Supplement: Creating DCIS digital cell lines from clinical immunohistochemical data

Protocol version: 1.0 (November 29, 2016)
Further updates: http://MultiCellDS.org and http://MultiCellDS.sf.net

Previously, Macklin and collaborators[2,3] developed a protocol to estimate simulation parameters for an agent-based model, based on morphometric and immunohistochemical measurements performed on ductal carcinoma in situ (DCIS) of the breast by Edgerton and co-workers[1]. Those calculations estimated cell birth, death, and size information, as well as (dimensionless) tissue oxygenation within the breast duct viable rims for 2-D simulations. (See prior works[2,3] for further details.) The calibrated model was able to predict DCIS growth rates consistent with the literature, match Ki-67 and cleaved Caspase-3 fractions of viable DCIS tissue, the higher prevalence of Ki-67 positive cells near the outer edge of the viable rim, and the size of the viable rim and necrotic core[2]. Coarse-graining these parameters[1] allowed Edgerton et al. to predict DCIS excision volumes in 12 of 17 measured cases; the poorest matches corresponded to cases where the steady state assumption of the coarse graining failed to hold[1]. We now improve and extend the technique to 3D to estimate cell phenotypic properties (for the standardized cell cycle models in MultiCellDS) in physioxic conditions for normal breast tissue, "standardized" physioxia, patient-specific *in vivo* ductal conditions, hypoxia, and in chronic hypoxia / necrotic conditions.

The available measurements by Edgerton et al.[1] include the tumor viable rim thickness (T_{rim}), duct radius (R_{duct}), the proliferative index (PI: percentage of cells staining positive for Ki-67), the raw apoptotic index (AI_{raw}, the percentage of cells staining positive for cleaved Caspase-3), the tumor cell density ρ (in 12 of the cases), and the DCIS subtype (cribriform, solid, or a mix of these types). All these measurements were recorded in several ducts for each of 17 patients; the mean values are given in Table 1.

The protocol below is our current best estimate of the mathematical model parameters. As measurement methods and mathematical analyses are improved, we will update this protocol and the digital cell lines accordingly. Indeed, this is one of the main ideas of curated digital cell lines: any data element can be replaced by a superior measurement or estimate as they become available, and shared with the broader research community.

Table 1: Mean DCIS patient measurements by Edgerton et al.[1] NA denotes "not available". 'C' = cribriform, 'S' = solid type, and 'M' = mixed type (partly solid, partly cribriform).

Case	MultiCellDS Digital Cell Name	MultiCellDS Digital Cell Line ID	Type (S, C, M)	T_{rim} (μm)	R_{duct} (μm)	ρ (cells/μm^2)	PI (%)	AI_{raw} (%)
8	DCIS_ACP2011_8	45.0.0.1	C	183.22	422.58	4.77e-3	9.37	0.24
13	DCIS_ACP2011_13	46.0.0.1	S	96.43	243.03	2.79e-3	25.90	8.59
14	DCIS_ACP2011_14	47.0.0.1	C	171.83	204.53	8.51e-3	7.87	0.04
15	DCIS_ACP2011_15	48.0.0.1	C	147.77	147.77	8.83e-3	0.56	0.10
17	DCIS_ACP2011_17	49.0.0.1	M	108.92	115.86	5.94e-3	3.08	10.07
18.1	DCIS_ACP2011_18.1	50.0.0.1	C	116.35	146.27	1.12e-2	0.11	0.04
18.2	DCIS_ACP2011_18.2	51.0.0.1	M	111.71	232.75	3.44e-3	13.99	0.86
19	DCIS_ACP2011_19	52.0.0.1	M	78.87	158.75	3.21e-3	17.43	0.64
21	DCIS_ACP2011_21	53.0.0.1	C	113.11	120.68	5.57e-3	3.64	0.00
22	DCIS_ACP2011_22	54.0.0.1	C	97.08	270.87	4.52e-3	16.08	0.77
23	DCIS_ACP2011_23	55.0.0.1	S	134.78	157.62	4.36e-3	17.07	2.81
28	DCIS_ACP2011_28	56.0.0.1	S	86.58	135.51	NA	19.78	1.410
39	DCIS_ACP2011_39	57.0.0.1	M	77.55	119.60	NA	3.30	0.18
40	DCIS_ACP2011_40	58.0.0.1	M	223.91	323.17	NA	4.39	0.34
42	DCIS_ACP2011_42	59.0.0.1	C	148.70	191.82	NA	3.33	0.25
48	DCIS_ACP2011_48	60.0.0.1	C	136.28	136.28	NA	5.05	0.23
51	DCIS_ACP2011_51	61.0.0.1	S	106.91	293.21	NA	16.24	1.81

