Cytoplasmic flows as signatures for the mechanics of mitotic positioning

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1 Abstract

The proper positioning of mitotic spindle in the single-cell Caenorhabditis elegans embryo is achieved initially by the migration and rotation of the pronuclear complex (PNC) and its two associated astral microtubules (MTs). Pronuclear migration produces global cytoplasmic flows that couple the mechanics of all microtubules, the PNC, and the cell periphery with each other through their hydrodynamic interactions (HIs). We present the first computational study that explicitly accounts for detailed HIs between the cytoskeletal components and demonstrate the key consequences of HIs on the mechanics of pronuclear migration. First we show that, because of HIs between the MTs, the cytoplasm-filled astral MTs behave like a porous medium with its permeability decreasing with increasing the number of MTs. We then directly study the dynamics of PNC migration under various force-transduction models, including the pushing or pulling of MTs at the cortex, and the pulling of MTs by cytoplasmically-bound force generators. While achieving proper position and orientation on reasonable time-scales does not uniquely choose a model, we find that each model produces a different signature in its induced cytoplasmic flow. We suggest then that cytoplasmic flows can be used to differentiate between mechanisms.

2 Introduction

The cytoskeleton is an ensemble of filaments and molecular motors immersed in the cytoplasmic fluid, and is involved in cellular processes such as cell division and migration. The energy required for the rearrangement of cytoskeletal components and organelle transport is typically provided by the force exchange between the cytoskeletal filaments –including microtubules and actin fibers– and motor proteins. These interactions are local, i.e. they occur over the length-scales of the molecular motors, which are significantly smaller than the length of the filaments. Nevertheless, since the

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structures are embedded in the cytoplasmic fluid, their motion can instantaneously induce flows on the scale of the cell [Shelley, 2016]. These nonlocal interactions between the cytoplasmic fluid and the structures within (fibers, nuclei, the cell cortex ...) are referred to as Hydrodynamic Interactions (HIs). Previous theoretical and computational studies of the mechanics of cytoskeleton mostly ignore HIs, often arguing that HIs are screened in the dense network/suspension of filaments [Broedersz & MacKintosh, 2014]. The purpose of this work is to revisit this assumption and use detailed simulations to demonstrate the importance of HIs in determining the mechanics of cytoskeletal assemblies.

For this purpose, we have developed a versatile and highly efficient numerical platform for studying the dynamics of active and flexible filaments in cellular assemblies [Nazockdast et al., 2017]. This method offers a major improvement from our earlier numerical study [Shinar et al., 2011] that did not explicitly model HIs between the microtubules (MTs) and their mechanical flexibility. This is, to our knowledge, the first attempt to incorporate many-body HIs between MTs and other intracellular bodies with the cytoplasmic fluid, while also accounting for the flexibility of MTs, their dynamic instability, and interactions with motor proteins. By accounting for HIs we can also compute the large-scale cytoplasmic flows generated by the movements of MTs and other immersed bodies within the cell.

As an example, we study pronuclear migration prior to the first cell division of C. elegans embryo; see the schematic in Fig. 1. Proper positioning of the mitotic spindle is indispensable to the successful segregation of chromosomes and to the generation of cell diversity in early development [Cowan & Hyman, 2004]. Prior to mitosis and after fertilization, the female pronucleus migrate towards (at $t = t_{-1}$ in the schematic) and meets the male pronucleus ($t = t_0$), and its associated astral array of MTs, at the cell posterior to form the pronuclear complex (PNC). The PNC then moves towards, and centers at, the cell center ($t = t_1$) and rotates 90° ($t = t_2$) to align the axis between its two associated centrosomes with the cell’s anterior-posterior (AP) axis. The mitotic spindle then forms, and the chromatid pairs are pulled towards the opposite sides of the cell ($t > t_2$).

HIs arise from several features of PNC migration. Centering and rotation of the PNC will push and rotate the cytoplasm, as will the astral MT arrays. Hence, each structure moves against the backdrop of flows produced by the other. Further, since the PNC and its associated MT arrays are on the scale of the cell itself, the confinement of the cell will have a very strong effect on the nature of the cytoplasmic flows. Finally, as we shall show here, the particular mechanisms of force transduction that position the PNC can have a qualitative—and in principle experimentally measurable—effect on these flows.

To start, we study the flow induced by the motion of the PNC and its attached astral MTs, irrespective of the force transduction mechanisms. For this, we perform the numerical experiment of pulling the PNC, and its astral MT array, with an externally applied force, from the posterior to the center of the cell and aligning it with AP-axis with an externally applied torque. By studying the resulting flows, we establish, as a consequence of HIs, that the cytoplasm-filled astral MTs behave like a porous medium surrounding the PNC, where the permeability of the medium decreases with increasing the number of MTs. To quantify the effect of HIs, we compare the computed translational and rotational drag on the PNC/MT-array complex with other estimates in the literature, including those based on the radius of the aster formed by the MTs [Reinsch & Gönczy, 1998; Kimura & Onami, 2005, 2007], those using a local drag model [Nedelec & Foethke, 2007], and with our own previous study that ignored the MT drag altogether (though not PNC drag or the effect of confinement) [Shinar et al., 2011]. We find in each case that these estimates drastically over- or under-estimate this drag by a large factor. We also find that confinement has a much stronger effect on translational drag than on rotational meaning that HIs cannot be lumped into a modified viscosity.

