Characteristics of swimming bacteria in flow

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**CHARACTERISTICS OF
SWIMMING BACTERIA IN FLOW**

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**SCHOOL OF MECHANICAL
AND AEROSPACE ENGINEERING**

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SWIMMING BACTERIA IN FLOW**

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Abstract

Many microorganisms utilize their flagella, which are hair-like appendages, to swim in fluid environments. The research of locomotion of flagellated microorganisms involves the study of flagella's behaviors. Since flagellated microorganisms swim by propelling their flagella through liquid environment, the propulsion of flagella plays a key role in hydrodynamics of the swimmers. In reality, the microorganisms are constantly subjected to flows and other external forces during swimming. Therefore, it is very important to understand how behaviors of the flagella contribute to the swimming in the presence of flow and external forces. In this thesis, I investigate the behavior of helically flagellated bacteria in external force field as well as the shape of a flagellum in shear flow.

First, I present a model to analyze the effect of dielectrophoretic (DEP) force on a swimming helically flagellated bacterium, particularly on its swimming direction and velocity. I consider simple DEP force that is acting along X -direction, and the force's strength varies with Y -positions. Both DEP force and rotational diffusion are considered when analyzing the swimming of the bacterium. In most cases, DEP force is main factor that determines the steady swimming orientation of the bacterium; however, the impact of rotational diffusion is more significant when the DEP force's strength varies strongly in the Y -direction. Interestingly, the variation in DEP force's strength in the Y -direction causes the bacterium to translate perpendicular to its primary axis. This phenomenon is the consequence of the interaction between helical shape of the flagellum and the external force, and it could be applied to focus the bacteria. The model developed here would contribute to our knowledge on how external force affects the swimming behavior of flagellated bacteria, emphasizing on the interaction between the helical geometry of the flagellum and the external force.

For the investigation of the flagellum's shape in shear flow, I derive the governing equation for the shape of a 2D beating flagellum in shear, and discuss the results at various shear strengths. The governing equation for the flagellum's shape allows us to obtain the shape of the flagellum at different degrees of shear strength as well as flagellum's flexibility. The numerical results show that shear flow has significant influence on the shape of the flagellum, especially when the flagellum is highly flexible, and in many cases, changes in the beating patterns of the flagellum positively promote the swimming speed of the swimmer. Furthermore, there exists an optimal shear rate that maximizes the effects of flexibility on the swimming speed. In most cases, the interaction between shear and flexibility of the flagellum plays a key role in determination of beating patterns. The results show that shear flow could be utilized to detect the difference in characteristics of flagellated swimmers, and to improve some existing sorting devices. I believe the analysis of the flagellum's shape in shear flow has great contribution as to the best of my knowledge, this kind of governing equation of the flagellum's shape in shear flow has not been mentioned in the literature.

On the experimental aspect, I conduct experiments to characterize the morphology of *B. subtilis* flagella and analyze the shape of flagella at different shear strengths. The proposed experimental method allows us to analyze the morphology of the bacterial flagella in great detail, including length, pitches, diameters, pitch angle. The experimental results show that external flow has different effects on different regions of the flagella. The regions of the flagella that are closer to the cells body are less influenced by the flow, and I believe the presence of the cells body reduces the effect of flow on the nearby regions of the flagella, and the bacteria have some mechanism to maintain the shape of the flagella in the regions close to the cells' body.

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List of figures

- Figure 3-1 Schematic of a bacterium and its orientation in 3-D space. (a) Geometric parameters of the bacterium in the body-fixed frame $oxyz$, which has its origin at the head's center and its z -axis is along the bacterium's primary axis. (b) The orientation of the bacterium is determined by (θ, ψ) , $0 \leq \theta \leq \pi, 0 \leq \psi \leq 2\pi$ [76]. 20
- Figure 3-2 Reorientation mechanism of the bacterium under different configurations of DEP force. DEP force is generated by: (a) positive C_0 ; (b) negative C_0 ; (c) positive C_1 ; (d) negative C_1 ; (e) positive C_2 ; and (f) negative C_2 . In each case, four typical orientations in four quadrants of space are considered, and the bacterium's head is at the origin. The red dashed and blue dashed positions are steady orientations [76]. 26
- Figure 3-3 Orientation distribution of the bacterium at different configurations of DEP force. θ is polar angle and ψ is the azimuthal angles ($0 \leq \theta \leq \pi, 0 \leq \psi \leq 2\pi$) . (a) $C_0 = 0.1$; (b) $C_0 = -0.1$; (c) $C_1 = 100$; (d) $C_1 = -100$; (e) $C_2 = 100$; (f) $C_2 = -100$ [76]. 28
- Figure 3-4 Mean swimming velocity $\bar{v}$ of the bacterium due to (a) C_0 and (b) C_2 . (c) Force and velocity analysis of one full turn of the left-handed helical tail due to positive C_2 . The solid red line, dashed blue line and dotted-dashed green line are velocity components in the X -, Y - and Z -direction, respectively. [76]. 31
- Figure 3-5 (a) Orientation of the bacterium when $\nabla|\mathbf{E}|^2 = [C_2 Y, C_2 X, 0]^T$ ($C_2 < 0$) . The arrows indicate the direction of DEP force exerted on the bacterium's head; (b) Orientation of the bacterium when $\nabla|\mathbf{E}|^2 = C_2 \{k_1 X + Y, X + k_2 Y, 0\}$ ($C_2 < 0$); (c) Mean velocity in the Z -direction of the bacterium at the origin at different values

of negative C_2 , for $\nabla \mathbf{E} ^2 = C_2[k_1X + Y, X + k_2Y, 0]^T$; (d) General shape of the electrodes when $C_2 = -100, k_1 = 1.3, k_2 = 1.5$, for an example [76].	33
Figure 4-1 a) Illustration of a swimming sperm in a high aspect cross-sectional ratio microchannel ($W \gg H$); b) Schematic of a sperm in shear flow; c) Force and moment analysis of an infinitesimal segment of the sperm's flagellum.	41
Figure 4-2 Comparison between analytical and numerical solutions of equation (4.43) at (a) $Sp = 2$, (b) $Sp = 7$, and (c) $Sp = 20$. On the left side, solid curves represent shape of the flagellum obtained from analytical method, and scattered curves are from Chebyshev collocation method. The maximum errors between solutions of exact method and Chebyshev collocation method show the quality of the numerical approximation.	54
Figure 4-3 Schematics of (a) positive and (b) negative shear flow configurations.	55
Figure 4-4 Shapes of beating flexible flagellum at different values of sperm number Sp and shear rates Z . The different line styles represent different time points in one cycle of oscillation.	56
Figure 4-5 Changes in beating patterns of the flagellum due to positive and negative shear flow. Flagellum with high flexibility is more sensitive to shear and there is significant change in its beating pattern.	57
Figure 4-6 Velocity gain at various levels of flexibility and shear strength, compared to the swimming velocity at no shear ($Z = 0$) with most rigid flagellum ($Sp = 5$). The red dot located at $(Sp, Z) = (5, 0)$ has velocity gain equal to 1. The color bar on the right shows range of velocity gain, starting from 0.45.	59
Figure 4-7 Velocity gain due to increasing flexibility (Sp) at different conditions of shear flow. There exists an optimal shear rate, $Z \approx 0.85$, that maximize the favorable effect of flagellum's flexibility on the swimming velocity	61

Figure 5-1 (a) Schematic of a microchannel and flow profile in the channel. The channel has high aspect cross sectional ratio, i.e. $W \gg H$, that generates parabolic flow profile. (b) Experimental setup for images acquisition.	67
Figure 5-2 A microscopic image showing single bacterial flagellum and bundle of flagella.	69
Figure 5-3 (a) (b) Tracing and (c) extraction of a flagellum image. The extracted image is binary and free of background noise.....	69
Figure 5-4 Rotation of flagella during tracing to make their roots on the left hand side. (a) Original orientations of four flagella. (b) Flagella are selectively rotated based on their original orientation.	71
Figure 5-5 (a) Image of a flagellum be imported to MATLAB as a logical matrix; (b) The flagellum image after being reoriented to be parallel with horizontal axis; (c) The flagellum and its mathematical representation (the red curve).	72
Figure 5-6 Morphological information of a flagellum obtained by geometrical analysis of the curve $y = f(x)$. The red dots are local maxima and the blue dots are local minima of the curve.....	73
Figure 5-7 Effect of the depth of field δz . The blue and green rods are wholly in focus while the red one is partially out of focus.	75
Figure 5-8 Visual comparison between traced flagella (binary images) and their mathematical representations (red curves) shows good performance of images processing script. The script is also successful in determine locations of extrema of the curves. Note that images of flagella shown above have different plotting scales.....	77
Figure 5-9 Mean length of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.	78

Figure 5-10 Mean diameter of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.	79
Figure 5-11 Mean helical pitch angle of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.	79
Figure 5-12 Mean pitch length of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.	80
Figure 5-13 Measurable and unmeasurable pitches of a flagellum. When a flagellum has one measurable pitch, its axial length might be longer than the only measurable pitch.	82
Figure 5-14 Sample images of bacteria under flow conditions. The images also show some flagella that are partially out of focus.	82
Figure 5-15 Pitches of the flagella at different flow rates. The lengths of the error bars are two times the standard deviation.	84
Figure 5-16 Diameters of the flagella at different flow rates. The lengths of the error bars are two times the standard deviation.	85
Figure 5-17 Pitch angles of the flagella at different flow rates. The lengths of the error bars are two times the standard deviation.	85

List of tables

Table 3-1 Effects of DEP components on the swimming bacterium.....	32
Table 5-1 Morphological properties of <i>B. subtilis</i> flagella.	81
Table 5-2 Number of measurable pitches of the flagella at different flow rates	83
Table 5-3 Number of measurable diameters of the flagella at different flow rates	83

List of symbols

Chapter	Symbol	Description
2	$\mathbf{u}$	Flow field
	$\mathbf{p}$	Pressure
	Re	Reynolds number
	ρ	Density of fluid
	L	Characteristic length
	U	Characteristic velocity
	μ	Dynamic viscosity of fluid
	$\mathbf{F}$	External force
	$\mathbf{M}$	External moment
	$\mathbf{U}$	Translational velocity
	Ω	Rotational velocity
	$\mathbf{f}_{\perp}$	Elemental force in the normal direction
	$\mathbf{f}_{\parallel}$	Elemental force in the tangential direction
	$\mathbf{u}_{\perp}$	Velocity in the normal direction
	$\mathbf{u}_{\parallel}$	Velocity in the tangential direction
	$\zeta_{\perp}$	Resistive force coefficient in the normal direction
	$\zeta_{\parallel}$	Resistive force coefficient in the tangential direction
	b	Radius of the flagellum's cross section
	λ	Wavelength
	θ	Pitch angle of flagellum's helical wave
3	X, Y, Z	Coordinates of the inertial frame
	x, y, z	Coordinates of the body-fixed frame
	$\mathbf{r}^b$	Position vector of flagellum in body fixed frame
	R	Radius of helical wave
	L	Total curve length of the helical flagellum
	α	Pitch angle of the helical flagellum
	n	Number of full turns of the helical flagellum
	a	Radius of the bacterial head
	s	Coordinate along curve length of the flagellum

	$\mathbf{v} = [v_x, v_y, v_z]^T$	Translational velocity
	$\boldsymbol{\omega} = [\omega_x, \omega_y, \omega_z]^T$	Rotational velocity
	ξ_{head}, ξ_{tail}	Resistance matrix of the head and the tail
	$\boldsymbol{\omega}_{rel}$	Rotational relative velocity
	$\delta \mathbf{f}_{tail}^{drag}$	Elemental drag force on the tail
	$\delta \mathbf{f}_{tail}^{DEP}$	Elemental DEP force on the tail
	K_T, K_N	Resistive force coefficients
	$\mathbf{v}_T^r, \mathbf{v}_N^r$	Relative velocities
	$\mathbf{t}$	Tangential vector of the flagellum
	L, Ω^{-1}	Characteristic length and characteristic time
	$\mathbf{F}_{tail}$	Total force exerted on the tail
	$\mathbf{M}_{tail}$	Total moment exerted on the tail
	$\mathbf{F}_{head}$	Total force exerted on the head
	$\mathbf{M}_{head}$	Total moment exerted on the head
	θ, ψ	Polar, azimuthal angles (2 typical Euler angles)
	D_R	Rotational diffusivity
	c	Probability of an orientation of the bacterium
	C_0, C_1, C_2	Coefficients that determine components of DEP force
	β	Angle resulted from competition between DEP and diffusion
4	W, H	Width, height of a microchannel
	$\dot{\gamma}$	Shear rate
	V_T, V_N	Velocities in the tangential and normal direction
	$\mathbf{t}, \mathbf{n}$	Unit vectors parallel and perpendicular to the flagellar segment
	EI	Flexural rigidity of the flagellum
	$\mathbf{V}_{rel}, \mathbf{V}_{swim}, \mathbf{V}_{wiggling}$	Relative, swimming, and wiggling velocities
	Sp	Sperm number
	Z	Dimensionless shear rate
	$y(s)$	Shape of the beating flagellum

Contents

Abstract.....	v
Acknowledgement.....	vii
List of figures.....	ix
List of tables.....	xiii
Contents	xvii
Chapter 1 Introduction.....	1
Chapter 2 Literature Review	7
2.1 Basic theory	7
2.2 Theoretical models for swimming flagellated microorganism	9
2.3 Previous studies on locomotion of flagellated microorganisms	11
2.3.1 Force acting on the flagella.....	12
2.3.2 Shape of the flagella.....	13
2.4 Flagella visualization techniques	17
2.5 Bacteria in external force field.....	17
2.6 Research objectives.....	18
Chapter 3 Effects of external force on the swimming of helically flagellated bacteria	19
3.1 Problem formulation	19
3.1.1 Model of a bacterium	19
3.1.2 Velocities of the bacterium	21

3.2 Results and Discussion.....	26
3.2.1 Effects of constant DEP force field on the swimming bacterium	27
3.2.2 Effects of variant DEP force on the swimming bacterium closed to the origin	28
3.2.3 Potential application in bacterial focusing	32
3.3 Summary	36
Chapter 4 Deformation of a flexible flagellum in flow: A two-dimensional study	39
4.1 Problem formulation	40
4.1.1 Model of a 2D swimmer.....	40
4.1.2 Governing equation of 2D flexible flagellum	41
4.1.3 Method for solving the flagellum's shape equation	47
4.1.3.1 Brief introduction to Chebyshev collocation method	48
4.1.3.2 Chebyshev collocation method for solving flagellum's shape equation	49
4.1.3.3 Quality of the numerical solver	53
4.2 Results and Discussion.....	55
4.2.1 Beating patterns of the flexible flagellum	55
4.2.2 Velocity of the swimmer	59
4.2.3 Potential application in sorting and separation	62
4.3 Summary	62
Chapter 5 Experimental analysis of bacterial flagella's morphology	65
5.1 Experimental methodology	65

5.1.1 Bacteria culturing	65
5.1.2 Bacteria fluorescent staining	66
5.1.3 Experimental setup	66
5.1.4 Images processing	68
5.1.4.1 Manual recognition and extraction of flagellum image	69
5.1.4.2 Morphological information extraction	71
5.1.5 Error analysis	74
5.1.5.1 Estimation of experimental uncertainties	74
5.1.5.2 Conversion of units	75
5.1.5.3 Effect of the depth of field	75
5.2 Experimental Results and Discussion	77
5.2.1 Performance of images processing by MATLAB script	77
5.2.2 Morphology of flagella under no flow conditions	78
5.2.3 Morphology of flagella under flow conditions	82
5.3 Summary	86
Chapter 6 Conclusion and Future work	89
6.1 Conclusion	89
6.2 Future work	90
Appendix: Bacteria growing protocols	93
References	97
Publications	107

Chapter 1

Introduction

Microorganisms are the earliest and simplest life form on earth that appeared more than 3.5 billion years ago. Throughout, microorganisms have played an extremely important role in the development of the earth's habitats and the evolution of other life forms. The study of microorganisms is termed microbiology, which is a specialized area of biology dealing with very tiny life forms that cannot be observed by naked eyes and without magnification. Microbiology has a long and rich history. In 1676, Antonie Philips van Leeuwenhoek (1632–1723), a Dutch scientist, became the first person who viewed a living microorganism employing a microscope of his own design, and this marked the beginning of microbiology. Today, the rapid development of modern microscopic technology allows scientists to continue exploring and revealing in depth the world of tiny beings.

There are many reasons for scientists to study microorganisms; most of them are from the unlimited benefits of microorganisms to our life. Microorganisms are part of the human environment, and they are deeply involved in the flow of energy and food through the earth's ecosystem. The interaction between microorganisms and other organisms helps maintain the balance of nature. Many microorganisms in the ocean and in fresh water receive energy from sunlight and store it in molecules that other organisms use as food. Microorganisms decompose the dead bodies of other organisms, waste material from living organisms and even some kinds of industrial wastes. They also produce nitrogen for plants. Microorganisms are helpful not only to other organisms but also to humans. They are essential links in the food chains that is nutrient supplier for

humans. The other major role of microorganisms is their ability to synthesize antibiotics; therefore, microorganisms can help cure diseases. Moreover, microorganisms are used extensively in genetic engineering to make important products to human such as interferon and growth hormones. In addition, microorganisms have a potential to improve our environment through bioremediation, a process of introducing microbes into the environment to restore its stability or to clean up toxic pollutants; and human is now even able to engineer new organisms in order to use in bioremediation process. On the other hand, there are certain microbes that cause disease; therefore, in depth knowledge of microorganisms is essential for diagnosis, treatment, and prevention of such diseases [1].

Microorganisms are classified into six major groups: bacteria, fungi, protozoa, algae, virus, and helminths. Among these groups, bacteria have been one of the most thoroughly studied, for their great benefits to human. The research interest in bacteria is not limited to biological aspects such as bacterial structure and their interaction with the environment, but extended to the physics of their motility, or aquatic locomotion. Aquatic locomotion refers to the propelled motion of microorganism through a liquid medium. In the case of bacteria, they move by propelling their hair-like appendages called flagella. A bacterial flagellum typically has helical structure and can rotate by a rotational motor located at its base. If a bacterium has more than one flagellum, each flagellum has its own motor that rotates independently of others. Multi-flagellated bacteria (such as *E. coli* and *B. subtilis*) swim forward, or “run,” by rotating their flagellar bundle, which is formed when all the flagella come together. When bacteria “sense” some positive nutrient gradient or wish to swim towards a higher food concentration area, they unbundle their flagella to change their swimming direction; this is known as “tumble.” Since the motion of bacteria is extremely dependent on the flagella, the research of flagellar behavior becomes the heart of bacterial motility studies.

The physics of swimming bacteria is investigated theoretically and experimentally. Since the swimming problem of bacteria involves the hydrodynamic interaction of bacteria and the surrounding fluid, theoretical research of bacteria swimming generally starts from the Navier-Stokes equation, which governs most problems that happen in fluid environment. At the microscale of bacteria, Reynolds number (i.e. the ratio between inertial force and viscous force) reaches the limit of zero; therefore, in this low Reynolds number regime, the Navier-Stokes equation can be simplified and the hydrodynamics of swimming bacteria is mainly governed by the Stokes equation, $-\nabla p + \eta \nabla^2 \mathbf{v} = 0$, where ∇p is the pressure gradient, $\mathbf{v}$ is velocity of the bacterial body and η is dynamic viscosity of fluid. Most studies in the literature attempted to determine the hydrodynamic force acting on the body and velocity of bacteria from the Stokes equation under different additional physical stimuli. For example, the motility of bacteria can be affected by a plane boundary, external flow or force field, or the material of the microchannel's walls. For this purpose, several mathematical theories were built to model the interaction of the flagella and the surrounding fluid. The most classical model is the resistive force theory [2], which is followed later by more advanced methods. By utilizing these mathematical theories, physicists have been exploring in depth the behavior of the flagella during swimming of bacteria, with or without the presence of external physical stimuli.

Many related experiments have been conducted to verify mathematical models. There are two main approaches. The first method is directly analyzing bacteria behavior. However, the microscale of bacteria sometimes brings challenges to setting up the experiment and visualizing bacterial flagella. Therefore, in the second approach, some researchers choose to do experiments in macroscale by making a physical model of a bacterial flagellum and put it into an environment that simulates the fluid environment surrounding the bacteria in reality [3, 4]. A macroscale physical model is utilized

especially for prediction of bacterial flagella behavior, since bacterial flagella are invisible even under a microscopy without the aid of special techniques. Recently, the development of fluorescent dyes makes it less challenging to visualize bacterial flagella under microscope [5, 6]. However, very few works that apply this technique to directly investigate the behavior of bacterial flagella have been reported in the literature. Another factor that makes experimental observation of bacteria challenging is the need to fabricate a microfluidic channels to provide swimming environment for them; however, this is now less difficult with the help of modern microfabrication techniques.

