Bacterial Flows: Mixing and Pumping in Microfluidic Systems Using Flagellated Bacteria

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This report is based on the PhD Thesis of MinJun Kim, supervised by Professor Kenneth Breuer, Division of Engineering, and submitted to the Graduate School at Brown University in May 2005. Correspondance should be addressed to Kenny Breuer: kbreuer@brown.edu
The objective of the proposed thesis is to demonstrate the use of bacteria as controllable elements of a microfluidic network in micro-engineered systems. We utilize flagellated bacteria (Escherichia coli and Serratia marcescens) whose motors are extremely powerful and reasonably well understood. We are strongly against the removal of the motor from the bacterium or the fabrication to mimic the microflagellar system since the full organism is already naturally designed to accomplish many engineering tasks. The core of the proposed study relies on the use of flagellated bacteria to actuate surrounding fluid in a controlled and directed manner. Flagellated bacteria are used both as individual actuators and in arrays (bacterial carpet), where the collective effort of the organisms can be applied to complete microfluidic systems such as a chaotic mixer and a fluidic pump. The effect of bacterial motion on the diffusion of a molecule of high molecular weight is studied by observing the mixing of two streams of fluid in a microfluidic flow cell. The presence of motile E.coli bacteria in one of the streams results in a remarkable increase in the effective diffusion coefficient. If a large number of Serratia marcescens are encouraged to dock to the walls of a microchannel, then a flagellar carpet is created with unique properties. The activity of the bacterial carpet can be used to enhance the mixing between two streams of fluid. Furthermore, we observe that the diffusion process is also shown to undergo a change from standard Fickian diffusion to superdiffusive behavior in both cases. Another exciting property of a bacterial carpet in a microfluidic system is the observation that it can pump fluid autonomously at speeds as high as 25 microns per second. The bacteria draw their propulsion energy from the nutrients in the motile buffer and hence the system is a self-contained microfluidic component. Factors governing the behavior of the bacteria-powered pump are tested to determine pumping characteristics as well as the durability of the bacterial motor array under a wide variety of external stimuli (food, temperature, geometry). Lastly, we investigate the mechanisms that lead to the onset and decay of self-organization and coordination of the flagella over length scales hundreds of times larger than the size of the individual cells.

Min Jun Kim has been experimentally investigating the mechanics of fluids at small scales including the behavior of biological materials in micron and sub-micron geometries and the characteristics of electrokinetic flows in microfluidic systems. Mr. Kim was born in Seoul, republic of Korea in 1972. After graduating from Whi-Moon High School, Seoul, he enrolled at Yonsei University. As a student of Mechanical Engineering he got his Bachelor of Science from Yonsei University in 1997. In the early stage of his academic career, he realized the necessity of higher education from a renown University in the field of Engineering and Technology. To achieve this goal, he came to Texas A&M University, College Station, TX in 1999 and obtained M.S.M.E in Microfluidics in 2001. Mr. Kim started his Doctoral degree in 2002 in the field of Microfluidics at Brown University, Providence, RI, and worked on Bacterial Microfluidics at Micro-Fluidics Laboratory. Mr. Kim is the recipient of a Simon Ostrach Fellowship from Brown University. Mr. Kim has also authored a number of articles in peer reviewed journal and conference.

This thesis considers the study of bacterial flows in a microfluidic system. The first chapter is a summary of the research presented in the papers included in the others. The summary includes an introduction to the basic concept, a review of previous works and a presentation discussion of the main results obtained. The set-up, procedure, and results of this experimental study are presented in several chapters and appendices in this document.
The thesis is based on and contains the following papers.


In Chapter 2, a macro-scale model for bacterial flagellar bundling is introduced to understand hydrodynamic interaction of the rotating helices. We also present particle image velocimetry (PIV) measurements of an extremely complex three-dimensional flow at a very low Reynolds number. My role in the research was to construct the macro-scale particle image velocimetry (PIV) system to measure the full-field velocity distribution for rotating helices. The works in Chapter 2 have been also published by Min Jun Kim, MunJu Kim, James C. Bird, Jinil Park, Thomas R. Powers, and Kenneth S. Breuer, *Proceeding of PIV03*, Busan, Korea, No 3313, 2003. The chaotic mixing due to motile bacteria is studied experimentally in Chapter 3. Moreover, the diffusion process is observed to undergo a change from standard *Fickian* diffusion to a superdiffusive behavior. The results presented in Chapter 3 have been simultaneously published by Min Jun Kim and Kenneth S. Breuer with help from Linda Turner, *Proceeding of 2003 ASME IMECE*, Washington D.C, IMECE2003-44014, 2003. In Chapter 4, we present results demonstrating the activity of the bacterial carpet propelled by global coordination of bacterial flagellar bundles, which is successfully utilized as actuators for microfabricated fluid systems. In addition, we show that the performance of the carpet-activated microfluidic system changes in response to modifications to the chemical and thermal environment of the bacteria. A particularly exciting result is shown that the pumping performance is affected by the global geometry of the pumping with narrower channels achieving a higher pumping velocity at an earlier time after the carpet creation. The works in Chapter 4 have been published by Min Jun Kim and Kenneth S. Breuer, *Proceeding of 2005 NSTI-Nanotechnology Conference and Trade Show*, Anaheim, CA, Vol 3, No 451, 2005. Finally, in Chapter 5, temporal and spatial correlation functions are introduced to explain how large-scale self-coordination of bacterial flagellar bundles evolve in a confined geometry. The results presented in Chapter 5 have been done by Min Jun Kim and Kenneth S. Breuer with help from Nicholas Darton.

I would like to express my deep gratitude and indebtedness to Prof. Kenneth S. Breuer for introducing me to this emerging field of bacterial flows and micro-fluidics. I would also like to thank him for his encouragement, continuous support and guidance during this work. Without him, this project would not have been possible.

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Chapter 1

Introduction

Recent developments in micro-fabrication technologies enabled a variety of miniaturized fluidic systems consisting of micro-mixers, pumps and various other actuating systems. Microfluidics, the study of the motion of fluids at the micron-scale, is a key factor in the miniaturization and integration of multiple functionality for chemical analysis and synthesis in micro-engineered systems. In addition, lab-on-a-chip technologies based on microfluidics are being used to sample multiple genes and DNA, to detect specific types of organisms, or to develop biosensor-like probes such as antibodies to detect molecules of interest. Advantages of microfluidics compared to conventional fluidic systems include low fabrication cost, enhancement of analytical performance, low power budget and low consumption of chemicals.

Due to the size and the surface force effects [1] [2], the micro-scale thermal/fluidic transport phenomena are different from their larger-scale counterparts, generally described by the Navier-Stokes equation [3]. Hence, the technological demands on microfluidic systems require a better understanding of the flow characteristics in micron and sub-micron level devices. For devices smaller than one millimeter in length, the origin of the fluid forces can be created by the surface forces or the body forces driven by pressures, based on short-range van der Waals forces and longer range electrostatic or Coulombic forces [1] [2]. As a result, fluids flowing in micro-fabricated systems and achievable flow speeds are accomplished using external actuators such as cumbersome syringe pumps, or unwieldy electric generators. One approach to manipulate fluids moving with ruthless efficiency in microfluidic devices is to utilize bio-molecular motors from flagellated bacteria as fluidic actuators [4] [5].

In this thesis, we demonstrate not only that flagellated bacteria, themselves propelled by several nano-scale bio-molecular motors, can be utilized both as individual actuators, and in arrays to generate local flow motion in a microfluidic network but also that the collective effort of the micro-organisms can be applied to achieve useful work in the micron scale world.

1.1 Flagellated Bacteria

The science of microbiology started with the invention of the microscope. In seventeenth century, van Leeuwenhoek examined a drop of rainwater and noticed it contained tiny creatures he called "animalicules" or little eels. These were in fact bacteria and so van Leeuwenhoek
became the first person to study bacteria [6]. In twentieth century, the development of optical microscopes, such as dark-field condenser and differential interference contrast (DIC), enabled us to investigate bacterial behaviors. In 1960s, Julius Adler [7] was successful in taking the first picture of *Escherichia coli* and demonstrating their chemotactic behaviors. Recently, Howard Berg [4] [6] has experimentally investigated that flagellated bacteria possess a remarkable motility system based on a reversible rotary motor which derives its energy from protons driven into the cell by chemical gradients or electrical fields.

Bacteria consist of only a single cell, but reproduce by dividing themselves. Bacteria are among the earliest forms of life that appeared on Earth billions of years ago. Bacteria live on or in just about every material and environment on Earth from soil to water to air, and from your house to arctic ice to volcanic vents. Most bacteria are small, about one micron in diameter. Some bacteria move about their environment by means of long, whip-like structures called flagella [8], and swim by rotating those flagella that lie on their surfaces. Some bacteria have one flagellum, some have a few, and some have many - depends upon the bacterial species. *Escherichia coli* and *Serratia marcescens* have several flagella, which act as independent units. When the flagella rotate in a counterclockwise direction (viewed from outside the bacterium), the separate flagella form a bundle that very efficiently propels the bacterium through solution. Using these flagella, bacteria can achieve remarkable performances under conditions of low Reynolds numbers [9].

*Escherichia coli* and *Serratia marcescens* are examples of flagellated bacteria, included in the *Enterobacteriaceae* [8]. *Escherichia coli*, living in our gut, was first indentified by the German pediatrician Theodor Escherich (1885), and named for Escherich in 1920 [6]. Bartolomeo Bizio, a pharmacist from Padua, Italy, discovered and named *Serratia marcescens* in 1819. Bizio named Serratia in honor of an Italian physicist named Serrati, who invented the steamboat, and Bizio chose marcescens from the Latin word for decaying because the
bloody pigment was found to deteriorate quickly [8].

The pattern of flagellation is an important feature in identification of motile bacteria. Polar flagella occur at one or both ends of the bacterium [10] [11]. Peritrichous flagella are randomly distributed around the surface of the organism [10] [11]. The bacterial cell wall serves to give the organism its size and shape as well as to prevent osmotic lysis. The material in the bacterial cell wall that confers rigidity is peptidoglycan [12]. The gram-positive cell wall appears thick and consists of numerous interconnecting layers of peptidoglycan [8] [12]. The gram-negative cell wall, on the other hand, contains a much thinner, single layer of peptidoglycan only two or three layers thick [8] [12]. *Escherichia coli* and *Serratia marcescens* are rod-shaped, peritrichously-flagellated, gram-negative bacteria about 1 µm in diameter by 2 µm long. *Escherichia coli* shown in Figure 1.1 swim at speeds of about 30 µm/s, propelled by the rotation of about 5 long (10 µm), thin (20 nm), helical filaments, each driven at its base by a flagellar motor [6]. When all of the motors spin counterclockwise, the filaments form a bundle that pushes the cell forward “run”. When one or more motors spin clockwise, their filaments leave the bundle, the cell body reorients “tumble”. In the absence of external stimuli, the motors switch from counterclockwise to clockwise at random times, and the cell executes a random walk [14].

Each flagellum is driven by a rotary motor embedded in the cell wall connected to the helical element via a short flexible hook. The nano-scale rotary motor (45 nm in diameter) shown in Figure 1.2 is built from about 20 different kinds of parts and is assembled from the inside out. There is an inner cytoplasmic ring (the C ring) with proteins that control the direction of rotation, a double-ring structure embedded in the inner membrane comprising the core of the rotor (the MS ring), a drive shaft (the rod) which passes through a bushing that gets the drive shaft through the peptidoglycan and outer membrane, and finally an extracellular flexible coupling (the hook) linked to a propeller (a long helical filament). Two proteins, MotA and MotB play the role of stator and the protein FliG, specific charged amino-acid residues, is the rotor [4].

The motor is powered by an electrochemical gradient that drives protons from the outside to the inside of the cell [15]. Passage of protons through a transmembrane channel drives a conformational change that allows MotA to ratchet along FliG, thus turning the rotor, drive shaft, and flagellar filament. The flagellar motor generates maximum torque at stall and continues to generate torque at 86-90% of this value up to about 200 Hz at room temperature [16]. Flagellar bundles and filaments spin at about 100 Hz, while tethered cells spin at about 10 Hz.

In order to create fluid motions in an intent manner using bacterial propulsion systems, we should understand the mechanism to control the direction of flagellar rotation. Indeed, it is very complex but reasonably well understood. Many flagellated bacteria can swim preferentially toward an increasing concentration of certain useful compounds and away from potentially harmful ones. This ability to control movement in response to chemical stimuli is termed chemotaxis [6]. Chemotactic bacteria contain receptors in the cell membrane that bind to certain chemicals and cause the basal body to direct either a run or tumble (or forward and reverse directions) [17]. When the chemical stimulus is an attractant, such as a rich nutrient source, the basal body is made to rotate so that the bacteria swim in straight lines toward the signal for long periods of time. If the stimulus is a repellant,
such as a poison, a signal is sent to the flagella motor which increases the probability of tumbling. For an example, there are transmembrane receptors sensitive to specific amino acids, dipeptides, and sugars coupled to a histidine kinase. The activity of the kinase depends upon receptor occupancy. When an attractant is added, the kinase activity falls and less CheY-phosphate is made. CheY-phosphate binds to a component of the motor’s C-ring stabilizing the clockwise state and destabilizing the counterclockwise state [4] [6]. Thus, when less CheY-phosphate is made, less binds to FliM, and the probability of the counterclockwise rotation increases, enhancing smooth swimming. So, one way that we can control the direction of flagellar rotation is to add or remove chemoattractants [18]. One other way is to change the temperature [19].

Flagellated bacteria are an ideal system to use for a wide variety of microfluidic systems. They have evolved extraordinary systems for the fabrication, operation, and maintenance of flagella. The flagellar motors are the most powerful molecular motors known to date and ideally suited for a chaotic micro-mixer and a self-sustained micro-pump. Conclusively, the bacterium represents a highly configurable system which can be incorporated into an engineered microfluidic system.
1.2 Bacterial Flagellar Bundling

Flagellated bacteria induce locomotion through the use of multiple flagella. Each flagellum is flexible and maintains roughly a left-handed helical shape [20]. At the base of each flagellum is a nano-scale rotary motor that can turn clockwise or counterclockwise. It is known that when the motors all turn counterclockwise (when viewed from behind the flagella), the flagella tend to gather together to form a single helix [13]. This movement, known as bundling, results in forward motion of the cell (shown in Figure 1.3). Here, interesting questions arise regarding the operation of a number of individually rotating helices in a bundle that involves a large overlap of the individual helical. As stated in the section 1.1, running and tumbling together enable the organism to move in one direction and reorient itself to move in another direction where there may be more favorable conditions for survival. This process may be in response to chemical stimuli (chemotaxis) [6] [18] or other factors such as temperature [19] and light intensity [21].

Most bacteria are difficult to see under the bright field microscope. Bacteria are almost colorless (remember, all cells are composed primarily of water) and therefore show little contrast with the broth in which they are suspended. To visualize bacteria, either dyes or stains, or an alternative source of illumination, such as dark-field microscope [22] or differential interference constrain [23], are used. Since staining of bacterial cells is relatively fast, inexpensive, and simple, it is the most commonly used technique to visualize bacterial cells. Staining not only makes bacteria more easily seen, but it allows their morphology (e.g. size and shape) to be visualized more easily. Using real-time imaging of fluorescent flagellar filaments [13], we can observe the motion of flagella on live bacteria. Cells run when pushed from behind by a flagellar bundle of the normal left-handed waveform. Cells tumble when the filaments turn clockwise (CW) and the bundle comes apart. Tumbles are complex and involve polymorphic transitions of the filament [13]. Turner et al. have found that cells can alter course by changing the direction of rotation of as few as one flagellar filament. However, since the radius of the flagellar filament (20 nm) is below optical wavelengths, and the motor rotation is relatively rapid (100 Hz), it is difficult to study the detailed mechanism of the bundling process directly.