We next establish that the cytoplasmic flows induced by HIs can be used as a diagnostic tool to
differentiate between different active mechanisms for pronuclear migration. For this, we instantiate three proposed mechanisms for pronuclear migration in \textit{C. elegans}: (i) \textit{the cortical pulling} model, where MTs impinging upon the cortex are pulled upon by dynein motors that are attached to the plasma membrane, in particular by association to the protein complex formed by the \( G_{\alpha} \) subunits, GPR-1/2, and LIN-5. An asymmetric distribution of PAR and LET-99 proteins on the cortex in prophase then produces an asymmetric association of dyneins with the protein complex, and larger pulling forces on the anterior, and so the pronuclear complex moves in that direction [Grill \textit{et al.}, 2001; Tsou \textit{et al.}, 2002; Labbé \textit{et al.}, 2004; Goulding \textit{et al.}, 2007; Kimura & Onami, 2007; Siller & Doe, 2009; McNally, 2013]; (ii) \textit{the cortical pushing} model, where the growth of astral MTs against the cell periphery induces repulsive forces on MTs that move the complex away from the periphery and thus opens space for further polymerization [Holy \textit{et al.}, 1997; Reinsch & Gönczy, 1998; Tran \textit{et al.}, 2001]. Perhaps the strongest evidence in support of the cortical pushing mechanism being involved in the positioning of the mitotic spindle in \textit{C. elegans} comes from the recent study by Garzon-Coral \textit{et al.} [2016], where the magnetic tweezers are used to directly measure the forces involved in the positioning of the mitotic spindle. Using these force measurements in different molecular and geometrical perturbations, Garzon-Coral \textit{et al.} [2016] argue that the cortical pushing forces maintain the position of the mitotic spindle during metaphase. (iii) \textit{The cytoplasmic pulling} model, where forces are applied by cargo-carrying dyneins attached on MTs and walking towards the centrosomes [Kimura & Onami, 2007]. As a consequence of Newton’s third law, the force applied by dynein on MTs is equal and opposite to the force required to move the cargo through the cytoplasm [Shinar \textit{et al.}, 2011; Longoria & Shubeita, 2013]. Since longer MTs carry more dyneins and produce larger pulling forces, the PNC moves in the direction of longer MTs, that is, anterior-wise.

First, we show that all three mechanisms can center and rotate the PNC on a reasonable time-scale, and so proper positioning alone cannot choose a unique model. However, we demonstrate that each mechanism produces its own fingerprint in the generated cytoplasmic flows, which can be used to differentiate between them. These flow signatures are generic features of each mechanism and do not depend on the details of its biochemical regulation and molecular pathways. Specifically, we show that the cytoplasmic flow generated in the cortical pulling model is analogous to the flow that arises from pushing a porous object with an external force. In the cortical pushing model, the cytoplasmic flow is the combination of that same driven porous object flow, with that produced by MT deformations induced by compressive polymerization forces at the periphery. Finally, we demonstrate that the flow induced by a cytoplasmic pulling model is fundamentally different as it can be interpreted as a porous structure that is moved by internal force generators, with its early time flows in the class of \textit{self-propelled Puller particles} [Saintillan & Shelley, 2013].

While this study focuses on the pronuclear migration process in \textit{C. elegans} embryo, the active mechanisms discussed here, including the polymerization forces or forces from cortically- or cytoplasmically-bound dyneins, are utilized in other stages of cell division and in other organisms [Howard, 2001]. Thus, the generic features of these mechanical models, including their flow signatures, can be useful in identifying or differentiating between force transduction mechanisms in other instances.

3 Results

3.1 A few remarks on the scale of a single microtubule drags and flows

The microscopic size of subcellular structures and the large viscosity of cytoplasm yields inertial forces of the cytoplasm negligible compared to viscous forces. Assuming for simplicity that the response of the cytoplasm is Newtonian, force balance in the fluid phase is governed by the
incompressible Stokes equations [Happel & Brenner, 1965]:
\[
\mu \nabla^2 \mathbf{u} - \nabla p = 0, \quad \nabla \cdot \mathbf{u} = 0,
\]
where \(\mu\) is the viscosity of cytoplasm, and \(\mathbf{u}\) and \(p\) are the fluid velocity and pressure fields, respectively. Due to the linearity of the Stokes equation, the induced velocity of the PNC is related to the net force on it by an instantaneous drag coefficient, \(\gamma\), of the structure composed of the PNC and its attached astral MTs.

An important consequence of being in the Stokesian regime is that the drag coefficients of the immersed objects scale with their longest dimensions, making the drag of very thin individual MTs nonetheless comparable to the drag on the PNC. To see this, consider an MT of length \(L = 10\mu m\), which is about the diameter of the PNC and on the scale of astral MT lengths, being moved transversely to itself at a constant speed, \(U\). Slender body theory [Tornberg & Shelley, 2004] estimates the drag force on the MT as \(F = 8\pi\mu LU/\ln(e^{-2}\epsilon^{-1})\) where \(\epsilon\) is the aspect ratio of the MT. The classical Stokes formula estimates the drag force on a spherical PNC moving at the same speed and diameter \(L\) as \(F = 3\pi\mu LU\). The ratio of these two drags (MT to PNC) is given by the formula \(8/\ln(e^{-2}\epsilon^{-1}) \approx 0.25\). Hence, despite having a diameter of only 24 nm a single MT nonetheless has 25\% of the PNC’s drag. This point can be seen more clearly by noting that the drag coefficient has only a weak logarithmic dependency \((\ln^{-1}(e^{-2}\epsilon^{-1}))\) on the thickness of the MT.

The large drag of a single MT is associated with the large volume of fluid that is transported by the motion of that MT. To visualize this long-range nature of the induced flows, in Fig. 2a we show the fluid velocity vectors around a single fiber (thick black line) being pulled in transverse direction, and the variations of the magnitude of the fluid velocity induced by this motion. The generated flow is three dimensional, but for visualization we only present the results in the plane of the fiber (and of the force). The solid white line is the contour line that corresponds to \(|\mathbf{u}|=0.20U\), where \(|\mathbf{u}|\) is the magnitude of the induced fluid velocity. We can see that at distances comparable to the length of the MT, the fluid velocity remains significant. In other words, as long as the separation distance between the astral MTs is less than or comparable to their length –as it typically is in cytoskeletal assemblies– the motion of an individual MT is strongly coupled to the other MTs through the cytoplasmic flows.

