Glioma and the microenvironment: remodeling and invasion

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Outline

- GBM and invasion; *in vitro* experiments
- The invasive phenotype.
- Invasion and the microenvironment.
- Remodeling the microenvironment.
- Recurrent tumors.

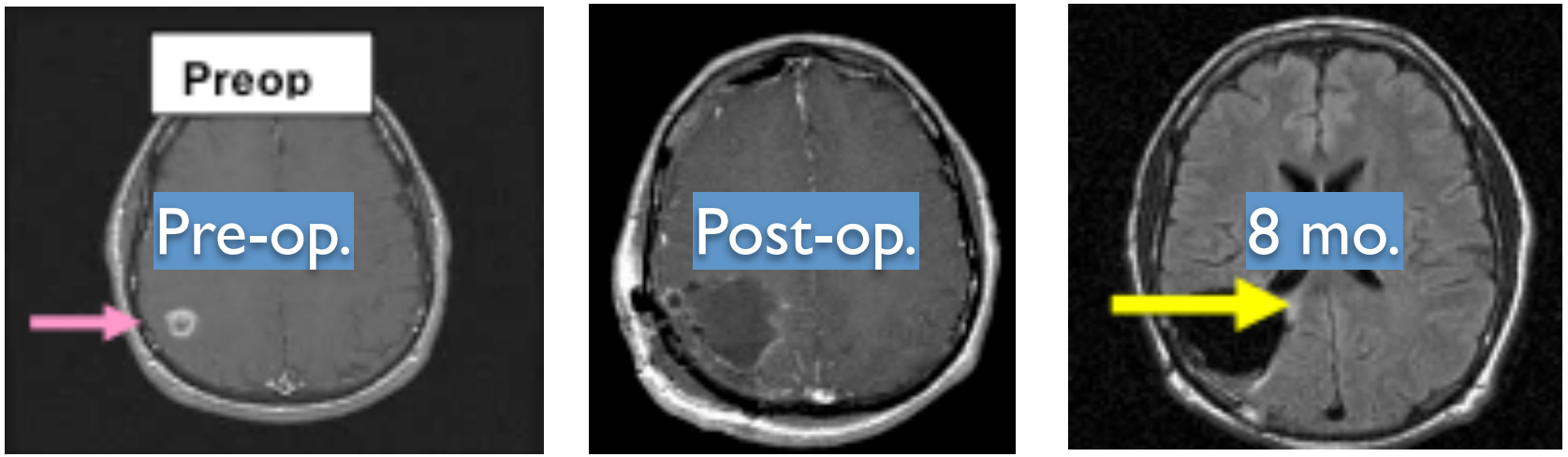
Collaborators

- Y. T. Lin, U Mich
- C. Schneider-Mizell, INI, Zurich
- A. Stein, Novartis
- D. Vader, Nine Point Medical, Boston
- D. Weitz, Harvard
- A. Marcus, Winship Cancer Center, Emory
- Mike Berens, TGen
- Tim Demuth, Merck
- E. Khain, Oakland U.
- M. Khasin, NASA Ames
- M. O. Nowicki, E. A. Chiocca, S. E. Lawler, OSU

Glioblastoma multiforme (GBM)

- Glioma affects glial cells in the brain, probably astrocytes.
- 18,000 people/year in the US are diagnosed with primary brain tumors.
- 9,000 have glioblastoma multiforme (GBM), the most malignant form.
- After diagnosis:
  - 50% of GBM patients die within 1 year.
  - 98% of GBM patients die within 5 years.
- No significant advances in the last 30 years.

GBM responds poorly to surgery

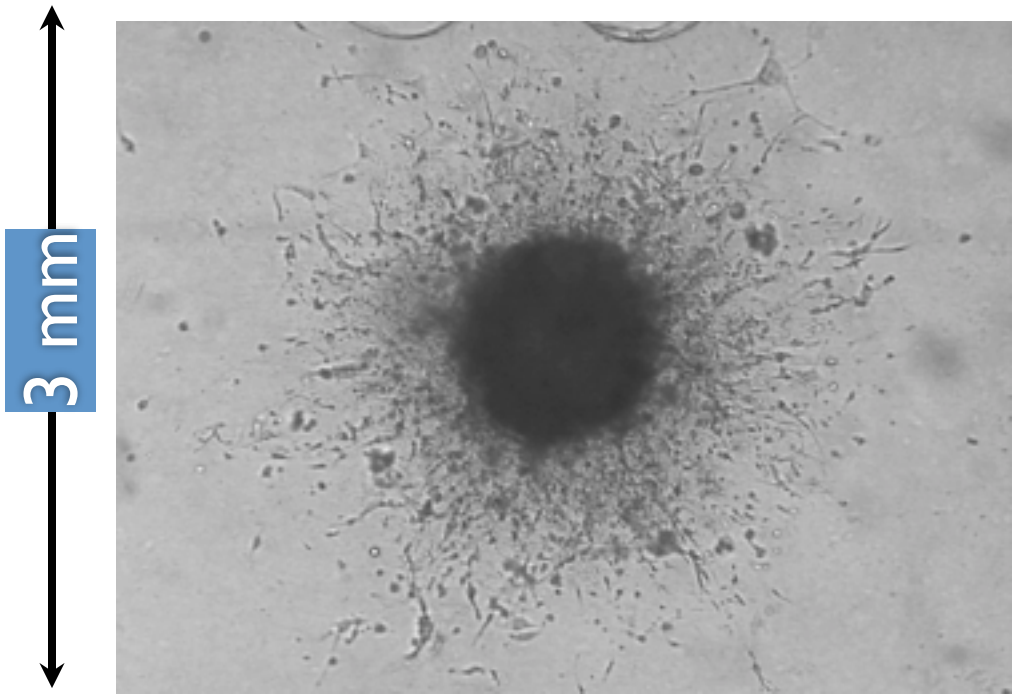


- This cancer is highly invasive; single cells leave the tumor surface and invade the surrounding tissue.
  - Many recurrent tumors near to the primary after resection.
  - Some recurrent tumors quite distant from the primary.
- Note that GBM is almost never metastatic, only invasive.
- Expanding resection to 'catch' the invasive cells always fails.
  - Some areas of the brain cannot be removed.
  - Even very extensive resection doesn't work.

GBM responds poorly to radiation and

- Blood-brain barrier blocks drug delivery.
- Invasive cells proliferate slowly.
- Current best practice: median survival of approximately 6 months if untreated. This rises to 14 months with the current standard of care, radiation plus temozolomide.

3d Tumor Spheroid Assay

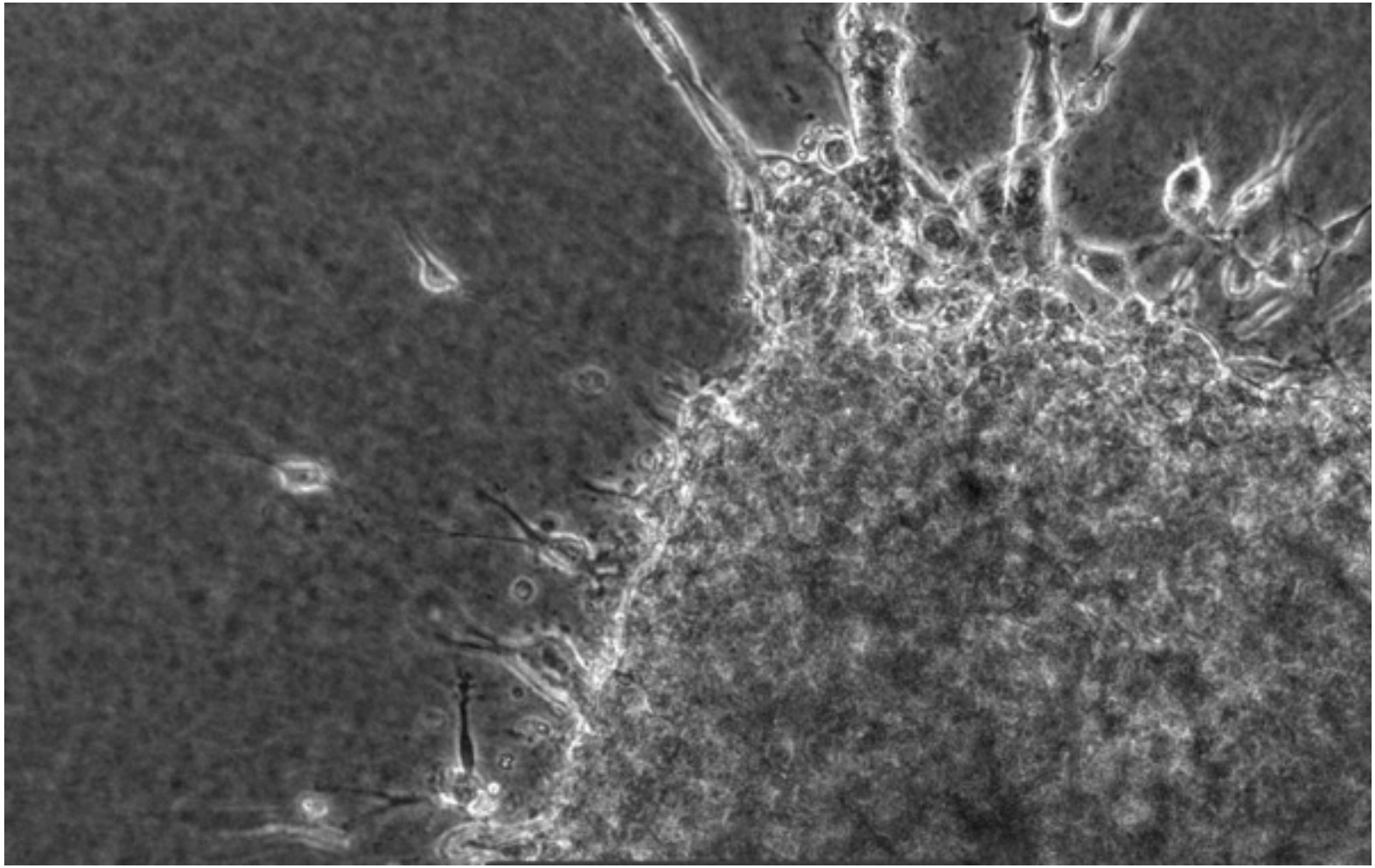


- Put a clump of cultured tumor cells (a tumor spheriod) in a gel. (We use matrigel or collagen-I).
  - Spheroid grows.
  - Single cells invade.
- May be a reasonable model for invasion in the brain.

T. S. Deisboeck et. al. (2001) Pattern of self-organization in tumour systems: complex growth dynamics in a novel brain tumour spheroid model. *Cell Prolif,* **34**, 115-134

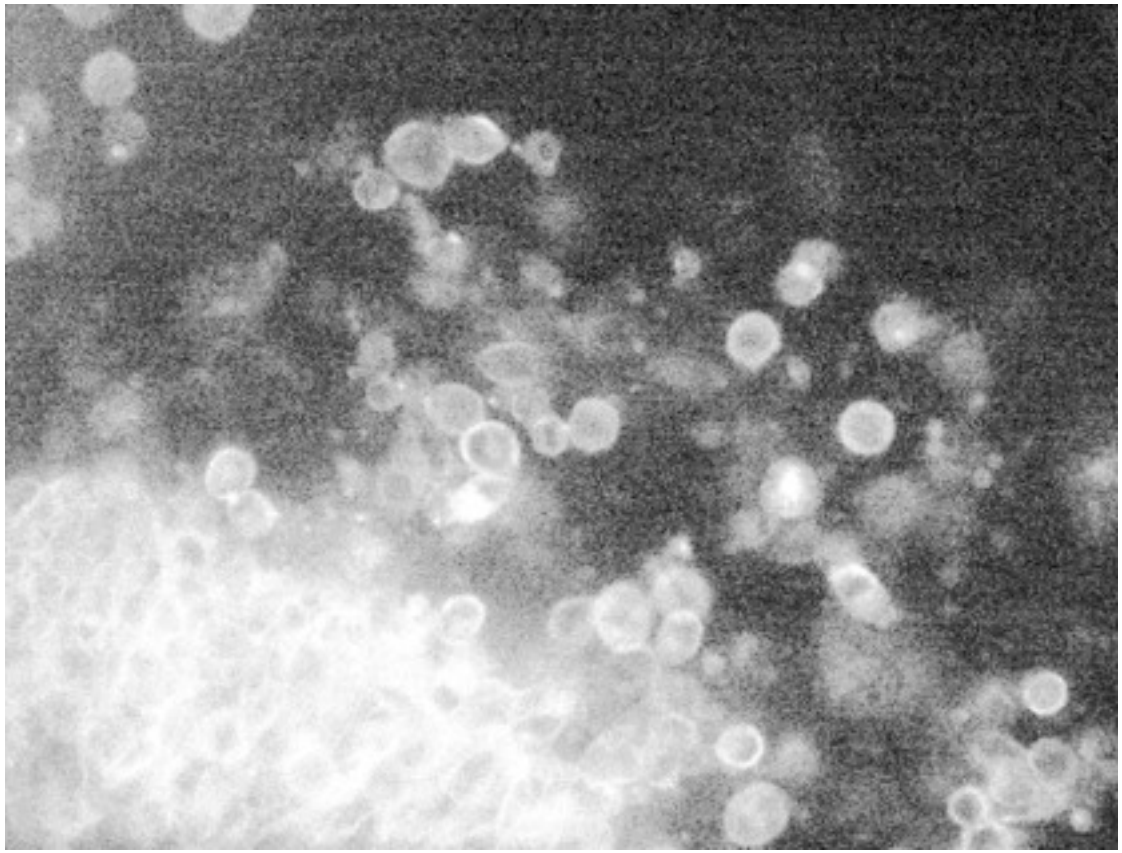
A. M. Stein, T. Demuth, D. Mobley, M. Berens, and L. M. Sander, *A Mathematical Model of Glioblastoma Tumor Spheroid Invasion in a Three-Dimensional In Vitro Experiment*, Biophysical Journal, **92**, 356 (2007).

Growth and invasion *in vitro*



New experiments in progress

- A. Marcus and collaborators have engineered inducible glioma cells. We can turn on (and off) motilty.



The invasive phenotype

- Invasive cells are phenotypically different from proliferative ones.
  - Grow vs. go: invasive cells proliferate far less than cells in the primary tumor, and have high motility:
    - A. Giese, M. A. Loo, N. Tran, D. Haskett, S. W. Coons, and M. E. Berens, *Dichotomy of astrocytoma migration and proliferation*, International Journal of Cancer, **67**, 275 (1996).
  - The nature of the signaling that leads to the switch is not understood (hypoxia? pH?..)

Invasive phenotype (cont.)

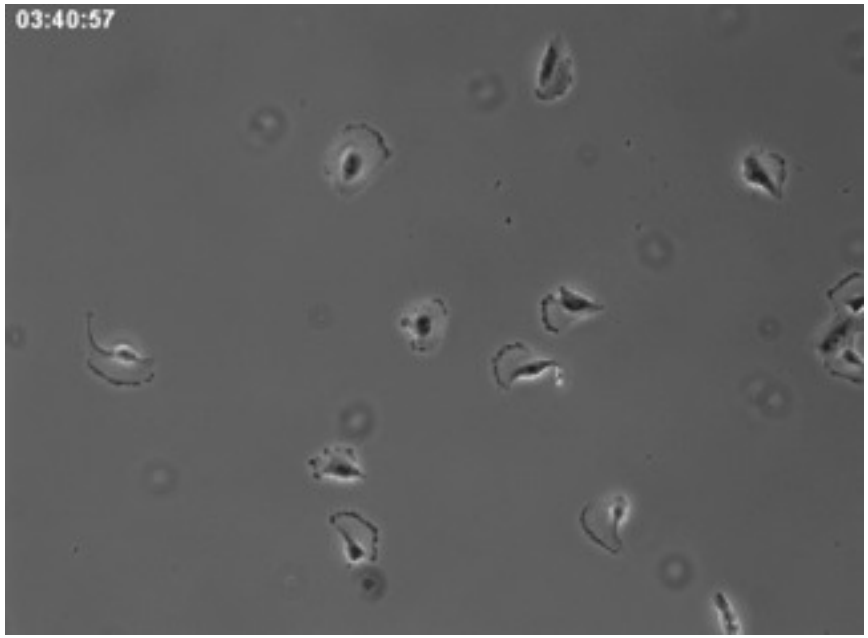
- Demuth et al: radial migration assay (i.e., let cells migrate, choose from inside and outside.)
  - Identify a 'stationary' signature and a 'migration' signature in profiles.
  - Gene that is most commonly upregulated in the migration signature is CTGF (connective tissue growth factor) which plays a role in migration and response to wounding in many cellular contexts.
    - Demuth et al. 2008 Glioma cells on the run the migratory transcriptome of 10 human glioma cell lines BMC Genomics **9** 54
- Godlewski, et al. identify microRNA-451 downregulated in migratory cells.
    - J. Godlewski, et al., *microRNA-451: A conditional switch controlling glioma cell proliferation and migration*, Cell Cycle, **9**, 2742 (2010).

Invasion

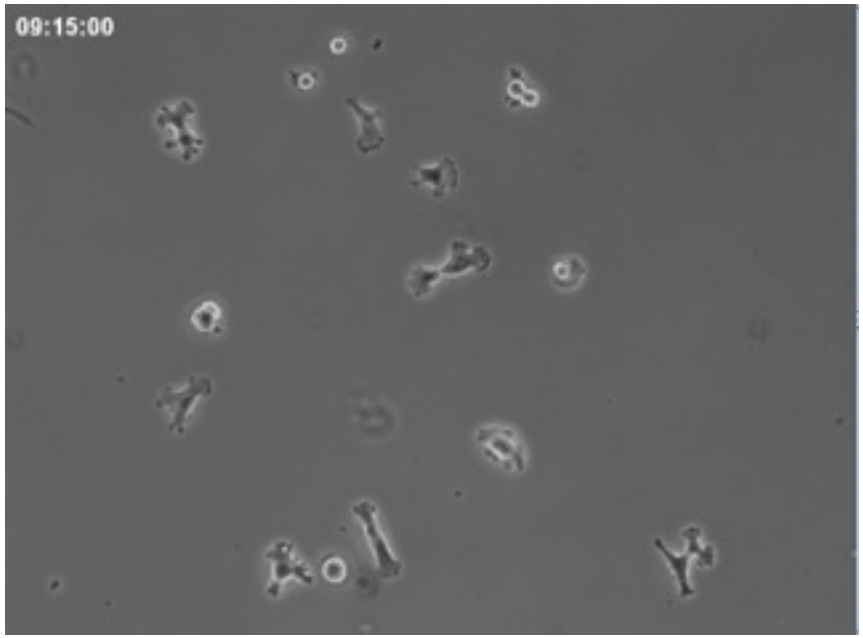
- What happens when a cell on the surface of a tumor switches to invasive phenotype?
  - Signaling from the stroma determines the mode of invasion.
  - Cells in the tumor remodel the stroma which facilitates invasion.
- We will focus on *mechanical* effects in the microenvironment.
  - We argue that a major phenomenon is *alignment of the ECM* which is made of a strain-stiffening biopolymers.
  - There is evidence for very similar effects in invasion in breast cancer.

