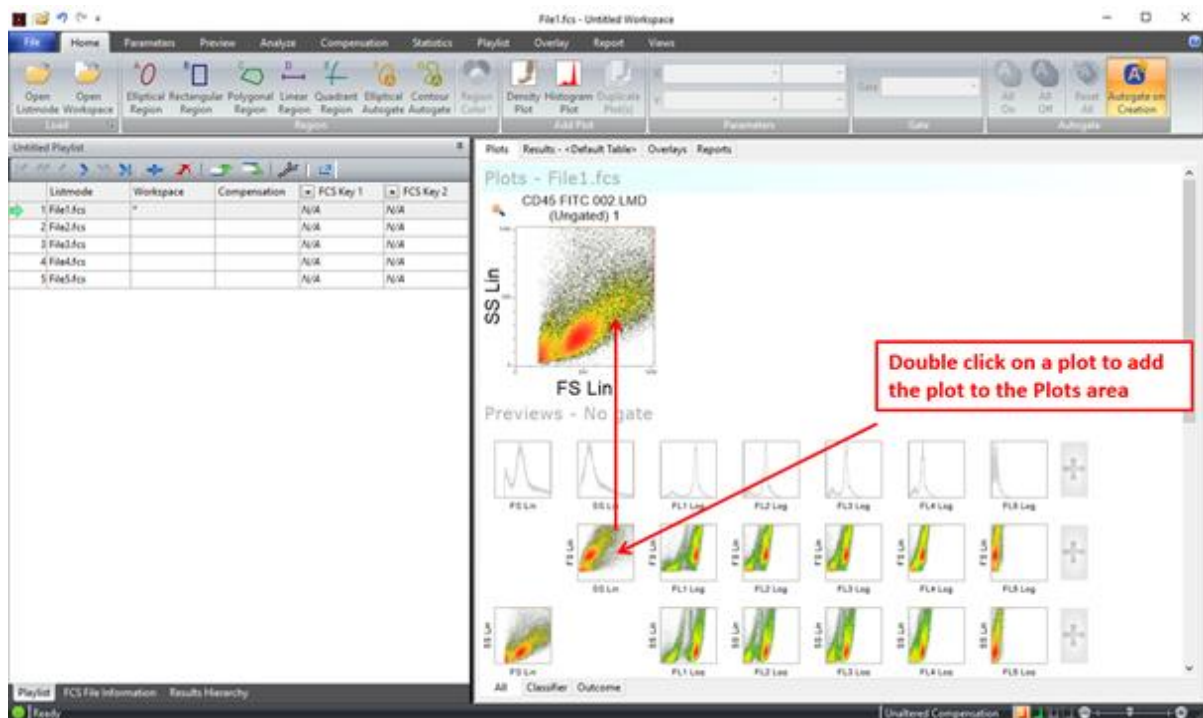
9 Preview Plots

Preview plots provide a fast way of creating a workspace.

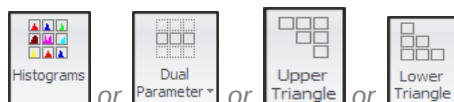
To display Preview Plots, make sure the **Preview** tab **Show Previews** option is enabled.

When an FCS file is opened, single and dual parameter plots are created for each parameter contained in the file. These are displayed in the **Previews** area of the **Workspace**.

Double click on a preview plot to add it to the **Plots** area for further analysis.

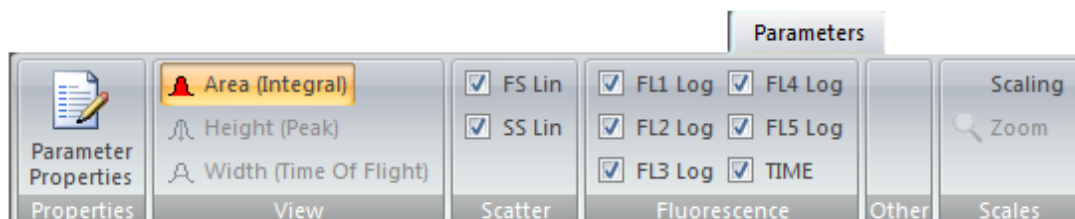


You can also add groups of preview plots with the **Add** buttons on the sides of each parameter in the **Previews** area.



Alternatively, select one of the buttons; **Histograms** or **Dual Parameter** or **Upper Triangle** or **Lower Triangle** from the **Plots** group in the **Analyze** tab to add the respective plots.

You can customize which parameters are displayed using the **Parameters** tab of the **Ribbon**. Check a checkbox to display the parameter; un-check it to not display it.



If the preview area displays parameters you do not wish to display, or does not show required parameters you can select or deselect individual or groups of parameters from those present on the **Parameters** tab.

Select the **Scaling** button on the **Parameters** tab to display the **Scales Properties** dialog. This controls the way your data appears including the setting of zoom values for each parameter. Zoom parameters by using the **Zoom** tool on the **Parameters** tab.

*Additional controls in the **Preview** group allow you to change the way VenturiOne displays parameters in the **Previews** area.*



10 Selection and Analysis of Plots in the Plots Area

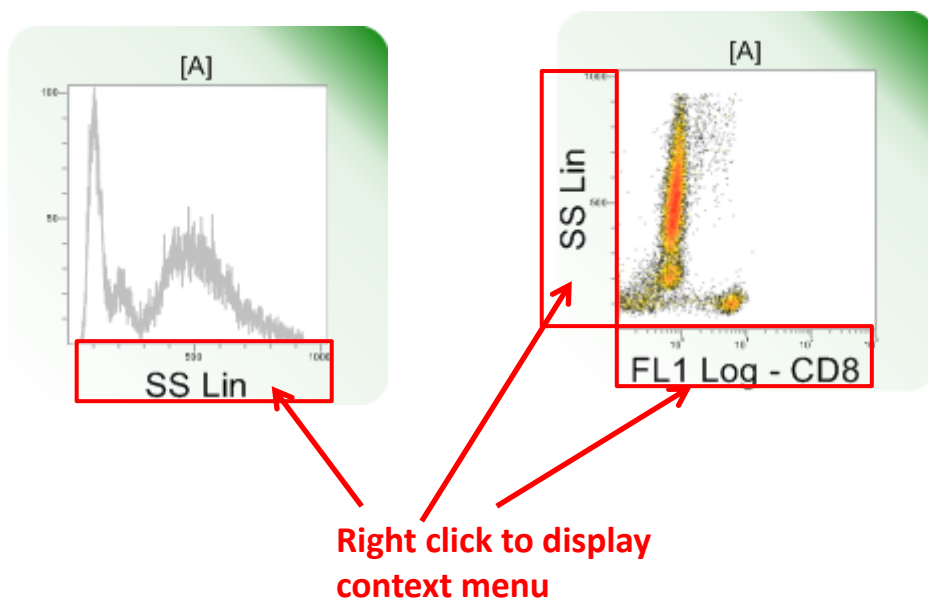
To analyze and modify the preview plots, they must be added to the **Plots** area.

To select a plot in the **Plots** area, click on the desired plot, multiple plots can be selected.

Press the **Delete** key to delete selected plots.

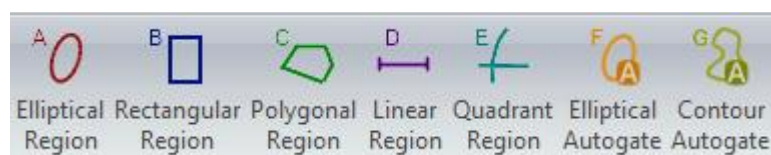
Double click the top margin of the plot to customize the plot title.

VenturiOne provides functionality for changing the parameters of the plots in the **Plots** area. You can right click anywhere in the area marked by the red rectangles as indicated in the figure below:



*You can rearrange plots within the **Plots** area by dragging and dropping the selected plot. A blue insert cursor highlights the new location of the plots.*

Regions can be created and edited from the **Region** group on the **Home** or **Analyze** tabs. There are seven region types as illustrated in the figure below:



To create a region on a plot, click the required region type to highlight the button. Then either click on a plot to set the region at the default size or draw out the required region size on the desired plot.

To cancel the creation of a region, press **Esc** before or during the dragging of that region.

When creating an autogate the rectangle you create will be used to determine the search area for a peak, the autogate will not move if the peak is outside the search area.

The controls on the Home tab allow you to control all autogating regions simultaneously.



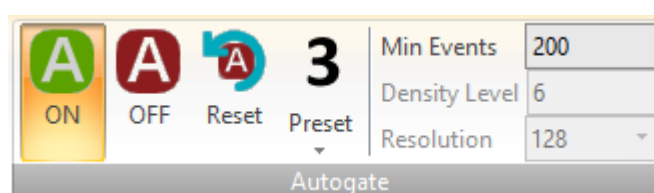
All On – Turns All auto gating regions on and recalculates their position.

All Off– Turns Off the automatic update for all autogates and allows manual adjustment.

Reset All – Turns Off all autogates and displays the search area for each autogate.

When the **Autogate on creation option** is active the autogate will automatically gate around the selected population. When inactive, the rectangular search area will be displayed.

Individual regions can be adjusted on the region format tab.



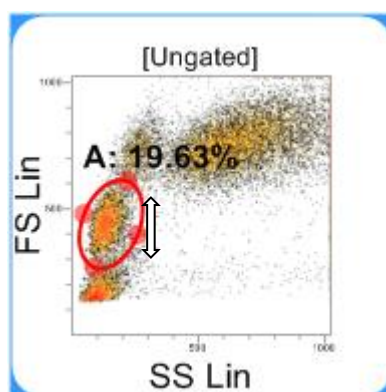
An autogate position cannot be modified without first turning the autogate function off for that region.

On – Turns the selected auto gating region on and recalculates its position.

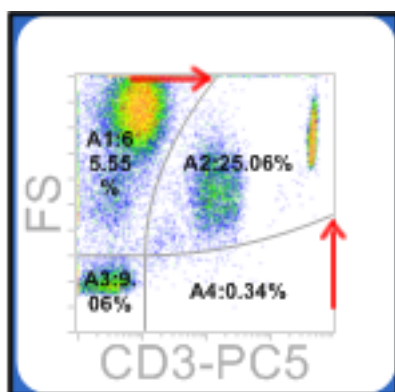
Off– Turns Off the automatic update for selected autogates and allows manual adjustment.

Reset – Turns Off the selected autogates and displays the search area for the autogate.

To edit the orientation, size and placement of regions, click on and drag the handles displayed on the selected region, as indicated by the cursor:



It is possible to create non-linear quadrant regions. Drag the end of each divider as shown below.