3.2 Cytoplasm-filled astral microtubules behave as a porous medium

The mechanical role of astral MTs in all three pronuclear positioning mechanisms is to transfer the force applied either on their plus-ends (by cortical pushing or pulling), or along their lengths (by cytoplasmic pulling), to their minus-ends anchored in the centrosomes, themselves attached to the PNC. This results in active forces and torques acting upon the PNC. Thus, the feature of the cytoplasmic flow that is common in all the cortically-based mechanisms—the cortical pushing and cortical pulling mechanisms—is the flow generated by the motion of the PNC and its anchored astral MTs under a given external force and/or torque. To understand the effect of HIs between MTs, we first study the flow induced by a much simpler MT assembly: a 5 × 5 microtubule array that moves with velocity \(U = 1\) along the transverse direction of MTs (x-axis) in free space; see Fig. 2(b). Figure 2(c) shows the resulting fluid velocity field and the spatial variations of its magnitude in x-z plane. Again, the fluid flow is 3D and the results are projected into x-z plane for visualization. The enclosed surface illustrated with a close dashed line is the fluid velocity magnitude contour corresponding to \(|\mathbf{u}|= 0.9U\). Hence, those fluid elements within this surface move roughly with the velocity of the MT array. In other words, HIs between the MTs significantly reduce the convective penetration of the fluid between the MTs and result in an effective hydrodynamic surface that is much larger than the total surface area of all MTs. The microtubule array therefore, can be seen as a porous volume in which the fluid permeability is decreased as more MTs are included in the array. The HIs also affect the drag on the MT-array. As we showed earlier, the drag of a single MT
moving transversely is roughly $1/4$ of a sphere with the same diameter as the MT length, $\gamma_{MT} = 0.25 \gamma_{sph}$. If HIs are neglected, the total drag on the MT-array would be the summation of the drag on the individual MTs: $\gamma_{array} = 5 \times 5 \times 0.25 \gamma_{sph} = 6.25 \gamma_{sph}$. However, when HIs are accounted for, the computed drag of the MT-array is only $\gamma_{array} = 0.66 \gamma_{sph}$, which is about 10 times smaller than the local drag prediction. Interestingly, increasing the number of MTs in the array to $10 \times 10$ only increases the total drag by less than 4% to $\gamma_{array} = 0.68 \gamma_{sph}$. The computed drag for a $15 \times 15$ remains almost unchanged from $10 \times 10$ array, as if the entire volume in the MT-array was filled with MTs. Note that if HIs are ignored, the predicted drag for $15 \times 15$ MT-array would be $\gamma_{array} = 56.2 \gamma_{sph}$, which is about 85 times larger than the computed value; see Table 1.

With that in hand, we now explore the flows generated by translation and rotation of the PNC and its associated astral MT arrays, first by pulling it with a given external force from the cell posterior to its center, and then rotating it to proper alignment by applying an external torque. The resulting flows are illustrated in Fig. 3 using $N_{MT} = 600$ astral MTs. Flows in the absence of astral MTs are also shown for comparison. Again, the flow is 3D and is projected to the plane of the applied force and AP-axis to aid visualization. The simulation parameters are given in Table 2. Two main translational flow features that are apparent in the presence and the absence of MTs are (1) the fluid flows in the direction of motion of the PNC and along the AP-axis, and (2) reversed flows induced by cellular confinement. Comparing Figs. 3(a) and 3(b) shows that the presence of the MT-array reduces the size of the reversing flow zone. In other words, the presence of MTs has increased the effective hydrodynamic radius of the PNC, illustrated by the dashed circle. This increase in hydrodynamic radius is also apparent in rotational motion (Figs. 3(c) and 3(d)) by noting that magnitude of the bulk velocity decays much more slowly away from the PNC when the astral MTs are present.

We observed the same trend in the earlier example of MT-arrays (see Fig. 2c), which can be described as follows. In pronuclear migration, the astral MTs act as a co-moving porous layer that surrounds PNC and abuts the cell periphery. In particular, the HIs between the MTs reduce the convective penetration of fluid into the porous layer and creates an effectively larger object moving through (or rotating in) the fluid. Note that the relevant dimension of MTs that defines the strength of HIs and the fluid flow into the astral MTs is the length of the MTs and not their thickness. Thus, as we saw in the earlier example of MT array, a small number of astral MTs can substantially increase the effective volume of the PNC and reduce the permeability of the fluid into the volume filled with astral MTs. See [Nazockdast et al., 2017] for a more quantitative study of the cytoplasm-filled astral MTs behaving as a porous medium.

3.3 Neglecting hydrodynamic interactions produce wrong estimates of pronuclear migration force magnitudes

To provide a more quantitative analysis of the effect of the astral MTs on the dynamics of pronuclear migration, we compute the translational, $\gamma_T = F/\Omega_{PNC}$, and rotational, $\gamma_R = F/\Omega_{PNC}$, drag coefficients as a function of the number of MTs. Here $\Omega_{PNC}$ and $\Omega_{PNC}$ are the computed translational and angular velocities of the PNC under external force $F$, and torque $T$, respectively. We consider three different conditions or models. In model (1), we neglect all HIs, and calculate drag on MTs using a local slender body drag formula [Tornberg & Shelley, 2004], and drag on the PNC using Stokes’ drag formula. This approximation closely follows the Brownian Dynamics simulation techniques used for modeling cellular assemblies, such as Cytosim [Nedelec & Foethke, 2007]. In model (2), we include HIs amongst the MTs and with the PNC, but neglect the backflow generated by the cell periphery through the no-slip boundary condition. That is, in model (2) cytoplasm flows in and out of the cell periphery without impedance. The confining presence of the cell wall is partially maintained by having MTs depolymerize upon reaching it. In model (3), we include all HIs; in particular, those that arise from applying the no-slip boundary condition at the periphery. A comparison between model (2) and model (3) allows us to separate the effect of
confinement flows induced by the cell wall from those induced by the aster.

