Swimming characteristics of gyrotactic microorganisms in low-Reynolds-number flow: *Chlamydomonas reinhardtii*

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Abstract The swimming characteristics of gyrotactic microorganisms are significant to understand the ecological activities in lakes, rivers and oceans. The swimming velocity of a typical motile microorganism, *Chlamydomonas reinhardtii*, was measured for both still water and low-Reynolds-number flow, based on a microfluidic system. Results show that the swimming speed is subject to Gaussian distribution for the still water, and corresponding mean swimming speed is 41 $\mu\text{m/s}$. The streamwise mean swimming velocity, 35 $\mu\text{m/s}$, in the moving water is slightly less than that in the still water. It is also shown that the swimming direction in the horizontal plane is dominated by cell randomness for the still water, and 80% of the cells are aligned with the ambient flow when the flow velocity exceeds 333 $\mu\text{m/s}$. The standard deviation of swimming direction and the percent of swimming direction in the streamwise direction can reach a stable status with the increase of the flow velocity.

Keywords Gyrotactic microorganisms · Low-Reynolds-number flow · Motile cells · Swimming velocity

1 Introduction

Phytoplankton, as the primary producers of aquatic ecosystems, play a significant role in the material and energy cycles (Heisler et al. 2008; Sengupta et al. 2017). The swimming behavior of phytoplankton species is essential to various ecological phenomena, including the

formation of bioconvection (Pedley et al. 1988; Hwang and Pedley 2014a, b), microscale patches (Durham et al. 2013; Goldstein 2015), phytoplankton thin layers (Durham et al. 2009), harmful algal blooms (HABs) (Durham and Stocker 2012), etc.

Regarding the swimming characteristics of motile phytoplankton, there have been various theoretical, numerical and experimental studies (Durham et al. 2009, 2013; Pedley et al. 1988; Pedley and Kessler 1990; Son et al. 2015), with focus on the swimming behaviors of *Chlamydomonas reinhardtii* (Martin et al. 2016; Sineshchekov et al. 2000), *Chlamydomonas nivalis* (Hill and Häder 1997), *Heterosigma akashiwo* (Durham et al. 2009, 2013) and *Volvox* (Drescher et al. 2009; Goldstein 2015). Two types of models, i.e. continuum model (Pedley et al. 1988; Pedley and Kessler 1990) and individual model (De Lillo et al. 2014; Durham et al. 2011) are used to describe the swimming behaviors, which are dependent on the intrinsic characteristics of cells and external conditions (light intensity, the vorticity of ambient flow, etc.).

With regard to the swimming characteristic of algal cells, lots of efforts have been made for the still water. The swimming velocity and trajectories were measured, mainly based on the microscope-based (Häder and Lebert 1985; Hill and Häder 1997) and the laser-based tracking methods (Vladimirov et al. 2000, 2004). The microscope-based tracking method can provide a high spatial resolution, while the laser-based tracking method can offer a large scope. Häder and Lebert (1985) investigated the positive phototaxis and negative phototaxis of *Euglena gracilis* under various light sources. Hill and Häder (1997) investigated the random walk model of swimming microorganisms. Vladimirov et al. (2004) measured the velocity

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distributions in populations of *C. nivalis*, using the laser-based tracking method.

There have been various studies on the response of motile microorganisms to flow. Kessler (1985) pointed out that the aggregation of gyrotactic cells was dependent on the flow shear. Marcos and Stocker (2006) studied the effects of vortices on the distribution of microorganisms in a microchannel. Durham et al. (2011, 2013) studied the distribution of motile cells in a steady vortex and the formation of phytoplankton layer triggered by shear. Croze et al. (2013) investigated the dispersion of motile microorganisms in laminar and turbulent channel flows. Lillo et al. (2014) reported the influences of acceleration of flow on the formation of algal patches in a rotating cylindrical tube. Martin et al. (2016) observed the focusing of *C. reinhardtii* in the flow through a microchannel under the combined action of vorticity and phototaxis. Rusconi et al. (2014) found that the flow shear hampered the chemotaxis of *Bacillus subtilis*.

This work is to understand the swimming characteristics of one typical gyrotactic microorganism, *C. reinhardtii*, in the low-Reynolds-number flow, based on the microscope tracking method. The specific objects are: (1) to measure

the swimming velocity and direction in the still water and low-Reynolds-number laminar flow, and (2) to examine the effects of flow on the swimming velocity of algal cells.

