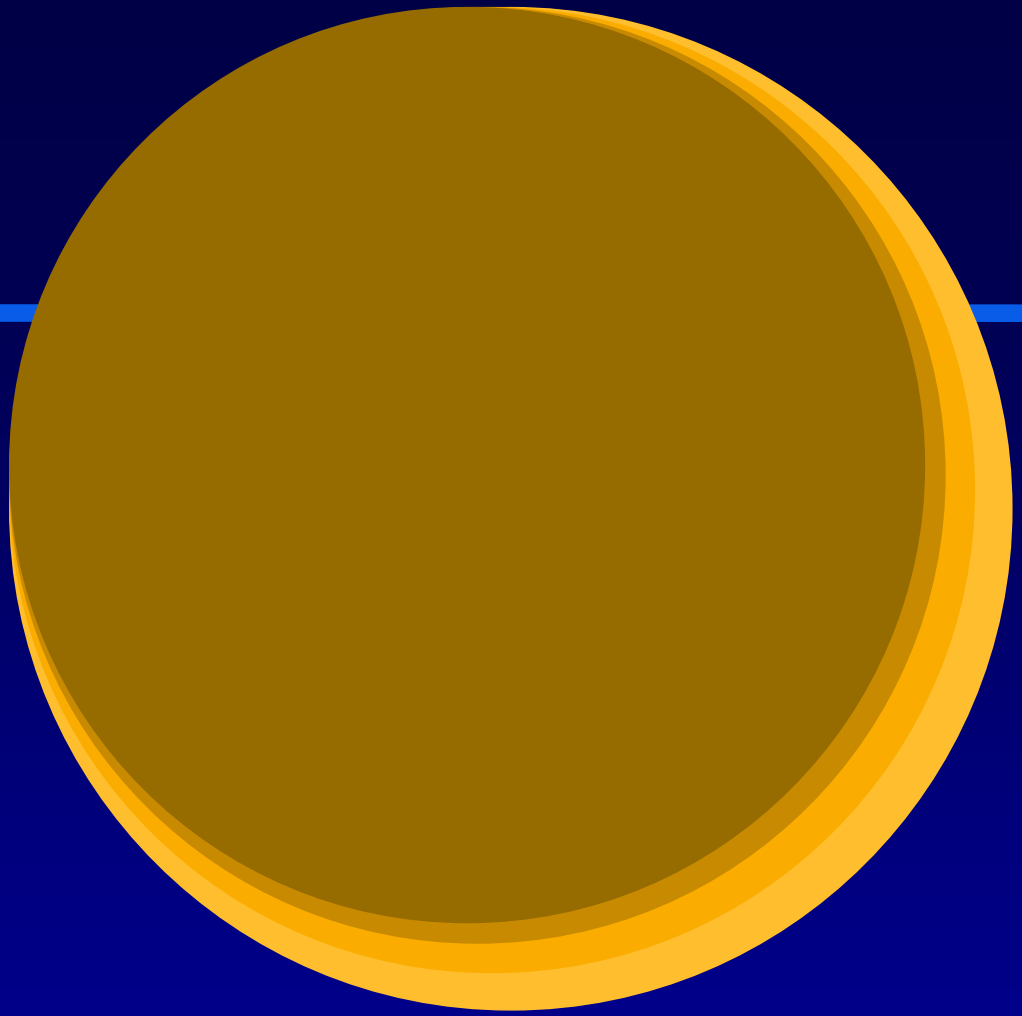


Lecture 3:
Parameter estimation & patient-specific calibration

Paul Macklin, Ph.D.
Lecturer
University of Dundee

23 August 2010

Motivation

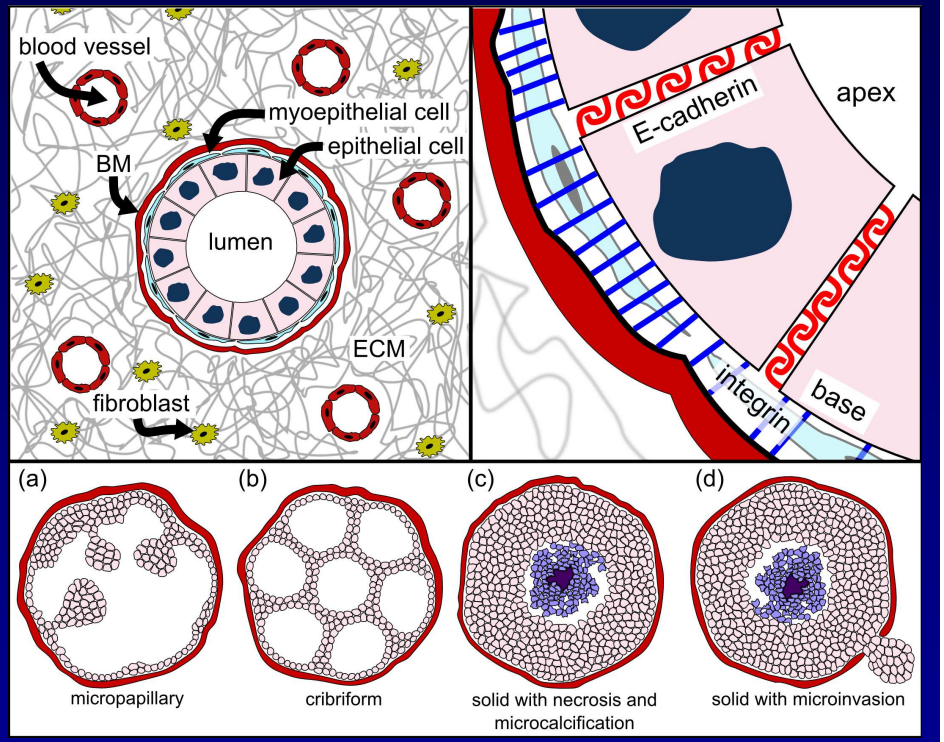
- Want to calibrate to patient data
- Want to make testable predictions on cancer biology
- Tautology: Complex models have many parameters. The apparent agreement between model behavior and data may be due to tuning free parameters. (“cheating”)
- To be predictive, we need to constrain the parameters.
  - Try to use simplest model possible with fewer parameters.
  - Estimate from experimental / theoretical biology.
  - Use mathematical analyses to extract more from the data.
  - For patient-specific behaviour, need to incorporate noisy data.
- Ideal: set the correct physical parameter values, and let the physics do the rest.

