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

Paul Macklin\textsuperscript{1,2,3}, Mary E. Edgerton\textsuperscript{4}, Alastair Thompson\textsuperscript{4,5}, Vittorio Cristini\textsuperscript{3,6}

Abstract

Ductal carcinoma in situ (DCIS)–an important precursor to invasive breast cancer–is typically diagnosed as microcalcifications in mammograms. These mammograms are also used to plan surgical resection of the DCIS, but repeated resection is often required to fully eliminate the DCIS.

This paper concludes a two-part series on DCIS modelling. In Part I, we developed a mechanistic, agent-based DCIS model. We now introduce the first patient-specific calibration method to fully constrain the model based upon immunohistochemistry and histopathology data available in typical breast biopsies at a single time point. We test and verify our calibration on data from an anonymised patient with DCIS.

After simulating 45 days of growth in our virtual patient, we generate testable predictions on the rate of DCIS growth and the relationship between the calcification size (as observed in a mammogram) and the actual tumour size (as measured by a pathologist). The model correctly predicts linear growth at approximately 1 cm per year. It also predicts a linear correlation between the calcification and tumour sizes, with an excellent quantitative fit to 87 clinical data points spanning several orders of magnitude in size. Actual 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.

The model sheds new light on the biophysical mechanisms underlying data in the clinical literature. Our results suggest that with rigorous calibration and validation, it should be possible to combine pre-operative biopsy data and mammographic images in a mathematical model to accurately predict the size and location of a patient’s DCIS, thereby improving the success of surgery, reducing the amount of healthy tissue removed, and allowing for better targeted radiotherapy.

Key words: agent-based modelling, patient-specific calibration, tumour simulation, necrosis, biomechanics, calcification, ductal carcinoma in situ (DCIS)

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

In Part I (Macklin et al., 2011), we introduced and analysed an agent-based cell model of ductal carcinoma in situ (DCIS)—a type of breast cancer where growth is confined to the duct lumen by the basement membrane. We conclude this work with an in silico study of 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). See Part I for a fuller description of DCIS biology and current clinical trends.

We rigorously estimate the “universal” model parameters (those common to all our DCIS simulations) by integrating a search of the experimental/theoretical biology literature with mathematical analyses. To determine the remaining parameters, we introduce a patient-specific calibration protocol, which fully constrains the model based upon tumour immunohistochemistry (IHC) and histopathology data. By leveraging our mathematical analyses from Part I, the calibration only requires data from a single time point, thus eliminating the need to estimate time derivatives from noisy image data. To our knowledge, this is the first patient-specific cancer calibration method that is based solely upon measurements that could reasonably be expected from a patient biopsy at a single time point.

Our model is mechanistic: we implement fundamental physical conservation laws, incorporate key molecular and cellular biology as constitutive relations, and restrict all parameters to physically reasonable values. Correct DCIS behaviour is not prescribed a priori, but instead becomes manifest as emergent phenomena that arise from the interactions of many cells. Such a mechanistic model is key to gaining insights on the biophysical mechanisms underlying the correlations found by data mining and other statistical approaches, as well as to providing patient-specific predictions that may one day improve surgical and therapeutic planning in patient-tailored therapy.

In this paper, we demonstrate and validate the predictive power of our model.
Based solely upon microscopic measurements, we make and test macroscopic predictions on: the rate of DCIS growth (on the order of 1 cm/year, consistent with clinical data); and the relationship between the size of the calcifications seen in patient mammograms at the time of diagnosis and the size of the actual tumour seen by pathologists (linearly correlated, with excellent fit to 87 published patient data points spanning two orders of magnitude). The model also makes microscopic predictions that match clinical data: heterogeneity in tumour cell proliferation correlates with oxygen availability, but cannot be fully explained without additional signalling heterogeneity; apoptosis can be characterised as a "background" exponentially-distributed process, with no signalling heterogeneity; fast necrotic cell lysis at the perinecrotic boundary creates the physical gap observed between the DCIS viable rim and comedonecrosis in the duct centre; and the distribution of nuclear debris and microcalcifications in the necrotic core arises due to the combined effects of (1) cell flux from the tumour viable rim into the necrotic core, and (2) the presence of several necrosis time scales spanning from hours to weeks.

Our model’s success in making quantitative predictions at the microscopic and macroscopic scales suggest that in the near future, it will be possible to use a well-calibrated model of DCIS to create a patient-specific map between the microcalcification geometry (as observed in mammography) and the actual tumour shape and size. This would allow surgeons to more precisely plan DCIS surgical margins while removing less non-cancerous tissue, and could improve targeting of intra- and post-operative radiotherapy.

The structure of this paper is as follows: We first briefly summarise the model (Section 1.1) and apply it to solid-type DCIS in Section 2. In Section 3, we estimate the “universal” parameters. In Section 4, we introduce a patient-specific calibration protocol for the remaining parameters with immunohistochemistry (namely Ki-67 and cleaved Caspase-3) and histopathologic measurements (from H&E staining). We demonstrate and validate the calibration on a specific (anonymised) mastectomy patient in Section 5. We briefly summarise our numerical technique in Section 6, with further details in the supplementary data. In recognition of the growing need for open, easily-parsed multicellular data formats to facilitate collaboration, we present an XML-based multicell data format as a potential draft for inclusion in the standard being developed by Sluka et al. (2011). See Section 6.1 and the supplementary material, where we provide benchmark datasets and open source C++ code.

In Section 7, we give a detailed analysis of a simulation using real patient-derived tumour data, in a 1.5 mm segment of breast duct over the course of 45 virtual days. As discussed above, this simulation gives numerous predictions that we test against independent clinical data in Section 7. We consider further simulation studies in Section 8 that illuminate the biomechanical basis of the model’s predictions. Animations of the key simulations are provided in
the supplementary material and online. We discuss the biological and clinical significance of our results, along with our ongoing work in Section 9.

1.1 Brief model summary

Each cell is an agent with (lattice-free) position \( x \), velocity \( v \), total volume \( V \) (and associated equivalent radius \( R \)), nuclear volume \( V_N \) (and equivalent nuclear radius \( R_N \)), solid fraction \( V_S/V \), maximum adhesive interaction distances \( R_{cca} \) (for cell-cell adhesion) and \( R_{cba} \) (for cell-basement membrane adhesion), and a phenotypic state \( S(t) \). Allowed cell states include quiescent (Q: cells in the G\(_0\) phase), proliferative (P: cells in G\(_1\), S, G\(_2\), or M phase), apoptotic (A), motile (M), hypoxic (H), necrotic, or (clinically-detectable) calcified debris (C). Transitions amongst the states are governed by exponentially-distributed random variables, where the transition parameters depend upon the cell’s internal state and its sampling of the local microenvironment, particularly the local oxygen level. Each non-quiescent cell state has a finite (but nonzero) duration, where cell activity is regulated by a “sub-model.” In particular, we model the change in cell volume during proliferation and necrosis.

