Supplementary Note: Mechanical description of focal adhesion-independent cell migration

Contents

We propose here a theoretical description of the mechanics of non-adherent blebbing Walker cells migrating in microchannels. Our assumptions on cell mechanics and the main equations are detailed in Section 1. We focus on the mechanics of the actomyosin cortical network, since it is the structure powering cell motion (Supplementary Fig. 1f), and describe the actin cortex as a thin layer of active, viscous fluid. This essential assumption is captured in Eq. 7, which states that the tension in the cortex is a sum of a viscous part, proportional to the gradient of flow, and an active part, reflecting internal contractile tension. The internal contractile tension is generated by myosin II motor proteins and thus depends on their concentration: the polarised myosin distribution observed in cell migration (Supplementary Fig. 1g, Supplementary Fig. 3b) translates into a gradient of internal tension, which we assume to be proportional to the gradient in myosin intensity (Supplementary Fig. 1i). A gradient in active tension can drive actomyosin flows in the cortical layer, and deformations of the cell surface. We assume that the cell shape is confined to a cylinder in the region where it is in contact with the microchannel walls, and that the rear and front pole regions are free to take the shape satisfying force balance (Fig. 3a, Supplementary Fig. 3b).
In addition to the stresses arising in the actomyosin cortex, two external forces act on the cell when it moves in the microchannel:

- (i) Forces between the cortex and the channel walls: we assume that cortical flows in the region in contact with the channel are resisted by a tangential friction force, proportional to the velocity of the flow relative to the channel wall. A friction coefficient α characterizes this proportionality relation (Eq. 11). Possibly, transient binding and unbinding events between proteins at the cell surface and the channel coating are contributing to the friction coefficient.
- (ii) Forces arising from the fluid surrounding the cell: to take into account forces arising from the external medium, we introduce an effective drag coefficient arising from the motion of the external medium, which must flow around or through the cell, or be pushed forward as the cell moves. In Section 3, we evaluate the different contributions to the effective drag acting against cell motion in a channel.

In Section 2, we solve the model equations analytically and obtain expressions for the cell shape, cell velocity and the cortical flow profile as a function of the mechanical parameters. Our central result, the expression for the cell velocity (Eq. 45), indicates that myosin-dependent cortical flows produce sufficient thrust to propel the cell through the surrounding fluid only above a critical threshold of substrate friction (see Fig. 3b and Supplementary Fig. 3f). The threshold friction coefficient depends on the drag coefficient and on the shape of the cell. For friction coefficients above the threshold, the mechanism for cell migration can be understood as follows: the active tension gradient gives rise to rearward cortical flows, which are opposed by friction at the cell-channel contact surface, giving rise to a force propelling the cell forward (Supplementary Fig. 3f). In addition, active tension dependent contraction of the cell rear and expansion of the front allow the cell to move, even in the limiting condition of infinite friction, when the cell body cannot move relative to the wall.
In Section 4, we proceed to compare the predictions of the theoretical description to experimental measurements of the cortical flow profiles and cell velocities in three different conditions of channel friction. A fitting procedure allows us to obtain estimates of the mechanical parameters introduced in the theoretical description. Remarkably, all our quantitative data on cell and cortex dynamics in three different friction conditions can be fitted very accurately with three parameters (Fig. 3b and c), yielding estimates consistent with previous studies[1–3] and additional validating experiments (see Section 4.3, and Supplementary Fig. 4e-h).
Finally, using parameters extracted from the fitting procedure, we obtain in Section 5 the distribution of forces created by the cells (Supplementary Fig. 4i). We measure the first moments of the force distribution along the axis of the channel and we find that in contrast with adhesion-based motility, the dipolar moment is positive (Fig. 4).

1 Model description and main equations

1.1 Cell shape parametrization

We start by introducing geometrical quantities characterizing the shape of the cell surface, in the reference frame of the cell. The cell shape is taken to be cylindrical where confined by the channel, and is free in the rear and front regions (see Supplementary Fig. 3b). The cell volume is assumed to be conserved during cell

movement. For simplicity, we describe a shape, which is averaged over the fluctuations induced by membrane blebbing at the front of the cell. We also do not include the uropod in our description, as laser ablation experiments show that most of the driving force is generated in the rear of the cell, in the region of the cortex adjacent to the uropod (Supplementary Fig. 1f). We consider the channel to be cylindrical and the cell shape to be axisymmetric around the central axis of the channel. The cell surface is parametrized by coordinates s and ϕ, such that a point on the surface is denoted by

$$\mathbf{X}(s,\phi) = x(s)\mathbf{e}_x + r(s)\sin\phi\mathbf{e}_y + r(s)\cos\phi\mathbf{e}_z, \tag{1}$$

where $\mathbf{e}_x$, $\mathbf{e}_y$ and $\mathbf{e}_z$ are unit vectors of the cartesian coordinates (Supplementary Fig. 3b).
To formulate constitutive equations and force balance on the curved cell surface, we first need to obtain the local basis of tangent and normal vectors to the surface, as well as the metric and curvature tensors. In what follows, the Einstein summation convention is used and lower and upper indices denote respectively covariant and contravariant tensors on the curved surface (for an introduction to differential geometry, please refer to e.g. [4]).

The vectors tangent to the surface are given by

$$\mathbf{e}_s = \partial_s\mathbf{X} = \begin{pmatrix} \partial_s x \\ \partial_s r\sin\phi \\ \partial_s r\cos\phi \end{pmatrix}, \quad \mathbf{e}_\phi = \partial_\phi\mathbf{X} = \begin{pmatrix} 0 \\ r\cos\phi \\ -r\sin\phi \end{pmatrix}. \tag{2}$$

In what follows, s is imposed to be an arc length coordinate, such that $|\mathbf{e}_s| = (\partial_s r)^2 + (\partial_s x)^2 = 1$. The normal vector to the surface, $\mathbf{n} = \dfrac{\mathbf{e}_s \times \mathbf{e}_\phi}{|\mathbf{e}_s \times \mathbf{e}_\phi|}$, is then given by

$$\mathbf{n} = \begin{pmatrix} -\partial_s r \\ \partial_s x\sin\phi \\ \partial_s x\cos\phi \end{pmatrix}. \tag{3}$$

