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# Analysis of Movement of a 3T3 Cell with a Lamellipodium on a Substrate with Variable Rigidity

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# **Analysis of Movement of a 3T3 Cell with a Lamellipodium on a Substrate with Variable Rigidity**

By

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A Thesis

Presented to the Graduate Research Committee of Lehigh University

In Candidacy for the Degree of

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Mechanical Engineering

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Alan Wopperer

## THESIS SIGNATURE SHEET

This thesis is accepted and approved in partial fulfillment of the requirements for the Master of Science.

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Date

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## **Abstract**

Live cells move in the body in response to physiological and mechanical stimuli. Cells move using lamellipodium which extend beyond the leading edge of the cell. This lamellipodium is part of the cytoskeleton of the cell which pulls the cell forward in cell migration. It is observed that cells will move directionally depending on the stiffness of the substrate the cell comes into contact with. It is hypothesized that cells probe their environment to test the stiffness of their substrate. As a cell comes into contact with a substrate, the resulting force is dependent on how rigid or soft the substrate is, which impacts cell deformation as has been observed in this simulation. If the cell probes a soft substrate, the resultant force is greater causing a larger deformation. If the substrate is stiffer, the resultant forces is less thus causing less deformation. These resultant forces are important because the surface integral of these forces is the strain energy of the cell. This is investigated using finite element analysis of the tensegrity model of the cell where the cell is modeled as a tensed cable network, which simulates the deformability of a live cell's cytoskeleton. The tensegrity approach is used to understand how the internal strain energy of the tensed cable network is affected by the substrate stiffness. Each member of this model carries either a tension or compression load to give the model a stable shape in space. This model reacts to various substrate stiffness values and prestress values, but it is seen that prestress has very little effect on the model's internal strain energy while substrate stiffness has a much greater effect on internal strain energy. Knowing that substrate stiffness constitutes a larger role on internal strain energy of a cell, models are created to observe what has been seen in lab experiments. As substrate stiffness increases, internal strain energy of the cell model decreases which has a direct effect on cell movement. It is hypothesized that live

cells prefer to stay in a lower strain energy state thus cells will move to the area of a substrate that will cause a lower strain energy. More rigid substrates will cause a lower strain energy compared to soft substrates therefore cells will move towards stiff substrates.

The purpose of this thesis is to investigate the internal strain energy of a 3T3 cell with a lamellipodium using a cell model based on the tensegrity approach. This model is connected to a substrate with varying stiffness values. The tensegrity approach is used on this model the same way it was used on a cell without a lamellipodium in previous computational experiments where a cell without a lamellipodium was attached to a substrate of varying stiffness. Studies found the strong relationship between substrate stiffness and internal strain energy. The computational results from this investigation are consistent with the results seen from lab experiments.

## 1. Introduction

Cell movement is dependent on many external factors, but for the purpose of this paper, the interaction between a cell and its substrate is investigated. It is hypothesized that cells have a process by which they choose the direction of their movement. Cells exert forces on substrates and learn from the resulting deformation of the substrate to determine which direction to move [1]. Cells probe and pull on the substrate to test its elasticity [2]. The cell transmits forces to the substrate through myosin-based contractility and transcellular adhesions [2]. Forces are generated by an interaction between actin and myosin microfilaments [2]. Forces from these cytoskeletal elements applied to the substrate are called traction forces which is a force per cross sectional area acting on a surface by the deformed cell. The cell responds to the rigidity of the substrate by adjusting its shape and adhesions [2]. The traction forces are found to be strong at the leading edge, lamellipodia, and sometimes present at the trailing edge [1]. It is seen that on a stiff substrate the traction forces were much stronger than traction forces on soft substrates [1]. How the traction forces affect the direction of a cell's movement and the pattern of traction forces in a cell on a substrate are shown in Figure 1. (Elsevier publishing company granted permission to use this image\*)

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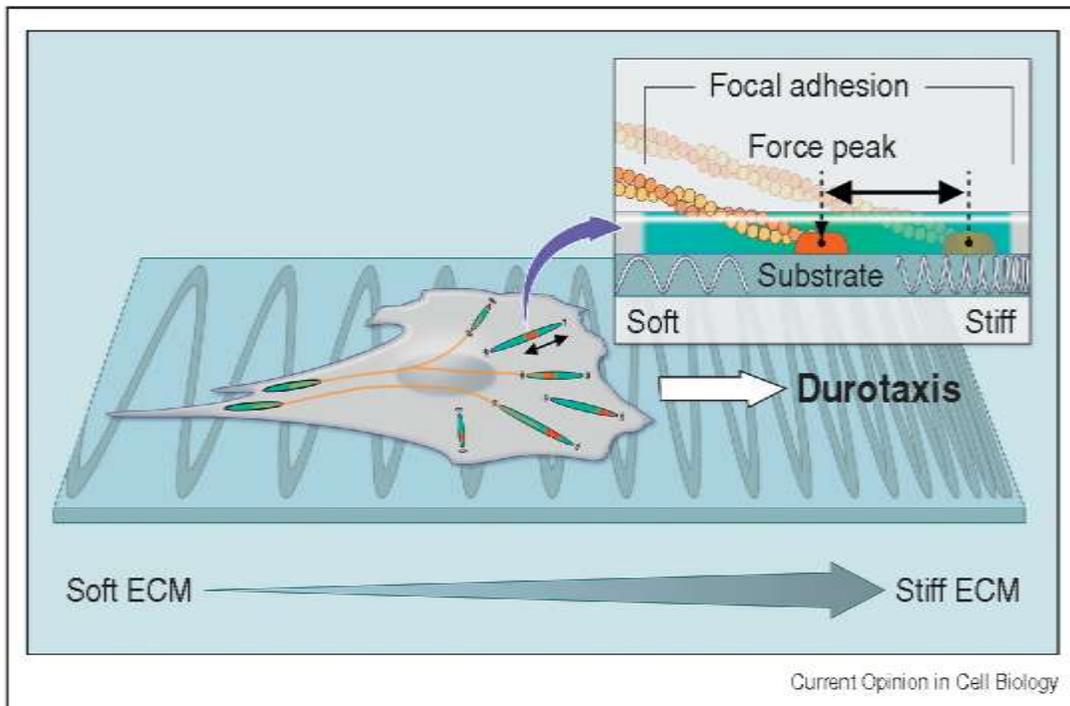


Figure 1. Nanoscale depiction of traction forces that guide the direction of cell movement towards a stiffer substrate. [ 3]

The traction forces are stronger on the leading edge and less prevalent at the trailing edge (Figure 1). These traction forces represent a “probing” process that the cell does to test the rigidity of its environment. This probing action on the substrate causes a force imbalance in the traction forces which requires the cell to retract on the soft side and extend on the stiff side of the substrate to achieve a new equilibrium as shown Figure 1 [4]. The process by which cells transduce a mechanical force into a biomechanical signal is called “mechanotransduction” [1]. Studies show that cells cultured on substrates with identical chemical makeup but different substrate stiffness values exhibit different shape and mobility [1]. The mechanism that dictates rigidity-guided cell movement is called “durotaxis” [1]. It is observed that the traction forces generated by cells on hard substrates was much higher than the traction forces generated by cells on softer substrates [1]. This happens because a cell probes the substrate to test the

substrate's rigidity. When a cell probes any substrate, the lamellipodia expands that will lead the cell to move towards the stiffer part of the substrate [1]. When a cell encounters a soft substrate, the lamellipodia retracts causing the cell to change directions [1]. Cell movement and cell growth is influenced by the mechanical environment because cells will move and grow along the substrates with the highest stiffness [5]. It is observed that cells are able to sense substrate rigidity because astrocytes, cells of the nervous system, exhibited small and round shapes on soft substrates and high cell spreading on hard substrates [6]. The topography of the substrate also plays a significant role in cell elongation. Lab results show topography of the substrate plays an important role in cell elongation [7]. This experiment studied how cells will react on a substrate with a corrugated surface [7]. Cell spreading is greatly affected by the depth of the corrugated surface in that the deeper the grating of the surface the more the cell elongated [7]. While this has all been observed in a lab setting, it is important to model the cell behavior in order to map a cell's movement given variable substrate stiffness values.

