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#### Review

## Mechanics of the spindle apparatus

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#### ABSTRACT

During mitosis microtubules self-organize to form a bipolar mitotic spindle structure, which positions the sister chromatids on the spindle mid-plane and separates them afterwards. Previous studies have identified many spindle associated proteins. Yet, we do not fully understand how these nanoscopic proteins lead to force generation through interactions of individual microtubules, motor proteins and chromosomes, and how a large number of these local interactions ultimately determine the structure and mechanics of the spindle in micron scale. Here we review the current understanding and open questions related to the structure and mechanics of the mitotic spindle. We then discuss how a combination of electron microscopy and computational modeling can be used to tackle some of these open questions.

#### 1. Introduction

Mitosis is a fundamental process of life. During its course microtubules assemble into a bipolar spindle that aligns the chromosomes on the metaphase plate and subsequently segregates them to the two arising daughter cells during anaphase. The faithful alignment and segregation of chromosomes is indispensable for the health and survival of any organism. Any error in the process of mitosis has severe consequences for the organisms. During embryonic development most of these errors will be lethal and result in miscarriage or still birth. In other cases, they can cause severe genetic conditions such as Monosomies and Trisomies, or cancer.

By today we have gathered a multitude of information about many proteins, such as nucleators, motor proteins, kinases, phosphatases and other MT associated proteins, which are involved in the assembly of spindles, quality control and the subsequent segregation of chromosomes. We have achieved a very good understanding of signaling cascades and checkpoints that monitor and control mitosis, yet we do not understand the force generation mechanisms that drive the self-organization of microtubules (MTs) into a robust and functional spindle structure and also coordinate the motion of chromosomes.

Since the first observation of microtubules in spindles by polarized light microscopy about 70 years ago, many studies have focused on understanding various aspects of spindle mechanics, most importantly (i) spindle assembly, and (ii) chromosome dynamics during congression and segregation. The findings of these studies are summarized in

several recent review papers [1-4].

One standing difficulty in understanding spindle mechanics has been measuring forces within cells. Although powerful tools such as microneedle manipulation [5–9], molecular tension-sensors [10,11] or laser-ablation techniques [12–14] have been developed that provided extremely valuable information, they are often limited to information about collective behavior in spindle length-scales. They do not allow us to measure forces generated or experienced by MT subgroups or even individual MTs and chromosomes.

In tandem with experiments, there have been many modeling studies of spindle mechanics. These studies can reproduce stable bipolar metaphase spindles with dimensions comparable to experiments [15–18] and predict chromosome capture, congression and segregation. The force generation mechanisms, namely the type of motors and microtubules involved, can vary significantly between these models. The underlying assumptions of these models cannot be tested with light microscopy. Thus, it is very difficult to choose the correct model or determine the contribution of each model.

Recent advances in electron tomography have resulted in a detailed picture of MT and chromosome arrangements in mitotic and meiotic spindles [19–22]. These results provide quantitative data on number, length, position, interactions and shapes of MTs, which can be used to test and modify the existing models for spindle mechanics or to develop new models that are consistent with microscopic data from tomography as well as the dynamic macroscopic results of light microscopy. Thus, possibly contributing to a more detailed analysis of forces within

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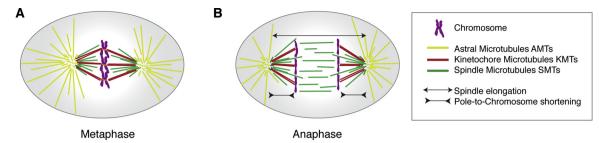


Fig. 1. Overview Mitosis. A, Cartoon of a mitotic spindle in metaphase. The different subclasses of microtubules are labeled by individual colors; Kinetochore microtubules (KMTs) are red, Spindle microtubules (SMTs) are dark green and Astral microtubules (AMTs) are light green. B, Cartoon of a spindle in Anaphase. The arrows indicated the different mechanisms contributing to chromosome segregation, such as the increase of the pole to pole distance during spindle elongation as well as the decrease of the pole to chromosome distance.

spindles.

This review takes a bottom-up approach to explore the different force generating mechanisms of spindle components and how those contribute to spindle assembly and chromosome segregation.

#### 1.1. Structural components of spindles

Microtubules are the main structural components of the spindle. Depending on the size of the spindle there can be somewhere between tens (2 µm long spindles budding yeast) and tens of thousands (60 µm long Xenopus spindle) of MTs [23]. MTs polymerize from alpha/betatubulin heterodimers and display a characteristic stochastic switch from slow growth to fast shrinkage, described as dynamic instability [24]. MTs show a distinct polarity with a relatively stable minus and a dynamic plus end. Most MT minus ends are associated with the centrosome. This non-membrane bound organelle is the major site of MT nucleation in animal cells, although other nucleation sites have been reported [25-27]. According to the direction of MT plus-end growth and interaction with a particular cellular target site, MTs of bipolar spindles can be grouped into different classes: Astral MTs (AMTs), Spindle MTs (SMTs) and Kinetochore MTs (KMTs) (Fig. 1A). AMTs are those MTs, which grow away from centrosomes towards the cellular cortex, thus mainly playing a role in positioning of the spindle apparatus [28]. SMTs are growing towards the center of the spindle and might play a role in spindle stability and KMTs are directly connected to the kinetochores on the chromosomes. Recent publications have described a fourth class of MTs, a subclass of SMTs, the so-called bridging fibers, which seem to play a role in chromosome segregation [29].

As the cell enters metaphase of meiosis or mitosis, all chromosomes are aligned on the spindle equator. The fidelity of the chromosome alignment and MT attachment is monitored by a rather complex checkpoint mechanism [30]. Once all chromosomes are aligned properly, the checkpoint is released and the cell will enter anaphase. The separation of chromosomes during anaphase can be divided into two different phases: Anaphase A and Anaphase B [31]. During Anaphase A the distance between the chromosomes and spindle pole shortens, moving the chromosomes towards the spindle pole. Anaphase B is characterized by a separation of the spindle poles (Fig. 1B). Once the chromosomes are segregated the cell will enter telophase. Subsequently the cell will undergo cytokinesis and abscission giving rise to two genetically identical daughter cells in mitosis or a haploid germline cell during the process of meiosis.

#### 1.2. Spindle assembly as a mechanical process

Mitotic and meiotic spindles can be viewed as mechanical entities that generate and exert forces to reorganize themselves and ultimately segregate the chromosomes. Within every mitotic cell there are a number of components that inevitably interact and exchange forces during the formation of spindles. There are MTs and MT associated

proteins, motor proteins and chromosomes, as well as membrane compartments, most obviously the cell cortex, but also the nuclear envelope, the endoplasmic reticulum (ER) and other organelles and vesicles. The forces that are generated and acting between individual microtubules and motor proteins play an essential role in the assembly and stability of spindles and are indispensable for the segregation of chromosomes.

