

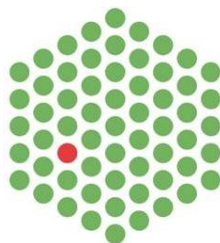
XXXI. OTDK Biológia Szekció

CONTRACTION MECHANISM IN THE
CYTOKINETIC RING

Lakatos Eszter



Pázmány Péter Catholic University
Faculty of Information Technology
Infobionics MSc, First year



European Molecular Biology Laboratory
Supervisor: Dr. François Nédélec

Contents

1	Introduction	4
1.1	Cytokinesis	4
1.2	Contractile ring in fission yeast	5
2	Cytosim	8
2.1	Calculations	10
2.2	Objects	10
2.2.1	Spaces	10
2.2.2	Fibers	11
2.2.3	FiberBinders	12
3	Contraction	14
3.1	One-dimensional model	14
3.2	An example from nature - Smooth muscle	16
	Implementation	16
3.3	Measuring contractility: Tension	17
3.3.1	Tension in smooth muscle	18
3.3.2	Tension in a ring model	20
3.4	Summary: Recipes of contraction	22
4	Ring formation	24
4.1	Ring formation in Cytosim	25
5	Reliable contraction	27
5.1	Two-dimensional model	27
5.2	Membrane mobility	30
5.3	Modelling the ring	31
6	Conclusion	37
	List of supplementary videos	38
	Acknowledgement	39
	References	41

Abstract

Cytokinesis is the final step of cell division, when the two daughter cells get physically separated by a newly formed membrane between them. The contractile ring is an actin based structure which forms along the cell equator in anaphase and decreases in diameter during cytokinesis. It is present in every animal cell and presumably plays an important role in cell separation. The ring has been extensively studied in fission yeast, already more than 50 proteins have been identified which are required for ring formation or constriction. However, the exact function of individual proteins, the mechanism of contraction and the connection with cytokinesis still offer many unanswered questions. Since the cytokinetic ring is a quasi one-dimensional structure, it is attractive for modelling, because relatively easy and fast to simulate. Additionally, actin contractility has great importance in many other cases during the cell-cycle, therefore understanding the ring may help us to shed light on the basics of several other cellular processes.

During my project I have learnt the use of **cytosim**, a C++ based program designed by François Nédélec, for the simulation of cytoskeleton. Using the tools given by **cytosim** and additional Python scripts, I designed model systems with simple geometry and minimal components. The simplest geometry had periodic boundary conditions which transformed the problem into a one-dimensional one, making the tests much faster. I simulated these systems to derive the necessary and sufficient conditions for efficient contraction. For the evaluation of certain mechanisms, I searched for a quantification method applicable for a geometry with periodic boundary conditions. I found that the tension within the system can be an appropriate measure and examined the properties of it. I ran simulations for a wide range of parameter values and described how contractility or tension depends on them.

Afterwards, I studied the reliability of contraction mechanisms and looked for a way to prevent the ring from breaking during constriction. Involving filament polymerization and depolymerization together with fiber nucleation, I established a model reliable and contractile at the same time. Changing certain parameters, I compared the model to systems with already known behaviour, and using them as controls I could determine a range where the model holds all the desired features. Finally, I could create a basic, realistic model able to reproduce experimental results, which can be used as a starting point for the examination of the dependencies on different parameter values.

1 Introduction

1.1 Cytokinesis

Cell division (mitosis) is one of the most crucial steps in the life cycle of every cell of every living organism. Numerous processes are needed for a perfect division of the original cell to two structurally - but not genetically - identical daughter cells. Each organism has its characteristic number of chromosomes which are necessary to produce ideal protein concentrations and keep the cell alive. Abnormal number of chromosomes - meaning under- or overrepresentation of given genes - results in under- or overexpression of proteins. Since every feature of a cell - therefore the organism - is determined by its protein concentration, if some errors move the concentrations from their natural equilibrium, serious diseases can occur. Therefore the perfect duplication of genetic information when the cell is about to divide (in phase S of the cell cycle), and the perfect, symmetric separation of these duplicated chromosomes are crucial for the survival of the cell. With a less functional protein set the cell usually dies - this process is called *apoptosis*. However, the main problem lies in the cells which somehow avoid apoptosis and continue growth and reproduction with an altered functionality.

After perfect segregation of chromosomes the separation of cells is of no less importance. It is not enough for a cell to have its two sets of DNA separated if they are still within the same compartment. A long-standing hypothesis on the mechanism of tumorigenesis states that the failure of cell division results in tetraploid cells - cells with four-fold chromosome set instead of the normal two-fold one - which are genetically unstable. [1]

The physical separation process of daughter cells is called **cytokinesis**. It is initiated after the duplicated centrosomes (called the *spindle pole bodies*) are clearly separated. In the meridional section of the cell - at equal distance from both poles - the diameter of the cell decreases, like if one would start pulling a string wrapped around the cell equator. {See fig. 1.} If we take a perpendicular plane the shape of the cell can vary along species (from rectangular over ellipse to circle), but in all cases the decrease in diameter means the upper and lower membrane of the equatorial section approaching. In practice membrane is continuously synthesized and degraded during cytokinesis. In the end the two new cells are completely separated by the newly formed membrane

between them, and start their individual cycle which ends with cell division.

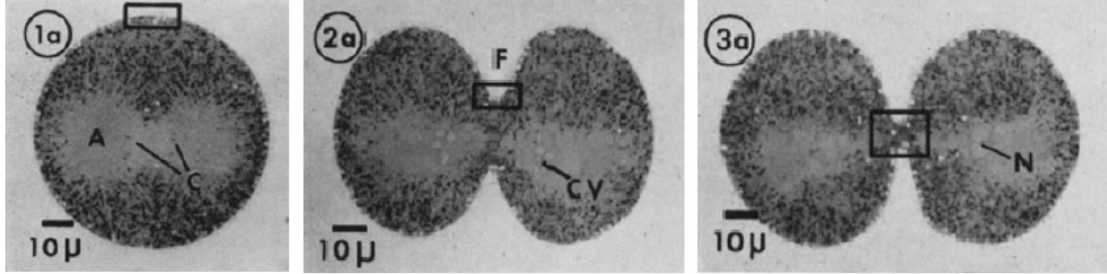


Figure 1: Meridional sections of an *Arbacia* egg, (1a) is at 80 s before cytokinesis begins, (2a) is 4 minutes, (3a) is 5 after the beginning of the cell separation process. C: daughter sets of chromosomes, CV: chromosomal vesicles, F: cleavage furrow, N: daughter nucleus. Images from [2].

The underlying mechanism of this crucial process is still mysterious. However, in every animal and fungal cell - plants, like in many other characteristics, are different - a ring is formed attached to the membrane at the division site in anaphase, which later contracts as the cell diameter decreases at the equator. The connection between the diameter change of the cell and the ring is not clear, but presumably the ring has a major role in cell separation. Moreover, since the main components of the ring are actin and myosin [2], the ring constriction can be similar to many other cellular processes based on actomyosin contractility. The most extensively studied example for this kind of process is striated muscle, but it has a very special spatial organization and differs very much from the other less known contractile cases. Hence focusing on the contractile ring can lead to important discoveries not only in cytokinesis but in other phases of the cell cycle.

1.2 Contractile ring in fission yeast

Several features of *Schizosaccharomyces pombe* (fission yeast) make it particularly attractive for studying cytokinesis. *S. pombe* is a rod-shaped cell that divides in the middle and expresses a structurally very compact ring suitable for imaging. In addition, because of its genetic tractability, fission yeast is also suitable for experiments

with null and temperature sensitive mutants to identify the function of different proteins. Moreover, there are several proteins in fission yeast playing an important role during cytokinesis which are present in higher organisms as well. [3]

The life of the cytokinetic ring contains three major stages all offering many unresolved questions.

In early mitosis, during interphase the key factors in ring initialization are gathered to more than 50 cortical spots (named *midsome structures*) in the middle of the cell. The mechanism behind this exact positioning became more clear during the last decade. First of all, the main player is *mid1p*, an anilin-like protein, which determines the localization of other factors arriving to the division plane later. The localization of *mid1p* is determined by both positive and negative signals. The position of the nucleus has great effect on *mid1p* accumulation, since moving the nucleus made the ring to form in a different position [4]. However, there are inhibitory factors preventing the ring from forming at cell tips. Computational models [5] suggested that only nucleus-related processes cannot reproduce the physiological *mid1p* distribution, while including inhibitory factors coming from the cell tips can solve the problem. Later it has been revealed that *pom1p* kinase is required to exclude *mid1p* from the non-growing cell end.

After more than 50 sites are specified by the gathering *mid1p*, other proteins are recruited to the contractile ring. After the main factors are recruited by *mid1p*, at the previous sites functioning *nodes* are formed able to nucleate actin filaments and move along them. In order to achieve movement, *type II myosin* localizes to the nodes, which requires *cdc4* and *rng2* to connect with *mid1p*. Later further proteins are recruited including an actin nucleator, *cdc12* formin. After the start of actin polymerization a set of *actin-binding proteins* (tropomyosin, fimbrin) enters the ring and contributes to bundling of actin.