Figure 4 shows the variation of computed translational and rotational drag coefficients with the number of MTs for all models (1,2,3). The values are normalized, respectively, by the drag coefficients of a rigid spherical PNC, \( \gamma_T^R = 6\pi \mu R_{PNC} \) and \( \gamma_R^R = 6\pi \mu R_{PNC}^2 \). The black reference lines at \( \hat{\gamma}_T = \frac{\gamma_T^R}{\gamma_T^R} = 2 \) and \( \hat{\gamma}_R = \frac{\gamma_R^R}{\gamma_R^R} = 8 \) are estimates that assume that PNC drag can be modeled using Stokes formula with an effective sphere of radius \( R = 2R_{PNC} \), as used by Kimura & coworkers [Kimura & Onami, 2005, 2007] in their modeling of pronuclear rotation and centering.

As expected for model (1), having no HIs between MTs, both drag coefficients increase linearly with \( N_{MT} \), showing no saturation. For model (2), \( \hat{\gamma}_{RT} \) increases monotonically with \( N_{MT} \), with \( \hat{\gamma}_{RT} \) saturating as \( N_{MT} \to \infty \). This behavior can be explained through our earlier finding that the astral MTs and cytoplasm form a porous medium. As the number of MTs is increased, the permeability of the porous medium is decreased while the effective hydrodynamic dimensions of the PNC are increased. In the limit of \( N_{MT} \to \infty \), the flow cannot penetrate the porous layer, which fills the cell volume. Since in model (2) the back flows induced by the cell confinement are neglected, in this limit the drag coefficients asymptote to those of a solid object filling the cell and moving in free space.

When confinement-induced back flows are included by accounting for HIs with the periphery [model (3)], the drag coefficients again show monotonic increase with \( N_{MT} \). However, for \( N_{MT} = 1200 \), the model (3) \( \hat{\gamma}_T \) is 6-fold larger than that for model (2), is several-fold smaller than for model (1), and is 6-fold smaller than for model (3) with \( N_{MT} = 0 \) (the red star), which corresponds roughly to our previous modeling [Shinar et al., 2011] of pronuclear migration in which MT drag was not included. Now as \( N_{MT} \to \infty \), the effective hydrodynamic dimensions of PNC approaches the size of cell periphery and we expect \( \hat{\gamma}_T \to \infty \) due to the no-slip condition.

### 3.3 Rotational dynamics of the PNC

Fig. 4b shows that the rotational drag coefficient qualitatively follows the same trend as the translational coefficient, with one key difference. Comparing model (2) and model (3) in Figs. 4a and 4b shows that confinement has a much smaller effect on the dynamics of rotation than on translational motion. This is expected, since rotation of the PNC and the astral MTs involves sliding an effectively larger PNC tangentially to the cell periphery, while translation involves moving the effective surface of the PNC and the astral MTs normal to the cell periphery’s surface, which induces larger resistance to motion [Happel & Brenner, 1965].

We can use the predictions of model (2), shown in Figs. 4a and 4b, to study a more fundamental question: Can the mixture of cytoplasm and astral MTs be modeled as a fluid with a simple effective viscosity? If that is true, then we expect the ratios of the translational drag coefficient to the rotational drag coefficient to be independent of the number of MTs i.e. \( \frac{\hat{\gamma}_{RT}}{\hat{\gamma}_T} = 1 \). Our model (2) predictions, however, show that \( \frac{\hat{\gamma}_R}{\hat{\gamma}_T} \) increases from 1 at \( N_{MT} = 0 \) to 7 at \( N_{MT} = 1200 \). This simple example demonstrates that HIs cannot be coarse-grained through a single effective viscosity, since HIs do not have the same dynamical effect on rotational and translational motions. Instead, our simulations show generally that the entire cytoplasm-filled MT array acts as a porous medium whose permeability decreases with an increasing number of MTs, thus giving an increased effective size of the PNC in response to an applied force (at least in the absence of force generators within the astral MT array producing active flows). The different scaling of translational and rotational drag coefficients with the effective radius of the PNC (\( \gamma_T \) scales with \( R \), while \( \gamma_R \) scales with \( R^2 \)) causes \( \frac{\hat{\gamma}_R}{\hat{\gamma}_T} \to R^2 \) to increase, as the effective radius of the PNC and its astral MTs increases with the number of MTs.
3.5 Three mechanisms of pronuclear migration yield proper positioning of the pronuclear complex

We now investigate the mechanics of pronuclear migration and positioning using three proposed positioning mechanisms in *C. elegans* embryo; namely (i) the cortical pulling, (ii) cortical pushing and (iii) cytoplasmic pulling models. To start, within our framework we have instantiated a cortical pulling model that Kimura & Onami [2007] developed for their study of pronuclear migration. Figure 5a shows a schematic of this model, which was motivated by experimental observations of Tsou *et al.* [2002]. In this model, pulling forces on astral MTs are generated by the asymmetric attachment of astral MTs to cortically-bound dyneins whose activation probability is inhomogeneously distributed along the posterior cortex with the greatest probability of attachment at the posterior pole, and the least to the immediate posterior of the mid-plane. Attached MTs are pulled upon, and simultaneously depolymerized, at the cortex; see Supplementary Materials for details. A snapshot of the simulation at long times is shown in the left panel of Fig. 5b. It demonstrates that this mechanism leads to centering and rotation to proper position. In this simulation, PNC translation and rotation are in temporal register, and proper position is achieved on a reasonable time-scale; see the left panel in Fig. 5c. Since the cortically-bound force generators in this model put the MTs under an extensional load, MT deformations are small and they remain relatively straight; see the left illustration in Fig. 5b. For the parameters used in this simulation (motor attachment distribution and number density, MT attachment and detachment rates, …) we find that the PNC robustly finds proper position. These chosen parameters were physiologically reasonable but also narrowly constrained, as other seemingly reasonable choices of these parameters can lead to lack of centering. More details are given in Supplementary Materials and the biophysical parameters corresponding to the figures are given in Table 2.