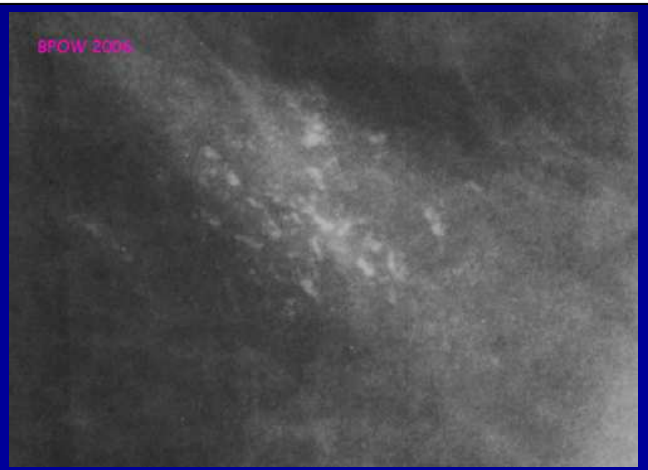
Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Brief introduction to DCIS

- Ductal carcinoma in situ (DCIS)
- Growth constrained to lumen by BM
- Not yet invasive
- Precursor to invasive carcinoma
- Growth fed by diffusion from outside duct
- Subtypes depend in part upon mechanics, cell polarisation
- Cribriform and micropapillary: cells polarised, but no anoikis
- Solid-type and comedo: cells unpolarised, oxygen diffusion limitations, necrosis
- Microinvasion: Cells break through BM and invade stroma
- Notable feature: calcification of necrotic material – prime detection method by mammogram





Lecture Outline

- Brief Introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Estimating easy parameters: Cell cycle length, G1 length

- Typical time scales are typically 18 to 24 hours
  - Byrne, Owen, Ward, King, others
- We separate G0 from cycle, so take shorter end
  - τ_P = 18 hours
- Experimental work by Smith & Martin (1973):
  - S + G2 + M = 9 hours (= ½ τ_P)
  - τ_P (their T_B) also in the range of 10 to 24 hours

Lecture Outline

- Brief Introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Estimating easy parameters: Some oxygen parameters

- Oxygen diffusion length scale $L \sim 100$ μm
  - Byrne, Owen, Ward, King, others, 1970s experiments …
  - D ~ 50 μm²/s = 3000 μm²/min ← Owen et al. 2004
- Use this and O2 diffusion coefficient to derive mean O2 uptake in the viable rim

$$L = \sqrt{\frac{D}{\langle \lambda \rangle}}$$

  - Get mean uptake ~ 0.1 1/min
- Compare Ward and King (1997) proliferation rate at the step function limit to our α_P, and get $\sigma_H \sim 0.2$

Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Estimating tricky parameters: Apoptosis time

- Can be a difficult process to observe from start to finish
- Not as much data out there as we'd like – clinical interest tends to end at the start of apoptosis
- Order of magnitude estimate: on the order of hours
  - Makes biological / chemical sense: inactivated Caspases are pre-positioned and ready to go
  - Makes intuitive sense: destruction is easier than creation
- Use mathematical mode + IHC to estimate
- Lee et al. 2006:
  - measured AI (TUNEL assay) and PI (Ki-67) in non-cancerous breast epithelium
  - Several hundred pre-menopausal women
  - Several hundred post-menopausal women
  - Large enough sample size that menstrual oscillations balance out
  - Assume that tissue is overall in homeostasis, when averaged across the menstrual cycle

