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iGEM Calibration Protocol - Flow Cytometry Fluorescence

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Abstract

This protocol can be applied to any strain of cell that can be safely run through a flow cytometer. For clarity, we have written it assuming E. coli DH5-alpha; to apply the protocol to another cell type, substitute the other cell type for any place where the protocol says [E. coli DH5-alpha].

This protocol has been written for measurement of GFP, YFP, or other yellow/green fluorescent proteins into MEFL units. To apply it to fluorescent proteins of other colors:

- Replace BBa_J364001 with a construct for strong expression of the other protein.
- For blue proteins (e.g., **mTagBFP**), measure with 405nm excitation and 450nm/50nm emission filter. Units will be MEC30.
- For red/orange proteins (e.g., **mCherry**), measure with 561nm excitation and 610nm/20nm or 620nm/15nm emission filter. Units will be MEPTR.
- For far-red / near-infrared proteins (e.g., IRFP), measure with 635nm excitation and 780nm/60nm or 750nm long-pass (LP) emission filter. Units will be MEAPCY7.

To apply the protocol to multiple colors, add a positive process control for each color and use one of the tools on the **iGEM Measurement Resources page** to compensate measurements for spectral overlap.

This protocol can be combined with bead-based cell size calibration.

Attachments

XLSX

iGEM Data Analysis T...

21KB

Materials

MATERIALS

Rainbow calibration beads **SpheroTech Catalog #**RCP-30-5A

Rainbow calibration beads **SpheroTech Catalog #**URCP-38-2K

Rainbow calibration beads **SpheroTech Catalog #**URQP-38-6K

- Only one set of rainbow calibration beads are necssary, options are listed.
- Negative process control: wild-type [E coli DH5-alpha]
- Positive process control: [E coli DH5-alpha] transformed with BBa_J364001 (or another similar construct for strong constitutive GFP expression)
- Experimental samples: [E coli DH5-alpha] transformed per your desired experimental procedure
- Flow cytometer with a channel that measures with a 488nm excitation and a 530nm/30nm emission filter. Measurements on this channel will be converted to MEFL.

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Before start

Read through this entire protocol carefully before you start your experiment and prepare any materials you may need. See the "Results" section for an example of a completed data analysis spreadsheet.

Acquisition of Data

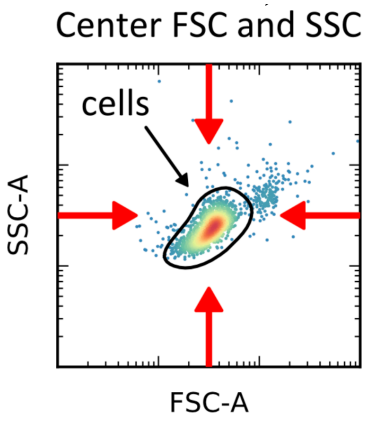
1 Prepare and culture experimental samples, negative process control, and positive process control according to your desired experimental procedure.

2 Prepare experimental samples, negative process control, and positive process control as needed for running through your flow cytometer.

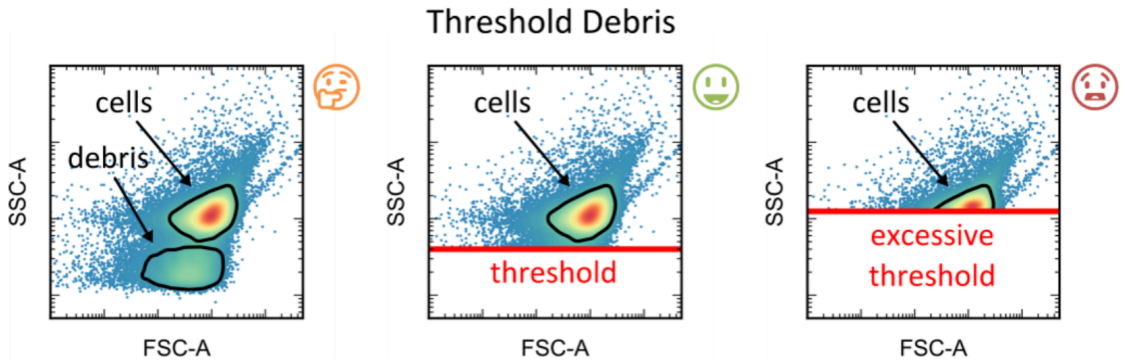
3 Shake SpheroTech rainbow calibration beads vigorously or vortex briefly. Prepare bead sample by diluting 15 µL of SpheroTech rainbow calibration beads into 200 µL of 1x PBS.

