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Patient-calibrated agent-based modelling of ductal carcinoma in situ (DCIS): From microscopic measurements to macroscopic predictions of clinical progression

Paul Macklin¹,²,³,⁴, Mary E. Edgerton⁵, Alastair M. Thompson⁵,⁶, Vittorio Cristini⁴,⁷

Abstract

Ductal carcinoma in situ (DCIS)—a significant precursor to invasive breast cancer—is typically diagnosed as microcalcifications in mammograms. However, the effective use of mammograms and other patient data to plan treatment has been restricted by our limited understanding of DCIS growth and calcification. We develop a mechanistic, agent-based cell model and apply it to DCIS. Cell motion is determined by a balance of biomechanical forces. We use potential functions to model interactions with the basement membrane and amongst cells of unequal size and phenotype. Each cell’s phenotype is determined by genomic/proteomic- and microenvironment-dependent stochastic processes. Detailed “sub-models” describe cell volume changes during proliferation and necrosis; we are the first to account for cell calcification.

We introduce the first patient-specific calibration method to fully constrain the model based upon clinically-accessible histopathology data. After simulating 45 days of solid-type DCIS with comedonecrosis, the model predicts: necrotic cell lysis acts as a biomechanical stress relief, and is responsible for the linear DCIS growth observed in mammography; the rate of DCIS advance varies with the duct radius; the tumour grows 7 to 10 mm per year—consistent with mammographic data; and the mammographic and (post-operative) pathologic sizes are linearly correlated—in quantitative agreement with the clinical literature. Patient histopathology matches the predicted DCIS microstructure: an outer proliferative rim surrounds a stratified necrotic core with nuclear debris on its outer edge and calcification in the centre. This work illustrates that computational modelling can provide new insight on the biophysical underpinnings of cancer. It may one day be possible to augment a patient’s mammography and other imaging with rigorously-calibrated models that help select optimal surgical margins based upon the patient’s histopathologic data.

Key words: agent-based model, tumour simulation, comedonecrosis, DCIS, ductal carcinoma in situ, biomechanics, calcification, patient-specific calibration

1991 MSC: 65C20, 92B05, 92C05
1 Introduction

Ductal carcinoma in situ (DCIS), a type of breast cancer where growth is confined within the breast ductal/lobular units, is the most prevalent precursor to invasive ductal carcinoma (IDC). Breast cancer is the second-leading cause of death in women in the United States. The American Cancer Society predicted that 50,000 new cases of DCIS alone (excluding other pre-invasive cancers such as lobular carcinoma in situ) and 180,000 new cases of IDC would be diagnosed in 2007 (Jemal et al., 2007; American Cancer Society, 2007). Co-existing DCIS is expected in 80% of IDC (Lampejo et al., 1994). While DCIS itself is not life-threatening, it is clinically important because it can be effectively treated and if left untreated, it has a high probability of progression to IDC (Page et al., 1982; Kerlikowske et al., 2003; Sanders et al., 2005). While the detection and treatment of DCIS have greatly improved over the last few decades, problems persist. DCIS can be difficult to detect by mammography (the principle modality in breast screening) or to distinguish from other aberrant lesions (Venkatesan et al., 2009). This can lead to “false positives” of DCIS and overtreatment, including unnecessary surgery. When excision is warranted, re-surgery is required in 20-50% of cases to fully eliminate all DCIS (Talsma et al., 2011), highlighting difficulties in estimating the full DCIS extent from patient imaging (Cheng et al., 1997; Silverstein, 1997; Cabioglu et al., 2007; Dillon et al., 2007). A solid scientific understanding of DCIS progression is required to improve surgical and therapeutic planning.

Open questions on DCIS biology contribute to current uncertainty in clinical practice. How does DCIS progress from a few proliferating cells to detectable lesions potentially including microcalcifications? Can immunohistochemistry (IHC) and histopathology be used to estimate important physiological constants? Can mathematical modelling provide new insight on interpreting these data? What is the relationship between the microcalcifications observed in mammography and tumour morphology? Can we calibrate patient-specific models to limited and noisy histopathologic data, often from only a single time point? These clinically-pertinent scientific questions motivate our work.

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Mathematical modelling has already seen use in understanding and predicting the growth and dynamics of DCIS. Franks et al. (2003a,b, 2005); Owen et al. (2004) used continuum models to investigate tumour growth in breast ducts, including the impact of volume loss in the necrotic core, ductal expansion, and the influence of basement membrane (BM) adhesion; this work can be traced to a long history of work (e.g., Ward and King (1997)) that includes matching to experiments. Rejniak (2007); Rejniak and Dillon (2007); Rejniak and Anderson (2008a,b); Dillon et al. (2008) applied an immersed boundary method to individual polarised cells; their model was able to reproduce several complex DCIS sub-types. Norton et al. (2010) conducted a similar investigation of the relationship between polarised cell adhesion, intraductal pressure, and DCIS morphology in 2D using a lattice-free agent model and were able to produce nontrivial (e.g., cribriform) tumour microstructures. Gatenby et al. (2007); Silva et al. (2010); Smallbone et al. (2007) investigated the role of hypoxia, glycolysis, and acidosis in DCIS evolution in 2D and 3D using cellular automata (CA) methods by including detailed metabolic sub-models. Mannes et al. (2002) used 2-D CA methods to investigate Pagetoid spread. Bankhead III et al. (2007) conducted early 3-D simulations of tumour cell hierarchy using CA techniques. Sontag and Axelrod (2005) combined population-scale models with machine learning techniques and statistical analyses to postulate new hypotheses on DCIS mutation pathways from benign precursors; Enderling et al. (2006, 2007) used continuum and CA methods to study mutations within DCIS and recurrence following radiotherapy. Very recently, Kim et al. (2011) used a detailed agent-based model to study interactions between DCIS cells and stromal cells via TGF-β and EGF signalling; their work included the effects of basement membrane expansion.

All this work has provided a degree of insight into DCIS, but has not fully answered the questions we posed. Typical CA methods cannot accurately model cell mechanics, particularly proliferation by tumour cells when fully surrounded by other cells; such proliferation is regularly observed in DCIS immunohistochemistry. Population-based ordinary differential equation (ODE) models do not account for spatial heterogeneity and cannot investigate the impact of heterogeneous mechanics, substrate transport, and their interaction. To date, none have modelled calcification, and existing necrosis sub-models have not considered the effects of cell swelling and lysis; many prevalent models ignore necrosis. The work by Norton et al. (2010) shows promise, but it has yet to predict tumour biophysics as emergent phenomena because it imposed many of its key properties a priori as algorithmic rules. The impressive morphological model of Rejniak and colleagues faces computational limits when applied to large numbers of cells. Continuum models can overcome these limits, but calibration to molecular- and cell-scale data is not straightforward (Macklin et al., 2010b). To our knowledge, there has been no prior patient-specific calibration to the proliferative and apoptotic indices generally measured in breast biopsies at any scale of modelling for DCIS (or for any type of cancer).
Modelling approach and advances: We presently develop a lattice-free, agent-based cell model that can be applied to many problems, exemplified by DCIS. The cells (agents) are modelled as objects subject to a balance of adhesive, repulsive, and motile forces that determine their motion. Cell-cell and cell-BM interaction mechanics are modelled using potential functions that account for finite interaction distances, uncertainty in cell morphology and position, and interaction between cells of variable sizes and types. Our potential functions are capable of both heterophilic and homophilic adhesion—an advance over current models. We introduce a level set formulation of the basement membrane morphology that provides a generalised framework for the exchange of forces between discrete cell objects and extended macroscopic objects with nontrivial, evolving geometries. Each cell is endowed with a phenotypic state, and phenotypic transitions are governed by exponentially-distributed random variables that depend upon the cell’s internal state and the local microenvironment. This modelling choice—a natural extension of constant probability per constant time step models in prevalent use today—is consistent with experimental biology (e.g., Smith and Martin (1973)), provides a rigorous method to vary the model’s probabilities with the microenvironment, allows for variable time step sizes, and lends itself to mathematical analysis.

We include detailed “sub-models” of cell volume change during proliferation and necrosis. Our necrosis model, which includes cell swelling and lysis, is the most biologically detailed to date. We are the first to model and investigate cellular calcification. We couple the agents to the microenvironment by solving reaction-diffusion equations for substrates that are altered by the cells. To make the model predictive, We constrain all major model parameters by surveying a broad swath of the experimental and theoretical biology literature.

We provide the first patient-specific model calibration protocol that estimates the population dynamic and mechanical parameters based upon IHC for proliferation (Ki-67), apoptosis (cleaved Caspase-3), and morphometric measurements from haematoxylin and eosin (H&E) histopathology images at a single time point, thus avoiding the inherently inaccurate problem of estimating time derivatives from noisy patient data. To our knowledge, this is the first patient-specific cancer calibration method that is based solely upon measurements that we could reasonably expect from a single patient biopsy. Our calibrated model is capable of making testable, quantitative patient-specific predictions of clinical behaviour (see below). Hence, an additional novelty of our work is that we fully document the process of developing a state-of-the-art agent model that is tailored to cancer biology, fully constraining it with biologically-relevant parameter estimates and a first-of-its-kind patient-specific calibration to pathology, and generating clinical predictions that are validated against the clinical literature. A preliminary version of this work appeared in Macklin et al. (2009a, 2010b,a); this paper refines the model, improves the calibration, and focuses on significant new results, with in-depth validation and analysis.
**Main results:** We use our model to study solid-type DCIS with comedonecrosis—a central necrotic core that is associated with more frequent recurrence of DCIS and poorer patient prognosis (Ottesen et al., 2000; Yagata et al., 2003). We calibrate our model to archived tissue data from Edgerton et al. (2011) and verify that the calibrated model successfully replicates our patient input data, thus demonstrating the feasibility of calibrating mathematical models based upon patient histopathologic data from a single time point. Away from the tumour’s leading edge, the simulated spatiotemporal dynamics reach a steady state after 7 to 14 days, consistent with a basic population dynamic model analysis and our prior continuum work (Macklin and Lowengrub, 2007).

Based solely upon calibration to microscopic measurements, we make and test macroscopic biological and clinical predictions. The model predicts that DCIS tumours grow at a constant rate through the duct, in agreement with mammographic data. Necrotic core biomechanics play a key role in this finding: necrotic cell lysis acts as a mechanical stress relief that directs proliferative cell flux towards the duct centre, rather than along the duct. Due to this mechanism, the model predicts that growth is slowest in large ducts with greater capacity to absorb proliferative flux. The model predicts DCIS growth rates between 7.5 and 10.2 mm/year, in quantitative agreement with published clinical data. While the “mammographic image error”–the distance between the calcification and the leading tumour boundary–increases over clinically relevant times, a DCIS tumour’s mammographic size is linearly correlated with its pathologic size; this is supported by the clinical literature. A linear extrapolation of the model-predicted correlation demonstrates an excellent agreement with 87 published patient data points spanning two orders of magnitude.

The model also makes microscopic predictions that match clinical data. Fast necrotic cell lysis at the perinecrotic boundary creates a physical gap between the viable rim and the necrotic core; this phenomenon is frequently observed in patient histopathology. The simulated tumours develop a stratified necrotic core, with increasing pyknosis (nuclear degradation) and calcification towards the duct centre; this is observed in patient histopathology. The model also predicts that calcification increases with distance from the tumour’s leading edge. The current model only predicts casting-type calcifications. Hence, we hypothesise that other biophysics—such as heterogeneous adhesive forces, cellular secretions, and degradation of the calcifications over long time scales—must be responsible for other types of calcifications observed in mammograms.