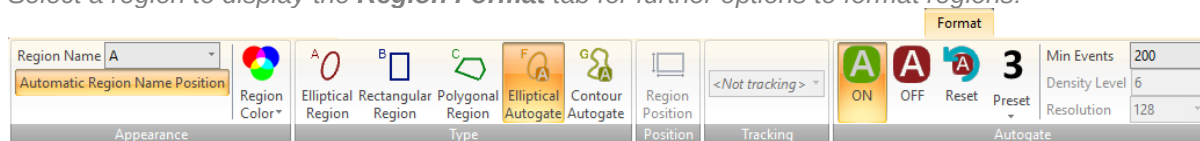


→ Denotes permitted direction of divider placement

When created, regions are given a default name and the name is displayed on the plot adjacent to the region. Regions are also assigned a default color. When a region is selected its name is displayed in the **Region Name** combo box and its color in the **Region Color** control.

To change the region name, select a default name from the drop-down list or type a name in the **Region Name** combo box. The color can be changed in the **Region Color** control. Both controls are located in the **Region** group of the **Analyze** tab.

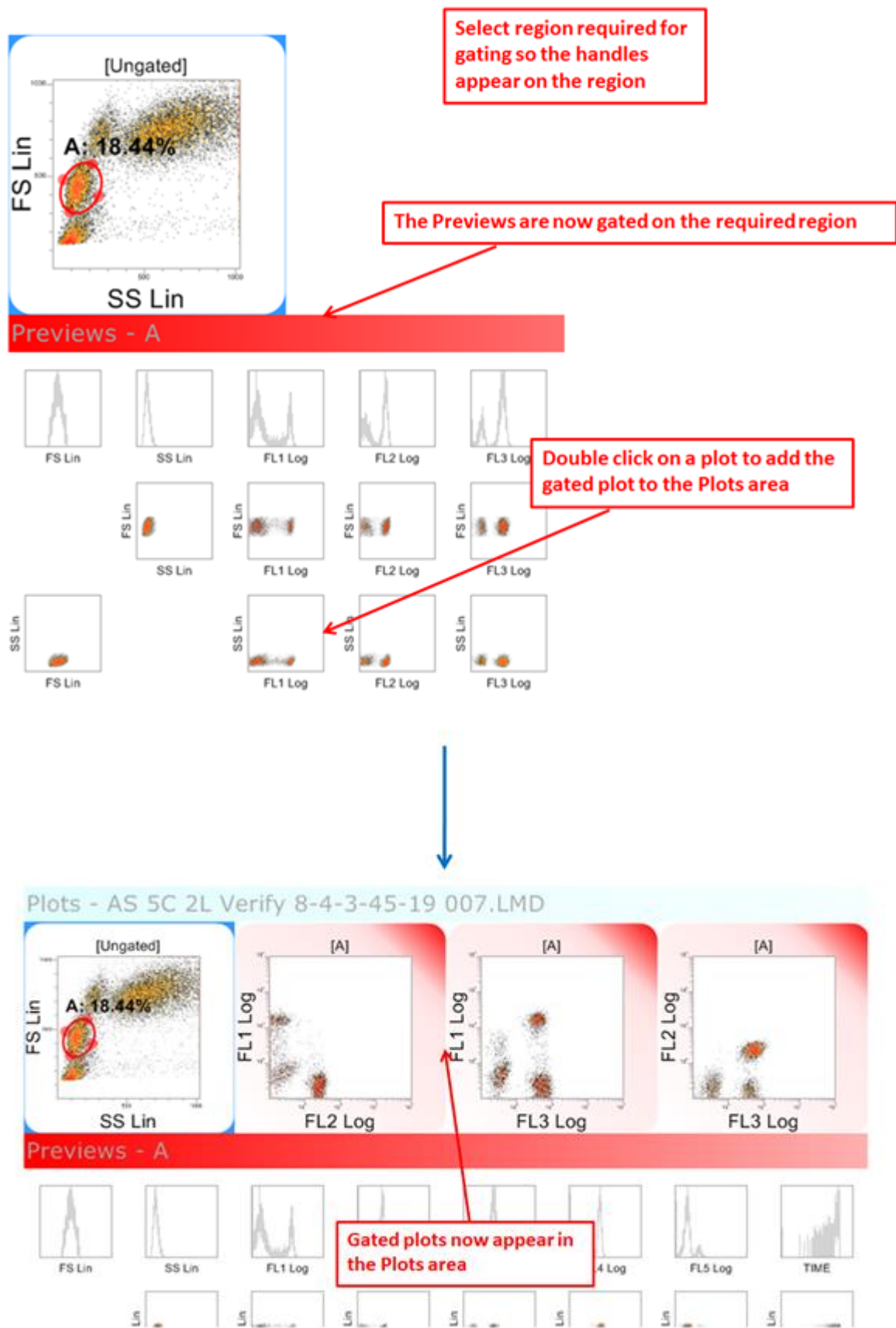
Select a region to display the **Region Format** tab for further options to format regions:



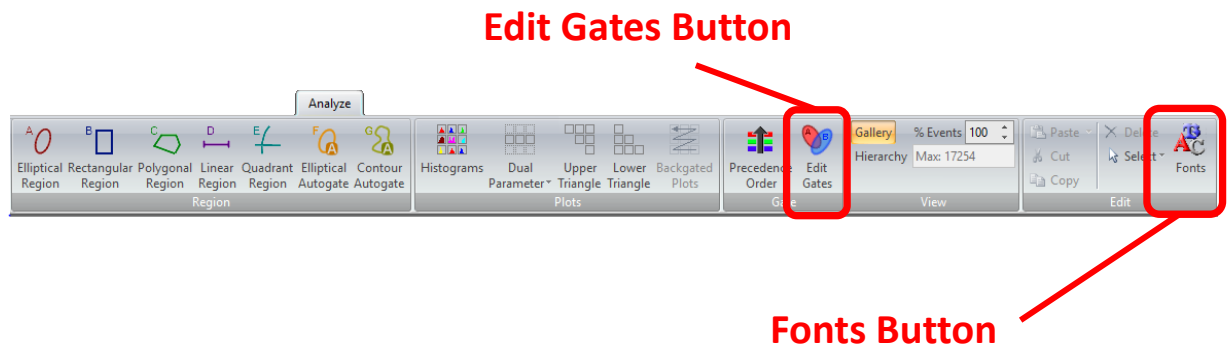
When a region is selected the plots in the **Previews** area become gated on the region. If you then double click on a preview plot, a gated plot is added to the **Plots** area.

When a gated plot is added to the **Plots** area, a gate associated with the region the plot is gated on, is automatically created. The gate has the same name and color as the region.

11 Creating Gated plots Using Preview Plots Area



To change the gate name and color select the **Edit Gates** button on the **Analyze** tab this launches the **Edit Gates** dialog.



Select the **Fonts** button on the **Analyze** tab to launch the **Edit Fonts** dialog. Here changes can be made to the font typeface and size for the plot titles, parameter names, axis scales, cell cycle names, region labels and statistics in the current Workspace.

12 Plot View Options – Hierarchy View

When you add plots to the **Plots** area they are added next to each other in a gallery format.

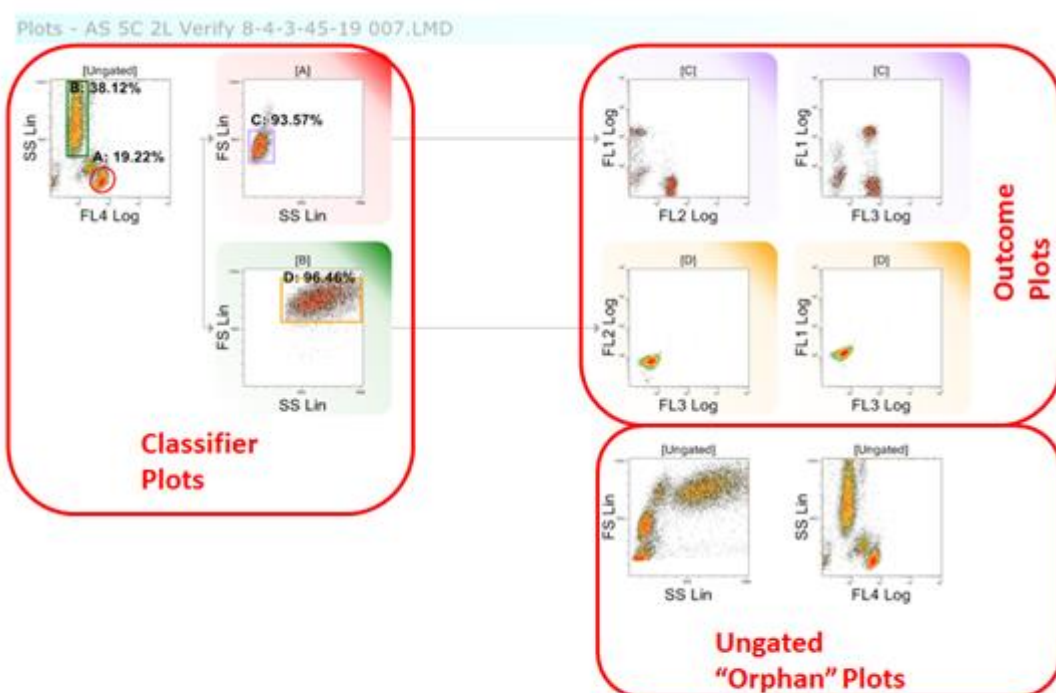
To change the display to a family tree style hierarchy format, click the **Hierarchy** button in the **View** group of the **Analyze** tab. In this format the plots are displayed in a tree-like structure with a separate branch for each gate. Select the **Gallery** button to return to the gallery plot view.

A plot with a region that is part of a gate equation is called a **Classifier** plot.

A plot that has no regions on it is called an **Outcome** plot.

A pathway is drawn between each classifier plot and between other classifier plots and the subsequent gated outcome plots,

Ungated plots with no gating regions drawn on them appear outside the hierarchy as orphaned plots.



13 Plot Format Options

When you select a plot or multiple plots, the **Plot Format** tab appears, this tab allows you to change the way plots are displayed in the **Plots** area.

Dual parameter plots in the **Plots** area can be displayed in one of the following formats:

- **Density** – plots are displayed as two-dimensional 256 x 256 bin histograms using color to represent the level of events within each bin
- **Color Precedence** - plots are displayed as two-dimensional 256 x 256 bin dot plots using color to represent the gating events that fall within bins on the plots
- **Precedence Density** - plots are displayed as color precedence plots incorporating density information

Single parameter linear plots in the **Plots** area can be displayed in one of the following formats:

- **Histogram** – plots are displayed as a standard 1024 channel histogram format
- **Cell Cycle** – plots are displayed with cell cycle results calculated.

To change the format of a plot, use the options in the **Plot Type** group of the **Plot Format** tab.



*Other options to format plots are also available on the **Plot Format** tab; these include the ability to change the gating and scaling of a plot. Histogram smoothing can also be applied here.*

14 Results

You can view the statistics associated with your analysis in the **Results** tab of the **Workspace**. The results table displays statistics from all the regions for all files in the **Playlist**.