Multiple studies have shown that motor proteins play crucial roles in organizing and sorting microtubules to guarantee a proper assembly into a functional bipolar spindle [32–34]. One common theme in these studies is that the force balance, that ultimately gives rise to a stable spindle, is a consequence of competing contractile (pulling) and extensile (pushing) forces arising from interactions of different types of motor-proteins with MTs [35–38], as well as other structures such as chromosomes [18] and the cell cortex [16,39,40]. We will review these interactions in the Micromechanics section 2.1; see also [41] for a review on the topic.

Another class of force balance models involves MTs polymerization/depolymerization forces [42–44]. In these models forces are in part generated by polymerization/depolymerization of KMTs against Chromosomes, which points to their key difference with MT-motor models, where KMTs generally do not play a role. The polymerization forces are explored in section 2.1.2.

Perhaps the simplest macroscopic measure of a stable spindle is its length. Previous studies show that the spindle length scales with the cell size and ultimately reaches a maximum length that is independent of the cell size. This scaling holds across different metazoans [45] and throughout successive cell divisions [46,47]. The role of active forces from polymerization and motor activity on spindle length are poorly understood. An alternative explanation that is entirely based on mass balance, and independent of force generation, posits that the cellular components and their rate of assembly become smaller in smaller cells, leading to smaller spindles [48,49].

An alternative model was recently proposed where the spindle length in *C. elegans* early divisions was proportional to the polymerization velocity of microtubules and their average length within the spindle; the stable length of spindles was mechanically established by the opposing polymerization forces acting on chromosomes from the poles [44]. In this model all KMTs directly connect the centromeres to chromosomes, which disagrees with our electron tomography observation that only a small fraction of KMTs are directly connected to chromosomes [50]. Moreover, based on recent findings that meiotic spindles in *Xenopus* and also *C. elegans* embryos are in fact composed of overlapping arrays of short microtubules [51,52] it does not seem obvious that short MTs necessarily have to result in the formation of shorter spindles.

Another important mechanical process involving forces and motion is the movements of chromosomes during mitosis; see [53,54] for reviews on the topic. Chromosomes movements can be divided into congression in prometaphase, maintenance in metaphase and

segregation in anaphase.

During prometaphase, MTs on each pole capture sister kinetochores. Forces applied to chromosomes include the pulling forces from MT depolymerization and cytoplasmic dyneins, and pushing, or polar ejection, forces generated by chromokinesins (i.e. CENP-E) that transport Chromosomes along MTs [36,37]. These forces along with forces from attachments of MTs from the opposing pole to the sister kinetochore ultimately control the chromosome congression and establishment of bioriented chromosomes in metaphase.

Chromosome motion in anaphase results from a combination of 1) microtubule depolymerization at kinetochores moving chromosomes towards spindle poles (Anaphase A), and 2) the separation of spindle poles (i.e. spindle elongation) dragging chromosomes along with them, with pole separation driven by motor proteins either generating external pulling forces or internally generated pushing forces (Anaphase B) (recently reviewed in [31])

In the past two sections we reviewed several force generation mechanisms involved in spindle assembly and movements of chromosomes during mitosis, which were a combination of forces from MT (de) polymerization and motor-proteins. These forces are balanced against passive forces, such the elastic deformation of MTs, their viscous drag. In the next two sections we will discuss the general aspects of these forces and their relationship to spindle mechanics. We will begin in the next section by discussing these forces in the scale of individual or pairs of MTs and motors.

#### 1.2.1. Microscopic mechanics

Because of the highly viscous cytoplasm and the microscopic size of the cellular structures, inertial forces become negligible on the cellular scale, which means the sum of forces at any point in time and space is zero. In the case of MTs this means that the active forces acting on them are balanced against two passive or reactionary forces: (i) MT elastic forces that arise from deformation of MTs, tensile forces along their length and stretching of their crosslinkers, and (ii) viscous (hydrodynamic) forces that arise because of the relative motion of MTs with respect to their fluid medium. Similarly, for chromosomes their motion is determined by the balance between forces from MTs and motors, their elastic deformation and the drag force acting on them, and their rearrangements and deformations.

We will in the following sections address the mechanisms of force generation by motor proteins and microtubules, as well as the contribution of so-called passive forces by elasticity, viscosity and subcellular barriers.

1.2.1.1. Motor proteins. MTs are considered as polar filaments, since they mainly polymerize from their plus-end, and the polarity, q, is typically defined by the normal vector connecting their minus-end to plus-end of a MT. Two major classes of MT associated motor-proteins recognize this polarity and, depending on their type, walk along plus- or minus-end directions. Thus, mechanical interactions of MTs and motorproteins can lead to MT polarity sorting. The idea of "motors" as agents for polarity sorting was first proposed before many of the MT associated motor-proteins were identified [55]. MT associated motor-proteins can bind to two structures simultaneously, one structure is a MT, while the other structure can be another MT, and other structures and organelles including the cortex and kinetochores. See Fig. 2 for a schematic. When a minus-end (plus-end) directed motor connects a pair of parallel MTs  $(q1 \ q2 > 0)$  it would bring together the minus-ends (plus-ends) of the paired MTs by walking towards the minus-end (plus-end) of each MT. Alternatively, if the motor connects a pair of antiparallel MTs, it would slide them apart; see Fig. 2A, B.

Within mitotic spindles we can find two major classes of motor proteins: plus-end directed kinesins and minus-end directed dyneins. The interplay of kinesins and dynein and thus the forces they generate by crosslinking and sliding MTs is important for the formation of meiotic and mitotic spindles [56–58]. The MT mins-ends are mostly

localized in MT organizing centers (poles). Minus-end directed cytoplasmic dynein has been associated with focusing the minus-ends, as well as sliding antiparallel MTs [59,60]. The plus-end directed kinesins have been reported to sort and arrange MTs during the formation of the meiotic spindle and are also involved in actively maintaining the spindle structure, in particular the spindle midzone in mitosis [33,56,61,62].

In many cells, i.e. *C. elegans* embryos, *Drosophila* neuroblasts and cell culture cells, dynein also localizes to the cell cortex, where it is part of a membrane anchored force-generating complex [63,64]. The cortical dynein captures MTs and generates a pulling force, which is essential for the positioning of the spindle within the cell (see Fig. 2C). In vitro experiments have shown that dynein can maintain the connection to a shrinking MT for several seconds against pulling forces up to 5 pN [65].

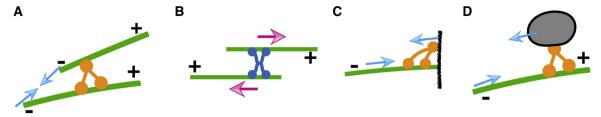
In addition to cortical dynein and dynein within the spindle, there is also a large cytoplasmic dynein population moving organelles along MTs (Fig. 2D). This transport of organelles is also capable of generating forces and could thus contribute to centrosome and spindle positioning [58,66,67].

