Miltenyi Biotec

Excite and inspire

Save time with the MACSQuant® Analyzer

Automated compensation

Background

Instrument compensation is crucial for the optimal acquisition and display of data obtained by flow cytometry. Compensation essentially accounts for the inherent overlap in emission spectra observed between different fluorochromes. This fluorescence spectral overlap, or 'spillover', will result in the detection of individual fluorochromes in more than one fluorescence channel. Spillover must be determined and corrected. This is especially important when multicolor analyses are performed—without proper compensation, results may be misinterpreted.

Performing auto-compensation on the MACSQuant® Analyzer

Most flow cytometer users would acknowledge that instrument compensation is a rather laborious and time-consuming procedure. This is not the case when using the MACSQuant® Analyzer, which can perform fully automated compensation on all channels at one time. Using either single-stained cells or compensation beads, the MACSQuant Analyzer can accurately and reliably perform automated compensation based on a 7×7 matrix (each channel compensated against the other 6 channels).

Materials and methods

Reagents and solutions

- Freshly prepared PBMC sample
- Suitable fluorochrome-conjugated antibodies
  For a comprehensive list of fluorochromes visit **www.miltenyibiotec.com**
- Sample buffer: Phosphate buffered saline, pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA
  Keep buffer cold (2–8 °C)

Materials

- MACSQuant® Analyzer with MACS® MiniSampler
- Seven 5 mL (12x75mm) polystyrene round bottom tubes tubes
- MACS® Chill 5 Rack

Cell preparation and staining

1. Label seven 5 mL polystyrene round bottom tubes from 1 to 7.
2. Freshly prepare peripheral blood mononuclear cells (PBMCs).
3. Determine the number of cells in the PBMC sample. Centrifuge cells (300×g for 10 minutes). Aspirate supernatant and resuspend cells in sample buffer at a concentration of 1×10^6 cells per 100 µL buffer.
4. Aliquot 100 µL of cells into all 7 tubes.
5. To tube 1 add an appropriate fluorochrome-conjugated antibody at the recommended titer, e.g., 10 µL of the CD8-VioBlue® antibody (human) [# 130-094-152]. Repeat the process for tubes 2–7 using suitable fluorochrome-conjugated antibodies depending on the number of channels that need to be compensated. Tubes 1–7 tubes should now contain single-stained cells.
6. Mix cells and incubate for 10 minutes in the dark at 4 °C.
7. Wash cells by adding 1–2 mL of sample buffer to each tube and centrifuge at 300×g for 10 minutes. Aspirate supernatant.
8. Resuspend cell pellets to a concentration of 1×10^6/mL.
9. Proceed to performing automated 7-color compensation on the MACSQuant Analyzer.

Note: It is recommended that the selected cell surface markers are brightly expressed on the cell population of interest, e.g., CD4, CD3 or CD8.

Seven color compensation on the MACSQuant® Analyzer

10. To open a saved instrument setting: click **File, Open** and highlight the tab **Instrument settings**. Select the appropriate file.

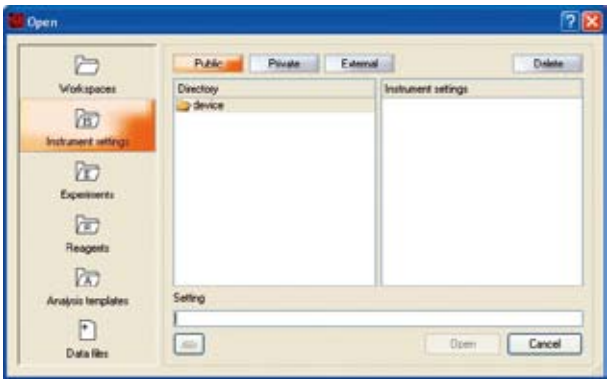


Figure 1: Opening an instrument setting

Note: It is possible to use current instrument settings rather than opening a pre-saved instrument setting. It is recommended to perform instrument calibration prior to auto-compensation.

11. Place a pre-cooled Chill 5 Rack onto the MACS MiniSampler and select **Chill 5 tube rack** from the **Rack** drop-down menu.

