

Microfabricated Structures Powered by Flagellated Bacteria

M. Selman Sakar^{1,*}, Edward Steager², A. Agung Julius¹,
Vijay Kumar³, Min Jun Kim², George J. Pappas¹

1. Department of Electrical and Systems Engineering, University of Pennsylvania, Philadelphia PA 19104, USA
 2. Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia PA 19104, USA
 3. Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia PA 19104, USA
- *email: sakarmah@seas.upenn.edu,

Introduction

As the field of engineered micro/nanoscale structures matures, a need has emerged for robust, controllable methods of actuation for miniaturized systems. In low Reynolds number hydrodynamics, the behavior of flagellated bacteria such as *Escherichia coli* and *Serratia marcescens* suggests that they could be used to manipulate suspended microbarges in microfluidic environments for potential applications. Flagellar motors offer many unique advantages as microactuators. Countless bacteria can be inexpensively cultured in a matter of hours. Those bacteria draw chemical energy from their environment, convert this energy to mechanical energy very efficiently, and can survive in a wide range of temperature and pH values. Bacteria have also been demonstrated to self-coordinate when patterned in monolayer carpets, and those carpets can be controlled by phototactic and chemotactic means [1]. However, the motion of the bacteria-driven microbarga throughout the microfluidic channel is unpredictable. It is not easy to determine the amount of chemicals or the intensity of ultraviolet(UV) light to be used for having the microbarga follow the desired trajectory. On the other hand, if we can successfully model both the bacteria-driven microbarga and the surrounding environment then we can find the optimal amount of external stimuli to be used in order to achieve the predetermined task. This approach will help us save tremendous amount of time and money.

We have studied self-coordinated transportation systems using SU-8 microstructures in open microchannels and here present a stochastic model for the bacteria-driven microbarga that is designed to mimic the motion of this system with and without chemical gradients or other external stimuli and compare experimental results with the outcomes of simulations. Our ultimate goal is to use our mathematical model as a workbench to propose various closed loop control schemes applicable for the real system.

Microfabrication

50x100 μm microbarges were fabricated utilizing standard photolithography techniques. SU-8 negative photoresist was chosen for its material properties and ease of use. SU-8 series 10 resists can be patterned to a thickness of 10m which provides a useful balance between structure thickness minimization and sufficient rigidity for micromanipulation. Additionally, SU-8 microbarges are relatively unaffected by troublesome surface and electrical forces that dominate the microfluidic environment. *Serratia marcescens* have also been observed to adhere favorably to SU-8 structures using a swarm blotting technique. Using this technique a monolayer of vigorous cells can be quickly patterned on the surface of several structures

in parallel. Such monolayer has been demonstrated to coordinate *en masse* inducing a net thrust on the microbarge.

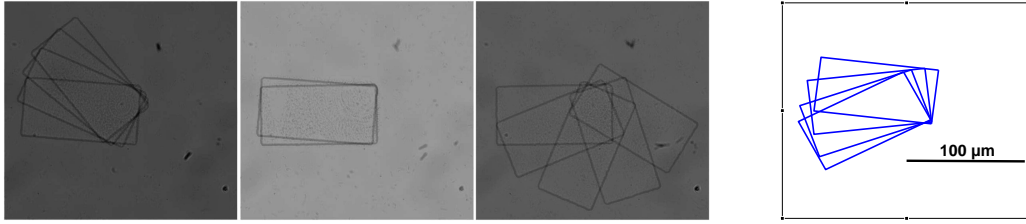
Mathematical Model

The body of *Serratia marcescens* is rod-shaped, about $1\mu m$ in diameter and $2\mu m$ in length, and, typically has several flagella (4-6). Each flagellum has a rotary motor that can turn at approximately 150 Hz, either clockwise(CW) or counterclockwise(CCW). When all the flagellar motors turn counterclockwise, the filaments rotate in parallel in a bundle that pushes the cell body forward, and the cell is said to "run". When one or more of the motors turns clockwise, the corresponding filaments unwind from the bundle, and the cell body moves erratically, or tumbles. The movement of *Serratia marcescens* can be characterized as a sequence of smooth-swimming runs interrupted by brief tumbles. During tumbling events, bacteria reorient themselves by randomizing the direction of the next run. The behavioral response of the bacteria is temporal and they compare current and past chemical environments. When, by chance, a cell moves up a gradient of attractants or down a gradient of repellents runs are extended. On the other hand, the tendency to tumble is enhanced when attractant concentrations decrease or repellent concentrations increase. This run-and-tumble strategy enables an individual bacterium to control its tumbling frequency and results in biased or directed random walk towards attractants and away from repellents.

