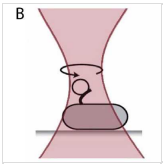

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BFM speed recording with back-focal-plane interferometry

Teuta Pilizota[1], Ekaterina Krasnopeeva[1], Jerko Rosko[1]

[1]The University of Edinburgh
Ekaterina Krasnopeeva: Currently at IST Austria
Jerko Rosko: Currently at Laboratoire Jean Perrin, Paris

1 *Works for me* dx.doi.org/10.17504/protocols.io.bch2it8e

Teuta Pilizota

Ekaterina Krasnopeeva

SUBMIT TO PLOS ONE

ABSTRACT

The speed of bacterial flagellar motor (BFM) is measured with back-focal-plane interferometry. Heavily attenuated optical trap (855 nm laser) is used to detect the rotation of a polystyrene bead attached to a truncated flagellar filament. Time course of the bead rotation is recorded with the position-sensitive detector.

EXTERNAL LINK

http://[1] https://www.pnas.org/content/114/38/E7969, [2] https://www.cell.com/biophysj/fulltext/S0006-3495(19)30392-3

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

[1] J. Rosko, V. Martinez, W. Poon, and T. Pilizota, “Osmotaxis in Escherichia coli through changes in motor speed,” Proc. Natl. Acad. Sci. U. S. A., vol. 114, no. 38, pp. E7969–E7976, 2017. [2] E. Krasnopeeva, C.-J. Lo, and T. Pilizota, “Single-cell bacterial electrophysiology reveals mechanisms of stress-induced damage,” Biophys. J., vol. 116, no. 12, May 2019.

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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MATERIALS TEXT

MATERIALS

Poly-l-lysine, 0.1% (wt/vol) **Sigma Aldrich Catalog #P8920**

Polybead Microspheres 0.50µm **Polysciences Catalog #07307-15**

SAFETY WARNINGS

A person performing measurements must be trained in laser safety

BEFORE STARTING

Bead assay for back-focal-plane interferometry requires the use of a strain with "sticky" mutation in *fliC* gene encoding flagellin (e.h. KAF84, EK01, EK07)

Preparing the cells 30m

1 Grow cells to the desired OD 5h

2 Truncate flagellar filaments by passing a bacterial suspension through two syringes with narrow-gauge needles (26 gauge) connected with a plastic tube (“shearing device”) 30-80 times 20m

3 Wash cells into the experimental medium 3 times by centrifugation at 8000*g for 2 minutes. We use microcentrifuge tubes to spin the cells down and resuspend the pellet in 1ml of the experimental medium. At the end of the wash resuspend the cells in the experimental medium to the desired concentration. 10m

Preparing the slide 20m

4 Coat the surface of the tunnel-slide (or flow-cell) with 0.1% poly-L-lysin (PLL) by flushing PLL through the flow-cell/tunnel-slide for 10-20 s followed by washing it out with the excessive volume of the experimental medium (~20 times the volume of the channel). 2m

5 Load sheared and washed cells into the flow-cell/tunnel-slide and incubate for 10 min to allow attachment. Wash excessive cells out with the experimental medium. 10m

6 Add 0.5 µm in diameter polystyrene beads (Polysciences, Inc, USA) resuspended in the experimental medium to the flow-cell/tunnel-slide and incubate for 10 min with consequent washing out of the non-attached beads. 10m

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Microscopy

7 Place a potential spinner into the focus of 855 nm laser.

Infrared laser
855 nm continuous wave diode laser
Blue Sky Research **855 laser**

8 Find the best position to see a circular trajectory and record the speed with 10 kHz sampling rate with the position sensitive detector with a 2.5 kHz cutoff anti-aliasing filter applied.

PSD Model 2931
Position sensitive detector
New Focus **PSD**

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