Active phase and amplitude fluctuations of flagellar beating

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The eukaryotic flagellum beats periodically, driven by the oscillatory dynamics of molecular motors, to propel cells and pump fluids. Small, but perceivable fluctuations in the beat of individual flagella have physiological implications for synchronization in collections of flagella as well as for hydrodynamic interactions between flagellated swimmers. Here, we characterize phase and amplitude fluctuations of flagellar bending waves using shape mode analysis and limit cycle reconstruction. We report a quality factor of flagellar oscillations, $Q = 38.0 \pm 16.7$ (mean±s.e.). Our analysis shows that flagellar fluctuations are dominantly of active origin. Using a minimal model of collective motor oscillations, we demonstrate how the stochastic dynamics of individual motors can give rise to active small-number fluctuations in motor-cytoskeleton systems.

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Systems far from equilibrium such as living matter display active, non-thermal fluctuations, as well as directed motion and oscillations, which are important for biological function. As a prominent example, molecular motors coupled to cytoskeletal filaments convert chemical energy into work and heat to generate motion at the cellular scale. Motor-filament systems can drive mechanical oscillations including spontaneous hair bundles oscillations in the ear [1, 2], mitotic spindle oscillations during cell division [3], sarcomere oscillations in insect flight muscle [4], and the regular bending waves of cilia and flagella, which propel cells in a liquid including sperm and green algae [5, 6], as well as clear mucus in mammalian airways [7]. Cilia and flagella are slender cell appendages of 10–100 $\mu$m length, ubiquitously found in non-bacterial cells, which comprise a reversed cylindrical scaffold of microtubules interspersed by ten-thousands of dynein molecular motors [8].

The collective dynamics of the motors working against a visco-elastic load can give rise to flagellar oscillations by virtue of a dynamic instability [9]. Force generation by individual motors relies on the stochastic progression through a mechanico-chemical cycle [10]. The stochastic nature of force generation should manifest itself in oscillations that display a characteristic level of noise, representative of active fluctuations. Intriguingly, previous work reported phase-slips in pairs of synchronized flagella [11], and Fourier peaks of finite width in power spectra of flagellar oscillations [12]. Both observations are indicative of flagellar fluctuations. A direct measurement of flagellar fluctuations is pending, let alone a mechanistic understanding. Flagellar fluctuations impart on biological function: Phase fluctuations of flagellar beating should counter-act synchronization in collections of flagella [11], which is important for fast swimming [6] and efficient fluid pumping [7, 13]. Amplitude fluctuations will result in noisy swimming paths of flagellated swimmers and impart on hydrodynamic interactions between swimmers [14].

Here, we report direct measurements of phase and amplitude fluctuations of the flagellar beat and discuss the microscopic origin of active flagellar fluctuations using a minimal model. We further illustrate the impact of flagellar fluctuations on swimming and synchronization. Our analysis contributes to a recent interest in driven, out-of-equilibrium systems and their fluctuation fingerprint [15–18] by characterizing noisy limit cycle dynamics in an ubiquitous motility system, the flagellum.

Flagellar shape analysis. We characterize flagellar beat patterns as a superposition of principal shape modes, which will form the basis of our subsequent fluctuation analysis. In the experiments [19, 20], sperm cells swam with nearly planar beat pattern parallel to a boundary surface and were filmed at 250 frames-per-second. We characterize two-dimensional tracked flagellar shapes by their centerline positions $r(s,t)$ as a function of time $t$ and arclength $s$ along the flagellum, see Fig. 1A. We define a material frame of the swimming cell by the center $r_h(t)$ of the sperm head and two ortho-normal vectors $e_1$ and $e_2$, where $e_1$ points along the long axis of the head.

