

Modelling collective mechanics of microtubule aster motility and positioning by molecular motors

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By

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Dedications

*This thesis is dedicated to my parents
Manmohan and Kamala Khetan
and my brother Aditya Khetan*

DECLARATION

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Abstract

Microtubules (MTs) and MT associated proteins form an essential component of the eukaryotic cytoskeleton. Asters are radial arrays of MTs, involved in vital cellular processes such as spindle assembly, chromosome segregation, intracellular transport, cell motility and organelle positioning. While several studies have addressed mechanisms for movement and positioning of a single or a pair of asters, only a few studies report the mechanical basis of multi aster positioning. Understanding how their numbers and position is mechanically regulated is the focus of this study. Using computational models, I have studied the mechanical basis of multiple-asters in confinement. In order to determine the general principles of multi-aster behaviour, the MT-motor parameter space was extensively explored and the key findings are discussed in the thesis.

The multi-aster centering in meiotic oocytes of mouse was recapitulated in a minimal model of dynein gradient, coupling dyneins, MT dynamics and cortical pushing. This suggests motor gradient as a mechanism for directed motility of small asters in large cells. While segregated arrangement of asters emerged with a hexagonal dominance, in presence of kinesin-5 motors and stabilized MTs at optimal system size. A similar hexagon ordering observed in ascidian meiotic oocytes, validated the simulation results. On cortical localization of dyneins, dynamic asters self-organized into collective rotational motion. This suggests possible mechanisms that may have evolved to suppress such motions *in vivo*. To explicitly simulate cortical dyneins, yeast dynein was modeled and the motor number dependent MT transport statistics was comparable to the MT gliding experiments.

The combined affect of MTs, motors and system size on mult-aster motility and position is demonstrated. This study provides a framework for theoretical understanding of the principles of multi-aster mechanics in spindle assembly, sub-cellular organization and embryogenesis.

Synopsis

Microtubules (MTs), molecular motors and the regulatory proteins form an essential component of the eukaryotic cytoskeleton. An aster is a radial array of MTs usually assembled by Microtubule Organizing Center (MTOC) in cells. The centrosomes or γ -tubulin foci functions MTOC in animal cells [1, 2]. MT asters are involved in vital cellular processes such as spindle assembly, chromosome segregation, cell polarization, cell movement, intracellular transport and sub-cellular positioning of the organelles. Hence understanding their spatiotemporal dynamics is essential and it could improve our understanding of sub-cellular mechanics. The radially emanating MTs are kinetically polarized filaments made of tubulin monomers that exhibit dynamic instability. Kinesin and dynein are the molecular motor proteins that walk towards the plus and minus end of the MTs respectively. Together with motor and MAP proteins, MTs undergo dramatic reorganization that result in diverse spatial patterns across cell types and stages of the cell cycle. Mixtures of MTs and molecular motors also self-organize to form patterns such as asters, bundles and vortices [3, 4]. The interactions between the MTs, motors and MAP proteins are responsible for the motility and positioning of the MT asters in space and time.

Mechanical models have been proposed for single aster positioning that are expected to vary with cell size and type such as (a) pushing and pulling at the cell boundary due to MT polymerization (b) dynein dependent pulling of depolymerizing MT and (c) MT length dependent pulling by immobilized cytoplasmic motors [5, 6, 7, 8, 9]. However, multiple asters exist in many systems such as syncytial cells, muscle cells, meiotic oocytes and cancerous cells. The mechanism of multi-aster movement and dynamics is less understood owing to the complexity of the system and redundant mechanisms which hinders the mechanistic deduction from the experiments. The aim of this study is to explore and understand the collective effects of microtubules and motors on multi-aster systems. The approach involved quantitative analysis of the experimental data, mathematical modeling and detailed MT-motor simulations.

In this study, the parameter space for MT-motor system was explored to find the general principles of self-organization and emergent behaviours that result in aster positioning, motility and interactions. The key findings from this study are summarized and discussed in the thesis under the following sections (i) mechanism of multi aster centering in the context of meiosis I in mouse oocytes, (ii) mechanism of multi-aster positioning and self-organized pattern formation, (iii) the spontaneous emergence of collective rotation and (iv) effect of collective motor mechanics in dynein-driven MT gliding assays.

The vertebrate oocytes at meiosis I lack centrosomes. Acentrosomal bipolar spindle is organized by molecular motors, RanGTP gradient, MTs and asters [10, 11, 12]. In mouse oocytes, bipolarization is preceded by centripetal convergence of multiple asters at the chromatin region, which nucleate randomly in the ooplasm prior to the nuclear envelope breakdown (NEBD) [10]. However, the known mechanisms of centering in oocytes such as biased search due to RanGTP gradient [13, 14, 15] or role of actin was eliminated in experiments [10, 11], but the precise mechanical basis for centripetal movement was not investigated. The aster trajectories from the time lapse data published earlier [10] on analysis suggested a space dependent bias in the directional movement of the asters. The form of the bias was inferred from a model of asters treated as random walkers in a non-homogeneous space. The shape and parameters of the form was optimized by comparison of the simulation outcomes to the experiment and it suggested the bias to result in pulling of the asters towards the cell-center. A stochastic mechanical MT-motor was developed in Cytosim [16] to deduce the molecular origins of the directional bias in the mouse cell. A spatial gradient of dynein motors originating from the cell-center was found to be the minimal model essential for the centripetal convergence of the asters. On addition of cytoplasmic diffusible coupling dyneins in the cell along with cortical pushing of MTs and MT dynamics the model could recapitulate the experimental trends and statistics. The study hypothesizes that a motor gradient can resolve the tug-of-war experienced by small asters and a gradient of MT dynamics is not

sufficient to result in the directional movement. It speculates that in the larger cells when boundary forces are insufficient to center, MT length asymmetry can center the larger asters while asymmetry in motor numbers is required for smaller asters. However, the existence and emergence of such motor gradients in cells is not known so far and needs to be tested. Preliminary results suggest similar spatial asymmetry in motor distributions can emerge in presence of perinuclear asters and MTs consistent with some reports that have shown presence of perinuclear asters prior to NEBD [17]. In future, a systematic study on the spatiotemporal control of aster nucleation may explain the emergence and maintenance of dynein gradient in the oocyte. This could also provide insights on general principles of motor distribution in various cells.

While the aster centering in mouse oocytes was shown to be independent of kinesin-5 activity [10], similar multi-aster centering in the mouse embryos during acentrosomal spindle assembly has been shown to involve kinesin-5, dynein and a gradient of RanGTP [18]. The antagonistic motors – dynein and kinesin-5 – are involved in multiple scenarios such as centrosome separation in somatic cells, nuclei positioning in muscle cells and spindle positioning in *Drosophila* syncytium. In multi-aster system the antagonistic motor kinesin-5 was incorporated along with dynein to explore the parameter space in presence of opposing motors, in terms of cell size, proportion of motors and perturbed MT dynamics. In the absence of implicit steric interactions and MT dynamics global patterns emerged from the kinesin-5 driven outward pushing of asters and inward pushing at the cortex due to confinement. Based on the proportion of motors and cell size, asters either coalesced, centered (only dynein) or formed a spatially segregated steady state network of asters (only kinesin-5). The regular positioning of asters in cells of intermediate size resembled the optimal packing of non-interacting circles in 2D. The dominance of hexagons in the polygon distributions from the tessellation of the aster centers confirmed the equilibrium packing of asters in simulations. Hexagonal ordering was observed in multiple scenarios, all of which satisfied the packing criterion. Such regular MT

aster patterns was found in the ascidian meiotic oocytes of *Phallusia mammillata*. Multiple asters emerged in the meiotic cytoplasm that formed a lattice of asters with a similar hexagonal dominance of ‘aster centered cells’. Such cytoplasmic asters were induced only on perturbation of RSK pathway however, the mechanism of its action for formation of aster patterns was not known. It is speculated that RSK inhibition slows down the MT dynamics which corroborates with loss of equilibrium packing and positioning of asters in simulations with dynamic astral MTs. MT mass collapsed at the center or formed sparse coalesced structures on kinesin-5 inhibition similar to the aster patterns in simulations in presence of only dynein. A kinesin-5 based model was optimized by testing the outcomes from dynein inhibited experimental data. On titrating the relative number of dynein and kinesin-5, a minimal model was proposed that recapitulated the experimentally observed patterns. The results suggest multiple asters can self-organize in confinement and form spatial patterns due to the local aster-aster interactions mediated by clustering of kinesin-5. Such patterns resemble aster and nuclei positioning in multi aster system and may provide insights on the mechanisms for their origin and maintenance. In future, it would be interesting to explore motor based spindle dynamics.

The antagonistic activity of dynein in the cytoplasm disrupts the equilibrium pattern due to the coalescence of asters. On uniform localization of the dyneins at the cortex, the kinesin-5 driven aster-aster interactions in the cytoplasm is restored however the equilibrium is perturbed. Collective and coherent rotation of astral network emerge in the presence of cortical dyneins, cytoplasmic kinesin-5 and MT dynamics. Such self-organized swarming behavior is reminiscent of the swarming in flocks of birds and schools of fish. The emergence of collective rotation on the exploration of parameter space was categorized into three types (a) coherent and persistent (b) coherent and not persistent (c) random rotations. To capture the physiological behaviour of dynein at the cortex due to the fluidity of the lipid membrane, flexibility of the anchors or actin scaffolds, dynein model was modified to incorporate these effects phenomenologically. Dyneins were allowed to diffuse when unbound and slip while stepping along the cortex. A transition in rotation order parameter is observed as a

function of number of motors per aster and the rate at which MT lengths fluctuate in time. The rotational movements cease when dyneins at the cortical sites cluster due to lack of diffusion driven re-distribution of dyneins along the cortex resulting in polarization of asters. This confirms that uniform distribution at a density beyond a threshold level is essential for the sustained collective rotation. These findings provide insights on the existing mechanisms of cortical MT-motor interactions that may have evolutionary implications. So far all the studies have shown dyneins to be localized as patches of clusters on the cortex. Secondly, end-on and not lateral MT-cortex interactions have been reported to function at the anaphase. In simulations (a) uniform distribution of dynein (b) lateral MT-cortex interactions and (c) fluctuations in MT lengths is essential for rotation of asters while kinesin-5 provides coupling of asters and is essential for the coherence. The observed phenomenology in this multi-aster system is analogous to the universal class of Viscek-like models for the active particles that undergo transition from random to ordered collective motion [19]. In future, this work can be extended to study the effects of cell shape, implications in cytoplasmic streaming, emergence of chirality and spindle dynamics.

In some systems such as budding yeast, the dyneins are non-essential with the only known function of it's role in spindle positioning during anaphase while localized at the cortex. In order to understand the molecular mechanism of cortical dyneins explicitly, a model for yeast dynein was developed. This dynein is well studied at a single molecule level, thus a minimal yeast stepping and detachment model based on published reports was developed and optimized according to the experiments from MT gliding assays in the lab. The asymmetric load-dependent detachment mechanics resulted in MT transport characteristics that recapitulated the experimental observations in terms of quantitative measures too. Simulations predicted a sharp increase in directionality at a threshold number of ~ 8 motors consistent with the experiments and propose that such motor number dependence could have a role in 'search and orient' of the spindle positioning in the budding yeast. The low motor

numbers allow astral MTs to search the cellular space that undergo motor number dependent transition to directional sliding movement along the cortex resulting in spindle orientation.

In conclusion, this study predicts the mechanical basis of motility and self-organized patterns in multi-aster systems. It reconciles the experimental observations and predicts in-vivo implications. A minimal model of spatial gradient and diffusible coupling dyneins resolved the force symmetry of asters resulting in centripetal movement, comparable to mouse meiotic oocytes. A model of multiple asters interacting with motors resulted in self-organized patterns of asters that recapitulate the regular positioning observed in-vivo. Such patterns have implications in aster based migration and positioning of sub-cellular organelles such as nucleus in the cell. Extensions of this model have revealed general principles of collective motion of multiple asters, similar to the swarming of active particles. That such self-organized aster rotations are not observed in cells, suggests additional mechanisms in vivo, which suppress such movement. Further, motor number dependent transition in linear MT filament transport suggests the emergence of collective behavior due to the single motor mechanics. This study provides a framework for theoretical understanding of the mechanics of MT-motor interactions in cells.

Thesis organization: This thesis is organized into 6 chapters and 4 appendices that contain supporting information, the contents are discussed briefly. The links to the video files is shared in the appendices.

Chapter 1: Introduction This chapter provides background information on MT-motor cytoskeleton. The components of an aster, their mechano-chemical properties and the functions are discussed here. It emphasizes the importance of spatio-temporal control of aster position and number in the cell and briefly highlights the key mechanisms responsible for positioning and motility of a single aster. The chapter introduces multi-aster systems and discusses the open questions in the field.

It then motivates the need for the computational exploration of such systems and states the objectives addressed in the thesis.

Chapter 2: A motor-gradient and clustering model of the centripetal motility of multiple asters in meiosis I of Mouse oocytes. In this chapter the mechanism of multi-aster centering of small asters in a large cell of mouse oocyte at meiosis I is discussed. Here, based on the detailed analysis of previously published aster mobility data the models were developed and optimized. A phenomenological was developed to constraint parameter search space and understand the phenomenology of the process. Based on the findings a mechanistically detailed model was developed to identify the molecular components responsible for the force generation. In this study, a gradient of dyneins along with diffusible dyneins is shown to be essential to recapitulate the centripetal convergence of the asters. In the chapter, the effect of motor numbers is explored. The end of the chapter highlights the limitation of the model and discusses the questions that can be addressed in future. Supporting information is included in the Appendix A.

Chapter 3: Self-organized patterns of multiple microtubule asters formed by kinesin-5 motors at optimal relative cell size. In this chapter, the role of antagonistic motors and MT dynamics on multi-aster centering and decentering is addressed and the effect of cell-size is explored. This chapter extends the previous study by incorporating the antagonistic motor, kinesin-5 complexes. A stable hexagonal lattice of asters is observed in the presence of kinesin-5 motors in simulations, similar to the patterns found in the ascidian meiotic oocytes of *Phallusia mammillata* on perturbation of RSK pathway (based on experiments by the team of Dr. Janet Chenevert). In this chapter the simulation studies provide a mechanical basis for the formation of stable aster patterns, which is confirmed in experiments. The chapter concludes with the limitations of this study and discusses the future prospects and implications of the finding in other multi-aster systems. Supporting information is discussed in the Appendix B.

Chapter 4: A microtubule-motor model for emergence of self-organized multi-aster rotation in confinement. In this chapter the collective rotation of multiple asters in the system is discussed. The motor numbers, their distribution and fluctuation in MT lengths contribute to the symmetry breaking that results in onset of collective motion. The transition from random to coherent and persistent multi-aster rotation is found to depend on the density of motors per aster and the noise in the system. The results have also been discussed in the context of Viscek type of models. The mechanisms that could have evolved to suppress the collective rotation in the cells speculated based on the findings are discussed. The chapter concludes with discussion on the future directions and the implications of this study.

Chapter 5: Effect of motor numbers and detachment mechanics on collective MT transport. In this chapter, the previous work has been extended to understand the behaviour of cortical dyneins explicitly. A minimal model of stepping and load dependent asymmetric detachment kinetics was developed for a well characterized yeast dynein. The model was optimized to the experimental data from MT gliding assays (performed by Kunalika Jain in the lab). In this chapter, the effect of MT lengths and motor numbers on the transport of linear MTs in simulations and its comparison to the experiments is discussed. The findings suggest the implications of motor number dependent transport in ‘search and orient’ of the aster driven nucleus in the budding yeast during the anaphase. The chapter concludes with discussion on the limitations and the future directions of this study.

Chapter 6: Summary and conclusions. In this chapter, the effect of microtubule and motor parameters and their behaviour in the multi-aster system is highlighted. The biological significance and insights on the existence of multiple mechanisms across such systems is discussed.

Publications

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Chapter 1

Introduction

1.1 Background

Microtubules (MTs) along with the molecular motor proteins and microtubule associated proteins (MAPs) form an essential component of the eukaryotic cytoskeleton. MT cytoskeleton is responsible for force generation in the cell essential for cell shape, cell physiology and cell division. Any disruption in their structure or dynamics result in range of genetic diseases such as ciliopathies [20, 21], dyneinopathies [22], genomic instability and cancer [23], neurodegenerative [24, 25] and bleeding disorders [26]. The work presented in this thesis focuses on microtubule asters; investigating the mechanical basis of their motility and positioning in multi aster systems that have implications during the cell division using computational approaches. In this chapter I will first introduce the biophysical aspects of MTs, motors and asters, followed by the discussion on multi-aster systems and the open questions in the field. I will then briefly introduce the existing simulation methods in the field and the specific aims addressed in this thesis.

1.2 Microtubule aster components

An aster is a radial array of MTs that is assembled by Microtubule Organizing Center (MTOC) in cells [1, 2]. MTOC is commonly known as centrosomes in animal cells, spindle pole body in yeast, basal body in cilia and atypical or acentrosomal MTOC in meiotic oocytes. Asters are involved in cellular processes such as cell polarization, cell shape and motility. They are also responsible for diverse sub-cellular processes such as spindle assembly, chromosome segregation, intracellular transport and positioning of organelles. The spatio-temporal control and coordination of dynamics is crucial to facilitate these processes. Thus understanding of the biochemical and mechanical basis that control their position, movements and numbers in a cell is essential. The findings would improve our understanding of the diseases and could have implications in identifying novel targets and therapeutics of medical relevance.

In the following section the components of an aster and their mechano-chemical properties are discussed briefly.

1.2.1 Microtubule organizing center

The MTOC comprises of large number of proteins that function as nucleators, scaffolds and anchors for the MTs that emanate from it. Centrosomes in animal cells, spindle pole body (SPB) in yeast and basal body in cilia functions as MTOC. It acts as a template that nucleates MTs and also stabilizes the minus end of the MTs [1, 2]. In centrosomes, MTs are nucleated by γ -tubulin ring complex (γ -TuRC) which caps the minus end and anchors it to the pericentriolar material (PCM) in association with many other proteins [27, 28]. In the absence of centrosomes in vertebrate oocytes and plant cells, PCM proteins such as pericentrin and γ -tubulin function as MTOC.

1.2.2 Microtubules

MTs that radiate from the MTOC are polar filaments formed by polymerization of tubulin heterodimer, α - β tubulin. The 8 nm long heterodimers polymerize longitudinally into protofilaments and ~ 13 such protofilaments close into a hollow tube of ~ 25 nm in diameter via the lateral interactions [29]. MT filaments exhibit structural and kinetic polarity. The MT end with β -tubulin exposed is termed as plus end while that with α -tubulin is termed as minus end, often the plus end is the faster growing end. MT filaments stochastically alternate between phases of growth and shrinkage, phenomenon known as dynamic instability [30]. Dynamic instability confers MTs the ability to re-model and reorganize the MT cytoskeleton in the cells [31]. The astral MTs can span from sub-micron to tens of microns in length, variation observed either across different cell-types or stages of the cell-cycle. The

MTs experience opposing forces that result in pushing or depolymerization or buckling or deformation on encountering an obstacle such as nucleus or cell membrane. The polymerizing MTs exert forces in the range of $\sim 1\text{-}5$ pN when obstructed by a barrier [32, 33], often the cell boundary. However, the MT filaments are rigid, the persistence length estimated to be around ~ 1000 μm [34] which confers mechanical stability to the cytoskeleton network. In cells, post translational modifications such as acetylation provides mechanical stability and resistance from mechanical stress to the microtubule cytoskeleton [35, 36]. The plus end of the astral MTs is dynamic while the minus end of the astral MT embedded in the MTOC is often non-dynamic. The association of the minus end at the MTOC is strong and can withstand forces upto 40-50 pN before getting ruptured from the SPB, which is 4 to 5 fold higher than the forces experienced due to MT polymerization at the MT-kinetochores [37]. Further, regulatory proteins (or microtubule associated proteins, MAPs) can modulate the MT dynamics in space which effects the lengths of astral MTs and the rate of MT dynamics [38, 39, 40, 14, 41]. The biochemical and mechanical forces together regulate microtubule properties important for cellular functions [42]. In addition to the MT polymerization forces and MT length fluctuations, depolymerization of MTs also result in force production when associated with the molecular motors.

1.2.3 Molecular motor proteins

Molecular motors are nanometer sized proteins that walk over the MT in steps of nano-metre scale by consuming energy from ATP hydrolysis. Motors associated with MTs are broadly classified into two types based on their directionality: Kinesins that walk towards the (+) end of MT while dyneins walk towards the (-) end of the filament. The motor proteins can bind to the MT filament, step on, stall and detach from the MT. Typically, motors can withstand forces upto $\sim 2\text{-}7$ pN before stalling or dissociating from the MT [43]. They can generate pulling forces while bound to a depolymerizing MT in the range from $\sim 5\text{-}50$ pN during positioning of organelles

and chromosome segregation [44, 45]. The binding of cofactors or accessory proteins can modulate the binding/unbinding kinetics of the motor, activity state and their localization in the cell [46, 47, 48]. Recently, the role of post translational modifications [49] along with MAPs [50, 51] has also been identified in activation, inhibition and recruitment of motors in the cell. The motor proteins transport cargoes such as vesicles, lipids, other motor proteins and MT assemblies in the cell. In addition to generating forces, some motor proteins are also known to act as MT polymerases and depolymerases [52]. Motor complexes such as tetrameric motors can bind to the two MT filaments simultaneously resulting in relative forces that can either cluster, coalesce or separate MTs (discussed in the section 1.5.2). These interactions have implications in spindle assembly and neuronal morphogenesis.

1.2.4 Cellular forces

The work done by MTs and motors is comparable to the thermal energy scale of $\sim k_B T$. The motors and MTs generate forces in the range of pico-newtons that operate at the nanometer length scales, while the thermal energy $k_B T$ corresponds to $\sim 4.1 \text{ pN} \cdot \text{nm}$. Thus, MT-motor activity is subject to the noise in the cells. For instance, the incorporation of tubulin dimers at the cell periphery is subjected to the Brownian fluctuations that create space for addition of tubulin dimers. The polarity of MTs, directionality of the molecular motors and the activity of cross-linkers result in utilization of energy to perform the work in the cells. Diffusion plays an important role in the transport and distribution of proteins and complexes inside the cell. The movement is obstructed and damped as the cells are extremely crowded and viscous. The Reynolds number for a micron sized protein is around $\sim 10^{-9}$ in the cytoplasm. (Reynolds number (R_e) is estimated assuming the following values: length scale (l) of $1 \mu\text{m}$, cytoplasmic viscosity (η) as $1 \text{ Pa}\cdot\text{s}$, density of the fluid (ρ) as $10^3 \text{ kg}/\text{m}^3$ moving at the velocity (v) of $1 \mu\text{m}$ from the equation $R_e = \frac{\rho \cdot v \cdot l}{\eta}$.) At such time and length scales, MT-motor activities are expected to be affected by the stochastic

forces and the viscous drag. As an example, the non-motor MAP protein such as Ase1 have been shown to generate entropic forces in the pN range, originating from diffusion when confined between the MTs resulting in sliding of the anti-parallel MTs [53].

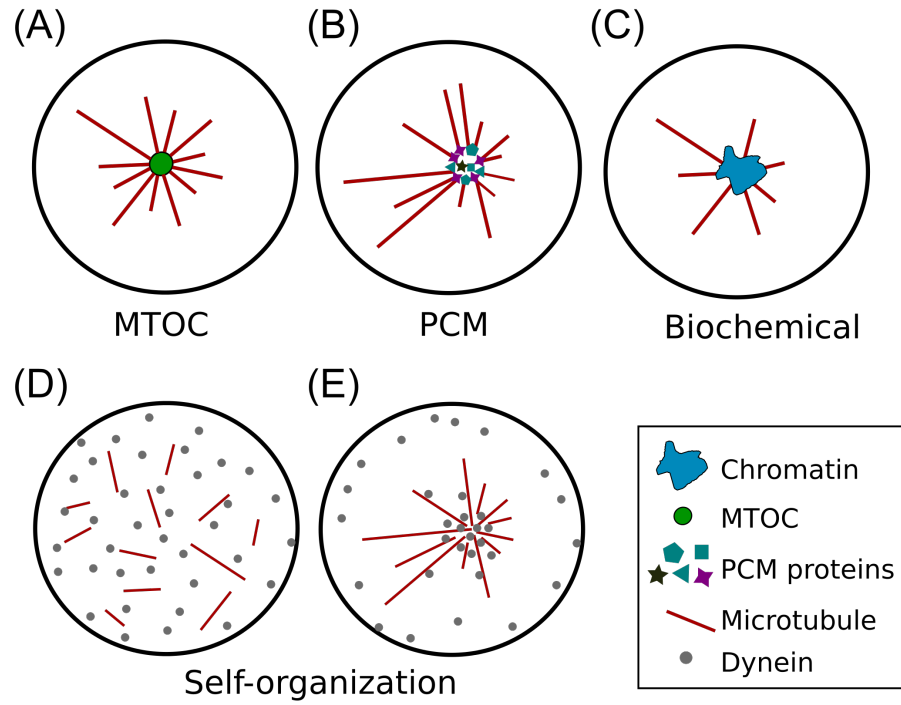


Figure 1.1: Multiple modes of aster formation. MT asters can be formed by multiple mechanism. (A) MTOC (green) nucleated MTs (red) form an aster. (B) In the absence of typical MTOC, peri-centriolar matrix (PCM) proteins can function as MTOC and result in formation of a MT aster. (C) The chromatin surface (blue) can nucleate MTs radially which is dependent on the RanGTP gradient. (D) MTs (red) and motor complexes (gray) self-organize to form (E) asters .

1.3 MT aster formation

Typically, asters are nucleated by MTOC such as centrosome in the animal cells. Centrosome duplicates prior to cell division at the inter-phase, forms a bipolar spindle and eventually segregates into daughter cells each. During fertilization in vertebrates, a centrosome is contributed by the sperm that results in formation of functional MT aster (Figure 1.1(A)) in the embryos as the oocytes lack centrosomes. In addition to the vertebrate oocytes, cells such as plant cells and mouse embryos lack a bonafide centrosome. In the absence of template based nucleation i.e. centrosomes during meiotic divisions in the oocytes proteins of pericentriolar material such as γ -tubulin, pericentrin and other PCM proteins function as MTOC that form MT asters (Figure 1.1(B)). Recently, self-assembly property of the PCM proteins such as PLK4, SPD-5 and γ -tubulin have been identified and the assembled PCM observed to function as MTOC [54, 55, 56, 57]. In addition, organelles like nucleus, golgi and plasma membrane can also function as MTOCs and result in the emergence of radial arrays of MTs [1]. The chromatin surface in *in-vitro* and chromosomes in cells are known to nucleate and organize MTs during the metaphase stage of the cell cycle, which is driven by the biochemical gradient, RanGTP originating from the chromatin [58, 59, 40] (Figure 1.1(C)). Multiple *in-vitro* reconstitution studies have demonstrated formation of star shaped MT structures by organization of MT tubulin and filaments by the activity of motors and cross-linker motor proteins that focus the MT ends resulting in a radial arrangement of MTs [3, 4, 60] (Figure 1.1(D)).

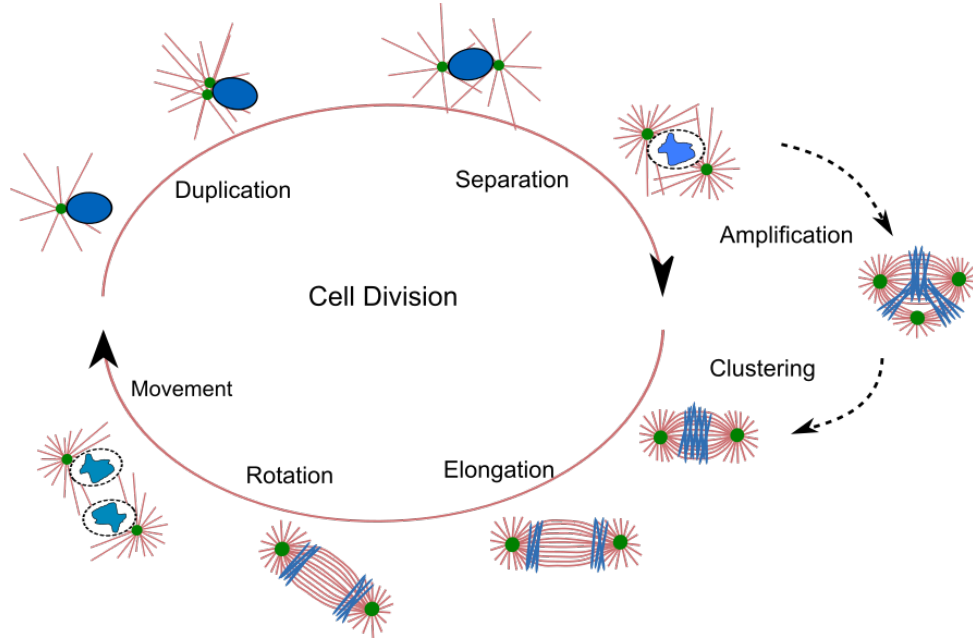


Figure 1.2: Schematic representation of aster dynamics during somatic cell division. In a typical somatic cell, the aster number, size and position change as the cell-cycle progresses. An aster exhibits different types of movement such as separation, centering, sliding, elongation and rotation during the process of spindle assembly and cell division. The number of asters also change during duplication, amplification and fusion as the cell cycle progresses. (Green: MTOC, Red: MT filaments, Blue: chromatin region)

1.4 Self-organization of MTs and motors

The spontaneous emergence of spatial MT patterns (i.e. ordered state) from a homogeneous mix (i.e. disordered state) of tubulin and motors in the presence of ATP and GTP is a self-organized process. Multiple patterns such as asters, vortices, bundles and networks emerged in *in vitro* experiments at varying density of motors and the size of chambers [3]. Based on the relative motor and MT concentrations and kinetic properties the different patterns as observed in micro-chambers were recapitulated in simulations [4, 61, 62]. The geometry and size of the system resulted in formation of different patterns [63] and asters [60] in confined droplets. The *ex vivo* studies resulted in emergence of contractile networks [64] and dynamic system

of rotating asters [65]. Tubulin and motors can self-organize into various spatial patterns that have implications in spindle assembly [66] and positioning [67, 68]. These aster-like shaped or radial structures lack a bonafide MTOC as they are formed by focusing and cross-linking activity of motor complexes and cross-linker proteins. Thus, MT and motors can be treated as active particles and the local interactions between them result in the emergence of global patterns without any additional information in the system. This is an out-of-equilibrium process that requires constant source of energy as an input in the form of ATP molecules. Thus, it is hypothesized that acentrosomal MT asters in the *in-vivo* systems can emerge from the self-organization of tubulin pool and motors in the cell [10]. Further, acentrosomal spindle assembly is an example of self-organized pattern formation in the system wherein multiple asters organize into bipolar structures.

1.5 Spatio-temporal dynamics of aster position and number

The position, number, MT nucleation potential and MT dynamics varies across cell cycle and cell type. The aster movement and inter-aster interactions dictate their number and position in the cell. In a typical cell, an aster is generally found at the center of the cell close to the nucleus and is responsible for maintaining the MT cytoskeleton that spans the entire cellular space (Figure 1.2). During, cell division the centrosome duplicates into two, that separate and move apart driven by the motor activity to occupy the diametrically opposite position as the cell cycle progresses. The two asters assemble into a bipolar spindle which is responsible for chromosome segregation into the two daughter cells. Asters undergo clustering and fusion in the case of aster amplification, failing which results in cell division defects. The bipolar spindle elongates during anaphase and it's position in the cell determines the plane of division whether the division is symmetric or asymmetric.

In some cells, spindles exhibit oscillatory movements due to pulling of astral MTs by cortical dyneins. On completion of the cell cycle, the two asters segregate into daughter cells, where each aster occupies a central position. All these movements are driven by MT forces, motor and MAP activity.

1.5.1 Aster motility

The aster position and number is coordinated in space and time due to the aster movement and inter aster interactions in the cell which is mediated by the activity of molecular motors and MAP proteins. However, an aster experiences a tug-of-war like scenario due to its radial geometry that results in random motility of the structure (Figure 1.3). The tug-of-war is depicted for a simplified geometry of an aster in 1D (Figure 1.3(A)). The stochastic binding and unbinding of motors from a MT result in transient force asymmetry that translates the movement of aster in a direction of more bound motors (Figure 1.3(B)). In addition, the stochastic fluctuations in MT lengths (MT dynamic instability) breaks the symmetry resulting in movement, in the direction of longer MT filament (Figure 1.3(B)). However, since these are fluctuations they result in only spontaneous symmetry breaking that are transient, and on an average result in no net movement of the aster.

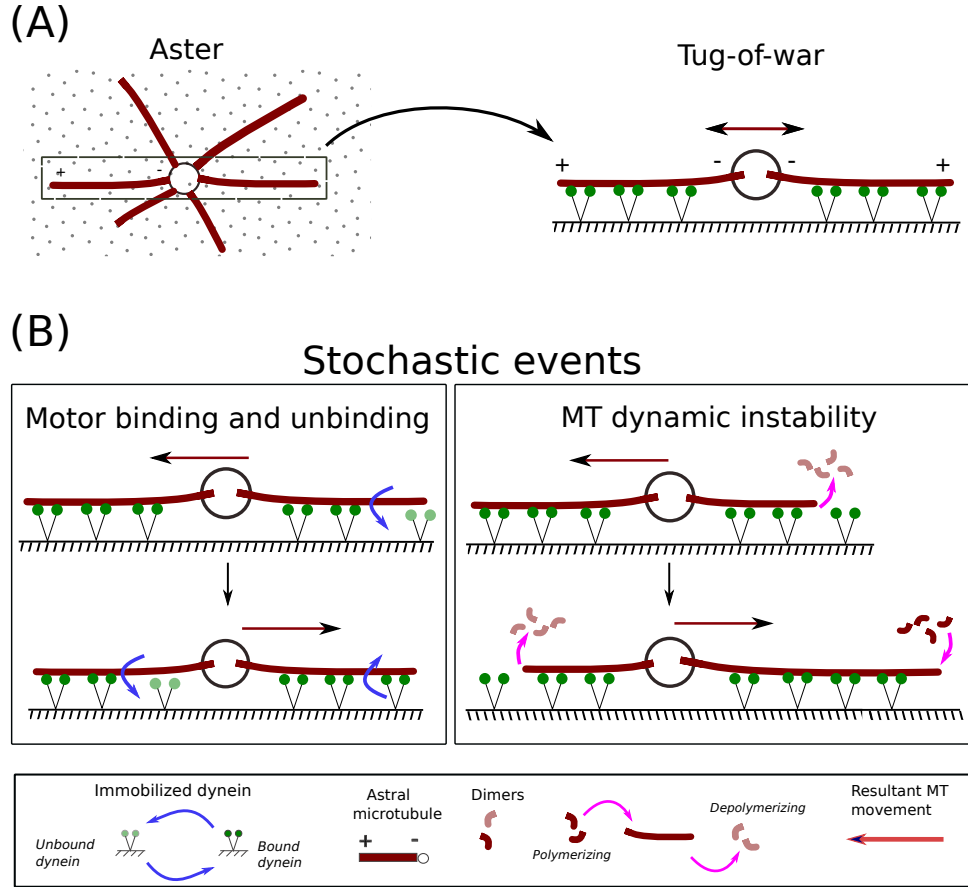


Figure 1.3: Schematic representation of tug-of-war in asters. (A) Representation of a radial aster gliding on a sheet of single type of motors (dynein, here). The geometry is simplified to a 1D geometry of anti-parallel MTs in the presence of uniform distribution of motors and MT lengths. (B) The stochastic events in binding and unbinding kinetics of motors results in uneven number of motors bound to each MT thereby translating into movement in a given direction (red arrows). The directions switch randomly based on the net force experienced and thus the net movement on an average is negligible. While, the fluctuations in MT lengths due to the MT dynamic instability results in more motors being bound to longer MTs and thereby more force and thus the movement in that direction. Since, the process is stochastic the MT lengths on either side fluctuate resulting in no net movement on an average.

Multiple mechanisms have been observed to center and decenter the aster in *in-vivo* and in *in-vitro* for different cell or chamber size. It is hypothesized that mechanisms vary based on the size of the cell and aster [7]. The most commonly observed mechanisms are summarized in a schematic (Figure 1.4) and described briefly. In

smaller cells, pushing forces generated by polymerization of MTs at the cell boundary result in aster centering [5, 9] (Figure 1.4(A)). The dynamic instability of MTs was shown to improve the centering ability of such *in vitro* asters [69]. In some interphasic cells, dynein pulling from the cortex centers the centrosome [70] while in cells such as budding yeast and *C. elegans* it is associated with spindle positioning by pulling the astral MTs [71, 72, 67, 6, 73]. In cells such as *C. elegans*, MT-length based pulling by immobilized dynein motors was observed in the centering movement of the male pronucleus at post-fertilization [74, 75]. Subsequent studies demonstrated the force generated by cytoplasmic streaming due to cargo transport [76]. Such streaming based centrosome centering was used in a theoretical study to explain the movement of the *Xenopus* sperm aster in meiosis II [77]. In larger cells like *Starfish* oocytes, acto-myosin contractility has been implicated in chromosome congression and spindle assembly [78]. In some cells, biochemical gradients modulate the MT dynamics spatially that result in length anisotropy in MT asters. Such asymmetry in length, translates into net movement of the aster in the direction of longer MT filaments. Observed length asymmetry was positively correlated to the distance of the MT aster from chromatin suggesting the existence of long range guidance cue in the meiotic extracts [58, 79, 40, 14]. The MT stabilization gradient of RanGTP originates from chromatin results in nucleation and MT stabilization by regulating MT polymerization [80, 79, 40, 81] via a downstream effector kinase Cdk11 [82] and MT nucleation through Tpx2 [83] and augmin [84]. Further, this was confirmed in simulations wherein a gradient of catastrophe frequency resulted in capture of all the chromosomes in time scales comparable to the experiments by reducing the search time [85]. Also, gradients of catastrophe and rescue frequency resulted in a two-fold length asymmetry (5-10 μm) in asters gliding on a sheet of dynein motors in presence of spatial gradient that translated into directional transport of asters towards the chromosomes [15].

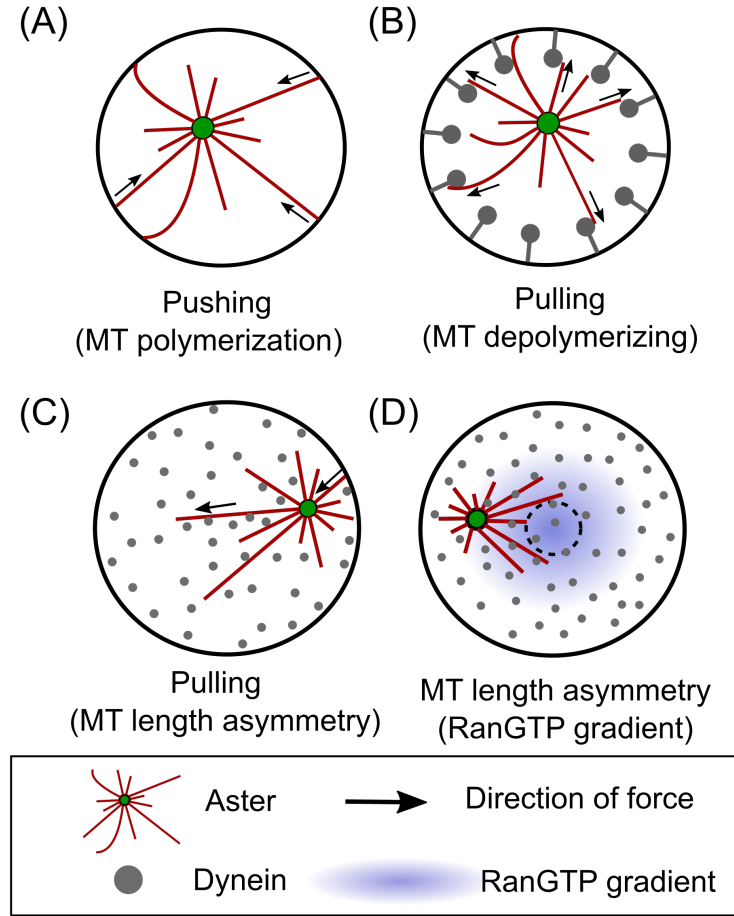


Figure 1.4: Schematic representation of mechanism of aster motility. (A) Polymerizing MTs experience opposing forces at the cortex that pushes the aster inwards. (B) Dynein at the cortex of the cell exerts pulling forces on the depolymerizing MTs while walking towards the minus-end of the aster. (C) Aster is pulled into the cytoplasm in the direction of longer MTs. Dynein motors are uniformly present in the cytoplasm and are immobilized. As dynein steps towards the minus end of the MT, the filament moves in a direction opposite to that of the motor. (D) The MT length asymmetry in the cytoplasmic aster can arise from the biochemical gradients that originate from the chromatin region.

1.5.2 Inter-aster interactions

In few systems, interaction between a pair of asters has been studied both experimentally and theoretically. Primarily, aster separation after duplication in inter-phase,

aster segregation in anaphase and fusion of asters during fertilization. The interaction between the astral MTs transition from being non-penetrative to inter-digitating during the cell cycle, speculated to be dictated by the regulatory proteins [8]. In simulations, two asters could fuse or separate based on the type of homo-tetrameric complexes while, a hetero-tetrameric complex resulted in stable anti-parallel overlaps in the absence of MAPs [62]. However, till date there is no evidence of hetero-tetrameric complexes in the *in-vivo* systems. Multiple studies have demonstrated dyneins capable of functioning like homo-tetrameric complexes, binding to two MTs simultaneously. Dynein complexes have been reported to be involved in fusion of spindles [86], cross-linking of MTs [87], MT-MT sliding [88] and MT focusing [89, 90]. While, kinesin-5 is a homo-tetrameric motor known to slide apart the anti-parallel MTs involved in centrosome separation, maintenance of spindle length and positioning of centrosomes in mitotic and interphasic cells [91, 92, 93]. Such motor mediated interactions between the astral MTs are depicted in the schematic (Figure 1.5). In cells, the activity of antagonistic motors and cross-linking motor proteins have been reported to form stable overlaps, or result in either fusion or separation of MTs and asters. Stabilized linear MTs were seen to be organized into asters that transitioned to a contractile network driven by the activity of dynein complexes [64]. In mammalian cells, the activity of kinesin-5 complexes and dimeric dyneins were shown to be responsible for centrosome separation [94]. However in some cell lines, kinesin-5 inhibited cells formed both bipolar and monopolar spindles, which was predicted to be dependent on the initial separation distance between the centrosomes in simulations [95]. Additionally, the relative concentration of the antagonistic motors could rescue the spindle morphology when dynein was depleted in the monastrol treated cells [94]. However, the time lapse studies indicate that coordination between the antagonistic motors is not a simple ‘push-pull’ mechanism as reported previously [96]. In addition, non-motor microtubule associated proteins such as Ase1, PRC1 and MAP65 are known to bundle MTs in the midzone of the spindles wherein they are involved in sliding of antiparallel MTs and have been reported to resist the

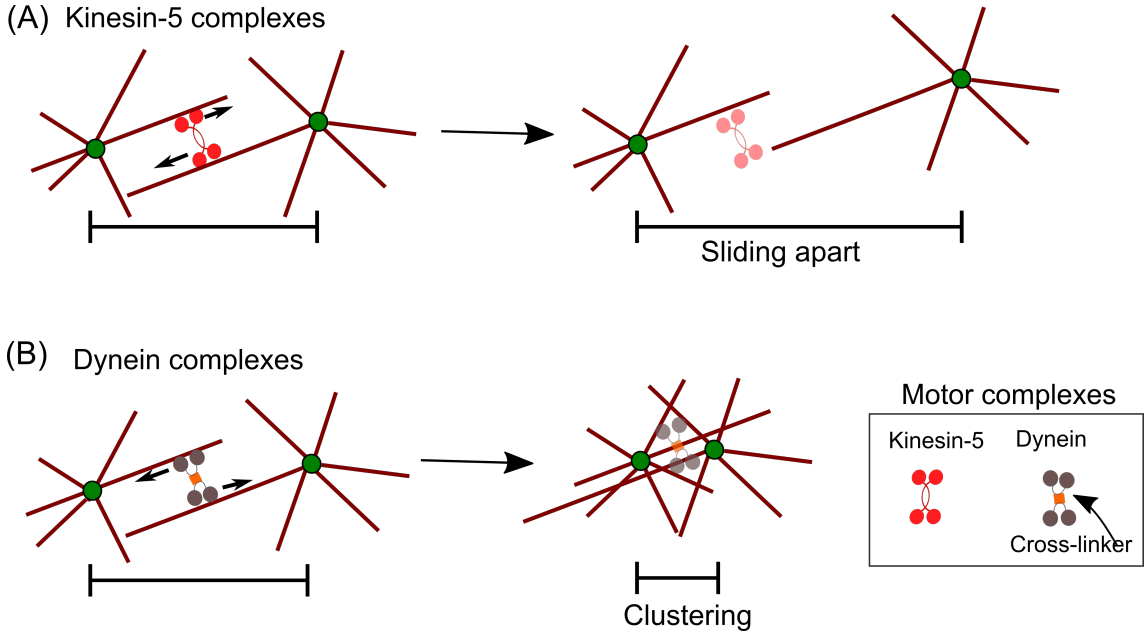


Figure 1.5: Motor mediated inter-aster interactions.(A) Tetrameric kinesin-5 complexes when simultaneously bound to two antiparallel MTs result in their separation. (B) Dimeric dyneins can be cross-linked via proteins such as NuMA forming a complex that when bound to two different MTs result in coalescence or focusing of the MTs [89].

kinesin-5 driven sliding apart of the MTs [53, 97, 98, 99]. A recent *in vitro* reconstitution study demonstrated a combination of cross-linker proteins, motor proteins and regulation of MT dynamics to result in formation of stable anti-parallel overlaps similar to that of spindle midzone [100], while self-organization of MTs into spindle like structures in the presence of only MT cross-linkers in the absence of motors has also been reported [101]. Taken together, it suggests that interaction between a pair of asters at different cell-cycle stage or in the *in vitro* reconstitutions in presence of antagonistic motors is not yet well understood.

1.5.3 Other roles of MTOCs and asters

The centrosome imparts structural organization and stability to the radial arrays by nucleation, anchorage and capping of the MT minus end in the pericentrosomal

matrix (PCM) [27, 28]. In addition it regulates the number of non-centrosomal MTOCs in the cell [102], number of centrosome cycles, the location of MTOC activity elsewhere in the cell [1] and the activity state of aster [103, 104]. The MTOC size and state effects the MT nucleation capacity that influence aster and thus the spindle length [105], compartmentalization of the space by sequestration of proteins [106] and organelles by MT network [107]. The MTOC can also control the recruitment of motor proteins, their activation and the dynamics of MTs emanating from it differentially based on their age [108]. In addition, MTOC is observed to nucleate actin along with MTs that forms a physical barrier thereby regulating the number of MTs at the MTOC in a cell cycle dependent manner [109, 110]. The recent findings suggests MTOC affects the functioning of an aster in the cell.

1.6 Multiple microtubule asters

Multiple asters exist in many systems such as syncytial cells, muscle cells, meiotic oocytes and cancerous cells. The number of asters can range from a few pairs to about 20 - 60 in the *in-vivo* scenarios. Supernumerary asters and multi-polar spindles in cancerous and non-cancerous cell lines have led to identification of motor and cross-linking proteins responsible for clustering of asters [111]. However, the mechanical and biochemical basis that maintains their spacing and distribution in normal cells is not clearly understood.

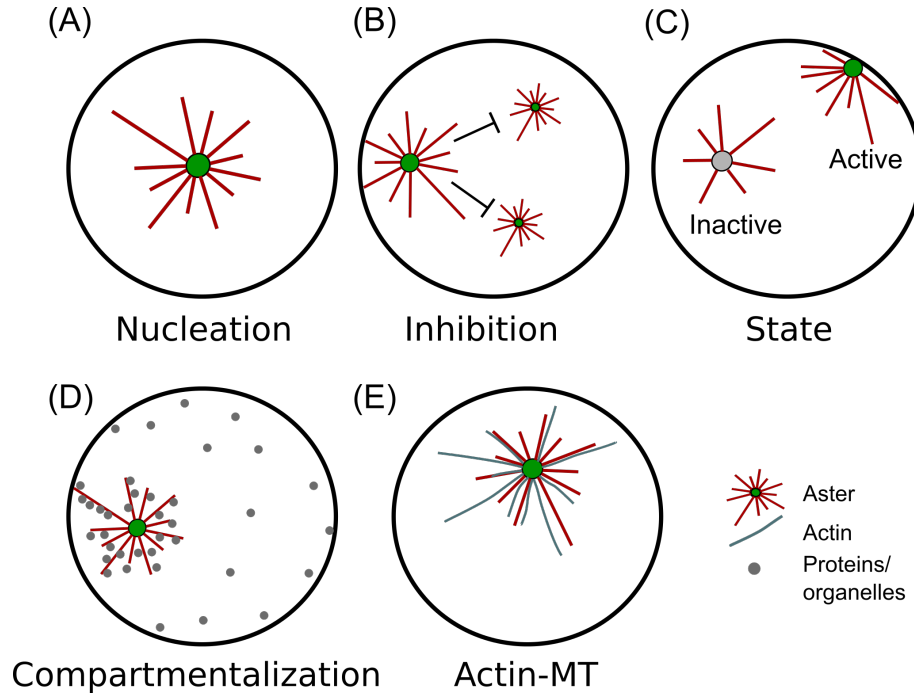


Figure 1.6: Illustration of the non-canonical roles of MTOC-nucleated asters. The MTOC in MT asters are responsible for (A) nucleation of MTs and organization of the MT cytoskeleton. (B) It inhibits the formation of non-centrosomal asters in the cytoplasm thereby controlling the aster numbers. (C) Can switch between the activity states and confer the MTOC functionality in the cell at some other location such as plasma membrane, golgi and nuclear envelope. (D) MT aster can result in compartmentalization of sub-cellular components that are MT-associated, the degree of which depends upon the symmetry of the aster and its nucleation ability. (E) MTOC also nucleate actin filaments along with the MTs and the proportion of the two depends on the biochemical state of the cytoplasm.

In *Drosophila* embryos, aster associated nuclei exhibit dynamic spatial ordering during the first 13 cycles of cell division which is rapid and occur in the common cytoplasm in the absence of cell boundaries [112]. The *in-vitro* experiments could recapitulate the spatial positioning between the nuclei and spindles, and showed aster migration to be responsible for the observed spacing [113]. The repulsive forces between the nuclei was shown to be essential in a phenomenological model [114], while recently kinesin-5 was identified as the force generator in the interphasic embryo for the maintenance of the order [93]. In simulations, MT dynamics and reducing

numbers of kinesin-5 motors for increasing number of nuclei due to doubling recapitulated the experimental observation of increasing nuclear order with successive cell divisions [115]. Likewise, in muscle cells of the fly, positioning of aster associated nuclei was demonstrated to be driven primarily by the repulsive forces generated by the polymerizing astral MTs against the neighbouring nuclei [116]. Collectively, the studies from *Drosophila* suggest a combination of factors such as motor types and their numbers, and MT dynamics dictate the aster associated nuclear ordering in the cells of length scales in millimetre range (embryos). While MT dynamics alone seems to drive positioning of multiple nuclei in the muscle cells which are in micron range [116].

Multiple non-centrosomal cytoplasmic asters (or cytasters) are induced in the ooplasm of meiotic eggs and cell lines on treatment with chemical agents such as taxol or hexylene glycol and physical perturbations such as alteration in pH and osmolarity conditions [117, 118, 119, 120, 121]. The emergent structures exhibit a spatial patterns in the oocytes, however the exact biochemistry and the mechanics that result in their formation is yet to be explored. While in most of the vertebrate oocytes that lack centrosomes, multiple *de novo* aster like structures emerge in the ooplasm that self organize to form a functional bipolar spindle [122, 123]. Such acentrosomal spindle assembly is observed in the oocytes of mouse, *Drosophila* and *Xenopus*. The experiments suggest RanGTP gradient to be essential for meiosis I in *Xenopus* [11] and *Drosophila* [124] oocytes and not so in the mouse oocytes [10]. However, meiosis II in mouse fails to progress on disruption of RanGTP gradient [11]. The mechanism that governs the nucleation, motility and interaction of these multiple asters is not well known. Studies have addressed how the multiple asters interact with chromosomes to form a bipolar structure [10, 125], however their nucleation, motility and interactions prior to the chromosome individualization is not well explored.

1.7 Open questions

The aim of this study is to investigate the motor-microtubule properties that result in collective multi-aster behaviour in confinement. Specifically, whether the mechanisms that resolve the single aster tug-of-war can be extended to a multi-aster scenario? Can a system of multiple asters self organize to form spatial-temporal patterns? How do multiple asters emerge in the cell? What regulates their nucleation, position and numbers in the cell? How do the multiple asters find their target and coordinate their movement in the cell? How is the distribution of sub-cellular structures maintained in such a multi-aster system? What are the motor and MT mechanics that effect the aster motility and inter aster interactions in a system?

Experimentally, it is quite challenging to track all the asters during live imaging in space and time during a process which limits the data available for the quantitative analysis. The perturbations either chemical such as drug inhibitions or physical such as laser ablations both have limitations as multiple treatments can effect the physiology of the process. The genetic perturbations are often commonly used in some model systems such as yeast and worms, however the complexity in terms of redundant pathways make the interpretation difficult. Additionally, microtubules and motors are often associated with proteins that either act as cofactors or the signalling pathways, both of which effect their state of activity and dynamics. The complexity of the system limits the quantitative analysis and the mechanical deductions is difficult. The *in vitro* and *ex-vivo* reconstitution studies address most of these concerns and provide insights on the mechanisms and effects of individual components in a precisely controlled system. Moreover, the multi-aster systems are fewer and much of the MT-motor behaviours has been inferred from cancerous cell lines and tumour samples or the large meiotic oocytes such as sea-urchin where physical and mechanical perturbations are easier. However, interactions of the parts in an *in vivo* scenario is greater than the sum of it's parts. Hence, theoretical and

modelling approaches provide a framework to test the role of every individual component and the principles of the phenomena that emerge in the cellular system. In this study, a computational and mathematical modelling approach was adopted to gain insights on the mechanics of the systems. The experimental data available or the experiments performed in collaboration are quantified and used to validate the simulation models or the proposed mechanisms. The complexity of the model was determined based on the resolution and precision of the experimental system and the objective of the study.

1.8 Simulation methods

The motor driven motility of single asters and filaments has been extensively studied in the *in silico* systems that span nano-meters to milli-meters in *in vivo* and *ex vivo* systems in space, and over seconds to minutes in time. The question under study and specifics on the spatiotemporal scales of the process under study, the level of model abstraction and system size determines the choice of modelling approach reviewed [126]. Analytical models, numerical simulations and agent based models span across methods of mean field, hydrodynamics, Langevin dynamics and kinetic approaches. The studies discussed in this thesis deals with aster motility, positioning and the cellular patterns at micron scales over minutes, which are driven due to the molecular motor activity in the presence of thermal and viscous forces. Thus the two types of modelling approaches adopted have been discussed here in the following sections:(a) phenomenological models and (b) discrete agent-based models.

1.8.1 Phenomenological model

The phenomenological models of active matter that treat particles as agents and simulate their movements without explicitly invoking any molecular details. Such class of phenomenological models are commonly referred to as Viscek type or active

particle models [127]. Such approach has been used to model the movement of asters in 2D off-lattice space. Aster is considered as a point particle, confined in a reflecting circular geometry to exhibit random walk in spatially varying drift fields. Motors and the interaction between the particles is not considered. The details of the model is discussed in Chapter 2 and Appendix D.

1.8.2 Discrete agent-based models

In order to explicitly simulate the molecular players such as motors and tubulin an agent based approach is adopted. One such simulation package of Langevin dynamics of particles is Cytosim, implemented in C++. Cytoskeletal elements such as MTs and motors can be explicitly modelled in a viscous environment. The thermal forces are also considered along with motor and filament dynamics. Filament behaviours such as bending of MTs, dynamic instability and motor behaviours such as diffusion, attachment, stepping and detachment can be modelled explicitly [16]. The experimentally measured single molecule properties of motors, tubulin and filaments can be considered and their emergent behaviours modelled. This tool has been extensively used for modelling of single aster [14, 15, 9] or a pair of asters [62, 128], MT gliding [129, 130] and spindle dynamics [67, 131, 132]. Since, the interest here was to explore the dynamics of multiple motors in multi-aster scenarios in confinement, cytosim was chosen as the platform to test the role of motors, their single motor properties and behaviour of MT dynamics explicitly. However, recently it has also been employed to model multi-aster systems such as muscle cells [116], fungi [133] and spindle assembly [125]. Modifications with respect to the MT and motor behaviour and localization was made and has been discussed in the respective chapters. The simulation objects in this thesis are microtubules, asters and motors and the general behaviour as implemented in Cytosim is briefly discussed. The scale of emergent MT behaviour in this study is in the range of microns and seconds which arises due to the motor interactions and thus such a coarse-grained models are a

reasonable assumption.

Microtubules are group of points, while an aster comprise of a solid at the center from which fibers emanate and nucleus is a spherical object. The motion of all the objects (which is group of points) is based on the over-damped langevin dynamics, wherein for every point the following is calculated at every iteration:

$$\delta x = \mu \cdot F(x, t) \cdot \delta t + \delta B(t) \quad (1.1)$$

where, F corresponds to all the forces acting on the object, B represents the Brownian force, μ the mobility coefficient calculated for the object under consideration at the time δt . Taken, together δx results in the instantaneous displacement at the time t [16].

Microtubules: MTs are modelled as semi-flexible fibers formed by basic units that correspond to tubulin dimers. MTs undergo probabilistic polymerization and depolymerization events that results in change to their lengths. They can also bend due to the thermal fluctuations, activity of motors or when encounter a rigid surface based on the flexural rigidity of the filament (κ). MTs experience pushing forces when the polymerizing MTs hit the rigid cell boundary (Equation 1.2). In cells MT filaments are observed to overlap and intersect with each other forming a mesh like network. MTs are modelled to be non-interacting filaments and steric interactions have been ignored in this study. In simulations, interactions of MTs are not explicit and can occur only via the activity of motor complexes. The scale of emergent MT behaviours in this study is in the range of microns and seconds which arises due to the motor interactions, thus such coarse-grained models are a reasonable assumption for the current study. Thus, in the absence of motors MTs exhibit diffusive movements based on the thermal ($K_B \cdot T$: Boltzmann constant and temperature) and viscous forces (η viscosity of the system and drag coefficient of the object under

consideration). The MT dynamic instability is modelled based on 2 state-4 parameter formalism provided by Verde et. al. [38] which depends on the velocities of MT growth (v_g) and shrinkage (v_s), transition frequencies of catastrophe (f_c) and rescue (f_r). The transition rates are determined stochastically for every filament at every iteration by implicit integration scheme.

$$F_{MT} = \frac{\pi^2 \cdot \kappa}{L^2} \quad (1.2)$$

where L is the filament length and κ is the flexural rigidity of microtubules and F_{MT} is the restoring force experienced by the MTs.

Motors: Motors are modelled as springs with a head and a tail. A dimer comprises of a single head while a tetramer comprises of two. Motors stochastically bind to the MTs if within the binding range with a probabilistic binding rate. Typically, motors assumed to step on the MT that follows a linear force-velocity relationship (Equation 1.3).

$$v = v_0 \cdot (1 - f_{ex}/f_s) \quad (1.3)$$

wherein, v is the instantaneous velocity of the motor, v_o is the unloaded single motor velocity, f_{ex} is the extension force parallel to the MT direction, while f_s is the single motor stall force.

While, the detachment from the MT is dependent on the force. The detachment of the motor is stochastically determined based on the kramer's law as in (Equation 1.4).

$$r = r_0 \cdot \exp^{-|f_{ex}|/f_d} \quad (1.4)$$

wherein, r is the instantaneous detachment rate of the motor, r_o is the unloaded single motor detachment rate, f is the magnitude of the extension force on the motor while f_d is the detachment force.

1.9 Specific aims

In this study the parameter space of the confined multi-aster system is explored along the following axes: (i) role of different motor types and their spatial distribution in the cell, (ii) role of MT dynamics and MT geometry and (ii) effect of cell and aster size as depicted in the Figure 1.7. The thesis summarizes the key findings from the study and discusses them under the following sections: (i) mechanism of multi aster centering, (ii) mechanism of ordered multi-aster positioning, (iii) emergence of collective rotational movement and (iv) effect of motor numbers and detachment mechanics on the collective MT transport.

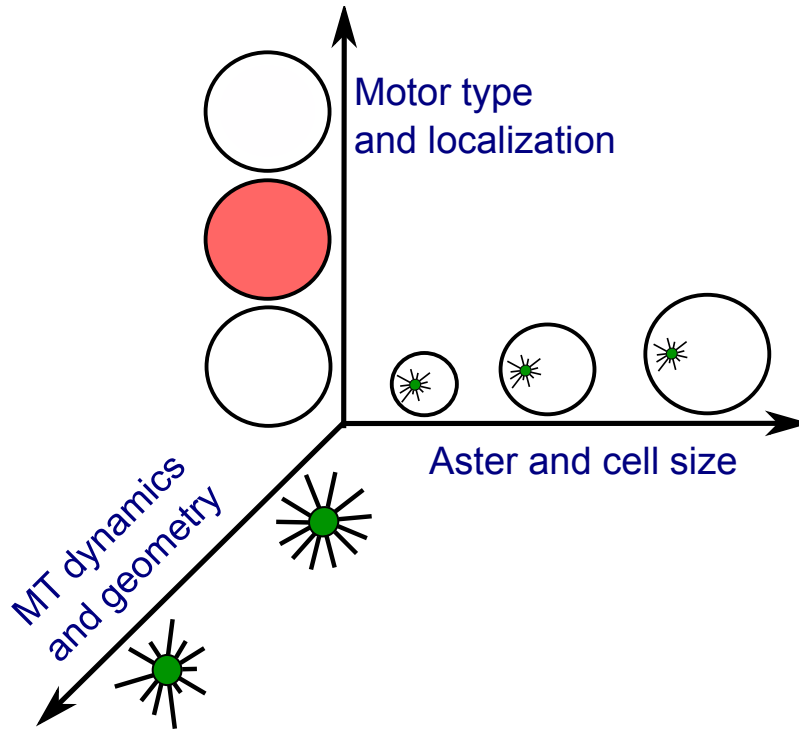


Figure 1.7: Exploration of multi-aster phase plane in simulations. The collective behaviour of multi-aster system was explored for the varying properties such as (x-axis) effect of aster and cell size, (y-axis) role of MT dynamics, astral MT distribution and MT geometry and (z-axis) motor types, their localization and numbers.

Chapter 2

A motor-gradient and clustering model of the centripetal motility of multiple asters in meiosis I of Mouse oocytes

Attributions. Primary aster trajectory data: Dr. Melina Schuh [[10](#)]. Parts of this chapter have been published as *Khetan, N, Athale, CA (2016). A Motor-Gradient and Clustering Model of the Centripetal Motility of MTOCs in Meiosis I of Mouse Oocytes. PLoS Comput. Biol., 12, 10:e1005102 [134]*.

