Microrheology of
Soft Matters and Active Materials

H. Daniel Ou-Yang
Lehigh University

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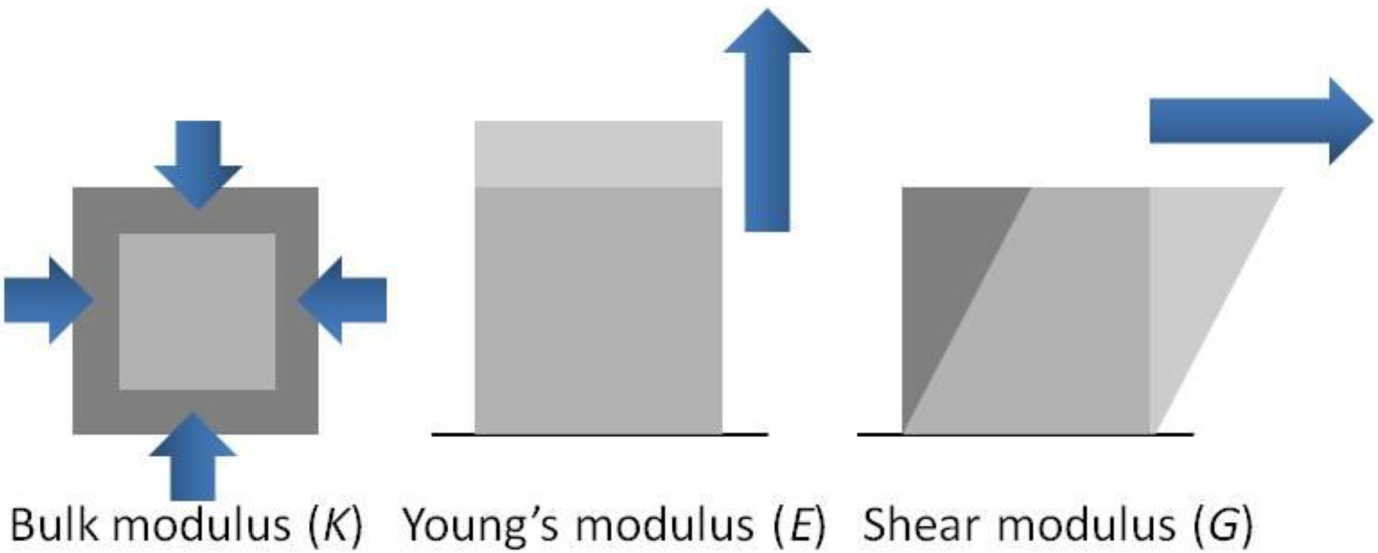
Introduction:

- In thermal equilibrium, mechanical fluctuations are related to the mechanical properties of the material through fluctuation-dissipation theorem (FDT).
- In an active system, noise and fluctuations can be generated by forces other than the thermal force, thus, the system does not necessarily follow the rules of FDT.
- We examine the nature of the mechanical noise in a non-equilibrium mechanical system inside a living cell to see what we can learn from it.

Outline

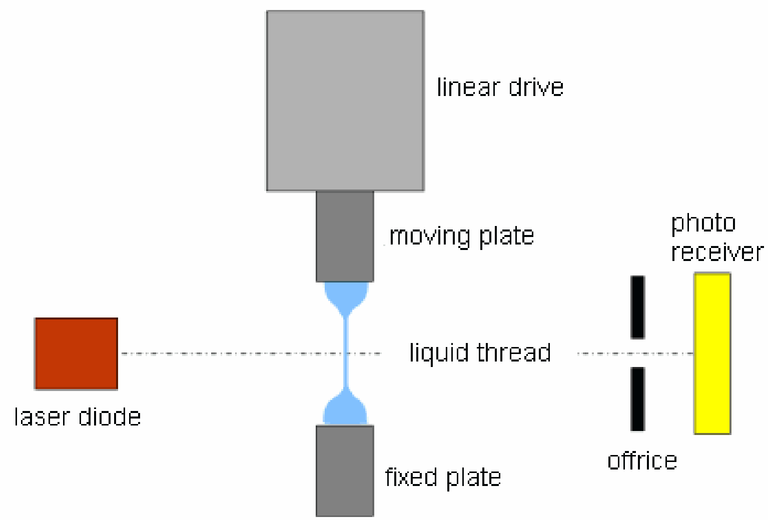
- Introduction
- Experimental tools
  - Optical tweezer-based microrheology
  - Passive single particle microrheology
- Viscoelastic polymer solutions
- Microrheology in living cell
- Intracellular mechanical properties of living cells are stress dependent
- Non-thermal noise in a non-linear mechanical system

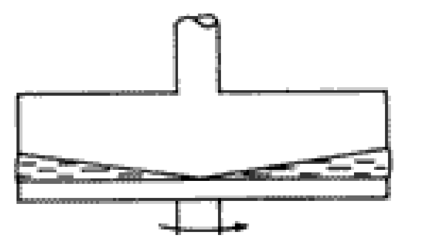
Mechanical Properties



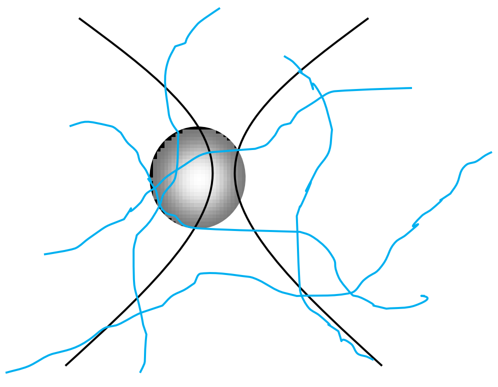
Rheology is the study of how materials deform and flow in response to externally applied force.

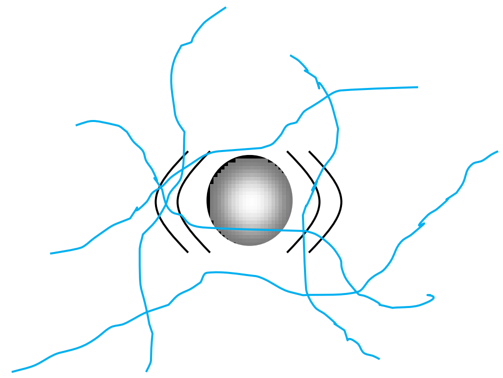
Conventional Rheometers





Microrheology: probes are small and often inside the materials





Active approach
Oscillating tweezers

Passive approach
Particle tracking

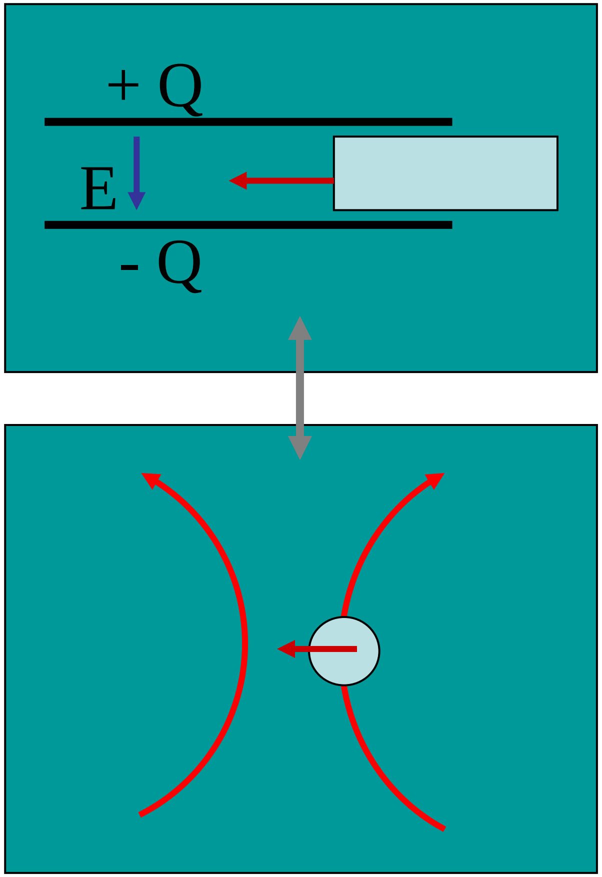
Fluctuation dissipation theorem

Optical Tweezers

$$\Delta U = -\frac{3\varepsilon_1\varepsilon_o}{2}\left[\frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1}\right]\left|E_1\right|^2 V_2$$

Dielectric particle near the focal point of a laser

$$U(r) = -\frac{3n_1^2 V_2}{c}\left[\frac{n_2^2 - n_1^2}{n_2^2 + 2n_1^2}\right] I_o e^{-r^2/R^2}$$



Optical tweezers act on the trapped micro-particle like a spring

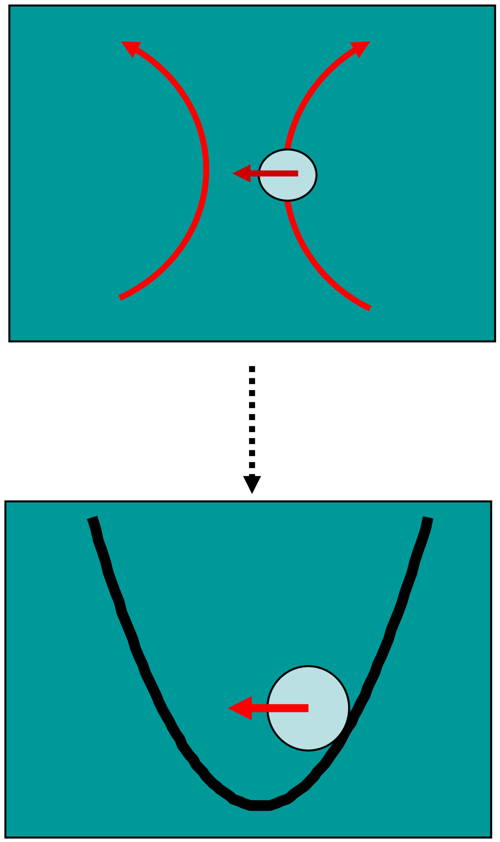
$$\vec{F} = -\Delta U = -\frac{6\, r\, V_2\, n_1}{c\, R^2}\, I_o \left(\frac{n_2^2 - n_1^2}{n_2^2 + 2\, n_1^2} \right) e^{-r^2/R^2}\, \hat{r}$$

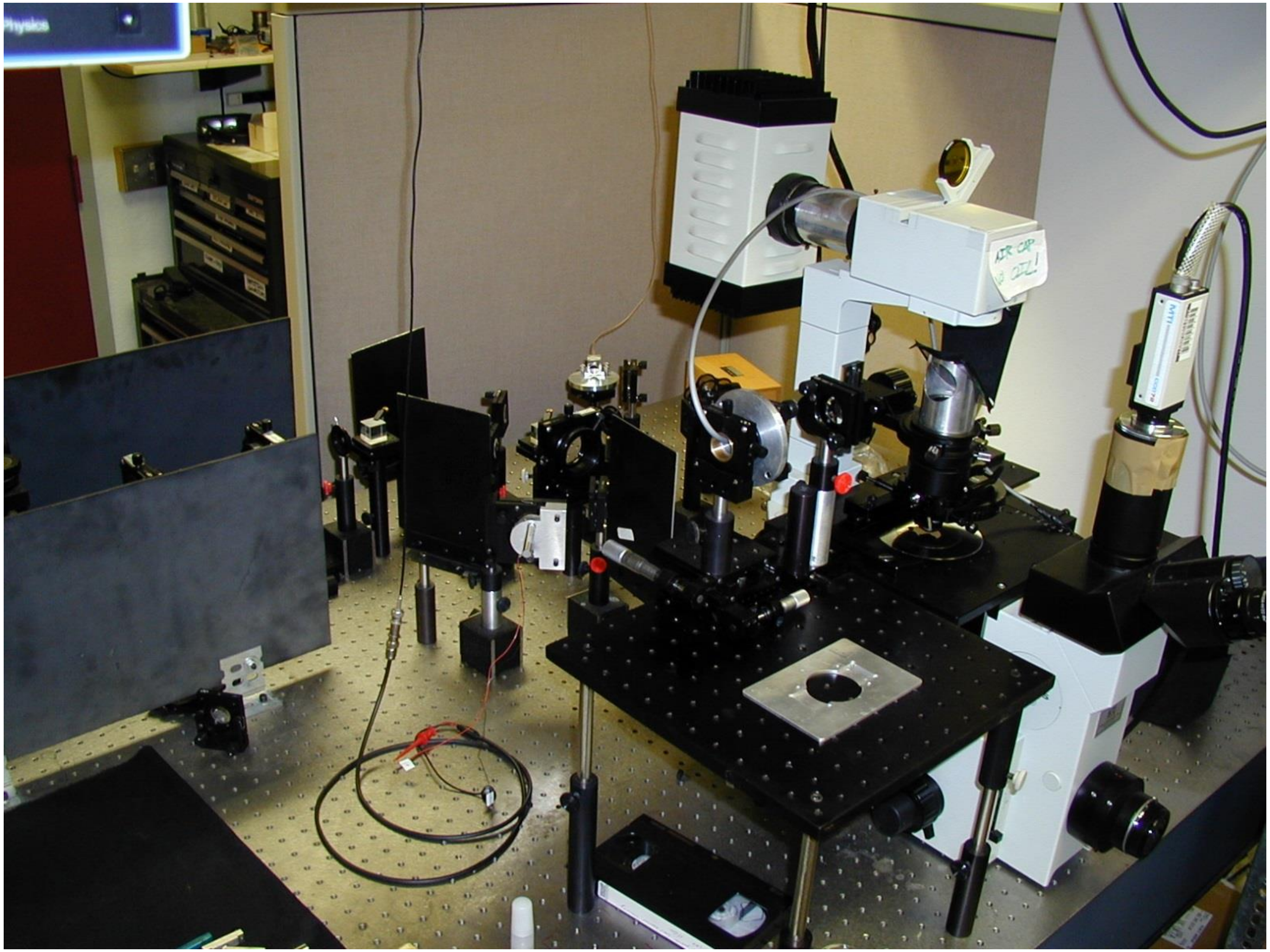
$$\equiv -k_{ot}\, r\, e^{-r^2/R^2}\, \hat{r}$$

