Methods of measuring

Cell Elasticity

Courtesy of Katja Taute, modified by Björn Stuhrmann

Motivation

- Apply laws of physics (derived from inanimate matter) to living objects
  → learn about amazing material properties of cells
- Mechanical properties of cells reveal structural characteristics
- How are cell function and mechanical properties correlated?
- Changes in cell elasticity often monitor physiological status of cell, e.g. cancer cells are less elastic than healthy cells.

Basics: What is Elasticity?

In the easiest case (homogeneous isotropic solid):

Compression/Stretching	Shear
Hooke's law: $F \sim \Delta x$	$F_T \sim \Delta x_T$
Stress: $\sigma = F/A$ Strain: $\varepsilon = \Delta x/x$	Shear stress: $\sigma_S = F_T/A$ Shear strain: $\varepsilon_S = \Delta x_T/x$
$\sigma = E\varepsilon$	$\sigma_S = G\varepsilon_S$
E: Elasticity or Young's Modulus	$G = E/(2+2\mu)$, μ: Poisson number G: Shear modulus

Does this apply to cells?

- Cells are neither homogeneous nor isotropic
  - Cells have internal structure
  - Cell components have different characteristics
- Cells are not purely elastic but show also viscous behavior
  - Cell material rather resembles a polymeric liquid or gel than a solid
  - Mechanical energy can be dissipated into heat
- Cells are active
  - Cell can respond to external forces e.g. by strengthening the cytoskeleton

➔ Some difficulties extracting E for a cell

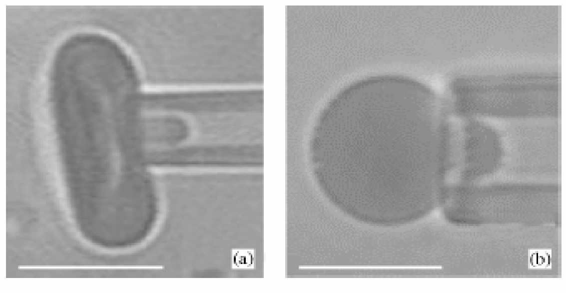
How to deal with the difficulties

- Inhomogeneity
  - → Use local measurements or averages over whole cell
- E not practicable
  - → Incorporate viscosity in the model
    - Use complex elasticity modulus $E^*(\omega)$ or shear modulus $G^*(\omega)$
    - Storage modulus $G'(\omega) = \mathrm{Re}(G^*)$ accounts for elasticity
    - Loss modulus $G''(\omega)=\mathrm{Im}(G^*)$ accounts for viscosity
    - Be aware that these quantities are frequency-dependent
- Living subject of study
  - → Allows for the study of biological response!

Techniques

- Basic mechanical methods
  - Micropipette Aspiration
  - Cell Poking
  - Silicon Micromachines
  - Atomic Force Microscopy
  - Biointerface Probe
  - Tensile Tester
  - Microplates
- Magnetic methods
  - Magnetic Twisting
  - Attached Magnetic Beads
  - Embedded Magnetic Beads
- Optical methods
  - Optical Tweezers
  - Optical Stretcher
- Other
  - Acoustic Microscopy
  - Laser Tracking Microrheology
  - Hydrodynamic Flow

Micropipette Aspiration

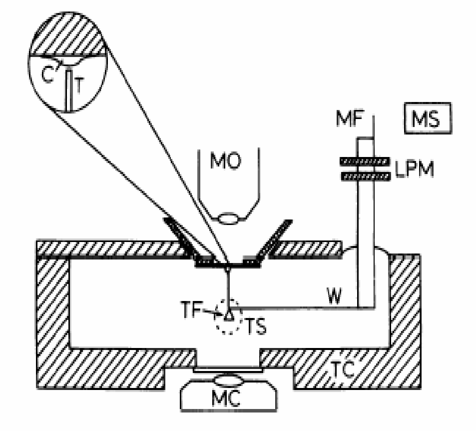


Principle
- suction pressure is created in micropipette
- leading edge of aspirated cell is tracked

Details
- tracking accuracy: ±25nm
- suction pressure range: 0.1-1000Pa
- force range 10pN-10mN
- pressure vs egde position yields E

Hochmuth (2000) J. Biomech. 33:15-22

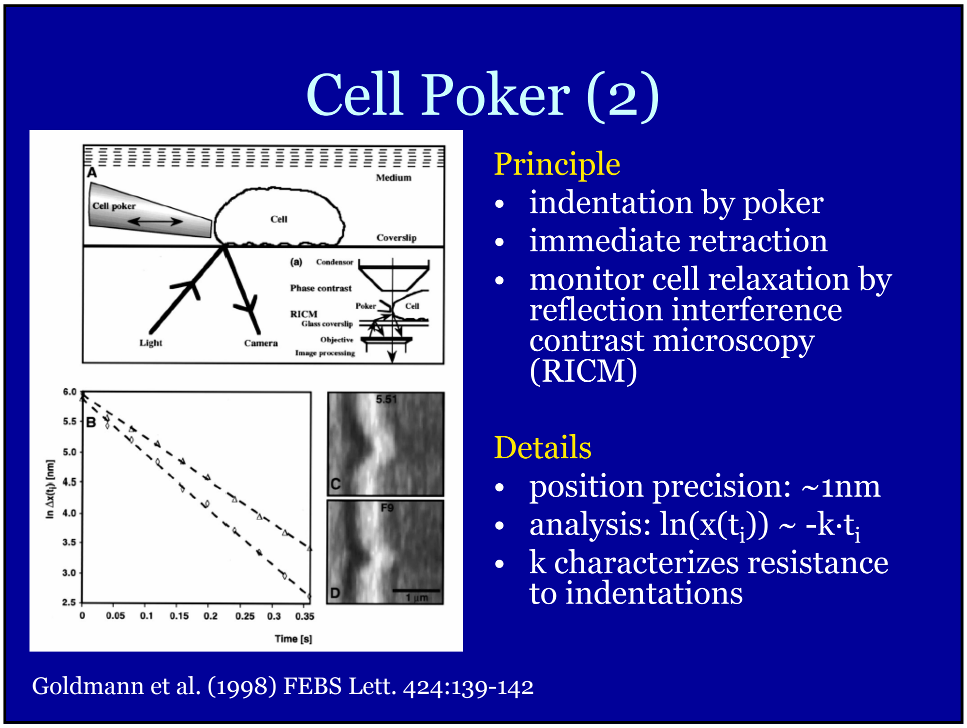
Cell Poker (1)



Principle:
- tip mounted on flexible wire
- position of both tip and wire arm are measured to determine deflection

Details
- tip: glass stylus d=2µm
- position precision: 0.1µm
- k_{wire}=38pN/nm
- force resolution ~4nN
- force range: <30nN

Petersen et al. (1982) Proc. Natl. Acad. Sci. 79:5327-5331



Cell Poker (2)

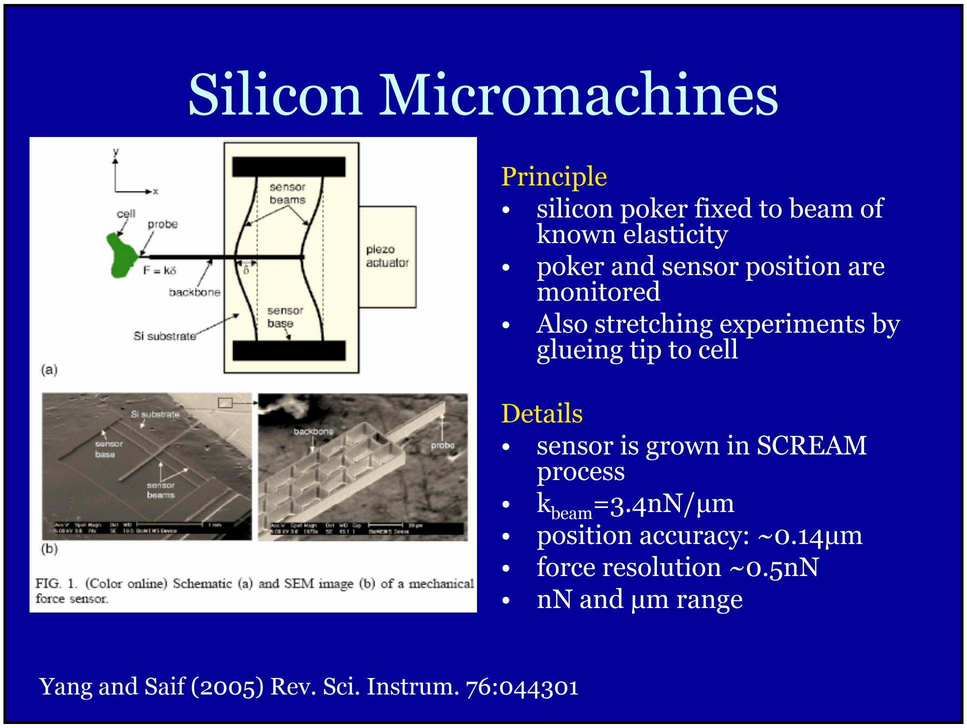
Principle

- indentation by poker
- immediate retraction
- monitor cell relaxation by reflection interference contrast microscopy (RICM)

Details

- position precision: ~1nm
- analysis: $\ln(x(t_i)) \sim -k \cdot t_i$
- k characterizes resistance to indentations

Goldmann et al. (1998) FEBS Lett. 424:139-142



Silicon Micromachines

FIG. 1. (Color online) Schematic (a) and SEM image (b) of a mechanical force sensor.