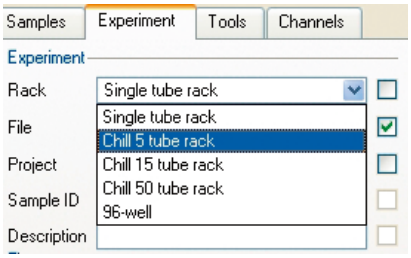


Figure 2: Selecting a Chill 5 tube rack

12. Position each of the seven tubes with single-stained cells in **columns** along the Chill 5 Rack . In this example the order was as follows: VioBlue (A1), FITC (B1), PE (C1), PE-Cy5 (D1), PE-Cy7 (A2), APC (B2), APC-Cy7 (C2).
13. Select and activate the corresponding rack positions. Click **Group** to group the samples into a single group as shown below.
14. Single-click on the **Racks** window to set all samples positions to **sample selected** (green circle).

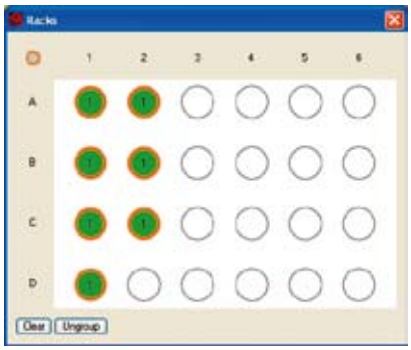
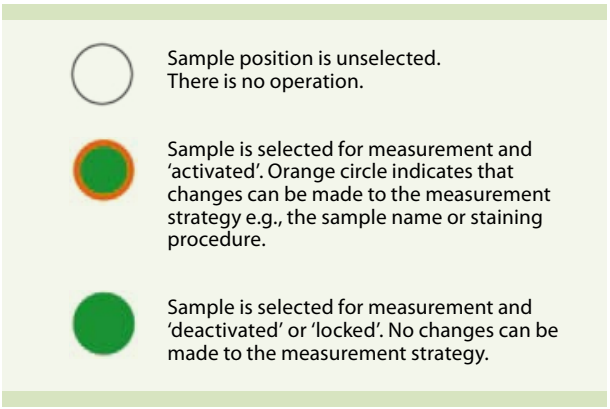


Figure 3: 'Activated' cell samples were grouped together



15. Check the **Express** radio button located under the **Settings** tab. Select **Setup** and **Compensation7Colors** from the drop-down menus.
16. Single-click on sample position A1 to activate it (green/orange circle). From the **Sample ID** drop-down menu, assign the relevant fluorochrome for compensation. In this example, cells in sample tube A1 were labeled by VioBlue.

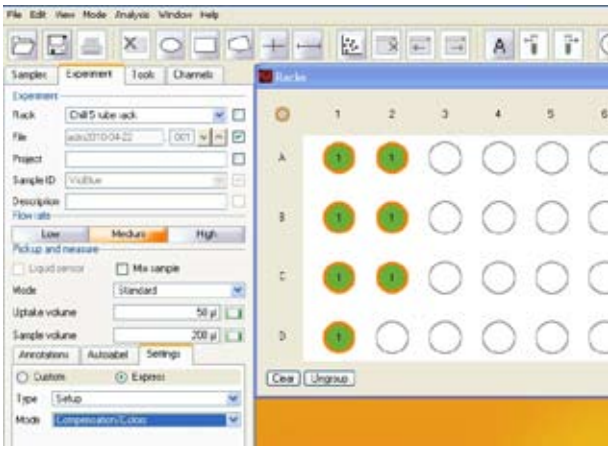


Figure 4: MACSQuantify™ Software will perform VioBlue auto-compensation on sample A1.

17. Select and activate sample position B1 and assign the relevant fluorochrome for compensation (e.g. in this example, FITC).
18. Continue this procedure for the remaining sample positions.

Note: Samples for compensation can be placed in any order. Please be sure to select the correct fluorochrome for each tube prior to starting the program

19. Click **Start** to begin compensation.
20. When prompted, draw a region around the cell population of interest.

Note: Selecting a region of interest is highly recommended. Depending on the experiment, a particular cell type or population may be selected for compensation.

