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.

Introduction

This short instruction describes the setup of detectors for multicolor flow cytometry experiments. It also guides the user through the automated 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: Click the **Clear** button on the instrument status bar to refresh the events.
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 appropriate name for recalled use.

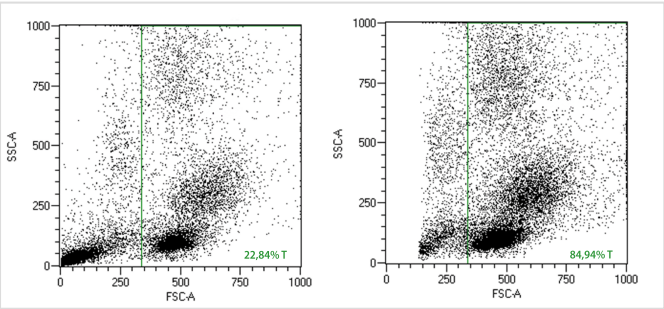


Figure 1: Adjustment of FSC and SSC PMT gains. Left: PBMCs prior to adjustment. Right: PBMCs after setting a trigger threshold to exclude debris.

Adjusting gains of fluorescence channels

1 Place a sample in 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 drop-down menu of the plot header 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 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.

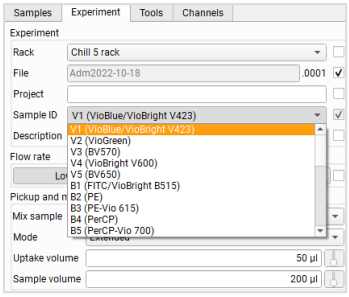
Sample material

- 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 Go to **File > New workspace**.
2 Open an instrument setting appropriate for the application.
3 Select an appropriate sample rack from the drop-down menu in the **Experiment** tab.
4 Go to the rack scheme in the **Experiment** tab.
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** radio button.
9 Select **Setup** from the **Type** drop-down menu.
10 Select **CompensationMultiColor** from the **Mode** drop-down menu.
11 Optional: Select mixing, change the flow rate, change the mode, or change the uptake and sample volume if 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**

drop-down menu 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 automated compensation. Do not clear unused channels.

13 Optional: If a dead cell discriminator is used that only labels dead cells, select the respective viability channel from the **Sample ID** drop-down menu for compensating the selected fluorescence from your dead cell discriminator (DCD) channel. Do not add the DCD.

14 Optional: If one blank sample is used for all stained controls, select **Blank** from the drop-down menu.

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

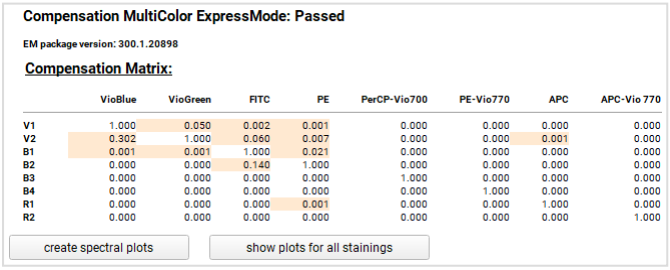
15 Check the levels of MACSQuant Running Buffer and waste.

16 Click the **Run** button to start the acquisition. A window opens.

17 Draw a region around the population of interest. Include only populations that are relevant for compensation and data analysis. At completion of the process, you are prompted to save as an instrument setting.

Spectral plots

After completion of the MultiColorCompensation the compensation matrix is shown in the analysis window. Applied compensation is highlighted orange in the matrix (**Figure 2**).



Compensation MultiColor ExpressMode: Passed

EM package version: 300.1.20898

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

- Select **create spectral plots** to show spectral overlap for each channel (**Figure 3**).
- Select **show plots for all stainings** to show spillover of one fluorescence channel against all other used channels (**Figure 4**).

Create spectral plots

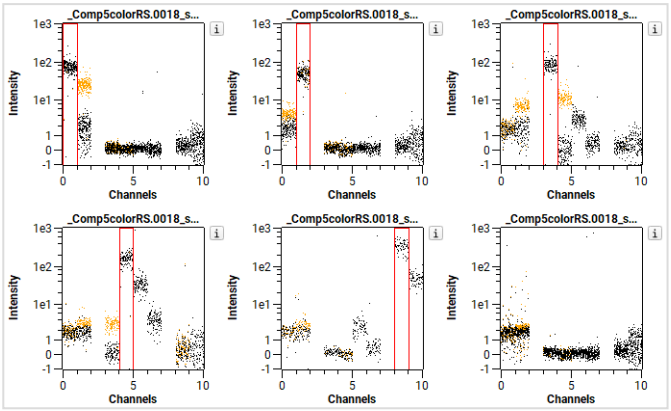


Figure 3: Spectral plots of a MultiColorCompensation sample. The red frame highlights the color that had spillover correction out of other channels. Fluorescence channels used for MultiColorCompensation Express Mode are designated in orange. Orange is signal prior to compensation; black is signal after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

Show plots for all stainings

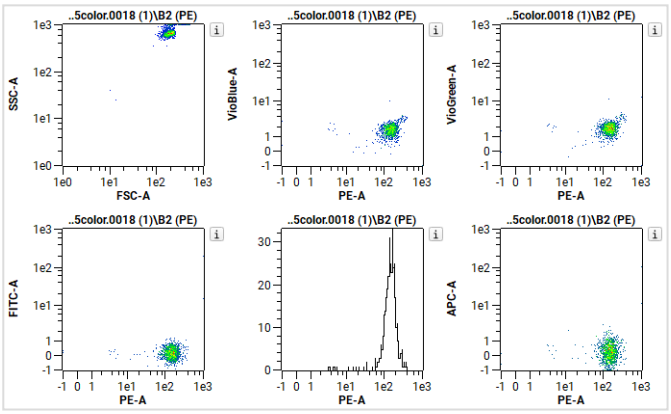


Figure 4: Plots of compensated PE channel against all other fluorescence channel used for MultiColorCompensation.

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