Direct Measurement of the Flow Field around Swimming Microorganisms

Knut Drescher, Raymond E. Goldstein, Nicolas Michel, Marco Polin, and Idan Tuval

Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge CB3 0WA, United Kingdom
(Received 3 May 2010; published 11 October 2010)

Swimming microorganisms create flows that influence their mutual interactions and modify the rheology of their suspensions. While extensively studied theoretically, these flows have not been measured in detail around any freely-swimming microorganism. We report such measurements for the microphytes Volvox carteri and Chlamydomonas reinhardtii. The minute (\(\sim 0.3\%\)) density excess of V. carteri over water leads to a strongly dominant Stokeslet contribution, with the widely-assumed stresslet flow only a correction to the subleading source dipole term. This implies that suspensions of V. carteri have features similar to suspensions of sedimenting particles. The flow in the region around C. reinhardtii where significant hydrodynamic interaction is likely to occur differs qualitatively from a puller stresslet, and can be described by a simple three-Stokeslet model.

Aided by advances in imaging techniques that allow detailed studies of the rotating flagella of bacteria [1] and the undulating flagella of spermatozoa [2] and algae [3], there is now a general consensus on how mechanical motions of microorganisms appendages generate propulsive forces in a viscous fluid [4]. No such consensus exists yet on the origins of collective behavior [5], transport [6,7] and forces in a viscous fluid [4]. No such consensus exists yet on the origins of collective behavior [5], transport [6,7] and rheological properties of suspensions [8], and the interaction of organisms with surfaces [9,10]. As hydrodynamics surely plays a key role in these effects, a detailed knowledge of the flow field around freely swimming microorganisms is needed, both in the near field and far away. Here we present the first such measurements.

The linearity of the Stokes equations implies that the far-field flow around a microorganism can be expressed as a superposition of singularity solutions [11], with the slowest decaying mode dominating sufficiently far away. Theories of fluid-mediated interactions and collective behavior typically assume neutrally buoyant swimmers which exert no net force on the fluid. The thrust \(T\) of their flagella and the viscous drag on their body are displaced a distance \(d\) apart (often comparable to the cell radius \(r\)), and balance to give the far-field flow of a force dipole, or stresslet [12], which decays as \(F_{\text{g}}/\eta r\), it is clear, if not appreciated previously, that there is a distance \(\Lambda \sim Td/F_{\text{g}}\) at which the nearby stresslet contribution crosses over to the distant Stokeslet regime. This is one of several crossover lengths relevant to swimmers; for ciliates, unsteady effects become important on scales smaller than the viscous penetration depth [15].

For a given organism, the relevance of the length \(\Lambda\) to a particular physical situation depends on the cell concentration and the observable of interest. At low concentrations the Stokeslet form suffices, but the near field is relevant to cell-cell interactions, especially in concentrated suspensions [16] and to tracer dynamics [7]. The notion of near field requires distinguishing between distances \(r\) satisfying \(R \ll r \ll \Lambda\), where a stresslet description may hold, and \(r \approx R\) where the multipole contributions may not be well ordered and the flow topology can differ from that of a stresslet.

A synthesis of tracking microscopy and fluid velocimetry is used here to quantify the competing force singularities and the near-field flow topology for the unicellular biflagellate green alga Chlamydomonas reinhardtii [17] (\(R \approx 5\ \mu\text{m}\)) and its larger relative Volvox carteri [18], a spherical alga (\(R \sim 200\ \mu\text{m}\)) which swims by the action of \(\sim 10^3\) Chlamydomonas-like cells on its surface. For Volvox our most significant finding is that the flow field is strongly dominated by its Stokeslet component, despite a density excess of a mere \(\sim 0.3\%\), much smaller than that of common unicellular organisms (\(\sim 5\%–10\%\)). Moreover, the high symmetry of Volvox results in a leading near-field correction in the form of a source doublet, and a smaller stresslet. The flow around Chlamydomonas is compatible with a simple “puller” stresslet only at distances \(\geq 7R\), where the fluid velocity is \(\leq 1\%\) of the swimming speed; closer to the cell, the flow topology reflects the finite separation of the flagellar and body forces.

V. carteri f. nagariensis (strain EVE) was grown axenically in standard Volvox medium (SVM) [19] with sterile...
air bubbling, whereas *C. reinhardtii* (strain UTEX 89) was grown axenically in tris-acetate-phosphate (TAP) medium [17] on an orbital shaker, both in a diurnal growth chamber with 16 h in artificial cool daylight (~4000 lux) at 28 °C, and 8 h in the dark at 26 °C. The large difference in organism size between *Volvox* and *Chlamydomonas* required two distinct methods to measure the flows they create [20]. A CCD camera (Pike, Allied Vision Technologies) mounted on a continuously focusable microscope (Infinivar, Infinity Optics) and connected to a vertical motorized XY stage (Thorlabs) followed individual *Volvox* colonies as they swim upwards [21] in a straight line along the central axis of a 5 × 5 × 50 mm sample chamber filled with SVM at 21 ± 1 °C. The stage was controlled by a custom LabView routine. The fluid was seeded at volume fraction 10⁻⁵ with 1 µm nille-red polystyrene microspheres (Invitrogen) illuminated by a vertical ~500 µm thick laser sheet (λ = 532 nm). *Volvox* is phototactic [22] at this wavelength, and at the intensities used here it swims smoothly along the laser sheet. We recorded the flow field of 19 different colonies at 30 fps for ~2–3 min each. The measured flow field was obtained by particle image velocimetry (Dantec Dynamics). Background flows in the chamber were <10 µm/s.

