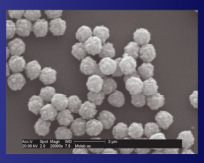
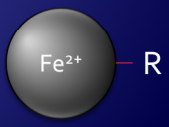
A technique that uses microscale beads to determine cell biomechanics…

Microbeads

- 1977, John Ugelstad succeeded in making spherical polystyrene beads of exactly the same size
- Two step swelling process:
  - Polymer seed particle in water
  - Grows by incorporating slightly water soluble organic compounds
- Magnetic beads: porous beads with oxidative groups mixed with Fe^{2+} salt solutions to form magnetic oxides
- Surface functionality used for separation applications





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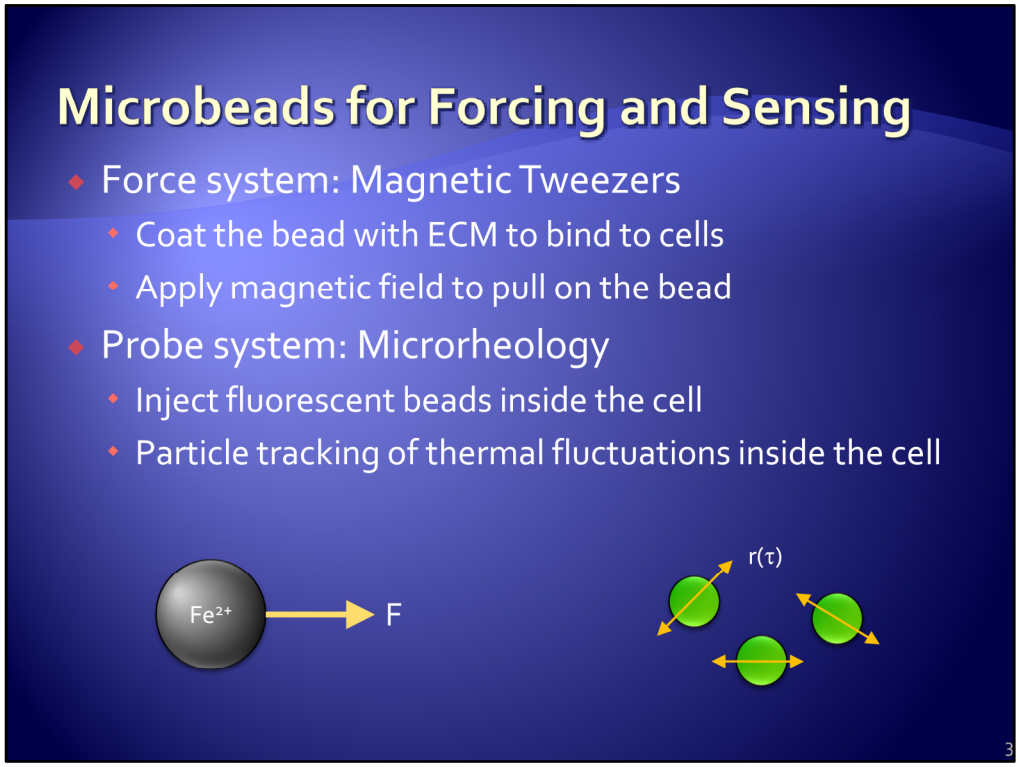
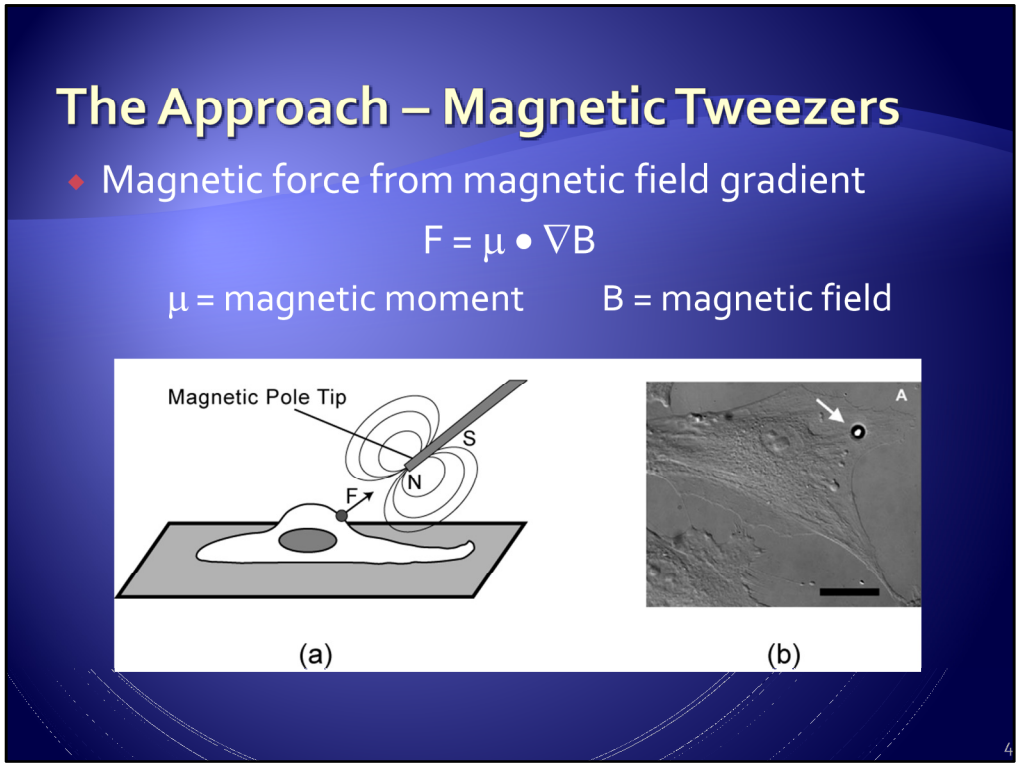
Professor John Ugelstad (Univ. of Trondheim, Norway) greatest scientific achievement was the preparation of large monodisperse polymer beads in 1977 (today known as DynaBeads). Previously, this was only achievable by NASA in the weightlessness of space.