When investigating the swimming of bacteria (such as *E. coli* and *B. subtilis*) and the behavior of their flagella during swimming, most of previous studies considered the bacterial flagellar bundle to be a rigid helix, and therefore did not take into account the effects of external factors on the morphology of the bundle. In reality, bacterial flagella are not rigid, and during swimming they are deformed not only by the rotational motion of the bacterial motor but also by external physical stimuli. Since the motility of bacteria strongly depends on the behavior of their flagella, a thorough understanding of flagellar behavior would deepen our knowledge about the swimming of bacteria, especially when they are influenced by external factors. In this thesis, I investigate the effect of shear flow on the shape of bacterial flagella, which in turn affects their motility during swimming. The effect of shear flow is worthwhile to examine since fluid flows normally involve not only advection but also velocity gradient, i.e. shear. Moreover, the interaction between bacteria and shear flow could influence the trajectory and motility of bacteria. The main aims of this work are to determine the effects of elasticity on the morphology change of bacterial flagellar bundle in shear flow, and to investigate if the bacteria possess ability to actively deform their flagella in response to the external shear flow.

I also extend our interest to include the behavior of bacteria in other external stimuli rather than flow, such as electric field. The study of bacteria in electric field were also well established; however, I observed that most previous studies are focused on the response of the whole bacterial population rather than the physics of one single bacterium, which is more fundamental. Therefore, our scope also includes the study of bacteria's behavior in response to the external force field, specifically force generated by dielectrophoresis. I pay attention to the role of flagella in the swimming of the bacteria in force field, and the interaction between the helical shape of the flagella and the external force. I also explore the feasibility of applying our findings to achieve bacterial focusing.

This thesis is organized as follows. In Chapter 2, I present the literature review of this research area. The literature review begins with the basic theory, followed by previous theoretical and experimental studies in locomotion of flagellated microorganisms. In Chapter 3, I build a model to predict the swimming behavior of helically flagellated bacteria in a dielectrophoretic force field. The model focuses on the role of the flagella in the swimming of one single bacterium when the bacterium is affected by both dielectrophoretic force and diffusion. Chapter 4 deals with the mathematical model to determine the shape of a 2D propulsion flagellum in shear flow and its influence on the swimming of the microorganism. I derive the governing equation of the flagellum's shape in shear, and discuss the results at various shear strengths. Chapter 5 presents the experiments to characterize the flagellar morphology of bacteria *B. subtilis*. Changes in the shape of the flagella due to external flow are also discussed. Finally, I conclude and present future work in Chapter 6. I expect that this study would significantly contribute to our knowledge of the behaviors of flagellated microorganisms in fluid environment and also lead to valuable applications.

Chapter 2

Literature Review

2.1 Basic theory

The swimming of microorganisms encompasses the hydrodynamics in viscous fluid. In order to determine the hydrodynamic parameters of the swimmer, there are two properties of the fluid surrounding the swimmer that need to be investigated: flow field and pressure. These hydrodynamic properties of the fluid have been well discussed in many previous studies [7-11]. Generally, these two properties are mathematically reflected in Navier-Stokes equation for an incompressible Newtonian fluid

$$\rho \left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = -\nabla \mathbf{p} + \mu \nabla^2 \mathbf{u}, \quad (2.1)$$
$$\nabla \cdot \mathbf{u} = 0$$

where $\mathbf{u}$ is flow field and $\mathbf{p}$ is pressure of the fluid, ρ and μ are density and dynamic viscosity of the fluids respectively. This equation can be non-dimensionalized by introducing the Reynolds number

$$\text{Re} = \frac{\rho L U}{\mu}, \quad (2.2)$$

where L and U are the characteristic length and velocity that govern the swimming problem, respectively. Physically, Reynolds number is a dimensionless parameter that indicates the ratio between inertial and viscous force. At the scale of bacteria and other flagellated microswimmers, the term $\rho L U$ is very small in comparison with μ ; therefore, Reynolds number reaches the limit of zero, that is, microswimmers are under low Reynolds number conditions. Low-Reynolds number means that the viscous force

exerted on the swimmer's body dominates over the inertial force, and all the inertial terms in the Navier-Stokes equation are negligible. For this reason, at low-Reynolds number the Navier-Stokes equation is simplified to the Stokes equation

$$\begin{aligned}\nabla \mathbf{p} &= \mu \nabla^2 \mathbf{u} \\ \nabla \cdot \mathbf{u} &= 0\end{aligned}\tag{2.3}$$

which governs the behavior of microswimmers in the low-Reynolds number world.

From equation (2.3), it is well expected that the force exerted on the body of the swimmer is linear to its velocity and can be expressed as [11]

$$\begin{pmatrix} \mathbf{F} \\ \mathbf{M} \end{pmatrix} = \begin{pmatrix} \mathbf{A} & \mathbf{B} \\ \mathbf{B}^T & \mathbf{C} \end{pmatrix} \cdot \begin{pmatrix} \mathbf{U} \\ \mathbf{\Omega} \end{pmatrix},\tag{2.4}$$

where $\mathbf{F}$ and $\mathbf{M}$ are external force and moment, respectively, $\mathbf{U}$ and $\mathbf{\Omega}$ are translational and rotational velocities respectively. Taking inverse of equation (2.4) we have

$$\begin{pmatrix} \mathbf{U} \\ \mathbf{\Omega} \end{pmatrix} = \begin{pmatrix} \mathbf{M} & \mathbf{N} \\ \mathbf{N}^T & \mathbf{O} \end{pmatrix} \cdot \begin{pmatrix} \mathbf{F} \\ \mathbf{M} \end{pmatrix}.\tag{2.5}$$

The matrices in equation (2.4) and equation (2.5) are called resistance and mobility matrices, respectively. In the review by Lauga *et al.* [11], three significant implications of equation (2.4) and (2.5) were mentioned. The first implication is that the matrices $\mathbf{A}$, $\mathbf{C}$, $\mathbf{M}$, $\mathbf{O}$ must not be isotropic, otherwise the locomotion cannot be performed at low-Reynolds number. Secondly, the matrices $\mathbf{B}$ and $\mathbf{N}$ are non-zero in the case of chiral bodies, which do not have a reflection symmetry plane. This happens to the bodies of bacteria swimming by rotating their helical flagellar bundle. The third point is that these matrices can be used to determine the diffusion coefficient of the swimmer.

Since at low-Reynolds number, the inertial force is totally dominated by the viscous force from the surrounding fluid; therefore the total force exerting on the body of the swimmer is equal to zero. The velocities of the swimmer, $\mathbf{U}$ and $\mathbf{\Omega}$, are computed so that they satisfy the free body force condition at low-Reynolds number.

2.2 Theoretical models for swimming flagellated microorganism

The study of locomotion of microorganism at low-Reynolds number has been being investigated for over fifty years. Many theoretical models were proposed in the past to address the swimming problem of microorganisms at low-Reynolds number. The main aim of these studies is to compute or approximate the resistance matrix of the microswimmers. Although these models have different levels of mathematical complexity, the general idea is using appropriate stokeslets distributed along a flagellum to represent the flow induced by the deformation of the flagellum.

Base on this general idea, in 1953, Hancock [12] proposed the first model and obtained the formulae for the velocity of a self-propulsion flagellum. However, the mathematical complexity of his model made it challenging to be computed numerically at that time. After that, Gray and Hancock [2] proposed the resistive force theory, also known as local drag theory for a slender rod. The resistive force theory determines the hydrodynamic force per unit length exerted on a segment of the flagellum by computing the component forces that are perpendicular and parallel to the primary axis of the segment using equation

$$\begin{aligned} \mathbf{f}_{\perp} &= -\zeta_{\perp} \mathbf{u}_{\perp} \\ \mathbf{f}_{\parallel} &= -\zeta_{\parallel} \mathbf{u}_{\parallel} \end{aligned} \quad (2.6)$$

where $\perp$ and $\parallel$ indicates the components perpendicular and parallel to segment's primary axis. Here, $\zeta_{\perp}$ and $\zeta_{\parallel}$ are known as the drag coefficients in the perpendicular and parallel direction, respectively. They were determined by Gray and Hancock [2] as

$$\zeta_{\perp} = \frac{2\pi\mu}{\ln \frac{2\lambda}{b} - \frac{1}{2}}, \quad \zeta_{\parallel} = 2\zeta_{\perp}, \quad (2.7)$$

where b is the radius of cross section of the flagellum and λ is the wavelength. However, the ratio $\zeta_{\parallel} / \zeta_{\perp} = 2$ proposed by Gray and Hancock just covers a limit range

of flagellum geometry. Later, studies of Brokaw [13] and Cox [14] together suggested a more realistic value for parallel drag coefficient

$$\zeta_{\perp} = \frac{2\pi\mu}{\ln \frac{2\lambda}{b} + \frac{1}{2}}, \quad (2.8)$$

which yields the ratio $\zeta_{\parallel} / \zeta_{\perp} \approx 1.7$. However, Brokaw showed in his work [15] that the best approximation of this ratio should be 1.8. The resistive force theory was then refined by Lighthill [16] and he suggested his own way to calculate the drag coefficients

$$\zeta_{\perp} = \frac{2\pi\mu}{\ln \frac{0.18\lambda}{b \cos \theta}}, \quad \zeta_{\parallel} = \frac{4\pi\mu}{\ln \frac{0.18\lambda}{b \cos \theta} + \frac{1}{2}}, \quad (2.9)$$

where θ is the pitch angle of flagellum's helical wave.

Another theory to model the flow induced by the motion of the flagellum is known as the slender body theory, pioneered by Hancock [12], which is considered to be more accurate than resistive force theory. Resistive force theory assumes that $1 / \log(\lambda / b) \ll 1$, where λ and b are wavelength and radius of cross section of the flagellum respectively. In reality, flagella have the ratio $b / \lambda \sim 10^{-2}$, therefore a higher development of resistive force theory is necessary [16, 17]. The slender body theory can fulfill this requirement, and therefore there are many previous studies involved in the development of the slender body theory [14, 18-24]. The main idea of the slender body theory is to model the flow induced by the motion of a flagellum by an appropriate distribution of flow singularities along the center line of that flagellum. In his review [11], Lauga *et al.* clearly classified previous works on slender body theory into two approaches. The first approach is an extension of resistive force theory, in which solution to the flow is expressed as a series of logarithmically small term [14, 22, 25]. The advantage of this approach is that all terms can be solved analytically; however, it requires a large number of terms in order to achieve a good model of the flow. The

second approach avoids the series solution by directly introducing an integral equation fulfilled by some unknown distribution of singularities along the flagellum, and the accuracy has order of b/λ [26, 27], which is more accurate than the first approach. However, it is technical demanding since one must solve for a large number of integral equations in order to compute the force distribution along the flagellum. Later, Johnson improved the model and reached the accuracy of order $(b/\lambda)^2 \log(b/\lambda)$ [28]. In order to derive such integral equation, Lighthill later proposed that the flow induced by the motion of the flagellum should be represented by a distribution of stokeslets and source dipoles along the center line of the flagellum [16, 29]. According to this method, the velocity of the flagellum is given by an integral equation which can be easily numerically computed and can be utilized to find the optimal drag coefficients of resistive force theory.

Based on the method suggested by Lighthill, Cortez developed the regularized stokeslets method [30, 31]. This method helps avoid singularities from distribution of stokeslets by representing each elements of the flagellum's surface by a regularized stokeslet. The regularized stokeslets method is based on Lagrangian mechanics and it is used to solve for Stoke flow induced by a distribution of forces at material points in the fluid. This method were also applied to analyze the motion of a helical body [31].

2.3 Previous studies on locomotion of flagellated microorganisms

The locomotion of bacteria has been investigated by many scientists in the past. Their research interest includes behavior of one flagellum and flagellar bundle during swimming, propulsive force exerting on the flagella, extension of flagella due to flagellar elasticity and instability, and change in trajectory under the influence of external physical stimuli.

2.3.1 Force acting on the flagella

The relation between force exerting on a flagellum and its extension as well as polymorphic changes are main focuses in the research of flagellated microorganisms' locomotion. Many experiments were conducted to reveal the influence of external forces on the polymorphic changes of the flagella. Darnton *et al.* [32] have studied the effects of applied force on polymorphic form of a flagellum, based on Calladine's model [33, 34]. Their experimental results showed that normal and coiled forms of *Salmonella* are transformed to the next polymorphic form by average forces of 5.5 pN and 4 pN, respectively. However, these transformations are not predictable since they are strongly influenced by how the force is applied on the flagellum. They also built the force-extension curves based on which one is able to determine the displacement of the flagellum at different values of force. Vogel *et al.* later utilized Kirchhoff's theory and resistive force theory to build a model for an elastic flagellum, which can be used to predict polymorphic transformation [35]. This analytical calculation was based on Brownian-dynamics and the force-extension curves generated from the model were consistent the experimental results of previous study [32]. In greater details, Vogel *et al.* found that the first coiled-to-normal transformation occurs at the values of force from 3 pN to 5 pN, and they also presented the influence of extensional rate on the transformation of the bacterial flagellum. Various transformations of the bacterial flagella were clearly shown in the study by Turner *et al.*, with the help of fluorescent staining of the flagella [5]. The experimental observations showed that during swimming, an individual *E. coli* cell has the tendency to change flagellar waveform in the sequence normal, semicoiled, curly 1; and change to semicoiled waveform occurs with the alternation in the swimming direction of the cell. The polymorphic transformation of the flagella were also studied in depth by Calladine [36], and it was shown that changes in waveform of the flagella are critical for the bacteria to change

their swimming direction in order to reach higher nutrient area (chemotaxis). Recently, Vogel *et al.* showed that the polymorphic transformations of bacterial flagella are triggered by a change in torque of the flagellar motors [37], and normal-to-curly 1 events happen when there is a reversal in the rotation of flagellar motors. On another approach, physical macroscale models were also considered to study the hydrodynamics of bacterial flagellum. Liu *et al.* [4] built a physical model to investigate the hydrodynamics of a swimming helical flagellum in both viscous and viscoelastic fluid. In their experiment, the low Reynolds number world was simulated by using very high viscosity fluid (high-molecular weight silicone oil) for the case of Newtonian fluid, and Boger fluid for the case of viscoelastic environment. Slender-body theories and boundary-element method were applied to predict the hydrodynamic parameters of the swimming helix in fluid, and the prediction from computational model well agreed with the experimental experiments. This study suggested that the shape of the flagellum during swimming strongly affects the dependence of swimming speed on Deborah number, which is the ratio of relaxation time and the time scale of the experiment.

2.3.2 Shape of the flagella

Since the flagellated microorganisms deform their flagella to move forward, it is well expected that the shape of the flagella plays an important role during swimming of these microorganisms. Therefore, not surprisingly the shape of the flagella of swimming microorganisms was also investigated in the literature. In 2008, Fu *et al.* [38] studied the beating patterns of flagella in both viscous and viscoelastic fluids. Both passive and active filaments were considered, and the analytical results of the shape of flagella well agreed with experimental observations done by Ishijima *et al.* [39] under certain conditions. The mathematical model, which is based on resistive force theory, showed

the influence of viscoelasticity on the hydrodynamic forces exerted along the flagellum, and how the force affects the beating patterns of the flagella.

Since swimming of flagellated microorganisms strongly depends on the shape of deforming flagella, it is interesting to investigate if there is an optimal shape of the flagella for swimming microorganisms. To address this point, Lauga *et al.* built a computational model in order to determine the optimal shape of active flagella [40]. In this study, Lauga *et al.* defined the optimal shape as the shape which helps the microorganisms to swim with lowest power consumption. The analytical results showed that at low Sperm number, the optimal shape looks like a combination of circular arcs with the same curvatures. When the sperm number gets higher, the optimal shape reaches the sawtooth shape as shown in previous study by Lighthill [24]. The results were then compared to other studies [41-43] to draw a more general idea about the optimal shape of the flagella. The optimal shape of flagella for swimming was also considered in the study done by Spagnolie *et al.* [44], in which they focused more on the energetic aspect. In their study, Spagnolie *et al.* had built a mathematical model which considers not only the energy needed to overcome the hydrodynamic drag; it also counts for the energy internally stored in the flagellum during bending.

Theoretical studies of the shape of helical flagella were also reported in studies of Takano *et al.* [45-47], in which the flagella of *Vibrio alginolyticus* and *Salmonella* were investigated using Kirchhoff rod model combined with resistive force theory. They studied how the helical shape of the flagellum changes with its rotation rate and flexural rigidity, paying strong attention to the changes in the pitch length. They found that at locations close to the root of the flagellum, the pitch is proportional to the rotational speed of the flagellar motor; however, far from the root, this linear relation is no longer maintained [46]. The analysis of the flagellar pitch is important, especially when resistive force theory is being employed, because it governs the resistive force

coefficients (see equations (2.7) - (2.9)). According to our observation, the studies of Takano *et al.* [45-47] are some of the few theoretical works that specifically pay very close attention to the morphology of the bacterial helical flagella. However, a major limitation of these studies is that the results are subjected to an initial swimming velocity obtained from the undeformed flagellum, and the authors did not re-compute the swimming velocity with the deformed flagellum to see how far the assumed initial velocity deviates from the velocity of the deformed flagellum. I believe a proper way to overcome this limitation is to iterate the computing steps until both flagellar shape and velocity converge.

Swimming direction or trajectory of microorganisms is also an interesting point in this field of research. The trajectory of helical flagellated bacteria is strongly influenced by external factors. In his study [48], DiLuzio *et al.* showed that the trajectory of an individual *E. coli* in a microchannel is affected by the distance between the bacterium and the walls of the channel. Near a solid glass surface, within a distance of 10 μm from the surface, a bacterium *E. coli* shows clockwise circular trajectory. It was also stated that *E. coli* have a preference to swim closer to the agar wall of the channel rather than the PDMS (oxidized polydimethylsiloxane) surface. This study suggested that the choice of microchannel's materials can drive the bacteria in the channel. Later, Lauga *et al.* used resistive force theory to address the clockwise circular motion of *E. coli* near a solid surface [49]. It was concluded that this behavior of *E. coli* resulted from the force-free swimming at low-Reynolds number, and from the hydrodynamic interaction between the bacteria and the solid wall. Experimental results also showed that bacteria with longer bodies have larger radius of circular trajectory. In the absence of external stimuli such as a nearby wall or external force, flagellated microorganisms change their swimming direction by "tumble." Tumbling happens when there is a change in direction of flagellar motors from counter-clock-wise to clock-wise, and then back to counter-

clock-wise [50-52]. When the cells swim forward straightly, it is called “run.” The hydrodynamics of “run” and “tumble” were also investigated in depth by Watari *et al.* [53]. “Tumble” mechanism for directional alternation is only possible for cells with reversible flagellar motors. When the flagellar motors of bacteria are irreversible, how can they change their direction? In a study done by Son *et al.* [54], it was shown that bacteria are able to utilize the instability of their hooks to change swimming direction. Bacterial hook is a joint that transmits torque from the flagellar motor to the filament [55]. When the load on the hook is higher than buckling threshold, it becomes unstable and this causes a change in the swimming direction of the cell [54].

In reality, bacterial hydrodynamics occurs in fluid flows that are neither steady nor uniform but turbulent. Due to their very small scale, bacteria “see” the surrounding turbulence as a simple shear flow. Therefore, effect of shear on the hydrodynamics of bacteria is an important topic. Shear flow could be understood as a flow that carries a velocity gradient. In their study, Marcos *et al.* [56] suggested that bacterial rheotaxis is resulted from the interaction between the helical geometry of the flagella and the velocity gradient of shear flow. Furthermore, bacteria with helical flagella are able to drift across the streamlines of the flow, and such phenomenon does not happen with many particles which have spherical or ellipsoidal shape. This mechanism was also utilized to separate chiral particles at microscale. Marcos *et al.* [57] showed that the drift of helical flagellated bacteria, which is perpendicular to the streamline, is caused by the tendency of the bacteria to align their helical flagella with the streamlines. The drift direction is dependent on the geometry parameters of the helix and direction of shear. This separation method proposed by Marcos *et al.* could achieve an efficiency of 80 % in a short time. Later, Ishikawa *et al.* attempted to apply the same mechanism to separate motile bacteria in a microchannel [58]. They built a microchannel to observe the drift phenomenon of motile bacteria, and built another one to perform separation. It was

shown that the height of the channel strongly affects the separation efficiency. However, the separation efficiency of the method proposed by Ishikawa *et al.* was relatively low.