The tensegrity approach is used to model the cytoskeleton of a cell. Since the cytoskeleton has a force balance stability, the tensegrity model can simulate those tension and compressive forces acting on elements of the cytoskeleton. This is done by modeling an interconnected network of compression and tension elements which will represent the microfilaments and microtubules, two main elements of the cytoskeleton responsible for mechanical force balance and stability [8]. The tensegrity model can explain the cell movement and shape change because force equilibrium is maintained through the actin filaments that are under tension and microtubules that are under compression [9]. A schematic of the tensegrity

model of a cytoskeleton [10] is shown in Figure 2 (Elsevier publishing company granted permission to use this image<sup>†</sup>):

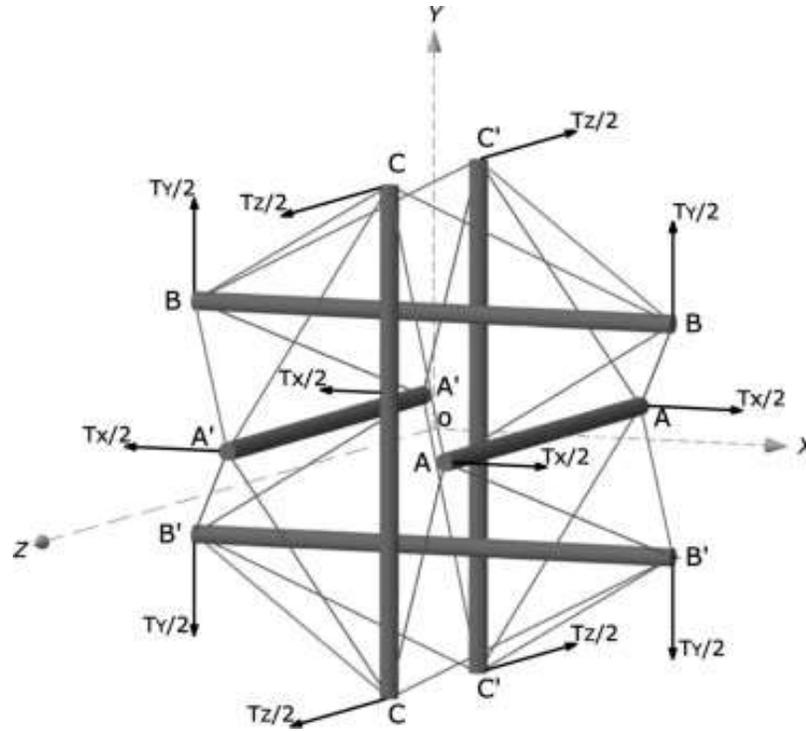


Figure 2. Schematic of the tensegrity cytoskeleton model. [11]

The microfilaments are modeled by cable elements due to their similarity to cable mechanical behavior [12]. Microfilaments respond to extensions like a stiff spring, have a small bending stiffness, and their cross sectional area is much smaller than their length [12]. Due to their mechanical characteristics, microfilaments are prone to buckling under a compressive load [12]. The microfilaments are modeled as cables that will only support a tensile force [12]. In Figure 2, the thin cables represent the cable network of the microfilaments and the thick beams represent the internal support elements provided by microtubules. Microtubules are hypothesized to be rigid beams that are unable to be extended compared to the highly

<sup>†</sup> Refer to Appendix 1 for proof of permission.

extendable cable network of microfilaments [13]. Compressive forces are applied to the microtubule elements that will resist extracellular forces that are seen in cell spreading, though it is unclear which mechanical forces influence which specific molecular responses [13]. The tensegrity model illustrates a cell's behavior on a surface with varying stiffness values. When a cell attaches to a substrate, the cell's geometry changes in the same fashion as applying external forces to the cell to create deformation [8]. Mechanotransduction is the reaction of the cell to external forces and the reaction of the cell to the change of surface stiffness which will explain molecular mechanisms such as cell spreading and alignment [8]. To understand the phenomenon of cell spreading, the total internal strain energy of the cell must be found since strain energy is the surface integral of the forces between the cell and the substrate. One study where the cytoskeleton was modeled as a prestressed cable network was able to predict the elastic properties and forces on the cell when the system was mechanically deformed [12]. However, the model failed to describe the cell response to twisting, but was able to offer insight to the tension mechanisms of the microfilaments that provide stability of cell shape [12]. A Monte Carlo method where the strain energy is to be minimized was used to create a tensegrity model of irregular structures [14]. This study was able to accurately predict cell shape and cell response which proved that strain energy is an important component to understanding cell movement. With the knowledge of these two [12 14] computational models, the cytoskeleton of a cell is modeled using the tensegrity approach and strain energy is calculated for the model to describe the cell's movement depending on the substrate stiffness value.

## 2. Methods

### 2.1 Tensegrity Model

The tensegrity model consists of a network of interconnected members carrying tension or compression to provide a mechanical force balance environment, stable volume, and shape in space [4]. The tensegrity approach can be used to find the internal strain energy of the cell model which can help illustrate cell motility and cell shape change. Cell motility and cell shape are found since the tensegrity approach is based on mechanical integrity being maintained and equilibrium is maintained through actin filaments under tension and microtubules under compression [15]. The tensegrity model is based on the set of members under compression embedded inside a net of tension members that separates the compressed members [4].

Tension and compression members carry “prestress” that is initial stress so that the model can support a load [4]. Figure 3 shows the tensegrity model of the cytoskeleton of a 3T3 cell, its nucleus, and lamellipodia. The model consists of 49 elements and is based on a 3T3 cell with a lamellipodia. 3T3 cells are mouse fibroblast cells [15]. In this model, a spring element is added between node 46 and 49 that will simulate the connection of a cell to the substrate. The spring element’s stiffness vary to simulate substrate stiffness so that strain energy of the model can be found at each stiffness. Voloshin’s study found the strain energy of the cytoskeleton of a 3T3 cell as a function of substrate stiffness [4]. Current study aims to find the strain energy of a cytoskeleton, nucleus, and lamellipodium of a 3T3 cell as a function of substrate stiffness. Node 3 is located at the origin of the coordinate system and is fixed. All the nodes can move in any direction to simulate cell spreading but cannot rotate to model the movement of a living cell

[4]. The length of the cables and struts of this model are able to change due to the applied prestress and deformation [4].

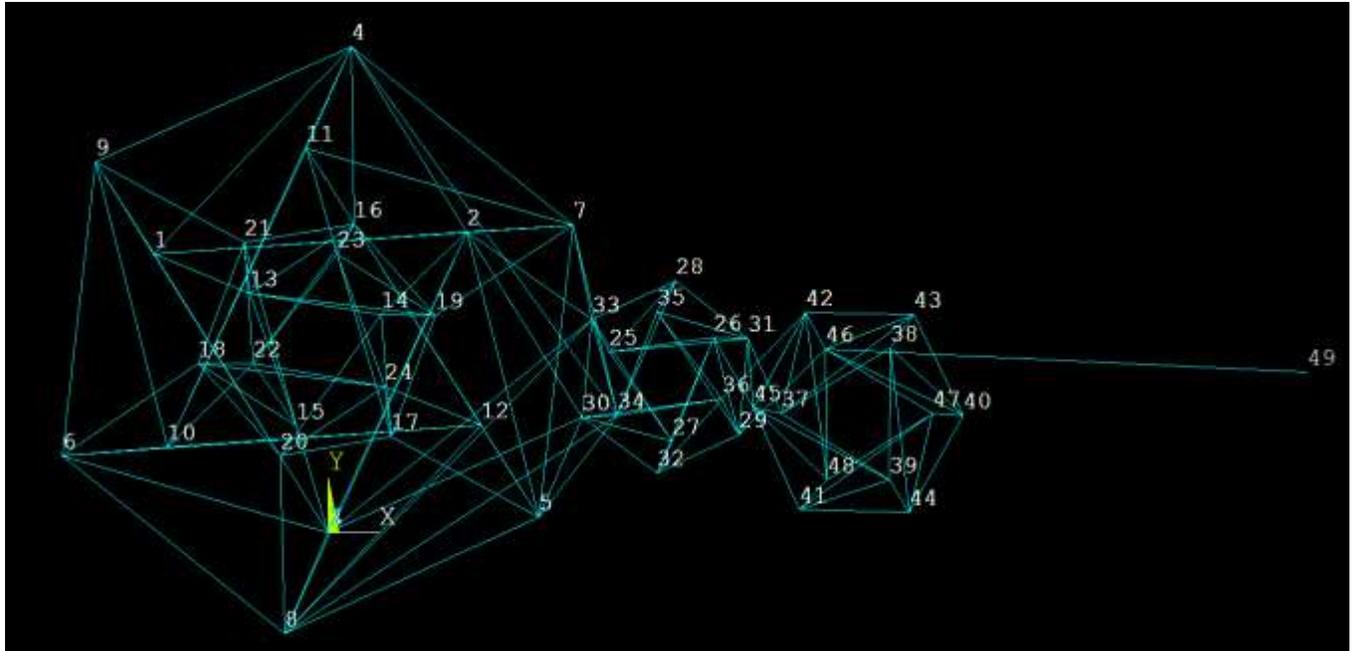


Figure 3. Tensegrity model with spring element (between node 46 and 49) which represents substrate stiffness.

Three structures are represented in Figure 3. The furthest left and largest structure is the tensegrity model for the cytoskeleton and nucleus of a 3T3 cell. The two smaller structures to the right represent the lamellipodium of the 3T3 cell. The spring element which is used to simulate substrate stiffness is the element that connects node 46 and node 49.

