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Decoding the hydrodynamic properties of microscale helical propellers from Brownian fluctuations

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The complex motility of bacteria, ranging from single-swimmer behaviors such as chemotaxis to collective dynamics, including biofilm formation and active matter phenomena, is driven by their microscale propellers. Despite extensive study of swimming flagellated bacteria, the hydrodynamic properties of their helical-shaped propellers have never been directly measured. The primary challenges to directly studying microscale propellers are 1) their small size and fast, correlated motion, 2) the necessity of controlling fluid flow at the microscale, and 3) isolating the influence of a single propeller from a propeller bundle. To solve the outstanding problem of characterizing the hydrodynamic properties of these propellers, we adopt a dual statistical viewpoint that connects to the hydrodynamics through the fluctuation–dissipation theorem (FDT). We regard the propellers as colloidal particles and characterize their Brownian fluctuations, described by 21 diffusion coefficients for translation, rotation, and correlated translation–rotation in a static fluid. To perform this measurement, we applied recent advances in high-resolution oblique plane microscopy to generate high-speed volumetric movies of fluorophore-labeled, freely diffusing *Escherichia coli* flagella. Analyzing these movies with a bespoke helical single-particle tracking algorithm, we extracted trajectories, calculated the full set of diffusion coefficients, and inferred the average propulsion matrix using a generalized Einstein relation. Our results provide a direct measurement of a microhelix’s propulsion matrix and validate proposals that the flagella are highly inefficient propellers, with a maximum propulsion efficiency of less than 3%. Our approach opens broad avenues for studying the motility of particles in complex environments where direct hydrodynamic approaches are not feasible.

Brownian motion | propulsion matrix | fluctuation-dissipation theorem | oblique plane microscopy | *E. coli* flagella

The original observations of Brownian motion (1, 2) and their connection to fluid drag coefficients described by Einstein’s seminal 1905 paper (3) provided a window into the deep connection between the equilibrium statistical properties and hydrodynamic response functions of a physical system. More generically, this connection is described by the fluctuation–dissipation theorem (FDT) (4). For a diffusing fluid, the FDT relates the experimentally accessible statistical signature of unconfined Brownian motion, the linear time dependence of the mean squared displacement (MSD), with the fluid drag coefficient and the fluid viscosity through the Stokes–Einstein relation. A common application of the Stokes–Einstein relation is the characterization of the size distribution, viscosity (η), and physical confinement of spherical particles at a low Reynolds number (Re ≪ 1) (Fig. 1A). In these experiments, single-particle tracking or spatiotemporal correlation functions characterize the motion of particles dispersed into a fluid by measuring the diffusion coefficient, D.

In contrast to spherical particles, complex, asymmetric particle shapes introduce rich new physics, including non-Gaussian Brownian motion (5) or propulsive coupling between rotational and translational motion (6) (Fig. 1B). To fully describe the position and orientation of an arbitrary-shaped particle, six total coordinates are required. The expanded parameter space allows for a more complicated set of “fluctuations” described as a matrix of correlation functions between displacements and rotations along the six coordinates.

The corresponding dissipation function takes the form of a matrix of drag coefficients, often called the propulsion matrix or the resistance matrix. The propulsion matrix describes fluid resistance to translations and rotations and fully characterizes the complex motility at low Re. The existence of the propulsion matrix is guaranteed at low Re because...
A key insight is that the propulsion matrix characterizes the efficiency of a rigid body at low \( \text{Re} \) when acting as a propeller (10). To obtain the propulsion matrix elements for any propeller geometry, it is possible to solve the Stokes equations using the Oseen tensor and flow singularities. However, the complexity of the boundary conditions makes exact analytic approaches intractable for all but the simplest geometries (e.g., spheres in a quasi-infinite fluid) (11). Similarly, experimental validation of theoretical predictions for complex geometries, such as \( \mu \)-scale helical particles, has not been possible due to the limited spatiotemporal bandwidth of available microscopy methods (12, 13).