2.4 Flagella visualization techniques

Investigating the behaviors of flagellated microorganisms, or flagella themselves, requires some techniques to make the flagella visible to human eyes. Many different methods for visualization of flagella have been reported in the literature [59-64]. However, the protocols of these methods are not simple and each of them has its own disadvantages, such as the instability of reagents, the side effects of reaction, etc. Recently, the developments in flagella visualization techniques made it easier to visualize the bacterial flagella under microscopy. In 2000, Turner *et al.* were successful in fluorescently staining the flagella of motile *E. coli* and make them visible clearly under dark-field microscopy [5]. The method proposed by Turner *et al.* is rapid and simple, using Alexa Fluor dye (Molecular Probes, Eugene, Oreg.). By this technique, it was possible to capture real-time video of the bacterial flagella. The same technique was also applied successfully to visualize the flagella of *S. typhimurium* and *S. marcescens* [65], *B. subtilis* [66] and *E. coli* [67]. In 2008, Blair *et al.* showed that some kinds of bacteria require genetically modification to the flagella in order to accept the fluorescent dye [6].

2.5 Bacteria in external force field

The main scope of this research is to the study of bacterial locomotion in fluid flow; however, I am also interested in behavior of bacteria in an external force field and its potential application in bacterial manipulation. In the literature, external sources for bacterial manipulation included optical source [68], magnetic and electric fields [69, 70], or fluid flow [56, 58]. Among these, I especially pay attention to the effects of

dielectrophoresis (DEP) on bacteria. When a particle is subjected to a non-uniform electric field, it is polarized. Because the electric field is non-uniform, one side of the particle experiences stronger force than the other side. As a result, the particle experiences a net dielectrophoretic force [71]. DEP had been used in many applications such as separation, lysing, detection, concentration [69, 72-75] and it has shown many advantages. With DEP, one can manipulate cells without labels, tags, and the samples are free from any contact with stimulation devices. However, most previous studies investigated effects of DEP on the bacterial population without paying attention to a more fundamental aspect, i.e. the role of flagellar morphology in the response of bacteria to external force field.

2.6 Research objectives

As discussed in the literature review above, the research of locomotion of flagellated microorganisms in physical aspect has many focusing points. The most interesting to us is the effect of shear flow on the hydrodynamic behavior of flagellated microorganisms. In order to further investigate the hydrodynamics of flagellated microorganisms in shear flow, it is important to know how shear flow influences the behavior or the shape of the flagella during their deformation in flow. However, to the best of my knowledge, this kind of study has not been found in the literature. Therefore, in order to fill the gap, our research objective is to investigate the effects of shear flow on the shape of flagella of flagellated microorganism, especially bacteria. I also extend our research to include the investigation of flagellated bacteria in an external force field such as dielectrophoretic force, emphasizing the role of the flagella. The ultimate goals of our research are to characterize the morphological parameters of the bacterial flagella, and to study how flagella and their shape influence the swimming of bacteria.

Chapter 3

Effects of external force on the swimming of helically flagellated bacteria

In this chapter, I build a model to study the effect of externally applied dielectrophoretic (DEP) force on the swimming of a single helically flagellated bacterium. Although in the nature bacteria are not necessarily affected by DEP force, I choose DEP force in this study because of its practical advantages, especially in the manipulation of bacterial cells. For example, using DEP, the cells are not required to be charged; and we can avoid physical contact between cells and stimulation devices. Through this model using DEP force, I also explore potential applications of DEP force in bacterial focusing.

In this theoretical study, the flagellum is modeled rigid such that its helical shape is maintained during swimming. I pay attention to the swimming orientation of the bacterium and the mechanism of how an external DEP force, together with rotational diffusion, affect the swimming direction. The probability distribution of the orientation is obtained numerically and the results are used to compute the bacterium's mean velocity at various DEP strengths. The role of helical shape of the bacterial flagellum in the response of the bacterium to external force is also analyzed. Finally, I explore the feasibility of applying the results in bacterial manipulation.

3.1 Problem formulation

3.1.1 Model of a bacterium

I model a bacterium as a combination of a spherical head of radius a and a single left-handed helical tail attached to the head (Figure 3-1a). The tail has radius R , thickness

$2b$, wavelength λ , number of wavelengths n , and pitch angle α . The total curve length L is defined as the distance from the fixed end to the free end of the tail, along the helical path. The relation between the wavelength and the total curve length of the tail is given by $\lambda = (L/n)\cos\alpha$, and the radius R of the tail is related to the pitch angle α by

Figure 3-1 Schematic of a bacterium and its orientation in 3-D space. (a) Geometric parameters of the bacterium in the body-fixed frame $oxyz$, which has its origin at the head's center and its z -axis is along the bacterium's primary axis. (b) The orientation of the bacterium is determined by (θ, ψ) , $0 \leq \theta \leq \pi, 0 \leq \psi \leq 2\pi$ [76].

$R = L \sin \alpha / (2\pi n)$. Here, I model the bacterial head as a sphere to avoid mathematical complexity, although it is not exactly the case in reality. The radius of the spherical head should be carefully determined as shown by Marcos *et al.* [56]. Also, this approximation is acceptable in the limit of this study because, as we will see later, I am focusing more on the behavior of the helical flagellum.

In the next step, I further define a body-fixed frame $oxyz$ such that the origin o is placed at the head's center and its z -axis is always parallel to the primary axis of the bacterium (Figure 3-1a). In this body fixed-frame, the tail is fully defined in the body-fixed frame by the position vector $\mathbf{r}^b$ as [76]

$$\mathbf{r}^b = [R \cos(2\pi ns/L), R \sin(2\pi ns/L), -a + (s-L) \cos \alpha]^T \quad (0 \leq s \leq L), \quad (3.1)$$

where s is the distance, along the helical path, from the free end of the tail to the interested point determined by vector $\mathbf{r}^b$. The superscript b indicates the body-fixed frame.

In Figure 3-1b, $OXYZ$ is the inertial frame which has its origin located at a reference point O . Any orientation of the bacterium is described by the polar angle θ and the azimuthal angles ψ , $(0 \leq \theta \leq \pi, 0 \leq \psi \leq 2\pi)$. For example, $(\theta = \pi/2, \psi = 0)$ results in the positive X -direction; and $(\theta = \pi/2, \psi = \pi/2)$ represents the positive Y -direction. The position vector $\mathbf{r}$ of the tail in the inertial frame can be determined by vector transformation from the body-fixed frame to the inertial frame.

3.1.2 Velocities of the bacterium

I denote translational and angular velocity of the bacterium's head in the inertial frame by $\mathbf{v} = [v_x, v_y, v_z]^T$ and $\boldsymbol{\omega} = [\omega_x, \omega_y, \omega_z]^T$, respectively. Let us denote $\boldsymbol{\omega}_{\text{rel}}$ as the angular velocity of the tail relative to the head, and $\boldsymbol{\omega}_{\text{tail}}$ as the absolute angular velocity of the tail. The angular velocities of the head and the tail are related by $\boldsymbol{\omega}_{\text{tail}} = \boldsymbol{\omega} + \boldsymbol{\omega}_{\text{rel}}$. In the body-fixed frame, $\boldsymbol{\omega}_{\text{rel}}^b = [0, 0, -\Omega]^T$ ($\Omega > 0$), where Ω is the tail's angular speed relative to the head. The bacterium's swimming velocity is determined by equating the total force acting on the bacterium to zero since at low Reynolds number, the inertial force is dominated by the viscous one. The effect of gravity on the bacteria is negligible because in reality, small size bacteria are neutrally buoyant [77].

The total force acting on the bacterium is derived by considering the force on the tail and the force on the head separately. I employ RFT to determine the hydrodynamic drag force on the tail, such that $\delta \mathbf{f}_{\text{tail}}^{\text{drag}} = -(K_T \mathbf{v}_T^r + K_N \mathbf{v}_N^r) \delta s$, where $\delta \mathbf{f}_{\text{tail}}^{\text{drag}}$ is the elemental

hydrodynamic force; K_T and K_N are resistive force coefficients, $\mathbf{v}_T^r$ and $\mathbf{v}_N^r$ are relative velocities parallel and perpendicular to the segment, respectively. Note that for a slender body, the resistance force coefficient is anisotropic, i.e. $K_N > K_T$. The hydrodynamic force on the segment δs can be further expressed as

$$\delta \mathbf{f}_{\text{tail}}^{\text{drag}} = -\left\{ K_T (\mathbf{v}^r \cdot \mathbf{t}) \mathbf{t} + K_N [\mathbf{v}^r - (\mathbf{v}^r \cdot \mathbf{t}) \mathbf{t}] \right\} \delta s. \quad (3.2)$$

In addition to the hydrodynamic force, the tail also experiences DEP force. Generally, the DEP force acting on a particle takes the form

$$\mathbf{F}_{\text{DEP}} = [F_{\text{DEPX}}, F_{\text{DEPY}}, F_{\text{DEPZ}}]^T = \Gamma \varepsilon_{\text{medium}} \text{Re}(f_{\text{CM}}) \nabla |\mathbf{E}|^2, \quad (3.3)$$

where Γ is the shape factor of the particle, $\varepsilon_{\text{medium}}$ is the permittivity of the medium, $\text{Re}(f_{\text{CM}})$ is the real part of the Clausius–Mossotti factor that relates the polarizability of the particle to its permittivity, and $\mathbf{E}$ is the electric field. $F_{\text{DEPX}}, F_{\text{DEPY}}, F_{\text{DEPZ}}$ are the DEP force components in the X -, Y - and Z -direction, respectively. The shape factor for a spherical particle of radius a is $\Gamma = 2\pi a^3$ and for a cylindrical particle of length l and radius r is $\Gamma = (1/6)\pi r^2 l$. Here, I consider the DEP force to act solely in the X -direction and its magnitude as well as direction varies with X - and Y -position, such that $\nabla |\mathbf{E}|^2 = [C_0 + C_1 X + C_2 Y, 0, 0]^T$. Physically, we can understand the coefficients C_0 as DEP force density, C_1 and C_2 as DEP gradient of force density in the X - and Y -direction, respectively. The DEP force is given by

$$\mathbf{F}_{\text{DEP}} = \Gamma \varepsilon_{\text{medium}} \text{Re}(f_{\text{CM}}) [C_0 + C_1 X + C_2 Y, 0, 0]^T. \quad (3.4)$$

For convenience, I introduce the following dimensionless variables using L as the characteristic length, Ω^{-1} as the characteristic time, ΩL as the characteristic velocity, $K_T \Omega L^2$ as the characteristic force, and $K_T \Omega L^3$ as the characteristic moment:

$$\tilde{a} = a/L, \tilde{b} = b/L, \tilde{R} = R/L, \tilde{\lambda} = \lambda/L, \tilde{s} = s/L, \tilde{t} = t\Omega, \quad (3.5)$$

$$\tilde{\mathbf{v}} = \mathbf{v}/(\Omega L), \tilde{\boldsymbol{\omega}} = \boldsymbol{\omega}/\Omega, \tilde{\mathbf{F}} = \mathbf{F}/(K_T \Omega L^2), \tilde{\mathbf{M}} = \mathbf{M}/(K_T \Omega L^3). \quad (3.6)$$

When performing non-dimensionalization equation (3.4), I combine $\varepsilon_{medium} \text{Re}(f_{CM})$ with C_0 , C_1 and C_2 such that $\varepsilon_{medium} \text{Re}(f_{CM})[C_0 + C_1 X + C_2 Y, 0, 0]^T$ becomes $[\tilde{C}_0 + \tilde{C}_1 \tilde{X} + \tilde{C}_2 \tilde{Y}, 0, 0]^T$. For a neat and tidy presentation, I keep all notation without tilde for dimensionless variables from now onwards. The dimensionless DEP force on the tail is given by

$$\delta \mathbf{f}_{tail}^{DEP} = [(1/6)\pi b^2 (C_0 + C_1 X_s + C_2 Y_s) \delta s, 0, 0]^T \quad (3.7)$$

Equation (3.2) is rewritten in the dimensionless form as

$$\delta \mathbf{f}_{tail}^{drag} = - \left[(\mathbf{v}^r \cdot \mathbf{t}) \left(1 - \frac{K_N}{K_T} \right) \mathbf{t} + \frac{K_N}{K_T} \mathbf{v}^r \right] \delta s. \quad (3.8)$$

The total dimensionless force and moment acting on the tail are given by

$$\mathbf{F}_{tail} = \int \delta \mathbf{f}_{tail} = \int (\delta \mathbf{f}_{tail}^{drag} + \delta \mathbf{f}_{tail}^{DEP}), \quad (3.9)$$

$$\mathbf{M}_{tail} = \int (\mathbf{r} \times \delta \mathbf{f}_{tail}). \quad (3.10)$$

Equations (3.9) and (3.10) can be written as

$$\begin{bmatrix} \mathbf{F} \\ \mathbf{M} \end{bmatrix}_{tail} = [\boldsymbol{\xi}]_{tail} \begin{bmatrix} \mathbf{v} \\ \boldsymbol{\omega} \end{bmatrix}_{tail} + [\mathbf{A}], \quad (3.11)$$

where

$$[\boldsymbol{\xi}]_{tail} = \begin{bmatrix} \xi_{11} & \xi_{12} & \xi_{13} & \xi_{14} & \xi_{15} & \xi_{16} \\ \xi_{21} & \xi_{22} & \xi_{23} & \xi_{24} & \xi_{25} & \xi_{26} \\ \xi_{31} & \xi_{32} & \xi_{33} & \xi_{34} & \xi_{35} & \xi_{36} \\ \xi_{41} & \xi_{42} & \xi_{43} & \xi_{44} & \xi_{45} & \xi_{46} \\ \xi_{51} & \xi_{52} & \xi_{53} & \xi_{54} & \xi_{55} & \xi_{56} \\ \xi_{61} & \xi_{62} & \xi_{63} & \xi_{64} & \xi_{65} & \xi_{66} \end{bmatrix} \quad (3.12)$$

is the resistance matrix of the tail whose elements are dependent on the tail's geometry only, and

$$[\mathbf{A}] = [F_{tail X}^{DEP}, 0, 0, 0, M_{tail Y}^{DEP}, M_{tail Z}^{DEP}]^T \quad (3.13)$$

is the force contribution due to external DEP acting on the tail. In equation (3.13), $F_{\text{tail } X}^{\text{DEP}}$

is DEP for acting on the tail in X -direction, $M_{\text{tail } Y}^{\text{DEP}}$ and $M_{\text{tail } Z}^{\text{DEP}}$ are moments in Y - and Z -directions generated by $F_{\text{tail } X}^{\text{DEP}}$. Note that $\boldsymbol{\omega}_{\text{tail}} = \boldsymbol{\omega}_{\text{head}} + \boldsymbol{\omega}_{\text{rel}}$.

The dimensionless hydrodynamic drag force and moment on the head are given by $\mathbf{F}_{\text{head}}^{\text{drag}} = -6\pi\mu a \mathbf{v}$ and $\mathbf{M}_{\text{head}}^{\text{drag}} = -8\pi\mu a^3 \boldsymbol{\omega}$ [78], where μ is the dimensionless viscosity of the fluid. The DEP force on the head is

$$\mathbf{F}_{\text{head}}^{\text{DEP}} = [2\pi a^3 (C_0 + C_1 X_h + C_2 Y_h), 0, 0]^T, \quad (3.14)$$

where X_h and Y_h are the centroid position of the head in the inertial frame. The total force and moment can be obtained by adding the hydrodynamic and DEP force and moment, respectively. We can express the force and moment on the head by

$$\begin{bmatrix} \mathbf{F} \\ \mathbf{M} \end{bmatrix}_{\text{head}} = [\boldsymbol{\xi}]_{\text{head}} \begin{bmatrix} \mathbf{v} \\ \boldsymbol{\omega} \end{bmatrix}_{\text{head}} + [\mathbf{B}], \quad (3.15)$$

where

$$[\boldsymbol{\xi}]_{\text{head}} = - \begin{bmatrix} 6\pi\mu a & 0 & 0 & 0 & 0 & 0 \\ 0 & 6\pi\mu a & 0 & 0 & 0 & 0 \\ 0 & 0 & 6\pi\mu a & 0 & 0 & 0 \\ 0 & 0 & 0 & 8\pi\mu a^3 & 0 & 0 \\ 0 & 0 & 0 & 0 & 8\pi\mu a^3 & 0 \\ 0 & 0 & 0 & 0 & 0 & 8\pi\mu a^3 \end{bmatrix} \quad (3.16)$$

is the resistance matrix of the head, and

$$[\mathbf{B}] = [F_{\text{head}}^{\text{DEP}}, 0, 0, 0, 0, 0]^T$$

is the force contribution due to DEP. At low Reynolds number, the total force and moment on the bacterium are zero, i.e.

$$[\boldsymbol{\xi}]_{\text{head}} \begin{bmatrix} \mathbf{v} \\ \boldsymbol{\omega} \end{bmatrix}_{\text{head}} + [\boldsymbol{\xi}]_{\text{tail}} \begin{bmatrix} \mathbf{v} \\ \boldsymbol{\omega} \end{bmatrix}_{\text{tail}} + [\mathbf{A}] + [\mathbf{B}] = \mathbf{0}. \quad (3.17)$$

The swimming velocity of the bacterium can be determined by

$$\begin{bmatrix} \mathbf{v} \\ \boldsymbol{\omega} \end{bmatrix} = [\xi_{\text{head}} + \xi_{\text{tail}}]^{-1} \left([\xi]_{\text{tail}} \begin{bmatrix} \mathbf{0} \\ \boldsymbol{\omega}_{\text{rel}} \end{bmatrix} - [\mathbf{A}] - [\mathbf{B}] \right), \quad (3.18)$$

where $[\xi_{\text{head}} + \xi_{\text{tail}}]^{-1}$ is the mobility matrix of the bacterium.

The orientation of the bacterium is not only influenced by DEP, but also affected by Brownian rotational diffusion. The probability of an orientation $c(\theta, \psi)$ can be obtained by solving the Fokker-Planck equation [79]

$$\partial_t c = D_R \nabla^2 c - \nabla \cdot (\dot{\boldsymbol{\omega}} c), \quad (3.19)$$

where D_R is the dimensionless rotational diffusivity of the bacterium, which is already non-dimensionalized with Ω , and $\dot{\boldsymbol{\omega}} = (\dot{\theta}, \dot{\psi})$ is the time rate of change of (θ, ψ) . The orientation distribution of the bacterium is obtained by solving equation (3.19). The boundary conditions for equation (3.19) come from the periodic characteristic of the orientation in the domain of interest, given by

$$c_{\psi=0} = c_{\psi=2\pi}, \quad \frac{\partial c}{\partial \psi}_{\psi=0} = \frac{\partial c}{\partial \psi}_{\psi=2\pi}, \quad \frac{\partial c}{\partial \theta}_{\theta=0} = 0, \quad \frac{\partial c}{\partial \theta}_{\theta=\pi} = 0. \quad (3.20)$$

The rotational diffusivity D_R can be determined from the mobility matrix [11]. According to equation (3.18), the mobility matrix is a 6×6 matrix which relates force on the bacterium to its velocity,

$$[\xi_{\text{head}} + \xi_{\text{tail}}]^{-1} = \begin{pmatrix} \kappa_{11} & \kappa_{12} & \kappa_{13} & \kappa_{14} & \kappa_{15} & \kappa_{16} \\ \kappa_{21} & \kappa_{22} & \kappa_{23} & \kappa_{24} & \kappa_{25} & \kappa_{26} \\ \kappa_{31} & \kappa_{32} & \kappa_{33} & \kappa_{34} & \kappa_{35} & \kappa_{36} \\ \kappa_{41} & \kappa_{42} & \kappa_{43} & \kappa_{44} & \kappa_{45} & \kappa_{46} \\ \kappa_{51} & \kappa_{52} & \kappa_{53} & \kappa_{54} & \kappa_{55} & \kappa_{56} \\ \kappa_{61} & \kappa_{62} & \kappa_{63} & \kappa_{64} & \kappa_{65} & \kappa_{66} \end{pmatrix}. \quad (3.21)$$

The rotational diffusivity can be approximated using the Stokes-Einstein equation, $D_R = k_B T \kappa_{44}$, where k_B is the Boltzmann's constant and T is the temperature. After obtaining the orientation probability, the bacterium's mean swimming velocity can be computed by

$$\bar{\mathbf{v}} = \int_0^{2\pi} \int_0^\pi \mathbf{v}(\theta, \psi) c(\theta, \psi) \sin \theta d\theta d\psi. \quad (3.22)$$

3.2 Results and Discussion

In this section, I discuss the effects of DEP force on the orientation and velocity of a swimming bacterium. Different components of DEP due to C_0 , C_1 and C_2 are considered independently. In our simulation, I choose $a = 1 \mu\text{m}$, $n = 4$, $\lambda = 2.5 \mu\text{m}$, $\alpha = 41^\circ$, $b = 50 \text{ nm}$, and $\Omega = 150 \text{ Hz}$ for an illustrative model, according to their typical ranges in previous studies [5, 80-83], and assume $K_N = 2K_T$ [2]. Using these parameters,

Figure 3-2 Reorientation mechanism of the bacterium under different configurations of DEP force. DEP force is generated by: (a) positive C_0 ; (b) negative C_0 ; (c) positive C_1 ; (d) negative C_1 ; (e) positive C_2 ; and (f) negative C_2 . In each case, four typical orientations in four quadrants of space are considered, and the bacterium's head is at the origin. The red dashed and blue dashed positions are steady orientations [76].

