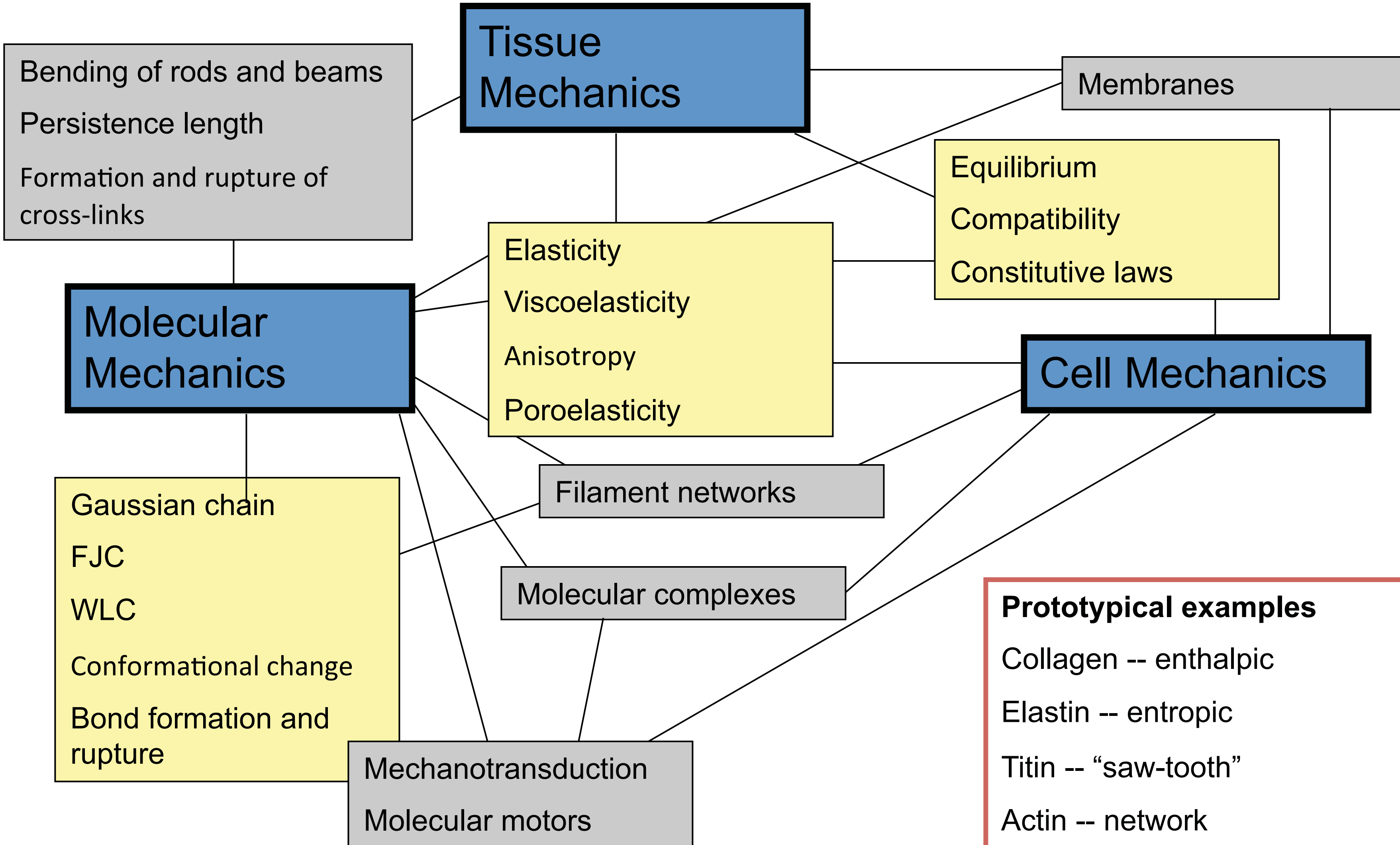
MCT Biomechanics -- Roadmap



Prototypical examples

Collagen -- enthalpic

Elastin -- entropic

Titin -- “saw-tooth”

Actin -- network

Talin -- mechanotransduction

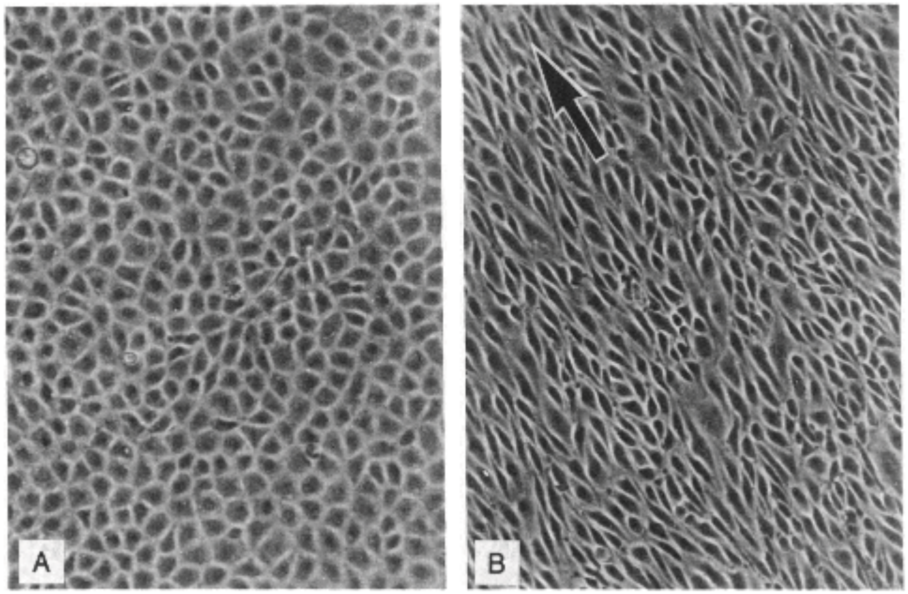
Kinesin -- molecular motor

Biomechanics in the news: Mechanism of the microtubule motor dynein

The cover of Science removed due to copyright restrictions.

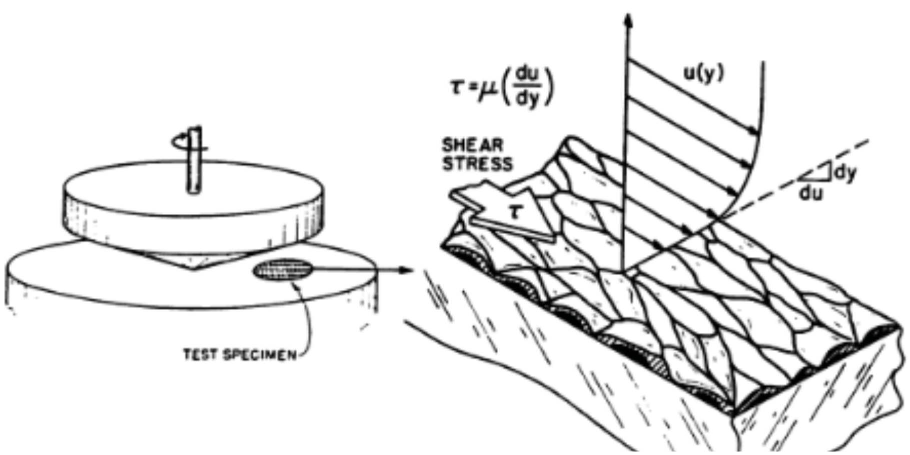
Image from *The Inner Life of the Cell* removed due to copyright restrictions.

The role of shear stress in cell alignment and atherosclerosis



Source: Davies, Peter F., et al. "Turbulent Fluid Shear Stress Induces Vascular Endothelial Cell Turnover in Vitro." *Proceedings of the National Academy of Sciences* 83, no. 7 (1986): 2114-7.

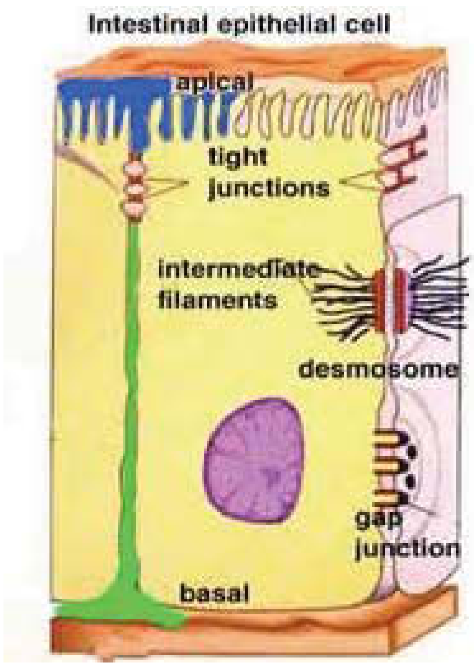
Davies, Gimbrone and Dewey, PNAS, 1986

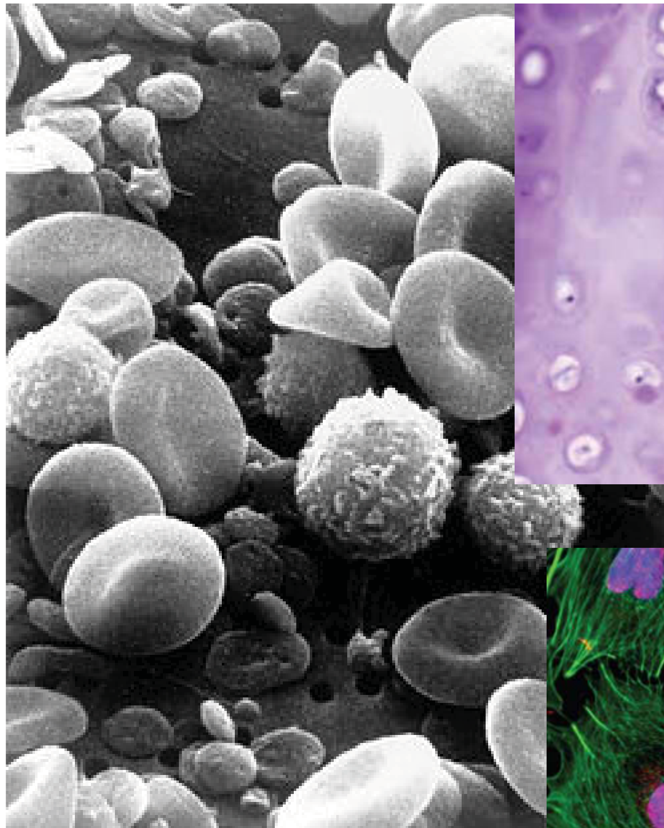


Many different cell types with different structure

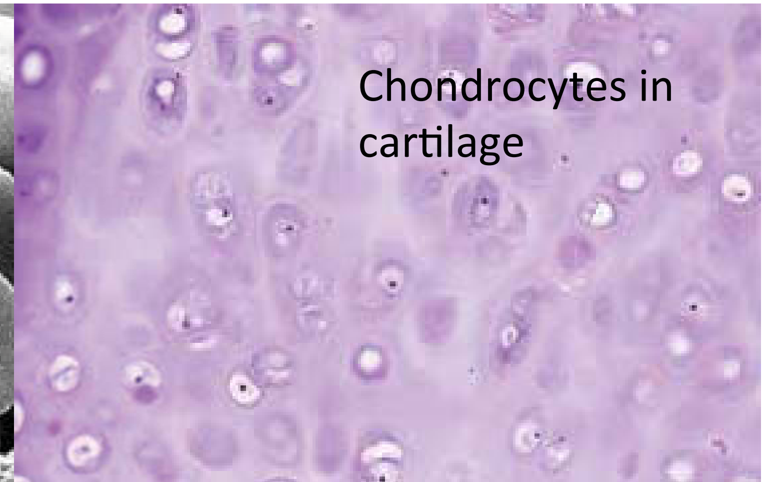
Red Blood Cells

Illustration courtesy of Blausen.com staff. "Blausen Gallery 2014". Wikiversity Journal of Medicine. DOI:10.15347/wjm/2014.010. ISSN 20018762. CC license BY.