Instead, prior work quantified the propulsion matrix directly from the hydrodynamic definition using larger helical particles, scaling laws, and applied external forces (Fig. 1 C–E) (6, 14–16).

Understanding the propulsion properties of asymmetric particles in low \( \text{Re} \), where viscous forces dominate over inertial forces, is important because bacteria and other microscale swimmers live in this regime. At low \( \text{Re} \), reciprocal motion cannot generate the net displacement required for propulsion (6, 11). As such, microscale swimmers must adopt different swimming strategies compared with larger organisms. For example, some swimmers have evolved to have flagella, propellers made of filaments that generate translation by rotation (17). Other swimmers utilize cilia, propellers made of filaments with a rigid power stroke to generate translation and a highly flexible, disordered return stroke to avoid reciprocal motion (18). Understanding the efficiency of these molecular propellers is an outstanding question in biology and fluid mechanics, dating back over half a century (19, 20).

In addition to single-swimmer hydrodynamics, propeller properties may play an important role in collective dynamics (21). For example, swarming cells typically grow additional flagella compared to their isolated counterparts, but the role of these additional propellers is not understood (22). Hydrodynamic interactions between cells or between cells and surfaces in complex fluids (polymer gels) are believed to play a role in biofilm formation (23) as well as the rheology of dense bacterial suspensions (24). Fully characterizing the hydrodynamic properties of single propellers is vital to understand these more complex phenomena.

Here, we present direct quantification of the propulsion matrix for one type of microscale helical propeller, specifically the *Escherichia coli* flagellum. We measure the Brownian fluctuations of individual flagella using oblique plane microscopy (OPM) (25). We pioneer the capability of quantifying rotational diffusion along the longitudinal axis at the microscale and demonstrate that our theoretical analysis and computational framework (SI Appendix, sections S1–S3) obtain propulsion matrix coefficients comparable to those measured by conventional hydrodynamic methods (6, 14–16). Our experimental measurements directly quantify the codiffusion coefficient that describes the coupling between the translation and rotation of the helix, confirming previous theoretical predictions about helical particle diffusion.

**Results**

To begin, we generated volumetric timelapse movies of freely diffusing, isolated, and Cy3B-labeled helical flagella separated from an *E. coli* body using a high-resolution OPM with fast volume imaging capability (25, 26) (Fig. 2A). OPM is a light sheet technique where only one objective interfaces with the sample, allowing light sheet imaging of samples mounted on slides or flowcells. Briefly, an oblique light sheet excites fluorescence in...
diffuses anisotropically in the body frame, anisotropic diffusion can only be observed in the laboratory frame over times that are short compared with $\tau_r$, where the axis $\hat{n}_1$ remains nearly fixed (SI Appendix, section S4). On longer time scales, the helix center of mass will diffuse with the rotationally averaged diffusion coefficient $S$.

For helical particles, symmetry considerations ensure that the propulsion matrix has only one off-diagonal term (SI Appendix, section S2). Therefore, a flagellum is expected to undergo independent translational and rotational diffusion about its transverse axes, $\hat{n}_2$ and $\hat{n}_3$, and coupled translational and rotational diffusion about its longitudinal axis, $\hat{n}_1$. The approximate helicoidal symmetry implies that the diffusion coefficients for the transverse axes will be nearly equal. In this case, the propulsion matrix drag equations reduce to four uncoupled equations and one coupled equation of the form

$$
\begin{pmatrix}
    F_1 \\
    \tau_1
\end{pmatrix}
= 
\begin{pmatrix}
    A & B & D \\
    B & D & A \\
    D & A & B
\end{pmatrix}
\begin{pmatrix}
    v_1 \\
    \psi_1 \\
    \omega_1
\end{pmatrix},
$$

where the forces, torques, and velocities all point along the helix’s longitudinal axis.