2.1 Introduction

Spindle assembly in higher eukaryotic cells involves organization of microtubules (MT) into a bipolar structure. During mitosis in animal cells, spindle poles are defined by a pair of centrosomes from which MTs nucleate. However bipolar structures emerge even in the absence of centrosomes during meiosis in vertebrates as well as mitosis in plants. In such acentrosomal spindles, the poles self-organize by the dynamic interactions of MTs with molecular motors, regulatory factors and chromatin resulting in a self-organized spindle. While multiple components of this cellular-scale pattern forming system have been identified, the precise nature of the interactions between the components are still not completely understood.

The meiotic maturation of mouse oocytes is a well studied example of such an acentrosomal spindle assembly system. The first meiotic division is characterized by germinal vesicle breakdown (GVBD) [135], before and after which small aster-like fibrillar structures are observed [136] that function as microtubule organizing center (MTOC) [10]. Asters which are nucleated both in the cytoplasmic and peri-nuclear spaces, both aggregate at the center to form a spindle by prometaphase I [137]. Such a convergence of radial MT arrays or asters was reported previously in *Xenopus* meiosis II oocytes [138]. Using cell-free *Xenopus* oocyte extracts, this convergence was shown to result from asymmetric centrosomal MT growth due to a gradient of RanGTP [79, 40, 14]- referred to as biased ‘search-and-capture’. However during meiosis I in mouse oocytes, experimental perturbation of RanGTP levels does not significantly affect spindle assembly [11, 10]. If RanGTP does not act as a guidance cue as reported previously [13], the nature of the directional cue and force generation remains to be understood.

The force required for convergence of multiple asters to the nuclear region is thought to originate from a combination of MTs, motors and anchorage points. Multiple mechanisms have been reported in the past to drive radial MT array transport in cells- (a) polymerization dependent pushing forces as seen during the centering of

asters *in vitro* [5, 139], (b) cortical force-generator based pulling [72], (c) cortical motors which both depolymerize and pull [67], (d) cytoplasmic minus-ended motors which pull asters in a length-dependent manner [74], (e) cytoplasmic streaming by cargo transport driving aster movement [76, 77] and (f) acto-myosin contractility as seen in starfish oocytes [78]. Contact with the cell cortex can move asters when the relative length of MTs is comparable to that of the cell radius [8]. Both active and passive mechanisms drive the movement of centrosome nucleated asters. However most of the cortical pushing and pulling models are unlikely to affect long-range movement of asters which have MT lengths $\sim 3 \mu m$ as compared to the cell-radius of $\sim 40 \mu m$. Transport of asters by cytoplasmic streaming based on cargo transport by one large aster [77], is also unlikely to drive mouse meiosis I oocyte asters due to their size and number (~ 80 to 100), which will prevent a coherent and directed flow. Inhibition of acto-myosin contractility has also been shown to have no effect on the centripetal movement of mouse asters [10]. While centrally-anchored asters and cross-linking motors have also been proposed by Schuh et al. [10] to drive the aster motility, the movement continues even after nuclear envelope breakdown (NEBD). Thus for a complete theoretical understanding of the mechanism by which asters converge in spindle assembly a mathematical model of the process is necessary to test multiple hypotheses that have been proposed.

Theoretical models have been used to probe the interactions of microtubules and motor complexes and are capable of reproducing *in vitro* self-organized patterns [3, 4, 61]. These simulations have been extended to understand the role of multiple components in spindle assembly such as antiparallel interactions [62], pole focussing by minus-end directed motors [90], gradients of stabilization [140] and intra-spindle nucleation and dynamic instability regulation [131]. Earlier in the lab, the biased movement of centrosomal MT asters towards the surface immobilized chromatin in *Xenopus* egg extracts was recapitulated by a gradient of polymerization dynamics and uniform motor distribution [15]. This is comparable to a model of length-dependent pulling by motors to translocate MT asters during *C. elegans*

embryogenesis [74] that results in a bias. However, neither of these models take into account the relatively shorter MTs seen in acentrosomal asters, and lack details specific to meiosis I. The theoretical modelling of the centripetal motility of small radial MT arrays may provide insights on the design principles of spindle assembly.

In this study, the spatial trends in aster motility was quantified and it suggests that the random and directional components of motility depend the position of aster from the cell center. The detailed quantitative analysis allowed to develop and test theoretical models of random walk with drift. Only a spatial gradient of drift could reproduce the experimental data. Such an optimized gradient was further used to model MT dynamic instability and motor distributions, to test the combination of mechanisms that can reproduce the experimental statistics of centripetal multi aster motility.

2.2 Models

The two kinds of models was developed- phenomenological and mechanistic to address both the general principles and specific mechanisms of the centripetal convergence of small MT asters. The models are:

1. 2D Random walk with drift (RWD) model
2. 2D MT-motor model

The outputs of both models were compared to 2D experimental motility measures of asters from mouse oocyte meiosis I reported previously by Schuh and Ellenberg [10]. The RWD model was used to optimize the functional form of the spatial drift field by comparison to experiment, while the MT-motor model was used to test molecular mechanisms which could generate the drift field. Mechanisms that have been previously hypothesized to drive asters to the center of cell involve MT-pushing

and pulling [141, 7]. A combination of pulling and pushing mechanisms has been experimentally tested in sand-dollar eggs [142] and *C. elegans* embryos [143, 76] and pulling alone in *Xenopus* egg extracts [79, 15], while pushing has been seen in fibroblasts [144]. The MT-motor model is used to test whether the reported self-organized clustering of asters (also referred to as MTOCs i.e. self-organized asters in the oocytes) in mouse oocytes [10] is sufficient to result in convergence of multiple asters, or whether additional mechanisms are necessary. These mechanisms are:

- (i) Self-organized mechanisms: not requiring explicit spatial localization
 - (a) Cortical pushing alone
 - (b) Clustering by dynein complexes.
- (ii) Spatial gradients: requiring explicit spatial localization
 - (a) MT dynamic instability gradient
 - (b) Dynein motor gradient
- (iii) Hybrid model: combination of self-organized and gradient mechanisms

An optimization routine was developed to compare the outputs of the ‘scenarios’, i.e. combinations of these mechanisms, to reproduce previous experimental reports of mouse meiotic MTOC motility and distribution [145, 10, 146].

The choice of a 2D model for a 3D spherical oocyte is determined by the need to compare simulated motility outputs with the only available experimental time-series dataset of MTOC/aster motility in mouse oocyte meiosis I, which is 2D over time [10] (Figure 2.1(a)). This compatibility of dimensions is essential since some of the RWD model input parameters are obtained from fits to experiment and MT-motor model mechanisms are optimized based on their ability to reproduce experimental statistics. Additionally, the choice of dimensionality of spatial models is considered to be determined by the balance between the need to capture the behaviour of the system sufficiently and the clarity of the model [147].

2.2.1 Random walk in a drift field

The mouse oocyte is modelled in a 2D circular geometry of radius r_{cell} , with concentric circular chromatin of radius r_{chr} . The outer cytoplasmic region has a radius r_{cyto} and $r_{cell} = r_{chr} + r_{cyto}$ (Figure 2.2(a)). Asters are modelled as point particles, nucleated uniformly in the cytoplasmic space of the oocyte. The motion of the simulated particles is a mixture of random Brownian and directed centripetal motion, depending on the position of the aster in the oocyte (Figure 2.2(a)), based on the previously observed ‘stop and go’ nature of the motility [10]. In this model, asters are transported to the cell center and ‘captured’ by the chromatin once they reach the central chromatin mass. The process resembles models of biased ‘search-and-capture’ used to describe spindle assembly [85, 14, 148]. This model was used to define the spatial properties of the bias of attraction by comparing the simulation outputs to spatial trends in aster motility seen in experiment.

Asters are modelled as non-interacting point particles in a 2D off-lattice space in a confined circular geometry and no explicit consideration of the mechanism of movement i.e. motors, viscous environment, particle interactions or temperature is considered. An explicit numerical method is implemented to simulate the movement of discrete particles in the system wherein at every time interval (δt), position of the particle is updated based on it’s position at the previous time point. The following scheme is implemented for ‘n’ iterations inferred from T (total simulation time) and time interval (δt) as, $T = n \cdot \delta t$ with T as 8000 s and δt as 0.1 s. The δt was optimized for the minimum deviation between the input diffusion coefficient and that obtained from the trajectories of particles in pure diffusive scenarios. Since the movement is modelled to be a combination of both diffusive and vectorial components, the motion during a time interval cannot be inferred from the velocity directly. The velocity of diffusing particle has no meaning, diffusion results in quadratic increase in distance with time. Thus, the aster trajectories from experiments were fit to Diffusion drift equation. The values of velocity ($v_{eff} = 0.008 \mu\text{m/s}$) and the effective diffusion

coefficient ($D_{eff} = 0.006 \mu\text{m}^2/\text{s}$) were estimated from the fits to Diffusion-drift model of the experimental tracks to determine the step size under diffusion and vectorial movement. The directed component of the step size is inferred from the constant velocity while the diffusive component computed from the diffusion (mean squared displacement) equation. The step size (δr) is discussed in the next section.

Step size: The Brownian component of the motion results from radial MTs in an aster interacting with motors in all directions resulting in uniform forces, which fluctuate due to thermal noise and microtubule dynamics [15]. The step size and the direction of motion of every particle is calculated at every time step based on the position of the particle at the previous time step. The step size (δr) comprises of random and directed components as in (Equation 2.1). The directed component of the step size is inferred from the constant velocity while the diffusive component computed from the diffusion (mean squared displacement) equation. The values of velocity (v_{eff}) and the effective diffusion coefficient (D_{eff}) were estimated from the fits to diffusion-drift model of the experimental tracks to determine the step size.

$$\delta r = \begin{cases} \delta r_{df} = \sqrt{4 \cdot D_{eff} \cdot \delta t}, & \text{Brownian component} \\ \delta r_{dr} = v_{eff} \cdot \delta t, & \text{Directed component} \end{cases} \quad (2.1)$$

where, δr_{df} and δr_{dr} correspond to magnitude of diffusive and directed step size, D_{eff} is the effective diffusion coefficient, v_{eff} is the effective velocity and δt is the integration time. The values of D_{eff} and v_{eff} are determined by fits to experimental data as described in the section on data analysis, the integration time was optimized and are listed in Table 2.1.

Direction: The net angle (θ_{net}) of motion comprises of diffusive (θ_{df}) and directed components (θ_{dr}), which governs the direction of motion and is determined by:

$$\theta = \begin{cases} \theta_{df} = \text{rand}(0, 2\pi), & \text{Brownian component} \\ \theta_{dr} = f(\overrightarrow{XC}), & \text{Directed component} \end{cases} \quad (2.2)$$

where θ_{df} is the diffusive and θ_{dr} the directed angle. The angle θ_{df} can take random values uniformly distributed between 0 and 2π , while θ_{dr} is determined by the angle of the vector $\overrightarrow{XC}$ between the particle ($\vec{X}$) and the center ($\vec{C}$) of the cell (Figure 2.2(a)).

Drift field: Sub-cellular transport has been successfully modelled in previous work by combining Brownian motion and directed transport [149]. In our model, the Brownian and directed components are spatially determined. Here, two radially symmetric fields of drift, one repulsive and one attractive, were modelled to represent the effective forces acting on the asters. The attractive field resulting in centrosomal aster convergence in *Xenopus* oocytes towards chromatin [79], was modelled by a sigmoid gradient [14, 15]. A similar function was tested for the model of mouse oocytes. The net step size of a particle (δr_{net}) is considered as weighted average of random and directed components defined as ϕ_a and $(1 - \phi_a)$. Since the attractive ($\phi_a(\vec{X})$) and repulsive fields ($\phi_r(\vec{X})$) determine the nature of motion, depending on the position of the particle in space, the net step size of a particle (δr_{net}) is given by:

$$\delta r_{net} = \phi_a \cdot \delta r_{dr} + (1 - \phi_a) \cdot \delta r_c \quad (2.3)$$

where δr_c is the effective step size at the cell cortex determined by the weighted average of repulsion and Brownian motion. δr_c is determined by (Equation 2.4).

$$\delta r_c = \phi_r \cdot \delta r_{dr} + (1 - \phi_r) \cdot \delta r_{df} \quad (2.4)$$

The net angle is weighed according the drift fields as in Equation 2.5:

$$\theta_{net} = \arctan \frac{\phi_a \cdot \sin(\theta_{dr}) + (1 - \phi_a) \cdot \sin(\theta_{df})}{\phi_a \cdot \cos(\theta_{dr}) + (1 - \phi_a) \cdot \cos(\theta_{df})} \quad (2.5)$$

The position of the particle at time, t is updated in the cartesian coordinates as in Equation 2.6.

$$r(t) = \begin{cases} x(t) = x(t-1) + (\delta r_{net} \cdot \cos(\theta_{net})) \\ y(t) = y(t-1) + (\delta r_{net} \cdot \sin(\theta_{net})) \end{cases} \quad (2.6)$$

In this model, attraction originates from the central chromatin, since the removal of the nucleus in previous experiments resulted in asters losing their directionality of motion [10, 150]. The repulsion from the cell cortex, is based on models of MT-aster pushing at the cell cortex as a mechanism of centering in multiple cell types [141, 7]. Asters undergo Brownian motion when neither attraction nor repulsion are acting on them. The net angle $\theta_{net}(X)$ is the circular weighted average [151] of θ_{df} and $\theta_{dr}(\vec{XC})$, weighted similarly to the velocity magnitude (Equations 2.3, 2.4).

The fields of $\phi_a(\vec{X})$ and $\phi_r(\vec{X})$ are modelled in 2D by radially symmetric functions dependent solely on the distance r from chromatin. A modified sigmoid gradient was tested, based on previous experimental measurements of long-range gradients acting on MTs around chromosomes in *Xenopus* spindle assembly [79, 40, 14] and theoretical models testing their form [140, 131] and their role in centrosome directional motility [15].

The field (ϕ) of either attraction (ϕ_a) or repulsion (ϕ_r) is determined by:

$$\phi = \frac{1}{1 + e^{[(r-r_{1/2})/s]}} \quad (2.7)$$

where r is the distance- for ϕ_a , $r = 0$ at the chromatin edge and for ϕ_r , $r = 0$ at the cell boundary. $r_{1/2}$ is the distance at which the function takes the half-maximal value and $1/s$ is the steepness factor. While exponential gradients were also tested, they failed to reproduce the spatial trend in experimental data and are not shown here. Representative gradients of ϕ_a and ϕ_r are plotted as scaled values between 0 and 1 (Figure 2.2(b)).

Parameter	Value	Reference
Model geometry		
Oocyte radius (r_{cell})	40 μm	[10]
Chromatin radius (r_{chr})	10 μm	[10]
RWD model		
Total simulation time (T)	8000 s	[11, 10]
Step time (δt)	0.1 s	Optimized for numerical accuracy
No. of MTOCs (N_p)	100	[11, 10]
Effective diffusion coefficient (D_{eff})	0.006 $\mu\text{m}^2/\text{s}$	Fit to experiment (Figure A2A, A2B)
Effective velocity (v_{eff})	0.008 $\mu\text{m}/\text{s}$	Fit to experiment (Figure A2A, A2C)

Table 2.1: RWD model parameters. Parameters are used from literature and fits to experimental data.

2.2.2 Mechano-chemical model of MT-motor interactions

A mechanistically detailed model was developed using Cytosim [16], a C++ Langevin dynamics simulation engine, by building on previously developed models of MT-mechanics [67, 152], polymerization kinetics [140, 14] and motor interactions [3, 4, 61, 62, 90, 131, 129, 15]. To test what minimal components will produce the observed centripetal motility of asters, a model of an MT stabilization gradient described previously [15] was developed by mapping the gradient shape optimized in the RWD model. This scenario was compared with scenarios where the gradient consisted of immobilized minus-ended molecular motors. These biased ‘search-and-capture’ scenarios were contrasted with self-organized scenarios which lacked any directional

bias, i.e. diffusible motor-complexes and aster pushing from the cell boundary. The model is implemented in an oocyte cell geometry with MT polymerization dynamics and mechanics as well as discrete stochastic molecular motors that are either immobilized or diffusible.

Cell geometry: As before the oocyte was modelled in a 2D circular geometry with a concentric chromatin region. The chromatin region here is not treated as an absorber in this model, unlike in the RWD model. In RWD model, point particles on reaching the chromatin region are captured and their positions are not updated any further. While, in this model the positions of the asters are updated until the end of the simulations.

MT polymerization dynamics and mechanics: MTs were modelled as discrete polymers undergoing dynamic instability- stochastic switching between growth and shrinkage phases- based on the four-parameter model [38, 153]. Since dynamic instability parameters of meiosis I mouse oocytes are not reported, values were taken from measurements made on centrosomal MTs in mitotic pig kidney cells, which have average MT lengths of $3.2 \mu m$ [154], very similar to the average length of MTs in mouse meiotic asters/MTOCs ($\langle L \rangle \sim 3 \mu m$) [136, 145, 137]. The flexibility of the simulated MTs is defined by a combination of bending modulus (κ), typical cytoplasmic viscosity and thermal energy. The values of these parameters were taken from literature (Table 2.2), from the mouse oocytes or related systems, if mouse data was not available.

MTOCs: The self organized asters are also referred to as MTOC here in the mouse oocytes based on the earlier reports [117, 10]. MTOCs were modelled as hollow circular structures of radius $0.2 \mu m$, initialized randomly throughout the cytoplasmic region. Each MTOC had N_{MT} number of MTs uniformly distributed radially around the centrosome, forming an aster. MTOCs can move throughout the cell interior. Steric considerations are not included in the model and MTs can interact with each other only via the motor complexes.

Motor mechanics: Molecular motors were modelled as described previously [152, 15] as discrete particles with properties taken from minus-end directed dynein-like motors moving at a velocity v_m . Motors bind stochastically to MTs at an attachment rate r_{attach} only if the distance between them is less than a threshold distance of attachment (d_{attach}). Detachment occurs at a rate r_{detach} . Motors bound to MTs behave like Hookean springs with a spring constant (k_{mot}), experiencing a extension force (f_{ex}) parallel to the filament if attached to a motile filament. The rate of detachment increases with extension as: $r_{detach} = r'_{detach} \cdot e^{|f_{ex}|/f_0}$ (based on Kramers theory [155]), where r'_{detach} is the constant basal detachment rate and f_0 stall force. A separate r_{detach}^{end} rate accounts for the rate at which motors detach from the end of a filament- here it is set to a value similar to r'_{detach} . As f_{ex} increases to values approaching f_0 , motor step sizes are expected to change, referred to as the ‘gear-like’ behavior of dynein [156]. This was modelled by a piece-wise approximation, as previously described for dyneins in Athale CA. et. al, 2014. In this model the probability of forward stepping per unit time (p_{forw}) is modelled to be dependent on the load experienced by the motor along the direction of MT while bound (f_{ex}) and the force thresholds f_1 and f_2 as described in Equation 2.8 [15]. The parameters of motor mechanics, when available, are taken from experimental measurements of dynein (Table 2.2). In the absence of reported values estimates were used. Immobilized motors bind to microtubules and generate forces on the MTs causing their movement. Conversely, diffusible motor complexes with diffusion coefficient D_c , are modelled as crosslinkers [62], which can bind two different microtubules. Since aster have minus-ends at the center, cross-linking minus-end directed motility of the motors results in coalescence of the MTOCs.

$$p_{forw} = \begin{cases} 0.9 & \text{if } , f_{ex} < f_1 \\ e^{(-a \cdot (f_{ex} - f_1))} - p_{back} & \text{if } , f_1 \leq f_{ex} < f_2 \\ p_{back} & \text{if } , f_{ex} \geq f_2, \end{cases} \quad (2.8)$$

where the exponent $a = [\ln(100 \cdot \frac{p_{forw}}{p_{back}}) + 1] / (f_2 - f_1)$, $p_{forw} = 0.9$ under no load, $p_{back} = 0.1$, $f_1 = 5$ pN and $f_2 = 8$ pN.

Aster motility: MTOC/asters move due to a net force calculated by resolving multiple forces acting on the MTs (using a finite difference scheme to solve the Langevin equation of motion in Cytosim [152]) which are: (a) f_{bend} , arising due to bending of growing MT filaments contact the rigid cell boundary and scaled by the bending modulus of MTs (κ) [5, 139, 6]; (b) f_{ex}^i , the extension force generated when immobilized motors bound to MTs walk on the filament and are stretched by length x , experience a restoring force $f_{ex}^i = k_{mot} \cdot x$, resulting in filament motion [152]; (c) f_{ex}^c , the motor-complex of diffusible tetrameric motors have two binding sites separated by a spring of stiffness k_{mot} . Complexes that are simultaneously bound to two MTs, undergo stretching. The restoring spring force brings the two MTs close and results in clustering [3, 61, 62]; (d) f_{diff} , the diffusive force is a random normally distributed force [152]; and (e) f_{drag} , the drag force acting on the MTs resulting from the translational and rotational drag forces on the individual points representing the filament [152] based on the cytoplasmic viscosity (η).

Parameter	Value	Reference
Filament unit size	$0.5 \cdot 10^{-6} \text{ m}$	[62]
Time step	0.01 s	[62]
Total time	1200 s	[10]
Cytoplasmic viscosity (η)	$0.05 \text{ Pa} \cdot \text{s}$	[157]
Microtubule bending modulus (κ)	$2 \cdot 10^{-23} \text{ N} \cdot \text{m}^2$	[34]
Thermal energy scale ($k_B T$)	$4.1 \cdot 10^{-21} \text{ N} \cdot \text{m}$	
Aster Parameters		
Number of asters per oocyte	80	[10, 146]
Number of MTs per aster (N_{MT})	20-120	”, estimate(Figure A9)
Mean MT length ($\langle L \rangle$)	$3.2 \mu\text{m}$	[137, 10, 146]
MT polymerization dynamics		
Growth rate (v_g)	$0.178 \mu\text{m/s}$	[154]
Shrinkage rate (v_s)	$0.205 \mu\text{m/s}$	”
	<i>Cyto; Stab</i>	
Catastrophe frequency (f_{cat})	0.075; 0.0397 s^{-1}	”, estimate
Rescue frequency (f_{res})	0.023; 0.0122 s^{-1}	”, estimate
Motor parameters		
Motor stiffness (k_{mot})	0.1 pN/nm	[158]
Motor (dynein) speed (v_m)	$2 \mu\text{m/s}$	[43, 159, 160]
Attachment rate (r_{attach})	12 s^{-1}	[15]
Basal detachment rate (r'_{detach})	1.5 s^{-1}	[15]
Motor stall force (f_0)	2 and 7 pN	[156, 159, 160, 161]
Attach distance (d_{attach})	$0.02 \mu\text{m}$	[15]
End detach rate (r_{detach}^{end})	1 s^{-1}	[15]
Diffusion constant of motor-complexes (D_c)	$20 \mu\text{m}^2/\text{s}$	[62]
Immobilized motors per oocyte (N_m^i)	10^2 to 10^4	Estimate
Diffusible motor complexes per oocyte (N_m^c)	10^3 to 10^5	Estimate

Table 2.2: MT-motor model parameters. The motor, microtubule and aster properties used are from the reported values in literature or estimated in this study. Cyto: Cytoplasm and Stab: stabilized.

2.3 Methods

2.3.1 Data analysis

Experimental 2D trajectories of multiple asters in mouse oocytes meiosis I have been described previously by Schuh and Ellenberg [10] with $\Delta t \approx 3$ to 4 minutes ranging between pre- and post-NEBD stages (data kindly shared by M. Schuh). The trajectories were normalized by shifting the origin to the respective end-points. Thus, multiple experimental trajectories could be compared. For comparison with experiment, simulated trajectories were down-sampled to a time-interval comparable to experiment ($\Delta t = 3.5$ minutes), and the following measures of motility were evaluated from both experimental and simulated data:

Motility statistics: The instantaneous velocity $v = \delta L / \delta t$ and directionality or tortuosity (χ) was estimated as $\chi = d_{net} / L$, where δL is instantaneous distance, L is the total distance or path length, δt is the instantaneous time and d_{net} is the displacement. A value $\chi = 1$ indicates directional motion, while $\chi \sim 0$ indicates a random (tortuous) path. The time taken by asters from their time of nucleation to arrive at the edge of the central chromatin region is used to calculate a ‘capture time’ from both experiments and simulations. The mean square displacement (msd) of experimental and simulated trajectories was estimated by:

$$\langle \Delta r^2 \rangle = \langle [r(t) - r(t + \delta t)]^2 \rangle \quad (2.9)$$

where Δr is the displacement, t is the time point, δt is the time increment. A sliding window of δt from the smallest simulated time step to 3/4th of the trajectory length was used [15]. This empirically determined cut-off reduces artefacts arising from the fact that the number of steps of time-length greater than this threshold are extremely small [162, 163].

In order to estimate input parameters for the RWD model (Equation 2.1), the msd profiles of experimentally measured asters were fit to the model of diffusion and transport:

$$\langle \Delta r^2 \rangle = 4 \cdot D_{eff} \cdot \delta t + (v_{eff} \cdot \delta t)^2 \quad (2.10)$$

On the other hand, the msd output from the motor-MT interaction model (Models section) were quantified by fitting to an ‘anomalous diffusion’ model:

$$\langle \Delta r^2 \rangle = 4 \cdot D' \cdot \delta t^\alpha \quad (2.11)$$

to estimate the apparent diffusion coefficient (D') and the anomaly parameter (α) as described previously [15] (fits to experimental data discussed in Appendix A.1). All data fitting was performed using the trust region reflective least square fitting algorithm implemented in the MATLAB.

2.3.2 Simulations

The RWD simulation time steps were optimized for numerical stability, by simulating a normal random walk ($\alpha = 1$) with no boundary conditions and minimizing the error in input and fit diffusion coefficient (D). The integration time step was varied from 10^{-3} to 1 s with 100 particles nucleated at the cell centre in the absence of any bias. Simulations at the optimized integration time step were performed with 100 point particles randomly nucleated in the cell region outside the nucleus for $8 \cdot 10^3$ seconds. The input parameters and model is discussed in section 2.2.1. The explicit MT-motor model (Cytosim) with 80 asters was run for $1.2 \cdot 10^3$ seconds with integration time step of 0.01 s. The model and parameters used in the MT-motor simulations is discussed in section 2.2.2.

2.3.3 Model optimization to experiment

The parameters of the model were optimized by a rank minimization method based on a modified weighted root mean square error (ϵ) of simulations as compared to experiment. Only one published dataset by Schuh and Ellenberg [10], has reported detailed XY over time trajectories of MTOC centering motility during mouse oocyte maturation, as a result of which quantitative model optimization was performed on this dataset (kindly provided by M. Schuh). The error ($\epsilon(k)$) for a parameter set k was estimated between the i^{th} experimentally measured (e_i) and simulated (s_i) values by:

$$\epsilon(k) = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^n w_i \cdot (e_i - s_i(k))^2} \quad (2.12)$$

The weight (w_i) was determined based on whether the simulated value was within one σ of the average experimental measure or outside that range:

$$w_i = \begin{cases} n_i/n_{max} & s_i \forall (e_i \pm sd) \\ w_m & s_i \sim (e_i \pm sd) \end{cases} \quad (2.13)$$

where $w_m = 2$, n_i is the number of experimental data-points for the i^{th} value and n_{max} is the maximal number of data-points in experiment. This weighting scheme allows us account for the increased uncertainty in experimental measurements with fewer data points. The choice of w_m was set empirically to 2, (two-fold of the maximal value) to ensure a higher penalty (since $n_i/n_{max} \leq 1$). For each parameter set the ϵ of multiple variables (v) were ranked ($R_v(k)$). The directionality (χ) distribution with distance (r) and frequency distribution of capture times (t_c) were used to calculate errors. The sum rank, $R_s = \sum_v R_v(k)$ was minimized to obtain a parameter set, referred to as optimal.

2.4 Results

2.4.1 Spatial bias in the centripetal motility of aster tracks

The experimental aster trajectories exhibit a distinct centripetal motion as seen in the time-projected trajectories (Figure 2.1(a)). The movements farthest from the convergence point appear directed and random in the mid region with a decreasing degree of randomness on approaching the chromatin region. Most of the tracked asters nucleate at or around the time of nuclear envelope breakdown (NEBD), while the time of capture at the chromatin periphery varies across the asters (Figure 2.1(b)). The asters that nucleate 150 minutes after the NEBD were ignored for the further analysis to eliminate the effects of RanGTP dependent nucleation at the later stages of acentrosomal spindle assembly. The asters appear to nucleate radially across the oocyte around the time of NEBD (Figure 2.1(c)). The distribution of instantaneous velocities of asters was fit to a log-normal distribution (Figure 2.1(d)). The mean velocity obtained from the fit is $0.53 \pm 0.4 \mu\text{m}/\text{min}$ with a five-fold value of $2.4 \mu\text{m}/\text{min}$ as the maximal instantaneous velocity. The decrease in the mean velocity of a trajectory (v_{av}) with observation time as well as the degree of directional movement (χ) suggest asters exhibit random walk like motion (Figure 2.1(e)) with increasing time-scales. In addition the spatial dependence of χ indicate degree of randomness to be dependent on the position of aster from the chromatin edge (Figure 2.1(f)). Taken together, aster motility is suggestive of random walk with a spatial bias.

2.4.2 RWD simulated aster motility compared to experiment

To deduce the form of spatial bias a minimal and simple phenomenological model was developed to simulate a biased random walk. The phenomenology of aster

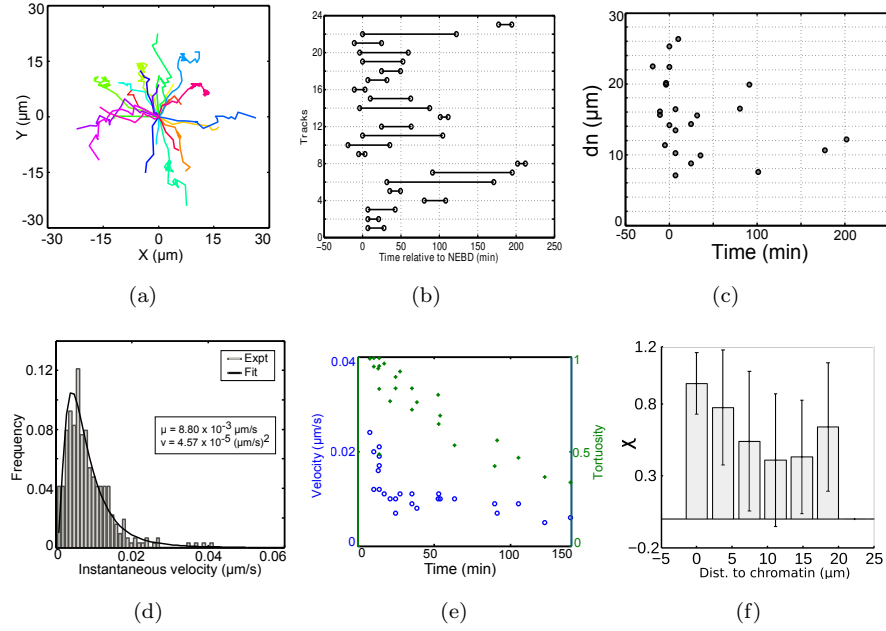


Figure 2.1: Spatio-temporal distribution of experimental asters. (a) The experimentally measured 2D trajectories of asters from mouse oocytes (from previous work [10], replotted based on raw data provided by the authors). (b) The track-wise distribution of the time at which asters were nucleated and reached the chromatin edge are plotted. (c) The distance from the chromatin edge (μm) at which the asters were nucleated are plotted as a function of the time at which they were nucleated. Here, time is measured in terms of time after NEBD (minutes). (d) The experimentally measured frequency distribution of instantaneous velocity is fit to a lognormal function. The parameters are the mean $\mu = 8.8 \cdot 10^{-3} \mu\text{m/s}$ ($n = 314$ pooled from 23 aster tracks). (e) The average velocity ($\mu\text{m/s}$) (blue circle) and tortuosity (χ) of each trajectory (green cross) is plotted as a function of the time length of the trajectory in minutes. (f) The trajectories were binned along the radial axis and the mean tortuosity (χ) was calculated for each bin as a function of distance from the chromatin edge. Error bars indicate standard deviation. This data was based on reanalysis of work by Schuh & Ellenberg.

movement was modelled as an effective diffusion with spatial drifts (Random walk with drift, RWD). The input parameters for diffusive and directed motion in the RWD model (Figure 2.2(a)) used were estimated from the fits to the experimental msd profiles (Figure A1(a), Equation 2.10). The mean effective diffusion, D_{eff} (Figure A1(b)) and velocity of drift, v_{eff} (Figure A1(c)) were constrained to the fit parameters while the form of spatial fields scanned. As a result, the nature of the centripetal motility depends solely on the parameters determining the field of drifts: attractive (ϕ_a) and repulsive (ϕ_r). Multiple shapes of the gradient were tested such

as step, linear, exponential and sigmoid functions. The sigmoid function with two parameters half-maximal ($r_{1/2}$) and slope (s) were optimized for both the fields of drift (Figure 2.2(b), Equation 2.7) i.e. $r_{1/2}$ is the distance at which the strength of the field is half the maximum value and s indicates the steepness of the curve. A total of 600 simulation scenarios were scanned for the fields: ϕ_a : $r_{1/2} = 2, 4, 5, 8$ and 10 and $s = 1, 2, 3$ and 5 while for the ϕ_a : $r_{1/2} = 20, 1, 2, 3, 4$ and 5 and $s = 1, 2, 3, 4$ and 5. The simulation outcomes from 600 possible simulation combinations were minimized for the sum rank of errors (Figure 2.3(a)) obtained from the ranked errors (ϵ) between simulated and experimental values of χ and t_c (scoring scheme discussed in section 2.3.3). The minimization scheme identifies the optimal gradient to be a long range attractive gradient ($r_{1/2} = 10 \mu m$ and $s = 1$) and a short range repulsive gradient from the cell boundary ($r_{1/2} = 0 \mu m$ and $s = 2$) based on directionality and capture time. While the error (ϵ) minima does not coincide (in terms of gradient parameters) between χ (Figure 2.3(b)) and t_c alone (Figure 2.3(c)), the sum-ranked scheme resulted in a global optimum. The outputs of such a gradient resulted in XY trajectories directed inwards at the cell boundary, random in the mid-zone and directed closer to chromatin (Figure 2.2(c)), qualitatively comparable to experiment (Figure 2.1(a)). Quantitative comparisons between experimental and simulated χ profile reflects this trend in motility- particles at the cell boundary and near chromatin are more directed, than those in the mid-zone (Figure 2.2(d)). The simulated capture time distribution also matches with experiment (Figure 2.2(e)). In order to understand the mechanism underlying the observed experimental motility, trajectories were further analyzed for their time-dependence.

2.4.3 Pushing and pulling profiles in aster transport

A distance time curve indicate the rate at which particle moves and thus the shape of the curve may provide insights on the phenomenology of the mechanism involved during the movement. In previous work, distance-time plots with a sigmoid profile

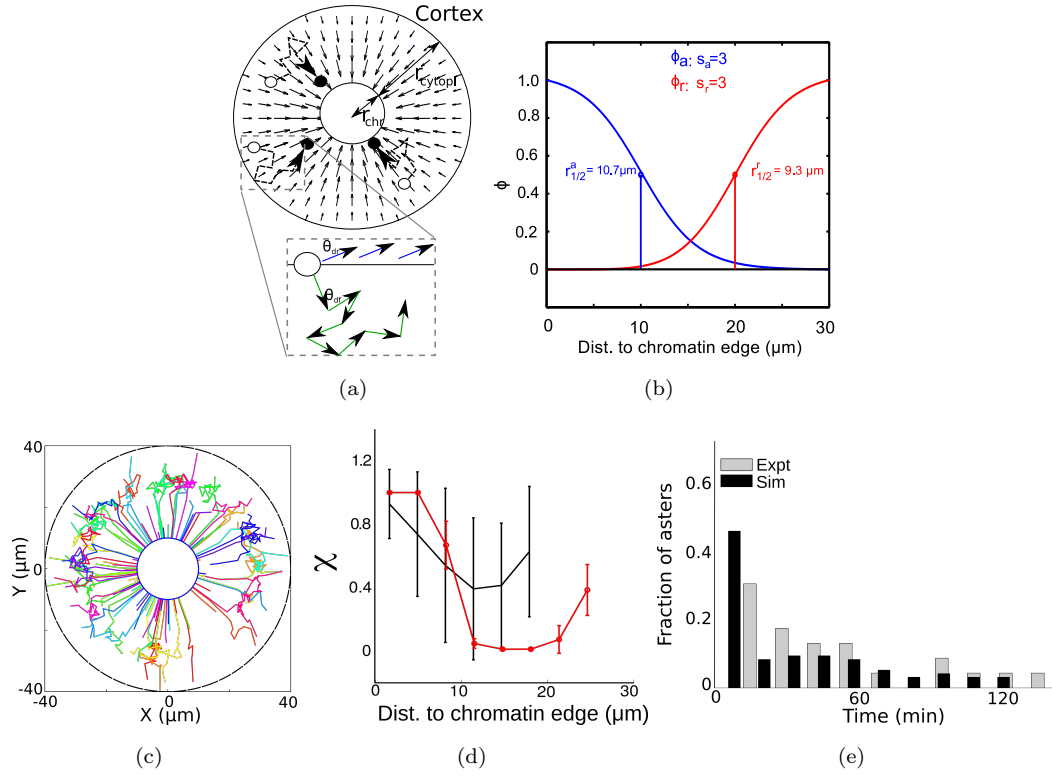


Figure 2.2: Optimizing the random walk with drift (RWD) gradient to experiment. The aster movement was modelled by (a) a 2D RWD model with the concentric cytoplasmic and chromatin regions of radius r_{cyto} and r_{chr} respectively. The cytoplasmic motility of asters from their initial positions (open circle) to final positions (closed circle) is driven by a field of drift (small arrows). (*inset*) The direction of motion is determined by the Brownian (θ_{df}) and directed (θ_{dr}) components. (b) The functional forms of the fields of attraction, $\phi_a(\vec{X})$ (blue) and repulsion, $\phi_r(\vec{X})$ (red), as a function of radial distance to chromatin (r) is determined by the respective $r_{1/2}$ and s values. The optimized gradient results in (c) XY trajectories from simulation (colors indicate individual tracks), (d) directionality (χ) (mean $\pm$ s.d.) as a function of radial distance from simulation (red) compared to experiment (black) and (e) the frequency distribution of capture time (t_c) from simulation (black) compared to experiment (grey).

have been interpreted to mean pulling forces are at work, while a parabolic has been interpreted to mean pushing is at play [74]. The parabolic shape of the curve is indicative of the pushing mechanism while the sigmoid curve suggests pulling. The distance time curves of aster trajectories in experiments exhibited both such shapes, which were also observed in simulations. The experimentally measured aster distance-time profiles were heterogeneous comprising of both parabolic and sigmoid

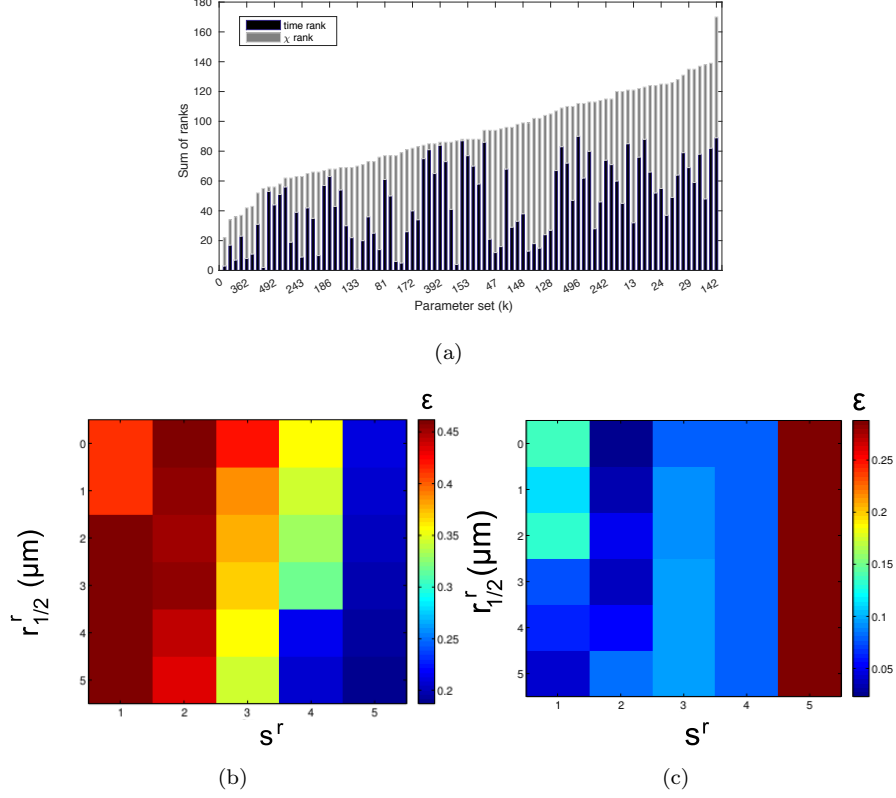


Figure 2.3: Optimizing gradient parameters. (a) All those parameter sets (k) which fell within the top 15% of the sum of scores ranking are plotted with the component rank for capture time ($R_t(k)$) (grey) and directionality ($R_\chi(k)$) (blue). The parameter sets (k) of the top-ten ranks are listed in Table A.1 with the values of $r_{1/2}$ and s for ϕ_a and ϕ_r . For a representative subset of the optimization scheme, the error (ϵ) in (b) χ and (c) t_c were evaluated keeping the attractive gradient constant ($r_{1/2}^a = 10 \mu m$ and $s^a = 1$) and varying the repulsive gradient parameters $r_{1/2}^r$ (y-axis) and s_2^r (x-axis). The colorbar indicates the value of ϵ .

shaped curves. Some asters moved rapidly in < 40 minutes, while others underwent a delayed (> 40 minutes) inward movement (Figure 2.4(a)). The optimized RWD model profiles qualitatively matched those from the experiment. Here, a function with three parameters were fit to the trajectories, n : a measure of the shape of the profile ($n > 1$: sigmoid and $n \leq 1$: parabolic), T_{half} : the time at which the distance travelled is half-maximal, and d_{max} : the maximal distance travelled, as follows:

$$d(t) = d_{max} \cdot t^n / (T_{half}^n + t^n) \quad (2.14)$$

The fit parameters d_{max} , T_{half} and n were obtained from the fits to the experiment data. A value of $n > 1$ indicated pulling, while $n \leq 1$ suggested pushing. Representative data from experiment (Figure 2.4(c)) chosen based on their nucleation position close to chromatin ($d_n = 7.05 \mu m$), mid-way in the cytoplasm ($d_n = 15.49 \mu m$) and close to the cell membrane ($d_n = 26.36 \mu m$), showed an apparent pattern in the n values- high in the mid-zone and low close to chromatin and near the cell boundary. Representative plots from the optimized RWD simulations (Figure 2.4(d)) with $d_n = 7.03 \mu m$, $d_n = 14.01 \mu m$ and $d_n = 24.88 \mu m$ showed a similar trend in the values of n . When the n from all experimental data (Figure A3) was compared to the simulated fits for different positions of nucleation, a qualitative match was observed (Figure 2.4(e)). The n value from simulations was higher in the mid-cell region as compared to near chromatin or at the cell boundary. This can be understood in terms of the sharp transition in the attractive gradient of drift (ϕ_a) at $r \approx 15 \mu m$, resembling a ‘pulling’ process. The phenomenological model however, does not allow for an interpretation of the origin of pushing or pulling forces. Therefore a detailed molecular-motor and MT polymerization dynamics was modelled to make experimentally testable predictions about the system.

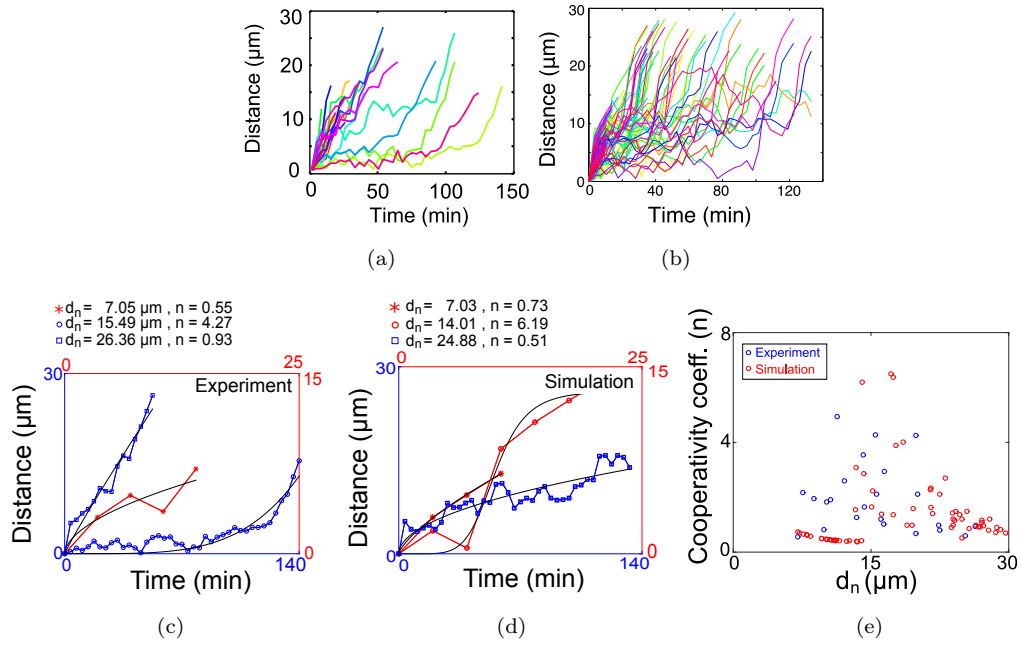


Figure 2.4: Distance travelled by asters in experiment and simulation.

The distance travelled from the site of nucleation is plotted as a function of time from (a) experiment and (b) optimized RWD gradient model. Colors indicate individual tracks. Representative fits (black line) to Equation 2.14 of data from (c) experiment and (d) simulation. Blue and red indicate the axes which scale the respective distance-time graphs. d_n is the distance from chromatin where the aster is nucleated and n is the cooperativity coefficient from the fit. (e) The fit values of n from experiment (blue) and simulation (red) are plotted as a function of d_n . The goodness of fit $R^2 > 0.7$ for all plots.

2.4.4 Mechanistic models of aster centering: Gradient and Self-Organized Models

The detailed mechanistic model combined single molecule parameters and reported behaviour of molecular motors, MT-dynamics and mechanics of MTs and membrane interactions, with the aim of developing a mechano-chemical understanding of the effective spatial drift fields. The mechanisms are categorized into the following two types:

- (i) Self-organized mechanisms: not requiring explicit spatial localization
 - (a) Cortical pushing alone
 - (b) Clustering by dynein complexes.
- (ii) Spatial gradients: requiring explicit spatial localization
 - (a) MT dynamic instability gradient
 - (b) Dynein motor gradient

The plausible mechanisms were systematically tested and evaluated for their ability to result in asters finding the center of the oocyte within an experimentally observed time-scale (~ 20 minutes).

(i) *Self-organized mechanisms:*

(a) *Cortical pushing:* In somatic cells the MT-asters find the center of a cell entirely based on pushing interactions at the cell cortex [144]. In simulations, it was explicitly tested if pushing from the cell boundary alone, could result in aster centering, while maintaining uniform MT polymerization and immobilized dynein-like motors (Figure 2.5(a)). Asters at the start of the simulation were localized randomly throughout the oocyte (Figure 2.5(b)). However even after 20 minutes, asters did not move centripetally (Figure 2.5(c), Video A1). Once an aster is displaced away from the cortex and all the MT contacts are lost, further centripetal movement due

to the pushing ceases. The pushing from the short astral MT lengths is not sufficient to center the asters in the large mouse oocyte cell.

(b) Clustering by dynein complexes: Based on the self-organized centripetal motility of asters observed experimentally in mouse oocytes, clustering motor complexes had been proposed to be the major driver of aster motility to the center of the cell [10]. As a result, diffusible minus-end directed (dynein-like) motor complexes were uniformly initialized in the cytoplasmic region (Figure 2.5(d), (e)). These complexes couple two nearby MT filaments and as a result of minus ended-motility, resulting in clustering of the asters (since asters have their minus-ends at the aster center). In this scenario, dynamic instability parameters were uniform and no immobilized motors were modelled. The complexes diffused through the whole cell including the chromatin region within ≈ 30 s, mimicking start of NEBD (Video A2). However even after 20 minutes of simulations, only a small fraction of the simulated asters were inside the chromatin region (Figure 2.5(f), Table 2.3).

(ii) Gradient-based mechanisms:

(a) MT dynamic instability gradient: Based on evidence of a gradient of dynamic instability in multiple cell types [79, 13, 85, 14], a gradient of f_{cat} and f_{res} as a mechanism for centering of asters was tested, while maintaining a uniform surface-immobilized motor distribution (Figure 2.5(g)) and random aster nucleation (Figure 2.5(h)). However, this too did not result in a perceptible increase in accumulation of asters at the chromatin center at the end of 20 minutes (Figure 2.5(i), Table 2.3), Video A3).

(b) Dynein motor gradient: It was only when motors were localized in a chromatin-centered sigmoid gradient and dynamic instability parameters were homogeneous (Figure 2.5(j)), did the randomly nucleated asters (Figure 2.5(k)) dramatically converge to the center (Figure 2.5(l), Video A5).

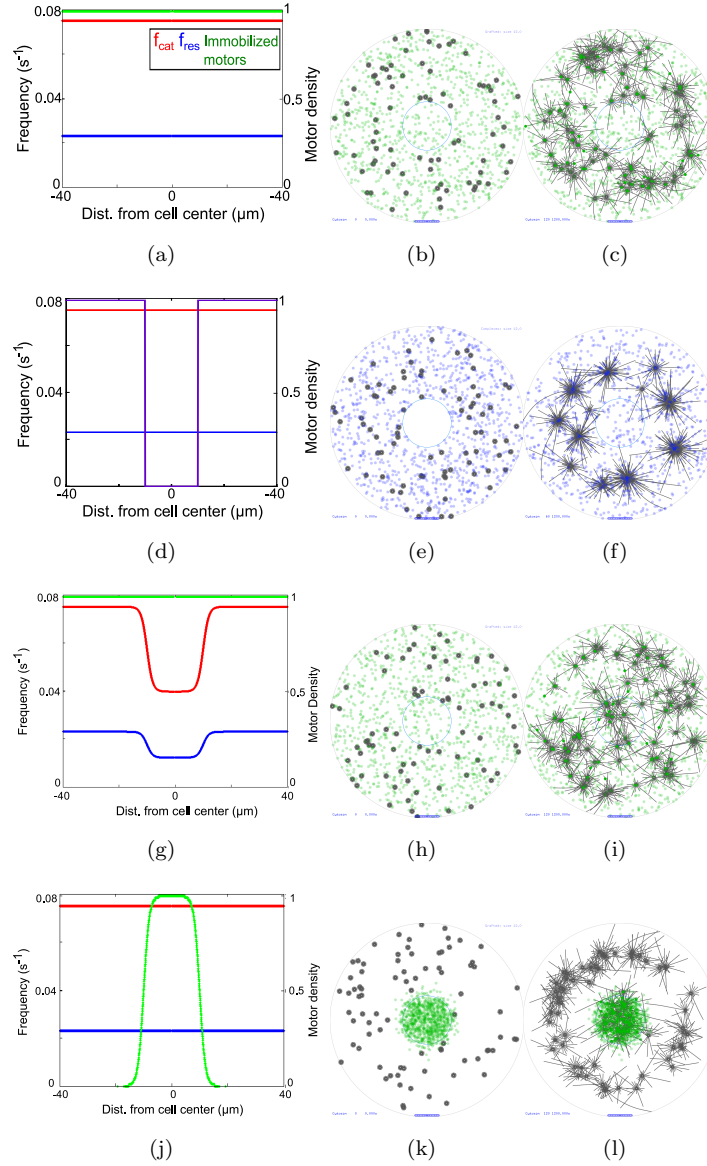


Figure 2.5: MT-motor model scenarios. (*Left column*) The distributions of f_{cat} (red), f_{res} (blue) and motor density of immobilized minus-ended motors (green) and (at $t=0$) diffusible minus-ended motor complexes (purple) are plotted as a function of radial distance from the oocyte center. (*Middle column*) Simulation output at $t=0$ minutes and (*Right column*) $t=20$ minutes with asters (grey) and a concentric nucleus (blue circle) with immobilized minus-ended motors (green dots) and diffusible tetrameric motor complexes (purple dots). Scenarios simulated: (a), (b), (c) Uniform distribution of f_{cat} and f_{res} and surface-immobilized motors $N_m^i = 10^3$ motors/oocyte, $f_0 = 7$ pN (green dots). (d), (e), (f) Diffusible minus-end directed motor complexes $N_m^c = 10^3$ motors/oocyte, $f_0 = 7$ pN (purple dots) and uniform f_{cat} and f_{res} . (g), (h), (i) Gradients of f_{cat} and f_{res} with uniform immobilized minus-ended motors $N_m^i = 10^3$ motors/oocyte, $f_0 = 7$ pN (green dots). (j), (k), (l) Gradient model of motor density with $N_m^i = 10^3$ motors/oocyte, $f_0 = 7$ pN and uniform f_{cat} and f_{res} values. Scalebar $10 \mu m$.

Model	Motor type	Localization	f_0	N_m	% captured
<i>Self-organized scenarios</i>					
No gradient	Minus-end directed	Uniform, immobilized	2 pN	10^3	0.5
			7 pN	10^3	3.13
Clustering	Minus-end directed, tetrameric	Uniform, diffusible	2 pN	10^4	0.38
			7 pN	10^4	0
<i>Gradient scenarios</i>					
Stabilization gradient	Minus-end directed	Uniform, immobilized	2 pN	10^3	2.38
			7 pN	10^3	8
Motor gradient	Minus-end directed	Gradient, immobilized	2 pN	10^3	18.5
			7 pN	10^3	32
Experiment					30.43

Table 2.3: Percentage of asters captured. After 20 min simulation time the proportion of asters captured were evaluated for the different scenarios in the mechanistic models and compared to experimental values on a similar time-scale. Values are estimated from 10 runs of a typical simulation with 80 asters each.

This suggests that for small asters, the asymmetry in forces induced by MT dynamic gradient is not sufficient to resolve the tug-of-war as experienced by the radial MT asters. A gradient of dynamic instability results in anisotropic asters- the astral MTs closer to the source of gradient on an average are longer than those relatively farther. For an aster gliding on a uniform sheet of dynein motors, larger number of motors are bound to longer MTs when compared to the shorter MTs. As a result, the net movement occurs in the direction of gradient. While in the presence of dynein gradient, on an average higher number of motors are bound to the astral MTs in the direction of the gradient. This results in a net movement in the direction of increasing motor numbers. The tug-of-war in small asters seem to be resolved by the uneven number of motors resulting in it's directional movement.

Thus, a minimal model of asymmetric pulling forces resulting from a gradient of immobilized motors moved asters to the center of the cell in a time-scale comparable to experiments (~ 20 minutes). MT-dynamic instability gradients are insufficient to generate the centripetal movement in the time-scales measured experimentally. Also, the addition of gradients of f_c and f_r along with the motor gradient, does not change the qualitative motility trends of asters and are comparable to only motor gradient scenario (Figure A8). However, a quantitative comparison of the simulation statistics with experiment is necessary to understand the critical parameters in this minimal model of motor gradient.

2.4.5 Model sensitivity to stall-force and density of motors and MTs per aster

Qualitatively, neither the tetrameric motor complexes, nor uniform surface immobilized motors and pushing at the cell boundary could result in a trend in χ comparable to the experiment. As expected from the qualitative trends, in the absence of any gradients the distance dependent mean binned directionality deviated from

the experimental trends (Figure (a)) with very few asters converging at the chromatin region (Table 2.2). Cross-linking by motor complexes of different stall forces ($f_0 = 2$ and 7 pN) and densities ($N_m^c = 10^3$ and 10^4 motors/oocyte) were tested and higher stall forces with high densities resulted in high values of χ throughout the cell (> 0.5). A directional bias in the form of a field of f_{cat} and f_{res} (based on Equation 2.7) resulted in asymmetric MT lengths and also failed to reproduce the directionality trends (Figure 2.6(b)). Confirming the qualitative observations from the simulation visualization, only a gradient of motors ($f_0 = 7$ pN, $N_m^i = 10^3$) could reproduce most of trends in directionality as a function of distance (Figure 2.6(c), 2.6(d)) and percentage of asters captured at the chromatin (Table 2.3).

However, the mean directionality trends in the mid-region showed a deviation from the experimental trend. Since there are no experimental estimates of the number of motors and their stall forces from the meiotic mouse oocytes, these parameters were scanned. The force experienced by the asters and thus their motility is strongly dependent on these parameters, motor numbers and their stall force. The parameters were explored for the two extreme values of f_0 (2 and 7 pN) reported in literature for dynein and scan N_m^i over three orders of magnitude. A high density ($N_m^i = 10^4$ motors/oocyte) of weak motors ($f_0 = 2$ pN) (Figure 2.6(c)) and a lower density ($N_m^i = 10^3$ motors/oocyte) of strong motors ($f_0 = 7$ pN) (Figure 2.6(d)), can both reproduce experimental profiles of directionality. The proportion of asters captured at the chromatin boundary was evaluated by following the distance of aster centers as they entered the chromatin mass. Strikingly the proportion of asters captured in the first 20 minutes of simulation from the motor gradient with immobilized motors of $f_0 = 7$ pN and $N_m^i = 10^3$ most closely matched experiment (Table 2.3). However, the mean velocity values from simulations were insensitive to either $f_0 = 2$ or 7 pN and motor densities over a range $N_m = 10^2$ to 10^4 motors/oocyte (Table 2.4). Similar trends is observed in the spatial velocity distribution (Figure A7). The msd profiles and spatial dependence are qualitatively comparable to the experiments (Figure A5). In order to further understand the role of motor-density changes and

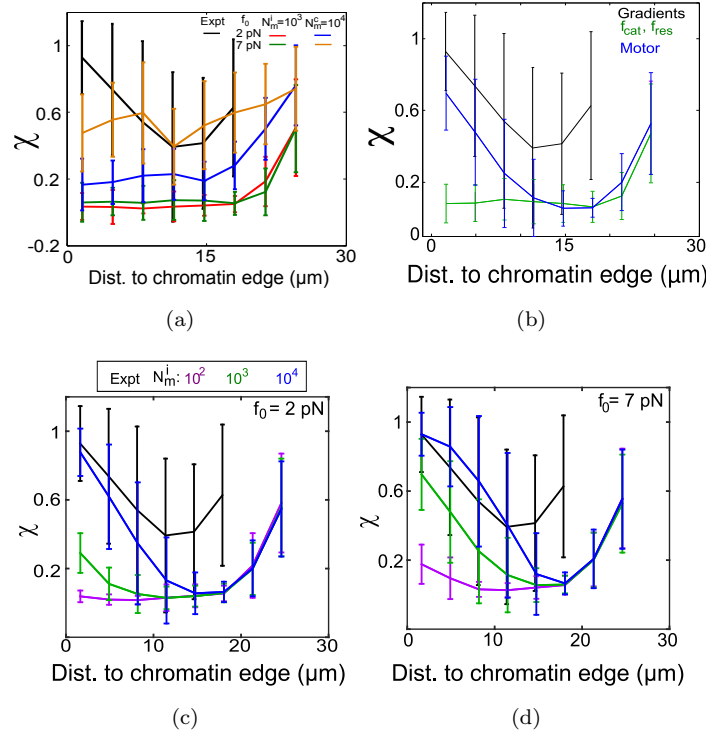


Figure 2.6: Directionality of simulated asters compared to experiment.

The distance dependence of binned directionality (χ) from experiment (black line) is compared to multiple simulated scenarios. (a) Uniformly distributed motors which are either immobilized weak ($f_0 = 2$ pN) (red) or strong ($f_0 = 7$ pN) (green) dynein-like motors with $N_m^i = 10^3$ motors/oocyte are compared to tetrameric diffusible minus-end directed dynein like motor complexes with $f_0 = 2$ pN (blue) and $f_0 = 7$ pN (orange), with $N_m^c = 10^4$ motors/oocyte. (b) The χ resulting from a gradient of f_{cat} and f_{res} (green) is compared to a gradient of the immobilized motors ($N_m^i = 10^3$ motors/oocyte, $f_0 = 7$ pN) (blue). In a gradient of immobilized motors with (c) $f_0 = 2$ pN and (d) $f_0 = 7$ pN the radial distribution of χ is plotted for increasing motor densities: $N_m^i = 10^2$ (purple), 10^3 (green) and 10^4 motors/oocyte (blue). All values of χ are mean $\pm$ s.d. Simulations were averaged from 10 runs with 80 asters each.

their effect on aster motility, the random walk statistics of the motility was evaluated and compared to the experiment.

The simulation outcomes are compared to the experiment for capture time, binned tortuosity and msd fit parameters and ranked as earlier to obtain an overall match to the experiments (Figure 2.7).

N_m^i	$f_0 = 2$ pN $\langle v \rangle$ ($10^{-3} \mu m/s$)	$f_0 = 7$ pN $\langle v \rangle$ ($10^{-3} \mu m/s$)
10^2	4.3 ± 6.3	4.8 ± 4.5
10^3	4.8 ± 4.6	5.4 ± 5.7
10^4	5.4 ± 5.7	5.4 ± 5.7
Experiment	8.7 ± 6.0	

Table 2.4: Aster velocity in a motor gradient. The mean instantaneous velocity ($\langle v \rangle \pm$ s.d.) with increasing motor density was calculated from 20 min simulation time and compared to experiment. N_m^i indicate total number of immobilized motors.

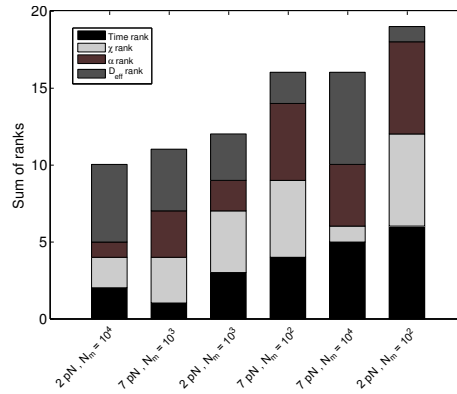


Figure 2.7: Optimization of motor parameters for gradient model. The simulation outputs for motor gradient mode for varying motor stall force and numbers was evaluated and ranked for measures of capture time, tortuosity, anomalous parameter and effective diffusion coefficient.

