6 Discrete cell modeling

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In this chapter, we: introduce discrete cancer cell modeling; assess the strengths and weaknesses of available discrete cell modeling approaches; sample the major discrete cell modeling approaches employed in current computational cancer modeling; and introduce a discrete, agent-based cell modeling framework currently being developed by the authors and collaborators that will be used to implement a next-generation, multiscale cancer modeling framework as detailed in Chapter 7.

6.1 A brief review of discrete modeling in cancer biology

Thus far, we have discussed continuum modeling efforts that model cancer at the tissue scale and average out the effects of individual cells. We now turn our attention to discrete models that address the behavior of one or more individual cells as they interact with one another and the microenvironment.

Discrete modeling has enjoyed a long history in applied mathematics and biology, dating as far back as the 1940s when John von Neumann applied lattice crystal models to study the necessary rulesets for self-replicating robots [677]. Perhaps the most famous early example of discrete biological modeling is John Conway’s 1970 “game of life,” a 2-D rectangular lattice of “cells” that changed colors according to rules based upon the colors of the neighboring cells [261]. Even simple rules can lead to complex emergent behavior, and Conway’s model was later shown to be Turing complete [598]. Today, discrete cell modeling has advanced to study a broad swath of cancer biology, spanning carcinogenesis, tumor growth, invasion, and angiogenesis.

Discrete, or individual-based, models are generally divided into two categories: lattice-based (including cellular automata) and lattice-free (agent-based). Both approaches track and update individual cells according to a set of biophysical rules. Typically these models involve a composite\(^1\) discrete-continuum approach

\(^1\) The agent model presented in this chapter is an extension of the work by Macklin et al. (2009) [435], and an advance copy of the work to be submitted by Macklin et al. in [436].

\(^2\) Many refer to these as hybrid models, but we reserve the term for models that simultaneously combine discrete and continuum representations for cells.
in the sense that microenvironmental variables (glucose, oxygen, extracellular matrix, growth factors, etc.) are described using continuum fields while the cells are discrete. See the reviews by Aber et al. [11], Moreira and Deutsch [478], Drasdo [183], Araujo and McElwain [36], Quaranta et al. [556, 555], Hatzikiriou et al. [309], Nagy [493], Abbott et al. [2], Byrne et al. [98], Fasano et al. [210], Galle et al. [253], Drasdo and Höhme [187], Thorne et al. [659], Anderson et al. [29], Deisboeck et al. [170], Anderson and Quaranta [30], and Zhang et al. [723]. We now review these two main approaches and give some key examples from the literature. Further review can be found in [427].

6.1.1 Lattice-based models

In lattice-based modeling, the cells are confined to a regular 2-D or 3-D lattice. Each computational mesh point is updated in time according to deterministic or stochastic rules derived from physical conservation laws and biological constraints. Some models use a high-resolution mesh to discretize the cells and the surrounding microenvironment with subcellular resolution, allowing a description of the cells’ finite sizes, morphologies, and biomechanical interactions. Cellular automata (CA) models, which describe each cell with a single computational mesh point, can be viewed as a special case of the approach.

The simple spatial arrangement of lattice-based models is relatively easy to implement, rendering them broadly accessible beyond the traditional scientific computing communities with less need for advanced computational expertise. The regular structure imposed by the computational mesh also eliminates the need for elaborate interaction testing between the discrete cells. This is particularly true for CA models on rectangular meshes, where cell-cell interaction is based upon the immediate neighboring mesh points. It is also straightforward to directly couple lattice-based methods to the microenvironment by assigning continuum variables to every mesh point (in the case of CA methods) or a coarsened sub-mesh (in the more general case).

The uniform spacing of lattice-based methods can be a weakness. While computationally efficient, low-resolution lattices (CA methods) can impose artificial constraints on the arrangement, orientation, and interaction of the cells, which can sometimes be observed as square or diamond-like artifacts. Such low-resolution lattice-based models cannot capture non-lattice cell patternings often found in both normal tissue (e.g., hepatic lobules) and cancer (e.g., cribriform ductal carcinoma in situ, or DCIS). Because the cells only have several possible orientations, these models can only crudely treat cell polarization; cell mechanics can only be modeled with great difficulty. Hence, low-resolution models are poorly-suited to the rigorous exploration of the balance of cell-cell adhesion, cell-BM adhesion, and cell mechanics (e.g., deformability). High-resolution (subcellular) meshes can better approximate cell mechanics, but are much more computationally expensive, hence impeding their ability to describe large systems of cells and microenvironment.
6.1.2 Lattice-free models

Lattice-free models, frequently referred to as agent-based models, do not restrict the cells’ positions and orientations in space. This allows a more complex and accurate coupling between the cells and their microenvironment, and imposes fewer artificial constraints on the behavior of multicellular systems. The cells are treated as distinct objects or agents and are allowed to move, divide, and die individually according to biophysically-based rules. For example, many models apply free-body force diagrams to the individual cells, allowing a mechanistic description of cell-cell and cell-ECM interactions. The level of detail in the cell size, volume, and morphology can vary from simple (e.g., infinitesimal points in 3-D space, such as in [1, 59, 427, 229]) to complex (e.g., evolving deformable spheres that develop cusps during mitosis, as in [188]). The agent interpretation of the cells makes modern object-oriented programming languages (e.g., C++ and Java) ideal for implementing these models.

Agent-based models are ideal for situations of freely-wandering and nonuniformly arranged cells, such as angiogenesis, carcinogenesis, immune system attacks on tumor cells, and metastasis. The level of detail of the agents can be tailored to the simulation. Each cell agent can be assigned individual protein and surface receptor signaling networks, a model of cell cycle progression, and genotypic and phenotypic characteristics; this makes agent-based modeling a powerful tool in multiscale frameworks. (Some agent-based models are restricted to motion on a regular lattice to save on computational cost. See [721, 722] for some examples.) The flexibility in detail, at times even down to the biochemical level, can make agent-based models easier to calibrate to biological data.

This flexibility comes at a price, however. Because each individual cell can be made almost arbitrarily complex, computational cost can be very high for non-lattice, agent-based models, limiting the approach to smaller systems of cells. The lack of a regular cell arrangement makes cell-cell interaction testing computationally expensive as well. In the worst case where there are $n$ cell agents that may each interact with all $n$ cells and there are no constraints on cell placement, one must test $n^2$ possible cell-cell interactions for each computational time step. In such a worst case, computational cost increases rapidly as more cells are added to the multicell system, rendering large-scale simulations infeasible.

6.1.3 Comparison with continuum methods

Continuum modeling can be too coarse-scaled to capture the spatial intricacy of tissue microarchitecture when cells are polarized (with visible apex, base, and anisotropic surface receptor distributions), or during individual cell motility. Furthermore, continuum models tend to lump multiple physical properties into one or two phenomenological parameters. For example, the models by [230, 439] lump cell-cell, cell-BM, and cell-ECM adhesion, motility, and ECM rigidity into a single “mobility” parameter, as well as forces on the tumor boundary. While
this eases the mathematical analysis of the physical systems, it can be difficult to directly match such lumped parameters to physical measurements. Some key patient-specific measurements occur at the molecular (e.g., immunohistochemistry, or IHC) and cellular scales, i.e., at a finer scale than continuum models.

In contrast, discrete cell models can in some cases be directly matched to such measurements. For example, Zhang, Deisboeck and colleagues have been very successful in tying intracellular signaling models to individual cell phenotype and motility in brain cancer [721]. Their work also made key advances in using molecular and cellular data to inform and calibrate the cell-scale model. Several groups (e.g., [719]) have made considerable advances at linking molecular- and cell-scale models, often with calibration to data at the appropriate scales. This is promising in the context of patient-specific cancer simulation (see Chapter 10) and multiscale modeling, allowing molecular-, cellular-, and tissue-scale data to be matched at their “native” scales; bi-directional data flow subsequently propagates this information to all the scales in the model. See Chapter 7.1, Section 10.4.3, and the Conclusions following Chapter 10 for more on this topic.

Discrete models have some drawbacks when compared with continuum approaches. Because they rely upon the behavior of individual cells to determine emergent system properties, they can be difficult to analyze. In addition, the computational cost of the methods increases rapidly with the number of cells modeled, the lattice resolution (for lattice-based methods), and the complexity of each cell object (for agent models). This can make the models difficult or impossible to apply to large systems, even with parallel programming.

Other difficulties relate to model calibration. Non-local effects (e.g., biomechanics) are often better described by continuum variables, making calibration more feasible at the continuum scale based upon macroscopic measurements. Hybrid modeling (Chapter 7) can address this issue by applying and calibrating discrete and continuum models alongside one another, followed by rigorous information flow between the scales.

Some model parameters, even if clearly related to cell-scale phenomena, may be difficult to measure in controlled experiments, whereas macroscopic measurements based upon the averaged behavior of many cells are simple to measure accurately and match to lumped parameters in continuum models. However, we note that analysis of volume-averaged agent models can sometimes provide further insight on how to motivate and interpret such matching. See Section 6.5 for such an example.

6.1.4 Some discrete modeling examples

We now discuss some discrete modeling examples that sample the range of cell-scale tumor modeling. Our survey is not exhaustive of the immense level of discrete modeling in cancer cell biology; the interested reader is encouraged to examine the reviews listed in Section 6.1, as well as [427].
Composite cellular automata modeling

In an illustrative example of cellular automata modeling, Anderson et al. developed a composite cellular discrete-continuum model of solid tumor growth with microenvironmental interactions [28, 26]. The microenvironmental variables (ECM and MMPs) are continuous concentrations, while tumor cells are discrete cellular automata. Cells move via a biased random walk on a Cartesian lattice; the movement probabilities are generated by discretizing an analogous continuum model of the tumor cell density. The transition probabilities are similar in spirit to the chemotaxis model developed earlier by Othmer and Stevens [512]. The cells respond haptotactically to the ECM density and produce MMPs that degrade the matrix. Cell-cell adhesion was not considered. The model predicts more extensive local tumor invasion in heterogeneous ECM than is predicted by the analogous continuum model.

Building on this work, Anderson et al. extended their model to include cell-cell adhesion by weighing the probability of motion by the number of desired neighbors [26]. Different cell phenotypes are modeled by varying the number of desired neighbors, the proliferation rate, and the nutrient uptake rate. The microenvironment plays an important role in the model: the nutrient supply is assumed to be proportional to the ECM as a model of the pre-existing vasculature, and so matrix degradation disrupts the nutrient supply as well. This model enabled evaluation of how individual cell-cell and cell-ECM interactions may affect the tumor shape. Anderson and co-workers further extended their model to provide a theoretical/experimental framework to quantitatively characterize tumor invasion as a function of microenvironmental selective factors [32]. In the extended model, they considered random mutations among 100 different phenotypes. In agreement with the findings of Cristini and co-workers [151, 725, 147, 231], hypoxia and heterogeneous ECM were found to induce invasive fingering in the tumor margins, with selection of the most aggressive phenotypes.