Estimating tricky parameters: Apoptosis time

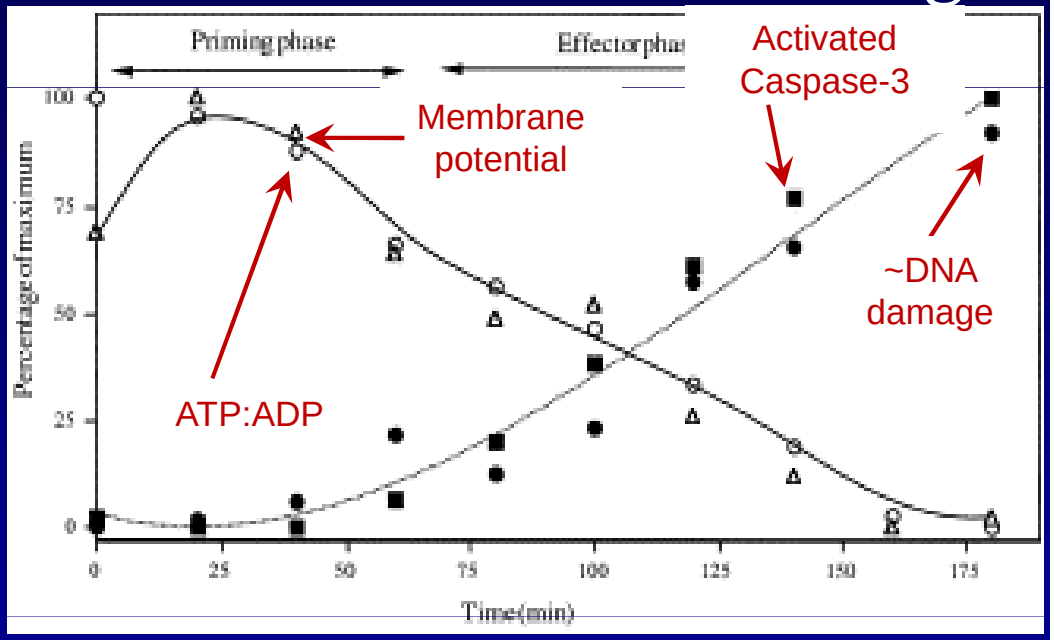
- Assume (a lá Hanahan & Weinberg) that tumour and normal cells use same processes with altered frequency
  - Assume same cell cycle length ~18h
  - same apoptosis length
- Use the volume-averaged estimate for normoxic tissue
  - $N' = PI / \tau_P - AI / \tau_A$
  - Solve for τ_A
- Get 5.71 hours from pre-menopausal data
- Get 5.62 hours from post-menopausal data
- Get similar results for differing hormonal situations
  - gives credence to the approach

Estimating tricky parameters: Apoptosis time

- Want to adjust the estimate to account for imaging and detection shortcomings
  - TUNEL assay: detects DNA fragmentation
  - Cleaved Caspase-3: detects an "executioner" protein present through most of apoptosis
  - Both detect apoptosis after initial processes – probably underestimate apoptosis
- Turn to experimental biology on individual cells
- Scarlet et al. 2003: apoptosis in Jurkat cells
  - Induction of apoptosis ("priming phase"):
    - Rapid loss of mitochondrial membrane voltage potential
    - Rapid change in ATP:ADP → visible marker of energy loss
    - Occurs on order of minutes

Estimating tricky parameters: Apoptosis time

- Scarlet et al. 2003: apoptosis in Jurkat cells – Later during apoptosis:
  - Cleaved caspase-3:
    - 0-60 min: ~0 detection
    - 50-60 min: 10% of peak
    - 180 min: peak level
    - → misses first 1-2 hours
  - TUNEL assay:
    - Little DNA laddering for 3 h.



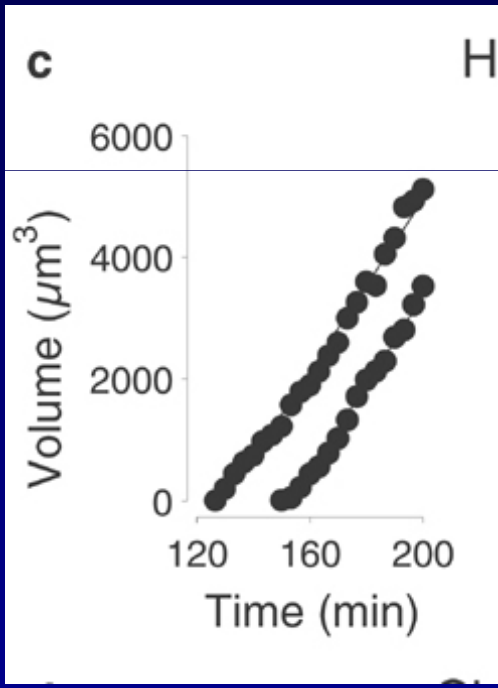
- Adjust estimate to about τ_A = 8.6h
- This is an example of combining experimental and theoretical biology literature with a mathematical understanding to improve modelling.
- Also shows need to understand the core biology and immunohistochemistry to better constrain the model.

