Miltenyi Biotec



MACSQuant® Instrument short instructions

Instrument settings and compensation

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.

Adjusting FSC and SSC gains

1. Ensure that the MACSQuant Instrument is in acquisition mode and calibrated.
2. Open a new analysis window or a saved analysis template.
3. Place a sample in the selected rack.
4. Click the **Run** button.
5. Adjust the gains for FSC and SSC in the **Channels** tab to bring the population of events into your desired area of the dot plot.
6. Optional: To refresh the events, click the **Clear** button on the instrument status bar.
7. Adjust the trigger threshold to minimize debris.
8. Continue to make adjustments until the population of interest is clearly defined.
9. Save as instrument setting with an appropriate name for recalled use.

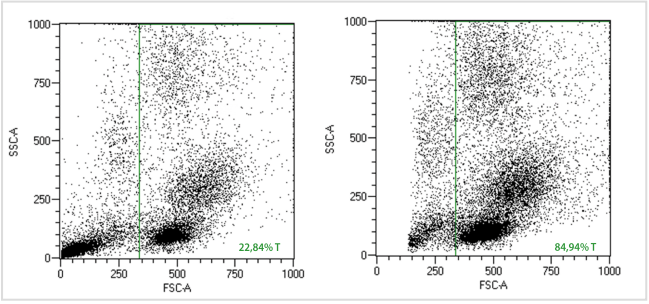


Figure 1: Adjustment of FSC and SSC PMT gains. Left: PBMCs prior to adjustment. Right: PBMCs after setting a trigger threshold to exclude debris.

Adjusting gains of fluorescence channels

1. Place a sample into the Single tube rack. An optimal sample should contain a negative and a positive population for each of the used fluorescence channels in the experiment.
2. Click the **Run** button.
3. Determine the position of positive and negative events. Optionally, draw a region around the appropriate population in the FSC versus SSC dot plot. Select the region from the dropdown list of the plot title in a fluorescence plot.
4. Increase or decrease the gains for the fluorescence channel in the **Channels** tab to have both negative and positive populations on scale, achieve an appropriate signal to noise, and ensure that the positive fluorescence signal is brighter in this channel than any spillover channel.
5. Continue with the next fluorescence channel.
6. Save as instrument setting with an appropriate name for recalled use.

Always adjust fluorescence channel PMT gains before running a compensation.

Express Mode CompensationMultiColor

The automated multicolor compensation corrects spillover for every channel.

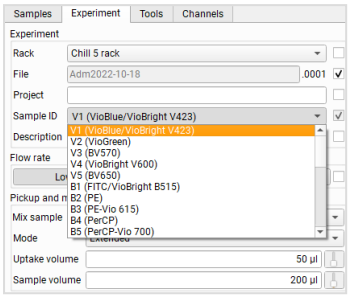
Sample material

- 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** from the **Mode** dropdown list.
11. Optional: Change the values for **Flow rate**, **Mix sample**, **Mode**, **Uptake volume**, or **Sample volume** as required.

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.

Once 5,000 events are measured, a pop-up window asking to draw your region of interest appears.

17 Draw a region around the population of interest. Include only populations that are relevant for compensation and data analysis. Click **OK**.

Spectral plots

After completion of the CompensationMultiColor, the compensation matrix is shown in the analysis window. Applied compensation values are highlighted orange in the matrix. See **Figure 2**.

Compensation MultiColor ExpressMode: Passed

EM package version: 1.0.0

Compensation Matrix:

	VioBlue	VioGreen	FITC	PE	PerCP-Vio700	PE-Vio770	APC	APC-Vio 770
V1	1.000	0.050	0.002	0.001	0.000	0.000	0.000	0.000
V2	0.302	1.000	0.060	0.007	0.000	0.000	0.001	0.000
B1	0.001	0.001	1.000	0.021	0.000	0.000	0.000	0.000
B2	0.000	0.000	0.140	1.000	0.000	0.000	0.000	0.000
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	0.001	0.000	0.000	1.000	0.000
R2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000

create spectral plots | show plots for all stainings

Figure 2: Compensation matrix

- To show spectral overlap for each channel, select **create spectral plots**. See **Figure 3**.
- To show spillover of one fluorescence channel against all other used channels, select **show plots for all stainings**. See **Figure 4**.

Create spectral plots

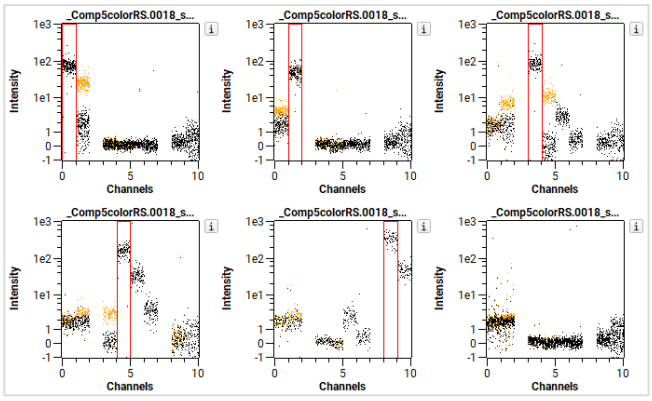


Figure 3: Spectral plots of a MultiColorCompensation sample. The red frame highlights the color that had spillover correction out of other channels. Fluorescence channels used for MultiColorCompensation Express Mode are designated in orange. The state before compensation is shown in orange; the state after applied compensation is shown in black

Show plots for all stainings

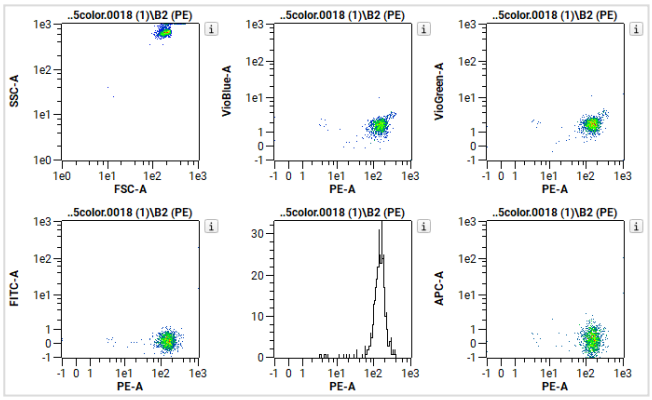


Figure 4: Plots of compensated PE channel against all other fluorescence channels used for MultiColorCompensation on a MACSQuant Analyzer 10

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