ECM affects cell motility

Motility on a surface



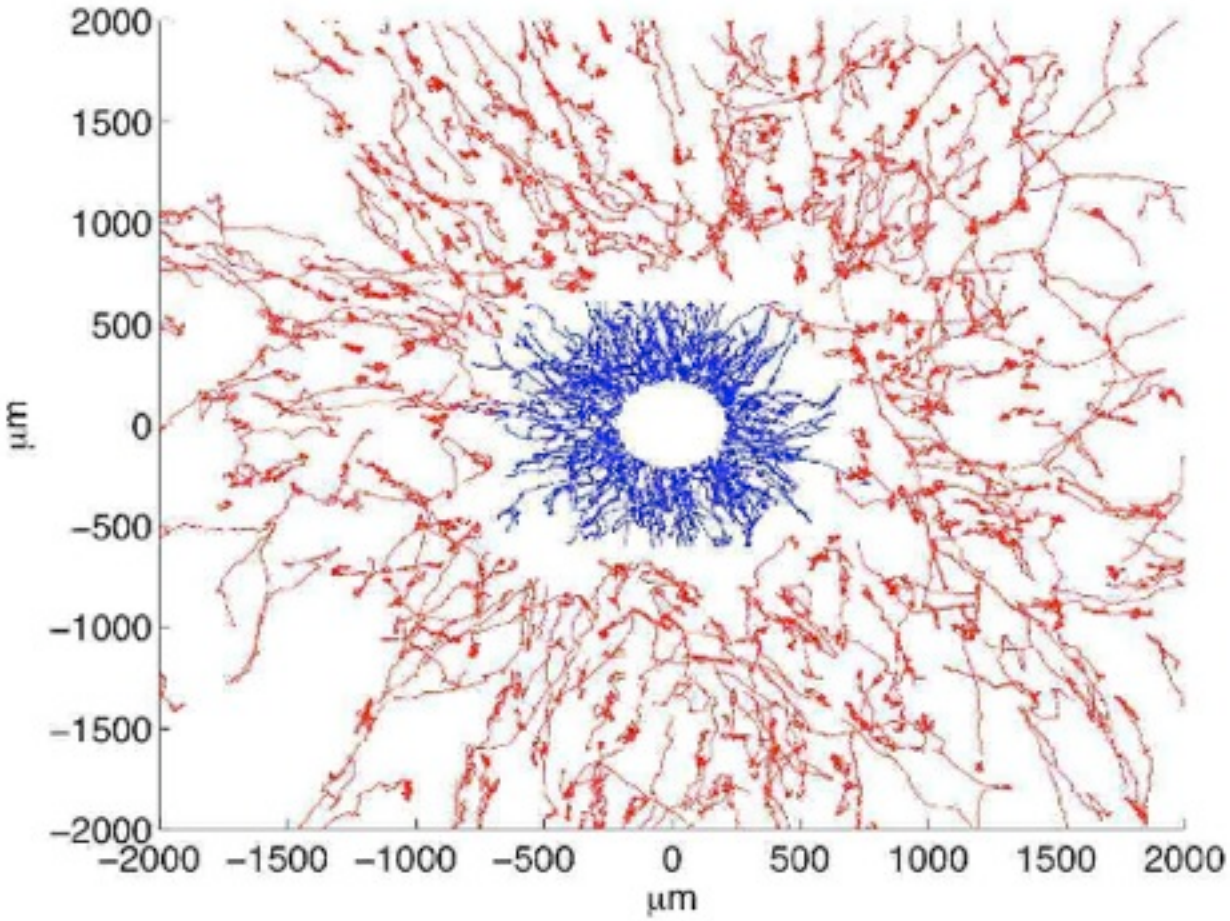
Glass



Soft gel

T. Ulrich, E. De Juan Pardo, and S. Kumar, *The Mechanical Rigidity of the Extracellular Matrix Regulates the Structure, Motility, and Proliferation of Glioma Cells, Cancer research,* ***69, 4167 (2009).***

In vitro invasion: cell tracking



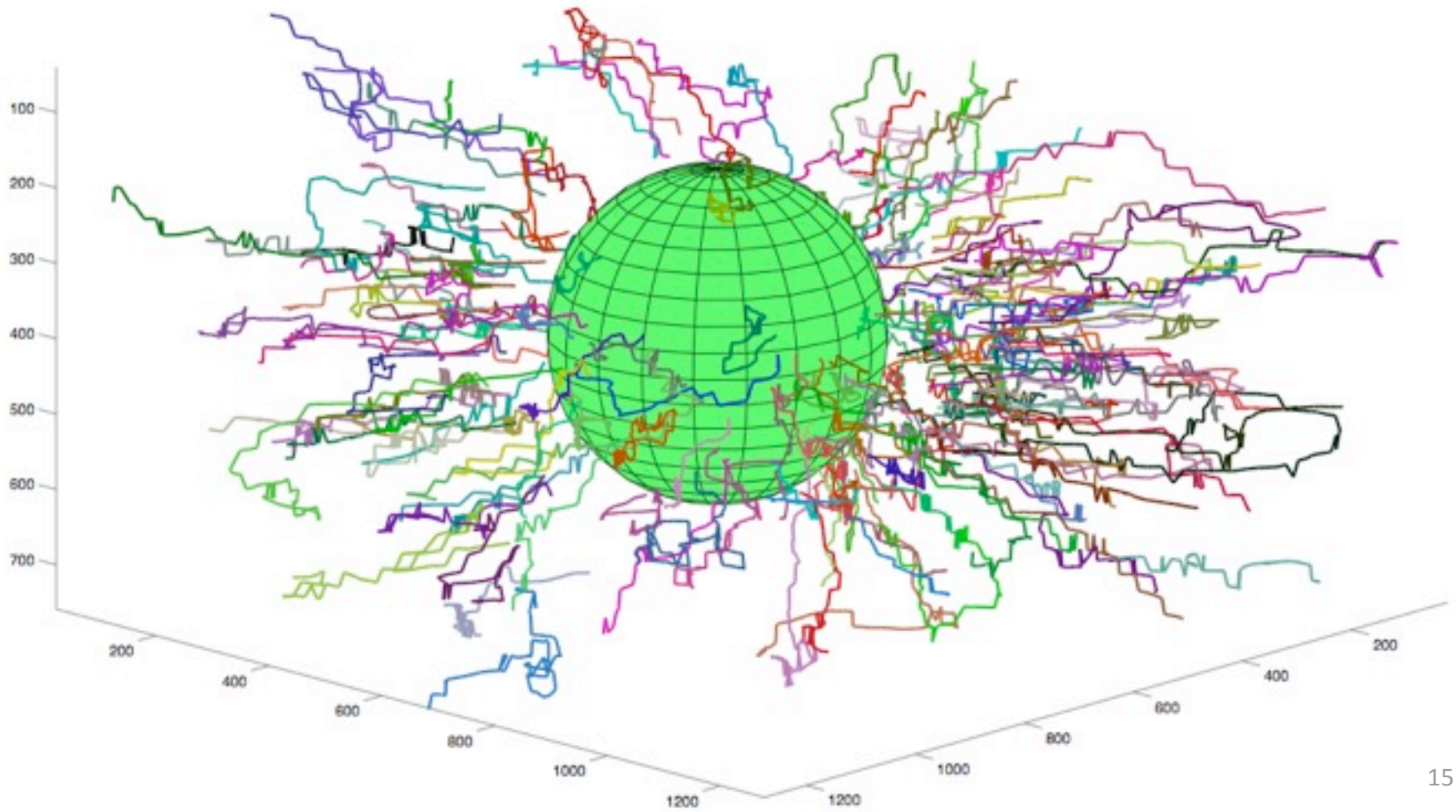
We tracked single cells streaming out from the tumor spheroid.

We showed that the paths are strongly biased radially outwards.

A. M. Stein, D. A. Vader, L. M. Sander, and D. A. Weitz. *Mathematical Modeling of Biological Systems*, volume I. Birkhauser, 2006.

3d cell paths from confocal microscopy

N.B. These are *not* random walks.



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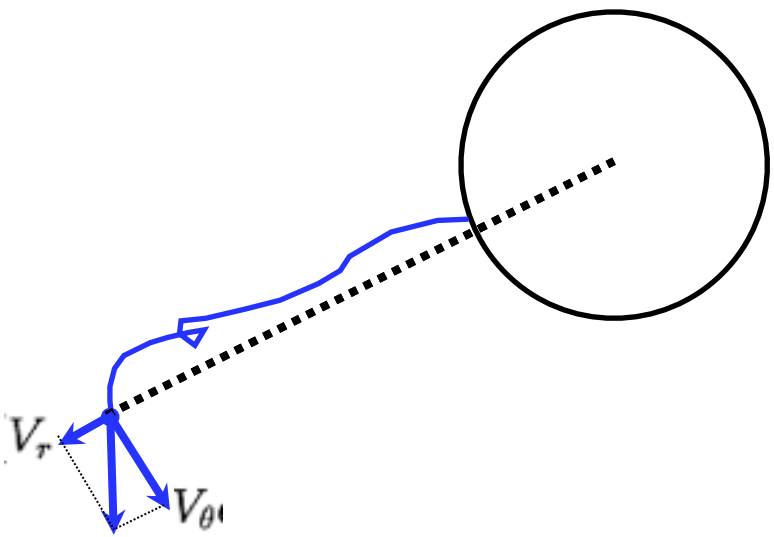
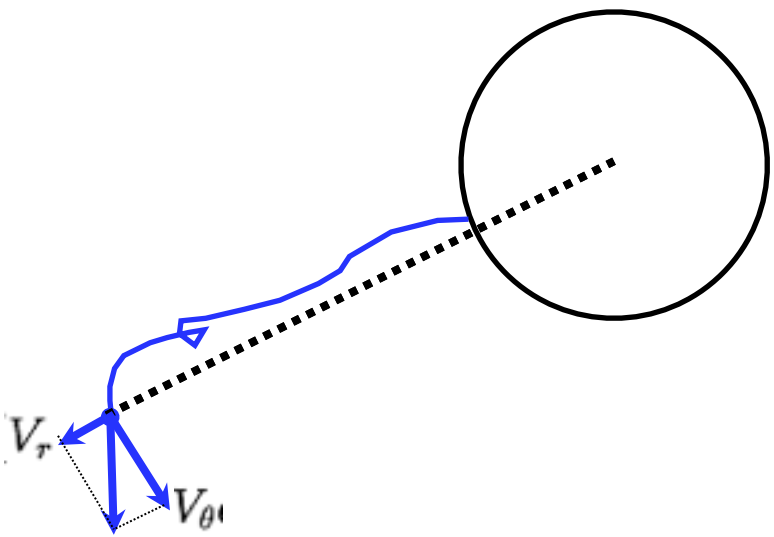
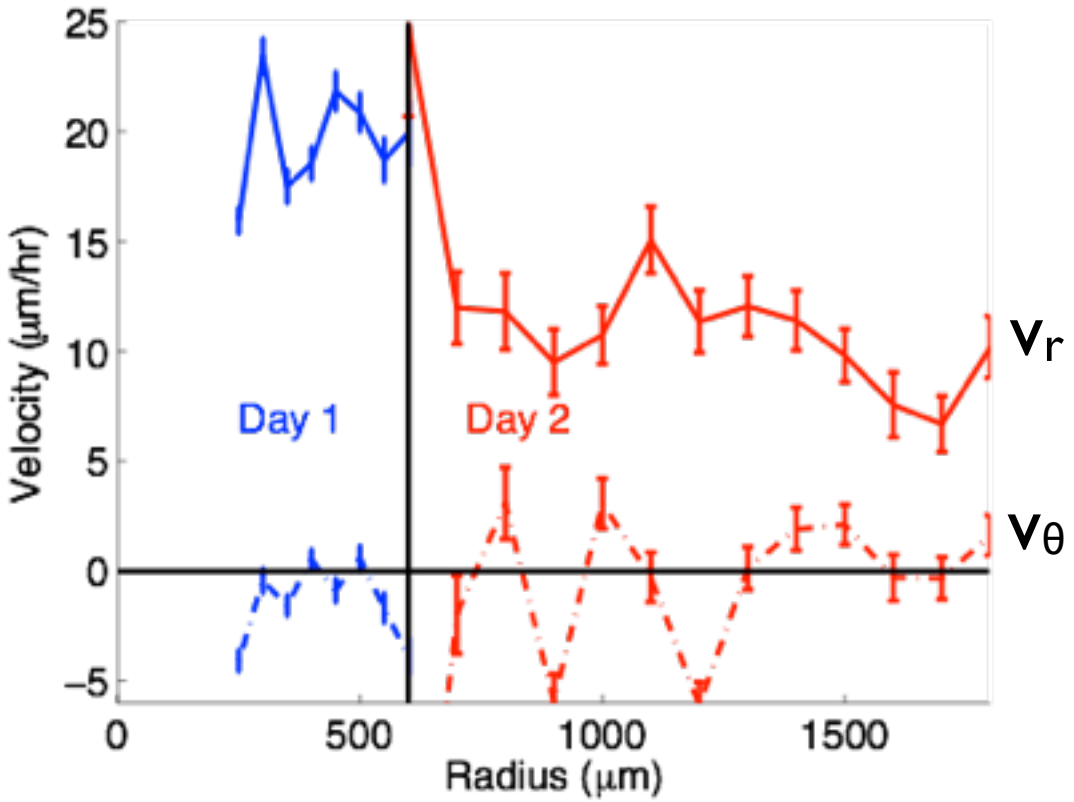
Cells are Biased Random Walkers



Model as a O-U process. Bias consistent with zero for azimuthal component.

Cells are Biased Random Walkers





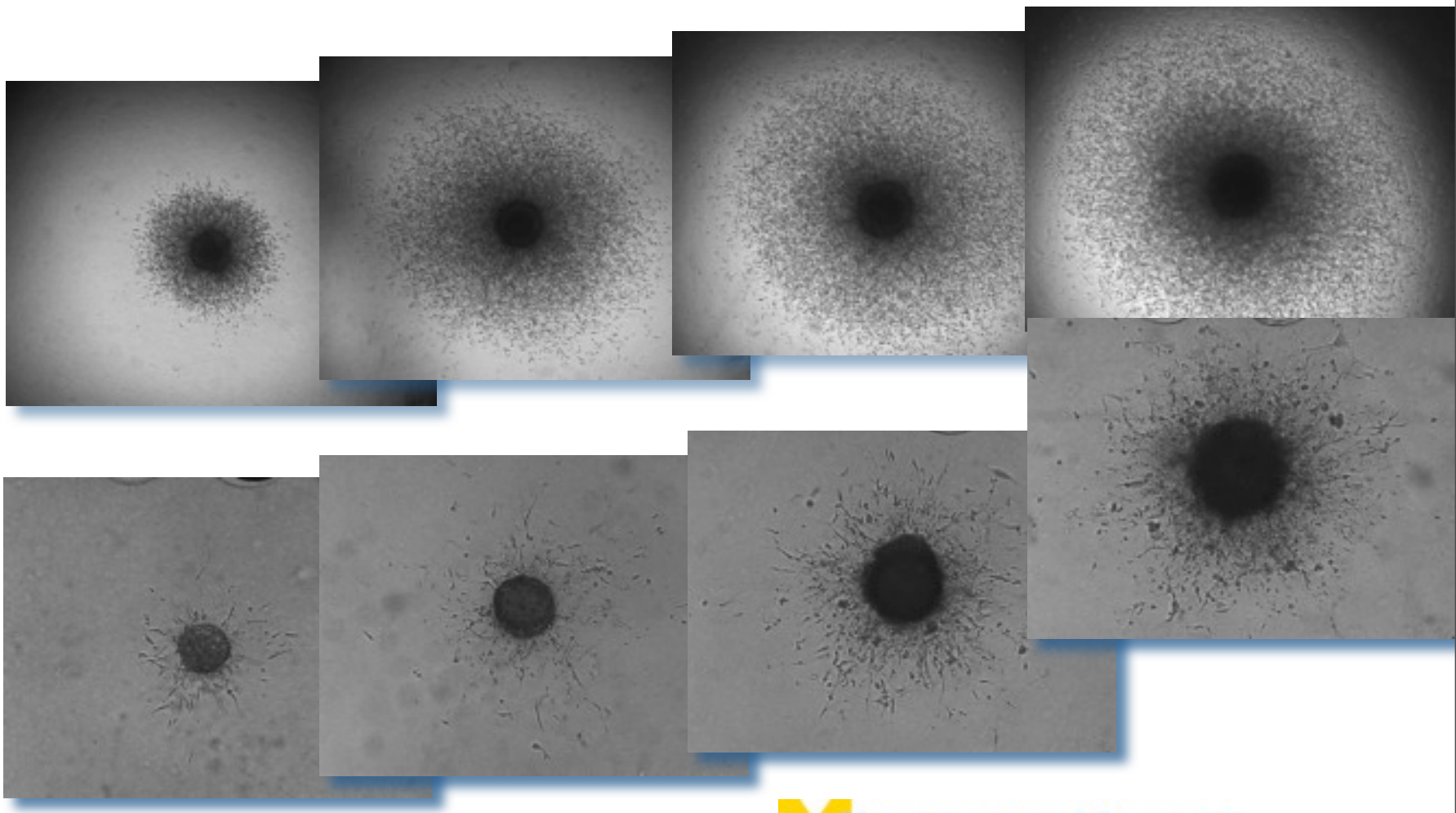
Model as a O-U process. Bias consistent with zero for azimuthal component.

Cell population dynamics

Day 1 Day3 Day5 Day7

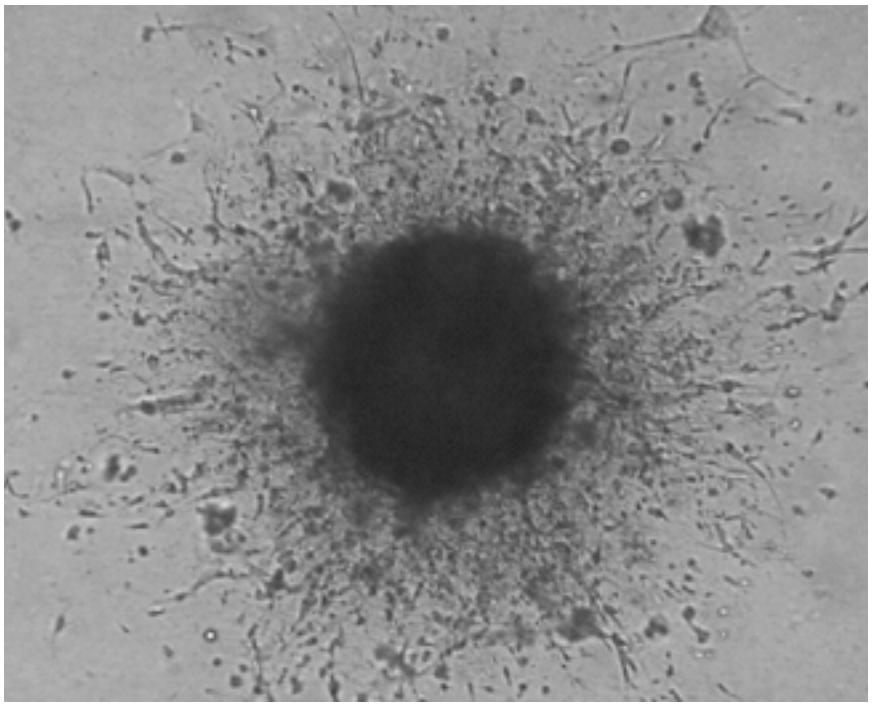
U87WT

U87dEGFR



Smoluchowski equation

$$\frac{\partial u}{\partial t} =$$

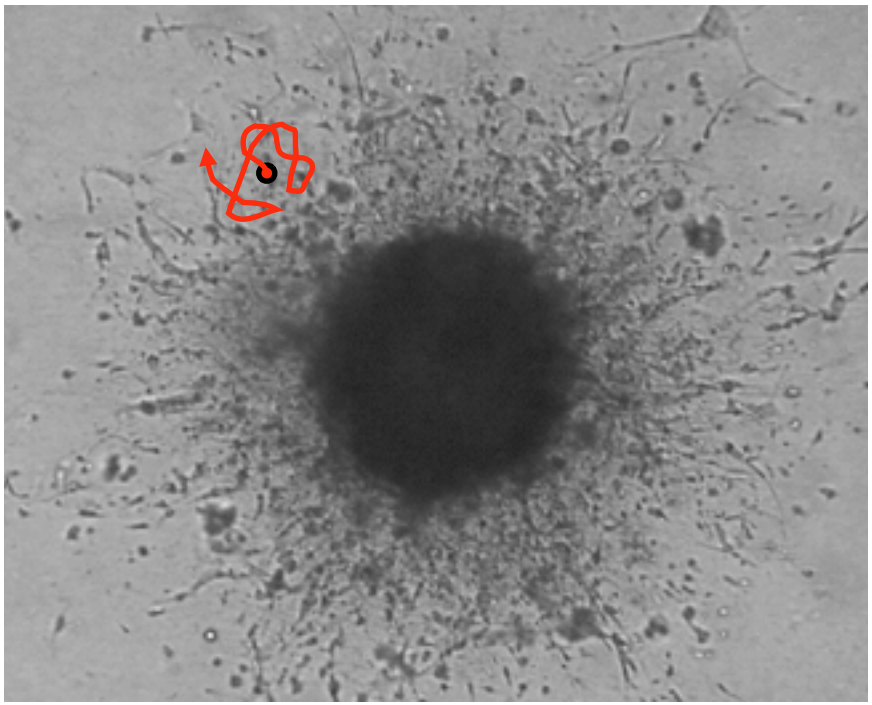


A. M. Stein, T. Demuth, D. Mobley, M. E. Berens, and L. M. Sander. A mathematical model of glioblastoma tumor spheroid invasion in a three-dimensional in vitro experiment. *Biophys. J.* 92:356–365, 2007.