The basic idea for this invention was a two-step swelling procedure. Small "activated" polymer particles are dispersed in water, and these will adsorb slightly water-soluble organic compounds and reach a volume more than 1000 times their own volume.

Further developments resulted in preparation of monodisperse paramagnetic beads in 1981. These may be prepared by dispersing porous beads covered with oxidative groups at the exterior and interior surfaces in an aqueous solution of Fe^{2+} containing salts. The ions react with the oxidative groups and form fine grains of magnetic oxides throughout the beads, resulting in supermagnetic beads, i.e. the beads are only magnetic in a magnetic field. Many applications of these beads lie within immunology, cellular biology, microbiology, molecular biology, medical diagnostics and DNA technology



Two different approaches for cell biomechanics measurements have relied upon the colloidal technology of microparticles. The first is a force application system that uses magnetic fields to pull on the ferrous microbead. The second uses the beads as markers of the local brownian motion within a cell. These particles are injected inside the cell and their positions over a set time frame are tracked to determine their degree of freedom and mobility. From the movement of the particles, the elasticity and viscosity of the cell can be approximated.



The general idea for magnetic tweezers is that a magnetic pole is brought into close proximity with the cell. The magnetic field is non-uniform and as a result, a magnetic body force acts on the magnetic particle. The direction and magnitude of this force depends on the dot product of the magnetic dipole moment of the magnetic particle (how much it can be magnetized) and the gradient of the magnetic field.

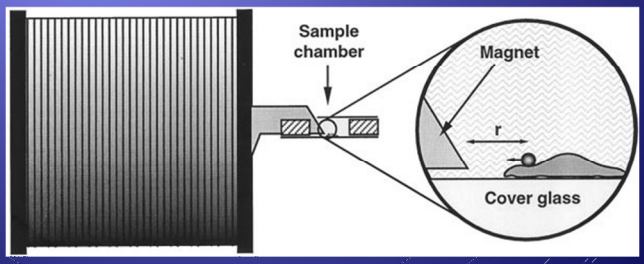
The System

- Electromagnetic Coil (Biot-Savart's Law)

$$|B| = N\mu_0 I/D$$

N = no. of loops μ_0 = permittivity

I = current D = coil diameter



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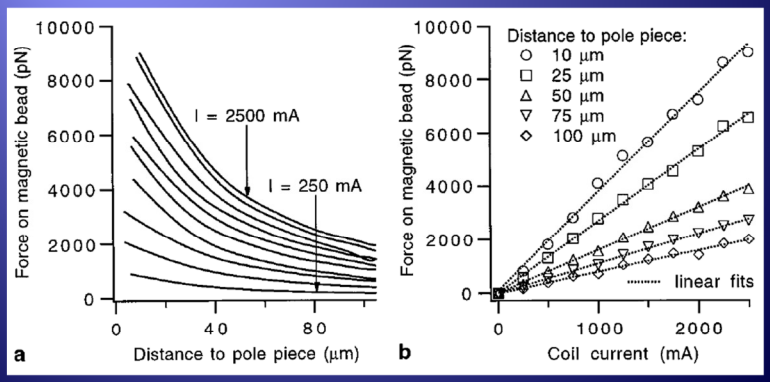
An electromagnetic coil is used to generate the magnetic field. The magnitude of the magnetic field from the coil can be found from Biot-Savart's Law for continuous loops. Basically, the number of loops in a coil, the current going their the wire and the compactness in size of the coil all help to generate appropriate magnetic fields for the magnetic tweezers. Biggest issues are positioning the pole tip of the magnetic tweezer as close as possible to the cell using a micromanipulator and the generation of heat from the current running through the magnetic coil.

