Cellular Automata Model of Angiogenesis

Formation of Blood Vessels on a Cellular Level -
A Final Project Report

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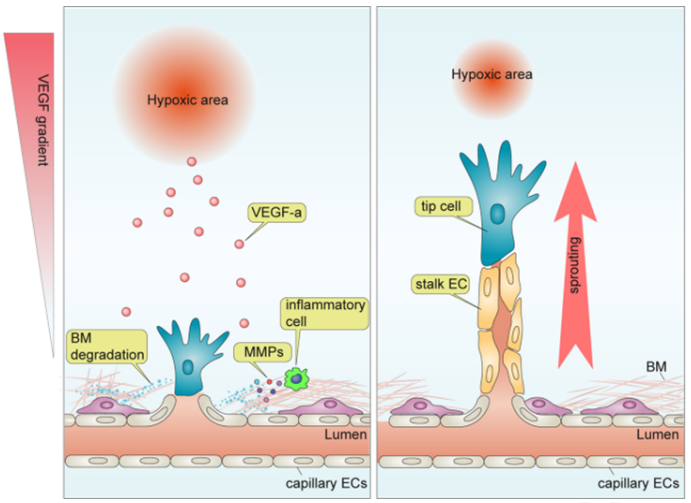
Introduction

Biological Problem - Angiogenesis

Angiogenesis is a biological process that involves the production of new blood vessels in mammals, specifically in response to nearby hypoxic tissue. There are several key concepts and rules which guide this mechanism, and our goal is to model this phenomenon and provide insights related to the driving forces of this process.

The key concepts that play a role in Angiogenesis, in chronological order, are
1. Growth factor signaling- VEGF-A (Vascular Endothelial Growth Factor A) is released by the hypoxic tissue and binds to the VEGFR2 receptors in the endothelial cells of an existing blood vessel. VEGF signaling promotes the formation of new vessels.
2. Sprouting - Differentiation into Tip and Stalk cells - One of the cells that intercept the VEGF strongly becomes a *Tip* cell.
The differentiation activates the Notch signaling pathway in the Tip cell, which causes adjacent cells to differentiate into *Stalk* cells. Tip cells are highly migratory. Stalk cells on the other hand are more stationary and are specialized in proliferating.
3. Vessel outgrowth - migration of the tip cell - The tip cell migrates in the general direction of the gradient created by the VEGF diffusion in the area. A physical link between the tip cell and the adjacent stalk cells is maintained, and the stalk cells proliferate to "fill the gaps" as the tip cell elongates the new vessel.



[4]

4. Anastomosis - Sprouting vessels eventually fuse to neighboring vessels and mature to become endothelial cells. This creates a closed, functioning vascular network.

VEGF signaling promotes sprouting by increasing the emergence of tip cells. Notch signaling and other regulatory networks, on the other hand, decrease sprouting in the area of the tip cell. Noguera-Troise et al. [2] showed the necessity of the phenomenon in the context of cancerous tumor growths, which are correlated with dense vascular networks surrounding them. Paradoxically, when VEGF is less negatively regulated, the growth of the tumor is eventually slowed down. Increased sprouting causes the network to become so dense that it loses its capability of effective oxygen supply, and subsequently causes the death of nearby hypoxic tissue. This strange effect ignited our motivation for using CA to model the phenomenon.

Computational Challenge

The computational challenge in modeling angiogenesis lies in the large amount and complexity of the factors related to the process. Attempts to model the problem in a purely analytical manner usually produced continuous models that did not provide results concerning the actual structure and morphology[3]. A more promising approach is cellular automata, which is a discrete approach based on a lattice (or a grid) containing individual "agents" and a set of localized rules for them. This model may provide bidirectional research of the

phenomenon, by producing a global pattern from the set of rules and allowing investigation of the pattern to obtain new rules. This approach is well-suited to study angiogenesis, since it bridges the micro and macro levels, and can be used to rapidly test different rulesets.

Methods

The cellular automata

To simulate angiogenesis, we implemented a cellular automaton based on two driving forces: The chemical gradient created by the concentrations of the growth factor (VEGF), and the mechanical pressure exerted by cells. We used a finite lattice, which hosted three different types of cells, each following a different set of probabilistic rules (see appendix 2.1) which were applied synchronously:

Attractor Cells: These cells represent locations that require blood such as new or injured tissue, or cancer cells. They increase the concentration of VEGF in their surroundings in relation to the distance. Meaning that as you get closer to the attractor, the concentration rises.

Stalk Cells: Proliferative cells that act as the main building block of the blood vessel. By proliferation, they are able to create Tip Cells during a process called sprouting.

Tip Cells: Migratory cells that migrate along the gradient of VEGF. In the biological system, the tip cells are physically attached to the stalk cells. In our model, we use a simplification in which the movement of tip cells creates a trail of stalk cells.