The default direction of rotation of the flagellar motor is counterclockwise. According to recent studies, the change in concentration of external chemicals determines the probability of CW or CCW rotation, and the switch is thrown by thermal fluctuations [2]. This assumption, together with the observation that CCW or CW rotation intervals are exponentially distributed, leads us to model each motor as a two-state continuous time Markov chain, with switching probabilities per unit time (λ^+ , λ^-) that depend on the external concentration gradient. Then the switching rates between those two states are $\lambda^+ = K_{CW} \exp(-\delta C/E)$ and $\lambda^- = K_{CCW} \exp(\delta C/E)$ where K_{CW} and K_{CCW} are the transition rates, δC is the concentration gradient and E is a proportionality constant.

Bacteria flagella on a single cell were found to switch asynchronously between CCW and CW senses of rotation so for the motion of a single bacterium with n flagella, we propose a variation of the unicycle model in 2D. In this model, the deviation angle from the previous run and the speed of the bacterium depend on the number of flagella turning CW. The mathematical formulation can be written as $w = w_m * f_{CW}/n$ and $v = v_m * (n - f_{CW})/n$ where w is the angular and v is the translational speed of the bacterium, w_m and v_m are chosen in such a way that the mean change in direction from run to run and the average speed of the bacterium are consistent with the previous observations and finally f_{CW} is the number of flagella rotating CW. We successfully simulated chemotaxis behavior of the bacteria with this model.

To simulate the motion of the bacteria-driven microbarge, we need to model each bacterium dynamically. As the bodies of the bacteria cannot move after they are attached, the motion of the barge is determined by the initial orientation and distribution of the bacteria, and the propulsive force applied by each bacterium. There are three basic assumptions we have used. First of all, a bundle of several flagella produces little more torque than a single flagellum produces [3]. Secondly, according to resistive force theory the bundle thrust decreases linearly



(a) Time lapse images of rotating barge Left: Rotational motion with 0.5 rad/s. Middle: During UV exposure rotation stops. Right: Rotation fully resumes after exposure.

(b) Screenshots taken during the computer simulation

Figure 1: Phototactic control of a 50x100 μm rectangular microbarge.

with the swimming speed. Lastly, as we are working at low Reynolds number the drag force is directly proportional to the velocity and inertial forces are negligible.

Experimental Results

The rectangular microbarge was first blotted on the swarm plate. We then rinsed off all agar and unattached bacteria to obtain a bacterial monolayer on the surface. Before releasing the microbarge, we analyzed the distribution and orientation of the bacteria and used this information in our simulations. We introduced this structure to an open channel of fresh motility buffer and the barge immediately started to turn around one of its corners in CCW direction (viewed from beneath) with an angular velocity of ~ 0.5 rad/s. After 5 s we exposed the microbarge to UV light, and it became inactive in 0.24 s after traveling 0.13 rad. Subsequent motion resumes when the UV light source is once again shuttered (Figure 1(a)). By using our mathematical model, we simulated the motion of the microbarge and reproduced the experimental results. The barge followed the same trajectory with an angular velocity of 0.52 rad/s (Figure 1(b)). To simulate the phototactic on/off control, we disabled all the flagellar propulsion during the virtual application of UV light. As a result, from the beginning until the end of the exposure, the only forces acting on the micro-structure are the inertial forces and the drag force. The drag force dominated the inertial forces as expected and the amount of time passed until the barge completely stopped was 0.27 s.

References

- [1] E. Steager, C.-B. Kim, J. Patel, S. Bith, C. Naik, L. Reber, and M. J. Kim, "Control of microfabricated structures powered by flagellated bacteria using phototaxis," *Appl. Phys. Lett.*, vol. 90, p. 263901, June 2007.
- [2] B. E. Scarf, K. A. Fahrner, L. Turner, and H. C. Berg, "Control of direction of flagellar rotation in bacterial chemotaxis," *Proc. Natl. Acad. Sci. USA*, vol. 95, pp. 201–206, January 1998.
- [3] N. C. Darnton, L. Turner, S. Rojevsky, and H. C. Berg, "On torque and tumbling in swimming escherichia coli," *J. Bacteriol.*, vol. 189, pp. 1756–1764, March 2007.