On-Lattice Agent-based Simulation of Populations of Cells within the Open-Source Chaste Framework

by

Grazziela P. Figueredo
Tanvi V. Joshi
James M. Osborne
Helen M. Byrne
Markus R. Owen

OCCAM Preprint Number 12/94
On-Lattice Agent-based Simulation of Populations of Cells 
within the Open-Source Chaste Framework

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Interface Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>RSFS-2012-0081</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>01-Nov-2012</td>
</tr>
</tbody>
</table>
| Complete List of Authors: | Figueredo, Grazziela; University of Nottingham, School of Mathematical Sciences 
Joshi, Tanvi; University of Nottingham, School of Mathematical Sciences 
Osborne, James; University of Oxford, Computer Science 
Byrne, Helen; University of Oxford, Computer Science 
Owen, Markus; University of Nottingham, Mathematical Sciences; |
| Subject: | Biomathematics < CROSS-DISCIPLINARY SCIENCES, Systems biology < CROSS-DISCIPLINARY SCIENCES, Computational biology < CROSS-DISCIPLINARY SCIENCES |
| Keywords: | agent-based modelling and simulation, tumour hypoxia, cell-cycle |

http://mc.manuscriptcentral.com/rsfs
On-Lattice Agent-based Simulation of Populations of Cells within the Open-Source Chaste Framework
Grazziela P. Figueredo\textsuperscript{1}, Tanvi V. Joshi\textsuperscript{1}, James M. Osborne\textsuperscript{2}, Helen M. Byrne\textsuperscript{2,3} and Markus R. Owen\textsuperscript{1}
\textsuperscript{1}Centre for Mathematical Medicine and Biology, School of Mathematical Sciences, University of Nottingham, NG7 2RD, UK
\textsuperscript{2}Oxford University Computing Laboratory, Department of Computer Science, University of Oxford, Wolfson Building, OX1 3QD, UK
\textsuperscript{3}Oxford Centre for Collaborative Applied Mathematics, OX1 3LB, UK

Abstract
Over the years, agent-based models have been developed that combine cell division and reinforced random walks of cells on a regular lattice, reaction-diffusion equations for nutrients and growth factors and ordinary differential equations (ODEs) for the subcellular networks regulating the cell cycle. When linked to a vascular layer, this multiple scale model framework has been applied to tumour growth and therapy. Here we report on the creation of an agent-based multiscale environment amalgamating the characteristics of these models within a Virtual Pysiological Human (VPH) Exemplar Project. This project enables re-use, integration, expansion and sharing of the model and relevant data. The agent-based and reaction-diffusion parts of the multiscale model have been implemented and are available for download as part of the latest public release of Chaste ("Cancer, Heart and Soft Tissue Environment"), (http://www.cs.ox.ac.uk/chaste/) version 3.1, part of the VPH Toolkit(http://toolkit.vph-noe.eu/). The environment functionalities are verified against the original models, in addition to extra validation of all aspects of the code. In this work, we present the details of the implementation of the agent-based environment, including the system description, the conceptual model, the development of the simulation model and the processes of verification and validation of the simulation results. We explore the potential use of the environment by presenting exemplar applications of the “what if” scenarios that can easily be studied in the environment. These examples relate to tumour growth, cellular competition for resources and tumour responses to hypoxia.

We conclude our work by summarising the future steps for the expansion of the current system.

Key words: agent-based simulation, multiscale model, cancer, lattice models, cell-cycle, tumour hypoxia

1 INTRODUCTION
In this work, we introduce a virtual research environment for 2D and 3D in silico simulation of the dynamics of lattice-based cell populations coupled to diffusible fields such as nutrients and growth factors. Our focus is to explain in details the steps of the simulation model development, regarding aspects such as the system description, the conceptual model, the simulation model development in Chaste\textsuperscript{9} as well as the system verification and validation.

The main purpose of this simulation system is to facilitate biological research in testing mechanisms such as interactions between different cell types (such as proliferating normal cells and cancer cells and non-proliferative macrophages) in a nutrient and growth-factor dependent environment. Furthermore, this simulation tool can be utilised to test potential new treatments for various pathologies, such as early-stage cancer.

The idea of incorporating this environment into the VPH toolkit came from the successful development of models for cell division, birth, death and movement within a lattice in 2D \cite{4} and 3D \cite{8}; regulation of cell cycle and factors, such as oxygen and other nutrients \cite{3}; tumour hypoxia and effects of hypoxia (low oxygen levels) in cell cycles of tumour and normal cells \cite{2}. These models represent the state-of-the-art multiscale model for tumour growth and cellular hypoxia, but their original implementation does not yet meet the standards of reproducibility, re-usability and interoperability that are required to enable the public release. Their implementation for the VPH toolkit within Chaste therefore allows for the release of a reliable, reusable and expandable code.

