A comprehensive model to predict mitotic division in budding yeasts

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Abbreviations: MTOC, microtubule organizing center; KT, kinetochore; MT, microtubule; SPB, spindle pole body; cMT, cytoplasmic microtubule; ipMT, inter-polar microtubule; kMT, kinetochore microtubule.

Abstract
High fidelity chromosome segregation during cell division depends on a series of concerted interdependent interactions. Using a systems biology approach, we built a robust minimal computational model to comprehend mitotic events in dividing budding yeasts of two major phyla: Ascomycetes and Basidiomycetes. This model accurately reproduces experimental observations related to spindle alignment, nuclear migration, and microtubule dynamics during cell division in these yeasts. The model converges to the conclusion that biased nucleation of cytoplasmic microtubules is essential for directional nuclear migration. Two distinct pathways, based on the population of cytoplasmic microtubules and cortical dyneins, differentiate nuclear migration and spindle orientation in these two phyla. In addition, the model accurately predicts the contribution of specific classes of microtubules in chromosome segregation. Thus we present a model that offers a wider applicability to simulate the effects of perturbation of an event on the concerted process of the mitotic cell division.

**Introduction**

Mitosis is a fundamental cellular process that enables faithful transmission of genetic material to the subsequent generation in eukaryotes. This process is well coordinated and requires the cumulative effort of several macro-molecular machineries including the centromere-kinetochore complex, the mitotic spindle, microtubule organizing centers (MTOCs), molecular motors and microtubule associated proteins (MAPs). The foundation for this process of chromosome segregation is provided by a specialized chromatin structure, the centromere, upon which 60 - 80 proteins assemble to form the kinetochore (KT). The KT connects centromeric chromatin to the mitotic spindle. The mitotic spindle, nucleated by MTOCs, is a bipolar array of microtubules
(MTs) that provides the force required to segregate chromosomes. This mitotic spindle is synergistically modulated by motor proteins (Mallik and Gross, 2004), the plus end directed kinesins and the minus end directed dyneins, and MAPs which dynamically alter the rate of microtubule stability. The unequal rate of MT polymerization and depolymerization provides the push-pull forces that mediate pole-ward movement of segregated chromosomes into two daughter cells. Apart from requiring of the assembly of the segregation machinery on the centromere and push-pull forces enable to chromosomes to segregate, proper spindle positioning and orientation is crucial to carry out faithful segregation of chromosomes (Segal and Bloom, 2001; Kusch et al., 2002).

MTs are largely localized to the cytoplasm, until the spindle formation begins during mitosis in most organisms. These cytoplasmic MTs (cMTs) emanate from either multiple cytoplasmic MTOCs like in metazoans or from a single nuclear envelope (NE)-embedded MTOC as in the budding yeast Saccharomyces cerevisiae. The cMTs along with motor proteins influence nuclear positioning and movement (Lee et al., 2000; Fink et al., 2006; Ten Hoopen et al., 2012). Upon the onset of mitosis, cMTs reorganize themselves to form the mitotic spindle between the two poles (spindle pole bodies (SPBs) in yeast or centrosomes in metazoans). The less dynamic minus ends of MTs are anchored to the SPBs, while the more dynamic plus ends radiate outward to facilitate interactions with other cellular components. Some of these MTs interact with KTs to become kinetochore microtubules (kMTs) and provide the pulling force on chromosomes during anaphase. Cytoplasmic (astral) MTs make contact with the cell cortex aiding in spindle positioning, while inter-polar microtubules (ipMTs) are formed when the plus ends of MTs originating from opposite poles interact via sliding, resulting in an anti-parallel array at the mid-
zone. The combination of pushing force provided by ipMTs on SPBs along with the pulling force from kMTs and cMTs aids in segregation during anaphase.

The spindle positioning is not only crucial for proper chromosome segregation but also defines the site of division. In general, the spindle is positioned centrally in the dividing cell and thus a mother cell gives rise to equal sized daughter cells by the fission mode of division. While most organisms undergo this type of division, few others show variations in spindle positioning and hence give rise to cell polarity (Horvitz and Herskowitz, 1992; Neumuller and Knoblich, 2009). This type of division is mostly observed during developmental stages of multicellular organisms and in stem cells (Knoblich, 2008; Neumuller and Knoblich, 2009). Budding yeasts also undergo a similar unequal cell division where the site of division is defined prior to spindle positioning (Fraschini et al., 2008). A number of studies have been carried out to identify the factors to understand the dynamics of spindle positioning. Some of these regulatory factors are shown to be different between budding yeasts and multicellular organisms (Fraschini et al., 2008; Neumuller and Knoblich, 2009).

The process of chromosome segregation during budding has been well studied in S. cerevisiae and Candida albicans, both belonging to the fungal phylum Ascomycota. Recently these processes were studied in another phylum of fungi Basidiomycota, represented by the yeasts Cryptococcus neoformans and Ustilago maydis. Although organisms mentioned above, belonging to two major fungal phyla, divide by budding, a striking variation is observed regarding the site of nuclear division which takes place in the mother cell in Ascomycetes but in the newly budded daughter cell in Basidiomycetes (Heath, 1980; Straube et al., 2005; Gladfelter and Berman, 2009; Kozubowski et al., 2013). In Ascomycetes, the nucleus moves close to mother-daughter cell junction (neck) and divides into two equal halves. One half then moves to
the daughter cell and other half is retained in the mother cell. In contrast, the nucleus moves completely to the daughter cell before division in Basidiomycetes. The nuclear division takes place in the daughter cell after which a divided nuclear mass moves back to the mother cell while the other half is retained in the daughter cell. To address the molecular basis for this observed variability in mitosis between these yeast species, first, we developed a common computational model that was subsequently modified to simulate the fungal phylum-specific nuclear dynamics during mitosis in Ascomycetes or Basidiomycetes. Mitosis has been studied extensively in several Ascomycetous yeasts. Thus, to begin with, we established a computational model with available parameters that are well characterized for Ascomycetes, followed by varying parameters measured \textit{in vivo} for both Ascomycetes and Basidiomycetes to develop two independent models. These models predict that both in Ascomycetes and Basidiomycetes, cMT bias is required for directional nuclear movement. Both the models also accurately simulate the altered conditions that prevail during mitosis upon treatment of cells with various microtubule specific depolymerizing drugs. We conclude that the models developed in this study offer a wider application towards understanding the consequences of not only short lived mitotic events but also evaluates the consequences of small perturbations in the entirety of the mitotic cycle.

