Mathematical Biology: from Individual Cell Behavior to Biological Growth and Form

Please write a report on the questions and hand them in on **25-11-2015** *by e-mail to* **merks@cwi.nl**, *Subject line: "HOMEWORK CPM".*

Lab session 4: Modelling biological development with the Cellular Potts model

Introduction

In this exercise you will experiment with Glazier and Graner's Cellular Potts model [1], which they originally developed to study the differential-adhesion hypothesis (DAH) [2]. The DAH states that tissues self-organize as a result of their adhesive properties.

The CPM represents biological cells as patches of lattice sites, $\vec{x}$, with identical indices $\sigma(\vec{x})$, where each index identifies, or "labels" a single biological cell. Connections between neighboring lattice sites of unlike index $\sigma(\vec{x}) \neq \sigma(\vec{x}')$ represent membrane bonds, with a characteristic *bond energy* $J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}$, where the cell types τ (*i.e.* endothelial, epidermal, *etc.*) determine the adhesion strength of the interacting cells. An energy penalty increasing with the cell's deviation from a designated target volume A_σ imposes a *volume constraint* on the biological cells.

We collect these effective energies in a *Hamiltonian*,

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2, \tag{1}$$

where λ represents resistance to compression, a_σ is the current cell volume, and the Kronecker delta is $\delta_{x,y} = \{1, x = y; 0, x \neq y\}$. The cells reside in a "medium" which is a generalized CPM cell without a volume constraint and with $\sigma = 0$, and $\tau = 0$.

To mimic cytoskeletally-driven membrane fluctuations, we randomly choose a lattice site, $\vec{x}$, and attempt to copy its index $\sigma_{\vec{x}}$ into a randomly chosen neighboring lattice site $\vec{x}'$. We often use the eight, first and second order neighbors, but we can reduce the effects of lattice anisotropy by using the twenty, first- to fourth-order neighbors on a square lattice (in the parameter file you can change this behavior by using `neighbors = 2` or `neighbors = 3` respectively). On average, we attempt an update at each lattice site once per Monte-Carlo step (*MCS*). We calculate how much the energy would change if we performed the copy, and accept the attempt with probability:

$$P(\Delta H) = \{\exp(-(\Delta H + H_0)/T), \Delta H \geq -H_0; 1, \Delta H < -H_0\}, \tag{2}$$

where $H_0 >= 0$ is an energy threshold which models viscous dissipation and energy loss during bond breakage and formation[3].

The cells' adhesivities are expressed in terms of the *bond energies* $J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}$, where *high* J-values imply *low* adhesivity and vice versa. It is convenient to describe the parameters in terms of the *surface tensions* $\gamma_{\tau_1,\tau_2} = J_{\tau_1,\tau_2} - (J_{\tau_1,\tau_1} + J_{\tau_2,\tau_2})/2$, which enable us to determine whether energetics favors homotypic ($\gamma_{\tau_1,\tau_2} > 0$) or heterotypic bonds ($\gamma_{\tau_1,\tau_2} < 0$) [1].

1 Exercises - Cellsorting with the CPM

1.1 Setting up a simulation

The Cellular Potts Model is implemented in the program `CompuCell3D`, which can be used to simulate cells and tissues. Start CompuCell3D using commandline: 'compucell3d' (or './compucell3d.sh' when in the CompuCell3D folder), or the icon. (to use CompuCell3D on your own computer, see the section Software at the end of the exercises.)

We are going to implement our own cellsorting simulation. This simulates a tissue in which two mixed celltypes sort themselves. Start the program `Twedit++` (CompuCell3D menu bar) for editing the simulation files. Click `CC3D Project→New CC3D Project` and enter cellsorting as `Simulation Name` and select the Desktop as `Simulation Directory`.

Set `XML only` as Simulation Type and click Next, select `Blob` as `Initial Cell Layout` and click Next. We will add cell two cell types: add cell type `Condensing` and `NonCondensing` to the list and click next untill you reach `Cell Properties and Behaviors`. Add `Contact` and `VolumeFlex` and finish the new project.