For $r \leq 0.15R$, in one dimentional motions

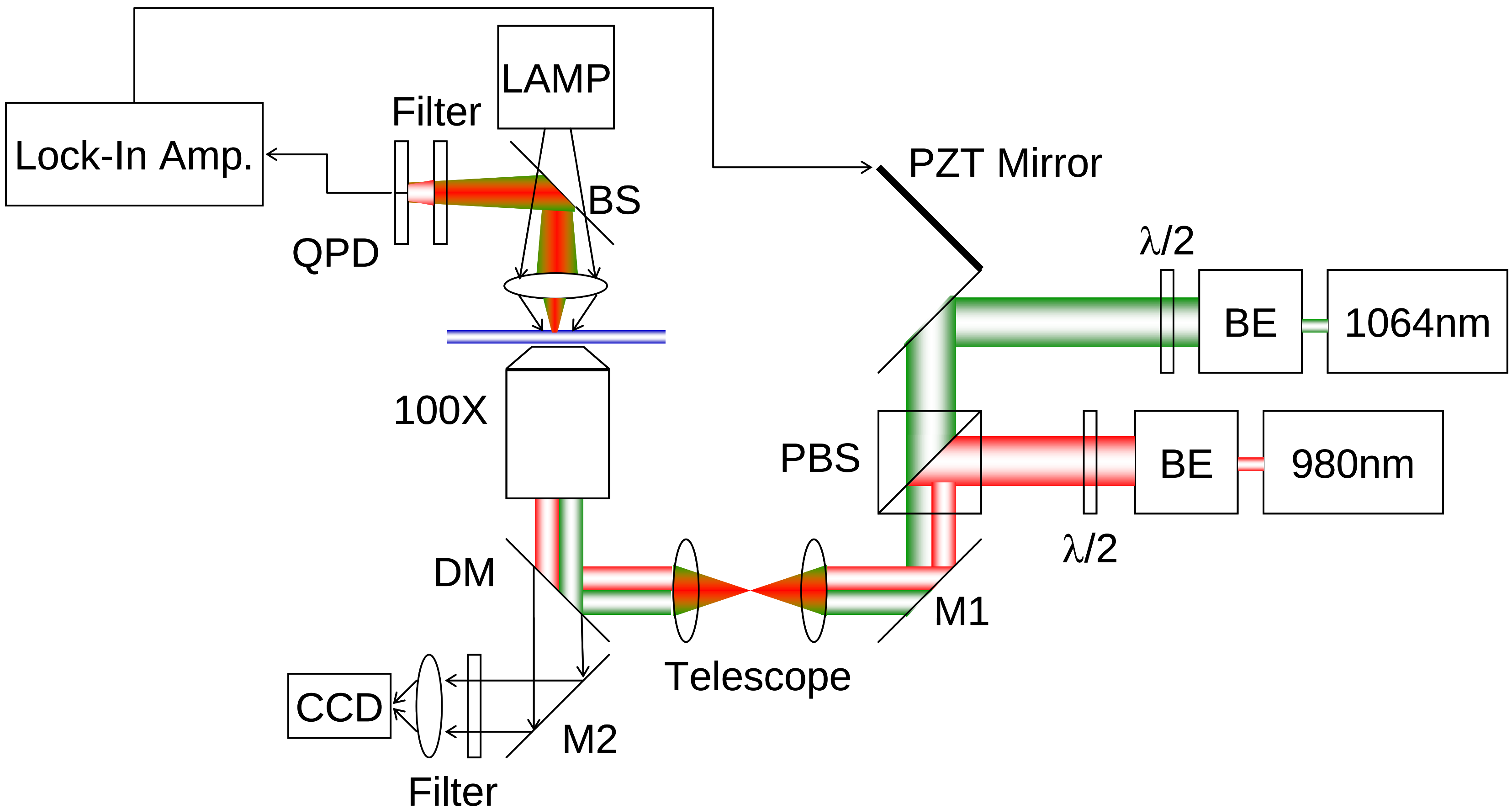
$$F \cong -k_{ot} x$$

$$k_{ot} \approx 10\, pN / \mu m = 1 \times 10^{-5}\, N / m$$

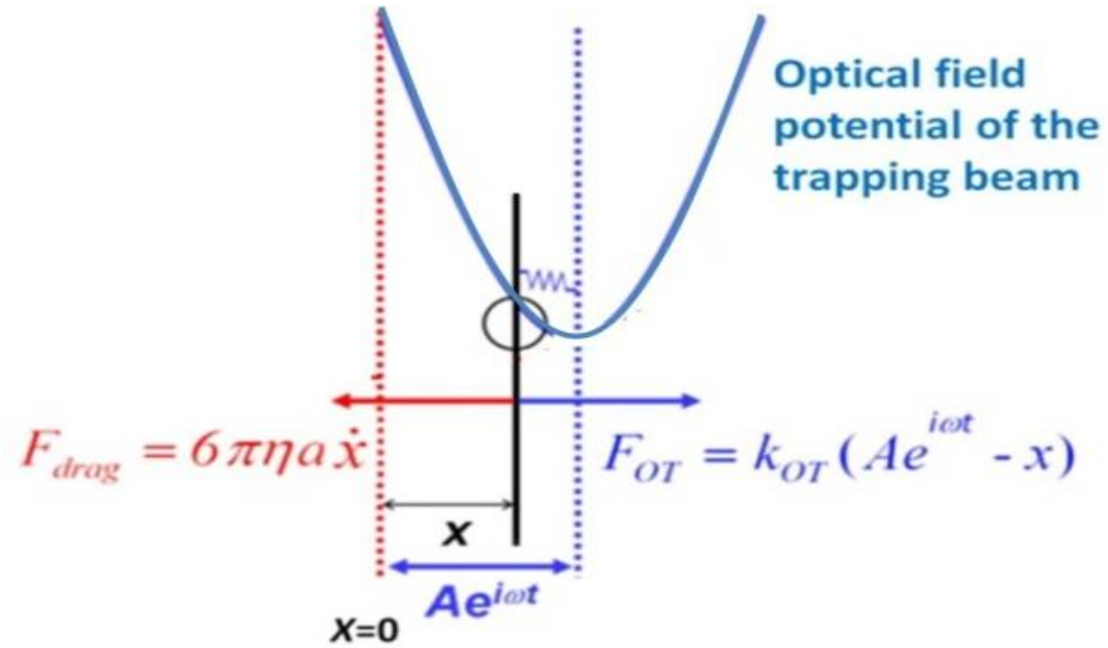



Physics
AIR CAP
NO OIL!
MTI

Setup of optical tweezers



Optical Tweezers as a Micro-spring



Valentine, UCSB

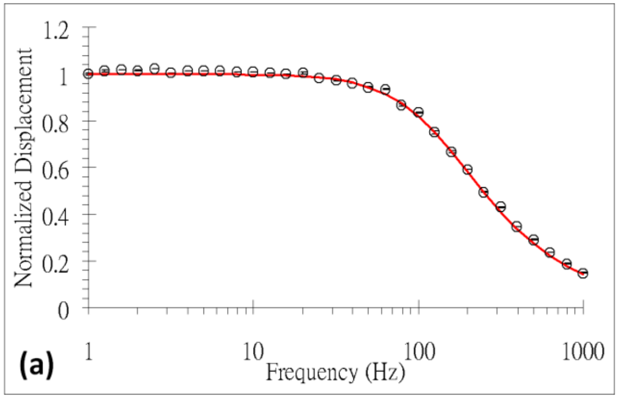
$$m\ddot{x}(\omega,t) = -6\pi\eta a\dot{x}(\omega,t) + k_{OT}(Ae^{i\omega t} - x(\omega,t))$$

Valentine, et al., *J. Phys.: Condens. Matter,* **8,** p. 9477–9482 (1996.).

Calibration of the Force Constant of Optical Tweezers

Polystyrene particle (diameter = 1.5 μm) in de-ionized water, 6 mW of laser power

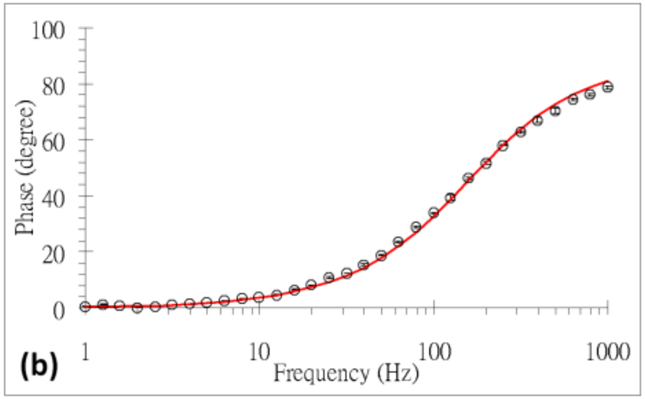
$$x(\omega,t) = D(\omega)e^{i(\omega t-\delta(\omega))}$$



15.65±0.52 pN/μm

$$D(\omega) = \frac{Ak_{OT}}{\sqrt{k_{OT}{}^2 + (6\pi\eta a\omega)^2}}$$

$$\delta(\omega) = \tan^{-1}\left(\frac{6\pi\eta a\omega}{k_{OT}}\right)$$



14.70±0.42 pN/μm

Lock-in Amplifier: how does it work?

Why Use a Lock-In?

Suppose the signal is a 10 nV sine wave at 10 kHz. Clearly some amplification is required to bring the signal above the noise.

Now try following the amplifier with a phase-sensitive detector (PSD). The PSD can detect the signal at 10 kHz with a bandwidth as narrow as 0.01 Hz! In this case, the noise in the detection bandwidth will be 0.5 μV (5 nV/√Hz × √.01 Hz × 1000), while the signal is still 10 μV. The signal-to-noise ratio is now 20, and an accurate measurement of the signal is possible.

Phase-Sensitive Detection

Typically, an experiment is excited at a fixed frequency and the lock-in detects the response from the experiment at the reference frequency.

Signal: $V_{sig}\sin(\omega_r t + \theta_{sig})$, where V_{sig} is the signal amplitude, ω_r is the signal frequency, and θ_{sig} is the signal's phase.

Reference: $V_L\sin(\omega_L t + \theta_{ref})$.

The output of the PSD: product of two sine waves.

$$V_{psd} = V_{sig}V_L\sin(\omega_r t + \theta_{sig}) \sin(\omega_L t + \theta_{ref})$$

$$= \tfrac{1}{2} V_{sig}V_L \{ \cos[(\omega_r - \omega_L) t + (\theta_{sig} - \theta_{ref})] - \cos[(\omega_r + \omega_L) t + (\theta_{sig} + \theta_{ref}] \}$$

The PSD output is two AC signals, one at the difference frequency $(\omega_r - \omega_L)$ and the other at the sum frequency $(\omega_r + \omega_L)$.

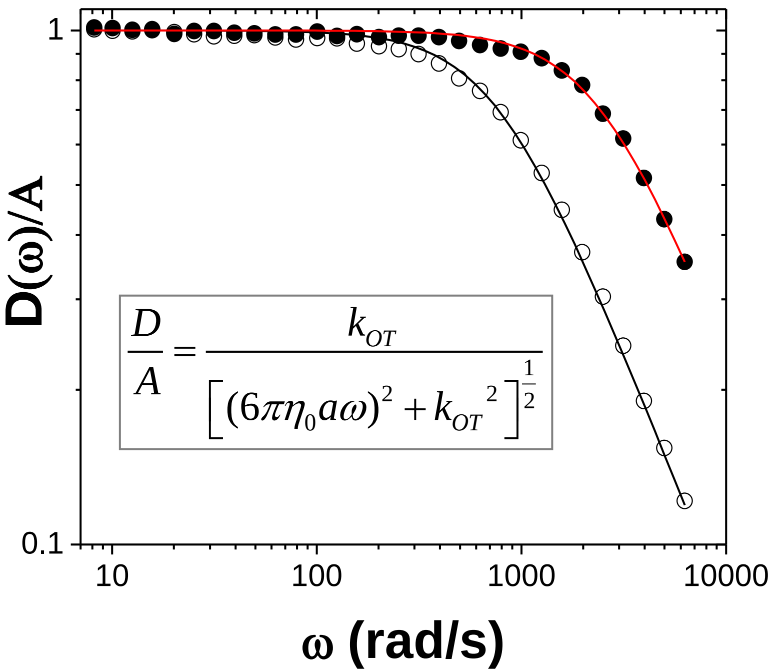
If the PSD output is passed through a low pass filter, the AC signals are removed. What will be left? In the general case, nothing.

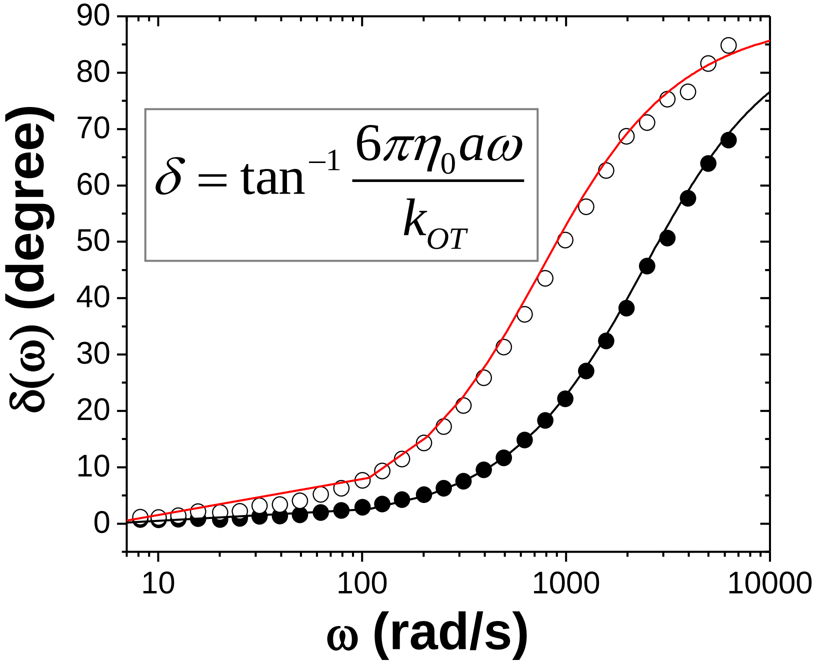
However, if ω_r equals ω_L, the difference frequency component will be a DC signal. In this case, the filtered PSD output will be:

$$V_{psd} = \tfrac{1}{2} V_{sig} V_L \cos(\theta_{sig} - \theta_{ref})$$

This is a very nice signal; it is a DC signal proportional to the signal.

Normalized Displacement D/A, and phase shift δ vs. frequency 1.6 μm silica particle in water.

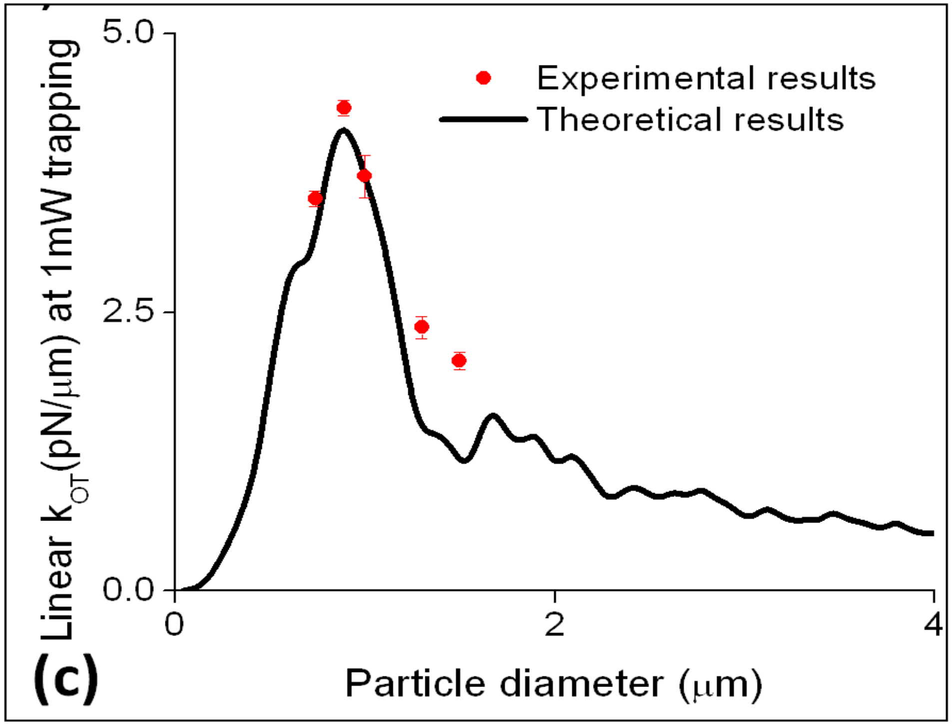




○: 48mW laser power at the optical trap, k_{OT} = 0.011 dyne/cm

●: 152mW laser power at the optical trap, k_{OT} = 0.034 dyne/cm

Calibration of the Force Constant of Optical Tweezers



M-T Wei *et al*. *SPIE Proc.* **8458** (2012)

Response Function for an Oscillating Particle in a Viscoelastic Medium

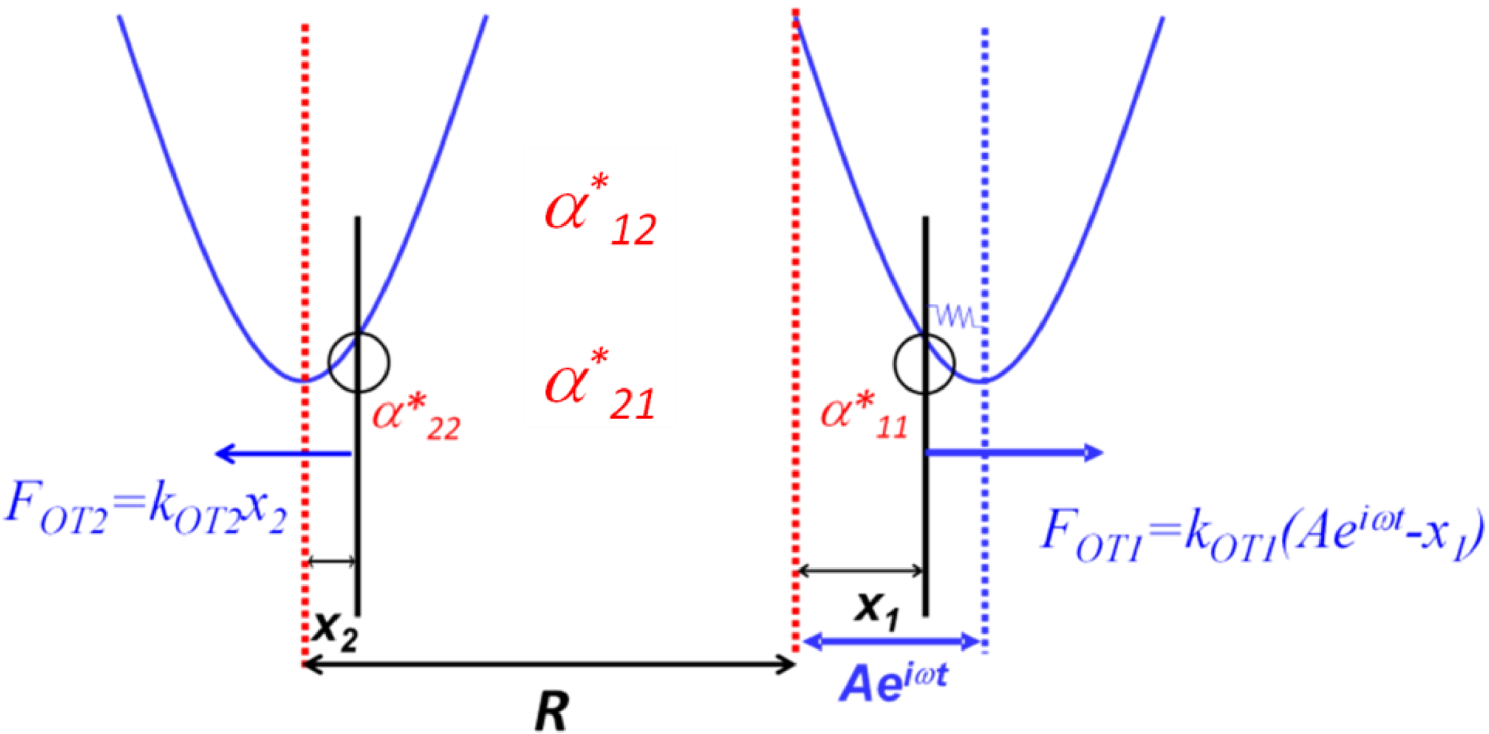
$$6\pi\eta^* a\dot{x}(\omega,t) + k_{OT}x(\omega,t) = k_{OT}Ae^{i\omega t}$$

$$G^*(\omega) = i\omega\eta^*(\omega) = G'(\omega) + iG''(\omega)$$

$$G'(\omega) = \frac{k_{OT}}{6\pi a}\left(\frac{A\cos\delta(\omega)}{D(\omega)} - 1\right) \quad and \quad G''(\omega) = \frac{k_{OT}}{6\pi a}\left(\frac{A\sin\delta(\omega)}{D(\omega)}\right)$$

Larry Hough, Solvay

Two-particle active microrheology for an inhomogeneous medium



Hough et al *Phys Rev E,* (2002).