\textbf{Results}

\textit{Generation of a computational model that replicates in vivo parameters of mitotic events}

In order to model mitosis in budding yeast, we considered simplified versions of several cellular components that are known to play a role in chromosome segregation including a) SPB/MTOCs, b) centromere-KT complex, c) cohesin complexes connecting sister chromatids prior to anaphase, d) MT network consisting of kMT, ipMT, and cMT, e) cell cortex and cortical dyneins
modulating cMT dynamics and f) kinesins involved in sliding overlapping ipMTs (Figure 1, A and B and Table 1). The mother cell was considered an ellipsoid while the nucleus was considered as a spherical object placed randomly within the mother cell at the onset of simulations. To mimic the experimental scenario, we resort to the same geometrical parameters of the mother cell as observed in our experiments (Table 1). Budding was initiated at a random location on the surface of the mother cell growing at the experimentally observed growth speed. MTs were modeled as straight filaments and MT dynamics was replicated by incorporating stochastic switching between growing (lengthening) and shrinking (shortening) states by using standard computational techniques (see Materials and Methods). The cell cortex was taken as a rigid wall that resists free polymerization of the cMTs by applying a resistive force at the cMT tip. During mitosis, cortically-anchored dynein motors that walk toward the minus end of the cMT, generate a pulling force on the SPB and provide directional movement of the nucleus/SPB towards the cMT tip (Figure 1, A and B, see Materials and Methods for detail). It is widely believed that cMT-cortex interactions play a vital role in nuclear migration in yeasts during mitosis (Carminati and Stearns, 1997; Adames and Cooper, 2000; Baumgartner and Tolic, 2014). We incorporated the idea of asymmetric loading of different protein molecules between the SPBs in order to ensure a biased nucleation of the cMTs in the model (Markus et al., 2012). Since we sought to understand the difference between the steady state positioning of the spindles in Ascomycetes and Basidiomycetes, we ignored their instantaneous dynamics in this particular study.

It is observed that KTs remain clustered in yeasts during mitosis (Jin et al., 2000; Anderson et al., 2009; Kozubowski et al., 2013; Varoquaux et al., 2015). Hence to avoid overlapping of individual KTs, each KT was modeled to have a hard-core excluded volume. Assembly of sister
KTs occurred immediately after centromere replication. In order to simplify the model, newly assembled sister KTs were assumed to be captured instantaneously. It is observed that in yeasts, the KTs always remain attached to the SPB during mitosis except for a few minutes during chromosome duplication (Tanaka et al., 2005; Tanaka and Tanaka, 2009; Tanaka et al., 2010; Gandhi et al., 2011). As compared to mammalian cells, the kinetochore capture process in Ascomycetes occurs faster. Considering that nuclear migration per se is a much slower process than the KT capture, instantaneous capturing of KTs is not expected to change our model prediction. At the ipMT overlap region, plus end directed molecular motors slide apart the MTs, generating a pushing force on the SPBs (Kapoor and Mitchison, 2001; Marco et al., 2013). The kMT-KT interaction is mediated by spring-like KT fibrils (McIntosh et al., 2008). Prior to anaphase, two opposing forces on the KTs, an outward pulling force towards SPBs, driven by motor proteins and depolymerizing kMTs and an inward cohesive force between the sister chromatids due to cohesin proteins, must be balanced to satisfy the spindle assembly checkpoint and subsequent entry to anaphase. To maintain the experimentally observed separation between the KT cluster and SPBs, a length-dependent catastrophe of kMTs was incorporated (Foethke et al., 2009; Sau et al., 2014).

The nuclear mass always divides close to the mother bud junction in budding yeasts

The nuclear division or mitotic spindle formation in Ascomycetes takes place in the mother cell, whereas it occurs in the daughter cell in Basidiomycetes (Heath, 1980; Straube et al., 2005; Gladfelter and Berman, 2009; Kozubowski et al., 2013) (Figure 1D; fourth and fifth row). Although the spindle positioning in Ascomycetes is a relatively well-studied process (Piatti et al., 2006; Merlini and Piatti, 2011), very little is known about the same in Basidiomycetes. We used fluorescence microscopy to understand the spindle and nuclear dynamics simultaneously in
these two classes of yeasts. In Ascomycetes, represented by *S. cerevisiae* and *C. albicans* henceforth, only one visible interphase MTOC serves as the SPB during mitosis (Figure 1D, (Segal and Bloom, 2001)). In contrast among Basidiomycetes, henceforth represented by *C. neoformans* and *U. maydis*, several MTOCs were seen spread throughout the cytoplasm during interphase (Figure 1D, (Straube *et al.*, 2003)). These MTOCs subsequently coalesced to form an active SPB during mitosis.

In pre-mitotic cells, SPBs are localized at a constant distance from each other after duplication, which segregates rapidly during the onset of mitosis (Figure 1D). The distance from the center of the mitotic spindle to the neck was measured in a number of cells (n= 30) during metaphase and early anaphase. The neck was taken as the origin and the distance was marked as (+) or (-) for the presence of spindle in the daughter cell or mother cell, respectively, during mitosis (Figure 1C). The net average neck-spindle distance for Ascomycetes (-1 ± 0.22 µm) was found to be similar in Basidiomycetes (+0.84 ± 0.23 µm). Thus, the nucleus was found to be positioned close to the neck during mitosis, irrespective of the dynamics of nuclear movement in pre-mitotic stages. In other words, the cellular machinery divides the nuclear mass into two equal halves across the neck in a well-conserved manner, irrespective of its earlier dynamics. The data obtained from these experiments as well as previously published results (Table 1) were incorporated into the universal model for mitosis described above to yield two working models, one each for Ascomycetes and Basidiomycetes (Figure 1, A and B and Supplementary Video1, Video2).

*Nuclear/spindle dynamics depends on the number of cMTs and dynein activity*

Having developed these models, we probed for the underlying variation in nuclear migration observed between Ascomycetes and Basidiomycetes. Differential migration patterns and a large
deformation of the nucleus during migration suggested that the magnitude of force pulling SPBs towards the bud is greater in Basidiomycetes as compared to Ascomycetes (Straube et al., 2005; Fink et al., 2006; Kozubowski et al., 2013). The larger force generated could either be due to an increased population of cMTs and/or a higher dynein activity at the cortical region. It is widely believed that Ascomycetes nucleate ~4 cMTs (Kosco et al., 2001), whereas the number of cMTs in Basidiomycetes is unknown. A previous study using U. maydis showed that the number of microtubules in this organism is 10-15 indicating a higher number of cMTs in Basidiomycetes (Straube et al., 2003).