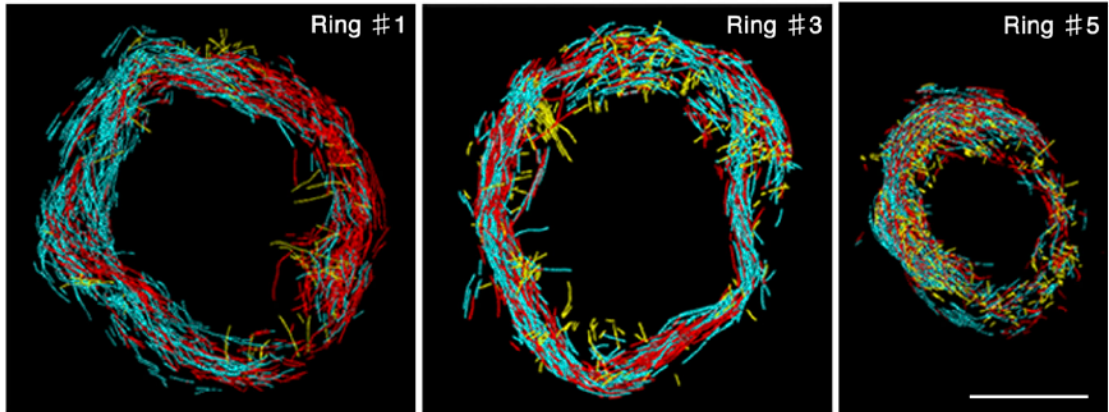


Figure 2: *Three-dimensionally reconstructed images of contractile rings of different stages. #1 is in early anaphase B, #3 is in late anaphase B, #5 is during cytokinesis. Filaments in red point clockwise, blue ones counterclockwise, other orientations are marked in yellow. Scale bar is $1\ \mu\text{m}$. Pictures from [6].*

During prometaphase the basic dimensions of the mature ring remain constant for approximately 30 minutes: there are 1000-2000 actin filaments of the average length of $0.6\ \mu\text{m}$ containing 50% of actin monomers present in the cell at all. However, most components in the ring are highly dynamic during this homeostasis. Then, in anaphase, the ring begins to contract, but the reason behind this sudden change is still unknown. The number of key components - except for myosin - decrease proportionately with ring diameter, e.g. actin filaments become shorter on average [6]. As the ring slowly contracts in 30 minutes, the dynamics of proteins remain constant.

There is evidence that ring constriction is essential for normal cell division [2], but the exact role it plays in fission yeast cell separation is still unclear. Although it is possible that ring contraction alone is capable of dividing fission yeast, it is equally possible that the ring merely marks the site for division and the assembly of *cell wall septum* is also necessary. [7] In large animal cells the ring is thought to exert significant contractile forces alone responsible for physical separation.

2 Cytosim

Cytosim is a C++ based program designed for simulations on cytoskeleton (for example its organization, networking and other processes) [8]. It is a free software and the previous versions are available for all operating systems on the website of the Nédélec group. The newest version which I used during my work is still under development, but a beta version (for Mac OSX) can also be downloaded from the website. The program can be run from command line, with three executables: **sim**, **play** and **play live**. **Sim** has its output in **.cmo** files - can be read by any text editors - containing all the necessary information about initial conditions and the simulated objects' coordinates during the simulation. This is a real simulation engine, therefore running **sim** with longer time-span or small time-step can take some time. **Play** is a visualization tool using the **.cmo** files from **sim** to help the user in understanding of the outcome of simulations, and with additional features like image capturing or changing the display parameters on the run. It requires **sim** to be previously run. **Play live**, as its name suggests, visualizes the simulated objects as soon as **sim** calculates the coordinates. It is useful for short simulations.

The input is a configuration file - also appropriate for all text editors -, which by default is named **config.cym**, but can be any other file with the extension **.cym**. All the features (objects, special properties, simulation conditions) of **cytosim** are available by editing this configuration file. The different cellular elements necessary for the simulations are classes within **cytosim**, their characterizing properties are also represented as individual classes linked to the object's class. All of these objects are biology-inspired, but simplified structures, with unique shape or behaviour. The configuration file mainly consists of the definition of objects - with command **new** - and setting parameter values (with command **set**). After initialization of simulation and object settings, the simulation is started with the run command - still within the configuration file -, and there is a possibility to change values afterwards or add different events to the simulation.

The newest version of **cytosim** will have a Doxygen generated documentation going into details about the classes and their properties, and listing several example **.cym** files for modelling different cell functions. The most basic example models a filament in a spherical space:

```
set simul example
{
    time_step = 0.005
    viscosity = 0.02
}
set space cell
{
    geometry = ( sphere 5 )
}
new space cell
set fiber microtubule
{
    rigidity = 20
    segmentation = 0.5
    confined = inside, 100
}
new 1 fiber microtubule
{
    initial_length = 8
}
run simul * 5000
```

In addition to the original code, there are complementary Python scripts making the use for systematic simulations easier. With the help of **run.py** and **pre-config.py**, multiple simulations can be started the same time with slightly different conditions, for example we can run 20 (or much more) simulations for a model with a given parameter value chosen randomly from $[0, 1]$ (or any other) interval. The simulations executed sequentially in different folders, therefore the results are not overwritten and can be read out easily. Further Python scripts utilize the functions of play and help saving multiple images (by **makeImage.py**) and generating a html page (**makeHTML.py**) or a video (**makeMovie.py**) from them.

2.1 Calculations

In **cytosim**, all objects are described by points. The coordinates of these points are collected in a vector x , of size $N \cdot d$, for a system of N points in d dimension. The equation of motion from Langevin dynamics [9] the program uses:

$$dx = \mu F(x, t)dt + dB(t),$$

where $F(x, t)$ contains all forces (interactions between objects and forces within the object) acting on the points in x at time t , while $dB(t)$ involves Brownian motion.

From an initial configuration, the system is calculated by discrete time steps (τ , given by the time-step property of **simul**), and **cytosim** uses implicit integration for calculating the next stage of the system. Therefore $F(x, t)$ is linearised as $A_t x + G_t$, where A_t contains the stiffness coefficients and G_t is a vector constructed from the constant forces acting on the system. After all non-linear interactions are also linearised, further transformations lead to a system of linear equations:

$$[I - \tau P_t \mu A_t](x_{t+\tau} - x_t) = P_t[\tau \mu (A_t x_t + G_t) + \delta B_t],$$

where $P_t = P(x_t)$ is an orthogonal projection from the linearisation of constraints and δB_t is a 'simulated Brownian'. This equation can be solved to calculate $x_{t+\tau}$ efficiently because the matrices describing the system are sparse.

2.2 Objects

Cytosim has many types of objects for the best description of the structural elements constituting the cytoskeleton. However, since I simulated not the behaviour of the whole cell but of a distinguished part of it, I used only a few but more common classes.

2.2.1 Spaces

Cells are very delimited units of living organisms, their inner functions are only indirectly connected to their environment. Although small uncharged molecules, like water, can freely diffuse through the limiting membrane, proteins participating in cytoskeleton are bigger in size and molecular weight, therefore bound inside or outside the

cell. The cell or membrane-limited cellular component - e.g. mitochondrion, nucleus - is represented by the class **Space**, because usually it determines the part of coordinate space any objects can move in. The membrane is the boundary of the space and the fact that it is impermeable for the simulated objects in **cytosim** is given by their **confined** property. The strength of confinement can be set in the configuration file, after determining its type: inside, outside, surface (representing a membrane-bound behaviour) or none (allowing free movement everywhere).

2.2.2 Fibers

The elements constructing the cytoskeleton are divided into three groups according to their diameter and cellular function: *microfilaments* ($d = 6nm$), *intermediate filaments* ($d = 10nm$) and *microtubules* ($d = 23nm$). All can be considered as a linear, elastic, non-extensible structure. In **cytosim** we do not take into consideration the diameter which is negligible from the point of view of most simulations, therefore all cytoskeletal elements can be merged into one class: **Fibers**. We can distinguish between different filaments by setting the appropriate parameters and by the use of three subclasses. The subclasses (ClassicFiber, DynamicFiber, Tubule) do not bear additional properties, but use different dynamic models on filament growth and shrinkage. In summary, fibers consist of segments given by their boundary points, where the length of a segment (distance between the equidistant model points) is set by the user defining segmentation value. The displacement of the fiber is calculated after all forces (Brownian forces, motor forces, etc.) acting on a fiber are taken into account for each segment and the resultant force is translated into movement or bending. In addition, the elongation or shortening of filament according to the selected dynamic model is executed. In each iteration the segmentation procedure is repeated to prevent from unequal distribution of modelling points.

DynamicFiber class uses a recent dynamic model, described for microtubules in previous works by the Nédélec group. [10] In this model a fiber can gain or loose units from its plus end while the minus end is static. Therefore a dynamic fiber left alone without Brownian motion would have its minus end in a fixed position. The dynamic end can be in a growing or shrinking state which are denoted by `state_green` and `state_red`, respectively. The switch between growing and shrinking phase (called *catastrophe*) happens with a rate calculated from the parameter hydrolysis rate and it determines

the time after a change between state green and red occurs. When there is no switch the fiber length increases or decreases based on growing- and shrinking-speed, respectively. The speeds are in unit/time-step, hence the absolute length gain in a step is given by $unit_length \cdot speed$. In a more complex system when different forces act on the fiber the resultant force at the plus end can radically affect the polymerization or depolymerization rate.

2.2.3 FiberBinders

Molecules which can bind to a fiber are formed from the general class **Hand**, and given specific attributes to represent some additional features we can observe in nature. Hand in itself is an object with the ability of binding to a fiber or diffuse in the *cytosol* according to Brownian dynamics. The most common example for this kind of objects are motor molecules, like *kinesin* and *dynein*, which walk along a cellular filament using the power gained from ATP hydrolysis, and carry other molecules (or even cell organelles) from one point to another.

