

Understanding tissue morphology: model repurposing using the CoSMoS process

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Abstract. Drawing inspiration from the CoSMoS project structure, we consider the assumptions made during the design and implementation of a software simulation of physical interactions during the formation of vascular structures from endothelial cells. We show how the abstract physical model and its software implementation can be adapted for a different problem – the growth of cancerous tissue under varying physical conditions. By identifying the changes that must be made to adapt the model to its new context, along with the gaps in our knowledge of the domain that must be filled by wet-lab experimentation when recalibrating the model, we maintain confidence in the repurposed model and achieve a satisfactory degree of model reuse.

Keywords: CoSMoS, model reuse, simulation, validation

1 Introduction

The CoSMoS process[1, 2] describes a principled approach to scientific modelling and simulation: it provides a structure for managing and documenting the iterative development of a simulation, and gives scientists and simulation developers tools to reason – with an appropriate balance of confidence and scepticism – about how their simulation’s results relate to the domain under study. CoSMoS is an agile approach based upon a pattern language: a user may organise their project entirely following the CoSMoS principles, or they may integrate some of the CoSMoS patterns as appropriate into an existing project.

Reusability of software components is a key concern of software engineering. Reusable components can – ideally – avoid the difficulty and expense of developing and validating substantial amounts of new software. But software developed for one purpose may not be reusable for a different purpose without substantial modification. In particular, a simulation component developed for one *in silico* experiment may rely on assumptions (parameter values, model simplifications, etc.) that are only valid within the context of that experiment. Adapting such a component for reuse in a different context requires careful consideration of the assumptions made during its design.

In this paper, we use the general structure of projects outlined by CoSMoS to organise our thinking around how a model of physical interactions among cells can be adapted from one context – the formation of vascular structures

from endothelial cells – to a different context – the effects of cancer treatment drugs on the growth of spheroid structures of cells. Neither of these projects was initially developed using a CoSMoS approach. To apply CoSMoS techniques, we must first effectively reverse-engineer our work to date, and attempt to organise the information we have about the systems under study and our models and simulations of them broadly in terms of the CoSMoS project structure. We expect that this step in itself will prove valuable.

Our objective is specifically to reuse the software components that implement this physical model within the simulation, as these required considerable development effort and are critical to the overall performance of the simulation. As the modes of physical interaction among cells are broadly similar between the two models, this seems *intuitively* to be an appropriate approach – but identifying and revalidating our assumptions will help to build our confidence in our simulation’s results, and enable the future reuse of the physical model in other contexts.

In addition, we are now at the point in the development of our cancer model where it is clear that some wet-lab experimentation is required in order to recalibrate the parameters of the physical model. Since wet-lab experimentation is expensive and time-consuming (in this case, time-series imaging requires several days’ commitment from a skilled researcher), we need to be confident that we are obtaining the correct data from the experiments to support our simulation development.

2 The Original Model: Vascular Formation

2.1 Research Context

The purpose of this simulation is to reproduce the results of an *in vitro* experiment from the literature, demonstrating the formation of capillary structures from endothelial cells [3].

The experiment explores how the physical interactions among the cells, and their low-level physical properties, affect the larger-scale structural patterns in the resulting capillary network. The effects of varying concentrations of growth factors – which have a direct effect on the low-level physical properties of the cells – are of particular interest.

2.2 Domain

Microvessels are formed within the body by the aggregation of endothelial cells, which themselves are formed by differentiation from stem cells. This formation process has three stages [4]:

- cell migration and early network formation;
- network remodelling, where cells connect to each other;
- further differentiation into tubular structures.

For the purposes of this experiment, we are only concerned with the first stage, which takes place between six and nine hours *in vitro* [3]. At the end of this stage, the basic network structure has formed, but cells have not yet begun to bind to each other or to differentiate further. All cells are similar in general terms during this stage, although their individual properties may vary – for example, we would expect to see a roughly normal distribution of cell sizes.

We believe that in this stage the most significant forces are those resulting from physical interactions: between pairs of cells, between cells and their surrounding medium, and between cells and the substrate (Matrigel film [3, p. 1778]). As the surrounding medium is relatively thin and the interactions with the substrate are strong, there is only limited potential for cell movement away from the substrate, and 2D imaging can be used effectively to capture cell positions in real-world systems. Time-series imagery can be used to characterise cell interactions – for example, [5] shows physical interactions among stem cells *in vitro*, including attraction between cells, and cell shape changes after differentiation and binding.