Calibration

- Stokes' Law for low Reynolds numbers

$$F = 6\pi\eta Rv$$

η = viscosity R = particle radius v = velocity



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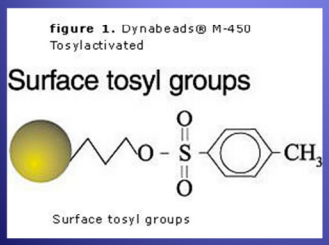
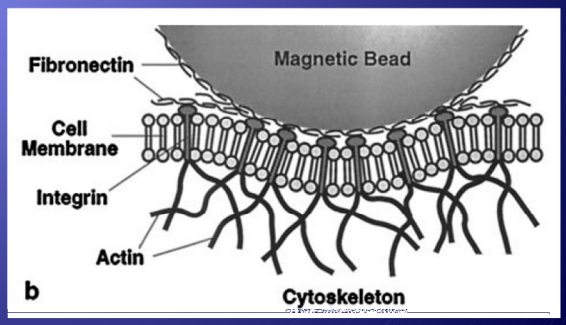
Since the magnetic field near the pole tip is not easily determined, you can model the field using computational software in order to determine the force applied to the bead. However, direct calibration of the force is possible through pulling on magnetic beads in a viscous liquid like silicone fluid. Here, the drag on the particle is extremely high and so there is a force balance between the magnetic force and the predictable drag force from Stokes' Law for low Reynolds number flow (Re < 1). The simple adage for Stokes' Law is: The bigger they are, the faster they fall. This is why chalk dust particles seem to float endlessly in a room while a piece of chalk drops quickly.

The velocity of the particles are tracked under the influence of the magnetic field from the current running through the coils. Here, the same particle was used for different coil currents so that the variability in the radius was minimized.

Adhesion-CSK Connection

- Magnetic particles functionalized by a p-toluene-sulfonyl (tosyl) group to fibronectin
- Allowed to bind to integrins that couple to actin





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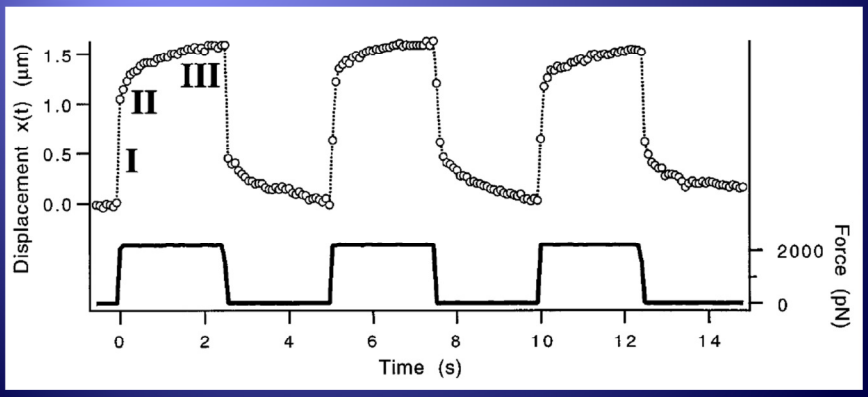
ECM proteins like fibronectin and be physically adsorbed (physorbed) onto the surface of the magnetic beads. However, a stronger covalent bonding can be formed. Invitrogen's Dynabeads have reactive sulfonyl esters that can react covalently with proteins (e.g. antibodies) or other molecules containing primary amino or thiol groups, e.g. the amino acid cystine. This allows for a direct coupling from the force of the bead to the actin cytoskeleton.

The Measurement

- "Classic" Creep Response
  - Elastic displacement (Regime I)
  - Relaxation (Regime II)
  - Steady-state Flow (Regime III)



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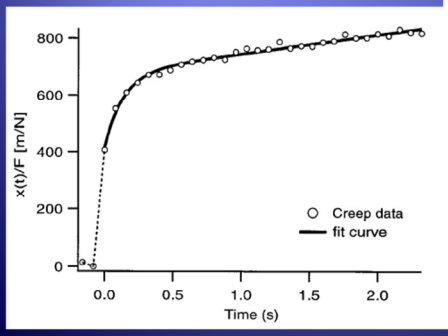
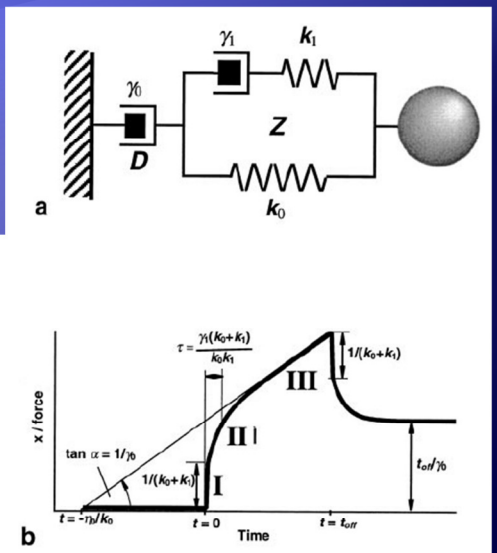
When the beads were added to the cells, the Bausch and Sackmann observed a classical creep behavior in the cell. I teach my undergraduates about the three observable parts of a creep response. The first is the elastic response where there is instantaneous displacement in the material due to the application of the load. After which, the creep relaxation occurs that dampens the displacement of the material. This is followed by steady state flow where the particle displaces the material on a constant velocity due to viscous flow within the material.