The attraction gradient

The VEGF created by the attractor cells is propagated through the lattice, resulting in a concentration gradient. The resulting concentration is used as "attraction" values that determine the direction of action for other cells. Equation (1) shows an exponential decay equation, inspired by Fick's law and the gaussian that is used to calculate the attraction exerted by one cell (S) on another cell (A).

$$1.\quad attraction_{s:a} = attraction_{src} \cdot e^{-D \cdot |S-A|_2}$$

Where $attraction_{src}$ is the attraction cell S exerts on itself (at source location), D is the decay coefficient, and $|S - A|_2$ is the euclidean distance between S and A.

Given that a cell is located in the tile T, it can feel attraction from multiple sources (cells). Thus, we can sum up the attraction generated by all the sources $s_1, s_2, \cdots, s_i$. Equation (2) shows the attraction of a tile T:

$$2.\quad attraction_T = \sum_i attraction_{s_i:T}$$

Directional actions

Each action performed by a cell, both migration and proliferation require a direction. This direction is affected by the chemical potential (attraction generated by attractors), and the mechanical pressure (presence of cells in the neighborhood of their neighbors). Equation (4) shows the attraction value of T, affected by the mechanical pressure of the neighbors.

$$3.\quad attraction^m_T = \frac{attraction_T}{\#neighbors\ of\ T}$$

Equation (3) shows the probability of a cell A, to move to a Tile T. The tiles in A's neighborhood are marked $T_1, T_2, \cdots, T_8$.

$$4.\quad P(A \to T) = \frac{attraction^m{}_T}{\sum_{i=1}^{8} attraction^m{}_T}$$

Code design

We have implemented the cellular automata using OOP in python 3.10.
Our code design (see appendix 2.2) consists of several objects that represent the different parts of the simulation. The simulation Engine contains the history of all grids. Each grid consists of a 2d array of tiles that can contain instances of cells. We have used inheritance to implement the cell API, and three inheriting classes: TipCell, StalkCell, and AttractorCell.
The next iteration (see appendix 2.3) is calculated using a well-defined chain of function calls, by each Grid on the cells. For each cell, the board uses get_context() to check what information the cell requires. The grid then calls get_cell_actions(context) to check which actions the cell wants to perform in this iteration (according to the automaton rules). After collecting all the actions of all cells, the grid creates a copy of itself and performs the actions on the new copy to generate the grid for the next iteration. This method was chosen to provide a clear and extensible simulation. This allowed us to modify and replace large parts of the model in a timely manner, which proved useful when trying different approaches as described in the results and discussion chapters.
Alongside this, a statistics module was developed to keep track of the results and provide options for quantitative analysis.
Simulation parameters were stored in an external JSON file and parsed using the built-in library.

Parameter name	Default	Meaning
tip_cell.p_migrate	0.9	Chance of a tip cell to move during each iteration
attractor_cell.attraction_generated	1e18	Attraction that the attractor cell exerts on itself (on distance 0)
stalk_cell.p_sprout	0.008	Chance of a stalk cell to sprout on each iteration. As shown in the *results* chapter, this lets us regulate the VEGF pathway.
attraction.decay_coef	0.7	How fast does the attraction decay (see equation (1))
attraction.update_precision	0.001	Used to optimize updating of board attraction. Changes smaller than this parameter will be ignored.
debug	false	If true, the simulation will display debug diagrams to visualize the direction decision of each cell.

Visualization was achieved using numpy and matplotlib, based on examples that were shown in tutorials.

Results

Default grid structure:

Shape	Colors
60x60	Tip cells: Yellow | Stalk cells: Red | Attractor cells: Blue

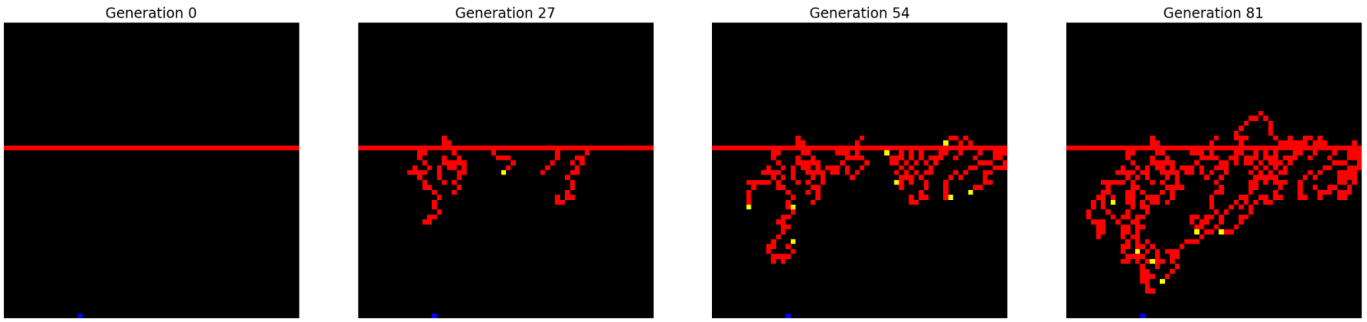
Criterion for assessment of result:
Each result is mainly judged visually. In addition to counting the amounts of different cells, we use a measure to estimate the density of the resulting shape. The *clustering coefficient* is defined as follows: $\frac{1}{\#cells}\sum_{i=0}^{\#cells}\frac{\#Neighbors(cell[i])}{8}$.