As cells follow growth factor gradients, the density of cells tends to be higher where there is a higher concentration of growth factors in the environment. The *in vitro* experiment examined the effects of an artificial reduction of growth factor levels across the environment, imaging control and reduced-factor experiments at 3 h intervals.

2.3 Domain Model

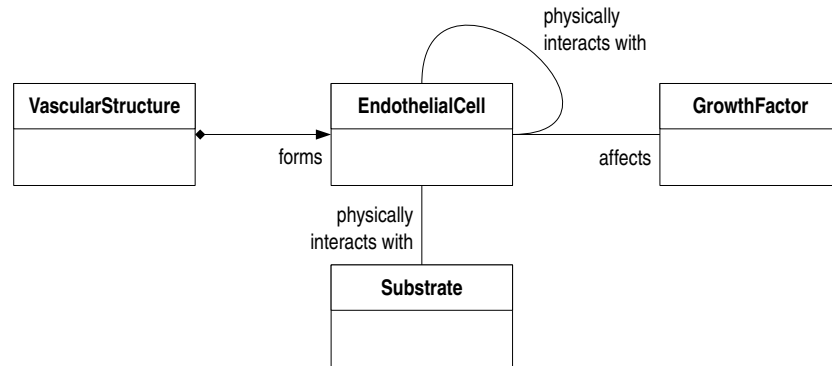


Fig. 1. Domain model: the entities of concern in the domain, shown as a UML class diagram

Fig. 1 shows the entities within the *in vitro* experiment that we are attempting to reproduce, and their interactions. This includes both the biological entities

under study and their experimental environment. Vascular structures are also included here as an emergent behaviour of the cells. Note that we have used UML in a rather informal way here, as we have in later figures – for example, while growth factors are indeed individual molecules, we would not generally think about them that way when modelling the system. While the semantics of the diagram are not correct, it is still useful as a “cartoon” in CoSMoS terms, capturing our (necessarily limited) understanding of the system in a convenient but loosely-specified notation.

2.4 Platform Model

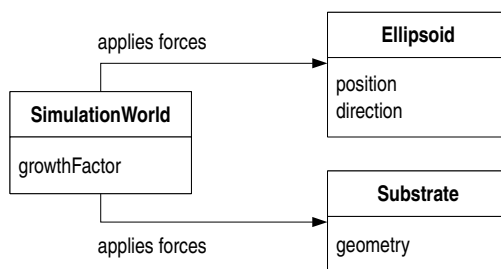


Fig. 2. Platform model: the entities of concern in the simulation, shown as a UML class diagram

Fig. 2 gives an overview of how the domain model has been simplified for the purposes of the simulation. We have chosen an agent-based modelling approach, so cells show a direct correspondence between the domain and platform models. This allows us to define interacting rules for single cells, and examine both the lower-level properties of individual cells and the higher-level behaviour of the system as a whole. The simulation proceeds in discrete timesteps, with all cells updating their positions and orientations atomically at the end of a timestep.

Vascular structures have been removed entirely, as these are the emergent property that we are attempting to reproduce. Other entities have been simplified, or introduced to allow implementation of the physical interactions within the system.

While cells can take a wide variety of shapes in the real world, we must model these as simpler shapes in order to practically simulate physical interactions at realistic scales. Modelling cells as simple spheres simplifies reasoning, but it does so by discarding information about the orientation of the cell, which limits the types of physical interactions that are possible. Initial prototyping showed that it was difficult to reproduce vascular formation behaviour using spheroid cells.

We therefore represent cells as ellipsoids. (Fig. 3). The shapes of cells observed in the *in vitro* experiment are roughly ellipsoidal (in the first phase). An

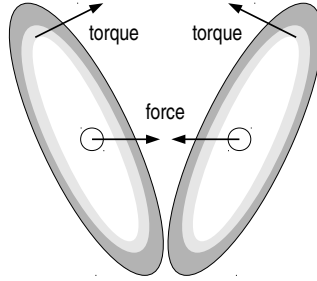


Fig. 3. Idealised ellipsoid cells within the platform model, showing torque and force

ellipsoid has three orthometric semi-axes, which can be used as a local coordinate system. The rotation of an ellipsoid can be represented by the change of this local coordinate system, and the direction of an ellipsoid can be represented by the transformation from the local coordinate system to the global coordinate system. The position of the centre of an ellipsoid represents the position of the whole ellipsoid.

We are only interested in simulating the first phase of vascular formation, during which cells do not divide or measurably change their physical properties. We do not therefore need to simulate cell differentiation or the cell cycle, and can assume that cells' sizes and shapes are constant over time.