For convenience, we introduce $\psi(s)$ the angle formed by $\mathbf{e}_s$ with the plane normal to the x-axis (Supplementary Fig. 3b), such that the coordinates $r(s)$ and $x(s)$ are related to ψ by $\partial_s r = \cos\psi$ and $\partial_s x = \sin\psi$. With these notations, the first fundamental form, the metric tensor is defined by $g_{ij} = \mathbf{e}_i\mathbf{e}_j$ and reads

$$g_{ij} = \begin{pmatrix} 1 & 0 \\ 0 & r^2 \end{pmatrix}. \tag{4}$$

The area element is thus $dS = \sqrt{\det g_{ij}}\ dsd\phi = r\ dsd\phi$. The curvature tensor of the surface, the second fundamental form, has the definition $C_{ij} = \mathbf{e}_i.\partial_j\mathbf{n}$. In the coordinates introduced above, the curvature tensor is given by

$$C_i{}^j = C_{ik}g^{kj} = \begin{pmatrix} \partial_s\psi & 0 \\ 0 & \dfrac{\sin\psi}{r} \end{pmatrix}. \tag{5}$$

Note that as a result of axisymmetry, the curvature tensor is diagonal and the radii of curvature can be immediately identified, $R_s = \dfrac{1}{\partial_s\psi}$ and $R_\phi = \dfrac{r}{\sin\psi}$.

Finally, we introduce the velocity field of the cell surface $\mathbf{v}$, which can be decomposed into a tangential and a normal part

$$\mathbf{v} = v^s\mathbf{e}_s + v^\phi\mathbf{e}_\phi + v^n\mathbf{n}. \tag{6}$$

The velocity component v^ϕ cancels because of the rotational symmetry. The normal component v^n is related to shape changes of the cell, and the tangential component of the velocity v^s represents cortical flows. Note that for the sake of clarity, the cortical velocity v^s is denoted V_{Cortex} in the main text.

1.2 Material equation for the actomyosin cortex

We describe the actomyosin network at the cell surface as a two-dimensional viscous fluid subjected to active tensions [5]. Migrating Walker cells indeed exhibit cortical flows on the timescale of minutes, exceeding the turnover rate of cortical crosslinkers ($\sim$ 20 s, Ref [6]), at which elastic stresses in the actin network are released. Furthermore, contractile stresses arising from the ATP driven activity of myosin motor proteins in the cortical actin network [7] give rise to an active contractile tension, which depends on the spatial density

of motor proteins. The constitutive equation for the total tension tensor in the actomyosin cortex ${t^i}_j$ has therefore a viscous and an active contribution

$${t^i}_j = \eta_\mathrm{s}\left[{v^i}_j - \frac{1}{2}{v^k}_k{\delta^i}_j\right] + \eta_\mathrm{b}{v^k}_k{\delta^i}_j + \zeta(c){\delta^i}_j, \tag{7}$$

where η_s and η_b are the shear and bulk viscosity of the actomyosin network, ${v^i}_j$ is the strain rate tensor, and $\zeta(c)$ is the active myosin induced tension, which depends on the myosin concentration c. For simplicity, we assume here that the shear and bulk viscosities are related through $\eta_\mathrm{s} = 2\eta_\mathrm{b} = \eta$, corresponding to a ratio of bulk to shear viscosity $\eta_\mathrm{b}/\eta_\mathrm{s} = 1/2$. Other choices for this ratio renormalize the viscosities in expressions given in these supplementary materials. In the coordinates introduced in Section 1.1, the linearized strain rate tensor is

$${v^i}_j = \begin{pmatrix} \partial_s v^s + \partial_s\psi v^n & 0 \\ 0 & \dfrac{\cos\psi v^s + \sin\psi v^n}{r} \end{pmatrix} \tag{8}$$

with v^s and v^n the flow velocity tangential and normal to the surface. Note that in the case of a stationary cell shape in the reference of the cell, the normal surface velocity has to vanish ($v^n = 0$), since the shape of the surface is modified by the normal velocity only.

1.3 Force balance equations

Newton's second law at low Reynolds number imposes that the sum of all forces acting on an element of the cell surface must vanish. Therefore, normal and tangential stresses arising from the intrinsic viscous and active tensions must balance pressures and shear stresses exerted by the medium surrounding the cortex. The tangential force balance reads

$$\nabla_i t^{ij} = \Sigma^j_\mathrm{in} - \Sigma^j_\mathrm{out} \tag{9}$$

where ∇_i denotes the covariant derivative on the curved surface indexed by i, j and $\mathbf{\Sigma}_\mathrm{in} - \mathbf{\Sigma}_\mathrm{out}$ is the external shear stress acting on the cell surface, respectively inside and outside the cell. The normal force balance is the Young-Laplace equation

$$C_{ij}t^{ij} = P_\mathrm{in} - P_\mathrm{out} \tag{10}$$

with P_in the intracellular pressure, P_out the extracellular pressure, such that $P_\mathrm{in} - P_\mathrm{out}$ is the pressure drop across the surface.

In what follows we assume that the shear stress Σ_in acting on the surface from inside the cell can be neglected compared to viscous and active stresses acting in the surface. This choice is consistent with our measurements of cortical flow fields, see section 4.2. The external shear stress Σ_out and the pressure drop $P_\mathrm{in} - P_\mathrm{out}$ have different values in the cylindrically confined part of the cell and in the pole regions, as detailed below:

- In the region where the cell is in contact with the channel walls, an external shear stress Σ_out acts on the surface. This shear stress is proportional to the relative velocity between the flowing cortex and the channel, $\Sigma_\mathrm{out} = -\alpha v^s$, with α the associated friction coefficient (Supplementary Fig. 3f). The tangential force balance then has the simple expression

  $$\partial_s {t^s}_s = \alpha v^s. \tag{11}$$

  The shape of the cell is constrained by the channel wall and the normal force balance Equation 10 thus yields the pressure exerted by the cell on the channel. Note that forces exerted by the channel wall normal to the cell surface have no direct mechanical effect on cell migration. Indeed, in the region where the cell is confined by the channel, forces normal to the cell surface are perpendicular to the direction of cell displacement, and therefore do not contribute in propelling the cell. In agreement with this, cells do not move in channels with low friction coefficient, despite the fact that normal forces arising from confinement are still present (Fig. 2 and 3).

