Mathematical modeling of cell adhesion and its applications to developmental biology and cancer invasion

CELL MECHANICS: FROM SINGLE SCALE-BASED MODELS TO MULTISCALE MODELLING

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10th December 2008

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Chapter 1

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Abstract Cellular adhesion is a key factor in many biological processes. Interactions of adhesion molecules at the molecular scale lead to cell rearrangements at the cellular scale and these may generate macroscopic patterns at the tissue scale. A multitude of discrete and continuous models of cell adhesion have been proposed which take into account effects at the various scales. Such models are reviewed and then a continuous model of cell adhesion [N.J. Armstrong et al., *J. Theor. Biol.*, (243), 98–113, 2006] is discussed in more detail. This model captures molecular and cellular scale effects in an integral (non-local) term defining a cell velocity due to adhesive effects. This velocity is then employed to drive rearrangements of cell densities at the tissue scale in an advection-diffusion-reaction system. The application of this framework to successfully model effects as observed in cell sorting experiments and cancer cell invasion demonstrates the suitability and generality of the approach. Analytical and numerical challenges of the framework are discussed and possible extensions are outlined.

Keywords Cell adhesion – Mathematical model – Pattern formation – Cancer Invasion – Morphogenesis

[1]AG gratefully acknowledges financial support by the Division of Mathematics, University of Dundee during a long-term visit in 2007 introducing him to the topic.

[2]KJP is supported in part by the Integrative Cancer Biology Program Grant CA113004 from the US National Institutes of Health, by a BBSRC grant BB/D019621/1 to the Centre for Systems Biology at Edinburgh and by the Mathematical Biosciences Institute, Ohio State University, Columbus, Ohio.

1.1 Introduction

From the earliest embryonic stages through to the complexity of the adult, the ability of cell populations to adhere with either each other or the surrounding extracellular matrix (ECM) is of critical importance to the survival of the organism. During embryonic development, carefully regulated adhesion plays a fundamental role directing the various cell populations into the developing organs while maintaining strong adhesive contacts is essential in preserving the integrity and structure of the adult tissues. The manifest importance of cellular adhesion is exposed due to its abnormal functioning in a wide variety of pathological conditions including malignant cancer growth (e.g. Mareel and Leroy [2003]) and cardiovascular diseases (e.g. Hillis and Flapan [1998]).

Adhesion can generally be classified into two principal forms, *cell-cell adhesion* and *cell-matrix adhesion*. The former defines the direct binding between cells through the creation of transmembrane protein-protein complexes, the prototype example of which are the strong contacts maintaining epithelial structures such as the epidermal skin layer. The latter describes the attachment of cells to the surrounding ECM, the scaffold support surrounding cells composed of a variety of molecules including collagens, fibronectins and laminins. While the ECM is present in all tissues, its prevalence in connective tissues such as the dermal skin layer makes cell-matrix adhesion particularly important for stromal populations such as fibroblasts and immune cells.

The control of cell-cell and and cell-matrix adhesion is fundamentally determined through the expression and regulation of a wide variety of membrane-based proteins, the cell adhesion molecules (CAMs); for a general review see Alberts et al. [2002]. Four principle families of CAM have been classified: the cadherins (e.g. E-cadherin, N-cadherin), the immunoglobin superfamily (e.g. NCAM, EpCAM), the integrins and the selectins. Members of these families generally consist of transmembrane molecules with an intracellular domain linking to intracellular signalling pathways and an extracellular domain connecting to other cells or the matrix. Adhesion is achieved through the protein-protein coupling of the extracellular domain to form either *homophyllic* interactions (i.e. binding between two proteins of the same type, such as E-cadherin – E-cadherin) or *heterophyllic* interactions (binding between two molecules of different type).

The cadherins form a large family of transmembrane adhesion molecules widely recognised for their capacity to mediate direct cell-cell adhesion, although their function extends to a host of other cellular processes ranging from apoptosis to signalling (for reviews on the behaviour and function of cadherins, see Patel et al. [2003], Gumbiner [2005]). Classic cadherins tend to form homophyllic interactions in the intermembrane space separating two cells, although heterophyllic interactions can also occur (e.g. E-cadherin – P-cadherin), albeit with different adhesive intensity [Duguay et al., 2003]. The transmembrane binding fastens cells in a zipper-like manner, conferring a key role to cadherins in all aspects of an organisms lifespan, from coordinating multicellular tissue movements during development to maintaining the tissue structure of the adult. A wide variety of cadherins have been identified, distributed across different cell populations. For example, the E-cadherins are mainly associated with epithelial cell populations, while more migratory, mesenchymal cells (e.g. fibroblasts) tend to favour N-cadherins [Wheelock et al., 2008].

The integrins form the dominant CAMs regulating adhesion to the extracellular matrix [Berrier and Yamada, 2007]. The extracellular domain couples the cells to ligands of the ECM to create various types of cell-matrix adhesion structures which, in turn, modulate

the intracellular component to interact with intracellular signalling. These adhesion structures have the capacity to recruit additional molecules, for example matrix proteases, and therefore locally alter the structure of the ECM. Dynamic control of cell-matrix adhesion is crucial to the migration of cells in ECM rich environments, such as connective tissue, where migration proceeds through a continuous cycle of attachment at the leading edge, extension and translocation of the cell body and detachment at the cell rear (e.g. Friedl and Wolf [2003]). Consequently, the structure of the ECM plays a significant role in directing migration: certain cells may migrate towards ligand-dense (i.e. more adhesive) regions of the matrix, a process termed *haptotaxis*, towards more rigid regions, *durotaxis* [Lo et al., 2000], or even along the aligned collagen fibres, *contact guidance* [Dunn and Heath, 1976].

1.1.1 Cell adhesion during pattern formation and development

In a series of classical experiments, Townes and Holtfreter [1955] demonstrated the intrinsic capacity for certain embryonic cell populations, when dissociated and randomly mixed, to spontaneously reorganise into their original embryonic relationship, a process attributed at the time to "tissue-affinity". The underlying mechanism(s) governing this "cell-sorting" have been the subject to a significant degree of speculation and experimentation over the years, with the Differential Adhesion Hypothesis (DAH) of Steinberg (see the reviews of Foty and Steinberg [2004], Steinberg [2007]) to the fore of theories. The series of experiments by Steinberg in the 1960s [Steinberg, 1962a,b,c] demonstrated that embryonic cell types obey strict rules: whatever the initial distribution for two separate populations was, the cells always rearranged into the same configuration, Fig. 1.1 (a). Furthermore, populations formed hierarchical relationships: if cells of type B are engulfed by cells of type A and cells of type C are engulfed by cells of type B, then C will always be engulfed by A, see Fig. 1.1 (b).

Based on these observations, the DAH employes thermodynamic principles, proposing that cell sorting derives from variation in cell surface tensions which, in turn, depend on the different adhesive properties between the cell types: cells are assumed to rearrange in a manner to minimize their free adhesive energy, analogous to the behaviour of two immiscible liquids. Through these arguments, a mixture of two cell populations, A and B, can be predicted to rearrange into four basic configurations according to the relative strengths of self adhesion (i.e. the binding between 2 cells of same type, S_{AA} and S_{BB}) and cross adhesion (i.e. binding between 2 cells of different type, C_{AB}): "mixing", "engulfment", "partial engulfment" and "complete sorting", see Fig. 1.1 (c).

Over the last decade or so, a series of thorough experiments have substantiated the DAH for sorting (see the reviews of Foty and Steinberg [2004], Steinberg [2007] for further details). Experiments with two cell lines expressing different levels of cadherins (and hence varying degree of adhesiveness) resulted in the population expressing higher cadherin levels aggregating to the centre with the other line confined to the periphery, consistent with the predictions of the DAH [Steinberg and Takeichi, 1994, Duguay et al., 2003]. Recent experiments of Foty and Steinberg [2005] have directly linked the surface tensions underlying sorting of tissues to differing strengths of cell-cell adhesion.

The capacity for differential adhesion to spatially sort out different populations implies an important role during the morphogenetic patterning of the embryo; indeed, examples of spatio-temporally controlled alterations to the adhesive properties of cells and the matrix include the whole-embryo tissue movements during gastrulation, the formation of the bound-

