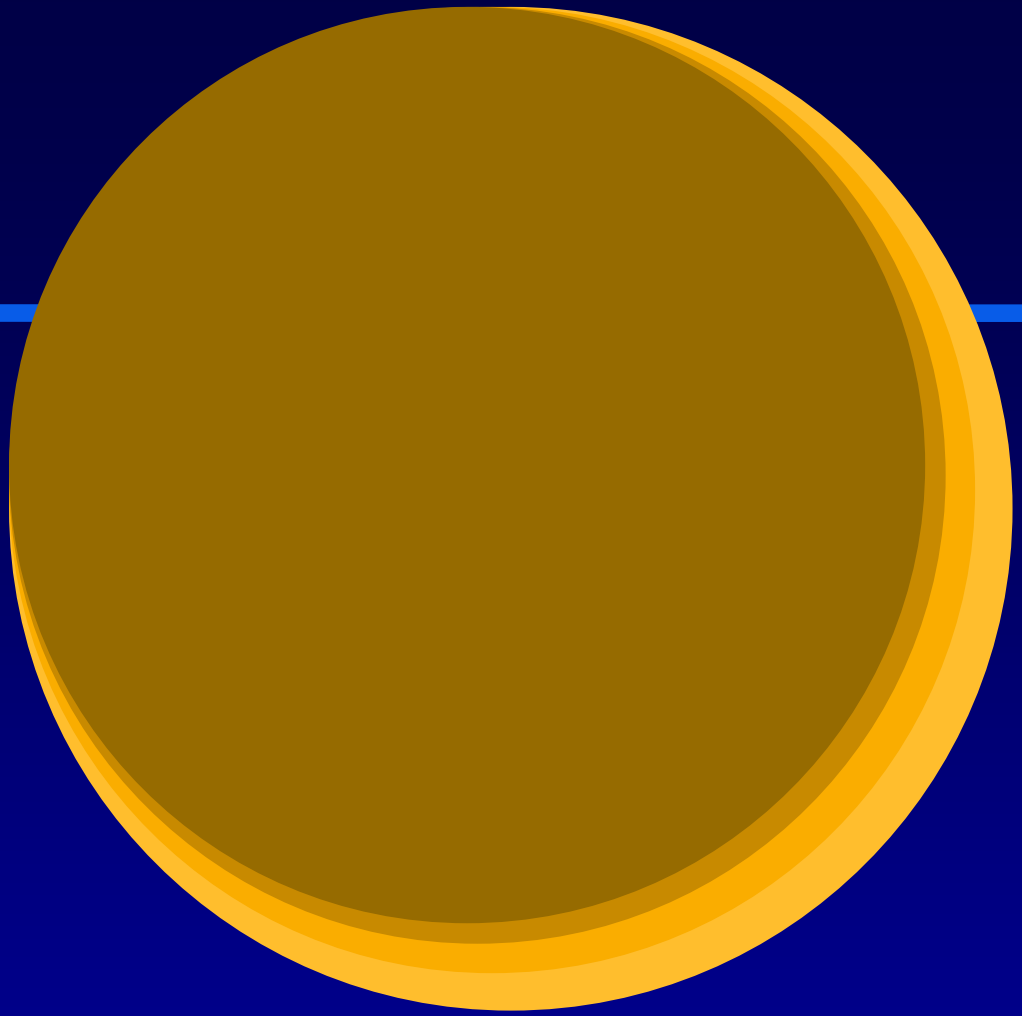

Lecture 4:
Numerical Method & Simulation Results

Paul Macklin, Ph.D.
Lecturer
University of Dundee

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Motivation

- Want to study microstructure, dynamics of necrotic core formation
- Want to understand impact of simple necrotic core mechanics on speed of tumour advance in duct
- Want to understand the relationship between microcalcifications and the actual position of the tumour leading edge
- Test bed for general cell agent framework.

Lecture Outline

- Programming and Architecture
- Algorithms
- Baseline Simulation
  - Simulation animation
  - Post-Processing / Analysis
  - Virtual Ki-67 IHC vs. Actual Ki-67
  - Virtual H&E (?) vs. Actual H&E
- Parameter studies
- Future Directions
- References

Programming and Architecture

- Standard ANSI standard C++:
  - Reasonably fast, reasonably flexible, reasonably powerful
  - write once, compile many places
    - Intel ICC, GNU g++, MS Visual C++, Portland, etc.
    - OSX, BSD, Linux, Unix, Solaris, Windows (MinGW or cygwin), etc.
    - PPC, x86, x86-64, Sparc, etc.
  - Separation of programming problems:
    - Setup by human-editable config files
    - Main routine saves data regularly
      - » Can recover from unexpected events
      - » Can continue interesting simulations
      - » Careful to save state of random number generator!
    - Graphics belong in post-processing of simulation data:
      - » No windowing to degrade cross-platform compatibility
      - » No GUI to degrade performance
      - » Separation of data from presentation & analysis
      - » Can replot / recolour / reanalyse data anytime

Programming and Architecture

- Object-oriented:
  - Cells (agents) are objects defined in a Cell class:
    - Member data:
      - » position, velocity, phenotype, etc.
    - Member functions:
      - » E.g., decide_behaviour(), progress_apoptosis(), etc.
      - » Easy to update each member function as a "sub-model"
      - » Update in one place, and automatically track through to all cells
    - Wrap all the properties together as a sub-class:
      - » Easy to pass arbitrary characteristics to daughter cells
      - » Easy to update without breaking the Cell class
      - » Can use similar sub-classes for Genes, Proteins
    - Well-defined interfaces between cells, program, environment:
      - » Protect "private" data, safer, more robust for collaborating ....
- Data:
  - Output cell internals to standards-compliant XML
    - Human-readable – won't forget your format!
    - Easy to parse by standardised, widely-available libraries
    - Better potential for trading data with others: CellML, SBML, etc.
  - Output field data (O_2, etc.) to matlab (.mat) format:
    - Widespread and well-documented, binary for smaller file size
    - Can be read by GNU Octave as well as MATLAB, personal C++ library, etc.

Programming and Architecture

- Doubly-linked list:
  - All cells have pointers to (memory addresses of):
    - Previous Cell
    - Next Cell
  - List structure managed automatically by default constructors and destructors Cell() and ~Cell();
  - Easy to insert new daughter cells into simulation (immediately after parent cell)
  - Easy to delete apoptosed cells (default destructor fixes the list)
  - No need to have fixed number of cells / memory allocated at compile time or program start-up time
  - List traversal can be expensive if you need to search for a specific cell or data item.
    - Introduce other data structures if you need to do such things!

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- Draw something on linked lists. ☺

Typical Cell Class, Typical Data

D:\data\Work\code\discrete_code\Cell.h - Notepad++

File Edit Search View Encoding Language Settings Macro Run TextFX Plugins Window ?

```
15
16   using namespace std;
17
18  #ifndef __Cell_h__
19  #define __Cell_h__
20
21  class Cell{
22   private:
23    friend ostream& operator<<(ostream& os, const Cell& c);
24   public:
25    cell_properties properties;
26    cell_state state;
27
28    Cell* pNextCell;
29    Cell* pPreviousCell;
30
31    bool UpdateVelocity( double dt );
32    bool UpdatePosition( double dt );
33
34    Cell();
35    void RandomInitialize( void );
36    ~Cell();
37
38    double tell_alpha_A( void );
39    double tell_alpha_P( void );
40
41    bool advance_cell_cycle( double dt );
42    bool apoptose_cell( double dt ); // return true if ready for removal
43    bool distress_via_anoxia( double dt ); // return true if transitions to necrosis
44    bool necrose_cell( double dt ); // return true if ready for calcification
45
46    Cell* Replicate( void );
47    bool Display( void );
48
49    bool decide_behavior( double dt );
50
51
52   bool read_from_xml( TiXmlElement* Root );
53  };
```

D:\data\Work\code\discrete_code\pp_2010\output00000720.xml - Notepad++

File Edit Search View Encoding Language Settings Macro Run TextFX Plugins Window ?

```
1  <data_set>
2      <program_version>1.38</program_version>
3      <globals>
4          <time>43200</time>
5          <next_output_time>43200</next_output_time>
6          <frame_number>720</frame_number>
7          <random_seed_state>2116596844</random_seed_state>
8          <Domain_width_in_microns>1000</Domain_width_in_microns>
9          <Domain_height_in_microns>340</Domain_height_in_microns>
10     </globals>
11
12     <cell_list>
13         <cell>
14             <cell_properties>
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16                 <cell_type_text>DCIS cell</cell_type_text>
17                 <mass>1</mass>
18                 <radius>4.61977316283</radius>
19                 <nuclear_radius>4.61977316283</nuclear_radius>
20                 <volume>413.000487398</volume>
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22                 <solid_volume>413.000487398</solid_volume>
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24                 <cell_adhesion_2_level>0</cell_adhesion_2_level>
25                 <matrix_adhesion_level>0</matrix_adhesion_level>
26                 <calcite_level>1</calcite_level>
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29                 <cadherin_sensitivity>0</cadherin_sensitivity>
30                 <mean_cell_cycle_time>1080</mean_cell_cycle_time>
31                 <mean_G1_time>540</mean_G1_time>
32                 <mean_time_to_apoptosis>9e+99</mean_time_to_apoptosis>
33                 <mean_time_to_mitosis>9e+99</mean_time_to_mitosis>
34                 <mean_time_to_migration>9e+99</mean_time_to_migration>
35                 <max_locomotion_speed>5</max_locomotion_speed>
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44                 <calcite_adhesion_max_distance>1.214</calcite_adhesion_max_distance>
45             </cell_properties>
46             <cell_state>
47                 <is_cycling>false</is_cycling>
```

