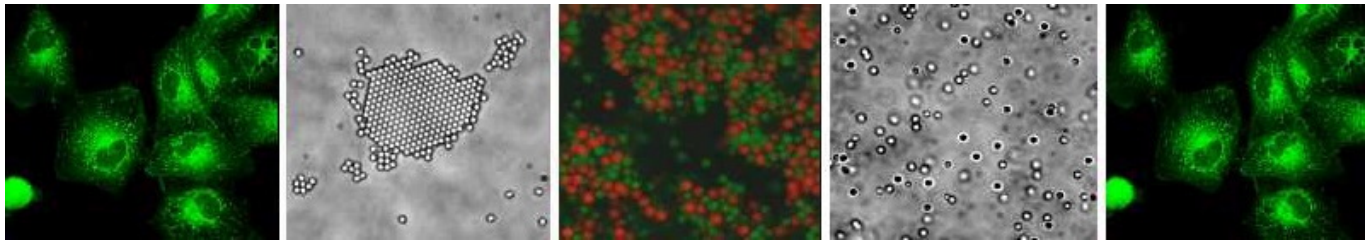
Dissecting Cell Rheology and Active Stress Fluctuations



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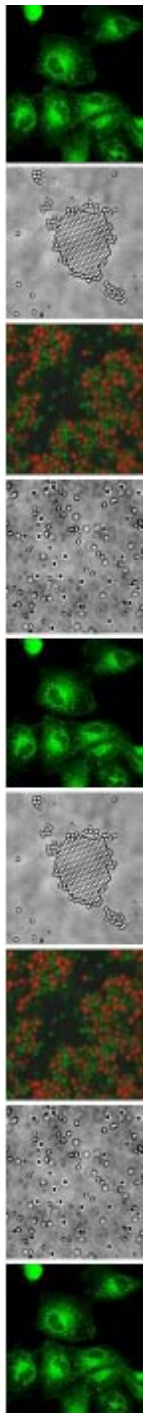
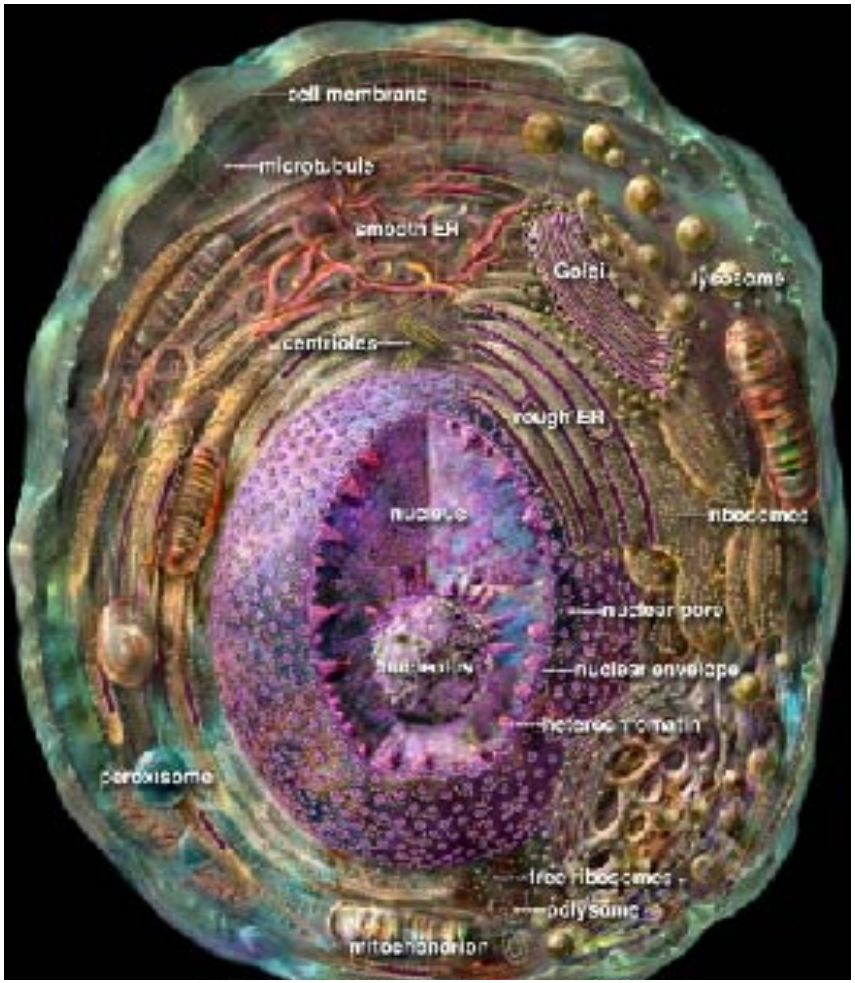
Penn
University of Pennsylvania

Krumhansl Symposium
Bangalore
7 FEB 2012

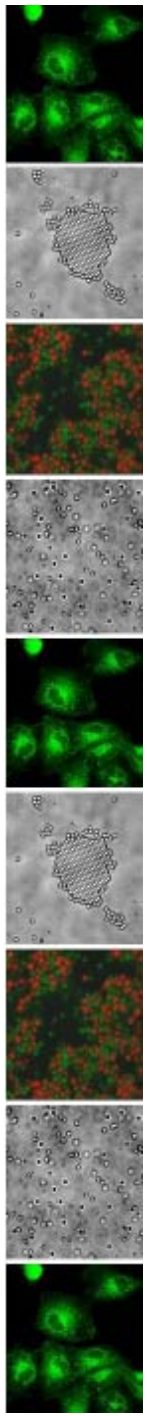


A cartoon cell

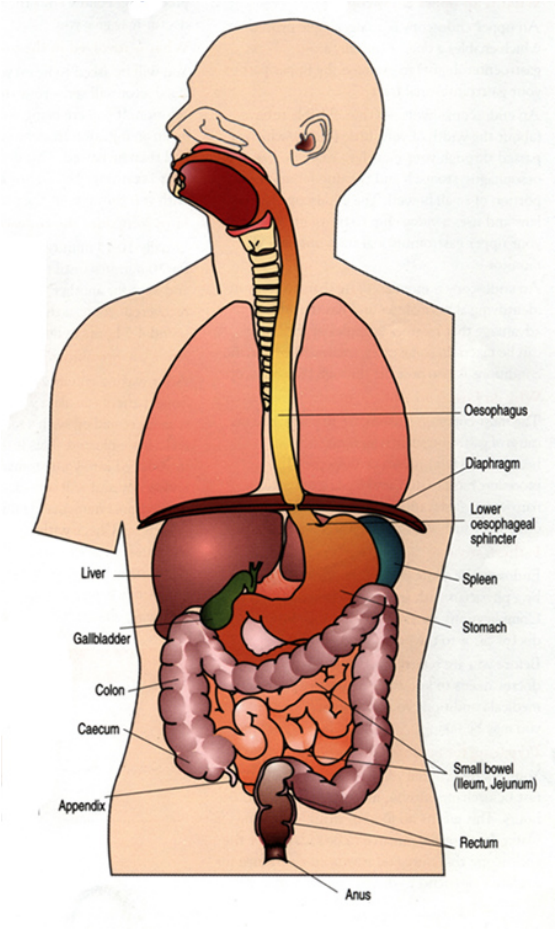


- A passive blob?
- A biochemical factory?
- A complex fluid or simple gel?

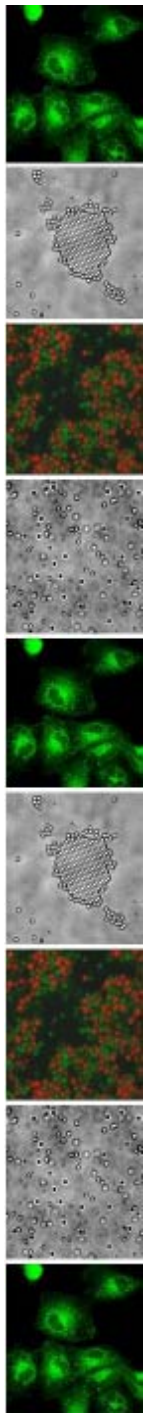
What is ‘cell biophysics’ good for?



A cartoon human

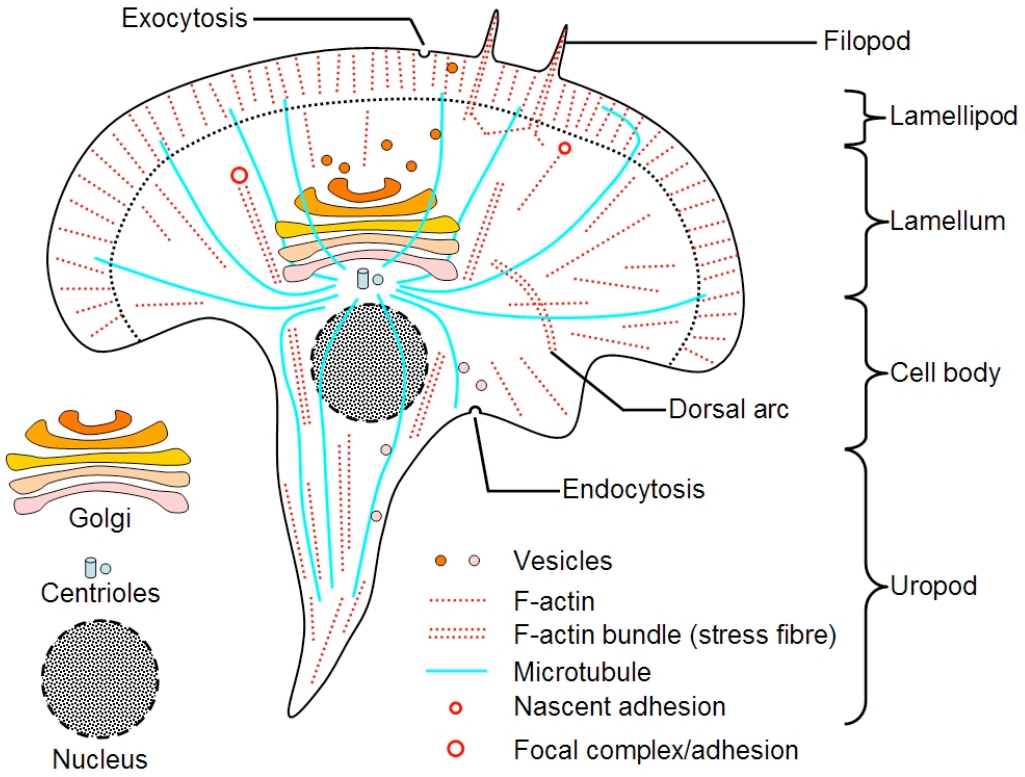


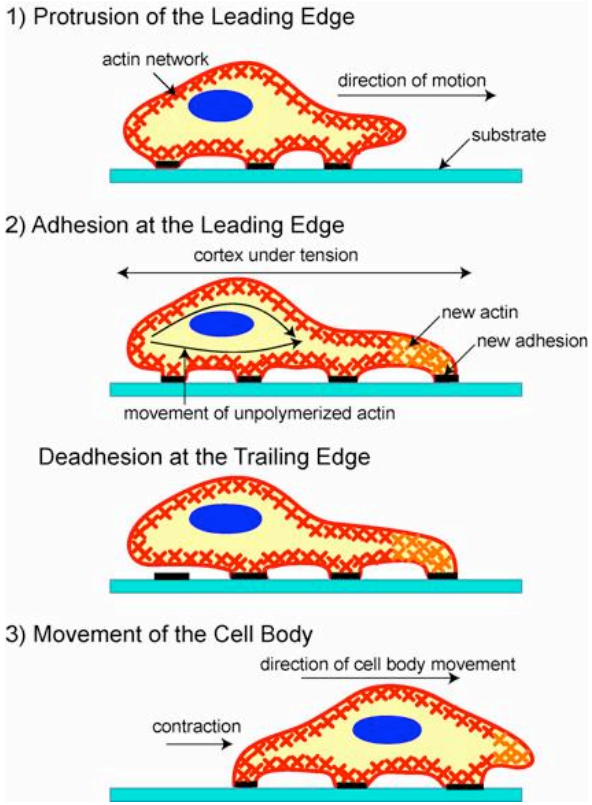
- Skeleton, musculature, brain, omitted
- Both cartoons show *biochemical apparatus*

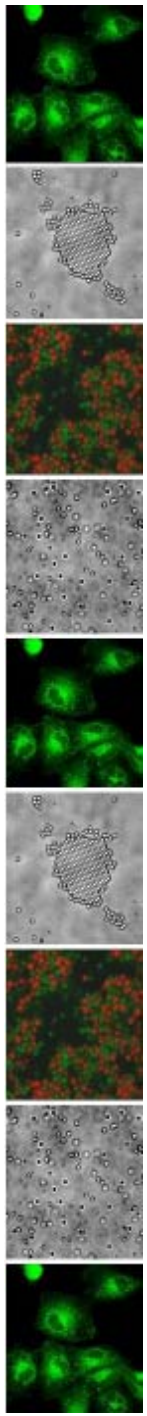


The Real Cell...

is a complex mechanical system driven by a biochemical metabolism, nanotech parts and ..... soft matter physics.

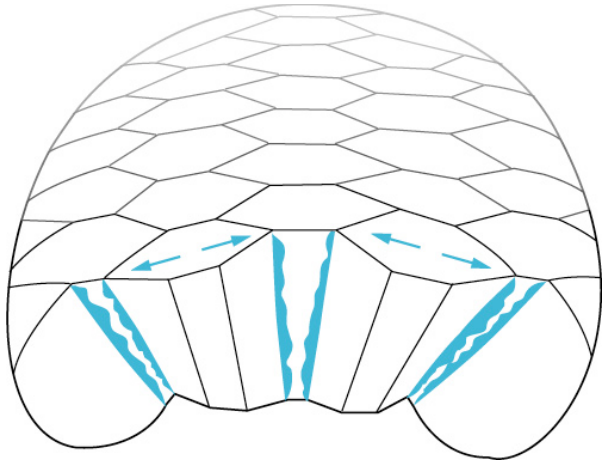


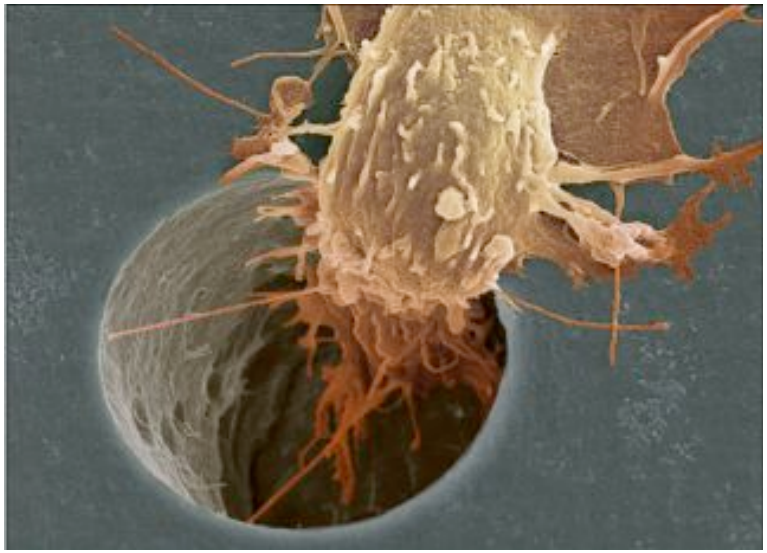


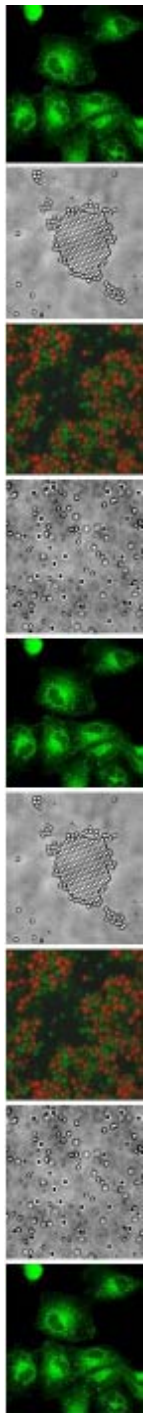


The Real Cell...

is a versatile, reprogrammable soft *robot*, capable of assembling complex tissues and navigating in 3d by integrating *sensory data* and performing simple computation....