Next switch to CompuCell3D and open the created project that simulates a tissue:
`file→open simulation file`
choose `Dektop→cellsorting`
and open `cellsorting.cc3d`
CompuCell3D now opened the cellsorting simulation. Run it using the play button. Describe what you see, and add an illustration.

1.2 Initial configuration and Behavior of cells

Return to the program `Twedit++` for editing the simulation files. The initial configuration and behavior of the cells is described in `cellsorting.xml`. Set the `NeighborOrder` in the basic properties (Potts section) and contact plugin to 2 and the number of `Steps` to 10000.
a). What do you expect the outcome of the simulation to be? Run the simulation in `CompuCell3D` and explain what you see. Does it meet your expectation?
b). What happens if we remove the volume constraint? Explain.

1.3 Cell sorting

Set the `BlobInitializer` radius to 40 to start with a bigger tissue. We want the cells to sort themselves such that `Condensing` cells clump together surrounded by `NonCondensing` cells. Change the values of the parameters in the cellsorting.xml file to change the behavior of the tissue.
a). Give an example of parameters that result in the cellsorting described above, and add an illustration. State the parameter conditions under which cellsorting should occur.
b). Reproduce the simulation results from figure 1. What are the parameter values? Add an illustration of your results.
c). What other types of behavior can be found by changing the parameter values? Think of other forms of cell sorting, such as mixing. Add images and parameter values to illustrate.
d). What are the conditions for the different kinds of behavior found in (b)? Can you think of their biological relevance?

1.4 Environmental variables

Also change the values of the temperature `T`, target area `A` and lambda λ.
a). How does the simulation respond to these changes? Does it meet your expectations?
b). Can you philosophize to which biological or physical parameters these parameters correspond?

Introduction to Vasculogenesis modeling with the CPM

In these exercises we will model different mechanisms of angiogenesis using the cellular Potts model. To do so, we need a partial differential equation layer to simulate the diffusion and decay of chemoattractants (*e.g.* VEGF-isoforms) in the ECM; also the CPM must be able to secrete chemicals into the ECM.

Our initial cell-centered model of vasculogenesis implements the basic assumption of the Gamba and Serini model [4, 5]: ECs migrate towards the chemoattract they themselves secrete. We use the basic *CPM*, and add a PDE layer which describes the diffusion and secretion of the chemoattractant in the uniform substrate underlying the cells:

$$\frac{\partial c}{\partial t} = \alpha\ \delta_{\sigma_{\vec{x}},0} - (1 - \delta_{\sigma_{\vec{x}},0})\epsilon\ c + D\nabla^2 c, \tag{3}$$

where $\delta_{\sigma_{\vec{x}},0} = 1$ inside the cells. α is the rate at which the cells release chemoattractant, ϵ is the decay rate of the chemoattractant, and D is the diffusion coefficient of the chemoattractant. Every site within the *CPM* cells secretes the chemoattractant, which only decays in the substrate. We solve this PDE numerically using a finite-difference scheme (forward Euler) on a lattice that matches the *CPM* lattice, using 15 diffusion steps per *MCS*. For these

parameters, the chemoattractant diffuses more rapidly than the cells, enabling us to ignore advection as the cells push the substrate forward.

We implement preferential extension of filopodia in the direction of chemoattractant gradients — which drives chemotaxis — by allowing for an extra energy drop at the time of copying [6]:

$$\Delta H_{\text{chemotaxis}} = -\mu(c(\vec{x}') - c(\vec{x})), \tag{4}$$

where $\vec{x}'$ is the neighbor into which site $\vec{x}$ copies its spin. We use a value of $\mu = 500$ to obtain sufficient chemotactic migration. In our initial simulations the cells do not adhere without chemotaxis ($J_{cc} = 2J_{cM}$).

