Swimming at a micrometer scale demands particular strategies. When inertia is negligible compared to viscous forces, hydrodynamics equations are reversible in time. To achieve propulsion, microswimmers must therefore deform in a way that is not invariant under time reversal. Here, we investigate dispersal properties of the micro-alga *Chlamydomonas Reinhardtii*, by means of microscopy and cell tracking. We show that tracked trajectories are well modeled by a correlated random walk. This process is based on short time correlations in the direction of movement called persistence. At longer times, correlation is lost and a standard random walk characterizes the trajectories. Moreover, high speed imaging enables us to show how the back-and-forth motion of flagella at very short times affects the statistical description of the dynamics. Finally we show how drag forces modify the characteristics of this particular random walk.

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Cell motility \[1\] is crucial to many biological processes including reproduction, embryogenesis, infection, etc. Many microorganisms are able to propel themselves, bacteria, sperm cells, microalgae, etc. A quantitative understanding of the hydrodynamics of flagella and cilia is thus of great interest \[2, 3\].

One of the peculiarities of the swimming of microorganisms is that it occurs at very low Reynolds numbers which is very different from our usual experience of swimming at our meter length scale \[4, 5\]. Indeed when inertia is negligible as compared to viscous forces (i.e. Reynolds number \(Re\) is lower than unity), in order to achieve propulsion, swimmers must deform in a way that is not invariant under time reversal. This is known as Purcell’s scallop theorem \[4\]. In living systems, several different strategies are used to achieve propulsion in such conditions: the *E. coli* bacterium uses a rotating flagellum at the “back” of its body, sperm cell propulsion relies on the asymmetry of their flagellar bending waves, the power and recovery strokes of the two front flagella of *Chlamydomonas Reinhardtii* (CR) algae are asymmetrical.

Flagellar propulsion in CR induces complex swimming behavior of cells. Over short time scales, the cells undergo an oscillating movement with changes in velocity direction occurring at the same frequency as the beating frequency of flagella. On a time scale longer than the period of beating, average swimming behavior is directional. Eventually, on larger time scales, direction is lost and swimming trajectories resemble a random walk.

CR is a 10µm motile bi-flagellated unicellular alga. The cell is spheroidal in shape with two anterior flagella \[6\]. It belongs to the puller type of swimmers as it uses its front flagella to propel itself, producing what a breaststroke-like movement. The swimming direction of the cells can be controlled by stimulus gradients, a phenomenon known as taxis, such as chemotaxis, rheotaxis or phototaxis. Gradients are not used in our experiments in order to avoid any external tropism on the motility.

Wild-type strains were obtained from the IBPC lab in Paris \[7\]. Synchronous cultures of CR were grown in a Tris-Acetate Phosphate medium (TAP) using a 12/12 hour light/dark cycle at 22°C. Cultures were typically grown for two days under fluorescent lighting before the cells were harvested for experiments.

We studied the swimming dynamics of this microor-
ganism by means of bright field microscopy imaging on
an Olympus inverted microscope coupled either to a CCD
camera (Sensicam, Photon Lines) used at a frame rate of
10Hz or to a high speed CCD camera (Miro, Phantom)
used at a frame rate of 400Hz. Long time experiments
used a ×10 magnification lens whereas we used a ×64
lens for high speed imaging. Two hundred micrometer
thick glass chambers were coated with bovine serum al-umine to prevent cell adhesion. The imaged cells were
located 30 to 60 micrometers from the glass walls. A
red light filter was used in order to prevent phototaxis.
Cell tracking [8] was performed using IDL (Interactive
Data Language) with a submicron precision in the detect-
tion of hundreds of cells (high speed experiments) and
thousands of cells for long time sequences. To quantify
the effect of drag on the cell dynamics, small amounts of
short chain dextran (Sigma Aldrich) were added to the
culture medium. The chains were short enough for non-
Newtonian effects to be absent and long enough to avoid
damaging the cells with osmotic effects. This allowed
the viscosity $\eta$ of the medium to be varied between 1.5
and 3.7mPa s. The range of viscosity is restricted to this
interval to ensure the viability of the cells.

Let us first recall the global dynamics of swimming,
i.e. over time scales of the order of a few seconds. Cell
trajectories are found to be correctly modelled by a per-
sistent random walk [9–11]. Cells swim in an almost fixed
trajectories are found to be correctly modelled by a per-
during long times. The correlation functions decay expo-
entially over a characteristic time $t_c$. This correlation
time $t_c$ is related to the mean time of persistence over
which the direction of swimming is preserved. The dif-
ferent symbols in figure 1(b) correspond to experiments
where the concentration of short chain dextran was var-
ed, hence modifying the viscosity of the medium from
1.5mPa s to 3.7mPa s. As viscosity increases, correlation
time $t_c$ increases (data not shown) from 1.5 to 3.9
seconds.

The global dynamics of swimming of $CR$ can thus be
described as a correlated random walk characterized by
a ballistic regime (with a mean velocity $V$) and a decor-
relation process (over a characteristic time $t_c$) due to the
turns made by the cells. As a consequence, a persistence
length $C$ is naturally defined as the product $V t_c$. From a
statistical point of view, such a behaviour is described by
the mean square displacement of cells $< r^2(t) >$ which
is linear for long times ($t \gg t_c$) and quadratic at shorter
times ($t \lesssim t_c$) [13, 14]. At even shorter times, the dynam-
ics reflect the consequences of low Reynolds swimming,
.i.e. a non-reciprocal movement of flagella. This then
leads to a zigzagging motion of cells due to the back-
and-forth movement of flagella [15].