Considering a conserved cMT-cortex interaction, our model revealed that the size of the cMT population must be ≥ 8 for producing sufficient force to pull the nucleus into the daughter cell (Figure 2A). Assigning the number of cMTs as 4 for Ascomycetes and 8 for Basidiomycetes, simulations predicted the mean distances between the neck and the spindle as -0.90 μm and +0.83 μm, respectively. These values are close to the experimental measurements (Figure 2B). Further, an increase in the density of cortical dyneins engaged in pulling the cMTs also provided enough pulling force for the migration of the nucleus into the daughter cell in the Basidiomycetes model when other parameters are kept constant (Figure 2C). To test the model’s prediction of requiring greater number of cMTs in Basidiomycetes for migration of the nucleus into the daughter cell, we counted the number of cMTs in C. albicans (Figure 2D) and C. neoformans (Figure 2E; see Materials and Methods). Our experiments revealed that C. neoformans has approximately at least 2 times higher number of cMTs than the C. albicans (Figure 2, D- F). It was observed that approximately 6 – 15 cMTs formed a dense mesh -like network in C. neoformans, with an average number of cMTs per cell being ~9 (Figure 2F), while each C. albicans cell has 3-5 cMTs with an average of ~4 cMTs per cell (Figure 2F). The results
presented above confirm the importance of cMT and dynein in positioning the spindle. Disruption of any of these components leads to severe mitotic defects (Markus and Lee, 2011; Laan et al., 2012; Xiang, 2012; Best et al., 2013). Thus, our model prediction supported by experimental validation confirms that an increased number of cMTs is required for migration of the nucleus/SPB into the daughter cell.

*A biased ‘search and capture’ by the cytoplasmic microtubules is required for proper nuclear migration and spindle alignment*

Next, we analyzed whether the nuclear migration was governed by a random or polarized nucleation of cMTs. To test this, simulations were performed on both the models keeping either an unbiased or a biased nucleation towards the daughter cell (Figure 3). The directional movement of the nucleus was impaired for an unbiased nucleation of cMTs, but the metaphase spindle length was found to be independent of cMT bias (Figure 3, A-D and Supplementary Figure S1, Video3, Video4). In both the cases, however, a biased dynamics of cMTs was crucial for proper nuclear migration (Figure 3, C and D and Supplementary Figure S1). The resulting spindle-neck distances were found to be similar to the experimental values. Basidiomycetes showed some directional movement of the nucleus even in the absence of biased nucleation which we attribute to a higher number of cMTs when compared to Ascomycetes (Figure 2F and 3D). An unbiased cMT dynamics also failed to align the spindle with the mother-daughter cell axis, adding to the severity of the defect in these cases (Figure 3, E and F).

Clearly, biased cMTs produce a directed force on the nucleus/SPB whereas uniformly nucleated cMTs generate force without any preferred directionality and hence often fails to move the nucleus/SPB to the predefined position. Many studies revealed that a cortical actin-dependent
mechanism, known as the ‘Kar9 pathway’, utilizes a myosin-V, Myo2-based machinery to guide the plus ends of cMTs along the cortex toward the neck at the early stage of the cell-cycle (Beach et al., 2000; Yin et al., 2000). Reports also suggest that asymmetric loading of Kar9 at the SPB can produce a chemical cue that leads to a biased nucleation of cMTs toward the neck (Liakopoulos et al., 2003; Cepeda-Garcia et al., 2010). The current model exploits these results providing an additional line of evidence for the same.

The model accurately reproduces experimental outcomes of various drugs affecting MT dynamics

Next, we tested the model by simulating the effects of two drugs that are known to affect the dynamics of specific classes of MTs in vivo. To depolymerize all MTs present in the cell, the MT depolymerizing drug nocodazole was used whereas methyl benzimidazole carbamate (MBC) (Akera et al., 2012) was used to disrupt ipMTs specifically. The depolymerizing kinetics for nocodazole treatment was simulated by increasing the catastrophe frequency of all the MTs in the model. Similarly, to simulate the effect of MBC, ipMTs catastrophe was increased without altering the dynamics of cMTs or kMTs.

Our model accurately simulates the effect of nocodazole treatment, resulting in shorter spindles which failed to move to the bud (Figure 4 and Supplementary Video5, Video6). For both Ascomycetes and Basidiomycetes, the spindle length was drastically shorter than untreated cells (Figure 4, A and B). We also observed that the spindle is mis-positioned and misaligned as depicted by the higher mean values for the neck to spindle distance and a higher spindle orientation angle (Figure 4, C-F). Misaligned spindles are identified as those making angles greater than 30° with the mother-daughter axis, while mis-positioned spindles position
themselves greater than 1 μm away from the neck. Simulations for higher nocodazole concentration were achieved by increasing the catastrophe frequency of the MTs (8-12/min), resulting in shorter MTs. Under these circumstances, initial MTOC and KT clustering in Basidiomycetes were highly affected due to altered cMT dynamics (Supplementary Figure S2). These simulated results correlated with the experimental results and accurately corroborated the in vivo observation of KTs failing to cluster upon nocodazole treatment as reported previously (Kozubowski et al., 2013).

After treatment with MBC, the spindle length was found to be shortened in Basidiomycetes (Figure 4B and Supplementary Video7). This effect was less drastic as compared to nocodazole, as the spindle length was longer for MBC treated cells. Similar to the nocodazole treatment, mis-positioned and misaligned spindles were also observed (Figure 4, D and F) when treated with MBC. These results suggest that the role of ipMTs is not crucial for SPB separation but they are required for spindle migration and orientation in Basidiomycetes. The effect of MBC treatment could not be examined in Ascomycetes because MBC does not affect the cell cycle events of C. albicans as reported earlier (Finley and Berman, 2005). Taken together, the consequences of experimental perturbation of various species of MTs on chromosome segregation are accurately simulated in the model. Based on these results, we conclude that the model developed is a robust one and can replicate the events observed in the in vivo experiments with high precision.

Discussion

In this study, we describe a model that accurately simulates the events of mitosis in distantly related budding yeasts belonging to the phyla Ascomycota and Basidiomycota. We also sought to understand the basis behind the differences in mitotic events observed in these two phyla. A universal model was developed for the budding mode of division following which mitotic events
were simulated and modeled both for Ascomycetes and Basidiomycetes by obtaining parameters either from literature or through experimental measurements. When compared, we observed that variations in the MT organization, orientation and dynamics account for most of the variations in mitotic events observed between these two classes of yeasts.