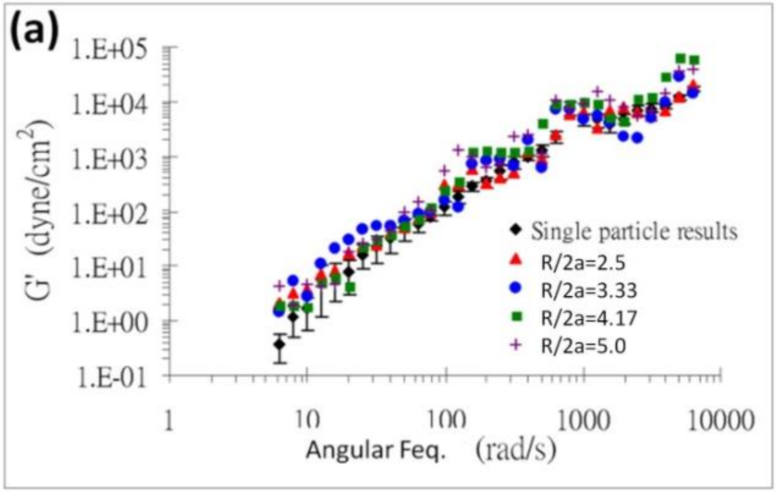
Two-particle active microrheology for an inhomogeneous medium

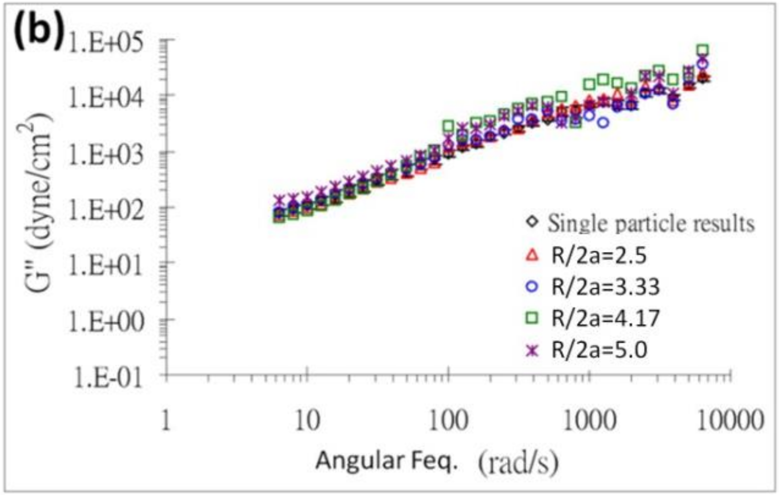
$$G_{12}{}^{*}(\omega) = \frac{k_{OT}}{4\pi R}\left[\frac{A}{x_2(\omega)} + \frac{x_1(\omega)}{x_2(\omega)}\left(\frac{x_1(\omega)}{A} - 2\right)\right]$$

$$G_{12}'(\omega) = \frac{k_{OT}}{4\pi R}\left[\frac{A\cos\delta_2(\omega)}{D_2(\omega)} + \frac{D_1{}^2(\omega)\cos(\delta_2(\omega) - 2\delta_1(\omega))}{AD_2(\omega)} - \frac{2D_1(\omega)\cos(\delta_2(\omega) - \delta_1(\omega))}{D_2(\omega)}\right]$$

$$G_{12}''(\omega) = \frac{k_{OT}}{4\pi R}\left[\frac{A\sin\delta_2(\omega)}{D_2(\omega)} + \frac{D_1{}^2(\omega)\sin(\delta_2(\omega) - 2\delta_1(\omega))}{AD_2(\omega)} - \frac{2D_1(\omega)\sin(\delta_2(\omega) - \delta_1(\omega))}{D_2(\omega)}\right]$$

Two-particle active microrheology for an inhomogeneous medium





A comparison between (a) the local storage modulus $G'_{11}(w)$ and the non-local storage modulus $G'_{12}(w)$; (b) the local loss modulus $G''_{11}(w)$ and the non-local storage modulus $G''_{12}(w)$, at several particle-distances for a 20 *wt%* solution of PEO. In the legends in the lower right, "R" is the distance between the two probe particles and "a" is the particle radius.

Hough et al, Phys Rev E, 2006

Passive microrheology:
Use of thermal fluctuation to determine viscoelasticity

Fluctuation-dissipation theorem

$$\alpha''(\omega) = \frac{\omega}{2k_B T} C(\omega)$$

$C(\omega)$ is the power spectral density of the thermal noise of a Brownian particle
α'' is the Imaginary part of 1/G

Passive microrheology:
Use of thermal fluctuation to determine viscoelasticity

Kramers-Kronig relation

$$\alpha'(\omega) = \frac{2}{\pi} P\int_0^\infty \frac{\xi\alpha''(\xi)}{\xi^2-\omega^2} d\xi = \frac{2}{\pi}\int_0^\infty cos(\omega t)\,dt \int_0^\infty \alpha''(\xi)\,sin(\xi t)\,d\xi$$

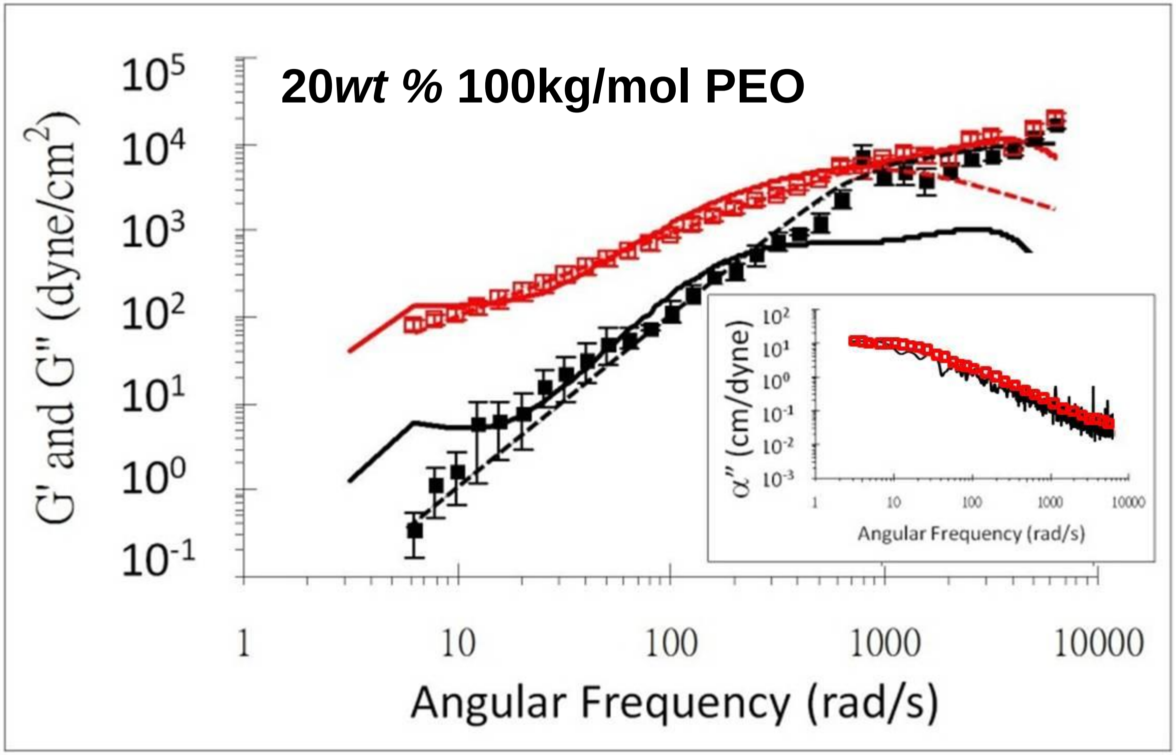
$$G'(\omega) = \frac{1}{6\pi a}\frac{\alpha'(\omega)}{\alpha'(\omega)^2+\alpha''(\omega)^2}$$

$$G''(\omega) = \frac{1}{6\pi a}\frac{-\alpha''(\omega)}{\alpha'(\omega)^2+\alpha''(\omega)^2}$$

B. R. Dasgupta, *et al.*, "Microrheology of polyethylene oxide using diffusing wave spectroscopy and single scattering," *Phys. Rev. E,* vol. 65, p. 051505, 2002.

Microrheology of Polymer Solutions

Viscoelasticity of PEO Solutions obtained
by active and passive methods

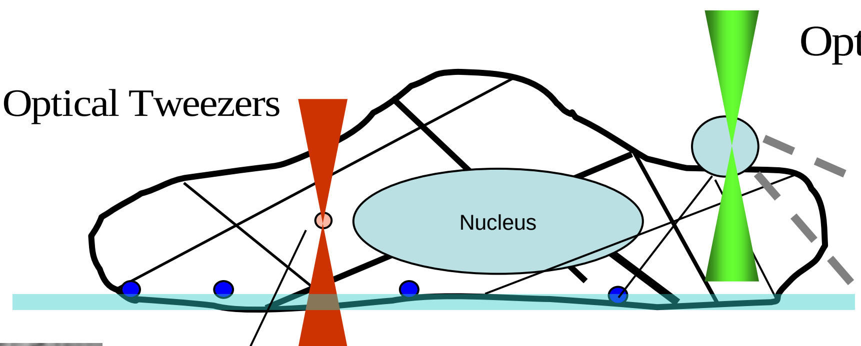


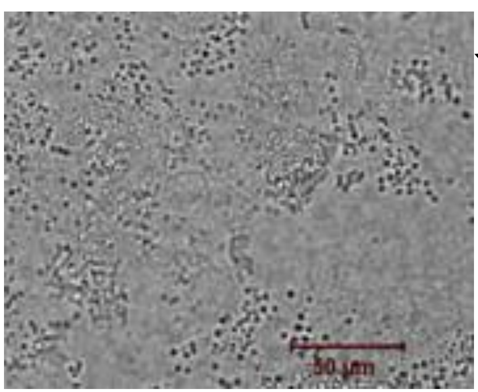
Hough et al J. Nanotechnology (1999).
Latinovic, Hough et al J *Biomechanics* (2010).

Latinovic, U. Maryland

Microrheology with intracellular and extracellular probes

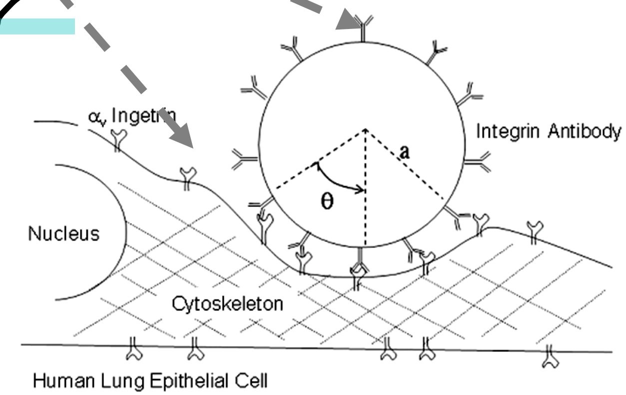
Optical Tweezers Based Cytorheometer





Granules: surfactant, lipid-protein complexes

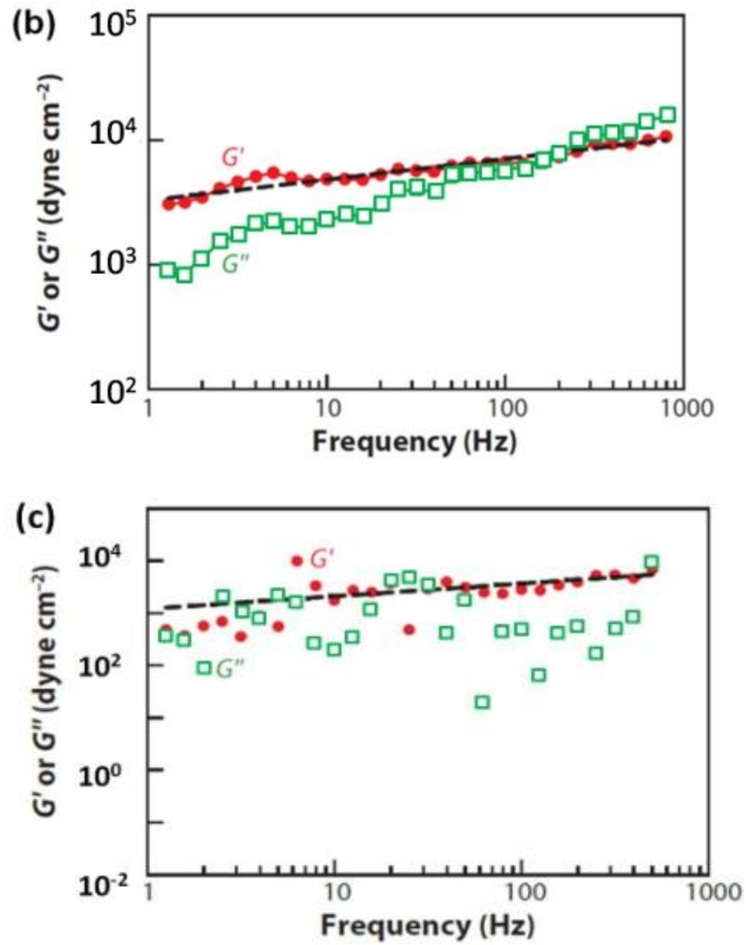
Alveolar Epithelial Monolayer



Microrheology of Living Cells

(b) Data probed by anti-integrin conjugated silica particles attached to the plasma membrane.

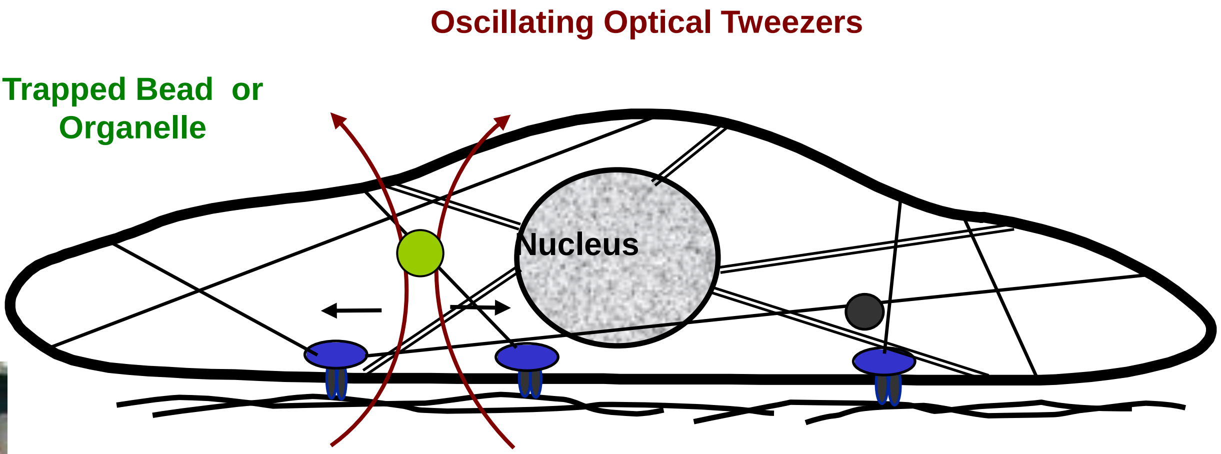
(c) Data probed by intracellular organelles



- The exponents of the power-law dependence of G on frequency.
- Differences in the magnitudes of the moduli from the two measurements may be caused by extensional stiffness of the plasma membrane