Combining the generalized Einstein relation of Eq. 2 with Eq. 3, we obtain the propulsion matrix coefficients in terms of the diffusion coefficients $D_{n_1}$ and $D_{\psi_1}$ and the codiffusion coefficient $D_{n_1\psi_1}$ (SI Appendix, section S1)

$$
A = \frac{D_{\psi_1}}{D_{n_1}D_{\psi_1} - D_{n_1}^2} k_B T,
$$

$$
B = -\frac{D_{n_1\psi_1}}{D_{n_1}D_{\psi_1} - D_{n_1}^2} k_B T,
$$

$$
D = \frac{D_{n_1}}{D_{n_1}D_{\psi_1} - D_{n_1}^2} k_B T.
$$

Here, $D_{n_1}$ describes translational diffusion along the longitudinal axis, $D_{\psi_1}$ describes rotational diffusion about the longitudinal axis, and $D_{n_1\psi_1}$ describes correlated translational and rotational diffusion. When propulsive coupling is absent, $D_{n_1\psi_1} = 0$, Eqs. 4–6 recover the familiar Einstein relations $A = k_B T/D_{n_1}$ and $D = k_B T/D_{\psi_1}$.

We measured the diffusion coefficients’ variation with viscosity using three concentrations of sucrose. Due to the linearity of the Stokes equations, we expect that all diffusion matrix coefficients scale inversely with viscosity $D \sim 1/\eta$. Intuitively, a more viscous fluid impedes the flagellar movement leading to reduced motion. As expected, the translational (Fig. 3B) and rotational diffusion coefficients (Fig. 3 D and F) decrease with increasing viscosity, approximately matching the expected scaling. (SI Appendix, Fig. S6 and section S7). As diffusion coefficients may also depend on flagellar length, we considered only flagella with lengths between 6 µm and 10 µm and an average length of 7.7(9) µm ($n = 79$).

We found that flagella diffuse faster along the longitudinal axis than the transverse axes for all viscosities (Fig. 3B). We quantified the anisotropy of the translational movement of the flagella by considering the ratios of the longitudinal to mean transverse diffusion coefficient, which were 1.49(7), 1.63(8), and 1.72(6) for the three viscosity values considered ($n = 24, 22$, and 33, respectively). This asymmetry can be qualitatively understood using resistive force theory (RFT), an approximation that replaces the fluid with two phenomenological drag coefficients describing drag along directions normal and tangent to the flagellar
centerline (16, 19, 20, 27). For a more precise estimate of the diffusion coefficient ratios, we can compare to the expected values for a slender cylinder with length equal to the flagellum arc length $L / \cos \theta$ and diameter equal to the filament diameter, which can be computed using Eqs. S60–64. For our parameters, this ratio is $\sim 1.7$, in good agreement with the measured values. In the long-cylinder limit, this ratio asymptotically approaches 2 (20).

We further found that the rotational diffusion along the longitudinal axis is two orders of magnitude faster than that along the transverse axes (Fig. 3 D and F). RFT again provides qualitative insight into the origin of this anisotropy. The fluid drag on a body is greater along the normal direction than in the tangential direction (19). When the helix rotates along the transverse axes, it presents a larger normal surface area than when it rotates along the longitudinal axis and hence experiences greater resistance. Indeed, RFT shows that the expected leading order scalings are $D_{n_1} \sim R^{-2} L^{-1}$ and $D_{n_2}, D_{n_3} \sim L^{-3}$; hence, the difference in the diffusion coefficients comes from the fact that $L$ is roughly one order of magnitude larger than $R$. The transverse rotational diffusion coefficients determine the tumbling time scales (5) $\tau_t = 1 \text{rad}^2 / 2D_{\perp} \sim 20, 40,$ and 100 s, respectively, which are of similar order to the length of our full tracks.

The codiffusion coefficients describe the correlation between a flagellum’s rotational and translational motion. In principle, there can be correlations between any pair of axes (SI Appendix, Fig. S4 and section S2). However, for a helix, we expect the dominant correlation between rotation about and translation along $\mathbf{n}_1$. Due to its chiral shape, a helix tends to move in one direction when it rotates in a positive sense and in the opposite direction when it rotates in a negative sense (Fig. 4 A and B).