Algorithms: Overall Program Flow

- Initialisation
  - Parse configuration XML file
  - Cell arrangement, initial states (randomly assigned to match IHC)
  - Initial BM geometry, initial steady solutions of field variables
- Main Loop
  - Update microenvironment
    - Includes impact of cells on substrates
    - Includes any evolution of BM morphology
  - Update cell-cell interaction data structure
  - Loop over all cells:
    - Progression in cell's current phenotype
    - Phenotypic decisions according to probabilities if Go (Q)
    - Update velocities
    - Set Δt
    - Update positions
      - Simplistic forward Euler – could easily use Runge-Kutta, etc.

$$\Delta t = \frac{1}{\max\left\{|\mathbf{v}_i|\right\}_{i=1}^{N(t)}}.$$

$$\mathbf{x}(t) = \mathbf{x}(t - \Delta t) + \mathbf{v}\Delta t$$

- Separate update of states, velocities, and positions precludes update order bias (all "see" the same state at each step)

Algorithms: Cell-Cell Interactions

- Build an interaction data structure: linked lists of cell memory addresses on a temporary lattice
  - Build the structure cell by cell (linear in #of cells):
    - Write own memory address within 2 RA
    - Bounded number of write operations per cell
  - Evaluate cell-cell interaction functions by truncating to the list at the nearest interaction lattice point.
    - Uniformly bounded number of "read" operations per cell
    - Transforms $O(n^2)$ operations to $O(n)$ operations

$$\text{for all cells } k \in \{k\}_{k=1}^{N(t)} \setminus \{\ell\} \text{ compute } f\left(\text{cell}_k, \text{cell}_\ell\right)(\mathbf{x})$$

$$\text{for all cells } k \in \left\{k_m^{N_{i,j}(t)}\right\}_{m=1}^{N_{i,j}(t)} \setminus \{\ell\} \text{ compute } f\left(\text{cell}_k, \text{cell}_\ell\right)(\mathbf{x}).$$

  - Alternate methods: Octrees, heirarchical meshes, explicit enumeration and update of neighbours per cell (still requires interaction testing), etc.

Baseline Simulation: Setup

- Instantaneous hypoxia
- Assume no ECM in the lumen
- No motility on duct wall either
- Necrotic swelling in 6 hours, double volume
- Calcification in 15 days
- Very low “background” O2 decay rate (0.001)
- Equal O2 uptake in all viable cells
- Cell-wall adhesion ~ 10 x cell-cell adhesion
- 30 days in a 1mm duct segment

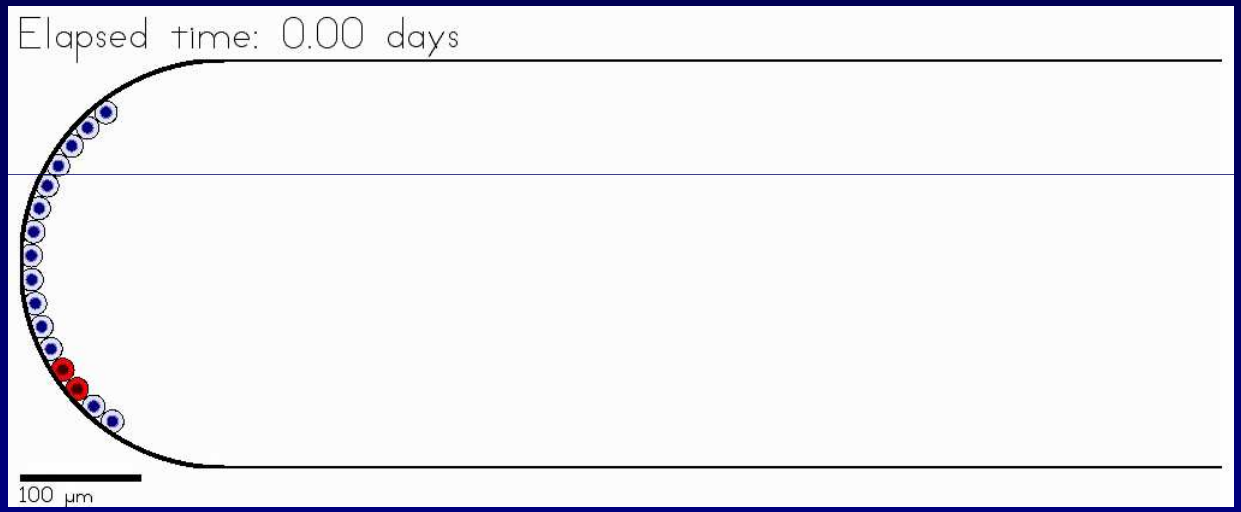
Baseline Simulation: Results

Pale blue = quiescent (G0) Red = apoptotic Green = cycling
Nuclei: darker, inner circles Cytoplasm: lighter, outer circles

Necrotic core:

- Grey expanding cells: cell swelling and lysis / bursting
- Dark points = nuclear debris
- Shades of red = degree of calcification
- Bright red points = fully-calcified debris

Baseline Simulation: Results



First necrosis: ~ 6.17 days (observe grey swelling cells that burst)
First fully-calcified nuclei: ~ 21.17 days

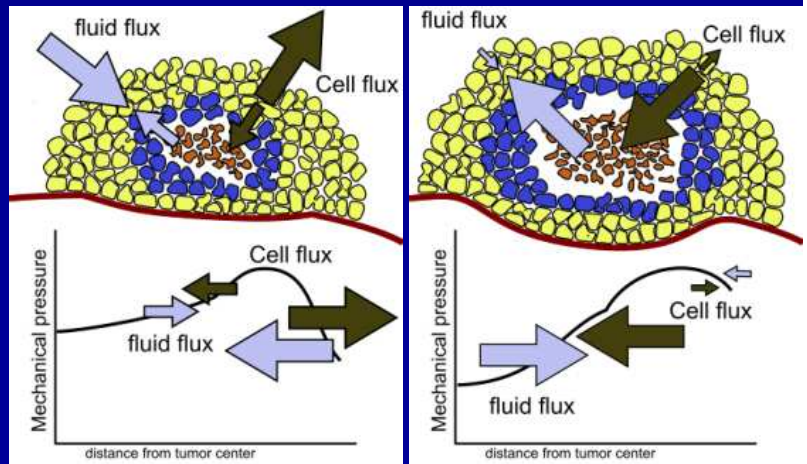
Holes in proliferative rim due to apoptosis – easy to misclassify as "cribriform" or "mixed" subtype
Mechanical "tears" in tissue due to growth strains, nonhomogeneous cell sizes – may be simulation artefact due to lacking morphological information!

Necrotic core microstructure:

- accumulated debris (dark nuclei), "gap" between viable rim and duct
- increasing calcification with distance from viable rim (shades of red)
- microcalcification in centre (bright red)
- "gap" might be real (requires cell swelling)

Necrotic core lysis is a *significant* stress relief and volume sink

- Much of the cell flux is towards necrotic core, rather than forward in duct
- Leads to much slower rate of tumour advance

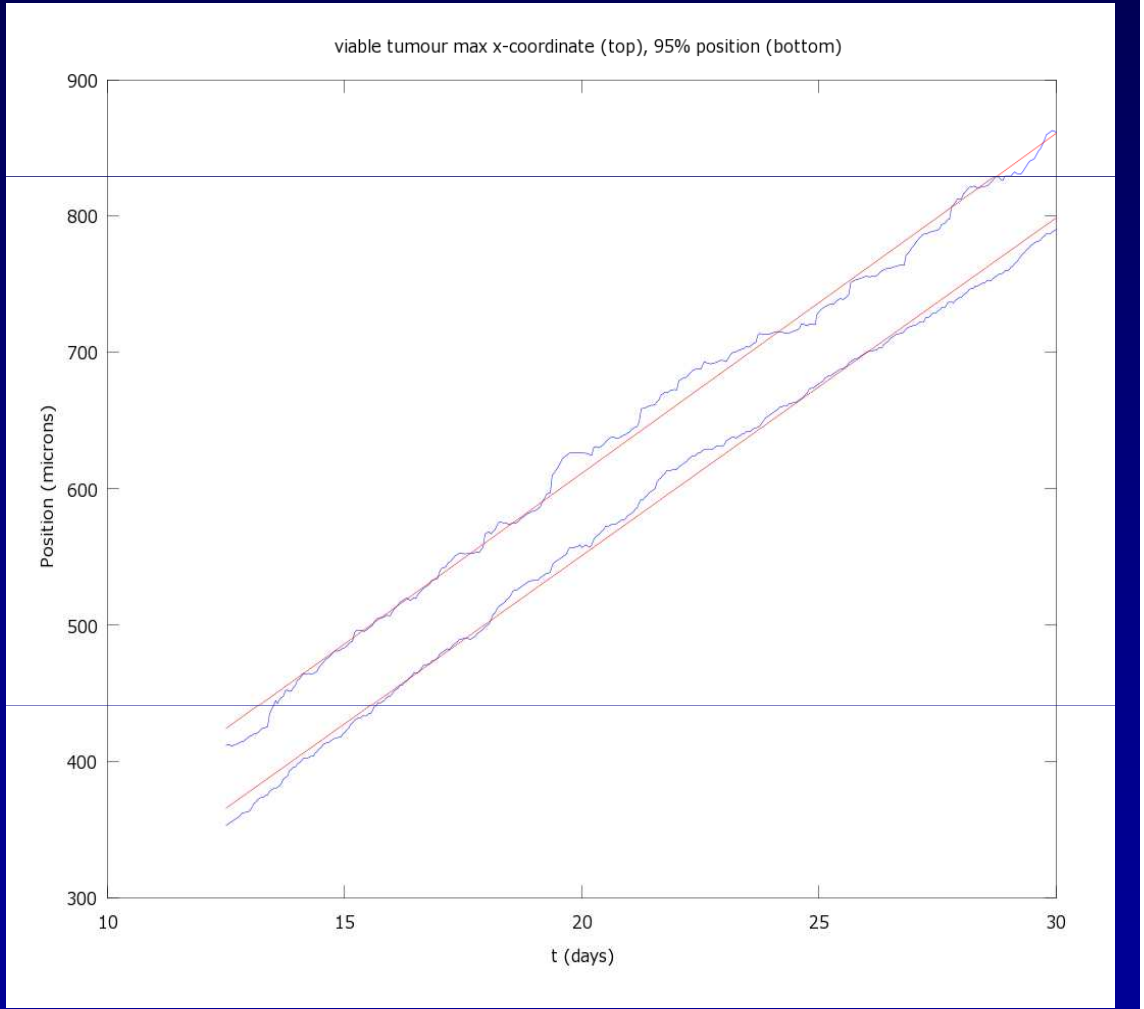


Baseline Simulation: Post-Processing

- Automated segmentation of viable rim (including hole-filling) in simulation data
- Calculate viable rim area
- Calculate position of farthest cell
- Calculate position of 95% of viable tumour bulk
  - (95% of viable tumour is left of this point)
- Calculate position of tip of calcification, center of mass
- Calculate PI, AI, viable rim thickness, density, etc.