Gerlee and Anderson simplified this model to investigate complex branched cell colony growth patterns arising under nutrient-limited conditions [274]. In agreement with earlier stability analyses (e.g., [151]), the stability of the growth was found to depend on how far the nutrient penetrates into the colony. For low nutrient consumption rates, the penetration distance was large, which stabilized the growth; for high consumption rates the penetration distance was small, leading to unstable branched growth. After incorporating a feed-forward neural network to model the decision-making mechanisms governing the evolution of cell phenotype, Gerlee and Anderson demonstrated how the oxygen concentration may significantly affect the selection pressure, cell population diversity, and tumor morphology [273, 275]. They further extended this model to study the emergence of a glycolytic phenotype [276]. Their results suggest that this phenotype is most likely to arise in hypoxic tissue with dense ECM. The group has further explored these themes recently with Quara and Rejniak [555]. Similar discrete modeling work on hypoxia has been pioneered in various col-
laborations amongst Gatenby, Smallbone, Maini, Gillies, Gavaghan and others (e.g., [264, 629, 265, 268, 630, 627, 628]).

**Lattice-gas cellular automata models**

Dormann and Deutsch developed a lattice-gas cellular automaton method for simulating the growth and size saturation of avascular multicell tumor spheroids [182, 175]. Unlike traditional cellular automaton methods where at most one cell can be at a single grid point (volume exclusion), lattice-gas models accommodate variable cell densities by allowing multiple cells per mesh point, with separate channels of movement between mesh points. The channels specify direction and velocity magnitude, which may include zero velocity resting states. Lattice-gas models typically require channel-exclusion: only one cell may occupy a channel at any time. Hatzikirou et al. [308] later used this approach to study traveling fronts characterizing glioma cell invasion. Very recently, they performed a mean field analysis of a lattice gas model of moving and proliferating cells to demonstrate that certain macroscopic information (e.g. scaling laws) can be accurately obtained from the microscopic model [308]. Accurate predictions of other macroscopic quantities that sensitively depend on higher-order correlations are more difficult to obtain.

**Immersed boundary model**

In [563, 564], Rejniak developed a highly detailed lattice-based approach to modeling solid tumor growth. Each individual cell is modeled using the immersed boundary method [534, 533] on a regular computational grid. The cell is represented as the interior of an elastic membrane with the nucleus represented as an interior point. Cell-cell adhesion and cell contractile forces are modeled using linear springs to mimic a discrete set of membrane receptors, adhesion molecules and the effect of the cytoskeleton on cell mechanics. The cytoplasm and interstitial fluid are modeled as viscous, incompressible fluids. The elastic, adhesive, and contractive forces impart singular stresses to the fluids. Cell growth is modeled with an interior volume source; once the cell grows to a threshold volume, contractile forces on opposite sides of the cell create a neck that pinches off to produce two approximately equal-sized daughter drops. Nutrient supply is modeled using continuum reaction-diffusion equations, with uptake localized in the cells. The method can describe individual cell morphology but is computationally expensive, thus restricting simulations to about 100 cells. Rejniak and Dillon recently extended the model to better represent the lipid bilayer cell membrane structure as two closed curves connected by springs [567]; sources and sinks placed in the simulated bilipid membrane modeled water channels.

The immersed boundary cell model has been applied to study preinvasive intraductal tumors, as well as the formation and stability epithelial acini (single-layered spherical shells of polarized cells attached to a BM) [567, 565, 566]; genetic mutations that disrupt cell polarity could lead to abnormal acini and ductal carcinoma. The model has also been used to study the interaction between

An extended Q-Potts model
A less detailed lattice-based method has been developed using an extended Q-Potts model, which originated in statistical physics to study surface diffusion grain growth in materials science, as in [34]. Graner and Glazier adapted the Q-Potts model to simulate cell sorting through differential cell-cell adhesion [292, 286]. In this approach, now referred to as the GGH (Graner-Glazier-Hogeweg) model, each cell is treated individually and occupies a finite set of grid points within a Cartesian lattice; space is divided into distinct cellular and extracellular regions. Each cell is deformable with a finite volume. Cell-cell adhesion is modeled with an energy functional. A Monte Carlo algorithm is used to update each lattice point and hence change the effective shape and position of a cell. Although the description of the cell shape is less detailed than in the immersed boundary approach described above, finite-size cell effects are incorporated.

Later work incorporated nutrient-dependent proliferation and necrosis in the GGH model to simulate the growth of benign, multicellular avascular tumors to a steady-state [642]; nutrient transport was modeled with a continuum reaction-diffusion equation. The steady state configuration consisted of a central necrotic core, a surrounding band of quiescent cells, and an outermost shell of proliferative cells; parameters were determined by matching the thickness of these regions to experimental measurements. Reference [667] extended the GGH model by incorporating the ECM-MMP dynamics, haptotaxis, and adhesion-controlled proliferation to study tumor invasion. The GGH model has also been extended to account for chemotaxis [594], cell differentiation [321], and cell polarity [718]. Others have modified the GGH model to include a subcellular protein signaling model to tie the cell cycle to growth promoters and inhibitors through continuum reaction-diffusion equations [353]. In that work, parameter values were selected such that model produced avascular tumors that quantitatively replicated experimental measurements of in vitro spheroids.

The GGH model has been used to simulate vasculogenesis and tumor angiogenesis [467, 466, 468] in heterogeneous tumor microenvironments [55], as well as the role of the ECM in glioma invasion [578]. Very recently, Poplawski and co-workers used the GGH method to study the morphological evolution of 2-D avascular tumors [541]; the work developed a phase diagram characterizing the tumor morphology and the stability of the tumor/host interface with respect to critical parameters characterizing nutrient limitations and cell-cell adhesion. In particular, they found that morphological stability depends primarily on the diffusional limitation parameter, whereas the morphological details depend on cell-cell adhe-
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The results are consistent with previous continuum [151, 147, 418, 439] and discrete [563, 274, 273, 31] modeling results.

**Some semi-deformable agent-based models**

Drasdo and co-workers developed an agent-based model that incorporates finite cell size to study epithelial cell-fibroblast-fibrocyte aggregates in connective tissue [188]. In their approach, simplified cells are modeled as a roughly spherical space containing a central region. The cells are slightly compressible and are capable of migration, growth and division. An undividing cell is taken to be spherical. As a cell mitoses, it deforms into a dumbbell shape until its volume roughly doubles, and then divides into two daughter cells. Adhesion and repulsion (from limitations on cell deformation and compressibility) among cells are modeled using an interaction energy that describes nearest-neighbor interactions. Mitosis and migration may induce pressure on neighboring cells. The cells respond by changing their mass or orientation to minimize the total interaction energy via a stochastic Metropolis algorithm [470]. Interaction potentials have also been used in agent models by Ramis-Conde and co-workers [560, 561]; in the model, cells move down the gradient of the potential, analogous to minimizing the interaction energy. Others have modeled cells as deformable viscoelastic ellipsoids [522, 163].

Drasdo and Höhme [185] adapted their approach to early-stage avascular tumor spheroids, where growth is not primarily limited by oxygen or nutrient supply, but rather by volume exclusion arising from limited cell compressibility. The biomechanical and kinetic parameters were estimated by comparison with tumor spheroid experiments from [225]. They later extended the model to account for glucose-limited growth, necrosis, and cell lysis, in order to simulate the spatiotemporal growth dynamics of 2-D tumor monolayers and 3-D tumor spheroids with biophysical and kinetic parameters drawn from experimental literature [186]. The results suggested that biomechanical growth inhibition is responsible for the transition from exponential to sub-exponential growth that is observed experimentally for sufficiently large tumors; glucose deprivation was found to primarily determine the size of the necrotic core but not the size of the tumor. Galle et al. extended the model to incorporate the effect of BM contact on cell cycle progress and apoptosis (see Section 2.1.1), and studied epithelial cell growth in monolayers [255]. They found that inactivation of BM-dependent cell cycle progression and apoptosis, or removal of contact-mediated growth inhibition (e.g., see Section 2.1.5) could lead to epithelial tumor growth. In similar monolayer simulations, Drasdo showed how the agent model can be used to determine the rules for a simpler cellular automaton model for use in deriving a continuum model with contact inhibition by coarse-graining, thereby providing a link between different scales and biophysical processes [184]. Byrne and Drasdo performed further analysis of the continuum model [104].
Recent examples of subcellular modeling

Individual cell agents can readily be endowed with subcellular models, making them ideal multiscale modeling platforms. Recently, Ramis-Conde and colleagues incorporated E-cadherin/β-catenin dynamics in an agent-based model to obtain a more realistic model of cell-cell adhesion mechanics [561]; β-catenin binds the membrane-bound E-cadherin to the cytoskeleton. Their detailed model could describe the detachment of cells from a primary tumor and the corresponding epithelial-to-mesenchymal transition. Galle et al. incorporated cell-ECM interactions and ECM contact-dependent cell regulation as well as cell differentiation [254]. They studied the effect of cancer stem cell organization on tumor growth, finding that tumors invade the host tissue much more rapidly when stem cells are on the tumor periphery, rather than confined to the interior.

Wang et al. developed a multiscale model of non-small cell lung cancer within a 2-D microenvironment, implementing a specific intracellular signal transduction pathway between the epidermal growth factor receptor (EGFR) and extracellular receptor kinase (ERK) at the molecular level [681]. Phenotypic changes at the cellular level were triggered through dynamical alterations of these molecules. The results indicated that for this type of cancer, downstream EGFR-ERK signaling may be processed more efficiently in the presence of a strong extrinsic chemotactic stimulus, leading to a migration-dominant cell phenotype and an accelerated rate of tumor expansion. Zhang et al. presented a 3-D multiscale agent-based model to simulate the cellular decision process to either proliferate or migrate in the context of brain tumors [721]. Each cell was equipped with an EGFR gene-protein interaction network module that also connected to a simplified cell cycle description. The results show that proliferative and migratory cell populations directly impact the spatiotemporal expansion patterns of the cancer. Zhang and co-workers later refined their model to incorporate mutations representing a simplified tumor progression pathway [722].

6.2 An agent-based cell modeling framework

To illustrate these concepts, we now introduce a discrete, cell-scale modeling framework that combines and extends the major features of the models introduced in Section 6.1. Our main objective is to develop a model that is sufficiently mechanistic that cellular and multicellular behavior manifest themselves as emergent phenomena of the modeling framework, rather than through computational rules that are imposed a priori. An additional design goal is that the model is modular (both in software and mathematics), allowing “sub-models” (e.g., molecular signaling, cell morphology) to be expanded, simplified, or outright replaced as necessary.

We use a lattice-free, agent-based approach to allows more accurate cell mechanics. We treat the cells (the agents) as physical objects subject to biophysically-justified forces. Cell-cell and cell-BM mechanical interactions are
modeled with interaction potential functions, similarly to [188, 185, 186, 184, 104]. The balance of these forces explicitly determines the cell’s velocity. The cells have nonzero, finite size, similar to the work by [188, 185, 186, 184]. We explicitly model mechanical interactions between the cells and the basement membrane, similarly to the work in [570]. As with many of the discrete models in Section 6.1.4, each cell has a phenotypic state, with transitions between those phenotypic states governed by stochastic processes. We note that we use the same model for both cancerous and non-cancerous cells. The cells differ primarily in the values of their proliferation, apoptosis, and other coefficients; this is analogous to modeling altered oncogenes and tumor suppressor genes [302].

We incorporate essential molecular biology through carefully-chosen constitutive relations. In particular, we attempt to model the mechanics, time duration, and biology of each phenotypic state as accurately as our data will allow; this should facilitate model calibration to molecular- and cellular data. As in preceding models (e.g., [28]), we incorporate the microenvironment using a composite discrete-continuum approach: the microenvironment is modeled as a set of field variables (e.g., oxygen concentration, ECM density) governed by continuum equations that can be altered by the discrete cells. Cell agents interact with this microenvironment both mechanically and biochemically through surface receptors that are part of a molecular-scale signaling model.