- In the unconfined pole regions, the cell is in contact with the external medium. Given the low viscosity of the medium (of the order of the viscosity of water, $\eta_c \simeq 10^{-3}$Pa.s), compared to cytoskeletal viscosities, we neglect viscous shear stresses arising in the fluid surrounding the cell. Therefore, the tangential and normal force balance equations Eqs. 9-10 read

  $$\partial_s {t^s}_s + \frac{\cos\psi}{r}({t^s}_s - {t^\phi}_\phi) = 0 \tag{12}$$

  $$\partial_s\psi {t^s}_s + \frac{\sin\psi}{r}{t^\phi}_\phi = P_\mathrm{in} - P_\mathrm{out}. \tag{13}$$

1.4 External fluid pressure and drag coefficient

The force balance equation 13 involves the external pressures acting on the rear and front of the cell, $P_{\text{out}}^{(\text{f})}$ and $P_{\text{out}}^{(\text{r})}$. In a fluid-filled channel subjected to a zero pressure difference at its boundaries, the only contribution to the difference of pressure acting on the cell $P_{\text{out}}^{(\text{r})} - P_{\text{out}}^{(\text{f})}$ results from the drag of the medium acting against the motion of the cell. We assume here that the external pressure opposing cell movement is proportional to the cell velocity and can be written in terms of an effective drag coefficient α_{D}

$$P_{\text{out}}^{(\text{r})} - P_{\text{out}}^{(\text{f})} = -\alpha_{\text{D}} U. \tag{14}$$

Here U denotes the velocity of the cell, which in the main article text is referred to by V_{Cell}. Note that such a proportionality relation between the pressure difference on the cell and the cell velocity does not necessarily hold. Indeed, the drag depends also on the motion of the fluid in the channel. For instance, an externally applied pressure difference at the channel boundaries, generating a fluid flow, would modify Eq. 14. We show however in Section 3 that under the experimental conditions in this work, the pressure acting on the cell can be expressed in the form of Eq. 14, and we detail how the value of α_{D} is related to the flow permeability of the channel and the migrating cells.

Combining the constitutive material equation 7 introduced in Section 1.2, the force balance equations 11-13 and the fluid drag equation 14 then allows to obtain the velocity and shape equations of the cell cortex.

2 Cortical velocity profiles and cell velocity

We now proceed to solve the model equations separately in the cylindrical and pole cell parts. In the following, the superscripts (r), (m) and (f) denote rear, middle and front cell region respectively (see Supplementary Fig. 3c). First, for convenience we introduce the following coordinates used in the different cell parts

$$\theta^{(\text{r})} = \frac{s}{R} \qquad \text{in the rear pole region,} \tag{15}$$

$$x^{(\text{m})} = s - R\frac{\pi}{2} \qquad \text{in the contact region,} \tag{16}$$

$$\theta^{(\text{f})} = \pi - \frac{s-L}{R}, \qquad \text{in the front pole region.} \tag{17}$$

We denote by R and L the radius of the microchannel and the length of the contact area between channel and cell (see Supplementary Fig. 3b).
In the next sections, we derive analytical expressions for i) the profile of cortical velocity v^s (Eq. 6) in the reference frame of the cell, and ii) the cell velocity in the reference frame of the channel, U.

2.1 Profile of active tension

Cell motility relies on the polarized distribution of myosin motors within the cell cortex. Experiments indicate that myosin accumulates at the cell rear (Fig. 2a, Supplementary Fig. 3b). The inhomogeneous distribution of myosin gives rise to a spatial profile of active tension $\zeta(x)$, decreasing from the cell rear to the front. For analytical calculation, we now postulate a simple form for this profile. We assume that the cell is subjected to a piecewise linear profile of active tension:

$$\zeta = \begin{cases} \zeta^{(\text{r})} & \text{in the rear pole region} \\ \zeta^{(\text{r})} - \frac{\zeta^{(\text{r})} - \zeta^{(\text{f})}}{L} x^{(\text{m})} & \text{in the contact region} \\ \zeta^{(\text{f})} & \text{in the front pole region} \end{cases} \tag{18}$$

This choice represents a good approximation of the experimentally measured myosin fluorescence profiles (Supplementary Fig. 1i). In Section 4, we evaluate more precisely the active tension profile by assuming a proportionality relation between the active tension and the myosin intensity profile $I(x)$, $\zeta(x) = \zeta_0 I(x)$, in line with previous studies[7–9].

2.2 Flow in the cylindrical region of the cell

We first calculate the flow field in the cylindrical part of the cell, which is in contact with the channel walls. In the reference frame of the moving cell, Eq. 11 can be written

$$\partial_x t^s{}_s = \alpha(v^s + U) \tag{19}$$

where v^s is now a velocity taken in the cell reference frame. Together with the constitutive equation 7, this yields the following equation for the cortical flow velocity

$$\eta \partial_x^2 v^s = \alpha(v^s + U) - \partial_x \zeta \tag{20}$$

which admits the general solution

$$v^s = C_1 \exp\left(\frac{x^{(\mathrm{m})}}{l}\right) + C_2 \exp\left(-\frac{x^{(\mathrm{m})}}{l}\right) - U - \frac{\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})}}{\alpha L} \qquad \text{for } s \in \left[R\tfrac{\pi}{2}, R\tfrac{\pi}{2} + L\right] \tag{21}$$

$$t^s{}_s = \frac{\eta}{l}\left(C_1 \exp\left(\frac{x^{(\mathrm{m})}}{l}\right) - C_2 \exp\left(-\frac{x^{(\mathrm{m})}}{l}\right)\right) + \zeta, \tag{22}$$

where $l = \sqrt{\frac{\eta}{\alpha}}$ is a hydrodynamic length characterizing the range of cortical flows. The integration constants C_1 and C_2 can be determined from boundary conditions on the velocity and tension at the contact points where the pole regions are connected to the cylindrical part of the cell (see Section 2.4).

2.3 Flow and deformation in the cell pole regions

Next, we work out the flow field and the shape of the free cell pole surfaces, in the reference frame of the cell and for a stationary shape, implying that the normal velocity v^n vanishes (see Section 1.2). Furthermore, taking the active tension to be uniform in the pole surfaces (18) implies $\partial_s \zeta = 0$. Without loss of generality, we explicitly solve here the equations in the rear part of the cell; however the solution in the front can be found by a simple symmetry transformation. Combining Equations 7 and 13, we obtain the velocity and shape equations

$$\partial_s^2 v^s + \frac{\cos\psi}{r}\left(\partial_s v^s - \frac{\cos\psi}{r} v^s\right) = 0 \tag{23}$$

$$\left(\partial_s \psi + \frac{\sin\psi}{r}\right)\zeta + \eta\left(\partial_s\psi\partial_s v^s + \frac{\sin\psi\cos\psi}{r^2} v^s\right) = P_{\mathrm{in}} - P_{\mathrm{out}}. \tag{24}$$