While making experimental measurements for Basidiomycetes using *C. neoformans*, it was observed that although the spindle migrates entirely to the daughter cell during mitosis, it is always positioned close to the neck at metaphase. Similarly in Ascomycetes, the nucleus moves very close to the neck, where the division takes place during mitosis. This indicates that the site of nuclear division with respect to the site of cytoplasmic division remains conserved in these two classes of yeasts in spite of the other observed differences. In metazoans, fission yeast and filamentous forms of many fungi (including the ones studied here), the site of nuclear division defines the site of cytoplasmic division (Balasubramanian *et al.*, 2000; Guertin *et al.*, 2002; Wang *et al.*, 2003; Gladfelter and Berman, 2009). Hence, cells seem to have developed a mechanism as the nuclear division takes place close to the pre-defined cell cleavage site in budding yeasts. The interaction of MTs with septin proteins and other cleavage elements plays a determinant role in this process (Castillon *et al.*, 2003; Rodal *et al.*, 2005). Indeed, several reports in *S. cerevisiae* have shown that positioning of the spindle close to the neck is important for accurate chromosome segregation (Piatti *et al.*, 2006; Merlino and Piatti, 2011). In absence of proper alignment and positioning of the mitotic spindle, spindle positioning checkpoint (SPOC) is activated in these cells delaying chromosome segregation (Piatti *et al.*, 2006; Fraschini *et al.*, 2008). Hence, this conserved distance observed between the neck to the spindle is possibly due the SPOC activity that employs cMTs to monitor the location of the spindle in dividing cells.
(Moore et al., 2009). Such a strict positioning of the spindle in Basidiomycetes might reflect the conservation of the regulatory process.

The cytoskeletal elements, primarily MTs and their accessory network of proteins, have been shown to influence nuclear migration (Hwang et al., 2003; Straube et al., 2003; Martin et al., 2004; Fink et al., 2006; Gladfelter and Berman, 2009; Markus et al., 2012; Kozubowski et al., 2013). Our model revealed that nuclear migration towards the daughter cell would occur only if cMTs organized themselves in a biased manner in the direction of the newly emerging daughter cell. This was found to be applicable for both Ascomycetes and Basidiomycetes. Previously, several reports indicated that actin and other cytoskeleton elements reorganize during budding in yeasts giving rise to cell polarity (Pruyne and Bretscher, 2000a, b). Here we show that polarized MT nucleation plays an important role during the process of mitosis. This hypothesis is supported by experimental observations suggesting that an asymmetric recruitment of proteins (Myo2, Bim1, Kar9 etc.) may guide the cMTs to grow and stabilize towards the emerging daughter cell (bud) (Miller et al., 1998; Miller and Rose, 1998; Yin et al., 2000; Huisman et al., 2004; Markus et al., 2012). Model simulations also predicted that at least 8 cMTs are required to provide the necessary force to migrate the entire nucleus to the daughter cell in Basidiomycetes. This was in agreement with our experimental observations that show each C. neoformans cell nucleates an average of ~9 cMTs. Thus a greater number of cMTs in Basidiomycetes (~9 cMTs/cell) as compared to Ascomycetes (~4 cMTs/cell) provides a larger pulling force on the SPB towards the emerging daughter cell resulting into a deeper penetration of the SPB in Basidiomycetes. However, our model also predicts a redundant pathway in which an increased activity (population) of dynein motors present at the cortical region of the daughter cell could also provide sufficient force to pull the nucleus/SPB into the bud.
The role of various MTs was determined by varying MT dynamics both by model simulations and by performing specific experiments, while scoring for the dynamics of the nuclear mass and SPBs through the progression of the cell cycle. The model predicted that disruption of all or only ipMTs results in the formation of short spindles without any nuclear movement towards the daughter cell. Experiments with depolymerizing drugs followed by measurement of the spindle length, neck to spindle distance and the spindle axis revealed cells are arrested at the short spindle stage. These experimental measurements, being in strong agreement with the model predictions, provide additional lines of validation for the developed models.

As our model can be used to simulate major transitions in mitosis, biological events spanning a short time scale can be incorporated to understand their global effects on the holistic process of mitosis. Although we focused solely on the role of MTs in this study, the model can also be used to address other contributing factors and their roles among these systems. For example, using this model we aim to further analyze the role of motor proteins during mitosis and define their roles more specifically. However, this model also has certain limitations which include consideration of only mechanical forces, absence of the nuclear envelope dynamics and lack of regulation by the mitotic checkpoint. DNA replication was considered as an instantaneous process and MT dynamics was taken as constant throughout the cell cycle further adding to the model constraints. However, this model lays the foundation for follow-up work which will help make a more refined and comprehensive model. It is important to mention here that the above said limitations/assumptions do not affect the quantitative conclusions presented in this study. The predictive nature and robustness also remain unaltered when model parameters were varied within a permissible window. Nevertheless, a parallel set of pathways based on novel assumptions may exist which could produce similar results as we have presented here.
In the present study, we developed a model to cover a large fraction of the mitotic cell cycle, which is the first of its kind to our knowledge. We could successfully characterize different mitotic events including the nuclear migration, spindle orientation and spindle length dynamics in a quantitative manner utilizing a holistic approach across two major fungal phyla. This type of systems biology approach to develop a predictive computational model may aid in identifying targets across human pathogenic yeasts for developing anti-fungal drugs.

**Materials and Methods**

**Model development**

In this section, we describe the model variables and governing equations in a simplified configuration to explain the mitotic mechanics in Ascomycetes and Basidiomycetes (Figure 1, A and B).

*Construction of mother and daughter cell*

We consider the mother cell as an ellipsoid of dimensions $a_{cell}$, $b_{cell}$ and $c_{cell}$ along the x, y and z direction respectively, whereas the nucleus is considered to be a sphere of radius $r_{nucleus}$ placed at a random location within the mother cell at the beginning of the simulation. In Ascomycetes, the SPB is embedded into the NE (Kahana *et al.*, 1995; Jaspersen and Winey, 2004), whereas, in Basidiomycetes, no active SPB is reported to exist in the early stages of mitosis. However, multiple MTOCs are found that wades along the nuclear surface in Basidiomycetes (Straube *et al.*, 2005; Fink *et al.*, 2006; Yamaguchi *et al.*, 2009). It is observed that during mitosis these MTOCs converge to a single mass leading to SPB activation (Straube *et al.*, 2003). Formation of the bud can occur anywhere on the cell surface. We considered that the final volume of the bud is ~80-90% of the mother cell volume as observed in our experiments. We chose the growth rate
of the daughter cell in our model such that it reflects the experimentally observed scenario. Our model predicts that the growth rate of the bud doesn’t play any significant role in spindle positioning (Supplementary Figure S3), however, cell size variation affects the spindle positioning (Supplementary Figure S4).

