MAY 08, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.2pegdje

Protocol Citation: Jacob Beal, Cheryl Telmer, Richard Tennant, Paul Rutten 2019. iGEM Calibration Protocol - Flow Cytometry Cell Size. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.2pegdje>

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Protocol status: In development

Created: May 07, 2019

Last Modified: Aug 07, 2019

PROTOCOL integer ID:
22982

🌐 iGEM Calibration Protocol - Flow Cytometry Cell Size

Jacob Beal¹, Cheryl Telmer¹, Richard Tennant¹,
Paul Rutten¹

¹iGEM Measurement Committee

iGEM Measurement

Tech. support email: pauljrutten@gmail.com

Paul Rutten

The University of Oxford

ABSTRACT

Conversion from forward scatter to $E_{\mu m}$ 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

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🔗 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 ($E_{\mu m}$).
- [TASBE Flow Analytics](#) - Analysis software

BEFORE START INSTRUCTIONS

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 $E_{\mu m}/a.u.$ scaling function from bead sample using [TASBE Flow Analytics](#) to create a TASBE color model.
- 6 Convert experimental data to $E_{\mu m}$ using [TASBE Flow Analytics](#) analysis functions.