1.2.1.2. MT polymerization/depolymerization forces. Polymerization and depolymerization of cytoskeletal filaments, like microtubules and actin, can generate forces in the absence of molecular motors (Fig. 3A). The presence of microtubule generated forces was first demonstrated by the landmark study of Bruce Nicklas where he measured the force required for stalling the motion of segregating chromosomes [68]. One of the earliest force generation hypotheses was motivated by the seminal study of Shinya Inoue, where he used polarized microscopy to show that the spindle is composed of filaments (later identified as microtubules) organized along the spindle axis [69]. It was hypothesized that chromosomes movements are driven by polymerization and depolymerization of these fibers [42].

MT polymerization forces were first measured by [70]. The first models for force generation through the polymerization of a single filament against boundaries, however, predates these measurements [71,72]. See [73] and [74] for reviews on the topic. The main predictions of these models that have been experimentally corroborated are the relationship between the pushing force applied to the plus-end and the polymerization velocity, in particular the force that stalls the polymerization reaction. Experimental studies estimate the stall force force to be in the order of ~ 5 pN [70,75]. In a recent study Garzon-Coral et al. measured the forces involved in maintaining the centered position of the mitotic spindle during metaphase, which they attribute to cortical pushing forces from MT polymerization [76]. Assembly and positioning of MT asters in microfabricated chambers also suggest that polymerization forces can properly position asters [77,78]. The forces generated by MT growth are also able to deform structures and vesicles [79]. As an example, MTs can deform the nucleus in mammalian cells [80,81].

In vitro studies show that MT depolymerization can also produce force [65,82]. Laan et al. [65] studied the positioning of MT asters in microfabricated chambers with their surface decorated with dyneins. They showed that the surface bound dyneins can capture MTs, inhibit their growth and trigger MT plus-end depolymerization. The interactions of depolymerizing MTs with dynein led to generation of pulling forces of several pN.

Depolymerization forces are key to chromosome segregation in anaphase [83]. In particular the shortening of the pole to chromosome distance during Anaphase A was suggested to be mainly driven by MT plus-end depolymerization [84–87]. In addition, MT pole-ward flux caused by minus-end disassembly contributes to pole-to-chromosome shortening in mammalian cells [88,89]. The stall force of chromosome motion was measured to be about 700 pN per chromosome [68,90]. Based on the observation that a mammalian kinetochore binds about 20–50 KMTs [91], this suggests that each KMT would have to generate



**Fig. 2.** Force generation by motor proteins requires cargo. **A**, A motor protein, i.e. dynein can generate the force to lead to a focusing of MT minus-ends. **B**, Motor proteins, such as kinesin, can generate forces resulting in an antiparallel sliding of MTs. **C**, Anchoring one end of motor-proteins to the immobile cell cortex can result in pulling forces on the MT. **D**, The transport of cargo along MTs can generate forces supporting the motion of MTs.

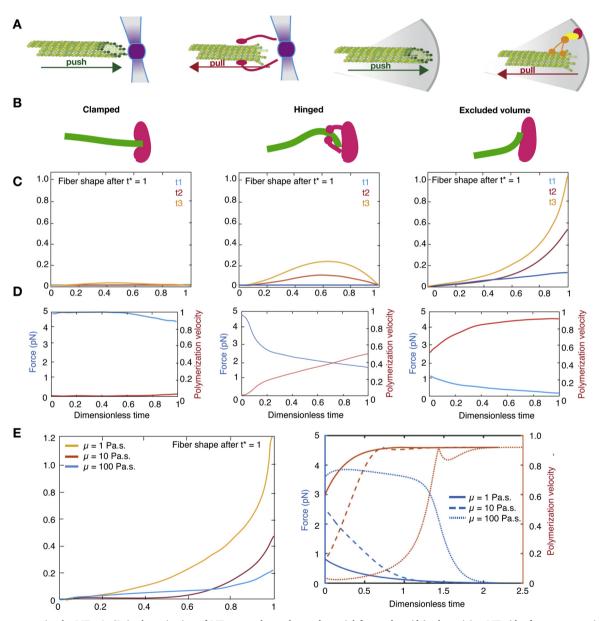


Fig. 3. Force generation by MTs. A, (De)polymerization of MTs can only produce substantial force when (de)polymerizing MTs (the force generator) polymerize against or interact with objects with comparable or larger drag coefficients of MTs, such as chromosomes and the cell boundaries. B, Boundary conditions of MTs from left to right (1) clamped: where the end of MT and its tangent angle remain fixed over time; (2) hinged, where the position remains fixed while the angle is free to change and moment is zero;(3) excluded volume (free sliding), where MTs cannot penetrate the object but they can bend and slide tangential to it. C, Dynamic simulation results of MT shape vs dimensionless time at t=t/=0, 0.5, 1 for the different conditions. Here t=L/Vp=17.5 sis the polymerization time-scale, L=7  $\mu$ m is the length of the MT and Vp=0.4  $\mu$ m/s is polymerization velocity. D, The end-force and polymerization velocity of MTs vs time under these conditions. E, left figure: Variations of MTs' shape after t=1 for viscosities viscosities: =1, 10, 100 Pa.s. Right figure: end-force and polymerization velocities in free sliding boundary condition at viscosities: =1, 10, 100 Pa.s.

a force of 14-35 pN during chromosome movement. Since the measured MT polymerization stall force is  $\sim 5$  pN, it is unlikely that the chromosome movements and stability are entirely controlled by polymerization forces. On the other hand, the theoretical maximum force a single MT can produce by depolymerization has been estimated to be about 75 pN per layer of dimer subunits removed [82]. Structurally, to generate a pulling force through depolymerization, the lattice of a depolymerizing MT has to be connected to the "Cargo", i.e. the kinetochore/ chromosome by an adaptor protein. Prominent examples for this are the Dam1 complex in yeast [92–94] or NDC-80 in mammals and nematodes [95–97].

1.2.1.3. Passive forces. In sections 2.1.1 and 2.1.2 we introduced two active force generation mechanisms inside the spindle: forces by motor-proteins and MT (de)polymerization. The dynamics of structural elements within the spindle (MTs and chromosomes) are determined by balancing these active forces against passive forces such as viscous friction due to motion through the cytoplasm and elastic forces that arise by resistance to deformation. Both of these passive forces will be discussed below. We will also discuss the role of cellular boundaries in this context.