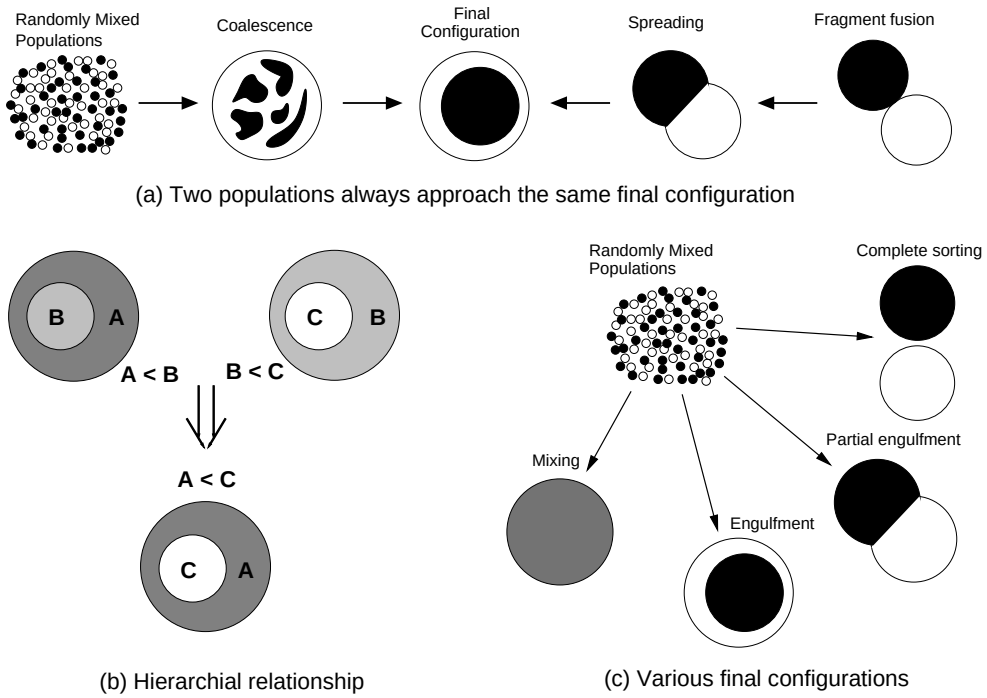

Figure 1.1: Sketches showing the behaviour of two adhesive cell populations, as predicted by the DAH. (a) The same populations always approach the same final configuration, regardless of initial distribution. Starting from left, populations of mixed and dissociated cells coalesce before evolving to a final configuration (shown here as "engulfment"). Starting from right, the same two populations, when placed together as fragments, spread over one another before reaching the same pattern. (b) Hierarchical relationships in adhesive populations. (c) Two populations, A and B evolve into various final configurations according to their self adhesion S_{AA}, S_{BB} (between A and A, between B and B) and cross adhesion C_{AB} (between A and B) strengths. For two populations, the observed patterns are *mixing* (in which the populations are uniformly distributed – requires dominant cross adhesion $C_{AB} > \frac{S_{AA}+S_{BB}}{2}$), *engulfment* (in which the more cohesive population is engulfed by the less cohesive population – requires or $S_{AA} < C_{AB} < S_{BB}$), *partial engulfment* (for which the cross adhesion strength is less than both the self adhesion strengths – $C_{AB} < S_{AA}$ and $C_{AB} < S_{BB}$) and *complete sorting* (for which $C_{AB} = 0$ and the two populations form separate aggregations). Figures adapted from Foty and Steinberg [2004].

aries during segmentation of the hindbrain and the precisely controlled movements of differentiated cell types during the patterning of the insect compound eye (for these and further examples see Takeichi [1995], Tepass et al. [2002], Keller [2002], Gumbiner [2005], Carthew [2007], Steinberg [2007]). In segmentation, the embryo is subdivided into a number of discrete blocks along the anterior–posterior axis, laying a blueprint for future development. For a number of organisms, including birds, fish and mammals, this proceeds through *somitogenesis*, in which parallel stripes of mesenchymal tissue (the paraxial mesoderm) metamerically pinch off to form somitic pairs either side of the developing neural tube. Compaction from the mesenchyme into epithelialised somites is thought to arise through increases to cell adhesion [Cheney and Lash, 1984] with a number of studies indicating roles for both matrix molecules such as fibronectin (e.g. Ostrovsky et al. [1983], Duband et al. [1987]) and cadherins (e.g.

Takeichi [1988], Linask et al. [1998]). Somites undergo subdivision first into distinct anterior and posterior portions (e.g. Pourquié 2001) before they subsequently sort into further embryonic subpopulations [Bagnall et al., 1989]. Differential cell-cell adhesion has been suggested to pattern somites into their anterior and posterior segments [Stern and Keynes, 1987], a theory strengthened by the distribution of various cadherins in the developing somite (e.g. Duband et al. [1987], Horikawa et al. [1999]).

1.1.2 Cell adhesion in cancer invasion

Understanding the processes regulating the control of adhesion during tissue development and homeostasis is crucial when it comes to determine the factors leading to tumour progression. The transition from a benign, compact tumour to an invasive, spreading tumour capable of forming metastases is a pivotal moment for prognosis and it is now widely accepted that modifications to the adhesive properties of the cells and surrounding ECM correlate with malignant development for a wide range of different cancer types (e.g. Christofori [2003], Mareel and Leroy [2003], Cavallaro and Christofori [2004]).

For many tumours of epithelial origin, a link between increased malignancy and progressive loss of function in the cell-cell adhesion molecule E-cadherin has been observed [Christofori, 2003], with forced expression of E-cadherin in cultures resulting in a reversal from an invasive to benign phenotype (e.g. Birchmeier [1994]). In a number of cancers, the loss of E-cadherin is accompanied by a gain in N-cadherin expression, a "cadherin-switching" mechanism [Wheelock et al., 2008] similar to those seen in various embryonic processes, for example ingression of cells through the primitive streak. Such transitions are believed to give rise to evolution to a more invasive/migratory form.

To infiltrate surrounding healthy tissue it is necessary for the tumour cells to interact with the surrounding ECM, a structure which can both provide a substrate through which cells can move as well as a physical barrier against migration. To migrate, cells must attach to the matrix through the formation of focal adhesions, mediated through the integrin family of CAMs. These focal adhesions provide a site for recruiting matrix proteases (e.g. MMPs) which degrade the ECM and hence provide space for tumour invasion and expansion to occur [Friedl and Wolf, 2003]. A wide number of *in vitro* and *in vivo* studies have investigated the importance of integrins and MMPs for cancer cell invasion, yet the precise impact on invasion (for example, promoting or inhibiting) varies widely according to cancer origin.

The form of the invasive front is also variable, with different tumours displaying diverse patterns of invasion, often resulting in an indistinct and diffuse tumour-host tissue interface [Friedl and Wolf, 2003]. Certain tumours (e.g. lymphomas, glioblastomas) tend to invade as individual cells, occasionally forming single-file cell chains known as "indian-chains" (e.g. in breast carcinomas). Other tumour types, particularly those of epithelial origin, tend to invade in a collective fashion in which multicellular strands of tumour cells known as "fingers" protrude into the host tissue or cell clusters migrate out from the tumour while maintaining close contacts. Once again, these distinct patterns of invasion correspond to different patterns of CAM expression with the individual-cell migration phenotypes, typified by mesenchymal/amoeboid cell types, expressing high levels of integrins and proteases while collective-cell invasion is characterised by epithelial cell types with strong cell-cell adhesion.

1.1.3 Chapter outline

Clearly, cellular adhesion plays a crucial role in many biological processes. While a wide range of models have incorporated adhesion at the discrete-level, the incorporation into continuous models has received relatively little attention, a fact that can mainly be attributed to a lack of models able to replicate the characterstic behaviours of adhesive populations. In this chapter, we first explore the history of modelling in this fundamental process. We proceed to review the derivation of the continuous model for cell-cell adhesion developed in Armstrong et al. [2006] and show how it captures the fundamental properties of aggregation and cell-sorting. In Section 1.5 we consider an application of this model to tumour invasion [Gerisch and Chaplain, 2008, Sherratt et al., 2009]. Finally, we raise a number of biological, modelling, analytical and numerical challenges stimulated by these works.

1.2 Mathematical modelling of cell adhesion

The recognition of cellular adhesion as a major driving force behind various biological processes has led to the development of a variety of modelling approaches and models. Naturally, the structure of a model will inevitably depend on the precise biological question to be addressed. However, it is reasonable to expect that for any model of cell-cell adhesion, at a *population* level it should capture core properties, such as an ability to predict the aggregation/coalescence of a population as the "adhesivity" of the cells is increased and, when expanded to include multiple populations, the various sorting properties predicted by the DAH. The mechanism of cell-cell adhesion—a non-local interaction between two cells through transmembrane receptor binding—naturally suggests the usage of discrete cell (i.e. individual cell or agent-based) approaches, which retain the finite cell size and permit relatively straightforward incorporation of the molecular interactions and/or forces that act between the cells. Weighed against such advantages, however, are the significant computational times required to simulate large populations and difficulties in obtaining analytical insight. Consequently, it is desirable to augment such methodologies with continuous models that capture the dynamics of population level behaviour.

1.2.1 Discrete models for cell adhesion

The last decade has witnessed the development of a wide variety of discrete models that incorporate cell adhesion and are of increasing sophistication. Generally, such models can be classified into two major classes: lattice-based and lattice-free approaches. We start this section with a brief discussion of a number of discrete lattice-based models, the book by Deutsch and Dormann [2005] reviews these models in greater detail with a specific focus on cellular adhesion in its Chapter 7, and consider cellular automaton, the discrete-continuum technique and Cellular Potts models.

In lattice-based approaches, the morphology of a cell is restricted according to some underlying discretisation of space, which can be either regular (for example, rectangular or hexagonal in two dimensions) or irregular (for example, a voronoi tessellation). These approaches can generally be further subclassified into those for which one cell correlates to one lattice site and spatially-extended approaches, with a cell defined by a connected set of sites.

Examples of the former class include many cellular automata models: for the evolution of cells under the influence of differential adhesion, see e.g. [Deutsch, 1999]; in Moreira and Deutsch [2005] a similar approach was employed to demonstrate how different adhesive properties can generate zebrafish pigmentation stripes. A second example of the single-site class is the discrete-continuum technique developed by Anderson and co-workers [Anderson, 2005, Anderson et al., 2006]. Here the discrete cells interact with each other and surrounding continuous fields representing extracellular matrix densities and growth factor concentrations. Movement probabilities are derived from these interactions, which include adhesion of cells to the extracellular matrix, and drive the reorganisation of the cell pattern in space and time. The primary application of this technique has been in models of tumour cell invasion.