I found that the swimming velocity under no DEP force is $v_0 \approx 58 \mu\text{m s}^{-1}$ and the diffusivity of the bacterium is approximately $5.3 \times 10^{-3} \text{rad}^2 \text{s}^{-1}$.

3.2.1 Effects of constant DEP force field on the swimming bacterium

In the following discussion on C_0 , I set C_1 and C_2 to zero. C_0 determines the constant DEP force in the X -direction; its magnitude reflects the strength and its sign determines the direction of the force. When C_0 is positive, the DEP force acts along the positive X -direction. Since the head has a larger volume than the tail, it experiences a larger DEP force. The unbalance between forces on the head and the tail generates a torque that turns the bacterium such that its head eventually points towards the positive X -direction (Figure 3-2a). When the bacterium is aligned with the X -axis, the DEP force no longer alters its direction and the bacterium achieves a stable orientation. This orientation is reflected in Figure 3-3a by the concentration spot located at $(\theta = \pi/2, \psi = 0)$, which represents the positive X -direction. The bacterium's velocity due to C_0 is shown in Figure 3-4a. The solid red line, dashed blue line and dotted-dashed green line are velocity components in the X -, Y - and Z -direction, respectively. The X -velocity is the only non-zero velocity component, and this agrees with the bacterium's orientation. Physically, if the orientation of bacteria in a population is initially isotropic, a DEP force in the positive direction generated by $C_0 < 0.1$ is not sufficient to reorient all bacteria in the positive X -direction. However, at higher values of C_0 (starting from 0.1), most bacteria are aligned in the positive X -direction. At large C_0 , $\bar{v}/v_0 > 1$ also suggests that the DEP force enhances the swimming of the bacteria.

Similarly, when C_0 is negative, the bacterium undergoes a torque that aligns it with the negative X -direction, due to DEP force in the negative direction (Figure 3-2b).

Therefore, the concentration spot in Figure 3-3b is located at $(\theta = \pi/2, \psi = \pi)$ and the mean X -velocity in Figure 3-4a is negative.

3.2.2 Effects of variant DEP force on the swimming bacterium closed to the origin

The direction and magnitude of the DEP forces generated by C_1 and C_2 depend on the bacterium's position. In the next section, I will show the potential of DEP force to focus the bacteria at the origin. Thus, in this part, I investigate the behavior of the bacterium at the origin, where $X_h = 0$ and $Y_h = 0$. At the origin, the DEP force on the head is negligible and any change in the swimming of bacterium is resulted from the DEP force on the tail.

When examining C_1 , I set C_0 and C_2 to zero. When C_1 is positive, the tail in the positive- X domain experiences DEP force in the positive direction (two cases on the right of Figure 3-2c). The DEP force generates a torque that turns the tail until it is aligned with the positive X -axis (blue dotted curve in Figure 3-2c). This orientation is shown in Figure 3-3c by the concentration spot located at $(\theta = \pi/2, \psi = \pi)$, which represents that the bacterium points at the negative X -direction. Similarly, when the tail

Figure 3-3 Orientation distribution of the bacterium at different configurations of DEP force.

θ is polar angle and ψ is the azimuthal angles ($0 \leq \theta \leq \pi, 0 \leq \psi \leq 2\pi$). (a) $C_0 = 0.1$; (b)

$C_0 = -0.1$; (c) $C_1 = 100$; (d) $C_1 = -100$; (e) $C_2 = 100$; (f) $C_2 = -100$ [76].

is in the negative- X domain, the force on the tail will turn it to the red dashed equilibrium orientation. Thus, the bacterium points to the positive X -direction as also shown in Figure 3-3c by the other concentration spot at $(\theta = \pi/2, \psi = 0)$. Since the bacteria are equally oriented either along the positive or negative X -direction, the mean velocities in all directions vanish (not shown in figure). However, if the head is off the origin due to perturbation, the bacterium will be pulled away from the origin because of the large DEP force on the head.

When applying negative C_1 , interestingly, the bacterium is not aligned with the X -axis although the DEP force acts in the X -direction. When the tail is located in the positive- Y domain (Figure 3-2d), since the DEP force is negative at the positive X -position and positive at the negative X -position, the bacterium is turned towards the YZ -plane. When the tail is in YZ -plane (blue dotted curve), half of the tail experiences DEP force in the negative direction while another half experiences force in the positive direction; therefore, the total force on the tail vanishes. Similar physics applies to those whose tail is in the negative- Y domain, and the final orientation of the bacterium is in the YZ -plane, indicated by the red dashed curve. As the bacterium is reoriented to the YZ -plane, ψ is focused to either $\pi/2$ or $3\pi/2$, and θ ranges from 0 to π as shown in Figure 3-3d. Since the bacterium's orientation is isotropic in the YZ -plane, the mean velocities in all directions vanish (not shown in figure). It should be noted that if the bacterium is perturbed away from $X_h = 0$, negative C_1 will stabilize it at $X_h = 0$ because the DEP force always points towards the YZ -plane.

For the investigation of C_2 , both C_0 and C_1 are set to zero. When C_2 is positive, the bacterium whose tail is in the positive- Y domain is rotated towards the positive X -axis until it reaches the equilibrium orientation in the ψ -direction (blue dotted curve in Figure 3-2e). The force continues to turn the bacterium towards the XY -plane until the tail is in

the XY -plane. Similar physics applies to the bacterium whose tail is in the negative- Y domain; however, the equilibrium in the ψ -direction (red dashed curve) is opposite to that of the bacterium whose tail is initially in the positive- Y domain. Furthermore, the equilibrium orientation in the ψ -direction due to positive C_2 is neither in the positive nor negative X -axis; instead, the stable orientation is $\psi = \beta$ or $\psi = \pi + \beta$ (Figure 3-2e), depending on the initial location of the tail. The angle β is resulted from the competition between the effects of DEP force and the rotational diffusion on the bacterium's orientation. The final bacterium's orientation due to positive C_2 is further shown in the two concentration spots at the location $(\theta = \pi/2, \psi = \beta)$ and $(\theta = \pi/2, \psi = \pi + \beta)$ in Figure 3-3e (here, $\beta \approx 15^\circ$ when $C_2 = 100$). Similarly, when C_2 is negative, the bacterium in the positive- Y domain reaches its stable ψ -position at the blue dotted curve, and those in negative- Y domain finally stays at the equilibrium ψ -position indicated by the red dashed curve. These equilibrium orientations are also indicated in Figure 3-3f by two concentration spots located at $(\theta = \pi/2, \psi = \pi + \beta)$ and $(\theta = \pi/2, \psi = \beta)$, where $\beta \approx -15^\circ$ when $C_2 = -100$. Since the bacterium's orientation due to C_2 is equally distributed either along the β -direction or $(\pi + \beta)$ -direction, the mean velocity in the X - and Y -direction vanishes (Figure 3-4b).

Interestingly, the DEP force generated by C_2 results in a non-zero translational velocity in the Z -direction (Figure 3-4b) although the bacterium is not aligned with the Z -axis. This phenomenon is the consequence of the drag anisotropy on an elongated body (such as the tail): the resistance when moving in the perpendicular direction to the body is larger than that in the parallel direction. Consider one full turn of the left-handed helical tail aligned in the X -axis as shown in Figure 3-4c. Each half of the turn can be considered as a moving body which experiences anisotropic drag. When the tail is

Figure 3-4 Mean swimming velocity $\bar{v}$ of the bacterium due to (a) C_0 and (b) C_2 . (c) Force and velocity analysis of one full turn of the left-handed helical tail due to positive C_2 . The solid red line, dashed blue line and dotted-dashed green line are velocity components in the X-, Y- and Z-direction, respectively. [76].

subjected to DEP force generated by C_2 , the red solid half experiences force in the positive direction while the blue dotted one experiences force in negative direction. Due to the drag anisotropy, each half of the tail does not move in the force's direction; instead, they move along a constant angle with the force's direction (Figure 3-4c). Subsequently, the total velocity of the tail is non-zero in Z-direction, and this Z-velocity increases with the strength of C_2 . Such lateral drift was also found in previous studies [56, 57], where a helical swimmer is exposed to a shear flow.

The effects of DEP components C_0 , C_1 , and C_2 are summarize in Table 3-1.

Table 3-1 Effects of DEP components on the swimming bacterium

Components		Descriptions	Effects on the swimming bacterium
C_0	Positive	Constant DEP force	Align the bacterium with positive X -direction.
	Negative		Align the bacterium with negative X -direction.
C_1	Positive	X -varying DEP force	Align bacterium in the positive/negative X domain with negative/positive X -direction.
	Negative		Align bacterium in the positive/negative Y domain with negative/positive Y -direction.
C_2	Positive	Y -varying DEP force	Almost align the bacterium in the positive/negative domain with the negative/positive X -direction. The bacterium has lateral velocity in Z -direction
	Negative		Almost align the bacterium in the positive/negative domain with the positive/negative X -direction. The bacterium has lateral velocity in Z -direction

3.2.3 Potential application in bacterial focusing

Bacterial focusing is necessary in the applications that require high density of bacteria at a certain location in the experimental devices such as microfluidic channels. The bacterium's reorientation due to DEP force points to the possibility of utilizing DEP force for bacterial focusing. When the bacterium is far from the origin, the head no longer has zero DEP force. Since the DEP force on the head is stronger than that on the tail, the bacterium's orientation and velocity are dominated by the force exerted on the head.

Among the DEP force components, the most interesting one is the force generated by C_2 since it causes the bacteria to move in the direction perpendicular to the force. If we can first focus the bacteria towards the origin, the force generated by C_2 can move the bacteria along the Z -axis, and eventually concentrate them. Motivated by this idea, I consider applying the force generated by C_2 in bacterial manipulation.

Figure 3-5 (a) Orientation of the bacterium when $\nabla|\mathbf{E}|^2 = [C_2Y, C_2X, 0]^T$ ($C_2 < 0$). The arrows indicate the direction of DEP force exerted on the bacterium's head; (b) Orientation of the bacterium when $\nabla|\mathbf{E}|^2 = C_2 \{k_1X + Y, X + k_2Y, 0\}$ ($C_2 < 0$); (c) Mean velocity in the Z-direction of the bacterium at the origin at different values of negative C_2 , for $\nabla|\mathbf{E}|^2 = C_2 [k_1X + Y, X + k_2Y, 0]^T$; (d) General shape of the electrodes when $C_2 = -100$, $k_1 = 1.3$, $k_2 = 1.5$, for an example [76].

To have the DEP force generated by C_2 , the X-component of $\nabla|\mathbf{E}|^2$ should be C_2Y , i.e. $(\partial/\partial X)|\mathbf{E}|^2 = C_2Y$. This can be satisfied by $|\mathbf{E}|^2 = C_2XY + K$, where K is an arbitrary constant. Thus, we have

$$\nabla|\mathbf{E}|^2 = C_2[Y, X, 0]^T. \quad (3.23)$$

Equation (3.23) suggests that if $F_{\text{DEPX}} \propto C_2 Y$, the Y -component must be non-zero and $F_{\text{DEPY}} \propto C_2 X$. When C_2 is negative, the bacteria which are in the first quadrant ($X_h > 0$ and $Y_h > 0$) or the third quadrant ($X_h < 0$ and $Y_h < 0$) move towards the origin (Figure 3-5a). However, those in the second and the fourth quadrants are directed away from the origin (Figure 3-5a). To overcome this, I consider additional force components in both X - and Y -direction such that the bacteria in the second and fourth quadrants experiences origin-oriented total force. For this purpose, $\nabla|\mathbf{E}|^2$ must take the form:

$$\nabla|\mathbf{E}|^2 = C_2[k_1 X + Y, X + k_2 Y, 0]^T, \quad (3.24)$$

where $k_1 > 1$ and $k_2 > 1$ are to determine the additional forces' strength. Since $C_2 < 0$, we have

$$F_{\text{DEPX}} \propto -|C_2|(k_1 X + Y), \quad F_{\text{DEPY}} \propto -|C_2|(X + k_2 Y). \quad (3.25)$$

Equation (3.25) shows that the direction of DEP force in the X - and Y -direction are determined by the sign of $(k_1 X + Y)$ and $(X + k_2 Y)$, respectively. Since k_1 and k_2 are both positive, they result in a greater magnitude of the forces in the first and third quadrants (region 1 and 5 in Figure 3-5b) without affecting the forces' direction. In the second quadrant, the DEP force points towards origin when the head's position satisfies

$$Y_h < -k_1 X_h \text{ and } Y_h > -(1/k_2) X_h \text{ (for } X_h < 0 \text{ and } Y_h > 0). \quad (3.26)$$

Similarly, the DEP in the fourth quadrant points towards the origin when

$$Y_h > -k_1 X_h \text{ and } Y_h < -(1/k_2) X_h \text{ (for } X_h > 0 \text{ and } Y_h < 0). \quad (3.27)$$

The DEP forces in regions 3 and 7 of Figure 3-5b point to the origin since X_h and Y_h satisfy equations (3.26) and (3.27), respectively. In region 2, the DEP force points away the origin; however, since the forces in the X - and Y -direction are both negative, the bacterium is guided to region 3 and eventually directed to the origin. Similarly,

bacteria in region 4 are pulled to region 3, those in region 6 and 8 are guided to region 7; finally, they are all directed to the origin. When the bacteria have been focused at the origin along the Z -axis, their Z -velocities due to C_2 further moves them along the Z -axis. Figure 3-5c shows the mean Z -velocity of a bacterium at different values of C_2 , when $k_1 = 1.3$ and $k_2 = 1.5$, for an example. Since the Z -velocity in Figure 3-5c is positive, if we apply this force configuration to a bacterial population swimming in a microchannel, they will be focused at one point on the ceiling of the channel.

I now discuss the general calculation to actualize the above DEP force. To achieve the DEP force field shown in Figure 3-5b, the dimensional electric field must take the form

$$|\mathbf{E}|^2 = C_2 \left(\frac{1}{2} k_1 X^2 + \frac{1}{2} k_2 Y^2 + XY \right) + K \quad (3.28)$$

where K is an arbitrary constant and the dimensional C_2 can be determined by multiple its dimensionless value with $K_T \Omega / [\varepsilon_{medium} \text{Re}(f_{CM}) L^2]$. If the electric field is generated by applying an electrical potential difference ΔV across two electrodes, then ΔV must satisfy

$$\Delta V = V_Q - V_P = \int_P^Q \mathbf{E} \cdot d\mathbf{l} \quad (3.29)$$

In the above equation, P and Q are any two points on the two electrodes such that PQ defines the distance between the electrodes at these points; V_P and V_Q are electrical potentials at two electrodes. For a given voltage difference ΔV , the distance between two electrodes is varying and such electrodes configuration generates a non-uniform electric field. A general electrodes shape is illustrated in Figure 3-5d using simulated parameters such that $K_T \approx 0.0015 \text{ N s m}^{-2}$, $\Omega \approx 942 \text{ rad s}^{-1}$, $L \approx 13 \mu\text{m}$, ε_{medium} has typical order of 10^{-9} F m^{-2} and $\text{Re}(f_{CM})$ takes the value of 0.5 [84, 85]. Generally, at any desired C_2 ,

both k_1 and k_2 must be larger than 1 to ensure the origin-oriented force. The ratio k_1/k_2 determines the correlation between the forces' strength in the X - and Y -direction, and it also shapes the regions in Figure 3-5b. The electric field strength can be adjusted through ΔV and K , and it should not cause any lethal effect to the bacteria. It has been demonstrated that the survival rate of the bacteria *E. coli*, for example, is 85% at up to 5 kV cm^{-1} [86], showing the potential of this method, while preserving cell viability.

3.3 Summary

In this chapter, I developed a model to study the effects of DEP force on the swimming direction of helically flagellated bacteria, assuming that the flagella of the bacteria are rigid. DEP force alters the swimming direction of the bacteria, and the reorientation of any bacterium depends on the correlation between the DEP forces exerted on its head and tail. Under the effect of DEP force as discussed in our study, if the force acting on the head is dominant, the bacterium is eventually aligned with the force's direction. However, if the force on the tail is stronger than that on the head, the final steady orientation of the bacterium depends on the initial angle between the bacterial primary axis and the force's direction. Rotational diffusion also contributes to the reorientation of the bacterium, and the contribution of the rotational diffusion is more significant when the DEP force varies more strongly along the direction that is perpendicular to the DEP force. More interestingly, when the gradient of the DEP force's strength is perpendicular to the force's direction, the DEP force causes the bacterium to move in the direction perpendicular to the force, and this is resulted from the interaction between the DEP force and the helical shape of the bacterium's tail. I further discussed how to apply our findings to achieve bacteria focusing and proposed a general calculation procedure to design the shape of electrodes that can generate the desired DEP force. Although the

study in this chapter is primarily about bacteria, its application in focusing can be extended to any helically shaped particle that possesses dielectrophoretic properties when being subjected to a non-uniform electric field.

Chapter 4

Deformation of a flexible flagellum in flow: A two-dimensional study

In the previous chapter, I showed that a better understanding of how an external force affects the swimming of bacteria opens the possibility to utilize the force in bacterial manipulation. So far, I assumed that the flagella of the bacteria are rigid. In reality, flagella of micro swimmers are flexible and they are subjected to deformation due to external force. Therefore, considering the flexibility of the flagellum when study bacterial hydrodynamics would reveal more realistic physics behind and may lead to some interesting application.

As mentioned earlier, many motile bacteria propel themselves forward in the fluid by rotating their helical-shaped flagella. Therefore, any change in the shape of the flagella due to some external stimuli, could affect the swimming behavior of the bacteria. If the flagella are flexible, the flexibility of the flagella would be one of the major factors that determines swimming behavior, and it is necessary to investigate how flexible flagella respond to external forces. In general, such investigation requires a three-dimensional mathematical model; however, I see the benefits in studying the two-dimensional case before moving forward to a more complicated one. The two-dimensional problem not only serves as foundation of the three-dimensional case, but it is also useful for studying the behavior of flexible flagellum that deforms itself in a plane.

At the microscale, a flagellum in unsteady flow “sees” the surrounding turbulence as a simple shear flow. In this chapter, I consider the deformation of a flexible 2D

flagellum being subjected to shear, specifically in a microfluidic flow. I focus our attention on microfluidic flow, in addition to DEP force, because many manipulation processes have been performed with the help of microfluidic devices, especially the manipulation of 2D flagellated swimmers such as sperms in the study of fertilization [87, 88]. This chapter provides an analysis of how microfluidic flow affects the flagellum's shape and velocity of a 2D swimmer, in particular a sperm. Here, "2D swimmer" refers to a single-flagellated swimmer whose flagellum is deforming in a plane.

In the experimental setups reported [88-90], the microfluidic channels have high aspect ratio cross-sectional area ($W \gg H$), and in such devices, the flow field is parabolic (Figure 4-1a). At the microscale, the swimmer does not recognize and respond to the whole parabolic profile of the flow field but simply "sees" it as a simply shear flow as shown in Figure 4-1b. Therefore, the effect of shear flow is essential in the study of swimming in microfluidic flow. The objectives of this chapter are to examine how 2D swimmer's velocity changes with various shear rates and to determine whether shear flow could support the motility of the swimmer.

