Numerical modelling of a branched cell network development interacting with a diffusive chemical gradient

UNIVERSIDAD DE CHILE

Author: Mathis Ray S.
Teachers: Rodrigo Arias F.
Ignacio Bordeu W.

July 2024
Santiago de Chile

Contents

1 Abstract

Cellular networks in animal organs seem to fill space remarkably efficiently: they leave no empty space, while creating no intersections between branches. In order to understand the associated mechanisms, numerical modelling of such a phenomenon is a favoured avenue. However, current models, some of which do provide satisfactory results in terms of the final architecture of networks, remain imperfect in terms of their methodology. In fact, the annihilation process (the one that stops the development of a branch before it intersects with another) does not seem to be consistent with the biological behaviour of cells.

This study proposes that it is a chemical interaction between branching cells and their environment that enables them to avoid collisions. It proposes a numerical model and then studies it.

2 Introduction

2.1 Context and challenges

2.2 State of the art

2.2.1 Biological process

The branches can be described in two parts, the stalk and the tip, as shown in figure 1. The tip corresponds to a set of cells, called 'active' cells, grouped together in a highly permeable membrane of fixed volume. Thanks to the permeable membrane, these cells can consume a chemical compound from the outside, enabling them to divide and thus remain active. Because they divide in a fixed volume, this space at the end is permanently saturated, pushing active cells further back towards a second zone, the stalk. The membrane there is less permeable, which hinders the consumption of the chemical compound, making them inactive. This second zone constantly receives new cells from the tip, causing it to elongate and pushing the tip forwards. This is the process of elongation. As it progresses, the tip accumulates adhesive molecules (those that hold the mesenchyme together) at its boundary. Figure 2 illustrates how these molecules end up separating the tip in two. This is the phenomenon of division, as explained in [4].

2.2.2 Branching Model

A numerical model of this behaviour was suggested in [1]. It accounts for the microscopic phenomena described in the previous part, but from a more macroscopic scale. The entire 'tip' is only modelled by one object called an 'active cell' (which does not correspond to a cell in the biological sense, but to a set of cells). The rest of the branches, i.e. the stalk (where the inactive cells in the biological sense accumulate) will be modelled by a succession of 'inactive cells' (in the numerical sense) linked by segments. Active cells will always be represented in red, and inactive cells in blue. In concrete terms, at each time step, the inactive cells remain inert, while each active cell carries out one of the following three actions: elongation, division and annihilation. Elongation corresponds to the forward movement of the active cell, leaving an inactive one behind: the branch lengthens. Division is a similar process, but involves an active cell producing two forward cells, always leaving an inactive one behind. Annihilation consists of the inactivation of the branching (the end becomes inactive). An inactive cell remains inactive for good. The choice between the first two processes is random according to a Poisson distribution. The third process is systematically activated when two branches intersect. Once implemented, this model gives results like figure 3. However, the annihilation process here does not seem to propose any biological mechanism for its activation. How does an active cell know that it should inactivate and not duplicate when it is too close to another? It is possible that this is due to an interaction between the network and a chemical compound present in the environment.

2.2.3 Interaction cells-chemical

The paper [2] proposes an interaction between cells and a chemical environment. It sets up a system of consumption by the cells, as well as a diffusive phenomenon. This paper will be of interest later, as it implements an interesting consumption function for cells.

2.3 Goal of this study

This study will therefore focus on linking the contributions of these two articles. It aims to model a branching model similar to the one proposed, while incorporating the notion of chemical gradient, so as to implement an annihilation process consistent with the biological behaviour of cells.

3 Network development through elongation and division mechanisms

3.1 Active Brownian Movement and persistence

In the paper [3], the cell dynamics follows an Active Brownian Movement, defined as follows:

$$\begin{cases} x_{n+1} = x_n + v.cos(\theta).\Delta t + \sqrt{2.D_t.\Delta t}.W_{x,n} \\ y_{n+1} = y_n + v.sin(\theta).\Delta t + \sqrt{2.D_t.\Delta t}.W_{y,n} \\ \theta_{n+1} = \theta_n + \sqrt{2.D_r.\Delta t}.W_{\theta,n} \end{cases}$$

The terms in *sin* and *cos* define a movement in the direction θ, to which we add a random component $\sqrt{2.D_t.\Delta t}.W_n$ where W_n is a Gaussian random variable, and D_t is a translational diffusion constant. This same Gaussian noise is applied to the direction angle θ, using a rotational diffusion constant D_r.