A prime example of a spatially-extended approach is the Cellular Potts Model (or Glazier-Graner-Hogeweg model). Originating in theoretical physics, it was adapted and applied to cell populations by Graner and colleagues in the 1990s, e.g. [Graner and Glazier, 1992, Glazier and Graner, 1993]. Here each (biological) cell is of a certain cell type and represented as a number of sites (vertices) of a regular lattice. For a given state of the system, a Hamiltonian function is defined based on the surface energy along the cell boundaries and deviations of cell sizes from typical values. The evolution of the system is then driven by a Monte Carlo like scheme which aims at reducing the value of the Hamiltonian by changing the cell association of a randomly chosen lattice site to that of one of its neighbouring sites. The surface energy depends on the cell types on either side of the cell surface and consequently accounts for self and cross adhesive effects. A background medium, e.g. representing the extracellular matrix, can also be included in the model. The generic model structure of this Potts model has been elaborated on by various authors to make it suitable for particular application areas, e.g. cellular slime mould morphogenesis [Maree and Hogeweg, 2001], vertebrate development [Popawski et al., 2007], epidermal homeostasis [Savill and Sherratt, 2003], solid tumour growth [Turner and Sherratt, 2002] and angiogenesis [Bauer et al., 2007].

The artificiality of the imposed grid can be countered through the adoption of a lattice-free approach, in which individual cells are allowed to move freely through continuous space. In a number of models of this type, cells are given variable, yet pre-defined, shapes such as deformable ellipsoids of fixed volume in a model for cell movement of *Dictyostelium discoideum*, [Palsson and Othmer, 2000, Dallon and Othmer, 2004, Palsson, 2008]. Another option, which allows for cell growth and division, is that the *average* cell shape at any point in the life cycle of a cell is predefined while the *actual* cell shapes are reconstructed from that by taking neighbouring cells into account. This approach, introduced by Drasdo et al. [1995], is followed in models of tumour growth, epidermal homeostasis, and early development; for a brief review and further references we refer to [Galle et al., 2006]. One recent extension of this approach has been the incorporation of intracellular and transmembrane molecular interactions, courtesy of an ordinary differential equation system for each cell that describes the regulation of E-cadherin through the β-catenin signalling pathway [Ramis-Conde et al., 2008]. In both these and the deformable ellipsoid model described above, movement of individual cells is driven by equilibrating forces, including adhesive ones; alternatively, as in [Drasdo et al., 1995] movement is governed by a Monte-Carlo algorithm based on a suitable interaction potential.

A number of further lattice-free models provide even greater flexibility to the manner in which cells refine their shape. The model of Schaller and Meyer-Hermann [2005] adopts a Voronoi-Delaunay method, permitting cells to shift between smoothly spherical and polyhedral with increasing tissue density, thereby providing greater control over the amount of cell-cell contact. The subcellular element model of Newman [2005] provides additional intracellular structure through subdividing each cell into a set of continuously deforming

elements, giving high malleability to the shape of a cell according to its interactions with neighbours and the environment. Finally, in the immersed boundary models for individual cells [Rejniak, 2007, Dillon et al., 2008] each cell is described as a fluid-elastic structure in which its membrane is represented by a deformable boundary immersed in a fluid. Force balances again are used to represent the adhesive forces that describe the movement and deformation of cells while channels at the membrane permit the influx of fluid into the cell required for growth.

1.2.2 Continuous models incorporating cellular adhesion

While discrete models for cells permit the straightforward incorporation of many intra-, extra-, and inter-cellular processes, they also have their drawbacks. Of particular concern is that the transition from the cellular to the tissue scale can require a formidable number of cells which in many models, and certainly for the more detailed ones, is computationally infeasible. In addition, discrete models often resist a thorough analytical investigation which can shed light on generic properties of the system under study. Both these issues can be relaxed by considering continuum scale (PDE) models where cells are represented through their density at the tissue level and events at the cellular level are accounted for by the particular choice of terms and parameter functions in those models. In the following we briefly review some continuous models which account for cellular adhesion.

The modelling of cell-extracellular matrix adhesion has been phenomenologically captured in a number of models through the idea of "haptotactic" migration (e.g. Anderson et al. [2000]). Here, cells are assumed to migrate up gradients in the density of an extracellular matrix through the incorporation of an advective-flux type term qualitatively the same to those traditionally employed in continuous chemotaxis models (e.g. Hillen and Painter [2008]).

Incorporation of cell-cell adhesion, however, has proved generally to be problematic at a continuous level. One approach, adopted in a number of models, e.g. [Hofer et al., 1995], has been to include cell-cell adhesion through a density-dependent cell diffusion coefficient. While this phenomenologically captures one aspect of adhesion, i.e., the restricted movement of cells in regions of high density, its capacity to describe more complex phenomena such as self aggregation and sorting of multiple populations is unknown. Byrne and Chaplain [1996] presented a model of cancer growth and invasion which accounts for cell-cell adhesion through the incorporation of a surface tension force at the tumour surface controlling the evolution of the tumour shape during growth. This idea has been taken up and extended in recent models [Cristini et al., 2003, Macklin and Lowengrub, 2007]. The single-phase approach in these models has been broadened to multi-phase using a diffuse interface framework in [Wise et al., 2008]. This model accounts for cell-cell and cell-matrix adhesive effects by incorporating them into a system energy which drives the system following an energy variation scheme. The non-local energy term is assumed to be sufficiently localised and the corresponding truncated expansion of that term leads to a fourth-order PDE model of Cahn-Hilliard type.

The modelling approach of Armstrong et al. [2006], which will be the focus of this chapter, also employs non-local terms to account for adhesive effects. In contrast to [Wise et al., 2008] no expansion of these terms is performed so that the resulting model equations are non-local or integro PDEs of second order. This approach has been employed to show that upregulated adhesion can drive both the formation and subsequent anterior-posterior compartmentalisation of somites [Armstrong et al., 2008] and, as we expand on below, incorporated into

models for tumour invasion [Gerisch and Chaplain, 2008, Sherratt et al., 2009].

A highly desirable objective is to develop continuous models for cellular adhesion as the appropriate limit from an underlying individual model for cell movement; in the case of chemotactic cell movement, this has been studied in detail (see Hillen [2002] for a review) and an obvious advantage lies in the determination of the macroscopic parameters (such as diffusion coefficients and chemotactic sensitivities) in terms of measurable microscopic parameters (e.g. cell velocities, turning rates). A number of recent attempts have been made to approach this problem. In Turner et al. [2004] a 1D representation of a Cellular Potts Model incorporating adhesion was taken, under specific scaling arguments, to its continuous limit, yet the resulting model is relatively unwieldy and it has not been shown whether sorting properties can be captured. Another approach adopted is to consider the evolution of a particle executing one-step jumps on a discrete lattice (e.g. Painter et al. [2003], Anguige and Schmeiser [2008]). While these models can capture self aggregation of a population, the ill-posed nature of the resultant continuum equations can create singular behaviour. Finally, Newman and Grima [2004] consider the limit of a Langevin-based individual model. Interestingly, the resulting continuum model incorporates non-local terms similar to those of the phenomenological model of Armstrong et al. [2006], described below.

1.3 Derivation of a non-local model for cell adhesion

We begin by reviewing and extending the phenomenological derivation for an integro-partial differential equation model for cell-cell adhesion first developed in Armstrong et al. [2006]. Here, a mass conservation approach was employed in which the cell density for an adhesive cell population $u(\mathbf{x}, t)$ ($\mathbf{x} \in \mathbb{R}^n$) was proposed to be governed by:

$$\frac{\partial u(\mathbf{x}, t)}{\partial t} = -\nabla \cdot \mathbf{J} + h(\cdot) \tag{1.1}$$

where $\mathbf{J}$ represents the cell flux and $h(\cdot)$ describes cell kinetics. A multitude of factors are known to dictate cell movement *in vivo*, ranging from long range chemoattractants to local cell-cell and cell-ECM interactions, indicating a flux of the form

$$\mathbf{J} = \mathbf{J}_{\text{random}} + \mathbf{J}_{\text{adhesion}} + \mathbf{J}_{\text{taxis}}\,, \tag{1.2}$$

where $\mathbf{J}_{\text{random}}$ is the flux due to "random cell movement" (typically modelled as a Fickian diffusion, $\mathbf{J}_{\text{random}} = -D_u \nabla u$, where D_u is the cell diffusion coefficient), $\mathbf{J}_{\text{adhesion}}$ is the flux due to adhesion and $\mathbf{J}_{\text{taxis}}$ is the flux due to long-range substances such as chemoattractants. For the latter, the classical assumption is to take $\mathbf{J}_{\text{taxis}} = u\chi(u, c)\nabla c$, where c represents the chemoattractant concentration and the function χ is referred to as the chemotactic sensitivity (Keller and Segel 1970, Hillen and Painter 2008).

To model the contribution of adhesion to the cell flux, $\mathbf{J}_{\text{adhesion}}$, we assume movement occurs due to the forces generated when cells bind with other cells or the surrounding matrix, the density of which we denote by $m(\mathbf{x}, t)$. For a cell at $\mathbf{x}$, binding with a cell at $\mathbf{x}+\mathbf{r}$ will create a *local* force $\mathbf{f}$ in the direction $\overrightarrow{\mathbf{r}}$ (equally, the cell at $\mathbf{x}+\mathbf{r}$ experiences the opposite force). To describe adhesion-based movement, we assume the size of this local force depends on the "adhesivity" of this site, namely the numbers and types of adhesion molecules. Rather than explicitly modelling the concentrations of such molecules, the adhesivity is taken to simply depend on the cell density (indicating the likelihood of forming a cell-cell bond) and the

matrix density (indicating the likelihood of forming a cell-matrix bond) at $\mathbf{x}+\mathbf{r}$ through the function $g(u(\mathbf{x}+\mathbf{r},t), m(\mathbf{x}+\mathbf{r},t))$. Note that the density of additional cell types can be included here, allowing for cross adhesion between cell types. The possibility of a cell at $\mathbf{x}$ forming a bond at $\mathbf{x}+\mathbf{r}$ is further expected to depend on the distance between the two sites: cells establish adhesive bonds at the membrane–substrate interface, yet their capacity to change shape (e.g. become elongated) or extend thin cell protrusions ranging from shorter range lamellipodia to longer range filopodia (occasionally up to 100 μm in length, Wood and Martin 2002) suggests that the probability of forming bonds may vary with distance.