Estimating cell geometrical properties:
As in the earlier protocol[3], we adjust the mean cell density (cells per slide viable rim area) to the confluent cell density (cells per confluent viable rim area), and use this to get the average cell cross-sectional area A_{cell}, radius R_{cell}, and volume V_{cell}:

$$A_{\mathrm{cell}} = \frac{f}{\rho} \quad (1)$$

$$R_{\mathrm{cell}} = \sqrt{\frac{A_{\mathrm{cell}}}{\pi}} \quad (2)$$

$$V_{\mathrm{cell}} = \frac{4}{3}\pi R_{\mathrm{cell}}^3 \quad (3)$$

In the equations above, f is the viable tissue confluence: the fraction of the viable rim occupied by tumor cells. (Note that $f = 1$ in fully confluent tissue, and $f = 0$ in open lumen.) Hyun and Macklin[3] estimated $f = 0.90$ for a mixed-typed DCIS case (mixed solid type and cribriform type). For this work, we set $f = 1$ for solid-type DCIS, $f = 0.9$ for mixed type, and $f = 0.80$ for cribriform. (Image analysis of several hematoxylin and eosin-stained samples of cribriform-type DCIS by Dong et al.[4] yielded f between 0.6 and 0.85, with many in the higher end of this range, so we chose 0.8 as an estimate consistent with this range and observations.) If better estimates for f become available, we can update the protocol and digital cell lines accordingly.

Estimating tissue oxygenation:
In physioxic (normal) breast tissue, oxygenation is approximately 52 mmHg pO_2 by prior measurements[5,6] (equivalent to culturing tissue at around 6.8% oxygenation). "Standardized" physioxic conditions are defined[5] to be 5% oxygenation or 38 mmHg pO_2. We define hypoxia to be 1% oxygenation or 8 mmHg pO_2: this is a representative, intermediate value between the half-maximum HIF-1α response (HIF-1α is a hypoxic response protein[5,7]) at 11-15 mmHg and the maximum response at 3.8 mmHg (0.5%)[5]. This is also consistent with reports that hypoxic proteomic and gene expression changes are observed below 7 mmHg pO_2 (approximately 1% oxygenation)[6]. See the summary in Table 2.

Tumor cells can vary widely in their survival in chronic low oxygenation conditions[5], complicating our effort to estimate typical oxygenation in necrotic tissues without direct measurements. We set the hypoxic tissue conditions discussed above (0.5% to 1.5%, or 3.8 to 11.4 mmHg pO_2) as an upper bound. McKeown reported that tumor cells are killed when exposed to 0.01% oxygenation for 24 hours, and most tumor cells are killed after 72 hours' exposure to 0.1% oxygenation[5]. This sets 0.01% to 0.1% (0.076 to 0.76 mmHg pO_2) as a lower-bound for necrotic tissues observed in pathology images. Under the assumption that most cells in necrotic ductal regions have been exposed to low oxygenation conditions for several days or more (chronic hypoxia), we shall initially set our necrotic threshold value to an intermediate value of 5 mmHg pO_2 (0.66%). This value is consistent with the observations by Vaupel that proteomic and genetic changes are observable in cells under 7 mmHg pO_2, and necrosis can result[6].

Table 2: Oxygenation values used for DCIS digital cell lines

Condition	pO_2 (mmHg)	% O_2
Physioxic (breast)	52	6.8
Physioxic (standard)	38	5
viable rim	patient-specific	
hypoxic	8	1
necrotic	5	0.66

We now estimate the patient-specific oxygenation in the viable DCIS tissue. Using the earlier protocol[3] but solving for a 3-D cylindrical duct geometry rather than a 2-D geometry, the mean tissue oxygenation ‹σ› in the viable rim is given by:

$$L = \frac{L_0}{\sqrt{f}} \quad (4)$$

$$\langle\sigma\rangle = \left(\frac{2\sigma_{\mathrm{N}}L}{I_0\left(\frac{R_{\mathrm{N}}}{L}\right)}\right)\left(\frac{2L}{2R_{\mathrm{duct}}T_{\mathrm{rim}} - T_{\mathrm{rim}}^2}\right)\left(R_{\mathrm{Duct}}I_1\left(\frac{R_{\mathrm{duct}}}{L}\right) - R_{\mathrm{N}}I_1\left(\frac{R_{\mathrm{N}}}{L}\right)\right) \quad (5)$$