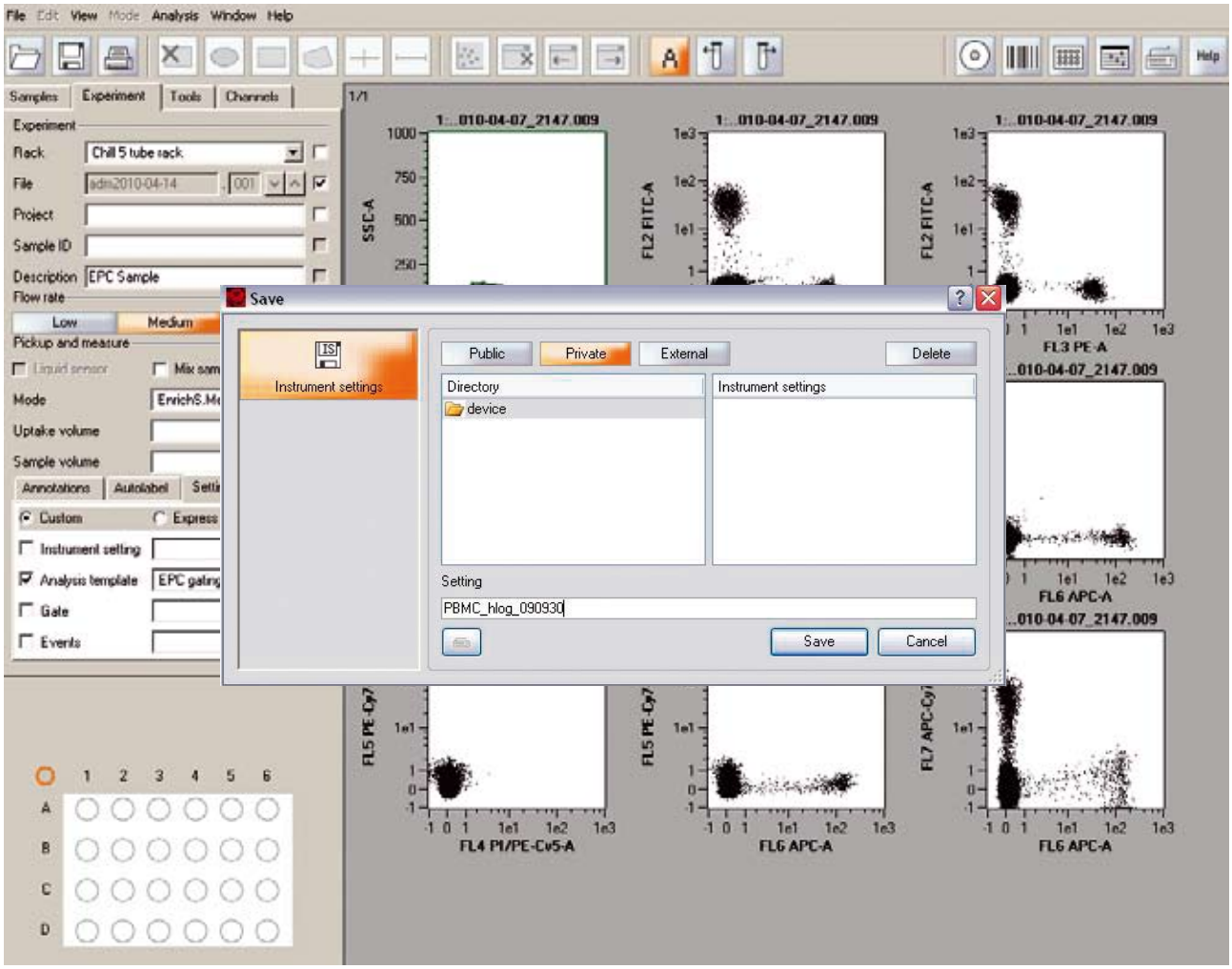


Figure 5: Seven color compensation was successfully completed and will be saved to the instrument settings by entering a name and selecting save.

21. The MACSQuant Analyzer will perform 7×7 color compensation on the selected region of interest.
22. When the compensation is completed, the dialog box to save instrument settings will open. Name the instrument settings and click **Save**. Click **Cancel** to abort the process.

Note: Single-stained beads can also be used for automated compensation. Please see the MACSQuant Analyzer User Manual for more details on compensation.

Conclusions

Flow cytometer compensation is often considered a tedious and time-consuming procedure that requires a significant degree of experience and expertise.
The MACSQuant Analyzer completely automates this procedure, making for reliable and reproducible results. Simply put: this walk-away convenience offers the researcher more time to focus on other aspects of the experiment.

For more information about additional cell analysis applications using the MACSQuant Analyzer, visit **www.macsquant.com**

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