Previous work on the analytical and numerical modeling of helical swimming motions has focused mainly on organisms with a single flagellum. Lighthill [24] [25] provided math-

Figure 1.3: The sequence of the bacterial flagellar bundling. Reprint from [13].
ematical analysis of the motion of a thin tube with helical shape using slender-body theory. Higdon [26] [27] combined mathematical and numerical analysis applied to the motion of an organism with a spherical body and a single flagellum. Higdon calculated the flow field near an undulating filament attached to a wall [27]. The flagellum was also modeled using slender-body approximations. The flows and interactions generated by several undulating or rotating filaments have received far less attention. Ramia et al. [28] used a computational approach based on the boundary element method to model the motion of an organism with spherical body and a single helical flagellum. Their study included motions near walls and near another organism of the same shape. Powers [29] considered a single straight but flexible filament which is rotated at one end in a circular fashion around an axis parallel but not coincident with the filament. The rotation simulates the cell body rotation and the filament represents a single flagellum. Based on steady states of the filament driven by various rotation frequencies, conclusions were drawn regarding the possibility of bundling. However, only a single filament was considered and the hydrodynamic interactions among neighboring flagella were not taken into account. Floresa et al. [30] determined conditions that tend to produce bundling of various prokaryotic flagella in close proximity to one another. They emphasized on the role of the hydrodynamic interaction of the flagella in the processes of bundling and tumbling. Due to the complexity of the geometry and the non-steady nature of the flow, the numerical works cited above have played a crucial role in understanding the bacterial flagellar mechanism. It is found that when all flagellar motors rotate counterclockwise, the hydrodynamic interaction can lead to bundling.

Macroscopic scale models (see Figure 1.4) were built to prove that the bacterial flagellar bundling purely arises from hydrodynamic interaction of the rotating helices [31] [32]. Macnab and Kim et al. perfected a manufacturing technique that could create large-scale model of the bacterial flagella with the appropriate stiffness and the helical geometry so that complete scaling could be achieved. This allows for detailed measurement and comparison with theory on the behavior of single and small arrays of flagella operating under a variety of conditions. The dynamic behavior of a set of helices originating on separate rotational axes was experimentally investigated by a variety of helical forms with changing amplitude, wavelength, and rotational speed [31] [32]. These dynamic helical parameters affect the overall hydrodynamic properties of the rotating helices (e.g., viscosity, flow, and mechanical stress). The macroscopic scale model demonstrates that the bundling of bacteria flagella is a purely mechanical phenomenon, arising from the interplay of hydrodynamic interactions, bending and twisting elasticity, and geometry [32].

It is very important to understand the hydrodynamic interaction in that the swimming of bacteria belongs to the Stokesian realm. One would assume that, given the small dimensions (1 $\sim$ 2 $\mu$m) and consequently, low Reynolds numbers of bacteria ($10^{-4}$ $\sim$ $10^{-5}$), the flow associated with them is completely laminar. When small things move through fluids slowly, viscous forces are dominant over inertial forces. In other words, the creature at high Reynolds number (e.g., brine shrimp and jellyfish) propels itself by accelerating water, the bacterium by using viscous shear. In short, the bacterium lives in a very different hydrodynamic world from which larger fish lives in. The net force on a cell swimming at a constant velocity is zero. If the net force were not zero, the cell would accelerate or decelerate. Torque generated by rotation of the flagella is balanced by viscous drag due to counter-rotation of the body
of the cell [14], and thrust generated by rotation of the flagella is balanced by viscous drag due to translation of the body of the cell [6] [14]. It is easy to see that torque is required to rotate a helical filament in a viscous medium, but it is not obvious why this rotation should generate thrust [14]. To clear this, it is essential to study velocity flow fields generated by rotation of the flagella.

The bacterial flagella rotating in bundles discussed above induce a variety of velocity fields in the surrounding medium. In Chapter 2, these fields have been measured using particle image velocimetry (PIV), in which particles are suspended in the ambient fluid and their motion is measured and analyzed using well-established PIV algorithms [33]. In addition to measurements of velocities we perform scaled-up measurements using a silicone oil (high viscosity) tank that replicates the low Reynolds number environment. The scaled-up flow measurements allow us to manipulate geometric parameter (e.g., rotational speed, helix shape, and initial helix separation) to map out the complete phase space of the bundling phenomenon. This is used to study the effects of flagellar arrays and the effects of chaotic mixing due to the random reversal of flagella. We also demonstrate that the bundling is result of hydrodynamic interaction between neighboring flagella.
1.3 Interactions with a Fluid (Mixing)

One of the fundamental properties of fluid mechanics at the micron scale is that swimming strategies which rely on imparting momentum to the surrounding fluid do not work. This feature follows from the kinetic reversibility property of Stokes flow, which states that if the external forces and motion of all boundary surfaces are reversed, then the flow velocity at every point in the bulk fluid reverses [14] [34]. A consequence of kinematic reversibility is that reciprocal motion generates no net thrust. However, microorganisms employ various strategies to elude this constraint on the net thrust. Flagellated bacteria, such as *Escherichia coli* and *Serratia marcescens*, use rotary motors at the cell wall to rotate their helical flagella. Since this motion always pushes fluid along the same axial direction for a given sense of rotation, the motion is not reciprocal and generates net thrust [10] [14].

*Escherichia coli* swim at about 30 µm/s (at room temperature, that is, about 30 diameters per second. The flagellar bundle spins rapidly one way (∼100Hz) and the cell body spins more slowly the other way (∼20Hz). From Stokes law, the net thrust is about 0.2 pN. To overextend the analogy, flagellated bacteria can be compared to the M1A2 Abraham Tank of the micron scale world.

The theoretical fluid-mechanical study of propulsion by flagella was investigated by G.I. Taylor who published a series of papers fifty years ago [35] [36] [37], in which the interaction between the flagella of two microscopic organisms [35] was addressed. Taylor took into account the flows induced by small-amplitude undulations of two infinite sheets, and found a strong reaction due to viscous stress which tends to force the wave trains into phase. Many of the theoretical tools for studying hydrodynamic interactions have since been developed [27] [38] [39], although they have almost been applied to interactions between segments of a single flagellum.

Distinct from the theoretical study of the fluid mechanics of the bacterial propulsion discussed above, there has also been a considerable real-scale experiment into the effect of bacterial motion on the fluid flows. Recently, Wu and Libchaber [40] studied the effect of bacterial motion on micron-scale beads in a freely suspended soap film. They monitored the motion of beads as an indirect way to investigate the dynamics of the bacteria. Indeed, large positional fluctuations are observed for beads as large as 10 µm in diameter, and the measured mean-square displacements indicate superdiffusive behaviors. Based on their experimental results, Grégoire et al. [41] showed a model that superdiffusive behavior should be generically observed in the transition region marking the onset of collective bacterial motion.

In a microfluidic environment, the small scale and consequently low Reynolds number leads to diffusion-limited, viscous dominated dynamics. As a result, the physical constraints fluid machines face in the microscopic world differs from the constraints faced by macroscopic machines. This has led to several engineering challenges, for example, how to pump fluids through small system with optimum efficiency and how to enhance mixing of parallel streams fluids. Mixing, for chemical systems continues to be a challenge, although several concepts for laminar mixers have been proposed. Most of these are associated with chaotic advection [42] in which the objective is to use a chaotic cycle to exponentially stretch the two fluid interface where mixing can occur. Spatial methods for generating chaotic mixing
Figure 1.5: The photographs illustrate typical intensity distributions showing the diffusion profiles that is established between the labelled and unlabelled streams: (a) the clean-walled microchannel, (b) the channel coated with a immotile bacterial carpet, and (c) the channel coated with an active bacterial carpet. The enhancement of mixing is apparent.

have been demonstrated with complex meandering channels [43] or ribbed channels that can generate a transverse rotation [44]. There have been attempts at making temporal chaotic mixers at the micron scale, however these have tended to fail due to previous difficulties in finding a compact rotational motor such as the bacterial flagellar system that can be incorporated into a microfluidic system.

A unique feature of flagellar motors in wild-type cells is that they alternate between clockwise and counterclockwise rotation in a random manner. We can take the chaotic nature of the spin direction as a given and exploit by assembling chaotic mixers based on the seminal work of Aref [42], and others who identified mechanisms for the mixing of fluids in low-Reynolds number environments. In this limit, laminar diffusion is the only mixing mechanism. Therefore, to reduce the mixing time, one needs to increase the material interface by means of a chaotic mixer. The bacteria provide a natural mechanism for achieving mixing - their motion is naturally chaotic and so by placing them in the appropriate places (bacterial carpet), or even by letting them swim in the reagent soup (motile bacteria), mixing enhancements can be achieved. This mechanism has been experimentally studied in Chapter 3 and Chapter 4. For example, if streams of fluids A and B are introduced into a microchannel, the two-stream mixing is enhanced due to the chaotic clockwise and counterclockwise reversal that the wild-type bacteria exhibit (see Figure 1.5). We have quantified the mixing as a function of control parameters (concentration of bacteria, concentration of glucose, and temperature).

1.4 Engineered Bacterial System

Due to the difficulty in fabricating nano-scale motors and developing micron-scale power sources, the pumping of fluids in microfluidic systems are currently only accomplished by external actuators such as pressurized pumping systems, or high voltage power supplies. Although these devices are effective, they are cumbersome and inefficient, often dominating in size and power consumption over the microfluidic network to which they are connected. An alternative approach to generating fluid motion using small-scale devices efficiently is to
employ flagellated bacteria as controllable actuation elements in microfluidic networks [4] [5], using external stimuli such as temperature [19], chemical concentration gradients [45], and geometrically restrictive environments [46] to control and direct bacterial behaviors.

The bio-molecular motors, discussed in the section 1.1 can be used in complex microfluidic systems based on bacterial actuation. A possible alternative approach to microflagellar systems might be to either mimic the bacterial system [47] or to isolate the bacteria motor, i.e., to devise a system that uses only motor assemblages. We firmly believe that this approach would not only be difficult, but also counterproductive, since the full organism is already naturally designed to accomplish many engineering tasks we desire. The bacteria contains all of the regulatory hooks necessary to build flagella, to repair or rebuild them if necessary, to switch their motors on and off, to control the direction and duration of their spin, and so forth. In short, the bacterium represents a highly configurable system which can be incorporated into an engineered microfluidic system. For example, although wild-type bacteria alternate their motor direction between counterclockwise (CCW) and clockwise (CW), “smooth swimming” bacteria (mutant) can be engineered whose motors spin continuously counterclockwise. Both of these bacteria can be employed in the microfluidic system.

If a large number of bacteria are encouraged to adhere to a substrate, then a bacterial carpet will be created with unique properties. Indeed this has already been observed by Darnton et al. [5], in which Serratia marcescens are observed to stick to PDMS materials, as well as to generate fluid flow motions. One can expect that the properties of the bacterial carpet will be quite unique and useful. If the bacterial flagella are, on average, spinning in one direction, a net thrust develops. If the bacterial carpet is fixed, the ambient fluid will be pumped (see Figure 1.6). Conversely, if the bacterial carpet is formed on a free chip, it will be moved by the bacterial engines [5]. Lastly, if the the carpet covers two distinct fluids, the random reversal of rotation can be used to mix fluids in confined geometries.

Instead of attaching the bacteria to a fixed surface and monitoring the net fluidic force, Darnton et al. could promote their docking to a PDMS chip or polystyrene bead [5]. The chip was submerged in the fluid medium and was free to move on a free surface. The translocation
of the PDMS chip by the bacteria could be measured as a function of cell density and chip geometry [5]. They propose to harness *Serratia marcescens* to an engineered cargo chip and to use the flagellar propulsors collectively to move this much larger system.

All of the previous discussion focuses on the generation of collective momentum from a bacterial flagellar system, and how we can use that momentum. However, bulk fluidic motion generated by the bacterial flagellar motion also suggests a second application to use them as sensors [18] [48]. Changes in temperature or the chemical environment can stimulate the bacteria’s sensory system and hence affect the flagellar motor performance (its mean clockwise and counterclockwise rotation intervals, rotation frequency, etc) [19] [20]. If we can detect global changes in the direction of rotation of the filaments of a flagellar carpet, then we can use this system to monitor a chemotactic response. More importantly, the chemotaxis receptors of the bacteria being used can be engineered to detect specific chemicals of interest. If the collective effort of the bacterial carpet can be used to amplify the chemotactic sensitivity of the bacterial system, we can detect a useful sensor output. This concept has been tested in Chapter 4.

Based on the feasibility of the use of flagellar bacteria, we can outline bacterial actuation elements in the microfluidic system such as a micro-pump, a micro-mixer, and a chemical sensor. As an extension of the bacterial carpet concept, fluidic pumping systems can be fabricated and tested. If a bacterial carpet is attached to the walls of a microchannel, a net flow generated by their rotating helical filaments will be induced in the channel. This has been fabricated using PDMS soft lithography to form a channel [49], coating the surface with a negatively charged *Serratia marcescens*. In Chapter 4, we have built up and tested this kind of pump (bacterial pump) to determine its pumping characteristics, as well as the durability of the bacterial motor array in service, the efficiency of the system.

All of the above microfluidic components rely on our ability to influence and control bacterial adsorption on PDMS and flagellar rotation using an external stimulus. We will demonstrate that the bacteria can be controlled by a variety of appropriate stimuli (thermal, chemical), and the engineering of a system can deliver those stimuli in a reconfigurable manner with the appropriate spatial resolution (geometrically restrictive environment) through this thesis.
Chapter 2

Paper #1: Particle Image Velocimetry Experiments on a Macro-scale Model for Bacterial Flagellar Bundling

*Escherichia coli* (*E. coli*) and other bacteria are propelled through water by several helical flagella, which are rotated by motors embedded at random points on the cell wall. Depending on the handedness and rotation sense, the motion of the flagella induces a flow field that causes them to wrap around each other and form a bundle. Our objective is to understand and model the mechanics of this process. Full-scale flagella are 10 µm in length, 20 nm in diameter, and turn at a rate of 100 Hz. To accurately simulate bundling at a more easily observable scale, we built a scale model in which 20-cm-long helices are rotated in 100,000 cp silicone oil (Poly-di-methyl-siloxane). The highly viscous oil ensures an approximately low Reynolds number. We developed a macro-scale particle image velocimetry (PIV) system to measure the full-field velocity distribution for rotating rigid helices and rotating flexible helices. In the latter cases, the helices were made from epoxy-filled plastic tubing to give approximately the same ratio of elastic to viscous stresses as in the full-scale flagella. Comparison between PIV measurements and slender-body calculations shows good agreement for the case of rigid helices. For the flexible helices, we find that the flow field generated by a bundle in the steady state is well approximated by the flow generated by a single rigid helix with twice the filament radius.

2.1 Introduction

Bacteria such as *Escherichia coli* use rotating helical flagella to swim. The body of *E. coli* is rod-shaped, about 1 µm in diameter by 2 µm long, and, typically, has several flagella. Each flagellum has a rotary motor which can turn at approximately 100 Hz, either clockwise or counter clockwise. The motor is embedded in the cell wall, and drives a short flexible hook connected to the helical filament, which is about 20 nm in diameter and approximately 10 µm long [14]. Recently, Turner, Rye, and Berg succeeded in fluorescently labeling the filaments, allowing detailed visualization of the flagellar motion in real time [13]. When all the flagella turn counterclockwise (when viewed from outside the cell body), they form a
bundle that pushes the body forward in a run. When one or more of the motors reverses, the corresponding filaments unwind from the bundle, and the cell body moves erratically, or tumble. Tumbles involve polymorphic transformations of the left-handed normal helices to the right-handed semi-coiled state, and then to the right-handed curly-1 state. The first transition reorients the cell body, and, once the motors reverse again, the curly-1 state transforms directly into the normal state, and the cell regains its initial speed once the complete bundle reforms [13]. Tumbles and runs alternate, causing the cell to execute a three-dimensional random walk [14]. In the presence of a gradient of desirable chemicals (such as sugar), the cell reduces its likelihood of tumbling when it swims up the gradient, leading to a drift toward higher concentrations. The random motion of the cells may be exploited: it has recently been shown that a suspension of motile bacteria enhances fluid mixing in films [40] and microchannels [50].

Because bacteria are so small, high-resolution microscopy methods are necessary for experimental studies. Direct visualization of the individual flagellar filaments in a rotating bundle is a daunting challenge, due to their 20-nm diameter. Also, micro-PIV approaches are currently incapable of resolving the flow pattern near the flagella, due to optical resolution and seeding limitations. To study the bundling process in more detail, Kim et al. [32] developed a macro-scale model consisting of stepper-motor-driven polymer helices rotated in a high-viscosity silicone oil, and successfully simulated flagellar bundling for the counterclockwise rotation of left-handed helices [51]. By varying the motor speed and helix stiffness, they also showed that the motor period controls the initial rate of bundling.