where σ_N is a necrotic oxygenation threshold estimated above (σ_N = 5 mmHg), $R_N = R_{Duct} - T_{rim}$ is the radius of the necrotic core, and f is the viable tissue confluence defined as above. (Note that $I_1(x)$ is modified Bessel function of the first kind.) As before[1-3], $L_0 = (D/\lambda)^{1/2}$ is the oxygen diffusion length scale in confluent tissue, where D is the oxygen diffusion coefficient in tissue ($D = 10^5$ μm^2/min by earlier experiments[8] and analysis[9]), λ is the

oxygen consumption rate in confluent tumor tissue, and we set L_0 = 100 μm. We note that this combination of f and L_0 yields effective diffusion length scales L ranging from 100 to 115 μm, consistent with prior measurements for breast[10] and other tumor tissues[11,12] giving L ~ 100 μm to 200 μm.

Estimating cell cycle and apoptosis parameters (Advanced Ki-67 model):

We update the earlier analysis[3] to use the "Advanced Ki-67" cell cycle phase model, where cycling cells stain positive for Ki-67 in the S, G_2, and M phases prior to mitosis (jointly, these are population K_1), and for a few hours after mitosis[1-3] (population K_2) before returning to a non-cycling, Ki-67-negative state (population Q). Any live cell can become apoptotic (population A). Because we have no data to specify the cell cycle phase at the onset of apoptosis, we use a constant background rate of apoptosis r_A for all cycle phases. We require T_1 (the mean duration of K_1), T_2 (the mean duration of T_2), the overall apoptosis rate r_A, the mean time spent in Q prior to cell cycle re-entry (T_Q), and the *in vivo* duration of apoptosis (T_A).

In this model, we require the following parameters:

Parameter	Physical meaning
T_Q	Mean time spent in (duration of) the quiescent Q state (Ki-67- cells)
T_1	The mean time spent in (duration of) the K_1 phase (pre-mitotic Ki-67+ cells)
T_2	The mean time spent in (duration of) the K_2 phase (post-mitotic Ki-67+ cells)
T_A	The mean duration of apoptosis
r_A	The mean rate of apoptosis across all non-apoptotic cells

We also seek to estimate the mean population doubling time (T_{double}) for cells in exponential growth.

Ki-67 is primarily expressed in the (particularly late) S, G_2, and M-phases[13,14], and less reliably in the G_1 phase[15]. Ki-67 is observed in post-mitotic daughter cells[1-3,16], but it is not produced post-mitotically[13,14]; instead, any remaining Ki-67 protein from the preceding M phase is degraded quickly, with a half-life of 60-90 minutes[11,12]. Thus, we set T_1 to be the combined duration of S, G_2, and M, which are relatively fixed compared to the duration of G_0/G_1 (T_2 + T_Q in the Ki-67 advanced model)[19,20]. As an estimate, we set T_1 = 13 hours, based upon typical estimates for the S, G_2, and M phases for eukaryotic cells[17], and consistent with reports where S+G_2+M can vary from 10 to 24 hours (e.g., 12.3 hours[18], 12 to 24 hours[19], and 10 to 10.5 hours[20]). We set T_2 to be on the order of two Ki-67 half-lives (we shall use the intermediate estimate of a 75 minute half-life), or 2.5 hours. We retain our prior estimate[2,3] of the duration of apoptosis T_A = 8.6 hours. The remaining data elements are T_Q and r_A.

Adjusting the apoptotic index for undercounting

As in prior work[21-23], we note that cleaved Caspase-3 only stains a fraction of apoptotic cells, and thus we adjust the apoptotic index to compensate via:

$$\mathrm{AI} = \frac{8.6}{6.6}\mathrm{AI}_{\mathrm{raw}} \tag{6}$$

Estimating the mean parameter values (T_Q and r_A) in the viable rim

The number of cells in the K_1, K_2, Q, and A states satisfy the following system of ordinary differential equations:

$$\frac{dK_1}{dt} = \frac{1}{T_Q}Q - \left(\frac{1}{T_1}+r_A\right)K_1 \tag{7}$$

$$\frac{dK_2}{dt} = \frac{2}{T_1}K_1 - \left(\frac{1}{T_2}+r_A\right)K_2 \tag{8}$$

$$\frac{dQ}{dt} = \frac{1}{T_2}K_2 - \left(\frac{1}{T_Q}+r_A\right)Q \tag{9}$$

$$\frac{dA}{dt} = r_A(K_1+K_2+Q) - \frac{1}{T_A}A \tag{10}$$

$$\frac{dN}{dt} = \frac{1}{T_1}K_1 - \frac{1}{T_A}A \tag{11}$$

where $N = K_1+K_2+Q+A$ is the total number of cells.

