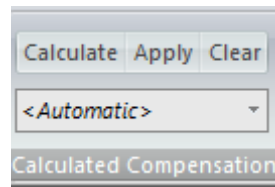


Calculating the Compensation Values

If you need to calculate the compensation matrix for a set of samples, the easiest way to do this is to use the **Compensation Wizard**.

If you need to use more complex gating than the **Compensation Wizard** allows then the **Calculated Compensation** group allows you to manually calculate the matrix.



Calculated Compensation Matrix

Compensation may be calculated by using a set of single stained LMD files.

Each LMD file should contain only one of the fluorochromes used in the experiment.

The value of a fluorochrome in its strongest channel is automatically determined and this is then used to compare to the values of that fluorochrome in each channel. The ratio created is then used to calculate the required compensation coefficient.

To generate a matrix follow the steps below:

- Load all the required LMD files into a single playlist.
- Open the first single-stained LMD file.
- Create a FS/SS dual parameter plot in the Plots area.
- Create a region around the required cells.
- Click on the region to gate the preview plots on this region.
- Click the **Calculate** button to calculate the compensation values for the current fluorochrome. These values will appear in the relevant column of the Calculated Compensation Matrix.
- Repeat these steps for each file and hence each fluorochrome.

The populations visible on the single parameter plots of the Preview area are used to calculate the compensation values. If there are more than one positive population present or if unstained cells are visible you must use further gating steps prior to calculation to ensure only the positive events from the correct fluorochrome are present in the preview plots before the **Calculate** button is selected.

5) Click Calculate button

1) Load all single color files into the Playlist

2) Create plots required for gating

3) Create region around population

4) Ensure Previews are gated on the region

As you proceed through each compensation sample the calculated compensation values will appear in the **Compensation** dialog; these can be viewed by selecting the **Compensation Calculations** radio button.

Compensation

Active Compensation
 Compensation Calculations

Print...
Size to Fit

	FL1 Log	FL2 Log	FL3 Log	FL4 Log	FL5 Log
FL1 Log	0.00	0.00	0.00	0.00	0.00
FL2 Log	14.72	0.00	0.00	0.00	0.00
FL3 Log	3.55	0.00	0.00	0.00	0.00
FL4 Log	0.48	0.00	0.00	0.00	0.00
FL5 Log	0.48	0.00	0.00	0.00	0.00

A completed Calculated Compensation Matrix, containing the compensation values for each stain, is illustrated below:

	FL1 Log	FL2 Log	FL3 Log	FL4 Log	FL5 Log
FL1 Log		0.72	1.06	0.78	0.41
FL2 Log	14.72		14.33	3.43	2.92
FL3 Log	3.55	25.95		2.09	0.97
FL4 Log	0.48	3.65	21.87		0.41
FL5 Log	0.48	0.89	6.10	25.71	

Once all coefficients have been calculated you can save the matrix in the form of a compensation file or use the **Apply** button to apply the calculated compensation values to any file in the playlist.

The calculated compensation matrix is lost if VenturiOne software is closed without saving a compensation file.

By default, the compensation applied to the currently opened file is the acquisition time compensation matrix from the current LMD file header.

Click the **Apply** button to apply the calculated compensation matrix to the current file.

You can select the **Active Compensation** option of the **Compensation** dialog at any point to view the currently applied compensation values.

Click the **Clear** button to reset the calculated compensation matrix.

	FL1	FL2	FL3	FL4	FL5
FL1		0.00	0.00	0.00	0.00
FL2	0.00		0.00	0.00	0.00
FL3	0.00	0.00		0.00	0.00
FL4	0.00	0.00	0.00		0.00
FL5	0.00	0.00	0.00	0.00	

This will only clear the calculated compensations values; the active compensation values for the current file will not be affected. To clear the active compensation values for the current file you should use the **Clear Compensation** button on the **Compensation** group.

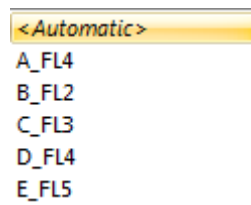
Compensation Region Combo Box

When calculating compensation values the value of a fluorochrome in its strongest channel is automatically determined and this is then used to compare to the values of that fluorochrome in each channel.

If the automatic selection is not the desired channel you can over-ride the automatic setting by creating a region around the correct population on a single parameter histogram, then selecting that region from the drop down list in the **Compensation Region** combo box.

The **Compensation Region** combo box is populated with all linear regions on fluorescence parameters.

Click the arrow to display a drop down list as illustrated below:



<Automatic> is the default selection.

! If there are more than one positive population present on the preview plots or if unstained cells are visible you must use further gating steps prior to calculation to ensure only the positive events from the correct fluorochrome are present in the preview plots before the **Calculate** button is selected.