We describe the flagellar waves with respect to this material frame, introducing the tangent angle $\psi(s,t)$ enclosed by the local tangent of the flagellar centerline and the $e_1$-direction

$$r(s,t) = r_h(t) - \int_0^s \cos \psi(s',t) e_1 + \sin \psi(s',t) e_2.$$  (1)

Fig. 1B shows a kymograph of the tangent angle, which reveals the periodicity of the flagellar beat. This high-dimensional data set can be projected on a low dimensional ‘shape space’ using shape mode analysis based on linear principal component analysis. We introduce the time-average of the tangent angle $\psi_0(s) =$

\[\text{...}\]
\[ \sum_{i=1}^{n} \psi(s, t_i)/n, \] which describes the mean shape of the beating flagellum, averaged over all \( n = 1024 \) frames of a single movie. We further define a two-point correlation matrix \( M(s, s') = \sum_i [\psi(s, t_i) - \psi_0(s)] [\psi(s', t_i) - \psi_0(s')] \), where \( s, s' \) range over \( m \) equidistant arc-length positions along the flagellum. The eigenvectors \( \psi_j(s) \) of the symmetric \( m \times m \)-matrix \( M \), sorted by decreasing magnitude of the corresponding eigenvalues, characterize principal shape modes of the flagellar beat. The first two shape modes account for 95% ±1% of the variance of the tangent angle data (unless stated otherwise, all measurements are mean±s.e., \( n = 7 \) cells). We project the full data set on a two-dimensional shape space spanned by these two shape modes

\[ \psi(s, t) \approx \psi_0(s) + \beta_1(t)\psi_1(s) + \beta_2(t)\psi_2(s) \] (2)

with shape coefficients \( \beta_1, \beta_2 \) obtained by least-square fit, see Fig. 1C,D. Flagellar beating implies periodic shape changes of the flagellum, and thus noisy oscillations of the shape coefficients with mean frequency \( \omega_0 = 2\pi/T \), where \( T = 32.4 \pm 1.9 \) ms. Individually, \( \beta_1(t)\psi_1(s) \) and \( \beta_2(t)\psi_2(s) \) describe standing waves; their combination results in a traveling bending wave propagating from the proximal to the distal end of the flagellum, which propels the cell.

**Limit cycle reconstruction.** The point cloud representing subsequent flagellar shapes in Fig. 1D forms a closed loop and allows us to define a limit cycle of noisy flagellar oscillations (red line). Specifically, we fitted a smooth curve \( (\overline{\beta}_1(\varphi), \overline{\beta}_2(\varphi)) \) with \( 2\pi \)-periodic coordinate functions to the point cloud, where \( \varphi \) denotes a phase. The phase parametrization of the limit cycle is defined by the requirement that the mean of the phase speed is independent of \( \varphi \) [21]. As a consequence, \( \varphi \) is slightly different from the polar angle in the \((\beta_1, \beta_2)\)-plane. We assign a unique flagellar phase to each tracked flagellar shape by projecting the corresponding point in the \((\beta_1, \beta_2)\)-plane radially onto the limit cycle. The shape trajectory \((\beta_1(t), \beta_2(t))\) avoids the singular origin and stays in an annulus around the limit cycle, which enables us to define a continuous phase dynamics \( \varphi(t) \) and instantaneous phase speed \( \dot{\varphi} \).

**Phase fluctuations.** The instantaneous phase speed \( \dot{\varphi} \) has mean equal to the frequency \( \omega_0 \) of the beat, but can fluctuate around this mean. Phase speed fluctuations cause a decay of the phase coherence correlation function \( C(t) = \langle \exp[i(\varphi(t_0 + t) - \varphi(t_0))] \rangle \), see Fig. 1E. This decay is insensitive to measurement noise that is uncorrelated from frame to frame. The frame-to-frame phase increments \( \Delta \varphi = \varphi(t_{i+1}) - \varphi(t_i) \) are approximately normally distributed (Fig. 1F, inset). Further, the correlation time \( \tau \) of phase speed fluctuations is on the order of our temporal resolution 4 ms or below, and thus short compared to the time-scale of phase decoherence. We can thus interpret the observed phase decoherence using an idealized model of \( \delta \)-correlated phase speed fluctuations,

\[ \dot{\varphi} = \omega_0 + \zeta, \] (3)

where \( \zeta \) is Gaussian white noise with \( \langle \zeta(t)\zeta(t') \rangle = 2D\delta(t - t') \) and \( D \) denotes a phase diffusion coefficient. In this idealization, \( |C(t)| = \exp(-D|t|) \). By fitting an exponential to measured \( |C(t)| \), we obtain the phase diffusion coefficient of sperm flagellar beating, \( D = 3.2 \pm 1.9 \) s\(^{-1} \), see Fig 1E. An alternative measure for the phase stability of oscillations is the quality factor, \( Q = \omega_0/(2D) = 38.0 \pm 16.7 \), where \( \omega_0/Q \) indicates the width at half-maximum of the principal peak in the power spectral density.