As in the prior protocol[3], we rewrite these as differential equations for $KI_1=K_1/N$, $KI_2=K_2/N$, and $AI=A/N$:

$$\frac{d}{dt}(KI_1) = \frac{1}{T_Q}QI - \left(\frac{1}{T_1}+r_A\right)KI_1 - KI_1\left(\frac{1}{T_1}KI_1 - \frac{1}{T_A}AI\right) \quad (12)$$

$$\frac{d}{dt}(KI_2) = \frac{2}{T_1}KI_1 - \left(\frac{1}{T_2}+r_A\right)KI_2 - KI_2\left(\frac{1}{T_1}KI_1 - \frac{1}{T_A}AI\right) \quad (13)$$

$$\frac{d}{dt}(AI) = r_A(1-AI) - \frac{1}{T_A}AI - AI\left(\frac{1}{T_1}KI_1 - \frac{1}{T_A}AI\right) \quad (14)$$

As before[3], we approximate these population fractions as near steady state at the time of biopsy, set d/dt = 0, and solve for T_Q and r_A. If KI_1 and KI_2 = PI-KI_1 are not measured independently, we can preliminarily estimate:

$$KI_1 \approx \frac{T_1}{T_1+2T_2}PI \quad (15)$$

$$KI_2 \approx \frac{2T_2}{T_1+2T_2}PI \quad (16)$$

(For any one cell leaving K_1 after T_1 time, there are two daughter cells spending T_2 time in K_2.) Solving Equation (14) to steady state, we can solve for r_A:

$$r_A = \frac{\frac{1}{T_A}AI + \frac{1}{T_1}AI\,KI_1 - \frac{1}{T_A}AI^2}{1-AI} \quad (17)$$

Using this and Equation (12) for KI_1 (after solving to steady state), we can estimate T_Q:

$$T_Q = \left(\frac{1-AI-PI}{(1+r_AT_1)KI_1 + KI_1^2 - \left(\frac{T_1}{T_A}\right)AI\,KI_1}\right)T_1 \quad (18)$$

Lastly, we iterate this process to refine our estimates as follows:

Step 1: Estimate T_Q and r_A based upon Equations (17) and (18).

Step 2: For the current estimates of KI_1, KI_2, T_Q, and r_A, and starting with AI(0) = AI, $KI_1(0) = KI_1$, $KI_2(0) = KI_2$, numerically solve the differential equations (12)-(14) for AI, KI_1, KI_2 to steady state. In our work, we solve with Δt = 0.1 hour until T_{max} = 365 days.

Step 4: Evaluate $PI_{numerical} = KI_1(T_{max}) + KI_2(T_{max})$, and $AI_{numerical} = AI(T_{max})$. If $|PI_{numerical} - PI| > \varepsilon \cdot PI$ or if $|AI_{numerical} - AI| > \varepsilon \cdot AI$ for some (relative) tolerance ε, then return to Step 1. In our work, we use $\varepsilon = 10^{-4}$.

Estimating the population doubling time

Equation (11) gives the total population versus time in the absence of spatial or other constraints. This can be rewritten as

$$\frac{dN}{dt} = \left(\frac{KI_1(t)}{T_1} - \frac{AI(t)}{T_A}\right)N. \quad (19)$$

If the population fractions have reached steady state, then

$$N(t) = N(0)e^{\left(\frac{KI_1}{T_1}-\frac{AI}{T_A}\right)t}, \quad (20)$$

so $r_{net} = (KI_1)/T_1 - AI/T_A$ is the net birth rate, and the population doubling time is given by

$$T_{double} = \frac{\ln 2}{\frac{KI_1}{T_1}-\frac{AI}{T_A}}. \quad (21)$$

Estimating the parameter values in other oxygenation conditions

Following the earlier protocol[1-3] and prior experimental evidence[24,25] that the cell cycle duration primarily varies in the duration of the G_0/G_1 phase (Q in the Ki-67 advanced model), we model a linear relationship between $1/T_Q$ (the rate of cell cycle entry) and oxygenation via:

$$\frac{1}{T_Q(\sigma)} = \frac{1}{T_Q(\langle\sigma\rangle)}\left(\frac{\sigma-\sigma_N}{\langle\sigma\rangle-\sigma_N}\right), \tag{22}$$

where σ_N is the necrotic oxygen value, and $\langle\sigma\rangle$ is the mean oxygenation estimated in the viable rim above.