4.1 Problem formulation

4.1.1 Model of a 2D swimmer

I model the swimmer as a rigid spherical head of radius a attached to a thin elastic flagellum of length L swimming in a simple shear flow $\mathbf{V}_{\text{flow}} = \dot{\gamma} y \mathbf{i}$, where $\dot{\gamma}$ is the shear rate (s^{-1}) and $\mathbf{i}$ is the unit vector in the x -direction (Figure 4-1b). In order to analyze the forces acting along the flagellum, the flagellum is discretized into infinitesimal segmental elements with length ds . The position of an infinitesimal segment with respect to the x - y axis is denoted by the displacement vector $\mathbf{r} = x \mathbf{i} + y \mathbf{j}$, where $\mathbf{j}$ is the unit

vector in the y -direction (Figure 4-1b). In this model, I assume that the swimmer is always aligned along the x -axis, which is consistent with the observation in the case that motile sperms are subjected to a parabolic velocity distribution [91, 92]. Furthermore, elongated objects, such as sperm cells, would spend most of their time aligned in the direction of the flow due to Jeffery's orbit [93].

4.1.2 Governing equation of 2D flexible flagellum

I employ the resistive force theory (RFT) to compute the hydrodynamic forces acting on the sperm's tail. Drag on a thin rod in low Reynolds number flow is anisotropic, with a

Figure 4-1 a) Illustration of a swimming sperm in a high aspect cross-sectional ratio microchannel ($W \gg H$); b) Schematic of a sperm in shear flow; c) Force and moment analysis of an infinitesimal segment of the sperm's flagellum.

greater resistance when the rod is oriented perpendicular rather than parallel to the flow. RFT expresses the drag force per unit length as $\mathbf{f} = k_T V_T \mathbf{t} + k_N V_N \mathbf{n}$, where V_T and V_N are velocities of the flagellum relative to the fluid parallel and perpendicular to the segment of the flagellum, respectively; k_T and k_N are the resistive coefficients parallel and perpendicular to the segment of the flagellum, respectively [94]. Here, $\mathbf{t}$ and $\mathbf{n}$ denote the unit vectors parallel and perpendicular to the segment of the flagellum, respectively. By utilizing the RFT, one is able to predict propulsive velocities how forces and

moments are distributed along the flagellum. Predictions of propulsive velocities have been experimentally verified [17]. Although RFT does not take into account long range, hydrodynamics interactions such as flagella-flagella interactions and body-flagella, it is an accurate theory when dealing with single flagellum, which has small radius compared to the length scale of flagellum's deformation and total flagellum's length [2, 94].

The forces and moments acting on an arbitrary infinitesimal flexible segment ds are illustrated in (Figure 4-1c). The segment experiences internal forces such as tension T , shear force Q , and bending moment M_b . The external hydrodynamic forces per unit length acting parallel and perpendicular on the segment are f_T and f_N , respectively. Since inertia is negligible in the low Reynolds number world, the total force and torque on a body equate to zero. Applying forces and torque balance on the infinitesimal segment gives

$$f_T = -\frac{\partial T}{\partial s} \quad f_N = -\frac{\partial Q}{\partial s} \quad Q = -\frac{\partial M_b}{\partial s}. \quad (4.1)$$

The bending moment M_b can be expressed in terms of the shape of the flagellum $y = y(s, t)$ and the bending stiffness EI using the constitutive moment relation [95]

$$M_b = EI \frac{\partial^2 y}{\partial s^2}, \quad (4.2)$$

where, E is the Young's modulus of the pertaining object and I denotes the cross-sectional second moment of area. Using Equations (4.1) and (4.2), we can obtain

$$f_N = EI \frac{\partial^4 y}{\partial s^4}. \quad (4.3)$$

As mentioned earlier, the external hydrodynamic forces are related to the velocities of the fluid relative to the flagellum. When a sperm swims in a shear flow, it experiences the velocity generated by the wiggling of the flexible flagellum $\mathbf{V}_{\text{wiggling}}$, the velocity due

to swimming of the flexible flagellum $\mathbf{V}_{\text{swim}}$, and the velocity due to the shear flow of fluid, $\mathbf{V}_{\text{flow}} = \dot{\gamma} y \mathbf{i}$. Here,

$$\mathbf{V}_{\text{wiggling}} = \frac{\partial \mathbf{r}(s, t)}{\partial t} = \frac{\partial x(s, t)}{\partial t} \mathbf{i} + \frac{\partial y(s, t)}{\partial t} \mathbf{j}, \quad (4.4)$$

and

$$\mathbf{V}_{\text{swim}} = V_x \mathbf{i} + V_y \mathbf{j} + \dot{\theta} \mathbf{k} \times \mathbf{r} = (V_x - \dot{\theta} y) \mathbf{i} + (V_y + \dot{\theta} x) \mathbf{j}, \quad (4.5)$$

where V_x and V_y are the swimming velocities at a reference point in the x - and y -directions, respectively, $\dot{\theta}$ is the rotational velocity, and $\mathbf{k} = \mathbf{i} \times \mathbf{j}$. The relative velocity of the fluid flow with respect to the flagellum is thus: $\mathbf{V}_{\text{rel}} = \mathbf{V}_{\text{flow}} - \mathbf{V}_{\text{swim}} - \mathbf{V}_{\text{wiggling}}$, or

$$\mathbf{V}_{\text{rel}} = \left(\dot{\gamma} y - V_x + \dot{\theta} y - \frac{\partial x}{\partial t} \right) \mathbf{i} + \left(-\dot{\theta} x - \frac{\partial y}{\partial t} - V_y \right) \mathbf{j}. \quad (4.6)$$

The external hydrodynamic forces that act on each segmental length can be analyzed in the normal and tangential directions, $\mathbf{n}$ and $\mathbf{t}$ respectively. Using RFT, the hydrodynamic force per unit length $\partial \mathbf{F} / \partial s$ is given by

$$\frac{\partial \mathbf{F}}{\partial s} = k_L (\mathbf{V}_{\text{rel}} \cdot \mathbf{t}) \mathbf{t} + k_N (\mathbf{V}_{\text{rel}} \cdot \mathbf{n}) \mathbf{n}, \quad (4.7)$$

where the tangential unit vector $\mathbf{t}$ is given by

$$\mathbf{t} = \frac{1}{\left| \frac{\partial \mathbf{r}}{\partial s} \right|} \frac{\partial \mathbf{r}}{\partial s} = \frac{\frac{\partial x}{\partial s} \mathbf{i} + \frac{\partial y}{\partial s} \mathbf{j}}{\left[\left(\frac{\partial x}{\partial s} \right)^2 + \left(\frac{\partial y}{\partial s} \right)^2 \right]^{1/2}}, \quad (4.8)$$

and the normal vector $\mathbf{n}$ can be obtained by $\mathbf{n} = \mathbf{k} \times \mathbf{t}$. Furthermore,

$$f_N = \frac{\partial \mathbf{F}}{\partial s} \cdot \mathbf{n} = k_N (\mathbf{V}_{\text{rel}} \cdot \mathbf{n}) ds. \quad (4.9)$$

Substituting equations (4.8) and (4.9) into equation (4.7), we obtain

$$\frac{k_N}{EI} \left[\frac{-\dot{\gamma} y \frac{\partial y}{\partial s} + V_x \frac{\partial y}{\partial s} - \dot{\theta} y \frac{\partial y}{\partial s} + \frac{\partial x}{\partial t} \frac{\partial y}{\partial s} - \dot{\theta} x \frac{\partial x}{\partial s} - \frac{\partial y}{\partial t} \frac{\partial x}{\partial s} - V_y \frac{\partial x}{\partial s}}{\left[\left(\frac{\partial x}{\partial s} \right)^2 + \left(\frac{\partial y}{\partial s} \right)^2 \right]^{1/2}} \right] = \frac{\partial^4 y}{\partial s^4},$$

$s \in [0, L], \quad t \geq 0.$

(4.10)

Equation (4.10) is the governing equation of motion required to obtain the shape of the flexible flagellum as it implies a direct relation to the shape equation of the flexible flagellum. For simplicity, I will limit our study to small amplitude flagellum and that the flagellum is non-extensible, such that $\partial x / \partial t \approx 0$, $dx \approx ds$ and $(\partial y / \partial s)^2 \approx 0$. Using the small amplitude approximation, equation (4.10) becomes

$$\frac{k_N}{EI} \left[-\dot{\gamma} y \frac{\partial y}{\partial s} + V_x \frac{\partial y}{\partial x} - \dot{\theta} y \frac{\partial y}{\partial s} - \dot{\theta} x - \frac{\partial y}{\partial t} - V_y \right] = \frac{\partial^4 y}{\partial s^4},$$

$s \in [0, L], \quad t \geq 0.$

(4.11)

We can non-dimensionalize equation (4.11) by consider L and ω^{-1} as the characteristic length and time scales, respectively, where ω is the beating frequency of the flagellum, and introduce the following dimensionless parameters:

$$\begin{aligned} \tilde{x} &= x/L & \tilde{y} &= y/L & \tilde{s} &= s/L & \tilde{t} &= t\omega \\ \tilde{V}_x &= V_x/(L\omega) & \tilde{V}_y &= V_y/(L\omega) & \Theta &= \dot{\theta}/\omega. \end{aligned}$$

(4.12)

Using the above dimensionless parameters in equation (4.11) and keep using the existing notations (without tilde) for the sake of neat and tidy presentation, we can rewrite equation (4.11) in the dimensionless form as follows:

$$Sp^4 \left[-Z y \frac{\partial y}{\partial s} + V_x \frac{\partial y}{\partial s} - \Theta y \frac{\partial y}{\partial s} - \Theta x - \frac{\partial y}{\partial t} - V_y \right] = \frac{\partial^4 y}{\partial s^4},$$

$s \in [0, 1], \quad t \geq 0,$

(4.13)

where $Z = \dot{\gamma}/\omega$ and $Sp = L(\omega k_N/EI)^{\frac{1}{4}}$. Here, Sp , known as sperm number, is a dimensionless parameter that relates the period of travelling wave of the flagellum to the bending relaxation time [11].

To solve for the shape of the flagellum, we can omit the contributions from the swimming velocities V_x , V_y , and Θ as these velocities exemplify rigid body motion and therefore they do not induce any relative motion between any parts of the flagellum. Hence, both the rotational and translational swimming velocities are insignificant in the calculations to acquire the shape of the flexible flagellum at least to the first order. Thus, equation (4.13) becomes

$$\frac{\partial^4 y}{\partial s^4} + Z Sp^4 y \frac{\partial y}{\partial s} + Sp^4 \frac{\partial y}{\partial t} = 0, \quad s \in [0,1], \quad t \geq 0. \quad (4.14)$$

We can supply appropriate boundary conditions for equation (4.14) at the fixed and free ends. At the fixed end ($s=0$), I consider a prescribed angle, and at the free end of the flagellum, force and torque must vanish. Therefore, the boundary conditions are

$$y(0,t) = 0; \quad \left. \frac{\partial y(s,t)}{\partial s} \right|_{s=0} = \varepsilon \cos t; \quad \left. \frac{\partial^2 y(s,t)}{\partial s^2} \right|_{s=1} = 0; \quad \left. \frac{\partial^3 y(s,t)}{\partial s^3} \right|_{s=1} = 0, \quad (4.15)$$

where ε is the amplitude of the prescribed angle. Here, ε must be carefully chosen to satisfy the small amplitude approximation, i.e. $|y(s,t)| \leq 0.1$.

After obtaining the shape of the flexible flagellum, the swimming velocity of the flagellum can be obtained through the force and moment analysis on an element. The total hydrodynamic force acting is given by equation (4.7), which in dimensionless form can be written as

$$d\mathbf{F} = k_N \left[(\mathbf{V}_{\text{rel}} \cdot \mathbf{t}) \left(\frac{k_T}{k_N} - 1 \right) \mathbf{t} + \mathbf{V}_{\text{rel}} \right] ds, \quad (4.16)$$

where the force $\mathbf{F}$ is already normalized with $k_N \omega L^2$. Similarly, the dimensionless moment, which is already normalized with $k_N \omega L^3$, on the element measured from the origin is given by

$$d\mathbf{M} = \mathbf{r} \times d\mathbf{F}. \quad (4.17)$$

The total force and moment acting on the flagellum can be obtained by integrating equations (4.16) and (4.17) with respect to ds along the flagellum, from the fixed end ($s = 0$) to the free end ($s = 1$), and they can be expressed in components as

$$\begin{pmatrix} F_x \\ F_y \\ M \end{pmatrix}_{\text{tail}} = \begin{pmatrix} \zeta_{11} & \zeta_{12} & \zeta_{13} \\ \zeta_{21} & \zeta_{22} & \zeta_{23} \\ \zeta_{31} & \zeta_{32} & \zeta_{33} \end{pmatrix} \begin{pmatrix} V_x \\ V_y \\ \Theta \end{pmatrix}_{\text{tail}} + \begin{pmatrix} A_1 \\ A_2 \\ A_3 \end{pmatrix}_{\text{tail}}, \quad (4.18)$$

or in a more compact form as

$$\mathbf{F}_{\text{tail}} = \boldsymbol{\zeta}_{\text{tail}} \mathbf{V}_{\text{tail}} + \mathbf{A}_{\text{tail}}, \quad (4.19)$$

where $\mathbf{F} = F_x \mathbf{i} + F_y \mathbf{j}$, $\mathbf{M} = M \mathbf{k}$, and the subscript “tail” refers to the flagellum.

The total force and moment acting on a sphere moving in a simple shear flow is given by [78]

$$\begin{pmatrix} F_x \\ F_y \\ M \end{pmatrix}_{\text{head}} = \begin{pmatrix} -6\pi\mu a & 0 & 0 \\ 0 & -6\pi\mu a & 0 \\ 0 & 0 & -8\pi\mu a^3 \end{pmatrix} \begin{pmatrix} V_x \\ V_y \\ \dot{\theta} \end{pmatrix}_{\text{head}} + \begin{pmatrix} 6\pi\mu a \gamma \dot{\gamma} \\ 0 \\ -8\pi\mu a^3 \dot{\gamma} \end{pmatrix}. \quad (4.20)$$

Equation (4.20) is non-dimensionalized by introducing $\tilde{a} = (a/L)$ and rewritten in dimensionless form (using notation without tilde) as

$$\begin{pmatrix} F_x \\ F_y \\ M \end{pmatrix}_{\text{head}} = \begin{pmatrix} -6\pi\mu a & 0 & 0 \\ 0 & -6\pi\mu a & 0 \\ 0 & 0 & -8\pi\mu a^3 \end{pmatrix} \begin{pmatrix} V_x \\ V_y \\ \Theta \end{pmatrix}_{\text{head}} + \begin{pmatrix} 6\pi\mu a \gamma Z \\ 0 \\ -8\pi\mu a^3 Z \end{pmatrix}, \quad (4.21)$$

or in a more compact form as

$$\mathbf{F}_{\text{head}} = \boldsymbol{\zeta}_{\text{head}} \mathbf{V}_{\text{head}} + \mathbf{A}_{\text{head}}, \quad (4.22)$$

where the viscosity μ in equation (4.21) is already non-dimensionalized with k_N . Here, it must be noted that forces and velocities are taken at a reference point fixed at the center of the head. In order to maintain the physical attachment between the head and the flagellum, $\mathbf{V}_{\text{head}}$ must be identical with $\mathbf{V}_{\text{tail}}$ during swimming. Therefore, combining equations (4.19) and (4.22), and noting that total force and total moment on the swimmer must vanish at low Reynolds, yields the instantaneous translational and rotational velocities $\mathbf{V}(V_x, V_y, \Theta)$ of the swimmer

$$\mathbf{V} = -[\zeta_{\text{head}} + \zeta_{\text{tail}}]^{-1} [\mathbf{A}_{\text{head}} + \mathbf{A}_{\text{tail}}], \quad (4.23)$$

where $\mathbf{V} = \mathbf{V}_{\text{head}} = \mathbf{V}_{\text{tail}}$. The time-average velocities can be obtained by integrating $\mathbf{V}$ over once cycle of oscillation:

$$\bar{\mathbf{V}} = \frac{1}{2\pi} \int_0^{2\pi} \mathbf{V} dt. \quad (4.24)$$

4.1.3 Method for solving the flagellum's shape equation

Obtaining the shape of the flagellum is key of this study, and this is done by solving the equation (rewrite equation (4.14))

$$\frac{\partial^4 y}{\partial s^4} + Z S p^4 y \frac{\partial y}{\partial s} + S p^4 \frac{\partial y}{\partial t} = 0, \quad s \in [0, 1], \quad t \geq 0, \quad (4.25)$$

and the solution must satisfy the following boundary conditions:

$$y(0, t) = 0; \quad \left. \frac{\partial y(s, t)}{\partial s} \right|_{s=0} = \varepsilon \cos t; \quad \left. \frac{\partial^2 y(s, t)}{\partial s^2} \right|_{s=1} = 0; \quad \left. \frac{\partial^3 y(s, t)}{\partial s^3} \right|_{s=1} = 0. \quad (4.26)$$

One can see this is a real mathematical challenge because equation (4.25) is a fourth order nonlinear partial differential equation, and the solution must be periodic in the time domain and satisfy the boundary values given by equation (4.26) in the space domain. Some analytical methods, such as generalized separation of variables and tanh methods

[96-99], are able to find exact or approximated exact solutions for equation (4.25); however, these solutions are not general enough and they do not satisfy the given boundary conditions. In such situation, numerical approach is a wiser choice to deal with the flagellum's shape equation.

4.1.3.1 Brief introduction to Chebyshev collocation method

To illustrate the Chebyshev collocation method for solving differential equations, let's consider the differential equation

$$Lu(x) - g(x) = 0, \quad -1 < x < 1 \quad (4.27)$$

with appropriated boundary conditions, where L is generally a linear differential operator. The basic idea of Chebyshev collocation method is to approximate the unknown function $u(x)$ by $N+1$ function values $u_j = u(x_j)$ at $N+1$ collocation points x_j , $j = 0 \dots N$, where the collocation points are given by [100]

$$x_j = \cos \frac{\pi j}{N}, \quad j = 0 \dots N. \quad (4.28)$$

Thus, the solution to equation (4.27) is sought in the discrete form $\mathbf{u} = [u_0(x) \dots u_N(x)]^T$. According to the method, the first order derivative $u^{(1)}(x) = du/dx$ is also approximated at the same collocation points, as

$$u_i^{(1)}(x) = \sum_{j=0}^N D_{i,j} u_j(x), \quad i, j = 0 \dots N, \quad (4.29)$$

where the first order differentiation matrix $\mathbf{D} = [D_{i,j}]$ is given by [100]

$$D_{i,j} = \frac{\bar{c}_i}{\bar{c}_j} \frac{(-1)^{i+j}}{(x_i - x_j)}, \quad 0 \leq i, j \leq N, \quad i \neq j$$

$$D_{i,i} = -\frac{x_i}{2(1-x_i^2)}, \quad 1 \leq i \leq N-1, \quad \text{and} \quad D_{0,0} = -D_{N,N} = \frac{2N^2+1}{6}, \quad (4.30)$$

where $x_i = \cos(\pi i/N)$, $\bar{c}_0 = \bar{c}_N = 2$, $\bar{c}_j = 1$ for $1 \leq j \leq N-1$. The first order derivative can be written in the vector form as $\mathbf{u}^{(1)} = [u_0^{(1)}(x) \dots u_N^{(1)}(x)]^T$, thus $\mathbf{u}^{(1)} = \mathbf{D}\mathbf{u}$ and it is

easy to observe that $\mathbf{u}^{(p)} = \mathbf{D}^p \mathbf{u}$, where $\mathbf{u}^{(p)} = [u_0^{(p)} \dots u_N^{(p)}]^T$. Using these approximations, the differential equation (4.27) can be discretized as

$$\mathbf{L}\mathbf{u} = \mathbf{g} \quad (4.31)$$

where $\mathbf{L} = [L_{i,j}]$ ($i, j \in 0 \dots N$) is the differential operator matrix, $\mathbf{u} = [u_0(x) \dots u_N(x)]^T$ and $\mathbf{g} = [g(x_0) \dots g(x_N)]^T$. Here, the Chebyshev collocation method approximates the solution of the differential equation (4.27) by the solution of the algebraic system (4.31). The boundary conditions of the differential equation can be implemented into (4.31) by directly modifying the matrix $\mathbf{L}$ and vector $\mathbf{g}$ to satisfy the function values on the boundaries.

