

Tracking settings with Rainbow Beads

Purpose: to ensure consistent signals over the course of a project, and verify proper function of the cytometers.

Rationale: The components of a cytometer can display variability from day to day. Fluctuations in laser power, changes in temperature and humidity affecting the PMT's, and changes in alignment on the instrument are just some examples of variables that can impact the output. In order to minimize these variations, a consistent fluorescence standard should be run with each experiment to make sure that the signal output of the instrument for a given level of fluorescence is kept consistent. To do this, we use a stable source of fluorescence (rainbow bead with a matched lot #), and then adjust the user controlled settings (voltages) to match the output.

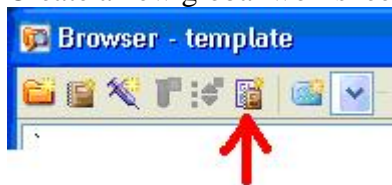
Unless there have been some major adjustments made to the instrument, settings should not vary by more than 20V or so. An added benefit of monitoring the instrument settings with beads is that it is readily apparent when something has happened to the instrument, such as a laser dying etc.

Procedure: After establishing settings for a given panel of reagents (explained elsewhere) such that all signals are on scale, compensation between channels is minimized, and separation of populations is either optimal (for dim labels) or sufficient (for bright ones), the operator collects a data file of a rainbow bead or other particle that gives positive signal for the established settings. For the next experiment, the first step would be to run the rainbow beads, and match the UNCOMPENSATED MFI from the initial run, to make sure the output of the instrument for the same level of fluorescence is the same.

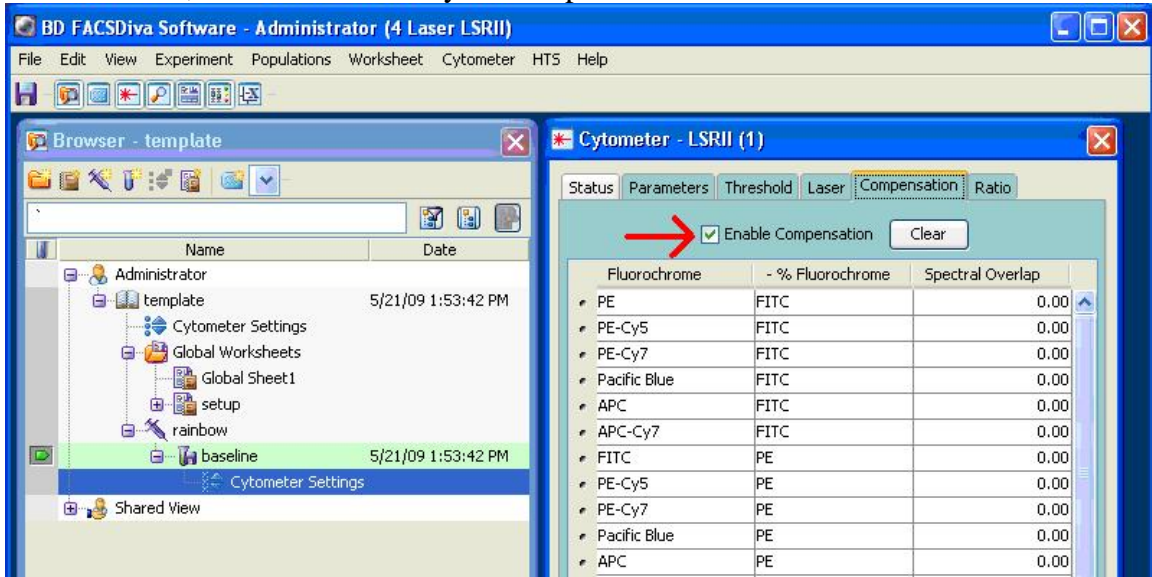
Step by step

On the initial day of your experiment:

1. Establish your panel settings (discussed elsewhere) and run your experiment (calculate compensation, collect your data files).
2. "Duplicate without Data" to create a copy of your experiment you just ran. Right click on the "Cytometer settings" directly beneath the experiment name, and "Unlink from setup" if that is an option. (Only happens if you link your compensation matrix instead of "apply only"). With "Cytometer settings" still selected, view the Compensation tab in the inspector. Select the first line, scroll to the bottom of the list, hold shift and select the last line, so that all compensation pairings are selected, and click "clear". This will set all of your values to 0.
3. Create a new global worksheet (button shown below), and label it "setup"



4. Draw a FSC/SSC dot plot, and a histogram for each color. See image at the end of this document.
5. Create a new tube, call it “baseline” and collect a data file of mid-range rainbow beads (Spherotech, RFP-30-5A) or another appropriate control particle. It may be necessary to change the FSC or SSC settings to be able to visualize the beads, and this is fine, and will not affect your compensation results.



6. On the “setup” worksheet, draw interval gates on the peak in each histogram. To accurately locate the peak, you can use the “+” magnifying glass tool and draw a box around the area to zoom in on (as shown in FITC below).
7. Rename experiment “4 color template” or whatever name will identify this experiment type.
8. “File>Export>Experiment”, and save the experiment to your D drive folder. This will create a template of your experiment with the baseline rainbow beads saved with it.

On subsequent experiments using the same layout:

1. “File>Import>Experiment” and import your template.
2. Change the name of the experiment to suit your method, and relabel any tubes as necessary.
3. Switch to the global sheet, and view the “setup” sheet. Activate the “Baseline” data file (specimen “Rainbows”) and verify the interval markers closely bracket the peak of signal.
4. “Next Tube”, label with the date, and run a tube of the SAME LOT of Rainbow beads. Check the instrument settings to make sure compensation is disabled (“Enable compensation” is de-selected or all values are 0).
5. Adjust voltages to center the peak in the interval marker (trying to match the MFI values in a stats view can be tricky as they refresh while the tube is running and can vary quite a bit).
6. Record a data file of the rainbows. Now you can go back and run your compensation tubes as usual, and no further voltage adjustments should be

necessary (other than FSC/SSC for your cells). Double check that compensation is “enabled” after calculating compensation.