Together, these assumptions lead us to propose the local force generated at $\mathbf{x}$ via adhesive binding at $\mathbf{x}+\mathbf{r}$ to be

$$\mathbf{f}(\mathbf{x},\mathbf{r}) = \frac{\mathbf{r}}{|\mathbf{r}|}\Omega(|\mathbf{r}|)g(u(\mathbf{x}+\mathbf{r},t), m(\mathbf{x}+\mathbf{r},t))\,,$$

where the right hand side terms break down into the direction of the force (a vector), the dependence of the force magnitude on the distance at which bonds are formed, Ω (a scalar), and the dependence of the force on the adhesivity, g (a scalar). We discuss various functional forms for these terms below.

The *total* force exerted at $\mathbf{x}$, $\mathbf{F}(\mathbf{x})$, will be the sum of all local forces, computed over a finite volume $\mathbf{x}+V$ indicating the "sensing region": the space over which the cell at $\mathbf{x}$ can make adhesion bonds. As described above, this V is minimally determined by the mean cell volume, yet is likely to be significantly larger due to cell shape change and protrusions. Thus, we compute the total force to be

$$\mathbf{F}(\mathbf{x}) = \int_V \frac{\mathbf{r}}{|\mathbf{r}|}\Omega(|\mathbf{r}|)g(u(\mathbf{x}+\mathbf{r},t), m(\mathbf{x}+\mathbf{r},t))\,d\mathbf{r}\,. \tag{1.3}$$

To incorporate the above into the mass balance equation (1.1), we note that at the low speeds of eukaryotic cell migration (typically 0.1-10 μm/min, according to cell type) we can reasonably expect inertia to be negligible and drag proportional to velocity and the cell radius R (Stokes law for a ball of radius R in a laminar flow). The adhesive flux will then be proportional to the cell density and the forces between them and therefore we take

$$\mathbf{J}_{\text{adhesion}} = \frac{\phi u}{R}\mathbf{F} \tag{1.4}$$

where ϕ is a constant of proportionality. Finally, we substitute Eq. (1.4) with $\mathbf{F}$ as given in (1.3) into Eq. (1.2), assume Fickian diffusion and a generic taxis cue $c(\mathbf{x},t)$ to obtain the following cell density evolution equation

$$\begin{aligned}\frac{\partial u(\mathbf{x},t)}{\partial t} &= \overbrace{D_u\nabla^2 u}^{\text{Random movement}} - \overbrace{\nabla\cdot(u\chi(u,c)\nabla c)}^{\text{Taxis movement}} \\ &\quad - \overbrace{\nabla\cdot\left[\frac{\phi u}{R}\int_V \frac{\mathbf{r}}{|\mathbf{r}|}\Omega(|\mathbf{r}|)g(u(\mathbf{x}+\mathbf{r},t), m(\mathbf{x}+\mathbf{r},t))\,d\mathbf{r}\right]}^{\text{Adhesive movement}} + \overbrace{h(\cdot)}^{\text{Cell kinetics}}\,.\end{aligned} \tag{1.5}$$

The above forms our basic model for cell adhesion which, when combined with appropriate dynamics for matrix and chemical signalling, can be applied to a wide range of biological

processes: a version of the above equation was first considered by Armstrong et al. [2006] to model the basic properties of an adhesive population and, through the incorporation of an extra adhesive population, extended to model cell sorting (see Section 1.4); an amalgamation of Eq. (1.5) into a chemical signalling system has been developed to model somite formation during embryonic development (see Armstrong et al. [2008]); the incorporation into the modelling of tumour invasion has been considered by Gerisch and Chaplain [2008] and Sherratt et al. [2009] (see Section 1.5).

1.3.1 Cohesion through adhesion

A fundamental test for any model for cell-cell adhesion is to determine its capacity to predict the organisation of a population of dispersed cells into aggregations: populations of cell lines aggregate rapidly into large and cohesive clumps with increasing cadherin expression (e.g. Foty and Steinberg 2005). To demonstrate the ability of Eq. (1.5) to allow this basic phenomenon, we neglect any effects from cell-matrix adhesion and chemoattractants and ignore cell kinetics (i.e. cell growth is assumed to be negligible on the timescale of adhesion-driven movement) to derive:

$$\frac{\partial u(\mathbf{x},t)}{\partial t} = D_u \nabla^2 u - \nabla \cdot \left[\frac{\phi u}{R} \int_V \frac{\mathbf{r}}{|\mathbf{r}|} \Omega(|\mathbf{r}|) g(u(\mathbf{x}+\mathbf{r},t))\, d\mathbf{r} \right] . \tag{1.6}$$

It remains to define appropriate functional forms for the various components in the non-local term. In the simulations that follow, we restrict to two spatial dimensions and therefore take the cell sensing region V to be the circle of radius R. The function Ω defines the dependence on the distance from $\mathbf{x}$: the simplest assumption is to assume Ω is constant throughout the sensing region, however a form in which Ω decreases due to the diminished likelihood of forming a bond with distance from the cell may be more appropriate. For the purposes here, we adopt the simplest form and take $\Omega(r) =$ constant: the impact of other forms have been considered in Sherratt et al. [2009] for a 1D version of the model.

For the adhesivity component, with *attractive* interactions we expect g to (at least initially) increase with the cell density u due to the increased likelihood of forming bonds within areas of higher cell densities (and hence more adhesion receptors). Yet at even higher cell densities, it is reasonable to expect the attractive force magnitude to either saturate (for example, due to all receptors becoming bound) or even decrease (due to an impedance against migrating into "crowded" regions). To explore the impact from different forms of g, we consider respectively a "linear", a "saturating" and a "logistic" form, all depending on an adhesion parameter α:

$$g(u) = \alpha u\,, \qquad g(u) = \tfrac{\alpha u}{K+u}\,, \qquad g(u) = \alpha u \max\left\{0, 1 - \tfrac{u}{U_{\max}}\right\} . \tag{1.7}$$

We have solved Eq. (1.6) for each functional form of g from (1.7) on a square domain $\Omega = (0,10)^2 \subset \mathbb{R}^2$ with periodic boundary conditions. The initial cell density $u(\mathbf{x},0) = 0.1 + \mathcal{U}(\mathbf{x})$ is constant with a uniformly distributed perturbation $\mathcal{U}(\mathbf{x}) \in 10^{-2}[-0.5, 0.5]$. The sensing region V is a circle of radius one and the other parameters used are

$$D = \phi = R = 1\,, \quad \Omega(r) = 1 \text{ for } r \in [0,1]\,, \quad \alpha = 30\,, \quad K = U_{\max} = 2\,.$$

