Hydrodynamics of a self-actuated bacterial carpet using microscale particle image velocimetry

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Microorganisms can effectively generate propulsive force at the microscale where viscous forces overwhelmingly dominate inertia forces; bacteria achieve this task through flagellar motion. When swarming bacteria, cultured on agar plates, are blotted onto the surface of a microfabricated structure, a monolayer of bacteria forms what is termed a “bacterial carpet,” which generates strong flows due to the combined motion of their freely rotating flagella. Furthermore, when the bacterial carpet coated microstructure is released into a low Reynolds number fluidic environment, the propulsive force of the bacterial carpet is able to give the microstructure motility. In our previous investigations, we demonstrated motion control of these bacteria powered microbiorobots (MBRs). Without any external stimuli, MBRs display natural rotational and translational movements on their own; this MBR self-actuation is due to the coordination of flagella. Here, we investigate the flow fields generated by bacterial carpets, and compare this flow to the flow fields observed in the bulk fluid at a series of locations above the bacterial carpet. Using microscale particle image velocimetry, we characterize the flow fields generated from the bacterial carpets of MBRs in an effort to understand their propulsive flow, as well as the resulting pattern of flagella driven self-actuated motion. Comparing the velocities between the bacterial carpets on fixed and untethered MBRs, it was found that flow velocities near the surface of the microstructure were strongest, and at distances far above, the surface flow velocities were much smaller. © 2015 AIP Publishing LLC.

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I. INTRODUCTION

In low Reynolds number microfluidic environments, the relative importance of forces for propulsion vastly differs from that of larger Reynolds number flows, as viscosity dominates over inertia in small scale flow regimes. To overcome the strong viscous forces at this scale, nature has developed nonconventional propulsion methods for microorganisms, namely nonreciprocal motion.¹ There are various ways to swim in viscous dominated fluids; one such way is the helical motion of bacterial flagella. Bacteria such as *Serratia marcescens* have several flagella, generally 10 μm in length and 20 nm in diameter, which can generate a forward thrust with the ability to change heading orientation. These flagella extended through the cell body from a reversible rotary motor allowing the flagella to bundle and unbundle due to counterclockwise (CCW) and clockwise (CW) rotation, resulting in run and tumble motions, respectively.^{2–5} During running, the bundled flagella act as a rigid helix, which upon rotation produce nonreciprocal corkscrew motion and converts viscous drag into forward thrust.

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Using this nonreciprocal rotational motion, bacteria, such as the *S. marcescens* used in this work, can swim up to 30 $\mu\text{m/s}$, and during a tumble, change their heading angle.^{4,5}

The hydrodynamics of flagella on an individual bacterium has been well studied through numerical modeling and experiments.^{3,6–10} Flagellar propulsion had also been studied using various models, such as by treating the flagellum as a long slender rod on the spherical cell body,^{7,9} or as a network of elastic springs.³ Additionally, experimental studies have been conducted to characterize the flow fields caused by bacterial flagella.^{9,10} However, these works investigated flagellar propulsion without considering intercellular interactions; which is an important aspect for harnessing the collective propulsive power of bacteria for engineering tasks. The generated propulsive force, by the collective flagellar motion of thousands of bacteria, has shown the capability to actuate microfluidic flows for mixing and pumping,^{11,12} and in the field of robotics, substrates coated with densely packed bacteria, termed bacterial carpets, have been shown to supply propulsive force for microrobots.¹³ Furthermore, when bacteria are packed densely together, as is the case in microbiorobots (MBRs), bacteria can generate substantial flows, resulting in bacterial “turbulence.”^{14,15}