1.2.1.3.1. Elastic MTs. MTs are very stiff polymers, with a flexural rigidity of  $\sim\!22$  pN  $\mu m2$ , a Young's modulus on the order of 2 GPa [98] and a persistence length in the mm range. Studies on single MT dynamics demonstrate that its mechanics can be described using the Euler-Bernouli beam theory for rods, where elastic forces are divided into bending, tensile and compressive forces [99]. Bending forces primarily act orthogonal to the MT orientation, while compressive and tensile forces are tangent to MTs. Large enough compressive forces can lead to the buckling of MTs, while they remain straight and can maintain their structural integrity under significantly larger tensile forces [99]. The shapes of MTs can be used to determine their mechanical properties and the force they are capable of generating [70,100].

A key factor in determining the time-dependent shape of polymerizing MTs and the force acting on them is the detailed mechanics by which MT ends interact with other objects i.e. their boundary conditions. Some common boundary conditions are (1) free end, where the end of the filament is not in contact with any other objects and no force or moment is applied to it; (2) clamped: where the end of a MT and its tangent angle remain fixed over time; (3) hinged, where the position remains fixed while the angle is free to change and the force moment is zero; (4) free sliding, where MTs cannot penetrate the object but they can bend and slide tangential to it. Fig. 3B gives a schematic presentation of the boundary conditions.

The panel in Fig. 3C shows simulation results of MT shape vs dimensionless time at  $t^* = t/\tau = 0$ , 0.5, 1, where  $\tau = L/Vp$  is the polymerization time-scale, L is the length of the MT and Vp is polymerization velocity. In these simulations the MT minus-end is assumed to be clamped, while the plus-end boundary conditions are clamped, hinged and free sliding. We have used  $L = 7 \ \mu m$ ,  $Vp = 0.4 \ \mu m/s$ , so  $t^* = 1$  correspond to  $t = 17.5 \ s$  in real time and cytoplasmic viscosity  $\mu = 1 \ Pa.s$  and polymerization stall force of  $F = 5 \ pN$ . The MTs take very different shapes in these three conditions. As Fig. 3D shows, the end-force and polymerization velocity of MTs also varies significantly between these conditions.

In addition to the shapes of individual MTs, the shape of the spindle itself can also inform about forces. Recent studies have shown that the human mitotic spindle is chiral due to twisting moments within MT bundles [100]. This twisting moment results in the rotation of the bundle cross-section along its length, suggesting that individual MTs within the bundle twist around each other like metal wires in a steel wire rope. This torque can be induced by mitotic motor proteins, such as kinesin-5, kinesin-8, kinesin-14 or dynein. In fact, recent in vitro experiment showed that kinesins -14 motors can drive a right-handed helical motion of antiparallel microtubules around each other [101].

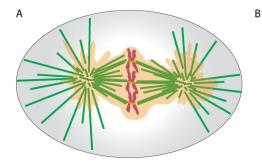
1.2.1.3.2. Viscous forces. Viscous forces arise from the relative motion of immersed bodies with respect to the bulk velocity of the cytoplasm. Due to small microscopic dimensions of MTs and other cellular structures, and the velocities being of order  $\sim$  m/s inertial forces can largely be ignored in cellular environments, and the fluid forces are described by Stokes equation [102,103]. In this limit the viscous force applied to the moving body is linearly proportional to its velocity difference with the fluid:  $F = R (U - u_f)$ , where U and U are the velocity vectors of the body and the fluid, and U is the so-called resistance (3  $\times$  3) tensor that is only a function of the geometry of the body. For the isotropic spherical particle U reduces to a scalar drag coefficient given by U0, where U1 is the sphere radius and is the viscosity of the fluid.

For slender MTs, which are anisotropic, the resistance to motion is different in directions parallel and perpendicular to MT axis and is given by:  $R = (8\pi\mu L) / \ln{(\epsilon^{-2}e^{-1})} [qq + 2(I-qq)]$  [SlenderBody]. Here  $\epsilon < 1$  is the aspect ratio of the MT, q is MT orientation and I is  $3 \times 3$  identity matrix. This formulation shows that the resistance is twice in the direction perpendicular to the filament than the parallel direction. More importantly, the viscous force is linearly proportional to the length of MT L and the aspect ratio of MT only affects the force through the weak logarithmic term  $\ln{(\epsilon^{-2}e^{-1})}$ . The consequence of this relationship is an interesting and perhaps counterintuitive fact: the viscous force generated by a MT of length L moving perpendicular to its axis is roughly  $\frac{1}{4}$  of a sphere of the same diameter. Any estimation of viscous forces must recognize this key feature.

The viscous force is proportional to the viscosity of the cytoplasm. In length-scales much larger than the size of their building blocks, the viscosity is determined by the ratio of the macroscopic shear stress to shear rate in simple and oscillatory shear flows. The macroscopic stress is the ensemble, or volume, average of the stress generated by the motion of the fluid as well as all the dispersed bodies [104]. Hence, it is fundamentally different from the viscosity of the fluid (cytoplasm) surrounding the bodies (MTs) i.e. the effective viscosity (or drag) on a body is very much dependent on the length- and time-scale of interest. For example, the average velocity of a MT under a given force before it collides with its neighbors (short time-and length-scales) can be significantly larger than its average velocity after a large number of collisions (large time- and length-scales). Understanding these physical aspects have been the subject of numerous studies and books in the fields of soft matter [104], and Suspension Mechanics [105]. We will discuss some of these aspects as they pertain to spindle mechanics in the Macroscopic Mechanics section.

Experimental studies show that even for structures as small as proteins, the crowdedness of the cytoplasm can reduce their diffusivity, which is inversely proportional to the viscosity, by three folds compared to water [106]. The effective viscosity corresponding to moment of needles inside the spindle estimate the spindle viscosity to be  $\mu \sim 1.9$  $\times$  10<sup>2</sup> Pa.s which is roughly 2  $\times$  10<sup>6</sup> times larger than water and more than 100 times greater than the viscosity of the cytoplasm outside the spindle, (1.2 Pa.s) [7]. Thus, to move a MT of length 2  $\mu m$  with velocities as low as 0.01  $\mu$ m/s within the spindle requires a force of ~ 8 pN, which is in the same order as the polymerization and motor-protein forces. As another example, in the original work of Nicklas [68] he argues that the force needed to stall chromosome movement is 2000-10,000 times smaller than the force needed for stalling chromosomes, and thus inconsequential for chromosome dynamics. These estimates were made taking  $\mu = 0.1$  Pa.s, which is 2000 times smaller than the measured values, in which case viscous forces may play an important role in determining chromosome segregation and spindle mechanics, as argued in [7]. This, once again, underscores the fact that the estimate for viscosity can change enormously across length-scales, which must be recognized when estimating the relative importance of different forces in the time- and length-scale of interest.