**Modeling of cMT and cMT-cortex based interaction**

The SPB nucleates cMTs that interact with the cell cortex via dynein motors (Carminati and Stearns, 1997; Adames and Cooper, 2000; Ten Hoopen et al., 2012). MTs are modeled as straight filaments elongating with velocity $v_g$ and shrinking with velocity $v_s$. Stochastic switching of MTs from a growing state to a shortening state and then shortening state to the growing state occur with catastrophe frequency $f_c$ and rescue frequency $f_r$ respectively (Mitchison and Kirschner, 1984). One can successfully simulate the dynamics of an MT using these four parameters. We assume that the cell cortex acts as a static wall that resists the growth of a cMT. The growth velocity of a cMT within the cortical region decreases as $v_g = v_g^0 \exp(-K_{cor}l_{dyn}/f_s)$ (Dogterom and Yurke, 1997; Janson et al., 2003), where $v_g^0$ is the unconstrained growth velocity of a MT, $K_{cor}$ is the stiffness of the cortex, $l_{dyn}$ is the length of penetration of the cMT tip within the cortex and $f_s$ is the stall force per MT. Dyneins engage with the cMTs, growing within the cortical region of width $l_{cor}$, to pull the SPB and the nucleus toward the cortex (Carminati and Stearns, 1997; Adames and Cooper, 2000; Lee et al., 2000; Ten Hoopen et al., 2012). We assumed that several proteins like Tem1, Bub2, Bfa1, Elm1, Num1 etc. assemble close to the cleavage apparatus at the neck during mitosis (Huisman et al., 2004; Rodal et al., 2005; Cuschieri et al., 2006; Baumgartner and Tolic, 2014). These protein molecules stabilize the cMTs and allow cMTs to interact with the cleavage apparatus (Castillon et al., 2003). In order to achieve stable cMTs, rescue frequency of the cMTs are adjusted as a function of the distance of
the cMT tip from neck as, $f_i(x) \propto \exp(-x/l)$, where $x$ is the distance between the tip of the cMT and neck and $l$ is a constant which is of the size of the cleavage apparatus (~0.2 µm). The pulling force due to the dyneins is calculated using the following expression:

$$f_{\text{dyn}} = l_{\text{dyn}} \lambda_{\text{dyn}} f_{\text{dyn}}$$

(1)

Here, $l_{\text{dyn}}$ is the penetration length of cMT within the cortical region, $\lambda_{\text{dyn}}$ is the number of dynein motors engaged per unit length of the cMT and $f_{\text{dyn}}$ is the magnitude of the force exerted by a single dynein motor. Summing $f_{\text{dyn}}$ over all the cMTs, we can estimate the total pulling force $F_{\text{dyn}}$ on the SPB/nucleus. The cMTs also exert a net pushing force $F_{\text{push}}$ (~1 pN) when the tip hits the cell periphery. Pushing force arises due to the polymerization of the cMT tip in contact with the cell cortex. In order to apply a bias to the cMTs, we explored several schemes, such as modulation of the dynamical parameters, differential cortical interaction between the mother and the daughter cells etc., independently.

**Modeling kinetochore and kinetochore-MT interaction**

During mitosis, KTs remain clustered and linked to SPBs through kMTs. To avoid any overlap among the KTs, we include inter-KT repulsion in a simplistic manner. Whenever two interacting KT spheres, each of radius $r_{\text{kt}}$, penetrate each other, a repulsive force keeps them separate. For the sake of simplicity, the repulsive force is considered as a linear function of the extent of overlap. The total repulsive force on the $i^{\text{th}}$ KT can be estimated as:

$$F_{\text{repul}} = \sum_{j=1,j\neq i}^{N_{\text{KT}}} C d^j$$

(2)

Here, $C$ is a constant, $N_{\text{KT}}$ is the number of KTs present in the nucleus and $d^j$ is the maximum overlap length between the $i^{\text{th}}$ and the $j^{\text{th}}$ KT.
The dynamics of the kMT plus end plays a crucial role in positioning the KT in yeast (Gardner et al., 2008). We assume that kMTs remain attached to the KTs throughout mitosis (Westermann et al., 2007). kMTs interact with the inner KT through spring-like KT fibrils (McIntosh et al., 2008). As the polymerizing kMT tip penetrates the KT, it applies a pushing force on the KT viz., $F_{\text{poly}} = l_{\text{pen}} K_{\text{fibrils}}$, where $l_{\text{pen}}$ is the length of penetration of the kMT tip within the KT and $K_{\text{fibrils}}$ is the effective spring constant of the KT fibrils. A depolymerizing kMT pulls the KT with a force $F_{\text{depoly}} = l_{\text{out}} K_{\text{c}}$ while trying to detach from the KT. Here $l_{\text{out}}$ is the separation between the kMT tip and the KT and $K_{\text{c}}$ is the force constant of the kMT-KT connecting springs (Wei et al., 2007; Powers et al., 2009). We calculate the total force acting between the SPB and KT as $F_{\text{SPB-KT}} = \sum (F_{\text{poly}} + F_{\text{depoly}})$, where the sum is over the number of MTs interacting with a single KT. To maintain a constant distance between the SPB and the KT we incorporated the notion of the length dependent catastrophe of the kMT (Varga et al., 2006; Foethke et al., 2009; Varga et al., 2009; Sau et al., 2014) i.e. catastrophe frequency of a kMT increases with its length $l_{\text{kMT}}$ as $f_c = hl_{\text{kMT}}$.

Modeling inter-polar microtubules

After the duplication of the SPB, overlapping inter-polar MTs (ipMTs) facilitate mechanical interaction between the SPBs. Due to the presence of kinesin 5 motors along the overlap (Kapoor and Mitchison, 2001; Marco et al., 2013), ipMTs tend to slide apart, essentially pushing the SPBs away from each other. The pushing force $F_{\text{ipMT}}$ reads as:

$$F_{\text{ipMT}} = l_{\text{overlap}} f_{\text{ipMT}}$$
where \( l_{\text{overlap}} \) is the total overlap length among all the ipMTs nucleated from the two SPBs, \( \lambda_{\text{ipMT}} \) is the linear density of kinesin motors engaged along the ipMTs and \( f_{\text{ipMT}} \) is the force produced by a single ipMT motor.

During SPB duplication, KTs detach from the kMTs and re-attach right after the successful duplication. We assume all the KTs are captured instantly by the MTs nucleated from the mother and the daughter SPB, such that chromosomes are bi-oriented. The actual capture process and achieving the biorientation, though, are far more complex and occurs over a finite time-scale (Marco et al., 2013). In the present study we ignore such details and focus on the spindle positioning and orientation during mitosis. It is noteworthy to mention that the kinetochores remain attached to the SPB throughout the cell cycle (except for 2-3 min during chromosome replication) and average nuclear migration time is long (~1 hour). Thus it is safe to assume the kinetochore capturing process as ‘instantaneous’. After chromosomal duplication, sister KTs remain attached to each other by cohesion springs. The cohesion springs, when stretched, generate tension between the sister-KTs:

\[
F_{\text{cohesion}} = K_{\text{cohesion}} x_{\text{KT}}
\]  

where, \( K_{\text{cohesion}} \) is the spring constant of the cohesion springs and \( x_{\text{KT}} \) is the separation between the sister-KTs.

