

Compensation Wizard

The **Compensation Wizard** simplifies the process of generating a full fluorescence compensation matrix.

The **Compensation Wizard** is launched from the **Compensation Wizard** button in the **Wizard** group of the **Compensation** tab:



The **Compensation Wizard** button is only enabled when there are at least two items in the playlist and there are no items that have no LMD file.

Initial Playlist setup

Before starting the **Compensation Wizard** you need to load all of the single stained samples and (if appropriate) the negative control sample into the **Playlist**.

For ease of use you should order the files with the negative control first, followed by the single stained samples in parameter order. E.g. FL1, FL2, FL3 etc. You will be able to re-allocate files later if required.

If a single LMD file contains more than one positive stain you must load in the file once for each stain present.

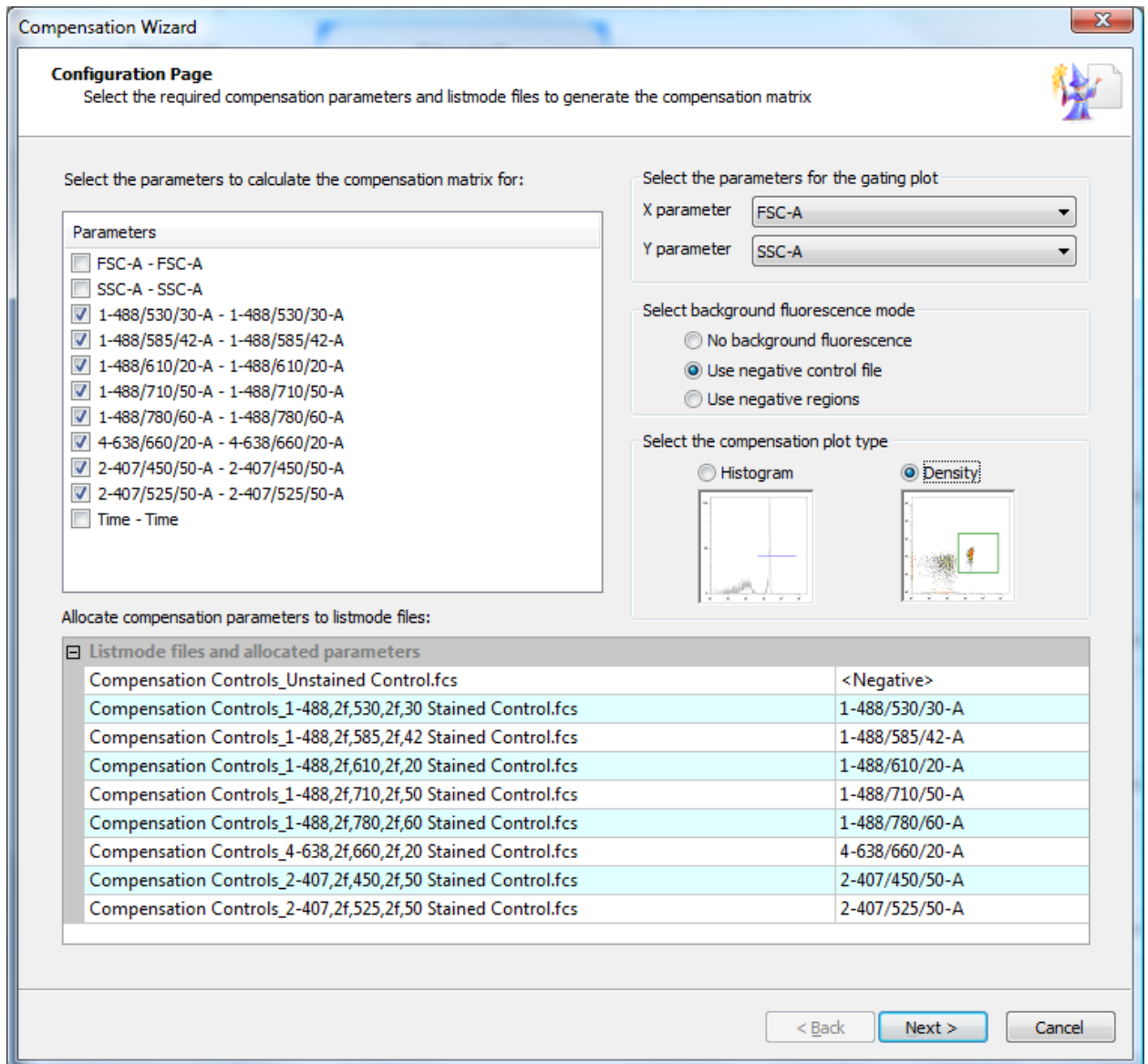
The wizard will allocate files based on the playlist starting from the first file. Additional files can be present in the Playlist but will not be recognized by the wizard.

