Biopolymer Network Mechanics, Part I

Basic Concepts in Rheology

Microrheology

Experimental Methods, Examples

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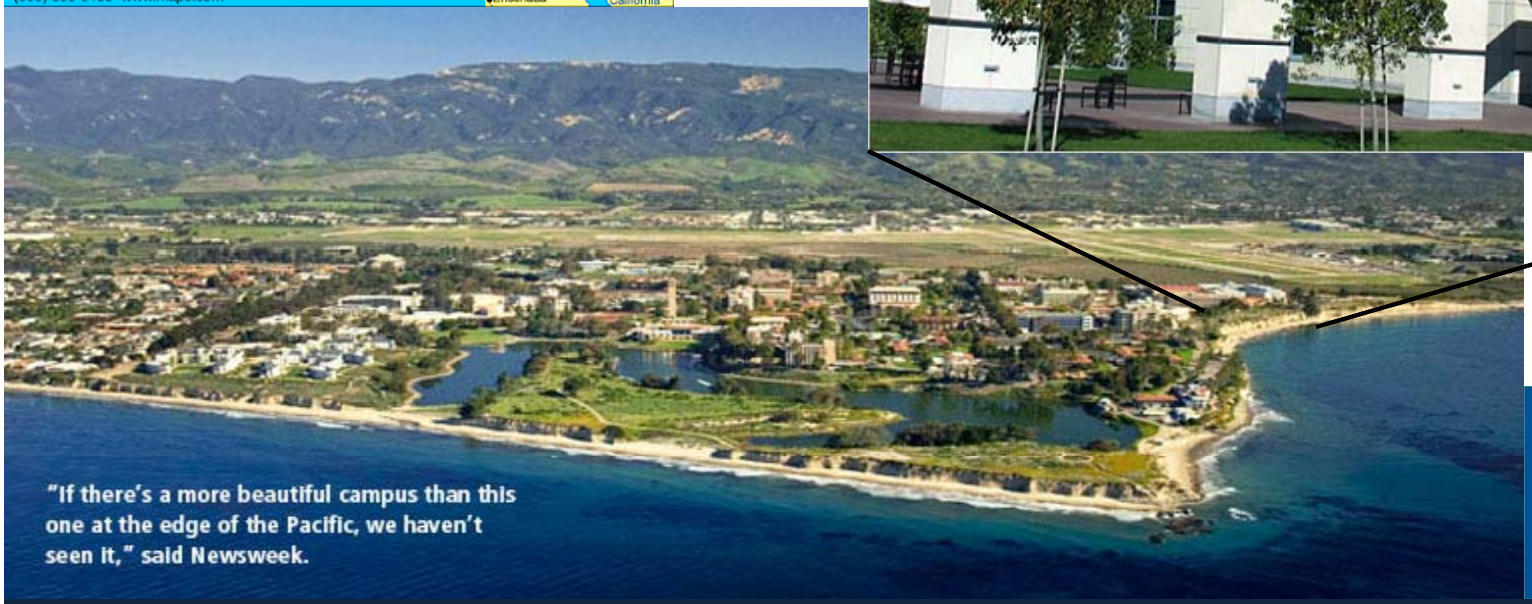
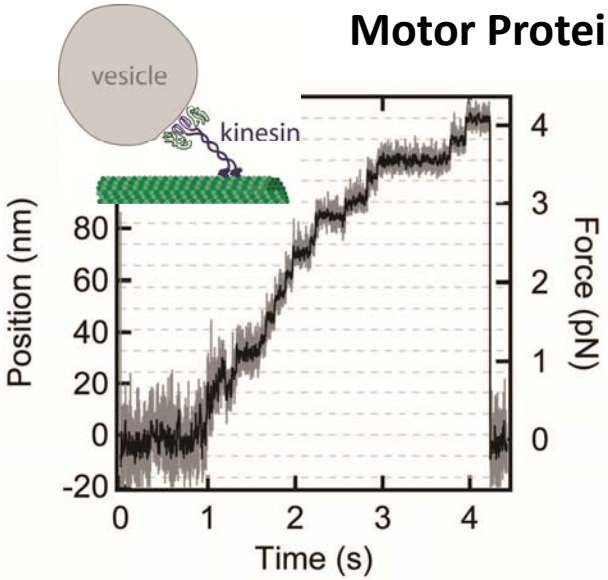
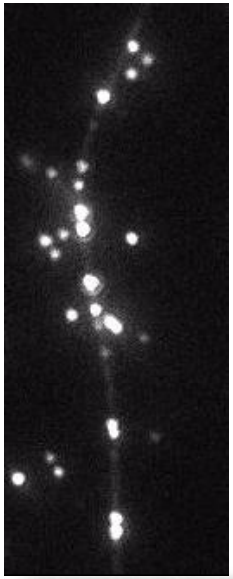


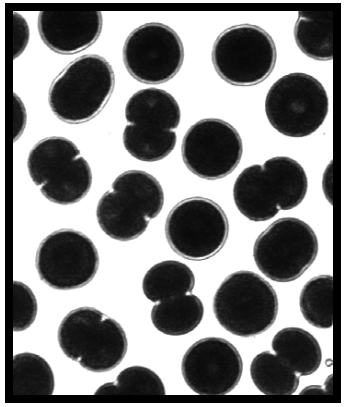
UCSB

Research focus: How are forces generated and transmitted in living materials?

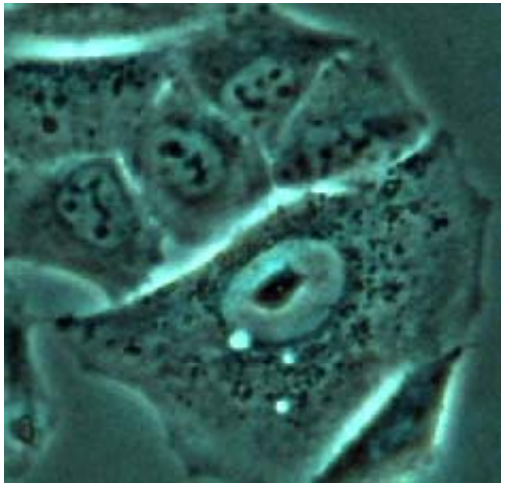


Motor Proteins

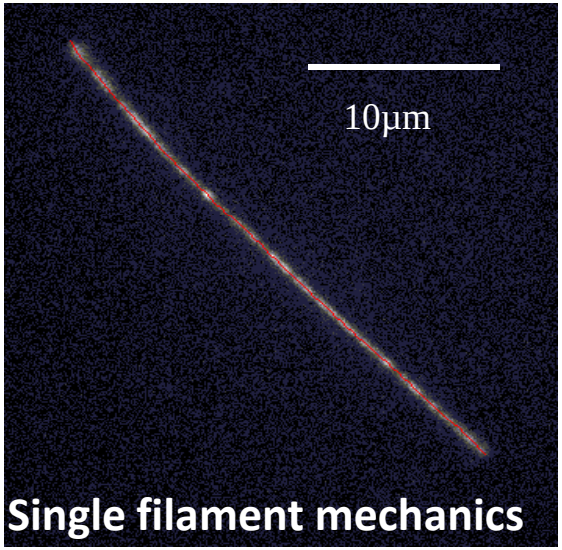




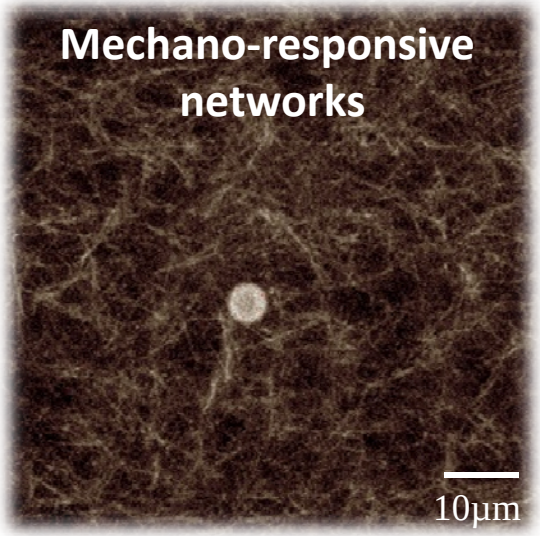
Oocyte development



Cell mechanics, adhesion



Single filament mechanics



Mechano-responsive networks

Marine-inspired adhesion

Biopolymer Network Mechanics, Part I

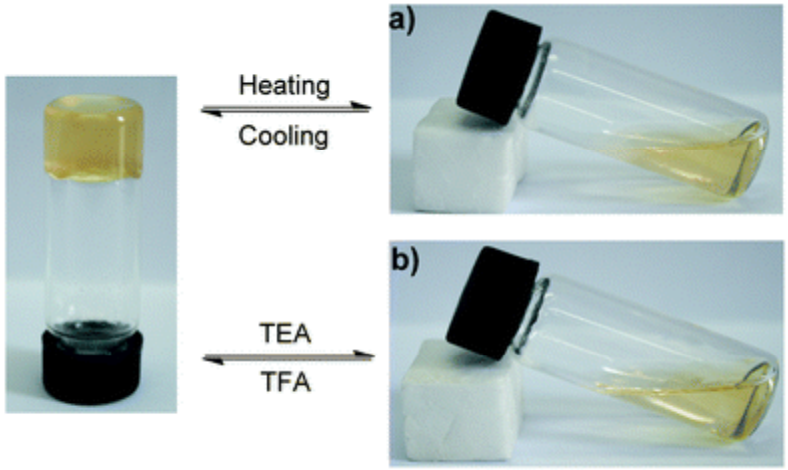
Basic Concepts in Rheology

Microrheology

Experimental Methods, Examples

How to we measure “squishy-ness”?

Do-To-Ri Mook ?? (Acorn Jelly/Jello)



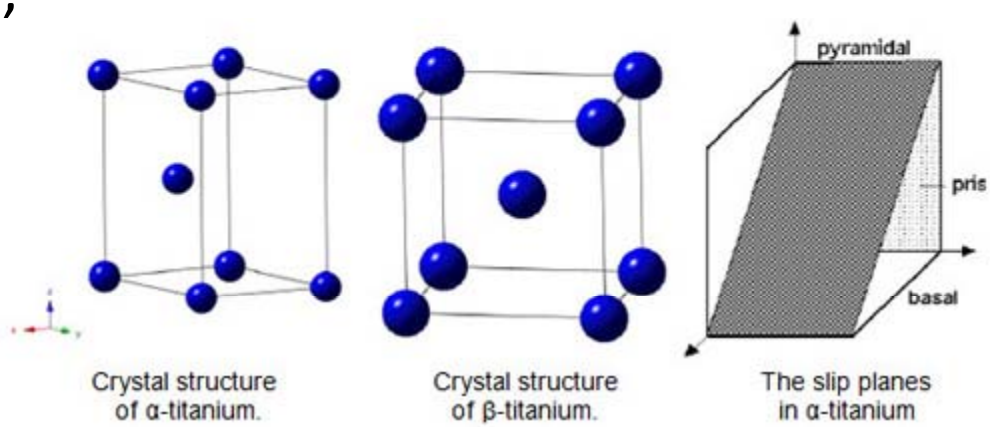
Polymers-→ Materials

F. Zeng, Y. Shen, C.-F. Chen
Soft Matter, 2013,**9**, 4875-4882

Classical engineering mechanics

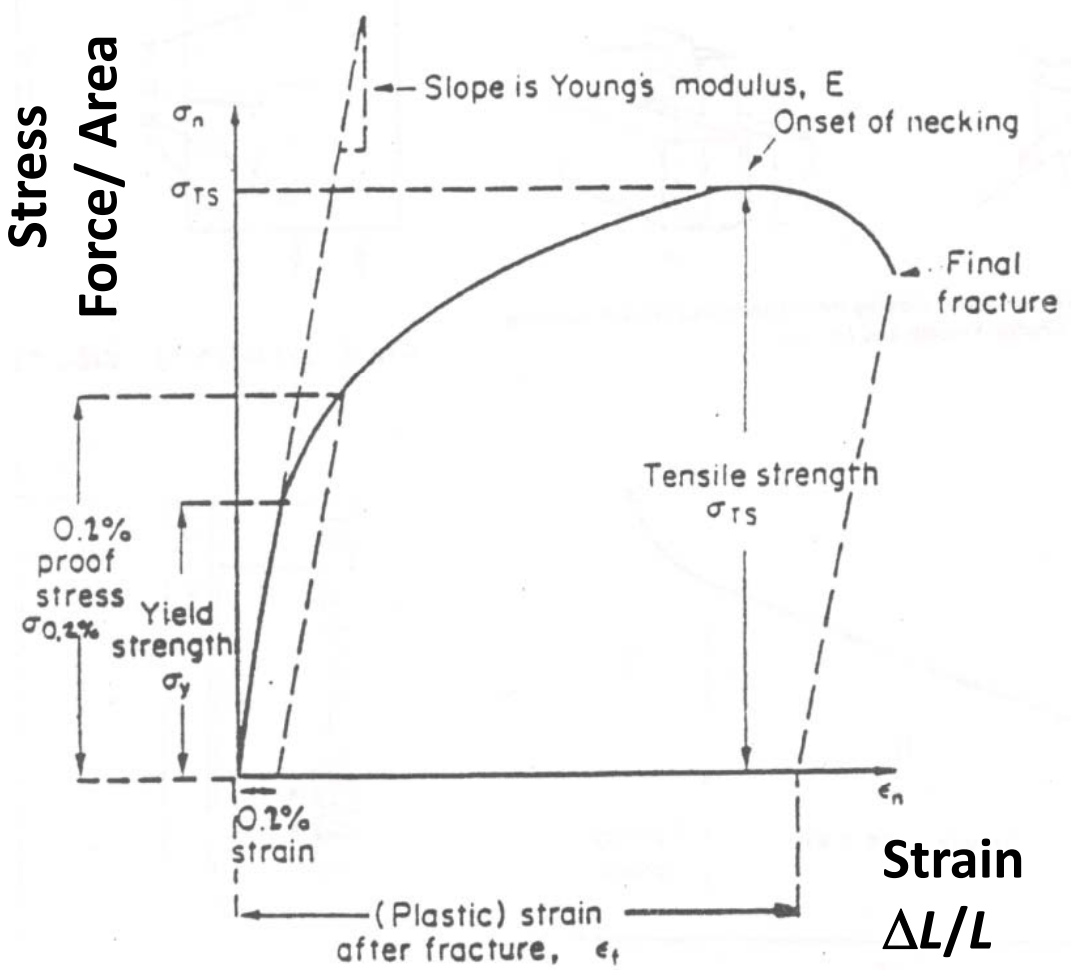
- Classical engineering materials are hard
  - Metals, alloys, ceramics, etc.
- Deformation of metals requires movement of atomic planes