Baseline Simulation: Results

- Nearly linear rate of tumour advance:
  - ~ 25 microns / day (by 95% position)
  - ~25 microns / day (by max. cell x-coord.)
  - →primarily convective (when no motility)
- Further highlights the importance of cell flux into necrotic core

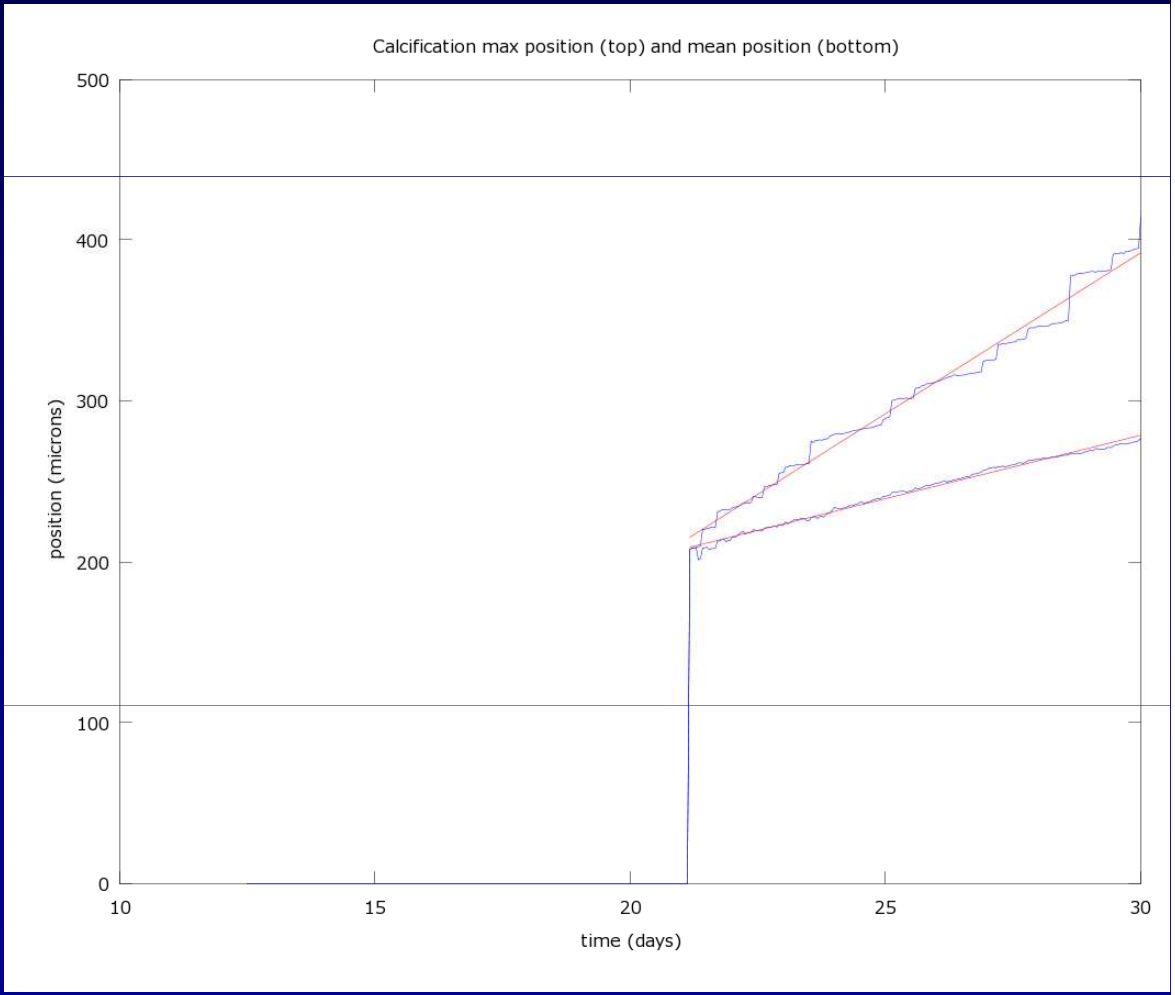


Top: maximum x-coordinate

Bottom: “95% position

Baseline Simulation: Results

- Calcification progression differs (?)
  - ~ 20 microns / day (by maximum x-coordinate)
  - ~ 7 microns / day (mean mean x-coordinate)
  - Really should calculate the 95% position