Interestingly, the response is quite repeatable for the same cell and same location. There is a little change in the creep response behavior. This suggests that the cell is a passive material, but the active remodeling of the cytoskeleton should drastically change this response.

The Model

- Effective elastic modulus $k = k_1 + k_2$
- Viscosity γ_0
- Relaxation time τ





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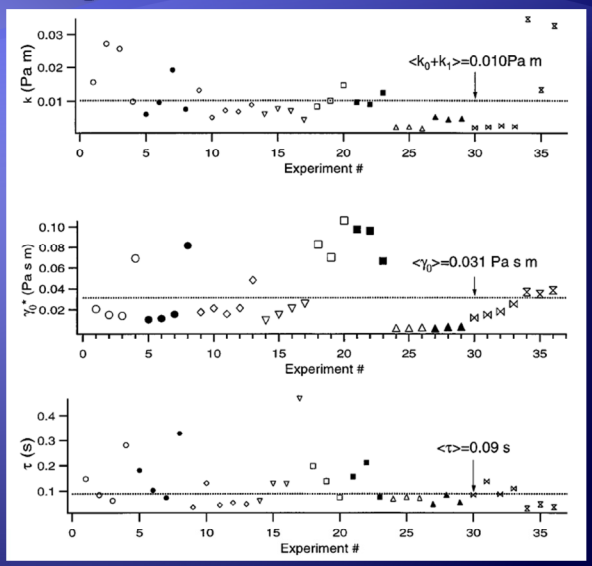
Sackmann proposed a simple four-element spring and dashpot model to explain the creep response. His model was is close agreement with the experimental results but we will cover this concept in more detail next week.

Viscoelastic Properties

- Same symbol: multiple measurements at one location
- Open, closed symbols: same cell, different locations
- Parameters vary up to an order of magnitude from cell to cell



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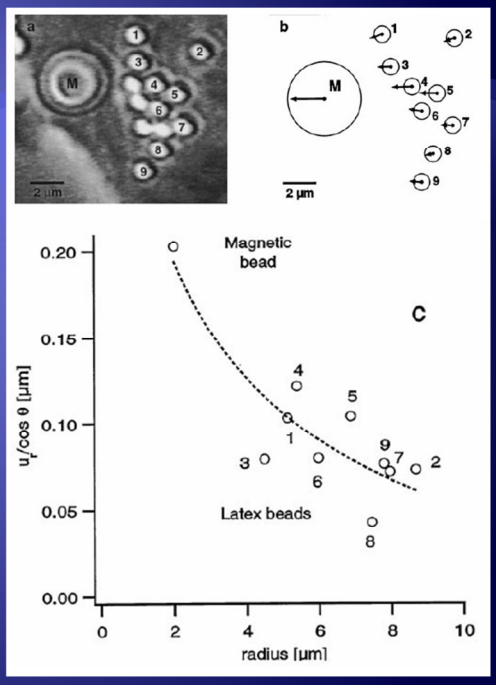
Conveniently, the authors made full disclosure on each of their individual measurements. The conducted 36 experiments on 7 cells. Outside of the first cell analyzed, the other cells show little variability with their measurement of elastic modulus, viscosity, and relaxation time. However, there is larger variability between measurements on different cells.