These successful quantitative predictions at the microscopic and macroscopic scales suggest that it may soon be possible to use a well-calibrated simulator to create a patient-specific map between the microcalcification geometry (as observed in mammography) and the actual tumour morphology. This could allow surgeons to more precisely plan DCIS surgical margins while removing less non-cancerous tissue, and could improve targeting of radiotherapy.
After detailing our agent-based model (Section 2), we apply it to DCIS with comedonecrosis (Section 3). We outline the computational method in Section 4. In recognition of the growing need for open, easily-parsed multicellular data formats to facilitate collaboration, we introduce MultiCellXML—an XML-based multicell data format as a potential draft for inclusion in the standard being developed by Sluka et al. (2011). See the supplementary material, where we provide benchmark datasets and open source C++ code. We estimate patient-independent DCIS parameters in Section 5; Section 6 details our patient-specific calibration protocol. After calibrating our model to archived DCIS patient tissue and verifying the calibration (Section 7), we simulate 45 days of DCIS growth and present our clinical predictions in Section 8; this section includes extensive validation against independent clinical data. Discussion and future directions are found in Section 9.

Supplementary material: We include a primer on DCIS biology, a sampling of significant prior agent-based modelling beyond DCIS, model generalisations, and further details on the relationship between exponentially-distributed phenotypic transitions and nonhomogeneous Poisson processes. We conduct a volume-averaging analysis (key to the patient calibration protocol) and apply it to clinical DCIS data in two ducts to examine earlier predictions of Michaelis-Menten population dynamics. We include full numerical implementation details (including MultiCellXML), expanded details our on parameter estimates, further analysis of our model calibration, insights on the significance of simulated variability in the apoptotic and proliferative indices, additional simulation results, and animations. See our dedicated webpage.

2 Agent-Based Cell Model

We now fully elaborate a discrete, cell-scale modelling framework that we preliminarily introduced in Macklin et al. (2009a, 2010b). See the supplementary material for a sampling of major agent-based modelling beyond DCIS, as well as recent reviews (Lowengrub et al., 2010; Macklin et al., 2010b). Our objective is a model that is sufficiently mechanistic that cellular and multicellular behaviour manifest themselves as emergent phenomena of the model, rather than through computational rules that are imposed a priori. The model is broadly applicable to the epithelial, stromal, and immune cells involved in carcinoma and sarcoma. We employ a modular design that allows “sub-models” (e.g., molecular signalling, cell morphology) to be expanded, simplified, or outright replaced as necessary. Where possible, we choose simple sub-models and test the model framework’s success in recapitulating correct DCIS behaviour.

http://www.mathcancer.org/JTB_DCIS_2011/
Cells are modelled as physical objects that exchange forces; essential molecular biology is incorporated through carefully-chosen constitutive relations. We attempt to model the mechanics, time duration, and biology of each phenotypic state as accurately as our data will allow; this should facilitate calibration to molecular- and cellular data. The agents interact with the microenvironment through coupled partial differential equations governing substrate transport. We use the same model for both cancerous and non-cancerous cells. Functionally, the cells differ primarily in the values of their proliferation, apoptosis, and other parameters; this is analogous to the downstream effects of altered oncogenes and tumour suppressor genes (Hanahan and Weinberg, 2000).

In this work, cells are not polarised. We do not focus on stem cell dynamics; this can readily be added by identifying agents as stem cells, progenitor cells, or differentiated cells, and assigning each class different characteristics. Thus, we focus on the tumour growth dynamics, rather than initiation. We do not model cell morphology, but rather total, nuclear, and cytoplasmic volume. Where cell morphology is necessary, we approximate it as spherical, similarly to Ramis-Conde et al. (2008a,b). This approximation is further discussed in Section 2.1. Basement membranes are modelled using level set functions (Section 2.2), which could model BM deformations (Macklin et al., 2010b).

2.1 Physical characteristics and mechanics

We endow each cell with a position $\mathbf{x}$, velocity $\mathbf{v}$, total volume $V$, cytoplasmic volume $V_C$, and nuclear volume $V_N$. In future work, we shall further divide $V_C$ into solid and fluid components. We assume that $\mathbf{x}$ and $\mathbf{v}$ are at the cell’s centre of mass and volume. While we do not explicitly track the cell morphology, we track the equivalent cell and nuclear radii (respectively $R$ and $R_N$) via

$$V = \frac{4}{3} \pi R^3, \quad V_N = \frac{4}{3} \pi R_N^3.$$  \hspace{1cm} (1)

See Fig. 1:left. For simplicity, we assume $V_N$ is fixed throughout the cell cycle.

Each cell has a maximum adhesion interaction distance $R_A \geq R$, which we use to express several effects. Because cells are deformable, they can stretch beyond $R$ to maintain or create adhesive bonds. As we do not explicitly track the cell morphology, there is inherent uncertainty as to maximum extent of the cell boundary relative to its centre of mass; $R_A$ needs to be sufficiently large to account for this. This effect is increased by random actin polymerisation/depolymerisation dynamics, which serve to randomly perturb the cell boundary (Gov and Gopinathan, 2006). See Fig. 1:right.

The cells are allowed to partly overlap to account for cell deformation. (Fig. 1: right.) We model the relative rigidity of the nucleus (relative to the cytoplasm).
by introducing increased mechanical resistance to compression at a distances less than $R_N$ from the cell centre; see Sections 2.3.1 and 2.3.4. Note that as $R_N \uparrow R$ (most of the cell resists compression) or $R_A \downarrow R$ (cells cannot deform to maintain adhesive contact), the cells behave like a granular material.

Fig. 1. **Cell morphology and mechanics:** Left: We track the cell volume $V$ and nuclear volume $V_N$ (with equivalent spherical radii $R$ and $R_N$, as labelled here); pale grey denotes the cytoplasm ($V_C$), and the darker grey denotes the nucleus ($V_N$). The unknown cell morphology (one possible realisation given as a dashed red curve) has an equivalent spherical morphology (solid blue curve). $R_A$ is the maximum adhesive interaction distance. Right: We account for uncertainty in the cell morphology by allowing the equivalent radii to overlap (left two cells), and by allowing adhesive contact beyond their equivalent radii (right two cells).

### 2.2 Basement membrane morphology

Let us denote the epithelium and lumen (the intraductal space when applied to DCIS) by $\Omega$ and the basement membrane by $\partial \Omega$. We represent $\partial \Omega$ implicitly with an auxiliary signed distance function $d$ (a level set function) satisfying

$$
\begin{cases}
  d(x) > 0 & x \in \Omega \\
  d(x) = 0 & x \in \partial \Omega \\
  d(x) < 0 & x \notin \overline{\Omega} = \Omega \cup \partial \Omega.
\end{cases}
$$

Additionally, $|\nabla d| \equiv 1$. See Fig. 2. This formulation can describe arbitrary BM geometries such as branch points in breast duct tree structures. The normal vector $n$ to the BM surface (oriented into the epithelium) is $n = \nabla d$, and $\nabla \cdot n$ gives the mean geometric curvature of the BM. This implicit representation is well-suited to describing a moving BM as it is deformed by mechanical stresses (e.g., due to proliferating tumour cells, as in Ribba et al. (2006)). See Macklin and Lowengrub (2005, 2006, 2007, 2008); Frieboes et al. (2007); Macklin et al. (2009b), where we used this method to describe moving tumour boundaries.
Fig. 2. BM morphology: Left: The BM separates the epithelium and lumen from the stroma. Right: The signed distance function $d$ represents the BM implicitly as its zero isocontour. $d > 0$ on the epithelial side, and $d < 0$ on the stromal side.

2.3 Forces acting upon the cells

Cells adhere to other cells (various cell-cell adhesion mechanisms: $F_{cca}$), the extracellular matrix (cell-ECM adhesion: $F_{cma}$), and the basement membrane (cell-BM adhesion: $F_{cba}$). Cells resist compression by other cells (cell-cell repulsion: $F_{ccr}$). The BM resists its penetration and deformation by cells (cell-BM repulsion: $F_{cbr}$). Motile cells experience a net locomotive force $F_{loc}$, and moving cells experience a drag force $F_{drag} = -\nu v_i$ by the luminal and interstitial fluids. See Fig. 3. We neglect any interstitial fluid pressure; this is equivalent to assuming the free flow of water, similarly to current continuum-scale mixture models (e.g., as in Wise et al. (2008).Newton’s second law gives the balance of forces acting on cell agent $i$:

$$m_i \ddot{v}_i = \sum_{j=1, j \neq i}^{N(t)} (F_{cca}^{ij} + F_{ccr}^{ij}) + F_{cba}^i + F_{cbr}^i + F_{cma}^i + F_{drag}^i + F_{loc}^i.$$

Here, $N(t)$ is the number of agents in the simulation at time $t$. The force terms are state-, time-, and microenvironment-dependent; apply to live and dead cell agents; and are governed by individual biological constitutive laws. We set $F_{loc} = 0$ to focus on the adhesive and repulsive forces. We set $F_{cma} = 0$ in any lumen; see the supplementary material for a more general form.

2.3.1 A simple family of potential functions

As in Drasdo et al. (1995); Drasdo and Höhme (2003, 2005); Drasdo (2005); Ramis-Conde et al. (2008a,b); Byrne and Drasdo (2009), we shall model cell-cell biomechanical interactions with potential functions ($\varphi$ for adhesion; $\psi$ for mechanical resistance/repulsion). We define $\varphi$ and $\psi$ by their gradients; the forms below are updated from Macklin et al. (2009a, 2010a,b). See Byrne
and Drasdo (2009) for a good discussion on modelling cell-cell interactions with potential functions. Ramis-Conde et al. (2008a,b) recently tied potential functions to detailed models of E-cadherin/β-catenin dynamics.

Let $R_A$ be the maximum adhesive interaction distance. For any $n \in \mathbb{N}$, define

$$\nabla \varphi(\mathbf{r}; R_A, n) = \begin{cases} 
(1 - \frac{|\mathbf{r}|}{R_A})^{n+1} \frac{\mathbf{r}}{|\mathbf{r}|}, & 0 \leq |\mathbf{r}| \leq R_A \\
0, & \text{else} 
\end{cases}$$

(4)

Note that $\nabla \varphi$ has compact support, to model the finite interaction distance between cells. The baseline case $n = 0$ is a linear ramping to the maximum force when $|\mathbf{r}| = 0$. For $n > 0$, $\varphi$ tapers off smoothly.

Similarly, if $m$ is a fixed nonnegative integer, $R_N$ is the nuclear radius, $R$ is the cell’s radius, and $M \geq 1$ is the cell’s maximum repulsive force, define

$$\nabla \psi(\mathbf{r}; R_N, R, M, m) = \begin{cases} 
-(c \frac{|\mathbf{r}|}{R_N} + M) \frac{\mathbf{r}}{|\mathbf{r}|}, & 0 \leq |\mathbf{r}| \leq R_N \\
-(1 - \frac{|\mathbf{r}|}{R})^{m+1} \frac{\mathbf{r}}{|\mathbf{r}|}, & R_N \leq |\mathbf{r}| \leq R \\
0, & \text{else} 
\end{cases}$$

(5)

where

$$c = \left( \left(1 - \frac{R_N}{R} \right)^{m+1} - M \right).$$

(6)

As with $\varphi$, $\psi$ and its derivatives have compact support; this models the fact that cells only repel one another when they are in physical contact. We make $\psi$ linear in the nuclear region (with $M \geq 1$) to model a stiffer material and allow the nuclear and cytoskeletal mechanics to be specified independently.

Although it is not necessary for our model, we can obtain $\varphi$ and $\psi$ by directly integrating $\nabla \varphi$ and $\nabla \psi$ with respect to $|\mathbf{x}|$ and setting $\varphi \equiv 0$ on $|\mathbf{x}| = R_A$ and $\psi \equiv 0$ on $|\mathbf{x}| = R$. In Fig. 4, we plot a linear combination of $\varphi$ and $\psi$ (left) and $\nabla \varphi$ and $\nabla \psi$ (right) that illustrates their use in the forces below.