We assume that the density of cells is even, so forces can be modelled as acting on the centre of the cell, and changes in cell orientation can be modelled as torques acting on the cell. This is a modelling convenience and difficult to validate against experimental data, as cell rotations are hard to distinguish in 2D time-series images.

We model physical interactions between cells in terms of forces between them. The *adhesion force* attracts cells to each other; the *contact force* repels them and prevents them from overlapping; there is also a *resistance force* resulting from cells' interactions with the surrounding medium. For each force, there is an corresponding torque that is computed in an analogous way.

The contact force only takes effect when cells are in physical contact; the greater the overlap, the greater the contact force. As the ellipsoid is not an isotropic shape, we cannot simply use the distance between two ellipsoids to calculate the contact force and torque. Instead, we compute a potential for each interaction: a path-independent potential energy. In Fig. 4, dotted lines represent potential surfaces around the cell – the potential is constant for any point on the same surface, although the distance to the cell centre will vary as a result of the cell shape. The potential is calculated following Perram and Wertheim's approach [6], using the direction, position and length of the semi-axes of the interacting ellipsoids.

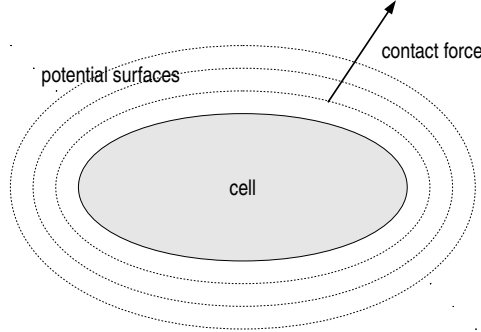


Fig. 4. An ellipsoidal cell showing potential surfaces, and the vector along which contact force is computed

The potential is then transformed into energy using the Hertz formula. The magnitude of the resulting force or torque is the same for all points on a potential surface; the direction is computed based on the partial derivative of the energy field towards the centre of the interacting ellipsoid (Fig. 4).

The adhesion force, however, is modelled as a constant force attracting the centres of every pair of cells in the same way, provided they are within a minimum distance of each other. This is the simplest approach that reproduces the behaviour observed in time-series images of the *in vitro* experiments. If cells are beyond the minimum distance they have no physical interactions; if they are within range, they move towards each other, until they become close enough to overlap, stopping at the point at which the adhesion force and contact force balance each other.

The relative strengths of the two forces may be calibrated so that this balance happens at a potential corresponding to that observed in cells *in vitro*. The potential will depend on the elasticity of the cells, with higher balancing potential levels indicating more rigid cells. Some elasticity is necessary to obtain realistic cell interactions: an early prototype of the model used a simpler approximation to the Hertz function which effectively gave inelastic collisions between cells, and resulted in cells visibly “bouncing off” each other – which did not match what we see in time-series images!

As cells move at relatively low speeds within the medium, their acceleration can be approximated as zero – which means the sum of the forces upon them is also zero:

$$\sum \mathbf{F} = 0 = \mathbf{F}_{\text{contact}} + \mathbf{F}_{\text{adhesion}} + \mathbf{F}_{\text{resistance}} \quad (1)$$

We can therefore compute the resistance force in terms of the contact and adhesion forces – and, from this, compute the velocity of the cell using Stokes’ law, based on the known size and shape of the cell and the properties of the

medium. The angular velocity can be found using a similar technique; from these, the position and orientation of the cell on the next timestep can be computed.

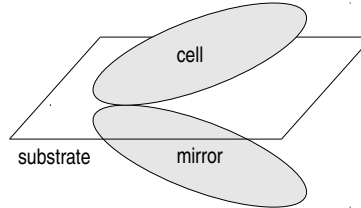


Fig. 5. Cell-substrate interaction, modelled as interaction with a copy of the same cell, mirrored in the substrate plane

The substrate itself is modelled as a plane. The physical interaction between a cell and the substrate is modelled as the interaction between a cell and its mirror image in the plane (Fig. 5). However, the adhesion force between a cell and its mirror image is scaled up to account for the stronger interactions between cells and the substrate than between cells and other cells.

The model is dimensionless, being defined in terms of a unit time (the simulation timestep) and a unit length (the radius of a typical cell). These two quantities are related, in that computing the velocity of a cell within the fluid medium depends on both the timestep and the shape of the cell. However, making an assumption about the maximum velocity of a cell allows us to find reasonable bounds for one unit knowing the other, and in our case choosing a unit timestep of 1 s gives a physically-plausible maximum velocity for endothelial cells.