Results										
Drag a column header here to group by that column.										
Filename	X Parameter	Y Parameter	Gate	Region	Count	%Gated	X Median	Y Median	X Mean	Y Mean
AS 5C 2L Verify 8-4-3-45-19 007.LMD	SS	FS	Ungated	A	2652	9.70	158.00	449.00	163.97	445.09
AS 5C 2L Verify 8-4-3-45-19 007.LMD	SS	FS	Ungated	B	5499	20.11	627.00	751.00	627.92	750.15
AS 5C 2L Verify 8-4-3-45-19 007.LMD	FL2 Log	FS	Ungated	C	12742	46.60	0.73	756.00	0.76	757.08
AS 5C 2L Verify 8-4-3-45-19 007.LMD	FL1 Log		Ungated	D	20726	75.80	1.31		2.31	

*If a **DNA Cell Cycle** plot is displayed additional DNA statistics are automatically generated.*

When you select the **Results** tab of the **Workspace**, the **Ribbon** automatically switches to the **Statistics** tab.



Only statistics for which check boxes on the **Statistics** tab are selected will be displayed in the results table.

Each statistic is displayed in a separate column in the table.

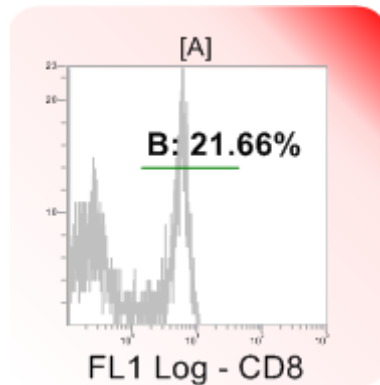
The name of the statistic is displayed as the column header.

Drag and drop the column headers to reorder the position of the columns.

Click the column header to sort the table by any column in an ascending or descending order.

Additional Statistics tables can be added, and the contents customized using the Filter Table option. When Exporting results only the results in the current results table are exported.

When on the **Plots** tab of the **Workspace** one statistic can be displayed on plots. The selected statistic is displayed on all regions and on all plots.



When you add statistics to a report (with or without the accompanying plot) the results selected in the **Statistics** tab are displayed on the report.

Results can be grouped using the column headers. Drag the column header from the results table onto the group control labeled **Drag a column header here to group by that column**.

15 Reports

VenturiOne can be used to create a customized page-based report of plots, statistics and other information.

In **VenturiOne** two types of reports can be created:

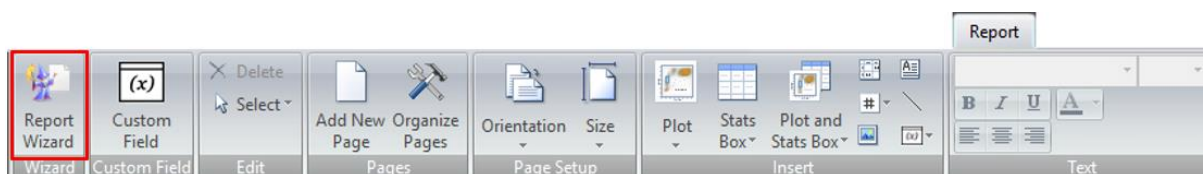
- **Playlist Report** – objects from any items in the **Playlist** can be added
- **Group Report** – use groups of samples to create a report.

Use groups to create a report for only one group of samples. This report is automatically used for all other groups in the **Playlist**. If the playlist report option is used it is necessary to specify which objects to add to the report for each item in the **Playlist**.

To generate reports with use groups, the same workspace should be applied to all files within a group to ensure that the plots required for the report are present for each sample.

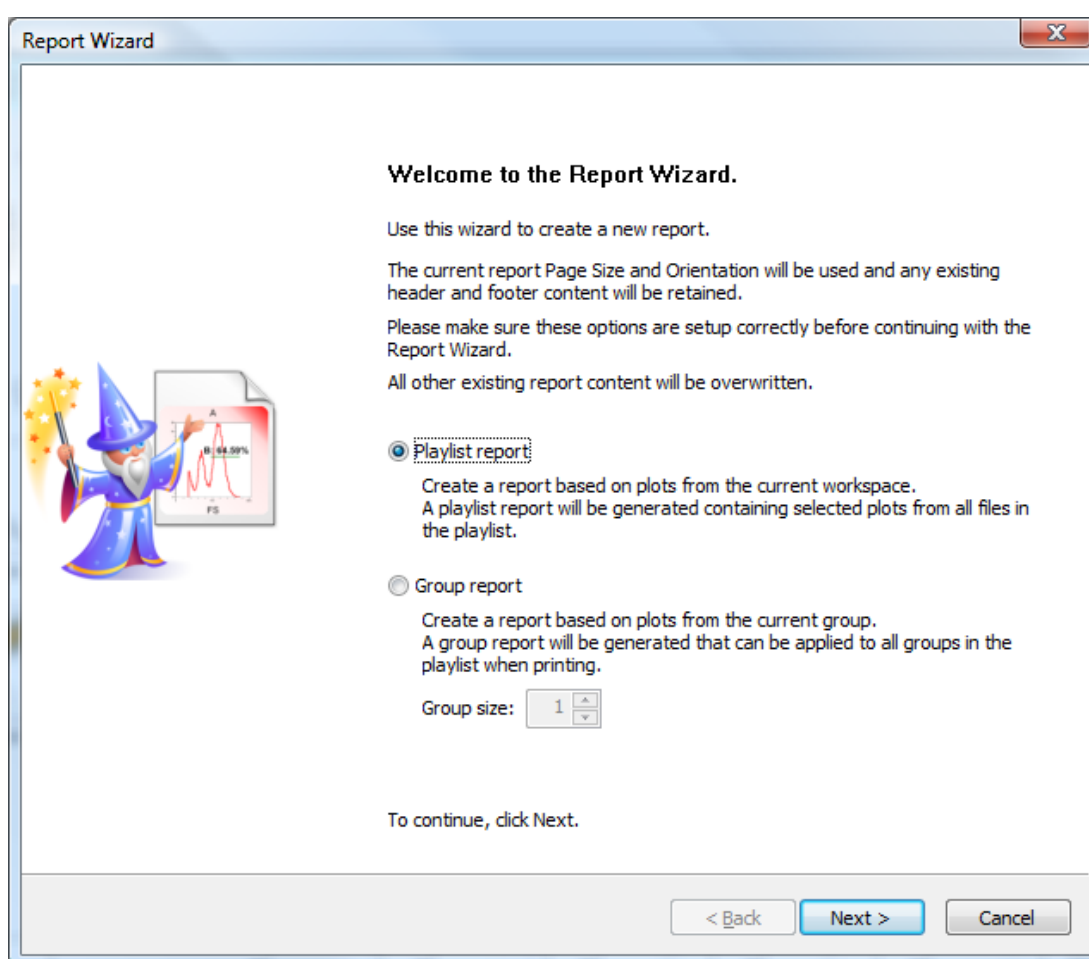
16 Create a Report with the Report Wizard

Create a report easily with the **Report Wizard**. To launch the wizard, click on the **Report Wizard** button located on the **Reports** tab of the **Ribbon**:



The wizard guides you through the creation of your report. Use it to create either a **Playlist Report** or a **Group Report**.

The first page of the **Report Wizard** is illustrated below:



The first page of the wizard allows you to decide if you want to use groups and set the group size.

The **Plots Selection** page contains a list of all the plots available in the current group or only the current workspace if you are not using groups.

Report Wizard

Plots Selection
Select the plots and the order in which they will appear on the report.
Highlight a row to display a preview of the plot.

☒	File No.	X Parameter	Y Parameter	Gate
☒	1	SS Lin	FS Lin	Ungated
☒	1	FL3 Log- CD45		Ungated
☒	1	FL4 Log - CD19		Ungated
☒	1	FL1 Log - CD8		A
☐	2	SS Lin	FS Lin	Ungated
☐	2	FL3 Log- CD45		Ungated
☐	2	FL4 Log - CD19		Ungated
☐	2	FL1 Log - CD8		A

Plot Preview:
[A]
B: 21.66%
FL1 Log - CD8

Plot Order:
▲ ▼
Reset

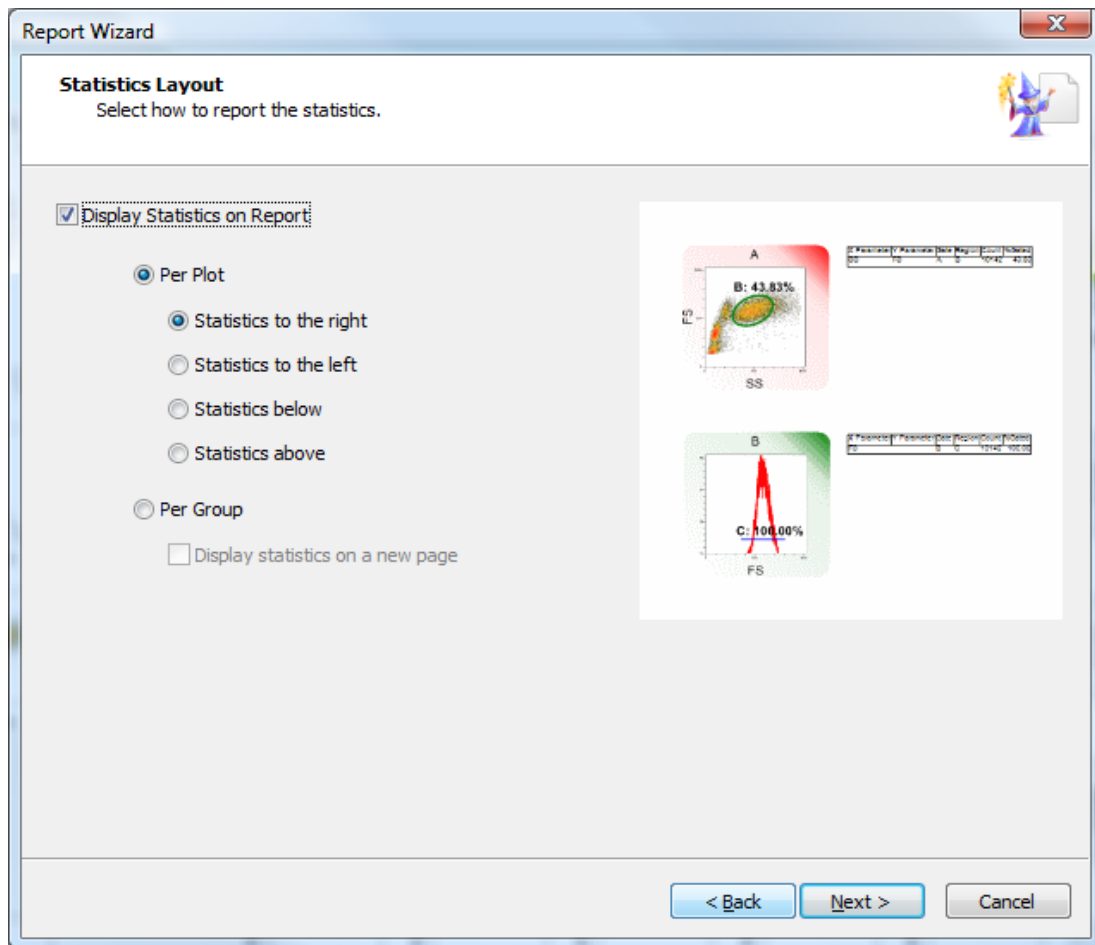
Plot Selection:
Select All Select None
☐ Show only selected plots

< Back Next > Cancel

Select the check boxes for all the relevant plots you wish to add to the report.