Once the **Playlist** contains the required compensation setup files, click the **Compensation Wizard** button to launch the wizard.

Configuration Page

The first page of the wizard, the **Configuration** page, is displayed when the wizard is launched.

On this page you can select the parameters you wish to compensate and allocate an LMD file to each of them. You can specify how negative control values should be calculated. You can also define the plot type required for definition of positive and negative populations.



Parameters List

The **Parameters** list displays all the parameters from the first LMD file in the **Playlist** in the format Parameter Name (\$PnN) - Stain name (\$PnS).

Parameters identified as fluorescence are automatically checked. You can uncheck the ones you do not want to compensate.

Parameters that are not recognized as fluorescence parameters cannot be checked.

Gating Plot Parameter Selection

The **X parameter** and **Y parameter** combo-boxes allow you to select the parameters for the primary bead / cell Gating Plot. They are populated with all the parameters shown in the **Parameters** list.

Background Fluorescence Mode

The radio buttons in the **Select background fluorescence mode** group allow you to select whether a separate background fluorescence control is being used (select the **Use negative control file** option). Alternatively you can use negative events within a positive sample to determine the background for each sample (select **Use negative regions**).

If no background control is present select the **No background fluorescence** radio button.

Compensation Plot Type

You can select to use either histogram plots or dual parameter density plots for the definition positive and negative events for each parameter, by selecting the **Histogram** or **Density** options in the **Select the compensation plot type** group respectively.

Allocating Parameters to Listmode Files

The **Listmode files and allocated parameters** list allows you to allocate one of the parameters selected for compensation to each LMD file.

The Listmode files (left-hand) side of the list shows the files from the **Playlist**, in their current order, starting from the first file. The list contains one LMD file for each selected Compensation Parameter. If **Use negative control file** radio button is selected, the list also contains a file to be used as the negative control file. When the selection of parameters changes the list updates accordingly.

The allocated parameters (right-hand) side of the list shows the allocated Compensation Parameters.

Each row consists of a drop-down list. The drop down list contains all the fluorescence parameters from the Parameters list selected for compensation.

When the **Use negative control file** radio button is selected the drop-down list also includes an item 'Negative' at the top.

<Negative>
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

Selecting an item from the drop-down list which is already selected for a different file clears the setting for that file.

The default allocation will be the Negative Control file first (if selected) followed by the selected parameters in the order of the **Parameter** list.

If the **Listmode files** list is updated (parameters selection or changing the **Use negative control file** radio button selection) the allocated parameters return to their default allocations.