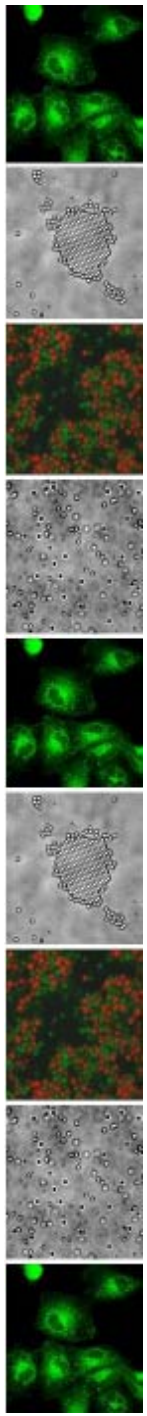


Big Picture

Cells interact with each other and their environment mechanically, sensing hydrodynamic stress, static tension, local stiffness .... by largely unknown means

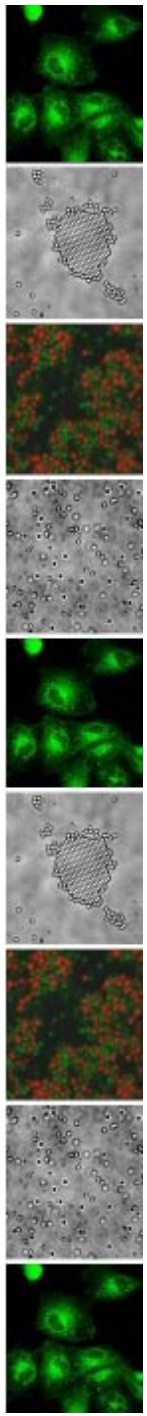
Our failure to understand cell mechanics and mechanosensing impedes progress on major biomedical problems...

Tissue engineering, stem cell therapy, cancer...



First Step

Understanding how stress propagates within and deforms cells is a *prerequisite* for understanding their bio-mechanics and ultimately their mechano-sensing.



Plan/Outline

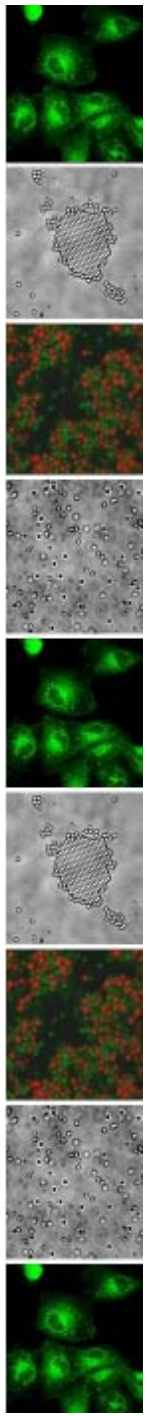
I. Background

II. Different Challenges

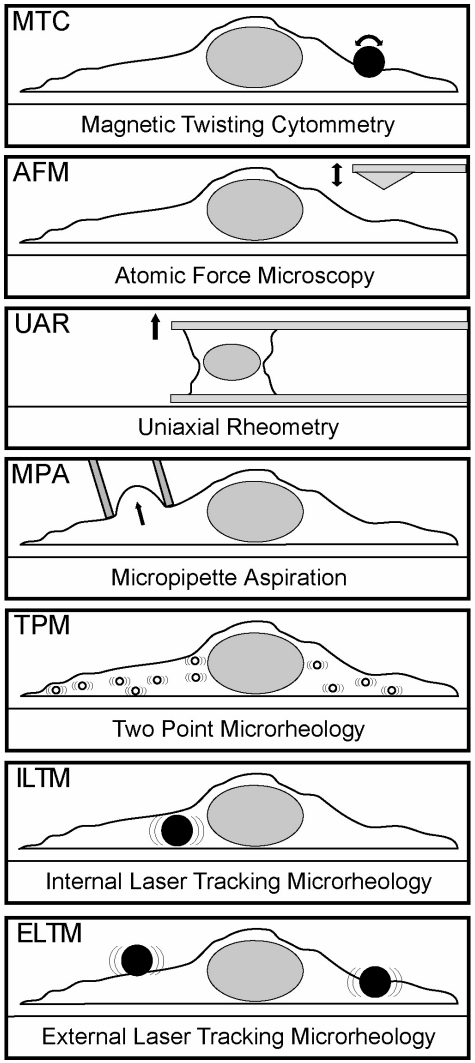
III. Find a consensus rheology

IV. Molecular roles in rheology

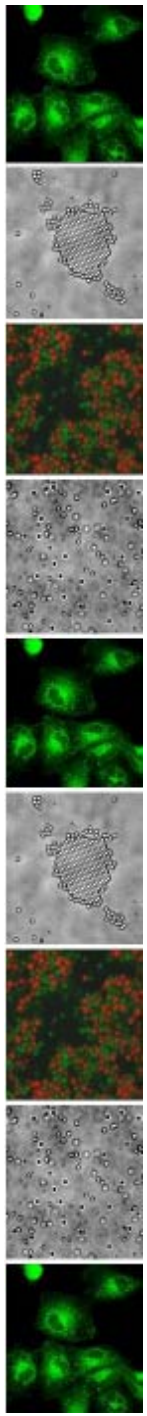
V. Active gel behavior



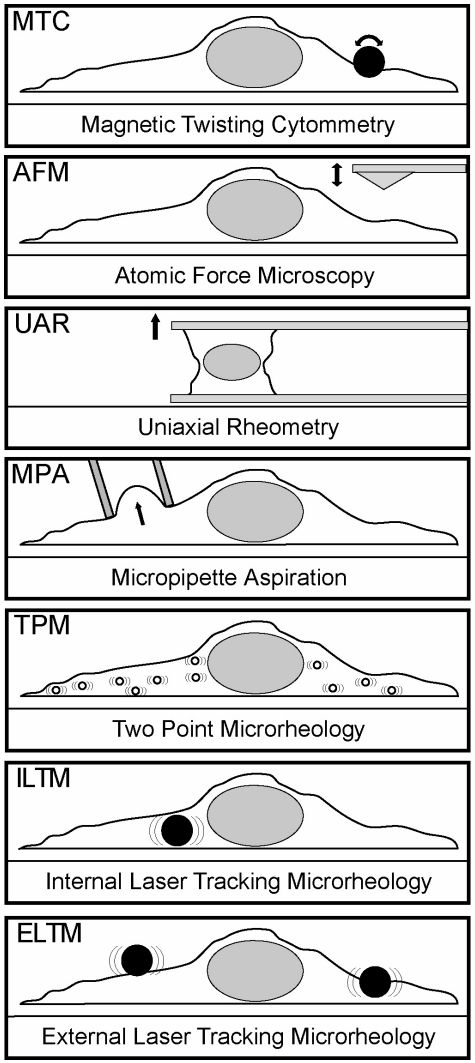
Cell Rheology Methods



- Many methods in use, some for a century (!)
- Geometries and deformation fields are ‘complicated’, requiring modeling
- Before ~2000: cells are either hard, soft, elastic, viscous, or viscoelastic (!)



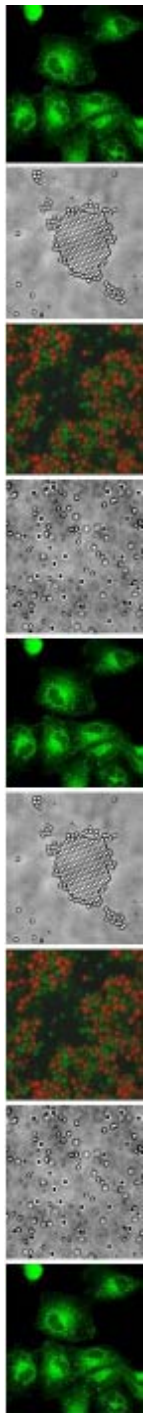
Cell Rheology Background



Last decade: ‘power-law fluid’, very little agreement on stiffness or treatment effects

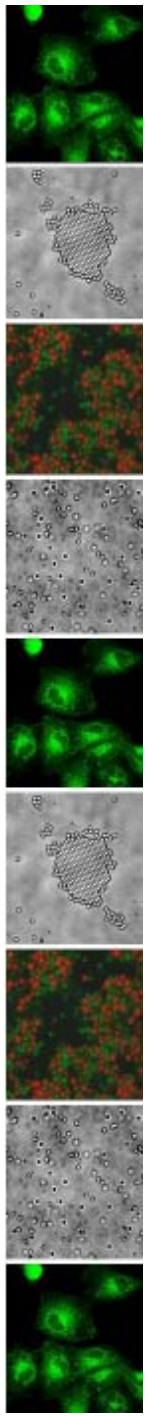
Results do NOT look like simple gels of purified filaments.

No proper physical model(s) (yet)!



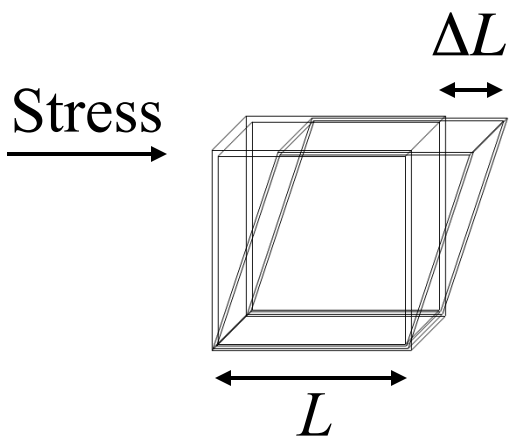
Toward a consensus

- Hypothesis: A cell contains distinct mechanical structures having different stiffness and rheology, and different methods may probe different structures.
- Approach: Compare multiple methods on a single cell population.



Rheology (Active Method)

- Measure dynamic shear modulus $G(\omega)$, a dynamic resistance to deformation



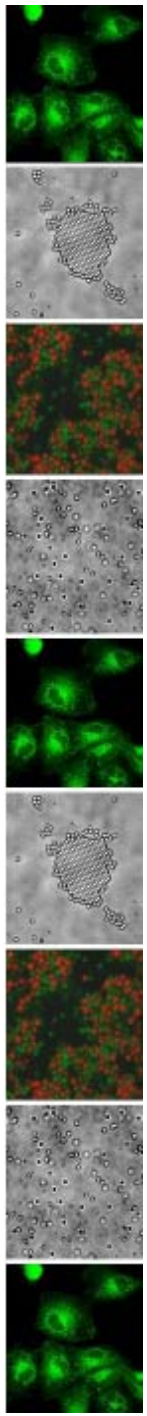
$$\gamma = \frac{\Delta L}{L}$$

$$\sigma e^{i\omega t} = G^*(\omega)\gamma e^{i\omega t}$$

$$G'(\omega) = \mathrm{Re}(G^*(\omega)) \quad \text{Elastic Component}$$

$$G''(\omega) = \mathrm{Im}(G^*(\omega)) \quad \text{Viscous Component}$$

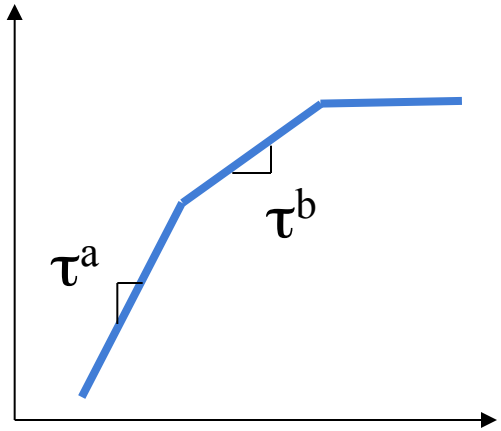
- "Spectroscopic" information about microscopic dynamics



Rheology (Passive Method)

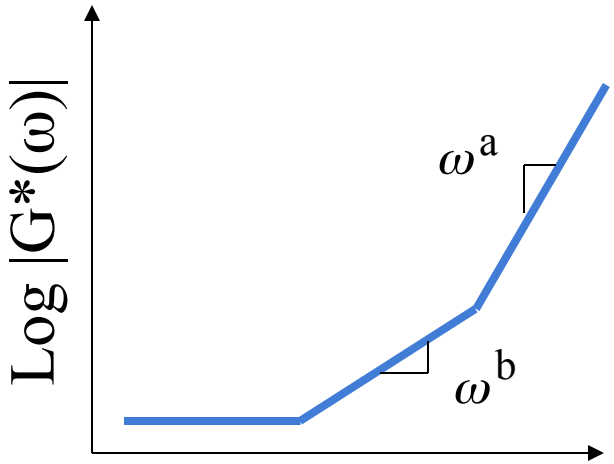
- Measure the Brownian motion of embedded or attached tracer particles.....

Mean-squared displacement:



Flip Both Axes!

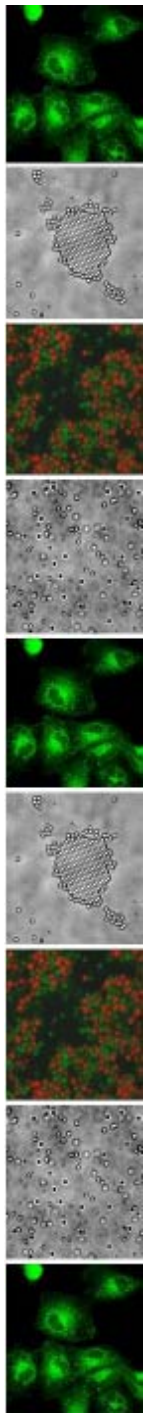
Rheology:



$$D = \frac{k_B T}{6\pi\eta a} \longrightarrow \left\langle \Delta r^2(\omega) \right\rangle = \frac{k_B T}{\pi\omega a G^*(\omega)}$$

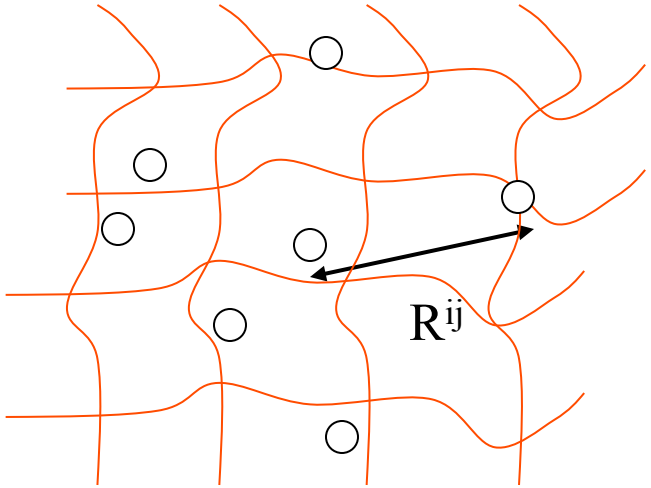
- NB: Assumes Brownian motion only and Stokes B/Cs

Mason/Weitz, Schmidt/Mackintosh, mid-90s



Two-point microrheology (TPM)

- Correlate the random motion of two tracers…



- Measures the motion of intervening network segment
- Insensitive to tracer b/c's!

Two Point Covariance Tensor (has 'Stokeslet' form):

$$D_{\alpha\beta}(R,\tau) = \left\langle \Delta r_\alpha^i(t,\tau)\Delta r_\beta^j(t,\tau)\delta(R - R^{ij}(t)) \right\rangle_{i\neq j,t}$$

Corresponding displacement

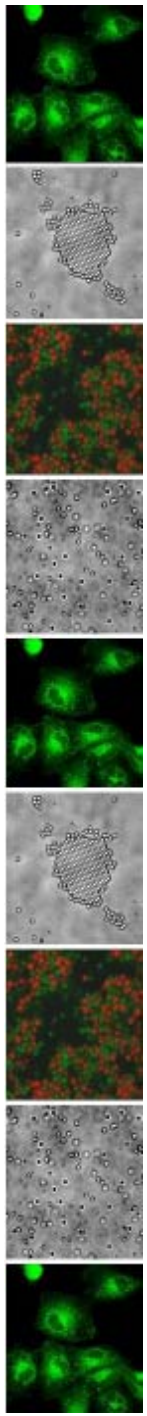
$$\left\langle r^2(\tau)\right\rangle_A = \frac{2R}{a} D_{rr}$$

Levine and Lubensky, PRL 85, 1774 (2000).

Stokes-Einstein Relation

$$D_{rr} = \frac{k_B T}{2\pi\omega R G^*(\omega)}$$

Crocker, et.al., PRL 85, 888 (2000).