For each living cell in the grid, we calculate its number of neighbors and divide it by the maximum possible number of neighbors, which is eight. We then average this measure across all cells.

In the following simulations, we use the default configuration (see code design) and change only the p_sprout and decay_coef parameters. Each simulation is composed of 81 iterations.
Full results comparison can be found in appendix 1.

Simulation #1 - Best result:

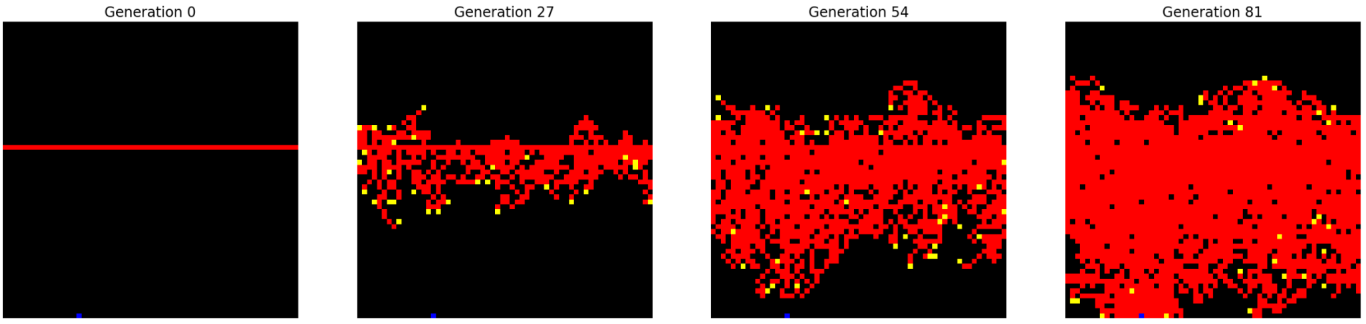
Input: p_sprout = 0.008 decay_coef = 0.7



Clustering Coefficient: 0.392
This result visually resembles real-world, typical angiogenesis, as the final shape looks like a web or a network of stalk cells, not too dense but not too spread out either, generally coming closer to the attractor region.

Simulation #2 - A “Blob”:

Input: **p_sprout = 0.016** decay_coef = 0.7

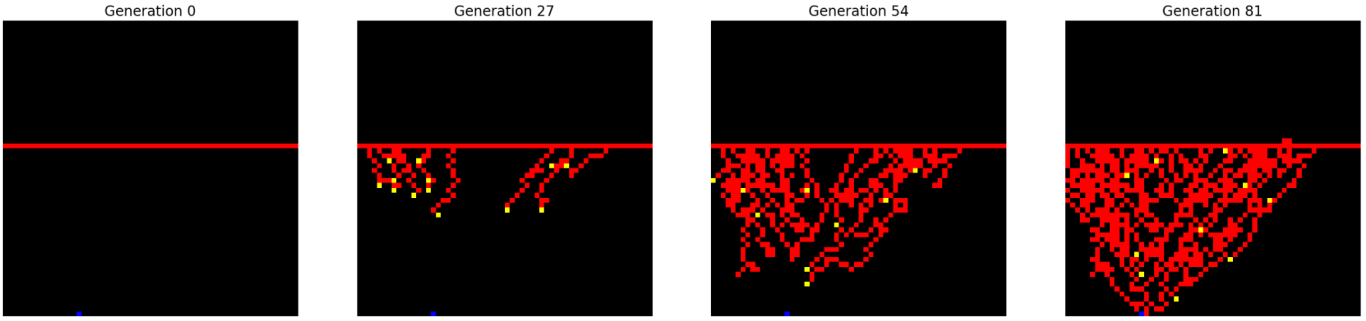


Clustering Coefficient: 0.624

This result shows the formation of an extremely dense unorganized network or a “blob”.