KTs always remain clustered in Ascomycetes, whereas in Basidiomycetes they are unclustered during interphase and each of them remain close to the NE in the beginning of mitosis. KT clustering process, ahead of mitosis, was shown to be mediated by MTs (Kozubowski et al., 2013). Further, the clustering of the MTOCs and the KTs occur at the same time prior to mitosis (Yadav V and Sanyal K, unpublished data). Hence, in this model, we assume a direct interaction
between the MTOCs on the outer surface of the nuclear envelope with the adjacent KTs present on the inner surface. All MTOCs in *C. neoformans* nucleate MTs in random directions and interact with each other via these MTs. If, by chance, a searching MT from one MTOC captures another MTOC, they migrate along the connecting MT toward each other and coalesce to form a unified MTOC, conserving the total volume. Number of MTs nucleated from the merged MTOC is proportional to its surface area. The ‘search & capture’ of MTOCs continues until all MTOCs merge together to form a single SPB. Since MTs can bend, the search extends along the nuclear periphery. In this way, two MTOCs situated on the diametrically opposite ends of the nucleus can interact with each other. Since self-assembly of MTOCs is an MT-driven phenomenon, efficiency of this process depends on the selection of the dynamical parameters that determines MT life-cycles. For instance, a very small catastrophe frequency leads to long MTs which are efficient in capturing distant MTOCs; however misdirected MTs waste valuable search time while completing life-cycles. Similarly, a very large catastrophe frequency leading to short MTs are also inefficient in finding distant MTOCs (Supplementary Figure S2). Thus MT dynamics are tuned and optimized to assemble MTOCs within experimental time frames.

The equation of motion for KT, SPB and nucleus can now be written as:

\[
\frac{dx_{KT}}{dt} = \frac{F_{SPB-KT}^{ij} + F_{cohesion}^{ij} + F_{repal}^{ij}}{\xi_{KT}}
\]  

(5)

\[
\frac{dx_{SPB}}{dt} = \sum_{i=1}^{N_{KT}} F_{SPB-KT}^{ij} + F_{dyn}^{ij} + F_{push}^{ij} + F_{upMT}^{ij}
\]

(6)

\[
\frac{dx_{Nu}}{dt} = \frac{F_{push} + F_{dyn}}{\xi_{Nu}}
\]

(7)
Here, the system of equations (Eq. 5, 6 and 7) is derived in accordance with the well-known Stokes law, \( v = F/\xi \); \( v \), \( F \) and \( \xi \) being the velocity, force and viscous drag of a moving particle, respectively. The viscous drag obeys the formula \( \xi = 6\pi \eta r \), where \( \eta \) is the coefficient of viscosity of the medium and \( r \) is the effective radius of the particle. Here \( \xi_{\text{KT}} \), \( \xi_{\text{SPB}} \) and \( \xi_{\text{Nu}} \) correspond to the effective drag on a KT, SPB and nucleus, respectively. In our model the medium in which the KTs, SPB and the nucleus move are nucleoplasm, NE and cytoplasm of the cell, respectively. The superscript \( i \) in Eq. 1 stands for the \( i^{\text{th}} \) KT and the all \( x \)'s in Eq. 5, 6 and 7 are the instantaneous coordinates of the objects considered here. After the SPB duplication, another set of similar equations of motion for sister KTs and daughter SPB are incorporated. The constrained motion of the SPBs along the NE is achieved using a tangential coordinate system. The constraint is relaxed, once the SPBs reach the diametrically opposite ends of the nucleus. At each time step all the forces are calculated using Eq. 1, 2, 3 and 4 and then Eq. 5, 6 and 7 are solved numerically to update the positions of KTs, SPBs and nucleus, respectively. We explore a range of values for the model parameters (Table 1) to evaluate the model predictions.

**Construction of fluorescent tagged strains**

The MTOC markers, Tub4 in *C. albicans* and Spc98 in *C. neoformans* were C-terminally tagged with GFP to visualize the dynamics of MTOC/SPB in live cells. In *C. albicans*, the 3’ part of *TUB4* gene (Orf 19.1238) was amplified from the genome and cloned in a plasmid carrying the GFP-URA3, as a SacII-SpeI fragment. The plasmid was digested using PacI and transformed into *C. albicans*, SN148, to generate the Tub4-GFP expressing strain. The resulting strain was
used to visualize the MTOC with GFP and nuclear mass by DAPI staining during the imaging.

For *C. neoformans*, histone H4 (ORF number CNAG_01648) was tagged with mCherry and Spc98 (ORF number CNAG_01566) was tagged with GFP using the overlap PCR strategy described earlier (Kozubowski et al., 2013). For this purpose, 1 kb each of the 3’ part of gene and 3’UTR was amplified from the genome. A GFP-NAT or mCherry-NEO fragment (approximately 3 kb) from was amplified from pCN19 or pLK25 plasmids, respectively, and all three fragments were fused by overlap PCR, generating the cassettes. First the Spc98-GFP-NAT cassette was transformed to get Spc98-GFP strain which was then transformed with H4-mCherry cassette to obtain a double tagged strain. Similarly, GFP-Tubulin strain (Kozubowski et al., 2013) was transformed with H4-mCherry cassette to obtain the strain.

**Microscopy and estimation of cMT number**

The dynamics of fluorescent tagged proteins within cells across various cell cycle stages were captured using Carl Zeiss confocal laser scanning microscope LSM 510 META (Carl Zeiss, Germany). The images were then processed using the LSM 5 Image Examiner software (Carl Zeiss, Germany) and/or Adobe Photoshop (Adobe systems, San Jose, CA).

The number of cMT per cell was determined by the method described previously (Kosco et al., 2001; Straube et al., 2003). Briefly, the GFP tagged Tub1 strain of *C. albicans* (YJB12856) or *C. neoformans* (CNVY109) was grown till log phase, harvested and mounted on an 2% agarose pad containing synthetic complete media (2% dextrose, 0.67% YNB w/o amino acids, 0.2% amino acid mix and 100 mg/l of uridine or uracil for *C. albicans* or *C. neoformans*, respectively). GFP tagged tubulin images of *C. albicans* and *C. neoformans* cells were captured with identical settings using Carl Zeiss LSM 510 META confocal microscope (Carl Zeiss, Germany). Images
were 3D rendered using Image J (NIH, USA). cMTs were tracked manually using 3D rendered images across all planes. Bright clustered signals of Tub1-GFP which represented MTOCs were excluded from counting. Subsequent processing was performed using Image J (NIH, USA) and Adobe Photoshop (Adobe systems, San Jose, CA). Cell number vs cMTs/cell was plotted using Prism (GraphPad Software, La Jolla, CA), with the calculated mean drawn for both *C. albicans* and *C. neoformans*.