## 2.2 Material Properties of the Elements

This model is created in ANSYS APDL [4] using Link180, Beam188, and Combin14 to model the microtubules, microfilaments, and substrate stiffness. Link180 modeled the cable system, Beam188 modeled the strut system, and Combin14 represented the substrate stiffness. The material properties of the microtubules and microfilaments are estimated from a study where

their rigidity was found [16] even though the exact properties of microfilaments and microtubules is not known. The Young's modulus and cross sectional area of the microtubules are 1.2GPa and  $45.2 \cdot 10^{-17} \text{ m}^2$  [16]. The Young's Modulus and cross sectional area of the microfilaments are 2.6 GPa and  $45.2 \cdot 10^{-17} \text{ m}^2$  [16]. It was hypothesized earlier that microtubules cannot be extended [13], but for the purpose of this investigation the microtubules and microfilaments are assumed to be completely elastic. Poisson's ratio for microtubules and microfilaments is used as 0.3 [16]. The length of the microtubule elements were found to be  $2.399 \cdot 10^{-5} \text{ m}$  [12]. Table 1 shows the material properties of the microtubules, microfilaments, and substrate.

*Table 1. Properties of Microtubules, Microfilaments, and Substrate*

	Microtubules	Microfilaments	Substrate
ANSYS APDL Element [3]	Link180	Beam188	Combin14
Cross Sectional Area [m <sup>2</sup> ]	$45.2 \cdot 10^{-17}$	$45.2 \cdot 10^{-17}$	N/A
Length [m]	$2.399 \cdot 10^{-5}$	$1.467 \cdot 10^{-5}$	$.2259 \cdot 10^{-5}$
Young's Modulus [Pa]	$1.2 \cdot 10^9$	$2.6 \cdot 10^9$	N/A
$\nu$	.3	.3	N/A
Stiffness Range [N/m]	N/A	N/A	$10^{-3} - 1000$

Equations 1, 2, 3, and 4 are used for this investigation and found the displacement (d), change in length for  $i^{\text{th}}$  microfilament or microtubule ( $e_i$ ), stiffness for the  $i^{\text{th}}$  microfilament or microtubule ( $k_i$ ), and total strain energy ( $U_T$ ) [17]. Displacement is found using Equation 1 where  $k$  is the elemental stiffness,  $d$  is the nodal displacement in the 3-D plane, and  $r$  is nodal loads in the 3-D plane [17]. Equation 1 is in a matrix form in order to find the solution for each node in the x,y, and z plane for each element.

$$[k]\{d\} = \{r\} \quad (1)$$

Change in length for the microfilaments and microtubules is found using Equation 2 where  $e$  is the change in length,  $F$  is the axial force,  $L$  is the initial length,  $A$  is the cross sectional area, and  $E$  is the Young's Modulus [17]. The displacement or change in length is found using the ANSYS APDL displacement solver. With this information new node positions can be calculated and reaction forces can be found using the ANSYS APDL solver.

$$e_i = \frac{F_i L_i}{A_i E_i} \quad (2)$$

The stiffness is found using Equation 3 [17].

$$k_i = \frac{A_i E_i}{L_i} \quad (3)$$

The total strain energy is found using Equation 4 where  $\{\sigma\}_m$  represents stress component of the microtubule and  $\{\epsilon\}_m$  represents the strain component of the microtubule.  $\{\sigma\}_a$  represents the stress component of the microfilament and  $\{\epsilon\}_m$  represents the strain component of the

microfilament.  $V$  stands for the volume of the elements and  $U_T$  represents the total strain energy [17].

$$U_T = \frac{1}{2} \int_V \{\sigma\}_m^T \{\epsilon\}_m dV_m + \frac{1}{2} \int_V \{\sigma\}_a^T \{\epsilon\}_a dV_a \quad (4)$$

Equations 1, 2, 3 and 4 are the governing equations used by ANSYS APDL to solve for strain energy. The strain energy of each element is found at a given stiffness value using the strain energy command in ANSYS APDL. These strain energy values are then summed to find the total strain energy of the model.

### 2.3 Prestress

A simple explanation of prestress is adding cables to a concrete beam which would counteract the applied forces of the beam. Prestress is used to improve a structure's performance under certain conditions because prestress allows a structure to resist external forces and maintain shape [4]. If a force is applied to a structure, elements move together to a new position of equilibrium between the structure and its environment [4]. Prestress is a very important part of the tensegrity model. The tensegrity model of a cell shows that the cell has internal tension of the cable network of microfilaments which is balanced by the internal compressive beams of microtubules [18]. Microfilaments are pretensed with a value of  $.8 \cdot 10^{-14}$  N, and microtubules are precompressed with a value of  $-1.92 \cdot 10^{-14}$  N [4]. Since prestress has little effect on the movement of the cell compared to substrate stiffness, any values can be chosen in the given range of  $1.0 \cdot 10^{-14}$  N to  $4.5 \cdot 10^{-14}$  N [4]. Prestress is an important factor that can dictate a cell's deformation [19]. An example of internal prestress in the body is the skin before an incision is

made [15]. Skin exists in a high tensile state, but when an incision is made, this is weakened [15]. When the cut is stitched adding reinforcement to the skin integrity, the tension is restored [15]. Since prestress influences the behavior of a living cell, it is important to include prestress in this model so that this model can accurately represent a living cell.

## **2.4 Simulation Procedure**

In this simulation, a node is extended which will represent the process by which the cell probes and senses the stiffness of its substrate. The internal strain energy changes during this probing process. The stiffness of the substrate is represented by a spring element attached between Node 46 and node 49. The strain energy is then calculated for the corresponding stiffness values. Since a cell prefers to stay in a low energy state, it is inferred that the lower the internal strain energy the likelier the cell will move towards that stiffness value so as to stay in the low energy state.

The simulation process is similar to the simulation of a 3T3 cell with different nodes being selected since the cell models are not the same [4]. To begin the simulation, prestress forces are applied to the elements which will cause a change in the node locations because the length of the microfilaments and microtubules will increase or decrease. Due to this extension or compression of the element, the node locations need to be redefined for the following step. The next step is to give a displacement to node 46 and node 49. The displacement of the spring element, the element between node 46 and node 49, is  $1 \cdot 10^{-6}$  meters in the X-direction. This displacement does not significantly change the shape of the cell and will not be applied towards the strain energy of the cell. This displacement however creates reaction forces at node 46. The

new node locations and reaction forces are stored from this second step to be used in the next steps. In the third step, node 49 is fixed which will fix one side of the spring element. The reaction forces from node 46 are then applied to find total strain energy of the cell. The following flow chart shows the procedure based on Voloshin's simulation [4].

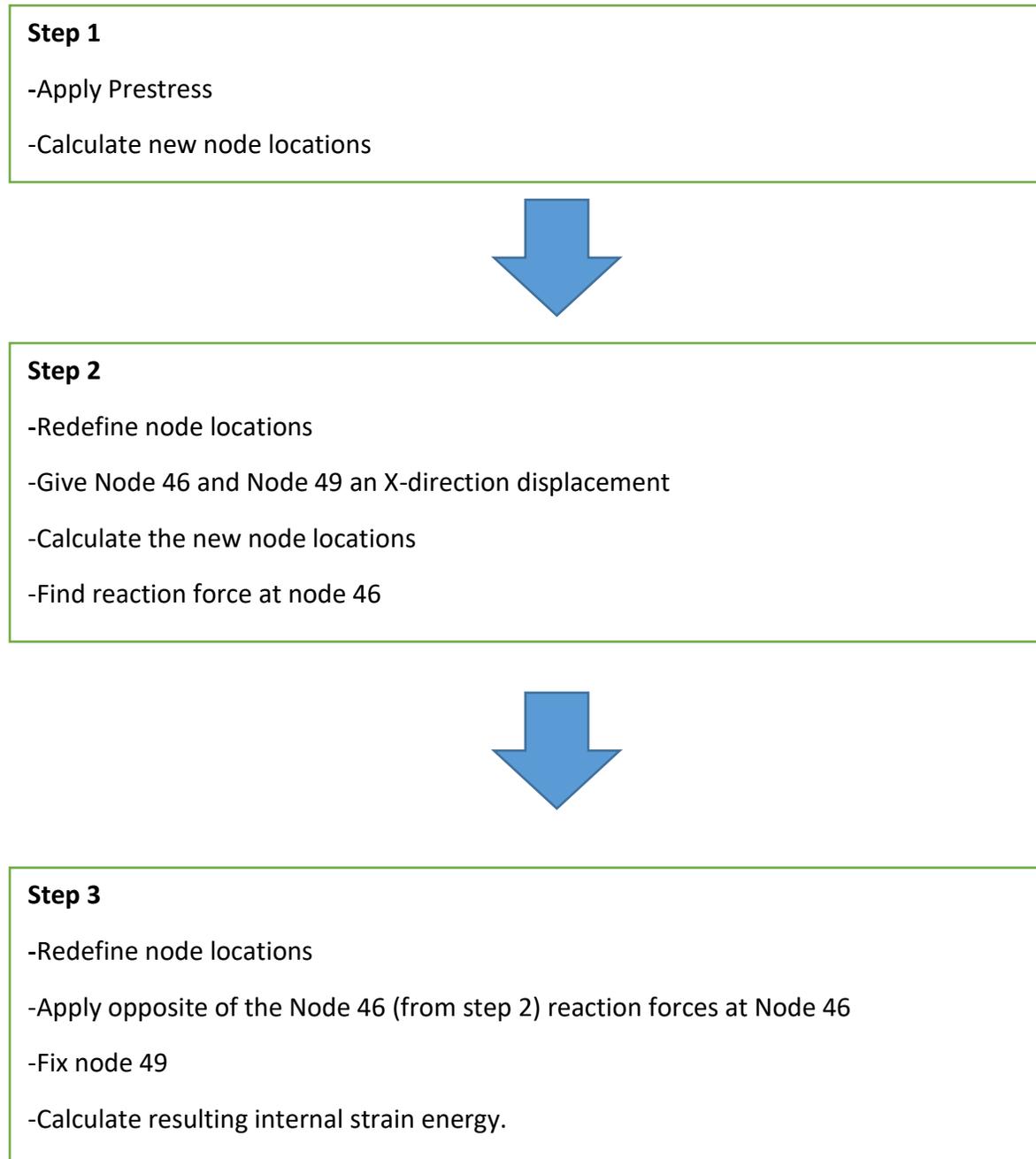


Figure 4. Flow Chart of Simulation Procedure [4]

