PY573 Report

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Introduction

In the past 50 years, there has been a shift from using radiation based cancer treatments to more invasive and less destructive methods, to preserve more of the underlying tissue. These treatments require a more fundamental understanding of cellular environment and the natural properties of the materials inside of cells[2]. Cellular biologists naturally investigated many of these properties, but need assistance from many other fields such as biochemistry, physics, and material science[5]. Each field is able to contribute their own specialty to understanding the complex cellular environment, such as advancements in chemical engineering, microscopy, and polymer theory from each field respectively. Many roadblocks arise when trying to measure cellular environments, mainly the nature of the system. Cells are held together via the cytoplasm, and so to investigate the cellular environment scientists need to look inside of the cytoplasm. This itself presents the problem, is the cellular environment changing as a result of this probing?

This does not change the amount of time and effort spent on trying to solve the fundamental questions underlying cell biology. Scientists have diligently worked to develop many different drugs and knockouts which can combat the ability for cancerous cells to grow and multiply[2]. The methodology of interest for this project is through the suppression of microtubules (MTs). These filaments have been observed through methods such as GFP tagging to be a dense network inside of cells, and play major roles during cell division and motility[2]. But it is extremely difficult to understand their mechanical properties as they are mainly found in cellular environments, and creating outside of the cell returns us to our main problem. But we can use electron microscopy track the positions of MTs to achieve an understanding of the main mechanical properties. This projects aims to use these mechanical properties to create a physical simulation of a cellular environment to compare to observed cellular environments. While mathematical models are still only approximations of the real world, they are significantly easier to perform perturbation analysis on justifying their existence.

Biological Considerations

Before getting trying to construct some kind of model, we need to discuss the underlying biological principles. First we need to investigate some ideas of what cells are. For this we will be considering tissue cells from large organisms. Cells are typically circular or pill shaped objects about $10\mu m$ long and $2\mu m$ in diameter[8]. They live on the timescale of a day, and divide in approximately 10 minutes. This provides the natural timescale of the system to be in seconds, a unit pleasing to our human worldview. We will be considering the cell as filled with some more viscous liquid, known as cytosol. The typical dogma informs us we have macroscopic structures called organelles floating in the cytosol, but for this work I am going to consider the cell to be mostly empty aside from the nucleus. Cells are densely populated by MTs, which are generally localized in the nucleus but are not restricted to that region in all cases.

Floating around in the cytosol is a slurry of subcellular biological building blocks such as proteins and amino acids. Given a proper temperature and concentration of biological material, there are Gibbs' free energy considerations which motivate the self organization of macroscopic structures[1]. This process allows for MTs to form around the cell. It is not well understood how this occurs, but tubulin can become localized at places around the cells, such as during division, to help the cell achieve a certain function. During mitosis, as shown in 1, MTs reach out from the centrosomes to the chromosomes and pull them apart. Thus something must be influencing the growth of these subcellular structures in some way.

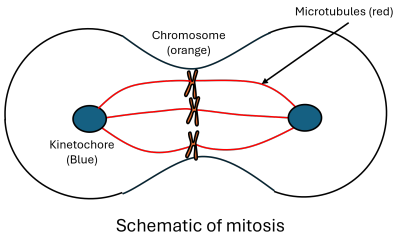


Figure 1: Schematic of Cell Undergoing Mitosis

Zooming in one more time, we want to consider what makes up microtubules themselves. MTs are made of a fundamental sub unit, a heterodimer of α and β-tubulin[2]. They organize off plane and form rod like structures typically $25nm$ in diameter and hollow, but this varies based on organism. This can be visualized with the picture shown below 2. It is know that the β-tubulin end hydrolyzes GTP during polymerization to bind more dimers onto the end. The $\alpha-$tubulin end is significantly more stable being held together by a GTP bond at the N site. Both bonds however are unstable, and both ends of the microtubules are prone to spontaneously shrinking or growing based on the free energy of the bond [1]. This behavior is characterized by a term dubbed dynamic instability. Since these filaments