As part of the Chaste test-driven approach to software development \cite{9}, extensive nightly and weekly tests are performed on all parts of our code, which means that functionalities are constantly being verified and should be preserved over time, allowing the generation of reproduceable simulation results. Furthermore, the functionalities that we have added to Chaste are verified against the original model implementation.

The object-oriented approach adopted by Chaste facilitates the expansion of the model to include different cell populations and diffusible substances. In the future, we aim to extend this environment to a vascular tissue modelling environment (VTME), encompassing models for fluid flow in a vessel network; transport, release and uptake of diffusible substances such as oxygen; and integration of angiogenic and vasculogenic endothelial cells into the vascular network \cite{1, 6}. The work presented here, therefore, represents the first step towards a VTME.

The remainder of this paper is organised as follows. First, we introduce the cellular, subcellular and diffusible components of the multiscale agent-based model (Section 2); subsequently, we introduce the conceptual model of the simulation, which defines the model scope and simplifications from the real-world biological system. In Section 3, we introduce the details of the model implementation as well as the verification and validation of each of the modules. Section 5 presents some applications that can be developed in the environment. Finally, in Section 6 we draw conclusions and present the future steps to expand our environment.
2 THE MULTISCALE AGENT-BASED SYSTEM

The system implemented comprises different elements from distinct time and length scales coupled together. The multiscale model features include competition between cell cycle-based cells (for example, cancer and normal cells which can divide) and lifespan-based cells (e.g., macrophages which can only die after a certain lifespan), cellular random walks and coupling to diffusible substances such as nutrients (e.g., via consumption and/or production by cells). Cell cycle models and cell death (apoptosis) can be dependent on the diffusible substances (e.g., nutrients such as oxygen). The model's general structure was therefore divided in three layers, corresponding to the diffusible nutrients, the cellular and the intracellular phenomena, as shown in the conceptual model of Figure 1.

[Figure 1 about here.]

The first layer contains the diffusible species existing within the environment. These species are the molecules that interact with cells, providing nutrition or stimulating cellular activity. One example of a diffusible shown in Figure 1 is oxygen, which directly interferes in some types of cellular proliferation and death.

The cellular layer focuses on the cellular interactions and their spatial locations. For our model, three types of cells are initially considered: normal and cancer cells, which have distinct nutrient-dependent cell cycles; and macrophages, which are immunocompetent cells responsible for mounting immune responses and aiding different types of immunotherapies [7]. Normal and cancer cells compete for space and nutrients, such as oxygen. Cancer cells, however, tend to be better competitors and take over the space of normal cells with time [5]. This is explained by the fact that cancer cells are more resistant to low levels of oxygen—they enter a quiescent state and survive longer than normal cells under such an unfavourable environment. The competition between these two types of cells is determined by rules that couple the cellular and the subcellular layers of the model. Likewise, the apoptosis (programmed cellular death) of normal and cancer cells is controlled by the level of oxygen (or any other type of nutrient) inside the cell. Macrophages, on the other hand, are marked for apoptosis with age. Finally, these cells are capable of moving randomly between neighbouring lattice sites.

The intracellular layer comprises the processes of cellular division and apoptosis. Cell division is regulated by the cell cycle, the set of events whereby a cell duplicates most of its components in order to replicate [4]. In this layer, the rules that determine how the cell cycle and cellular apoptosis should respond to the levels of nutrient are implemented. The details of the implementation of these layers will be further discussed in the next section (Section 3).

3 DEVELOPMENT OF THE SIMULATION ENVIRONMENT

The simulation environment was implemented using a multi-method approach comprising (1) 2D and 3D lattices containing the objects from the system; (2) agents, representing the cells that lie in the lattice; (3) random-walk rules for the agents' movement; (4) ordinary differential equations (ODEs) for subcellular networks that regulate the cell cycle; and (5) partial differential equations for the transport, release and uptake of diffusible species such as oxygen. Each of these elements and their integration will be described in greater detail below.

3.1 On Lattice Simulations

Our on-lattice model can accommodate empty lattice sites with no cells associated. Additionally, multiple cells can be placed in each lattice site, according a pre-defined carrying capacity. This implementation allows the simulation models to overcome some of the limitations of existing traditional cellular automaton models. First, the maximum population size is not constrained to the size of the lattice. Rather, the number of biological cells in the simulation model is controlled by the carrying capacity of each lattice site. This characteristic allows for lattice sites to correspond to a user-defined tissue volume whose natural carrying capacity will depend on that volume and on the properties of the cells under consideration (e.g., the cell volume and degree of contact inhibition). Furthermore, different carrying capacities can be defined for each lattice site, allowing, for example, for the simulation of areas in a tissue with different microenvironmental conditions (e.g., extracellular matrix density). The lattice sites do not have rules associated with them. Instead, they are just possible locations where the biological cells may lie, with the advantage that this type of lattice is capable of containing simultaneously heterogeneous populations with distinct rules associated to each type of biological cell. The concept of lattice neighbourhood is also used, in order to allow cell movement in space. Following some concepts of the traditional approach of cellular automata models, two types of neighbourhoods are considered, the von Neumann and Moore neighbourhoods. These neighbourhoods are also implemented in three dimensions.