### 2.2 Experimental Setup

*E. coli* usually has several filaments per cell, but for simplicity we consider the case with only two filaments. To accurately simulate the system at a more easily observable scale, the flagellar length was scaled up to approximately 10,000 times its normal value resulting in flagella that were about 20 cm in length. Two series of experiments were performed. The first used rigid helices, made from copper tubing (4.0 mm diameter) bent around an aluminum mandril to match the shape of the full-scale flagella. The second series of measurements used flexible helices made from thin plastic tubing (4.0 mm diameter). The tubing was filled with epoxy to achieve the desired bending stiffness. To match the shape of the full-scale flagella, each flexible helix was wrapped around a cylindrical mandril during the curing process [32]. The helical pitch ($P$) was 6.6 cm and the helix radius ($R$) was 1.27 cm. Reynolds number similarity was achieved (approximately) by rotating the helices at a low speed (0.25 Hz instead of 100 Hz) in high viscosity (100,000 cp) silicone oil (Poly-di-methyl-siloxane). A second important non-dimensional parameter that must be preserved in the scale test is the ratio of viscous to elastic stresses, $M = \mu \omega L^4 / EI$, where $\mu$ is the viscosity, $\omega$ is the rotation speed, $L$ is the axial length of the helices, and $EI$ is the bending stiffness. For bacteria, we estimate $M \approx 150$, while in our scale model $M \approx 140$ for the flexible helices [32].

A pair of model flagella were placed into a rectangular tank (42 cm × 42 cm × 32 cm) of silicone oil and attached to a pair of synchronized stepper motors. Starting from a parallel position, they were rotated at the same speed. The rotation leads to flow, which in the case of flexible helices causes bundling for sufficiently large $M$ [32]. Black plates were also installed
on the tank walls to minimize reflections from the laser flashes. To measure the flow, we seeded the oil with 23 µm diameter silver-coated glass spheres (of density 1.6 g/cm³), and illuminated a plane in the tank with two pulses of a sheet of laser light, in rapid succession (see Figure 2.1). The velocity field was then computed using standard techniques [33]. We used chaotic mixing to disperse the tracer particles in the highly viscous silicone oil. The tank was mounted on a motorized turntable and rotated counterclockwise (when viewed from above) at 3 rpm, while a fixed impeller with a nearly vertical shaft rotated clockwise (when viewed from above) at 33 rpm [52]. The anchor shape of the impeller was chosen for its suitability for mixing highly viscous liquids. From time to time, we manually varied the angle of the shaft of the impeller with the vertical from 0° to 20°. The primary difficulties were clumping of the powder of tracer particles and the slow rate of dispersion. To minimize clumping, a small amount of tracer particles (5 g) was measured out and uniformly scattered on the top of the silicone oil. Additional tracer particles were added periodically to the silicone oil until the volume fraction of the tracer particles reached 0.04%. It took fourteen hours to complete the seeding of particles in the silicone oil tank.

The PIV system consisted of a Q-switched twin Nd:YAG laser (Quantel, Les Ulis Cedex, France), which provided frequency-doubled (λ= 532 nm) pulsed emissions of up to 150 mJ/pulse, and a pulse duration of approximately 5 ns. The time delay Δt between the two successive pulses was chosen to be 10 ms. A combination of a cylindrical lens and a

Figure 2.1: Left: Schematic of the arrangement for seeding the particles by chaotic mixing. Note the anchor shape of the impeller. Right: Geometry of rotating helices and planes illuminated by pulses of the sheet of laser light; we either illuminate a vertical plane containing the initial axes of the two helices, or a horizontal plane bisecting the two helices.
spherical lens collimated the laser light to a sheet of approximately 1 mm thickness at the measurement regions. Images were captured from two vantage points. In the first view, the laser sheet was aligned with the axes of the helices and images were taken from the front of the tank. In the second view, the laser sheet was perpendicular to the axes of the helices, and images were taken from below, via a mirror inclined at 45 degree. A full-frame interline-transfer $1,300 \times 1,030 \times 12$-bit cooled CCD camera (IDT, Tallahassee FL) was used for recording the particle images. The CCD chip has an image plane measuring 8.7 mm (horizontal) $\times$ 6.9 mm (vertical), and each pixel is square with side length $d_r = 6.7 \, \mu$m. The field-of-view of PIV images was set as $(l_x = 165 \, \text{mm}) \times (l_y = 130.7 \, \text{mm})$ for the flow measurement. A Nikon 24 mm manual lens with $f^\# = 2.8$ was attached to the CCD camera with magnification $M_o = 0.053$. Commercial software (IDT ProVision, Tallahassee FL) was used for the image recording, time synchronization control between the laser and the CCD camera, and subsequent data processing. The velocity vectors were calculated using $32 \times 32$ pixel interrogation window with adjacent windows overlapping by 50%.

The experimental uncertainties in the velocity field are determined by the accuracy of the measure of particle displacements. The root-mean-square (rms) error in the velocity measurement is given by

$$\sigma_u = \sigma_{\Delta x} / (M_o \Delta t), \quad (2.1)$$

where the image magnification $M_o = 0.053$ and $\sigma_{\Delta x}$ is the rms error of the displacement on the pixel plane. We take the rms error $\sigma_{\Delta x}$ to be 4% of the recorded image diameter [53],

$$\sigma_{\Delta x} = 0.04 \sqrt{d_e^2 + d_r^2}, \quad (2.2)$$

where $d_e$ is the optical diameter of the image prior to being recorded on the pixel plane, and $d_r = 6.7 \, \mu$m represents the resolution of the recording medium, and is taken to be equivalent to the pixel size. Assuming that the particle image is diffraction limited and its image intensity is Gaussian, the diameter $d_e$ of the diffracted particle image [33], obeys the following:

$$d_e^2 = M_o^2 d_p^2 + (2.44(1 + M_o) f^2 \lambda)^2, \quad (2.3)$$

where for the current case, the tracer particle diameter $d_p = 23 \, \mu$m, the $f$-number of the imaging lens $f^\# = 2.8$, and the laser wavelength $\lambda = 532 \, \text{nm}$. Thus, the recorded image diameter $\sqrt{d_e^2 + d_r^2}$ is calculated to be $7.8 \, \mu$m, a little more than 1 pixel. Raffel et al. [33] estimated an ideal particle image diameter, for minimum uncertainties, as $\sim 1.5$ pixels by using double exposed PIV recording data. Substituting equation (2.3) into equation (2.2) gives $\sigma_{\Delta x} = 0.31 \, \mu$m, and subsequently, the rms velocity measurement error $\sigma_u = 0.59 \, \text{mm/s}$. Thus, the minimum resolvable velocity fluctuation is reasonably small in comparison with the typical velocity of 1 cm/s. More accurate results would be possible with a greater time delay between pulses. However, this was not possible with the current synchronization system used at the time. In addition, the highly three-dimensional nature of the flow required a relatively short $\Delta t$ so that the particles remained within the laser sheet between images.
2.3 Results and Discussions

2.3.1 Validation

We tested our PIV system against the flow induced by a rotating cylinder with a 12.7 mm radius, rotating at the center of the silicone oil tank. For analytic solution for the azimuthal velocity at the radius $r$, $v_\theta$, induced by a cylinder of radius $R$, rotating at the center of a circular tank of radius $\sigma R$ is given by

$$\frac{v_\theta}{\omega R} = \frac{1}{\sigma^2 - 1} \left( \frac{\sigma^2}{r/R} - \frac{r}{R} \right)$$

(2.4)

where $\omega$ is the rotational speed [54]. Although the cross-section of our tank is square, not circular, the solution (2.4) accounts for the effects of the walls accurately enough. Figure 2.2 shows a comparison of the analytic solution and the velocity measured at several locations in the tank for $\omega = 0.2$ Hz. The measured velocity field was confirmed to be rotationally
symmetric, and agreed very closely with the analytic approximation (2.4) in the region near the cylinder ($R < r < 110$ mm).

### 2.3.2 Rigid Helices

The rigid helices were rotated at 0.25 Hz and image pairs were acquired at 2 Hz, resulting in eight velocity fields per rotation. Four cycles were recorded, and the resulting thirty-two velocity fields were checked for consistency position by position. Any vectors that statistically deviated from the others (typically due to a high error in the cross-correlation) were eliminated from the ensemble and then an average was taken over each interrogation window. In two regions of the flow—near the shadows of the helices and near the bright spots caused by reflected laser light from the surface of the helices—spatial averaging was used to smooth the velocity vectors. For perfectly aligned rigid helices exactly in phase, the velocity fields would be periodic. Figure 2.3 shows the $z$-components of four different instantaneous velocity fields, captured at the same phase angle and viewed from below. The figure also shows a raw sample image and a sketch of the orientation of the laser sheet relative to the helices. Note the shadow cast by the helix, originating at the point the helix cuts through the laser sheet. The velocity fields are calculated using a standard cross-correlation technique without any type of vector validation or smoothing applied to the field. The velocity measurements in Figure 2.3 are not identical and exhibit a standard deviation (averaged over the entire field) of 0.09 cm/s, compared to the maximum velocity of about 2 cm/s. However, the similarities between each realization are strong. The ensemble average of the four instantaneous velocity fields in Figure 1.3 is shown in Figure 1.4, and compared with results from a numerical slender body calculation run at the same conditions and geometry [55]. The agreement is good, both quantitatively and qualitatively except in the region where the shadow appears in the physical image and hinders the PIV processing. The PIV field shows a maximum velocity which is about 9% smaller than that of the simulation. This discrepancy is slightly higher but still comparable to that of the validation flow. In order to minimize the shadow effects on the PIV data, a larger interrogation cell ($36 \times 36$ pixels) was used in this region. In addition, black plates were installed on the tank walls to minimize reflections. In case of flexible helices, the shadow effects were less severe since the flexible helices are translucent, and thus scatter less light than the solid copper helices.

Figure 2.5 shows a comparison between a PIV measurement and the corresponding numerical simulation for the vertical plane; the rotating speed is 0.25 Hz. This view shows the bands of positive and negative velocity generated by the helix pair rotation. Both figures show qualitative agreement in pattern. However, the velocity magnitudes are not quite matched between the simulation and experiment. The maximum velocity in the side view PIV field is as much as 14% lower than the corresponding maximum in the simulation. The greater discrepancy between experiment and simulation for the side view is expected, because in the side view, each helix pierces the sheet of light several times, leading to more shadows than the bottom view, where each helix only pierces the sheet of light once.
Figure 2.3: PIV results of four separate measurements at the same periodic position for rigid helices rotating at 0.25 Hz. The view is looking up the helices towards the motors (which are at $y = 4P$, where $P$ is the helical pitch), and the color code represents the $z$-component of velocity in cm/s in the plane at $y = 2P$. Note that the helices can be discerned in the velocity field plots. The panels on the far left show the helices and tracer particles (top) and the sense of rotation and orientation of the light sheet (bottom).
Figure 2.4: Comparison of the $z$-component of the velocity in the plane $y = 2P$ measured by PIV (left) and computed numerically (right). The PIV results are the average of four figures in Figure 2.3. The units for the color bar are cm/s.

2.3.3 Flexible Helices

The flexible helices generate a more complex flow field since they deflect as the experiment progresses. Furthermore, small changes in the initial orientation of the flagella mean that, although the overall characteristics of the flow and the bundling are repeatable, the flows are not exactly repeatable and thus ensemble averaging is impossible. However, the previous results indicate that the instantaneous flow field is a reliable measurement and that averaging does not significantly improve our understanding of the velocity field structure. Figure 2.6 shows the velocity field in the $xz$-plane (bottom view) during the several stages of bundling process. The positions of the helices are clearly shown in the particle images (top panels) at four times during the bundling. Since the motors run at a common constant velocity (0.25Hz) and do not slip, the phase difference between the motor shafts is constant. When the motors turn counterclockwise, the helices rotate about each other, eventually entangling in a bundle, which persists indefinitely as long as the motor speeds are sufficiently low.

The flow fields of Figure 2.6 are complex and difficult to interpret. Furthermore, we cannot use numerical simulations as a guide to understanding the flow since simulations for flexible helices are currently not available. However, we can use the PIV flows to answer a simple but important question: is the flow field of the fully developed steady-state bundle similar to the flow induced by a single rigid helix? To make a quantitative comparison, we averaged the azimuthal velocity (in the plane bisecting the two initial helix axes) over circles of constant radius, $r$, for the PIV measurements of the flow generated by the flexible helices, and for the slender-body theory simulation of the flow generated by a single rigid helix. We
Figure 2.5: Side view of flow induced by the rotation of two rigid helices. Upper left: Raw image. Upper right: Orientation of sheet of laser light for the side view. Lower left: PIV measurement of the $z$-component velocity. Lower right: Corresponding numerical result. The units for the color bar are cm/s.
Figure 2.6: Raw images and corresponding PIV measurements of z-component velocity due to a bundling flexible helix pair ($y = 2P$, rotated at 0.25 Hz) shown at 5 s, 25 s, and 45 s during the bundling process and viewed from below. The units for color bar are cm/s.
Figure 2.7: Comparison between PIV measurements and slender body calculations of the azimuthal velocity (averaged over a circle around the helix) as a function of radius. Experimental results from two rotational speeds are shown, normalized by the angular speed $\omega$, and helix radius, $R$. The PIV and numerical results in the lower curve are from the flow due to a single (rigid) helix of thickness 4.0 mm. The experimental data that form the upper curve are from the velocity induced by a fully formed two-helix bundle, while the corresponding numerical solution corresponds to the flow due to the rotation of a single rigid helix with the thickness doubled (8.0 mm).

used helices with thicknesses of 4.0 mm and 8.0 mm in the simulations; the double thickness was chosen since when the flexible helices entangle, the helix thickness is effectively doubled. Figure 2.7 shows the results and indicates excellent agreement between measurement and simulation. The lower of the two curves is the velocity due to a single helix, and PIV data taken at two speeds is shown. The agreement is excellent both inside and outside the helix ring. There is some discrepancy in the immediate vicinity of the helix ring ($r/R = 1$). PIV measurements in this region are difficult due to reflections and shadows near the solid surface. In addition, the numerical simulation is also not completely reliable close to the solid surface since the singular solutions of slender body theory lead to diverging velocities at points just inside the surface. (Note that the lines drawn through the numerical results in Figure 2.7 are to guide the eye; the sharp cusp at the maximum would be smoothed out if we calculated more points in this region.) Thus, the discrepancy is likely due to errors in both the theory and measurement. The upper curve shows azimuthally-averaged PIV data.
of the velocity due to a helix bundle formed from two flexible helices (Figure 2.7). These measurements are compared with the numerical solution of the flow due to a single rigid helix with thickness double that of the baseline case (8.0 mm instead of 4.0 mm). As before, the agreement is remarkable with the exception of the region near the helix body where the measurements indicate a lower and more smoothly varying velocity distribution to that predicted by the simulation. The data suggest that the geometric complexities of the bundle do not contribute significantly to the overall flow, and that the flow of the steady-state two helix bundle is thus closely approximated by the flow of a single rigid helix with twice the thickness. Note that there is a measurable difference between the flow in the single-thickness and double-thickness simulations, indicating a weak but significant dependence of the flow on thickness. This dependence implies that since our helices do not have exact geometric similarity to the full-scale flagella, there is a small but significant discrepancy between the model flows and the flagella-generated flows. Since the flow in slender-body theory depends logarithmically on the aspect ratio (see e.g. [55] and references therein), this discrepancy should get smaller as the aspect ratio of the model helix increases. However, for a given material, the bending stiffness of a helix decreases rapidly as the thickness decreases. Thus, faced with a compromise, we chose to match \( M \) accurately and the aspect ratio only roughly.