Unlike the translational and rotational diffusion coefficients, the codiffusion coefficient is not strictly positive, and the sign indicates the directional coupling between translational and rotational motions. Our measured codiffusion coefficients are negative, indicating that when the helix rotates in the right-handed sense about $\mathbf{n}_1$, it translates along the $-\mathbf{n}_1$ direction (Fig. 4C). The negative value is expected as E. coli flagella have a left-handed helical shape that rotates counterclockwise during steady motion when viewed from the propeller’s distal end (28, 29). If the vector $\mathbf{n}_1$ points toward the cell, the motor rotates the flagella “negatively,” driving the cell forward.

Discussion

With these experimentally derived diffusion coefficients, we determined the coefficients of the propulsion matrix using Eqs. 4–6. The propulsion matrix coefficients are expected to depend only on the viscosity and helical geometry and hence can be parameterized by the helical radius $R$, length $L$, filament radius $a$, and helical pitch $\lambda$. To allow comparison of our work with prior results, we define nondimensionalized propulsion matrix coefficients $\bar{A}^r \equiv A / \eta L$, $\bar{B}^r \equiv B / \eta LR$, and $\bar{D}^r \equiv D / \eta LR^2$ that remove all dependence on viscosity and first-order dependence on the flagellar length and helical diameter based on RFT. We note that these nondimensional coefficients do not remove dependence on the helical pitch (equivalently $R/\lambda$ or $\lambda/a$.

To determine the nondimensional propulsion matrix elements from our measured diffusion coefficients, we relied on experimentally measured geometric parameters of E. coli flagella. From the image analysis of the flagella, we find $l = 7.7(9) \mu m$.
and \( \lambda = 2.5(1) \mu m \), values that are consistent with previous measurements (30, 31). As the helical radius is on the order of the diffraction limit, we rely on the previously reported value \( R = 0.25 \mu m \) (30), which implies that the helical pitch angle, \( \theta = \tan^{-1}(2\pi R/\lambda) \), is \( \theta = 32^\circ \). As the filament radius is much smaller than the diffraction limit in our setup, we rely on the value obtained by x-ray diffraction, \( a = 0.01 \mu m \) (32, 33).

We compared nondimensionalized propulsion matrix elements obtained from Brownian motion versus conventional hydrodynamics experiments for helices with \( \theta \sim 32^\circ \) (Fig. 4D). We considered values from three experimental approaches, which studied either \( E. coli \) in optical tweezers (14) or millimeter scale helices in highly viscous fluids (10, 16), and two theory techniques, slender body theory (34) (SBT) and the regularized Stokeslet approach (16, 35).

We find that our measured propulsion matrix coefficients are in reasonable agreement with previous experiments. In particular, our measured \( A^* \) and \( B^* \) coefficients agree with optical tweezer measurement on whole \( E. coli \), while our measured \( D^* \) is somewhat larger. The discrepancies between our measured \( D^* \) and the optical tweezer results may be related to the fact that in ref. 14, the measured rotational drag coefficient is the sum of flagellar drag and cell body drag. As the cell body drag is an order of magnitude larger than the flagellar drag, we expect the systematic uncertainty in extracting the flagellar drag to be large. Our approach avoids this complication. Additionally, the approach of ref. 14 makes the assumption that only local hydrodynamic effects are important (inspired by the local RFT drag coefficients), which implies that the total drag coefficient is the sum of the \( E. coli \) body and the flagella, neglecting the hydrodynamic interactions between the body and the flagella (36). This approximation is expected to introduce less than 10% error (20, 37).