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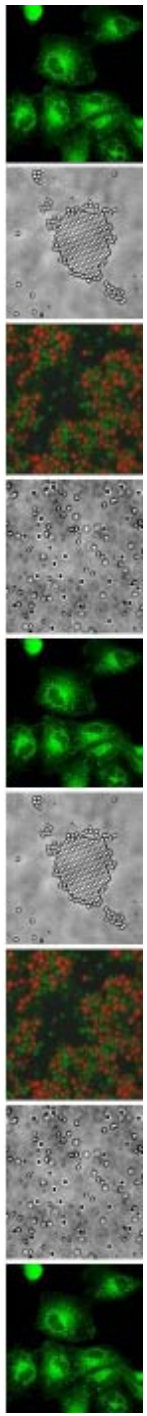
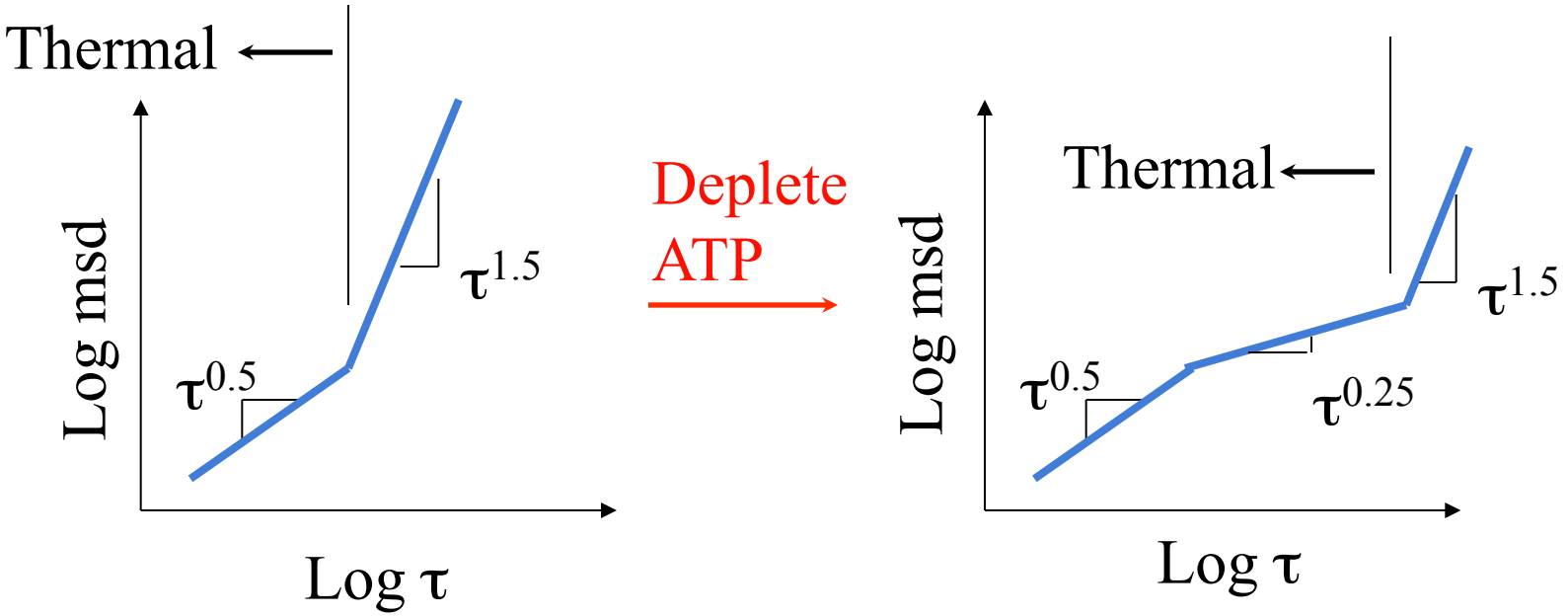


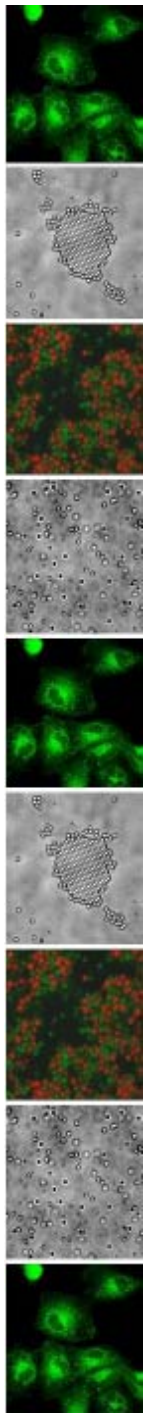
Challenge 1: Cells are alive

- Molecular motors drive ‘super-diffusive’ motion

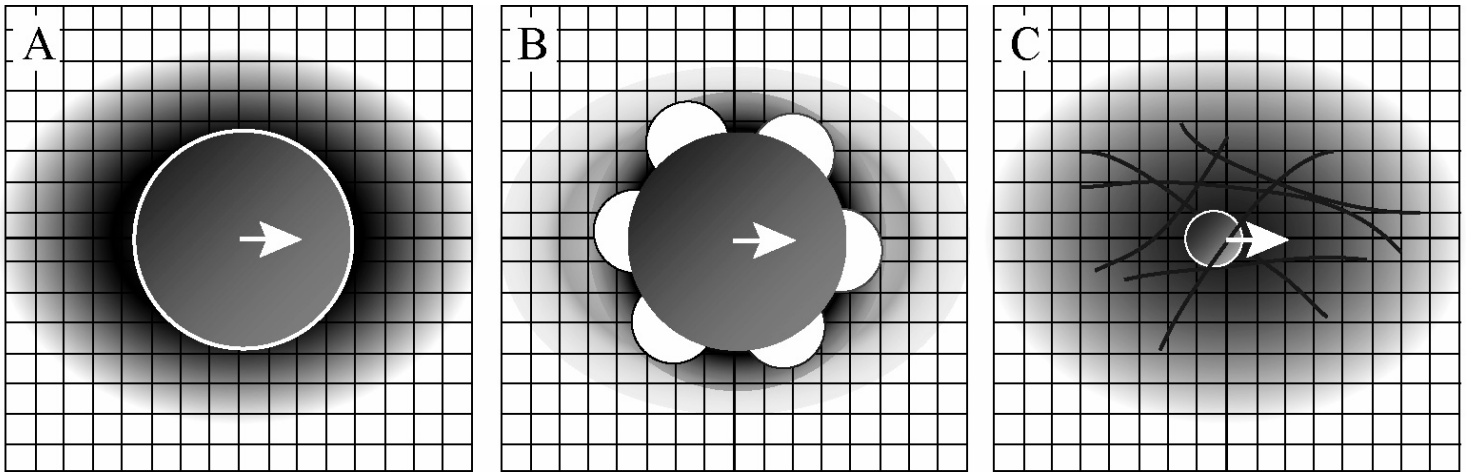


Resolution:

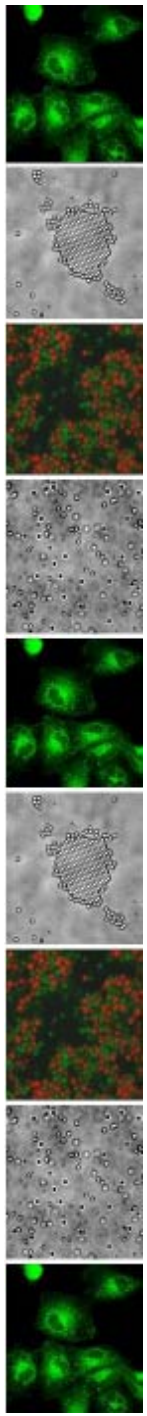
- Deplete ATP using azide and deoxyglucose
- ‘Super-diffusion’ disappears or moves to much longer lag times.
- Use ‘thermal’ part to compute rheology, do controls.



Challenge 2: 'Heterogeneity'



- Probes can report 'right' stiffness in A, under-report in B, over-report in C.
- All three cases can have identical frequency dependence



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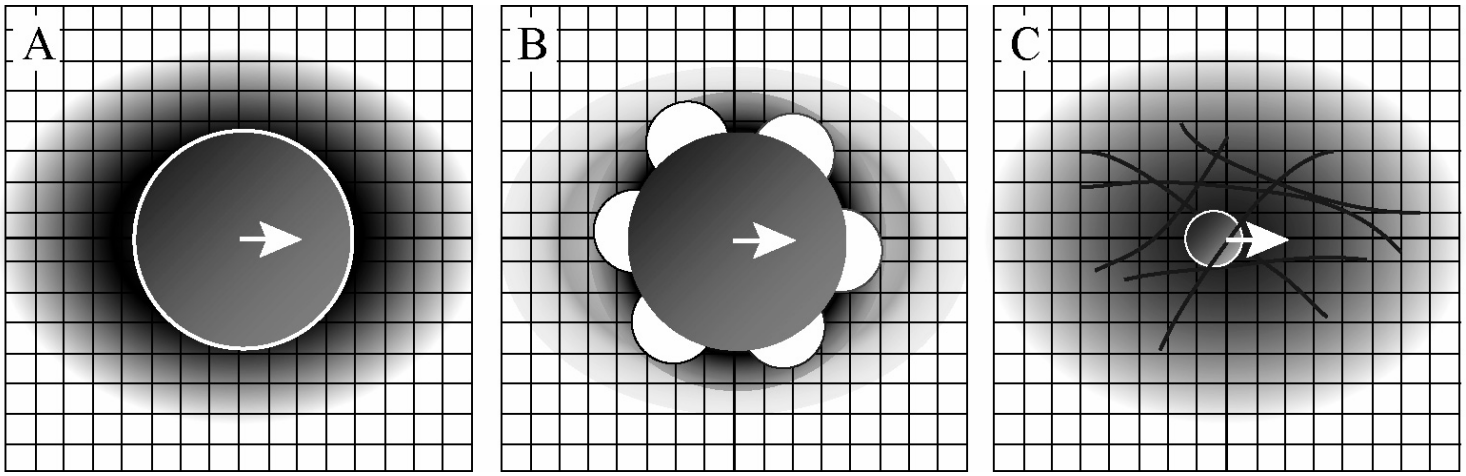
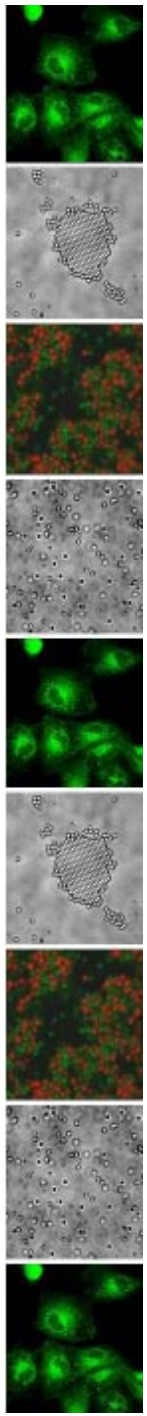


- Probes can report 'right' stiffness in A, under-report in B, over-report in C.
- All three cases can have identical frequency dependence

Resolution:

- Rely on frequency dependence, discount stiffness.
- Use deformation mapping or 'two-point' microrheology



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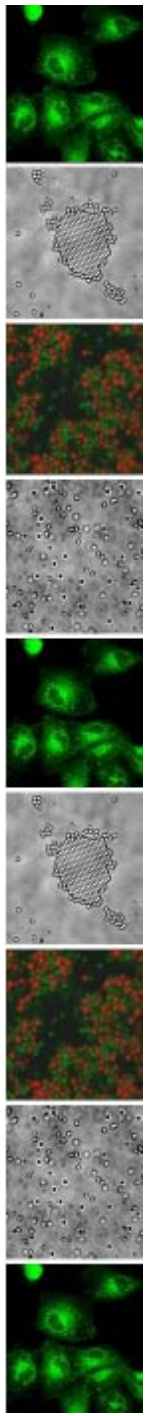


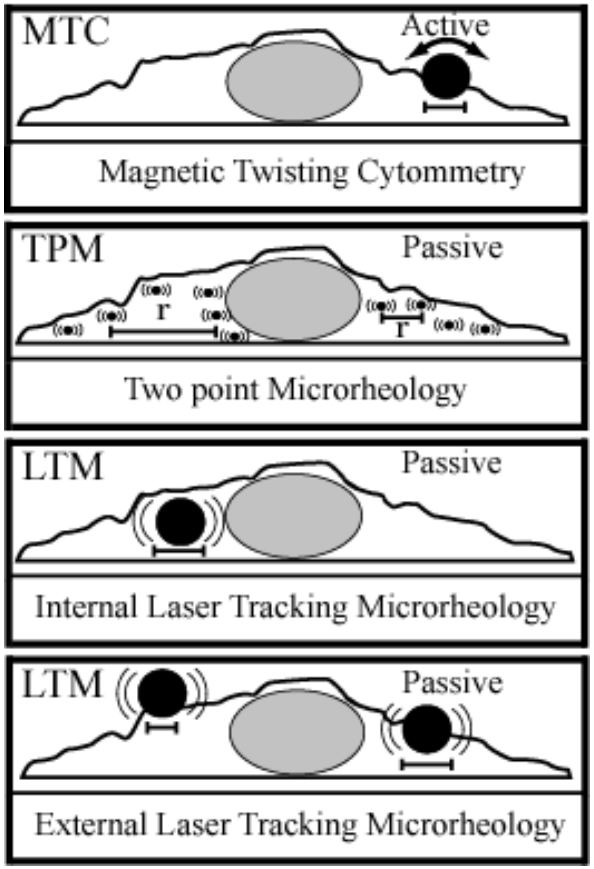
Experimental Approach

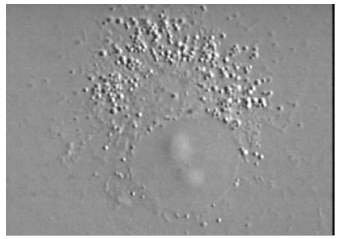
Use multiple methods:

- Internal/External
- Active/Passive
- Wide frequency range

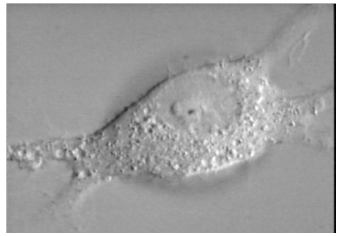
Tools:

- Image particle tracking
- Laser deflection tracking
- Applied magnetic field

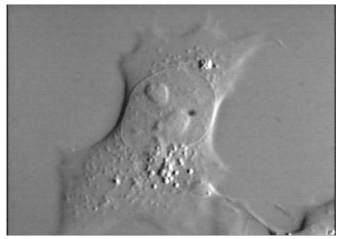




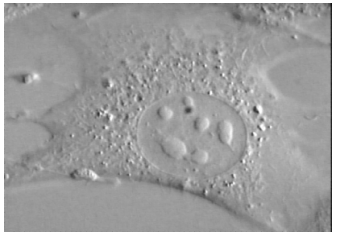
TC7 Epithelial



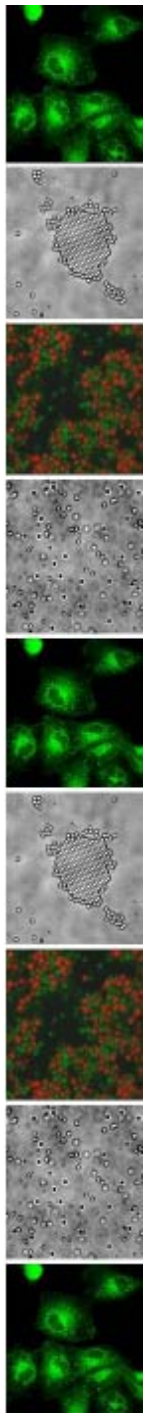
J774A.1 'Macrophage'



F9 Carcinoma

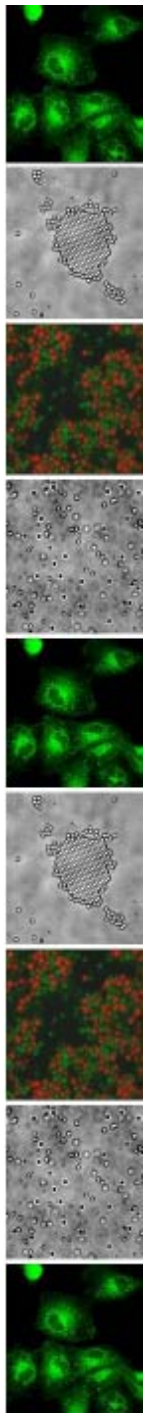


NIH 3T3 Fibroblast

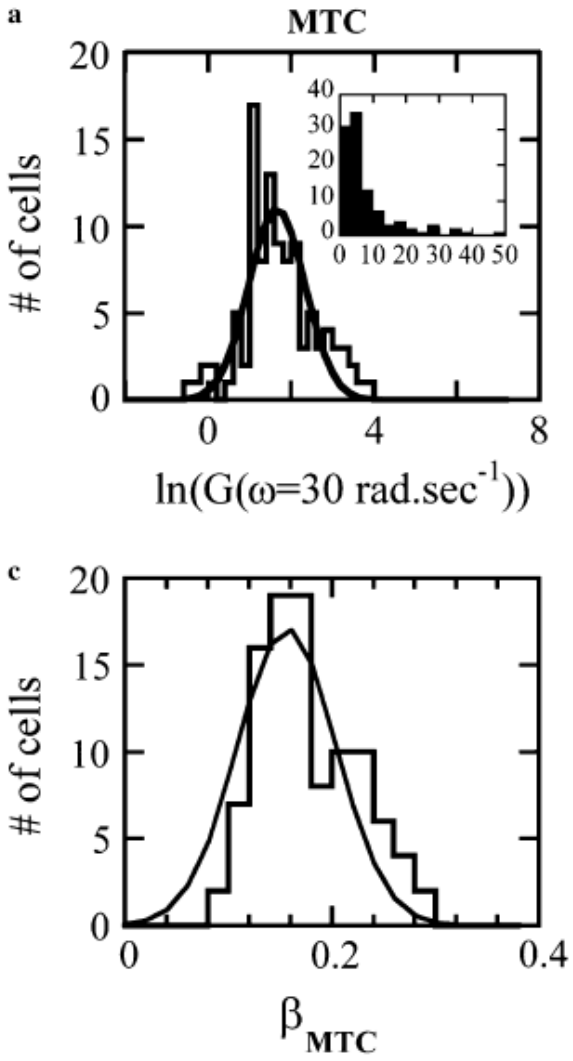


Questions

- Does a given method give reproducible results?
- Do different methods agree?
- Compare to literature?