## 2.5 Cell Movement Mapping

After the relationship between the cell's internal strain energy and substrate stiffness is understood, mapping the cell movement on a substrate with an increasing stiffness can show the cell's tendency to move towards the higher stiffness value. This is done by conduction of Monte Carlo simulations in MATLAB (Appendix 2). A 3-D plane is created by setting a diagonal of increasing values. From there values are based on a random number generator that will increase or decrease depending on the preceding cell. This is done by creating an increasing diagonal for the stiffness matrix. From there the cells around the diagonal are populated depending on the diagonal value that is closet. The values of stiffness either increase or decrease the further away from the diagonal they go. This will create a surface that linearly increases the further away from the origin it moves. This continues until a square matrix is created. An example of pseudocode to create this plane is shown below where "n" is the size of the given square matrix and "s" is the substrate matrix:

```
1.   for i = 1:n-1
2.       for j = i:n-1
3.           s(i,j+1) = s(i,j) + 12*(rand()-0.5); %
4.       end
5.   end
6.   for i = 2:n
7.       for j = 2:i
8.           s(i,j-1) = s(i-1,j-1) + 12*(rand()-0.5);
9.       end
10.  end
```

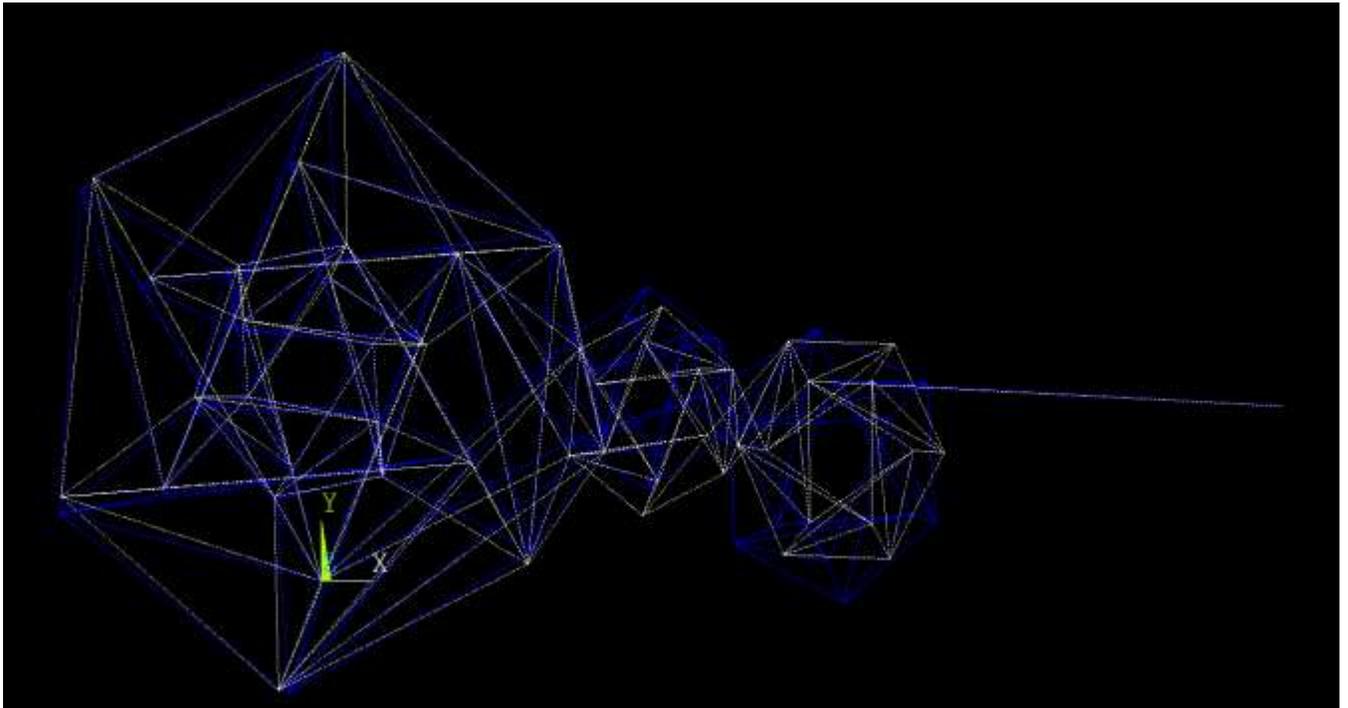
*Figure 5. Pseudocode for Creating Substrate Plane*

After the substrate surface is created, the algorithm to model the cell moving on the substrate is built. This algorithm is almost identical to how it is hypothesized that a cell will move since a cell will probe the surrounding substrates to find the stiffest substrate to move to as previously mentioned. Given that the nodes are able to move and interact with the substrate that have

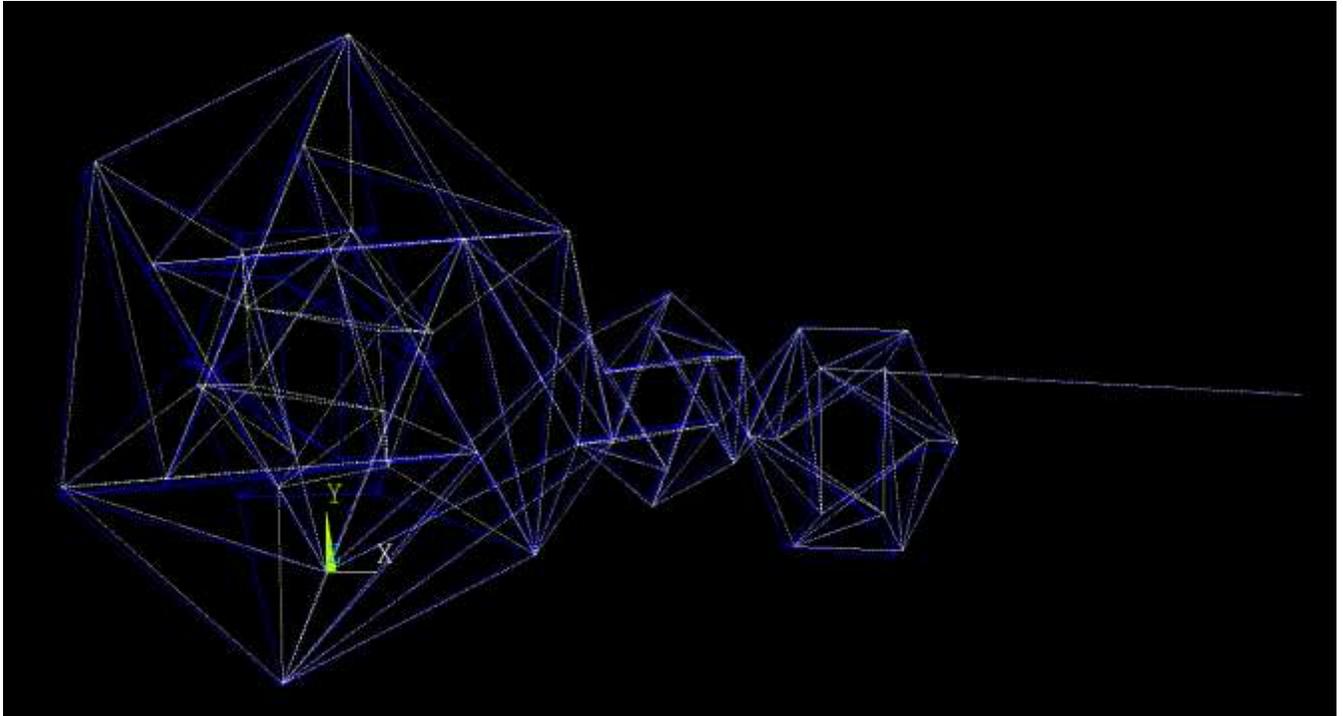
been created in this matrix of increasing stiffness, the force calculations can be used to plot where a cell will likely probe and the move to. At a given position, the cell will probe the surrounding substrates and move towards the substrate with the highest stiffness value [1]. In this model, values of stiffness of the surrounding cells in the matrix are checked which represents the probing, and the position will move to the cell in the matrix with the highest value of stiffness. The rules that govern the cell movement in the code is that the position will be saved and the values of the closest cells of the matrix are surveyed in a similar way to how a living cell will probe its environment. From there the position moves to the cell in the matrix that has the highest value. This is the same way a living cell would move. This continues in a loop until the position of the cell is in the position of the highest value of the stiffness matrix.