Compared with the millimeter-scale helix experiments, our values for \( B^* \) and \( D^* \) agree well, but our value for \( A^* \) is somewhat too large. However, we note that the wire helix filaments have \( \lambda/a \sim 150 \), whereas we estimate that the flagella have \( \lambda/a \sim 254 \). This difference in filament thickness almost entirely explains the discrepancy between the millimeter-scale helix results and the theory results in Fig. 4D.

On the other hand, our measured propulsion matrix coefficients are \( \sim 20\% \) to \( 50\% \) larger than predicted by SBT and the regularized Stokeslet approach. As discussed above, a larger effective value for the filament radius \( a \) would lead to larger drag coefficients in the experiment. An alternative explanation could be that various material properties of real flagella differ from the idealizations used in the theoretical approaches. For example, these assume that the helix is perfectly rigid, but it is well known that flagella are elastic as their elasticity is critical for bundling during \( E. coli \) swimming (38). The effect of elasticity is complex (39) and has not been characterized for diffusing flagella.

Another interesting property that we can extract from the propulsion matrix is the maximum propeller efficiency, typically quantified as the ratio of the power that would be required to pull the object divided by the power required to propel it using a rotary motor. This efficiency is related to the propulsion matrix coefficients by \( \epsilon_{\text{max}} = B^*/(4A^*D^*) \). \( \epsilon_{\text{max}} \) only depends on the shape of the propeller, independent of the viscosity or the cargo (10, 14), such as the \( E. coli \) body. Our measurement finds that the propulsion efficiency is \( \epsilon_{\text{max}} = 0.91(16)\% \), which is close to both the theoretical values (20, 27) and other experimental measurements (6, 14), as shown in Fig. 4D.

It is initially surprising that the propulsion efficiency is so small, considering the conservation of the flagellar structure across many bacterial species. Some authors have argued that the low efficiency is unimportant because \( E. coli \)'s power expenditure during swimming is roughly \( 1 \times 10^{-16} \) W (6, 14), a small fraction of its total metabolic power consumption \( 1 \times 10^{-13} \) W (40). Recent work suggests that considering hydrodynamic efficiency alone is too simplistic, as the mechanical efficiency (41) and metabolic cost of assembling a propeller may be equally critical (42). In this view, swimmers must solve an optimization problem that balances several different terms, including hydrodynamic efficiency, mechanical efficiency, and construction cost. This optimization is also subject to various constraints, such as total metabolic output and material limitations.

**Fig. 4.** Correlation between translation and rotation. (A) Schematic of translation induced by rotation of a left-handed helix in a viscous fluid. Counterclockwise and clockwise rotations cause the helix to move downward (Left) and upward (Right), respectively. (B) Free-body diagram of filament segments in their rest frames while helix rotates clockwise (panel A, Right). Due to the rotation, the segments see fluid flow (blue) in opposite directions. The forces in the horizontal direction cancel, while those in the vertical direction add causing the helix to translate in the \( n_1 \) direction (upwards). Green and pink insets correspond to the regions shown in A. (C) Codiffusion coefficients, \( D_{n_1 n_1}^* \), for flagella in different viscosity solutions. Diffusion coefficients determined from each measurement (circles) and average diffusion coefficients (plusses) are shown. Error bars are SEM. (D) Nondimensionalized propulsion matrix elements obtained from Brownian motion versus conventional hydrodynamics experiments for helices with \( \theta \sim 32^\circ \).
The solution found by E. coli trades low hydrodynamic efficiency for high mechanical efficiency and low construction cost. The E. coli motor is up to 80% efficient (43), and the estimated raw material and energy cost to assemble an E. coli flagellum are significantly less than for a Eukaryotic cilium (42). The relative simplicity of bacteria flagella, which are polymers of flagellin, limits construction costs but also constrains the range of available helical shapes. It is not possible to arbitrarily modify the flagellar geometry to increase efficiency by, for example, choosing the optimal helix angle \( \theta \), because flagellin can polymerize in only 12 different helical shapes (44).