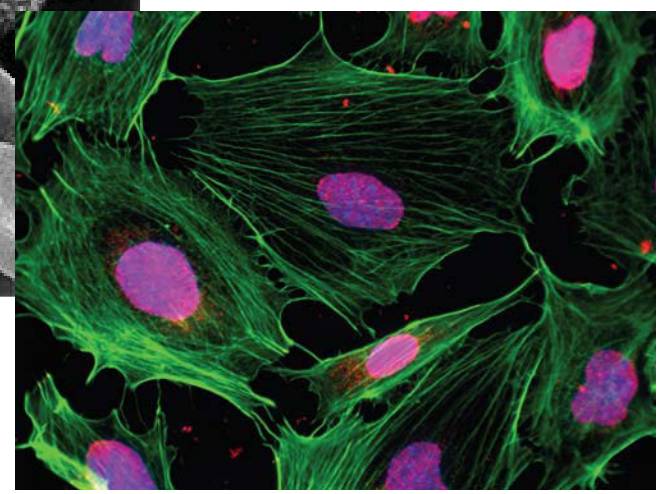




RBCs, leucocytes, and platelets



Chondrocytes in cartilage



Fibroblasts

Cell mechanics: Structure

Transmission electron micrograph showing pituitary cell removed due to copyright restrictions.

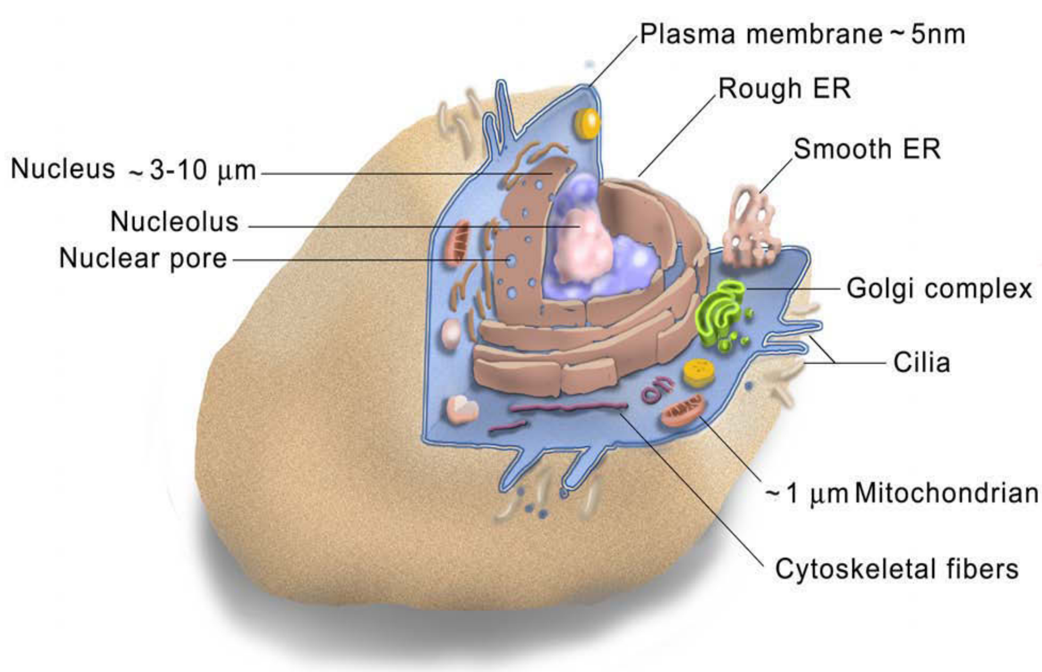


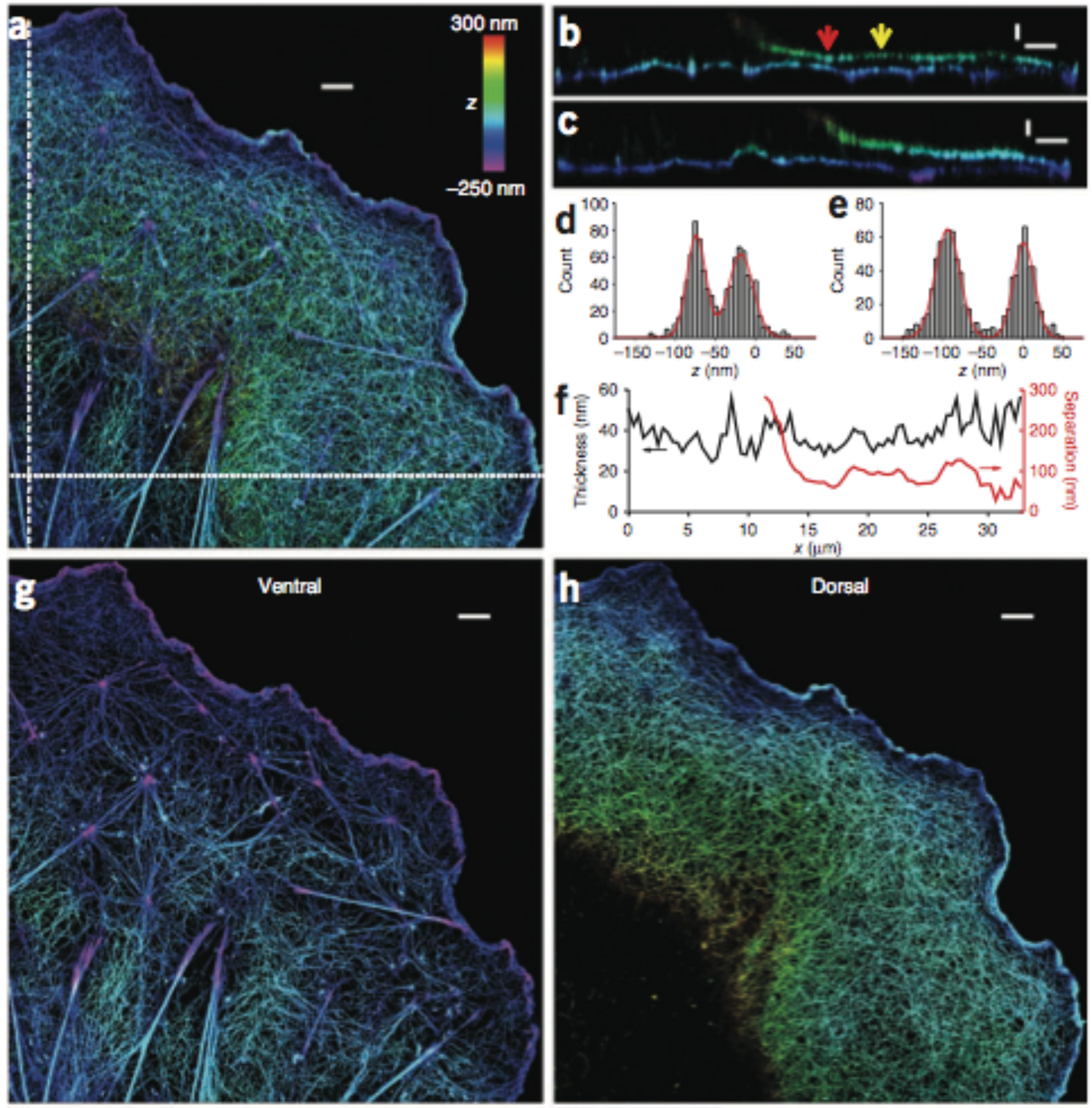
Figure by MIT OpenCourseWare.

H. Lodish et al. (2004)

Super-resolution imaging of cytoskeletal structures

Figure 3 | Sheet-like cell protrusion comprises two layers of actin networks with distinct structures. (**a**) Dual-objective STORM image of actin in a BSC-1 cell. The *z* positions are color coded (color bar). (**b**,**c**) Vertical cross sections (each 500-nm wide in *x* or *y*) of cell in **a** along dotted and dashed lines, respectively. When far from cell edge, *z* position of dorsal layer increases quickly and falls out of imaging range. (**d**,**e**) The *z* profiles for two points along vertical section (red and yellow arrows in **b**, respectively). Each histogram is fit to two Gaussians (red curves), yielding apparent thickness of ventral and dorsal layers and peak separation between the two layers. (**f**) Quantification of apparent thickness averaged over two layers and dorsal-ventral separation obtained from *x*–*z* cross-section profile in **b**. (**g**,**h**) Ventral and dorsal actin layers of cell in **a**. (**i**,**j**) Ventral and dorsal actin layers of a COS-7 cell treated with blebbistatin. (**k**,**l**) Vertical cross sections (each 500-nm wide in *x* or *y*) of cell along dotted and dashed lines, respectively. (**m**) Actin density of ventral and dorsal layers along yellow box in **i**,**j**, measured by localization density. Scale bars, 2 μm (**a**,**g**–**j**); 100 nm for *z* and 2 μm for *x* and *y* (**b**,**c**,**k**,**l**).

We observed two vertically separated actin layers in the sheet-like cell protrusion despite its small thickness (**Fig. 3a–c**). The apparent thickness of each layer was



Stochastic optical reconstruction microscopy (STORM)

Source: Xu, Ke, et al. "Dual-objective STORM Reveals Three-dimensional Filament Organization in the Actin Cytoskeleton." *Nature Methods* 9, no. 2 (2012): 185-8.

http://www.nature.com/nmeth/video/moy2008/index.html

Actin/spectrin structure in axons

Figure 4 removed due to copyright restrictions.
Source: Xu, Ke, et al. "Actin, Spectrin, and Associated Proteins form a Periodic Cytoskeletal Structure in Axons." *Science* 339, no. 6118 (2013): 452-6.