### 3. Results

Using ANSYS APDL, the internal strain energy of the model is found on a substrate with varying stiffness values. Given a stiffness value of the spring element which represents the substrate rigidity, the strain energy of the model is calculated. Since substrate stiffness was the only factor being varied, the cell's motion is a function of the substrate's stiffness. To show the relationship between cell movement and substrate rigidity, Figure 6 and Figure 7 represent the deformation of a cell with a low stiffness value (Figure 6) and high stiffness value (Figure 7).



*Figure 6. Deformation at Stiffness Value .001 N/m*



*Figure 7. Deformation at Stiffness Value 1000 N/m*

The white lines represent the original cell, and the blue lines represent the deformed cell due to applied displacement. One can see that the cell in Figure 6 is more deformed than the cell in Figure 7. This makes sense since there is more deformation of a soft substrate causing more deformation of the cell. Conversely, the more rigid the substrate the less deformation of the substrate causing less deformation of the cell. Figure 8 shows the relationship between displacement of Node 46 and substrate rigidity. A cell moves more on a less rigid substrate compared to a more rigid substrate which supports the notion that a cell will stay on a rigid substrate to stay in a low energy state. The relationship between internal strain energy and substrate is shown in Figure 9, Figure 10, and Figure 11. The prestress stayed the same for all simulations because this investigation dealt solely with the stiffness of the substrate. Figures 9,

10, and 11 shows the trend that the higher the stiffness the lower the internal strain energy which is consistent with previous findings [4].

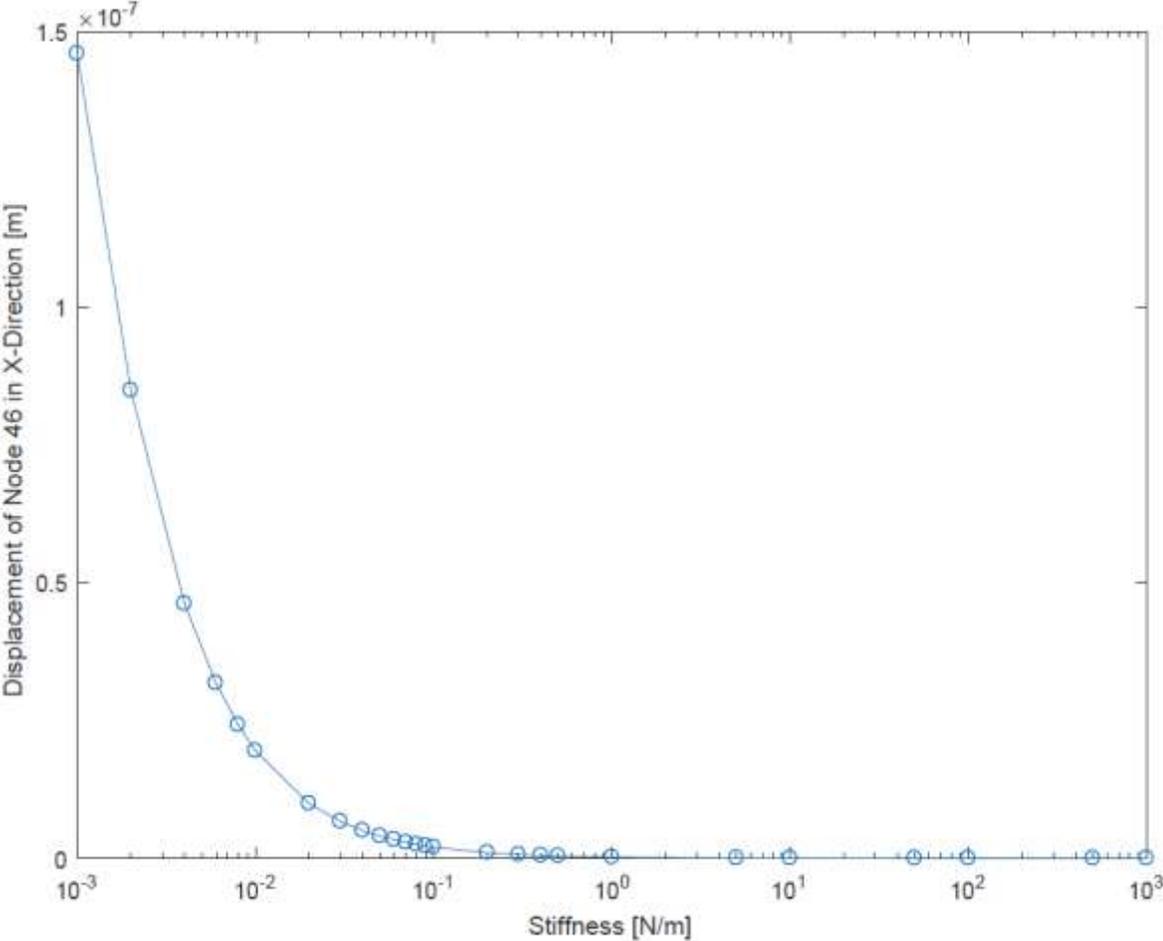


Figure 8. Displacement of Node 46 in X-Direction for Stiffness Values form  $10^{-3}$  N/m to  $10^3$  N/m

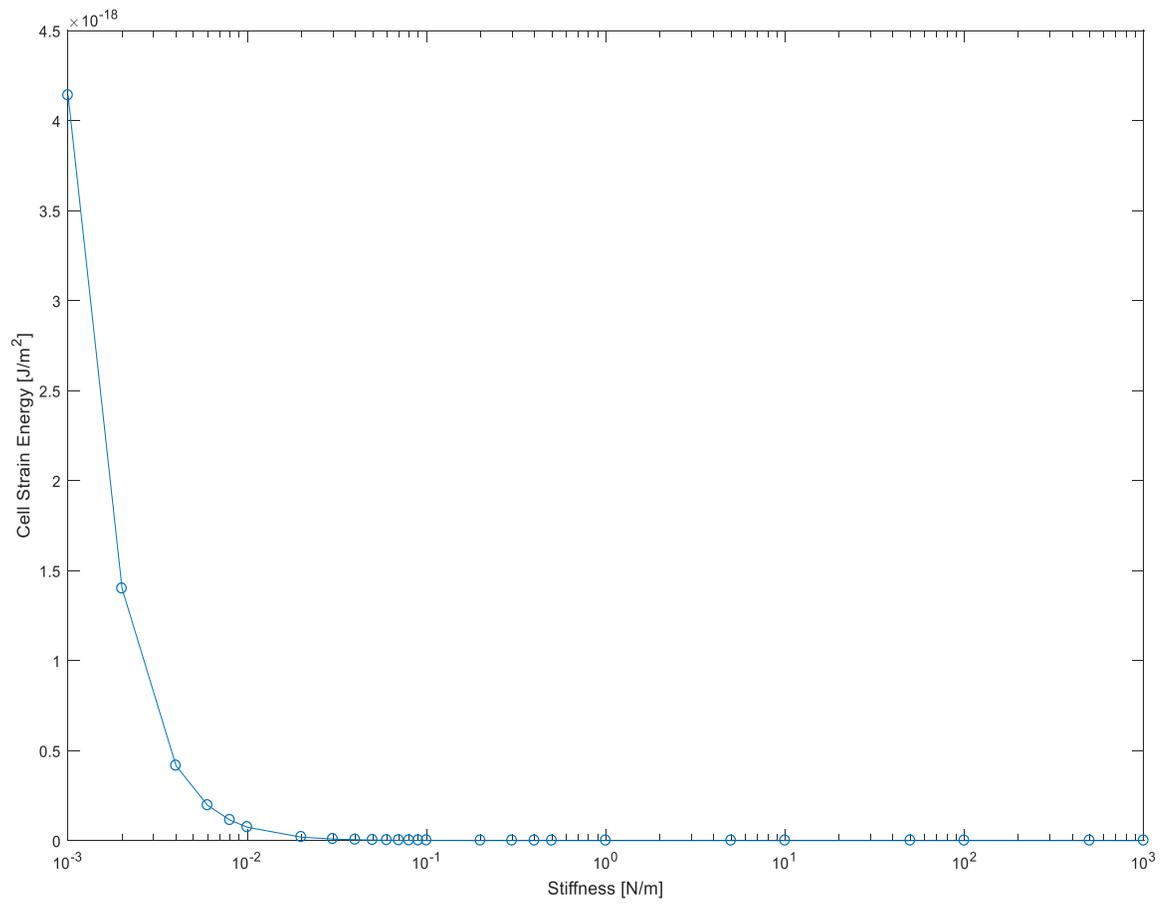


Figure 9. Internal Strain Energy for Stiffness Values from  $10^{-3}$  N/m to  $10^3$  N/m