Additionally to test how the motility was affected by the total number of MTs per aster in the scenario of a motor gradient, we examined the χ and $\langle v \rangle$ of simulated asters which were initialized at a distance of $15 \mu m$ from the chromatin edge. As a result these asters it is expected that asters would experience the maximal force asymmetry, as they are at the lower end of the motor gradient. For increasing motor densities (N_m^i) directionality χ continued to increase, but increasing N_{MT} per aster appeared to rapidly saturate the χ value for any given N_m^i value for both $f_0 = 2$

and 7 pN (Figure 2.8(a),(b)). Increasing N_m^i lead to a marginal increase in the mean velocity ($\langle v \rangle$) for a fixed N_{MT} value. However increasing the value of MTs per aster, surprisingly, does not affect $\langle v \rangle$ (Figure 2.8(c),(d)). It suggests that the uniform radial distribution of MTs in the aster and a tug-of-war arising from it results in such an effect.

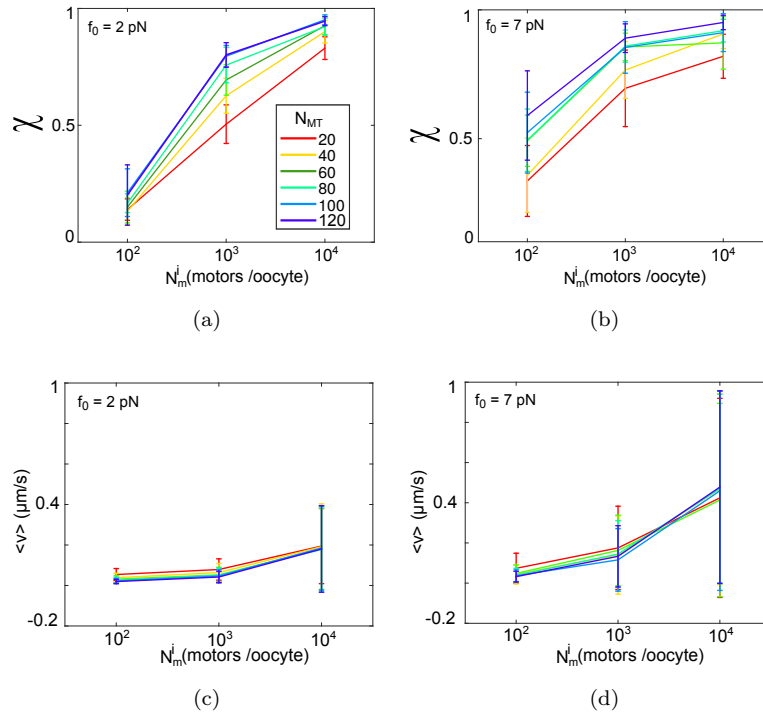


Figure 2.8: Effect of varying MTs per aster and motor density. Asters in the optimized motor gradient nucleated at $d_n = 15 \mu\text{m}$ from the chromatin edge, were simulated for increasing MTs per aster (N_{MT}): 20, 40, 60, 80, 100 and 120 (colors). (a), (b) The directionality χ and (c),(d) mean velocity ($\langle v \rangle$) are plotted for $f_0 = 2$ pN and, $f_0 = 7$ pN for motor densities ranging between $N_m^i = 10^2$ to 10^4 motors/oocyte (x-axis). All values are mean $\pm$ s.d. from 10 asters.

2.4.6 A hybrid model of a motor-gradient and self-organized clustering enhances aster centering

Simulations of multiple gradient forms, motor types and densities in this work suggest a motor gradient as a minimal model to understand the centering motion of asters in mouse meiosis I oocytes. However, the experimentally observed $\chi \sim 0.5$, when asters were 10-20 μm from the chromatin edge could not be reproduced. Interestingly, the presence of the self-organized clustering motors ($f_0=7$ pN, $N_m^c = 10^4$ motors/oocyte) alone resulted in simulated χ values that matched experiment, in the radial distance between 10 and 20 μm (Figure 2.6(a)), due to aster aggregation. Thus it was hypothesized that a hybrid mechanism combining an immobilized motor-gradient with diffusible clustering motor-complexes might reproduce the complete experimental distance-dependent directionality profile. Visually the asters appear to find the center more efficiently (Figure 2.9(a), (b)). Here 10^4 clustering motors per oocyte of stall force 2 pN were combined with 10^4 immobilized motors per oocyte and stall force 2 pN. A systematic screen of the effect of increasing tetrameric complex density while keeping the density of immobilized of motors constant was evaluated in terms of the measure of directionality. The radial distance profile of χ increases in the mid-range when clustering complex density is increased from 10^3 to 10^5 motors/oocyte (Figure 2.9(c)). Either ‘weak’ diffusible dynein-like complexes ($f_0 = 2$ pN) with a high density ($N_m^c = 10^5$) or ‘strong’ motors ($f_0 = 7$ pN) with a lower density ($N_m^c = 10^4$ motors/oocyte), both result in χ matching the experimental values in the mid-zone of the cell (10-20 μm) (Figure 2.9(c), 2.9(d), Video A6). This result suggests that while clustering alone cannot center asters in oocytes, it improves the fit to experiment.

Thus, a minimal model reproduces both the qualitative and quantitative nature of the aster centering motility and provides novel insights into the sensitivity of this model. It demonstrates how a combination of directional cues and self-organized clustering can center small MT asters in a large cell such as an oocyte. In addition

it highlights the general relevance of emergence of motor gradients in the cells and the role of immobilized and diffusible complexes. It also suggests a potential link between spatio-temporal organization of the dyneins which can be explored. A few studies in the meiotic mouse oocytes report the presence of dynein motors at the surface of the nucleus prior to the nuclear envelope breakdown (NEBD) [164, 17].

2.5 Conclusion and Discussion

Meiotic spindle assembly in mammalian cells in the absence of centrosomes involves the nucleation of asters in cytoplasm and their coalescence and sorting around chromosomes, resulting in bipolar spindle assembly. The nucleation of asters in cytoplasm and the centripetal motility of small radial MT asters has been previously observed during the first meiotic division in mouse oocytes [137]. Similar convergence was also observed in *Drosophila* oocytes [165] and quantitative analysis and model calculations were used to infer that directed transport of asters was essential for their coalescence [166]. In *Drosophila* the unconventional kinesin Ncd (minus-end directed) was implicated to play a role in this inward motility [124]. In mouse oocytes the aster coalescence by cytoplasmic dyneins or comparable minus-end directed motor have been implicated in force generation for the centripetal movement [10]. Here, the experimental data from the experiments at the early stages of the meiotic maturation of mouse oocytes was analyzed. The experimental analysis is used to constrain a field of spatially inhomogeneous drift in a random walk. The optimized model of drift is used to model gradients of the motor dynein and MT dynamic instability. By comparing the simulation outputs with experiments, a minimal model of a gradient of motors, essential to reproduce the experimentally observed statistics is proposed.

The experimental statistics of the aster motility from mouse oocytes are mostly representative of post-NEBD dynamics, allowing us make the simplifying assumption

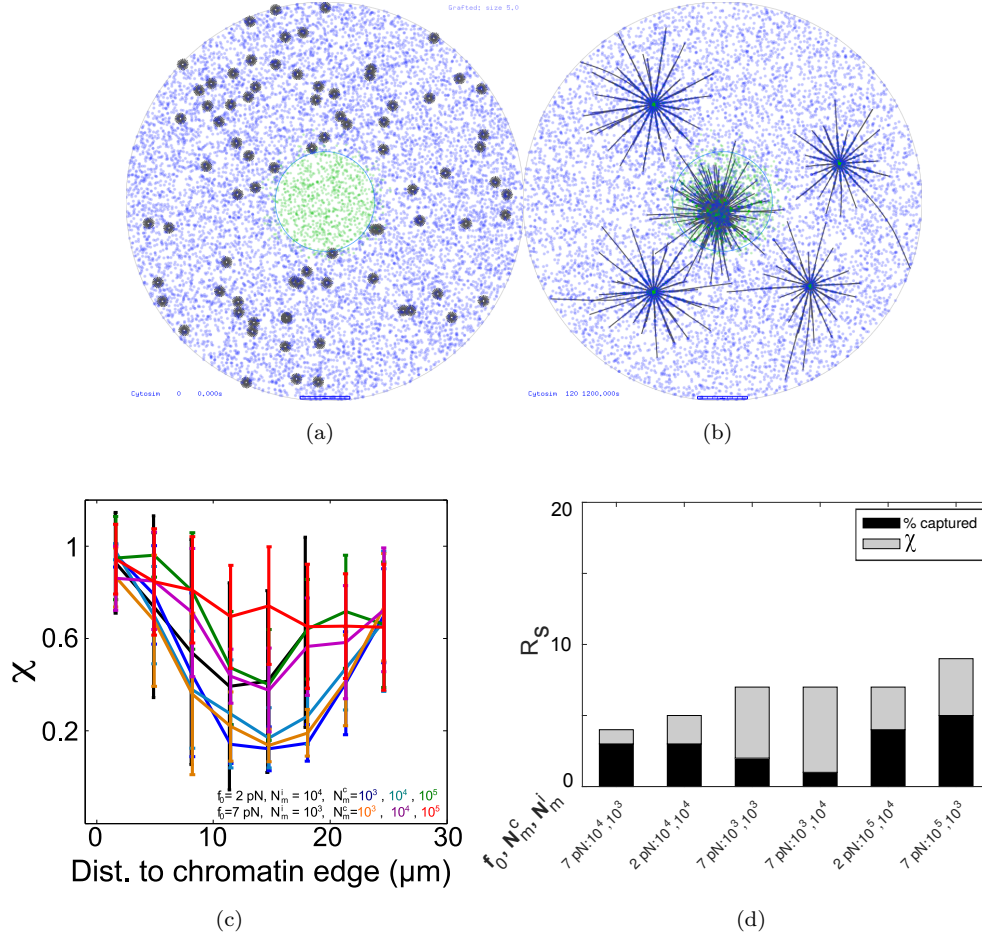


Figure 2.9: Combining immobilized and clustering motors. Simulation outputs of asters (grey) motility in the presence of a combination of diffusible tetrameric minus-ended motor complexes ($N_m^c = 10^4$, $f_0 = 7$ pN) with chromatin-centered gradient of immobilized motors ($N_m^i = 10^3$ motors/oocyte, $f_0 = 7$ pN) at (a) $t=0$ and (b) $t=20$ minutes. (c) The χ values of aster movement resulting from increasing densities of weak ($f_0 = 2$ pN) and strong ($f_0 = 7$ pN) clustering motors ($N_m^c = 10^3$ to 10^5 motors/oocyte) in the presence of a gradient of immobilized motors with the same f_0 values ($N_m^i = 10^4$ for $f_0 = 2$ pN and $N_m^i = 10^3$ for $f_0 = 7$ pN). (d) The sum rank (R_s) based on the sum of square errors (ϵ) from χ distributions (grey) and % asters captured in 20 minutes (black) from experiment are plotted in an ascending order, with the parameters of the density of immobilized motors (N_m^i) and diffusible motor complexes (N_m^c) plotted, assuming the f_0 is constant for both.

that the nuclear envelope plays no explicit role in the process. The movement of these radial MT arrays appears visually to have both an effectively diffusive and a transport component (Figure 2.1(a)). The frequency distribution of velocity from

experiment is long tailed and fit to a lognormal function (Figure 2.1(d)), suggesting anomalous super-diffusive transport. While the motility had been previously described as ‘stop-and-go’ [10], the evidence of ‘stop’ or pause events in the motility was low. The quantification of aster motility in cells, demonstrates the motility is qualitatively comparable to previous estimates of centrosomal aster movement observed in *C. elegans* fertilization [74], asters in *Drosophila* oocyte meiosis I [166] and centrosomal asters in *Xenopus* meiotic extracts [79, 15]. This suggests a common theme underlying the transport of radial MT arrays in meiotic spindle assembly.

The distance travelled or displacement from the start-point plotted over time of MT arrays have been used previously [74] to distinguish between “pulling” and “pushing” modes of motility of centrosomal MT arrays during pronuclear migration. In the case of mouse meiotic MTOCs, these plots also help to distinctly separate trajectories into two sub-populations- those which show an initial rapid rise followed by capture (< 40 min), while others do not move much for a long time (> 40 min) and after this delay are captured at chromatin (Figure 2.4(a)). We further improve on the work of Kimura et al. (2005) [74] by fitting the data with a saturation model with cooperativity (Equation 2.14), and use it quantitatively to distinguish between pushing ($n \sim 1$) and pulling ($n > 1$) mechanisms (Figure 2.4(c)). Every trajectory from experiment was fit to obtain a profile shape (sigmoid or parabolic) term n (Figure A3) and comparing n from simulation and experiment shows a pushing mode of transport ($n \sim 1$) close to chromatin and the cell boundary, while those in the mid-range are pulled ($n > 1$) (Figure 2.4(e)). The distance travelled plots from the mechanistic motor-gradient model with MT asters sorted by nucleation distance demonstrate an increase after a short delay near chromatin ($d_n = 0$ to $10 \mu m$) due to pulling (Figure A4(a)). Those in the intermediate range ($d_n = 10$ to $20 \mu m$) have a longer delay and then appear to be pulled (Figure A4(b)). Close to the cell-boundary ($d_n > 20 \mu m$) they increase rapidly and then saturate, due to MT-pushing at the membrane (Figure A4(c)). Thus a model of a gradient of chromatin-centered motors and a rigid cortex can reproduce the qualitative differences observed in the

distance-time profiles of aster transport. However in these experiments, the absence of clear ‘pulling’ in experiment ($n > 1$) near chromatin might have been missed, since the aster identity is lost as it nears the chromatin. While cell cortex-based pushing mechanisms have been demonstrated to generate centripetal movement [139, 6], the nature and localization of minus-end directed pulling motors in the oocyte remains to be determined. Recent evidence of from mouse oocytes during aster fragmentation [17] and meiotic maturation [146] suggest dynein anchored at the nuclear envelope might influence both processes. A careful study of dynein localization dynamics in this and related systems, could be used to test our model prediction.

A ‘tug-of-war’ in the transport of anti-parallel MTs moving on a surface coated with motors arises from the action of the same species of motor acting against each other, with small asymmetries in length, amplifying the velocity of transport [167]. Two-fold length asymmetries (10 to 20 μm) have been previously observed in centrosomal asters [79, 14] and simulations of such asymmetric asters on sheets of dynein motors resulted in aster transport towards chromosomes [15]. However, the mouse aster radius is in the range of 2 to 3 μm and no appreciable asymmetry of lengths has been reported [10]. In this work, neither homogeneous motor distributions (Video A1), nor tetrameric motor-complexes (Video A2), nor a dynamic instability gradient (Video A3) result in convergence to chromatin in the ~ 20 min time scale seen in experiment. Taken together, a gradient of motors is necessary in a minimal model with a mouse oocyte geometry for asters to converge to the chromatin center (Video A4, Video A5). This suggests aster motility in meiosis I of mouse oocytes differs from that in meiosis II of *Xenopus* oocytes. In the latter, length asymmetry can result in directional transport, however in mouse oocytes the asters are ~ 4 fold shorter in MT length, resulting in smaller forces being generated. The motor numbers would then be insufficient to successfully resolve the tug-of-war in mouse oocytes by a simple ~ 2 -fold changes in MT length, as seen in *Xenopus* oocytes. In contrast, the gradient of immobilized motors result in a comparable spatial distribution of velocity for minus-ended motors with $f_0 = 2$ pN (Figure A7(a)) and $f_0 = 7$ pN

(Figure A7(b)).

Spatial gradients of RanGTP [40, 41] are thought to direct centrosomal MT asters to chromatin during spindle assembly in meiotic extracts [79, 40, 14]. However the outcome of our simulations predicts that in meiotic aster motility, motor gradients are necessary. Such a gradient could arise from self-organized diffusion and attachment of minus-ended motors on MTs nucleated at the chromatin periphery. In addition, the motors could be immobilized on intracellular organelles, as shown in centrosomal aster centration in *C. elegans* [76]. It remains to be seen which of these specific mechanisms results in the chromatin centered gradient of anchored motors. Some evidence from experiments in mouse oocytes serves to support our motor gradient model, where pre-NEBD maturing oocytes showed a high-concentration of dynein motors around the nucleus [164]. More recently, evidence of dynein localized at the nuclear periphery fragmenting asters [17], suggests a validation of our model of a chromatin centered gradient of dynein-like motors. Further testing is however required to examine its dynamics. The role of chromosomes in the centering activity of asters in maturing mouse oocytes during meiotic I spindle assembly had been tested by experimentally removing the nucleus [10, 150]. The asters of such oocytes have a scattered appearance and fail to assemble bipolar spindles. While chromosomes are considered essential for meiotic spindle assembly [168], we hypothesize that they also serve as the primary guidance cue of centripetal aster motility, in a manner comparable to other aster cell centering systems [79, 85, 15]. To test this, the spatial organization of asters in enucleated oocytes was measured using published experimental data reported by Schuh and Ellenberg [10]. The image analysis of aster positions from experiment (Figure A6(a), A6(b)) resulted in a radial density distribution around the cell center (Figure A6(c)). This distribution is comparable to a simulation of randomly localized asters, suggesting the chromatin serves as an important guidance cue for the centripetal motility of asters, and in its absence the directional motility is lost. The molecular mechanism which converts the positional information of the chromatin into a gradient of molecular motors, still remains to

be understood.

The msd profiles of asters from simulations that were close to chromatin transition from super-diffusive to sub-diffusive motility (Figure A5), as estimated by α , the measure of anomalous diffusion (Figure A5(f)). This transition results purely from changing the single dynein motor stall force from that reported for bovine brain cytoplasmic dynein ($f_0 = 2$ pN) [156] to the yeast cytoplasmic dynein ($f_0 = 7$ pN) [160] value. The aster convergence in the 2 pN stall force calculations is slow (Video A4) as compared to when the motors had a higher stall force (7 pN) (Video A5) at the same motor density ($N_m^i = 10^3$ motors/oocyte). Once the asters reach the center of the motor gradient, they undergo effectively sub-diffusive movement, unable to generate enough force asymmetry to escape. With an additional centrosomal fluorescence label in mouse aster motility, the intra-nuclear motility and reorganization of asters could be studied in future. This will also help better understand the subsequent bipolar spindle formation and its connection with chromosome biorientation in meiosis I of mouse oocytes [169, 170].

The sensitivity analysis of the gradient model demonstrates that MT number per aster does not affect velocity and directionality of the asters. On the other hand the model is sensitive to motor density. The motor density in 2D area-density ranging between $N_m^i = 10^3$ and 10^4 motors/oocyte corresponds to a physical density of ≈ 0.2 to 2 motors/ μm^2 for an oocyte of radius $40 \mu m$. The addition of motor complexes, which cross-link MTs and walk to the minus-ends of the respective MTs, also change the dynamics of aster transport. At high densities of motor complexes, the asters coalesce to a few (~ 10) clusters and centripetal convergence results. Yet the directionality profiles are qualitatively different from the experimental measures (Figure 2.9(c)). While the densities of both immobilized and diffusible motors tested are mean area densities, over-expression of dynein motor proteins could serve as an experimental test of our model predictions.

A limitation of the models described here is that both the phenomenological and

mechanistic models assume a 2D circular geometry, while the mouse oocyte is a 3D sphere. The simulation system was extended to 3D wherein the multiple asters were initialized randomly and the localization of motors and MT dynamics were tested for different conditions as in 2D. The aster trajectories from a single run as depicted in (Figure 2.10) confirms that in the absence of any gradient, the tug-of-war cannot be resolved in the uniform conditions of the cell. The gradient of MT dynamics, as in 2D scenarios is a weak source of force asymmetry in the system, while localization of dyneins in a spatial gradient in the cell results in significant asters moving towards the cell-center. However, the convergence of most of the asters at the chromatin region within 20 minutes is not observed. The combination of MT dynamic and motor gradient does not improve the centering ability as in 2D. Thus, it suggests that asters finding the cell center in 3D is expected to take longer due to dimensionality in biological search problems [171], and a further parameter optimization would be required. The effort here serves to reduce the search for ‘scenarios’, i.e. combinations of mechanisms such as motors, MT-dynamic instability, gradients and clustering motors, by optimizing simulations to experimental data. The preliminary result captures the qualitative convergence of asters at the chromatin region and suggests either more time or scaling of the system components such as motor numbers is essential to generate forces required for directional movement of the asters. In future, a full 3D model would then only require parameter optimization to a 3D experimental dataset. At the other extreme, a 1D model could have further simplified the geometry based on the radial-symmetry of the system, as has been assumed in the ‘slide and cluster’ model of linear MT filaments in spindle assembly [172]. However this would ignore the orthogonal interactions between MTs of multiple asters, which are only possible in an explicit 2D geometry. Thus our choice of spatial dimensions is driven by an attempt to capture the important qualitative behaviour of the system, while keeping the model simple enough for clarity and calculation speed. While a 3D simulations of cellular processes are more ‘complete’, it has been suggested the choice of spatial dimensions should simplify the system to sufficiently capture the spatial dynamics [147, 173]. A further limitation of both the phenomenological RWD and

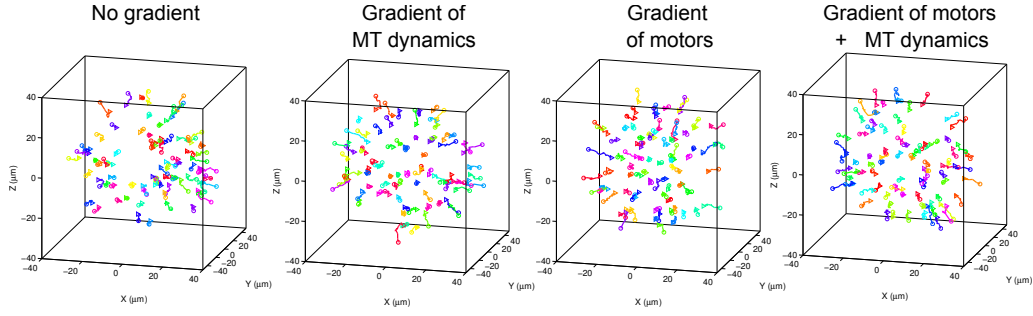


Figure 2.10: Aster trajectories from different simulation scenarios in 3D. XYZ tracks from a single simulation run over a period of 20 minutes is represented from scenarios of (a) uniform motor and MT dynamics in the cell, (b) gradient of MT dynamics originating from the cell center, (c) gradient of immobilized motors originating from the cell center and (d) combination of MT dynamic and motor gradients as in (b) and (c). Colours correspond to individual trajectories. The simulation parameters are similar to those in 2D as in the Figure 2.5.

MT-motor model is the availability of only one time-series dataset. In future, additional experiments with mouse oocytes could help test the model predictions. While this model has been developed to test the generality of mechanisms on the mouse aster motility, it would be useful in future to explore the relevance of this model to other aster-centering systems such as in *C. elegans* [74], *Xenopus* [15], *Drosophila* [174] and sea urchin [75]. Additional mechanisms such as a contractile actin network has been shown to drive spindle assembly in meiosis I of starfish oocytes [78], but are ignored in this model since in experiments, it does not affect the process [10]. Additionally the potential role of MT-dependent MT nucleation [84] remains to be explored as a self-organized mechanism. Testing alternative cellular geometries and additional mechanisms could in future result in a more complete exploration of MT aster centripetal transport and the physical constraints. This in turn might help us better understand the physical basis of the evolutionary diversity of aster-centering mechanisms. The models of aster centripetal motility explored in this study, provide insights into several aspects of the early stages of self-organized spindle assembly. For instance, the RWD model demonstrates that for a cell of diameter $\approx 80 \mu m$, a

simple random-walk strategy of aster particles ‘search-and-capture’ at the chromosomes is insufficient within the time-scale of spindle assembly in meiosis (20-30 min) [10], and a long-range bias in motility is essential. The mechanistic model demonstrates that a bias of motors is minimally capable of reproducing the dynamics. MT mass increase which could arise due to increasing MT lengths (stabilization) [79, 40] or number (nucleation) [84], both appear to be insufficient to affect the dynamics of motility. We also find, dynein-like clustering motor complexes, which diffuse and cross-link MTs, at a high density result in an aggregation of asters to the cell interior. Such a self-organized mechanism however is inefficient in resulting in aster capture percentages comparable to experimental values (Table 3) in comparable time-scales (≈ 20 min). Additionally MT density per aster does not affect their centripetal motion but the density of immobilized motors (N_m^i) localized in a gradient does change the dynamics. Such parameters are likely to be adjusted by cells, depending on the specific geometry and time-constraints. The results of this study could be used to predict the nature of centripetal transport of asters, when the size ratios of asters to cell sizes are comparable to the mouse oocyte. A more complete picture of the evolutionary constraints on mechanisms driving radial MT arrays to find the center of a cell will however require further quantitative studies from acentrosomal spindle assembly from more organisms, as well as calculations that explore a greater parameter space.

The model presented here predicts the functional form of a drift field in agreement with experimental data of aster motility from meiotic mouse oocytes. A mechanistic model of the gradient with immobilized minus-ended motors minimally reproduces experimental dynamics and allows us to test the effect of single molecule characteristics of motors on collective transport statistics. A hybrid model with a gradient of immobilized motors and diffusible clustering dynein-like complexes, combined with cortical pushing (Figure 2.11), satisfies all experimental measures available. Measuring the localization, density and mobility of dyneins in the mouse oocyte during meiosis I, would be a useful test of the model predictions. While the model is fit

to spindle assembly during meiosis I in mouse oocytes, it also predicts the design constraints in terms of MT lengths and the localization, mobility and density of molecular motors when MT radial arrays are required to search space and undergo capture at the cell center.

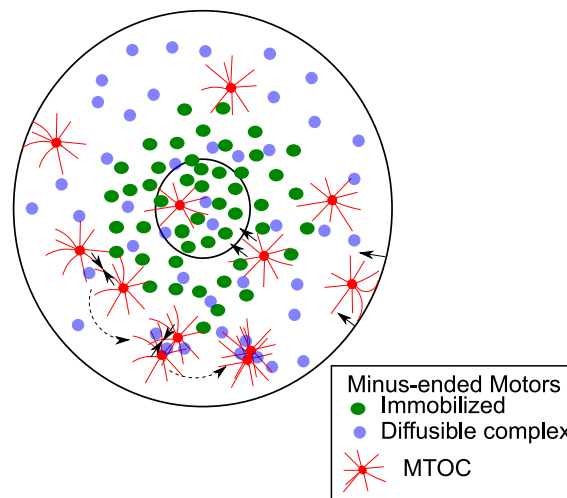


Figure 2.11: Mechanism of MT centering. An overview of our model of the centering motility of asters (red) in a mouse-oocyte geometry. Net forces (solid arrows) arise from: inward pushing due to MT bending at the cell boundary, inward pulling due to a gradient of immobilized motors around the chromatin space (green) and clustering by uniformly distributed diffusible clustering motors (purple). The dotted arrows indicate the process of clustering.

2.6 Outlook

A minimal model of dynein gradient and diffusible clustering dyneins reconciles with experimental data. A gradient of dynein is essential to resolve the aster tug-of-war in the meiotic mouse oocytes. A gradient of MT dynamics is insufficient for centripetal convergence of asters and a combination of both the gradients exhibit motility statistics comparable to those from only the motor gradient scenario. This study further raises multiple interesting ideas that can be explored.

Emergence of motor gradient

In the current model of multi-aster centering, a steady state spatial gradient of dynein originating from the cell center was assumed at the time of initialization. The existence of such a gradient in the mouse oocyte or in any other *in-vivo* system remains to be tested. Multiple studies have shown spatial dynamics of the motors to vary across the cell stage or processes, however the mechanism that govern their localization is not well understood. Fewer studies report presence of dyneins at the nuclear envelope prior to the NEBD in the mouse oocytes [164, 146, 17] however whether such spatial distribution exists is not explored. Additionally, although RanGTP gradient has been demonstrated to be not essential for the convergence of multiple asters at meiosis I, the presence of nucleus was shown to be essential. The degree of centripetal convergence observed showed a direct correlation to the time of enucleation [10]. In budding yeast, kinesins have been shown to be involved in delivery of dyneins to the bud cortex [175, 176, 108]. In order to find the mechanism for the emergence of spatial gradient of dynein originating from the cell-center in the mouse oocyte at meiosis I prior to the NEBD multiple scenarios were explored. The preliminary results suggest accumulation of dynein motors at the pre-existing MTs or asters at the nuclear envelope to amplify the motor numbers. Multiple configurations of MT patterns at the initialization was explored such as anti-parallel linear arrays, random MT array and asters at the nuclear periphery. The geometry, number and orientation of MTs influenced the kinetics and profile of the motors in the space which was used to compare it to the simulations data to. The pre-existing MT mass provide sites for accumulation of dyneins, which further organizes MTs and recruit more dyneins as the dynamic MTs explore the space resulting in a positive feedback in the system. The preliminary result suggest perinuclear asters (i.e. asters spanning the nuclear periphery prior to NEBD) result in formation of gradients that qualitatively resemble the input gradient (Figure 2.12). However, further calculations are required to systematically evaluate the parameter space and

time-dependence of the process to gain complete understanding of the mechanism for the emergence of a spatial motor gradient in the cells.

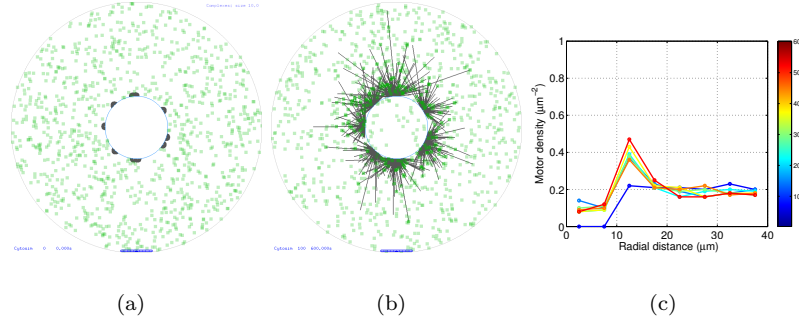


Figure 2.12: Accumulation of dyneins at perinuclear region. Simulation snapshots at (a) the start of simulation with asters initialized at the nuclear periphery and (b) at the end of simulation. (c) The radial distribution of dyneins is plotted at different time points. Colors indicate time. $N_A = 10$, $N_m^c = 10^3$ and total simulation time = 600 s.

General principles of directed motility of asters

The study suggests that a gradient of motor is essential for resolving force symmetry of the small asters while gradient of MT dynamics are essential for the larger oocytes. This finding motivates to find the general principles that dictate the mechanism for directed motility of multi-asters i.e. how much force asymmetry is essential and when gradient of MT dynamics and gradient of motors results in that threshold force asymmetry. It is speculated that gradient of MT dynamics is efficient for large asters gliding on a sheet of single species of motors. The two fold increase in MT lengths is significant. While, asymmetry in motor numbers can resolve ‘tug-of-war’ in a system of small asters. Thus, the aster motility trends for different relative length scales i.e. ratio of the cell radius to the mean MT length of an aster was scanned. Some of these results are discussed in the **Chapter 3** while the rest remains to be tested systematically. The relative length scale in the mouse oocytes corresponds to ~ 12.5 ($R = 40\ \mu\text{m}$, $L_{MT} = 3.2\ \mu\text{m}$) while in *Xenopus* ~ 120 ($R = 500\ \mu\text{m}$, $L_{MT} = 4.25\ \mu\text{m}$). It would be interesting to explore the parameter

space in which gradient of MT dynamics, gradient of motors and both exists as the mechanism for the directed movement of the asters. The biochemistry behind the origin of MT dynamics gradient is well understood. It is also known that biochemical networks scale with cell sizes and result in properties that show dependence on the concentrations, reaction-diffusion rates and boundary conditions. Thus the mouse model can be extended to explore the effects of cell size, motor concentrations and MT dynamics to gain insights on the general principles of directed motility.

Role of antagonistic motors in centering and spacing

In the model of multi aster convergence only dyneins were considered and the effects of antagonistic motor, kinesin ignored. This was motivated by: (a) the process of interest (i.e. centering) which is primarily dynein driven and (b) kinesin-5 inhibition had no effect on the aster motility at this stage [10]. Interestingly, inhibition of kinesin-5 increased the centering speed of multiple asters in the mouse embryos during the acentrosomal spindle assembly [18]. The antagonistic motors: (a) kinesin and dynein (b) kinesin-5 and dynein are known to act together in multiple systems associated centering, decentering and positioning of asters or aster driven organelles. The role of antagonistic motors in the cytoplasm for varying relative concentrations and cell size was explored. The key results from this work is discussed in the next chapter **Chapter 3**. The mechanistic findings from the individual processes such as directional movement of asters, spatio-temporal regulation of motors, the collective behaviour of multiple motors, effect of motor numbers, their single motor properties and interplay of MT dynamics and motors will help in understanding of biophysical principles of cellular scale patterns such as acentrosomal spindle assembly and spindle positioning.

Chapter 3

Self-organized spatial patterns of microtubule asters formed by kinesin-5 motors at optimal relative cell size

Contributions: Experimental collaborators: Janet Chenevert and Gérard Pruliere at LBDV, France. The chapter is part of the manuscript in preparation *Kinesin driven self-organized packing patterns of multiple microtubule asters scale with cell size*.

3.1 Introduction

Radial arrays of microtubules (MTs) or MT asters are typically assembled in cells either from centrosomes or microtubule organizing centers (MTOCs) in a template-dependent manner or self-organized by the motor-driven clustering of linear MTs. In addition, cytoplasmic asters or cytasters can be induced by changing the pH [118], osmolarity of the medium [119] or addition of MT stabilizing drugs like taxol [117] in large cells such as sea urchin eggs. Indeed varying concentrations of taxol and hexylene glycol have resulted in size changes in cytasters as well as emergence of spirals [120]. Taxol induced cortical cytasters have also been seen to emerge in mammalian cell lines, in a NuMA and minus-ended kinesin HSET dependent manner [121]. In leech zygotes taxol treated oocytes resulted in orderly movement of cytasters [177]. Asters are critical for cell division, motility, intra-cellular transport, polarization and positioning of organelles [178] and thus spatial and temporal control of asters is essential for cell physiology. Their number and position in the cell appear to be governed by controlling MT motility and interactions through mechanisms that depend on both cell size and whether the aster is to be centered, de-centered or polarized. MT asters can find the center of small cells by simple inward radial pushing forces that are generated when polymerizing MTs contact the cell boundary, a principle that has been demonstrated using asters assembled in vitro in micron-sized confinements [5] as well as in simulations based on cells in culture [9]. Pulling forces generated by minus-end directed motors like dynein, anchored at the cortex can also result in aster centering [179]. Such radially anchored motors, produce symmetric forces that pull asters towards the cell boundary from all directions resulting in aster centering, by walking towards minus ends of MTs that lie at aster center, as demonstrated in vitro [180, 6]. On the other hand, non-uniform distributions of motors or spatial differences in their mechanics can result in off-center localization of asters as seen in vivo during *Caenorhabditis elegans* spindle positioning [181, 182, 183, 184]. However, in larger cells with diameters exceeding 30 μm , where asters do not always contact the cell boundary, intracellular forces originating from cytoplasmic motors,

typically dyneins, can drive aster positioning as seen during *C. elegans* fertilization [74, 76] and in *Xenopus* oocyte extracts [15] and during sperm aster centering in sea urchin eggs [75]. The asymmetric force acting on asters in large cells can originate from MT length regulation by biochemical gradients [79, 14, 15, 185] or from spatial gradients of motors combined with self-organized clustering [10, 134]. More recently, a theoretical model of how pushing and pulling forces drive single aster positioning has been developed and applied to the *C. elegans* embryo [186]. However, this theory does not take into account collective effects of multiple interacting asters and the patterns formed.

Indeed mixtures of linear MTs and molecular motors are known to spontaneously form diverse spatial patterns ranging from self-organized asters to vortices [3, 61, 187]. MT asters however are expected to produce ‘geometrical frustration’ of forces when acted on by one species of motor, due to their radial geometry [15]. While single and pairs of asters have been widely studied in the context of center finding in interphasic cells and during mitosis respectively, multiple aster organization by motors is less well understood. In a simulation screen to identify the minimal components required for a pair of asters to form stable bipolar structures resembling mitotic spindles, motor complexes were required in a combination of diffusible homo-tetrameric dyneins or kinesins which drive fusion or separation of aster pairs respectively, and hetero-tetrameric complexes (made of two motors of opposite directionality) for stable antiparallel MT overlaps [62]. Understanding the collective mechanics of systems with more than two asters, i.e. multi-aster systems, is also due to the role of motors and cross-linking proteins that are known to cluster supernumerary (more than two) centrosomal asters and reduce the effect of multi-polar spindles, that drive genome instability in some cancer cell lines [174, 188]. On the other hand, the antagonistic activities of plus-end and minus-end directed motors are both required for aster separation and organization into bipolar structures during spindle assembly as seen in *Xenopus* extracts [189, 190], *Drosophila* embryos [191] and mammalian cell culture [192]. Confirming this, while the inhibition of kinesin-5

during centrosome separation in mammalian cells resulted in both monopolar and bipolar spindles [95], kinesin-5 inhibition in a dynein depleted cell rescued spindle bipolarity [94]. Recently, time lapse studies have confirmed that the coordination of three motors- Eg5, Kif15 and dynein- is required for spindle assembly, based on a balance of ‘push-pull’ inward and outward forces [96]. In addition, aster migration speeds are reported to be affected by both dynein and kinesin-5 motors in developing mouse embryos [18]. The regular spacing of syncytial spindles as seen during *Drosophila* embryogenesis is driven by a combination of aster transport and inter-aster interactions [193] that can be recapitulated ex vivo using embryo extracts [113]. While kinesin-5 driven forces have been shown to regulate the spacing observed in *Drosophila* embryonic syncytia, it remains to be seen how the combined effects of MT lengths and motor mechanics, together with concentration and spatio-temporal distributions affect the aster patterns. Indeed given the non-linear nature of single motor mechanics, it is likely that an explicit model of collective multi-aster mechanics could produce counter-intuitive patterns.

Here, a computational model of multiple asters combined with diffusible motor complexes was developed to explore the potential self-organized pattern formation ability of the multi-aster systems in simulations. The emergence of packing patterns of multiple asters as a result of interactions with varying proportions of opposing motor types is observed, which emerged based on the individual properties of molecular motors and their local interaction with MTs. The packing patterns of multi-asters emerged at global scale as a result of local interactions of the opposing motors and MTs. The proportion of motors and orientation of MTs result in segregation or coalescence of asters in a system. Surprisingly, at a critical size of asters relative to cell size, model predicts the spontaneous emergence of hexagonal optimal packing of asters. Further, the model could explain the hexagonal lattices of induced cytasters observed in oocytes of the ascidian *Phallusia mammillata* treated with BiD1870, a ribosome S6 kinase (RSK) inhibitor. This minimal model of kinesin-5 and dynein driven multi-aster patterns matches the observed pattern of cytasters and predicts

the quantitative distribution of MT lengths and relative ratio of antagonistic motors required to produce steady state patterns to recapitulate observations in vivo.

3.2 Methods

3.2.1 Animal and oocyte collection

Adults of the ascidian *Phallusia mammillata* were collected in the Etang de Tau (Mediterranean coast, France) and maintained in aquaria with running seawater for a maximum of 4 months. Oocytes were collected by dissection and dechorionated using trypsin protease as described (McDougall et al 2015). After dechoriation, washed oocytes were stored in gelatin-coated petri dishes at 18 °C for up to 8 hours.

3.2.2 Pharmacological treatments

All chemical inhibitors were prepared as 1000 X stock solutions in DMSO. Stock solutions: Bi-D 1870: 10 mM (RSKII inhibitor, Calbiochem - CAS 501437-28-1); Ciliobrevin D: 25 mM (Dynein ATPase inhibitor, Sigma-Aldrich 250401); Monastrol: 100 mM (mitotic kinesin EG-5 inhibitor, Calbiochem - CAS 254753-54-3); Taxol: 20mM (paclitaxel, Sgma-Aldrich). Treatment with Bi-D 1870 inhibitor was carried out by incubating oocytes in sea water supplemented with the drug at the desired concentration at 18 °C for one hour. For double inhibitor experiments, *Phallusia* eggs were incubated in either Ciliobrevin D (final concentration 25 μ M) or Monastrol (final concentration 100 μ M) for 30 minutes prior to addition of BI-D 1870 (final concentration 10 μ M) and further incubation for 60 min.

3.2.3 Immunostaining and microscopy

The following primary antibodies purchased from Sigma-Aldrich were used: Anti-alpha tubulin: mouse monoclonal DM1A, dilution 1:1000, anti-gamma tubulin: mouse monoclonal GTU 88, dilution 1:200. Secondary antibodies coupled to fluorescein isothiocyanate or to tetramethyl rhodamine isothiocyanate (Jackson ImmunoResearch Laboratories, West Grove, PA) were used at a dilution 1:200. For fixation, inhibitor treated or control (DMSO-treated) *Phallusia* eggs were plunged rapidly into cold (-20 °C) methanol containing 50 mM ethylene glycol tetra-acetic acid (EGTA) pH 7.5. For immunolabeling, fixed samples were rehydrated via three 5-minute washes in phosphate-buffered saline (PBS) and blocked by three 15-minute washes in PBT (PBS, pH 7.4, containing 3 % bovine serum albumin and 0.05 % Triton X-100), before incubations with primary antibodies (diluted in PBT) for either 2 h at room temperature or overnight at 4°C. Eggs were then washed three times in PBT, incubated with secondary antibodies supplemented with 1 $\mu\text{g}/\text{ml}$ Hoechst 33342 (Sigma-Aldrich) to label DNA, washed again in PBT, and mounted on glass slides in antifading reagent Citifluor (Chem Lab, Canterbury, United Kingdom). Fluorescent images of fixed immuno-labeled samples were acquired with a Leica SP5 confocal microscope equipped with a 40x oil objective, using a z step size of 1 μm .

3.2.4 Image analysis

The frames with visible cytasters (upper to mid plane) from the z-stack of the *Phallusia* oocytes were projected in 2D based on maximum intensity in ImageJ [194]. Along with, the images were cropped such that a single oocyte fits the image frame entirely i.e. cell-boundary was inferred from the background-level staining of the tubulin. In order to achieve this, the brightness of the image was varied manually in ImageJ to increase the clarity. *Astral MT length*: In order to estimate MT lengths from individual cytasters, central cytasters in the images were selected.

We developed a script in MATLAB R2017b (Mathworks Inc., MA, USA) based on a previously described method [14], where the user interactively selects the aster center and the outermost limit of the individual aster to generate a radial intensity profile that is fit to an exponential distribution to obtain the decay length.

3.2.5 Polygon analysis

Voronoi tessellation [195] for the experimental and simulation data was performed using aster centroids as the inputs based on an in-house developed Python (ver.2.7) script. The xy coordinates at the end of the simulations were read from a file while the aster centroids were interactively selected from the images during the analysis (Figure 3.1(H)). Minimal points were added at the cell periphery (~ 6 -11) to include the peripheral asters which otherwise yields infinite regions (Figure 3.1(B,E,I)). All the points were tessellated using Voronoi module from the SciPy package [196]. From tessellations area, polygon and near neighbour distances were extracted. Each Voronoi region was triangulated [195] using Delaunay module. Area of each region was obtained by summing the area of all the triangles. The polygonality was calculated as the number of edges in the region and the near-neighbour distance was calculated as the distance between the aster centroids that share a common edge. These steps were repeated ten times to randomize the position of boundary points in order to eliminate any effects that may arise due to their positions. For visual representation higher number of boundary points (~ 35) were initialized (Figure 3.1(C,F,J)). All the plots were generated using MATLAB while Voronoi plots in python.

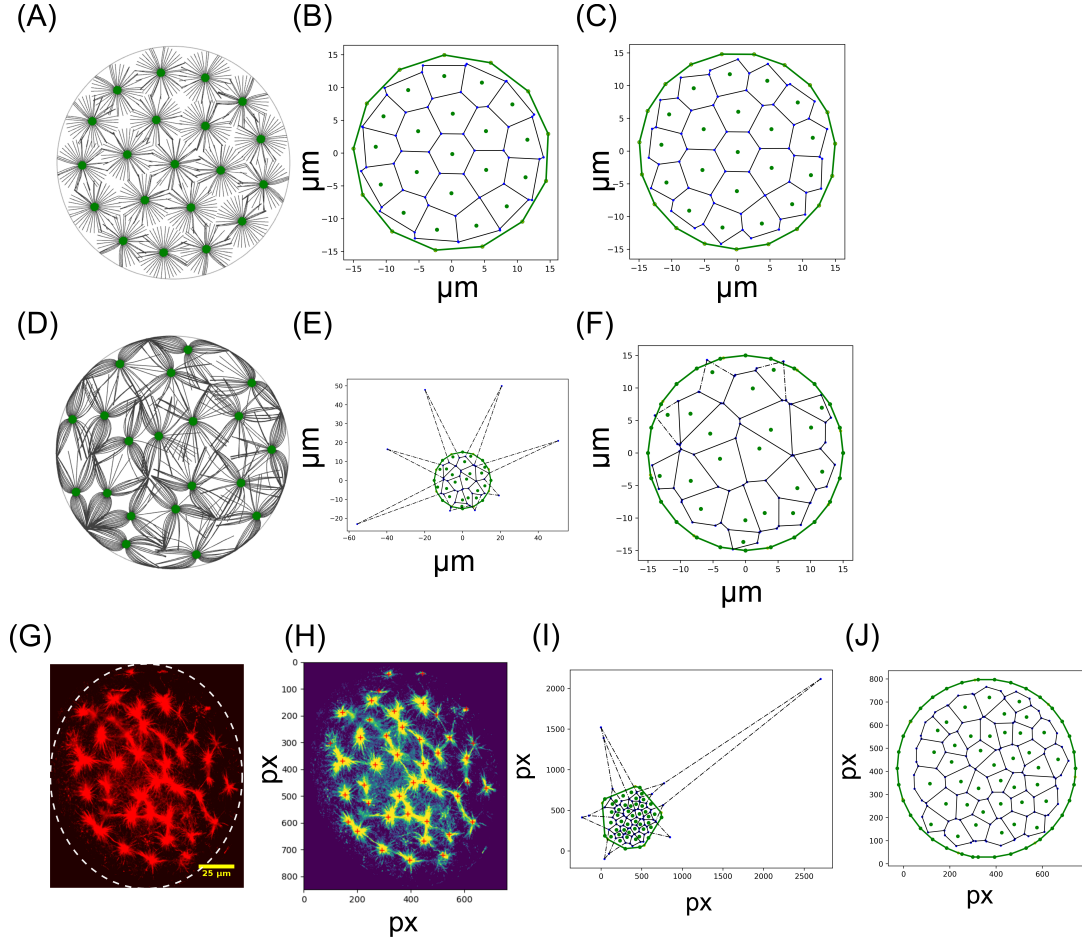


Figure 3.1: Voronoi tessellation of asters in simulations and experiments. (A,D) End point simulation snapshots represent aster positions. (B,E) Aster centroids along with minimal number of boundary points is Voronoi tessellated and its statistics is computed. (C,F) For representation, additional boundary points is initialized and is Voronoi tessellated along with aster centroids. (G) The maximum intensity projected image of cytasters in *Phallusia* oocytes is represented. White dashed line represents the cell boundary. Scale bar corresponds to 25 μm . (H) Aster centroids are interactively selected during the analysis (red crosses). (I) Aster centroids along with minimal number of boundary points is Voronoi tessellated and its statistics is computed. (J) For representation, additional boundary points is initialized and is Voronoi tessellated along with aster centroids.

3.2.6 Computer simulations of collective aster-motor mechanics

All simulations were performed in 2D using cytosim, a C++ based simulation engine based on Langevin dynamics [16] where simulations of motor-microtubule interactions are affected by the thermal energy, $k_B T$ of 4.2×10^{-21} N.m. The simulation parameters used are in Table 3.1.

Model geometry: The cell was modelled as a 2D circular space with a confined boundary. Microtubules on contacting the cortex experienced an inward force. The cortex was modelled as a spring. The restoring force was calculated using Hooke's law where, (k_c) the spring constant determined the stiffness of confinement [9].

Symbol	Description	Value	Reference
δt	Time step	0.001 s	This study
T	Total time	600 s	This study
η	Viscosity of cell	0.05 N·s/ μm^2	[15, 16]
$k_B T$	Thermal energy	4.2 pN·nm	-
R	Cell radius	4 to 40 μm	This study

Table 3.1: Simulation parameters. Simulation environment parameters.

Microtubule and aster mechanics: Microtubules are simulated as flexible rods with a flexural rigidity (κ) based on previous experimental reports [34]. The length of MTs is kept constant during the simulation. In calculations where MTs are dynamic the change in lengths of the MTs are modelled as the two state four parameter model [38] and the parameters are based on the estimates from the *Xenopus in-vitro* studies [14, 15]. The aster is modelled as a radial array of microtubule filaments nucleating isotropically from the center. A central circle is represented in simulations for the visual purpose alone. At initialization, asters distributions are determined by a uniform random density in the cell. Aster positions are updated based on the net forces experienced due to the combination of microtubule dynamics, forces experienced by the MTs at the cortex and the MT-motor interactions [134]. The

MTs are non-interacting in the absence of motor complexes as the sterics interactions have not been modelled. The inter MT interactions are mediated by the activity of motor complexes.

Symbol	Parameter	Value	Units	Reference
N_A	Number of asters	Varied (10,20,40)		This study
N_{MT}	Number of MTs per aster	40 and 100		This study
κ	Microtubule bending modulus	20	pN· μm^2	[45]
δL	Filament unit size	0.5	μm	[15, 16]
L_{MT}	Length of astral MTs	Varied (3,4,25,6)	μm	This study
<i>MT dynamics</i>				
v_g	MT growth velocity	0.196	$\mu\text{m/s}$	[14, 15]
v_s	MT shrinkage velocity	0.325	$\mu\text{m/s}$	[14, 15]
f_c	MT catastrophe frequency	0.049	s^{-1}	[14, 15]
f_r	MT rescue frequency	0.0048	s^{-1}	[14, 15]

Table 3.2: Aster and MT parameters. Parameters of the aster and astral microtubules.

3.2.7 Estimation of transition frequency parameters

The degree of suppression in MT dynamics was mimicked by modulating the rate at which MTs switch between their states while the mean MT length is constant. The two state-four parameter MT dynamic instability model provides a relationship between MT parameters and properties for the centrosome nucleated MTs [38]. The MT parameters: velocity of MT growth (v_g), shrinkage (v_s), frequency of catastrophe (f_c) and rescue (f_r) provide an estimate for mean MT length, $\langle L_{MT} \rangle$ (Equation 3.1).

$$\langle L_{MT} \rangle = (v_g \cdot v_s) / (v_s \cdot f_c - v_g \cdot f_r) \quad (3.1)$$

While the net MT velocity or flux is estimated from the formulation as in Equation 3.2. The f_c and f_r values were obtained by solving the system of two equations simultaneously for varying MT flux values at constant velocity of growth (v_g), shrinkage (v_s) and mean length ($\langle L_{MT} \rangle$) (Chapter 3, Table 3.3).

$$J = (v_g \cdot f_r - v_s \cdot f_c) / (f_c + f_r) \quad (3.2)$$

The parameters obtained are summarized in Table 3.3.

f_c (s^{-1})	f_r (s^{-1})	J ($\mu m/s$)
<i>Stabilized:</i>		
None	None	0
<i>Dynamic:</i>		
20.2683	33.5317	-0.0003
2.0527	3.3273	-0.0028
0.2312	0.068	-0.0279
0.049	0.0048	-0.2785

Table 3.3: Transition frequencies and MT flux rates. The parameters of MT dynamic instability: frequency of catastrophe (f_c , s^{-1}) and frequency of rescue (f_r , s^{-1}) was varied to obtain different MT flux rates (J) at constant value of growth velocity ($v_g = 0.196 \mu m/s$), shrinkage velocity ($v_s = 0.325 \mu m/s$) and mean MT length ($L_{MT} = 4.25 \mu m$). The values were reported previously in [38, 14].

Motor model: Coupling motors such as kinesin (kinesin-5) and dynein are modelled as tetrameric motors that can bind to two MTs simultaneously. Two motor complexes form a tetramer, modelled as a hookean spring with a stiffness constant of (k_m). A complex can bind stochastically (r_a) to the MT if it is within the binding range (d_a). The binding of a second complex is non-specific to the orientation of the MTs i.e. irrespective of parallel or anti-parallel orientation of the MTs. Each complex can walk on the MT with a speed which is linearly dependent on the load

force experienced by the complex (Equation 3.3) [131, 62].

$$v = v_0 \cdot \left(1 - \frac{f}{f_s}\right) \quad (3.3)$$

where, v_0 is the single molecule load free velocity of the motor, v is the force dependent speed of the motor at an instant, f is the load force experienced by the motor and f_s corresponds to the single molecule stall force of the motor. The bound complex can detach stochastically with unbinding rates that increase exponentially with the load force of the motor (Equation 3.4).

$$r = r_0 \cdot e^{(|f|/f_d)} \quad (3.4)$$

where, r_0 is the single molecule unbinding rate of motor under no load, r is the force dependent unbinding rate of the motor at an instant, $|f|$ is the magnitude of the load force experienced by the motor and f_d corresponds to the single molecule unbinding force of the motor. Kinesin detaches spontaneously on reaching the plus end [131] while the unbinding rate of dynein is the same across the MT. Single motor properties used are in Table 3.4.

Symbol	Description	Dynein	Kinesin	Units	References
v	Motor speed	2	0.04	$\mu m / s$	[16, 18] ; [65]
d_a	Attachment distance	0.02	0.05	μm	[16, 18] ; [65]
r_a	Attachment rate	12	2.5	1/s	[16, 18] ; [65]
k_m	Linker strength	100	100	pN/ μm	49 ; [65]
f_s	Stall force	1.75	5	pN	65 ; [65]
f_d	Detachment force	1.75	1.6	pN	[67]
r_o	Detachment rate	1	0.05	1/s	49 ; [65]
r_0^{end}	End detachment rate	1	immediate	1/s	49 ; [65]
D	Diffusion coefficient	10	10	$\mu m^2 / s$	This study
ρ_m	Motor density	1,10	1,10	motors/ μm^2	This study

Table 3.4: Motor parameters. Single molecule motor parameters.

3.3 Results

3.3.1 Modelling collective mechanics of multiple asters and antagonistic motors

Multiple asters (N_A : number of asters) in the computational model interact with one another through complexes of diffusible motors (dynein and kinesin) confined in a circular cell of radius R , with a rigid boundary in a 2D geometry (Figure 3.2(A)). Two kinds of forces drive aster movement: (a) An inward force, F_{MT} and (b) Antagonistic forces due to motor activity.

We model pushing by astral MTs on a rigid cell boundary, based on evidence from experiments demonstrating the mechanical basis of how confined asters spontaneously center both *in vitro* [197, 139] and in cells [70, 198, 199]. This ability to find the center has been modelled to result from an inward pushing force acting on asters [197], based on a standard model of beam mechanics of MTs [34]. MTs bend when they contact the rigid cell boundary, and the restoring forces from all the MTs result in a net force on an aster center pushing it to the cell center (Figure 3.2(B)). The degree of bending of MTs in our simulations is determined by the flexural rigidity (κ) of MTs, the value of which is taken from previous experimental reports (Table 3.2). In large cells with a cell radius ten-fold larger than the aster radius, asters are unable to reach the cell center, since MT pushing at the boundary depends contact with the cell boundary [200, 134]. In such cases, complexes of coupling motors in the cytoplasm have been found to play a major role in centering and organizing asters. Tetrameric kinesins like Eg5 (Kinesin-5) that slide MTs apart [190], and dynein complexes that coalesce MTs [134, 95] form a minimal antagonistic pair of force generators. We model these two motor types as tetrameric complexes of either minus- or plus-end directed motors (henceforth referred to as kinesins and dyneins, respectively), which when bound to two microtubules originating from neighboring asters, produce coupling forces (Figure 3.2(C)). The relative orientation of astral

MTs combined with the type of motor complexes, together determine the forces acting on asters: kinesin complexes separate asters, when walking on anti-parallel MTs, but ‘zipper’ them together, when MTs are parallel. Dyneins on the other hand result in either aster ‘coalescence’, when MTs are anti-parallel or ‘zippering’, if MTs are parallel (Figure 3.2(D)).

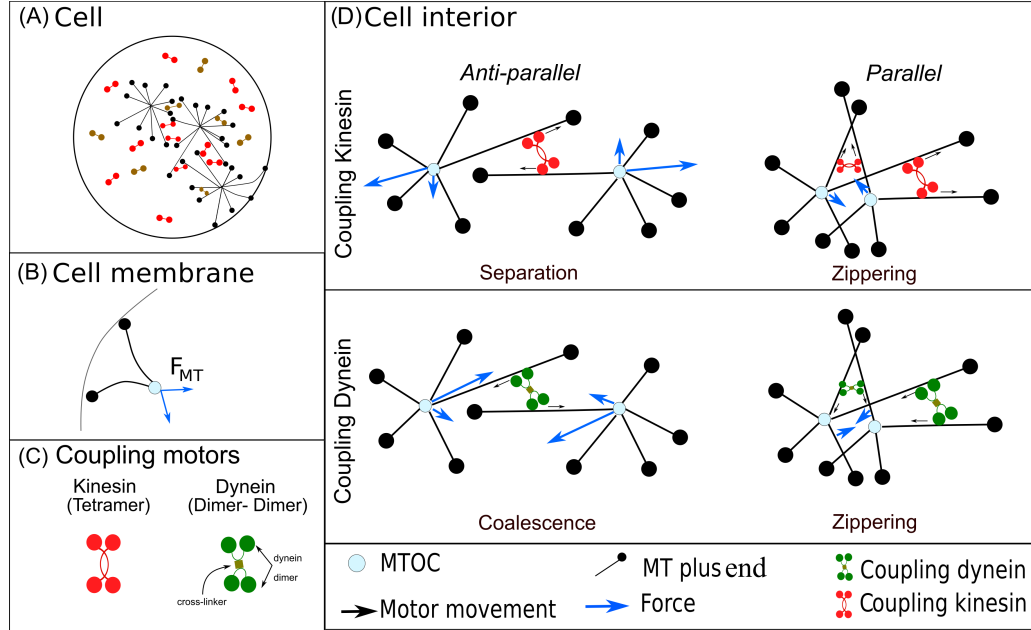


Figure 3.2: Model of multi-aster interactions in a cell. (A) The minimal 2D model of multiple asters in a circular cell consisting of two kinds of antagonistic motor complexes (red and green dumbbell shapes) and microtubules (MT, black lines) arranged as radial arrays or asters with their minus ends embedded in the center and free plus-ends (black circles). (B) Inward forces act on aster centers (blue arrows) due to a restoring force generated when MTs bend (F_{MT}) on contact with the rigid cell boundary. (C) The two kinds of coupling motors- tetrameric kinesin-5 like complexes (red) and dimers of dynein complexed by cross-linkers (green). (D) When two asters are cross-linked by walking motors, forces generated depend on both the type of motor and the orientation of the MTs. Rows: kinesin- or dynein-complexes. Columns: The orientation of cross-linked MTs is anti-parallel or parallel. Motor movement on MTs (black arrows) generates forces on aster centers (blue arrow) resulting in aster separation, zippering or coalescence.

(a) The inward force acting on asters results from a restoring force based on elastic beam theory, that arises due to MT bending when they encounter the rigid cell boundary (Figure 3.2(B)). The magnitude of the inward force F_{MT} is determined by

the variable radius of curvature and filament length and the flexural rigidity κ , characteristic for the material [16] with values taken from previous measurements (Table 3.2). This model of inward forces generated by MTs pushing at the boundary and bending is consistent with previous reports of confined asters that can spontaneously find the center in vitro [5, 139], in cells [199, 198, 70] and in-silico in the geometry of the mouse meiotic oocyte [134] or *C.elegans* first embryonic division [186].

(b) Antagonistic forces are generated by tetrameric motor complexes of either minus- or plus-end directed motors, referred to henceforth as dyneins and kinesins respectively, that bind to pairs of MTs and produce forces on neighboring asters (Figure 3.2(C)). In large cells with radius ~ 10 fold larger than the aster radius, MTs will rarely contact the cell boundary as a result of which MT bending based inward forces are expected to play only a minor role [134, 200]. In such cases, forces generated by cytoplasmic motor complexes that bind to pairs of MTs from two different asters dominate. Tetrameric kinesins have been shown previously to separate linear MTs apart [190] while dynein complexes coalesce linear MTs [95]. In the model, the nature of forces acting on asters due to motor activity depends on the relative orientation of MTs from a pair of asters and the type of motor complex bound to the MTs (Figure 3.2(D)): kinesin complexes are expected to generate separating forces when walking on anti-parallel MTs, but ‘zipper’ them together when MTs are parallel, while dyneins are expected to produce either forces that ‘coalesce’ asters when MTs are anti-parallel or ‘zipper’ them if MTs are parallel.

In such a model, varying the cell size and proportion of motor complexes spontaneously results in different steady state spatial patterns of multiple asters (Figure 3.3), (Video A1,A2,A3). In small cells, asters always find the center of the cell based on pushing forces alone, independent of the type of motors (Figure 3.3, bottom row), consistent with previous models of aster pushing based centering. At the other extreme, in very large cells asters have minimal overlaps, and neither cell boundary nor motor type affects them, analogous to the ‘dilute limit’ in gases where

inter-molecular interaction can be ignored (Figure 3.3, top row). However, at an intermediate cell size and in presence of kinesin-complexes alone, ‘separating’ forces acting on antiparallel MTs result in uniformly segregated lattices of asters that resemble optimal packing with a hexagonal geometry (Figure 3.3, right column). The hexagonal lattice-like arrangement is independent of the number of microtubules per aster (Figure B1). This suggests that aster density and the relative length scales of MTs and cells are important factors that drive these patterns. On the other hand, in the presence of dynein-complexes alone, ‘coalescence’ dominates and local clusters of asters are observed. Finally, motor binding kinetics and diffusion are modelled as stochastic processes, but MT lengths are fixed. Modelling MTs as dynamic filaments that stochastically transition between growth and shrinkage (dynamic instability) results in a break-down of the regular patterns of segregation driven by kinesins, at optimal cell sizes, while dynein driven clustering is enhanced (Figure 3.4). Based on the observations of regular lattice-like patterns of asters in presence of kinesins and their apparent cell-size dependence, we proceeded to analyze if this effect had any relation to more general packing problems. An intermediate cell and aster radius was chosen in the absence of MT dynamic instability to test the scale-dependence of the spatial patterns.

