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 affect the output. In order to minimize these variations, we should apply a consistent fluorescence standard with each experiment to make sure that the signal output of the instrument for a given level of fluorescence is consistent. To do this, we use a stable source of fluorescence - Rainbow Calibration Particles (8 peaks)

Rainbow Calibration Particles (8 peaks)
Ref: **559123** Enzifarma
A mixture of 3.0 - 3.4 µm Rainbow Particles that are dyed to eight different fluorescent intensities. Every Rainbow Particle contains a mixture of fluorophores that are excited at any wavelengths from 365 - 650 nm.

Procedure: After establishing settings for a given panel of reagents 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 rainbow beads that gives positive signals for the established settings. For the next experiment, the first step would be to run the rainbow beads, and make sure that the uncompensated MFIs are <±15% different from the initial run.

Step by step

On the initial day of your experiment

Establish your panel settings and run your experiment (set voltages; calculate compensation; collect your data files);

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"Duplicate without Data" to create a copy of the experiment you just ran. Call it *Setup Experiment.*

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Delete the specimen and tubes from the original experiment and create a new specimen. Then create the first tube, call it "baseline" and collect a data file of 8-peak rainbow beads (It may be necessary to change the FSC or SSC settings to be able to visualize the beads).

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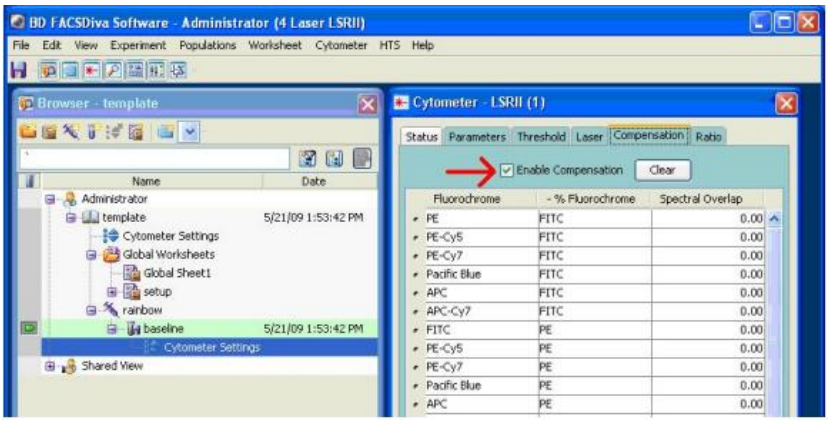
Select “Cytometer settings” to view the Compensation tab in the inspector. Clear all the compensation values or de-select the “Enable compensation” bottom.



On the global worksheet, draw a FSC/SSC dot plot, and a histogram for each color.

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On each histogram, draw interval gates on the 8th or 7th peak and evaluate the MFI for each one of the colors

(Ask for the Statistics Table, right clicking on a plot; Edit Statics, right clicking on the statistics table and ask for the Mean value for all the colors).

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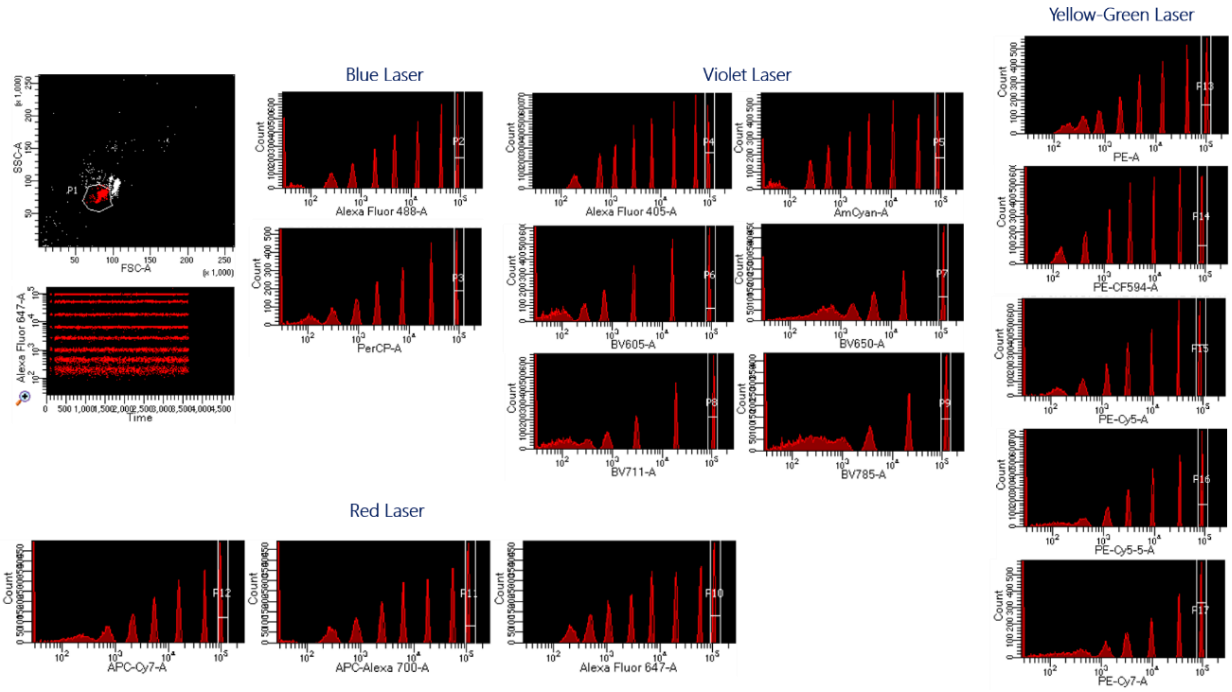
Export the experiment and save the template.

Rainbow Calibration beads



On subsequent experiments

Open the *Setup Experiment*

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"Next Tube", label with the date, run and record the 8-peak Rainbow beads.
(Every time, check the instrument settings to make sure compensation is disabled ("Enable compensation" is de-selected or all values are 0)).

MFI values of the 7th or 8th peak are inside the target range (<±15%)

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You are ready to acquire your samples and compare them with all the other time points

MFI values of the 7th or 8th peak are out of the target range (>±15%)

The equipment might be dirty or have any other fluidic or optical problem

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Ask technical staff to troubleshoot and solve the issue

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Re-run the beads: MFI values of the 7th or 8th peak are inside the target range (<±15%)

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You are ready to acquire your samples and compare them with all the other time points

The Preventive Maintenance was performed or some other optical alterations such as laser replacement

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Adjust voltages to center again the 7th or 8th peak in the target range by matching the target MFI values. Record that data file

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With these new voltages, re-run your compensation tubes (unstained + single colors) and link the new compensation matrix

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You are ready to acquire your samples and compare them with all the other time points

Reference peak target MFI				
Date:			**RB LOT #**	
Channel	**Lower MFI (-15%)**	**Target MFI**	**Upper MFI (+15%)**	**Rainbow bead peak #**

Reference peak target MFI				
Date:			**RB LOT #**	
Channel	**Lower MFI (-15%)**	**TARGET MFI**	**Upper MFI (+15%)**	**Rainbow bead peak #**