Light Control of the Flow of Phototactic Microswimmer Suspensions

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Some microalgae are sensitive to light intensity gradients. This property is known as phototaxis: The algae swim toward a light source (positive phototaxis). We use this property to control the motion of microalgae within a Poiseuille flow using light. The combination of flow vorticity and phototaxis results in a concentration of algae around the center of the flow. Intermittent light exposure allows analysis of the dynamics of this phenomenon and its reversibility. With this phenomenon, we hope to pave the way toward new algae concentration techniques (a bottleneck challenge in biofuel algal production) and toward the improvement of pollutant biodetector technology.

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Nature presents a wide and fascinating array of organisms that can propel themselves in a fluid. Among them, a lot of microorganisms [1,2] like spermatozoa, bacteria, or microalgae can move with the help of flagella or cilia [3,4] and are classified as microswimmers [5]. Thanks to the 19th century seminal work of Engelmann [6] and Pfeffer [7,8], it is well known that some bacteria, like *Escherichia coli*, move toward or away from certain chemicals [9], a phenomenon known as chemotaxis. For example, *E. coli* consumes oxygen and swims along oxygen gradients [10]. Another phenomenon discovered a long time ago on *paramecium*—a ciliate protozoa—is gravitaxis, i.e., sensitivity to gravity, which makes the cells swim vertically [11], a property also observed on some algae in combination with flow vorticity, together called gyrotaxis [12,13]. The phototaxis property describes the motion of microswimmers along gradients of light intensity [14] and is used by some marine phytoplankton to move vertically in water columns in the euphotic zone [15].

A very active field of research involves understanding how artificial or natural microswimmer suspensions can self-organize under the influence of externally controlled fluid-mediated interactions. For example, some light-powered autonomous micromotors have shown the ability to provoke schooling behavior in water [16]. In the 1980s, Kessler [17] combined gravity with the flow of a gravitactic alga suspension. The torque exerted by gravity on each alga leads to their self-focusing or to their migration toward the walls of the container depending on the vertical orientation of the Poiseuille flow. Self-focusing or remixing was observed, depending on the vertical direction of the Poiseuille flow (downward for self-focusing and upward for mixing). Even in nature, in a marine ecosystem, thin layers of phytoplankton are found in the coastal ocean, a few meters below the surface, and contain cell concentrations up to 2 orders of magnitude above ambient concentrations. This separation phenomenon is due to the hydrodynamic flow that causes gyrotactic trapping [13].

In our study, we show that, by combining light and a Poiseuille flow, we can provoke easily controlled and reversible self-focusing of an alga suspension of *Chlamydomonas reinhardtii*. The advantage of light is that it controls self-focusing or remixing regardless of flow direction. Furthermore, by switching the light on and off, we can reverse from self-focusing to remixing. After analyzing the dynamics of this phenomenon with a periodical modulation of light exposure, we measured the dynamic response of *C. reinhardtii* to the presence of light. Finally, we demonstrated that the migration of a cell through the flow lines can be simply understood and qualitatively described and computed with a simple nonlinear model.

Chlamydomonas reinhardtii (CR) [14] is a genus of green alga. It is a biflagellated unicellular organism. CR is used as a model organism in molecular biology, especially for studies of flagella motion, chloroplast dynamics, biogenesis, and genetics. It is spheroidal in shape with two anterior flagella moving in a back-and-forth movement, producing a jerky breast stroke with a mean swimming speed of $V_0 \sim 50 \pm 20\ \mu$m/s in a waterlike medium [18]. Since the cell radius is $R \sim 5\ \mu$m, Brownian motion is negligible. CR is phototactic thanks to an eyespot [14], which is a structure made of carotenoid-filled granules in the cell membrane. Under light exposure, the beating of the cis-flagellum (close to the eyespot) is inhibited, unlike the beating of the trans-flagellum (opposite the eyespot) which is enhanced: This means the alga orients itself and swims toward the light source. CC124-strain cells were obtained from the IBPC lab in Paris [19]. Synchronous cultures of CR were grown in a Tris-acetate phosphate medium by using a 14/10 h light/dark cycle at 22 °C. Cultures were typically grown for 2 days under fluorescent lighting before cells were harvested for experiments.