Unlike a classical Brownian motion, it allows a certain persistence in the cell motion to be taken into account. For example, an abrupt turn is very unlikely, whereas extension in a direction that varies more continuously is much more probable.

3.2 Implementation of the first two processes

Initially, only the elongation and division processes are implemented, with, for simplicity, a probability rate per unit time for division σ, and $(1-\sigma)$ for elongation. Annihilation will not be implemented until later, as it must result from an interaction with the chemical concentration. The result is shown in figure 4. As there is no annihilation process, there are a lot of intersections.

4 Chemical diffusion

4.1 Finite differences method

The diffusion equation is as follows (inspired by [2]):

$$\frac{\partial \rho}{\partial t} = D\nabla^2\rho - c_{max}Q(x,y,\rho)$$

The term ρ represents the concentration of chemical. The c_{max} is a maximal rate of consumption (in $mol.L^{-1}s^{-1}$). The consumption term Q can be developed as follows :

$$Q(x,y,\rho(t)) = h(\frac{\rho}{\rho_0})\sum_{i=1}^{N(t)}\delta(x - x_i(t))$$

where the x_i represent the cell positions and h is Hill's function, using a threshold for concentration. The Hills function is used to constrain the model so that concentrations do not become negative, by imposing increasing consumption as a function of local concentration.

The concentration values will be represented in a matrix. This matrix represents a 2D grid, in which the value associated with each pixel corresponds to its concentration. The ideal is to ensure that the dimension of each pixel is well below the elongation length scales of a branch over a unit of time. This discretization of the plane encourages the use of the finite difference method. At first order, the following discretization is obtained

$$\rho_{i,j}^{k+1} = \rho_{i,j}^k + \frac{D.\Delta t}{\Delta x^2}(\rho_{i+1,j}^k + \rho_{i-1,j}^k + \rho_{i,j-1}^k + \rho_{i,j+1}^k - 4\rho_{i,j}^k) - c_{max}Q(x,y,\rho)$$

4.2 Matrix optimisation

Calculating the Laplacian is fairly costly. It is advisable to matrix its calculation, and not to iterate the 5-points stencil on each pixel one-by-one. An easy solution is to use the numpy function *np.roll*, as explained in [5].

4.3 Edge condition management

By default, the matrix calculation (and in particular the np.roll function) imposes periodic conditions at the edges. In this case [justification!!!] it is possible to add zero flux conditions at the edge, to simulate a closed environment.

4.4 Relative speed between elongation and diffusion

This method imposes strict conditions on the value of the constant $D.dt/dx^2$. If it is too large, the solution will diverge. This imposes fairly low values for D. It may therefore be worth choosing to carry out several iterations for diffusion between each elongation/division iteration. Two distinct variables then appear: dt_{elong} and dt_{diff}. It is possible to do away with these two variables by referring only to the factor n, defined as $n = \frac{dt_{elong}}{dt_{diff}} > 1$. The apparent diffusion constant is then $D_{app} = n.D$, which accelerates diffusion without diverging the model.

5 Network-Chemical interaction

5.1 First attempt: best angle method

Intuitively, an effective method for the branches to develop best in areas of low network density would be to assume that the cells are capable of remotely measuring the concentration value of the chemical in the vicinity, and then to direct themselves preferentially into these areas. After implementation and reflection, it appears that the cells are only capable of measuring concentrations locally, not remotely. This process has been eliminated.

5.2 Second attempt: back to active brownian movement, and annihilation process

The preferred approach would be to use an active brownian movement to fill the space while maintaining the persistence of the branches, and to add an annihilation system consistent with biological reality. The process is as follows: digitally, before each elongation/division stage, each active cell takes a local measurement of chemical concentration. If the value is too low, we can assume that this zone is close to another branch, which has already partly consumed the chemical. Hence the idea of introducing a minimum concentration level variable c_{anni} which, if not exceeded, leads to the inactivation of the cell.

5.3 Adjustment: boundary condition between intersections and survival

If this variable c_{anni} is chosen to be very low, this means that an active cell remains active even under conditions of very low concentration. This means that, even in the vicinity of another branch, it will not inactivate. There will therefore be intersections, as shown in Figure 5.