As another example, we explore the effect of change in viscosity in the problem of a polymerizing MT against obstacles (see subsection



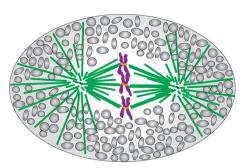


Fig. 4. Intracellular boundaries.
Cartoons based on observations from electron tomography showing the "crowdedness" surrounding the mitotic spindle. A, Extensive membrane compartments, such as the endoplasmic reticulum span the cytoplasm. B, Cells are filled with numerous vesicles and organelles that surround the spindle but are excluded from inside the spindle.

Elastic MTs), while keeping the rest of physical parameters the same.

If the viscosity is increased from 1 to  $\mu=10$  Pa.s, MTs remain almost straight in both clamped and hinged boundary conditions and forces remain close to stall force. The behavior is significantly changed for free sliding MTs. Fig. 3E shows the large variations of MT shape, end-force and polymerization velocities in free sliding boundary condition at viscosities:  $\mu=1,10,100$  Pa.s, underscoring the key role of viscosity on MT dynamics. In some scenarios the minus-end of KMTs may be free and not crosslinked to other MTs or the centrosome. Simulations of this scenario show that if the viscosity of the fluid is sufficiently large the MTs may undergo buckling due to viscous pushing forces that arise by polymerization against chromosomes [107].

1.2.1.3.3. Chromosomes as boundaries. Through the theoretical work of Mogilner & Oster [108] and experimental studies [79,109] we know that the flexibility of the obstacle against which the MTs (or actin filaments) are polymerizing can have a major effect on the generated forces and polymerization velocities. In that light it is reasonable to assume that the mechanical properties of chromosomes and their compactness as well as their kinetochore domains can alter the force generated by (de)polymerization. This may be one of the factors involved in the reported missegregation of chromosomes when their compaction is reduced [110-112]. Moreover, chromosomes display different structural appearances, i.e. some chromosomes are very small like chromosome 21 in humans, others are very big, such as the human chromosome 1. In addition, the kinetochores to which MTs attach can be localized in the center of the chromosome (metacentric), asymmetrically (acrocentric) or at the end of the chromosome arms (telocentric)). We do not know if and to which extend the structure and size of chromosomes impact the transmission of MT generated forces. Interestingly, recent research has reported a bias in the segregation fidelity of individual chromosomes [113,114] and it is tempting to speculate that this bias could be driven by changes induced by force generation structural differences of individual chromosomes.

1.2.1.3.4. Membranes as intracellular boundaries. Membranes, such as the cortex and intracellular membranes like the nuclear envelope, endoplasmic reticulum and organelles can act as a barrier. Depending on the membrane, these barriers are more or less elastic and deformable.

The cell cortex is the outer boundary of the cell and MTs from the spindle arrive at the boundary, where they can either experience a pulling force when interacting with cortical dynein or generate a pushing force through polymerization [63,64,76]). These forces are important for the positioning of the spindle within the cell, but they also might have implications for the forces generated within spindles [115].

A good example for this is the one-cell embryo of the nematode *C. elegans*. During the first cell division the mitotic spindle has to be positioned asymmetrically in order to give rise to a larger anterior and smaller posterior cell, thus laying the foundation for future cell fate. The positioning is coordinated by a trimeric cortical force-generating complex composed of  $G\alpha$ , GPR-1/2 and Lin-5 (or  $G\alpha/LGN/NuMA$  in other organisms) that recruits dynein and exerts a pulling force on astral MTs [116]. Studies using laser-ablation have shown that the spindle

is under tension in anaphase as the severing of the spindle midzone results in a fast separation of the spindle poles. This observation also suggested that cortical pulling forces could be the main drivers of chromosome segregation in *C. elegans* embryos [63,64,117]. However, chromosome segregation is not prevented upon depletion of the force generating complex, and the rate and extent of chromosome segregation remain very similar to wildtype embryos [115].

As mentioned above, MTs also generate pushing forces on the cell cortex and the force balance of those pushing and pulling forces is essential for spindle centering and positioning during mitosis. Experiments using magnetic beads to probe the centering stiffness and mechanisms have shown that the force-generating machinery that maintains the spindle at the cell center has spring-like properties [76]. The centering stiffness is high enough to ensure the precise maintenance of spindle position against thermal fluctuations while spindle assembly is completed and the cell segregates the chromosomes. The centering stiffness is however low enough to allow the force generators to fine-tune the position of the spindle to allow asymmetric cell division.

In summary, the cell cortex acts as a boundary against which MTs polymerize or depolymerize to position the spindle. The rigidity of the cortex is an essential feature during this process and it was shown that softening the cortex strongly affects the centering of the spindles [118]. In addition, the force generated on microtubules at the cortex also impacts the forces that are generated within spindles in order to segregate the chromosomes [115].

In addition to the cell cortex, there are large membrane compartments within the cell, as well as many vesicles and organelles (Fig. 4). Not much is known yet about their role in the process of spindle mechanics, it seems however plausible that these inter-cellular membrane compartments can act in a similar way as the cell cortex does. Those membranes could move MTs towards them, away or be moved along MTs. All these processes can generate forces.

There is still very limited research accessing the role of inter-cellular membranes for forces during mitosis. Maybe a good example of how inter-cellular membranes could influence the mechanics is the observation that the nuclear envelope and surrounding endoplasmic reticulum (ER) function as a boundary during spindle formation [119] (Fig. 4A). The classic search and capture model, in which MTs emanate from the centrosome, encounter a kinetochore by chance and then move the chromosome around, has been calculated to take several times longer than the typical duration of mitosis [40]. Introducing a spatial barrier into this model that restraints the directional growth of MTs and positioning of chromosomes, can significantly enhance the chance that stochastic encounters between MTs and kinetochores result in timely incorporation of all chromosomes into the mitotic apparatus.

### 1.2.2. Macroscopic mechanics

Thus far we discussed the active force generation mechanisms and passive forces that respond to these forces on a single MT scale. The key to understanding spindle mechanics is to understand how these forces are integrated on the single MT level and ultimately determine the mechanical properties of the spindle composed of hundreds to hundreds

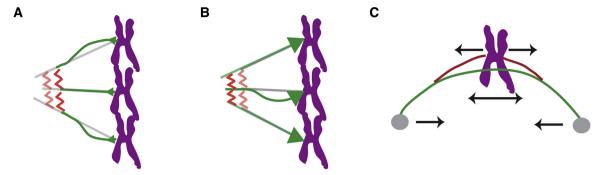


Fig. 5. Crosslinking between MTs causes depolymerizing MTs to buckle and polymerizing MTs to remain straight. A, Crosslinkers (red zig zag lines) that are strong enough to maintain the distance between the minus-ends of the MTs (green), will push MTs that have a larger angle, resulting in a buckling of those MTs. The grey line indicates the initial shape of the microtubule B, If a few polymerizing kinetochore MTs are strongly crosslinked, maintaining the distance between the minus-ends, the MT with the largest angle will be under tensile forces from crosslinkers causing it to remain straight. The crosslinkers will apply an equal and opposite pushing forces to the neighboring MTs causing them to bend/buckle. C, Adapted from Pavin & Tolic 2016 [3] This model includes bridging microtubules (green) as a link between sister k-fibers. K-fibers (red) are under tension near the kinetochores (arrows at the chromosomes) and under compression near the spindle poles (arrows at the gray spheres). These forces are balanced by the compression in the bridging fiber (arrows in the middle).

of thousands of MTs, motors and crosslinkers.