4.1.3.2 Chebyshev collocation method for solving flagellum's shape equation

To solve for the shape of the flagellum using Chebyshev collocation method, first we need to map the spatial domain of equation (4.25) from $[0,1]$ to $[-1,1]$, since Chebyshev approximation is only valid on $[-1,1]$. This mapping is done by the coordinate transform $z = 2s - 1$; consequently, equation (4.25) becomes

$$16 \frac{\partial^4 u(z,t)}{\partial z^4} + 2ZSp^4 u(z,t) \frac{\partial u(z,t)}{\partial z} + Sp^4 \frac{\partial u(z,t)}{\partial t} = 0, \quad z \in [-1,1], \quad t \geq 0 \quad (4.32)$$

where $\partial u(z,t)$ gives the shape of the flagellum and satisfies the boundary conditions

$$u(-1,t) = 0, \quad \left. \frac{\partial u(z,t)}{\partial z} \right|_{z=-1} = \frac{\varepsilon}{2} \cos t, \quad \left. \frac{\partial^2 u(z,t)}{\partial z^2} \right|_{z=1} = \left. \frac{\partial^3 u(z,t)}{\partial z^3} \right|_{z=1} = 0. \quad (4.33)$$

We approximate the solution $\partial u(z,t)$ by $\mathbf{u} = [u_0(x) \dots u_N(x)]^T$ at $N+1$ collocation points $z_j = \cos(\pi j/N)$, $j = 0 \dots N$, and approximate the first order derivative $\partial u(z,t)/\partial z$ as $\mathbf{u}^{(1)} = [u_0^{(1)}(x) \dots u_N^{(1)}(x)]^T = \mathbf{D}\mathbf{u}$, where $\mathbf{D}$ is first order differentiation matrix obtained by (4.30). Here, it is important to keep in mind that now $\mathbf{u}$ is also time-dependent. The

fourth order derivative $\partial^4 u(z, t) / \partial z^4$ can be approximated by $\mathbf{u}^{(4)} = \mathbf{D}^4 \mathbf{u}$. However, such approximation amplifies the round-off error; therefore, it is reasonable to convert equation (4.32) to a system of first order differential equations, so that we can eliminate the high order derivative. This can be done by introducing a set of immediate unknown discrete functions $\mathbf{u}_1, \mathbf{u}_2, \mathbf{u}_3, \mathbf{u}_4$ such that

$$\mathbf{u}_1 = \mathbf{u}, \quad \mathbf{u}_2 = \frac{\partial}{\partial z} \mathbf{u}, \quad \mathbf{u}_3 = \frac{\partial^2}{\partial z^2} \mathbf{u}, \quad \mathbf{u}_4 = \frac{\partial^3}{\partial z^3} \mathbf{u}, \quad (4.34)$$

where

$$\mathbf{u}_1 = [u_{1_0}, \dots, u_{1_N}]^T, \quad \mathbf{u}_2 = [u_{2_0}, \dots, u_{2_N}]^T, \quad \mathbf{u}_3 = [u_{3_0}, \dots, u_{3_N}]^T, \quad \mathbf{u}_4 = [u_{4_0}, \dots, u_{4_N}]^T \quad (4.35)$$

and realizing that

$$\begin{aligned} \frac{\partial}{\partial z} \mathbf{u}_1 &= \mathbf{D} \mathbf{u}_1 = \mathbf{u}_2, & \frac{\partial}{\partial z} \mathbf{u}_2 &= \mathbf{D} \mathbf{u}_2 = \mathbf{u}_3, \\ \frac{\partial}{\partial z} \mathbf{u}_3 &= \mathbf{D} \mathbf{u}_3 = \mathbf{u}_4, & \frac{\partial}{\partial z} \mathbf{u}_4 &= \mathbf{D} \mathbf{u}_4 = \frac{\partial^4}{\partial z^4} \mathbf{u}_1. \end{aligned} \quad (4.36)$$

Consequently, equation (4.32) is equivalent to the system

$$\begin{cases} \mathbf{D} \mathbf{u}_1 - \mathbf{u}_2 = \mathbf{0} \\ \mathbf{D} \mathbf{u}_2 - \mathbf{u}_3 = \mathbf{0} \\ \mathbf{D} \mathbf{u}_3 - \mathbf{u}_4 = \mathbf{0} \\ \mathbf{D} \mathbf{u}_4 = -\frac{Sp^4}{16} \frac{d}{dt} \mathbf{u}_1 - \frac{ZSp^4}{8} \mathbf{u}_1 \mathbf{u}_2, \end{cases} \quad (4.37)$$

where $\mathbf{u}_1 \mathbf{u}_2$ is point-wise product of $\mathbf{u}_1$ and $\mathbf{u}_2$, and the set of boundary conditions:

$$u_{1_N} = 0, \quad u_{2_N} = \frac{\varepsilon}{2} \cos t, \quad u_{3_0} = 0, \quad u_{4_0} = 0. \quad (4.38)$$

Due to (4.28), the fixed end and the free end of the flagellum correspond to u_{1_N} and u_{1_0} , respectively. The system (4.37) is solved by performing time-stepping such that

$$\begin{cases} \mathbf{D}\mathbf{u}_1^{n+1} - \mathbf{u}_2^{n+1} = \mathbf{0} \\ \mathbf{D}\mathbf{u}_2^{n+1} - \mathbf{u}_3^{n+1} = \mathbf{0} \\ \mathbf{D}\mathbf{u}_3^{n+1} - \mathbf{u}_4^{n+1} = \mathbf{0} \\ \mathbf{D}\mathbf{u}_4^{n+1} = -\frac{Sp^4}{16} \frac{\mathbf{u}_1^{n+1} - \mathbf{u}_1^n}{\Delta t} - \frac{ZSp^4}{8} \mathbf{u}_1^n \mathbf{u}_2^{n+1}, \end{cases} \quad (4.39)$$

where the superscript n and $n+1$ indicate that functions are evaluated at the current and the next time steps ($n \geq 0$). When $n = 0$, the functions are at their initial conditions. Here, when performing time stepping, I treat the nonlinear term $\mathbf{u}_1 \mathbf{u}_2$ using semi-implicit scheme as $\mathbf{u}_1^n \mathbf{u}_2^{n+1}$ to avoid solving nonlinear algebraic system. The system (4.39) can be rewritten in matrix form as

$$\begin{bmatrix} \mathbf{D} & -\mathbf{I} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{D} & -\mathbf{I} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{D} & -\mathbf{I} \\ A\mathbf{I} & B \text{diag}(\mathbf{u}_1^n) & \mathbf{0} & \mathbf{D} \end{bmatrix} \begin{bmatrix} \mathbf{u}_1^{n+1} \\ \mathbf{u}_2^{n+1} \\ \mathbf{u}_3^{n+1} \\ \mathbf{u}_4^{n+1} \end{bmatrix} = \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \\ A\mathbf{u}_1^n \end{bmatrix}, \quad (4.40)$$

where $A = Sp^4 / (16\Delta t)$, $B = ZSp^4 / 8$, $\mathbf{I}$ is $(N+1) \times (N+1)$ identity matrix and $\text{diag}(\mathbf{u})$ is $(N+1) \times (N+1)$ diagonal matrix which has $\mathbf{u}$ being its main diagonal.

Here, when we decompose equation (4.32) into system (4.40), not only can we reduce the round-off error, but we can also handle the boundary conditions in a more straightforward way. It is because the complicated set of boundary conditions in (4.33), which includes first, second, and third orders derivatives, are now transformed into four simple Dirichlet boundary conditions applying to four unknown functions $\mathbf{u}_1, \mathbf{u}_2, \mathbf{u}_3, \mathbf{u}_4$. To see how boundary conditions are imposed on system (4.40), we express the unknown functions as $4(N+1)$ -vector

$$\begin{aligned} \mathbf{U} &= [\mathbf{u}_1^{n+1} \quad \mathbf{u}_2^{n+1} \quad \mathbf{u}_3^{n+1} \quad \mathbf{u}_4^{n+1}]^T \\ &= \left([u_{1_0}, u_{1_1} \dots u_{1_{N-1}}, u_{1_N}, u_{2_0}, u_{2_1} \dots u_{2_{N-1}}, u_{2_N}, u_{3_0}, u_{3_1} \dots u_{3_{N-1}}, u_{3_N}, u_{4_0}, u_{4_1} \dots u_{4_{N-1}}, u_{4_N}]^{n+1} \right)^T \end{aligned} \quad (4.41)$$

and “zoom in” the system (4.40)

$$\begin{bmatrix}
D_{1,1} & D_{1,2} & \cdots & D_{1,N-1} & D_{1,N} & -1 & 0 & \cdots & 0 & 0 \\
D_{2,1} & D_{2,2} & \cdots & D_{2,N-1} & D_{2,N} & 0 & -1 & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\
D_{N-1,1} & D_{N-1,2} & \cdots & D_{N-1,N-1} & D_{N-1,N} & 0 & 0 & \cdots & -1 & 0 \\
D_{N,1} & D_{N,2} & \cdots & D_{N,N-1} & D_{N,N} & 0 & 0 & \cdots & 0 & -1 \\
\\
D_{1,1} & D_{1,2} & \cdots & D_{1,N-1} & D_{1,N} & -1 & 0 & \cdots & 0 & 0 \\
D_{2,1} & D_{2,2} & \cdots & D_{2,N-1} & D_{2,N} & 0 & -1 & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\
D_{N-1,1} & D_{N-1,2} & \cdots & D_{N-1,N-1} & D_{N-1,N} & 0 & 0 & \cdots & -1 & 0 \\
D_{N,1} & D_{N,2} & \cdots & D_{N,N-1} & D_{N,N} & 0 & 0 & \cdots & 0 & -1 \\
\\
D_{1,1} & D_{1,2} & \cdots & D_{1,N-1} & D_{1,N} & -1 & 0 & \cdots & 0 & 0 \\
D_{2,1} & D_{2,2} & \cdots & D_{2,N-1} & D_{2,N} & 0 & -1 & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\
D_{N-1,1} & D_{N-1,2} & \cdots & D_{N-1,N-1} & D_{N-1,N} & 0 & 0 & \cdots & -1 & 0 \\
D_{N,1} & D_{N,2} & \cdots & D_{N,N-1} & D_{N,N} & 0 & 0 & \cdots & 0 & -1 \\
\\
A & 0 & \cdots & 0 & 0 & Bu_{i_0}^n & 0 & \cdots & 0 & 0 \\
0 & A & \cdots & 0 & 0 & 0 & Bu_{i_1}^n & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\
0 & 0 & \cdots & A & 0 & 0 & 0 & \cdots & Bu_{i_{N-1}}^n & 0 \\
0 & 0 & \cdots & 0 & A & 0 & 0 & \cdots & 0 & Bu_{i_N}^n
\end{bmatrix}
\begin{bmatrix}
u_{i_0} \\
u_{i_1} \\
\vdots \\
u_{i_{N-1}} \\
u_{i_N} \\
u_{2_0} \\
u_{2_1} \\
\vdots \\
u_{2_{N-1}} \\
u_{2_N} \\
u_{3_0} \\
u_{3_1} \\
\vdots \\
u_{3_{N-1}} \\
u_{3_N} \\
u_{4_0} \\
u_{4_1} \\
\vdots \\
u_{4_{N-1}} \\
u_{4_N}
\end{bmatrix}^{n+1}
=
\begin{bmatrix}
0 \\
0 \\
\vdots \\
0 \\
0 \\
0 \\
0 \\
\vdots \\
0 \\
0 \\
0 \\
0 \\
\vdots \\
0 \\
0 \\
Au_{i_0}^n \\
Au_{i_1}^n \\
\vdots \\
Au_{i_{N-1}}^n \\
Au_{i_N}^n
\end{bmatrix}
\quad (4.42)$$

The size of coefficient matrix on the left hand side of equation (4.42) is $4(N+1) \times 4(N+1)$ and the forcing vector on the right hand side has $4(N+1)$ elements.

To impose the boundary conditions, we do the following steps:

- To impose $u_{i_0} = 0$, remove u_{i_0} from the vector $\mathbf{U}$, delete $[3(N+1)+1]$ -th row and $[3(N+1)+1]$ -th column from the matrix, and delete $[3(N+1)+1]$ -th element from the forcing vector.
- To impose $u_{3_0} = 0$, remove u_{3_0} from the vector $\mathbf{U}$, delete $[2(N+1)+1]$ -th row and $[2(N+1)+1]$ -th column from the matrix, and delete $[2(N+1)+1]$ -th element from the forcing vector.
- To impose $u_{2_N} = (\varepsilon/2)\cos t$, remove u_{2_N} from the vector $\mathbf{U}$, delete $2(N+1)$ -th row from the matrix, delete $2(N+1)$ -th element from the forcing vector, and move $2(N+1)$ -th column from the matrix, which is already multiplied with $[-(\varepsilon/2)\cos t]$, to the right hand side (without any other change of sign).
- To impose $u_{i_N} = 0$, remove u_{i_N} from the vector $\mathbf{U}$, delete $(N+1)$ -th row and $(N+1)$ -th column of the matrix, and delete $(N+1)$ -th element of the forcing vector.

The size of coefficient matrix reduces to $4(M+1) \times 4(M+1)$ where $M = N-1$, after imposing boundary conditions, the length of forcing vector reduces to $4(M+1)$, and the unknown function $\mathbf{U}$ has $4(M+1)$ elements. The time-dependent solution is found by performing time stepping (or discrete integration) as follows:

- Start with $n = 0$ at $t = 0$, we have $\mathbf{u}_1^0 = \mathbf{0}$. Solve the system for $\mathbf{u}_1^1, \mathbf{u}_2^1, \mathbf{u}_3^1, \mathbf{u}_4^1$.
- Update $\mathbf{u}_1^n$ in the system with $\mathbf{u}_1^1$, solve for $\mathbf{u}_1^2, \mathbf{u}_2^2, \mathbf{u}_3^2, \mathbf{u}_4^2$. Note that both coefficient matrix and forcing vector need to be updated after each time step.
- Repeat until the solution reaches periodically steady state. The shape of the flagellum is finally obtained by extracting the first $M+1$ elements of $\mathbf{U}$, combined with another zero element at the boundary.

4.1.3.3 Quality of the numerical solver

In the absence of shear flow, the second term in equation (4.32) vanishes and the shape equation becomes

$$16 \frac{\partial^4 u(z, t)}{\partial z^4} + Sp^4 \frac{\partial u(z, t)}{\partial t} = 0, \quad z \in [-1, 1], \quad t \geq 0, \quad (4.43)$$

which can be solved analytically using separation of variables method. Hence, a comparison between analytical and numerical solutions to equation (4.43) would give us some idea about the quality of numerical solver based on Chebyshev collocation method. Figure 4-2 plots the comparison between analytical and numerical solutions to equation (4.43) at three different values of sperm number. The shapes of the flagellum obtained from analytical method and from Chebyshev collocation method are shown on the left side of Figure 4-2. The solid curves represent shapes of the flagellum at different time points in one cycle of oscillation obtained by analytical method, while the scattered

Figure 4-2 Comparison between analytical and numerical solutions of equation (4.43) at (a) $Sp = 2$, (b) $Sp = 7$, and (c) $Sp = 20$. On the left side, solid curves represent shape of the flagellum obtained from analytical method, and scattered curves are from Chebyshev collocation method. The maximum errors between solutions of exact method and Chebyshev collocation method show the quality of the numerical approximation.

curves are from numerical approximation. Here, the spatial domain is discretized with $N = 70$, and time-integration is performed at time step $\Delta t = 0.001$. In all three cases, the maximum relative errors in amplitude between analytical and numerical solutions in one cycle of oscillation, which are shown on the right side of Figure 4-2, do not exceed 6×10^{-5} . This clearly shows the good quality of numerical approximation by Chebyshev collocation method. Furthermore, by obtaining the shape of flagellum at these three values of sperm number (i.e. $Sp = 2$, $Sp = 7$, and $Sp = 20$) in the absence of shear flow, I confirm the results presented by Fu *et al.* [38] for the case of Newtonian fluid.

4.2 Results and Discussion

In this section, I present the shapes of the beating flexible flagellum and the corresponding swimming velocities at various values sperm number and shear rates. I choose to examine the flagellum's shape in the limits $5 \leq Sp \leq 20$ and $-3 \leq Z \leq 3$, which allows us to investigate behaviors of flagella with different levels of flexibility and to study the effects of both negative and positive shear rates on the beating pattern of the flagellum. Figure 4-3 distinguishes between positive and negative shear flow configurations, and this will be useful when I discuss, in the next section, the effects of positive and negative shear rates on the beating pattern of flexible flagellum. Here, at each value of sperm number, I carefully choose ϵ such that the maximum dimensionless amplitude of the beating flagellum does not exceed 0.1 when Z is varying in $[-3, 3]$, in order to satisfy the small amplitude approximation.

Figure 4-3 Schematics of (a) positive and (b) negative shear flow configurations.

4.2.1 Beating patterns of the flexible flagellum

Figure 4-4 shows the shapes of beating flexible flagellum at different values of sperm number and shear rates. The beating pattern of the flagellum is illustrated by the flagellum's shapes at six different time points in one cycle of oscillation. The different line styles, including red solid, blue dotted, green dashed, orange dashed-dotted, purple dashed-dotted-dotted, and black dashed-dashed-dotted, represent the different time sequences (in second) that the flagellum undergoes, in ascending order: zero (beginning

of a cycle), $\pi/3$, $2\pi/3$, π , $4\pi/3$, and $5\pi/3$. According to the definition of sperm number, $Sp = L(\omega k_N / EI)^{1/4}$, any change in sperm number can result from several causes, including changes in total curve length L , beating frequency ω , resistive force coefficient

Figure 4-4 Shapes of beating flexible flagellum at different values of sperm number Sp and shear rates Z . The different line styles represent different time points in one cycle of oscillation.

k_N , or rigidity EI of the flagellum. When discussing the shape of the flagellum in this section, I consider the flexibility of the flagellum as the major factor that causes changes to sperm number.

Comparing the three columns of Figure 4-4 shows that a more flexible flagellum, which has greater value of Sperm number, is more sensitive to shear flow. In the first column, when $Sp = 5$, the change in beating pattern is slight when shear rate is varying. This is because at $Sp = 5$, the flagellum is rigid and strong enough to maintain its beating pattern even at high shear rate. However, when the flagellum is more flexible, $Sp = 12$ and $Sp = 20$, I observe very different beating patterns at high shear rates, comparing to

Figure 4-5 Changes in beating patterns of the flagellum due to positive and negative shear flow. Flagellum with high flexibility is more sensitive to shear and there is significant change in its beating pattern.

the beating pattern at no shear. When there is no shear, the wave propagation mainly happens close to the fixed end, while at strong shear, wave propagation takes places

along the length of the flagellum. This is reasonable because flagellum with higher flexibility is more easily deformed due to external force.

When the flagellum is flexible and therefore its shape sensitive to shear flow, it is interesting to study the physics governing how beating pattern of the flagellum changes with shear rate. In Figure 4-5, I show the shear flow profiles together with the flagellum's beating patterns when relatively rigid ($Sp = 5$) and highly flexible ($Sp = 20$) flagella are exposed to weak ($|Z| = 0.5$) and strong shear ($|Z| = 3$). In general, the beating patterns of the flagellum always have characteristics of a travelling wave in the case of swimming in Newtonian fluid [38]. In the absence of shear, the beating patterns has little characteristics of travelling wave and more characteristics of standing wave, which can be observed from Figure 4-5 at $Z = 0$. The presence of shear, which is mathematically governed by the second term in equation (4.14), amplifies the “travelling” properties of the flagellum's beating pattern. Since the source of beating wave is located at the fixed end of the flagellum, the wave must always travel from the left to the right in Figure 4-5. When the shear rates are positive, flow in the upper half-plane, where $y > 0$, is flowing from the left to the right; consequently, the travelling wave effect “chooses” to occur in the upper half-plane and flagellum is shifted towards the positive y -direction, as clearly shown in Figure 4-5. Note that the travelling wave properties are more significant and noticeable at stronger shear and with a more flexible flagellum. Similar physical argument applies to explain the case of negative shear.

Since the swimmer moves forward by beating its flagellum, any change in the beating pattern of the flagellum would result in changes of swimming behavior. Therefore, it is well expected that swimming velocity is also dependent on sperm number and shear rate, and we will see, in the next section, how swimming velocity responds to changes in sperm number and shear rate.

