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Active Vertex Model of Epithelial Monolayer Mechanics

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ACTIVE VERTEX MODEL OF EPITHELIAL
MONOLAYER MECHANICS

By

Daniel Barton

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SCHOOL OF SCIENCE AND ENGINEERING

I certify that Daniel Luke Barton has satisfied all the terms and conditions of the relevant Ordinance and Regulations to qualify in submitting this thesis in application for the degree of Master of Science by Research.

Dated: April 2017

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I declare that the work described in this thesis is my own; that I am the author of this thesis; that it has not previously been put forward in submission for any other degree of qualification; that where I have consulted the published work of others, this is always clearly attributed.

Daniel Barton
Abstract

An Active Vertex Model (AVM) for cell-resolution studies of the mechanics of confluent epithelial tissues consisting of tens of thousands of cells, with a level of detail inaccessible to similar methods is presented. The AVM combines the Vertex Model for confluent epithelial tissues with active matter dynamics. Cell contacts are generated dynamically from positions of cell centres which leads to a natural description of the T1 transition events responsible for local tissue rearrangements. The AVM also includes cell alignment, cell-specific mechanical properties, cell growth, division and apoptosis. The AVM introduces a flexible, dynamically changing boundary of the epithelial sheet allowing for studies of phenomena such as the fingering instability or wound healing. We illustrate these capabilities with a number of case studies. An expression for mechanical stress in the model is then derived from the Hardy stress for atomistic simulations. Calculation of stress is important for understanding how cell-level events coordinate to drive morphogenic processes.
Chapter 1

Introduction

The tissue composed of thinly layered sheets of cells which lines the walls of blood vessels, cavities and organs is called Epithelium. Epithelial cells arrange into sheets by forming various types of cell-cell junctions. Among them are *Tight* junctions, designed to be an impermeable barrier to fluid and *Adherens* junctions which connect cell membranes as well as link cytoskeletons [1]. Adherens junctions are firm enough to transmit forces while still maintaining the ability to be broken and reformed by the cell molecular machinery [2]. Linking cell cytoskeletons together has the effect of extending the sturdy nature of the cytoskeleton over the whole epithelium and stabilises the tissue against external forces. With these properties epithelial tissue can maintain its structure while bending to form the highly curved structures of the human body [3] like skin or the internal surfaces of the gut. At the cellular scale, individual epithelial cells can divide, die, detach from the tissue or migrate past each other, continuously breaking and reforming their cell-cell junctions [4]. Examples of Epithelial tissue are shown in Figure [1.1](#).

In this thesis we build a model as well as some analytical tools for the study of
single-cell thick epithelial sheets or epithelial monolayers. Using the term monolayer rather than tissue we include in our scope any dense collections of epithelial cells such as those cultured on an artificial substrate. We also specify that the monolayer should be confluent, that is, having no gaps between cells. This confluent property is central to the impermeable character of epithelial tissue and is observed in epithelial monolayers in vitro as well as in vivo. Generally one side of an epithelial sheet is supported by an extracellular matrix or substrate, we call this the basal side. Opposite is the apical side and the mutually connected faces of cells are their lateral sides. A view of the apical side shows a detailed network of cell-cell contacts while along the apical-basal axis the cells resemble straight columns, shown in Figure 1.2. The apical surface is of particular interest. Firstly, because a portion of the molecular machinery which controls epithelial cell contacts is concentrated there, in a ring pattern that encircles the cell [4] and secondly, because this surface is typically
Figure 1.2: Schematic showing the geometry of a columnar epithelial tissue. Three cells are shown with coloured rings close to their apical faces to indicate where the cell machinery which is responsible for binding cells together is located. The green ring represents adhesive proteins (i.e. Cadherins), the red ring indicates the presence of bundles of cytoskeletal filaments and the blue ring indicates that special ‘motor’ proteins are present which can use chemical energy to apply forces on cytoskeletal elements. Adapted from [4].

the most accessible to modern imaging techniques. This machinery localised at the apical surface can actively contract in a uniform way to bend an epithelial sheet in three dimensions [7, 8, 9]. Alternatively individual cell-cell junctions can contract leading to cell neighbour rearrangements [10, 11] which coordinate to drive tissue level morphogenesis behaviours seen in the chick embryo [12] and Drosophila wing tissue [13] for example. The recent development of specialised experimental techniques for tracking the movements of many thousands epithelial cells in vivo [14] is a driving force behind the modern study of epithelial cell dynamics. We discuss an example in Section 1.1.1.
1.1 Collective Cell Migration

Biological cells are motile in that they may propel themselves by consuming their own reserves of chemical energy. Some cells like white blood cells migrate independently to track down invaders while others migrate collectively to close wounds or during embryo development. In fact, collective migration plays a role in the construction of essentially all tissues with complex shapes or functions. Methods of motility vary depending on the environment such as ‘swimming’ through liquid or ‘crawling’ on a surface. Although the crawling motion of cells is not exactly understood, we know that cells extend protrusions in the direction of motion and that cells have fine control over the strength of their cell-substrate adhesion sites. Cells in a confluent epithelial monolayer with a fixed boundary can gain traction from each other and move by contracting their shared cell-cell contacts. The problem of how cells collectively determine their desired direction of travel is under intensive study. Cells may migrate in response to chemicals in their environment often by orienting themselves to point up a concentration gradient. Alternatively densely clustered cells have been shown to orient towards the available free spaces or along directions in which they are being physically pulled by adjacent cells. In some cases the cell can be said to have a polarity which corresponds to the internal structures of the cell being organised for movement in a specific direction. Note that since epithelial cells have distinct apical and basal sides they are said to have apical-basal polarity but they also move in the plane of the tissue, and if they do so by crawling we may expect them to have an independent planar-polarity. The motility mechanisms of cells in epithelial sheets are
poorly understood compared to that of individual cells \cite{24} because the epithelial cells may have complex interactions with the substrate layer as well as each other. It is interesting that densely packed cells can cooperate with each other to migrate more efficiently than individual cells \cite{25}. But how is it that cells take advantage of the monolayer structure to migrate more effectively? By what methods do they communicate and over what length-scales do they cooperate? These are fundamental questions with broad implications in biology and medicine, for example in tissue engineering and regenerative medicine. We hope that the methods developed in this thesis will aid in answering such questions. Initially we examine two experimental configurations being used to study collective and cooperative cell migration and mention some of the modern tools used to study them. The systems under study are the chicken embryo and cultured epithelial cells from the inner lining of blood vessels (RPME cells). When discussing cultured epithelial cells in this work we are always referring to experiments on RPME cells.
1.1.1 Chick Embryo

In the initial stages of chicken embryo development, cells proliferate and form an epithelial tissue. The embryo is at first one-cell thick and remarkably flat, making it a convenient system for study in vivo. During development, a pair of large-scale vortex-like flows span the width of the embryo and carry cells towards a central ‘streak’ region where they are forced out of the sheet in the basal direction, a process called \textit{ingression}. This is the formation of the ‘primitive streak’ which marks a key stage in the development of many types of animal embryo, including mammals. It is not known what sets this pattern of cell migrations. Certainly, chemical gradients are important, but the response of cells to mechanical forces from their neighbours is also likely to play a role.

Experimental tools have been developed and applied recently to record cell-level resolution images of the whole chick epithelium over periods of many hours. The improvement on the scale of tissue that can be studied this way comes from effective use of light-sheet microscopy \cite{5,20}. A light-sheet microscope scans across the sample, illuminating successive planes at 45° to the surface. The planes are then reconstructed into a 3D image of the epithelium. This reconstructed image is targeted at the apical side of the tissue and loses resolution towards the basal side so that the basal faces of cells are not always possible to resolve. To finally obtain a two-dimensional image of the tissue, like the one shown in Figure 1.1a, a cut is made through the 3D data just below the apical surface.

The chick embryos come from a genetically modified chicken which has its cell membranes tagged with green fluorescent marker. This causes cell boundaries to
fluoresce brightly so that an image analysis algorithm can track the shapes, trajectories, divisions and ingressions of each individual cell to a high degree of accuracy [5]. During primitive streak formation the chick embryo starts developing separate layers and three dimensional processes become increasingly relevant. Still, we expect that most of the important cell dynamics can be seen from the apical surface.

1.1.2 In-vitro Monolayer

The second example of cell migration occurs in epithelial cells cultured on an artificial substrate. We describe the behaviour of epithelial cells with regard to a specific set of experiments [23, 22], it is yet to be seen whether these behaviours are general features of crawling epithelial cells. Firstly, these cells adhere strongly to each other and are tightly packed together just as they would be in vivo. Ideal conditions for growth are provided and the cell patch allowed to naturally proliferate. It is reasonable to propose that the expansion of the monolayer is driven by cell growth and division which passively pushes out the patch boundaries. In fact when the monolayer is left to grow freely, cells actively migrate away from the centre while maintaining tight boundaries with each other. Cells naturally elongate in their direction of motion, then divide along this axis resulting in progeny that are initially more rounded and the process repeats [23].

To make good progress, nearby cells should align their direction of motion and cooperatively mediate the strength of their adhesive junctions with each other and with the substrate. In order to do this, cells must be able to sense each other and transmit signals over distances of several cell lengths. As with the chick embryo it is unclear whether mechanical or chemical signalling or some combination thereof is
Figure 1.4: The three types of topological changes on the apical network corresponding to (a) Exchange of neighbours as one pair of cells move together to become adjacent while another pair of cells are pushed apart, i.e. a T1 transition. (b) A single cell splits into two, of approximately equal area, in a division event. They then relax into more natural shapes. (c) Cell ingression or apoptosis leaving a 5-fold rosette where 5 cells meet at a point in the network.
of primary importance for this coordination. With regard to mechanical signalling, stresses on the cell membrane have been known to induce not only a sophisticated mechanical response \cite{27} but also a change in the cell biochemistry via chemical binding sites that are sensitive to being stretched or to local curvatures \cite{28,29}.

### 1.1.3 Measuring Stresses

To fully understand collective migration it is critical to have the tools to measure the ‘traction’ forces exerted on the substrate as well as the forces between cells in the monolayer. One direct method is to cut the tissue with a laser and observe the way it relaxes to a new force balance. The edges of an individual junction severed by the laser are pulled apart if the surrounding cell aggregate is under tension. The rate of displacement of the cut edges can be used to calculate the tension force in the junction prior to cutting. Alternatively, tension in individual junctions can also be revealed without damaging them by probing with optical tweezers and measuring the response \cite{30}. Finally we mention that some information about the stresses in the tissue can be inferred from the apical network geometry under certain assumptions \cite{31,32}.

A recently developed method for measuring stress in-vitro involves adding many small fluorescent beads close to the surface of a substrate. As the cells crawl, they apply a gripping and pulling action which deforms the substrate and displaces the beads slightly. These displacements are captured by a camera and used to recover the forces acting between the cells and the substrate. The procedure is then taken one step further and the forces between cells in the monolayer are calculated \cite{33}. Using deformations of a substrate to determine cell-substrate forces is a long standing
method known as Traction Force Microscopy [34] but it has only recently been extended to correctly measure inter-cell forces [33, 35]. The method is non-destructive unlike laser cutting, more scalable than optical tweezers and yields more information than stress inference but is still not perfect. It only applies to crawling cells and cannot be done in vivo. In the next chapter we develop a computational model for epithelial sheets which supports active cell migration and in the final chapter we derive an expression for stress in this model and discuss how to compare it with Traction Force Microscopy data.

1.2 The Apical Network

In preparation for developing a model for these epithelial sheets we focus on the apical network of cell-cell contacts, seen clearly in Figure 1.1a. Cells are in contact if they share a common edge in the apical network and we call the set of contacts a network topology. We would like to describe the dynamics of the system purely in terms of this apical network and not be concerned with any complicated dynamics happening towards the basal side. An ingression corresponds to all of a cell’s apical edges shrinking completely, the apical face shrinks to a point and its neighbours now form a temporary rosette structure, see Figure 1.4c. From the perspective of the apical network we make no distinction between apoptosis and cell ingression. Cell division, by comparison, is a more complex process; cells have been known to divide along the out-of-plane axis and then rearrange to be side by side columns in the monolayer[4]. However from the perspective of the network topology a cell division merely corresponds to the addition of a new edge which bisects the cell.
Finally, pairs of cells can exchange neighbours by the shrinking away of a single cell contact and subsequent extension of a new edge in the network. This particular topological move was first described in the study of foam bubbles and is called a T1 transition \cite{36}. Cells in the chick embryo are observed to collectively migrate large distances (many cell lengths) by sequences of T1 transitions. The key features of any dynamics of the cells in the plane of the monolayer can be understood as a combination of these three topological moves, Figure \ref{fig:11}. Cells at the periphery of the embryo grip the underlying membrane and actively pull so as to hold the whole epithelium under tension. The biological significance of the epithelial sheet being under tension is not clear but one suggestion is that maintaining tension in the cell-cell contact network helps facilitate the migration of cells by sequences of T1 transitions.