Titanium



Structure ←→ Mechanics Relationships

Classic tensile tests

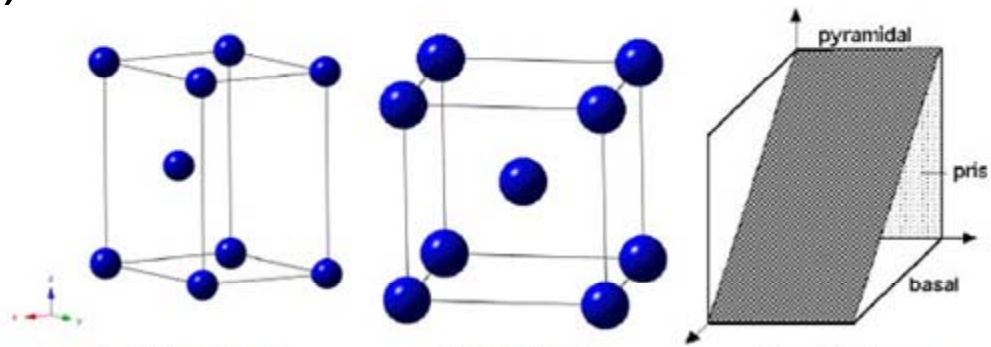


Quasi-static loading

Classical engineering mechanics

- Classical engineering materials are hard
  - Metals, alloys, ceramics, etc.
- Deformation of metals requires movement of atomic planes

Titanium

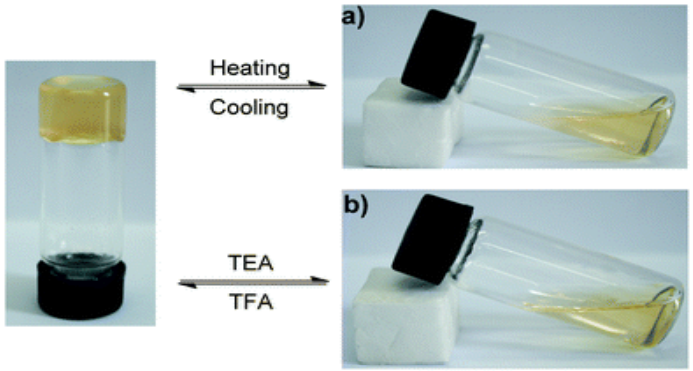


Crystal structure of α-titanium. Crystal structure of β-titanium. The slip planes in α-titanium

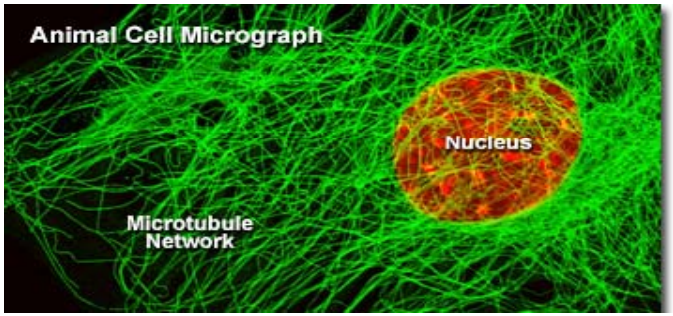
$$Modulus \sim \frac{Energy}{Volume} \sim \frac{eV}{(10^{-10}m)^3} \sim \frac{1.6\times10^{-19}J}{10^{-30}m^3} \sim 100GPa$$

Soft materials….not so easy…..

- For harder plastics, stiffer biomaterials tensile testing possible
- But….clamps can damage materials, gels slip/flow
- Typically, time-dependent response, so loading rate matters.



Soft Matter, 2013,**9**, 4875-4882

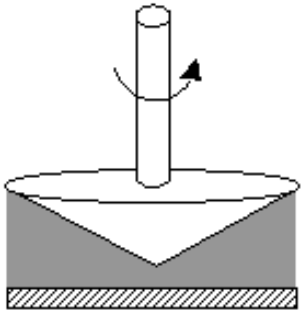


http://micro.magnet.fsu.edu

$$Modulus \sim \frac{Energy}{Volume} \sim \frac{k_B T}{(10^{-7} \rightarrow 10^{-8} m)^3} \sim \frac{4 \times 10^{-21} J}{10^{-21} \rightarrow 10^{-24} m^3} \sim 4 - 4000 Pa$$

Alternative: Shear Rheometry

- Material is placed onto plate or cup and sheared
- Several tool geometries are common

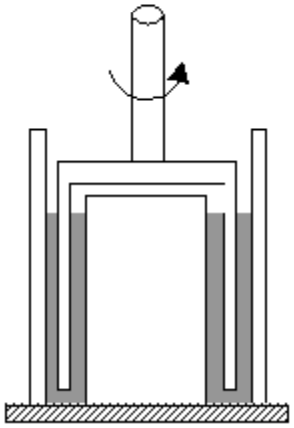


Cone/plate

Constant shear rate

Plate/plate

Minimal volume



Double Cylinder

Maximal sensitivity

Cylinder Or Couette

Transducer

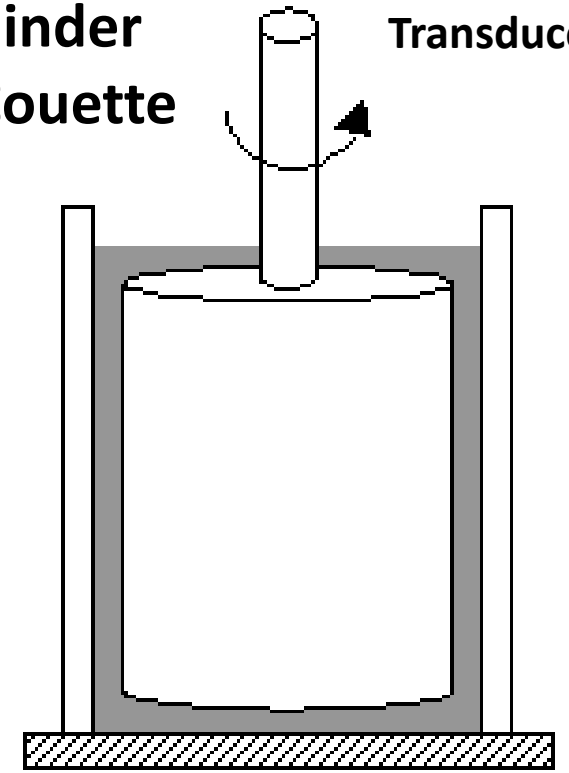


Plate is on motor

Drives at fixed amplitude and frequency

Rheometer requirements/capabilities

- 0.5-10 mL of sample
- Control/change temperature
- Control humidity
- Stress or strain control possible
- Minimum strain depends on torque sensitivity
- Maximum strain depends on material

Bohlin Rheometer

Oscillatory Measurements

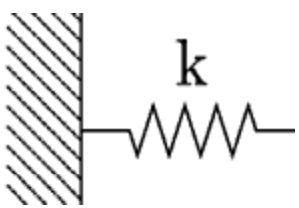
Apply oscillatory strain: $\gamma = \gamma_0 \sin(\omega t)$

Measure oscillatory stress:

$$\sigma = \gamma_0 \left[G' \sin(\omega t) + G'' \cos(\omega t) \right]$$

In-phase elastic component
"Storage modulus"

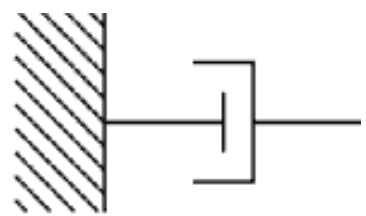
Out-of-phase viscous component
"Loss modulus"