### 2.3.2 Cell-cell adhesion ($F_{cca}$):

Adhesion receptors on a cell’s surface bond with adhesive ligands (target molecules) on nearby cells. Hence, the strength of the adhesive force between the cells is (to first order) proportional to the product of the receptor and ligand expressions. The adhesion strength increases as the cells are drawn more closely together, bringing more surface area (and receptor-ligand pairs) into direct contact. We model the force imparted by cell $j$ on cell $i$ by

$$F_{cca}^{ij} = -c_{cca} f_{ij} \nabla \varphi \left( \mathbf{x}_j - \mathbf{x}_i; R_A^j + R_A^i, n_{cca} \right),$$

(7)
Fig. 3. **Agent model forces:** On Cell 5, find labelled the cell-cell adhesive \( F_{\text{cca}}^{ij} \) and repulsive \( F_{\text{ccr}}^{ij} \) forces, and the cell-BM adhesive \( F_{\text{cba}}^5 \) and repulsive \( F_{\text{cbr}}^5 \) forces. We label the net cell locomotive force \( F_{\text{loc}}^i \) for Cell 6 (undergoing motility along the BM) and Cell 7 (undergoing motility within the ECM). We show the cell-ECM adhesive force \( F_{\text{cma}}^7 \) and fluid drag \( F_{\text{drag}}^7 \) for Cell 7. An earlier version of this figure appeared in advance in Macklin et al. (2009a, 2010b).

![Diagram of cell-cell and cell-BM forces](image)

Fig. 4. **Potential functions and derivatives** for \( m = n = 1, M = 1, R = 10, R_A = 12, R_N = 5, c_{\text{ccr}} = 1, \) and \( c_{\text{cca}} = 0.5184; s = 7 \) is the equilibrium spacing between two interacting cells, where \(-\nabla (c_{\text{ccr}} \psi + c_{\text{cca}} \varphi) = 0.\) \( c_{\text{ccr}} \) and \( c_{\text{cca}} \) are defined in the following sections. **Left:** \( c_{\text{ccr}} \psi + c_{\text{cca}} \varphi.\) **Right:** \(-\frac{\partial}{\partial r} (c_{\text{ccr}} \psi + c_{\text{cca}} \varphi).\)

where \( f_{i,j} \) describes the specific molecular biology of the adhesion, \( R_A^i \) is cell \( i \)'s maximum adhesion interaction distance, and \( c_{\text{cca}} \) is constant. Note that this form takes into account the deformability of both cells by using \( R_A^i + R_A^j.\)

In homophilic adhesion (e.g., Panorchan et al. (2006)), adhesion receptors \( \mathcal{E} \) bond with identical ligands \( \mathcal{E}.\) Hence,

\[
f_{i,j} = \mathcal{E}_i \mathcal{E}_j, \tag{8}
\]

where \( \mathcal{E}_i \) is cell \( i \)'s (nondimensionalised) \( \mathcal{E} \) receptor expression.
Calcite crystals in partly- and wholly-calcified necrotic cells remain strongly bonded in microcalcifications. We model this as homophilic cell-cell adhesion. If \( C_i \) is the nondimensional degree of calcification (see Section 2.5.4), then the general homophilic cell-cell adhesive form is

\[
f_{i,j} = \mathcal{E}_i \mathcal{E}_j + C_i C_j. \tag{9}
\]

Note that \( \mathcal{E}_i \) and \( C_i \) are time- and state-dependent: \( C_i = 0 \) in non-necrotic cells; \( \mathcal{E}_i \) is degraded and \( C_i \) increases in necrotic cells, allowing simultaneous E-cadherin- and calcite-based adhesion during necrosis (Section 2.5.4). See the supplementary material for the analogous heterophilic cell-cell adhesion form.

2.3.3 Cell-BM adhesion (\( \mathbf{F}_{cba} \)):

Integrin molecules on the cell surface form heterophilic bonds with specific ligands \( \mathcal{L}_B \) (generally laminin and fibronectin (Butler et al., 2008)) on the basement membrane (with density \( 0 < B < 1 \)). We assume that \( \mathcal{L}_B \) is distributed proportionally to the (nondimensional) BM density \( B \). Hence, the strength of the cell-BM adhesive force is proportional to its integrin surface receptor expression and \( B \). Furthermore, the strength of the adhesion increases as the cell approaches the BM, bringing more cell adhesion receptors in contact with their ligands on the BM. We model this adhesive force on cell \( i \) by

\[
\mathbf{F}_{cba}^i = -c_{cba} \mathcal{L}_B d \nabla \varphi \left( d(\mathbf{x}_i) \mathbf{n}(\mathbf{x}_i); R_A^i, n_{cba} \right), \tag{10}
\]

where \( c_{cba} \) is a constant, \( d \) is the distance to the basement membrane, \( \mathbf{n} \) is normal to the basement membrane (see Section 2.2), \( n_{cba} \) is as described above, and \( R_A^i \) and \( \mathcal{L}_B \) are cell \( i \)'s maximum adhesion interaction distance and (nondimensionalised) integrin receptor expression, respectively. Notice that setting the maximum interaction distance to \( R_A^i \) is consistent with our modelling simplification that the basement membrane is non-deformable.

2.3.4 Cell-cell repulsion (\( \mathbf{F}_{ccr} \)):

Cells resist compression by other cells due to the structure of their cytoskeletons, the incompressibility of their cytoplasm, and the surface tension of their membranes. We introduce a cell-cell repulsive force that is zero when cells are just touching, and increases rapidly as the cells are pressed together, particularly when their nuclei are in close proximity. We approximate cell deformation by allowing partial cell overlap; see Section 2.1. We model \( \mathbf{F}_{ccr} \) by

\[
\mathbf{F}_{ccr}^{ij} = -c_{ccr} \nabla \psi \left( \mathbf{x}_j - \mathbf{x}_i; R_N^i + R_N^j, R_i + R_j, M, n_{ccr} \right), \tag{11}
\]

where \( c_{ccr} \) is a constant, \( R_N^i \) and \( R_i \) are cell \( i \)'s nuclear radius and radius, respectively, and \( M \) and \( n_{ccr} \) are described above.
2.3.5 Cell-BM repulsion ($F_{cbr}$):

We model the basement membrane as rigid and thus resistant to deformation and penetration by the cells and debris. We model this force by

$$F_{cbr}^i = -c_{cbr} B \nabla \psi \left( d(x_i) n(x_i); R_N^i, R_i, M, n_{cbr} \right),$$  \hspace{1cm} (12)

where $c_{cbr}$ is a constant, $d$ is the distance to the BM, $R_N^i$ and $R_i$ are described earlier, and $M$ and $n_{cbr}$ are described above. We discuss planned work to model viscoplastic membrane expansion in Macklin et al. (2010b).

2.4 “Inertialess” assumption

Similarly to Drasdo et al. (1995); Galle et al. (2005); Ramis-Conde et al. (2008b) and as discussed in Lowengrub et al. (2010), we make the “inertialess” assumption that the forces equilibrate quickly, and so $|m_i \dot{v}_i| \approx 0$. Hence, we approximate $\sum F = 0$ and solve for the cell velocity from Eq. 3:

$$v_i = \frac{1}{\nu + c_{cma} \mathcal{I}_{E,i} E} \left( \sum_{j=1}^{N(t)} \left( F_{cca}^{ij} + F_{ccr}^{ij} \right) + F_{cba}^i + F_{cbr}^i + F_{loc}^i \right). \hspace{1cm} (13)$$

This has a convenient interpretation: each term $\frac{1}{\nu + c_{cma} \mathcal{I}_{E,i} E} F_{\square}$ is the “terminal” (equilibrium) velocity of the cell when fluid drag, cell-ECM adhesion, and $F_{\square}$ are the only forces acting upon it. Here, “$\square$” represents any individual force above, e.g., cba, cca, etc., and $N(t)$ is the number of simulated cells at time $t$. The coefficient $1/ (\nu + c_{cma} \mathcal{I}_{E,i} E)$ can be directly related to Darcy’s law in several tumour models; see the supplementary material.

2.5 Cell phenotypic states

We endow each agent with a phenotypic state $S(t)$ in the state space $\{Q, P, A, H, N\}$ (introduced below). Quiescent cells ($Q$) are in a “resting state” ($G_0$, in terms of the cell cycle); this is the “default” state in the framework. We model transitions between cell states as stochastic events governed by exponentially-distributed random variables that are linked to the cell’s genetic and proteomic state, as well as the microenvironment. These exponentially-distributed variables can be regarded as arising from nonhomogeneous Poisson processes; a brief discussion is in the supplementary material.
For a transition to state $S_2$ from the current state $S_1$, and for any interval $(t, t + \Delta t]$, we use the general form

$$\Pr (S(t + \Delta t) = S_2 | S(t) = S_1) = 1 - \exp \left( - \int_t^{t+\Delta t} \alpha_{12}(S, \bullet, \circ)(s) \, ds \right),$$  (14)

where $\alpha_{12}(S, \bullet, \circ)(t)$ is the intensity function, $\bullet$ represents the cell’s internal (genetic and proteomic) state, and $\circ$ represents the state of the surrounding microenvironment sampled at the cell’s position $x(t)$. Note that for small $\Delta t$,

$$\Pr (S(t + \Delta t) = S_2 | S(t) = S_1) = \alpha_{12}(S, \bullet, \circ)(t) \Delta t + O(\Delta t^2);$$  (15)

when $\alpha_{12}$ is constant, we recover (to second order) the commonly-used constant transition probabilities for fixed step sizes $\Delta t$; these may be regarded as approximations to our more general model here. This linearisation may be used in numerical implementations for small $\Delta t$ to improve performance.

In our phenotypic state space, quiescent cells can become proliferative ($P$) or apoptotic ($A$). Non-necrotic cells become hypoxic ($H$) when oxygen $\sigma$ drops below a threshold value $\sigma_H$. Hypoxic cells can recover to their previous state or become necrotic ($N$). Cell calcification (previously denoted $C$ in Macklin et al. (2009a, 2010b,a)) is included in the necrotic state. See Fig. 5. We include the subcellular scale by varying the transition parameters with the cell’s internal state and the local microenvironment.

Cell cycle models have also been used to regulate the $P \rightarrow Q$ transition (e.g., Abbott et al. (2006); Zhang et al. (2007)), and signalling networks have been developed to regulate $Q \rightarrow \{P, A, M\}$ (where $M$ is motile) transitions. These can be directly integrated into the agent framework by modifying the
stochastic parameters or by outright replacing the exponential random variables with deterministic processes. Excellent examples of agent-based modelling with subcellular signalling components include Chen et al. (2009b,a); Kharait et al. (2007); Wang et al. (2007); Zhang et al. (2007, 2009).

2.5.1 Proliferation (\(P\)):

As suggested by experimental and theoretical work (e.g., Smith and Martin (1973)), quiescent cells enter the proliferative state (i.e., progress from \(G_0\) to \(S\)) with a probability that depends upon the microenvironment. We model the probability of a quiescent cell entering the proliferative state in the time interval \([t, t + \Delta t]\) via an exponential random variable:

\[
\Pr(S(t + \Delta t) = P|S(t) = Q) = 1 - \exp\left(-\int_t^{t+\Delta t} \alpha_P(S, \bullet, \circ)(s) \, ds\right) 
\approx 1 - \exp(-\alpha_P(S, \bullet, \circ)(t)\Delta t),
\]  

(16)

where the approximations best hold for small \(\alpha_P\Delta t\).

Assuming a correlation between the microenvironmental oxygen level \(\sigma\) (non-dimensionalised by the far-field oxygen level in non-diseased, normoxic tissue) and proliferation (see the supplementary material and the excellent exposition in Silva and Gatenby (2010)), we expect \(\alpha_P\) to increase with \(\sigma\). Hence:

\[
\alpha_P(S, \sigma, \bullet, \circ)(t) = \overline{\alpha_P}(\bullet, \circ)\frac{\sigma - \sigma_H}{1 - \sigma_H},
\]  

(17)

where \(\sigma_H\) is a threshold oxygen value at which cells become hypoxic, and \(\overline{\alpha_P}(\bullet, \circ)\) is the cell’s \(Q \rightarrow P\) transition rate when \(\sigma = 1\) (i.e., in normoxic tissue), which depends upon the cell’s genetic profile and proteomic state (\(\bullet\)) and the local microenvironment (\(\circ\)). In tumours, low oxygenation is the norm (Gatenby et al., 2007; Smallbone et al., 2007), and so \(\sigma\) is far below 1; typically, \(\sigma_H \sim 0.2\) and \(\sigma < 0.4\) in the lumen; see the supplementary material.