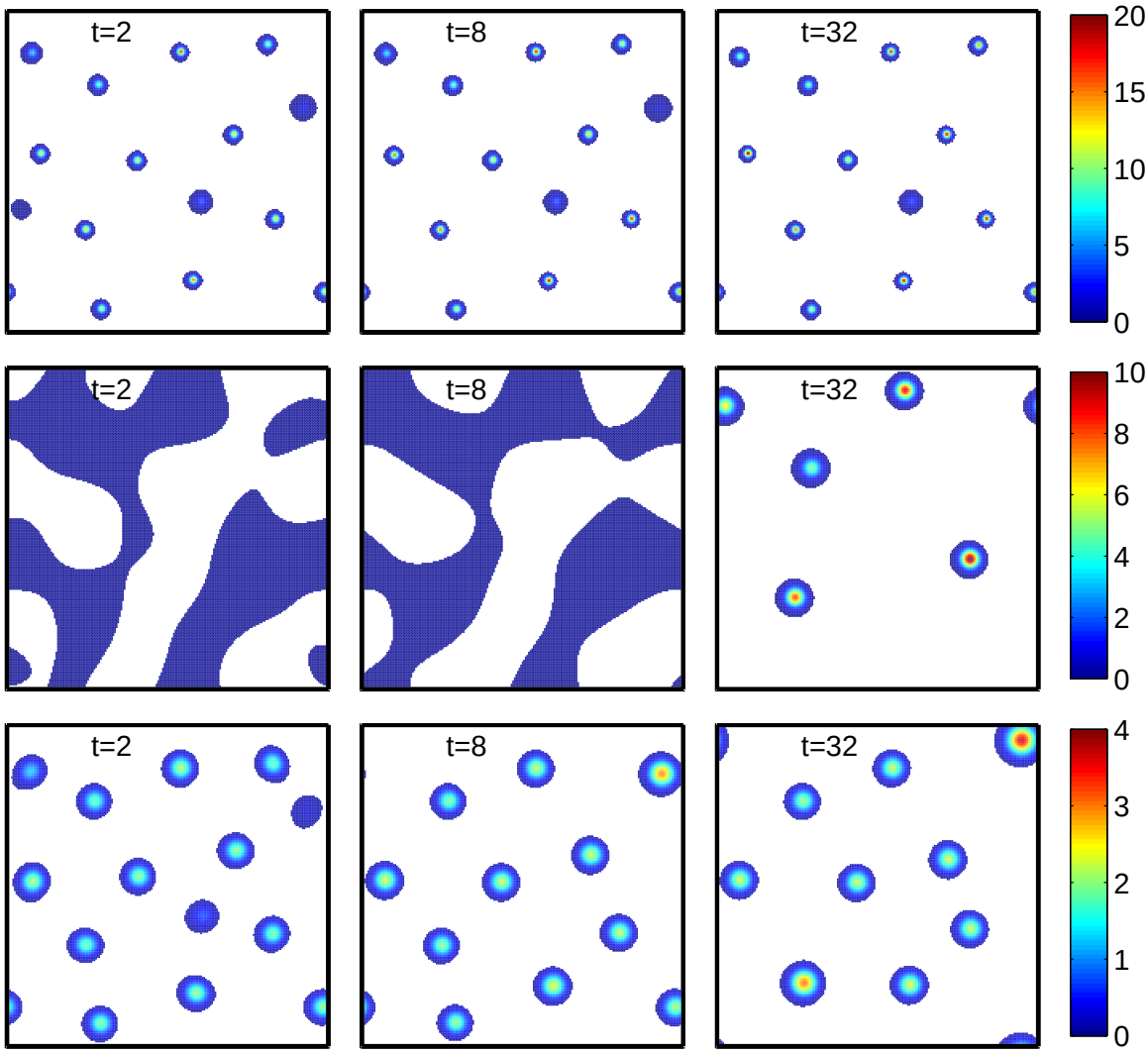

Figure 1.2: Simulation results $u(\mathbf{x}, t)$ for Eq. (1.6) with linear (top row), saturating (middle row), and logistic (bottom row) form of function g, see (1.7), at three output time points t. Note the different colour scalings for the three functional forms of g; values of u below 0.1 are suppressed in the plot.

The numerically computed cell density $u(\mathbf{x}, t)$ at three output times t is shown in Fig. 1.2. With the setting described above, we observe aggregation of cells for all three functional forms of g given in (1.7). With the linear form of g we obtain a very fast aggregation process leading to many small cell clusters with large cell density in the region of up to 20. As time proceeds, some of these clusters coalesce leading to a further increase in cell density, see Fig.1.3. The diffusion in the model prevents a further increase (also the finite grid width contributes to this; on finer grids the maximum solution value becomes even larger). With the saturating form of function g the onset of aggregation becomes visible only much later than with the other two forms. This can be understood from observing that at the low initial

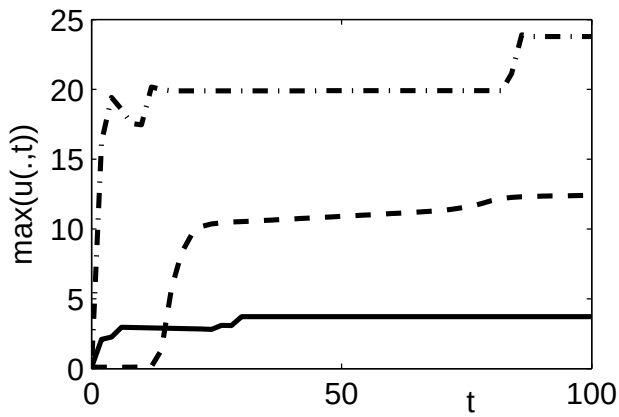

Figure 1.3: The maximum cell density as a function of time for the numerical experiments shown in Fig. 1.2 using a function g which is linear ($- \cdot - \cdot$), saturating ($- -$), or logistic (—). [Maybe also add image about maximum density depending on alpha value (does it stay below $U_{\max}$ for smaller α)?] [Good idea!]

cell densities (≈ 0.1), the saturating form gives $g \sim \alpha u/2$ whereas $g \sim \alpha u$ for the other two functional forms: the adhesive pull driving aggregation is therefore much lower. Once the clusters have formed, a slow but steady increase in the maximum density occurs which only flattens off as the density increases above 10 and the impact of the saturation in g takes hold. Finally, the logistic form for function g leads, like the linear form, to a quick formation of cell aggregates. However, unlike in the linear case, the maximum cell density is much smaller here and appears to be bounded by ≈ 4. This value is larger than the parameter $U_{\max} = 2$. In a reduction of the two dimensional case to a quasi-one dimensional problem, Sherratt et al. [2009] have shown that the density is bounded by $U_{\max} = 2$, provided the adhesion parameter α is below some critical value; consequently this result appears either not to generalise to the genuinely 2D setting or imposes additional constraints on the size of α for boundedness by $U_{\max}$.

Based on the reasonably fast aggregation and the capacity to bound cell densities at lower levels, the choice of the logistic form for function g is recommended and will be considered in the remainder of this paper.

1.4 Modelling cell-cell sorting

In this section we aim to demonstrate whether the continuous framework developed in Section 1.3 can replicate the predictions of the DAH for cell sorting, cf. Fig. 1.1. The prototypical setting here is to consider two cell populations which differ only in their adhesive properties. Initially, the two cell populations are distributed more or less arbitrarily and one is interested in the long-term configuration of the system, see Fig. 1.1. We denote the densities of the two cell populations by $u_A(\mathbf{x}, t)$ and $u_B(\mathbf{x}, t)$. It is reasonable to assume that cell proliferation is negligible on the time scale of cell sorting and we further assume that the random motility coefficient is approximately the same for each population. Under these simplifications we

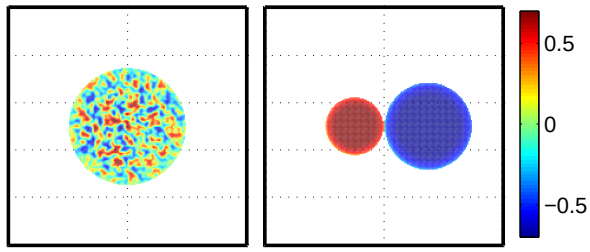

Figure 1.4: Initial cell distributions shown as the difference $u_A(\mathbf{x},0) - u_B(\mathbf{x},0)$; difference values between ± 0.05 are suppressed in the plots [Do you mean when the differences are between... AND that the total cell density is below some critical value? Otherwise, why obtain the green values?]. (left) A pellet of radius 2.5 in the centre of the domain with a random mixture of cells of type A and B such that $u_A(\mathbf{x},0) + u_B(\mathbf{x},0) = 0.8$ in the pellet's centre and slightly decreasing towards the periphery; densities are zero outside the pellet. (right) Two adjacent cell pellets, of radii 1.25 (type A) and ≈ 1.87 (type B), containing one cell type each at a density of ≈ 0.8; densities are zero outside the pellets.

obtain the following set of PDEs describing the spatio-temporal evolution of the system

$$\frac{\partial u_i(\mathbf{x},t)}{\partial t} = D\nabla^2 u_i - \nabla \cdot \left[\frac{\phi u_i}{R} \int_V \frac{\mathbf{r}}{|\mathbf{r}|} \Omega(|\mathbf{r}|) g_i(u_A(\mathbf{x}+\mathbf{r},t), u_B(\mathbf{x}+\mathbf{r},t))\, d\mathbf{r} \right], \quad i = A, B\,. \tag{1.8}$$

This system is considered on the 2D spatial domain $\Omega = (0,10)^2 \subset \mathbb{R}^2$ and complemented with periodic boundary conditions for both species. We consider two sets of initial conditions $u_A(\mathbf{x},0)$ and $u_B(\mathbf{x},0)$, corresponding to the left- and right- most frames of Fig. 1.1 (a): a single pellet of randomly mixed cell types, Fig. 1.4 (left), and a pellet of cell type A juxtaposed to a pellet of cell type B, Fig. 1.4 (right). The initial masses of cell types A and B are approximately equal for the initial condition shown in Fig. 1.4 (left) whereas there is a larger initial mass of cell type B in Fig. 1.4 (right).

The functions g_i in the cell adhesion term are parametrised by the self and cross adhesion parameters, $S_{AA}, S_{BB}, C_{AB} = S_{AB} = S_{BA}$ of the two cell types and we employ a logistic functional form, cf. Sec. 1.3.1, Armstrong et al. [2006], Sherratt et al. [2009],

$$g_i(u_A, u_B) := (S_{iA}u_A + S_{iB}u_B) \max\left\{0, 1 - \frac{u_A + u_B}{U_{\max}}\right\}, \quad i = A, B\,.$$

The contributions $S_{ii}u_i$ account for self adhesion whereas $S_{ij}u_j$ with $i \neq j$ account for cross adhesion. The factor $\max\{0, 1 - \frac{u_A+u_B}{U_{\max}}\}$ is employed to limit the density to which an aggregate can reach, see the effect of the various forms for g in Fig. 1.2; under this form, the adhesive pull of a region increases at lower cell densities before decreasing at higher densities. The sensing region V is a circle with radius one and we use

$$D = \phi = R = 1\,, \quad \Omega(r) = 1 \text{ for } r \in [0,1]\,, \quad U_{\max} = 1\,.$$

Our first test is to demonstrate the capacity of Eqs. (1.8) to predict various final configurations according to the self and cross adhesion parameters, as illustrated in Fig. 1.1.