The observed phase fluctuations of the flagellar beat are dominantly of active origin and surpass passive, thermal fluctuations by orders of magnitude (as suggested by earlier, indirect measurements [11]): For a simple estimate, we consider a flagellar beat that is constrained to move along the shape limit cycle with \( \varphi \) as only degree of freedom. The friction force \( F_\varphi \) conjugate to \( \varphi \) comprises hydrodynamic friction \( \gamma \varphi \) and dissipation within the flagellum. Using a simple theory of flagellar hydrodynamics known as resistive force theory [20, 22], we estimate \( \gamma \approx 3 \) pN ms\(^{-1} \). We thus obtain an upper bound \( k_B T/\gamma \approx 0.0015 \) s\(^{-1} \) for the contribution of thermal fluctuations to phase diffusion \( D \), which is a thousand-fold smaller than the value measured.

**Amplitude fluctuations.** We define a real-valued, instantaneous amplitude of the flagellar beat, \( A(t) = |\beta_1(t) + i\beta_2(t)|/\rho_0(\varphi(t)) \), normalized by \( \rho_0(\varphi) = |\overline{\beta}_1(\varphi) + i\overline{\beta}_2(\varphi)| \), and introduce the complex oscillator variable \( Z(t) = A(t) \exp[i\varphi(t)] \), which maps the shape limit cycle onto the unit circle. In our data, the amplitude \( A(t) = |Z(t)| \) is approximately normally distributed with \( \sigma_A^2 = (A(t)^2) - 1 = 0.0070 \pm 0.0023 \) [33]. The autocorrelation function of amplitude fluctuations decays with time-constant \( \tau_A = 5.9 \pm 1.8 \) ms. Interestingly, we find a negative correlation between \( A(t) \) and \( \Delta \varphi/\Delta t \), see Fig. 1F; the slope \(-\omega_1 \) of a linear regression gives \( \omega_1/\omega_0 = 0.37 \pm 0.09 \).

**Noisy normal form.** Previous theoretical work described the onset of flagellar oscillations as a supercritical Hopf bifurcation [23] with normal form \((\mu > 0) \) [24]

\[ \dot{Z} = i(\omega_c - \omega_1)|Z|^2 Z + \mu(\Lambda - |Z|^2)Z + \Xi. \] (4)

In the absence of noise, \( \Xi = 0 \), as considered originally [23], the complex oscillator variable \( Z(t) = A(t)\exp[i\varphi(t)] \) exhibits spontaneous oscillations with amplitude \( \Lambda = \Lambda^{1/2} \) for effective motor activity \( \Lambda > 0 \). In this case, we may assume \( \Lambda = 1 \) after a parameter transformation.

To study the role of fluctuations, we add a multiplicative noise term \( \Xi = Z(\zeta_1 + i\zeta_2) \) with uncorrelated Gaussian white noise variables satisfying \( \langle \zeta_j(t)\zeta_k(t') \rangle = 2D\delta(t - t') \), \( j, k \in \{A, \varphi\} \), and use Stratonovich interpretation. This choice represents the simplest...
D phase diffusion coefficient is that in the special case
the full parametrization of eq. (4) (with \( \Lambda = 1 \)). Note
on microscopic parameters, we now discuss a mini-
additive noise. Inset: phase increments are approximately normally
ellar shape by projection onto this limit cycle.

A minimal model for noisy motor oscillations. We exemplify how a finite collection of motors drives spontaneous oscillations with characteristic small-number-fluctuations using the classical rigid two-state model [9, 26] in its most simple form: A collection of \( N \) motors, rigidly (and equidistantly) attached to an inextensible backbone interacts with a filament through an effective interaction potential, \( W(x) = U[1 - \cos(2\pi x/l)] \), see Fig. 2A. Here \( x \) is the coordinate of the motor along the filament, and \( l \) the periodicity of the filament. Individual motors can bind to and unbind from the filament with transition rates \( \omega_{on}(x) = \Omega[\eta - \alpha \cos(2\pi x/l)] \) and \( \omega_{off} = \Omega - \omega_{on} \), respectively. Here, \( \eta \) denotes the mean fraction of attached motors (“duty ratio”). Importantly, the binding rates are spatially inhomogeneous as characterized by \( \alpha \) and break detailed balance. If the filament is now coupled to the backbone by a visco-elastic element with viscosity \( \xi \) and elastic stiffness \( k \), we obtain a force-balance equation for the position \( X(t) \) of the filament, \( kX + \xi X = F_{in} \) with \( F_{in} = -\sum_i \delta X W(x_i - X) \), where the sum extends over all bound motors and \( x_i = il/N \) is a simple choice for the positions of the motors along the backbone.