In *cytosim* hands can be in a single molecule form or form a couple by 'carrying' another hand. From the point of a fiber a **Single** can act passively (when it is only walking and not exerting any force on the fiber) or actively (when it is fixed by its binding site), and make the fiber move. A **Couple** also can act two ways: if only one hand is attached, it acts like a free single; with both hands attached its function is similar to the fixed single's, only this time the two fibers - which the binders are attached to - move relative to each other.

By default, hands own the ability to bind to a fiber and stay at that point (described by a pointer to the fiber and the position's distance from the minus-end) until detachment occurs. Beside binding, hands can have different additional properties set by their activity.

A **Nucleator** can nucleate one fiber of given kind (e.g. *actin*, *tubulin*) with specific properties (e.g. initial-length, orientation). The nucleated fiber is attached to the nucleator by its minus end. However, as in the case of every fiberbinder, the connection between the fiber and the nucleator can break, and the supervision of this event follows an exponential distribution based on the unbinding-rate property of the binder. Appropriately chosen unbinding-rate can influence the overall characteristics of the whole system, as discussed later.

Motors can move on a fiber with a maximal speed given by the parameter max-speed, but if they are attached to something they exert force on the fiber to make it move - this force is set by the stall-force value. The relationship between the speed and the force of a motor is assumed to be linear and the aforementioned two parameters are the two intercepts. An additional feature which can be set is the ability to hold to the fibers' end instead of automatic detachment. This way at the end of a fiber the binder couple can act like a single and hold the fiber end on a fixed location until detachment occurs.

3 Contraction

In the first part of my project, I examined the different mechanisms which can lead to efficient contraction within the ring.

To simplify the problem as much as possible, I used periodic boundary conditions which allowed me to handle the ring as a one-dimensional structure - a line with its left and right ends connected. Moreover, for this first model I used simple fibers instead of microtubule-like dynamic ones, therefore the number and length of the objects were constant during the simulation. Since periodic boundary conditions made the length of structure (the circumference of the ring) fixed, I could not see explicit contraction, I had to find a way to read out the potential for contraction. The will to contract generates tension within the fibers, which I could easily measure by the lagrangian value assigned to each segment. I found that the averaged lagrangian provides a useful measure of contractility, therefore made multiple simulations on simple systems to get a full description of this property. The model systems helped us to two radically different recipes of contraction: motors with end-holding ability and smooth muscle like organization.

3.1 One-dimensional model

Already more than 50 proteins have been identified to play a role in ring formation and constriction. However, it also has been proven that many of them are responsible for similar functions, and numerous null mutants have a perfectly functioning cytokinetic ring. Therefore, I had no intention to model every protein, only the major roles fulfilled by them. I simply used one kind of motor and one of cross-linker molecules and neglected the fact that there are several different proteins which can act these ways. For both cross-linkers and motors I used **couples**, because only they can make connections between filaments. Linkers consisted of two identical hands called **binders**, while motors were formed from two **myosins** with motor activity. Since I modeled a slice of the fission yeast cell following the equator, I used a rectangular shaped space, but with periodicity in one direction, and arranged the fibers to a narrow horizontal line in the middle. The filaments were static with a constant length of $0.6 \mu m$.

First I did an exhaustive screening to examine different behaviours of this system. Most of the results were predictable but still informative, since I could estimate how the distribution of fibers look in **cytosim** under the effect of a given number of cross-linkers and motors. In each simulation I used 500 fibers and 0, 2000 or 5000 cross-linkers/motors in different combinations. I also changed two important properties: whether the linkers were antiparallel and whether the motors held to fiber ends or detached from them. With `makeImage.py` I could easily examine the final distribution, and using the options available during replay (different colouring schemes of filaments), I could also study the tensions within the filament bundle.

The main conclusions are: without motors, the displacement of fibers is negligible; without any type of linkage between fibers (linkers or end-holding) only 'passive' sliding occurs which cannot lead to change in overall length; and combination of motors and linkers lead to a combination of aforementioned reactions. For example when the number of motors was at least two times of the number of cross-linkers, sliding (with low tension) occurred, while with a low number of motors the fibers barely moved.

There was only one case when contraction could be observed, the final filament distribution differed from the initial conditions. This way I could get the first recipe for contraction: **end-holding motors**. When they stayed attached to the filament ends for a while, after a time various number of shorter filament aggregations formed with small empty spaces between them {figure 3}. I ran the exact same configuration file without periodic boundary conditions, and the bundle clearly shortened during the simulation.

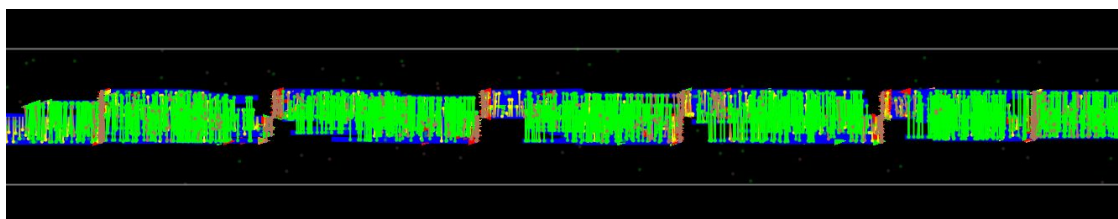


Figure 3: *Final state after simulating the system with end-holding motors. Display is shifted to help visualizing the inter-filament connections. Fibers are in blue, crosslinkers in green, motors in brown and yellow.*

3.2 An example from nature - Smooth muscle

The other recipe was provided by nature, since we can find many cases in which contraction is caused by the interactions in an actomyosin system. The most well-known and understood is the striated muscle, but in this case the interaction is supported by a very strict arrangement of actin and precisely localized myosin thick filaments. The cytokinetic ring seems completely structure-less compared to muscle. Fortunately, the other type of muscle, *smooth muscle* also lacks the strict spatial organization typical for striated muscle. In this case actin filaments are paired up by a still mysterious part called *dense body*, while myosin not necessarily form thick filaments. It is enough if myosin molecules are coupled, and then, as they both try to walk towards the central part of a given actin pair, they can move the filaments relative to each other. Because of this activity the centres of gravity of the pairs become closer, therefore the side-to-side distance of the whole muscle cell is decreasing. {See figure 4.}

Since smooth muscle has a proven highly efficient contraction mechanism, it was inevitably useful to implement it, because for later models it could serve as a control.

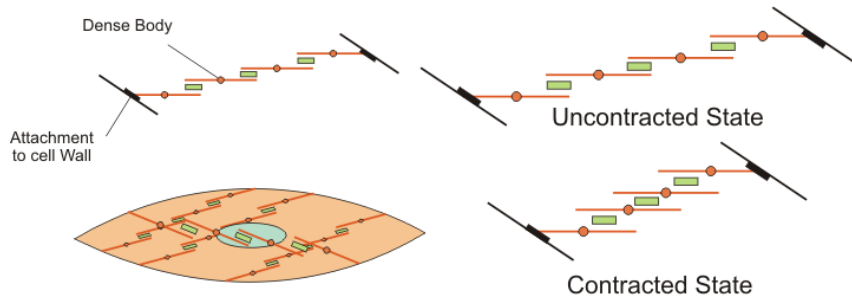


Figure 4: *The mechanism behind smooth muscle contraction. Orange lines are actin filaments, red spots are dense bodies and green rectangles represent individual or grouped myosin couples.*

Implementation

In **cytosim** there are several ways to organize fibers into a structure instead of randomly placing them within the cell space. For implementing smooth muscle the most suitable solution was a bundle of two antiparallel fibers, automatically attached by their minus-ends. This way each actin pair was a bundle of two $1\ \mu\text{m}$ long static fibers, and then the bundles were randomly placed with horizontal orientation. Motors were made of two coupled myosin molecules without any further organization, unlike

in striated muscle.

In the model of muscle cell it had non-periodic boundaries, and in a short time the side-to-side distance decreased from initial $10\ \mu m$ to $2\ \mu m$. Furthermore, the contraction was so strong, that even in the periodic case, the bundle could not handle the tension generated by motor activity, and after a time the ring broke and acted like the bundle in the nonperiodic case. {See movie 1 in supplementary videos.}

However, not even this simple structure has been observed in the cytokinetic ring. Therefore I wanted to reproduce this behaviour without the constraint on filament organization, only with the help of specific cross-linkers which are more probable to play a role in ring constriction. Some motor molecules can track the end of a fiber and stick only to that point, without any interaction with other points of the filament. I used this end-tracking ability to create cross-linker couples which could only hold together fibers by their minus-ends. Besides this end-tracking I had no additional constraints on the couples - they could attach to two parallel and antiparallel fibers. Finally, instead of bundles I added single fibers with initial length $1\ \mu m$, these linkers and, after some steps, the myosin motors. The system acted very similar to 'real' smooth muscle, clearly contracted without periodic boundaries and also contracted after breakage in periodic case. {See movie 2.} As a control I repeated this simulation without cross-linkers: this model did not show any organized behaviour and the overall length of the bundle remained constant.

3.3 Measuring contractility: Tension

In the one-dimensional model the ring was represented by a periodic line, its circumference was given by the length of the rectangular space I used to model the cell. Its main difference from a nonperiodic system is that the ring is not able to change its diameter, therefore we cannot see contraction. With periodic boundaries there are three ways to test the system's ability to contract: running the same simulation without periodicity, cutting the ring or measuring the tension caused by the will to contract. The third option seemed to be the most reliable, especially because it offered not only a binary - contractile or not - decision, but a certain value to describe a model.