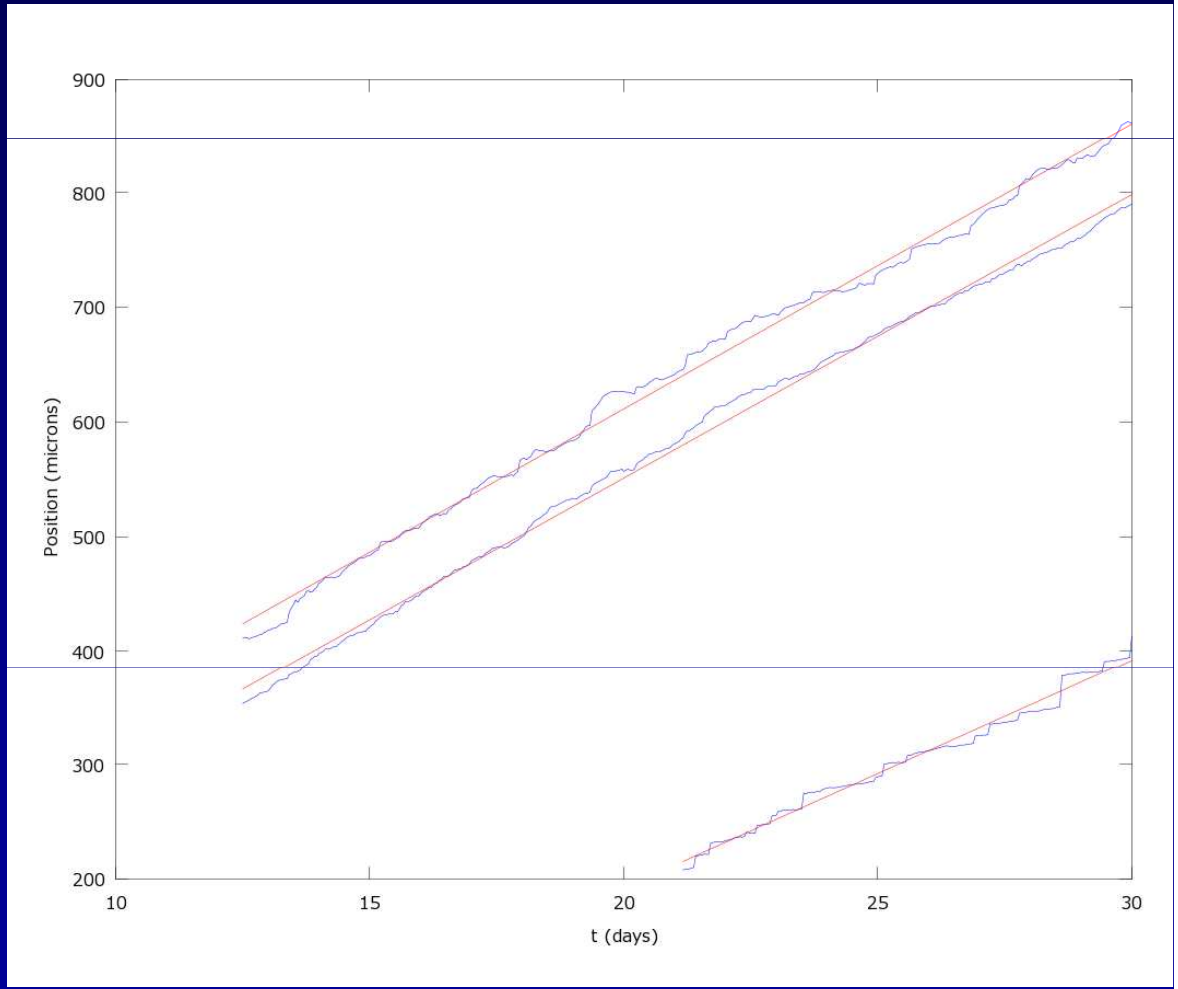


Top: max calcification x-coordinate

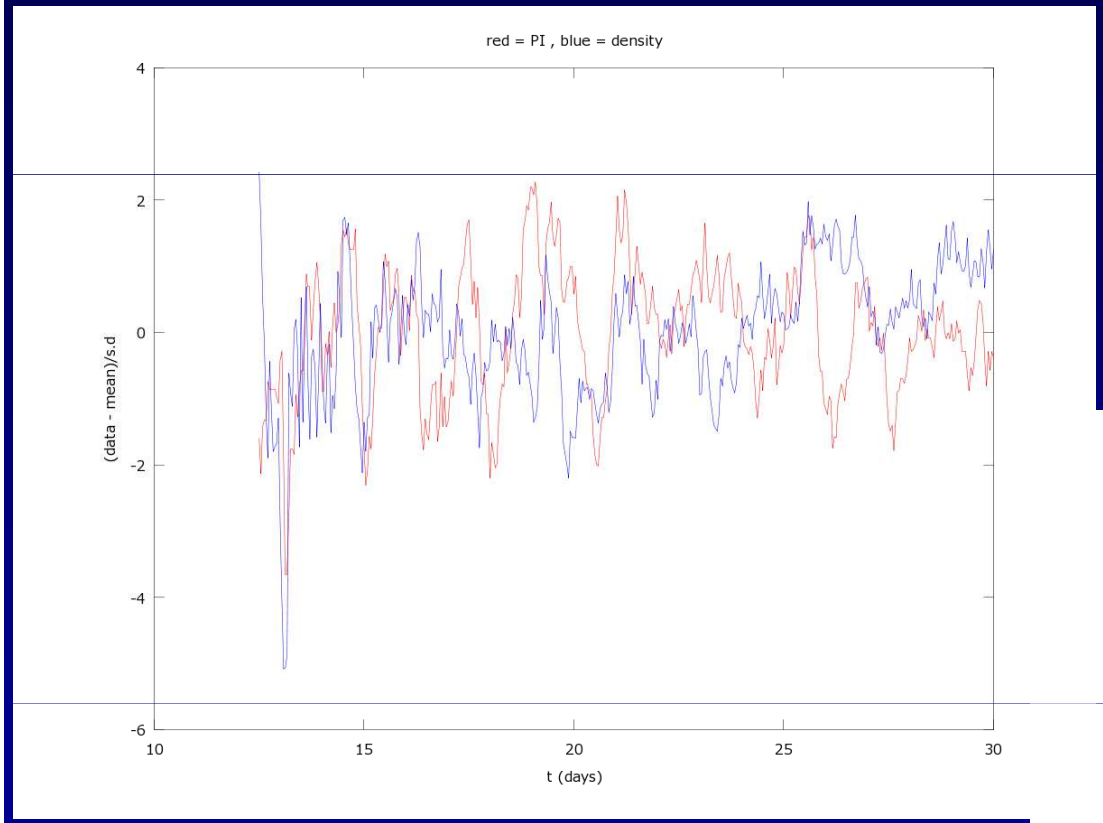
Bottom: mean calcification x-coordinate

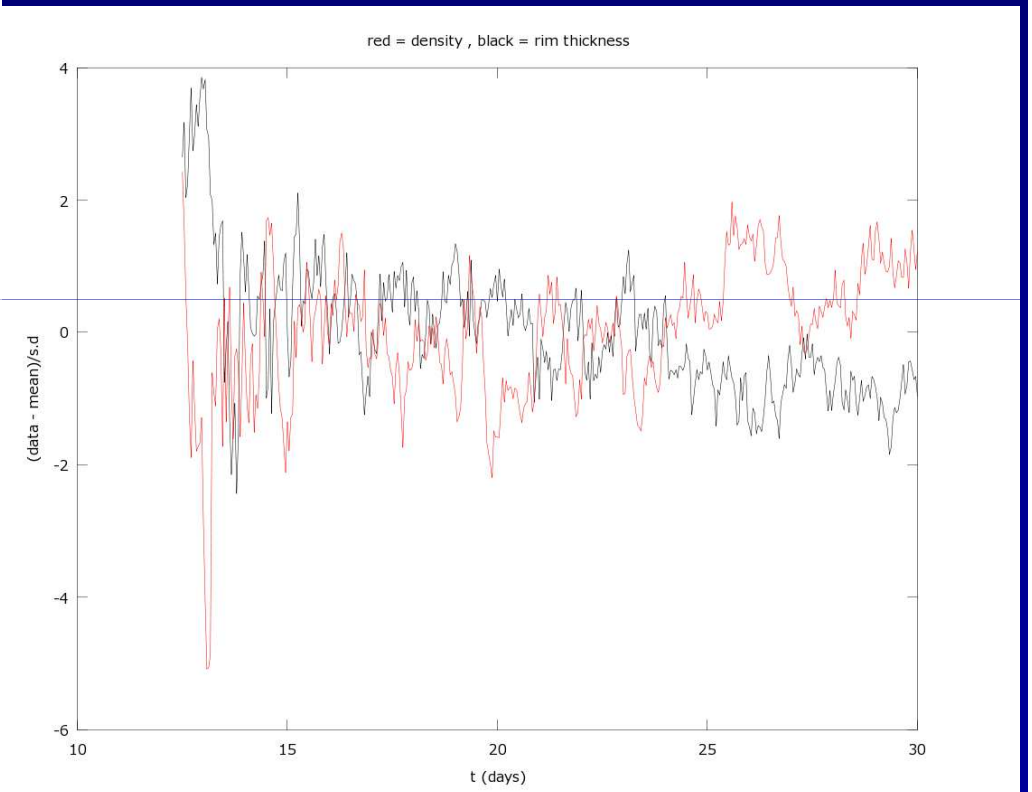
Baseline Simulation: Results

- Relationship between tumour viable rim and calcification not yet clear
- May be non-constant, growing distance between detectable calcification and the actual tumour boundary
- On the other hand, the rates of advance aren't so far apart (20 microns/day vs 25 microns/day)
- Question: Will this approach a steady value as the inner part of the tumour reaches its steady dynamic and the necrotic core fills up?



Baseline Simulation: More P.P.



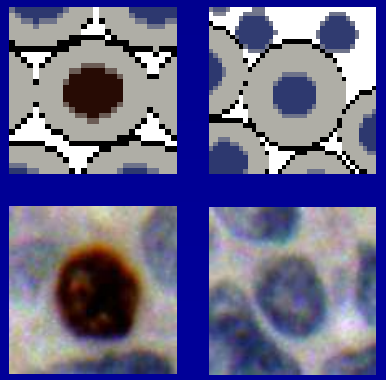


Baseline Simulation: Some Predictions

- Once the tumour cross-sectional profile "settles down" away from the leading edge, growth seems to be primarily linear
  - Most cell flux away from leading edge towards necrotic core
  - Forward advance primarily by cells at the leading edge that "see" more oxygen diffusing from the lumen
  - Similar to spheroids
- Density slowly rising → adding contact inhibition in rear of tumour should lead to slowing rate of progression?
- There may eventually be a bounded distance between the max viable tumour position and the max (and 95%) calcification position
  - Predict as a function of duct radius, PI, AI, and time scales?

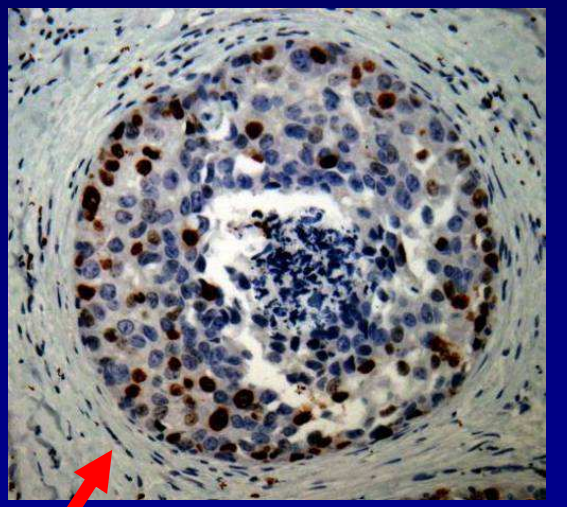
Baseline simulation: Virtual Ki-67 IHC

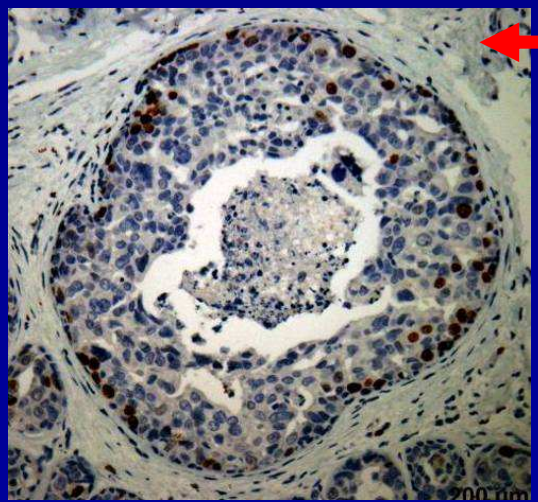
- Virtual Ki-67 immunostaining:
  - (Phenomenologically) mimic the biochemistry of the stains
    - Facilitates direct matching to image data: morphology, qualitative traits
    - A more physical stain model could be used to study the correspondence between stains and what's actually there (e.g., sub-threshold items, nonlinear response, stain decay, …)
    - Computer-generated histopathology for beginning students down the road?
  - Virtual DNA stain of nuclear content (dark blue)
    - Mimics hemotoxylin counterstain
  - Virtual stain of Ki-67 in nuclei (dark red)
    - Mimics immunostain process
  - Virtual stain of cytoplasm (pale blue)
    - Mimics lower-affinity counterstain activity
  - Compare to actual IHC (next slide)
  - Can evidently stain $CaPO_4$ later on