To summarise, we have made the following assumptions when constructing the platform model:

- Cells can be represented as ellipsoids.
- Cell size and shape do not change during the experiment.
- Matter is evenly distributed within a cell.
- Only contact force, adhesion force and resistance force are significant.
- Contact force can be computed using the Perram-Wertheim approach.
- Adhesion force can be modelled as a step function on distance (i.e. the growth factor gradient does not have a significant effect on attractive force).
- Contact and adhesion forces balance at a defined point when cells are in contact, and the strengths of the forces can be calibrated based on this.
- Cells move at very low speed, so their acceleration approaches zero and the forces upon them are balanced.
- Resistance force can be computed using Stokes' law, and the known properties of the fluid medium.
- Interactions with the substrate can be modelled as interactions with mirrored cells.

The physical parameters of the model (the unit time and length, and the constants involved in computing the forces) depend on the following values:

- the typical size of a cell;
- the range of ellipsoidal shapes a cell may adopt;
- the mean density of a cell;
- the dynamic viscosity of the fluid medium;
- the maximum speed at which a cell may move in the medium.

2.5 Simulation Platform

The simulation implementation follows the structure described in the platform model (Fig. 2). The simulation world object maintains the set of agents, and computes and applies the forces among them. In addition, it provides the ability to import simulation parameters, and to export the state of the simulation to a file for visualisation and analysis by external tools.

Model parameters were calibrated as described above. However, testing the simulation with these constants resulted in cells moving unrealistically rapidly. Reducing the strengths of the cohesion force and adhesion force by an order of magnitude resulted in more realistic cell movement – but the cause of this has not yet been traced back to the model.

2.6 Results Model

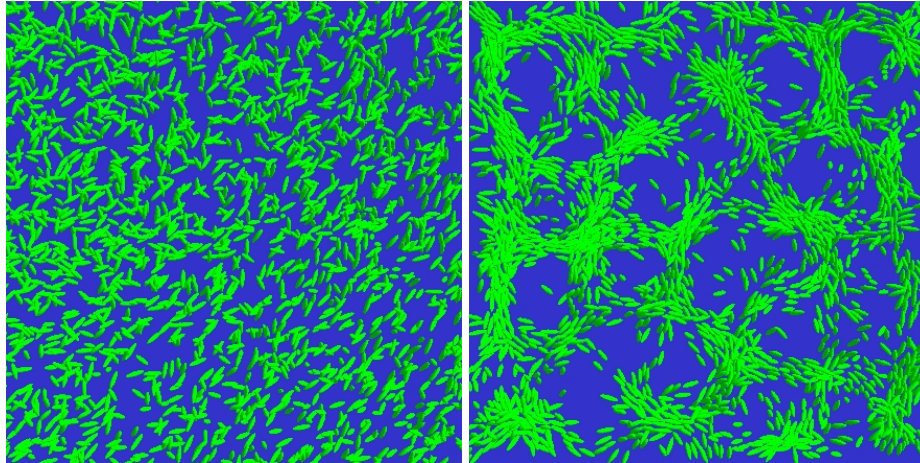


Fig. 6. Visualisation showing cell positions and orientations at the start (left) and end (right) of the simulation; “unstable” pattern

Fig. 6 shows the starting and ending conditions of the simulation. This certainly resembles the vascular network we are trying to reproduce – but we need

a quantitative measure of this, in order to relate the results back to the changes in the level of growth factor.

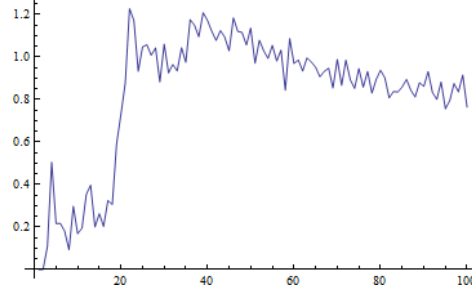


Fig. 7. Radial distribution of cells in Fig. 6 (right); X axis is distance between cells in simulation units, and Y axis is normalised probability of finding another cell at that distance

There is a quantised method to describe the pattern of this structure, which is called the radial distribution function. The radial distribution function is a tool to describe space distribution of a system that consists of particles, by describing the chance of finding another particle within an arbitrary distance from the reference particle. In the form of the distribution curve, normally the X axis is distance, and the Y axis is the function value. If the function value is bigger than 1.0 at a certain distance, it means the cell density is higher than average at that distance; if the function value is smaller than 1.0, it means the cells are more sparse at this distance. Fig. 7 shows the radial distribution of cells at the endpoint of the simulation.