3.3.1 Tension in smooth muscle

Since during the component tests I already studied tension inside the filaments, there was an obvious solution to quantify this attribute. I used the **lagrangian** coefficient of each fiber segment, summarized this values to get the tension of a fiber and consequently to get the average for all actin filaments.

In order to extract this parameter of the system, I wrote a new function for Fiber_Set, called **InfoTension**. Then, I added another case to display statistics for live following of the tension, and used **custom5** function to write out the averaged values into a text file, called tension.txt.

First, to calibrate my new measuring system, I applied custom5 function on the periodic smooth muscle configuration. The time between two consecutive sample points was 0.04 s. I could clearly observe an increase in tension for the first time, then a short equilibrium with a typical plateau, finally a break in the ring - a weakening in fiber-fiber connections - which rapidly decreased the tension to zero. I used MATLAB for post processing and analysing the data.

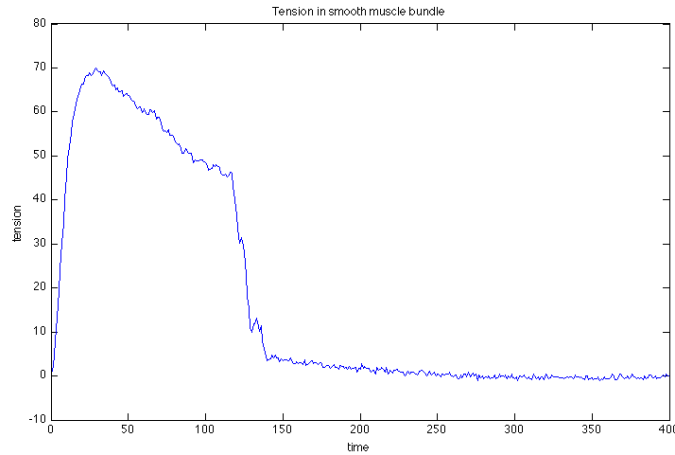


Figure 5: *Typical tension curve of periodic smooth muscle simulation. Time-unit is 0.04 s, tension is in pN.*

I defined many values which could altogether describe a graph {Fig. 5}: the maximal value, the time until breakage and spent in tense state, and the time needed for contraction. Then I wanted to study how these values depend on one parameter -

because I was interested in the strength of the system's will to contract, I chose the motors' stall-force value. I used `run.py` and `pre-config.py` to start multiple simulations with force chosen from the $[0.5, 5.5]$ interval. (Other parameters were the same for all simulations.) Although the general shape of the curve remained, the most characterizing values changed according to force. {Fig. 6} As expected, time of breakage and the timespan of high tensions are in inverse proportion to stall-force. The maximal tension also clearly depends on stall-force, while the speed of contraction varies in a wide range, since it rather depends on motor speed. {Fig. 7}

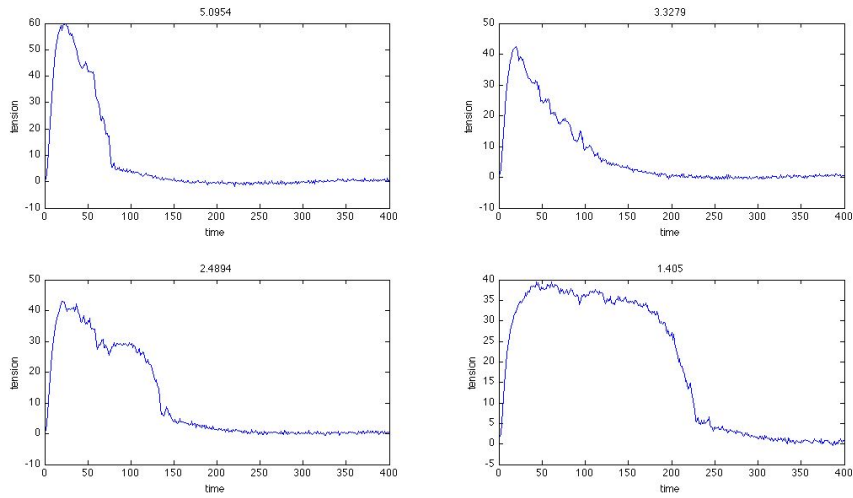


Figure 6: *Different tension curves for different stall-force values.*

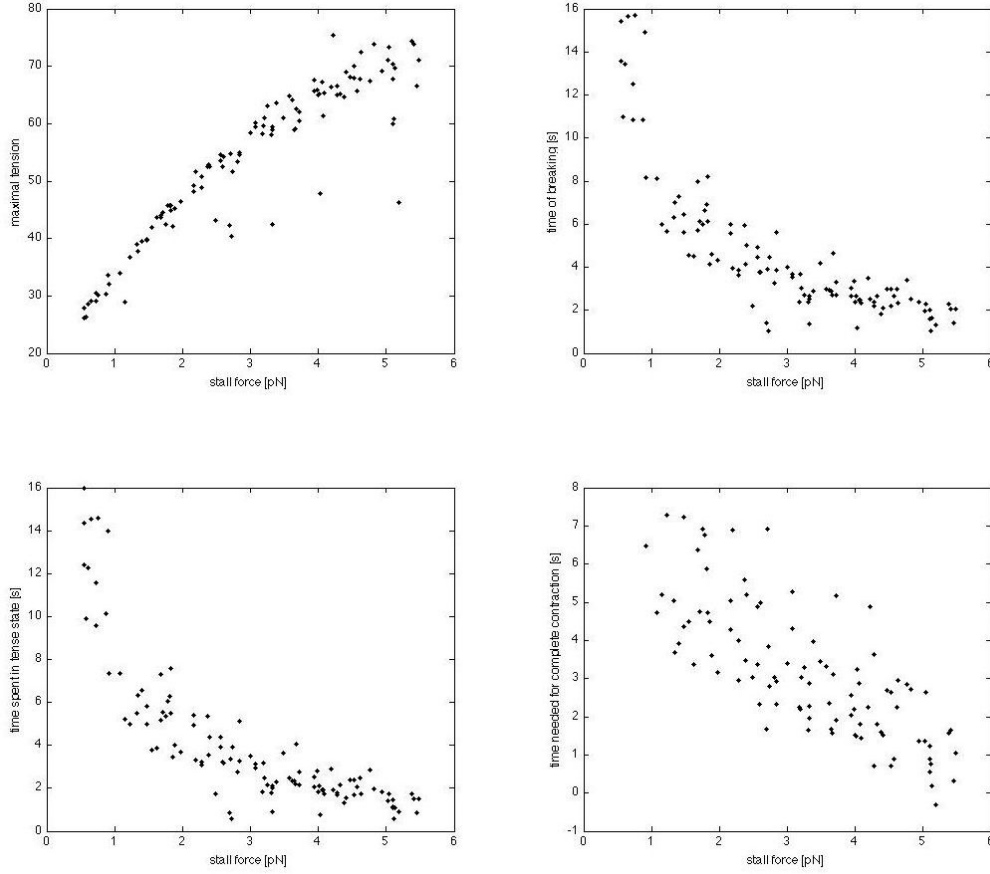


Figure 7: *Force dependency of tension attributes.*

3.3.2 Tension in a ring model

Second, I used my tension measurement as a tool to examine the necessity of end-holding property implied by previous simulations. I further simplified the ring model by decrement in the number of filaments, so the simulations could be performed within a few minutes. I still used a horizontal, periodic line for the ring, which now consisted only 24 fibers (12-12 pointing to each direction) randomly placed along the x axis. This way the lack of spatial organization and the 1/1 ratio of the two orientations still held. I added myosin motors to the system and applied custom5 to measure tension within the formed bundle (with 0.04 s between sample points). When motors had end-holding property the graphs were similar to the first part of the previous figure {5}: tension building up in the bundle. {Fig. 8, see also movie 3} If the motors did not own this special feature tensionless sliding occurred, coincidentally with the near zero

values of the graph. {Fig. 9} Though naturally the simulations were too short for the bundle to break under tension, I could test another contractility visualization method: cutting the fibers. After the cut at time 100, the tension immediately disappeared. {Figure 10} Furthermore, according to the theoretical work of Kruse et al. [11], the tension generated by end-holding motors is greater if all fibers are parallel (point to the same direction). Simulations on the 24-fiber model system confirmed this theory: the maximal tension was unequivocally higher in this case. {Fig. 11}

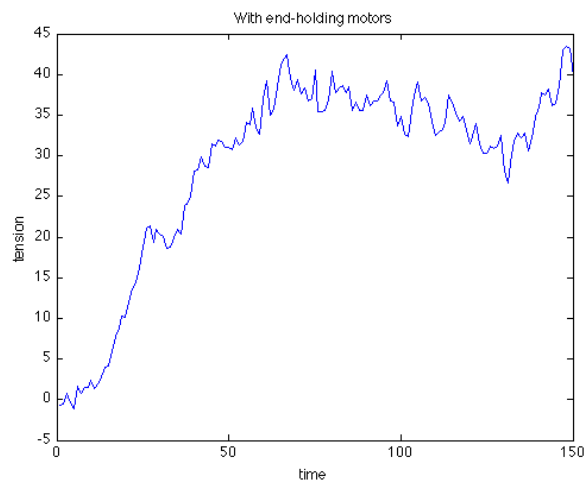


Figure 8: *Tension graph of ring model, motors holding to end of fibers. Units of the horizontal and vertical axes are 0.04 s and 1 pN, respectively.*