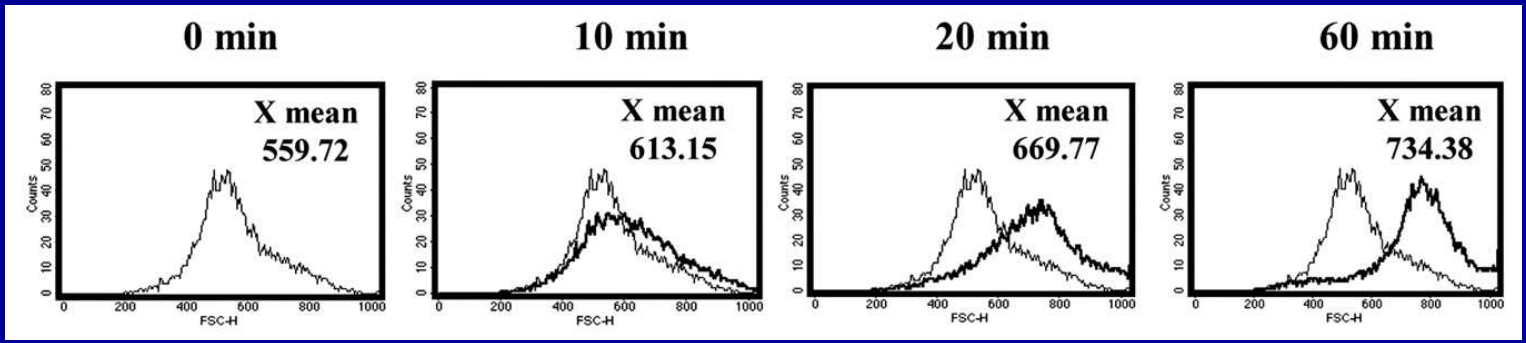
Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Estimating tricky parameters: Necrosis and calcification

- Early swell swelling (and magnitude), lysis
  - Ion pumps lose energy early
  - Ion pumps ordinarily necessary for apoptosis
  - Expect swelling and lysis faster than apoptosis
    - Estimate around 6 hours to swell and lyse
  - Jun et al. 2007: 30% swelling in 60 minutes
  - Barros et al. 2003: necrotic blebs grow linearly, 200 min
  - Grönroos et al. 2005: 1.5-fold increase in 12 h
  - Wu et al. 2010: 2- to 5-fold volume in 24 h.
    - Estimate a conservative f_{NS} 0.3 to 1.0 for start
- Time to lose functional adhesion receptors
  - No easy estimate, but necrotic material seems to aggregate even prior to calcification
  - Some sort of adhesion (possibly not by E-cadherin) on order of days





Estimating tricky parameters: Necrosis and calcification

- Very little data on calcification
- Difficult to study *in vitro*
- Best data: aortic calcification, heart disease
- Jian et al. 2003: significant calcification in *post morten* cardiac valves in 7 days (10% increase in Ca) to 14 days (40% increase)
- Lee et al. 2006: gradual elastin calcification in rad subdermal model in 2-3 weeks
- Gadeau et al. 2001: calcified rabbit aorta after balloon angioplasty injury, calcium deposits in 2-4 days, increased through 8 days, approached steady in 8-30 days
- Our estimate: on the order of weeks
- Hone estimate by simulation, compare percentage of necrotic core occupied by calcification to comedo DCIS (Macklin et al. 2009)
  - 15-day parameter value gave best match (for older model)
  - Newer model with necrotic volume changes: value may even be higher?

τ_C (days)	0.5	1	5	15	30
Fraction of core calcified after 30 days (%)	94.0	83.7	51.1	6.9	0.0

Lecture Outline

- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters (L and lambda, s_H)
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Estimating tricky parameters: Mechanics parameters

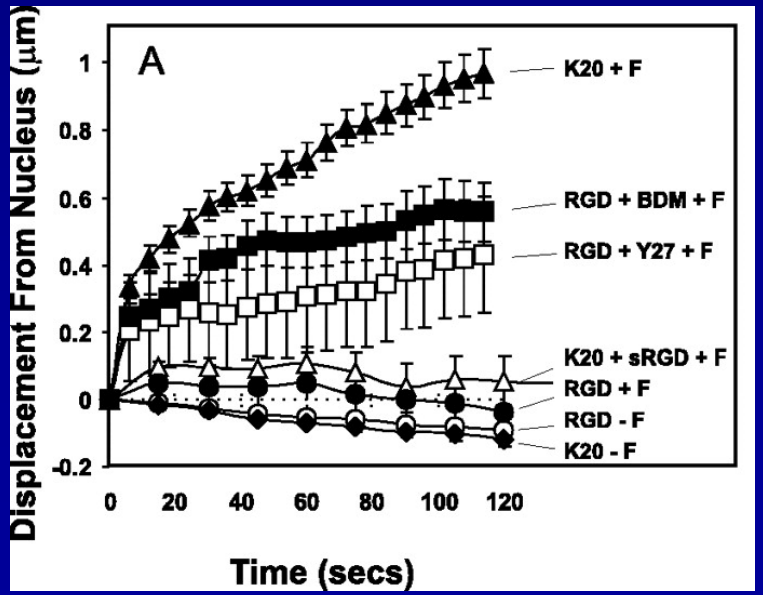
- Solid fraction:
  - experimental data from Macknight et al. (1971) +
  - Assumption solid materials are 1-10 denser than H_2O
  - → $V_S \sim 10\%$
- Cell interaction distance:
  - Byers et al. 1995: MCF-7 and MCF-10A deform 50-70% in sheer flow
  - Guck et al. 2005: After 60 second of stress:
    - MCF10 (benign cell line) deform 10.5%
    - MCF7 (adhesive, moderately aggressive) deform 21.4%
    - MCF7 + weakened cytoskeleton: 30.4%
    - MDA-MB-231 (aggressive): 33.7%
    - For DCIS → estimate $R_{cca} = R_{cba} \sim 1.214\ R$
    - Might be larger due to morphological uncertainty, but good start

