

A Discrete Cellular Automaton Model Studies Major

Factors in Tumor Growth And Development

Abstract

Cancer is one of the deadliest diseases known to mankind, yet many of its mechanisms of development are still unknown. In vivo and in vitro experimentation have been both employed in order to elucidate the growth patterns of cancer. Additionally, mathematical and computational models can also provide data on the growth patterns. In order to study the influence of environmental and biological conditions on the growth of cancer, this paper attempts to unify many of the factors influencing tumor growth, such as the availability of nutrients, the double hit hypothesis, and the cell and nutrient migration rate, into one mathematical model. Each of these factors can have their effects controlled by varying the parameters involved in the model, which results in a highly variable simulation procedure that can be adapted to various biophysical environments. Through this model, many previous results from in vivo experimentation, including the effects of angiogenesis and the autophagy induced by cancer cells, have been confirmed. This model's results of tumor growth can additionally be extrapolated and used in order to predict the type of environment which cancer cells grow in, as well as the most prevalent factors promoting cancer growth, based on cancer's depicted growth patterns.

A Discrete Cellular Automaton Model Studies Major

Factors in Tumor Growth And Development

I. Introduction

Cancer development is an intricate process involving thousands of genes and millions of possible outcomes, as well as chemical and physical signals between cells, but understanding the growth of a tumor is an equally daunting task. Normally, the development of a tumor requires a combination of genetic mutations, which can be caused by radiation, chemical compounds, or viruses.¹ Several critical aspects of tumor growth are well known including The Theory of Angiogenesis,² The Double Hit Hypothesis,³ the effects of Cellular Migration on the growth of tumors,⁴ the autophagy of neighboring cells,¹⁴ and the effects of cell signaling on the growth of cancer⁵.

Tumor suppressor genes and oncogenes expressed through epigenetic relationships and the binding and unbinding genes causes the primary development of the disease. Overexpression of oncogenes such as the Ras gene and lack of expression of tumor suppressor genes like P53 can cause cancer cells to divide uncontrollably, without regard to external growth signals. Thus, cancer cells that have a combination of both active oncogenes and inactivate tumor suppressor genes are especially prone to forming cancer.⁶ The development of a tumor is additionally governed by the double hit hypothesis, which states that there has to be at least 2 mutations before the cancer can develop. The explanation for a tumor's ability to grow in size comes from the theory of angiogenesis, showing another factor in the expression of tumor growth.² The Theory of Angiogenesis claims that cells that do not undergo resource acquisition cannot

proliferate beyond a certain point and this type of cell is shown through less invasive types of cancer. Angiogenesis is a key topic in creating a simulation to model cellular growth. Another process that facilitates the growth of tumors is in the migration of cancer cells. When cancer cells migrate, they allocate space between them for more cells to fill in, thus, spreading the tumor out and allowing cells on the interior of the tumor to grow while still receiving nutrients, preventing necrosis.⁷ Necrosis found in some very aggressive tumors and the following ring structure of living cells on the outside and dead cells on the inside can be explained by the large amount of resources necessary for the rapid growth of cells. The more cells migrate, the more cells will proliferate and the larger a tumor will become.

After cancer develops, signals that are accepted by normal cells and apoptosis signals are ignored, meaning that their growth is uncontrollable.⁴ Additionally, they also starve other cells by inducing them to undergo autophagy, which allows them to quickly proliferate by using the nutrients generated by the autophagic cells.¹⁴ Cancer cells however do not perform normal cellular functions, and due to their ability to quickly divide, they replace useful cells that lead to the loss of organ functionality. The lethality in cancer is derived from a tumor's ability to replace functional with non-functional cells.

Taking into account for all the aspects, intermediates, and rules to cancer growth, it is possible to create a cellular automaton model that uses probabilistic methods to determine the growth pattern of tumor cells. Using a mathematical model in Java to replicate the conditions of cells before the emergence of cancer and the factors that are necessary in the growth of a tumor, it was possible to analyze the growth patterns of cells within the simulations using MatLab, which allows us to confirm the in vivo experimentation as well as further extrapolate results.^{8,14}

The model allocates the properties of cancer cells into adjustable parameters and enables us to correctly model the growth patterns of cancer. With variations on the code, it is possible to produce models of growth that elucidates the complex development of cancer with a 2-dimensional cross section of the tumor. By using this model, cancer growth patterns with defined external conditions can be predicted, by varying the parameters within the program. With this knowledge, it is possible to additionally determine the most critical aspects of cancer development and which variables are the most prominent in the spread of the disease. This model can thus be used in extrapolating and predicting cancer growth patterns and growth conditions, resulting in an advancement in understanding tumors.