Simulation #3 - Heightened Sensitivity to VEGF:

Input: p_sprout = 0.008 **decay_coef = 1.4**

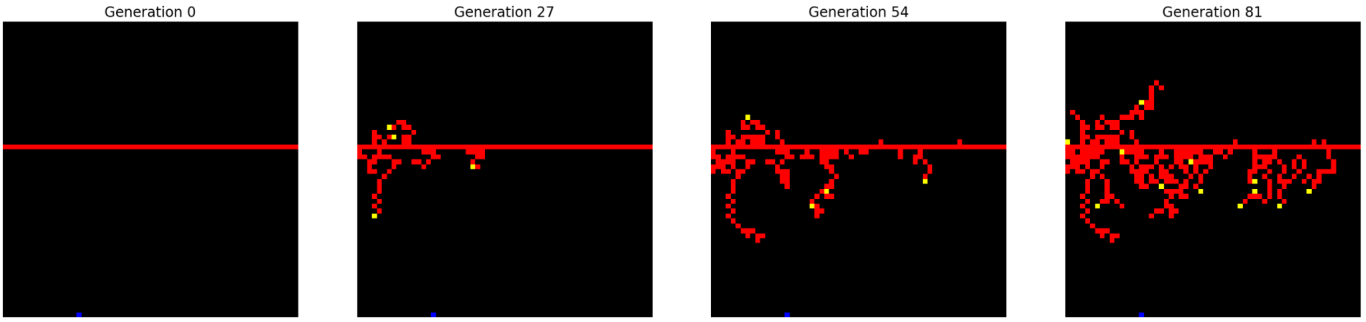


Clustering Coefficient: 0.464

This result shows the sprouts having more linear shapes as they approach the attractor cell.

Simulation #4 - Lowered Sensitivity to VEGF:

Input: p_sprout = 0.008 **decay_coef = 0.35**



Clustering Coefficient: 0.404

In this result, we see that the vessels spread but not necessarily towards the attractor cell.

Discussion

The model we present here shows the necessity and effect of several factors on angiogenesis. For one, our final model shows how a VEGF concentration gradient can alter a tip cell's stochastic migration to be a non-random and even guided movement toward its source.
As seen in simulations #3 and #4, toying with the *decay_coef* parameter caused the gradient to change its steepness, and hence also the tip cell's behavior. A bigger slope made the cells more sensitive to the direction of the VEGF source, causing them to migrate almost in a straight line, with much less variance in their choice of direction. On the other hand, a smaller gradient made the cells less sensitive to the potential, and thus much more varied in their directions, never reaching the attractor cell in the given timeframe.
In addition, our model shows the importance of regulation on sprouting; The *p_sprout* parameter decides the rate of the sprouting process, and thus works as a simplified regulatory pathway in our model. As we've shown in simulations #1 and #2- it correlates strongly with network density, fitting what research suggests[1].

One issue that was observed in the current model, is the way probabilities are normalized when choosing directions for the actions. The probability is calculated in a way that might cause very small attraction values and large attraction values to behave the same because only the *ratio* of attraction affects the probability. To address this issue, future models could normalize the probabilities proportionally to the total attraction on the grid, or implement other features such as movement speed to represent the difference in the amount of attraction.
In addition, our model lacks many other factors and features of angiogenesis, such as tissue density, lumen width, the mechanical effect of blood flow on proliferation, genetic expression variance between the cells, and many more.

Our model underwent numerous changes and additions of parameters until it showed good results, and each modification had shown us the importance of another key concept. For example, the addition of mechanical pressure made tip cells move away from dense areas. Another major change had been the permanent attachment of stalk cells to tip cells by making them form in place of a migrating tip cell.
At first, we let the stalk cells proliferate towards the tip cells with varying probabilities, but as time passed we concluded that stalk cells must have a 100% chance of proliferating towards their tip cell, which is equivalent to them being physically attached. And indeed, all research on the topic we've encountered assumes this physical attachment.

Author contribution

Literature review: MR & MH
Code design & implementation: MR & MH
Results visualization: MR, based on examples from the tutorials
Simulation execution and result analysis: MR
Parameter optimization: MH
Presentation: MR & MH
Biological background summary: MR
Methods description: MH