Communities of flagellated bacteria collectively swim or swarm due to chemical and hydrodynamic influences. Ishikawa *et al.* evaluated the interaction between two swimming bacteria using a numerical method and found that the final heading orientations of the bacteria are strongly influenced by flagellar hydrodynamic interactions between neighboring cells.¹⁶ Furthermore, it has been observed that the swarming behavior of densely packed bacteria can lead to a vastly different type of motility which highlights the distinction between individual cells and a community.^{17–20} In an effort to harness a large number of bacteria for engineering applications, swarming bacteria have been blotted onto surfaces, creating bacterial carpets for mixing, pumping,^{11,12} and robotic tasks.^{13,21} These hybrid structures, consisting of an inorganic surface (usually SU-8) coated with bacteria, have been termed as MBRs. The thrust generated by the bacterial carpets of MBRs results in microstructure movement and allows for the possibility to perform tasks, such as microscale transportation, with these structures.²² When a MBR is released into a fluid, the attached bacterial carpet actuates the MBR, due to the random motion of their flagella on the bottom glass substrate. The self-coordinated motions of untethered pieces of PDMS propelled by bacterial carpets have been previously demonstrated.²³ To quantify the force generated from bacterial carpets, flow fields above the structures were characterized in unbounded conditions by tracking tracer particles on the surface of the wide bacterial carpet.²³ While cell orientation was not controlled in these experiments, it has been previously reported that cell orientation does not greatly impact generated flow fields.²³ However, this work did not investigate the flow field related to self-actuated motion near the surface of the bacterial carpet. In this paper, we investigate the flow field strength caused by interactions between rotating flagella near and away from the substrate surface.

There are a number of studies showing that flagellar motion, and the corresponding hydrodynamic interactions, is different near the surface of the bacterial carpet, and that the swimming velocity increases when a flagellated microorganism swims near this region.^{7,10,24,25} However, no studies thus far have been conducted on near boundary interactions of bacterial carpets; such studies can elucidate the bacterial carpet’s capability as a driving force for MBRs motion. Here, we have applied microscale particle image velocimetry (μPIV) to understand the flow generated by bacterial carpets. Specifically, we observed the flow field generated by bacterial carpets in 80 μm high PDMS chambers. In case of the bacterial carpets on static structures, all experimental videos were recorded at separate times sequentially. Only the 2-dimensional flow field was analyzed due to experimental limitations.

II. MATERIALS AND METHODS

A. Cell culture and swarming agar plate

Experiments were conducted using the bacterium *S. marcescens* (ATCC 274, American Type Culture Collection, Manassas, VA), 1–2 μm in length, grown overnight on an agar plate for swarming. The agar used was composed of 2.5 g Difco Bacto tryptone, 1.25 g yeast extract, 1.25 g NaCl, and 1.5 g Difco bacto agar in 250 ml of deionized water. The solution was poured

into 14 cm Petri dishes and allowed to solidify after adding 600 μl of 25% glucose solution. The swarm plate was inoculated on the edge of the plate as shown in Fig. 1(a) with 5 μl of *S. marcescens* culture LB broth. The inoculated plate was incubated for 8–15 h at 34 °C. *S. marcescens* on the swarm plate produces polysaccharide on the body, and this pink material helps *S. marcescens* to stick the surface.^{26,27}

B. Fabrication of static and moving microstructures and blotting bacteria

The microscale structures for attachment of bacteria were made by a general photolithographic process. Figure 2 illustrates how the static and the moving MBR structures were fabricated. Figure 1(b) shows a bright field image of static structures, fabricated on cover glass substrates using SU-8 2035, blotted with *S. marcescens*; the thickness was 35 μm . A dark field chrome (Cr) mask used for patterning micro scale structures had 30 $\mu\text{m} \times 34 \mu\text{m}$ rectangular patterns. Individual structures were separated 40 μm apart so that each bacterial carpet on the respective structure did not interfere with bacterial carpets on neighboring structures. In case of moving structures, a sacrificial layer of dextran was spun onto the glass slide before SU-8. After patterning of SU-8 and blotting bacteria, this sacrificial layer was removed by gentle agitation in water, releasing untethered microstructures. This released structure was subsequently self-actuated by the propulsive force of the bacterial carpet. The fabricated structure in this case has the same dimension with the static structure due to the same Cr mask. However, the final thickness of the moving structure was 3 μm . Fabricated microstructures were then blotted with swarming *S. marcescens* by inverting the glass slide onto the active swarming region. (Fig. 1(a))