We observed a dilute suspension (~3 × 10⁶ cells/cm³) of *Chlamydomonas* in TAP on a Nikon inverted microscope at 40 × (NA 0.6) by exciting their chlorophyll autofluorescence with a laser (635 nm, ~60 mW), which also excited 1.6 µm fluorescent polystyrene microspheres (FS04F, Bangs Labs) used as tracers. Cylindrical polymethylsiloxane sample chambers (5 mm radius, 0.4 mm height) were prepared, pacified, and filled following [7]. Experiments were performed at 21 ± 1 °C, with the laser providing the only light source. We focused on a plane 150 µm inside the chamber to minimize surface effects, and recorded movies at 250 fps (Fastcam SA3, Photron). Movies were analyzed with standard algorithms to track cells and tracers. For each cell swimming along the focal plane for more than 1 s (~10 body lengths), we collected the instantaneous velocity of all tracers at r < 14R, normalized by the swimmer’s speed. The resulting 3.3 × 10⁶ velocity vectors were binned into a 2.5 µm square grid (shown in Fig. 4), and the mean of the well-resolved Gaussian in each bin was used for the flow field.

In both experiments *U₀* indicates the swimmer velocity, while *u*(r) and *v*(r) = *u*(r) − *U₀* are the velocity field in the laboratory and comoving frames, respectively.

A typical experimental flow field around *Volvox* is shown in Fig. 1(a). We fit these fields to a superposition of a uniform background velocity (*U₀*), a Stokeslet (St), and a stresslet (str) and a source doublet (sd):

\[
\mathbf{v}_{\text{fit}}(\mathbf{r}) = -U_0 \hat{y} - \frac{A_{St}}{r} (\mathbf{I} + \mathbf{r} \hat{r}) \cdot \hat{y} - \frac{A_{str}}{r^2} [1 - 3(y/r)^2] \mathbf{r} \cdot \hat{y} - \frac{A_{sd}}{r^3} \left( \mathbf{I} - \frac{1}{3} \mathbf{r} \mathbf{r} \right) \cdot \hat{y}
\]

where \( \mathbf{I} \) is the unit tensor, \( \hat{y} \) is the upward vertical unit vector, \( \mathbf{r} = r \hat{r} \), and \( r \) is measured from the center of the organism \((x_c, y_c)\). The orientation of all multipoles is fixed to be along the vertical, and we are left with six parameters: \( U_0, A_{St}, A_{str}, A_{sd}, x_c, y_c \). The fits, obtained by minimizing the integrated squared difference between the model and the experimental flows, describe remarkably well the experimental flow, almost down to the surface of the organisms [see Figs. 1(b) and 1(c)]. Typical values for the parameters are \( U_0 \sim 10^2 \mu m/s, A_{St} \sim 10^4 \mu m^2/s, A_{str} \sim 10^6 \mu m^3/s \) (indicating a pusher-type stresslet), \( A_{sd} \sim 10^6 \mu m^3/s \), with the actual magnitude depending on the colony radius \( R \). From the Stokeslet component, we can calculate the average colony density as \( \Delta \rho = 6 \eta A_{St}/g R^3 \), where \( \eta = 10^{-3} \) Pa s and \( g \) is the gravitational acceleration. The dependence of both \( \Delta \rho \) and \( U_0 \) on \( R \) (Fig. 2) compares well with previously published data [21] obtained by different means, thereby validating the measurements and analysis procedures. Removing the Stokeslet contribution from the experimental flow field [Fig. 3(a)] reveals that the near field is dominated by the source doublet component, with the stresslet responsible only for a slight forward-backward asymmetry [Figs. 3(b) and 3(c)]. The orientation of the source doublet is opposite to that around a translating solid sphere, and is compatible with a model that assigns a constant force density to the colony.
pulling the cell body through the fluid at speeds mostly in a synchronous breast stroke at $50 \, \text{Hz}$.

Volvox from the high degree of anterior-posterior symmetry of the organism. The near complete dominance of the flow field around Volvox by the Stokeslet term found here provides ex post facto justification for the neglect of higher moments. Perhaps more importantly this result shows that in terms of interparticle hydrodynamic couplings a suspension of Volvox is like a sedimenting suspension [27], except that the velocity of each colony is the sum of a self-propelled contribution and mutual advection in the flow field of other spheres. Elsewhere we illustrate this correspondence in detail [28].

The correspondence between the measured time- and azimuthally-averaged flow field of Volvox and the three-Stokeslet model illustrates how well such a simplification captures the complex flow topology, lending support to this approximation in modeling ciliary interactions [29]. Our results indicate that the simple puller-type description for Volvox is only valid at distances $\geq 7R$, where the flow field is already $\lesssim 1\%$ of $U_0$. We then expect interactions with other swimmers, boundaries or tracers, to be influenced mostly by the flow structure at shorter separations, where the full time dependence of the
flow may be important [30]. We are currently investigating whether similar conclusions hold for the flow field around bacteria, the prototypical "pusher" microorganisms.

We thank K. C. Leptos for suggesting the use of auto-fluorescence to track Chlamydomonas cells, S. B. Dalziel, V. Kantsler, and T. J. Pedley for discussions, D. Page-Croft and N. Price for technical assistance, and acknowledge support from the EPSRC, the BBSRC, the Marie-Curie Program (M. P.), and the Schlumberger Chair Fund.

[28] I. Rushkin et al. (to be published).