Spring

$$F = kx$$

Hooke's Law



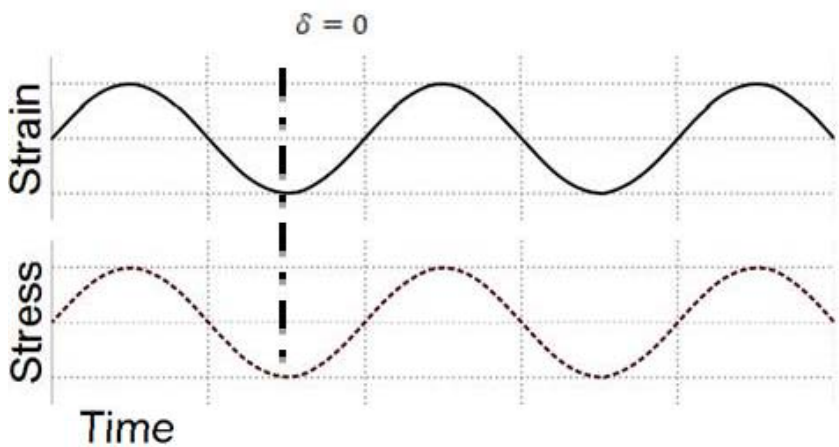
Dashpot

$$F \sim \eta \frac{dx}{dt}$$

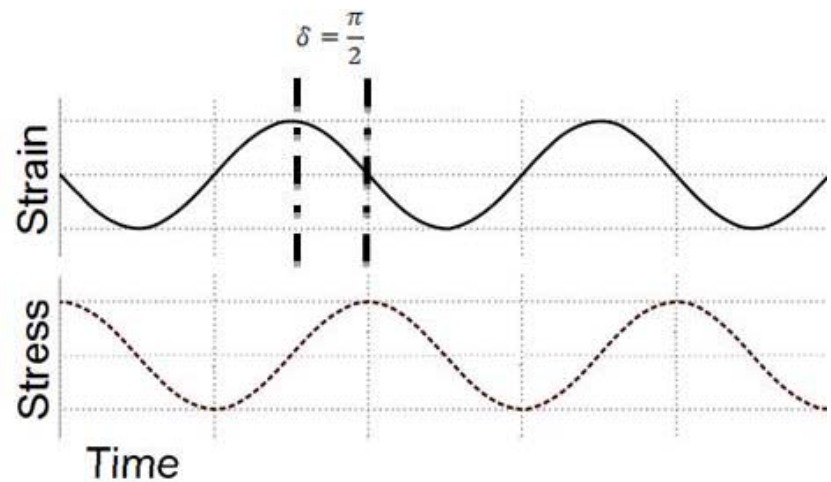
Stokes' Law

Oscillatory Measurements

Pure elastic response



Pure viscous response



$$\sigma \sim \gamma_0 \sin(\omega t + \delta)$$

Phase Angle

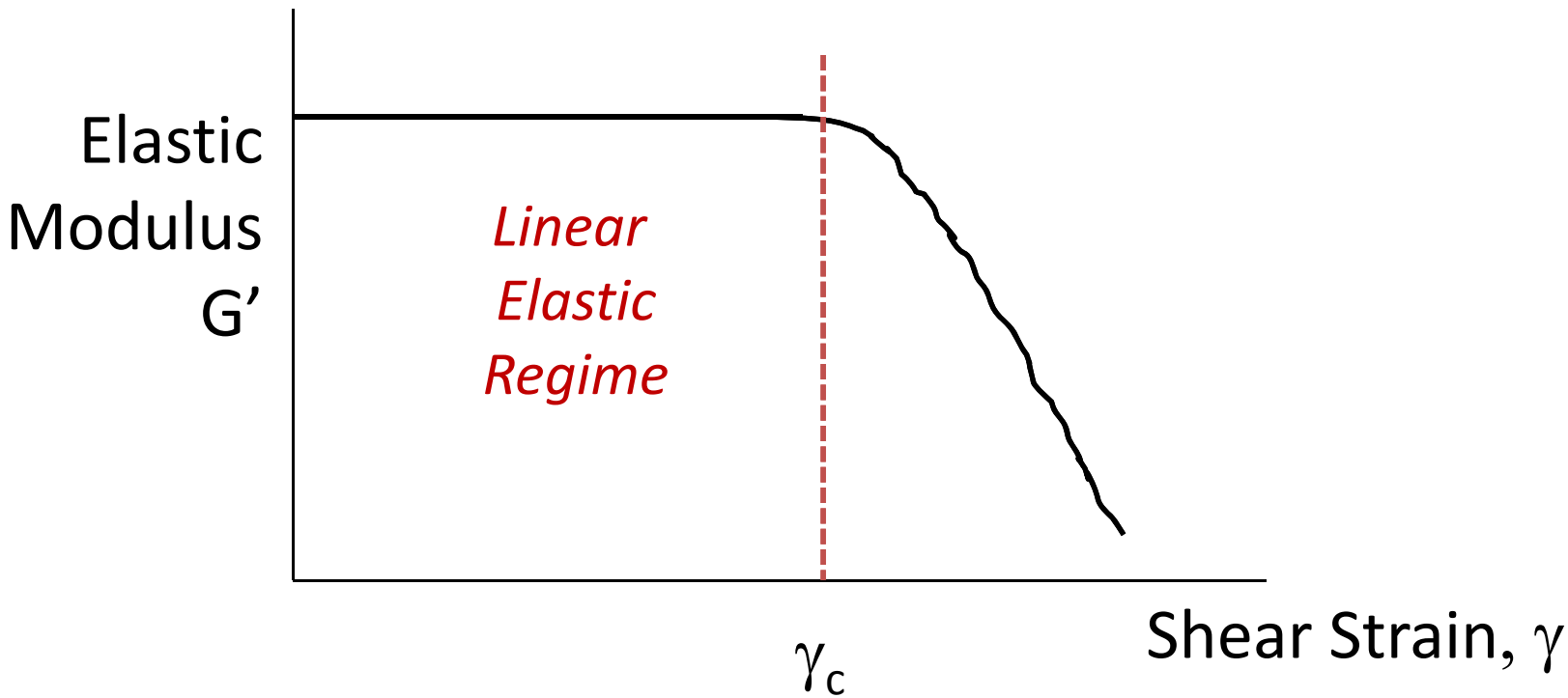
$$\tan\delta = \frac{G''}{G'}$$

$G' > G''$ **Viscoelastic Solid**

$G'' > G'$ **Viscoelastic Liquid**

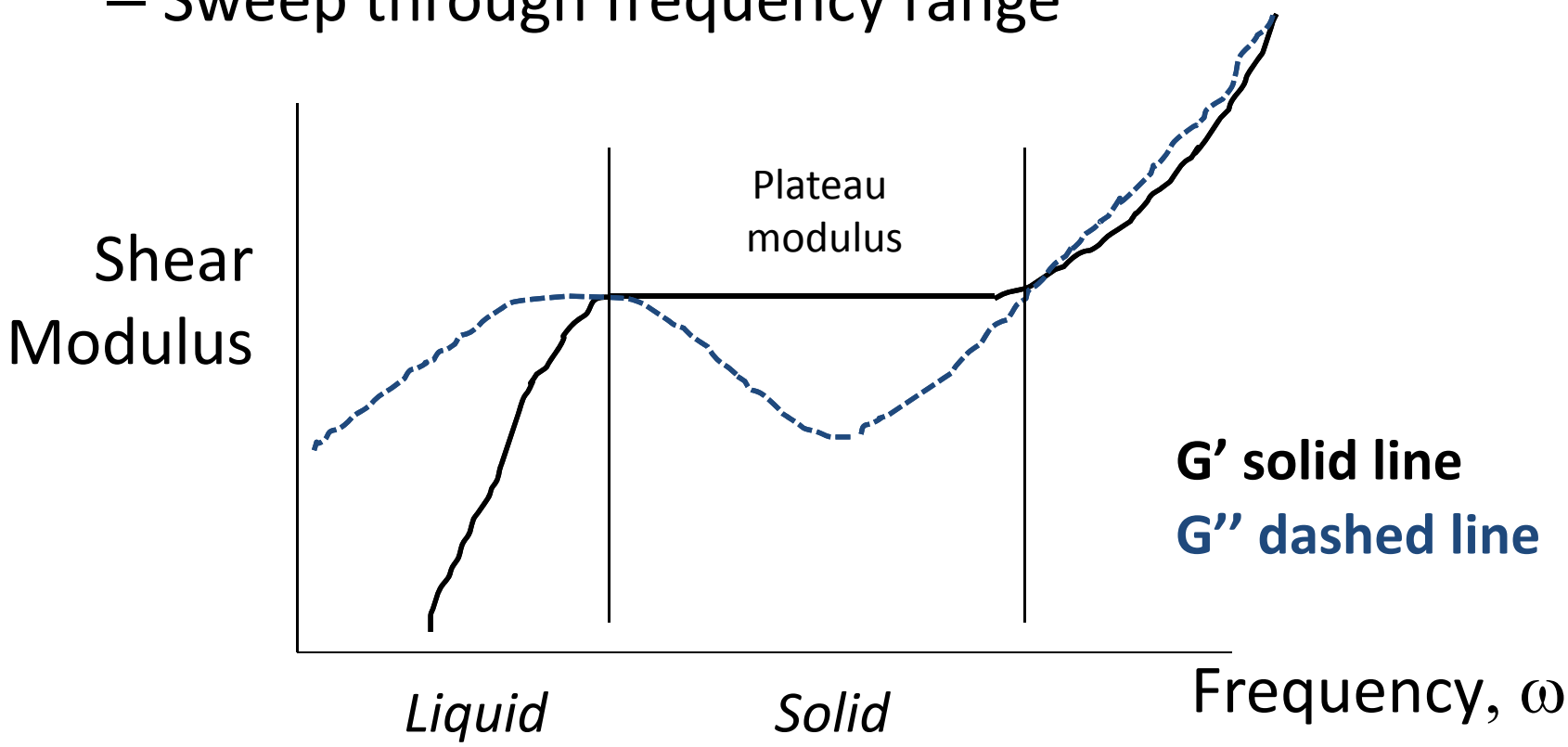
Common Tests

- Strain Sweep
  - Fix frequency
  - Sweep through strain range



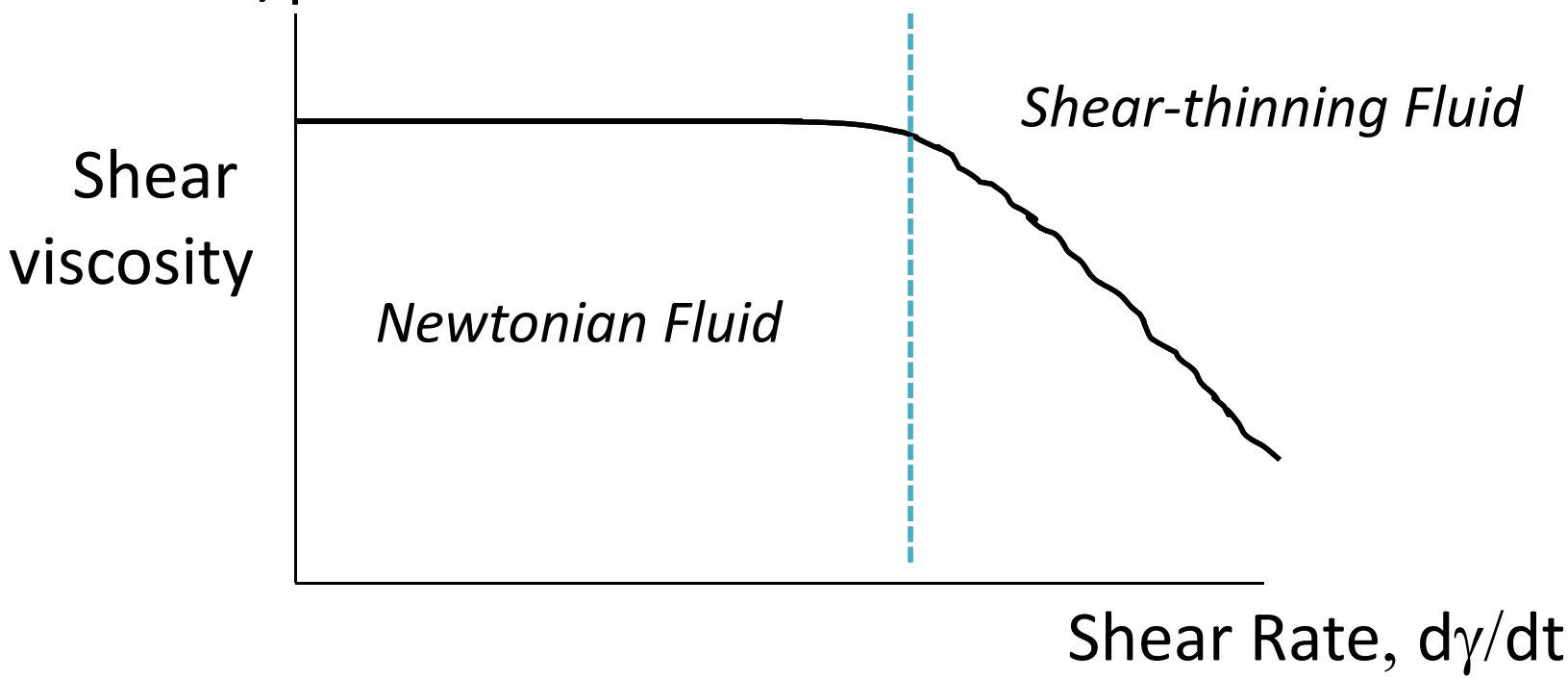
Common Tests

- Frequency Sweep
  - Fix strain in linear regime
  - Sweep through frequency range



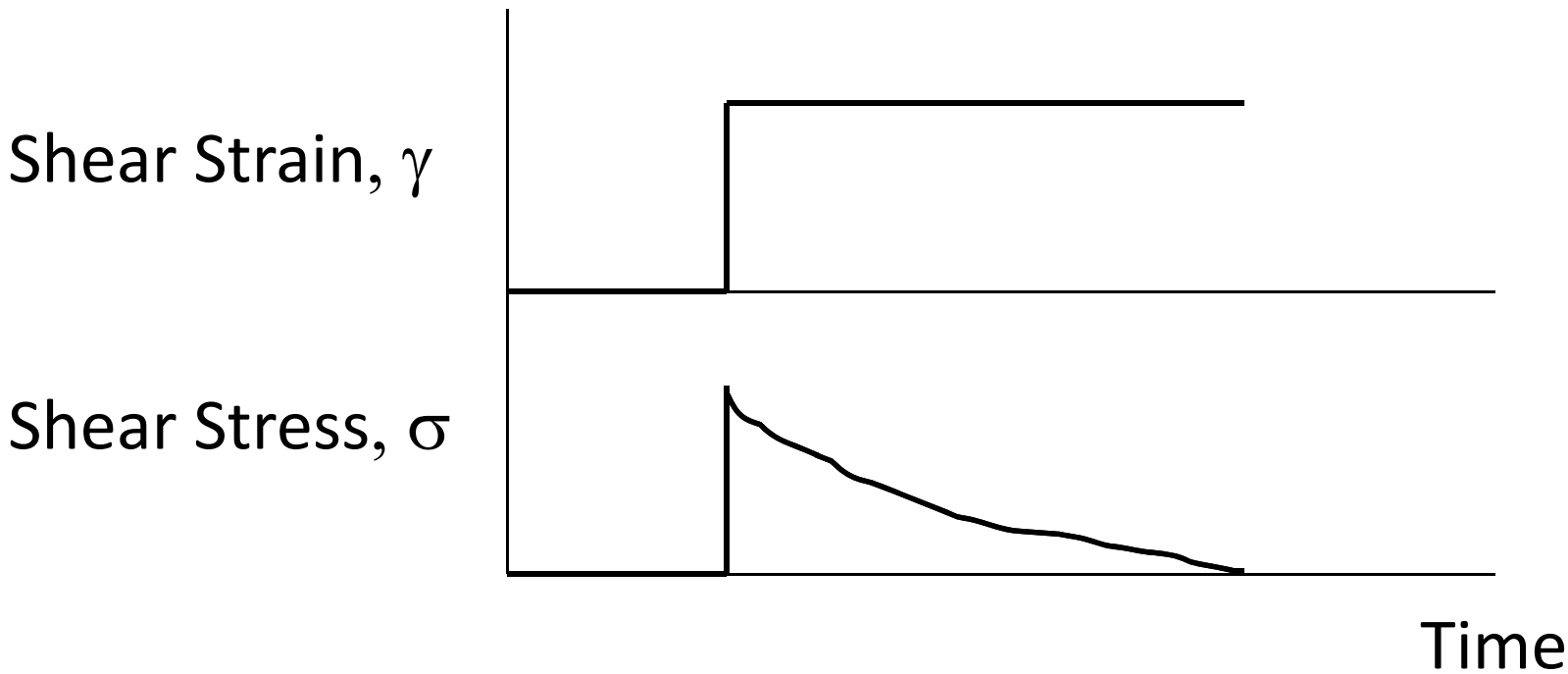
Common Tests

- Strain Rate Sweep
  - Continuous rotation, with increasing shear rate
  - Liquids only
  - Cone/plate best tool