4 Measure all samples in flow cytometer

- Using the bead sample, adjust MEFL channel voltage so that the top bead peak is below detector saturation.
- Using the negative process control sample, adjust forward-scatter and side-scatter voltages to place the strong cell peak as close to the center of the detector range as possible (see example illustrated below).



- Instrument gating should be set to ensure that no cell events are discarded (see example illustrated below).



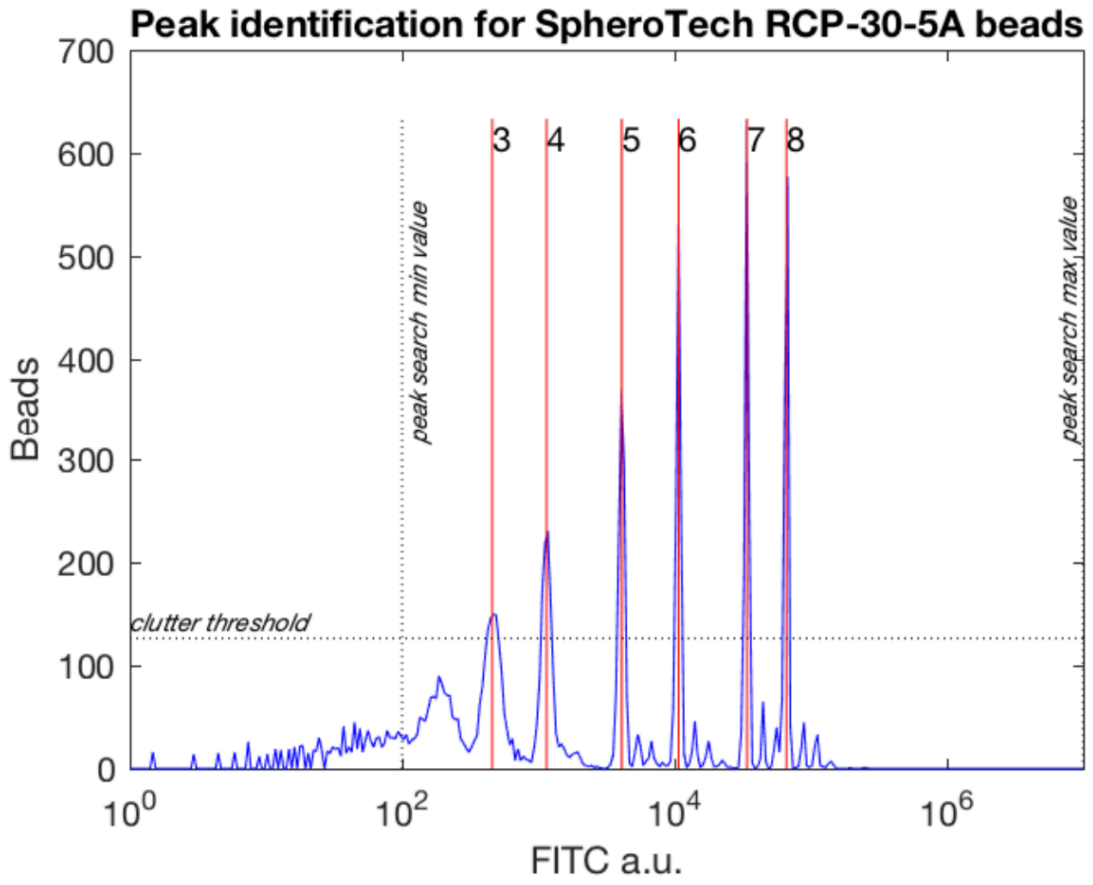
- Collect at least 10,000 events per sample.

5 Compute the MEFL/a.u. scaling factor from bead sample either using one of the tools on the **Measurement resources page** or following the Excel-based scaling factor computation below.

6 Multiply all fluorescence measurements by MEFL/a.u. scaling factor to convert from a.u. to MEFL.

Excel-based scaling factor computation

7 Compute a histogram of GFP channel measurements for the rainbow calibration bead sample; this should show some number of distinct high peaks (as in the example below).



8 Identify the center of each distinct, narrow, high peak, and record these locations in the provided cell of the Fluorescence Calibration spreadsheet, starting with the highest peak. Leave all other locations blank. iGEM Data Analysis Template - Flo...

9 Select the model and channel for your calibration beads from the menu in the sheet.

10 Record the lot number for your calibration beads (e.g., “AJ02”) in the provided cell. This should cause a set of bead values to appear. The lot number must precisely match the lot in the “Bead Catalog” sheet.

11 The MEFL/a.u. scaling factor and the graph on the sheet should now fill in.

12 The graph shows one point for each peak and a line for the MEFL/a.u. scaling factor.

13 Check that the points are all close to the line (as in the example below). If this is not the case, check to be sure that you have identified and recorded your peaks correctly.