For simplicity, we model \(\overline{\alpha_P}\) as constant for and specific to each cell type. In Macklin et al. (2010b), we discuss how to incorporate \(\bullet\) (i.e., a cell’s internal protein expression) and \(\circ\) (as sampled by a cell’s surface receptors) into \(\alpha_P\) through a subcellular molecular signalling model. Note that models have been developed to reduce the proliferation rate in response to mechanical stresses (e.g., Shraiman (2005)); in the context of the model, a cell samples these stresses from continuum-scale variables (i.e., “\(\circ\)”) to reduce \(\alpha_P\).

Once a cell has entered the proliferative state \(P\), it remains in that state until dividing into two identical daughter cells of half volume, which themselves remain in \(P\) until “maturing” into full-sized cells at the end of \(G_1\). Thereafter,
the daughter cells are placed in the “default” quiescent state \( Q \) to simulate the transition from \( G_1 \) to \( G_0 \). We now describe these events in greater detail.

Define \( \tau \) to the elapsed time since the cell entered the cell cycle from \( Q \). Similarly to Ramis-Conde et al. (2008b), we divide the cell cycle (with duration \( \tau_P \)) into the S-M phases and the \( G_1 \) phase (with duration \( \tau_{G1} \)). While \( \tau_P \) and \( \tau_{G1} \) may generally depend upon the microenvironment and the cell’s internal state, we currently model them as fixed for any given cell type.

\[ V(\tau) = \begin{cases} V_0 & 0 \leq \tau \leq \tau_P - \tau_{G1} \\ \frac{1}{2} V_0 \left(1 + \frac{\tau_{G1} + (\tau - \tau_P)}{\tau_{G1}}\right) & \tau_P - \tau_{G1} \leq \tau \leq \tau_P, \end{cases} \]  

where \( V_0 \) is the cell’s “mature” volume; \( V_N \) is fixed through the cycle.

To position the daughter cells, let \( R_{\text{parent}} \) be the radius of the parent cell (with position \( x_0 \)), and \( R_{\text{daughter}} \) that of the two daughter cells (with positions \( x_1 \) and \( x_2 \)). Pick \( \theta \in [0, 2\pi) \) with uniform distribution. Define \( \mathbf{u}_{\text{rand}} = (\cos \theta, \sin \theta) \), and set

\[ x_1 = x_0 + (R_{\text{parent}} - R_{\text{daughter}}) \mathbf{u}_{\text{rand}} \]
\[ x_2 = x_0 - (R_{\text{parent}} - R_{\text{daughter}}) \mathbf{u}_{\text{rand}}. \]

Thus, the cells partially overlap after mitosis; cell-cell repulsive forces (see Section 2.3.4) subsequently push them apart. This overlap partly accounts for the non-spherical cell geometry following mitosis.
2.5.2 Apoptosis ($A$):

Apoptotic cells undergo “programmed” cell death in response to signalling events. We model entry into $A$ using an exponentially-distributed random variable with parameter $\alpha_A(S, \bullet, o)(t)$. We assume no correlation between apoptosis and oxygen:

$$\Pr(S(t + \Delta t) = A|S(t) = Q) = 1 - \exp\left(-\int_{t}^{t+\Delta t} \alpha_A(S, \bullet, o)(s) \, ds\right), \quad (20)$$

where

$$\alpha_A(S, \bullet, o)(t) = \alpha_A(\bullet, o), \quad (21)$$

and where $o$ does not include oxygen $\sigma$, but may include other microenvironmental stimuli such as proximity of the BM (anoikis), chemotherapy, or continuum-scale mechanical stresses that increase $\alpha_A$ as in Shraiman (2005). Cells remain in the apoptotic state for a fixed amount of time $\tau_A$; afterward they are removed from the simulation to model phagocytosis of apoptotic bodies. Their previously-occupied volume is made available to the surrounding cells to model the release of the cells’ water content after lysis.

2.5.3 Hypoxia ($H$):

Cells enter the hypoxic state at any time that $\sigma < \sigma_H$. In this paper, we use the simplification that hypoxic cells cannot recover to their previous state, and instead immediately become necrotic ($\beta_H \to \infty$ in Fig. 5). See Macklin et al. (2010b) and the supplementary material for a more generalised form.

2.5.4 Necrosis (including calcification) ($N$):

For any cell in the $N$ state, its surface receptors (particularly E-cadherins and integrins) and subcellular structures degrade, it loses its liquid volume, and calcium is deposited (primarily) in its solid fraction. Let $\tau$ denote the elapsed time spent in the necrotic state. Define $\tau_{NL}$ to be the length of time for the cell to swell, lyse, and lose its water content, $\tau_{NS}$ the time for all surface receptors to become functionally inactive, and $\tau_C$, the time for calcification to occur. We assume that $\tau_{NL} < \tau_{NS} < \tau_C$. In Macklin et al. (2009a) we found that a simplified model (where $\tau_{NL} = \tau_{NS} = \tau_C$) could not reproduce certain morphological aspects of the viable rim-necrotic core interface in breast cancer.

We assume a constant rate of calcification, reaching a radiologically-detectable level at $\tau = \tau_C$. If $C$ is the nondimensional degree of calcification, then

$$C(\tau) = \frac{\tau}{\tau_C} \quad \text{if } \tau < \tau_C, \quad \text{and } \quad C(\tau) = 1 \quad \text{otherwise.} \quad (22)$$
We model the degradation of any surface receptor species $S$ (scaled by the non-necrotic expression level) by exponential decay with rate constant $\log 100/\tau_{NS}$; the constant is chosen so that $S(\tau_{NS}) = 0.015(0)$. We set $S(\tau) = 0$ for $\tau > \tau_{NS}$. After $\tau_{NS}$, adhesion is primarily to other partially- and fully-calcified cells; this is a simplified model of the calcium phosphate bonds in the calcification.

To model the necrotic cell’s volume change, let $f_{NS}$ be the maximum percentage increase in the cell’s volume (just prior to lysis), and let $V_0$ be the cell’s volume at the onset of necrosis. Then we model:

$$V(\tau) = \begin{cases} V_0 \left(1 + f_{NS} \frac{\tau}{\tau_{NL}}\right) & \text{if } 0 \leq \tau < \tau_{NL} \\ V_N & \text{if } \tau_{NL} < \tau. \end{cases} \quad (23)$$

To model uncertainty in the cell morphology during lysis, we randomly perturb its location $x$ such that its new radius $R(\tau_{NL})$ is contained within its swelled radius $R(0) (1 + f_{NS})^{\frac{1}{3}}$.

2.6 Dynamic coupling with the microenvironment with upscaling

We integrate the agent model with the microenvironment as part of a discrete-continuum composite model. We do this by introducing field variables for key microenvironmental components (e.g., oxygen, signalling molecules, ECM, etc.) that are updated according to continuum equations. The distributions of these variables affect the cell agents’ evolution as already described; simultaneously, the agents impact the evolution of the continuum variables. We demonstrate by coupling to oxygen transport. All cell agents uptake oxygen as a part of metabolism. At the macroscopic scale, this is modelled by

$$\frac{\partial \sigma}{\partial t} = \nabla \cdot (D \nabla \sigma) - \lambda \sigma, \quad (24)$$

where $\sigma$ is oxygen, $D$ is its diffusion constant, and $\lambda$ is the (spatiotemporally variable) uptake/decay rate. Suppose that viable (non-necrotic, non-calcified) tumour cells uptake oxygen at a rate $\lambda_t$, host cells at a rate $\lambda_h$, and elsewhere oxygen “decays” (by reacting with the molecular landscape) at a low background rate $\lambda_b$. Suppose that in a small neighbourhood $B$ of $x$, tumour cells, host cells, and stroma (non-cells) respectively occupy fractions $f_t$, $f_h$, and $f_b$ of $B$, where $f_t + f_h + f_b = 1$. Then $\lambda(x)$ is given by

$$\lambda(x) \approx f_t \lambda_t + f_h \lambda_h + f_b \lambda_b, \quad (25)$$

i.e., by averaging the uptake rates with weighting according to the tissue composition near $x$. This is consistent with the uptake rate model by Hoehme and Drasdo (2010), which they based upon the experimental literature.
We could further decompose \( f_t \) and \( f_h \) according to cell phenotype, if the uptake rates were expected to vary. In numerical implementations, we compute \( \lambda \) at a scale that resolves the cells (e.g., mesh size \( \sim 1 \mu m \)) and then upscale it to the computational mesh. Thus, the cell uptake rate varies with the tumour microstructure, which, in turn, evolves according to substrate availability.

Boundary conditions vary by the biology of the problem. We set \( \sigma = \sigma_B \) (for constant \( \sigma_B \)) on the BM and inside the stroma (wherever \( d \leq 0 \)) to model the release of oxygen by a pre-existent vasculature in the stroma. Wherever the simulation boundary intersects lumen, we use Neumann boundary conditions.

3 Model application to Ductal Carcinoma in Situ (DCIS)

We now adapt the model to solid-type DCIS, where tumour cells are non-polarised (with uniformly-distributed adhesion receptors) and are assumed to ignore E-cadherin signalling for contact inhibition. Tumour cells in the viable rim can be quiescent (\( Q \)), apoptotic (\( A \)), or proliferative (\( P \)). In hypoxic regions (\( \sigma < \sigma_H \)), cells immediately become necrotic (\( N \)), and eventually become calcified. See the Macklin et al. (2010b) and the supplementary material for a less simplified hypoxia model. We assume that there is no ECM in the duct lumen, and so \( E \equiv 0 \). Cell-cell adhesion is assumed homophilic between E-cadherin molecules, and cell-BM adhesion is heterophilic between integrins and uniformly-distributed ligands on the BM. We do not focus on cell motility in this study. For simplicity, we neglect molecular-scale signalling and membrane deformation and degradation, allowing us to instead focus upon the effects of the various cell states and forces. We also neglect the presence of non-cancerous epithelial cells lining the duct.

For simulation in 2D, consider cells growing in a fluid-filled domain \( \Omega \) (a rigid-walled duct) of length \( \ell \) and width \( 2R_{duct} \). We “cap” the left edge of the simulated duct with a semicircle of radius \( R_{duct} \). Cells are removed from the simulation if they cross the right edge of the computational boundary. We represent the duct wall with a signed distance function \( d \) as discussed above. We model oxygen transport within the duct by Eq. 24 where \( D \) is now constant, and \( \lambda \) is the locally-averaged oxygen uptake rate discussed above. We set \( \partial \sigma / \partial n = 0 \) on the righthand side of the duct.

4 Numerical methods

We implement the model using object-oriented ANSI C++, where each agent is an instance of a \texttt{Cell} class. Each cell object is endowed with an instance of
a **CellState** class, which contains the cell phenotypic parameters \((\pi_P, \alpha_A, \tau_P, \text{etc.})\), volumes \((V_C, V_N, V)\), radii \((R_N, R)\), maximum interaction distance \((R_A, \text{recorded as a multiple of } R)\), position \(x\), and velocity \(v\). We discretise microenvironmental field variables (e.g., oxygen \(\sigma\)) on an independent Cartesian mesh with uniform spacing \(\Delta x = \Delta y = 0.1L\), where \(L\) is the oxygen diffusion length scale. We represent the BM morphology with a level set function, and we use an auxiliary data structure to reduce the computational cost of cell-cell interaction testing and evaluation. The overall computational cost of the algorithm scales linearly in the number of cells (per computational time step). See the supplementary material for full computational details.