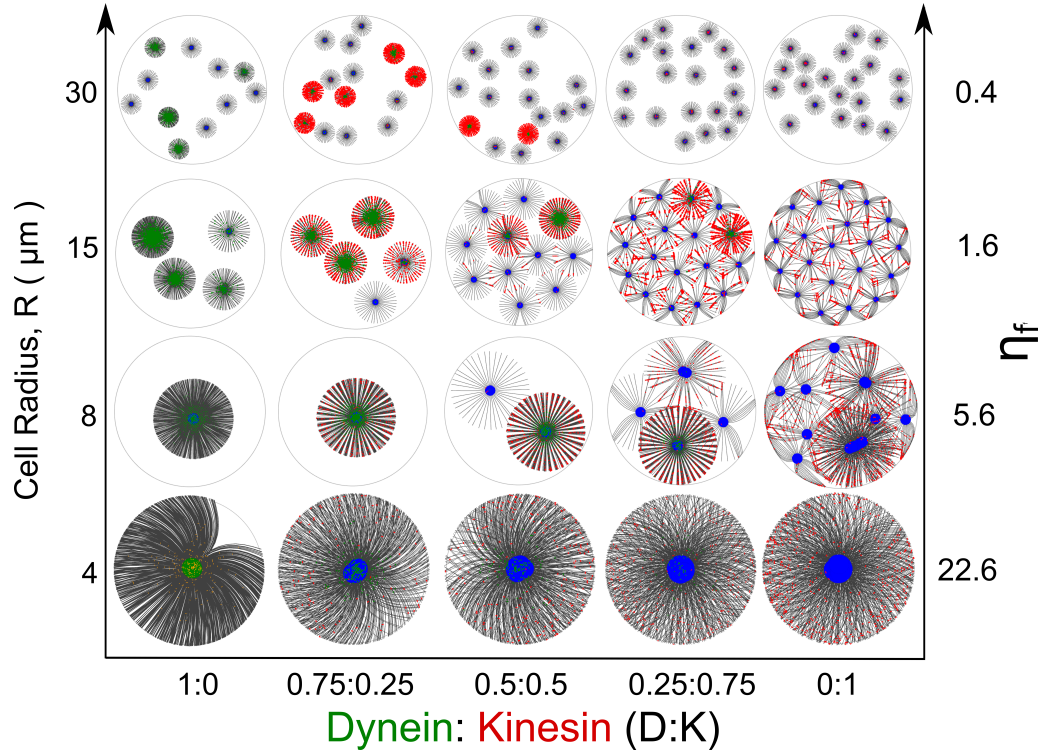


Figure 3.3: Effect of cell size and proportion of antagonistic motors on multi-aster patterns. Steady state outputs of aster (grey) simulations performed with different ratios of kinesin (red circles) and dynein (green circles) motors (x-axis) and changing cell radius, R (μm) (left y-axis) with a constant number of asters (N_A), resulting in differences in packing fraction, η_f (right y-axis) with a constant aster size, determined by MT lengths (L_{MT}). The packing fraction decreases as the cell size (R) increases, L_{MT} and N_A are constant (Equation 3.5). The sum motor density (dynein + kinesin) is ρ_m of 10 motors/ μm^2 . Diffusion coefficient of motors (kinesin and dynein) is 10 $\mu\text{m}^2/\text{s}$, N_A is 20, L_{MT} is 4.25 μm and total simulation time 600 s.

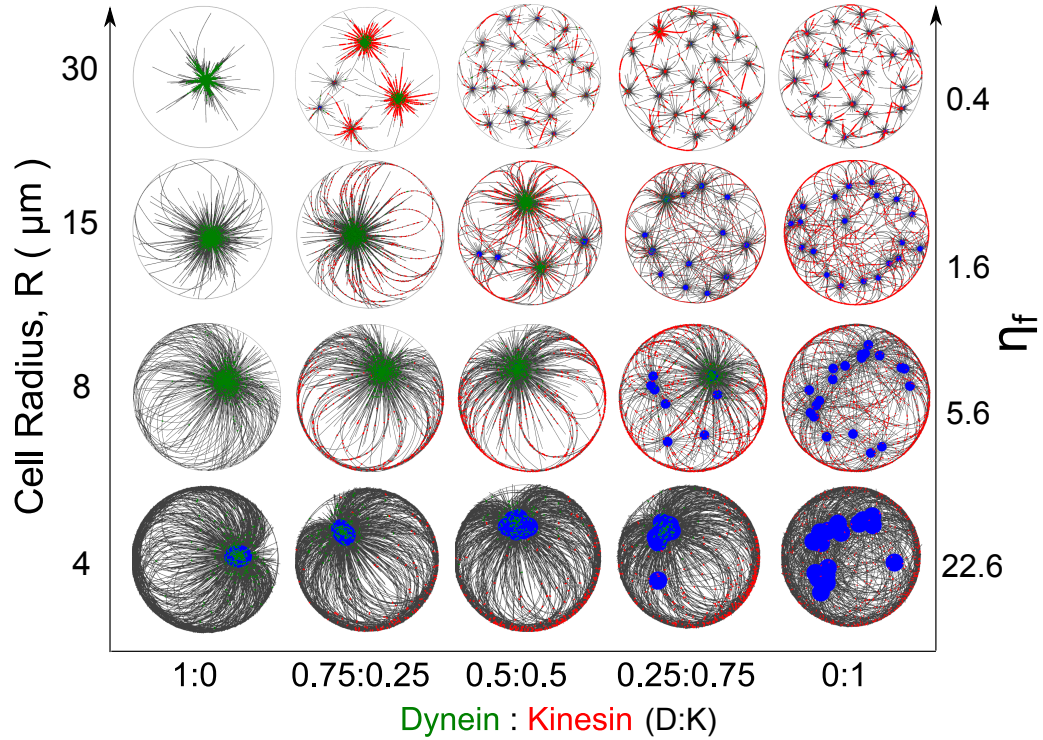


Figure 3.4: Effect of dynamic instability on multi-aster patterns. Results of simulations obtained using variable proportions of opposing motors, kinesin- and dynein-complexes (x-axis) and increasing cell radius, R (μm) (left y-axis) and relative cell size R' (right y-axis). Here L_{MT} is the length of MT filaments, $4.25 \mu\text{m}$. The sum motor density (dynein + kinesin) is maintained constant at $\rho_m = 10 \text{ motors}/\mu\text{m}^2$, while the proportion is varied (x-axis). Motor diffusion coefficients for both motors are $10 \mu\text{m}^2/\text{s}$ and number of asters is 20. Scale bar: $10 \mu\text{m}$ (vertical blue bars).

3.3.2 Packing regularity of asters induced by kinesin complexes at optimal cell size

In contrast to the self-organized patterns formed by linear MTs interacting with motors [120, 121], this model predicts the emergence of steady-state hexagonal lattice-like structures from a mixture of multiple asters and kinesins, in addition to the previously reported aster centration. The hexagonal lattice-like patterns are driven by the separating forces of kinesins, interacting with neighboring asters in

the confinement of a cell. Thus the motor activity appears to result in an effective repulsion of asters to one another, comparable to the interactions driving the arrangement of atoms, cannonballs and circles in a geometric sense. Therefore, we proceeded to compare such kinesin-driven aster packing to the general circle packing problem i.e. what arrangement of uniformly sized circles in two dimensions will maximize the proportion of space covered? J. L. Lagrange proved in 1773, followed by Carl Gauss in 1831, that uniform circles will maximally fill a circular space if they are arranged in a hexagonal lattice, like a bee's honeycomb, occupying 90.69% of the total area being packed (Weisstein, E.W. Sphere Packing. Available at: <http://mathworld.wolfram.com/SpherePacking.html>). Here, each polygon contains the center of the circles being packed and the polygon edges define the lines separating adjacent circles (Figure 3.5(A)). We tested the hypothesis that our segregated asters quantitatively follow the rules governing the hexagonal packing patterns, by assuming the asters of radius corresponding to the microtubule lengths L_{MT} can be treated similarly to circles to be packed. The confining cell boundary has a radius R and kinesin-complexes generate the repulsive force between asters, analogous to the self-avoidance effect of the circle packing problem. We define the packing fraction of asters (η_f) as the ratio of the total area of all asters to area of the cell (Equation 3.5) and estimate the value hexagonally packed asters to be 1.6 (Figure 3.6).

$$\eta_f = \frac{N_A \cdot (\pi L_{MT}^2)}{\pi R^2} \quad (3.5)$$

where, η_f is the packing fraction, i.e. the proportion of area occupied by the circles or asters as a fraction of the total area. The fact that this value is greater than the maximal packing fraction of circles in a hexagonal arrangement, $\eta_f = 0.906$ and exceeds a theoretical value of 1 in terms of area-ratios of contained objects to the confining area, clearly points to the intercalating nature of the asters, as opposed to the rigid, non-overlapping nature of circles. Indeed, for asters to form the patterns, MT overlaps from neighboring asters, i.e. intercalations, are essential for motors to act. To test this hypothesis, we examine the spatial patterns of asters

at steady-state, if N_A , the number of asters, is increased while maintaining η_f of 1.6 (Equation 3.5). In our simple scenario, this can only be achieved by reducing L_{MT} , the length of MTs, i.e. we ask what is the result of aster density increase while aster size decreases, for a constant confining cell size (R). We find that a constant packing fraction for increasing aster density compensated by reduced MT lengths results in asters being arranged in a lattice with the frequency of hexagons dominating, followed by pentagons and heptagons (Figure 3.5(B,C)). This suggests a scaling relation with aster size and density, which is remarkably similar to scaling relations of spindle sizes with changing cell size based on astral microtubule lengths. For instance, previous work has shown decreasing cell sizes during successive cell divisions in early embryonic development of the nematode *C. elegans* and the sea urchin *Paracentrotus lividus* lead to smaller spindles with corresponding reduction in MT lengths [201]. A similar spindle scaling with cell size has also been reported across metazoans [202]. One mechanism by which such scaling might occur has been suggested in syncytial divisions in *Drosophila* embryos in which kinesin-complexes interact with astral-MTs to determine spindle spacing [193]. Indeed recent work with ex vivo reconstitution of *Drosophila* spindles in extracts supports the mechanical nature of spacing of spindles in the syncytium [113]. To test the effect of cell-division, we simulate the effect of halving of cell sizes, while satisfying Equation 1 (Figure 3.5(D)) and find that only if MT length are also linearly scaled (halved), do we observe the emergence of hexagonal packing of the asters (Figure 3.5(E,F)). Indeed either halving MT lengths with constant cell size (Figure 3.5(D), vertical axis) or halving cell size while MT lengths are held constant (Figure 3.5(D), horizontal axis), both result in abrogation of the regular ‘hexagonal’ spacing pattern. This suggests the existence of a unique parameter space (Figure 3.5(D), diagonal and 3.5(E)), that determines regular aster segregation patterns. On systematic variation of the astral MT lengths in a cell of constant size and aster numbers, aster positioning with hexagon dominance is observed upto η_f of 1.8 (Figure 3.6). While simulation predictions are promising idealizations, evidence from experimental condition in vivo could be used to test this theoretical prediction.

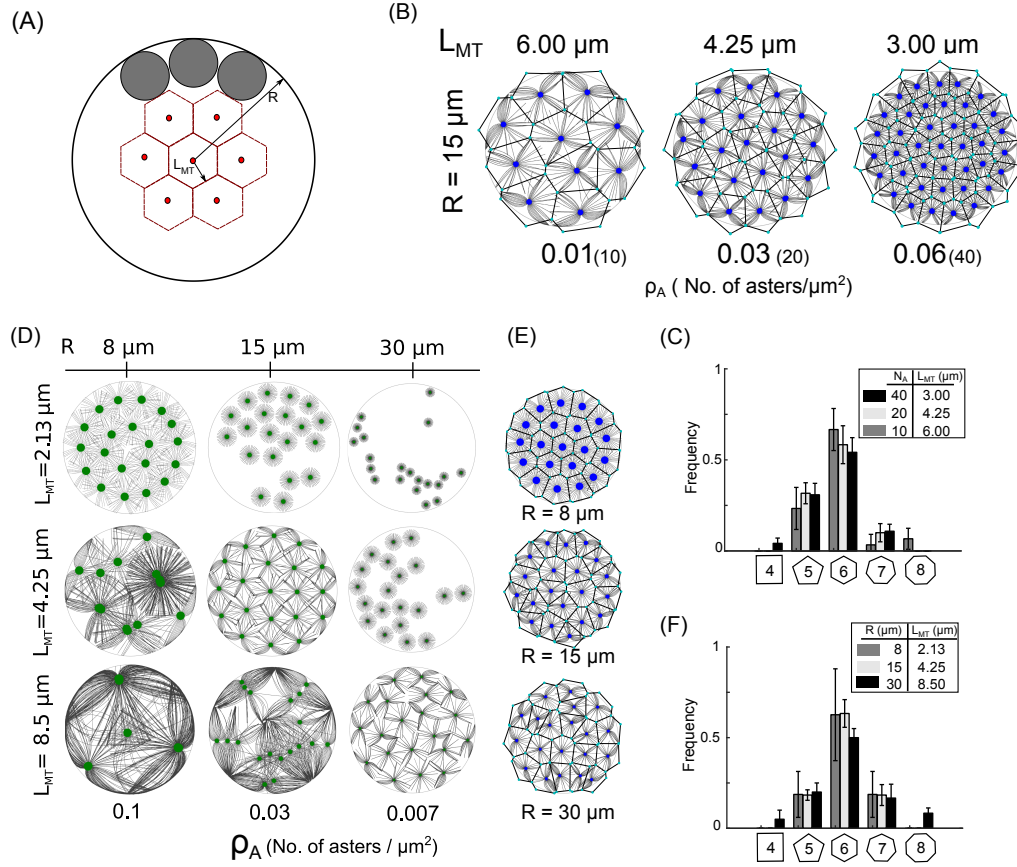


Figure 3.5: Multi-aster patterns in terms of the circle packing problem.

(A) The schematic depicts optimal packing of circles in a hexagonal lattice (red dashed lines) when confined in a larger cell of radius R , with the centers of the circles (red dots) forming the polygon centers. We approximate asters as rigid circles by setting circle radii to the length of MTs (L_{MT}) to estimate the packing fraction η_f . (B, C) The packing fraction η_f of 1.6 results in hexagonal arrangement of asters. To test whether this packing fraction always results in the same patterns, increasing aster numbers, N_A , and proportionately decreasing MT lengths (aster radius) in a cell of constant size ($R = 15 \mu\text{m}$) by observing (B) steady state aster patterns and (C) polygon frequency distributions in simulations. (D) The steady state aster patterns driven by kinesin are observed when cell radius, R (x-axis) and aster sizes, i.e. L_{MT} (y-axis) were independently varied for a constant number of asters ($N_A = 20$) and kinesins. (E, F) The simulation outputs along the diagonal of (D) and (E) with packing fractions of η_f (top to bottom) 1.41, 1.6 and 1.6 were Voronoi tessellated and (F) the polygon frequency distribution plotted. Total simulation time: 600 s, kinesin density: 10 motors/ μm^2 . Aster core (green circle), MTs (grey lines).

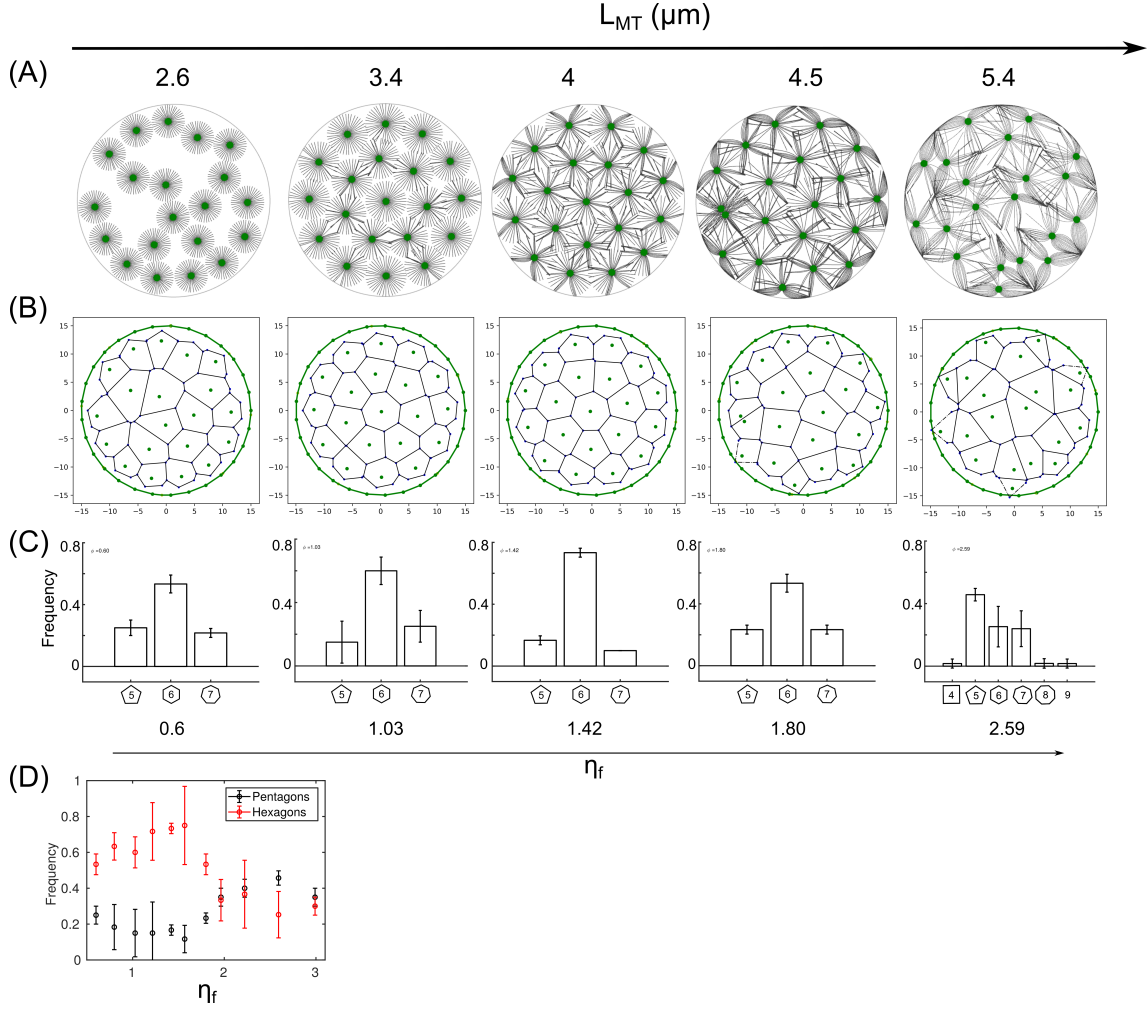


Figure 3.6: Hexagon dominance persists until η_f of 1.8. The packing ratio was varied by increasing the MT length (L_{MT}) at constant cell radius ($15 \mu\text{m}$), kinesin-5 density ($10 \text{ motors}/\mu\text{m}^2$) and aster numbers (N_A). (A) Simulation end-point snapshots, (B) aster centered Voronoi cells and (C) frequency distribution of polygons is plotted for increasing packing fraction, η_f . (D) Mean occurrence of hexagons and pentagons for increasing η_f is plotted. $N_{Runs} = 3$. Error bars indicate s.d.

3.3.3 Inducible cytasters in oocytes recapitulate the computational model

Cytoplasmic asters or cytasters have been previously shown to be induced in oocytes of multiple species, independent of maturation, by drug treatments that work by apparently reducing the critical concentration of polymerization of tubulin. In sea

urchin oocytes (unfertilized eggs) for example, MTs have been observed to transition from loose-networks and spiral arrays to multiple cytasters when triggered by temperature, pH, taxol or hexylene glycol [119, 117, 120]. We find that oocytes of the ascidian *Phallusia mammillata* rapidly form multiple aster-like structures in the cytoplasm when treated with 10 μ M Bi-D 1870, an inhibitor of the ribosomal S6 kinase (RSK) (Figure 3.7). RSK is a component of the MAP kinase pathway, which is known to play a role in suppression of microtubule dynamics in unfertilized oocytes of diverse species [203, 204, 203]. These asters consist of radial MTs with a central γ -tubulin staining observable by immunofluorescence (Figure 3.7(A)), comparable to conventional MTOC asters [205]. The MTs form a regular lattice with bundles between asters that form spindle-like structures even in the absence of chromosomes (Figure 3.7(A)). Voronoi tessellation of these asters using the same approach used for simulated asters (Figure 3.7(B)) results in predominantly hexagons separating the aster centers (Figure 3.7(A)), comparable to our results from simulations. Increasing the concentration of Bi-D 1870 (hereafter, “BiD”) results in proportionately increased bundling of MTs that connect asters (Figure B4(A)), while maintaining the hexagonal pattern (Figure B4(B)), suggesting MT nucleation is enhanced. Consistent with the nucleation effect of BiD, we find increasing the drug concentration leads to a slight increase in the mean number of aster centered Voronoi cells (Figure B4(C)) with a corresponding but slight decrease in area per Voronoi cell (Figure B4(D)). However the statistics of the tessellation indicate hexagons continue to be the most frequent polygons (Figure B4(E)). Thus, we infer that BiD might affect the number of MTs but not their lengths. We find that multiple cytasters also emerge when *Phallusia* oocytes are treated with taxol (10 and 20 μ M) or 10 mM ammonium chloride (Figure B3). While these cytasters do not form spindle like connections as seen on BiD treatment, they can also be tessellated (Figure B3(B)) with the number of Voronoi cells (Figure B3(C)) and the area per cell (Figure B3(D)) varying with taxol concentration, while hexagons are the major polygon (Figure B3(E)). The subtle differences between these results and BiD treatment, could be attributed to the potential role of the RSK-inhibitor in both nucleation and stabilization.

The polygon tessellation of cytaster patterns seen in *Phallusia* oocytes (Figure 3.7) are strikingly similar to results from simulations with kinesin driven multi-aster patterns (Figure 3.6). BiD induced cytasters lose their regular arrangement if oocytes are also treated with monastrol, a kinesin-5 inhibitor, appearing dispersed and irregular (Figure 3.7). Taken together, kinesin is essential for the formation of the regular lattice-like pattern of cytasters in the oocytes, and this is demonstrated in simulations of multiple asters where a similar loss of lattice-like arrangement is observed in response to reducing the kinesin motor density (Figure 3.7(E)).

Thus, the cytaster patterns in BiD-treated *Phallusia* oocytes appear to be self-organized and aster distribution patterns at the scale of a cell (~ 10 micrometers) emerge from the interactions of kinesin motors walking on neighboring MTs at the scales of molecules (~ 0.010 micrometers), over three orders of magnitude. While the role of kinesins in the cytaster patterns are demonstrated in experiment and simulation, the role of MT lengths in the patterning process is less clear and we proceed to examine this by comparing model to experiment.

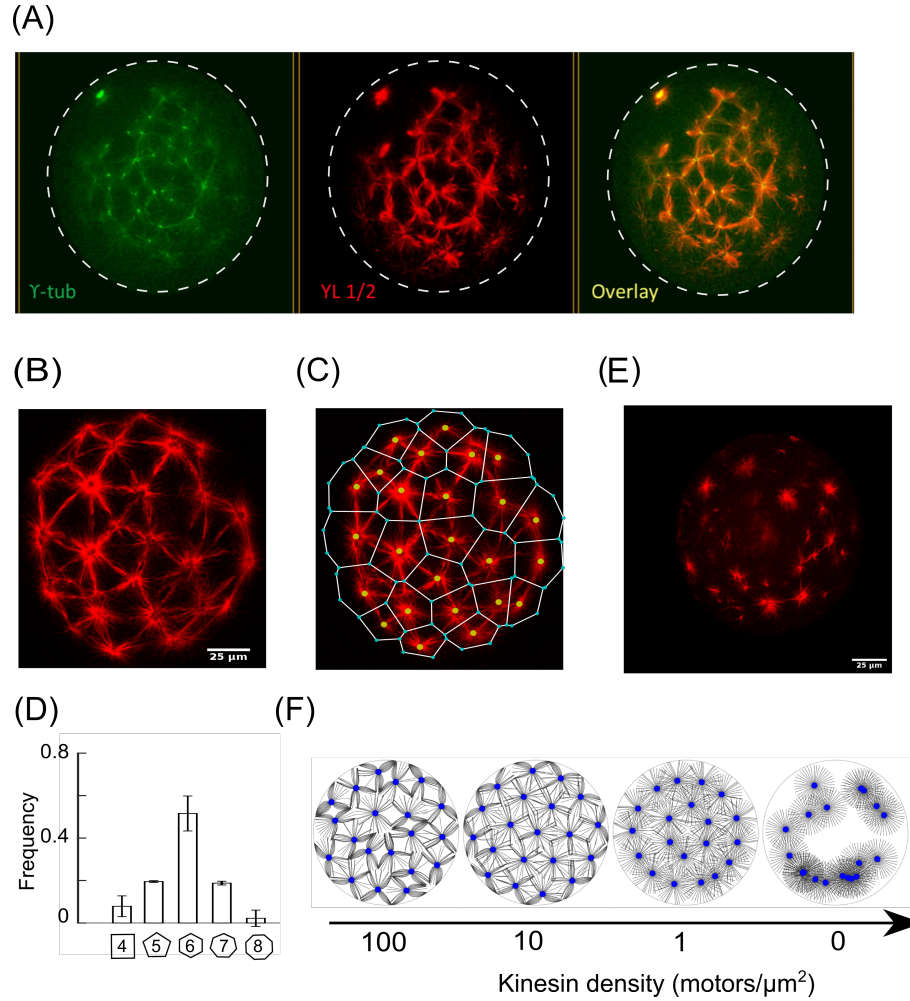


Figure 3.7: Kinesin dependent patterns of BiD induced cytasters in ascidian oocytes. (A) Oocytes of *Phallusia mammillata* treated with 10 μM Bi-D 1870 (RSK inhibitor) were fixed and labelled with antibodies to γ -tubulin (green) and α -tubulin (red), with the overlay image on the right (yellow). The cell outline is marked in white. Z projection of confocal images containing MT structures (surface view); bottom images are magnified regions. (B, C) A representative Bi-D treated oocyte stained for tubulin (B) was Voronoi tessellated (C) with polygon edges (white) separating neighbouring aster centers (yellow). (D) The frequency distribution of polygon sides for the oocyte in (C). (E) An oocyte was first treated with the kinesin-5 inhibitor monastrol and then with Bi-D 1870 and immunostained to label tubulin. (E) Simulation snapshots at steady state for a decreasing proportions of kinesin-complexes represent the positions of multiple asters that have lost the packing pattern. Motor density was varied from 100 to 0 motors/ μm^2 . N_A is 20 μm , R is 15 μm , simulation time 300 s

3.3.4 Evaluating alternative estimates of MT length distribution in BiD treated *Phallusia* oocytes

The in vivo results from ascidian oocyte cytasters validate and confirm the principle of our computational model. We therefore proceed to use the experimental data to further refine the model. One variable in the model is MT length (Table 3.2). Since the lengths of individual MTs cannot be directly measured due to the limits of diffraction, we use the interplay between experiment and theory to determine the best method to estimate MT length. We use two alternative approaches to estimate MT lengths. We then put these numbers into the simulations and compare the emergent tessellation patterns to those observed in experiments. To prevent a potential coalescence of asters due to dynein, we use data from *Phallusia* oocytes treated with BiD and ciliobrevin (CB), a dynein inhibitor (n= 7 asters from 3 oocytes) and tessellate them (Figure 3.8(A)). We find the number of tessellated polygons is the unchanged for BiD and BiD+CB treated oocytes, suggesting a minor role for dynein (Figure 3.8(B)). We therefore estimated MT lengths from BiD+CB treated oocytes since they are more likely to represent the characteristic aster sizes, as compared to BiD alone. Our estimates are based on two alternative models: (i) M1: mean inter-aster distance or (ii) M2: exponential distribution of MT lengths. (i) Mean inter-aster distance (M1): Since many MTs appear to extend from cytasters to neighboring cytasters, nearest neighbor distances (NND) between Voronoi cell centers can be used to estimate the inter-aster distance (Figure 3.8(C)). The mean NND is estimated from averaging the distances (d_i) between tessellated aster-centered cells and MT lengths would then be half the mean NND. (ii) Exponential distribution of MT lengths (M2): MT lengths are exponentially distributed both in vitro [130] and in cytoplasm as seen with asters in *Xenopus* oocyte extracts [206], consistent with predictions from equilibrium polymer theory [207]. Thus, the decay length ($1/b$) of the radial tubulin-intensity can be taken as the mean MT length (Figure 3.8(D)). Based on M1, the NND appears to be normally distributed (Figure 3.8(E)), while

the intensity profile of asters fits an exponential decay, as expected from M2 (Figure 3.8(F)). We therefore have two estimates of L_{MT} of $8.9 \mu\text{m}$ (M1) and $2.34 \mu\text{m}$ (M2) (Figure 3.8(E,F)). The mean estimated MT lengths from the models is $8.9 \pm 2.6 \mu\text{m}$ and $2.9 \pm 1.3 \mu\text{m}$ when measurements were on multiple different oocytes (Figure 3.8(G)). We also expect the differences in length distribution would result in qualitative differences in MTs overlaps between neighboring asters (Figure 3.8(H)).

Using these two estimates as inputs to simulations, while keeping all other parameters of the model constant- aster and kinesin density, cell size and MTs per aster, comparable to values obtained in *Phallusia* oocytes- we obtain aster distribution patterns that are visually indistinguishable for the two different inputs (Figure 3.9(A,B)). However, quantification of the patterns based on tessellation demonstrates differences between M1 and M2 in the distributions of the nearest neighbor distances, NND (Figure 3.9(C,D)) and polygons (Figure 3.9(E)) compared to experiment. We measured the deviation of simulations from experiments using a score ϵ , such that a smaller value of ϵ would indicate a better match to experiments. The metric takes into consideration the frequency distribution of (a) polygon sides and (b) NND based as follows:

$$\epsilon = \sum |e - s| = \sum_i^{N_{polygon}} |e_i - s_i| + \sum_j^{N_{nnd}} |e_j - s_j| \quad (3.6)$$

where, $N_{polygon}$ and N_{nnd} refer to the total number of polygon and NND classes while ‘ i ’ and ‘ j ’ correspond to the data in the i^{th} and j^{th} bin and e and s correspond to the experimental and simulation data respectively. We find the exponential distribution of MT lengths (M2) minimizes the error to experiment (Figure 3.9(F)), as compared to the alternative model M1.

The polygon distribution in simulations with M1 and M2 are comparable and score similarly for the deviation from the experiments unlike the NND distribution. The M1 model corresponds to $\eta_f \sim 0.9$ ($L_{MT} = 9 \mu\text{m}$), consistent with the optimal packing of asters (Figure 3.6). All the asters on an average are equidistant from each

other (Figure 3.9(A)) with a narrow spread in their distribution (Figure 3.9(C)). A similar hexagonal dominance is observed in M2 with $\eta_f = 0.1$ (based on the exponential mean of astral MTs) while the inter aster distance compares better to the experiment in terms of the spread (Figure 3.9(B,D)). The asymmetry in the aster due to the exponentially distributed MT lengths result in variability of inter-aster distances. Although the MT filaments are shorter in M2, the maximal lengths range from 9 to 23 μm (Figure B6(A,B)). As a consequence, based on the lengths of interacting MTs the variation in the inter-aster distances emerge. For instance, asters that are $\sim 9 \mu\text{m}$ apart could have interacting MTs that are 15 and 3 or 9 and 9 μm each resulting in variation and spread in the distribution. We find the exponential distribution of MT lengths (M2) minimizes the error to experiment (Figure 3.9(F)), as compared to the alternative model M1. We therefore retain Model 2 as best representing the in vivo situation for estimation of microtubule length. We then used this approach of comparing experiment and theory to determine the relative contributions of antagonistic motors, dynein and kinesin, in cytaster patterning.

3.3.5 Predicting the effect of motor stoichiometry on cytaster patterns in experiment

The collapse of the central microtubule mass and the presence of fewer and larger asters when *Phallusia* oocytes were treated with monastrol followed by BiD confirm the indispensable role of kinesin-5 in aster spacing in the ooplasm. When kinesin-5 is absent, asters appear to fuse forming clusters (Figures B5 and 3.7(D)), and do not form the ordered patterns observed when kinesin is present (3.7(B)). Such an effect is observed in simulations when dynein motors are also included (Figure 3.3). Taken together, it suggests that the coalescence of cytasters in the absence of kinesin-5 could be due to the activity of coupling dynein-complexes. In order to understand the likely in vivo stoichiometry of dynein, images of oocytes treated with BiD alone were tessellated (Figure 3.9(A)) and compared to simulations of asters

with kinesin to dynein stoichiometry (K:D) ranging from 1:0.5 to 1:10 (Figure 3.9(B-E)). While the mean NND values obtained from analyzing simulation outputs for different K:D values do not vary greatly (Figure 3.9(F)), the frequency distribution for K:D of 1:1 matches experiments more closely (Figure 3.9(G-J)). This is also reflected in the frequency distribution of polygons, with K:D of 1:1 resembling experiments better than other K:D ratios (Figure 3.9(K)). Finally, the number of Voronoi cells decreases in simulations with increasing proportion of dynein, due to increased aster coalescence (Figure 3.9(L)). Using the deviation metric described in Equation 2 we conclude that an equal stoichiometry of antagonistic motors agrees best with experiment (Figure 3.9(M)).

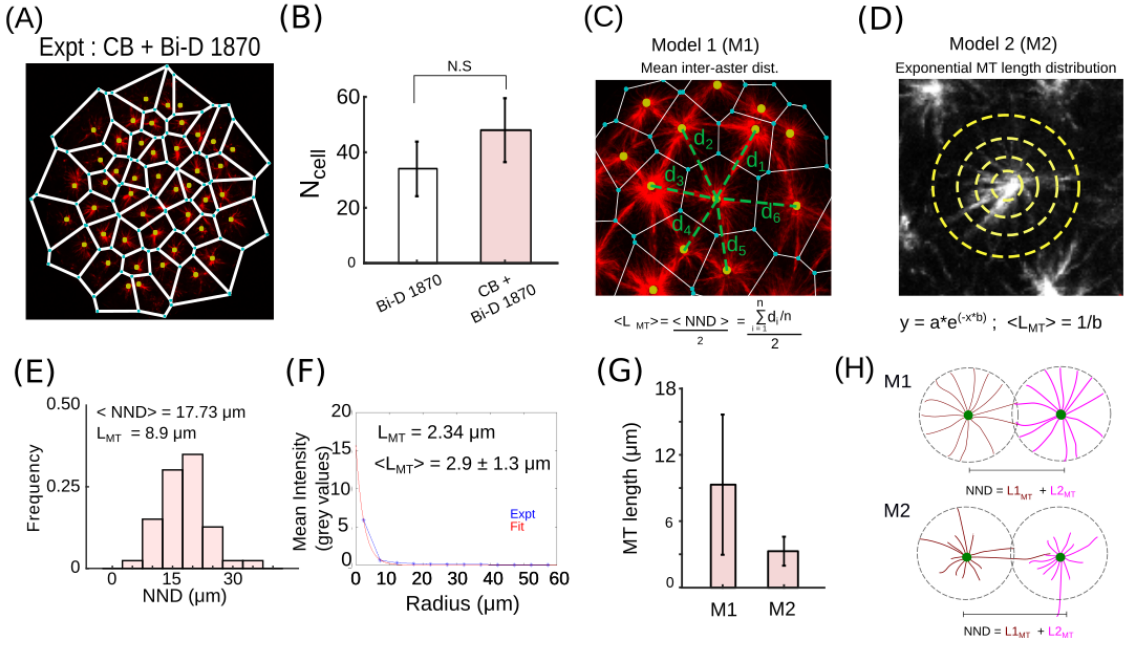


Figure 3.8: Two alternative models to estimate microtubule length. (A) A representative image of a *Phallusia* oocyte treated with BiD and ciliobrevin (CB) was immunostained for tubulin (red) and Voronoi tessellated (edges: white lines, centers: yellow dots). (B) The number of aster centered Voronoi cells, N_{cells} estimated from BiD cells that undergo treatment alone ($n=2$) or BiD+CB ($n=3$). N.S.: the difference between the means is not significant. (C,D) Aster sizes were estimated from BiD+CB oocytes based on two alternative models M1 and M2. (C) M1: Mean MT length $\langle L_{MT} \rangle$ is half the mean nearest neighbor distances (NND), which is estimated from d_i ($i=1$ to n), the distances (green dotted line) between aster centers (yellow circles) for pairs of neighbouring tessellated Voronoi cells (white lines). (D) M2: Mean radial fluorescence intensity from the concentric bins (dashed yellow lines) is fit an exponential function with the decay length $1/b$ used as the mean aster radius. (E-G) $\langle L_{MT} \rangle$ is estimated from (E) M1 based on the frequency distribution of NND and (F) and M2 based on the exponential fit (red) to experimental data (blue) of the radial intensity profile of tubulin (oocytes = 3, $N_A = 7$), resulting in (G) $8.9 \mu\text{m}$ (M1) and $2.34 \mu\text{m}$ (M2). The inset represents the mean MT lengths. (H) While uniform MT lengths predict multiple and symmetric overlaps between neighbouring asters, exponential MT lengths predict sparse overlaps and anisotropy in MT asters.

Thus, we find the minimal model can predict the roles of two kinds of motor complexes- kinesin and dynein, the exponential MT length distributions and MT

dynamics in generating highly ordered patterns of cytasters in BiD *Phallusia mam-millata* oocytes.

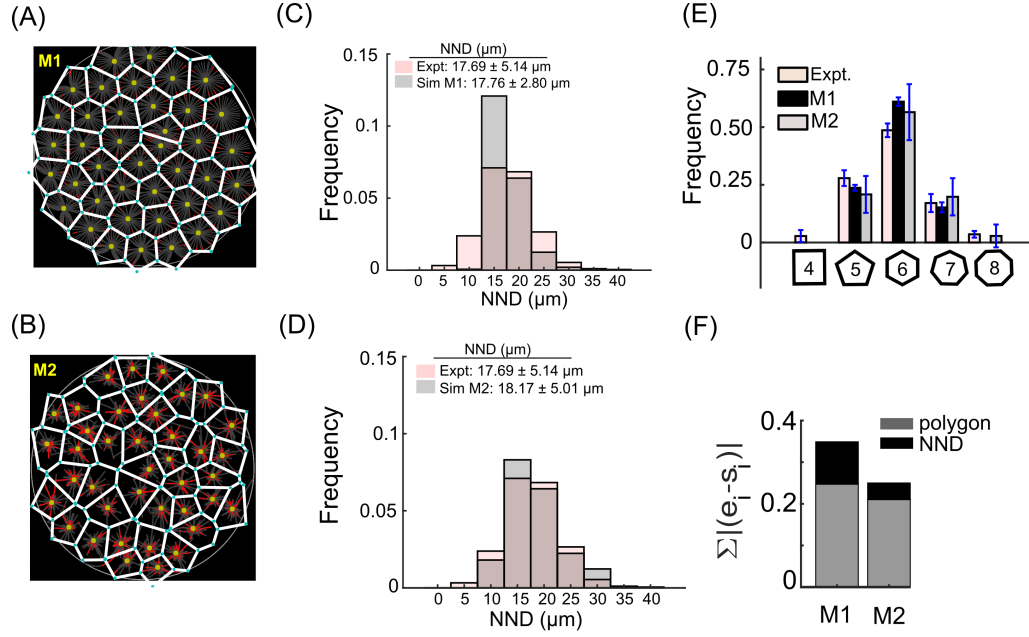


Figure 3.9: Reconciling the in silico effect of MT lengths on aster patterns with those in vivo. (A,B) Simulation output aster centers were tessellated from simulations performed using MT lengths obtained from either (A) M1 or (B) M2. (C,D) Tessellation was used to compare the frequency distribution of NND from in vivo data (Expt) to simulation outputs based on (C) M1 or (D) M2. Gray bars: simulations. (E) Frequency distribution of polygon sides (mean ± s.d.) from experimental data (n=3) is compared to simulation results with M1 or M2 as inputs (n=3). (F) Sum deviation scores (ϵ) quantify the deviation of outputs of the two simulation conditions M1 and M2 from experiment based on polygon (gray) and NND frequency (black) distributions. Simulation results were compared for the same values of number of asters $N_A = 40$, cell radius R of $60 \mu\text{m}$ and the kinesin density of $1 \text{ motor}/\mu\text{m}^2$.

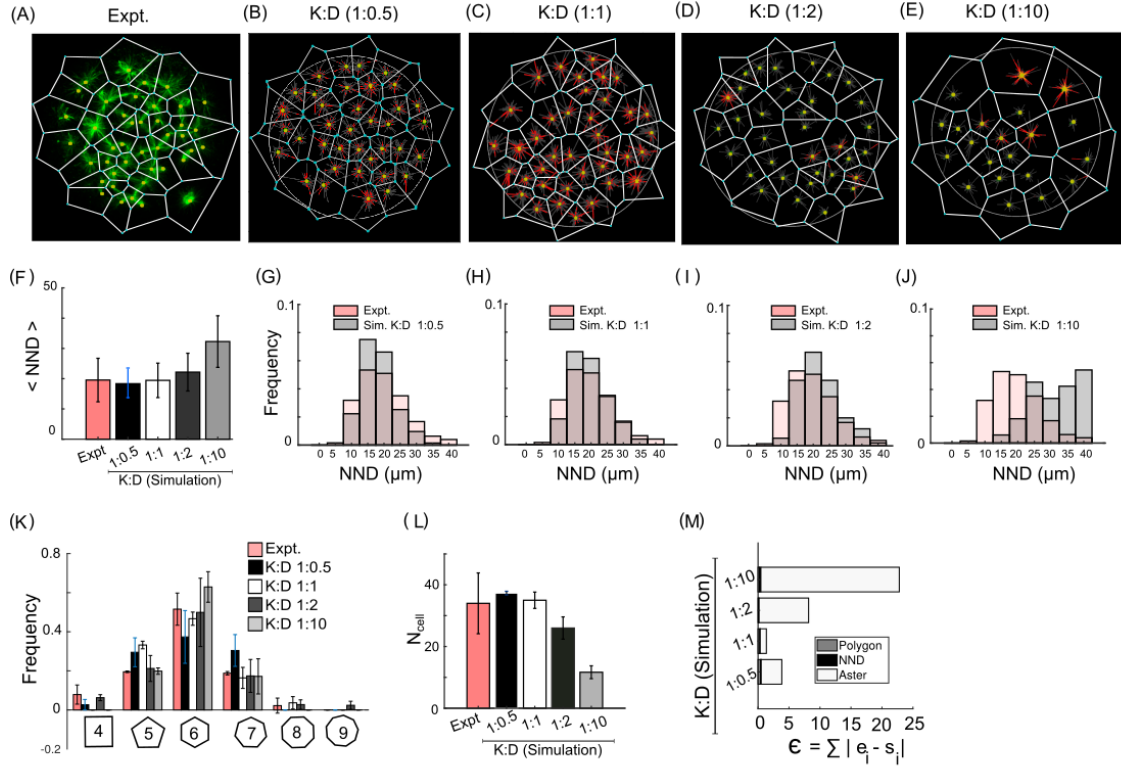


Figure 3.10: Predicting dynein proportion by fitting simulations to BiD treated oocytes. (A-E): Aster positions indicated by overlays of Voronoi cells (white lines) on (A) BiD treated *Phallusia* oocyte, or (B-E) simulation end points obtained with varying ratios of kinesin to dynein (K:D). The simulation outcomes are compared to the experiment (Expt) for (F) mean NND and (G-J) distribution of NND, (K) polygon distribution, and (L) number of aster centered cells (N_{cells}). (M)) The absolute sum deviation between experiment and different simulation scenarios calculated over number of aster centered cells, polygon and NND distribution. Total simulation time: 600 s, N_A : 40, N_{MT} : 100, R :60 μ m.

3.3.6 Interplay between degree of MT dynamic suppression and motor activity

The molecular effects of RSK inhibition is not well understood. It is speculated that it enhances MT nucleation and stabilization due to the patterns that emerge in the oocytes are similar to that obtained from taxol perturbations, which is known to slow down the MT dynamics. The centrosome nucleated astral MTs are also known

to follow an exponential distribution at equilibrium due to the dynamic plus end [38]. Since, the cytoplasmic asters in the *Phallusia* oocytes do contain central γ -tubulin foci and form stable steady state patterns on perturbation of RSK pathway we hypothesized that suppression of MT dynamics is essential and not complete stabilization which may not be physiologically relevant. Thus we explicitly tested for the effect of lowering the rate of MT dynamics on aster positions in the presence of kinesin-5 motors (Figure 3.11). With increasing flux rates (J), asters tend to organize themselves irregularly. While in the presence of kinesins and dyneins clustering of asters is observed as expected in (Figure 3.4). This further confirms that cytasters formed are non dynamic or have very slow dynamics thus the length distribution tends towards the exponential with emergence of stable hexagonal domains in time. This further forms the basis as to why the comparison of experimental data treated with BiD-1870 and observed after 60 minutes to simulation outcomes from exponentially distributed non-dynamic MTs at the end of 10 minutes is reasonable. Along with MT lengths and degree of MT dynamics the motor activity also influence the position and number of asters in a cell.

3.4 Conclusion and Discussion

Here, we have developed a mechanical model of pattern formation by MT asters driven by antagonistic motor complexes in a rigid boundary. These patterns appear to be influenced by cell size. In simulations, asters interacting with kinesin-complexes form a hexagonal lattice at steady state that is related to the patterns seen in the more general circle packing problem. The hexagonal patterns are observed upto the packing ratio of 1.6, higher than that achieved for optimal packing of rigid circles. This can be understood to arise from the intercalating nature of asters, as compared to ‘hard shell’ interactions of circle packing. Indeed the aster arrangements are sensitive to the packing fraction, as seen when either cell sizes were reduced to mimic cell division, or MT lengths increased. This is in turn consistent with the scaling

of spindle size with the size of the cell observed in embryogenesis. We find MT asters in oocytes of the marine animal *Phallusia mammillata* to form qualitatively similar patterns of cytoplasmic asters or cytasters in hexagonal arrangements, when the oocytes are treated with a ribosomal s6 kinase (RSK) inhibitor BiD. Confirming the predictions of the minimal model, we find monastrol based inhibition of kinesin-5 results in a disruption of the patterns of aster organization. Using comparisons with in vivo observations, two aspects of the computational model are refined: the effect of MT lengths and the role of antagonistic dynein motors. This study shows exponential MT length distributions in simulations fit the statistics of cytaster patterns better than uniform MT lengths. Additionally, the model demonstrates the need to include an equal proportion of dynein complexes that antagonize the kinesin complexes in simulations, based on optimization to cytaster statistics obtained from experiments.

While self-organized pattern formation by linear MT filaments and motors have been widely reported [3, 4, 61], it remained to be seen if asters can self organize. Previously we have shown that asters transported by immobilized motors of either plus- or minus-ended nature, i.e. a gliding assay, experience a tug of war due to their inherent radial geometry [15, 134]. Reports of a kinesin gliding assay with antiparallel MT doublets (comparable to a "1D aster") demonstrated how a tug-of-war due to geometry was resolved through length asymmetry resulting in a phase-transition in transport velocity [167]. Experiments that demonstrated how single asters find the geometric center of artificial cells without any signalling, through MT bending mechanics and length dynamics [5, 139] in combination with cortical force generators like dynein [6] have improved our general understanding of the role of mechanics in aster spatial patterns in small and large cells [200]. Indeed, to position asters large cells require the additional activity of immobilized motors [8], gradients of MT regulators resulting in asymmetric pulling forces [15, 79, 40, 14], dynein-like motors localized on intracellular organelles [76], diffusible dyneins that cluster asters [10] and diffusible kinesins that separate astral MTs [113], amongst other

mechanisms. We have modelled the self-organized ability of multi-aster systems to form patterns without spatial heterogeneity based on a few of these mechanisms—clustering dyneins, separating kinesins and MT mechanics interacting with the cell boundary and find the model can explain the structures seen in a large cell, BiD treated oocytes of *Phallusia*. In addition to this system, the model could have some relevance to understanding the spindle alignment and spacing in syncytial systems such as the embryo of *Nematostella* [208] and the *Drosophila* embryo, in which aster spacing patterns show a similar dependence on MT length [193]. In the future a more complex model that invokes spindle mechanics could be used to address the quantitative nature of scaling of spindle separation with division and MT lengths, with some relevance to other syncytial systems.

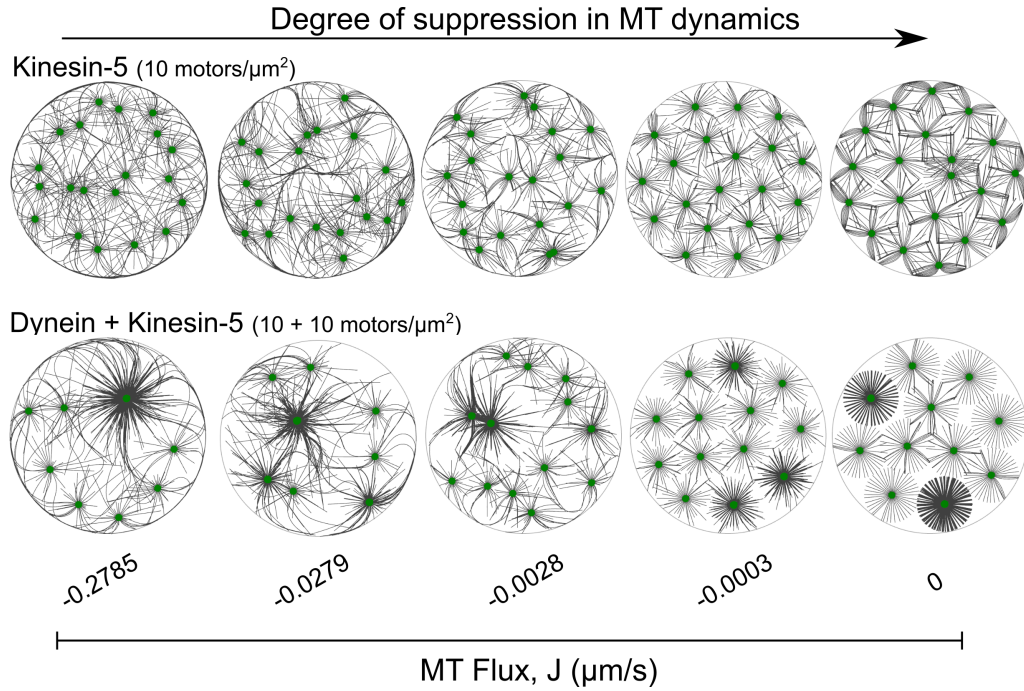


Figure 3.11: Degree of MT dynamics dictate aster-positioning in simulations. In a cell of constant size ($R = 15 \mu\text{m}$) with constant number of asters ($N_A = 20$), rate of MT fluctuations (i.e. Flux, J) was varied. Simulation snapshots represent position of aster centroids at the end of simulations in the presence of (a) only kinesin-5 motors and (b) dynein and kinesin-5 motors. The range of values scanned and the corresponding transition rates are included in Table 3.3.

MT lengths play an important role in cell physiology as seen in their regulation between interphase and mitosis. From equilibrium polymer theory we expect MT lengths to be exponentially distributed [207], with a high number of short filaments. Due to the aster geometry, with area depending quadratically on filament length, short filaments nucleated from aster centers cannot be resolved in light microscopy. Instead they produce a diffraction limited bright spot at the center, often used to identify asters in images. Previous work modelling MT length distributions in asters predicted that whether MTs were radially nucleated or grew as branches from pre-existing MTs, they would be indistinguishable in microscopy images [209]. We find that cytaster lattice patterns- quantitated by measurements of polygon sidedness distributions and aster spacing- observed in BiD treated *Phallusia* oocytes in this study are better explained by a model of exponential rather than uniform length distributions. This result appears to suggest that MT lengths of asters that make up the lattice arrangement are consistent with the expectations from equilibrium polymer theory, even though BiD treatment appears to enhance nucleation, stabilization and bundling processes. Indeed, the consistency of polygon statistics of oocytes that were treated with taxol or ammonium chloride (FigureB3), with those of BiD treated oocytes appears to validate our model predictions, since taxol is known to result in exponentially distributed MT lengths [130]. It remains to be seen the biochemical basis of how BiD inhibition of RSK activity drives cytaster formation.

The activity of antagonistic molecular motors in driving MT patterns has been implicated in the intrinsic ‘self-organizing’ ability of such collective interactions [190, 210] and shown to be relevant to acentrosomal spindle assembly in generating a bipolar structure [211, 212]. This suggests that in the presence of a pair of centrosomes during mitotic spindle assembly, where bipolarity is ensured, MTs may be less likely to self-organize. However, MT-motor mixtures do have an intrinsic ability to ‘self-organize’ mixtures of motors and multiple asters, as seen in previous work on mouse meiotic spindle assembly [10]. In contrast to the spontaneous patterns of linear MT

filaments and motors in confinement: semi-asters, asters, vortices- formed in experiment [3, 4, 60] and predicted in simulations [3, 210, 62]- we find distinct patterns produced by multi-aster systems and motors; coalesced asters, centered structures, hexagonal lattices, polarized asters and vortices. We observe a few of these patterns in the *Phallusia* oocyte system and it remains to be seen if other predicted patterns will be observed in other experimental systems.

Thus, in this study a a mechanically detailed model of radial MT arrays or asters, interacting with motors has been developed. In presence of kinesin-complexes, the system self-organizes into a hexagonal lattice which tessellates the confining space of the simulated cell. We find cytasters induced in large oocytes from *Phallusia* produce similar kinesin motor driven patterns of cytasters in a hexagonal lattice. Through the model, MT length distribution in the experimental system is predicted to be exponential and requiring a proportion of antagonistic dynein activity. Ther model simulations not only quantitatively reproduce the experimental patterns, but also predict a novel pattern forming system, based purely on dissipative mechanics of motors acting on MT asters in confinement, that provides a special case of the general ‘circle packing problem’. We believe these results will help drive further experimental studies in diverse cellular systems and result in an improved understanding of the role of cytoskeleton-motor collective mechanics in sub-cellular pattern formation.

3.5 Outlook

In this study the kinesin-5 is modelled as a simple plus end directed motor and doesn’t have binding preference towards parallel or anti-parallel MTs as reported in some studies. The multi aster patterns that emerge from kinesin-5 and aster interaction is consistent with that of a pair of two MTs. The sliding activity of kinesin-5 is a consequence of the MT orientation and motor polarity thus, there is no need to bias the binding or implementation of trans-activity in the model.

MT bundling, sliding and zippering emerge as a consequence. Surprisingly, this model also recapitulates the *in-vivo* cytaster patterns in experiments. However, in some systems such as yeast cross-linkers like Ase are known to cause bundling and sliding of interacting MTs during the spindle assembly. Thus it would be essential to explore the effects of diffusible cross-linkers during the kinesin-5 driven spatial patterns formed by the asters. The type and properties of the diffusible cross-linker can be studied in simulations. This would provide insights whether the motor and cross-linker activities are redundant *in-vivo* or complimentary to each other. It will also allow to dissect the possible molecular mechanism by which RSK inhibition results in such patterns in the presence of motors and other molecular associated proteins, if any.

During the acentrosomal spindle assembly in the mouse embryos, convergence of multiple small asters is driven by combined activity of kinesin-5 and dynein [18]. Thus it opens questions about the mechanistic details of directional movement of multiple small asters in the presence of antagonistic motors in large cells. How their proportions in space and time along with the relative scales of asters and cells result in different motility or static patterns which may have biological implications. In addition, the antagonistic motors in cells may not be present uniformly in space and time. The asymmetry in their spatial distribution can be explored which would also provide insights on assembly of acentrosomal spindles.

Interestingly, in *Drosophila* embryonic syncytium, multiple non-cellular centrosome associated nuclei exists in regular order in the absence of membrane defined cells [193]. Activity of antagonistic motors was shown to be essential for maintaining spindle positioning in these embryos [191] and recently kinesin-5 was identified in the interphase responsible for maintaining the regular order between the nuclei [93]. This current system can be extended to address the mechanisms of cortical migration of the nuclei to the cell periphery from the center which is not clearly understood. *In-vitro* reconstitution studies showed that length scale of spindle separation is conserved across the species of *Drosophila* and is set by aster migration [113]. Thus the

current set up can be used to address the cortical migration of nucleus associated asters and their positioning in the syncytium system. This work can be further extended to understand the spatial dynamics of aster associated organelles and spindle positioning. Additionally, the role of vesicles and lipid droplets in the embryo can be explicitly tested in simulations. It further, opens questions on the effect of cell shape and boundary effects/processes on aster positioning and packing.

In this work, we show rate of MT dynamics does effect length distribution of astral MTs which in turn leads to self-organization in the presence of molecular motors to form spatial patterns. However, in experiments we do not yet know the exact mechanism by which RSK inhibition in the *Phallusia* oocytes result in emergence of such patterns. The microtubule-mechanics can be integrated with the biochemical pathways to understand and predict the molecular details of the process. Such a understanding would provide insights based on which the possible drug targets can be designed, help us understand the evolutionary implications of such biochemistry and might provide insight on the mechanism by which in some systems MT-motors are not effected by certain agents like taxol, BiD-1870. In past, MT-motor mechanics have been integrated with biochemical pathways using reaction-diffusion systems [14, 213]

Here, we have studied the patterns that emerge at the steady state. It would be interesting to explore the effects of perturbing the system and to ask whether the system can re-organize itself. In this study, the phenomenon in experiment and simulation has been studied and analyzed under nearly steady state condition. However, it would be interesting to follow the time course which will provide information on kinetics and time scales. Such understanding will have implications in the applied sciences wherein dose, time and effect of MT suppression and motor inhibition can be monitored and translated into possible drug targets. The extent and time of motor inhibition, recovery and MT dynamic suppression can help develop a series of sequential events which can shift the equilibrium of the system and slow down the entire MT-motor driven processes.

In this work, we show the effect of antagonistic motors: dynein and kinesin-5 complexes that are diffusible in the cell. While, motors anchored to the organelle surfaces or cortex can be modelled as immobilized motors. The preliminary results suggest in presence of cytoplasmic kinesin-1 motors and dyneins, due to motor polarity, aster geometry and boundary effect polarization of asters is observed when MTs are non-dynamic which is much more pronounced in the presence of MT dynamics (Figure 3.12). It remains to be tested if combination of these motors act in the *in-vivo* scenarios especially in the cases where cortical cytasters are observed in the absence of cortical dynein motors.

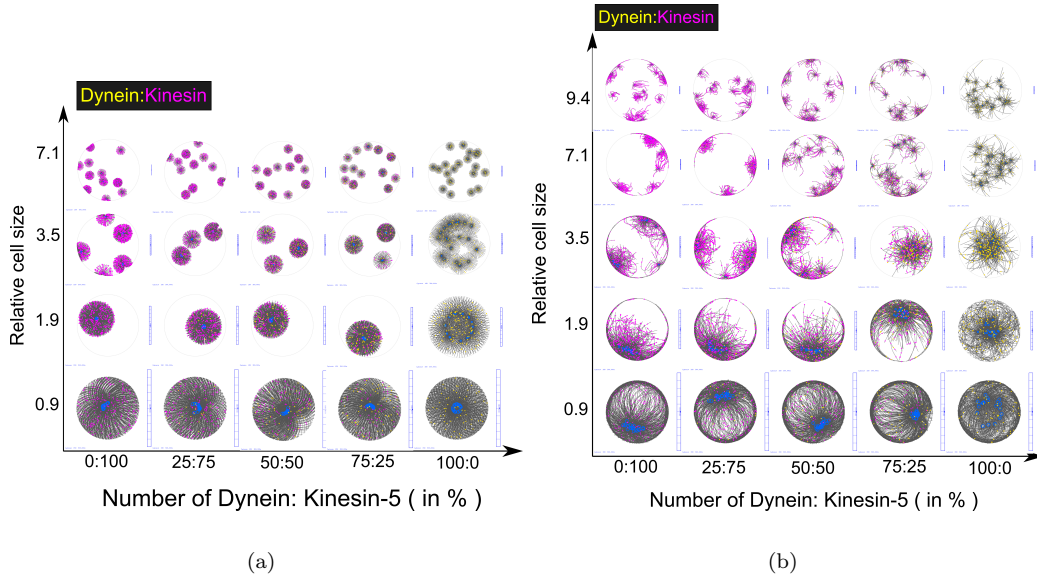


Figure 3.12: Effect of immobilized motors on clustering and polarization of asters. Dynein motors (yellow) and kinesins (purple) were immobilized in the cytoplasm at varying proportions across different cell sizes keeping motor density at $10 \text{ motors}/\mu\text{m}^2$. The end point of simulations are compared when MT lengths (a) are fixed and (b) fluctuating. The number of asters is 20 and cell radius is $15 \mu\text{m}$.

Chapter 4

**A microtubule-motor model for
emergence of self-organized
multi-aster rotation in confinement**

4.1 Introduction

Self-organized pattern formation resulting in the emergence of order from simpler interactions of many individual components at an underlying level of complexity is observed almost universally in biological systems [214, 215]. Such patterns range in scales from swarming birds and bee honeycombs [216], through collective cell migration [217, 218, 219, 220, 221, 222] and spontaneous cell polarization by biochemical networks [223, 224, 225] to self-organized patterns seen *in vitro* in mixtures of microtubules (MT) and motors [3, 61, 4, 226] and actin and myosin [227, 228]. The combination of MT-motor activity and circular boundary conditions has been shown to produce a breaking of symmetry in such systems to produce vortices [3, 65]. Evidence that such vortices are not restricted to *in vitro* reconstituted systems, has come from *in vivo* studies of *Caenorhabditis elegans* [229] and *Drosophila* [230] embryos during development and the cells of the plant *Chara* [231]. A general model of self propelled particle (SPP) motion has been proposed for such collective motion by Vicsek that is qualitatively comparable to cytoskeleton-motor mixtures [19]. The role of specific molecular interactions of the components, could however result in deviations from the general properties of such SPP models. The relative ease of control over and mechanistically detailed understanding of the components, compared to whole cells, has resulted in multiple studies demonstrating the emergence of higher-order patterns of *in vitro* reconstituted microtubule-motor systems [3, 65]. Detailed models of self-organized patterns of cytoskeleton-motor formed by linear MT filaments in presence of kinesin [3, 61, 4] or dynein [226] and filaments of actin with myosin activity [227, 232, 228] have been well studied. However, radial MT arrays or asters will form similar patterns, since motor-based transport is expected to result in a tug-of-war due to the radial geometry of the filaments [167, 15, 134]. A systematic examination of the ability of such aster-like geometries to self-organize due to the activity of motors remains to be explored.

The control over copy number of centrosomes that nucleated asters is an important

regulatory process in animal cells, and a dysregulation results in chromosome segregation defects as seen in some cancers [121]. Additionally MTOC asters in meiotic oocytes [10, 17] and astral MTs of spindles in syncytial embryos [113] also the effect of motors and MT dynamics on aster positioning. Thus, an improved understanding of the effect of collective mechanics of asters on spatial organization is relevant to *in vivo* scenarios in development and disease.

Here, we develop a numerical model of semi-flexible MT arrays that are nucleated from simulated centrosomes. We model the effect of diffusible kinesins that serve to locally couple the asters, cortically localized dyneins that provide an outward force and noise in the form of experimentally reported dynamic instability parameters. We find, akin to general classes of swarming models, our system can undergo spontaneous rotation. The specific details of our model deviate from the simpler generalization, and aspects of diffusion limitation and density effects suggest a novel class of collective motion.

