Supplementary Materials for

**Human sperm uses asymmetric and anisotropic flagellar controls to regulate swimming symmetry and cell steering**

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The PDF file includes:

- Legends for movies S1 to S6
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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/31/eaba5168/DC1)

- Movies S1 to S6
Video S1: Original 3D microscope stack for the sperm sp6 swimming near to the coverslip and depicted in Fig. 2(a) Main Text. See Material and Methods section for more details on the 3D imaging.

Video S2: 3D microscope stack and flagellar reconstruction showing both head spinning and flagellar rolling around the swimming axis for the sperm sp6 swimming near to the coverslip, and depicted in Fig. 2(a) Main Text. Red marker tracks the trajectory of the mid-flagellar point of the sperm, also known as flagelloid. See Material and Methods section for more details.

Video S3: Flagellar waveform relative to the laboratory fixed frame of reference \((x, y, z)\) (Methods) for the sperm sp6 swimming near to the coverslip, as depicted in Fig. 2(a) Main Text. Colour progression of the waveform through the flagellar rolling cycle is indicated by the cyclic colormap inset in Fig. 2 Main Text.

Video S4: Flagellar waveform relative to the comoving frame of reference \((x_c, y_c, z_c)\) (Methods) for the sperm sp6 swimming near to the coverslip, as depicted in Fig. 2(c) Main Text. Colour progression of the waveform through the flagellar rolling cycle is indicated by the cyclic colormap inset in Fig. 2 Main Text.

Video S5: Flagellar waveform relative to the comoving-rolling frame of reference \((x_{cr}, y_{cr}, z_{cr})\) (Methods) for the sperm sp6 swimming near to the coverslip, depicted in Fig. 3(e) Main Text. Colour progression of the waveform through the flagellar rolling cycle is indicated by the cyclic colormap inset in Fig. 3 Main Text. See Material and Methods section for more details.

Video S6: Original video-microscopy of a non-progressive and rolling spermatozoa. Middle imaging plane showing the non-progressive sperm sp29 prevented to swim forward due to the presence of an obstacle in its path immediately above the coverslip (Methods). Flagellar rolling is not suppressed by the obstacle, however the flagellar rolling direction is opposite to the rolling direction of free swimming cells, as discussed in the Main Text.

The 3D flagellar wave envelope at the comoving frame of reference

The 3D wave envelope has a symmetric bullet-shape SI-Fig. 1(a), in contrast with the conical helix often postulated [9, 10, 42]. Spermatozoa swimming far from the coverslip displayed a more symmetric flagellar envelope (red in SI-Fig. 1(a)), with a nearly circular cross-section (bottom row), when compared with cells swimming near to the coverslip (blue in SI-Fig. 1(a)), which is characterised instead by a “bent” bullet-shape envelope resembling a bird’s beak. The nearby coverslip is thus a weak source of asymmetry to the flagellar beat. The amplitude of the flagellar wave is also reduced for spermatozoa swimming near to the coverslip, potentially due to the hydrodynamic interactions between the flagellum and the solid boundary of the coverslip [6, 43, 61]. The volume ratio of the wave envelope observed between cells swimming near to and far from the coverslip was \(V_{near}/V_{far} = 0.75\).

The flagellar beat oscillates between a purely planar and weakly non-planar waveforms