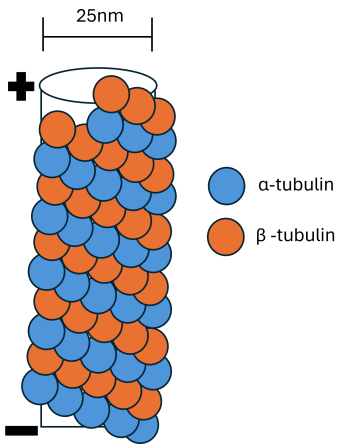


Figure 2: Microtubule Subunits

have two different molecules on the end, they are thought of as polarized, which means the two ends behave differently. This leads the plus end, the β-tubulin end, and the negative end, the α-tubulin end. MTs start through a process called nucleation where multiple dimers start to organize protofilaments which can link up to become MTs. It is important to understand the mechanics of MTs, but they will not be discussed in detail any further aside from how the macroscopic system should behave.

Given observation, we have a few parameters describing MT growth. MTs spontaneously form, through the process known as nucleation. MTs grow at a rate given by the polymerization rate, and shrink given by the depolymerization rate. Lastly MTs switch from polymerization to depolymerization given by a catastrophe frequency, and from depolymerization to polymerization through a rescue frequency. During polymerization and depolymerization tubulin sub-units are added to the end of the MT, and GTP is hydrolyzed to strengthen the bond, with a similar process for depolymerization[2]. It has been found that polymerization is a process that depends on the local amount of free tubulin, while depolymerization does not depend on it. There is some relation between the catastrophe/rescue frequency and the tubulin concentration but it is not well understood. Additionally these properties all hold for the plus end, but are less understood for the minus ends.

Physical Modeling

With our biological considerations out of the way, we can begin to model the system. For this work, I am considering a system shown by 3 in which we have effectively a band of MT elements being added onto the end, and not adding individual elements. This is not an apparent issue however, as this is the view cell biologists have. Microscopes aren't able to continuously image protein interactions, they can only see what appears to be pixel sized additions to MTs.

It has been shown that the polymerization and depolymerization rates are similar [6]

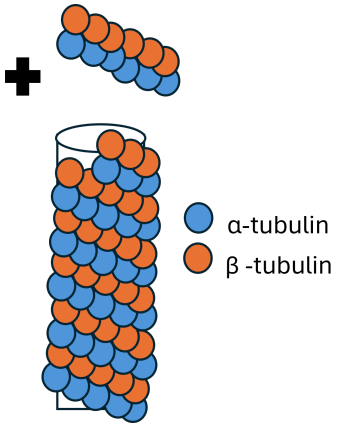


Figure 3: Subunit Visualized

[8], and thus for this model I am considering them to be the same and the MT grows with unit step size addition to the length. This gives us 4 main parameters for our model: nucleation($\frac{1}{s}$), polymerization/depolymerization ($\frac{\mu m}{s}$), catastrophe ($\frac{1}{s}$), and rescue ($\frac{1}{s}$). Inspecting these variables, we have 3 variables which are frequencies and 1 velocity like term. This leads to the conclusion that this system should be treated with a Markov-chain Monte-Carlo type setup [7]. Our system will have some probability for events to occur, given input frequencies, and then evolve using the results of whether or not the events occurred. We can simulate this by considering

$$P(k) = 1 - e^{-k_i \cdot dt},$$

with k_i being the associated frequency to an event[7]. We use the natural timescale of 1 sec for dt in this model, which corresponds to the units provided for the frequencies in the literature. We then see if a random number, picked with np.random.random(), is larger than this event probability, and if it is not we accept the event as happening.

Using the Monte-Carlo framework, we need to now consider what our variable space looks like. Underlying all of this is a physical environment, so a framework must be picked which can be analogous to cellular environments. It has been shown previously that cells can be described to sufficient accuracy using a 2-dimensional system, since two dimensions are typically significantly larger than the third, resulting in a pancake like shape [4]. Additionally we know that MTs must grow on some plane and be able to interact with the local free tubulin, which implies we need some form of grid for the MTs to live on along with the tubulin. Thus a finite element approach could accommodate both of these considerations with little logical overhead. The size of the grid is then picked based on the polymerization rate, so our MTs grow one unit length in one unit time. This restricts our diffusion grid to live on the same scale.

