Jul 19, 2019

iGEM Calibration Protocol - Flow Cytometry Cell Size

Forked from iGEM Calibration Protocol - Flow Cytometry Cell Size

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1 Works for me dx.doi.org/10.17504/protocols.io.5ncg5aw

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ABSTRACT

Conversion from forward scatter to Eμm is not a linear function, so data cannot be converted simply by multiplying with a scaling factor. We thus recommend use of software tools for processing data with size calibration.

This protocol can be combined with bead-based fluorescence calibration.

MATERIALS

NAME	CATALOG #	VENDOR
Size calibration beads	PPS-6K	SpheroTech

MATERIALS TEXT

- Experimental samples: transformed per your desired experimental procedure
- Flow cytometer with a forward scatter channel (typically a 488nm excitation and a 488nm/10nm emission filter). Measurements on this channel will be converted to equivalent μm diameter (Eμm).
- TASBE Flow Analytics - Analysis software

BEFORE STARTING

Read through this entire protocol carefully before you start your experiment and prepare any materials you may need.

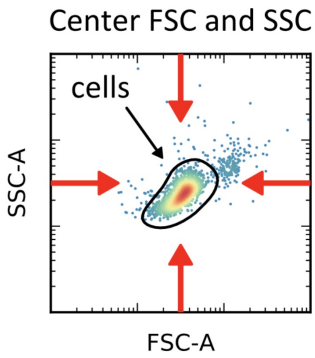
1 Prepare and culture experimental samples according to your desired experimental procedure.

2 Prepare experimental samples as needed for running through your flow cytometer.

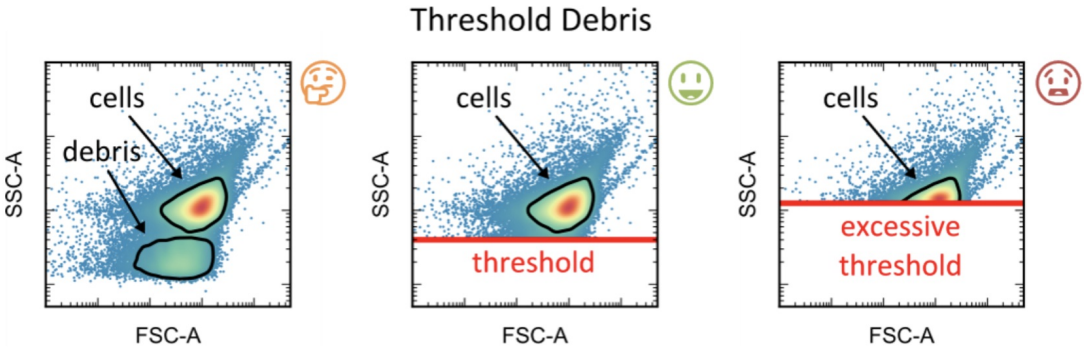
3 Follow SpheroTech directions for preparation for PPS-6K bead sample.

4 Measure all samples in flow cytometer

- Using the bead sample, adjust FSC 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 Eμm/a.u. scaling function from bead sample using TASBE Flow Analytics to create a TASBE color model.

6 Convert experimental data to Eμm using TASBE Flow Analytics analysis functions.