1.3 Vertex Model

The initial focus of this thesis is on our computational model which is adapted from the common vertex model for epithelial sheets \cite{4}. It is the network structure of the apical cell-cell junctions, shown in Figure \ref{fig:11}, that forms the basis of the vertex model. Although it may not be a general property of epithelia, all the examples of real epithelial tissue we discuss are under a net tension. The cell membranes have naturally high tensile strength and so it is reasonable to expect that a significant part of the tensile load is borne by cell-cell junctions which will attempt to straighten under tension. Furthermore, a key function of epithelial tissue is to maintain an impermeable barrier with no holes between cells. With this in mind we make the
approximation that all junctions in the apical network are perfectly straight and that all cells are ideal columns with flat surfaces. Several studies have been made which relax one of these assumptions [37, 38, 39, 40] but we do not discuss them here. For straight edges and ideal columns, the positions of the network vertices and their connectivity is the only information needed to represent the monolayer state, hence the name ‘vertex’ model. While cell vertex positions can drift smoothly as cells move and grow, the network topology can only change in discrete steps by one of the events described in the previous Section 1.1. That is, by ingression, division or T1 transition (neighbour exchange). Of course, such networks or meshes are simple mathematical objects and hence straightforward to operate on using a computer.

To complete the vertex model we include dynamics – rules by which the cell shapes and positions change over time. We know that cells have a preferred shape and that stretching or compressing cells costs energy. The idea is that, in the absence of external forces, the monolayer should relax to a state where the deviations of the cells from their preferred characteristics are minimised. We define a quantity which reflects these cell characteristics [4],

$$E_v = \sum_{i=1}^{N} \left[ \frac{K_i}{2} \left( A_i - A_i^0 \right)^2 + \frac{\Gamma_i}{2} P_i^2 \right] + \sum_{\mu,\nu} \Lambda_{\mu \nu} l_{\mu \nu},$$  \hspace{1cm} (1.1)

and call it the vertex model energy. The term ‘energy’ is used loosely, the energy in a real cell colony is gathered and expended continuously by each cell so as to maintain its shape and function. $A_i, A_i^0$ are the actual area and preferred area respectively of cell apical face $i$. $K_i$ is a free parameter which sets the resistance to deviations in cell area. Similarly $\Gamma_i$ measures the resistance to lengthening the
perimeter of the cell \( P_i \). Throughout this thesis, Latin indices are used to denote apical faces and Greek indices are used to denote vertices. Thus \( l_{\mu\nu} \) is the length of the edge connecting vertices \( \mu \) and \( \nu \) and \( \Lambda_{\mu\nu} \) is the line tension, see Figure 1.5. The first sum is over all \( N \) cells, the last sum is over all cell edges. This energy is based on empirical observations, it is by no means the only choice and can be tuned to the specific system of interest. In this case we reason that the cell perimeter stretches like an elastic spring whose energy changes with length squared. On the other hand the energy cost of a cell-cell junction is associated with the formation of adhesive sites and changes linearly based on the length of the two membranes in contact.

The line tension parameter, \( \Lambda_{\mu\nu} \) is expected to take a negative value and as such it balances the contracting effect on cells of the \( P_i^2 \) term. At equilibrium, the monolayer cells will attain a compromise between achieving their desired area \( A_0 \) and balancing their perimeter and line tension costs. If the parameter \( \Lambda_{\mu\nu} \) is constant for all edges then we may write the last two terms together as \( \sum_{i=1}^{N} \frac{\Gamma_i}{2} (P_i - P_i^0)^2 \) where \( P_i^0 \) is a target perimeter analogous to the target area \( A_i^0 \) (this is demonstrated in Section 3.1). Allowing the \( \Lambda \) parameter to vary between edges enables the study of a wider range of behaviour such as the tendency for cells to expand along a single shared axis \[41\] and the organisation of cells into compartments \[42\]. We consider both the area and perimeter terms to include, in part, active forces generated in the cell membrane which regulate cell shape. The idea that cell dynamics can be modelled by assigning an energy value to the network originally comes from the physics of foams and other non-living materials \[43\].
1.4 Equation of Motion

Taking the negative of the gradient of $E_v$ with respect to each vertex $\mu$ will give a set of forces, $F_\mu = -\nabla r_\mu E_v$, that drive the system towards a local minimum energy state. To determine the dynamics we start by writing down an *overdamped* equation of motion for the vertex $\mu$,

$$\gamma \frac{dr_\mu}{dt} = F_\mu,$$

(1.2)

where $F_\mu$ depends on the positions of each vertex connected with $\mu$. Hence the dynamics is described by a system of $N_v$ coupled differential equations with $N_v$ being the number of vertices in the apical network. At this point standard numerical integration tools take over to iteratively step the system through time and obtain the new system states $r_\mu(t)$. To better understand the origin of equation (1.2) consider the Langevin Equation for the erratic motion of a particle in a fluid, also known as Brownian Motion,

$$m \frac{d^2 r_i}{dt^2} = -\gamma \frac{dr_i}{dt} + \nu_i(t),$$

(1.3)
where $m$ is mass, $\gamma$ is coefficient of friction and $\nu$ is the random force or ‘noise’ term. This random force is sampled at each time step from a gaussian distribution with no temporal or spatial correlation and zero mean. In other words the random forces on each particle at each instant of time have no correlation with each other. Formally, the mean $\langle \nu_i \rangle = 0$ and variance $\langle \nu^a_i(t)\nu^b_j(t') \rangle = 2D\delta_{ij}\delta_{\alpha\beta}\delta(t-t')$, where $D$ is the parameter which sets the average magnitude of the random force and the indices $(\alpha, \beta)$ are used to indicate the components of the random vectors $\nu_i$ and $\nu_j$. The angle brackets $\langle \cdots \rangle$ denote the statistical average. A common approximation for dynamics on the intracellular length scale and to a somewhat weaker extent the cellular length scale is that the friction term, $\gamma \dot{r}$ is much larger than the inertial term, $m\ddot{r}$ [44]. By taking the limit $|m\ddot{r}| \ll |\gamma \dot{r}|$, introducing our vertex model force $F_p$ and neglecting $\nu$ for the moment we obtain equation 1.2.

By reducing each cell to a simple polygon and a few parameters, vertex models neglect the intricate complexity of real cells so that the collective behaviour of thousands, perhaps tens of thousands, of cells can be studied. In contrast the chick embryo surface layer, 24 hours after the egg is laid, is made up of more than 200,000 epithelial cells. A key feature of vertex models then is their compromise of sophistication and efficiency. Models which are extended to three dimensions [45] or include other modifications [43, 46] provide valuable new insights but also lead to reductions in the feasible system sizes and as a result, the emergent features of interacting cells on the length- and time-scales of real tissues are obscured. Since so many variants of the vertex model have been developed, effort has been made in support of a fast and extensible open-source library, CHASTE [47], to ease development of new models. However, since our own variation of the vertex model shares similarities with
particle-based models we find it more appropriate to extend a modular and efficient library for particle-based simulations, SAMoS [48].

We wrap up the section with some examples of vertex models being used in support of experimental work. Landsberg et. al. [49] study a compartment boundary between regions of an epithelial sheet made up of two distinct cell types. They show that particularly high tension along the boundary junctions is important for maintaining the compartment boundary during growth. Also on the mechanical features of growing epithelia, Mao et. al. [50] study a monolayer with growth concentrated at the centre. The central, fast dividing, cells push radially on the boundaries which stretches cells near the periphery in the circumferential direction. As a result central cells and peripheral cells tend to divide along perpendicular axes. Finally we mention the topology of the apical network itself is of interest because, (i) the topology is known to change during development, for example from a disordered state towards an ideal hexagonal packing [51] and (ii) the network topology is relatively straightforward to measure, the only requirement is high quality cell-resolution images. The most common measure for the network topology is the distribution of the number of neighbours of each cell. For example in a perfect hexagonal lattice all cells have a neighbour number of 6. Li et. al. compared neighbour number distributions between real tissue and vertex model simulations [52].

1.5 Active Matter

Organisms consume energy continuously in order to maintain their function. At the molecular scale this involves breaking down food and storing the energy chemically
in small molecules (e.g. ATP). The vast majority of cell processes require energy to function and so they interact with these energy carrying molecules. For example, manipulating adhesive sites, contraction of cell-cell junctions and cell motility in general are all energy-consuming processes \[53\]. We describe processes that use energy as *active* and refer to forces generated at the molecular scale by consuming chemical energy as *active forces*. In general our energy, \( E_v \), does implicitly include active processes which maintain cell shape but it cannot capture cell motility or any of the other rich, out-of-equilibrium behaviour of real biology.

Our contribution is to add active forces to the vertex model which drive the movement of cells. The aim is to model the collective cell migrations that are prevalent in tissue morphogenesis \[17\]. Crawling cells move by a combination of pushing against a substrate to extend protrusions from their bodies and pulling on the substrate with these protrusions. Chick embryo epithelial cells appear to move primarily by contracting or extending their shared cell-cell junctions. We neglect the specific details of these motility mechanisms and apply forces to the cells a generic way, which takes inspiration from the field of physics concerned with the collective behaviours of individually propelled elements – Active Matter. It is possible to gain some insight into what features of active epithelial cells are, and are not, sensitive to their specific motility mechanism using this approach. One basic Active Matter model is a system of spherical particles interacting via steric repulsion. Each particle has an independent polarity vector \( \mathbf{n} \) along which they apply their constant motility force \( f_a \). For this to be physically realistic for cells, they must be able to grip and gain traction from their environment.

The polarity vector then updates with some heuristic rule. There is significant
difficulty in choosing an appropriate rule which is compatible with the behaviour of biological cells while also reproducing features of real biological systems. Nonetheless we may choose to study more generic properties of systems of active cells such as their rheology, see Section 3.1. In this case we can start with a very simple rule such as a random rotational diffusion where the $i$-th particle polarity $\mathbf{n}_i = (\cos \theta_i, \sin \theta_i)$ updates according to

$$\gamma_r \frac{d\theta_i}{dt} = \nu_i^r(t),$$

where $\gamma_r$ is a rotational friction and $\nu_i^r(t)$ is a random term and is equivalent to $\mathbf{\nu}_i$, the spatial noise.

In the next chapter we construct model cells with an activity force of this type by formulating a mapping between particle-based and vertex model representations of cells. This active matter model for cell-generated active forces is not intended to be biologically realistic for all (or even most) types of epithelial monolayers and tissues. Nonetheless it includes the minimal ingredients to describe motile cells and thus should reproduce some features of real cell dynamics, particularly over long length and time scales.
Chapter 2

Active Vertex Model

It has been noted that epithelial tissues resemble a Voronoi diagram. Voronoi diagrams are constructed from a finite set of scattered ‘seed’ points, \( \{ r_p \} \), by assigning to each one a local region of space made up of all the points which are closer to that seed than any other. The result in two dimensions is a partitioning of the plane into simple convex polygons, which are suggestively referred to as Voronoi cells. Since each seed represents one cell we refer to seeds and cells interchangeably. By definition then, Voronoi cells fill a two dimensional plane with no gaps while epithelial cells do the same thing because it aids their function as an impermeable barrier. What’s more, there is a unique Voronoi diagram for each set of seed points \( \{ r_p \} \). In this chapter we show that, starting with a particle-based model of cells, we can still associate an approximate shape to each cell under the assumption that the monolayer geometry is close to a Voronoi diagram. The vertex model dynamics described in section 1.3 can then be incorporated in the model alongside the active motility force described in section 1.5 and we name the result an Active Vertex Model (AVM). In this chapter we explicitly derive the vertex model force as it applies to
Voronoi seed points, $\mathbf{F}_p = -\nabla_{\mathbf{r}_p} E_v$, using the fact that $\frac{\partial}{\partial \mathbf{r}_p} E_v = \frac{\partial E_v}{\partial \mathbf{r}_\mu} \frac{\partial \mathbf{r}_\mu}{\partial \mathbf{r}_p}$ where $\mathbf{r}_\mu$ is the position of the vertices of the Voronoi cells and $\mathbf{r}_p$ is the position of the cell seed points. Note that the seed points at the edge of the patch of cells have infinite Voronoi regions, we deal with this problem in section 2.3.

2.1 Delaunay - Voronoi Mapping

To progress, we need to calculate the Voronoi diagram from its seed points on every timestep so that the vertex model force can be calculated. This is a computationally intensive procedure but there is a shortcut to accelerate the computation that is reliant on another mathematical construct for a set of scattered points – the Delaunay Triangulation. A triangulation on a set of points connects all the points with edges such that the edges form triangles which tessellate the surface. While there are many possible triangulations for the same set of points, the Delaunay triangulation has a special property that it eliminates as many highly acute angles as possible. The result is a triangulation which looks more even and homogeneous. We reserve the word *mesh* to describe the network of Voronoi cells where cells can have any number of sides as opposed to a triangulation where each element is a triangle.