Next we consider a variation of the cortical pushing model. In this model we constrain the position of the MT plus-ends which reach and polymerize at the cortex to remain fixed (and hinged) at the cortex, as long as they are in growing state (see supplementary materials). In an idealized system where the PNC is pushed by an up-down symmetric set of astral MTs, the intercentrosomal axis would remain orthogonal to the AP-axis throughout the centering process (having started that way). However, a combination of dependency of polymerization forces on MT length (shorter MTs are harder to deform, resulting in larger polymerization forces) and the elongated shape of the cell periphery makes this orientation mechanically unstable and susceptible to the fluctuations which MT dynamic instability can provide. Once seeded this "torque instability" produces a self-reinforcing, rotating torque upon the PNC, moving it towards proper position, which is a mechanically stable equilibrium (see Supplementary Materials).

As shown in the middle panel of Fig. 5c, this model results in proper centering and rotation of the PNC on a reasonable timescale. Also, the middle panel in Fig. 5b shows that for this particular instantiation of the cortical pushing model, the MTs are substantially buckled near the cortex. We have also considered another variation of cortical pushing where –rather than fixing the plus-ends of MTs– pushing forces are applied in the normal direction to the cell boundary (pointing inwards) so that MT plus-ends cannot penetrate the boundary. MTs are nonetheless allowed to grow or slide freely tangentially. Our simulations show that, while the PNC properly centers in this model, it fails to rotate the PNC to the proper alignment with the AP-axis; this variation of the model is discussed in detail in Supplementary Materials.

Finally, a schematic of the cytoplasmic pulling model is shown in the right panel of Fig. 5a. This model was initially proposed to explain observations in newly fertilized sand-dollar eggs [Hamaguchi & Hiramoto, 1986]. Later, through modeling and experimental study, Kimura and coworkers proposed this as a mechanism for pronuclear positioning in *C. elegans* embryo [Kimura & Onami, 2005; Kimura & Kimura, 2011]. This model was studied in our own earlier work on pronuclear migration [Shinar *et al.*, 2011], where minus-end directed cargo-carrying dyneins walk on MTs and so apply a pulling force on them towards their plus-end. Applying proper force
balance [Shinar et al., 2011], the cargo exerts an equal and opposite force on the fluid. If dyneins are uniformly distributed along the MTs, as is assumed here, the PNC moves in the direction of the longest MTs as these contribute the greatest pulling forces; see Supplementary Materials for detail. As shown in Fig. 5c right panel, this model can yield both centering and rotation on a time-scale that is comparable to experimental observations [Kimura & Onami, 2005]. In similarity to the cortical pulling model, the MTs are under extension and their deformations are small, which is evident in the long-time 3D snapshot of the simulation in Fig. 5b (right panel).

3.6 Cytoplasmic flows during pronuclear migration

Our simulation results, thus far, show that all three positioning mechanisms can produce the expected alignment and position of the pronuclear complex within a reasonable time-scale and choice of biophysical parameters. We now discuss their induced cytoplasmic flows and the generic features specific to each. Our motivation is that different mechanisms of force transduction will exert different forces upon MTs and hence should be associated with generically different cytoplasmic flows. To demonstrate these differences, we start by studying the flows induced by a single MT growing against a barrier, and by a single cargo carrying dynein motor walking along a MT. These are the simplest representations of cortical pushing and cytoplasmic pulling flows, respectively. These flows are shown in Fig. 6. The flow induced by a MT being pulled by cortical force generators –the simplest model of the cortical pulling mechanism– is the same as in Fig. 6a, but in the opposite direction, and, thus, is not presented here.

There are general physical principles that underlie the gross cytoplasmic flow structures that we observe for the different models. In both cortically-based models, the MTs are pushing or being pulled against a fixed boundary (cell periphery), while an opposite force is applied from the cell boundary or the cortically bound dynein motors to the MTs. In such a case –since the force on the outer boundary does not generate any internal flows– the cytoplasmic flows are associated with the motion of the MT under an external force within the cellular confinement. In the case of the cortical pushing mechanism –since the MT plus-end is fixed at the cortex– the polymerization forces push the MT away from the cortex, to open space for adding the newly formed microtubule materials. This outward flux creates a flow by dragging cytoplasm from the cell periphery into the cell volume (see Fig. 6a). In case of the cortical pulling mechanism, the applied force and the resulting cytoplasmic flows are in the opposite direction.

In the cytoplasmic pulling model, the pulling force applied on the MTs by a dynein motor is balanced by the equal and opposite force applied by that motor’s cargo onto the cytoplasm through which it is being dragged. Unlike the cortical models, where the applied forces at the cell boundary do not induce internal flows, the force applied by the dynein motors to the cargo generates a flow in the opposite direction of the MT motion. The flow is then a combination of the flow induced by the motion of the MT as well as the flow induced by the cargo transport. Since equal and opposite forces are applied to the cargo and the MT, the flow roughly corresponds to the flow induced by a force-dipole [Happel & Brenner, 1965]. Figure 6b shows the 3D velocity streamlines and spatial variations of velocity magnitude, induced by a single dynein motor carrying a 0.1 µm spherical cargo along a 2 µm long microtubule. The results are projected to the plane of motion of the MT. Note that the flow strength in this model decays much faster than the flow induced by growing/shrinking MTs against barriers and becomes negligible at distances less than the length of the MT.