The waveform oscillates between a purely planar and weakly non-planar beating at each instant, SI-Fig. 1(b,c), in agreement with earlier observations [12]. The average ratio between the minor and major axis of the flagellar inertia ellipsoid for the cells “near to” and “far from” the coverslip (Methods) appears to be conserved across the population with \(c/a = 0.06\), characterised by flat ellipsoids. Thus only a small non-planar component of the waveform can induce large flagellar excursions in 3D, see Fig. 2(c,d) Main Text. The proximity to the coverslip does not appear to influence the weak non-planarity of beat, as show in SI-Fig. 1(c). We also emphasise that the quasi-planar oscillations observed here, and in Ref. [12], occur in the time-scale as the flagellar beat cycle. This is in contrast with the persistent two-dimensional slithering motion reported by Nosrati et al. [42], in which 2D slithering episodes lasted for approximately 1s and for distances of 70\(\mu m\) for free-swimming human sperm near to surfaces. The observed slithering motion, however, only occurred for human sperm swimming in high viscosity, as no slither swimmers were detected in low viscosity for human sperm [42]. This is in further agreement with our
experiments which focused on human sperm migrating in low viscosity (Methods). Human sperm is well-known to modulate its flagellar beat to nearly planar waveforms when swimming in high viscosity [4], thus slithering motion is expected in thicker fluids [4, 42].

The swimming angle of attack, however, is regulated by the proximity to the coverslip [43, 61], as shown in SI-Fig. 1(d). Spermatozoa far from the coverslip are able to swim both towards and away from the boundary, whilst all spermatozoa swimming near to the coverslip displayed a conserved angle of attack of $-7^\circ$, directed towards the surface, SI-Fig. 1(d). Interestingly, whilst the angle of attack oscillates around its mean, the amplitude of oscillation allows sign changes over the course of time even for the sperm swimming near to the surface. This indicates that the sperm flagellum is directed away from the coverslip for short periods when swimming close to the boundary, blue bars in SI-Fig. 1(d).

### 3D vs 2D waveform characteristics

SI-Figs. 24, 25 shows the typical discrepancy between the Fourier spectrum of the 3D curvature, $k$, and the 2D curvature, $k_{2d}$, as seen with a 2D microscope (grey curves in Fig. 2(c,d) Main Text). The symmetry of the beat in 2D renders the static mode of $k_{2d}$ negligible, SI-Fig. 15, thus the asymmetry of the beating is not detectable. The 2D curvature displays the usual two-frequency peaks (SI-Fig. 25), instead of the single-peaked frequency observed for the 3D curvature (SI-Fig. 24). The 1st and 2nd frequency peaks of the 2D curvature are postulated to be related with, respectively, the head spinning and the beating frequencies. The beating frequency is strongly correlated with 2nd peak-frequency of $k_{2d}$ ($R=0.82$, SI-Fig. 2(d)) though, in average, the 2nd peak-frequency underestimates the 3D beating frequency (SI-Fig. 2). Furthermore, no correlation was found between the 1st frequency-peak of $k_{2d}$ and the head spinning, SI-Fig. 2(b), also in agreement with [4]. The 1st frequency-peak $\omega_{1,2d}$ correlates instead with the head spinning frequency SI-Fig. 2(c), as expected from the 2D projection of the 3D beat. No correlation is observed between the head spinning and flagellar rolling in SI-Fig. 2(a), as expected from the asymmetry of the beat in Fig. 6 Main Text, as detailed in Discussion.

A correction was proposed by Rikmenspoel [62], for the head spinning and beating frequencies, respectively, given by $\omega_{\text{spin}}^{r,k} = (\omega_{1,2d}^2 - \omega_{2,2d}^2)/2$, $\omega_{\text{rk}}^{r,k} = (\omega_{1,2d}^2 + \omega_{2,2d}^2)/2$, where $\omega_{1,2d}^2$, $\omega_{2,2d}^2$ are respectively the first two peaks of the 2D spectrum of the beat. The corrected $\omega_{\text{spin}}^{r,k}$ and $\omega_{\text{rk}}^{r,k}$ correlates significantly, respectively, with the head spinning frequency in SI-Fig. 2(e) and beating frequency SI-Fig. 2(f). However, the estimated beating frequency, $\omega_{\text{rk}}^{r,k}$, has a slightly lower correlation ($R=0.78$) with the beating frequency than the 2nd frequency-peak of the 2D curvature without the correction ($R=0.82$) in SI-Fig. 2(d).