Stochastic optical reconstruction microscopy (STORM)

Xu, Science, 2013

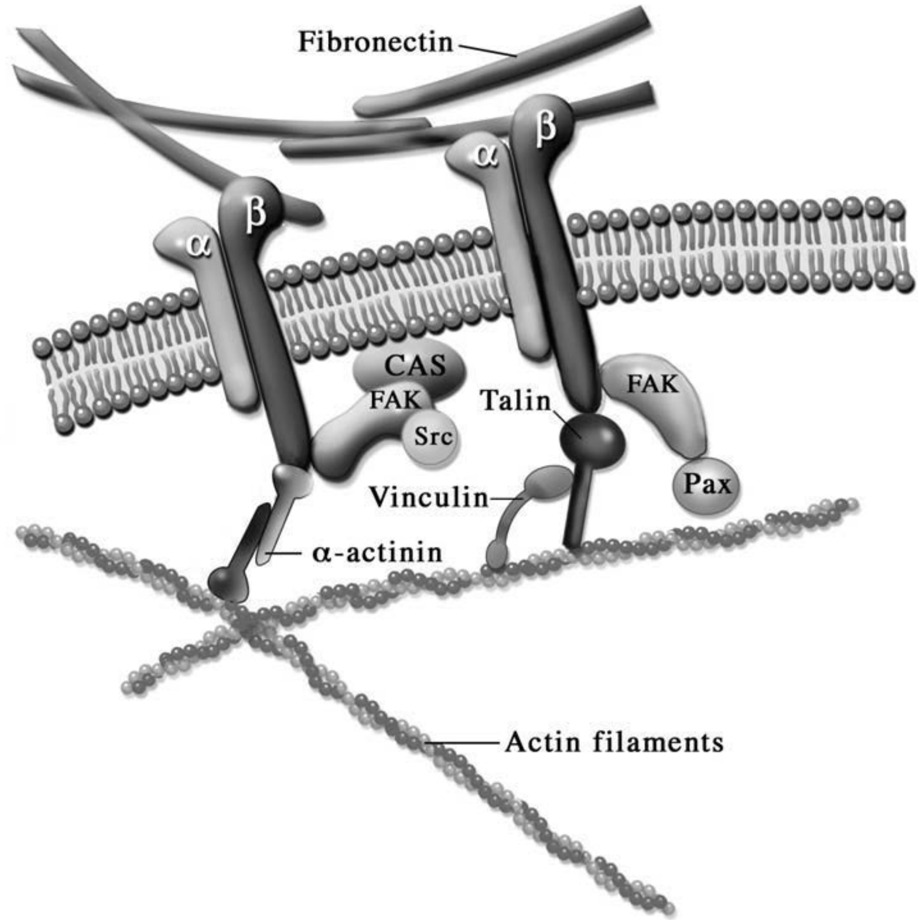
Force Transmission Pathways

ECM

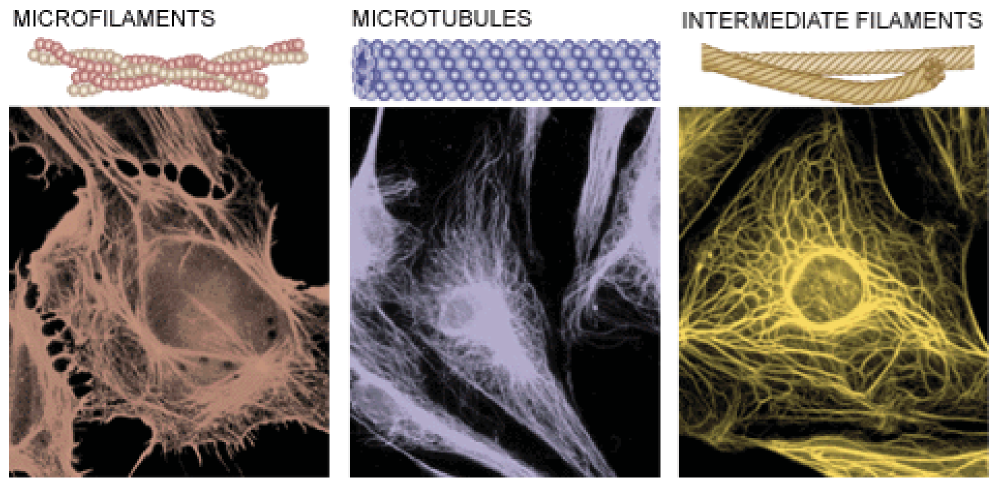
Transmembrane proteins

Integrin-CSK linking proteins

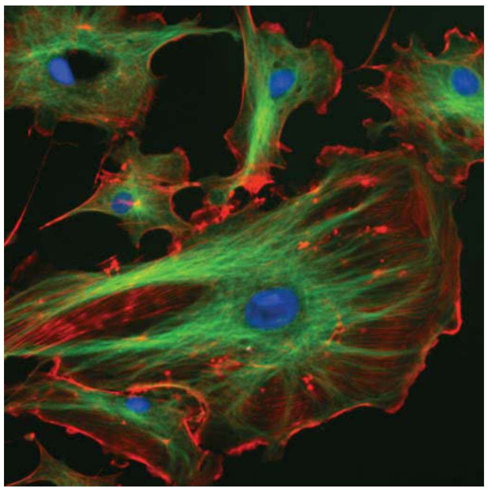
Actin and cross-linking proteins



Primary structural filaments of the cytoskeleton



Source: Ingber, Donald E. "The Architecture of Life." *Scientific American* 278, no. 1 (1998): 48-57.



Actin (phalloidan)
microtubules (FITC),
nuclei (DAPI)

Ingber, Scientific American

	Diameter (nm)	Persistence length (μm)	Bending stiffness (Nm^2)	Young's modulus (Pa)
Actin filament	6-8	15	$7x10^{-26}$	$1.3\text{-}2.5x10^{9}$
Microtubule	25	6000	$2.6x10^{-23}$	$1.9x10^{9}$
Intermediate filament	10	1-3	$4\text{-}12x10^{-27}$	$2\text{-}5x10^{6}$

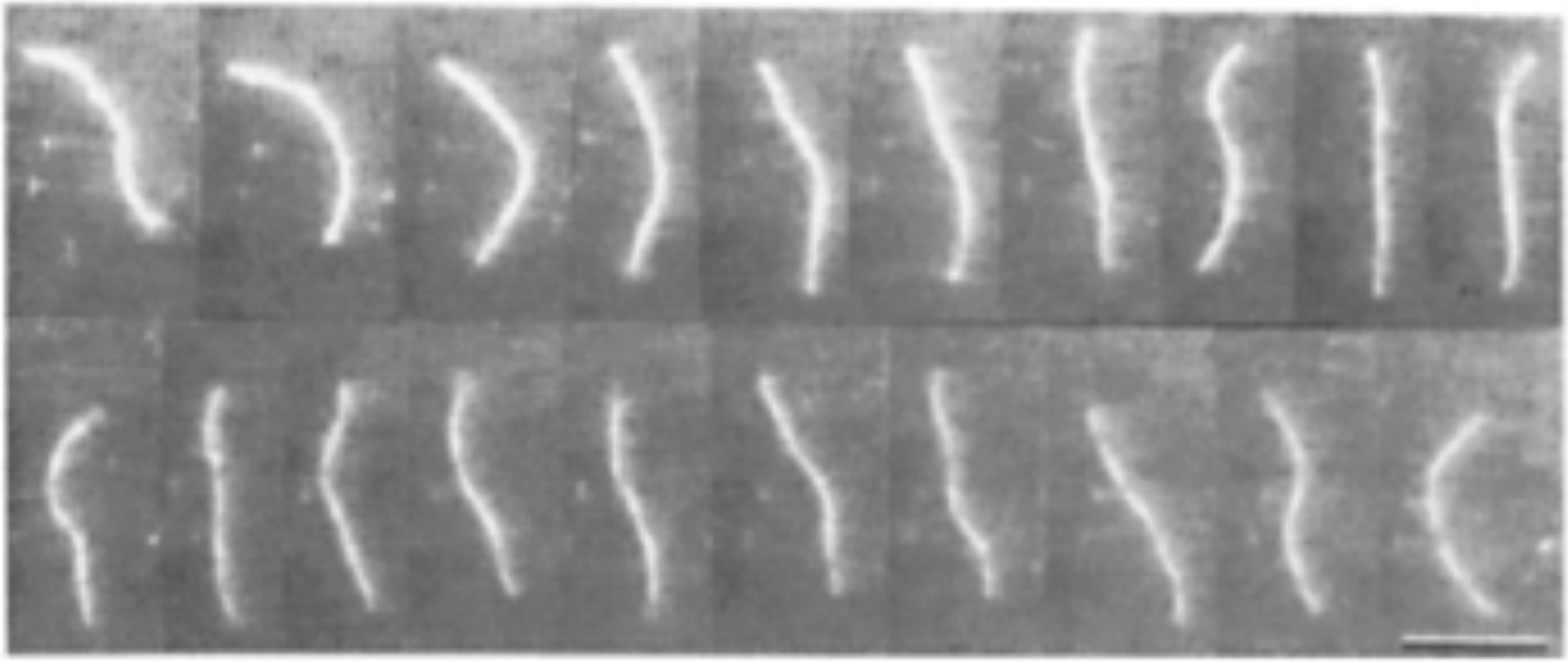
$$l_p = \frac{K_b}{k_B T} \qquad K_b = EI = E\frac{\pi}{4}a^4$$

Mechanical factors in integrin adhesion

Figures 1 & 2 removed due to copyright restrictions.
Source: Roca-Cusachs, Pere, et al. "Finding the Weakest Link–exploring Integrin-mediated Mechanical Molecular Pathways." *Journal of Cell Science* 125, no. 13 (2012): 3025-038.

Image from *The Inner Life of the Cell* removed due to copyright restrictions.

Fluctuations of an actin filament



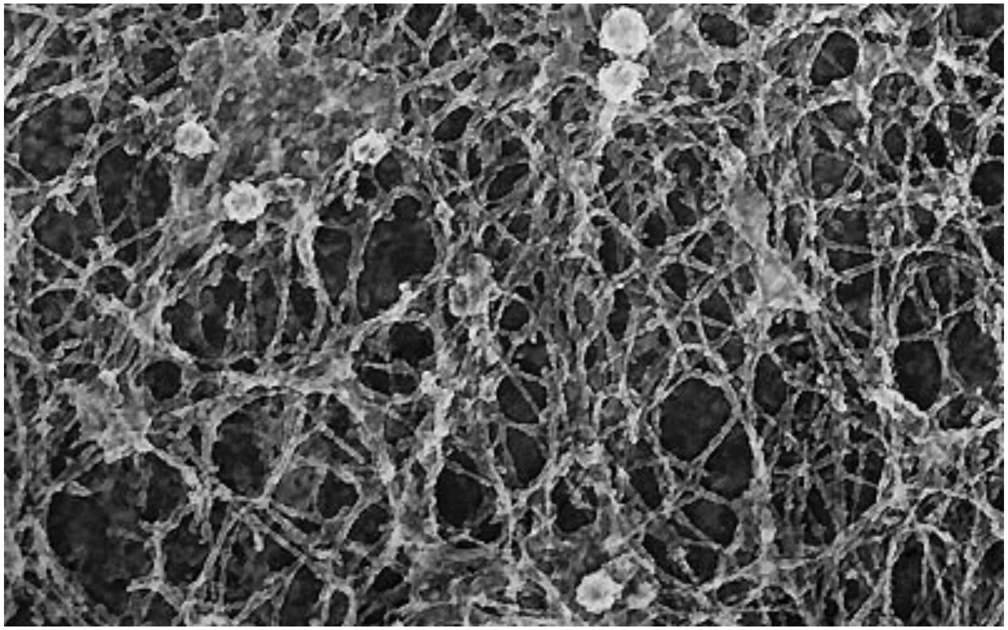
scale bar = 10 microns

Source: Gittes, Frederick, et al. "Flexural Rigidity of Microtubules and Actin Filaments Measured from Thermal Fluctuations in Shape." *The Journal of Cell Biology* 120, no. 4 (1993): 923-34.