Smoluchowski equation

$$\frac{\partial u}{\partial t} = D\nabla^2 u$$

Diffusion



- Invasive cell motion has a random component and

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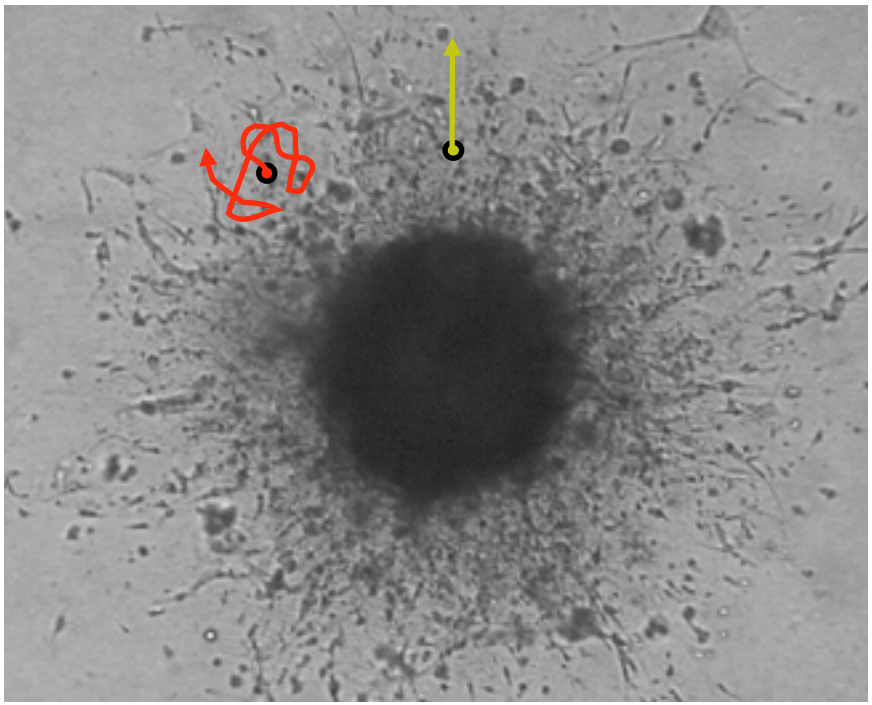
Smoluchowski equation

$$\frac{\partial u}{\partial t} = D\nabla^2 u - v\nabla_r u +$$

Diffusion Directed Motility



- Invasive cell motion has a random component and a directed component

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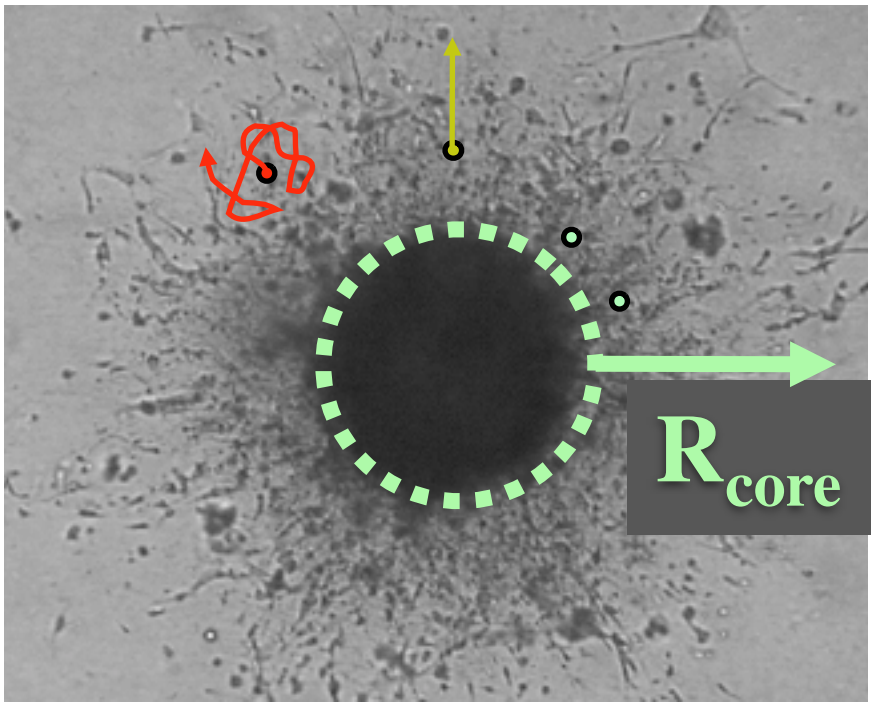
Smoluchowski equation

$$\frac{\partial u}{\partial t} = D\nabla^2 u - v\nabla_r u + s\delta(r - R_{core}(t))$$

Diffusion Directed Motility Cell Shedding



- Invasive cell motion has a random component and a directed component
- Core radius expands at a "slow", constant velocity.
- Invasive cells are shed from the core surface

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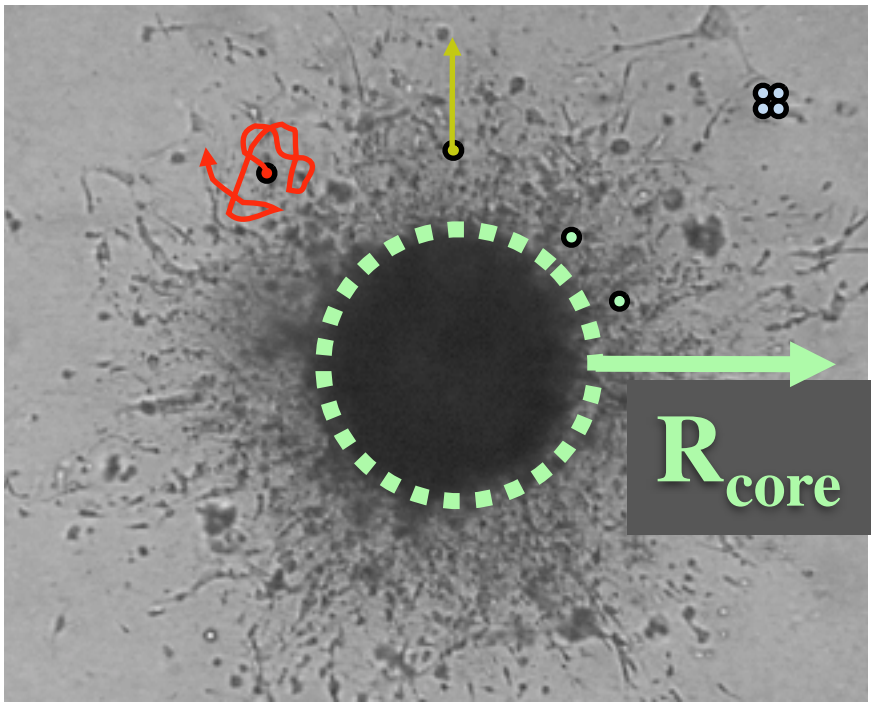
Smoluchowski equation

$$\frac{\partial u}{\partial t} = D\nabla^2 u - v\nabla_r u + s\delta(r - R_{core}(t)) + gu(1 - u/u_{max})$$

Diffusion | Directed Motility | Cell Shedding | Proliferation



- Invasive cell motion has a random component and a directed component
- Core radius expands at a "slow", constant velocity.
- Invasive cells are shed from the core surface
- Invasive cells proliferate

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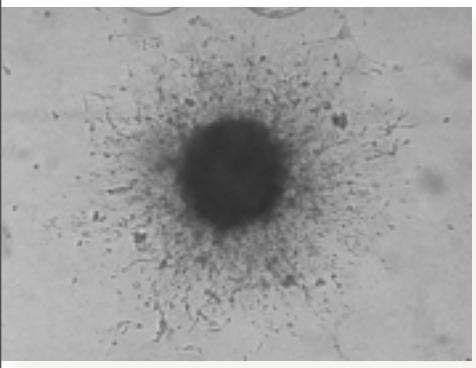
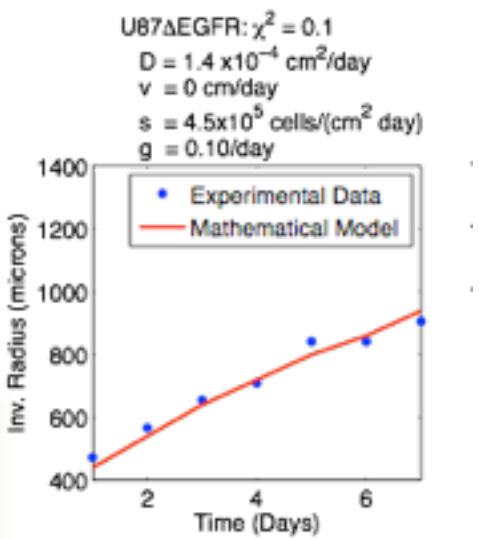
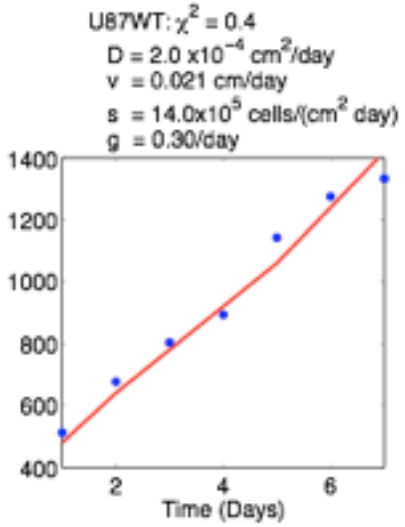
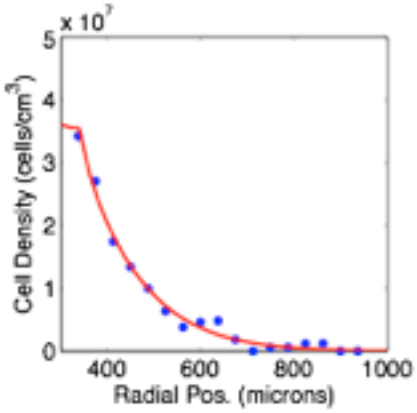
Fit Model to Different Cells

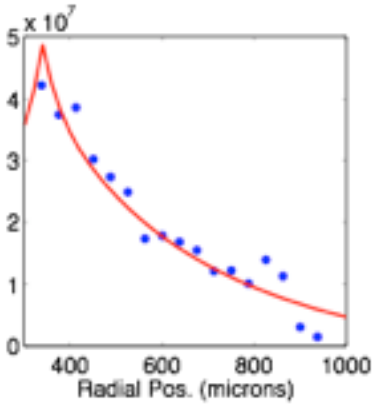


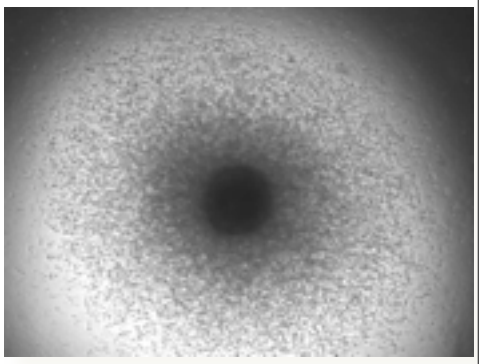
More malignant





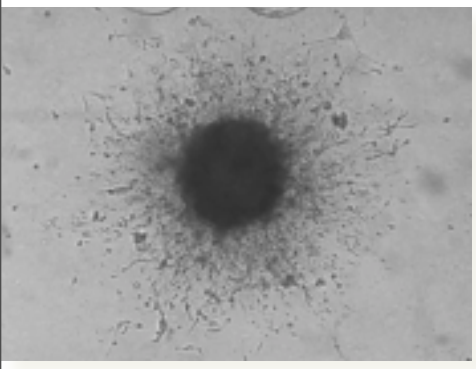




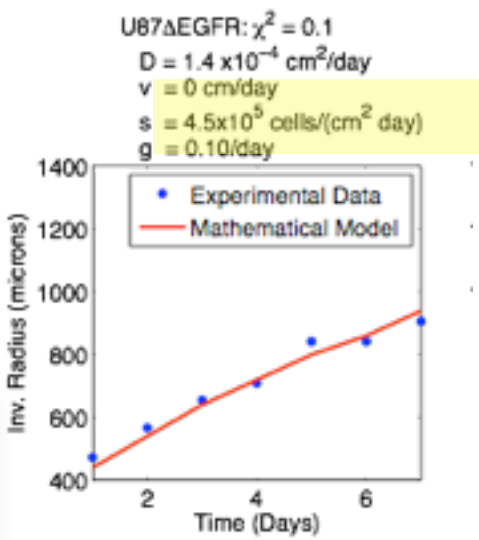


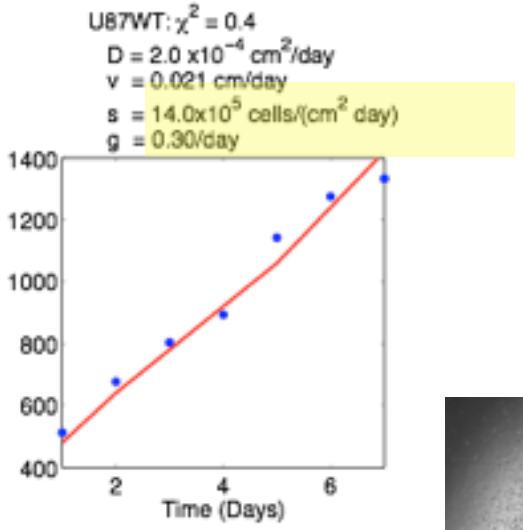
Less malignant

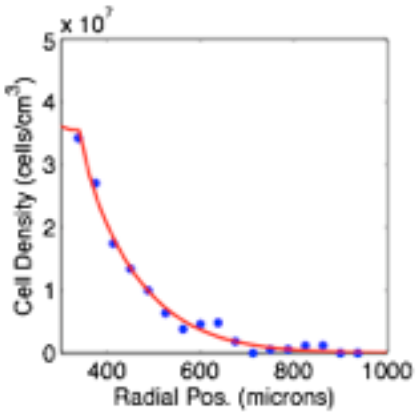
Fit Model to Different Cells

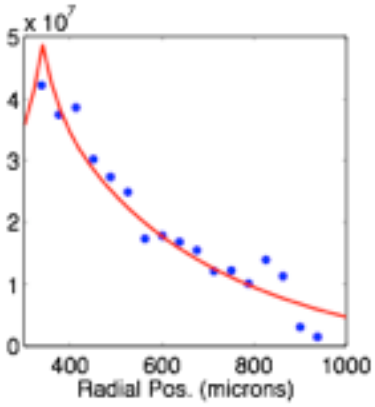


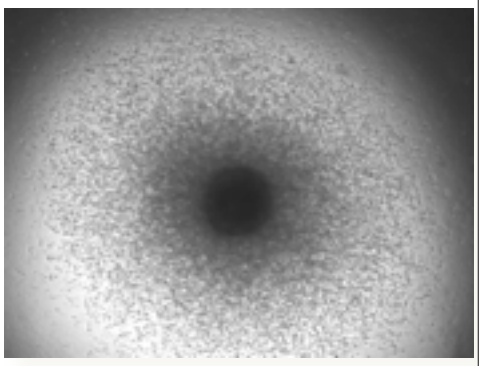
More malignant









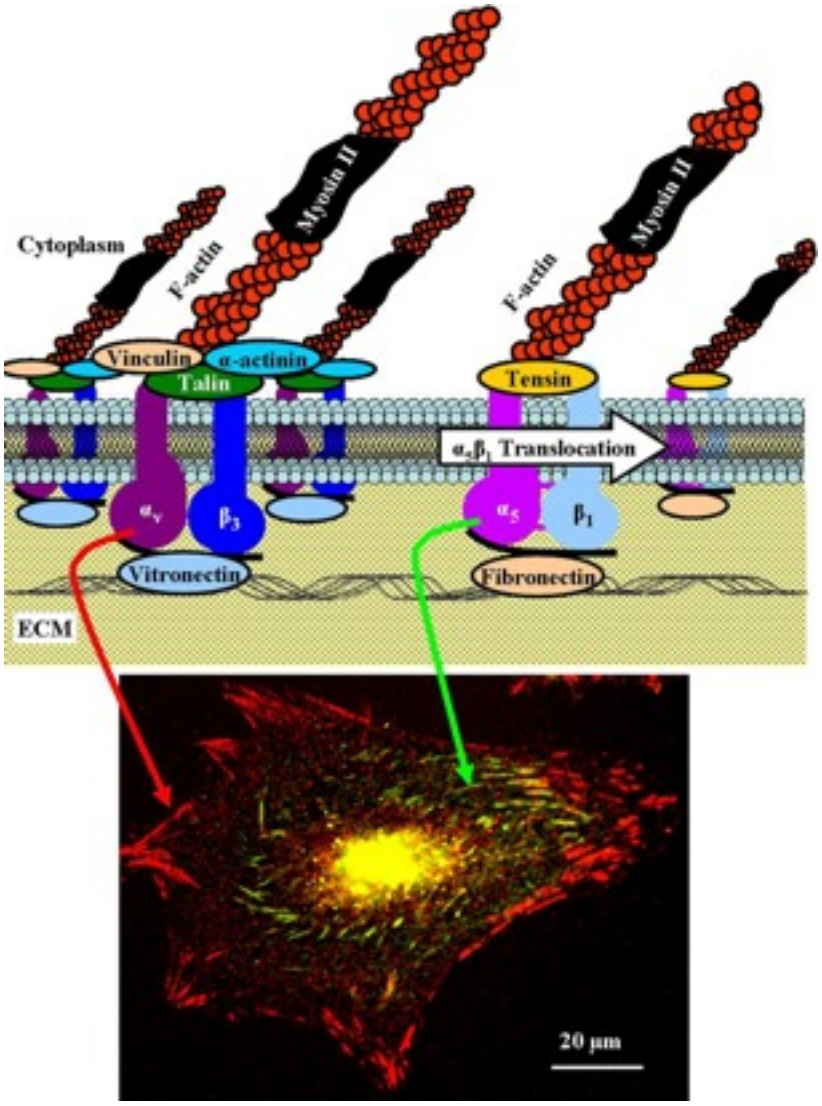


Less malignant

Results of experiments

- Cell-tracking and population tracking show:
  - Cell shedding from spheroid.
    - Rate presumably depends on competition between cell-cell adhesion and cell-matrix adhesion.
  - Random motion ("diffusion").
  - Directed motion away from spheroid at about 10μm/hr.

Cell motility: basic facts



- Cells attach to a substrate or matrix via a transmembrane protein.
  - In mammalian cells adhesion is via integrins.
  - Attachment to ECM at one end, actin network at the other.
- Cell contraction followed by detachment at the back leads to motion.

Cells affect the matrix

- Degradation: Cells produce enzymes that degrade the matrix (particularly important for fibrin).
- Production: Cells can produce their own collagen.
- Deformation: Cells deform matrix by exerting forces.
  - compaction
  - alignment

Cells Align Matrix

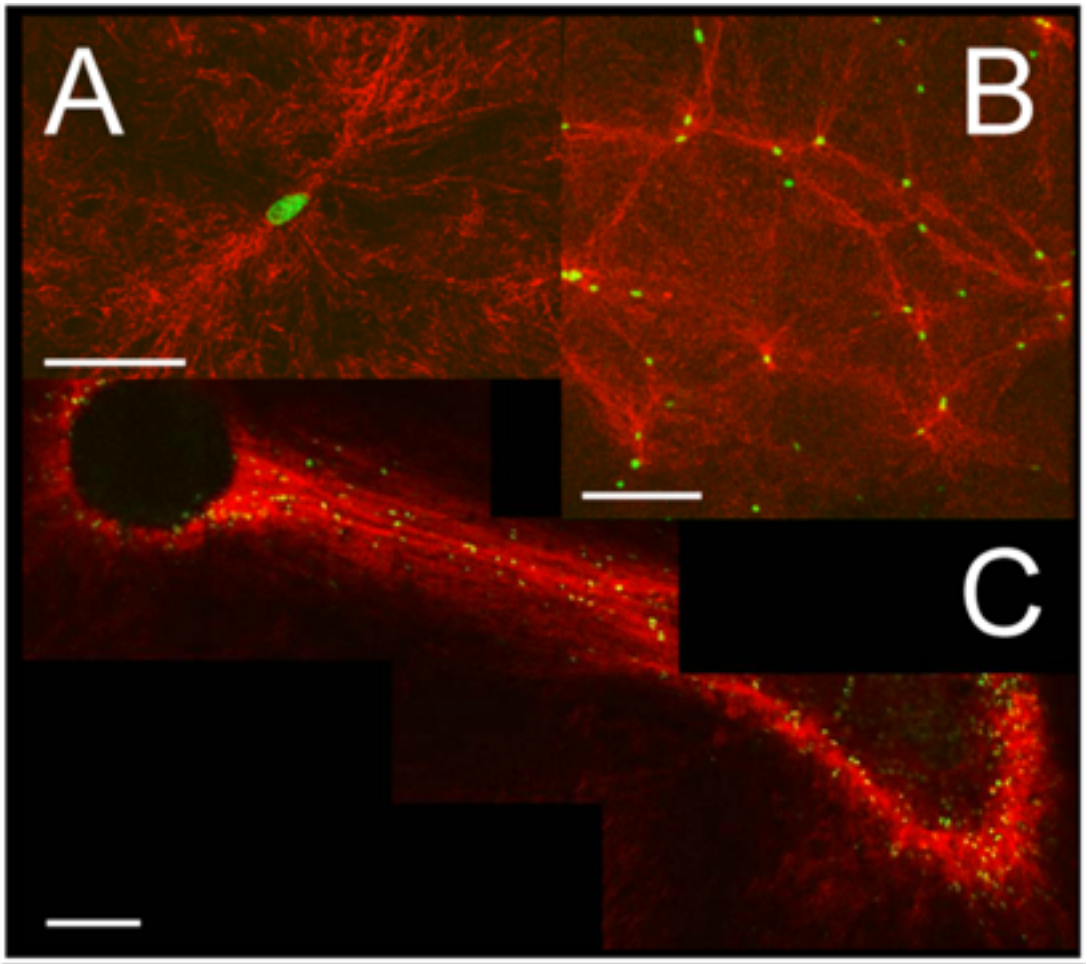
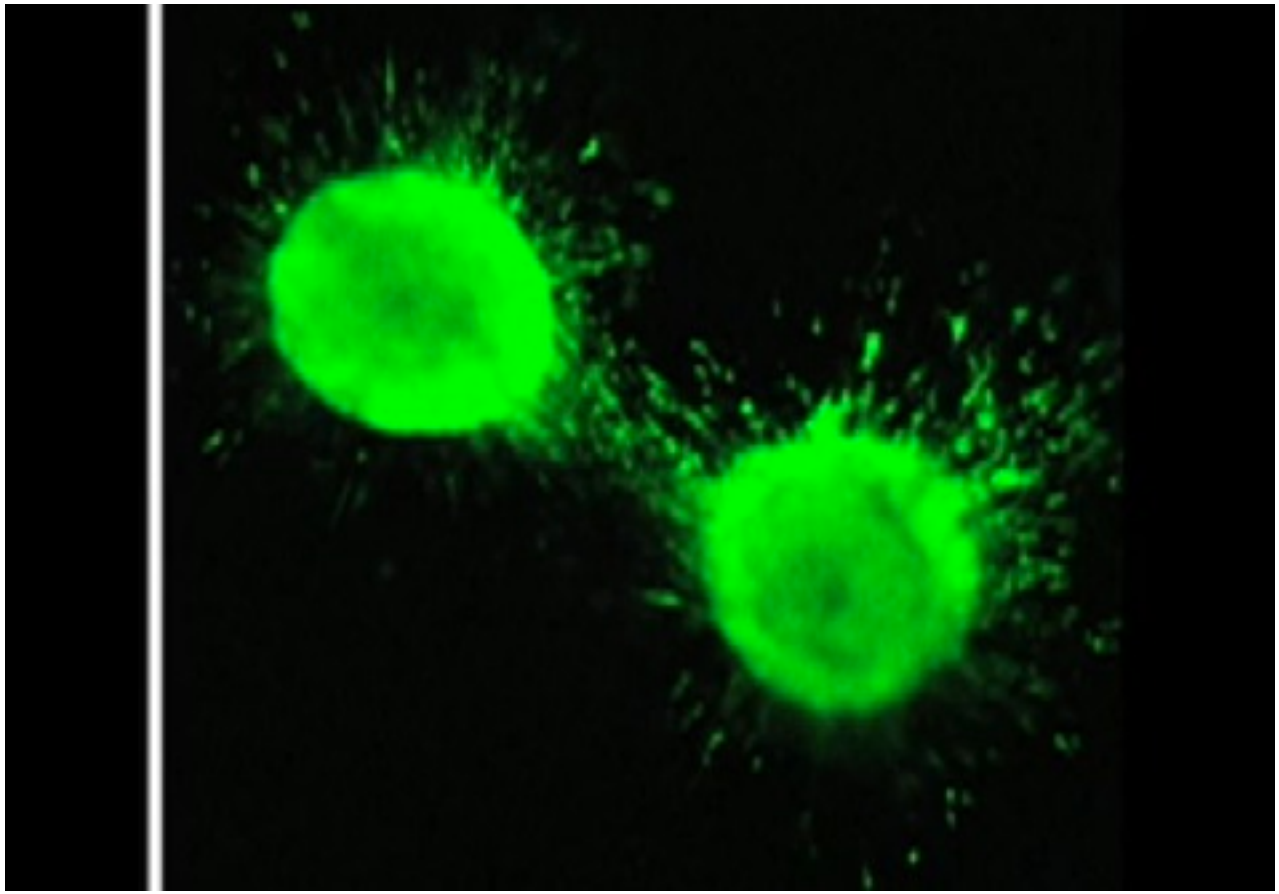