2 Exercises - Vasculogenesis modeling with the CPM

2.1 Chemotaxis to autocrinically secreted factors

We will now create a model bades on the assumptions of the Gamba and Serine model. We will create a spheroid of endothelial cells that all secrete a chemoattractant and react to it. First we set up a new simulation. Again start `Twedit++` and create a new project:
-Add one new celltype to the project (e.g. `cell`)
-Chemical field: `Field Name = VEGF`, with `solver = FastDiffusionSolver2DFE`. Enable multiple calls of PDE solvers.
-`Cell Properties and Behaviors`: Add `Contact`, `Chemotaxis`, `VolumeFlex` and `Connectivity`.
-`Chemotaxis Plugin`: Give λ=1000 for chemotaxis towards VEGF. Click `regular` and add entry.
Finish the project, and adapt all values of the parameters. (Temperature=50, neighborOrder=2, TargetVolume=100, LambdaVolume=50, Jcell-cell=40, Jcell-Medium=20, connectivityPenalty=1000000,)
Pay extra attention to the diffusion field (is there decay of VEGF in the Medium or in cells in the paper?) (secretionrate=1.8e-4, decay rate=1.8e-4, DeltaT=2, DeltaX=2e-6, DiffusionConstant=1e-13, extra steps PDESteppableCaller = 14)

The XML of the FastDiffuionSolver2DFE will look like:

```
<Plugin Name="PDESolverCaller">
<CallPDE ExtraTimesPerMC="14" PDESolverName="FastDiffusionSolver2DFE"/>
</Plugin>


   <Steppable Type="FastDiffusionSolver2DFE">
<DiffusionField>
<DiffusionData>
<FieldName>morph</FieldName>
<DiffusionConstant>1e-13</DiffusionConstant>
<DecayConstant>1.8e-4</DecayConstant>
<DoNotDecayIn>cell</DoNotDecayIn>
<DeltaT>2</DeltaT>
<DeltaX>2e-6</DeltaX>
</DiffusionData>
<SecretionData>
<Secretion Type="cell">1.8e-4</Secretion>
</SecretionData>
<BoundaryConditions>
<Plane Axis="X">
<ConstantValue PlanePosition="Min" Value="0"/>
<ConstantValue PlanePosition="Max" Value="0"/>
</Plane>
<Plane Axis="Y">
<ConstantValue PlanePosition="Min" Value="0"/>
<ConstantValue PlanePosition="Max" Value="0"/>
</Plane>
</BoundaryConditions>
</DiffusionField>
```

`</Steppable>`

As in the PDE model by Gamba *et al.*, the cells secrete a chemoattractant (You can make de chemoattractant visible by selecting `VEGF` as projection in the graphics window. The concentration is shown in colors with legend), and move towards higher concentrations of the attractant.
a). What patterns do you see? Does this meet your expectations, based on what you know from the Gamba-Serini continuum model?

Now experiment with the parameters, including the decay rate of the chemoattractant (`DecayCoefficient`), the diffusion coefficient (`DiffusionCoefficient`), and the number (by editing the Region of the cell box of the UniformInitializer and `width` of the cells) and size of the cells (`TargetVolume`). You can edit the configuration of the simulation using `Twedit++`.
b). What behavior do you find? Add illustrations.

2.2 Making vascular networks

In the lectures we have discussed several mechanisms that help form vascular networks, including cell elongation and contact-inhibition of motility.

To mimic cell elongation due to cytoskeletal remodeling we add a cell- length constraint to the free energy:

$$H' = H + \lambda_L \sum_{\sigma} (l_\sigma - L_\sigma)^2, \tag{5}$$

where l_σ is the length of cell along its longest axis, L_σ its target length, and λ_L is the strength of the length constraint. Assuming that cells are ellipses, we can derive their length from the largest eigenvalue of their inertia tensor I [7, 8]. The length constraint could cause cells to split into disconnected patches. We prevent this artifact by introducing a connectivity constraint, which reflects the physical continuity and cohesion of the actual cell [8].

2.2.1 Cell elongation

Use paper: Merks, R.M.H., et al., Cell elongation is key to in silico replication of in vitro vasculogenesis and subsequent remodeling. Dev Biol, 2006. 289(1): p. 44-54.

Create a new simulation. This simulation is the same as for the Serini and Gamba model (also same parameters), but with the addition of a `LengthConstraint` in the Cell Properties and Behaviors. Finish the project, and adapt all values of the parameters and the PDE field again. (TargelLength=60, LambdaLength=5)

(Make a 'clean' copy of this XML file for later).

a). Play with the target length and volume and their coefficients. What happens? How are cells organized? What seems to be the main driving force behind this mechanism?
b). Also try experimenting with small numbers of cells (say 10, by limiting the cell box size). What happens to the polygonal pattern over time? If you are patient, you might also be interested in trying larger fields `Dimensions (x=y=200)`. Do not forget to move the cell box to the middle of the screen.