II. Materials and Methods

In order to better model tumor growth, a mathematical model was created through the Java language that built upon the work of Phan.⁴ In this discrete model, nutrients were implemented for control of cell growth, as well as tumor cells which ignored cellular controls on growth and could divert nutrients away from normal cells. The specifics of this mathematical model are displayed in the flowchart directly below and further aspects of the parameters the program uses can be found below the flowchart. The source code can be found in reference 17 below.

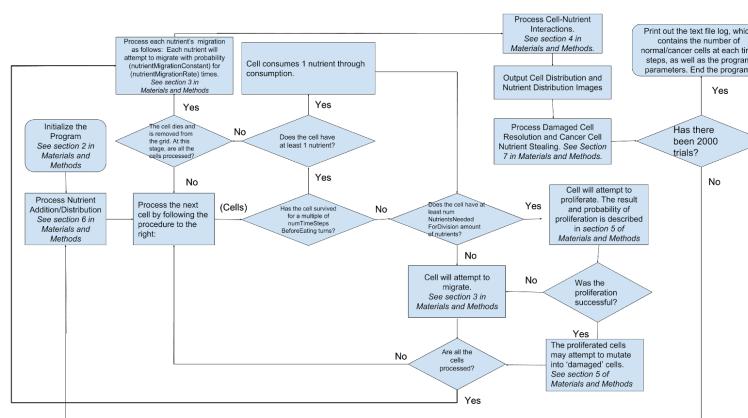


Figure 1: Program Procedure Flowchart²¹

nutrientKProportionOfGrid	Sets maximum number of nutrients in the grid, as a percentage of grid size. Can be any nonnegative number.
nutrientStartProportionOfGrid	Sets the starting number of nutrients in the grid, as a percentage of grid size. Can be any nonnegative number.
cellMigrationRate	Controls cell migration rate, varies between 0 [never migrate] and 1 [always migrate].
nutrientMigrationRate	Controls nutrient migration rate, varies between 0 [never migrate] and 1 [always migrate].
nutrientUptakeRate	Controls the probability of nutrients being uptaken by the cell, varies between 0 [never pick up] and 1 [always pick up].
damageRate	Controls the probability of cells becoming damaged, in accordance with the two-hit hypothesis. Varies between 0 [never becomes damaged] and 1 [always becomes damaged]
damagedToNormalProbability	Controls the probability of the cells becoming normal, after becoming damaged [cellular repair]. Varies between 0 [never recovers] and 1 - damagedToDeathProbability.
damagedToDeathProbability	Controls the probability of the cells becoming normal, after becoming damaged and being unrecoverable [apoptosis]. Varies between 0 [never dies] and 1 - damagedToNormalProbability.
damagedToCancerProbability	Controls the probability of the damaged cells becoming cancer cells. The chance of becoming a cancer cell is equal to 1 - damagedToDeathProbability - damagedToNormalProbability.
effectOfNeighboringCellsOnCellDivision	Controls the rate of cell division. See section 5 (in materials and methods) for more details.
numNutrientsNeededForDivision	Controls the number of nutrients that cells need in order to proliferate. Needs to be an integer that is ≥ 2
numTimeStepsBeforeEating	Controls how often cells consume their nutrients. Nutrients will be consumed every [value]th turn the cell is alive.
cancerCellStealingRadius	Controls the radius that cancer cells can steal nutrients from normal cells. Needs to be an integer that is ≥ 0 .
nutrientReplenishRate	Controls the frequency of nutrients being added to the grid. Nutrients will be added every [value]th turn.
nutrientAddLimitPerTurn	Controls the maximum number of nutrients that can be added to the grid per turn.
numTurnsBeforeCancerGrowth	Controls how many time steps need to pass before cells can become damaged. Represents a period of time in which the rate of cancer development is insignificant and normal cells are allowed time to form a developed piece of tissue.
nutrientMigrationConstant	Controls how often the nutrients migrate, relative to the cell migration speed. Nutrients will move [value] times faster than cells.

Table 1: Parameters and their functions

1. Introduction to the Grid Model

The grid is where the whole simulation takes place. It is divided into 256 x 256 grid spaces [each represented by one pixel], and each grid space includes both nutrients and cells. Each grid space can contain one cell, but can contain infinitely many nutrients.

2. Initialization Procedure

To start off the discrete cellular automaton model, one cell is created in the center of the grid [represented by one pixel]. The nutrients will also be distributed across the grid, with the number of nutrients across the grid being equal to nutrientStartProportionOfGrid times the total grid size.