C. Data acquisition and analysis

In order to measure the flow velocity using particle image velocimetry, a laser system is required to obtain accurate particle movement through consecutive images. Our system consisted of a He-Ne continuous wave laser, which provides a bacteria safe 633 nm wavelength light source. According to Wright *et al.*²⁸ and Taylor *et al.*,²⁹ blue light influences the motility of bacteria because of the perturbation of electron transport in the body. Thus, the use of lower wavelength, high intensity light sources, e.g., 390–530 nm, leads to slow swimming. However, it was observed that bacterial motility is normal under 633 nm wavelength laser light, and even with 3 s exposure times does not have a significant impact on bacteria motion.³⁰ We applied a combination of optical devices to create a uniform expanded beam employing a 10 $\times$ beam expander and a cylindrical lens in front of the beam expander. The image was captured by a high speed camera, Photron FASTCAM SA3, which is equipped with CMOS chip. The CMOS chip has 1024 $\times$ 1024 pixels with 12 bits, and each pixel size is 17 μm . Red fluorescent beads (0.2 μm in

FIG. 1. Bacteria culture and bacterial carpet after blotting method on pillar static structures. (a) Swarming bacteria cultured on an agar plate, where the outer edge of the swarming culture yields the most active bacteria used to create bacterial carpets and MBRs. (b) Static structures after blotting process.

FIG. 2. Schematic diagrams of the microfabrication procedure required to fabricate a SU-8 platform for bacterial attachment. (a) Process to fabricate a static structure used to create bacterial carpet. (b) Process to fabricate a detachable structure via dextran sacrificial layer to create a moving MBR.

diameter) were used for measurement of particle velocity. After recording images continuously at 250 Hz, a commercial software package (DaVis 8.0) was used to calculate velocity vectors through a 32×32 pixel interrogation window with adjacent windows overlapping by 50% using consecutive images. In processing of PIV-time series, image processing and vector post processing, such as smoothing, were applied. Figure 3 shows our experimental setup. The viewing window had dimensions of $5 \text{ mm} \times 10 \text{ mm} \times 80 \mu\text{m}$. For visualization of the moving structure, low intensity bright field was used, and was later removed through post processing.

III. RESULTS

A. Validation of experimental setup

We performed two experiments to characterize the flow field generated by bacterial carpets. For the first experiment, we blotted bacteria onto stationary microstructures attached on a substrate. We then seeded the fluid above the bacterial carpet with fluorescent tracer particles with a mean diameter of 200 nm (Life Techn. Co., F8807) and measured the velocity flow field on

FIG. 3. Schematic representation of the μ PIV measuring system. (a) Optics assembly for the laser system used to direct a collimated light beam into the microscope for uniform illumination. (b) Image acquisition setup capable of high speed recording at 250 fps under $100\times$ magnification.

the plane of the bacterial carpet, and at incremental heights above the carpet via μ PIV. For the second experiment, we blotted bacteria onto the bottom of untethered microstructures which effectively created 1 μm in thick bacterial carpet on bottom of the microstructures; we then used μ PIV to measure the flow field of this layer. Both experiments were performed in a sealed chamber filled with deionized water.

To check the experimental uncertainties in velocity fields, root-mean-square (rms) error σ_u in the velocity measurement was calculated with the following equation:

$$\sigma_u = \frac{\sigma_{\Delta x}}{M_0 \Delta t}, \quad (1)$$

where M_0 is the objective magnification ($100\times$) and $\sigma_{\Delta x}$ is the rms error of the displacement on the pixel plane. We chose the rms error to be in the range of 4% of the recorded image diameter³¹

$$\sigma_{\Delta x} = 0.04(d_e^2 + d_r^2)^{1/2}, \quad (2)$$

where d_e is the optical diameter of the image before being recorded on the pixel plane. When d_r is 17 μm , the resolution of the recording medium is the same as the pixel size of the CMOS chip. The diameter d_e of the diffracted particle image is given by³²