*Microtubule depolymerization experiments*

We performed microtubule depolymerization experiments using nocodazole (Sigma) and MBC (Sigma), microtubule depolymerizing drugs, to disrupt either MTs or ipMTs respectively. Both *C. albicans* (LSK111) and *C. neoformans* (CNVY197) GFP-tagged MTOC strains were grown overnight. The overnight culture was transferred to fresh media with an initial OD$_{600}$ of 0.2. The culture was grown for 3 h to get the cells in log phase (OD$_{600}$ = 0.5-0.6). The cells were then treated with nocodazole (100 ng/ml for *C. neoformans* and 20 µg/ml for *C. albicans*) or MBC (1 µg/ml for *C. neoformans*) for 4 h. An aliquot of cells were treated with only DMSO as a control. The cells were harvested after 4 h and washed with 1 ml of distilled water. Finally, cells were suspended in water and images were captured using a microscope (DeltaVision) equipped with a CoolSnap HQ2 CCD. The images were then processed using ImageJ (NIH, USA) and Adobe Photoshop (Adobe systems, San Jose, CA).

**Acknowledgment**

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References


Figure 1. Model development to study mitotic progression in Ascomycetes and Basidiomycetes. (A and B) Schematic of bi-orientation of chromosomes that occurs within (A) the mother cell in *C. albicans* (B) the daughter cell in *C. neoformans*. Various forces are responsible for proper bi-orientation in both these organisms and are depicted in detail as follows: De-polymerization of kMTs at the kinetochore enables pole-ward movement of
chromosome (circle I). Kinetochores interact with kMTs through spring-like attachments which regulate kMT dynamics. MT de-polymerization at the kinetochore pulls chromosomes towards the SPB. Prior to anaphase this pole-ward force is countered by the sliding force generated by the positive end directed kinesins acting along the ipMTs (circle II) and the cohesive force between the sister chromatids which is considered as a spring between sister chromatids (circle III). cMTs interact with the cell cortex, where dynein pulls SPBs towards the cortex (circle IV). (C) The representative sign convention for labeling the spindle distance from the neck. (D) Cell cycle phase-specific dynamics of nucleus, MTOCs and MTs in Ascomycetes (C. albicans) and Basidiomycetes (C. neoformans) was monitored by imaging a GFP-tagged component of MTOC or MTs along with nuclear dynamics, represented by DAPI stained nuclei in C. albicans and mCherry tagged histone H4 in C. neoformans. In C. albicans, a single MTOC, visible in unbudded cells, forms two active SPBs during S-phase (small budded cells). The duplicated SPBs then migrate away from each other to establish a bipolar spindle (~ 1.2 μm) in the mother cell during metaphase (large budded cells). In C. neoformans, multiple foci of MTOCs are observed at the beginning of the cell cycle. Observed MTOC foci merge together towards the onset of mitosis forming an active SPB. After duplication, the SPBs migrate into the daughter bud and then establish a bipolar spindle evidenced by an increase in the distance (~1.6 μm) between the SPBs. The nucleus, MTOCs and MTs are false colored as magenta, cyan and yellow, respectively. Bars, 5μm.
Figure 2. Dependence of nuclear migration on the number of cytoplasmic MTs and dyneins activity in Ascomycetes and Basidiomycetes. (A) In silico measurements of the neck to spindle distance upon altering cMT numbers per cell during mitosis. We observed that, for a fixed density of cortical dynein, a higher number of cMTs leads to a deeper penetration of the spindle into the daughter cell (bud). With 4 cMTs, the observed spindle distance from the neck is close to -1.0 µm, which is similar to that observed in Ascomycetes. As we increase the number of cMTs, the spindle moves closer to the neck and when there are 6 cMTs, the spindle just crosses over into the daughter cell. The spindle is strongly pulled and moved deep into the daughter cell when the number of cMTs is 8 or more, resembling what is observed in experiments. (B) Mean distance of the spindle from the neck is plotted as observed in simulation (n=100) and experiment (n=30) for both C. albicans and C. neoformans. Experimental measurements were carried out in a strain that had MTOCs tagged with GFP. DIC was used as a reference point for calculating spindle mid to neck distance. The mean distance of the spindle from the neck in C. albicans
estimated as -1.0±0.22 µm in experiments while our *in silico* model prediction with 4 cMTs turns out to be -1.0±0.02 µm. On the other hand, the spindle to neck distance in *C. neoformans* with greater or equal to 8 cMTs is found to be +0.84±0.23 µm and +1.0±0.05 µm from experiments and *in silico* measurements, respectively. (C) The spindle migration can also be affected by an alternative pathway involving cortical dyneins. An increase in the cortical dynein density, for a fixed number of cMTs, results in similar nuclear dynamics obtained previously by altering the cMT number. Standard error of mean (SEM) is shown in red bars. (D) and (E) *C. albicans* (YJB12856) and *C. neoformans* (CNVY109) strains expressing Tub1-GFP were used to monitor and estimate cMTs. To rule out false positives in counting, high resolution 3D rendered images were used to trace cMTs prior to estimation of their numbers. cMTs in all stacks were taken into consideration. Two different views over the y axis (0°, top panels, and 160°, bottom panels) of the 3D rendered images are shown to improve the visibility of cMTs that may be masked by others in a given orientation. Bar, 2µm. (F) The number of cMTs were counted in large number of cells of *C. albicans* and *C. neoformans*. These values were plotted and the calculated mean of cMTs per cell in each case was represented by a grey line. *C. neoformans* was found to contain 6-15 cMTs per cell with an average of 8.95, while the same in *C. albicans* ranges from 3 -5 with an average cMTs per cell being 3.9.
Figure 3. Biased vs. unbiased MT dynamics in maintaining spindle length, position and orientation in Ascomycetes and Basidiomycetes. (A and B) The spindle length at metaphase was calculated in the model with biased or unbiased cMTs for both *C. albicans* and *C. neoformans*. Experimental measurements were carried out during mitosis to measure the spindle length. The model predicts that the spindle length at metaphase remains unaltered irrespective of cMT bias as experimentally observed. (C and D) Neck to spindle distance is measured with or without cMT bias, while simultaneously comparing it with experimental data (wild-type). With unbiased cMT, the spindle often failed to move to the pre-defined spatial positions in both Ascomycetes and Basidiomycetes. The spindle-neck mean distance changes from -1.0 µm to -3.35 µm during unbiased nucleation in Ascomycetes. The spindle to neck mean distance in Basidiomycetes, changes from +1.0 µm to -2.2 µm during unbiased nucleation. (E and F) Orientation of the spindle is calculated by measuring its tilt with respect to the mother-daughter