We have developed a human-readable, XML-based data format, which includes the random seed state, global variables, information on (and filenames of) microenvironmental field variables, and a list of each cell object and its current state. This structure allows us to easily parse the data, simplifying visualisation and post-processing. The list of cells in the XML file is very similar to the object-oriented **Cell** data structure in the simulator, making the format well-suited to resuming simulations from saved states. Model parameters can be readily modified during a simulation using simple plaintext search/replace operations in the XML files. In recognition of the growing need for open, easily-parsed multicellular data formats to facilitate collaboration, we fully document **MultiCellXML** in the supplementary material\(^8\) and at the project website.\(^9\) We include sample data sets and open source C++ code for post-processing and visualisation with the format.

## 5 Patient-independent parameter estimation

We estimated patient-independent parameters based upon an extensive search of the theoretical and experimental biology and clinical literature; we summarise those estimates here, and provide full details in the supplementary material; the estimates are improved beyond Macklin et al. (2009a, 2010a).

**Cell cycle time** \(\tau_P\): 18 hours by modelling literature.

**G\(_1\) time** \(\tau_{G1}\): \(\frac{1}{2}\tau_P = 9\) hours by theoretical biology literature.

**Apoptosis time** \(\tau_A\): 8.6 hours by population dynamic analysis of immunohistochemical stains of terminal ductal lobular units in non-cancerous women in the clinical literature. Estimate accounts for detection shortcomings in TUNEL assay and cleaved Caspase-3 immunohistochemistry.

**Necrotic cell lysis time** \(\tau_{NL}\): 6 hours by experimental literature.

**Necrotic cell volume increase** \(f_{NS}\): 1.0 by experimental literature.

\(^9\) [http://multicellxml.sourceforge.net](http://multicellxml.sourceforge.net)
Necrotic cell calcification time $\tau_C$: 15 days by clinical and experimental literature, and preliminary simulations.

**Oxygen diffusion length scale** $L$: 100 $\mu$m by modelling literature.

**Mean tumour cell oxygen uptake rate** $\langle \lambda \rangle$: 0.1 min$^{-1}$ by modelling literature and $L = \sqrt{D/\langle \lambda \rangle}$. Value also used for $\lambda_p$ and $\lambda_{np}$, based upon experimental biology literature, examination of quiescent cell morphologies in histopathology images, and supplemental simulation results.

**Oxygen uptake/decay rate for non-viable cells and background** $\lambda_b$: 0.01$\langle \lambda \rangle$ by model simplification.

**Hypoxic oxygen threshold** $\sigma_H$: 0.2 by modelling literature and analysis.

**Maximum adhesion interaction distance** $R_A$: 1.214 $R$ ($R$ is the equivalent cell radius) by experimental literature on breast cell deformations.

**Cell-cell repulsive force coefficient** $c_{ccr}$: 10.0$\mu$m/min by comparing potential functions to experimental literature on tensional forces applied to magnetic microbeads embedded in cell cytoskeletons.

**Cell-BM repulsive force coefficient** $c_{cbr}$: $c_{ccr}$ by model simplification.

**Cell-cell adhesion and repulsion potential exponents** $n_{cca}$ and $n_{ccr}$: Set to 1 by model simplifications.

**Cell-BM adhesion and repulsion potential exponents** $n_{cba}$ and $n_{cbr}$: Set to $n_{cca}$ and $n_{ccr}$, respectively, by model simplification.

$M$, the maximum value of $|\nabla \psi|$: 1 by model simplification.

6 **Patient-specific model calibration**

We now present a patient-specific calibration protocol for DCIS. The technique can be applied more generally to tumours with clearly visible viable rims; we point out these generalisations wherever possible. The following DCIS patient data are available (full methodological details given in Edgerton et al. (2011)):

- Average duct radius $\langle R_{duct} \rangle$ and viable rim thickness $\langle T \rangle$, measured on the IHC images. In a tumour spheroid, we would use its radius in place of $R_{duct}$.
- Average cell density $\langle \rho \rangle$ in the viable rim, calculated by counting nuclei and dividing by the computed viable rim size.
- Average cell nuclear radius $R_N$.
- Cell confluence $f$ in the viable rim, defined to be the area fraction of the viable region occupied by cell nuclei and cytoplasm.
- Proliferative index PI, measured by staining images for Ki-67, (a nuclear protein marker for cell cycling), and then counting the total number of Ki-67-positive nuclei versus the total number of nuclei in the viable rim.
• Apoptotic index AI, measured by staining for cleaved Caspase-3, an “executioner” caspase reflecting the apoptosis process. As Caspase-3 is a cytosolic protein, we identify positive cells by comparing the whole cell staining intensities. AI is then computed across the viable rim as with PI.

The patient-specific parameters and their physical meanings are in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Physical Meaning</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R$</td>
<td>cell radius</td>
<td>9.53 $\mu$m</td>
</tr>
<tr>
<td>$R_N$</td>
<td>cell nuclear radius</td>
<td>5.295 $\mu$m</td>
</tr>
<tr>
<td>$\sigma_B$</td>
<td>oxygen value on the BM</td>
<td>0.263717</td>
</tr>
<tr>
<td>$\langle \sigma \rangle$</td>
<td>mean oxygen level in viable rim</td>
<td>0.221065</td>
</tr>
<tr>
<td>$\langle \alpha_P \rangle$</td>
<td>mean $Q \to P$ transition rate</td>
<td>0.013705 hour$^{-1}$</td>
</tr>
<tr>
<td>$\alpha_P^{-1}$</td>
<td>mean waiting time prior to $Q \to P$ transition when $\sigma = 1$</td>
<td>115.27 min</td>
</tr>
<tr>
<td>$\alpha_A$</td>
<td>$Q \to A$ transition rate</td>
<td>0.00127128 hour$^{-1}$</td>
</tr>
<tr>
<td>$s$</td>
<td>cell spacing</td>
<td>18.957 $\mu$m</td>
</tr>
<tr>
<td>$c_{cca}$</td>
<td>cell-cell adhesive force coefficient</td>
<td>$0.0488836 c_{ccr}$</td>
</tr>
<tr>
<td>$c_{cba}$</td>
<td>cell-BM adhesive force coefficient</td>
<td>$10 c_{cca}$</td>
</tr>
</tbody>
</table>

Table 1
Patient-specific parameters for Patient 100019 for the DCIS model.

**Duct and cell geometry:** We match the simulated duct radius to the mean measured duct radius $\langle R_{\text{duct}} \rangle$. We obtain the average (equivalent) cell radius $R$ from the mean viable rim cell density $\langle \rho \rangle$ and measured confluence $f$ (where $0 \leq f \leq 1$) by the relation:

$$f = \langle \rho \rangle \pi R^2.$$  \hspace{1cm} (26)

We measure the cell nuclear radius $R_N$ in histopathology.

**Oxygen:** To solve for $\lambda_p$, $\lambda_{np}$, and $\lambda_b$, we separate the viable rim into fluid (with fraction $1 - f$), proliferating cells (with fraction $f\text{PI}$), and non-proliferating cells (with fraction $f(1 - \text{PI})$), and apply the defined uptake rates in each of these regions as a volume fraction-weighted uptake rate. Hence:

$$\langle \lambda \rangle = f (\text{PI} \lambda_p + (1 - \text{PI}) \lambda_{np}) + (1 - f) \lambda_b.$$ \hspace{1cm} (27)

If we obtain two additional constitutive assumptions on $\lambda_p/\langle \lambda \rangle$ and $\lambda_{np}/\langle \lambda \rangle$, we can uniquely determine $\lambda_p$, $\lambda_{np}$, and $\lambda_b$. See the supplementary material.

We use the mean viable rim thickness $\langle T \rangle$ as an indicator of oxygenation to determine the boundary value $\sigma_B$. In 2D (for 3D, see the supplementary material), the steady-state oxygen profile away from the leading edge is
\[
\begin{align*}
0 &= \begin{cases}
D\sigma'' - \langle \lambda \rangle \sigma & 0 < x < \langle T \rangle \\
D\sigma'' - \Lambda_b \langle \lambda \rangle \sigma & \langle T \rangle < x < \langle R_{\text{duct}} \rangle
\end{cases}
\end{align*}
\]  

(28)

with the boundary and matching conditions

\[
\begin{align*}
\sigma(0) &= \sigma_B, \quad \sigma(\langle T \rangle) = \sigma_H, \quad \sigma'(\langle R_{\text{duct}} \rangle) = 0 \\
D \lim_{x \uparrow \langle T \rangle} \sigma'(x) &= D \lim_{x \downarrow \langle T \rangle} \sigma'(x).
\end{align*}
\]

(29)

Here, \( x \) is the distance from the duct wall, and \( \Lambda_B = \lambda_B / \langle \lambda \rangle \). After applying all conditions except \( \sigma(0) = \sigma_B \), solving Eq. 28 analytically, and evaluating at \( x = 0 \), we obtain the boundary condition \( \sigma_B \):

\[
\sigma_B = \sigma_H \left[ \cosh \frac{\langle T \rangle}{L} + \sqrt{\Lambda_b} \tanh \left( \frac{\langle R_{\text{duct}} \rangle - \langle T \rangle}{L/\sqrt{\Lambda_b}} \right) \sinh \frac{\langle T \rangle}{L} \right].
\]

(31)

Similarly, the mean oxygen value across the viable rim is

\[
\langle \sigma \rangle = \sigma_H \frac{L}{\langle T \rangle} \left[ \sqrt{\Lambda_b} \tanh \left( \frac{\langle R_{\text{duct}} \rangle - \langle T \rangle}{L/\sqrt{\Lambda_b}} \right) \left( \cosh \frac{\langle T \rangle}{L} - 1 \right) + \sinh \frac{\langle T \rangle}{L} \right].
\]

(32)

For tumour spheroids, we would replace \( \langle R_{\text{duct}} \rangle \) with the mean tumour spheroid radius. For fingering tumours, we would use mean “finger” radius.

**Population Dynamics:** By solving ODEs for PI and AI (supplementary material) to steady state, given \( \tau_P, \tau_A, \) PI and AI, we obtain \( \langle \alpha_P \rangle \) and \( \alpha_A \) via:

\[
\begin{align*}
\langle \alpha_P \rangle &= \left( \frac{1}{\tau_P} \left( \text{PI} + \text{PI}^2 \right) - \frac{1}{\tau_A} \text{AI} \cdot \text{PI} \right) / (1 - \text{AI} - \text{PI}) \\
\alpha_A &= \left( \frac{1}{\tau_A} \left( \text{AI} - \text{AI}^2 \right) + \frac{1}{\tau_P} \text{AI} \cdot \text{PI} \right) / (1 - \text{AI} - \text{PI})
\end{align*}
\]

(33)

(34)

We calibrate the functional form for \( \alpha_P \) by combining this result with the computed mean oxygen in the previous step and solving for \( \bar{\sigma}_P \):

\[
\langle \alpha_P \rangle = \frac{\bar{\sigma}_P \langle \sigma \rangle - \sigma_H}{1 - \sigma_H}.
\]

(35)

**Cell-cell mechanics:** We first estimate the equilibrium spacing \( s \) between cell centres. For confluent cells \(( f = 1 \) in non-hypoxic tissue), we determine \( s \) by converting the mean density \( \langle \rho \rangle \) to an equivalent hexagonal cell packing:

\[
s = \sqrt{2 / \left( \sqrt{3} \langle \rho \rangle \right)}.
\]

(36)
Next, for two cells \(i\) and \(j\), we solve for the ratio of the (homophilic) adhesive and repulsive forces that enforces the cell spacing \(s\) by equilibrating the cell-cell adhesive and repulsive forces at \(r = s\):

\[
\mathcal{E}_i \mathcal{E}_j \frac{c_{\text{cca}}}{c_{\text{ccr}}} = \left| \frac{\partial}{\partial r} \psi \left( s; \mathbf{R}_N^i + \mathbf{R}_N^j, R^i + R^j, M, n_{\text{ccr}} \right) \right| = \left( 1 - \frac{s^2}{R^i + R^j} \right)^{n_{\text{cca}} + 1} \left( 1 - \frac{s^2}{R_A^i + R_A^j} \right)^{n_{\text{ccr}} + 1}, \tag{37} \]

Heterophilic adhesion is handled analogously. If \(i\) and \(j\) are of the same cell type with identical radii, nuclear radii, and interaction adhesion distances, and if we set \(\mathcal{E} = 1\) for both cells, then this simplifies to

\[
\frac{c_{\text{cca}}}{c_{\text{ccr}}} = \left( 1 - \frac{s}{2R} \right)^{n_{\text{ccr}} + 1} \left/ \left( 1 - \frac{s}{2R_A} \right)^{n_{\text{cca}} + 1} \right. \tag{38} \]

This leaves a free parameter: in effect, \(\langle \rho \rangle\) determines the equilibrium spacing but does not stipulate the time scale at which the forces operate to maintain the density. We apply our estimate of \(c_{\text{ccr}}\) (supplementary material) to fully constrain the cell-cell mechanics. It may also be possible to constrain the mechanics by matching the simulation to the variance in \(\rho\). Lastly, we can apply this technique in multiple tissue types and regions if the cell-cell mechanics were expected to vary (e.g., decreased cell-cell adhesion in hypoxic regions).