2.4 Summary

In this chapter we have presented PIV measurements of an extremely complex three-dimensional flow at very low Reynolds number. The flow field is carefully constructed to match both the Reynolds number and ratio of elastic and viscous stresses so that comparisons can be made with the flows generated by flagellated bacteria swimming through water at the micron scale. The velocity fields around rigid helices are compared with results from slender body computations, and are found to agree qualitatively at the level of vector fields and quantitatively for the average azimuthal velocity, validating the technique. For the case of flexible helices, the deformation of the helices significantly complicates the flow field and since numerical computations, though feasible, have not yet been produced, no direct comparisons are possible. However, the measured rotational flow induced by the bundle in the steady state agrees well with calculated flow induced by a single rigid helix with twice the radius. The highly three-dimensional nature of the flow also complicates the accuracy of the velocity measurements. The time separation between images was perhaps shorter than was desired for optimal PIV accuracy, but was restricted both by the specifics of the current synchronization system and by the need to keep particles inside the laser sheet between image pairs. These compromises likely added to the discrepancies between the measurements and their corresponding simulations. Nevertheless, these results do help considerably in our understanding of the micro-hydrodynamics of bacterial motion. In addition, we have established an experimentally validated theoretical understanding of the flow generated by individual cell and the interaction among the cells, which will help understand the collective effects of the suspension or the carpet.
Chapter 3

Paper #2: Enhanced Diffusion Due to Motile Bacteria

The effect of bacterial motion on the diffusion of a molecule of high molecular weight is studied by observing the mixing of two streams of fluid in a microfluidic flow cell. Flagellated bacteria are used both as individual actuators and in arrays, where the collective effort of the organisms can be applied to complete a chaotic mixer in a microfluidic system. We present that the presence of motile *E.coli* bacteria in one of the streams results in a remarkable increase in the effective diffusion coefficient of Dextran, which rises linearly with the concentration of bacteria from a baseline value of $0.2 \times 10^{-7}$ to $0.8 \times 10^{-8}$ (cm$^2$/sec) at a concentration of $2.1 \times 10^9$/ml (approximately 0.5 % by volume). The activity of the bacterial carpet is shown to enhance the mixing between two streams of fluid as well. Furthermore, we observe that the diffusion process is also observed to undergo a change from standard Fickian diffusion to a superdiffusive behavior in both cases. Using motile bacteria, the diffusion exponent rises from 0.5 to 0.55 as the concentration of bacteria rises from 0 to $2.1 \times 10^9$/ml.

3.1 Introduction

Flagellated bacteria, such as *E.coli* or *Serratia Marcescens*, propel themselves by means of several helical flagella, each approximately 6-10 microns long, that are rotated at speeds of approximately 100 Hz by bio-molecular motors embedded in the cell membrane [56]. When the motors all rotate counterclockwise, the flagella coalesce together to form a bundle which propels the organism through the medium at speeds of up to 30 $\mu$m/s. When one or more of the rotary motors change direction, the flagellar bundle disperses and the bacteria tumbles, changing direction. Thus the organism executes a random walk [57]. The cell motion induces motion in the surrounding fluid [32] and one would expect that this might have an effect on the transport and diffusion of a passive scalar in the fluid. Wu and Libchaber [40] studied the effect of *E.coli* on micron-scale beads in a freely suspended soap film. They showed that the diffusion of beads is proportional to the concentration of *E.coli*. Through their experiments, large positional fluctuations were observed for beads grew fast than $\sqrt{t}$ for short times suggesting a superdiffusive behavior. Grégoire *et al* [41] have introduced a
simple model for the motion of passive beads in a noisy bath of active "boids" interacting only locally. Their results not only indicate that true superdiffusive motion of both bacteria and bead tracer is present in the bacterial bath but also suggest that superdiffusive behavior should indeed be generically observed in the transition region marking the onset of collective motion. The present experiments are aimed at exploring this phenomenon in some more detail, but rather than looking at the diffusion of relatively large particles, we are concerned about the diffusion of macro-molecules (in this case Dextran) uniformly distributed in the bulk fluid.

### 3.2 Experimental Setup

In the experiment, a PDMS (poly-dimethyl-siloxane) microchannel is fabricated using standard soft-lithography techniques [58] [59] (Figure B.1). The channel is in the form of a "Y", with two arms each feeding a stream of fluid into a main channel which measures 28 mm long, 40 microns high and 200 microns wide. One arm carries a biological buffer solution, plus a low concentration (0.02 % by volume) of FITC-labelled Dextran (MW 77,000). The second side contains the same buffer and Dextran, except that the Dextran is not fluorescently labelled. As the two streams flow down the main channel, the clear boundary that exists between the two streams spreads gradually due to molecular diffusion between the fluid in the two streams. The local concentration of labelled Dextran is proportional to
the fluorescence intensity across the channel \( I(y) \), measured optically using an inverted microscope state at 20 × magnification using a high-resolution (1,300 × 1,030 pixels) cooled charge coupled device (CCD) camera.

### 3.3 Results and Discussions

#### 3.3.1 Enhanced Diffusion Due to Swimming Bacteria

Standard diffusion theory [60], in which we ignore the variations that exist through depth of the channel and assume that the channel width is large compared to the width of the diffusion zone, predicts that intensity profile across the channel is given by the complimentary error function

\[
c(y) = \text{erfc}(\eta),
\]

where \( \eta \) is a similarity variable:

\[
\eta = \frac{y}{\sqrt{Dx/U}},
\]

\( D \) is the molecular diffusion coefficient, \( U \) is the average velocity and \( x \) is the distance from the mixing origin. The gradient of the intensity profile should behave like

\[
\frac{\partial c}{\partial y} = \frac{1}{2\sqrt{\pi Dx/U}} e^{-\left(\frac{y}{2\sqrt{Dx/U}}\right)^2}
\]

from which one observes that the maximum in the intensity gradient should decay proportional to \( x/U^{-1/2} \), and that the width of the diffusion zone, measured by the standard deviation of the intensity gradient, should grow proportional to \( \sqrt{x/U} \).

Live and highly motile \( E.coli \) bacteria were introduced at low concentrations into the fluorescent stream and the changes in the diffusion characteristics of the FITC-Dextran are recorded. A sample pair of images from the baseline (no bacteria) and a case with bacteria is shown in Figure 3.2. The enhanced diffusion due to the presence of bacteria is apparent. Images such as these were captured using two strains of bacterial: wild type \( E.coli \) (HCB 33) and tumbly \( E.coli \) (RP 1616). The tumbly mutant has active flagella similar in size and performance to the wild-type strain. However, the motors in the tumbly mutant are biased to favor clockwise rotation more than the wild type, and for this reason the flagella do not form a coherent bundle and consequently the cells are trapped in a permanent tumble mode and are rarely observed to run. After testing with wild type \( E.coli \), 0.001 % of FCCP was added to the buffer, immobilizing the bacteria. The experiments were then repeated using the de-energized bacteria. A range of dilute bacterial concentrations (0 ~ 2.1 × 10⁹/ml) and flow rates (0.5, 0.75, 1.00, 1.25 µl/min) were tested. Measurements were taken at seven \( x \)-stations along the mixing channel (\( x = 0, 4, 8, 12, 16, 20 \) and 24 mm, distances measured from the Y-junction). Intensity profiles, \( I(y) \), were generated from the images by averaging over 300 pixels (107 microns) in the streamwise direction and over 10 separate images. The profiles were acquired at different combinations of axial location, flow rate and bacterial concentration.
Figure 3.2: Optical micrographs of diffusion profiles for the baseline condition (1), and conditions in which non-motile *E. coli* (2), tumbly *E. coli* (3), and wild type *E. coli* (4) have been introduced to the upper (fluorescent) stream at a concentration (0.16 % by volume) of $1.05 \times 10^9$/ml. In these cases the flow rate is 0.5 $\mu$l/min and the images captured 24 mm (section 7) from the Y-junction. The enhancement of mixing is apparent.

As an example, Figure 3.3(a) shows the gradient of the intensity profile, $dI/dy$, at several $x$-locations for a fixed bacterial concentration and flow rate of 1.25 $\mu$l/min. The position, $x$, and average speed, $U$, can be combined to form a combined variable, $\tau = x/U$, which measures the average time taken by a fluid element to advect at speed $U$ to point $x$. The maximum of the intensity gradient is observed to be solely a function of $\tau$, for the full range of $x$ and $U$ tested [Figure 3.3(b)], confirming that the diffusion enhancement is independent of the average shear rate established in the system by the flowing streams (which is constant with $x$, but varies with $U$), and that the one-dimensional assumption in our analysis (in which we ignore both the shear through the depth of the channel and the weak shear across the width of the channel) is valid.

If we assume that the system follows Fickian diffusion dynamics as discussed above, we can extract the apparent diffusion coefficient, $D$, by least-squares regression analysis. Doing this for all the cases tested results in Figure 3.4 which shows $D$ as a function of the bacteria concentration for three different cases: wild type, tumbling, and inactive *E. coli*. The effective diffusion coefficient is observed to increase linearly with bacterial concentration and is enhanced by a factor of four at the highest bacterial concentration tested (which is still a relatively low volume concentration of bacteria, corresponding to a volume concentration of less than 0.5 %). Placing the bacteria in the non-fluorescent stream (instead of the fluorescent stream) has the identical effect, indicating that the FITC tag does not affect the mixing process or the bacterial effectiveness. Finally, seeding both streams with bacteria approximately doubles the effective value of $D$. All three configurations suggest that the
Figure 3.3: (a) Diffusion profiles at different distances from the Y-junction, generated from images for $Q = 1.25 \, \mu l/min$. (b) The variation of the maximum of the diffusion distribution, from all seven $x$-locations and four different flow rates, plotted using the combined variable: $\tau = x/U$. 
Figure 3.4: Variation of the apparent diffusion coefficient, $D$ as a function of the concentration of bacteria, assuming standard diffusion. The effects of adding wild-type *E. coli*, tumbly *E. coli*, and inactive *E. coli* are shown. The baseline case has no *E. coli* present. Three configurations are shown: (1) bacteria are added only to the FITC + Dextran stream while the nonfluorescent stream is not changed: (2) the fluorescent stream is left untouched while *E. coli* are added to the nonfluorescent stream. The results are identical with the previous configuration, confirming that the presence of the FITC does not affect the results. Lastly, (3) *E. coli* are added to both streams. In this case, the diffusion coefficient is approximately double the case in which bacteria at the same concentration are present in only one stream. The error bars represent the uncertainty in the determination of $D$. 
mixing enhancement is due to the local concentration of bacteria in the mixing interface, and not affected by the particular manner in which they are brought there.

The de-energized *E. coli* are also observed to promote an enhanced diffusion of the Dextran which, although much weaker than the enhancement observed with active cells, is nevertheless present. As mentioned above, the results are independent of the average shear rate in system, which would suggest that solute transport due to shear-induced diffusion (either of the Dextran, or of the bacterial cells) is negligible [61] [62] [63]. The source of the enhanced transport, which increase linearly with bacterial concentration, might be due to Brownian motion of the inactive cells and their flagella. Enhanced transport due to particles suspended in a flow without shear has been observed [64], although we are not aware of extensive investigation of this phenomenon and this needs to be investigated further. The difference between the enhancement due to wild type and tumbly *E. coli* argues for one of two possible mechanisms responsible for the mixing enhancement: First, the clockwise flagellar motion (more common in the tumbly strain) may be less efficient than the counter-clockwise flagellar motion (more common in the wild type strain). Second, it may be that the motion of the cell body during the coordination run (only exhibited by Wild type strain) generates a more efficient mixing field than the uncoordinated tumble phase (dominant in the tumbly strain).

A more careful examination of the decay in the intensity gradient [Figure 3.3 (b)] shows that the slope of the diffusion profile evolution (plotted on log-log axes) deviates from the theoretical value of -0.5 in a small, but consistent manner and that this slope varies with bacterial concentration. This suggests that a non-conventional diffusion process may be present. Dimensional analysis suggests that the thickness of the diffusion layer, $\delta$, normalized by the thickness at some reference location, can be modeled as

$$\frac{\delta}{\delta_o} \propto \left[\frac{\tau}{\tau^*}\right]^\alpha,$$

(3.4)

where $\alpha$ is the diffusion exponent and $\tau^*$ is a time scale. For Fickian diffusion, $\alpha$ is equal to 0.5, and $\tau^*$ is determined solely by the molecular diffusion coefficient. In the case of non-conventional diffusion, $\tau^*$ will also be influenced by non-molecular scales such as the characteristic time between bacteria-bacteria interactions measured approximately as the mean bacterial separation divided by their average swimming speed. Adopting this model for the diffusion process, we have fit the measured data to this model, finding optimal values for both $\alpha$ and $\tau^*$. The results are shown in Figure 3.5 which shows the values of $\alpha$ and $\tau^*$, both as a function of bacteria concentration, $c$. We find that there is a clear trend of increasing superdiffusion ($\alpha > 0.5$) as the concentration of bacteria increases. A indication of the global error in the experiment is given by the fact that the baseline case ($c = 0$) should be characterized by $\alpha = 0.5$, but instead is found to have $\alpha = 0.49$ - a discrepancy of only 2%. In contrast, is observed to increase smoothly and linearly as $c$ increases, rising to a value of $\alpha = 0.55$ for $c = 2.1 \times 10^9$ /ml. Extrapolating this trend predicts $\alpha = 0.74$ at a concentration of $10^{10}$ /ml - in excellent agreement with the lower estimate for observed by Wu and Libchaber [40]. The corresponding value of $\tau^*$ is also observed to fall, suggesting that as the bacteria become more densely packed, their interaction time falls. However, more detailed measurements of the bacterial motion are needed before one can say much.
Figure 3.5: Variation of (a) the diffusion rate, $\alpha$, and (b) the associated time scale, $\tau^*$, as a function of the bacterial concentration. The effects of wild type $E. coli$, tumbly $E. coli$, and de-energized $E. coli$ are shown. The three wild-type configurations are as described in Figure 3.4. The error bars represent the uncertainty in the determination of $\alpha$ and $\tau^*$. 
Figure 3.6: The variation of the maximum of the diffusion distribution, from all seven $x$-locations and four different flow rates. Fluorescence intensity distributions are extracted from photographs shown in Figure 1.5, and plotted against $\tau = x/U$ where $U$ is the average flow velocity. Three sets of data are shown: (i) the clean-walled microchannel, (ii) the channel coated with a non-motile bacterial carpet, and (iii) the channel coated with an active bacterial carpet and the downward shift in each line indicates the increased diffusion due to presence and motion of bacterial flagella.

more about this. However, direct measurement of the actual bacterial motion are needed before one can infer anything about the onset of larger scale cooperative motions that might be responsible for the superdiffusive behavior.

### 3.3.2 Enhanced Diffusion Due to Bacterial Carpets

A microfluidic system is coated with a bacterial carpet comprised of a dense layer of motile flagellated bacteria. The bacteria cell bodies stick to the surface with most of flagella free to rotate in the fluid. The depth of the microchannel was 15 $\mu$m. The motion of flagella from a bacterial carpet has been shown to substantially enhance fluid motion and the random diffusion of tracer beads in the near vicinity of the carpet [5]. We suspect that the flow inside a microchannel coated with a bacterial carpet may demonstrate similar properties (Figure 1.6). This was tested using a technique previously used to explore enhanced mixing due to freely-swimming bacteria in solution [50].

Figure 3.6 shows the behavior of the maximum of the intensity gradient for three cases: (i) a baseline channel with clean walls, (ii) a channel coated with an inactive bacterial carpet, and (iii) a channel coated with an active bacterial carpet. For this particular carpet (80%
The effective diffusion coefficient of the dextran, $D$, is observed to increase above the baseline value by a factor of four, rising from 21.3 to 85.4 $\mu m^2/s$. The channel coated with an immotile carpet also results in an observed increase in $D$ (to 41.8 $\mu m^2/s$) indicating that the mere presence of the passive flagella in the flow enhances fluid transport (table 3.1). Applying the dimensional analysis model to the current data yields $\alpha = 0.499$ for the baseline (clean channel on the 15 $\mu m$ deep microchannel) while $\alpha = 0.524$ and 0.542 for the immotile and active carpets respectively clearly indicating a superdiffusive process (table 3.1), and comparable to the values found for freely swimming bacteria in a microchannel [50].