The dynamical mode of the 2D curvature (2nd peak-frequency mode) is overestimated by 35% when compared with the 3D case, SI-Fig. 15. Nevertheless, because of the absence of the static mode in the 2D case, the overall amplitude of the 2D curvature is overestimated by approximately fivefold, when compared with the maximum of the average 3D curvature (SI-Fig. 15). This biases the waveform in such a way that the abrupt increase in 3D curvature at the midpiece is missed (SI-Fig. 15 top-row). The 2D curvature thus shows a misleading linear increase in arclength (SI-Figs. 15, last column, top and middle-rows), since the static mode is approximately zero. This linear curvature modulation in 2D has been the basis of numerous model assumptions, as well as postulations of the mechanical properties the mammalian sperm flagellum [2, 4, 6, 15]. Furthermore, the wave characteristics of $k_{2d}$ underestimated the beating frequency by 12%, and overestimated the wavelength by 34%, and wavespeed by 26% for the spermatozoa swimming near to the coverslip, in which the planar projection is possible.

### 2D microscope projection fails to capture flagellar beating asymmetry

The 2D projection of the waveform is unable to detect the intrinsic asymmetry of the waveform and biases the general waveform characteristics (SI-Fig. 15). Despite the good qualitative agreement between the 2D and 3D dynamic modes of the curvature SI-Fig. 15, the lack of static mode for the symmetric bending waves in 2D implies that the amplitude of 2D curvature increases linearly [4, 6], which contradicts the observations in 3D. More importantly, the symmetry of the 2D microscope projection leads to the acceptance of the symmetry of the beat, partly straight-line swimming in human spermatozoa is a direct result of symmetric bending waves with a linear increase in amplitude along the flagellum. Therefore, it is necessary to reassess observations of symmetric beating in 2D for rolling spermatozoa, and observations of asymmetric waveforms, such as during sperm hyperactivation.

The beating symmetry is the cornerstone in all sperm swimming analysis to date, from computer assisted semen analysis (CASA) parameters [2, 4, 8, 51], such head trajectories, linear and curvilinear paths, amplitude of lateral displacement, from which progressive and non-progressive categories are defined, often associated with broken-symmetry of the beat, including modern kinematic analysis of 2D flagellar wave characteristics [5, 7, 8, 63], in which biased waveform symmetry would entail circular swimming trajectories and non-progressive motion. Equally, human sperm reactivation, capacitation and hyperactivation assays [50] rely on deviations from control symmetric beating patterns. Despite the ongoing debate on how to measure sperm hyperactivation [50], the asymmetry of bending waves is still widely used. The intrinsic asymmetric bending of human sperm observed
here may thus have an impact on the study of hyperactivation.

Theoretical models invoke beating symmetry widely [6, 15, 16, 18, 20, 64], from flagellar waveform analysis, to computational fluid mechanics simulations from imaging data [2, 8], mathematical predictions of swimming behaviour, derived from either waveform observations or via mathematical prescription [48], and even, molecular motor control hypotheses [15–18, 23, 65], in which symmetric spatial distribution of molecular motors along the flagellum is assumed, for both predictions and models fitting with experiments. Likewise, symmetry arguments are also commonly hypothesised for the 3D helical beating [9, 10, 42], thus deeming spermatozoa rolling self-organization as a trivial outcome. More research is thus needed to elucidate the consequences of the observed beating anisotropy and asymmetry in both empirical and theoretical studies conducted thus far.

The flagellar beats of free-swimming and rolling vs tethered sperm

Tethered sperm experiments are commonly employed to study flagellar beat, especially when involving intricate flagellar reactivation and hyperactivation procedures [5, 11, 50, 66, 67]. Extrapolations of tethered sperm beating to freely swimming sperm behaviour have been widely accepted, driven by the abundance of 2D microscope observations showing straight-line swimming for apparently symmetric side-to-side movement of the flagellum [68]. However, the planar and symmetric bending waves of tethered spermatozoa [5, 50] are markedly distinct from the 3D asymmetric and anisotropic flagellar movement observed here (Fig. 2 Main Text). This indicates that flagellar self-organisation in sperm is modulated by mechanical constraints imposed to the cell. The physical forces and torques required to tether sperm to the coverslip suppress both head swimming and 3D flagellar rolling intrinsic to freely swimming cells (Fig. 2 Main Text). Furthermore, although 2D observations of tethered sperm have raised questions about the impact of higher beat frequency components on the steering of free swimming sperm [5, 68], the major differences between tethered and free-swimming and rolling cells have not been taken into account.