For a more rigorous understanding we introduce the *circumcircle* of a triangle as the circle that passes through each triangle vertex. A *circumcentre* then, is the centre of a circumcircle. Formally the Delaunay triangulation is the one in which the circumcircle of every triangle passes through exactly three triangulated points and contains no others. A more natural but less constructive definition is that the Delaunay triangulation is the one which maximises the smallest angle of any
triangle. We wish to make use of the fact that from the Delaunay triangulation to
the Voronoi diagram there is a nearly exact one-to-one mapping. In the rare case
that more than three points lie perfectly on a circumcircle there is some degeneracy
in the Delaunay triangulation but it does not matter which triangulation we choose,
since they all correspond to the same Voronoi diagram. Obtaining the Voronoi
diagram from the corresponding Delaunay triangulation is straightforward, simply
find the circumcentre of every triangle and connect them according to which triangles
are adjacent. In this way three-sided triangular faces map onto polygon vertices
and triangulation vertices of degree $n$ onto $n$-sided polygonal faces, Figure 2.1.
In our Vononoi mesh all vertices connect exactly three edges but edges are free
to shrink until they are vanishingly small at which point multiple vertices sit on
top of eachother, thus higher order vertices can develop naturally in the AVM. As
mentioned in Section 1.1, higher order vertices where more than three cells meet at
a point, called ‘rosettes’, are observed in real Epithelia. They are observed following
cell ingression and also as part of collective cell migrations [56].
We want to quickly calculate the Voronoi diagram from the Delaunay triangulation on each timestep. Calculating the Delaunay triangulation from scratch is also costly but the advantage is that it only changes when there is a change in topology of the cell network. Usually there are at most a few minor network changes needed on each step and usually none at all are needed. We can apply these changes directly to the existing triangulation by the equiangulation algorithm [57], which iteratively ‘flips’ edges. Each edge is associated with a pair of triangles, if the sum of the pair of angles opposite to an edge is greater than 180° then the edge is flipped so that it now connects an alternate pair of vertices, see Figure 2.2. Flips always occur between a pair of approximately right-angled triangles whose circumcircles coincide and, as a result, the flip is experienced by the Voronoi mesh as a smooth T1 transition. This procedure complements the idea that the Delaunay triangulation avoids highly obtuse triangles. Crucially, it can be shown that the equiangulation procedure always converges [57]. As the cell seeds travel relative to each other, T1 transitions in the network occur naturally unlike in standard vertex models which have specific rules to determine how edges reconnect when those edges become sufficiently small.

In general whenever Voronoi seeds approach very close together, the Voronoi diagram we construct is highly sensitive to small displacements, see Figure 2.3. To
ensure stability of the system we use a soft repulsive interaction between seeds. The radius of this interaction should be made smaller than the typical cell radius, the idea being that this repulsive interaction should only play a role for a few extreme cases. The soft repulsion does a good job of gently preventing very sharp triangles without setting any hard limits. Figure 2.3 shows a certain kind of undesirable behaviour for highly obtuse triangles which is mitigated by the soft repulsive potential. The repulsive potential defined for neighbouring cell seeds $i$ and $j$ is

$$V_{soft}(r_{ij}) = \begin{cases} \frac{1}{2}k(r_{ij} - 2a)^2 & \text{for } r_{ij} < 2a \\ 0 & \text{otherwise} \end{cases}$$ (2.1)

where $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$. The range of the repulsive force is defined by the length $a$ which we take to be 0.5 unless otherwise stated. The corresponding force on cell $i$ due to cell $j$ is

$$\mathbf{F}_{i}^{soft} = -\nabla_{\mathbf{r}_i}V_{soft}(r_{ij}) = \begin{cases} -k(r_{ij} - 2a)\hat{r}_{ij} & \text{for } r_{ij} < 2a \\ 0 & \text{otherwise} \end{cases}$$ (2.2)

with $\hat{r}_{ij} \equiv |\mathbf{r}_i - \mathbf{r}_j|/r_{ij}$.

Combining the vertex model force $\mathbf{F}_p$ and active forces $f_a \mathbf{n}_p$, the complete equations of motion for the Active Vertex Model are

$$\gamma_r \frac{d\mathbf{r}_p}{dt} = \mathbf{F}_p + f_a \mathbf{n}_p + \mathbf{\nu}_p,$$

$$\gamma_r \frac{d\theta_p}{dt} = \nu^r_p(t).$$ (2.3)
Figure 2.3: For frames (a) and (b). A single Voronoi cell (green) with a single vertex, $\mu$, highlighted (blue dot). The specific triangle that is associated with the highlighted cell vertex is drawn in grey and its circumcircle is the large blue circle. An active force $f_a$ (red arrow) is applied to the cell seed point and drives it towards cells $j$ and $k$. Since this causes an unnaturally pointed cell shape the vertex model force $F_p$ will counteract the active force and prevent further movement in this direction. However it is important to consider that, because the grey triangle in frame (b) is highly obtuse, a small displacement in the position of cell seed $i$ causes a large displacement in the position of vertex $\mu$. This behaviour is undesirable for simulations. A soft repulsive interaction between cell seeds helps to maintain stability, shown by black circles with broken lines in frame (c).

$f_a$ is a variable parameter that represents the magnitude of the active motility force applied by each cell. It is constant for each cell and throughout the simulation but the direction $n_p = (\cos \theta_p, \sin \theta_p)$ changes on each time step according to the second equation. We usually continue to neglect the positional noise term $\nu_p$ and focus on the rotational noise, $\gamma_r$, as the source of stochasticity in the system.

The ratio of edges, faces and vertices for an infinite hexagonal network is 3 edges : 1 face : 2 vertices. Although our mesh is finite and not hexagonal we can still expect roughly half as many cell faces as cell vertices. By exchanging full vertex model cells for Voronoi cells we only have half the number of degrees of freedom remaining. This can be good for computational efficiency but clearly we are imposing subtle restrictions on the shape that each cell can attain, the exact effect of these restrictions on the dynamics is not clear. It is apparent though, that individual Voronoi cells
cannot become arbitrarily highly elongated and no cells can have concave shapes.
The active vertex model is not an effective model for sheets of cells that do not closely resemble Voronoi cells. This still leaves many Voronoi-like tissues to choose from [55].

2.2 Force Calculation

To complete the equation of motion we derive an expression for $F_p$ by taking the derivative $-\nabla r_p$ of each term in the energy equation in turn, stopping when we arrive at a form which is amenable to efficient computation. Firstly though we need to break down the geometric quantities, $A_p, P_p$ and $l_{\mu\nu}$ in terms of vertex positions, $r_{\nu}$. We name each term in the vertex model as follows, $E_v = E_{\text{area}} + E_{\text{perim}} + E_{\text{junct}}$. The area of a polygonal cell can be calculated from its vertices,

$$A_p = \frac{1}{2} \sum_{\nu} (r_{\nu} \times r_{\nu+1}) \cdot N,$$

(2.4)

where $r_{\nu}$ is the position of vertex $\nu$ and $N$ is the unit normal of the cell face. We deal only with flat geometry so $N$ is identical for each cell and coincides with the unit vector along the z-axis, $e_z$. Using symbol $L_i$ for the set of vertices of cell $i$ we may also write

$$P_i = \sum_{\nu \in L_i} |r_{\nu} - r_{\nu-1}|, \quad l_{\mu\nu} = |r_{\mu} - r_{\nu}|.$$

(2.5)

To construct the mapping from Delaunay to Voronoi diagrams we use barycentric coordinates, a natural coordinate system for triangular domains [58]. The position
of a Voronoi seed in the coordinate frame of its associated triangle can be written as

\[ \mathbf{r}_\mu = \lambda'_i \mathbf{r}_i + \lambda'_j \mathbf{r}_j + \lambda'_k \mathbf{r}_k = \lambda'_p \mathbf{r}_p \] (2.6)

where \( \mathbf{r}_i, \mathbf{r}_j, \mathbf{r}_k \) are vertices of the triangle and \( \lambda'_p \) are normalized barycentric coordinates. Now in terms of the triangle side lengths,

\[ \lambda_i = l_i^2 (l_j^2 + l_k^2 - l_i^2) , \]
\[ \lambda_j = l_j^2 (l_k^2 + l_i^2 - l_j^2) , \] (2.7)
\[ \lambda_k = l_k^2 (l_i^2 + l_j^2 - l_k^2) , \]

are the non-normalized barycentric coordinates where \( l_i = |\mathbf{r}_k - \mathbf{r}_j| \), the length of the side opposite to the vertex \( i \) and \( \mathbf{l}_i \) would be the corresponding vector, \( \mathbf{l}_i = \mathbf{r}_k - \mathbf{r}_j \).

We normalize barycentric coordinates by setting \( \lambda'_i + \lambda'_j + \lambda'_k = 1 \) so

\[ \lambda'_p = \frac{\lambda_p}{\lambda_i + \lambda_j + \lambda_k} , \] (2.8)

for \( p \in \{i, j, k\} \).
2.2.1 Area Term

We now take the derivatives of each term with respect to the seed positions $\mathbf{r}_p$, for each term on the right hand side of equation 1.1, starting with the area term,

$$F^\text{area}_p = -\nabla_{\mathbf{r}_p} E_{\text{area}} = -\nabla_{\mathbf{r}_p} \frac{K}{2} \sum_p (A_p - A^0_p)^2 = -\frac{K}{2} \sum_j (A_j - A^0_j) \nabla_{\mathbf{r}_p} (A_j - A^0_j)$$

$$= -\frac{K}{2} \sum_j (A_j - A^0_j) \nabla_{\mathbf{r}_p} A_j.$$  

(2.9)

The area $A_p(\mathcal{L}_p)$ is a function of the set of vertices of cell $\mathcal{L}_p = \{\mu_1, \mu_2, ..., \mu_n\}$. Therefore

$$\nabla_{\mathbf{r}_p} A_j = \sum_{\mu \in \mathcal{L}_p} \frac{\partial A_j}{\partial \mathbf{r}_\mu} \cdot \frac{\partial \mathbf{r}_\mu}{\partial \mathbf{r}_p}$$  

(2.10)

where the product denoted by the $(\cdot)$ is a row vector multiplying a matrix to give a column vector.

We start by calculating $\frac{\partial \mathbf{r}_\mu}{\partial \mathbf{r}_p}$ which is the Jacobian matrix that maps forces on cell vertices to forces on cell seeds. We calculate it once and reuse it several times in the final expression for $\mathbf{F}_p$. To start with, vertex $\mathbf{r}_\mu$ is expressed as a sum of three barycentric coordinates $\lambda'_{iq} \mathbf{r}_q = \lambda'_{ir} \mathbf{r}_i + \lambda'_{jr} \mathbf{r}_j + \lambda'_{kr} \mathbf{r}_k$ (Equation 2.6). The specific triangle $\triangle ijk$ depends on $\mu$ but we continue for an arbitrary vertex $\mu$ and seed $p$ without loss of generality. We choose indices $n$ and $m$ to label the components of
vectors \( \mathbf{r}_\mu \) and \( \mathbf{r}_p \) respectively. Finally define \( \Lambda = \lambda_i + \lambda_j + \lambda_k \) then,

\[
\left[ \frac{\partial \mathbf{r}_p}{\partial \mathbf{r}_p} \right]_{nm} = \left[ \frac{\partial}{\partial \mathbf{r}_p} \right]_m \left[ \frac{\lambda_q \mathbf{r}_q}{\Lambda} \right]_n
\]

\[
= \frac{1}{\Lambda^2} \left[ \Lambda \left[ \frac{\partial}{\partial \mathbf{r}_p} \right]_m (\lambda_q \mathbf{r}_q)_n - (\lambda_q \mathbf{r}_q)_n \left[ \frac{\partial \Lambda}{\partial \mathbf{r}_p} \right]_m \right]
\]

\[
= \frac{1}{\Lambda^2} \left[ \Lambda \left[ \frac{\partial \lambda_q}{\partial \mathbf{r}_p} \right]_m (\mathbf{r}_q)_n + \Lambda \lambda_q \left[ \frac{\partial \mathbf{r}_q}{\partial \mathbf{r}_p} \right]_{nm} - (\lambda_q \mathbf{r}_q)_n \left[ \frac{\partial \Lambda}{\partial \mathbf{r}_p} \right]_m \right]
\]

\[
= \frac{1}{\Lambda^2} \left[ \Lambda \mathbf{r}_q \otimes \frac{\partial \lambda_q}{\partial \mathbf{r}_p} + \Lambda \lambda_q \delta_{nm} - (\lambda_q \mathbf{r}_q) \otimes \frac{\partial \Lambda}{\partial \mathbf{r}_p} \right],
\]

where \((\otimes)\) is the outer product of two vectors. Define \( L = l_i^2 + l_j^2 + l_k^2 \) and writing \((\lambda_i, \lambda_j, \lambda_k)\) in terms of a single subscript \( q \in \{i, j, k\} \) we obtain,

\[
\lambda_q = l_q^2 (L - 2l_q^2).
\]

The derivative with respect to \( \mathbf{r}_p \) is

\[
\frac{\partial \lambda_q}{\partial \mathbf{r}_p} = \frac{\partial l_q^2}{\partial \mathbf{r}_p} (L - 2l_q^2) + l_q^2 \left( \frac{\partial L}{\partial \mathbf{r}_p} - 2 \frac{\partial l_q^2}{\partial \mathbf{r}_p} \right)
\]

\[
= l_q^2 \frac{\partial L}{\partial \mathbf{r}_p} + (L - 4l_q^2) \frac{\partial l_q^2}{\partial \mathbf{r}_p}.
\]

We make a minor simplification by expanding \( \frac{\partial \Lambda}{\partial \mathbf{r}_p} \),

\[
\frac{\partial \Lambda}{\partial \mathbf{r}_p} = \sum_q \frac{\partial \lambda_q}{\partial \mathbf{r}_p} = L \sum_q \frac{\partial l_q^2}{\partial \mathbf{r}_p} + \frac{\partial l_q^2}{\partial \mathbf{r}_p} \sum_q l_q^2 - 4 \sum_q l_q^2 \frac{\partial l_q^2}{\partial \mathbf{r}_p}
\]

\[
= 2L \frac{\partial L}{\partial \mathbf{r}_p} + 4 \sum_q l_q^2 \frac{\partial l_q^2}{\partial \mathbf{r}_p}.
\]
Now all we need is $\frac{\partial l^2_q}{\partial r_p}$. Taking the derivatives,

$$\frac{\partial l^2_i}{\partial r_p} = \frac{\partial}{\partial r_p} |r_k - r_j|^2 = \frac{\partial}{\partial r_p} (r_k - r_j) \cdot (r_k - r_j)$$

$$= \frac{\partial}{\partial r_p} (r_k \cdot r_k - 2r_k \cdot r_j + r_j \cdot r_j)$$

$$= 2 \begin{bmatrix} 0 \\ r_j - r_k \\ r_k - r_j \end{bmatrix}_p = 2 \begin{bmatrix} 0 \\ -l_i \\ l_i \end{bmatrix}_p$$

where we wrote down a column vector by explicitly putting $i,j$ and $k$ back into $p$.