$$d_e^2 = M_0^2 d_p^2 + [2.44(1 + M_0)f^\# \lambda]^2, \quad (3)$$

where d_p is the diameter of the fluorescent tracer particle diameter (0.2 μm), f is the f -number of the imaging lens 0.4, and λ is wavelength of the laser (633 nm). Therefore, the one particle diameter in a recorded image $(d_e^2 + d_r^2)^{1/2}$ is calculated as 18.88 μm , which means that one particle occupied more than one pixel. Substituting Eq. (3) into Eq. (2) results in $\sigma_{\Delta x} = 0.75 \mu\text{m}$ and $\sigma_u = 1.88 \mu\text{m/s}$. The rms error of velocity is acceptable because the magnitude of the error is smaller than the normal velocity of bacteria, normally 50 $\mu\text{m/s}$, and therefore, Δt (0.004 s) is enough time to capture the induced flow field caused by flagellar rotation (100–240 Hz). In the experiment, we changed the observation focal plane from the surface of bacterial carpet to different heights for investigating the velocity flow depending on height. The depth of field was calculated in order to determine an increment rate. The equation for the depth of field in Refs. 33 and 34 is written as

$$\delta z = \frac{3n\lambda}{\text{NA}^2} + \frac{2.16d_p}{\tan \theta} + d_p, \quad (4)$$

where n is the index of refraction of the immersion medium between the microfluidic system and the lens immersion medium. In our case, n was 1.515. The numerical aperture (NA) of our objective lens is 1.25. The small light collection angle θ was 55.6° , and d_p was the same as the rms error in the velocity measurement. As a result of Eq. (4), the field of depth was 2.47 μm . We increased the height of the focal plane by an increment of 2 μm , up to 50 μm , for the first experiment with stationary structures. The schematic in Fig. 4 depicts the μ PIV measurements for both experiments.

B. Motion of MBR by self-actuation

Untethered MBRs generally show clockwise rotational motion as a result of the flagellar motion of the bacterial carpet, which is characterized as self-actuated motion,^{21,22} as shown in Fig. 5(b). Self-actuation is caused by the thrust generated from the bacterial carpet on the bottom of the microstructure, as described in a kinematic model by Julius *et al.*²¹ The geometry of the structure does not play an important role in the motion.^{13,21} For each individual blotted structure, the self-actuation is different due to uncontrollable biological factors of the bacteria, which can be modeled using various parameters in the kinematic model.²¹ Previous experiments

FIG. 4. Schematic of the μ PIV experimental setup. (a) Bacterial attachment on the static structure surface creating a bacterial carpet. The μ PIV experiment sought to capture the flow field created by the flagellar motion of the attached bacterial carpet. (b) Experimental setup showing the MBRs, objective lens, and focal planes (S_{obs} is observation space in experiment).

measured the average velocity of MBRs, due to self-actuation, as $5 \mu\text{m/s}$. In two different experiments, we seek to investigate the flow field generated from the bacterial carpet using μ PIV in order to understand the flow patterns and the hydrodynamic interactions that result in the actuation motion. From our first experiment using a stationary structure, we investigate the flow in bulk fluid by observing the fluid above the bacterial carpet (Fig. 5(a)). From our second experiment using untethered structures, we investigated the hydrodynamic interaction at the lubrication layer where flagella-induced flow interactions with the substrate results in self-actuation (Fig. 5(b)).

C. Velocity flows induced by flagellated bacterial carpets

As a control experiment, we first investigated Brownian motion without the presence of bacterial carpets by performing μ PIV measurements on unblotted fixed microstructures with the same dimension as the ones used for the other experiments. The presence of these fixed microstructures provided a controlled environment for studying flagella generated flow fields by

FIG. 5. Bacterial carpet on static structure and self-actuated MBR. (a) Bacterial carpet attached on the structure from Fig. 1(b). (b) Self-actuation of a MBR resulted from flagellar motion of the attached bacteria.

maintaining the same near-wall conditions for the tracer particles. We hypothesized that the flow induced by the bacterial carpet would be significantly greater than that from the Brownian motion observed from the control experiment. For all experiments, we observed the flow field at the surface of the microstructure, or bacterial carpet, and at planes up to $22\text{ }\mu\text{m}$ above that surface, with an increment of $2\text{ }\mu\text{m}$. The surface was determined by the first plane where the microstructure surface was visible under bright field imaging.