On the other hand, if c_{anni} is chosen to be very large, then each active cell requires a high chemical concentration to remain active. In other words, the consumption of its own branching will be enough to inactivate it: we'll say that the network dies, i.e. that all the active cells become inactive, and that the network definitively stops its development process, as shown in Figure 5.

It is therefore advisable to choose an ideal value for c_{anni}, i.e. a value for which there is an absence of intersection (c_{anni} high enough) without causing the network to die (c_{anni} low enough). It is shown in Figure 7. Thus, we assume a phase diagram similar to Figure 8.

The ideal value of c_{anni} is of course dependent on the $cmax$ consumption rate of the cells. For example, if c_{max} is high (resp. low), the cells will have a high (resp. low) consumption and will impose a low (resp. high) general level of chemical concentration on the environment. It is therefore advisable to choose a low (resp. high) c_{anni} as well.

This extremely fine adjustment between these two parameters seems interesting, and will be the subject of further study in this paper.

5.4 Evolution of the phase transition

The $c_{anni,ideal}$ should therefore be plotted as a function of the c_{max}, for different values of n. The relative speed between elongation and diffusion also seems to have an important influence. The figure 9 seems to show the existence of an exponential law linking $c_{anni,ideal}$ and c_{max}.

To highlight this, the first and last points are removed, giving three lines that appear to have the same slope. After calculating three linear regressions, we obtain three slopes: $p_1 = -76.68 mol/L$, $p_2 = -86.89 mol/L$ and $p_3 = -82.75 mol/L$. We can consider them to be equal by using $p_{mean} = -82.1 mol/L$, which is equivalent to a relative deviation of less than 6.6%.

By imposing the value of the slope on p_{mean}, we can now optimise b to minimise the mean square error. The results appear satisfactory in Figure 10. The equations of the lines are:

$$\begin{cases} (n=10) \ : \ c_{anni,ideal} = p_{mean}.ln(\frac{c_{max}}{c_0}) + b_{10} \\ (n=20) \ : \ c_{anni,ideal} = p_{mean}.ln(\frac{c_{max}}{c_0}) + b_{20} \\ (n=50) \ : \ c_{anni,ideal} = p_{mean}.ln(\frac{c_{max}}{c_0}) + b_{50} \end{cases} \tag{1}$$

Where $c_0 = 1\, mol.L^{-1}.s^{-1}$ and $b_{10} = 1024\, mol/L$, $b_{20} = 1052\, mol/L$, $b_{50} = 1095\, mol/L$.

Eventually, for $n \in \{10, 20, 50\}$:

$$\boxed{c_{max} = c_0.exp(\frac{c_{anni,ideal} - b_n}{p_{mean}})}$$

Studying the evolution of b_n as a function of n would also seem to be an interesting approach, but impossible to carry out with so few data values.

6 Conclusions

The first objective seems to have been achieved: it is possible to model a biological network that fills the space without intersections using only processes that are consistent with the biological mechanisms of the cells, i.e. using a cell inactivation process activated only by a local measurement of a chemical concentration.

This discovery raises the question of how to adjust this survival threshold for active cells. This study seems to prove that the threshold value must be chosen with great precision according to the rate of cell consumption, so as not to result in the death of the network or intersections between branches. From a mathematical point of view, this corresponds to an exponential law linking these two quantities.

However, this model remains numerical, and its validation cannot be rigorous without experimental verification of the various behaviours observed.