Common Tests

- Creep Test
  - Apply step strain, measure stress change over time



Conventional Rheometry: Summary

Advantages:

- Direct mechanical measurement
- Strain- and frequency-dependent measurements are possible
- Probes average behavior of complex materials
- Fairly easy, fast

Disadvantages:

- Relatively large sample volumes are required (>500 µL)
- Fragile materials can be damaged during loading
- Frequency range is limited by tool inertia
- Difficult to study heterogeneous, or phase-separated materials

Particular challenges for biopolymers

- Very soft materials → signals are small
  - Hard to obtain in large quantities
  - Small linear regime (often)
- Samples are fragile, easily dry out or denature
- Samples are heterogeneous
  - And the heterogeneity matters!
- Very limited temperature range available
  - Prevents use of time/temperature superposition

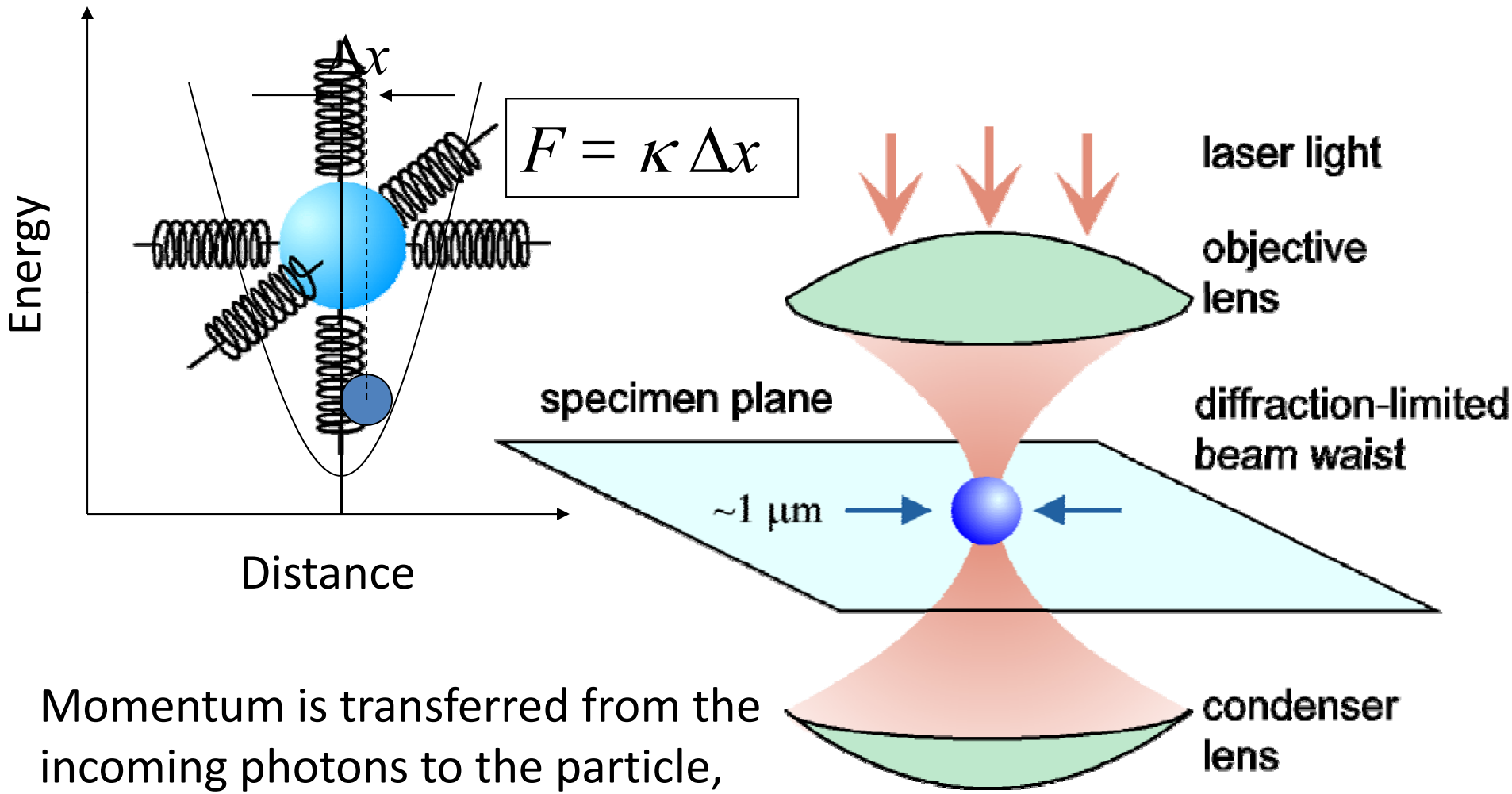
Microrheology is a good alternative, especially for biomaterials

1. **<u>Active Methods</u>:** **Material is locally deformed, and microscopic viscoelastic response recorded. (Magnetic or Optical Tweezers, AFM, etc.)**

Note: unrelated to *active materials*…..

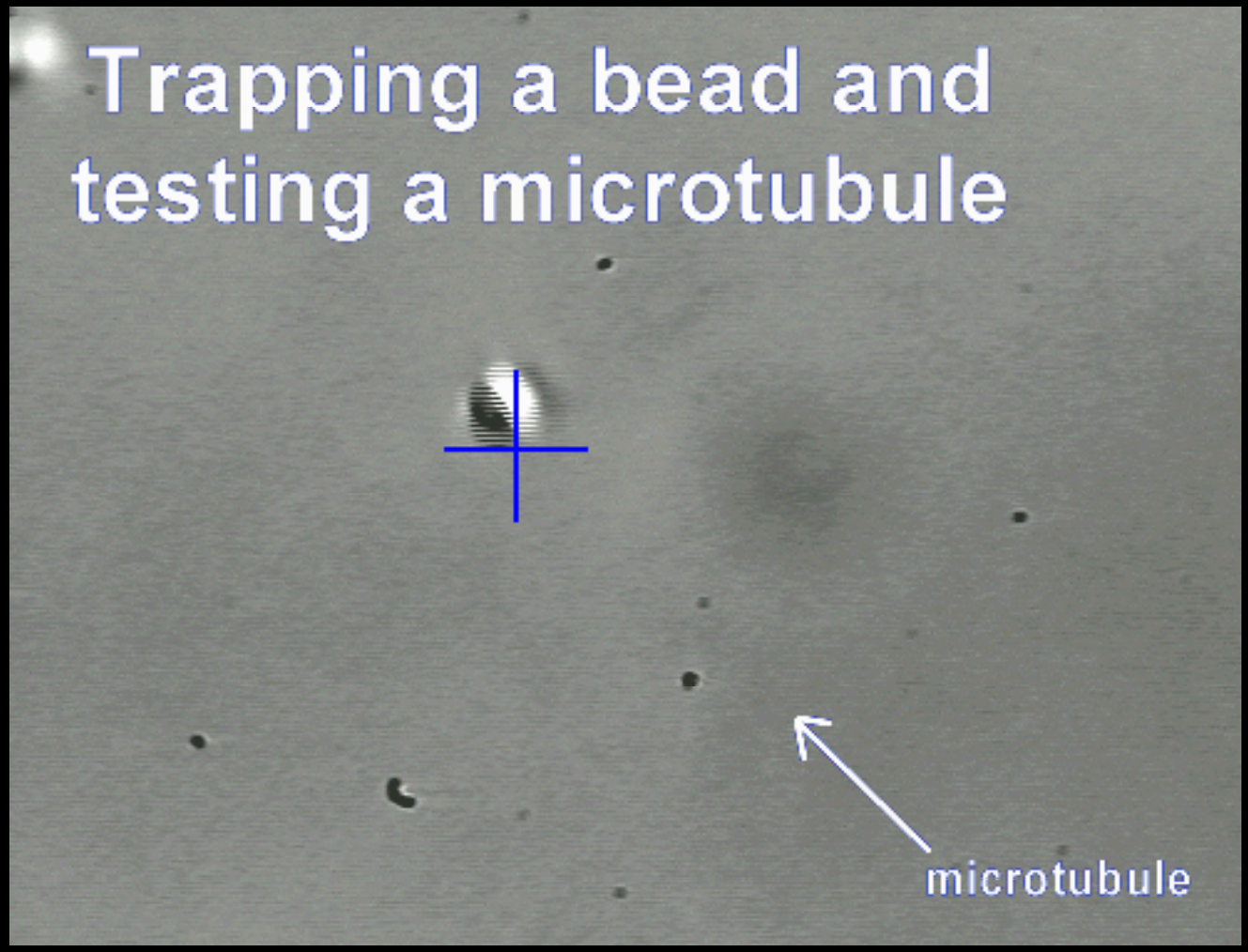
2. <u>Passive Methods:</u> Tracer particles are embedded in a complex material, and their thermal displacements measured.

Active microrheology using optical traps

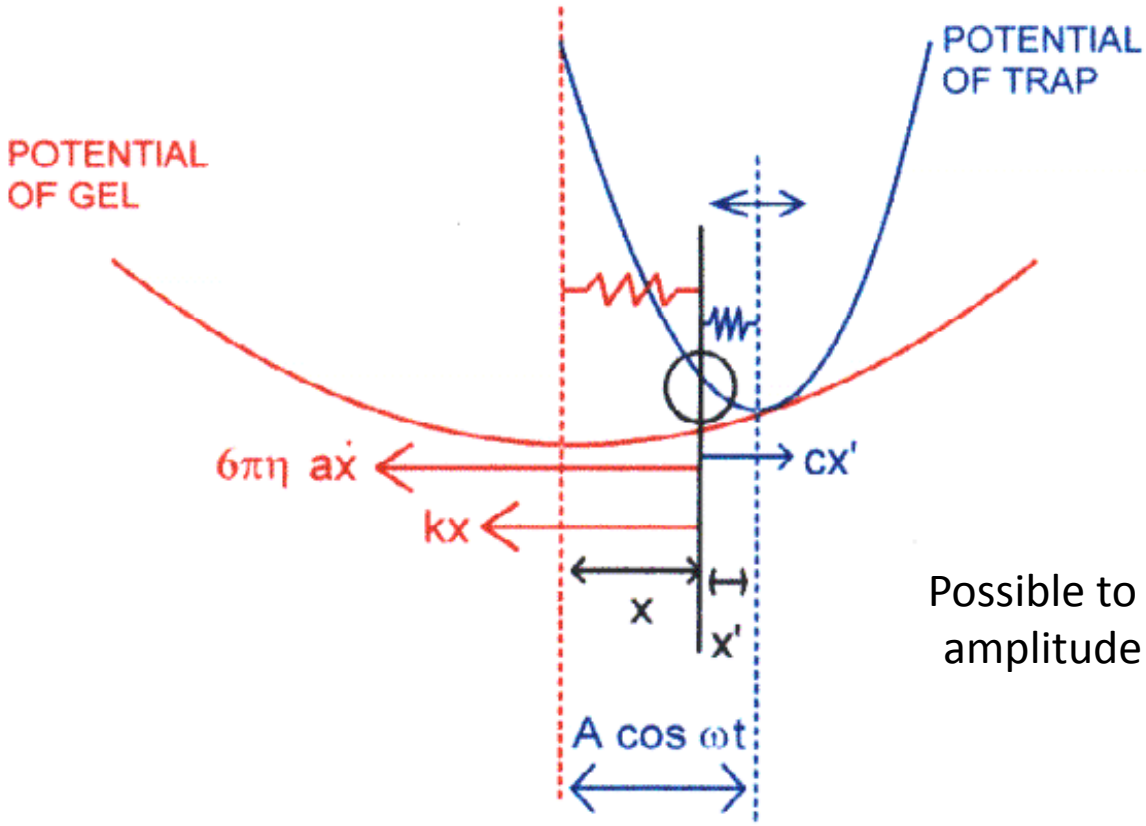


Momentum is transferred from the incoming photons to the particle, creating an attractive force.