For the static structure experiment, the thickness of the microstructures was $35\text{ }\mu\text{m}$, which was chosen to give sufficient separation between the bacterial carpet on the microstructures' top surface and the bacteria attached on the glass substrate. Even though the body of bacteria was fixed on the surface, the flagellar hook between the flagellum and the motor is very flexible, enabling the filaments to rotate CCW and CW independently. The recording time for each video was 2.42 s with a rate of 250 frames per second (fps). The velocity flow direction and streamline from one static structure experiment are shown in Fig. 6 at 0.02 s, 1.00 s, and 2.00 s, respectively. Figure 6 shows the instantaneous flow field found using the PIV data at three different times (0.02, 1, and 2 s). The magnitude of velocity vectors in Figs. 6(a)–6(c) is represented by color map in Figs. 6(d)–6(f). The vectors in regions corresponding to blue regions in Figs. 6(d)–6(f) have zero velocity and most of that area has weak velocity. However, the strongest velocity vectors are located in the region directly above the MBR structure. Several microvortices were created overtop of the static structure area where the bacterial carpet exists (white rectangles in Fig. 6), similar to the results found by Darnton *et al.*²³ The locations of the microvortices and dissipation of the microvortices are shown in Figs. 6(d)–6(f). In Fig. 6, it is clear that at 0.02 s, a microvortex was present above the bottom portion of the structure, and after 1 s dissipated. It is also evident from Fig. 6 that at the 1 and 2 s marks, three microvortices formed that were not present at 0.02 s. Fluid flow above the active surface of the static structure seemed to consist of several microvortices, rotating either CW or CCW. The distance between microvortices inside of the structure was $16.8 \pm 6\text{ }\mu\text{m}$ during the recording time. As a result, the microvortex located in middle of the structure became weak at 2.0 s. In order to quantify the propulsive force, the instant ensemble average velocity for three frames was calculated using the generated velocity field (rectangle in Fig. 6). The instantaneous ensemble average

FIG. 6. Velocity flow fields on the static structure. (a) Velocity flow by μPIV result data with same scale arrows on the region of the structure at 0.02 s. (b) Velocity flow by μPIV result data with same scale arrows on the region of the structure at 1.00 s. (c) Velocity flow by μPIV result data with same scale arrows on the region of the structure at 2.00 s. (d) Streamline and vortex at 0.02 s (e) Streamline and microvortices at 1.00 s. (f) Streamline and microvortices at 2.00 s.

velocity represents the average magnitude of flow on the surface. The ensemble average velocities for each frame were $11.4 \mu\text{m/s}$, $6.9 \mu\text{m/s}$, and $6.2 \mu\text{m/s}$, respectively.

For each height, we calculated the mean-square displacement (MSD) using

$$\langle \Delta \vec{r}^2(\Delta t) \rangle = \langle (\vec{r}(t + \Delta t) - \vec{r}(t))^2 \rangle, \quad (5)$$

where $\vec{r}$ is the representative particle position calculated by total displacement of all interrogation windows using the velocity flow results obtained by DaVis software. The MSD calculated for the control experiment (Brownian motion of tracer particles) and the MSD from the bacterial carpet were compared in Figs. 7(a)–7(c). Each experiment was performed using two different cases for Brownian motion and bacterial carpet respectively. In our control experiment, where we measured the Brownian motion of 200 nm fluorescent tracer particles, there was a deviation between our experimental data and the theoretical MSD. Comparing the two, we obtained a difference of $14.61 \mu\text{m}^2$ for the last time point (2.5 s). This deviation between the experimental and theoretical values can be attributed to measurement noise. In the case of Brownian motion, the change in mean-square displacement, as the height of the observation view increased, remained nearly constant (Fig. 7(a)). At 2.42 s , the MSDs for Brownian motion were $59.5 \pm 5.9 \mu\text{m}^2$ (Fig. 7(a)). However, in cases of bacterial carpets, MSD increased until $10 \mu\text{m}$ height as shown in Figs. 7(b) and 7(c). Moreover, the MSD at 2.42 s was much bigger than Brownian motion. $10 \mu\text{m}$ above the surface of the static structure, the slope of the mean-square displacement began to decrease as is the focal plane moved further away from bacterial carpet. However, it did not show a gradual drop in comparison to previous work.²³ A possible explanation for this discrepancy is that our PDMS chamber had a smaller distance from bacterial carpet to the top surface of the chamber ($80 \mu\text{m}$) than the chamber used by Darnton²³ (more than $160 \mu\text{m}$). The maximum MSD for two bacterial carpets was around $170 \mu\text{m}^2$ at $6 \mu\text{m}$ height.