3. Cell and Nutrient Movement

At each time step in the discrete model, cells will migrate with cellMigrationRate probability. If the cell is going to migrate, then it will migrate with equal probability to the unfilled spaces in the grid. Nutrients likewise will migrate with nutrientMigrationRate probability, and have an equal probability of migrating into any space that it surrounds, due to the fact that multiple nutrients can occupy a single space. Nutrients will migrate faster than cells do (exact rate is controlled by nutrientMigrationConstant), due to the fact that nutrients are constantly being circulated around the organism, and that cells are localized in the organism. Both cells and nutrients will not be able to migrate outside of the grid border (which is set at 256 x 256 pixels).

Cell-Nutrient Interactions [4]

When a cell and nutrients occupy the same grid space, then the cell has a chance of uptaking all the nutrients in that grid space with probability nutrientUptakeRate and can then utilize the nutrients in its cellular processes. The cell will periodically consume a nutrient in order to

survive - if it doesn't have any spare nutrients, then it will die. Additionally, when the cell has enough nutrients, it can proliferate (see section 5).

Cell Proliferation [5]

When a cell has enough nutrients, then it has a chance of proliferating in that time step. However, cell-cell interactions as well as space limitations will lower the chance of the cell proliferating. Overall, a normal cell with sufficient nutrients will have a $N^{4-E}(1 - \frac{P}{S})$ chance of proliferating in each time step, where N = effectOfNeighboringCellsOnCellDivision (a constant), E = number of empty cells around the proliferating cell, P = total population of cells in the whole grid, and S = total size of the grid (a constant). This expression is derived from the fact that more neighboring cells will result in increased anti-growth signals, as well as the space limitations posed by the presence of multiple cells. These limitations do not apply to tumor cells, such that the tumor cells will attempt to proliferate whenever it has enough nutrients, and when there is space available around it. After a normal cell proliferates, it has a chance of becoming damaged, and these cells will be resolved later in the program (see Section 7). On the other hand, when a tumor cell proliferates, then they remain as tumor cells, and cannot revert back. After any cell proliferates, both of the new cells will start off with one nutrient (the minimum necessary for survival).

Nutrient Refilling [6]

Nutrients will be refilled every few turns, in accordance with blood circulation and the diffusion of nutrients from the bloodstream into the cellular grid. There is a limit of nutrients that can be added per turn, as well as a limit to the number of nutrients on the grid, which simulates the homeostatic effect of nutrient distribution across the organism.

Abnormal Cell Processes [7]

Damaged cells will undergo one of three possible outcomes: mutation, apoptosis, or repair, which will respectively change the damaged cell into a cancer cell, make the cell die, or turn the damaged cell into a normal cell. If the damaged cell undergoes apoptosis, then its nutrients will be expelled to the spot on the grid (Note: normal/tumor cells can only die through lack of nutrients, so no nutrients will be expelled when they die). Other than these, there are no differences between normal and damaged cells. On the other hand, cancer cells will be able to divide uncontrollably (see sec. 5), and steal nutrients from neighboring cells. At every time step, the cancer cell will check each cell within a set radius (cancerCellStealingRadius) and take one nutrient from each normal cell that has nutrients. As a result, it will be able to quickly accumulate nutrients and proliferate quickly.

Work and Acknowledgements

These modifications to preexisting models were developed independently by the students involved in the project. The data analysis and the data collected was done independently by the students as well. Acknowledgements go towards the Mentor of this project, who was extremely helpful in guiding this project, and also allowed the students to use the Mentor's work area.

III. Results, Illustrations, and Discussion:

In order to document the effects of various environmental and biological conditions, one control was used, and one parameter was varied at a time. The control parameters can be found below:

nutrientKProportionOfGrid = 1	nutrientStartProportionOfGrid = 0.5	nutrientMigrationConstant = 50
nutrientMigrationRate = 0.8	nutrientUptakeRate = 0.8	cellMigrationRate = 0.8 damageRate = 0.1
damagedToNormalProbability = 0.5	damagedToDeathProbability = 0.499	effectOfNeighboringCellsOnCellDivision = 1.25
numNutrientsNeededForDivision = 2	numTimeStepsBeforeEating = 100	cancerCellStealingRadius = 1
nutrientReplenishRate = 1	nutrientAddLimitPerTurn = 200	numTurnsBeforeCancerGrowth = 500

In the resulting images from the simulation, a specific color code to depict the growth conditions, which apply to all the data generated. The specifics of this color code are shown here:

Cells	Graph	Nutrients
Green	Normal Cell with 1 nutrient.	Green
Pink	Normal Cell with > 1 nutrient.	Red
Blue	Damaged Cell	Black
Red	Tumor Cell	Yellow
Table 2: Color Code		Yellow
		Pink
		Gray
		Cyan
		Magenta
		> 4 nutrients in that grid space

In the below snapshots of the control data, the nutrient distribution maps were included in all the snapshots (though some show very little nutrients). As is apparent from the below images, the nutrient maps closely models the cellular growth areas. Thus, the nutrient maps will be omitted from the non-control figures.