### 3.4 Summary

The enhancement of diffusion using the addition of live bacteria and the formation of bacterial carpet has been studied experimentally. Assuming standard “Fickian” diffusion, the effective diffusion coefficient has been observed to increase linearly with bacterial concentration and is enhanced by a factor of four at the highest bacterial concentration tested. Even de-energized *E.coli* swimming in the stream and *Serratia marcescens* on the carpet have been shown to promote an enhanced diffusion of the Dextran, although it is much weaker than the enhancement observed with active cells. Due to the addition of a second length scale, most likely associated with the density of the bacteria, the mixing process has been observed to progress in a superdiffusive manner which becomes more apparent as the bacteria concentration increases.
Chapter 4

Paper #3: Bacterial Microfluidics: Mixing and Pumping Using Bacterial Carpets

We present results demonstrating the successful use of live bacteria as actuators for microfabricated fluid systems. The flow-deposition of bacteria can create a live bacterial carpet which can generate local fluid motion inside a micro-fabricated system. The carpet-activated microfluidic system can be used not only to enhance mixing but also to pump fluid autonomously through the microfabricated channel at speeds as high as 25 microns per second. Furthermore, the mixing and pumping performance of the system changes in response to modifications to the chemical and thermal environment of the bacteria. The mixing performance, measured by tracking the dispersion of small particles can be increased by the addition of glucose (food) to the surrounding buffer, or by increasing the flagellar motor activity by raising the buffer temperature. Increasing the glucose concentration also results in an increase of the maximum pumping velocity, presumably due to increased cellular activity. In addition, the pumping performance is affected by the global geometry of the pump with narrower channels achieving a higher pumping velocity at an earlier time after the carpet creation.

4.1 Introduction

The practical development of so-called lab-on-a-chip systems for performing compact chemical analysis, DNA sequencing and other tasks is often held up by difficulties encountered in the relatively mundane operations of mixing and pumping picoliter quantities of fluid. Candidate micro-Total Analysis Systems (µTAS) usually rely on either external devices such as syringe pumps, or relatively large and inefficient systems based on piezo-electric, pneumatic, electrokinetic or electromagnetic effects [65] [66] [67] [68]. While these approaches work, they often rely on bulky and inefficient hardware which often negates the advantages of the micron-scale chemical system which they support. One potential solution to this problem is to use the nanometer-scale motors from biological systems. This concept has been demonstrated in very simple systems, such as using the F$_1$-ATPase rotary motor to
turn a nano-rod [69] [70] and moving microtubules along patterned tracks of kinesin molecules [71] [72]. In both of these examples, although the potential for a useful device that marries a biomolecular motor to an engineered system has been demonstrated, no useful work (such as fluid pumping or mechanical shaft power) was extracted from the system.

An extremely powerful biological motor is the bacterial flagellar motor found in organisms such as *E. coli* or *Serratia marcescens* [6]. These bacteria are propelled through water at speeds as high as 50 microns per second by three or four helical flagella each approximately 10 microns in length and driven by a rotary motor which measuring approximately 100 nm diameter. These motors strongly affect their surrounding fluid environment, and the activity of *E. coli* freely swimming in solution has been shown to enhance the diffusion of tracer beads [40] and of molecules [50] dissolved in water. If the bacterial cells are immobilized on a surface, the rotation of the flagella can also be utilized to generate useful work. Active surfaces, or “bacterial carpets”, have been formed by blotting a Polydimethylsiloxane (PDMS) film onto a swarm plate of *Serratia marcescens* [5]. In such carpets, the cell bodies are stuck to the PDMS, while the flagella are free to rotate, inducing fluid motion and enhanced diffusion of tracer particles in the region near the active surface. The properties of this bacterial carpet with their flagella oriented in one direction can not only pump fluid as a plug-like flow [73] [74] [75] but also transport PDMS chips through a fluid medium [5]. It is advantageous for microfluidic networks to use the bacterial motor system in that the bacteria draw their propulsion energy (protons) from the nutrients in the motile buffer [5] [15] and hence the system requires no external components for operation.

Flagellated bacteria are exquisitely sensitive to a wide variety of external stimuli. They respond to thermal and chemical gradients which directly influence their motive characteristics and form the basis for chemotactic and thermostatic responses [4]. Changes in factors such as temperature, the concentration of certain sugars, or the spatial environment can stimulate the bacteria’s sensory pathway and hence affect aspects of their motor performance, including the counterclockwise and clockwise rotation intervals and the rotation frequency [19] [45] [46]. In this paper we apply the concept of the bacterial carpet to the wider class of closed systems, and have fabricated microfluidic devices in which both the mixing and pumping of fluids is performed by the motion of bacterial flagella. We first demonstrate that bacteria can be flow-deposited on the inside of a closed microfluidic system to form an active bacterial carpet. Furthermore we show that this system can be used to enhance mixing in the sealed system and to pump fluid unassisted for more than an hour at speeds of approximately 25 µm/s. Most importantly, we can efficiently produce global changes in the direction of rotation of the filaments of a flagellar carpet by utilization of bacterial chemotactic characteristics, which promote mixing or pumping performance in a microfluidic system. The present chapter shows that the collective effect of the bacterial carpet corresponding to certain stimuli can be used to amplify the chemotactic sensitivity of the bacterial system and thus generate a useful output. Bulk fluidic motion generated by the bacterial carpet (mixing & pumping) will be quantified as a function of control parameters (temperature, concentration of glucose, pH, spatial restriction, etc.).
4.2 Experimental Setup

After depositing Serratia marcescens on the surface of a straight microchannel (200 µm (w) × 15 µm (d) × 20 mm (l)), the motile buffer in which fluorescent particles (490-nm) were diluted to keep the motility of the bacterial carpet was supplied into the channel. Next, the inlet and the outlet were blocked by RTV glue to measure effective diffusion coefficient of tracer beads in the closed system. In order to study the quantitative analysis for the pumping phenomenon, the inlet and the outlet were connected to polyethylene tubes. Care was taken to ensure that the inlet and outlet tubes were at the same level to prevent hydrostatic pressure gradients, that there were no bubbles, and that the menisci at the ends of the inlet and outlet tubes were identical (so that there were no capillary effects).

The motion of fluorescent particles (490 nm dia.) suspended in the ambient buffer solution was measured using standard fluorescence microscopy techniques. Several image-pairs, each pair consisting of two CCD images (5 ms exposure, separated by 50 ms) were acquired. Particle displacement between the two images was computed using a custom particle tracking velocimetry (PTV) algorithm written using MATLAB [76]. Velocity vectors were then calculated from each displacement vector. Typically 100 images pairs were recorded at a fixed point in the channel over the course of few minutes. Approximately 3000 velocity vectors were computed at each condition. Using a 100 objective lens, the image resolution is 67 nm/pixel, and assuming a tracking accuracy of 0.05 pixels, the velocity resolution is 67 nm/s.

4.3 Mixing Enhancements in a Sealed System

4.3.1 Effects of Glucose Concentration

Once the bacterial carpet was created on the inside of a microchannel, beads moved much more rapidly, and over much larger distances, than beads after a long time from formation of bacterial carpet. Motion was recorded at a series of times in the middle of active surfaces (about 7 µm from the bottom) to measure variation of the initial diffusion coefficient and the decay rate of bacterial carpets in response to changes in concentration of glucose. One may describe the long time behavior of the particle displacements as a diffusion process in the frame co-moving with the settling particles [77]. The diffusion can be characterized by its mean square displacements in two dimensions. Figure 4.1 shows mean-square displacement (a) and effective diffusion coefficient of the fluorescent particle \( D_p \) as a function of time \( t \) (b). The mean-square displacement in two dimensions is given by

\[
< r^2(t) > = 4D_p(t - \tau + \tau \exp(-t/\tau)),
\]

where the diffusion coefficient of the fluorescent particle \( D_p \), correlation time \( \tau \). The solid lines in Figure 4.1 (a) are fit to the equation (4.1), yielding \( D_p = 22.16 \pm 1.21 \mu m^2/s \) and \( \tau = 0.22 \pm 0.01 \) sec at 5 min, and \( D_p = 1.31 \pm 0.21 \mu m^2/s \) and \( \tau = 0.02 \pm 0.01 \) sec at 180 min. After a long time from formation of bacterial carpet in the microchannel (180 min), the mean-square displacement is uniformly linear in time, which is consistent with small diffusion coefficient of the tracer beads. However, the mean-square displacement begins as
Figure 4.1: (a) Mean-square displacement of tracer beads as a function of time $t$ (No glucose), in the middle of the microchannel at 5 min (blue square) and 180 min (black circle) from the formation of the bacterial carpet, (b) Effective diffusion coefficient of the fluorescent particle $D_p$ as a function of time $t$. The upper line was obtained with diffusion coefficients in 20 mM glucose concentration (red square), and the lower line with ones in no glucose solution (blue circle).
ballistic motion in the bacterial carpet, but soon changes over to Brownian motion at the initial time from formation of bacterial carpet (5 min). In addition, correlation over time spans (1/12 s) produces a curvature in $\langle r^2(t) \rangle$ for $t < 0.5$ s. The initial curvature occurs because particle motion is correlated over approximately 0.22 sec.

$$D_p = D_0 \exp(-\alpha t) + D_b,$$
(4.2)

Each of the mean-square displacement curves was fit as shown in Figure 4.1 (a), yielding $D_p(t)$ shown in Figure 4.1 (b). Solid lines are fits to the equation (4.2). $D_0$ is the initial diffusion coefficient of the bacterial carpet, $\alpha$ is the decay rate of the bacterial carpet, and $D_b$ is the diffusion coefficient of Brownian motion at the room temperature respectively. The upper curve was obtained with diffusion coefficients in 20 mM glucose concentration (red square), and the lower curve with ones in no glucose solution. The maximum of the initial diffusion coefficient was 28.49 $\mu$m$^2$/s at 20 mM glucose. The effective diffusion coefficient could be enhanced by an optimum concentration of glucose for a specific time because the rate of an oxygen consumption and the formation of a pH gradient (Figure 4.2) by the bacteria should be affected by the addition of a glucose in the sealed system (inlet & outlet were blocked by RTV silicone adhesive sealant).
Figure 4.3: Variation of the initial diffusion coefficient (a) and the decay rate (b) of bacterial carpets in response to changes in concentration of glucose. Higher glucose concentration increases the initial motility but also increases the decay rate. A plateau is reached at approximately 30 mM.
Figure 4.3 shows the dependence of the initial diffusion coefficient and the decay rate of the particle diffusion due to bacterial carpets in response to changes in concentration of glucose. A slight increase in the buffer glucose concentration quickly increases the initial diffusion coefficient, although as the concentration increases the diffusion coefficient approaches a plateau. This behavior is due to the fact that, although the presence of small concentrations of glucose does increase the metabolic rate of the bacteria, resulting in higher motor rotation rates [15] [20], the glucose consumption rate per cell quickly saturates at a buffer concentration of approximately 20 mM above which the increased concentration has no additional effect. The same behavior is seen with the decay rate of the diffusion coefficient. This may be explained by the fact that bacterial catabolism (destructive metabolism) of glucose rapidly produces lactic, acetic, citric, and pyruvic acids in the buffer [78], and since the system is sealed, there is no mechanism to remove these waste products and they cause the buffer pH to drop (Figure 4.2). Cellular activity, including motor motion is driven by trans-membrane pH gradient and significantly influences the rotation of bacterial flagellum [15] and as the pH drops, the cell motility (and thus the carpet motility) falls [4]. Thus in these sealed systems, the carpets activity directly leads to its own decline and at the elevated metabolic rates induced by the superabundant glucose concentrations, this process is only made more extreme.

4.3.2 Effects of Temperature

The flagellar motion on the bacterial carpet can be affected immensely by temperature because an individual bacterial’s motility pattern is determined by the frequency of tumbling and rotation [45] [20]. Tumbles occur when each flagellar motor independently alternate between clockwise (CW) and counterclockwise (CCW) rotation in the absence of large stimuli [6]. The specific responses in an individual bacterium on the carpet occur when a spatially uniform stimulus is applied to the bacterial carpet. Indeed, the response to temperature changes are similar to the chemotactic response, if the high temperature is replaced with an attractant. In result, the mixing has been enhanced due to the chaotic clockwise (CW) and counterclockwise (CCW) reversal that the wide-type Serratia marcescens on the carpet exhibit. We have quantified the mixing enhancement as a function of temperature.

The relationship between temperature and effective diffusion coefficient was analyzed from the tracks of fluorescent beads. The evolution of the effective diffusion coefficients as a function of time were measured at different ambient temperatures (Figure 4.4). Solid lines were fits to the equation (4.2), yielding a variation of the initial diffusion coefficient and the decay rate of bacterial carpets in response to different temperatures. The percentage deviation from the baseline measurement (at 23 °C) of the initial diffusion coefficient, \( D_0 \), and the decay rate, \( \alpha \), is shown in Figure 4.5.

In general, the initial diffusion coefficient increases with increasing temperature until it reaches a peak at 35 °C. The dip at 40 °C might be related to the fall in the tumbling frequency at higher temperatures [19] which may lead to reduced hydrodynamic effectiveness. As with the glucose dependence, the behavior of the diffusion coefficient decay rate follows closely the behavior of the initial diffusion coefficient and strengthens the hypothesis that the reduction in carpet effectiveness is directly caused by the production of metabolic side-
products, and that at higher metabolic rates (due to glucose or temperature effects), higher carpet activity is accompanied by a faster decline in motility.

The bacterial behavior in the temperature difference is attributed to the effect of viscosity on bacterial motility [79]. Figure 4.5 (b) shows an apparent increase in the decay rate of the bacterial carpet. A decrease in viscosity of a motile buffer is caused by increasing temperature. The flagellar motion on the bacterial carpet can be influenced by viscosity because the conformation of the flagella helix affects efficiency of propulsion, although it is a little [80]. The viscosity of the motile buffer was varied from 1.53 to 0.9 cp in the range of temperatures between 15 °C and 40 °C. As the helix radius increases and the wavelength decreases with decreasing viscosity [80], the propulsive efficiency of the flagellum is responsible for the increase in the decay rate of bacterial carpets when temperature is raised.

4.3.3 Effects of Oxygen

The power required to swim bacteria is actually quite small. It is equal to the viscous drag times the velocity [14]. For the bacterium, the power is about $8 \times 10^{-18}$ watts ($8 \times 10^{-11}$ ergs/sec) [14]. For a bacterium burning glucose, this corresponds to the consumption of oxygen at the rate of about 25 molecules/sec [14]. In general, an amount of dissolved oxygen at 25 °C is $1.58 \times 10^{20}$ molecules/liter [81]. Taking into account an amount of dissolved oxygen in the microchannel ($7.11 \times 10^{12}$ molecules) and a number of bacteria ($1.98 \times 10^{6}$)
Figure 4.5: Variation of the initial diffusion coefficient (a) and the decay rate (b) of bacterial carpets in response to different temperatures. The increase in temperature of a buffer shows an apparent increase in the initial motility and the decay rate up to 35 °C.
based on a fill factor of bacteria carpet (83 % \(\approx\) 33 bacteria/100 \(\mu m^2\)), we can calculate a time that bacteria consume all amount of oxygen in the microchannel completely, which is 39.9 hours. As a result, we may assure that there is no chemotactic response to the amount of dissolved oxygen in the microchannel and the oxygen consumption rate by bacteria during the experiments (3 hours) in the sealed system.

4.4 Enhancements of Pumping Performance

An exciting property of the microfluidic channel functionalized with the bacterial carpet is the observation that it can pump fluid autonomously. Velocity measurements were made at a fixed point in the channel, sampling at several times following the formation of the carpet \((t = 0)\). The average streamwise velocity through the microchannel was observed to build over the course of several minutes (Figure 4.6) reaching a peak velocity of approximately 25 \(\mu m/s\), 20 min. after the carpet formation. At later times, the average velocity decays slowly, falling to 5 \(\mu m/s\) by \(t = 150\) min. The velocity was uniform throughout the channel, characteristic of a surface-driven flow (similar to electrosmotic pumping [73] [74] [75]). We confirmed that this autonomous pumping was due to the activity of the bacterial carpet by repeating the experiment numerous times under different conditions, and by observing that the pump did not operate if the carpet was immobilized using FCCP (which inhibits the bacterial motor rotation).