To properly define a thermodynamic limit for large motor numbers \( N \), we rescale stiffness and viscosity as \( k = k_0N \) and \( \xi = \xi_0N \). In the limit \( N \to \infty \), the system can exhibit spontaneous oscillations by a supercritical Hopf-bifurcation, when the normalized motor activity \( \xi_0/\xi = 2\pi^2\alpha NU/(\Omega^2\xi) \) exceeds the threshold \( 1 + \nu \), where \( \nu = k/(\xi \Omega) \) \( [26] \). For a finite motor number, we numerically observe noisy oscillations, see Fig. 2.

In the following, we will analytically compute the quality factor \( Q \) in the limit of large (yet finite) motor number \( N \), assuming that we are close to the Hopf bifurcation with \( \varepsilon = \xi_0/\xi - 1 - \nu \) positive and small. Following [26], we first approximated the discrete jump processes that describe the binding and unbinding dynamics of the individual motors by a diffusion approximation, thus arriving at a Fokker-Planck equation for the joint probability distribution of filament position and motor binding states. This density \( \rho(x) \) of bound motors can be expanded into an infinite series of spatial Fourier modes. From the Fokker-Planck equation, we obtain a coupled system of stochastic dynamic equations for these Fourier coefficients. Due to the simple choice of potential \( W(x) \), which comprise only the first Fourier mode, and the constant rate approximation, the first Fourier mode of \( \rho(x) \) decouples from the higher modes, resulting in a 3-dimensional stochastic system [26]. Using a near-identity transformation and center manifold technique as described in [24], this system can be brought into Hopf normal form eq. (4) with oscillator variable \( Z \) satisfying \( \Re Z = X/l + O(\varepsilon^{3/2}) \) and phase-dependent noise term \( \Xi = i\zeta \), where \( \langle \zeta(t)\zeta(t') \rangle = 4\Lambda \delta(t - t') \) \( [34] \). The qual-
The quality factor $Q = \frac{\omega_0}{2D}$ is found to scale with $N$

$$Q \approx \frac{\omega_0}{2\Omega} \frac{N\lambda}{\eta(1-\eta)} \left( \frac{2\pi\alpha}{\sqrt{\nu} + 1/\sqrt{\nu}} \right)^2 .$$

Furthermore, $\Lambda \approx \varepsilon(1+4\nu)/(3\pi^2\nu(1+2\nu))$, $\mu \approx \Omega\varepsilon/(2\Lambda)$, $\omega_0 = \omega_c - \omega_1 \approx \Omega\sqrt{\sigma^2 + \varepsilon^2/(2+4\nu)}$. Interestingly, the motor duty ratio $\eta$ controls oscillation quality, although $\eta$ affects neither amplitude nor frequency (for $N \to \infty$). To understand this, note that the total number of bound motors fluctuates with mean $\eta N$ and variance $\eta(1-\eta)N$. This number characterizes a spatially homogeneous “background” of bound motors, which does not contribute directly to the oscillations, but sets the amplitude of motor density fluctuations responsible for phase diffusion. Oscillations become also more regular for increasing amplitude. The approximation eq. (5) and simulations of the full model agree well close to the Hopf bifurcation, see Fig. 2. This minimal motor model recapitulates the experimental observation of phase diffusion in a minimal setting and illustrates how noisy oscillations can arise from small-number-fluctuations.