The minimum near distance 0 shows that cells tend not to have very close neighbours; the second minimum near distance 100 shows the typical size of hole in the net-shaped structure. This minimum corresponds to the typical net-size in [3], which is determined by the concentration of growth factor. As the distance from the reference cell increases, the value of the distribution function varies around 1.0, which means over longer distances the cells tend to be distributed evenly. Comparing with the distribution curve obtained from the *in vitro* experiment [3], we can say that our physical interaction has similar effects to the growth factor in the experiment.

If we allow the simulation to continue past the state shown in Fig. 6 – i.e. past the period of time covered in the original model design – the pattern will collapse into a few large clusters of cells. Fig. 8 shows the results of an simulation where the physical parameters have been adjusted to produce a stable pattern that does not collapse; while some network structure is visible, it is not as clear as the original model. This is echoed in the radial distribution, shown in Fig. 9, which no longer shows a clear minimum.

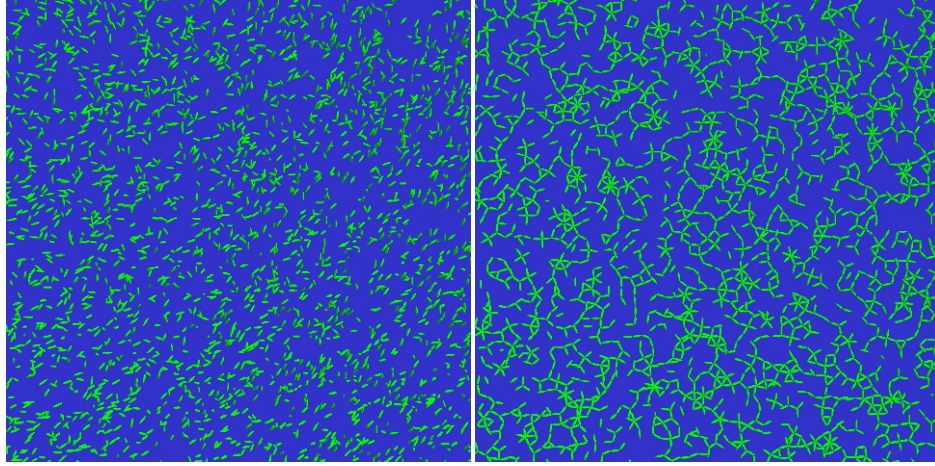


Fig. 8. Visualisation showing cell positions and orientations at the start (left) and end (right) of the simulation; “stable” pattern

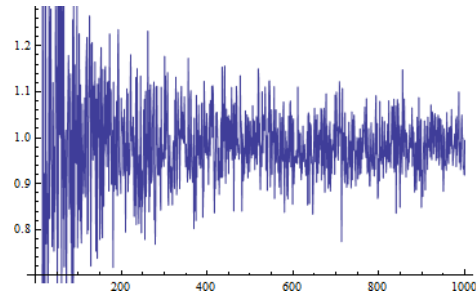


Fig. 9. Radial distribution of cells in Fig. 8 (right); X axis is distance between cells in simulation units, and Y axis is normalised probability of finding another cell at that distance

3 The New Model: Spheroid Growth

3.1 Research Context

As with the vascular development model, our objective is to relate lower-level physical interactions to higher-level structural behaviours: we want to explore the effects of

- certain cancer treatment drugs,
- hypoxia (low concentrations of oxygen), and
- different cell lines (types of cell grown for experimental purposes)

upon the growth of tumours. This work forms part of a wider programme of activity developing techniques for cancer drug discovery and development [7]. Our domain experts are cancer researchers who are interested in making use of models and simulations to direct experimentation.

Tumours develop distinctive patterns of cells, which can be classified by domain experts either manually or using automated image processing. It is specifically these spatial patterns that we are interested in reproducing within a simulation.

Our existing physical model has already demonstrated the ability to reproduce spatial patterns of cell growth resulting from physical interactions within an agent-based simulation, and we have existing tools to visualise and analyse the output from the model. We would like to reuse as much of this infrastructure – both the model and the simulation code – as possible to reduce development time, but to do this we must identify the changes that need to be made by reevaluating our original assumptions within the new research context.

In addition, we must identify what information necessary for reengineering and calibrating the model needs to be obtained by wet-lab experimentation. We aim to maximise the value obtained from this experimentation.