**Cell-BM Mechanics:** To ensure attachment of the epithelial cells to the duct wall (even when adhered to multiple cells in the lumen), set \(c_{\text{cba}} = 10c_{\text{cca}}\). In Video S2 and the supplementary material, we show that if \(c_{\text{cba}} \leq c_{\text{cca}}\), too many cells pull away from the BM; this is not consistent with typical patient histopathology. For simplicity, we set \(c_{\text{cbr}} = c_{\text{ccr}}\). In future work, we plan to calibrate \(c_{\text{cba}}\) by measuring the mean distance between the cell centres and the BM and then setting \(|F_{\text{cba}}| = |F_{\text{cbr}}|\) at that distance.

### 7 Sample calibration for Patient 100019

We demonstrate the calibration protocol on immunohistochemistry and histopathology data obtained from archived mastectomy tissue from an anonymised DCIS patient at the M.D. Anderson Cancer Center (anonymised case number 100019) from Edgerton et al. (2011)\(^\text{10}\). The patient had nuclear grade III (high-grade), mixed cribriform/solid-type DCIS with comedonecrosis; the patient measurements for this case (see below) are typical for mixed-type and

\(^{10}\)Preliminary data may deviate from the finalised values in Edgerton et al. (2011).
solid-type cases in Edgerton et al. (2011). The measurement techniques for these data are described in detail in Edgerton et al. (2011). In addition to these data, we measured the size of several nuclei in the viable rims in Fig. 7. We use the same case as in Macklin et al. (2009a, 2010b) to facilitate direct comparison with our earlier modelling results. The measurements for this case are given in Table 2. We point out that the variation in patient data is the combined effect of measurement errors and genuine intratumoural heterogeneity; see the supplementary material, where we discuss the implications for signalling heterogeneity in the proliferative and apoptotic pathways.

This case had no measurements of \( f \), so we approximate it as solid-type with \( f \approx 1 \). We set the patient-independent parameters as determined in Section 5. By applying the calibration protocol in Section 6 to these values and the patient-specific data, we obtain the parameter values in Table 1.

### 7.1 Verification of the Calibration

To verify the success of the calibration, we ran a simulation using the numerical methods in Section 4 for 30 days. We computed the simulated AI and PI, mean viable rim thickness, and viable rim cell density at 1-hour increments for the last 15 days of simulated time. (The full time-course evolution is examined in Section 8.) Full post-processing source code is described in the supplementary material\(^8\) and provided at the MultiCellXML project website.\(^9\)

In Table 3, we present the mean and standard deviation of these computations for the last 15 days of the simulation and compare to the patient data; these are plotted in Fig. 8 as intervals [mean − std. dev., mean + std. dev.], for the simulated data (left bars) and actual patient data (right bars). Because apoptosis is a rare stochastic event (< 1%) in a region with fewer than 500 cells, we expect considerable variability; indeed, this is observed in the patient AI.
Fig. 7. **Ki-67 immunohistochemistry of two DCIS duct cross sections** in case 100019. Nuclei of cycling cells (P: S, G₂, M, and G₁) stain dark red, while nuclei of non-cycling cells (Q: G₀) counterstain blue. In each duct, the viable rim is clearly visible, with greatest proliferation along the outer edge. In the duct centres, necrotic cores are filled with partly-degraded nuclear debris (red arrows, pointing up and right), mostly-degraded nuclei (green arrow, pointing down and left), and possibly microcalcifications in the degraded region. Note the physical gap (black horizontal arrows) between the viable rims and the necrotic cores. A colour version of this figure is available in the online edition.

As well. Because all the numerical targets (outlined in Table 2) are within the range of patient variation, the calibration can be considered as successful. The discrepancy in the PI can be eliminated by better accounting for the length of G₁ in the calibration; see the supplementary material.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Patient Data</th>
<th>Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI (%)</td>
<td>17.43 ± 9.25</td>
<td>24.04 ± 4.587</td>
</tr>
<tr>
<td>AI (%)</td>
<td>0.831 ± 0.572</td>
<td>0.7378 ± 0.7146</td>
</tr>
<tr>
<td>Viable rim thickness (µm)</td>
<td>76.92 ± 12.51</td>
<td>80.73 ± 1.10</td>
</tr>
<tr>
<td>Cell density (cells/µm²)</td>
<td>0.003213 ± 5.95e-4</td>
<td>0.002950 ± 6.09e-5</td>
</tr>
</tbody>
</table>

Table 3

**Verification of the patient-specific calibration**: Comparison of the patient (second column) and computed (third column) mean and standard deviation for the proliferative index, apoptotic index, viable rim thickness, and cell densities. All computed quantities are within the range of patient variation.
8 Patient-calibrated DCIS simulation: Hypothesis testing and model validation by clinical data

We now simulate DCIS in patient 100019 using the patient-independent parameters in Section 5; the patient-specific parameters are as in Section 6. The dynamic simulation is presented in Fig. 9. In this and all subsequent figures, small dark circles are cell nuclei, pale blue cells are quiescent (Q), green cells are cycling (P), red cells are apoptosing (A), dark grey cells are necrotic but not yet lysed (N), and the dark circles in the duct centres are necrotic cellular debris. The shade of red indicates the degree of calcification; bright red circles are clinically-detectable microcalcifications (N with τ > τC). An animation of this simulation is available as Video S1 in the supplementary material.

8.1 Overall spatiotemporal dynamics

In the simulation, a small initial population begins proliferating into the duct (0 days). As the tumour grows along the duct, oxygen uptake by the cells leads to the formation of an oxygen gradient (not shown). At 6.17 days, the
Fig. 9. **Agent-based simulation of DCIS in a 1 mm length of duct.**

Legend: The black curve denotes the basement membrane. Cell nuclei are the small dark blue circles, quiescent cells (Q) are pale blue, proliferating cells (P) are green, apoptosing cells (A) are red, and necrotic cells (N) are grey until they lyse, after which their solid nuclear fraction remains as debris (dark circles in centre of duct). The shade of red in the necrotic debris indicates the level of calcification; bright red debris are clinically-detectable microcalcifications (N with \( \tau > \tau_C \)). Simulated times (from top to bottom): 0, 7, 14, 21, and 28 days. Bar: 100 \( \mu \)m. A colour version of this figure is available in the online edition.

Oxygen level drops below \( \sigma_H \) in the centre of the duct near the leading edge of
the tumour, causing the first instance of necrosis; this cell lyses at 6.42 days. By 7 to 14 days, a viable rim of nearly uniform thickness (approximately 80 µm) can be observed, demonstrating the overall oxygen gradient decreasing from $\sigma_B$ at the duct boundary to $\sigma_H$ at the edge of the necrotic core.

Consistent with the assumed functional form of the $Q \rightarrow P$ transition, proliferating cells are most abundant near the duct wall where the oxygen level is highest, with virtually no proliferation at the perinecrotic boundary. Because oxygen can diffuse into the tumour from the duct lumen, viable cells are also observed along the tumour’s leading edge near the centre of the duct. Apoptosis occurs with approximately uniform distribution throughout the duct. See 7 days and onward in Fig. 9. These spatiotemporal dynamics emerge by 7 to 14 days and remain throughout the simulation. This is consistent with our analysis that the cell state dynamics reach a local steady state in 10 to 100 days in regions away from the leading edge (supplementary material).

The first clinically-detectable microcalcification appears at 21.17 days. By 22 days, a new characteristic length emerges: the trailing edge of the microcalcification maintains a distance of approximately 180 µm from the end of the duct. (See 28 days in Fig. 9 and Video S1 in the supplementary material.) Several features combine to cause this. We do not model contact inhibition, and so cells at the end of the duct continue to proliferate and push cells towards the tumour’s leading edge. Because the end of the duct has reached a local dynamic equilibrium by this time, a steady flux of tumour cells into the necrotic region has emerged. Because the calcification time ($\tau_C$) is fixed, the cells are pushed a fixed distance along the necrotic core before lysing and calcifying, leading to the observed “standing wave” pattern.

The necrotic core biomechanics play a key role in the tumour’s advance through the duct. Whenever a necrotic cell lyses, its former volume is converted to a small core of cellular debris and a large pocket of (released) fluid, which is easily occupied by other cells. Thus, necrotic cell lysis acts as a mechanical stress relief, analogously to the mechanical pressure sink terms used in the necrotic core in Macklin and Lowengrub (2005, 2006, 2007, 2008) and Macklin et al. (2009b). As a result, a pattern of cell flux emerges, where proliferating cells on the outer edge of the duct push interior cells towards the necrotic core, diverting much of the overall cell flux inwards rather than towards the tumour leading edge. See Video S1 in the supplementary material. This is a characteristic emergent feature of our model, and it has important implications for the rate of tumour advance through the duct. See Section 8.2.

A notable feature is the physical tear or gap between the tumour’s viable rim and the necrotic core. (See 14 days and onward in Fig. 9.) This phenomenon is observed in stained tissue slides. See Fig. 7 and Section 8.4. It has been attributed to dehydration, but it was unclear whether the dehydration is an
Fig. 10. **Tumour and microcalcification positions in the duct:** The top curve plots the maximum position of viable tumour tissue; the second curve (from the top) plots the maximum calcification position. The lighter line is the least-squares fit of the tumour advance from 11 to 45 days.

artefact due to tissue processing or a natural part of necrosis. The emergence of this phenomenon in a mechanistic model supports the hypothesis that the observed separation, while perhaps exacerbated during specimen preparation, is a *bona fide* result of DCIS tissue biomechanics. We note that an earlier version of our model—where necrotic cells gradually lost volume, rather than abruptly lysing—did not predict large gaps (Macklin et al., 2009a, 2010b). Fast cell swelling (over the course of $\tau_{NL} = 6$ hours) and subsequent bursting act as a perturbation of the perinecrotic tumour boundary. This is consistent with our earlier hypothesis that the physical gap must be due in part to necrotic cell volume loss over a fast time scale (Macklin et al., 2009a).

### 8.2 Constant rate of tumour advance – confirmation with clinical data

To quantify and understand the tumour’s growth, we post-processed our data to obtain the time evolution of the maximum position (extent) of viable tumour cells along the duct ($x_V(t)$) and the maximum position of the calcification ($x_C(t)$). To obtain better statistics on the growth dynamics, we extended our virtual duct to 1.5 mm, and continued the simulation to 45 days. C++ post-processing source code and pre-compiled binaries are provided in the supplementary material to compute these and other statistics.

In Fig. 10, we plot $x_V$ (top curve) and $x_C$ (bottom curve) for the first 45 days of growth. For the first 10 to 11 days, the simulation exhibits transient dynamics due to the left computational boundary. After this time, the tumour has developed a sufficiently large region between the left boundary and the leading edge for the dynamics to begin reaching a steady state as discussed above. See 11 days in Fig. 11. From 11 to 45 days, $x_V$ increases linearly at
Fig. 11. **Additional timepoints for the baseline simulation:** From top to bottom, 11, 24, and 45 days. Cells are coloured as in Fig. 9. Bar: 100 µm. A colour version of this figure is available in the online edition.