D. Flow velocities generated by bacterial carpets on self-actuating structures

In the second experiment, we observed moving structures with self-actuation by the bacterial carpet (see supplementary material³⁵). There were three main differences in this experiment compared to the first experiment with static structures: (1) The structures were not fixed to the substrate, thus, free to move when subject to external forces; (2) the bacterial carpet was on the bottom of the SU-8 structure; therefore, the bacterial carpet was close to wall, and boundary effects on the flagella-induced flow field must be taken into account; (3) the structures observed exhibit self-actuated motion, which means they changed the position throughout the duration of the experiment. It is important to note that, although the bacterial carpet of the untethered MBR was face down, because of the bacteria secreted lubrication layer, a thin layer of liquid, approximately $1 \mu\text{m}$ in depth, separated the bacterial carpet from the glass substrate. The depth of lubrication layer was estimated by moving an observation plane of microscope up from the focal plane of the bottom substrate to the focal plane of the bacterial carpet. In this experiment,

FIG. 7. Comparison of mean square displacements (MSDs) for Brownian motion and bacterial carpet generated particle flow. (a) MSD for Brownian motion, (b) bacterial carpet measured at different depths for case 1, and (c) bacterial carpet measured at different depths for case 2.

we performed μ PIV measurements at this lubrication layer. As mentioned, the layer is only approximately $1\text{ }\mu\text{m}$; hence, we only measured one plane.

The self-actuated structure rotated clockwise with translation motion as shown in Fig. 8. Using μ PIV analysis, we studied the streamlines and microvortices that developed in the flow field, as shown in Figs. 8(d)–8(f). A strong velocity flow profile was observed above the middle of the MBR, where traced beads were transported outward from the structure area by the strong flow. Like the static structure, the microvortices in the flow field were shown on the surface of bacterial carpet. However, the strong streams of flow were much stronger in the moving MBR at relatively the same area on the static structure surface at each time in Fig. 8(a)–8(c). A small microvortex was observed left bottom corner between 1.14 s to 2.02 s. We hypothesize that these microvortices result from the interaction between flagella and boundary. Additionally, the ensemble average velocity vectors were calculated for the three timescales with magnitudes of $8.9\text{ }\mu\text{m/s}$, $21.9\text{ }\mu\text{m/s}$, and $18.3\text{ }\mu\text{m/s}$, respectively. These ensemble average velocities were larger than the ones of the static structure experiments, which indicates that the displacement of the untethered structures contributes to an increase of flow.

To compare the strength of flow fields generated by the blotted static structures and the untethered structures, we evaluated their respective MSDs (Fig. 9). The static structure, for which the MSD was calculated using the experimental data at $6\text{ }\mu\text{m}$, presented the strongest flow field. The MSD of pure Brownian motion was significantly smaller than both the static and untethered cases (Fig. 9), and the MSD of the static structures was smaller than the untethered cases by $36.3\text{ }\mu\text{m}^2$ at 2.42 s. The difference of MSDs for the static and untethered cases seemed to be caused by the presence of a near-boundary effect on untethered bacterial carpet. We have performed the experiment numerous times and see a general increase in the average MSD for untethered cases.³⁵

In case of self-actuated MBRs, the displacement of moving structure might need to be accounted for in our MSD calculations. However, we found that the difference of MSD using a displacement of moving structure had, on average, only 0.09 less than the MSD calculated without subtracting the displacement of self-actuated structures by bacterial carpet, shown in

FIG. 8. Velocity flow fields on the moving structure MBR. (a) Velocity flow by μ PIV result data with same scale arrows on the region of the structure at 0.14 s. (b) Velocity flow by μ PIV result data with same scale arrows on the region of the structure at 1.14 s. (c) Velocity flow by μ PIV result data with same scale arrows on the region of the structure at 2.02 s. (d) Streamline and microvortices at 0.14 s. (e) Streamline and microvortices at 1.14 s. (f) Streamline and microvortices at 2.02 s.