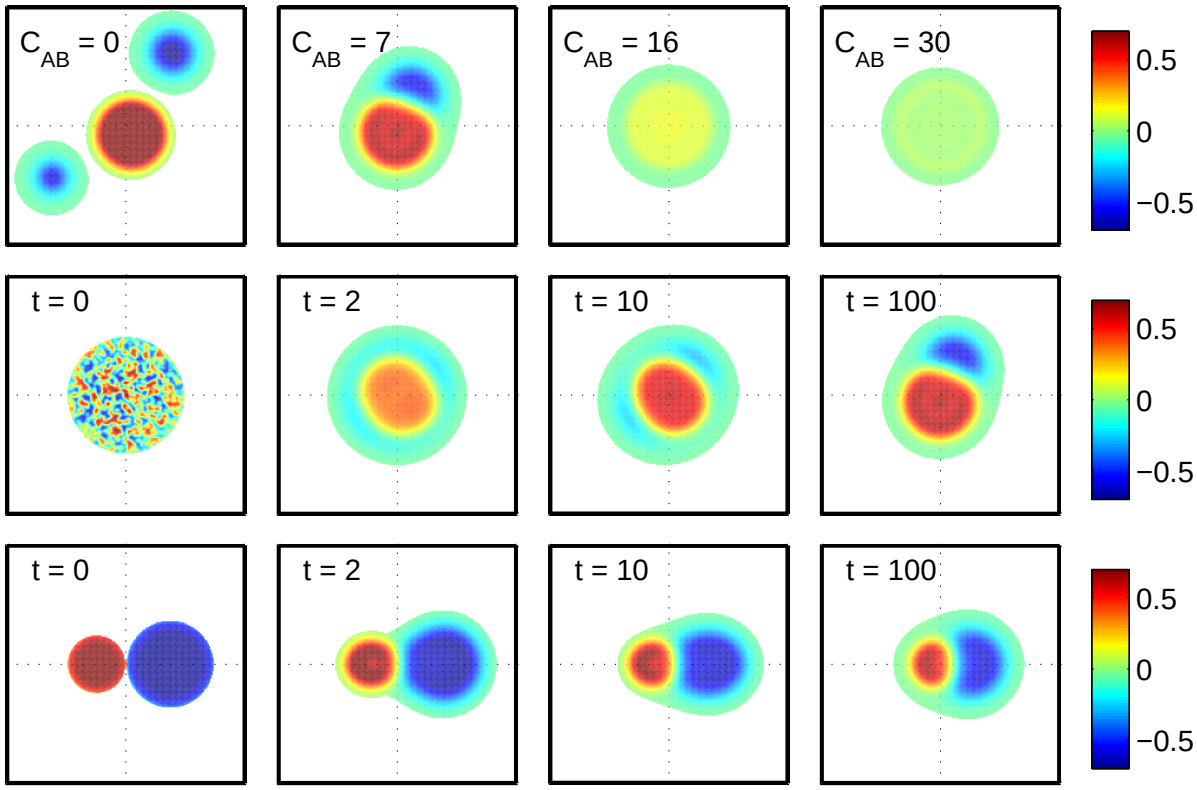

Figure 1.5:

Accordingly, we start with a random mixture of cells of the two types in a pellet centered in the domain, see Fig. 1.4 (left). The self adhesion coefficients are fixed at $S_{AA} = 30$ and $S_{BB} = 15$ (i.e. population A has stronger self adhesion) and we consider the impact of variation in the cross adhesion strength C_{AB}. The results of the simulations are represented by plotting the differences of the cell densities u_A and u_B at large times (i.e. at numerical steady states), shown in Fig. 1.5 (top row). Depending on the value of C_{AB}, the steady state distribution attained corresponds to that predicted by the DAH based on the relative size of the adhesion coefficients, cf. Fig. 1.1 (c). In particular, it is noted that the more strongly adhesive cell type A tends to accumulate in the centre of the pellet except for the case without any cross adhesion, $C_{AB} = 0$, i.e. total separation.

As a second exploration, we investigate the relatively insensitive nature of the final configuration with respect to the initial distribution of the populations, cf. Fig. 1.1 (a). Here we choose $S_{AA} = 30$, $S_{BB} = 15$ and $C_{AB} = 7$; according to the DAH this predicts the partial engulfment of A by B at the steady state. Starting from the two sets of initial conditions illustrated in Fig. 1.4, the timecourses as solutions evolve to the steady state distribution are plotted in the middle and bottom rows of Fig. 1.5. Clearly, we observe evolution to the same pattern phenotype at the steady state; the differences in the right-most configurations stem from the smaller proportion of A used in the bottom row.

As a final test of the continuous cell sorting model, we explore whether Eqs. (1.8), when extended to three cell populations, can predict the hierarchical relationship of adhesive populations, similar to Fig. 1.1 (b). For three populations A, B and C obeying the self adhesion

hierarchy $S_{AA} > S_{BB} > S_{CC}$, simulations predict that population A becomes engulfed at the centre, population C is confined to the periphery and population B is sandwiched between A and C, Fig. 1.6.

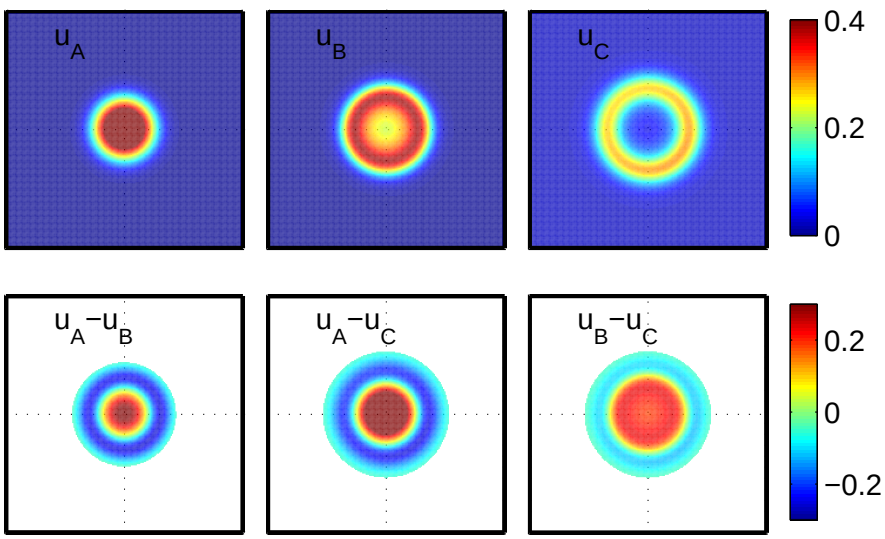

Figure 1.6: The plots show numerical solutions of Eqs. (1.8) extended to three cell types A, B, and C. (top row) Solutions u_A, u_B, and u_C at time $t = 50$. (bottom row) Pairwise solution differences at time $t = 50$. The initial condition is a randomly mixed pellet of the three cell types. The adhesion parameters are $S_{AA} = 45$, $S_{BB} = 30$, $S_{CC} = 15$, $C_{AB} = 31$, $C_{AC} = 0$, $C_{BC} = 16$.

1.5 Modelling adhesion during cancer invasion

In this section we demonstrate the applicability of the continuous framework for cellular adhesion by considering a simple and minimalist model of cancer cell invasion into healthy tissue, cf. Gerisch and Chaplain [2008]. The model consists of three equations describing the cancer cell density, c, the extracellular matrix (ECM) density, v, and the concentration of a (generic) matrix degrading enzyme (MDE), m. The model equations are given by

$$\frac{\partial c(\mathbf{x},t)}{\partial t} = D_1 \nabla^2 c - \nabla \cdot \left[\frac{\phi c}{R} \int_V \frac{\mathbf{r}}{|\mathbf{r}|} \Omega(|\mathbf{r}|) g(c(\mathbf{x}+\mathbf{r},t), v(\mathbf{x}+\mathbf{r},t))\, d\mathbf{r} \right] + \mu_1 c(1-c-v)\,, \tag{1.9a}$$

$$\frac{\partial v(\mathbf{x},t)}{\partial t} = -\gamma m v + \mu_2 (1-c-v)\,, \tag{1.9b}$$

$$\frac{\partial m(\mathbf{x},t)}{\partial t} = D_3 \nabla^2 m + \alpha c - \lambda m\,. \tag{1.9c}$$

For simplicity, we restrict our attention to a 2 dimensional geometry and consider the above equations on a domain $\Omega = (-1.5, 1.5)^2 \subset \mathbb{R}^2$, subject to periodic boundary conditions. In this model, both the cancer cells and ECM occupy physical space while the volume occupied

by MDE is assumed negligible. The cancer and ECM density equations above have been normalised such that the total density $c + v = 1$ characterises fully occupied physical space.

In this simple model, cancer cell migration is assumed to arise (i) from random motility (the corresponding coefficient D_1 is rather small) and (ii) from a directed movement due to adhesive effects of cancer cells with themselves and the surrounding ECM. The sensing region V for the adhesion term is a circle of radius $R = 0.1$ and for the function $\Omega(r)$ we select a linearly decaying function

$$\Omega(r) = \frac{3}{\pi R^2}\left(1 - \frac{r}{R}\right) \quad \text{for} \quad r \in [0, R]\,. \tag{1.9d}$$

The linear decay of $\Omega(r)$ models a diminishing influence of adhesive bonds towards the periphery of the sensing region. The leading factor in $\Omega(r)$ follows from a normalisation ensuring that the integral of $\Omega(|\mathbf{r}|)$ over the sensing region V is equal to one, stipulating a fixed maximum capacity of cells to form adhesive bonds within the sensing region independent of its actual size. The magnitude of that capacity is captured in the (parameters of the) function g which takes the form

$$g(c, v) = (S_{cc}c + S_{cv}v)\max\{0, 1 - (c + v)\}\,. \tag{1.9e}$$

This functional form implies that cancer cells adhere to themselves (self adhesion parameter S_{cc}) and to the matrix (cross adhesion parameter S_{cv}). Again, we include the limiting term such that g becomes zero if the total density $c + v$ approaches the value one. In addition to cell migration, cancer cell proliferation is also incorporated through the employment of a logistic growth type law with growth rate μ_1 and "carrying capacity" dependent on the locally available space, $1 - c - v$.

