

DYNAMIC INSTABILITY

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Introduction

The purpose of this work is to model the dynamics of microtubule polymerization and depolymerization in cells. Dynamic instability is the switching between growing (polymerizing) and shrinking (depolymerizing) states in vivo. Microtubules are parts of many important cellular processes including reproduction and gameteo-genesis. The fundamental importance and diversity of the roles of microtubules in cells provokes one to study this polymer of tubulin in an exciting realm where physics and biology converge.

Classically four parameters are used to describe this process, the frequency of switching to a shrinking phase f_{cat} (frequency of catastrophe), the frequency of switching to a growth phase f_{res} (frequency of rescue), the growth velocity of the microtubule while in the growth phase v_+ and the shrinking velocity of the microtubule while in the shrinking phase v_- . Based upon the formulation of Dynamic instability [1]:

$$(1) \quad f_{cat}v_- > f_{res}v_+$$

$$(2) \quad n_o = \frac{v_-v_+}{(f_{cat}v_- - f_{res}v_+)}$$

$$(3) \quad p(n) = \frac{1}{n_o} e^{-\frac{n}{n_o}}$$

If equation (1) is satisfied then the mean length of a large population of microtubules will be n_o and the lengths of the microtubules in a large population will be distributed according to eqn. (3) [figure 4,5].

This formulation of microtubule dynamics though important is over-simplified, it is believed that more than four parameters are needed to truly describe the dynamic of microtubules in vivo [3]. In order to understand how more than four parameters could be needed to describe dynamic instability in vivo, we need to take a direct look at what is really happening in vivo.

One Particle Driven System

In biological cells there are numerous different groups of proteins that bind and affect the microtubules and protofilaments that make up microtubules in many different ways. In this work we will consider proteins to only have certain simplified interactions with a 1-Dimensional microtubule. Initially, we consider the case of a single type of motor protein that moves with directional bias toward one end of the chemically polar microtubule (toward the plus end, for example).

In biological systems, a multitude motor protein molecules are present and they collide with each other while moving on the microtubule. In addition, motors can bind to and unbind from the one-dimensional "track" formed by the microtubule (MT). We assume a simple exclusion model, where each MT binding site (indexed by integer i) only one motor can bind. Motors are injected at the left side of the system (site zero) at rate α and are removed from the right side of the system (site N) at rate β [Figure 2].

This model was studied in ref. [2], assuming that the MT track has a fixed length, so dynamic instability was neglected. Their starting equation for the motors is:

$$(4) \quad \frac{\partial}{\partial t} \hat{n}_i(t) = \hat{n}_{i-1}(t)[1 - \hat{n}_i(t)] - \hat{n}_i(t)[1 - \hat{n}_{i+1}] + \omega_A[1 - \hat{n}_i(t)] - \omega_D \hat{n}_i(t)$$

Protein Movements in Totally asymmetric simple exclusion process with Langmuir kinetics [2]

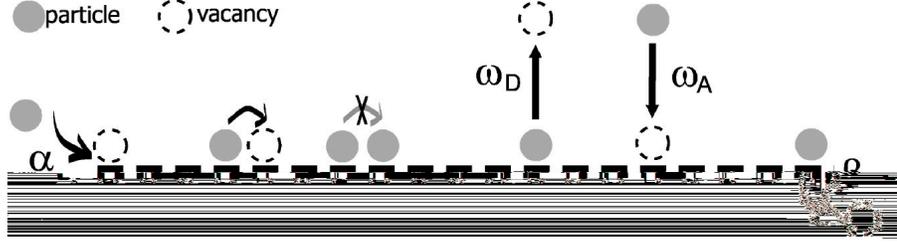


FIGURE 1. Protein movements in which a particular driven protein can move only with left to right directional bias. Here α denotes an on rate at the end_- (left end) where β denotes and off rate at the end_+ (right end). ω_A denotes an attachment rate while ω_D denotes a detachment rate. The microtubule is split into discrete sites $i = 0, 1, 2, \dots$

There are several ways to analyze the model presented above in Figure 1. as studied in [2]. Among which, a stochastic network theory and a Fock space formulation of the stochastic dynamics were explored. Of the following the Fock space formulation is worth mentioning.

In equation (4) $\hat{n}_i(t)$ is a variable that takes on a value of zero if there is no particle in the site denoted by the subscript i or a value of one if there is a particle in the site denoted by i . This equation is suitable for the sites in the bulk but not for the sites on the ends, here I am just focused on the bulk for simplicity.