We evaluate this expression for the mean time spent in the Q state for oxygenation values of 5 mmHg (chronic hypoxia / necrotic tissue), 8 mmHg (hypoxic), 38 mmHg (standard physioxia), and 52 mmHg (breast physioxia). All other parameter values are left unchanged. To estimate the population doubling time, we solve Equations (12)-(14) to steady state using the new value for T_Q and the prior values for T_1, T_2, T_A and r_A, obtain the steady state values for KI_1 and AI, and use Equation (21).

Estimating cell cycle and apoptosis parameters (Basic Ki-67 model):

In this model, Ki-67 positive cells are included in a proliferative state P (with duration T_P), Ki-67 negative cells are in a non-proliferative state Q (with duration T_Q), and apoptotic cells are in the A state (with duration T_A). As before, we also seek the population doubling time. In this section, we assume that the parameters have already been determined for the advanced Ki-67 model (in each oxygenation condition), so we seek parameters that are as consistent with that model as possible. Thus, we choose T_Q and r_A to fit the measured Ki-67 positive fraction (PI) and corrected apoptotic fraction (AI), and we choose T_P so that the population doubling time matches the Ki-67 advanced model. We use the same apoptosis duration T_A.

In this model, we require the following parameters:

Parameter	Physical meaning
T_Q	Mean time spent in (duration of) the quiescent Q state (Ki-67 negative cells)
T_P	The mean time spent in (duration of) the K phase
T_A	The mean duration of apoptosis
r_A	The mean rate of apoptosis across all non-apoptotic cells

Estimating the mean parameter values (T_Q, T_P and r_A) in the viable rim

The number of cells in the P, Q, and A states satisfy the following system of ordinary differential equations:

$$\frac{dP}{dt} = \frac{1}{T_Q}Q - \left(\frac{1}{T_P}+r_A\right)P \tag{23}$$

$$\frac{dQ}{dt} = \frac{2}{T_P}P - \left(\frac{1}{T_Q}+r_A\right)Q \tag{24}$$

$$\frac{dA}{dt} = r_A(P+Q) - \frac{1}{T_A}A \tag{25}$$

$$\frac{dN}{dt} = \frac{1}{T_P}P - \frac{1}{T_A}A \tag{26}$$

where $N = P + Q + A$ is the total number of cells.

Similarly to the work for the Ki-67 advanced model above, we rewrite these as differential equations for PI=P/N and AI=A/N:

$$\frac{d}{dt}(PI) = \frac{1}{T_Q}QI - \left(\frac{1}{T_P}+r_A\right)PI - PI\left(\frac{1}{T_P}PI - \frac{1}{T_A}AI\right) \tag{27}$$

$$\frac{d}{dt}(AI) = r_A(1-AI) - \frac{1}{T_A}AI - AI\left(\frac{1}{T_P}PI - \frac{1}{T_A}AI\right). \tag{28}$$

Continuing, if the population fractions AI and PI have reached steady state, then the population doubling time is given by

$$T_{\text{double}} = \frac{\ln 2}{\frac{PI}{T_P}-\frac{AI}{T_A}} \tag{29}$$

We match this to Equation (21) to set the value of T_P, so that both Ki-67 models give consistent population doubling times:

$$T_P = \frac{\mathrm{PI}}{\mathrm{KI}_1} T_1. \tag{30}$$

We use this value of T_P throughout the remaining calculations. We solve Equations (27)-(28) to steady state to determine r_A and T_Q:

$$r_A = \frac{\frac{1}{T_A} AI + \frac{1}{T_P}\mathrm{AI}\cdot\mathrm{PI} - \frac{1}{T_A}\mathrm{AI}^2}{1-\mathrm{AI}} \tag{31}$$

$$T_Q = \left(\frac{1-\mathrm{AI}-\mathrm{PI}}{(1+r_A T_P)\mathrm{PI} + \mathrm{PI}^2 - \left(\frac{T_P}{T_A}\right)\mathrm{AI}\cdot\mathrm{PI}} \right) T_P \tag{32}$$

Estimating the parameter values in other oxygenation conditions

We model T_A and r_A as fixed in all other oxygenation conditions. Because KI_1 varies with oxygenation (as calculated by the Ki-67 advanced model), we re-calculate T_P for each oxygenation condition based on Equation (30), and then T_Q based on Equation (32). We evaluate these for oxygenation values of 5 mmHg (necrotic tissue), 8 mmHg (hypoxic), 38 mmHg (physioxic_standard), and 52 mmHg (physioxic_breast).