Microscopy imaging of chlamydomonas suspensions was carried out with an Olympus inverted microscope coupled with a fast camera used at frame rates up to 200 Hz. Square section channels of 1×1 mm were

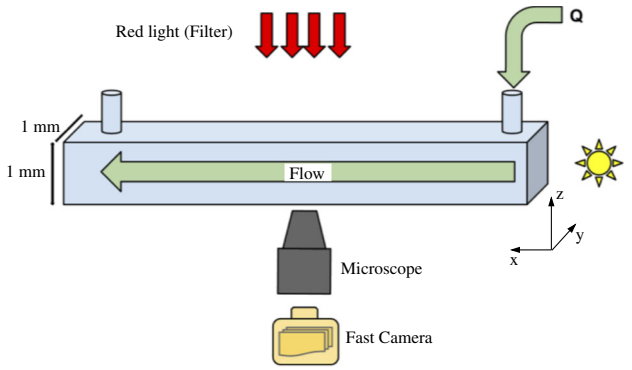

FIG. 1 (color online). The setup of the experiment.

made of polydimethylsiloxane by using soft lithography techniques [20]. The experimental setup is shown in Fig. 1.

The Reynolds number $\mathrm{Re} \sim V_0 R/\nu$ associated with swimming alga in water is very weak (about 2.5×10^{-4} where kinematic viscosity is $\nu \approx 10^{-6}\ \mathrm{m^2\,s^{-1}}$). It is well known that for vanishing Reynolds numbers, because of Stokes flow reversibility, passive spherical particles rotate in a Poiseuille flow (except at the center) but do not experience any migration across the flow lines [21]. In the case of microswimmers, the situation is quite different: Their motion in a Poiseuille flow follows an oscillating trajectory [22] due to flow vorticity combined with swimming velocity. Therefore, each cell undergoes time periodic cross-stream migration but with no net migration averaged over the cell's period of rotation. Figure 2(a) shows 40 ms microswimmer tracks in such a Poiseuille flow: The cells are homogeneously distributed over the width of the channel. Although the trajectories experience some oscillations, they are not visible at this scale, as the spatial wavelength of the oscillations is expected to be approximately a few channel widths (see below).

When a light source is switched on on the right-hand side of the flow—i.e., oriented upstream since the flow goes from right to left—the situation is noticeably different: The microswimmers migrate to the center of the flow, resulting in strong self-focusing. Figure 2(b) shows 40 ms microswimmer tracks in the presence of light. It represents the final stage where the cells have migrated to the center of the channel. Figure 3 shows the corresponding distributions of cells though the channel (without and with light) for different values of the imposed flow rate. When the light source is on the left-hand side—oriented downstream—with the same flow direction, cells migrate toward the walls of the channel.

Let us now simply sketch the phenomenon. In the presence of light, the swimmer rotates due to flow vorticity, but it also regularly reorients itself toward the light source within a typical time of about 1 s. Because of local vorticity, the swimmer will be more frequently oriented by the flow toward the center where it moves to by swimming [Fig. 4(a)]. Note that if the light source is on the opposite side, the cells are more frequently oriented toward the walls to which they migrate [Fig. 4(b)]. In the model below, we have solved numerically a simple nonlinear model based on Ref. [22] but including regular swimmer reorientations toward the light. It clearly shows motion toward the center or the walls depending on the direction of reorientation, i.e., toward the position of the light source.

Experimentally, the self-focusing is observed in the range of flow rate $0.03\ \mathrm{ml/min} < Q < 0.09\ \mathrm{ml/min}$, which in terms of shear rate averaged between the center of the squared channel and the walls gives $1.0\ \mathrm{s^{-1}} \lesssim \overline{\dot{\gamma}} \lesssim 3.0\ \mathrm{s^{-1}}$. Below this range, the flow is too weak to force the cells to rotate, since a CR can resist the flow rotation [23], and the algae are not oriented by the flow. Above this