In-order to transform this equation to solve if we consider the steady state where $\frac{\partial \hat{n}_i(t)}{\partial t} = 0$ and average. Therefore:

$$(5) \quad 0 = \frac{\partial}{\partial t} \langle \hat{n}_i(t) \rangle = \langle \hat{n}_{i-1}(t) \rangle [1 - \langle \hat{n}_i(t) \rangle] - \langle \hat{n}_i(t) \rangle [1 - \langle \hat{n}_{i+1}(t) \rangle] + \omega_A [1 - \langle \hat{n}_i(t) \rangle] - \omega_D \langle \hat{n}_i(t) \rangle$$

Next we use a mean-field approximation:

$$(6) \quad \langle \hat{n}_i(t) \hat{n}_{i+1}(t) \rangle = \langle \hat{n}_i(t) \rangle \langle \hat{n}_{i+1}(t) \rangle$$

And then we take:

$$(7) \quad \rho_i = \langle \hat{n}_i \rangle$$

After the above we look at the equations at a continuum limit as (the number of sites on the microtubule) $N \gg 1$. Which leads us to the the non-linear partial differential equation:

$$(8) \quad \frac{\epsilon}{2} \frac{\partial^2 \rho(x)}{\partial^2 x} + (2\rho(x) - 1) \frac{\partial \rho(x)}{\partial x} + \Omega_A(1 - \rho(x)) - \Omega_D \rho(x) = 0$$

$$(9) \quad \rho(0) = \alpha$$

$$(10) \quad \rho(1) = 1 - \beta$$

The results above for the one particle drive system were derived in [2]. Here $0 < x < 1$ and the boundary conditions of this system are seen by equation (9) and equation (10). These results are interesting to mention because the authors of the paper were able to find certain analytical approximations to the system that match simulation of this scenario. Here $\Omega_A = \omega_A N$, $\Omega_D = \omega_D N$ and $\epsilon = L/N$. As part of my project, I reproduced the simulations done in reference [2] and compared the simulation results to the analytic approximations in the paper. My results agree well with the previously published work, which was a useful check that the simulation framework is correct.

Two Particle Driven System

In biological systems, more than one type of motor may be present. Therefore I studied the generalization of the one-particle system and extended it to include two types of particles with different parameters. The model is a straightforward extension of that discussed above and shown in figure 2 to include two particle types. In the two particle system each particle has its own set of characteristic parameters.

	Particle 1	Particle 2
Velocity	V^A	V^B
α	α_1	α_2
β	β_1	β_2
ω_A	R_A	R_B
ω_D	S_A	S_B
<i>FockVector</i>	$\hat{n}_i(t)$	$\hat{m}_i(t)$

The same general idea that was developed by [2] in the previous section as extended here. First we will define our Fock space vectors for each particle.

$$\begin{aligned} \frac{\partial \hat{n}_i(t)}{\partial t} = & V^A \hat{n}_{i-1}(t)[1 - \hat{m}_i(t) - \hat{n}_i(t)] - V^A \hat{n}_i(t)[1 - \hat{n}_{i+1}(t) - \hat{m}_{i+1}(t)] \\ & + R_A(1 - \hat{n}_i(t) - \hat{m}_i(t)) - S_A \hat{n}_i(t) \end{aligned} \quad (11)$$

$$\begin{aligned} \frac{\partial \hat{m}_i(t)}{\partial t} = & V^B \hat{m}_{i-1}(t)[1 - \hat{n}_i(t) - \hat{m}_i(t)] - V^B \hat{m}_i(t)[1 - \hat{m}_{i+1}(t) - \hat{n}_{i+1}(t)] \\ & + R_B(1 - \hat{m}_i(t) - \hat{n}_i(t)) - S_B \hat{m}_i(t) \end{aligned} \quad (12)$$

As before the vectors \hat{m}_i and \hat{n}_i can only take on values of zero or one. For vector \hat{n}_i if particle one is occupying discrete site i then the vector at i has a value of one. For \hat{m}_i , if particle two is occupying site i , then vector \hat{m}_i will take on a value of one at i .