Repeating for $l^2_j$ and $l^2_k$ gives,

$$\frac{\partial l^2_j}{\partial r_p} = 2 \begin{pmatrix} 0 & 1_j & -1_k \\ -1_i & 0 & 1_k \\ 1_i & -1_j & 0 \end{pmatrix}_{pq},$$

where the elements of this matrix are 2-vectors for our planar system. Summing over $q$ gives,

$$\frac{\partial L}{\partial r_p} = 2 \begin{bmatrix} 1_j - 1_k \\ 1_k - 1_i \\ 1_i - 1_j \end{bmatrix}_p$$

We calculated all the derivatives we need for $\frac{\partial r_p}{\partial r_p}$, that is $\frac{\partial l^2}{\partial r_p}$ and $\frac{\partial L}{\partial r_p}$ which are both written in terms of $\frac{\partial l^2}{\partial r_p}$ and $\frac{\partial L}{\partial r_p}$. Having calculated it once, we will reuse $\frac{\partial r_p}{\partial r_p}$ for the
perimeter and contact length terms. It remains to calculate \( \frac{\partial A_j}{\partial r_\mu} \) for the area term,

\[
\frac{\partial A_j}{\partial r_\mu} = \frac{1}{2} \sum_\nu \frac{\partial}{\partial r_\mu} [(r_\nu \times r_{\nu+1}) \cdot N_j] \\
= \frac{1}{2} \frac{\partial}{\partial r_\mu} (r_\nu \times r_{\nu+1}) \cdot N_j - \frac{1}{2} \frac{\partial}{\partial r_\mu} (r_\nu \times r_{\nu-1}) \cdot N_j .
\]

(2.14)

In order to evaluate the cross product we switch to Levi-Civita symbols and use Einstein summation convention on Latin indices.

\[
\left[ \frac{\partial}{\partial r_\mu} (r_\mu \times r_{\mu+1}) \cdot N \right]_i = \partial_i r_\nu \epsilon_{lmn} N^l r_m^{\mu+1} = \epsilon_{lmn} N^l r_m^{\mu+1} \frac{\partial}{\partial r_\mu} N^m \\
= \epsilon_{lmn} N^l r_m^{\mu+1} \delta_{im} = \epsilon_{lmn} N^l r_m^{\mu+1} = [r_{\mu+1} \times N]_i .
\]

(2.15)

Hence

\[
\frac{\partial A_j}{\partial r_\mu} = \frac{1}{2} (r_{\mu+1} \times N - r_{\mu-1} \times N) \\
= \frac{1}{2} \sum_\mu \frac{\partial P_j}{\partial r_\mu} \cdot \frac{\partial r_\mu}{\partial r_j} .
\]

(2.16)

2.2.2 Perimeter and Contact Length terms

Taking the derivative of the cell perimeter term in the vertex model energy,

\[
\nabla_{r_j} P_j^2 = 2P_j \nabla_{r_j} P_j \\
\]

(2.17)

where

\[
\nabla_{r_j} P_j = \sum_\mu \frac{\partial P_j}{\partial r_\mu} \cdot \frac{\partial r_\mu}{\partial r_j} .
\]

(2.18)
We already calculated $\frac{\partial r_\mu}{\partial r_j}$ for the area derivative. That leaves

\[
\frac{\partial P_j}{\partial r_\mu} = \sum_\nu \frac{\partial}{\partial r_\mu} |r_\nu - r_{\nu-1}|
\]

\[
= \left. \frac{\partial}{\partial r_\mu} |r_\nu - r_{\nu-1}| \right|_{\nu=\mu} + \left. \frac{\partial}{\partial r_\mu} |r_\nu - r_{\nu-1}| \right|_{\nu=\mu+1}
\]

\[
= \frac{r_\mu - r_{\mu-1}}{|r_\mu - r_{\mu-1}|} - \frac{r_{\mu+1} - r_\mu}{|r_{\mu+1} - r_\mu|}
\]

(2.19)

For each vertex $\mu$ of cell $i$ we get a pair of non-zero terms corresponding to the pair of edges of cell $i$ connected at this vertex. A useful notation is $r_{\mu\nu} = r_\mu - r_\nu$ then

\[\hat{r}_{\mu\nu} = \frac{r_\mu - r_\nu}{|r_\mu - r_\nu|} \text{ and } \frac{\partial P_j}{\partial r_\mu} = \hat{r}_{\mu-1} - \hat{r}_{\mu+1}.\]

(2.20)

For the final term, the force due to cell junctions, $F^\text{junct}_p$ is

\[-\nabla r_p \sum_{\nu,\xi} \Lambda_{\nu\xi} l_{\nu\xi} = -\sum_{\nu,\xi} \Lambda_{\nu\xi} \nabla r_p l_{\nu\xi} = -\sum_{\nu,\xi} \Lambda_{\nu\xi} \sum_\mu \frac{\partial l_{\nu\xi}}{\partial r_\mu} \cdot \frac{\partial r_\mu}{\partial r_p}\]

(2.21)

where

\[\frac{\partial l_{\nu\xi}}{\partial r_\mu} = \frac{\partial}{\partial r_\mu} |r_\nu - r_\xi| = \frac{r_\nu - r_\xi}{|r_\nu - r_\xi|} (\delta_{\mu\nu} - \delta_{\mu\xi}) = \hat{r}_{\nu\xi} (\delta_{\mu\nu} - \delta_{\mu\xi}).\]

(2.22)

The edges, represented by a pair of adjacent vertices $(\nu, \xi)$, which give contributions to the sum are the edges of the cell, $\{(\nu, \nu + 1)\}$, plus the edges which connect to vertices of the cell, call them $\{\nu, \nu_\xi\}$, for a total of $2n$ contributions where $n$ is the
number of cell edges. In general then,

\[
\sum_{\nu,\xi} \Lambda_{\nu\xi} \sum_{\mu} \hat{r}_{\nu\xi} (\delta_{\mu\nu} - \delta_{\mu\xi}) \cdot \frac{\partial r_{\mu}}{\partial r_p} = \sum_{\nu,\xi} \Lambda_{\nu\xi} \hat{r}_{\nu\xi} \cdot \left( \frac{\partial r_{\nu}}{\partial r_p} - \frac{\partial r_{\xi}}{\partial r_p} \right).
\]

(2.23)

If the cell vertices are denoted by \( \nu \) then it is sufficient to sum over \( (\nu, \nu - 1) \) and \( (\nu, \xi_{\nu}) \) where \( \xi_{\nu} \) is the vertex connecting to vertex \( \nu \) which is not part of the cell \( p \). In other words we sum over the edges of cell \( p \) as well as the edges directly connected to cell \( p \).

\[
\mathbf{F}_{\text{junct}}^p = -\sum_{\nu \in L_p} \Lambda_{\nu\nu-1} \hat{r}_{\nu\nu-1} \cdot \left( \frac{\partial r_{\nu}}{\partial r_p} - \frac{\partial r_{\nu-1}}{\partial r_p} \right)
- \sum_{\nu \in L_p} \Lambda_{\nu\xi_{\nu}} \frac{\partial r_{\nu}}{\partial r_p}
- \frac{1}{2} \sum_{\nu \in L_p} [\Lambda_{\nu\nu-1} \hat{r}_{\nu\nu-1} - \Lambda_{\nu+1\nu} \hat{r}_{\nu+1\nu}] \frac{\partial r_{\nu}}{\partial r_p}
- \frac{1}{2} \sum_{j \in \text{n.n.p}} \sum_{\nu \in L_p \cap L_j} [\Lambda_{\nu\nu-1} \hat{r}_{\nu\nu-1} - \Lambda_{\nu+1\nu} \hat{r}_{\nu+1\nu}] \frac{\partial r_{\nu}}{\partial r_p}.
\]

(2.24)

2.2.3 Total Force

The total force on cell \( p \) due to change in cell areas has contributions from cell \( p \) as well as the adjacent cells which we will denote as n.n.p for nearest neighbours of \( p \). For the contact length terms we already took into account all the neighbouring contributions in Equation 2.24 so we leave that part as is.

\[
\mathbf{F}_{\text{area}}^p = \sum_{j \in \{\text{cells}\}} K_j \left( A_j - A_j^{(0)} \right) \sum_{\mu \in L_j} \frac{\partial A_j}{\partial \mu} \cdot \frac{\partial r_{\mu}}{\partial r_p}
\]

(2.25)
Where the sum over $j$ is over all cells but only cell $p$ and its nearest neighbours contribute because $\frac{\partial r_p}{\partial r_p}$ only has non-zero components for these cells. We find it convenient to break the sum into a direct component and a set of components from adjacent cells as follows

$$F_{\text{area}} = -\frac{K_p}{2} \left( A_p - A_p^{(0)} \right) \sum_{\mu \in L_p} \frac{\partial r_\mu}{\partial r_p} \cdot \left[ r_{\mu+1} \times N_p - r_{\mu-1} \times N_p \right]$$

$$- \frac{1}{2} \sum_{j \in \text{n.n.p}} K_j \left( A_j - A_j^{(0)} \right) \sum_{\mu \in L_j \cap L_p} \frac{\partial r_\mu}{\partial r_p} \cdot \left[ r_{\mu+1} \times N_p - r_{\mu-1} \times N_p \right].$$  \hspace{1cm} (2.26)

Similarly for $F_{\text{perim}}$

$$F_{\text{perim}} = -\Gamma_p P_p \sum_{\mu \in L_p} \frac{\partial r_\mu}{\partial r_p} \cdot [\hat{r}_{\mu-1} - \hat{r}_{\mu+1}]$$

$$- \sum_{j \in \text{n.n.p}} \Gamma_j P_j \sum_{\mu \in L_j \cap L_p} [\hat{r}_{\mu-1} - \hat{r}_{\mu+1}]_n \left[ \frac{\partial r_\mu}{\partial r_p} \right]_{nm}. \hspace{1cm} (2.27)$$

Finally we note that $F_p = F_{\text{area}} + F_{\text{perim}} + F_{\text{junct}}$ which concludes our calculation.

We go on to discuss a strategy to handle the boundary of the monolayer.

### 2.3 Boundaries

A basic Voronoi diagram with a finite number of seed points includes Voronoi regions around the edges which extend out to infinity. We lack complete information to define the shape of these boundary cells. We can solve the problem by surrounding the cells with an unbroken loop of boundary markers which are passively pushed and
pulled. These boundary markers do not generate active forces, they cannot divide, die or ingress. The boundary markers are allowed to be created and destroyed as necessary to maintain an unbroken line at the monolayer edge. We distinguish cells from boundary markers and define the connectivity of the markers in the initial configuration. The boundary can then still grow, shrink or bend according to a rule which dynamically adds or removes markers, see Figure 2.4. This section describes the boundary handling rules in detail.

