Control of microfabricated structures powered by flagellated bacteria using phototaxis

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Flagellated bacteria have been employed as microactuators in low Reynolds number fluidic environments. SU-8 microstructures have been fabricated and released on the surface of swarming *Serratia marcescens*, and the flagella propel the structures along the swarm surface. Phototactic control of these structures is demonstrated by exposing the localized regions of the swarm to ultraviolet light. The authors additionally discuss the control of microstructures in an open channel powered by bacteria which have been docked through a blotting technique. A tracking algorithm has been developed to analyze swarming patterns of the bacteria as well as the kinematics of the microstructures. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2752721]

As the field of engineered micro-nanoscale structures matures, a need has emerged for robust, controllable methods of actuation for miniaturized systems. The biomolecular motors embedded in the cell bodies of various strains of bacteria may be employed as actuators for such applications. Flagellated bacteria such as *Escherichia coli* and *Serratia marcescens* use rotating helical flagella to swim.[1,2] Flagellar motors offer many unique advantages as microactuators. Through well-established cell culturing techniques, countless bacteria can be inexpensively cultured in a matter of hours. They draw chemical energy directly from their environment and are able to survive in a wide range of temperature and *p*H. Additionally, bacteria are controllable *en masse* through light and chemical sensory mechanisms. The ability of cells to respond to chemical concentration gradients is referred to as chemotaxis,[3] while phototaxis refers to the ability of cells to respond to light stimuli. Bacteria have also been demonstrated to self-coordinate when patterned in monolayer carpets, creating effective microfluidic pumps and mixers.[4] Taken together, these attributes provide convincing motivation for the use of bacteria as configurable microactuators.

In the current experiment, a 50 μm equilateral triangular structure is fabricated using conventional microfabrication techniques,[5] which has been chosen for all experiments to maintain consistency. A thickness of 10 μm offers a balance between scale for bacterial actuation and structural rigidity for release from the substrate. Negative photoresist SU-8 Series 10 (MicroChem, Newton, MA) has been used for the microstructures based on observations that *Serratia marcescens* ATCC 274 (American Type Culture Collection, Manassas, VA) attach favorably to this material.

The bacteria *Serratia marcescens* are cultured using a swarm plate technique. Swarming bacteria are especially useful as actuators due to their rigor and size.[6] These bacteria are hyperflagellated, elongated, and migrate cooperatively.[7] An agar plate is inoculated on one edge with 2 μl of *Serratia marcescens* saturated culture, and swarming begins within 8–16 h. The inoculation site will generally turn pink slightly after the swarming motion develops. The swarm itself progresses across the plate in waves that appear as concentric rings with the most active bacteria along the outermost edge of the swarm.

A tracking algorithm was developed to study the velocity field of *Serratia marcescens* swarming on a soft agar plate. Average velocities for local regions regularly arranged over the entire flow field were investigated rather than those for individual bacteria. Consecutive images describing the minute motions of the swarming bacteria were captured and digitized, and imported into MATLAB for analysis. The 600 $\times$ 600 pixel investigation region was swarmed with thousands of bacteria, but due to difficulties with recognition of individual bacterium swarming in close proximity or having unclear boundaries limited by image resolution, local interrogation windows (10 $\times$ 10 pixel) for locally averaged velocity analysis were defined over the entire investigation field, with 5 pixel shifts in both vertical and horizontal directions of the image. Each interrogation window included several bacteria heading toward similar or different directions, and along the edges of these windows only the fraction of the individual cell bodies present in the window was included for calculation. To investigate an averaged velocity to compensate all bacterial motions involved in an interrogation window, two temporally consecutive but spatially similar interrogation windows were compared by shifting the latter window by 1 pixel in every major or diagonal direction with respect to the center of the former window to find the minimum change in pixel values by subtracting one window from the other and consequently obtain the new center of the shifted window. This method is equivalent to tracking the centroids of the moving bacterial carpet with coordinates given by

$$x_c = \frac{\sum A_i x_i}{A_{\text{total}}},$$

$$y_c = \frac{\sum A_j y_j}{A_{\text{total}}},$$

where x_c and y_c represent centroid coordinates and A represents area.