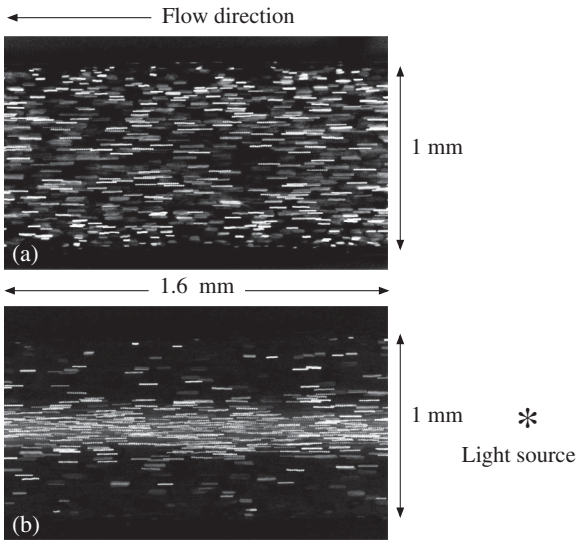

FIG. 2. CR trajectories (obtained by superposing 10 images). (a) With no light. CR are transported by the Poiseuille flow from right to left. (b) With light on the right-hand side. The CR move toward the center.

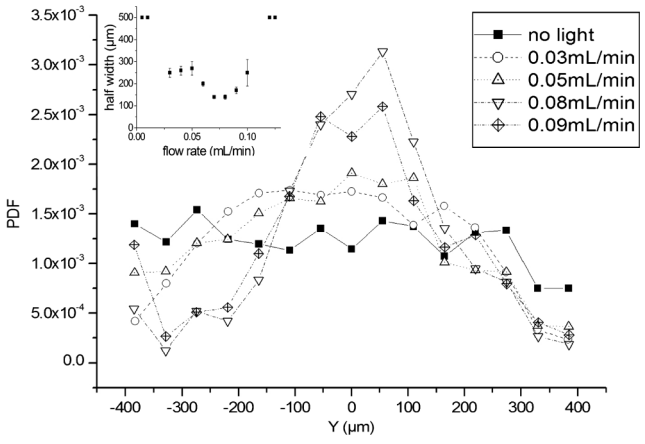

FIG. 3. Probability distribution of CR in the Poiseuille flow. (a) Without light, (b) with the light source on the right. Inset: The full band width at half maximum as a function of the flow rate.

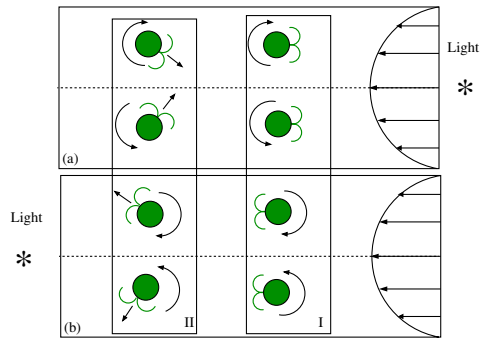

FIG. 4 (color online). Schematic view of the phenomenon. Cells orient themselves toward light (I) and are rotated by the vorticity (II). (a) Light source upstream, resulting in a self-focusing. (b) Light source downstream resulting in a migration toward the wall.

range, the cell's rotation is too fast, and it has no time to orient itself toward the light, a phenomenon similar to the gyrotactic trap observed in phytoplankton [13], and, consequently, the cell cannot migrate. The concentration of cells in the center results in a reinforcement of the hydrodynamic interactions between cells and, thus, in a broadening of the distribution of CR around the center where hydrodynamic interactions prevail. As shown in Fig. 3, the band width depends on the flow rate. The band width is measured at the half maximum value of the cell distribution. The minimum band width obtained at a flow rate of 0.09 ml/min ($\overline{\dot{\gamma}} \approx 3.0\ \mathrm{s}^{-1}$) represents 22% of the channel width [see the inset in Fig. 3].

Self-focusing is associated with hydrodynamic forcing which orients the cells toward the center of the flow where they migrate at their own velocity perturbed by their mutual interactions. When the light is switched off from the focusing state, hydrodynamic interactions between oriented CR [24] reinforced by the concentration of algae at the flow center cause the cells to mix back in the fluid. We analyze the dynamics of such a phenomenon (see Fig. 5) by

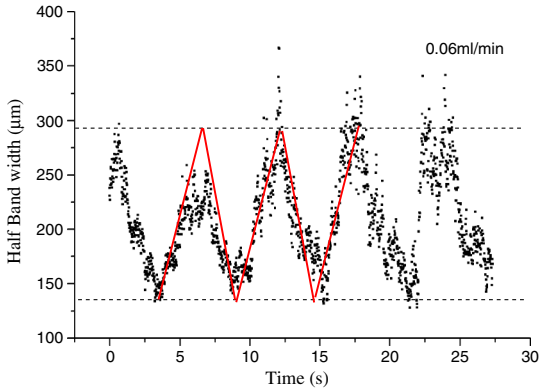

FIG. 5 (color online). Band width of CR as a function of time under light exposures of 2.5 s separated by dark periods of 2.5 s. A linear variation is observed with a transverse velocity equal on average to $\sim 60\ \mu\mathrm{m\,s}^{-1}$ (indicated by the red lines).