With these simple examples in hand, we now discuss the computed cytoplasmic flows during pronuclear migration in complete instantiations of the three mechanisms. We start with the cortical pulling mechanism. Since the MTs are hardly deformed in this model, the motion of entire
PNC/MT-array complex generates cytoplasmic flows similar to that of a porous body towed through a confined space. Figure 7a (top) shows a snapshot of the cytoplasmic velocity field at early times, which, at this instant, is dominated by translation and bears a striking resemblance to that shown in Fig. 3a, where the PNC/MT-array is translated by an external force. At an intermediate time, the cytoplasmic flow has become primarily rotational [see Fig. 7a (middle)]. In these conditions the flow begins to resemble that seen in Fig. 3c, where the centered PNC/MT-array is rotated by an external torque. At late times [e.g. Fig. 7a (bottom)] the cytoplasmic flows are weak and arise from fluctuations in PNC position due to the stochastic attachment and detachment of MTs from the cortex. As the MTs remain straight under their extensive loading from cortical force generators, the cytoplasmic velocity fields arise almost entirely from the translations and rotations of the PNC/MT array complex.

For the cortical pushing model shown in Fig. 7b, the cytoplasmic flow is a combination of the flows generated by the motion of the PNC/MT-array complex (similar to the flow shown in Fig. 3a) and flows due to MT deformations near the periphery. After the MTs reach the cortex, and continue polymerizing, they are pushed away from the boundaries with the same speed as they grow. Thus the generated flows near the cortex scale with the plus-end polymerization rate and are primarily in the direction opposite the polymerization direction; see Fig. 6a. For the range of biophysical parameters used in our simulations, see Table 2. The flow induced by MT deformation near the periphery is comparable in magnitude to that induced by the PNC motion for most of the migration process. The middle and lower panels of Fig. 7b show that the same flow patterns are also observed near the periphery after completion of PNC centering and rotation.

Finally, we discuss the flows in the cytoplasmic pulling mechanism. Unlike the cortical pushing and pulling models, which use the cell periphery as the mechanical substrate against which to exert forces through MTs, the force substrate is now the cytoplasm in which the cargos are immersed. Hence, the generated flow arises from two sources: (1) the flow induced by the motion of the PNC/MT-array, and (2), the flow generated by the motion of cargos towards the minus-ends of MTs. Figure 7c shows that the cytoplasmic flows thus produced are fundamentally different than those observed in the two previous models. The key flow signature that is present in all stages of migration is that, unlike the cortical pushing and pulling mechanisms, the cytoplasmic flow in the anterior is in the opposite direction of the motion of the PNC and is along the direction of cargo transport towards the centrosomes in the volume occupied by the astral MTs. The strength of the flow in the cytoplasmic pulling model is determined by the total active force applied by the dynein motors, which in our model scales with the total length of the MTs. Consequently, the strength of flow throughout and after the migration process does not change significantly. On the other hand, the velocity of the PNC monotonically decreases as it approaches the center of the cell. Hence, as we see in Fig. 7c (middle and bottom panels), after centering the strength of cytoplasmic flows is much stronger than the small fluctuating velocity of the PNC.

The flows arising from cytoplasmic pulling resemble those generated by “Puller” microswimmers [Saintillan & Shelley, 2013] such as Chlamydomonas reinhardtii [Drescher et al., 2010]. The reason lies in the ensemble behavior of the PNC/MT-array and the immersed dynein
motors. As shown in Fig. 7a, for any force applied by the attached dynein motors on the MTs there is an equal and opposite force applied by the cargos to the cytoplasmic fluid. Thus, while the force on the PNC/MT-array is not zero, the net force on the PNC/MT-array and the cytoplasmic fluid is identically zero. In particular, the motion of each cargo on the MT-array generates a force-dipole, described by a tensor, whose symmetric part gives the net stress on the system, which unlike the net force is not zero. For straight MTs and a uniform distribution of the motors the net stress tensor induced by motor activity is

\[ \sigma_{\text{motor}} = \frac{1}{2} \sum_{i=1}^{N} L_i (p_i r_i + r_i p_i) f_{\text{motor}}, \]

where \(L_i\) and \(p_i\) are the length and unit tangent vector of the \(i^{th}\) MT, respectively, and \(r_i\), taken as orthogonal to \(p_i\), gives the relative position of the cargo with respect to its attachment point on the \(i^{th}\) MT. The antisymmetric part of the force dipole determines the net torque on the system, which is also zero. Zero net force and torque and a finite active stress are the two hallmarks of active, self-propelled particles [Saintillan & Shelley, 2013]. As a result, the flow far from such particles is that generated by the symmetric part of a force dipole. While in our system the size of the “active particle” – the PNC/MT-array – is similar to the scale of its confinement, the flow still closely resembles that generated by a Puller particle in an open flow (see Fig. 5 of [Saintillan & Shelley, 2013]).

The cortically-based models both generate grossly similar cytoplasmic flows (though different MT deformations) as they share the feature that the force is transduced to the PNC by MT pushing/pulling on/from an immobile cell boundary. In these conditions the net force on the PNC/MT-arrays and the cytoplasm is non-zero. The cytoplasmic flows then resemble those generated by a force monopole, or a Stokeslet, within a confinement.

4 Discussion

The motion of cytoskeletal components and payloads within the cytoplasm can generate global flows and long-ranged hydrodynamic interactions (HIs) between them. Most previous studies related to the mechanics of cytoskeleton ignore the effect of HIs; see [Shinar et al., 2011] for an exception. In this study we have used detailed, dynamic simulations that explicitly accounts for many-body HIs to demonstrate several important consequences of HIs in the mechanics of pronuclear migration stage of the first cell division in \textit{C. elegans} embryo. Nevertheless, the findings of this study are quite generic and extendable to other cytoskeletal assemblies.

