# Dynamics of a motile bacterium in an optical trap

Ashwini V Bhat,[1] Praveen P,[1] Shruthi S Iyengar,[1] Balaji Yendeti,[2]
Chetana D,[1] Mohana D C,[3] Ashok V S,[4] and Sharath Ananthamurthy[1,4,*]

[1] *Department of Physics, Bangalore University, Bangalore-560056, India*
[2] *ACRHEM, University of Hyderabad, Hyderabad-500046, India*
[3] *Department of Microbiology, Bangalore University, Bangalore-560056, India*
[4] *School of Physics, University of Hyderabad, Hyderabad-500046, India*

An optical trap can trap micron sized objects when it is passed through a high numerical aperture objective[1].The optical tweezer consist of a single laser beam focused to a diffraction limited spot (typically about $\lambda/2$, $\lambda$ being the wavelength of the laser beam used).We optically trap a gram-positive bacterium Bacillus subtilis- in an optical trap and study the flagellar rotation. Bacillus subtilis is a non-pathogenic bacterium which uses flagella rotation to move in a liquid environment [2]. The rotation of the flagella in an anticlockwise direction constitutes a run sequence and in a clockwise direction constitutes a tumble sequence. The rotating movement of bacterium flagellum has an important role in cell motility and chemotaxis. B.subtilis flagellum consists of three architectural domains: the basal body, the hook and the filament. The filament is a helical structure made up of repeated units of protein flagellin. The hook is a distinct structure located at the base of the filament and also attached to the basal body. The basal body is located inside the cell envelope. The motor included in the basal body contains two functional entities; the rotor and the proton conducting stator. The proton-motive force generates a torque at the rotor interface in the basal body. This torque is imparted to the filament through the hook, causing the flagellum to rotate [3]. In order to balance this torque, the cell body of the bacterium rotates in opposite direction (the cell rotates clockwise during run sequence (as shown in fig1) and anticlockwise during tumble sequence). When the bacterium is trapped in an optical trap, we observe the rotational motion of the cell body and are recorded by a high frame rate camera. The trajectory of the bacterium is obtained by video analysis and the data is processed to obtain the power spectrum of the trajectory ( fig 2). The power spectrum consists of two peaks, the larger peak corresponds to cell body rotation frequency and the smaller peak corresponds to flagella rotation frequency. This experiment helps us to determine the propulsion coefficicents of the motile bacterium in low Reynolds number, which are useful in determining forces and torques exerted on the flagellum [4, 5]. Previously, several experiments are performed to determine the torque exerted on the flagellum of the bacterium by attaching beads to the hook of the flagellum to get the torque-speed relations [6]. We can consider this experiment as a non- contact way of determining the torque exerted on the flagellum without attaching any other external geometries to the bacterium by using a simple optical trap and can further be applied to study the torque-speed relations at different environmental conditions.

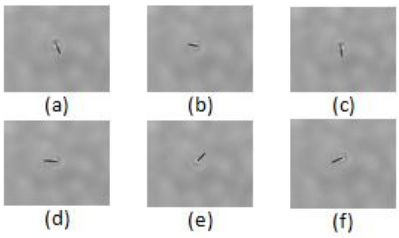


FIG. 1. (a-f) Rotation of the cell body observed in an optical trap. Each frame has an interval of 13 ms .The line is drawn to show the sense of rotation

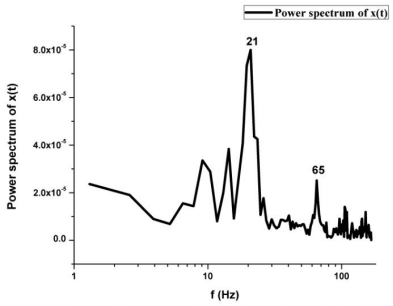


FIG. 2. Power spectrum of the trajectory x(t) of the bacterium, the tall peak corresponds to cell body rotation and the small peak corresponds to flagella rotation.

Ashwini V Bhat acknowledges a DST-INSPIRE fellowship. The experiment set up was enabled through a previous DST (Nanoscience and Technology Initiative) project

* Corresponding author: sasp@uohyd.ac.in

[1] A Ashkin, J M Dzeidzic, J E Bjorkholm and S Chu Opt. Lett. **11(5)** 288-90 (1986)
[2] S B Guttenplan, S Shaw, and D B Kearns Mol. Microbiology **87(1)** 211-29 (2013)
[3] C Diethmaier, R Chawla, A Canzoneri, D B Kearns ,P P Lele and D Dubnau Mol. Micro. **106(3)**, 367-80 (2017)
[4] E M Purcell *Proc. Natl. Aca. Sci. USA.* 94 11307 (1997)
[5] S Chattopadhyay, R Moldovan, C Yeung and X L Wu *PNAS* 103 13712-17 (2006)
[6] N Terahara, Y Noguchi, S Nakamura, N Kami-ike, M Ito, K Namba and T Minamino Scientific Reports **7** 46081 (2017)