Our 3D observations demonstrate that the sperm flagellum is modulated differently when the head is tethered. The flagellar waveform is a direct result of the balance of a triad of contributions, namely hydrodynamic drag, flagellum elasticity and molecular-motor activity [2]. Molecular-motors are mechanotransducers well known to self-regulate spontaneous oscillations according to external loads [69–73], such as the additional forces/torques introduced by sperm head tethering [74]. For this reason, flagellar systems are incredibly sensitive to mechanical changes in the environment. Notable examples include bending modulation of the human sperm flagellum by fluid viscosity [4, 75], flagellar synchronisation via hydrodynamic interactions [69, 76, 77], and the 3D amplitude modulation reported here due to the proximity of the sperm to the coverslip (Fig 5, Main Text).

The sperm flagellum elastohydrodynamics [20, 48, 78], in which the fluid drag and flagellum elasticity are mathematically intertwined, also depend dramatically on the nature of external forces and torques at flagellum end points, known as boundary conditions of the elastohydrodynamic system [15, 16, 23, 79]. These boundary conditions define whether, for example, the sperm head is clamped/pinned (requiring external forces/torques to enforce this constraint), or free from external forces/torques, as in the free swimming case [15, 16, 18, 20, 23, 48, 74, 78, 79]. Most importantly, each of different head condition leads to very different waveforms [ibid], and even dictates the travelling wave direction of self-organisation control models [15, 16, 18, 23]. The same is true for the fluid flow around a free swimming sperm, and associated viscous stresses and hydrodynamic forces, which differ substantially between free and tethered sperm [6, 80–83]. These discrepancies are equally pronounced for period-averaged fluid flow measurements around a free swimming sperm, which displays the characteristic dipolar structure, as expected from a force-free Green’s function of the Stokes equation, while tethered sperm is given by a direct “point force” or stokeslet [ibid].

As a result, large mechanical effects are introduced at all levels by physically constraining the sperm motion. This is instigated by additional (external) forces and torques imparted by the coverslip to the sperm flagellum via the tethered head. As such, this causes dramatic changes in the fluid mechanics, solid mechanics and molecular-motor dynamics, and leads to the modulation of the beat patterns in spermatozoa, due to their combined action. Therefore, tethered sperm experiments alone cannot be immediately extrapolated to inform free-swimming behaviour in human spermatozoa.

Flagellar buckling may explain bidirectional rolling in human sperm

Ishijima reported that 57% of human sperm cells rolled counterclockwise (relative to the posterior end), with the remainder of the cells rolling in the opposite direction [44]. This appears to contradict earlier reports on purely unidirectional rolling [13], and unidirectional rolling with short intermissions in the opposite direction [12], as well as unidirectional rolling [14], though in bull spermatozoa and hamster spermatozoa [37]. Only two human sperm cells were observed to display counterclockwise rolling in our experiments (sp29 and sp30 in SI-Fig. 4). Interestingly, they were prevented from swimming freely due to obstacles in their path, though they were still able to roll (see video).