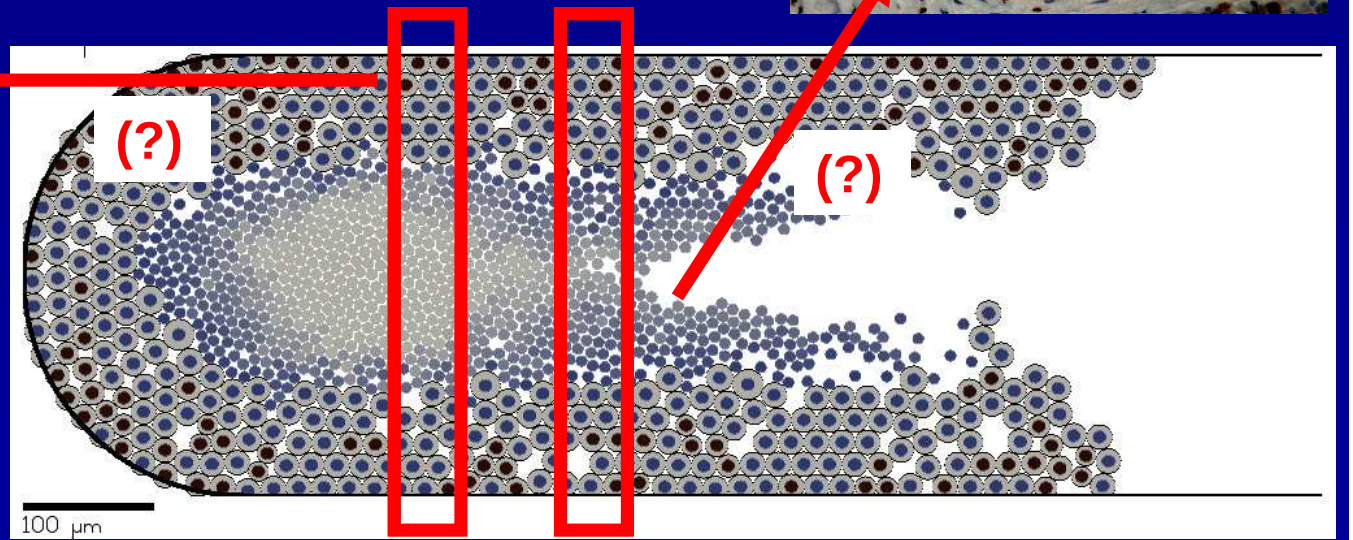


Baseline simulation: Virtual Ki-67 IHC

- Correct general distribution of proliferation
  - Proliferation within the interior as well as at edges
  - Peak proliferation near duct wall
- Correct necrotic core microstructure:
  - Nuclear debris in center
  - Fewer lysed cells around perinecrotic zone
  - Variable degree of calcification (gradual degradation of nuclei)
  - "gap" between the viable rim and necrotic core – may not be an artefact after all
- Suggests hypothesis for testing by breast pathologists:
  - Cross-section appearance may correlate with:
    - Distance to advancing tumour "front"
    - "Age" of the necrotic core in the slice

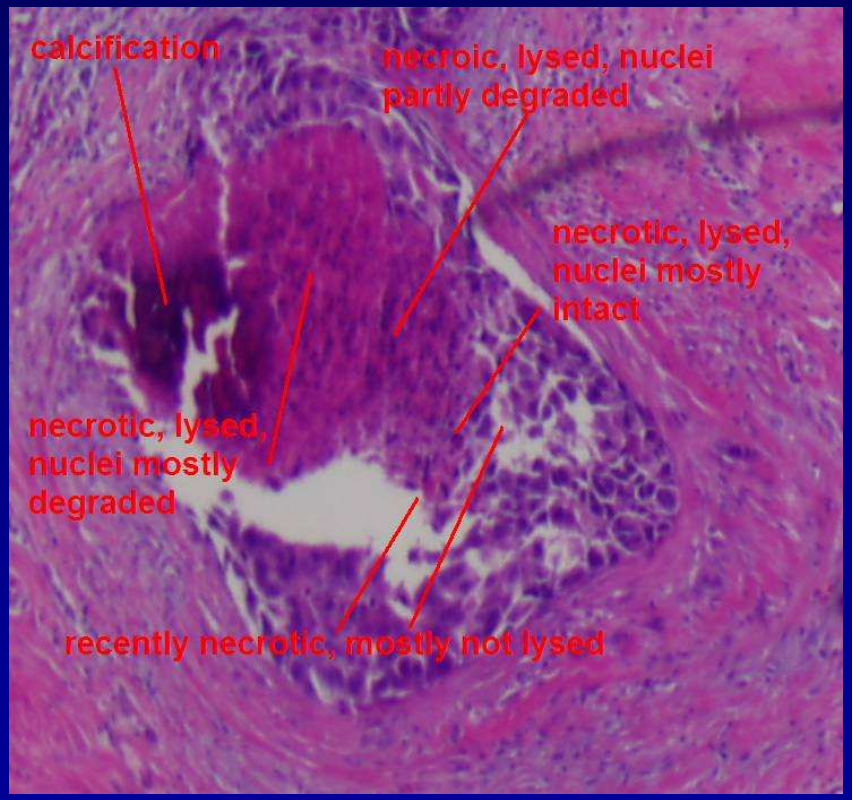






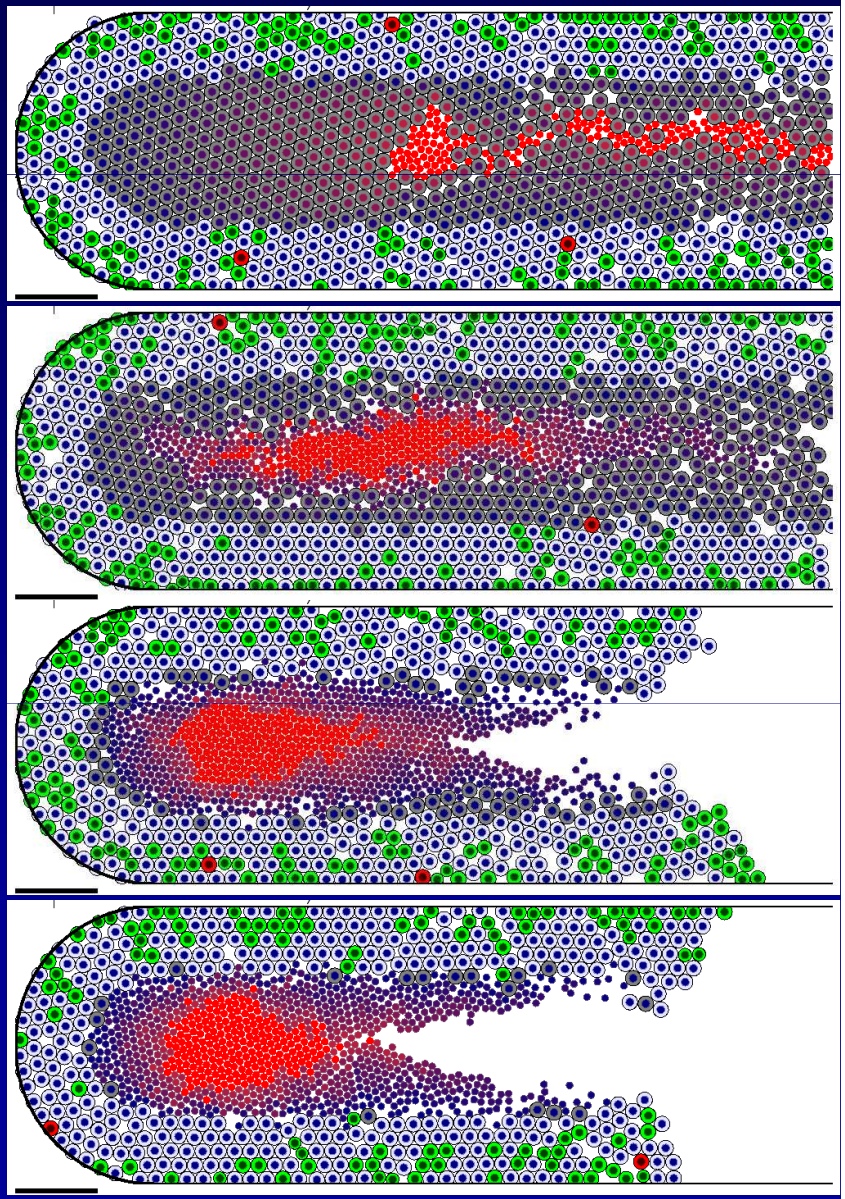
Baseline Simulation: More Questions

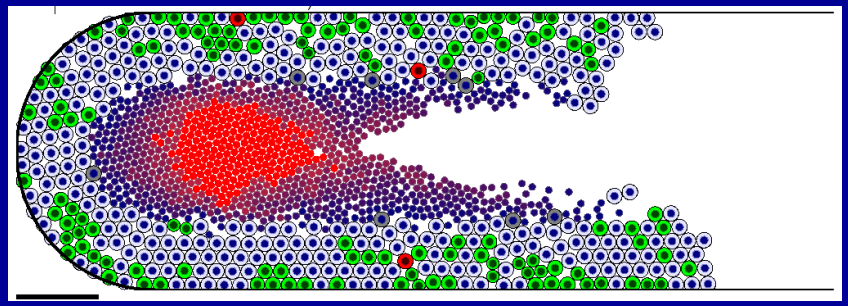
- Virtual H&E (on my task list):
  - Hematoxylin (purple) stains DNA *and* calcium phosphate (via PO_4)
    - (Difference in stain intensity?)
  - Eosin (obnoxious pink) stains cytoplasm, stroma, etc.
  - Colour the output accordingly.
- Expected result:
  - Not much gap between (simulated) nuclear debris and calcification
  - Reality: regions of intact nuclei, degraded nuclei, then calcification
- New hypotheses to test:
  - Separate time scales for nuclear degradation and calcification
  - Need a better calcification model – model the biochemistry of the crystal aggregation
  - Mechanical tears in the necrotic core and calcification:
    - Weaker mechanics!
- Theme:
  - Simulation + Virtual H&E / IHC → New hypotheses on interpreting these images, as well as the tumour biophysics