To solve these equations, we perform an expansion around an unpolarised cell state where the active tension is uniform and no flow arises in the cortex. In this case, the rear and front surfaces take the shape of hemispherical caps (in the rear, $v_0^s = 0$, $r_0 = R\sin(s/R)$, $\psi_0 = s/R$). We linearize the shape and flow equations around this solution ($\psi = \psi_0 + \delta\psi$, $r = r_0 + \delta r$, $P_{\mathrm{in}} - P_{\mathrm{out}} = 2\zeta/R + \delta P$). This expansion is valid in the limit where the variation of active tension is small compared to the average cellular surface tension $(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})/\bar{t} \ll 1$, where the average cellular surface tension $\bar{t}$ includes both the average cortical tension and the cell membrane tension. For the Walker cells studied here, the total surface tension has been measured to be 279 ± 50 pN/μm (Ref.[3]). Our final estimate of $\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})} = 68 \pm 7$ pN/μm (see Section 4, Table 2) is thus consistent with the above assumption. Furthermore, the observed time-average cell shape in the pole regions indeed corresponds approximately to a hemisphere (see Supplementary Fig. 3b).
Neglecting higher order terms and using 15, Equations 23-24 become

$$\left[\partial_\theta^2 + \cot\theta\partial_\theta - \cot^2\theta\right] v^s = 0 \tag{25}$$

$$\left[\partial_\theta^2 + 1\right]\delta r = -\frac{\delta P R^2}{\zeta}\sin\theta + \frac{\eta}{\zeta}\partial_\theta(v^s \sin\theta). \tag{26}$$

Here, θ stands for $\theta^{(r)}$. The solution of Equation 25 is a linear combination of the associated Legendre polynomials $\mathcal{P}_\nu^1(\cos\theta)$ and $\mathcal{Q}_\nu^1(\cos\theta)$, with $\nu(\nu+1) = 1$. The solution cannot diverge in $\theta = 0$, therefore, $\mathcal{Q}_\nu^1(\cos\theta)$ is not part of the solution and the velocity and tension profiles are given by

$$v^s = v^{(\mathrm{r})}\frac{\mathcal{P}_\nu^1(\cos\theta)}{\mathcal{P}_\nu^1(0)} \tag{27}$$

$$t^s{}_s = \zeta + \eta\frac{v^{(\mathrm{r})}}{R}\frac{\partial_\theta \mathcal{P}_\nu^1(\cos\theta)}{\mathcal{P}_\nu^1(0)} \tag{28}$$

where we denote by $v^{(\mathrm{r})}$ the cortical velocity at the contact point between the cell surface and the channel wall (see Supplementary Fig. 3d).

Integrating Equation 24 yields the following solution for the shape equation

$$\begin{aligned}\delta r = &\frac{\eta}{\zeta} v^{(\mathrm{r})} \left(a \left(\cos\theta (\theta - \frac{\pi}{2}) - \sin\theta \right) - \left(\int_0^{\frac{\pi}{2}} \cos\theta' \sin\theta' \frac{\mathcal{P}_\nu^1(\cos\theta')}{\mathcal{P}_\nu^1(0)} d\theta' - 1 \right) \cos\theta \right) \\ &+ \frac{\eta}{\zeta} v^{(\mathrm{r})} \int_0^\theta \cos(\theta - \theta') \frac{\mathcal{P}_\nu^1(\cos\theta')}{\mathcal{P}_\nu^1(0)} \sin\theta' d\theta',\end{aligned} \tag{29}$$

where we have introduced the numerical coefficient $a = \int_0^{\frac{\pi}{2}} d\theta' \sin^2\theta' \frac{\mathcal{P}_\nu^1(\cos\theta')}{\mathcal{P}_\nu^1(0)} \simeq 0.56$. The following three boundary conditions have to be satisfied by the shape profile:

- The shape has to be smooth at the cell apex ($\partial_\theta \delta r = 0$ at $\theta = 0$).
- At the contact point with the cylinder, the shape has to be tangent to the wall ($\partial_\theta \delta r = 0$ at $\theta = \frac{\pi}{2}$).
- At the contact point with the cylinder, the cortex cannot penetrate the wall ($\delta r(\frac{\pi}{2}) = 0$ at $\theta = \frac{\pi}{2}$).

These conditions were used to determine the integration constants in Eq. 29 and yield the following expression for the pressure drop across the cell surface:

$$P_{\mathrm{in}} - P_{\mathrm{out}} = \frac{2\zeta}{R} + \frac{2\eta}{R^2} v^{(\mathrm{r})} a. \tag{30}$$

Thus, we have derived the expressions for the flow velocity, the tension and the shape, as well as for the difference of pressure across the cell surface.

2.4 Cortical velocity and tension at the contact points

To summarise, we give below the full solutions for the velocity, tension and pressure drop of the pole regions on each side:

- Rear pole region:

$$v^s = v^{(\mathrm{r})} \frac{\mathcal{P}_\nu(\cos\theta^{(\mathrm{r})})}{\mathcal{P}_\nu(0)} \tag{31}$$

$$t^s{}_s = \zeta^{(\mathrm{r})} + \eta \frac{v^{(\mathrm{r})}}{R} \frac{\partial_{\theta^{(\mathrm{r})}} \mathcal{P}_\nu(\cos\theta^{(\mathrm{r})})}{\mathcal{P}_\nu(0)} \tag{32}$$

$$P_{\mathrm{in}}^{(\mathrm{r})} - P_{\mathrm{out}}^{(\mathrm{r})} = \frac{2\zeta^{(\mathrm{r})}}{R} + \frac{2\eta}{R^2} v^{(\mathrm{r})} a, \tag{33}$$

- Front pole region:

$$v^s = v^{(\mathrm{f})} \frac{\mathcal{P}_\nu(\cos\theta^{(\mathrm{f})})}{\mathcal{P}_\nu(0)} \tag{34}$$

$$t^s{}_s = \zeta^{(\mathrm{f})} - \eta \frac{v^{(\mathrm{f})}}{R} \frac{\partial_{\theta^{(\mathrm{f})}} \mathcal{P}_\nu(\cos\theta^{(\mathrm{f})})}{\mathcal{P}_\nu(0)} \tag{35}$$

$$P_{\mathrm{in}}^{(\mathrm{f})} - P_{\mathrm{out}}^{(\mathrm{f})} = \frac{2\zeta^{(\mathrm{f})}}{R} - \frac{2\eta}{R^2} v^{(\mathrm{f})} a. \tag{36}$$

Five unknown constants remain to be determined from boundary conditions: C_1, C_2, $v^{(\mathrm{r})}$, $v^{(\mathrm{f})}$ and U. Four of them can be obtained by imposing that the velocity and tension fields have to be continuous at the contact

points:

$$C_1 + C_2 - U - \frac{\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})}}{\alpha L} = v^{(\mathrm{r})} \tag{37}$$

$$\frac{C_1 - C_2}{l} = \frac{a}{R} v^{(\mathrm{r})} \tag{38}$$

$$C_1 \exp\left(\frac{L}{l}\right) + C_2 \exp\left(-\frac{L}{l}\right) - U - \frac{\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})}}{\alpha L} = v^{(\mathrm{f})} \tag{39}$$

$$\frac{C_1 \exp\left(\frac{L}{l}\right) - C_2 \exp\left(-\frac{L}{l}\right)}{l} = -\frac{a}{R} v^{(\mathrm{f})}. \tag{40}$$

A fifth condition is required to close the system of equations and obtain an expression for the cell velocity U. This last condition can be obtained by considering the balance of pressure inside the cell, which we discuss in the next section.