7 Figures

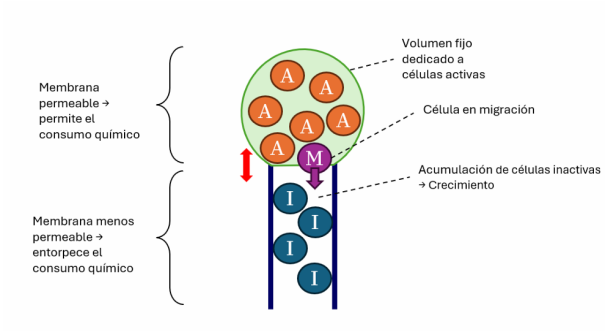

Figure 1: Body and tip of a ramification

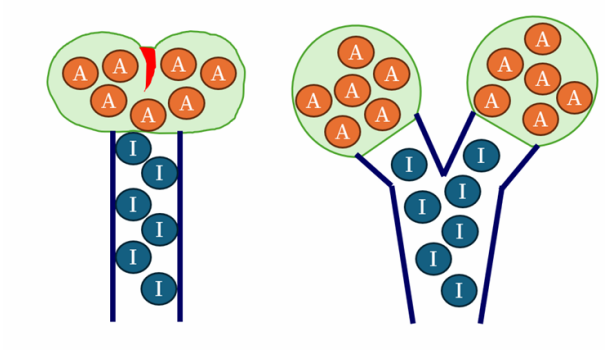

Figure 2: Accumulation of adhesive cells, before division

Figure 3: Branching Model in [1]

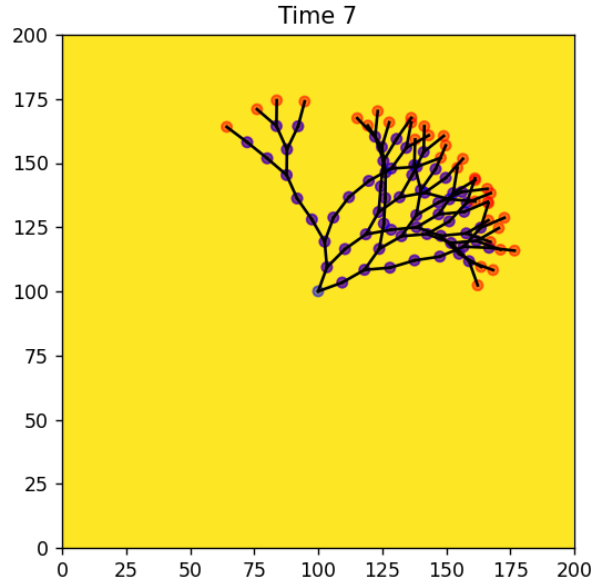

Figure 4: Branching model without annihilation, $\sigma.\Delta t = 0.5$

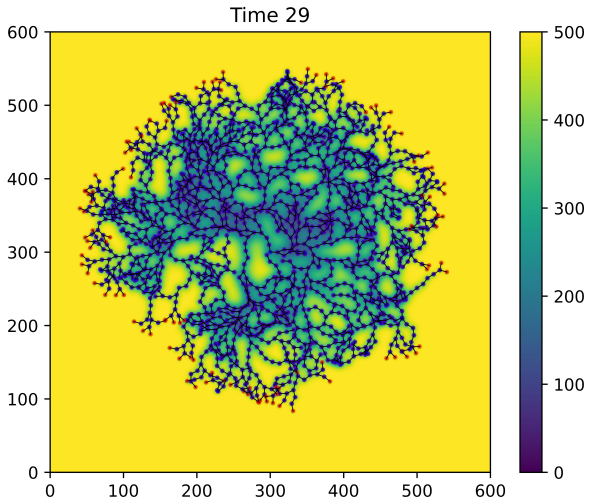

Figure 5: Network which c_{anni} is to low: there are many intersections

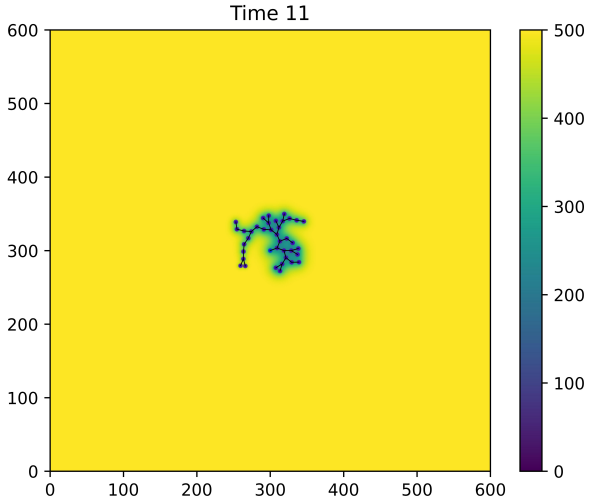

Figure 6: Network which c_{anni} is to high: the network 'dies' (no remaining active cells)