This agent model introduces several new features to discrete modeling as part of our design philosophy. The cell states are chosen specifically to facilitate calibration to immunohistochemistry in patient-specific simulations. We explicitly link the phenotypic state transitions to the microenvironment and signaling models through functional relationships in the stochastic parameters. Our model differentiates between apoptosis and necrosis and introduces a model for necrotic cell calcification. To better facilitate a mechanistic understanding of the model and matching to experimental biology, we separate the forces into separate potential functions, rather than lump them together into a single function. We use interaction potential functions with compact support (are zero outside some finite maximum interaction distance) to more realistically model the finite interaction distances between cells and their neighbors. The adhesion model can differentiate between homophilic and heterophilic adhesion, and separates the effects of cell-cell, cell-BM, and cell-ECM adhesion.

In this discussion, cells are not polarized, and in particular, we assume an isotropic distribution of cell surface receptors; we discuss model extensions to address this in [436]. Also, we do not currently focus on stem cell dynamics, although this can readily be added by identifying cells as stem cells, progenitor cells, or differentiated cells, and assigning each class different proliferation and other phenotypic characteristics. We treat the cells as mostly-rigid spheres, with growth both in 2D and 3D; basement membranes are currently modeled as sharp boundaries using level set functions [437, 438, 439, 440, 230, 441]. We present applications of the model to breast cancer in Section 6.6 and Chapter 10.
A brief review of exponential random variables and Poisson processes

Because we model transitions between cell states as stochastic processes, we begin with a brief review of the necessary preliminaries. This discussion necessarily only introduces the key concepts and does not explore the full richness of measure theory-based probability and stochastic processes. The interested reader can find more in widespread references (e.g., [612, 507]).

A random variable \( T \) is exponentially distributed with parameter \( \alpha \) if for any \( t > 0 \), the probability \( \Pr (T < t) \) is given by

\[
\Pr (T < t) = 1 - e^{-\alpha t}.
\]

(6.1)

Also, \( T \) has expected value \( \text{Ex} [T] = \frac{1}{\alpha} \) (i.e., the mean \( \langle T \rangle \) is \( 1/\alpha \)) and variance \( \text{Var} [T] = \frac{1}{\alpha^2} \). The simple relationship between the mean \( \langle T \rangle \) and \( \alpha \) is useful for calibration by limited data. Exponential random variables are memoryless. For any \( 0 \leq t, \Delta t \), the probability that \( T > t + \Delta t \) given that \( T > t \) is

\[
\Pr (T > t + \Delta t | T > t) = \Pr (T > \Delta t),
\]

(6.2)
i.e., if the event \( T \) has not occurred by time \( t \), then the probability that the event occurs within an additional \( \Delta t \) units of time is unaffected by the earlier times, and so we can “reset the clock” at time \( t \). This is useful for modeling cell decision processes that depend upon the current subcellular and microenvironmental state, and not on previous times. Even if the current cell decisions do depend upon past times, that information can be built into the time evolution of the internal cell state.

A stochastic process \( N_t \) is a series of random variables indexed by the “time” \( t \). In particular, \( N_t \) is a counting process if:

1. \( N_0 \geq 0 \). (The initial number of events \( N_0 \) is at least zero.)
2. \( N_t \in \mathbb{Z} \) for all \( t \geq 0 \). (The current number of events \( N_t \) is an integer.)
3. If \( s < t \), then \( N_t - N_s \geq 0 \). (\( N_t \) is the cumulative number of events, and the number of events occurring within \( (s, t] \) is \( N_t - N_s \).)

A Poisson process \( X_t \) is a particular type of counting process satisfying:

1. \( X_0 = 0 \). (The initial count is 0.)
2. If \( [s, s + \Delta s] \) and \( [t, t + \Delta t] \) are non-overlapping, then \( X_{s+\Delta s} - X_s \) and \( X_{t+\Delta t} - X_t \) are independent random variables. (What happens in the interval \( (t, t + \Delta t) \) is independent of what happened in \( (s, s + \Delta s) \).)
3. For any \( 0 \leq s < t \), the distribution of \( X_t - X_s \) only depends upon the length of the interval \( (s, t) \) (stationary increments), and in particular, if \( n \in \mathbb{N} \),

\[
\Pr (X_t - X_s = n) = \frac{e^{-\alpha(t-s)} \alpha(t-s)}{n!}.
\]

(6.3)

Poisson processes have a useful property that we rely upon in the model. If \( A_n = \inf \{ t : P_t = n \} \) is the arrival time of the \( n^{th} \) event (i.e., the first time at which \( X_t = n \)), and \( T_{n+1} = A_{n+1} - A_n \) is the interarrival time between the \( n^{th} \)
and \((n + 1)^{st}\) events for \(n \in \mathbb{N}\), then \(T_n\) is an exponentially-distributed random variable with parameter \(\alpha\), and

\[
\Pr (X_{t+\Delta t} - X_t \geq 1) = \Pr (A_n \in (t, t + \Delta t] | A_n > t) = \Pr (T_n < \Delta t) = 1 - e^{-\alpha \Delta t}.
\]

Thus, for sequence of events governed by a Poisson process, the time between consecutive events are simple exponentially-distributed random variables. In the context of stochastic cell models, if \(X_t\) is a Poisson process that gives the cumulative number of phenotypic state changes experienced by the cell by time \(t\), the time to the next phenotypic state change is exponentially distributed.

Lastly, we note that if \(\alpha = \alpha (t)\) varies in time, then \(X_t\) is a \textit{non-homogeneous} Poisson process with interarrival times given by

\[
\Pr (X_{t+\Delta t} - X_t = n) = \frac{e^{- \int_{t}^{t+\Delta t} \alpha (s) \, ds} \left( \int_{t}^{t+\Delta t} \alpha (s) \, ds \right)^n}{n!}.
\]

\[
\Pr (T_n < \Delta t) = \Pr (X_{t+\Delta t} - X_t \geq 1) = 1 - e^{- \int_{t}^{t+\Delta t} \alpha (s) \, ds} \approx 1 - e^{-\alpha (t) \Delta t}, \quad \Delta t \downarrow 0.
\]

In our work, the Poisson processes are non-homogeneous due to their dependencies upon microenvironmental and intracellular variables that vary in time. However, these can be approximated by homogeneous processes on small time intervals \([t, t + \Delta t]\) as above [435, 436].

### 6.2.2 A family of potential functions

As in the work by [188, 185, 186, 184, 104], we shall use potential functions to model biomechanical interactions between cells and the microenvironment. We now introduce the family of interaction potential functions \(\varphi (x; R, n)\) used in the agent model. Parameterized by \(R\) and \(n\), they satisfy

\[
\varphi (x; R, n) = \begin{cases} 
- \frac{R}{n+2} \left(1 - \frac{|x|}{R}\right)^{n+2} & \text{if } |x| < R \\
0 & \text{else,}
\end{cases}
\]

\(\varphi' (x; R, n) = \begin{cases} 
\left(1 - \frac{x}{R}\right)^{n+1} & \text{if } x < R \\
0 & \text{else,}
\end{cases}\)

\[
\nabla \varphi (x; R, n) = \begin{cases} 
\left(1 - \frac{|x|}{R}\right)^{n+1} \frac{x}{|x|} & \text{if } |x| < R \\
0 & \text{else,}
\end{cases}
\]

where \(R\) is the \textit{maximum interaction distance} of \(\varphi\), and \(n\) is the \textit{exponent} of the potential. We use this form of potential function because:
1. The potential (and its derivatives) has compact support: it is zero outside a closed bounded set (in this case, the closed ball $B(0, R)$). This models finite cell-cell and cell-BM interaction distances.

2. For any $R$ and $n$, and for any $0 < |x| < R$, we have

$$0 = \varphi'(R; R, n) < \varphi'(|x|; R, n) < \varphi'(0; R, n) = 1.$$  \hspace{1cm} (6.8)

The baseline case $n = 0$ is a linear ramping, and for higher $n$, the function tapers off to zero gradient smoothly.

A good discussion of the use of potential functions to mediate cell-cell adhesion and interaction for individual-based models can be found in [104].

### 6.2.3 Cell states

![Figure 6.1](image-url)

**Figure 6.1** Flow between the cell states in the agent-based model. Reprinted with permission from [436].

We emulate the cells' biological function by endowing each agent with a state $S(t)$ in the state space $\{Q, P, A, H, N, C, M\}$. Quiescent cells ($Q$) are in a “resting state” (G0, in terms of the cell cycle); this is the “default” cell state in the agent framework. We model the transitions between cell states as stochastic events governed by exponentially-distributed random variables. (Transition events are interarrival times modeling the elapsed time between proliferation and apoptosis events. See [436] for a discussion of the mathematical theory of this modeling construct.) Quiescent cells can become proliferative ($P$), apoptotic ($A$), or motile ($M$). Cells in any state can become hypoxic ($H$); hypoxic cells can recover to their previous state or become necrotic ($N$), and necrotic cells are degraded and replaced by calcified debris ($C$). See Figure 6.1. The subcellular scale is built into this framework by making the random exponential
variables depend upon the microenvironment and the cell’s internal properties. Cell cycle models have also been developed which can regulate the $P \rightarrow Q$ transition [2, 721], and protein signaling networks have been developed to regulate the $Q \rightarrow P$, $Q \rightarrow A$, and $Q \rightarrow M$ transitions. These can be directly integrated into the agent framework presented here by modifying the stochastic parameters; see Section 6.3. Further discussion of agent-based modeling with subcellular signaling components are in Section 6.1.4 and the excellent work done by [404, 176, 692, 697, 46, 719, 347, 691, 378, 681, 721, 134, 133, 170, 723].

**Proliferation ($P$):**

Quiescent cells $Q$ enter the proliferative state $P$ with a probability that depends upon the microenvironment. We model the probability of a quiescent cell entering the proliferative state in the time interval $(t, t + \Delta t]$ as an exponential interarrival time with parameter $\alpha_P(S, \bullet, \circ)$; $\bullet$ represents the cell’s internal (genetic and proteomic) state, and $\circ$ denotes the local microenvironmental conditions. Hence,

$$
\Pr(S(t + \Delta t) = P|S(t) = Q) = 1 - e^{-\alpha_P \Delta t} \approx \alpha_P \Delta t. \tag{6.9}
$$

Assuming a correlation between the microenvironmental oxygen level $\sigma$ (nondimensionalized by $\sigma_\infty$, the far-field oxygen level in non-diseased, “well-oxygenated” tissue) and proliferation (See Section 10.4.2), we expect $\alpha_P$ to increase with $\sigma$.² We model this by

$$
\alpha_P = \alpha_P(S(t), \sigma, \bullet, \circ) = \begin{cases} 
\overline{\alpha}_P(\bullet, \circ) \frac{\sigma - \sigma_H}{\sigma - \sigma_\infty} & \text{if } S(t) = Q \\
0 & \text{else},
\end{cases} \tag{6.10}
$$

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 “well-oxygenated” tissue), which depends upon the cell’s genetic profile and protein signaling state ($\bullet$) and the local microenvironment ($\circ$). For simplicity, we model $\overline{\alpha}_P$ as constant for and specific to each cell type. In Section 6.1.4, 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 signaling model. We note that models have been developed that reduce the proliferation rate in response to mechanical stresses (e.g., see the excellent description by [613]); in the context of the model above, the cell samples these stresses from continuum-scale field variables or tensors (i.e., “$\circ$”) to reduce $\alpha_P$.