1. Control Group

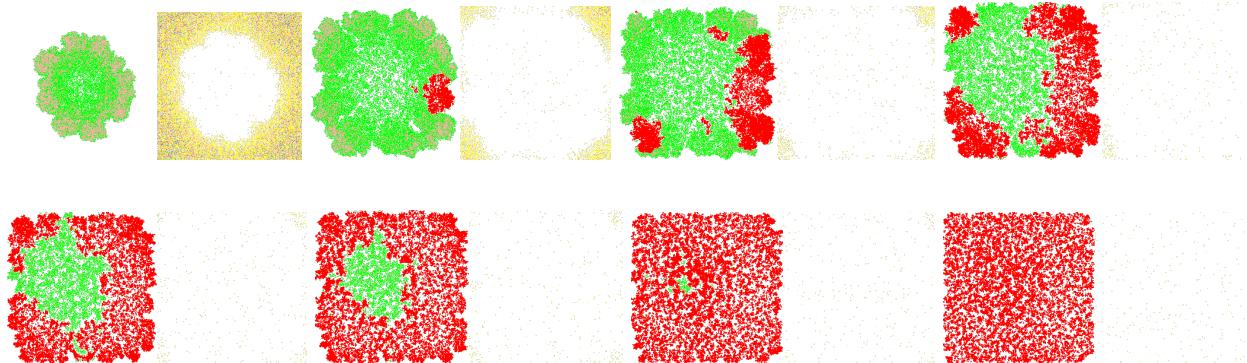
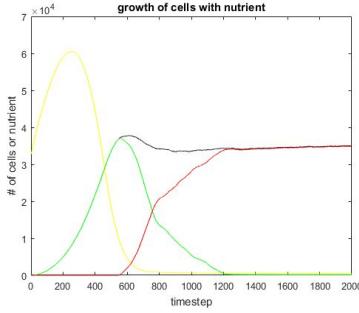


Figure 2: Control Figures, Snapshots taken at timesteps 400, 600, 700, 800, 900, 1000, 1200, 1600. See Table 2 for Legend.^{19,20}



In the control group experiment, the population develops in a method typical of cellular and cancer growth.^{10,11} In the beginning, it starts with one normal cell in the center and a 50 percent randomly filled nutrient map. At the 400th time step, it develops a filled cluster of normal cells covering around 50 percent of the cell map. The pink pixels at the circular edge of the cell population mark the normal cells with more than one nutrient; the fact that the pink cells concentrate on the edge suggests that the cells at the edge of the population obtain more nutrients than the cell in the inside, thus they proliferate at a faster rate. After 600 time steps, the first cancer cell develops on the side of the normal cell population. It proliferates rapidly by stealing nutrients from the surrounding normal cells through steps 600, 700, 800, 900, and 1000. The cancer cell clusters develop first around the edge of the normal cell population; this fact suggests that the cancer cells tend to proliferate faster around the location where the nutrients are plentiful and where the cells have plentiful nutrients that the cancer cells can steal. Also note that multiple cancer cells develop on multiple locations of the map, suggesting that the cancer cells in the model grow in a multifocal manner. After the cancer population completely surrounds the normal cell population, the cancer cells start to develop inward, gradually occupying the central area. As the cancer cells develop towards the center, the area where the cancer population had previously occupied becomes less concentrated, as more small hollow areas show up in the middle of the cancer population. After 1200 time steps, the cancer population occupies the

majority of the cell map, and after 1600 time steps, the cancer population occupies the whole map, reaching an equilibrium state, as the horizontal line on the curve of the total population suggests. Note that the number of normal cells does not reach zero in the end, but rather, stays at a low number ranging from 114 to 138.