Coincidently, the experimental setup used by Ishijima
to identify the cell rolling direction required spermatozoa to be pinned at the coverslip with the wave axes of the beating flagellum oriented nearly vertical to the coverslip, similarly to the configurations of sp29 and sp30. Thus, the discrepancy between the observations of the direction of cell rolling may be related to the specific modulation of the sperm flagellum when the cell is pinned. This could be achieved, for example, via molecular motor mechano-transduction, given the increase in hydrodynamic load experienced by the flagellum when the sperm is not swimming freely (as also discussed in the section above), or equally via induced snap-through buckling of the the static asymmetric mode (SI-Fig. 13), as additional compression forces arising from the head attachment could induce the necessary sign change in the spiral handedness and consequently switching the cell rolling direction, as discussed in the Main Text. This might explain, for example, the observed switch in rolling direction during rheotaxis of human spermatozoa. Indeed, localised buckling at the midpiece was found to correlate with the turning direction during rheotaxis sperm reorientation [12]. These results emphasise the critical importance of the static asymmetric helical mode in determining the rolling direction, and subsequent sperm response to changes in the environment [10].
SI-Fig. 1: **Human sperm flagellar beating near to and far from the coverslip in 3D.** (a) compares the 3D flagellar envelope relative to the comoving frame of reference for sperm near (blue) to and far (red) from the coverslip across the free-swimming population (see Methods). Flagellar modulation induced by the proximity to the coverslip is apparent. (b-d) The flagellar Inertia ellipsoid capturing the three-dimensionality of the beat as described in the Methods: (b) The inertia ellipsoid and its ellipsoidal axes at a given instant for the spermatozoa sp6 and sp23 depicted in Fig. 2(a,b) Main Text, respectively, in red and blue. (c) The variation of ratio between the minor and major axis of the inertia ellipsoid $c/a$ at each instant for free swimming sperm sp1-sp28, respectively, for each horizontal bar from bottom to top. Sperm immediately above the coverslip (sp1-sp20) and far from the coverslip (sp21-sp28) are shown, respectively, by the blue and green bars, the yellow marker shows the temporal average. If $c/a = 0$ the waveform is a planar curve at this instant. The flagellar beat oscillates between a planar and a weakly non-planar waveform. (d) The vertical orientation of the major axis of the inertia ellipsoid relative to the coverslip ($xy$-plane), as shown by the red vectors in (b), defines the angle of attack of the flagellum at each instant: negative (positive) angles indicate that the sperm is swimming towards (away from) the coverslip. (d) uses the same colour scheme as in (c). Spermatozoa swimming near to the coverslip displayed a conserved angle of attack of $-7^\circ$, with the average orientation of the sperm flagellum directed towards the coverslip.
SI-Fig. 2: 3D vs. 2D curvature, head spinning and flagellar rolling. (a) Very weak correlation between the head spinning frequency, $\omega_{\text{spin}}$, and the rolling frequency of the sperm flagellum around the swimming axis, $\omega_{\text{roll}}$. (b) No correlation exists between the $1^{\text{st}}$ frequency-peak of 2D curvature, $\omega_{1,2d}^\kappa$, and the head spinning $\omega_{\text{spin}}$. (c) The $1^{\text{st}}$ frequency-peak of 2D curvature, $\omega_{1,2d}^\kappa$, correlates instead with the flagellar rolling around the swimming axis, $\omega_{\text{roll}}$. (d) The $2^{\text{nd}}$ frequency-peak of 2D curvature, $\omega_{2,2d}^\kappa$, is strongly correlated with the beating frequency, defined by the $1^{\text{st}}$ peak of the Fourier spectrum of the curvature $\omega_{\kappa}$. (e,f) Corrections proposed by Rikmenspoel for the head spinning frequency $\omega_{\text{spin}}^{R}$ (e) and beating frequency $\omega_{\kappa}^{R}$ (f), derived from the two-peaked frequency spectrum of the 2D curvature $\kappa_{2d}$ in SI-Fig. 24, as detailed in SI text.
SI-Fig. 3: The flagellar waveform relative to the comoving frame of reference $(x_c, y_c, z_c)$. Red curve depicting the trajectory of the mid flagellar point, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Transformation between the frames of reference is detailed in Methods.
SI-Fig. 4: **Flagelloid patterns.** Flagelloids are defined as the projection of the mid-flagellar trajectory at the rolling plane (Methods). Flagelloid patterns are shown for, respectively, spermatoza near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip (Methods). Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were still able to rotate around their rolling axis (see SI video). Spermatozoa sp1-28 rotate clockwise (when seen from the posterior end of the flagellum), whilst sp29-30 rotate counterclockwise. A bewildering array of geometrical patterns is apparent, from rotating star-shapes to triangles, squares and looping patterns with polar symmetry. Flagelloid patterns are not a distinguishing feature of cells swimming near to and far from the coverslip.
SI-Fig. 5: The flagellar waveform relative to the comoving-rolling frame of reference \((x_{cr}, y_{cr}, z_{cr})\). Waveforms, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Transformation between the frames of reference is detailed in Methods.
SI-Fig. 6: The principal component analysis (PCA) reconstruction of the waveform at the comoving frame of reference. First two PCA modes, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Red curves depict the trajectory of the mid-flagellar point. Principal Component Analysis is detailed in Methods.
SI-Fig. 7: The principal component analysis (PCA) reconstruction of the waveform at the comoving-rolling frame of reference. First two PCA modes, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Principal Component Analysis is detailed in Methods.
SI-Fig. 8: **PCA surfaces for the comoving frame of reference.** The surfaces generated by the 1\(^{st}\) PCA mode (blue), $X_{pca1}(s)$, and 2\(^{nd}\) PCA mode (orange), $X_{pca2}(s)$, in the course of time, for two different views (left and right plots in each subplot) for the waveform reconstruction at the comoving frame of reference, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Principal Component Analysis is detailed in Methods.
SI-Fig. 9: **PCA surfaces for the comoving-rolling frame of reference.** The surfaces generated by the 1st PCA mode (blue), $X_{pca}^1(s)$, and 2nd PCA mode (orange), $X_{pca}^2(s)$, in the course of time, for two different views (left and right plots in each subplot) for the waveform reconstruction at the comoving-rolling frame of reference, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Principal Component Analysis is detailed in Methods.
SI-Fig. 10: Amplitude of the PCA modes for the comoving frame of reference. Shape scores \( A_1(t), A_2(t) \) of the first two principal component modes for the waveform reconstruction at the comoving frame of reference, i.e. \( X_c \approx A_1(t)X_{pca}^1(s) + A_2(t)X_{pca}^2(s) \), where \( X_{pca}^1(s) \) is the first and \( X_{pca}^2(s) \) is the second PCA spatial modes, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Principal Component Analysis is detailed in Methods.