FIG. 9. Mean-square displacement comparison between the flow field of self-actuated MBRs and the flow field of the bacterial carpet at 6 μm height on the static structures, and Brownian motion.

Fig. 9. Thus, subtracting the displacement of the moving structures did not have a significant impact on the results.

E. Diffusion coefficient of bacterial carpet

In order to evaluate the strength of the flow field, depending on height, we evaluated the diffusion coefficient D using the diffusion equation

$$\Delta \bar{r}^2(\Delta t) = 2dD\Delta t, \quad (6)$$

where d means a dimension of flow field (2 in this case). The diffusion coefficient for Brownian motion and the bacterial carpet on static structure are shown in Fig. 10. The bacterial carpet has much higher diffusion coefficient than Brownian motion, and the largest value exists at the height of 6 μm . Figure 10 indicates a strong collective flow induced by bacterial carpet presented within the flagellum length, which is nominally 10 μm . Note that the diffusion coefficient is determined using PIV measurements and, therefore, is subjected to random errors due to large Brownian motion displacement and small particle size. According to Olsen and

FIG. 10. Diffusion coefficients for Brownian motion and bacterial carpet measured at different heights.

Bourdon,³⁶ a 600 nm particle with 1.63 pixels of displacement due to Brownian motion (3–4 particles per interrogation window) is approximately 74%. In our experiments, the particles are 200 nm with an average Brownian motion displacement of 1.41 pixels (3–4 particles per interrogation window); thus, we can expect a base line error due to Brownian motion. Another potential source for discrepancies is the small particle size leading to pixel locking. In general, the seeding particles should occupy at least three or four pixels in diameter; however, our particle images (after image processing) occupy 1–2 pixels.

Based on the work of Darnton *et al.*,²³ the effective diffusion coefficient decreases gradually with increasing height from the surface of bacterial carpet to 20 μm . Similarly, the result for the static structure experiments also showed a drop in diffusion coefficient by a maximum of 4 $\mu\text{m}^2/\text{s}$ after reaching a peak at the height of 10 μm as shown in Fig. 10. However, the rate of decrease in diffusion coefficient gradually becomes smaller, and then the diffusion coefficient eventually began to increase, reaching another peak at 20 μm . After 20 μm , the diffusion coefficient increased again. This increase is most likely due to the MBRs interaction with the upper boundary of the test chamber. Due to the need to reduce background noise from the focus of fluorescent particles, we restricted the height of the test chamber to 80 μm . With the additional height from the microstructure, and the bacteria monolayer, the first planes (0 μm) for μPIV measurement have a distance of approximately 40 μm from the chamber's upper boundary. The flow field of the bacterial carpet has an effective range of approximately 50 μm ,²³ so it is assumed that the flow of the bacterial carpet, in the static structure experiment, was due to flow fields in the bulk fluid interacting with the chamber's upper wall. Therefore, the increase in diffusion coefficient before the peak at 20 μm is most likely the result of near-wall hydrodynamic interactions, leading to dissipation which created a stronger planar (xy) flow at the expense of confinement in the z -directional flow. These near-wall hydrodynamic interaction are significantly affected by a thin lubrication layer—produced by *S. marcescens*, which reduces the surface tension of water—and enable *S. marcescens* to easily migrate and swarm.²⁶ The diffusion coefficient value of the moving MBR structures at the lubrication layer was $22 \pm 2 \mu\text{m}^2/\text{s}$, which is larger than the maximum value from the static structures.