The physical mechanisms that lead to the observed bulk pumping of fluid are difficult to confirm directly. However, the statistics of the tracer particle motion does lead to several insights. Brownian motion of the tracer particles appears in the PTV analysis as a Gaussian distribution of “diffusion velocities” [14], characterized by zero mean velocity, random orientation and a standard deviation (for the experimental conditions tested), \(\sigma_r\), of 4.9 \(\mu m/s\). The measured particle velocity distributions show this character after long times when the bacteria have all died although during the period when the carpet is actively pumping, the velocity fluctuations are observed to be significantly higher than this baseline, as well as highly anisotropic, with the fluctuations in the streamwise direction, \(\sigma_x\), as much as three times larger than the fluctuations in the cross-stream direction, \(\sigma_y\), (Figure 4.6). The overall pumping performance peaks well before the streamwise velocity fluctuations reach their maximum implying that the pumping is not solely a function of the amplitude of flagellar-driven flow. The growth and subsequent decay in pumping performance of the system suggests that the pumping arises due to a global coordination in the flagellar orientation throughout the channel. The flagella motor rotates predominantly counterclockwise, with randomly occurring events during which the motor rotates clockwise for short times [13] [57]. Since the control of the sense of rotation for each flagellar motor is independent of its neighbor (even within the same cell), it seems unlikely that the clockwise and counterclockwise rotations become coordinated across the carpet. However, hydrodynamic interactions between closely spaced rotating flagella [13] [32] does lead to coordination and the formation of flagella bundles, and we hypothesize that this coordination spreads so that a net flow is established in the microchannel. Such areas of organization were observed in the open carpet in the form of coherent “rivers” along which fluorescent beads were transported at speeds as high as 60 \(\mu m/s\) [5]. In the microchannel considered here, the fluid is constrained to flow in one
Figure 4.6: Evolution of the average and fluctuating velocities (in both the streamwise and spanwise directions) in the microchannel as a function of the time following the creation of the carpet-coated microchannel (no external stimulus). The average velocity reaches its maximum at approximately $t = 20$ min, which is close to the maximum in the anisotropy of the velocity fluctuations. Even as the average pumping velocity declines, the fluctuations remains above the velocity measured by the PTV analysis due to Brownian diffusion "velocities" ($4.9 \mu$m/sec for water at this temperature and $\Delta t$).

direction, and so a coherent river arising at one point in the channel forces fluid in the same direction throughout the entire channel. The net flow will entrain other flagella in the same direction and thus provides a positive feedback mechanism which might serve to assist in the coordination of other regions of the bacterial carpet. In this manner, larger areas of coordinated pumping motion become established and the average pumping speed rises. The role of a mean flow in seeding the pumping ability of the bacterial carpet is supported by the observation that the pump always operates in the same direction as the flow-deposition process that was used to form the carpet.

The pumping performance of the system decays approximately exponentially from its peak value, although the anisotropy in the velocity fluctuations lags somewhat, reaching a maximum at about 40 min. after the carpet formation. The fluctuations become isotropic after about 80 minutes although they remain higher than what would be expected for purely Brownian motion suggesting that the bacteria still exhibit motile activity. In addition, a weak pumping continues for well over two hours. Several hours later, after the bacteria have died, the pumping motion was no longer observed, and the diffusion velocities were found to be in line with that predicted by Brownian motion. The reasons for the decay in the
pumping performance are still unclear, particularly since the decline in motility does not appear to be the primary culprit. This issue will be focused in chapter 5.

Figure 4.6 shows evolution of the average flow velocity as a function of the time following the creation of the carpet-coated microchannel. In order to characterize differences of glucose effects in the pumping performance, we do define two periods in the velocity profile: one is a period of growth (red linear line), the other is a period of decline (blue exponential line). The average pumping velocity is linearly increased to the maximum one in the period of growth, while it is exponentially decreased to Brownian motion. The pumping velocity declines slowly, most likely due to breakdown of the flagellar coordination throughout the carpet, since the overall bacterial motility remains high.

\[
\bar{v} = \varphi t, \quad (t < t^*) \tag{4.3}
\]

\[
\bar{v} = v_{max}e^{\frac{-\zeta}{t^*-t^*}}, \quad (t > t^*) \tag{4.4}
\]

The solid line in the period of growth and that of decline can be fits to the equation (4.3) and the equation (4.4), yielding growth rate rate (\(\varphi\)) and decay rate (\(\zeta\)) in each concentration of glucose respectively. An intersection between a linear red line (in a period of growth) and an exponential blue one (in a period of decline) can be expressed as a maximum pumping velocity (\(v_{max}\)) and a peak time (\(t^*\)) that an average pumping velocity reaches a maximum point (asterisk: *).

4.4.1 Effects of Glucose Concentration

The performance of the pump, as define by the maximum pumping velocity, the time to maximum performance and the velocity decay rate, was characterized as a function of the concentration of glucose in the buffer solution. As shown in Figure 4.7, maximum pumping velocities are significantly enhanced by increases in the glucose concentration in the motile buffer. As before, the performance increases do tend to saturate, although the concentration at which the saturation occurs appears to be higher than was the case for the sealed system. This is likely due to the fact that, since fresh buffer is continuously entering the system, and since waste products are continually being pumped out of the system, the exposure of an individual cell to the products of catabolism are reduced and hence the carpet is more resilient to the effects of its own activity. The time at which the peak velocity is reached (\(t^*\)) is not affected by the glucose concentration (remaining constant at approximately 16.5 min) suggesting that the enhanced flagellar motion does not result in faster self-organization and that this global coordination has little to do with the local cellular processes, but more to do with the overall structure of the carpet.

Figure 4.8 shows the growth rate and the decay rate of average pumping velocities in response to changes in concentration of glucose. With growth rate, we can explain how much a maximum pumping velocity in each concentration of glucose can be produced by the flagellar carpet. The decay rate can be interpreted how long the pumping performance can be maintained by the bacterial carpet in each concentration of glucose. If the decay rate is high (no glucose), it means that the pumping performance goes to decay fast. With glucose effects on the bacterial carpet, the life time of the bacterial pump (the duration of the bacterial motor array in service) could be extended.
4.4.2 Effects of Pump Geometry

The microchannels were designed to measure the spatial dependence of the pumping performance in geometrically restrictive environments. Due to the spatial limitation, bacterial flagellar motion on the carpet may be influenced by the cross-sectional area of the microchannel. Berg and Turner [46] studied motility of bacteria in an array of fine glass capillaries. They found that the net migration of flagellated bacteria in a 10 µm capillary was faster than in a 50 µm capillary. It was also reported that *Escherichia coli* cells swam persistently in only one direction in a 6 µm capillary because of the effect of the geometrical restriction [82]. In our experiments, we made use of straight microchannels with a 15 µm depth and a length of 15 mm. However, a range of widths of microchannel (50, 100, 200, 400, and 800 µm) were tested, so that enhancements of the average pumping velocity in geometrically restrictive environments (Figure 4.9) could be investigated. The profiles of average pumping velocities were dramatically changed by widths of microchannel.

On the carpet, *Serratia marcescens* rotate their flagella freely with the rotation rate of 140 ± 30 Hz, and they also bundle and unbundle [5]. In the absence of a spatial limitation, bacterial flagellar motion on the carpet could persistently produce many vortices (rotating both clockwise and counterclockwise) and relatively linear streams, which lasted more than 10 minutes. In geometrically restrictive environments, the interaction between a cell and a surface or between two cells (shown in Chapter 2) may play an important role in fluid flow with bacterial carpets. The geometry of the microchannel affects the performance of the bacterial pump in two ways. Firstly, it is known that the behavior and motion of bacteria
Figure 4.8: Variation of the growth rate (a) and the decay rate (b) of average pumping velocities in response to changes in concentration of glucose. A plateau is reached at approximately 40 mM.
Figure 4.9: Enhancement of the average pumping velocity in geometrically restrictive environments. Wider channels achieve lower pumping speeds, presumably due to poorer global coordination at such large scales.

...can be influenced by their geometric environment [46] [82]. Secondly, even if the bacterial behavior remains unchanged, the self-organization of the carpet may be a function of the pump geometry. The results shown thus far were performed in a microchannel measuring 15 µm deep 200 µm wide and 15 mm long.

Figure 4.10 shows the maximum pumping velocity and the time to maximum pumping, $t^*$, as a function of the channel width (keeping the length and depth constant). Five channel widths were tested: $w = 50, 100, 200, 400, \text{ and } 800 \mu m$. The results are quite striking, and we see that both the maximum pumping velocity achieved and the time to peak performance are strongly affected by the channel width with improved performance achieved with narrow channels. For the 50 µm channel, a maximum speed of 25 µm/s is achieved immediately after the channel is created (to within our ability to measure), while the widest channel tested, $w = 800 \mu m$, barely pumps at all and requires over 25 minutes to reach its best performance. In the wide channels (400 and 800 µm wide), however, there were small differences (approximately 5 µm/s) between maximum average pumping velocities and minimum ones due to poorer global coordination at such large scales. As a result, the growth rate was rather smaller than one in the narrow microchannel (Figure 4.11). Since we assume that the individual cell behavior is not affected by the pump geometry, we can only conclude that the geometry affects a nature of the flagellar coordination that leads to the global pumping behavior, and that the narrower channels coordinate faster and with more efficiency leading to the observed global pumping performance. Decreasing the pump
Figure 4.10: Variation of the maximum pumping velocity and its peak time as a function of width of microchannel.
Figure 4.11: Variation of the growth rate (a) and the decay rate (b) of average pumping velocities in response to changes in the width of microchannel. A plateau in the growth rate is approximately started at the 400 \( \mu \text{m} \) wide channel and the maximum value of the decay rate is appeared at the 200 \( \mu \text{m} \) wide channel.
length also increased the maximum pumping speed and decreased $t^*$, however the effects were moderate (improvements of approximately 10 % for a three-fold reduction in channel length).

4.5 Summary

The performance of microfluidic devices powered by bacterial carpets has been shown to be a sensitive function of both the environment in which the bacteria live as well as the global device geometry. Factors that enhance bacterial motility, such as the concentration of glucose and the system temperature, similarly affect the carpet motility and hence the overall particle diffusion (mixing) and transport (pumping). The decline of the device performance is thought to be related to the effects of catabolism in which by-products of the carpet metabolism reduces the buffer pH and hence leads to a reduction in motility and device performance. Other chemical effects will also influence pump performance. Most importantly the role of dissolved oxygen will need to be explored, although simple calculations suggest that there is more than enough oxygen in the standard buffers to support the carpets over the few hours that each experiment lasts. Perhaps the most intriguing result, is the finding that the geometry of the pump, specifically the channel width, affects its global performance dramatically, and that in the narrow channels, where coordination is achieved almost immediately, the pumping velocity approaches a maximum speed that is comparable to that achieved by freely-swimming cell.
Chapter 5

Paper #4: Global Coordinations of Bacterial Flagellar Bundles in Microchannels

We have found that wide-type *Serratia marcescens* can be patterned onto the surface of a microchannel in a dense-packed manner to form a bacterial carpet. The global activity of bacterial flagella induces a net flow, creating a self-pumping channel. In order to study the flow generated by the collective motion of the bacterial carpet, methods based on the temporal and spatial correlation functions of the velocity field in the microchannel are exploited.

5.1 Introduction

Motile bacteria propel themselves through the surrounding fluid by rotating their flagella in one direction or the other. If a large number of *Serratia marcescens* are flow-deposited on the inside surfaces of a confined microchannel, a flagellar carpet with unique properties [5] can be patterned onto the surface. As stated in the section 4.4.2, the motion of the bacterial flagella on the carpet can generate coordinated fluid motion, including vortices and relatively linear streams. Darnton *et al.* [5] observed similar flow patterns in the open channel.

Interesting questions arise regarding the large-scale self-coordination of a number of bundling flagella that develops a net thrust. We found that the most probable orientation of the cells on the flow-deposited carpet was parallel to the major axis of the channel, and that 55% of the cells were aligned to within 30 degrees of the channel’s x-axis. However, it has recently been reported that the alignment between flow direction and cell orientation on the carpet does not match particularly well, although the carpet is not created in a confined micro-geometry, but in an open channel [5]. Hence, further investigation is needed to understand global coordination of bacterial flagellar bundles in the carpet. The best way is to employ real-time visualization techniques. As stated in Chapter 1, it is extremely difficult to visualize self-coordinated flagellar networks directly on the bacterial carpet. Even polarized light microscopy using the birefringence associated with thin filaments [83] is inadequate for this application. The reason for this is that the magnitude of background retardance created by the cells that lie on the carpet is neither small nor uniform over a significant area in the
5.2 Experimental Procedure

The image acquisition system was used with the same experimental setup described in Chapter 4. However, the strategy to capture images was different from that in Chapter 4. The fluorescent beads were imaged with a 1500-EX charged-coupled digital (CCD) camera (IDT, Tallahassee, FL) with 6.45 m × 6.45 m pixel resolution and 12-bit dynamic range with 1x1 binning, for an effective 64.5nm x 64.5nm per pixel resolution for the optical system. We
captured 12 images/s in external trigger mode and used 700 × 700 pixels video format. A 100× oil-immersed objective lens was used for magnification of the images, and the field of view of our experiment was approximately 45 µm × 45 µm. PTV image processing of up to 96 images consecutively captured for 8 sec was used to obtain estimates of the velocities in the microchannel. From these velocities, temporal and spatial correlation functions were computed and analysed.

Figure 5.1 shows an optical micrograph of the bacterial carpet during its pumping phase. Here a small fraction of the bacteria have been fluorescently labelled [13] so that we can see the individual flagella. Several remarkable features are worth pointing out. Firstly, we can see flagellar systems both in their tumbling state (figure 5.1a) as well as in their bundled state (figure 5.1b). Secondly, we see (figure 5.1c) that in the cases when the flagella are bundled (i.e. when we only see a single flagellum) that the bundle is always oriented in the direction of pumping. However, during a tumbling phase, the flagellar appear to be randomly oriented without regard to the pumping direction. This is the first direct evidence that the flagellar are collectively oriented during the pumping phase of the carpet-covered microchannel. However, more analysis will be required to fully understand this. As a first step, we use global correlations of the velocity field to assess the level and structure of the carpet’s coordination.

5.3 Temporal Correlation Function

The PTV technique (described in the section 5.2) generates the trajectory and velocity of single particles in the flow but, due to the light seeding of the microchannel, does not produce a velocity field throughout the entire field of view. If we follow an ensemble of single particles in space and time, we can compute the temporal correlation function of the entire field, defined as

$$\Psi_{CC}(t, \tau) = \langle C(t)C(t+\tau) \rangle,$$

where the angle brackets represent an ensemble average, $C$ is the dynamic variable of interest (e.g. $x$-velocity), and $t$ is the time, measured from the point of carpet formation. In case that the starting time is arbitrary, the ensemble average starting at any time, $\tau$, can be expressed as $\langle C(t)C(t+\tau) \rangle$ in equation 5.1. The temporal correlation of the streamwise velocity component can be represented by an integral or by a summation in equation 5.2.

$$\Psi_{uu}(t, \tau) = \langle u(t)u(t+\tau) \rangle = \frac{1}{T} \int_0^T u(t)u(t+\tau)dt = \frac{1}{T} \sum_j u(t_j)u(t_j+\tau).$$

Normalization is applied by dividing by $\langle u(t)u(t) \rangle$. Thus for short times, $\Psi_{uu}$ will have values close to unity, while for long times, much longer than the system correlation time, randomization of the particle’s trajectory by thermal fluctuations will drive $\Psi_{uu}$ towards zero. Sample correlation functions ($t = 20$ min) are shown in Figure 5.2 which illustrate two key points: firstly that the streamwise correlation is much larger than its spanwise counterpart, but that their decay rate (Figure 5.2(b)) is comparable - approximately like $t^{1/2}$.
Figure 5.2: (a) Temporal correlation function and (b) plotted on a log-log scale, evaluated at $t = 20$ minutes (measured from the time of the carpet formation). Circles: streamwise velocity auto-correlation function, square: spanwise velocity auto-correlation function.
To characterize the correlations for streamwise and spanwise velocities induced by the bacterial carpet, we define a correlation time based on the lagrangian correlation function as

$$
\tau_{uu} = \int_0^T \frac{\Psi_{uu}(t, \tau)}{\Psi_{uu}(t, 0)} dt = \int_0^T \frac{\langle u(t)u(t+\tau) \rangle}{\langle u(t)u(t) \rangle} dt,
$$

which estimates how long the global coordination of the system lasts to persist the flow patterns. The correlation time for a particle in a simple fluid (i.e. no bacterial carpet) can be theoretically estimated using a Langevin model [84], balancing the particle’s mass with the damping due to viscous forces, $m/\xi$. The friction coefficient, $\xi$, for spherical particles is given by Stokes law: $6\pi\eta a$, where $\eta$ is the viscosity of the solvent and $a$ is the radius of the sphere. The correlation time for the motion of a fluorescent particle (490 nm dia.) of mass $m = 6.87 \times 10^{-17}$ kg is in the nanosecond time range: $m/\xi = 0.15 \times 10^{-9}$ sec. In our experiments the image acquisition system provided the capture of two successive images with 83 ms (12 Hz) interframe time ($\tau$), which far exceeds the system’s correlation time. Hence, any correlations observed in the present system should be due to coordination imposed by the bacterial carpet.