4.2 Model

Components and interactions: A multi-aster system in a cellular compartment in the overdamped regime with mechanics and stochastic binding kinetics of kinesin and dynein (Figure 4.1) was modelled using a previously developed computational agent-based model of MT-motor interactions [134] in Cytosim [16]. The MTs emanate from MTOC of as aster and undergo dynamic instability. Neighboring asters are cross-linked by molecular motors that bind to and move on their MTs. This results in mechanical coupling of micrometer sized asters, acted upon by motor-driven forces acting over a few nanometer length scales. The net force experienced by a complex of coupled asters determines whether they are transported or static. Stochasticity in the model originates from three sources: MT length fluctuations, diffusion and the

binding-unbinding kinetics of motors. The mechanical interactions considered in the model can be broadly classified into (a) cortical and (b) cell interior or cytoplasmic.

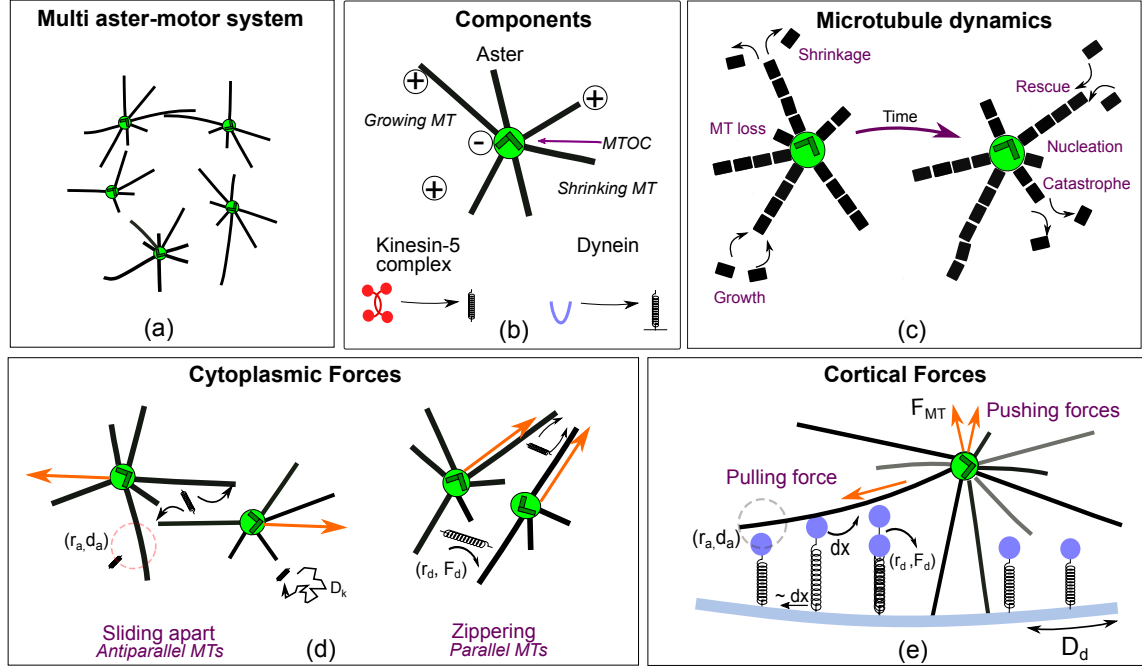


Figure 4.1: Model of forces generated on microtubule (MT) asters with two kinds of motors. (a) The simulation setup of multi aster and motors in confinement. It depicts the three primary model components. (b) Microtubule organizing center (MTOC) nucleated asters with minus-ends of MTs embedded in the MTOC and plus-ends pointing radially out. MTs can switch between growing (black) and shrinking (gray) states. Dynein (blue) is modeled as springs with a motor domain while Kinesin-5 complex (red) is modeled as two motor domains joined by a spring. (c) MTs exhibit dynamic instability. Growing MTs can continue to polymerize or switch to the shrinking state and a shrinking MT can continue to depolymerize or switch to the growing state. Number of MTs nucleating from MTOC is held constant. (d) In the cell interior unbound kinesin-5 diffuse in the cytoplasm and stochastically bind to MTs. When bound to two MTs they simultaneously generate forces that separate or aggregate asters, based on MT orientation. (e) Asters experience pushing and pulling forces at the cortex. Dyneins localized at the cortex, stochastically binds to MT, walks towards the minus end resulting in pulling of aster. Free dyneins diffuse along the cortex. The polymerizing MT bends at the rigid cell cortex and generates inward pushing forces (F_{MT}). Orange arrows: force on the asters. Black arrows: direction of motor movement.

(a) *Cortical*: At the cell cortex we have modeled dynein-like motors that bind to and

walk on filaments resulting in aster transport along the cell boundary. Such movement is comparable to a ‘gliding assay’ that we have previously described in Jain et al [130]. The circular geometry also results in MT bending due to confinement with the cell boundary, generating an inward force (Figure 4.1). In addition, MT-bound motors can also be dragged through the membrane. When multiple motors bind to oppositely oriented MTs of the aster a tug-of-war arises in the MT-motor system. Such tug-of-wars emerge from the radial geometry of asters. Dyneins that are not bound to MTs, can diffuse in the membrane.

(b) *Cell interior*: In the cell interior, diffusible tetrameric kinesin motors are modeled based on the mitotic kinesin-5 complexes. They can bind MTs and walk towards the plus end and when bound to astral MTs from neighbouring asters simultaneously, they produce forces on asters. The resultant force depends on MT orientation- parallel or anti-parallel, leading to either ‘coalescence’ or ‘separation’ of asters respectively (Figure 4.1). Model parameters are taken from experimental reports where possible (Table 4.1).

MT asters are modeled as radial structures at the center of which is a microtubule organizing center (MTOC), that nucleates a finite number of MTs. MTs are modelled as semiflexible rods with a bending modulus κ of 20 N/m^2 , based on previous reports [34]. When MTs at the cell boundary bend, they experience a restoring force based on beam-theory, $F_{bend} = \pi^2 \cdot \kappa / L^2$, where L is the filament length and κ is the flexural rigidity of microtubules. Bending can occur either because the aster is held at the rigid cell boundary, or MTs are bound to multiple dyneins. MT lengths (L_{MT}) are modelled as either uniform and of fixed lengths (stabilised) or fluctuating (dynamic instability) with a mean length. We use the mean length of asters from measurements made on *Xenopus* oocytes [38] of $\langle L_{MT} \rangle = 4.25 \text{ } \mu\text{m}$. The length dynamics are described by the frequencies of catastrophe (f_c) and rescue (f_r) and velocities of growth (v_g) and shrinkage (v_s) with values taken from those reported for *Xenopus laevis* oocyte extracts [38, 14]: $f_c = 0.049 \text{ 1/s}$ and $f_r = 0.0048 \text{ 1/s}$, $v_g = 0.196 \text{ } \mu\text{m/s}$ and $v_s = 0.325 \text{ } \mu\text{m/s}$. The MTs do not interact in the absence of

motor complexes as steric interactions have not been modelled here. All the inter MT interactions are the consequence of activity of the complexes.

Motor mechanics: The motors modeled are of two kinds, cortical dyneins and diffusible kinesin-5 complexes. Cortical dyneins are modelled as single walking motors with a spring-like stalk domain (Figure 4.1), the spring constant k_d of which determines the stretch force $F_s = -k_d \Delta h$ for a Δh change in stalk length [15, 134]. Bound motors are modeled to step with a step size that is constant in the absence of a load force, and reduces piecewise for increasing opposing forces, as we have described previously [15, 134]. Additionally, the anchored dyneins attached to the MT are dragged along the cell-boundary based on the stretch on motor due to the opposing force originating from the spring stretch force (F_s). The detachment and stall forces are identical ($f_d = f_s$) for dynein. Dyneins not attached to the MT diffuse in the membrane with an effective diffusion coefficient D_d , the value of which is varied (Table 4.1). Kinesin-5 motor complexes are modeled as two motor heads joined by a Hookean spring like connector representing the stalk (Figure 4.1), based on the stalk-linked dimer-of-dimers structure of this motor, that walks on two MTs simultaneously, thus coupling them [190]. The complexes are diffusible throughout the interior of the cell with an effective diffusion coefficient D_k , the value of which we take to be $20 \mu\text{m}^2/\text{s}$ based on typical cytosolic proteins (Table 4.1). Motors stochastically bind to MTs within a distance d_a based on an attachment rate of r_a . The second motor-domain can similarly bind another MT, independent of the MT orientation. A bound motor walks on the MT under no opposing load and in the presence of an opposing load as in (Equation 4.1).

$$v(f_{\parallel}) = \begin{cases} v_0 & \text{if } f_{\parallel} \geq 0 \\ v_0 \cdot (1 - \frac{|f_{\parallel}|}{f_s}) & \text{if } f_{\parallel} < 0 \end{cases} \quad (4.1)$$

where, v_0 is the unloaded velocity of the motor, f_s is the stall force and $f_{\parallel}$ is the projection of the extension force (f_{ex}) along the MT (f_{ex} is calculated along the

direction of the MT (minus end to the plus end) hence the modulus of the $f_{parallel}$ is considered for hindering loads). In the case of the multi-aster system, a kinesin complex bound to two filaments simultaneously, will experience a load, resulting in a restoring force that drives MTOC movement. This is based on similar collective motor mechanics models used to model kinesin-5 in previous work [131]. Both dynein and kinesin-5 motors detach based on Kramers theory [155] with a rate r_d as in (Equation 4.2). When a kinesin-5 motor walks to the end of the MT it is modelled to detach immediately [233, 131].

$$r_d = r_0 \cdot e^{|f_{ex}|/f_d} \quad (4.2)$$

where r_0 is the load-free basal detachment rate and f_d is the detachment force. When a kinesin-5 motor walks to the end of the MT it is modelled to detach immediately [233, 131]. Thus the position of asters is determined by the net force that results from all these sources (Figure 4.1) i.e. inward pushing force due to MT bending at the cortex (F_{MT}), a force that pulls the asters to the cell boundary due to dyneins and separating or ‘zippering’ forces in the cytoplasm due to kinesin-5 motility when bound to pairs of parallel or anti-parallel MTs respectively (Figure 4.1).

4.3 Analysis

4.3.1 Measure of rotation and coherence in rotation

The degree of rotational movement was quantified as rate of change in angular displacement ($\delta\theta$) calculated at time intervals (δt) of 1 s over the entire trajectory. The mean angular velocity ($\langle\omega\rangle$) calculated is an average over the last 30 s of the trajectory. The ϕ_R is the order parameter for rotational movement comparable to previous reports for cells [235] and active particles [236]. The rotational order parameter ϕ_R can take values from -1 for counter clock wise motion (CCW) to 1 for

Parameter	Description	Cortically anchored dynein	Cytoplasmic Kinesin-5	Reference
D	Diffusion coefficient	0 to 20 $\mu\text{m}^2/\text{s}$	20 $\mu\text{m}^2/\text{s}$	[234]
v_0	Motor velocity	2	0.04 $\mu\text{m}/\text{s}$	[43]
d_a	Attachment distance	0.02 μm	0.05 μm	[15, 134, 131]
r_a	Attachment rate	12 s^{-1}	2.5 s^{-1}	"
k	Linker strength	100 pN/ μm	100 pN/ μm	"
f_s	Stall force	1.75	5 pN	"
r_d'	Basal detachment rate	1 s^{-1}	0.05 s^{-1}	"
$r_{d,end}$	Basal end-detachment rate	1 s^{-1}	immediate	"
f_d	Detachment force	0.5 pN	1.6 pN	[233], this study

Table 4.1: Model parameters. The parameters that determine the mechanics and dynamics of motors are taken from experimental measurements reported in literature, and where missing estimated.

Parameter	Description	Value	Reference
f_c	Frequency of catastrophe	0.049 (s^{-1}), varied	[14], this study
f_r	Frequency of rescue	0.0048 (s^{-1}), varied	[14], this study
v_g	Growth velocity	0.196 ($\mu\text{m}/\text{s}$)	[14]
v_s	Shrinkage velocity	0.325 ($\mu\text{m}/\text{s}$)	[14]
κ	MT bending modulus	20 (N/m^2)	[34]
<i>Aster:</i>			
N_a	Number of asters	40, varied	This study
N_{MT}	Number of MTs/aster	20	This study

Table 4.2: MT parameters. The parameters that determine the mechanics and dynamics of MT are taken from experimental measurements reported in literature, and where missing estimated.

clock-wise motion, and zero if there is no rotation. The ϕ_R is calculated as Equation 4.3.

$$\phi_R(t) = \frac{1}{N} \cdot \sum_{i=1}^N \hat{e}_{\theta_i}(t) \cdot \hat{v}_i(t) \quad (4.3)$$

where, the $\phi_R(t)$ is the mean rotational order parameter at time t over N asters, the index i refers to the particles, N is the total number of particles (asters), $\hat{v}$ is

the unit velocity vector and $\hat{e}_{\theta_i}$ is the unit angular direction vector of the aster. As chirality in the motion is not observed when simulation were run for multiple times, the modulus of the ensemble ϕ_C is considered and it varies between 0 and 1, independent of CW and CCW orientation. ϕ_C corresponds to the degree of coherence, during the rotational movement calculated as in Equation 4.4.

$$\phi_C = \sum_{t=t_s}^T \langle |\phi_R(t)| \rangle \quad (4.4)$$

where, ϕ_C is the mean coherence measure: time average of $\phi_R(t)$ over the last 30 s (t_s is 270 s), The (ϕ_C) is measured over the last 30 (t_s) seconds, which is an approximate steady state phase in case of sustained rotations.

4.4 Results

4.4.1 Spontaneous emergence of collective aster rotation

Aster motility results from symmetry breaking in forces that arise from a combination of multiple forces: (i) kinesin-5 complexes that either zipper or separate asters based on the orientation of astral MTs, (ii) the bending forces from polymerizing MTs at the cell boundary, (iii) dynein pulling forces at the cell boundary (iv) stochasticity in astral MT lengths and (v) Brownian forces corresponding to the energy $k_B T$. However, none of these individually have any innate ability to drive directional motion due to the radial symmetry of asters and Brownian motion, as illustrated in Figure 4.1. Three distinct spatial patterns of asters emerge out of the interactions between MTs and diffusible kinesin-5, boundary based pulling by cortical dyneins and noise originating from dynamic instability (Figure 4.2). The simulation images depict spatial pattern of asters at the end of 300 s, while their trajectories demonstrate motility. The trajectories are plotted over the last 30 s of

the simulation for clarity.

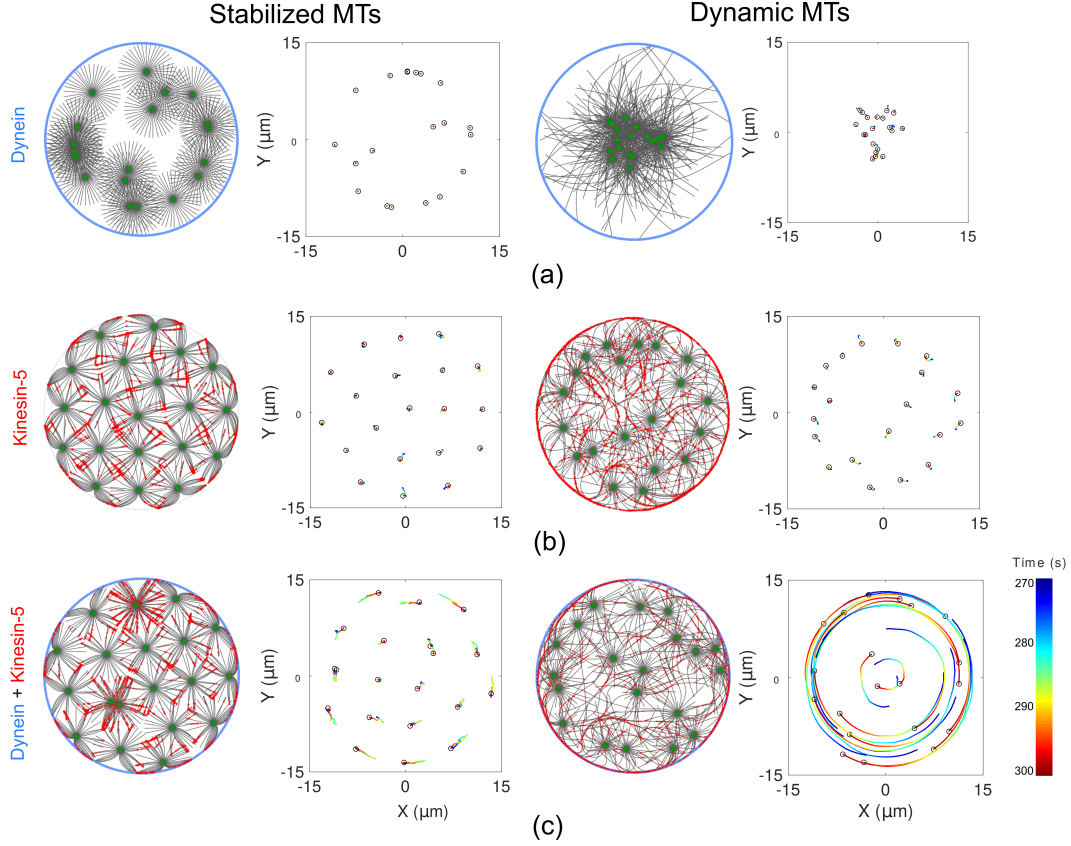


Figure 4.2: Spontaneous emergence of collective aster rotation. Simulation outputs of centrosome (green circle) nucleated asters in a simulated cell in presence of either (a) diffusible cortical dyneins (blue circles) or (b) cytoplasmic tetrameric kinesin-5 motors (red circles) or (c) both motor types are depicted. The the last 30 s of the trajectories of aster centers of the corresponding simulations are plotted (the colorbar represents time). MT lengths are modeled as either (*left*) stabilized (fixed in length) or (*right*) stochastically fluctuating due to dynamic instability. Kinesin density $10 \text{ motors}/\mu\text{m}^2$, cortical dynein density $10^2 \text{ motors}/\mu\text{m}$, $\eta = 0.05 \text{ N} \cdot \text{s}/\text{m}^2$, $N_a = 20$, $N_{MT} \text{ per aster} = 40$, $R_{cell} = 15 \mu\text{m}$ and total time 300 s. MT flux, $J = 0$ for stabilized MTs and $-0.3 \mu\text{m}/\text{s}$ for dynamic MTs. (Video C1).

Centering: Cortical dyneins exert an outward pulling force on multiple confined asters, opposing the inward force generated by MT bending. Asters with fixed lengths of MTs localize at steady state at the cell boundary, without any particular arrangement (Figure 4.2a). The introduction of noise in the form of MT dynamics on the other hand results in the asters converging to the center of the cell. While

the mean lengths $\langle L_{MT} \rangle$ of dynamic MTs are the same as those of fixed-length MTs as determined by f_c , f_r , v_g and v_s , the fluctuations in MT length occasionally result in long filament bending against the cell membrane to produce inward-forces sufficient to overcome the outward pulling force of the cortical dyneins.

Aster segregation pattern: Diffusible kinesin-5 motors simulated with asters without filament length dynamics result in a steady state lattice, that resemble a hexagonal packed structure with asters at their center. Such structure emerges due to the dominance of kinesin-5 acting on anti-parallel MTs generating pushing forces (Figure 4.2b). The addition of ‘noise’ through MT dynamic instability distorts this structure and results in aster separation without any particular order.

Spontaneous rotation: Combining cortical dyneins and diffusible kinesin-5 motors produces a well separated structure, comparable to the hexagonal packing that arises in presence of just kinesin-5 (Figure 4.2c). However, short range back-and-forth sliding motion is observed due to the pulling by cortical dyneins. The addition of ‘noise’ in form of MT dynamic instability surprisingly results in spontaneous, steady-state rotation, analogous to ‘swarming’ dynamics, driven by kinesin and dynein motors.

4.4.2 Dynein density dependent onset of rotation patterns

The cortical dyneins form the minimal system for onset of sliding and rotation movements while kinesin-5 results in onset of collective motion in the cell. Thus number of dyneins was systematically varied to test the effects of dynein density on rotation. The XY trajectories of asters transit from short and random movements at low density to long and directed at high dynein density (ρ_d) (Figure 4.3a). The instantaneous angular velocity (ω) in time exhibit density dependent trends which suggest three distinct patterns of collective aster rotation i.e. sustained, coherent and persistent (Figure 4.3b). The traces of individual asters undergo short and rapid

fluctuations in time at low dynein density. With increasing densities the fluctuations decrease while the magnitude of angular velocity increases. The instantaneous velocity, ω traces from individual asters exhibit fluctuations in time that decrease with increasing ρ_d along with an increasing synchronization between the individual traces. All the velocities, ω across time and asters were pooled into a histogram. The distribution at low dynein density is Gaussian-like, the width of the Gaussian increases with increasing densities of dynein from 1 to 10 motors/ μm . At an intermediate density of 10^2 dyneins/ μm the distribution is approximately symmetric and bi-modal which transits into a single-peak at high density indicative of persistent motion. Switching frequency was calculated (total number of switch events by the total rotation time) to quantify the persistence in the direction, which decreases with increasing density (Figure 4.3c). Rotating asters are not chiral since the time spent by individual asters moving in clockwise (CW) and counter-clockwise (CCW) orientations is equal between multiple iterations. Individual simulations result in a spontaneous choice of an orientation and the collective movement is entrained (Figure 4.3(d)). However, due to the shorter sliding events at low densities, the proportion of time spent in either state is comparable which begins to diverge for increasing dynein densities. To quantify the emergent collective order in a system of rotating asters, the degree of coherence between the multiple asters was evaluated by an order parameter (ϕ_C) (Equation 4.4). In order to do so rotational order parameter (ϕ_R) was calculated (as described in section 4.3.1). The ϕ_C is measured over an approximate steady state phase (t_s) in simulations for sustained rotations which corresponds to last 30 s. The coherence increases with ρ_d and saturates at high densities (Figure 4.3e). The mean angular velocity over the last 30 s also transits from a low to high value at a ρ_d of ~ 100 motors/ μm . Taken together, it suggests that a minimal number of dyneins (10 dyneins/ μm , optimal) is sufficient for the onset of rotation while a sustained, coherent and persistent rotation emerge only at a higher dynein density (10^2 dyneins/ μm , critical). Thus the density of dynein is critical for the onset of sustained collective multi-aster rotation.

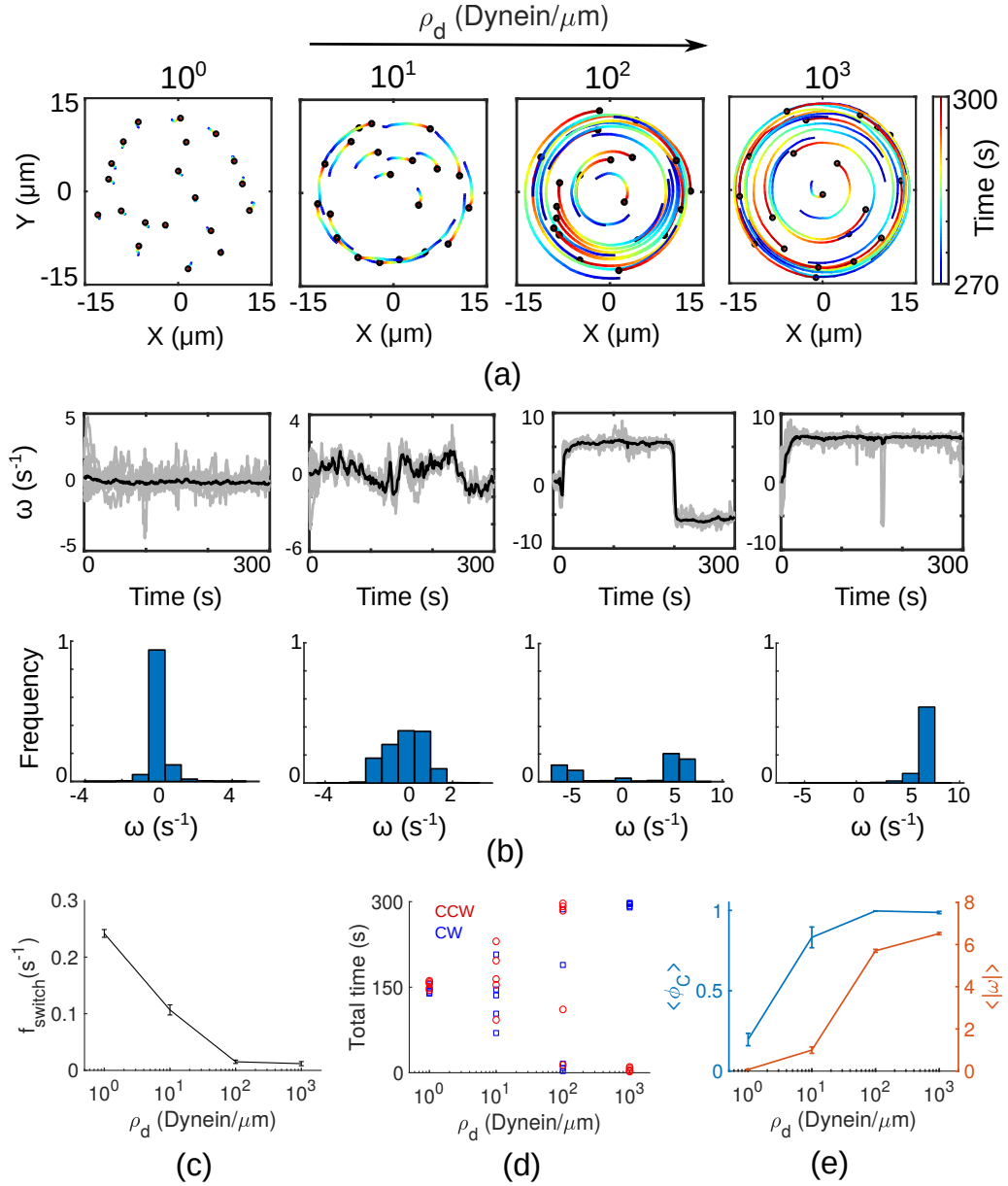


Figure 4.3: Onset of sustained aster rotation at threshold dynein density. (a) Aster trajectories over the last 30 s are plotted for varying densities of cortical dynein ($\rho_d = 10^0$ to 10^3 motors/ μm). Color encodes for time. (b) The instantaneous angular velocity (ω) for each aster (gray) and the mean angular velocity (black) in time is plotted for increasing ρ_d . The corresponding frequency distribution of angular velocities (ω) is plotted. (c) The switching frequency and (d) total time spent in either clockwise or counter-clockwise direction is plotted for increasing densities of dynein over the entire trajectory. (e) The mean coherence in rotation (ϕ_C , Equation 4.3) and the mean angular velocity ($\langle|\omega|\rangle$) over the last 30 s is plotted as a function of increasing dynein density. Cell radius: $15 \mu\text{m}$, $N_a=20$, surface density of kinesin is ($\rho_k = 10^0$ motors/ μm^2) and diffusion coefficient (d_d) of dyneins is $10 \mu\text{m}^2/\text{s}$, $J = -0.3 \mu\text{m}/\text{s}$. $N_{\text{runs}} = 5$. Error bars represent SEM.

4.4.3 Homogeneous distribution of dyneins at the cortex is essential to sustain aster rotation

The role of dynein mobility on the onset and maintenance of rotation was tested to address whether the absolute number of motors or their relative distribution at the cortex effects the process. Since fluctuations drive the symmetry breaking, movement of dyneins along the cortex was implemented by varying the ‘effective diffusion coefficient’, (D_d) of free dyneins. At critical dynein density ($\rho_d = 10^2$ dynein/ μm), decrease in dynein diffusivity from 10 to 0 $\mu\text{m}^2/\text{s}$ results in transition of asters from swarming to the static state (Figure 4.4(a)). However, an increase in D_d beyond 10 has no effects in the motility patterns. The angular velocity traces of all asters show random or potentially local oscillations, when the diffusion coefficient of dynein is less than 1 $\mu\text{m}^2/\text{s}$ (Figure 4.4(b)), also captured in switching frequency profiles (Figure 4.4(c)) and the steady state angular velocities (Figure 4.4(d)). The coherence in rotational order parameter ϕ_C undergoes a switch like transition with increasing D_d (Figure 4.4(e)). The variability in ϕ_C between runs is the greatest at an intermediate value of 1 $\mu\text{m}^2/\text{s}$, as the system transitions from static asters to sustained rotation. The similar diffusion dependent trend is observed at higher densities of dynein. At low densities D_d has no effect and asters continue to be static. This suggests that a minimal motility ($D_d = 10 \mu\text{m}^2/\text{s}$) is essential to sustain coherent aster rotations that emerge at and beyond the optimal dynein density. The diffusivity of free dyneins restore the uniformity at the cortex which is lost due to the effective drag experienced by the motors. At low D_d diffusion is not sufficient to re-distribute dyneins resulting in formation of clusters irrespective of dynein density. The homogeneity in the dynein distribution determines whether the rotation is maintained or not while, the minimal number dictates the onset of rotation from the sliding events. Further, to understand whether the collective motion in this system falls in the realm of general principles of swarming behaviour as described by Viscek [127], the effect of particle density i.e. aster and kinesin-5 and noise i.e. MT dynamics was explored.

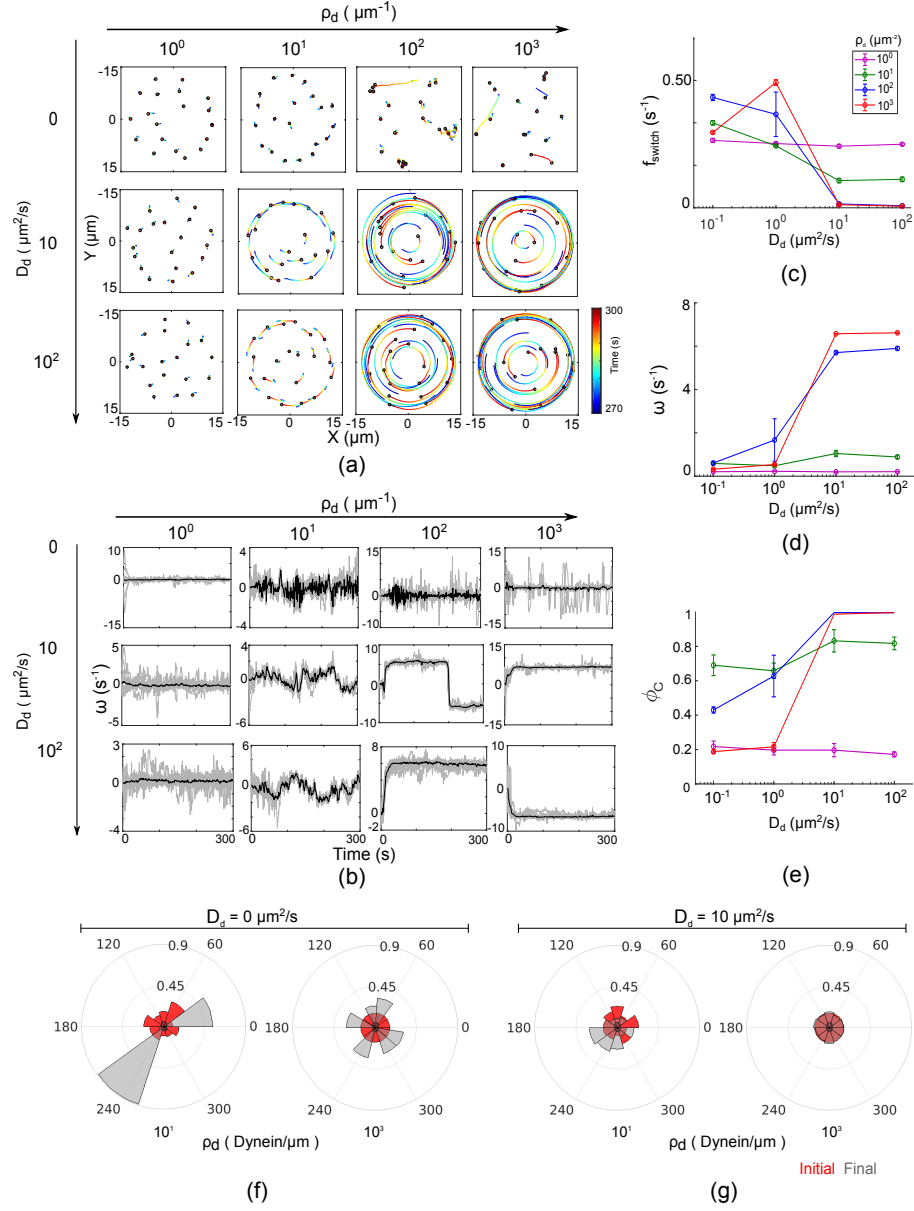


Figure 4.4: Diffusion of dynein restores the uniform redistribution at the cortex essential for the sustained rotation. (a) The aster trajectories over the last 30 s and (b) the instantaneous angular velocity (ω) in time is plotted for varying dynein diffusivity, D_d (column) and dynein density, ρ_d (row). (c) The switch frequency over the entire trajectory is plotted as a function of dynein diffusion coefficient. Colours indicate varying dynein densities. The ensemble average over last 30 s for (d) mean angular velocity ($\langle \omega \rangle$) and (e) mean coherence measure (ϕ_C) is plotted for increasing values of dynein diffusion coefficient, (D_d). Distribution of dyneins at the cortex from a single run at the start (red) and end (gray) of the simulation for low and high dynein diffusivity. $N_a = 20$, $J = -0.3 \mu\text{m}/\text{s}$. $N_{\text{runs}} = 5$. Error bars indicate SEM.

4.4.4 Loss of rotational order with increasing aster density

The local interactions between the asters emerge from the activity of kinesin-5 and MT dynamics. Asters close to the cortex experience pulling forces from the dynein while the cytoplasmic kinesin-5 are the coupling motors that affect the inter-aster positions. The fluctuations due to MT dynamics influence the aster pulling at the cortex and push-pull between them in the cytoplasm. Such a model of local interactions of self-propelled objects, with alignment at a smaller scale than the whole system has been described for a general class of swarming models by Vicsek et al. [19]. In particular, in Vicsek class of models collective ordered transport of the individuals can emerge when the strength of local coupling and ‘noise’ or stochasticity are both optimal. The strength of aster-aster interactions was modulated by varying the aster density (ρ_a) in the cell with optimal values of dynein density and diffusivity as in (Figure 4.4) for a constant number of kinesin-5 motors ($\rho_k = 10$ motors/ μm^2). The number of asters was varied over two order of magnitudes (1 to 114) in a cell of $11 \mu\text{m}$ radius with dynein density (ρ_d) of 10^2 motors/ μm and ‘effective diffusion coefficient’ (D_d) of $10 \mu\text{m}^2/\text{s}$ (Figure 4.5 (a,b), middle row). The choice of the cell size was a trade-off between the system size and the maximal range of aster densities. A cell radius of $11 \mu\text{m}$ spanned asters from 1 to 114 with size of the system in terms of MT and motor numbers being in a computationally feasible framework. The system transits from a network of rotating asters to a static system for increasing number of asters at constant number of motors. The collective motion is sustained in the range of D_d from 1 to $100 \mu\text{m}^2/\text{s}$, and the asters exhibit persistence in their direction, attain maximal angular velocity and rotate coherently up to the aster density of 0.1 asters/ μm^2 (Figure 4.5(c-e)). The data in the shaded region corresponds to a single aster thus is not considered under the collective rotation.

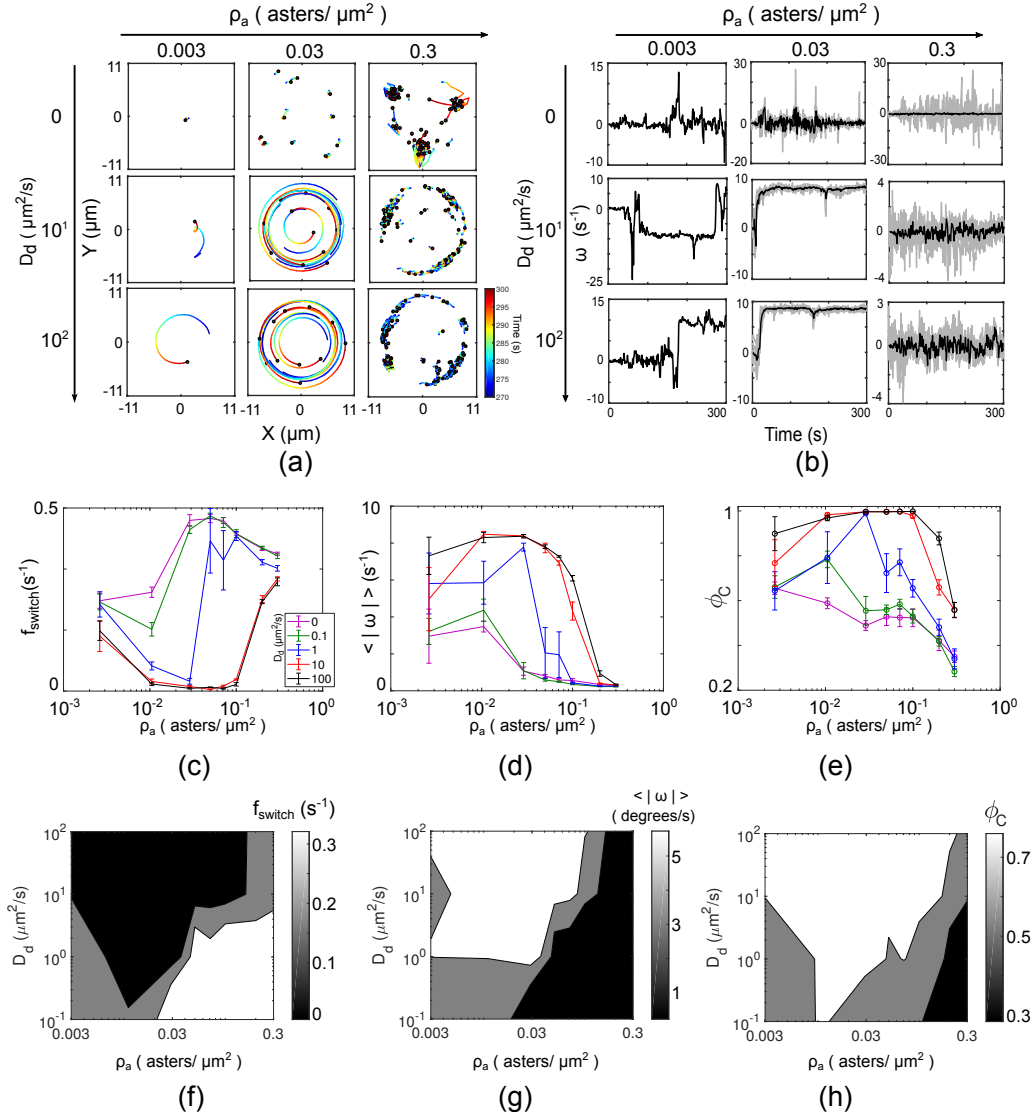


Figure 4.5: Aster density dependent multi-aster motility patterns. (a) The aster trajectories over the last 30 s and (b) instantaneous angular velocity (ω) in time is plotted for varying aster density, ρ_a (column) and dynein diffusion coefficient, D_d (row). (c) The switch frequency over the entire trajectory is plotted as a function of aster density. Colours indicate dynein diffusion coefficient. The ensemble average over last 30 s for (d) angular velocity and (e) measure of coherence in rotation is plotted for increasing values of aster density (X-axis, log scale). The gray shaded area in (c-e) corresponds to data with 1 aster in the cell. The motility patterns across aster densities and dynein diffusivity based on (f) switching frequency, (g) angular velocity and (h) measure of coherence is broadly categorized into 3 distinct types as depicted in contour plots for the data in (c,d,e) respectively. $\rho_d = 100$ motors/ μm , $\rho_k = 10$ motors/ μm^2 , $J = -0.3 \mu\text{m/s}$. $N_{\text{runs}} = 5$. Error bars represent SEM.

Additionally, the variability between the runs reduce at the optimal density of around 10^{-1} aster per μm^2 . The observed density dependence deviates from the kinetic phase transitions in the Viscek type models. The drop beyond a density in the system could be simply due to the non-linear increase in forces with aster numbers which is insufficient to drive large-scale rotation in the presence of constant motor numbers. The absence of sustained rotations at low densities for low values of diffusion coefficient is due to the clustering of motors that fail to restore uniformity at the cortex. However, the absence of sustained rotation at high aster density and high dynein diffusivity confirms that here, rotation is not limited by diffusion driven uniform density of dyneins at the cortex. It is expected that, with increase in number of MT-dynein encounters, more motors would undergo drag and fewer/shorter episodes of diffusion, limiting the redistribution of dyneins at the cortex. Since, redistribution and uniform distribution of dyneins is retained at the cortex, it indicates that the motor forces begin to dilute at high aster numbers and are not sufficient to sustain the aster rotations.

4.4.5 Kinesin-5 numbers dictate strength of inter-aster interactions

Since the inter aster coupling is mediated by kinesin-5 motors, their numbers too could effect the strength of inter aster forces. Kinesin-5 complexes generate pushing or pulling forces between the asters based on the orientation of the astral MTs to which it is bound. A pair of cytoplasmic aster in a multi-aster scenario experiences multiple push-pull forces at any instant due to the many such interacting pairs. Thus these interactions can give rise to an effective noise in the system which would then depend on the number of kinesin complexes. Interestingly, at a density of 10 motors/ μm^2 collective rotation emerged (Figure 4.6(a-b)). The collective rotations were coherent and persistent (Figure 4.6(c-e)) at and beyond the density of 10 motors/ μm^2 . However, at this threshold density the diffusion coefficient of kinesin-5

did not effect the collective rotation patterns. This suggests kinesin-5 density is an additional source that determines the strength of aster-aster interactions. Increase in their density results in increased pushing forces and thus their spatial positions.

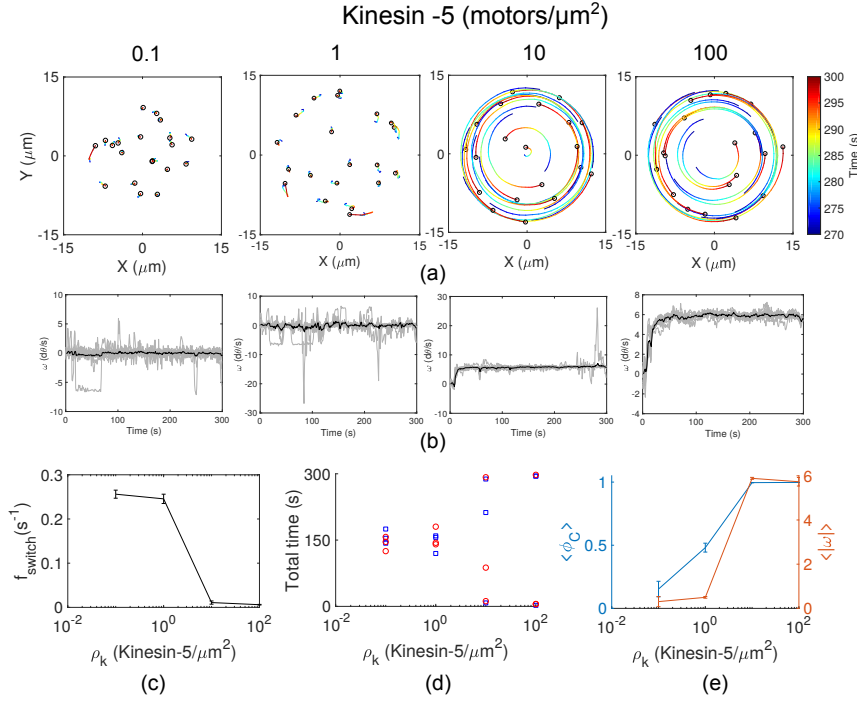


Figure 4.6: Strength of inter-aster interactions dictate onset of sustained collective rotation. (a) Representative XY trajectories of asters for varying densities of kinesin-5 motors in the cytoplasm is plotted over the last 30 s. Color encodes for the trajectory time. (b) The instantaneous angular velocity, ω is plotted in time. Gray curves represent individual aster track while the ensemble mean is represented in black. (c) The switching frequency over the entire trajectory is plotted for varying kinesin-5 densities on (X-axis, log scale). (d) The total time spent in either clockwise (blue) or counter-clockwise (red) direction is plotted for increasing kinesin-5 density over the entire trajectory. (e) The measure of coherence in rotation, ϕ_c averaged over the last 30 s is plotted over the varying values of kinesin-5 density (X axis- on the log scale) on the left y-axis and the ensemble mean over the last 30 s for the angular velocity is plotted on the right y-axis. $\rho_d = 100$ motors/μm, $\rho_a = 0.03$ asters/μm², $N_{\text{runs}} = 3$. Error bar indicates SEM.

4.4.6 Order increases with increasing degree of stochasticity in MT lengths

The noise in the system influence motor mediated aster-aster interactions. The stochastic changes to MT lengths is essential for the symmetry breaking as it results in increased exploration of space and thus interaction with motors. The average rate (MT flux, J in $\mu\text{m/s}$) of MT length dynamics is considered as a proxy for the strength of noise in the system. The J value is varied by changing the transition frequencies (f_c , f_r) (as described in section 3.2.7 and table 3.3). In the presence of cortical dyneins at the optimal density, MT dynamics have a minimal effect on the onset of coherent rotation. However, it does affect the onset of persistence in the direction of rotation and the angular velocity being higher at increased flux rates (Figure 4.7). The onset of coherent collective rotation exhibits a saturation for increasing noise. This is contrary to the results observed in the Viscek like models wherein, the order decreases in the system with increasing noise. In the motor-aster systems higher noise allows for faster binding and unbinding events that drive the coherence in the system. Increase in flux, also increases the encounters at the cell cortex and kinesin-5 in the cytoplasm thereby increasing the order in the system.

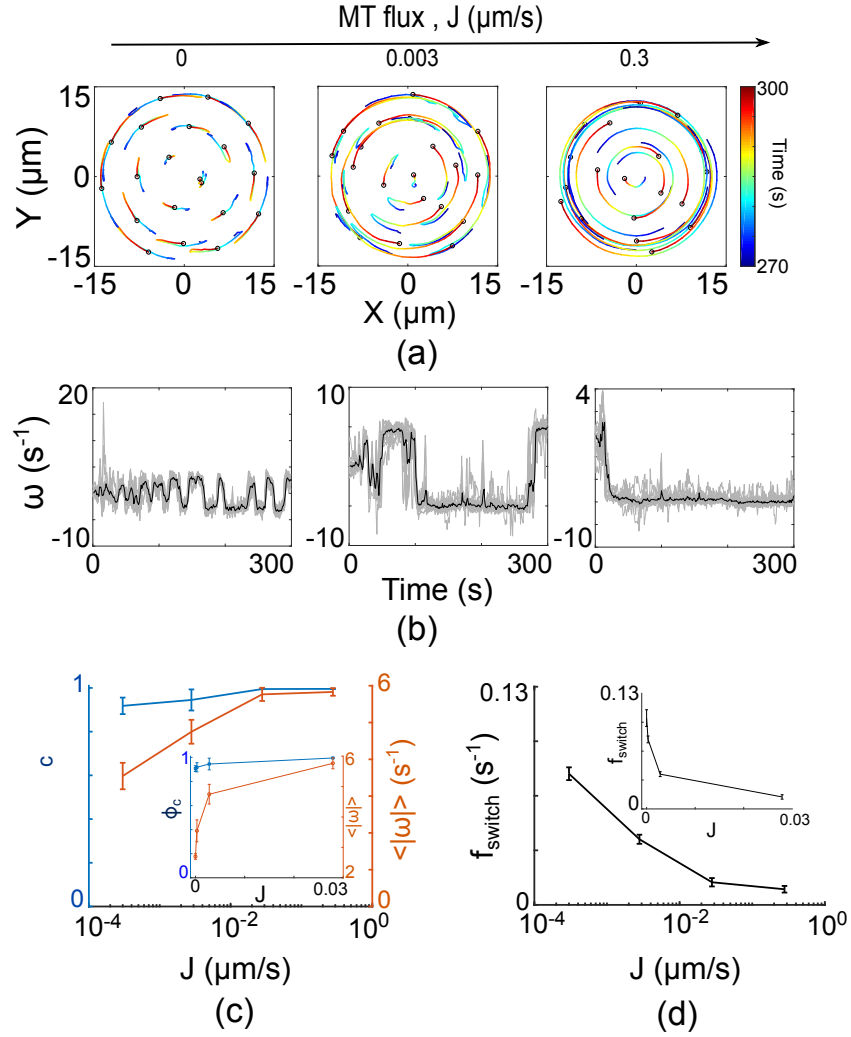


Figure 4.7: Fluctuations in MT length results in persistent rotation.(a) The aster trajectories over the last 30 s is plotted for increasing values of MT flux (noise). Colour encodes for the trajectory time. (b) The instantaneous angular velocity, ω is plotted in time. Gray curves represent individual aster track while the ensemble mean is represented in black. (c) The measure of coherence in rotation, ϕ_c averaged over the last 30 s is plotted over the varying flux values (X axis- on the log scale) on the left y-axis and the ensemble mean over the last 30 s for the angular velocity is plotted on the right y-axis. The inset plot is on a linear scale. (d) The switching frequency over the entire trajectory is plotted for varying flux values on (X-axis, log scale) while the inset represents the data on the linear scale. Different flux values (J) were obtained by varying the values of f_c and f_r (Table 3.3), $\rho_d = 100$ motors/ μm , $\rho_k = 10$ motors/ μm^2 , $N_a = 20$. $N_{\text{runs}} = 3$. Error bars represent SEM.

4.4.7 Immobilized dyneins result in similar emergence of collective rotation

In this study, the dynein behaviour was extended from the standard stepping model as reported earlier for the mammalian oocytes [15, 134] to incorporate the complex cortical interactions in the most simplistic manner. Thus, to confirm whether the observed motility patterns is not an artifact, system was explicitly tested with dynein model as used previously. The dynein motors can only step along the MT and do not undergo any displacement from its point of anchorage at the cortex when either bound or diffuse when free (referred as immobilized dyneins). The aster density was varied as in (Figure 4.5) at optimal dynein density at the cortex, $\rho_d = 10^2$ motors/ μm . Rotations with smaller angular velocities at low detachment force of, $F_d = 0.5$ pN are observed (Figure 4.8) similar to the qualitative trends in (Figure 4.5). The angular velocity and degree of coherence increase when the single motor detachment force is increased to 1.75 pN (Figure 4.8). However, rotation of asters is not observed at high aster densities. This confirms that loss of rotation is due to the insufficient pulling forces from the cortex since the immobilized motors cannot move, cluster and diffuse. The motor numbers need to be scaled with aster numbers to generate forces that can sustain the symmetry breaking events. The relationship that scales motor and aster numbers in order to generate forces sufficient to break the symmetry has not been explored systematically here. The detachment force determines the forces that a motor can exert before detaching from the MT which was modeled to be dependent on the load experienced by the motor (Equation 4.2). In order to test the effect of load independent detachment of motors, motor were modeled to detach stochastically at a detachment rate (r_d) chosen arbitrarily. At low detachment rates, short sliding events are observed which transit into collective and persistent rotation at higher rates (Figure 4.9). Taken together, it suggests that transient and strong MT-dynein interactions is essential to translate the stochastic MT length fluctuations into sustained directional sliding events. The frequent and multiple such MT-dynein encounters at a single motor level generates pulling forces

that drive the sliding events while the transient interactions due to multiple binding-unbinding events at the cortex maintains the kinesin-5 mediated aster-aster coupling in the cytoplasm resulting in collective movement of the aster network (Figure 4.9, at $r_d = 10 \text{ s}^{-1}$) as opposed to sliding of all the asters at the cortex (Figure 4.9, at $r_d = 1 \text{ s}^{-1}$).

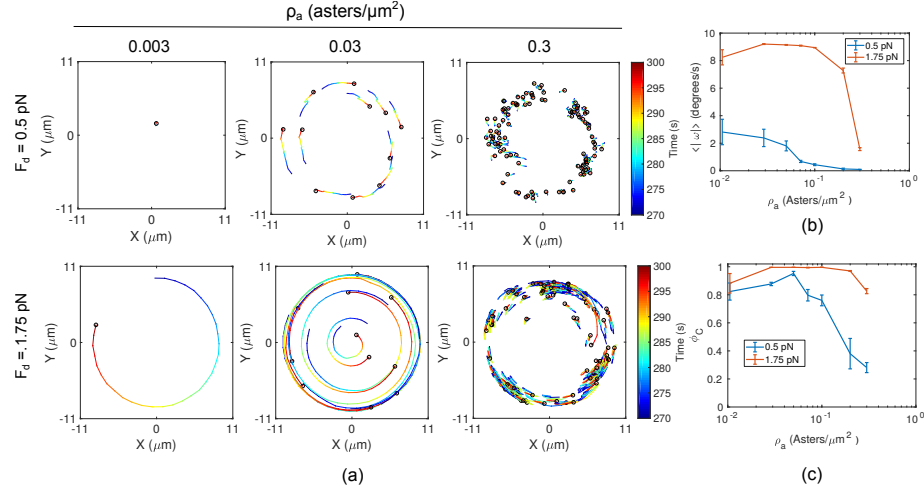


Figure 4.8: Decreased rotational movements at high densities of asters in the presence of immobilized dyneins at the cortex. (a) XY trajectories from the representative runs over the last 30 s across aster densities. (*Top:*) low detachment force ($F_d = 0.5 \text{ pN}$) and (*Bottom:*) high detachment force ($F_d = 1.75 \text{ pN}$). Colors correspond to time. (b) The mean angular velocity ($\langle |\omega| \rangle$) and (c) mean coherence (ϕ_C) in rotation over the last 30 s is plotted for increasing aster densities at low (blue) and high (orange) detachment rates. $\rho_d = 10^2 \text{ motors}/\mu\text{m}$, $N_{runs} = 5$. Error bar corresponds to SEM.

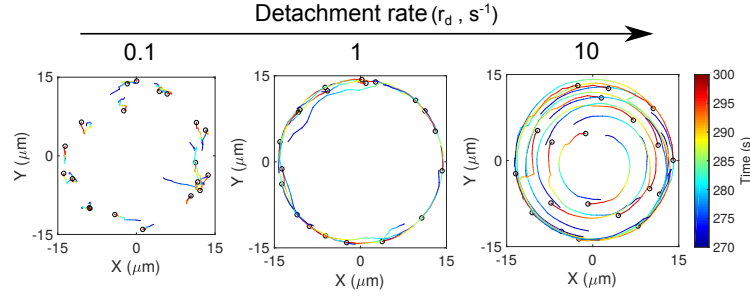


Figure 4.9: Transient MT-dynein interactions at the cortex maintains the coherent cytoplasmic motion of asters. The dyneins were modeled to undergo stochastic detachment independent of the force experienced by the motors. The representative XY trajectories of aster centroids over the last 30 s is plotted for increasing values of detachment rates (r_d). Color encodes time.

Further, the scenarios as in (Figure 4.2) was tested with the dyneins immobilized at the cortex with higher detachment force of 1.75 pN. A striking difference is observed when kinesin-5 motors is absent in the cytoplasm (4.10(a)). Asters closer to the periphery with stabilized MT filaments are pulled towards the cortex and undergo back-and-forth movement along the cortex. While in the presence of dynamic instability, asters closer to the periphery undergo sliding along the cortex and asters at the interior center and de-center. In the presence of kinesin-5 motors (4.10(b)) the motility patterns in the presence and absence of dynamic instability is similar to that observed with diffusible dyneins in (Figure 4.2). Strikingly when the dynein density is varied with the immobilized base at the cortex, onset of sustained collective and coherent rotation is observed at lower density of motors when the detachment force is 1.75 pN (Figure 4.11).

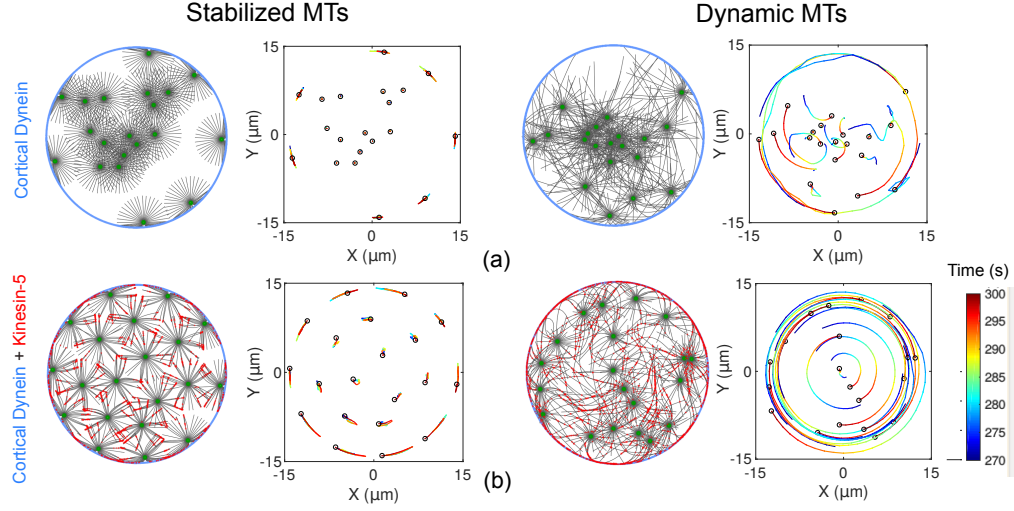


Figure 4.10: Emergence of collective aster rotation with dyneins immobilized at the cortex. Simulation outputs of centrosome (green circle) nucleated asters in a simulated cell in presence of either (a) immobilized cortical dyneins (blue circles) or (b) both dyneins (blue) and diffusible kinesin-5 (red) motor types. The last 30 s of the trajectories of aster centers of the corresponding simulations are plotted (the colorbar represents time). MT lengths are modeled as either (left) stabilized (fixed in length) or (right) stochastically fluctuating due to dynamic instability. Kinesin density $10 \text{ motors}/\mu\text{m}^2$, cortical dynein density $100 \text{ motors}/\mu\text{m}$, $N_a = 20$, $N_{MT} \text{ per aster} = 40$, $R_{\text{cell}} = 15 \mu\text{m}$ and total time 300 s.

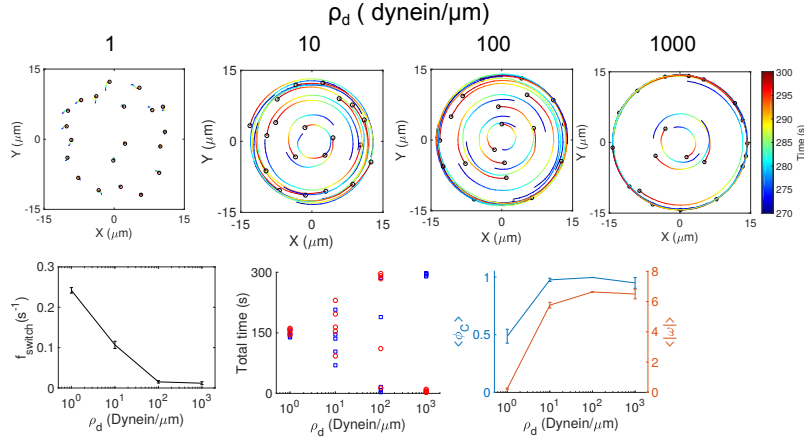


Figure 4.11: Onset of multi-aster rotation at lower density of immobilized dyneins.(a) The XY trajectories of aster centroids over the last 30 s, (b) the switching frequency and (c) the total time spent in CW (blue) and CCW (red) direction over the entire trajectory for the increasing dynein densities is plotted. (d) The mean angular velocity and mean coherence in rotation over the last 30 s as a function of dynein density is plotted. $F_d = 1.75$ pN, $N_a = 20$, $N_{runs} = 5$. Error bar corresponds to SEM.

Taken together it suggests that mobility of dynein motor at the cortex is not essential for onset and maintenance of aster rotation. The ability of the motor to drive the collective coherent rotation depends on single motor properties such as detachment force and detachment rate. However, the uniform presence of dyneins is essential for sustained rotation. The local inhomogeneity in the motor numbers act as brake due to its inability to translate into directional movement. When the stepping and detachment behavior is different the similar patterns emerge at different parameter space. Thus our model predicts the combination of a uniform outward force from cortical motors, separating activity of kinesin-5 and noise due to dynamic instability can produce spontaneous rotations in multiple MT asters in a confined system. The multi-asters exhibit phase transition like trend for increasing dynein and kinesin-5 density and MT dynamics that saturate at optimal range. While, dependence on the order decreases with increasing aster density due to the low number of motors available per MT aster.

4.5 Conclusions and Discussions

In summary, the cortical dynein step towards the minus end of the astral MT generating a force perpendicular to the line joining the cell-center and the point of contact at the cortex resulting in sliding in of the astral MTs (Figure 4.12(A)). Further, kinesin based outward pushing and/or inward pushing forces at the cell boundary result in bending of the astral MTs along the cortex that increases their lateral MT-dynein contacts and thus the force along the cortex. This results in further sliding in of the MT filaments. Motors undergo stochastic binding and force dependent unbinding from astral MTs. The stochastic fluctuations in MT length (i.e. MT dynamic instability) affect the rate of MT-dynein encounters and thus their interactions which results in sustained sliding. The symmetry breaking event is spontaneous and the choice of direction is random. However, once the symmetry is broken the direction can be maintained for some parameter space while switch back and forth in some others. The number of motors available per aster along with MT dynamics and activity of coupling motors determine whether the rotation is coherent and persistent. At large number of motors (dynein and kinesin) per aster, the asters near the cortex exhibit sliding while the MT dynamics. When the number of motors (dynein and kinesin) per aster is large, the asters near the cortex undergo sliding while MT dynamics dictate the direction of movement (Figure 4.12(B)). In the case of low number of motors per aster, the direction of sliding is a combined effect of direct inter aster interactions due to kinesin-5 motors and indirect inter-aster interactions due to the availability of cortical dyneins due to close proximity of multiple aster nearby (Figure 4.12(C)).