As a portion of the monolayer boundary expands, boundary edges in the triangulation are extended. The angle opposite the boundary edge usually increases (red angle in Figure 2.4a). To make boundaries flexible, firstly we require that the angle opposite to every triangulation boundary edge is less than 90°. In terms of Voronoi cell shapes this constraint is roughly equivalent to preventing very sharp, spiky cell shapes at the boundary. We then prevent any such angle from exceeding 90° by tactically introducing new boundary markers. When the angle increases to approximately 90° we mirror the cell seed in the boundary edge to obtain the new marker position. The single boundary edge is then replaced by two new boundary edges connected to the new marker (2.4). The old boundary edge is now an internal edge and can be flipped by the equiangulation algorithm as usual. In fact we can think of this procedure for adding boundary markers simply as a rule for flipping boundary edges, the only difference being that for these edges we have add a new marker to make the flip possible. The process happens in reverse when an edge near the boundary is naturally flipped by the equiangulation algorithm so that a boundary marker becomes obsolete at which point it is simply deleted. Boundary markers are obsolete when they travel far enough away from any cell seed points
Figure 2.4: The rule for smoothly adding and removing boundary particles. As the cell (marked in green) approaches the boundary the angle marked in red exceeds 90°. At this moment we flip the associated boundary edge, adding a new boundary marker in the process (red square). The new marker is added as if it is the reflected image of the cell seed \( i \) in the boundary edge prior to the flip. This move leaves the shape of the Voronoi cell intact and works in reverse. In the reverse case, a triangulation edge connected to the boundary is flipped by the equiangulation algorithm so that the flipped edge connects two boundary markers then the excess marker is deleted. Frames a and b are immediately before and after addition of the new boundary marker while frame c shows how the boundary has relaxed a short time later.

that they no longer influence the Voronoi diagram.

The boundary marker rule maintains unbroken lines of boundary markers throughout the whole simulation. We opt to use these boundary lines as a proxy for the real boundary edges of the cells when it comes to deciding how the monolayer edge interacts with the external environment. Note that relatively little is understood regarding the general properties of the free boundaries of cell monolayers [59]. We define an energy associated with the boundary line which includes a line tension term to control boundary stretching as well as a bending term.

Line tension energy, \( E_{lt} \), is

\[
E_{lt} = \frac{1}{2} \sum_{(i,j)} \lambda_{ij} (l_{ij} - l_0)^2
\]  

(2.28)
where $\lambda_{i,j}$ is the line tension of the edge connecting boundary vertices $i$ and $j$, $l_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$ is the length of that edge and $l_0$ is its native length (We usually choose $l_0 = 0$). The bending term controls the resistance to boundary bending

$$E_{\text{bend}} = \frac{1}{2} \sum_i \zeta_i (\theta_i - \pi)^2,$$

where $\zeta_i$ is the stiffness of angle $\theta_i$ at the boundary particle $i$, determined as

$$\theta_i = \arccos \frac{\mathbf{r}_{ji} \cdot \mathbf{r}_{ki}}{|\mathbf{r}_{ji}| |\mathbf{r}_{ki}|},$$

where $\mathbf{r}_j$ and $\mathbf{r}_k$ are the positions of the pair of boundary particles connected to particle $\mathbf{r}_i$.

We justify adding these two new parameters to the model on the basis that the dynamics of cells in the monolayer can be highly sensitive to boundary conditions, see Figure 2.5. Our boundary handling method works in practice but it should be considered a temporary solution to the problem. An alternative would be to assign cells on the boundary a maximum radius and let their free edges be represented
by circle segments [12, 38]. The dynamics of monolayer boundaries is particularly important for morphogenesis and wound healing, and as such it has been steadily attracting interest [64, 65, 22]. The ability to simulate a flexible boundary is therefore a valuable property of the Active Vertex Model.

2.4 Growth and division

Cell proliferation is another type of active process that can be added to the vertex model. We do so simply by adjusting the preferred area of each cell on each timestep. Having cells grow by a constant factor $\mu$ is an appropriate starting point,

$$A_0(t + \delta t) = (1 + \mu \delta t)A_0(t), \quad (2.31)$$

where $\delta t$ is the simulation timestep.

Cell division is modelled based on the ideas of Bell and Anderson [66]. The cell can only divide once its area is larger than some critical area $A_c$, in which case it divides with probability proportional to $A - A_c$,

$$p_{\text{div}} = \begin{cases} 
\chi (A - A_c) & \text{for } A > A_c \\
0 & \text{otherwise}
\end{cases}, \quad (2.32)$$

where $\chi$ is a constant with units of inverse area times time. Upon division the two daughter cells are placed along the direction of the active force orientation vector $n_i$.

Division in our Active Vertex Model is somewhat different to the standard vertex
model due to our reliance on Voronoi diagrams. Division in the standard vertex model can happen smoothly by the addition of an edge, shown in Figure 1.4b. The shape of the dividing cell changes dramatically from a single polygon to a pair but the surrounding cells are untouched. Subsequently, the system relaxes locally over a short period of time to, for example, the state shown in the final frame of Figure 1.4b. However, when cells divide in the Active vertex Model the original cell seed is removed and two new seeds added a short distance apart along the division axis of the original cell. The shape of the cell network jumps over any intermediate states to find a state which is a valid Voronoi diagram. As an immediate result the geometries of all adjacent cells change discontinuously to accommodate both new cells. Although this behaviour is undesirable, our simulations are sufficiently robust to the sudden addition of new cells. For the times when these discontinuities cause problems we allow the vertex model forces associated with the pair of new cells to be ‘phased in’ over a period of tens or hundreds of timesteps so that the full impact of the division is not felt by the system all at one instant. We test the capabilities of our implementation by simulating a growing system and discuss the results in Section 3.2.

2.5 Implementation

The Active Vertex Model is implemented in a software package originally written for simulations of active particles constrained on curved surfaces. The Soft Active Matter on Surfaces or SAMoS software is open source and freely available [48]. It includes both the fast C++ simulation code and codes for constructing initial
conditions and analysing results which is written in Python. The analysis code can
reconstruct the Delaunay and Voronoi meshes in halfedge data structures \[67\] so
they can be analysed in detail. For more details on how the simulation code is
organised to be modular and extensible see \[68\]. Detailed instructions and examples
are given for simulations of active particles and epithelial monolayers in the hope
that other researchers will find the model and implementation useful.

2.6 Discussion

Our Active Vertex Model combines cell growth, division and death with a simple
model of active cell migration. Switching between particle based and vertex model
representations of the monolayer is prevented from becoming prohibitively expensive
by utilising the mathematical duality between Delaunay and Voronoi tesselations.
T1 transitions between cells arise naturally as a consequence of flipping Delaunay
triangulation edges according to the equiangulation algorithm. The AVM is partic-
ularly suited for studying phenomena that occur on large length and time scales.
By retaining cell-level resolution we can attempt to connect the cell-level processes
with global collective behaviours. Including active forces with the vertex model in
an efficient manner enables investigation of the morphogenic processes driven by
collective migration on the scale of tens of thousands of cells. Such biological sys-
tems are typically only accessible to continuum models. Still, there are several ways
in which the model can be improved.

We discuss the applicability of the model to the example biological systems
described in section \[1.1\] Cells in the early developing chick embryo \[5\] do not
migrate by crawling, instead we think of them as forming a tight network in which cells do not migrate individually, rather they move by contracting their shared cell-cell junctions. Cells at the edge of the embryo sheet grip and pull on the underlying membrane in order to hold the monolayer under tension and provide a support for this type of network contractions \[69\]. In this case there is no reason to think that cells themselves have a preferred direction or *polarity* in the monolayer plane. The action of a force \(f_a\) along a single direction \(n_i\) would be inappropriate in this case, instead active forces should be primarily restricted to act on cell vertices and be oriented along cell junctions.

The restriction of cells to Voronoi shapes makes it difficult to study how collective migration arises as a consequence of individual edge contractions. We do not have the freedom in the Active Vertex Model to contract edges arbitrarily. The mechanical forces exerted by cell ingressions may also be important in context of the chick embryo but these can only be modelled as discrete processes in the AVM, whereby a cell simply disappears instantaneously and the surrounding cells adjust their shapes to fill the gap. This is similar to the way that cell division is implemented whereby the removal of one cell and the addition of two new cells causes an immediate shift in the geometry of each neighbour.

The monolayers of crawling cells which we have discussed are more appropriate systems for the AVM, but they still have their own challenges. Although our Voronoi cells can become elongated in certain arrangements, the AVM may have trouble capturing general patterns of cell elongation. We discuss the importance of cell shape for calculating stresses in Section 4.3. One solution would be to augment the model to allow two particles to represent a single cell. Each cell body would then
be the union of a pair of Voronoi regions. During cell elongation the pair of two Voronoi seeds would diverge from each other.

Finally, an investigation of the cell boundary instability as seen in Figure 2.5 requires no additions to the model. Even so, extending the boundary handling rules so that separate protrusions or cell patches can merge together or break apart would be a significant improvement with regards to studies of the boundary dynamics.
Chapter 3

Case Studies

To demonstrate the capabilities of the Active Vertex Model we show several examples, linking the observed behaviours to either experiment or other computational models. The intention is to show what kind of systems could be investigated with this model and to demonstrate the validity of the implementation. In particular we show that the AVM can cope with system sizes on the order of 10,000 proliferating cells and reproduce behaviour observed in the older models of Graner et. al. [42] which relates to the ability of cells to self-organise into compartments. Firstly though we discuss a key result of the Active Vertex Model – that depending on the parameters $A_0$ and $\Lambda/\Gamma$ the system can be defined as being in a fluid-like or solid-like state. We will define what these terms mean in the context of cells.

The result is first demonstrated using the Self-Propelled Voronoi (SPV) model [70] which precedes our Active Vertex Model. The SPV model also combines actively propelling Voronoi cells with the vertex model but is restricted to periodic boundary conditions. It also has no additional features such as cell growth and division or distinct cell types and no implementation is freely available.
3.1 Solid-Fluid Transition

We define our model monolayer as being a solid if the individual cells must overcome some energy barrier in order to slip past each other – acquiring new cell-cell contacts and losing old ones. Cells that slip past each other like this are said to intercalate. A solid-like state would be expected for epithelial tissue where cells resemble a hexagonal packing [55]. What is perhaps not so obvious is that the model also permits a liquid-like state in which the energy barrier for intercalation events approaches zero. The fluid-like behaviour can be seen in the AVM by choosing successively lower active driving forces $f_a$ and observing that cells are still able to exchange neighbours, albeit rarely. In the simple case that all the cells have identical parameters, by changing the ratio of $\Lambda/\Gamma$ to $A_0$ one can observe a transition between these states. Phase diagrams showing the two states have been published for the SPV model [70] and reproduced using the Active Vertex Model with different boundary conditions [68]. We discuss these results in this section without reproducing them again. Note that the SPV model uses an alternative form of the vertex model energy,

$$E_{i,SPV}^{\text{SPV}} = \sum_{i=1}^{N} \left[ \frac{K_i}{2} (A_i - A_i^0)^2 + \frac{\Gamma_i}{2} (P_i - P_i^0)^2 \right], \quad (3.1)$$

where $P_i^0$ is the preferred perimeter of cell $i$, analogous the preferred area $A_i^0$.

If $\Lambda_{\mu\nu}$ in equation ?? is identical for every cell-cell contact and $P_i^0 = P^0$ is the same for every cell then equation [3.1] is the same as the original form of the Vertex Model energy. Note that this is the only case we consider in the context of the fluid-solid transition. To show both equations are identical we need that
\[ \sum_i \Gamma (P_i - P^0_i)^2 / 2 \equiv E_{\text{perim}} + E_{\text{junct}} \], with \( E_{\text{perim}} \) and \( E_{\text{junct}} \) being the second and third terms respectively in Equation 1.1.

Perimeter term in SPV model. Just contact term in AVM.

\[
\sum_i \Gamma (P_i - P^0_i)^2 = \sum_i \left[ \Gamma^2 (P_i)^2 - \Gamma P_i P^0_i + \Gamma^2 (P^0_i)^2 \right] = E_{\text{perim}} - \sum_i \Gamma P_i P^0_i \\
\sum_{\mu,\nu} \Lambda_{\mu\nu} l_{\mu\nu} = \frac{1}{2} \sum_i \Lambda \sum_{\nu \in L_i} l_{\mu\nu+1} = \sum_i \frac{\Lambda}{2} P_i
\]

We scored out a constant term on the second line because shifting the energy by a constant factor has no effect on the dynamics. Comparing the equations gives \( \Lambda = -2\Gamma P^0_i \) so in this case the \( \Lambda \) parameter effectively sets a preferred perimeter \( P^0_i \) for each cell. \( P^0_i \) and \( \Gamma \) are naturally positive which implies that \( \Lambda \) is negative, thus the \( \Lambda \) term acts to extend the cell-cell edges.

### 3.1.1 Shape Index

To characterise the fluid-solid transition Bi et al. [71] introduce a shape index \( p_0 \),

\[
p_0 = \frac{P_0}{\sqrt{A_0}} = \frac{-\Lambda}{2\Gamma \sqrt{A_0}}.
\]  

(3.2)

The shape index is a dimensionless number that indicates the ratio of a polygonal cell’s native perimeter \( P_0 \) and the square root of its native area \( A_0 \). For example a regular hexagon has a shape index of 3.722 while a square has an index of 4.0. However, this doesn’t imply that a patch of cells with \( p_0 = 4.0 \) will relax into a perfect square grid in the absence of external or active forces. If left to relax the
system will find the first minimum in the energy landscape and rest there. It may look something like the disordered state shown in Figure 3.2b which corresponds to $p_0 = 3.93$. On the other hand, a representative energy minimising state for a much lower $p_0 = 3.35$ is shown in Figure 3.2a.