Optical Trapping in Action



Oscillating optical traps measure local rheology



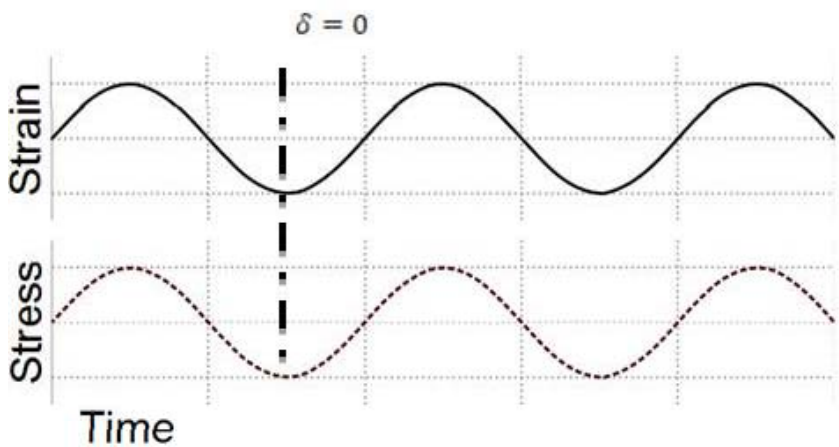
Possible to vary frequency & amplitude of bead motion.

Figure 1. The force diagram of a particle trapped by optical tweezers in a viscoelastic medium.

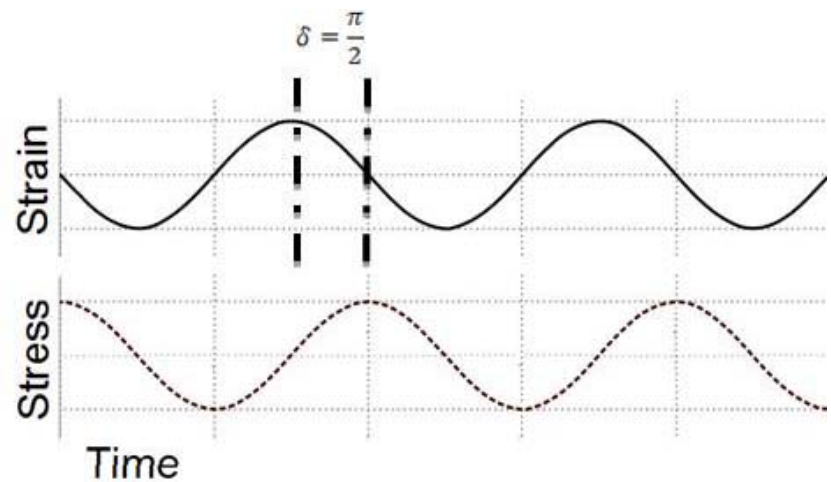
Valentine, Dewalt, et al. *J. Phys. Cond. Matt* (1996).

Oscillatory Measurements

Pure elastic response



Pure viscous response



Compare motion of trap with motion of the bead
Look for in phase and out of phase response
Measure amplitude shift

Damped driven harmonic oscillator problem

$$m\ddot{x} + 6\pi\eta a\dot{x} + (k+c)x = cA\cos(\omega t)$$

Steady-state solution:

$$x(t) = D\cos(\omega t - \delta)$$

$$D(\omega) = \frac{c}{m}\frac{A}{\sqrt{\left(\omega_o^2 - \omega^2\right)^2 + 4\omega^2\beta^2}}$$

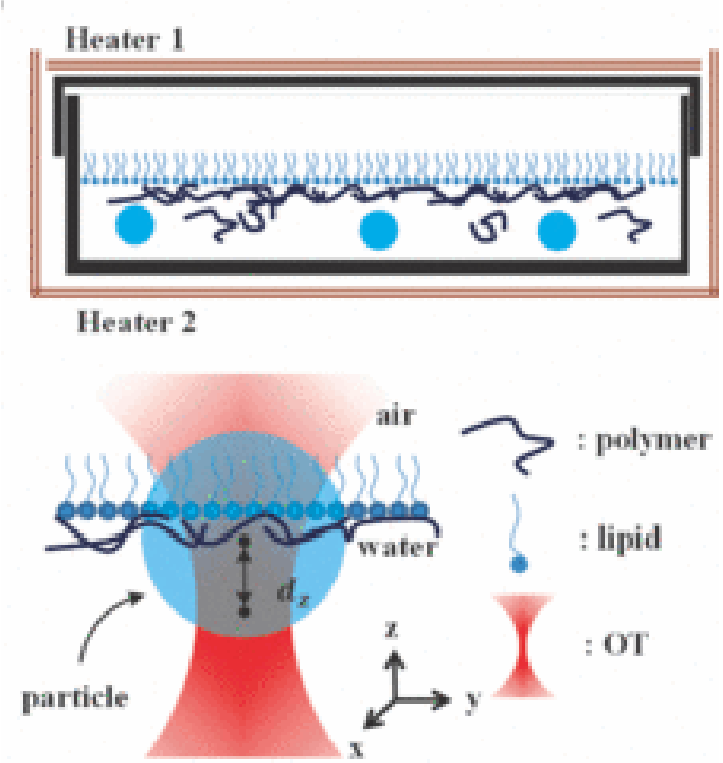
$$\delta(\omega) = \tan^{-1}\left(\frac{2\omega\beta}{\omega_o^2 - \omega^2}\right)$$

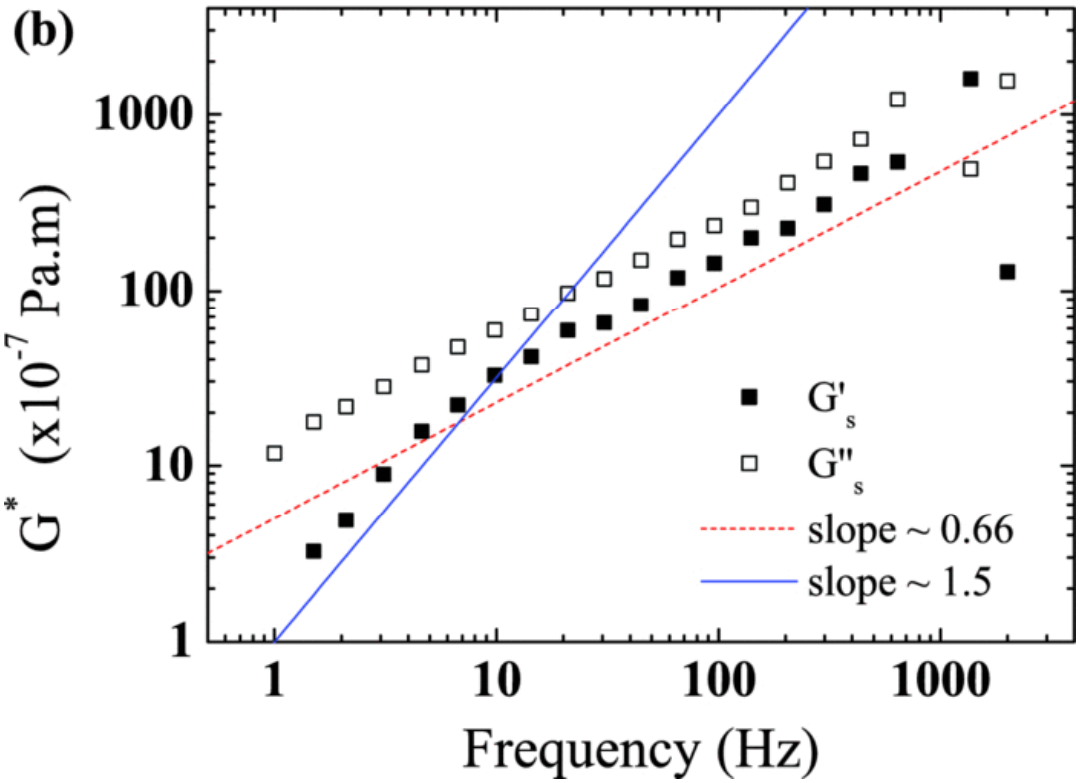
$$\omega_o^2 = \frac{k+c}{m}$$

$$\beta = \frac{3\pi\eta a}{m}$$

Experimentally, need phase-locked detection technique, local measurement of temperature, some assumptions about mass of entrained fluid.

Example: Lipid bilayer rheology





Polyelectrolyte/Lipid Monolayer

C.Y. Park, H.D. Ou-Yang, M.W. Kim
Rev. Sci. Instrum. 82, 094702 (2011).

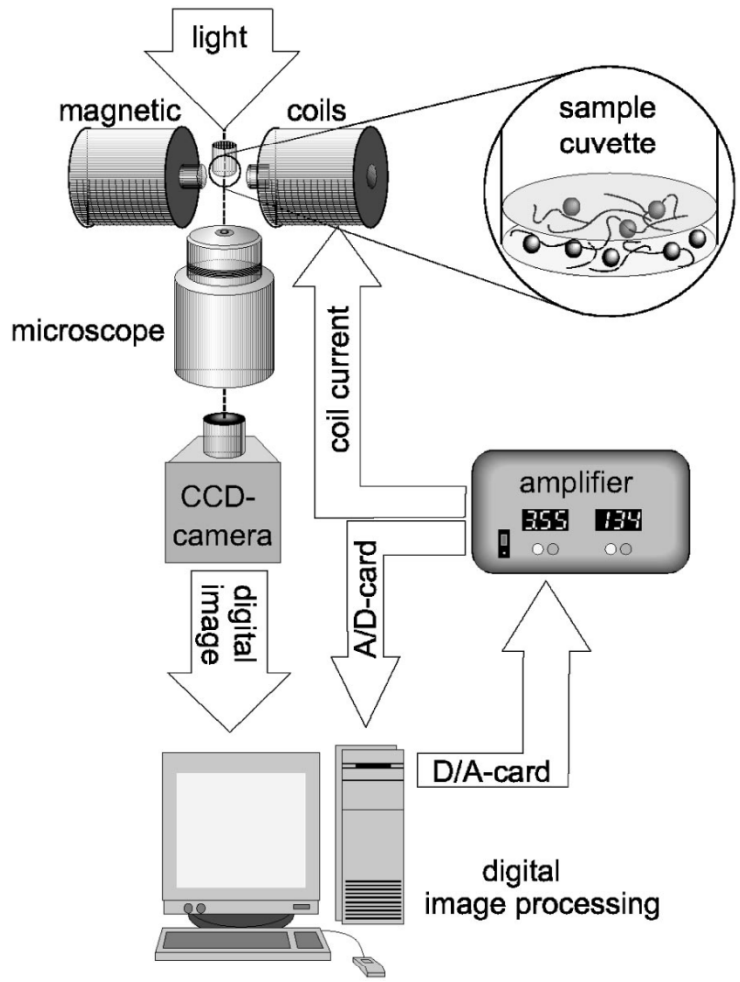
Oscillatory microrheology with magnetic tweezers

- Electromagnets can apply time-varying magnetic fields

- Requires a control system

Force on a super-paramagnetic bead:

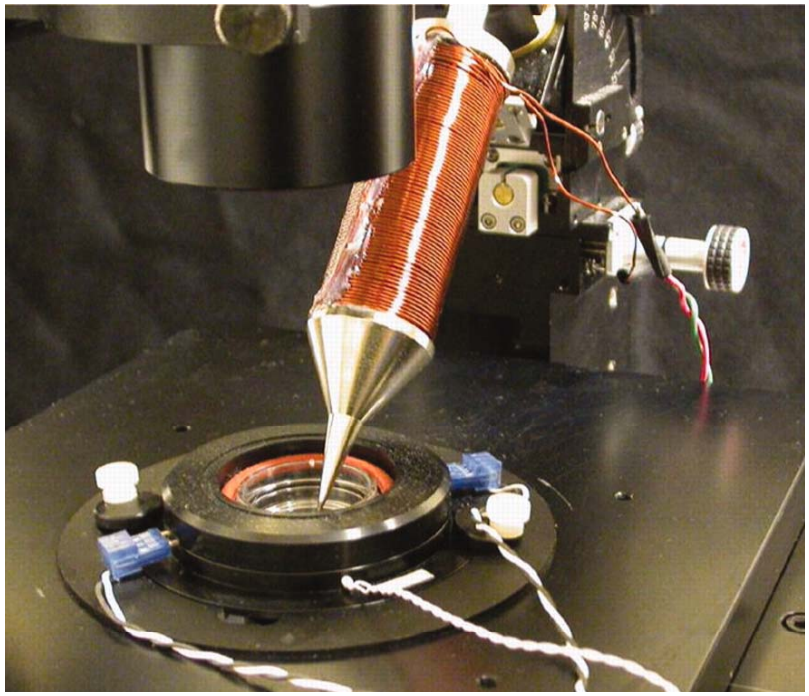
$$\vec{F} = \tfrac{1}{2}\vec{\nabla}\left(\vec{m}(\vec{B}) \bullet \vec{B}\right)$$