2 Materials and methods

2.1 Experimental setup

The experimental system consists of a microfluidic system, an imaging system and a microfluidic chip, as shown in Fig. 1. The microfluidic system contains microfluidic flow control system (MFCS-EZ, Fluigent Company) and flow-rate platform (FRP, Fluigent Company). The imaging system includes microscope (IX83, Olympus), camera (DP80, Olympus) and imaging analysis software (CellSens, Olympus). The microfluidic chip is made of PDMS and a standard glass slide. The main microchannel in the chip has a length of 58,500 μm , a width of 500 μm and a depth of 50 μm . A 120- μm -wide and 7500- μm -long channel is connected to the main channel to check the flow pattern in the main channel. The diagram of experimental setup and the enlarged view of the microfluidic are shown in Fig. 1.

Fig. 1 Experimental sketch

2.2 Experimental materials

The experimental materials include algal cells (*C. reinhardtii*, Institute of Hydrobiology, Chinese Academy of Sciences), ultrapure water and tracers (potassium permanganate). The algal cells were incubated at 25 °C in selenite enrichment (SE) medium, and the daily cycle was 12 h of white light (2000 lux) and 12 h in the dark. The cells used for experiments were taken from the upper layer of suspensions which were vibrated for 5 min and stood for 30 min. The tracers were injected from the Inlet B to observe the flow pattern through the main channel, and the ultrapure water and algal cells were injected from Inlet A.

2.3 Statistical analysis

The velocity of cell migration (U_x , U_y) is calculated by

$$U_x = k_1 \frac{x_i - x_{i-1}}{\Delta t} \quad (1)$$

$$U_y = k_2 \frac{y_i - y_{i-1}}{\Delta t} \quad (2)$$

where (x_i, y_i) and (x_{i-1}, y_{i-1}) are the pixel positions of algal cells in the i -th frame and $i-1$ -th frame, k_1 (1.48 $\mu\text{m}/\text{pixel}$) and k_2 (1.48 $\mu\text{m}/\text{pixel}$) are the conversion coefficients between pixel and physical coordinates in the x and y directions, respectively, and Δt (0.125 s) is the time interval between two continuous images. The resultant speed, U_r , is defined as

$$U_r = \sqrt{U_x^2 + U_y^2} \quad (3)$$

which represents the speed of migration of cells in the horizontal plane. For the case of quiescent water, the swimming velocities of cells (V_x , V_y) are equal to the migration velocity (U_x , U_y). For the moving water, the swimming velocity (V_x , V_y) are given by

$$V_x = U_x - u_{yz} \quad (4)$$

$$V_y = U_y \quad (5)$$

where u_{yz} is the velocity of ambient flow. The z -axis is perpendicular to the x - y plane. The vertical position of focal plane is determined by adjusting the focal length and recording the z -axis step value of the microscope. u_{yz} and corresponding discharge Q through the cross section of the main channel are given by (Shah and London 1978; White 2005),

$$u(y, z) = \frac{16a^2}{\mu\pi^3} \left(-\frac{d\hat{p}}{dx} \right) \sum_{i=1,3,5,\dots}^{\infty} (-1)^{(i-1)/2} \times \left[1 - \frac{\cosh(i\pi z/2a)}{\cosh(i\pi b/2a)} \right] \times \frac{\cos(i\pi y/2a)}{i^3} \quad (6)$$

$$Q = \frac{4ba^3}{3\mu} \left(-\frac{d\hat{p}}{dx} \right) \left[1 - \frac{192a}{\pi^5 b} \sum_{i=1,3,5,\dots}^{\infty} \frac{\tanh(i\pi b/2a)}{i^5} \right] \quad (7)$$

where a (250 μm) and b (25 μm) are half-width and half-height of the channel, respectively, $\hat{p}$ is the pressure and μ is the dynamic viscosity of water. The convergence of Eq. (6) is numerically shown very fast, and the first 10 terms ($N = 10$) of the series are qualified for a reasonable approximation to the exact solution, as shown in Fig. 2. The swimming direction is defined as the angle (θ) between the positive y -axis and the cell axis. θ is calculated by Eqs. (8)–(12). The positive angle represents the clockwise rotation, and the negative angle represents the counter-clockwise rotation, as shown in Fig. 3.