3.2 The Cell Population

A cell population is a computational representation of a collection of cells for example a tissue. The cell population exhibits the following features:

1. Cells are associated with only one lattice site. The implementation considers only on-lattice simulations.
2. Each lattice site (and cell) has a neighbourhood associated with it.
3. Lattice sites may have no cell associated with them.
4. Cells are added (cellular birth) and removed (cellular death) from the lattice during the course of a simulation.
5. Cells move randomly in the lattice and the probabilities of movements can be specified to include certain cell behaviours, such as chemotaxis.

6. Different cell types occupy the lattice (e.g. normal cells, tumour cells, macrophages).

7. There can be more than one cell per lattice site. Each lattice site has a carrying capacity that determines the maximum number of cells which can be located at that site.

These features will be described in more detail in the sections below.

### 3.2.1 The Cell Agents

There are two main types of agents in the system: those that have a cell cycle (Figure 2) and those who die with age (Figure 5). Here, we illustrate these concepts using State charts from the unified modelling language (UML), where it is possible to define and visualise the agents’ states (round squares), transitions between the states (arrows), events that trigger transitions, timing and agent actions.

Figure 2 introduces the state chart for cell cycle-based agents. Normal and cancer cells belong to this class of agents. In the figure, the cycle-based cell assumes two main states: Alive or Dead, which is the final state where the cell is removed from the system. When the cell is alive, it moves randomly in the lattice. This cell movement is dictated by the transition Move, which occurs probabilistically. In addition, when the cell is alive, it enters its nutrient-dependent cell-cycle state for division and, if it runs low of nutrients, it is considered to enter an apoptotic state, that precedes its death. The general action chart for the cell cycle is illustrated in Figure 3. As shown in the state chart of Figure 2, the cell cycle is a cyclic procedure that occurs until the cell is labelled for apoptosis. Once the cell is marked for apoptosis, the conditional transition Check Apoptosis is triggered and the cell assumes the Apoptotic state.

The general apoptosis action chart, which determines when low nutrient causes a cell to be labelled for apoptosis, is presented in Figure 4. Specific processes of cellular death for normal, cancer cells and macrophages are explained in Section 3.4.

As described in Section 2, some types of cells, such as cancer cells, can enter a quiescent state that means they are more resistant to the lack of nutrients (e.g. oxygen) [5]. This cell quiescence is established when the conditional transition Check Apoptosis is updated. Further details on the cell cycle for cancer and normal cells are given in Section 3.6.

[Figure 2 about here.]

[Figure 3 about here.]

[Figure 4 about here.]

Figure 5 presents the state chart for cells that die after a certain lifespan, such as macrophages. Similarly to the other cell types, these cells are either dead or alive. While they are alive, they move and age. They die after age reaches a certain threshold.

Although for our *in silico* experiments we considered only three types of cells, our environment within Chaste is easily extensible to consider other types of cells, diffusion rules and cell-cycles.

### 3.2.2 Adding Daughter Cells to the Population

When the subcellular (cell cycle) model indicates that a cell is ready to divide, a check is made whether there is space available to place a daughter cell in a neighbouring lattice site. If space is available, a new cell (the daughter cell) is added to a randomly selected available site in the parent cell’s neighbourhood. If there is no space available, the daughter cell is not created and the parent cell cycle is reset.

The pseudo-code for adding new cells to the population is shown in Algorithm 1.

**input**: The cell to divide (parent cell)

**output**: The new cell created

Get location of the lattice site corresponding to the parent cell;

Get neighbouring locations of the parent cell;

\[ \text{found_available_neighbour} \leftarrow \text{false}; \]

foreach \( n \) in the neighborhood do

  if \( n \) is available then
    \[ \text{found_available_neighbour} \leftarrow \text{true}; \]
    // Stop searching
    break for;
  end

if \( \text{found_available_neighbour} \) then
  create a new cell;
  place the new cell in \( n \) location;
end

**Algorithm 1**: Adding cells to the population pseudo-code

The verification and validation of this module will be discussed in section 3.5, where we introduce the methods used for cellular proliferation.