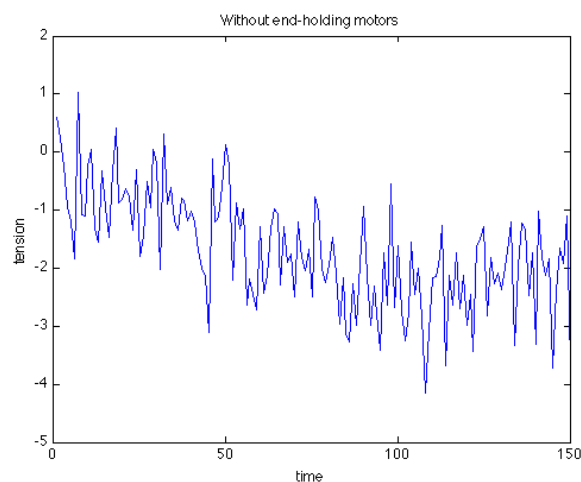


Figure 9: *Tension graph of ring model with motors detaching at fiber ends. Units as before.*

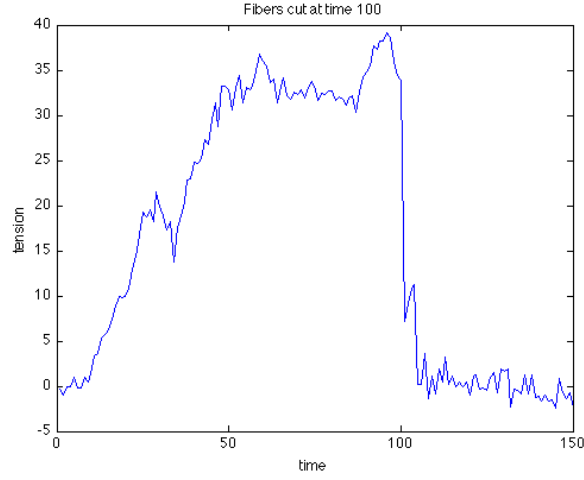


Figure 10: *Tension graph of ring model if fibers are cut during the simulation. Units as before.*

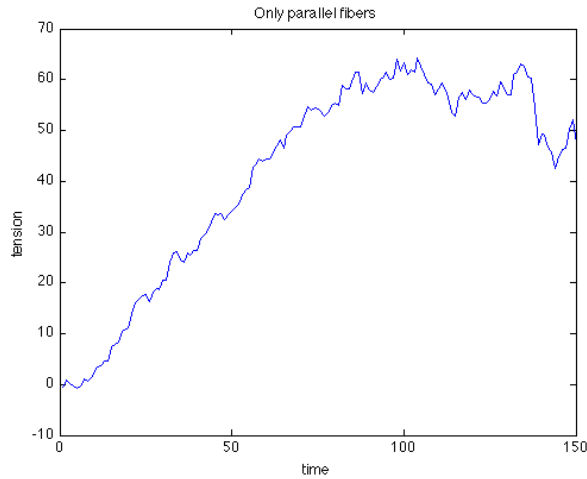


Figure 11: *Tension graph of ring model with all fibers pointing to positive direction. Units as before.*

3.4 Summary: Recipes of contraction

There are two radically different mechanisms of contraction, both require some kind of constraints on the system. In the first case fibers can lack any organization beside their confinement to the cell-membrane along the equator. On the other hand, motors had to hold to the fiber endings, otherwise no contractile forces are generated within the ring.

The other possible explanations for contraction was a structure similar to smooth muscle. At first glance the model seems to be too restrictive to characterize a mechanism present in every animal and fungal cell. However, as previous simulations proved, there is no need for strict bundles, a random filament distribution with specific end-tracking proteins can result in an appropriate organization. Furthermore, during prophase, when the ring is formed from precursor nodes gathered in the division plane, nodes nucleating multiple filaments can lead to the formation of sufficient antiparallel actin pairs. Therefore I decided to study and simulate ring formation.

Another point important to emphasize is that both mechanisms lead to an alignment of fiber endings. Either because end-holding motors keep them in position, or because the central parts connecting actin-pairs are aligned in a given distance from all fiber ends. This brings up interesting questions, as electron micrographs did not suggest this kind of structure would exist in real, and it would imply undesirable inhomogeneity leading to instability in the ring.

4 Ring formation

There are two different but non-exclusive classes of theories on the formation of the cytokinetic ring. [12] The 'leading cable' hypothesis assumes a single actin nucleation site (consisting *cdc12p formin*) attached to the membrane somewhere along the equator. This site, called the *cdc12p* 'spot', nucleates all the ring consisting actin filaments during the first part of ring formation. As the filaments elongate by the membrane, it is assumed that they search for filaments with opposite polarity, while motor-containing nodes help their transport and anchor them to the cortex. If two oppositely oriented fibers can contact, they are pulled into a ring orthogonal to the long cell axis, the shortest structure possible on the surface of a cylinder (what is the shape of fission yeast cells). Moreover, possibly the tension generated in the filaments by pulling forces upregulates formin activity to produce more actin. This formation model implies that for the first time the ring consists of two half-rings made of actin filaments of opposite polarity, which seems to be the case according to electron microscopic images. [6]

According to the second theory, as a first step, several (60-80) nodes are formed by the recruitment of actin nucleator (*cdc12p formin*), actin binder (*myosin-II*) and additional proteins. These nodes are capable of filament nucleation and capture of filaments passing within a range of binding. In addition, the motor function of myosin-II results in the nodes moving on the captured filament to the direction of the pointed end, which makes two adjacent nodes move towards each other. Vavylonis et al. [13] used fluorescent microscopy to image myosin containing nodes, and observed an initial broad distribution which over 10 minutes evolved into a ring-like structure through stochastic motions. As proposed in [13] the motion of nodes starts a few minutes after SPB separation, by the time *cdc12p* is recruited to the nodes. The presence of *cdc12p* enables actin filament formation, each node nucleating averagely 2-3 cables to random directions (on a two-dimensional surface parallel to the membrane). Filaments grow with a growing speed of about $0.2 \mu m/s$, until a maximum length of $4 \mu m$, meaning the lifetime of a filament does not exceed 20 s. As actin grows the filament may pass within the capturing range of another node, therefore myosin-II can attach to the filament and start walking towards the pointed end connected to the nucleator node. Filaments randomly break (depolymerize) after a τ_{turn} lifetime, so connecting filaments and the ones searching for connection are also temporarily present. After the

connection between two nodes broke, the nodes stops moving towards each other and stay still until they manage to connect with another node, which is consistent with the burst-like nature of node movement observed in fluorescence microscopy experiments.

4.1 Ring formation in Cytosim

If we consider the second (node, pull-and-release) ring formation theory, and Vavylonis' assumptions, the polymerizing nodes provide a smooth muscle like structure: ~ 2 fibers nucleated from the same spot with their minus-ends attached to it. Therefore I worked on a configuration to simulate the formation of the ring and see how the resulted ring acted under the activity of motor couples.

For the first time I used numerous simplification of the model to fit best with the already existing features of **cytosim**. I did not want to reproduce the simulations in Vavylonis' article, only get a realistic state which I could use as a starting point for ring constriction simulations. My simplified model contained couples of **nucleators**, which therefore were capable of forming two actin filaments, and couples of cross-linkers, called **catchers**, which only bound to actin. The force which helped ring formation only resulted from filament dynamics.

Later I found that I needed nodes with a complex set of abilities (nucleating multiple fibers while binding the ones nucleated by others), it was not enough to use aforementioned nucleators and catchers. Hence I used filaments with negligibly small length and bound the different hands to them. This structure could nucleate and capture multiple filaments at the same time and because of the random initial distribution, the number of nucleators (therefore nucleated actins) varied amongst node-like structures. Moreover, to prevent fibers from connecting multiple nodes at the same time, I had to switch off the filament's growth if it already found a node to connect its nucleator node. I altered the step function of Fiber class to search for binders attached to the fiber and change the polymerization rate accordingly. Actins attached to more than one node could not gather additional units to their pointed ends, therefore stopped growing after finding a connection.

During the simulation, the nodes initially distributed in a wider stripe around the equator collapsed into a narrow line. {See movie 4.} Filaments were pointing to different directions, but formed a solid line in the middle. I saved the final state and

used it to initialize another simulation when the system contained motor molecules beside the aforementioned components. The system's behaviour was not as clear as in {movie 2}, but still the horizontal line along the virtual cell equator contracted like in smooth muscle.

5 Reliable contraction

In the followings, I tested the previously found mechanisms on their reliability and stability: meaning whether they could contract without breakage.

I used a two-dimensional simulation, where fibers form a ring instead of a periodic line, the system is enabled to contract, therefore we can distinguish between breaking which we saw instead of contraction, and breaking which actually tears the two-dimensional form. Observing contraction directly also gives us the possibility to make measurements on the speed of contraction, a rather important property of a system.

As we saw, both contraction providing mechanisms have the tendency to align fiber ends, and change the density distribution of fibers, which causes instability and easily results in the opening of the ring at one point. The solution for this problem lies within the dynamic nature of actin filaments: there has to be a much faster mechanism responsible for the redistribution of fibers. It has been shown in several studies that dynamic actin turnover rather than stable actin filaments are needed for normal cytokinetic ring. [11] In line with this, the ring functions as a site of actin polymerization both *in vitro* and *in vivo*, and inactivation of factors which promote actin growth causes serious defects in cytokinesis. [14] Pelham and Chang [15] observed that complete inhibition of actin polymerization lead to the disappearance of actin in 60 s, which means that actin turnover is an about 30 times faster process then constriction.