Fig. 3 Definition of swimming direction

Fig. 2 Velocity profiles for $N = 5, 10$ and 15 at: **a** $y = 0$ and **b** $z = 0$

$$\theta = \arctan \frac{U_x}{U_y} \quad (U_y > 0) \quad (8)$$

$$\theta = \pi + \arctan \frac{U_x}{U_y} \quad (U_x \geq 0, U_y < 0) \quad (9)$$

$$\theta = -\pi + \arctan \frac{U_x}{U_y} \quad (U_x < 0, U_y < 0) \quad (10)$$

$$\theta = \frac{\pi}{2} \quad (U_x > 0, U_y = 0) \quad (11)$$

$$\theta = -\frac{\pi}{2} \quad (U_x < 0, U_y = 0). \quad (12)$$

3 Results

3.1 Swimming characteristics in still water

C. reinhardtii under the microscope is shown in Fig. 4. The diameter of cells ranges from 7 to 11 μm in the present work. The trajectory of *C. reinhardtii* was observed at 26 °C in a count box with a length and width of 20,000 μm , and a depth of 250 μm . The average swimming speed is approximately 41.0 $\mu\text{m/s}$, and the maximum swimming speed can reach 160 $\mu\text{m/s}$. The probability distribution function (PDF) of the swimming speed V_r is approximately subject to Gaussian distribution $A_1/(\sqrt{2\pi}\sigma_1)e^{-(V_r-V_{rc})/(2\sigma_1^2)}$, where $A_1 = 0.97$, $\sigma_1 = 19.67$ and $V_{rc} = 38.30$, as shown in Fig. 5a. In the still water, no obvious tendency was observed in the horizontal plane, as shown in Fig. 5b.

Fig. 4 *C. reinhardtii* under the microscope

3.2 Flow in a microchannel

Four flow conditions, adjusted by the discharge module of microfluidic system, were adopted in this experiment, as shown in Table 1. The flow pattern and cell swimming in the main channel were observed and analyzed at the observation position depicted in Fig. 1. The Reynolds number (Re) in the main channel is defined as $Re = \bar{u}L/\nu$, where $\bar{u}$ is the average flow velocity, L (50 μm) is the depth of the microchannel and ν ($0.877 \times 10^{-6} \text{ m}^2/\text{s}$ at 26 °C) is the kinematic viscosity.

A laminar flow was observed in the channel when the potassium permanganate solution and ultrapure water were injected into the channel from the inlets B and A, respectively. The flow patterns near the inlet B are shown in Fig. 6. The results showed that there was no vortex formation at the interface between the potassium permanganate solution and the ultrapure water, indicating the flow in the main channel was a low-Reynolds-number laminar flow.

The width-to-depth ratio of the main channel is 10 in present experiment. The velocity distribution in the cross section is shown in Fig. 7.

3.3 Swimming velocity in low-Reynolds-number flow

Figure 8 presents the probability density distribution of U_r , V_x and V_y . The U_r corresponding to the peak of probability distribution increases with the flow velocity, as shown Fig. 8a. The mean swimming velocity in the streamwise direction enhances as the velocity of ambient flow increases for the current flow conditions, as shown in Fig. 8b. The randomness of cell swimming still exists even for the case of moving water. The maximum swimming velocity in the x direction in the moving water is larger than that in the still water. The average of V_x is approximately 35 $\mu\text{m/s}$ for conditions A1–4, which is slightly less than the average swimming speed in the still water. V_y is subject to the Gaussian distribution with expectation value approximately equal to 0.0, as shown in Fig. 8c.

3.4 Swimming direction in low-Reynolds-number flow

The swimming direction of more than 80% cells is in the range of $90^\circ \pm 34^\circ$ for conditions A1–4, and the influence of cell randomness on cell migration becomes weak with the increasing flow velocity in the main channel, as shown in Table 2 and Fig. 9a. The swimming direction is approximately subject to Gaussian distribution, $A/(\sqrt{2\pi}\sigma)e^{-(\theta-\theta_c)/(2\sigma^2)}$, where θ_c depends on flow little,

Fig. 5 Swimming speed and direction of *C. reinhardtii* cells: **a** PDF of swimming speed and **b** distribution of swimming direction