3.2 Domain

In the real domain, cancer cells develop and grow into tumours within surrounding tissue [8]. In the lab, growth experiments may be conducted on a Petri dish – in which case cells can grow into a flat structure – or in a larger volume of gel, in which case spheroid structures can form (Fig. 10).

Petri-dish experiments are easier to collect data from, since 2D images can be taken non-destructively; spheroids must be sectioned before imaging in order to obtain data at a cellular resolution. A typical Petri-dish experiment contains around 5,000 cells; a spheroid contains on the order of 10^6 cells. A single section through a spheroid is comparable in size to a Petri-dish experiment.

Experiments are conducted using cell lines: cells grown for experimental use which have well-understood properties, such as the activation of particular oncogenes, or the ability to form structures such as spheroids.

The shape and volume of cells varies as they progress through their developmental cycle (Fig. 11); the rates at which the cycle progresses varies somewhat

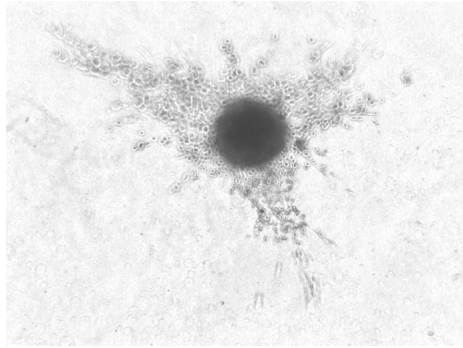


Fig. 10. 2D side-view image of a three-dimensional spheroid growing within a gel medium

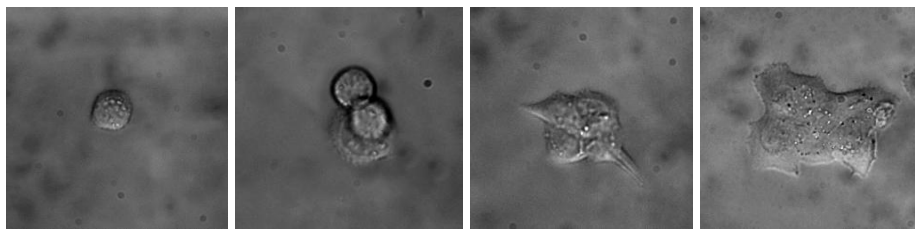


Fig. 11. HCT-116 (p53+/+) cells, growing on a glass plate, imaged at 6 h intervals. The diameter of the initial cell is 10 μm .

among cell lines. The HCT-116 cells we are using typically have diameter $10\text{ }\mu\text{m}$ immediately after division, and can be observed to grow over a period of approximately 24 h before dividing. Cells only remain healthy under experimental conditions for a limited period of time; it is therefore impractical to run experiments for more than 72 h, and images are typically taken every 6–8 h.

Some cancer drugs limit cell growth by arresting the cell cycle at a particular stage [9]. The progression of the cell cycle within the individual cells is therefore important when understanding the effects of drugs upon a tumour: if the cell cycles are synchronised (as can happen under experimental and *in silico* conditions), then a drug can arrest many cells simultaneously, whereas cells at a mix of developmental stages will be less strongly affected.

For spheroid structures, we are particularly interested in the effects of hypoxia, which can have a suppressive effect on cell growth [10]. The high density of cells within a spheroid structure means that cells become increasingly hypoxic towards the centre of the spheroid.

3.3 Domain Model

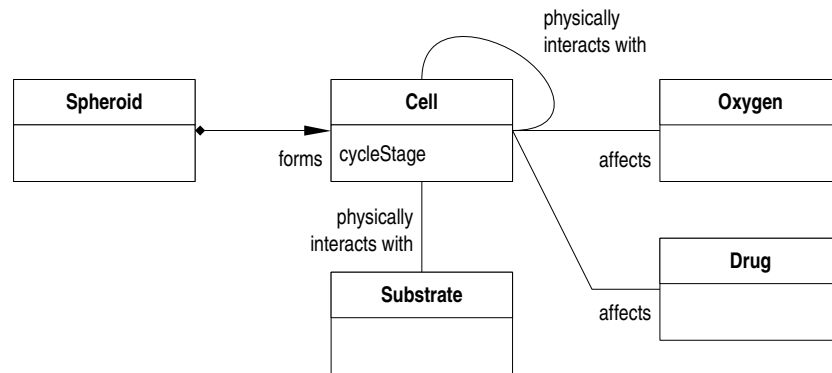


Fig. 12. Domain model: the entities of concern in the domain, shown as a UML class diagram

Fig. 12 shows the entities within the domain model. While the domain is substantially different from the previous one, the way cells are modelled retains a level of similarity, because the emergent behaviour of interest still results from physical interactions among cells. However, the physical properties of the cells themselves are somewhat different from our previous model – in particular, the cells’ properties are known to change over time, and we are interested in the effects of this on the emergent properties.