When a MT grows it incorporates the local tubulin dimers into itself, which means our MT grid must be able to take information from the tubulin grid. It is also know this tubulin diffuses, being in an aqueous material, and has been shown to be vital to the model.

This supports the use of finite difference, as this is makes implementing diffusion relatively simple [3].

Many authors consider a mean field approximation for considering small volumes of cellular mass [5]. I instead consider to look at a larger picture of cells, which makes the grid elements much larger than some authors prescribe, but contains useful information. Doing this, different cellular boundaries can be investigates. Cells typically form pill like shapes, but are frequently modeled using squares or circles, which is less physical. I will be implementing a ellipse type boundary parameterization for the cellular environment. One more consideration I take is to assume MTs start from one of two circular centrosomes in the cell, such as the system seen in cells that are undergoing mitosis, to see if this influences how the cells grow. This additionally provides motivation to consider the minus ends of the MTs to be stable[2].

Lastly we have to consider the movement around the cell. We decided MTs move at unit step, but we need them to move intelligently, as the polymerization is dependent on the tubulin. For this I have picked to use a biased random walk. This is mediated where a walker samples the diffusion grid around it, and adds up all of the concentrations, then the probability of walking in a direction is:

$$P(x_i) = \frac{c_i}{\sum_k c_k}.$$

The directions with less tubulin should be chosen less frequently. This choice was largely motivated by the knowledge that cellular environments are largely dependent random walks, as most particles are small enough that they behave to randomly walk unless bound.

Putting all of this together we can construct a complex decision tree as shown below:4

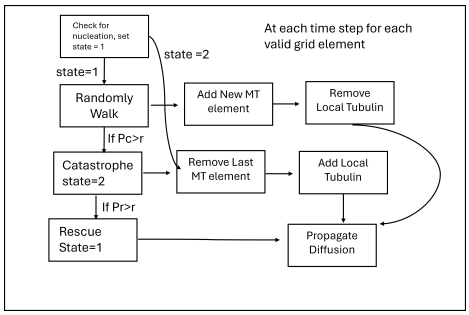


Figure 4: Decision Tree

So for each time step, we see if a grid point is inside of our ellipse. Then if the grid point is inside of the centrosome, we check if it starts nucleating. Then if there are nucleated MTs, they start polymerizing and grow in a biased direction inside of the bounding ellipse. After moving, they check if they start depolymerizing. If they are depolymerizing, they shrink, and then check if they undergo rescue. After all of these processes, we propagate the results to the diffusion grid and change the local tubulin, and run a diffusion step.

Before finding our biological constants, we need to determine our actual model constants. It is not easy to estimate the diffusion constant inside of cells, but there seems to be agreement that cells are significantly more viscous than water, and things diffuse much slower. Using what [5] used for their model, I am estimating it is $1\mu^2/s$. I am using that we are moving at $0.2\mu m/s$ which based on the methodology described above[8] means our grid should be in ticks of $0.2\mu m$. Then we require our cell to have a volume of around $1000\mu m^2$ so we pick our grid to be $50x50$ pixels and draw an ellipse with $a = 30$ and $b = 40$ denoting the semi-minor and semi-major axes respectively in pixels. The kinetochores are draw using a circle of radius 12 pixels and shifted 10 pixels from the center.

Lastly, to enforce the system is dependent on the free tubulin, k_{nuc} and k_{res} have the relation:

$$k_{nuc,t} = k_{nuc} \cdot T_{conc},$$

with T denoting our diffusion grid evaluated at the point in question. The catastrophe is fixed at the value given in the table. This should motivate us to grow when there is free tubulin, and leave our shrinking constant. Then when we grow or shrink, we will lose some amount of tubulin. Since the diffusion grid is a concentration amount for simplicity of modeling, we have to input a starting concentration of tubulin which will be $35\mu M$, which is typical and found in [8]. Then the amount the tubulin grid is changed during a grow or shrink step is a ratio of the input concentration.