Principle

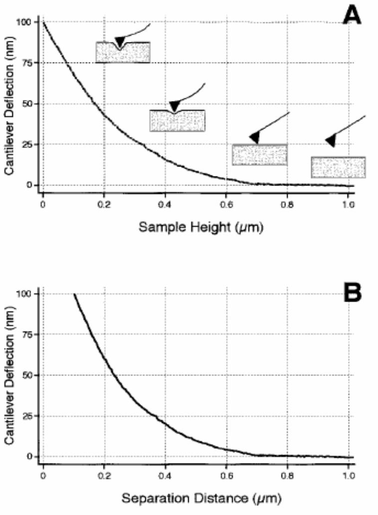
- silicon poker fixed to beam of known elasticity
- poker and sensor position are monitored
- Also stretching experiments by glueing tip to cell

Details

- sensor is grown in SCREAM process
- k_{beam}=3.4nN/µm
- position accuracy: ~0.14µm
- force resolution ~0.5nN
- nN and µm range

Yang and Saif (2005) Rev. Sci. Instrum. 76:044301

Atomic Force Microscopy (AFM)



Principle

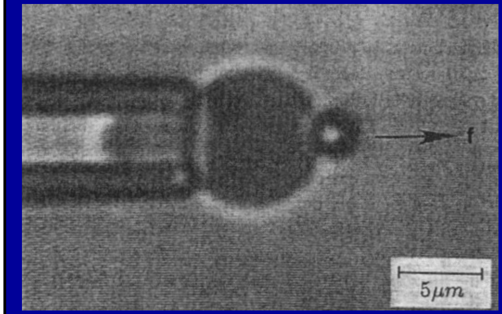
- Record player principle: cantilever scans sample
- Cantilever deflection is recorded by laser reflection
- Force-distance-maps can be produzed for each x-y-position

Details

- $k_{cantilever}$~10-50pN/nm
- Deflection of laser beam is recorded with 2-segment photo diode
- Cantilever tip can be modified with beads for more convenient geometry and nondestructive imaging (Mahaffy et al. (2000). Phys. Rev. Lett. 85:880:883)

Al-Hassan et al. (1998) Biophys. J. 74:1564-1578

Biointerface Probe



Principle

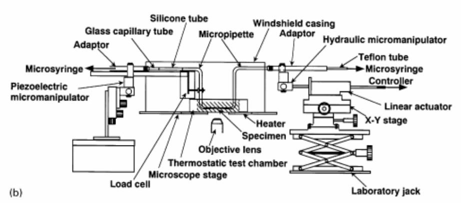
- bead probe glued to red blood cell (RBC) held by micropipette
- RBC acts as variable force transducer

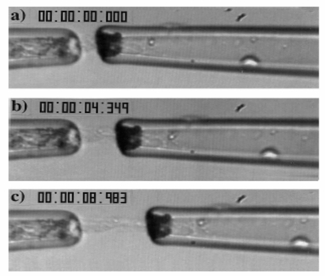
Details

- latex bead d=2-3μm
- probe position precision ~5nm
- suction pressure ~ RBC surface tension ~ RBC stiffness
- k=1fN/nm-10pN/nm
- force range 10^{-2}-10^{3}pN

Evans et al. (1995) Biophys. J. 68:2580-2587

Tensile Tester





Principle:

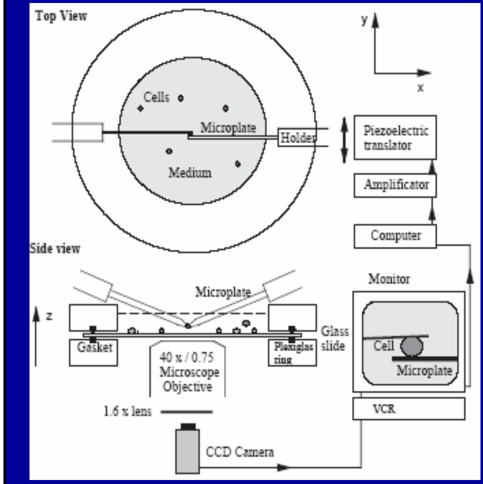
- cell is stretched between two micropipettes
- distance between pipette tips measures elongation

Details

- displacement resolution: 0.24μm
- forces are measured with semiconductor strain gauge, accuracy ±50nN

Miyazaki et al. (2000) J. Biomech. 33:97-104

Microplates



Principle

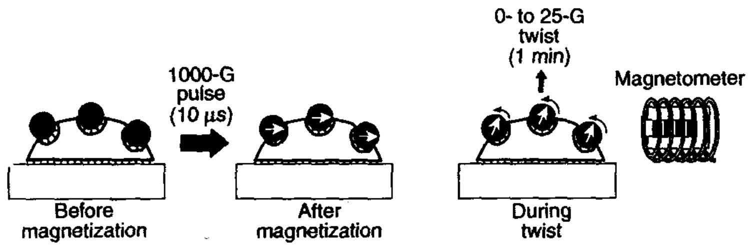
- cell is squeezed/stretched between a solid and a flexible microplate
- cell deformation is observed

Details

- solid microplate is moved with piezo
- force range: 10-100nN
- $k_{microplate}$=1-10nN/μm
- position accuracy: 0.4μm
- Analysis: $\sigma(t)=\varepsilon_0(k_0+k_1e^{-t\tau})$

Thoumine and Ott. (1997) J. Cell Sci. 110:2109-2116

Magnetic Twisting



Principle

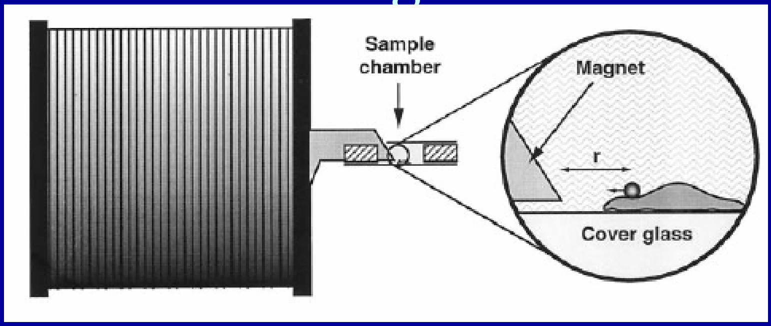
- surface-bound ferromagnetic beads are magnetized by strong field pulse
- then twisted by small perpendicular field
- twisting angle reflects shear response

Details

- field pulse: 0.1T for 10µs
- twisting field: $<25 \cdot 10^{-5}$T
- twisting angle is inferred from magnetometer measurements

Wang et al. (1993) Science 260:1124-1127

Attached Magnetic Beads



Principle

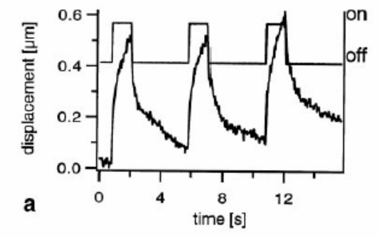
- force pulses are applied to paramagnetic beads bound to cell surface
- creep response and relaxation are recorded

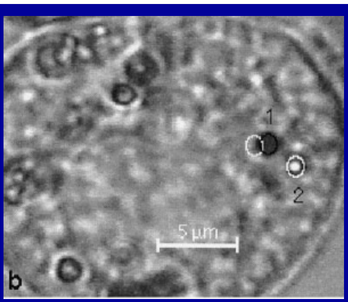
Details

- bead d=4.5µm bound to integrin receptors
- force range: up to 10nN
- position accuracy: ~10nm
- time resolution: 0.04s

Bausch et al. (1998) Biophys. J. 75:2038-2049

Embedded Magnetic Beads





Principle

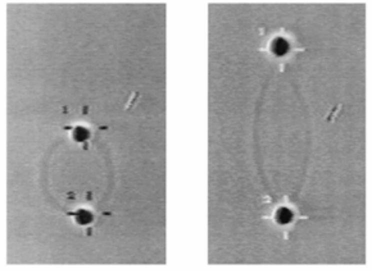
- field pulses are applied to ferromagnetic beads in cells (macrophages)
- creep response and recovery curves determine viscoelasticity

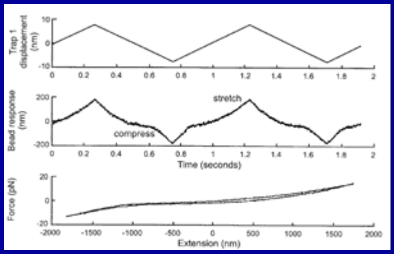
Details

- bead size: d=1.3µm
- force range: 300-700pN
- time resolution: 0.04s

Bausch et al. (1999) Biophys. J. 76:573-579

Optical Tweezers





Principle

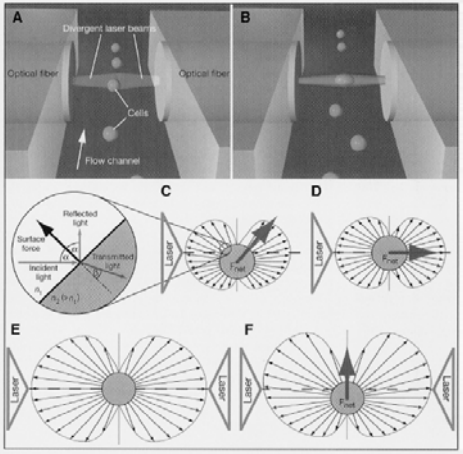
- beads glued to cell are held in optical traps
- one is moved and the response of the second is measured

Details

- k_{trap}=0.08pN/nm
- forces < 25pN
- bead d=1µm
- bead 1 is moved using acousto-optical modulator
- bead 2 response is monitored with quadrant detector

Sleep et al. (1999) Biophys. J. 77:3085-3095

Optical Stretcher



Principle

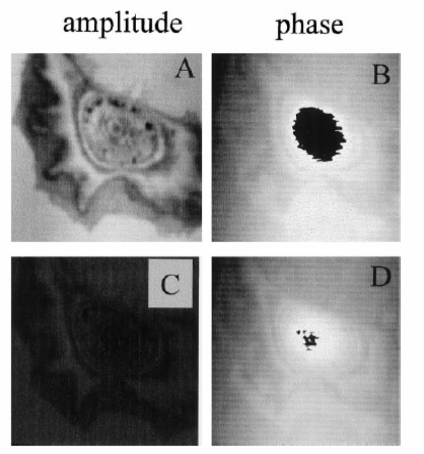
- No absorption → laser beam passes through cell
- Momentum conservation → surface stress
- Cell deformation is measured in terms of aspect ratios
- No physical contact required!

Details

- surface force scales with n_{cell} and laser intensity
- force range: ~10^2pN

Guck et al. (2005) Biophys J. 88:3689-3698

Acoustic Microscopy



Principle

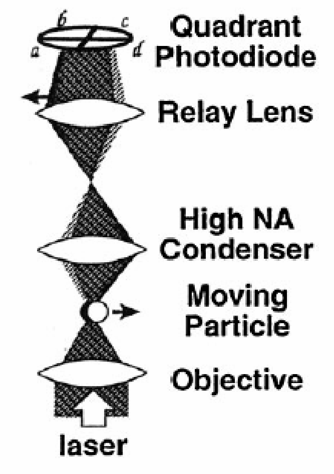
- acoustic lens scans sample
- phase and amplitude of reflected wave encode sound velocity and attenuation
- sound velocity is a measure of elasticity

Details

- VHF ultrasound: ~1Ghz
- scan: 512×256 pixels
- spatial resolution: 3μm^2

Kundu et al. (2000) Biophys. J. 78:2270-2279

Laser Tracking Microrheology (LTM)



Principle

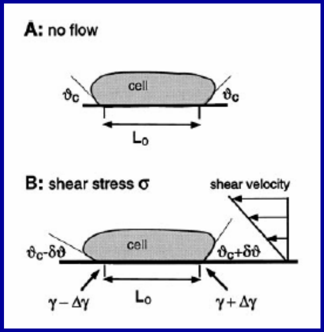
- some cells contain granules (lipid droplets), $d \approx 0.3 \mu m$
- their Brownian motion in cytoplasm is observed by light scattering
- mean square displacement is measure for viscoelasticity of surrounding medium

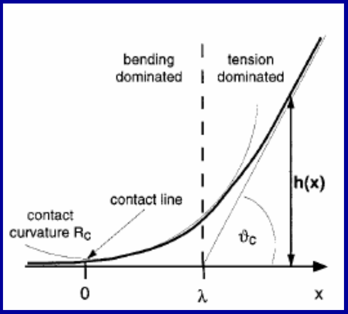
Details

- laser: $P = 0.13 mW$, $\lambda = 670 nm$
- displacement calibration with polystyrene beads (similar optical properties)
- displacement resolution: <1nm
- MSD: $\langle R^2(\tau) \rangle = \langle (r(t+\tau) - r(t))^2 \rangle$

Yamada et al. (2000) Biophys. J. 78:1736-1747

Hydrodynamic Flow





Principle

- Hydrodynamic flow deforms cell
- Cell profile is determined by membrane bending modulus

Details

- Shape is analyzed using reflection interference contrast microscopy (RICM)
- λ yields bending modulus

Simson et al. (1998). Biophys.J. 74:514-522

Brief Review of Techniques

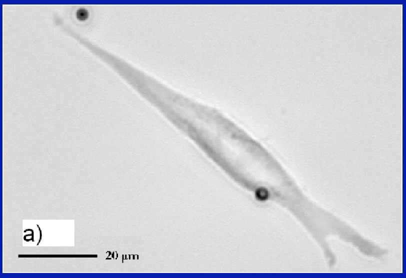
- Basic mechanical methods
  - Micropipette Aspiration
  - Cell Poking
  - Silicon Micromachines
  - Atomic Force Microscopy
  - Biointerface Probe
  - Tensile Tester
  - Microplates
- Magnetic methods
  - Magnetic Twisting
  - Attached Magnetic Beads
  - Embedded Magnetic Beads
- Optical methods
  - Optical Tweezers
  - Optical Stretcher
- Other
  - Acoustic Microscopy
  - Laser Tracking Microrheology
  - Hydrodynamic Flow

Summary

- Different techniques measure different aspects
- Quantity characterizing elasticity needs to be chosen with care
- Results obtained with different techniques may be difficult to compare
- Choice of technique must depend on the specific aim of the experiment

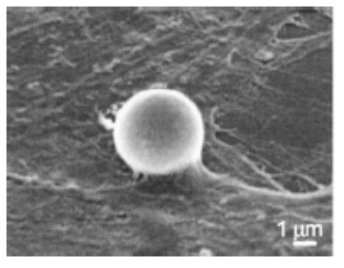
Experimental Results

Techniques for Attached Cells



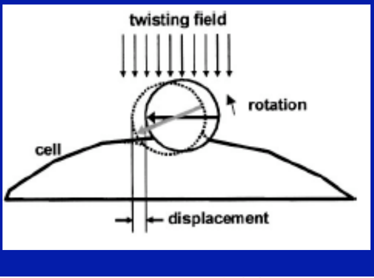
Adherent cells

Beads as handles

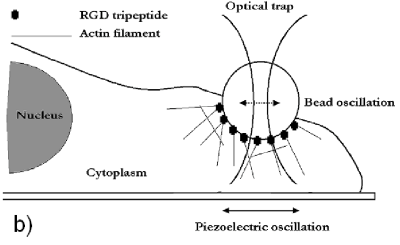


Techniques for Attached Cells

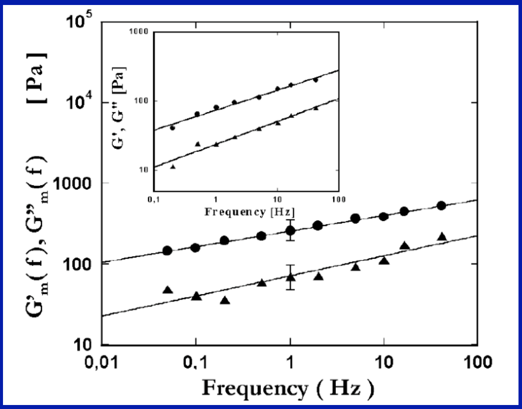
Magnetic bead twisting
(Fabry et al., *PRL* 2001; *PRE* 2003)



Optical Tweezers
(Balland et al., *Eur. Biophys. J*, 2005)



Rheological Results: Attached Cells

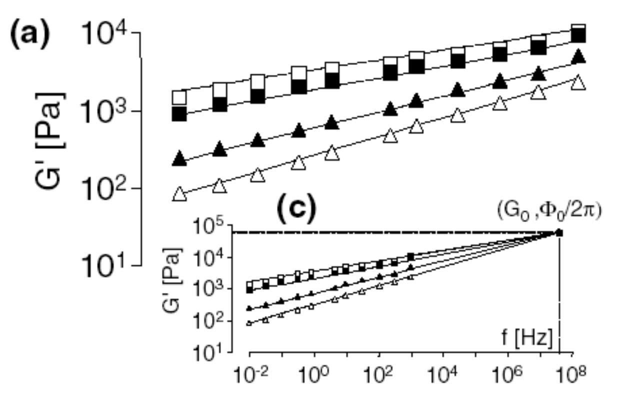


$$G' \propto \omega^x$$

$$\log(G') = x \cdot \log(\omega) + c$$

Balland et al., *Eur. Biophys. J.* (2005)

Rheological Results

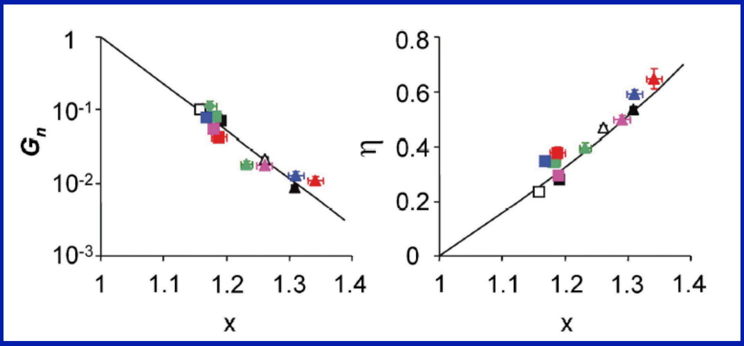


$$\log(G') = x \cdot \log(\omega) + c$$

All experiments with all kinds of different cells can be described by a Master Curve; only difference is x!

Fabry et al., *PRL* 2001; *PRE* 2003

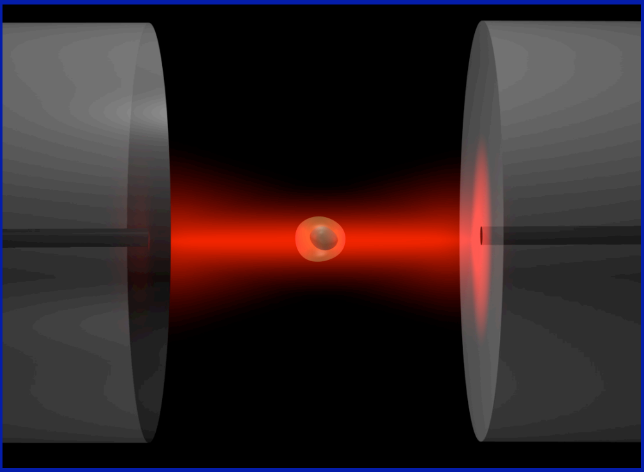
Rheological Results



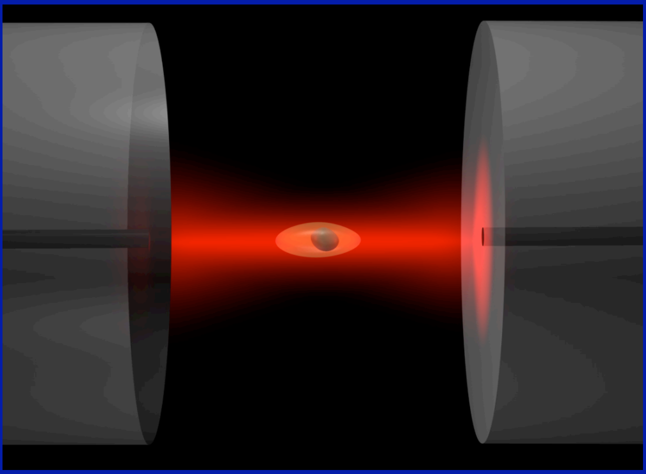
Can be described by theory of "soft glassy materials" (Sollich, 1997, 1998)
(Foams, pastes, colloids, slurries, ...)

- very soft (Pa – kPa)
- G' and G" follow the same power law (no single relaxation time)
- x is an effective noise temperature
- microscopic origin???

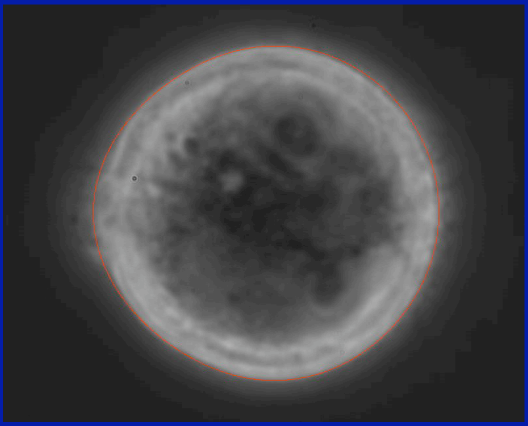
Deforming Cells in Suspension?



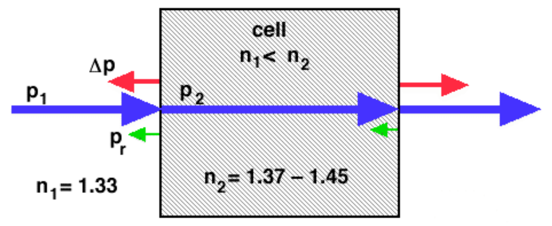
The Optical Stretcher



The Optical Stretcher



Optical Surface Forces



Momentum of a light ray

$$p = \frac{n_i E}{c}$$

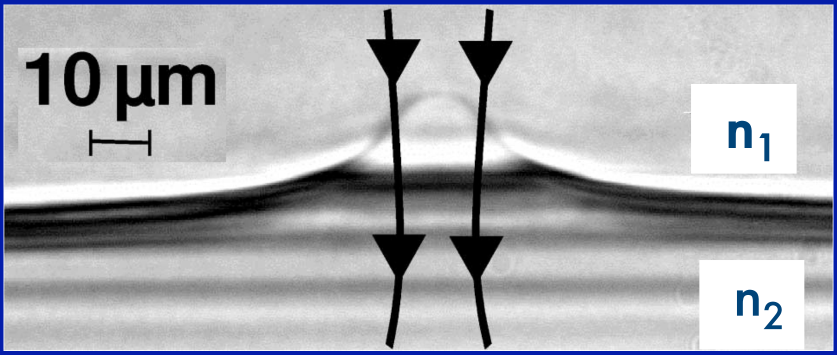
Conservation of momentum at surface

$$\Delta p = \frac{E}{c}\left(n_1 + Rn_1 - (1-R)n_2\right)$$
$$= \frac{2n_1E}{c}\left(\frac{1-n}{1+n}\right) < 0$$
$$n = \frac{n_2}{n_1} > 1$$

- Whenever light enters or exits a dielectric medium it exerts a force AWAY from the denser medium and NORMAL to the surface

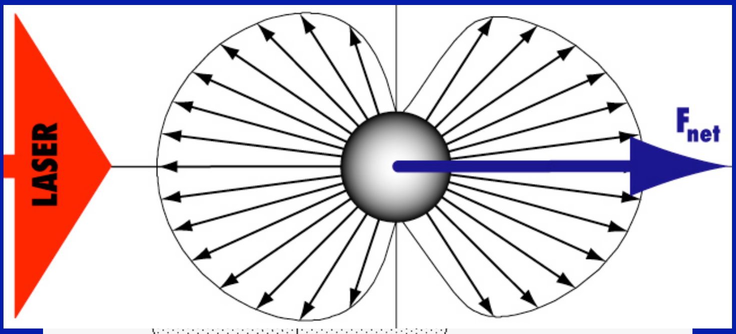
J. Guck, et al. *Phys. Rev. Lett.* **84**:5451, 2000

Surface Deformation

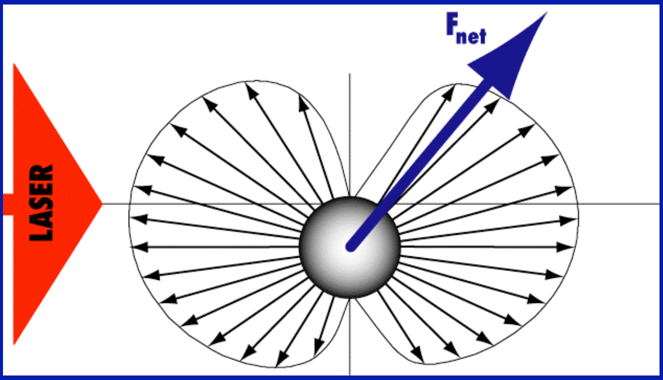


Casner and Deville. *Phys. Rev. Lett.* 2001

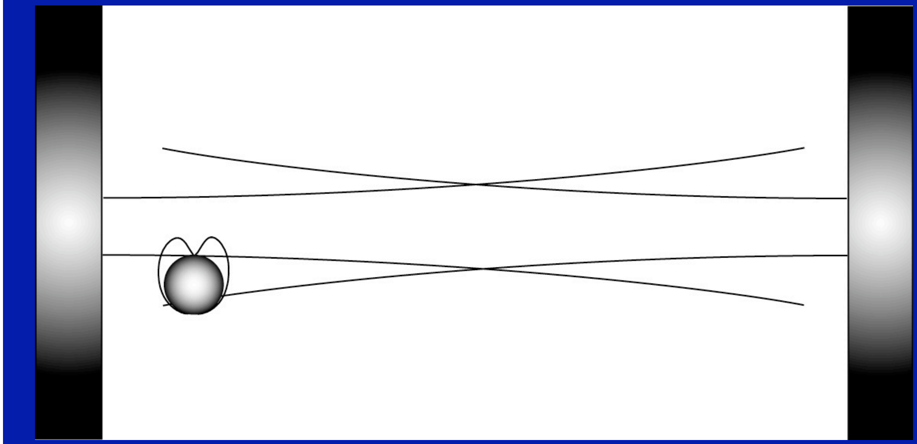
Surface Forces in Gaussian Beams



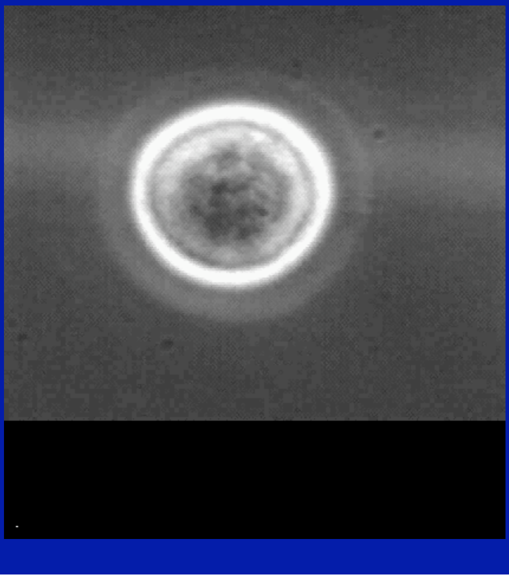
Surface Forces in Gaussian Beams



Optical Stress in DBLT



Microrheology on Fibroblasts



Step-stress experiment

Deformation ($\gamma_{\max}$ = 7.5%)

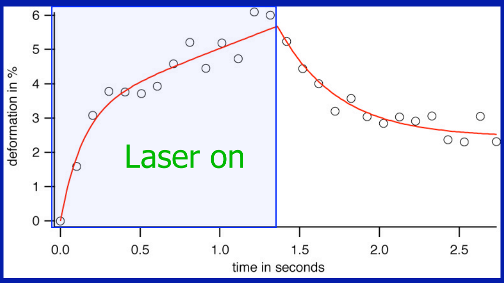
Viscoelastic Characterization

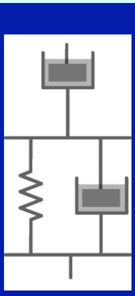
$$\sum_{i=0}^{n} a_i \partial_t^i \gamma(t) = \sum_{j=0}^{m} b_j \partial_t^j \sigma(t)$$

$$a_1 \partial_t \gamma(t) + a_2 \partial_t^2 \gamma(t) = \sigma(t) + b_1 \partial_t \sigma(t)$$

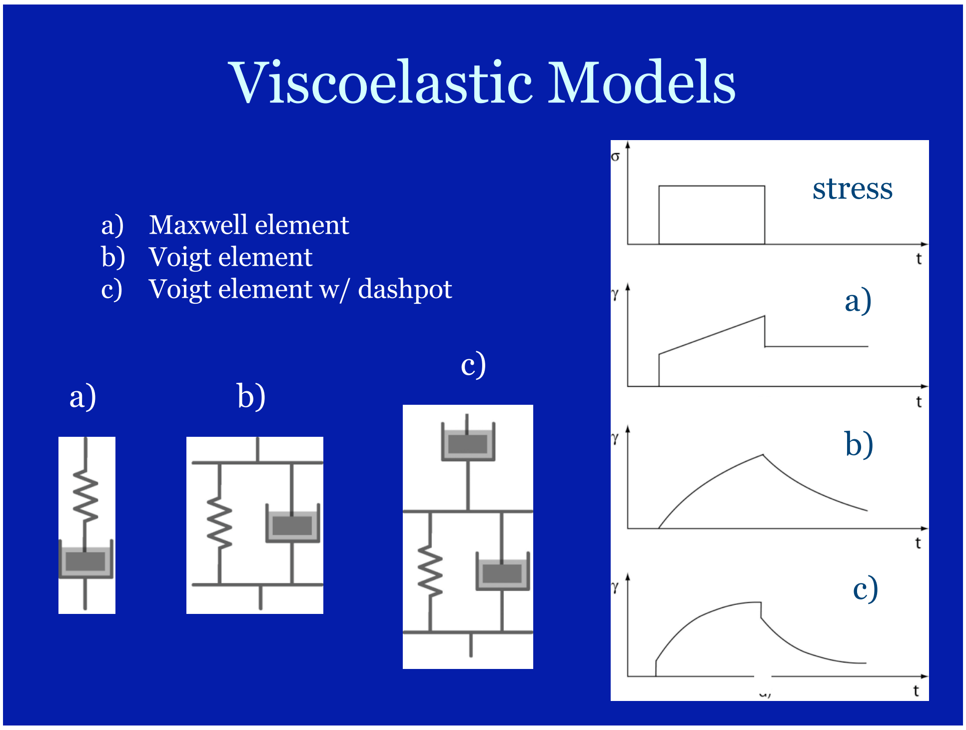
$$\gamma_{ext}(t) = F_G \sigma_0 \left(\frac{b_1}{a_1} - \frac{a_2}{a_1^2} \right) \left(1 - e^{\frac{a_1}{a_2} t} \right) + \frac{F_G \sigma_0}{a_1} t$$

$$\gamma_{rel}(t) = F_G \sigma_0 \left(\frac{b_1}{a_1} - \frac{a_2}{a_1^2} \right) \left(1 - e^{\frac{a_1}{a_2} t_1} \right) e^{\frac{a_1}{a_2}(t - t_1)} + \frac{F_G \sigma_0}{a_1} t_1$$



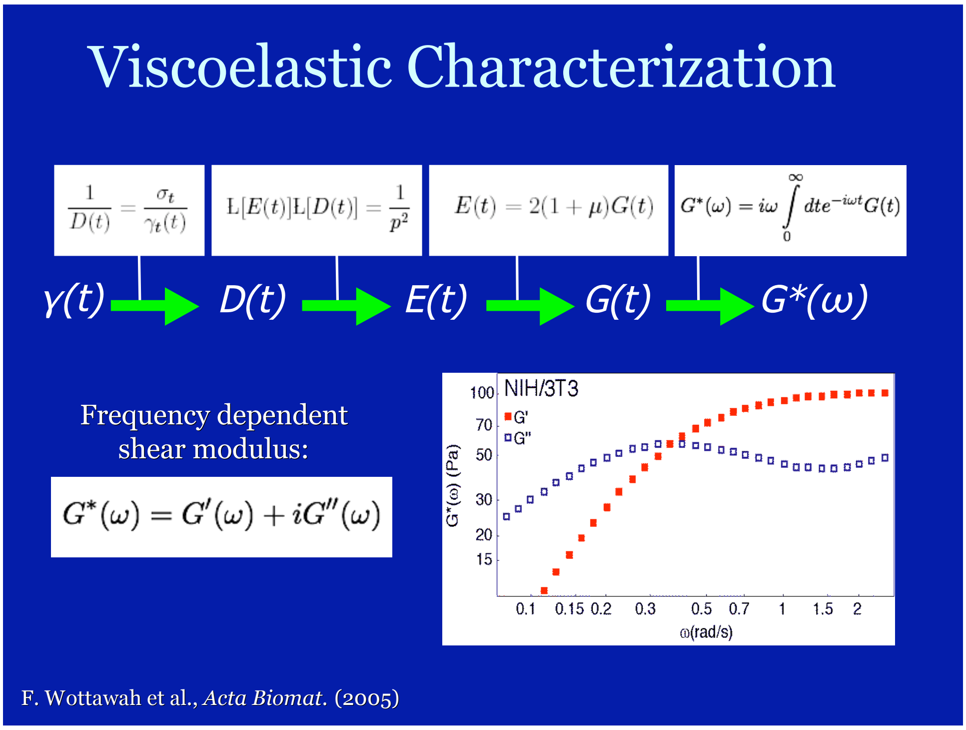


F. Wottawah et al., *Phys. Rev. Lett.* **95** (2005)



Viscoelastic Models

a) Maxwell element
b) Voigt element
c) Voigt element w/ dashpot



Viscoelastic Characterization

$\frac{1}{D(t)} = \frac{\sigma_t}{\gamma_t(t)}$

$L[E(t)]L[D(t)] = \frac{1}{p^2}$

$E(t) = 2(1+\mu)G(t)$

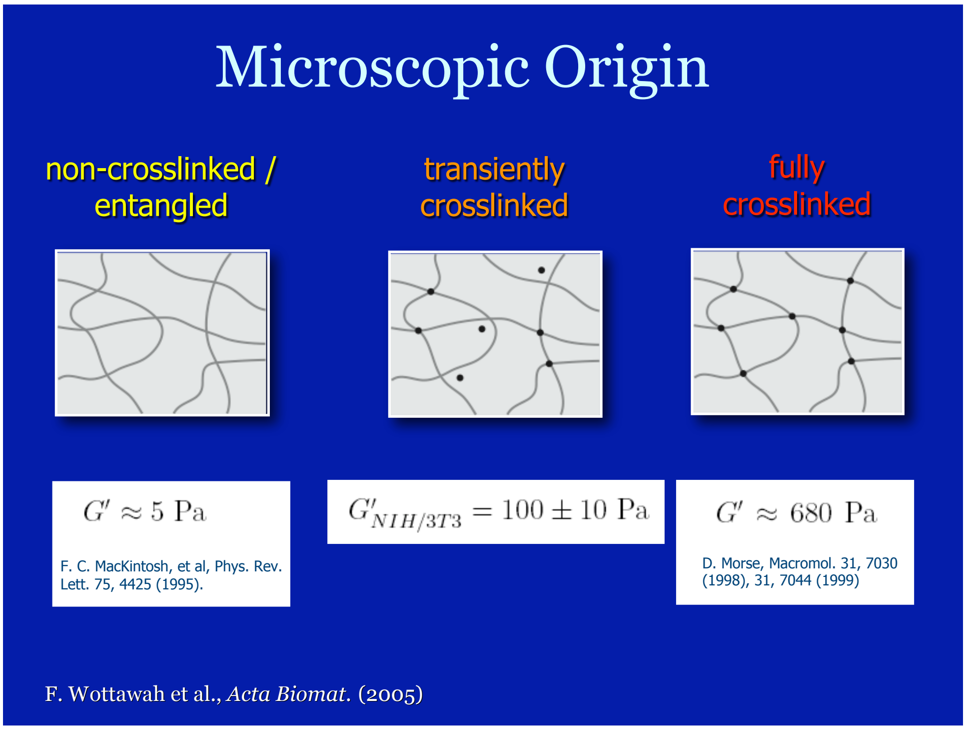
$G^*(\omega) = i\omega \int_0^\infty dt e^{-i\omega t} G(t)$

$\gamma(t) \rightarrow D(t) \rightarrow E(t) \rightarrow G(t) \rightarrow G^*(\omega)$

Frequency dependent shear modulus:

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

F. Wottawah et al., *Acta Biomat.* (2005)



Microscopic Origin

non-crosslinked / entangled

transiently crosslinked

fully crosslinked

$G' \approx 5\ \mathrm{Pa}$

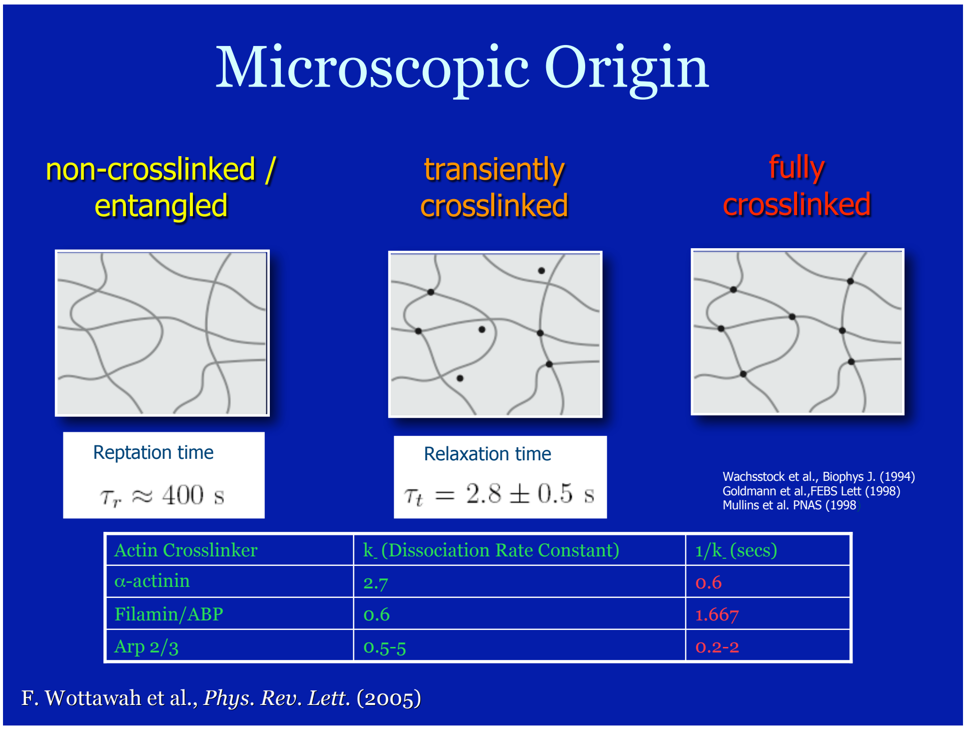
F. C. MacKintosh, et al, Phys. Rev. Lett. 75, 4425 (1995).

$G'_{NIH/3T3} = 100 \pm 10\ \mathrm{Pa}$

$G' \approx 680\ \mathrm{Pa}$

D. Morse, Macromol. 31, 7030 (1998), 31, 7044 (1999)

F. Wottawah et al., *Acta Biomat.* (2005)



Microscopic Origin

non-crosslinked / entangled

transiently crosslinked

fully crosslinked

Reptation time

$\tau_r \approx 400\ \mathrm{s}$

Relaxation time

$\tau_t = 2.8 \pm 0.5\ \mathrm{s}$

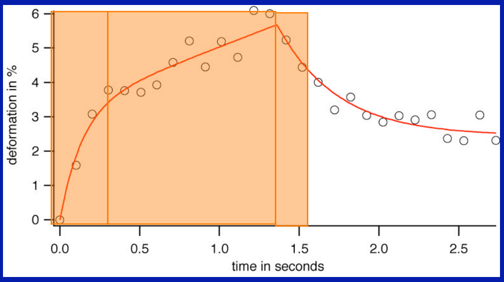
Wachsstock et al., Biophys J. (1994)
Goldmann et al.,FEBS Lett (1998)
Mullins et al. PNAS (1998)

Actin Crosslinker	k_- (Dissociation Rate Constant)	$1/k_-$ (secs)
α-actinin	2.7	0.6
Filamin/ABP	0.6	1.667
Arp 2/3	0.5-5	0.2-2

F. Wottawah et al., *Phys. Rev. Lett.* (2005)

Microscopic Origin

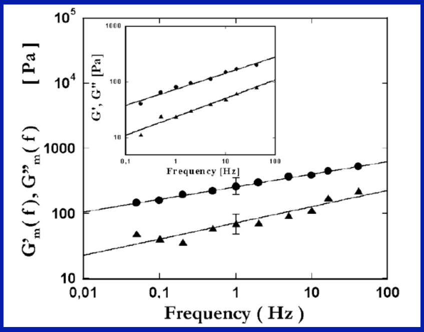
Transiently crosslinked: beyond $1/k_-$ the cell behaves like a fluid

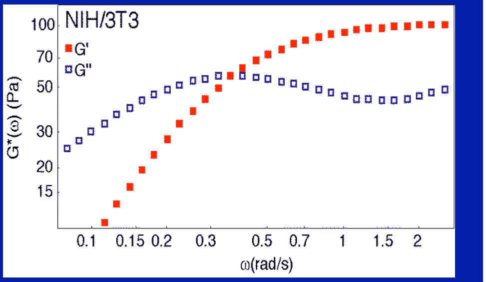


Explanation of the Difference?

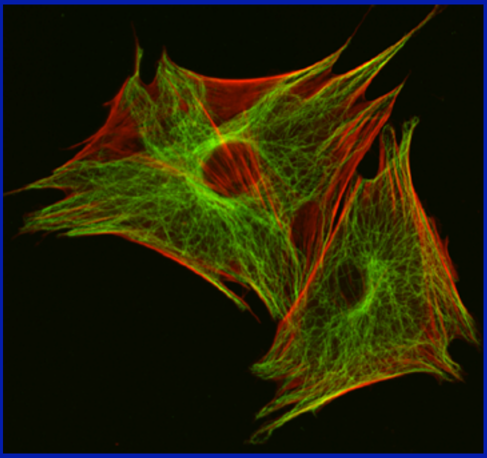
Attached

Suspended

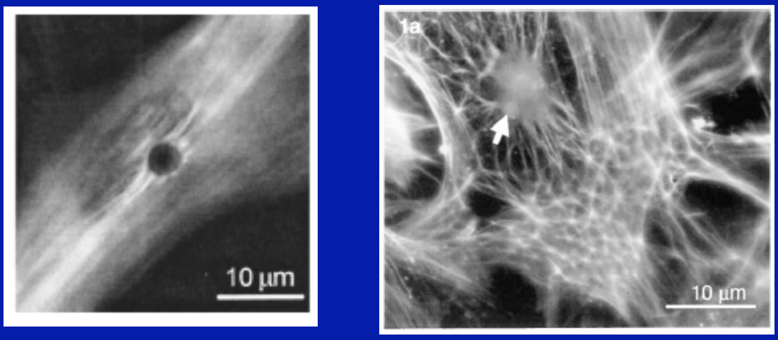




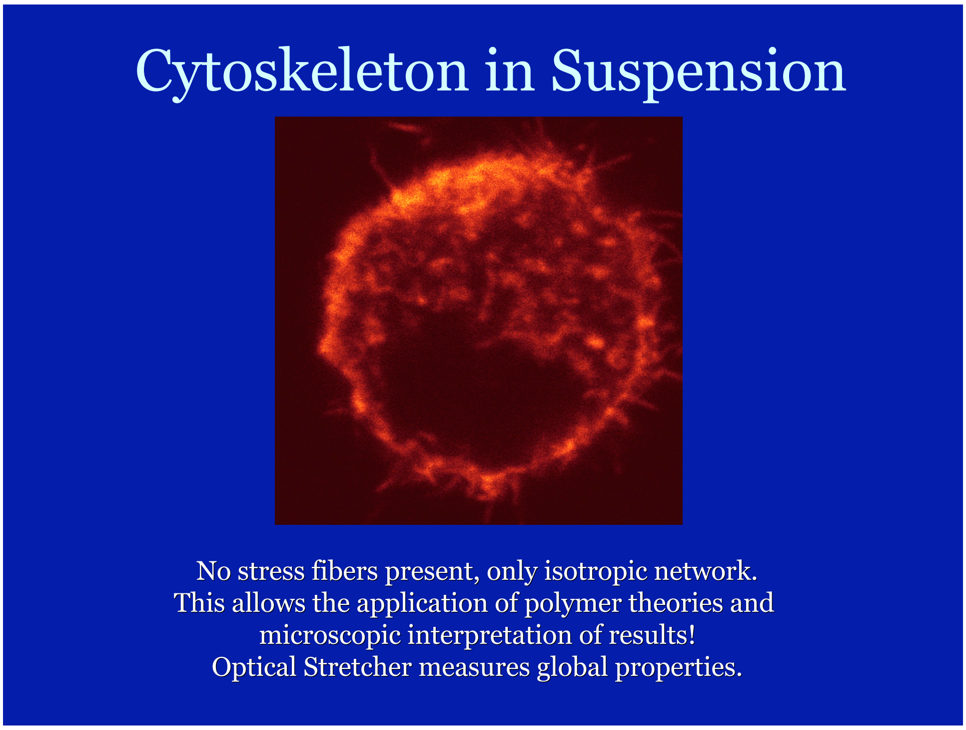
Cytoskeleton in Adherent Cells



Handles attach to Stress Fibers

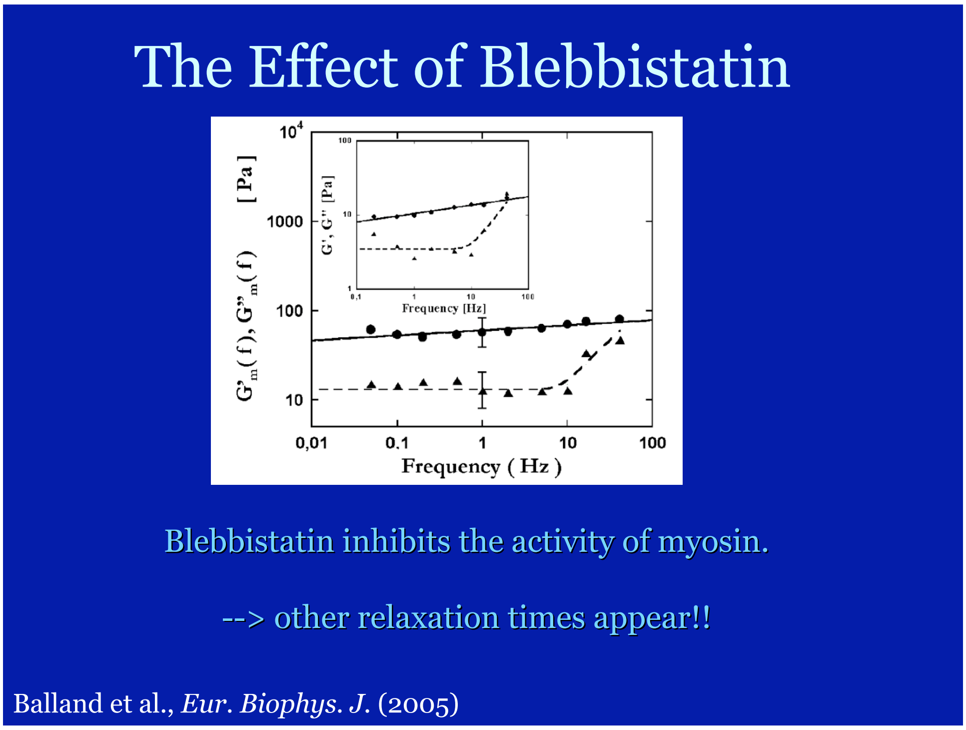


Methods probe dynamics of the contractile
actin-myosin stress fibers (very local measurement).
Response on many time-scales!



Cytoskeleton in Suspension

No stress fibers present, only isotropic network.
This allows the application of polymer theories and
microscopic interpretation of results!
Optical Stretcher measures global properties.



The Effect of Blebbistatin

Blebbistatin inhibits the activity of myosin.

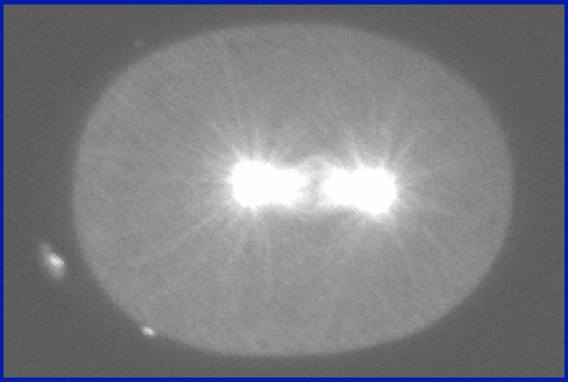
--> other relaxation times appear!!

Balland et al., *Eur. Biophys. J.* (2005)

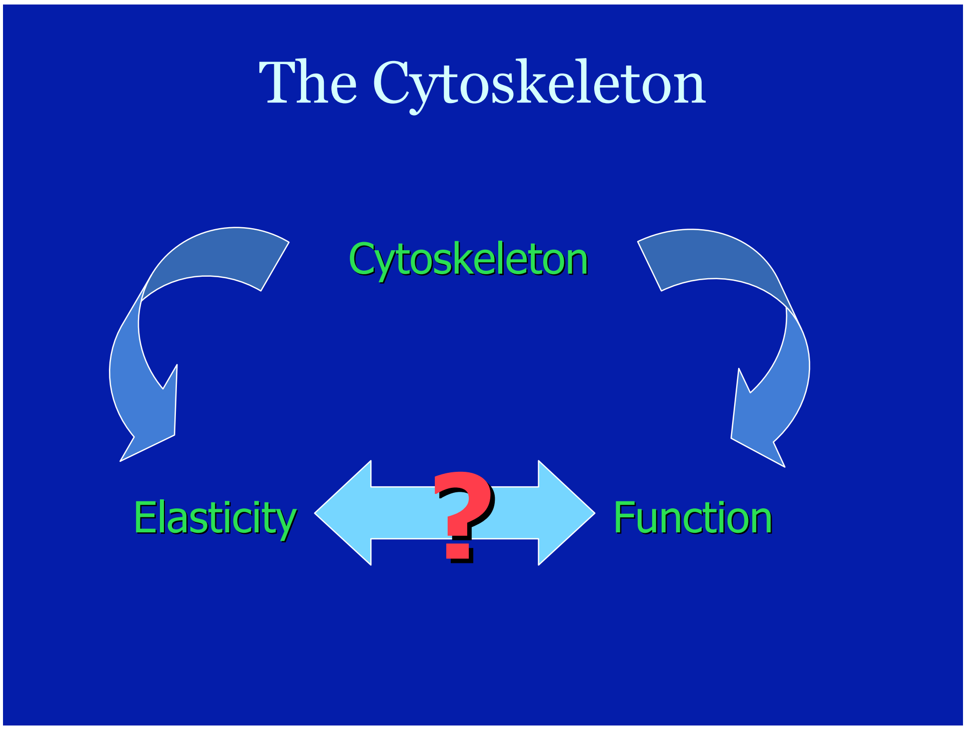
Cell Mechanics as a Cell Marker

The Cytoskeleton

Far from being static and passive, it is a very dynamic system that fulfills many important cell functions.



Courtesy of Dr. Stefan Grill, MPI f. MCB Dresden



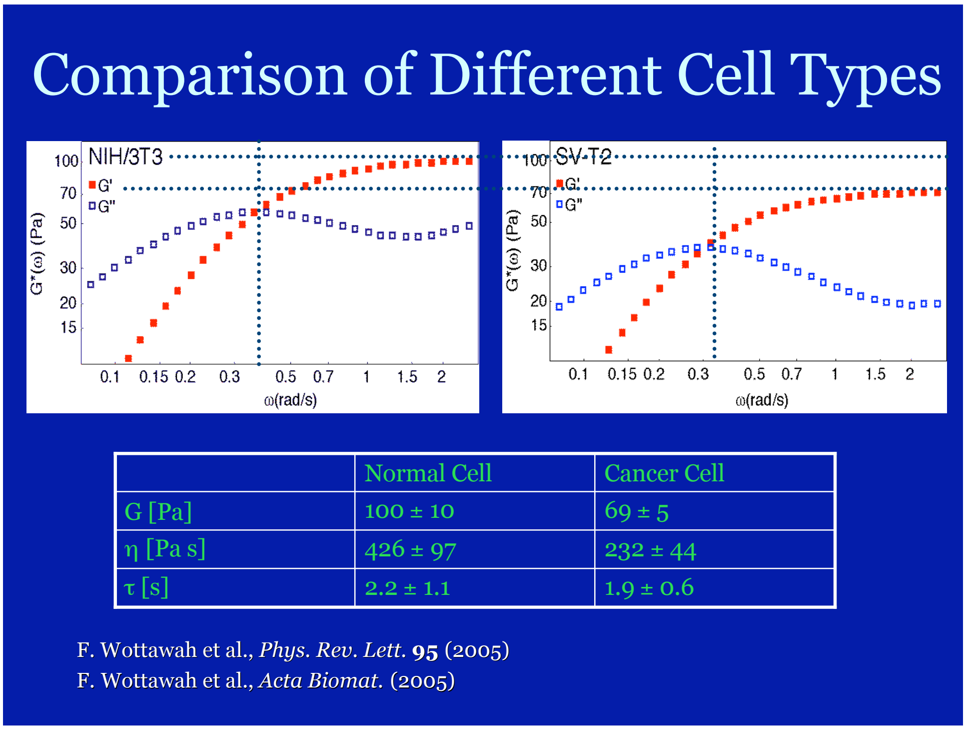
The Cytoskeleton

Cytoskeleton

Elasticity

?

Function

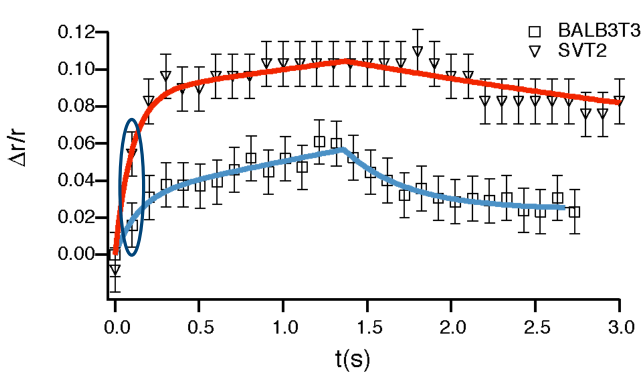


Comparison of Different Cell Types

	Normal Cell	Cancer Cell
G [Pa]	100 ± 10	69 ± 5
η [Pa s]	426 ± 97	232 ± 44
τ [s]	2.2 ± 1.1	1.9 ± 0.6

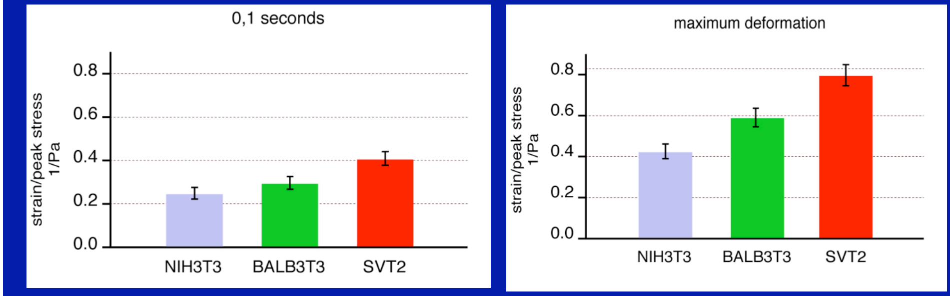
F. Wottawah et al., *Phys. Rev. Lett.* **95** (2005)
F. Wottawah et al., *Acta Biomat.* (2005)

Deformability of Cells



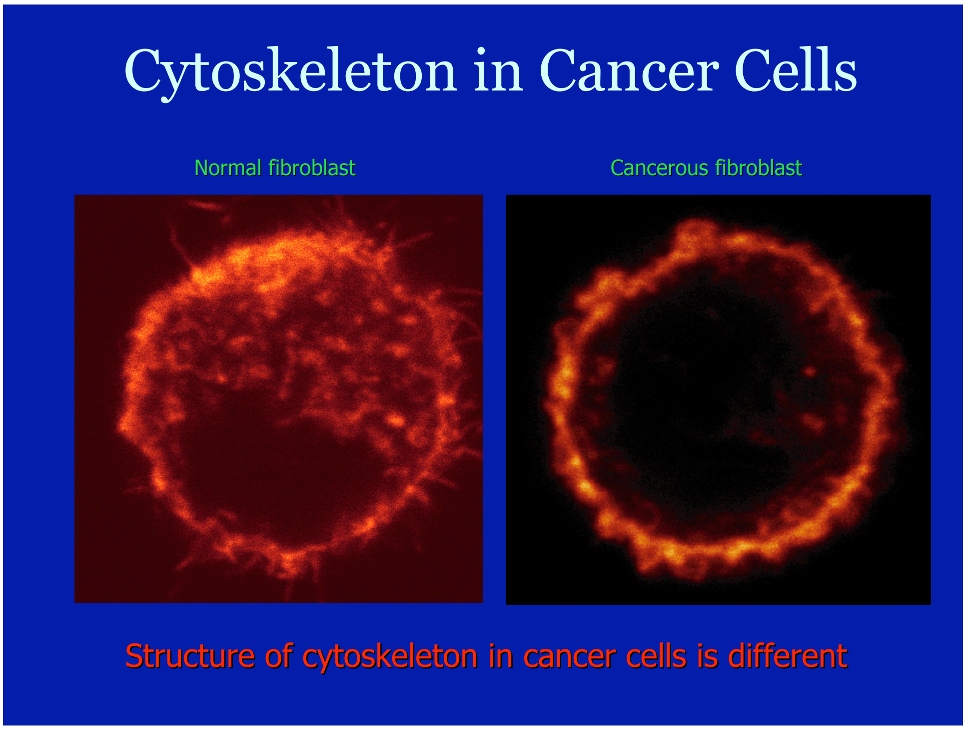
S. Schinkinger et al., *J. Biomed. Opt.* (submitted)

Optical Deformability



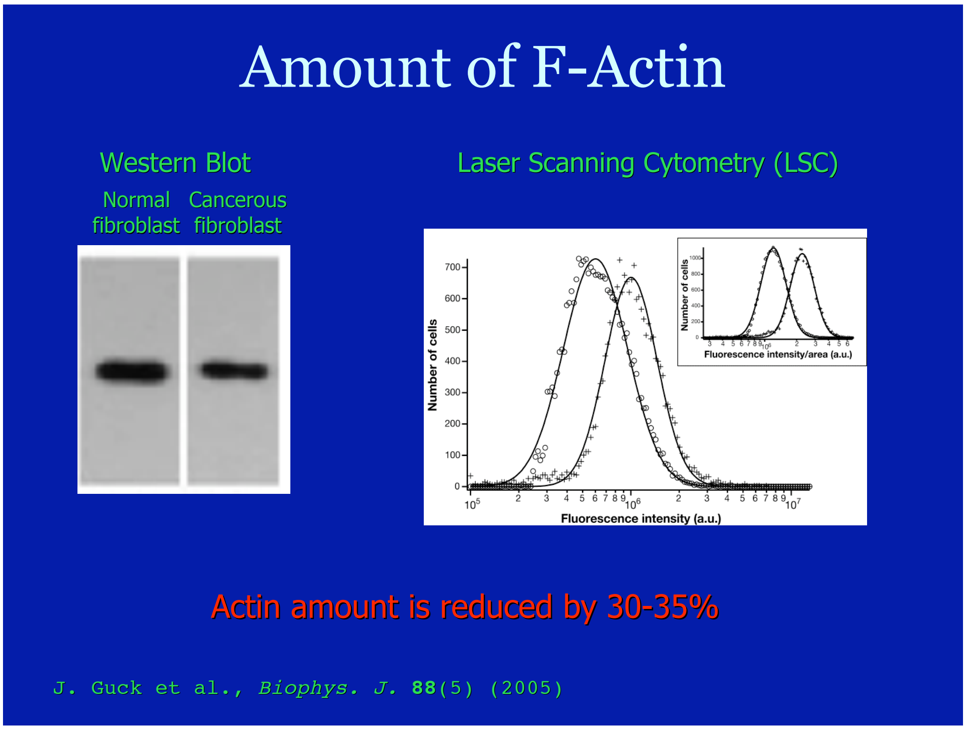
Only 30 cells each measured.
Deformabiliity of cells is a tightly regulated cell marker

S. Schinkinger et al., *J. Biomed. Opt.* (submitted)



Cytoskeleton in Cancer Cells

Structure of cytoskeleton in cancer cells is different

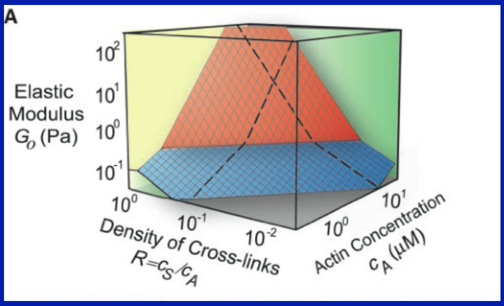


Amount of F-Actin

Actin amount is reduced by 30-35%

J. Guck et al., *Biophys. J.* **88**(5) (2005)

Deformability as Cell Marker



M. Gardel et al., *Science* (2004)

$$G' \propto c_A^{2\text{–}6.7}$$

Cell deformability provides built-in, strong amplification of molecular changes in single living cells.