Next button

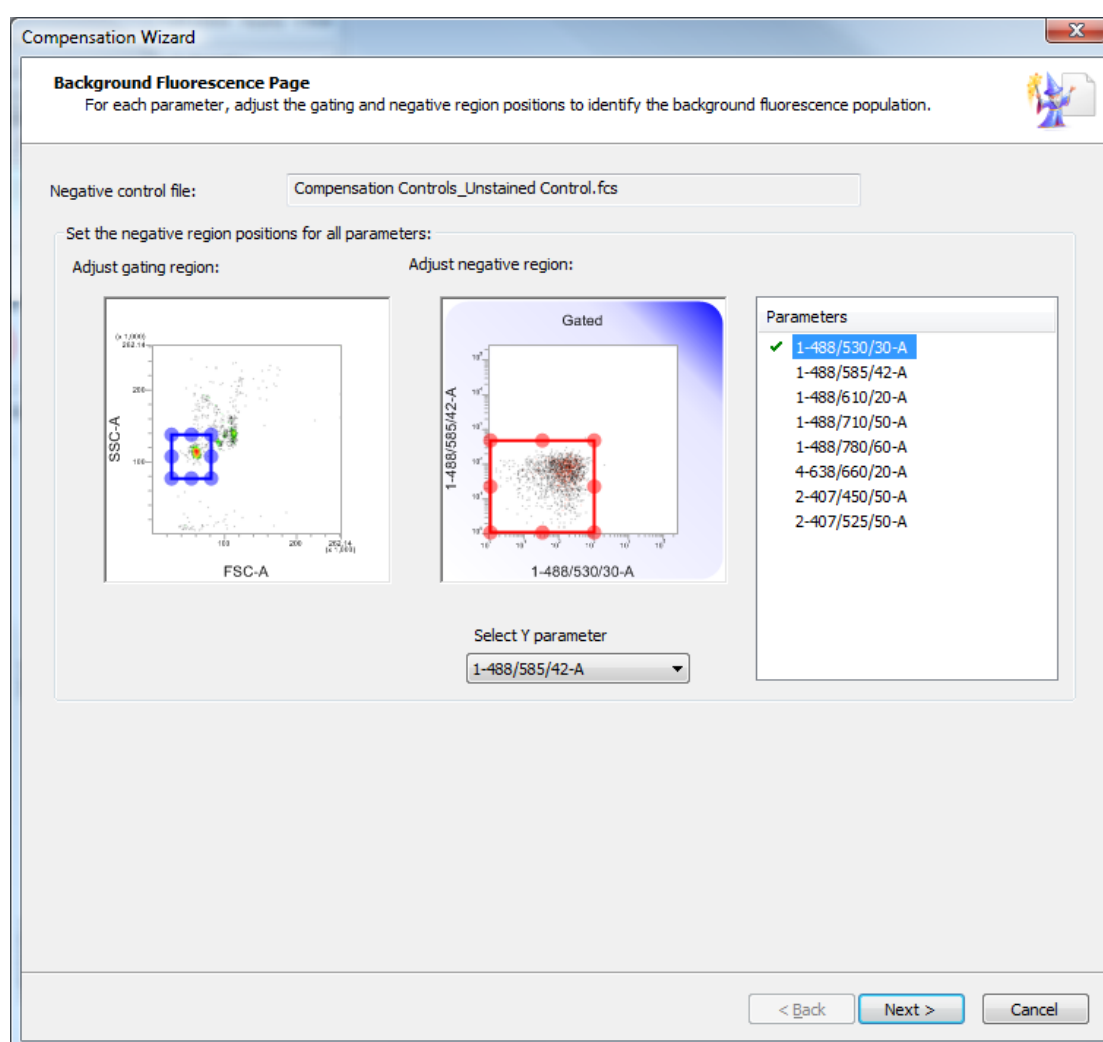
Select the **Next** button to proceed to the next page of the wizard. If the **Negative control file** option is selected the next page is the **Background Fluorescence** page, otherwise it is the first **Compensation** page.

If the selections made on the **Configuration** page are incorrect you will not be allowed to proceed to the next page and an appropriate error message will be displayed.

Background Fluorescence Page

The **Background Fluorescence** page of the **Compensation Wizard** is only displayed if the **Use negative control file** option is selected on the **Configuration** page.

On this page you will define a default gate position for subsequent pages, and then use a region to define the negative events for each parameter.



The **Negative control file** field displays the name of the negative control LMD file that will be used for the background fluorescence computation.

Gating Region Plot

The gating region plot is an ungated dual parameter density plot. The X and Y parameters on the plot are those selected on the **Configuration** page.

The plot contains a single rectangular blue colored region.

You can move and resize the region as required. Any adjustment of the region automatically updates the background fluorescence plot.

Negative Region Plot

The negative region plot is used to define the negative population for the X parameter of the current plot. A region needs to be defined for each parameter to be compensated.

The plot will be either a histogram or dual parameter density plot depending on the selection on the Configuration Page. The plot is gated on the Gating region.

The plot contains a single region, colored red that can be moved and resized as required to define the negative events.

If the plot is a dual parameter plot you can change the Y parameter in the **Select Y parameter** combo box. The combo box is only visible when the negative region plot is a dual parameter plot.

Parameters List

The **Parameters** list contains all fluorescence parameters that are selected for compensation on the **Configuration** page.

The currently selected parameter is highlighted in the list.

Parameters which have had the negative region configured are indicated by a tick in the checkbox.

Next Button

If not all of the parameters listed in the **Parameters** list have been configured (checkbox ticked), then clicking the **Next** button will select the next un-checked parameter in the list.

When all parameters have been configured then the **Next** button will proceed to the first **Compensation** page of the wizard.