2.5 Cell velocity

The cytoplasm has been described as a poroelastic material with a rheological timescale below one second (e.g. in [10]). Accordingly, equilibration of intracellular pressure differences can be assumed fast compared to the timescales of cell motion, and the hydrostatic pressure inside the cell can be taken uniform:

$$P_{\mathrm{in}}^{(\mathrm{r})} = P_{\mathrm{in}}^{(\mathrm{f})}. \tag{41}$$

From this relation and Equations 33 and 36, an expression can be obtained for the the cell velocity of the following form

$$U = U_0 + \frac{1}{\chi}(P_{\mathrm{out}}^{(\mathrm{r})} - P_{\mathrm{out}}^{(\mathrm{f})}). \tag{42}$$

with U_0 the intrinsic velocity the cell would achieve in the absence of any external resistance to cell motion, given by

$$U_0 = \frac{L(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})}{\eta} \left(\frac{l}{2L} \frac{\exp\left(\frac{L}{l}\right) + 1}{\exp\left(\frac{L}{l}\right) - 1} + \frac{R}{2aL} - \frac{l^2}{L^2} \right), \tag{43}$$

and χ an effective cell friction,

$$\chi = \frac{a4\eta \left(1 - \exp\left(\frac{L}{l}\right)\right)}{R\left(al - R + \exp\left(\frac{L}{l}\right)(al + R)\right)}. \tag{44}$$

The flow-velocity relationship predicted by the theory (Eq. 42) is shown in Supplementary Fig. 3e. The cell moves with a spontaneous cell velocity U_0 in the absence of any external force. Under the presence of an external force proportional to the cell velocity Eq. 14, we can solve Eq. 42 for the cell velocity U, obtaining

$$U = \frac{2(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})(al(2l + L + \exp\left(\frac{L}{l}\right)(L - 2l)) + (\exp\left(\frac{L}{l}\right) - 1)LR)}{L(alR\alpha_{\mathrm{D}} - R^2\alpha_{\mathrm{D}} - 4a\eta + \exp\left(\frac{L}{l}\right)(R(al + R)\alpha_{\mathrm{D}} + 4a\eta))}. \tag{45}$$

This expression is the central result of our calculations and describes the cell velocity as a function of the hydrodynamic length l, the cell geometric parameters R and L, the gradient of active tension $(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})/L$ and the external drag α_{D}. Fig. 3b and Supplementary Fig. 3f show the predicted cell velocity as a function of the friction coefficient α. The cell velocity U exhibits two different regimes: for small friction compared to the external drag, the velocity U vanishes, as the resistance to cortical flow is not sufficient to propel the cell. For infinite friction $\alpha \to \infty$, the cell velocity is given by the simple expression

$$U = \frac{(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})R}{2a\eta}. \tag{46}$$

In this limit, a no-slip boundary condition holds between the cortex and the channel, the cortex cannot flow relative to the wall in the cylindrical cell region. However, cell movement is still possible due to the contraction and expansion of the cell rear and front respectively (Video S11). As a result, in that limit, the cell velocity depends on the ratio $\frac{\eta}{\zeta}$, which is a characteristic timescale for contraction of the cortex under the active tension ζ.

2.6 Threshold friction for cell movement

As shown in Fig. 3b, the cell velocity U exhibits two different regimes for small and large friction α. In this section, we perform an approximate calculation of the threshold friction enabling cell movement. To this end, we expand U around the limit of zero friction ($\frac{l}{L} \to \infty$)

$$U = \frac{(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})L(aL+6R)}{6aR\alpha_{\mathrm{D}}l^2} - \frac{(\zeta^{(\mathrm{r})} - \zeta^{(\mathrm{f})})L^2\left(30\alpha_{\mathrm{D}}R^3 + 10a(LR\alpha_{\mathrm{D}} + 12\eta) + a^2L(LR\alpha_{\mathrm{D}} + 20\eta)\right)}{60\left(aR\alpha_{\mathrm{D}}\right)^2 l^4} + O\left(\frac{1}{l^5}\right). \tag{47}$$

The second term is negative and is responsible for the cell velocity levelling off as the friction coefficient α is increased. The ratio of the first two terms in the expansion therefore defines a critical hydrodynamic length l^*, below which friction is not sufficient to drive cell movement

$$\left(\frac{l^*}{L}\right)^2 = \frac{R}{2La} + \frac{1}{60}\left(5 + \frac{aL}{aL+6R}\right) + \frac{2\eta}{LR\alpha_{\mathrm{D}}}. \tag{48}$$

For the fitted values of η and α_{D} (see Section 4 and Tables 1 - 2), the last term dominates $\left(\frac{l^*}{L}\right)^2 \approx \frac{2\eta}{LR\alpha_{\mathrm{D}}}$. Thus, we find the following expression for the critical friction coefficient enabling cell movement

$$\alpha^* \approx \frac{R\alpha_{\mathrm{D}}}{2L}. \tag{49}$$

To estimate more precisely the critical friction for cell motion, we numerically evaluated the first inflexion point of $U(\alpha)$ for increased friction, for different values of α_{D} (Supplementary Fig. 3g). We find that the approximate estimate Eq. 49 actually yields a very good estimate for the inflexion point of $U(\alpha)$ in the regime of small drag coefficients.

Eq. 49 indicates that the cell velocity is critically dependent on the ratio of the friction to drag coefficient, $\alpha^*/\alpha_{\mathrm{D}}$. Cell locomotion can only be achieved provided that the friction coefficient is of the same order or larger than the drag coefficient. This very simple criterion provides a quantitative prediction for conditions enabling cell motion driven by cortical flow and friction.