Bi et al. [71] show that the critical value of $p_0$ which divides the solid-like and liquid-like phases is 3.81. Collections of cells with $p_0 > 3.81$ are in a liquid-like state as they have potential to intercalate even for tiny active driving forces, whereas the strength of active forces $f_a$ required to force intercalations to occur increases as $p_0$ drops below 3.81. If sufficient active forces are present to drive intercalations in the $p_0 < 3.81$ regime we say the system is fluidised by those active forces. Figure 3.1 shows a portion of the phase diagram for varying $p_0$ and $f_a$ for three different boundary conditions. The initial configuration for each simulation is a circular patch of 1000 cells. The initial patch has a packing fraction $\phi = 1$, in other words the total patch area is $1000 A_0$. On average cells start with an area close to their preferred area but not necessarily their preferred perimeter, $P_0 = -\Lambda/2\Gamma$. The patch can contract and expand slightly as it accommodates a balance between $A_0$ and $P_0$ terms. In Frame 3.1h however contractions or expansions of the monolayer as a whole are prevented by a fixed boundary. Frames 3.1b and 3.1c have flexible boundaries with boundary line tension $\lambda = 0$ and $\lambda = 0.1$. With no boundary line tension and high $p_0$, the cell patch tends to start breaking apart (see Figure 2.5) shortly after which the AVM model fails because no method is implemented of stitching together groups of cells separated by the monolayer boundary. The white region of 3.1b corresponds to this type of unstable boundary.
Figure 3.1: Phase diagrams showing solid-like and liquid-like regions for (a) fixed boundary, (b) flexible boundary with no boundary line tension and (c) flexible boundary with line tension $\lambda = 0.1$. The dashed white line is a rough boundary between these solid and liquid behaviours and for each boundary condition the critical value of $p_0$, where this line hits the x-axis, is 3.81.

Irrespective of the boundary conditions, the solid-like to fluid-like transition occurs consistently at $p_0 = 3.81$ as $f_a \to 0$. In addition, the state of the system at each point is characterised by the **alpha-relaxation time** $\tau_\alpha$ [68]. $\tau_\alpha$ indicates the time scale over which cells rearrange, thus in the solid-like regime where cells never intercalate it diverges. The alpha-relaxation time is defined according to the self-intermediate scattering function

$$F(q,t) = \langle \exp[ i q \cdot (r(t) - r(0)) ] \rangle.$$  \hspace{1cm} (3.3)

$F(q,t)$ measures the decay of the autocorrelation function, $\langle r(t) \cdot r(0) \rangle$, for some wave vector $q$ which is taken to be the inverse cell size $q \equiv |q| = 2\pi/A$ so that $q \cdot r(t) = q |r(t)|$. Then $\tau_\alpha$ is the time at which $F(q,t)$ has decayed by half.

The critical shape index $p_0 = 3.81$ has a curious feature, namely this value is the shape index of a regular pentagon. In contrast a regular hexagonal lattice of cells with $p_0 = 3.722$ is clearly solid-like. The first step of a T1 transition is an existing
Figure 3.2: Cell patches in the active vertex model are relaxed to a local minimum energy state for (a) $p_0 = 3.35$ and (b) $p_0 = 3.93$. A low $p_0$ the system finds a state which shows broadly hexagonal order while for high $p_0$ the local minimum energy state is a more disordered network.

cell edge contracting completely, therefore one pair of cells in a hexagonal network would be momentarily reduced to 5 sides. Hence there is subtle connection then between $p_0$ and the number of cell edges.

To better understand this fluid-like behaviour we set the active force $f_a = 0$ and investigate the low-energy, relaxed states. For groups of cells making up a hexagonal lattice structure, like those seen in Figure 3.2a, each cell side has a similar length there is little flexibility. Less obvious is that certain edges in Figure 3.2b can be contracted while others are extended with either none, or very little change in the vertex model energy. As a result, the monolayer gains the liquid-like property that cells can rearrange in response to a minimal driving force. Using the language of classical mechanics we describe this high $p_0$ state as having a low shear stress.
3.2 Growth and Division

Division and death processes are important in any living tissue. It is important to note that the removal of one cell during apoptosis or ingression and the addition of two new cells during division in the AVM causes a discrete change in the Voronoi tessellation which implies a discontinuous change of the local forces derived from the vertex model energy. We have simulated the long term growth of a small cluster of cells to assess whether this discrete change in geometry can lead to any instabilities in the model. We find that some care should be taken to avoid extreme conditions for dividing cells.

In order to illustrate the growth process, we choose a shape index, $p_0 = 3.10$, corresponding to $\Gamma = 1$ and $\Lambda = -5.5$ and no active driving, in other words we set $f_a = 0$. The simulation runs for $10^6$ time steps at $\delta t = 0.005$, corresponding to 5000 time units, starting from 37 cells and stopping at about 24,000 cells. To balance computational efficiency with a smooth rate of division, cells are checked for division every 25 time steps. That is, cells are only permitted to divide at 25 timestep intervals but that the average number of cells which divide following each 25-step increment is significantly less than 1, even for several thousand cells. We show snapshots of different stages of the tissue growth in Fig. 3.3a-e.
Figure 3.3: Snapshots of a growing epithelial tissue. Frames (a), (b), (c), (d) and (e) have 37, 63, 124, 4633 and 23787 cells and are at times 50, 500, 1000, 3500 and 5000, respectively. Cells have a chance to divide if their area is greater than a critical area $A_c = 2.8(a^2)$ after which the probability of a cell to divide increases linearly with its area. $a$ is the range of the soft repulsion between Voronoi seeds (Equation 2.1) and we use $(2a)$ to set the lengthscale. In this simulation, the shape factor was set to $p_0 = 3.10$. (f) Log-linear plot of the total number of cells as a function of simulation time. The growth rate of the patch is initially exponential but starts to slow at around 3000 cells. This is due to cells in the centre of the cluster being prevented from expanding by the surrounding tissue. (g) Tissue after 5000 time units ($10^6$ time steps) with each cell coloured by pressure. Pressure has built up in the centre of the tissue while close to the edge the average pressure is low. (h) Average pressure (averaged over the polar angle) as a function of the radial distance from the centre of the tissue. (i) Distribution of the number of neighbours for cells in the system shown in (g).

We note that the stability of simulations which involve growth are quite sensitive to the values of the parameters used in the AVM. For example, divisions of highly irregularly shaped cells, as commonly observed in the high $p_0$ regime, can put a
significant strain on the simulation and even make it numerically unstable. Helpfully, some of these problems are alleviated by the soft repulsive potential between cell centres. This in addition to a smaller time step is used to mediate the impact of cell divisions for growing systems with high $p_0$.

In Fig. 3.3f we show the tissue size as a function of the simulation time. In this simulation there are no apoptosis or cell ingression events and, as expected, the tissue size grows exponentially. However, at long times, the growth slows down and deviates from exponential growth. This is easy to understand, as the centre of the tissue is prevented from expanding by the surrounding cells. The effect can be seen in Fig. 3.3e, where cells located towards the centre have, on average, smaller areas and in Fig. 3.3g, which shows a clear pressure buildup in the centre. The radially averaged pressure is plotted in Fig. 3.3h to show the effect clearly. We see that in the later stages, the simulated tissue is no longer in mechanical equilibrium since the pressure at the centre is increasing faster than it can be dissipated towards the edges. Pressure is computed as the trace of the Hardy stress tensor [72], we discuss in detail how this stress calculation is adapted for systems of polygonal cells in the vertex model in Chapter 4. We also see clear heterogeneities in the local pressure shown in Fig. 3.3g. Note that pressure (and stress) inhomogeneities are a persistent feature of epithelial cell monolayers that have been investigated by traction force microscopy [73, 33], the method described in detail in Section 1.1.2. Finally, in Fig. 3.3i we show the distribution of the number of neighbours for this model system. The observed distribution is in a good agreement with the observations in actual tissues [74, 75].
3.3 Cell Sorting

The AVM is equipped to allow for cell-specific parameters, which enables us to investigate tissues with locally varying mechanical properties. A commonly studied example of the effects such heterogeneities is cell sorting, where cells self-organise into compartments of the same type.

We show simulations that display sorting of two distinct cell types. This is achieved by setting the junction tension $\Lambda$ for each type of cell-cell and cell-boundary contacts. All our simulations consist of 1000 cells with half chosen randomly to be of the “red” type and the others being of the “blue” type. In these simulations, boundaries have been kept fixed. We observe sorting behaviour akin to that found in other tissue models [76, 42]. Using $r$, $b$ and $M$ to denote red, blue and the boundary respectively, we start by fixing $K = 1$ and $\Gamma = 1$ and set $-6.8 = \Lambda_{rr} < \Lambda_{rb} < \Lambda_{bb} = -6.2$, corresponding to $p_0$ in the range 3.58 - 3.93. We note however that for cells with a combination of different types of neighbours, $p_0$ may be different for each one. In this context we use $p_0$ only as a estimate of whether a collection of different types of cells display fluid-like of solid-like behaviour.

For cell sorting simulations we set the active driving, $f_a$, to zero but to facilitate sorting behaviour we still want some random fluctuations in the system which allows for T1 transitions that can bring initially distant cells into contact.

We have chosen different values for $\Lambda_{rr}$ and $\Lambda_{bb}$ to reflect the idea that the surfaces of these cells have different adhesive properties [77]. Note that the $\Lambda$ parameter for a particular contact is proportional to its energy per unit length. Sorting of cells into groups of the same type occurs when the energy of two red-blue contacts is greater
than the energy of one red-red contact and one blue-blue contact, corresponding to
\[ \Lambda_{rb} > (\Lambda_{rr} + \Lambda_{bb})/2, \] see Fig. 3.4a-c. In this regime, for cells of the same type it is energetically favourable for the new contact to elongate while local red-blue contacts are shortened. Conversely, if \( \Lambda_{rb} < (\Lambda_{rr} + \Lambda_{bb})/2 \) then cells maximise their red-blue contacts forming a ‘checkerboard’ pattern (Fig. 3.4d). The final pattern is not without defects, the number and location of which depend on the initial conditions.

The tissue boundary consists of contacts between cell centres and boundary particles so \( \Lambda_{rM} \) and \( \Lambda_{bM} \) need also to be specified to reflect the way in which the cell types interact with the extracellular matrix or surrounding medium. Initially we set \( \Lambda_{rM} = \Lambda_{bM} = -6.2 \) and observe that blue cells cover the boundary enveloping red cells because this facilitates lower energy red-blue and red-red contacts being formed in the bulk. If we incentivise red-boundary contacts by setting \( \Lambda_{rM} < \Lambda_{rr} + \Lambda_{bM} - \Lambda_{rb} = -6.6 \) we make red-boundary contacts preferable \[42\]. This case is shown in Fig. 3.4e for \( \Lambda_{rM} = -6.8. \]

Figure 3.4: (a-c) Snapshots of a system of two cell types at times 10, 500 and 5000 with \( \Lambda_{rb} = -6.4. \) (d) A ‘checkerboard’ pattern forms immediately (at time 10) when red-blue cell-cell contacts are energetically favourable compared with pairs of red-red and blue-blue contacts, \( \Lambda_{rb} = -6.7. \) (e) Same initial system as (a-c) but with red-boundary contacts slightly favoured over blue-boundary contacts. The system gradually separates into compartments of each cell type. The uncorrelated random fluctuations are sufficient to drive neighbour exchanges within the bulk of both the red and blue cell compartments. Cells on the compartment boundary can sometimes move parallel to it but meet strong resistance when trying to move across it.
3.4 Additional examples

In the previous discussions, all examples assumed a patch of cells with the topology of a disk. However, the AVM is not restricted to the circular geometry and can be applied to systems of arbitrary shapes, provided that all monolayer boundaries are specified in advance. Monolayers with multiple boundaries and non-trivial topology often arise when modelling experimental systems where cells surround an obstacle, or in studies of wound healing. Figure 3.5 is a gallery of non-circular shapes produced using the AVM. The annular geometry shown in Fig. 3.5a would be suited for modelling wound healing problems as well as situations where cells migrate in order to fill a void. A common experimental setup where cell colonies are prepared as rectangular strips [60] is shown in Fig. 3.5b, where three separate patches grow towards each other. Finally, in Fig. 3.5c we show an example of another interesting situation where cells are grown in a confined region of space. The subject of cell colonies growing in confined spaces is relevant when cells invade another tissue or grow in porous environments and may be important in many developmental processes [78].