Once a cell has entered the proliferative state $P$, it remains in that state for time $\beta_P^P$, which may generally depend upon the microenvironment and the cell’s

---

³ The interarrival time normally gives the probability of having at least one proliferation event (rather than precisely one) during $(t, t + \Delta t]$. Our $\alpha_P$ in Eq. (6.10) precludes this because $\alpha_P \downarrow 0$ until completing proliferation.

⁴ In Chapters 6 and 10, $\sigma$ and $g$ denote oxygen and glucose, which are generalized by the substrate $n$ in the remainder of the book. In these chapters, $n$ denotes an integer.
internal state, but which we currently model as fixed for both tumor and epithelial cells (with the same value). This models our assumption that both tumor and noncancerous cells use the same basic cellular machinery to proliferate, but with differing frequency due to altered oncogene expression [302]. Once the cell exits the proliferative state, we replace it with two identical daughter cells. Both inherit the parent cell’s phenotypic properties, are randomly positioned adjacent to one another while conserving the parent cell’s center of mass, and placed in the “default” quiescent state.

**Apoptosis (A):**

Apoptotic cells are undergoing “programmed” cell death in response to internal protein signaling. As with proliferation, we model entry into $A$ as an exponential interarrival time with parameter $\alpha_A(S, \bullet, \circ)$. We assume no correlation between apoptosis and oxygen [195]. Hence, $\alpha_A(S, \bullet)$ is fixed for each cell population:

$$\Pr(S(t + \Delta t) = A | S(t) = Q) = 1 - e^{-\alpha_A \Delta t},$$  \hspace{1cm} (6.11)

where

$$\alpha_A = \alpha_A(S(t), \bullet, \circ) = \begin{cases} \pi_A(\bullet, \circ) & \text{if } S(t) = Q \\ 0 & \text{else,} \end{cases}$$  \hspace{1cm} (6.12)

where $\circ$ does not include oxygen $\sigma$, but may include other microenvironmental stimuli such as chemotherapy or continuum-scale mechanical stresses that increase $\alpha_A$, as in the work by [613]. Cells remain in the apoptotic state for a fixed amount of time $\beta_A^{-1}$. Cells leaving the apoptotic state are deleted from the simulation to model phagocytosis of apoptotic bodies by surrounding epithelial cells. Their previously-occupied volume is made available to the surrounding cells to model the release of the cells’ water content after lysis.

**Hypoxia (H):**

Cells enter the hypoxic state anytime $\sigma < \sigma_H$. Hypoxic cells have an exposure-dependent probability of becoming necrotic:

$$\Pr(S(t + \Delta t) = N | S(t) = H) = 1 - e^{-\beta_H \Delta t},$$  \hspace{1cm} (6.13)

where we currently model $\beta_H$ as constant. If $\sigma > \sigma_H$ (i.e., normoxia is restored) at time $t + \Delta t$ and the cell has not become necrotic, it returns to its former state ($Q$, $P$, $A$, or $M$) and resumes its activity. For example, if the cell transitioned from $P$ to $H$ after spending $\tau$ time in the cycle, and normoxic conditions are restored before the cell transitions to $N$, then it returns to $P$ with $\tau$ time having elapsed in its cell cycle progression. Because $\Pr(S(t + \Delta t) = N | S(t) = H) \approx \beta_H \Delta t$, the probability that a cell succumbs to hypoxia is approximately propotional to $\ell(t : S(t) = H)$, its cumulative exposure time to hypoxia. This construct could model cell response to other stressors (e.g., chemotherapy), similarly to “area under the curve” models (e.g., [197, 198]).
Necrosis ($N$):
In our model, a hypoxic cell has a probability of irreversibly entering the necrotic state, simulating depletion of its ATP store. We can also simplify the model and neglect the hypoxic state by letting $\beta_H \to \infty$.

We assume that cells remain in the necrotic state for a fixed amount of time $\beta_N^{-1}$, during which time their surface receptors and subcellular structures degrade, they lose their liquid volume, and their solid component is calcified (replaced by calcium deposits). We define $\beta_{NL}^{-1}$ to be the length of time for the cell to swell, lyse, and lose its water content, $\beta_{NS}^{-1}$ the time for all surface adhesion receptors to degrade and become functionally inactive, and $\beta_C^{-1}$ the time for the cell to fully calcify. Generally, $\beta_{NL}^{-1} \leq \beta_{NS}^{-1} < \beta_C^{-1}$. In [435], we found that a simplified model ($\beta_N^{-1} = \beta_{NS}^{-1} = \beta_{NL}^{-1} = \beta_C^{-1}$) could not reproduce certain aspects of the breast cancer microarchitecture.

If $\tau$ is the elapsed time spent in the necrotic state, we model the degradation of the surface receptor species $S$ (scaled by the non-necrotic expression level) by exponential decay with rate constant $\beta_{NS} \log 100$; the constant is chosen so that $S(\beta_{NS}^{-1}) = 0.01 S(0) = 0.01$, i.e., virtually all of the surface receptor is degraded by time $\tau = \beta_{NS}^{-1}$.

For a preliminary model of the necrotic cell’s volume change, we neglect its early swelling and instead model it’s volume change after lysis:

$$V(\tau) = \begin{cases} V & \text{if } 0 \leq \tau < \beta_{NL}^{-1} \\ V_S & \text{if } \beta_{NL}^{-1} < \tau, \end{cases} \quad (6.14)$$

where $V_S$ is the cell’s solid volume.

Lastly, we assume a constant rate of cell calcification, with the necrotic cell 100% calcified by time $\beta_C^{-1}$. If $C$ is the nondimensional degree of calcification, then $C(t) = \beta_C \tau$.

Calcified debris ($C$):
Cells leaving the necrotic state $N$ irreversibly enter the calcified debris state $C$. Lacking functional adhesion receptors, these cells only adhere to other calcified debris. This is a simplified model of the crystalline bonds between calcium phosphate and/or calcium oxalate molecules in the microcalcification.

Motility ($M$):
The transition of cells to the motile state (via $\alpha_M$) is a complex “decision” that depends upon the cell’s microenvironment as communicated to the cell by surface receptors and its internal signaling network. Once the cell enters $M$, its speed (and hence $\beta_M$) and direction of motion depend upon its interaction with the microenvironment. Depending upon the sophistication of the model, the duration of motility can be fixed (constant $\beta_M$) or determined by the motility model (e.g., $\beta_M = 0$ until the cell reaches its destination, at which time we “force” an immediate $M \to Q$ transition). We discuss this further in Section 6.2.4.
6.2.4 Forces acting on the cells

Each cell is subject to competing forces that determine its motion within the microenvironment. Cells adhere to other cells (cell-cell adhesion: \( F_{\text{cca}} \)), the extracellular matrix (cell-ECM adhesion: \( F_{\text{cma}} \)), and the basement membrane (cell-BM adhesion: \( F_{\text{cba}} \)), calcified debris adheres to other calcified debris (debris-debris adhesion: \( F_{\text{dda}} \)), cells and calcified debris resist compression by other cells and debris (cell-cell repulsion: \( F_{\text{ccr}} \)), and the basement membrane resists its penetration and deformation by cells and debris (cell-BM repulsion: \( F_{\text{cbr}} \)). Motile cells experience a net locomotive force \( F_{\text{loc}} \) along the direction of intended travel. See Figure 6.2, where we show the forces acting on cell 5. In addition, moving cells and debris experience a drag force \( F_{\text{drag}} \) by the luminal and interstitial fluids, which we model by \( F_{\text{drag}} = -\nu v_i \). We express this balance of forces by Newton’s second law acting on cell \( i \):

\[
m_i \ddot{v}_i = \sum_j \left( F_{\text{cca}}^{ij} + F_{\text{ccr}}^{ij} + F_{\text{dda}}^{ij} \right) + F_{\text{cma}}^i + F_{\text{cba}}^i + F_{\text{cbr}}^i + F_{\text{loc}}^i + F_{\text{drag}}^i.
\]

(6.15)

Here, the sum is over all cells \( j \) in the computational domain.

**Figure 6.2** Basic schematic of the model. Key forces acting on cell 5 are labeled. Reprinted with permission from [436].

**Cell-cell adhesion (\( F_{\text{cca}} \))**: Cell-cell adhesion can be both homophilic [524] and heterophilic [633, 657, 429].
Homophilic adhesion:
Adhesion molecules $E$ on the cell surface bond with $E$ molecules on neighboring cells. Hence, the strength of the adhesive force between the cells is proportional to the product of their respective $E$ expressions. Furthermore, the strength of the adhesion increases as the cells are drawn more closely together, bringing more surface area and hence more adhesion molecules into direct contact. We model this adhesive force between cells $i$ and $j$ by

$$F_{cc}^{ij} = \alpha_{cca} E_i E_j \nabla \varphi \left( x_j - x_i; R_{cca}^i + R_{cca}^j, n_{cca} \right),$$

(6.16)

where $E_i$ and $R_{cca}^i$ are cell $i$’s (nondimensionalized) adhesion receptor expression and maximum cell-cell adhesion interaction distance, respectively, $r_i$ is the cell’s radius, and $n_{cca}$ is the cell-cell adhesion power for our potential function family in Section 6.2.2. We typically set $R_{cca}^i > r_i$ to approximate the cell’s ability to deform before breaking all adhesive bonds, with the strength of force decreasing as the cells are separated.

Heterophilic adhesion:
Adhesion molecules $I_A$ on the cell surface bond with $I_B$ molecules on neighboring cells. Hence, the strength of the adhesive force between the cells is proportional to the product of their $I_A$ and $I_B$ receptor expressions. Furthermore, the strength of the adhesion increases as the cells are drawn more closely together, bringing more surface area and hence more receptors into direct contact. We model this adhesive force between cells $i$ and $j$ by

$$F_{cc}^{ij} = \alpha_{cca} (I_{A,i} I_{B,j} + I_{B,i} I_{A,j}) \nabla \varphi \left( x_j - x_i; R_{cca}^i + R_{cca}^j, n_{cca} \right),$$

(6.17)

where $I_{A,i}$ and $I_{B,i}$ are cell $i$’s (nondimensionalized) $I_A$ and $I_B$ receptor expressions, $R_{cca}^i$ is the cell’s maximum cell-cell adhesion interaction distance, and $n_{cca}$ is the cell-cell adhesion power as before. As with homophilic cell-cell adhesion, we typically set $R_{cca}^i > r_i$ to approximate the ability of cells to deform before breaking all adhesive bonds, with the strength of force decreasing as the cells are separated.

Cell-ECM adhesion ($F_{cma}$):
Integrins $I_E$ on the cell surface form heterophilic bonds with suitable ligands $L_E$ in the ECM. We assume that $L_E$ is distributed proportionally to the (nondimensional) ECM density $E$. If we assume that $L_E$ is distributed uniformly across the cell surface and $E$ varies slowly relative to the spatial size of a single cell, then cells at rest encounter a uniform pull from $F_{cma}$ in all directions, resulting in zero net cell-ECM force. For cells in motion, $F_{cma}$ resists that motion similarly to drag due to the energy required to overcome $I - L$ bonds:

$$F_{cma} = -\alpha_{cma} I_{E,i} E v_i.$$

(6.18)

If $E$ or $L_E$ varies with a higher spatial frequency, or if $I_E$ is not uniformly distributed, then the finite half-life of $I_E - L_E$ bonds will lead to net haptotactic-
type migration up gradients of \( E \) [586]. We currently neglect this effect except by including it into the cell’s (active) locomotive force \( \mathbf{F}_{\text{loc}} \).