The cell’s velocity is determined explicitly by applying an inertialess assumption to the balance of cell-cell adhesive (\( F_{cca} \)) and repulsive (\( F_{ccr} \)) forces, cell-basement membrane (BM) adhesive (\( F_{cba} \)) and repulsive (\( F_{cbr} \)) forces, fluid drag (\(-\nu v\)), net locomotive force (\( F_{loc} \)), and cell-extracellular matrix (ECM) adhesive force (\( F_{cma} = \alpha_{cma} I_{E,i} E v \), where \( I_{E,i} \) is the cell’s integrin expression, and \( E \) is the local ECM density). Hence, for cell \( i \),

\[
v_i = \frac{1}{\nu + \alpha_{cma} I_{E,i} E} \left( \sum_{j=1}^{N(t)} \left( F_{cc}^{ij} + F_{dd}^{ij} + F_{ccr}^{ij} \right) + F_{cba}^{i} + F_{cbr}^{i} + F_{loc}^{i} \right),
\]

where \( N(t) \) is the number of simulated cells at time \( t \). Adhesive and repulsive forces are modelled using potential functions \( \varphi \) and \( \psi \) (respectively) with compact support. The basement membrane morphology is described using a signed distance function \( d \) such that \( d = 0 \) on the BM, \( d > 0 \) in the epithelium and lumen, and \( d < 0 \) in the stroma. See Part I for full model details.

2 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 (σ < σ_H), cells become hypoxic (H), necrose (N), and eventually become calcified debris (C). We simulate the simplified case where hypoxic cells immediately transition to the necrotic state (β_H^{-1} = 0). We assume that there is no ECM in the duct lumen, and so E ≡ 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 Ω (a rigid-walled duct) of length ℓ 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

\[
\begin{cases}
    \frac{\partial \sigma}{\partial t} = D \nabla^2 \sigma - \lambda \sigma & \text{if } x \in \Omega \\
    \sigma = \sigma_B & \text{if } x \notin \Omega,
\end{cases}
\]

where λ is the locally-averaged oxygen uptake rate discussed in Section 2.6 in Part I. We set \( \partial \sigma/\partial n = 0 \) on the righthand side of the duct.

3 “Universal” parameter estimation

We first estimate parameters that are common to all patients, based upon literature searches of theoretical and experimental biology, mathematical analysis, and prior modelling efforts. The remaining parameters are calibrated to patient-specific data, as detailed in Section 4. The full list of non-specific parameters and their physical meanings is given in Appendix A.

3.1 Cell cycle timescales

We estimate that the cell cycle time \( \tau_P \) is 18 hours by the modelling literature (e.g., Owen et al. (2004)). We estimate that \( \tau_{G1} \approx \frac{1}{2} \tau_P = 9 \) hours (e.g., see the S + G2 + M time in Smith and Martin (1973)).
The time course from the initial signal to commence apoptosis to final cell lysis has been difficult to quantify (Hu et al., 1997). Early reviews estimated the early cellular events in apoptosis comprise a fast process on the order of minutes, with digestion of apoptotic bodies occurring within hours of phagocytosis (Kerr et al., 1994). Hu et al. (1997) conducted a detailed in vivo observation of apoptosis in the rat hippocampus, observing cells breaking up in 12–24 hours and the complete elimination of apoptotic bodies within 72 hours. Experimental work by Scarlett et al. (2000) similarly observed most apoptotic processes on the order of hours. These provide a bound for $\tau_A \leq 24$ h. These figures also suggest that apoptotic bodies are absorbed by surrounding cells in under 48 hours after cell lysis. In total, the experimental observations in the literature lead us to estimate $\tau_A \approx O(10h)$.

We estimate $\tau_A$ for breast epithelial cells based upon the hypothesis that cancerous and noncancerous cells use the same basic mechanisms of proliferation and apoptosis, only with altered frequency (Hanahan and Weinberg, 2000). Hence, we postulate that $\tau_A$ and $\tau_P$ are the same for DCIS cells and noncancerous breast epithelial cells. By Part I (Macklin et al., 2011), the total number of cells $N(t)$ in a fixed region of breast epithelium is given by

$$\dot{N} = \left(\frac{1}{\tau_P} \text{PI} - \frac{1}{\tau_A} \text{AI}\right) N,$$

where PI and AI are the proliferative and apoptotic indices (the fractions of proliferating and apoptosing cells), respectively. If we assume that noncancerous breast epithelial tissue is in homeostasis (when averaged through the duration of the menstrual cycle), then $\dot{N} = 0$, and

$$\tau_A = \frac{\text{AI}}{\text{PI}} \tau_P.$$ 

In Lee et al. (2006b), the mean proliferative and apoptotic indices of noncancerous breast epithelial cells in several hundred pre-menopausal women were measured at $0.0252 \pm 0.0067$ and $0.0080 \pm 0.0006$, respectively. While the AI and PI can vary considerably in time due to hormone fluctuation during the menstrual cycle (Navarrete et al., 2005), when averaged over many women (who fall at different points in this cycle), the effects of the monthly variation should cancel. Assuming that $\tau_P = 18$ h, we estimate $\tau_A \approx 5.71$ h. This is consistent with our estimated order of magnitude.

Since DCIS occurs predominantly in postmenopausal women, any effect of monthly variation with the menstrual cycle is not pertinent for the majority of women who develop DCIS. However, and reassuringly, in the same study (Lee et al., 2006b), the PI and AI were measured over several hundred post-
menopausal women at 0.0138 ± 0.0069 and 0.0043 ± 0.0007, respectively. Using these data gives a similar estimate $\tau_A \approx 5.62$ h. The similarity of the figures in pre- and post-menopausal women supports our working hypothesis that $\tau_A$ and $\tau_P$ are relatively fixed for the cell type, even when apoptosis and proliferation occur with differing frequencies and in different hormonal environments. We also note that conducting the same calculation with the data from Navarrete et al. (2005), while using a much smaller sample size, gives an estimated apoptosis time $\tau_A \approx 3.85$ h, consistent with our estimate.

We now account for detection shortcomings in the immunostaining. (See Duan et al. (2003) for a good overview of the current apoptosis marking methods in histologic tissue samples.) The AI measurements in Lee et al. (2006b) were obtained by TUNEL assay, which detects DNA fragmentation. According to the detailed work on Jurkat cell apoptosis in Scarlett et al. (2000), there was an approximately 3-hour lag between the inducement of apoptosis (observed as rapid changes in mitochondrial membrane voltage potential and the ratio of ATP to ADP) and the detection of DNA laddering and chromatin condensation. Cleaved Caspase-3 activity was negligible for the first 60 minutes and steadily climbed thereafter, peaking after 180 minutes and reaching approximately 10% of that peak in 50-60 minutes. On this basis, we would expect that TUNEL-assay-based AI figures fail to detect approximately the first 3 hours of apoptosis, and cleaved Caspase-3-based AI stains could underestimate the first one-to-two hours. Thus, we increase our estimate for $\tau_A$ to 8.6 hours. This also gives “correction factors” to account for undetected apoptotic cells by TUNEL assay and cleaved Caspase-3 immunostaining:

$$\frac{\text{AI}_{\text{actual}}}{\text{AI}_{\text{TUNEL}}} \approx \frac{8.6}{5.6}$$

and

$$\frac{8.6}{7.6} \leq \frac{\text{AI}_{\text{Caspase-3}}}{\text{AI}_{\text{actual}}} \leq \frac{8.6}{6.6}$$