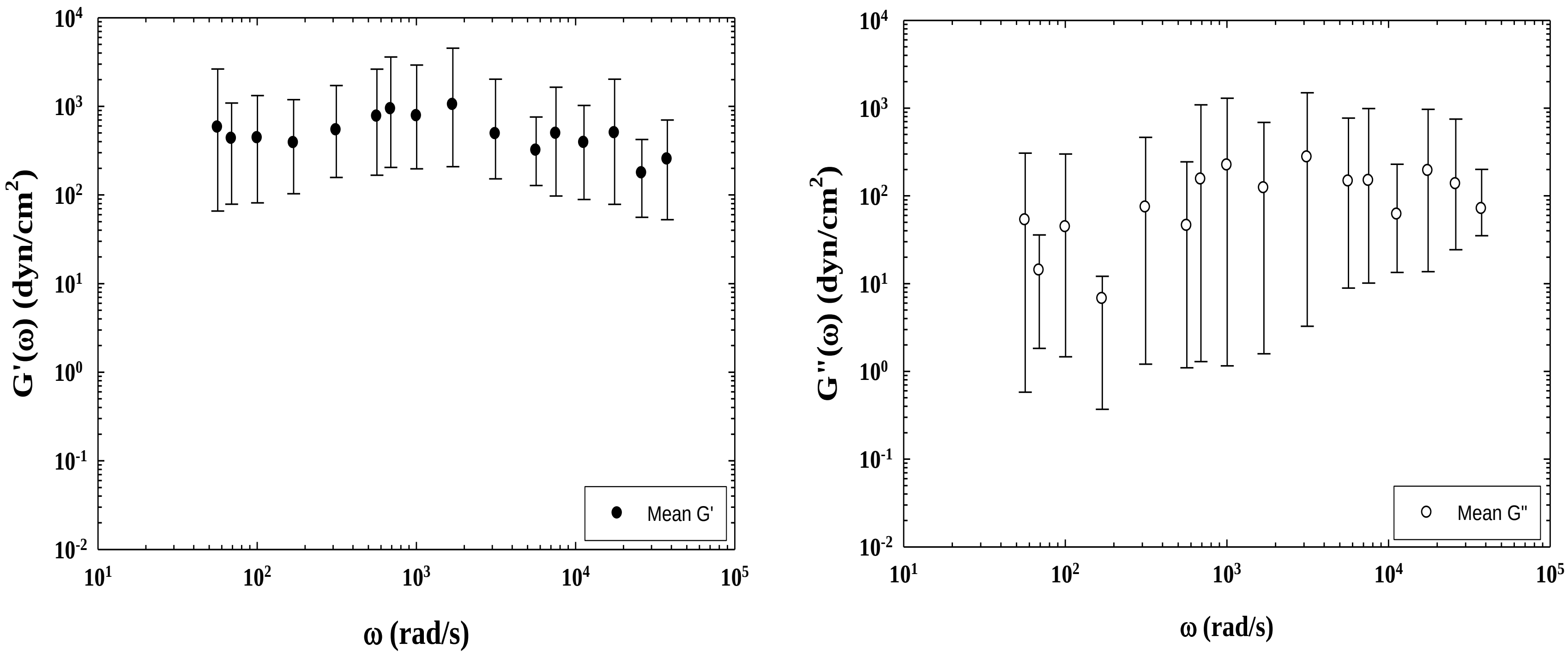
Wei *et. al. OE* **16** 8594 (2008)

Intracellular Measurements of the Cell Mechanical Properties

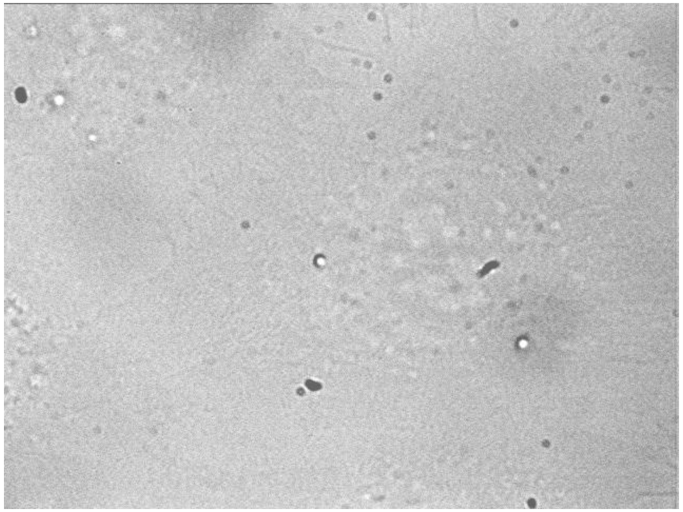


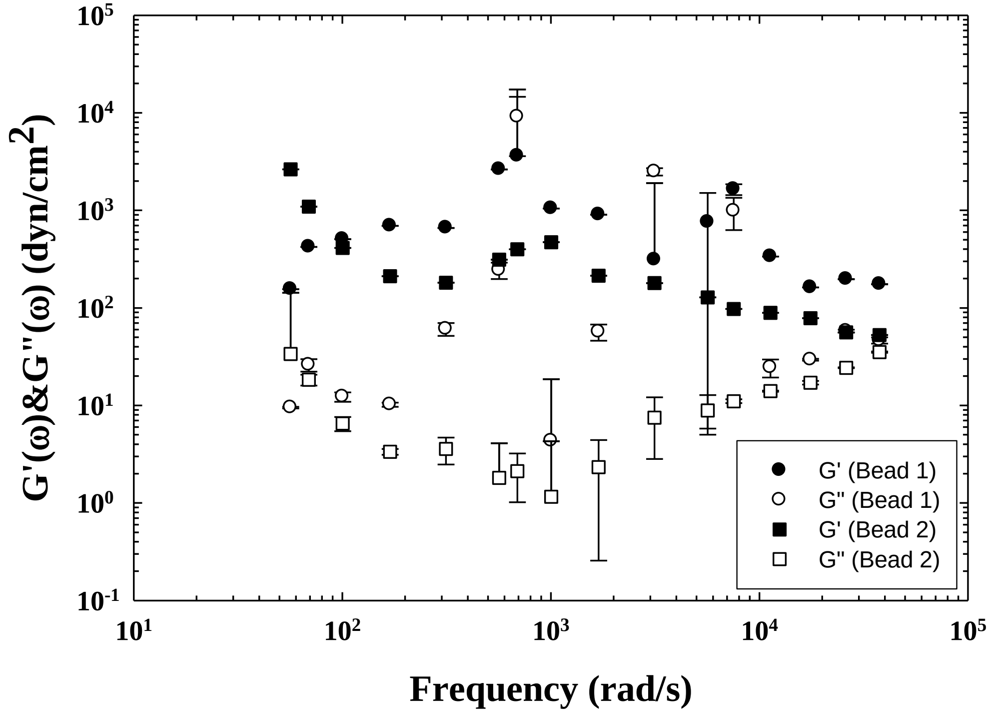
Maron Mengistu, U. Maryland

Frequency-dependent G' and G" of Bovin Vascular Endothelial Cells (BVEC) using endocytosed polystyrene beads as probes



Intracellular inhomogeneity probed with 2 polystyrene beads





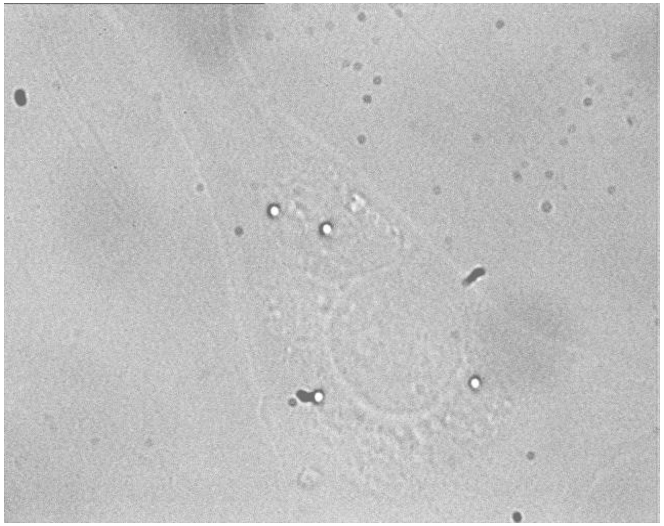
- Large dynamic fluctuations are intrinsic to live cells
- Spatial inhomogeneity seems to be caused by these dynamic fluctuations

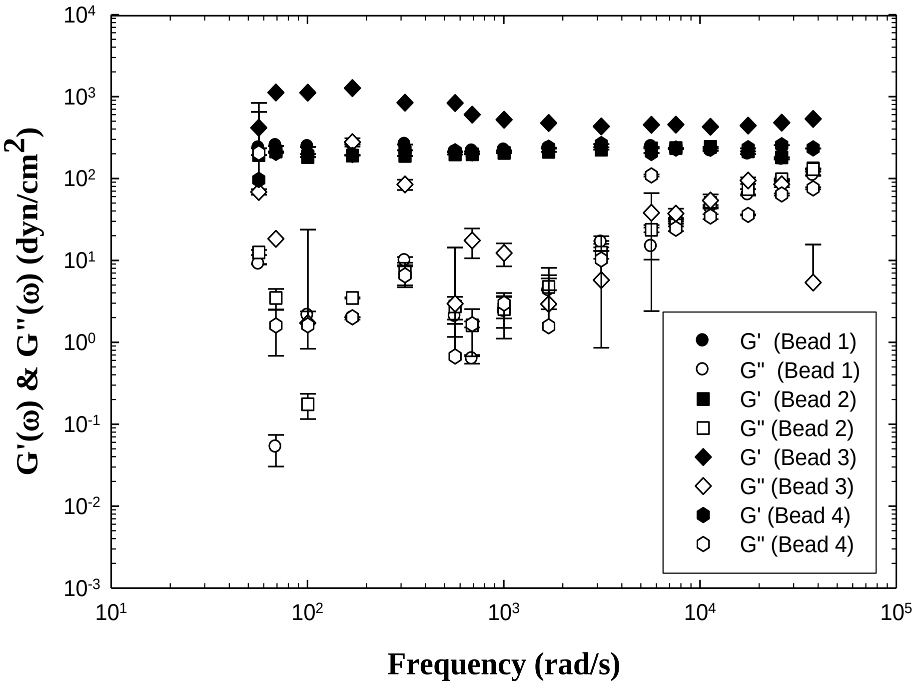
Hypothesis:

- F-actin network and the associated molecular motors are responsible for the fluctuations

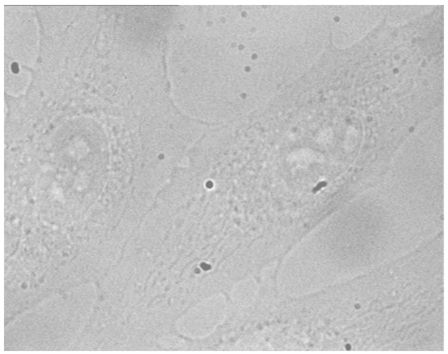
- Nutrient depletion
- Depolymerization of cytoskeleton protein network

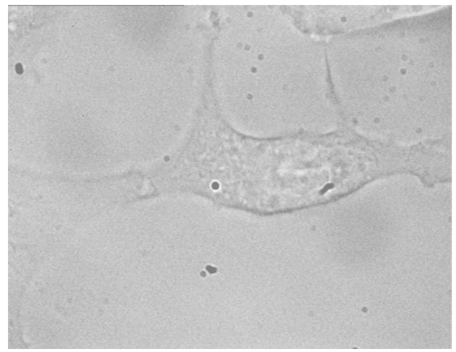
Endocytosed polystyrene beads were used to probe the effect of nutrient depletion on G' and G'' fluctuations

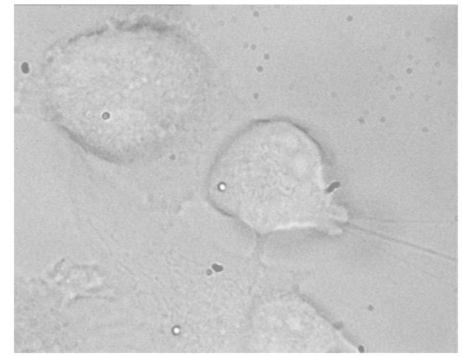


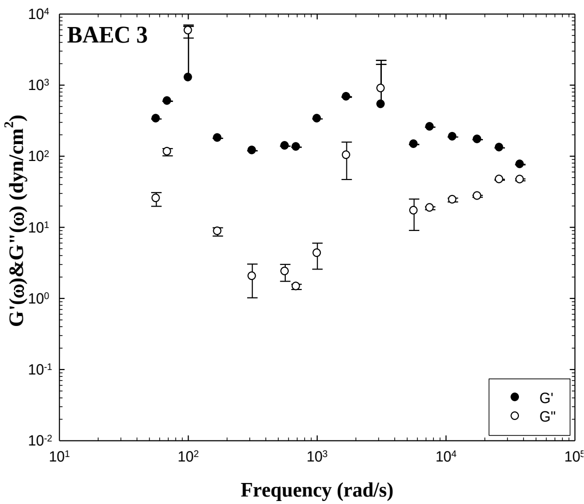


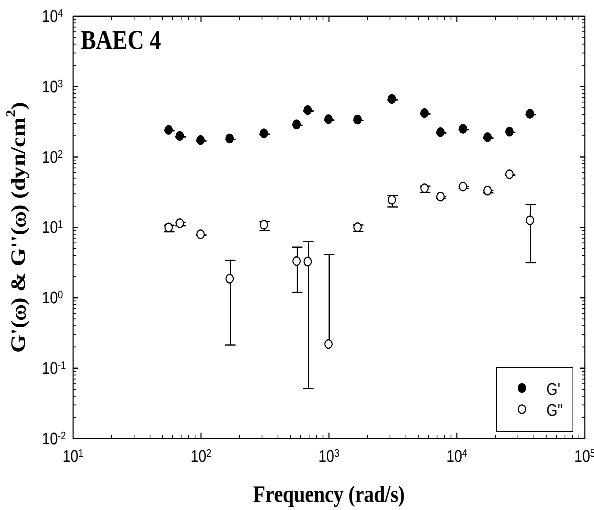
Effect of cytochalasin B treatment (actin filaments depolymerized)