To summarize, we have calculated the cell velocity U and the rearward cortical flow field v^s resulting from a gradient of active cortical tension, and we find that cortical flows enable cell movement at a finite velocity only if sufficient substrate friction is available to overcome fluid drag. Our predictions on cortical flows and cell velocities at different friction coefficients can be directly compared to experimental measurements (Section 4).

3 Fluid drag coefficient in a channel

In this section, we relate the fluid drag coefficient α_{D} introduced in Eq. 14 to the hydraulic properties of the channel and the cell. In general, the external medium can flow from the front to the rear of the cell, either due to fluid crossing the cell membrane, or due to fluid flowing in the narrow space between the cell and the channel wall (Supplementary Fig. 4e). Depending on the cell permeability and the effective permeability of the space between the cell surface and the channel wall, this flow gives rise to a difference of pressure between the medium in front of the cell and the medium at the rear of the cell. For a single cell, this pressure difference can be written

$$P^{(\mathrm{r})}_{\mathrm{out}} - P^{(\mathrm{f})}_{\mathrm{out}} = \xi S(\bar{v}_{\mathrm{c}} - U), \tag{50}$$

where ξ is the effective fluid flow resistance of a channel segment containing a cell, S is the cross-sectional area of the channel, and $\bar{v}_{\mathrm{c}}$ is the average fluid flow. No pressure difference is created, when the velocities of the fluid $\bar{v}_{\mathrm{c}}$ and the cell U are equal. In addition, the microchannel offers resistance to fluid flow (Supplementary Fig. 4e), such that the relation between a pressure drop across some distance in the channel and the fluid flow is given by Poiseuille's law. Considering a single cell moving in a channel, the pressure difference between channel inlet and cell rear on one side of the cell and channel outlet and cell front on the other side of the cell are thus given by

$$P^{(\mathrm{r})}_{\mathrm{applied}} - P^{(\mathrm{r})}_{\mathrm{out}} = \frac{8\eta_{\mathrm{c}}L^{(\mathrm{r})}_{\mathrm{c}}}{R^2}\bar{v}_{\mathrm{c}} = \xi^{\mathrm{r}}_{\mathrm{c}}S\bar{v}_c \tag{51}$$

$$P^{(\mathrm{f})}_{\mathrm{out}} - P^{(\mathrm{f})}_{\mathrm{applied}} = \frac{8\eta_{\mathrm{c}}L^{(\mathrm{f})}_{\mathrm{c}}}{R^2}\bar{v}_{\mathrm{c}} = \xi^{\mathrm{f}}_{\mathrm{c}}S\bar{v}_c \tag{52}$$

where $L_c^{(r)}$ and $L_c^{(f)}$ are the channel lengths in the rear and in the front of the cell, and $P_{\text{applied}}^{(r)}$ and $P_{\text{applied}}^{(f)}$ are the pressures applied to the two ends of the channel. When these two pressures are equal, the difference of pressure acting on the cell is simply

$$P_{\text{out}}^{(r)} - P_{\text{out}}^{(f)} = -\xi_c S \bar{v}_c, \tag{53}$$

where $\xi_c = \xi_c^r + \xi_f^r \simeq 8\eta_c L_c/(R^4\pi)$ is the overall resistance of the channel, with $L_c \simeq L_c^{(r)} + L_c^{(f)}$.

Combining Eq. 50 and 53, we obtain a relation between the fluid flow created by the cell and the cell velocity:

$$\bar{v}_c = \frac{\xi}{\xi + \xi_c} U. \tag{54}$$

The pressure acting on the cell can then be determined self-consistently from Eqs. 50 and 54:

$$P_{\text{out}}^{(r)} - P_{\text{out}}^{(f)} = \left(\frac{\xi}{\xi + \xi_c} - 1\right) \xi S U, \tag{55}$$

which has the form of Eq. 14 with $\alpha_D = \left(\frac{\xi}{\xi+\xi_c} - 1\right) \xi S$.

We now investigate the case of a cell migrating in a channel containing $N-1$ other motile cells (Supplementary Fig. 4e) and derive the expression for the fluid drag coefficient α_D for that case, since we analyzed experiments where several cells were moving in each channel. We assume here that individual cells differ predominantly in their spontaneous velocities U_0, which scale with the active tension differences between cell rear and front (see Eq. 43) and thus depend on cell polarity state. For N simultaneously migrating cells, the induced flow velocity $\bar{v}_c$ reads

$$\bar{v}_c = \frac{\xi}{\xi_c + N\xi} \sum_{i=1}^{N} U_i, \tag{56}$$

where U_i is the velocity of cell i. Using eqs. 42 and 50, U_i can be rewritten as

$$U_i = \frac{\chi}{\chi + \xi S} U_{i,0} + \frac{\xi S}{\chi + \xi S} \bar{v}_c, \tag{57}$$

and we obtain for the external pressure difference acting on cell i

$$P_{i,\text{out}}^{(r)} - P_{i,\text{out}}^{(f)} = \xi S U_i \left(\frac{\chi + S\xi}{\chi \dfrac{U_{i,0}}{\bar{v}_c} + S\xi} - 1 \right). \tag{58}$$

Analysis of the distribution of cell velocities indicates that cells can be grouped into fast and slow moving cells (Supplementary Fig. 4h). For fast moving cells, $U_{i,0}$ is large compared to $\bar{v}_c$. In channels where the friction coefficient is large, we find $\bar{v}_c/U_0 \simeq 0.3$. Then, $\bar{v}_c/U_i \ll 1$ and Eq. 58 can be simplified to $P_{i,\text{out}}^{(r)} - P_{i,\text{out}}^{(f)} \simeq -\xi S U_i$, such that the drag coefficient can be approximated by

$$\alpha_D \simeq \xi S. \tag{59}$$

We find that the drag coefficient depends mainly on the effective fluid flow resistance ξ of the cell. This can be understood from the fact that for a sufficiently large number of cells N, the resistance to fluid flow of the cells dominates over the channel flow resistance ξ_c.