3.3 Necrosis parameters

Necrotic cells lack sufficient energy to maintain ion pumps that regulate intracellular H$^+$, K$^+$, Na$^+$ and Ca$^+$ concentrations. K$^+$ and Na$^+$ play key roles in modulating cell volume; pumps for these ions are active during apoptosis to promote orderly cell shrinking and prevent premature lysis (Majno and Joris, 1995; McCarthy and Cotter, 1997; Barros et al., 2001; Cantoni et al., 2005). On this basis, we estimate $\tau_{NL} < \tau_A$ (8.6 h). This is consistent with experimental reports of necrotic cell lysis times ranging from “immediate” (e.g., Cantoni et al. (2005)), 6-7 hours (e.g., in Majno and Joris (1995)), and “overnight” (e.g., Mattes (2007)). We use $\tau_{NL} = 6$ hours for our initial estimate.
There has been a wide range of reported cell volume increase \( (f_{\text{NS}}) \) in necrotic cells prior to lysis. Jun et al. (2007) reported cell volume increase of approximately 30% within 60 minutes of the onset of necrosis in SN4741 neuron cells. Necrotic “blebs” on cultured liver cells were reported to increase their volume linearly in time for over 200 minutes in Barros et al. (2003), which supports our linear necrotic core volume increase, and suggests \( \tau_{\text{NL}} \) is on the order of hours. Grönroos et al. (2005) observed a 1.5-fold increase in cell volume in necrotic renal tubular cells in approximately 12 hours. Wu et al. (2010) observed necrotic cells swelling between two- and five-fold \((1 \leq f_{\text{NS}} \leq 4)\) after 24 hours in rat adrenal medulla cells. We use \( f_{\text{NS}} = 1 \) as our initial estimate; other values are briefly discussed in the supplementary material, but do not significantly affect the long-term rate of tumour growth.

### 3.4 Calcification timescale

Little-to-no literature data are available on the calcification process for necrotic breast epithelial cells. In the absence of data on the calcification process for necrotic breast epithelial cells, the best available experimental data are generally animal time course studies of arterial calcification; we use these to estimate the order of magnitude of \( \tau_C \). Time course studies on post mortem cardiac valves by Jian et al. (2003) observed significant tissue calcification between 7 days (10% increase in Ca incorporation) and 14 days (40% increase) after injection by TGF-\( \beta_1 \). Lee et al. (2006a) examined a related process (elastin calcification) using a rat subdermal model, demonstrating calcification to occur gradually over the course of two-to-three weeks. Gadeau et al. (2001) measured calcium accumulation in rabbit aortas following oversized balloon angioplasty injury. Calcified deposits appeared as soon as 2-4 days after the injury, increased over the course of 8 days, and approached a steady state between 8 and 30 days. Hence, we estimate \( \tau_C \) is on the order of days to a few weeks.

To sharpen our estimate, we conducted a parameter study on \( \tau_C \) using a simplified form of the model in Macklin et al. (2009a). We varied \( \tau_C \) from 12 hours to 30 days and calculated the percentage of the necrotic core occupied by calcified debris (calculated by area). The results are in Table 1. Calcification times under 15 days lead to necrotic cores that were nearly entirely calcified; this is not typically observed in H&E images of DCIS. On the other hand, the 30-day calcification time lead (as expected) to a complete absence of microcalcifica-

<table>
<thead>
<tr>
<th>( \tau_C ) (days)</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of core calcified after 30 days (%)</td>
<td>94.0</td>
<td>83.7</td>
<td>51.1</td>
<td>6.9</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 1

Fraction of the necrotic core occupied by calcified debris after 30 days of simulation.
tions in the core at time 30 days. Edgerton et al. (2008) hypothesised that DCIS tumours may grow to steady state in as little as two-to-three months, and so we expect microcalcifications by this time. Hence, our sharpened estimate of $\tau_C$ is 15 days, consistent with the literature.

3.5 Oxygen transport parameters

By Owen et al. (2004), the oxygen diffusion length scale $L$ is $\sim 100 \text{µm}$, and the mean cellular oxygen uptake rate $\langle \lambda \rangle$ (in the viable rim) can be estimated at approximately $0.1 \text{ min}^{-1}$ via $L = \sqrt{D/\langle \lambda \rangle}$ and their published value of $D$. Other values of $D$ (e.g., from Grote et al. (1977) and Evans et al. (1981)) give $0.1 \text{ min}^{-1} \leq \langle \lambda \rangle \leq 10 \text{ min}^{-1}$. This does not majorly impact our results because (1) we calibrate the proliferation and oxygenation sub-models in a self-consistent manner, and (2) $\langle \lambda \rangle$ acts as an oxygen transport time scale, and all these values yield fast equilibration relative to the proliferation timescale.

To estimate the hypoxic threshold $\sigma_H$, we examine the mitosis function $k_m(\sigma)$ in Ward and King (1997). At the step function limit, $k_m(\sigma) \propto H(\sigma - \sigma_c)$, where $H$ is the Heaviside function. The authors determined experimentally that $\sigma_c \approx 0.2$ when $\sigma$ is nondimensionalised by the far-field substrate value in non-pathologic, well-vascularized tissue. Because the step function limit is similar to $\alpha_P$, our $\sigma_H$ is analogous to $\sigma_c$ in Ward and King (1997), and as we have nondimensionalised oxygen similarly, we set $\sigma_H = 0.2$.

We observe in our immunohistochemical and histological images that the quiescent and proliferating viable tumour cells have the same general size; this suggests that the quiescent tumour cells are relatively metabolically active compared to non-cancerous, long-term quiescent cells that generally are smaller with condensed nuclei (relates to lack of transcriptional activity), reduced mitochondrial populations (Freyer, 1998), and less cytosol. Hence, we estimate that $\lambda_p \sim \lambda_{np}$. This is consistent with experimental evidence that cell mitochondrial volume is linearly proportional to cell cytoplasmic volume (James and Bohman, 1981), and that cell oxygen uptake (Hystad and Rofstad, 1994) correlates with mitochondrial volume. Similarly, experiments report a linear correlation between glucose uptake and cell volume (Miller, 1964; Low et al., 1996). For simplicity, we set $\lambda_p = \lambda_{np}$ and $\lambda_b = 0.01 \langle \lambda \rangle$. In Video S3 and the supplementary material, we show that setting $\lambda_p/\lambda_{np} > 1$ destabilises the perinecrotic border, and is not consistent with typical patient histopathology.
We estimate the cells’ solid volume fraction ($V_S/V$) at approximately 10% by combining the published data of Macknight et al. (1971) with the assumption that the solid cell component is one-to-ten times denser than water (Macklin et al., 2009a). When patient-specific nuclear size measurements are unavailable, we consider nuclear grade, where the tumour cell diameter is compared to the size of a red blood cell (RBC: generally 6 to 8 µm (e.g., Dao et al. (2003))). Low-grade DCIS nuclei are 1.5 to 2.0 RBCs across (4.5 µm ≤ $R_N$ ≤ 7 µm), high-grade are 2.5 RBCs or more ($R_n ≥ 7.5$ µm); intermediate grade lies between these (Schwartz et al., 1997; Tan et al., 2001).

We estimate the maximum cell-cell and cell-BM interaction distances $R_{cca}$ and $R_{cba}$ using published measurements of breast cancer cell deformations. Byers et al. (1995) found the deformation of MCF-7 (an adhesive, moderately aggressive breast cancer cell line) and MCF-10A (a non-malignant but transformed cell line) breast epithelial cells to be bounded around 50% to 70% of the cell radius in shear flow conditions; this is an upper bound on $R_{cca}$ and $R_{cba}$. Guck et al. (2005) measured breast epithelial cell deformability (defined as additional stretched length over relaxed length) after 60 seconds of stress. Deformability increased with malignant transformation: MCF10 deformed 10.5%, MCF7 deformed 21.4%, MCF7 deformed 30.4% after weakening the cytoskeleton, and MDA-MB-231 (an aggressive, often motile cancer cell line) deformed 33.7%. Because solid-type DCIS is adhesive but not yet invasive, we use the MCF7 estimate and set $R_{cca} = R_{cba} = 1.214R$.