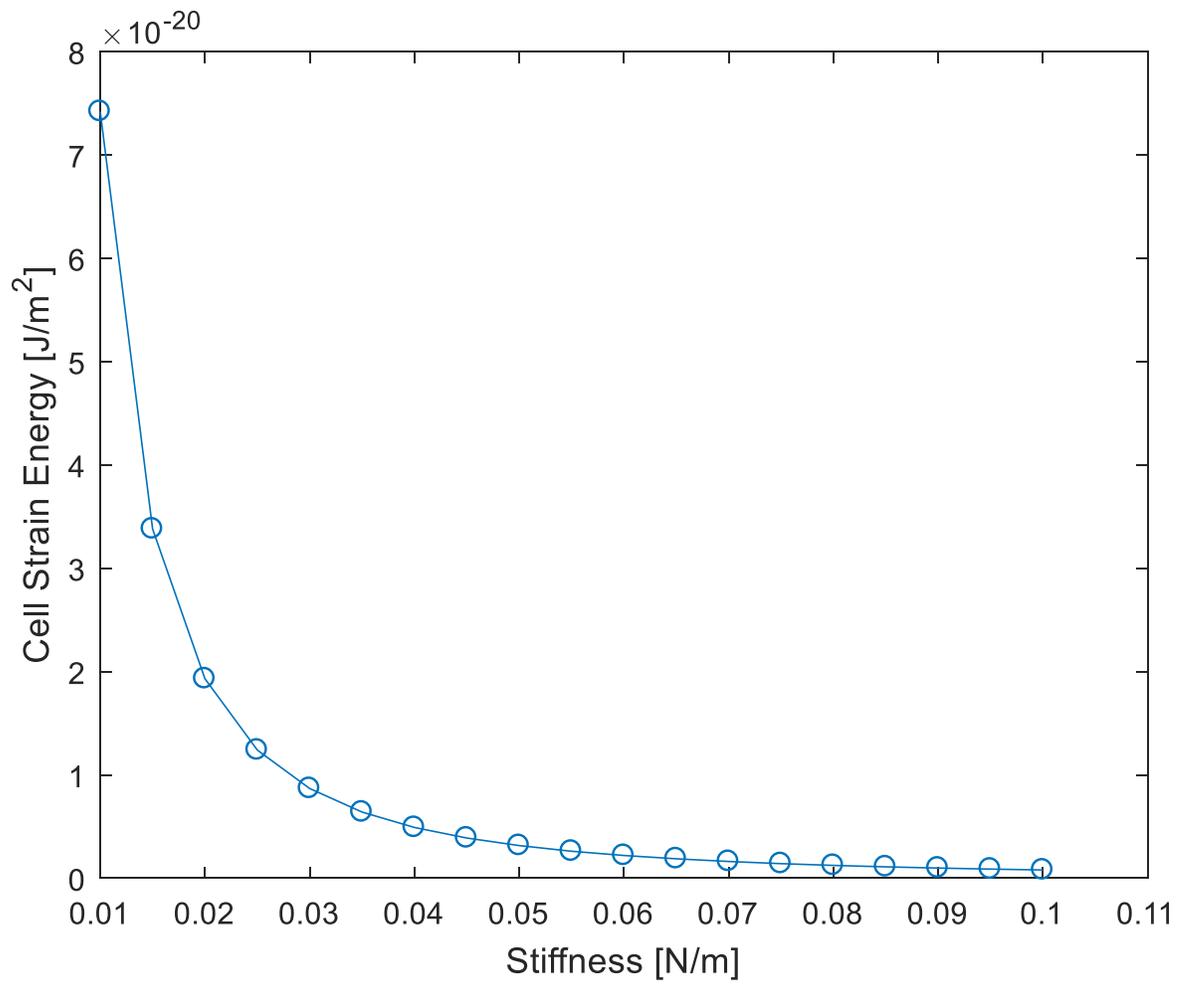


Figure 10. Internal Strain Energy for Stiffness Values from .01 N/m to .1 N/m

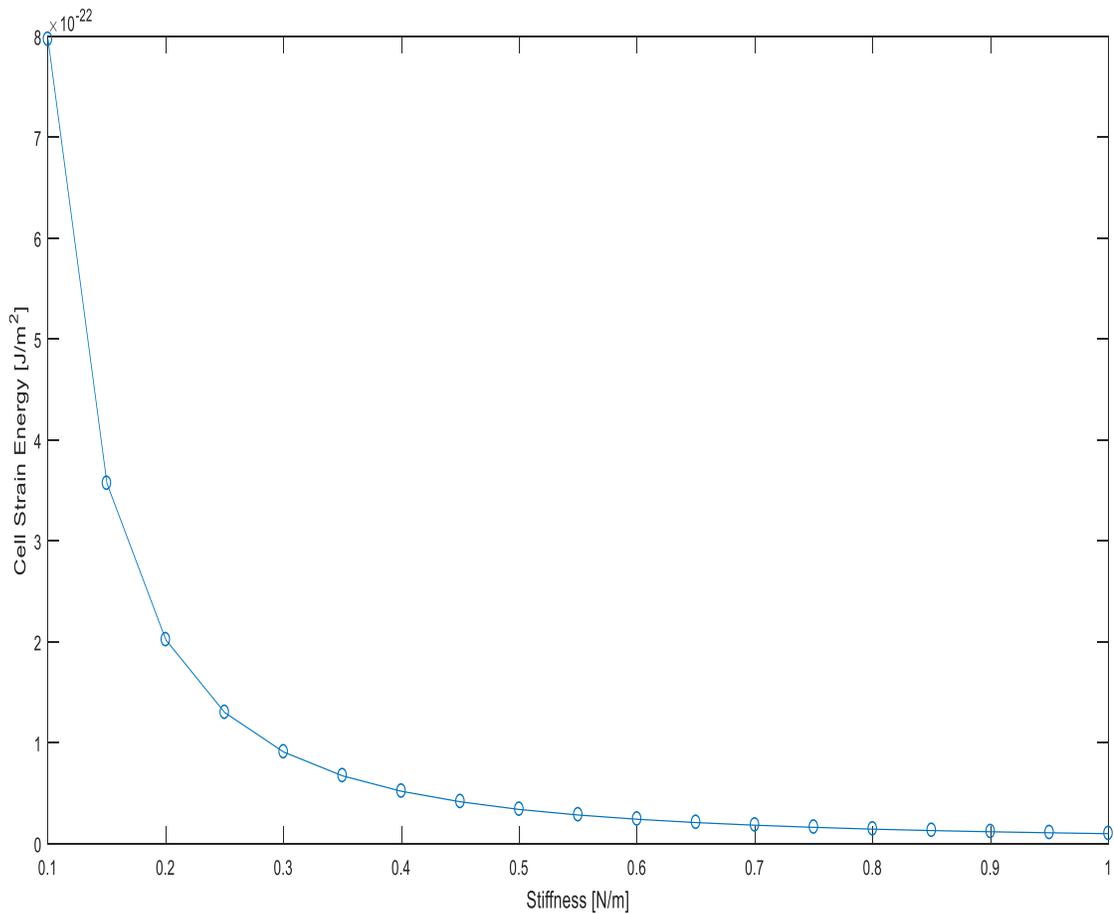


Figure 11. Internal Strain Energy for Stiffness Values from .1 N/m to 1 N/m

Figure 9, 10, and 11 show the cell's internal strain energy will decrease as substrate stiffness increases. As the substrate stiffness increases, the cell prefers to stay in a lower energy state. This supports the idea that a cell probes its substrates stiffness to find the highest stiffness which shows the cell's direction of movement. Figure 8 is the relationship between the displacement of Node 46 in the x direction and substrate stiffness. From Figure 8, it is seen that as substrate stiffness increases the displacement decreases. The displacement of Node 46 is the focal adhesion of the cell to its substrate which is a function of substrate stiffness. The values

for the strain energy and displacement of Node 46 for each value of stiffness are found in the table below.