1.2.2.1. Mechanical coupling through active and crosslinking. Forces are integrated when multiple MTs are attached to a structure such as chromosomes or centrosomes, or when MTs are cross-linked throughout the spindle. This mechanical coupling can lead to a number of complex behaviors. Let us begin with a simple physical example. We know that the depolymerization of a single KMT against chromosomes generates pulling forces, causing the KMT to be straight. However, if several depolymerizing microtubules that attach with different angles with respect to the main axis of the spindle are crosslinked, the MTs with a larger angle will buckle outwards, assuming that the cross-linkers are strong enough to maintain the distance between the minus-ends of the MTs. This is shown schematically in Fig. 5A. Similarly, if a few polymerizing KMTs are strongly cross-linked (maintaining the distance between the minus-ends), the MT with the largest angle will be under tensile forces from cross-linkers causing it to remain straight. The cross-linkers will apply an equal and opposite pushing force to the neighboring MTs causing them to bend/buckle; see Fig. 5B. In other words, KMT polymerization and depolymerization do not necessarily lead to net pulling/contractile and pushing/extensile forces, respectively.

Now let us consider a related problem in spindle mechanics. We know that in HeLa cells both interpolar MTs and KMTs are curved near chromosomes [120,121], implying that these MTs are under compressive forces. On the other hand, tension between sister kinetochores is required for passing the spindle checkpoint, suggesting that the plusends of KMTs are under tension. The model proposed by Pavin and Tolic resolves this paradox of coexistence of tensile and compressive forces by *bridging* k-fibers of two poles by MTs in between [3]. In this model the crosslinking of k-fibers with bridging fibers allows k-fibers to bend in response to pushing forces from the poles, while their plus-ends remain under tension from kinetochores (Fig. 5C)

MTs within the spindle are frequently crosslinked by minus-end and plus-end directed motors. The collective behavior of large assemblies of MT and motor-proteins depends on several physical parameters of motor-proteins and MTs, including the number of minus-end and plusend motors, the number of MTs, the force applied by each class of motors, their walking speed, and rates of detachment and how they compare against MT rates of polymerization and catastrophe. A controlled perturbation of these parameters in live cells is very challenging. Studies on biosynthetic suspensions of MTs and a few purified proteins provide a roadmap for understanding the physics of MT assemblies in cells. These studies show that if motor-proteins are sufficiently fast to reach MT ends and gather other MT ends, suspensions of MTs organize into asters [122,123]. Other experiments show that adding depleting

agents to short stabilized MTs induce MT bundling and MT assemblies that exhibit active liquid crystal structure and large-scale surface flows [124]. Recently Roostaloo et al. [125] showed, through experiments and computer simulations that low ratios of motor/MTs and large ratios of MT polymerization speed/motor speed result in formation of active liquid crystals, while the opposite results in formation of MT asters for both minus-end and plus-end directed motors. The next step in these studies is to form a spindle-like assembly, which requires a coexistence of MT aster (poles) and nematic liquid crystal (near chromosomes).

1.2.2.2. Mechanical coupling through the generated fluid flows. Previous light microscopy experiments using photoactivation of fluorescent tubulin show a poleward flux of MTs, which are believed to be produced by polymerization of kinetochore MTs against chromosomes [126,127], or sliding motions generated by motor-proteins [128]. These MT movements are expected to generate flows that scale with the velocity of MTs. These flows can change the MT transport, including the distribution of MT density, minus-ends and lengths within the spindle [52]. These long-range interactions of MTs through their cytoplasmic flows are referred to as Hydrodynamic Interactions (HIs) [129]. To put it in the context of viscous forces discussed earlier, HIs cause the drag coefficient of a MT to be dependent on the position of the neighboring MTs, since movement of one MT induced cytoplasmic flows that affect the movements of other MTs. Hence, HIs is expected to play an important role in determining several mechanical aspects of the spindle, including its viscosity, the rate deformation of MTs, and transport of chromosomes.

Advancements in high speed imaging, and image processing in the past decade has led to several studies involving accurate measurements of cytoplasmic flows [130]. Since the cytoplasmic flows are determined by distribution of forces and stresses in cellular structures and their ensued motions, they can be used as noninvasive diagnostic tools for studying active force generation mechanisms in cellular processes [131,132].

On the computational front, tools for simulating HIs between cytoskeletal flexible filaments have only recently been developed [133]. This platform has been used to study the effect of HIs and cytoplasmic flows on the positioning of the mitotic spindle in cell division [131] and the effect of HIs on forces generated by polymerization of cytoskeletal filaments against boundaries [131]. These studies highlight several consequences of HIs that were previously ignored; some of these predictions were corroborated in a recent experimental study on pronuclear migration [107].

The role HIs in self-organization of MT assemblies has been studied to a much greater extent in biosynthetic systems. The interested reader is referred to [134] and [135] for reviews on the recent experimental

and theoretical developments.

1.2.2.3. Surface forces. Surface tension between two fluids is a direct consequence of sharp gradients of intermolecular forces across the interface. Because of the presence of the nuclear envelope prior to spindle formation, the concentration of spindle constituents, including MTs and motor-proteins, can be very different within the former nucleoplasm and in the cytoplasm. For example, since a large fraction of organelles and vesicles are too large to be transported inside the nucleus, their density is significantly less within the spindle than the outer domain (See Fig. 4B). This gradient in density may lead to a pressure/stress gradients and interfacial forces across the spindle that works to minimize spindle's surface area. The balance of these stresses against the spindle's bulk active and passive stresses determines its shape and dimensions. An effective interfacial stress can also be produced by the gradient in concentration of crosslinkers and motors across spindle; see Brugues 2014 [136] for a detailed theoretical and experimental implementation of these forces in Xenopus egg extracts.

## 1.3. Spindles as materials

In the past few sections we explored different ways that the forces generated in the scale of a single MT or a pair can be integrated to act on the spindle scale. This integration ultimately determines how the forces are transported within the spindle and how the spindle responds to external forces. Determining the ensemble average mechanical behavior of an assembly based on its microstructure and microscopic interactions of its building blocks i.e. MTs is the central objective of statistical mechanics. The past several decades have brought about statistical mechanical theories for a wide range of complex materials, including polymers, colloids, and liquid crystals. In tandem with these theories many experimental techniques have also been developed to test and classify the mechanics of these materials. Studying the spindle as a complex active material allows us to utilize this repository of knowledge and techniques to develop theories for macroscopic behavior of spindle matter based on simple microscopic rules of interactions and test those theories using the experimental methods.