Figure 1. Collagen gel morphological changes induced by presence of cells. (A) Single U87 glioblastoma cell in a collagen network 10 hours after gel polymerization. bar = 50 mm. (B) Several U87 cells on the surface of a collagen gel 10 hours after gel polymerization. bar = 200 mm. (C) Two cell colonies embedded in a collagen matrix 48 hours after gel polymerization. bar = 200 mm. Fibers (artificial red color) are imaged through confocal reflectance; cell nuclei (green) are labeled with a GFP-histone heterodimer.
doi:10.1371/journal.pone.0005902.g001

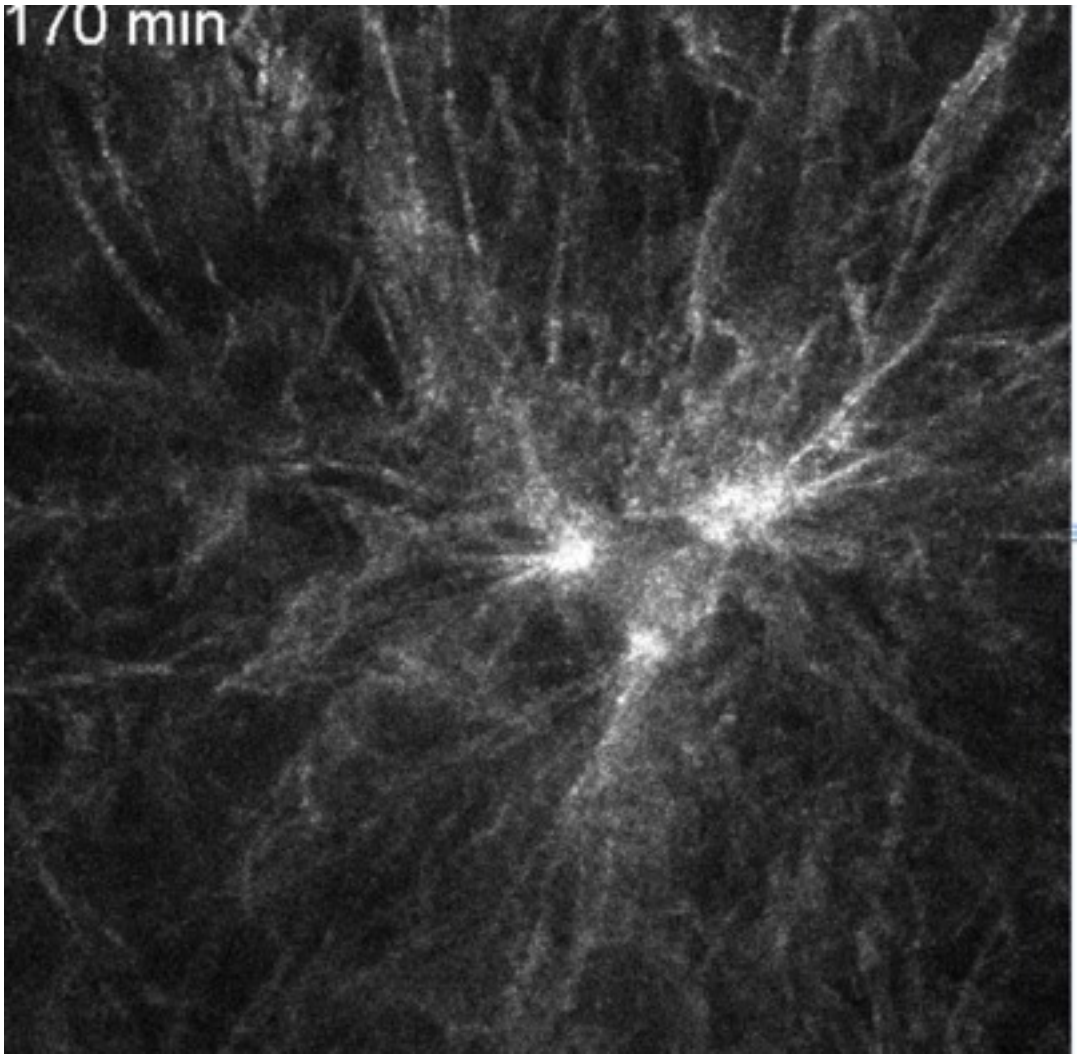
D. Vader, A. Kabla, D. Weitz, and L. Mahadevan, *Strain-induced alignment in collagen gels, PloS one, **4** (2009**).***

Cells follow alignment



- Demuth & Berens

Single glioma cell in collagen



Force measurements

- Seed collagen with fluorescent beads, measure displacements.
  - V. Gordon, et al. *Measuring the mechanical stress induced by an expanding multicellular tumor system: a case study*, Experimental cell research, **289**, 58 (2003).
  - Invading cell tip pulls nearby gel inward with a force in the range 10 –100 nN

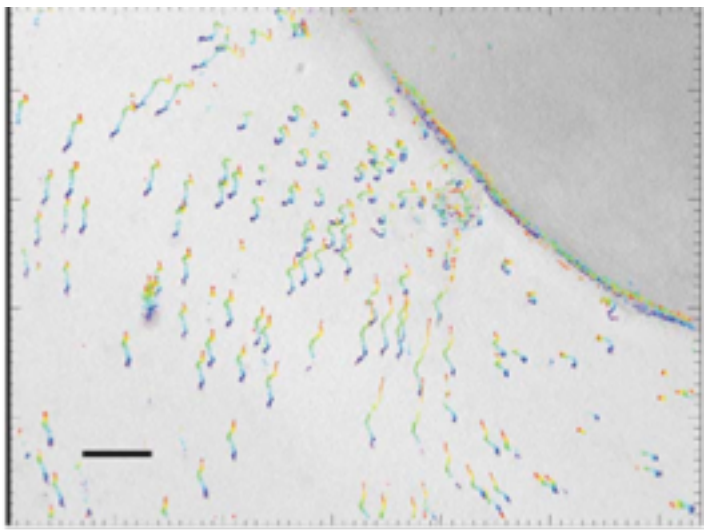
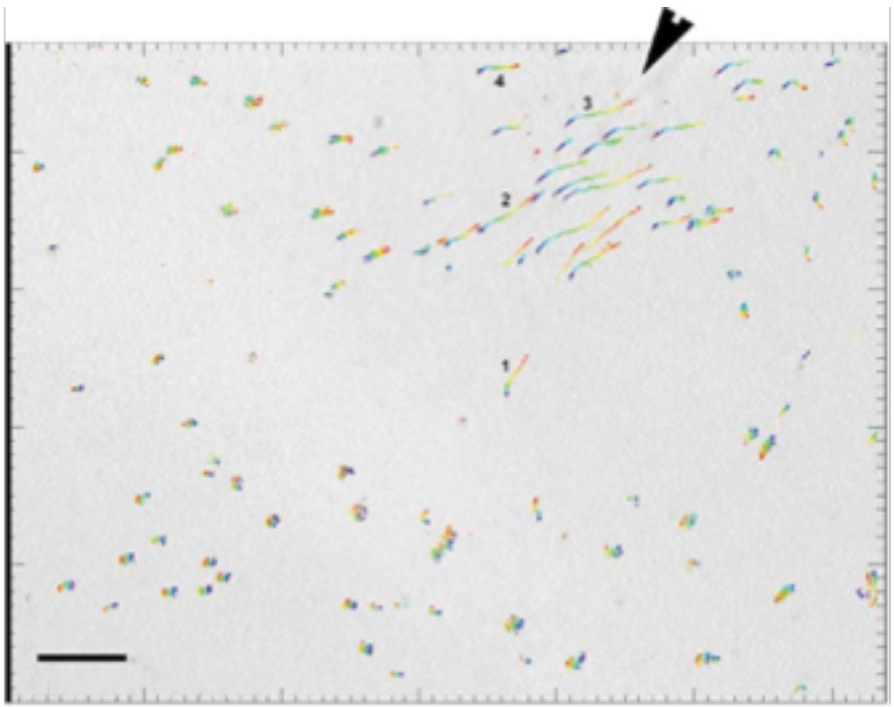


Fig. 4. Time-lapse multiparticle tracking (Day 1). Tracks are drawn to trace the paths of moving beads as color is used to time stamp bead positions: early times are indigo blue and colors shade along the spectrum to red at late times. The tracks have been superimposed on the first frame in the time-lapse sequence. Note darkened multicellular tumor spheroid (top right). Ticks indicate acquired image dimensions, 10 pixels per tick mark (bar = 10 μm; original magnification: ×40; time-lapse duration: 110 min).



Modeling cell interactions with ECM

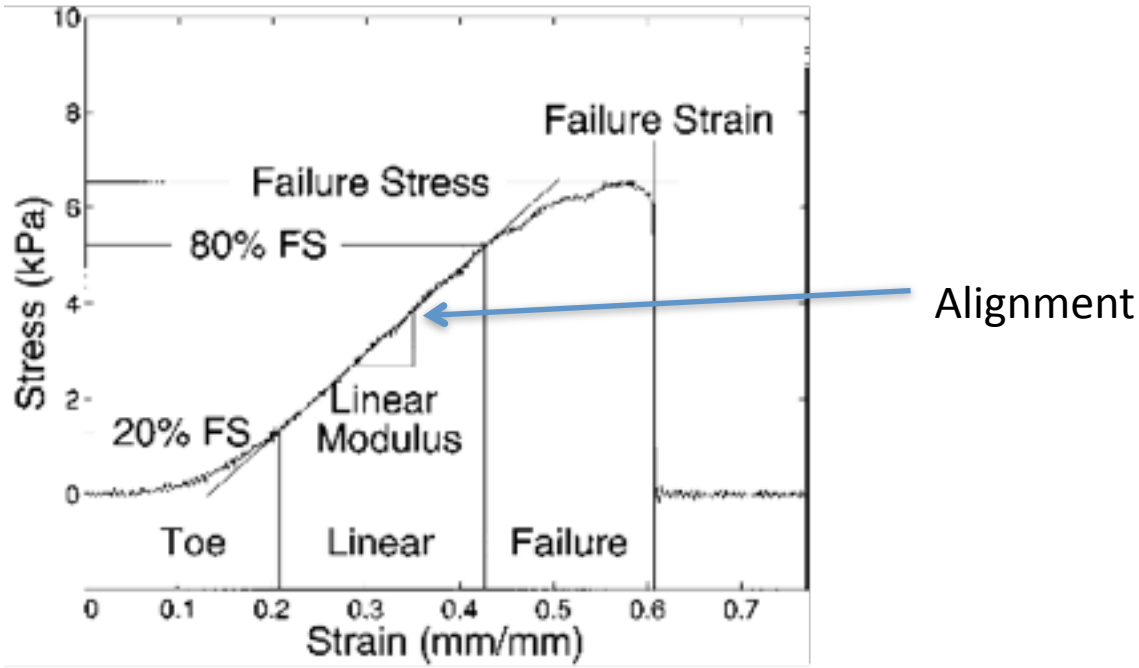
- Interactions of cells with the matrix in which they move.
  - Matrix is a tangled net of polymers -- a *gel.*
  - Cells can deform the gel, and the presence of the gel can impede or guide cell motion.
- We try to understand these effects by investigating the mechanics of the gel.

Alignment and cell motion

- Near a spheroid there may be massive alignment of fibers.
- We think that the aligned regions make “highways” for cell motion.
- We need to understand the collagen medium in which the cells move.

Collagen remodeling

- Remodeling and alignment occur because cells pull collagen together. We need to understand collagen mechanics.
- Collagen and most biopolymers, have non-linear elasticity, and fibers *align at large strains*; this leads to strain-stiffening.



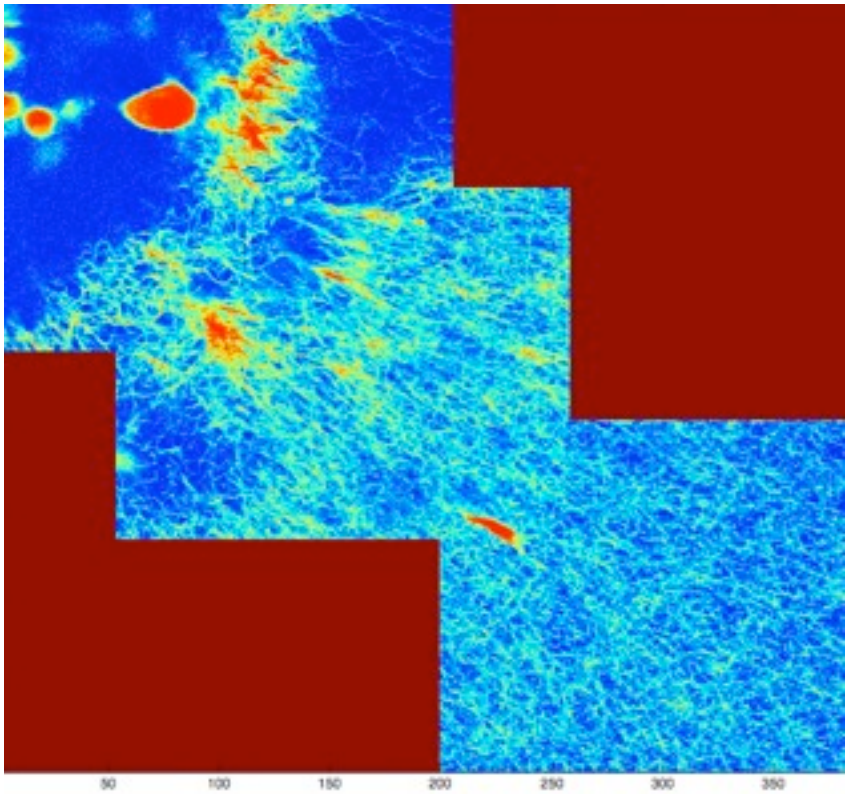
Roeder et. al., 2002

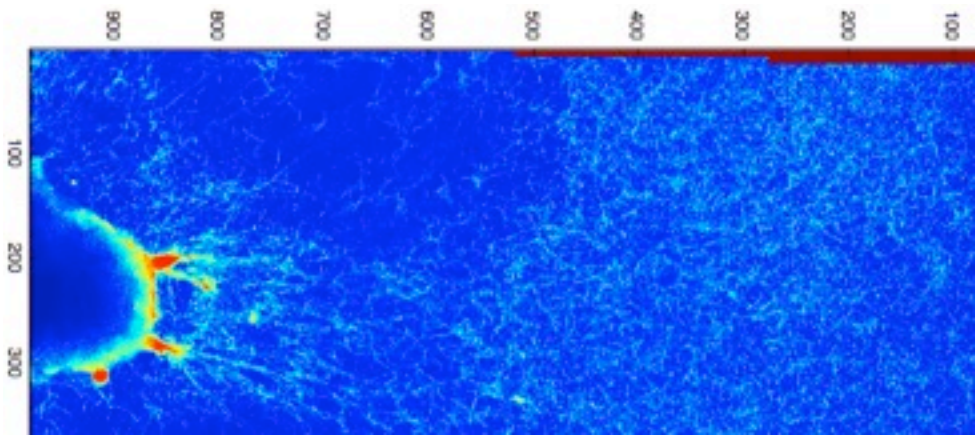
Confined alignment

- Since alignment only occurs at *large* strain, it will be confined to a region very near the cell.
- We argue that this 'sphere of influence' has been indirectly observed, and we compute its size using a model for the non-linear elasticity of collagen.
- This is *very unlike* linear elasticity where all effects of point deformations lead to power laws with no natural scale.
  - For example, for an elastic medium with a spherical inclusion that contracts, $\sigma_{rr} \sim 1/ r^3$.

Evidence for confined alignment

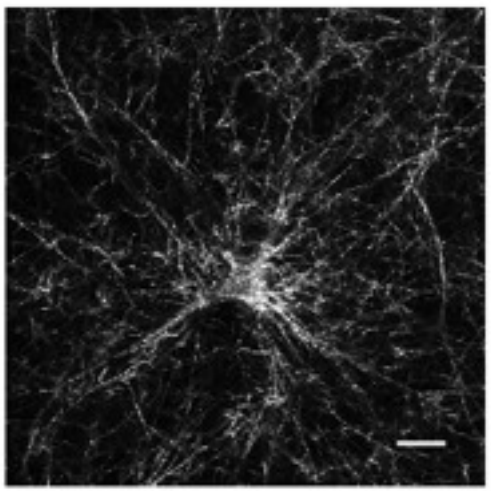
- Experiments on tumor spheroids in a 3d collagen assay.
  - Here it is glioma cells that pull on the medium, and they are in a large lump about 250μ across.





Fibroblast-populated collagen gels

- In a popular method, the *fibroblast-populated collagen microsphere assay*, the compaction of collagen gels is studied.
- Evans & Barocas, 2009 studied mechanics of the gel:
  - Propose a model in which 'the gel compaction is not homogeneous but consists instead of extreme densification near the cells in an otherwise unchanged matrix.'
  - Good fit to mechanics of the microsphere from spherical inclusions around each fibroblast of about 100-150 μ diameter.



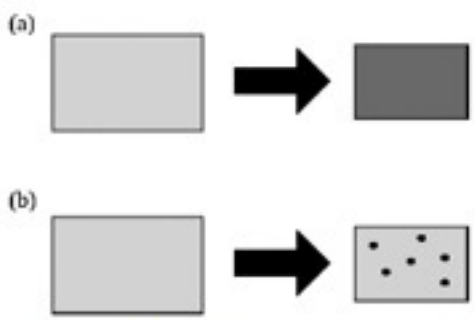


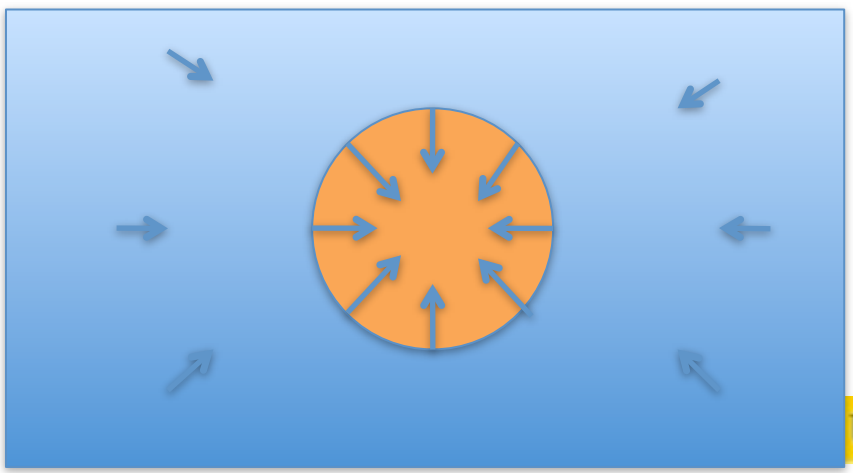
Fig. 6 Schematic of former and proposed view of cell-driven compaction. (*a*) Old view. Cells drive homogeneous compaction, increasing the density of the gel throughout. (*b*) Proposed view. Cells create small, very dense inclusions in an otherwise largely unaltered gel.