Estimating tricky parameters: Mechanics parameters

- Cell relaxation: varied time scales (glassy material) Bursac et al. 2005
  - Tenths of seconds: immediate viscoelastic response
  - Minutes, days or more: prolonged stresses
  - Can combine these with magnitude of deformation to estimate α_{ccr}/υ
  - Matthews et al. 2006: measured motion of magnetic microbeads in cytoskeleton
    - Displacement velocities ($\alpha_{ccr}/\upsilon\,|\nabla\,\psi|$) of 0.1 to 10 μm / min
  - We use $\alpha_{ccr} = 10\,\upsilon$ to give comparable magnitudes at comparable membrane displacements
  - Just a preliminary estimate

distance from cell center r (μm)	$\frac{\alpha_{ccr}}{\nu}\,\lvert\nabla\psi(r)\rvert$ (μm/min)	distance from cell center r (μm)	$\frac{\alpha_{ccr}}{\nu}\,\lvert\nabla\psi(r)\rvert$ (μm/min)
$R - 0.50 = 9.4530$	0.02524	$R - 0.50 = 9.4530$	0.05047
$R - 1.00 = 8.9530$	0.10095	$R - 1.00 = 8.9530$	0.20189
$R - 2.00 = 7.9530$	0.40379	$R - 2.00 = 7.9530$	0.80757
$R - 3.00 = 6.9530$	0.90852	$R - 3.00 = 6.9530$	1.81704

Table 2
Cell relaxation rate given by $\nabla\psi$ for $R = 9.953$ μm, $n_{ccr} = 1$, and $\alpha_{ccr}/\nu = 10.00$ μm/min (*left*) and 20.00 μm/min (*right*), for small and intermediate deformations. The value of M does not play a role when $r > R_N$ (typically 4 to 7 μm).

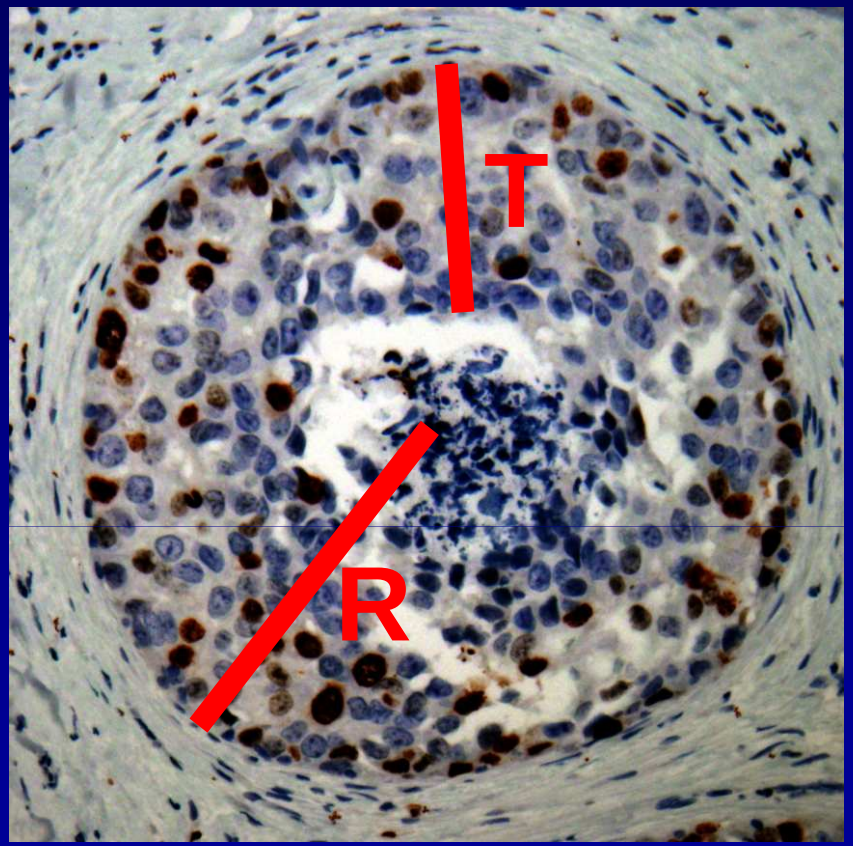


Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

What patient data are available?