Strain Field

- Magnetic bead (M)
- Latex beads (#1-9)
  - Nonmagnetic, 1 μm
  - Fibronectin coated
  - 3 beads untrackable
- F = 3.7 nN, 1 sec



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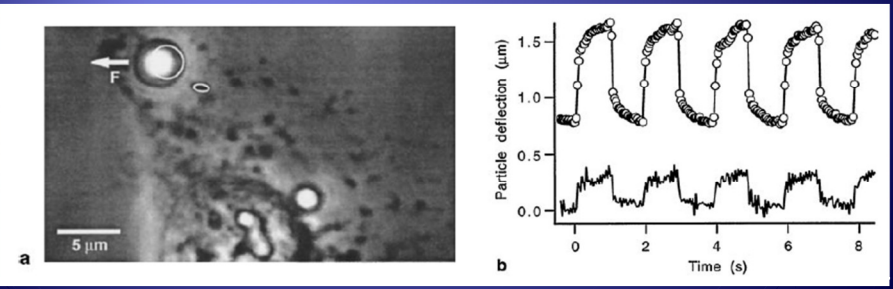
A typical strain field experiment is shown. A magnetic bead (M) is firmly bound on a surface region of a 3T3 fibroblast. Surrounding the magnetic particle are latex, nonmagnetic beads (#1-9). The latex beads were coated with fibronectin and allowed to bind to the cell at the same time as the magnetic beads. A rectangular force pulse of 3700 pN for 1 second was applied and the displacement of the latex probes were tracked with ImageJ using a custom particle tracking algorithm. The authors report that three of the latex beads were not able to be measured because of the their close-proximity to each other and overlap. The amplitude of the deflections is normalized by the polar angle cosine. The authors claim that the displacement decreases exponentially from the point of applied load.

Deep strain fields

- Cell vacuoles show displacement under magnetic bead force.
- Shear on the cell surface penetrates partially into the cytoplasm



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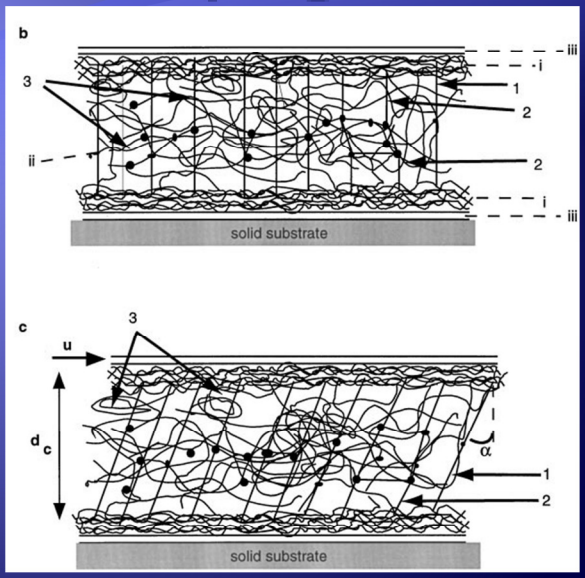
Interestingly, these strain fields are not confined to the surface cortex of a cell but can cause intracellular displacement. This is illustrated by tracking the deflection of vacuoles inside the cell.

Cell Mechanical Coupling Model

- Composite shell composed of lipid-protein bilayer and associated cortical actin



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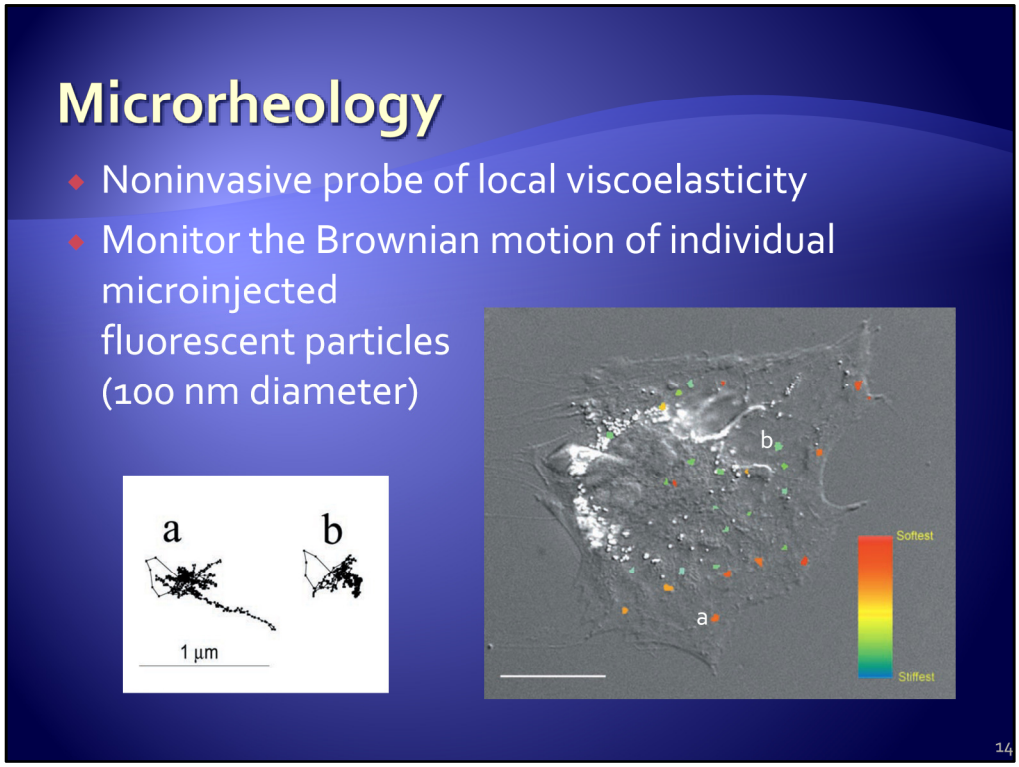


Multiple-particle micro-rheology mapping of a living cell. This process is non-invasive because cells continue to grow and divide normally more than 16 h after microinjection.