Other controls on this page allow you to change the plot order and select/deselect all plots.

On the **Statistics Layout** page of the wizard you can choose if and how you would like statistics to be reported. To report statistics, check the **Display Statistics on Report** check box.

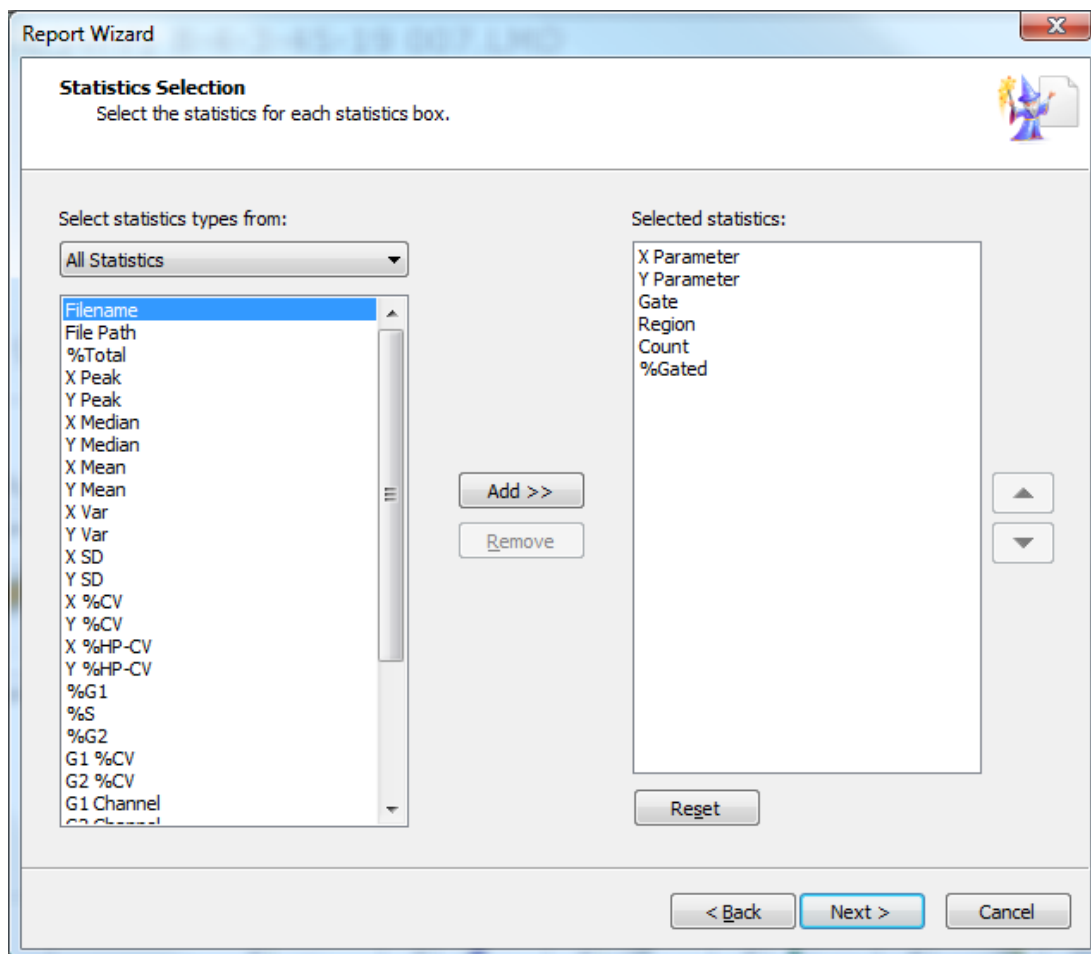


Choose **Per Plot** if you want the statistics for each plot to be displayed adjacent to it.

Choose **Per Playlist** (or **Per Group** if displayed) if you would like all the statistics for all plots displayed in a single table at the end of the report.

You can choose to display statistics above or below a plot, and to the left or right of a plot.

On the **Statistics Selection** page shown below you can select the statistics required for the report and the order in which they are displayed on the report. (The **Statistics Selection** page is skipped if you chose not to have statistics reported.)



Select statistics from the list on the left and use the **Add >>** button to add them to the **Selected statistics** list on the right to be reported. When all desired statistics are added, use the **Move Up** and **Move Down** buttons to arrange the statistics in the desired order.

*To delete statistics, click on one of the currently selected statistics and then click the **Remove** button.*

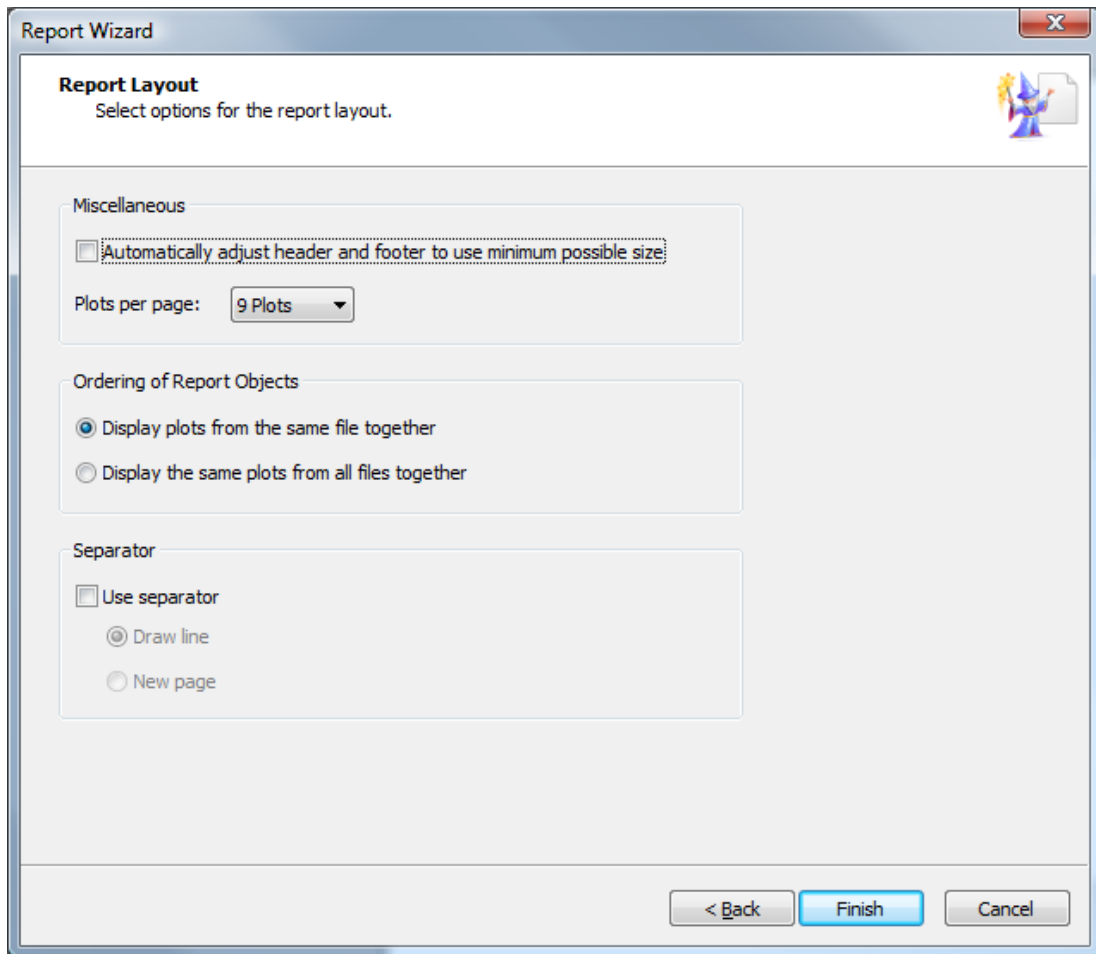
*Note: Instead of adding individual plot result tables a complete results table can be added to the report using the **Send Table To** option on the **Statistics tab** of the ribbon.*

The final page of the **Report Wizard** is the **Report Layout** page. Here you can decide how to lay out the plots in the report.

The desired number of plots per page can be selected and also if plots should be arranged by file or by plot.

Select **Display plots from the same file together** to group plots from the same file together, this is the default option.

Selecting **Display the same plots from all files together** will cause plots with the same parameters from all files to be grouped together.

The image shows a screenshot of the 'Report Wizard' dialog box, specifically the 'Report Layout' tab. The title bar says 'Report Wizard' with a close button. The tab is labeled 'Report Layout' with a subtitle 'Select options for the report layout.' and a wizard icon. The dialog is divided into three sections: 'Miscellaneous', 'Ordering of Report Objects', and 'Separator'. In the 'Miscellaneous' section, there is a checkbox for 'Automatically adjust header and footer to use minimum possible size' which is currently unchecked, and a 'Plots per page:' dropdown menu set to '9 Plots'. In the 'Ordering of Report Objects' section, there are two radio buttons: 'Display plots from the same file together' (which is selected) and 'Display the same plots from all files together'. In the 'Separator' section, there is a checkbox for 'Use separator' which is unchecked, and two radio buttons: 'Draw line' (selected) and 'New page'. At the bottom right, there are three buttons: '< Back', 'Finish' (highlighted in blue), and 'Cancel'.

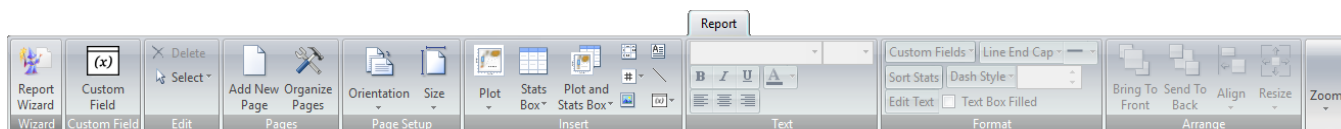
To maximize the space available for plots, select the **Automatically adjust header and footer to use minimum size** checkbox.

To force the report to use a new page for each set of plots or use a line to discriminate between sets of plots. By default, a continuous report with no separator will be created.

Click the **Finish** button to create the completed report which is displayed in the **Report** tab.

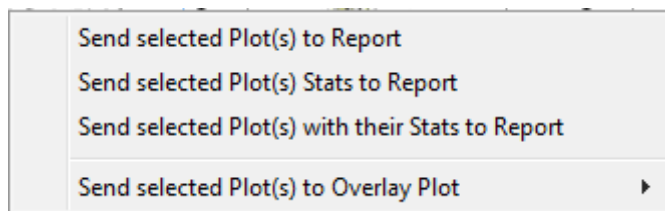
17 Modify a Report

To modify an existing report or manually create a new report use the options contained in the **Report** tab.