After including dynamic instability and establishing a two-dimensional, dynamic model of the contractile ring, I had to revise the space used for modelling the cell membrane. I searched for a suitable method to make the membrane sensitive to pulling and pushing forces acting on it. First method was to simply change the radius of membrane as if it followed the ring immediately. Later I refined this method by adding another property to the cell called mobility, which determined the 'reaction time' of the membrane.

5.1 Two-dimensional model

The space represented a slice in the cell equator, but in this case perpendicular to the main axis of the cell, hence it was spherical with radius R . The center of this circle was the location of the pre-divisional nucleus and the ring is gathered around this point, close to the cortex. [12] For the first simulations the radius of the ring was $3\text{ }\mu\text{m}$, later I decreased it according to the data from [6] and [11], but the results did

not depend on the size of the cell.

Since in the real system fibers are constrained to grow parallel to the cortex and, according to electron microscopy data [6], only a small percent of filaments point towards the center, while none of them can grow through the phospholipid layer, I confined the filaments to the **surface**. With an appropriate stiffness value, the fibers easily followed the curvature of the membrane (considering that actin filaments are much more flexible than microtubules [16]), and the ring could form an almost perfect bundle.

To involve dynamic turnover on the above model, this time I used the class `DynamicFiber` which is originally a model for microtubule polymerization and depolymerization dynamics. Because of their rapid turnover, fibers initially added to the system could disappear in a minute, so continuous nucleation of fibers was needed to keep the number of filaments approximately constant. As in the previous implementation, I used a couple of nucleators (**nodes**) to nucleate replacement actin filaments permanently, so each node could nucleate at most 2 fibers at a time. Furthermore, because of the restricted growth of fibers, I introduced a new orientation setting called **tangential**. This can be set in the `nucleation_spec` property of a nucleator and make the filaments grow along a vector perpendicular to the radius (the node's position vector), to clockwise or counterclockwise direction (with equal chance). Therefore the (maximum) 2 nucleated filaments were either parallel or antiparallel, and attached to each other until the node released one/both of them (which occurred according to unbinding-rate).

Due to nucleating activity after a given time (depending on the fiber's parameters) the system arrived to an equilibrium of filament growth and depolymerization, and had an approximately constant number and length later on. Therefore I could search for an adequate set of parameters (e.g. growing-speed of actin) to reproduce some of the experimental data: the average number and length of actin filaments [6] and turnover time [15]. In the interest of this, I created a simple model with a few nucleators. I ran simulations when I fixed the hydrolysis-rate (0.6) and changed growing-speed or vice versa (growing-speed = 0.03). Because of the smaller amount of nodes, the configurations when the number of nucleated filaments were between 100 and 180 matched with the number of actin filaments in the ring. [17] In most cases there is a square proportionality between the changed and the measured parameters {figure 12 and 13}.

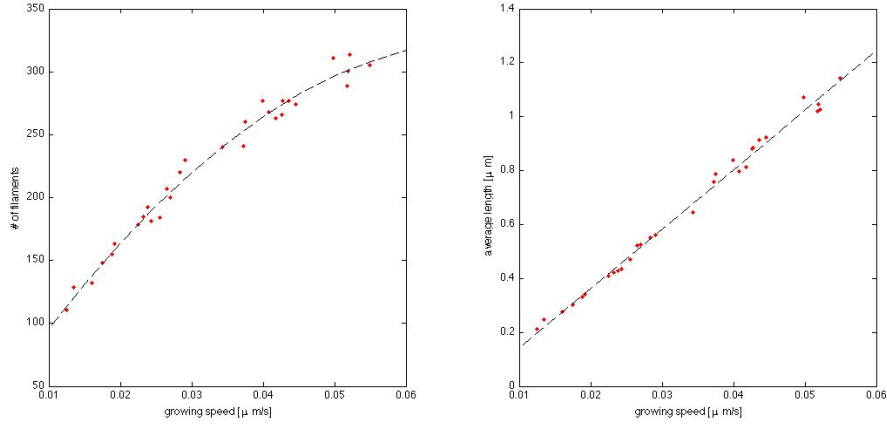


Figure 12: *Dependency of average filament number and length on growing-speed with hydrolysis-rate = 0.6.*

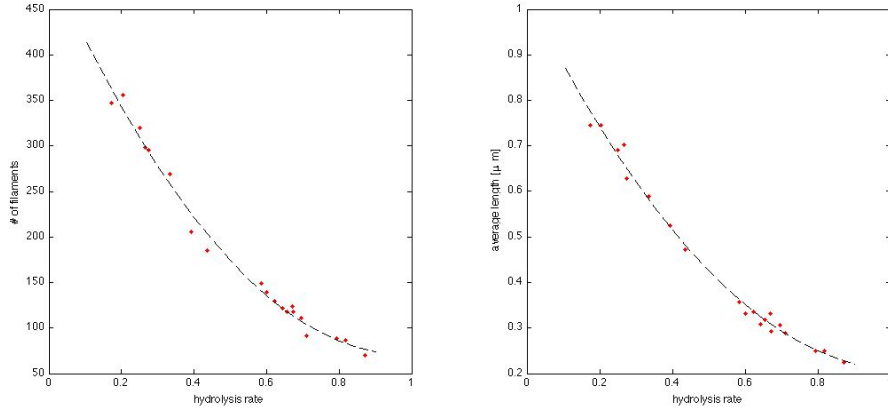


Figure 13: *Dependency of average filament number and length on hydrolysis rate with growing-speed = 0.03.*

As in the one-dimensional simulation, I needed a contraction quantification method for further comparison between different simulations on the cytokinetic ring. Measuring the radius of the ring was an obvious solution, but the way to derive this measure from object positions had some difficulties. Since there was some randomness in the direction of fibers, I calculated the distances between the center of the space and each model point, and finally averaged over all points and all fibers to get r , the ring's radius. To do so, I wrote an additional function for `Fiber_Set` called **InfoRadius**, which calculated the distance of each model point from the origo (0,0), averaged them and gave also the standard deviation.

5.2 Membrane mobility

Since the fibers were confined to the surface, after a given radius (depending on the stiffness of the confinement) the F force exerted by the membrane became too high and the ring could not contract any further. {See movie 5.} However, in the real system the interaction between the ring and the membrane is mutual, the diameter of cell also decreases which then allows the ring to contract more and more. Giving this feature to the spheric space simulating the cell was essential.

Therefore, after measuring the radius of the ring, I used the results to change the cell radius accordingly. This method was a strong simplification of the real process, which assumed not only a linear connection between the membrane-ring distance and the pulling force on the membrane, but also that the membrane follows immediately the ring. In this case membrane movement depended on the stiffness of confinement only because it restricted how far individual filaments could diverge from the surface. This dependency made configurations with reasonable confinement stiffness unable to contract. The model needed further refinement to realistically incorporate the membrane's behaviour.

Therefore I assumed that filaments exert force on the membrane according to the distance between membrane and ring ($\delta R = R - r$), and a force pointing towards the center of the cell makes it decrease in radius. The distance and the force (F) are proportional, as well as the force and the shift of the membrane. The connection between them is characterized by the confinement stiffness and the membrane mobility, respectively.

$$\xi \frac{dR}{dt} = F = k \cdot \delta R$$

Which in the case of discrete time-steps (τ) becomes:

$$\Delta R = k/\xi \cdot \delta R \cdot \tau,$$

where $1/\xi$ is defined by mobility and ΔR is the (negative or positive) change in radius from time t to time $t + \tau$. A system can be characterized by k/ξ ratio which describes how fast the membrane reacts after the ring pulls - or pushes - it. $k/\xi = 1/\tau$ was the

case in the first model, which assumed an extremely high mobility.

I used another custom function (**custom2**) to implement these calculations and write out cell and ring radius to a text file in each time the function is called. Then I applied custom2 in every iteration, therefore calculated the above equation for $\tau = 5ms$. I set the stiffness (k) to 2 and for the following simulations $k/\xi = 1$ with $\xi = 0.5$.

5.3 Modelling the ring

After this mobility is added to the space, the ring is allowed to contract further, but the direction of movement is not restricted by the interaction above (ΔR can be negative or positive), so besides the desired contraction the system has the ability to act contrariwise if the circumstances imply.

By varying the parameters of nodes, I examined the efficiency and reliability of different mechanisms. In all the following cases I tried to keep the number of fibers between 1400 and 1500 (average length $0.5\mu m$) by changing the number of nodes if it was necessary. Besides I simulated only the first 50 s of contraction, which was enough to see the general tendencies in the system, but did not take more than 30 minutes to run. Like during tension measurements, I used MATLAB for visualizing and analysing the data.

As mentioned before, smooth muscle is a highly efficient contractile system, but as a ring-like structure, it lacks the sufficient reliability. Nevertheless, for our simulations, a smooth muscle like system can provide a good control as its behaviour is already known. A similar organization could be found in the ring, if nucleation did follow these rules: nodes nucleate two antiparallel actin filaments, which stay attached to the node by their minus ends, and every disappearing filament is immediately substituted by a new nucleation. Because of the dynamic instability of actin filaments this model gives a less regular structure than stable filaments in smooth muscle, but still owns the features most important for efficient contraction.