cell axis. Unbiased cMT dynamics results in a larger angular orientation (~42° in Ascomycetes and ~41° in Basidiomycetes) with the mother-daughter cell axis reflecting the misaligned spindles for unbiased cMT dynamics. For biased cMT dynamics, the spindle aligned with mother-daughter cell axis and the angular orientation is measured as ~21° ± 2.1° for Ascomycetes and ~10.25° for Basidiomycetes. Red bars indicate SEM.
Figure 4. Comparison of *in vivo* and *in silico* results upon altering dynamics of MTs in *Ascomycetes* and *Basidiomycetes*. (A and B) Metaphase spindle lengths upon treatment with nocodazole or MBC are plotted for Ascomycetes and Basidiomycetes along with the unperturbed (DMASO control) numerical and experimental data. For Ascomycetes, we observed that upon nocodazole treatment the spindle length becomes ~0.50 ±0.04 µm which is in accordance with our model prediction ~0.52 ±0.01 µm. Similarly in Basidiomycetes, the spindle length is reduced to ~0.61 µm from its wild-type spindle length of ~1.66 µm. This is in accordance with the experimental value 0.62 ± 0.04 µm. For MBC treatment, in Basidiomycetes, the spindle length is shortened to 0.9 µm from its native value of 1.6 µm. This *in silico* result is in agreement with the experimental data for Basidiomycetes as shown. (C and D) Measurements of spindle to neck distances for Ascomycetes (with nocodazole) and Basidiomycetes (either with nocodazole or MBC) revealed inability of the spindle to move to their unperturbed spatial locations. The spindle always remained in the mother cell with an increased mean distance (~ -2.7 µm for
nocodazole) from the neck as compared to its wild-type value (~ -1.0 µm) in Ascomycetes. In Basidiomycetes, the spindle failed to move to the bud and always remained in the mother cell during either nocodazole or MBC treatment. (E and F) The spindle orientation in the presence or absence of drugs was measured and it was found to misalign with mother-daughter cell axis as shown by the higher spindle orientation angle in both the cases. Red bars indicate SEM.
Table 1. Various parameters used to develop the model.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Meaning</th>
<th>Values for Ascomycetes</th>
<th>Values for Basidiomycetes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{KT}$</td>
<td>Number of KTs in haploid cell</td>
<td>16 ($S. cerevisiae$)</td>
<td>14 ($C. neoformans$)</td>
<td>Our experiment</td>
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<tr>
<td>$N_{cMT}$</td>
<td>Number of cMTs</td>
<td>4</td>
<td>$\propto 4\pi r_{MTOC}^2$</td>
<td>(Kosco et al., 2001)</td>
</tr>
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<td>$a_{cell},b_{cell},c_{cell}$</td>
<td>Dimension of the Cell</td>
<td>2.5-3.0 $\mu$m</td>
<td>2.50-3.0 $\mu$m,</td>
<td>Our experiment</td>
</tr>
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<td>$l_{cor}$</td>
<td>Width of cortex</td>
<td>0.2 $\mu$m</td>
<td>0.2 $\mu$m</td>
<td>(Rodal et al., 2005)</td>
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<td>$K_{cor}$</td>
<td>Spring constant of the cortex</td>
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<td>5.0 pN/$\mu$m</td>
<td>This study</td>
</tr>
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<td>$r_{nu}$</td>
<td>Initial radius of the nucleus</td>
<td>1.0 $\mu$m</td>
<td>1.0 $\mu$m</td>
<td>Our experiment</td>
</tr>
<tr>
<td>$r_{SPB}$</td>
<td>Radius of single SPB</td>
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<td>0.125 $\mu$m</td>
<td>(Seybold and Schiebel, 2013; Lee et al., 2014)</td>
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<td>$r_{KT}$</td>
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<td>0.05 $\mu$m</td>
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<td>$v_{g},v_{s}$</td>
<td>growth, shrink velocity of MT</td>
<td>6.4 $\mu$m min$^{-1}$,</td>
<td>10.4 $\mu$m min$^{-1}$,</td>
<td>(Fink et al.,</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Reference</td>
</tr>
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<tr>
<td>$f_c, f_r$</td>
<td>catastrophe, rescue frequency of MT</td>
<td>0.34 min$^{-1}$, 0.02 min$^{-1}$</td>
<td>1.0 min$^{-1}$, 0.02 min$^{-1}$</td>
<td>(Fink et al., 2006; Finley et al., 2008)</td>
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<td>$f_s$</td>
<td>Stall force of MT</td>
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<td>1.7 pN</td>
<td>(Dogterom and Yurke, 1997)</td>
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<td>1.0 pN</td>
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<td>6.0 /μm</td>
<td>(Civelekoglu-Scholey et al., 2006)</td>
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<td>Density of ipMT motor per unit length</td>
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<td>1.0 /μm</td>
<td>(Civelekoglu-Scholey et al., 2006)</td>
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<tr>
<td>$f_{ipMT}$</td>
<td>Force produced by single ipMT motors</td>
<td>1.0 pN</td>
<td>1.0 pN</td>
<td>This study</td>
</tr>
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<td>$\eta_{cell}$</td>
<td>Viscosity of cytoplasm</td>
<td>5.0 pN s/μm$^2$</td>
<td>5.0 pN s/μm$^2$</td>
<td>(Civelekoglu-Scholey et al., 2006)</td>
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<td>$\eta_{nu}$</td>
<td>Viscosity of nucleoplasm</td>
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<td>10.0 pN s/μm$^2$</td>
<td>(Civelekoglu-Scholey et al.,</td>
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<td>$\eta_{\text{NE}}$</td>
<td>Viscosity of NE</td>
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<td>10.0 pN s/μm$^2$</td>
<td>(Civelekoglu-Scholey et al., 2006)</td>
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<td>$K_{\text{cohesion}}$</td>
<td>Spring constant of the cohesion springs</td>
<td>0.1 pN/μm</td>
<td>0.1 pN/μm</td>
<td>(Joglekar and Hunt, 2002)</td>
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<td>(Civelekoglu-Scholey et al., 2006; Sau et al., 2014)</td>
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<td>$K_{\text{fibrils}}$</td>
<td>Spring constant of the KT fibrils</td>
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<td>5.0 pN/μm</td>
<td>(Civelekoglu-Scholey et al., 2006; Sau et al., 2014)</td>
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<td>Repulsion strength of KTs</td>
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