Add plots and statistics with the controls in the **Insert** group of the **Report** tab. Here also insert additional objects such as lines, text boxes and fields to the report.

When in the **Plots** area of the **Workspace**, 'send' objects from the **Workspace** to the report. Right click on a plot in the **Plots** area and use the context menu displayed:



Plots and statistics sent in this way are added to a new page at the end of the report.

18 Print a Report

To print the current report only, select the **Print** menu item in the **VenturiOne Menu**.

When using groups, to print reports for all groups select the **All Group Reports** option in the **Print what** drop down list of the **Print** dialog.

19 Creating a Custom Field

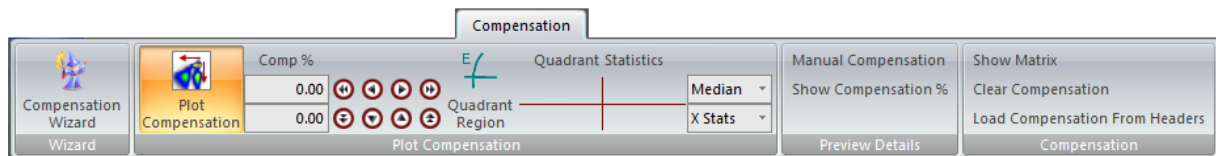
Custom Fields allow you to create calculated results and create rules based on standard results, calculated results or FCS headers. These sample fields, calculations and rules can be placed on a report, they dynamically update as new samples are loaded.

20 Compensation

VenturiOne allows you to obtain the correct compensation for a file in a number of ways. Compensation values can be restored from the FCS file header values (if the values were stored with the file). You can also calculate the compensation within the **VenturiOne** software and save these calculated values for future use.

The software automatically loads compensation values from the FCS file header.

Controls for compensation are located on the **Compensation** tab.



Use FCS files from compensation samples to calculate compensation values; these are usually in the form of single stained cells or beads.

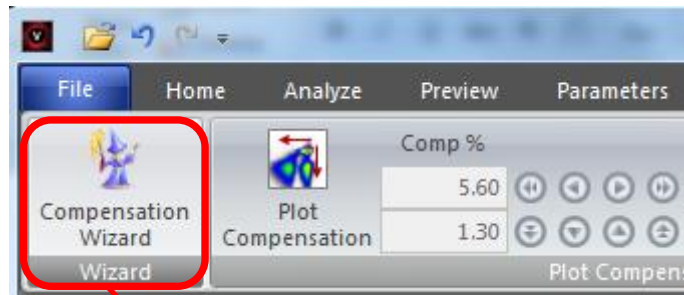
The ratio of a fluorochrome in its primary channel compared to the value in all other channels is used to calculate the compensation values.

For easy calculation of a compensation matrix **VenturiOne** provides the **Compensation Wizard**.

21 Calculate Compensation Values with the Compensation Wizard

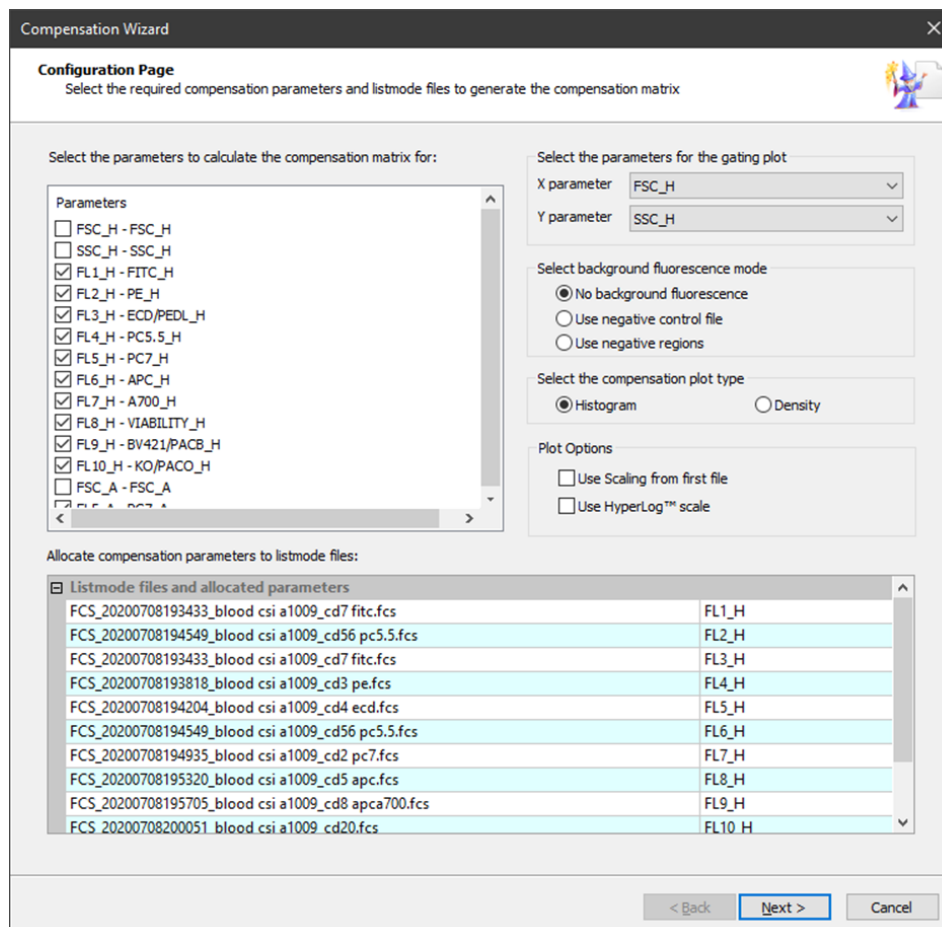
To begin, place the FCS files being used to calculate compensation in the **Playlist**.

To launch the wizard, click the **Compensation Wizard** button on the **Compensation** tab.



**Click to launch the
Compensation Wizard**

The first page displayed is the **Configuration Page** of the wizard.



All the parameters from the first FCS file in the **Playlist** are displayed in the **Parameters** list. All the compensatable parameters are checked by default. You can uncheck any parameters you do not wish to calculate compensation for.

Select which parameters are required to gate the plots (commonly Forward Scatter and Side Scatter). These can be selected from the list of parameters in the **Select the parameters for the gating plot** drop down lists.

To use a separate negative control sample to calculate the background fluorescence select the **Use negative control file** option.

Or alternatively to use negative cells within a positively stained sample, select the **Use negative regions** option.

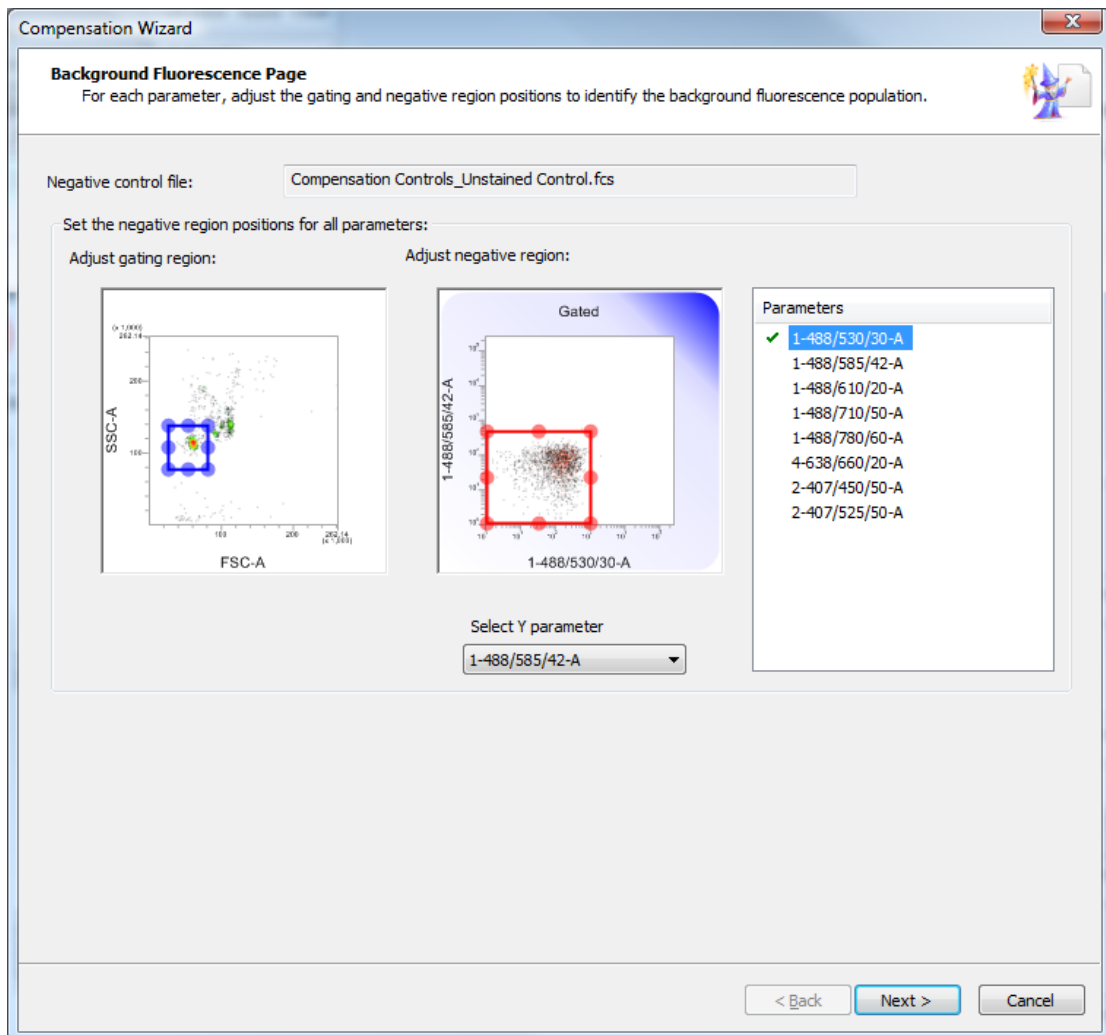
On this page the type of plot used for compensation calculations is selectable in order to clearly identify the target fluorescence population for each file. Single parameter **Histogram** format or dual parameter **Density** plots can be used.

By default, the plots in the compensation wizard will be displayed at the full range of the data. If you have customized the scaling of the workspace parameters, you can apply the parameter scaling settings from the first file in the playlist to all compensation plots by selecting the **Use Scaling from first file** option.

You can also select the **Use HyperLog scale** option to use HyperLog scaling in the compensation calculation plots.

Allocate the compensation parameters to each file from the **Playlist**; use the **Allocate compensation parameters to FCS files** list control. Click on a parameter stain name to view the list of all selected compensation parameters in the file and select the appropriate stain for the file.