Impact of Lysis Time τ_{NL} (No swelling) All plots at t = 30 days

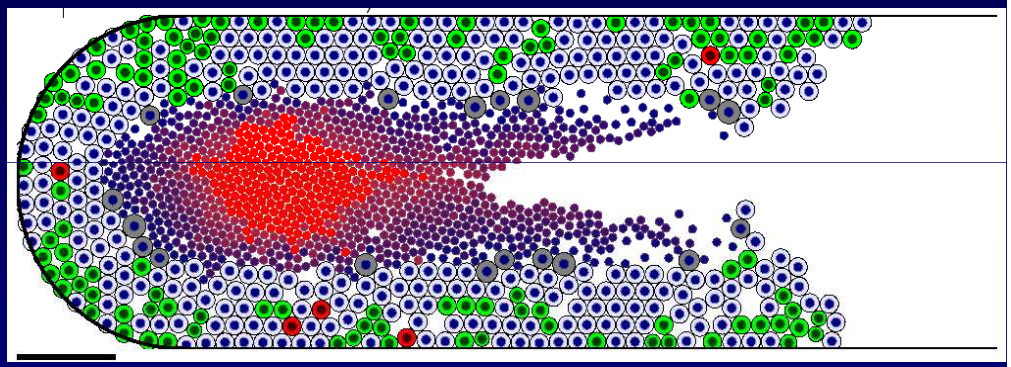
- From top to bottom:
  - 15 days
  - 5 days
  - 1 day
  - 6 hours
  - 2 hours
- Faster lysis → greater stress relief in necrotic core
- 6-24 hours seem to match IHC best
- Next step: compare to H&E, try to get statistics on morphology in non-calcified cross sections

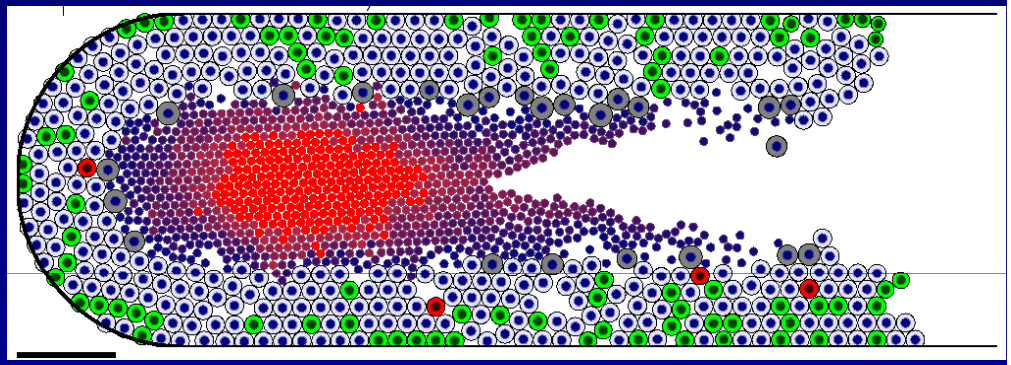


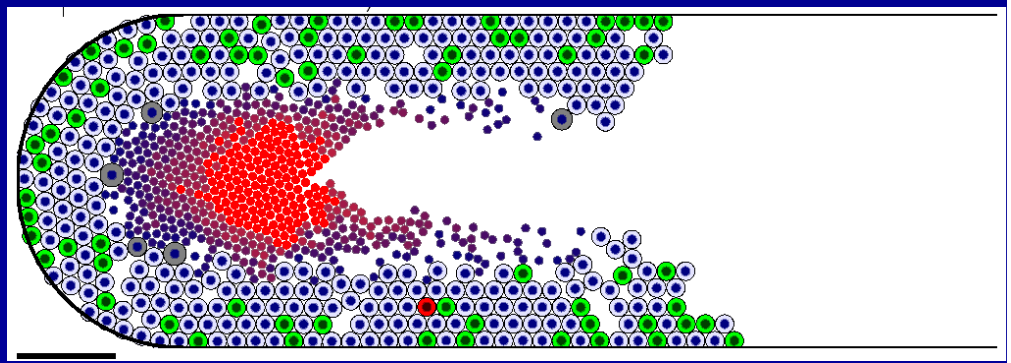


Impact of Growth Time (τ_{G1}) All plots at t = 30 days

- From top to bottom:
  - 9 hours
  - 3 hours
  - 1 minute
- Effect is unclear. Nonlinear?
  - Instantaneous division increases local density faster, reduces O2, and thus reduces PI?
- Next step: double-check simulation
- After that: try to get statistics on distribution of proliferating cell sizes in IHC, relate to estimated distribution in simulations

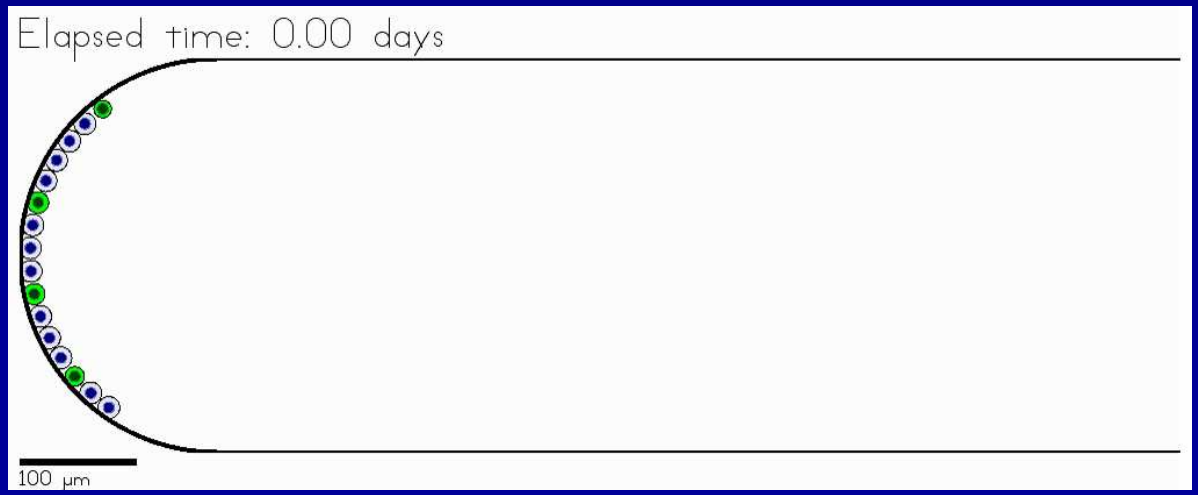


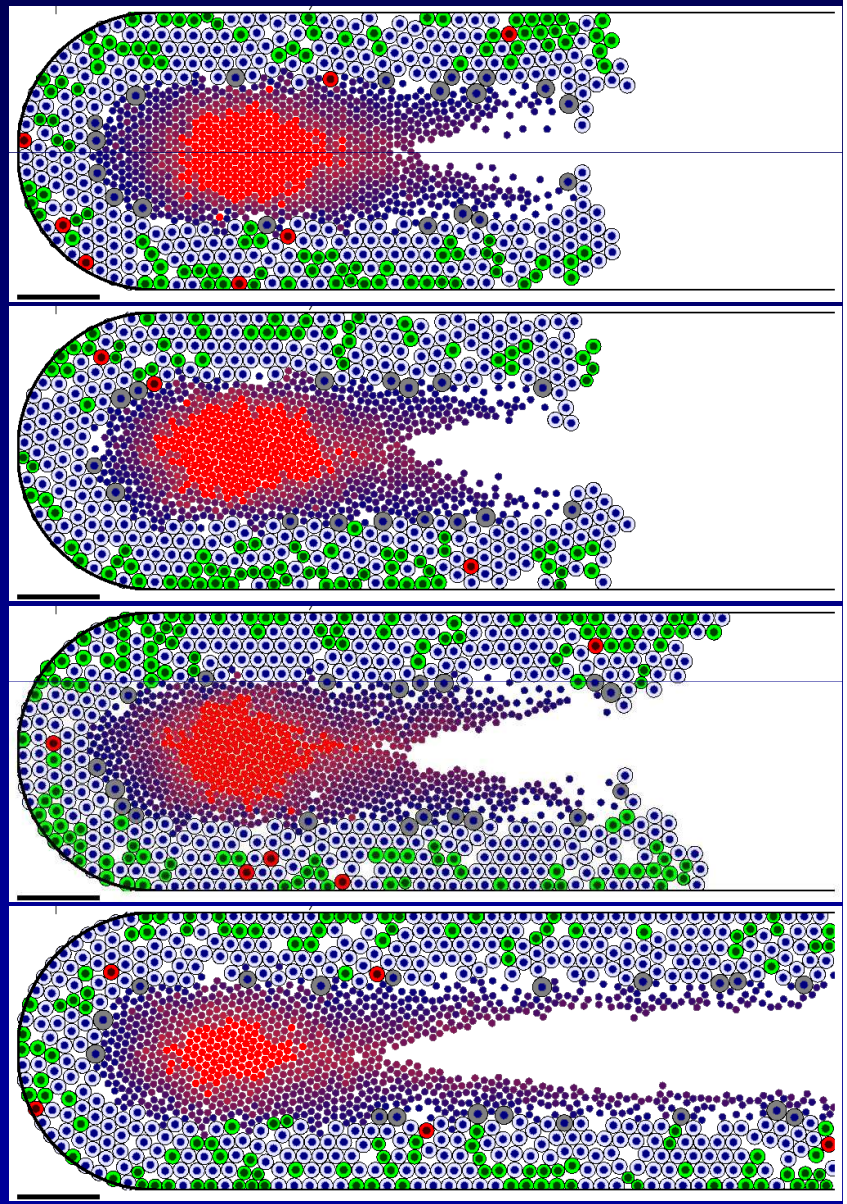




Impact of Cell-BM to Cell-Cell strength
All plots at t = 30 days*

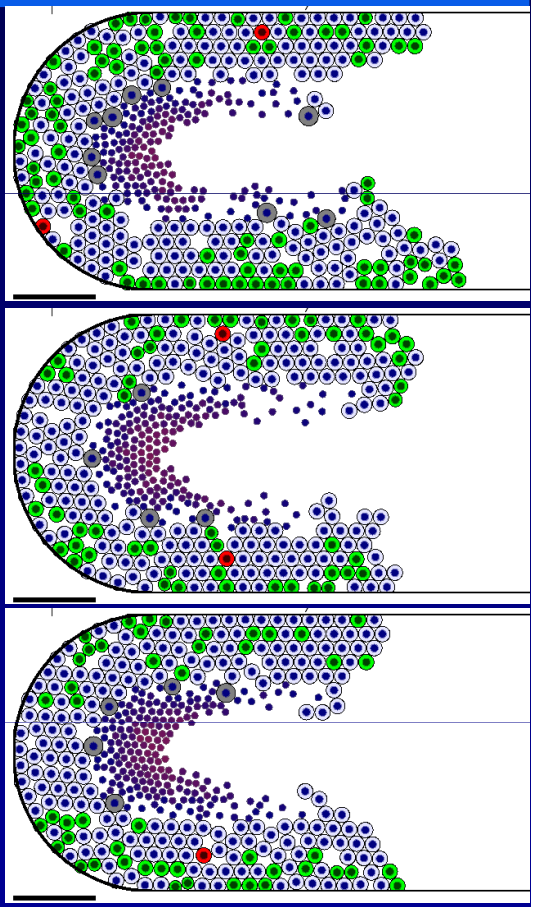
- From top to bottom:
  - 0.1
  - 1
  - 10
  - 100
- For low ratios, cells pull off wall, form convex surface
- For high ratios, wicking / wetting behaviour → speeds rate of advance through the duct!
  - Compounded effect: more cells in high O2