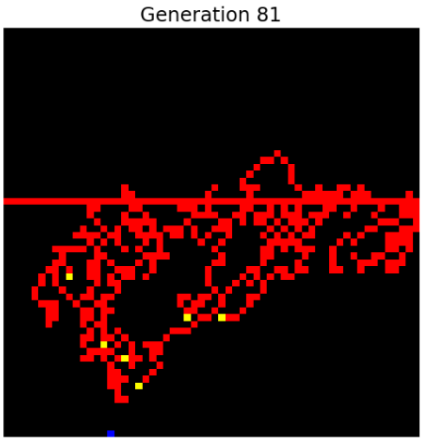
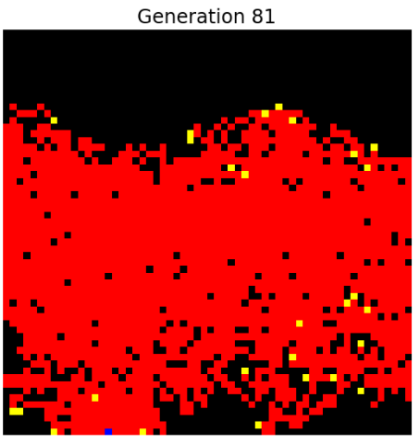
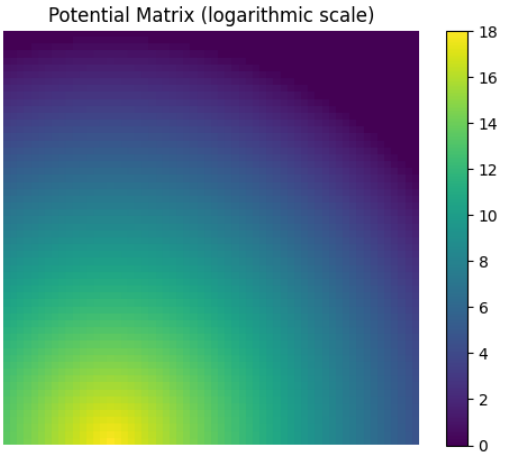
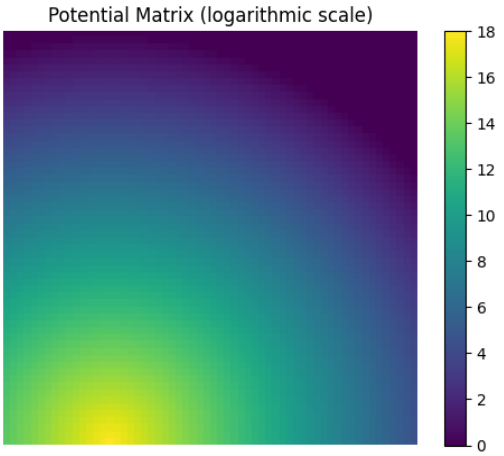
References

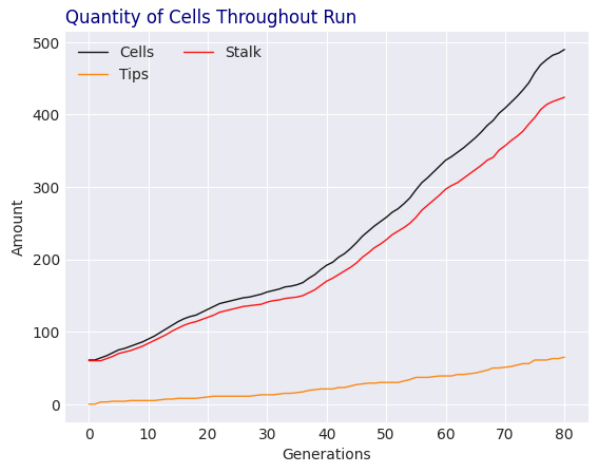
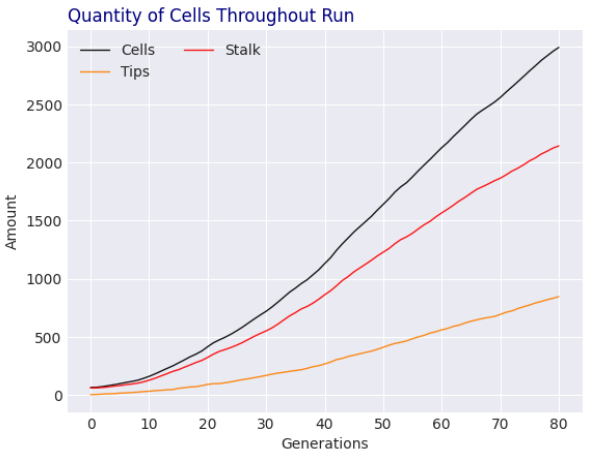
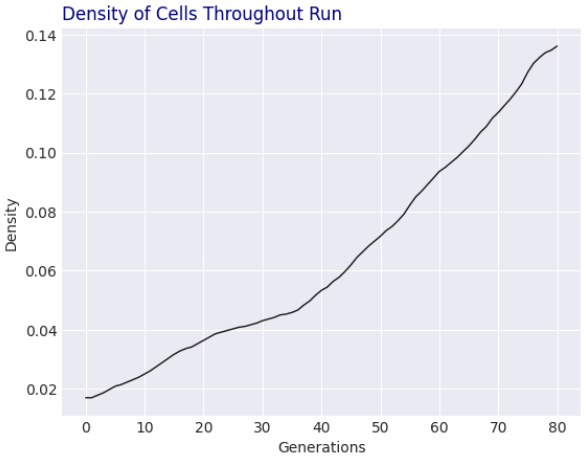
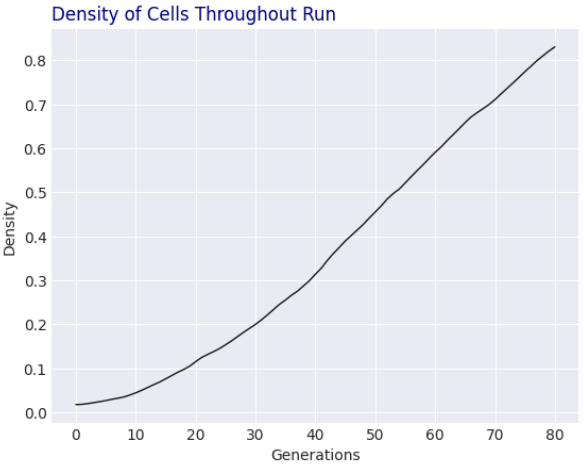
[1] Udan, R. S., Culver, J. C., & Dickinson, M. E. (2013). Understanding vascular development. Wiley interdisciplinary reviews. Developmental biology, 2(3), 327–346. https://doi.org/10.1002/wdev.91
[2] Noguera-Troise, I., Daly, C., Papadopoulos, N. et al. (2006). Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Nature 444, 1032–1037 https://doi.org/10.1038/nature05355
[3] Guidolin, D., Rebuffat, P., & Albertin, G. (2011). Cell-oriented modeling of angiogenesis. TheScientificWorldJournal, 11, 1735–1748. https://doi.org/10.1100/2011/586475
[4] Lee, HW., Shin, J.H. & Simons, M. Flow goes forward and cells step backward: endothelial migration. *Exp Mol Med* **54**, 711–719 (2022). https://doi.org/10.1038/s12276-022-00785-1