The **Background Fluorescence Page** is only displayed if the **Use negative control file** option was selected.



On this page, move and/or resize the region on the **Adjust gating region** plot to gate on the correct population. Then adjust the background fluorescence region on the **Adjust negative region** plot.

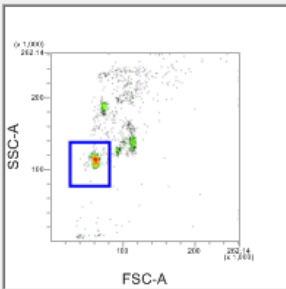
The next page of the wizard is the **Compensation Page**. It is displayed once for each compensation parameter.

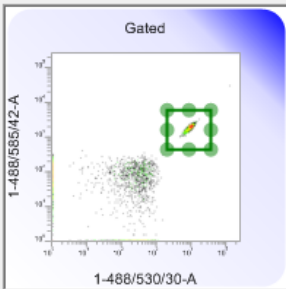
Compensation Wizard

Compensation Page
Adjust the gating and compensation region positions to calculate the compensation coefficients for this stain

Stain for compensation: 1-488/530/30-A

Stain listmode file: Compensation Controls_1-488,2f,530,2f,30 Stained Control.fcs

Adjust gating region: 

Adjust compensation and negative regions: 

Select Y parameter: 1-488/585/42-A

Calculated compensation matrix:

	1-488/530/30-A	1-488/585/42-A	1-488/610/20-A	1-488/710/50-A	1-488/780/60-A	4-638/660/20-A	2-407/450/50-A	2-407/525/50-A
1-488/530/30-A		0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-488/585/42-A	19.44		0.00	0.00	0.00	0.00	0.00	0.00
1-488/610/20-A	14.10	0.00		0.00	0.00	0.00	0.00	0.00
1-488/710/50-A	1.25	0.00	0.00		0.00	0.00	0.00	0.00
1-488/780/60-A	0.13	0.00	0.00	0.00		0.00	0.00	0.00

< Back Next > Cancel

First gate on the correct population, move and/or resize the region on the **Adjust gating region** plot. Then adjust the fluorescence region and the background region, if present, on the **Adjust compensation and negative regions** plot.

The fluorescence region should be adjusted to only include events that are stained just with the target fluorescence stain.

If the target fluorescence population is not clearly visible using the default parameters provided, if necessary change the Y-axis parameter to better visualize the population of interest.

The **Calculated compensation matrix** is dynamically updated as the regions are moved and compensation values are calculated.

Click the **Next** button to repeat the procedure for the next compensation parameter.

After all parameters have been compensated click the **Next** button to display the **Review Page**:

Compensation Wizard

Review Page

Review, print and save the compensation settings file

Compensation matrix:

	1-488/530/30-A	1-488/585/42-A	1-488/610/20-A	1-488/710/50-A	1-488/780/60-A	4-638/660/20-A	2-407/450/50-A	2-407/525/50-A
1-488/530/30-A		0.20	0.00	0.60	0.03	0.08	0.00	0.00
1-488/585/42-A	19.55		5.73	0.62	2.80	0.48	0.00	0.62
1-488/610/20-A	14.03	66.71		0.59	2.03	0.13	0.00	1.61
1-488/710/50-A	1.24	8.47	0.07		1.80	2.31	0.00	0.36
1-488/780/60-A	0.15	1.03	0.02	22.02		0.56	0.03	0.12
4-638/660/20-A	0.04	0.01	0.01	2.35	0.01		0.00	0.36
2-407/450/50-A	0.00	0.00	0.01	0.00	0.01	0.00		5.76
2-407/525/50-A	1.58	0.02	0.00	0.00	0.01	0.00	14.37	

Print...

Click 'Finish' to save the compensation file and to optionally apply it to the playlist

Compensation file:

Use the Browse button to select a destination for the compensation file

Browse...

☒ Apply compensation file to playlist

< Back

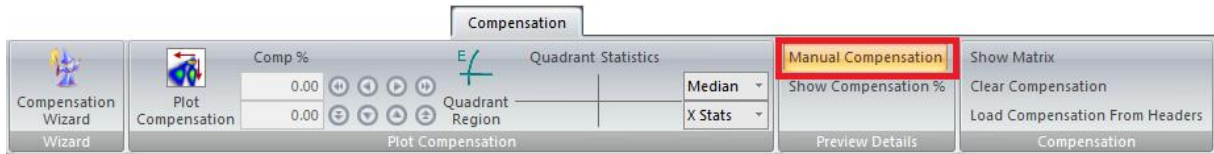
Finish

Cancel

From the **Review Page** you can save the calculated compensation matrix in a file for future use. You can also apply the saved compensation settings to the **Playlist**.

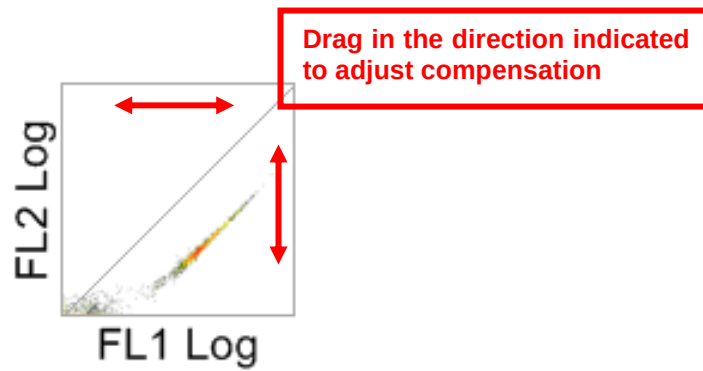
22 Manually Adjust Compensation Using the Preview Area

To adjust compensation manually using the Preview plots; first select the **Manual Compensation** button on the **Compensation** tab so it becomes highlighted:



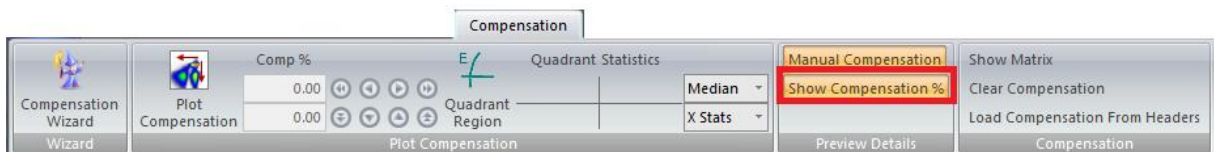
Then on the preview plots, drag populations to adjust the compensation coefficients.

The diagonal line from lower left to upper right distinguishes the two coefficient sections. Drag the population above the line in a horizontal direction to adjust one coefficient. Drag the population below the line in a vertical direction to adjust the other coefficient. The populations can be dragged to adjust compensation coefficients from 0-100%.

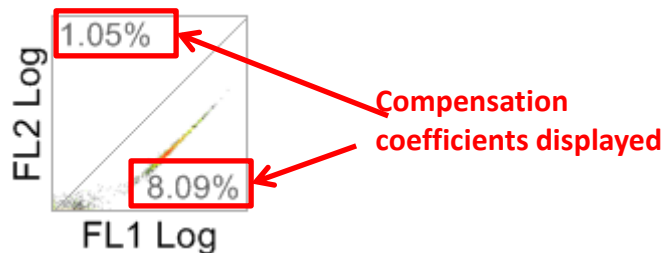


Only the current plot is updated during adjustment of compensation coefficients. When complete, all plots and preview plots affected by the change are updated.

To display the compensation coefficients on the preview plots select the **Show Compensation %** button on the **Compensation** tab.

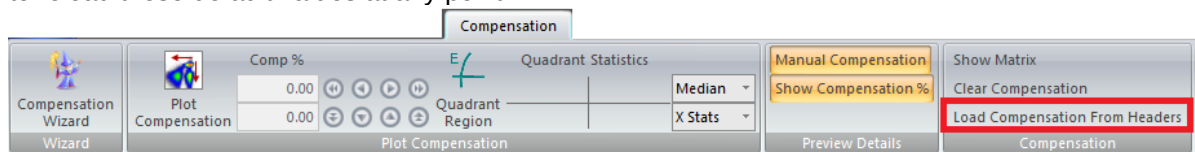


The compensation coefficients are now displayed on the plots in the **Previews** area as illustrated below:



To view the full compensation matrix select the **Show Matrix** button on the **Compensation** tab. To clear the compensation matrix and set all values to zero select the **Clear Compensation** button.

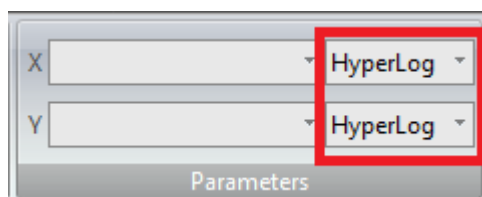
If the FCS file contains compensation values, select the **Load Compensation From Headers** button to reload these default values at any point:



23 HyperLog™ Scaling and HyperLog™ Sliders

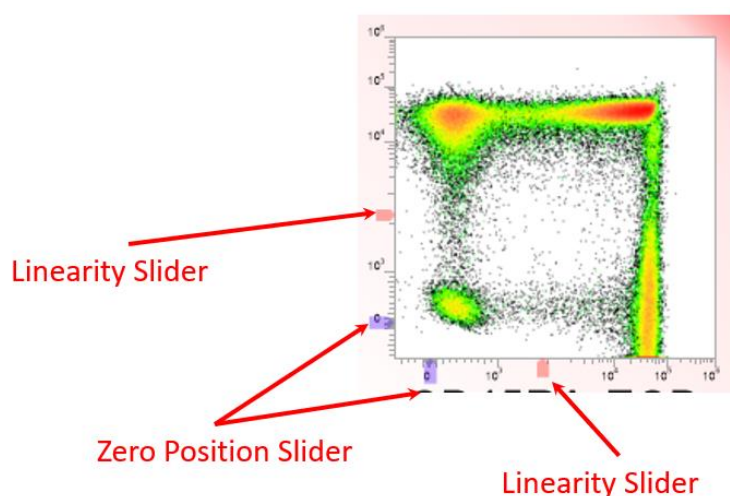
HyperLog scaling is a display that combines linear and logarithmic scaling allowing clearer definition of negative populations and aids the manual compensation process.

To display HyperLog scaling on a plot select one or more plots then select the HyperLog option from the Home Tab Parameters section for the X and / or Y axes. This will change the selected axes for all selected plots to display the HyperLog scaling.



When a single plot displaying a HyperLog parameter is selected, sliders appear which allow you to adjust the position of the zero point (and hence the number of negative decades displayed) and the Linearity.

Changing the value for a parameter changes the value for all plots displaying that parameter.



The Parameter Tab Scales Properties dialog allows the manual entry of Linearity and Negative section values.