Estimating cell cycle and apoptosis parameters (Live-Dead model):

This model tracks live cells L (with mean cell birth rate b and cell cycle duration $T_P = 1/b$, and with death rate d) and dead (assumed apoptotic) cells A (with duration T_A, with no change in value from the more detailed cell cycle models). As before, we also seek the population doubling time. We set the parameters to match the live fraction 1-AI, and to match the population doubling time of the advanced Ki-67 model. The Live-Dead model is:

$$\frac{dL}{dt} = (b-d)L \tag{33}$$

$$\frac{dA}{dt} = dL - \frac{1}{T_A} A \tag{34}$$

$$\frac{dN}{dt} = bL - \frac{1}{T_A} A = \left(b(1-\mathrm{AI}) - \frac{1}{T_A}\mathrm{AI} \right) N \tag{35}$$

where $N = L+A$ is the total number of cells. If AI reaches steady state, then the population doubling time is

$$T_{\mathrm{double}} = \frac{\ln 2}{b(1-\mathrm{AI}) - \frac{1}{T_A}\mathrm{AI}}. \tag{36}$$

If we match to the population doubling time for the Ki-67 Advanced model in Equation (21), then we obtain a matched estimate for the birth rate b:

$$b = \frac{\mathrm{KI}_1}{T_1} \cdot \frac{1}{1-\mathrm{AI}}. \tag{37}$$

We now require an estimate for the death rate d. Following the earlier analyses, we can write an equation for the apoptotic fraction AI:

$$\frac{d\mathrm{AI}}{dt} = d(1-\mathrm{AI}) - \frac{1}{T_A}\mathrm{AI} - b\mathrm{AI}(1-\mathrm{AI}) + \frac{1}{T_A}\mathrm{AI}^2 \tag{38}$$

If the population fractions have reached steady state, then

$$d = \frac{\frac{1}{T_A}\mathrm{AI} + b\mathrm{AI}(1-\mathrm{AI}) - \frac{1}{T_A}\mathrm{AI}^2}{1-\mathrm{AI}} \tag{39}$$

These parameters should be separately matched for each phenotype dataset.

Estimating cell cycle and apoptosis parameters (Total Cells model):
This model tracks the total number of cells N with net birth rate r_{net}. The basic equation to parameterize is

$$\frac{dN}{dt} = r_{\text{net}} N. \tag{40}$$

By matching directly with Equation (19) in the advanced Ki-67 model, we obtain

$$r_{\text{net}} = \frac{\text{KI}_1}{T_1} - \frac{\text{AI}}{T_{\text{A}}}. \tag{41}$$

We repeat this conversion for each phenotype dataset.

Estimating necrosis parameters (order of magnitude analysis):
We wish to estimate (to order of magnitude, given the relative lack of direct measurements) the necrotic cell death rate r_{nec} in chronic hypoxic conditions at σ = 5 mmHg pO_2 (and equivalently, the mean tumor cell survival time $T_{survival}$ in these conditions conditions), so that the tumor cell population satisfies

$$\frac{dL}{dt} = -r_{\text{nec}}(\sigma) L \tag{42}$$

$$\frac{dN}{dt} = r_{\text{nec}}(\sigma) L - \frac{1}{T_{\text{necrosis}}} N \tag{43}$$

where L is the live cell population, N is the necrotic cell population, $r_{necrosis}$ is the (pO_2-dependent) necrotic death rate, and $T_{necrosis}$ is the mean time for a necrotic cell to fully degrade. By prior estimates[2,26], $T_{necrosis}$ ~ 60 days.

By prior modeling work[2], the population-scale model in Equation (42) is consistent with a cell-scale model where the individual cell's survival time $T_{survival}$ is exponentially distributed with parameter r_{nec}:

$$T_{\text{survival}} \sim \text{Exp}\big(r_{\text{nec}}(\sigma)\big), \quad \text{where} \quad r_{\text{nec}}(\sigma) = \frac{1}{\langle T_{\text{survival}}(\sigma) \rangle}, \tag{44}$$

and whose cumulative probability distribution is given by

$$\text{Prob}(T_{\text{survival}}(\sigma) \le t) = 1 - e^{-r_{\text{nec}}(\sigma) t}. \tag{45}$$