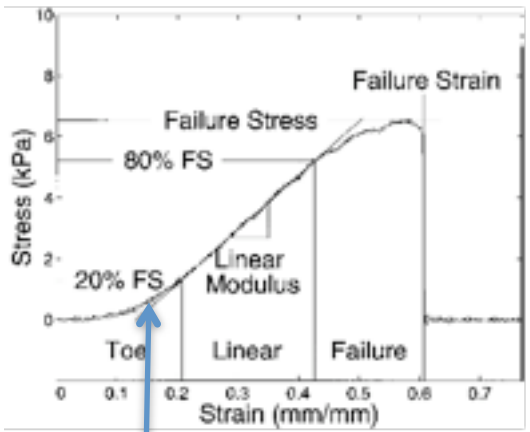
M. C. Evans and V. H. Barocas, *Journal Of Biomechanical Engineering* ***131 (10), 101014 (2009).***

Estimate confinement radius

- Outside of the confined region the medium is linear, so we can use standard methods.
  - Assume the aligned region is a sphere of radius R with negative pressure, p.
  - We take the displacement to be radial, a function of r alone.
  - Simple calculation: $\sigma_{rr} = pR^3/r^3$,

$$\sigma_{\theta\theta} = \sigma_{\phi\phi} = -pR^3/2r^3$$





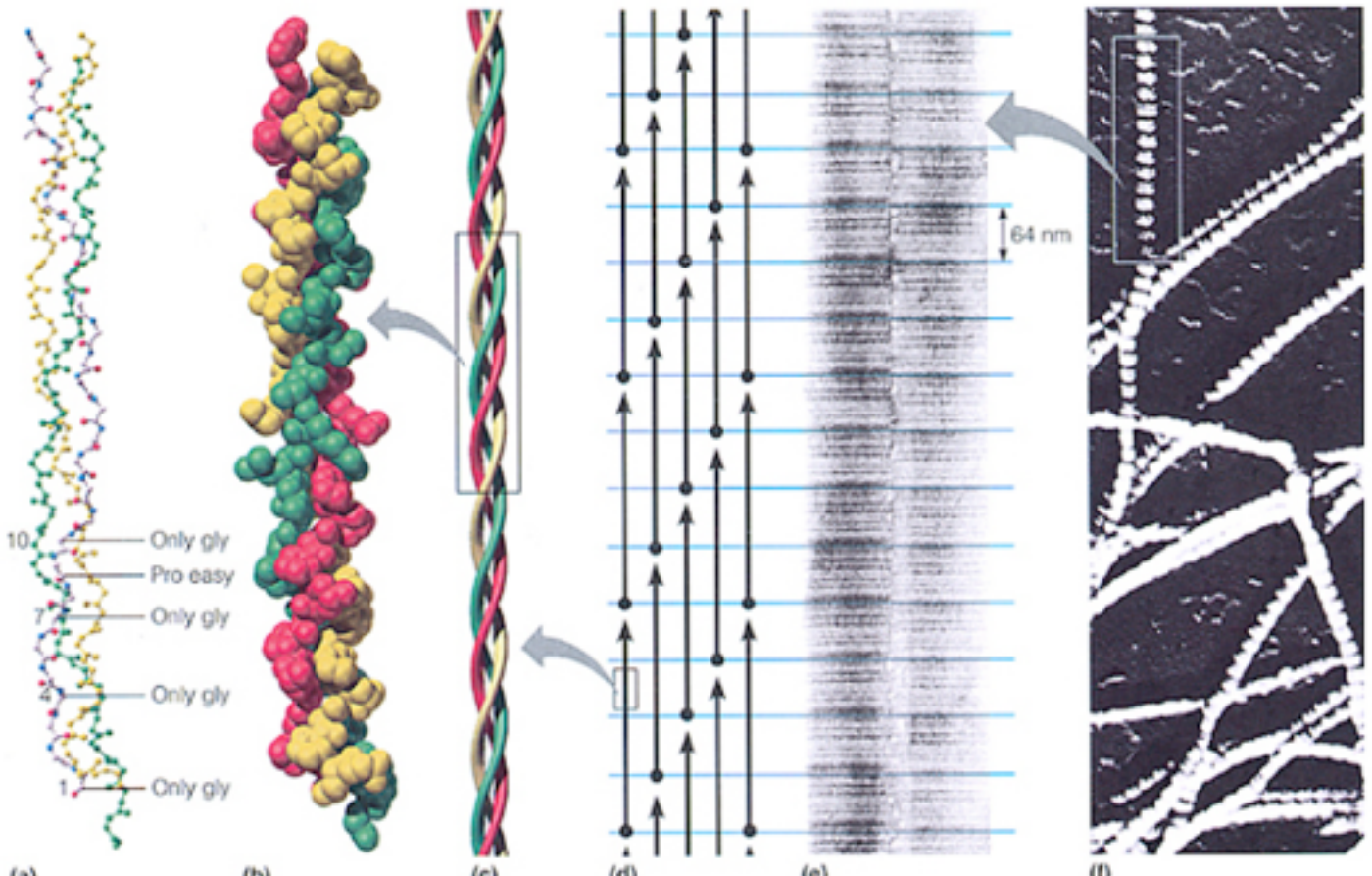
Onset of non-linearity

Confinement radius, cont.

- Determine p: Assume that the cell pulls on rigid aligned fibers so that the total force applied by the cell, f_o, is transferred to the medium at radius R.
  - Glioma are known to pull with about 10 nN (A bit more for fibroblast).
  - This gives, e.g., $\sigma_{rr} = -f_o R/4\pi r^3$.
- Determine R: the non-linear regime sets in at stresses of order 1 Pa. So 1 Pa $= f_o/4\pi R^3$.
- This gives R ~ 30μ, within a factor of 2 or 3 of the observation.

Modeling collagen mechanics

- Collagen is the primary animal structural protein. It is found in bone, cartilage, tendons, ECM, and Jello.



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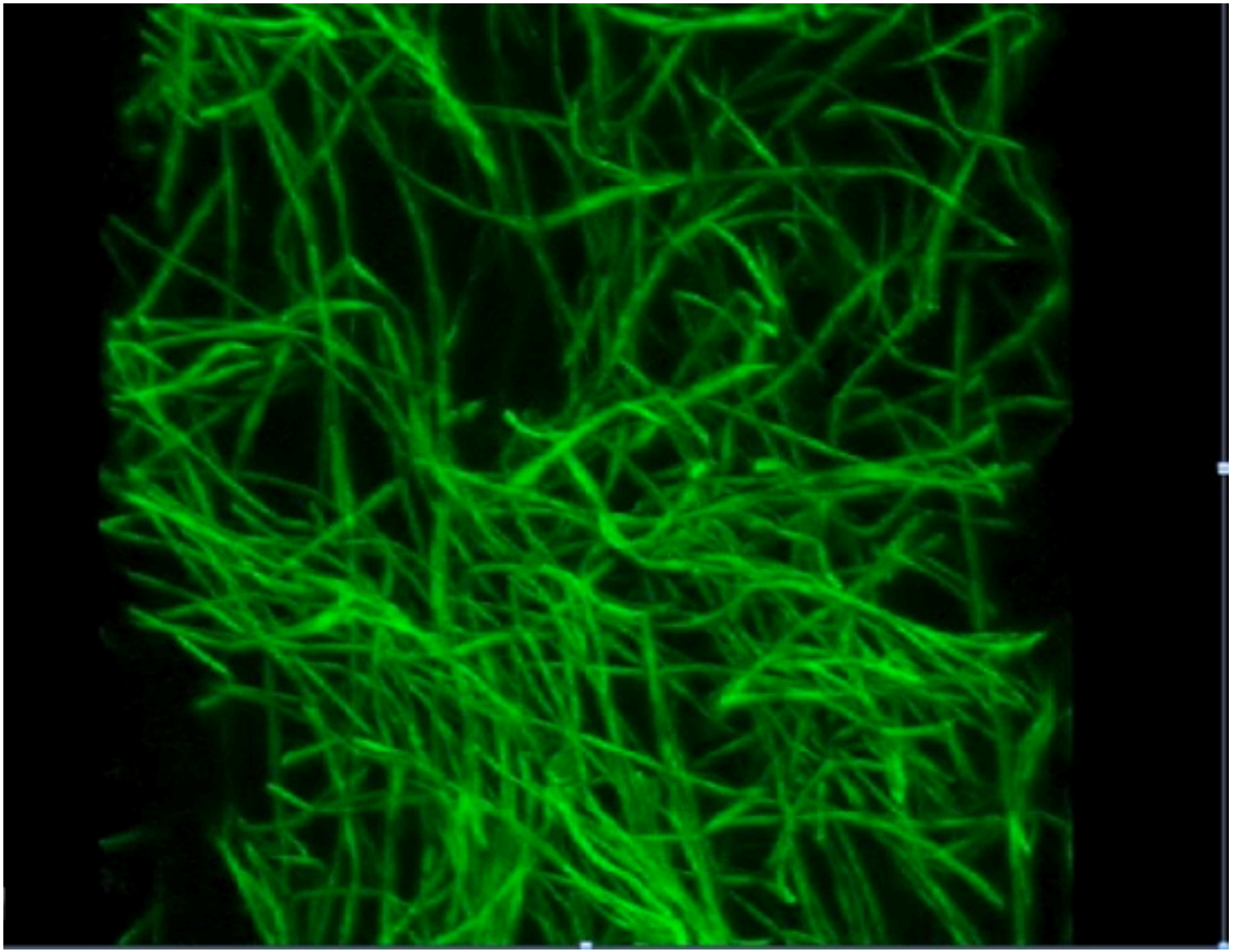
Collagen-I Gel

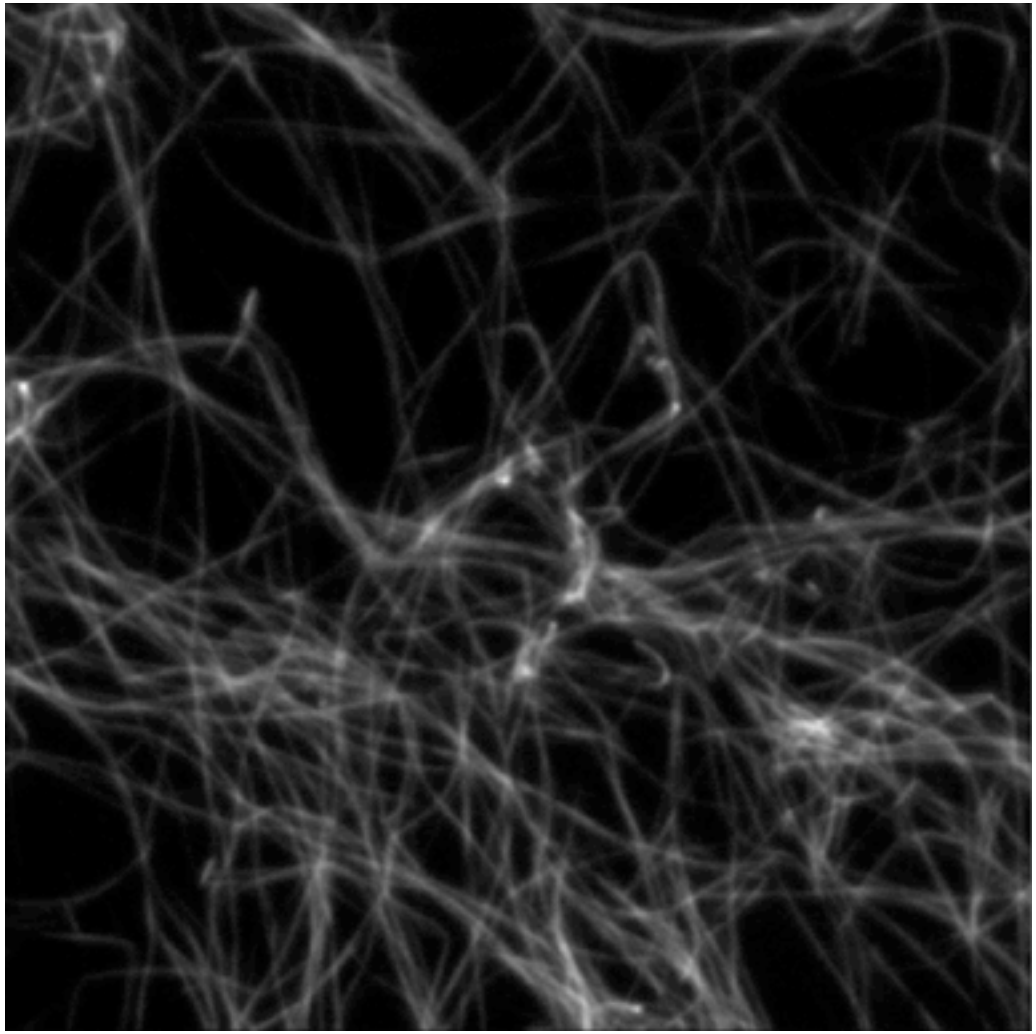


1.5 mg/ml

Collagen Gel Physics

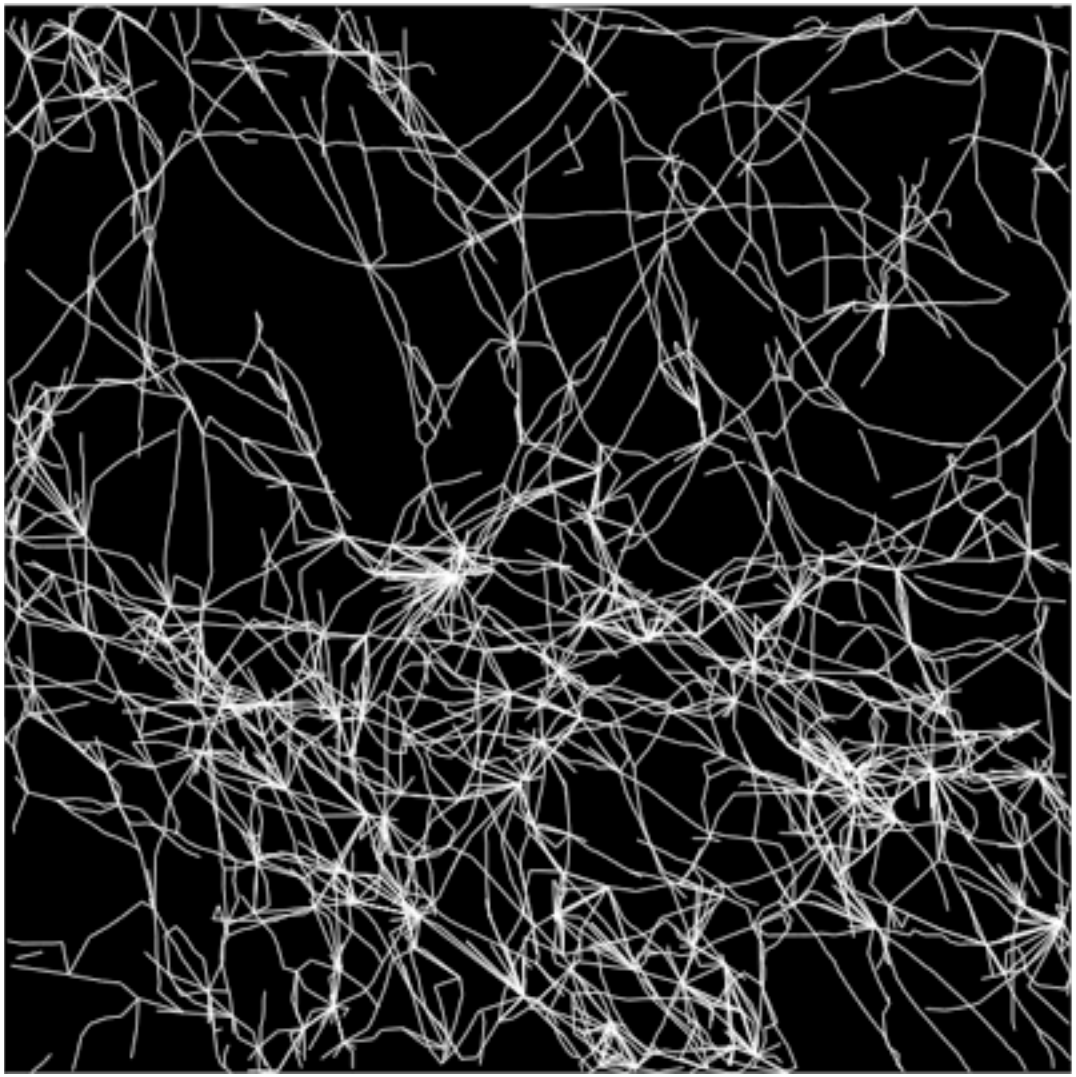
- Collagen is viscoelastic up to 10-15% strains.
- Significant strain stiffening and plastic deformation occur at larger strains.
- Many other biological gel networks have these properties, e.g. actin.
- A micromechanical model is needed to understand strain stiffening and plasticity.

Actual Networks



A. M. Stein, D. A. Vader, L. M. Jawerth, D. A. Weitz, and L. M. Sander, *An algorithm for extracting the network geometry of three-dimensional collagen gels*, Journal of microscopy, **232**, 463 (2008).

Actual Networks



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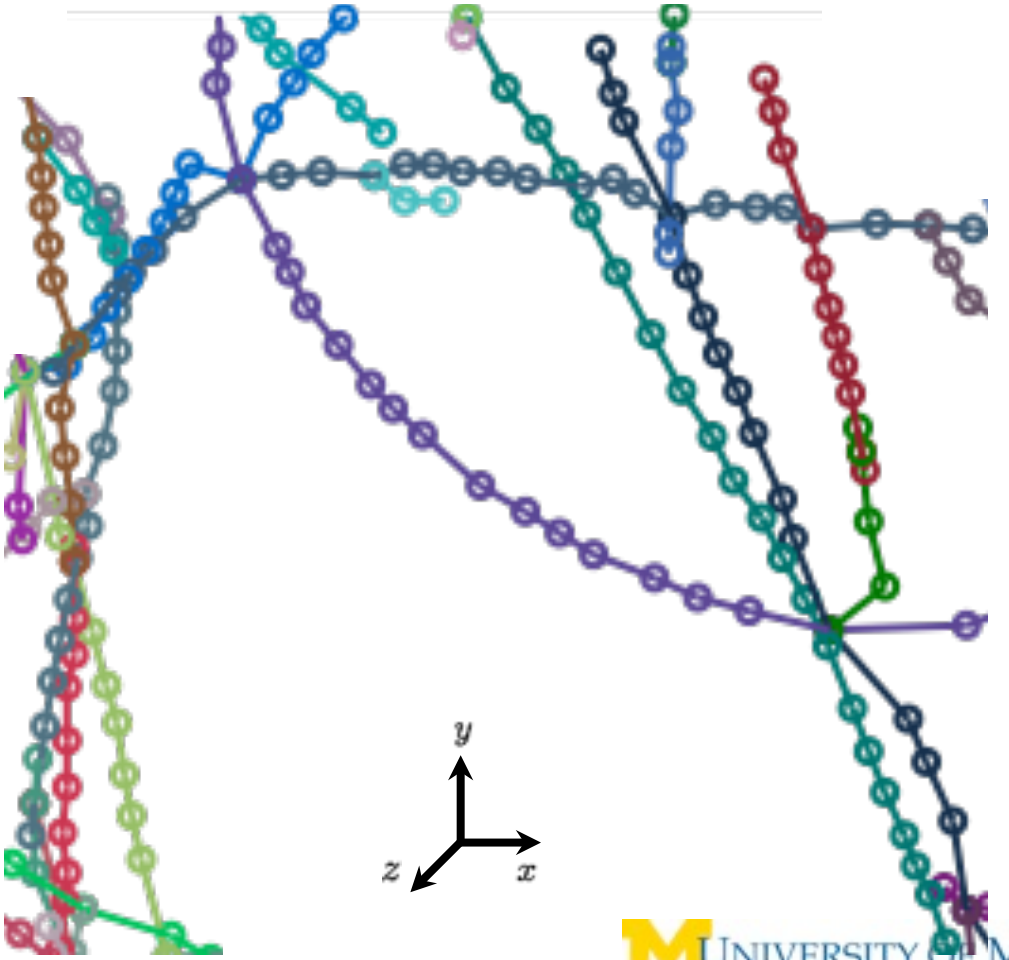
Mechanical Modeling of Networks

Stein, A. M., Vader, D. A., Weitz, D., and Sander, L. (2010). The micromechanics of three-dimensional collagen-I gels. Complexity *16, 22-28.*