In this work, we demonstrated a direct quantification of the hydrodynamic properties of a molecular propeller, specifically the isolated E. coli flagellum, in the low Reynolds number regime. We combined recent advances in high-resolution, high-speed volumetric fluorescence imaging with a theoretical and data analysis approach that relies on the fluctuation–dissipation theorem and computational tracking of extended 3D particles. Our work introduces a general method for characterizing molecular propellers without the need to enforce external fluid flows or forces, as previously required. The approach we detail here has broad applications for studying the propulsion of other bacterial species and Eukaryotic flagella as well as potential extensions to understanding hydrodynamic interactions between systems of multiple bacteria, multiple propellers, and the design of artificial microswimmers for targeted drug delivery and other medical applications (45).

Materials and Methods

Sample Preparation.

E. coli flagellum. Glycerol stock of E. coli strain MG1655WT was grown overnight in 1.5% agar with T-broth [1% Tryptone (211705; BD) and 0.5% NaCl (S7653; Sigma-Aldrich)] at 37 °C. Subsequently, a single colony was taken for inoculation with 10 ml T-broth in a sterile 125-ml flask. The culture was grown overnight in a rotary shaker, moving at a speed of 150 RPM at 30 °C until it reached saturation. Then, 100 μl of this culture was diluted in another 10 ml T-broth in a 125-ml sterile flask and then grown in a rotary shaker moving at a speed of 150 RPM at 30 °C until it reached OD600 = 0.6. The motility of the bacteria was confirmed using a bright-field microscope. Although the flagella were not visible, the body movement of the bacteria indicated that it had successfully developed long-propelling flagella. Subsequently, the bacteria solution was washed three times by centrifugation (2,000 g for 10 min) at room temperature to separate E. coli from its culture medium. The precipitate was gently resuspended using 10 ml of motility buffer (10 mM KPO4 (P0662; Sigma–Aldrich), 67 mM NaCl (S7653; Sigma-Aldrich), and 0.1 mM EDTA (A15161; Sigma-Aldrich), final pH 7.0). The final suspension was stored at 500 μL.

The amine-coated surface of E. coli, including its flagella, was stained with organic dyes tagged with N-hydroxysuccinimide (NHS) ester (PA63101; GE)-30. Since active esters are unstable in moisture, the Cy3B NHS ester was first prepared in small 3 μl aliquots of 25 mM in DMSO (85190; ThermoFisher Scientific) and kept in −20 °C. Of the final bacteria suspension, 50 μl was mixed with a 3 μl aliquot of Cy3B. Then, 2.5 μl 1 mM sodium bicarbonate (S6041-500G; Sigma-Aldrich) was added to the new sample to shift its pH to ~7.8. The sample was then incubated on a slow rotator at room temperature for 1 h. Subsequently, the sample was washed three times with motility buffer added with 1 × 10−4% Triton X-100 (786-513; G-Biosciences) to remove excess dye and prevent any labeled cells from sticking to the test tube. The washed sample was kept at a volume of 50 μl. The stained flagella were extracted by vortexing and pipetting followed by centrifugation at 8,000 g for 5 min at room temperature. Then, 5 μl of the resulting supernatant was mixed with 40%, 50%, and 70% (w/v) sucrose (S0389; Sigma-Aldrich) for imaging.

Microtubules. Cycled unlabeled tubulin (O32005; PurSolutions) and labeled tubulin-Alexa Fluor 647 (O64705; PurSolutions) were mixed in a 4:1 ratio. Single-cycle microtubules were then synthesized by mixing 20 μM of the tubulin mixture and 1 mM GMPCPP (NU-4055; Jena Bioscience) in BRBB0 buffer (O32003; PurSolutions) and 10 mM DTT (10708984001; Sigma-Aldrich) for 10 min on ice followed by a 2 h incubation period at 37 °C (46). This incubation time led to microtubules with an average length of 6.5 μm (47). The sample, diluted to 200 nM, was then mixed with 90%(w/v) sucrose. An additional 10 μM Taxol (I7402 5MG; Sigma-Aldrich) was added to stabilize these GMPCPP microtubules. To delay photobleaching, the mixture was supplemented with GLOX oxygen scavenging buffer [0.02 mg/mL, 0.05 mg/mL catalase from the bovine liver (C1345-1G; Sigma-Aldrich), 0.05 mg/mL glucose oxidase from Aspergillus niger (G2133-10KU; Sigma-Aldrich), and 3 mg/mL glucose (0643-1K; WWR)].