**Flagellar fluctuation imply non-deterministic swimming:** Using measured noise strengths, we simulated realistic beat patterns and corresponding stochastic swimming paths, see Fig. 3A. Specifically, we (i) use eq. (4) to simulate $Z(t) = A(t) \exp[i\varphi(t)]$, (ii) construct shape coefficients $\beta_1(t) + i\beta_2(t) = A(t)\rho_0[\varphi(t)]$, and tangent angles $\psi(s,t)$ by eq. (2), (iii) compute the path $r_N(t)$ using resistive force theory [22] as described in [20]. We find that the center $R(t)$ of sperm swimming circles diffuses with diffusion coefficient $D_R = 3.3 \mu m^2/s$, which is on the same order of magnitude, albeit smaller, than a value $D_R = 9 \pm 2 \mu m^2/s$ measured for sea urchin sperm [12]. Our analysis includes amplitude and phase fluctuations, but neglects additional shape fluctuations; thus our value is a lower bound.

Interestingly, amplitude and phase fluctuations affect the swimming path differently. Phase fluctuations, in the absence of amplitude fluctuations, cause fluctuations in swimming speed, but not change the shape of the path. This is because the Stokes equation governing self-propulsion at low Reynolds numbers [27] is invariant under (stochastic) re-parametrizations of time.

Phase fluctuations cause phase slips in pairs of synchronized flagella, e.g. in the green algae *Chlamydomonas* [11]. *Chlamydomonas* swims with two flagella, which can synchronize their beat. Analysis of phase-slips allowed a previous estimate of the phase diffusion coefficient of individual flagella $D \approx 6 s^{-1}$ [11]. These earlier, indirect measurements match our direct measurements, if we account for differences in beat frequency (*Chlamydomonas*: 50 Hz, sperm: 30 Hz), yielding $Q = \omega_0/(2D) \approx 25$ and $Q = 38 \pm 16.7$, respectively. Note that differences in flagellar length $L$ may also affect $D$ (*Chlamydomonas*: 10 µm, sperm: 50 µm). Interestingly, flagellar synchronization in *Chlamydomonas* seems to operate just below a tolerable level of noise: Consider the approximate dy-
ynamics of the phase difference $\delta$ between two identical, coupled oscillators, $\delta = -\lambda/T\sin\delta + \zeta$, where $\zeta$ is Gaussian white noise with $\langle\zeta(t)\zeta(t')\rangle = 4D\delta(t - t')$ [11, 28]. Here $\lambda$ denotes a synchronization strength, which had been estimated as $\lambda \approx 0.3$ [11]. Using these values, we find $AQ \approx 10$, which yields robust synchronization. A ten-fold higher noise level, however, implies failure of synchronization, see Fig. 3B.

Conclusion. The beating flagellum is a noisy oscillator, driven by $N \approx 8 \cdot 10^4$ dynein motor domains [8]. Here, we precisely measured its phase and amplitude fluctuations, using a novel method of shape mode analysis [29, 30]. We obtain a quality factor $Q = 38 \pm 16.7$ of flagellar oscillations. Values estimated in other cytoskeletal oscillators are $Q = 2.2 \pm 1.0$ ($N \approx 2500$) for spontaneous hair bundle oscillations [31, 32], and $Q = 1.4 \pm 1.1$ ($N = 10 - 100$) for an in-vitro acto-miosin system [16]. We find that the strength of flagellar phase fluctuations is several orders-of-magnitudes above the level corresponding to thermal noise, highlighting the active origin of flagellar fluctuations.

We compute the quality factor $Q$ in a minimal model of motor-filament oscillations, and find that $Q$ is proportional to the number of motors. A simple numerical example with parameters given in [35], leads to noisy oscillations with amplitude, frequency, and quality factor, $A_l \approx 68$ nm, $\omega_0 \approx 228$ s$^{-1}$, $Q \approx 33$, respectively, which roughly match measured values ($A_l \approx 100$ nm, $\omega_0 \approx 200$ s$^{-1}$) [19], $Q \approx 38$. Our analytic approximation eq. (5) is not applicable for these large-amplitude-oscillations. Note that the model does not fully capture flagellar oscillations quantitatively because it strongly simplifies flagellar geometry and motor dynamics.

We show that phase and amplitude fluctuations affect sperm swimming differently: Whereas amplitude fluctuations cause an effective diffusion of sperm swimming circles, phase fluctuations imply speed fluctuations, but do not change the shape of the path. Additionally, phase fluctuations introduce phase-slips in collections of synchronized flagella [11].

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