4 Comparison of theoretical profiles to experimental measurements

4.1 Cortical flow profiles and cell velocities at different frictions

To directly compare the model predictions to experimental measurements of Walker cells migrating in microchannels, we measured the distribution of myosin II fluorescence intensity and the cortical flow velocity profiles in moving cells. Measurements were performed for three different surface coatings providing large, small and intermediate friction conditions. The actomyosin flow field was estimated from time-lapse images

Parameter	Units	Value
Large friction α	Pa s/m	$27 \pm 2\ 10^6$
Intermediate friction α	Pa s/m	$18 \pm 3\ 10^3$
Small friction α	Pa s/m	$15 \pm 6\ 10^2$
Contact length L	μm	24.7 ± 0.5
Channel radius R	μm	4.4
Viscosity η_{2D}	**Pa s m**	$\mathbf{27 \pm 3\ 10^{-4}}$
Cell flow resistance ξ	**Pa s/m^3**	$\mathbf{3.5 \pm 0.5\ 10^{15}}$
Myosin scaling factor ζ_0	**pN/ μm**	$\mathbf{53 \pm 5}$

Table 1: Model parameters. Quantities obtained from the fitting procedure are highlighted in bold.

of cells with fluorescent myosin II using particle image velocimetry (see Materials and Methods for details). We use the density distribution of myosin to provide an estimate of the active tension field driving cortical deformation and flow (see Supplementary Fig. 1g-i and Materials and Methods). Specifically, we introduce a scaling factor ζ_0 connecting the relative myosin fluorescence intensity to the active tension ζ

$$\zeta(x) = \zeta_0 \frac{I_{\mathrm{top}}(x) - I_{\mathrm{middle}}(x)}{\bar{I}} \tag{60}$$

where $I_{\mathrm{top}}(x)$ and $I_{\mathrm{middle}}(x)$ are the fluorescence intensity profiles in the focal plane adjacent to the channel wall and at the center of the cell (see Supplementary Fig. 1g). $\bar{I}$ is the average fluorescence intensity in a region of the image at the front of the cell where both middle and top planes are devoid of bright myosin foci. We remove the cytoplasmic fluorescence signal contained in $I_{\mathrm{top}}(x)$ by subtracting $I_{\mathrm{middle}}(x)$, thereby achieving a better readout of the active, cortical myosin density.
The cortical velocity in the cylindrically confined cell part reads for an arbitrary active tension profile $\zeta(x)$ (as follows from Eq. 19)

$$\begin{aligned} v^s = {} & C_1 \exp\left(\frac{x^{(\mathrm{m})}}{l}\right) + C_2 \exp\left(-\frac{x^{(\mathrm{m})}}{l}\right) - U \\ & - \frac{1}{2\eta}\left(\exp\left(\frac{x^{(\mathrm{m})}}{l}\right)\int_{x_0}^{x^{(\mathrm{m})}} \exp\left(-\frac{x'}{l}\right)\zeta(x')dx' + \exp\left(-\frac{x^{(\mathrm{m})}}{l}\right)\int_{x_0}^{x^{(\mathrm{m})}} \exp\left(\frac{x'}{l}\right)\zeta(x')dx'\right). \end{aligned} \tag{61}$$

To obtain a noise-reduced estimate of $\zeta(x)$, we approximated the myosin fluorescence intensity profile in Eq. 60 with a fifth-degree polynomial, where the six free coefficients were determined by minimising the sum of squared errors between the curve and the fluorescence intensity data points. The resulting expression was used to analytically evaluate the integrals of $\zeta(x)$ in Eq. 61.
Finally, from microfluidic measurements in the three different friction conditions, we obtained estimates of the friction coefficients α as functions of the cell flow resistance ξ (see Materials and Methods and Table 1). Particle image velocimetry analysis provided us with $k = 3$ datasets of the average cortical flow velocity $\hat{v}_{ki}$ at points x_i along the cell, with $i = 1...N_k$, where $N_k = 15, 14, 15$ for large, intermediate and small friction conditions respectively. Furthermore, we measured the average migration velocity $\hat{U}_k$ of cells in the three different conditions. To fit the remaining unknown parameters η, ξ and ζ_0 to match the theoretical cortical flow field and predicted cell velocities to the experimental data, we projected the expression for the cortical velocity (Equations 31, 34 and 61) onto the x-axis using the transformations

$$\theta^{(\mathrm{r})} = \arccos\frac{R - x}{R} \tag{62}$$

$$x^{(\mathrm{m})} = x - R \tag{63}$$

$$\theta^{(\mathrm{f})} = \arccos\frac{x - R - L}{R}. \tag{64}$$

The fitting was performed by minimizing the objective function

$$S(\eta, \xi, \zeta_0) = \sum_{\text{friction condition k=1}}^{3} \left(\frac{1}{N_k}\sum_{i=1}^{N_k}\left(v_k(x_i;\eta,\xi,\zeta_0) - \hat{v}_{ki}\right)^2 + \left(U(\alpha_k;\eta,\xi,\zeta_0) - \hat{U}_k\right)^2\right), \tag{65}$$

with the hat denoting measured data. The resulting estimates of η_{2D}, ξ and ζ_0 are given in Table 1. Assuming a thickness of the cortical layer of $h = 200$ nm (Ref.[11]), the 2D cortical viscosity would yield a 3D viscosity

Parameter	Units	Value
Bulk viscosity η_{3D}	kPa.s	13.4 ± 1.4
Active tension drop $(\zeta^{(r)} - \zeta^{(f)})$	pN/μm	68 ± 7
Fluid drag coefficient α_D	kPa.s/m	208 ± 29

Table 2: Mechanical parameters derived from fitted estimates.

$\eta_{3D} = \eta_{2D}/h = 13.4 \pm 1.4$ kPa.s. Using Eq. 59, the fluid drag coefficient can be obtained from the fitted value of ξ; we find $\alpha_D = 208 \pm 29$ kPa.s/m. A summary of physical quantities obtained from the fitting procedure is given in Table 2.

4.2 Role of internal friction

While in some systems, the cell nucleus appears to have an important influence on cell migration mechanics in confined environments (see e. g. Refs. [12], [13]), we see no indication of a dissipating effect due to the presence of the nucleus in Walker cells. Indeed, cell migration velocity does not correlate with nuclear size in both large and intermediate friction conditions, suggesting that nuclear properties do not affect cell migration mechanics (see Supplementary Fig. 4a-c). Furthermore, cortical flows are not slowed at the location of the nucleus, which would be expected in the case of mechanical coupling between nucleoskeletal and cortical elements (see Fig. 3c). Accordingly, as stated in section 1.3, we assume that internal shear stresses acting on the cortex from the intracellular material are negligible compared to external shear stresses acting along the contact surface between the cell and the channel walls. To further test this assumption, we introduced in our physical description an internal friction force resisting cortical flows in the reference frame of the cell. Such a friction force can arise from the dissipation in the cytoplasm entrained by the cortical flow, or from possible links between the cortex with internal organelles, such as the nucleus. Accordingly, we modified Eq. 19 to