The next step as outlined before is to take the system of equations into consideration when $\frac{\partial \hat{n}_i(t)}{\partial t} = 0$ and average while taking into consideration a Mean-field approximation. In a mean-field approximation we ignore correlations.

$$\begin{aligned} \langle \hat{n}_{i-1}(t) \hat{n}_i(t) \rangle &= \langle \hat{n}_{i-1}(t) \rangle \langle \hat{n}_i(t) \rangle \\ \langle \hat{n}_{i-1}(t) \hat{m}_i(t) \rangle &= \langle \hat{n}_{i-1}(t) \rangle \langle \hat{m}_i(t) \rangle \\ \langle \hat{n}_{i+1}(t) \hat{n}_i(t) \rangle &= \langle \hat{n}_{i+1}(t) \rangle \langle \hat{n}_i(t) \rangle \\ \langle \hat{m}_{i-1}(t) \hat{n}_i(t) \rangle &= \langle \hat{m}_{i-1}(t) \rangle \langle \hat{n}_i(t) \rangle \\ \langle \hat{m}_{i-1}(t) \hat{m}_i(t) \rangle &= \langle \hat{m}_{i-1}(t) \rangle \langle \hat{m}_i(t) \rangle \\ \langle \hat{m}_{i+1}(t) \hat{n}_i(t) \rangle &= \langle \hat{m}_{i+1}(t) \rangle \langle \hat{n}_i(t) \rangle \end{aligned} \quad (13)$$

We also take $\rho_i^n = \langle \hat{n}_i(t) \rangle$ and $\rho_i^m = \langle \hat{m}_i(t) \rangle$.

After this we find the following system of equations:

$$(14) \quad 0 = V^A[\rho_{i-1}^n - \rho_{i-1}^n \rho_i^m - \rho_i^n \rho_{i-1}^n] - V^A[\rho_i^n - \rho_{i+1}^n \rho_i^n - \rho_i^n \rho_{i+1}^m] + R_A(1 - \rho_i^n - \rho_i^m) - S_A \rho_i^n$$

$$(15) \quad 0 = V^B[\rho_{i-1}^m - \rho_{i-1}^m \rho_i^n - \rho_i^m \rho_{i-1}^m] - V^B[\rho_i^m - \rho_{i+1}^m \rho_i^m - \rho_i^m \rho_{i+1}^n] + R_B(1 - \rho_i^m - \rho_i^n) - S_B \rho_i^m$$

Now considering a continuum limit, $\epsilon = L/N$ where $L = 1$. For large microtubules with a large number of sites $N \gg 1$, and $1 \gg \epsilon$. Here $\rho^n(x) \equiv \rho_i^n$ and $\rho^m(x) \equiv \rho_i^m$. Therefore:

$$(16) \quad \begin{aligned} \rho^n(x \pm \epsilon) &= \rho^n(x) \pm \epsilon \frac{\partial \rho^n(x)}{\partial x} + \frac{\epsilon^2}{2} \frac{\partial^2 \rho^n(x)}{\partial^2 x} + O(\epsilon^3) \\ \rho^m(x \pm \epsilon) &= \rho^m(x) \pm \epsilon \frac{\partial \rho^m(x)}{\partial x} + \frac{\epsilon^2}{2} \frac{\partial^2 \rho^m(x)}{\partial^2 x} + O(\epsilon^3) \end{aligned}$$

After applying the Taylor expansions to our system of equations and going to the continuum limit we find the equations to be:

$$(17) \quad 0 = V^A(1 - \rho^n(x)) \frac{\partial \rho^n(x)}{\partial x} + V^A \frac{\partial}{\partial x}(\rho^n(x) \rho^m(x)) + \frac{\epsilon V^A}{2} [(1 - \rho^m(x)) \frac{\partial^2 \rho^n(x)}{\partial^2 x} + \rho^n(x) \frac{\partial^2 \rho^m(x)}{\partial^2 x}] + R_A(1 - \rho^n(x) - \rho^m(x)) - S_A(\rho^n(x))$$

$$(18) \quad 0 = V^B(1 - \rho^m(x)) \frac{\partial \rho^m(x)}{\partial x} + V^B \frac{\partial}{\partial x}(\rho^m(x) \rho^n(x)) + \frac{\epsilon V^B}{2} [(1 - \rho^n(x)) \frac{\partial^2 \rho^m(x)}{\partial^2 x} + \rho^m(x) \frac{\partial^2 \rho^n(x)}{\partial^2 x}] + R_B(1 - \rho^m(x) - \rho^n(x)) - S_B(\rho^m(x))$$