Results

Before showing any results, we need to make some choices for our parameters going into our model. Some of the logic for this project was based on [8], so I will try using their parameters first to see what kind of results I arrive at. They are summarized in the following table:

	Frequency (1/s)
Nucleation	0.0005
Catastrophe	0.7
Rescue	0.4
Polymerization	0.2 μm

Putting all of this in, we arrive at a picture like shown in 5:

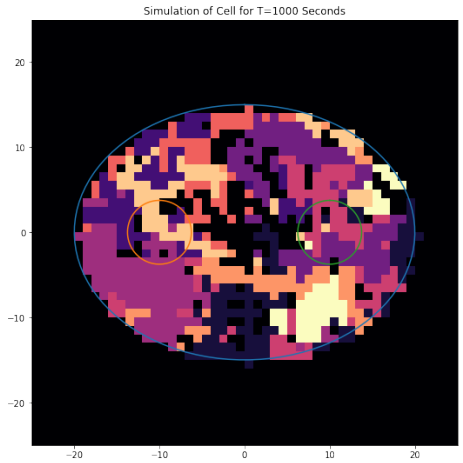


Figure 5: Snapshot of MTs

We are looking at is the model of the cell outlined in blue, the kinetochores in green and orange, and the MTs in various colors according to the magma color scheme. This was chosen to be able to identify different MTs across the experiment. We can also visualize the distribution of tubulin around the cell at the end of the experiment where yellow denoted $T_{conc} = 1$ and blue $T_{conc} = 0$ 6:

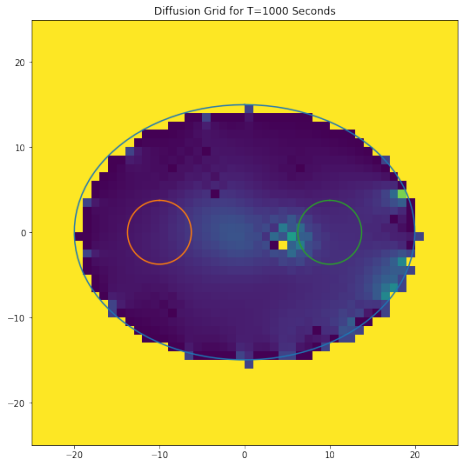
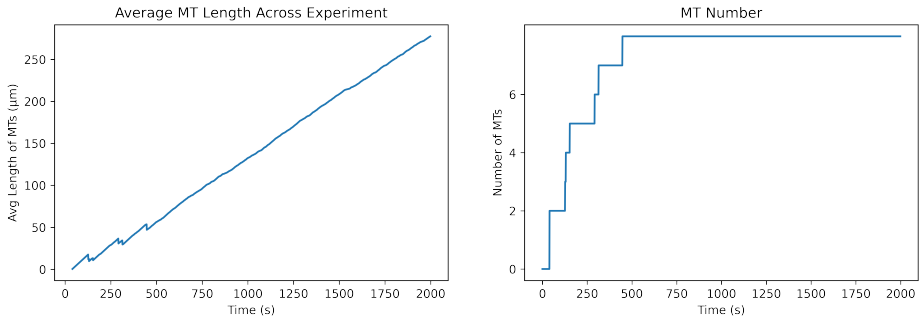


Figure 6: Tubulin Concentration Across Experiment

Now these are interesting to look at, but we can't really extract any quantitative information from them. We can visually tell that MTs have populated the entire cell, matching what is seen in [8] figure 1. We can try to extract a little more information by considering two parameters of interest, the average length across the experiment and the number of MTs. These are important to think about as these are empirical measurements by which we can measure and compare the results of experiments. Below are our comparisons 7a, where we

see for this parameter set the number of MTs shoots up, and then plateaus at some value.



