May 08, 2019

🌐 iGEM Calibration Protocol - Flow Cytometry Cell Size
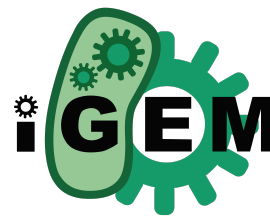
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Protocol status: In development

Created: May 07, 2019

Last Modified: August 07, 2019

Protocol Integer ID: 22982

Keywords: flow cytometry cell size conversion from forward scatter, flow cytometry cell size conversion, fluorescence calibration, based fluorescence calibration, igem calibration protocol, data with size calibration, size calibration, calibration protocol, eµm, forward scatter

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

MATERIALS

⊗ Size calibration beads **SpheroTech Catalog #**PPS-6K

- 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).

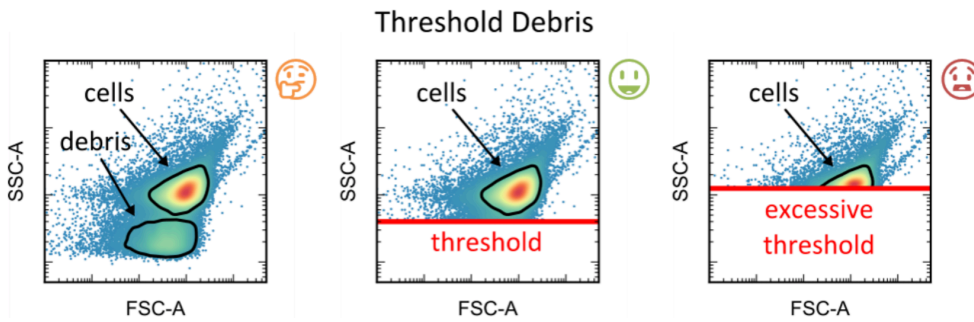- **TASBE Flow Analytics** - Analysis software

Troubleshooting

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.

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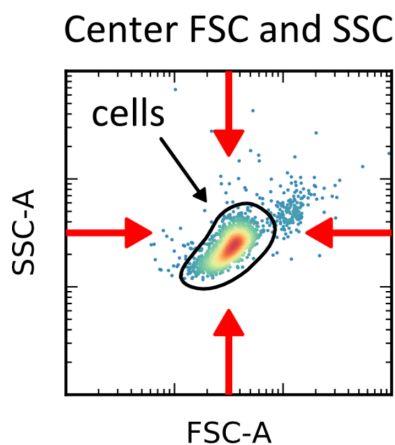
- Instrument gating should be set to ensure that no cell events are discarded (see example illustrated below).

Threshold Debris

- 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.