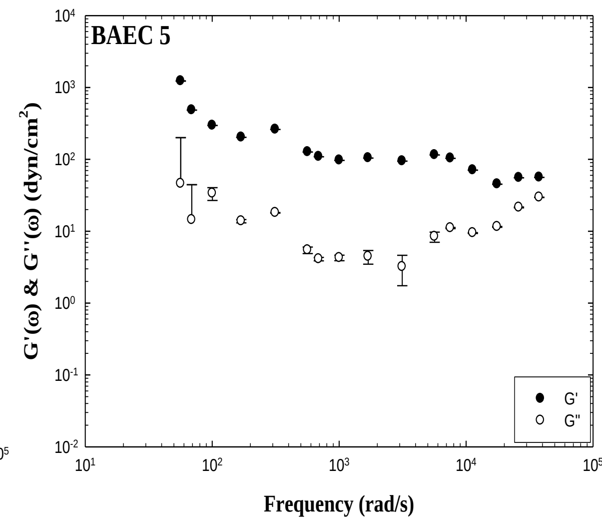




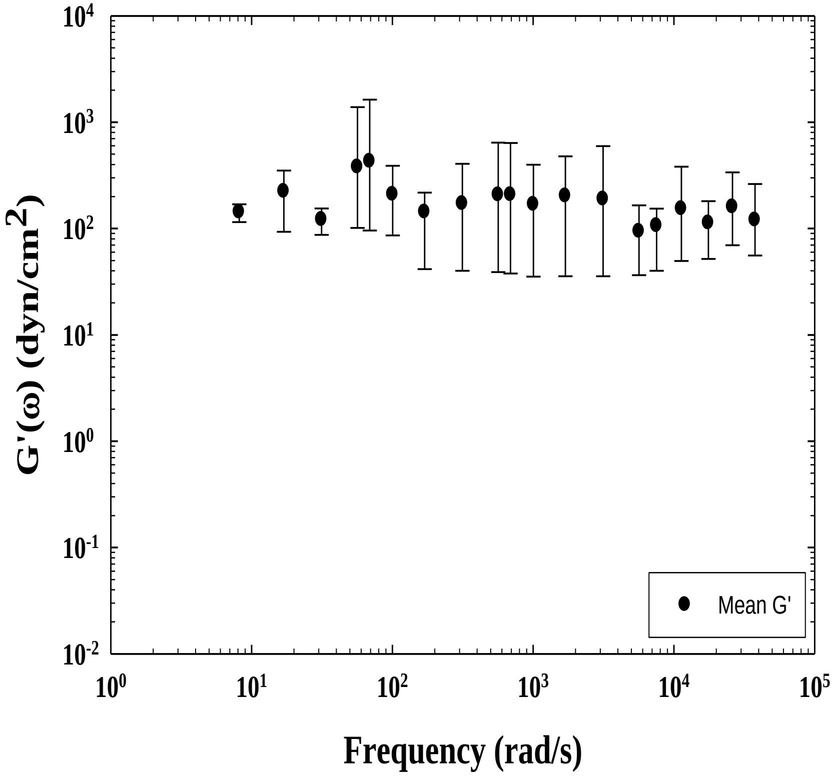


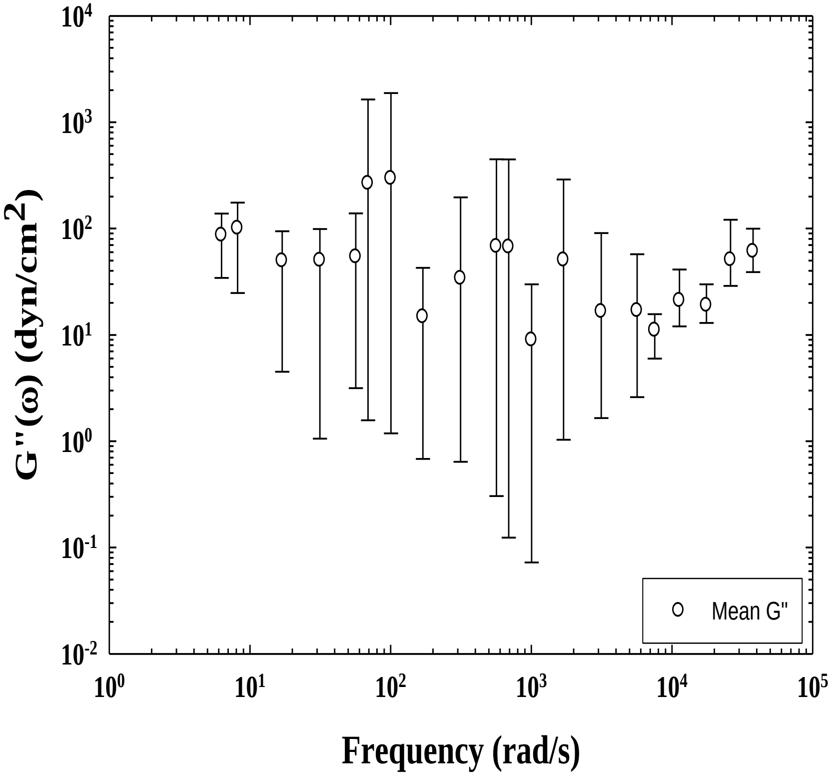




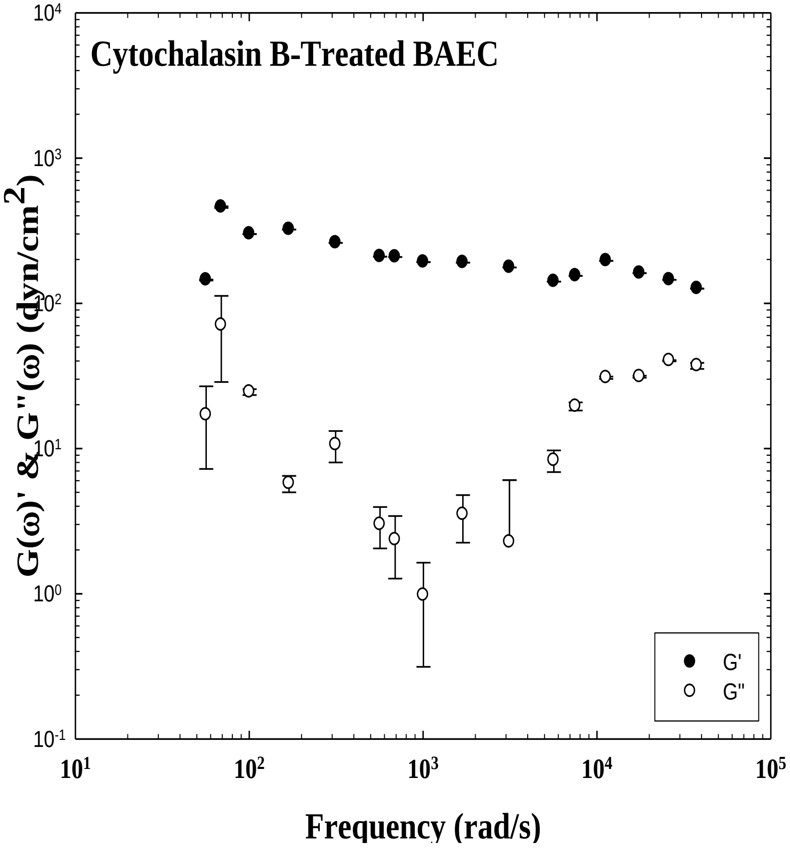


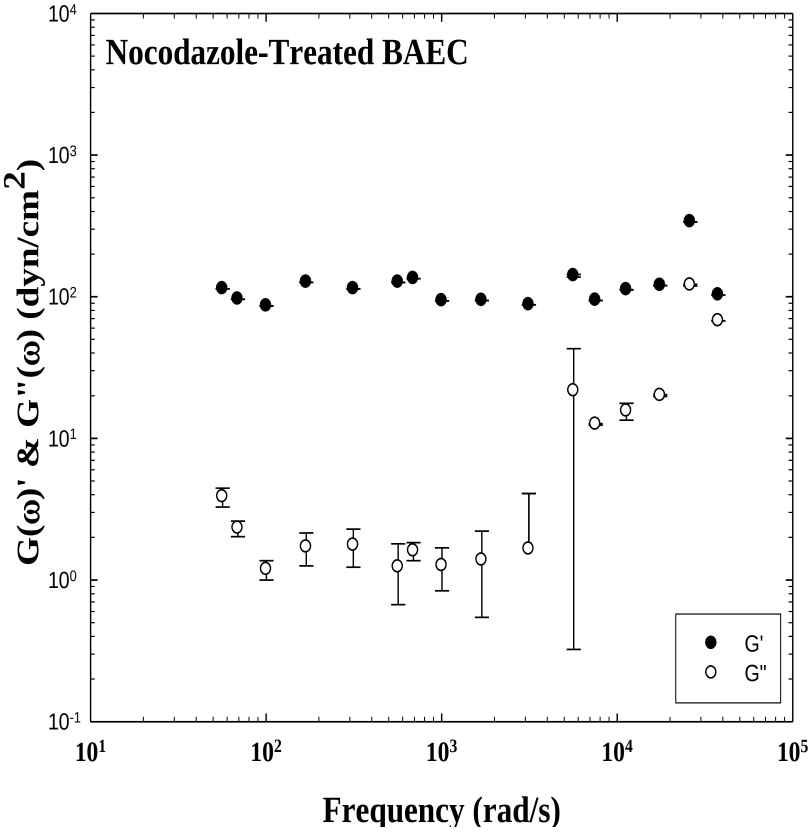
Effect of nocodazole treatment on viscoelastic properties (microtubules depolymerized)



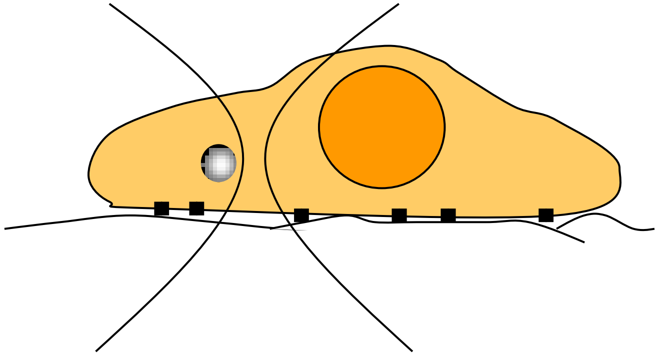


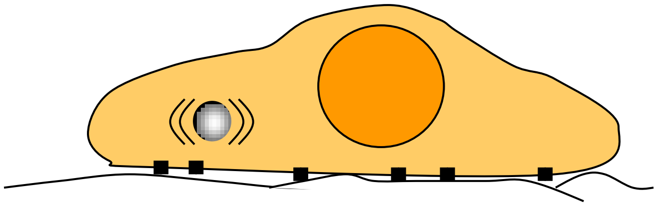
Nutrient depletion of drug treated BAECs





Comparison of Active and Passive Microrheology in Living Cells





Active approach
Oscillating tweezers

Passive approach
Particle tracking

Fluctuation-dissipation theorem

Passive Microrheology

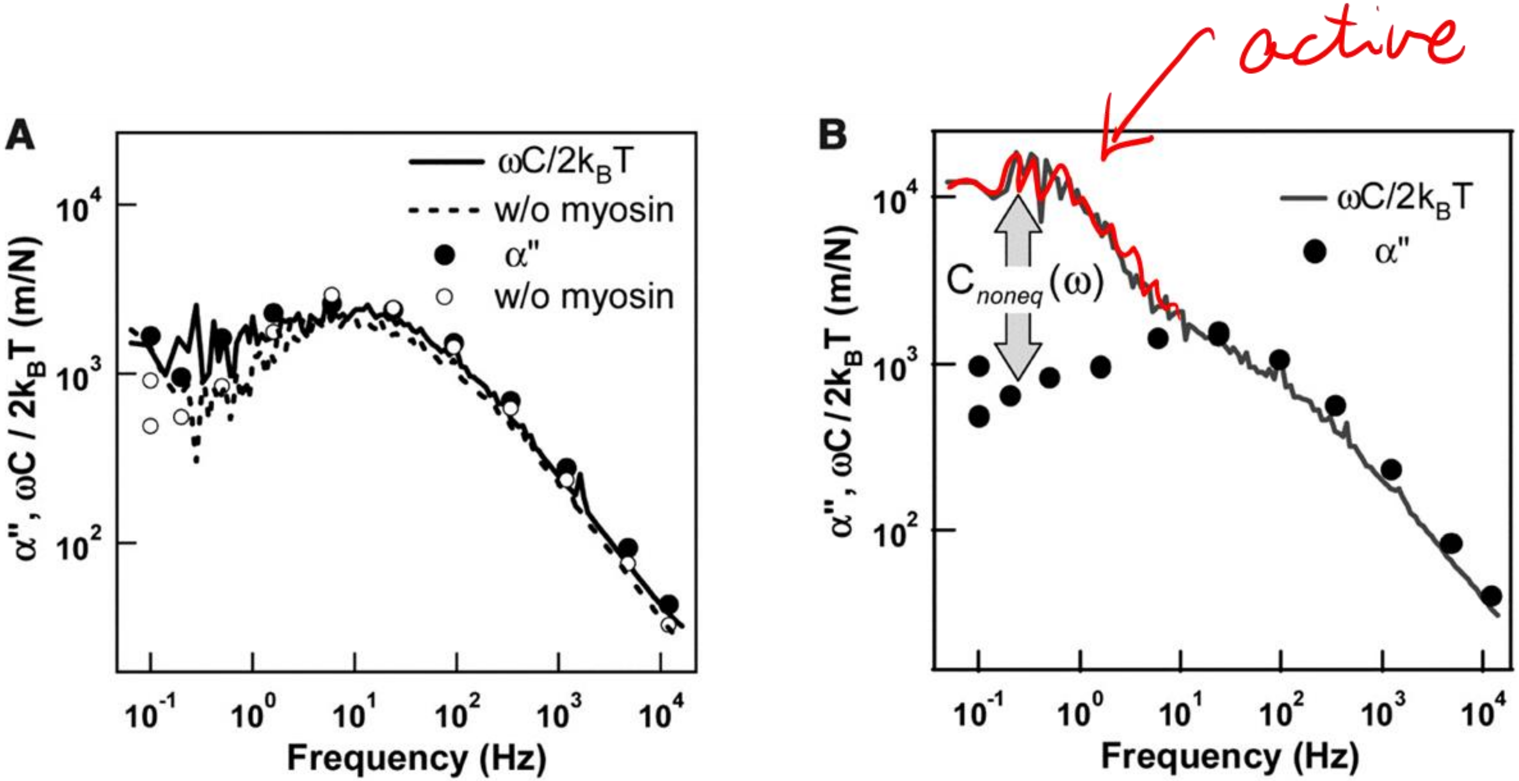
$$C(\omega) \equiv \int \langle X(t) X(0) \rangle \exp(i\omega t) dt$$

$$Fluctuation - dissipation:$$

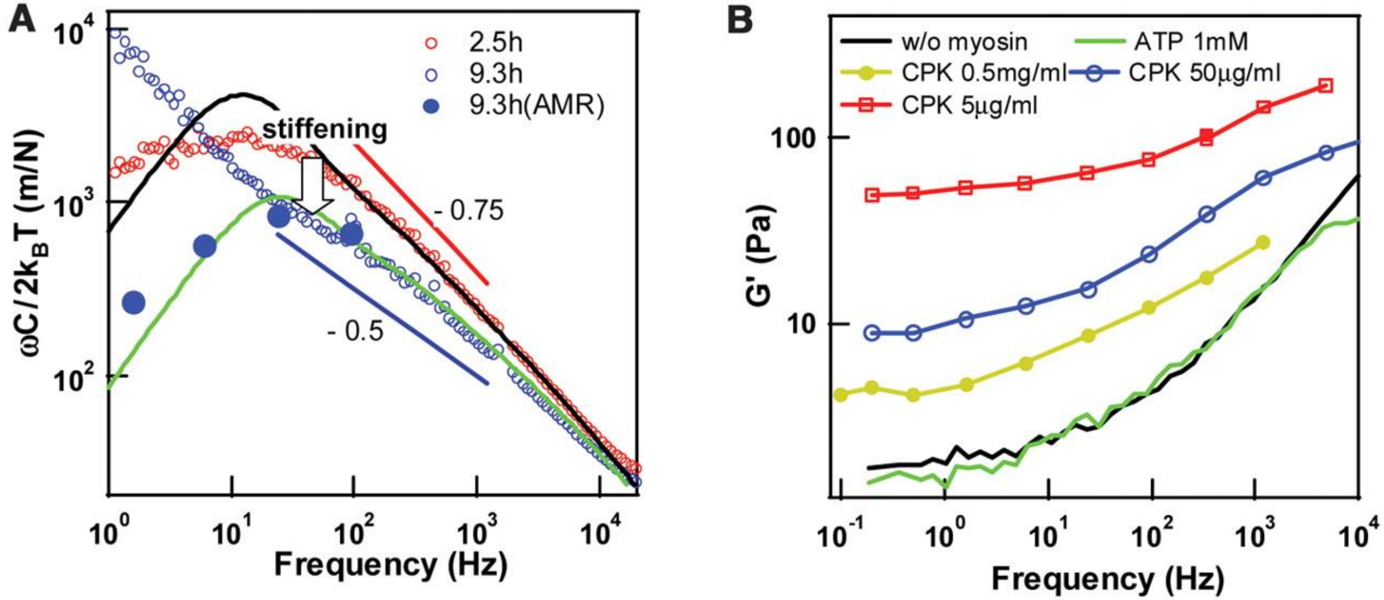
$$\alpha''(\omega) = \frac{\omega}{2k_B T} C_{eq}(\omega) \qquad : \alpha \equiv \frac{X}{F} = \frac{1}{6\pi a G}$$

A model bioactive system composed of actin, myosin, and biotin/neutravidin cross-links

D. Mizuno et al., Science 315, 370 -373 (2007)

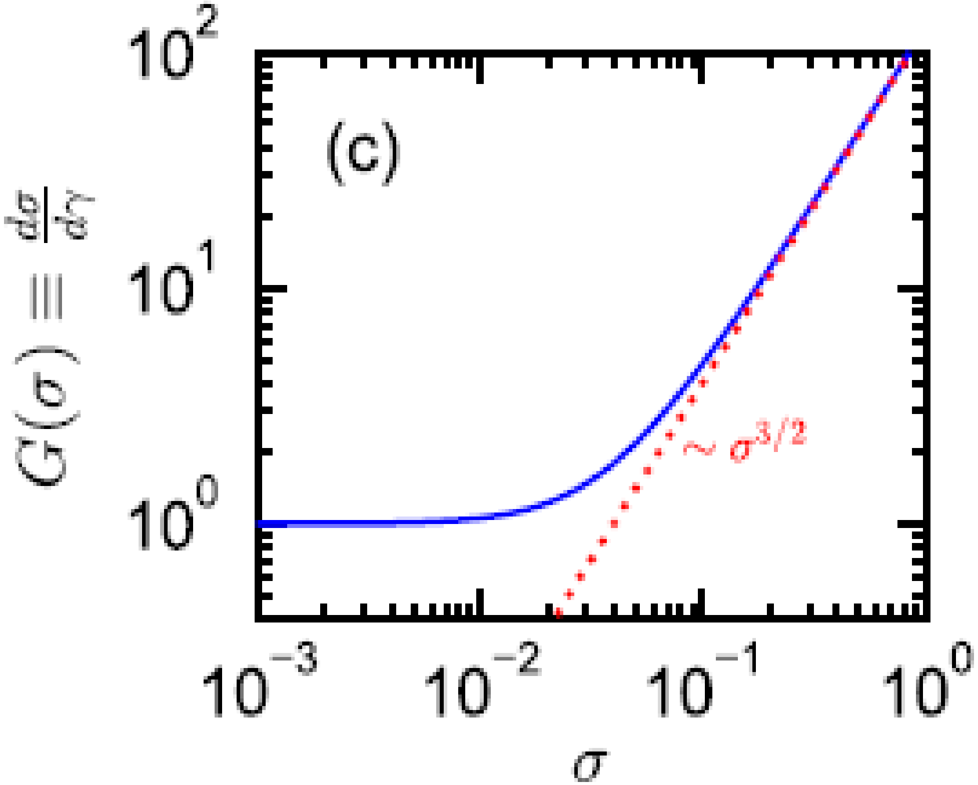


Effect of filament tension on the response of the active networks (actin and myosin concentrations as in Fig 1)



