Miltenyi Biotec



MACSQuant® Instrument short instructions

Compensation MultiColor Flex

Before using the instrument for the first time, read the MACSQuant Instrument user manual and MACSQuantify™ Software user manual.

These short instructions are valid for the MACSQuantify Software 3.1 and later. They guide the user through the automated multicolor compensation process to correct fluorescence spillover.

Express Mode CompensationMultiColor_flex

The automated multicolor compensation corrects spillover for every channel. The CompensationMultiColor_flex Express Mode expands the CompensationMultiColor Express Mode process and measures the whole uptake volume. It allows the user to adjust the automatically generated gating for each channel individually. This enables compensation of both cells and beads simultaneously, in the same experiment.

Sample material

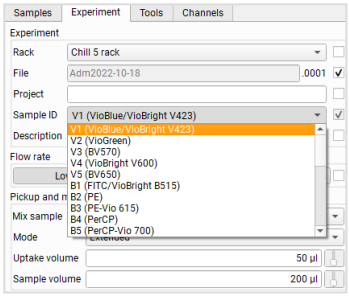
- Single-stained controls representing all fluorochromes used in the experiment. Use cells or compensation beads as sample material.
- One unstained sample as blank for all samples or a negative (unstained) population in each tube.
- Optional: one unstained sample for compensation of viability dye channel, for example, propidium iodide (PI).

Protocol

1 Click **File** > **New workspace**.
2 Open the appropriate instrument setting for the application.
3 In the **Experiment** tab, select an appropriate sample rack from the dropdown list.
4 To open the rack scheme, click the **Rack** button.
5 Select the appropriate number of sample positions to match the number of samples. Note the rack processing order indicated by the symbol in the upper left corner.
6 Click the **Group** button.
7 Place the samples in the rack accordingly.
8 From the **Experiment** tab, go to the **Settings** tab and select the **Express Mode** radio button.
9 Select **Setup** from the **Type** dropdown list.
10 Select **CompensationMultiColor_flex** from the **Mode** dropdown list.
11 Optional: Change the values for **Flow rate**, **Mix sample**, **Mode**, **Uptake volume**, or **Sample volume** as required.

When running the CompensationMultiColor_flex Express Mode, the entire uptake volume defined in the experiment settings is measured.

12 Select a single sample position in the **Rack** window. Select the channel that represents the fluorescent stain in the sample from the **Sample ID** dropdown list matching the order of the samples in the rack. The fluorochromes in brackets are based on the entries in the **Annotations** tab.

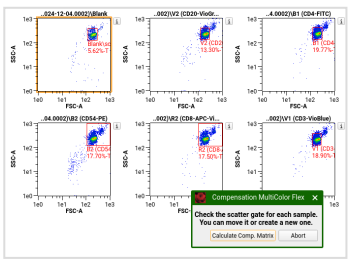


Ensure that all channels are selected in the **Channels** tab before starting an automated compensation. Do not deselect unused channels.

13 Optional: If a viability dye is used that only labels dead cell, select the respective viability channel from the Sample ID dropdown list. The spillover of other selected fluorescence channels is compensated out of the viability dye channel. Do not add the viability dye.
14 Optional: If one blank sample is used for all stained controls, select **Blank** from the dropdown list.

If different blanks are needed due to different sample material/autofluorescence, ensure that a blank and positive control is present in each sample.

15 Check the levels of MACSQuant Running Buffer and waste.
16 Click the **Run** button to start the acquisition.
Results are automatically shown on the analysis page. An analysis page is created for each compensation sample.



17 Review, modify, create, or delete the automatically created gates as needed. Ensure that there is one gate per compensation sample to allow clear identification of the relevant population.

Delete the existing gate and draw a new one as needed. The newly drawn gate can be of each shape, not only a polygon.