I implemented this by setting the **unbinding-rate** to **0** and using 800 pair of nucleators (800 nodes). This way, the actin filaments attached to the same node were not necessarily of the same length, nor antiparallel (because the nodes randomly nucleated

clockwise and counterclockwise fibers). Still, the similarities to smooth muscle made the distribution of filaments becoming more and more inhomogeneous, along with the distribution of nodes, so continuous renucleation cannot rescue the ring from breaking. After a short period of quick contraction, the ring broke, the nodes gathered into several clumps and the radius remained constant. {Fig. 14, movie 6 and 7.}

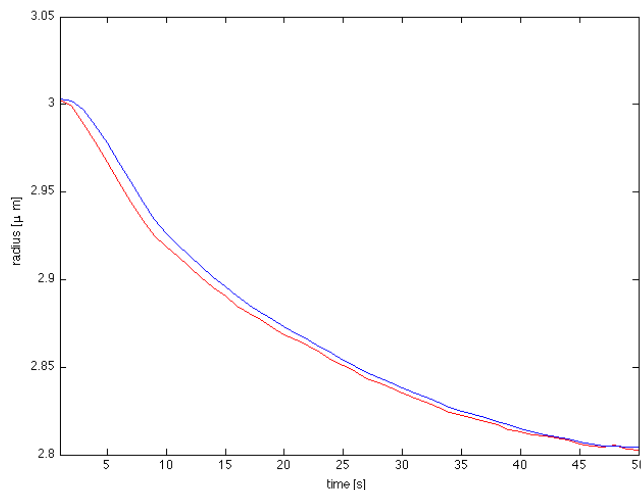


Figure 14: *Ring (red) and cell (blue) radius with zero unbinding-rate.*

Another special case is when the filaments are not attached to each other by any kinds of cross-linkers, therefore the system contains only single fibers pointing to one or another direction. This case I could implement in two ways. First, I kept the unbinding-rate 0, and used only single nucleators (1600). Second, by having a relatively **high unbinding-rate** value (10), I made the fibers detach quickly from their nucleator and move as single, independent filaments. This system was the two-dimensional representation of the one examined in my first screening and modelled by {fig. 8} and {movie 3}. As before, the filaments could easily slide on each other without any forces moving the centres of gravities closer. The nodes were carried around the cell with constant speed or stayed at their initial location with no fibers attached to them. The ring was not contracting in either implementations, the radii slightly fluctuated around the initial 3 μm . {See figure 15 and movie 8 & 9.}

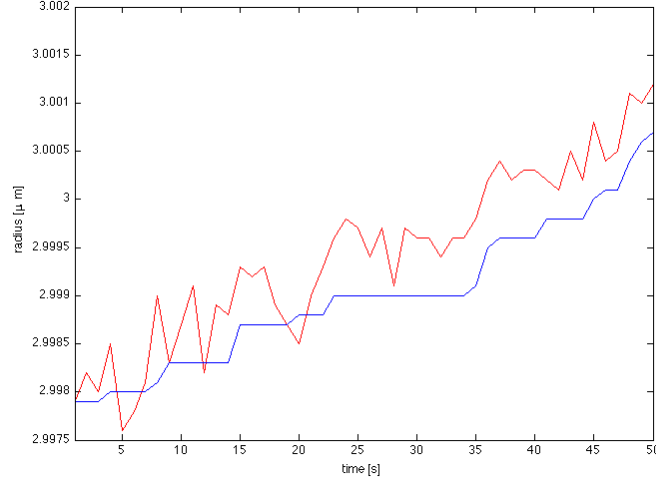


Figure 15: *Ring (red) and cell (blue) radius with high unbinding-rate.*

Between the previous two extremes there has to be an interval of unbinding-rate values, when the two combination of the two behaviours results in a contractile system with relatively homogeneous filament distribution. To determine this range, I ran multiple simulations with unbinding-rates between 0 and 10, and observed that (for a system bordered by a membrane for which $k/\xi = 1$) unbinding-rates higher than 0.1 make the ring unable to contract. Therefore I concentrated on the unbinding-rate $\ll 1$ case. The ring was contracting and the decrease in radius followed a linear curve in all cases, but the speed of contraction - which I could obtain from the final radius after 50 s - depended on the unbinding rate value. {Fig. 16 and 17.}

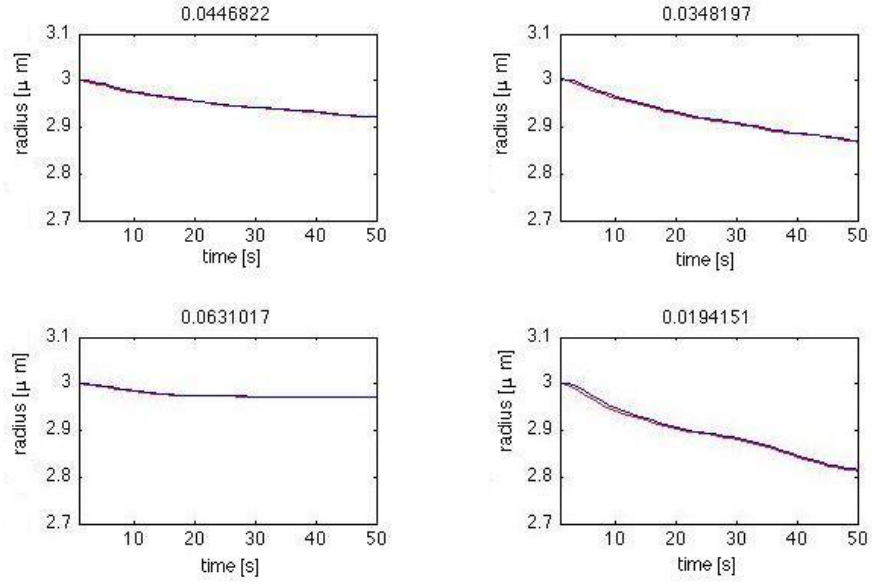


Figure 16: Ring (red) and cell (blue) radius for different unbinding-rate values (denoted by the title of each graph).

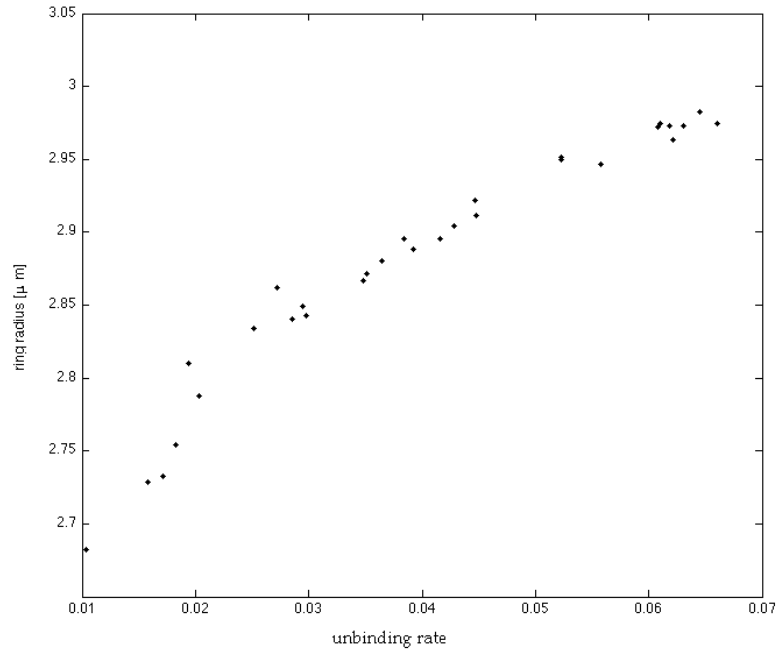


Figure 17: Ring radius after 50 s as a function of unbinding-rate.

Since the ring contracts (in fission yeast) in 20-30 minutes, I needed a relatively slow contraction, therefore I chose the unbinding-rate to be 0.05 for the model of the ring. The radius decreased following a linear curve, meaning the contraction velocity was constant in line with [15]. {Figure 18, movie 10 and 11.}

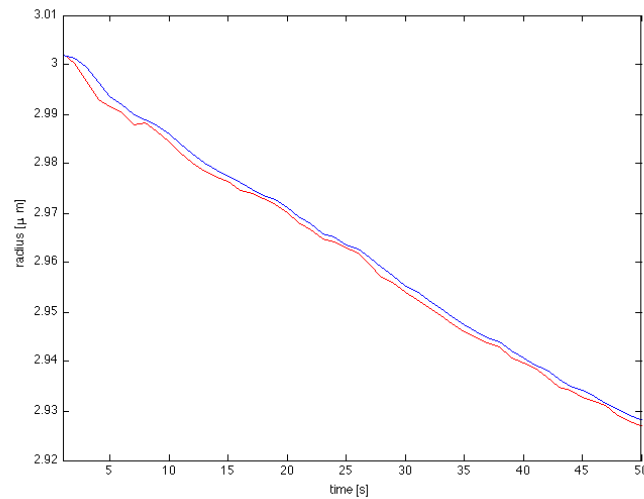


Figure 18: *Ring (red) and cell (blue) radius with unbinding-rate = 0.05.*

Furthermore, to prove that contraction is not (falsely) implied by the geometry and functions I used but the result of motor activity, I changed the direction of myosin motors. As motors walking towards the center of attached actin pairs caused contraction in both smooth muscle and the ring, myosins walking with a speed of opposite sign resulted in elongation. The filaments were pushing the membrane and both radii increased. {Fig. 19, movie 12.}

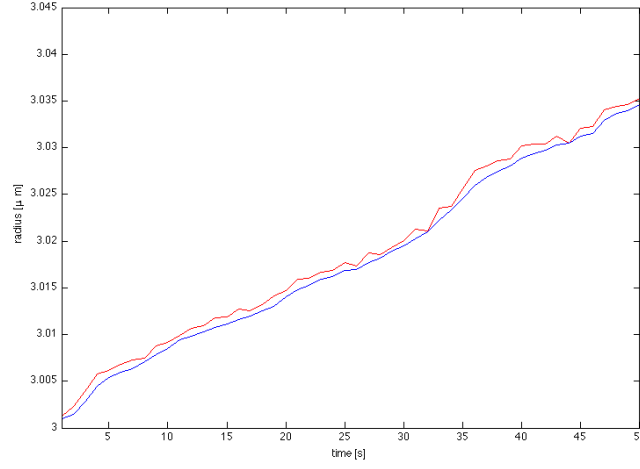


Figure 19: *Ring (red) and cell (blue) radius, if motors move to opposite direction.*

Finally, I tried a model for a longer timespan of 1200 s (20 minutes). The ring contracted with approximately constant velocity until the diameter became too small compared to the average filament length ($0.5 \mu m$), which was constant during the simulation. {See fig. 20 and movie 13.} In the real ring the amount of actin in the ring decreases during constriction, which also contributes to complete contraction.