Flexural Rigidity of Microtubules and Actin Filaments Measured from Thermal Fluctuations in Shape

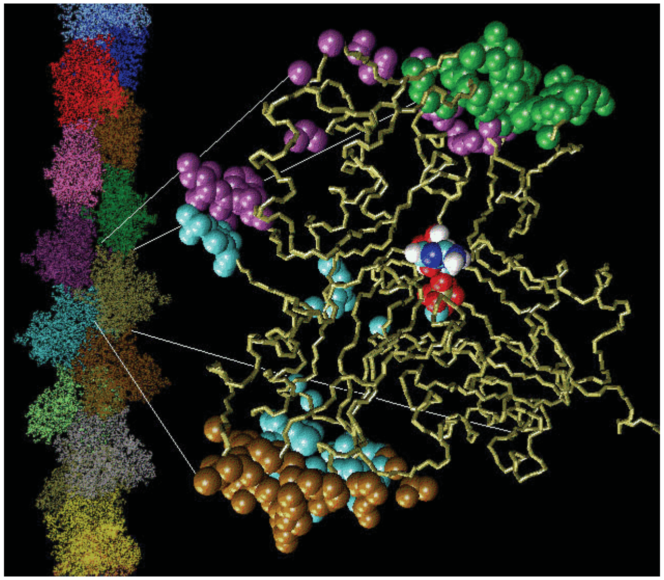
Frederick Gittes, Brian Mickey, Jilda Nettleton, and Jonathon Howard

Actin microfilaments



Courtesy of John Hartwig. Used with permission.

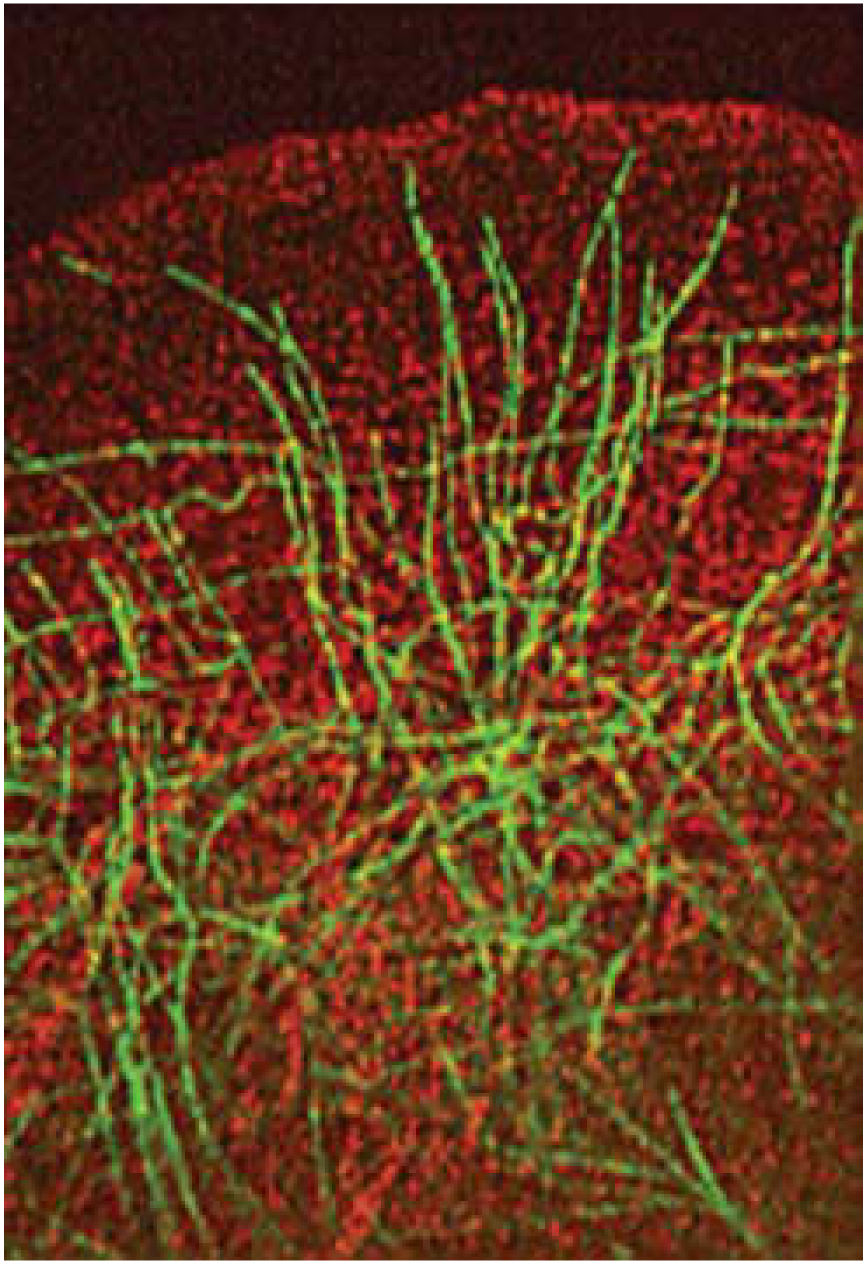
Image of G-actin monomers polymerizing into F-actin filaments removed due to copyright restrictions.



Structure of actin. Image courtesy of Dr. Willy Wriggers. Used with permission.

Cytoskeletal structure

Figure 1 removed due to copyright restrictions.
Source: Hirokawa, Nobutaka. "Kinesin and Dynein Superfamily Proteins and the Mechanism of Organelle Transport." *Science* 279, no. 5350 (1998): 519-26.

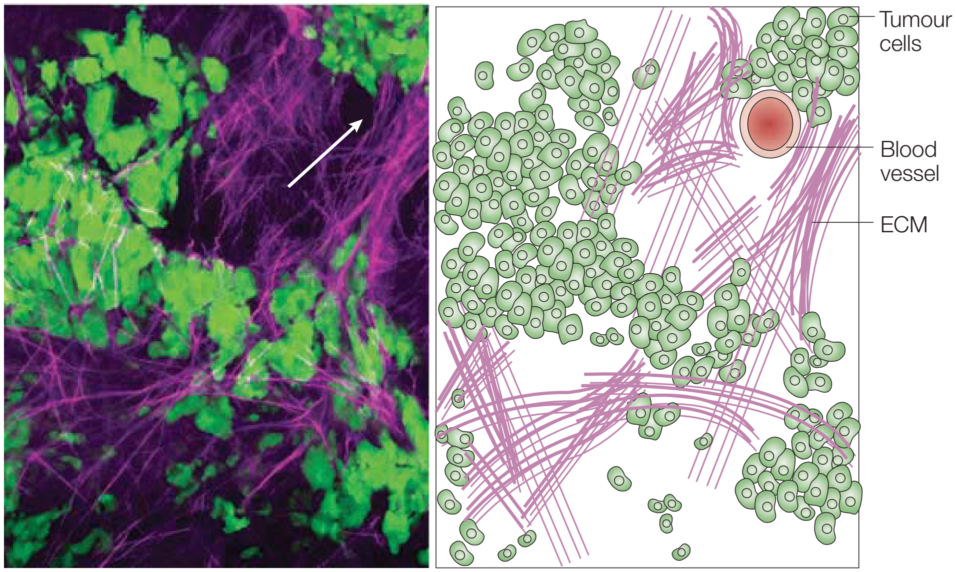


Actin filament and microtubule dynamics – FSM (fluorescent speckle microoscopy)

Salmon, et al., J Cell Biol, 158:31-37, 2002

Source: Salmon, Wendy C., et al. "Dual-wavelength Fluorescent Speckle Microscopy Reveals Coupling of Microtubule and Actin Movements in Migrating Cells." *The Journal of Cell Biology* 158, no. 1 (2002): 31-37.

Tumor microenvironment governs cell migration



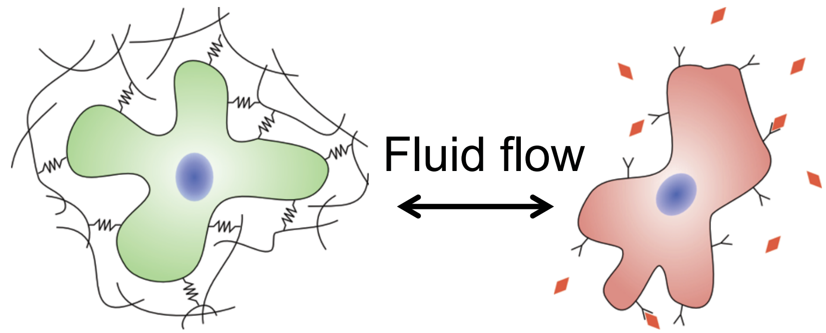
Source: Condeelis, John, and Jeffrey E. Segall. "Intravital Imaging of Cell Movement in Tumours." *Nature Reviews Cancer* 3, no. 12 (2003): 921-30.