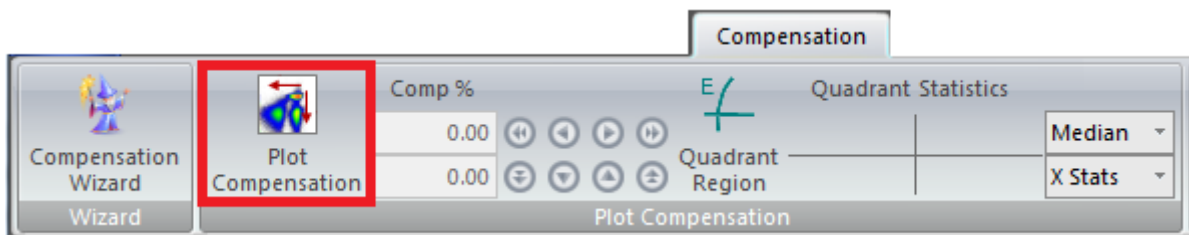
24 Manually Adjust Compensation on an Individual Plot

It is possible to compensate using plots in the Plots area rather than Preview plots if so desired.

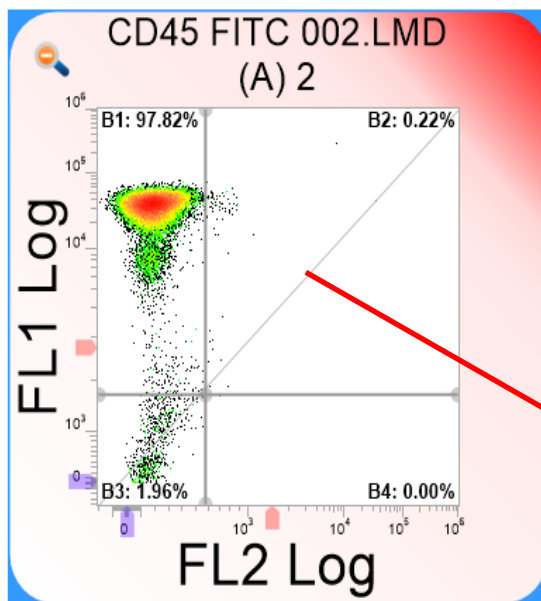
This method allows more accurate setting of compensation values, allows the manual editing of compensation values and allows quadrant statistics to be used to help set correct compensation values.

Ensure all plots that are to be used for compensation have quadrant regions drawn on them.

Select a plot in the **Plots** area that contains the parameters required for compensation and then press the **Plot Compensation** button on the **Compensation** tab.



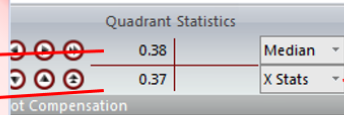
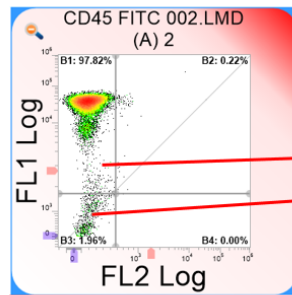
The currently selected plot will display a 45degree line to show compensation is active on that plot.



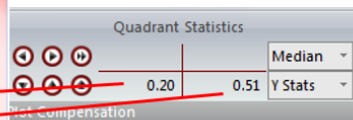
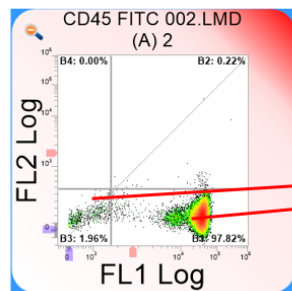
45deg line shows compensation is active for this plot

Select the statistic to display in the compensation **Quadrant Statistics** boxes, Mean or Median can be selected.

Select the required combination of statistics to display, X or Y statistics can be displayed.

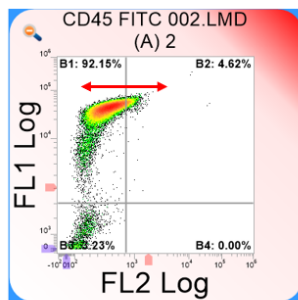


Select X-Stats to display the X-Axis statistics on Quadrant 1 and Quadrant 3 of the currently selected plot



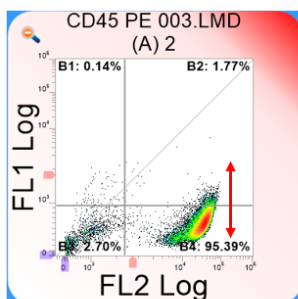
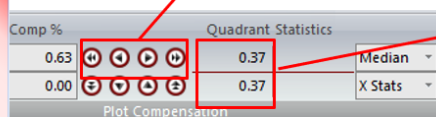
Select Y-Stats to display the Y-Axis statistics on Quadrant 3 and Quadrant 4 of the currently selected plot

The arrow buttons are used to move the required population into the correct position. This process is then repeated for each plot that compensation is to be adjusted.



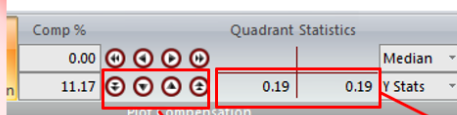
Use the left and right arrows to adjust the population in Quadrant 1

Adjust the population until the X-Axis statistics on Quadrant 1 and Quadrant 3 are the same



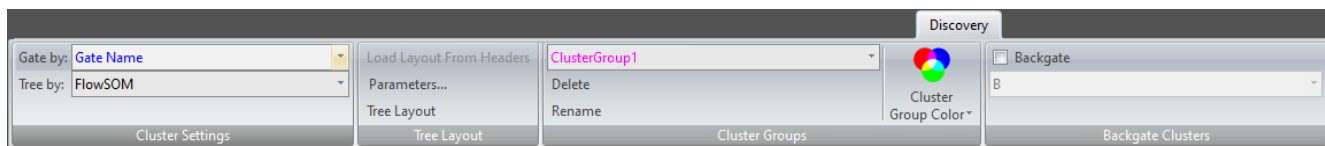
Use the up and down arrows to adjust the population in Quadrant 4

Adjust the population until the Y-Axis statistics on Quadrant 3 and Quadrant 4 are the same



25 Getting Started with Discovery

The **Discovery** tab of the **Ribbon** contains controls that allow you to pick a cluster parameter to display in the **Cluster Tree** display, pick a gate for the Cluster Tree display, control the tree layout, create **Cluster Groups** for display on the Cluster Tree and track populations from a gate on the standard plots on to the Cluster Tree by selecting a **Backgate**.

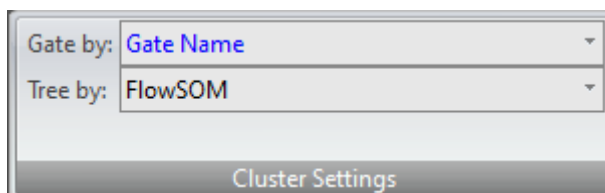


In order to function correctly the FCS files must contain an embedded cluster parameter (such as FlowSOM).

If your file does not contain such cluster parameters, you can create them using the Applied Cytometry CytoSwarm application.

For details of the CytoSwarm application see <https://www.appliedcytometry.com/software/cytoswarm/>

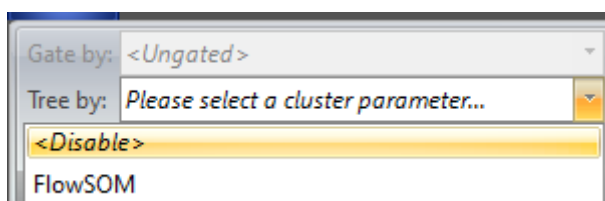
The Cluster Settings allow selection of the gating of the Cluster Tree plot and the Parameter to be used for display on the Cluster Tree plot.



The **Tree by** drop-down list displays potential Cluster parameters. The list may contain non cluster parameters as these cannot be automatically detected.

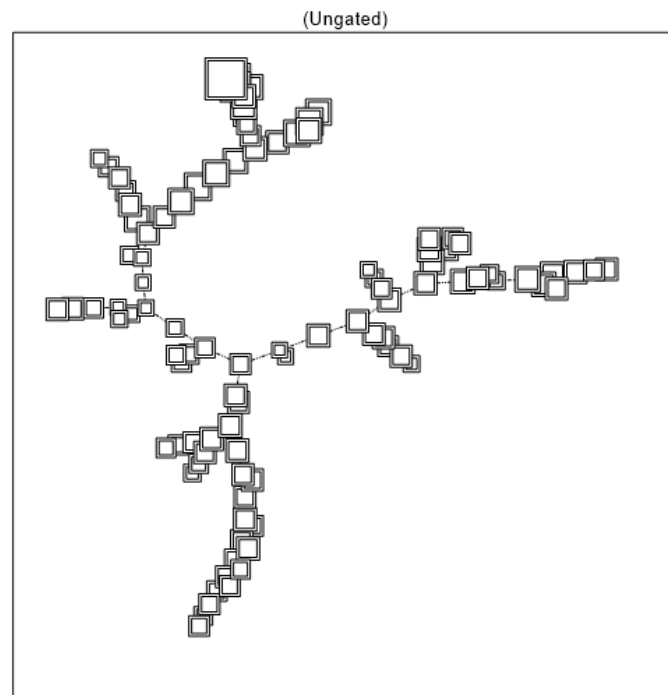
Select a suitable cluster parameter (such as FlowSOM or phengraph) from the list.

If no cluster parameters are listed the FCS file does not contain a recognized possible cluster parameter.

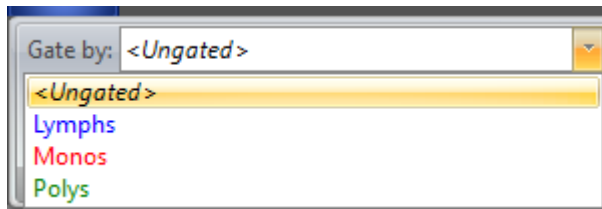


If you select a non-cluster parameter from the list, you may get unexpected results.

The selected cluster parameter will be displayed in the Cluster Tree plot.

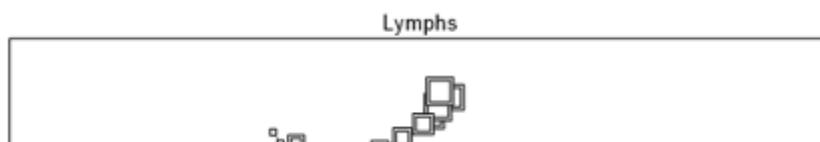


The **Gate by** option is only enabled when a cluster parameter has been selected in the **Tree by** control. Here you can select a gate from the current workspace to gate the **Cluster Tree** view.