**Cell-BM adhesion (\( \mathbf{F}_{\text{cba}} \)):**
Integrin molecules on the cell surface form heterophilic bonds with specific ligands \( \mathcal{L}_B \) (generally laminin and fibronectin [92]) 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

\[
\mathbf{F}_{\text{cba}}^i = \alpha_{\text{cba}} I_{B,i} B \nabla \varphi (d(\mathbf{x}_i); R_{\text{cba},i}, n_{\text{cba}}),
\]

where \( d \) is the distance to the basement membrane, \( I_{B,i} \) and \( R_{\text{cba},i} \) are cell \( i \)’s (nondimensionalized) integrin receptor expression and maximum cell-BM adhesion interaction distance, respectively, and \( n_{\text{cba}} \) is the cell-BM adhesion power. (See Section 6.2.2.) As with cell-cell adhesion, we typically set \( R_{\text{cba},i} > r_i \) to approximate the cell’s limited capacity to deform before breaking all its adhesive bonds.

**Calcified debris-calcified debris adhesion (\( \mathbf{F}_{\text{dda}} \)):**
We model adhesion between calcified debris particles similarly to homophilic cell-cell adhesion; hence calcite crystals in the interacting calcified debris particles remain strongly bonded as part of the microcalcification. We model this adhesive force between the calcified debris particles \( i \) and \( j \) by

\[
\mathbf{F}_{\text{dda}}^{ij} = \alpha_{\text{dda}} C_i C_j \nabla \varphi \left( \mathbf{x}_j - \mathbf{x}_i; R_{\text{dda},i} + R_{\text{dda},j}, n_{\text{dda}} \right),
\]

where \( C_i \) and \( R_{\text{dda},i} \) are cell \( i \)’s (nondimensionalized) degree of calcification and maximum debris-debris adhesion interaction distance, and \( n_{\text{dda}} \) is the debris-debris adhesion power. The \( \alpha_{\text{dda}} \) can be interpreted as the adhesive force between two fully-calcified debris particles.

**Cell-cell repulsion (including calcified debris) (\( \mathbf{F}_{\text{ccr}} \)):**
Cells resist compression by other cells due to the structure of their cytoskeletons, the incompressibility of their cytoplasm (fluid), and the surface tension of their membranes. We introduce a cell-cell repulsive force that is zero when cells are just touching, and then increases rapidly as the cells are pressed together. We approximate any pressure-induced cell deformation by allowing some overlap between cells. We model \( \mathbf{F}_{\text{ccr}} \) by

\[
\mathbf{F}_{\text{ccr}}^{ij} = -\alpha_{\text{ccr}} \nabla \varphi (\mathbf{x}_j - \mathbf{x}_i; r_i + r_j, n_{\text{ccr}}),
\]
where \( n_{ccr} \) is the cell-cell repulsion power (Section 6.2.2) and \( \alpha_{ccr} \) is the maximum repulsive force when the cells are completely overlapping.

**Cell-BM repulsion (including debris) \( (F_{cbr}) \):**

We model the basement membrane as rigid and non-deformable due to its relative stiffness and strength. Hence, it resists deformation and penetration by the cells and debris particles. We model the force by

\[
F_{cbr}^i = -\alpha_{cbr} B \nabla \varphi \left( d(x_i); r_i, n_{cbr} \right),
\]

where \( n_{cbr} \) is the cell-BM repulsion power, \( d \) is the distance to the BM, and \( \alpha_{cbr} \) is the maximum repulsive force when the cell’s center is embedded in the basement membrane. Cells can secrete matrix metalloproteinases (MMPs) that degrade the BM (Section 2.2.3) and hence reduce \( F_{cbr} \); we model this effect by making the cell-BM repulsive force proportional to the remaining BM density \( B \). We discuss this further in Section 6.4.

**Motile locomotive force \( (F_{loc}) \):**

If \( S \neq M \), then \( F_{loc} = \mathbf{0} \). Otherwise, we can model the locomotive force with various levels of detail:

1. **Imposed chemotaxis and haptotaxis:**
   For a simple motility model, we choose a deterministic direction of motion based upon biological hypotheses, such as chemotaxis in response to growth factors \( f \) and haptotaxis along gradients in the ECM \( E \):
   \[
   F_{loc} = \alpha_1 \nabla f + \alpha_2 \nabla E.
   \]
   The coefficients \( \alpha_1 \) and \( \alpha_2 \) can be either fixed or altered to model energetic concerns such as oxygen availability, level of receptor activation, and expression of adhesive ligands necessary for traction. For instance, one could model
   \[
   \alpha_1 = \bar{\pi}_1(\sigma, g) f f_r \mathcal{I}_{E,i} E,
   \]
   where \( g \) is the cell’s internal glucose level, \( \bar{\pi}_1(\sigma, g) \) models the rate of locomotion as a function of oxygen and glucose, \( f_r \) is the cell’s surface expression of receptors to \( f \); \( f f_r \) denotes overall activation of \( f_r \). Similarly, \( \mathcal{I}_{E,i} E \) gives the overall level of binding of adhesion receptors \( \mathcal{I}_{E,i} \) to their ligands in the ECM. We apply \( F_{loc} \) for a fixed amount of time \( \beta_{M}^{-1} \); afterwards, we set \( S = Q \) and \( F_{loc} = \mathbf{0} \). One could use similar functional forms for \( \alpha_2 \).

2. **Biased random motion:**
   Increasing the complexity somewhat, we choose a random direction of motion when the cell enters the motile state and fix it for the duration of motility, with distribution dependent upon the gradients of microenvironmental factors such
as oxygen ($\sigma$), growth factors ($f$), and ECM ($E$). One such method is

$$
\mathbf{w} = r_1 \nabla \sigma + r_2 \nabla f + r_3 \nabla E, \quad -1 \leq r_1, r_2, r_3 \leq 1
$$

(6.25)

where the distributions of the random variables $r_1, r_2, \text{ and } r_3$ are chosen according to the desired weighting of oxygen taxis, chemotaxis, and haptotaxis. Random weightings can be used to model more complex signaling dynamics, where the cell must “choose” among competing signals, but the internal decision process is unknown. We apply the vector of travel for pseudotime $0 \leq \tau \leq \beta_1^{-1}$, after which $S = Q$ and $F_{\text{loc}} = 0$.

**Motion along the BM:**
We can model motility along a basement membrane by extension and contraction of lamellipodia as follows: at any time $t$ that the cell state changes to $M$, we choose a random direction $\mathbf{w}$ (with $|\mathbf{w}| = 1$; see Figure 6.3a), and test the line segment between $\mathbf{x} + r \mathbf{w}$ (on the cell membrane) and $\mathbf{x} + (r + \ell_{\text{podium}}) \mathbf{w}$ (the maximum extension of a lamellipodium) for intersection with the BM (labeled as $x_{\text{loc}}$ in Figure 6.3b). If there is no intersection, we set $S = Q$. We assume that the cell adheres to the BM with a lamellipodium at $x_{\text{loc}}$ and pulls towards this location until either (i) the cell’s boundary reaches $x_{\text{loc}}$ (Figure 6.3c), or (ii) the cell “gives up” at a maximum time $\beta_1^{-1} \text{max}$. Until then, we model

$$
F_{\text{loc}} = \alpha_{\text{loc}} \frac{x_{\text{loc}} - x}{|x_{\text{loc}} - x|}
$$

(6.27)

where $\alpha_{\text{loc}}$ gives the cell’s speed of lamellipodium contraction. To avoid “double-counting” cell-BM adhesion, we set $F_{\text{cba}} = 0$ during motility. See Figure 6.3. Once motility is complete, we set $F_{\text{loc}} = 0$ and $S = Q$.

**Figure 6.3** To simulate motility along a basement membrane, we (a) choose a random unit vector $\mathbf{w}$, (b) test for intersection with the BM along that direction, and apply $F_{\text{loc}}$ towards that intersection point until (c) the cell membrane reaches the BM. Reprinted with permission from [436].

**Mechanistic motility:**
In a more rigorous motility model, we recognize locomotion as the combined effect of directed actin polymerization (drives protrusion of the cell membrane)
and differential cell-ECM adhesion [405]. Suppose that $a(\theta, \varphi, g, \sigma, f, f_r)$ gives the distribution of actin polymerization activity across the cell’s surface, where $0 \leq a \leq 1$, and $f$ and $f_r$ also vary with position $(\theta, \varphi)$ on the cell surface. Let $I(\theta, \varphi)$ and $E(\theta, \varphi)$ denote the distributions of surface adhesion receptor and nearby ligand, respectively, where these also vary between 0 and 1. Then the distribution of successful adhesion of cell membrane protrusions to the ECM can be modeled by

$$M(\theta, \varphi) = a(\theta, \varphi, g, \sigma, f, f_r) I(\theta, \varphi) E(\theta, \varphi).$$

(6.28)

We set

$$F_{loc} = \alpha_{loc} a_{\text{max}} \text{ OR } F_{loc} = \alpha_{loc} \nabla M,$$

(6.29)

where $a_{\text{max}}$ is the direction that maximizes $a$ (or is randomly chosen if $\nabla M = 0$), and we apply this force until reaching a maximum motility duration $\beta^{-1}$. This approach could replace the explicit treatment of cell-cell, cell-BM, and cell-ECM adhesion. We are investigating this approach in a modified agent model that explicitly discretizes cell membrane extension and retraction [586].

This general form should capture oxygen/glucose taxis, chemotaxis and haptotaxis as emergent properties, as well as the effects of ECM anisotropy: if the rate of actin polymerization is assumed to correlate with local ATP availability (proportional to glucose and oxygen), then $\nabla a$ should correlate with $\nabla g$ and $\nabla \sigma$, thereby recovering nutrient taxis. If actin polymerization is assumed to occur as a result of an internal signaling response to a chemotactant $f$ and if the receptor $f_r$ is roughly uniformly distributed, then $\nabla a$ should correlate with $\nabla f$, giving chemotaxis as an emergent behavior. If $a$ is uniform but the ECM has a local gradient, then $F_{loc} \parallel \nabla E$, thus recovering haptotaxis. By incorporating a signaling model into $a$ that connects with actin polymerization activity (e.g., as in [476, 456, 216, 707, 369, 593]), we can help make the model more mechanistic and less phenomenological, thereby improving the predictivity of the model. This is a key strength of multiscale modeling.

"Inertialess" assumption:

We assume that the forces equilibrate quickly, and so $|m_i \dot{v}_i| \approx 0$. Hence, we approximate $\sum \mathbf{F} = 0$ and solve for the cell velocity from Eq. (6.15):

$$v_i = \frac{1}{\nu + \alpha_{\text{cma}} I_{E, i} E} \left( \sum_j \left( F_{cca}^{ij} + F_{dda}^{ij} + F_{ccr}^{ij} \right) + F_{cha}^{ij} + F_{chr}^{ij} + F_{loc}^{ij} \right).$$

(6.30)

This has a convenient interpretation: each term $\frac{1}{\nu + \alpha_{\text{cma}} I_{E, i} E} \mathbf{F}_{\square}$ is the “terminal” (equilibrium) velocity of the cell when fluid drag, cell-ECM adhesion, and $\mathbf{F}_{\square}$ are the only forces acting upon it. (Here, "\(\square\)" represents any individual force above,
e.g., cba, cca, etc., and the summation is over all cells \( j \) in the computational domain.) This will be useful when calibrating cell motility in future work, as motility is generally measured as a cell velocity (e.g., \([304]\)).