Magnetic Twisting Cytometry



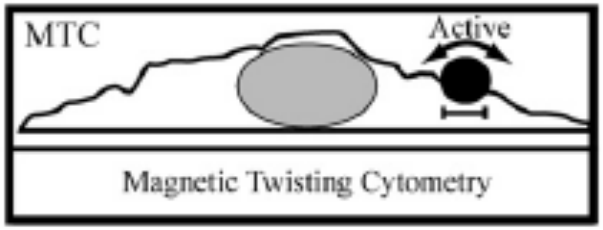
- Rocking amplitudes are log-normal distribution

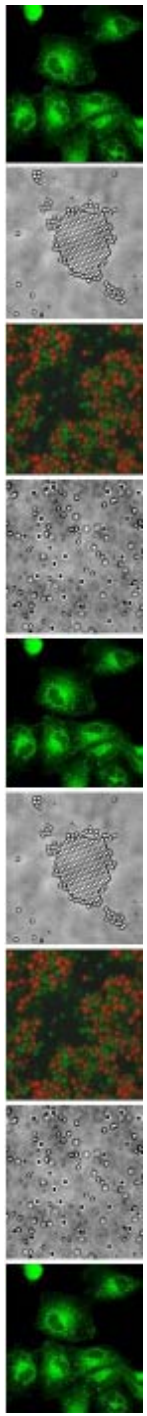
  amplitudes vary by > decade

- Power-law fluid:

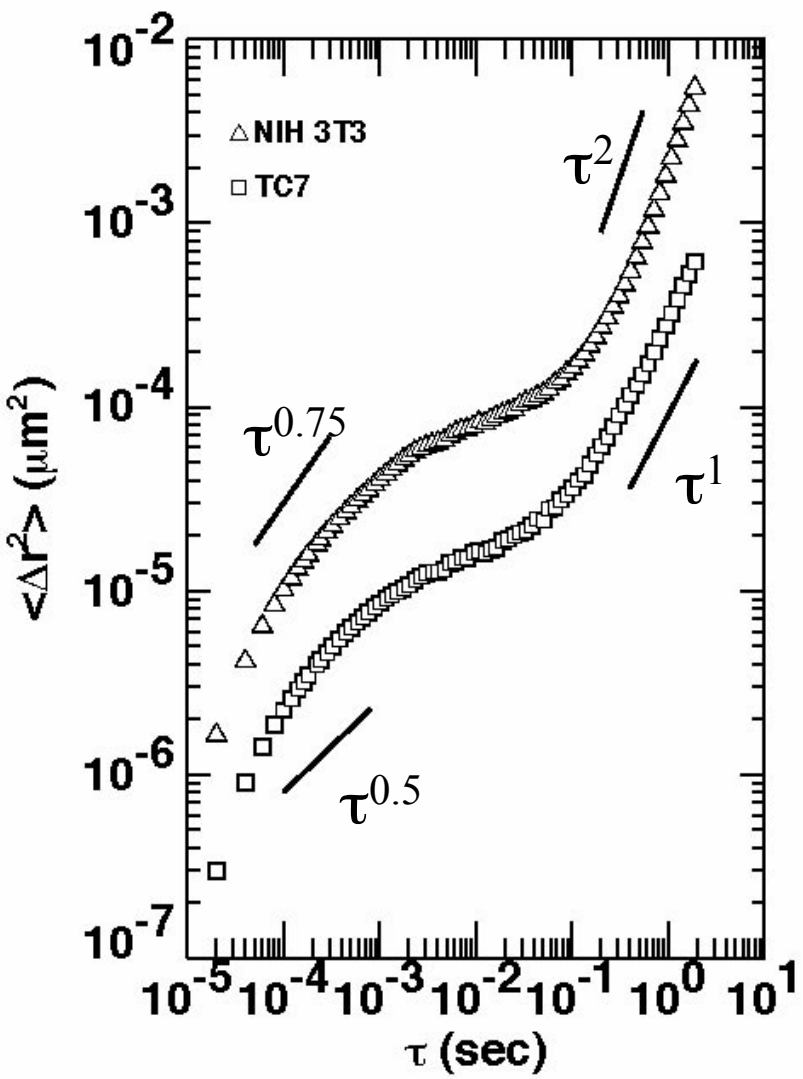
$$G(\omega) \sim \omega^{\beta}$$

- Frequency dependence IS consistent from tracer to tracer and over time





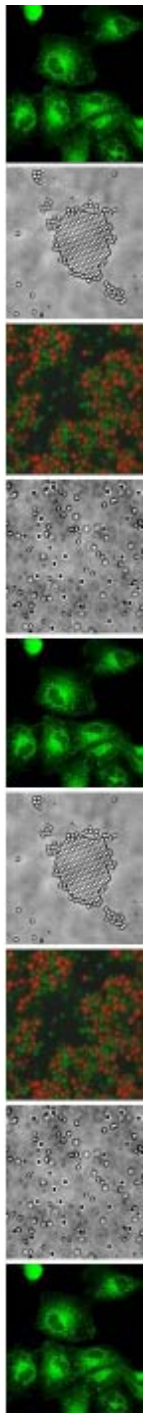
External LTM (passive motion)



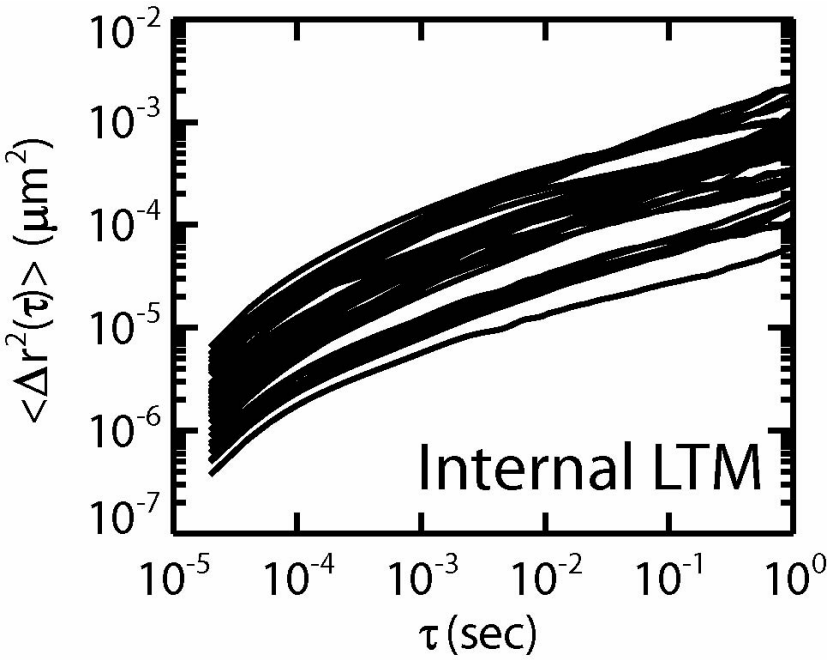
- Long time super-diffusion: random crawling or drift

  (need to deplete ATP)
- Frequency depend-ence NOT consistent from tracer to tracer

amplitudes vary by > decade



Internal LTM (passive motion)



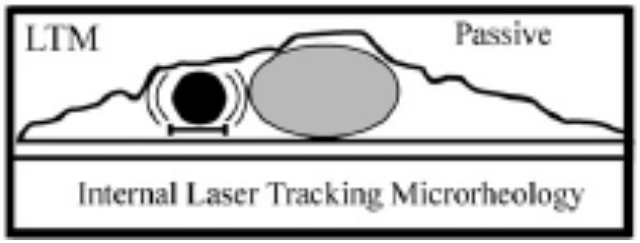
ATP depleted

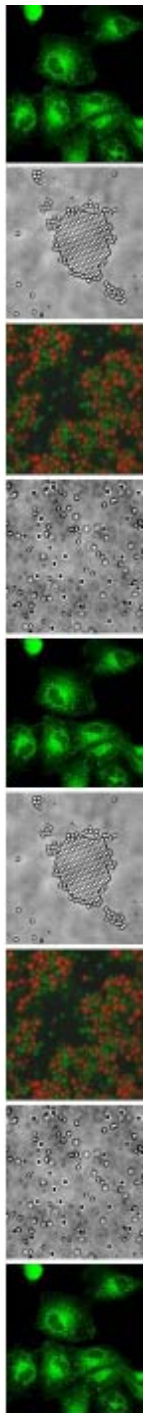
- Intermediate times consistent with power-law fluid:

$$msd \sim t^{\beta}$$

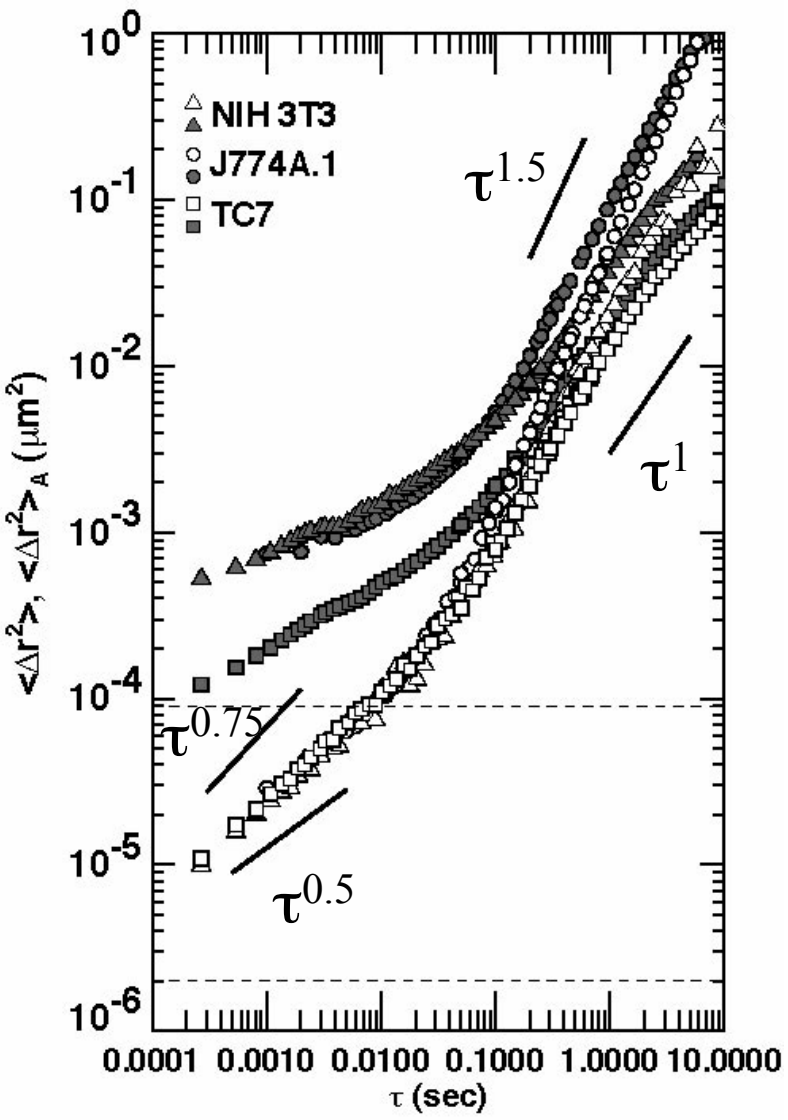
- Frequency dependence IS consistent from tracer to tracer

amplitudes vary by > decade





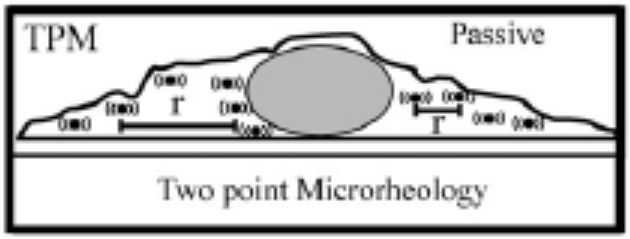
Two-Point Microrheology

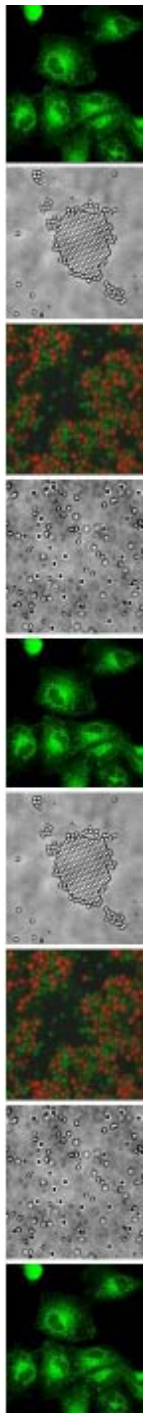


- Large 1-point/ 2-point difference.....

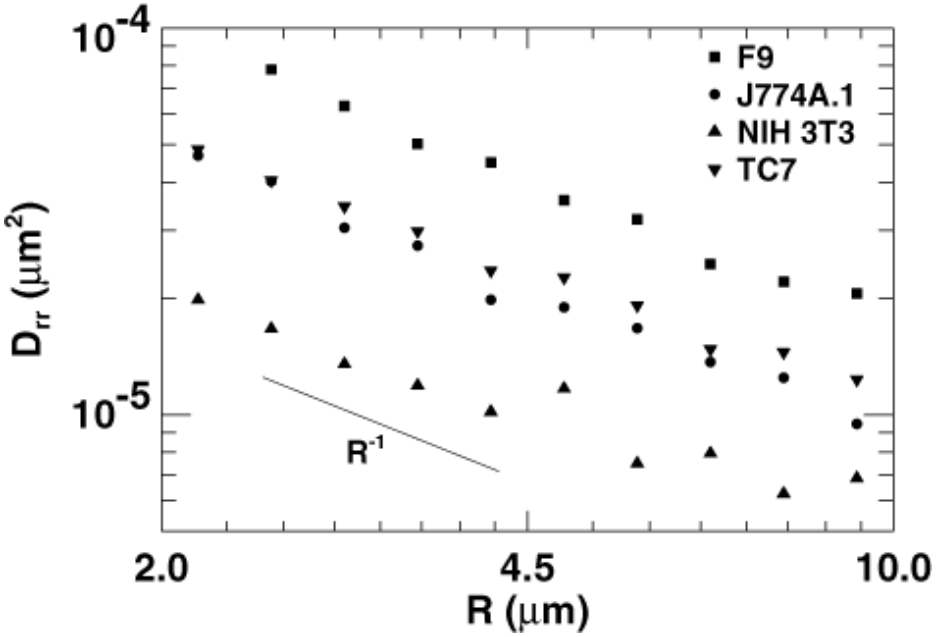
  heterogeneity: either spatial or of B/Cs

- Amplitude similar b/w cells and cell types!
- Super-diffusive at long times: ATP depletion

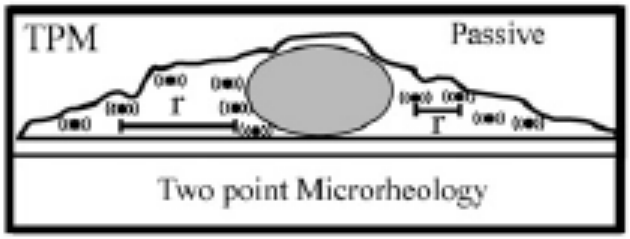


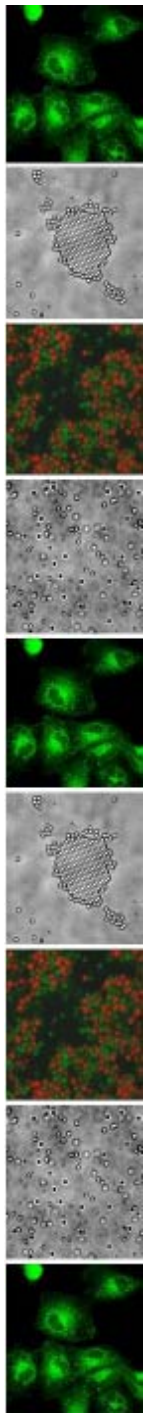


TPM Continuum check



- 1/R decay in D_{rr} indicates 3D continuum
- Not sensitive to micron scale heterogeneity or weak spatial gradients