F. Ensemble average velocity of bacterial carpets

The ensemble average velocities for each experiment (Fig. 11), which serve as good measures of the strength of the flow created by the bacterial carpet's flagellar motion, were compared for static structures and moving MBRs. The average ensemble velocity of the bacterial carpet on static structures is 10.2 $\mu\text{m}/\text{s}$, which is larger than that of Brownian motion. In the case of moving MBR structures, the mean of ensemble velocity was 20 $\mu\text{m}/\text{s}$, which is larger than that of the static structures. The standard deviations for each case are 5.3 and 10.4,

FIG. 11. Comparison of ensemble average velocities for each experiment. (a) Ensemble average velocity comparison between bacterial carpet on the static structures and Brownian motion. (b) Comparison between ensemble average velocity of self-actuated MBRs and velocity of the body structure.

respectively. This can be attributed to the dissipation of flow due to a thin lubrication layer composed of secreted surface-active cyclodepsipeptides, e.g., *Serrawettin W1*, produced by *S. marcescens*, which act as wetting agents.³⁷ Comparing with the average velocity of the moving MBR, which is $5.63 \mu\text{m/s}$, the ensemble average velocity of the flow field was higher, by a factor of 2, which supports the hypothesis that the self-actuation motion was generated by strong flow fields interacting with the boundary at the bio surfactant lubrication layer. The fluctuation of the ensemble average velocities in both experiments, shown in Fig. 11, can be explained by the random switching of flagellar motion on individual bacteria. The independent flagellar rotation change, from CCW to CW, creates peaks and transient oscillations in the flow and the instantaneous strong velocity flow might result from strong hydrodynamic interactions between flagella. Also, the frequency of the velocity for the static structure case was 14 Hz using power spectra.³⁵ However, in case of untethered structure, the highest peak occurs at a frequency of 118 Hz.³⁵ This computation indicates that the bacterial carpets on moving structures generate strong flows more frequently than the bacterial carpets on static structure. The resulting hydrodynamic effects between the untethered bacterial carpet and the boundary plate may affect the measured swimming velocities.

The velocity flow profile was shown to be affected by the boundary for both the static and moving structure. As the separation distance between the bacterial carpet and the boundary decreased from $42 \mu\text{m}$ to $1 \mu\text{m}$, the ensemble average velocity in flow increased by 90% as shown in Fig. 11. The hydrodynamics of flagellar motion can be approximated by a resistive force that is proportional to the difference between the fluid velocity and the local flagellar velocity.^{38,39} When the flagellar motions occur near a wall, the important linear dimension in the resistance coefficients becomes the effective separation distance from the wall with same beating of flagellum.³⁸ The individual bacteria that adhered on the surface increased the resistive force of the MBR, and consequently imparted translational and rotational drag on the surrounding fluid, as seen through the fluorescent particles moving faster. As the cell body is close to the boundary surface, the resistive force becomes stronger because the short distance between flagellar motion and boundary surface makes large difference of velocity field of flagellar velocity from the fluid velocity. Therefore, the effect of the fluid near-boundary leads to the increase of the resistance coefficients, resulting in stronger propulsive forces and the MBR self-actuation.

Using the ensemble velocities, we plotted the accumulated displacement for each experiment in Fig. 12; as expected, the accumulated displacement of the moving structures showed a wider motion range, as compared to the other two experiments with static structures and

FIG. 12. Ensemble motion of moving MBRs, bacterial carpet on static structures, and Brownian motion calculated using their respective velocity flow fields.

Brownian motion. The individual bacteria on the adhered surface increased those ratios consequently, and this advantage gave an effect to translational and rotational drag on the cell body as the motion of fluorescent particles moved faster.

IV. CONCLUSION

The collective motion of microorganism serves an important role in low Reynolds locomotion and as examples for generating propulsive force for robotic systems. MBRs utilize bacterial carpets which are composed of multiple bacteria that generate strong thrusts through coordinated flagellar motion. While the kinematics of MBRs has been thoroughly studied, the flow interactions that generate their self-actuation motion are unclear. To elucidate this matter, we used μ PIV to measure the velocity flow fields created by bacterial carpets on static structures to study the flagella-induced flow generated by fixed and untethered structures with a lubrication layer. In the first case with static structures, the flagella-induced flow fields diminished at heights higher than $35\text{ }\mu\text{m}$, which matches favorably with previously published reports. In the second case with untethered structures, the boundary effects in the lubrication layer yielded a stronger in-plane velocity field, resulting in self-actuated motion. Further analysis using MSD and diffusion coefficient demonstrated the increased flow from the bounded condition of the lubrication layer. Finally, our μ PIV analysis provides insights into the flagellar hydrodynamics of bacterial carpets and enhances understanding of the effects of flagella driven boundary flows.

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