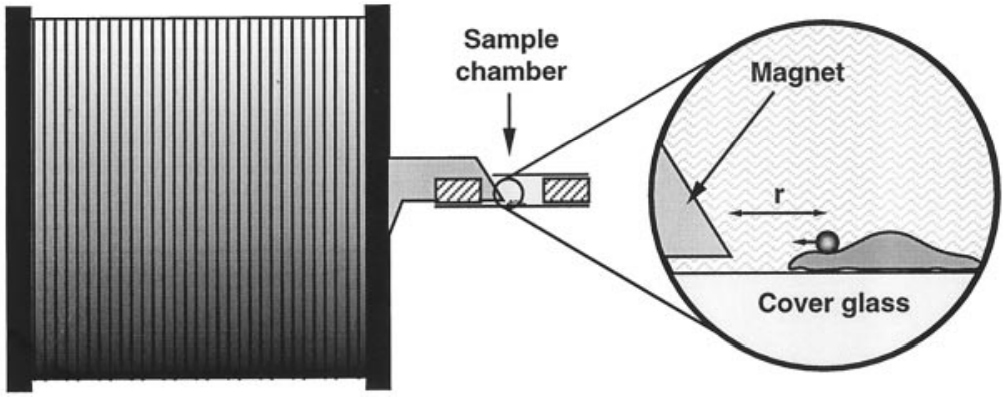
M. Keller, J. Schilling, and E. Sackmann
Review of Scientific Instruments **72, 3626 (2001)**

Electromagnets can also apply constant force for creep measurements

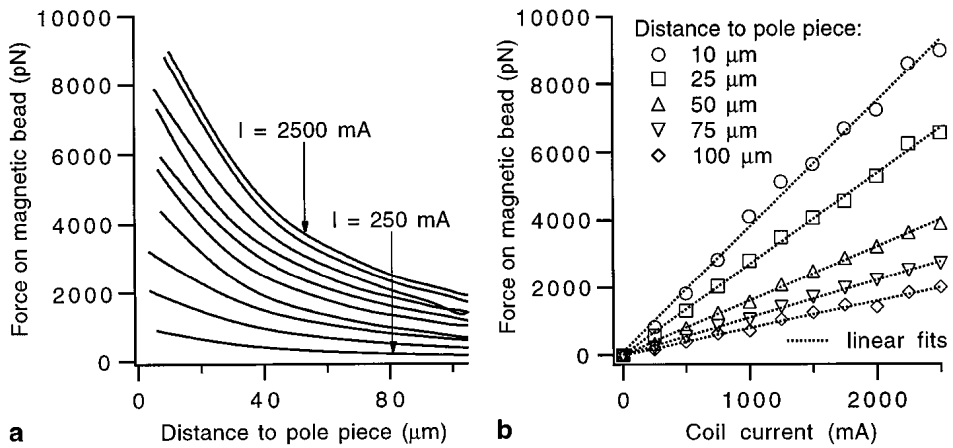
Magnetic Tweezers



Huang H et al. *Am J Physiol Cell Physiol* (2004)



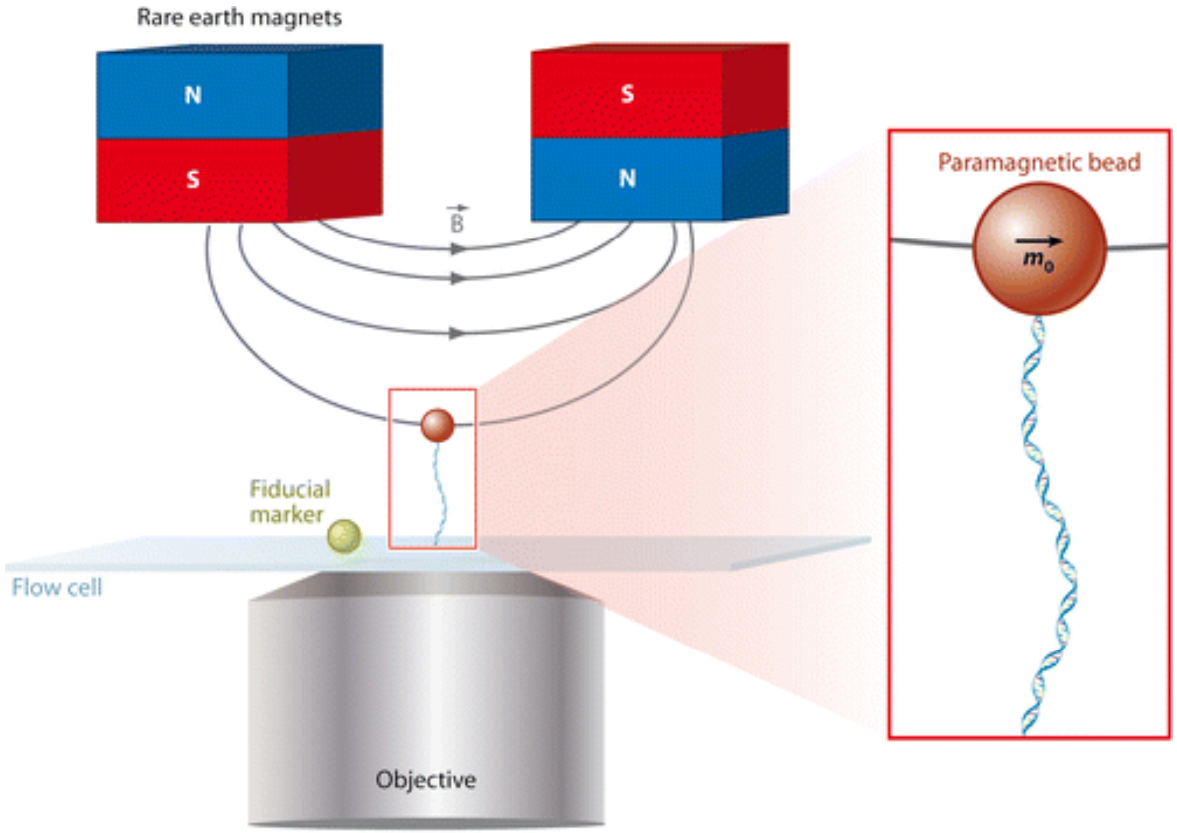
Bausch et al. *Biophys. J.* (1998)



Rare-earth permanent magnets are a good alternative for magnetic tweezers applications

Magnetic Tweezers: Rare earth magnets

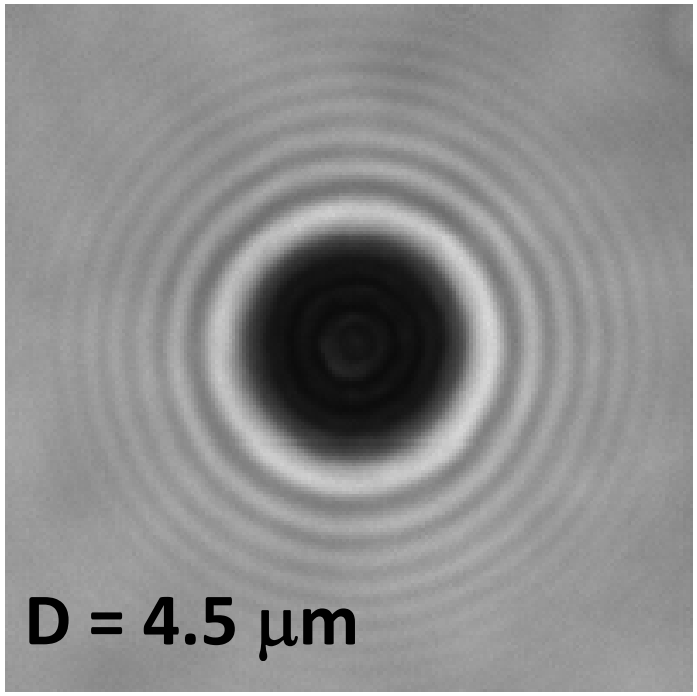
- NdFeB-based magnets
- Inherently constant force measurements
- No external power supplies, no hysteresis
- Magnet-bead distances are typically 1-30 mm
- Typically used for single molecule manipulation
- Nanometer position determination
- -- Possible to achieve high forces



De Vlaminck I, Dekker C. 2012. Annu. Rev. Biophys. 41:453–72

Automated tracking of diffraction pattern gives particle position in three dimensions

Top view



10 μm

Standard centroid tracking in *x,y*

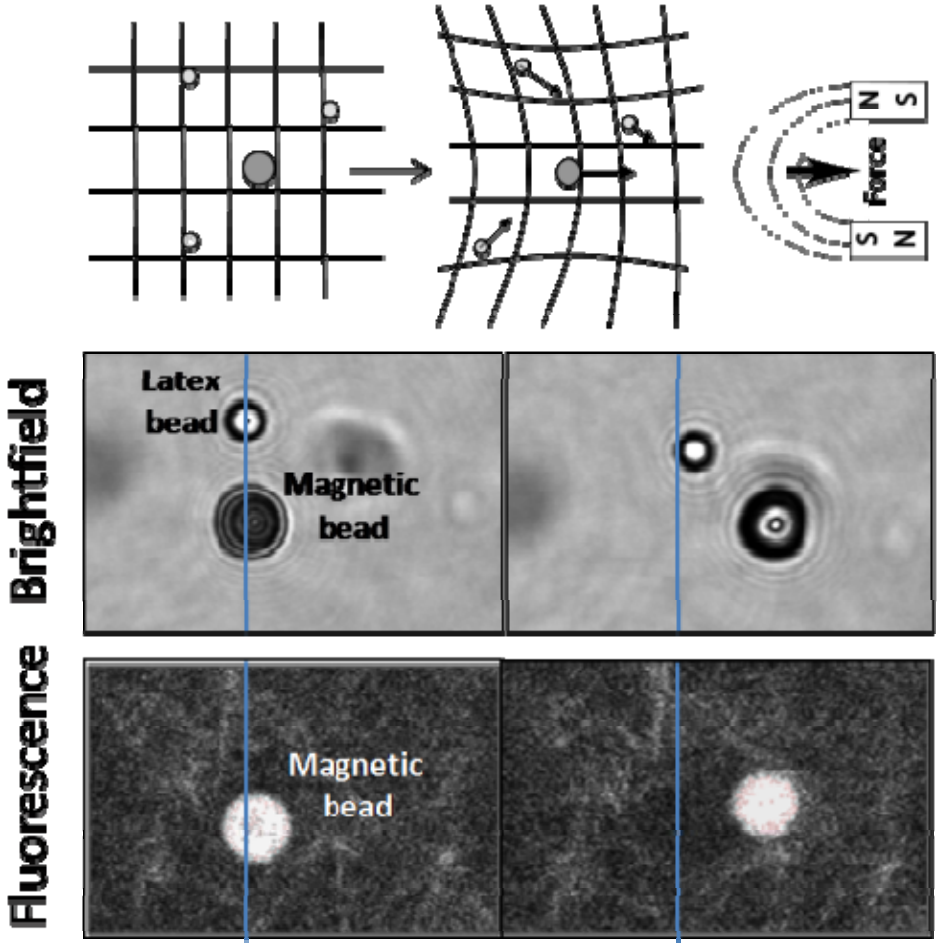
The measured diffraction ring radius, *r*, depends on bead height *z*

Subtract off motion of coverslip by tracking stuck "reference" beads.

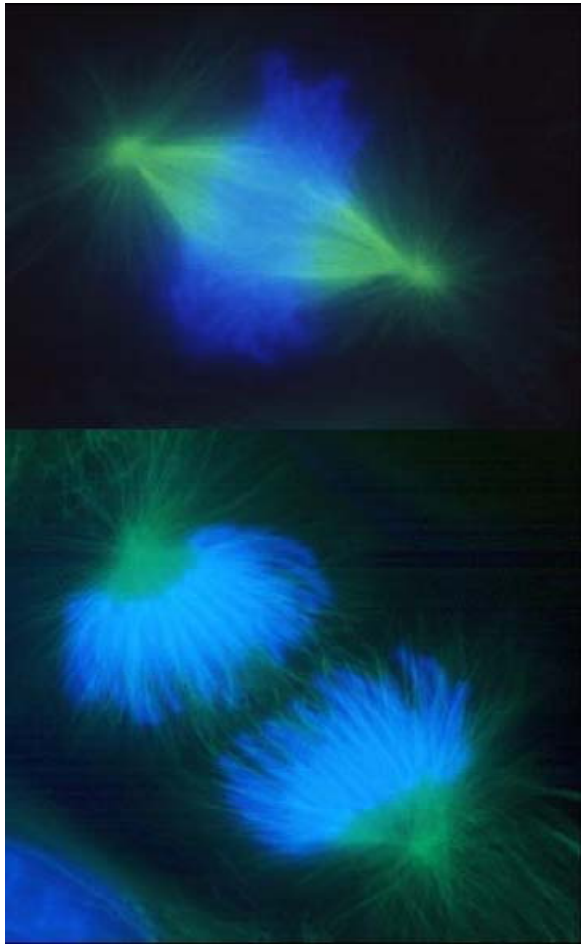
Instrinsic resolution in *x,y,z* ~ 1-2 nm