First, through direct simulation, we showed that the most fundamental effect of HIs in pronuclear migration is that the astral MTs array behaves effectively as a porous medium; see [Nazockdast et al., 2017] for a more quantitative study. Increasing the number of astral MTs reduces the permeability of the fluid resulting in larger hydrodynamic dimensions of the PNC and a larger net force/torque associated with moving the PNC to the proper position/orientation. We showed that previous approximations of the PNC drag coefficient, that either ignore HIs or include them partially, over-predict or under-predict active forces by a large factor. Through comparing the translational and rotational motion of the PNC and its astral MTs we showed that the effect of astral MTs cannot be simply reduced to an effective viscosity. This observation has important consequences that go beyond the pronuclear migration problem. For example, consider measuring the effective viscosity of the cell’s interior using active microrheology, where a probe is externally driven through the cytoplasm using...
magnetic forces and/or optical tweezers with a given force or torque [Wirtz, 2009]. The measured translational or angular velocity of the probe is then used to compute the effective viscosity. For a spherical probe the viscosity is computed using $\mu = F/(6\pi U a)$ or $\mu = F/(8\pi \Omega a^3)$ for cases of applying a fixed external force $F$ or torque $L$, respectively. If the cellular interior acts as a porous medium – since the hydrodynamic effect of the astral MTs cannot be modeled with an effective viscosity – the computed viscosities from these two modes (applying a force vs a torque) may yield viscosities that differ considerably. Instead, if the astral MTs were not attached to the PNC and were freely suspended in the cytoplasm, these two experiments are expected to give similar results for the viscosity.

We studied the dynamics of PNC/MT-array positioning using simple instantiations of the cortical pulling, cortical pushing and cytoplasmic pulling mechanisms. Our results show that all three mechanisms can center and rotate the PNC within reasonable times and range of biophysical parameters. Thus, proper positioning alone cannot identify the main mechanism of PNC migration. We propose that the structure of cytoplasmic flows may select between the different possible active mechanisms involved in cellular processes such as pronuclear migration. We show, through simulation, that each of these force transduction mechanisms leaves its own specific fingerprint in the generated cytoplasmic flows; these features are directly related to how the force is transferred from molecular motors and cell boundaries to the MTs. Specifically, we show that when the active forces are applied from an immobile substrate to the MTs, such as in the cortical pushing and pulling mechanisms, the basic feature of the cytoplasmic flows is that of the flow generated by a point force (Stokeslet) in a confined geometry. In the cytoplasmic pulling mechanism where the substrate is mobile (cargo carrying dynein), the generated flow is the combination of the flow generated by the average motion of the PNC/astral MT and flow induced by cargo transport, which resembles the flows induced by a force dipole.

These aspects of flow signatures are generic features of each active mechanism, and do not depend on details such as the values of shear viscosity of the cytoplasm, the MTs bending rigidity, stall force of the molecular motors, or their force-velocity relationship. Thus, they can be used to study the possible contributions of these differing force transduction mechanisms in other stages of cell division and possibly other cytoskeletal structures. We are currently following up on these ideas by directly measuring the cytoplasmic flows by particle tracking methods.

**Acknowledgement**

We thank Sebastian Füerthauer, Hassan Masoud, Tong Gao, Michael O’Neal and Calos Garzon-Coral for helpful discussions. We acknowledge support from National Institutes of Health Grant 1R01GM104976-01. Ehssan Nazockdast and Michael Shelley acknowledge support from National Institutes of Health grant 1R01GM104976-01, and National Science Foundation grants DMS 1463962 and DMS-1620331. Abtin Rahimian acknowledges the support of the US National Science Foundation (NSF) through grant DMS-1320621.

**References**


**List of Figures**

![Diagram](image)

**FIGURE 1**: A schematic illustrating the modeled structural components and dynamics of pronuclear migration in the single-cell *C. elegans* embryo. (a): the structural components. The pronuclear complex –here modeled as a rigid sphere—contains the male (red) and female (yellow) pronuclei, and is attached to two arrays of MTs (green lines) that polymerize from two centrosomes (green bodies). These structures are immersed in the cellular cytoplasm (light blue) and confined within an ellipsoidal eggshell. (b): The dynamics of pronuclear migration and positioning. At $t = t_1$, the female pronucleus moves to the posterior to
combine with the male pronucleus ($t=t_0$) to form the pronuclear complex (PNC). This initial period of female nuclear migration is not modeled here [Payne et al., 2003]. Between $t=t_0$ and $t_1$ the PNC moves anterior-wise to the center while rotating into “proper position” with the centrosomal axis along the anterior-posterior (AP) axis ($t=t_2$).

FIGURE 2: The 3D cytoplasmic flow induced by moving (a) a single MT and (c) a $5 \times 5$ MT-array, in their transverse direction (x-axis) and with a net velocity $U = 1$, projected in the plane of the motion of the MT(s), x-z plane. The arrows show the fluid velocity vectors. The size of the arrows and the background color-plots correspond to the local magnitude of the fluid velocity. The 3D configuration of MT array is shown in Fig. 2(b). The solid white line in Fig. 2(a) corresponds to the contour with velocity magnitude $|u| = 0.20U$. The dark dashed line in Fig. 2(c) is the contour with $|u| = 0.90U$, roughly corresponding to the effective hydrodynamic size of the MT-array.
FIGURE 3: The cytoplasmic flows induced by (a) translational and (c) rotational motion of the PNC and its attached astral MTs. Subfigures (b) and (d) show the translational and rotational flows in the absence of astral MTs.

FIGURE 4: (a) The normalized translational, $\tilde{\gamma}_T = \gamma_T / \gamma_T^0$, and rotational, $\tilde{\gamma}_R = \gamma_R / \gamma_R^0$, drag coefficients vs the number of MTs ($N_{MT}$), where $\gamma_T^0 = 6\pi\mu_R P_{\text{PNC}}$ and $\gamma_R^0 = 8\pi\mu_T P_{\text{PNC}}$ are the translational and rotation drag coefficients, respectively, of a rigid spherical PNC in the absence of MTs. The solid straight lines correspond to the estimates used in [Kimura & Onami, 2005, 2007]; the “◊” symbol shows the predictions of model (1), where only the local drag coefficient of individual MTs are accounted for—as used by Cytosim [Nedelec & Foethke, 2007]; the “□” symbol is the predictions of model (2), where HIs with the cell cortex (hydrodynamic confinement) is
neglected; the "

" symbol roughly corresponds to our own earlier study that neglects the drag on the MTs [Shinar et al., 2011]; the " ○ " symbol presents the simulations result of model (3) that fully accounts for all HIs. The solid lines going through the prediction of model (2) are linear fits to the data. These equations are 

\[
\hat{\beta}_T = 1 + 0.12 N_{NT} \\
\hat{\beta}_R = 1 + 0.17 N_{NT}
\]

for the translational and the rotational drag, respectively.