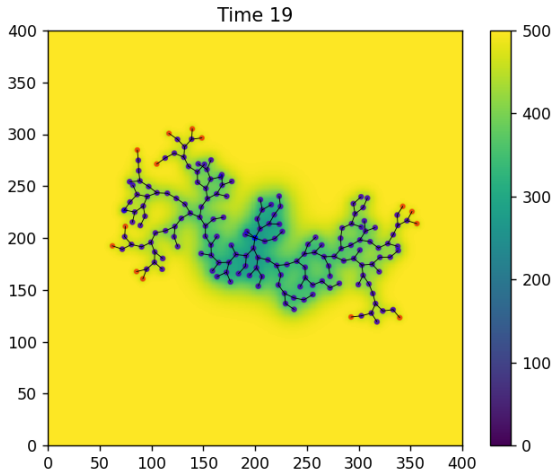

Figure 7: Limit case of c_{anni}, just before reaching its network death value. The number of intersections is minimal.

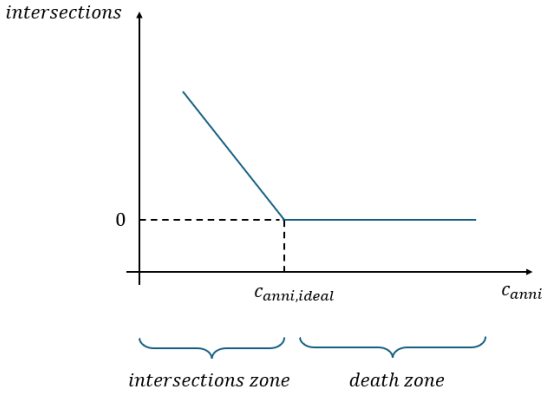

Figure 8: Rough form of the survival phase diagram for a network, as a function of c_{anni}

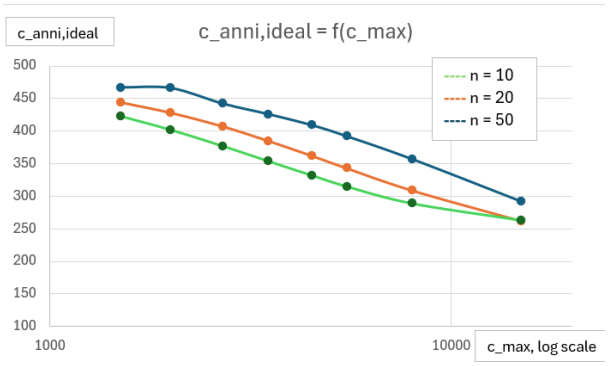

Figure 9: Plot of $c_{anni,ideal}$ as a function of c_{max} (in log scale) for different values of n.

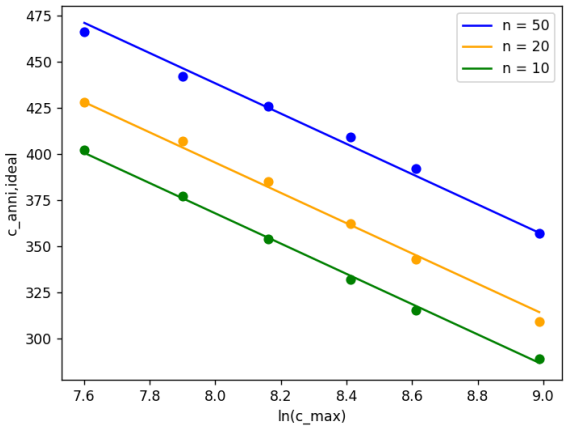

Figure 10: Linear regression with a fixed slope value of $c_{anni,ideal}$ as a function of $ln(c_{max})$ (in log scale) for different values of n.

8 References

References

[1] E. Hannezo et al. *A Unifying Theory of Branching Morphogenesis*. Elsevier, 2017.

[2] Hannezo et al. *Stem Cell Populations as Self-Renewing Many-Particle Systems*. Annual Review of Condensed Matter Physics, 2021.

[3] A. Callegari and G. Volpe. *Flowing Matter, Numerical Simulation of Active Brownian Particles*. Soft and Biological Matter, 2019.

[4] E. Hannezo and B. D. Simons. *Multiscale dynamics of branching morphogenesis*. Elsevier, 2019.

[5] K. Reuter S. Kehl S. Ohlmann. *2D diffusion problem*. 2022. URL: https://mpcdf.pages.mpcdf.de/python-for-hpc/08--Diffusion.html.