6.3 Subcellular modeling

We can incorporate a molecular-scale signaling model to improve the cell agent’s “decision process.” For example, in the context of the EGFR pathway, Deisboeck et al. modeled the PLC\( \gamma \) and ERK proteins to drive cell decisions on quiescence, proliferation, and motility based upon thresholding on the time derivatives of these proteins \([169, 46, 45, 681, 721, 133, 723]\).

Taking an analogous approach, we may derive a generalized signaling model by introducing a set of proteins \( P = [P_1, P_2, \ldots]^T \) governed by a (nonlinear) system of ODEs. Because the cell’s protein state depends upon sampling the microenvironment, we define a “stimulus” vector \( S = [S_1, S_2, \ldots] \), where the \( S_i \) may be oxygen, receptor ligands, and so forth. Furthermore, the signaling network topology depends upon the cell’s genetic makeup, which we generically refer to as \( G \). Hence:

\[
\dot{P} = f(G, P, S). \tag{6.31}
\]

The phenotypic transition probabilities then depend upon \( P \) and \( \dot{P} \).

We illustrate this idea with a rudimentary E-cadherin/\( \beta \)-catenin signaling model. Let \( P_1 \) be the cell’s unligated E-cadherin, \( P_2 \) the ligated E-cadherin (bound to E-cadherin \( S_1 \) on neighboring cells, considered an external stimulus), \( P_3 \) the free cytoplasmic \( \beta \)-catenin, and \( P_4 \) the ligated E-cadherin-\( \beta \)-catenin complexes. Then a basic system of nonlinear ODEs that includes protein synthesis, binding, dissociation, and proteolysis is given by [436]

\[
\dot{P}_1 = \frac{\text{synthesis}}{c_1} - \frac{\text{homophilic binding}}{c_2 S_1 P_1} + \frac{\text{dissociation}}{c_3 P_2} - \frac{\text{proteolysis}}{c_4 P_1}. \tag{6.32}
\]

\[
\dot{P}_2 = c_2 S_1 P_1 - c_3 P_2 - \frac{\text{proteolysis}}{d_2 P_2 P_3} + \frac{\beta\text{-catenin binding}}{d_3 P_4}. \tag{6.33}
\]

\[
\dot{P}_3 = d_1 - d_2 P_2 P_3 + d_3 P_4 - \frac{\text{proteolysis}}{d_4 P_3}. \tag{6.34}
\]

\[
\dot{P}_4 = d_2 P_2 P_3 - d_3 P_4 - \frac{\text{proteolysis}}{d_5 P_4}. \tag{6.35}
\]

The first two equations describe E-cadherin receptor trafficking, and the second two give the interaction of cytoplasmic \( \beta \)-catenin with ligated E-cadherin. We assume that transcription of downstream targets of \( \beta \)-catenin is proportional to \( P_3 \) (see Section 2.1.5) and incorporate this molecular signaling into the pheno-
typic transformations by altering the $Q \rightarrow P$ transition parameter $\alpha_P$:

$$\alpha_P = \frac{\sigma - \sigma_H}{\sigma_H}$$  \quad \text{(6.36)}

Here, $f_P$ satisfies $f_P' > 0$ (transcription increases with $P_3$), $f_P(1) = 1$ ($\beta$-catenin is not a limiting factor if there is no E-cadherin binding), and $f_P(0) = 0$ (there is no downstream transcription if all $\beta$-catenin is bound).

### 6.4 Dynamic coupling with the microenvironment

As discussed throughout Section 6.2.3, a cell agent’s behavior is inexorably linked to the microenvironment. We now 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, signaling molecules, extracellular matrix, etc.) that are updated according to continuum equations. The distribution of these variables affects the cell agents’ evolution as already described; simultaneously, the agents impact the evolution of the continuum variables. We give several key examples to illustrate the concept.

**Oxygen:**

All cell agents uptake oxygen as a part of metabolism. At the macroscopic scale, this is modeled by

$$\frac{\partial \sigma}{\partial t} = \nabla \cdot (D \nabla \sigma) - \lambda \sigma,$$  \quad \text{(6.37)}

where $\sigma$ is oxygen, $D$ is its diffusion constant, and $\lambda$ is the (spatiotemporally variable) uptake rate by the cells\(^5\). Suppose that tumor cells uptake oxygen at a rate $\lambda_t$, host cells at a rate $\lambda_h$, and elsewhere oxygen “decays” (by interacting chemically with the molecular landscape) at a low rate background rate $\lambda_b$. Suppose that in a small neighborhood $B$ (a ball $B_\epsilon(x)$ of radius $\epsilon$ centered at $x$), tumor 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 \text{(6.38)}

i.e., by averaging the uptake rates with weighting according to the combination of cell types and stroma present near $x$. Note that we could further decompose $f_t$ and $f_h$ according to cell phenotype, if the uptake rates are expected to vary.

In a numerical implementation, $\lambda$ is calculated first on a high-resolution mesh (e.g., 1 mesh point is 1 cubic micron in 3D) and subsequently averaged to obtain $\lambda$ at the continuum scale (e.g., 1000 cubic micron mesh points) [436]. In this

\(^5\) In Chapters 6 and 10, $\sigma$ and $g$ denote oxygen and glucose, which are generalized by the substrate $n$ in the remainder of the book. In these chapters, $n$ denotes an integer.
formulation, the cell uptake rate varies with the tumor microstructure, which, in turn, evolves according to nutrient and oxygen availability.

**Extracellular matrix:**

Cell agents adhere to the extracellular matrix and require ECM for certain types of cell locomotion (Section 6.2.4). Cells can also affect the ECM by secreting matrix metalloproteinases that degrade the extracellular matrix, and possibly by also depositing new ECM (Section 2.2.3). If $E$ is the concentration of ECM and $M$ is the concentration of MMPs secreted by the cells, then we model this at the continuum scale similarly to our prior work in [441]:

$$\frac{\partial E}{\partial t} = \lambda^E_{\text{production}}(x) - \lambda^E_{\text{degradation}} EM$$

(6.39)

$$\frac{\partial M}{\partial t} = \nabla \cdot (D_M \nabla M) + \lambda^M_{\text{production}}(x) - \lambda^M_{\text{decay}} M,$$

(6.40)

where $\lambda^E_{\text{production}}$ are production rates ($\Box = E, M$), $\lambda^E_{\text{degradation}}$ is the rate ECM degradation by MMPs, $\lambda^M_{\text{decay}}$ is the MMP decay rate, and $D_M$ is the MMP diffusion constant (generally low).

The production rates $\lambda^E_{\text{production}}$ are upscaled from the cell scale analogously to the oxygen uptake rate, and generally are functions of $E$ and $M$. Reference [441] used production rates of the form $\lambda^\Box_{\text{production}}(1 - \Box)$, $\Box = E, M$ for a constant value of $\Box$ within the tumor viable rim. This was an approximation of subcellular signaling under the assumption that ECM and MDE production decrease as $E$ and $M$ approach an equilibrium value, here scaled to 1. In a multiscale, composite modeling context, however, these assumptions are not required, as the phenomena can emerge directly from subcellular signaling models.

**Basement membrane:**

In Section 6.2.4, the basement membrane impacts the cell agents through a balance of adhesive and repulsive forces. The cells can also impact the BM by deforming and degrading it (Section 2.2.3). We model the BM as a sharp, heterogeneous, deformable interface. The cell agents require information on (1) their distance to the basement membrane, and (2) the properties of the BM at that closest location. We satisfy these requirements using an augmented level set approach. For simplicity, we describe this method for simulations in 2D; the 3-D approach is analogous.

Let $\{x^{BM}(s) : 0 \leq s \leq 1\}$ parameterize the basement membrane, let $n(s)$ be normal to the BM at $x^{BM}(s)$ for all $s$ (facing towards the epithelial side of the BM), and let $b(s) = (b^1(s), b^2(s), \ldots)$ be a set of properties along the basement membrane at position $x(s)$. In our work, $b^1$ is the BM density and $b^2$ is the integrin concentration. For any point $x$ in the computational domain, define

$$s(x) = s \text{ that minimizes } \{|x - x^{BM}(s)| : 0 \leq s \leq 1\},$$

(6.41)
that is, $x_{BM}(s(x))$ is the closest point on the BM to $x$. Note that the minimum value in Eq. (6.41) is equal to $|d(x)|$. For notational simplicity, let $s_i = s(x_i)$, $x_{BM}^{BM}(s_i)$, and $n_i = n(s_i)$ for any cell $i$.

Here, $d(x)$ is the signed distance function (first referenced in Section 6.2.4), satisfying:

- $d = 0$ on the basement membrane
- $d > 0$ on the epithelial side of the BM
- $d < 0$ on the stromal side of the membrane
- $|\nabla d| \equiv 1$ ($d$ is a distance function).

We modify the cell-BM adhesive and repulsive forces to account for the heterogeneity introduced by $b(s)$. The modified cell-BM adhesive force acting on cell $i$ is

$$F_{cba}^i = \alpha_{cba} I_{B,i} b^2(s_i) \nabla \varphi (d(x_i); R_{cba}, n_{cba}).$$  (6.42)

We modify the cell-BM repulsive force on cell $i$ to

$$F_{cbr}^i = -\alpha_{cbr} b^1(s_i) \nabla \varphi (d(x_i); r_i, n_{cbr});$$  (6.43)

note that this assumes that the BM stiffness is proportional to its density.

The MMPs secreted by the cells ($M(x)$; see the previous section) degrade the BM. We model this by

$$\frac{db^1(s)}{dt} = -\lambda_{degradation}^E b^1(s) M(x_{BM}(s)) \text{ for each } 0 \leq s \leq 1.$$  (6.44)

Cells in contact with the basement membrane impart forces that deform it. In the simplest deformation model, the membrane acts like a semi-plastic material whose deformations remain even if the cell-imparted stresses are eliminated. Any cell contacting the BM with velocity directed towards it contributes locally to the membrane normal velocity $v_{BM}(s_i)$. This membrane velocity varies according to the heterogeneous membrane stiffness. We model this by first defining the BM normal velocity wherever the cells are touching it:

$$v_{BM}(s_i) = -\max (-v_i \cdot n_i, 0) b^1(s) \text{ for any } i \text{ such that } d(x_i) < r_i.$$  (6.45)

We then smoothly interpolate this function to obtain $v_{BM}$ for other values of $s$. We update the boundary position using the extended normal velocity (e.g., by extending $v_{BM}$ off the basement membrane and using level set techniques, as in [437, 438, 439, 440, 230, 441]).

More sophisticated models can also be applied to the membrane deformation. We could discretize $x_{BM}$ and $b$, connect the discrete membrane points with springs, and balance the forces at each membrane mesh point to model an elastic boundary; gradually reducing the strain in each virtual spring could then model relaxation in a viscoelastic material.
More sophisticated still, we could upscale the cells’ velocities \( v_i \), to obtain the mean mechanical pressure in a coarsened spatial grid using Darcy’s law 
\[
\langle v \rangle = \mu \nabla p,
\]
as in Chapter 3. Combined with proper material properties and boundary conditions, we could then solve for the basement membrane velocity. Ribba and co-workers have used similar approaches for continuum descriptions of membrane deformations under viscoelastic stresses \[570\]. Such an approach would be very well-suited to hybrid models, where both discrete and continuum representations of the cell velocities are already present. In fact, membrane deformations are better suited to continuum descriptions that are well-developed for solid material mechanics, while membrane heterogeneity can be well-characterized by the localized alterations by the discrete cells; this is a good example where a hybrid model would be stronger than either a discrete or continuum approach alone. Chapter 7 will explore hybrid models in greater detail.