2. Nutrient Effects

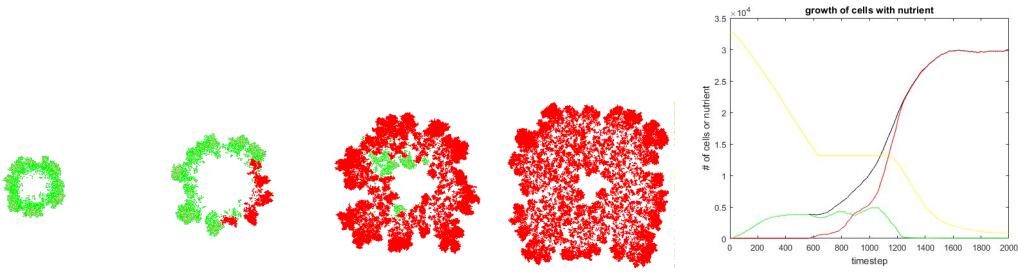


Figure 3: $\text{nutrientKProportionOfGrid} = 0.2$. Snapshots taken at timesteps 400, 800, 1200, and 1600. See Table 2 for Legend.^{19,20}

In the first experiment, the maximum proportion of nutrients that can be refilled to the grid is set to 0.2 instead of 1, meaning that if the nutrients cover more than 20 percent of the nutrient map, no nutrients are added. This influence is illustrated by the horizontal yellow curve approximately from time step 600 to time step 1200, where the nutrient level is kept at around 20 percent of the map until the cell population grows large enough to consume the nutrients faster than the nutrient replenish rate. After 400 time steps, the cell population grows into a ring structure. The normal cells with more than one nutrients are on the outside of the ring, the normal cells with only one nutrient lay in the inner of the ring, and a large hollow ring with few cells appears in the center. After 800 time steps, the cancer population develops at the bottom right of the ring composed of normal cells. The cancer develops along the ring by stealing nutrients from the normal cells rather than growing into the outer area where the nutrient concentration is high; this fact suggests that under the same circumstances, the cancer population grows faster where it can steal nutrients

from the cells, and this factor may have a greater impact than the surrounding nutrient concentration.¹⁴ After 1200 time steps, the cancer cells replace almost all the normal cells on the ring structure, and the cancer cells start to grow inward to the center of the map. After 1600 time steps, the cancer population almost fills the center hollow area, leaving only a small area of a low concentration of cells. Note that the cancer cells still have a ring structure, although it is wider than before (at the 1200th time step). On the outer edge of the cancer population, the cancer cells tend to be more concentrated, while on the inner area, the cancer cells tend to scatter and to be less concentrated. This result is explained by the commonly observed characteristics of cancer cells, such as necrosis of faster developing cancer cells⁷. When the cancer population grows too fast, the inner area of the cancer population cannot get enough nutrients due to a lack of nutrient supply; thus, there is a less concentrated area of cancer cells in the center of the cancer population.

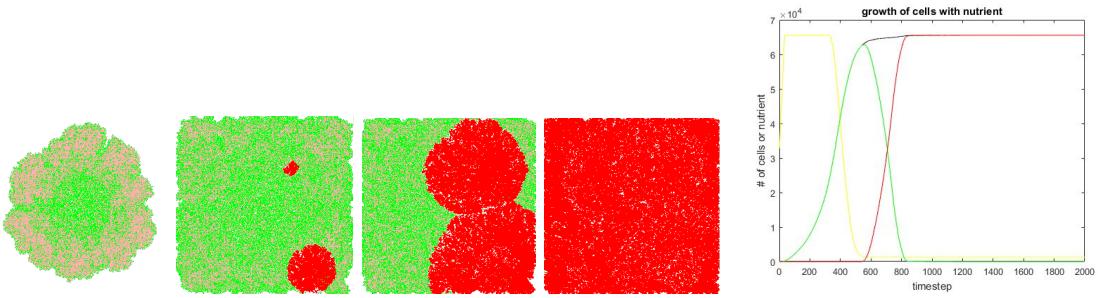


Figure 4: $\text{nutrientAddLimitPerTurn} = 1000$. Snapshots taken at timesteps 400, 700, 800, and 1000. See Table 2 for Legend.^{19,20}
In the second experiment, the maximum number of nutrients that can be added to the map each time is set to 1000 instead of 200. This change results in a much faster growth rate for both normal cells and cancer cells, which is representative of a homeostatic well-vascularized tissue. After 400 time steps, the normal cells still grow in a ring structure as the pink cells with more nutrients still lie on the outside, however, the inside area is filled and no hollow structure shows

up in the center. After 800 time steps, the normal cells already occupy the whole map, and the cancer cells starts to develop from two locations. This time, the cancer population arises from a fully developed normal cell population, compared with the control, which was not completely covered at the time cancer started to develop. The cancer cluster develops in a circular pattern and soon occupies the whole cell map after 1000 time steps (compared with ~ 1600 for the control). The steep slope of the tangent line to the curve representing the cells also suggests that the increase of nutrient replenish capability drastically increases the growth rate for both cells.