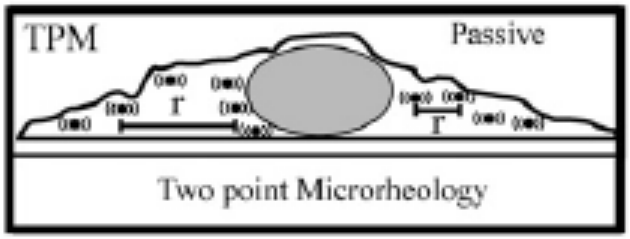


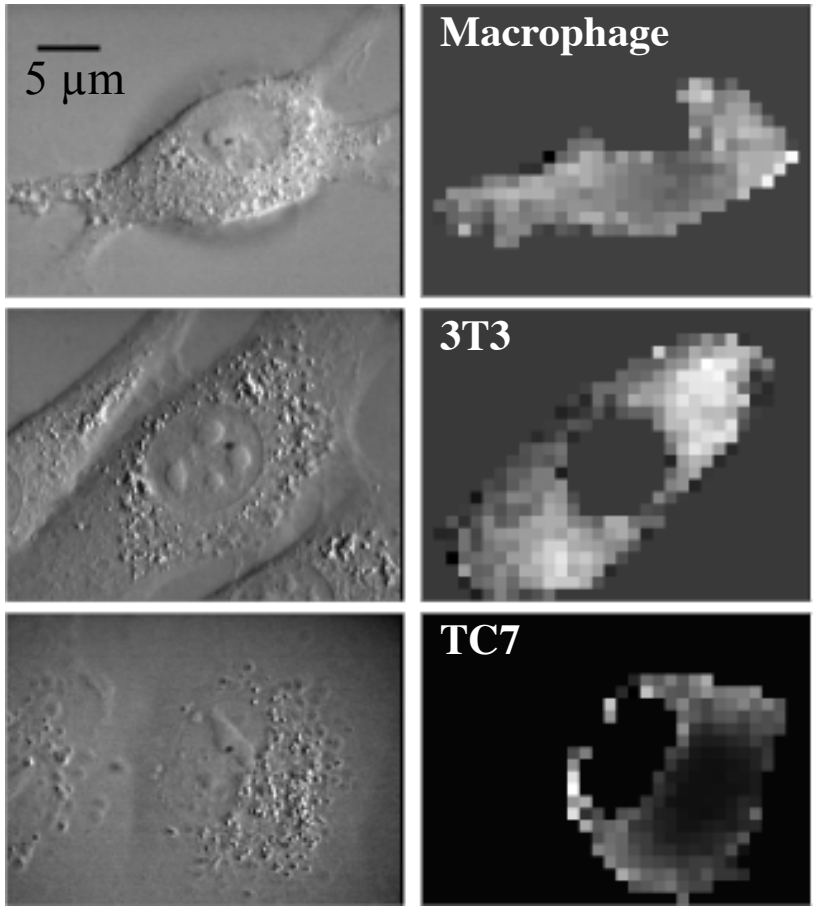


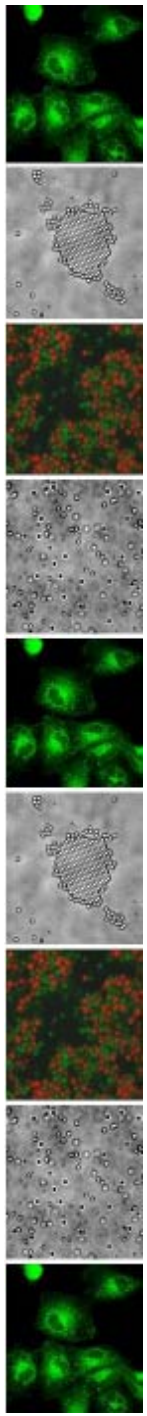
Two-point mapping

- Strain maps differ among cell types
- Contrast between interior and edge

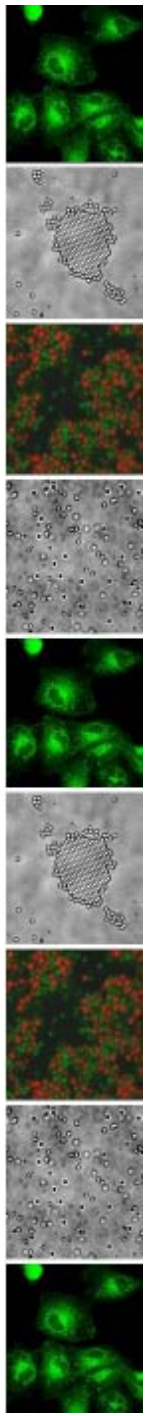
  factor of 2x or so
- Dark region is rough endoplasmic reticulum (RER)



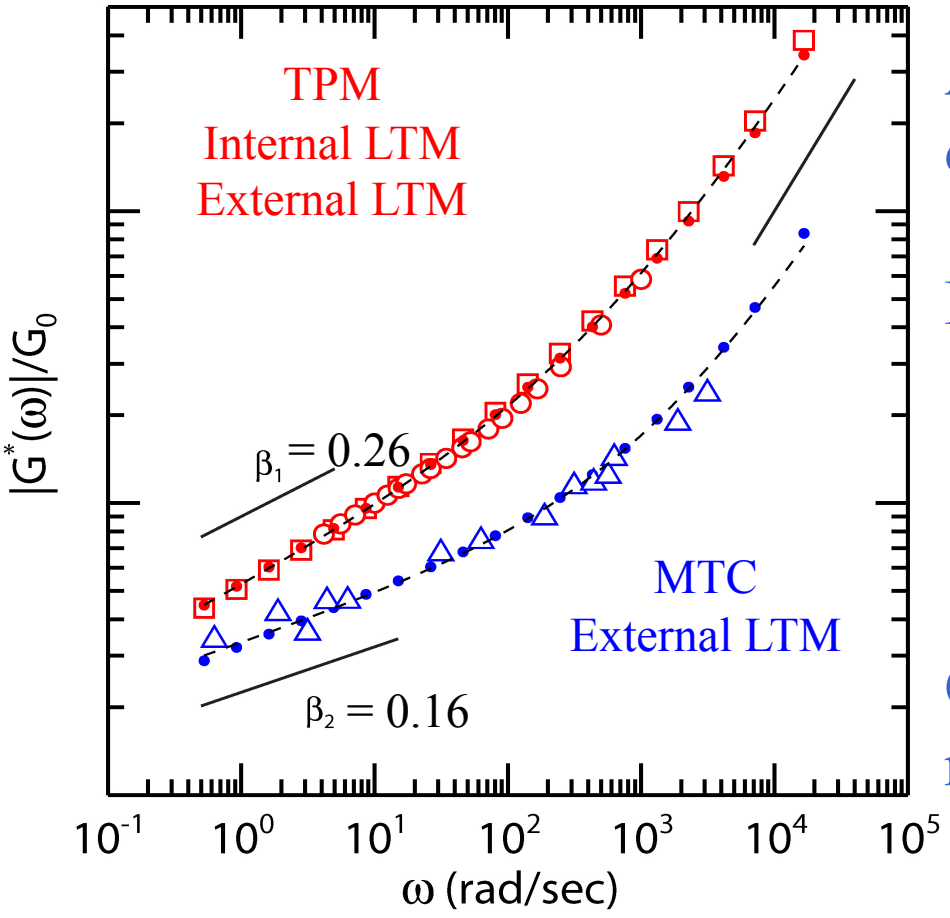




Do the four techniques agree?



Two *Different* Responses….

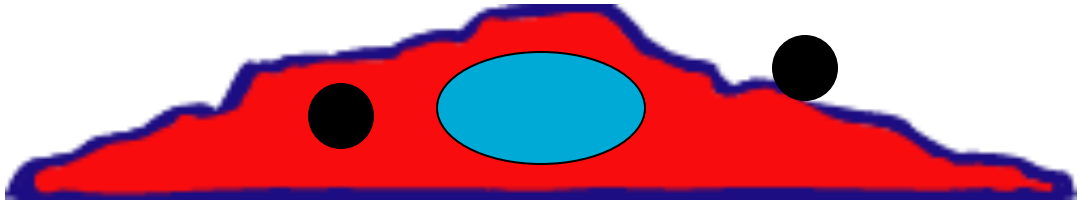


Amplitude (stiffness) divided out

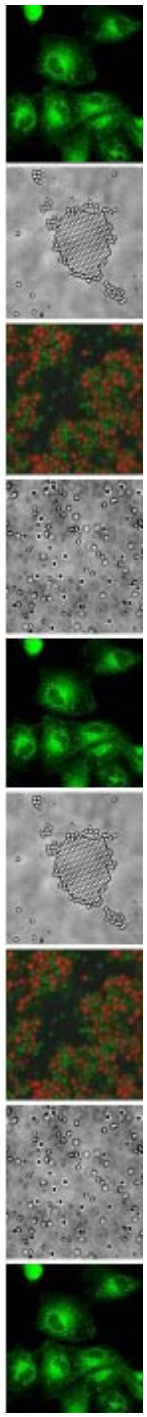
Both responses fit by:

$$\frac{|G^*(\omega)|}{G_O} \propto \omega^{\beta} + \omega^{3/4}$$

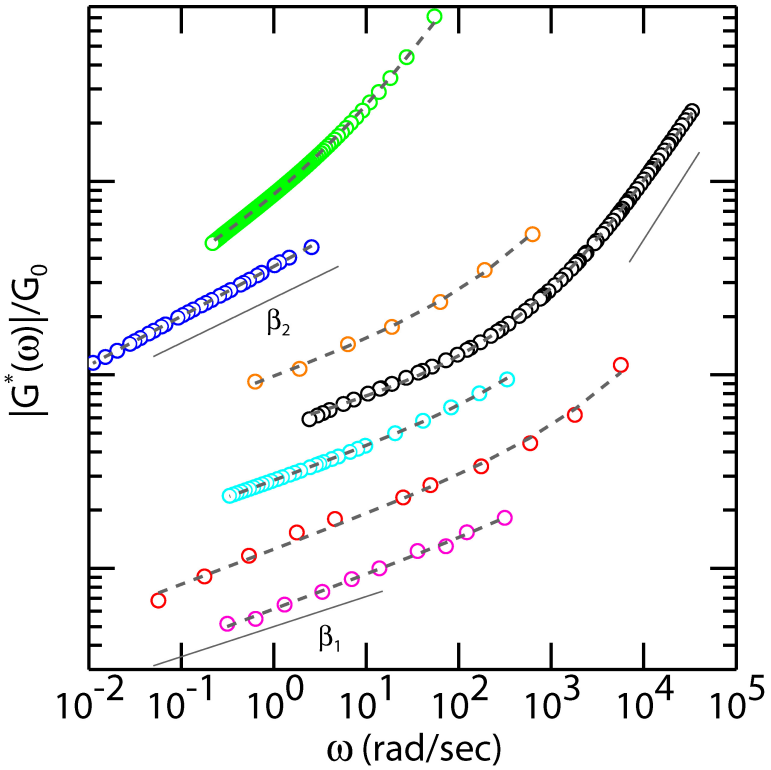
Cortical vs. Intracellular response.....



Hoffman, B.D., Massiera G., Van Citters K. Crocker, J., *PNAS* **103**, 10259, 2006.



Comparison to Literature

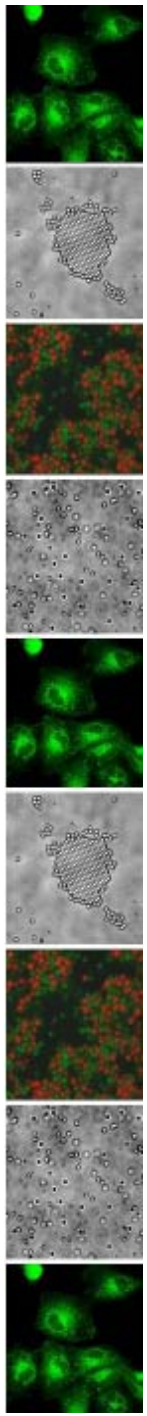


All active experiments:

- External Bead Pulling
  Feneberg, et. al BJ, 2004
- Uniaxial Rheometer
  Desprat, et. al., BJ, 2005
- AFM
  Alcaraz, et. al., BJ, 2003
- 'Lamella' LTM
  Yamada, et. al., BJ, 2000
- External Bead Twisting Creep
  Lenormand, et. al., J. R. Soc. Lond. Interface
- MTC
  Fabry, et. al., PRL, 2001
- External Bead Laser Trapping
  Balland, et. al., European BJ, 2005

- All fit by: $\left|G^*(\omega)\right| \propto \omega^{\beta} + \omega^{3/4}$
- Also fit into two groups of exponent values, cortical/ deep interior
- Caveat: Grouping may be spurious: two different structures may have similar rheology.

Hoffman, B.D., Massiera G., Van Citters K. Crocker, J., *PNAS* **103**, 10259, 2006.



A Universal description?

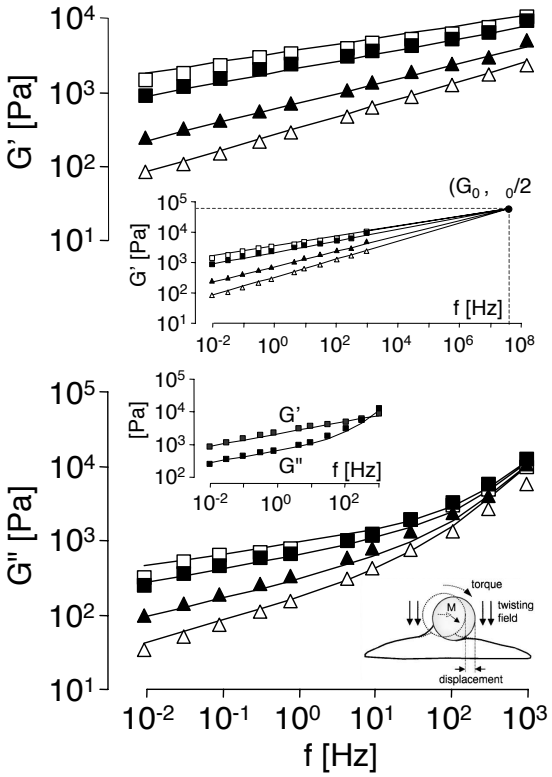
- Cells are “power-law fluids”:

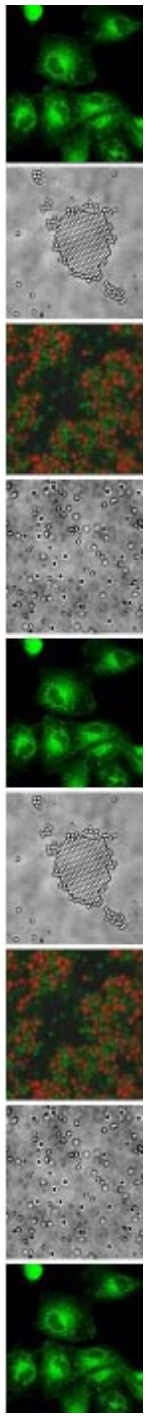
$$G^*(\omega) = G_0\left(\frac{\omega}{\omega_0}\right)^{\beta} + \text{viscosity}$$

- *Five decade range*
- *Exponent is continuously distributed:* $0.1<\beta<0.25$

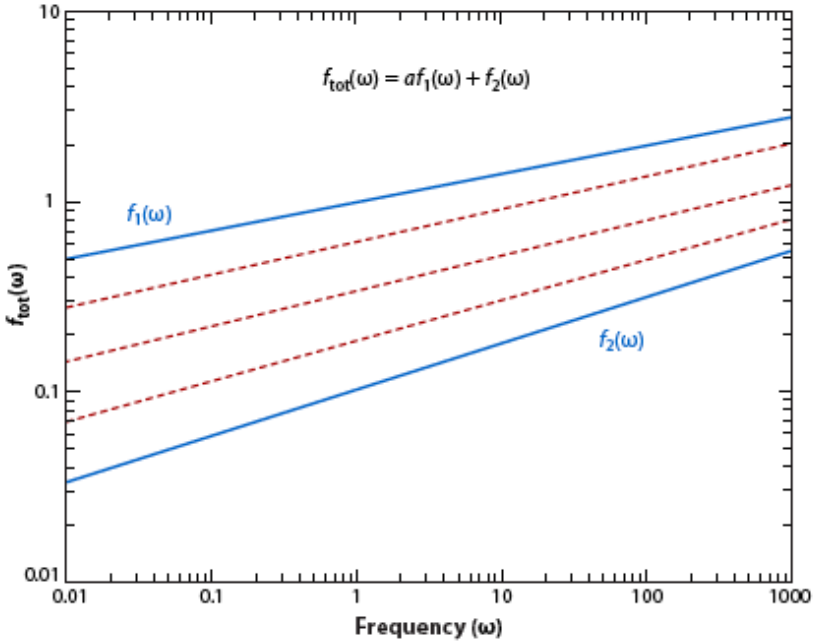
- SGR Theory ?!

Fabry *et al.*, *Phys. Rev. Lett.* 87, 148102 (2001).



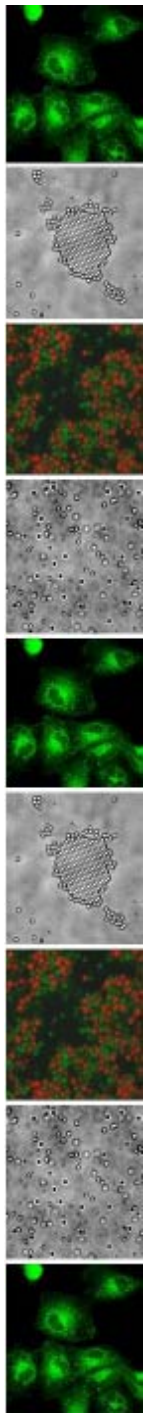


Challenge 3: Power-law math



Linear superposition of two weak power-laws creates 'intermediate power-law exponents'!

- Family of cross-over curves extrapolate to a point
- May be unreliable to use exponents as 'fingerprints' for identifying mechanical structures.
- Might see a composite result -> need to 'dissect'.