The next chapter describes how to calculate pressure and stress for vertex models.
Figure 3.5: (a) Snapshots of the simulation of a cell sheet with an annular geometry used to illustrate how cells divide and fill the circular void in the centre. The system configuration was recorded at times 0, 1000 and 1700. The initial configuration has \( p_0 = 3.46 \), i.e., it was in the solid-like phase. While it is not simple to define \( p_0 \) for a growing system due to a constant change in the cell target area \( A_0 \), we note that throughout the simulation, cell shapes remain regular. (b) Illustration of a common experimental setup where cells are grown in rectangular ‘moulds’ for a system with initial \( p_0 = 3.35 \). Once the entire region is filled with cells, the mould is removed and the colony is allowed to freely grow. Images on the right show two of the strips about to merge. In (c), we model tissue growth in confinement using a system with initial \( p_0 = 3.46 \). Grey beads form the boundary of the confinement region that constrain the cell growth. Initially, cells do not touch the wall and freely grow. As the colony reaches the wall, one starts to notice pressure buildup. Finally, the entire cavity is filled with cells and any subsequent division leads to increase of the pressure in the system. Snapshots were recorded at times 1200, 1480 and 1600.
Chapter 4

Stress in Epithelial Monolayers

Stress is the quantity in the field of mechanics which measures internal forces on an element of a solid body due to the surrounding elements. One can learn about the properties of materials by measuring their deformation following the application of a known force. A distinction is made between elastic deformations where the material will immediately evert back to its original shape of the external force is removed and plastic deformations where this does not happen. For example, in the simple case where the stress in an elastic material is proportional to the applied deformation, the material is said to be linearly elastic. Metals used in engineering commonly have linearly elastic and plastic regimes, separated by a ‘yield’ point [79]. Materials forced to deform beyond their yield points can lead to catastrophic failures in engineering applications. It is similarly useful to understand how biological materials respond to forces, both externally applied forces and internal cell-generated forces. In this chapter we describe how to calculate stress in the Active Vertex Model.

Stress can be defined as a tensor which relates an element \( dS \) of an imaginary closed surface \( S \) within the material with the force applied to that element from
the surrounding material. We call this force $T^{(n)}$ where the superscript $n$ is the normal vector which defines the surface element $dS$. Therefore, stress is defined as the tensor quantity $\sigma$ that relates vectors $n$ and $T^{(n)}$. We can write this succinctly as $T^{(n)}_j = \sigma_{ij} n_i$, using Einstein summation convention [80].

By convention the surface normal $n$ vector for a material element points outwards from the material surface so a tensile (positive) force applied at the material surface induces a positive stress in the material. Despite having many unique properties, biological tissue is a material and is still studied with many of the tools of materials science. The chick embryo is under tension because the cells at its periphery pull outwards on the underlying membrane. Likewise tensile stresses can be generated in a cultured monolayer by the accumulation of active forces of all the crawling cells pulling towards the edges. Stress in the monolayers measured by Tambe et al. [33] is predominantly tensile but there are also sparse regions of slightly compressive stress as measured by Traction Force Microscopy (Section 1.1.2). In contrast, the process of cell growth and division generates compressive stresses. Cells use the materials and energy from nutrients they absorb in order to push on their neighbours and continue extending their internal structures. Therefore in these examples, cell growth acts to relieve tensile stresses while cell deaths or ingressions compound tensile stresses.

It is often useful to decompose the stress tensor into hydrostatic and deviatoric parts,

$$\sigma = \frac{1}{d} \text{Tr}(\sigma) \hat{I} + \left( \sigma - \frac{1}{d} \text{Tr}(\sigma) \right)$$

(4.1)

where $d$ is the dimension of the system and $\text{Tr}(\sigma) = \sigma_{ii}$ is the trace of $\sigma$. The hydrostatic part of stress for a material element represents internal forces in the material.
that are exerted evenly in all directions. Hydrostatic stress is closely related with the concept of pressure. In fact we can define pressure as \( P = -\frac{1}{2} \text{Tr}(\sigma_{\text{Hydrostatic}}) \) [81].

The convention that negative (compressive) stress corresponds to a positive pressure may be confusing. An example is the positive atmospheric pressure. Gravity compresses the atmosphere, inducing a negative stress. In response, the atmosphere attempts to expand by applying its positive pressure force on any surface that it contacts. We use this definition of pressure to characterise the simulated monolayers in Section 3.2 and Figure 3.5c.

The deviatoric part of the stress tensor is also informative. It can be shown that the stress tensor is symmetric for systems in mechanical equilibrium [81]. We assume the epithelial monolayer is close to mechanical equilibrium on the timescales of minutes or hours. Real symmetric matrices have the convenient property that they can always be decomposed into a set of \( n \) orthogonal eigenvectors and \( n \) eigenvalues where \( n \) is the dimension of the matrix. Therefore in our two dimensional monolayer there are a pair of orthogonal axis \( n_1 \) and \( n_2 \) for which \( \sigma n_1 = \sigma'_{11} n_1 \) and \( \sigma n_2 = \sigma'_{22} n_2 \) where \( \sigma'_{11} \) and \( \sigma'_{22} \) are the components of a diagonal matrix. Finding the eigenvalue with the greater absolute magnitude \( \sigma_{\text{max}} = \max(\{|\sigma'_{11}|, |\sigma'_{22}|\}) \) we call its associated eigenvector, \( n_{\text{max}} \), the direction of maximal principal stress [33] and its orthogonal counterparts are \( \sigma_{\text{min}} \) and \( n_{\text{min}} \). Switching to the coordinate frame defined by \( n_{\text{max}} \) and \( n_{\text{min}} \):

\[
\sigma_{\text{Deviatoric}} = \begin{pmatrix} \sigma_{\text{max}} & 0 \\ 0 & \sigma_{\text{min}} \end{pmatrix} - \frac{\sigma_{\text{max}} + \sigma_{\text{min}}}{2} \hat{I} = \frac{1}{2} \begin{pmatrix} \sigma_{\text{max}} - \sigma_{\text{min}} & 0 \\ 0 & \sigma_{\text{min}} - \sigma_{\text{max}} \end{pmatrix}.
\] 

(4.2)
The stress is preferentially oriented along $n_{\text{max}}$ with the strength of this preference quantified by the value of the local shear stress, $(\sigma_{\text{max}} - \sigma_{\text{min}})/2$. Results from Traction Force Microscopy experiments indicate that epithelial cells can align with the local principle direction of stress in the monolayer and travel in that direction. The alignment effect is more prominent the greater the local shear stress. This connection between cell motility and stress is a useful tool for understanding patterns of collective migration.

### 4.1 Computing Stress

Stress is defined as a continuous quantity. When describing macroscopic materials with an order of say, $10^{23}$ atoms, it is normal to construct a continuous stress field by considering ‘infinitesimal’ volume elements. Each volume element should contain a sufficiently large number of particles so that macroscopic quantities like density or stress are well defined. At the same time the volume element must be much smaller than the system size so that the material can be considered continuous.

In reality, computer simulations can only represent materials as finite collections of discrete elements. Likewise experimental measurements of stress can only yield finite datasets. To make comparisons between simulation and experimental data it is often necessary to compute continuous fields approximately for both datasets and compare those fields. The rest of this section is devoted to constructing approximate continuous stress fields. The AVM is composed of three sets of discrete elements; cells, vertices and edges. However, when we come to sum up contributions to the stress from discrete elements we choose to use cells because they turn out to be the
most convenient.

To compute an approximate continuous stress field we need some kind of averaging procedure which ‘smoothes out’ contributions from the discrete cells into a continuous field. The Hardy stress defines one such averaging procedure [72],

$$\sigma_\omega(r) = \frac{1}{2} \sum_{\alpha \neq \beta} f_{\alpha\beta} \otimes (r_\beta - r_\alpha) b(r, r_\alpha, r_\beta)$$  \hspace{1cm} (4.3)

where

$$b(r, r_\alpha, r_\beta) = \int_{s=0}^{1} \omega \left( (1 - s)r_\alpha + sr_\beta - r \right) ds.$$  \hspace{1cm} (4.4)

The position vector $r$ is any point in the system and indices $\alpha$ and $\beta$ denote elements of the system, in our case cells and cell vertices. The quantities $f_{\alpha\beta}, \omega, r_\alpha$ and $r_\beta$ are defined as follows.

- The forces in the system can be decomposed into pair contributions from the elements of the simulation. The contribution of the force on element $\alpha$ due to element $\beta$ is $f_{\alpha\beta}$ and acts along the bond vector $r_{\alpha\beta} = r_\beta - r_\alpha$. The force decomposition is constructed by defining the partial derivative $\frac{\partial E_v}{\partial r_{\alpha\beta}}$ so that

$$f_{\alpha\beta} = -\frac{\partial E_v}{\partial r_{\alpha\beta}} \frac{r_\alpha - r_\beta}{|r_\alpha - r_\beta|} \text{ where } r_{\alpha\beta} = |r_\alpha - r_\beta| \text{ and } E_v \text{ is our usual vertex model energy.}$$

- A smoothly varying value of stress $\sigma(r)$ can be calculated for any point $r$ by adding contributions from bonds $r_{\alpha\beta} = r_\beta - r_\alpha$ between elements $\alpha$ and $\beta$ of the system which are close to $r$. Contributions are computed according to a smoothing function $\omega$ which is centred around $r$ and satisfies $\int_{\mathbb{R}^2} \omega(r) dr = 1$.

- The bond function $b(r, r_\alpha, r_\beta)$ assigns a weight to the contribution of the force
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\(f_{\alpha\beta}\) to the stress at \(r\) by integrating the smoothing function \(\omega\) over the length of the bond \(r_{\alpha\beta}\). The value of \(b(r, r_\alpha, r_\beta)\) is always \(0 \leq b \leq 1\).

To complete our stress calculation we need to make choices for \(\omega\) and the definition of \(\frac{\partial E_v}{\partial r_{\alpha\beta}}\). Note that these choices can give wildly varying results for the stress. The Hardy stress only gives a prescription for generating possible stress tensors all of which converge to the same ‘real’ stress field only in the purely theoretical limit where the number of particles in the simulation tends to infinity, i.e. in the thermodynamic limit [72]. We need to choose \(\omega\) as well as a force decomposition which is computationally efficient and reflects the natural symmetry of the system. We will find that our choices reduce the calculation to a version of the more common Virial stress. Note that in general the Virial and Hardy stresses have a second, kinetic, term. We discard this term because it is, in practice, several orders of magnitude smaller than the potential term which is defined in Equation 4.3.

4.1.1 Force decomposition

Calculation of \(\frac{\partial E_v}{\partial r_{\alpha\beta}}\) is simple for pairwise forces but \(E_v\) depends on cell areas and perimeters so it is not pairwise. It turns out that the force associated with any realistic energy potential can be decomposed into a sum of pairwise and central contributions [72]. That is, energy contributions which only depend on the distances \(r_{\alpha\beta}\) between elements of the system \((\alpha, \beta)\). The number of distances between \(N\) particles is \(N(N - 1)/2\) but because of the local nature of the vertex model force we will only need a fraction of these distances. We want to choose a minimal set of distances \(\{r_{\alpha\beta}\}\) that completely defines the geometry of each cell. We can then
write down an expression for \( E_v = E_v(\{r_{\alpha\beta}\}) \) which depends only on this set of distances. The perimeter and cell contact terms are already written in terms of cell edges \( \{r_{\mu\nu}\} \) but this set of distances is not sufficient to determine the cell areas \( A_i \).

We opt to break the polygonal cell areas into triangles. For a polygon with \( n \) vertices we are free to choose one of the vertices \( \mu \) and break the polygon into \( n - 2 \) triangles by adding \( n - 3 \) new bonds, see Figure 4.1a. However, more generally we can choose any point inside the cell and divide the cell into \( n \) triangles around that point. The area of a triangle with sides \( (a,b,c) \) can be computed using Henon’s formula \( A = \sqrt{s(s-a)(s-b)(s-c)} \) where \( s = (a+b+c)/2 \). Some experimentation reveals that the choice of this point does not effect the result. This is consistent with other recent work [82].

Because Voronoi seeds are the degrees of freedom in our model it is convenient to choose Voronoi seeds to divide the cell areas (Figure 4.1b). The area of a cell \( i \) can then be computed as,

\[
A_i = \frac{1}{4} \sum_{j \in n.n.i} |r_{ij}| |r_{ij}^{(j)}|, \quad (4.5)
\]

where \(|r_{ij}^{(j)}| \) is the length of the contact edge between cells \( i \) and \( j \) and \(|r_{ij}| \) is the distance between the Voronoi seeds of cells \( i \) and \( j \). Using the properties of the Voronoi diagram we identified each triangle has having a base of length \(|r_{ij}^{(j)}|\) and height of \(|r_{ij}|/2\). We go on to discuss our choice of smoothing function.
Figure 4.1: Potential methods for breaking down the area term into bonds which fit in the Hardy stress formula. (a) Splitting the cell into the minimal number of triangles. (b) Splitting the cell around the Voronoi seed. The yellow highlighted triangle has area of $|r_{ij}|r_{\nu\nu-1}|/4$.