Substrate Stiffness [N/m]	Displacement of Node 46 in X Direction [m]	Internal Strain Energy [J/m <sup>2</sup> ]
.001	$.146 \times 10^{-6}$	$.415 \times 10^{-17}$
.002	$.849 \times 10^{-7}$	$.140 \times 10^{-17}$
.004	$.462 \times 10^{-7}$	$.416 \times 10^{-18}$
.006	$.318 \times 10^{-7}$	$.196 \times 10^{-18}$
.008	$.242 \times 10^{-7}$	$.114 \times 10^{-18}$
.01	$.195 \times 10^{-7}$	$.742 \times 10^{-19}$
.02	$.995 \times 10^{-8}$	$.193 \times 10^{-19}$
.03	$.668 \times 10^{-8}$	$.868 \times 10^{-20}$
.04	$.502 \times 10^{-8}$	$.491 \times 10^{-20}$
.05	$.403 \times 10^{-8}$	$.316 \times 10^{-20}$
.06	$.336 \times 10^{-8}$	$.220 \times 10^{-20}$
.07	$.288 \times 10^{-8}$	$.162 \times 10^{-20}$
.08	$.252 \times 10^{-8}$	$.124 \times 10^{-20}$
.09	$.225 \times 10^{-8}$	$.982 \times 10^{-21}$
.1	$.202 \times 10^{-8}$	$.797 \times 10^{-21}$
.2	$.101 \times 10^{-8}$	$.201 \times 10^{-21}$
.3	$.676 \times 10^{-9}$	$.907 \times 10^{-22}$

.4	.507*10 <sup>-9</sup>	.519*10 <sup>-22</sup>
.5	.406*10 <sup>-9</sup>	.339*10 <sup>-22</sup>
1	.203*10 <sup>-9</sup>	.994*10 <sup>-23</sup>
5	.406*10 <sup>-10</sup>	.229*10 <sup>-23</sup>
10	.203*10 <sup>-10</sup>	.205*10 <sup>-23</sup>
50	.406*10 <sup>-11</sup>	.197*10 <sup>-23</sup>
100	.203*10 <sup>-11</sup>	.197*10 <sup>-23</sup>
500	.406*10 <sup>-12</sup>	.197*10 <sup>-23</sup>
1000	.203*10 <sup>-12</sup>	.197*10 <sup>-23</sup>

*Table 2. Strain Energy and Node 46 Displacement for Stiffness Values 10<sup>-3</sup> N/m to 10<sup>3</sup> N/m*

These results show the relationship between the cell's internal strain energy and the substrate stiffness. The relationship between internal strain energy and substrate rigidity is that the strain energy will decrease as the substrate becomes more rigid. Since a cell prefers to stay in a lower energy state, the cell prefers to move in the direction of the stiffer substrate. In addition, as substrate rigidity increases the displacement of focal adhesion also decreases due to the stronger mechanical feedback for the cell-substrate system. With the knowledge that a cell moves towards the substrate with the highest stiffness, the movement of the cell can be mapped in MATLAB by creating a stiffness plane using the random number generator discussed in the methods section.

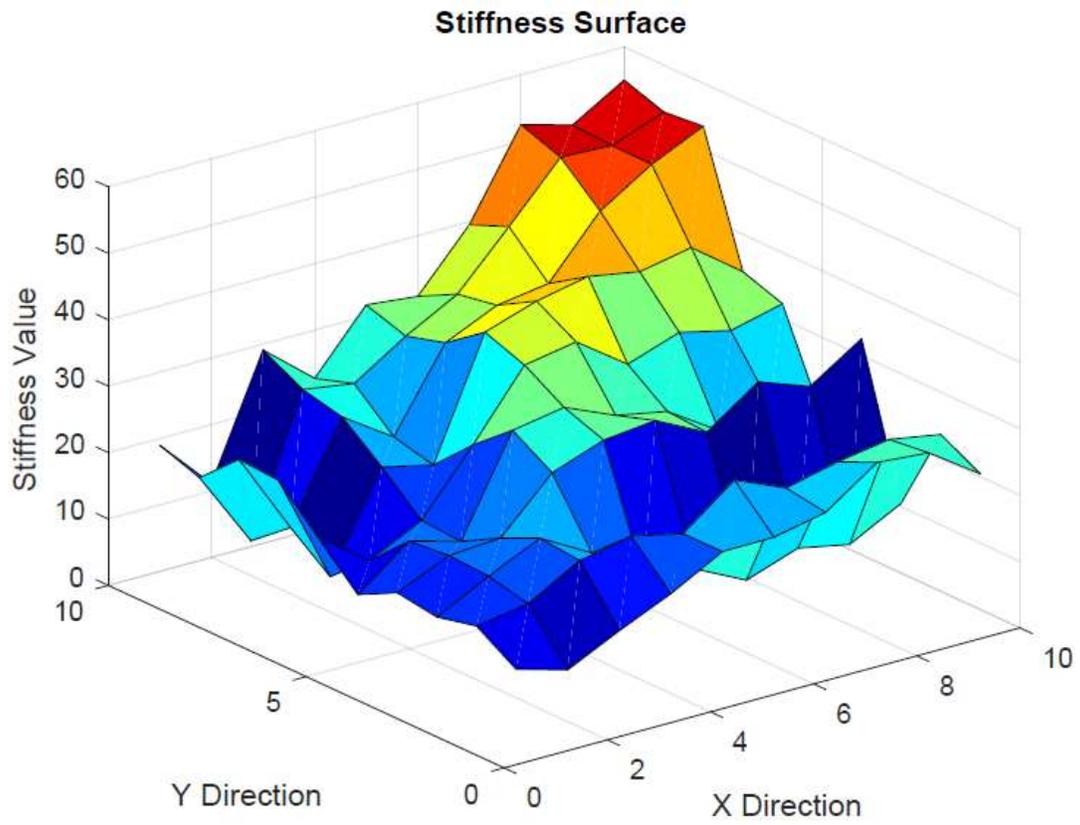
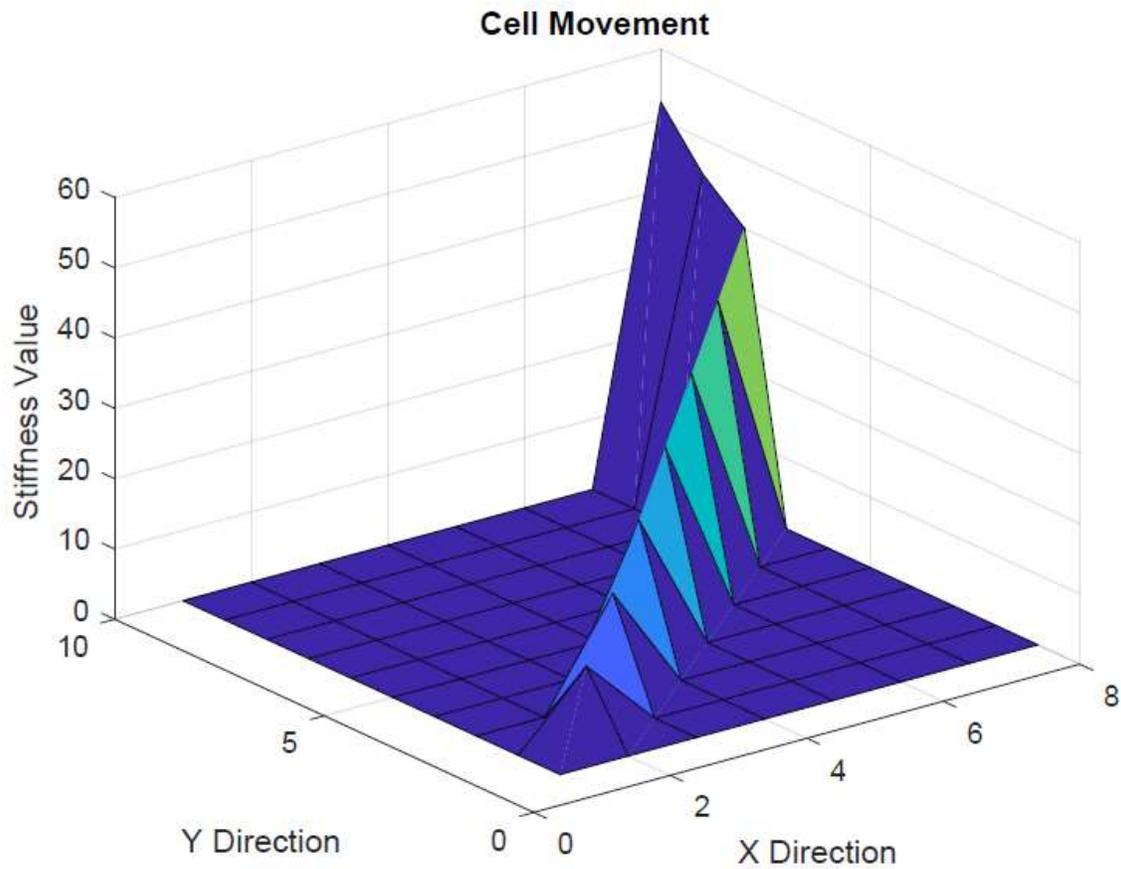


Figure 12. Simulated Substrate Stiffness



*Figure 13. Movement of a Cell based on Figure 12.*

Figure 12 shows a 3-D plot of a random stiffness plane that increases the further away it goes from the origin. Knowing that a cell moves towards the highest stiffness value, Figure 13 depicts a representation of a cell moving on a substrate with stiffness values similar to Figure 12. While these results are not nearly as robust as the results found from ANSYS APDL, more work can be done to improve predicting where a cell will move on a given substrate.