27.97 µm/day (obtained by least-squares linear fitting); see the lighter line in Fig. 10. The constant rate of tumour advance is due to the combined effects of substrate transport limitations and necrotic cell lysis in the duct interior. Because lysis acts as a mechanical stress relief, a significant portion of the proliferative cell flux is directed towards the duct interior, rather than towards the leading edge. Hence, the only forward-directed flux occurs along the leading tumour edge. In additional simulations, we found that setting $\tau_{NL} = 15$ days results in convex, exponential-like growth curves (supplementary material). This further supports the vital role of necrotic cell lysis in linear DCIS growth. Interestingly, recent modelling by Astanin and Preziosi (2009) with inverted geometry—a blood vessel surrounded by a growing tumour cord—also predicted linear tumour advance along the nutrient source.

Linear growth is consistent with mammographic measurements; Carlson et al. (1999) analysed the relationship between the maximum DCIS diameter and the elapsed time between mammograms, finding a near-linear relationship between the elapsed time between mammograms and the median DCIS size.

The rate of tumour advance in the duct—27.97 µm/day, or 10.2 mm per year—is consistent with DCIS growth estimates obtained by analysis of mammograms.
Fig. 12. **Inverse correlation of the duct radius and rate of tumour advance:**
For small ducts, little lumen is available for mechanical relaxation, leading to rapid tumour advance. Conversely, growth is slower for larger ducts, with a threshold minimum rate of advance (approximately 20.52 µm/day). The mean proliferative and apoptotic indices were fixed for all simulations.

Thomson et al. (2001) analysed changes in microcalcifications in mammograms to determine that high-grade DCIS tends to grow at about 7.1 mm per year (along an axis to the nipple). The group also analysed the data published by Carlson et al. (1999) and determined 13 mm/year and 6.8 mm/year mean and median growth rates, respectively. Simulating with $\tau_{G1} = 1\ \text{min}$ (for a better fit to the patient $\langle PI \rangle$—see the discussion in the supplementary material) yields a rate of tumour advance of 7.86 mm/year (result not shown). It is encouraging that a mechanistic cell-scale model—with calibration solely by molecular- and cell-scale data—can accurately predict emergent, macroscopic behaviour.

### 8.2.1 Inverse relationship between duct radius and rate of tumour advance

The link between necrotic cell lysis and linear DCIS growth suggests that the rate of tumour advance is inversely correlated with the duct radius—larger ducts have a greater “reservoir” of lumen available for mechanical stress relief, thereby directing more cell flux into the lumen. Smaller ducts should exhaust this mechanism more quickly, leading to a faster overall advance.

To test this, we simulated DCIS with the same phenotypic parameters as our main simulation, in virtual ducts with $R_{\text{duct}} \in \{100, 125, 150, 170, 11\}$. To eliminate the effect of differing oxygenation, we set the boundary condition $\sigma_B$ to maintain $\langle \sigma \rangle$ (and hence $\langle PI \rangle$) constant in each simulation, as given in Eq. 31. All simulations had $\langle PI \rangle$ between 22 and 24%, and mean viable rim thicknesses between 80 and 81 µm (result not shown).

For the duct of radius 100 µm, cells reach the edge of the computational domain at 1 mm after just 20.58 days, with a mean rate of advance (from
10 to 20 days) of 53.65 µm/day. For the duct of radius 125 µm, cells reach 1 mm by 27 days, and advance 37.75 µm/day (from 10 to 27 days). For the 150 µm duct, the tumour advanced 29.80 µm/day (from 10 to 30 days). In our baseline case with radius 170.11 µm, cells advance at 25.87 µm/day from 10 to 30 days. See Fig. 12; these data indicate a relationship of the form

\[ x_V' = a + e^{b-cR_{duct}} \]

for positive constants \( a, b, \) and \( c. \) To estimate these, we chose \( a \) that minimises \( \| x_V' - \left( a + e^{-p_a(R_{duct})} \right) \|_2 \) on \{100, 125, 150, 170.11\}, where \( p_a(R_{duct}) \) is the linear least-squares fit to \( \ln(x_V' - a) \). By this procedure, we estimate:

\[ x_V' \approx 20.52 + e^{6.085-0.02584R_{duct}} \text{ µm/day}. \]

See the red fitted curve in Fig. 12. Notice that as \( R_{duct} \uparrow \infty \), the rate of tumour advance (for fixed oxygenation and cell phenotypic parameters) saturates at a nonzero minimum (estimated here at approximately 20.52 µm/day, or 7.5 mm per year). This has important implications for clinical planning, as it provides a range as well as a lower boundary for the rate of growth of DCIS.

8.3 Calcification size and tumour size are linearly correlated – confirmation with clinical data

Prior to breast-conserving surgery, surgeons use mammographic images of microcalcifications to plan the correct surgical volume; for impalpable lesions, the planning is guided by stereotactically-placed localisation wires. Pathologists evaluate the success of the resection by examining the surgical resection margin: the outer edge of the excised specimen. The definition of an adequate margin width for DCIS, i.e. the distance from the tumour boundary to the surgical margin, varies by guideline. Smaller margin widths typically correlate with increased residual disease in the patient and a greater risk of local recurrence (Boland et al., 2003; Macdonald et al., 2006). However, the goal of breast-conserving surgery is to minimise the amount of normal tissue that is excised while fully eliminating the DCIS. Several studies have addressed these competing goals to determine an adequate post-operative radiation field based on margin width and other tumour characteristics (e.g. Vicini et al. (2004)). However, there has been little attention given to improving the pre-operative estimate of the optimal surgical volume.

To investigate this, we define a “mammographic image error” \( e(t) = x_V - x_C \) to be the distance between the true edge of the viable tumour (\( x_V \)) and the edge of a radiographically detectable calcification (\( x_C \)). If the desired margin width per institutional surgical protocols is added to \( e(t) \), then the distance from visible DCIS-associated microcalcifications to the desired surgical mar-
Fig. 13. **Simulated error in mammographic images:** We plot the time-course evolution of $x_V - x_C$—an estimator of the discrepancy between the mammographic measurement of a comedonecrosis microcalcification and the actual, pathology-measured tumour size. This discrepancy grows slowly and roughly linearly in time.

Origin can be estimated from a mammographic image. (This requires that the microcalcifications are confirmed to arise from DCIS and are not benign.)

In Fig. 13, we plot $e(t)$ from 21.17 days (the time of the first microcalcification) to 45 days. We see that $e(t)$ grows at a slow, roughly linear rate. When attempting to fit $e(t)$ to the form $e_\infty - e^{a-rt}$ (for $e_\infty, a, r > 0$), we found no evidence that $e$ reaches $e_\infty$ in time scales under four years (result not shown). We conclude that $x_V$ and $x_C$ are linearly correlated over clinically-relevant time scales. See Fig. 14: left. This relationship is confirmed in the clinical literature. de Roos et al. (2004) compared the maximum calcification diameter in mammograms (corresponds to $x_C$) with the measured pathologic tumour size (corresponds to $x_V$) in 87 patients, and found a significant linear correlation between these measurements.

To predict the quantitative relationship between the mammographic and pathologic tumour sizes, we compute the linear least-squares fit between $x_V$ and $x_C$:

$$x_V \approx 0.4203 + 1.117x_C \text{ mm.} \quad (41)$$

We plot this against our simulated DCIS data (blue points) and the data (red squares) from de Roos et al. (2004) in Fig. 14: right. Our model not only correctly predicts a linear correlation between a DCIS tumour's mammographic and pathologic sizes, but also quantitatively fits published clinical data *two orders of magnitude larger* than our simulation data.
Fig. 14. Comparison of mammographic and pathologic DCIS sizes: Left: Our DCIS simulation predicts a linear correlation between the mammographic calcification size \( x_C \) and the actual pathology-measured tumour size \( x_V \). Right: A linear least-squares fit of our simulation data (blue circles) fits clinical data (red squares) from de Roos et al. (2004), further demonstrating our model’s predictivity.

Fig. 15. Selected DCIS cross-sections at 45 days. a: Close to the leading edge, very little necrotic debris is visible, although the viable rim thickness is comparable to other cross sections. b: Farther from the leading edge, a band of intact necrotic debris surrounds a hollow duct lumen. c: As the distance increases, the lumen is filled with necrotic debris. Nuclei on the outer edge is newer and less degraded; material in the centre is more degraded. d: Farther still, a band of degraded nuclei surrounds a calcified core. e: With increasing distance, the microcalcification occupies a greater portion of the necrotic core. Bar: 100 \( \mu \)m. Cells are coloured as in Fig. 9. A colour version of this figure is available in the online edition.

8.4 Predicted necrotic core microstructure – comparison with histopathology

The microstructure of the simulated necrotic core affords us further opportunity to generate hypotheses on DCIS, which can be tested by comparison against histopathology. In Fig. 15, we highlight several typical DCIS cross sections in our simulation at time 45 days.

In Slice a, there is a viable rim of thickness comparable to the remainder of the tumour, but with little visible evidence of necrosis. This suggests that
in cases where too few ducts are sampled, a pathologist may not observe evidence of comedonecrosis. This could lead to mischaracterisation of the tumour as without comedonecrosis, whereas the biological mechanisms (particularly hypoxia) are the same as those with necrosis. This would be particularly true in cases where \( \langle PI \rangle / \tau_p \approx \langle AI \rangle / \tau_A \), as little net cell flux from the viable rim to the necrotic core would be expected.

Farther from the tumour leading edge in Slice b, we see a ring of necrotic debris, surrounding a hollow duct lumen. In cross sections like this, there has not yet been sufficient tumour cell flux from the viable rim to completely fill the lumen with necrotic debris. Farther still from the leading edge in Slice c, there has been sufficient cell flux to fill the lumen with necrotic material; we also see an outermost band of intact necrotic nuclei, encircling a central region of mostly degraded nuclei (modelled here simply as partly calcified). Ducts like these are observed in our patient’s H&E stains (Duct 1 in Fig. 16).

Moving farther from the leading edge in Slice d, we see a thin outermost band of relatively intact necrotic nuclei surrounding an inner band of mostly degraded necrotic material, and an inner core of microcalcification. Cross sections like these are observed in our patient histopathology (Duct 1 in Fig. 16 and the left duct in Fig. 7). In Slice e, the microcalcification is larger, and the outermost band of intact necrotic nuclei is largely gone. This is seen in Ducts 2 and 3 in Fig. 16. In general, the fraction of the necrotic core occupied by calcification increases with distance from the tumour leading edge.

The overall model prediction on the necrotic core microstructure is that the oldest material is in the centre, and is surrounded by increasingly newer, less-degraded, and less-calcified necrotic material; this age-ordered structure arises due to the overall flux of cells from the viable rim into the necrotic core. This observed ordering suggests that there is in fact an additional necrosis time scale beyond our current model, separating the rates of necrotic nuclear degradation and calcification. As an initial estimate, we might surmise that nuclear degradation occurs on the time scale comparable to our current estimate of \( \tau_C \), and calcification may be somewhat slower than our initial estimate.

9 Discussion and ongoing work

In this work, we developed and analysed an agent-based model of ductal carcinoma in situ (DCIS) of the breast. Our model refines and makes more explicit the biological underpinnings of current agent-based cell models, particularly for finite cell-cell interaction distances, the need for partial cell overlap to account for uncertainty in cell positions and morphology, and a rigorous way to vary phenotypic transition probabilities with the time step size, the cell’s in-
Fig. 16. H&E staining of DCIS in several ducts in case 100019. In each labelled duct, a readily visible outer viable rim (with faintly haematoxylin-stained nuclei) is separated from the necrotic core by a physical gap (black horizontal arrows). **Duct 1 necrotic core:** An outer band of partly degraded nuclei (red arrow, pointing up and right) surrounds a region of partly- or mostly-degraded nuclei (green arrow, pointing down and left). **Duct 2 necrotic core:** A region of mostly-degraded nuclei (green arrow, pointing down and left) surrounds a microcalcification (white vertical arrow). **Duct 3 necrotic core:** An outer band of partly degraded nuclei (red arrows, pointing up and right) surrounds a region of partly- or mostly-degraded nuclei (green arrows, pointing down and left), with a central core of microcalcifications (vertical white arrows). This duct is likely the intersection of two or more ducts near a branch point. A colour version of this figure is available in the online edition.

ternal state, and the microenvironment. We provide the most detailed necrosis model to date—including the impact of volume changes over time scales ranging from hours to weeks. We are the first to model necrotic cell calcification.