We also turn to the experimental literature to estimate the overall magnitude of the mechanical forces. Cell mechanics can operate over a large range of time scales (Bursac et al., 2005), ranging from ~ 0.1 seconds for immediate viscoelastic responses to 1 minute or more when exposed to prolonged stresses (Matthews et al., 2006). Matthews et al. (2006) applied magnetic forces to microbeads attached to cultured endothelial cells to measure their cytoskeletal
response to mechanical stress. For longer-duration stresses, they observed bead displacement velocities on the order of 0.1 µm/min to 10 µm/min (after early transient dynamics). (See Figs. 6 and 7 in Matthews et al. (2006).) We find that \( \alpha_{ccr}/\nu = 10 \mu m/min \) gives \( |F_{ccr}|/\nu \) within this range for typical cell-cell interaction distances \((R - 3) \mu m < r < (R - 0.5) \mu m\); see Table 2. This is consistent with Macklin et al. (2009a), where setting \( \alpha_{ccr}/\nu = 8 \mu m/min \) and \( \alpha_{cbr}/\nu = 5 \mu m/min \) prevents unreasonable simulation behaviour (overlapping cell nuclei and cell penetration of the BM). In the supplementary material, we show that our simulation results are resilient to error in \( \alpha_{ccr} \): the cell density and rate of tumour growth exhibit little change over a broad range of \( \alpha_{ccr} \). For simplicity, we set \( M = 1, \alpha_{ccr} = \alpha_{cbr} = 10 \nu \mu m/min, \) and \( n_{ccr} = n_{cbr} = 1 \) (to model anticipated nonlinear but smooth cell mechanical responses).

4 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, as measured and described by Edgerton et al. (2011):

- Average duct radius \( \langle R_{duct} \rangle \) and viable rim thickness \( \langle T \rangle \), measured directly on the IHC images. In a generalised 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 images for cleaved Caspase-3, an “executioner” caspase reflecting the apoptosis process. Because Caspase-3 is a cytosolic protein, we identify cleaved Caspase-3 positive cells by comparing the whole cell staining intensities. The apoptotic index is then computed across the viable rim as with PI.

The full list of the patient-specific parameters and their physical meanings is given in Appendix B. Full methodological details on these patient measurements are beyond the scope of this paper, but can be found in the primary source (Edgerton et al., 2011).
4.1 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 \( f \) \( (0 \leq f \leq 1) \) by the relation:

\[
f = \langle \rho \rangle \pi R^2. \tag{7}\]

We measure the cell nuclear radius \( R_{N} \) in histopathology.

4.2 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 \( f_{PI} \)), and non-proliferating cells (with fraction \( f(1 - PI) \)), and apply the defined uptake rates in each of these regions as a volume fraction-weighted uptake rate. Hence:

\[
\langle \lambda \rangle = f_{PI} \lambda_p + (1 - f_{PI}) \lambda_{np} + (1 - f) \lambda_b. \tag{8}\]

If we obtain two additional constitutive assumptions on \( \lambda_p / \langle \lambda \rangle \) and \( \lambda_{np} / \langle \lambda \rangle \), then we can uniquely determine \( \lambda_p \), \( \lambda_{np} \), and \( \lambda_b \). See Section 3.5.

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 Section 4 in Part I), the steady-state oxygen profile away from the leading edge reduces to

\[
0 = \begin{cases} 
D \sigma'' - \langle \lambda \rangle \sigma & 0 < x < \langle T \rangle \\
D \sigma'' - \Lambda_b \langle \lambda \rangle \sigma & \langle T \rangle < x < \langle R_{\text{duct}} \rangle
\end{cases} \tag{9}
\]

with the boundary and matching conditions

\[
\sigma(0) = \sigma_B, \quad \sigma(\langle T \rangle) = \sigma_H, \quad \sigma'(\langle R_{\text{duct}} \rangle) = 0 \tag{10}
\]

\[
D \lim_{x \uparrow \langle T \rangle} \sigma'(x) = D \lim_{x \downarrow \langle T \rangle} \sigma'(x). \tag{11}
\]

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. 9 analytically, and evaluating at \( x = 0 \), we obtain the boundary condition \( \sigma_B \):

\[
\sigma_B = \sigma_H \left[ \cosh \frac{\langle T \rangle}{L} + \sqrt{\Lambda_b} \tanh \left( \frac{\langle R_{\text{duct}} \rangle - \langle T \rangle}{L/\sqrt{\Lambda_b}} \right) \sinh \frac{\langle T \rangle}{L} \right]. \tag{12}\]
Similarly, the mean oxygen value across the viable rim is
\[
\langle \sigma \rangle = \sigma_H \frac{L}{\langle T \rangle} \left[ \sqrt{\Lambda_b} \tanh \left( \frac{\langle R_{\text{duct}} \rangle - \langle T \rangle}{L/\sqrt{\Lambda_b}} \right) \left( \cosh \frac{\langle T \rangle}{L} - 1 \right) + \sinh \frac{\langle T \rangle}{L} \right].
\] (13)

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.

4.3 Population Dynamics:

By the analysis of the steady-state ODEs for PI and AI in Section 3 in Part I (Macklin et al., 2011), given \( \tau_P, \tau_A, \) PI and AI, we obtain \( \langle \alpha_P \rangle \) and \( \alpha_A \) via:

\[
\langle \alpha_P \rangle = \frac{1}{\tau_P} \left( PI + PI^2 \right) - \frac{1}{\tau_A} AI \cdot PI \left( 1 - AI - PI \right)
\] (14)
\[
\alpha_A = \frac{1}{\tau_A} \left( AI - AI^2 \right) + \frac{1}{\tau_P} AI \cdot PI \left( 1 - AI - PI \right)
\] (15)

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

\[
\langle \alpha_P \rangle = \frac{\langle \sigma \rangle - \sigma_H}{1 - \sigma_H}.
\] (16)

4.4 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 via

\[
s = \sqrt{\frac{2}{\sqrt{3} \langle \rho \rangle}}.
\] (17)

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 \( r = s \):
\[
E_i \frac{\alpha_{\text{cca}}}{\alpha_{\text{ccr}}} \frac{\partial}{ \partial r} \psi \left( s; R_N^i + R_N^j, R^i + R^j, M, n_{\text{ccr}} \right) \bigg\vert \\
= \frac{\partial}{ \partial r} \varphi \left( s; R_{\text{cca}}^i + R_{\text{cca}}^j, n_{\text{cca}} \right) \bigg\vert \\
= \left( 1 - \frac{s}{R + R} \right)^{n_{\text{ccr}}+1} \left( 1 - \frac{s}{R_{\text{cca}} + R_{\text{cca}}} \right)^{n_{\text{cca}}+1}. 
\] (18)

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 \(E = 1\) for both cells, then this simplifies to

\[
\frac{\alpha_{\text{cca}}}{\alpha_{\text{ccr}}} = \frac{\left( 1 - \frac{s}{2R} \right)^{n_{\text{ccr}}+1}}{\left( 1 - \frac{s}{2R_{\text{cca}}} \right)^{n_{\text{cca}}+1}}. 
\] (19)

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 the estimate of \(\alpha_{\text{ccr}}\) from Section 3.6 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).