Compensation Page

The **Compensation** page will be displayed once for each selected 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:

SSC-A

FSC-A

Gated

1-488/585/42-A

1-488/530/30-A

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	0.00
1-488/585/42-A	19.44	0.00	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	0.00
1-488/710/50-A	1.25	0.00	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	0.00

< Back Next > Cancel

The **Stain for compensation** field specifies the stain that is being currently compensated.

The **Stain listmode file** field specifies the name of the listmode file used for the compensation calculation.

Gating region plot

The gating region plot is an ungated dual parameter density plot. The X and Y parameter on the plot are those selected on the **Configuration** page.

The plot contains a single rectangular blue colored region.

You can move and resize the region as required. Any adjustment of the region automatically updates the compensation plot and the **Calculated compensation matrix**.

Compensation plot

The compensation plot is a single or dual parameter plot depending on the selection on the Configuration Page. The plot is gated on the Gating region plot.

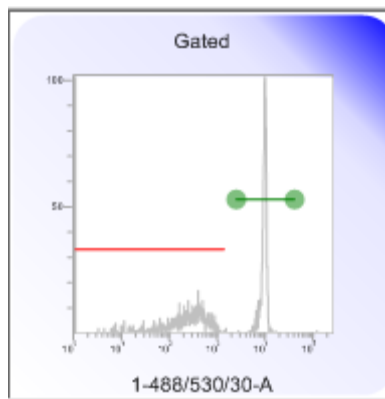
The X parameter on the plot is the current compensation parameter.

If the plot is a dual parameter plot you can change the Y parameter in the **Select Y parameter** combo box. The combo box is only visible when the compensation plot is a dual parameter plot.

The compensation plot contains a green colored region used to specify the positive population for that stain.

You can move and resize the region. Adjusting the region updates the **Calculated compensation matrix**.

If you have selected the **Use negative regions** option on the **Configuration** page the compensation plot also contains a red colored region which is used to specify the background fluorescence population.



You can move and resize the region. Adjusting the region updates the **Calculated compensation matrix**.

Calculated compensation matrix

The **Calculated compensation matrix** displays the currently calculated compensation coefficients.

The **Calculated compensation matrix** formatting is the same as for the **Compensation** dialog.

! The top and bottom channels of the plot are excluded from the calculations.

Back and Next Buttons

Selecting the **Back** button returns you to the previous page of the **Compensation Wizard**.

The **Back** button on the first **Compensation** page is disabled if the **Use negative control file** option was not selected.

Selecting the **Next** button proceeds to the next **Compensation** page or to the **Review** page when on the last **Compensation** page.

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

The **Compensation matrix** displays the completed matrix of compensation values.

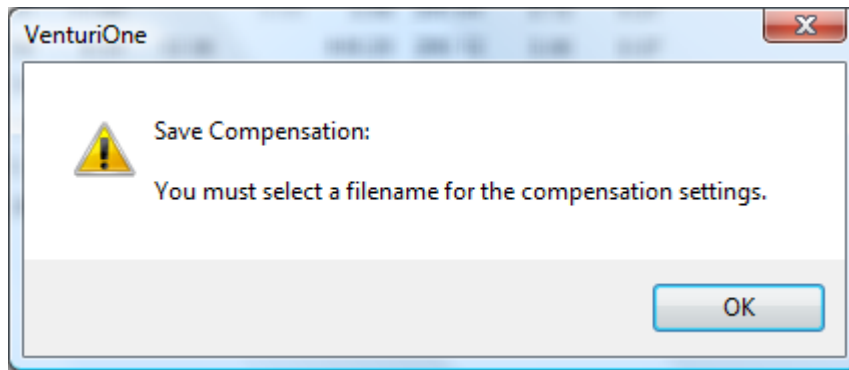
Click the **Print** button to print the compensation matrix.

The **Compensation file:** field displays the destination path and filename for the compensation file. Click the **Browse** button to specify the destination and name for the compensation file. The compensation file is not saved at this time.

Check the **Apply compensation file to playlist** checkbox if you wish to replace the current compensation values in the workspace with the newly calculated compensation coefficients.

Select the **Finish** button to complete the Compensation Wizard and save the compensation file with the specified name.

If no filename has been specified for the compensation file, a warning message is displayed when you click **Finish**:



Select **OK** on the message to close it and launch the Windows **Save As** dialog allowing you to specify a file name for the compensation file

If you then specify a valid filename in the **Save As** dialog, the **Compensation Wizard** will complete once you click **Save** in the dialog.