SI-Fig. 11: Amplitude of the PCA modes for the comoving-rolling frame of reference. Shape scores \( A_1(t), A_2(t) \) of the first two principal component modes for the waveform reconstruction at the comoving-rolling frame of reference, i.e. \( X_{cr} \approx A_1(t)X_{pca}^1(s) + A_2(t)X_{pca}^2(s) \), where \( X_{pca}^1(s) \) is the first and \( X_{pca}^2(s) \) is the second PCA spatial modes, respectively, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Principal Component Analysis is detailed in Methods.
SI-Fig. 12: The coverslip modulates the flagellar beat anisotropically in 3D in the comoving frame of reference. (a-c) each row, from top to bottom: amplitude of the static and dynamic modes, and phase, respectively, of $y_c$ for sperm near to (a) and far from (b) the coverslip, with black curves depicting averages in the sperm population. (c) compares the averages of $y_c$ across the population for spermatozoa near to (blue) and far from (red) the coverslip. (d-f) each row, from top to bottom: amplitude of the static and dynamic modes, and phase, respectively, of $z_c$ for sperm near to (d) and far from (e) the coverslip, with black curves depicting averages in the sperm population. (f) compares the averages of $z_c$ across the population for spermatozoa near to (blue) and far from (red) the coverslip. Fourier analysis is detailed in Methods.