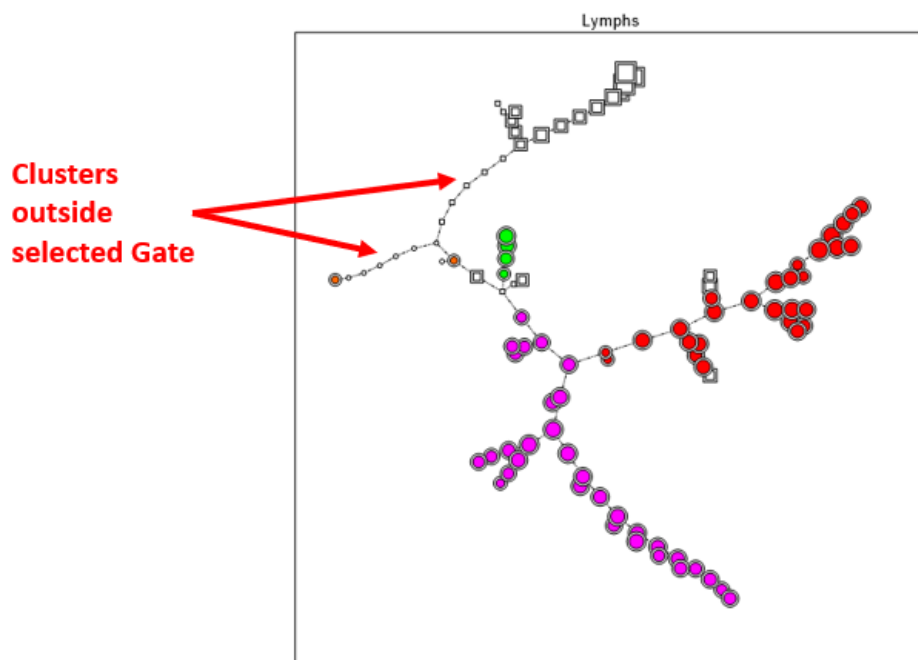


Select the **<ungated>** option to remove any gating from the Cluster Tree.

The name of the selected gate appears in the title of the cluster plot



Clusters not in the selected gate appear as small uncolored squares.



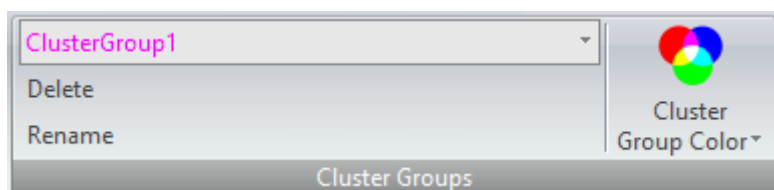
The layout of the cluster tree may be calculated by the original algorithm and may be stored within the FCS file. VenturiOne can also calculate positions for the clusters based on their properties.

Options in the Tree Layout group allow you to load the layout from the FCS file (if present) or recalculate the cluster links using selected parameters.

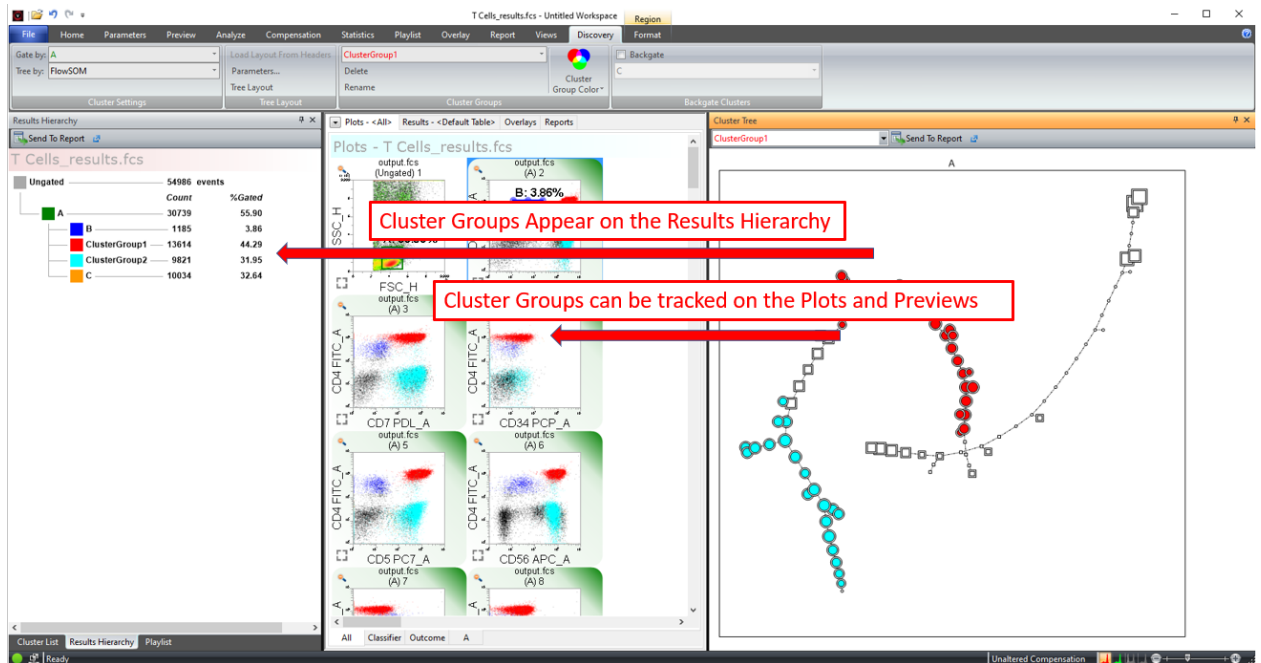
Note: Re-calculating the cluster tree will by default remove any defined cluster groups.

The **Tree Layout** button will create a new Cluster Tree based on the currently available clusters and cluster links. It creates a new layout for the existing information, all cluster groups and the selection of clusters associated with a group will be retained.

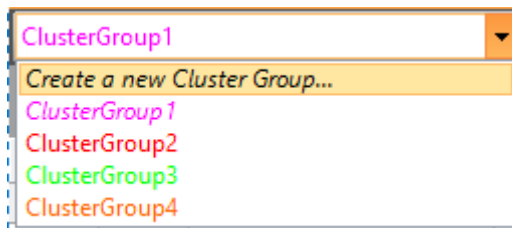
Cluster Groups can be created and modified using options in the Cluster Groups section of the Discovery tab.



Cluster Groups can be tracked by color or by gating using plots and Previews, Cluster Group statistics are displayed in the Results Hierarchy and the Results tab of the workspace



The **Cluster Group** drop down list allows you to select a cluster group to view or modify. Alternatively click the **Create a new Cluster Group** option to create a new cluster group.

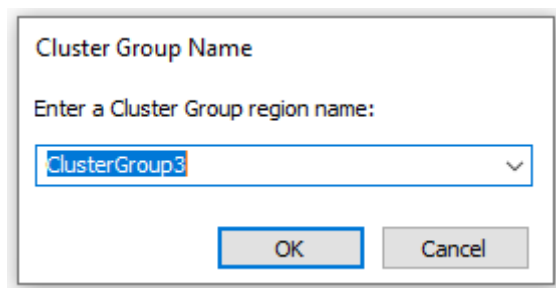


Clicking on a cluster, or dragging a selection area around a number of clusters within the cluster tree display adds that cluster to the currently selected cluster group.

Clicking on an already assigned cluster will remove the cluster from any cluster group.

This option will be disabled when the Cluster Plot is in back gating mode.

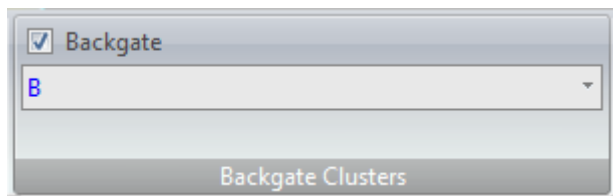
Click the delete button to delete the currently selected cluster group or click Rename to open the edit cluster name dialog, allowing renaming of the selected cluster.



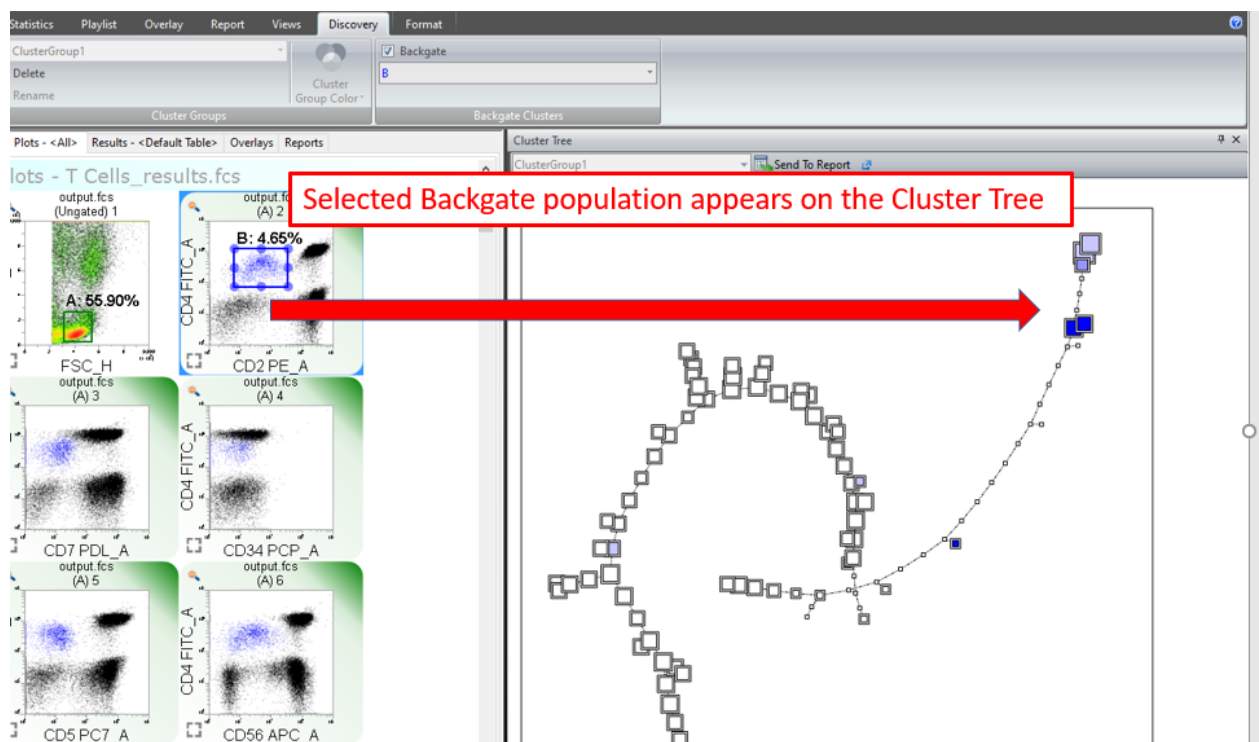
Click the **Cluster Group Color** button to open the color picker dialog, allowing selection of a new color for the selected cluster.



The Backgate option allows you to select a population from the plots area to display on the cluster tree.



Select the **Backgate** checkbox to enter backgate mode and select a gate from the dropdown list to display events within that gate on the cluster list. Only one gate can be displayed at a time.



Mar 2022