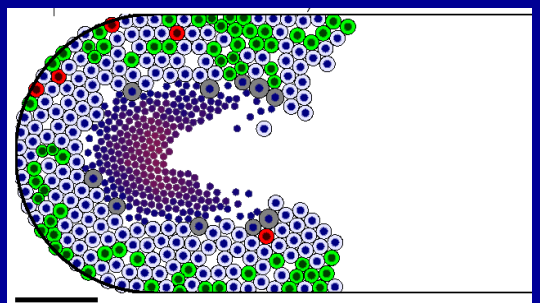


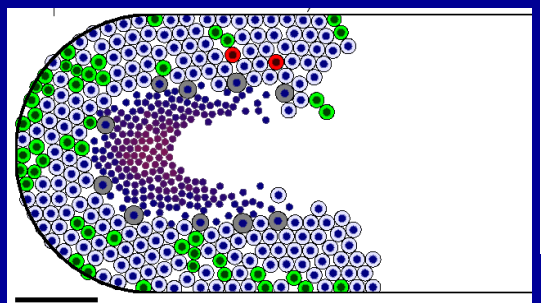


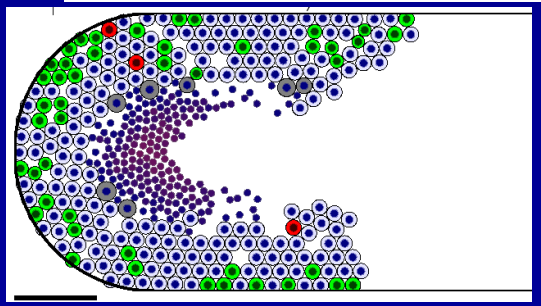
Impact Mechanics time scale
All plots at t = 15 days

- Multiply all mechanics parameters by (top to bottom CW):
  - 10
  - 5
  - 2
  - 1
  - .5
  - .1
- For very high, can spread cells out faster and contribute to faster spread
- For very low values, adhesion of many cells overcomes pairwise repulsion, and you get cell crowding
- For intermediate values, generally identical behaviour. (But high values compute **very slowly** due to Δt cond.)
- Next steps:
  - implement E-cadherin/β-catenin and see what's most realistic
  - compare to theoretical results of Drasdo et al.



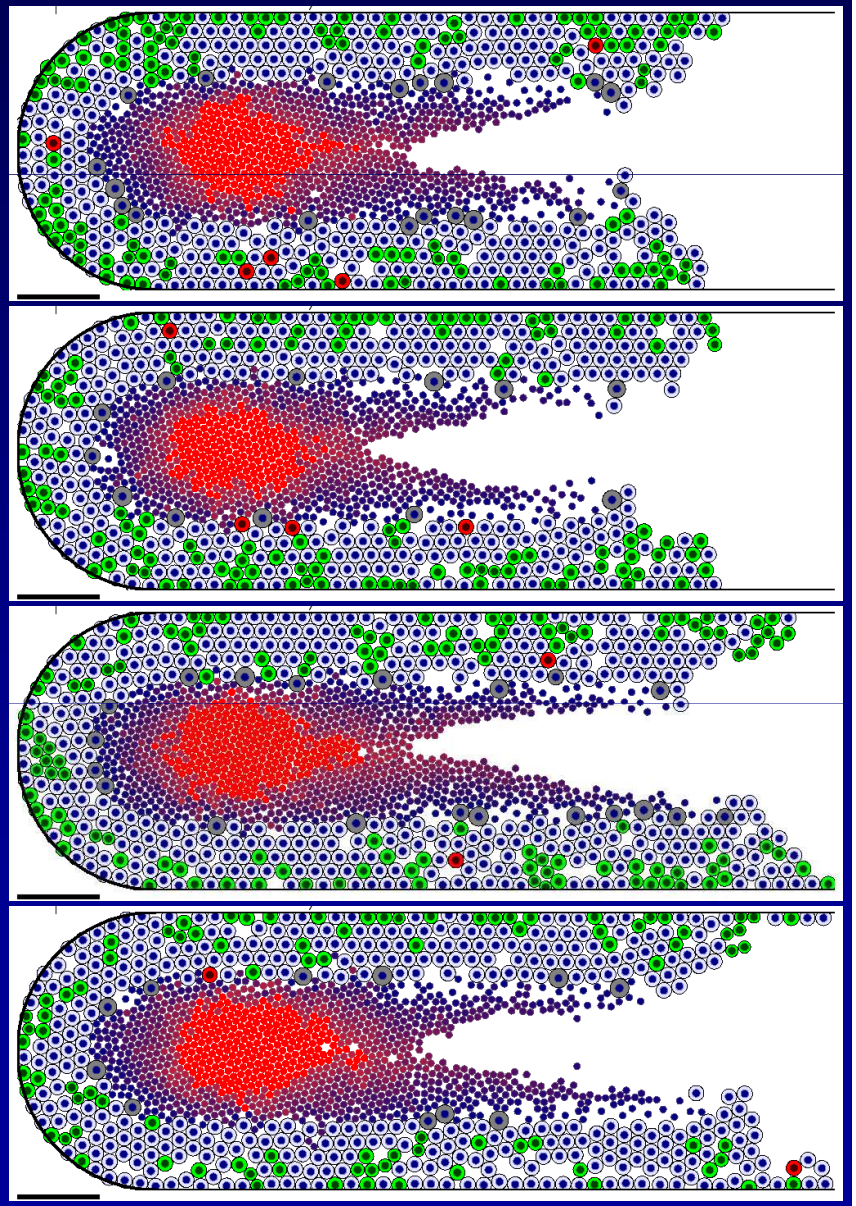






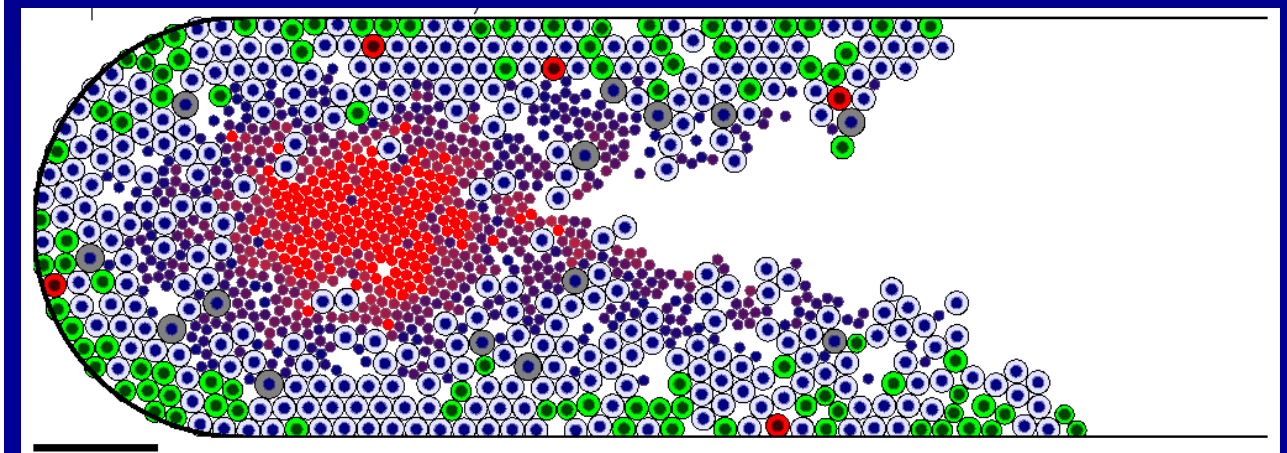
Impact of Random Motility (along BM) All plots at t = 30 days