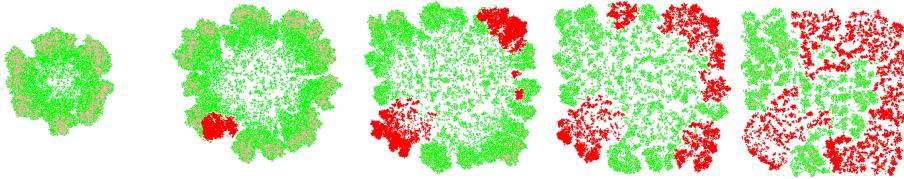
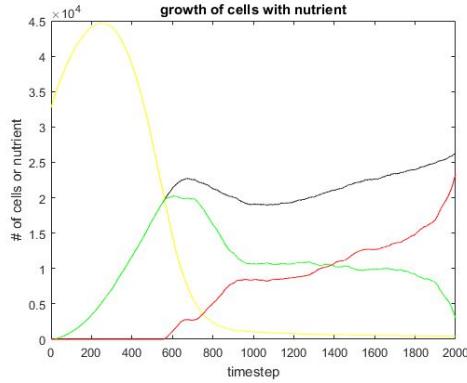


Figure 5: $\text{nutrientAddLimitPerTurn} = 100$. Snapshots taken at timesteps 400, 600, 800, 1000, 1600. See Table 2 for Legend.^{19,20}



In the third experiment, the maximum number of nutrients that can be added to the map each time is set to 100 instead of 200. This change results in a much slower growth rate for both normal cells and cancer cells. Due to a lack of nutrient replenishment, the existing nutrient concentration cannot support large group of connected cell population; thus, the cells tend to grow in isolated islands, that is, small individual clusters of cells, which tend to spread out from each other. This is in contrast to the relatively well-connected cancer seen in the control. The

cancer cells still invade into the normal cell clusters, but in a much slower rate, as the cancer population still does not occupies the cell map after 1600 time steps. The cancer invasion is also obstructed by this isolated cell structure, as the squiggly curve of the cancer population suggests.

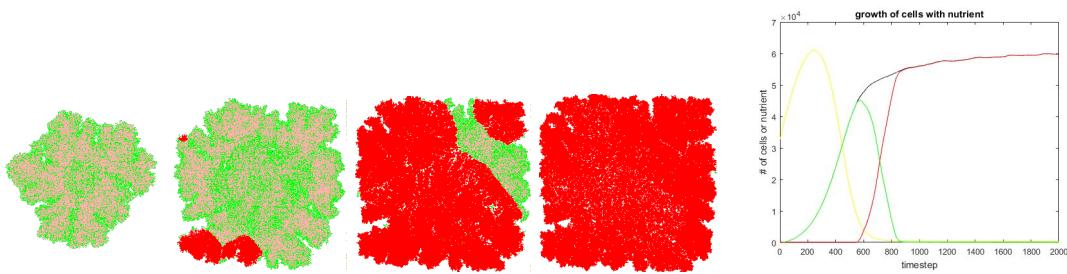


Figure 6: numTimeStepsBeforeEating = 250. Snapshots taken at timesteps 400, 600, 800, and 1000. See Table 2 for Legend.^{19,20}

In the fourth experiment, the number of time steps that passes before the cell consumes one unit of nutrient is changed to 250 instead of 100, thus increasing the lifetime that one cell can survive without additional nutrients. After 400 time steps, the normal cell population grows larger than the one in the control group and the pink cells with more than one nutrient are spread out evenly throughout the cell population. After 600 time steps, the cancer cells appear and quickly occupy the whole map after 800 and after 1000 time steps. The slope of the line tangent to the cancer cells' growth curve is very steep, suggesting faster growth than the control. Since the nutrient supply sustains the cells for a longer time, the final population of cancer cells tends to form a large continuous block with few blank areas inside, suggesting a high concentration of cancer.

3. Migrational Effects

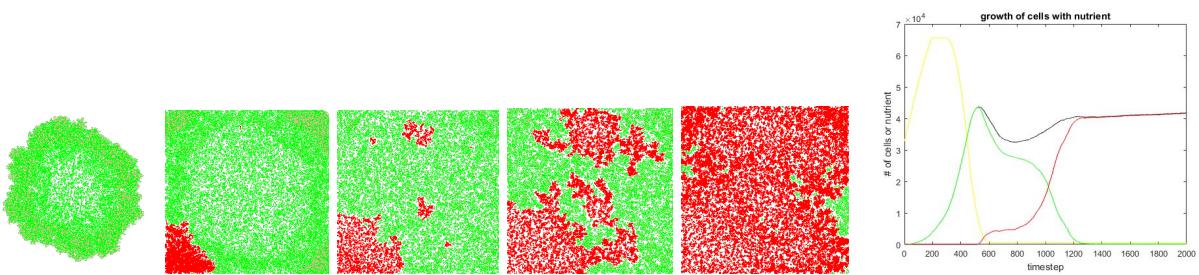


Figure 7: nutrientMigrationConstant = 5. Snapshots taken at timesteps 400, 600, 800, 1000, 1600. See Table 2 for Legend.^{19,20}