4.5 Cell-BM Mechanics:

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

5 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
<table>
<thead>
<tr>
<th>Quantity</th>
<th>Measurement (Mean ± std. dev.)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duct Radius $R_{duct}$</td>
<td>170.11 ± 76.37</td>
<td>µm</td>
</tr>
<tr>
<td>Viable Rim thickness $T$</td>
<td>76.92 ± 12.51</td>
<td>µm</td>
</tr>
<tr>
<td>PI</td>
<td>17.43 ± 9.25</td>
<td>%</td>
</tr>
<tr>
<td>raw AI</td>
<td>0.638 ± 0.424</td>
<td>%</td>
</tr>
<tr>
<td>corrected AI</td>
<td>0.831 ± 0.553</td>
<td>%</td>
</tr>
<tr>
<td>Cell density $\rho$</td>
<td>0.003213 ± 5.95e-4</td>
<td>cells/µm²</td>
</tr>
<tr>
<td>Nuclear diameter $2R_N$</td>
<td>8.48 to 12.70 (typical range)</td>
<td>µm</td>
</tr>
</tbody>
</table>

Table 3
Key data for anonymised case 100019 in Edgerton et al. (2011).

100019) from Edgerton et al. (2011)⁷. 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 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. 1. 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 3. We point out that the variation in patient data is the combined effect of measurement errors and genuine intratumoural heterogeneity; see the discussion in Section 4 in Part I.

This case had no measurements of $f$, so we approximate it as solid-type with fully-confluent cells ($f \approx 1$). We set the “universal” parameters as determined in Section 3. By applying the calibration protocol in Section 4 to these values and the patient-specific data, we obtain the parameter values in Table B.1.

5.1 Verification of the Calibration

To verify the success of the calibration, we ran a simulation using the numerical methods in Section 6 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 7.) Full post-processing source code is described in the supplementary material and provided at the MultiCellXML project website⁸.

⁷ Note: Preliminary data used here may deviate slightly from the finalised measurements published by Edgerton et al. (2011).
⁸ http://multicellxml.sourceforge.net/
Fig. 1. **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.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Patient Data</th>
<th>Simulated: $\tau_{G1} = 9\text{ hr}$</th>
<th>Simulated: $\tau_{G1} = 1\text{ min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI (%)</td>
<td>17.43 ± 9.25</td>
<td>24.04 ± 4.587</td>
<td>18.25 ± 4.25</td>
</tr>
<tr>
<td>AI (%)</td>
<td>0.831 ± 0.572</td>
<td>0.7378 ± 0.7146</td>
<td>1.204 ± 0.1102</td>
</tr>
<tr>
<td>Viable rim thickness (µm)</td>
<td>76.92 ± 12.51</td>
<td>80.73 ± 1.10</td>
<td>80.57 ± 1.68</td>
</tr>
<tr>
<td>Cell density (cells/µm²)</td>
<td>0.003213 ± 5.95e-4</td>
<td>0.002950 ± 6.09e-5</td>
<td>0.002923 ± 7.67e-05</td>
</tr>
</tbody>
</table>

Table 4

**Verification of the patient-specific calibration:** Comparison of the patient (second column) and computed (third and fourth columns) 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.

In Table 4, 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. 2 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
Simulated statistics (left bars) vs. patient data (right bars)

(All bars are mean ± standard deviation)

Fig. 2. **Verification of the patient-specific calibration:** We compare the simulated (left bars) and patient (right bars) PI (column one), AI (column two), cell density (column three), and viable rim thickness (column four) over the last 15 days of our simulation. Notice that (1) the bars overlap for each datum, and (2), the simulated mean (left triangles) are within the patient variation for each datum. Hence, the calibrated model matches the calibration data within tolerances.

cells, we expect considerable variability; indeed, this is observed in the patient AI as well. Because all the numerical targets (outlined in Table 3) are within the range of patient variation, the calibration can be considered as successful.

5.2 **Analysis of calibration discrepancies**

To understand the discrepancy in the mean PI between our simulation and the patient data for future improvement in our calibration protocols, we examined our proliferation model more closely. Our protocol was based upon an earlier version of the proliferation “sub-model”, where the cells do not divide until spending $\tau_P$ time in the cell cycle (Macklin et al., 2009a); in our current model, cells divide after $\tau_P - \tau_{G_1}$ and continue cycling and growing for $\tau_{G_1}$ time. For a given set of $\alpha_A$ and $\alpha_P$ parameters, this should increase the simulated proliferative index. To test this, we first modify our system of ODEs to include $P_{SG_2M}$ (cycling cells in S, G2, and M phases), $P_{G_1}$ (cycling cells in G1 phase), $A$ (apoptotic cells), and $Q$ (quiescent cells in $G_0$):
\[
P_{SG2M} = \langle \alpha_p \rangle Q - \frac{1}{\tau_P - \tau_{G1}} P_{SG2M}
\]

\[
\dot{P}_{G1} = \frac{2}{\tau_P - \tau_{G1}} P_{SG2M} - \frac{1}{\tau_{G1}} P_{G1}
\]

\[
A = \alpha_A Q - \frac{1}{\tau_A} A
\]

\[
\dot{Q} = \frac{1}{\tau_{G1}} P_{G1} - (\langle \alpha_p \rangle + \alpha_A) Q
\]

If \( P = P_{SG2M} + P_{G1} \) and \( N = P + A + Q \), then

\[
PI = \frac{P}{N}, \quad \text{and} \quad AI = \frac{A}{N}.
\]

If we solve the system for \( 0 \leq t \leq 720 \) hours with \( Q(0) = 1 \) and \( A(0), P(0) = 0 \) with the parameter values in Table B.1, then we should be able to predict the simulation’s mean AI and PI. By this calculation, the simulated PI and AI should approach 24.45\% and 0.761\%, respectively; both of these limits match our simulated mean PI (24.04\%) and AI (0.738\%) very well.

Conversely, setting \( \tau_{G1} = 1 \) min minimises the impact of the G1 phase, and the simulated PI matches the calibration target very closely; see Table 4. Because we can fully account for the discrepancy between the patient and simulated data with our improved understanding of the model, we can safely conclude that the calibration is performing well, and should match patient data exceedingly well once taking into account the division of the cell cycle into volume growth (G1) and non-growth (S-G2-M) phases. This provides new potential for matching bimodal distributions of cell sizes and cell cycle states often observed \textit{in vitro} (e.g., Tzur et al. (2009)). We plan improvements to our calibration based upon these observations in ongoing work.