Appendix

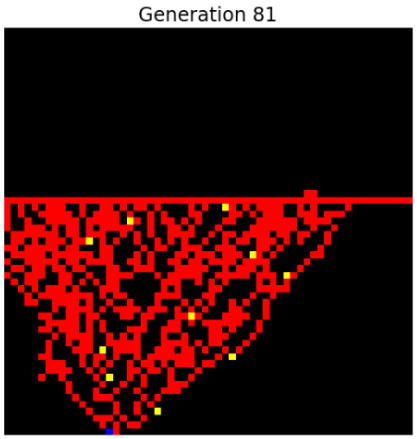
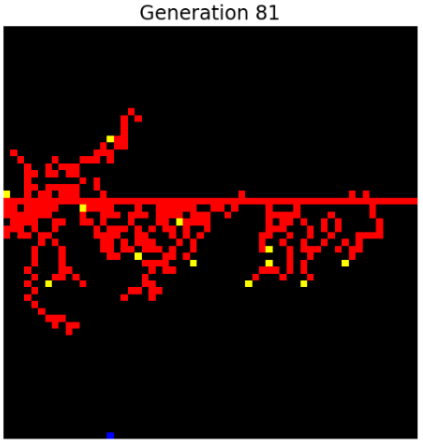
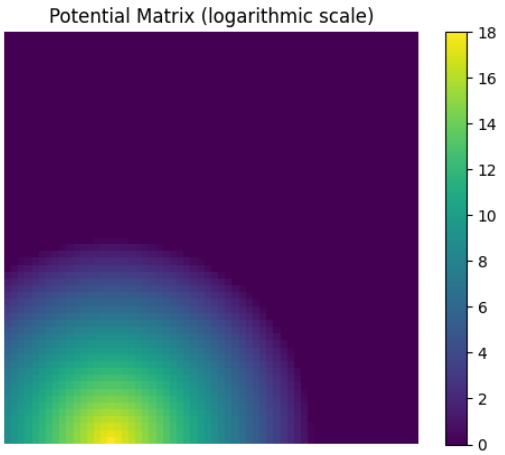
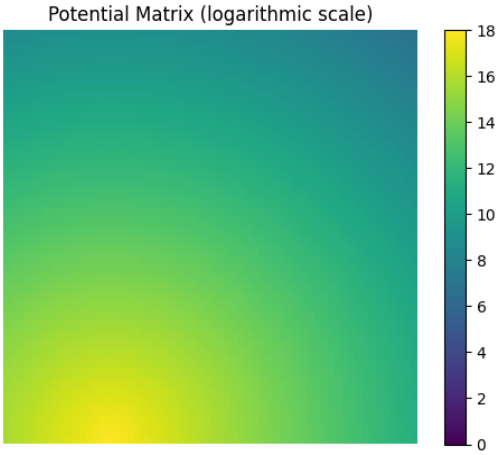
1. Results