varying light exposure time: The light is switched on and off alternately with a time period $T = 5$ s. Fast camera visualization clearly shows that the half band width varies linearly and reversibly with time. We found that the corresponding average velocity for both self-focusing and remixing is $60\ \mu\mathrm{m\,s}^{-1}$ for a flow rate of 0.06 ml/min (i.e., $\overline{\dot{\gamma}} \approx 2.0\ \mathrm{s}^{-1}$). This velocity value emerges from the dynamics of the collective motion of the cells. It is close to the swimming speed of a single CR which plays an important role in the phenomenon (see the model below). However, the hydrodynamic interactions between the cells are known to generate hydrodynamic diffusion (especially in concentrated suspensions) that strongly perturb the motion of a single cell and can thus modify the collective dynamics and its velocity.

We then developed a very simple nonlinear model that describes the motion of a swimmer within a Poiseuille flow in a cylinder of radius R with regular orientation toward a light source situated on the right-or left-hand side of the channel. We do not describe the remixing obtained experimentally which is due to a strong repulsion between oriented CR. This model is inspired by Ref. [22] and has been modified in order to take into account reorientation of the microswimmer upstream or downstream toward a light source. We define $\mathbf{e}_\rho$ and $\mathbf{k}$ the unitary radial and longitudinal vectors, respectively. The microswimmer situated in $\mathbf{r} = \rho\mathbf{e}_\rho + z\mathbf{k}$ is simply seen as moving at velocity $\mathbf{V} = \mathbf{V_0} + \mathbf{u}(\rho)$, where $\mathbf{V_0}$ is the intrinsic velocity of the microswimmer ($\|\mathbf{V_0}\| = V_0$) moving in a fluid flowing at velocity $\mathbf{u}(\rho) = u_{\max}(\rho^2 - R^2)\mathbf{k}$ (Poiseuille flow), $u_{\max}$ being the maximum value of $\mathbf{u}(\rho)$ at the center of the flow ($\rho = 0$). The direction of $\mathbf{V}_0$ (i.e., the direction of the swimmer) is determined by local flow vorticity $\omega(\rho) = 1/2\nabla \times \mathbf{u} = -u_{\max}\rho/R^2\mathbf{e}_\theta$. The trajectory of the microswimmer is thus given by

$$\mathbf{r}\,(t) = \int_0^t \mathbf{V}(\rho)dt', \tag{1}$$

with $\mathbf{V} = V_0 \sin\theta(\rho)\mathbf{e}_\rho + [V_0 \cos\theta(\rho) + u(\rho)]\mathbf{k}$ and

$$\theta(\rho) = \int_0^t \omega(\rho)dt'. \tag{2}$$

By solving this system of equations numerically, we obtain trajectories which experience oscillations but no net migration [see Fig. 6]. Note that the oscillations have a wavelength much larger than the channel radius R [22] and are not visible in Fig. 2. The oscillations result from the combination of flow vorticity and swimmer velocity. In order to take into account the effect of light, the microswimmer is regularly reoriented upstream. This can be done by adding a torque; for example, Williams and Bees [25] use a phototactic torque balancing a gravitactic torque and resulting in a mechanical equilibrium (the swimmer is an isolated body and must be torque-free). Instead here we simply reorient the swimmer by changing the time varying angle $\theta(t)$ to 0 (upstream) or π (downstream) at