Gosse and Croquette, 2002

Microscale creep measurements with magnetic tweezers

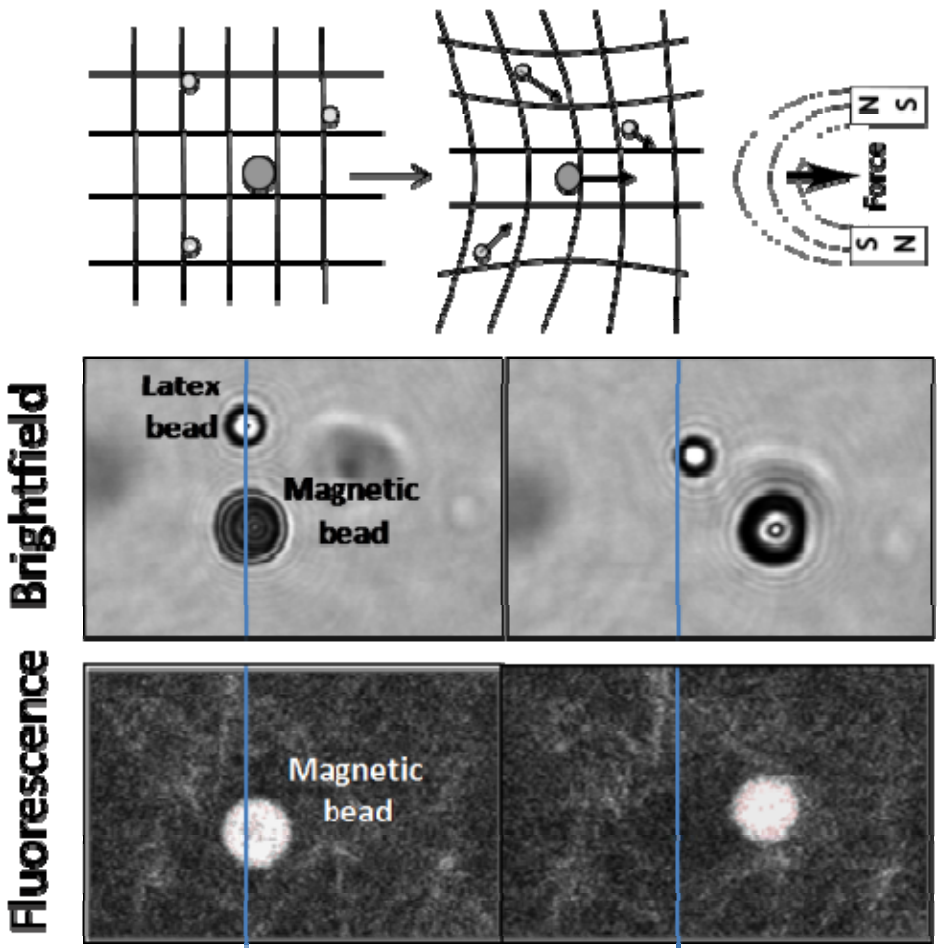


Entangled network of microtubules

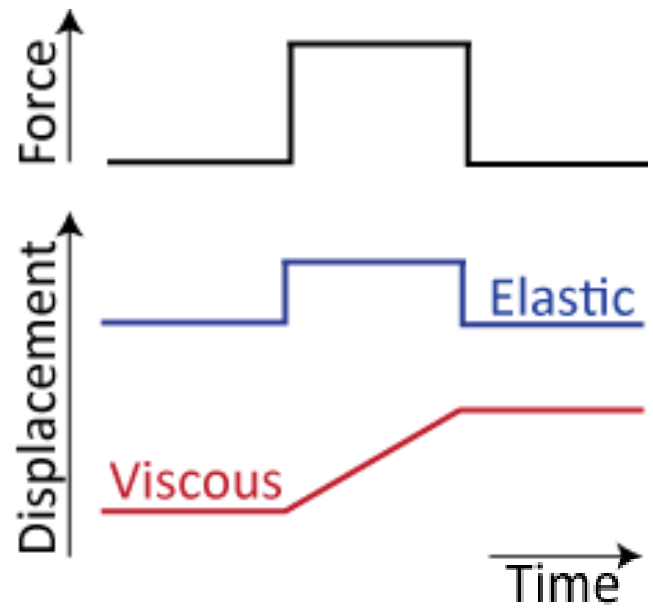


http://www2.warwick.ac.uk/fac/sci/bio/ug/courses/biolsci

Microscale creep measurements with magnetic tweezers



Entangled network of microtubules



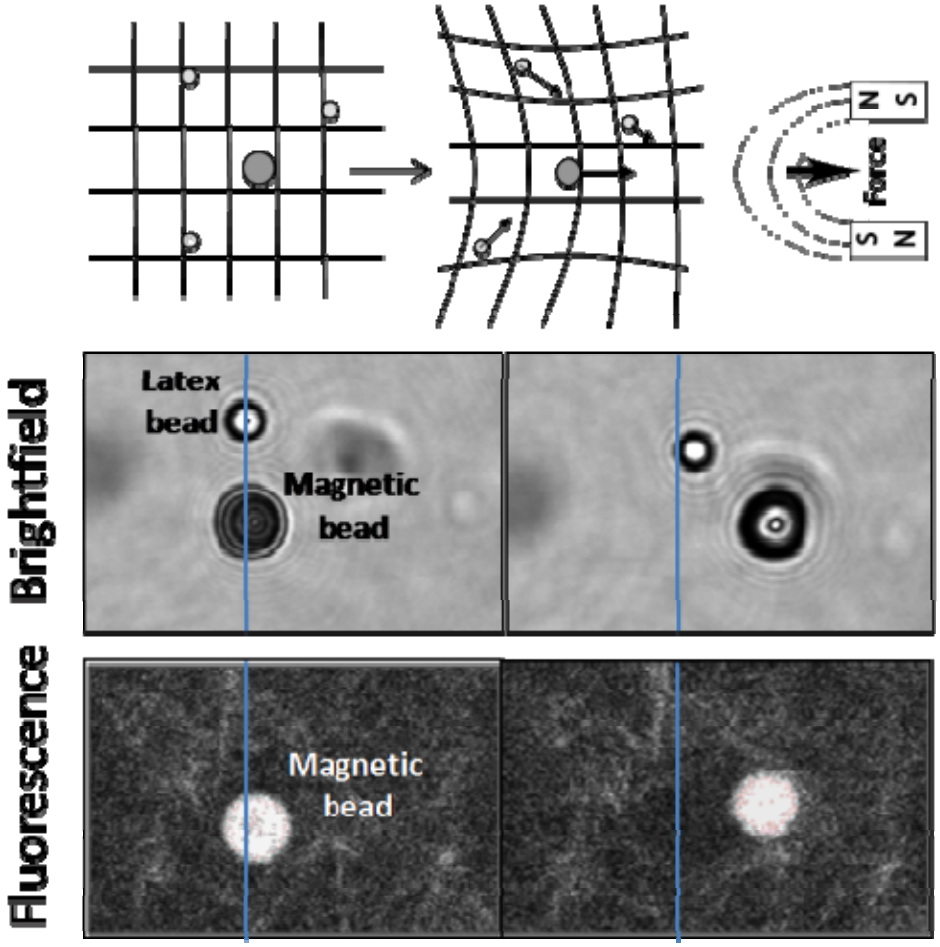
Elastic Solids: Hooke's Law

$$F = \kappa x$$

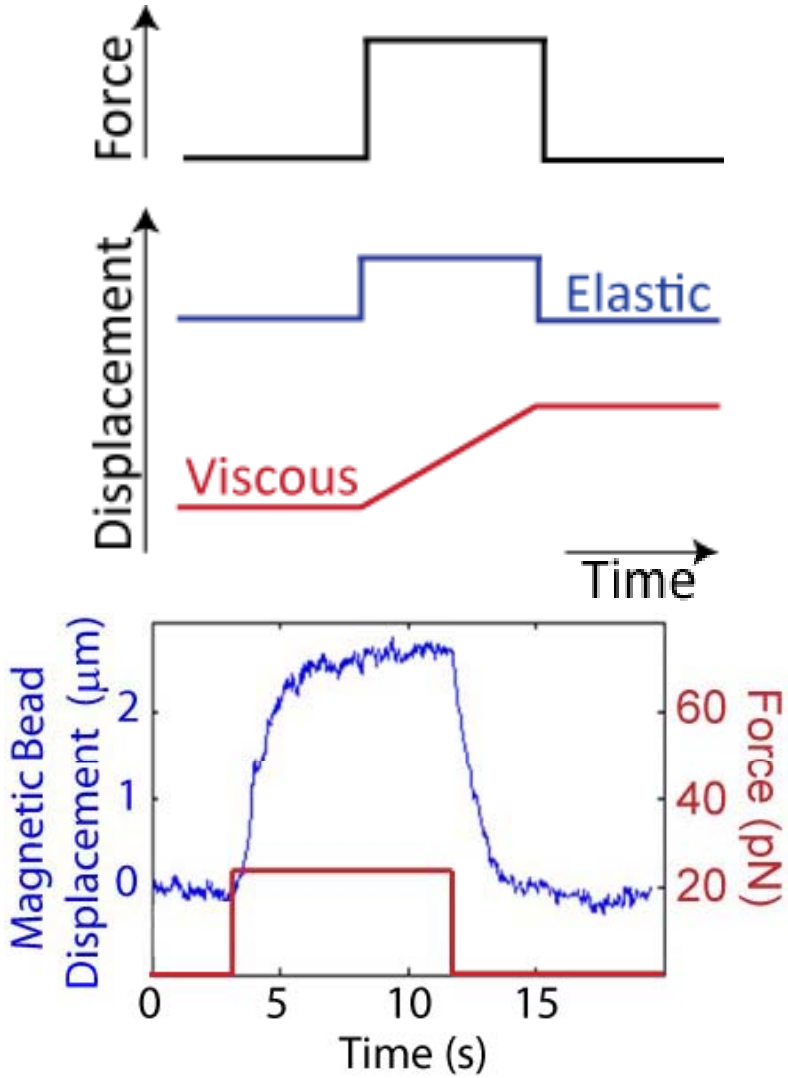
Viscous Fluids: Stoke's Law

$$F = 6\pi\eta a \frac{dx}{dt}$$

Microscale creep measurements with magnetic tweezers



Entangled network of microtubules



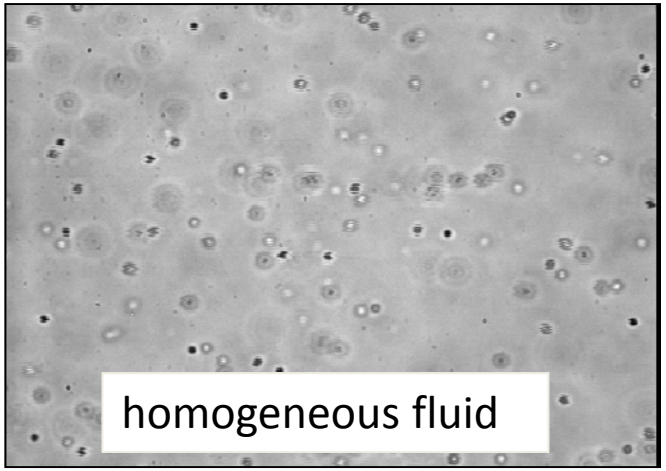
Microrheology is a good alternative, especially for biomaterials

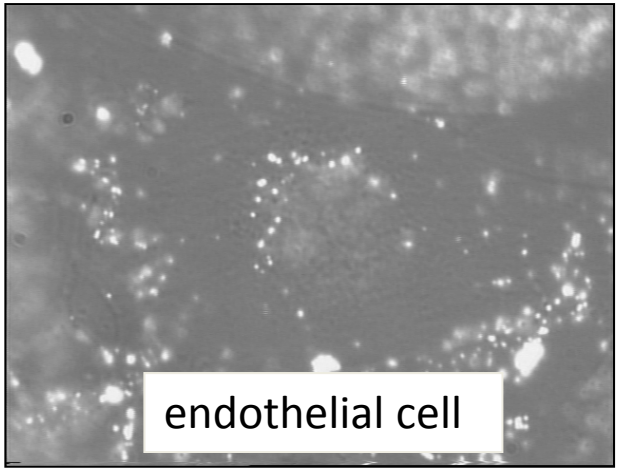
1. Active Methods: Material is locally deformed, and microscopic viscoelastic response recorded. (Magnetic or Optical Tweezers, AFM, SFA)

2. **Passive Methods:** **Tracer particles are embedded in a complex material, and their thermal displacements measured.**