Shown is a DIC micrograph superimposed to a fluorescence micrograph of the trajectories of microinjected microspheres. The trajectories, which were recorded for 20 s, were transformed into local compliances, which were normalized by the maximum compliance and color coded between red (most compliant microenvironment within the cell) to blue (least compliant microenvironment). Scale bar, 30 m.

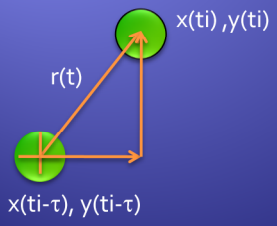
Polystyrene-based spheres are internally dyed using solvent swelling, dye entrapment. The highly hydrophobic fluorescent dyes remain trapped in the beads.

For 2D loading, the tip of an automated femtoliter pipette is loaded with a high concentration of the nanoparticles in buffer. The tip is placed above a target cell and the injection processes is activated. The tip goes into the cell at a slight angle and delivers its payload.

For the ballistic nanoparticle delivery system (1) helium gas is accelerated through a gas chamber through a rupture disk, which impacts into (2) macrocarrier disks coated with fluorescent nanoparticles and causes them crash into a (3) stopping screen. The momentum of the macrocarrier disk is transferred to the nanoparticles, which penetrate the (4) target cells. Of the cells ballisticaly injected, 30-40% survived

Particle Tracking

- Mean squared displacement (MSD), $\langle \Delta r^2(\tau) \rangle$
  - τ is the time lag (a/k/a frame rate)
  - t is the elapsed time (a/k/a video length)
  - $\langle \Delta r^2(\tau) \rangle = MSD_x(\tau) + MSD_y(\tau)$