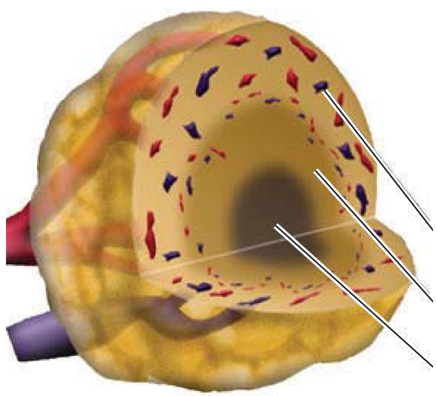
MECHANICAL (20.310)

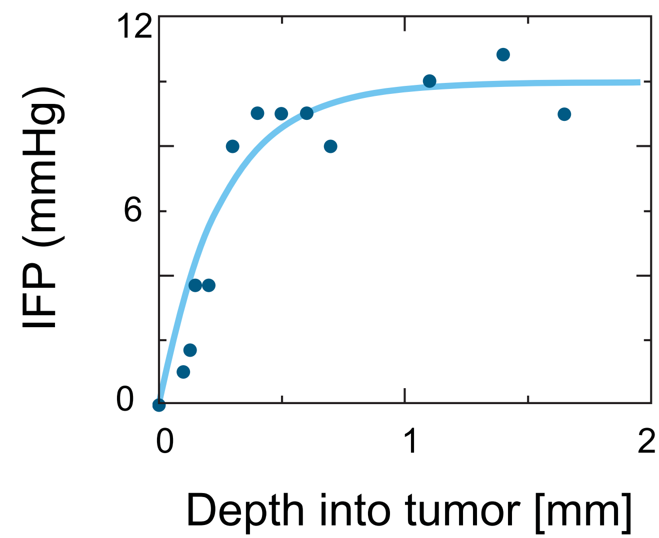
- ECM Topography
- ECM Stiffness
- Force/Pressure

CHEMICAL (20.330)

- Autocrine Signals
- Paracrine Signals
- Oxygen Tension

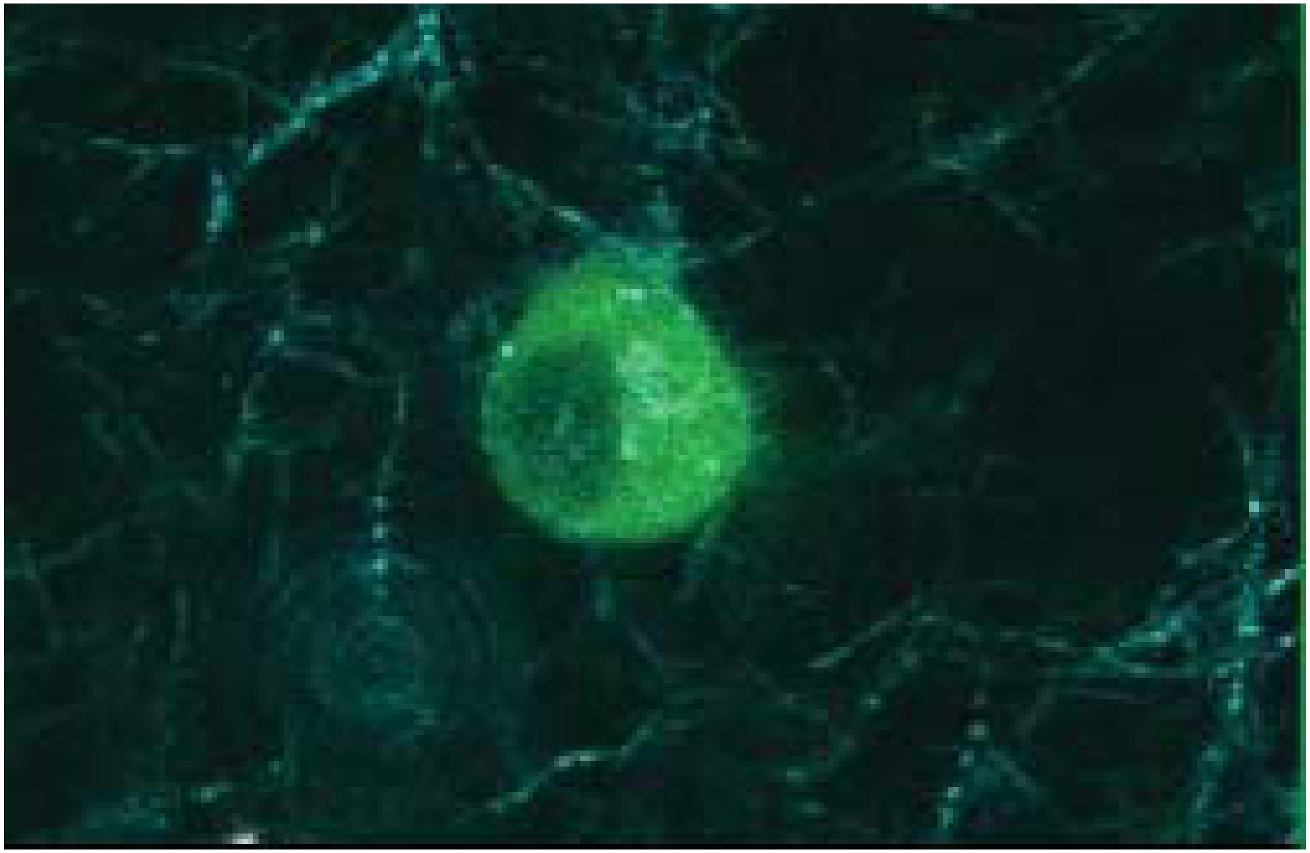




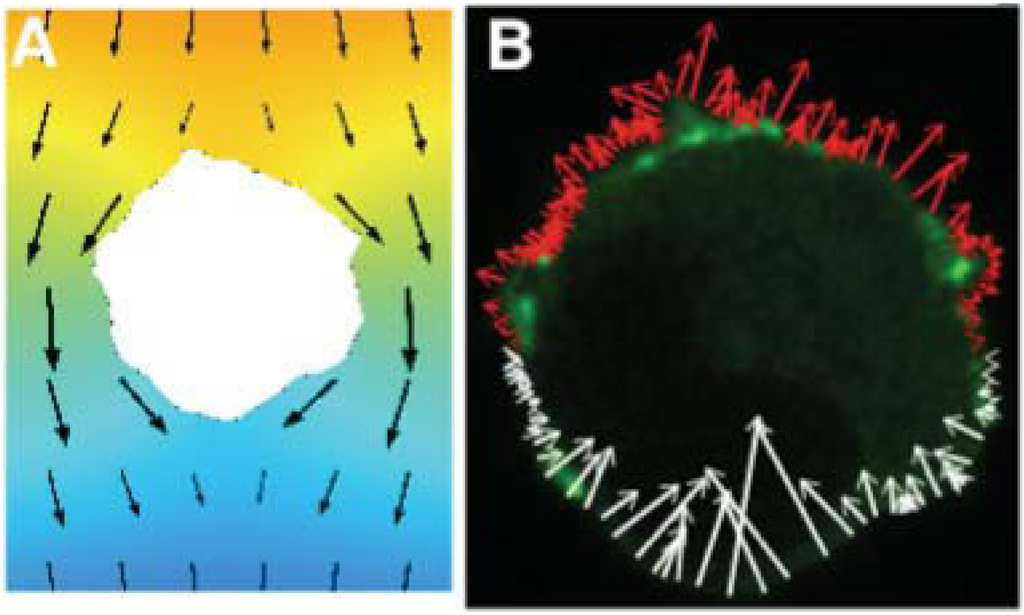


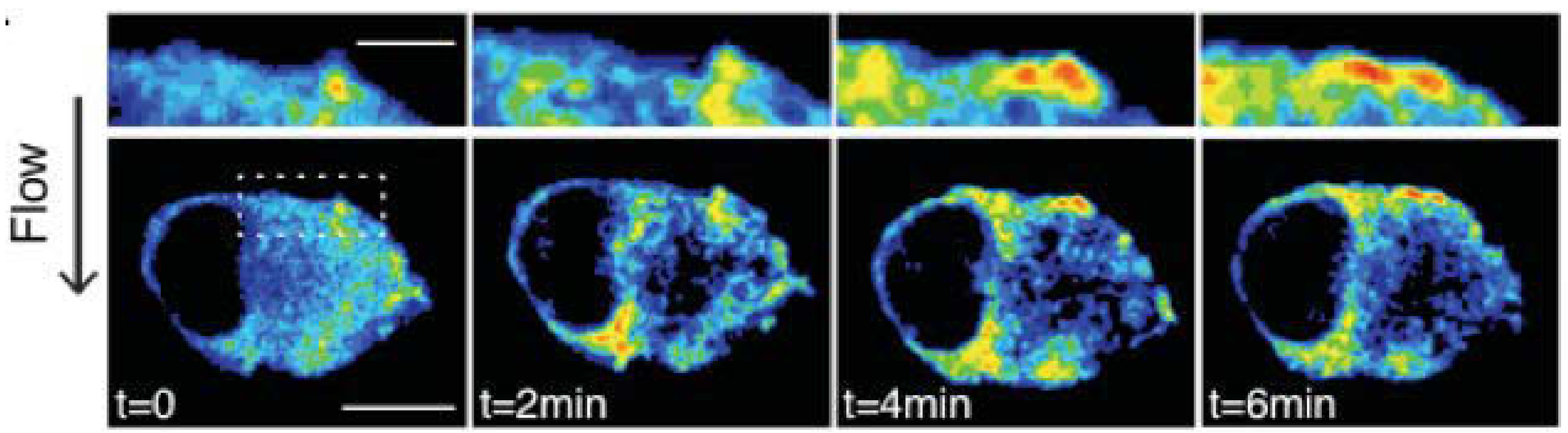
Source: Chauhan, Vikash P., et al. "Delivery of Molecular and Nanoscale Medicine to Tumors: Transport Barriers and Strategies." *Annual Review of Chemical and Biomolecular Engineering* 2 (2011): 281-98.

Forces on a single tumor cell during interstitial flow



Interstitial flow alters tumor migration characteristics

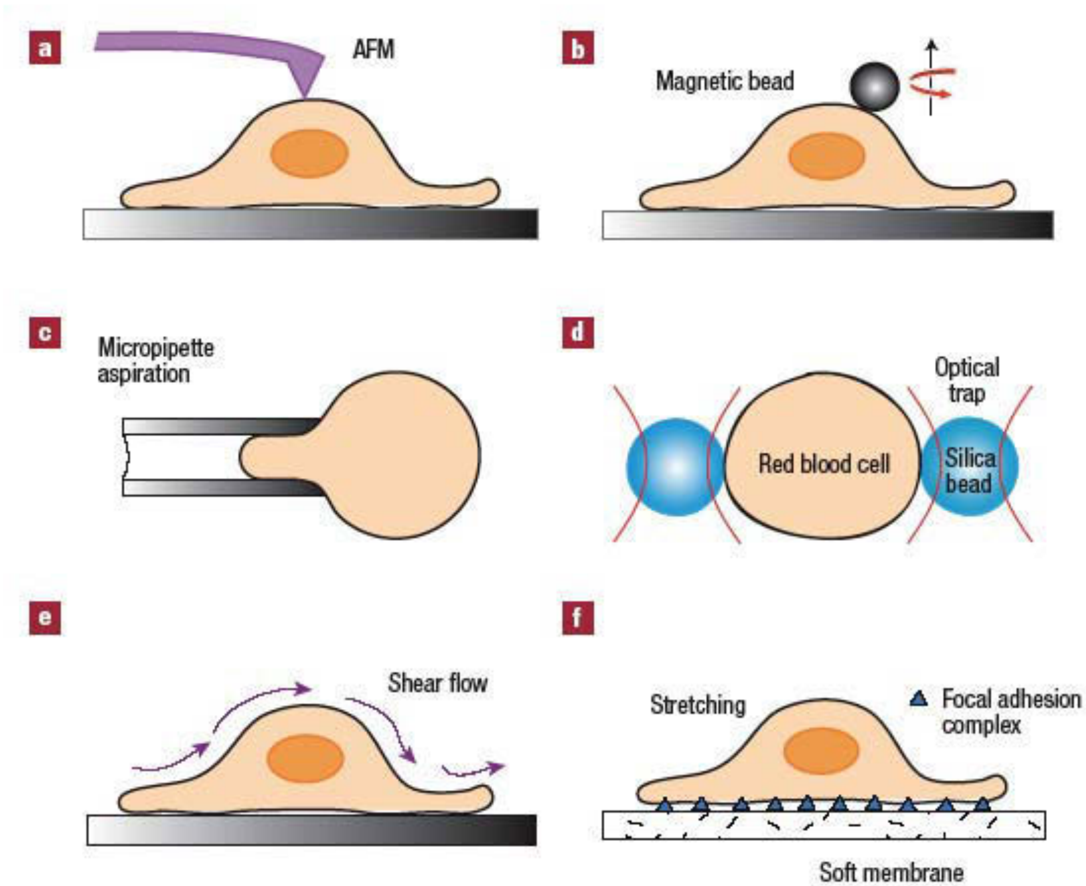




Source: Polacheck, William J., et al. "Mechanotransduction of Fluid Stresses Governs 3D Cell Migration." *Proceedings of the National Academy of Sciences* 111, no. 7 (2014): 2447-52.