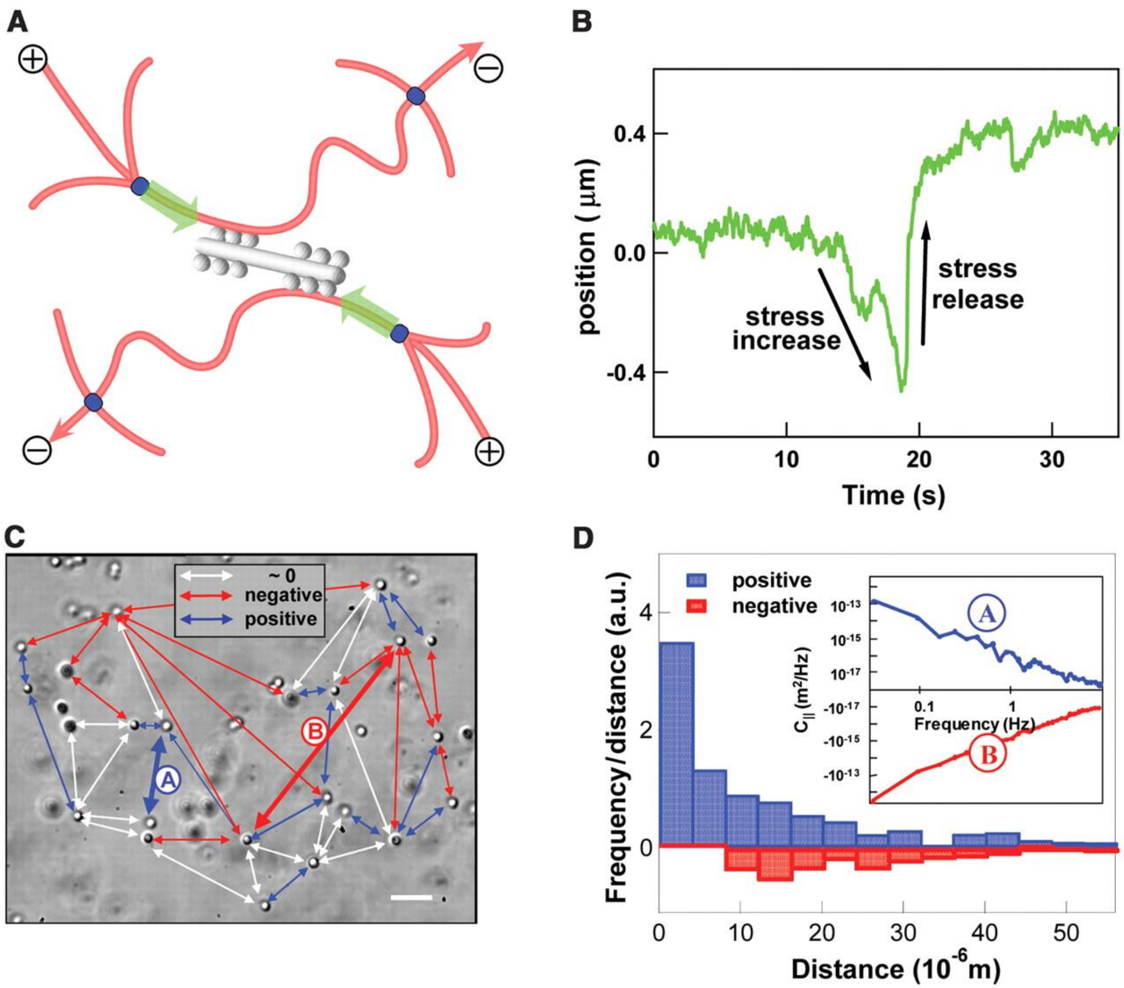
D. Mizuno et al., Science 315, 370 -373 (2007)

Stress-dependent cell stiffness: a theoretical model



Y Shokef et al. PRL **108** 178103 (2012)

Fig. 4. (A)
Schematic illustration of tension development in actin filaments (red)



D. Mizuno et al., Science 315, 370 -373 (2007)

The apparent mechanical properties in living cells are modulated by active biological forces!
The dynamical fluctuation in the mechanical properties are caused by fluctuating biological forces!

$$m\ddot{x}(\omega,t)+6\pi\eta[F_2]a\dot{x}(\omega,t)+k[F_2]x(\omega,t)$$

$$=k_{OT}Ae^{i\omega t}$$

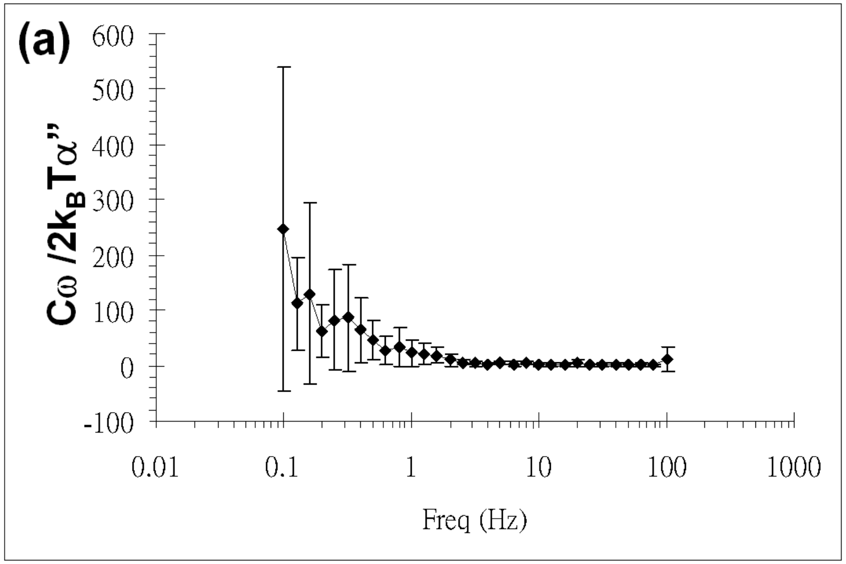
$$+\mathrm{F}_1\text{(thermal fluctuation)}$$

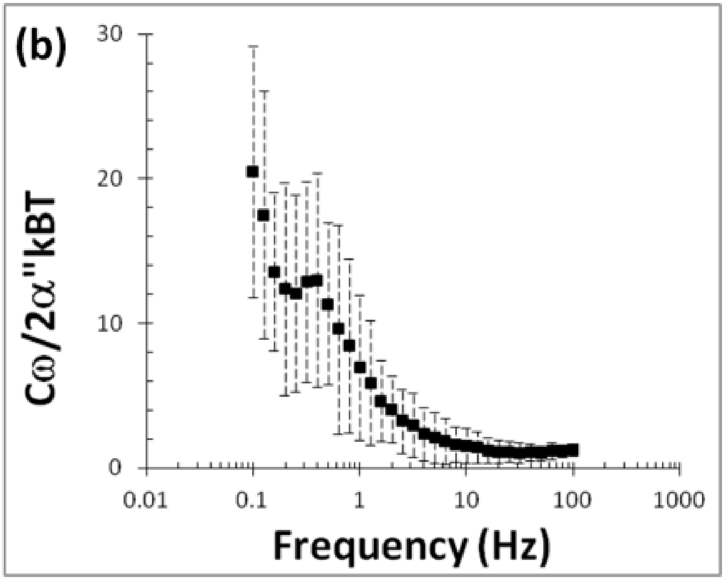
$$+\mathrm{F}_2\text{(active biological foces)}$$

- Relationship of fluctuations and dynamic tension?
- Response to defined external perturbations: pump and probe experiments
- Relate biological functions to these fluctuation dynamics

Mechanical fluctuations in living cells do not follow FDT

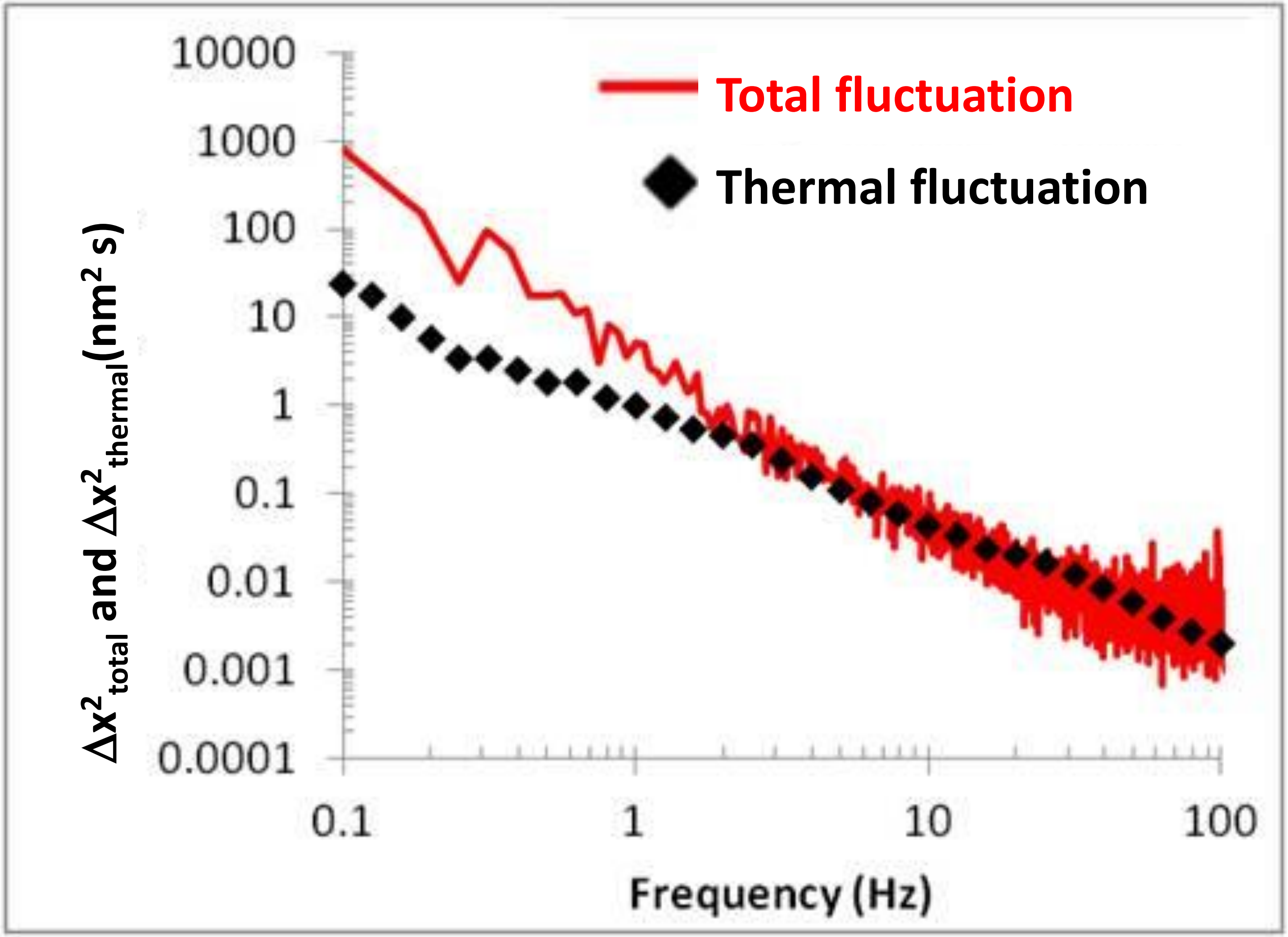
The normalized total fluctuation noise power spectral density ($2k_B T\alpha''/\omega$) probed by
(a) an endogenous endosome (more active)
(b) an engulfed micro-particle (less active).





M-T Wei *et al. SPIE Proc.* **7762** (2010)

Total fluctuation *vs.* thermal fluctuation power spectra densities



$$\Delta x^2_{nonthermal} = \Delta x^2_{total} - \Delta x^2_{thermal} - 2\Delta x_{nonthermal}\Delta x_{thermal}$$

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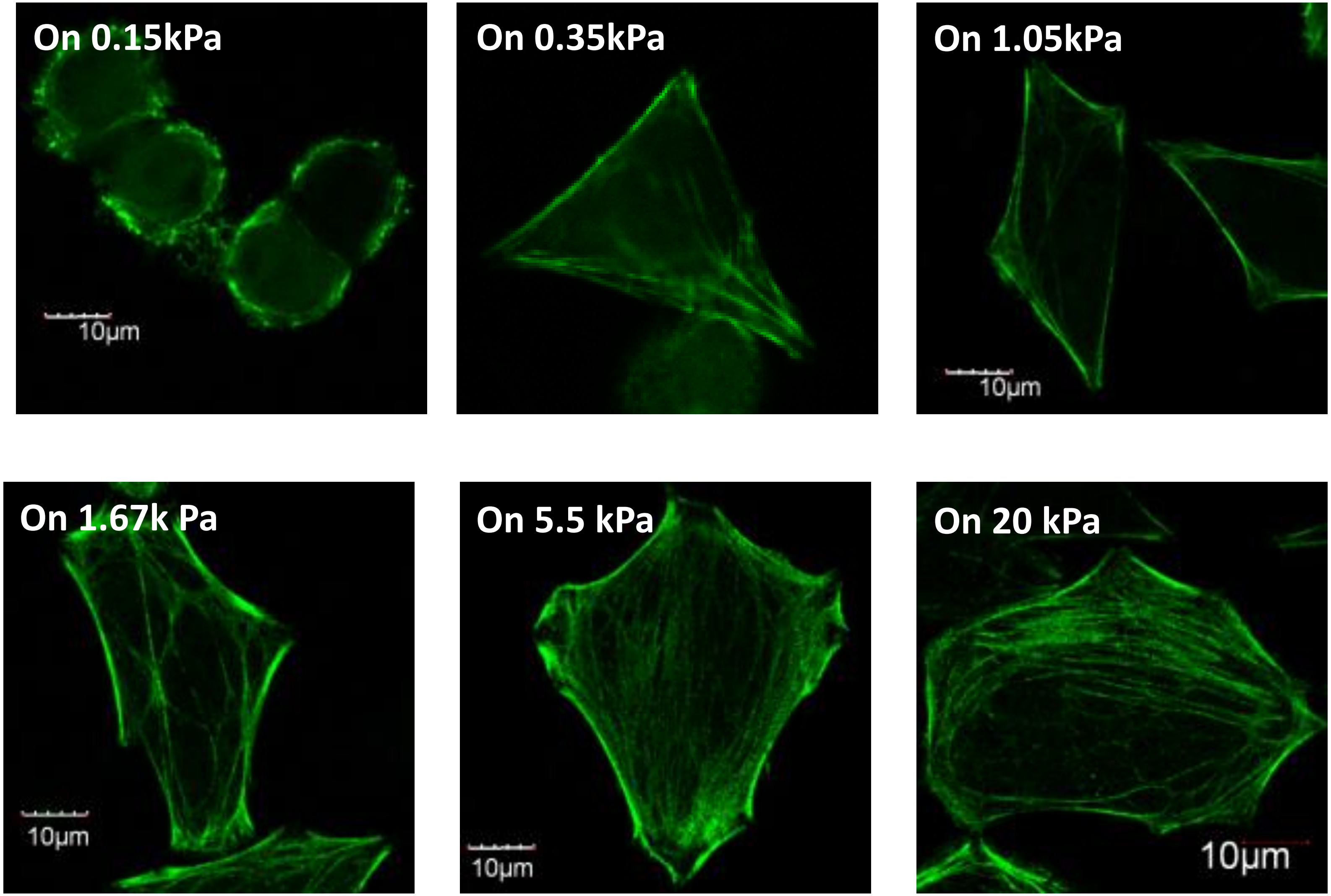
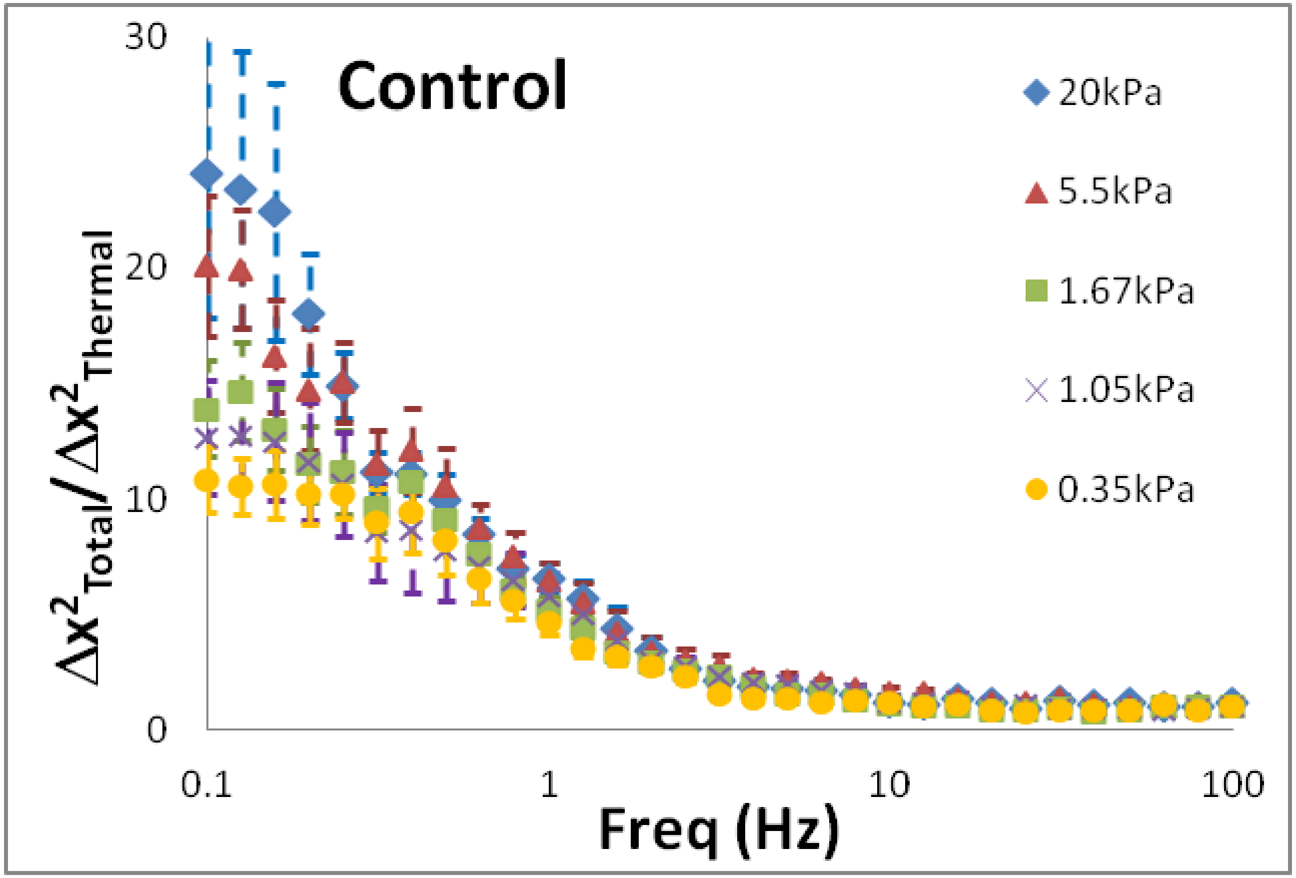
Intracellular stiffness of HeLa cells increases with increasing substrates stiffness