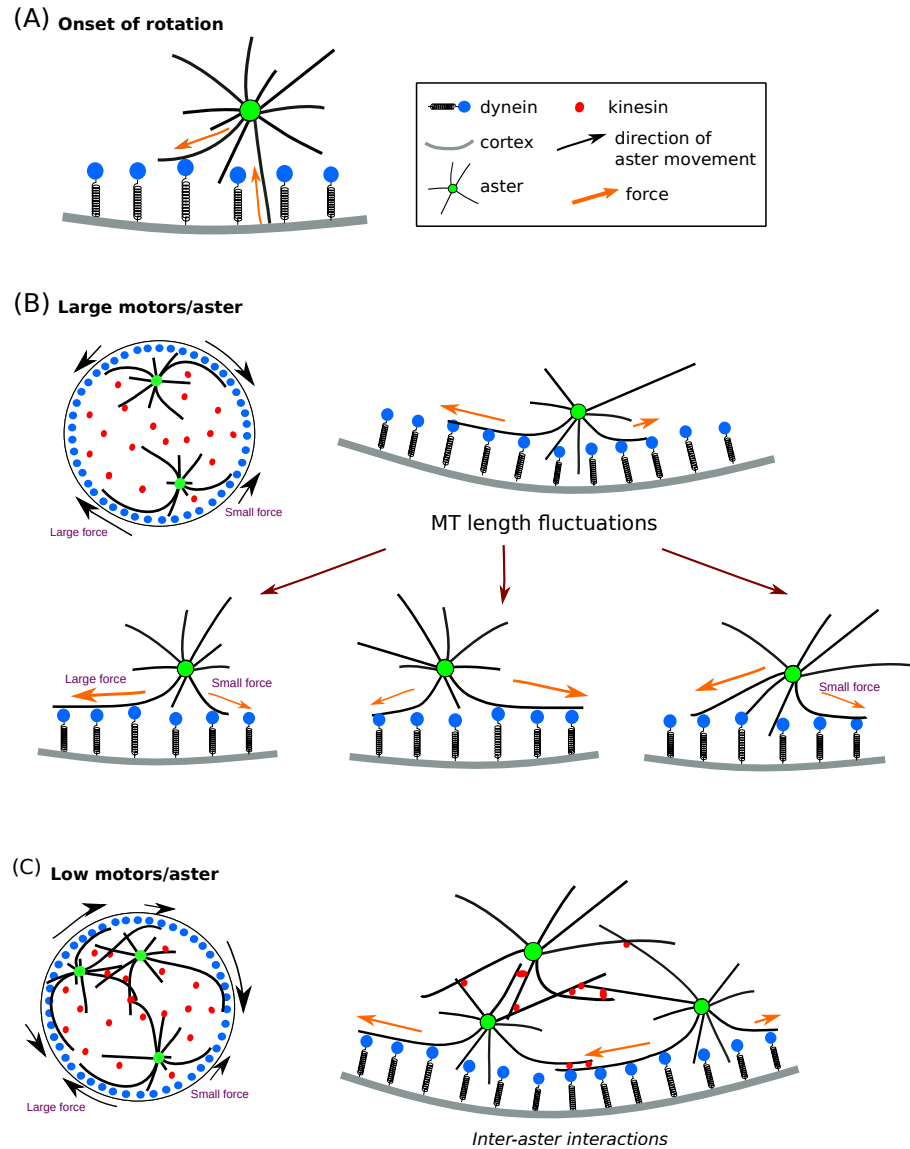


Figure 4.12: Schematic representation of the multi-aster rotation. (A) Onset of rotation due to the pulling by cortical dyneins. The binding and unbinding of MT filaments along with MT dynamics result in aster rotations. (B) In a system with high number of motors per aster result in direction of rotation primarily determined by MT dynamics. MT length asymmetry and fluctuations in the number of interacting MTs at the cortex is a consequence of MT dynamics and result in change in direction of sliding. (C) In a system, with low number of motors per aster the combined effects of MT dynamics, inter-aster interactions due to kinesin-5 and availability of cortical dyneins result in maintenance of the direction of initial symmetry breaking.

The emergence of self-organized multi-aster swarming in confinement is comparable

to a wide range of biological systems that span several scales from molecules, through bacterial populations to large animal swarms. The specific properties of molecular motors and their critical role in cell physiology, growth and division makes their study particularly important. The role of radial MT arrays seen here in the spontaneous emergence of patterns depend critically on four factors: (a) rigid boundary resulting in bending of MTs, (b) pulling and sliding by cortical motors at the cortex, (c) coupling kinesin-5 like motors that result in coalescence and separation of multiple asters based on local interactions and (d) stochasticity due to filament polymerization kinetics that break the symmetry of the system. Depending upon the parameter space, different motility patterns emerge that can be broadly classified as coherent, coherent and persistent rotation and transient sliding events that do not undergo collective rotation. Individually the effect of increasing (a) dynein density results in sustained rotation, (b) kinesin-5 strengthens inter aster coupling and results in coherence and (c) MT dynamics results in increased persistence in aster trajectories. Collectively, the average number of motors interacting per aster determines the effect of length fluctuations on multi aster movement (Figure 4.13).

The model suggests that such systems should be general and appear at multiple scales [237]. Mixtures of microtubules (MT) and motor with an energy source, have been previously shown to show similar properties to such active systems and linear MT filaments were seen to self-organize to centered aster-like structures and transition to vortices depending on the system size and coupling motor densities [3, 61, 4]. This would suggest a generality of the model. Indeed using purified components, similar patterns have been reported in actin-myosin systems which also demonstrate density dependent phase transition and swirling of filaments [232], demonstrating the relative independence of these patterns from the biochemistry.

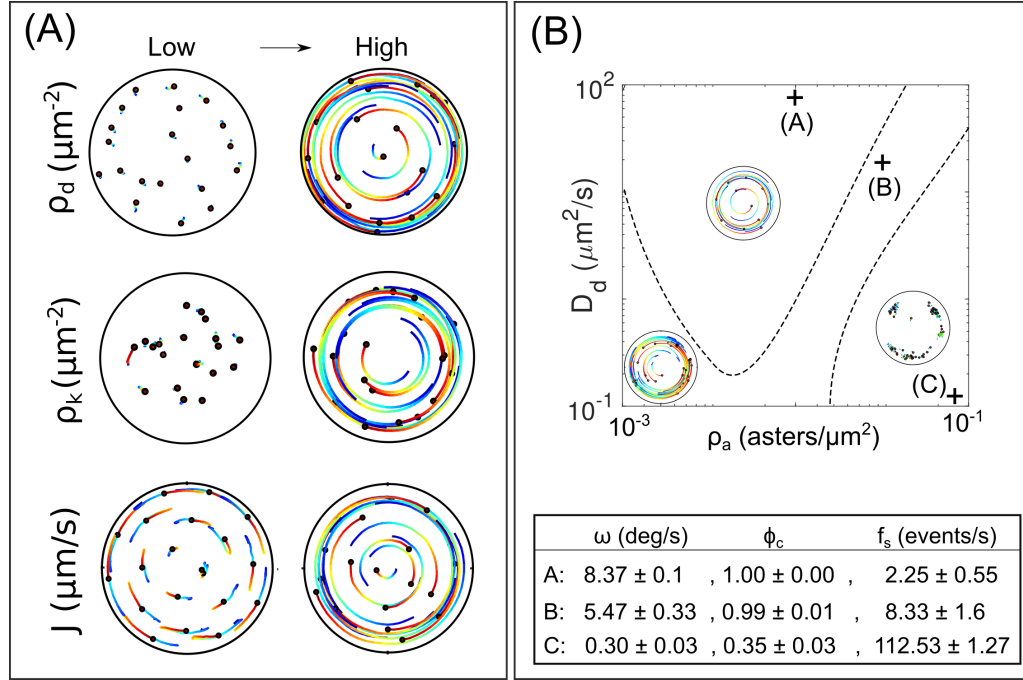


Figure 4.13: Patterns of collective aster rotation. The effect of motor density and rate of MT dynamics at the two extreme conditions scanned is represented. XY trajectories represent movement of asters over the last 30 s for scenarios of (*Top row:*) dynein density, (*Middle row:*) kinesin-5 density and (*Bottom row:*) MT dynamics. (B) The three distinct motility patterns that emerge for varying aster density and dynein diffusivity. Representative XY trajectories of aster centroids over the last 30 s from a simulation scenario is depicted in the phase space. The values of the representative scenarios are included in the table.

The dynein behaviour was modelled to incorporate the complex interactions of the motor at the cortex observed in *in-vivo* cells. Without explicitly invoking the underlying mechanistic details of the several processes the effective phenomenology was captured. In addition to the binding-unbinding kinetics and stepping of motor heads, motors were modelled to be mobile along the cortex. Unbound motors could diffuse with a step size determined by an effective diffusion coefficient and the MT bound motors dragged based on the motor stretch, independent of the motor stepping. Such, approximation was done to account for the multiple possible interactions at the cortex phenomenologically.

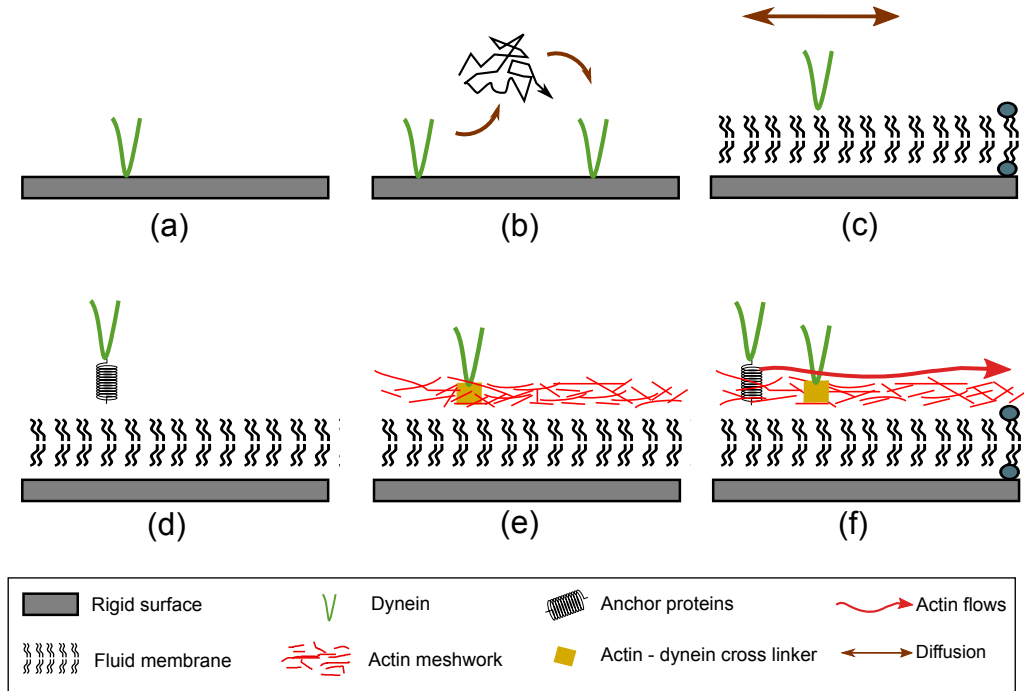


Figure 4.14: Schematic representation of dynein movements along the cortex. (a) Immobilized dynein at the rigid cortex exerts pulling force while walking onto the MT. (b) Dynein can unbind from the cortex, diffuse in the cytoplasm and bind elsewhere on the cortex. (c) Dynein is anchored onto the lipid membrane at the cortex which can be dragged due to the diffusion of lipids in the membrane. (d) The anchoring at the lipid membrane can be via the anchor proteins which themselves may be rigid or flexible (indicated as spring) thus resulting in motor movements. (e) Dynein may be attached to the cortex via proteins onto the actin meshwork beneath the fluid membrane and (f) the anchor-motor complex or actin-crosslinker-motor complex may experience drag due to the actin induced flows resulting in dynein displacement.

(i) In *in-vivo* cells, dyneins were observed to be transiently associated with cortex for few seconds that rapidly changed its position along the cortex [238]. Also, dyneins were observed to detach from the cortex while bound to the MT first, then detach from the MT, diffuse in the cell and bind elsewhere at the cortex [239, 240]. This is different from the conventional gliding assays wherein the motors are immobilized on the glass cover-slip and remain fixed at the anchored point (Figure 4.14(a)). Additionally, dynein localization and re-distribution at the cortex was also observed to be mediated by MTs [238, 241, 242]. Taken together, it suggests that there can

be net change in the position of the dyneins at the cortex in time conceptualized as in (Figure 4.14(b)). (ii) Secondly, the cell cortex is not rigid. It comprises plasma membrane and actin network beneath it. MT gliding experiments with motors anchored on the lipids report differences in MT transport features and motor behaviours as opposed to the conventional gliding assays [243, 244] due to the motor slipping and diffusion. The measured diffusion coefficients are in the range of 0.5 to 1.5 $\mu\text{m}^2/\text{s}$ for kinesins and myosins [245, 244, 243]. A recent study reported ATP independent clustering of kinesins at the MT due to the lateral diffusion of membrane lipids to which it is bound in a gliding assay set up [246]. However, in *in-vivo* cells, dyneins are not bound to the plasma membrane directly. Dynein anchoring at the cortex is mediated via adaptor proteins such as Num1, dynactin, mcp5 or ternary complexes such as NuMA/LGN/G α i which have membrane binding domains [247, 248, 249, 250, 251]. Anchoring via such complexes could further affect the dynein stepping and drag at the membrane based on their localization, anchor-lipid coupling, stiffness and the membrane composition that effects the local viscosity and fluidity (Figure 4.14(c-d)). (iii) Further, the local cortical environment is heterogeneous and known to effect the behaviour of lipids. Cortical regions are enriched in actin (Figure 4.14(e-f)). Actin results in hindered diffusion of the lipids of plasma membrane [252, 253] which may result in the affecting the dynein behaviour at cortex in an *in-vivo* scenario. Recent studies are beginning to shed light on role of actin-associated proteins in dynein dependent spindle positioning processes [250, 254]. Some of these proteins regulate the distribution of dynein anchor proteins such as NuMA and dynactin complexes thereby regulating the dynein localization at the cortex such as Afadin [255], Huntingtin and MISP [256, 257]. Additionally, directional flows arising from actin networks displace motors along the cortex during the pronuclear migration in the *C. elegans* embryos [128] while the myosin is known to effect the positioning of the anchors which recruit dyneins at the cortex [247]. Taken together, the dynein localization and behaviour at the cortex is complex which we have modelled phenomenologically by allowing for the movement of dyneins. In future, aster reconstitution studies can be coupled to the lipid-motor [244, 246] and

lipid-actin [253, 252] reconstitution studies which would be extremely challenging and complex. It would help in understanding the collective behaviour of motors at the cortex *in-vitro* with higher degree of complexity but, still keeping the system separate from the effects of signalling pathways such as cdc42, cdk1 which is known to effect the lipid-actin and motor behaviours in the cell.

We also speculate that biological systems may have evolved mechanisms to suppress rotations. Our results provide mechanistic insights on the observed interactions between astral MTs and cortical dyneins. Dynein based cortical pulling is evolutionary conserved mechanism across cells during spindle positioning however, rotations are not often observed. Many *in-vivo* studies indicate cortical dyneins to be localized in clusters as patches at the cortex [238, 258, 247, 240, 251]. Additionally, the non-uniformity in dynein distribution can arise from heterogeneity in (a) active/inactive state or (c) single motor properties such as binding-unbinding kinetics. In simulations, we observe loss of rotation in presence of clustered dyneins irrespective of the dynein density. Interestingly, sliding of free MTs along the cortex was observed in the budding yeast [71], that suggests cortical forces are sufficient to pull free MTs but not the entire SPB-nucleus complex. In simulations, a uniform dynein density at the cortex results in onset of sliding events while a sustained collective aster rotation emerge at optimal densities. Secondly, MT length fluctuations and lateral sliding along the cortex of MTs is essential in simulations. However, in *in-vivo*, ‘end-on’ and ‘lateral’ encounters of MTs at the cortex is observed which seems to be dependent on the shape of the cell, stage of the cell-cycle and/or the accessory proteins that are engaged with MTs or dyneins. Typically, end-on interactions are observed during the centering processes while off-centering movements are driven by the lateral interactions. The geometry of the cell from round to a elongated shape tends to shift the MT-cortex interaction from end-on to the lateral [259] irrespective of the cytoplasmic state of the cell. While, stabilization of MTs at the onset of anaphase was reported to be essential for correct spindle positioning [260]. MT stabilization implies slower MT dynamics. We observe transient back-and forth sliding events in the absence of

MT dynamics and sustained persistent rotation with increase in MT dynamics in simulations. An increase in catastrophe rates is also observed for the MTs sliding along the cortex in budding yeasts at the anaphase [261, 73]. Such behavior would result in length reduction of MTs and thereby suppress the lateral interactions. Additionally, the MT interaction at the cortex could be end on or lateral based on the anchor proteins to which it binds [251]. Thus, the multiple mechanisms responsible for precise positioning of spindles could have been an optimization to suppress the sustained lateral interaction of MTs at the cortex thereby preventing the rotations in the cell. The system described here is based on experimentally reported parameters of MT and motor mechanics and kinetics. While it is qualitatively comparable to the general model of density and noise-driven global ordered motion seen in locally coupled active particles [19], the details of the system make it distinct. In particular, in Vicsek class of models collective ordered transport of the individual, self propelled objects can emerge when the strength of local coupling and ‘noise’ or stochasticity are both optimal. The analogy of our detailed biological cytoskeleton-motor system can be formulated in terms of equilibrium model properties, where temperature can be compared to the random fluctuations in MT length and pressure compared to aster density. However, unlike density dependent swarming patterns of collectively migrating cells in experiment and theory [218], aster swarming was lost at higher densities of asters. Although the trends were similar at lower diffusion coefficients of dynein, the degree of rotation was reduced significantly. Such a diffusion limitation arises because the dyneins fail to re-distribute themselves and restore the uniform motor density. As a consequence, both asters and dyneins aggregate locally around the cell boundary. This is further confirmed based on the observed loss of rotation with immobilized dyneins at high aster densities. Taken together, it suggests that rotational order increases with increasing number of motors (dynein and kinesin-5) per aster wherein length fluctuations coupled with frequent motor-MT binding and unbinding events result in sustained collective aster rotation.

Our study is distinct from the multiple reports of cytoskeletal filament vortices and

sustained flows due to the MT geometry and multiplicity of asters. In contrast, cytoplasmic streaming in *Drosophila* oocytes driven by kinesin-dependent MT transport [262] due to MTs sliding on other, cortically immobilized MTs [230], requires linear MTs. More recently, a combination of experiment and theory have demonstrated large motor driven MT vortices in space on either 2D surfaces [226] or in 3D confined compartments with *Xenopus* extracts [65], both of which were based on taxol stabilized linear MTs. MT asters have been seen to oscillate during the first embryonic division of *Caenorhabditis elegans* due to pulling by cortical dyneins and a force asymmetry arising from MT bundling and motor density. However, these involve only a pair of asters, coupled at the metaphase plate [67], but do not address the properties of multiple asters. In contrast, the centering ability of asters based on pushing at the cell membrane and pulling by cytosolic dynein motors seen in sea urchin embryos [263], suggests such cellular processes are robust to positioning errors arising from thermal noise. Since our predictions of spatial patterns are based on similar mechanistic components- filaments, diffusible and cortically anchored motors and a rigid boundary-arising from the presence of many asters and multiple, diffusible motors. The choice of parameters of motor and MT mechanics is based on experimentally measured values, with the aim of predicting the outcome of potential experiments. Indeed recent developments with linear MTs encapsulated in lipid droplets together with motors, demonstrate a transition from random to astral geometries dependent on collective mechanics [264, 265]. In future, the encapsulations of multiple asters could be used to test some of our model predictions.

Thus we believe these results point to a novel self-organized pattern forming system based on the optimal densities and noise.

4.6 Outlook

Emergence of collective multi-aster rotation in the system deviates from the universal models of self-propelled particles wherein, coherence emerges at high density and low noise. While in the minimal system in this study, collective rotation emerge at low aster density and high noise. This model has three active entities instead of one when compared to the classic models of collective movement: dynein dynamics that forms an active boundary, diffusive kinesin-5 complexes and multiple microtubule asters with MT dynamics. Fluctuating MT lengths and MT-motor binding/unbinding events contribute to the noise in the system. These events influence MT-MT interactions and motor-MT encounters while, dynein driven cortical pulling and kinesin-5 driven inter- aster pushing contribute to the local directional movements. Increasing kinesin-5 and cortical dynein density result in emergence of order while the order is observed only for intermediate range of aster densities. In the present study, the understanding of mapping and the deviation from the universal class of model is lacking. It would be interesting to develop a simple phenomenological model that would capture the complexity of cytoskeleton system and provide a generalized model for self organization of particles based on ratio of motors to asters and proportions of antagonistic interactions (push and pull from boundary, push and pull between the asters) in the presence of active boundary.

In this study, the effect of membrane and actin on dynein activity at the cortex in an *in-vivo* scenario is explored. The combined effect is phenomenologically captured by allowing the motors to be dragged from its base while stepping, diffuse along the cortex when free while the stepping and undergo force dependent detachment as implemented earlier [15, 134]. Coherent collective multi-aster rotation emerge even if stepping or detachment behaviour is varied but at different values of single motor parameters. Conversely, the single molecule parameters and properties can differ based on how the measurements were made in an *in-vivo* scenario. This study

allows us to explicitly test the forces experienced under the different scenarios and identify the underlying common principle that results in emergence of such patterns. A systematic test of individual scenarios would provide a basis to compare with the experimental quantitative data. Such an understanding would provide insights on the multiple processes such as spindle oscillations and positioning inside the cells. In *in-vivo* studies spindle oscillations are observed in multiple systems and the most commonly studied model system is one-celled embryo of *C. elegans*. Multiple hypotheses exist in the field and some suggest asymmetry in motor localization while some suggest differences in their kinetic properties or existence of different dynein populations at the cortex [72, 266]. This study can be further extended to systematically dissect the effects of every single component and understand their collective effects at the MT-motor level that govern the cellular process. For instance, on asymmetric localization of the motors at the two halves of the cortex in terms of their densities, the aster trajectories deviated from a smooth circular trajectory (Figure 4.15). While, the rotation was predominantly confined to the half in which the cortical motors were localized as opposed to the half that did not have motors (Figure 4.15). This effect is different from the scenario wherein, motors were allowed to cluster and re-distribute via diffusion (Figure 4.4). In yeast cells, cortical dyneins involved in spindle positioning have been reported to have properties that are strikingly different from mammalian systems at a single molecule level such as higher stall force (~ 7 pN, [160]). Thus this study prompts us to explore the behaviour of cortical dyneins explicitly in yeast and mammalian systems in order to identify the mechanisms that result in such collective properties in an *in-vivo* context. Further, the contribution of length and motor numbers for transport of symmetric MT structures would help understand the regimes of transient and sustained symmetry breaking events.

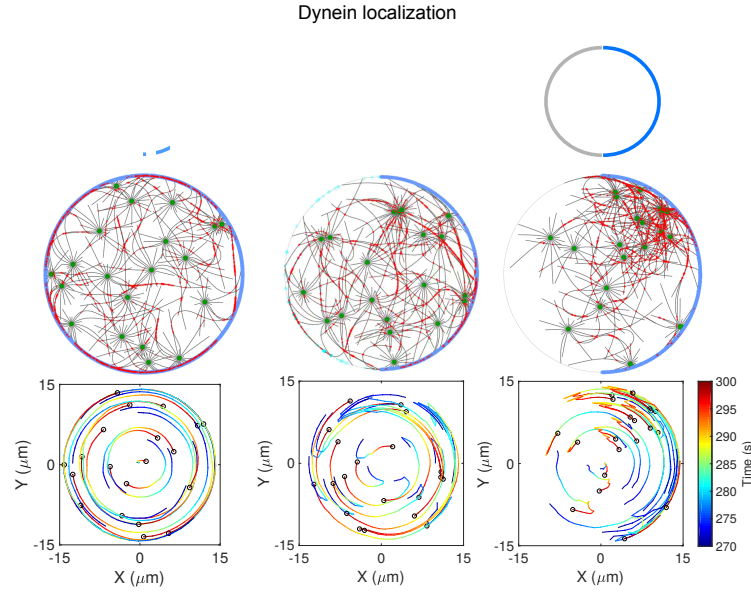


Figure 4.15: Restricted aster movements due to the motor asymmetry at the cortex. (a) Schematic representation of dynein localization at the cortex: uniformly present, the density of motors on the right is more than the left. (b) Snapshots at the end of simulations are represented for the corresponding scenarios. (aster centroids: green, kinesin-5 complexes: red and dyneins: blue). (c) The corresponding XY trajectories of the last 30 s is represented. Color encodes for time.

It would be interesting to explore the spatio-temporal dynamics of dynein behavior at the cortex that govern the spindle positioning and bipolarity in *in-vivo*. In the *in-vitro* dynein gliding assays or droplet encapsulation studies, dyneins have been immobilized on the glass cover-slip or at the boundary via the antibody linkages. Recently, experiments have been reported that test the motor activity of kinesins and myosins on lipid surfaces [243, 244, 246]. The *in-vivo* studies are primarily based on genetic mutants of dyneins or the accessory proteins during the spindle positioning in *C. elegans* and the budding yeast. Thus, this study forms the basis to test the multiple hypothesis that can be systematically explored computationally and tested in the context of spindle positioning and cell-division. Can the asymmetries in the dynein numbers, binding-unbinding kinetics, localization of the anchor proteins influence the spatial-temporal dynamics of spindles and what is the the molecular

mechanism of the observed patterns. Further, how exactly does the *in vivo* environment effect the behaviour of dyneins and whether the emergent properties of dynein in the *in-vivo* scenarios are any different from the single molecule measurements made in the *in-vitro* set up. Is the behaviour of dyneins different when active at the cytoplasm versus when active at the cortex due to the complexity arising from lipid and actin interactions or there are two distinct populations of dynein that operate differentially remains to be tested.

Chapter 5

Motor number dependent collective effects of yeast dynein on MT transport

Contributions. Experiments: Dr. Kunalika Jain, IISER,Pune. Parts of this chapter have been published as *Jain, K, Khetan, N, Athale, CA (2019). Collective effects of yeast cytoplasmic dynein based microtubule transport. Soft Matter, 15, 7:1571-1581 [130].*

5.1 Introduction

Microtubule (MT) transport by molecular motors plays a vital role in regulating cell physiology and mechanics as seen in eukaryotic cell division [154, 172], polarization [267] and migration [268]. While single-molecule *in vitro* assays have increasingly provided a detailed understanding of the mechanochemistry of kinesins [269, 270, 271] and dyneins [272, 273, 274], cellular processes typically involve multiple motors acting together. Transport processes involving many motors acting together have been well studied in vesicle transport where motors work in teams to carry cargoes on immobile filaments [275, 276]. However, filament transport by immobilized motors, has also been reported in physiologically relevant processes such as spindle assembly [172, 277, 190], neuronal axon development [278, 279], *Caenorhabditis elegans* fertilization [76, 75], mouse oocyte maturation [10], nuclear oscillations in *Schizosaccharomyces pombe* [280, 281, 282] and mitotic nuclear positioning in *Saccharomyces cerevisiae* [283]. The regulation of the number of motors (N) transporting MTs and their localization result in spindle oscillations in *S. pombe* [282, 239, 240] and *C. elegans* [284]. Thus, understanding the effect numbers of motors (N) on collective transport properties could provide insights into MT transport *in vivo*. Dyneins play an important role during the *in vivo* transport of filaments. However, they are relatively less well understood at a single molecule level compared to kinesins, due to their greater structural complexity [158]. One such well studied dynein is the highly processive yeast cytoplasmic non-essential dynein. At a single molecule level it has been shown to stochastically step backwards and forwards with variable step sizes ranging from 8 to 32 nm in the absence of load [273] and increasing in backward steps under load [160]. The processivity of the dimeric motor has been attributed to a lack of inter head co-ordination and asynchronous stepping when the two motor domains are close to each other [285, 286], while coordinated stepping can arise with increasing inter-head separation through linker tension gated release of the motor from MTs [287]. Load sharing between the heads allows dynein to step processively even under large loads [288]. Additionally, single molecule detachment

rates for this yeast dynein have asymmetric force dependence - assisting forces increase detachment, while hindering forces result in persistent binding (Figure 5.1) [287, 289]. The effect of single-molecule dynamics on multi-molecular transport by teams of this dynein is not apparent from current theoretical models of collective transport that are based on ‘loose coupling’ of motors [290, 291, 292], fluctuation based load-sharing [293, 294] and negative cooperativity of detachment [295, 296]. A mathematical model specific to the yeast dynein would thus help better understand the *in vivo* role of this ‘model’ dynein.

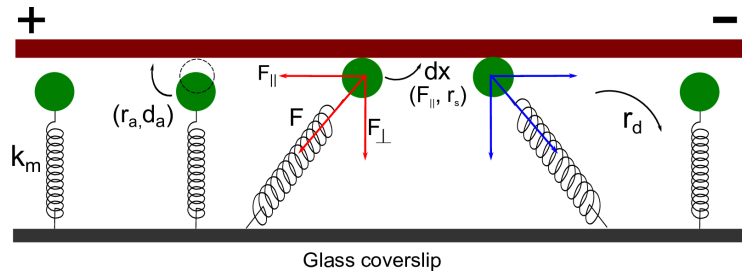


Figure 5.1: Simulation set up of MT gliding assay. A schematic representation of dynein driven MT gliding in 2D simulations. The dimeric motors (green) are modeled as Hookean springs with a stiffness k_m . The motor can stochastically attach to the MT with an attachment rate (r_a) if the MT is within the reach distance of the motor (d_a). Motor stepping along MTs produce a parallel force $F_{||}$ in forward (blue arrow) and backward (red arrow) directions, affecting the motor stepping size (δx), direction and the detachment rates (r_d).

The collective transport velocity of the yeast cytoplasmic dynein has been shown to be comparable to the single molecule velocity in experiments [273]. While a DNA-origami cargo has been used to precisely control the effect of increasing dynein team sizes on run lengths and times [297], it involved maximally 7 motors moving a cargo on static MTs. Studying the number dependence of MT transport by anchored motors over a wider range, could be more representative of the *in vivo* scenario during *S. cerevisiae* mitosis, where increasing lengths of astral MT are thought to ‘glide’ on cortically anchored dyneins, driving nuclear positioning (Figure 5.2) [298, 283, 299, 300]. At the same time, the astral MTs act as tracks transporting more dynein to the cortex, increasing the local density [301]. Thus, ‘search and

capture' of astral MTs in nuclear positioning involves changes in motor numbers and MT lengths (Figure 5.2(a)). This suggests, a minimal system which quantitatively address the role of MT lengths and motor numbers, could improve our understanding of nuclear positioning *in vivo*.

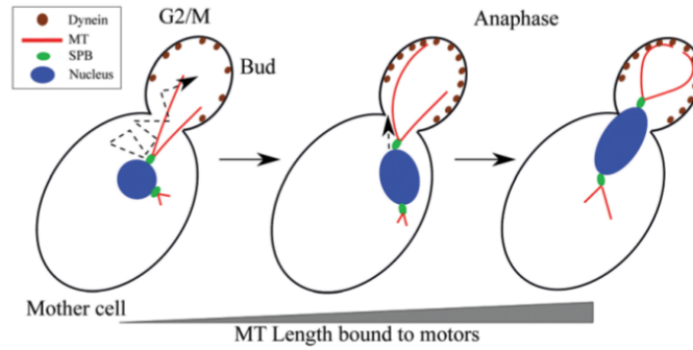


Figure 5.2: Gliding of astral MT along the cortex during mitosis in budding yeast. A schematic representation of positioning of (dashed arrow) yeast nucleus (blue) by dynamic astral MTs (red) nucleated from SPBs (green) pulled by cortical dyneins (brown) localized in the bud cell during mitosis. The scale represents the increasing length of MTs interacting with the motors (*Jain. et. al. 2019*)

In this study a minimal stepping and detachment model for the non-essential truncated yeast dynein is developed based on the previously published data from single molecule experiments. To address the effect of motor numbers on MT transport, dynein numbers per MT was varied by modulating (i) MT lengths and (ii) surface density of motors. MT transport statistics such as MT diffusivity, velocity and directionality in simulations were compared to the experiments for varying MT lengths and dynein surface densities. The results are then interpreted in terms of the role of collective effects in dynein driven MT transport and its potential role in the *S. cerevisiae* cell.

5.2 Model

5.2.1 Model of dynein driven collective MT transport

A gliding assay simulation was developed within the framework of a stochastic, discrete particle based simulator Cytosim, that has been extensively used to model collective cytoskeleton-motor mechanics [62, 16, 14, 152, 131, 15]. In the simulation, motors are assumed to be immobilized on a surface by their stalks and the heads can bind MTs based on stochastic attachment-detachment kinetics. The average effective velocity of MT transport ($\langle v_{eff} \rangle$) results from a combination of (i) attachment and detachment dynamics of individual motors, (ii) stochastic stepping of individual motors at a velocity v_i where i is the index of motors, (iii) the passive stretch force experienced by a static motor bound to a filament, due to the transport of the filament by other motors and (iv) the thermal and drag forces due to the medium (Figure 5.1).

5.2.2 Motor geometry

The motors are immobilized by their tails on the surface, stochastically attach and detach to filaments, and when attached can walk on them resulting in MT transport. Each motor is modeled as a discrete, stochastic mechanical entity, which algorithmically determines the probability of attachment if unattached, detachment probability if attached and if attached the stepping probability and the size and direction of the discrete step (Figure 5.3(a)).

5.2.3 Attachment-detachment kinetics

Free motors stochastically bind MTs within a distance of attachment, d_a if a random number is less than the probability of attachment given by the attachment rate, r_a

(Figure 5.1). Motors that were already attached are tested for motor detachment by estimating the component of the force acting on the motor along the MT, i.e. the parallel component of the force, $F_{\parallel}$ (Figure 5.3(a)). When the parallel force acts in the same direction as the direction of motor-motion, towards the minus-end of the MT, it is referred to as the forward force F_{forw} and backward force (F_{back}) when it acts opposite to the direction of motor-motion towards the plus-end of the MT, as previously described [287, 289]. Based on measurements of the force-dependent detachment dynamics of individual yeast dynein motors [287, 289], we have implemented a novel model parametrized from experiments where the rate of detachment (r_d) changes asymmetrically, depending on the direction of the load force. Thus, r_d with increasing load force has two different forms for forward and backward loads respectively. (i) For a backward load, detachment depends on a saturation function (Figure 5.3(b)), dependent on the magnitude of the parallel force, $|F_{\parallel}|$, as:

$$r_d(F_{\parallel}) = a + (b - a) \cdot e^{-c \cdot |F_{\parallel}|} \quad (5.1)$$

with parameters $a = 2.3159$, $b = 0.0400$, $c = 0.8414$. (ii) The forward load dependence is determined by a piecewise function determined by a threshold force $F_T = 3.51728$ pN: below the threshold force ($0 < |F_{\parallel}| < F_T$) detachment saturates according to Equation 5.1 with $a = 7.2768$, $b = 0.0400$, $c = 0.6426$ ($R^2 = 0.90$), and above the threshold ($|F_{\parallel}| > F_T$) detachment is linear, $r_d(|F_{\parallel}|) = m \cdot |F_{\parallel}| + c'$, with $m = 3.5814$, $c' = -6.4016$ ($R^2 = 1$) as seen in Figure 5.3(b). A load free motor detaches according to a basal detachment rate (r_d^0).

5.2.4 Motor stepping

Motors are modeled to step stochastically, with the probability dependent on the rate of stepping and time interval

$$p_{step} = 1 - e^{-r_{step} \cdot \delta t} \quad (5.2)$$

where the stepping rate r_{step} is 8 s^{-1} taken from the wild-type yeast dynein construct [287] and δt is the time interval between simulation steps. The motor was simulated to take discrete steps of size δx , with the force-dependent step-sizes based on the predominant peak reported in experiments [273, 160] as follows:

$$\delta x(t) = \begin{cases} 8 & \text{nm, if } F_{\parallel} < F_s \\ 4 & \text{nm, if } F_{\parallel} \geq F_s \end{cases} \quad (5.3)$$

where F_s is the stall force of the dynein motor and $F_{\parallel}$ is the magnitude of the parallel force. The direction of stepping is stochastic and force dependent (both direction and magnitude), determined by probability of forward (p_f) and backward (p_b) stepping, where $p_b + p_f = 1$. The magnitude of the backward force (F_{back}) reduces the likelihood of forward stepping. We model it using multiple thresholds (f_1 to f_4) which are inferred from single molecule level force experiments with this yeast dynein [160] as follows:

$$p_f = \begin{cases} 0.80 & \text{if } F_{back} \leq f_1 \\ 0.74 & \text{if } f_1 < F_{back} \leq f_2 \\ 0.66 & \text{if } f_2 < F_{back} \leq f_3 \\ 0.60 & \text{if } f_3 < F_{back} \leq f_4 \\ 0.48 & \text{if } f_4 < F_{back} < F_s \\ 0.25 & \text{if } F_{back} \geq F_s \end{cases} \quad (5.4)$$

Symbol	Parameter	Value	Reference
<i>MT and motor mechanics</i>			
κ	MT bending modulus	$2 \times 10^{-23} \text{ N} \cdot \text{m}^2$	[34]
δ	MT diameter	$25 \times 10^{-9} \text{ m}$	[43]
k_m	Motor stiffness	$2 \times 10^{-4} \text{ N/m}$	This study
d_a	Attachment distance	$2 \times 10^{-8} \text{ m}$	[15]
r_a	Attachment rate	5 s^{-1}	[302]
r_d^0	Basal detachment rate	0.04 s^{-1}	[273]
v_0	Basal motor velocity	$1.02 \times 10^{-7} \text{ m/s}$	[273]
F_s	Stall force	$4.8 \times 10^{-12} \text{ N}$	[160, 303]
ρ_m	Motor density	$10^{-1} \text{ to } 10^2 \text{ motors}/\mu\text{m}^{-2}$	This study
<i>Simulation parameters</i>			
δt	Simulation time step	0.01 s	This study
L_{box}	Simulation box length	$50 \mu\text{m}$	This study
$k_B \cdot T$	Thermal energy	$4.07 \times 10^{-21} \text{ N} \cdot \text{m}$	-
η	Viscosity (10 % glycerol)	$1.3 \times 10^{-3} \text{ Pa} \cdot \text{s}$	[304]

Table 5.1: Simulation parameters of MTs, dynein and the environment.

where $f_1 = 0 \text{ pN}$, $f_2 = 1 \text{ pN}$, $f_3 = 3 \text{ pN}$ and $f_4 = 4 \text{ pN}$.

Thus, the detachment and stepping behavior of the simulated motors depend on the magnitude and direction of load forces acting on the motors along the MT ($F_{\parallel}$) to which they are bound (Figure 5.3(b)), resulting in mechanical coupling during collective transport.

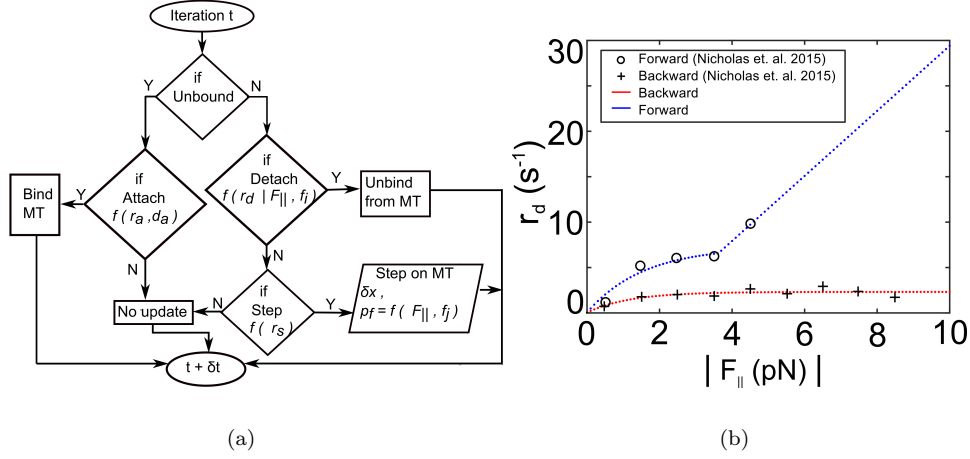


Figure 5.3: Motor model. (a) The flow diagram represents the algorithmic sequence of the stochastic model of the motor. The model determines the attachment of free motors, detachment of bound motors, followed by stepping rate and step size. The model description (Section 5.2) includes detailed expressions for each of the variables (Jain *et. al.* 2019 [130]). (b) The experimentally measured detachment rate (r_d) with an increase in the parallel load force ($F_{||}$) in forward (o) and backward (+) directions is taken from a previous single-molecule study on yeast dynein [289] and fit by custom functions (Equation 5.1), that depend on forward (blue) and backward (red) load forces (Jain *et. al.* 2019 [130]).

5.2.5 Filament transport

The transport of filaments is modelled by numerical integration of a constrained Langevin equation as previously described [16, 133] and briefly summarized here. Filaments are divided into i points separated by rigid segments of $0.5 \mu\text{m}$ (smaller by orders of magnitude than the persistence length). The net force acting on a single MT is calculated by summing all points of each filament and solving a Langevin equation of motion with deterministic and stochastic components to determine the position ($\vec{x}$) based on:

$$d\vec{x} = \mu \cdot F(\vec{x}, t) \cdot dt + dB_t \quad (5.5)$$

where the deterministic terms are μ : the mobility constant of the fiber, $F(\vec{x}, t)$: the matrix of forces acting on every point at time t . The stochastic term is represented

by dB_t that result in Brownian motion. The mobility (μ) of a filament is calculated by assuming it to be cylinder and the term is simplified by considering an average between the longitudinal and transverse components (a two-fold difference). The mobility of the fiber is approximated as:

$$\mu = (p + 1) \cdot \mu_i \quad (5.6)$$

where p is the number of model points of the filament, i corresponds the model points and μ_i is the mobility value at each point. The mobility of all the points on a filament (μ_i) is assumed to be equal and is calculated as:

$$\mu_i = \log(L_h/\delta)/3 \cdot \pi \cdot \eta \cdot L \quad (5.7)$$

where μ_i is the mobility constant of the i^{th} point, i corresponds to the model points on the filament, L is the MT length, δ is the diameter of the rod, L_h is the length scale that corresponds to the smaller value between the length of the filament or the hydrodynamic cut-off, the term $\log(\frac{L_h}{\delta})$ corresponds to the hydrodynamic correction term for the filament and η is the viscosity of the fluid.

The Brownian motion is implemented as dB_t which follows a normal distribution of mean 0 and variance β^2 , and calculated as sum over all the model points (dB_i) for a filament as:

$$dB_i = \beta \cdot \theta_i \quad (5.8)$$

where θ_i is a normal random number. For every model point in the fiber, a random number from a standard normal distribution (mean 0 and variance 1) is obtained by transformation of uniform pseudo random number. While the step size (β) for every point is calculated as $\beta = \sqrt{2D\tau}$ where D is determined by the Stokes-Einstein relation as $D = \mu_i k_B T$.

The parameters of the system are taken from experimental reports and in the case of motor density varied over a range (Table 5.1).

5.2.6 Simulations

2D simulations were performed using Cytosim [16], an agent-based simulation engine written in C++. MT and motor dynamics and mechanics were modeled as before [14, 15, 134] in a square simulation box of length L_{box} with periodic boundary conditions. The periodic boundary is implemented. The integration time was chosen to be smaller than the fastest time-scale (Table 5.1). The system consists of MT filaments of a fixed length (L) and a fixed number of motors determined by the density (ρ_{2D}). Both filaments and motors were randomly distributed in simulation space at the time of initialization. In all the calculations in this study in a single simulation run 100 MTs are randomly initialized in a square box of 50 μm in length. The resulting MT density is 0.04 MTs/ μm^2 and thus MTs assumed to be non-interacting while gliding on a sheet of immobilized dynein motors. MTs were modelled to be non-interacting with each other and do not experience sterics.

5.3 Analysis

5.3.1 MT trajectory analysis

The plus tip (leading tip) coordinates were used for the MT trajectory analysis sampled at every 1 s interval, comparable to the MT trajectories in experiments. The detailed description on the filament tracking is described in *Jain et. al. 2019*.

5.3.2 Speed and ‘effective velocity’ analysis

MT speed is the mean of all instantaneous speeds from a MT trajectory. If (x_n, y_n) are the coordinates of the MT track at a time instant (t_n), then the instantaneous speed (represented as speed or v) calculated as (Equation 5.9) depicted as $(|\vec{OB} -$

$\vec{OA}|)/dt$ (Figure 5.4(a)).

$$v = \frac{\sqrt{(x_n - x_{n-1})^2 + (y_n - y_{n-1})^2}}{t_n - t_{n-1}} \quad (5.9)$$

The instantaneous effective velocity is defined as the difference between the magnitude of the displacement vectors with respect to the origin divided by the instantaneous time. This method assumes linearity in the MT path and estimates the velocity along one dimension. This is also implemented in the MT tracking software such as FIESTA [305]. The effective velocity (represented as velocity or v_{eff}) is defined as (Equation 5.10) depicted as $(|\vec{OB}| - |\vec{OA}|)/dt$ in Figure 5.4(a)).

$$v_{eff} = \frac{d_n - d_{n-1}}{t_n - t_{n-1}} \quad (5.10)$$

where, d_n are the magnitude of vectors with respect to the origin calculated as

$$d_n = \sqrt{(x_n - x_0)^2 + (y_n - y_0)^2} \quad (5.11)$$

where d_n corresponds to the displacement of the MT plus tip at time instant 'n' from the first the plus tip at the first time point as depicted in (Figure 5.4(a)). The effective instantaneous velocity as calculated here, is comparable to the instantaneous speed when the MT trajectory follows a linear path.

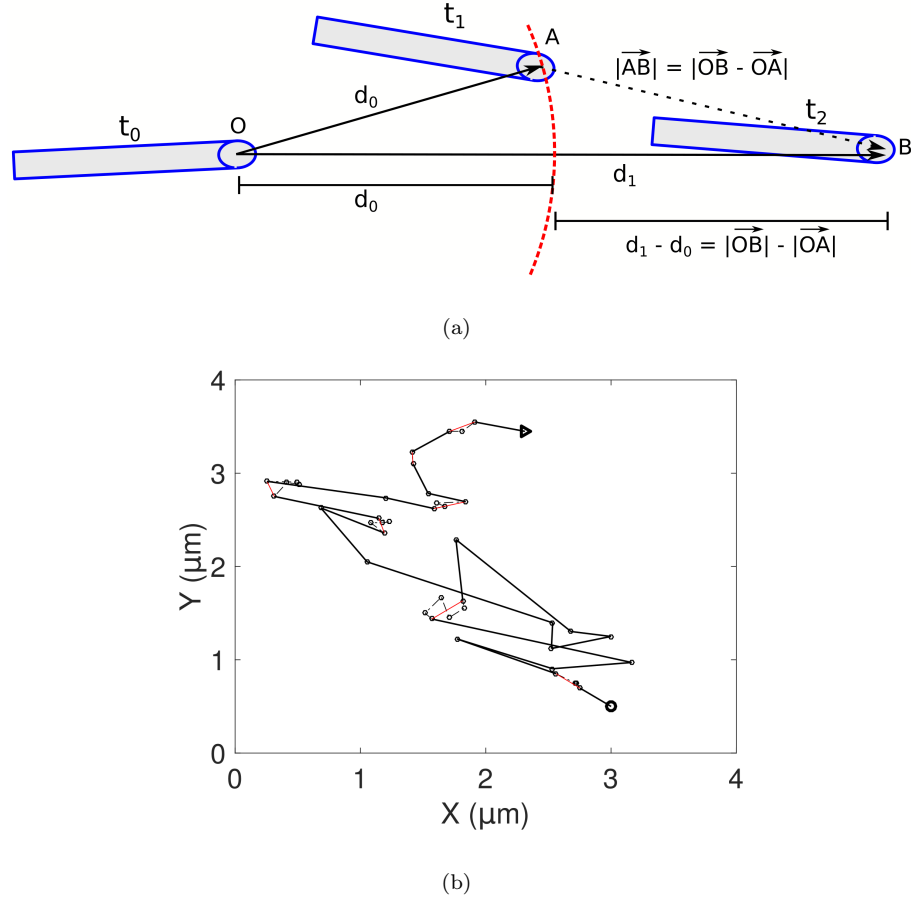


Figure 5.4: Schematic representation for measure of speed, effective velocity and directionality. The MT filaments are represented as cylinders with plus ends depicted as circles (O, A and B) at three consecutive time frames i.e. t_0, t_1 and t_2 . The displacement of plus tip at every time instant is calculated from its plus end at the start point (i.e. d_0 and d_1 at time t_1 and t_2 respectively) as in equation 5.9. The effective velocity (v_{eff}) is calculated as the difference between the successive displacements from the start point divided by the instantaneous time interval (Equation 5.8). In the schematic, v_{eff} corresponds to $(d_1 - d_0)/\delta t$. The red dashed line corresponds to the locus of the vector $\vec{AB}$ i.e. magnitude d_0 . While the speed is calculated as the magnitude of the velocity vector i.e. instantaneous displacements divided by time i.e. $|\vec{AB}|/\delta t$ (Section 5.3.2). (b) A representation XY track (black) with start point (circle), end point (triangle) and smaller circles indicate instantaneous time points. The instantaneous displacements that do not satisfy the criterion are shown as black dotted lines while the interpolated distances are shown in red.

5.3.3 Motor density estimation

In simulations, the linear density of immobilized motors was estimated similar to the approach used in the experiments. A fluorescence calibration method was used to determine the surface density of dyneins in 2D, described in detail in *Jain. et. al. 2019* [130]. Assuming, distribution of motors to be uniform and homogeneous in 2D space, the linear density (ρ_{1D}) of motors per MT was estimated based on the square lattice approximation. The 2D density (ρ_{2D}) was translated into 1D as $\rho_{1D} = \sqrt{\rho_{2D}} \cdot L$, where L is length of the MT .

5.3.4 MSD and diffusivity analysis

The mean square displacement (MSD) is calculated from the 2D positional coordinate of the filament ($\mathbf{r}$) as previously described [306, 15, 134] for increasing each time interval (δt) and averaged based on the expression:

$$MSD(\delta t) = \frac{1}{N} \sum [\mathbf{r}(t) - \mathbf{r}(t + \delta t)]^2_{t \leq 3T/4} \quad (5.12)$$

, where t is the time point, T is the total time and N is the total number of displacements in a time interval. The analysis used $\delta t \leq 0.75 \times T$, based on previous reports for improved statistics [306, 163]. If the net displacement of a trajectory was 154 nm or less (equivalent to a pixel in the experiments), those trajectories were ignored, assuming the filament did not move to avoid artefacts from analyzing static filaments in both experimental and simulation. Simulation data were fit to two models of effective diffusion: (a) anomalous diffusion and (b) diffusion with transport. The anomalous diffusion model allows us to test whether MT length or density resulted in any deviation from diffusive behaviour (super- or sub-diffusive)

[307, 308, 15], while the transport velocity (v) can be extracted from the diffusion-transport model [306, 134, 244]. The (a) anomalous diffusion model is:

$$MSD = 4 \cdot D' \cdot t^\alpha + c_1 \quad (5.13)$$

,where D' is the anomalous diffusion coefficient, t is time, α is the anomaly parameter and c_1 is the error in detection for the experimental tracks. The (b) diffusion with transport model is:

$$MSD = 4 \cdot D_{eff} \cdot t + (v \cdot t)^2 + c_2 \quad (5.14)$$

,where D_{eff} is the effective diffusion coefficient, t is time, v is the transport velocity and c_2 is the error in detection for the experimental tracks. The c_1 and $c_2 = 0$ while fitting the MT tracks for simulation data because of the absence of imaging and detection errors.

5.3.5 Tortuosity analysis

Tortuosity (χ) is an independent measure of the degree of randomness in MT motion, defined as a ratio of displacement (D) to path length (PL). A value of 0 suggests track is random while 1 is indicative of directed transport. The experimental trajectories required an additional step. MT trajectories with $D \leq d_c$ was discarded for the analysis wherein, d_c was equal to 1 pixel i.e. 154 nm. Additionally, the instantaneous displacement (d_i) between the consecutive time frames in the MT trajectory was skipped and not added to the path length if $d_i \leq d_c$ (dotted lines in black) . The skipped distances were accounted for by a simple linear interpolation of the skipped stretches (red) (Figure 5.4). The interpolated distances were added to calculate the path length of the trajectory.

5.4 Results

5.4.1 MT length and motor density affect MT transport

The XY tracks of MT plus tips exhibit qualitative differences across MT lengths and motor densities (Figure 5.5). The MT trajectories suggest four distinct MT motility patterns (i) Brownian motion, (ii) swivelling with occasional diffusive movements, (iii) linear transport interspersed with stretches of diffusion and (iv) persistent directional transport. At a low motor density of $1 \text{ motor}/\mu\text{m}^2$ MT trajectories appear random for short filaments ($L \leq 5 \mu\text{m}$) as compared to long filaments ($L > 5 \mu\text{m}$) that exhibit swivelling motion with reduced degree of randomness. In contrast, at high motor density ($1000 \text{ motors}/\mu\text{m}^2$) filament transport appears to be linear and persistent, independent of the MT length. At the intermediate densities MT trajectories undergo MT length dependent transition from random to swivelling followed by combination of random and directed movements to complete directed transport. The MT length at which these transitions occur decreases with increasing motor densities. The swivelling movements are completely absent at high dynein densities irrespective of MT lengths. Similar qualitative differences in MT tracks at a low ($6.8 \text{ motors}/\mu\text{m}^2$) and high density ($72.4 \text{ motors}/\mu\text{m}^2$) of motors is observed in experiments (Figure 5.5(b)). In experiments, short MTs swivel and diffuse away in a few frames. Intermediate lengths ($\sim 3 \mu\text{m}$) of filaments are transported with intermittent changes in direction at low motor density and directionally persistent at high dynein density.

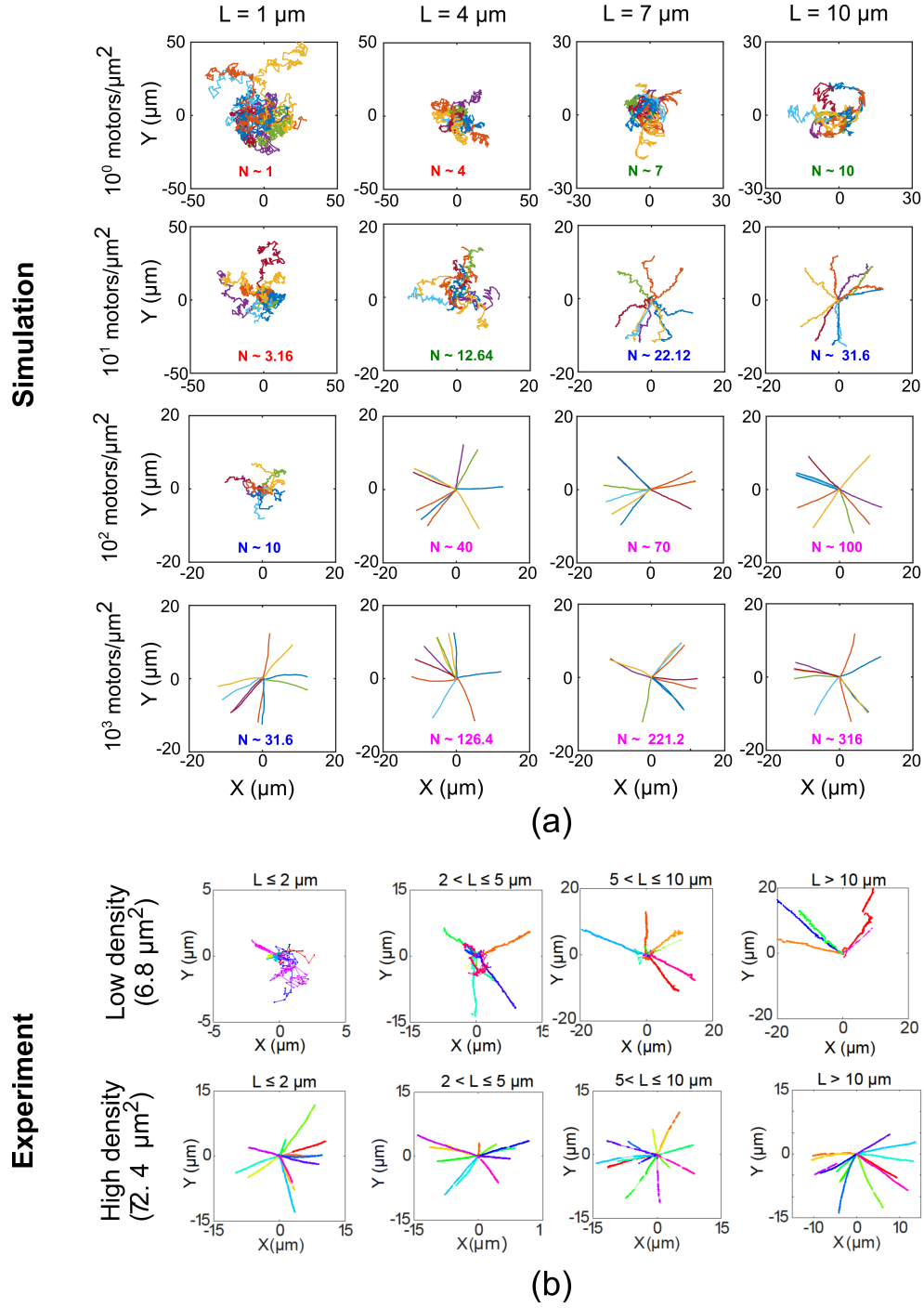


Figure 5.5: MT length and motor density dependent MT transport patterns. Representative MT XY trajectories of varying lengths and motor densities in (a) simulations and (b) experiments is plotted. (a) The expected number of motors per MT (N) encodes for the pattern of MT motility (red: free diffusion, green: swivelling, blue: diffusion and transport, magenta: directed transport). (b) XY trajectories of MTs in experiments is represented for the two motor densities 6.8 and 72.4 motors per μm^2 for increasing MT lengths. Colors indicate different tracks. (Adapted from [130]).

Since MT length and motor densities both qualitatively affect MT transport, we attempted to estimate the number of motors interacting with an individual MT. The motor number per MT (N) was calculated as discussed in (Section 5.3.3) and it suggests MT motility patterns can be best understood in the terms of motor numbers across varying MT lengths and motor densities. Both in experiments and simulations a transition in MT transport from diffusion to swivelling to directional motion of MT is observed for increasing number of motors per MT consistent with literature on collective motor transport [309, 310, 311]. Although when similar number of motors (~ 10) interact with MTs at a low density (1 motor/ μm , $L = 10 \mu\text{m}$) the MT motility appears to be qualitatively different from high density (100 motor/ μm , $L = 1 \mu\text{m}$) in (Figure 5.5(a)). This is consistent with the experiments (*Jain et. al. 2019* [130]). This appears to suggest that along with motor number dependence on MT transport, subtle effects of motor density may also play a role. Thus to systematically examine the effect of similar number of motors per MT on the MT transport at varying MT lengths and motor densities, the trajectories in simulations and experiments were analyzed quantitatively and the statistics compared.

5.4.2 MT speed and velocity converge to 40 nm/s above a threshold number of motors

MT speed decreases with increasing MT lengths (a) gradually at low densities, (b) sharply at intermediate motor densities and, (c) remains independent of MT length at high motor densities (Figure 5.6(a)). Also the fluctuations in speed decreases with increasing motor densities across the MT lengths. The length dependent differences in MT speeds diminishes with increasing motor densities (Figure 5.6(b)). As expected from (Figure 5.5(a)), the MT speeds independent of MT lengths and motor densities collapse onto a curve when plotted as a function of number of motors per MT (Figure 5.6(c)). MT velocity was also computed for each of the scenario which shows a similar profile to that of the MT speeds (Figure 5.6(d)). Although the magnitudes

are 100 fold lesser, the saturation is observed at 40 nm/s similar to the saturation at 40 nm/s observed in MT speeds (Figure 5.6(b)). However, the MT velocities for the similar number of motors from motor densities lower than 100 motors μm^2 exhibit subtle differences (Figure 5.6(f)) suggesting that along with absolute number of motors engaged with MT the surface density also effects the net transport of MT. We speculate such a discrepancy may arise either due to pure Brownian motion or swivelling events that occur at low number of motors per MT. We next examined the MT motility patterns in MT gliding assays and compared the simulation outcomes to the outcomes from experiments.

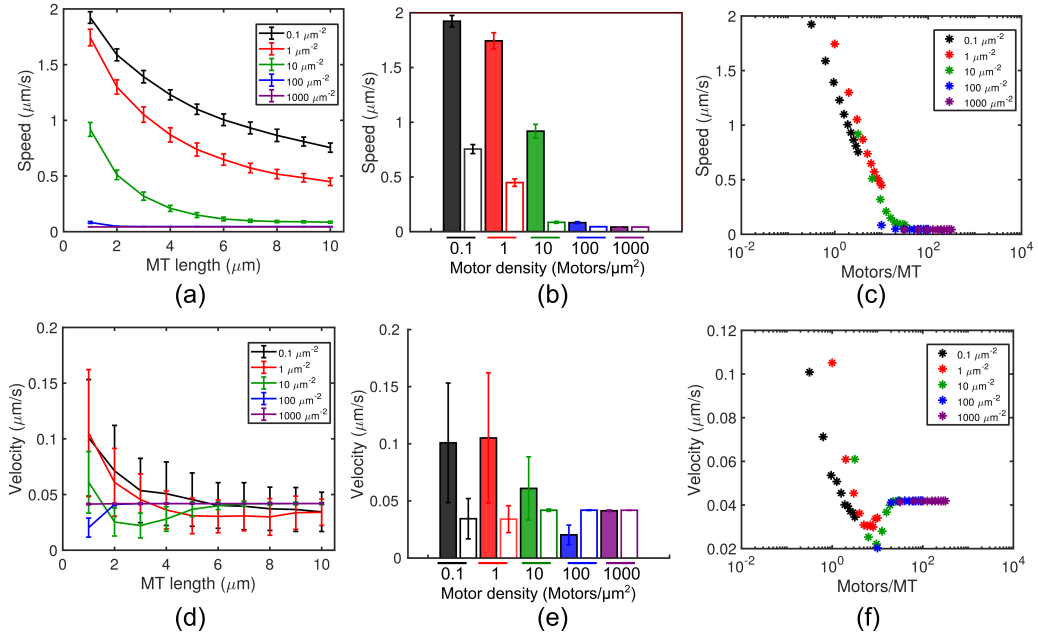


Figure 5.6: MT speed and velocity converge at high motor numbers. (a) The instantaneous speed of gliding MTs is plotted for varying MT lengths across motor densities. (b) The speeds of short (filled bar) and long MTs (unfilled bars) across the motor densities is compared. (c) The MT speeds are plotted as a function of number of expected motors (N) interacting per MT from (a). Similarly, (d) MT velocity as a function of MT length for varying motor densities, (e) comparison of MT velocities for short and long MTs across motor densities and (f) MT velocities as a function of N_m is plotted. Colors correspond to motor densities. Number of MT tracks for each scenario is 100.

5.4.3 Simulations capture MT speed and velocity profiles in experiments

The MT speeds in experiment at low and high motor density show a weak dependence on MT lengths (Figure 5.7(a)). The fluctuations in the mean speeds is higher at low motor density than at the high density. However, the tracks for short MT filaments wasn't sufficient to extract speeds and velocities as they diffuse from the field of view when not bound to motors and swivelling results in MTs being out of focus. The speeds in simulations from 1 motor/ μm^2 for longer MTs and 10 motors/ μm^2 are comparable to the low density while, MT speeds from motor densities of 50 to 70 motors/ μm^2 are comparable to the high density data which is comparable to estimates of motor density in experiments (low density: 6.8 motors/ μm^2 , high density: 72.4 motors/ μm^2 -Jain. et al. 2019 [130]).