- Histopathology hematoxylin and eosin (H&E)
  - Viable rim thicknes (T)
  - Duct radius (R_{duct})
  - Viable rim confluence (f), cell density (ρ)
  - Tumour microstructure
    - Calcifications
- Immunohistochemistry:
  - Ki-67: proliferation marker : images and PI
  - Nuclear size (R_N)
  - Cell density, viable rim confluence
  - Cleaved Caspase-3: apoptosis marker : images and AI

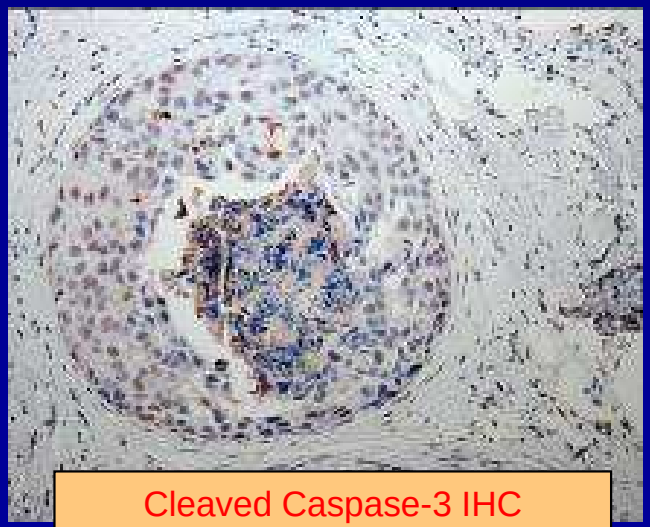


Data Sources

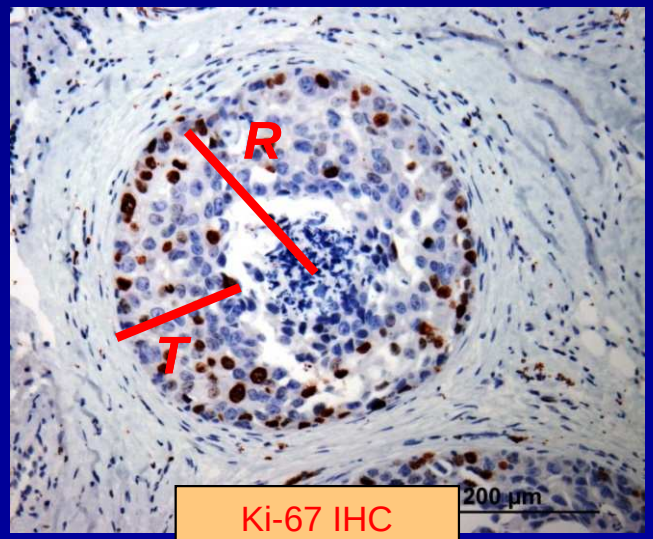
- **Cleaved Caspase-3 IHC**
  - Often under 1%
  - Terrible stain – cytoplasmic – makes segmentation difficult
  - Must also correct for undercounted cells
- **Measure Proliferative Index (PI) with Ki-67 IHC**
  - Nuclear stain – easier to segment / count
  - Present through most of G1, S, G2, M
  - Ranges from 5 to 20%

Quantity	Measured Mean	Units
Duct Radius r_{duct}	158.737	μm
Viable Rim thickness T	78.873	μm
PI	17.429	%
raw AI	0.638	%
corrected AI	0.831	%
Cell density ρ	0.003217	cells/μm^2

Table 2: Key data for de-identified patient 100019.



Cleaved Caspase-3 IHC



Ki-67 IHC

H & E Staining

Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Patient-Specific Calibration: Geometry

- Relate cell cross-sectional area to density:
  - (confluence) (Area/cell count) = f/ρ = area of 1 cell
- Relate to spherical approximation to get equivalent radius :
  - $f/\rho = \pi R^2$
- Get duct radius, viable rim thickness, duct geometry from direct measurements

Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Patient-Specific Calibration: Oxygen transport

- Viable rim thickness gives an indirect measurement of oxygen penetration
  - Solve O_2 in idealised cylinder away from tumour leading edge
  - Evaluate to get duct boundary condition

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

  - Evaluate to get mean oxygen in viable rim

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

  - If using different proliferative and non-proliferative uptake rates, apply additional constraints with volume-averaged rate

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

Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Patient-Specific Calibration: Population dynamics

- Four main population dynamic parameters in the viable rim governing $\mathcal{P} \leftrightarrow \mathcal{Q} \rightarrow \mathcal{A}$ network
- We have AI and PI measurements from IHC
- We estimated the cycle time and apoptosis time
- Assume steady-state population dynamic as in the preceding analysis:
  - We can fully determine the parameters
  - No need to estimate time derivatives from noisy data – temporal information already given by τ_A, τ_P
  - Tie to the functional form for α_P + oxygen estimate to finish

$$\langle \alpha_P \rangle = \frac{\frac{1}{\tau_P}\left(\mathrm{PI} + \mathrm{PI}^2\right) - \frac{1}{\tau_A}\mathrm{AI}\cdot\mathrm{PI}}{1 - \mathrm{AI} - \mathrm{PI}}$$

$$\alpha_A = \frac{\frac{1}{\tau_A}\left(\mathrm{AI} - \mathrm{AI}^2\right) + \frac{1}{\tau_P}\mathrm{AI}\cdot\mathrm{PI}}{1 - \mathrm{AI} - \mathrm{PI}}.$$

$$\langle \alpha_P \rangle = \overline{\alpha}_P \frac{\langle \sigma \rangle - \sigma_H}{1 - \sigma_H}$$