The spindle building blocks are micron-sized MTs, and the thermal forces are insufficient to transport them within the required time to form a functional spindle. Hence, the cell uses active force generators such as motor-proteins and polymerization forces for directed motion of MTs and overall formation and maintenance of the cytoskeleton. These forces are significantly larger than thermal forces, which render these assemblies far from equilibrium. Statistical mechanics of these active materials are far less understood than their passive material counterpart, and have been the subject of many studies over the past two decades [103,136–139]. Below we review some of the experimental studies on characterizing and measuring the material properties and forces within the spindle.

#### 1.3.1. Measuring spindle mechanics

Early studies addressing the forces acting on chromosomes were using glass microneedles to displace individual chromosomes in grass-hopper spermatocytes [90]. These experiments showed that kine-tochore fibers were stiff under tension but elastic under compression and that chromosomes in metaphase could be detached from kine-tochore fibers by pulling forces. Detached metaphase chromosomes could also be reintegrated into the spindle. Interestingly, chromosomes in anaphase could not be detached [5,90]. In comparison to this, the tensile forces acting on a prometaphase chromosome were measured to be around 50 pN, suggesting that the spindle generates much larger forces than the force required to move a chromosome.

Experimental evidence that entire mitotic spindles are under tension was provided by microneedles. Kronebusch and Borisy [139] used microneedles to sever the central spindles in PTK1 cells at early anaphase. Spindle poles of severed spindles continued to separate with higher

velocities than intact spindles, suggesting that AMTs might pull on the spindle poles.

The first insight into the spindle as a material arose from centrifugation experiments that suggested the spindle had gel-like properties [140]. This was further investigated by applying hydrostatic pressure on spindles [141]. High pressure stopped chromosome motion, suggesting that forces might be transmitted to the chromosomes by two phase transitions: a sol to gel transition, which added spindle fiber material and a solation of chromosomal fibers, occurring at the spindle poles. Observations of spindle fusion in Xenopus egg extract also provide information about the material properties of spindles. If two spindles are brought close together (10  $\mu m$  or less), they begin to interact and over time the two spindles merge and form a single bipolar spindle of normal shape and size. The fusion of spindles has been shown to be dependent on cytoplasmic dynein [9,60].

Further studies on spindles in Xenopus extract showed that the spindle's response to small deformations is viscoelastic, while larger deformations result in more plastic responses [142]. In addition, the forces required to compress the spindle along the pole-to-pole axis were found to be higher than those required to compress the spindle along its width [6,120,142]. This difference in forces is possibly due to the orientation of MTs, which are aligned along the pole-to-pole axis. This agrees with the observation that the spindle's response to deformations along its long axis is viscous [7], while the response to forces along the short axis was found to be viscous on timescales of up to tens of seconds but more elastic at slower or faster timescales [143].

Recently, microneedles were used to test the mechanical coupling of MTs across different positions along the spindle in Xenopus extract [8]. The results show that SMTs mechanics change with respect to their distance from the poles and the equator. MT-arrays near the poles and the spindle equator were found to be robust against forces, consistent with earlier observation by [144] using static microneedles, while MT arrays in the middle between poles and chromosomes were compliant and fluid like. The mechanical heterogeneity of the MT network was linked to motor proteins kinesin-5 and dynein [8].

In addition to microneedles also magnetic beads have been used to mechanically perturb and apply forces to spindle structures. Garzon-Coral et al. [76] have used this strategy to determine the forces acting on centrosomes during spindle positioning by measuring the displacement in response to applied force as well as the repositioning after cessation of force application.

Apart from mechanical perturbations using physical devices, ablation of spindle structures by laser has significantly contributed to our understanding of forces within spindles. Further evidence that the spindle is under tension, generated by polar pulling forces, was provided by severing of spindle midzones using laser microbeams [64,117]. These pulling forces were later, also by laser ablation, shown to be generated by cortical force generators, localized by polarity markers that generate a dynein mediated pulling force on AMTs and play an important role for asymmetric cell division [63,64].

Recent advances in laser ablation technology using femto-lasers have resulted in minimized damage induced by irradiation and allow for better spatially resolved severing of structures. This has enabled the ablation of individual k-fibers at distinct positions within the spindle [12,14]. Systematic ablation of k-fibers along the spindle length in mammalian cell culture cells suggested that a constant number of molecular-scale force generators per unit length are located along k-fibers. Furthermore, these experiments suggested that the protein NuMA distributes the load of chromosome movement from k-fibers to nearby spindle MTs through NuMA based MT-MT crosslinking.

The ablation of individual k-fibers also led to the discovery of pushing forces that are exerted by bridging fibers that are able to segregate chromosomes independently [87]. This agrees with recent observations in U2OS cells and *C. elegans* embryos suggesting that the spindle midzone is able to generate pushing forces that contribute to the segregation of chromosomes [115].

In addition to mechanical perturbations to study spindle forces, approaches for direct force measurements have also been developed. Fluorescence based tension-sensors have provided important insight into local forces acting on kinetochores. There are two main classes of current tension sensors, which are FRET based sensors or Talin/Vinculin based sensors. The first provides information of forces based on FRET efficiency, the second by the amount of vinculin heads binding to a talin rod. In both cases the sensor has to be integrated into a protein that is involved in force generation, i.e. NDC-80, a protein establishing kinetochore to MT attachment, or CENP-C, a protein that links the inner and outer kinetochore. In addition, the tension sensors have to be calibrated for quantification of forces.

Integration of a FRET sensor into CENP-C in a Drosophila S2 cell line by Ye et al. [10] suggested an average force of about 12–62 pN per kMT and 144–764 pN/kinetochore.

These numbers are of the same order of magnitude as measured by Nicklas for kinetochores in meiotic spermatocytes.

Integration of a FRET biosensor into the NDC-80 of budding yeast by Suzuki et al. [11] suggested forces in the range of 6 pN/ KMT at metaphase, 2.5 pN in late anaphase and 4 pN in interphase. These measurements are comparable to in vitro measurements of 7–9 pN for the detachment force of isolated yeast kinetochores from MT ends [145] and are similar to the 7.5 pN obtained from the stiffness measurements of stretched metaphase centromeres in budding yeast.

# 1.4. Integrating electron tomography with light microscopy and modeling to study spindle mechanics

While many years of mitosis research have brought essential and important insight into the mechanics of spindles, we still have a limited understanding of how molecular forces are integrated in vivo to serve complex cellular processes such as spindle assembly and chromosome positioning and segregation during mitosis. One reason for this is the lack of structural information, such as the number and length of MTs composing the spindles or the geometry of MT and chromosome interaction.