$$MSD_x(\tau) = \frac{\sum_{i=1}^{N} (x(t_i - \tau) - x(t_i))^2}{N+1}$$

$$MSD_y(\tau) = \frac{\sum_{i=1}^{N} (y(t_i - \tau) - y(t_i))^2}{N+1}$$

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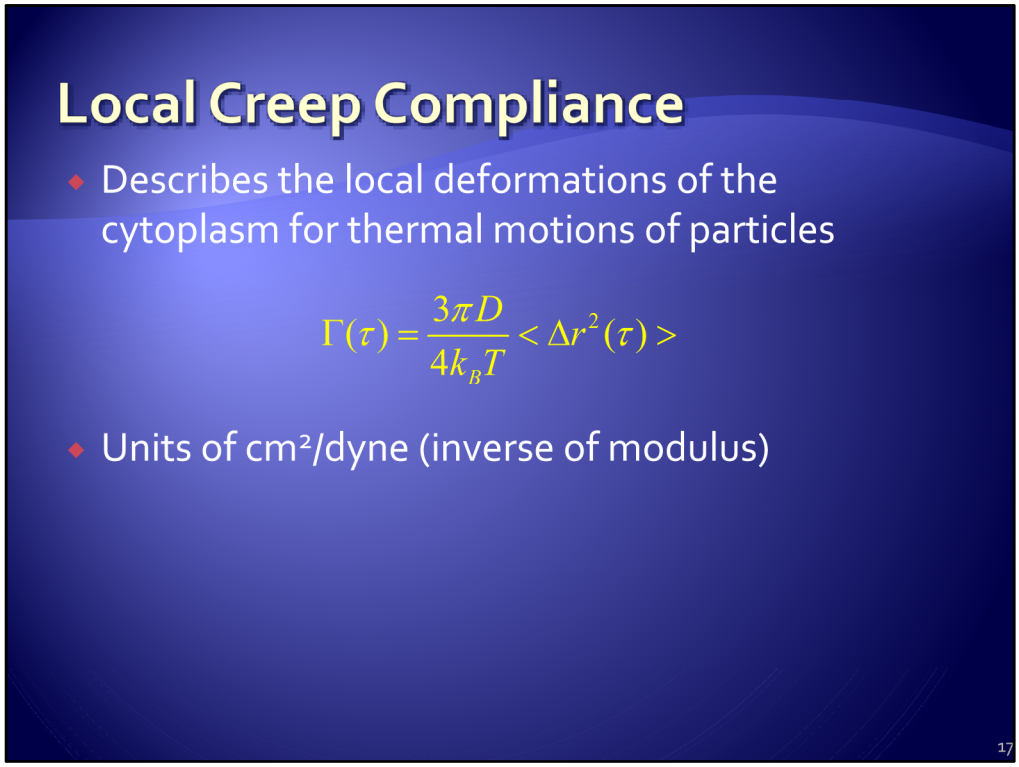
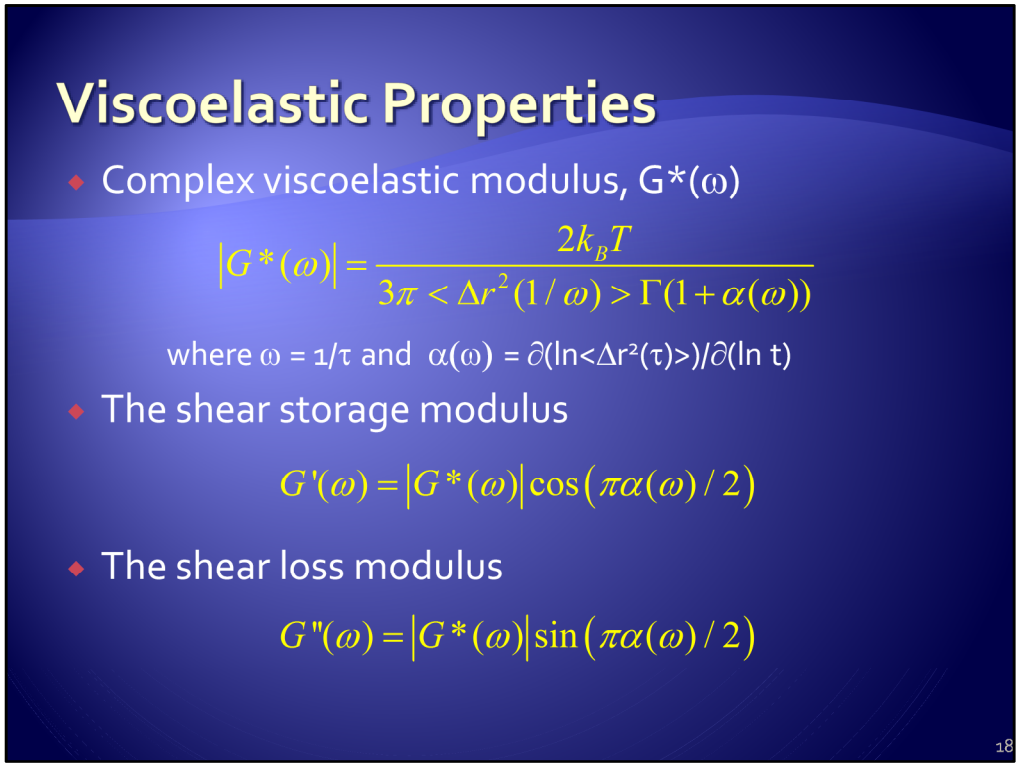
From the time-dependent coordinates, (x,y), the projections of the MSD for each nanoparticle can be calculated. From this parameter, we can then determine the viscoelastic properties of the cell.

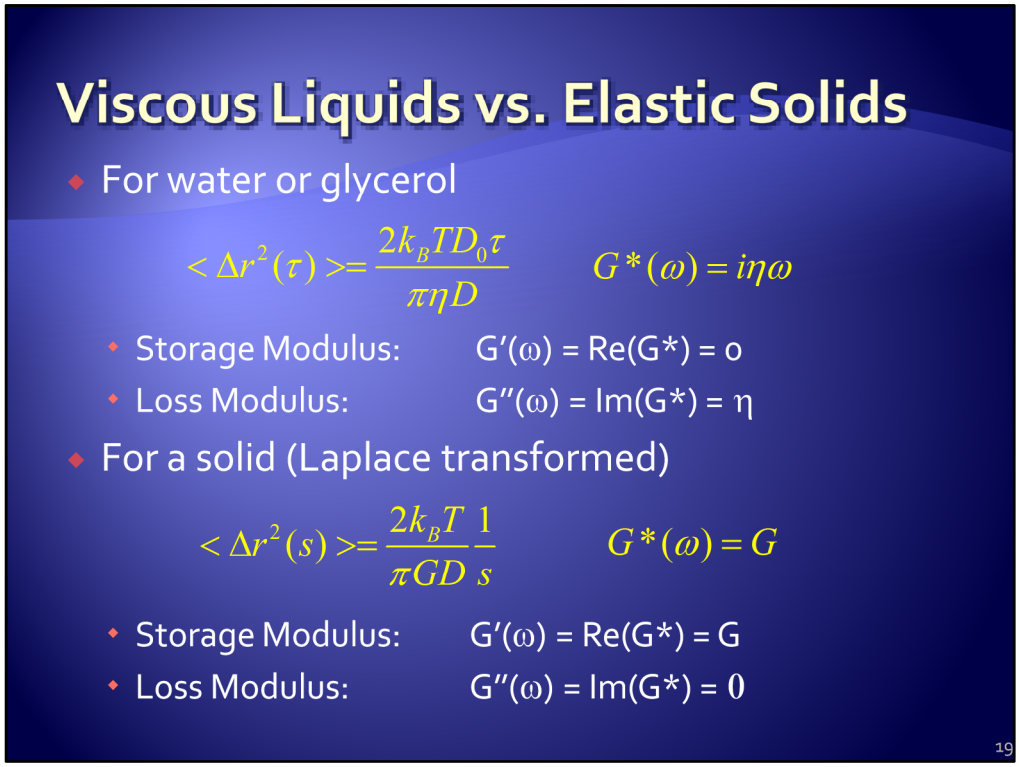
Note that this measurement does not consider the Brownian displacement in the z direction.