In the fifth experiment, the nutrient migration speed, relative to cell migration speed, is changed to 5 instead of 50. This change slows down the process of cell development as the nutrient concentration around the cell population is always lower than the nutrient concentration of the area with fewer cells. At the 600th time step, the cancer population is concentrated at the bottom left corner. After 800 time steps, however, the cancer population starts to scatter and it has a lower overall cell concentration. This change is because the nutrients in the original location are used up and few nutrients are able to migrate to these cells due to a low migration speed. After 1000 time steps, the cancer population is developed in an irregular shape towards the center rather than a circular cluster or a ring structure. This contrasts with the control, in where the cancer cells are seen to occupy most of the space. This shape may be due to the fact that the low nutrient motility does not increase the fitness of circular structures which are favored by plentiful nutrient diffusion, but rather, the low nutrient motility gives a fitness advantage to those irregular shapes with large perimeters which can get into touch with more nutrients¹⁵.

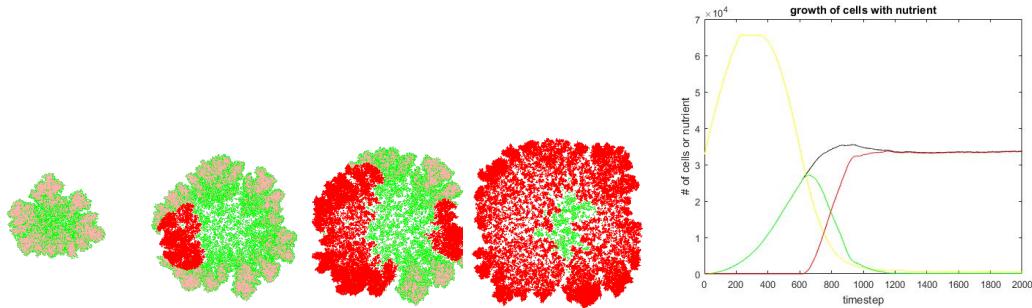


Figure 8.0 cellMigrationRate = 0.2. Snapshots taken at timesteps 400, 700, 800, and 1000. See Table 2 for Legend.^{19,20}

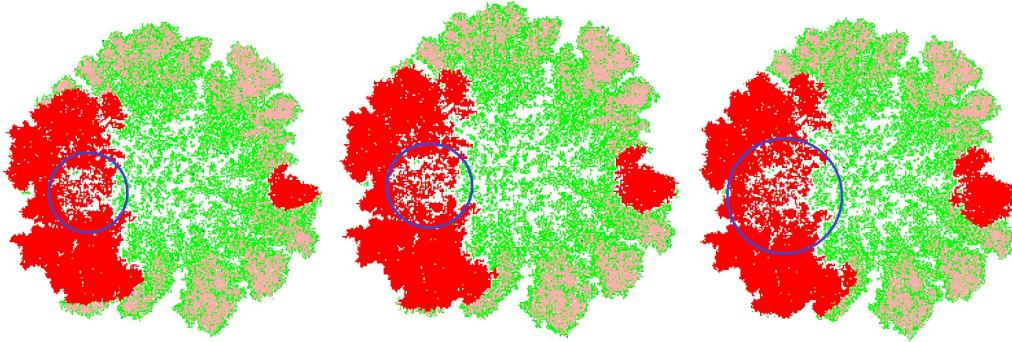


Figure 8.1 $\text{cellMigrationRate} = 0.2$. Snapshots taken at timesteps 750, 760, 770 to show inner area. See Table 2 for Legend.^{19,20}

In the sixth experiment, the probability at which the cells migrate is changed to 0.2 from 0.8, which decreases the motility of cells. In the graph generated in figure 8.0, the maximum number of nutrients reached is around 65000, compared to the number in the controlled group, around had approximately 60000 nutrients. This increase in the maximum amount of nutrient suggests an decrease in the nutrient consumption rate, which is caused by a lower cellular motility. This result is in accordance with Phan's model in where the cellular motility increases the cellular growth rate. This decrease of cellular motility also brings in a closer look to the changes of the inner area of the cancer population, since the pattern is more recognizable with less disruption from cell migration. In figure 8.1, three consecutive snapshots are taken at time steps 750, 760, and 770 with the artificial blue circle to indicate the area of interest. There is an area of low cancer concentration in the center of the cancer cluster, and as time passes, this area of low concentration expands in an approximately circular pattern. This pattern contrasts with the growth pattern of the cancer population in the control, in where the cancer cells are distributed in the cell map with approximately equal density. This inner low-density area is in accordance with the cancer cells' lack of angiogenesis. With low motility, the inner cancer cells tend to not be able to obtain enough nutrients, and they die together in the inner area.