## 4. Discussion

The results found in ANSYS APDL show a clear pattern that a cell will have a lower strain energy on a more rigid substrate. Since a cell prefers to stay in a lower energy state, the cell moves towards the substrate with the highest stiffness value. A mathematical model based on thermodynamics was created to link substrate rigidity to cell stability and cell movement [20]. It was found that the stability of a cell stuck to a substrate is governed by the minimal total energy in the cell-substrate system [20]. The internal strain energy of the cell generated by internal and external forces due to the substrate rigidity destabilizes cell morphology and movement which means as the stiffness increases the lower the internal strain energy [20]. The internal strain energy is found by taking the surface integral of the forces along the cell [1]. When the cell probes the substrate, the cell deforms more on a soft substrate compared to a rigid substrate. Experimental results show that a cell probes the environment to check the elasticity of the substrate [21]. The cells then moves towards the substrate with the highest stiffness value and moves away from a substrate with a lower stiffness value [1]. Figure 9, Figure 10, and Figure 11 show that the internal strain energy of the cell decreases as substrate stiffness increases. The cell prefers to stay in a lower energy state, thus it moves towards the substrate that yields the lowest internal strain energy which corresponds to the higher stiffness values. In summation, cells are able to sense the stiffness of the substrate by probing for its elasticity. It is unclear exactly how cells transduce the substrate rigidity into a mechanical response, but it is possible that the cell can sense a small adhesion displacement which results in a strong mechanical feedback [1]. This is understood because strain energy is the integration of the forces along the surface of the cell which can also explain that with the same internal

strain energy, the cell will have a larger adhesion displacement and a weaker mechanical feedback [1]. Since the mechanical feedback is stronger on rigid substrates, this can lead to the activation of stress-sensitive ion channels which can regulate the stability of focal adhesions and strength of contractile forces [1]. This is able to explain why the deformation of the cell on a softer substrate is higher than the deformation of a cell on a rigid substrate. This can be seen in Figure 6 and Figure 7 where the deformation of the cell is shown on a soft substrate and a rigid substrate. Figure 8 shows the focal adhesion displacement of the cell as the stiffness increases. On a rigid substrate, the deformation is smaller since the forces are a function of the substrate stiffness [4]. The cell adheres to the rigid substrate easier since it produces a more stable cell-substrate system which shows the cell's preferential movement to a rigid substrate instead of a soft substrate [4]. In addition, the simulations are able to show the relationship between cell movement and substrate rigidity based on the internal strain energy of the cell whereas cell movement is most likely stimulated by a complex combination of chemical and physical stimuli [4]. These stimuli can include substrate rigidity as well as others [4].

Mapping the movement of a cell on a substrate with an increasing stiffness, Figure 12, as seen in Figure 13 can be the next step in understanding where a cell will move. The parameters used to create the substrate were chosen through trial and error, but if the stiffness values of the substrate are known, the directional movement of the cell is known. Since it has been verified that a cell's movement is a function of internal strain energy, mapping the cell's movement should be possible as long as the substrate is known. The cell moves towards the more rigid substrate to stay in a lower energy state. This can lead to understanding of macroscopic

directional movement of cells in response to mechanical changes in their environment even if microscopically the reason for directional movement isn't completely understood.

## 5. Conclusions

Experimental and computational data shows that a cell moves towards a substrate with a higher stiffness value because the more rigid a substrate, the lower the internal strain energy. In addition, cells probe their environment as a means to understand their environment and respond to the mechanical properties of their environment. Cells move towards an area where the internal strain energy is the lowest. The lower the internal strain energy, the smaller the deformation of the cell. When a cell probes its substrate, if the substrate is softer the greater the displacement of the focal adhesion and weaker mechanical feedback which will increase the strain energy. For a more rigid substrate, the displacement is less because of the strong mechanical feedback. The relationship between cells with lower internal strain energy and more rigid substrates are the most stable because of the lower deformation. Therefore, the cell's efficiencies and formations increase [4]. The model used in this investigation is a tensegrity structure of a 3T3 nucleus, cytoskeleton, and lamellipodium attached to a substrate based on the simulation that was ran in a previous [4] study. In addition, this investigation further validated the tensegrity model as an accurate representation of the living cell. Microfilaments are modeled as a cable network carrying tension while microtubules are modeled as beams carrying compression which provide support. The internal force balance can be seen in live cells and can computationally be used to model live cells. Continued use of the tensegrity approach can further explain cell mechanics and mechanical phenomena. The findings from this model are consistent to findings of experimental and computational data. As substrate stiffness increases, cell deformation will decrease, and as substrate stiffness increases, the internal strain energy of the cell decreases. Since it is accepted that a cell moves

towards the position where its internal strain energy is the lower, the cell will move towards a more rigid substrate.

# Appendix

## 1. Proof of Permission



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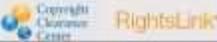
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## 2. Matlab Code

```
clc
clear all

% s = randi([0 1000],5,5);

a = [10 15 20 25 30 35 40 45 50 55];

s = diag(a);

%matrix builder
for i = 1:9
    for j = i:9
        s(i,j+1) = s(i,j) + 12*(rand()-0.5); %
    end
end

for i = 2:10
    for j = 2:i
        s(i,j-1) = s(i-1,j-1) + 12*(rand()-0.5);
    end
end

cpos = zeros(5,5);

%plot stiffness surface
figure(1)

surf(s)
colormap(jet)
title('Stiffness Surface')
xlabel('X Direction')
ylabel('Y Direction')
zlabel('Stiffness Value')

cx = 1;
cy = 1;
n = 0;
z = 1;

c = length(s);

while n < 1

    nx = cx;
    ny = cy;

    c_num = s(cx,cy);
```

```

if nx + 1 > c
    b_num = 0;
elseif nx + 1 < c
    b_num = s(nx+1,ny);
end

if nx - 1 < 1
    t_num = 0;
elseif nx - 1 > 1
    t_num = s(nx-1,ny);
end

if ny + 1 > c
    r_num = 0;
elseif ny + 1 < c
    r_num = s(nx,ny+1);
end

if ny - 1 < 1
    l_num = 0;
elseif ny - 1 > 1
    l_num = s(nx,ny-1);
end

if nx - 1 < 1 || ny + 1 > c
    tr_num = 0;
else
    tr_num = s(nx-1,ny+1);
end

if nx + 1 > c || ny + 1 > c
    br_num = 0;
else
    br_num = s(nx+1,ny+1);
end

if nx - 1 < 1 || ny - 1 < 1
    tl_num = 0;
else
    tl_num = s(nx-1,ny-1);
end

if nx + 1 > c || ny - 1 < 1
    bl_num = 0;
else
    bl_num = s(nx+1,ny-1);
end

if c_num > r_num && c_num > tr_num && c_num > br_num && c_num > tl_num &&
c_num > bl_num && c_num > l_num && c_num > t_num && c_num > b_num
    cx = cx;
    cy = cy;
    n = 1;

```

```

elseif r_num > l_num && r_num > t_num && r_num > b_num && r_num > tr_num &&
r_num > br_num && r_num > tl_num && r_num > bl_num
    cx = nx;
    cy = ny + 1;
elseif tr_num > l_num && tr_num > t_num && tr_num > b_num && tr_num > r_num
&& tr_num > br_num && tr_num > tl_num && tr_num > bl_num
    cx = nx-1;
    cy = ny+1;
elseif br_num > l_num && br_num > t_num && br_num > b_num && br_num > r_num
&& br_num > tr_num && br_num > tl_num && br_num > bl_num
    cx = nx+1;
    cy = ny+1;
elseif l_num > r_num && l_num > t_num && l_num > b_num && l_num > tr_num &&
l_num > br_num && l_num > tl_num && l_num > bl_num
    cx = nx;
    cy = ny - 1;
elseif tl_num > l_num && tl_num > t_num && tl_num > b_num && tl_num > r_num
&& tl_num > br_num && tl_num > tr_num && tl_num > bl_num
    cx = nx-1;
    cy = ny-1;
elseif bl_num > l_num && bl_num > t_num && bl_num > b_num && bl_num > r_num
&& bl_num > br_num && bl_num > tl_num && bl_num > tr_num
    cx = nx+1;
    cy = ny-1;
elseif t_num > r_num && t_num > l_num && t_num > b_num && t_num > tr_num &&
t_num > br_num && t_num > tl_num && t_num > bl_num
    cx = nx - 1;
    cy = ny;
elseif b_num > r_num && b_num > l_num && b_num > t_num && b_num > tr_num &&
b_num > br_num && b_num > tl_num && b_num > bl_num
    cx = nx + 1;
    cy = ny;
end
c_num
cpos(cx,cy) = c_num;
z = z + 1;

end

```

```

figure(2)
surf(cpos)
title('Cell Movement')
xlabel('X Direction')
ylabel('Y Direction')
zlabel('Stiffness Value')

```

## **Vita**

Alan Wopperer was born in Buffalo, New York on March 3<sup>rd</sup>, 1995. He enrolled at Lehigh University in January of 2014 and graduated with a Bachelor of Science in Mechanical Engineering in May of 2017. After graduation, he continued his studies in graduate school at Lehigh University pursuing a Master of Science in Mechanical Engineering.

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