If one were to want to analyze the system of two particles they would want to start from these equations. There are potentially many ways to analyze these equations. In particular we were interested in the homogeneous steady state of the system where there are no gradients in the bulk. Here it can be shown that:

$$(19) \quad \rho^n = \frac{R_A}{R_A + S_A + \frac{S_A R_B}{S_B}}$$

$$(20) \quad \rho^m = \frac{R_B}{R_B + S_B + \frac{S_B R_A}{S_A}}$$

The next step was to take a look at the particle interactions, in-order to do this I solved eqn. (8) in-order to get the homogeneous steady state of the system of one particle and found our analog with our new parameters:

$$(21) \quad \rho^1 = \frac{R}{R + S}$$

Where R and S denote binding and unbinding constants of one particle. To find a one particle density equivalent to a two particle system I found the following equations had to be solved.

$$(22) \quad \rho^1 = \rho^n + \rho^m$$

$$(23) \quad \alpha = \alpha_1 + \alpha_2$$

$$(24) \quad (1 - \beta) = (1 - \beta_1) + (1 - \beta_2)$$

The motivation behind equation (23) and equation (24) was that both boundary conditions had to match up unless of coarse $\alpha > 1$ or $1 - \beta > 1$ which are cases not looked at here. During this study it was seen through Monte Carlo simulations that when proteins where nearly the same or near homogeneity they behaved in a manner that a one particle system could easily mimic. Though when they were off homogeneity the proteins began to interact with each other in curious ways which could possibly be explained by the eqns 17 and 18.

Figure 4. is an interesting figure and it motivates the importance for studying the protein density partial differential equations. Here in Figure 4. for the curve of ρ^n near zero it is apparent that the density is above the concentration it should be. Also near the end of the x-axis, the density goes down. Perhaps the particles of type one get stuck at the begining and cannot make it to the end. There are many interesting phonenmena that have been observed in the computer simulation.

Now that we have investigated density profiles of a microtubule with two driven particles, we will move on to a microtubule that has two diffusive particles moving across it which affect the MT length in various ways.

Dynamic Instability Mediated by Two Proteins

Each particle has 6 parameters:

Two Protein Movements in Totally asymmetric simple exclusion process with Langmuir kinetics (near Homogeneity)

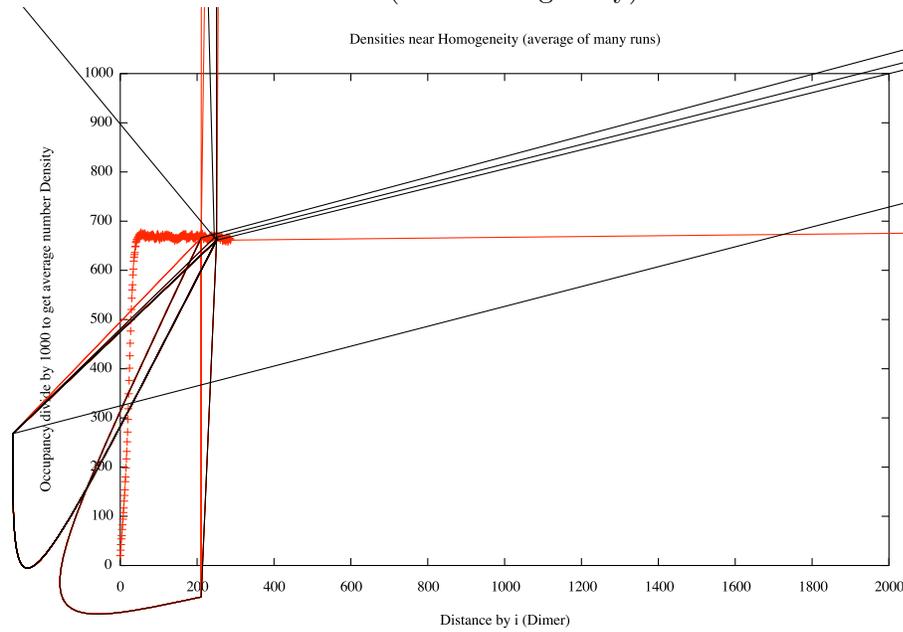


FIGURE 2. Here ρ^n is in blue and ρ^m is purple ρ^1 is in green and the addition of the two particle densities ρ^n and ρ^m is in red. Here we see that if the particles were the same or near the same that the densities should add up exactly to ρ^1 or $\rho^m + \rho^n = \rho^1$ which is seen to be true.