Imaging Chamber Preparation. A 18 × 18-mm coverslip (Thorlabs) and a 24.5 × 76.2-mm microscope slide (WWR) were cleaned with acetone (LC1042044; LabChem) and isopropanol (BDH1133-4LP; WWR), dried, and plasma-cleaned using a plasma cleaner (Harrick Plasma) for 10 min. To create a flow chamber, 100-μm thick double-sided Kapton tape was sandwiched between the coverslip and the microscope slide. The chamber was then treated with 1 mg/mLBSA (A4503-10G; Sigma-Aldrich) or 10 mg/mL casein from bovine milk (C7078-50G; Sigma-Aldrich) for 10 min. Then, 20 μl of the labeled flagella–sucrose sample was flowed into the chamber. After the sample was drained, the flow chamber was sealed by applying epoxy to both chamber openings to prevent evaporation. The epoxy-sealed coverslip was then incubated for an hour at room temperature.

Oblique Plane Microscopy. We modified our previously described stage-scanning high numerical aperture oblique plane microscope (OPM) for high-speed, volumetric, low-inertia acquisition (26) using a lens-based galvanometric mirror scan/descan unit (48–50).

Briefly, a remote focus microscope consisting of a 100 × NA 1.35 silicone oil primary objective (MRD73950; Nikon Instruments), 200-mm primary tube lens (MXA22018; Nikon Instruments), lens-based galvanometric mirror scan/descan unit (2 × CLS-SL and GV5201; Thorlabs), custom 357-mm secondary tube lens (AC508-500-A and AC508-750-A; Thorlabs) (50), custom pentaband dichroic (zt405/488/561/640/730pc-uf3; Chroma Technology Corporation), custom pentaband laser barrier filter (zet405/488/561/640/730m; Chroma Technology Corporation), and 40 × NA 0.95 air secondary objective (MRD70470; Nikon Instruments) with a glued antireflection coated coverslip (Applied Scientific Instrumentation). A tertiary imaging system imaged the remote image volume, tilted at 30° to the optical axis, consisting of a bespoke solid immersion objective (AMS-AGY v1.0; Special Optics) (50), custom pentaband laser barrier filter (zet405/488/561/640/730m; Chroma Technology Corporation), 200-mm tertiary tube lens (MXA22018; Nikon Instruments), and camera (OrcaFlash BT, Hamamatsu Corporation).

Excitation light was provided by a set of five solid-state lasers (OBIS LX 405-100, OBIS LX 488-150, OBIS LS 561-150, OBIS LX 637-140, and OBIS LX 730-30; Coherent Inc.) contained within a control box (Laser Box: OBIS; Coherent Inc.). The lasers were steered into a colinear path using kinematic mirrors and laser combining dichroic mirrors (zt405rdc-U1, zt488rdc-U1, zt561rdc-U1, and zt640rdc-U1; Chroma Technology Corporation). All five beams were filtered using a 30 μm pinhole spatial filter (P30D, Thorlabs), with the size selected to best filter the 561-nm laser line. After spatial filtering, the beams were expanded and focused to a line by a cylindrical lens (ACY254-075-A; Thorlabs) onto a mirror conjugate to the front focal plane of the primary objective. The line focus was reflected into the optical train of the OPM by the dichroic mirror between the secondary tube lens and the secondary objective. The tilt of the mirror at the line focus controlled translation at the back focal plane of the primary objective and, therefore, the light sheet angle in the sample. The numerical aperture of the light sheet was controlled using an adjustable slit (V10100C; Thorlabs) placed in the back focal plane of the cylindrical lens.