Control
20kPa
5.5kPa
1.67kPa
1.05kPa
0.35kPa
30
20
10
0
0.1
1
10
100
Freq (Hz)
$\Delta x^2_{Total}/\Delta x^2_{Thermal}$

A "non-thermal force" $< f^2 >$ can be obtained from the fluctuations spectra by PMR and the cell stiffness by AMR.

$$\left\langle f^2 \right\rangle \alpha^{*2} = C - C_{thermal} = C - \frac{2k_B T \alpha''}{\omega}$$

A. Levine, F. MacKintosh, PRL 2009

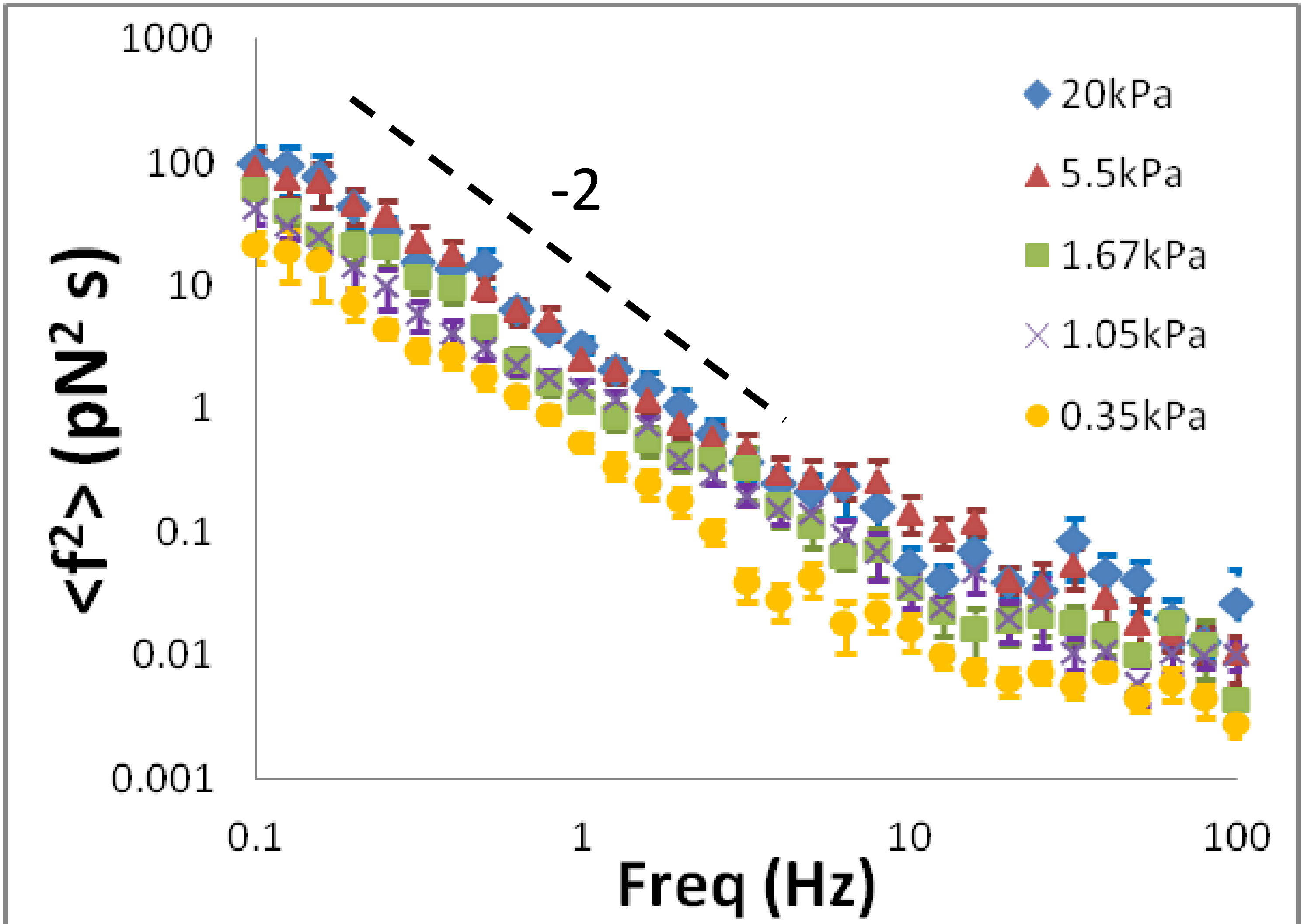
C. Wilhelm, *PRL,* **101**, p. 028101, 2008.

F. Gallet, D. Arcizet, P. Bohec, and A. Richert, *Soft matter*, **5**, p. 2947, 2009

D. Mizuno, R. Bacabac, C. Tardin, D. Head, and C. F. Schmidt, *PRL* **102**, p. 168102, 2009

D. Robert, T. Nguyen, F. Gallet, and C. Wilhelm *Plos One,* **5**, p. e10046, 2010.

Non-thermal force spectrum increases with increasing substrate rigidity



$$\langle f^2 \rangle = K^2 \Delta x^2_{nonthermal}$$

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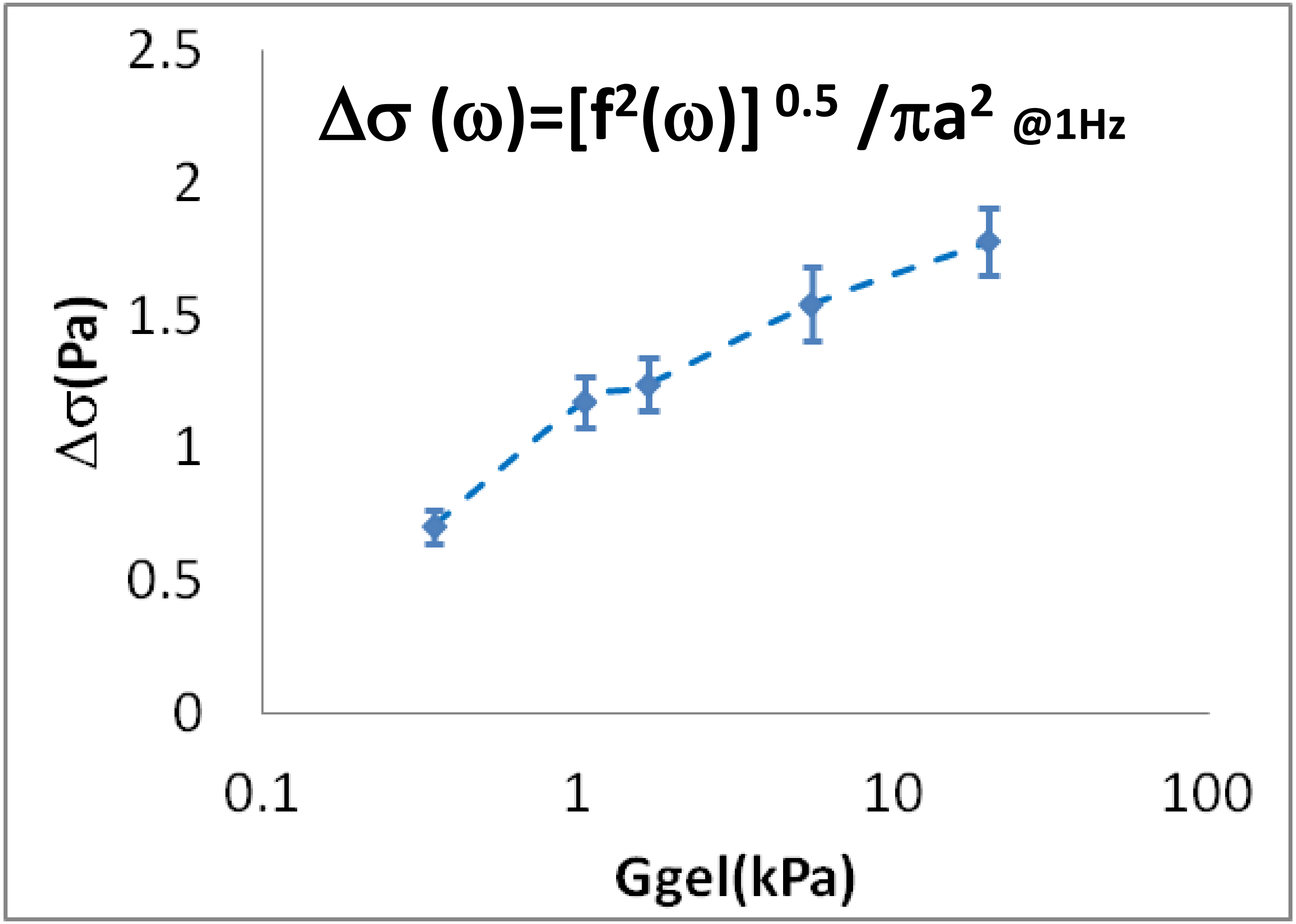
Intracellular dynamic pulling stress ($\Delta\sigma$) increases with increasing substrate rigidity



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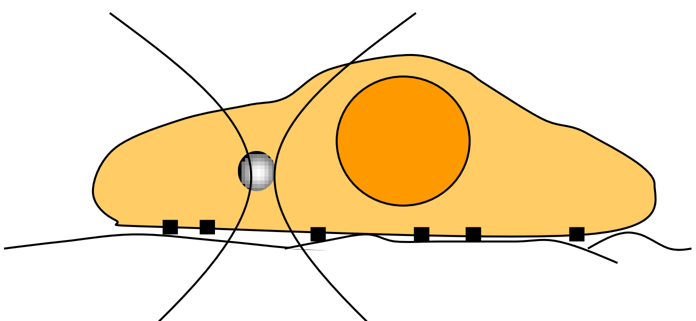
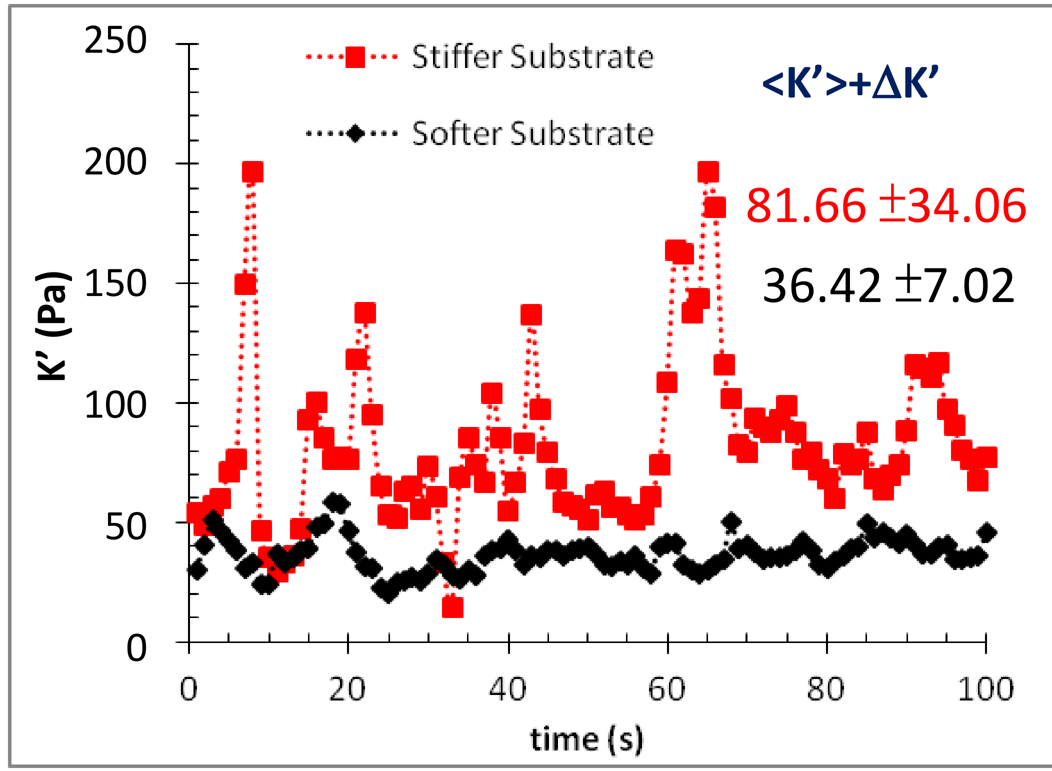
Assumption:
fluctuations in intracellular differential stiffness are caused by fluctuations in intracellular stress

Active approach
Optical tweezers



Directly measure the mechanical properties



$$\Delta K' \equiv \Delta K'_{rms} = \frac{1}{\tau}\int_0^{\tau}\left(K'(\mathrm{t})-\left\langle K'\right\rangle\right)^2 dt$$ **@1Hz**

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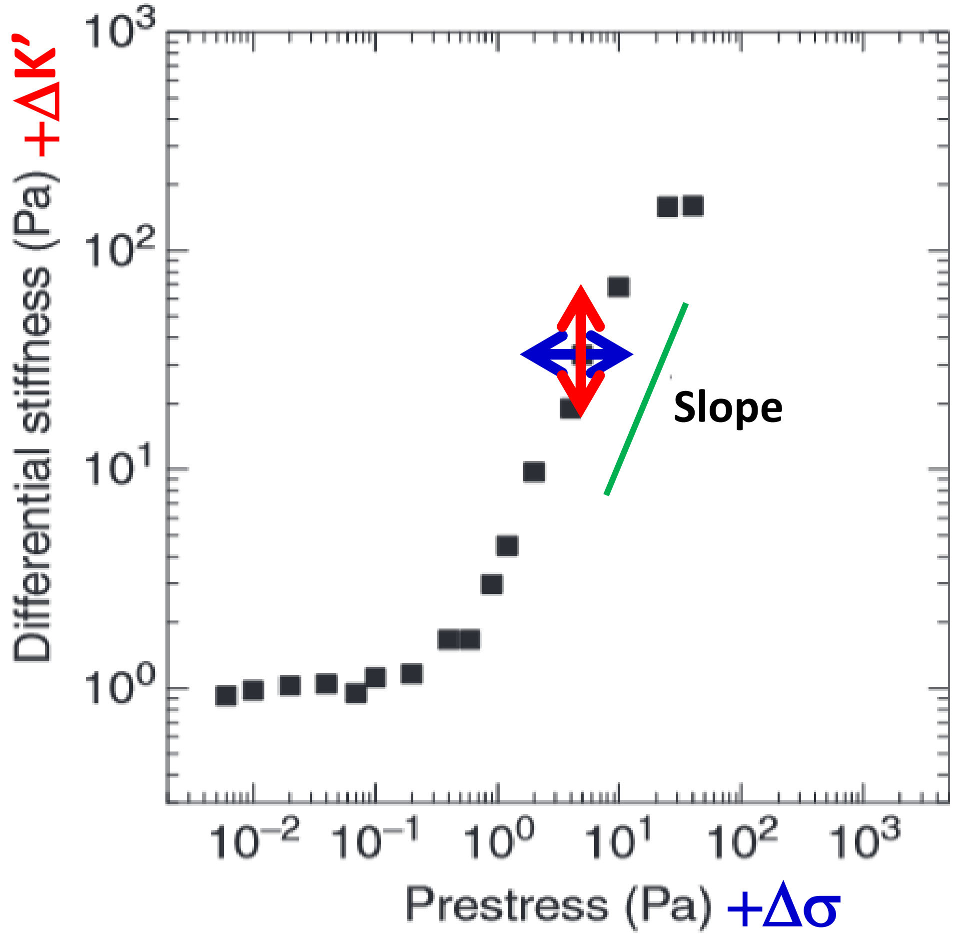
Difficult to determine local stress level in cells: can one use $\Delta K'/\Delta\sigma$ to determine intracellular stress?