Polacheck et al., PNAS, 2012

Various methods used to probe cell mechanics



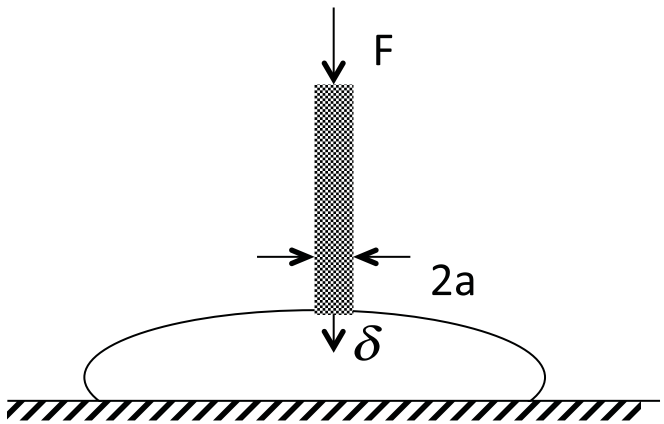
Bao & Suresh, 2003

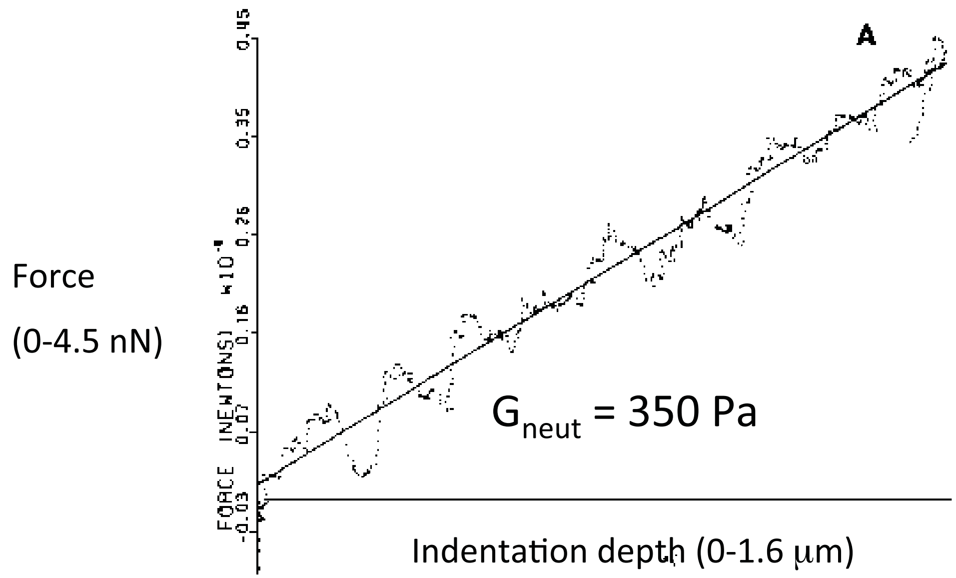
Source: Bao, Gang, and S. Suresh. "Cell and Molecular Mechanics of Biological Materials." *Nature Materials* 2, no. 11 (2003): 715-25.

Experiment #1: Indentation

Neutrophils

(Zahalak et al., 1990)





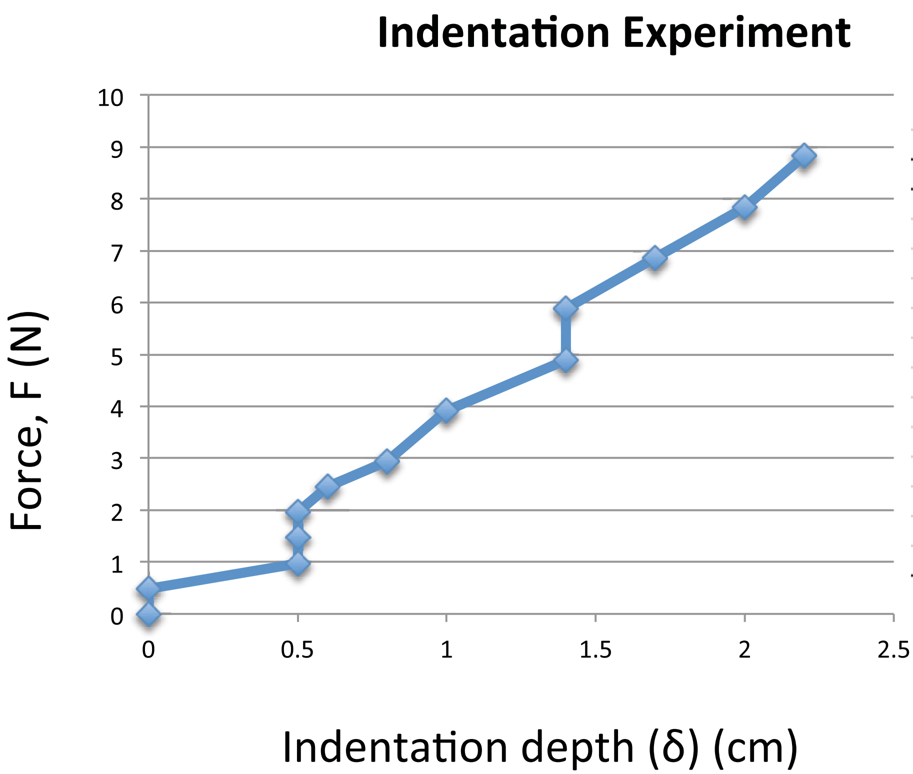
$$F \sim G\delta a$$

$$Exact:$$

$$F = 8G\delta a$$

Source: Zahalak, G. I., et al. "Determination of Cellular Mechanical Properties by Cell Poking, with an Application to Leukocytes." *Journal of Biomechanical Engineering* 112, no. 3 (1990): 283-94.

Indentation experiment on sponge



20.310 Spring 2014 - Lecture 12		DRY sponge	
Indentation Experiment			
Water volume [ml]	Sponge height [cm]	Change in height	Force (N)
0	23.5	0	0
50	23.5	0	0.49
100	23	0.5	0.98
150	23	0.5	1.47
200	23	0.5	1.96
250	22.9	0.6	2.45
300	22.7	0.8	2.94
400	22.5	1	3.92
500	22.1	1.4	4.9
600	22.1	1.4	5.88
700	21.8	1.7	6.86
800	21.5	2	7.84
900	21.3	2.2	8.82

$$F = 8Ga\delta \Rightarrow G = 6{,}700 \text{ Pa}$$

Cell Squashing

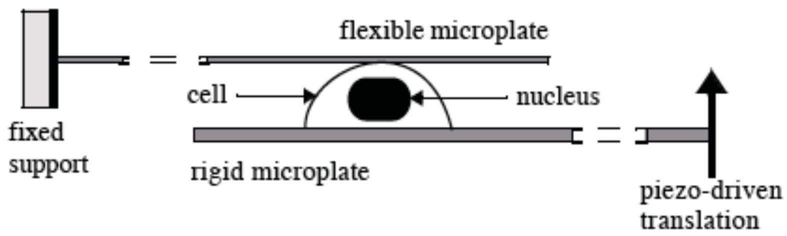
Expt. #2

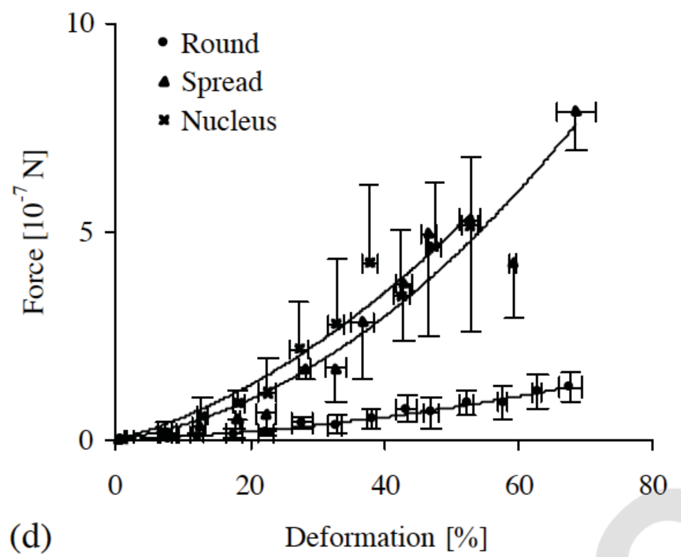
Scaling solution

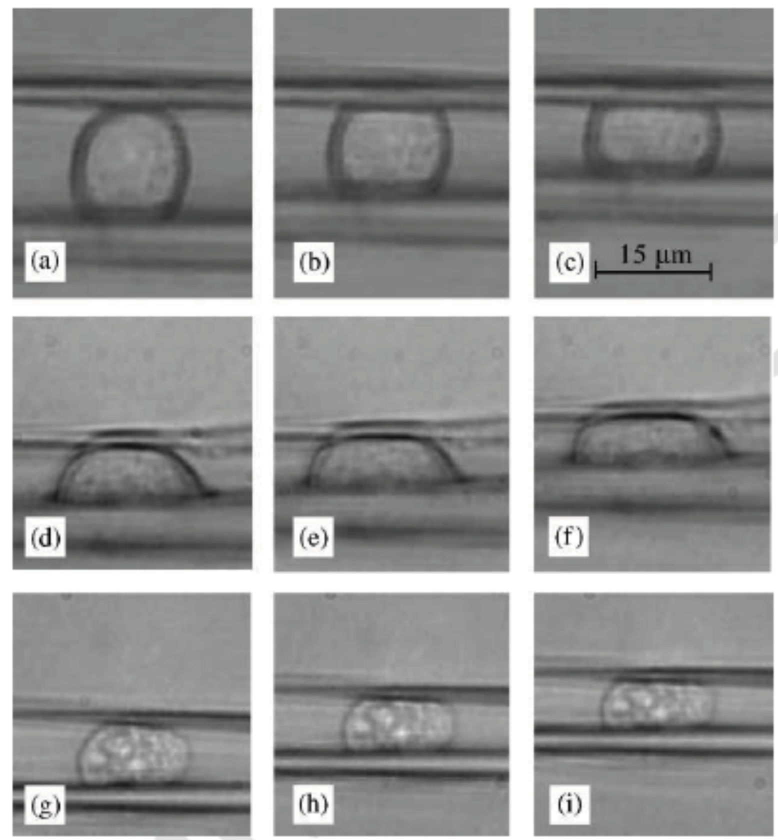
$$F \sim GR^{1/2}\delta^{3/2}$$

Exact solution for a sphere

$$F = \frac{4}{3}\frac{ER^{1/2}}{1-\nu^2}\delta^{3/2}$$







From top, a round cell, a spread cell and a nucleus. (Caille, et al., J. Biomech, 2002)