4.1.2 Smoothing function $\omega$

The uniform smoothing function is the simplest choice,

$$\omega(r) = \begin{cases} 
1/A_\Omega & \text{if } r \in \Omega \\
0 & \text{otherwise}
\end{cases}$$

(4.6)

where $A_\Omega$ is the area of a local region of space $\Omega$ around $r$. This choice replaces the bond function with $b_\Omega(r_{\alpha\beta}) = \chi/A_\Omega$ where $\chi$ is just the fraction of the bond $r_{\alpha\beta}$ in $\Omega$. It is common to choose a radially symmetric function $\omega(r)$ however, on the short lengthscale of a few cells our cellular system is not radially symmetric. It very convenient to instead choose the region $\Omega$ to coincide with actual cells. The cell edges $\{r_{\mu\nu}\}$ now lie on the boundaries of the $\Omega$ regions and we take half of the force part $f_{\mu\nu}$ to be associated one cell and half for the other cell. Similarly we divide the bond $r_{ij}$ into two bonds, one in each cell. If we then restrict ourselves to calculating the stress on a collection of whole cells then we can replace the bond function with a constant value for each region $b_\Omega = 1/A_\Omega$. 
4.1.3 Virial stress

Substituting our choice of $\omega$ into the Hardy stress gives,

$$\sigma_\omega(r) = \frac{1}{2A\Omega} \sum_{\alpha,\beta} \frac{\partial E_v}{\partial r_{\alpha\beta}} \hat{r}_{\alpha\beta} \otimes (r_\beta - r_\alpha), \quad (4.7)$$

and we can now derive an explicit expression for stress on a single cell. Starting with the area term in the vertex model, $E_{v,\text{area}}^\omega = \frac{K}{2} (A_i - A_i^0)^2$,

$$\sigma_{\omega,\text{area}}(r) = -\frac{1}{2A_i} \sum_{\alpha,\beta} K_i \left( A_i - A_i^0 \right) \frac{\partial A_i}{\partial r_{\alpha\beta}} \hat{r}_{\alpha\beta} \otimes (r_\beta - r_\alpha)$$

$$= \frac{K_i}{4A_i} \left( A_i - A_i^0 \right) \sum_{j \in \text{n.n.i}} \left[ |r_{\nu\nu-1}| \hat{r}_{ij} \otimes r_{ij} + |r_{ij}| \hat{r}_{\nu\nu-1} \otimes (r_{\nu\nu-1}) \right]$$

$$= \frac{K_i}{4A_i} \left( A_i - A_i^0 \right) \sum_{j \in \text{n.n.i}} |r_{\nu\nu-1}| |r_{ij}| \hat{r}_{ij} \otimes \hat{r}_{ij} + \hat{r}_{\nu\nu-1} \otimes \hat{r}_{\nu\nu-1}$$

$$= K_i (A_i - A_i^0) \hat{I}, \quad (4.8)$$

where the cell edge $r_{\nu\nu-1} = r_{\nu\nu-1}^{(j)}$ depends on $j$ just as it does in Equation 4.5. In the first line the sum is over all bonds $(\alpha, \beta)$ which are the cell edges plus bonds which connect the cell Voronoi seed $r_i$ and its neighbours $\{r_j\}$ and the factor of $\frac{1}{2}$ accounts for double counting of the same bond $r_{\alpha\beta}$ and $r_{\beta\alpha}$. We convert this sum into a sum over the pair of bonds associated with each neighbouring cell $j$. We also substitute for $A_i$ using Equation 4.5. In the third line we used the fact that for two perpendicular unit vectors $\hat{r}_a$ and $\hat{r}_b$ is can be shown that $\hat{r}_a \otimes \hat{r}_a + \hat{r}_b \otimes \hat{r}_b = \hat{I}$.

The contribution to stress of the area term is isotropic; it has no shear stress.
component. In other words this term just contributes a pressure of \(-K_i (A_i - A_i^0)\) to the system. This fact is not a new result, rather it should be understood that vertex-model cells have no internal structure and the pressure from the area term is applied evenly along all cell edges. The shear part of the stress tensor is captured by the other two vertex model terms which we calculate now for constant \(\Lambda_{\mu\nu} = \Lambda\).

\[
\sigma^{\Gamma + \Lambda}_\nu (r) = \frac{1}{A_i} \sum_{\nu \in \mathcal{L}_i} \left( \Gamma_i P_i \frac{\partial}{\partial r_{\nu \nu - 1}} \sum_{\mu \in \mathcal{L}_i} (r_{\mu \mu - 1}) + \frac{\Lambda}{2} \right) r_{\nu \nu - 1} \otimes r_{\nu \nu - 1}
\]

\[
= \frac{1}{A_i} \left( \Gamma_i P_i + \frac{\Lambda}{2} \right) \sum_{\mu \in \mathcal{L}_i} \frac{r_{\nu \nu - 1} \otimes r_{\nu \nu - 1}}{|r_{\nu \nu - 1}|}.
\]

(4.9)

We add contributions from each cell edge by summing over the vertices \(\mu\) of the cell \(i\). We dropped the factor of \(\frac{1}{2}\) in Equation 4.3 and introduced a factor of \(\frac{1}{2}\) in front of the \(\Lambda\) term to avoid separate instances of double counting. Total stress on a single cell \(i\) is then,

\[
\sigma_i = K_i (A_i - A_i^0) \hat{I} + \frac{1}{A_i} \left( \Gamma_i P_i + \frac{\Lambda}{2} \right) \sum_{\mu \in \mathcal{L}_i} \frac{r_{\nu \nu - 1} \otimes r_{\nu \nu - 1}}{|r_{\nu \nu - 1}|}.
\]

(4.10)

We refer to this stress as the Virial stress since it contains no mention of a smoothing function and all bonds \(\{r_{\alpha\beta}\}\) which contribute to the stress at \(A_i\) are contained (or exactly half-contained) in \(A_i\). We can think of this cell-level stress as having the same value within each cell and changing discontinuously between cells. The calculation is also straightforward for non-constant \(\Lambda_{\mu\nu}\).
4.2 Coarse graining

The development of shape and structure of an embryo is characterised by large-scale processes of hundreds of thousands or more cells. At the same time these large-scale processes are the result of individual cell rearrangements, divisions and ingressions. The stresses in the monolayer seem to be relevant for understanding all of these cell-level processes [83, 84]. For example, mechanical feedback on cell growth the division can work to control the size and shape of the Drosophilia wing during development [85]. Traction Force Microscopy experiments have established a correlation between the orientation of principle stresses on a cell and its direction of motion [33].

When studying large-scale processes the stress should be calculated by averaging over regions which are small compared with the processes of interest but still large enough to span several cell lengths so that the global stress patterns are not obscured by stochasticity at the cell-level. Meanwhile the extreme case of a single-cell stress calculation (Equation 4.10) is still useful for studying cell-level processes, e.g. for comparing principle stresses with the cell orientation. In addition, when making comparisons with experimental data, one should also tailor their averaging lengthscale to the resolution of that data.

Several approaches to calculating smooth, coarse-grained stress fields are shown in Figure 4.2. Method (a) calculates the stress associated with a cell by summing contributions from the cell’s neighbours and neighbours of neighbours recursively \( n \) times. The selected region \( \Omega \) is the sum of the area of each cell in it, so \( A_\Omega = \sum_i A_i \). Contributions of the forces in the system to stress and contributions of the cell areas
Coarse graining are added up separately. To clarify this point we write down the equation,

$$\sigma_\Omega = \frac{1}{A_\Omega} \left( \sum_{i \in \Omega} \sigma_i A_i \right),$$

(4.11)

where $\sigma_i$ is the single-cell stress so $(\sigma_i A_i)$ is just the same but prior to division by $A_i$. This method of coarse-graining is both efficient to compute and deals well with monolayers that have a strong anisotropy, that is, monolayers in which the cells are preferentially elongated along a specific direction.

The method indicated in Figure 4.2b is an alternative which averages over cells if their Voronoi seeds are contained in a region of radius $R$. This method is also efficient to compute and works for any point $r$ in the system, however, the resulting stress does not vary smoothly with varying $r$. Methods (a) and (b) are both perfectly valid approaches to calculating Virial stress. The final method, Figure 4.2c, is similar to (b) but keeps partial contributions from bonds which cross the edge of the region and uses the original Hardy stress formula, Equation 4.3. This significantly increases the computational cost but gives a continuous stress field for any averaging radius $R$. Note that for increasingly large $R$ methods (b) and (c) converge and the additional computation involved in (c) is wasted. On the other hand for small $R$, i.e. the radius of a single cell, the method (c) gives a smooth stress field even though we do not have sufficient information density in the AVM to justify it. To understand the last point, consider sampling the Hardy stress over several positions within the same cell. For a small radius $R$ we can get highly varied results depending on the shape of the surrounding cells. We are measuring differences in stress at an intracellular scale but we only have as many degrees of freedom in the model as cells so these
Figure 4.2: Coarse graining approaches. (a) Choose a cell and select its neighbours and neighbours of neighbours recursively $n$ times, in this case $n = 2$. (b) A circular region is drawn around any point and stress is averaged for the set of cells with their Voronoi seeds inside the region. (c) The same circular region but use the Hardy stress to include partial contributions from cells at the edges.

Differences are not meaningful.

An example of a coarse-grained stress is given in Figure 4.3. Pressure is used as a proxy for the individual components of the stress tensors for visualisation purposes. The method (a) was the preferred method and the pressure shown for $n = 0, 1$ and $3$. In this simulation the monolayer started close to its relaxed state and allowed to relax for a short 1000 timesteps. A clear trend can be seen for $n = 1$ and $n = 3$ that the monolayer is contracting slightly and it does so first at the edges where there is almost no pressure (or stress). In contrast for the single-cell stress ($n = 0$) the random fluctuations in pressure dominate the picture. However we can see from the $n = 0$ graph which individual cells are bearing the largest and smallest pressures. In more complicated systems this information is may be relevant for predicting cell intercalations or ingestions. This example illustrates the key idea that coarse-graining stress in the monolayer reveals different information on different lengthscales.
Discussion

Since we derived our stress equation (4.10) from the Hardy stress we can replace our choice of $\omega$ or include contributions from partial bonds (Figure 4.2c) if a smooth and continuous stress field is necessary. This approach to calculating stress is not unique for the AVM and works for any vertex model or any system with complex structure and complex potential energy.

In future work it would be interesting to compare stress in simulations to that calculated by Traction Force Microscopy or any of the other techniques mentioned in section 1.1.3. In general active forces in a system can be modelled on the macroscopic lengthscale as an extra term in the mechanical stress tensor $\sigma_\text{total} = \sigma_\text{passive} + \sigma_\text{active}$. In other words, the total stress is the sum of passive and active parts. Experimental measurements of stress in active systems naturally take into account all the forces in the system, including active forces. Unfortunately to our knowledge, a general mechanism for coarse-graining generic active forces at the atomistic (cell)
level to obtain a macroscopic active stress tensor has not been elucidated. Nor is it clear under precisely what conditions such a coarse-graining procedure would be possible. Such problems are beyond the scope of this thesis.

Instead we make the reasonable assumption that $|\sigma_{\text{passive}}|$ is significantly larger than $|\sigma_{\text{active}}|$ where we use $\cdots$ to indicate comparison between the absolute values of the components of the stress tensor. The assumption is justified by the fact that the timescale associated with cell intercalations is much longer than the relaxation time of the system following laser ablation.
Chapter 5

Summary

We have described a model for studying the dynamics of epithelial cells in monolayers based on the vertex model \[43\]. Epithelial cells communicate and cooperate to move in collective patterns which are of central importance of wound healing and morphogenic processes \[17\]. The Active Vertex Model is implemented efficiently which allows for simulations of systems containing tens of thousands of cells on a single CPU core. Because the AVM retains a cell-level description of the monolayer is an appropriate tool for investigating how coordinated behaviours at the cell level are experienced on lengthscale of thousands of cells. In addition, the AVM is also able to handle multiple cell types and type specific cell contacts, which allows simulations of mechanically heterogeneous systems, Section \[3.3\].

The model has been used to reproduce the transition between fluid-like and solid-like states described by Bi et. al. \[70\], Section \[3.1\]. When assessing this transition between states the boundary conditions were shown to be important, as they are for many other studies \[22, 78\]. One direction of future work is to improve boundary handling to allow holes to form or patches of cells break apart and reform.
Activity is introduced in the AVM in a rudimentary way – by assuming that cells self-propel in the direction of their polarity vector. In future work this aspect of the model should be further developed with attention to the details of specific biological systems. We discuss in Section 2.6 with reference to specific examples of biological systems, how our model could be improved.

In Chapter 3 we demonstrate the capabilities of our implementation with a number of examples intended to show the diversity of applications. Including growth of cells in confined environments [78] and studies of cell compartments, Sections 3.3 and 3.4. We hope that the AVM will provide a useful and complementary tool for probing the aspects of the epithelial tissue mechanics that are not available to other methods, as well as serve as an independent validation for the results obtained by other methods.

Finally we derive an expression for the Virial stress in the AVM. We demonstrate the importance of choosing an appropriate lengthscale for smoothing out the individual contributions of each cell to obtain an approximately continuous stress field. The calculation needs only slight modifications to be generally applicable to any vertex model and not just our active vertex model which uses Voronoi cells. Stress in epithelial monolayers is important for understanding collective migration patterns [33] and other processes [85, 83, 84].
Bibliography