Here, kB is Boltzmann's constant and T is the absolute temperature (in Kelvins). For large particles, they are more restricted in the cytoplasm and so any motion of the particles means that the creep compliance has to be very large. For high temperatures, there is more thermal energy and so there would be more thermal motion in the particles that needs to be accounted for.

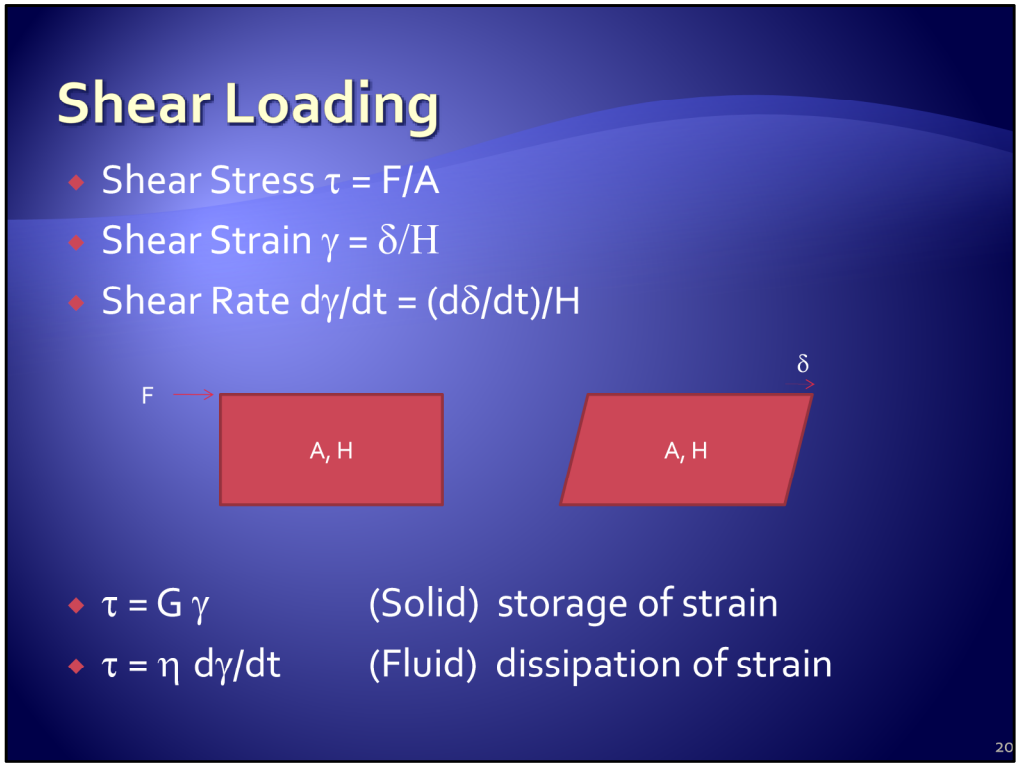


From particle tracking, we can find determine the viscoelastic moduli of the cell at our frequency of interest (ω). The complex viscoelastic modulus is a complex number and contains both the "elastic" storage modulus in its real part and the "viscous" shear modulus in its imaginary part. To better understand this, we will consider the difference between a viscous fluid and a Hookean solid.



For a viscous fluid like water or glycerol with diffusivity D_0 and viscosity η, the storage modulus is zero and the loss modulus is the viscosity.

For an elastic solid with constant shear modulus G, the storage modulus is G and the material does not dissipate the displacement because the loss modulus is zero.



Here are some elementary definitions of shear to help with understanding viscoelastic properties of cells. Solids will store the strain and release it once the load is removed. Fluids will dissipate the strain and when the load is removed, they will hold the same configuration.

We will see in the next session that cells, like some materials, are more elastic at low frequencies (G'>G'') but are more dissipative at high frequencies (G''>G').