Plan/Outline

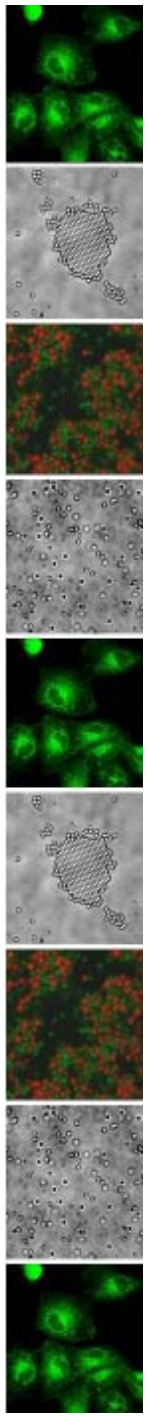
I. Background

II. Different Challenges

III. Find a consensus rheology

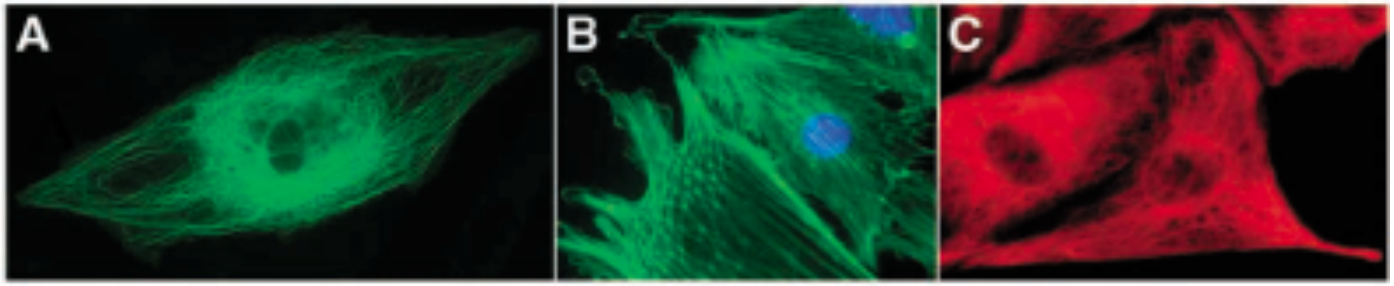
IV. Molecular roles in rheology

V. Active gel behavior



The Choices....

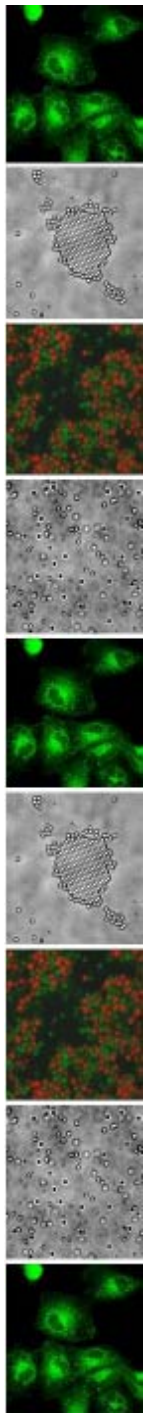
Ingber, JCS, 2003



Microtubules Actin Intermediate Filaments

- Actin Network
- Microtubules
- Intermediate filaments (keratin, vimentin, etc)
- Stress fiber network (actin-myosin)
- Smooth ER (bicontinuous lipid tubule network)
- Rough ER, Golgi (lamellar lipid stacks)

Approach: biochemical perturbation rather than mechanical 'dissection'



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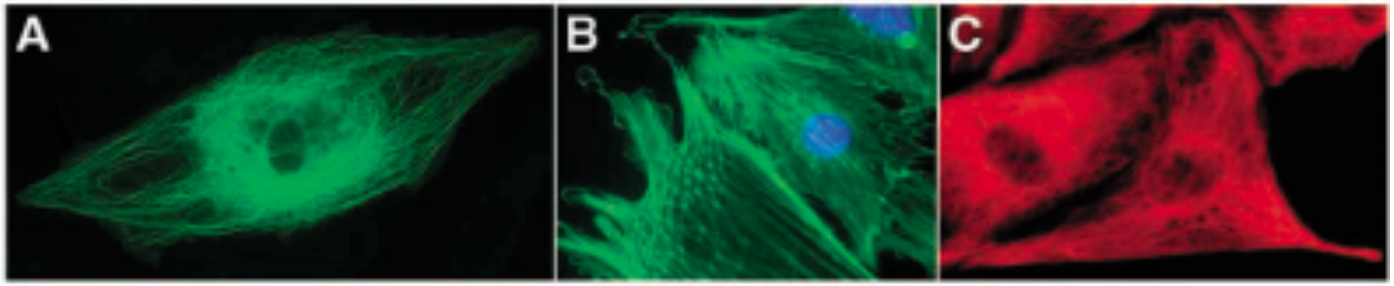
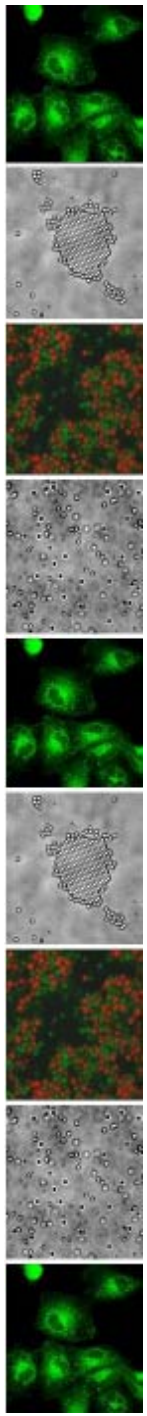


Microtubules Actin Intermediate Filaments

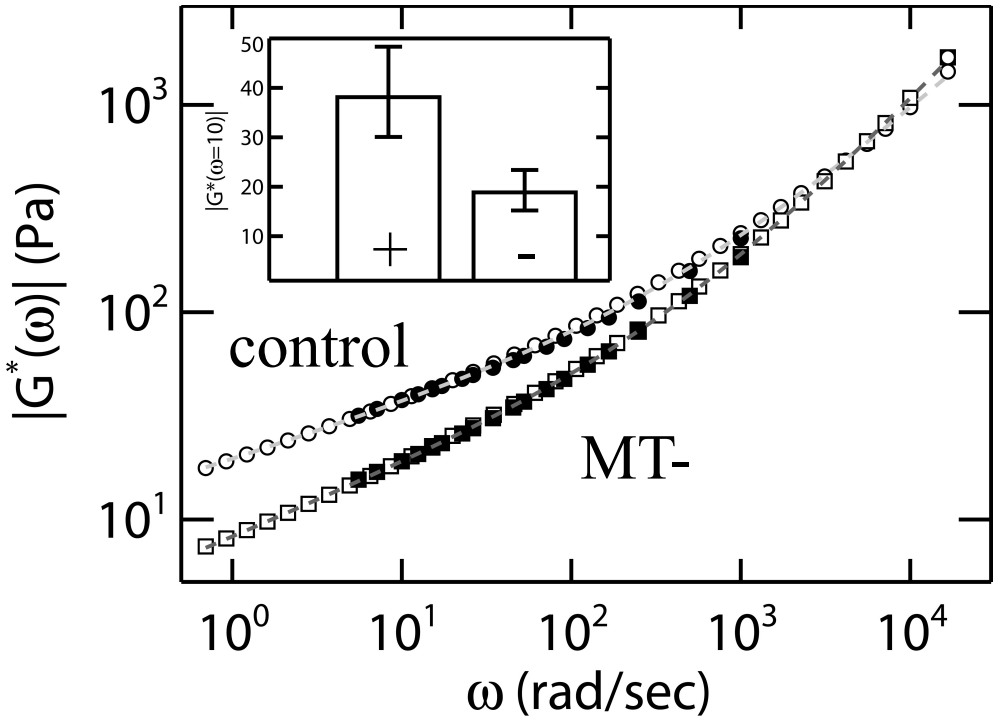
Actin Network	Latrunculin A
Microtubules	Colchicine
Intermediate filaments	Gene knockout ?
Stress fiber network	Blebbistatin
Smooth ER	MβCD ?
Rough ER, Golgi	Brefeldin A ?

Challenges: 2+ separate structures/compartments, networks are interconnected, cells can 'adapt'.....

Do 4 measurements per treatment and a lot of controls/interpretation.

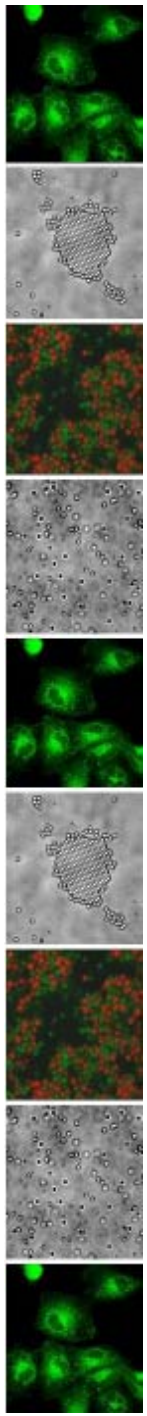


MT disruption (interior)

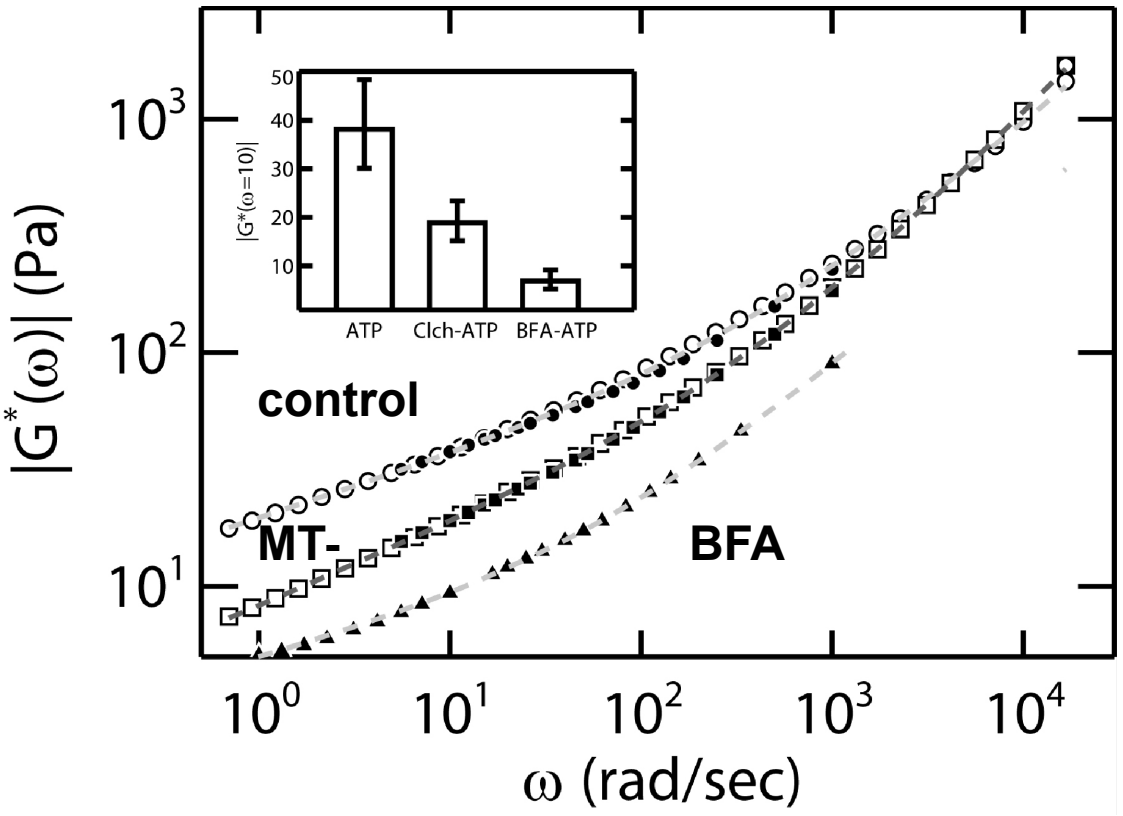


TPM +
LTM (int)

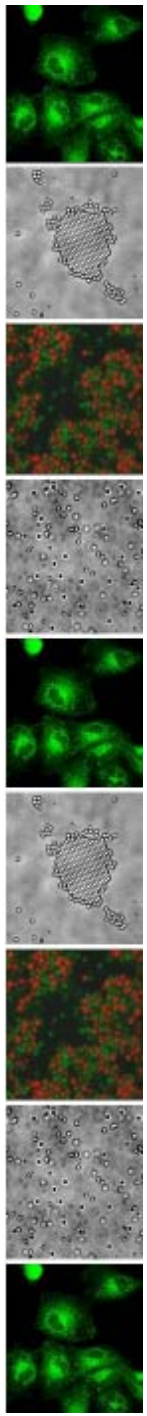
- Colchicine 10 μM, ATP depleted. (Staining not shown)
- Roughly a 2X softening at 10 rad/sec, ($p<0.01$).
- Different exponents in fit, 0.26->0.33.
- Suggests a mechanical role for MTs, but not only MT.
- No cortical effect (not shown)



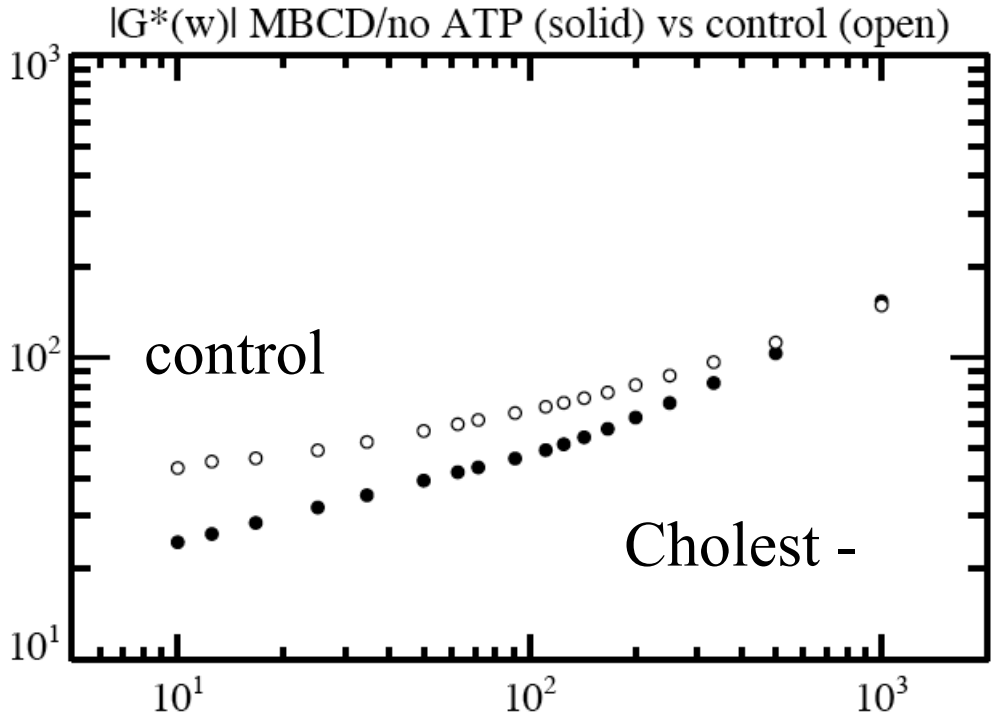
ER perturbation (BFA)



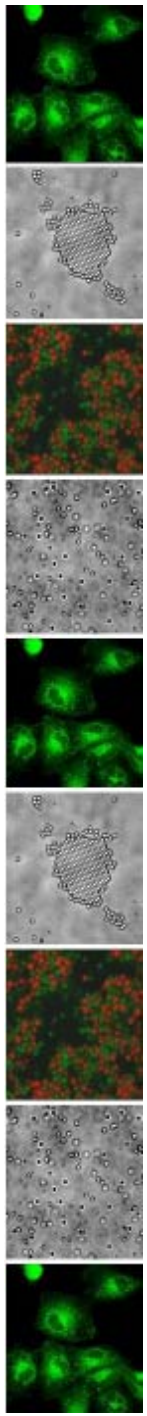
- ER perturbation with BFA leads to 3X softening (interior).
- Softening appears frequency independent.
- NB: dense membrane systems have power-law rheology.