In the present case, cells of diameter $2R \sim 10 \mu m$
are moving at a velocity $V$ around 50$\mu m/s$ in a wa-
with the long time swimming behavior are visible. In

such a CR propulsion strategy of microorganisms such as this micro-alga. The other consequence of the fact that swimming is restricted during the backward movement. The symmetry under time reversal is thus broken and propulsion is ensured. However, because inertia has no role in this regime, this kind of propulsion leads to a back-and-forth movement of the cell in which the velocity is alternatively positive and negative. High speed imaging (400Hz) allows us to resolve the very short time dynamics due to flagella beating and thus to study the consequences of this back-and-forth movement on the properties of the swimmers' random walk.

The insets in figure 2 show typical cell trajectories imaged at 400Hz: the back-and-forth movement of swimmers due to the absence of inertia (Re ≪ 1) together with the long time swimming behavior are visible. In these examples, the cells are swimming either in a nutritive medium of viscosity $\eta = 1$ mPa s (top left inset) or in a dextran-rich medium of viscosity 3.7 mPa s (bottom right inset). Cells have a net forward movement corresponding to the power stroke, followed by the recovery stroke that propels the cell backward. As the distance traveled forward is longer than the backward movement, the cells ultimately progress forward. However, these fluctuations in the direction of the velocity have consequences on the measured statistical quantities [17] that we will discuss now.

The measured mean square displacement $< r^2(t) >$ shows a plateau region at very short time ($t \ll t_c$) that reflects the transition between two quadratic regimes: on the one hand, a fast ballistic regime characterized by the instantaneous velocity $u$ of swimmers and, on the other hand, a slower ballistic regime corresponding to the mean velocity $V$ of swimming which is the resulting forward velocity over several back-and-forth movements.

The position of the plateau therefore corresponds to the beating frequency $f$ of the swimmer, which depends on the viscosity of the surrounding medium. To quantify the back-and-forth swimming motion of the cells, we measured the angle probability distribution function. Figure 3.a shows distribution functions for different times. For a given short time $t$, the distribution of angles $\theta(t)$ as defined earlier, peaks at around zero, reflecting a given direction at very short time. For longer time scales (close to $1/2f$), anti-correlation in cell direction resulted in new distribution peaks at values of around $\pm \pi$. When a new stroke is produced, the measured angle is again close to zero giving rise to a peak around zero. Hence, angle distributions have a periodicity which reflects the beating frequency. This is shown in figure 3.a as the distributions are very similar at times shifted by $1/(2f)$, where the typical frequency of the beating $f$ is deduced from the periodical nature of the correlation function of direction. Figure 3.b shows such correlation functions at varying $f \times t$, the product of time multiplied by the fitted frequency of the signal. Data are well described by an exponentially attenuated cosine function. The different symbols correspond to different viscosities of the medium. The exponential decay of the correlation function should reflect the turns in direction the cells eventually perform forward. However, due to the 3D nature of the trajectories and the 2D geometry of our setup, correlation was attenuated faster than that.

The other consequence of the fact that swimming is produced at low Reynolds number is that propulsion requires non-zero drag forces. Viscous friction is thus crucial in the dynamics of microswimmers. By varying the viscosity of the medium, we were able to draw some conclusions about the effects of friction forces on the locomotion of microorganisms such as this micro-alga.

Here, short time dynamics of swimming can be fully described by few mean quantities: flagella frequency $f$ and the viscosity of the surrounding medium $\eta$.
beating \( f \) (deduced from a cosine fit in figure 3(b)) and the mean modulus of instantaneous velocity \( u \), which is the velocity achieved during a power or a recovery stroke. We studied the effects of viscous forces on these quantities. Velocities and beating frequency are found to be inversely proportional to the viscosity of the bath (figure 4). As viscosity increases, the beating frequency decreases, varying from 30Hz to 13 Hz with a viscosity variation from 1.5 to 3.7mPa s (figure 4(a) giving a slope \( \eta f = 0.045 \pm 0.01 \text{Pa} \)). Accordingly, velocity decreases from 135 to 75µm/s (figure 4(b) giving a slope \( \eta u = 0.15 \pm 0.04 \text{Pa} \)). These results supports the idea of imposed-force locomotion [11]. The corresponding stall force, which is proportional to the product \( \eta \times u \), is then constant.

The velocity \( u \) can be related to the mean propulsion force on the cell body by Stokes’ law. Let’s now assume that a power stroke (respectively a recovery stroke) results from the friction length \( \xi_\perp \) (resp. \( \xi_\parallel \)) of the flagella moving perpendicular (resp. parallel) to its long axis. Moreover, the beating frequency \( f \) can be related to the friction of flagella acting on a typical distance of one cell diameter: \( 6\pi \eta R_u = 2Rf \eta (\xi_\perp + \xi_\parallel) \). Using measurements of the slopes \( \eta u \) and \( \eta f \), we can estimate a sum \( \xi_\perp + \xi_\parallel \sim 32 \mu \text{m} \). Using the friction coefficient expressions of a cylinder given in [18], this leads to an aspect ratio of 200 for a 10µm long flagellum with a radius of 25nm. This is a reasonable estimate [19] considering that flagella are not exactly perpendicular and parallel to the flow during power and recovery strokes.

In this work, we quantified the complex dynamics of swimming at short time scale using high speed microscopy imaging and particle tracking techniques. This study allowed us to analyze the breaststroke like swimming of a CR cell in a fluid at low Reynolds number and how this swimming is influenced by the viscosity of the ambient fluid. It showed how the friction acting on a CR cell can be qualitatively extracted from the back-and-forth motion of a thin and elongated pair of flagella. This means that a description in terms of time averaged flows is not to be encouraged for such systems [19].

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