6.5 A brief analysis of the volume-averaged model behavior

Let us fix a volume \( \Omega \) contained within the viable rim (i.e., any cell \( i \) in \( \Omega \) satisfies \( S_i \notin \{H,N,C\} \)). We analyze the population dynamics in the simplified \( Q-A-P \) cell state network (we assume no motility); this analysis is the basis of the model calibration in Section 10.3. Let \( P(t) \), \( A(t) \), and \( Q(t) \) denote the number of proliferating, apoptosing, and quiescent cells in \( \Omega \) at time \( t \), respectively. Let \( N(t) = P + A + Q \) be the total number of cells in \( \Omega \). If \( \langle \alpha_P \rangle(t) = \frac{1}{|\Omega|} \int_{\Omega} \alpha_P \, dV \) is the mean value of \( \alpha_P \) at time \( t \) throughout \( \Omega \), then the net number of cells entering state \( P \) in the time interval \( [t, t + \Delta t] \) is approximately
\[
\dot{P}(t + \Delta t) = P(t) + Pr(S(t + \Delta t) = P|S(t) = Q)Q(t) - \beta_P P(t)\Delta t
\]
whose limit as \( \Delta t \downarrow 0 \) (after some rearrangement) is
\[
\dot{P} = \langle \alpha_P \rangle Q - \beta_P P. \tag{6.47}
\]
Similarly,
\[
\dot{A} = \alpha_A Q - \beta_A A \tag{6.48}
\]
\[
\dot{Q} = 2\beta_P P - (\langle \alpha_P \rangle + \alpha_A) Q. \tag{6.49}
\]
Summing these, we obtain
\[
\dot{N} = \beta_P P - \beta_A A. \tag{6.50}
\]
Next, define \( PI = P/N \) and \( AI = A/N \) to be the proliferative and apoptotic indices, respectively. We can express the equations above in terms of \( AI \) and \( PI \) by dividing by \( N \) and using Eq. 6.50 to properly treat \( \frac{d}{dt} (P/N) \) and \( \frac{d}{dt} (A/N) \).
After simplifying, we obtain a nonlinear system of ODEs for PI and AI:

\[
\dot{P}_I = \langle \alpha_P \rangle (1 - AI - PI) - \beta_P (PI + PI^2) + \beta_A AI \cdot PI
\]
(6.51)

\[
\dot{A}_I = \alpha_A (1 - AI - PI) - \beta_A (AI - AI^2) - \beta_P AI \cdot PI.
\]
(6.52)

These equations are far simpler to compare to immunohistochemical measurements, which are generally given in terms of AI and PI.

Lastly, let us nondimensionalize the equations by letting \( t = \hat{t} \), where \( \hat{t} \) is dimensionless. Then if \( f' = \frac{df}{dt} \), we have

\[
\frac{1}{\hat{t}} \dot{P}_I = \langle \alpha_P \rangle (1 - AI - PI) - \beta_P (PI + PI^2) + \beta_A AI \cdot PI
\]
(6.53)

\[
\frac{1}{\hat{t}} \dot{A}_I = \alpha_A (1 - AI - PI) - \beta_A (AI - AI^2) - \beta_P AI \cdot PI.
\]
(6.54)

The cell cycle length \( \beta_P^{-1} \) is on the order of 1 day (e.g., as in [515]), and in Section 10.4.1, we determine that \( \beta_A \) is of similar magnitude. Thus, if we choose \( \hat{t} \sim \mathcal{O}(10 \text{ day}) \) or greater, then we can assume that \( \frac{1}{\hat{t}} \dot{P}_I = \frac{1}{\hat{t}} \dot{A}_I = 0 \) and conclude that the local cell state dynamics reach steady state after after 10-100 days. This is significant, because it allows us to calibrate the population dynamic parameters \( \alpha_A, \alpha_P, \beta_A, \text{ and } \beta_P \) without the inherent difficulty of estimating temporal derivatives from often noisy \textit{in vitro} and immunohistochemistry data.

### 6.6 Numerical examples from breast cancer

We now present numerical examples of the agent model applied to ductal carcinoma in situ: a type of breast cancer where the cells are confined to growth and motion in a breast duct. We focus on the simulation results and defer a biological discussion of breast cancer and model calibration to Chapter 10.

For simulation in 2D, consider cells growing in a fluid-filled domain \( \Omega \) (representing a rigid-walled duct) of length \( \ell \) (1 mm in our simulations) and width \( 2R \) (340 \( \mu \)m in our simulations). We “cap” the left edge of the simulated duct with a semicircle of radius \( R \). 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 \( d \) satisfying \( d > 0 \) inside the duct.

Cell states (see Figure 6.1) include the proliferative (\( P \)), quiescent (\( Q \)), apoptotic (\( A \)), hypoxic (\( H \)), necrotic (\( N \)), calcified debris (\( C \)) and motile (\( M \)) states. We use the simplified model of motility along a basement membrane in randomly-selected directions. See Figure 6.3. For simplicity, we neglect membrane degradation, membrane deformation, and molecular-scale signaling, allowing us to instead focus upon the effects of the various cell states and forces. We assume there is no ECM in the duct (\( F_{cma} = 0 \)), and cells adhere to one another with E-cadherin (homophilic adhesion) and to the BM with integrins. We assume the surface adhesion receptors and BM adhesion ligands are distributed uniformly on the cell surfaces and BM, respectively.
We model oxygen transport within the duct by

\[
\frac{\partial \sigma}{\partial t} = D \nabla^2 \sigma - \lambda \sigma \quad \text{if } x \in \Omega
\]
\[
\sigma = \sigma_B \quad \text{if } x \in \partial \Omega,
\]

where \( \lambda \) is the locally-averaged oxygen uptake rate discussed in Section 6.4. We use a Neumann condition \( \partial \sigma / \partial n = 0 \) on the righthand side of the duct.

The parameter values for the simulations are given in Table 6.1. The duct size, population parameters (\( \alpha_A, \alpha_P, \beta_A, \) and \( \beta_P )\), balance of cell-cell adhesion and repulsion (\( \alpha_{cca} \) vs. \( \alpha_{ccr} \)), and oxygen boundary value \( \sigma_B \) are calibrated to the patient data presented in Chapter 10; more detail is given there. Further detail on the numerical implementation can be found in Section 10.2.4 and [436].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Physical Meaning</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_{duct} )</td>
<td>duct diameter</td>
<td>340 ( \mu \text{m} )</td>
</tr>
<tr>
<td>( L_{duct} )</td>
<td>duct length</td>
<td>1 mm</td>
</tr>
<tr>
<td>( R )</td>
<td>Cell radius</td>
<td>9.953 ( \mu \text{m} )</td>
</tr>
<tr>
<td>( \sigma_H )</td>
<td>hypoxic oxygen threshold</td>
<td>0.2</td>
</tr>
<tr>
<td>( \sigma_B )</td>
<td>duct boundary ( \text{O}_2 ) value</td>
<td>0.263717 if ( \lambda_b = 0 ) ( 0.277941 ) if ( \lambda_b = 0.001 ) ( 0.386095 ) if ( \lambda_b = 0.1 )</td>
</tr>
<tr>
<td>( \langle \lambda \rangle )</td>
<td>mean oxygen uptake rate</td>
<td>0.1 ( \text{min}^{-1} )</td>
</tr>
<tr>
<td>( \lambda_t )</td>
<td>tumor cell ( \text{O}_2 ) uptake rate</td>
<td>0.1 ( \text{min}^{-1} )</td>
</tr>
<tr>
<td>( \lambda_b )</td>
<td>background ( \text{O}_2 ) decay rate</td>
<td>0.001, 0.01, or 0.1 ( \text{min}^{-1} )</td>
</tr>
<tr>
<td>( \lambda^{-1}_A )</td>
<td>oxygen diffusion length scale</td>
<td>100 ( \mu \text{m} )</td>
</tr>
<tr>
<td>( \alpha^{-1}_A )</td>
<td>mean time to apoptosis</td>
<td>786.61 hours</td>
</tr>
<tr>
<td>( \lambda^{-1}_P )</td>
<td>mean time to proliferation (when ( \sigma = 1 )</td>
<td>115.27 min if ( \lambda_b = 0 ) ( 151.21 ) min if ( \lambda_b = 0.001 ) ( 434.53 ) min if ( \lambda_b = 0.1 )</td>
</tr>
<tr>
<td>( \beta^{-1}_A )</td>
<td>time to complete apoptosis</td>
<td>8.6 hours</td>
</tr>
<tr>
<td>( \beta^{-1}_P )</td>
<td>time to complete cell cycle</td>
<td>18 hours</td>
</tr>
<tr>
<td>( \beta^{-1}_H )</td>
<td>time to complete calcification</td>
<td>15 days</td>
</tr>
<tr>
<td>( \beta^{-1}_N )</td>
<td>mean survival time for hypoxic cells</td>
<td>0 or 5 hours</td>
</tr>
<tr>
<td>( \beta_{N,L} )</td>
<td>time for necrotic cells to lyse</td>
<td>2, 24, 120, or 360 hours</td>
</tr>
<tr>
<td>( \beta_{N,S} )</td>
<td>necrotic cell surface receptor degradation time</td>
<td>5 or 15 days</td>
</tr>
<tr>
<td>( V_s/V )</td>
<td>solid cell fraction</td>
<td>10%</td>
</tr>
<tr>
<td>( \alpha_{cca}, \alpha_{cha}, \alpha_{dda} )</td>
<td>cell-cell, cell-BM, debris adhesive forces</td>
<td>0.39 ( 153 \nu ) ( \mu \text{m/min} )</td>
</tr>
<tr>
<td>( \alpha_{cre} )</td>
<td>cell-cell repulsive force</td>
<td>8( \nu ) ( \mu \text{m/min} )</td>
</tr>
<tr>
<td>( \alpha_{cbr} )</td>
<td>cell-BM repulsive force</td>
<td>5( \nu ) ( \mu \text{m/min} )</td>
</tr>
<tr>
<td>( \alpha_{loc} )</td>
<td>locomotive (motile) force</td>
<td>5( \nu ) ( \mu \text{m/min} )</td>
</tr>
<tr>
<td>( \alpha_{M}^{-1} )</td>
<td>mean time between migration events</td>
<td>120, 300, or ( \infty ) min</td>
</tr>
<tr>
<td>( \beta_{M \text{_max}} )</td>
<td>maximum motility time</td>
<td>15 min</td>
</tr>
<tr>
<td>( \ell_{\text{podium}} )</td>
<td>maximum lamellipodium length</td>
<td>15 or 20 ( \mu \text{m} )</td>
</tr>
<tr>
<td>( \alpha_{c:a}, \alpha_{cha}, \alpha_{dda} )</td>
<td>adhesion potential parameters</td>
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</tr>
<tr>
<td>( \alpha_{cre}, \alpha_{cbr} )</td>
<td>repulsion potential parameters</td>
<td>1</td>
</tr>
<tr>
<td>( R_{cca}, R_{cha}, R_{dda} )</td>
<td>maximum interaction distances</td>
<td>12.083 ( \mu \text{m} )</td>
</tr>
</tbody>
</table>

Table 6.1. Main parameters used for the agent model DCIS simulations.