(a) Length of MTs Across Exp.

(b) Number of MTs Across Exp.

Figure 7

Our MT length however keeps growing with no sense of stopping. To interpret these, MTs can only form in the kinetochore, so after a couple have already formed there is no more room for new ones to form. Additionally since the growth is certain to happen if a MT is in the growth state, it doesn't matter if there is a lack of local tubulin, it will continue to grow. This explains why they keep growing ad nauseam. The source for these parameters chose a maximum number of MTs and artificially cut off growth when reaching this number, which explains why this model leads to nonphysical results. This could be fixed by reincorporating the dependence of the polymerization on the local tubulin concentration which should cause MTs to continuously slow down as they get longer.

Now to remedy what we have seen in the previous model, we can window shop some other models for the parameters which may explain the physical nature more sufficiently. Using now [6] we have to adjust our model slightly. Here they specify

$$k_{i,t} = k_{i,1} \cdot T_{conc} + k_{i,2}.$$

where the $k_{i,j}$ are given for each parameter in the model. This is similar to what was used previously, but on the bounds of $0 < T_{conc} < 1$ our parameter is restricted from getting especially small when there is no tubulin. They additionally consider $k_{i,t} > r$, with r being a random number between 0 and 1, instead of the exponential function. These parameters are summarized:

	k_1 $(1/\mu m$ s)	k_2 (1/s)
Nucleation	-0.1	0.2
Catastrophe	-10^{-6}	0.06
Rescue	0.01	0.02
Polymerization	0.035 μ	

Now we can look at some of the same results as the previous model, snapshots at the end of

the experiment and the lengths and number of MTs as a function of the experiment time.

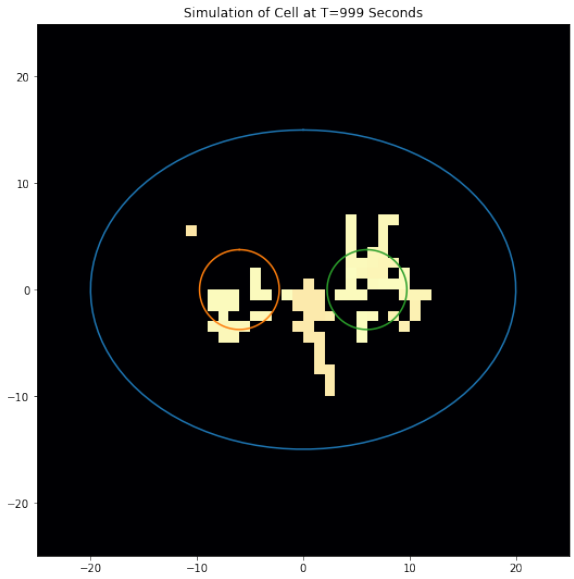
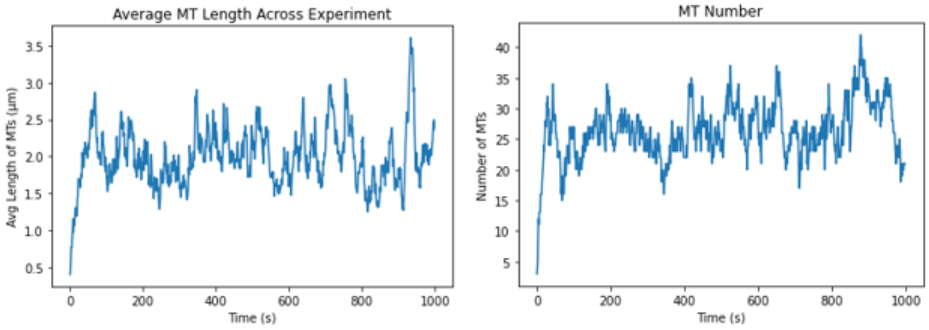


Figure 8: Snapshot of MTs With New Parameters



(a) Length of MTs Across Exp.

(b) Number of MTs Across Exp.