SI-Fig. 13: The coverslip modulates the flagellar beat anisotropically in 3D in the comoving-rolling frame of reference. (a-c) each row, from top to bottom: amplitude of the static and dynamic modes, and phase, respectively, of $y_{cr}$ for sperm near to (a) and far from (b) the coverslip, with black curves depicting averages in the sperm population. (c) compares the averages of $y_{cr}$ across the population for spermatozoa near to (blue) and far from (red) the coverslip. (d-f) each row, from top to bottom: amplitude of the static and dynamic modes, and phase, respectively, of $z_{cr}$ for sperm near to (d) and far from (e) the coverslip, with black curves depicting averages in the sperm population. (f) compares the averages of $z_{cr}$ across the population for spermatozoa near to (blue) and far from (red) the coverslip. Fourier analysis and cell populations are detailed in Methods.
SI-Fig. 14: **The coverslip modulates the flagellar beat curvature in 3D.** Each row, from top to bottom: amplitude of the static and dynamic modes, and phase, respectively, of 3D curvature $\kappa$ for sperm near to (1st-column) and far from (2nd-column) the coverslip, with black curves depicting averages in the sperm population. 3rd-column compares the averages across the population for spermatozoa near to (blue) and far from (red) the coverslip. Fourier analysis and cell populations are detailed in Methods.

SI-Fig. 15: **2D microscopy fails to capture beating asymmetry in the curvature.** Each row, from top to bottom: amplitude of the static and dynamic modes, and phase, respectively, for 3D curvature $\kappa$ (1st-column) and the 2D curvature $\kappa_{2d}$ (2nd-column), with black curves depicting averages in the sperm population. 3rd-column compares the averages across the population for the 3D curvature $\kappa$ (blue) and the 2D curvature $\kappa_{2d}$ (red). Fourier analysis and cell populations are detailed in Methods.
SI-Fig. 16: **Comoving frame of reference** $y_c$ **coordinate**. Kymographs as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 17: Comoving frame of reference $z_c$ coordinate. Kymographs as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 18: Comoving-rolling frame of reference coordinate is a travelling wave. Kymographs as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 19: Comoving-rolling frame of reference $z_{cr}$ coordinate pulsates in time. Kymographs showing the pulsations of $z_{cr}$ as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. Note that $z_{cr}$ for sp29 and sp30 are sign-inverted in respect to the free swimming cells.
SI-Fig. 20: Travelling waves of curvature in 3D. Kymographs of the curvature $\kappa$ as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 21: Travelling waves of perversion and waveform torsion in 3D. Kymographs of the torsion $\tau$ as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 22: Curvature waves of the flagellar spiral. Kymographs of the spiral’s curvature $\kappa_s$ (waveform projection in the rolling plane) as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 23: Travelling waves of chirality show the lack of persistent handedness of the 3D flagellar beat. Kymographs of the chirality $\psi$ as a function of arclength $s$ and time $t$ for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4.
SI-Fig. 24: Fourier spectrum of the flagellar beat in 3D. Average Fourier spectrum of the 3D curvature $\kappa$ in arclength, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. The Frequency spectrum is characterised by a single main frequency-peak $\omega_{\kappa}$.
SI-Fig. 25: 2D projection of the flagellar waveform introduces low frequency peaks in the Fourier spectrum of the beat. Average Fourier spectrum of the 2D curvature $\kappa_{2d}$ in arclength, obtained by projecting the 3D waveform in the $xy$-plane at the comoving frame of reference, for spermatozoa near to (sp1 to sp20) and far from (sp21 to sp28) the coverslip. Sperm cells sp29 and sp30 were prevented to swim freely due to obstacles in their paths, though they were able to rotate around their rolling axis, see SI-Fig. 4. The Frequency spectrum is characterised by two frequency-peaks $\omega_{1,2}$. Static modes observed for cells swimming far from the coverslip are due directional bias introduced by projecting waveforms with a large angle of attack in the $xy$ plane (SI-Fig. 1).
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