### 3.3 Cellular Motility

Cells migrate along the lattice randomly to available sites belonging to their neighbourhood. The probability of a cell at site \( x \) moving to site \( y \) in its neighbourhood in a simulation time step \( \Delta t \) is given by

\[
Pr(x, y, t) = \frac{D \Delta t}{2d_{x,y}} \left( N_m - N(x,t) \right), \quad \text{for } x \neq y, \tag{1}
\]

where \( N(x,t) \) is the number of cells at site \( x \), \( D \) is the maximum cell motility in the absence of chemotaxis, \( N_m \) is the carrying capacity for movement of the cell type attempting to move and \( d_{x,y} \) is the distance between sites \( x \) and \( y \). Thus, if the site \( y \) is occupied by a number of cells equal to its carrying capacity, then the probability to move to this site is set to zero. We remark that Equation (1) is

[Figure 5 about here.]
equivalent to the form used in [7], but without chemotaxis. Future extensions to the Chaste implementation will include chemotaxis.

3.3.1 Random Movement Verification

The verification of the random movement implementation asserts whether diffusion is accurately implemented. What is expected from the random movement calculation is that, given a cell neighbourhood and a sufficient number of random samples, there is about an “equal” probability of moving to each of the available lattice sites (according to the neighbourhood used—for the Moore neighbourhood, the probability of moving in vertical and horizontal directions is higher than in diagonal directions). In order to verify the movement rule, two tests were defined using the Moore neighbourhood to determine the cells’ moving directions. The first test checks that the probability calculation is returning the correct value [7]. In the second test, we checked that movement, in the eight directions corresponding to the Moore neighbourhood, occurs with the frequency expected (results not shown).

3.3.2 Random Movement Validation

Results for the movement of a single cell are validated against those from the original implementation established in [7]. In order to validate the results, 100 realisations of both implementations were run and the results were collected. The parameters used for the simulations are presented in Table 1 and the results are shown in Figures 6 and 7.

Figure 6 shows the mean cell displacement against time for the original model implementation (blue line) and our implementation in Chaste (green line). Furthermore, the red line shows the mathematical prediction of the mean displacement, calculated as:

\[
\text{prediction} = 2 \times \sqrt{1440 \times \text{Dcell.time}}
\]

The outcomes are very close and follow the same pattern, which suggests that both implementations have equivalent outputs. The differences observed in the graphs are due to the different random numbers used for each implementation. Figure 7 presents trajectories of single cell random movement for the original implementation (Figure 7(a)) and our environment (Figure 7(b)) for one hundred realisations.

3.4 Cell Cycle Model

In this section we expand the Cell_Cycle_Process action chart from Figure 3 to the specific types of cells implemented (normal and cancer cells) and the diffusible substance considered (oxygen). Although our implementation in Chaste permits the expansion of the system to incorporate different types of cell cycles, currently we adopt an oxygen-dependent ODE-based cell-cycle model, proposed by Owen et al., Cancer Research; 71(8), 2011 [7] as a simplification of the model of Alarcón et al. [2]. This cell cycle is a simple phase model given by Equation (2) below:

\[
\frac{d\phi}{dt} = \frac{C}{T_{\text{min}} (C_{\phi} + C)},
\]

where \(\phi \in [0, 1]\) represents the phase of the cell cycle. The oxygen concentration at the cell’s current location is given by \(C(x, t)\). \(T_{\text{min}}\) is the minimum period of the cell cycle and \(C_{\phi}\) is the oxygen concentration at which the speed is half maximal.

Since \(\phi = 0\) represents the start of the cycle, and \(\phi = 1\) is the completion of one cycle, when \(\phi\) reaches one, it is then reset back to zero. As shown in Equation (2), the speed of progress through the cell cycle depends on the oxygen concentration. Table 2 shows the default parameter values used for normal cells and cancer cells in our simulations.

Over the course of the simulation, the above ODE is solved over each time step. If \(\phi = 0\) at any stage, the cell is assumed to be ready to divide. In this case, the parent cell divides forming daughter cells, only if there is sufficient room to place the newly formed daughter cells. \(\phi\) is then reset to zero and the cycle is repeated. \(\phi\) is also reset when there is no space to divide.

In case of cancer cells, if the oxygen concentration at the cell’s current location drops below a certain threshold, the cell is labelled to be quiescent. When in quiescent state, cancer cells do not progress through the cell cycle (i.e. the ODEs are not solved). When the oxygen concentration rises above another minimum threshold value, the cells stop being quiescent and the cell cycle progresses again.

An equivalent cell cycle model has also been implemented and tested, which exploits the fact that an explicit solution to Equation (2) is available under the assumption (used throughout) that the diffusible variables are constant over each simulation time step \(\Delta t\). In this model, \(\phi\) the phase of the cell cycle is updated according to:

\[
\phi(t + \Delta t) = \phi(t) + \frac{C}{T_{\text{min}} (C_{\phi} + C)} \Delta t,
\]

where \(\phi(t = 0) = 0\) and \(t\) is the current time.