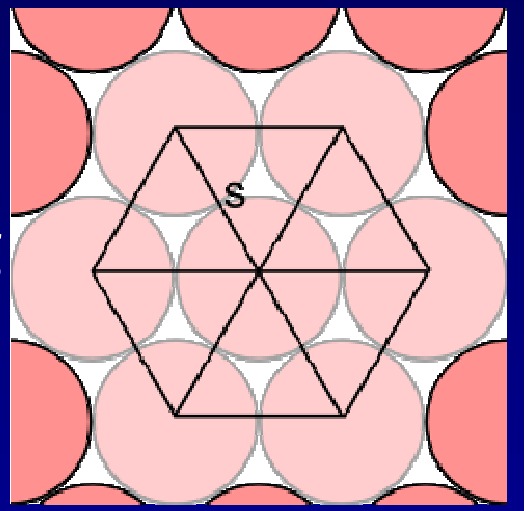
Lecture Outline

- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration and Verification
- Coming next
- References

Patient-Specific Calibration: Cell mechanics

- Density is an indicator of the balance of adhesive and repulsive forces
- Use hexagonal cell packing approximation to determine equilibrium cell spacing

$$s = \sqrt{\frac{2}{\sqrt{3}\langle\rho\rangle}}$$



- Set repulsive and adhesive forces equal at this spacing

$$\mathcal{E}_i\mathcal{E}_j\frac{\alpha_{\text{cca}}}{\alpha_{\text{ccr}}} = \left|\frac{\frac{\partial}{\partial r}\psi\left(s; R_N^i + R_N^j, R_{\text{cca}}^i + R_{\text{cca}}^j, M, n_{\text{ccr}}\right)}{\frac{\partial}{\partial r}\varphi\left(s; R^i + R^j, R_{\text{cca}}^i + R_{\text{cca}}^j, n_{\text{cca}}\right)}\right|$$

- Two coefficient parameters, and we already estimated repulsion → system is determined
- Use tumour microstructure (H&E images) to try to estimate cell-wall mechanics
  - Equilibrium cell-wall distance could be used for similar analysis
  - Without additional forces, need higher cell-wall adhesion for attachment

Lecture Outline

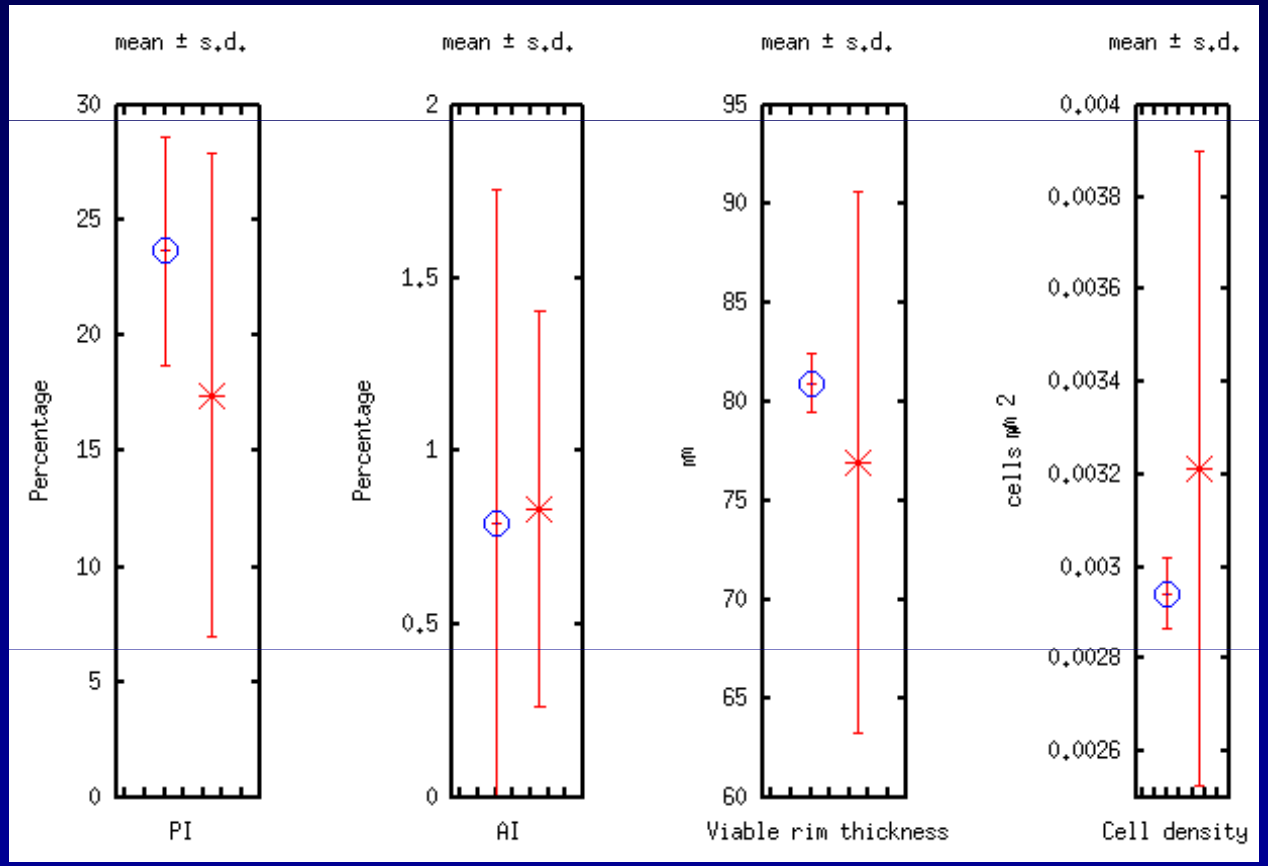
- Brief introduction to DCIS
- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample Calibration & Verification
- Coming next
- References