$$\partial_x t^s{}_s = (\alpha_{\text{ext}} + \alpha_{\text{int}}) v^s + \alpha_{\text{ext}} U, \tag{66}$$

where α_{int} is an internal friction coefficient, and the friction coefficient corresponding to dissipation between the cortex and the channel wall has been renamed α_{ext} (previously α). For simplicity, we consider internal friction to act only in the region where the cell is confined by the microchannel (see Supplementary Fig. 4d). By action-reaction principle, the friction force exerted by the intracellular material on the cortex results in a counterforce, acting from the cortex on the intracellular material. In addition, the sum of forces acting on the intracellular material has to vanish for force balance to be satisfied. We therefore find

$$\left(P_{\text{in}}^{(r)} - P_{\text{in}}^{(f)}\right) \pi R^2 = -\alpha_{\text{int}} 2\pi R \int_0^L v^s dx, \tag{67}$$

stating that the forces acting on the intracellular material arising i) from the difference of pressure at the front and rear of the cell, and ii) from the friction force with the cortex, sum to zero. Eq. 67 replaces Eq. 41 in section 2.5.

We then proceeded to derive the solutions for the cortical flow velocity and the cell velocity as described in the previous sections. We fitted the resulting equations to the cortical flow data measured in three different friction conditions as in section 4, now with the additional unknown parameter α_{int}. We find that the error between the experimentally measured velocity points and the model curve is minimised with the following set of parameter values: $\eta_{2D} = 27 \pm 3\ 10^{-4}$ Pa.s.m, $\xi = 3.5 \pm 3\ 10^{15}$ Pa.s/m^3, $\zeta_0 = 53 \pm 5$ pN/μm and $\alpha_{\text{int}} = 5.1 \pm 0.3\ 10^{-8}$ Pa.s/m. The fit values for the viscosity, the cell flow resistance and the myosin scaling factor correspond closely to what we find in the absence of internal friction (see Table 1), while the ratio of internal to external friction is found to be very small ($\alpha_{\text{int}}/\alpha_{\text{ext}} \simeq 0$ for the small friction condition). Thus, we conclude that the measured cortical flow fields are consistent with a small effect of internal friction compared to other dissipative processes.

4.3 Fluid flow in the channel

In order to directly assess the value of the cell flow resistance ξ yielded by the fit of the model to the data, we investigated the fluid flow induced by migrating cells in large friction channels and we compared it to the theoretical expression for the average fluid flow velocity in the channel (Eq. 56). To estimate the fluid

velocity from Eq. 56, we quantified the average number of cells per channel $N = 14 \pm 1$ and the average cell velocity $\bar{U} = 2.22 \pm 0.36$ μm/min (see Supplementary Fig. 4f-h). We then predict that the cells induce an average fluid flow of $\bar{v}_c = 1.4 \pm 0.2$ μm/min in the channel. To test this prediction and experimentally measure the average fluid flow, we injected microspheres into channels together with migrating cells and tracked their position over time in a bright-field microscope (Supplementary Fig. 4f). We proceeded to relate the average velocity of microspheres $\bar{v}_{\rm bead}$ to the average fluid flow velocity $\bar{v}_c$ in the following way:

- Assuming Hagen-Poiseuille flow in the channel, $v_c(r)$ is related to the average fluid velocity $\bar{v}_c$ by

$$v_c(r) = 2\bar{v}_c \frac{R^2 - r^2}{R^2}. \tag{68}$$

- The average velocity of the microspheres is related to the fluid flow $v_c(r)$ by

$$\bar{v}_{\rm bead} = 2\pi \int r dr \;\; p(r) v_c(r), \tag{69}$$

  where $p(r)$ is the radial distribution of bead positions.

- The distribution of bead positions obtained in the bright-field microscope is projected onto the y-axis. To estimate the radial distribution $p(r)$ from the projected distribution of bead positions $p(y)$, we used the inverse Abel transform [15].

From Equations 68-69, we then find $\hat{\bar{v}}_c = 2.61 \pm 0.31$ μm/min, close to our prediction $\bar{v}_c = 1.4 \pm 0.2$ μm/min.

5 Force density on the cell surface

Finally, we describe the calculation of the forces exerted by the cells on the channel walls during migration (see Supplementary Fig. 4i and Fig. 4a). The 2D force density on the wall is given by the product of the friction coefficient α and the relative velocity between the cortex and the wall:

$$f = \alpha(v^s + U). \tag{70}$$

The resulting spatial force pattern f is plotted in Supplementary Fig. 4i, and can be characterized by computing the first three terms of a multipole expansion:

$$\tau = \int f dS \tag{71}$$

$$\rho = \int x f dS \tag{72}$$

$$\gamma = \int x^2 f dS. \tag{73}$$

where the integrals are taken over the contact surface between the channel and the cell, τ is a force monopole, ρ is a force dipole and γ a force quadrupole. At low Reynolds number, the total force transmitted by the cell to its environment is zero. Thus, the force transmitted to the substrate by a crawling cell equals the drag force exerted on it by the surrounding fluid. The drag forces in a narrow channel are comparable to forces exerted by the cell on the substrate; therefore, the total force exerted by the cell on the channel does not vanish and gives rise to a non-zero force monopole τ (Table. 3). We find that the next term in the multipole expansion, the force dipole ρ, is positive for all friction conditions (Table. 3), indicating that a higher propulsive force is generated at the cell rear. This observation is in sharp contrast with adhesion-based motility, where the force dipole has been measured to be negative [16]. Finally, we note that the quadrupole γ has a significant contribution to the force distribution for cells moving in large friction conditions. The ratio of quadrupole to dipole moments in this case yields a length much larger than the size of a single cell ($\gamma/\rho \simeq 2$ mm). Thus, flow and/or deformation fields induced by the cellular forces at large friction conditions are predominantly characterized by the quadrupole moment of the force distribution at distances below a few millimetres.

Multipole	Large friction	Intermediate friction	Low friction
τ (monopole)	$-1.55\ 10^{-12}$ N	$-1.04\ 10^{-12}$ N	$-1.31\ 10^{-13}$ N
ρ (dipole)	$7.7\ 10^{-17}$ N.m	$2.5\ 10^{-18}$ N.m	$1.5\ 10^{-19}$ N.m
γ (quadrupole)	$1.42\ 10^{-20}$ N.m^2	$-3.9\ 10^{-23}$ N.m^2	$-5.7\ 10^{-24}$ N.m^2

Table 3: Multipole moments of the force density exerted by migrating Walker cells on large, intermediate and small friction channels.

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