5.3 Testing biological hypotheses: heterogeneity in PI is not solely due to oxygenation gradients; apoptosis is a “background” process

Because our calibration protocol only prescribed the mean behaviour, we can use the simulated variation to test the model’s underlying biological hypotheses. First, we note that the simulated variation in PI is significantly smaller than the actual variation; this suggests that our current model (which varies proliferation solely with the local level of oxygenation) does not fully characterise the signalling mechanisms responsible for heterogeneity in DCIS proliferation. This is therefore additional confirmation of our hypothesis in Part I that while oxygenation dominates the variation in proliferation, other signalling mechanisms (e.g., contact inhibition by E-cadherin/\( \beta \)-catenin pathways) play a significant role in controlling DCIS proliferation as well.
In Part I, we assumed that apoptosis occurs at a low “background” rate that is independent of oxygenation and any other signalling, and hence occurs with probability that is independent of position within the viable duct. We see that our standard deviation in AI is quite similar to the actual standard deviation in the patient AI, which supports this biological hypothesis. In fact, the patient’s mean and standard deviation are of comparable magnitude, which is consistent with an exponentially-distributed random variable, just as in our model.

6 Numerical methods

We implement the model using object-oriented ANSI C++, where each agent is an instance of a Cell class. Each cell object is endowed with an instance of a CellState class, which contains the cell phenotypic parameters ($\alpha_P$, $\alpha_A$, $\tau_P$, etc.), volumes ($V_S, V_N, V$), radii ($R_N, R$), maximum interaction distances ($R_{cca}, R_{cba}$, recorded as multiples 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 an auxiliary level set function (see Part I), 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). Full details of the computational algorithm are given in the supplementary material.

6.1 MultiCellXML data format

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 (using standardised XML parsers, such as Expat (Clark, 2007), xmlParser (Berghen, 2009), and TinyXML (Thomason et al., 2010)) for use in data 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. Modifying simulation parameters during a simulation can be readily achieved with 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 our open data format in the supplementary material and at the
MultiCellXML project website\(^9\), and we include (1) sample data sets and (2) open source C++ code for post-processing and visualisation based upon the data format. We put forth our data format as a potential draft for inclusion in the standard being developed by Sluka et al. (2011).

### 7 Patient-calibrated DCIS simulation: Hypothesis testing and model validation by clinical data

We now simulate DCIS in patient 100019 using the “universal” parameters in Section 3; the patient-specific parameters are as in Section 4. The dynamic simulation is presented in Fig. 3. 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 gray 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 (\(C\)). An animation of this simulation is available as Video S1 in the supplementary material.

#### 7.1 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 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 \(\mu\)m) can be observed, demonstrating the overall oxygen gradient decreasing from \(\sigma_R\) 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. On the other hand, apoptosis occurs with approximately uniform distribution throughout the duct. See 7 days and onward in Fig. 3. These spatiotemporal dynamics emerge by 7 to 14 days and remain throughout the simulation. This is consistent with the prediction in Part I that the cell state dynamics reach a local steady state by 10 to 100 days in regions away from the leading edge.

\(^9\) [http://multicellxml.sourceforge.net](http://multicellxml.sourceforge.net)
Fig. 3. 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 gray until they lyse, after which their solid 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 (C). Simulated times (from top to bottom): 0, 7, 14, 21, and 28 days. Bar: 100 µm. A colour version of this figure is available in the online edition.
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. 3 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 7.2.

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. 3.) This phenomenon is observed in stained tissue slides. See Fig. 1 and Section 7.4. It has been attributed to dehydration, but it was unclear whether the dehydration is an artifact 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).
Fig. 4. **Tumour and microcalcification positions in the duct:** Left: The top curve plots the maximum position of viable tumour tissue; the second curve (from the top) plots the maximum calcification position. The lighter lines are the least-squares fitted lines of the tumour advance from 11 to 45 days. Right: Plot of $\log_{10}(x_V)$ from 24 to 35 days, demonstrating exponential tumour advance when the mechanical relaxation mechanism is partly exhausted.

### 7.2 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. 4: left, 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. 5.

From 11 to 24 days, $x_V$ increases linearly at 27.48 $\mu$m/day (obtained by least-squares linear fitting). The constant rate of tumour advance, which notably occurs without need for modelling contact inhibition, 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. 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. 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.

By 24 days, a significant portion of the necrotic core has filled with debris, thereby exhausting the lysis-based mechanical relaxation mechanism in those regions. (See 24 days in Fig. 5.) Because this model does not incorporate contact inhibition, cells in these regions continue to proliferate. With little interior mechanical relaxation possible, the majority of this flux is now directed towards the tumour leading edge, leading to exponential (rather than linear) tumour advance through the duct. See the plot of $\log_{10}(x_V)$ in Fig. 4: right; the linear curve indicates near-exponential tumour advance. However, there is a negative feedback on this behaviour: as exponential growth pushes cells beyond the filled portion of the necrotic core, these can now flux into the lumen as before. The rate of tumour advance decelerates, and the tumour resumes its linear progression. We anticipate that this cycle continues throughout the duration of growth, leading to (on average) linear tumour advance through the duct. Indeed, from 11 to 45 days, $x_V$ fits a linear least-squares curve (lighter
line in Fig. 4: left) quite well, advancing roughly 27.97 \( \mu m \) per day.

This oscillatory phenomenon, where the tumour alternates between exponential and (sub-)linear growth, is partly an artifact of neglecting cell-cell contact inhibition. We anticipate that incorporating E-cadherin/\( \beta \)-catenin signalling dynamics (e.g., as by Ramis-Conde et al. (2008a,b)) would reduce or eliminate the effect, because proliferation would cease in regions near densely-packed necrotic cores. Nonetheless the rate of tumour advance in the duct–27.97 \( \mu m/day \), or 10.2 mm per year–is consistent with DCIS growth estimates obtained by analysis of mammograms. 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 \( \tau_{G1} = 1 \) min (for a better fit to the patient \( \langle PI \rangle \)–see Section 5.2) yields a rate of tumour advance of 7.86 mm per 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.

7.3 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
Fig. 6. **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.

from visible DCIS-associated microcalcifications to the desired surgical margin can be estimated from a mammographic image. (This requires that the microcalcifications are confirmed to arise from DCIS and are not benign.)

In Fig. 6, 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. 7: 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 $x_C$:

$$x_V \approx 0.4203 + 1.117x_C \text{ mm.} \quad (25)$$

We plot this against our simulated DCIS data (blue points) and the data (red squares) from de Roos et al. (2004) in Fig. 7: 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. 7. **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), giving further evidence of our model’s predictivity.

Fig. 8. **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: Further 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 µm. Cells are coloured as in Fig. 3. A colour version of this figure is available in the online edition.

7.4 **Comparison of the simulated necrotic core microstructure with histopathology – predicting the tumour size from histopathology appearance**

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. 8, 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 \( \langle P_l \rangle / \tau_p \approx \langle A_l \rangle / \tau_A \), 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. 9).

Moving farther from the leading edge in Slice d, we see a very 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. 9 and the left duct in Fig. 1). Farther still 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. 9. 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.

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: (1) a viable rim with greatest proliferation towards the basement membrane, (2) a gap or mechanical separation between the viable rim and necrotic core, (3) an outer band of the necrotic core with relatively intact necrotic nuclei, (4) an inner necrotic band of relatively degraded nuclei, and (5) a central core of microcalcification. The closer a cross section is to the leading edge, the fewer of these elements it contains.
Fig. 9. **H&E staining of DCIS in several ducts** in case 100019. In each labelled duct, a readily visible outer viable rim (with faintly hematoxylin-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 necrotic 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 necrotic 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.

This leads us to hypothesise that the microstructure of a given duct cross section in a histopathology slide can 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. More importantly, if the model can be calibrated with 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 allowing more accurate surgical planning.