Figure 5.3 shows the correlation times for streamwise and spanwise velocities in the bacterial pumps. As suggested by Figure 5.2, the correlation times in the cross-stream direction are significantly smaller than those in the streamwise, or pumping direction. This suggests that fluid elements (tagged by tracer particles) are able to move for significantly longer times in a “balistic” manner along the streamwise direction. Given the fact that fluid is not confined in the streamwise direction, this result is perhaps not so surprising. Although both $\tau_{uu}$ and $\tau_{vv}$ decay as time progresses, they maintain a strong anisotropic character, as shown by Figure 5.4 which shows the ratio of the two as a function of time. What is particularly interesting is the fact that anisotropy reaches a maximum at approximately $t = 20$ minutes, which coincides with the time at which the system achieves its maximum pumping velocity.

a significant difference between the two correlation times. The pumping evolved from uncoordinated initial condition, in which both correlation times were relatively high. Then, the spatial limitation induced by the confined micro-geometry allowed much more effective decay in the correlation time for spanwise velocities. As a result, after 20 minutes, the correlation time decay in Figure 5.3 (b) was very small. With the results of the correlation times, we could infer that the large-scale self-coordination was fully-developed at around 20 minutes. Indeed, the mean pumping velocity is found to be approximately linearly related to the anisotropy between the streamwise and spanwise correlation times. As one would expect, the anisotropy continues to decrease with increasing time, eventually reaching a state of full isotropy with a ratio of 0.98 ($\pm 0.13$) for $t \approx 5$ (hours).

5.4 Spatial Correlation Function

Many discussions of the definition and properties of spatial correlation functions are available in the literature [5] [86] [87]. The underlying principle is that if you have a field of velocity vectors at an instant in time, they are “aligned” if their dot product is large and positive.
Figure 5.3: (a) Evolution of the correlation time for streamwise velocity components ($\tau_{uu}$) and (b) one for spanwise velocity components ($\tau_{vv}$) in the bacterial pump.
Conversely, two orthogonal vectors will have a dot product equal to zero, and two vectors pointing in opposite directions will have a large and negative dot product. If we compute the dot product between every pair of velocity vectors, we would expect that the velocities of particles that are close together will have highly correlated velocities, while that are far apart will, on average, be uncorrelated. If we average over dot products resulting from particles falling into a range of separations, \( r_1 < r < r_2 \), we can compute an average spatial correlation function for the entire flow.

Mathematically, if you have a vector field \( \vec{\upsilon}(\vec{r}) \),

\[
\Omega(|\vec{s}|) \equiv \langle \vec{\upsilon}(\vec{r}) \cdot \vec{\upsilon}(\vec{r} + \vec{s}) \rangle_{\vec{r}},
\]

where the average \( \langle \rangle_{\vec{r}} \) is computed over all space. If you have discrete sampling of velocities at a discrete number of positions, you compute \( \vec{\upsilon}(\vec{r}) \),

\[
\Omega(|\vec{s}|) \equiv \langle \vec{\upsilon}_i \cdot \vec{\upsilon}_j \rangle_{\vec{r}_i - \vec{r}_j = \vec{s}}.
\]

Or, more practically,

\[
\Omega(|\vec{s}|) \equiv \frac{1}{N(s)} \sum_{\vec{r}_i - \vec{r}_j = \vec{s}} \vec{\upsilon}_i \cdot \vec{\upsilon}_j,
\]

where \( N(s) \) is the number of pairs of vectors (i.e. the number of data point) that obey the restriction \( \vec{r}_i - \vec{r}_j = \vec{s} \):

\[
N(|\vec{s}|) \equiv \sum_{\vec{r}_i - \vec{r}_j = \vec{s}} 1,
\]
In practice, we plot the results in a histogram, so we allow the data for the $n^{th}$ bin to include all distances that fall within a certain range: $s_n < |\vec{r}_i - \vec{r}_j| < s_{n+1}$. In order to analyze the spatial correlation of a vector field, we started with a set of bins of distance; generally we used about 100 bins covering the range from 0 to the total length of the field of view. Then, we computed the distance vectors $(x_1, y_1)$ and $(x_2, y_2)$ and the dot product between the velocities. All the distances based on their histograms were examined to estimate how many data points fell into each distance bin. Next, we summed all $\vec{\nu} \cdot \vec{\nu}$ that fell into a given distance bin. Lastly, we computed the average over all pairs of vectors measured at relative distances and relative times. We should note that this does not take into account the orientation of the particle separation (i.e. in the streamwise or cross-stream direction), and this could be an important factor. However, for the time being it has not been considered. The spatial correlation between measured velocities was normalized to 1 at $r = 0$. However, we could not rely on our correlations in the range $0 < r < 2.5 \, \mu m$, due to a proximity limitation in the PTV algorithm and the possibility of hydrodynamic interactions between spheres at such small separations [88] [89] [90].

Figure 5.5 shows the evolution of the normalized spatial correlation function of velocities in the bacterial pump, measured at four times: (a) immediately after the carpet’s formation but before the global pumping behavior has built ($t = 5$ min), (b) at the peak of the pumping performance ($t = 20$ min), (c) after the decay of the global pumping ($t = 80$ min) and finally long after the bacteria have died and the carpet is immotile. Darnton et al. reported that the observed motion of tracer beads in an open channel above a bacterial carpet revealed reproducible flow patterns that included relatively linear “streams” as well as rotational pools.
or “vortices” [5]. They described that the linear streams were well-correlated spatially, while the vortices caused anti-correlation, reflected in humps of a variety of sizes in the semilog plot of the global correlation function; the hump width was the average diameter of a vortex. In the present case we observe very similar results to those of Darnton et al. for the non-pumping, motile cases (a and c in Figure 5.5) indicating that during these phases the carpet has similar kinematic properties to the open system studied by Darnton et al. and, in particular, that there exist both streams and vortices. The vortex size varies, but appears to be approximately 5 and 16 microns in diameter, with larger vortices appearing as the pumping action decays.

The spatial correlation function during the times of effective pumping (b in Figure 5.5) is quite different from the correlation functions before and afterwards, and does not show any significant humps, suggesting the absence of any vortical structures in the carpet flow. This suggests that the carpet-induced fluid flow is highly coordinated and thus the velocity field is highly oriented in the pumping direction. This is in full agreement with the observed anisotropy observed in the temporal correlations as well as the global performance history. As time progresses, however, the coordination deteriorates such that the carpet, while still active, does not exhibit the extreme degree of alignment present at its peak. At such time, the vortical structures appear again and, since fluid is being pumped inefficiently in circles, the global pumping performance decreases.

After long times, the non-motile carpet shows no correlation beyond a length scale of 10 microns (which is approximately the length of the flagella, now floating passively in the buffer but presumably still imposing some organization via their inactive presence).

As with the temporal correlation functions, we can define an average correlation length by integrating the normalized spatial correlation function (from 0 to 40 microns). As shown by Figure 5.5, the correlation length through the channel is observed to build over the course of several minutes reaching a peak of approximately 11.62 µm, 20 minutes after the carpet formation (corresponding to the time of maximum average pumping velocity). Following this maximum, the correlation length decays slowly, falling to 4.5 µm at 140 minutes after the carpet’s creation. This history is quite similar in shape to that seen by the ratio of temporal correlation functions (Figure 5.4) and by the average pumping velocity (Figure 4.4). The relatively large standard deviations at the initial stage of pumping action and over the range of times 60 to 100 min because of the existence of vortices in the flow which leads to large variations from instant to instant. The non-motile carpet had a correlation length of 2.2 µm.

The high degree of coordination of bacterial flagellar motion indicates that the surface-driven flow generated by the bacterial carpet can dominate flow patterns over a significant time. Once the bacterial carpet is formed on the inner walls of the microchannel, the cells on the carpet immediately begin to self-coordinate their flagellar motion, most likely due to the hydrodynamic interaction between the flagella. Hence, even though the bacterial pump is at the initial stage (5 min after formation), the average pumping velocity reaches \( \approx 10 \mu m/s \) (about 25 % of \textit{Serratia marcescens}'s average swimming velocity). Subsequently some additional time, usually approximately 10 minutes, is apparently required before the collective activity of the flagella reaches its maximum efficiency with the flow optimally aligned along the major axis of the channel. At that point, the flow rate reaches about 25 \( \mu m/s \) (approximately 60 % of \textit{Serratia marcescens}'s average swimming velocity). As we have
seen (section 4.4.2), this additional time required for the build up of the optimal coordination is strongly related to the channel geometry. We can hypothesize that in the early stages of the carpet’s formation the vortices form spontaneously and that the net motion resulting from their independent action has no global consequences since fluid elements are simply pushed around at random. However, with time, adjacent vortices interact and coalesce, and as they get larger and, it becomes easier for fluid elements to chart a direct path along the axis of the channel, leading to the observed rise in the pumping performance. In a narrower channel, less vortex coalescence is required to enable coherent global pumping, hence the observed dependence on channel width. For large times, the decline of the pumping performance after the peak is most likely due to degraded bacterial motility over the course of the experiment (about 2 hours).

5.5 Summary

Temporal and spatial correlation functions are presented in order to examine the large-scale global coordinate imposed by bacterial flagellar motion in a microchannel. Experiments utilized a PTV technique, and we have shown that the motion of tracer beads induced by the carpet correlates both temporally and spatially and is closely associated with the growth and decay of the flagellar coordination. In addition, the result of the ratios of correlation times and the correlation lengths links strongly with the pumping performance, and this would seem to be a good measure of the underlying coordination in the flagellar carpet. Overall,
a good agreement between the ratios of correlation times and the correlation lengths is observed, and both of these are quite similar in character to the average pumping velocities. When the average pumping velocity is at a peak, not only does the ratio of correlation times reach its maximum value, but also the correlation length reaches a maximum. This suggests that the flagellar coordination is highly aligned along the channel axis. However, that coordination deteriorates over time such that the carpet, while still active, can not self-organize to maintain its pumping action. We also observe that the flow field associated with the presence of the passive flagella in the immotile carpet has a little correlation between measured velocities unlike one in pure Brownian motion.
Chapter 6
Concluding Remarks and Recommendations

Through this research we have demonstrated the use of bacteria as controllable elements of a microfluidic network by incorporating them into micro-fabricated systems. It has been proven that flagellated bacteria are an ideal system to use for a wide variety of microfluidic systems, because the flagellar motors are the most powerful bio-molecular motors known to date in the micron scale world and ideally suited for microfluidic environments. Using different bacterial propulsion systems, such as *Escherichia coli* and *Serratia marcescens*, we have developed the necessary understanding and technical infrastructure to accomplish the following:

- to flow-deposit bacteria in a dense-packed manner on the inner surfaces of a microfluidic channel with their flagella oriented in one direction along the channel. The properties of this “bacterial carpet” have been studied, including the ability of the carpet to generate bulk fluid flow in response to modifications to the chemical and thermal environments.

- to use bacteria both as individual actuators and carpets to build chaotic mixers for micro-fluidic systems. The alternation between CW and CCW rotation in a random manner is a unique feature of flagellar motors and has been used in a confined micro-geometry.

- to use bacterial carpets to pump the fluid in a controlled and directed manner. The bacterial pump has been fabricated and tested to determine its pumping characteristics, as well as the durability of the bacterial motor array in service, including geometrically restrictive environments.

- to elucidate large-scale self-coordinations of bacterial flagella in the carpet based on temporal and spatial correlation functions.

Distinct from the study of bacterial actuation elements discussed above, there has also been a considerable investment into the fluid mechanics of micron-scale engineered systems, especially low Reynolds number flows. The measurement of fluid motion at micron-scales...
has been limited by optical access and the diffractive limits of optical microscopy. However, we have developed several advances in applying Particle Tracking Velocimetry (PTV) techniques and image processing techniques to micron-scale flows.

6.1 Impact on Microfluidics

With the demonstration of performance in microfluidic systems, this work serves as a breakthrough in developing microfluidic components based on microbiological cells. This has led to several engineering challenges, for example, how to enhance mixing of parallel streams of fluids using both as individual actuators and in arrays, how to pump fluids through small systems with optimum efficiency, and how to measure the effects at such small scales. An ambient fluid in microfluidic systems based on bacterial actuation can be transported through micron sized channels, where they undergo chaotic mixing. By operating the autonomous fluidic pump for several hours, we have shown that a self-sustained bio-molecular motor can be incorporated into a microfluidic system.

The progress in microfluidic technologies and bacterial actuation techniques significantly contributes to the notion of microelectromechanical systems (MEMS) for biological applications - BioMEMS. We have also established a method for creating a bacterial carpet for enhancing mixing and pumping to a flow, and simultaneously providing an optimal stimuli to control and direct its performances in a variety of microfluidic environments.

Such bacterial systems are (to our knowledge) the first demonstrations of biological actuation of an engineered microfluidic system. The robustness, ease of “manufacture” and the ability to genetically modify their behavior make such systems highly attractive for powering microfluidic devices.

6.2 Recommendations for Future Work

Most studies in this thesis focus on the generation of collective momentum from flagellated bacteria, and how we can use that momentum in a microfluidic network. However, bulk fluid motion generated by the bacteria also implies their use as sensors. If we can detect changes in the rotary motor performance by monitoring the global microfluidic effect, we can use this system to monitor a chemotactic response. Furthermore, the chemotaxis receptors of the bacteria can be engineered to detect specific chemicals of interest.

It is also realized that the bacterial carpet developed in this work can be promoted to a “bacterial barge” - an engineered chip that is free to move. A barge, constructed by a PDMS, may be controllable in a number of ways - chemically, electrically, or optically. In the future, we can employ flagellated bacteria as transportation elements in the unseen worlds of the microspace to deliver a micron-scale MEMS device. Indeed, they are easily “manufactured”, self-contained and easy to fuel. Therefore, the flagellar motors are ideally suited for this transportation system.

Finally, we strongly encourage one to develop numerical simulation tools to aid in interpreting and complementing effective flow fields generated by flagellar carpets in complex confined micro-geometries. A numerical approach for this work is requisite to understand the
non-steady nature of the flow. It will show how hydrodynamic interactions in a microfluidic network lead to large-scale global coordination of the bacterial flagella, a phenomenon that is not visualized.
Appendix A

Masks

Masks are an integral component in the photolithographic process of microchannel fabricating. Using an illustration software (Adobe Illustrator v10), the designed two-dimensional micro-geometry was drawn with precision, and printed onto a transparency with high resolution (3600 dpi). Then, the high resolution image served as the mask for the photolithographic procedure. Figure A.1 and A.2 are the printed micro-geometries of an actual transparency mask for the chaotic mixer and the bacterial pump. Using negative photoresist SU-8 5 or 50 (MicroChem, Newton, MA) the unexposed, or masked, portion of this material is then removed so it can either be etched to form channels or be deposited with other materials. Thus, our masks are positive. If the process is reversed using positive photoresist, we should use a negative mask.
Figure A.1: Mask for the chaotic mixer.
Figure A.2: Mask for the bacterial pump.
Appendix B

Fabrication of Microchannels

Fabrication, shown in Figure B.1, starts with a polished silicon wafer, which is first cleaned surface using the piranha cleaning technique ($\text{H}_2\text{SO}_4$ and $\text{H}_2\text{O}_2$) to remove residues accumulated on the silicon wafers during storage. A layer of 40 µm layer of negative-tone photoresist (SU-8 50, MicroChem, Newton MA) is spun onto the cleaned silicon wafer and soft-baked at 65 °C on the surface of a hotplate for 5 minutes and at 95 °C for an additional 15 minutes. The channel manifold is patterned onto the coated wafer using standard photolithography techniques using a mask made out of a high-resolution photographic negative film (3600 dpi) printed at full scale (shown in Figure A.1 and A.2). The exposed wafer is baked at 65 °C for 2 minutes and 95 °C for 4 minutes and developed to create the master mold. The master mold is covered by a thin layer of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technology, Bristol PA) by placing the mold in a vacuumed desiccator with a vial containing a few drops of the silane which acts as a mold release for the PDMS. Sylgard 184 silicon elastomer and its curing agent (Dow Corning, Midland MI) are mixed at a 10:1 mass ratio, and placed in a vacuumed desiccator for degassing. The PDMS mixture is then poured onto the master mold and cured at 75 °C on a hotplate for 45 minutes. Once the PDMS has solidified and cooled, it can be peeled off from the master mold, with the microchannel structure engraved. The patterned PDMS surface is sealed against a clean microscope glass slide to create the flow channels. The 170 micron glass slide is rinsed in heptane and then in ethanol to remove organic residues. The PDMS is cleaned with ethanol. Both surfaces are dried under a nitrogen stream, and placed in a plasma etcher and plasma-oxidized for 1 minute. The two surfaces bond irreversibly upon immediate contact. Fluid access into the channels is made by insertion of plastic tubing (0.965 mm O.D. polyethylene tubing, Becton Dickinson, Sparks MD) through the pre-punctured holes. RTV silicon sealant (Permatex, Solon OH) is applied around the insertion point to seal the junction.
Figure B.1: Schematics of the photolithography sequence: (1). Spin-casting process for photoresist, (2). Etch a channel manifold on the substrate using the photolithography technique, (3). Developing process to peel off the excess photoresist, (4). PDMS molding process to replicate the pattern on the silicone wafer, (5). Cleaning PDMS microchannel with Ethanol, (6). Oxygen plasma treatment for bonding PDMS to glass substrate.
Appendix C

Cells

*Escherichia coli* and *Serratia marcescens* (ATCC 274, American Type Culture Collection, manassas, VA) shown in Figure C.1 were used in this study, which were provided by Linda Turner and Howard Berg of the Rowland Institute at Harvard University.