2.2.2 Passive cell shape changes

Reset the simulation by putting back the text of the 'clean' XML file (Or make a new cell elongation simulation again).
a). Experiment with the cell adhesion between endothelial cells as we did in the cell sorting experiment. What do you see? Is this what you expected?

Now, release the shape constraint from the endothelial cells, by setting $\lambda =$ `0.0` for the `TargetLength`, and run a new simulation. You might also want to use small target areas.
b). What happens? What could be responsible for the change you see?

2.2.3 Contact-inhibition of motility

Use paper: Merks, R.M.H., et al., Contact-inhibited chemotaxis in de novo and sprouting blood-vessel growth. PLoS Comput Biol, 2008. 4(9): p. e1000163.

Create a new simulation as in exercise **2.1** for the Serine and Gamba model, with the following exception for the `Chemotaxis Plugin`: Give λ=500 for chemotaxis towards VEGF. Click `regular` and click `Chemotact towards` (The word `Medium` appears in the box), and add entry.
As a result, we have turned off chemotaxis at cellular interfaces (we hypothesize that phosphorylation of VEGFR-2 receptors by vascular-endothelial cadherin causes such contact-inhibition of chemotaxis); the cells are only sensitive to the chemoattractant at cell-ECM interfaces.
The paramater values are: Temperature=50, neighborOrder=2, TargetVolume=50, LambdaVolume=25, Jcell-cell=40, Jcell-Medium=20, connectivityPenalty=1000000, secretionrate=1e-3, decay rate=1e-3, DeltaT=2, DeltaX=2e-6, DiffusionConstant=1e-13, extra steps PDESteppableCaller =14).
a). Run the simulation. What happens? Can you explain the resulting behavior?
b). You can experiment with different initial cell distributions by varying the `gap, width` parameters in the BlobInitializer, or the UniformInitializer:

```
<Steppable Type="UniformInitializer">
<Region>
<BoxMin x="35" y="0" z="30"/>
<BoxMax x="135" y="1" z="430"/>
<Gap>0</Gap>
<Width>5</Width>
<Types>YOUR CELLTYPES</Types>
</Region>
</Steppable>
```

What happens? Do your observations help explain the role of contact-inhibition in vasculogenesis (see also [9]).

2.3 Software (for home use)

Download the program CompuCell3D from the website:
(`http://www.compucell3d.org/SrcBin`) and install. Consult the installation instructions if needed. (Easy install for linux-users:
download the .deb file from the website
change folders to the file location in terminal, and enter:

```
sudo dpkg -i [FILE NAME].deb
sudo apt-get -f install
```

to install the program)

References

[1] J. A. Glazier and F. Graner. Simulation of the differential adhesion driven rearrangement of biological cells. *Phys. Rev. E*, 47:2128–2154, 1993.

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[4] A. Gamba, D. Ambrosi, A. Coniglio, A. De Candia, S. Di Talia, E. Giraudo, G. Serini, L. Preziosi, and F. Bussolino. Percolation morphogenesis and burgers dynamics in blood vessels formation. *Phys. Rev. Lett.*, 90:118101, 2003.

[5] G. Serini, D. Ambrosi, E. Giraudo, A. Gamba, L. Preziosi, and F. Bussolino. Modeling the early stages of vascular network assembly. *EMBO J.*, 22:1771–1779, 2003.

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[7] M. Zajac, G. L. Jones, and J. A. Glazier. Simulating convergent extension by way of anisotropic differential adhesion. *J. Theor. Biol.*, 222:247–259, 2003.

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[9] Roeland M H Merks, Erica D Perryn, Abbas Shirinifard, and James A Glazier. Contact-inhibited chemotaxis in de novo and sprouting blood-vessel growth. *PLoS Computational Biology*, 4(9):e1000163, Sep 2008.