The drugs and hypoxia condition are added to environment conditions, forcing cells to enter or quit certain cell cycle phases. For the two-dimensional experi-

ment, we still need to consider the substrate. But for the spheroid experiment, as the tumour cells grow in 3D in agar gel, we will no longer consider the substrate.

3.4 Platform Model

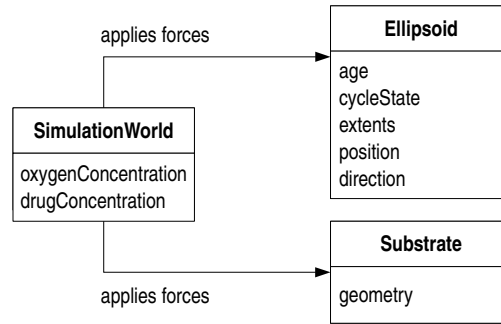


Fig. 13. Platform model: the entities of concern in the simulation, shown as a UML class diagram

We know from the domain model that the individual development of the agents – e.g. the growth of cells over time – will be important to the behaviours we are trying to replicate, and must be taken into account in the simulation. As a result, we have chosen again to use an agent-based modelling approach. Fig. 13 shows the entities within the simulation platform.

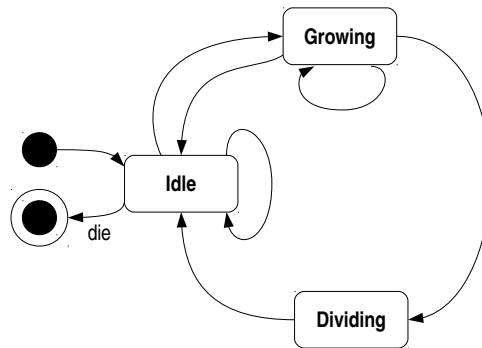


Fig. 14. Platform model: simplified cell cycle, shown as a UML state diagram

Fig. 14 shows the state machine that models a simplified cell cycle and drives the behaviour of the simulated cell. This represents the observed behavioural modes of the cell – growth, reproduction, apoptosis – rather than the biological markers that would normally be used to describe cell cycle stages.

In the construction of the physical aspects of this platform model, we aim to reuse, as far as possible, our previous approach. In order to evaluate whether this is appropriate, we must reconsider our previous assumptions, listed in Sect. 2.4, based on our knowledge about the new research context.

While the physical properties of the cells and medium are somewhat different, we believe that most assumptions remain valid. One assumption, however, is no longer reasonable: cell size and shape *do* change during the simulation. This requires changes to how the interaction potentials and their resulting forces and torques are computed, since these must now take changes to cell size and shape into account.

We must also ensure that we have sufficient information to allow calibration of the physical parameters. We no longer just need the typical size and shape of a cell: we need a profile showing how cell size and shape can change as the cell cycle progresses. This information will need to be obtained by time-series imaging under the experimental conditions we wish to simulate, as in Fig. 11. We will then give each simulated cell an interpolated growth curve based on the measured points. The other information we need for calibration is available in the literature (e.g. the dynamic viscosity of the medium).

The choice of timestep size (i.e. unit time in the model) is a concern. The timestep must be short enough to obtain results at a comparable temporal resolution to the *in vitro* experimental data. However, smaller timesteps require more calculation steps to simulate the same length of real-world time; the 1 s timesteps used in the previous simulation would result in *in silico* experiments taking an impractically long time to run with typical simulation sizes (10^3 – 10^6 cells).

In the *in vitro* experiment, the typical treatment time is 48 h to 72 h, and the sampling rate varies with different phases of the experiment. For example, in the first 2 h, the cells may be imaged every 10 min, then the time between each sample increases as the experiment goes on. The simulation timestep does not therefore need to be any less than 10 min, and 1 h would probably be reasonable. The other constants will need to be adjusted to suit – for example, this results in the simulation’s unit length being considerably smaller (which does not affect the outcome of the simulation).

3.5 Simulation Platform

The simulation platform is currently under development, following the structure described in the platform model. As we have reused the physical aspects of the platform model, we have similarly been able to reuse much of the code from the previous simulation platform – with the adaptations we have just described.