Typical hypoxia measurements (e.g., these prior works[27,28]) give the time to reach a cell surviving fraction of 10% or less. In one study[27] (see Figure 1 of the study[27]), ~85% of murine fibroblast cells died after exposure to 0.01% (0.076 mmHg pO_2) conditions. In another study[28], the authors worked with several prostate cancer lines, and found that in 0.1% oxygenation conditions, cell proliferation slowed after 1 day of exposure, death could be observed after 2 days, and ~95% of cells were killed by 3 days. (See Figure 2 of the study[28].) We use these reports to estimate $T_{survival}(0.01\%)$ and $T_{survival}(0.1\%)$ based upon Equation (45):

$$0.85 = 1 - e^{-r_{\text{nec}}(0.01\%) 1.25} \Longrightarrow r_{\text{nec}}(0.01\%) = -\frac{\ln(0.15)}{1.25 \text{ day}} \text{T} \tag{46}$$

$$T_{\text{survival}}(0.01\%) = \frac{1}{r_{\text{nec}}} \approx 0.7 \text{ days}$$

and similarly (with rounding to the nearest 0.1 day),

$$0.95 = 1 - e^{-r_{\text{nec}}(0.1\%) 3} \Longrightarrow r_{\text{nec}}(0.1\%) = -\frac{\ln(0.05)}{3 \text{ day}} \tag{48}$$

$$T_{\text{survival}}(0.1\%) = \frac{1}{r_{\text{nec}}} \approx 1 \text{ day}. \tag{49}$$

Thus, cancer cells survive (on average) on the order of one day in very low oxygen conditions. Our earlier calculations use a necrotic oxygen threshold σ_N = 5 mmHg (0.67%), about 7 times higher than the experimental

conditions in the prostate cancer cell experiment[28], but with comparable order of magnitude. Thus, we estimate the cell survival time in 0.67% oxygenation is on the order of 1 to 7 days. As an intermediate estimate, we set $T_{survival}(0.67\%) = 4$ days and $r_{nec}(0.67\%) = 0.25$ day^{-1}. We note that Papandreou et al.[27] observed a decrease in net cell proliferation (birth-death) at 0.5% (3.8 mmHg pO_2) after 3 days of exposure, and even at 15 mmHg pO_2 (2%) oxygenation, cell death has been reported after 4 days of exposure for several prostate cancer cell lines[29], consistent with this estimate. Lastly, we point out that these estimates can be improved (and the digital cell lines can be updated) as more direct cell tracking measurements of necrotic cell death come available.

REFERENCES

1 Edgerton, M. E. *et al.* A novel, patient-specific mathematical pathology approach for assessment of surgical volume: Application to ductal carcinoma in situ of the breast. *Anal Cell Pathol* **34**, 247-263, doi:10.3233/Acp-2011-0019 (2011).

2 Macklin, P., Edgerton, M. E., Thompson, A. M. & Cristini, V. Patient-calibrated agent-based modelling of ductal carcinoma in situ (DCIS): from microscopic measurements to macroscopic predictions of clinical progression. *J Theor Biol* **301**, 122-140, doi:10.1016/j.jtbi.2012.02.002 (2012).

3 Hyun, A. Z. & Macklin, P. Improved patient-specific calibration for agent-based cancer modeling. *Journal of theoretical biology* **317**, 422-424, doi:10.1016/j.jtbi.2012.10.017 (2013).

4 Dong, F. *et al.* Computational pathology to discriminate benign from malignant intraductal proliferations of the breast. *PloS one* **9**, e114885, doi:10.1371/journal.pone.0114885 (2014).

5 McKeown, S. R. Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. *The British journal of radiology* **87**, 20130676, doi:10.1259/bjr.20130676 (2014).

6 Vaupel, P. Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. *The oncologist* **13 Suppl 3**, 21-26, doi:10.1634/theoncologist.13-S3-21 (2008).

7 Macklin, P. in *Multiscale Modeling of Cancer: An Integrated Experimental and Mathematical Modeling Approach* (eds Vittorio Cristini & John S. Lowengrub) Ch. 2, 8-23 (Cambridge University Press, 2010).

8 Grote, J., Susskind, R. & Vaupel, P. Oxygen diffusivity in tumor tissue (DS-carcinosarcoma) under temperature conditions within the range of 20--40 degrees C. *Pflugers Archiv : European journal of physiology* **372**, 37-42 (1977).

9 Ghaffarizadeh, A., Friedman, S. H. & Macklin, P. BioFVM: an efficient, parallelized diffusive transport solver for 3-D biological simulations. *Bioinformatics* **32**, 1256-1258, doi:10.1093/bioinformatics/btv730 (2016).

10 Groebe, K. & Vaupel, P. Evaluation of oxygen diffusion distances in human breast cancer xenografts using tumor-specific in vivo data: role of various mechanisms in the development of tumor hypoxia. *International journal of radiation oncology, biology, physics* **15**, 691-697 (1988).

11 Olive, P. L., Vikse, C. & Trotter, M. J. Measurement of Oxygen Diffusion Distance in Tumor Cubes Using a Fluorescent Hypoxia Probe. *Int J Radiat Oncol* **22**, 397-402 (1992).