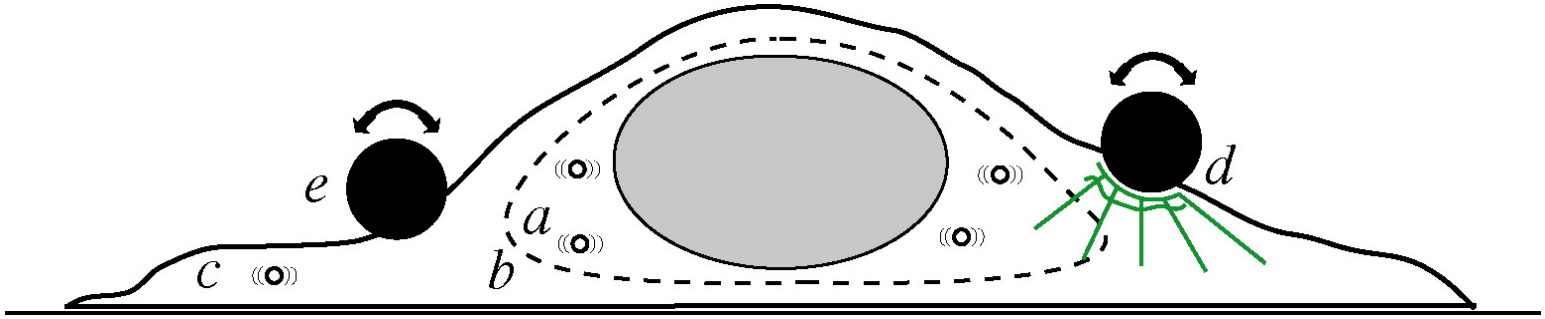
ER perturbation (MβCD)



- Cellular cholesterol depleted by methyl-β-cyclodextrin (MβCD)
- Roughly a 2X softening ($p<0.05$).
- Rheology exponent increases, similar to MT disruption.
- Suggests endomembrane (EndoM) role in stiffness



Summary (so far)

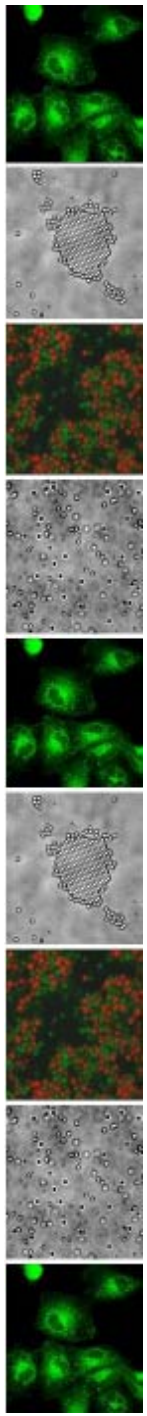


- Three structures: cortex/lamellipodium (b,c), interior (a), and stress fiber network (d).
- Cortex is actin; stress fibers are actin/myosin
- Interior is a complex composite of microtubules and lipid-based structures.

Other studies.....

- Little/no IF role in linear rheology
- Nuclear envelope is stiff and has power-law rheology

Hoffman BD, Crocker JC, *Ann Rev of Biomed Eng*, **11**, 259-288, 2009.



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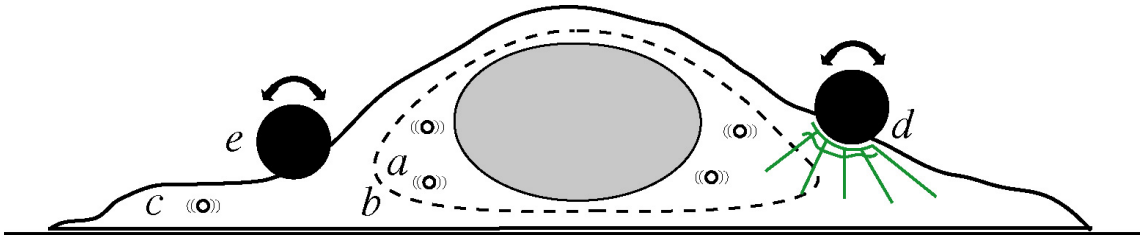
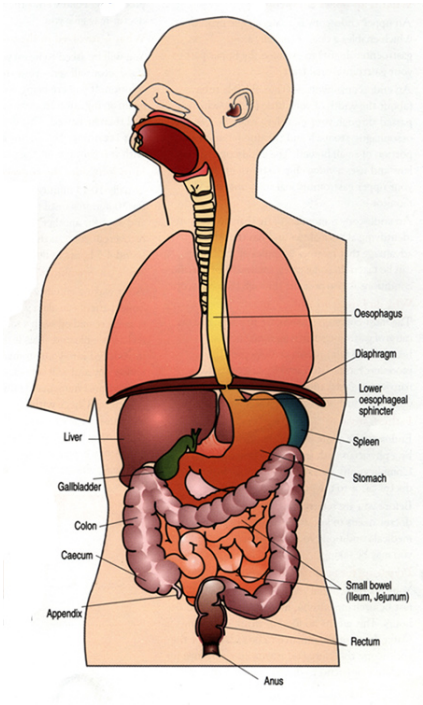
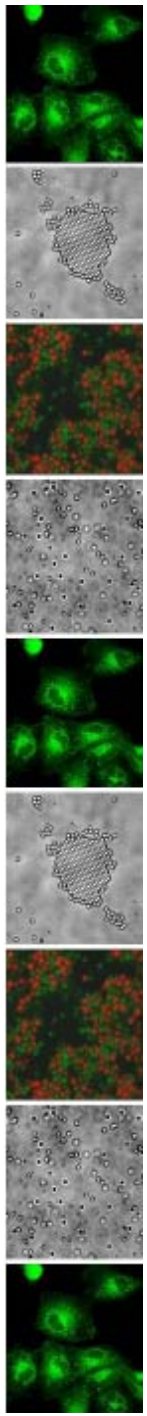




The functional biomechanical organization of cells resembles that of metazoans:

- Delicate control/shell: brain/skull : nucleus/envelope
- Passive biochemical apparatus: thorax : perinucleus
- Dedicated motility apparatus: musculature : cortex

Hoffman BD, Crocker JC, *Ann Rev of Biomed Eng*, **11**, 259-288, 2009.



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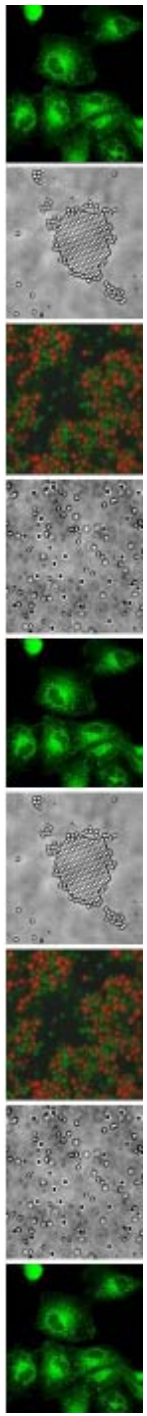
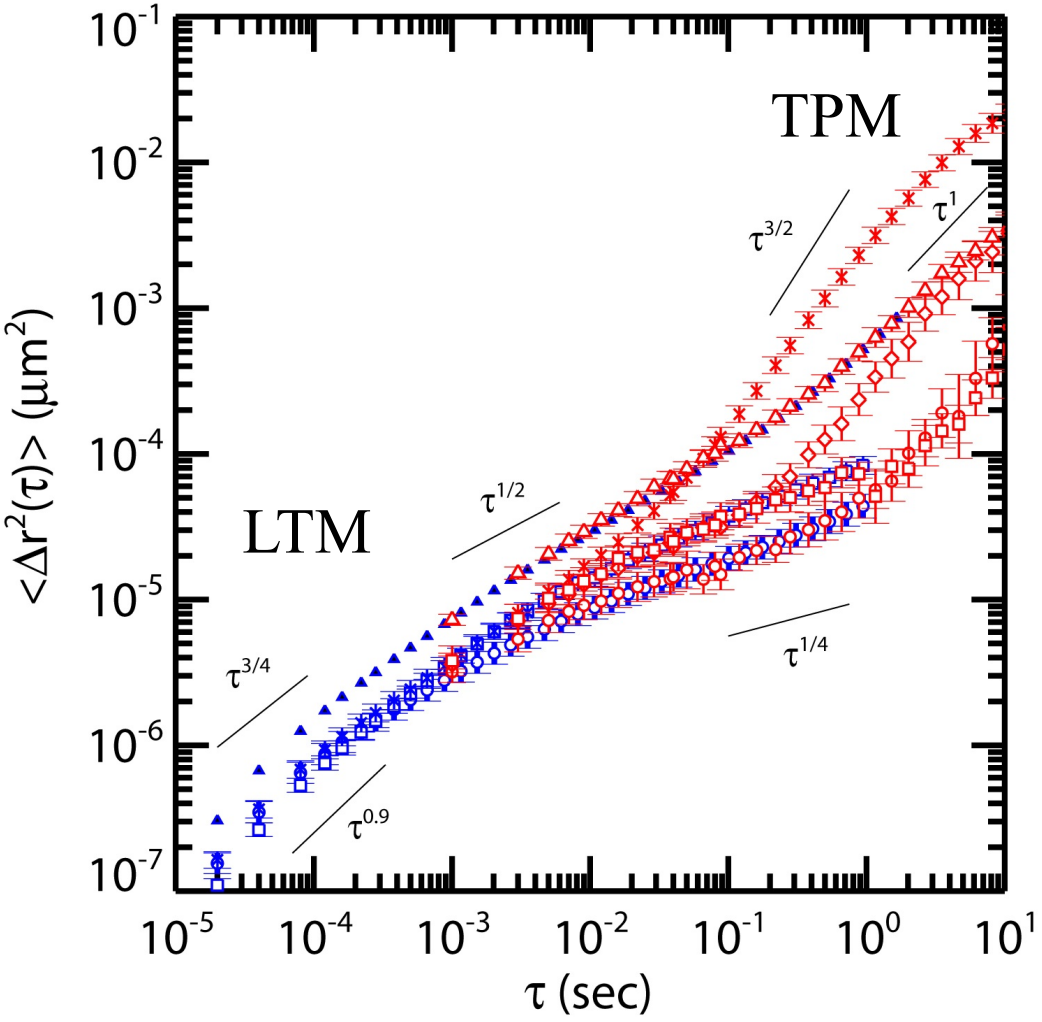


Active gel behavior of cells



controls cells (X

colchicine (△

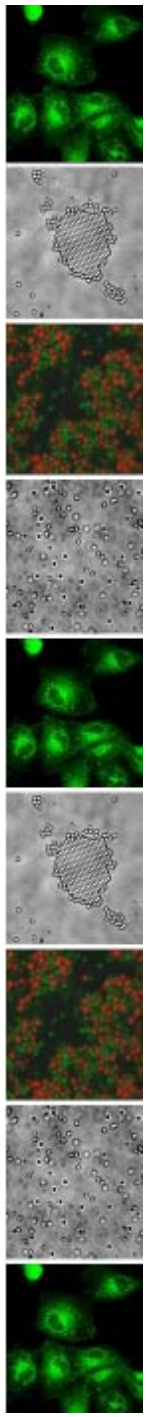
colchicine and latrunculin (▽

ATP depletion(○

ATP Depletion and colchicine(□

- Increased MSD is either:
  (1) softer
  (2) more motors
- How to disentangle?

Lau, Hoffman, Crocker, and Lubensky, PRL 91, 198101 (2003)



Quantifying active fluctuations...

- GSER no longer valid in a non-thermal regime

$$D_{rr}(\omega, r) = \frac{k_b T}{2\pi\omega r G^*(\omega)}$$

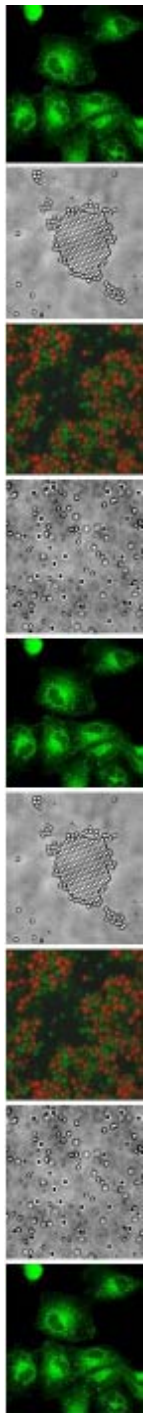
- Two point correlation still valid in an active, driven medium

- Brownian driving replaced by $\Delta(\omega)$, Fourier power spectrum of the fluctuating stresses (assume stresslet force dipoles)

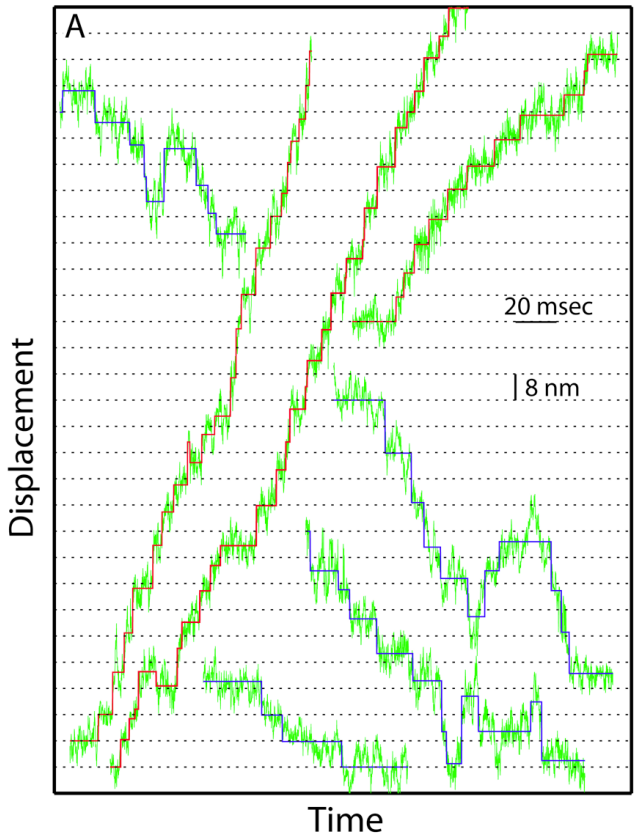
Linear Response $\longrightarrow$ $strain^2 \propto \frac{stress^2}{|G^*|^2}$ $\longrightarrow$ $D_{rr} = \frac{\Delta(\omega)}{6\pi r |G^*(\omega)|^2}$

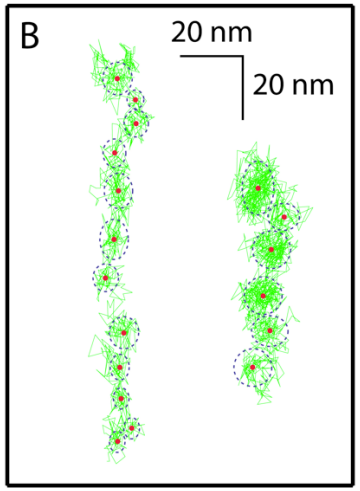
- If we know $G^*(\omega)$ and D_{rr} then can compute $\Delta(\omega)$ ....
- Use ATP depleted cells to get rheology, as before.

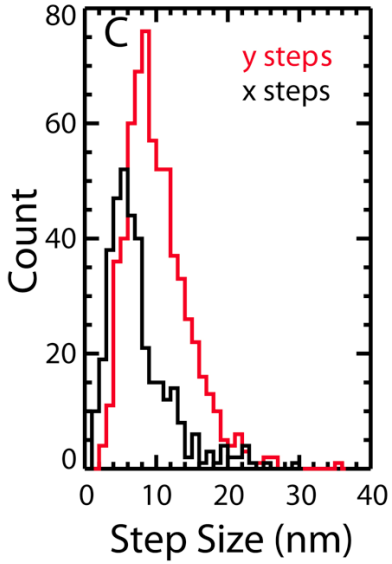
Lau, Hoffman, Crocker, and Lubensky, PRL 91, 198101 (2003)



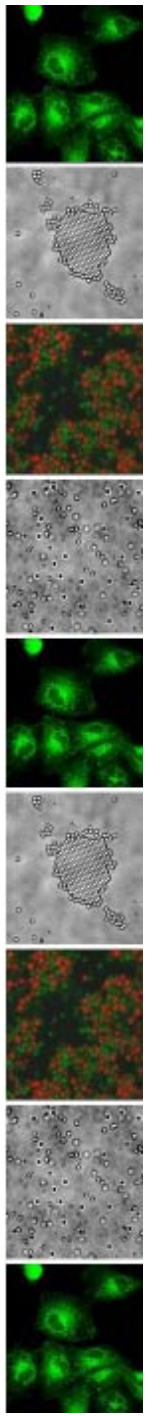
Contribution #1 Molecular Motors







- Step size and rates match expectations for MT motors
- Inbound (dynein) take larger steps than outbound (kinesin)
- Bidirectional motion from multiple motors ?
- 'Jiggling' between steps is >> tracking error



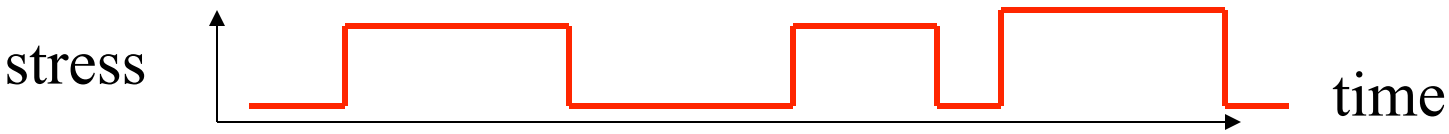
Contribution #1 Molecular Motors

- Molecular motor stress steps have ~ ω^{-2} spectrum,

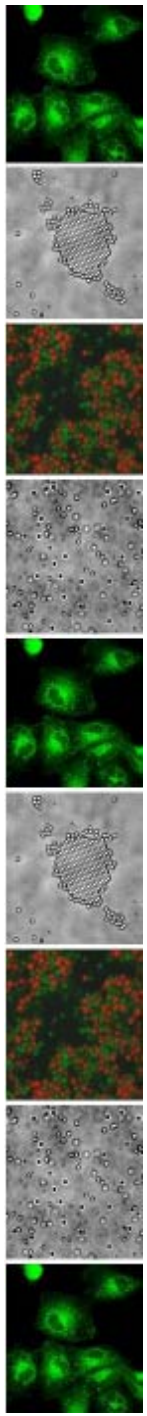
  stress kicks have ~ ω^{0} spectrum.