Since a low density of motors in experiments show a qualitative change in the transport with length (Figure 5.5), we quantified the mean gliding velocity ($\langle v_{eff} \rangle$, referred to as velocity hereafter) of MT motility with increasing MT lengths (Figure 5.7(b)) and numbers of motors (Figure 5.7(c)), obtained from binning MT filaments at lengths of 1 μm . Interestingly, the $\langle v_{eff} \rangle$ i.e. velocity increases five-fold when ~ 8 -10 motors are encountered. This threshold could be smaller, based on the assumptions inherent in the motor number estimation and the expectation that the number of bound motors will always be lower than those encountered due to the binding and unbinding events. Additionally, the mean velocity of MTs for a saturating number of motors encountered is ~ 40 nm/s, in qualitative agreement with the reported velocity of 41 nm/s of yeast spindle translocation during mitosis [312]. Furthermore, the simulation outputs for the motor densities comparable to the experimental estimates show a similar transition and saturation at ~ 40 nm/s (Figure 5.7(d)). The absence of higher values for low motor numbers in experiments could simply be due to the absence of data in that range due to diffusion and swivelling of MTs. Additionally, in simulations at low motor density, MT speeds and velocities

saturate at values different from 40 nm/s for varying motor stepping rates (Figure 5.8). While the saturation velocity remains robust for varying motor attachment rates and reach distances with an exception to the shorter MT filaments. However, since the saturation values are comparable to the experimental observations we proceeded with minimal model for dynein.

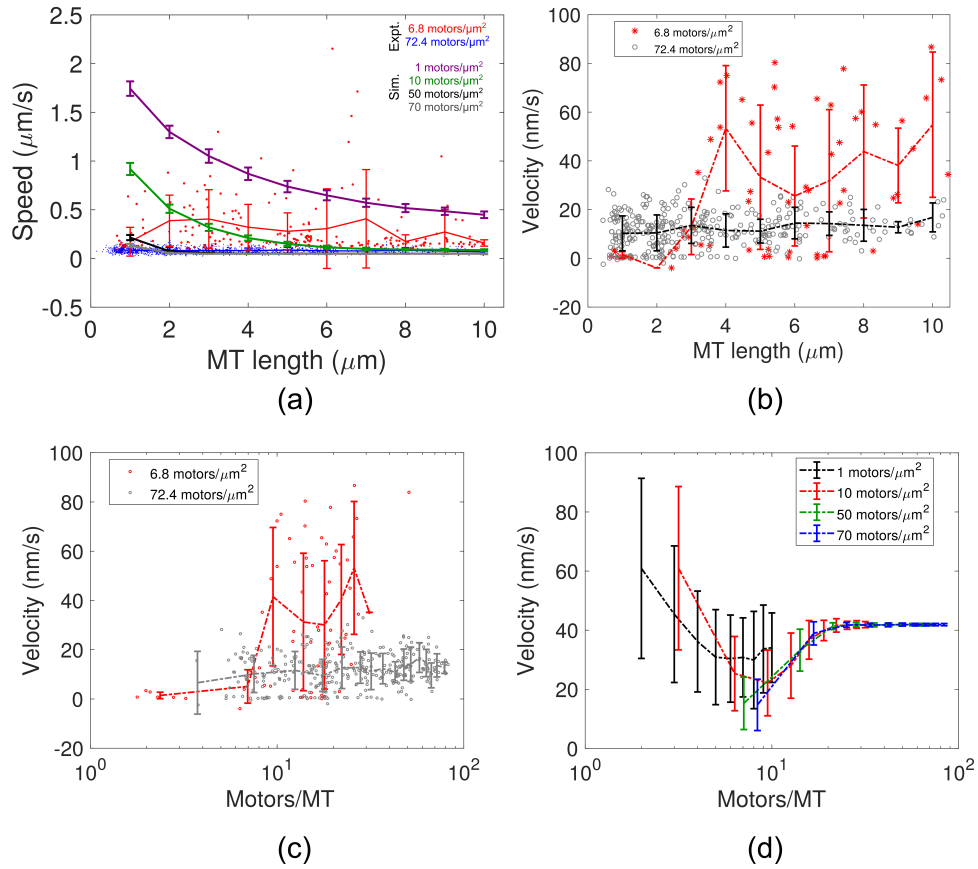


Figure 5.7: Simulations capture MT speed and velocity profiles in experiments. The mean statistics in experiments at low and high motor densities is compared to simulations at comparable motor densities for varying MT lengths. (a) The mean MT speeds for binned MT lengths at low (red) and high (blue) motor density in experiments is over-laid with MT speeds from simulations. The mean MT velocity in experiments from low (red) and high (gray) densities is plotted as a function of (b) MT lengths and (c) motors per MT (Adapted from [130]). (d) The MT velocity from simulations with motor densities comparable to the experimental estimates is plotted. Colors indicate motor densities. Error bars indicate standard deviation. Mean in simulations is over 100 MTs for each MT length.

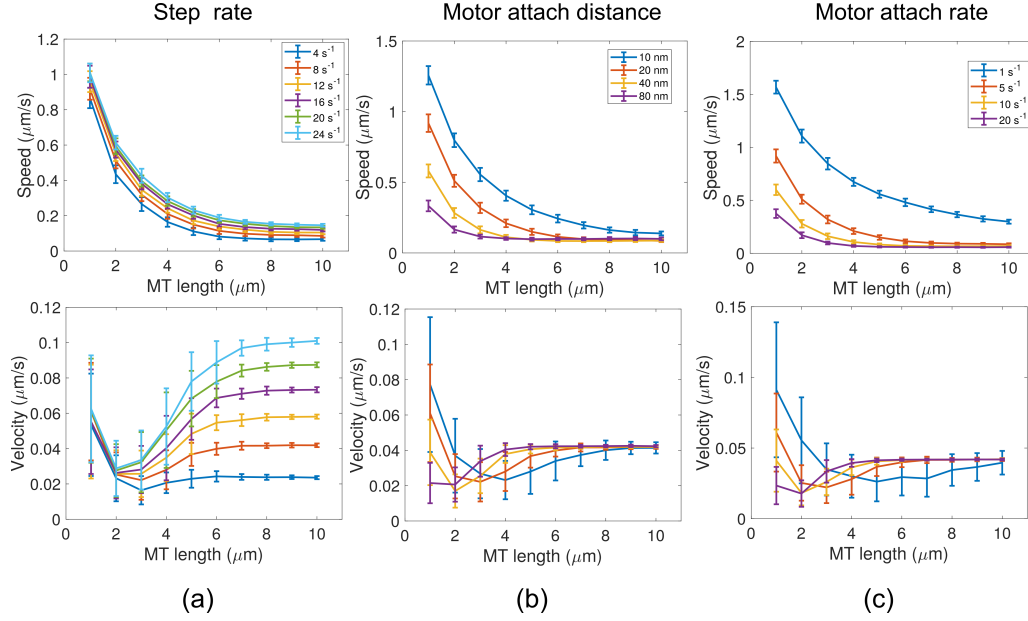


Figure 5.8: Parameter scan of single motor binding and stepping properties. The effect of motor parameters on MT speeds and velocities at low motor density for varying MT lengths at a constant surface density of $10 \text{ motors}/\mu\text{m}^2$ for (a) stepping rate (s^{-1}), (b) reach distance, (nm) and (c) rate of attachment (s^{-1}) is plotted. Mean and s.d. are plotted over 100 MTs for each MT length.

5.4.4 Motor number dependence on MT diffusion and transport velocity

The transition in MT velocity in Figure 5.7 appears to coincide with the qualitative transition from random to vectorial transport in Figure 5.5. Thus, to explicitly identify the random and vectorial components of the transport Mean Square Displacement (MSD) was computed for the MT trajectories. MSD analysis of MT plus tips (the leading tip) provides a quantitative framework to distinguish between the diffusive like and directed movements.

Thus to quantitate the qualitative trends, MSD was computed for each MT track as discussed in the Methods section 5.3.4. The mean MSD and individual MSDs were fit to the two models to extract the transport features: (i) a phenomenological anomalous diffusion model (Equation 5.13) with the anomaly parameter α used to

distinguish between diffusive, super- and sub-diffusive motility and (ii) a diffusion with transport model (Equation 5.14) to quantify the random and directed components of the motility. The MSD curves indicate transition from free diffusion at low motor density to super-diffusion at high density independent of MT lengths (Figure 5.9(a)). While at an intermediate motor density, MT length dependent transition is observed from diffusion to super-diffusion. The individual MSDs exhibit greater variability at low densities and sub-diffusive profiles suggestive of corralling effect due to swivelling motion. The α obtained from fitting the phenomenological model shows a motor number dependent transition from diffusive to super-diffusive transport at ~ 10 motors. The sub-diffusive transport is observed for motor densities lesser than 10 motors/ μm^2 (Figure 5.9(b), $\alpha \leq 1$). In accordance, the effective diffusion coefficient (D_{eff}) follows an inverse motor number dependent trend further confirming reduction in the diffusive mobility of the MT tracks with increasing motor numbers (Figure 5.9(c)). The reduction in mean D_{eff} with the number of motors ($1/N$ dependence) resembles the inverse length dependence, $1/L$ of diffusivity of MTs transported by kinesins reported previously [310]. In experiments, $\alpha > 1.5$ is observed across all the motor densities and MT lengths and a decreasing spread in experimental D_{eff} with increasing motors as described in the experimental section of Jain *et. al.* 2019 [130]. The drift or transport velocity (v) in simulations exhibit a motor number dependent transition from 0 to 40 nm/s around which saturation is observed (Figure 5.9(d)). In experiments, the transport velocity from individual MSDs show a spread from 0 - 45 nm/s for the low density data. At high motor density, the velocity is observed to be independent of motor numbers but saturates around a lower transport velocity of around $\sim 15 - 20$ nm/s. The quantitatively lower velocity obtained from experiments as compared to simulations, at high motor densities, could result from crowding effects, as reported previously for kinesin [244]. Such effects are not observed in simulations, since the model here does not invoke steric and crowding effects, for simplicity.

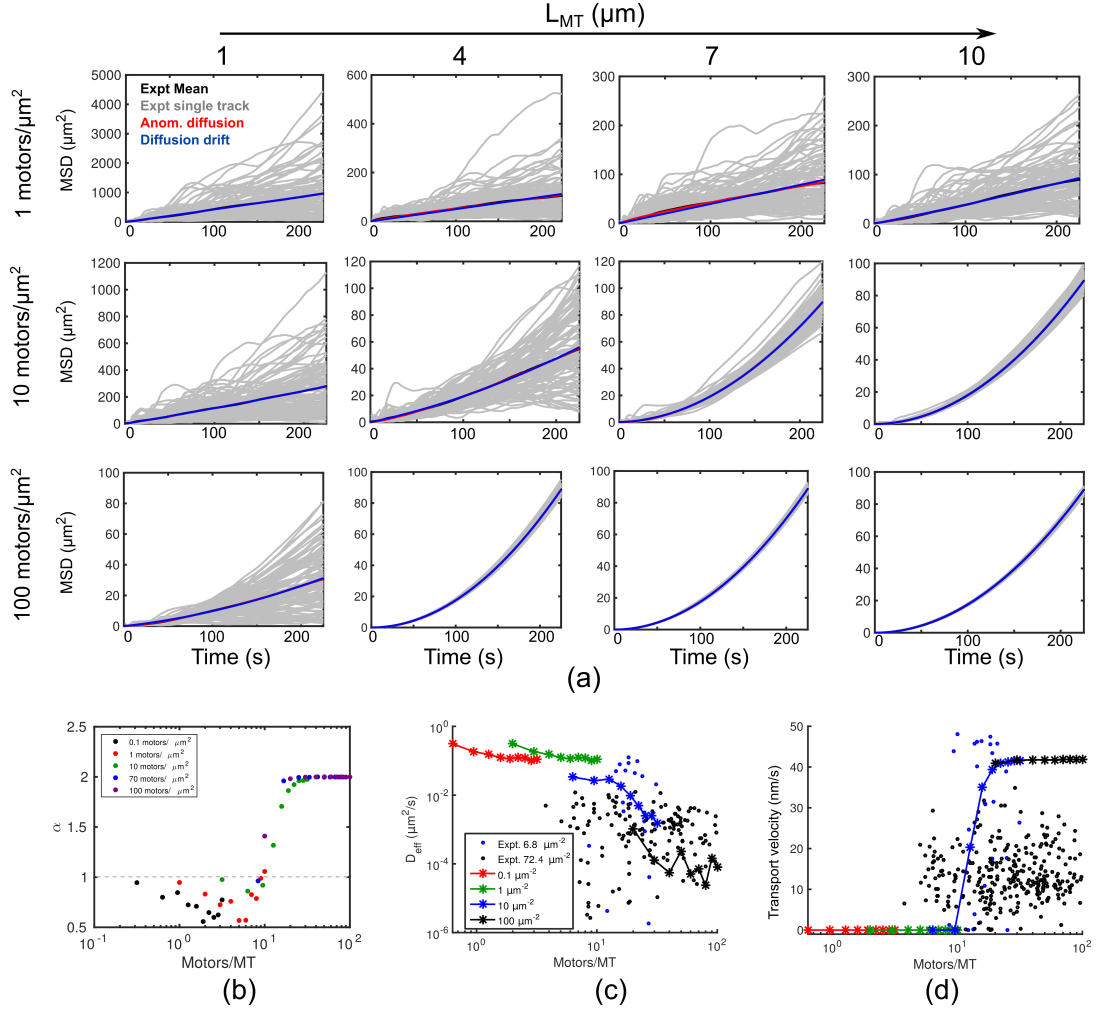


Figure 5.9: Motor number dependence of effective diffusion and transport velocity. (a) The mean MSD as a function of time (black) for increasing MT filament lengths (row) and motor densities (columns) is plotted. Data was fit to anomalous diffusion (red, Equation 5.13) and diffusion with transport (blue, Equation 5.14). MSD of individual MTs in gray. (b) The anomolay parameter (α) is plotted as a function of motor numbers in the simulation. The fits to the diffusion with transport model (c) the effective diffusion coefficient (D_{eff}) and (d) transport velocity (v) of MTs is plotted as a function of the expected motor numbers. Simulation data corresponds to the median values over 100 MTs from a MT of given length. The experiment data in (c,d) represents the median values over the MTs for the binned filament categories. Colors represent motor densities. ((c and d) adapted from *Jain et. al. 2019*).

Additionally, the possibility of statistical uncertainty in estimating D_{eff} and v from MSD fits cannot be completely ignored. It suggests the need for an alternative measure of directionally persistent motion. Thus a simpler and more robust measure of

directionality χ , which is independent of any fitting [15, 134] was implemented as described in the method section 5.3.5. In experiments, the mean directionality (χ) is length dependent for low motor densities but not for high density with a four-fold change in χ seen when MT lengths increase from $3 \mu\text{m}$ to $L \geq 4 \mu\text{m}$ only at low motor densities (Figure 5.10(a), [130]). Interestingly, motility changes quantified by χ correspond to three qualitative modes of filament transport for increasing length at low motor densities, also captured in time projections of the MTs: (a) swivelling by short filaments ($\sim 1 \mu\text{m}$) when 1-2 motors are expected to interact, (b) transport and intermittent changes in direction of intermediate length ($\sim 3 \mu\text{m}$), when interacting with ~ 7 motors and (c) directionally persistent transport of long filaments ($5 \mu\text{m}$), when interacting with ~ 10 motors (Figure 5.10(b), [130]). Similar MT length dependent increase in χ is captured in simulations at a motor density of 10 motors/ μm^2 (Figure 5.10(c)). In contrast, directionality at motor densities of 70 to 100 motors/ μm^2 which is comparable to the high density estimate in experiments suggests higher degree of directionality in its transport. MTs exhibit directionally persistent motion independent of the MT lengths at motor density 100 fold higher than the estimate in experiments. The motility patterns at 10 motors/ μm^2 , comparable to the low density estimate in experiments shows similar trends in time projections of the MTs (Figure 5.10(d)): (a) swivelling and occasional free-diffusion when filaments encounter 1-2 motors, (b) 2D search interspersed with stretches of transport with 3-5 motors and (c) persistent directional transport when the number of motors encountered exceeds 5. When the data from different motor densities and MT lengths is combined, in terms of the expected number of motors interacting with a filament, all the data falls onto a trend of increase and saturation of χ above a threshold of 8 to 10 motors (Figure 5.10(e), [130]). This motor number threshold above which transport becomes directionally persistent is based on the expected number of motors a filament encounters, while binding-unbinding kinetics would result in a lower number of bound motors based on single molecule mechanochemistry of this yeast non-essential dynein.

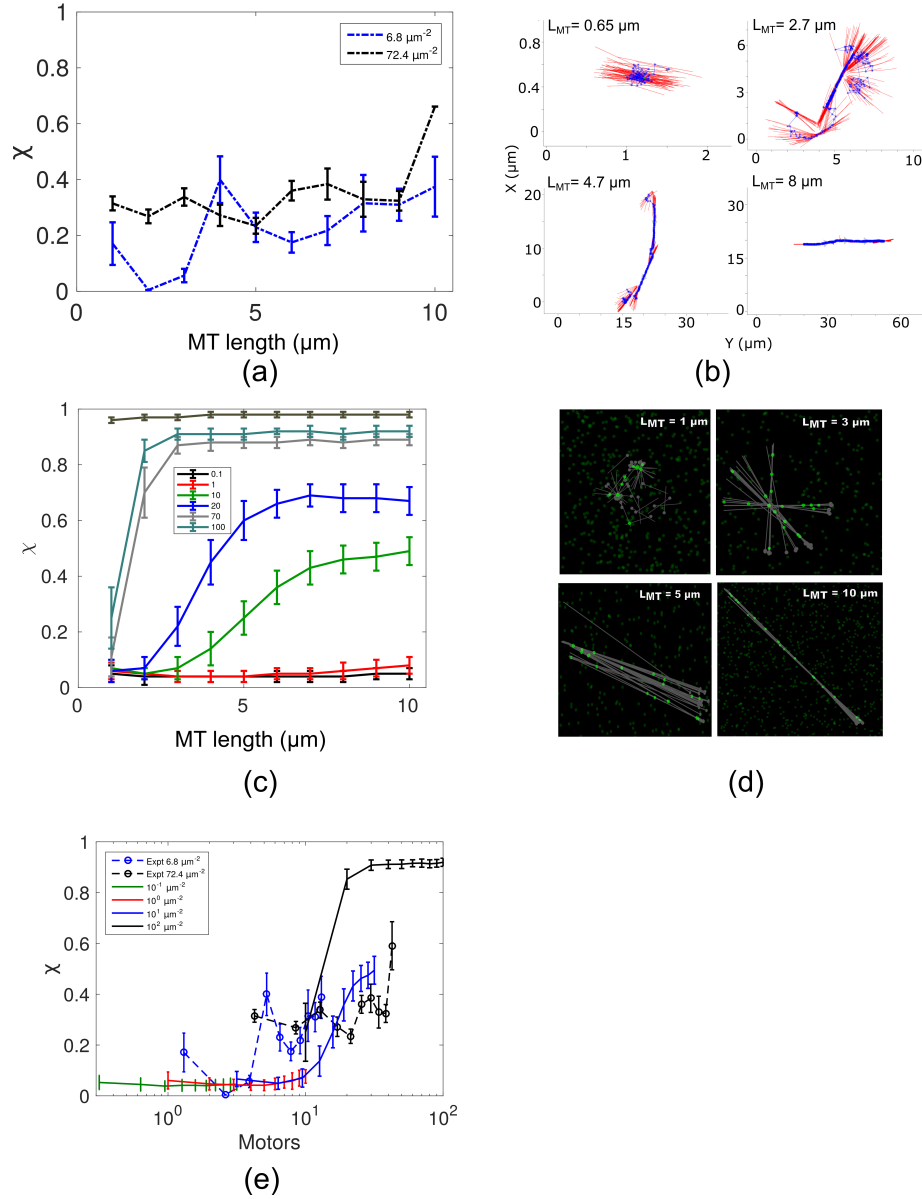


Figure 5.10: Length and motor-dependence of MT transport directionality in experiment and simulation. (a) The mean directionality, χ ($\pm$ s.e.) of MT transport is plotted as a function of MT length in presence of two different motor densities (colors) from experiments. (b) Representative time-projections of filament contours (red lines) are overlaid with trajectories of centroids (blue *) from experiments for a low density of motors ($6.8 \text{ motors}/\mu\text{m}^2$) for increasing lengths. The time-interval between frames is 1 s. (c) The mean χ from simulations for MT lengths at varying motor densities is plotted. Colors correspond to motor densities. (d) Time projections of MT filaments (grey) in simulations at 0.25 s per frame for a motor density of $10 \text{ motors}/\mu\text{m}^2$ is represented. Two states of motors are indicated: bright-green: MT-bound, dark-green: free. (e) The value of χ from experiment (o-, mean $\pm$ s.e.) and simulation (-, mean $\pm$ s.d. from 100 MTs each) are plotted in terms of motors encountered per filament. Colors represent motor density in units of $\text{motors}/\mu\text{m}^2$.

5.4.5 Switch in directionality predicted with up to half of motors active

In order to understand the deviation in experiments and simulations at high density, the effect of motor inactivity on the MT transport was explored. In experiments multiple motor densities were used. To achieve high densities, motors were non-specifically adsorbed onto the glass surface. This could result in a mixture of orientations of motors. Additionally, the percent activity of motors is unlikely to be 100%. To consider these factors, decreasing percentage of activity for the same density was simulated. Simulations were performed to test the effect of varying percentages of motor activity from 10 to 100% at a constant surface density of 10 motors/ μm^2 , to address the potential role of inactive motors on MT transport and directionality statistics as a function of motor numbers (Figure 5.11). The inactivity is based on the inability of the modelled motor to step, which does not affect the binding and unbinding kinetics, comparable to the reported behaviour of inactive dynein in vitro [285]. The simulation outcomes from 50% active motors at a motor density of 10 motors/ μm^2 for increasing lengths indicate a decrease in the net transport as visualized qualitatively in the XY tracks (Figure 5.11(a)) when compared to the space explored by the MTs at 100% motor activity for the same motor density (Figure 5.11(a)). Also, the lowered mean MSD profiles with higher variability in the individual MSDs (Figure 5.11(b)) as compared to 100% active motors (Figure 5.9(a)) at the motor density of 10 motors/ μm^2 . As expected from the MSD curves, the averaged MSD profiles across representative MT lengths exhibit lower values at 50 % activity of motors when compared to the values from 100 % active motors (Figure 5.11(c)). The length dependent effective MT velocities exhibit saturation at values lower than ~ 40 nm/s for increasing proportions of inactive motors (Figure 5.11(d)). A similar trend is observed for the transport velocity (v) obtained from the fits to diffusion with transport model (Figure 5.11(e)).

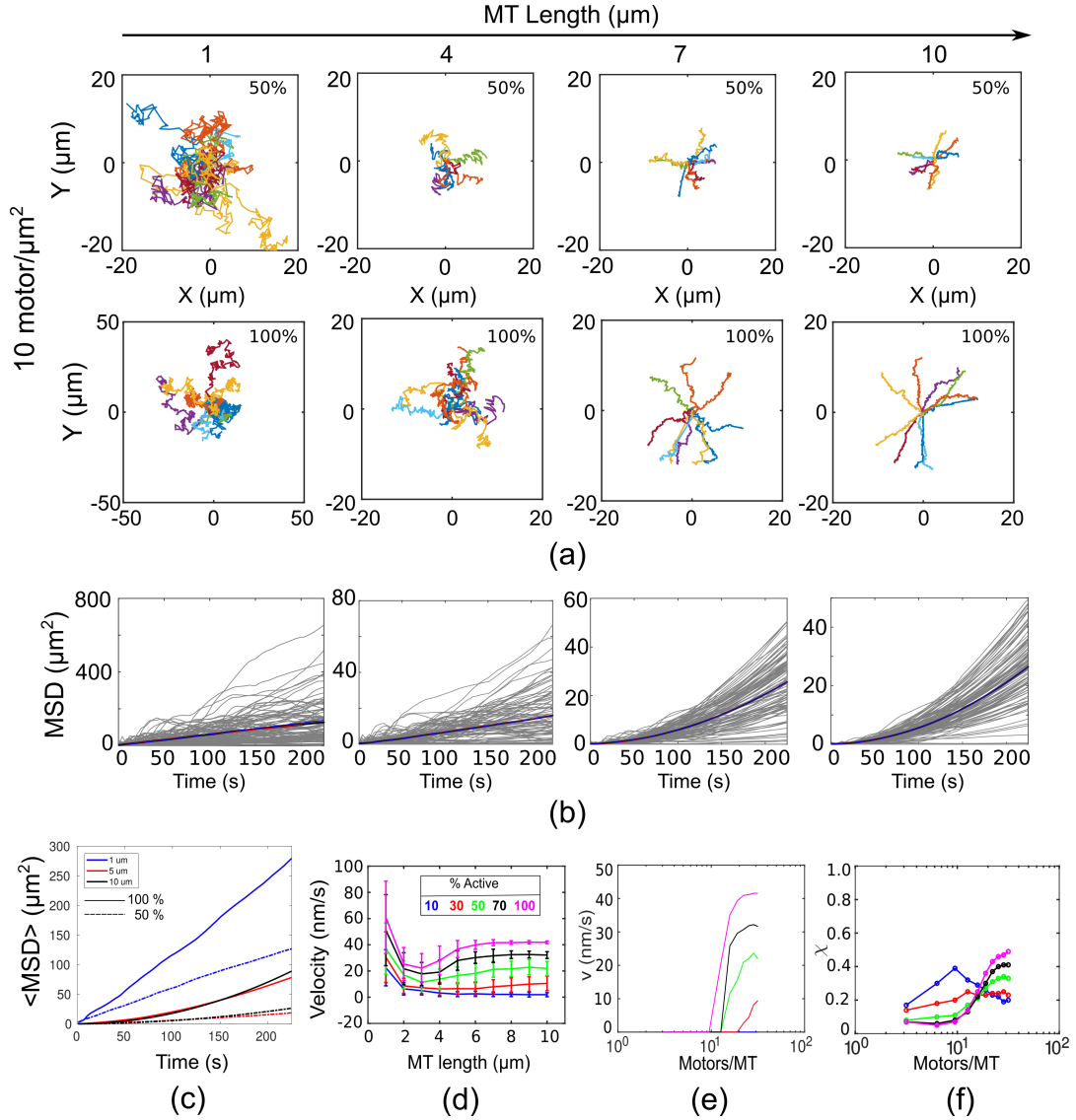


Figure 5.11: Effect of motor inactivity on MT transport. (a) XY trajectories for increasing MT lengths at a motor density of 10 motors/ μm^2 is represented. Colors indicate tracks. (b) MSD profiles of MT tracks corresponding to the xy tracks in (a). Gray corresponds to MSDs of individual MT, black: mean MSD and blue (fit to anomalous diffusion model) and red (fit to diffusion transport model). (c) The averaged MSD curve for 3 representative MT lengths is compared at 100% (-) and 50% (- -) activity of motors. (d) Length dependent MT velocities for increasing proportion of active motors. Color correspond to differences in % activity of the motors. (e) Transport velocity (v), obtained from fits to the diffusion with transport model and (f) directionality in MT transport is plotted as function of motors/MT for increasing proportions of active motors. Colors correspond to percentage of inactive motors.

Surprisingly, the number of motors at which the MT transport undergoes the transition in directionality (inflection point) remains a constant value, even when just half the motors are active. Here, motors were modeled as being defective in stepping alone (immobile), with all other properties same as the ‘wild-type’ dynein model. Additionally, for lower than 50% activity, the qualitative trend in the directionality (χ) is dramatically different, potentially serving to validate our assumption of the activity of the motors. A possible physiological explanation for the insensitivity of the percent inactive fraction of motors, is also to be found in published reports of the single-molecule dynamics of yeast dynein dimers that report the ability of one active motor head to drive the motility as fast as two active heads, provided its inactive partner has moderate affinity for MT [287, 285, 313]. It suggests that the affinity may further decrease with increasing motor densities due to the orientation defects. This suggests, simulations with lower binding rates of motors would mimic the high density experiments better. The MT speed for decreasing motor binding rates at 100 % active motors already indicate increased component of diffusion (Figure 5.8) suggestive of decrease in directionality and transport velocity.

5.4.6 Simulating the effect of alternative detachment mechanics on MT transport directionality

The asymmetric model of dynein detachment mechanics developed in this study is based on the single molecule report [289]. Whether this asymmetric detachment model is essential to reproduce the length and motor-number dependence of 2D MT transport directionality, alternative detachment models were tested. Parameter values of the two alternative models were identical to the more complex asymmetric detachment rate model as described and they are: (a) constant ($r_d = r_d^0$), i.e. load-independent detachment, (b) symmetric load-dependent detachment based on Kramers law [155] ($r_d = r_d^0 \cdot e^{|F_{\parallel}|/F_s}$), keeping all other parameters identical to the asymmetric detachment rate model. and (c) the asymmetric detachment rate model

described in methods section 5.2.3. None of the alternative models predicted the shape of change in χ with N that could reproduce the experimental data. Although, the directionality increases with motor numbers in all the three models (Figure 5.12(a-c)), the threshold number at which the switch is observed differs (Figure 5.12(d)). This result appears to point to motor-detachment as a determinant of the onset of directionally persistent transport, and not density and spacing, which are common to all three motor models. We speculate, this mechanical property might have evolved to exploit the low density of available dyneins at the yeast bud cortex during ‘search and capture’ of astral MTs, to enable correct orientation of the nucleus by an initial random phase, followed by a sharp transition to directionally persistent motility. Such an idea could be tested in experiments in future. We can show in simulations that the additional mechanism that causes an increase in directionality above a threshold results from a combination of 2D ‘search’ by MTs for motors (Figure 5.10) and an asymmetric force-dependent detachment rate model of single motors are responsible for the seemingly higher value (> 2 motors) of this transition.

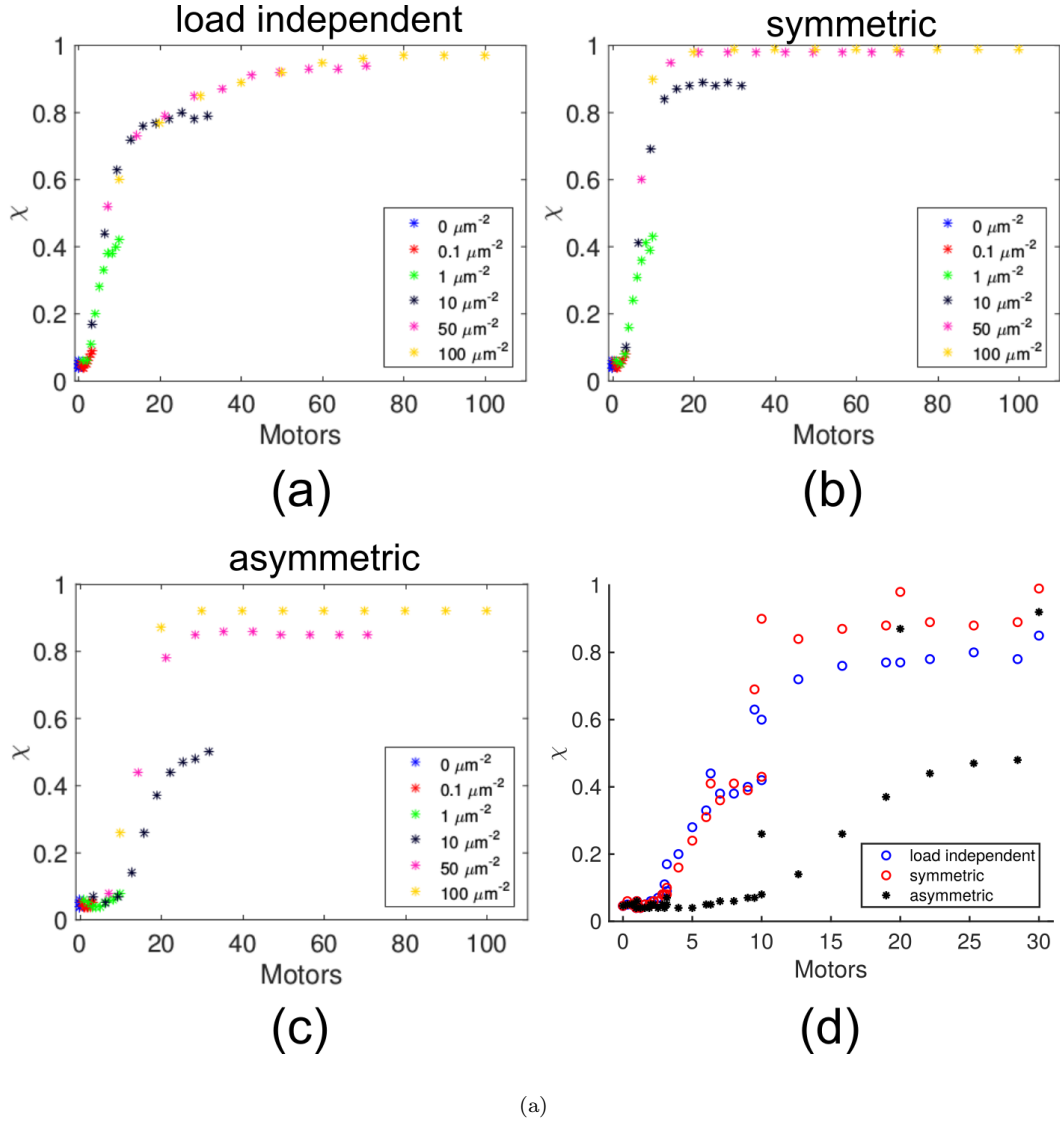


Figure 5.12: Simulations of the effect of motor detachment models on directionality. The change in directionality χ as a function of average motors acting per MT are compared for a range of MT lengths and densities. The detachment models compared are: (a) constant detachment rate of r_d^0 , (b) symmetric detachment rate based on Kramers law ($r_d = r_d^0 \cdot e^{|F_{\parallel}/F_s|}$) [155, 15, 134] and (c) asymmetric detachment rate that differ in forward and backward directions. Colors indicate motor density (ρ_{2D}) and each point corresponds to the mean directionality for a given length ($N_{MT} = 100$). (d) The data in (a-c) is plotted for a smaller range of motor numbers. Colors indicate the detachment model. Model parameters in Table 5.1. Adapted from Jain et. al. 2019

5.5 Conclusion and Discussions

A minimal model for the yeast dynein is developed based on the multiple single molecule data reported previously. The simulation outcomes recapitulates the MT transport behaviour observed in experiments. A wider range of motor numbers was explored in simulations. We observe both MT length and motor density dependent MT motility trends in simulations consistent with the experiments. The distinct MT motility trends that emerge in simulations are: (i) a predominant motor independent free diffusion of MTs, (ii) motor dependent swivelling of MTs with occasional diffusion, (iii) combination of motor driven directional transport and diffusive events and (iv) motor driven directionally persistent transport. The data across MT lengths and motor densities was combined into a single parameter i.e. number of motors encountered per MT (N) based on the square lattice approximation. The qualitative and quantitative measurements in experiments and simulations clearly indicate motor number dependent change in motility patterns.

The transport velocity exhibits a number dependent sharp increase when ~ 10 or more motors are expected to interact with MT (Figure 5.9(d)). Surprisingly, such collective transport pattern emerge in simulations just based on the single molecule parameters and the model of stepping and detachment behaviour. The symmetric models tested also suggest that only a few (1-2) motors with symmetric detachment can result in directionally persistent transport, while the asymmetric detachment model requires a larger ‘team’ of motors to encounter MTs (Figure 5.12). We speculate this may point to a property of this yeast dynein to ‘switch’ from random to persistent transport of MTs through an N -dependent (collective) mechanical coupling, rather than a simple 2D ‘search’. This would suggest that such a motor number based threshold dependence of persistent filament transport might differ for a full length dynein construct, due to a quantitative difference in the load dependence as compared to the truncated dynein used in this study.

Simulations predict that while the directionality of transport over three orders of magnitude of motor density appears to fall on one curve resembling saturation kinetics, the transition is not ‘smooth’ as a function of motors per MT. For instance the directionality of filaments of increasing lengths in presence of $10 \text{ motors}/\mu\text{m}^2$ do not appear to increase above 0.4, while higher values of directionality are only seen in case of an order of magnitude higher motor density. This could be due to the fact that increasing MT lengths for a given motor density could result in filament bending and result in defects in motion (kinks, spiral etc.) as previously reported for myosin and kinesin [314], thus reducing directionality. Additionally, simulations also predict the point of inflection of directionality as a function of motors (N-dependence) will be seen even if as little as half the motors are active (Figure 5.11(d)), suggesting a potential experimental test of the model.

Simulations predict a decrease in diffusion coefficient with increasing motors per MT with MT lengths ranging from 1 to $10 \mu\text{m}$ and densities ranging from 10^{-1} to $10^3 \text{ motors}/\mu\text{m}^2$ (Figure 5.9(c)). In simulations, MTs that undergo free diffusion due to the absence of bound motors is captured while in experiments such filaments are lost during imaging, while filaments that exhibit short stretches of directional transport or swivelling motions do show similar trends as in simulations. This can further explain the observed discrepancy in MT speeds and velocities between the experiments and simulations especially at the low motor densities (Figure 5.7). The α from experimental MT tracks is always ≥ 1.5 (data not shown, [130]) suggesting absence of MT tracks that are purely sub-diffusive ($\alpha \leq 1$) and diffusive ($\alpha = 1$) as opposed to simulations where $\alpha \leq 1$ is observed (Figure 5.9(b)). However, MT tracks that undergo transient diffusion and swivelling is included in the analysis, if detected by the tracking algorithm. The MT velocity in simulations for shorter filaments yield higher velocities as diffusing and swivelling filaments deviate from linear path resulting in an over-estimate. Additionally, the high density data in experiments deviate from the quantitative simulation outcomes too. The qualitative XY trajectories in experiments are comparable to the tracks in simulations from 10^2

times the high motor density while the velocities are lower than the saturation velocity observed in simulations. Interestingly, the single motor velocity under no load condition is reported to be around 80 -100 nm/s for this dynein while the speed of spindle movement driven by the full length of this dynein is around 40 nm/s [312, 315]. This would imply that the MT velocity and speed decreases with increasing load. Whether with increasing MT lengths and motor densities, load experienced by the individual motors per MT increase or decrease. Also, in the *in-vivo* the force thresholds would change based on the full length v/s truncated yeast dynein.

In order to test whether the deviation at high density arises from the reduced proportion of active motors due to the immobilization method or crowding effects in experiments simulations with inactive motors were performed. In simulations, steric effects was excluded for the sake of simplicity eliminating any crowding effects. The simulations with inactive motors (Figure 5.11) suggest the motor inactivity upto 50 % of the total population is sufficient to capture the observed trend of motor number dependent switch in directionality with the transition occurring at a number $\sim 8 - 10$ motors as observed for the active motors. However, the MT velocity and transport velocity (v) decrease with increasing proportion of inactive motors that saturate at lower velocities as a function of MT length and motor numbers respectively (Figure 5.11(b,c)). It would be interesting to explore the effects of sterics in simulations and role of motor inactivity in experiments at high motor densities.

Interestingly, the observation of a sharp transition in gliding velocity (v_{eff}), transport velocity (v) and directionality (χ) from low values of (less efficient transport) to high values above a threshold of motors, in simulations and in experiments, resembles a phase-transition. Phase transitions in directional motility of motor-driven transport, have been predicted in theory for symmetric and asymmetric motors [316, 317, 318]. They have also been experimentally demonstrated in the case of antagonistic kinesins transporting anti-parallel MT-doublets [167]. Similarly, a simple

dynein driven collective MT transport system could also fall into this general category. The finding implies cells could regulate the qualitative nature of multi-motor transport by simply modulating the number of motors.

This result suggests the motor numbers of this dynein could have a role to play during *S. cerevisiae* mitosis in pulling MTs *in vivo*. The native yeast cytoplasmic dynein is localized to the daughter cell during mitosis [319] as part of the machinery that positions the nucleus to the bud neck between the daughter and mother cells [298, 283, 299]. During the G2/M to anaphase transition, astral MTs emanating from SPB embedded in the nucleus, are thought to ‘search’ and are ‘captured’ by dynein motors localized to the bud-cell cortex which then ‘reel in’ the nucleus [283]. The captured MTs are then transported in a manner reminiscent of a gliding assay, with both MT lengths and motor numbers changing in time. The reported number of dyneins present on cortical foci in budding yeast 6 ± 2 dimers [312]. We observe a transition in the directionality of MT transport when ≈ 10 motors are expected to interact with an MT. We speculate that the number dependence of random to directed MT transport, could affect the ‘search and orientation’ of the nucleus (Figure 5.2). Since the process involves collective motor transport, a transition to directed transport with increasing motor numbers might improve the accuracy and reduce the time taken for spindle alignment during division, while maintaining a constant velocity. Our experimental and theoretical observations suggest that the mechanics of the yeast dynein, might be adapted to ensure robust positioning of the nucleus in anaphase. Further quantitative analysis of yeast nuclear migration will be required to test this hypothesis.

5.6 Outlook

This study can be extended to simulate spindle positioning in yeast cells during the pre-anaphase stage. The motor number dependent (N) transition in directionality

and transport velocity of astral MTs emanating from nucleus associated SPB can be tested. Further, it's proposed functional implication in 'search-capture and orient' during the spindle positioning can be tested. It necessitates for an explicit validation as the current outcomes are from a set up which is different from the *in-vivo* context such as: (a) MTs are non-dynamic in experiments and simulations, (b) viscosity is comparable to the *in-vitro* assay, (c) geometry of the MT is different from linear MTs and (d) dyneins are anchored at the cortex instead of uniform surface distribution. Additionally, it remains to be tested whether the minimal construct which is functional and like wild-type in the *in-vitro* experiments [273] can result in spindle positioning under physiological conditions. Recent studies suggest stepping rates of single motors to be dependent on the magnitude and direction of the load experienced by the motor [320, 321]. However, in the minimal model here, stepping rate (r_{step}) is constant [287] and independent of the load direction. While a scan over the r_{step} indicates saturation at higher values for MT speed and velocity beyond a threshold number of motors with increasing stepping rates (Figure 5.8(a)). In contrast, the model predicts no significant effect of increased binding probabilities and reach distance on MT speeds and velocities (Figure 5.8(b)). It remains to be tested whether the motor driven MT transport at non-physiological high densities and long MT lengths is similar to the load experienced by motors during spindle positioning. The potential source of high loads in *in-vivo* other than the high motor numbers or long MT filaments could be from the geometry of MTs and drag due to the nucleus embedded SPBs, cellular viscosity and crowding in the cell and the effects from the cell geometry. Taken together, it suggests an *in-vivo* modeling of the spindle positioning may help corroborate multiple reports across systems and improve the mechanistic understanding of the physiological process and their implications.

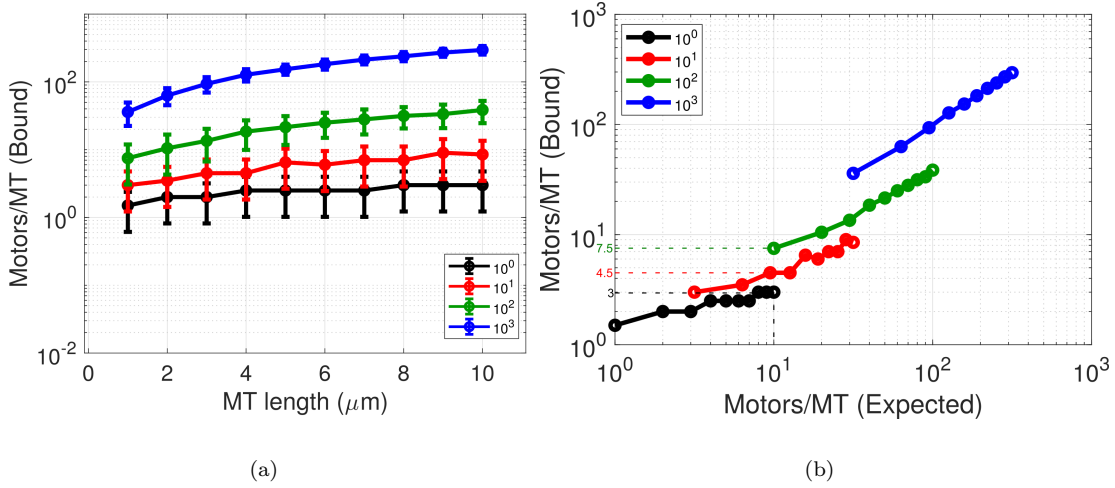


Figure 5.13: MT Bound motors in simulations. (a) Mean number of bound motors per MT for increasing MT length (X-axis) across the varying motor densities (colors) is plotted. Error bar corresponds to S.D. (b) The mean number of bound motors per MT in simulations is plotted as a function of expected motors calculated based on square lattice approximation. Colors correspond to motor densities (motors/ μm^2). Means are calculated over 100 MTs for each length class.

Although *in vivo* experiments have shown the role of dyneins in spindle positioning [283, 73] and estimated the number of dynein foci at the cortex [319], the quantification of exact number of motors interacting with the MT at the cortex is difficult to estimate experimentally. Additionally, the number of MT bound motors per MT was calculated in simulations explicitly. Surprisingly, the average number of bound motors remain nearly constant for increasing MT lengths at motor densities of 0.1 and 1 motors/ μm^2 . The motor numbers between the two densities for MTs of same length are also comparable. An increase in number of bound motors per MT is observed with increasing MT lengths at higher densities (Figure 5.13(a)). The number of motors/MT calculated from the square lattice assumption (Expected number of motors/MT) was compared to those calculated in the simulations (Actual number of motors/MT). We observe that square lattice approximation results in an overestimate of motor numbers at low densities and tends towards the expected numbers only at the high densities of 1000 motors/ μm^2 (Figure 5.13(b)). For instance, an expected number of 10 motors/MT corresponds to 3, 4.5 and 7.5 motors/MT from

simulations at motor densities of 0.1, 1 and 10 motors/ μm^2 . It remains to be tested if this deviation is due to the method adopted for estimating linear density from the surface density or an effect that arises due to the load dependent stepping and detachment behaviour of the dynein motor. Since the directionality trends exhibit differences across motor detachment models (Figure 5.12), the understanding of the load dependent behaviours in the collective remains to be addressed. Since, dyneins are anchored at the cortex in yeast during nucleus and spindle positioning, the asymmetric detachment model may provide directional bias which is dependent on the force experienced by the motors interacting with astral MTs. Thus it is essential to address the load experienced by the other bound motors and whether it confers any advantage during the pulling of astral MTs. A systematic evaluation of the duration for which a motor is engaged to the MT and the average number of bound motors across the different detachment model would provide insights on the forces generated by the motors at the collective level.

This study suggests MT lengths and motor densities both the MT transport and can be best understood in terms of motor number per MT. However, the effect of motor density is more pronounced as it strongly influences the search process in 2D while MT lengths has a linear effect. It suggests any deviation from homogeneous distribution of motors on the 2D surface and any anisotropy in the motor distribution in space would definitely bias the search process and the encounter of MT-motor interactions. Thus, the effect of MT lengths and motor numbers require explicit exploration in higher dimension and non-homogeneous surfaces. In addition, it asks whether the collective MT transport statistics would be similar in a system wherein the MTs are dynamic and how does the motor models effect the collective behaviour. Interestingly, when this dynein is localized at the cortex of a 2D circular cell, in a multi-aster set up with astral MTs undergoing length fluctuations in time due to dynamic instability as discussed in Chapter 4, loss of collective multi-aster rotation is observed (Figure 5.14(a,b)). The XY tracks and angular velocities suggest random fluctuations and absence of directional sliding at the cortex. In contrast, in presence

of mammalian cytoplasmic dyneins at the cortex (Chapter 4, [15, 134]) the system of asters spontaneously self-organize into a rotating system of asters (Figure 5.14(c,d)). All other system components and parameters were held same as in Chapter 4, Figure 4.9, with a linear dynein density of 100 motors/ μm and cytoplasmic kinesin-5 motors at 10 motors/ μm^2 . The motor number dependent trend in directionality is strikingly different with the mammalian dynein modelled wherein transition occurs at a low number of motors/MT (Figure 5.14(e)). Such difference could be due to the variation in the motor properties such as, the single motor velocity of yeast dynein is 10 times and the binding rate is half of the mammalian dynein. In order to test the role of motor detachment models, the force experienced by the motors needs to be evaluated.

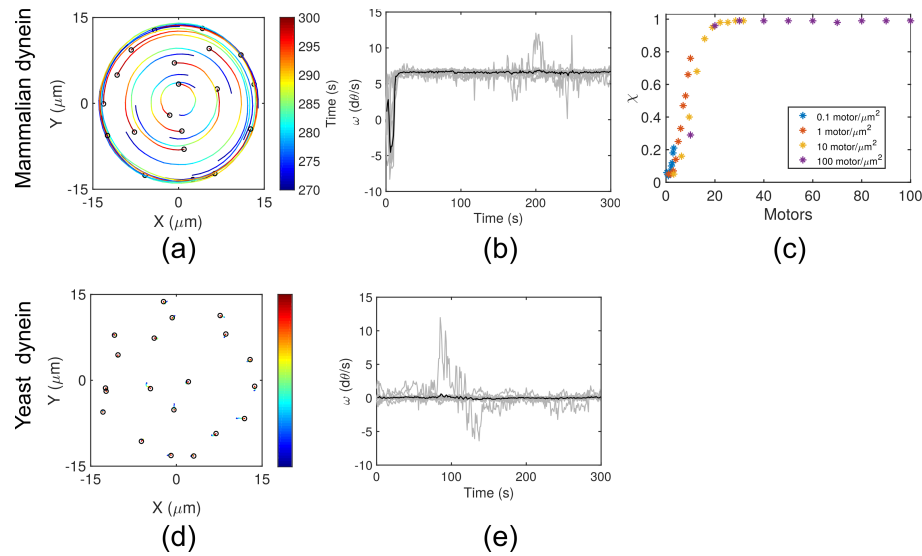


Figure 5.14: Aster motility in confinement with mammalian and yeast dynein at the cortex. Multiple asters were randomly initialized with kinesin-5 motors in a 2D circular cell in the presence of cortical dyneins. XY trajectories of asters over the last 30 s is represented with dyneins modelled as (a) mammalian and (d) yeast dyneins. Color encodes for time. The instantaneous angular velocity (ω) of asters in time (gray) and the mean over the asters (black) is plotted for the (b) mammalian and (e) yeast dynein. (c) The dependence of directionality (χ) on the motor numbers is plotted. This is calculated from the simulations of linear MTs gliding over the sheet of immobilized mammalian like dyneins. Colors correspond to different surface densities of dyneins. Radius of the cell is 15 μm , N_a is 20 asters, kinesin-5 complexes = 10 motors/ μm^2 and cortical dynein density 10² motors/ μm .

In summary, this study suggests single motor behaviour can effect the nature of MT transport when multiple such motors are involved. The switch from diffusion to directed transport and transport velocity depends on the number of motors bound per MT. We propose such N dependent transition may allow for spindle positioning by diffusion driven search and controlled MT sliding along the cortex under motor number limiting scenario of the budding yeast cell at pre-anphase. We speculate different motor behaviors might have evolved across systems in nature based on the (a) size and shape of the cell, (b) geometry and dynamicity of MTs, (c) location and the properties of the environment where present, and (d) their numbers in the context of biological function.

Chapter 6

Summary and Conclusion

6.1 Summary

The aim of this study was to explore and understand the mechanical basis of multi-aster and multi-motor systems in confinement. In order to determine the general principles of multi-aster motility and positioning, the parameter space consisting of microtubules and motor properties were explored (Figure 6.1). The key findings as a consequence of varying these parameters are highlighted in this chapter. More than ~ 250 scenarios were scanned and the key results discussed in these chapters highlight the effect of combination of such parameters. In **Chapter 1**, the effect of motor localization, their numbers and properties pertaining to the directional movement of asters is addressed (y-axis in Figure 6.1). A model of spatial gradient of immobilized dyneins and diffusible coupling dyneins are proposed, which recapitulates the experimental statistics from the mouse oocytes. **Chapter 2** summarizes the effects of relative size of the system, role of antagonistic motors and MT dynamics in pattern formation and aster positioning in cells (x,y,z -axis in 6.1). It demonstrates that kinesin-5 driven pushing in the absence of MT dynamics, result in an ordered arrangement of asters for a range of packing fraction. The simulation outcomes are validated experimentally in the *Phallusia* oocytes. **Chapter 3** discusses the emergence of self-organized motion arising due to the interplay of MT dynamics, motor types and aster numbers. Here, the onset of collective and coherent rotation was observed at specific combination of cortical dyneins, cytoplasmic kinesin-5 motors and MT dynamics. In **Chapter 4** the collective behaviour of motors due to the motor mechanics on MT transport is addressed. The effect of motor numbers and MT lengths on the transport of linear MTs is discussed.

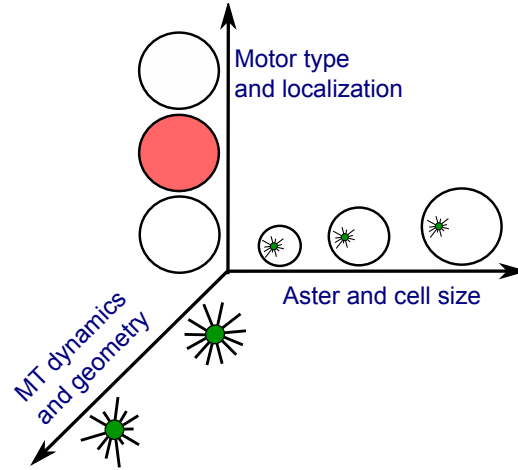


Figure 6.1: Summary of the multi-aster exploration space. The collective behaviour of multi-aster system was explored for the varying properties such as (x-axis) effect of aster and cell size, (y-axis) role of MT dynamics, astral MT distribution and MT geometry and (z-axis) motor types, their localization and numbers.

6.2 Significance

This study attempts to find the general principles of cellular organization that would help in improving our current understanding of cellular processes such as acentrosomal spindle assembly, spindle positioning and self organized pattern formation largely based on the single aster studies. The understanding of microtubule cytoskeleton also helps understand the cytoskeleton dynamics in oocytes and cancerous cells which have medical implications. Multiple studies have addressed mechanisms involved in movement and positioning of a single aster in the cell. However, fewer systems exist in which multiple asters are found to occur naturally. In most of the cases multi-aster scenarios are associated with diseased conditions or chemical and physical perturbations in the system. Multiple asters are found in all the vertebrate oocytes and syncytial embryos wherein, they are involved during the spindle assembly, faithful segregation of chromosomes and cell division. Thus the mechanisms by which their numbers, spatio-temporal position and dynamic movements

are controlled and maintained (as discussed in the chapters) inside a cell is of great importance. The results, provide insights on the mechanisms of MT-motor interactions that could have evolved based on the mechanical constraints of the system and the processes associated with it. The findings raise yet more questions in the field that can be addressed in theory and in experiments in future, has been discussed at the end of each chapter under the outlook section.

6.3 Conclusion

Every aster in a multi-aster system experiences tug-of war in forces due to it's radial geometry similar to a single aster. A simplified view of an aster in the presence of uniform distribution of single type of motor, dyneins is depicted in 1D (Figure 6.2(a)). Asters undergo transient symmetry breaking event resulting in short directional movements that arise due to stochastic binding and unbinding events of the motors and the MT dynamics. In confinement, type of motor can dictate the effective position of the asters based on their polarity and directionality of the motors. For instance, multiple asters gliding on a sheet of immobilized dyneins appear random and do not move much in the absence of MT dynamics while in the presence of kinesin-1 motors move towards the periphery where the boundary results in their confinement (Chapter 3 Figure 3.12(a)).

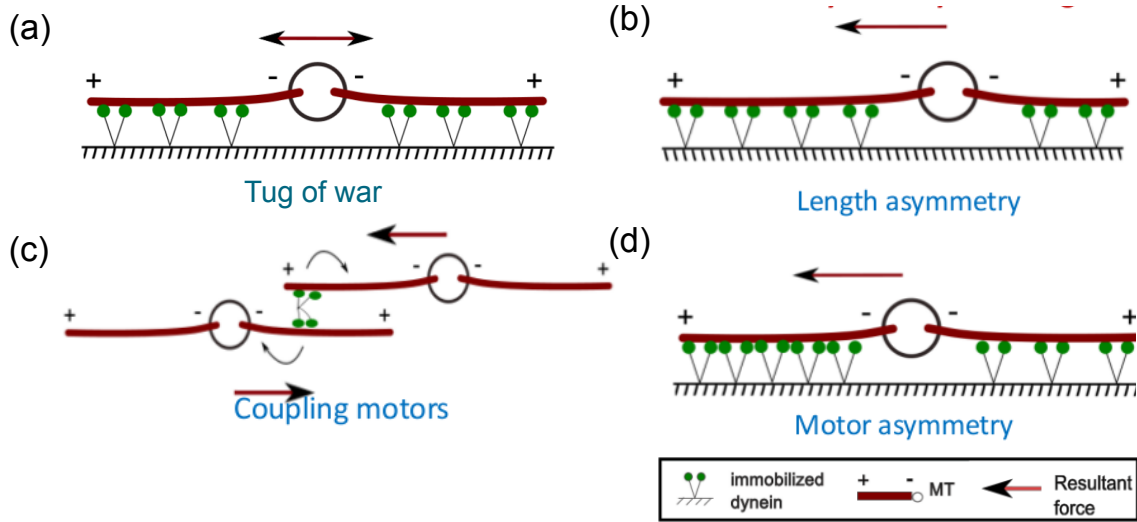


Figure 6.2: Mechanisms of symmetry breaking. Aster is depicted as a simplified 1D structure with a central MTOC and two emanating MTs from it (red) gliding on a sheet of immobilized dynein motors (green). (a) Tug-of-war of symmetric aster results in random movement of the structure. (b) Biased net movement in the direction of longer MTs due to the length asymmetry. (c) Clustering of motors result in local directional transport of the MTs. (d) Asymmetry in the motor numbers results in directional transport of a symmetric aster.

The number of transient symmetry events increase, when astral MTs undergo dynamic instability. Due to the stochastic length fluctuations, more number of motors interact with some of the longer astral MT filaments resulting in a directed movement at shorter time and spatial scales (Figure 6.2(b)). The stochastic fluctuations in the length gets averaged out due to the symmetric MT geometry, resulting in random motility of the structure. The type of immobilized motor and MT dynamics determines whether the net movement over the longer time and spatial scale is effectively random or biased due to the system properties. In the presence of MT dynamics, the effect is more pronounced (Chapter 3, Figure 3.12(b)) resulting in peripheral movement of the asters in presence of kinesin-1 while centering in smaller to mid cells in presence of dyneins. The degree of peripheral movement and centering depends on the proportion of the two motors that are uniformly immobilized in the cytoplasm. This suggests that multiple asters in confinement exhibit apparent symmetry breaking dictated by the motor type and availability of motor numbers

per aster. In a larger cell of mouse oocyte at meiosis I, multiple smaller asters gliding on a sheet of only immobilized dyneins do not center even in the presence of MT dynamics and cortical pushing (Chapter 2, Figure 2.5(c)). A spatial bias in the transition frequencies of MT dynamics (Figure 6.2(b)) biases the fluctuations in MT lengths such that the average astral MT lengths differ along the aster, resulting in an asymmetric aster. Surprisingly, the biased aster asymmetry was not sufficient to drive the directional movement of these asters (Figure 2.5(i)). On introducing force asymmetry in terms of the motor numbers in the cell, the multiple asters converged to the center of the cell (Chapter 2, Figure 2.5(l)). This suggests, for the multiple small asters length asymmetry is not sufficient to break the symmetry in forces as reported in multiple studies. Instead non-homogeneous spatial distribution of force generators translates force asymmetry into the directional movement of the asters (Chapter 2, Figure 6.2(d)). The mechanisms that result in emergence of such motor gradients in the cells needs to be addressed systematically. The result from this study in the light of previous results suggest that the mechanism that result in force asymmetry would depend on the relative size of the aster to the cell. Additionally, the combined effects of both the gradients did not improve the statistics of directed movement in the mouse oocytes (Chapter 2, Figure A8). Thus a systematic investigation between the asymmetry in the MT structure and the forces generated that translates to the directional movement in the presence of gradients would provide a formalism that can be explicitly tested in the *in vitro* systems. Such combined effects could arise due to the cell-cycle control of biochemical gradients and proteins in the cell.

The cross-linking or coupling motor proteins result in local directional movements relative to the interacting MTs. The direction of the MT movement depends on the motor type and the orientation of the interacting MTs as described in Chapter 3, Figure 3.2. In multi-aster systems, in addition to the role of immobilized motors, coupling motors play a significant role as they mediate inter-aster interactions. The

convergence of multiple asters in mouse oocyte require diffusible clustering motors in the cytoplasm along with the gradient of immobilized dyneins to recapitulate the experimental trends quantitatively. While the tetrameric kinesin-5 complexes are essential for the aster segregation and positioning, the stable aster patterns emerge only when the packing fraction of asters is optimal dictated by aster size, cell size and the number of asters in the cell. The addition of dynein had an antagonistic effect, resulting in more number of aster clusters as the proportion of dyneins increase in the cell (Chapter 3 , Figure 3.10). Under, optimal packing fractions the segregation effect of kinesin-5 motors tend to maintain the connectivity between the asters that result in onset of collective and coherent motion in the presence of dyneins at the cortex. The increasing number of clustering motors result in increased directionality due to stronger local pull and coalescence of asters in case of dyneins in the mouse oocytes, while increased degree of separation and coherence is observed in the presence of kinesin-5 motors. The relative proportion of the two diffusible motors show a size dependent spatial patterns in the system. Multiple asters center in cells of smaller size by mere pushing at the cortex independent of the motor type and undergo centering up till the mid sized cells based upon the proportion of dynein motors (Chapter 3, Figure 3.3). The centering ability under the similar conditions is enhanced in the presence of MT dynamics (Chapter 3, Figure 3.4). The MT dynamics along with the kinesin-5 motors perturb the regular packing of the asters via the motor interactions which is striking and stable in the absence of MT dynamics (Figure 3.11). Similarly, back and forth sliding movement of the asters along the cortex is observed when the dynein motors are immobilized at the cortex in the absence of MT dynamics (Chapter 4, Figure 4.2) that transition into a dynamic collective movement of multiple asters in the cell on introducing the dynamic instability (Chapter 4, Figure 4.2). The dynamic instability of MT filaments result in frequent stochastic symmetry breaking event that translates into directional movement, the persistence of direction however depends on the number of motors interacting per aster. Since, the MT dynamics is completely stochastic higher number of motors per aster often result in pulling of asters on either sides along the cortex in 2D thereby

resulting in frequent changes in direction of the aster motility (Chapter 4, Figure 4.12). While for low numbers due to the fluctuations in their lengths, on an average the initial direction of spontaneous symmetry breaking is maintained, as observed in outcomes of varying aster and dynein densities independently (Chapter 4, Figure 4.5) and (Chapter 4, Figure 4.3). Contrarily, increasing the total number of immobilized dyneins in the gradient of dyneins, result in an increased directionality as the aster encounters increasing number of motors towards the cell-center (Chapter 2, Figure 2.8(b)) or locally in Figure 2.6(a). The increasing number of motors per MT in simulations of gliding assays also result in increased directionality of linear MTs irrespective of the motor models (Chapter 5, Figure 5.12, 5.14).