4.2.2 Velocity of the swimmer

After obtaining the shape of the flagellum, the average swimming velocity in one cycle of oscillation is computed according to equation (4.24). Here, it is more meaningful to study the velocity gain when there are changes in flexibility of the flagellum and strength of shear flow. For this purpose, I normalize the average swimming velocities with the swimming velocity at $Sp = 5$ (most rigid flagellum in the study range) and $Z = 0$ (no

Figure 4-6 Velocity gain at various levels of flexibility and shear strength, compared to the swimming velocity at no shear ($Z = 0$) with most rigid flagellum ($Sp = 5$). The red dot located at $(Sp, Z) = (5, 0)$ has velocity gain equal to 1. The color bar on the right shows range of velocity gain, starting from 0.45.

shear), which is indicated by the red dot at $(Sp, Z) = (5, 0)$ in Figure 4-6. Hence, at this red dot, the velocity gain is 1. At first glance, the velocity gain shown in Figure 4-6 is symmetric about $Z = 0$, indicating that positive and negative shear rates, with the same strength, affect the swimming velocity in opposite directions. This is consistent with the effects of positive and negative shear rates on the flagellum's shape as mentioned earlier,

and suggests a very solid relation between flagellum's beating pattern and swimming velocity.

When moving away from the location $(Sp, Z) = (5, 0)$, we achieve higher velocity gain in general. However, for an exceptional case, if we are traveling along the line $Z = 0$ from low to high Sp , we will find that velocity gain is dropping from 1 to 0.45. To explain this, we revisit Figure 4-4 and look at the beating patterns at different values of Sp when there is no shear. In the absence of shear flow, at high Sp , the wave propagation of the flagellum only occurs in a small portion close to the fixed end of the flagellum, causing a drop in total power that the beating flagellum generates to push the swimmer forward. In particular, at $Sp = 12$, wave propagation mainly happens from $s = 0$ to $s \approx 0.75$; and at $Sp = 20$, this range is even more limited, from $s = 0$ to $s \approx 0.5$. In contrast, at $Sp = 5$, we do not have any range of zero amplitude along the length of the flagellum.

In Figure 4-6, the velocity gain goes very high when we reach the top-right and bottom-right corners of the Sp - Z domain. This suggests higher flexibility and stronger shear are more favorable to the swimming velocity. The physics behind is revealed by examining the beating pattern of the flagellum at these conditions. As mentioned earlier, the beating patterns of the flagellum possess properties of a travelling wave, and the flexibility of the flagellum, together with shear strength, determine whether the beating patterns have more or less characteristics of travelling wave. For example, the beating pattern at $(Sp, Z) = (5, 0)$ has little characteristics of travelling wave and admits more properties of standing wave, while at $(Sp, Z) = (20, 3)$, we clearly see a wave is travelling from the fixed end to the free end of the flagellum over time in one cycle of oscillation. This travelling wave towards the free end of the flagellum boosts the forward velocity of the swimmer. The beating patterns in Figure 4-4 and color gradient in Figure 4-6 also suggest that when the flagellum is more flexible, shear strength has stronger influence

in promoting the travelling wave characteristics of beating pattern and forward swimming velocity of the swimmer. It is interesting that the velocity gain in Figure 4-6 is positive in the whole domain, which means the sign of shear rates never alters the swimming direction.

Figure 4-7 Velocity gain due to increasing flexibility (Sp) at different conditions of shear flow. There exists an optimal shear rate, $Z \approx 0.85$, that maximize the favorable effect of flagellum's flexibility on the swimming velocity

To further investigate collective effect of flexibility and shear strength on the swimming velocity, we look at the velocity gain due to increasing flexibility at different conditions of shear flow in Figure 4-7. At any given shear rate, I compare the velocities at various flagellum's flexibility with the velocity of the most rigid flagellum ($Sp = 5$). Figure 4-7 suggests that Z must be greater than 0.2 to guarantee any increase in flexibility results in faster swimming speed. More interestingly, as observed from Figure 4-7, there exists an optimal shear rate that maximizes the favorable effect of flagellum's flexibility on the swimming. This is exciting when we consider, for example, two swimmers A and B whose swimming are characterized by two different sperm numbers, $Sp^A < Sp^B$. We already knew that, from earlier discussion, swimmer B is swimming faster when Z is

large enough, and the difference in swimming speeds of A and B becomes significantly noticeable when they are exposed to the optimal shear rate, conditioned by $Z \approx 0.85$. Here, it is important to remind that the difference between Sp^A and Sp^B is not only resulted from difference in flagellum's flexibility, but it also comes from the differences in total length of two flagella, swimmers' beating frequencies, and the resistive force coefficients. With this in mind, one can think about using this optimal shear rate to detect differences in characteristics of swimmers by comparing their velocity. Note that in Figure 4-7, I only show positive shear rates because at negative shear rates, we arrive at the same physics due to the symmetric effects of positive and negative shear.

4.2.3 Potential application in sorting and separation

Now we can think of a potential application of the discovered physics, for example in sorting and separation of motile and non-motile sperms in infertility treatments. Most sperm cell separation and sorting devices reported in the literature rely on the behavior of motile sperms under the effect of microfluidic flow: Motile sperms align their primary axis axes with the flow, and they swim upstream in a specific range of flow velocity [89, 90]. As discussed, shear flow could support swimmer's motility and we can utilize this to enhance previous sperm sorting and separation devices. At a favorable shear rate, the motile sperms swim upstream at a higher speed because its beating pattern is enhanced by shear flow, while the non-motile ones are carried downstream; thus we can separate the motile sperms from the non-motile ones in less time. Therefore, this mechanism could improve the efficiency of the existing separation devices.

4.3 Summary

In this chapter, I discussed the swimming of single-flagellated swimmer in shear flow, where the beating and deformation of the flagellum is assumed to occur in a plane. I

investigated the beating patterns of the flagellum at various levels of flagellum's flexibility and shear strength, and the velocity generated by these beating patterns. Shear flow positively affects the swimming velocity by causing the flagellum to deform in more favorable patterns to the velocity; however, the flagellum must be flexible enough in order to allow the shear effect to take place. The physical mechanism of how shear flow alters the beating patterns was also established and discussed throughout. Furthermore, there is an optimal shear rate that maximizes the effect of flexibility on swimming speed. In any case, the swimming velocity is resulted from the physical interaction between shear flow and flagellum's flexibility, and it is essentially determined by the beating pattern of the flagellum. Our model showed that shear flow could be utilized to detect difference in characteristics of swimmers and to improve some existing sorting devices. The current model is limited to small amplitude deformation of the flagellum; however, this is necessary to avoid a too complicated mathematical model and, more importantly, it does not break the nature of physical link between shear flow and beating pattern of the flagellum. Therefore, I believe the analysis in this chapter is a strong foundation for further theoretical and experimental investigations.

Chapter 5

Experimental analysis of bacterial flagella's morphology

In this chapter, I present an experimental work to quantify the morphological characteristics of bacterial flagella, which include length, pitch, and radius. The bacteria are injected to a microchannel and the experiments are conducted with and without flow in order to detect the response of bacteria to external flow. The bacteria used in our experiments are *B. subtilis*, strain DK2187 [6], which is a smooth-swimming strain and already mutated to allow the bacterial flagella to be fluorescently stained. The fluorescent staining of the bacterial flagella is necessary to make them visible under fluorescent microscopy, since they are unobservable in bright field mode. After acquiring microscopy images of the bacteria, the images of bacterial flagella are manually digitalized in preprocessing step and the morphological information of the flagella are automatically extracted from the preprocessed images using MATLAB scripts.

5.1 Experimental methodology

5.1.1 Bacteria culturing

I received the *B. subtilis* (strain DK2187) from Professor Kearns, Daniel B. [6] as a dried spore on a Whatman[®] filter paper. The bacteria were activated by wetting the dried spore with Lysogeny broth (LB) medium with 100 $\mu\text{g ml}^{-1}$ spectinomycin (from Sigma-Aldrich[®]) and placed it on fresh agar plate, incubated overnight at 30 °C. After that, the bacteria were streaked off the agar plate using sterile inoculating loop and transferred to

5 ml of liquid medium (Cap Assay Minimal with $100\ \mu\text{g ml}^{-1}$ spectinomycin) for growing for 6 hours at $37\ ^\circ\text{C}$, shaking at 250 revolutions per minute (rpm). Then, the frozen stocks were made by well mixing 300 μl of sterile glycerol 50 % to every 450 μl of liquid culture, and freezing at $-80\ ^\circ\text{C}$. The frozen stocks should remain in good condition for years.

Prior to each experiment, a small amount of bacteria is transferred from frozen stock to 5 ml of liquid medium using sterile inoculating loop and grown at $37\ ^\circ\text{C}$, shaking at 250 rpm in 6 hours. After that, bacteria are streaked on an agar plate and incubated overnight at $30\ ^\circ\text{C}$ in order to form isolated single colonies. Then, one single colony of bacteria is streak off the agar plate and transferred to 5 ml of liquid medium using sterile inoculating loop, and grown for 6 hours at $37\ ^\circ\text{C}$, shaking at 250 rpm. After 6 hours, the bacterial suspension is ready for flagella fluorescent staining.

5.1.2 Bacteria fluorescent staining

To stain the flagella of the bacteria, 2 ml of bacteria suspension is centrifuged at 3000 rpm in 2 minutes to remove the medium from the bacteria. After that, 50 μl of liquid medium containing $0.1\ \mu\text{g}\ \mu\text{l}^{-1}$ Alexa Fluor[®] 488 C₅ Maleimide (A10254 from ThermoFisher Scientific) is added to the bacterial sedimentation and incubated at room temperature in dark for 10 minutes to allow the staining reaction to take place. Then, the bacteria suspension is washed three times in 1 ml of liquid medium to remove free dyes from the suspension. The final bacterial suspension (1 ml) is diluted fivefold to reduce the background noise when observing under fluorescent microscope.

5.1.3 Experimental setup

Figure 5-1 shows the experimental setup for the observation of bacterial flagella's behavior during swimming in a microchannel. The microchannel, as shown in Figure

5-1a, has high aspect ratio cross sectional area, i.e. $W \gg H$, and the velocity of fluid flow in such channel admits a parabolic distribution

$$u(y) = (3U_F/2)[1 - (2y/H)^2], \quad (5.1)$$

where U_F is the mean flow speed, and $y = 0$ is the mid-depth of the microchannel [57].

Such parabolic fluid flow generates simple shear determined by

$$\dot{\gamma} = du/dy = -12yU_F/H^2. \quad (5.2)$$

The microchannel is fabricated by soft lithography technique [101], which has dimensions $W \times H \times L = 1000\mu\text{m} \times 100\mu\text{m} \times 20\text{mm}$, where L is the length of the microchannel. In Figure 5-1b, the glass side has thickness of 0.17 mm to meet the requirement of oil immersion objective using in this experiment.

Experiment is set up as shown in Figure 5-1b. Bacterial suspension, which contains fluorescently stained bacteria, is transferred into a 1ml Hamilton glass syringe, and the syringe is loaded into a syringe pump (KD Scientific Legato 210P). The syringe is connected to the inlet of the microchannel by a plastic tube. The flow velocity introduced into the microchannel is controlled by adjusting the flow rate generated by the syringe

Figure 5-1 (a) Schematic of a microchannel and flow profile in the channel. The channel has high aspect cross sectional ratio, i.e. $W \gg H$, that generates parabolic flow profile. (b) Experimental setup for images acquisition.

pump according to the formula $U_F = Q/6$, where U_F is measured in $\mu\text{m s}^{-1}$ and Q is the flow rate input to the syringe pump and being measured in nl min^{-1} , given the cross section of the microchannel is $W \times H = 1000\mu\text{m} \times 100\mu\text{m}$. The shear rate at any value of U_F is then calculated according to equation (5.2).

The swimming bacteria in the microchannel were observed under an inverted microscope (Zeiss AxioObserver.Z1) equipped with a high speed, high sensitive CCD camera (Rolera EM-C², QImaging) operating in Easy EMGain mode. Images were acquired using a 63 $\times$ objective (Zeiss Plan-Apochromat 63 $\times$ /1.40 Oil DIC) and Optovar 1.6 $\times$, which yields a total magnification of 100.8 $\times$. The images were captured with exposure time 10 ms, pixels binning 2 $\times$ 2. This configuration yields images resolution of 502 $\times$ 501 pixels and the pixel size is 0.159 $\mu\text{m} \times$ 0.159 μm .

5.1.4 Images processing

Figure 5-2 shows a microscopic image of bacterial flagella. The bacterial cells' bodies are not visible in the image because the dyes used in this experiment do not stain the cells' bodies, therefore cells' bodies do not reflect light. The bacterial flagella can either stay isolated in the case of a single flagellum or attach together to form a flagellar bundle, as shown in Figure 5-2.

The most challenging task of the images processing is to detect and extract the image of each flagellum from the acquired microscopic images. Since the flagella have no specific pattern and their shapes vary from one to another, recognition and extraction are not practical without human intervention. Therefore, a manual processing step to extract the image of each flagellum is necessary from the original images. After image extraction of flagella, each image that contains one single flagellum is automatically processed by MATLAB script to extract morphological information.

Figure 5-2 A microscopic image showing single bacterial flagellum and bundle of flagella.

5.1.4.1 Manual recognition and extraction of flagellum image

The manual recognition and extraction of the flagella are done by tracing each flagellum using an Intuos Pen Tablet (Wacom®). An advantage of manual recognition and

Figure 5-3 (a) (b) Tracing and (c) extraction of a flagellum image. The extracted image is binary and free of background noise.

extraction is that non-flagellum objects, which can be accidentally recognized as flagella in automatic method, are completely removed from the data, and therefore noise due to misrecognition is avoided. Furthermore, after being traced, the flagella images are saved as binary (black and white), and therefore the background noise in the original images are completely removed.

Figure 5-3 shows the tracing and extraction of a flagellum image from the original one. It is observed from Figure 5-3 that not every flagellum's image is ideal for tracing because some of them are broken into parts. Such images of flagella must be excluded when tracing in order to avoid introducing errors to the data, and this task can only be achieved with the human intervention. The extracted images are free of noise and are ready for the next stage of processing.

For the determination of flagellar roots and tips as well as their orientation during images processing, a flagellum might be rotated after tracing such that its root is always on the left hand side compared to the tip. Figure 5-4a shows four flagella at four different orientations in xy -plane, where the filled dots indicate the roots of the flagella. The four angles $\alpha_1, \alpha_2, \alpha_3$ and α_4 are undetermined at the tracing step, in which α_1 and α_2 are positive, α_3 and α_4 are negative. The roots of flagellum 1 and flagellum 4 already point to the left hand side, therefore no rotation is required. After tracing, flagellum 2 and flagellum 3 are rotated 90 degrees clockwise and counterclockwise, respectively, in order to make their roots on the left hand side, as shown in Figure 5-4b. The orientations of flagellum 2 and flagellum 3 after being rotated are α_2' and α_3' . The four angles $\alpha_1, \alpha_2', \alpha_3'$ and α_4 are determined during the analysis of extracted images of flagella using MATLAB scripts. The original orientations of the four flagella are determined by $\alpha_1, \alpha_2' + 90^\circ, \alpha_3' - 90^\circ$ and α_4 .

Figure 5-4 Rotation of flagella during tracing to make their roots on the left hand side. (a) Original orientations of four flagella. (b) Flagella are selectively rotated based on their original orientation.

5.1.4.2 Morphological information extraction

The morphological information is extracted from the flagella images using MATLAB® Images Processing Toolbox. First, each flagellum image is read into memory as a rectangular logical matrix, whose elements are either 0 (low) or 1 (high). The shape of the flagellum is determined by positions of 1s (white) in the matrix, while the 0s (black) determine background (Figure 5-5a). The orientation angle (α_1 , α'_2 , α'_3 or α_4 in Figure 5-4b) is determined by querying the property `Orientation` via the function `regionprops` in MATLAB® Image Processing Toolbox. After having the orientation angle, the flagellum is re-oriented such that its major axis parallel to the x -axis, as shown in Figure 5-5b. Note that after being reoriented, the root of the flagellum in Figure 5-5b is always on the left hand side.

The reoriented flagellum image in Figure 5-5b allows us to represent the shape of the flagellum by a mathematical wave-like curve. The column and row indices of each point along the flagellum are converted to x - and y -coordinates of a discrete

Figure 5-5 (a) Image of a flagellum be imported to MATLAB as a logical matrix; (b) The flagellum image after being reoriented to be parallel with horizontal axis; (c) The flagellum and its mathematical representation (the red curve).

mathematical curve $y = f(x)$ at that point (Figure 5-5c), and morphological information is obtained by geometrically analyzing the curve $y = f(x)$. Morphological information of a flagellum includes length, pitches, diameters, and orientation angle. The length of the flagellum is obtained by integrating $y = f(x)$ from the root to the tip of the flagellum. Given that every flagellum has wave-like shape, the pitches and diameters are obtained by locating of local maxima and local minima of the curve $y = f(x)$, which are shown in Figure 5-6 by red dots and blue dots, respectively. After having the locations of the local maxima and minima of the flagellum, a pitch length is defined by the horizontal distance between two consecutive maxima or between two consecutive minima, as shown in Figure 5-6 by λ_1 and λ_2 , respectively. The number of pitches of a

flagellum depends on its waveform; for example, the flagellum shown in Figure 5-6 has three maxima and four minima, thus it has five pitches in total. A diameter of the flagellum is defined by the vertical distance between two consecutive extrema; for example, d_1 and d_2 in Figure 5-6. The number of diameters along the flagellum also depends on the number of extrema. For the flagellum in Figure 5-6, there are six

Figure 5-6 Morphological information of a flagellum obtained by geometrical analysis of the curve $y = f(x)$. The red dots are local maxima and the blue dots are local minima of the curve.

diameters in total. Considering multiple pitches and diameters, as just mentioned, allows us to quantify the morphology of the flagellum along its length, from the root to the tip. This is helpful in analyzing the behaviors of parts the flagellum in relation to their distances measured from the root of the flagellum.

The local maxima (red dots) and the local minima (blue dots) of the curve $y = f(x)$ are determined by employing the function `findpeaks` from MATLAB® Signal Processing Toolbox. The function `findpeaks` returns the locations of local maxima of the signal, which is the shape of flagellum in our case, and therefore it can be directly applied to find the locations of red dots in Figure 5-6. To find the x -locations of the blue dots, the curve $y = f(x)$ is vertically flipped and input to `findpeaks`. The y -locations of blue dots are then obtained with the knowledge of their x -locations and $y = f(x)$.

5.1.5 Error analysis

Errors in our measurement include systematic errors and random errors. Systematic errors arise in all measurements in the same manner due to precision of instruments, data acquisition and processing techniques, etc.; while random errors are unpredictable and can be revealed by repeating the measurements [102].

5.1.5.1 Estimation of experimental uncertainties

For any quantity x that contributes to the determination of flagellar morphology, the measurements are repeated N times. The best estimate of x is determined by

$$\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i, \quad (5.3)$$

and the average uncertainty of $x_1, x_2, \dots, x_N$ is determined by the standard deviation

$$\sigma_x = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}. \quad (5.4)$$

Assuming the quantity x obeys the normal distribution, I can be confident that approximately 68% of our measurements will be in the range $x_{\text{true}} \pm \sigma_x$, where x_{true} is the true value of x [102].

If the system error of the experiments is δx_{sys} , the final measured result of x obtained from N measurements $x_1, x_2, \dots, x_N$ is given by

$$(\text{value of } x) = \bar{x} \pm \delta x_{\text{total}} = \bar{x} \pm \sqrt{\delta x_{\text{sys}}^2 + \delta x_{\text{ran}}^2}, \quad (5.5)$$

where δx_{total} is the total uncertainty of the best estimate $\bar{x}$, and $\delta x_{\text{ran}} = \sigma_x$ is the random error. In our experiments, one potential source of error is the flagella tracing. In the acquired images, the thickness of the flagella is approximately 4 pixels; therefore, if the flagella are carefully traced, the error due to tracing is not larger than 2 pixels, and this is considered as part of random error. The systematic error is

$\delta x_{\text{sys}} = 0.159/2 \approx 0.08 \mu\text{m}$, which is equal to half of the size of one pixel in the acquired images.