The ECM is assumed to be non-motile and degraded upon contact by MDEs at a rate γ. In the general formulation, a simple ECM production term permits regeneration of the ECM with rate μ_2 (note that in the simulations below, it is assumed that $\mu_2 = 0$). Finally, MDEs diffuse throughout the tissue (with diffusion constant D_3), are produced by the cells at rate α and decay at rate λ. The following set of parameters is used in the simulation

$$D_1 = D_3 = 10^{-3}\,, \mu_1 = 0.1\,, \mu_2 = 0; \alpha = 0.1\,, \gamma = 10\,, \lambda = 0.5\,, R = 0.1\,, S_{cc} = 0.05\,, S_{cv} = 0.1. \tag{1.9f}$$

Clearly, the model (1.9) is highly simplified in its nature and excludes many pertinent biochemical interactions. However, the focus here is on the incorporation and effect of the adhesion term in a model of tumour invasion and, consequently, we wish to retain the simplicity of the model. Crucial questions for any model of cancer invasion are whether it permits the breakage of cancer cells from a central tumour mass and how cancer cell migration is affected by a heterogeneous tumour environment. To address these issues, we consider an initial tumour population concentrated at the centre of the domain (representing the central tumour mass) and lying within a spatially structured ECM matrix. The initial MDE concentration is chosen to be proportional to the cell density. Simulations for a striped distribution in the initial ECM densities are shown in Figs. 1.7 and 1.8.

In Fig. 1.7 we observe the preferential accumulation and invasion of cancer cells along stripes of higher ECM density, in concert with degradation of that ECM. Cell migration obeys the restriction of physical space, i.e. cells do not move into densely packed tissue. Cells at the tumour periphery do not accumulate in regions of low ECM density, but rather quickly cross these areas to concentrate at the front of the next ECM barrier. The variation in ECM

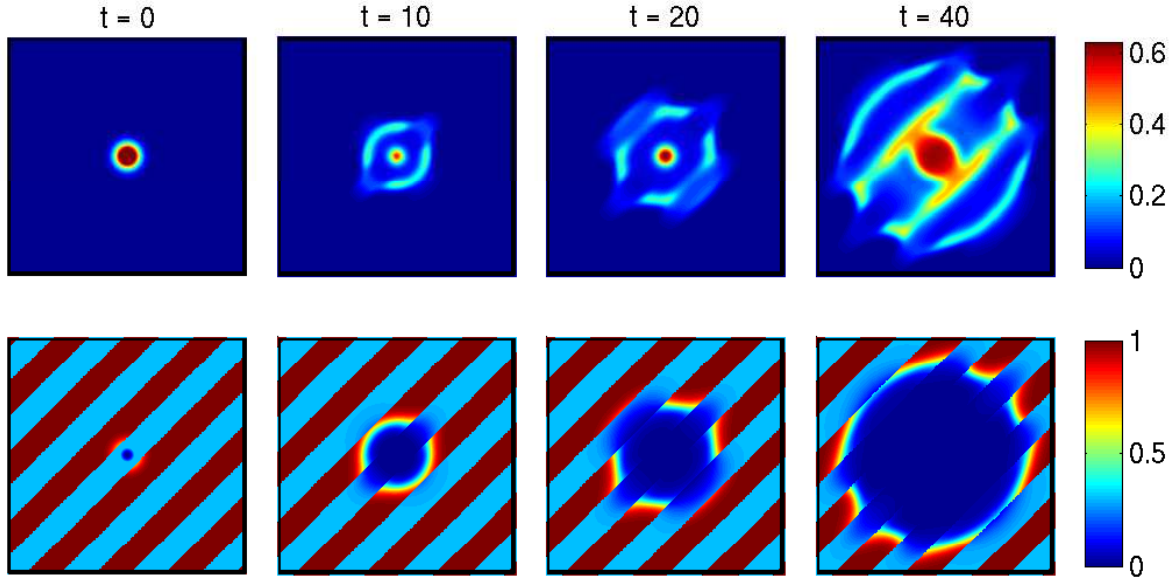

Figure 1.7: Simulation results for model (1.9) with a diagonally striped initial ECM distribution. Shown are the tumour cell density (top row) and the MDE density (bottom row) in the central part $(-1, 1)^2$ of the spatial domain at 4 time points. The MDE concentration displays similar features as the cell density and is not shown.

density also leads to the formation of protrusions which stretch from the cancer mass into the healthy tissue. Due to the regular structure of the ECM these protrusions are also regular. Similar results apply when cell proliferation is excluded, however the protrusions now take the form of high density tumour clumps extending along the ECM stripes, Fig. 1.8.

1.6 Discussion and outstanding questions

In this chapter we have discussed the critical role played by cellular adhesion during a wide spectrum of biological processes, highlighting the need for mathematical models to capture this fundamental phenomenon. A brief review of existing models, discrete and continuous, has demonstrated their individual strengths and weaknesses. Of particular note is the lack of continuous models that can replicate the "sorting" behaviour of multiple adhesive populations. As far as we are aware, the only continuous model that has been demonstrated to capture this property is that developed in Armstrong et al. [2006]. Here we expanded the derivation of this model and corroborated its suitability through an extended numerical analysis that replicates the wide variety of cell sorting "experiments", as predicted by the Differential Adhesion Hypothesis (DAH). The ultimate success of this approach lies in its capacity for integration with existing continuous models; as a demonstration of its suitability, we considered its extension into a model for cancer invasion as originally studied in Gerisch and Chaplain [2008] (see also [Sherratt et al., 2009]). A second application, considered in Armstrong et al. [2008], has been to the process of chick somitogenesis. There it was shown that upregulation of cellular adhesion, regulated through an underlying chemical signalling network, could drive both the epithelialisation and subsequent sorting of pre-somitic cells

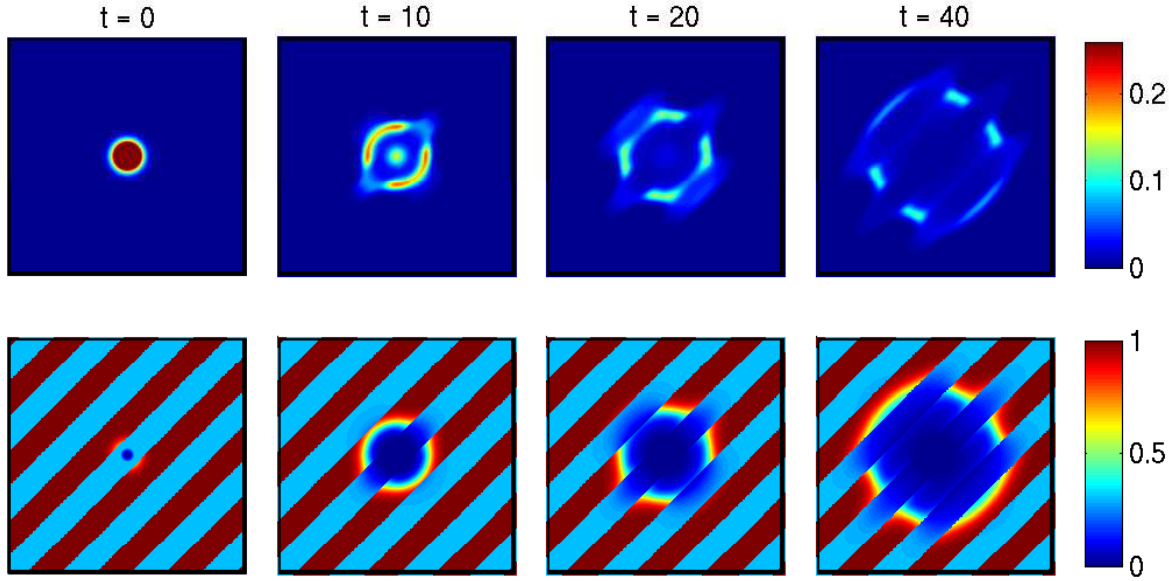

Figure 1.8: Simulation results for model (1.9) as in Fig. 1.7 but without cancer cell proliferation, i.e. $\mu_1 = 0$.

into somites. The ubiquity of cellular adhesion would allow a catalogue of potential applications to be listed: some typical examples, based on the history of modelling in these areas, include angiogenesis, wound healing, development of the slime mold *Dictyostelium* and skeletal patterning.

In the continuous adhesion model, the microscopic processes (for example, receptor binding) can be accounted for by a suitable choice of cell adhesion parameters. A crucial extension of this work lies in the development of truly multi-scale models of cell adhesion, in which the sizes of adhesion parameters in the continuous model can be determined from the processes occurring at a microscopic scale. To achieve this, it will be necessary to derive models for cell adhesion from a realistic underlying description of individual cell behavior; as discussed in Section 1.2, a number of attempts have been made at exploring some of these issues (e.g. see [Turner et al., 2004, Newman and Grima, 2004]).