The spatial distribution of motors along with MT dynamics, clustering motors and motor numbers effect the motility patterns of asters. The gradient on dynein originating from the cell-center result in convergence of multiple asters at the center due to the force asymmetry in that direction, while the same dynein localized at the cell-cortex result in pulling of asters towards the periphery and their sliding along the cortex (Figure 4.10). In some cells such as budding yeast, dyneins are non-essential and are only involved in spindle positioning during anaphase. To test whether there could be differences in the dynein behaviour that are explicitly localized at the cortex and have evolved as functional adaptation to the system, a minimal stepping and detachment model based on the reported single molecule parameters and behaviour was developed for the yeast dynein. On localization of yeast dynein at the cortex the striking sustained sliding movements failed to emerge in the system (Chapter 5, Figure 5.14). This suggests that forces generated by the yeast dynein at the same density, does not generate sufficient pulling forces required for the sustained rotations. This arises due to the detachment kinetics of the yeast dynein which captured the linear transport of MTs in the *in vitro* gliding assays.

Taken together, we can speculate that probably different mechanisms have evolved for directional movement of the MT structures based on the MT geometry and shape of the cell that regulate the positioning of aster and their numbers essential

for the cellular processes (Figure 6.3). The motor distributions in the cellular space (Figure 6.3) and the biochemical pathways could have evolved to tune the force-generating machinery in space and time to enable the cellular functioning. For instance, the detachment behavior of cytoplasmic motors could be different from that of the cortical motors, and may also be influenced by the shape of the cell and the size of the aster. Thus it would be interesting to test whether the minimal developed model of the yeast dynein that captured the *in-vitro* experimental trends could result in spindle pole body-aster positioning in the yeast cell. It would be worthwhile to test, if the sliding events can be sustained in their presence at the cellular cortex, if the geometry of cell is changed from being circular to elliptical as in the *C.elegans* embryo to a cylinder in case of fission yeast to incorporate the curvature of the cell. In addition, reports from *in-vivo* studies suggest dynein can result in increased catastrophe rates of the bound MTs [73, 67]. Thereby suggesting that decrease in MT polymerization at the cortex and localization of motors in clusters at cortex could be an evolutionary adaptation to suppress the sustained sliding of symmetric asters that exhibit dynamic instability of MTs.

In-vivo studies suggest that motors at the cortex are anchored via multiple anchor proteins, interact with lipid proteins and actin network as discussed in the Chapter 4. The complex interactions at the cortex was modelled phenomenologically to incorporate the effects arising from the complex environment. Further, it would be interesting to implement actin interactions at the cortex. All these studies together have implications in cellular processes such as acentrosomal spindle assembly and cell morphogenesis that require interplay of motors, MT dynamics and biochemical regulatory networks. In this study, the role of MT nucleation and branching of MTs has not been considered. Also, the biochemical pathways have not been incorporated in the study discussed in this thesis, while only their possible effects have been captured in the models and the parameters (Chapter 2 and Chapter 3). It would be worth to integrate the regulatory networks with the microtubule mechanics to gain complete understanding on the functioning of the system.

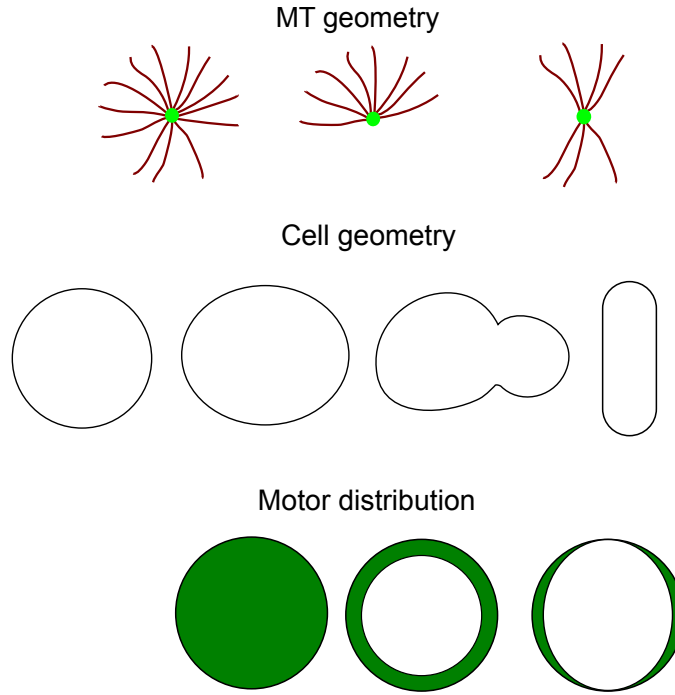


Figure 6.3: Schematic represents different forms and organization. Cells exhibit diverse shapes, organization and motor distributions. Here, it is speculated that such forms could have evolved based on the geometric constraints of the system and the functions associated with them.

The mechanics and biochemistry together result in self-organization of the microtubules and motors in the cell that are of functional significance. For instance, the bipolar spindle that assembles in the mouse oocytes at meiosis I involves self-organization of microtubule cytoskeleton and the interplay biochemical gradients. Prior to nuclear envelope breakdown (NEBD), multiple asters nucleate in cytoplasm and converge to the central chromatin region in the absence of RanGTP gradient [11, 10]. Although RanGTP disruption does not effect the centripetal movement it is observed to reduce the MT mass and delay the spindle assembly [10]. Enucleated oocytes prior to NEBD have been observed to form asters that interact, fuse and move however, failed to converge at the centre of the oocyte [150]. While the degree of convergence correlated with the time at which oocytes were enucleated [150, 10]. Inhibition of actin and kinesin-5 did not affect the centripetal motility [10]. About ten percent of the asters were observed to be peri-nuclear prior to the NEBD, after

the central congression of the asters, interact with chromosomes to form a homogeneous ball of MTs. Simultaneously, bivalent chromosomes undergo centrifugal sorting during chromosome individualization in the presence of kinesin-5 motors to occupy peripheral positions in the MT ball. The velocities of centrifugally moving chromosomes were comparable to the velocity of the chromokinesins as observed in *Xenopus*, however the knock out of Kid at this stage did not affect the process, implying presence of other chromokinesins in the mouse oocytes [169]. After the formation of apolar circular mass of MTs with individualized bivalents, ejection of multiple poles occur which collapses into a bipolar spindle by progressive clustering of the poles. Polar ejection does not occur on inhibition of kinesin-5 resulting in collapse of the spindle at this spherical stage [10]. Clustering of poles result in formation of bi-polar baralled shaped spindles responsible for chromosome segregation and cell-division. Interestingly, in mouse embryos RanGTP gradient and kinesin-5 is involved in centripetal movement of the asters and assembly of a bipolar spindle wherein with subsequent cell divisions the acentrosomal spindle assembly transits into centrosomal spindle assembly [18]. Most of the studies till date assume an anti-parallel arrangement of MTs as the start point for spindle assembly [172, 131]. The findings from this study provides insights on each of these modules of spindle assembly and can be combined together to understand the bipolar spindle assembly via self-organization.

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Appendix A

Supporting material to Chapter 2

A.1 Msd analysis and anomalous diffusion

MT-transport results in a tug-of-war of motors when radial MT arrays are transported by a sheet of single type of immobilized molecular motors [15]. Such a tug-of-war has been quantified by evaluating the nature of the msd profiles, and qualitatively distinct msd profiles can be identified based on the fit of msd to the model of anomalous diffusion (Equation 2.11) where D' is the apparent diffusion coefficient and α the anomaly parameter. Experimentally measured msd plots of MTOCs appear to be directed in their motion, near the cell center (Figure A.5(a)), in the mid-cell region (Figure A.5(b)) and at the cell periphery (Figure A.5(c)). The radial distance of nucleation does not change experimental D' significantly (Figure A.5(d), A.1(a)) and simulations with low-densities of both motor types ($f_0 = 2$ and 7 pN) reproduce this trend. The experimental anomalous diffusion exponent α is consistently > 1 , indicating of anomalous super-diffusion, and is also invariant with nucleation distance (Figure A.5(f), A.1(b)). The short-lived trajectories appear to have higher apparent diffusion coefficients, as compared to long-lived ones and the α value decreases with increasing time for which the trajectory was followed (Figure A.1(b)). The deviation (ϵ) between simulations and experiments of the averaged D'

(Figure A.5(e)) and α (Figure A.5(g)), suggest a gradient of weak ($f_0 = 2$ pN) motors with a density of 10^4 motors/oocyte are qualitatively closest to experimental data. The α from the motor-gradient model is consistently lower than experiment. This deviation between simulation and experiment in the mid-cell region is also observed in the χ measure in the mid-cell region, where the experimental value is higher than simulation (Fig 2.6). This would suggest potentially short-range directional motions in the mid-cell region, might cause this effect.

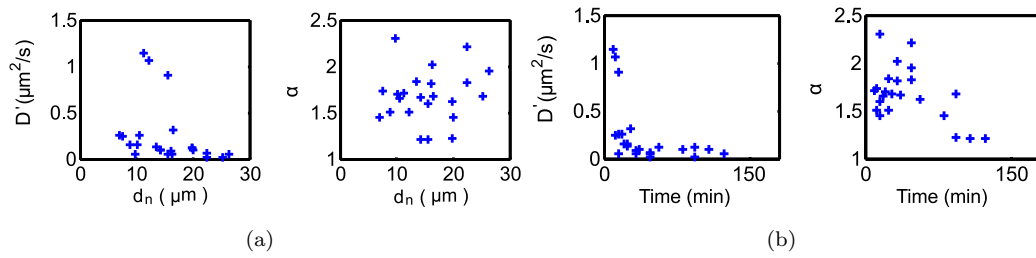


Figure A1: Spatio-temporal trends in apparent diffusion coefficient and anomaly parameter from experiment. The apparent diffusion coefficient (D') and the measure of anomalous diffusion (α) were obtained from fitting the anomalous diffusion model (Equation 2.11) to experimental msd profiles. (a) D' and α are plotted as a function of nucleation distance and (b) time duration of the trajectory.

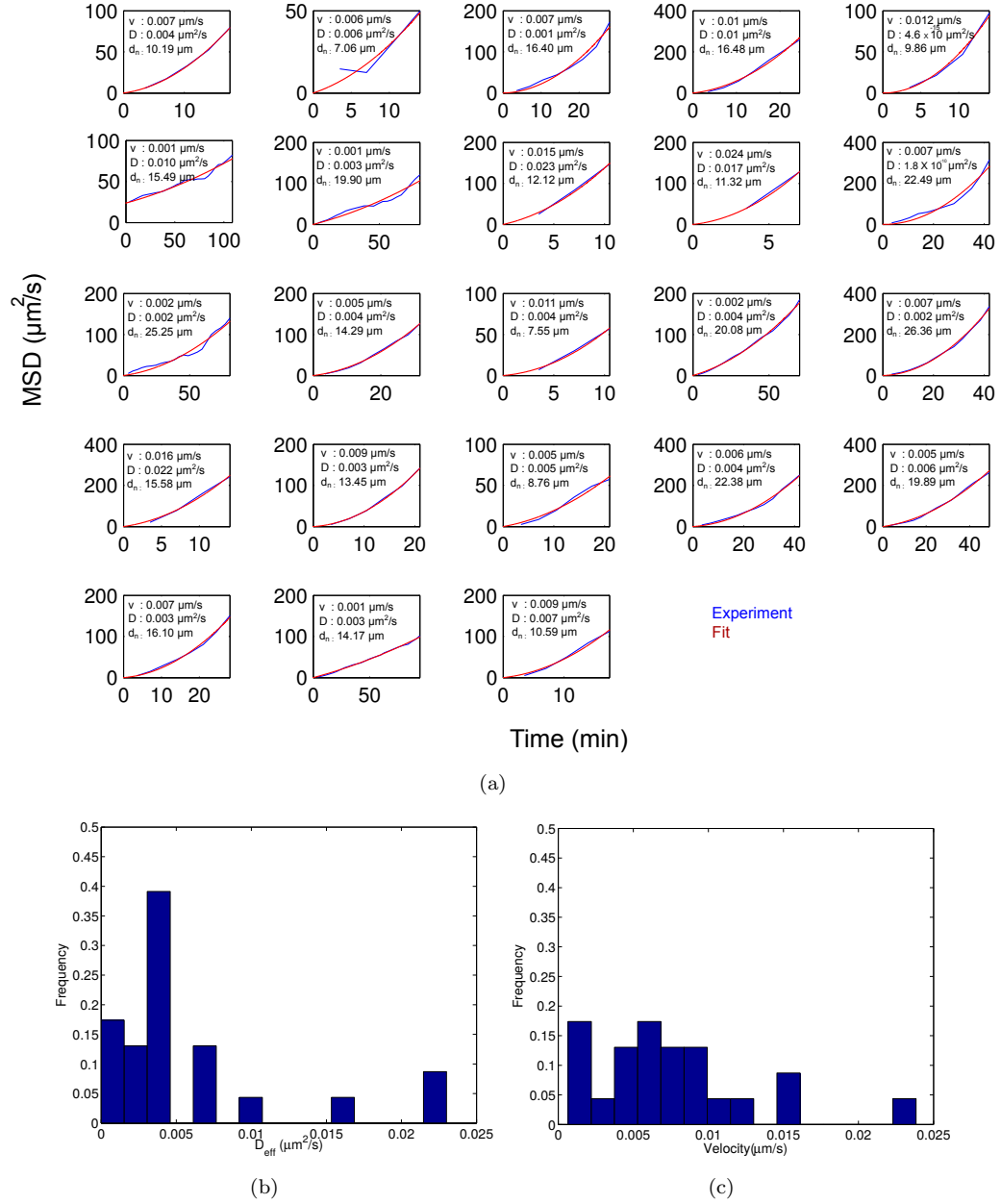


Figure A2: Msd of asters measured in experiment. (a) The msd (μm^2) (blue line) as a function of time is calculated (based on Equation 5.12) for all available MTOC trajectories from Schuh & Ellenberg [10] and fit to an effective diffusion and drift velocity model (Equation 2.10). The frequency distribution of the fit parameters (b) effective diffusion coefficient (D_{eff}) (mean $0.006 \pm$ s.d. $0.006 \mu m^2/s$) and (c) drift velocity (v_{eff}) (mean $0.008 \pm$ s.d. $0.005 \mu m/s$) are plotted.

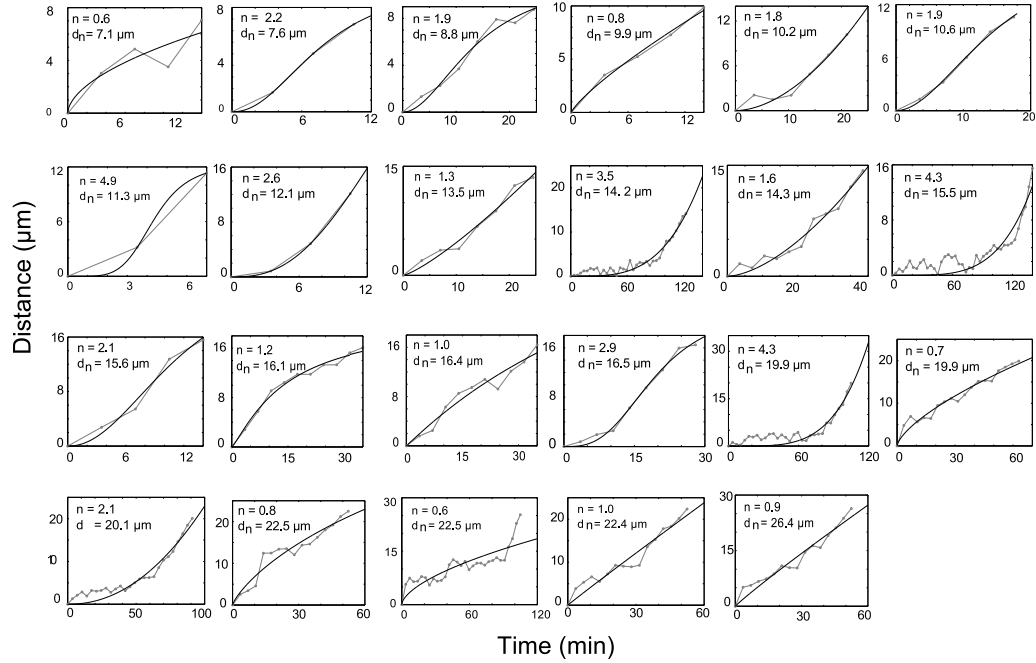


Figure A3: Fits to distance travelled by MTOC from experiment. The distance travelled of experimentally measured MTOCs were plotted as a function of time in minutes. The plots are sorted based on increasing distance of nucleation (d_n) from chromatin. Each profile was fit to the effective model (Equation 2.14) to obtain a ‘cooperativity parameter’(n).

Parameter set (k)	Sum of ranks ($R_s(k)$)	$r_{1/2}^a$	s^a	$r_{1/2}^r$	s^r
482	22	10	1	0	2
487	34	10	1	1	2
377	36	8	1	3	2
497	37	10	1	3	2
362	42	8	1	0	2
372	43	8	1	2	2
506	52	10	1	5	1
267	55	6	1	5	2
363	56	8	1	0	3
492	56	10	1	2	2

Table A.1: Optimized RWD gradient parameters: The top ten sum ranks are listed in ascending order, based on the ranks from the directionality and capture time error ($\epsilon(k)$), with the corresponding parameters of the attractive ($r_{1/2}^a$ and s^a) and repulsive ($r_{1/2}^r$ and s^r) gradients (see Figure(2.3(a))).

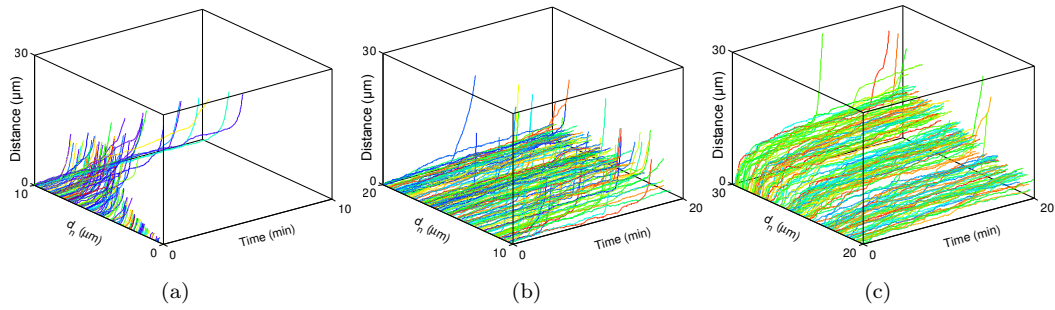


Figure A4: Cumulative displacements in simulation: MT-motor simulations in the presence of a motor gradient ($f_{stall} = 2$ pN) were used to plot the cumulative distance moved by MTOCs (z-axis) as a function of time in minutes (x-axis) and nucleation position (y-axis). The plots represent the subset of MTOCs nucleated (a) close to chromatin (0-10 μm), (b) at an intermediate distance (10-20 μm) and (c) far from the chromatin edge (20-30 μm).

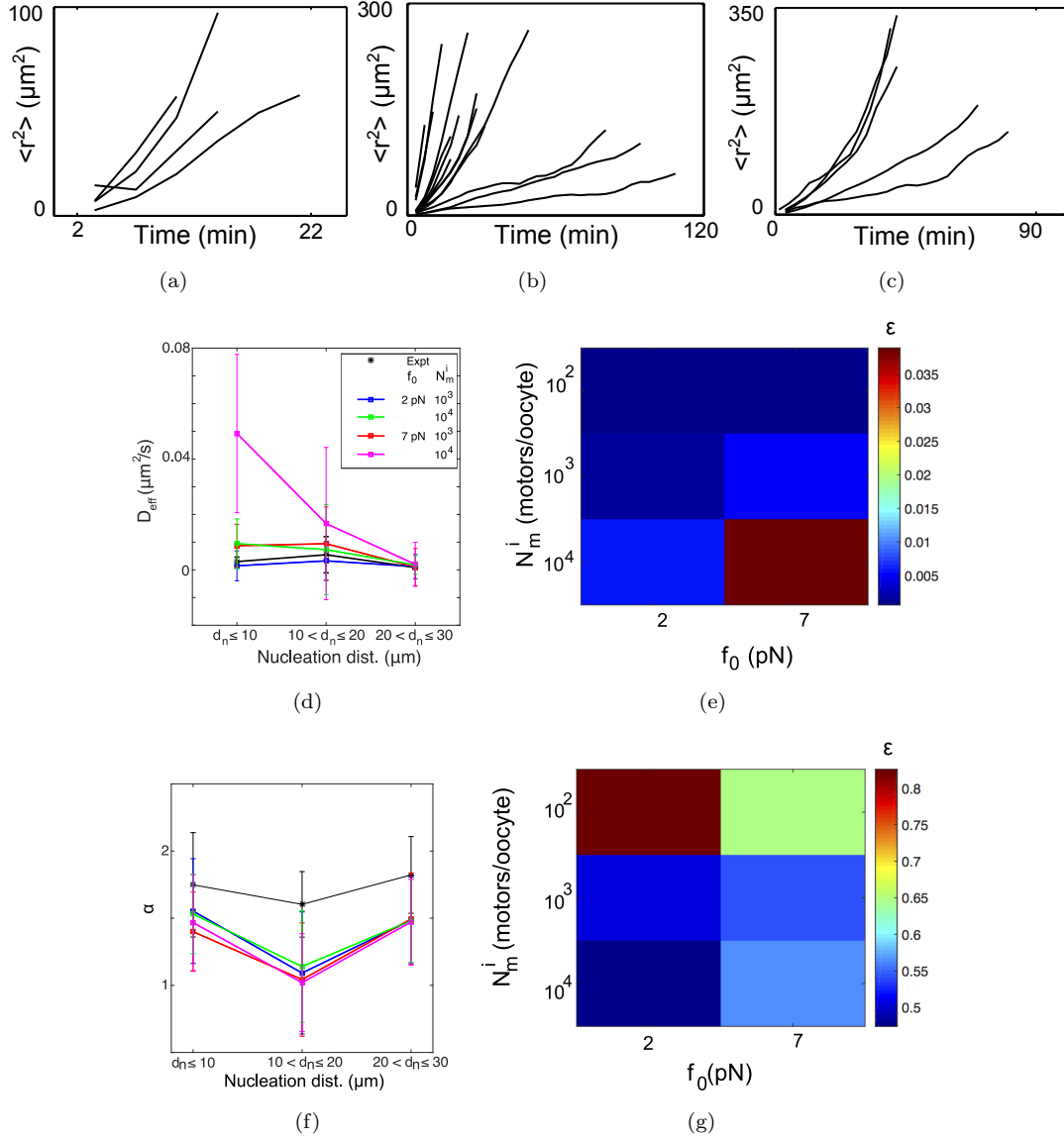


Figure A5: Msd as a function of nucleation distance from experiment and simulation. Msd profiles were calculated for the experimentally measured MTOC trajectories and sorted by their nucleation distance (d_n) where ((a)) $d_n \leq 10 \mu m$, ((b)) $10 < d_n \leq 20 \mu m$ and ((c)) $20 < d_n \leq 30 \mu m$. ((d)) The D_{eff} and ((f)) α values obtained from fits to simulated msd trajectories are plotted as a function of d_n . Experimental profiles (black line) are compared to multiple scenarios in simulation (800 trajectories per scenario) with different stall forces (f_0) and motors per cell (N_m^i). The values are mean $\pm$ s.d. The error between simulation and experiment, ϵ (colorbar) is plotted for ((e)) D' and ((g)) α as a function of stall force (f_0) and motor density (N_m^i).

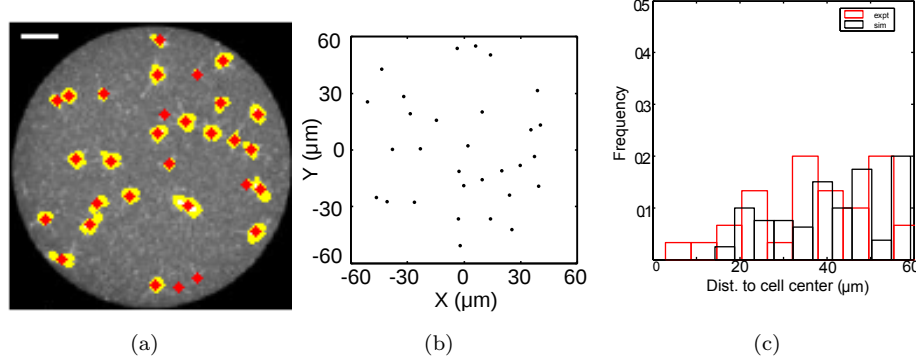


Figure A6: Enucleation and MTOC distributions. (a) Previous data of mouse oocyte enucleation prior to NEBD by Schuh & Ellenberg [10] was used to automatically detect MTOCs (yellow outlines) and their centroids (red asterisk). Scale bar = 10 μm . (b) The 2D coordinates of these experimental MTOC positions were used to (c) compare the radial density distribution of experimentally measured MTOCs (red) with simulated MTOCs (black) that were randomly localized, assuming the density of MTOCs is uniform throughout the oocyte.

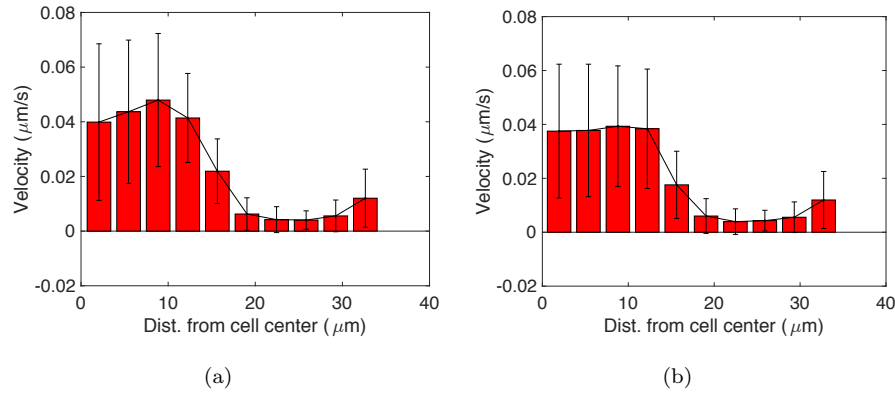


Figure A7: Spatial velocity distribution. The mean velocities in $\mu\text{m/s}$ (y-axis) radially binned as a function of distance from the cell center in μm (x-axis) calculated from the MT-motor model in an immobilized motor gradient with motor stall forces and densities: ((a)) $f_0 = 2$ pN, $N_m^i = 10^4$ motors/oocyte and ((b)) $f_0 = 7$ pN and $N_m^i = 10^3$ motors/oocyte.

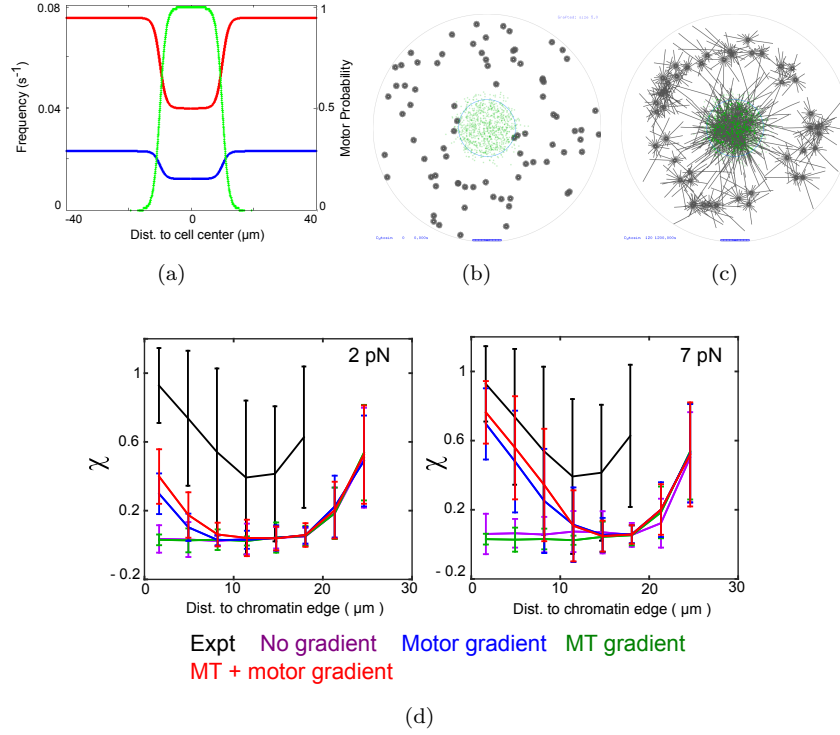


Figure A8: Gradient of dynein motors and MT dynamic instability. (a) The spatial profile of f_{cat} (red), f_{res} (blue) and motor density of immobilized minus-ended motors (green) (at $t=0$) are plotted as a function of radial distance from the oocyte center. (b) Simulation output at $t=0$ minutes and (c) $t=20$ minutes with MTOCs (grey) and a concentric nucleus (blue circle) with immobilized minus-ended motors (green dots). (d) The tortuosity profiles as function of the radial distance compared for the scenarios from gradient of MT dynamics, motor gradient and their combined effects.

A.2 Estimation of parameters

Transition frequencies The four-parameter dynamic instability model parameters for the cytoplasm were set from literature and for stabilized values optimized based on Equations A.2,A.1. MTs were modeled as two state dynamic polymers with spatially varying transition rates based on the four parameter model of dynamic instability [38]- velocities of growth (v_g) and shrinkage (v_s) and frequencies of catastrophe (f_c) and rescue (f_r). Dynamic instability parameters were modulated between cytoplasmic and stabilized values based on gradients. Cytoplasmic values were derived from a mitotic epithelial cell [154], due to the similarity in mean MT lengths with mouse meiotic MTOCs (Figure 2.5). The stabilized MT-lengths were assumed to be two-fold greater than cytoplasmic lengths based MT length measurements in presence of RanGTP concentrations resembling those expected near chromatin [58, 80, 79]. The f_c and f_r parameters corresponding to the stabilized MTs were calculated by simultaneously solving the equations for the flux rate (J):

$$J = \frac{v_g \cdot f_r - v_s \cdot f_c}{f_c + f_r} \quad (\text{A.1})$$

and mean length ($\langle L \rangle$) at steady state:

$$\langle L \rangle = \frac{v_g \cdot v_s}{v_s \cdot f_c - v_g \cdot f_r}. \quad (\text{A.2})$$

Estimation of MT numbers

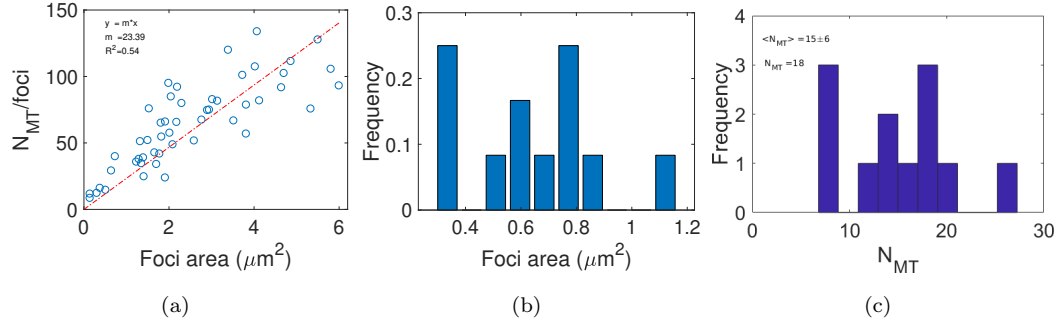
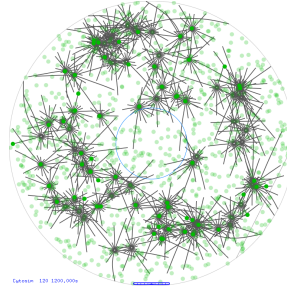


Figure A9: Estimation of microtubule numbers per microtubule organizing center. (a) Scatter plot depicts number of microtubules nucleated from a pericentrin foci of a certain size. This data is extracted by digitizing the plot from a previously reported study in the mouse oocyte [146]. The data was fit to a linear function with zero intercept to obtain a relation between the pericentrin size and the number of microtubules nucleating from it. (b) The frequency distribution of the foci size extracted from the pericentrin labelled microtubule organizing centers. The images used were published previously in mouse oocytes ([10]). (c) Frequency distribution of microtubule numbers per MTOC in the mouse oocyte, inferred by multiplying the slope as obtained in (a) to the foci size as in (b). A value of 20 MTs per MTOC was chosen as an input in the simulations.

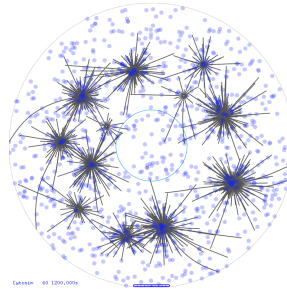
A.3 Supporting Videos

Videos chapter 2: Multi-aster centering in mouse oocytes at meiosis I

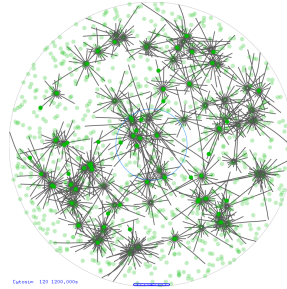
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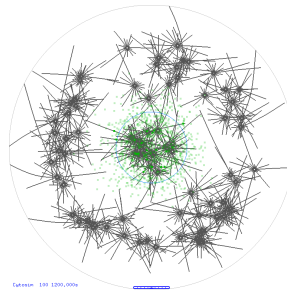
Video A1: MTOC motility in the absence of a gradient. 80 MTOCs (grey) in the oocyte are pushed inwards by the cell boundary (outer blue circle) with $N_m^i = 10^3$ immobilized minus-end directed motors (green dots) with $f_0 = 7$ pN resulting in random MTOC motility. No gradient originates from the chromatin (inner blue circle). [Link to SV 1.](#)



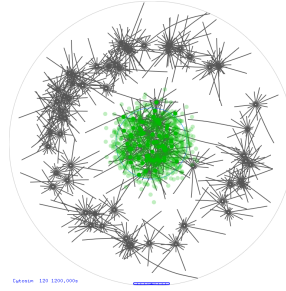
Video A2: MTOC motility in the presence of diffusible minus-ended motor complexes. 80 MTOCs (grey) were simulated in cytoplasmic space of the 2D oocyte geometry marked by the outer cell boundary (outer blue circle) and inner chromatin region (blue circle) in the presence of minus-end directed motor complexes (purple dots) initialized in the cytoplasm with density $N_m^c = 10^3$ motors/oocyte. These diffusible motors with stall force $f_0 = 7$ pN, can cross-link MTs and result in clustering by walking towards the minus-ends of neighboring asters that they crosslink. [Link to SV 2.](#)



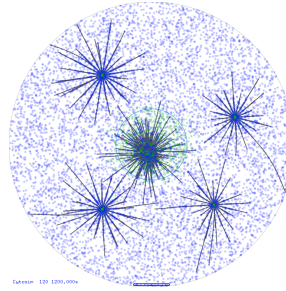
Video A3: MTOC motility in a gradient of MT dynamic instability. 80 MTOCs (grey) were simulated in the 2D oocyte geometry with the outer cell boundary (outer blue circle) and $N_m^i = 10^3$ uniformly distributed surface immobilized motors with $f_0 = 7$ pN. The f_{cat} and f_{res} parameters were distributed in a sigmoid gradient (Fig 2.5(g)) originating from the center of the chromatin region (inner blue circle). [Link to SV 3.](#)



Video A4: MTOC motility in a gradient of weak motors. 80 MTOCs (grey) were simulated in the 2D oocyte geometry with the outer cell boundary (outer blue circle) and $N_m^i = 10^3$ surface immobilized motors with $f_0 = 2$ pN distributed in a sigmoid gradient (Fig 2.5(j)) originating from the center of the chromatin region (inner blue circle), and homogeneous dynamic instability. [Link to SV 4.](#)



Video A5: MTOC motility in a gradient of strong motors: 80 MTOCs (grey) were simulated in the 2D oocyte geometry with the outer cell boundary (outer blue circle) and $N_m^i = 10^3$ surface immobilized motors with $f_0 = 7$ pN, distributed in a sigmoid gradient (Fig 2.5(j)) originating from the center of the chromatin region (inner blue circle). [Link to SV 5.](#)



Video A6: MTOC motility in a gradient of immobilized motors and diffusible motor-complexes: 80 MTOCs (grey) were simulated in the 2D oocyte geometry with the outer cell boundary (outer blue circle) and motors which are immobilized at a density of $N_m^i = 10^3$ motors/oocyte (green dots) in a sigmoid gradient originating from the center of the chromatin region (inner blue circle). The diffusible minus-end directed motor-complexes with $N_m^c = 10^4$ motors/oocyte (purple dots) bind to 2 MTs and walk simultaneously on them, generating a clustering force on the MTOC asters. For both kinds of motors $f_0 = 7$ pN. [Link to SV 6.](#)

Appendix B

Supporting material to Chapter 3

B.1 Supporting figures

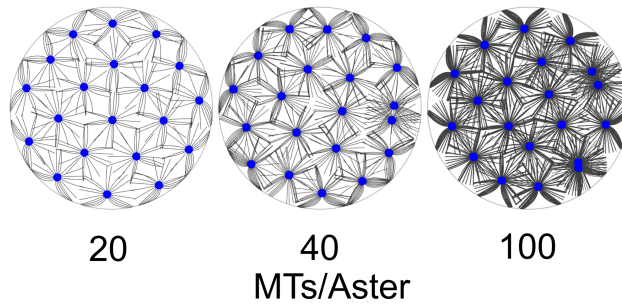


Figure B1: Effect of number of MTs per aster. Snapshots at the end of simulations represent the aster centroids (blue) for increasing numbers of MT (N_{MT} : 20, 40, 100). (N_A : 20, L_{MT} : $4.25 \mu\text{m}$, R : $15 \mu\text{m}$, and the density of kinesin motors: $10 \text{ motors}/\mu\text{m}^2$ with a random initialization of aster positions.

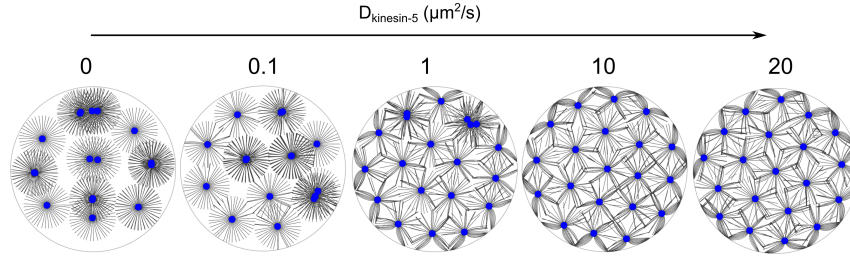
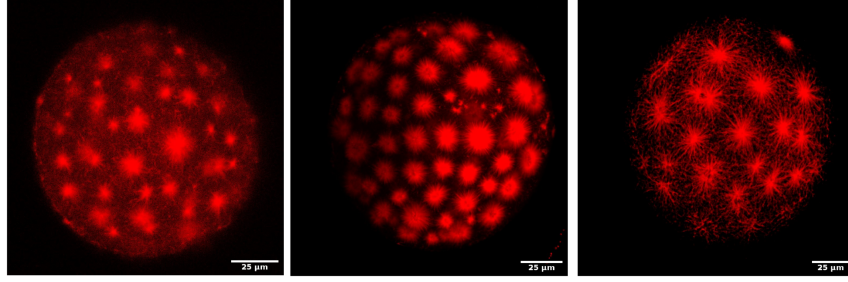
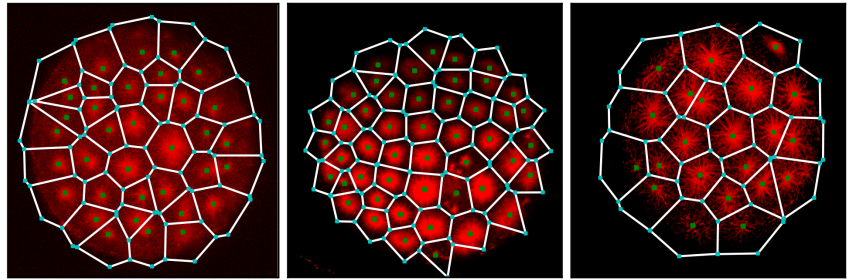


Figure B2: Effect of diffusivity of kinesin-5 motors. Simulation snapshots at the end of simulations represent the aster centroids (blue) for increasing diffusion coefficient of kinesin-5 motors (D_K). Here, number of asters N_A is 20, the radius of the aster L_{MT} is $4.25 \mu\text{m}$, the radius of the cell R is $15 \mu\text{m}$, and the density of kinesin motors is $10 \text{ motors}/\mu\text{m}^2$ with a random initialization of aster positions.

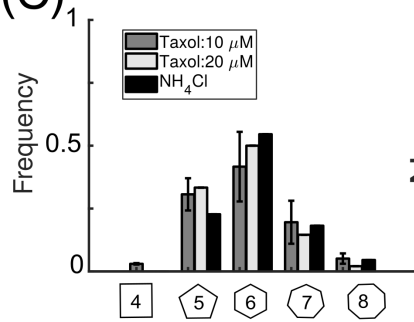
(A)

Taxol: 10 μM Taxol: 20 μM NH_4Cl

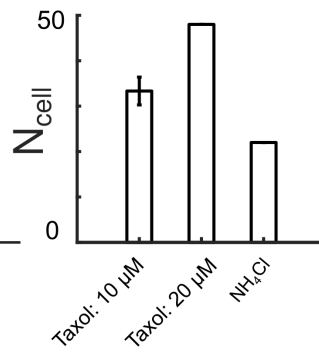
(B)

Taxol: 10 μM Taxol: 20 μM NH_4Cl

(C)



(D)



(E)

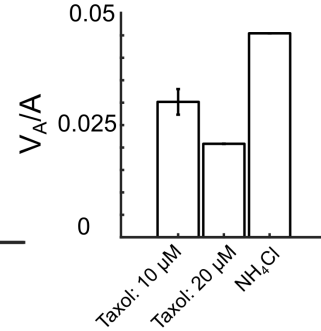


Figure B3: Cytaster formation by taxol and NH_4Cl treatment. (A, B) *Phallusia* oocytes were treated for one hour with Taxol (10 or 20 μM) or NH_4Cl (10 mM), then fixed and labelled with anti-alpha tubulin (red). (B) Voronoi tessellation of images in (A), showing the overlay of aster centroids (green circles), Voronoi vertices (cyan circles) and polygon edges (white lines). (C-E) The effect of MT stabilization is quantified in terms of (C) the polygon distribution, (D) the mean number of Voronoi detected cells and (E) relative Voronoi cell area V_A as a fraction of total oocyte area A . Scale bar: 25 μm . Error bars: $\pm$ s.d.

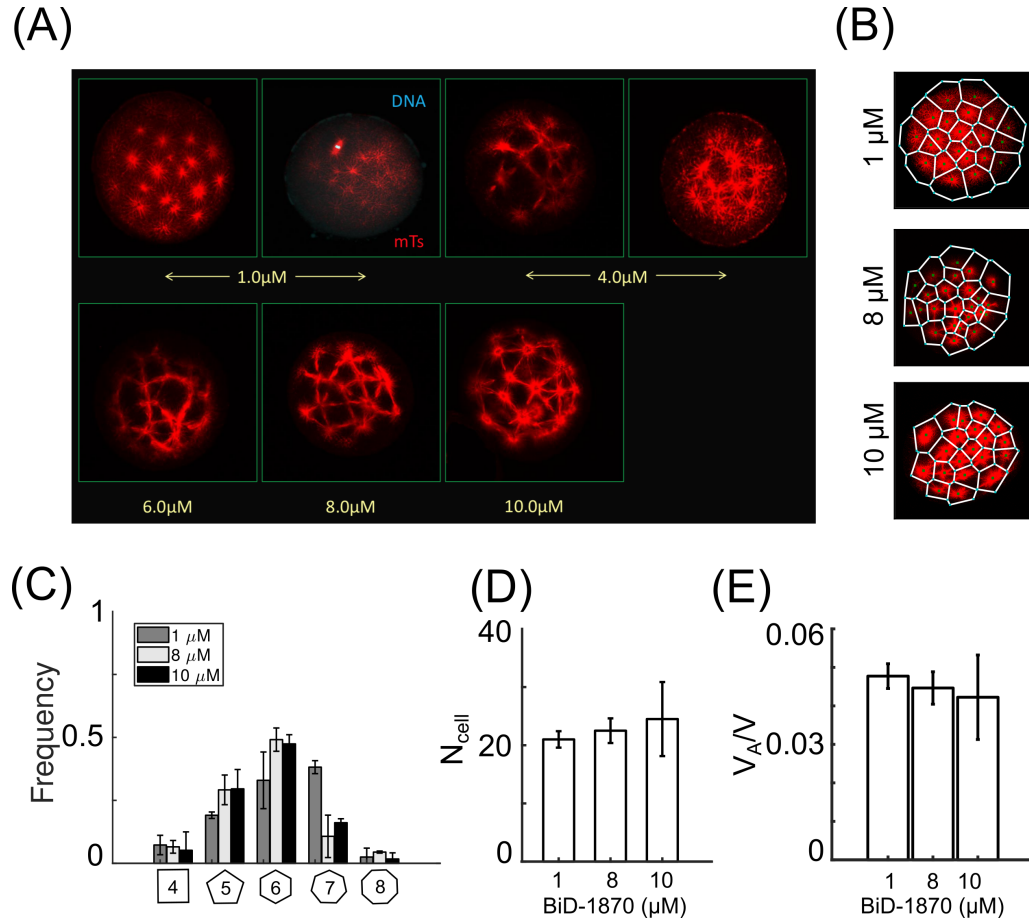


Figure B4: Dose response of oocyte cytasters to BiD treatment. (A) Representative images of *Phallusia* oocytes treated with 1, 4, 6, 8 and 10 μM BiD 1870 and stained with anti-alpha tubulin antibodies marking MTs (red) and stained for chromatin (blue). (B) Voronoi tessellation for 3 concentrations of the BiD-treated oocytes, overlaid on maximum intensity projections of the top and bottom 9 slices of each confocal z-stack. Aster centroids are green circles, Voronoi vertices are cyan dots and polygon edges are white lines. For each condition we quantitated the (C) polygon distribution, (D) mean number, and (E) mean area of the Voronoi cells. Error bars: $\pm$ s.d.

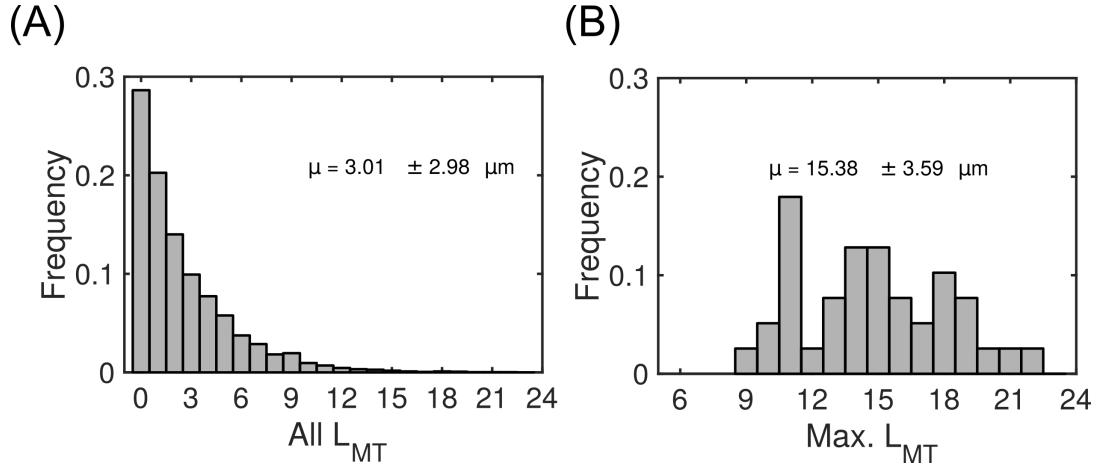


Figure B6: Astral MT length distributions in M2 model of astral MT lengths. The length distributions of astral MT lengths in a single simulation with conditions similar to the M2 model i.e. $R_{\text{cell}} = 60 \mu\text{m}$, $N_A = 40$ and dynein density at $1 \text{ motors}/\mu\text{m}^2$. (A) All the MT filament lengths and (B) longest MT filament from each aster. The inset represents the mean length. $N_{\text{MT}}/\text{aster} = 40$.

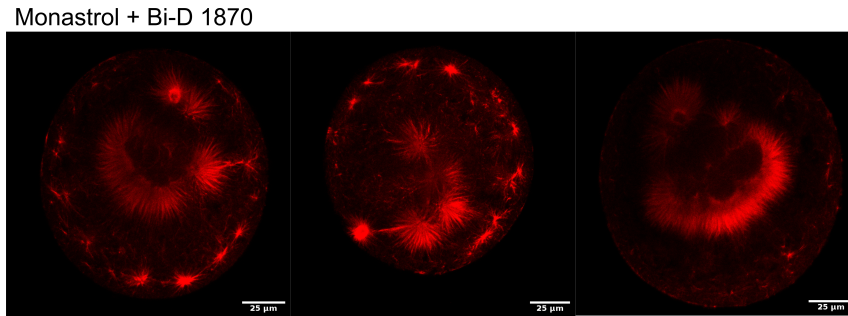
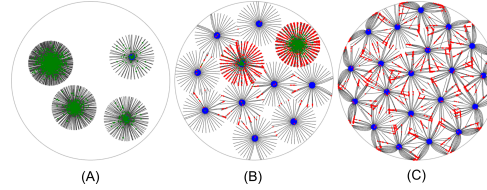


Figure B5: Effect of inhibiting kinesin-5 motors followed by microtubule stabilization. Examples of oocytes first treated with monastrol to inhibit kinesin-5, followed by treatment with the RSK inhibitor BiD-1870. Red: anti- α tubulin antibody staining. Scale bar: $25 \mu\text{m}$.

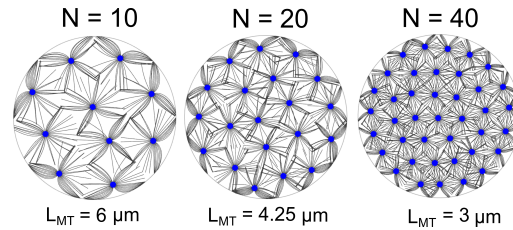
B.2 Supporting Videos

Videos of chapter 3: of multi-aster positioning

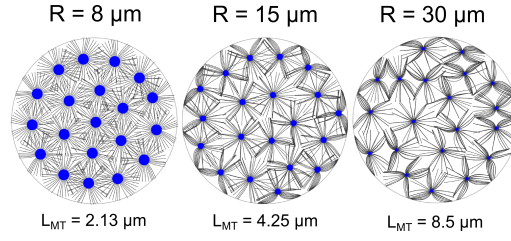
URL link- <https://figshare.com/s/e8186e1b4216c4fd952e>



Video B1: Multiple asters initially localized randomly in a cell of radius $15 \mu\text{m}$ evolve to form multiple patterns, based on mechanics of microtubules (grey lines) and presence of motor activities (green circles: dynein complexes, red circles: kinesin complexes, blue circles: aster centers). Simulation time-series correspond to cells with multiple asters and (a) dynein complexes alone, (b) a ratio of 0.5 of dynein to 0.5 kinesin complexes (for a constant total motor density), or (c) only kinesin complexes. The spatial steady state emerges from an initial random state. Number of asters N_A : 20, MTs per aster: 40, total motor density: 10 motors/ μm^2 , total simulation time: 600 s. Only those motors that cross-link two MTs are displayed for clarity. [Link to SV 1.](#)



Video B2: The simulation time series were used to test the ‘aster packing’ model (Equation 1) by increasing aster numbers (N_A) while decreasing the aster radius (L_{MT}), for a constant packing fraction η_F of 1.6. The numbers of asters per cell, N_A are (Left to right:) 10, 20 and 40 while the corresponding L_{MT} values are 6 , 4.25 and 3 μm , respectively. Kinesin density: 10 μm^2 , R: 15 μm and total simulation time: 600 s. This video corresponds to Figure 3B in the main text. [Link to SV 2.](#)



Video B3: The doubling of cell size does not affect hexagonal arrangement of multi-aster patterns for a constant number of asters, if astral MT lengths L_{MT} are linearly scaled to maintain the optimal circle packing fraction (η_F). The time series for such cell size doubling with η_F is 1.6 are depicted for (*left to right:*) cell radius: 8, 15 and 30 μm with L_{MT} : 2.13, 4.25 and 8.5 μm , respectively. Here, $N_A = 20$, density of kinesin complexes: 10 motors/ μm^2 and total simulation time: 600 s. These videos correspond to the diagonal elements in Figure 3D. [Link to SV 3.](#)

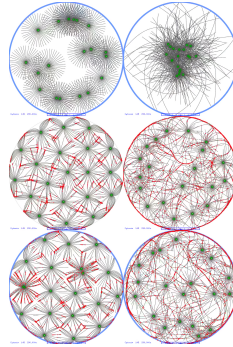
Appendix C

Supporting material to Chapter 4

C.1 Supporting Videos

Videos of chapter 4: [Multi-aster rotation](#)

URL link- <https://figshare.com/s/6e5653b0028d1d62b3b5>



Video C1: The effect of (*top row*) cortical force generators, (*mid-row*) kinesin complexes and (*bottom row*) both kinds of motors on MT asters with the two columns simulating MTs lengths as (*left*) fixed (*right*) dynamically unstable. The video corresponds to results depicted in Figure 4.2. Time 300 s, N_a 20, ρ_d 100 motors/ μm , ρ_k 10 motors/ μm^2 and $J = 0$ and $-0.3 \mu\text{m/s}$ for stabilized and dynamic MTs respectively. [Link to Video](#).

Appendix D

Simulation methods

D.1 Random walk models

The random walk model was developed in C++ and Python for simulating the ‘search and capture’ of particles. The movement of objects is simulated in an off-lattice 2D and 3D space. In Chapter 2, asters were modelled as particles that exhibit random walk in spatially varying drift fields. The scope of this model was extended to generate various types of movements such as centripetal, circular, oscillatory and random. In addition, these were extended to simulate the ‘search, capture and cleavage’ of restriction enzymes in the presence of DNA filaments.

D.1.1 Random walk with spatial drift fields

The equations 2.1 to 2.4 in Chapter 2 correspond to the phenomenological model and not the Langevin dynamics based model. The phenomenological model developed simulates an off-lattice random walk of point particles in a spatially varying drift fields. This is similar to the formalism of models for self-propelled active particles. The simplest model amongst these class of models is provided by Viscek et.

al. 1995 [127]. The particle position at every time step is updated based on the constant velocity, while the randomness is determined by the orientation angle.

Here, asters are considered as point particles in a confined circular geometry and no explicit consideration of the mechanism of movement i.e. motors, viscous environment, particle interactions or temperature is considered. The step size and the direction of motion of every particle is calculated at every time step based on the position of the particle at the previous time step. The step size (δr) comprises of random and directed components as in (Equation D.1). The directed component of the step size is inferred from the constant velocity while the diffusive component computed from the diffusion (mean squared displacement) equation. The values of velocity (v_{eff}) and the effective diffusion coefficient (D_{eff}) were estimated from the fits to diffusion-drift model of the experimental tracks to determine the step size.

$$\delta r = \begin{cases} \delta r_{df} = \sqrt{4 \cdot D_{eff} \cdot \delta t}, & \text{Brownian component} \\ \delta r_{dr} = v_{eff} \cdot \delta t, & \text{Directed component} \end{cases} \quad (\text{D.1})$$

where, δr_{df} and δr_{dr} correspond to magnitude of diffusive and directed step size, D_{eff} is the effective diffusion coefficient, v_{eff} is the effective velocity and δt is the integration time.

Similarly, the direction of movement was calculated as a combination of random and directed components (Equation D.2). The random component was sampled from a uniform distribution of angles while the directed component was calculated based on the position of the particle with respect to the target.

$$\theta = \begin{cases} \theta_{df} = rand(0, 2\pi), & \text{Brownian component} \\ \theta_{dr} = f(\overrightarrow{XC}), & \text{Directed component} \end{cases} \quad (\text{D.2})$$

where θ_{df} is the diffusive and θ_{dr} the directed angle. The angle θ_{df} can take random

values uniformly distributed between 0 and 2π , while θ_{dr} is determined by the angle of the vector $\overrightarrow{XC}$ between the particle ($\vec{X}$) and the center ($\vec{C}$) of the cell (Figure 2.2(a)).

The weighted mean of the random and directed components was calculated for both the step-size and the net angle of movement. The proportion of each was weighed by a factor determined by the drift function (Equation D.3) based on the radial position of the particle.

$$\phi = \frac{1}{1 + e^{[(r-r_{1/2})/s]}} \quad (\text{D.3})$$

where r is the distance- for ϕ_a , $r = 0$ at the chromatin edge and for ϕ_r , $r = 0$ at the cell boundary. $r_{1/2}$ is the distance at which the function takes the half-maximal value and $1/s$ is the steepness factor.

Now, the net step size of a particle (δr_{net}) is considered as weighted average of random and directed components defined as ϕ_a and $(1 - \phi_a)$ (Equation D.4).

$$\delta r_{net} = \phi_a \cdot \delta r_{dr} + (1 - \phi_a) \cdot \delta r_c \quad (\text{D.4})$$

where δr_c is the effective step size at the cell cortex determined by the weighted average of repulsion and Brownian motion. δr_c is determined by (Equation D.5).

$$\delta r_c = \phi_r \cdot \delta r_{dr} + (1 - \phi_r) \cdot \delta r_{df} \quad (\text{D.5})$$

The net angle is weighed according the drift fields as in Equation D.6:

$$\theta_{net} = \arctan \frac{\phi_a \cdot \sin(\theta_{dr}) + (1 - \phi_a) \cdot \sin(\theta_{df})}{\phi_a \cdot \cos(\theta_{dr}) + (1 - \phi_a) \cdot \cos(\theta_{df})} \quad (\text{D.6})$$

The position of the particle at time, t is updated in the cartesian coordinates as in Equation D.7.

$$r(t) = \begin{cases} x(t) = x(t-1) + (\delta r_{net} \cdot \cos(\theta_{net})) \\ y(t) = y(t-1) + (\delta r_{net} \cdot \sin(\theta_{net})) \end{cases} \quad (\text{D.7})$$

D.2 Cytosim: MT-motor models

The mechanistically detailed MT-motor models was developed in Cytosim. Cytosim is a simulation package developed in C++ by Francois Nedelec and the group [16]. For the work in Chapter 2, 4 and 5 the Cytosim PI version 2003-2005 was used while in Chapter 3 the Cytosim version as [available on the github 2017 was used](#). Modifications with respect to the MT and motor behaviour and localization was made and has been discussed in the respective chapters.

The specific modifications made are discussed in each of the chapters separately. The table (Table D.1) summarizes all the sections and equations pertaining to the Cytosim.

Description	Reference
Motor behaviour	Chapter 5, Figure 5.3(a)
Motor localization	Chapter 2, Section 2
MT filament motion	Chapter 5, Equation 5.5
Motor binding	Chapter 5 , Section 5.2.3
Motor stepping	Chapter 2, dynein, Equation 2.8 Chapter 3, dynein and kinesin-5, Equation 3.3 Chapter 4, kinesin-5: Equation 4.1 , dynein: Equation 2.8 Chapter 5, yeast dynein, Equation 5.2,5.3, 5.4
Motor unbinding	Chapter 2, dynein, Section 2.2. Chapter 3, dynein and kinesin-5, Equation 3.4 Chapter 4, dynein and kinesin-5, Equation 4.2 Chapter 5, dynein, Section 5.2.3, Equation 5.1

Table D.1: Simulation equations for MT-motor models. The equations for MT-motor models used in the various chapters for the cytosim simulations.

D.3 Filament motion in the absence of motors

In order to test the motility pattern of MTs in the absence of molecular motors in the system, MTs of a fixed length was initialized randomly in a 2D box with a periodic boundary conditions. The filament motion is expected to be driven by thermal and viscous forces in such a scenario. In chapter 5, section 5.2.5 describes the model for filament movement. In independent simulations MT lengths were varied for a constant viscosity to address the effect of drag forces on the filaments which depends on the length of rods and viscosity of the medium. Further, the viscosity of the medium was scanned across 10^{-3} to 10^3 Pa.s. The thermal energy was held constant as 4.07×10^{-21} N.m (Table 5.1). The mean squared displacement of the trajectories was computed and fitted to the anomalous diffusion model as described in chapter 2 and chapter 5. The anomaly parameter (α) was ~ 1 suggestive of random motion.

While only at the highest value of viscosity a low value of α was observed suggestive of sub-diffusive confined motion due to the high resistance making the filaments nearly immobile. The effective diffusion coefficient (D_{eff}) extracted shows a length dependence which further confirms that the drag experienced by filaments increase with increasing lengths thereby reducing the motion. clarity.

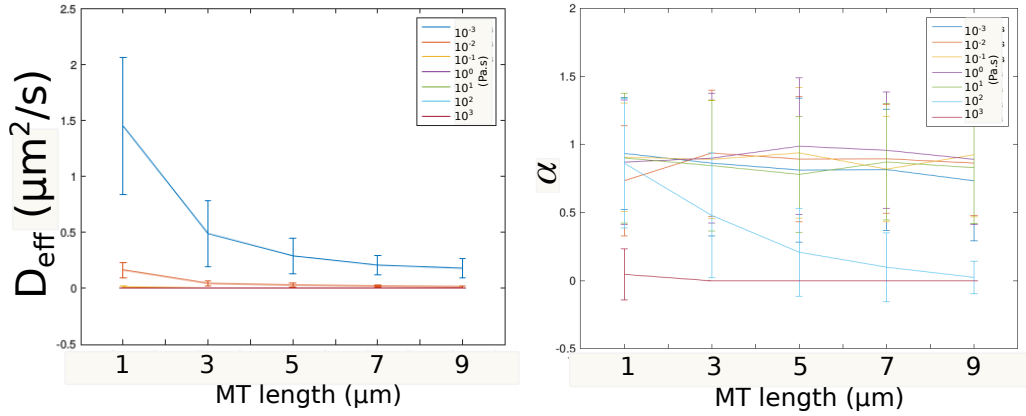


Figure D1: Random motion of the filaments in the absence of molecular motors. The MT filament motion is characterized by MSD analysis for effective diffusion coefficient (D_{eff}) and anomaly parameter (α). The D_{eff} decreases with increasing the MT lengths for a constant system viscosity due to the increasing drag force. The D_{eff} also decreases for increasing values of viscosity. Colors represent viscosity values. The α values of 1 correspond to diffusive motion. At high viscosities, filaments experience large drag and fairly immobile thus exhibiting a sub-diffusive motion.