Two Protein Movements in Totally asymmetric simple exclusion process with Langmuir kinetics (off Homogeneity)

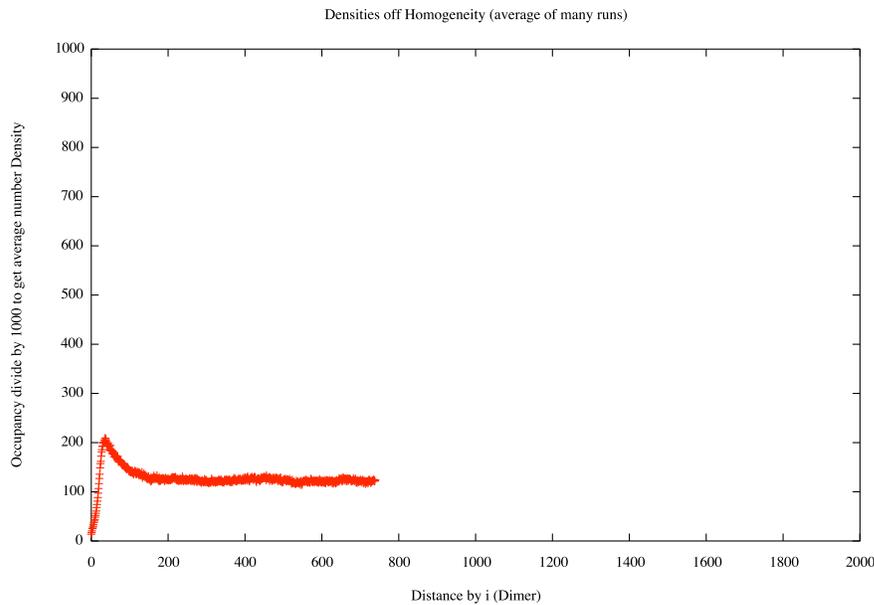


FIGURE 3. Here ρ^n is in red and ρ^m is green ρ^1 is in purple and the addition of the two particle densities ρ^n and ρ^m is in blue. Here we see that if the particles are not near the same particle they do not add up ($\rho^m + \rho^n \neq \rho^1$).

	Particle 1	Particle 2
Depolymerization	$v_- * 5$	$v_- * 220$
Polymerization	$v_+ * 5$	$v_+ * 1$
α	α_1	α_2
β	β_1	β_2
ω_A	R_A	R_B
ω_D	S_A	S_B

Here each particle is assumed to be diffusive based upon a certain diffusion constant for each particle. I was able to come up with a model that mimics protein dynamics and their interactions with the microtubule given f_{cat} , f_{res} , v_- , v_+ . I tried to correctly model the conditions in [3] but I found that it is hard to predict what average length n_o that will come out of the simulation also it is almost impossible to determine v_- and v_+ with-out further analysis and a deeper understanding of the experimental data found in [3]. I will move on to the data from my Monte Carlo simulation that mimics the physical circumstance so that I can prove first that the model is working.

Computer Simulations and Future Considerations

From the computer simulations one can see that the data fits the expected distribution functions and the traces are like those seen in many other simulations and observations of Dynamic Instability. The next step in this project is to find a way to better interpret the data from [3] which we are trying to model. In specific, one needs to figure out how exactly protein crowding from depolymerization affects the shrinking rates of the microtubules elicited by the different proteins. If one is able to do this, a better guess of v_- can be had.

Other considerations that could be implemented into the simulation are ways of determining which phase the microtubules are in by determining what state the tubulin dimers are in GTP+ or GDP-. This would be interesting so that the model could be self inclusive but as far I know the mechanism for this is only slightly understood.

Protein interactions need to be better understood at the end of the microtubule when the microtubules are in the shrinking states. This could be done by better understanding the results from [3].