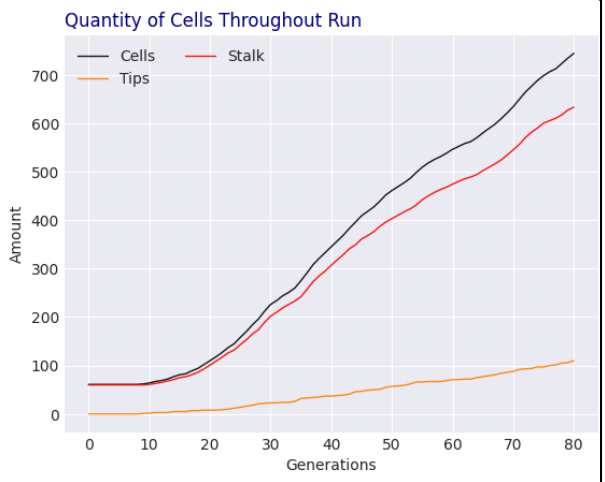
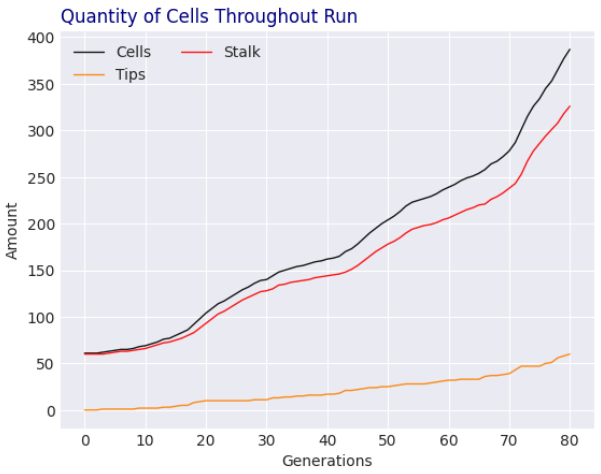
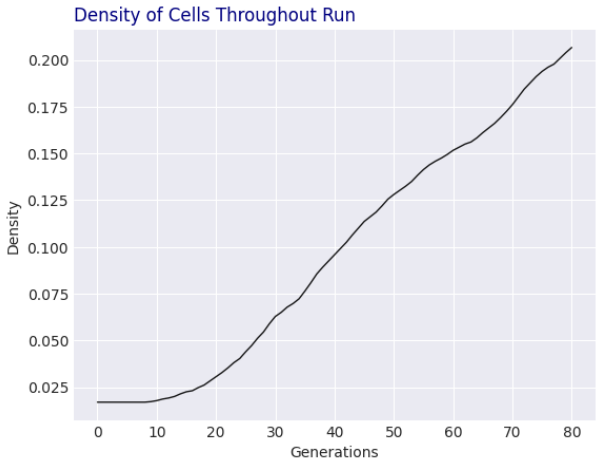
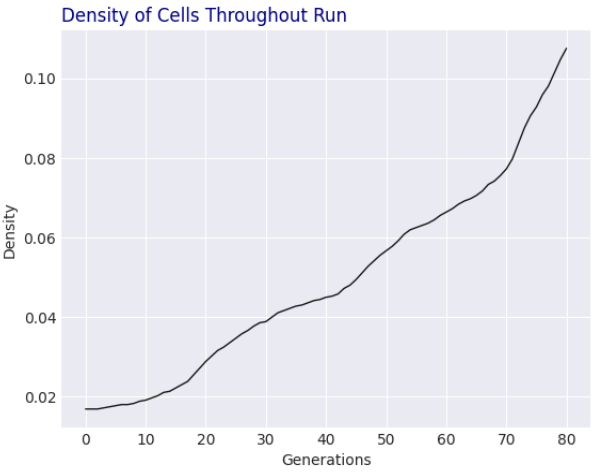
1.1 Comparison of Simulation #1 and #2

	Simulation #1	Simulation #2
Parameters	**p_sprout = 0.008** | decay_coef = 0.7	**p_sprout = 0.016** | decay_coef = 0.7
Final result		
Potential gradient		
Clustering coefficient	**0.392**	**0.624**