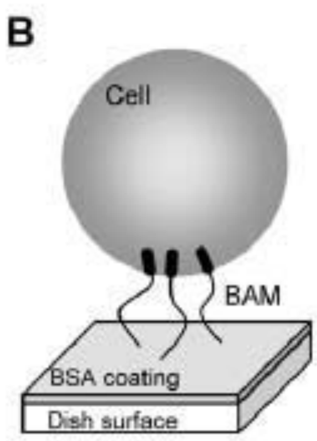
Source: Caille, Nathalie, et al. "Contribution of the Nucleus to the Mechanical Properties of Endothelial Cells." *Journal of Biomechanics* 35, no. 2 (2002): 177-87.

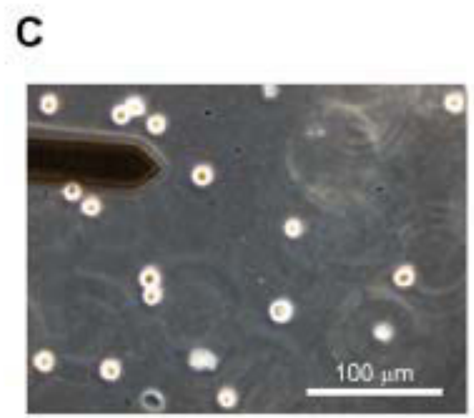
OPEN ACCESS Freely available online PLoS one

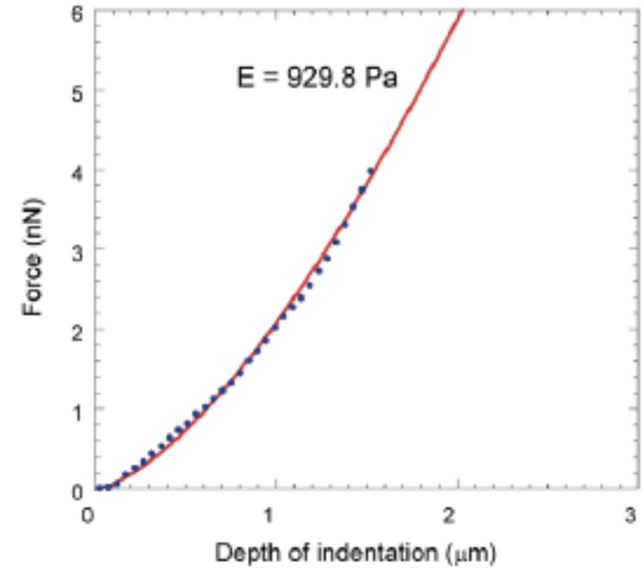
Simple Display System of Mechanical Properties of Cells and Their Dispersion

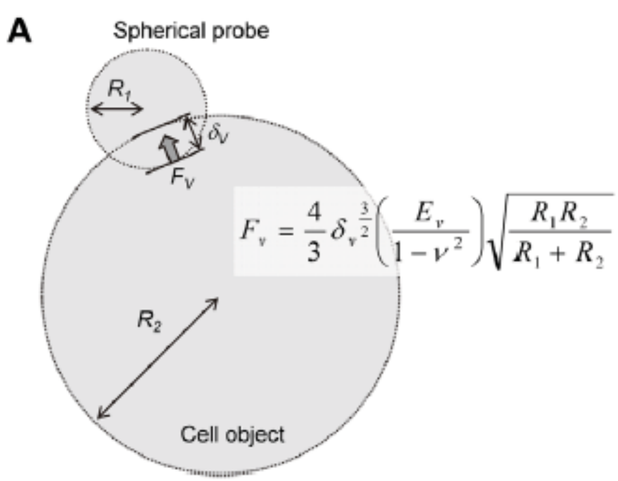
Yuji Shimizu[1], Takanori Kihara[1*], Seyed Mohammad Ali Haghparast[1], Shunsuke Yuba[2], Jun Miyake[1]

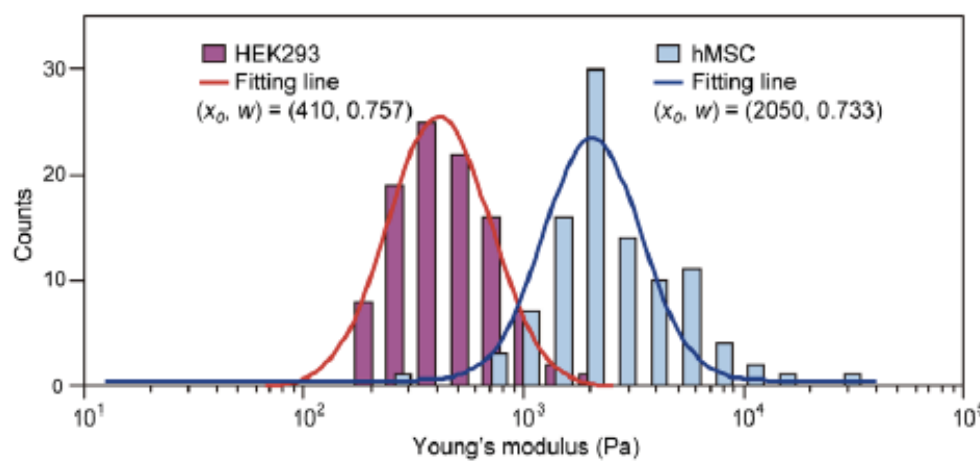
March, 2012





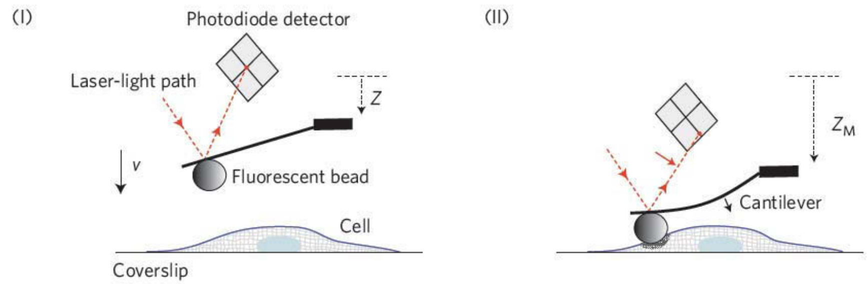


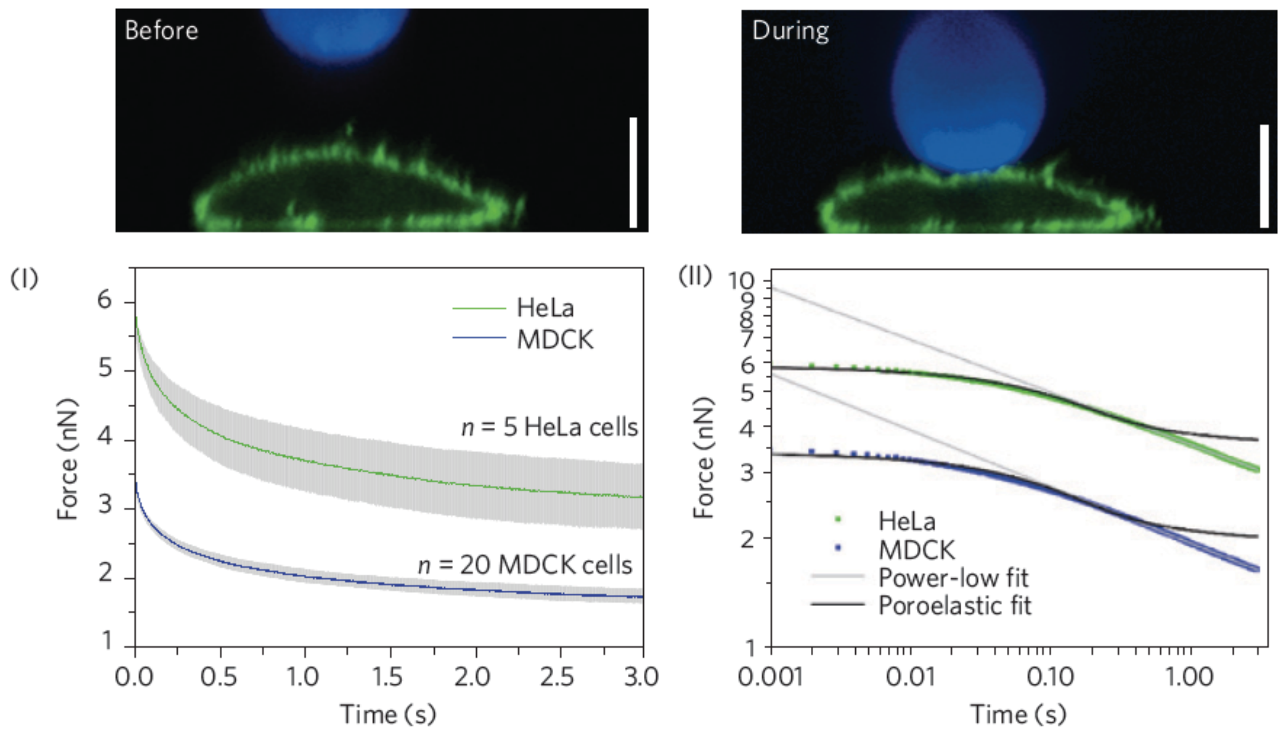




Source: Shimizu, Yuji, et al. "Simple Display System of Mechanical Properties of Cells and their Dispersion." *PloS ONE* 7, no. 3 (2012).