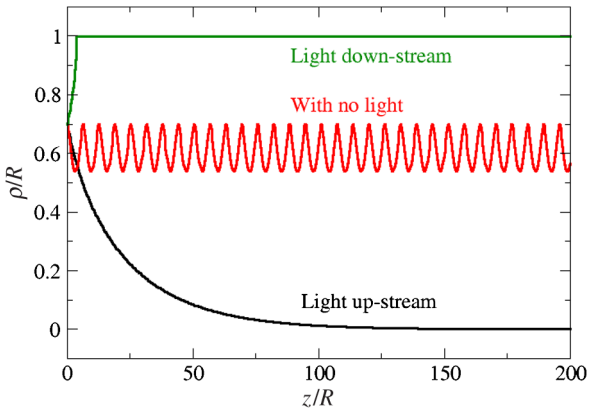

FIG. 6 (color online). Trajectories of a microswimmer within a Poiseuille flow. $\rho/R = 0$ is the center of the Poiseuille flow. The microswimmers experience oscillations in the absence of orientation toward light (red curve). Light can be up- or downstream oriented. The microswimmers migrate toward the walls if they are regularly oriented downstream, or they migrate toward the center if oriented regularly upstream.

a frequency τ^{-1}. The time τ corresponds to the response time of a CR to a light stimulus (τ is of the order of 1 s). In the model, we choose $\omega_{\max}\tau \sim \mathcal{O}(1)$, where $\omega_{\max}$ is the maximum absolute value of the vorticity at the wall (in the experiments $\omega_{\max} \sim 1\ \mathrm{s}^{-1}$, since $\omega_{\max} \approx \overline{\dot{\gamma}}$ in a squared geometry). The swimmer is still rotated by vorticity; however, at each time τ it is oriented upstream (respectively, downstream), vorticity makes the swimmer turn toward the center of the flow (respectively, the walls) where it moves to. As a result, the model shows that $\rho(t)$ (in the self-focusing case) is close to an exponential decay (data not shown) with a time constant $\tau^* \sim \rho_0/[V_0\omega(\rho_0)\tau]$, where $\rho_0 = \rho(t = 0)$. Therefore, at a short time scale, the radial velocity of the swimmer is typically $V_\rho \sim \rho_0/\tau^*$ and, thus, $V_\rho/V_0 \sim \omega(\rho_0)\tau$. It states that a single swimmer, initially close to the wall, migrates to the center with the typical velocity V_0 when $\omega_{\max}\tau \sim \mathcal{O}(1)$. The model also shows that when $\omega_{\max}\tau > 1$ a bifurcation occurs: The vorticity is too high, cells seldom orient themselves toward the light (i.e., $\tau > \omega_{\max}^{-1}$), and no migration is obtained since the radial velocity cannot exceed V_0. This is consistent with the experiments where, at too high flow rate, the self-focusing is not observed above $\omega_{\max} \gtrsim 3\ \mathrm{s}^{-1}$, and, thus, the model provides a typical time $\tau \sim 0.3$ s. This value should be taken with care because of the absence of interactions between swimmers in the model. However, the model gives reasonable orders of magnitude and, mainly, provides new insights on the nonlinearities of the phenomenon, since the upper limit of the self-focusing effect could be seen as a bifurcation. Future experimental investigations on a single cell will be analyzed and compared with this model. It could help to separate the contribution of the hydrodynamic interactions from individual motion of a cell. For instance, in our experiments, for the self-focusing effect, due to repulsive hydrodynamic and steric interactions, the cells cannot of course concentrate in $\rho = 0$ but are scattered in a band width around the center of the flow (Fig. 3). Therefore, we believe that only a full description of the hydrodynamics coupled with a suspension of modelized swimmers could help to predict the precise value of the band width which depends on the flow rate and on the cell concentration. This will be done in the future.

In this work, we show that the coupling of the phototaxis property of the *Chlamydomonas reinhardtii* microalga with a Poiseuille flow can lead to reversible self-focusing of microalgae at the center of the flow. This phenomenon could pave the way toward new algal concentration or separation processes in pipe flows, a field of particular interest in hydrogen production by algae [26,27], for example. The advantage of self-focusing is to avoid cell accumulation at the walls where adhesion can occur, leading to irreversible alteration of the suspension. Another possible application could be found in biodetectors of river pollutants. These devices use microalgae with phototaxis properties that are very sensitive to traces of pollutants such as copper ions or pentachlorophenol [28]. The velocity associated with self-focusing being averaged on all the cells, it can help to diagnose the phototaxis efficiency of the whole suspension of swimming CR. However, this phenomenon also opens several more fundamental questions that should be addressed in the future by more refined models. Indeed, the hydrodynamic interaction between cells as well as the fluid disturbance created by the swimmers should be taken into account in order to better describe the competition between self-focusing and hydrodynamic interactions resulting in a band of concentrated cells at the center of the channel.

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