### 6.6.1 Baseline calibrated run

We first simulate DCIS with simplified hypoxia (\( \beta_H^{-1} = 0 \)) and no motility (\( \alpha_M = 0 \)). We apply a simplified model of necrosis: \( \beta_{N,S}^{-1} = \beta_{N,L}^{-1} = \beta_{N,C}^{-1} \). We set \( \lambda_b = 0.01 \lambda_t \). The dynamic simulation is presented in Figure 6.4.
Figure 6.4 Dynamic agent-based simulation of DCIS in a 1 mm duct. **Legend:** quiescent cells are white, apoptotic cells are black, proliferating cells are diagonally striped, necrotic cells are light gray, and calcified cell debris is dark gray. Reprinted with permission from [436].

In the simulation, a small initial population begins proliferating into the duct (0 days). As the tumor grows along the duct, oxygen uptake by the cells leads to the formation of an oxygen gradient (not shown). At time 5.04 days, the oxygen level drops below $\sigma_H$ in the center of the duct near the leading edge of the tumor, causing the first instance of necrosis (light gray cells). By 7 to 14 days, a viable rim of nearly uniform thickness (approximately 80 $\mu$m thick) 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. (See also Section 10.3.3.) This is consistent with the prediction in Section 6.5 that the cell state dynamics reach a local steady state by 10 to 100 days.

Consistent with the assumed functional form of the $Q \rightarrow P$ transition, proliferating cells (diagonally striped) are most abundant near the duct wall where
the oxygen level is highest, with virtually no proliferation at the peri-necrotic boundary. Because oxygen can diffuse into the tumor from the duct lumen (with low decay rate $\lambda_b$), viable tumor cells are also observed along the tumor’s leading edge near the center of the duct. See Figure 6.4 at times 7, 14, and 21 days.

Cells begin to lyse at 20.04 days, and because $\beta_{NC} = \beta_{NL}$, they immediately calcify (dark gray cells). By 28 days, another characteristic length emerges: the trailing edge of the microcalcification maintains a distance of approximately 500 $\mu$m from the end of the duct. 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 toward the tumor’s leading edge. Because the end of the duct has reached a local dynamic equilibrium by this time (see the discussion above and [439]), a steady flux of tumor cells into the necrotic region has emerged. Because the lysis and calcification times ($\beta^{-1}_{NL}$ and $\beta^{-1}_{NC}$) are fixed, the cells are pushed a fixed distance along the necrotic core before lysing and calcifying, leading to the observed “standing wave” pattern.

The viable rim of the tumor reaches the end of the simulated 1 mm stretch of the duct by time 22.5 days, and the necrotic core reaches this computational boundary by approximately 24 days. Cells that exit the computational domain are removed from the simulation, leading to an artificial drop in mechanical resistance to growth, particularly as cells from the trailing edge of the tumor continue to proliferate and push into the lumen. Thus, the simulation is more valuable for examining the radial tumor dynamics than the rate of progression through the duct after this time.

6.6.2 Impact of hypoxic survival time

We next examined the impact of $\beta^{-1}_H$, the mean time hypoxic cells survive before necrosing. We found that increasing $\beta^{-1}_H$ from 0 to 5 hours has a minimal impact on the simulation, principally by delaying calcification (because cells wait a few more hours before necrosing). (Results not shown.)

The minimal impact of $\beta_H$ on the current model behavior is not surprising: hypoxic cells are not motile in this model implementation, and the overall flux of cells is from the proliferating rim towards the center of the duct. Hence, there is no opportunity for hypoxic cells to take advantage of their increased survival time to return to the viable rim. Thus, increasing $\beta^{-1}_H$ merely delays necrosis (in this model formulation). Furthermore, the hypoxic cells are not glycemic in this model, and so they do not lower the pH (acidosis) in the nearby viable rim; hence, the increased hypoxic survival time does not impact the survivability and behavior of cells in the viable rim. We expect that $\beta_H$ would have a greater impact if:

1. hypoxic cells were allowed to switch to anaerobic glycolysis, thus making the viable rim microenvironment acidic and giving the hypoxic cells a competitive advantage over nearby non-resistant tumor cell strains;
2. hypoxic cells were allowed to migrate out of the hypoxic region, thereby propogating their phenotypic adaptations into the tumor viable rim;
3. continuing the previous point, hypoxic cells could reach the basement membrane and initiate invasive carcinoma; and
4. hypoxic cells may experience increased genetic instability due to the harsh environment. This tends to accelerate the previous points.

Taken together, hypoxia would be expected to increased tumor invasiveness if these aspects were incorporated in the model.

6.6.3 Impact of the cell lysis time

Next, we investigate the impact of the necrotic cell lysis time $\beta_{NL}^{-1}$ on the overall tumor evolution. We varied $\beta_{NL}^{-1}$ with values 15 days, 5 days, 1 day, and 2 hours. Because surface receptor degradation is likely a faster process than cell calcification, we set $\beta_{NS}^{-1}$ to 5 days. However, we found that varying $\beta_{NS}$ had little impact on the mechanical behavior due to the dense cell packing in the simulated tumors.

![Figure 6.5 Impact of the necrotic cell lysis timescale](image)

Figure 6.5 Impact of the necrotic cell lysis timescale: We plot the DCIS progression at time 25 days for $\beta_{NL}^{-1} = 15$ days (top), 5 days (second from top), 1 day (second from bottom), and 2 hours (bottom). Reprinted with permission from [436].
In Figure 6.5, we plot the DCIS simulations at time 25 days for each of these lysis parameter values. The parameter has a great effect on the rate of tumor advance through the duct: as the lysis time is decreased, the rate of tumor advance through the duct slows. This is because the necrotic core acts as a volume sink when the cells lyse, thereby relieving mechanical pressure. In turn, more of the cell flux is directed towards the center of the duct, rather than forward towards the tumor leading edge. Once $\beta_{NL}^{-1} < 1$ day, further reductions have little additional impact on the tumor progression, because the time the cells spend in an unlysed state is much smaller than the growth time scale.

In addition, the size of the microcalcification grows as $\beta_{NL}^{-1}$ decreases. This is because more cells are pushed into the necrotic core rather than forward for small values of $\beta_{NL}^{-1}$, whereby a greater number of cells have been in the necrotic region longer than the calcification time $\beta_{NC}^{-1}$. Furthermore, the microcalcification appears to have a rounder morphology for the shorter lysis times. This topic is still under investigation, but it appears to be due to the more uniform packing of the lysed necrotic cells, owing to their occupying a greater percentage of the necrotic core for small values of $\beta_{NL}^{-1}$. Furthermore, the microcalcifications appear to occupy a larger percentage of the duct cross section due to the slower rate of tumor advance through the duct.

Similarly to the “standing wave” calcification pattern in the baseline simulation (and for similar reasons), a characteristic length emerges between the end of the duct and the start of the lysed cell region. This length is shorter than the distance from the end of the duct to the trailing edge of the calcification. These lengths both decrease as $\beta_{NL}^{-1}$ decreases, due to the faster progression from viable cells to necrotic cells to lysed cells to calcified cells.

In a detailed comparison of these simulation results to breast histopathology, we find that 1 day $\leq \beta_{NL}^{-1} \leq 5$ day yields the best match between the simulated and actual necrotic core morphological features, including the rough distribution of lysed and unlysed cells and the general appearance of the necrotic cross sections [436].

6.6.4 Impact of background oxygen decay rate

We varied $\lambda_b$ with values 0.001 min$^{-1}$, 0.01 min$^{-1}$, and 0.1 min$^{-1}$ to investigate its impact on the simulation results. Aside from eliminating the viable cells from the center of the tumor’s leading edge (see the baseline run), there was very little impact on the simulations (not shown). This is because the tumor necrotic centers were densely packed for all values of $\lambda_b$, and since necrotic cells uptake oxygen at rate $\lambda_t = \langle \lambda \rangle$, the oxygen uptake rate is equal throughout the bulk of the computational domain. Thus, the oxygen profile is similar for all three cases, leading to comparable amounts of cell proliferation in all cases; i.e., the extra viable cells in the leading edge did not contribute substantially to the tumor’s advance through the duct. However, $\lambda_b$ may have a greater impact in less dense tumors, such as cribriform DCIS (see Section 10.1.2).
6.6.5 Impact of cell motility

When next allowed random cell motility along the basement membrane, as described in Section 6.2.4. We set $\beta_{NL}^{-1} = 2$ hours, $\beta_{H}^{-1} = 5$ hours, and investigated the role of the mean time to migration ($\alpha_{M}^{-1} = 300$ min, $120$ min) and that of the maximum lamellipodium length (15 or $20 \, \mu m$). We set the maximum migration speed ($\alpha_{loc}/\nu$) to $5 \, \mu m/min$ and the maximum duration of migration to $15$ min. All simulations set $\lambda_0 = 0.001 \, min^{-1}$.

In Figure 6.6, we plot the simulations at time 30 days for $15 \, \mu m$ maximum lamellipodium length, for $\alpha_{M} = 0$ (top), $\alpha_{M}^{-1} = 300$ min (middle), and $\alpha_{M}^{-1} = 120$ min (bottom). In additional to the earlier figure legends, hypoxic cells are light gray with black dots. As $\alpha_{M}^{-1}$ is decreased from infinite (top) to $120$ min (bottom), the tumor leading edge morphology becomes less blunt, and the tumor advances slightly farther along the duct. Thus, even random motility can increase the rate of a tumor’s advance through a breast duct. We expect that this effect will be more pronounced if the cells instead move in a directed manner (e.g., chemotactically, as in Paget’s disease [166]).

We also increased the maximum lamellipodium length $\ell_{podium}$ from $15 \, \mu m$ to $20 \, \mu m$ for $\alpha_{M}^{-1} = 120$ min. Similarly to decreasing $\alpha_{M}^{-1}$, increasing $\ell_{podium}$ accentuates the effect of motility because the motile cells can travel farther per migration event (simulation not shown). Note that these effects are subtle, and so further analysis and simulations (with different random seeds) are required to fully quantify them and confirm their statistical significance.

Figure 6.6 Impact of cell motility: As the mean time to migration is decreased from infinite (top) to $300$ minutes (middle) to $120$ minutes (bottom), the rate of tumor advance through the duct is slightly increased, with the leading edge more spread out. Reprinted with permission from [436].
6.7 Conclusions

In this chapter, we reviewed the major discrete modeling approaches currently employed in mathematical cancer cell biology, with particular focus on a generalized agent-based cell modeling framework. After a brief analysis of the model’s population dynamics, we closed by presenting numerical examples of the model applied to breast cancer; this application is explored in greater depth in Chapter 10. We found that the specific modeling of the necrotic volume loss can have a major impact on the rate of tumor advance through the duct. Furthermore, even random cell motility along the basement membrane can speed a tumor’s spread through the duct system, and the effect should be much more pronounced in cases of directed motility, such as Paget’s disease (chemotactic motion along the ducts towards chemoattractants released by keratinocytes in the breast nipple [166]). On the other hand, we found that if hypoxia is modeled merely as a delayed progression to necrosis, then it has little impact on DCIS progression; instead, glycolysis, acidosis, and motility must be considered. This finding is consistent with previous modeling results by Gatenby and co-workers (e.g., [264, 629, 265, 268, 630, 627, 628]).
References


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