12 Thomlinson, R. H. & Gray, L. H. The Histological Structure of Some Human Lung Cancers and the Possible Implications for Radiotherapy. *Brit J Cancer* **9**, 539-&, doi:Doi 10.1038/Bjc.1955.55 (1955).

13 Bruno, S. & Darzynkiewicz, Z. Cell-Cycle Dependent Expression and Stability of the Nuclear-Protein Detected by Ki-67 Antibody in Hl-60 Cells. *Cell Proliferat* **25**, 31-40, doi:DOI 10.1111/j.1365-2184.1992.tb01435.x (1992).

14 Urruticoechea, A., Smith, I. E. & Dowsett, M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol* **23**, 7212-7220, doi:10.1200/Jco.2005.07.501 (2005).

15 Aoyagi, M. *et al.* Immunohistochemical Detection of Ki-67 in Replicative Smooth-Muscle Cells of Rabbit Carotid Arteries after Balloon Denudation. *Stroke* **26**, 2328-2331 (1995).

16 du Manoir, S., Guillaud, P., Camus, E., Seigneurin, D. & Brugal, G. Ki-67 labeling in postmitotic cells defines different Ki-67 pathways within the 2c compartment. *Cytometry* **12**, 455-463, doi:10.1002/cyto.990120511 (1991).

17 Cooper, G. M. *The Eukaryotic Cell Cycle. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA)*, <http://www.ncbi.nlm.nih.gov/books/NBK9876/> (2000).

18 Sutherland, R. L., Hall, R. E. & Taylor, I. W. Cell proliferation kinetics of MCF-7 human mammary carcinoma cells in culture and effects of tamoxifen on exponentially growing and plateau-phase cells. *Cancer Res* **43**, 3998-4006 (1983).

19 Larsson, S., Ryden, T., Holst, U., Oredsson, S. & Johansson, M. Estimating the variation in S phase duration from flow cytometric histograms. *Mathematical biosciences* **213**, 40-49, doi:10.1016/j.mbs.2008.01.009 (2008).

20 Lin, W. & Arthur, G. Phospholipids are synthesized in the G2/M phase of the cell cycle. *The international journal of biochemistry & cell biology* **39**, 597-605, doi:10.1016/j.biocel.2006.10.011 (2007).

21 Edgerton, M. E. *et al.* A novel, patient-specific mathematical pathology approach for assessment of surgical volume: Application to ductal carcinoma in situ of the breast. *Anal. Cell. Pathol.* **34**, 247-263, doi:10.3233/acp-2011-0019 (2011).

22 Macklin, P., Edgerton, M. E., Thompson, A. M. & Cristini, V. Patient-calibrated agent-based modelling of ductal carcinoma in situ (DCIS): From microscopic measurements to macroscopic predictions of clinical progression. *J. Theor. Biol.* **301**, 122-140, doi:10.1016/j.jtbi.2012.02.002 (2012).
23 Hyun, A. Z. & Macklin, P. Improved patient-specific calibration for agent-based cancer modeling. *J. Theor. Biol.* **317**, 422-424, doi:10.1016/j.jtbi.2012.10.017 (2013).
24 Streichan, S. J., Hoerner, C. R., Schneidt, T., Holzer, D. & Hufnagel, L. Spatial constraints control cell proliferation in tissues. *Proc Natl Acad Sci U S A* **111**, 5586-5591, doi:10.1073/pnas.1323016111 (2014).
25 Smith, J. A. & Martin, L. Do cells cycle? *Proc Natl Acad Sci U S A* **70**, 1263-1267 (1973).
26 Macklin, P., Mumenthaler, S. & Lowengrub, J. in *Multiscale Computer Modeling in Biomechanics and Biomedical Engineering* Vol. 14 *Studies in Mechanobiology, Tissue Engineering and Biomaterials* (ed Amit Gefen) Ch. 150, 349-380 (Springer Berlin Heidelberg, 2013).
27 Papandreou, I. *et al.* Anoxia is necessary for tumor cell toxicity caused by a low-oxygen environment. *Cancer Res* **65**, 3171-3178, doi:10.1158/0008-5472.CAN-04-3395 (2005).
28 Butterworth, K. T. *et al.* Hypoxia selects for androgen independent LNCaP cells with a more malignant geno- and phenotype. *International journal of cancer. Journal international du cancer* **123**, 760-768, doi:10.1002/ijc.23418 (2008).
29 Ma, T. *et al.* Effects of oxygen on the antigenic landscape of prostate cancer cells. *BMC research notes* **8**, 687, doi:10.1186/s13104-015-1633-7 (2015).