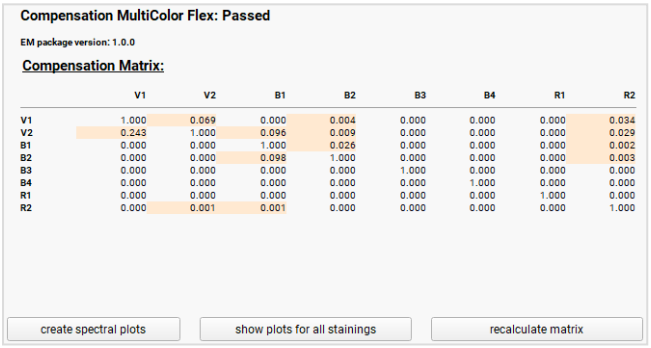
18 If needed to set the same gate for all regions of interest, first delete the gates in the individual plots, then set the gate on the selected plot. The gate appears in all plots.

19 Click on **Calculate Comp. Matrix**.

20 When compensation is completed, a dialog box opens to save the instrument setting. Enter a name for the instrument settings file and click **Save**.

Spectral plots

After completion of the CompensationMultiColor_flex the compensation matrix is shown in the analysis window. Applied compensation is highlighted orange in the matrix. See **Figure 1**.



	V1	V2	B1	B2	B3	B4	R1	R2
V1	1.000	0.069	0.000	0.004	0.000	0.000	0.000	0.034
V2	0.243	1.000	0.096	0.009	0.000	0.000	0.000	0.029
B1	0.000	0.000	1.000	0.026	0.000	0.000	0.000	0.002
B2	0.000	0.000	0.098	1.000	0.000	0.000	0.000	0.003
B3	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
B4	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
R1	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
R2	0.000	0.001	0.001	0.000	0.000	0.000	0.000	1.000

Figure 1: Compensation matrix

- To show spectral overlap for each channel, select **create spectral plots**. See **Figure 2**.
- To show spillover of one fluorescence channel against all other used channels, select **show plots for all stainings**. See **Figure 3**.
- To change the gate for all groups or selectively for a specific group and repeat the compensation, select **recalculate matrix**.

Create spectral plots

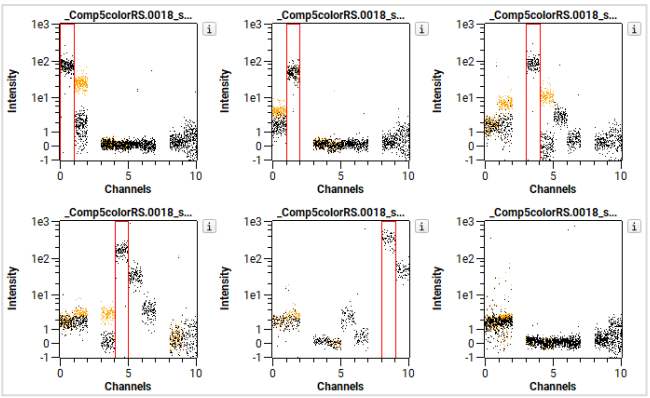


Figure 2: Spectral plots of a MultiColorCompensation_flex sample. The red frame highlights the color that had spillover correction out of other channels. Fluorescence channels used for MultiColorCompensation_flex Express Mode are designated in orange. The state before compensation is shown in orange; the state after applied compensation is shown in black

Show plots for all stainings

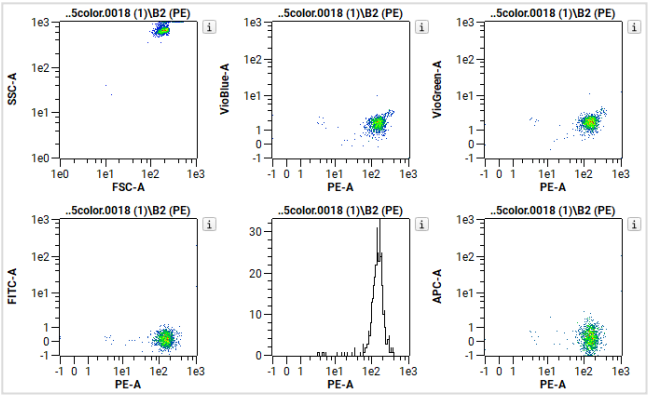


Figure 3: Plots of compensated PE channel against all other fluorescence channel used for MultiColorCompensation_flex on a MACSQuant Analyzer 10.

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