3.6 Results Model

We are primarily interested in the shape of structure tumour cells can form. We can expect cell density changes across a slice through the spheroid. Typically as hypoxia often happens in the centre of spheroids, the cell density in centre part should be lower than the cell density near the surface of the spheroid. If we slice the tumour tissue, the cell density should be lowest in the middle of the slice.

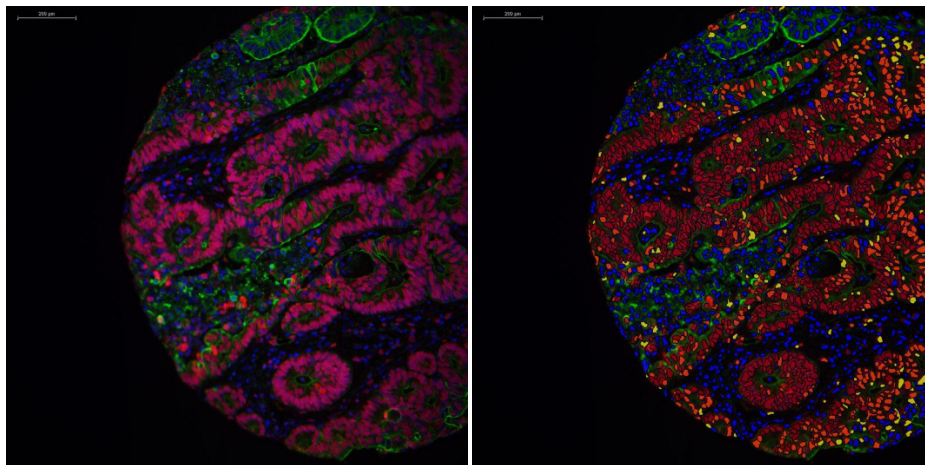


Fig. 15. Left: Ki67 expression in colorectal carcinoma tissue microarray data. Right: cell outlines and activity levels automatically identified from the previous image using Definiens.

We are also interested in the overall shape of the spheroid. There are existing image analysis tools that can be used for this. We will use them to extract information from wet-lab experimental imagery, including the position and direction of all the cells. We will then have directly compatible data from both wet-lab experiments and our simulation that can be analysed using a consistent approach. With this information we may use methods such as fractal geometry[11] to analyse the overall structure of both experimental and simulation data. Based on our experience with our physical model, we expect to be able to obtain reasonably good correspondence for 2D data – but we suspect that extending the spatial interactions into three dimensions will require further elaboration of the model.

4 Conclusion

Through initial analysis guided by the CoSMoS project structure, we have identified that the physical aspects of the new domain do indeed have considerable similarities with those of the original domain – so reuse of the physical model

should be appropriate, provided that the assumptions in the model – which we have explicitly identified – are reevaluated appropriately within the new context.

Other aspects of the two domains are substantially different; for example, the cancer simulation requires an implementation of the cell cycle in order to accurately simulate the effects of different treatments and environmental conditions, whereas this was unnecessary in the vascular simulation owing to the initial stage limitation.

We have also identified the gaps in our knowledge about the domain, necessary to appropriately calibrate the physical model, which must be filled by web-lab experimentation. This gives us confidence that we are asking the right questions when conducting experiments in support of calibration.

What we have ended up with is emphatically *not* “a CoSMoS project” – we have simply made use of a few aspects of CoSMoS to structure our thinking about model reuse. In effect, we have only made use of some of the large-scale CoSMoS patterns that describe concepts such as “domain model”, and that in a rather sketchy and informal way – but we feel that even this first step towards CoSMoS has been valuable in terms of forcing us to think in a principled way about our existing work. As this work continues, we intend to make increased use of CoSMoS techniques; for example, to more effectively structure our interactions with domain experts during the calibration of the spheroid model. We feel, in general, that the ability to adopt patterns as appropriate is a significant strength of the CoSMoS approach in terms of adoption by existing projects – as it is for other pattern languages.

While it is important to emphasise that this project is still work in progress, we feel that we have achieved a satisfactory degree of model and software reuse – and, more importantly, we are confident that this reuse has been achieved in a way that is *appropriate* and *useful* within our new research context. In the future, we would like to consider strategies and patterns for this kind of reuse within the CoSMoS process – in particular, how a validity argument might be constructed and updated as a model is reused.

In addition, by documenting this process, we now have a framework in place that would allow us to reuse the physical model within new research contexts in future projects. Once the cancer cell growth model has been demonstrated in two dimensions, we plan to extend it to simulate three-dimensional spheroid structures – which will require further reevaluation of the physical model, particularly relating to interactions with a gel medium.

5 Acknowledgements

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