FIGURE 5: (a) Schematic presentations of the force transduction mechanisms. The left figure shows the cortical pulling model where the blue, yellow and red strips on the cortex correspond to the lowest, average and highest density, respectively, of the cortical force generators. See Supplementary Materials for details. The middle panel shows the schematic of the cortical pushing model. The larger number of MTs polymerizing against the cortex on the posterior side and their shorter lengths (corresponding to larger bending forces) result in larger pushing forces on the posterior pole (compared to the anterior) and pronuclear migration from posterior to the center of the cell. The right panel shows the schematic of the length-dependent cytoplasmic pulling mechanism, where cargo carrying dynein motors apply pulling forces on the astral MTs. Longer MTs have more dyneins attached to them, which result in pronuclear migration towards the longest MT i.e. from posterior to the center of the cell. (b) Long-time 3D snapshots of the simulations in the three mechanisms (Movie S1, S2, S3). The fibers are color-coded with respect to the local tension; red, blue and white colors denote compressional, extensional, and no forces respectively. In the cortical and cytoplasmic pulling models the MTs are under extensile forces and thus remain straight; while in the cortical pushing simulations, the MTs are buckled due to the compressional forces from their polymerization against the
cortex. (c) the variations of the PNC position and angle between the intercentrosomal axis and the AP-axis with time.

FIGURE 6: (a) Flow induced by a MT growing against a barrier, and (b) the flow induced by a cargo carrying dynein walking on a MT, as simple representations of the cortical pushing and the cytoplasmic pulling mechanisms. The induced flows are all three dimensional and are only presented in the plane of the MT and the direction of its motion for visualization. The solid curved lines in both figures are the flow streamlines. The arrows tangent to the streamlines are the velocity vectors. The size of the arrows and the background contour plot correspond to the magnitude of fluid velocity. Those vectors with only arrow head in Fig. 6b correspond to nearly zero fluid velocity, as evident from the contour plot. The maximum and minimum in Fig. 6b colorbar (1 and -0.2) correspond to the velocity of the cargo and the MT in x direction respectively.
FIGURE 7: Snapshots of cytoplasmic flows at different stages of pronuclear migration: (a) the cortical pulling, (b) cortical pushing and (c) cytoplasmic pulling mechanisms. Numbers denote time in minutes: seconds after the nuclei meeting. The results are projected onto the $xy$-plane to aid visualization, where the AP-axis is the x-axis and the intercentrosomal axis is initially aligned with the y-axis. Note that both the shape of the MTs and the flow are defined in three dimensions. The fainted colors of the velocity vectors in the bottom panel of the cortical pulling flows, and the middle and bottom panels of the cortical pushing flows represent the "weakness" of the cytoplasmic flows in those instances, when compared against the flow strength at the initial stages of the PNC migration. In contrast, the flows in the cytoplasmic pulling model remain "strong" throughout the entire migration process.

<table>
<thead>
<tr>
<th>Number of MTs</th>
<th>Drag on the MT-array without HIs</th>
<th>Drag on the MT-array with HIs</th>
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<tr>
<td>$3 \times 3$</td>
<td>2.25</td>
<td>0.63</td>
</tr>
<tr>
<td>$5 \times 5$</td>
<td>6.25</td>
<td>0.66</td>
</tr>
<tr>
<td>$10 \times 10$</td>
<td>25</td>
<td>0.68</td>
</tr>
<tr>
<td>$15 \times 15$</td>
<td>56.25</td>
<td>0.68</td>
</tr>
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</table>

Table 1: The predicted drag on the MT-array, $y_{array}$, in the presence and absence of HIs. The values are normalized by the drag on a sphere with a diameter equal to the length of the MTs in the array.
Table 2: The biophysical parameters used in our Simulation. These values are taken from Kimura and Onami [Kimura & Onami, 2005, Table 1]. The references related to each measurement is also given in that article.

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Values used in simulations</th>
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<tbody>
<tr>
<td>MT growth velocity ((V_R))</td>
<td>(0.12 \mu m \cdot s^{-1})</td>
</tr>
<tr>
<td>MT Shrinkage velocity ((V_d))</td>
<td>(0.288 \mu m \cdot s^{-1})</td>
</tr>
<tr>
<td>MT rate of catastrophe ((f_{cat}))</td>
<td>(0.014 s^{-1})</td>
</tr>
<tr>
<td>MT rate of rescue ((f_{res}))</td>
<td>(0.014 s^{-1})</td>
</tr>
<tr>
<td>MT Bending modulus ((E))</td>
<td>(10 pN \cdot m^2)</td>
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<tr>
<td>MT stall force for polymerization reaction ((F_{mt}^{p}))</td>
<td>(4.4 pN)</td>
</tr>
<tr>
<td>Cytoplasmic dynein’s stall force ((F_{mt,all}))</td>
<td>(1 pN)</td>
</tr>
<tr>
<td>Viscosity of the cytoplasm ((\mu))</td>
<td>(1 p\alpha.s)</td>
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<td>Long axis of the cell</td>
<td>(50 \mu m)</td>
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<tr>
<td>Short axis of the cell</td>
<td>(30 \mu m)</td>
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<tr>
<td>Radius of the pronuclear complex ((a_{PNC}))</td>
<td>(5 \mu m)</td>
</tr>
</tbody>
</table>