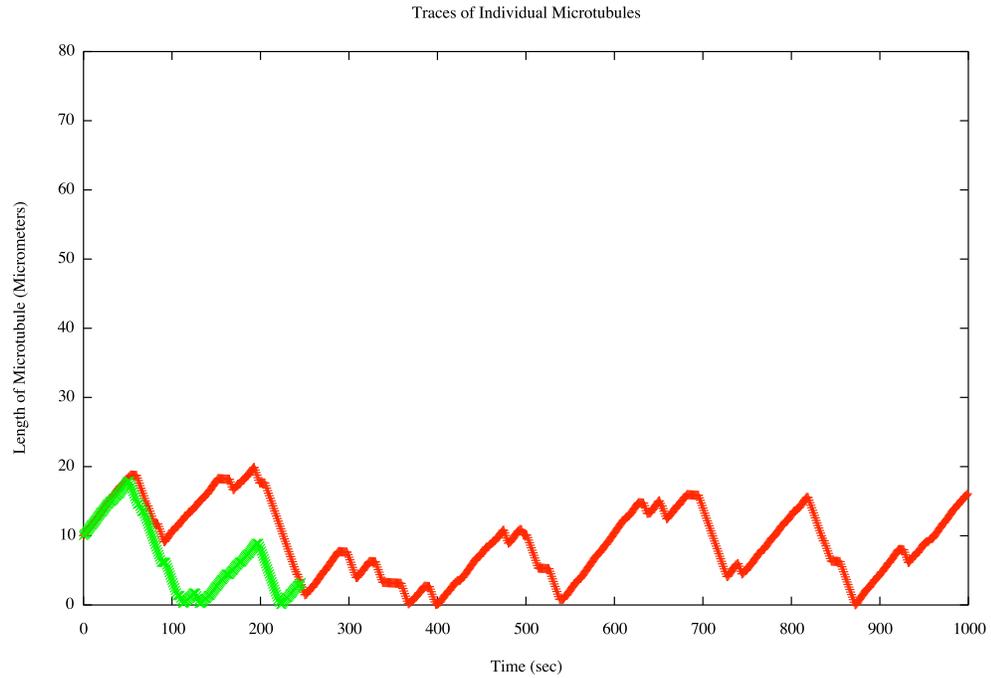


FIGURE 4. Here each traces is a run of a computer simulation with biological parameters for each protein.

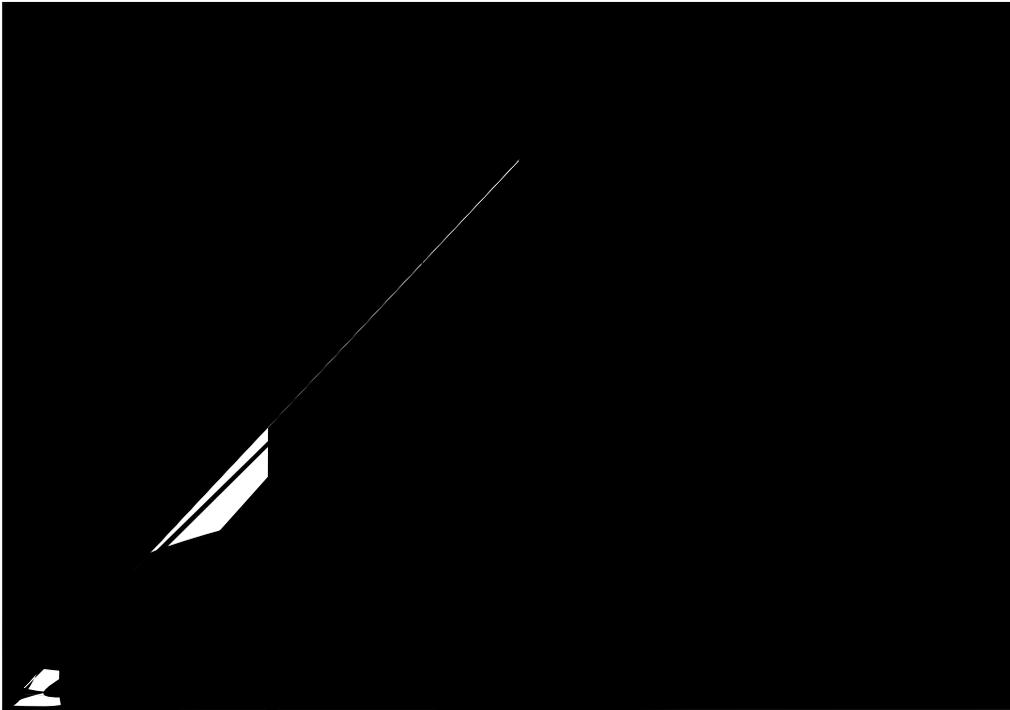


FIGURE 5. Here is the distribution of the lengths of all the microtubules (1000) after a time of 500 seconds for 500 seconds, notice that the green curve is an exponential fit like [1] predicts and the average here is 23.395 the factor of 500,000 is due to the fact that I did not normalize the distribution to one.

- [1] Howard, Jonathon. *Mechanics of Motor Proteins and the Cytoskeleton*. Sinauer Associates, Inc., 2001. Print.
- [2] Parmeggiani, A., T. Franosch, and E. Frey. "Totally asymmetric simple exclusion process with Langmuir kinetics." *Phys. Rev. E* 70 (2004): 046101. Web.
- [3] Kazuhisa Kinoshita, et al. "Dynamics Using Purified Components Reconstitution of Physiological Microtubule." *Science* 294, 1340 (2001);