Like in the ODE model, \(\phi = 1\) marks the completion of one cycle. The cell is ready to divide into daughter cells provided that there is sufficient room for the newly formed daughter cells. The value of \(\phi\) is then reset back to zero and the cycle repeats. For quiescent cancer cells, the value of \(\phi\) remains constant throughout the quiescent phase.

Macrophages do not have a cell cycle. Instead, they have a fixed lifespan, after which they die (Section ??). Biologically, macrophages typically do not proliferate and they enter tissues in vivo from the vasculature and or would be introduced in an in vitro situation. Therefore, there is no need for this cell type to divide. As the vascular parts are still to
be added to our environment, for now the macrophages, as well as the other cells are created and placed in the lattice in the start of the simulation.

3.5 Cellular Proliferation
As mentioned in the previous section, the proliferation of normal cells and cancer cells is controlled by a cell cycle model. Our cell cycle demands a minimum level of oxygen, and the proliferation occurs if there is available space within a certain region. Results for cellular proliferation are validated against those from the original implementation [7]. The growth curves for both implementations are similar (see Figure 8).

[Figure 8 about here.]

3.6 Cell Death
In this section we explain how the action chart for cell apoptosis (Figure 4) was implemented in our agent-based environment. The death of normal and cancer cells depends on how they respond to hypoxia. The cell cycles of normal and cancer cells exhibit remarkable differences [3]: normal cells undergo apoptosis in response to persistent periods of hypoxia, whereas cancer cells appear to be more resistant to the lack of oxygen. This resistance is partially due to their ability to enter a quiescent state in response to severe hypoxic stress [10]. In cases from which the oxygen levels remain low, the cancer cells will also eventually die. The rules associated with the death of normal and cancer cells are based on characteristics of cellular responses to hypoxia, as outlined in the sections below.

3.6.1 Normal Cell Death
A normal cell is marked for apoptosis if the oxygen concentration within its neighbourhood falls below a prescribed threshold. This threshold increases when a normal cell is surrounded by more cancer cells. The threshold is defined as (Algorithm 2):

```plaintext
Algorithm 2: Normal cell threshold calculation
input: Cell ratio
if ratio > ρ then
    threshold ← lower value;
else
    threshold ← higher value;
Algorithm 2: Normal cell threshold calculation
```

The cell ratio is calculated via the pseudo-code outlined in Algorithm 3:

```plaintext
Algorithm 3: Cell ratio calculation
input: Current cell
output: ratio
Get location of the cell;
if Location (lattice site) has more than one cell then
    normal_count ← number of normal cells;
cancer_count ← number of effector cells;
else
    Get the set of the cell’s neighbours;
    Iterate over the set of neighbouring sites to count the total number of normal cells and cancer cells surrounding the target cell;
end
ratio = normal_count/(normal_count + cancer_count);
```

3.6.2 Cancer Cell Death
Cancer cells die if they remain quiescent for a time that exceeds a predefined critical value. As mentioned previously, this time is determined in the cell cycle model. At each time step the interval of quiescence is updated or reset, depending on the local oxygen cone. If the time exceeds the critical value, the cell is labelled for apoptosis.

3.6.3 Cell Killer
The elimination of cells is performed by a cell killer method, which identifies those cells from the population those cells that should be labelled for death, following the rules (introduced above) for each cell type. The pseudo code for this method is shown in Algorithm 4.

3.6.4 Cell Killer Verification
We verify that the cell killer method has been implemented correctly by considering the following scenarios:

1. Normal cell death:
   (a) **Death caused by low oxygen concentration:** This scenario describes a normal cell with no cancer cells in its neighbourhood and an oxygen concentration which is lower than the hypoxic threshold at the current location of the normal cell. In this case, the rules dictate that the cell should die.
   (b) **Death with cancer cells in the neighbourhood:** A normal cell is placed on the lattice and surrounded by cancer cells in such a way that the cell ratio, as calculated in (Algorithm 3), falls below the parameter ρ. The oxygen concentration threshold is therefore increased. This verification process asserts that the cell dies in this case.
   (c) **Death with normal cells in the neighbourhood:** Let us now consider the case where a normal cell is surrounded by only normal cells. As before, if the cell ratio is greater than ρ then the threshold oxygen concentration will be set to the lower value. If we assume further that the oxygen concentration at the cell’s location is higher than the hypoxic threshold then the cell should not die in this case.
input : The cell population

foreach cell c in the population do
    if c is cell cycle based then
        Determine the current nutrient (oxygen) level;
        if c is a normal cell then
            ratio ← RatioCalculation(c);
            threshold_oxygen ← NormalCellThresholdCalculation(ratio);
        if oxygen level of cell c < threshold_oxygen then
            Kill c;
        Update quiescent_time;
    if c is quiescent for a long time then
        Kill c;
    end
    if c is a cancer cell then
        if oxygen level of cell c < threshold_quiescence then
            Leave quiescence; Reset quiescent_time;
        else
            if oxygen level of cell c > threshold_leave_quiescence then
                Leave quiescence; Reset quiescent_time;
            end
        end
    end
end

Algorithm 4: Pseudo-code for the cell killer

(d) Death with no cells in the neighbourhood:
Next we consider a normal cell surrounded by empty space. Then the cell ratio is set to one, which is greater than ρ and the threshold oxygen concentration is once again set to the lower value. The oxygen concentration at the cell’s location is set to exceed the hypoxic threshold and hence the cell will not die.