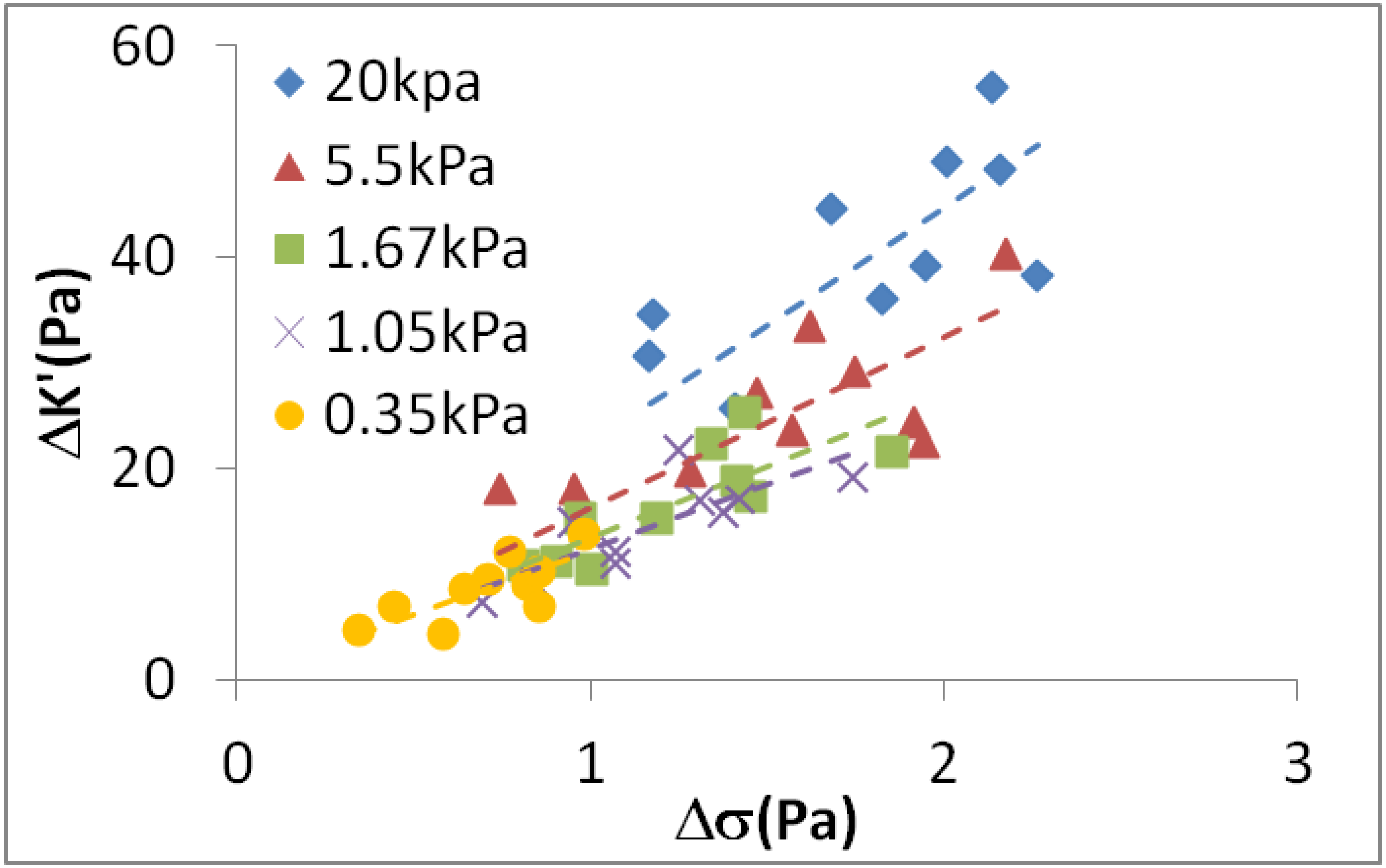


$\sigma(K') = \int (1/slope)\, dK'$

M.L. Gardel *et al*. *PNAS* **103** 1762 (2006)

Assumption:

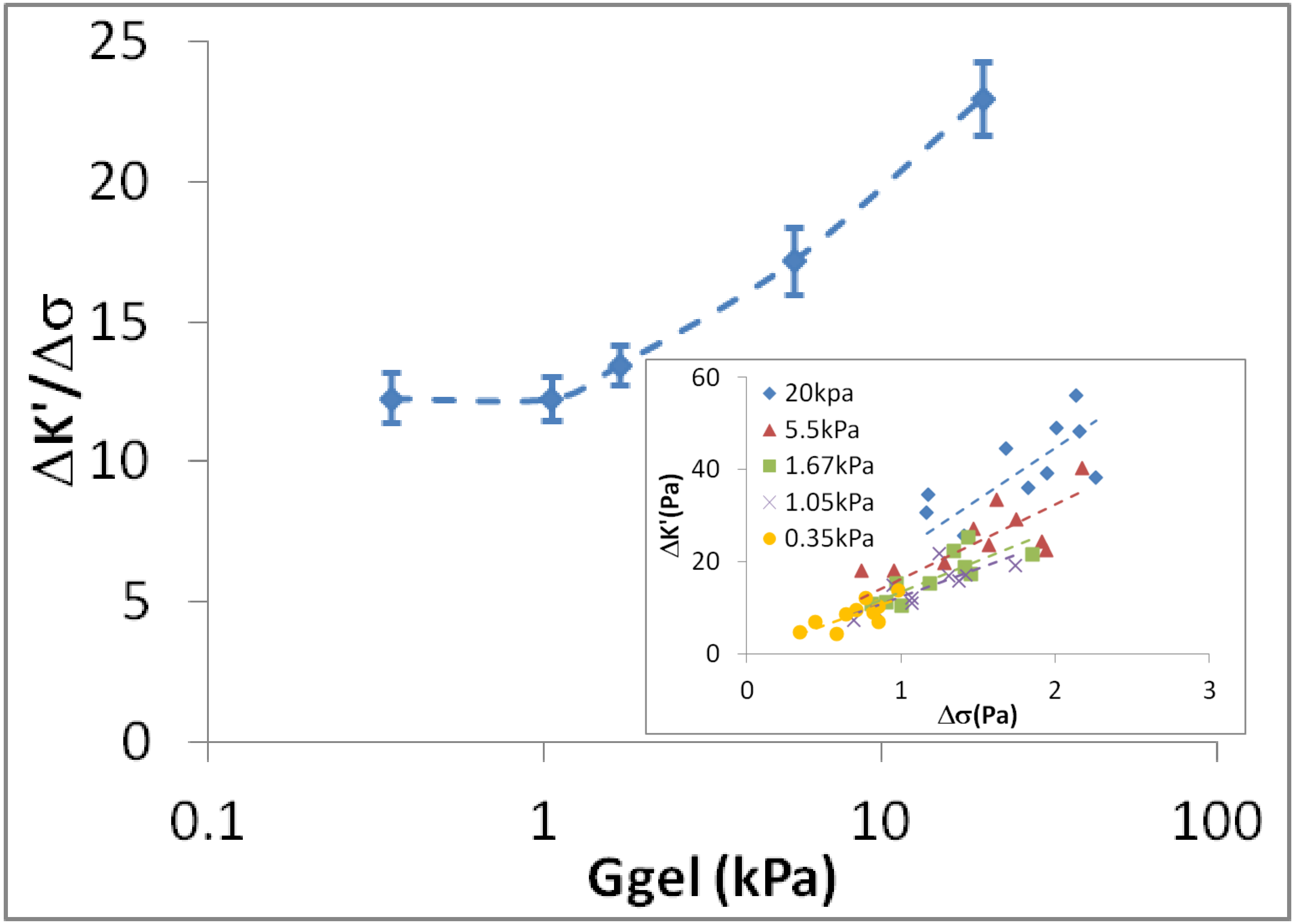
Fluctuations in intracellular differential stiffness are caused by fluctuations in intracellular stress .



60
40
20
0
0
1
2
3
ΔK'(Pa)
Δσ(Pa)
20kpa
5.5kPa
1.67kPa
1.05kPa
0.35kPa

$\Delta K'_{rms}/\Delta\sigma$ @1Hz

increases with increasing substrate rigidity



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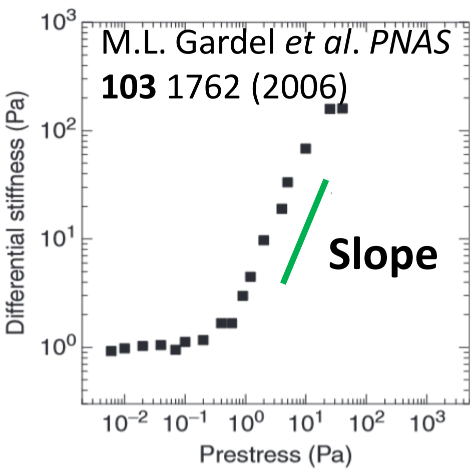
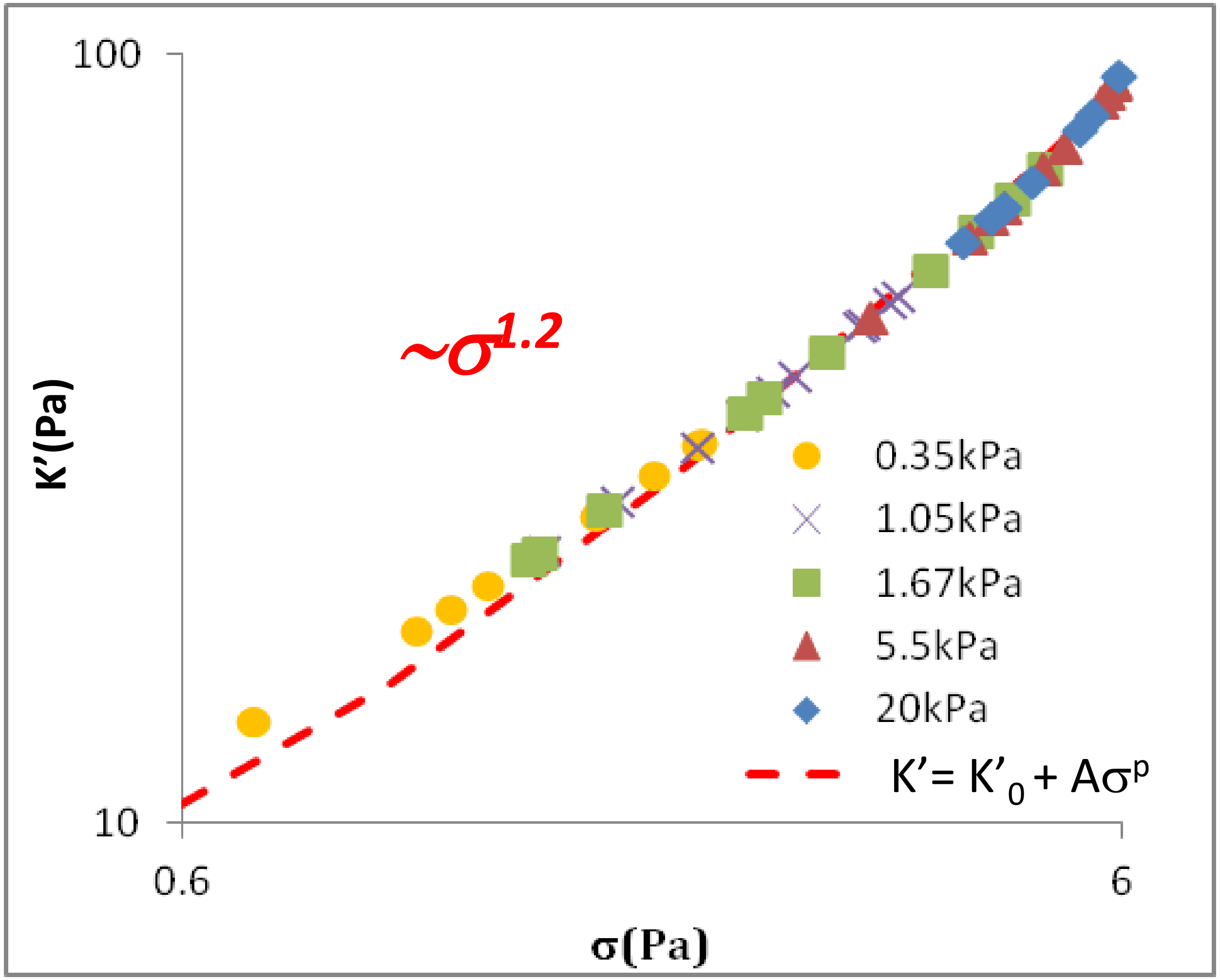
Integrating ΔK'/Δσ yields a master K' (σ): different substrates rigidities merely shift stress level





$$\sigma\,(K') = \int (\Delta\sigma/\Delta K')\,dK'$$

K'_0 in the absence of intracellular stress is assumed to be 5Pa, which is reported by Soft Matter **7** 3127 (2011)

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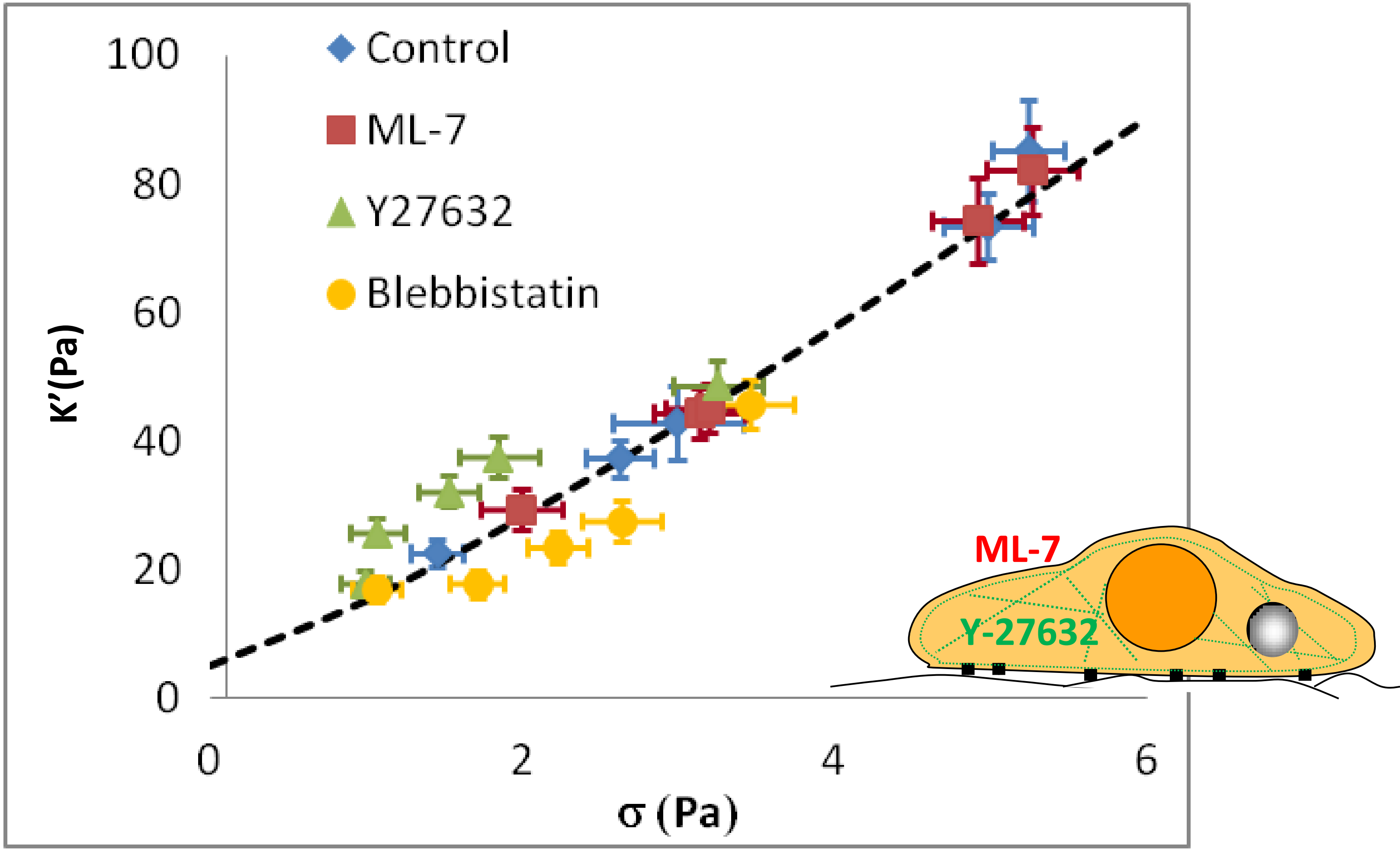
Drug treatments follow the same K’ (σ): merely shift intracellular stress level



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Conclusions

- Mechanical noises in living cells do not obey the Fluctuation-Dissipation relationship
- Non-thermal force spectrum increases with increasing substrate rigidity. Force ~ frequency^{-2}
- Fluctuations in intracellular differential stiffness are caused by fluctuations in intracellular stress
- Use $\Delta K'/\Delta\sigma$ to determine intracellular stress
- Integrating $\Delta K'/\Delta\sigma$ yields a master K' (σ):
  - different substrates rigidities merely shift stress level
- Drug treatments follow the same K' (σ):
  - Drugs merely shift intracellular stress level

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