Video-based multiple particle tracking

δx ~ 10 nm
δt ~ 1/30 sec



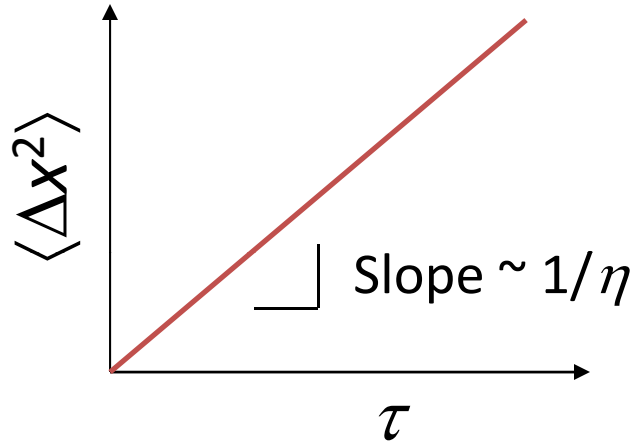


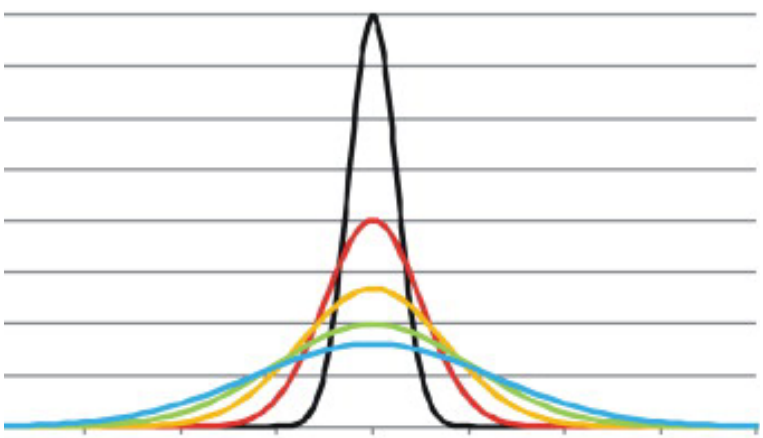
Passive Microrheology: Thermal fluctuations of tracers measure rheology for homogeneous materials

1-d Particle Diffusion in a homogeneous fluid:

$$\langle \Delta x^2(\tau) \rangle \equiv \left\langle \left| x(t+\tau) - x(t) \right|^2 \right\rangle_t$$

$$\langle \Delta x^2(\tau) \rangle = 2D\tau$$





Gaussian Distribution of particle displacements

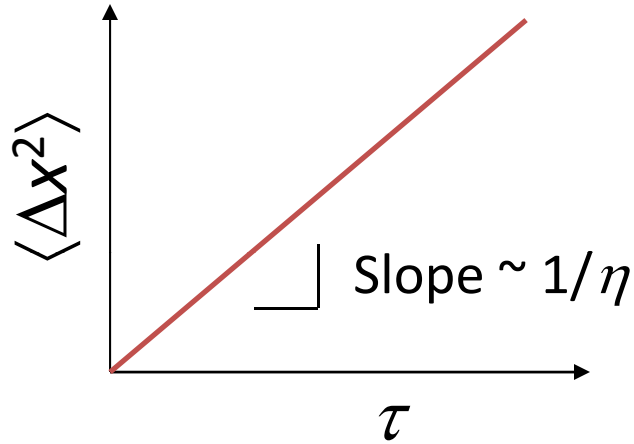
Mean doesn't change
Standard Deviation grows as $\sqrt{\tau}$

Passive Microrheology: Thermal fluctuations of tracers measure rheology for homogeneous materials

1-d Particle Diffusion in a homogeneous fluid:

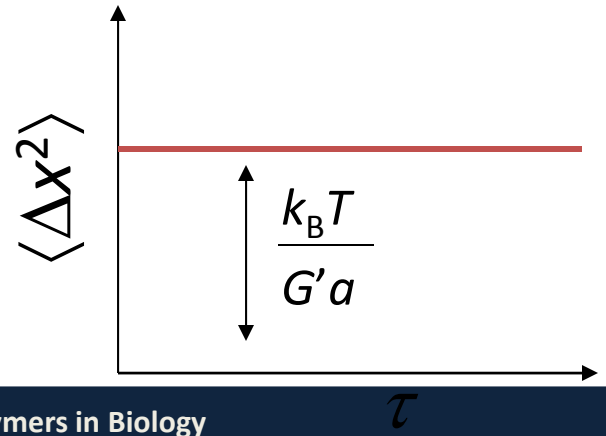
$$\langle \Delta x^2(\tau) \rangle \equiv \left\langle \left| x(t+\tau) - x(t) \right|^2 \right\rangle_t$$

$$\langle \Delta x^2(\tau) \rangle = 2D\tau = \frac{k_B T \tau}{3\pi\eta a}$$



Particle Diffusion in a homogeneous solid:

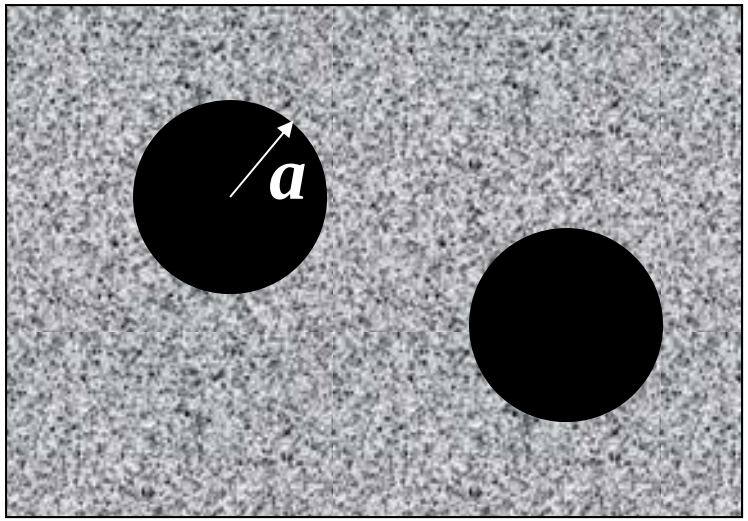
$$\frac{1}{2} k_B T = \frac{1}{2} \kappa \langle \Delta x^2 \rangle$$



Passive measurements are inherently in linear regime.

Thermal Fluctuations can also measure local frequency-dependent viscoelastic moduli G', G''

Homogeneous Material



Particles move as network fluctuates

$a >>$ structural length, ξ

$$\left\langle \Delta x^2(\tau) \right\rangle = 2D\tau = \frac{k_B T \tau}{3\pi\eta(\tau)a}$$

Mason and Weitz (1995) PRL

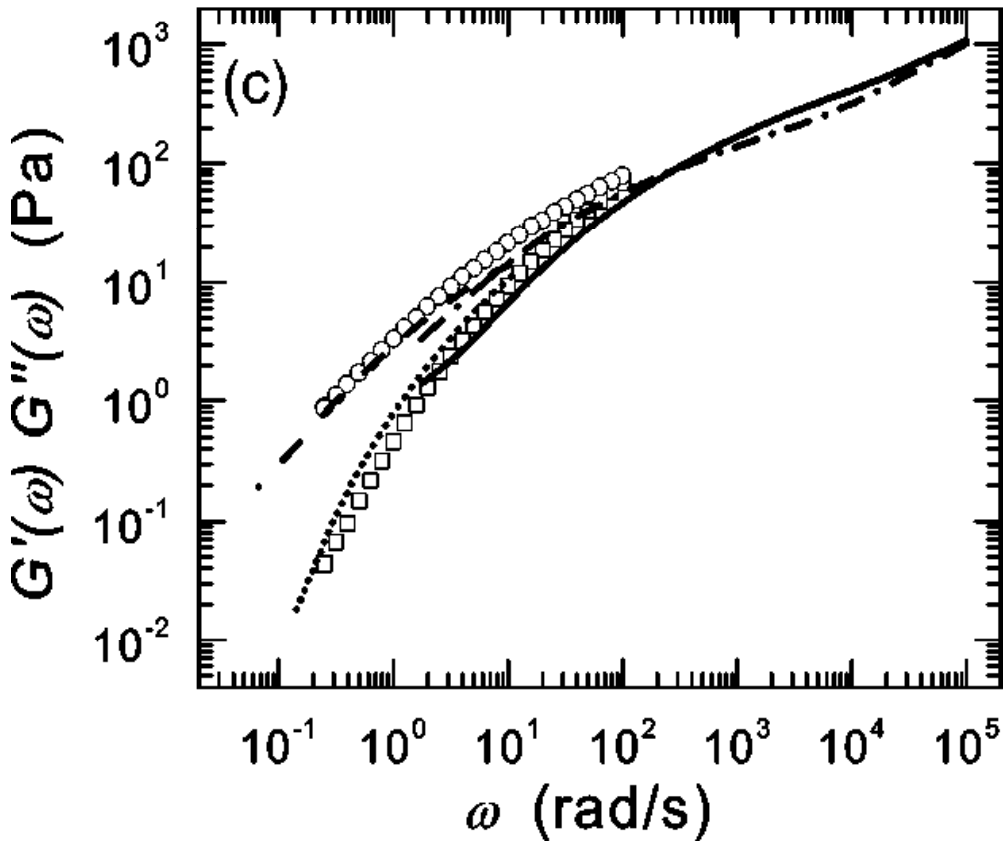
$$\eta(\tau) = \frac{k_B T \tau}{3\pi a \left\langle \Delta x^2(\tau) \right\rangle}$$

$$G(s) = \frac{k_B T}{3\pi a s \left\langle \Delta x^2(s) \right\rangle}$$

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

Generalized, frequency-dependent Stokes-Einstein relation

Example: Microrheology of Poly(EthyleneOxide)



900 kDa PEO

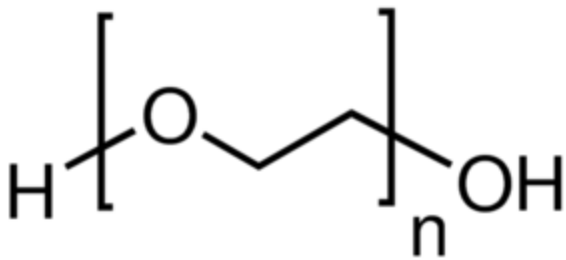
Symbols – Bulk rheology

Lines – Microrheology using light scattering (DLS, DWS)

Data are identical with different particle sizes and chemistries

BR Dasgupta, SY Tee, JC Crocker, BJ Frisken and DA Weitz
PHYSICAL REVIEW E, VOL. 65, 051505 (2002)

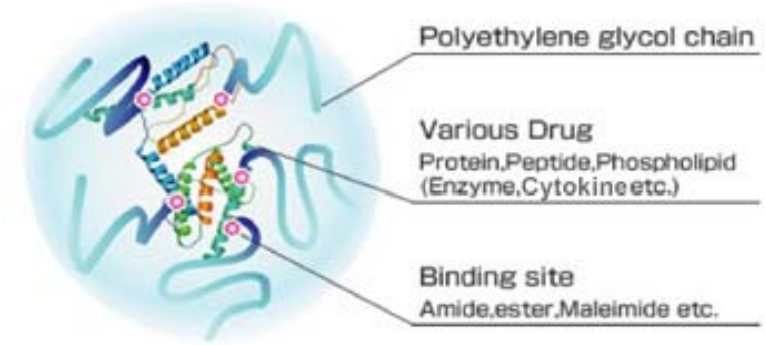
Example: Microrheology of Poly(EthyleneOxide)



High Mw – Poly(ethylene oxide) – PEO
Low Mw – Poly (ethylene glycol) – PEG

Extremely hydrophilic, biocompatible

Drug Delivery



Toothpaste

Laxative

Limits of resolution for passive methods

Using video-based detection methods:

δx ~ 10 nm
δt ~ 30 msec

a ~ 100 nm
T ~ 21 °C

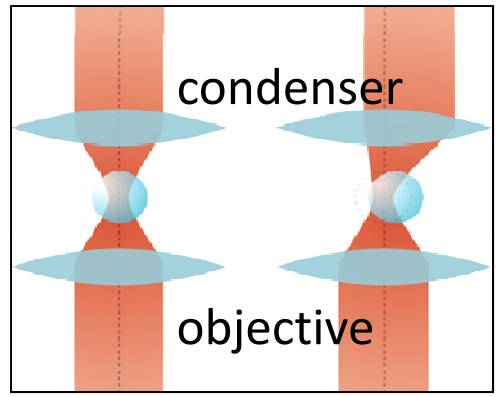
$$\eta = \frac{k_B T}{3\pi a}\frac{\delta t}{\delta x^2} \sim 1500\ \mathrm{mPa \cdot s}$$

Scales with δt, so longer times are better…
Need to eliminate/control for instrument drift

$$G' = \frac{k_B T}{a}\frac{1}{\delta x^2} \sim 400\ \mathrm{Pa}$$

Alternative: Light Scattering (DLS, DWS)

Using laser deflection methods:



δx ~ 0.5 nm
δt ~ 0.1 msec

a ~ 100 nm
T ~ 21 °C

$$\eta = \frac{k_B T}{3\pi a}\frac{\delta t}{\delta x^2} \sim 2000\ \mathrm{mPa \cdot s}$$

$$G' = \frac{k_B T}{a}\frac{1}{\delta x^2} \sim 200\ k\mathrm{Pa}$$

For next time….

- Find an interesting material
  - Fluid, Soft solid, Something in between…
- Develop some method to measure its rheology
  - Use everyday tools
- Report back
  - Do your measurements make sense?
  - What classes of biomaterials might be similar to your material of choice?