2. Cancer cell death: Verification for cancer cells asserts that they die if they have been in a quiescent state for a prolonged period of time. For this purpose, we set the oxygen concentration, at the cell’s location, such that the cell is labelled to be in the quiescent state. The amount of time that the cell has been quiescent is updated at each time step as mentioned in (Section 3.6.2). We continue to increment the time step in the simulation until the amount of time the cell has been quiescent exceeds the critical value. Also, at every time step we assert that the cell does not progress through the cell cycle, that it continues to remain quiescent and that it is not labelled apoptotic. Once the cell has been quiescent for longer than the critical value, the cell should be labelled for apoptosis and thus be killed.

4 Diffusibles

The distribution of diffusibles such as oxygen is modelled using reaction-diffusion equations, which are assumed to be in quasi steady state. Hence, the governing partial differential equations (PDEs) are of the form

\[ 0 = \partial_{t} C + \nabla U C = \partial_{t} C + \nabla \cdot \left( \nabla C \right) = \partial_{t} C + \nabla C \nabla, \]

where, \( D_u \) is the diffusion coefficient of diffusible \( U \), \( S_u = S_u(U, ...) \) is cell-dependent production/removal rate and \( \delta_u \) is \( U \) decay rate. For all diffusible species, we impose zero flux boundary conditions.

The PDE for each diffusible is updated at each time step. The solution at each site is then used to update the cell cycle phase, and/or to determine whether a cell should undergo apoptosis.

4.1 Oxygen, C(x,t)

The environment acts as a source of oxygen and cells act as the spatially distributed sinks, as they consume oxygen. If the rate of oxygen consumption by a cell at site \( x \) is given by \( k_{cell} \), then we get:

\[ S_C(x,t) = - \sum_{cell \ at \ x} k_{cell} C, \]

and \( \delta_{c} = 0. \)

5 EXAMPLES

In this section we present examples of in silico experiments relating to cellular proliferation and nutrient consumption that can be performed in our environment.

5.1 Oxygen-Dependent Cell Proliferation

We illustrate cellular proliferation by inserting a single tumour cell in an environment with a square grid of size \( 50 \times 50 \) (see Figure 9(a)). The cells are set to consume oxygen at the rate \( k = 0.1 \). The diffusion coefficient was set to \( D_c = 1 \). The PDE used to determine the oxygen distribution is given by Equation (6) below and Dirichlet boundary conditions \( (C = 30) \) were imposed on all the boundaries. In more detail, we have

\[ 0 = D_c \nabla^2 C - k I(x, y) C \]
where

\[ I(x, y) = \begin{cases} 1, & \text{if a cell is present at } (x, y) \\ 0, & \text{otherwise} \end{cases} \quad (7) \]

Simulation results showing the evolution of the system over 1000 hours are presented in Figure 9. We observe the initial proliferation of the cells (Figure 9(b)), which consume the oxygen available at the centre of the tumour. This consumption generates hypoxic areas and consequent cellular death due to lack of oxygen (Figure 9(c)). As there is still space and oxygen towards the edges of the lattice, cells there continue to proliferate and the tumour increases in size (Figure 9(d)). With the increase of the number of cells and oxygen consumption rate, the number of cells towards the centre of the tumour undergoing cell death due to hypoxia also increases (see Figure 9(d)).

[Figure 9 about here.]

5.2 Oxygen-Dependent Cell Proliferation with Alternative Boundary Conditions

We now consider the case identical to that in the previous section, except that we impose a zero flux boundary condition on the bottom boundary.

The simulation was run for a period equivalent to 1000 hours. The results for this simulation, are presented in Figure 10. We observe that hypoxia region forms in the centre as cells consume oxygen (Figure 10(d)). The oxygen concentration is also low near the bottom boundary, due to absence of any source of oxygen there. (Figure 10(c)). The cells continue to proliferate near the top, left and the right boundaries, where \( C = 30 \) (see Figures 10(a) to 10(d)).

[Figure 10 about here.]