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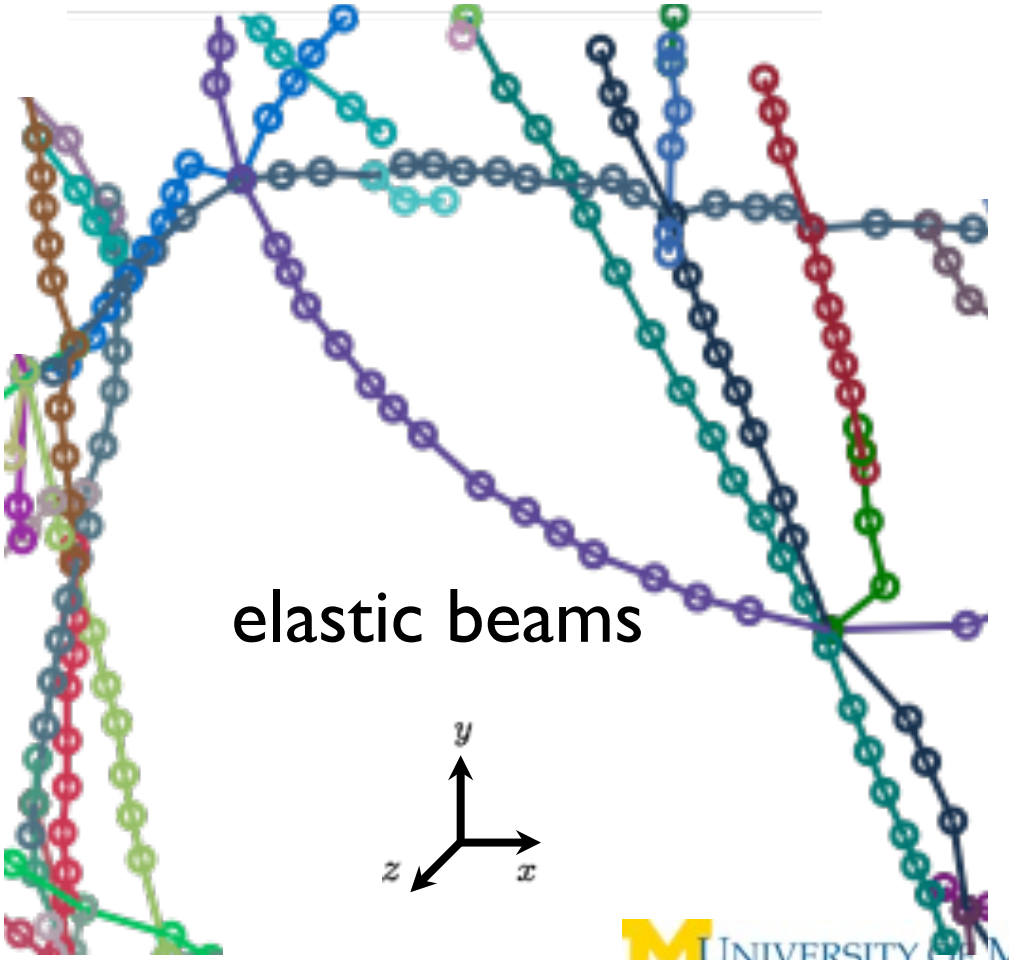

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Mechanical Modeling of Networks

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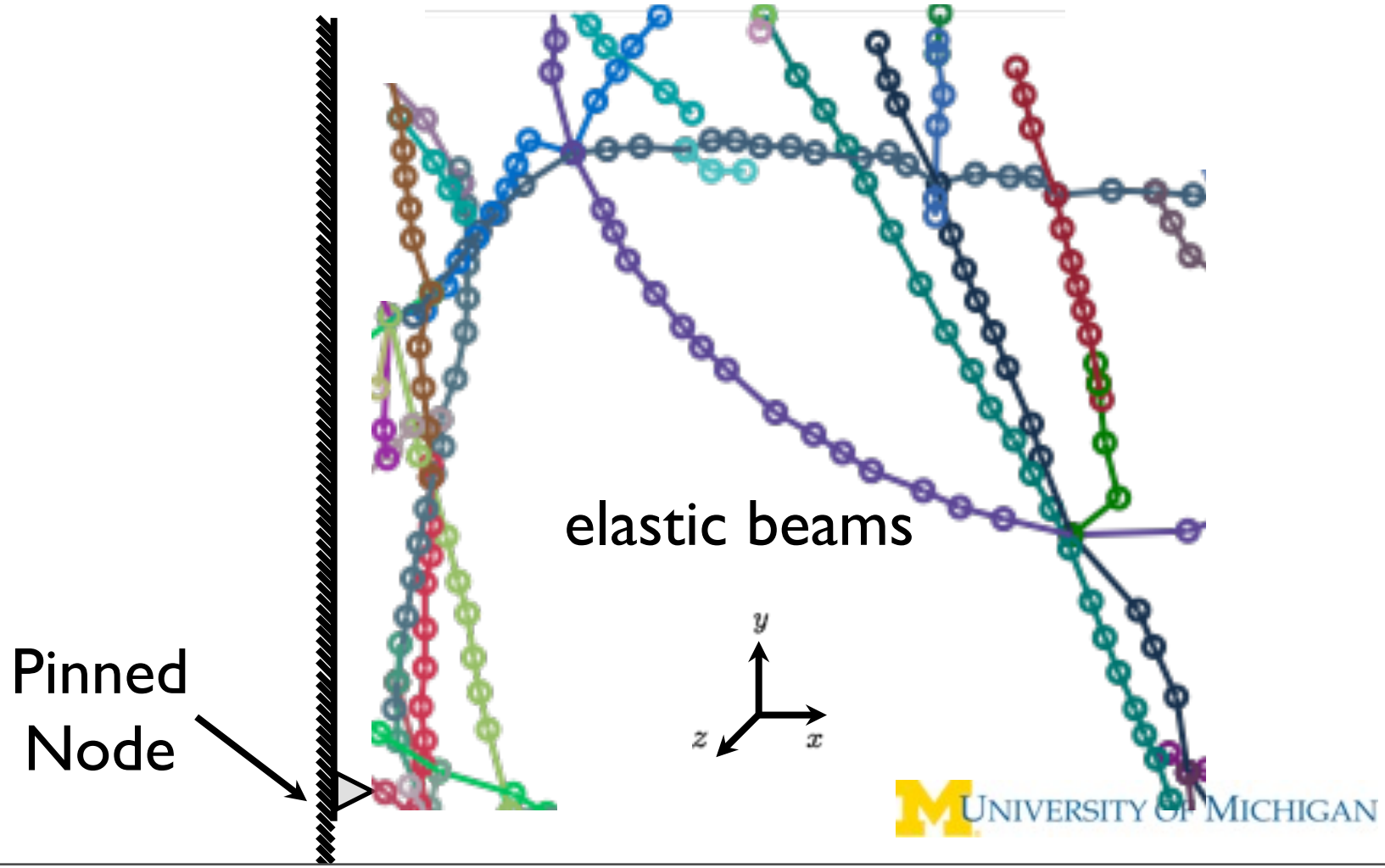

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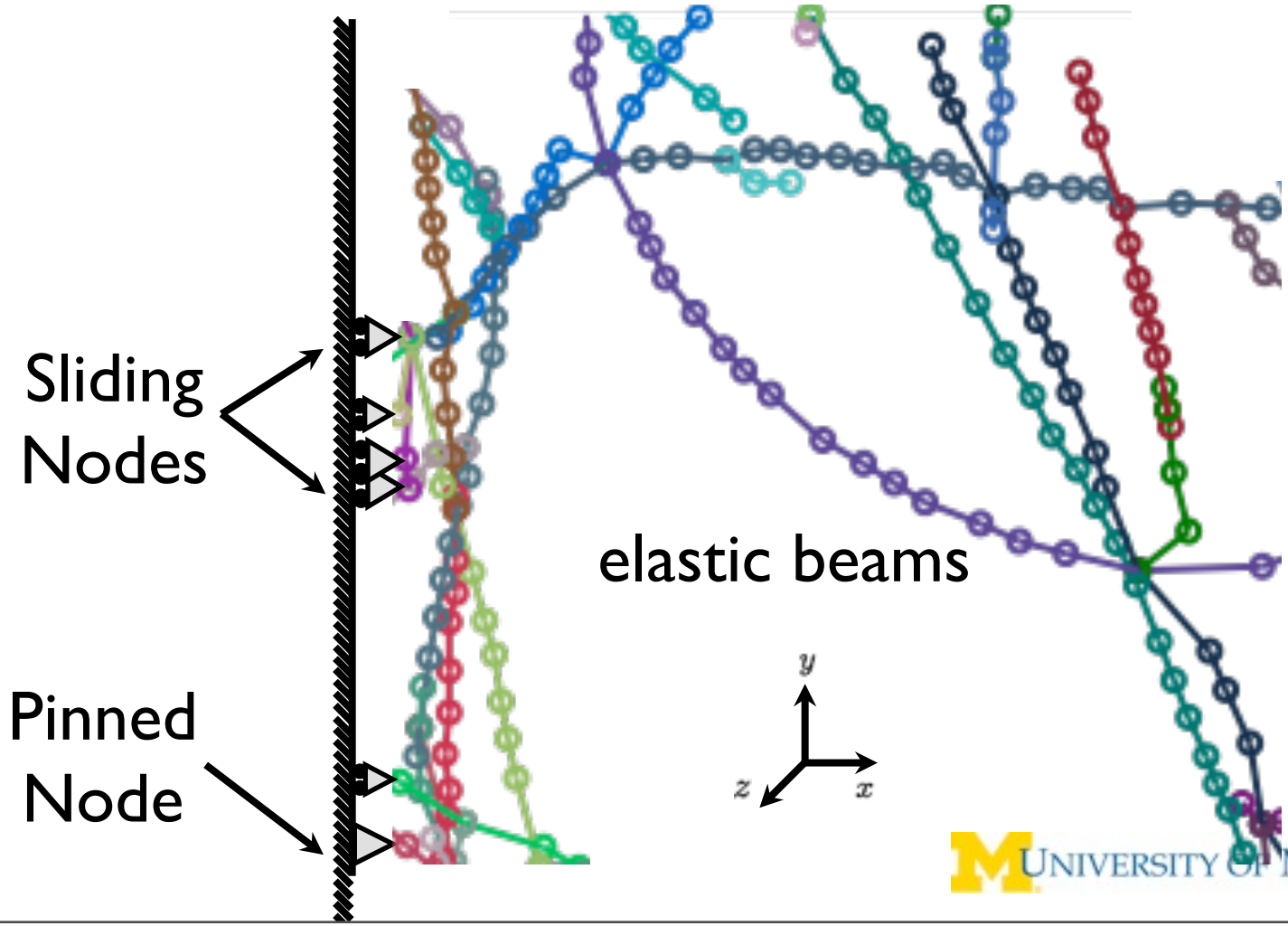

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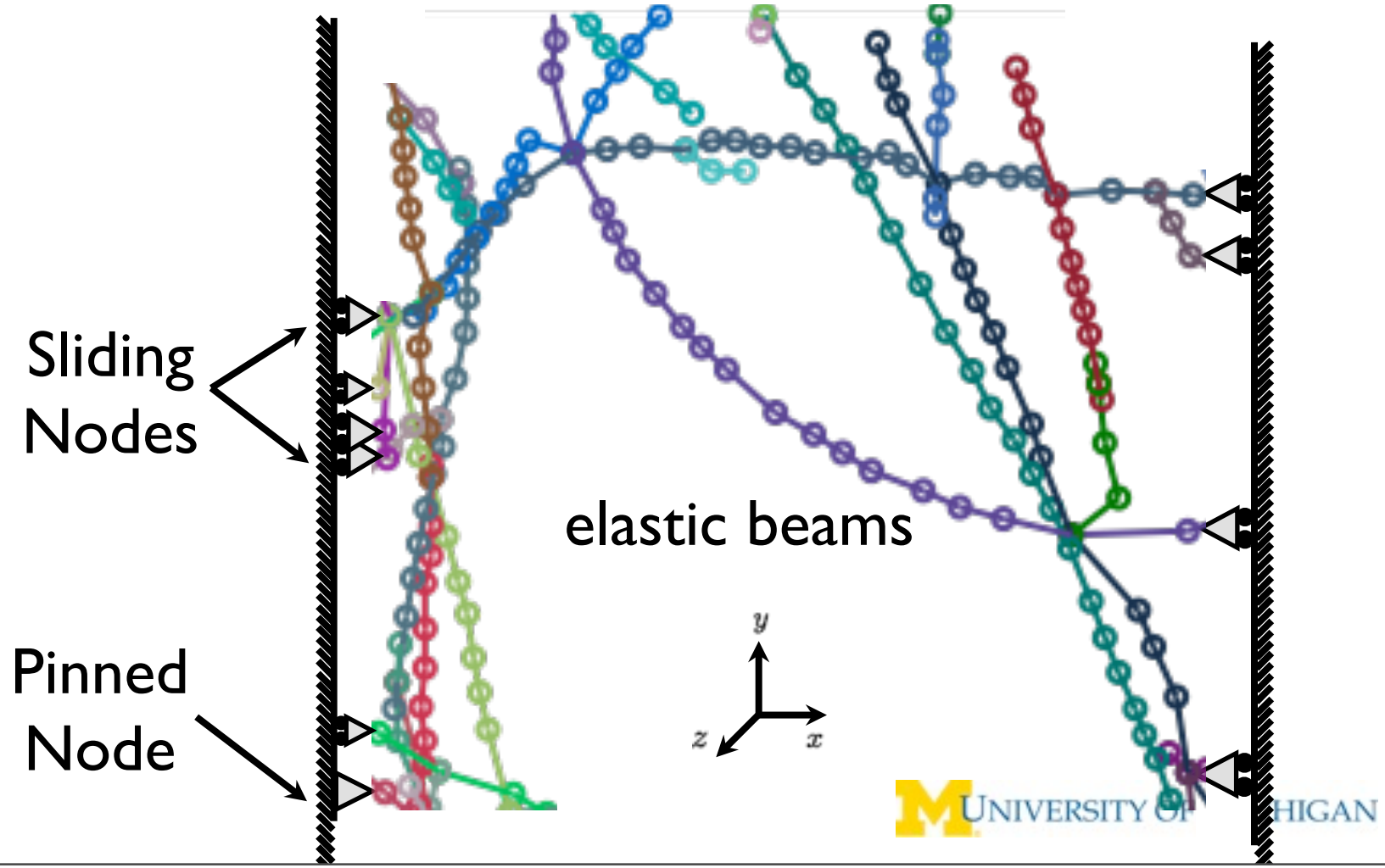

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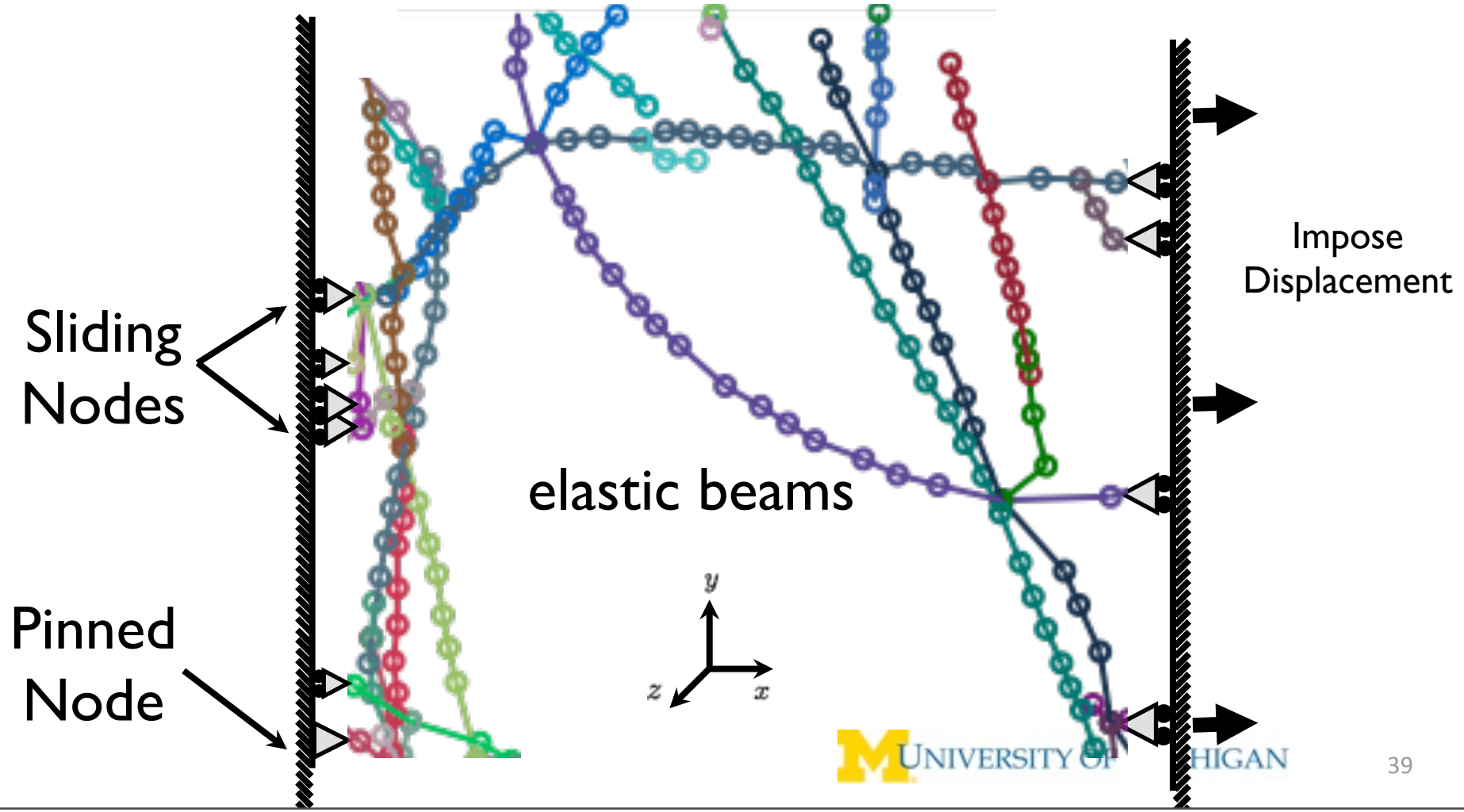

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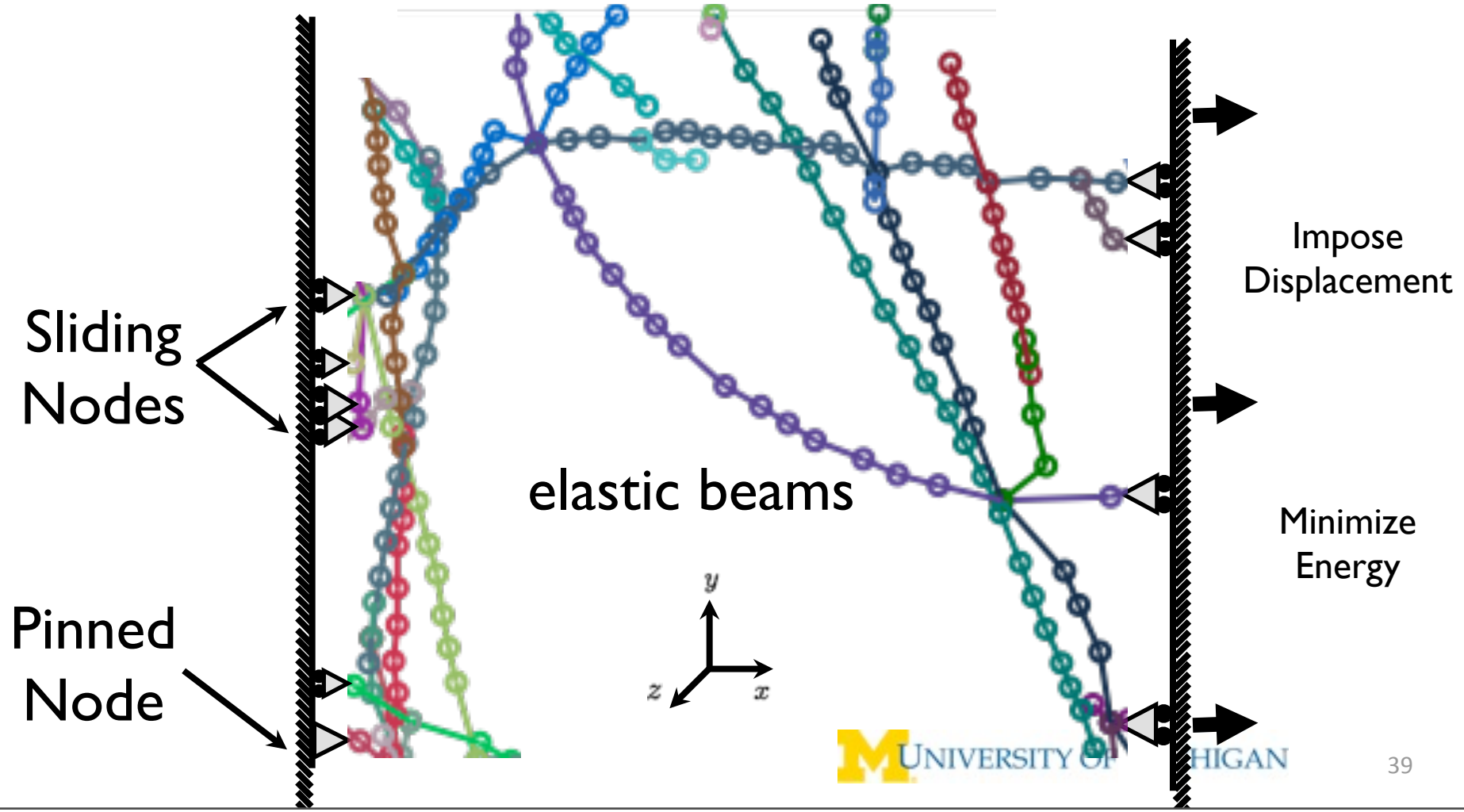

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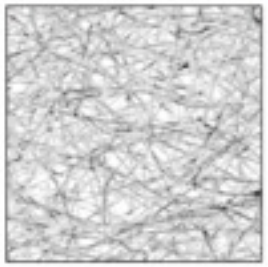
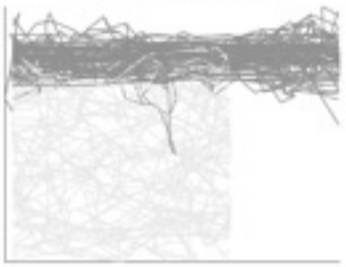
Results of modeling

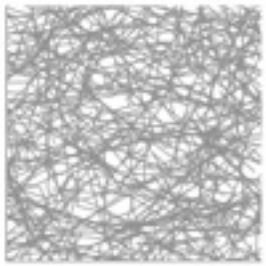
a) Max. Intensity Proj.