Sample Calibration and Verification

- Apply the calibration with the following data:
- Run the simulation for 30 simulated days in a 1 mm segment of duct
- Calculate AI, PI, density, and viable rim thickness once per hour
  - Calculate simulated mean and standard deviation
    - Simulated s.d. mostly shows stochasticity, and should decrease as cell number increases
    - But it's also indicative of the heterogeneity across the simulated tumour
  - Restrict away from simulation edges to best match the calibration conditions
  - Compare to mean +/- standard deviation of patient measurements
  - If simulated mean is within [mean – s.d., mean + s.d.], we consider the calibration a "success" (consistent with the data)

Sample Calibration and Verification: Did we hit our targets?

- PI and AI are within the ranges
- Density is within the range
- Viable rim thickness is within the range
- Perfectionism / Learning:
  - PI a bit high
  - V.R. a bit thick
  - Density a bit low
- Likely scenario:
  - Overestimated confluence
  - Underestimated adhesion strength
  - Underestimated cell uptake rate
  - → increased mean O2
  - → larger viable rim
  - → increased proliferation
- Problem is sensitive to O2, which interacts with density
- Next time, better measurements of confluence



Blue / Left on each series: simulation
Red / right on each series: patient data

Sample Calibration and Verification: Any better with simpler O2?

- Do the same calculations with no difference in o2 uptake rate, to eliminate
- PI and AI are within the ranges
- Density is within the range
- Viable rim thickness is within the range
- All are a better fit, when the dependence of O2 upon density is eliminated
  - → method is okay, and highlights importance of confluence measurement

All figures given as mean ± standard deviation

Quantity	Patient Data	Simulated
PI (%)	17.43 ± 10.48	17.193 ± 7.216
AI (%)	0.831 ± 0.572	1.447 ± 3.680
Viable rim thickness (μm)	76.92 ± 13.70	80.615 ± 4.454
Cell density (cells/μm^2)	0.003213 ± 6.89e-4	0.003336

Sample Calibration and Verification: A Caution

- Don't get too excited yet. This *isn't* a prediction. – It's a validation that your programming and calibration are correct / self-consistent.
- You need to predict something outside your calibration data and model assumptions (e.g., predict behaviour in a different geometry) to claim predictivity.

Lecture Outline

- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample calibration
- What's next
- References

Coming Next:

- **Lecture 1:**
  - **Cancer biology for modellers**
- **Lecture 2:**
  - **An agent-based cell model; application to DCIS**
- **Lecture 3:**
  - **Parameter estimation, patient-specific calibration**
- **Lecture 4:**
  - **Numerical method, simulation results**

Lecture Outline

- Estimating easy parameters
  - Cell cycle length, G1 length
  - Some oxygen parameters (L and lambda, s_H)
- Estimating tricky parameters
  - Apoptosis time
  - Necrosis and calcification parameters
  - Mechanics parameters
- What patient data are available?
- Patient-specific calibration
  - Geometric parameters
  - Oxygen transport
  - Population dynamics
  - Cell-cell adhesion mechanics
- Sample calibration
- What's next
- References

Some References

- The calibration presented here is published in:
  - P. Macklin et al. Agent-based modeling of ductal carcinoma in situ: Application to patient-specific breast cancer modeling. In: T. Pham (ed). *Computational Biology: Issues and Applications in Oncology*. Springer, New York, NY USA, 2009. Chapter 4, pages 77-112. ISBN 978-1-4419-0810-0.
  - P. Macklin et al. Agent-based cell modeling: application to breast cancer. In: V. Cristini and J. Lowengrub. *Multiscale Modeling of Cancer*. Cambridge University Press, Cambridge, UK, 2010. Chapter 10, pages 216-44. ISBN 978-0521884426. (in press)
  - P. Macklin et al. A composite agent-based cell model, with application to breast cancer-II: Calibration, Numerical Method and Simulation Results. *J. Theor. Biol.* 2010. (in review)
- Some great DCIS calibration work:
  - See references in publications above by:
    - Owen, Ward, King, Byrne and colleagues: cell energetics, transport
    - Smallbone, Gatenby and colleagues: cell energetics, hypoxia, glycolysis, acidosis

Contact Information:

- **Email:**
  - macklin@maths.dundee.ac.uk
- **Web:**
  - http://www.maths.dundee.ac.uk/macklin
    - (new but under construction)
  - http://biomathematics.shis.uth.tmc.edu
    - (old but already built)