We developed the first patient-specific model calibration protocol to use immunohistochemical and histopathologic measurements from a single time point to simulate cancer in individual patients—an advance that could improve patient-tailored surgical and therapeutic planning. This calibration technique is broadly applicable to current agent-based models for multiple cancer types. Our model made numerous quantitative predictions on DCIS that we successfully tested against clinical data. We predicted that DCIS grows linearly, with a constant rate of approximately 1 cm per year (7.5 to 10.2 mm per year). These findings are quantitatively consistent with the clinical literature. We predict that the difference between the mammographic and pathological tumour size increases slowly with time. Our model generates a linear correlation between the mammographic and pathological tumour sizes that quantitatively fits clinical data spanning several orders of magnitude. Observing such an excellent match over a broad range of scales suggests that the model mechanics are biologically sound, and that our parameter estimates are accurate.
The model also correctly predicts the DCIS microstructure: a proliferative rim (with greatest proliferation on its outer edge) surrounds a stratified necrotic core. The viable rim and necrotic core are mechanically separated by a small gap—a feature that emerges from the mechanics of necrotic cell swelling and fast lysis, rather than being wholly attributable to tissue processing artefacts. The necrotic core has a layered structure that closely correlates with the “age” of the material. Relatively intact necrotic nuclei are observed in the outermost regions where cells have recently lysed. Closer to the duct centre, these nuclei start to disappear, and microcalcifications are found in the innermost region. These features are all observed in patient images, as illustrated in Fig. 16.

**Biological insight:** Because we calibrate to the means of patient data but not their standard deviations, we can use the simulated variation to test the model’s underlying biological hypotheses. Because the simulated variation in PI is significantly lower than the actual variation (Fig. 8), we conclude that the heterogeneity in DCIS proliferation is likely due to signalling variations (e.g., along the E-cadherin/β-catenin pathway), rather than oxygenation gradients alone. On the other hand, the simulated and actual standard deviations in the apoptotic index are quite similar (Fig. 8), supporting our biological hypothesis that apoptosis occurs at a low “background” rate that is independent of oxygenation and any other signalling. In fact, the patient’s mean and standard deviation are of comparable magnitude, which is consistent with the exponentially-distributed random variables used in our model.

Necrotic core biomechanics drive DCIS development. The constant rate of tumour advance is due to the combined effects of substrate transport limitations and the mechanical stress relief provided by cell lysis in the necrotic centre. Remarkably, we recover a quantitatively reasonable growth rate without need for modelling contact inhibition. Galle et al. (2005) used a rigorously-calibrated agent model to assess the impact of contact inhibition and growth substrate transport limitations on 2-D and 3-D cell cultures, finding that contact inhibition alone was responsible for growth limitations in 2D, but substrate transport limitations are also significant in 3D. They also found that cell-cell contact inhibition is further reduced when cells lose contact with the BM. This is consistent with cells growing in a lumen, such as in DCIS. More recently, Galle et al. (2009) further validated their agent-based model by comparing its predictions to a well-calibrated multiphase (continuum) model, with excellent model agreement in predicting 2-D in vitro Widr cell colony growth as a function of contact inhibition (where oxygen transport limitations do not apply). Their results—also consistent with continuum models such as Chaplain et al. (2006) that include ECM-MMP dynamics that should be typical of DCIS microinvasions—show the importance of refining our model in the future to include contact inhibition signalling. Indeed, we found that cell proliferation varies with density, which suggests that contact inhibition plays a role in
the finer details of DCIS progression (supplementary material).

The gap between the viable rim and the necrotic core is due to the relatively fast time scale of necrotic cell lysis. Analysis of the morphology and size of this gap may give insight on the progression of necrotic cell swelling and lysis, as well as the relative adhesive properties of lysed necrotic cells. The stratified necrotic core structure emerges due to (1) the net outflux of cells from the viable rim into the necrotic core, resulting in an age structuring, and (2) the relatively slow time scale of cell calcification. Hence, the relative distribution of these structures within the necrotic core may shed further insight as to the relative magnitudes of the time scales of pyknosis (nuclear degradation), water loss following lysis, and calcification. Indeed, the existence of a layer where the nuclei are mostly degraded with little evidence of calcification suggests that the time scale of pyknosis is between that of lysis (hours) and calcification (weeks). We plan in vitro studies to further investigate these key time scales.

It is interesting to note that the model predicts a linear/casting-type calcification, where the calcification forms a long “plug” that fills the duct centre. Other calcification morphologies (e.g., granular and fine pleomorphic) are not predicted by the biophysical assumptions of our model. While casting-type calcifications correlate with comedonecrosis (Stomper et al., 1989), they are only present in approximately 30%-50% of DCIS (Evans et al., 2010; Hofvind et al., 2011). Furthermore, casting-type calcifications can be absent from small, high-grade DCIS, while present in larger, low-grade DCIS (Evans et al., 2010). Hence, additional biophysics (e.g., secretions, heterogeneous adhesive properties, and degradation of the calcifications over very long time scales) are required to model the broader spectrum of observed calcifications in DCIS. Our H&E images support the notion of long-time degradation. The centres of many calcifications—which we have demonstrated are associated with the “oldest” necrotic material—demonstrate significant cracks that suggest extensive degradation and weak cohesion. See Fig. 16.

Phospholipids—such as those from subcellular structures that likely form a “backbone” for the formation of microcalcifications—degrade with half-lives on the order of 80 (Ayre and Hulbert, 1996) to 300 hours (Krause and Beamer, 1974) in non-pathologic tissue. If the degradation is two-to-ten times slower in necrotic tissue, we would expect degradation to progress over the course of a few months. This may partly explain rare cases of spontaneous resolution of calcifications in mammograms, where calcifications become smaller or occult without alternative explanations (e.g., invasive foci) (Seymour et al., 1999): in slow-growing DCIS (e.g., with both high PI and AI, as is observed in high-grade DCIS (Buerger et al., 2000)), calcifications may be degraded more quickly than they are replaced by new necrotic material.
Clinical insights: Because the “mammographic error” \( x_V - x_C \) (the difference between the mammographic and pathologic sizes) increases over time, it is unlikely that there is a single fixed “safe” surgical margin for all affected tumour ducts for all times. Instead, the margin size should vary with the tumour pathological properties, the duct size, oxygenation, and time. Given proper calibration to accurate measurements of a patient’s proliferative index, apoptotic index, cell density, duct sizes, and other related histopathologic and radiographic data, it should be possible to create a patient-specific map between the microcalcification geometry and the actual tumour shape and size. This could allow surgeons to use modelling based on data from the diagnostic core biopsy to more precisely plan DCIS surgical margins while removing less non-cancerous tissue, and could improve targeting of intra- and post-operative radiotherapy. Our calibration protocol can be combined with upscaling methods to calibrate multiscale cancer models. As a proof of concept, we applied this approach to histopathology data from 17 patients to calibrate a simplified continuum model of DCIS, with the goal of predicting surgical excision volumes in individual patients (including both the ducts and the surrounding tissue) (Edgerton et al., 2011). Although the continuum model used a steady state simplification and neglected necrosis, the model-predicted volumes were consistent with patient mammographic measurements in 14 of 17 cases. Hence, there is great promise in using our agent model and patient-specific calibration to incorporate patient pathology data into multiscale models.

The model predicts a general trend for the cross-sectional structure of a DCIS tumour. Moving from the basement membrane towards the duct centre, we see the following layers: a viable rim with greatest proliferation towards the basement membrane, a gap between the viable rim and necrotic core, an outer band of the necrotic core with relatively intact necrotic nuclei, an inner necrotic band of relatively degraded nuclei, and a central core of microcalcification. Cross sections closer to the leading edge contain fewer of these elements. We hypothesise that the microstructure of a given duct cross section in a histopathology slide could be used to estimate its position relative to the leading tumour edge in that duct; this could be tested by comparing the slide’s position to any known geometric information on the patient’s tumour. Moreover, if we can obtain sharper estimates of the various necrosis time scales, then we could potentially use the model to quantitatively predict the distance from each histopathology cross section to the actual tumour boundary, thereby further assisting surgical and therapeutic assessment.

Ongoing work: We chose DCIS as our initial modelling test bed because it is a clinically and scientifically significant problem in and of itself, it is tractable to patient-specific simulation with currently-available data, and it is a necessary step in modelling progression to invasive ductal breast carcinoma (IDC). In future work, we plan to integrate molecular-scale models
of invasion-related pathways (e.g., the ErbB family), hypoxia pathways (e.g., HIF-1α), BM deformation and degradation, and cell motility. These additions will allow us to extend our investigations to IDC. Given the critical role played by necrosis in determining the DCIS growth rate, we are extending our model to better account for changes in the fluid and solid content and the time scale for pyknosis (nuclear degradation). The calcification model will be refined to describe the formation and degradation of calcium crystals in phospholipids (e.g., in the cell membrane and degraded organelles) that remain after pyknosis. These improvements will be accompanied by advances in the model calibration to account for variations in the cell size throughout the cell cycle.

A potential weakness of this work is a lack of unicity in the data used for parameterisation, with in vitro and in vivo data combined from human and animal models, across multiple cell types. This may result in subtle incompatibilities, such as inconsistencies in the cell microenvironments, and variations in key biophysical processes. In effect, the assumption that cells use the same fundamental processes with altered frequency may only hold to leading order, and may affect the quantitative accuracy of our model predictions. We are addressing this concern by conducting appropriate in vitro experiments to measure single-cell properties in breast cell lines (in various phenotypic states), and are reviewing the state-of-the-art in cell biomechanics experiments.

We are working to further validate the model and bring it closer to clinical application. We are refining the calibration protocol to more accurately accounts for the impact of τ_{G1}; it should better recapitulate the input PI and density data. We are conducting a patient-specific model validation, where we obtain pathology from multiple patients, determine the model-predicted growth rates and correlations between mammographic and pathology sizes, and compare these to the case histories and mammograms from at least two time points. We plan to leverage our early model successes to study the impact of inadequate surgical margins on tumour regrowth and microinvasion, and the effect of adjuvant chemo- and radiotherapy in ameliorating these phenomena.

**Final thoughts:** Our model is based upon physical conservation laws, with the key molecular and cellular biology of DCIS integrated through constitutive relations. We have taken particular care to not prescribe DCIS behaviour; these instead become manifest as emergent phenomena—a trait of a scientifically sound predictive model. By carefully calibrating the model to the experimental and clinical literature, we can use its successful predictions to gain insight into the underlying mechanisms of DCIS. This is a key advance over phenomenological and statistical models, which can make predictions on DCIS behaviour but not on the underlying mechanisms. Furthermore, because statistical models generate correlations that apply to broad classes of patients, they cannot make quantitative predictions on DCIS in specific patients.
tic models, on the other hand, have this potential when rigorously calibrated.

We have demonstrated that a carefully-calibrated, mechanistic model of DCIS can make quantitative, testable predictions at the macroscopic scale, based solely upon microscopic, patient-specific measurements. Once validated and integrated into highly-efficient hybrid multiscale modelling frameworks (Lowengrub et al., 2010; Deisboeck et al., 2011; Edgerton et al., 2011), this work has the potential to improve the precision, disease-focused, and cosmetic outcome of patient-tailored breast-conserving surgery and radiotherapy.

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