Cell count		
Density		

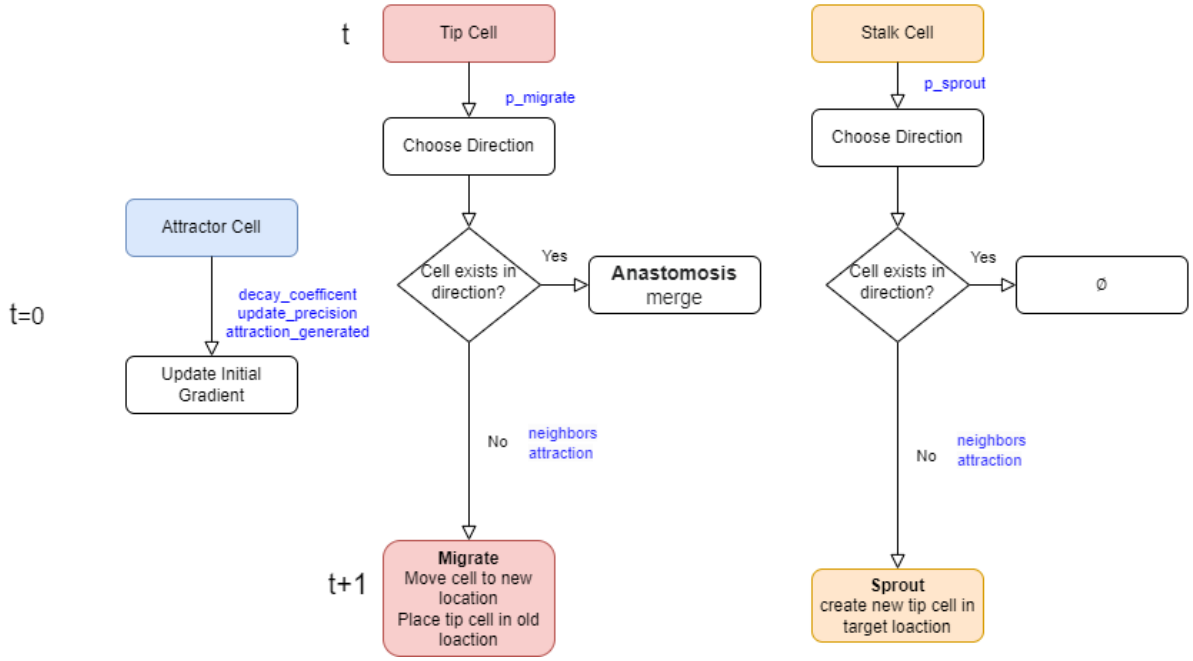
1.2 Comparison of Simulation #3 and #4

	Simulation #3	Simulation #4
Parameters	p_sprout = 0.008 | **decay_coef = 1.4**	p_sprout = 0.008 | **decay_coef = 0.35**
Final result		
Potential gradient		
Clustering coefficient	**0.464**	**0.404**

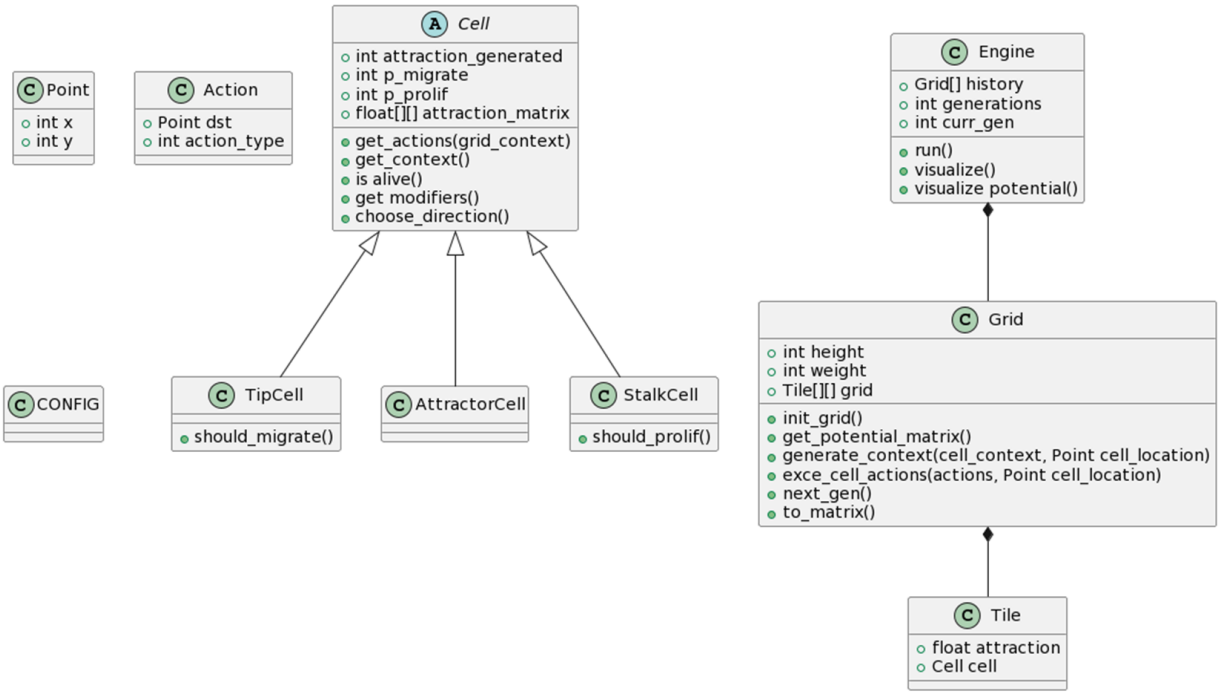
Cell count		
Density		

2. Methods

2.1 The states of different cell types



2.2 OOP UML



2.3 Chain of calls during iterations