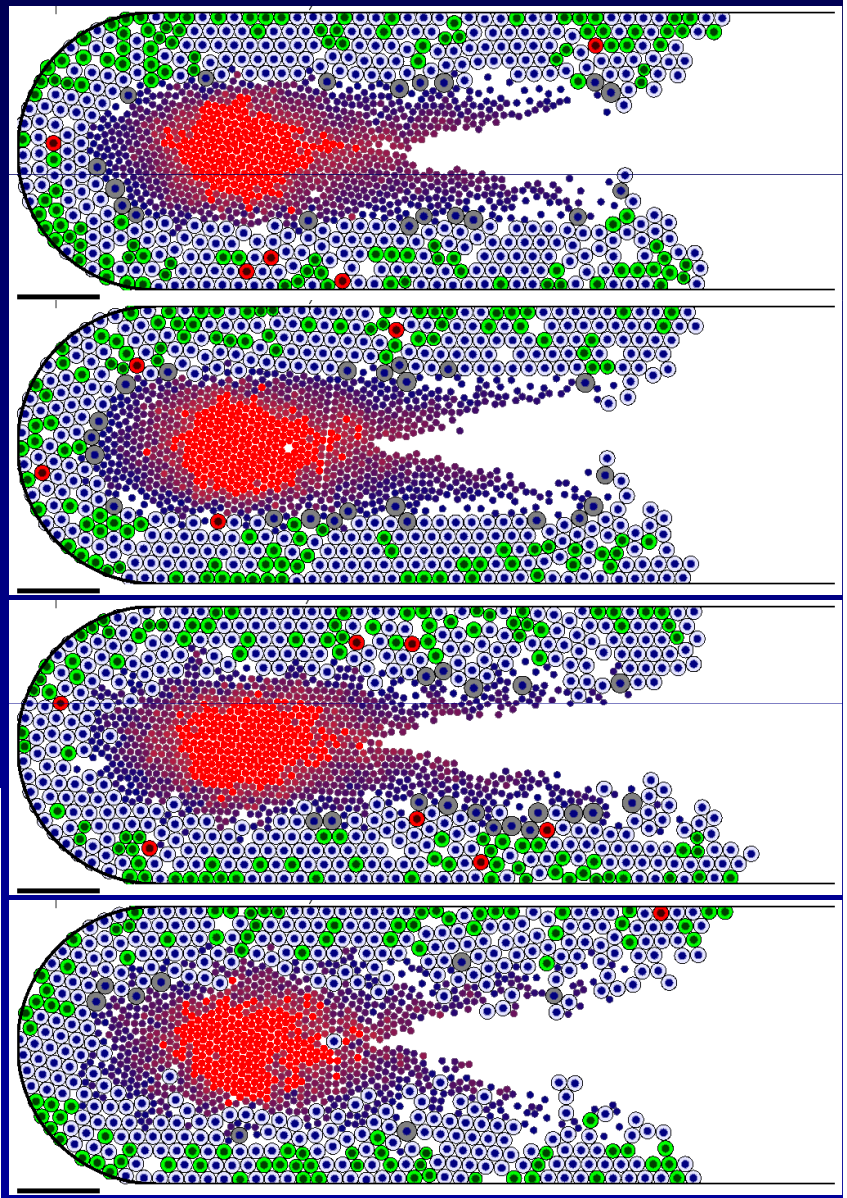
- Mean wait time (top to bottom):
  - Infinite
  - 300 min
  - 120 min
  - 60 min
- Increased motility increases rate of spread through duct even if undirected
  - Also helps pull the tumour along, similar to wetting behaviour
- Next step: directed motility



Impact of Proliferative O2 uptake rate
All plots at t = 30 days

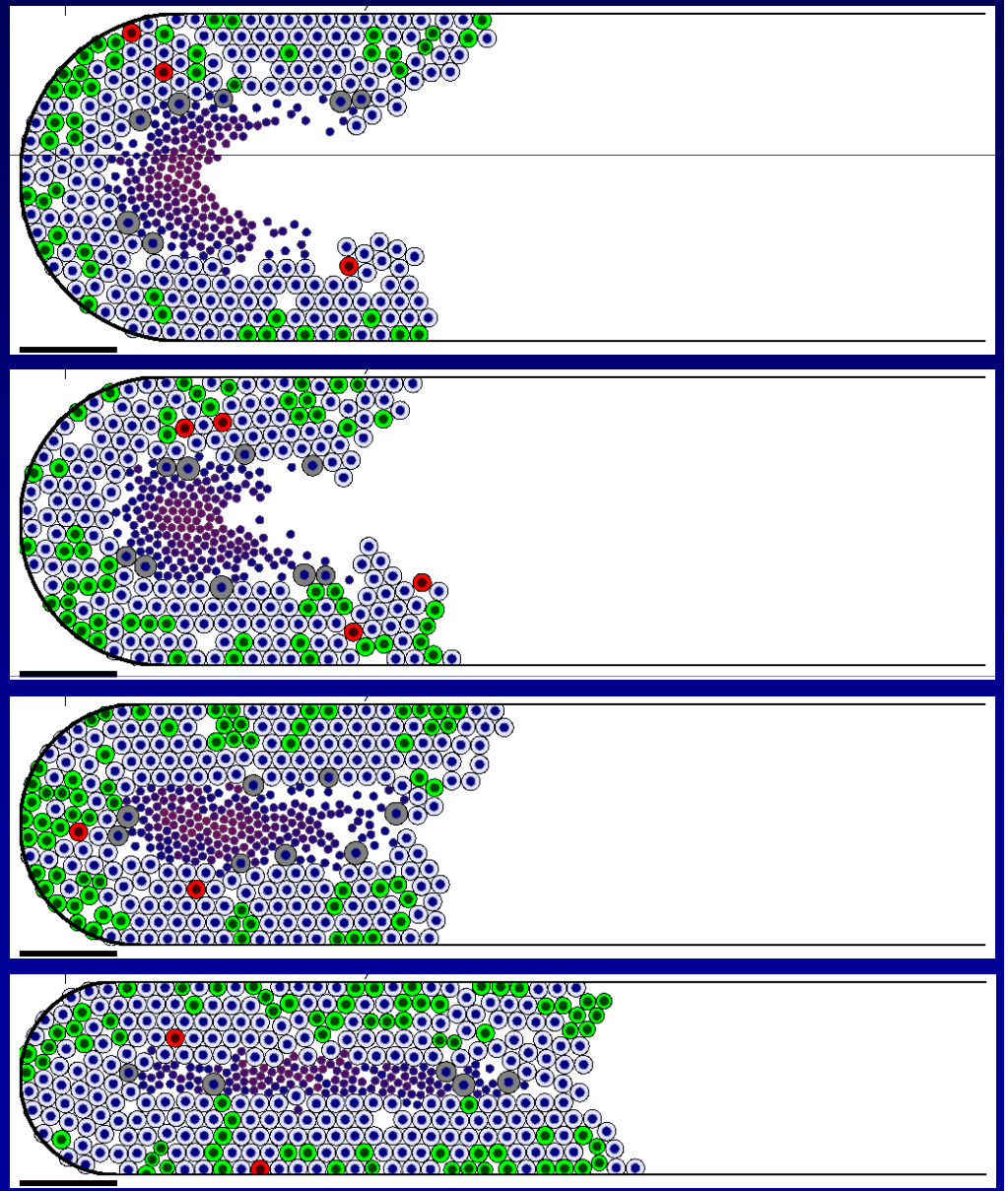
- $\lambda_P : \lambda_{np}$ (top to bottom CW):
  - 1, 2, 5, 10 , 100
- As the value increases, more cell mixing, instability in perinecrotic boundary
  - Proliferating cells uptake more 02, decrease local O2, change local viable rim thickenss
- Note: does not require anything special in hypoxic modelling!
- testable hypothesis!





Impact of Duct Radius
All plots at t = 15 days

- From top to bottom:
  - 170, 150, 125, 100 microns
  - All big enough for necrotic core
  - All calibrated to have same PI
- Faster rate of advance in smaller ducts
  - Due to necrotic core mechanics



Ongoing (Still Postprocessing)

- Numerical studies on:
  - Mechanics parameters
    - Ratio of adhesion strengths
    - Repulsion parameters
    - Interaction distance
  - Necrosis / calcification parameters
    - Lysis time, calcification time
    - Swelling amount
  - Oxygen parameters
    - Impact of differing uptake rates
    - Phenotype-specific uptake rates
  - Duct geometry

Future Directions: Upscaling

- Use a rudimentary upscaling to calibrate a simplified continuum model
- Testable predictions on patient-specific tumour sizes
- Compare with pathology measurements of resected tumours
- Reasonably good given the noisy input data and model simplifications
- Dynamic upscaling in true multiscale simulation might well do the trick

- In preparation for submission to *Cancer Research* (Edgerton et al.)

Future Directions: Numerics

- Shared-memory architecture parallelisation (OpenMP)
- Optimisation of the cell-cell interaction data structure
- "Asynchronous" and adaptive cell updates
  - Similar philosophy to adaptivity in NAGSI for continuum quasi-steady problems (Macklin and Lowengrub 2008)
- Equation-free approach (Kevrekidis et a.) to accelerate simulations away from the leading edge?
- Hybrid discrete-continuous treatment of necrotic core:
  - Treat the calcification as a mass with level set potentials, similar to the BM

Future Directions: Modelling

- Necrosis & Calcification:
  - Separate time scales for nuclear degradation, calcification
  - Separate time scale for liquid volume loss from lysed necrotic cells
  - Better model of calcification biochemistry
- Viable rim biology and biophysics:
  - Integrate rudimentary E-cadherin/β-catenin model for contact inhibition
  - Solve for interstitial fluid pressure
  - Selective pressures and motility in hypoxic regions
  - ECM secretion, ECM-MMP dynamics, invasion
  - Acidosis
- Duct geometry:
  - Elastic duct expanded by growing tumour

Future Directions: Analysis & Investigations

- Adjust viable rim population equations for necrotic core flux
- Impact of more complex duct morphologies?
- Impact of initial tumour position?
- Impact of normal epithelium?
- Impact of directed motility
- Investigate tumour advance “markers” as a function of:
  - PI, AI, duct radius, oxygenation, mechanics

Future Directions: Agent Model

- Cell polarisation
- Variable cell morphologies
- Cytoskeletal mechanics
- Relationship of cytoskeletal mechanics to:
  - anisotropic adhesion receptor distribution
  - heterogeneous intracellular signalling

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- M.D. Anderson Cancer Center
  - Mary E. Edgerton, M.D. Ph.D. ← Pathologist
- Graduate students, undergraduate interns, pathology interns, etc. ← gathered images, developed and ran image processing software

…

Some References

- The agent model 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.
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  - 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 preparation)

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)