8 **Further simulation studies**

To gain a deeper understanding of the biomechanics underlying the model’s predictions, we now turn our attention to focused parameter studies.
Fig. 10. The tumour advances exponentially (red and blue curves) until the viable rim is well-established and cell lysis has begun. Thereafter, the tumour advances through the duct at a constant rate (magenta curves).

### 8.1 Necrotic cell lysis is critical to linearity of tumour advance

In Section 7, we found that necrotic cell lysis acts as a mechanical stress relief, and leads to a constant rate of tumour advance through the duct. To better understand this effect, we varied the necrotic cell lysis time scale $\tau_{NL} \in \{2, 6 \text{ hours}, 1, 5, 15 \text{ days}\}$, with all other parameters as in the baseline simulation. To characterise the impact, we first examine the time evolution of the maximum tumour cell extent $x_V$ for $\tau_{NL} = 15 \text{ days}$. See Fig. 10: left.

For the first 6 days, there is no necrosis, and the tumour grows exponentially; see the plot of $x_V$ (black and white curve) versus the red fitted exponential curve on $[0, 6]$ in Fig. 10: left. (All exponential fits are linear least squares fits to $\log_{10} x_V$.) At 6.08 days, the first cells necrose, and the viable region undergoes a topological change, splitting into upper and lower viable rims; in 3D, this would correspond to a hollow tube. Once this topological change is well-established (by approximately 10 days), growth continues exponentially at a lower rate; see the blue fitted exponential curve on $[10, 21]$ in Fig. 10: left. At 21.04 days (15 days after the first instance of necrosis), the necrotic cells begin to lyse, and the tumour’s growth becomes linear as discussed earlier; see the magenta fitted line in Fig. 10: left on $[21, 22.08]$. At 22.08 days, the cells reach the edge of the computational domain at 1 mm.

The dynamic is the same for $\tau_{NL} = 5 \text{ days}$: growth is exponential at a high rate until the first instance of necrosis around 6 day; see the red fitted exponential curve on $[0, 6]$ in Fig. 10: right. From 6 days to approximately 10 days, the tumour viable region is undergoing a topological change to a hollow tube; this can be observed by its transitional behaviour from 6 to approximately 10 days. The first necrotic cells begin to lyse at 11 days, and the tumour growth is linear until cells leave the simulation domain (1 mm) around 27 days; see
For sufficiently small necrotic cell lysis times ($\tau_{NL}$), the tumour’s linear advance is roughly identical, with linear growth after the onset of necrotic cell lysis (around 5 to 6 days) that becomes clearer once the viable rim topology is well-established (around 10 days).

the magenta fitted line in Fig. 10: right. Note that while growth is linear from 11 to 15 days, it appears to be at a faster rate, due to the dominance of the unlysed necrotic cells for these earlier times.

For lysis times under 1 day, growth is exponential for approximately the first 5 to 6 days, followed by a transitional period from approximately 6 to 10 days while the viable rim undergoes its topological change. Linear growth follows from 10 days until the end of the simulation at 30 days. This is the expected dynamic, given that that necrotic cells begin lysing well before the end of the viable rim topological change. See Fig. 11. Note that all three tumours advance at approximately the same rate.

8.2 Inverse relationship between duct radius and rate of tumour advance

The tumour undergoes temporary periods of exponential advance whenever the necrotic mechanical relaxation mechanism is partly exhausted (as the duct lumen fills with necrotic cellular debris). This leads us to hypothesise 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 much 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 in Section 7, in virtual ducts ducts with $R_{duct} \in \{100, 125, 150, 170.11\}$. 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. 12. All simulations had $\langle PI \rangle$ between 22 and 24%, and mean viable rim
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.

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

\[ x'_V = a + e^{b - c R_{\text{duct}}} \]  

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

\[ x'_V \approx 20.52 + e^{6.085 - 0.02584 R_{\text{duct}}} \mu m/day. \]  

See the red fitted curve in Fig. 12. Notice that as \( R_{\text{duct}} \to \infty \), 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.
In this work, we applied an agent-based model (developed in Part I) to ductal carcinoma in situ (DCIS) of the breast, making numerous advances over preceding work. We are the first to model necrotic cell calcification. We provide the most detailed model of necrosis to date—including the impact of time scales ranging from hours to weeks—to better understand the microstructure of the necrotic core. These biophysics impact how a DCIS tumour advances through a duct. We developed the first model calibration protocol to use immunohistochemical and histopathologic measurements to simulate cancer in *individual patients*—an advance that could improve patient-tailored surgical planning. By combining our volume-averaged analysis of the phenotypic state space network with rigorous estimates of several key time scale parameters, we can calibrate to data from a *single time point*—thereby eliminating the need to estimate derivatives from noisy patient image data. This calibration technique is broadly applicable to current cutting-edge agent-based models.

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 consistent with the clinical literature. We predict that the difference between the mammographic and pathological tumour size increases slowly and linearly with time; this, too, is in agreement with clinical data. 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.

An analysis of the simulated standard deviation in the proliferative index shows that proliferation is governed by multiple limiting factors, and not by oxygen (or glucose) alone. Heterogeneous cell signalling (e.g., E-cadherin/β-catenin) must play a significant role in determining the fine details of proliferation. This is consistent with our analysis of proliferation in multiple breast ducts in Part I. On the other hand, the standard deviation of the tumour apoptotic index closely matched our simulation, suggesting little apoptotic signalling heterogeneity across the tumour.

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 under-
lying 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 in the mean, they cannot make quantitative predictions on DCIS in specific patients. Mechanistic models, on the other hand, have this potential when rigorously calibrated.

The model ties the tumour’s advance through a duct to its biomechanical underpinnings. The constant rate of DCIS advance results from the balance of cell outflux from the viable rim (arising from proliferation) with mechanical relaxation in the necrotic core (due to cell lysis). The rate of cell outflux is determined by the net balance of proliferation and apoptosis, which, in turn, depends upon oxygen availability and heterogeneities in tumour cell signalling. The rate of mechanical relaxation depends upon the time required for cell lysis, the fluid fraction of cells (which is lost during cell lysis), and the size of the duct. Based upon the emergent relationship between the rate of tumour advance and the duct radius, the model predicts that any tumour should have a minimal, nonzero rate of growth. Interestingly, DCIS growth is fastest in small ducts with less potential for necrotic mechanical stress relaxation. However, small ducts as terminal ductal/lobular units anatomically merge into ducts of larger diameter, thus rapidly reaching ducts of typical size as exemplified by Fig. 9.

The model 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 artifacts. 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. This stratified 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. These features are all observed in patient images, as illustrated in Fig. 9.

Our work has important clinical applications. 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 would 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.
Furthermore, it may also improve estimates of the extent of DCIS based upon the appearance of the necrotic core microstructure in histopathology samples (e.g., the percentage of the necrotic core that is calcified, the mean level of nuclear degradation, etc.).

In future work, we plan to refine our proliferation model by including cell contact inhibition through the E-cadherin/β-catenin pathway, similarly to recent work by Ramis-Conde et al. (2008a,b). We also plan to investigate the impact of cell growth in the G₂ phase. In the necrosis model, we plan to introduce a new time scale for nuclear degradation and improve the calcification model by describing the formation of calcium crystals in phospholipids (e.g., in the cell membrane and degraded organelles) that remain after nuclear degradation. Improvements in these sub-models will be accompanied by advances in the model calibration to account for (bimodal) variations in the cell size throughout the cell cycle. We plan to test our calibration method against further examples of resected DCIS.