An XYZ positioning stage (FTP-2000; Applied Scientific Instrumentation) and controller (Tiger; Applied Scientific Instrumentation) were used to position the sample into the focal volume of the primary objective.

The microscope was controlled using a Windows 11 Pro 64-bit computer (Thinkstation P620; Lenovo) running custom Python software based around the C++ core of Micromanager via pymmcore-plus and a Napiar graphic.
Numerical Simulation of Helix Diffusion. Simulation of propulsion matrix performed on raw data using the native OPM point spread function for coverslip. After the acquisition, each image stack was "deskewed" by orthogonal interpolation into the coverslip coordinate system, was (301 ± 21) nm, (340 ± 19) nm, (755 ± 18) nm. Raw image data were stored as a Zarr file with custom metadata stored as a text file.

Volumetric Timelapse Acquisition. The flagella and microtubule solutions were imaged with the 561-nm laser of the OPM setup using a light sheet angle of 30°. The camera region of interest was cropped to 1,800 px × 256 px, and the exposure time was 2 ms. Each volume was acquired using a galvanometric mirror scanning approach (48, 49), where we collected 37 images separated by 400 nm at a scanning rate of 0.5 kHz. This resulted in 3D images over a 180 × 30 × 15 μm³ parallelepiped shaped volume acquired at a rate of 13.3 volts.

The OPM images were acquired in a coordinate system tilted relative to the coverslip. After the acquisition, each image stack was "deskewed" by orthogonal interpolation onto an isotropic 3D grid aligned with the coverslip (26, 53). Optional GPU-accelerated Richardson-Lucy deconvolution (Microvolution) was performed on raw data using the native OPM point spread function for microtubule, but not flagella, experiments.

Numerical Simulation of Helix Diffusion. Simulation of propulsion matrix parameters using slender body theory (34) and the method of regularized Stokeslets (35) was performed using the Matlab code developed in ref. 16, which is available at https://www.mathworks.com/matlabcentral/fileexchange/39265-helical-swimming-simulator. All parameters must first be normalized to the helix radius, so the simulations were performed with R = 1, L = 30.9, λ = 10.2, and a = 0.04.

Nondimensional Propulsion Matrices from Prior Works. Optical tweezer values (14) have been nondimensionalized using their parameter estimates R = 0.21 μm, L = 6.5 μm, λ = 1.5 μm, θ = 41°, and τ = 10.0. Load cell values from ref. 16 were taken from the figure 2 data point with R = 6.3 mm, L = 130 mm, λ = 61.8 mm, θ = 32.6° and a = 0.397 mm. Gravity values from ref. 10 were taken from the second entry in Table 1, with R = 0.2 cm, L = 7.8 cm, λ = 1.56 cm, θ = 39°, and a unreported. They quoted a maximum efficiency of 0.78%, but using their stated propulsion matrix values, this should instead be 0.85%. Johnson SBT and regularized Stokeslet values were obtained using the code developed in ref. 16 with the flagellar parameters given in the main text.

Computational Resources and Data Storage. All computation, except for data acquisition, was performed using a Linux Mint 19 server with 48 computing cores, 1 terabyte of RAM, and two dedicated GPU computing cards (Titan RTX, NVIDIA). All data were stored in a dedicated network-attached storage with 780 terabytes of redundant disk storage (Diskstation DS3018XS with 2 × DX1215 expansion units; Synology). A local fiber network connected the acquisition PC, computational server, and network-attached storage.

Data, Materials, and Software Availability. Microscope control and processing code is available at https://github.com/QI2lab/OPM. Data analysis code is available at https://github.com/fgdjunta/flagella3D. Deskewed images, thresholded images, and data analysis associated with this paper are available on Zenodo at https://doi.org/10.5281/zenodo.7662806. Images used for the viscosity calibrations are available on Zenodo at https://doi.org/10.5281/zenodo.7637551 (54).

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