Such data can be extremely helpful to establish and test models of how spindles generate and respond to forces. Large-scale electron tomography has the ability to deliver such high-resolution data about MTs and chromosomes [20–22,51,115,146,147] (Fig. 6A–C). It allows us to measure the number of MTs, the location of their ends, and to a lesser extent if these ends are plus- or minus-ends based on their endmorphology (open vs closed). We can also determine the length, orientation and density distribution of MTs within the spindle and distinguish between different subclasses of MTs, such as KMTs, SMTs and AMTs.

Although data obtained by electron microscopy provides high resolution information, it remains static. Information about spindle dynamics needs to be obtained by light microscopy. In particular, polymerization velocities of MTs within and outside of the spindle are determined by MT plus-end tracking and measurements of time-dependent shape changes (length and width) of the spindle and spatial-temporal analysis of chromosome positions.

The high-resolution data obtained by tomography can be integrated with dynamic information gathered by light microscopy to refine current models of spindle assembly or to develop new hypotheses.

Two fundamental equations that ultimately determine the physical properties of the spindle (and any other material) are the *conservation of mass and momentum* within the spindle. Conservation of mass is determined by parameters such as rates of nucleation, (de)polymerization, dynamic instability and MT transport for SMTs and KMTs. Assuming that the metaphase spindle is in a dynamic steady-state, the balance between these rates ultimately determines the spatial distribution of KMT and SMT density, and their minus- and plus-ends, as well as their length distribution within the spindle. Comparing the predictions of different models of nucleation, growth and transport with the direct

measurements from electron tomography provides a powerful mathematical framework for choosing between these models and highly constraining their parameters.

Our earlier work took the initial steps in this direction. Tomographic reconstructions revealed that KMTs do not reach the spindle poles. Based on this observation we proposed three models for SMT and KMT nucleation and (de)polymerization dynamics. We then used the nucleation and length distributions of SMTs and KMTs from electron tomography, and polymerization velocity measurements from plus-end tracking of MTs from light microscopy to identify the only model that gave consistent predictions of the observations [20].

The extension of such an analysis to studying spindles under biochemical perturbations that affect MT nucleation and polymerization dynamics as well as studying spindles of different sizes, would allow us to quantify the correlation between spindle dimensions and MT polymerization dynamics. However, identifying these correlations is not sufficient for developing a mechanistic understanding of spindle structure. Doing so requires understanding force generation and transport within the spindle i.e. conservation of momentum. Also, here electron tomography can provide valuable structural information that is not accessible by light microscopy. Most importantly, we can visualize the shape of each individual MT, and use this shape to compute the bending forces along each MT. In our earlier discussion of elastic forces in section 2.1.3.1 we observed that different mechanical interactions of the polymerizing ends of MTs with boundaries can lead to clear differences in their shapes (Figs. 4 and 5). Within these lines, studying the shape of KMTs may help us determine if KMTs are pushing against chromosomes, and even go so far as to identify the nature of mechanical interactions of the ends of MTs with chromosomes.

However, structural information from tomography is not sufficient for determining the force distribution within the spindle. Determining the contribution of viscous forces requires measuring the velocity of MTs, which cannot be done by static data from tomography. Here the use of different methods such as photoactivation of fluorescent tubulin for measuring MT flux inside the spindle can provide at least an estimate of the relative importance of viscous and elastic forces. A key difficulty in measuring elastic forces from the shape of MTs is that they remain straight under pulling forces, irrespective of the magnitude of those forces. Thus, we cannot determine the tensile forces.

Finally, to fully utilize the structural information about the shape of individual MTs, their spatial orientation and organization, we need to use this data in tandem with simulation platforms that account for flexibility of MTs, their (de)polymerization dynamics and interactions with other objects such as chromosomes and motor-proteins, as well the ensued fluid-structure interactions. Cytosim is a popular software that accounts for several of these features and has been used to study spindle formation [15] [15 which -as we argued- can qualitatively change the organization and mechanics of spindles. We have recently developed a highly efficient large-scale computational platform that accounts for these fluid-structure interactions, while also accounting for other factors [Kruse, 2005, Generic theory of active polar gels: a paradigm for cytoskeletal dynamics] and have deployed this platform to study spindle positioning [145] (Fig. 6D). Combining these computational tools with tomography, and light microscopy provides the ground for developing a mathematical framework that can recapitulate different force generation mechanisms and choose between these models and highly constrain their physical parameters through comparison with experimental observation. The ultimate result is a microscopic model of interactions of spindle and kinetochore MTs, chromosomes and motors that can accurately predict the spindle microstructure from the shape of single MTs to the emergent shape of the spindle in large-scales.

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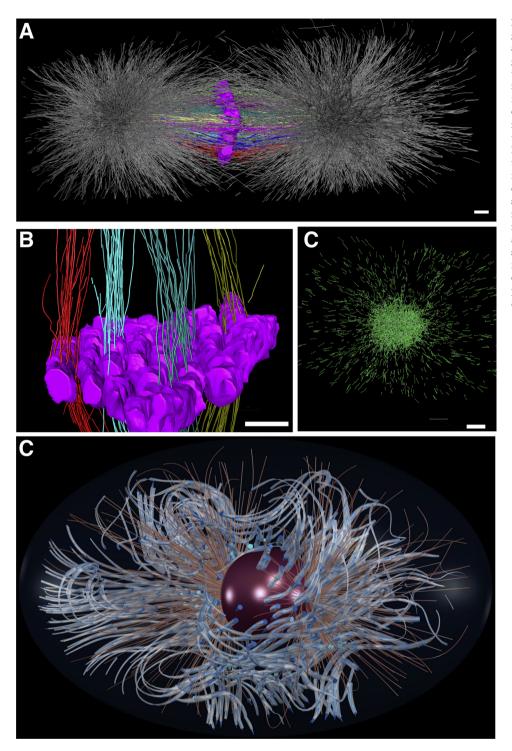


Fig. 6. Studying spindle mechanics by tomography and modeling. A, 3D reconstruction of a mitotic spindle in metaphase of the C. elegans one-cell embryo. Kinetochore microtubules that are attaching to individual chromosomes are differently colored B, A zoomed in view of the metaphase plate of the reconstruction shown in A. Selected groups of microtubules (shown in different colors) attaching to individual chromosomes are shown. For a better view of microtubules connecting to the other chromosomes are not shown. C, A filtered tomographic reconstruction focusing on the left centrosome of the reconstruction shown in A. Only microtubules that are < 500 nm are shown. D, A snapshot of dynamic simulation of 600 MTs (light red filaments) that are anchored to pronuclear complex (red sphere) that is centered within the cell, and the flows (velocity vectors and streamlines) generated by the motion of this structure and the polymerization of MTs against cellular bound-

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