  Pulses, mean length τ_m : Lorentzian w/ corner freq $1/\tau_m$

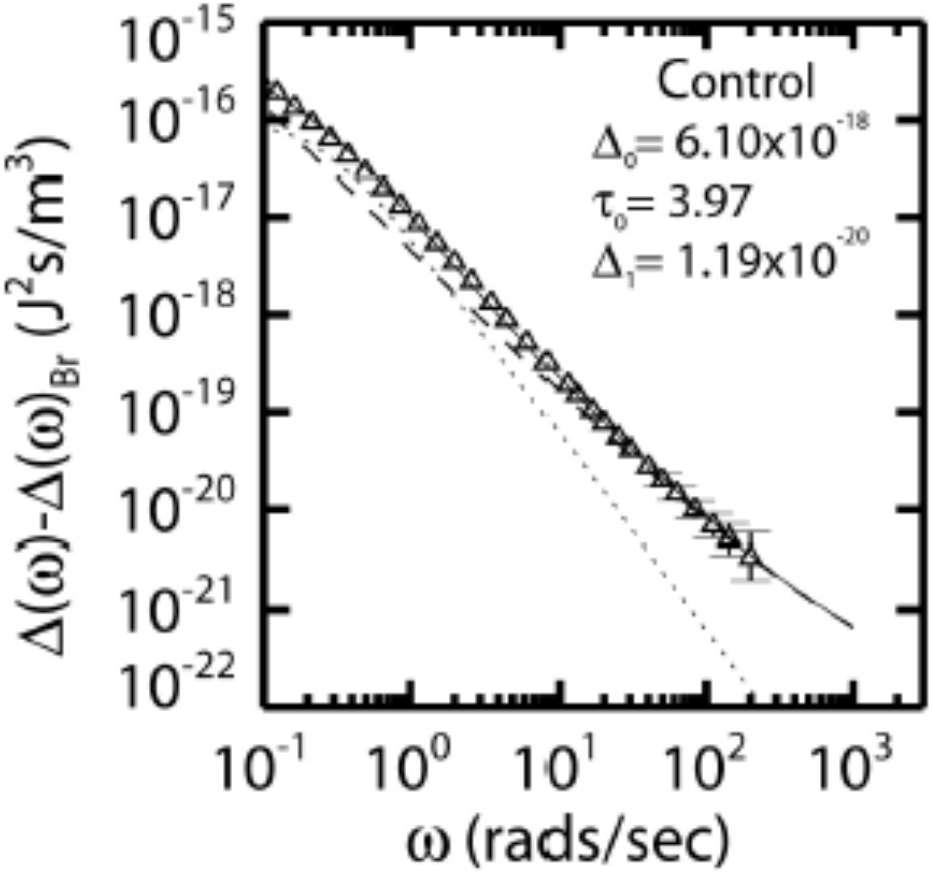
$$\Delta_m(\omega) \sim 1/(1+(\omega\tau_m)^2)$$



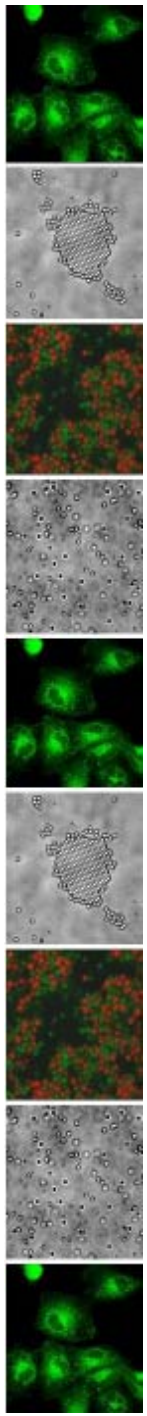
Contains information about motor timescales...



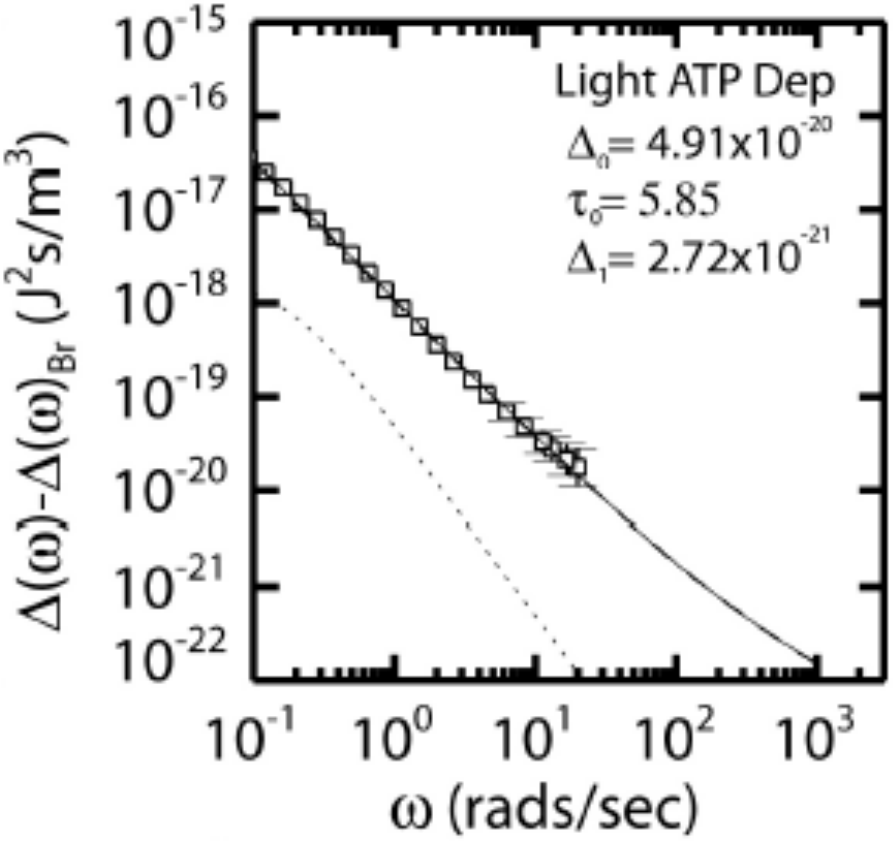
Stress spectra (normal cells)



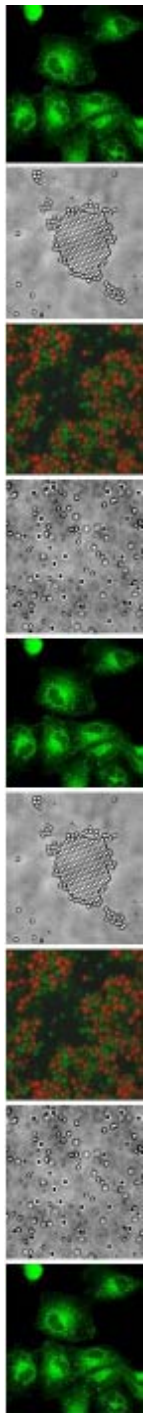
- Normal cells require a superposition of Lorentzian and a power law $\sim \omega^{-3/2}$ — two sources? (Brownian subtracted)
- Motor timescale is ~ 4 sec, about right for MT motors such as kinesin



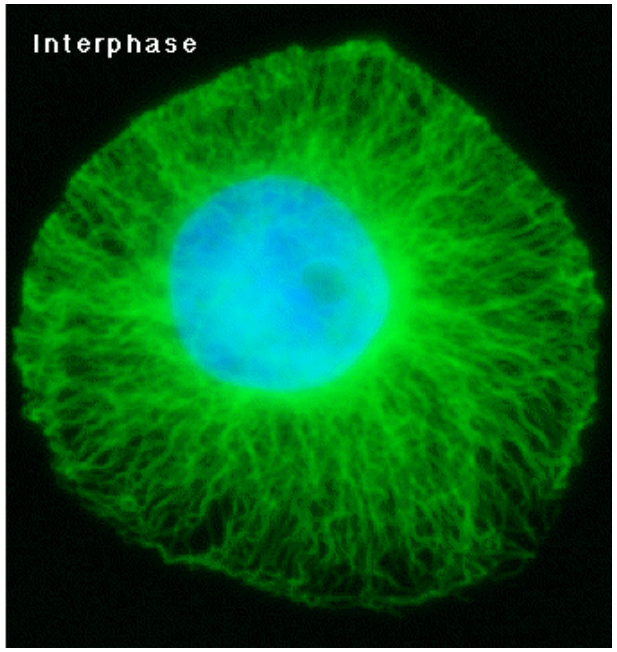
Stress spectra (slight ATP-)

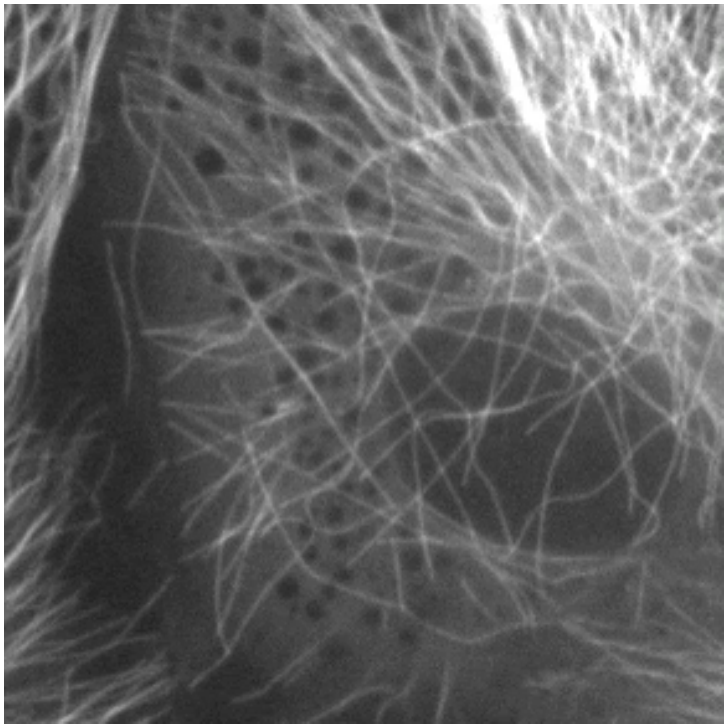


- Partial ATP depletion confirms two distinct sources
- Kinesin processivity is very sensitive to [ATP]
- $\sim \omega^{-3/2}$ fluctuations remain, dominate the spectrum.



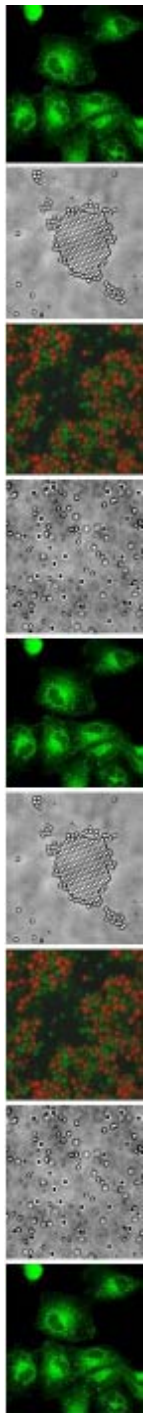
Contribution #2 MT remodeling





P Wadsworth, UMass

- Bending indicates MT are in stressed configurations
- Stress is applied (released) when MT (de)polymerize



Contribution #2 MT remodeling

Andy WC Lau, Florida Atlantic University

- Assume an active polar gel, having a spatio-temporally varying mean polarization field, obeying the PDE:

$$\partial_t p_i(\mathbf{x},t) = -\tau_p^{-1}[1 - \xi_p^2 \nabla^2]\, p_i(\mathbf{x},t) + s_i$$

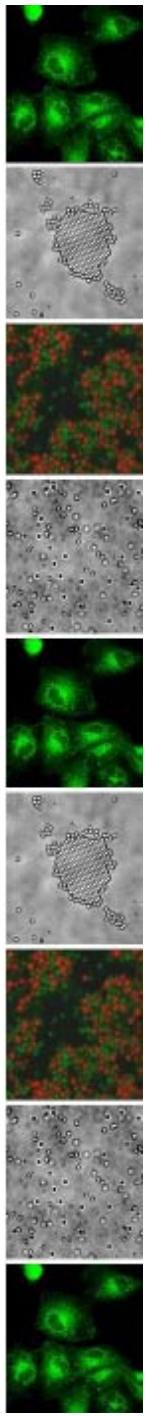
where τ_p is a relaxation time due to MT remodeling, s_i, and ξ_p is a correlation length due to MT bending.

- It can be shown that the TPM stress fluctuations have the form:

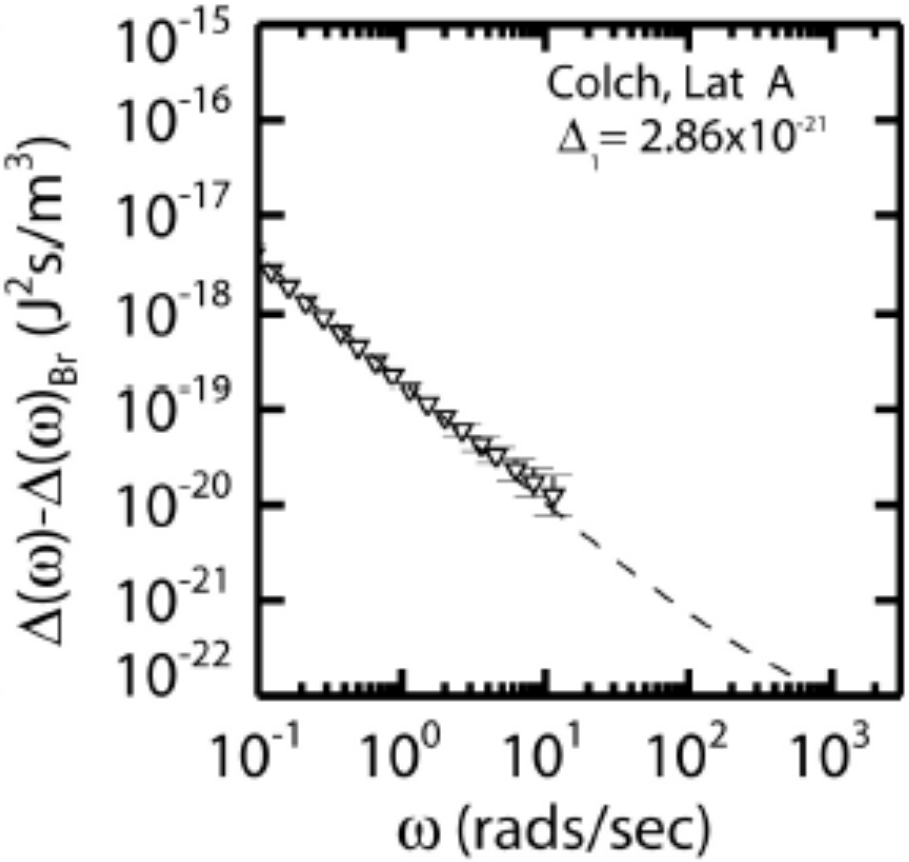
$$\Delta_p(\omega) \sim J(\omega\tau_p/2) \qquad \text{where} \qquad J(x) = \frac{\sqrt{1+\sqrt{1+x^2}} - \sqrt{2}}{x^2}$$

which reduces to $\Delta_p \sim \omega^{-3/2}$ at high frequencies

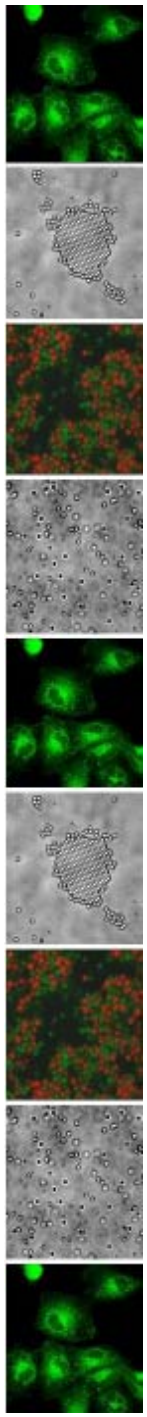
Hoffman, Van Citters, Lau, Crocker, submitted



Stress spectra (MT/actin negative)



- Colchicine and Latrunculin A disrupt MT and actin.
- Weak, ATP dependent residual stress fluctuations....
- Unknown origin, corresponds to simple diffusion in time



Conclusions

- Animal cells in culture are structured objects, with spatially distinct mechanical subunits seen by different methods.
- Deep interior is a composite of microtubules and lipid structures.
- Stress fluctuations in cells have three distinct sources: MT motors, MT remodeling, and a third unknown source
- Cell subunits all have (different) power-law rheology, suggesting soft glassy dynamics is ubiquitous (but not universal)
- Need new soft matter theories/models.

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http://crocker.seas.upenn.edu