Population averaged force–relaxation curves showed similar trends for both HeLa and MDCK cells, with a rapid decay in the first 0.5 s followed by slower decay afterwards (Fig. 1d(I)). In Fig. 1d(II), we see that force–relaxation clearly exhibited two separate regimes: a plateau lasting ~0.1–0.2 s followed by a transition to a linear regime (Fig. 1d(II)). Hence, at short timescales, cellular force–relaxation does not follow a simple power law. Comparison with force–relaxation curves acquired on physical hydrogels[22,23], which exhibit a plateau at short timescales followed by a transition to a second plateau at longer timescales (Supplementary Fig. S3A,B), suggests that the initial plateau observed in cellular force–relaxation may correspond to poroelastic behaviour. Indeed poroelastic





Source: Moeendarbary, Emad, et al. "The Cytoplasm of Living Cells Behaves as a Poroelastic Material." *Nature Materials* 12, no. 3 (2013): 253-61.

Homogeneous??

Cells in 3D matrix

Figures removed due to copyright restrictions.

MDA-MB-231 breast cancer cells migrating inside a collagen gel.

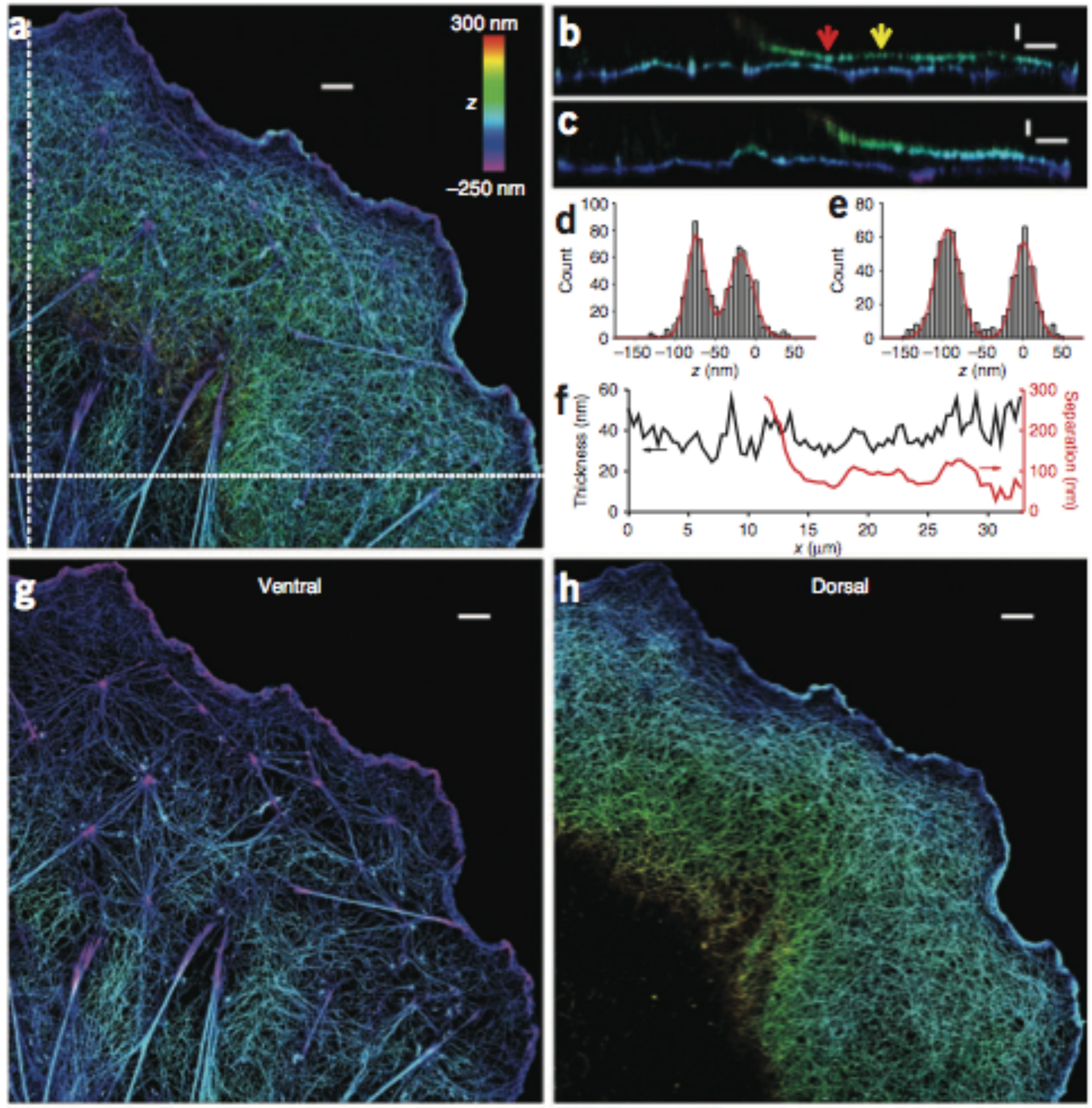
- Dense cortical actin with myosin.
- Cross-linkers more homogeneously distributed

Rajagopalan, unpublished

Isotropic??

Figure 3 | Sheet-like cell protrusion comprises two layers of actin networks with distinct structures. (**a**) Dual-objective STORM image of actin in a BSC-1 cell. The *z* positions are color coded (color bar). (**b**,**c**) Vertical cross sections (each 500-nm wide in *x* or *y*) of cell in **a** along dotted and dashed lines, respectively. When far from cell edge, *z* position of dorsal layer increases quickly and falls out of imaging range. (**d**,**e**) The *z* profiles for two points along vertical section (red and yellow arrows in **b**, respectively). Each histogram is fit to two Gaussians (red curves), yielding apparent thickness of ventral and dorsal layers and peak separation between the two layers. (**f**) Quantification of apparent thickness averaged over two layers and dorsal-ventral separation obtained from *x*–*z* cross-section profile in **b**. (**g**,**h**) Ventral and dorsal actin layers of cell in **a**. (**i**,**j**) Ventral and dorsal actin layers of a COS-7 cell treated with blebbistatin. (**k**,**l**) Vertical cross sections (each 500-nm wide in *x* or *y*) of cell along dotted and dashed lines, respectively. (**m**) Actin density of ventral and dorsal layers along yellow box in **i**,**j**, measured by localization density. Scale bars, 2 µm (**a**,**g**–**j**); 100 nm for *z* and 2 µm for *x* and *y* (**b**,**c**,**k**,**l**).

We observed two vertically separated actin layers in the sheet-like cell protrusion despite its small thickness (**Fig. 3a–c**). The apparent thickness of each layer was



Source: Xu, Ke, et al. "Dual-objective STORM Reveals Three-dimensional Filament Organization in the Actin Cytoskeleton." *Nature Methods* 9, no. 2 (2012): 185-8.

http://www.nature.com/nmeth/video/moy2008/index.html

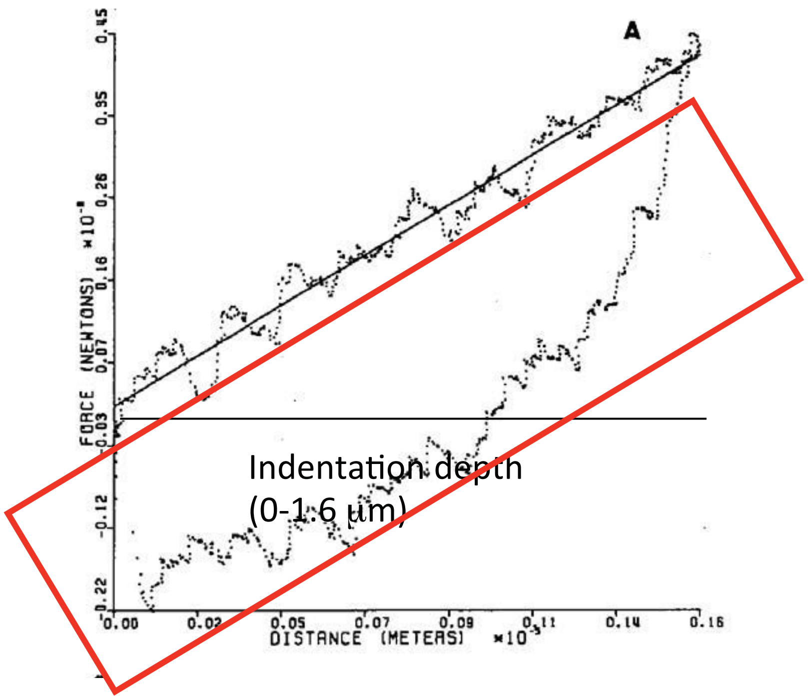
Elastic or viscoelastic??

Micropipette Aspiration

Cells are viscoelastic

Force

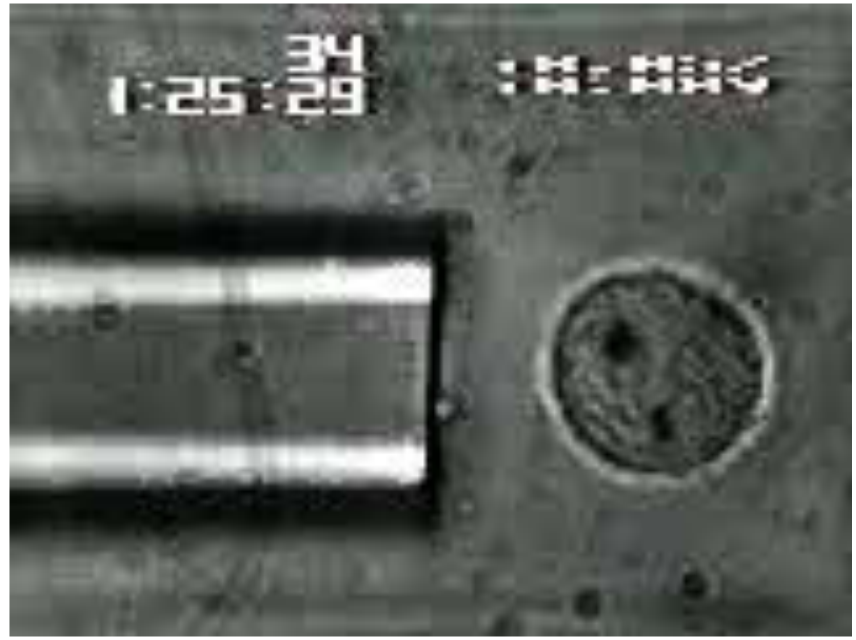
(0-4.5 nN)



Indentation

Source: Zahalak, G. I., et al. "Determination of Cellular Mechanical Properties by Cell Poking, with an Application to Leukocytes." *Journal of Biomechanical Engineering* 112, no. 3 (1990): 283-94.

(Zahalak et al., 1990)



20.310J / 3.053J / 6.024J / 2.797J Molecular, Cellular, and Tissue Biomechanics
Spring 2015