5.3 Multiple cell types

We consider now, different types of cells, i.e. normal cells and cancer cells. In this example, the diffusion coefficient was set to 0.02 and each lattice site carrying capacity was set to 4. The lattice dimensions were set to \([50 \times 50]\). Dirichlet boundary condition \( (C = 100.0) \) was imposed on all boundaries. The oxygen uptake rate for both types of cells was set to \( k = 0.1 \). The simulation was run for a period equivalent to 500 hours. Results are shown in Figures 11(a) to 11(d). Figure 11(a) shows the initial disposition of the cells in the system – there is a cluster of normal cells with two cancer cells inside it. As simulation progresses, normal cells start replicating and moving along the lattice (see Figure 11(b)). Tumour cells also start replicating and take over the space originally occupied by normal cells (Figure 11(c)). There is also an increase in the hypoxic areas, due to higher rates of oxygen consumption 11(d).

[Figure 11 about here.]

5.4 Cell Proliferation in 3D

In this section, we illustrate cell proliferation in 3D. For this purpose, we consider a single cell in a cube of size \([20 \times 20 \times 20]\) (Figure 12(a)). A cell consumes oxygen at the rate \( k = 1.0 \). The diffusion coefficient \( D_x = 1 \). Dirichlet boundary condition \( (C = 30) \) is imposed on all boundaries. The simulation was run for a period equivalent to 500 hours. The results for this simulation are presented in Figure 12. Similarly to the 2D example, we observe the initial cell proliferation (Figure 12(b)), which consumes the oxygen at the centre of the tumour. This consumption generates hypoxic areas and consequent cellular death due to lack of oxygen (Figure 12(d)).

[Figure 12 about here.]

6 CONCLUSIONS

We have introduced an open-source, extensible, agent-based virtual research environment for 2D and 3D in silico simulation of the dynamics of cell populations and their responses to nutrient shortage. Our environment was developed within Chaste [9], as part of the VPH toolkit. The system development was based on models representing the state-of-the-art multiscale models of cellular proliferation and its response to nutrient shortage. The simulation environment was implemented using a multi-method approach comprising (1) 2D and 3D lattices containing the cells and molecules from the system; (2) agents, representing the biological cells that lie in the lattice; (3) diffusion rules for the agents’ motility; (4) ODEs for subcellular networks that regulate the cell cycle; and (5) PDEs for the transport, release and uptake of nutrients. Following Chaste philosophy, nightly and weekly testing have been extensively performed on our code in order to verify and preserve the functionalities implemented. Further verification and validation were performed against the original multiscale models in which our environment is based, ensuring the reproducibility of the simulation results.

In the future, we intend to extend our environment to include a vascular layer. We aim to develop a vascular tissue modelling environment (VTME) for curated and sustainable multiscale models to investigate the complex interplay between subcellular signalling (e.g. the cell cycle, responses to hypoxia), growth factor and nutrient distributions, and vascular dynamics at the network level.

7 ACKNOWLEDGEMENTS

This work was supported in part by Award No. KUK-013-04, made by King Abdullah University of Science and Technology (KAUST).

References


List of Figures

1. The conceptual model of the multiscale environment. There are three layers in the model: the diffusibles, the cells and the intracellular phenomena (such as the cell cycles and apoptosis).

2. Cycle-based cell state chart.

3. Cell cycle action chart.


5. Lifespan-based cell state chart.

6. Results comparison for single cell random movement.

7. Single cell random movement for one hundred realisations.

8. Cell proliferation for one hundred realisations, in a 2D lattice of size $[100 \times 100]$, with carrying capacity per lattice site equals to one. The simulation was run for a period equivalent to 480 hours and the cells’ diffusion coefficient $D$ was set to $0.3 \times 10^{-9}$. The simulation starts with a single cell placed in the middle of the lattice. The graphs show the mean value of one hundred realisations. The random element to these simulations is caused by the rules for cellular motility (Section 3.3).


10. Oxygen-Dependent Cell Proliferation with Alternative Boundary Conditions.

11. Evolution of a cluster containing multiple cell types. In the figure, the white spheres are tumour cells while the blue spheres are the normal cells.

12. Cell Proliferation in 3D.
Figure 1: The conceptual model of the multiscale environment. There are three layers in the model: the diffusibles, the cells and the intracellular phenomena (such as the cell cycles and apoptosis).
Figure 2: Cycle-based cell state chart.
Figure 3: Cell cycle action chart.
Figure 4: Cell apoptosis action chart.
Figure 5: Lifespan-based cell state chart.
Figure 6: Results comparison for single cell random movement
Figure 7: Single cell random movement for one hundred realisations.
Figure 8: Cell proliferation for one hundred realisations, in a 2D lattice of size $[100 \times 100]$, with carrying capacity per lattice site equals to one. The simulation was run for a period equivalent to 480 hours and the cells' diffusion coefficient $D$ was set to $0.3 \times 10^{-9}$. The simulation starts with a single cell placed in the middle of the lattice. The graphs show the mean value of one hundred realisations. The random element to these simulations is caused by the rules for cellular motility (Section 3.3).
(a) Initial stage – time = 0h