4. Combined discussion:

Compared to the control group, the sets of experiments each display a unique pattern of tumor growth. By decreasing the maximum nutrient replenish amount to 20 percent of the grid space, both the normal cells and the cancer cells grow in a ring structure (figure 3). By increasing the nutrient replenishment rate to 1000, both the normal cells and the cancer cells grow dramatically faster, and the clusters of cell population are dense and continuous (figure 4). On the other hand, by decreasing the nutrient replenish amount to 100, both the normal cells and the cancer cells grow dramatically slower, and the clusters of cell population are sparse and scattered (figure 5). By increasing the amount of time steps that a cell can sustain itself with just one unit of nutrient, the cancer cells also grows in a faster rate, but the cancer population grow in an irregular shape (figure 6) rather than the circular shape displayed in figure 4. These changes of the variables regarding the distribution of nutrients suggest that a higher nutrient replenishment rate will lead to a faster growth rate in both normal cells and cancer cells; however, the exact way in which the nutrient distribution is changed can yield different growth patterns for cancer cell population. By decreasing the nutrient migration speed to 5 times as fast as cell migration, the cancer cell population grows in a way such that it has a large perimeter, instead of expanding towards the area with plentiful nutrients (figure 7). By decreasing the cell migration probability, the cancer population displays an unique property in that the cancer cells in the inner area are less dense, and continuously die in an organized pattern, creating a sparse area in the middle of the cancer cluster. Those quantitative changes of the extent of motility of cells and nutrients create completely different types of cancer development.

These results not only suggests that the motility of cancer cells have an effect on the tumor growth pattern⁴, but also that the motility of nutrients can directly affect the pattern of tumor growth. This model builds up on the work of Phan, who demonstrated that cell motility increases fitness in solid tumors, but goes beyond it by considering multiple new environmental factors of tumor growth. Based on the biological information provided, the model created predicts many realistic characteristics of tumor growth, such as the effects of the lack of angiogenesis² (figure 8.1), necrosis⁷ (figure 3), migrational effects, multifocal development¹³ (figure 2) of cancer cells, and autophagy (figure 3).¹⁴ The model is also unique as it models the tumor growth in the environment of a fast growing normal cell structure. It also demonstrates the tumor growth patterns in epithelial tissues such as breast cancer. The model is additionally able to yield various patterns of cancer development from different inputs of environmental variables. The various parameters regarding nutrient allocation yields completely different results, which can be matched to specific tumor growth conditions.

IV. Conclusion and Further Work

Overall, the experimental results demonstrate that a biophysical model can be built to respond to various biophysical conditions, including cell growth rate and nutrient concentration. Though this model cannot be characterized as 100% accurate, it still agrees with much of the in vivo experimentation data presented in many previous articles.^{2,3,4,14} Due to the fact that the data obtained agrees with many previous works, it is highly likely to be accurate at the simulation of tumor growth. As such, it can also provide a way for predicting the conditions (such as nutrient concentration and migration) in where actual tumor growth occurred, based on its growth patterns.

To refine our conclusions and to increase the validity of our methods, further implementation of more biological features into our model, such as immune cells or blood vessels, can be done. These methods have biological roots and have been well-documented in the field of cancer biology.^{2,12,16} Additionally, the implementation of a 3D model, instead of simply a 2D cross-section, can also be done in order to more realistically model the cellular growth conditions. As a result, in order to make our results more significant, adding immune cells, blood vessels, and extending the model to 3D would be our next steps in refining our cellular automaton model to make it more accurate.

During the development of this project, not many problems were ran into during the actual execution, but the start was somewhat unorganized. If this project was restarted today, then we would start off by reading more papers in the area of interest, and have a more specific question to answer, before actually beginning to write the program and running test cases. With these factors in mind, our research could have become more efficient and more impactful.

Despite the results created through this model, there are still many unanswered questions regarding tumor growth. For example, we still don't know about the effects posed by implementing blood vessels completely, implementing immune cells and the effects of immunotherapy, or the changes that will result in the cellular growth structure if the model was extended to three dimensions.

V. References

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