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 [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.

🌐 BFM speed recording with back-focal-plane interferometry

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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.

MATERIALS

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 START INSTRUCTIONS

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)

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID:
33050

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-lysine (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
- Load sheared and washed cells into the flow-cell/tunnel-slide and incubate for 10 min to allow 10m

- 5 attachment. Wash excessive cells out with the experimental medium.
- 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

Microscopy

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

Equipment

Infrared laser

NAME

855 nm continuous wave diode laser

TYPE

Blue Sky Research

BRAND

855 laser

SKU

- 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.

Equipment

PSD Model 2931

NAME

Position sensitive detector

TYPE

New Focus

BRAND

PSD

SKU