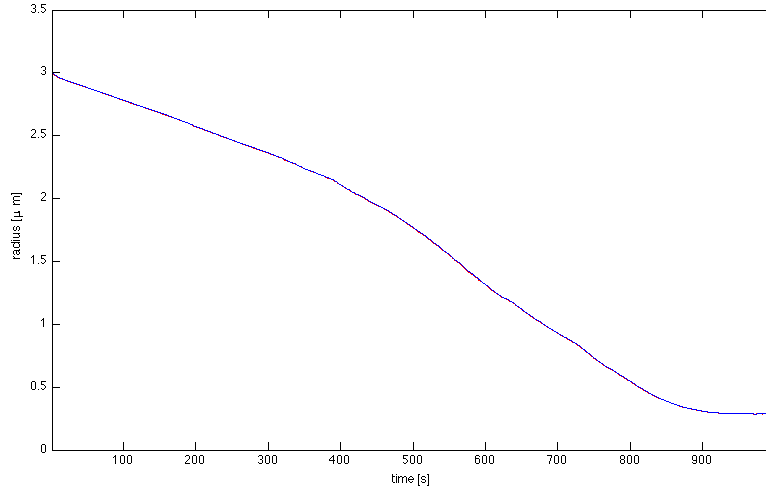


Figure 20: *Ring (red) and cell (blue) radius, first 1000 s is plotted.*

6 Conclusion

During the elaboration of my project I found two possible contractile mechanisms and examined their efficiency. I also simulated the formation of the ring to prove the biological relevance of the smooth muscle like mechanism. Then I created a two-dimensional space representing a section of the cell at the division plane. In order to simulate the membrane which is not a fixed, rather flexible border, I assumed that there is a linear connection between the force acting on the membrane and the shift of the membrane. I introduced a property to the membrane called mobility characterizing the ability to change shape under pulling or pushing forces. In this case shape change meant the change of radius because of the spherical form of the space. In this system I confined the fibers close to the membrane, and used rapid filament dynamics to prevent instability caused by inhomogeneities in the ring. Using a mobile membrane, actin nucleators and myosin motors, I could establish a model of the cytokinetic ring able to reproduce reliable and linear contraction.

The model uses many simplifications, takes into account only the most important key components of the ring and makes some assumptions on filament organization. Therefore the discussed mechanism may differ from the real one behind cytokinetic ring contraction. However, at the moment there are no results contradicting with these starting points, and some of them suggest the correctness of the model (e.g. [13]). Nevertheless, the main idea that smooth muscle like contraction can occur in 'everyday' cells, may be worth considering in connection with other cellular processes based on contraction. Hopefully this work will contribute to future experimental and theoretical researches.

List of supplementary videos

- Movie1 - Smooth muscle with periodic boundary conditions. Actins are white filaments, myosins are brown points between them. The periodicity is denoted by dotted lines at the right and left borders of the space.
- Movie2 - Smooth muscle reproduced from single filaments with end-tracking cross-linkers. Linkers are in green, actin in white, myosin in brown.
- Movie3 - A model of the ring with 24 filaments (in blue) and end-holding motors (in brown).
- Movie4 - Formation of the ring from a broad band of nodes. Nucleators are in orange, catchers in brown, fibers not shown.
- Movie5 - Ring confined to the fixed membrane surface, unable to contract. Nucleator nodes in yellow, filaments in white, motors in red.
- Movie6 - Ring with zero unbinding-rate (smooth muscle like case). Fibers are in white, motors in red.
- Movie7 - The same as in 6, but filaments are invisible. Bigger yellow spots are nucleator nodes, red points are myosin motors.
- Movie8 & 9 - Ring with high unbinding-rate (single fibers case). Motors in red, nodes in yellow, filaments are white (8) or invisible (9).
- Movie10 & 11 - Ring with intermediate unbinding-rate, reliable and contractile. Motors in red, nodes in yellow, filaments are either white (10) or invisible (11).
- Movie12 - Ring with inverted motor directions, both cell and ring radius are increasing. Actin filaments are white lines, nodes and motors are yellow and red points, respectively.
- Movie13 - Longer simulation on the model of the contractile ring. As before, filaments are in white, motors in red and nucleator nodes in yellow.

Acknowledgement

I would like to thank to **Dr. François Nédélec**, who welcomed me to his lab in the European Molecular Biology Laboratory (EMBL), and for three months helped me asking questions and finding the answers with his advice and programming skills. I hope we will continue working on this or other projects in the future.

Moreover, I would like to thank to all the previous and current members of the **Nédélec group**, for their work on developing **cytosim** and for the friendly, helpful environment I worked in during my stay.

I also would like to thank to the **Faculty of Information Technology**, for all the support given me during my studies, including an Erasmus scholarship for the time I spent working abroad. Furthermore, I am thankful to **Dr. Szabad János** for his kind advice and letter of recommendation leading to my fruitful work in the EMBL.

References

- [1] T. Boveri, “Concerning the origin of malignant tumours by theodor boveri. translated and annotated by henry harris,” *Journal of Cell Science*, vol. 121, pp. 1–84, Jan. 2008.
- [2] T. Schroeder, “The contractile ring. ii. determining its brief existence, volumetric changes, and vital role in cleaving arabacia eggs,” *J Cell Biol*, vol. 53, no. 2, pp. 419–34, 1972.
- [3] M. K. Balasubramanian, E. Bi, and M. Glotzer, “Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells,” *Current biology : CB*, vol. 14, Sept. 2004.
- [4] R. R. Daga and F. Chang, “Dynamic positioning of the fission yeast cell division plane,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, pp. 8228–8232, June 2005.
- [5] N. Padte, S. Martin, M. Howard, and F. Chang, “The Cell-End factor pom1p inhibits mid1p in specification of the cell division plane in fission yeast,” *Current Biology*, vol. 16, pp. 2480–2487, Dec. 2006.
- [6] T. Kamasaki, M. Osumi, and I. Mabuchi, “Three-dimensional arrangement of f-actin in the contractile ring of fission yeast,” *The Journal of Cell Biology*, vol. 178, no. 5, pp. 765–771, 2007.
- [7] J. Jochova, I. Rupes, and E. Streiblova, “F-actin contractile rings in protoplasts of the yeast schizosaccharomyces,” *Cell Biol Int Rep*, vol. 15, no. 7, pp. 607–10, 1991.
- [8] F. Nedelec and D. Foethke, “Collective langevin dynamics of flexible cytoskeletal fibers,” *New Journal of Physics*, vol. 9, no. 11, p. 427, 2007.
- [9] D. S. Lemons, *An Introduction to Stochastic Processes in Physics (Johns Hopkins Paperback)*. The Johns Hopkins University Press, illustrated edition ed., May 2002.

- [10] L. Brun, B. Rupp, J. J. Ward, and F. Nédélec, “A theory of microtubule catastrophes and their regulation,” *Proceedings of the National Academy of Sciences*, vol. 106, pp. 21173–21178, Dec. 2009.
- [11] A. Zumdieck, K. Kruse, H. Bringmann, A. A. Hyman, and F. Julicher, “Stress generation and filament turnover during actin ring constriction,” *PLoS ONE*, vol. 2, no. 8, p. e696, 2007.
- [12] M. Bathe and F. Chang, “Cytokinesis and the contractile ring in fission yeast: towards a systems-level understanding,” *Trends in Microbiology*, Dec. 2009.
- [13] D. Vavylonis, J.-Q. Wu, S. Hao, B. O’Shaughnessy, and T. D. Pollard, “Assembly mechanism of the contractile ring for cytokinesis by fission yeast,” *Science*, vol. 319, no. 5859, pp. 97–100, 2008.
- [14] J. R. Bamburg, “Proteins of the ADF/cofilin family: essential regulators of actin dynamics,” vol. 15, pp. 185–230+, 1999.
- [15] R. J. Pelham and F. Chang, “Actin dynamics in the contractile ring during cytokinesis in fission yeast,” *Nature*, vol. 419, no. 6902, pp. 82–86, 2002.
- [16] A. Ott, M. Magnasco, A. Simon, and A. Libchaber, “Measurement of the persistence length of polymerized actin using fluorescence microscopy,” *Physical Review E*, vol. 48, pp. R1642–R1645, Sept. 1993.
- [17] J.-Q. Wu and T. D. Pollard, “Counting cytokinesis proteins globally and locally in fission yeast,” *Science*, vol. 310, pp. 310–314, Oct. 2005.