For the best motility, the 100 µl frozen aliquot of *Escherichia coli* or *Serratia marcescens* was put into 10 ml of LB growth medium and incubated for 3 - 4 hours at 30 - 34 °C. One liter of culture medium (LB broth) contained 10 grams of tryptone, 5 grams of yeast extract, and 10 grams of NaCl in distilled pure water. The cultures were aerated by gently shaking the tube at about 180 rpm. The bacteria were removed from the incubator during the exponential phase of their growth for use in experiments. After longer incubation times, the density of the culture saturates and motility quickly decreases. The bacteria were separated from the nutrient broth, by centrifugation at 2200 g for 10 minutes. On our fixed-angle rotor this corresponds to a centrifuge speed of 3000 rpm. At the end of a centrifuge spin, we could obtain the pellet that was soft not tightly packed. we poured off the supernatant in one motion, and then re-suspended in 0.5 ml of buffer and gently mixing. Buffer was then added to bring the total volume to 10 ml. This separation process was repeated three times to ensure that all the growth medium was removed. Usually, we gently jiggled the centrifuge tube at about a 45 degree angle such that the fluid sloshed gently over the pellet. The pellet should easily resuspend within about 3 - 4 min. If it breaks up into clumpy pieces, then it was packed too densely hence centrifuged with too much g or for too long time. It is very important to gently resuspend the bacteria in pellets so as not to break off flagellar filaments. After culturing, the cells were diluted into a pH 7 motile buffer, consisting of 0.01M KPO₄, 0.067M NaCl, and 10⁻⁴M EDTA. The motility of the bacteria can degrade in the anaerobic environment of the microchannel and supporting tubing. So, the motion of the cells was observed carefully both before and after the experiment to ensure that the cells maintained their motility.
Figure C.1: (a) A *Serratia marcescens* colony swarming across an agar plate. (b) Phase-contrast image of a culturing *Serratia marcescens* on the swarm plate. An asterisk mark in a colony (a) approximates a location to be captured an image and the bar is 10 µm.
Appendix D

Formation of Bacterial Carpets

Since the microchannel network is fully enclosed, we cannot form bacterial carpets using the blotting technique [5]. Instead, bacterial carpets were formed on the inner surfaces of a microfluidic channel using a flow deposition procedure. Using a syringe pump, a buffer containing a high concentration (2 - 5 × 10^9/ml) of motile Serratia marcescens was pumped through the channel at a low flow rate (0.05 µl/min). After five seconds, the pump was switched off, and the system was allowed to settle for five minutes. During this time, the bacteria swim randomly through the channel, sticking on contact to bare spots on the PDMS surface. Some bacteria produce slime to help them to stick to surfaces. The slime produced by Serratia marcescens enables them to stick to the surface of the microchannel, which is usually made up from polysaccharides [91]. The flow-and-settle cycle was repeated until the surface was coated to the desired density. To counteract gravitational sedimentation effects, the system must be rotated to ensure an even coating of all surfaces. The formation of the carpet was tracked optically using Differential Interference Contrast (DIC) microscopy (Figure D.1 and Figure A.1).

Figure D.2 shows the growth of the fraction of the surface covered by bacteria. Note that that the PDMS surface coats more quickly than the glass surface, consistent with the fact that the Serratia are observed to adhere more easily to PDMS than to glass. Unlike

Figure D.1: Micrographs of the bacterial carpet as it develops inside the microfluidic system. The pictures are taken at (1) t = 20, (2) 200, and (3) 2000 seconds after the initiation of the flow deposition procedure. The bar is 10 µm.
Figure D.2: Development of the “fill factor” - the percentage of the surface covered by the bacterial carpet - as a function of time. Note that the glass surface coats more slowly than the PDMS due to the lower adhesion between the *Serratia marcescens* and the glass. Points (1), (2), and (3) correspond to the images in Figure D.1.
Figure D.3: Probability density function of cell orientation. 54.9 % of the cells are aligned to between - 30 and 30 degrees with respect to the channel’s x-axis. The percentage of the cells oriented between - 40 and 40 degrees, and between -50 and 50 degrees are 64.3 % and 74.5 % respectively.

the smooth and densely packed carpets formed on an open slide by blotting from a swarm plate [5], the flow-deposited carpet does have some gaps and some surface roughness due to cells adhering to the surface at odd angles. Approximately 80 % of the cells adhere to the surface as a single isolated cell, while the remaining were in contact with or partially on top of another bacterial cell. The cell bodies are elongated spheroids (approximately 3 microns in length, 1 micron in width). Due to the alignment by the flow, the cells tend to adhere with a clear orientational preference, with 55 % of the cells aligned to within 30 degrees of the channel’s x-axis (Figure D.3). Although a few cells were observed to stick to the surface by their flagella most of the cells stuck to the surface by their bodies with their flagella free to rotate in the flow.
Fluorescence is the property of some atoms and molecules to absorb light of a particular wavelength and after a brief interval, to re-emit light at longer wavelengths [92]. In our experiments, we made use of fluorescein isothiocyanate (FITC)-dextran (Sigma-Aldrich Co, St. Louis, MO) and red fluorescent microspheres (Duke Scientific Corp, Palo Alto, CA), whose excitation maximums were 490 nm and 542 nm and their emission maximums were 520 nm and 612 nm respectively. Figure E.1 illustrates an example of the separation of blue excitation light from green fluorescence.

Many microbiological specimens are colorless, nearly transparent, and relatively thick, such as bacteria, which do not give a clear image in the ordinary microscopy. In order to produce contrast in this type of specimen, Differential Interference Contrast (DIC) requiring several special optical components was used, which enabled specimens that had a refractive index similar to their surroundings to be visually differentiated [93]. Figure E.2 illustrates the apparatus commonly used for Normaski DIC microscopy.
Figure E.1: Fluorescence microscopy system for image acquisitions: separation of fluorescence excitation light from fluorescence emission light by a dichromatic beam splitter (epifluorescent filter cube). Blue excitation light of 490 nm is generated when the excitation light passes through the short-pass filter. This filter allows light below 490 nm to pass through the filter unattenuated. The excitation light then hits the chromatic beam splitter and light below 510 nm is reflected downward through the objective and on to the sample. The emitted fluorescence and scattered excitation light then re-enters the objective and hits the beam splitter. The splitter allows light above 510 nm to pass through the mirror, while reflecting light below 510 nm, preventing this light from reaching the detector. A long-pass barrier filter, which allows only light of wavelengths of at least 515 nm to pass through the filter, removes the residual excitation light.
Figure E.2: Differential Interference Contrast (DIC) microscopy system for bacteria [94]: a illumination starts as a polarized beam of light. This is divided into two beams that have different polarization angles and that are separated horizontally by a distance roughly equal to the resolution of the objective lens. As these two beams pass through bacteria, changes in refractive index due to the fine structure of the bacteria affect one beam more than the other. After passing through the bacteria and the objective lens, the horizontal separation between the two beams is removed. In the final step, the two beams are converted to the same polarization angle. At this point, the two beams can interface with one another to produce amplitude contrast. This contrast reflects the optical path difference between the two beams that was introduced by the bacteria. The differential in DIC is the difference in optical path length of the two beams. This difference results from different horizontal refractive index gradients in the bacteria.
Appendix F

Data Acquisition and Image Processing for Mixing Experiments

The images were obtained using a Nikon TE200 inverted epi-fluorescent microscope with a 20× objective and recorded with an IDT SharpVision 12-bit cooled CCD camera, with 1,300 × 1,080 pixels (Figure E.1). Ten images were recorded at each x-station and each flow rate. The intensity profiles across the channel were computed by averaging the ten frames and averaging over 300 pixels in the streamwise direction (corresponding to 107 microns). At each pixel, the gradient of the intensity profile was computed by fitting a quadratic polynomial to five pixels straddling the pixel of interest (two to the left, two to the right) and then differentiating the polynomial function. Similarly, the maximum value of the intensity gradient and its location was determined by sub-pixel interpolation in which a quadratic polynomial was fit to the five pixels surrounding the maximum in the gradient peak. The critical point was then determined analytically from the polynomial coefficients. Determination of the standard deviation of the intensity gradient distribution was computed by integration of the zeroth, first and second moments of the intensity gradient using Simpson’s rule.
Appendix G

Particle Tracking Velocitmetry (PTV) Processing for Pumping Experiments

Velocity distributions were obtained using particle tracking velocimetry (PTV), which is the low particle concentration mode of particle image velocimetry (PIV) [33]. Since, the required seeding density is lower in comparison with PIV, tracking the movement of the individual particles within the area of interest yields better results than the conventional statistical analysis methods due to a higher detection rate which can be achieved within the measurement window [76].

The fluid was seeded (0.04 % by volume) with 490 nm fluorescent particles. Image pairs consisting of two successive CCD images (5 ms exposure) separated by 50 ms were acquired using standard fluorescence microscopy techniques. The fluorescent particles were identified and tracked from the first image to the second, yielding a velocity vector. Approximately 3000 velocity vectors were computed at each condition. Using a 100× objective, the image resolution is 60 nm/pixel, and assuming a tracking accuracy of 0.05 pixels, the velocity resolution is 60 nm/s. Typically 100 image pairs were recorded at a fixed point in the channel in the course of several minutes. The recorded image pair was analyzed with a PTV algorithm in the MATLAB programming environment.

The particle tracking procedures are as follows. First, to measure particle velocities, several image pairs were taken for subsequent PTV image analysis. Generally, a lot of background noises can be observed because of the light scattering from the out-of-focus particles in the bulk of the fluid [Figure G.1 (a)]. A global threshold algorithm was applied to find the particles in the particle image using the grey-level intensity and pixel size information [Figure G.1 (b)]. This algorithm is fast and easy to adapt and robust to the noise. After finding all particles, the Gaussian curve fit [95] [96] [97] was applied again to calculate the location of particle centroids in the sub-pixel range. To track each particle, particle centroids should be extracted from the particle image. For each image pair, the identified particles, counted from each image ranged from 10 to 30, were matched using a simple particle tracking algorithm of smallest particle displacement.

Particle displacement vectors were computed from the displacements of particle centers. Using the image resolution, and the applied interframe time, one velocity vector can be calculated from each displacement vector. For average pumping velocity analysis, the streamwise
Figure G.1: Images of the same group of particles. (a) Particle image as viewed through the epi-fluorescent filter. (b) Particle image applied threshold for PTV processing. The scale bar is 10 µm.
velocities were produced by vector projection onto the mean velocity direction. Because of
the random nature of Brownian motion caused by the sub-micron size of the seed particles,
the streamwise velocities were extracted by finding the mean of all velocity vectors. The
distribution of the spanwise velocities can reveal interesting characteristics of diffusion gen-
erated by bacterial carpet. The displacements of particles in the cross-stream direction had
a Gaussian distribution with mean and skewness close to zero.
Appendix H

Temperature-controlled Test Cell

The swimming velocity of flagellated bacteria at various constant temperatures is found to increase with increasing temperature because of the frequency of tumbling in response to changes in temperature [19]. For *Serratia marcescens*, the optimum temperature for their high activity is not at room one but at 37 °C [98]. The normal body temperature of a healthy, resting adult human being is stated to be at 98.6 °F or 37.0 °C. In order to investigate effect of temperature on the bacterial carpet, we developed a special iso-thermal heating system to measure effective diffusion coefficient of tracer beads in the buffer temperature. The main components of the system shown in Figure H.1 were composed of a heating holder and plate for a 100 × objective lens and a heating cover for a microchannel, which were made of copper, thermoelectric coolers equipped with connectors, and thermo-couples digitally to measure temperatures on the plate and the cover. Thermoelectric coolers (TECs) (Melcor, Trenton, NJ, USA) are solid state heat pumps that utilize the Peltier effect. During operation, DC current flows through the TEC causing heat to be transferred from one side of the TEC to the other, creating a cold and hot side. TECs could be used to fulfill the demand of a large temperature range in our study. We needed a specific adhesive for thermally bonding TECs to copper surfaces and heat sinks, while electrically isolating one from the other. To do this, DeltaBond “152” (Wakefield Engineering Inc, Pelham, NH, USA) was applied to produce a rigid, high strength bond to the materials when cured. Two LDT-5525 temperature controller (ILX Lightwave Corp, Bozeman, MT, USA), a microprocessor-based temperature controller designed for temperature control of sensitive thermoelectric coolers, were used to accurately control temperatures, which were connected to the TECs in the heating holder and the heating cover. Temperature range to be controlled by a LDT-5525 temperature controller were -20 °C ~ 70 °C and long term temperature stability was ±0.01 °C.

A schematic of the set-up is shown in Figure H.2. A microchannel positioned on the heating plate was covered with another heating plate. The gap between two copper plates was sealed with high thermal conductivity paste, omegatherm “201”, (Omega Engineering Inc, Stamford, CT, USA), which exhibited important characteristics necessary for accurate, fast, reliable temperature measurement: good adhesion and strength, high thermal conduction, thixotropic consistency, fast cure, and easy application. Two thermo-couples were adhered to the system: one was on the heating plate, the other was in the inside of the heating cover. It enabled us to double-check whether or not the temperature inside the system is isothermal.
Figure H.1: Components of an isothermal heating system to measure bacterial motility in response to changes in temperature.
Figure H.2: Experimental set-up with an isothermal heating system.
With this system, we could accurately increase and decrease temperature that we wanted to reach for the experiment, which could be kept reliably for a long time (approximately 5 hours). Five different temperatures (15, 23, 30, 35, and 40 °C) were used to measure bacterial motility in response to changes in temperature.

**H.1 Validation**

The PTV system for thermal effects on bacterial carpets was tested by measuring diffusion coefficients induced by different temperatures. For validation, five different temperatures (23, 30, 35, 40, and 43 °C) were applied to the iso-thermal heating system. In order to compute theoretical diffusion coefficient at each temperature, the Stoke-Einstein equation was employed as an analytic solution:

\[
D_{sphere} = \frac{kT}{6\pi \eta a},
\]  

\text{(H.1)}
where $k$ is Boltzmann’s constant, $T$ is the absolute temperature, $\eta$ is the viscosity of the solvent, and $a$ is the radius of the sphere [84]. Figure H.3 shows the comparison between PTV measurements and analytical calculations of Stoke-Einstein equation at each temperature. The measurement results show about 4.1% discrepancy from the analytic solution at 23 °C (room temperature) and below 2.0% at other temperatures. Overall the accuracy was deemed acceptable and it can be believed that the PTV results in the iso-thermal heating system are reliable.
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