The velocity field of bacteria typically featured the combination of linear translation and vortex modes.[8] The bacteria

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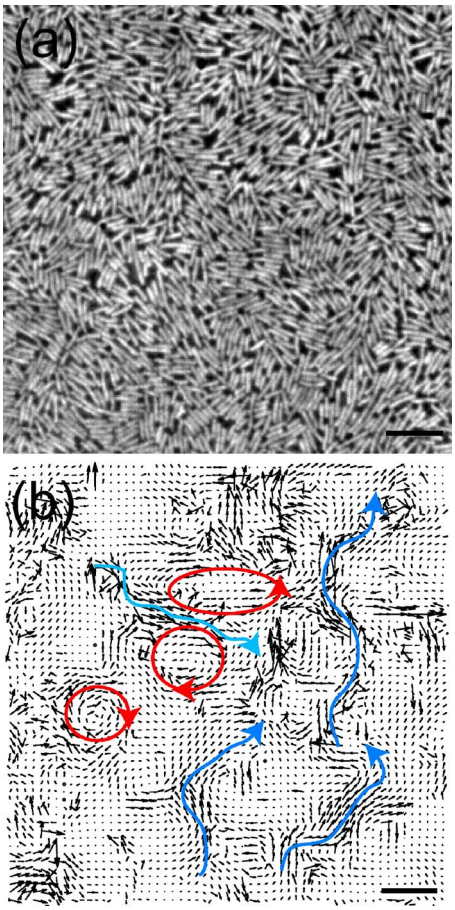

FIG. 1. (Color online) Swarming *Serratia marcescens* and (b) instantaneous velocity field of swarming bacteria. Clear examples of translating streams and rotating vortices are delineated. The scale bars are 10 μm.

formed several groups over the entire investigation region for a short time period, mostly translating along rather curved paths. They joined a temporarily main stream but shortly after branched off in all directions and joined other streams forming another translational stream or sometimes vortices. They repeated these patterns and the density of the bacteria in the investigated region seemed to be quite constant. The magnitude of the velocity ranged from 20 to 30 μm/s, and the diameter of the vortices appeared to be two to four bacterium lengths. The vector field of the investigated region indicates the averaged magnitude and direction in each local interrogation window (Fig. 1). The computed result showed good agreement with the original images.

To further investigate the flow patterns generated by the collective motion of the swarming bacteria, methods based on the temporal and spatial correlation functions of the velocity field on the swarm are exploited.[9] The spatial correlation function offers an insight to the degree to which the swarming bacteria are coordinated in some direction for a specific area of interest, while the temporal correlation function indicates the tendency of swarming bacteria to maintain motion in a particular direction over time. The spatial correlation function Ω is given as

$$\Omega(|\vec{s}|) \equiv \frac{\langle \vec{u}(\vec{r}+\vec{s}) \cdot \vec{u}(\vec{r}) \rangle}{\langle \vec{u}(\vec{r}) \cdot \vec{u}(\vec{r}) \rangle},$$

where $\vec{u}$ represents the velocity at location $\vec{r}$, and $\vec{s}$ represents the vector between each velocity vector. Correlation length

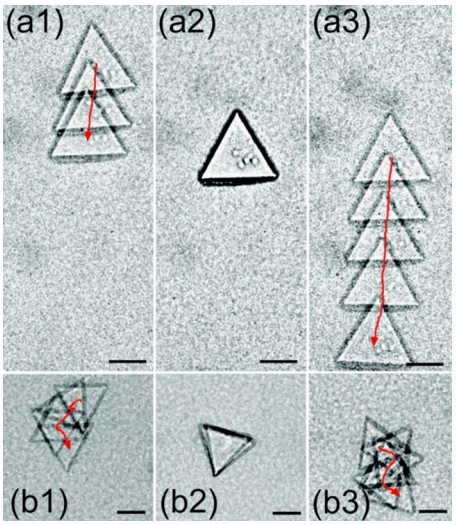

FIG. 2. (Color online) Phototactic control of a 50 μm triangular microstructure: (a1) translation with the average velocity of 9.15 μm/s before UV exposure, (a2) during 5 s exposure translation stops, (a3) after exposure translation fully resumes within 8 s, and (b1) rotation with the average angular velocity of 1.05 rad/s on swarm plate before UV exposure; centroid follows path shown (b2) rotation stops during 5 s exposure and (b3) rotation fully resumes within 7 s after exposure. The scale bars are 25 μm.

can be determined by integrating Ω. The correlation time for a generalized direction τ_{aa} can be found by integrating the temporal correlation function,

$$\tau_{aa} = \int_0^\infty \frac{\langle a(t)a(t+\tau) \rangle}{\langle a(t)a(t) \rangle} d\tau,$$

where a represents a generalized direction of interest, τ represents the time interval between interrogation frames, and t is the time. Using the bacterial tracking algorithm the mean correlation length is 8.47 ± 0.85 μm and the mean correlation time is 0.28 ± 0.056 s.