We chose DCIS as our initial modelling testbed because (1) it is a clinically and scientifically significant problem in and of itself, (2) it is tractable to patient-specific simulation with currently-available data, and (3) its proper modelling is a necessary step in modelling later progression to invasive ductal breast carcinoma (IC). In future work, we plan to integrate molecular-scale models of ErbB-family pathways necessary to invasive transformation, HIF-1α and related pathways for improved assessment of hypoxic stress and glycolysis, basement membrane deformation and degradation, and cell motility. These additions will allow us to extend our investigations to IC. Because the physics and biology we have incorporated in the cell agents are fundamental, our model is broadly applicable to most in situ cancers and sarcomas; this is a strength of a truly mechanistic approach. With further enhancements, the model should apply to invasive carcinomas as noted above, and biological problems where cell morphology and polarisation are significant (e.g., developmental biology, squamous cell carcinomas, and cribriform-type DCIS). Work is currently underway to integrate computationally-feasible individual cell morphologies.

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 (see the preliminary work by Edgerton et al. (2011) and the reviews by Lowengrub et al. (2010) and Deisboeck et al. (2010)), 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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P. Macklin, M. E. Edgerton, J. Lowengrub, and V. Cristini. Discrete cell


A  “Universal” Parameter Estimates

The key “universal” model parameters—those common to all simulations, as estimated in Section 3, are summarised in Table A.1 for reference.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Physical Meaning</th>
<th>Value</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tau_P$</td>
<td>cell cycle time</td>
<td>18 hours</td>
<td>3.1</td>
</tr>
<tr>
<td>$\tau_{G1}$</td>
<td>length of G1</td>
<td>9 hours</td>
<td>3.1</td>
</tr>
<tr>
<td>$\tau_A$</td>
<td>apoptosis time</td>
<td>8.6 hours</td>
<td>3.2</td>
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<tr>
<td>$\tau_{NL}$</td>
<td>necrotic cell lysis time</td>
<td>6 hours</td>
<td>3.3</td>
</tr>
<tr>
<td>$f_{NS}$</td>
<td>necrotic cell volume increase</td>
<td>1.0</td>
<td>3.3</td>
</tr>
<tr>
<td>$\tau_C$</td>
<td>necrotic debris calcification time</td>
<td>15 days</td>
<td>3.4</td>
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<tr>
<td>$L$</td>
<td>oxygen diffusion length scale</td>
<td>100 $\mu$m</td>
<td>3.5</td>
</tr>
<tr>
<td>$\langle \lambda \rangle$</td>
<td>mean cell oxygen uptake rate</td>
<td>0.1 min$^{-1}$</td>
<td>3.5</td>
</tr>
<tr>
<td>$\sigma_H$</td>
<td>hypoxic oxygen threshold</td>
<td>0.2</td>
<td>3.5</td>
</tr>
<tr>
<td>$V_s/V$</td>
<td>cell solid fraction</td>
<td>0.1</td>
<td>3.6</td>
</tr>
<tr>
<td>$R_{cca}$</td>
<td>maximum cell-cell adhesion distance</td>
<td>1.214 $R$</td>
<td>3.6</td>
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<tr>
<td>$R_{cba}$</td>
<td>maximum cell-BM adhesion distance</td>
<td>$R_{cca}$</td>
<td>3.6</td>
</tr>
<tr>
<td>$\alpha_{ccr}$</td>
<td>cell-cell repulsive force coefficient</td>
<td>10.0$\nu$ $\mu$m/min</td>
<td>3.6</td>
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<td>$\alpha_{cbr}$</td>
<td>cell-BM repulsive force coefficient</td>
<td>$\alpha_{ccr}$</td>
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<td>$n_{cca}$</td>
<td>cell-cell adhesion potential exponent</td>
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<td>3.6</td>
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<tr>
<td>$n_{ccr}$</td>
<td>cell-cell repulsion potential exponent</td>
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<td>cell-BM adhesion potential exponent</td>
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<td>cell-BM repulsion potential exponent</td>
<td>$n_{cbr}$</td>
<td>3.6</td>
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<tr>
<td>$M$</td>
<td>maximum value of $</td>
<td>\nabla \psi</td>
<td>$</td>
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Table A.1

“Universal” parameters shared by all simulations in the DCIS model.
The key patient-specific model parameters, as estimated in Section 4, are summarised in Table B.1 for reference.

<table>
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<th>Parameter</th>
<th>Physical Meaning</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>$R$</td>
<td>cell radius</td>
<td>9.953 µm</td>
</tr>
<tr>
<td>$R_N$</td>
<td>cell nuclear radius</td>
<td>5.295 µm</td>
</tr>
<tr>
<td>$\lambda_p$</td>
<td>oxygen uptake rate for proliferating cells</td>
<td>$0.1 \text{ min}^{-1}$ if $\lambda_p = \lambda_{np}$, $0.38930 \text{ min}^{-1}$ if $\lambda_p = 10\lambda_{np}$, $0.54777 \text{ min}^{-1}$ if $\lambda_p = 100\lambda_{np}$</td>
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<tr>
<td>$\lambda_{np}$</td>
<td>oxygen uptake rate for non-proliferating, viable cells</td>
<td>$0.1 \text{ min}^{-1}$ if $\lambda_p = \lambda_{np}$, $0.038930 \text{ min}^{-1}$ if $\lambda_p = 10\lambda_{np}$, $0.005478 \text{ min}^{-1}$ if $\lambda_p = 100\lambda_{np}$</td>
</tr>
<tr>
<td>$\lambda_b$</td>
<td>oxygen uptake/decay rate for non-viable cells and background</td>
<td>$0.001 = 0.01 \langle \lambda \rangle \text{ min}^{-1}$</td>
</tr>
<tr>
<td>$\sigma_B$</td>
<td>oxygen boundary value</td>
<td>0.263717 if $\lambda_b = 0.01\langle \lambda \rangle$</td>
</tr>
<tr>
<td>$\langle \sigma \rangle$</td>
<td>mean oxygen level in viable rim</td>
<td>0.221065 if $\lambda_b = 0.01\langle \lambda \rangle$</td>
</tr>
<tr>
<td>$\langle \alpha_P \rangle$</td>
<td>mean $Q \rightarrow P$ transition rate</td>
<td>0.013705 hour$^{-1}$</td>
</tr>
<tr>
<td>$\sigma_P^{-1}$</td>
<td>mean waiting time prior to $Q \rightarrow P$ transition in normoxic tissue ($\sigma = 1$)</td>
<td>115.27 min if $\lambda_b = 0.01\langle \lambda \rangle$</td>
</tr>
<tr>
<td>$\alpha_A$</td>
<td>$Q \rightarrow A$ transition rate</td>
<td>0.00127128 hour$^{-1}$</td>
</tr>
<tr>
<td>$s$</td>
<td>cell spacing</td>
<td>18.957 µm</td>
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<tr>
<td>$\alpha_{cca}$</td>
<td>cell-cell adhesive force coefficient</td>
<td>0.0488836 $\alpha_{ccr}$</td>
</tr>
<tr>
<td>$\alpha_{cba}$</td>
<td>cell-BM adhesive force coefficient</td>
<td>0.1, 1, 10 or 100 times $\alpha_{cca}$</td>
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Table B.1
Patient-specific parameters for the DCIS model.