Table 1 Experimental conditions

Conditions number	Discharge (nl/min)	Velocity ($\mu\text{m/s}$)	Re
A1	100	66.66	3.80E-03
A2	300	200.00	1.14E-02
A3	500	333.33	1.90E-02
A4	700	466.66	2.66E-02

Fig. 6 Flow patterns in the main channel: **a** $\bar{u} = 200 \mu\text{m/s}$, **b** $\bar{u} = 2667 \mu\text{m/s}$ and **c** $\bar{u} = 4667 \mu\text{m/s}$

Fig. 7 Velocity distribution: **a** velocity contours corresponding to condition A1, **b** velocity profile at $y = 0$ for conditions A1–4 and **c** velocity profile at $z = 0$ for conditions A1–4

and σ decreases with the increase of flow velocity, as shown in Fig. 9a, b. The migration of algal cells is influenced by the ambient flow, as shown in Fig. 9c. It is shown that 80% of the cells were aligned with the ambient flow when the flow velocity is greater than 333 $\mu\text{m/s}$. σ and the percent of swimming direction in the streamwise direction ($90^\circ \pm 3^\circ$) can reach a stable status, when the flow velocity in the main channel increases up to 333 $\mu\text{m/s}$.

4 Discussion

The measured mean speed of *C. reinhardtii*, based on 2407 cells, in the present work is approximately 41 $\mu\text{m/s}$ for the still water. Sineschekov et al. (2000) reported that the mean velocity of *C. reinhardtii* was 47.5 and 60 $\mu\text{m/s}$ in the light-adapted and dark-adapted cultures for a red background illumination, respectively, as well as 36 and

Fig. 8 PDF of U_r , V_x and V_y for conditions A1–4

Table 2 Swimming direction

Conditions number	Percent (%)	Swimming direction (°)
A1	80	$90^\circ \pm 34^\circ$
A2	80	$90^\circ \pm 14^\circ$
A3	80	$90^\circ \pm 3^\circ$
A4	80	$90^\circ \pm 3^\circ$

44 $\mu\text{m/s}$ in the absence of red background illumination, respectively. The swimming speed in the present work is close to that in the absence of red illumination. The probability distribution measured in the present study is similar to that by Leptos et al. (2009). The mean speed, 41 $\mu\text{m/s}$, observed in this work, is less than that (about 80 $\mu\text{m/s}$) measured by Leptos et al. (2009). The difference is possibly caused by the differences of cell growth environment. The culture in the present work is SE medium, which is different from that (Tris-Acetate-Phosphate medium) used by Leptos et al. (2009). Furthermore, the

mean swimming speed of *C. nivalis* and *H. akashiwo*, similar to *C. reinhardtii* in the shape and swimming behavior, is 55 $\mu\text{m/s}$ (Vladimirov et al. 2004) and 40 $\mu\text{m/s}$ (Bearon and Grünbaum 2008) in the still water, respectively.

The flow velocity is obtained by an analytical solution rather than a direct experimental measurement. In the present work, the focal plane is considered as a fixed plane with zero thickness, which may lead to a small position difference due to the existence of focal depth.

5 Conclusions

The swimming characteristics of a typical gyrotactic microorganism, *C. reinhardtii*, were studied for a low-Reynolds-number flow, based on a microfluidic system. The measured swimming speed of *C. reinhardtii* for the still water in the present work, ranging from 0 to 160 $\mu\text{m/s}$, is subject to Gaussian distribution. The swimming direction

Fig. 9 Swimming direction in various flow conditions: **a** PDF of swimming direction, **b** standard deviation σ corresponding to the PDF and **c** percent of swimming direction

is still subject to Gaussian distribution for the still water. The ambient flow can influence the swimming behavior of cells, which results in a steeper variation of probability density distribution of swimming velocity in the cross-flow direction. It is also found that the maximum of swimming velocity aligned with the flow direction increases to some extent for the moving water. The streamwise mean swimming velocity in the ambient flow is slightly less than that in the still water. It is also shown that the swimming direction in the horizontal plane was dominated by cell randomness for the still water, and 80% of the cells were aligned with the ambient flow when the flow velocity exceeds $333 \mu\text{m/s}$. σ and the percent of streamwise swimming direction ($90^\circ \pm 3^\circ$) can reach a stable status, when the flow velocity in the main channel increases up to $333 \mu\text{m/s}$.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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