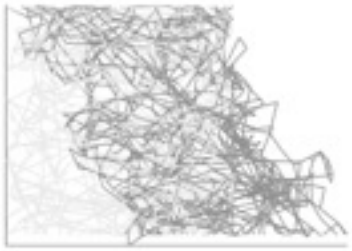
d) 50% Tension



b) FIRE skeletonization

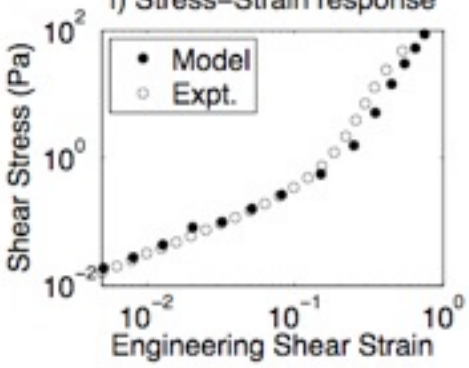


e) 50% Shear



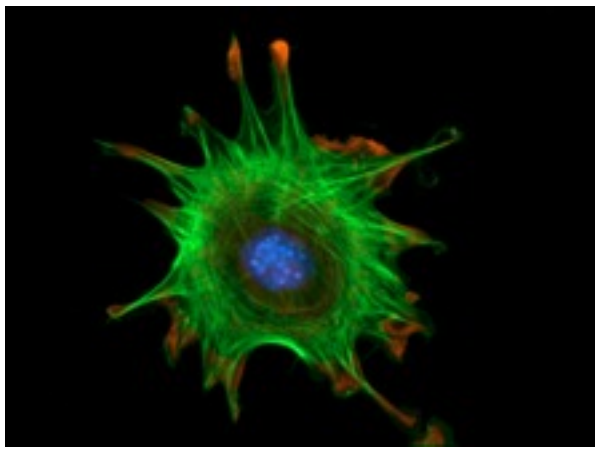
c) reduced network

f) Stress–Strain response

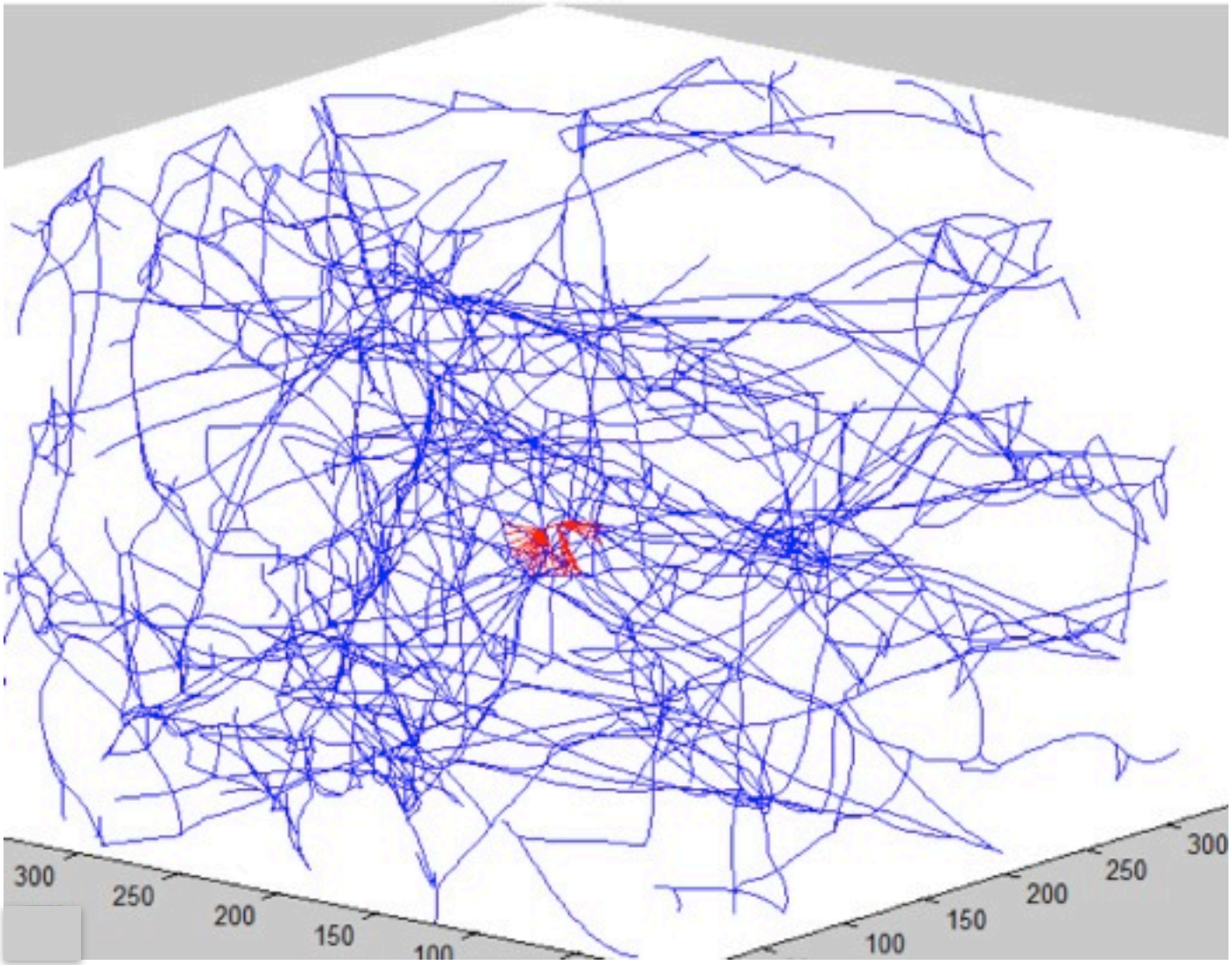


Insert a ‘cell’

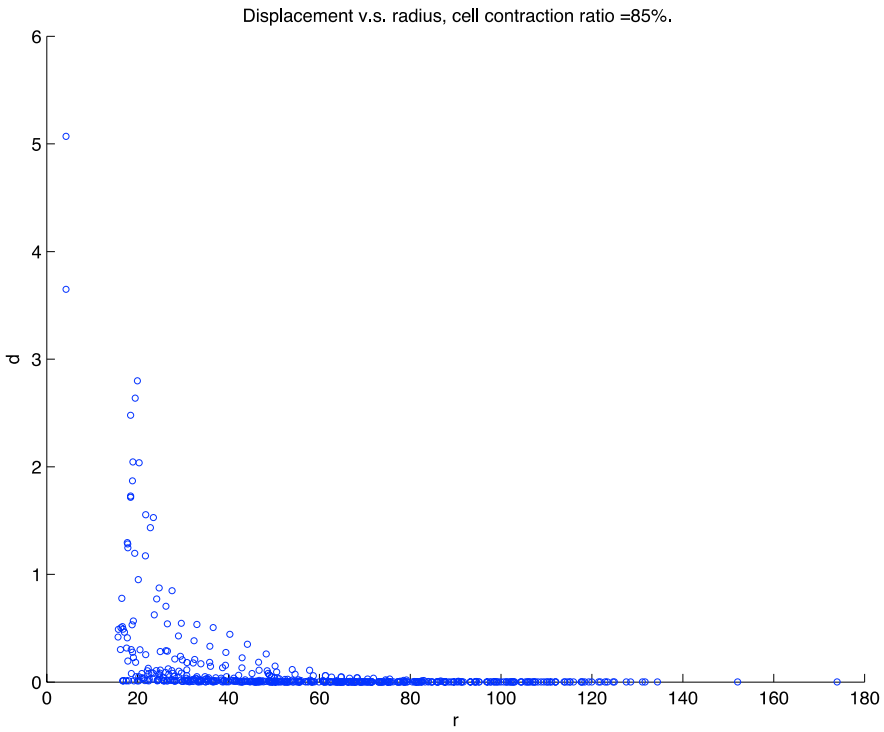
- Use a realistic network extracted from confocal microscopy.
- Model a ‘cell’ as a center with ~5-10 filopods which attach to the network at random places.
- Dynamics: shorten the filopods by 15%, figure out the deformation of the fibers.

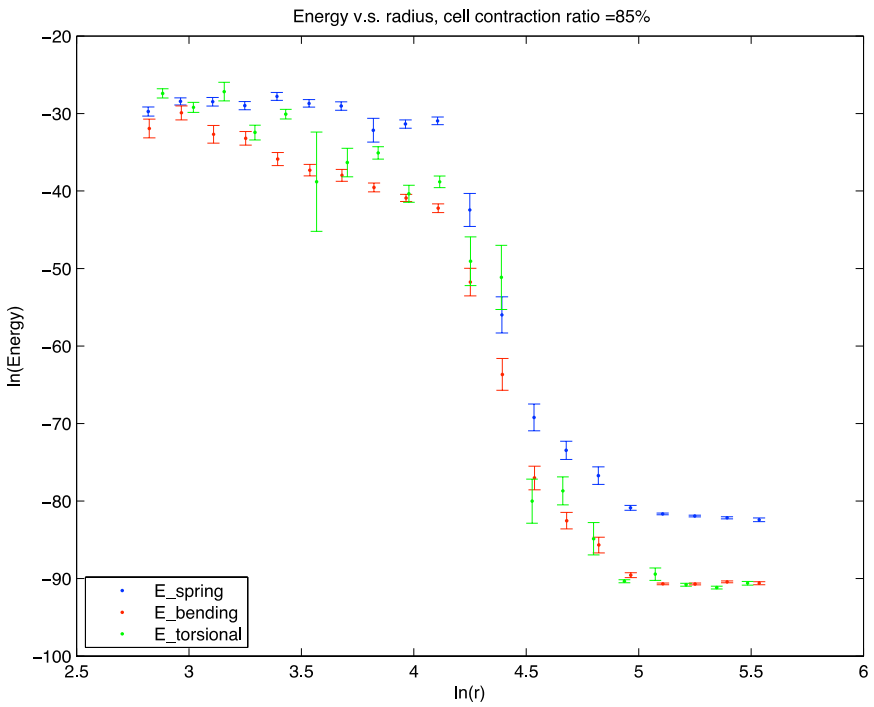


Model fibroblast in network



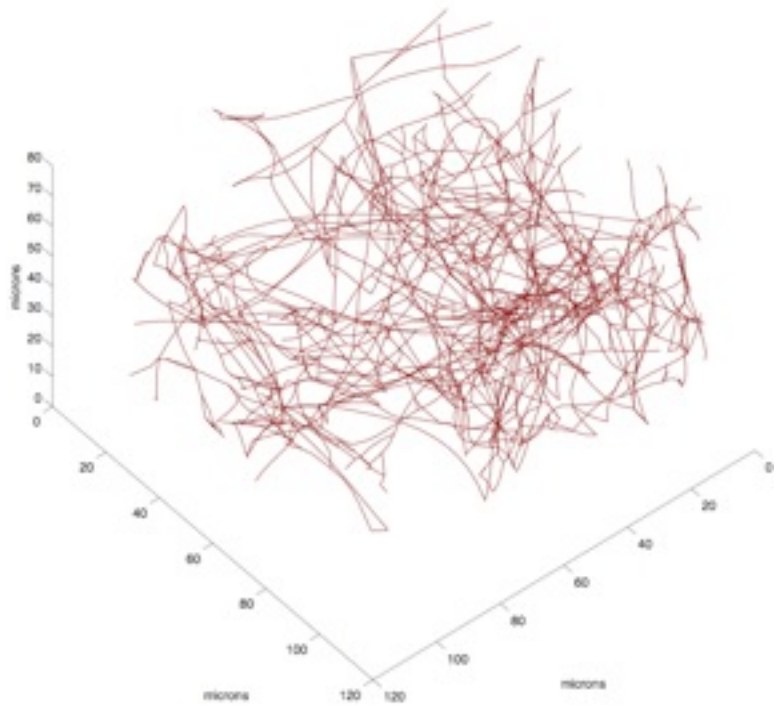
Range of deformation



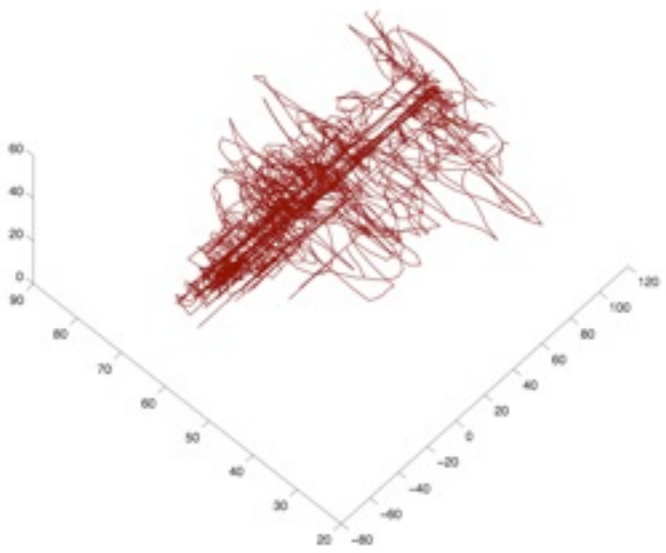


- We computed displacement of nodes and stored elastic energy as a function of radius.
- We find a rapid fall off of all quantities at