Mathematically, a striking feature in the modelling approach is that cellular adhesion is accounted for via a non-local (integral) advective type term. As such, this fits coherently into the typical taxis-diffusion-reaction frameworks frequently employed in the modelling of pattern-formation type phenomena. Similarly, existing simulation packages for diffusion-reaction systems can be extended in modular fashion to allow the incorporation of such non-local terms. One difficulty, however, is the additional computational effort required to evaluate the non-local term. A suitable solution to this problem is outlined in the Appendix, the scheme in which provides high-resolution simulations within reasonable computing times (at least for the case of a rectangular spatial domain with periodic boundary conditions). However, extra work will be required to extend the numerical techniques to more general situations, such as irregular geometries or three dimensions.

Analytically, a number of results are available on the properties of solutions. In Sherratt et al. [2009], the boundedness of solutions was addressed under particular forms of the model.

Specifically, it was shown (in one dimension) that for $g(u) = \alpha u \max\{0, 2-u\}$, boundedness of u below 2 is possible under specific restrictions for the size of α and form of $\Omega(|r|)$. In Hillen et al. [2007] a related non-local model for chemotaxis was derived and global existence of solutions was proven for all finite sampling radii. A considerable number of questions, however, remain unanswered. Of particular interest are an extension of the boundedness results to spatially two-dimensional settings, the proper incorporation of other boundary conditions from both an analytical as well as a modelling point of view, and an analysis of the limiting scenario as the sampling radius $R \to 0$.

The formulation of the model presented here clearly simplifies many crucial components regarding the behaviour of adhesive populations *in vivo*. For example, the dynamics of adhesive binding are assumed to correlate to overall cell/matrix densities rather than the concentrations of adhesive molecules at the cell membrane, the dynamics of which can vary both spatially and temporally according to intra- and extra- cellular signals. Further, while adhesion is assumed here only to generate forces resulting in cell migration, the signalling initiated through binding interplays with many facets of cell behaviour, including division and apoptosis. Extending the model to include some of these complexities will further advance its relevance to understanding the role of adhesion in a wide variety of biological processes.

1.7 Appendix: Numerical Method

The models in this chapter are all solved following the Method of Lines (MOL). The rectangular spatial domain is covered with a uniform grid where each grid cell, or finite volume, is a square of side length h. In a first step of the MOL, the spatial derivatives are discretised on that grid and we employ a Finite Volume Method (FVM) of order two, see e.g. [Gerisch and Chaplain, 2006]. This transforms the PDE model into a large and in general stiff system of ordinary differential equations (ODEs), the MOL-ODE system

$$\frac{\mathrm{d}\mathbf{U}(t)}{\mathrm{d}t} = \mathcal{F}(t, \mathbf{U}(t)), \quad \mathbf{U}(0) = \mathbf{U_0}. \tag{1.10}$$

As is customary when using the FVM, the components of this ODE system represent approximations to the averages of the PDE solution in each finite volume. The numerical solution of the MOL-ODE (1.10) constitutes the second step of the MOL and an appropriate time integration scheme must be selected. Implicit time integration schemes can deal efficiently with the inherent stiffness of the MOL-ODE. We favour the linearly-implicit, 4th order Runge-Kutta method ROWMAP [Weiner et al., 1997]. The multiple Arnoldi process used within this method for the solution of the linear equation systems in each time step makes this scheme particularly suited for the large ODE system at hand. Furthermore, the method does not require any computation of the Jacobian of the MOL-ODE by the user; the required Jacobian-times-vector products are computed automatically by a suitable finite difference approximation using the right-hand side $\mathcal{F}$ of the MOL-ODE.

The FVM described in Gerisch and Chaplain [2006] has been applied to taxis-diffusion-reaction systems. There difficulties arise in regions of strong variation of the solution of the PDE, e.g. near moving fronts. These are due to the taxis term of the model and special attention was given to ensure that the discretisation of that term does not introduce oscillations or negative solution values in the solution of the MOL-ODE. This goal can be

achieved, while maintaining the order two of the FVM as much as possible, using a second-order upwind discretisation together with a nonlinear limiter function. The models of this chapter have a non-local adhesion term which is similar to the taxis terms in Gerisch and Chaplain [2006]. So we apply the same discretisation to that term, with the added difficulty of the approximation of the integral. The adhesion term, in general, takes the following form for the adhesive species u_i of a vector $\mathbf{u}$ of concentrations or densities in the models

$$-\nabla \cdot [u_i(\mathbf{x},t) \underbrace{\frac{\phi}{R} \int_V \frac{\mathbf{r}}{|\mathbf{r}|} \Omega(|\mathbf{r}|) g_i(\mathbf{u}(\mathbf{x}+\mathbf{r},t))\, d\mathbf{r}}_{\text{the adhesive velocity in } (\mathbf{x},t)}] \,. \tag{1.11}$$

The adhesive velocity, and consequently the integral must be approximated on each edge of the spatial grid for each evaluation of the right-hand side of the MOL-ODE. This task constitutes the computational bottleneck of the whole numerical solution process. If we assume that (i) the sensing region V at each $\mathbf{x}$ is the same and time-independent and (ii) that we solve the PDE system on a rectangular domain with periodic boundary conditions, then the adhesive velocity in (1.11) can be approximated on all vertical (or all horizontal) edges of the spatial grid simultaneously by evaluating a matrix-vector product $M\mathbf{G}$. Here, the matrix $M \in \mathbb{R}^{N,N}$, N the number of grid cells, and each row of M corresponds to the approximation of the non-local term on one edge. We arrive at this by, first, evaluating function g_i at the approximation $\mathbf{U}(t)$ yielding $\mathbf{G} \in \mathbb{R}^N$, second, reconstructing a function $\tilde{g}_i(\mathbf{x})$ from that data using bilinear interpolation and, third, approximating the integral with $\tilde{g}_i$ instead of g_i. Thanks to a suitable basis representation of $\tilde{g}_i$, the matrix M will be independent of the data $\mathbf{G}$ and can be pre-computed before the time integration of (1.10) commences. Furthermore, the third step can be performed to any desired accuracy so that the overall accuracy of the non-local term evaluation hinges solely on the quality of the reconstruction of function g_i by $\tilde{g}_i$, i.e. can be controlled by the spatial grid width h. Typically, the sensing region V is much smaller than the spatial domain of the PDE model. Consequently, the matrix M contains many zeros. However, in contrast to the approximation of derivatives, the fraction of non-zero elements of M remains constant with decreasing spatial grid width h. In that sense, sparse matrix techniques can only have a limited impact for the efficient evaluation of the matrix-vector product $M\mathbf{G}$. At this point the periodic boundary conditions become important which give M the structure of a block-circulant matrix with circulant blocks. Matrix-vector products with such matrices can be evaluated efficiently with Fast Fourier Transform (FFT) techniques. This substantially reduces the computational complexity and hence CPU time requirements for the evaluation of $M\mathbf{G}$. More details of the integral approximation and evaluation can be found in [Gerisch, 2008].

In the discussion above we have assumed periodic boundary conditions for the PDE problem. This is not always suitable from the point of view of modelling. No-flux boundary conditions are frequently encountered and in the following we describe how they can be included in the computational framework. For the non-local term, the boundary conditions become only important in points $\mathbf{x}$ where $\mathbf{x}+V$ intersects the boundary of the spatial domain of the PDE. For such $\mathbf{x}$ we follow the approach taken in Armstrong et al. [2008], that the integral of the non-local term is taken only over those $\mathbf{r} \in V$ such that $\mathbf{x}+\mathbf{r}$ is within the spatial domain. This modification implies that the matrix M changes from a block-circulant matrix with circulant blocks to a block-Toeplitz matrix with Toeplitz blocks. FFT techniques cannot be applied directly to such matrices but any such matrix can be embedded into a block-circulant matrix $\tilde{M}$ with circulant blocks. The size of $\tilde{M}$ will be larger than the size of M but for our application the increase will be modest. The vector $\mathbf{G}$ must also be padded with zeros in appropriate places to yield the extended vector $\tilde{\mathbf{G}}$. Now, $M\mathbf{G}$ can be extracted from the

efficiently to evaluate product $\tilde{M}\tilde{\mathbf{G}}$. We illustrate this for the Toeplitz to circulant case, i.e. no block structure, which is applicable for the simulation of spatially 1D models. In that case, the matrix M is a banded Toeplitz matrix with, say, upper bandwidth m and lower bandwidth n

$$M = \begin{pmatrix} l_0 & l_1 & \dots & l_m & 0 & \dots & 0 \\ l_{-1} & \ddots & \ddots & & \ddots & \ddots & \\ \vdots & \ddots & & & & & \\ l_{-n} & & & & & & \\ 0 & \ddots & & & & & \\ \vdots & \ddots & & & & & \\ 0 & & & & & & \end{pmatrix} \in \mathbb{R}^{N,N} .$$

The corresponding circulant matrix $\tilde{M}$ then has $N + \max\{n, m\}$ rows and columns and is defined by its first column

$$(l_0, l_{-1}, \dots, l_{-n}, 0 \dots, l_m, l_{m-1}, \dots, l_1)^\mathsf{T} \in \mathbb{R}^{N+\max\{n,m\}} .$$

The extended vector $\tilde{\mathbf{G}}$ is given by $\tilde{\mathbf{G}} = (\mathbf{G}, \mathbf{0})^\mathsf{T} \in \mathbb{R}^{N+\max\{n,m\}}$ and then holds

$$M\mathbf{G} = \left[\tilde{M}\tilde{\mathbf{G}}\right]_{1,\dots,N} ,$$

i.e. only the first N entries of $\tilde{M}\tilde{\mathbf{G}}$ are used.

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