5.1.5.2 Conversion of units

The images processing requires the conversion of units from pixel to micrometer. In our experiment, the acquired images, which were exported from Zen acquisition software (Zeiss), have the resolution of 72 dpi (dot per inch). In order to improve the accuracy of morphological information extraction in MATLAB, I saved the traced images of flagella at the resolution of 300 dpi. Therefore, the conversion of units from pixel to micrometer is given by

$$x_{(\text{in } \mu\text{m})} = 0.159 \times \frac{72}{300} \times x_{(\text{in pixel})} = 0.03816 \times x_{(\text{in pixel})} . \quad (5.6)$$

Applied to measurement results, we have

$$(\text{value of } x \text{ in } \mu\text{m}) = 0.03816 \times \bar{x}_{(\text{in pixel})} \pm \sqrt{\delta x_{\text{sys}}^2 + (0.03816 \times \delta x_{\text{ran (in pixel)}})^2} . \quad (5.7)$$

5.1.5.3 Effect of the depth of field

In microscopic system, an object is in focus when it is axially located within the depth of field, and that object appears sharp in the acquired image. In Figure 5-7, δz is the

Figure 5-7 Effect of the depth of field δz . The blue and green rods are wholly in focus while the red one is partially out of focus.

depth of field and any object axially located in this range will be sharply captured. Imagine we have three rods, blue, green and red ones, that have the same length l being captured using an objective as shown in Figure 5-7. The blue rod is wholly located within the depth of field and parallel with the focal plane; therefore, its length is fully and accurately captured as $l_1 = l$. The green rod is also wholly located within the depth of field; however, because it is not parallel with the focal plane, the captured length of the green rod is $l_2 = l \cos \beta_2$, which is shorter than its true length, and we know that l_2 is the length of the projection of the rod on the focal plane. In the case of the red rod, however, the angle between the rod and the focal plane is too large; consequently, part of the rod is out of focus and the captured length of the red rod is $l_3 = l \cos \beta_3$, which is neither the true length of the rod nor the length of its projection on the focal plane. The same situation happens to the flagella when I am performing images acquisition. A flagellum can be wholly or partially located within the depth of field; but it is not possible to know, unfortunately, which case applies during experiments. In such situation, we can assume that the captured length l of a flagellum is the projection of its true length on the focal plane, and the more appropriate length should be

$$L = l / \cos \beta, \quad (5.8)$$

where $\beta = \tan^{-1}(\delta z / l)$ and δz is the depth of field given by [103]

$$\delta z = \frac{n \lambda_0}{NA^2} + \frac{n e}{NA M}. \quad (5.9)$$

In equation (5.9), n is the refractive index of the oil applied on the objective, λ_0 is the wavelength of light, NA is the numerical aperture of the objective, M is the total magnification, and e is the smallest distance detected by the CCD camera. In our system, $n = 1.518$, $\lambda_0 = 0.488 \mu\text{m}$, $NA = 1.4$, $M = 100.8$, and $e = 8 \mu\text{m}$ yield $\delta z \approx 0.46 \mu\text{m}$. It is necessary to note that the use of equation (5.8) can help the measured length closer to

the true value but it does not improve the errors due to flagella that are partially out of focus. Equation (5.8) also applies to the pitch lengths of the flagella, i.e. $\Gamma = \lambda / \cos \beta$, where λ is the measured pitch from the acquired images and Γ is the improved value of the pitch.

5.2 Experimental Results and Discussion

5.2.1 Performance of images processing by MATLAB script

Here, I present the visual results of images processing by MATLAB script. Figure 5-8 shows 10 flagella images randomly selected from the data for the visualization of images

Figure 5-8 Visual comparison between traced flagella (binary images) and their mathematical representations (red curves) shows good performance of images processing script. The script is also successful in determine locations of extrema of the curves. Note that images of flagella shown above have different plotting scales.

processing outputs. The binary (black and white) images in Figure 5-8 are the traced images of flagella being imported to MATLAB. The red curves are the mathematical curves $y = f(x)$ representing the flagella. The figures confirm that our script can accurately reproduce the shapes of the flagella as mathematical functions, and the errors are less than the thickness of traced flagella (~ 3 pixels). Our script is also successful in detection of extrema of the curves, as shown in Figure 5-8, which gives high confidence in the determination of pitches, radii, and length of the flagella using automated script.

5.2.2 Morphology of flagella under no flow conditions

In this part I present the morphological properties of *B. subtilis* flagella at no flow condition. Experiments were repeated five times with five different cultures of bacteria being grown at the same condition, and the sample size in each experiment is 310 flagella. Figure 5-9 shows the mean length of the flagella in five sets of data. Over the five sets of experiments, the mean length ranges from $6.32\ \mu\text{m}$ to $6.70\ \mu\text{m}$, with standard deviation ranging from $1.12\ \mu\text{m}$ to $1.31\ \mu\text{m}$ (Table 5-1). The insignificant difference

Figure 5-9 Mean length of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.

Figure 5-10 Mean diameter of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.

between the lowest mean and the highest one among five sets of experiment suggests the robustness of our experiments, data acquisition and processing methods.

Figure 5-10 shows experimental results for average diameters of the flagella. As mentioned earlier, the morphology of the flagella is analyzed by finding locations of their extrema and calculating multiple diameters along the flagella. The average

Figure 5-11 Mean helical pitch angle of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.

diameter of a flagellum is determined by taking the average of all diameters along its length. Among the five sets of experiment, the mean of average diameters ranges from 0.45 μm to 0.53 μm , and the standard deviation ranges from 0.09 μm to 0.11 μm (Table 5-1).

Figure 5-12 Mean pitch length of the flagella over five sets of experiment. The lengths of the error bars are two times the standard deviation.

The average pitch lengths of the flagella are obtained in the same manner with obtaining average diameter. The results for the average pitch lengths are shown in Figure 5-12, where we have mean average pitch length ranges from 2.36 μm to 2.44 μm , and the standard deviation ranges from 0.22 μm to 0.38 μm (Table 5-1) over the five sets of experiment. The results of average helical pitch angle of the flagella are shown in Figure 5-11. The pitch angle is measured by the angle between horizontal axis and the segment that connects two consecutive extrema. Experimental results show that the mean average helical pitch angle falls into the range 19.6 degree to 24.6 degree, where the standard deviation ranges from 2.3 degree to 3.4 degree over five sets of experiment. The morphological parameters of *B. subtilis* flagella are summarized in Table 5-1, combining

all five sets to form a total sample of size $N = 1550$. Our measurement results show that the curve length of the flagella is $6.48 \pm 1.25 \mu\text{m}$, the average pitch length is $2.39 \pm 0.31 \mu\text{m}$, the average diameter is $0.49 \pm 0.14 \mu\text{m}$, and the average helical pitch angle is 22.0 ± 3.2 degree. From the curve length and the average helical pitch angle, the average axial length can be estimated by $6.48 \times \cos(22^\circ) = 6.01 \mu\text{m}$, and the results well agree with previous studies where the flagella of *B. subtilis* (strain OI1085) were reported to have axial length of $6 \mu\text{m}$ and diameter of $0.48 \mu\text{m}$ [56, 104]. The knowledge of flagella length, diameter, pitch length and pitch angle is specifically important in modeling the bacterial flagellum as a helix.

Table 5-1 Morphological properties of *B. subtilis* flagella.

Mean value	Standard deviation	Total error	Final measurements results
Curve length (μm)			
6.48	1.25	1.25	6.48 ± 1.25
Average pitch length (μm)			
2.39	0.30	0.31	2.39 ± 0.31
Average diameter (μm)			
0.49	0.11	0.14	0.49 ± 0.14
Average helical pitch angle (degree)			
22.0	3.2	3.2	22.0 ± 3.2

5.2.3 Morphology of flagella under flow conditions

In this part I present the experiment results and discuss the morphological changes in bacterial flagella when they are subjected to flow. Sample microscopic images of

Figure 5-13 Measurable and unmeasurable pitches of a flagellum. When a flagellum has one measurable pitch, its axial length might be longer than the only measurable pitch.

bacteria in flow are shown in Figure 5-14. According to our measurement as mentioned earlier, each flagellum has multiple values of pitch and diameter, and the number of pitches and diameters depends on the waveform the flagella. Table 5-2 and Table 5-3 show the number of measurable pitches and diameters of the flagella. Here, the term “measurable pitch” refers to pitch that is measured by the method described earlier. The difference between “measurable” and “unmeasurable” pitches is shown in Figure 5-13.

Figure 5-14 Sample images of bacteria under flow conditions. The images also show some flagella that are partially out of focus.

Note that when a flagellum has only one measurable pitch, its axial length is not necessarily the same as the only pitch length.

Table 5-2 Number of measurable pitches of the flagella at different flow rates

Minimal number of measurable pitches	$Q_0 = 0$ ($N = 1550$ flagella)		$Q_1 = 120 \text{ nl min}^{-1}$ ($N = 217$ flagella)		$Q_2 = 240 \text{ nl min}^{-1}$ ($N = 205$ flagella)	
	Number of flagella	Percentage	Number of flagella	Percentage	Number of flagella	Percentage
1	1550	100 %	217	100 %	205	100 %
2	1384	89 %	194	89 %	182	88 %
3	622	40 %	83	38 %	69	34 %
4	217	14 %	33	15 %	27	13 %
5	63	4 %	10	5 %	6	3 %

Table 5-3 Number of measurable diameters of the flagella at different flow rates

Minimal number of measurable diameters	No flow ($N = 1550$ flagella)		120 nl/min flow rate ($N = 217$ flagella)		240 nl/min flow rate ($N = 205$ flagella)	
	Number of flagella	Percentage	Number of flagella	Percentage	Number of flagella	Percentage
1	1550	100 %	217	100 %	205	100 %
2	1550	100 %	217	100 %	205	100 %
3	1384	89 %	194	89 %	182	88 %
4	622	40 %	83	38 %	69	34 %
5	217	14 %	33	15 %	27	13 %
6	63	4 %	10	5 %	6	3 %

In Table 5-2 and Table 5-3, the number of analyzed flagella drops under flow conditions due to images quality issue. When flow is applied, there are less traceable flagella in the acquired images because fluid flow promotes the diffusion of free fluorescent dye particles and causes the stained dyes detached from the flagella, and consequently reduces signal to noise ratio of the flagella images. This is the major difficulty I face in our experiment. Free dyes in the bacterial suspension can be further removed by washing more times (by centrifugation) after staining; however, this also

washes out the stained dyes and reduces the signal intensity. The same problem arises if one tries to reduce the amount of fluorescent dyes used for staining. Thus, the quality of images acquired in the presence of fluid flow strongly depends on the balance among many factors, and this needs to be improved for future development of the experiment.

Based on the results from processed images of flagella, as shown in Table 5-2 and Table 5-3, fluid flow does not affect the general waveform of flagella significantly. This is observed from comparing the percentage of flagella that have the same minimal number of pitches and diameter at different flow rates. Data shown in Table 5-2 and Table 5-3 also suggest that most helical flagella of *B. subtilis* have three turns in average.

Figure 5-15 and Figure 5-16 show multiple pitches and diameters along the flagella at different flow rates $Q_0 = 0$, $Q_1 = 120 \text{ nl min}^{-1}$, and $Q_2 = 240 \text{ nl min}^{-1}$. With the current experimental setup, the quality of acquired images is best when the bacteria have vertical positions ranging from 0 to 10 μm measured from the bottom of the microchannel. Therefore, according to equation (5.2), the flow rates Q_1 and Q_2 generate shear rate ranges as S_1 from 0.96 s^{-1} to 1.2 s^{-1} and S_2 from 1.92 s^{-1} to 2.4 s^{-1} . In Figure 5-15, we can see flow rates Q_1 and Q_2 have insignificant effect on pitch length of the

Figure 5-15 Pitches of the flagella at different flow rates. The lengths of the error bars are two times the standard deviation.

Figure 5-16 Diameters of the flagella at different flow rates. The lengths of the error bars are two times the standard deviation.

flagella at the locations close to their roots. However, the changes in pitches are more noticeable at locations closer to the tips of the flagella. A similar trend also occurs in the diameters of the flagella, as shown in Figure 5-16, where we observed more significant changes in the diameters of the flagella at locations closer to their tips. These

Figure 5-17 Pitch angles of the flagella at different flow rates. The lengths of the error bars are two times the standard deviation.

observations suggest the cells body plays certain role in determining the changes in flagellar shapes due to flow. A possible hypothesis is that the presence of the cells' body blocks the flow in some manner and reduces the influence of the flow on the nearby parts of the flagella. Furthermore, comparing Figure 5-15 and Figure 5-16 suggests that diameters of the flagella are more sensitive to the external flow. If there are changes in diameters of the flagella due to flow while their pitches are insignificantly affected, there must be changes in the pitch angles because for a helix with fixed curve length, diameter, pitch, and pitch angle of a helix are closely related to others. The changes in pitch angles of the flagella are shown in Figure 5-17. Again, pitch angles that are closer to the tips of the flagella are more significantly affected by flow. Different behaviors of pitches, diameters, and pitch angles at different regions of the flagella suggest that the bacteria might be able to maintain the shapes of their flagella in the region close to the flagellar motors.

5.3 Summary

In this chapter, I presented the experimental work performed to study the *B. subtilis* flagellar morphology and behaviors in flow. The bacteria flagella were fluorescently stained and images of flagella were captured using a fluorescent microscope. The acquired microscopic images of flagella were manually traced to remove background noise, and flagellar morphological information was extracted from the traced images in subsequent images processing steps. Although manual tracing of the flagellar images is time consuming and demands high level of carefulness, it yields excellent extracted images of flagella that are completely free of background noise while avoiding non-flagella objects in the original images.

By analyzing the images of flagella at no flow conditions, I studied the morphological parameters of the helical flagella such as pitch length, radius, pitch angle,

and curve length. For *B. subtilis*, the curve length of the flagella is $6.48 \pm 1.25 \mu\text{m}$, the average pitch length is $2.39 \pm 0.31 \mu\text{m}$, the average diameter is $0.49 \pm 0.14 \mu\text{m}$, and the average helical pitch angle is 22.0 ± 3.2 degree. I also exposed the bacteria to external flow to investigate their behavior due to shear. I observed that the tips of the flagella are more sensitive to the external flow. Possible explanations to this are that (1) the bacteria are able to maintain their flagellar shapes at the region close to the flagellar motor, and (2) the presence of the cells body reduces the effects of flow on the region of flagella close to the cells body.

I believe the proposed experimental methodology represents a valuable contribution to the investigation of bacterial flagellar morphology. Since motion of bacteria, in many cases, is originated from the rotating of helical flagella, a good knowledge about flagellar morphology is essential to study of bacterial swimming, and a detailed analysis of bacterial morphological behaviors is important to study how bacteria react to external stimuli.

Chapter 6

Conclusion and Future work

6.1 Conclusion

To conclude, external forces and shear flow influence the behavior of swimming bacteria. The effects of DEP force on the bacterium depend on the correlation between the forces acting on the bacterium's head and tail. If the DEP force exerted on the head is stronger than that on the tail, the bacterium is eventually aligned with the force's direction. However, when the DEP force on the tail is dominant, the steady orientation of the bacterium depends on its initial orientation. Rotational diffusion also contributes to the reorientation of the bacterium, and the rotational diffusion has higher impact when the DEP force's strength varies stronger with Y -position. More interestingly, I found that the interaction between the helical shape of the flagellum and the variance of DEP force in Y -direction cause the bacterium to translate perpendicular to the direction of DEP force. I also discussed how different components of DEP force can be combined to achieve bacterial focusing and proposed calculation steps for the design of electrodes that can generate the desired DEP force field.

When the flagella are flexible, shear flow has significant influence on its beating pattern and swimmer's velocity. For a swimmer with 2D beating flagellum, I found that shear flow could positively promote the swimming speed when the flagellum is flexible enough to allow the effect of shear to take place. Shear supports the swimming speed of the swimmer by affecting the beating patterns of the flagellum such that the flagellum waveform admits more characteristics of a "travelling wave" (wave that is "travelling" from the root to the tip of the flagellum), as a result generating more power to push the swimmer forward. There exists an optimal shear rate that maximizes the effect of

flagellum's flexibility on the swimming speed, and such mechanism could be employed to detect differences in flagellum's flexibility among different swimmers. I also showed that our findings could be applied to enhance performance of existing sorting devices.

On the experimental aspect, I proposed a methodology for characterizing the morphology of bacterial flagella in great detail. Our method can measure length, pitch, diameter, and pitch angle of helical flagella. The flagella of *B. subtilis* have curve length of $6.48 \pm 1.25 \mu\text{m}$, diameter of $0.49 \pm 0.14 \mu\text{m}$, pitch length of $2.39 \pm 0.31 \mu\text{m}$, and pitch angle of 22.0 ± 3.2 degree. Our experiment setup allowed us to analyze the shape of bacterial flagella at different shear strength, I found that the regions close to the tips of the flagella are more sensitive to shear. Our hypothesis is that the presence of the cells body of the bacteria block the flow and therefore reduces the effect of flow on the nearby region of the flagella; and that the bacterial flagella are able to maintain their shapes in the regions close to the cells body. At the current stage our results are still limited to low flow rates ($< 250 \text{ nl min}^{-1}$); nevertheless, I believe this study has great contribution to the characterization of the flagellar morphology as well as the analysis of flagellar behaviors in flow.

6.2 Future work

I consider this project can be further developed and improved on both theoretical and experimental aspects. At the moment, I am in the process of developing a theoretical model to predict changes in the shape of a helical flagellum in shear flow and putting effort to improve our current experimental setup.

The frame work for theoretical model has been proposed in Chapter 4 when I were considering two-dimensional flagellum. One way to implement the proposed method in three-dimensional helical flagellum problem is to decompose the three-dimensional

flagellum into two simpler ones by projecting the helical flagellum onto two planes. Thus, instead of directly considering three-dimensional geometry, I solve for two coupled two-dimensional problems. The major challenge of this approach is that I have to deal with very large linear system (double in size of the system in two-dimensional problem) and therefore time-stepping might be very slow. The theoretical models could be also improved by considering large amplitude deformation of the flagella, in which I expect a very highly mathematical demanding.

On the experimental aspect, I am trying to improve the quality of image acquisition. The quality of the images is highly dependent on the amount of free fluorescent dye in the bacterial suspension, and I aim to reduce it as much as possible, without affecting the signal to noise ratio of the images. The quality of images when applying flow rates is also affected by the flow itself. Strong flow rates wash the dyes off the flagella rapidly and significant reduce the signal to noise ratio of the images. One approach to obtain high shear rates without introducing strong flows is to reduce the height of the microchannel; however, this could strengthen the effects from the walls of the microchannel on the bacteria. In short, the improvement in our experiment requires a good balance among many factors and needs a high number of trial tests.

Appendix: Bacteria growing protocols

Growing *B. subtilis* from Spores on an agar plate

To grow strain from spores that are on a sterile filter paper, just cut a piece of the filter paper and place it on an agar plate, and let it grow overnight at 30 °C. Alternatively, you can wash the spores off the filter paper with sterile water, and then spread the water on an agar plate, and let it grow overnight at 30 °C.

Growing *B. subtilis* in liquid medium from an isolated colony

Culture medium: LBr or Cap Assay Minimal

After having the bacteria growing on an agar plate overnight, pick a single isolated colony from the plate and grow in 5 ml culture medium at 37 °C, shaking at 250 rpm.

The culture should be in exponential phase in around 6 hours.

Culture medium

Lbr

10 g Tryptone

5 g Yeast Extract

5 g NaCl Dissolve in 1 L water

Autoclave

Cap Assay Minimal (1L)

50 mM KPO₄ (6.805 g KH₂PO₄ + 8.71 g K₂HPO₄) pH=7

1 mM MgCl₂ (0.203 g MgCl₂·H₂O)

1 mM (NH₄)₂SO₄

1:1000 Sporulation Salts

Autoclave

For every 20 ml, add:

60 µL HMT (5 mg/mL each of Histidine, Methionine and Tryptophan, filter sterilized)

200 µL TBr

200 µL 1M Sorbitol (filter sterilized)

TBr

10 g Tryptone

5 g NaCl

Dissolve in 1 L water

Autoclave

1X Sporulation Salts

0.14M CaCl₂

0.01M MnCl₂

0.20M MgCl₂

Preparing Glycerol stocks

First grow the bacteria in liquid medium in around 6 hours. For every 3 ml of liquid culture, add 900

µl of

glycerol 50%. Freeze at -80 °C.

Growing bacteria from frozen glycerol stock

Transfer a small amount of the bacterial stock to 5 ml liquid medium (using sterile inoculating loop) and grow at 37 °C, shaking at 250 rpm. Normally just one drop of stock is enough. Alternately, streak out bacterial stock on an agar plate and grow overnight at 30 °C. Do it fast to prevent the stock from thawing.

Preparing agar plate (LB Agar)

Follow the instruction of the agar powder manufacturer for how much powder to be dissolved in 1 L of DI water.

Autoclave at 121°C in 15 minutes (using “Agar” mode of the autoclave machine).

Quickly distribute the warm liquid agar (at 60 °C) to petri dishes before it solidifies. 1 L of agar can be well distributed to 20 petri dishes (60 mm × 15 mm in sizes). Let the agar solidify in petri dishes, after that store them at 4 °C.

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