Alignment near the tumor spheroid

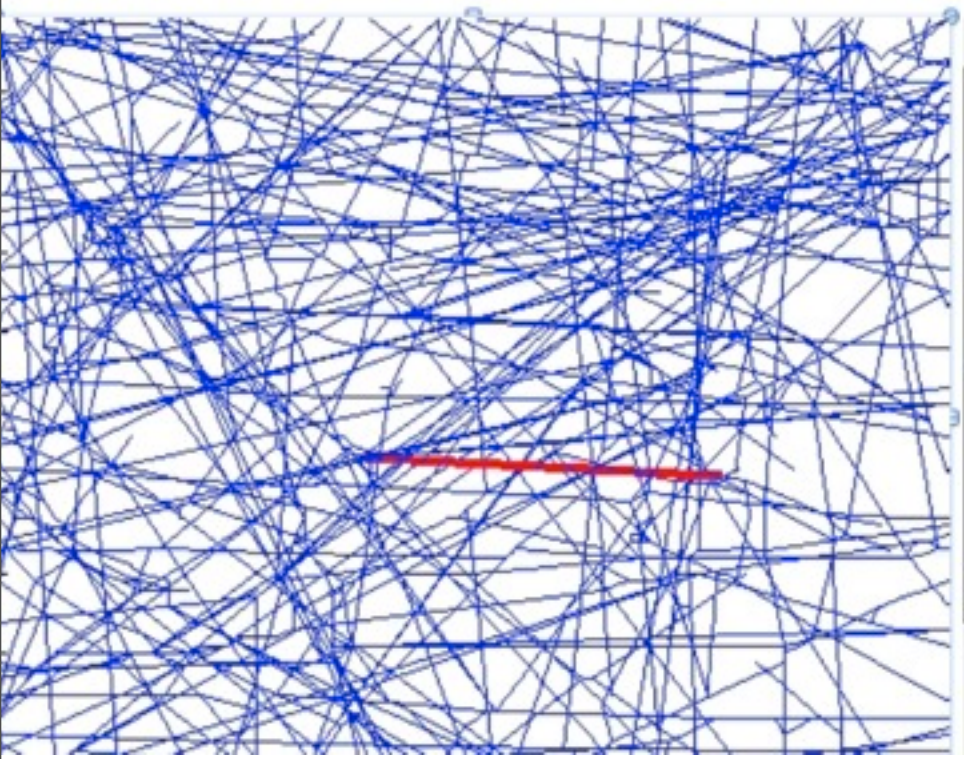


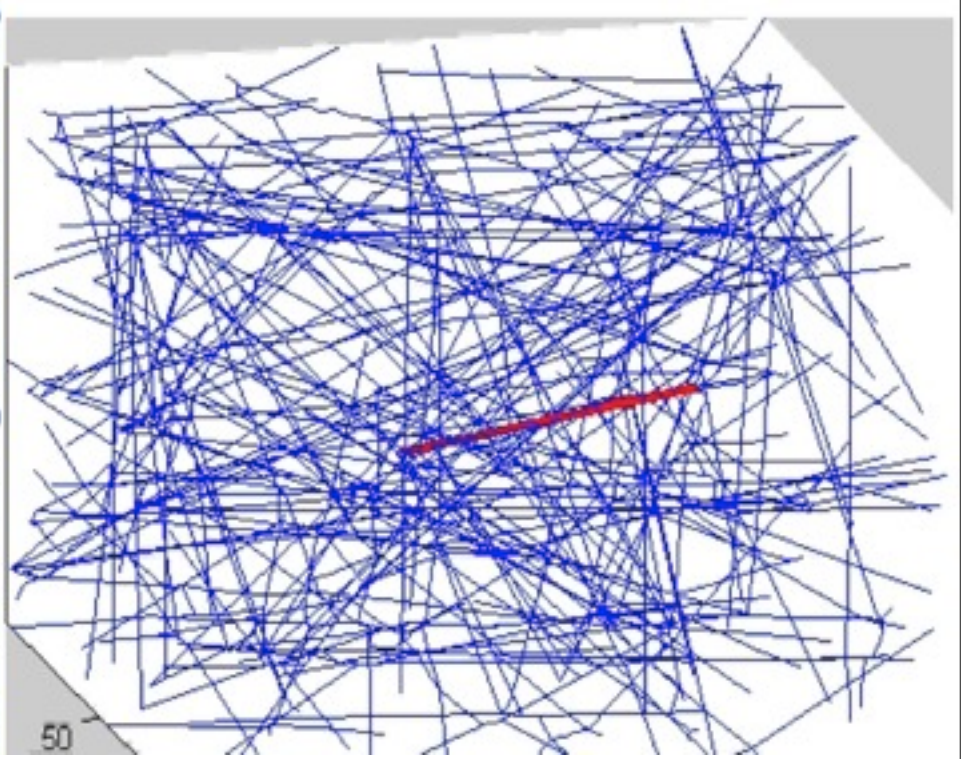
Unstressed network



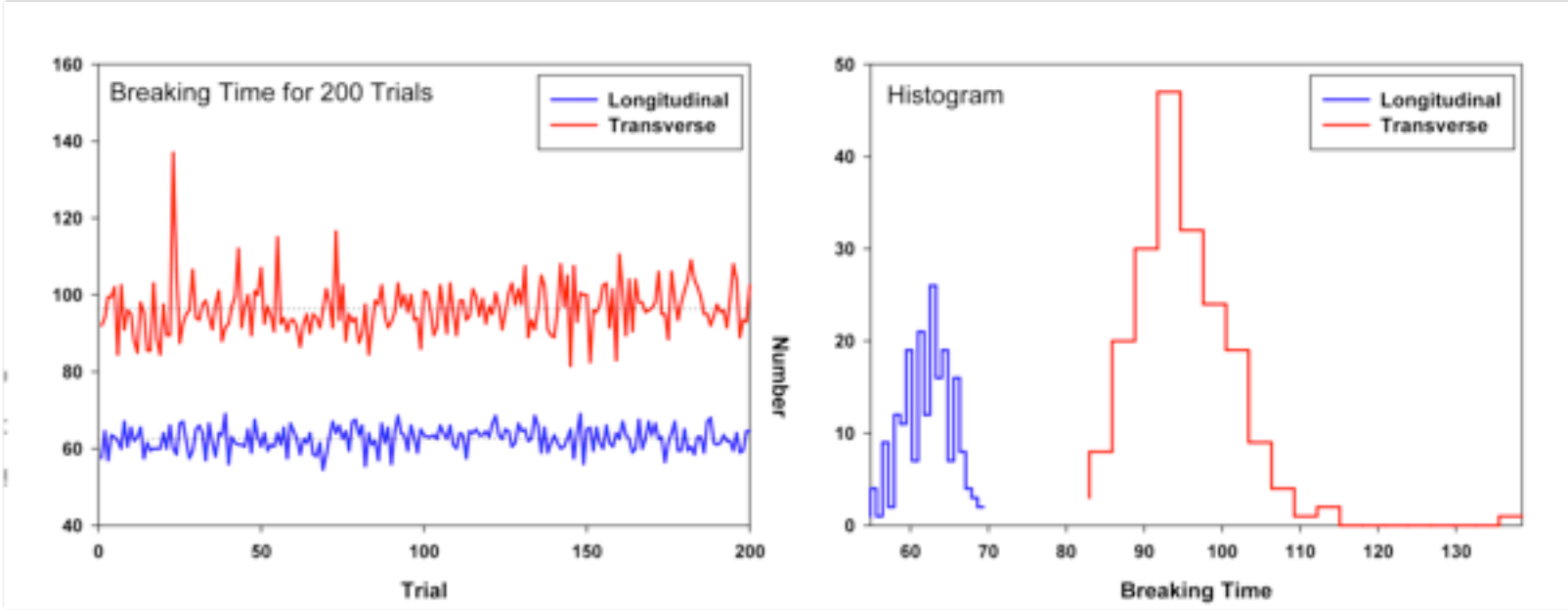
Stressed by plane of cells

Model cells & network





Detachment versus Alignment



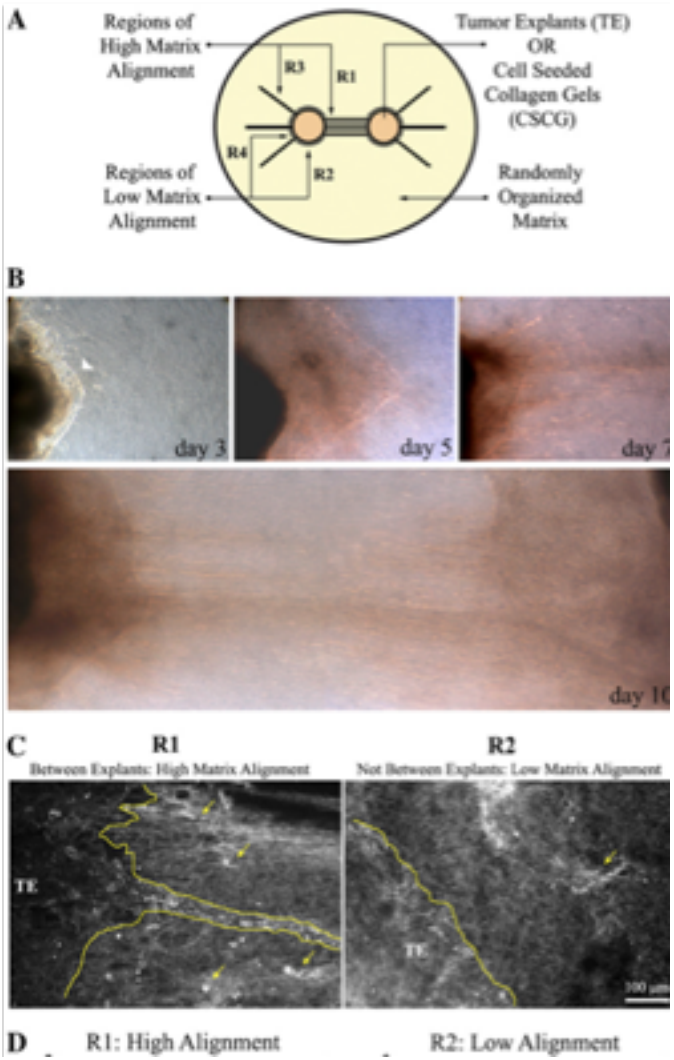
More rapid detachment, faster cell motion, along fibers compared to across them.

This is the beginning of a mechanical model for contact guidance.

Alignment in vivo, breast cancer

- A group looked at alignment of the stroma near breast tumors.
  - P. P. Provenzano, D. R. Inman, K. W. Eliceiri, S. M. Trier, and P. J. Keely, *Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization*, Biophys J, **95**, 5374 (2008).
- They propose alignment (measured by second harmonic generation on biopsy specimens) as a prognostic signature for invasiveness.
  - M. W. Conklin, J. C. Eickhoff, K. M. Riching, C. A. Pehlke, K. W. Eliceiri, P. P. Provenzano, A. Friedl, and P. J. Keely, *Aligned Collagen Is a Prognostic Signature for Survival in Human Breast Carcinoma*, The American Journal of Pathology, **178**, 1221 (2011).

Breast cancer experiment



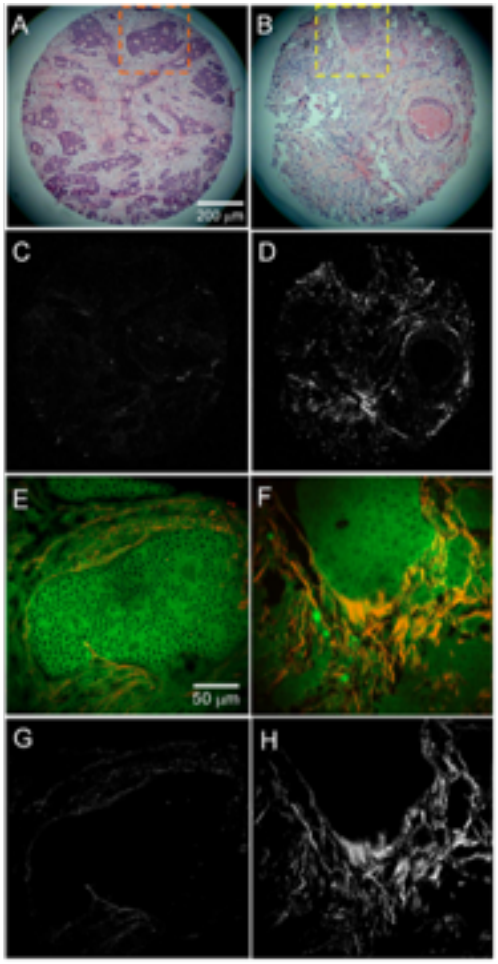


Figure 1. Second harmonic generation imaging of human breast cancer biopsies. **A** and **B**: Two representative examples of H&E-stained slices that demonstrated varying histopathological findings and tumor grade. **C** and **D**: The corresponding second harmonic generation (SHG) images illustrate the complexity and variability of collagen localization in these samples. **E–H**: **Insets** in **A** and **B** show that endogenous fluorescence intensity from cells and stroma was preserved in histopathology slides where the corresponding SHG image can either be overlaid with the fluorescence image (**E**, **F**) or examined by itself (**G**, **H**) to visualize the relationship of collagen to cells. Scale bars (**A–D**) = 200 μm, and (**E–H**) = 50 μm.

Recurrent tumors

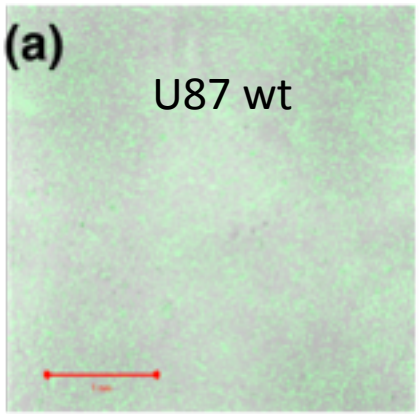
- Spontaneous clustering of glioma cells as a trigger for recurrent tumor formation.
  - With Evgeniy Khain, Michael Khasin, in prep.
- Proposal for the formation of multifocal glioblastoma tumors: *clustering* of invasive cells outside of a primary tumor could make a favorable microenvironment for a recurrent tumor.
- The spontaneous clustering is treated using the methods of large deviations theory from statistical physics.

Phenotypic switch

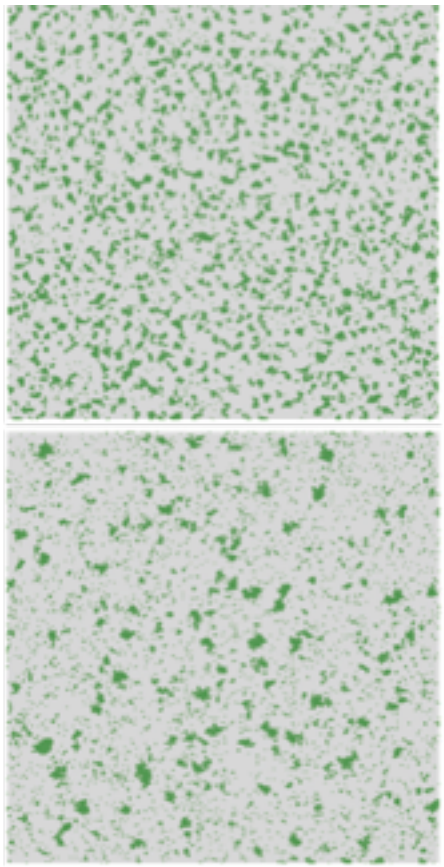
- In order to form new (recurrent) tumors, invasive cells need to stop migrating, start proliferating again.
- We propose that cell signaling by neighbors via adhesive contacts, the production of growth factors, or the presence of matrix proteins produced by neighbors could cause the switch.
- Indirect evidence via an *in vitro* experiment.

Cell clustering

- Experiment on clustering:
  - Plated two cell lines on substrate, waited for cluster formation
  - Analyzed cluster distribution using a simple model for cell diffusion, proliferation, adhesion.
  - Need enhanced proliferation on clusters to give correct distribution.
  - Khain, Schneider, Nowicki, Chiocca, Lawler, and Sander, *Pattern formation of glioma cells: Effects of adhesion*, EPL (Europhysics Letters), **88**, 28006 (2009).



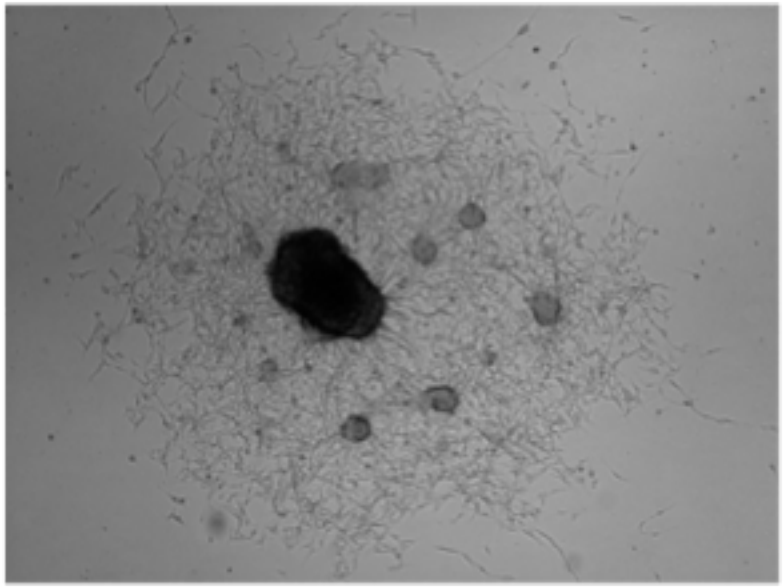
Snapshots of the system for the two cell lines five days after the beginning of the experiment (see footnote [1]). ΔEGFR cells form clusters (b), while WT cells are homogeneously distributed over the system (a). The typical cell diameter is 10 μm, so each cluster in (b) contains hundreds of cells.



Simulation: top, small proliferation, Bottom, large proliferation

Clustering and cadherins

- Spontaneous formation of clusters depends on adhesion exceeding a certain critical level.
- However, cell lines (e.g. U87wt) do not exceed that level, but still form recurrent tumors.



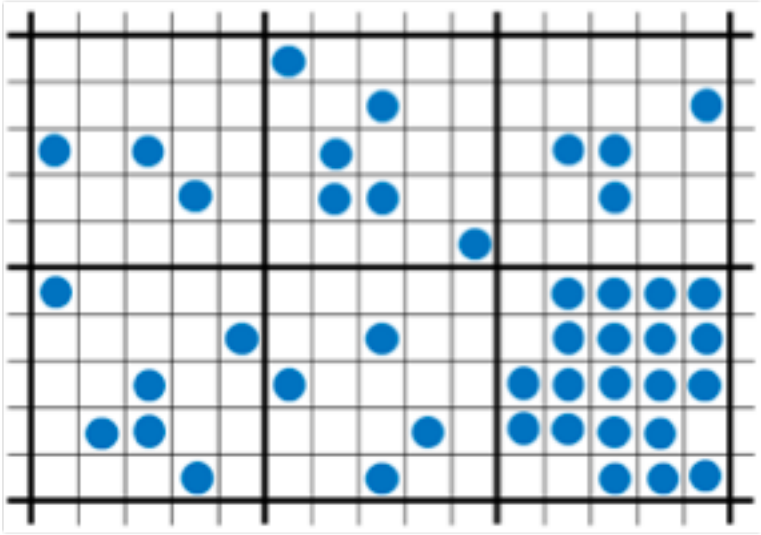
U87 wt with N-cadherins upregulated. (M. Chopp, et al.)

Cell model

- Square two-dimensional lattice; each lattice site can be empty or occupied by one cell.
- A cell is picked at random, and one of the four neighboring sites is also picked at random.
  - If this site is empty, the cell can proliferate there (a new cell is born there) with probability α, or migrate there.
  - $p_{migr} = (1 - \alpha)(1 - q)n$, where $0 < q < 1$ is the adhesion parameter, and n is the number of nearest neighbors.
  - We can show that for $q<0.82$, clusters are not stable.

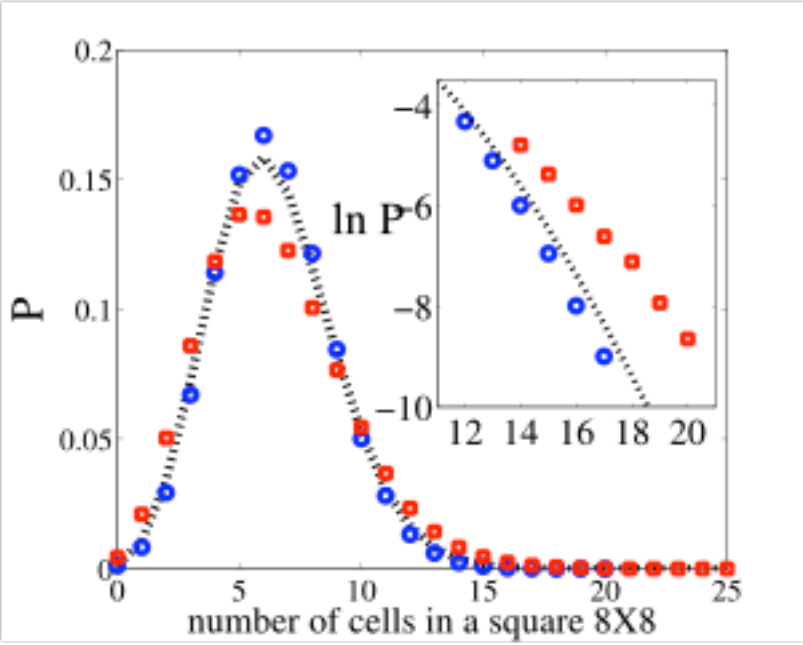
Clustering via fluctuations

- Divide lattice into squares, look for occupancies, m_i.
- If number in a square exceeds a value, say 20, this is a fluctuation large enough to start a new tumor.



Discrete lattice model for cell migration. Lattice sites can be occupied by one cell or be empty. The cell dynamics is described in the text. The average cell density is small, but rare, large density fluctuations can lead to cluster formation, as in the lower right square. We assume that some cells in that cluster become proliferative.

Simulations



Numerical results for the density distribution function, $P(m)$. Here m is a number of cells in a 8×8 square for average area fraction $\bar{\nu} = 0.1$ (no proliferation). Blue circles, $q = 0$, red squares, nonzero (but subcritical) adhesion, $q = 0.6$. Black dotted line, Poisson distribution. Inset: tail region: the probability of large cluster formation increases exponentially with the adhesion parameter q.

Master equation

- Master equation for P(m):

$$\dot{P}(m) = \sum_{r=\pm 1} W(m-r;r)P(m-r) - W(m;r)P(m)$$

$\lambda(m) \equiv W(m;1)$ rate to add a cell,

$\beta(m) \equiv W(m;-1)$ rate to lose a cell.

- Assume different regions independent.
- Get λ, β by an 'equation-free' approach.

Estimate rates

- Define reduced rate of migrating into the square, λ_0:

$$\lambda(m) = \left(1 - \frac{m}{L^2}\right) \lambda_0$$

  - The average area fraction inside the square, m/L^2 is equal to the probability of having an empty site on the boundary, so that a particle have a space to move in.

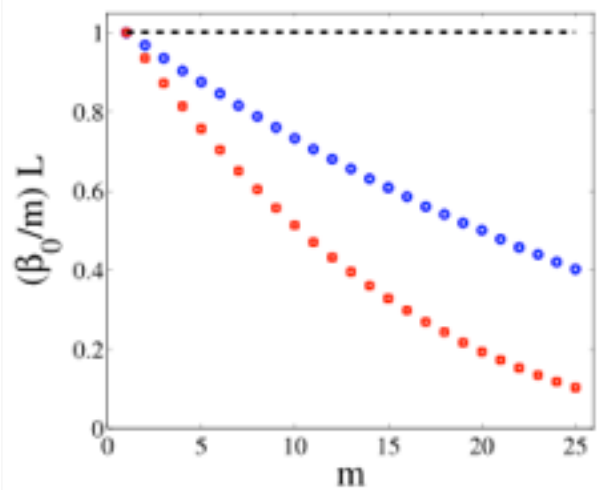
- Similarly,

$$\beta(m) = \left(1 - \frac{\bar{m}}{L^2}\right) \beta_0$$

--

Measure rates

- Sample over many configurations, measure rate in, rate out.
- Note that this is much easier than doing a full simulation.
- It is not clear that this will work, i.e., m may not be the only relevant variable.



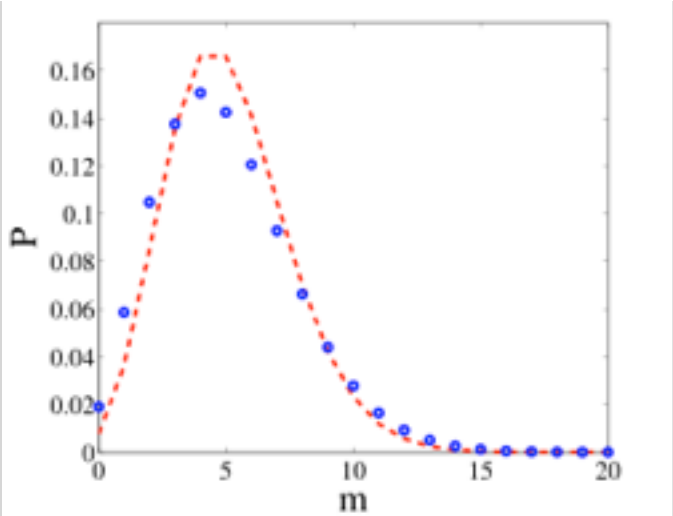
Scaled rate of migration out of square β_0 as a function of number of particles in the square for $L = 5$ and zero proliferation. The black dashed line, zero adhesion, $q = 0$, blue circles, $q = 0.3$, red squares, $q = 0.6$.

Solution of master equation

- We get the stationary probability distribution from detailed balance:

$$\lambda(m-1)P(m-1) = \beta(m)P(m) \qquad \Sigma_m P(m) = 1.$$

- Somewhat surprisingly, the method works very well.



Density distribution function $P(m)$, where m is a number of cells in a square L by L ($L = 5$) for the average area fraction $\bar{\nu} = 0.2$ and zero proliferation. Blue circles correspond to nonzero (but subcritical) adhesion, $q = 0.6$; red dashed line is computed from Eq. (2) using the measured rates for the same adhesion, $q = 0.6$.

Estimates

- See if this is reasonable:
  - For average density 0.1, q=0.6, P ~ 10^{-6}.
  - Need to wait approximately

    $T = 106 \times t_{diff} = 5 \times 10^6$ minutes,

    to get a cluster of 20 particles in a square 100 μm on a side (i.e. 5 cells wide).
  - In a two-dimensional system of size 3 x 3 mm we will need to wait

    $T = 5 \times 10^6 / 900$ minutes ~ 4 days.
  - But q = 0.3, T ~ 38 months

Results

- We need to know lots of things from experiment to make this into more than speculation:
  - What is the minimum size (if there is one) for a recurrent tumor to flourish?
  - What are the signals that lead migratory cells to start proliferating again?
  - What are the effects of an inhomogeneous environment?

Summary

- We have argued that invasion by tumor cells is accompanied by remodeling, in particular, alignment, of the ECM
  - Cells align the ECM
  - Aligned ECM provides 'highways' for cell motion.
- Alignment of a biopolymer has an unexpected feature, *stress confinement.*
- We give a speculation about the formation of recurrent tumors by spontaneous clustering of invading cells.