Figure 9

Here 8, 9a, we see a significantly different conclusion to what we had previously seen. Now we have that MT number rises after the experiment, but then appears to approach equilibrium, which is notably not stable. Additionally we see a similar story for the length. This behavior much more closely matches a typical experiment *in vitro*, as we see MTs being bounded and spontaneously falling apart after some amount of time. Additionally the $3\mu m$ length being the average makes it about a quarter of the size of a cell, which is accurate to what we see in cells normally. It seems these parameters create a much more physically realistic scenario than the previous one.

Failures of the Model

There are numerous failure of the model, warranting their own section, so I will discuss them here. One glaring issue from looking at the grids is that there are clearly sharp corners to both the diffusion grid and the cell grid. This was done based on the supposed growth rate provided by [8], put in balance with the length of a supposed cell. This left both grids being inaccurate at the boundaries, along with causing large boundary interference from the finite element diffusion method. This can be dealt with by a more thoughtful implementation of both grids. MTs could instead live in some abstract space and their positions projected down onto the viewing plane and the diffusion grid. Both grids would run into slight errors as the projection would not be perfect, resulting in the end of the MT possibly being halfway between two squares defined by the grid. However this should go away in some large limit.

The second issue I would like to discuss is the polymerization rates. I had thought it was wise to consider the polymerization and depolymerization rates equal, however this does not seem to be the case [6]. Since these were chosen to be equal, there is no restriction on growth when the tubulin is almost fully absorbed by the MTs. At this point the amount lost should start to balance the growth, as there is less material to use to grow.

Something else to consider is the lack of collision between MTs. Based on the MTs biased walk, it was a less probable chance of happening, but there is nothing from preventing MTs from being on the same grid element. This presents a problem when they shrink, they could remove an element from inside of another MT, which may allow for a new one to grow. Aside from the larger issue of this being physically impossible as two classical objects cannot occupy the same space.

Another issue is the random walk implementation. This algorithm picks a random direction by sampling the adjacent spaces. This runs into some issues as this means our walker is only restricted to not return to the square it was just on. This should work by looking in a $3x3$ grid to get an idea about many more squares to provide more information for the walk. Another method to fix this would be to treat each concentration as either $+T_{conc}$ and $-T_{conc}$ depending on direction. This approach could provide a unit vector walking away from areas with low concentration magnitudes, and would be best in a non grid environment.

Lastly I want to discuss the physicality of the MTs. MTs are known to have very large persistence lengths and be rigid. However my model allows them to randomly walk as in 10:

We can see immediately this is an impossible scenario by considering what we know about rigid rods. The work by [5] uses the reference of Landau and Lifschitz 5th book on elasticity to inform free energy arguments requiring rods to not bend, and only allowing energetically acceptable states. This would greatly reduce the issues with the random walk and create a more physically acceptable system. This methodology could also repair issues with the random walk.

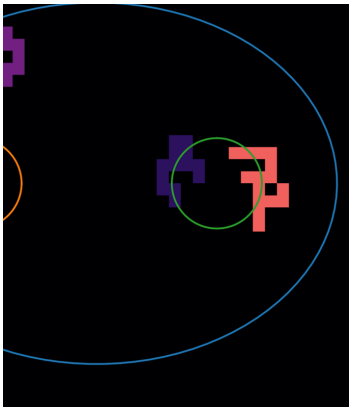


Figure 10: Nonphysical MT

Conclusion

What we can see from these simulations is a way to understand and explain the physical world. In the simulations, different parameters were tried to achieve 2 very different results for some steady state of how the cellular environment may behave. This can be used in conjunction with changing parameters such as the amount of tubulin in the cell, the catastrophe rate, and the polymerization rate to investigate whether certain medications or treatments may result in shorter MTs, which may prevent cells from diving properly. Another application for this prospective modeling would be for understanding forces. Shorter MTs may produce less force due to being smaller in mass, thus giving an idea as to the intercellular forces. There were many ways to improve the model here, but that sentiment can be applied to all models, and any model with any credibility is useful to help interpret and understand the physical world.

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