Microstructures were released from the substrate by applying minimal force along the edges with a scalpel blade. SU-8 adheres very strongly to the silicon surface; however, with such a small contact area the structures can be removed fully intact. Observations were first performed along the leading edge of the swarm. The microstructure motion generally translates in the direction of the swarm edge, and becomes immotile after the most active portion of the swarm passes over. The microstructure motion may be accelerated by adding a thin layer of motility buffer (0.01M potassium phosphate, 0.067M sodium chloride, $10^{-4}M$ ethylenediaminetetraacetic acid, 0.01M glucose, and 0.002% Tween 20, pH 7.0). The motility buffer, a solution rich in nutrients necessary for sustained bacterial motion, both accelerates the swarming motion on the surface of the agar and introduces a buoyancy force allowing the surface flagellar motion on the microstructure to be more easily studied. The higher density of SU-8 ($\sim$1.2 g/cm^3) relative to motility buffer keeps the microstructure in contact with the swarm surface.

Phototactic control of the microstructure is demonstrated by exposing the localized swarm region to ultraviolet (UV) light (Fig. 2). This is accomplished by introducing an unfiltered mercury vapor light source to the region, which is otherwise observed under phase contrast. After exposing the region temporarily to UV light, the local observation region

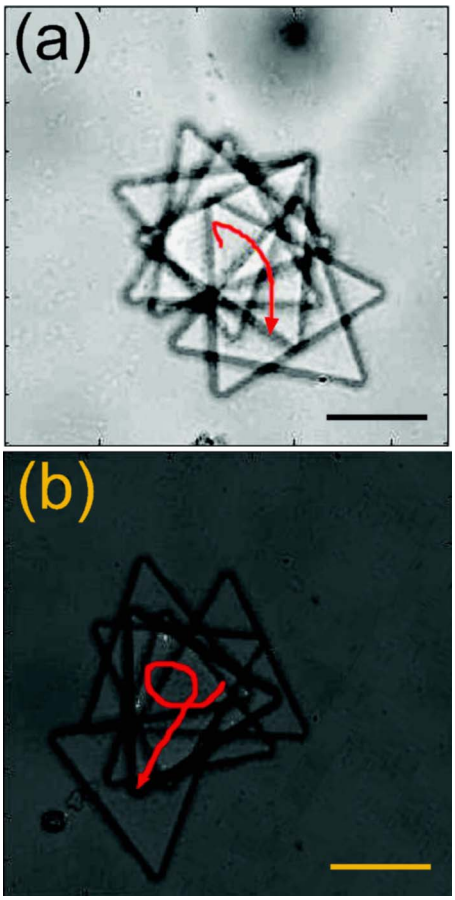

FIG. 3. (Color online) 50 μm triangular microstructure rotating in an open channel motility buffer; (a) rotational motion with 0.21 rad/s and (b) coiled motion with 0.31 rad/s. The scale bars are 25 μm.

becomes inactive within 5 s. Swarming and subsequent motion of the microstructure resume when the UV light source is once again shuttered, typically within 8 s.

Microstructures are also observed in the open channel of motility buffer utilizing a blotting technique.[4] The microstructures are first blotted on the swarm plate and rinsed off all agar and unattached bacteria. This process leaves a bacterial monolayer on the surface of the microstructure. These structures are introduced to an open channel of fresh motility buffer. The structures are free to move in any direction and, with the obvious exception of the bottom of the Petri dish, are unaffected by near-wall viscous interactions or other fluidic forces. By creating a bacterial monolayer on the surface of the structure, a velocity field similar to that found on the swarm surface is replicated on one face of the structures. The major difference is simply that the bacteria are fixed in place and the flagella self-coordinate.[4,10] This natural global self-coordination of the blotted bacteria exerts a net thrust on the microstructure causing rotational and/or translational movement of the microstructure (Fig. 3). Phototactic on/off control can again be demonstrated for microstructures in the open channel.

Phototactic control has been for these experiments due to its clear advantages over chemotactic control. Firstly, the response of the bacteria is immediate and uniform throughout the exposure region. Perhaps just as importantly, phototactic stimulus is easily removed without causing fluidic disturbances such as those introduced by refreshing chemicals.

Our results show that microstructures may be fabricated and actuated using bacteria on the swarm plate or in the open channel microfluidic environment. The phototactic response of bacteria to UV light may be utilized to exert on/off control of microstructures in either environment. The scale of the microstructure (50 μm) was chosen based on the result of the observation of Dombrowski *et al.*[8] Microstructure motion reflecting both the translational streams and the rotational vortices that are typical of bacterial swarming movement is observed and controlled through phototaxis. By replicating the velocity field on the surface of the microstructure by employing a blotting technique and global flagellar self-coordination, a self-powered microscale transportation system in an open channel has been demonstrated.

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