(b) Cells begin proliferation – time = 600h

(c) Death of cells in the hypoxic region – time = 650h

(d) Further cell proliferation and growing hypoxic region = time = 1000h

Figure 9: Oxygen Based Cell Proliferation
(a) Initial stage – time = 0h
(b) Cells begin proliferation – time = 600h
(c) Death of cells in the hypoxic region – time = 650h
(d) Further cell proliferation and growing hypoxic region – time = 1000h

Figure 10: Oxygen-Dependent Cell Proliferation with Alternative Boundary Conditions
(a) Initial stage – time = 0h
(b) Cells begin proliferation – time = 100h
(c) Death of cells in the hypoxic region – time = 300h
(d) Further cell proliferation and growing hypoxic region – time = 500h

Figure 11: Evolution of a cluster containing multiple cell types. In the figure, the white spheres are tumour cells while the blue spheres are the normal cells.
(a) Initial stage – time = 0h

(b) Cells begin proliferation – time = 100h

(c) Further cell proliferation – time = 300h

(d) Further cell proliferation and growing hypoxic region – time = 500h

Figure 12: Cell Proliferation in 3D
List of Tables

1. Parameters for the first experiment: single cell random movement. ................. 23
2. Parameters used for the cell cycle model (2). ........................................... 24
Table 1: Parameters for the first experiment: single cell random movement.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulation end time</td>
<td>28800 time steps</td>
</tr>
<tr>
<td>Time step length</td>
<td>0.25 min</td>
</tr>
<tr>
<td>Diffusion coefficient</td>
<td>$0.3 \times 10^{-9} \text{cm}^2/\text{min}$</td>
</tr>
<tr>
<td>Lattice size $\Delta_x$</td>
<td>$5 \times 10^{-4} \text{cm}$</td>
</tr>
</tbody>
</table>
Table 2: Parameters used for the cell cycle model (2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Cell</th>
<th>Cancer Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{min}$</td>
<td>3000 min</td>
<td>1600 min</td>
</tr>
<tr>
<td>$C_\phi$</td>
<td>3 mmHg</td>
<td>1.4 mmHg</td>
</tr>
</tbody>
</table>
RECENT REPORTS

12/72 Some observations on weighted GMRES G"uttel Pestana

12/73 Bounds on the solution of a Cauchy-type problem involving a weighted sequential fractional derivative Furati

12/74 Static and dynamic stability results for a class of three-dimensional configurations of Kirchhoff elastic rods Majumdar Goriely

12/75 Error estimation and adaptivity for incompressible, nonlinear (hyper)elasticity Whiteley Tavener

12/76 A note on heat and mass transfer from a sphere in Stokes flow at low Péclet number Bell Byrne Whiteley Waters

12/77 Effect of disjoining pressure in a thin film equation with non-uniform forcing Moulton Lega

12/78 A Review of Mathematical Models for the Formation of Vascular Networks Scianna Bell Preziosi

12/79 Fast and Accurate Computation of Gauss-Legendre and Gauss-Jacobi Quadrature Nodes and Weights Hale Townsend

12/80 On the spectral distribution of kernel matrices related to radial basis functions Wathen Zhu

12/81 Inner product computation for sparse iterative solvers on distributed supercomputer Zhu Gu Liu

12/82 A new pathway for the re-equilibration of micellar surfactant solutions Griffiths Breward Colegate Dellar Howell Bain

12/83 Object-Oriented Paradigms for Modelling Vascular Tumour Growth: a Case Study Connor Cooper Byrne Maini McKeever

12/84 Chaste: an open source C++ library for computational physiology and biology Mirams Arthurs Bernabeu Bordas Cooper Corrias Davit Dunn Fletcher Harvey
12/86  Boolean modelling reveals new regulatory connections between transcription factors orchestrating the development of the ventral spinal cord

Lovrics Gao Juhász Bock Byrne Dinnyés Kovács

12/87  Asymptotic solutions of glass temperature profiles during steady optical fibre drawing

Taroni Breward Cummings Griffiths

12/88  The kinetics of surfactant desorption at the airsolution interface

Morgan Breward Griffiths Howell Penfold Thomas Tucker Petkov Webster

12/89  An experimental and theoretical investigation of particlewall impacts in a T-junction

Vigolo Griffiths Radl Stone

12/90  Transitions through Critical Temperatures in Nematic Liquid Crystals

Majumdar Ockendon Howell Surovyatkina

12/91  Biaxial defect cores in nematic equilibria: an asymptotic result

Majumdar Pisante Henao

12/92  The Three Sphere Swimmer in a Nonlinear Viscoelastic Medium

Curtis Gaffney

12/93  Diffusion of multiple species with excluded-volume effects

Bruna Chapman

12/94  The Mechanics of a Chain or Ring of Spherical Magnets

Hall Vella Goriely

Copies of these, and any other OCCAM reports can be obtained from: