Experimental Techniques in Small Scale Manipulation of Biological Systems

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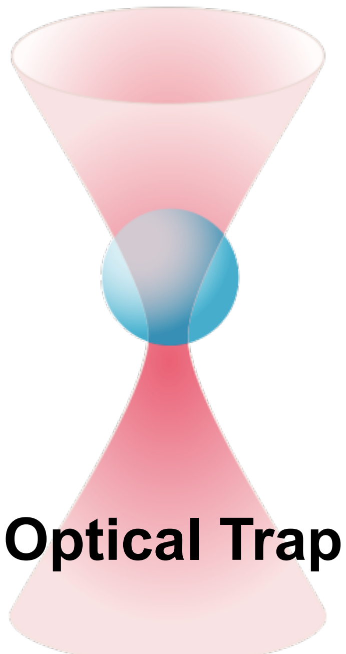
Ovijit Chaudhuri (UC Berkeley) and more ...

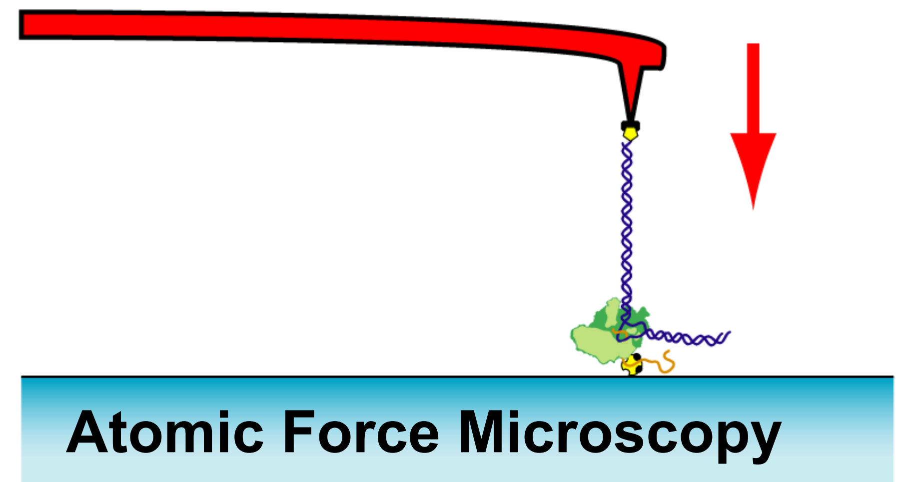
Tools for measuring dynamics and forces

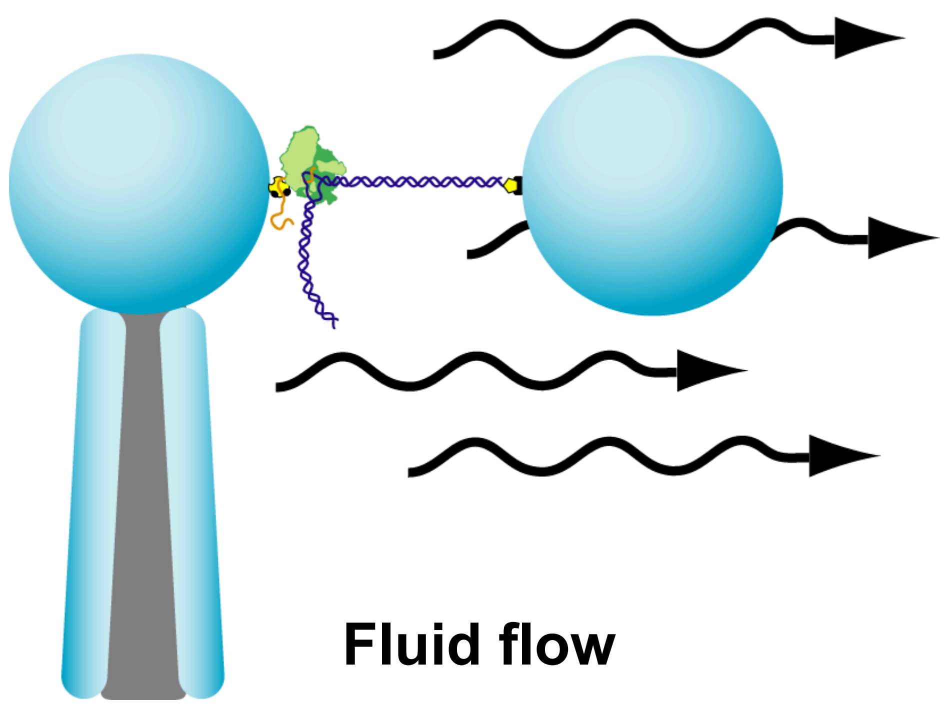
"You can learn a lot by watching"

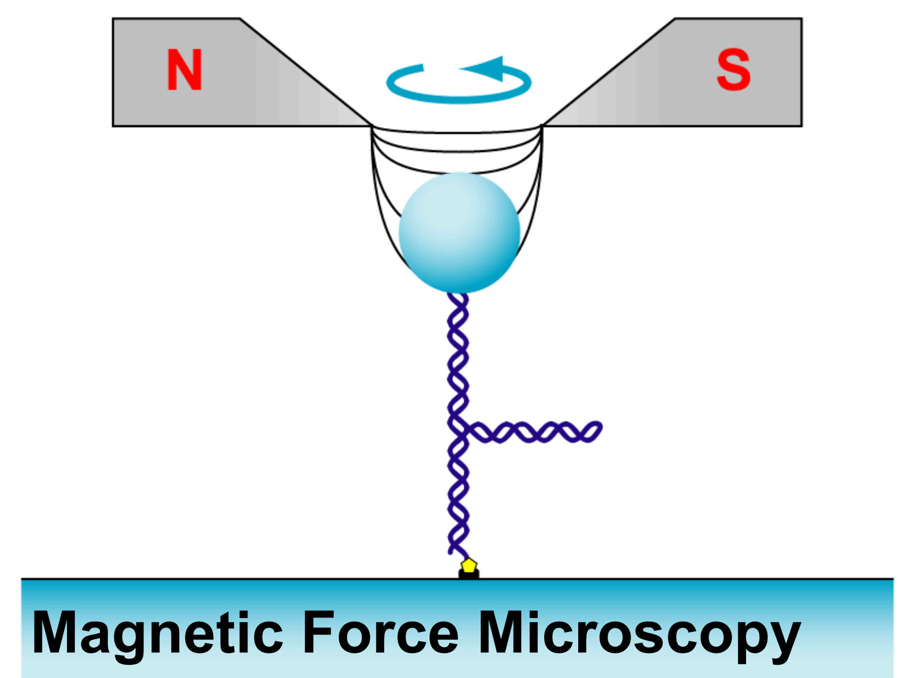
-Yogi Berra

Tools for measuring dynamics and forces



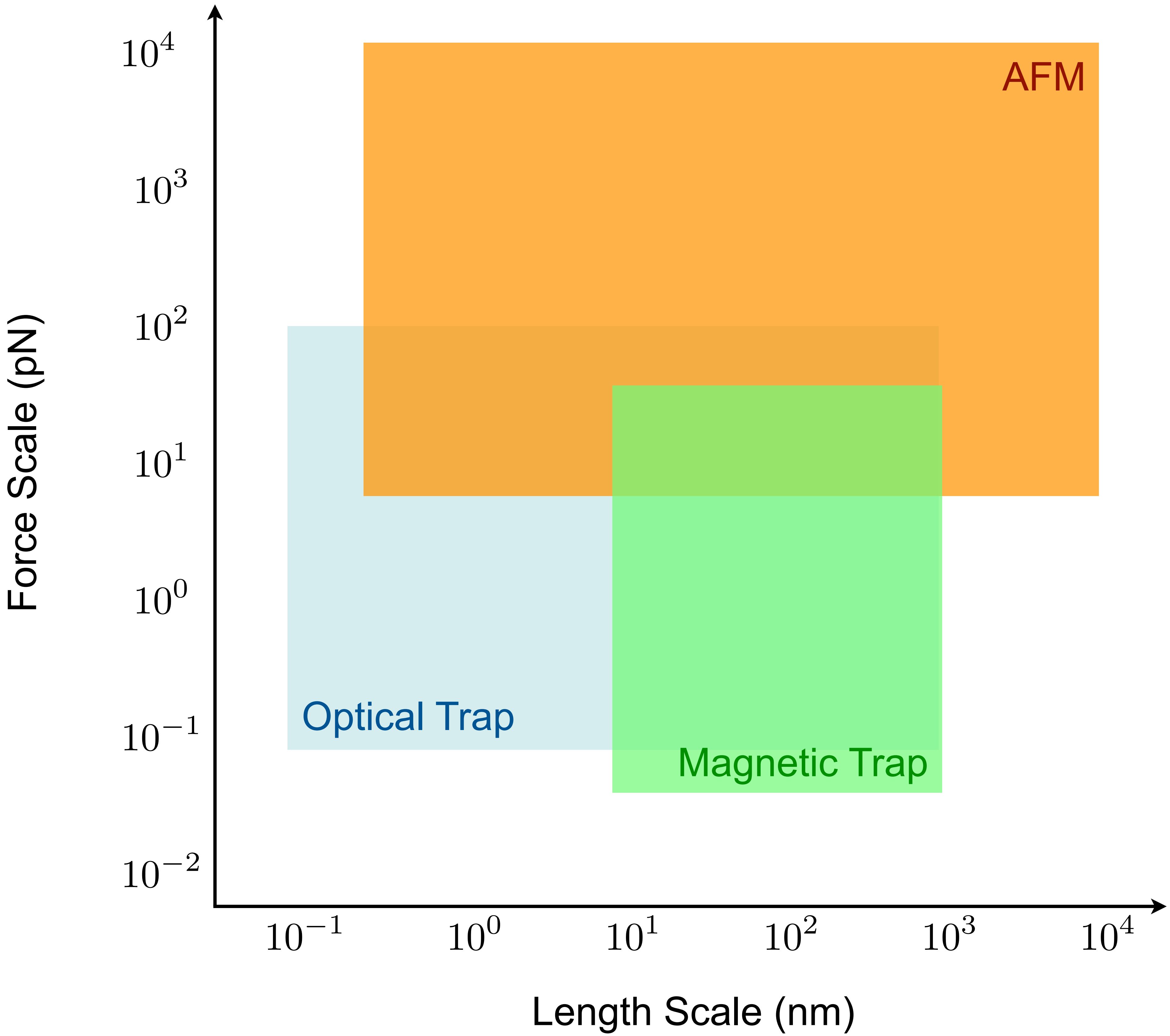






and many more …

Force and position scales for different techniques



Light has momentum, but its small!

"A very short experience in attempting to measure these forces is sufficient to make one realize their extreme minuteness – a minuteness which appears to put them beyond consideration in terrestrial affairs…"

- J. H. Poynting ($\vec{S} = \vec{E} \times \vec{B}$) 1905

The force on a reflecting mirror

The momentum of a single photon is $h\nu/c$.

For a laser of power, P, there are $P/h\nu$ photons per second striking the mirror.

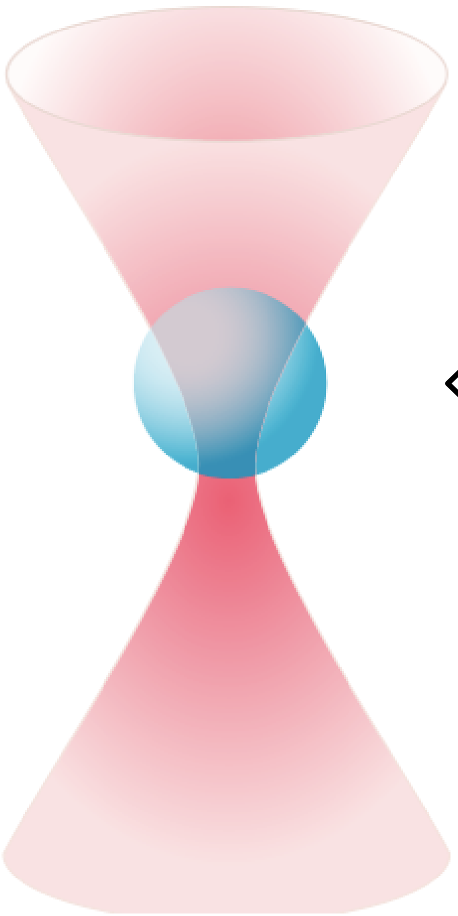
The total change in momentum of the light per second is $(2P/h\nu)(h\nu/c)=2P/c$.

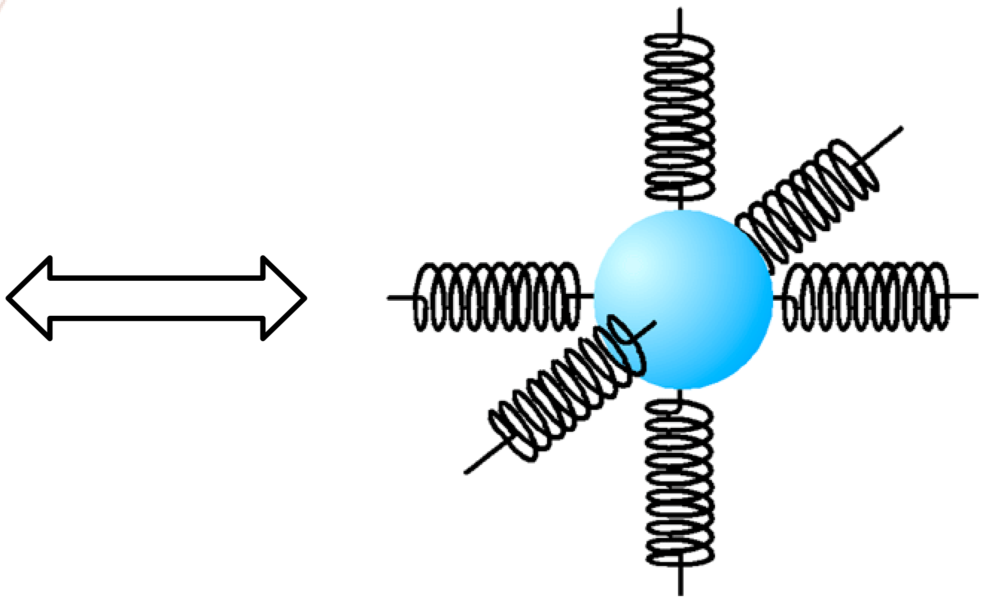
By conservation of momentum the mirror feels an equal and opposite momentum change per second, which is a force!

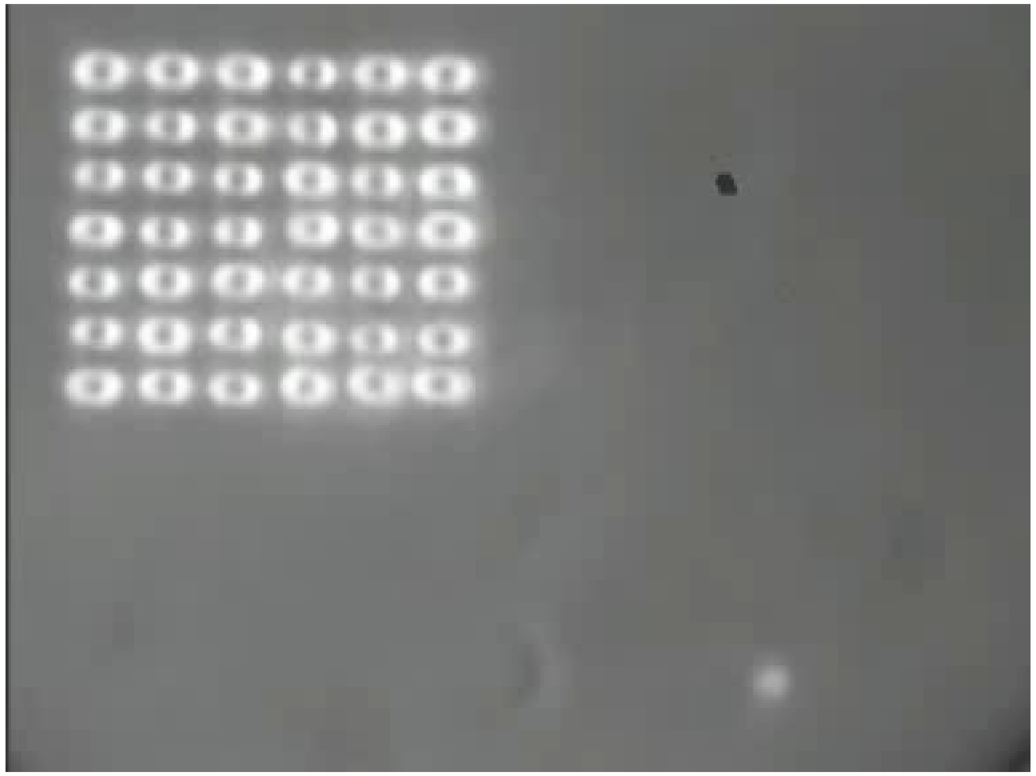
e.g. if P = 1 watt → Force = 10^{-11} Newtons = 10 nN!

My laser pointer is 1 mW → Force = 10 pN

Optical traps are 3D springs made of light







C.Schmidt lab

Optical traps can . . .

- manipulate the position of micron-sized objects (like bacteria or glass beads)
- apply forces up to ~100 pN
- measure the motions produced by biological molecules with high 3D spatial (~1 Å) and temporal (<100 μs) resolutions
- readily combined with other optical microscopy techniques

At a focus there is a refractive restoring force

The trapping laser imparts a force onto the particle directed towards the laser focus.

The magnitude of this force is:

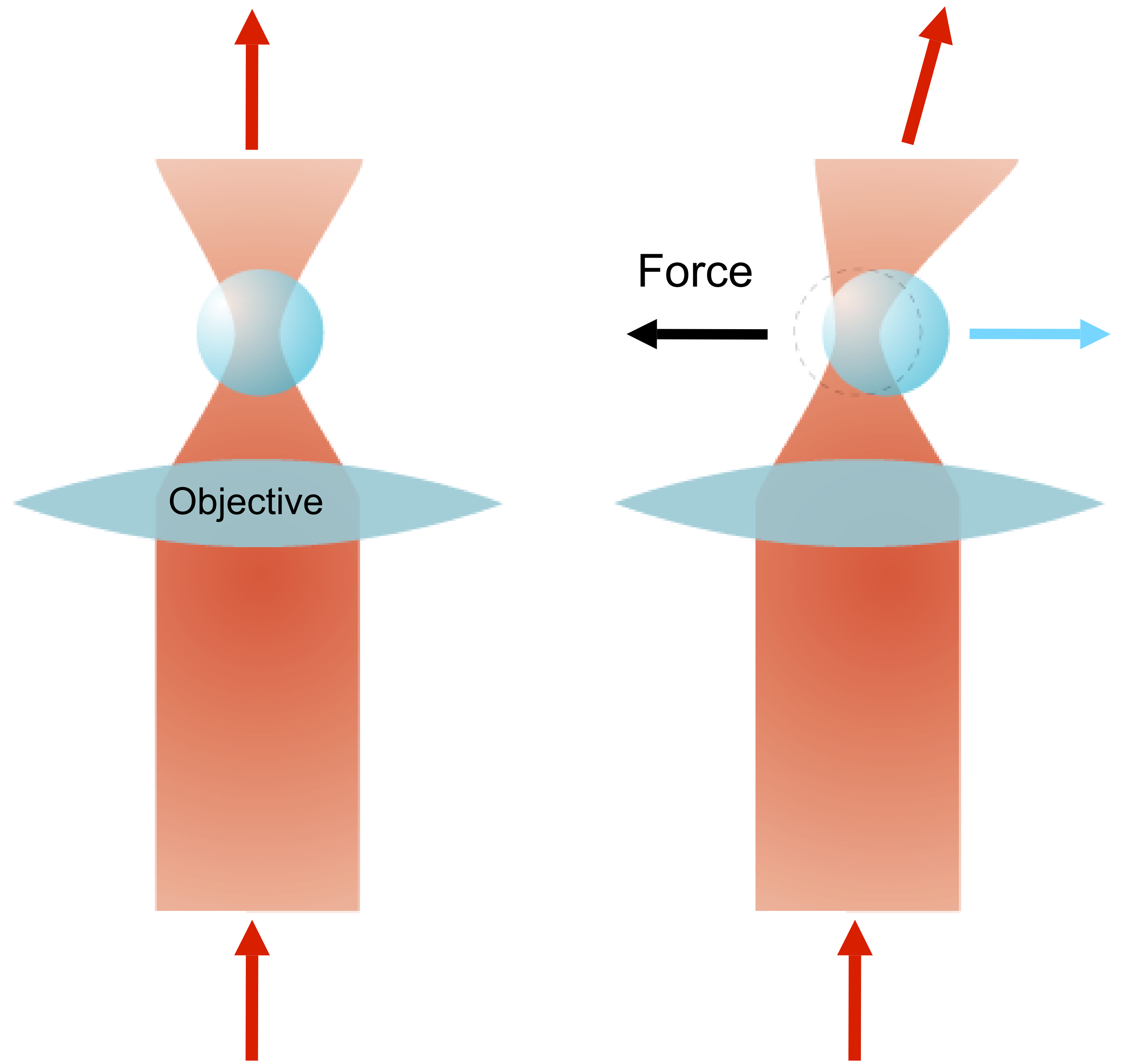
$$F = \frac{Qn}{c} P$$

P = laser power

n = particle refractive index

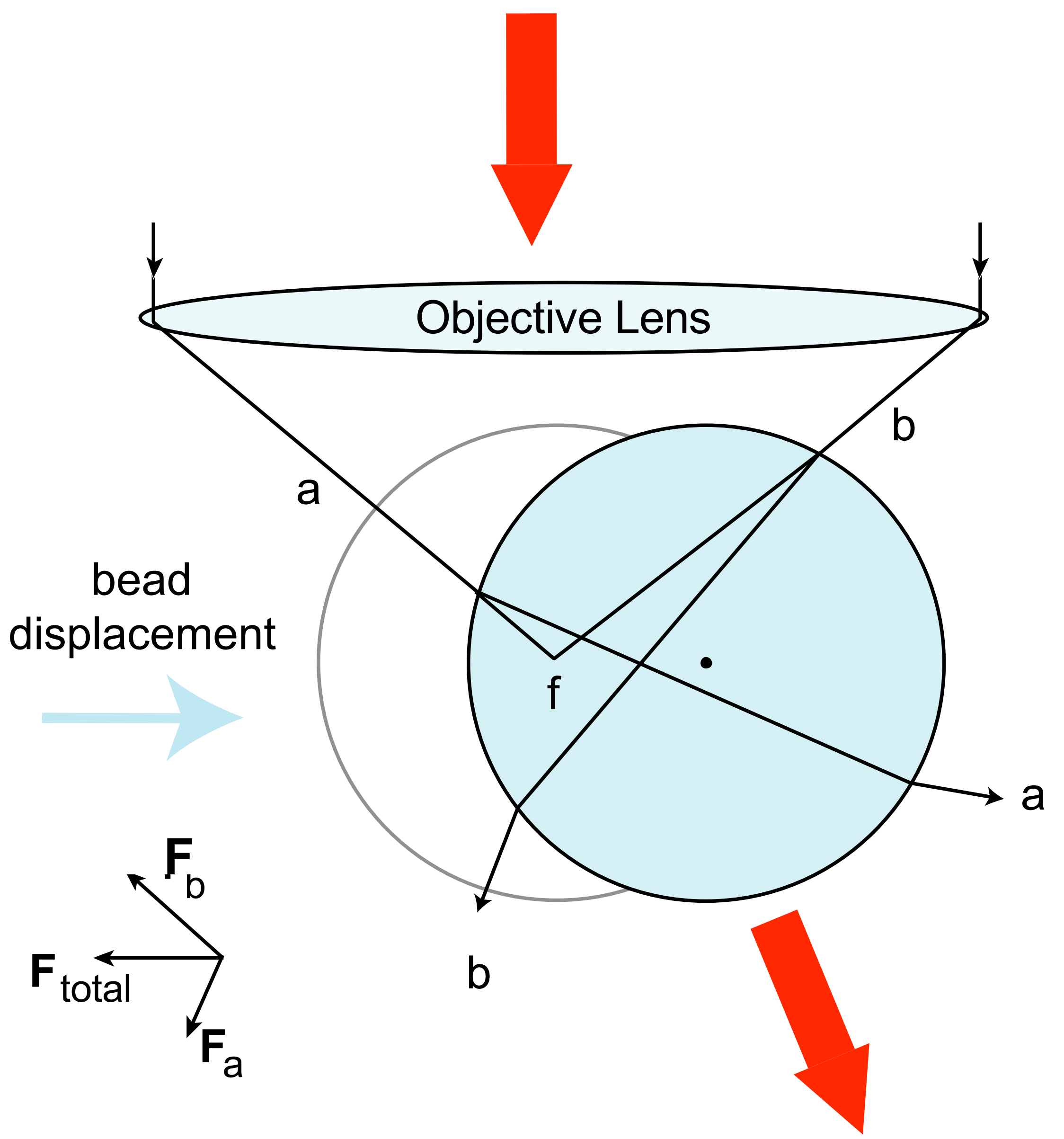
c = speed of light

Q = trapping efficiency

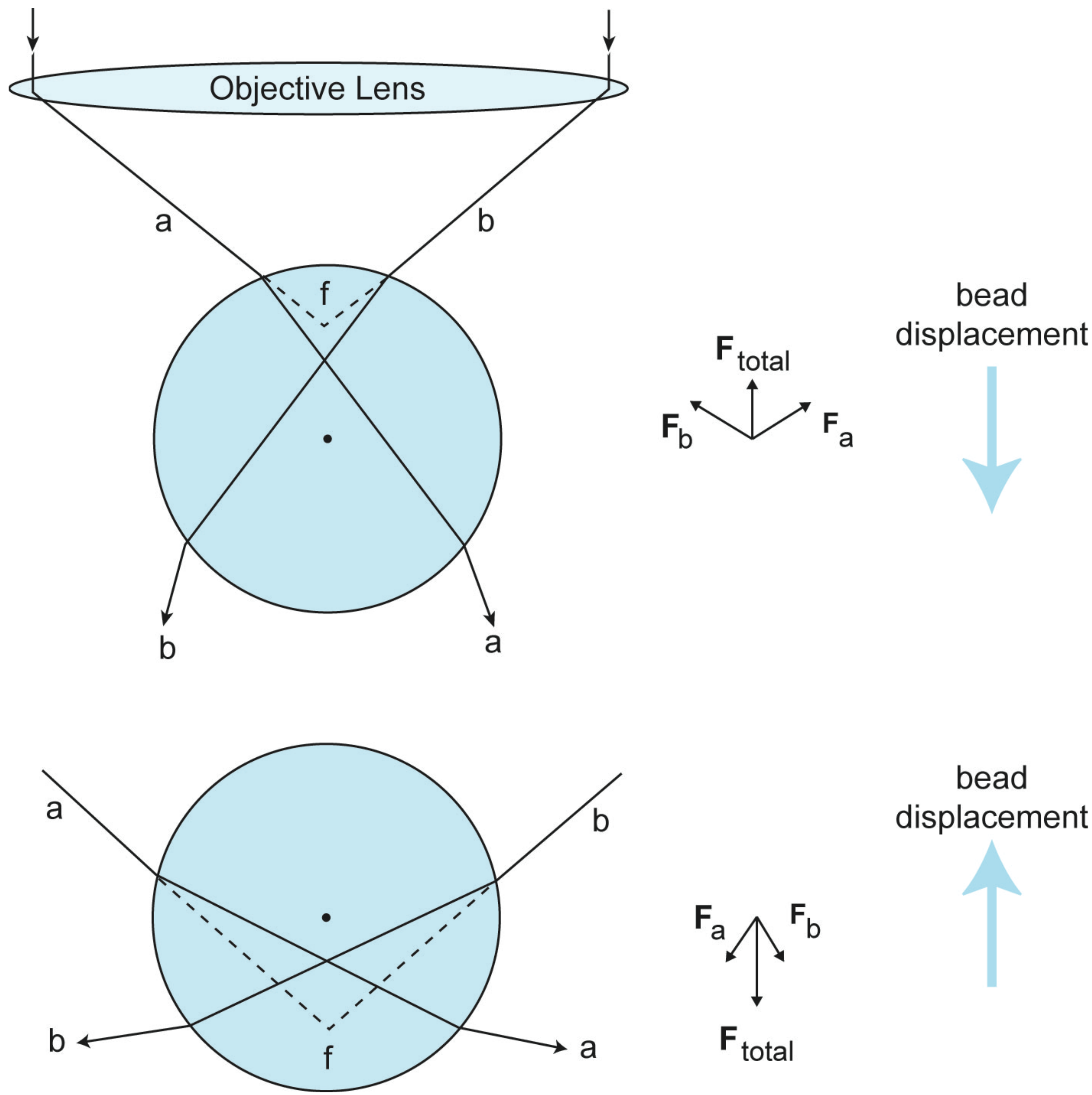


Svoboda and Block, *Annu Rev Biophys Biomol Struct* (1994)

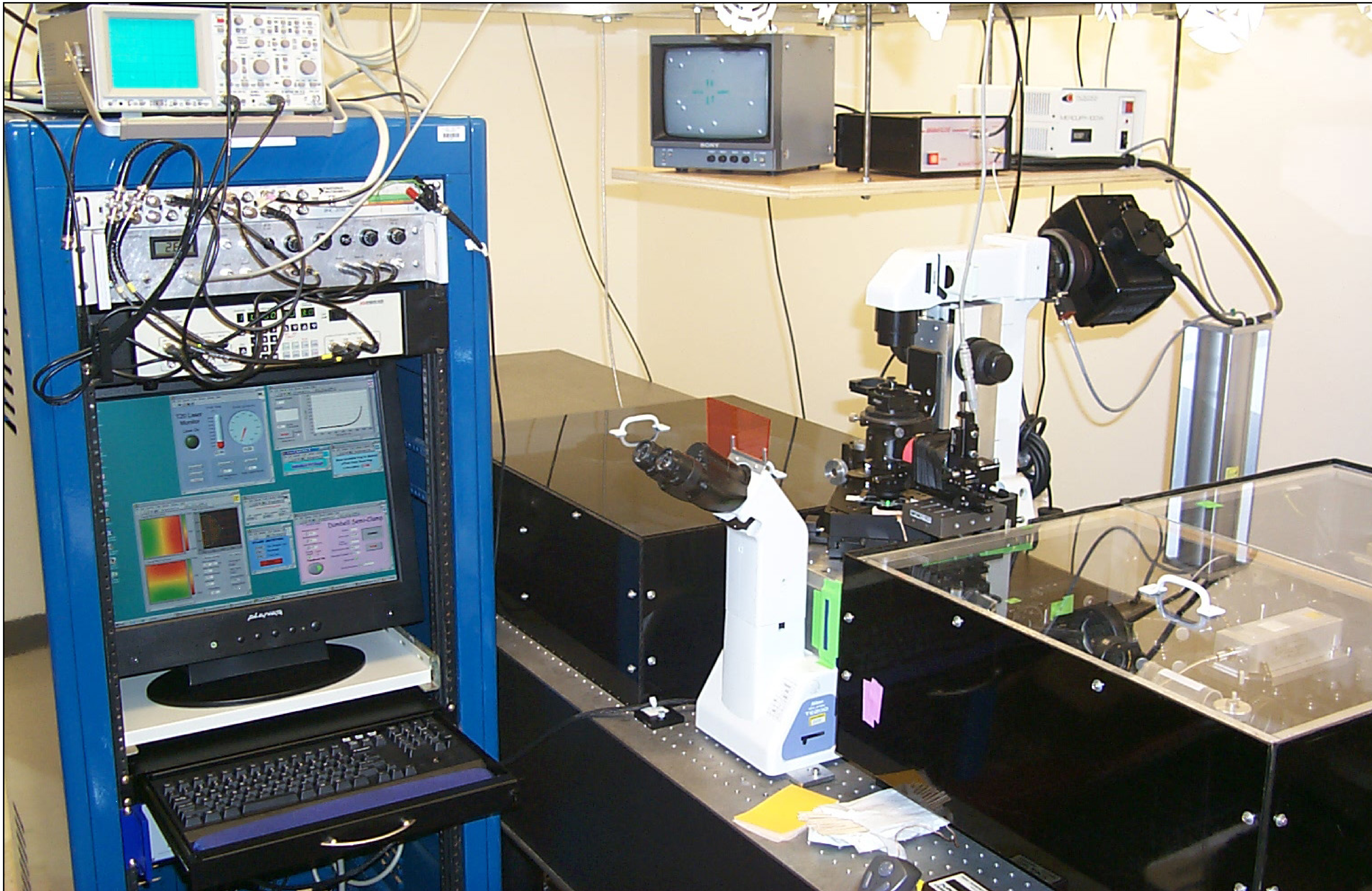
One lens, and two rays is all you need



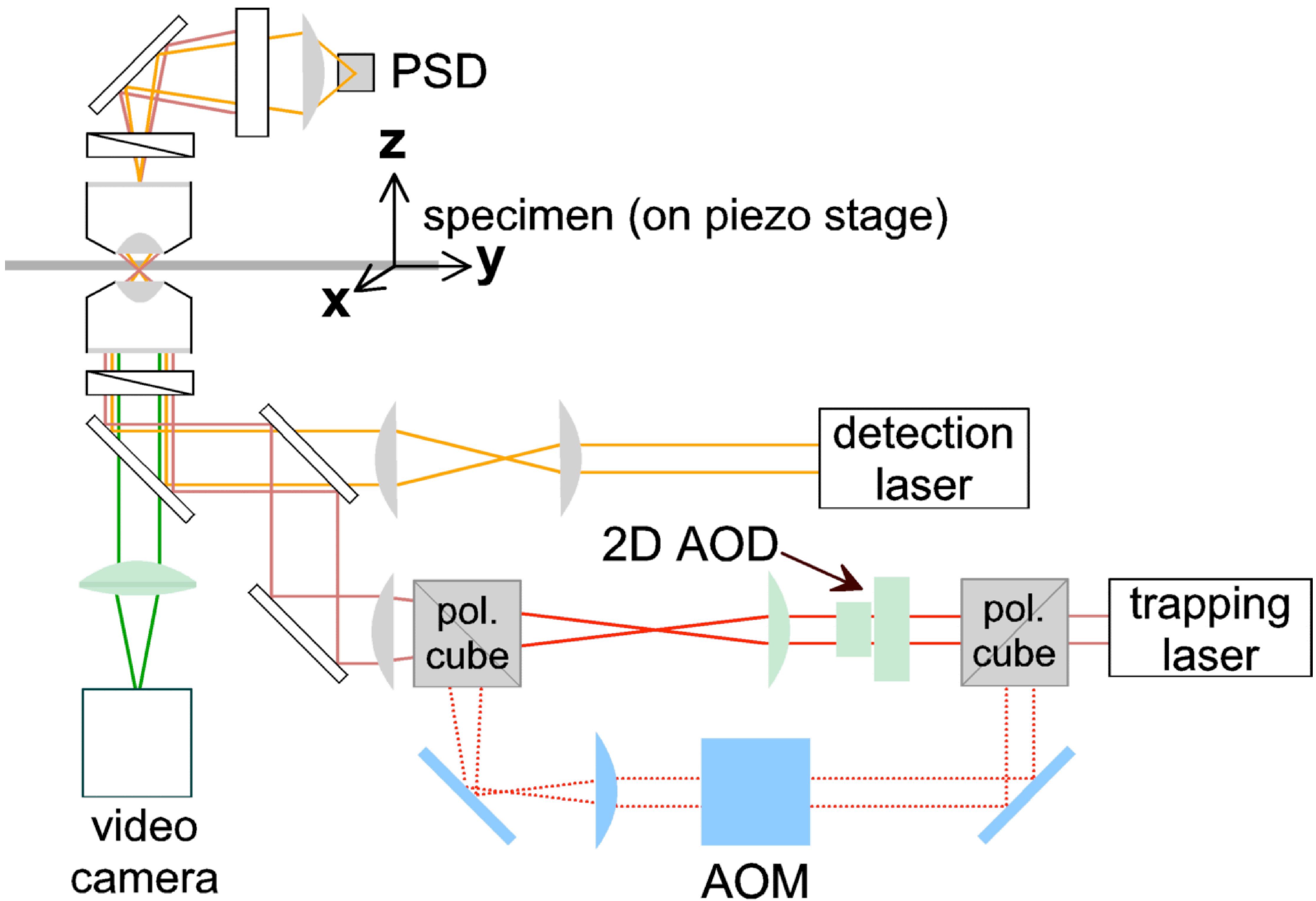
The restoring force acts in the axial direction too



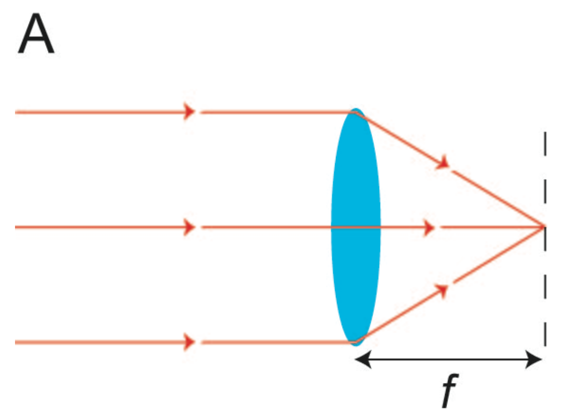
The optical trap used for the RNAP work

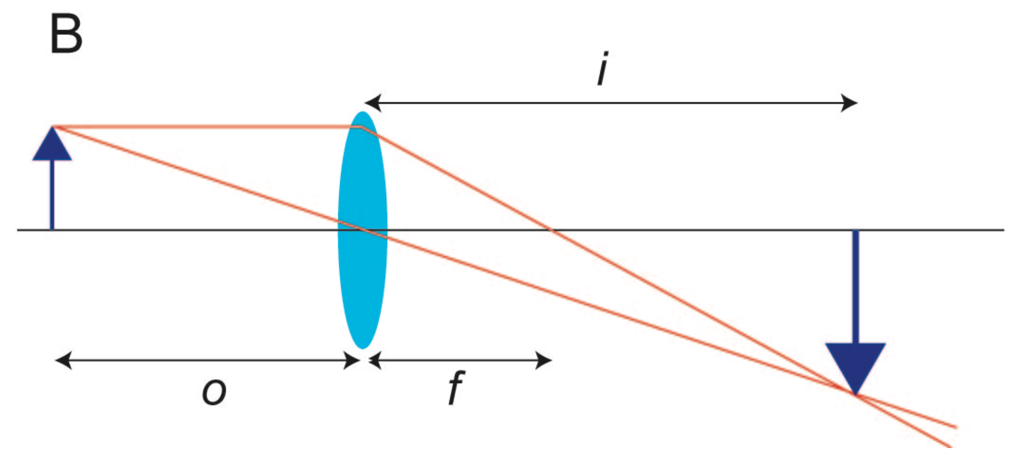


Optics Diagram

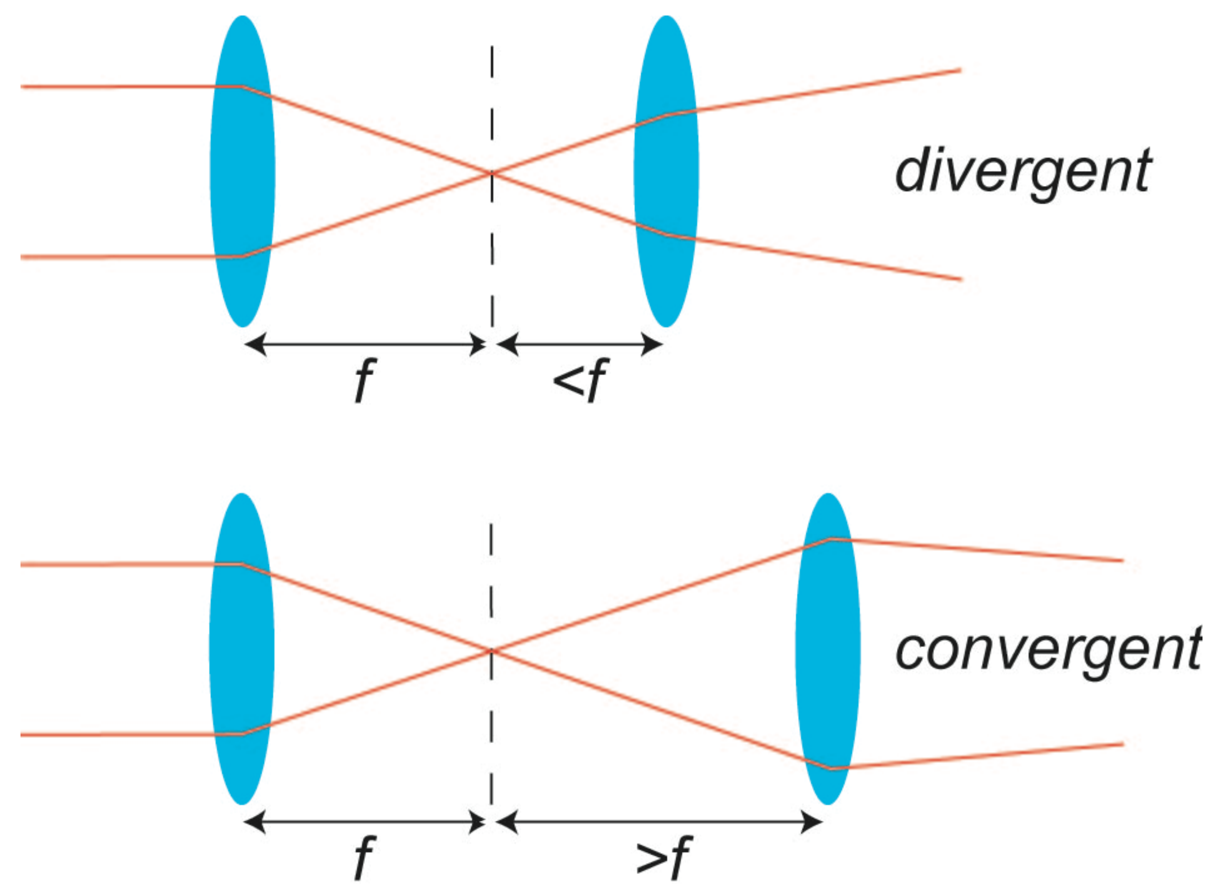


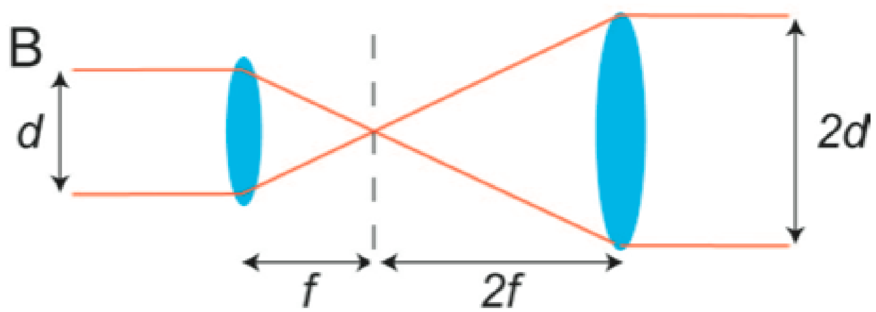
Basic ray optics



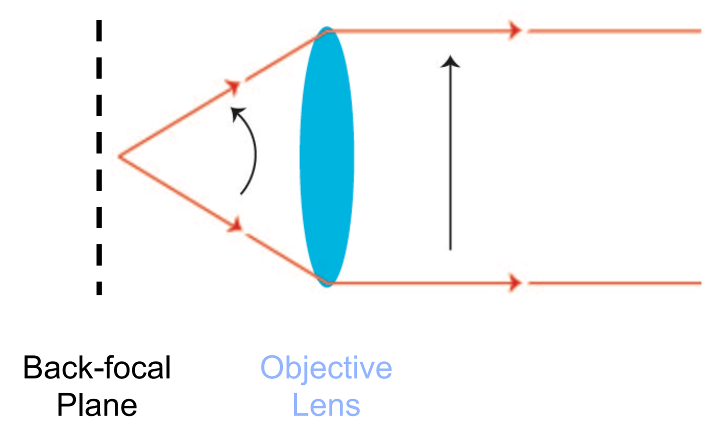


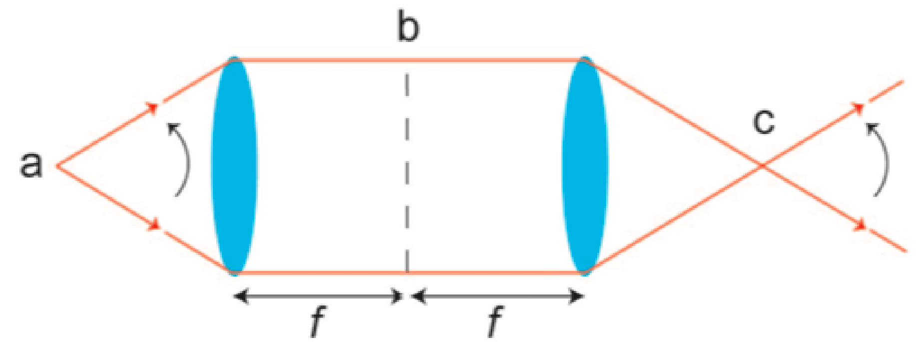
$$\frac{1}{o} + \frac{1}{i} = \frac{1}{f}$$

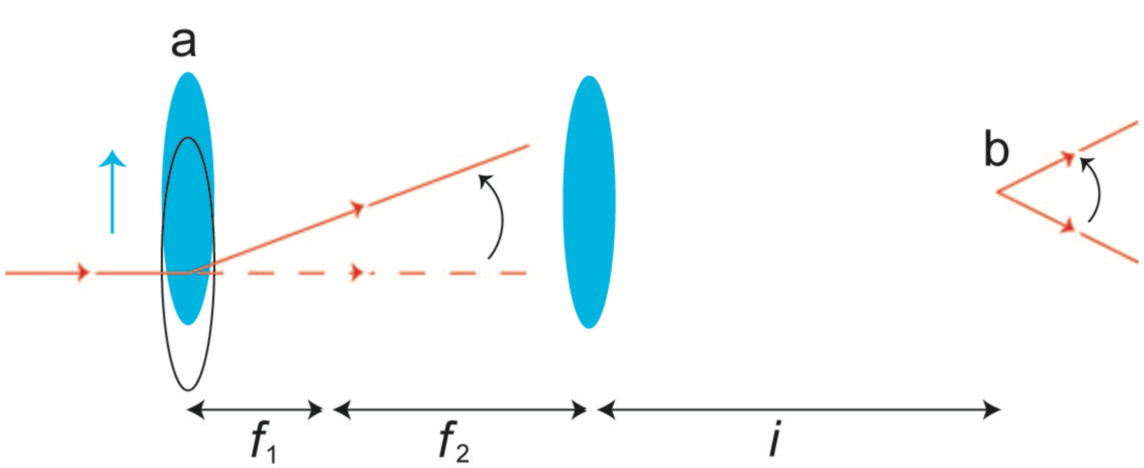


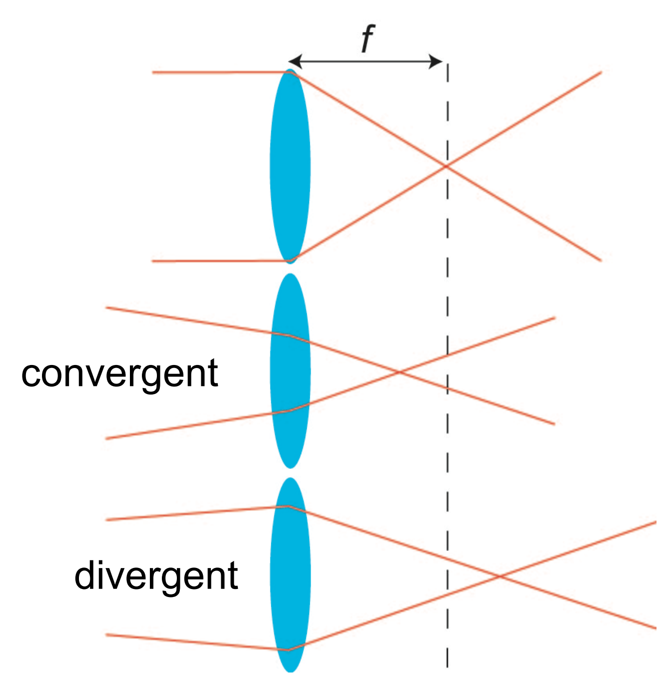


Beam steering is achieved by rotations



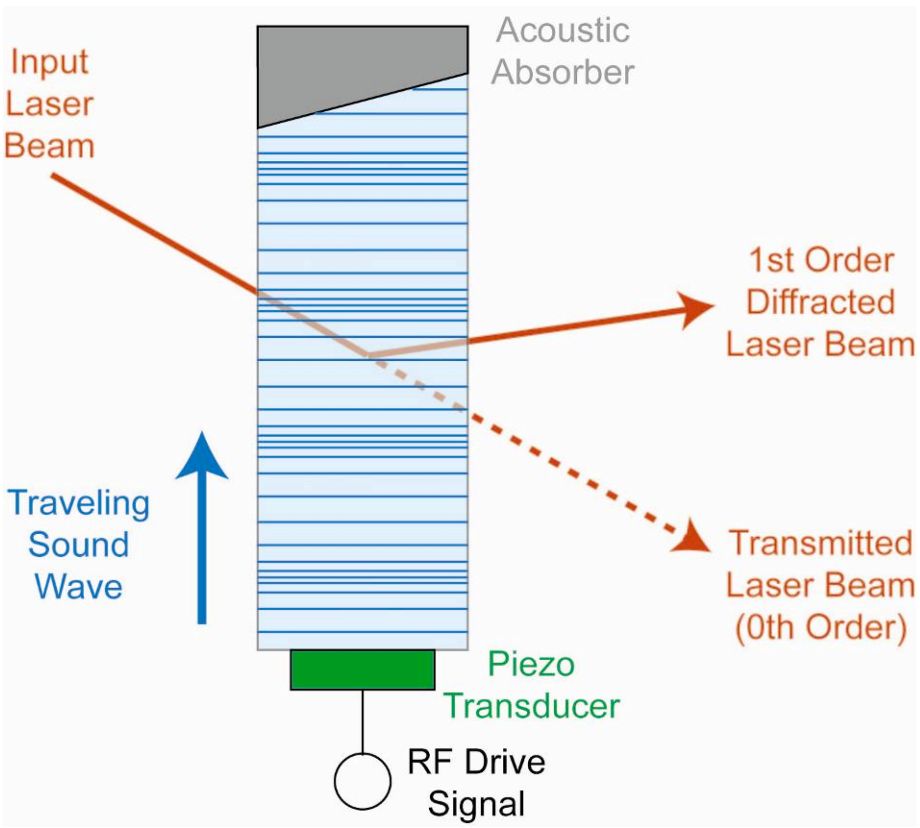






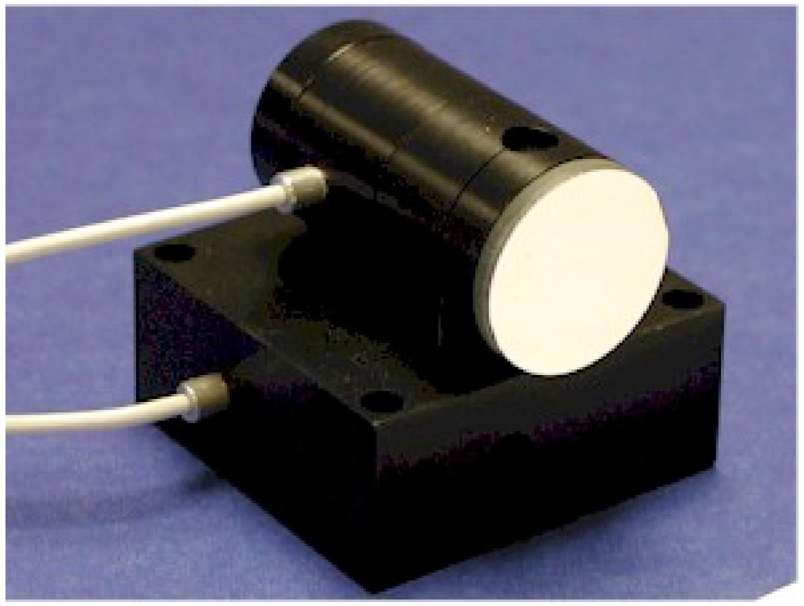
Computer controlled steering technology

Acousto-optic deflector



$$\Delta\theta \approx \lambda \frac{\Delta f}{V_{sound}}$$

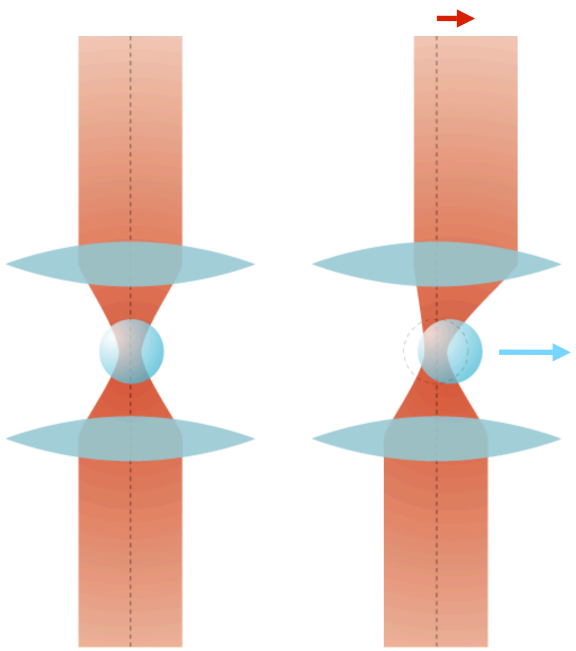
Piezo-driven mirror

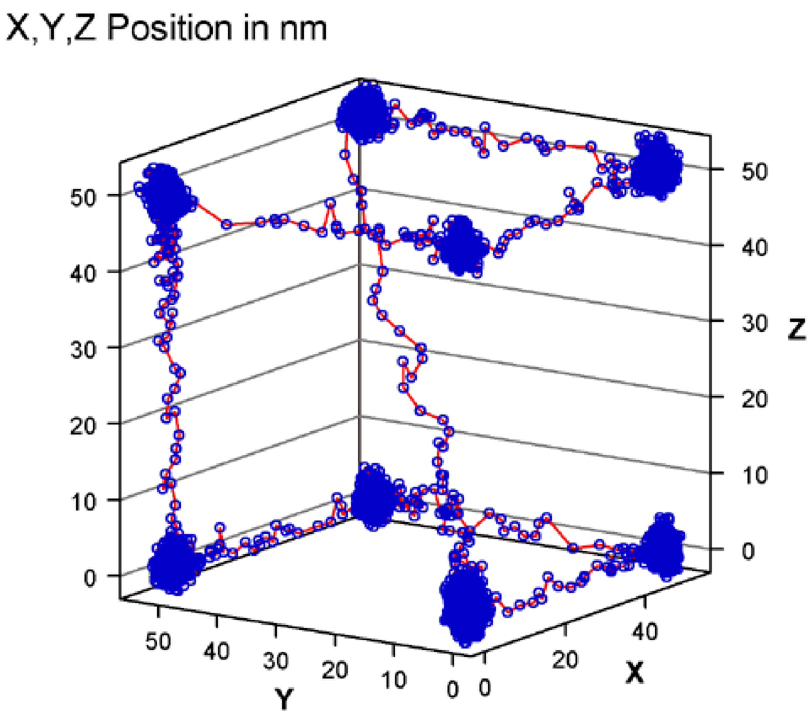


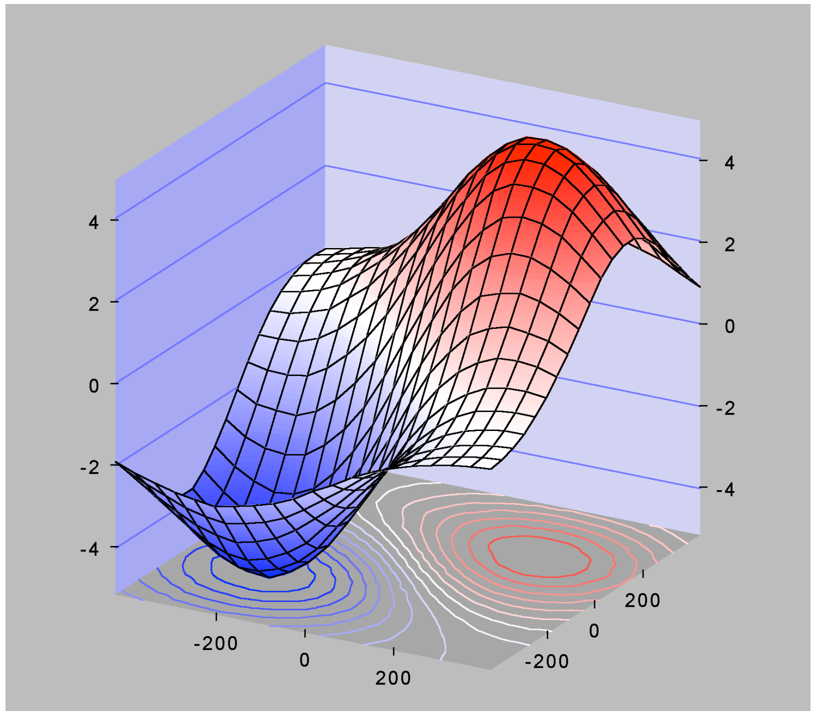
Electro-optic deflector

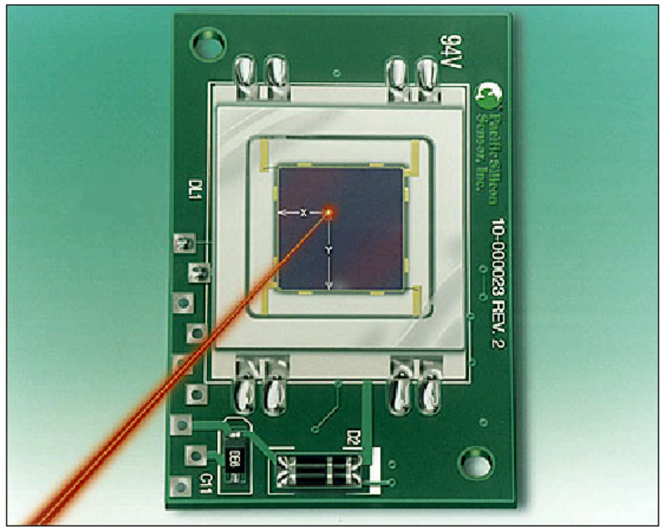
$$\theta = K \frac{LV}{a^2}$$

Bead position detection

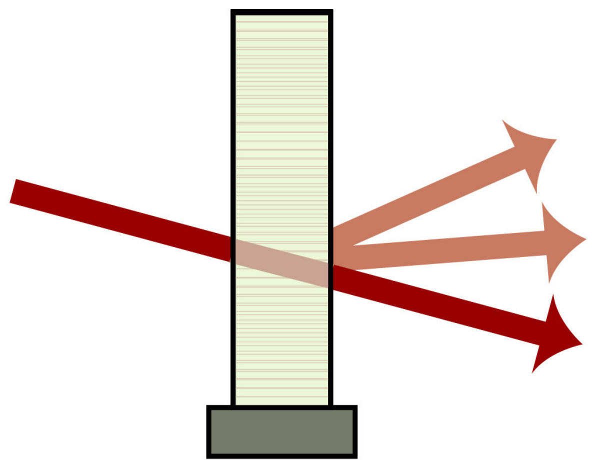








Techniques for making two traps

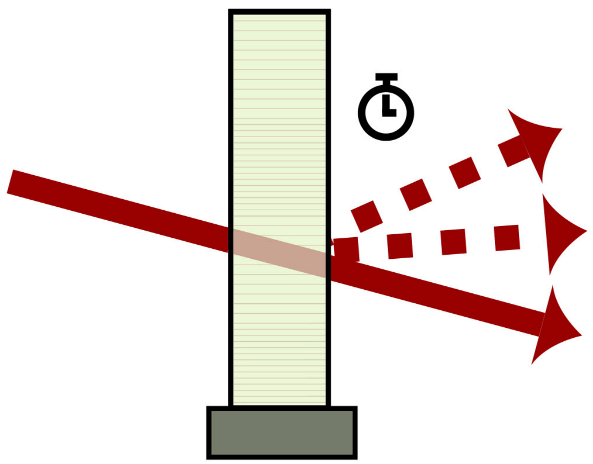


AOD splitting: simultaneous multiple frequencies

Two frequencies are fed into an acousto-optic crystal at the same time creating two first-order diffracted beams.

Pros: Easy to implement; More than two traps can be created

Cons: Beam intensities fluctuate as position changes; Traps can be moved independently in only one dimension; “Ghost” traps created

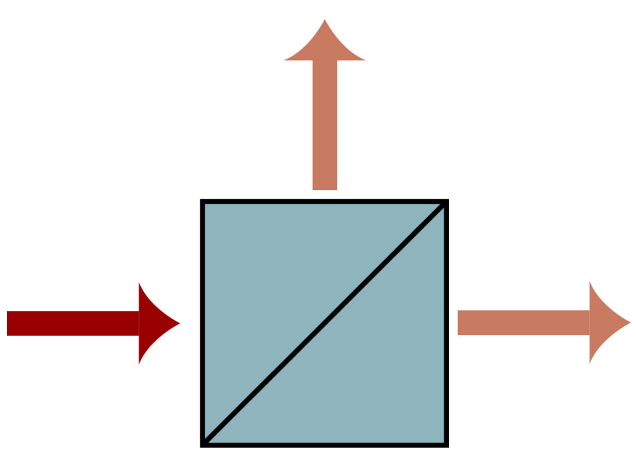


AOD splitting: time shared multiple frequencies

AOD rapidly alternates between two different frequencies (beam positions).

Pros: Traps can be moved independently in two dimensions; More than two traps can be created, Traps intensities are independent of each other

Cons: Requires a fast computer or RF capable electronics; Non-linear and harmonic effects distort trap; “Ghost” traps created

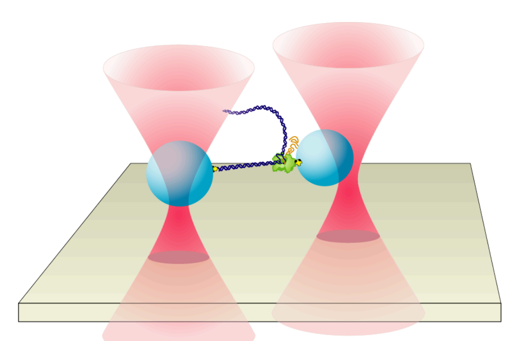


Polarization splitting

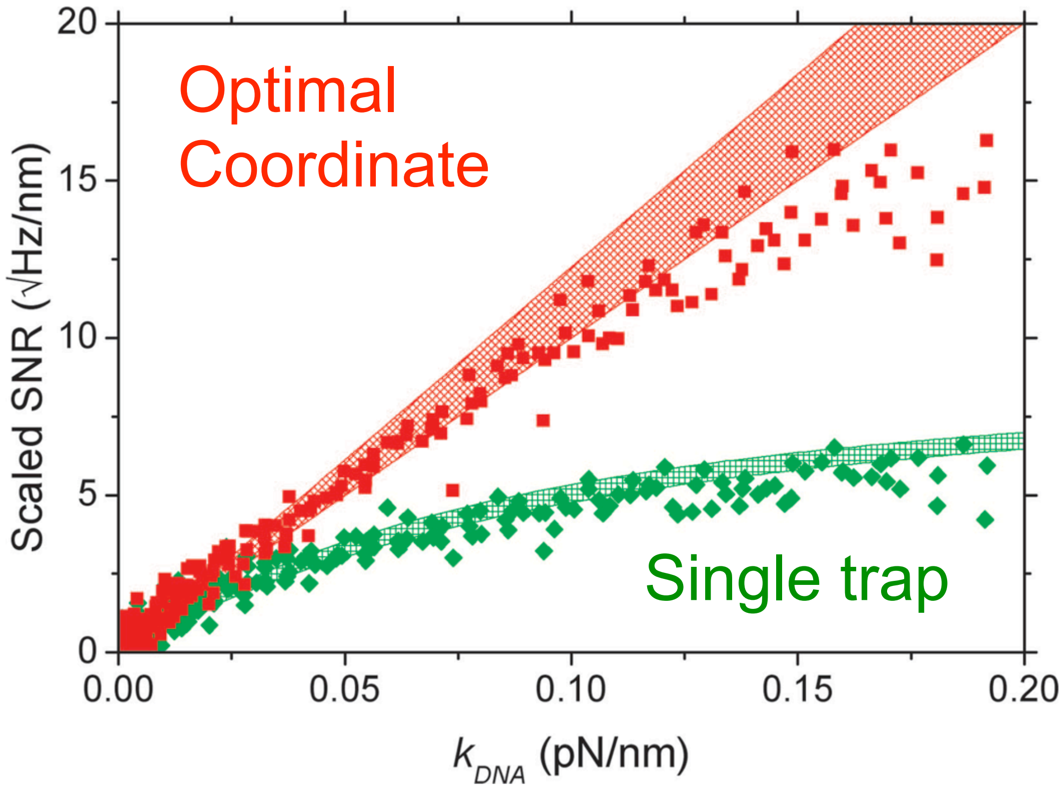
Beam is split into two orthogonal polarizations

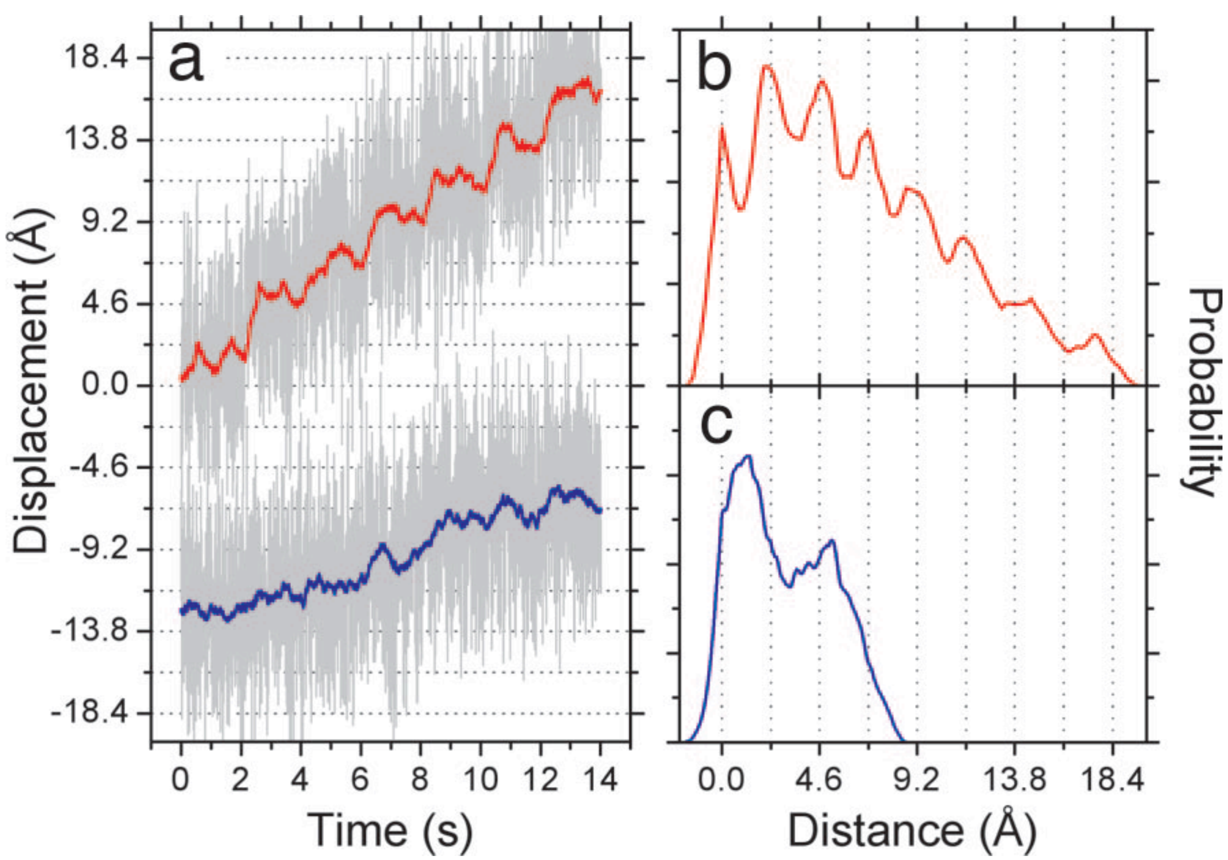
Pros: No non-linear AOD effects; Traps can be steered independently in two dimensions; Traps intensities are independent of each other

Cons: Requires more table space and optics; Difficult to add additional traps; Requires two sets of AOD crystals and associated electronics.



Two traps are better than one

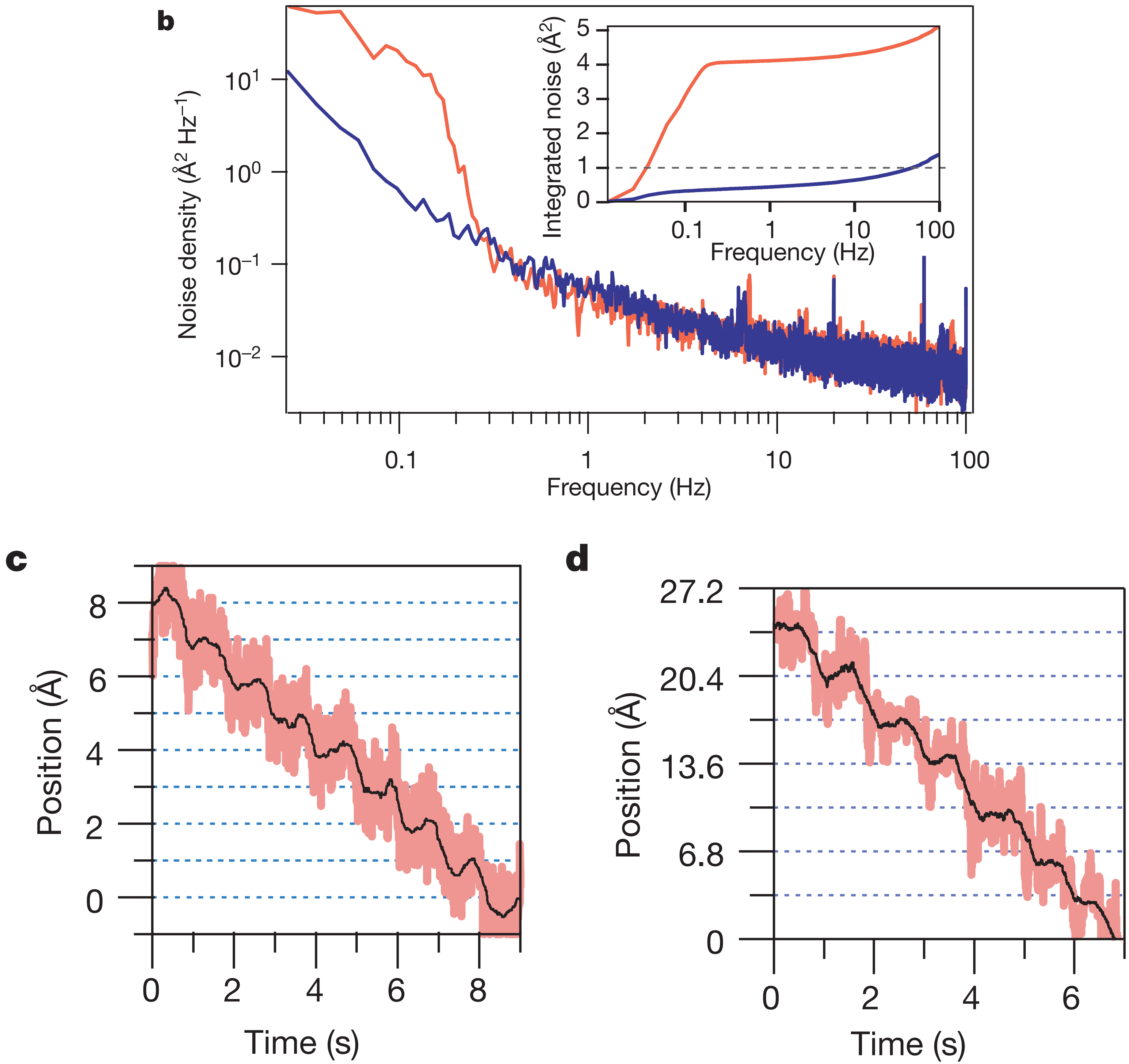




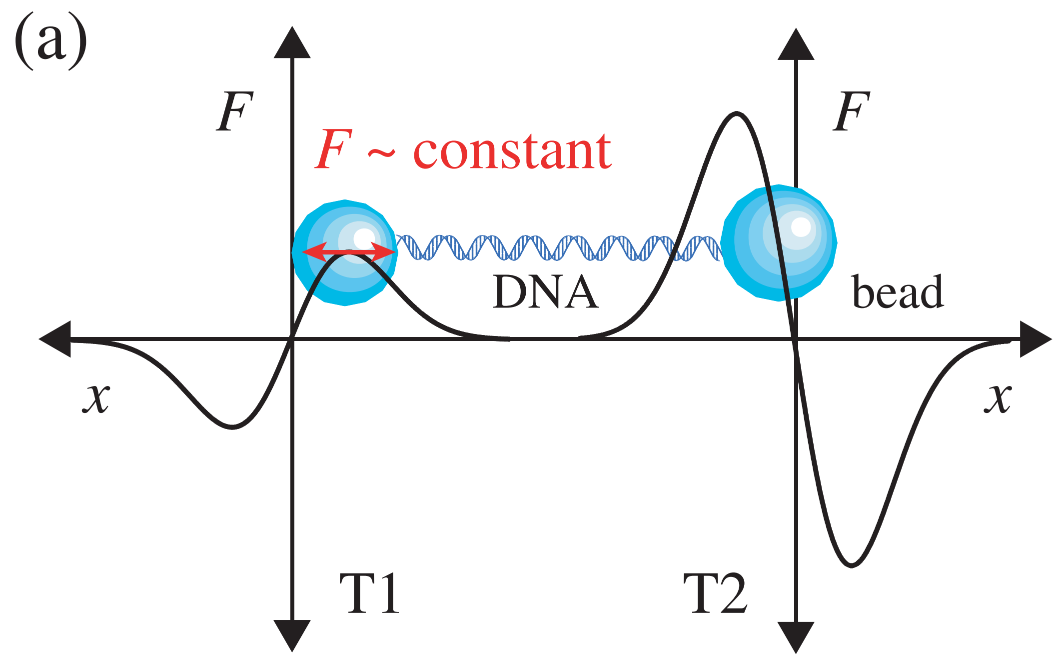
By taking into account the correlations in bead motion for a bead-DNA-bead dumbbell you can can increase the signal-to-noise, especially at large DNA stiffnesses (i.e. large streching forces).

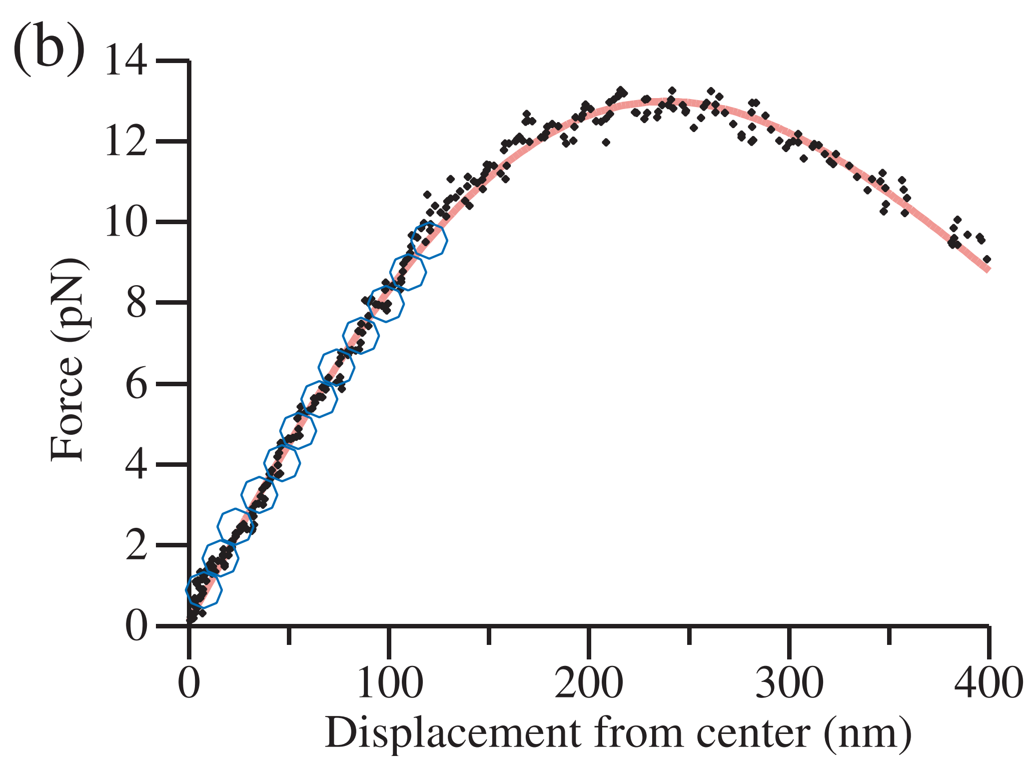
Moffitt et al. *PNAS* (2006).

Angstrom precision aided by helium

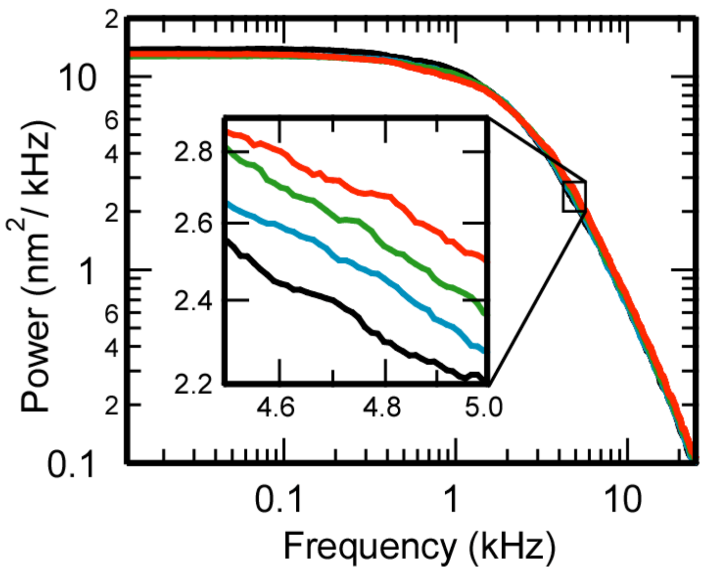


A passive force clamp increases bandwidth

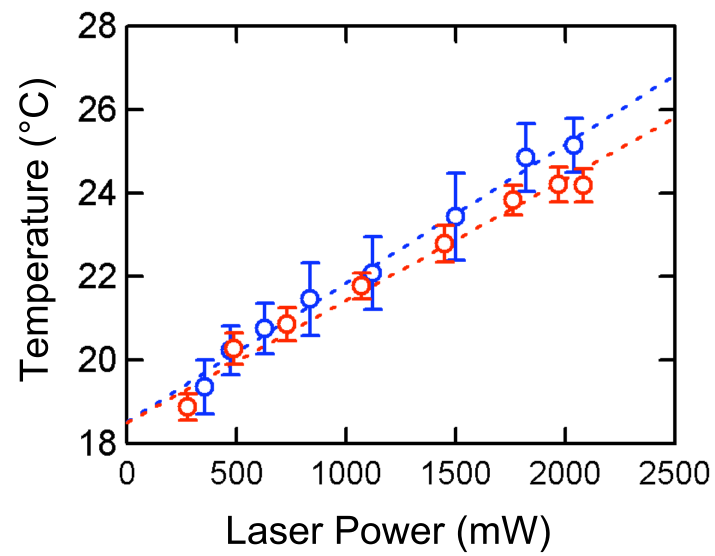


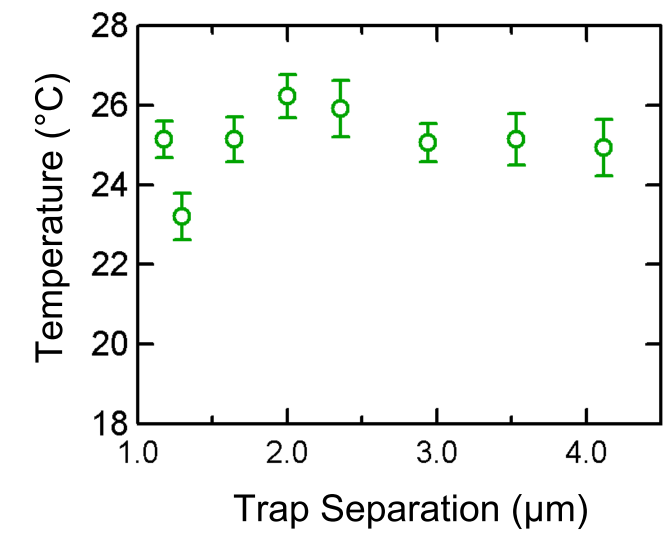


Using an optical trap as a heater

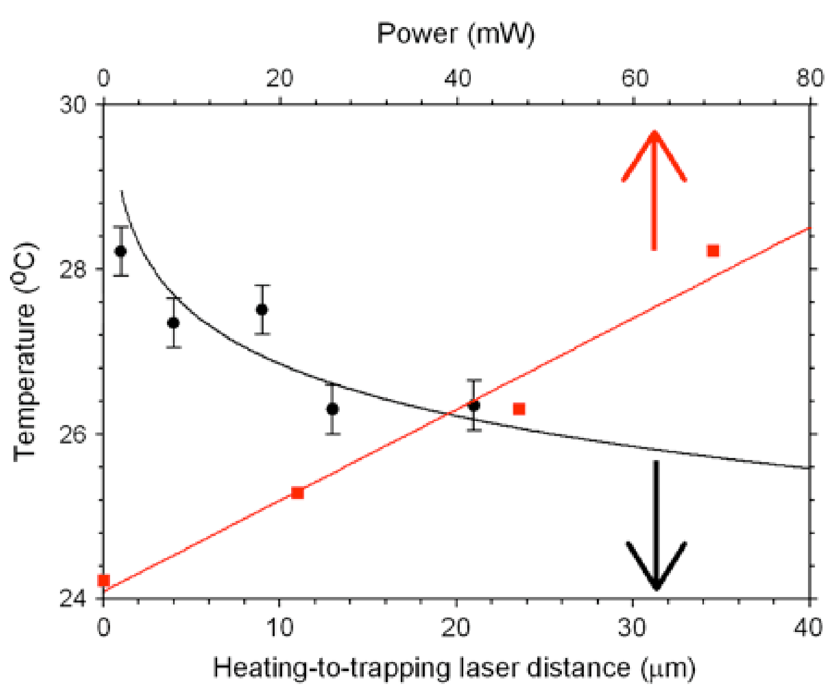


$$S_{yy}(f) = \frac{k_B T}{6\pi^3 \eta(T) r \left[\left(\frac{\alpha}{12\pi^2 \eta(T) r} \right)^2 + f^2 \right]}$$

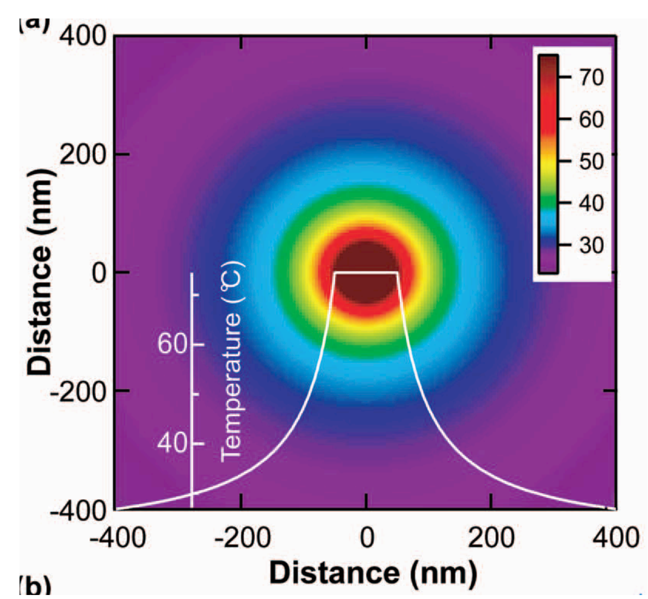




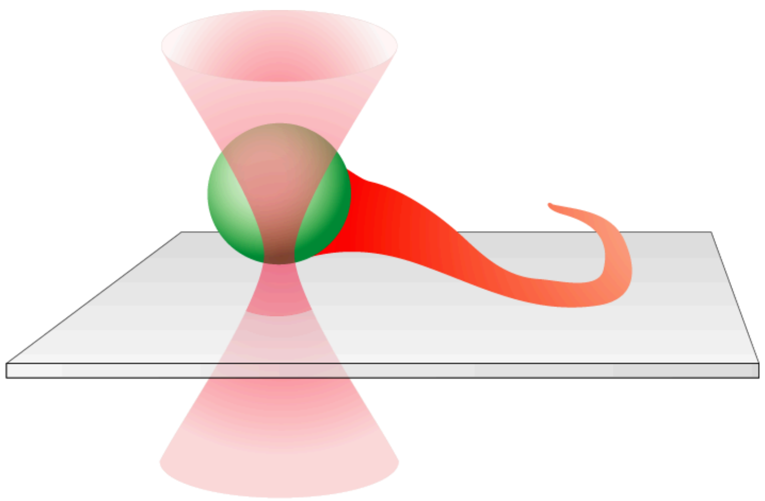
Decay distance ~10-20 microns
Mao et al. *Biophys J* (2005)

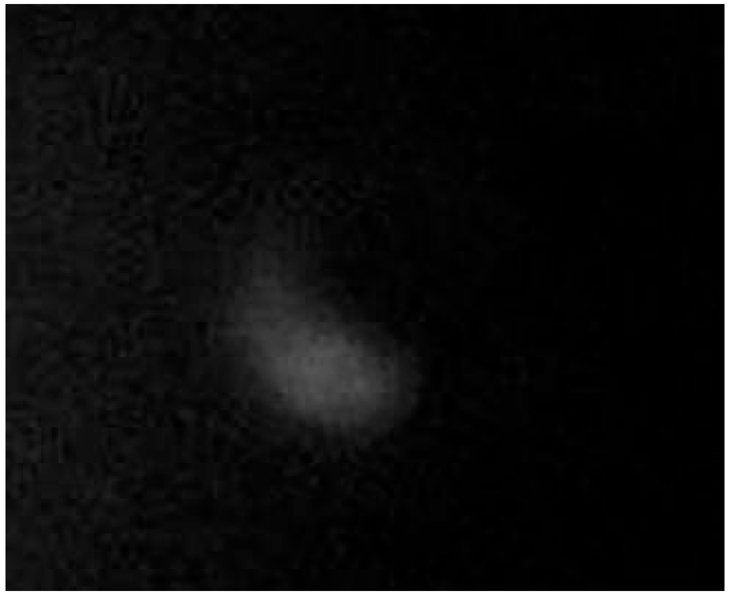


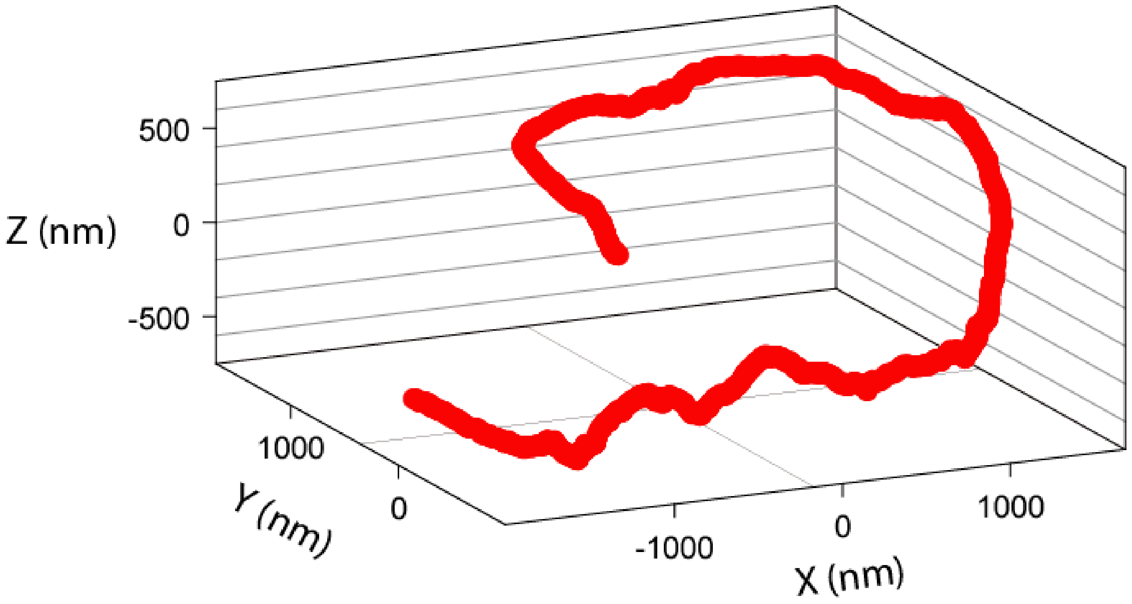
Gold particles heat up alot more:
~250 degC / Watt
Seol et al. *Optics Letters* (2006)

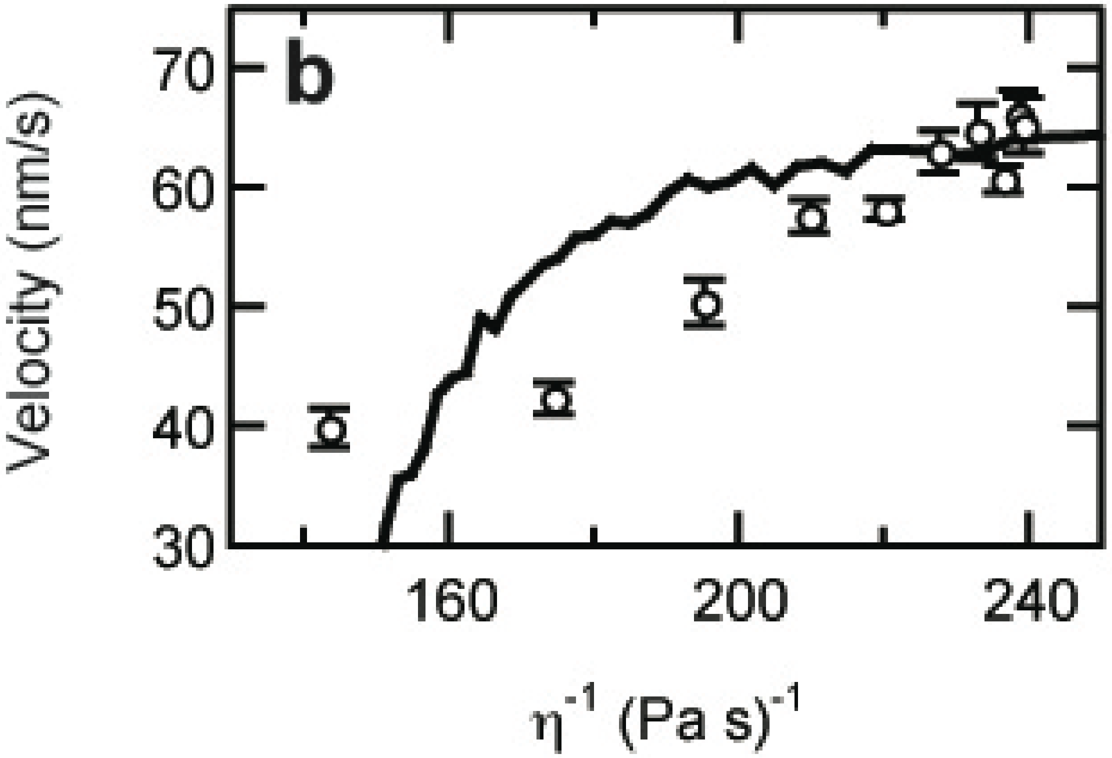


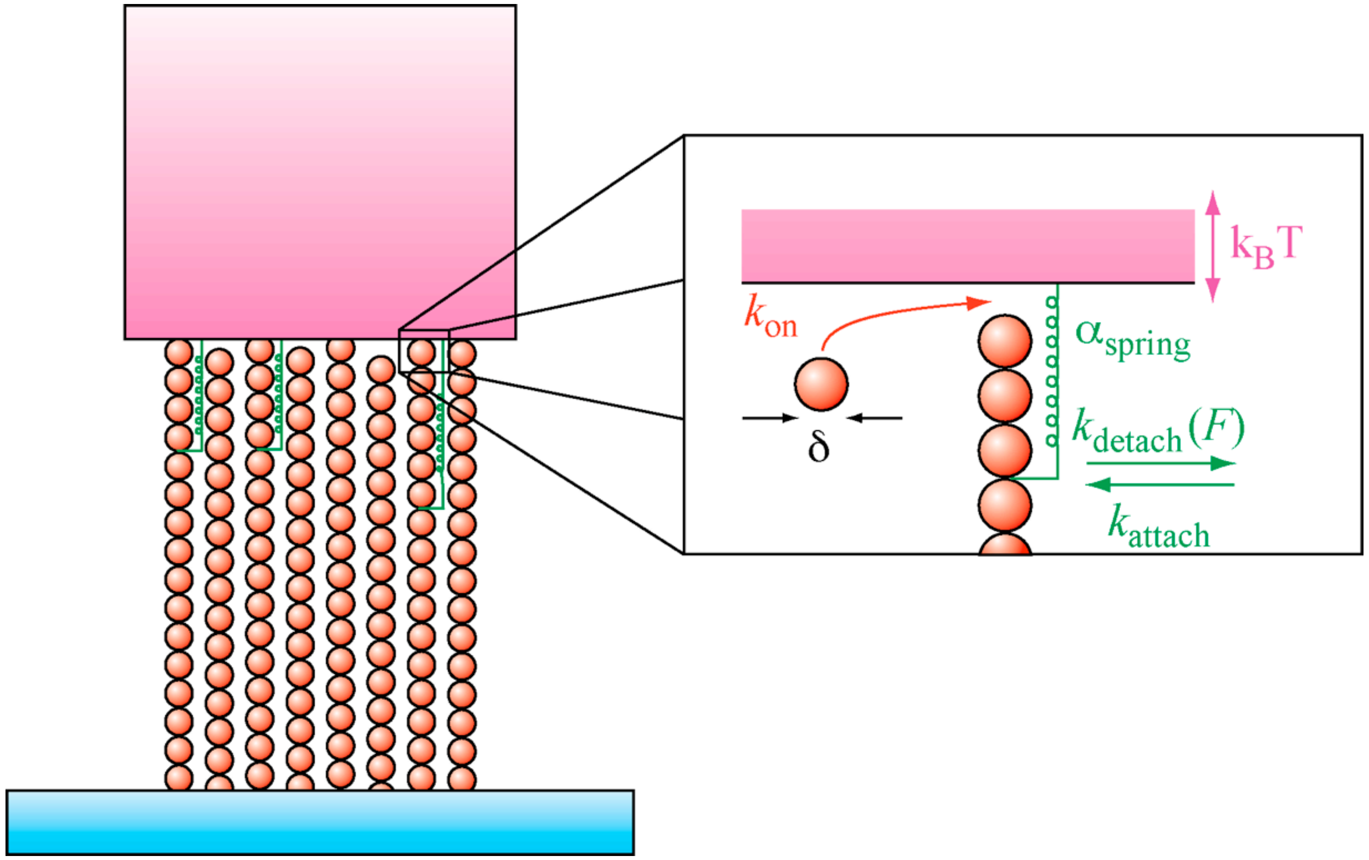
3D tracking sheds light on a brownian ratchet



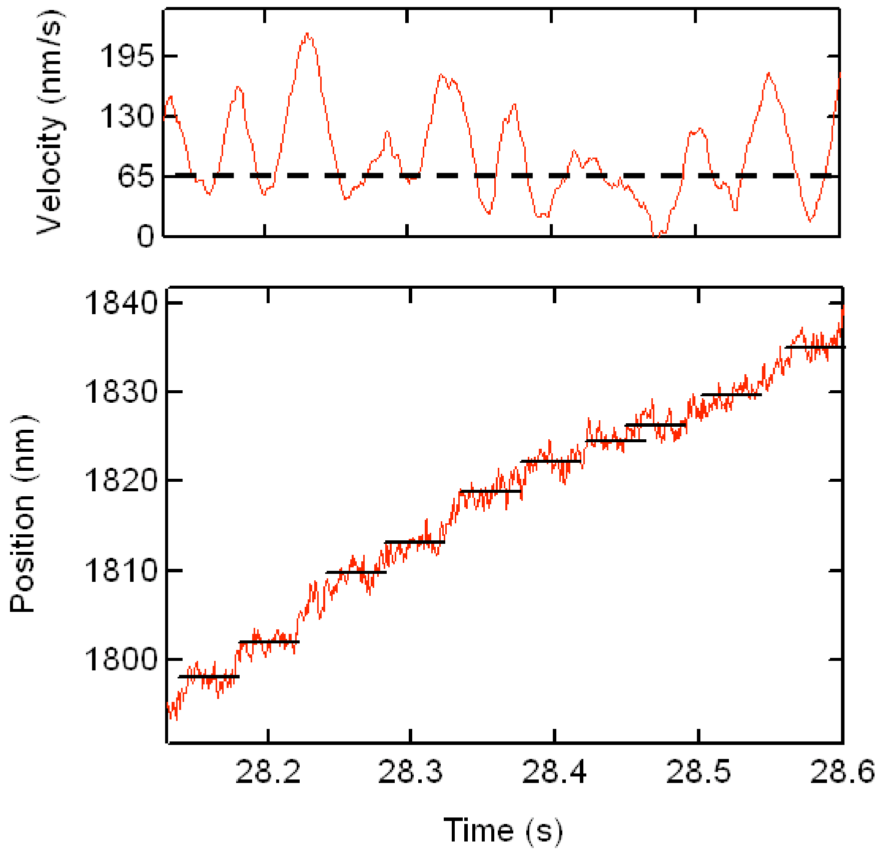


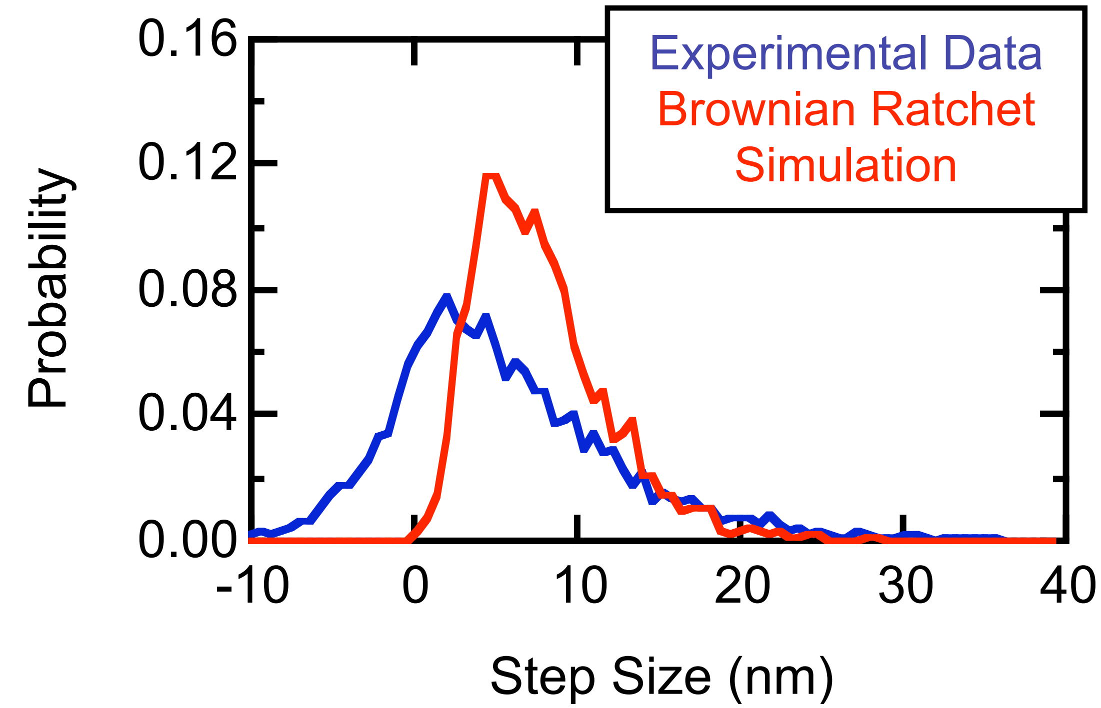


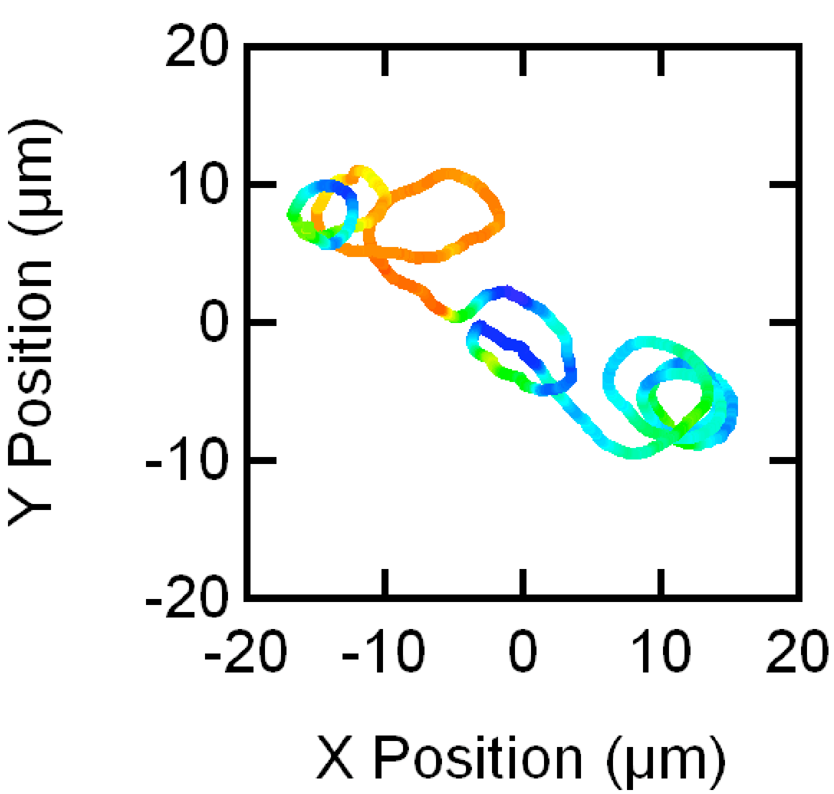


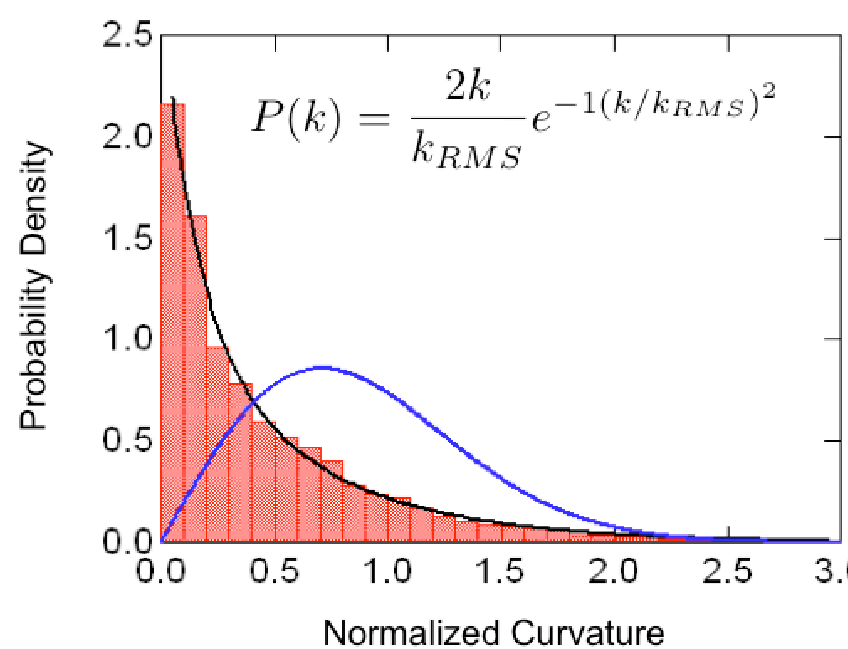


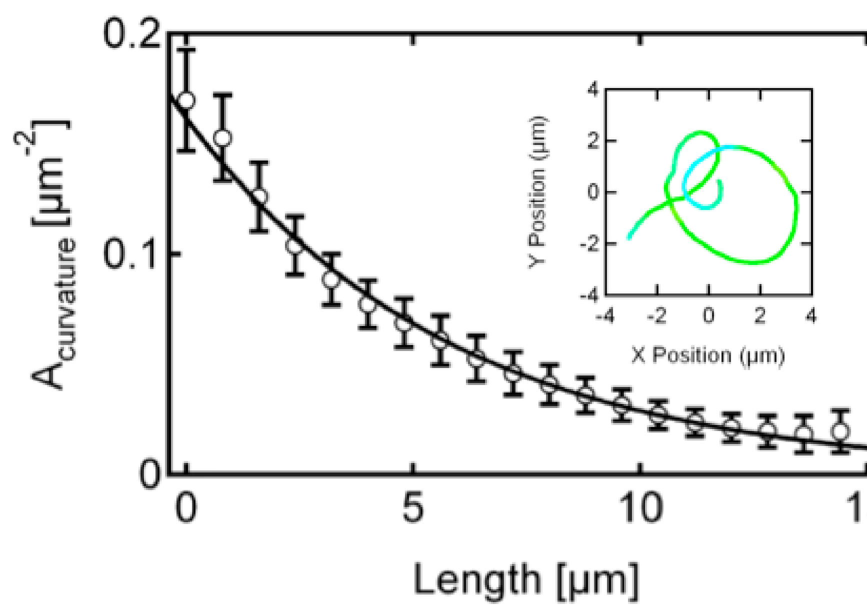
Trajectory shapes yield details of ratchet motion



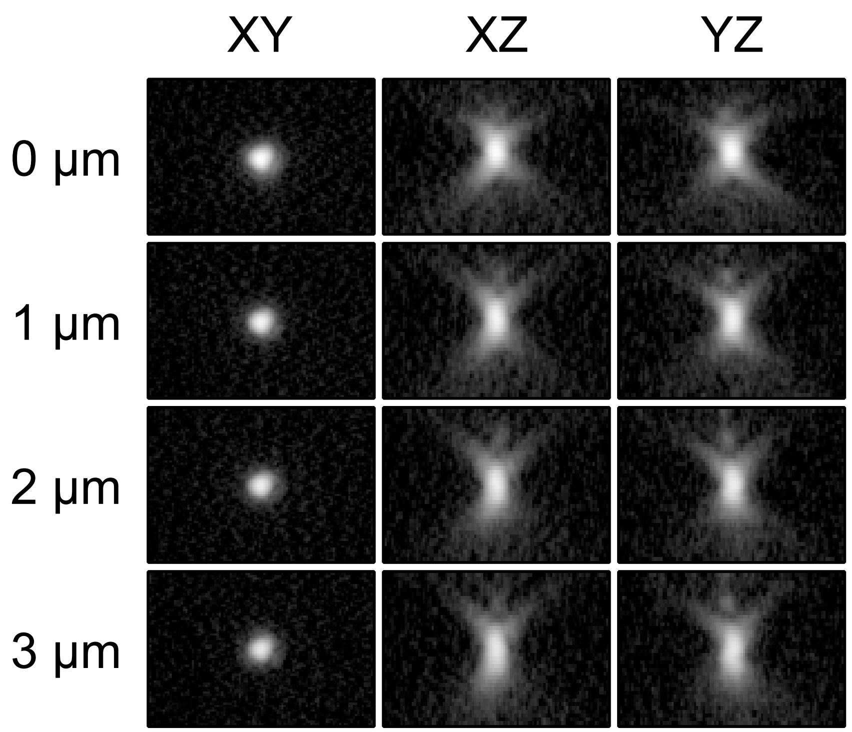


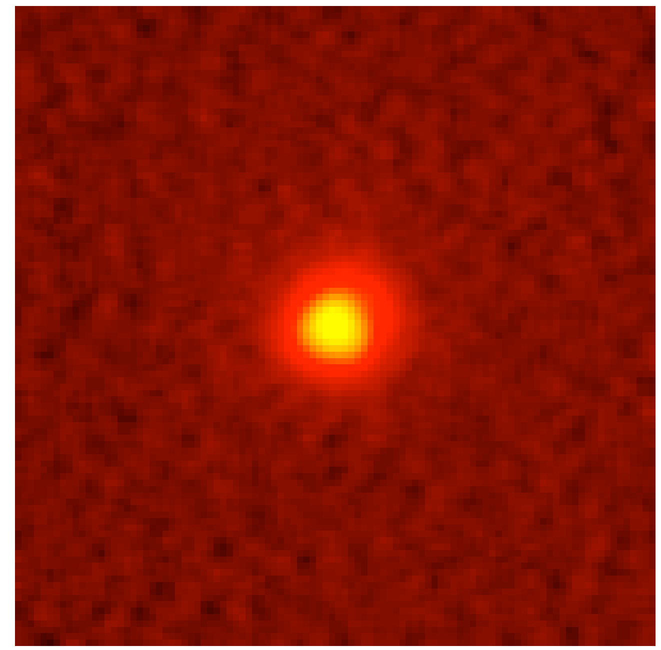


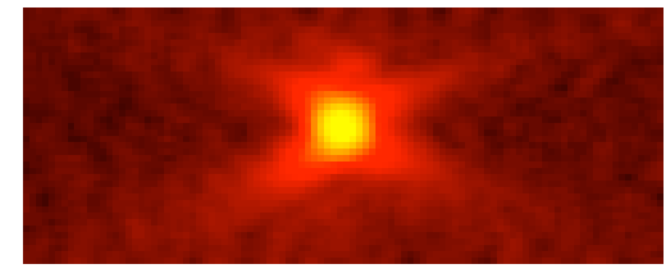


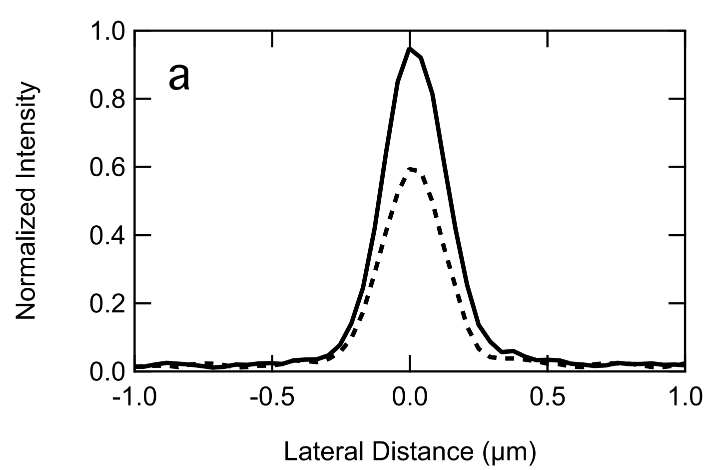


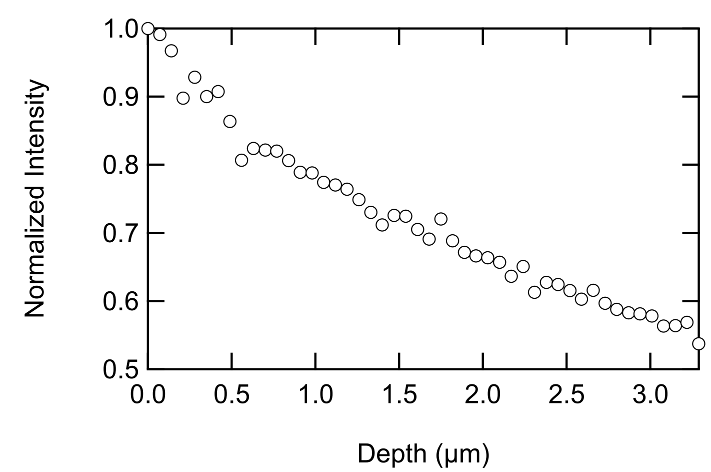
Measuring 4D PSF

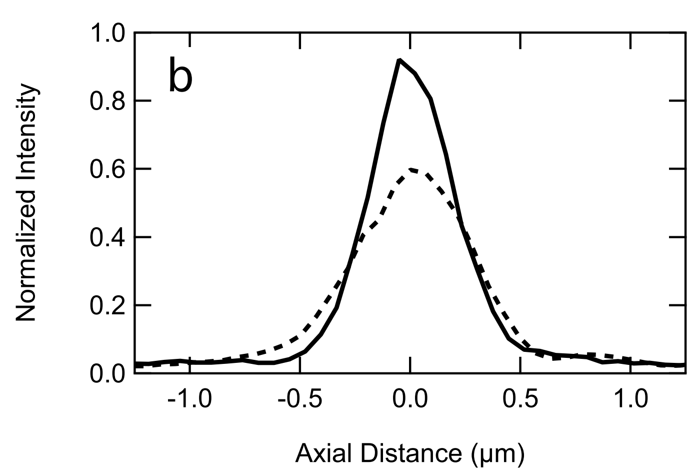


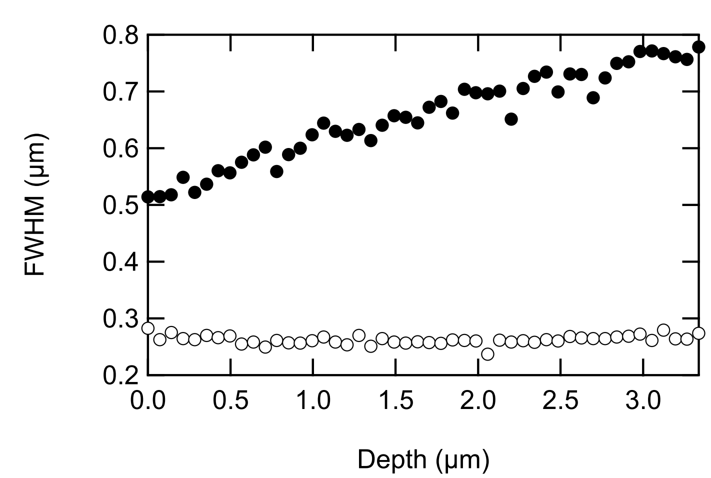




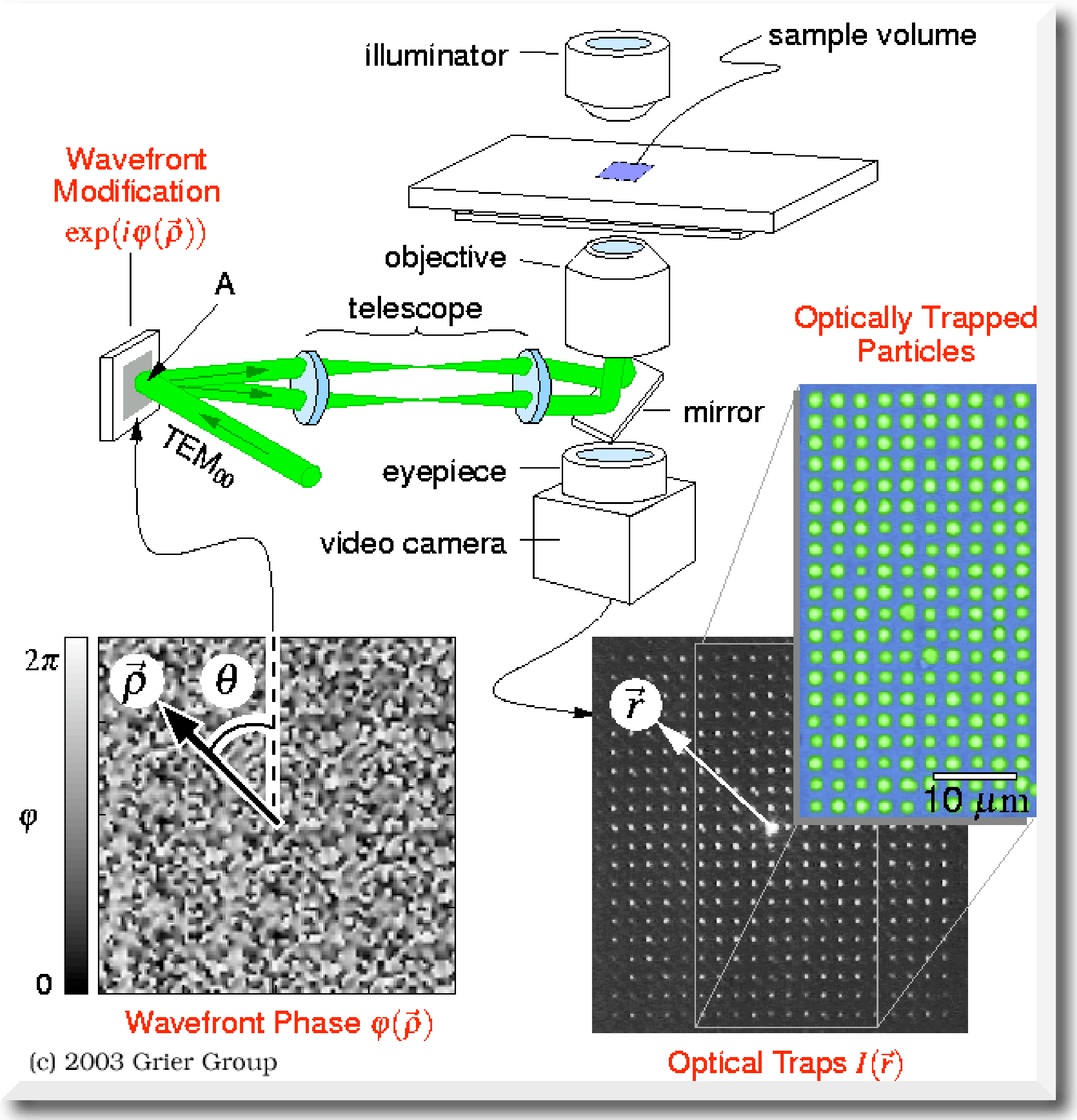


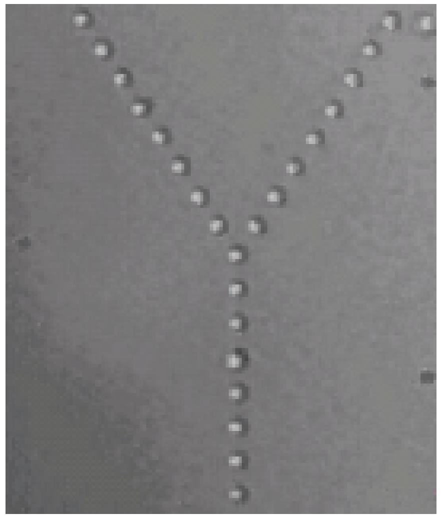


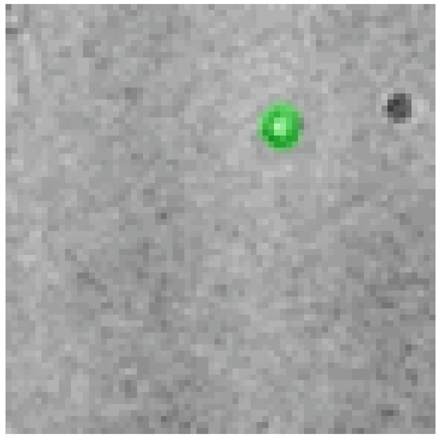


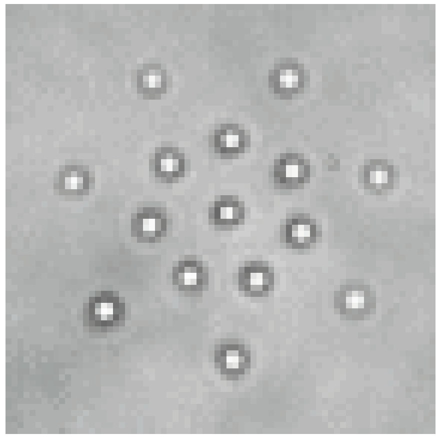


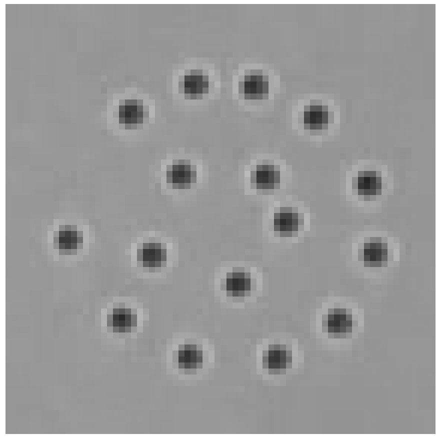
Holographic optical traps

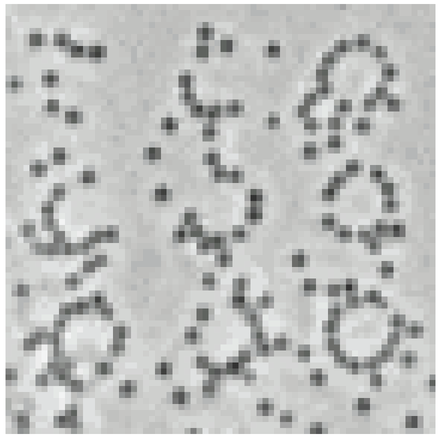




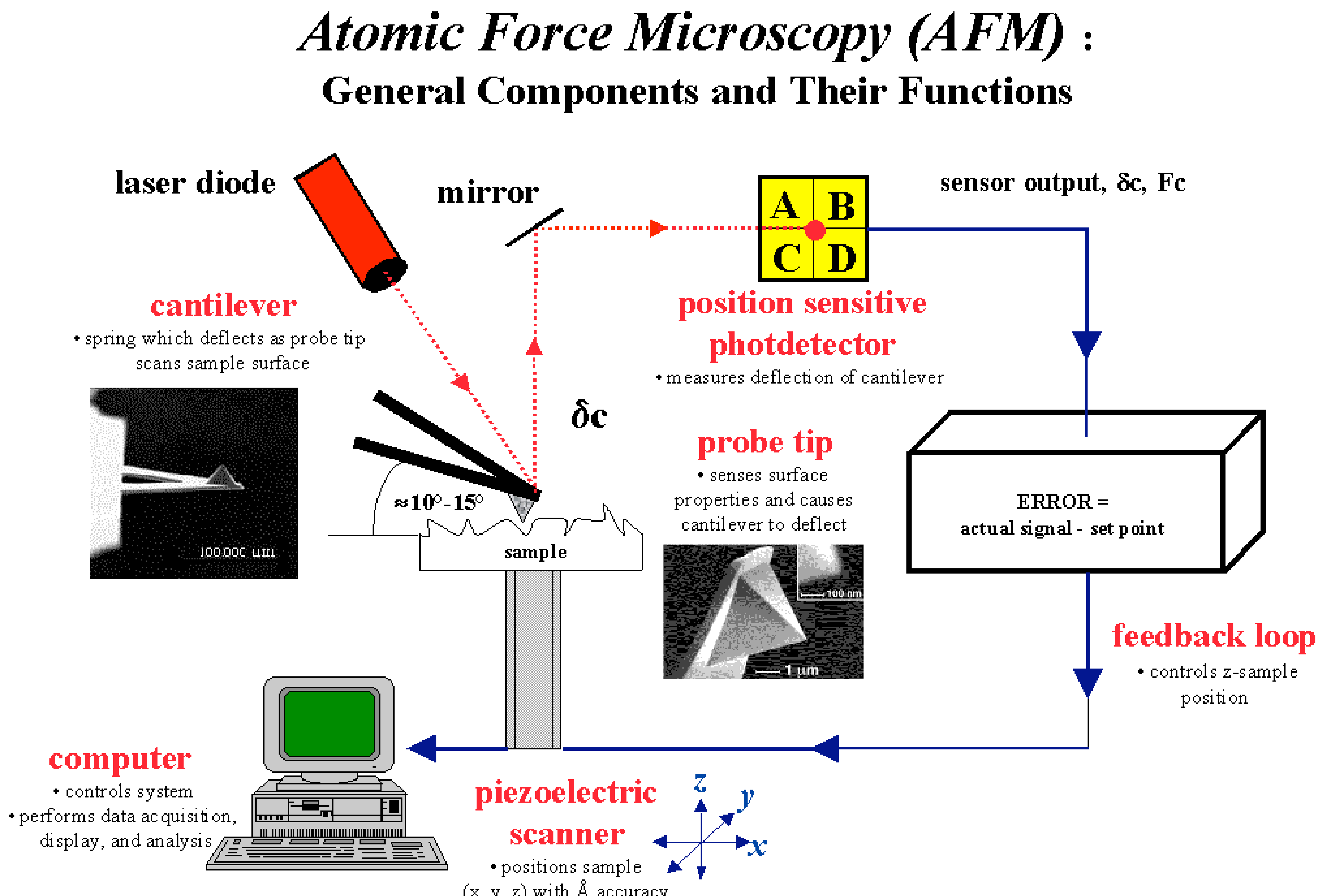






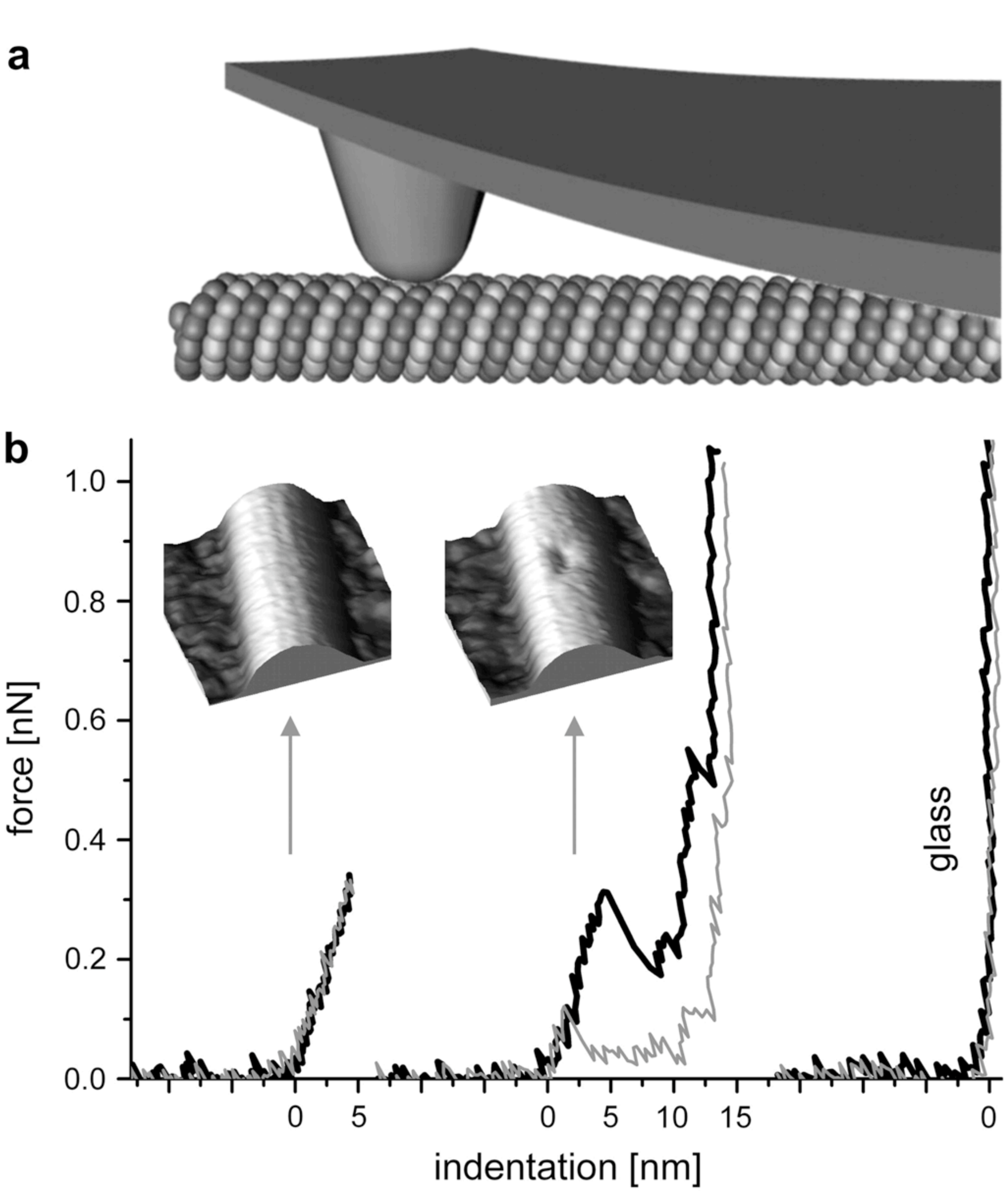


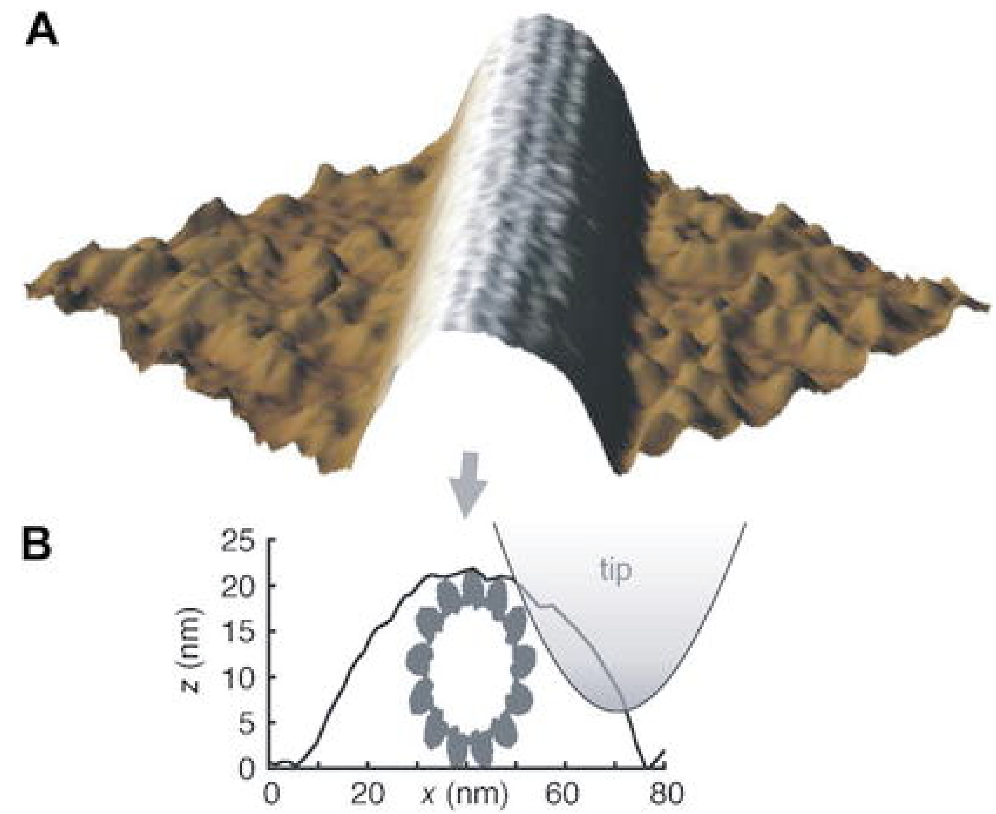
AFM as a tool for proteins and cells

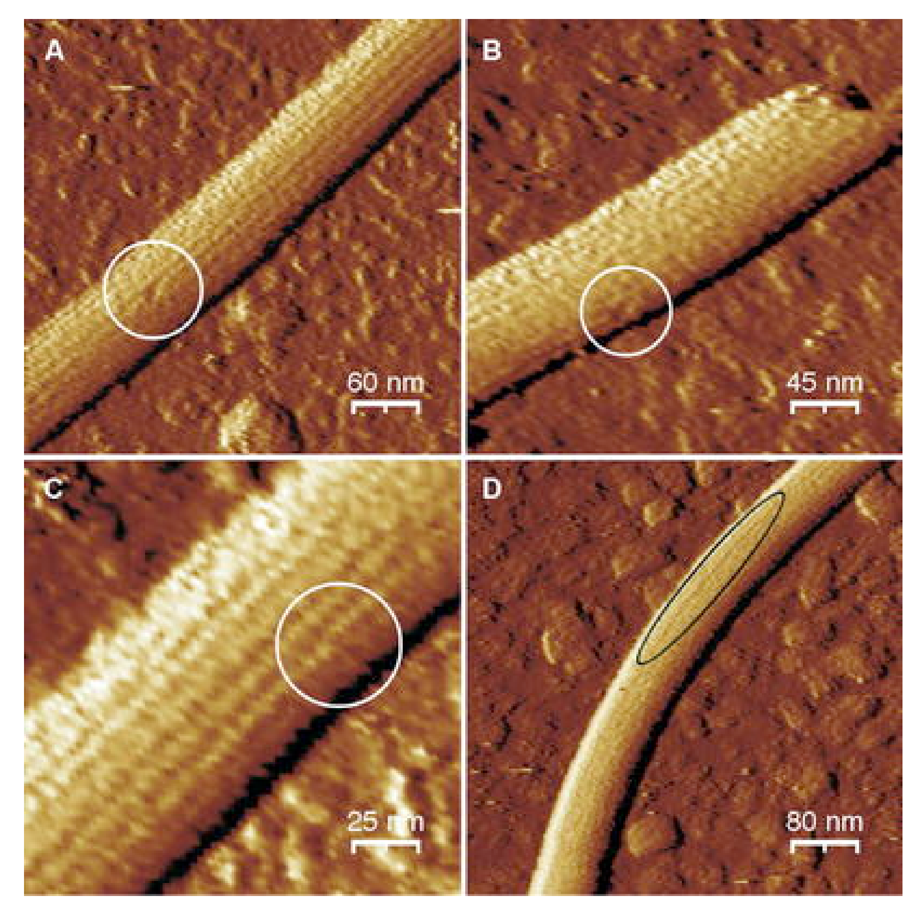


Ortiz Lab @ MIT

Imaging and perturbing microtubules

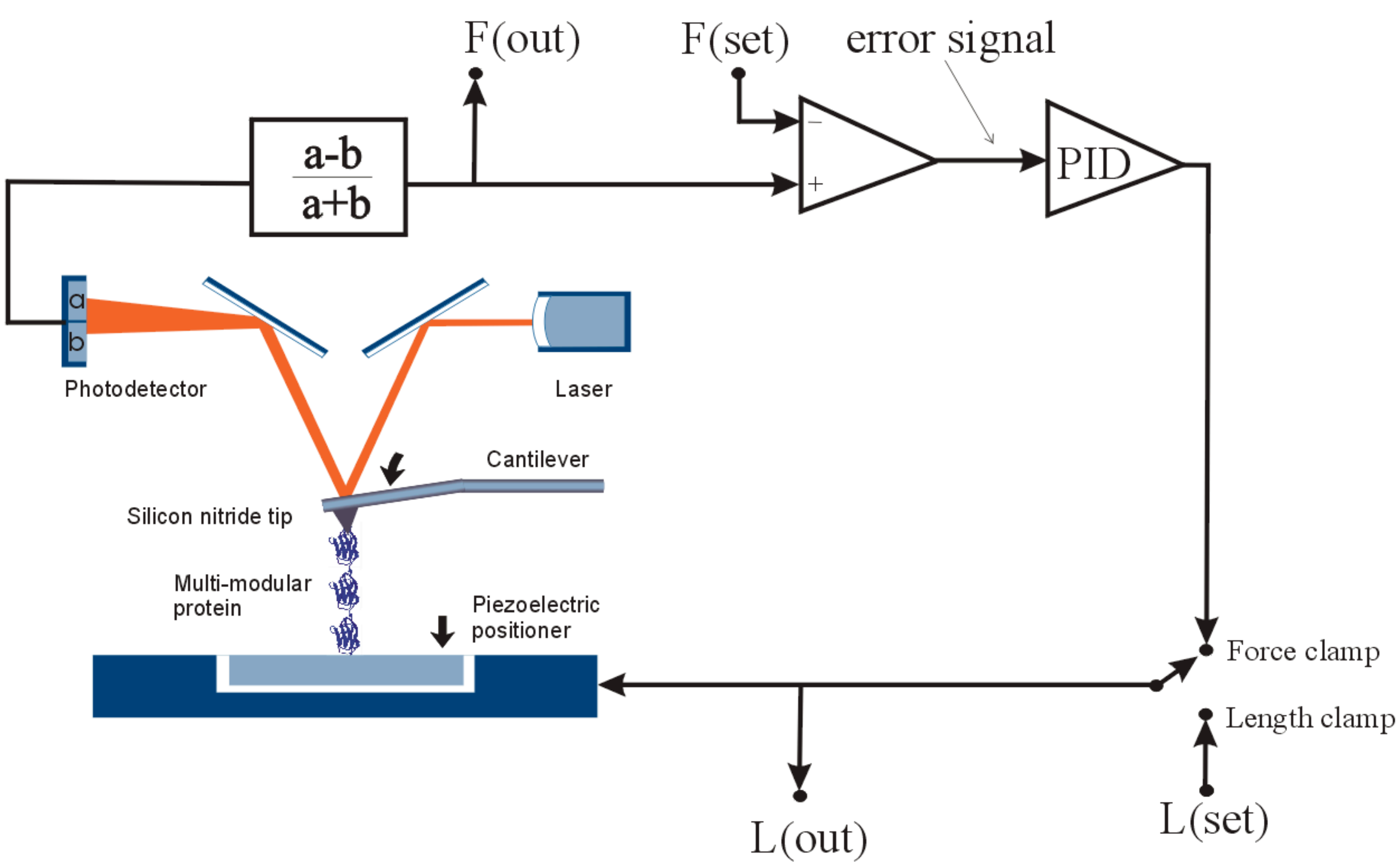


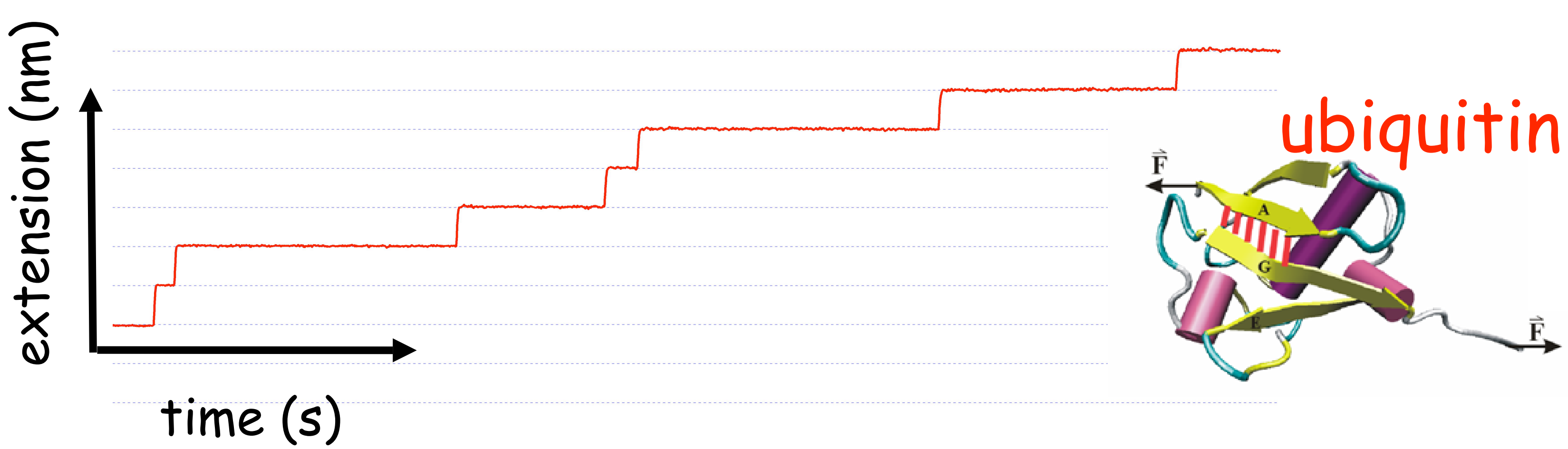




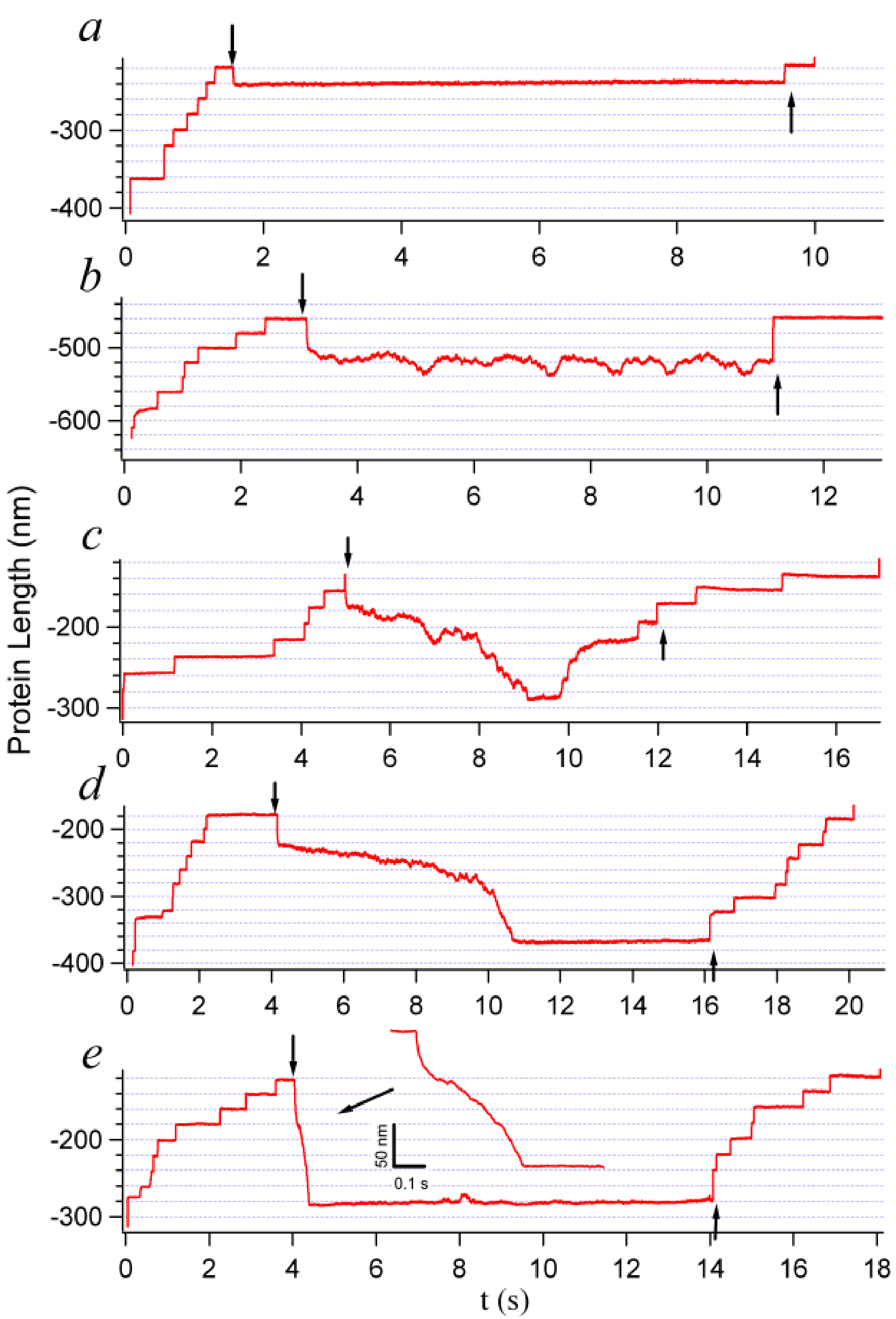
de Pablo et al. *PRL* (2003)
de Pablo et al. *Nanotech* (2003)
Schaap et al. *Eur. Biophys* (2004)
Schaap et al. *Biophys J* (2006)

AFM used for stretching proteins





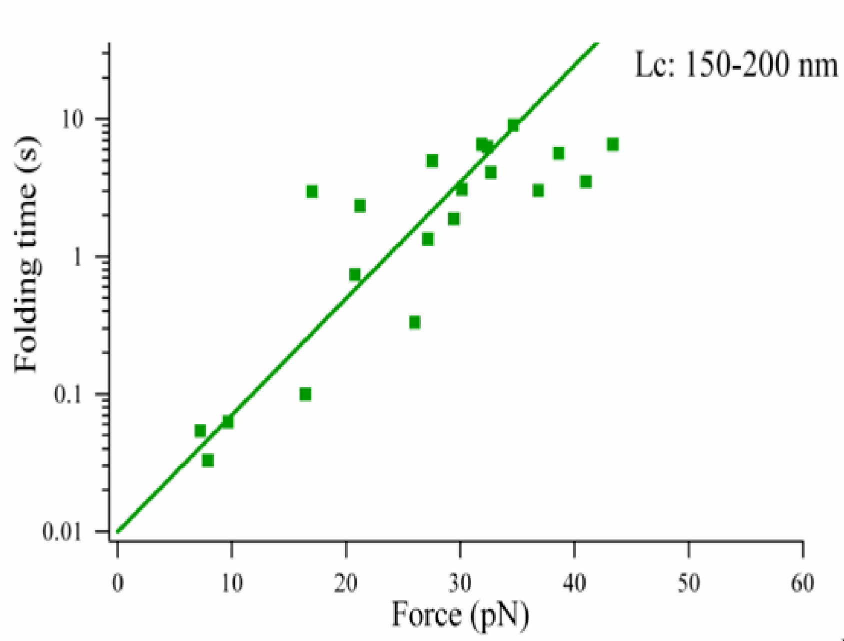
Can study unfolding and folding kinetics



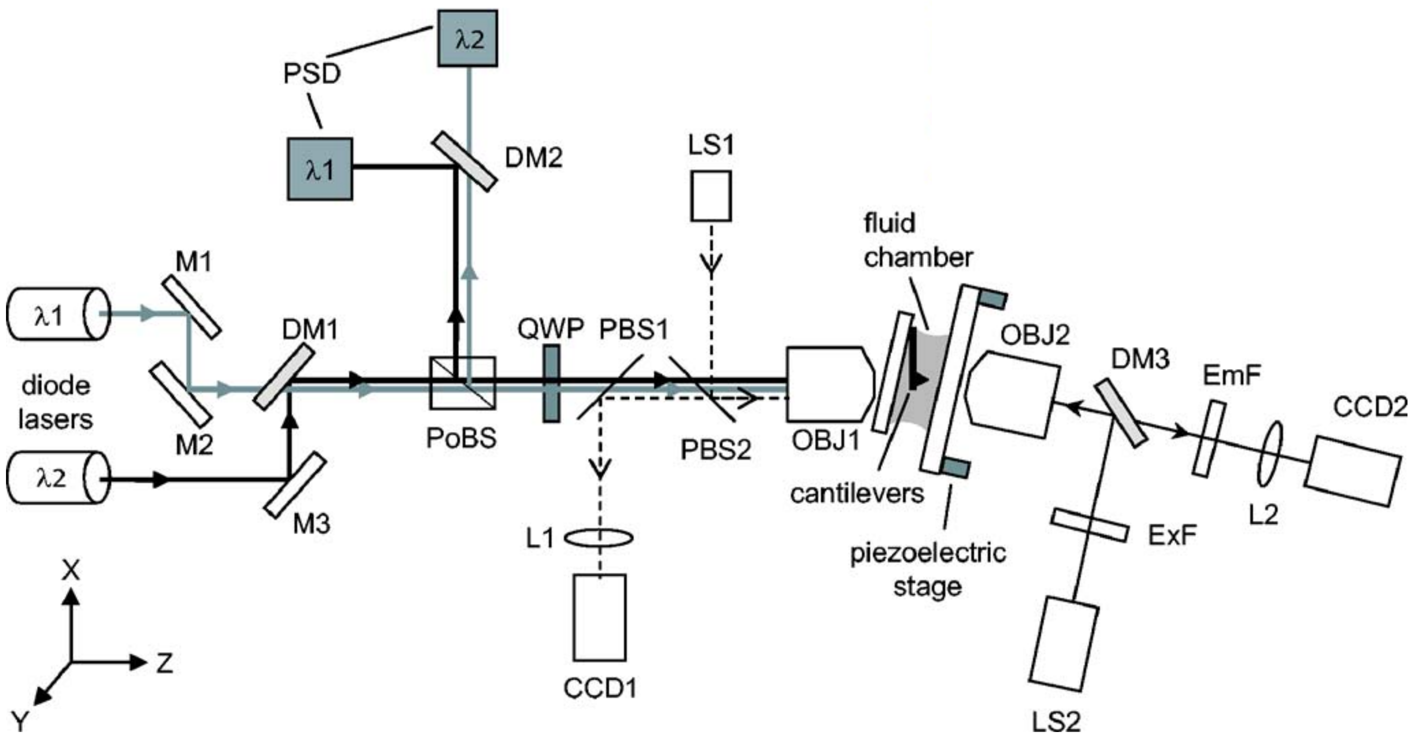
Folding is slowed by force

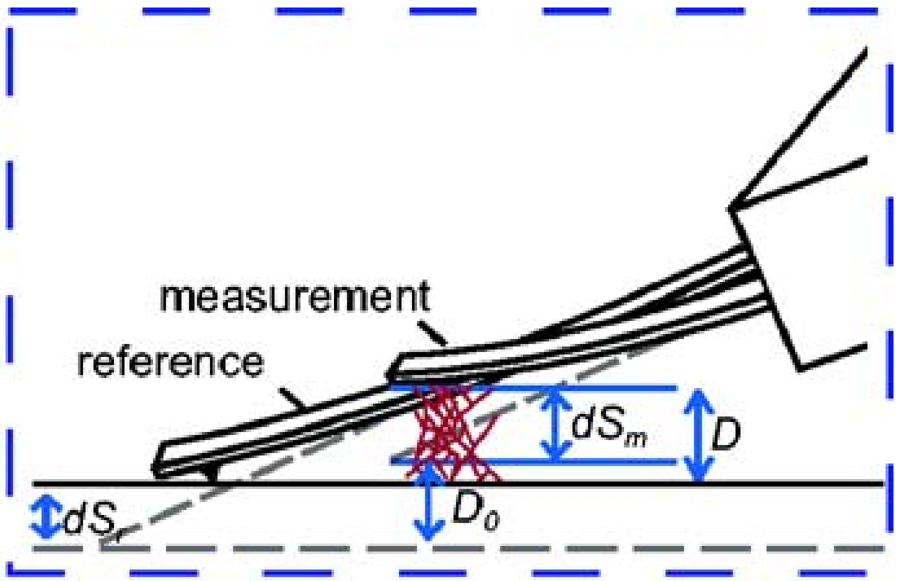
$k_0 = 100\ s^{-1}$

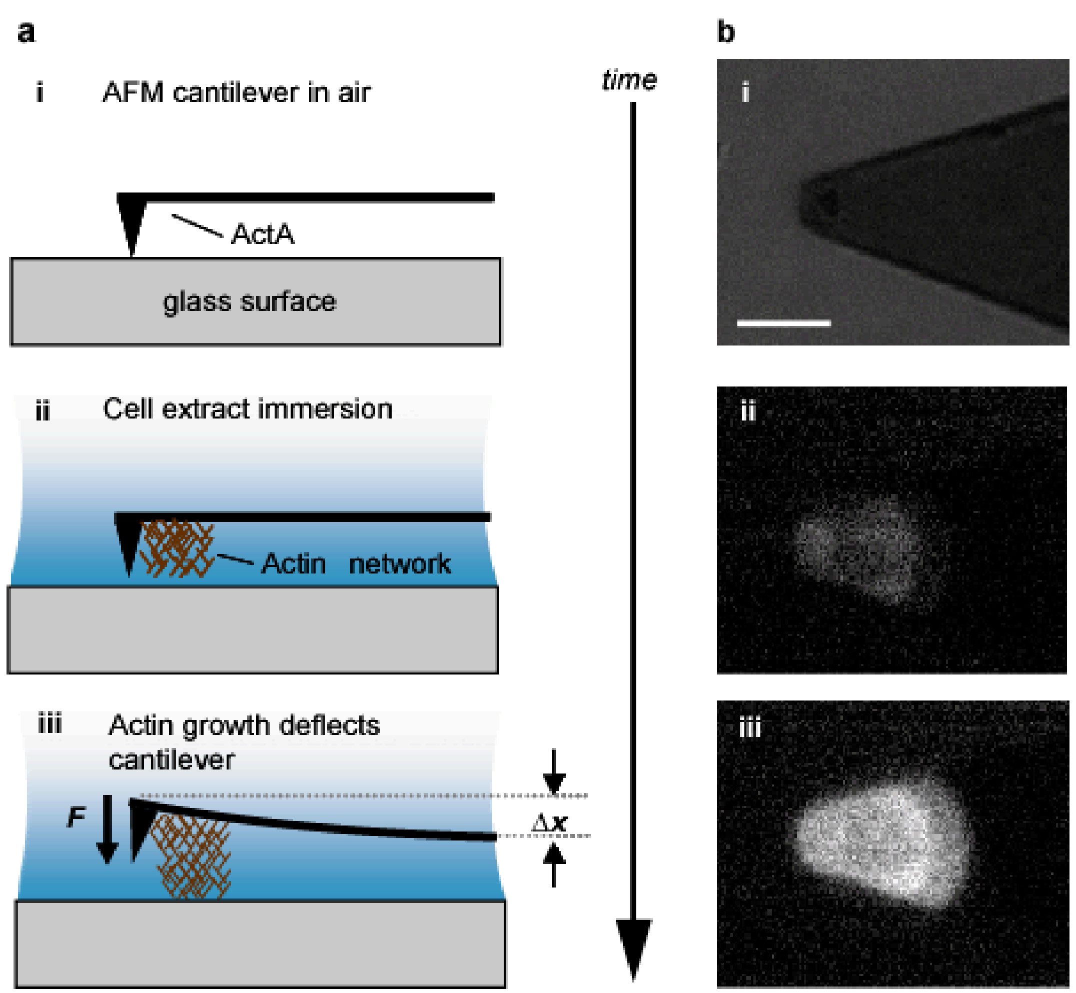
$\Delta x_f =$ **8.2 Å**

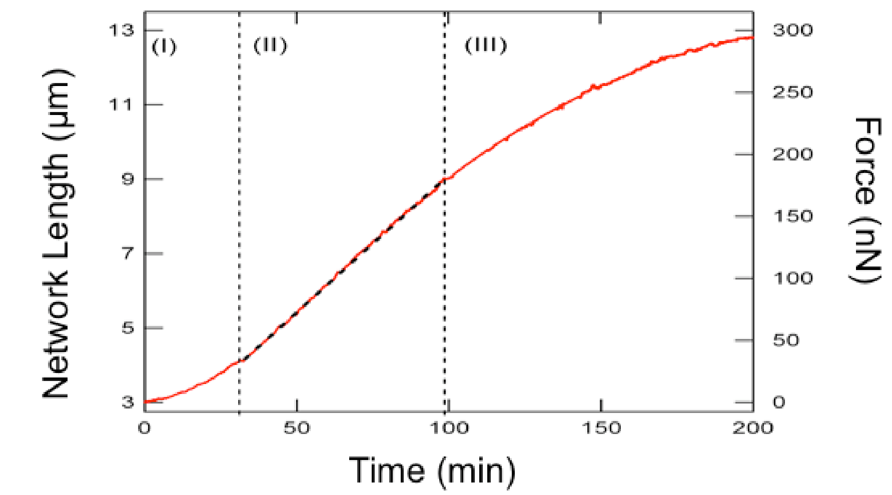


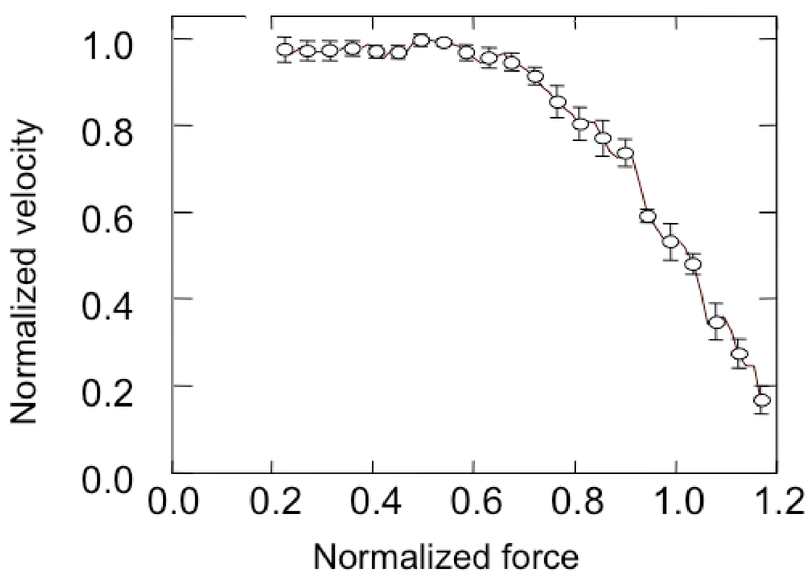
Measuring the FV curve of a growing actin network



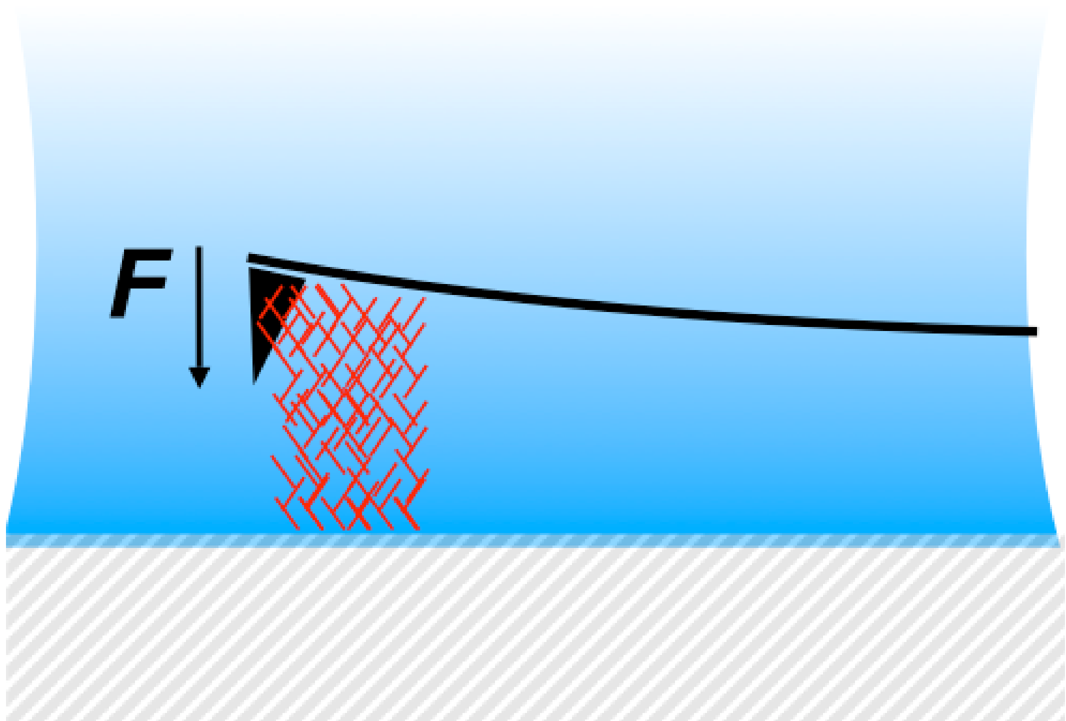


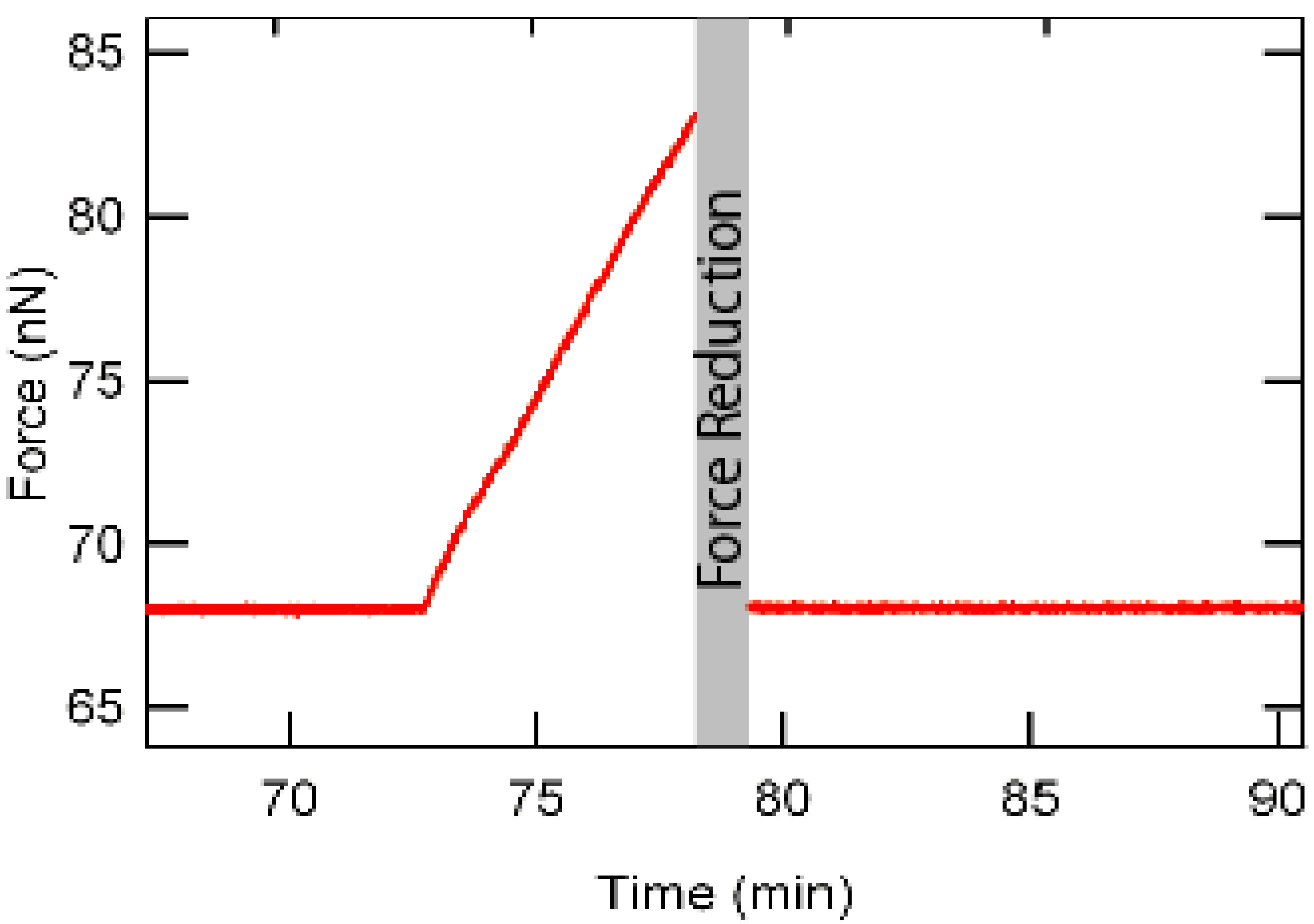






Loading history determines growth velocity





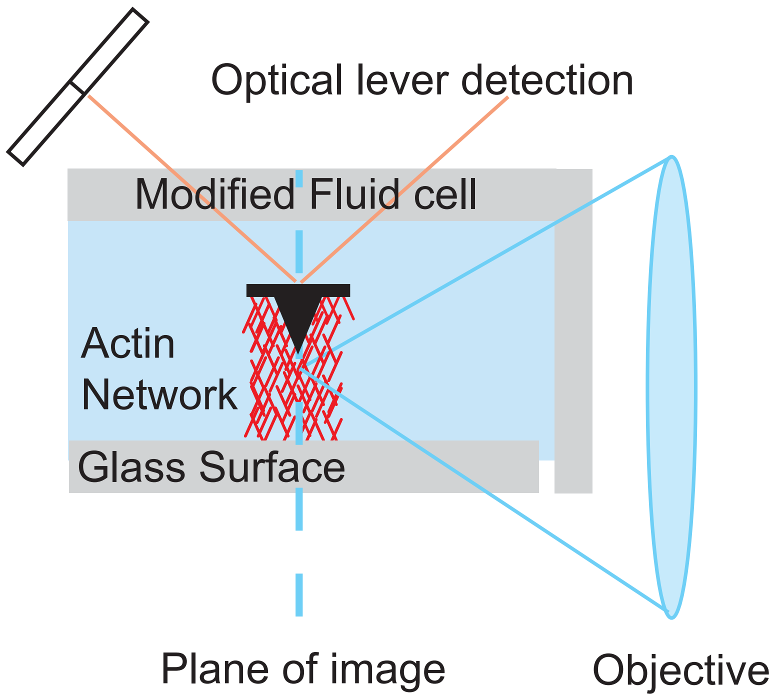
1. Grow actin network under a constant force.
2. Allow network to increase the applied force as it grows.
3. Return network to the original constant force.
4. Quantify network growth rates.

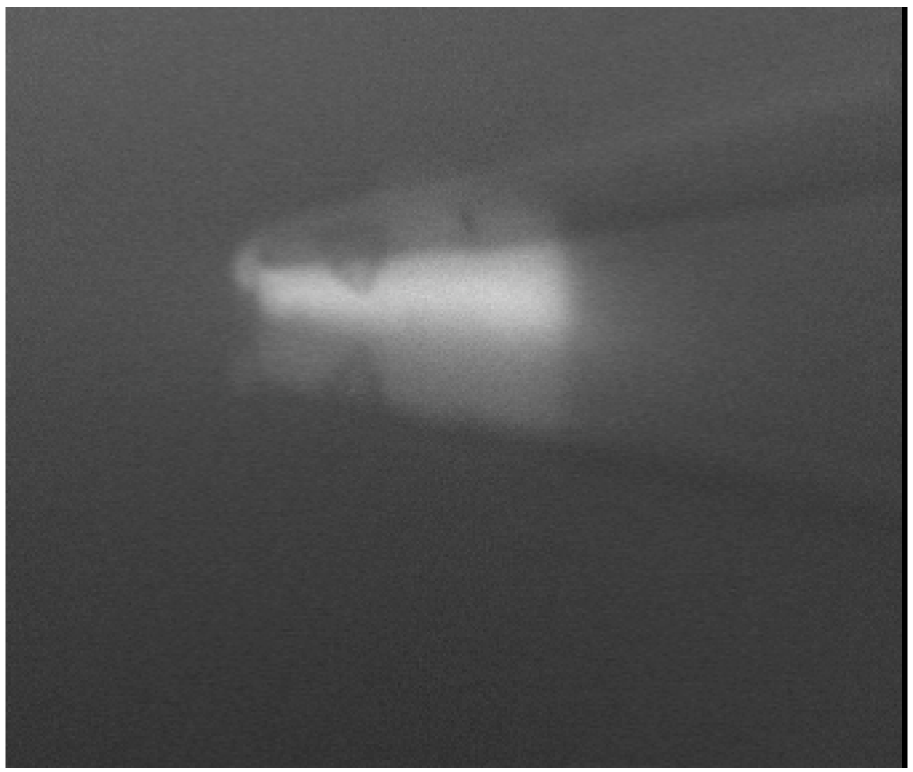
Growth velocity before and after loading is different

Remodeling of network in response to load

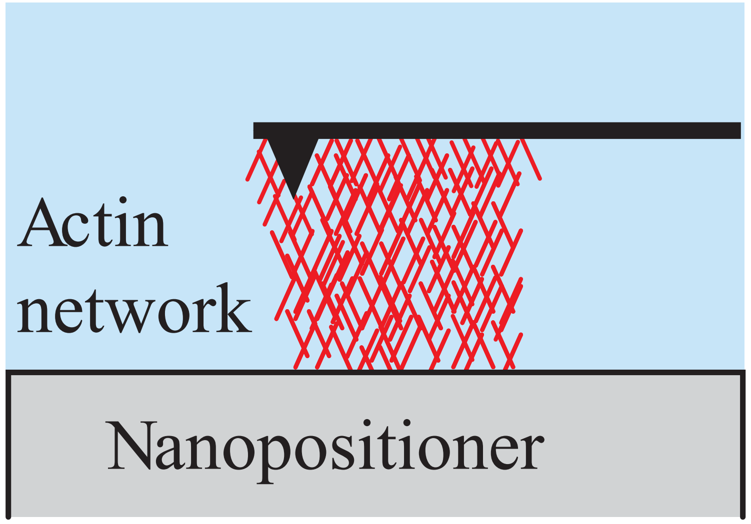
Parekh et al, *Nature Cell Bio*, 2005

Side view allows you to view actin density



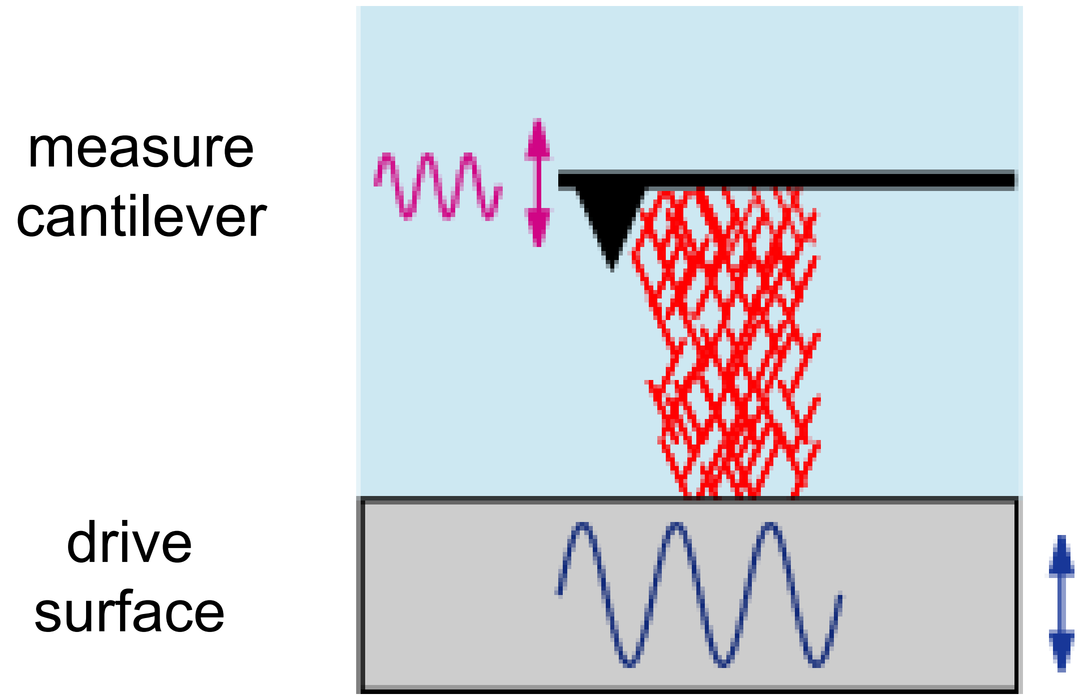


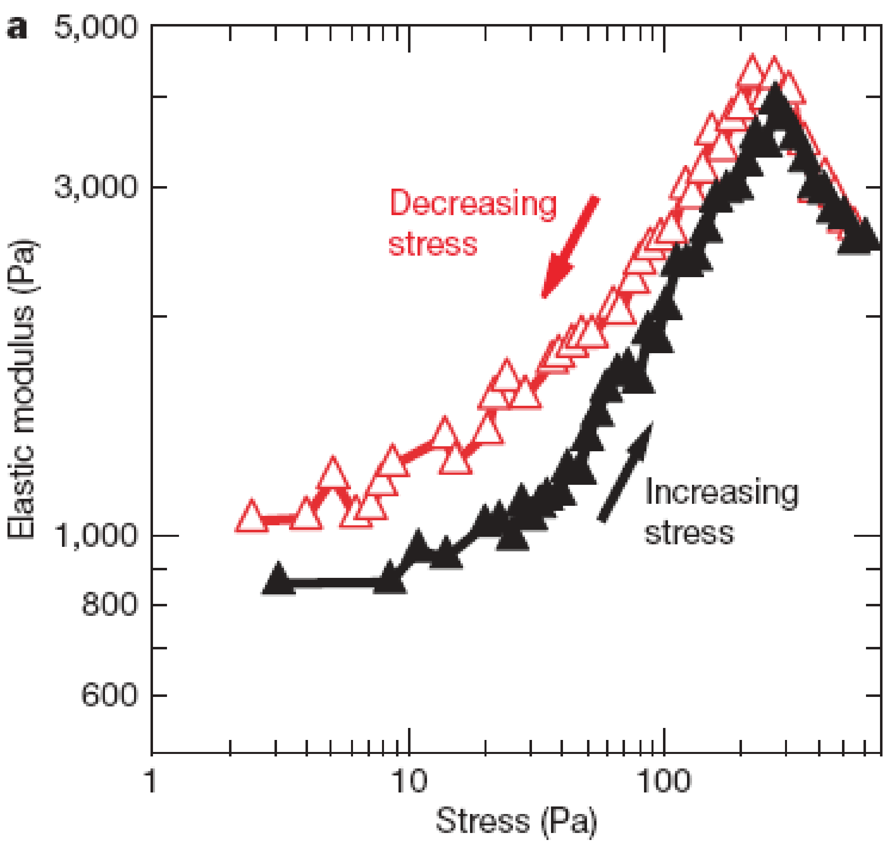
Desired sideview image:

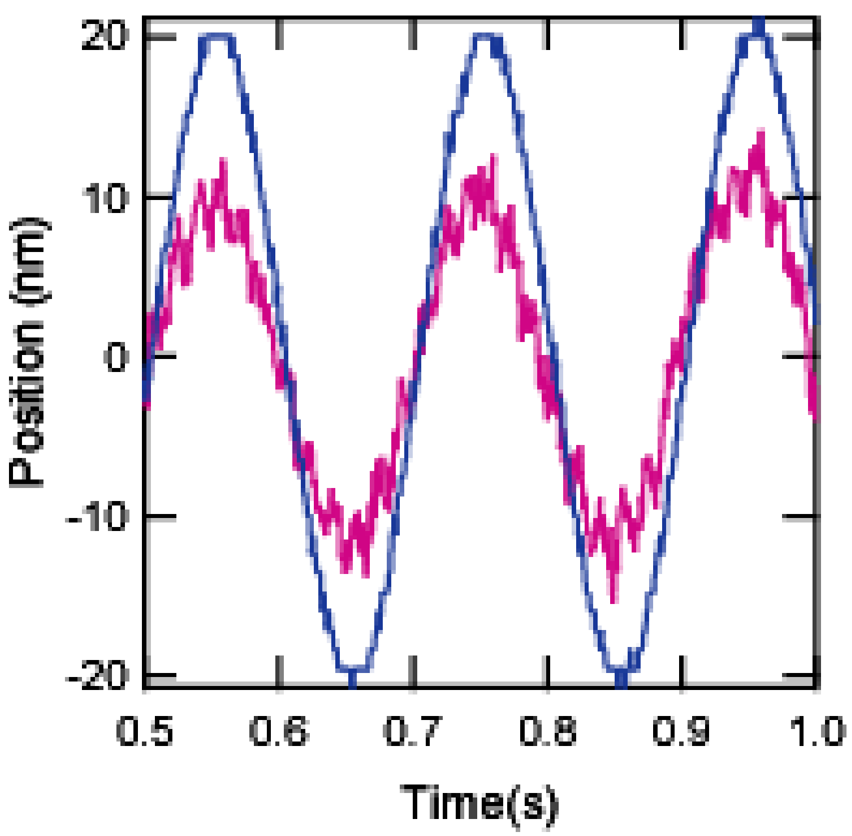


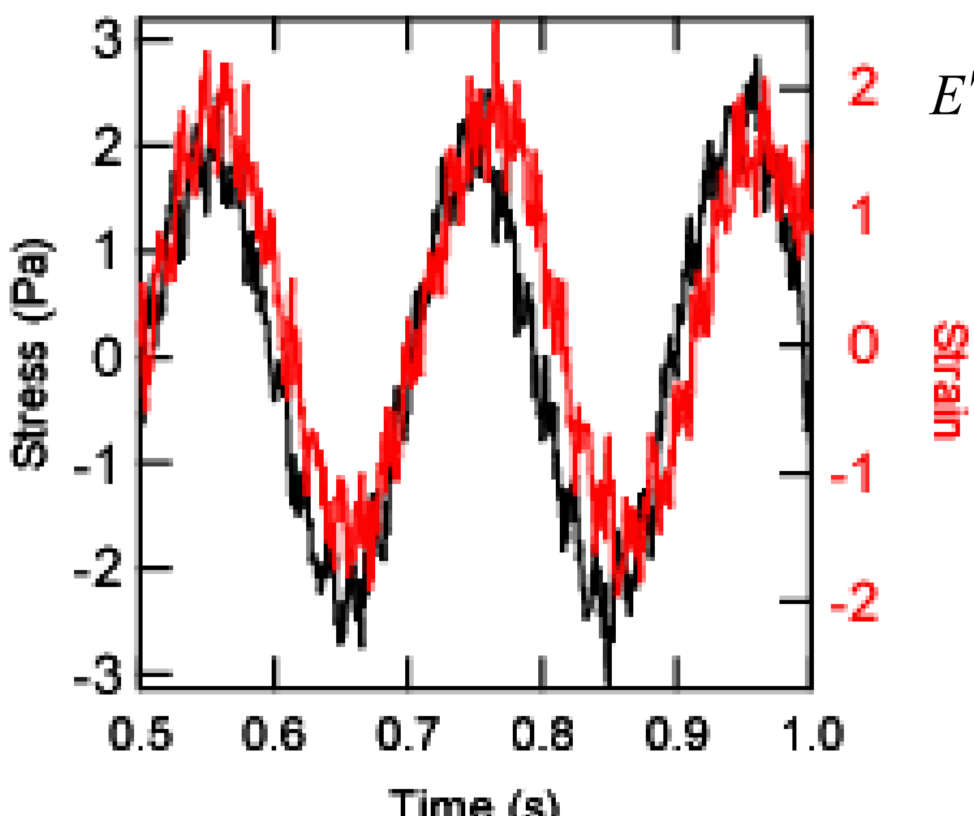
Does the network get denser with increasing force?

AFM as a local rheology probe



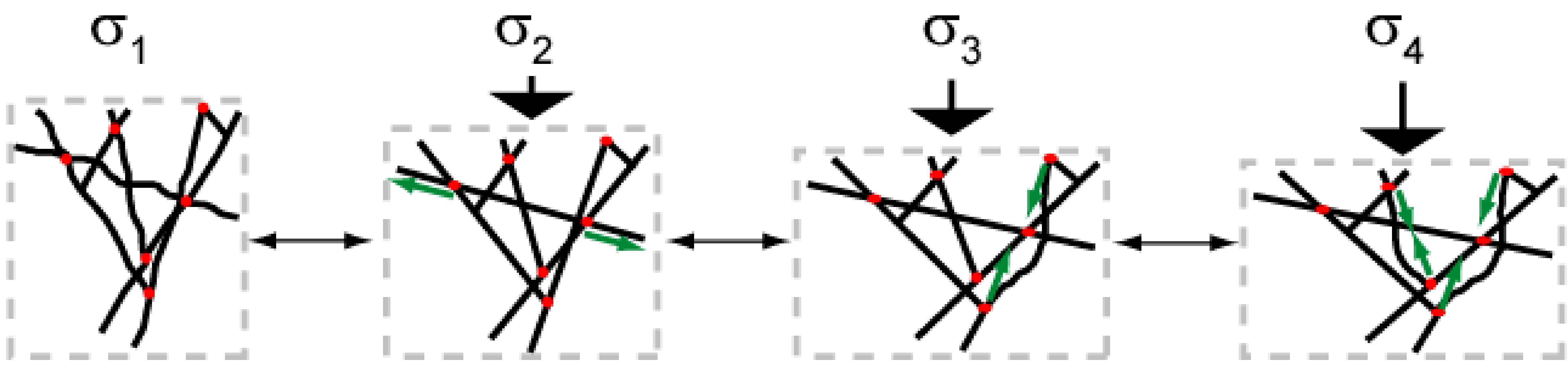


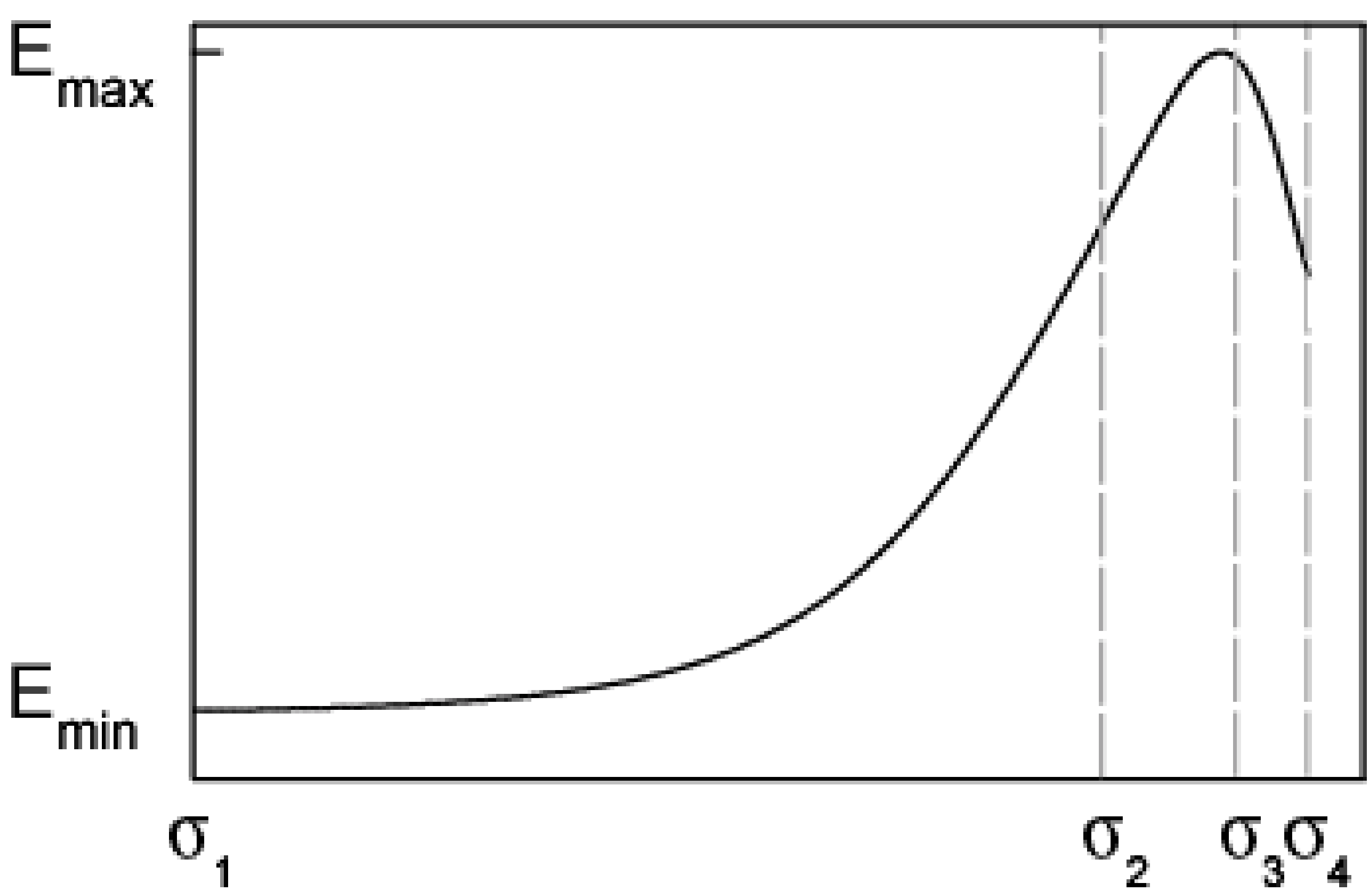




$$E'(f) + iE''(f) = \frac{\tilde{\sigma}(f)}{\tilde{\varepsilon}(f)}$$

A model of actin network elasticity

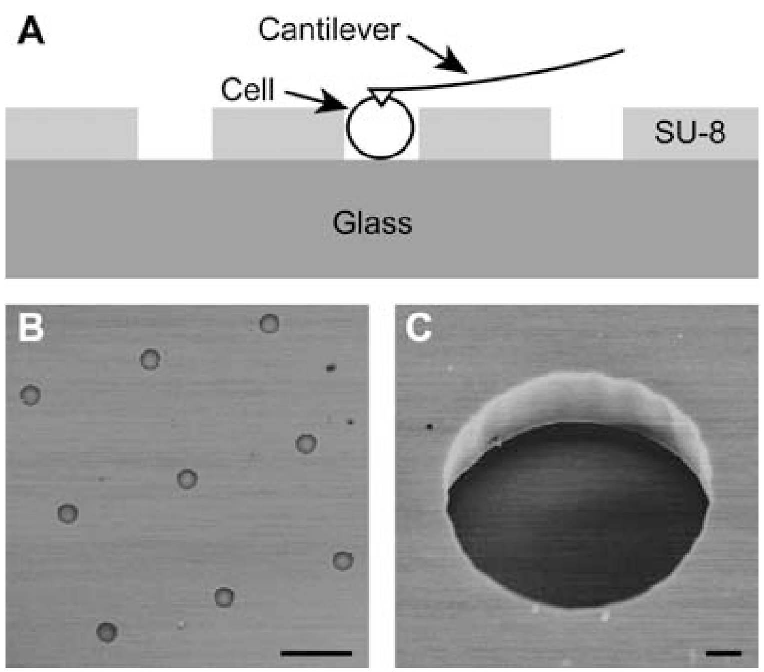


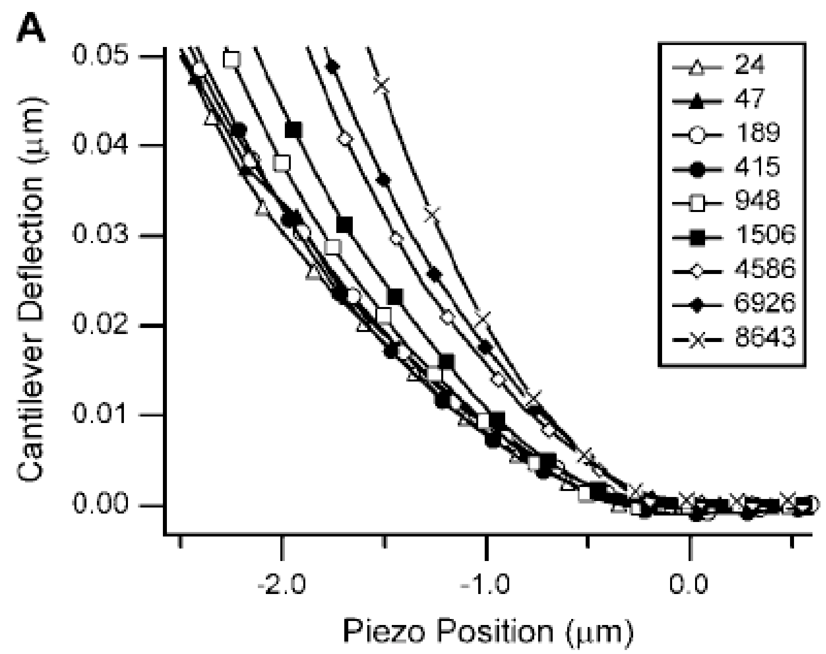


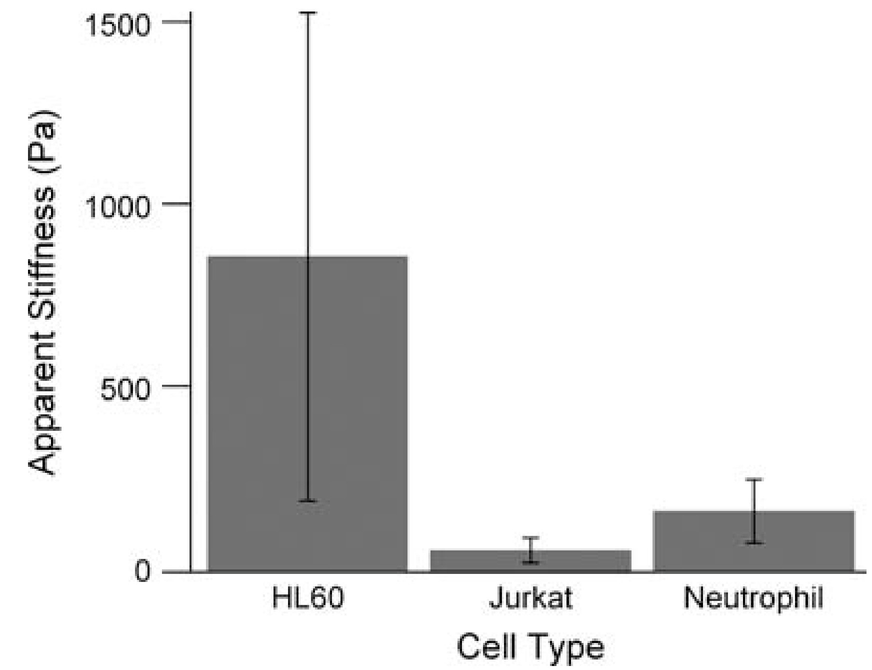
1. linear elastic
2. stress stiffening
3. critical stress
4. stress softening due to buckling

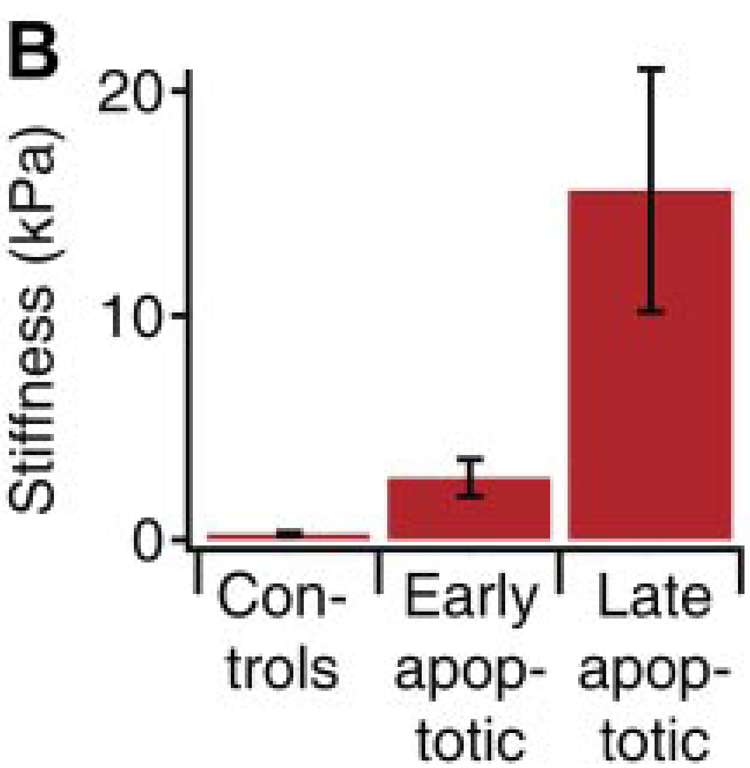
Both entropic & enthalpic contributions play a role

Measuring cell stiffness with an AFM

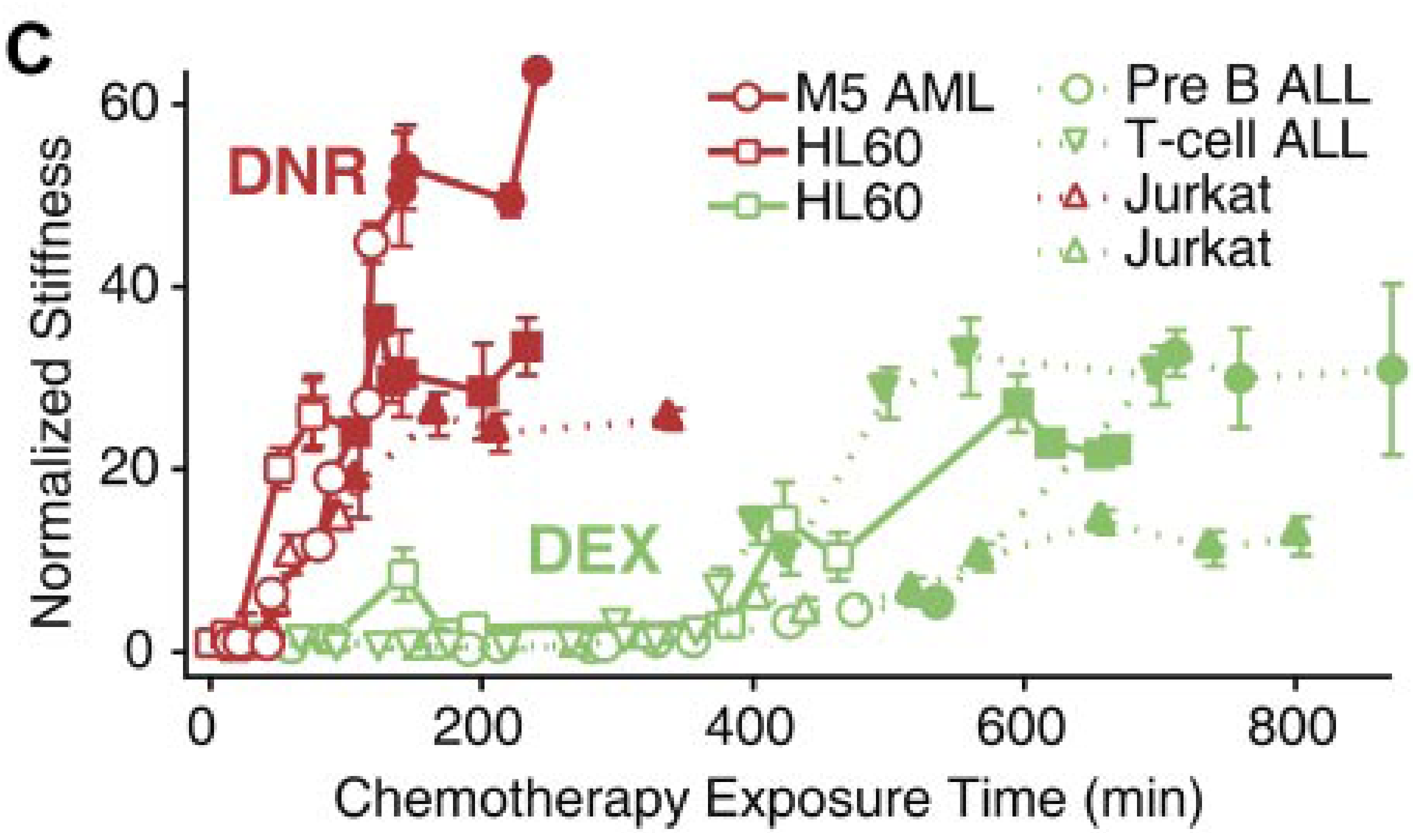






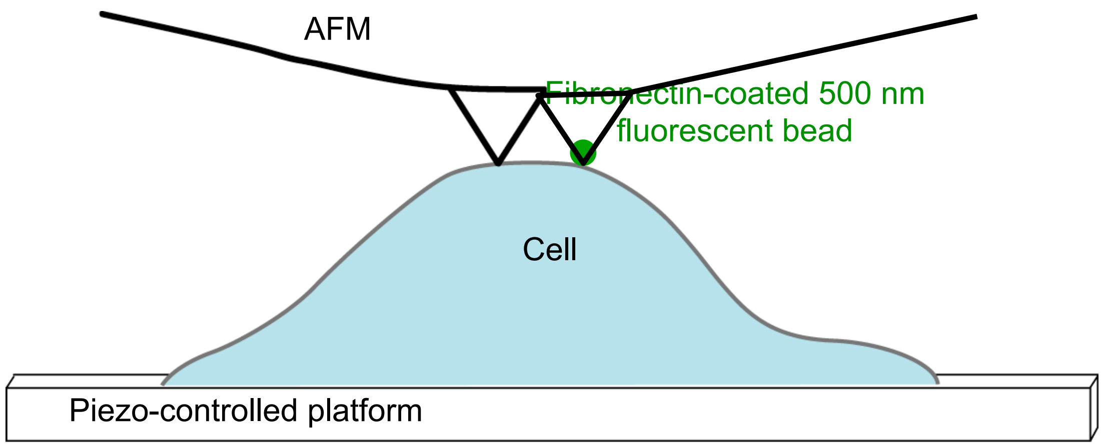


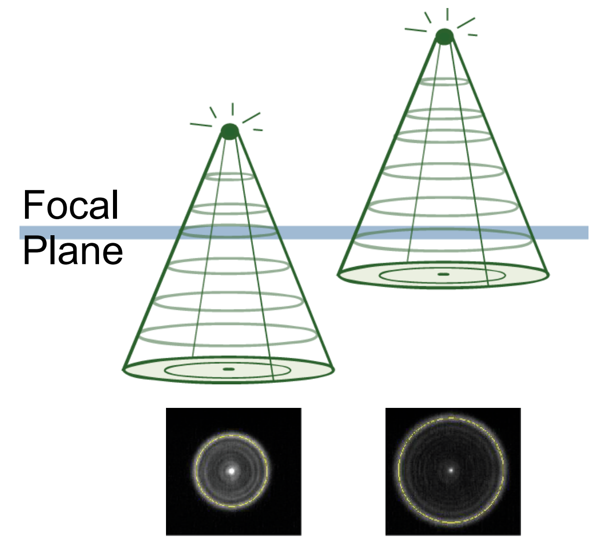
Rosenbluth et al., *Biophys J* (2006)

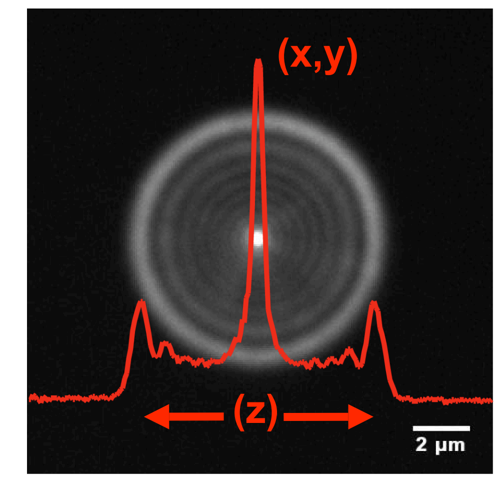


Lam et al., *Blood* (2007)

Mechanical coupling on short distance scales







Documented in Speidel et al., *Optics Letters* 2003