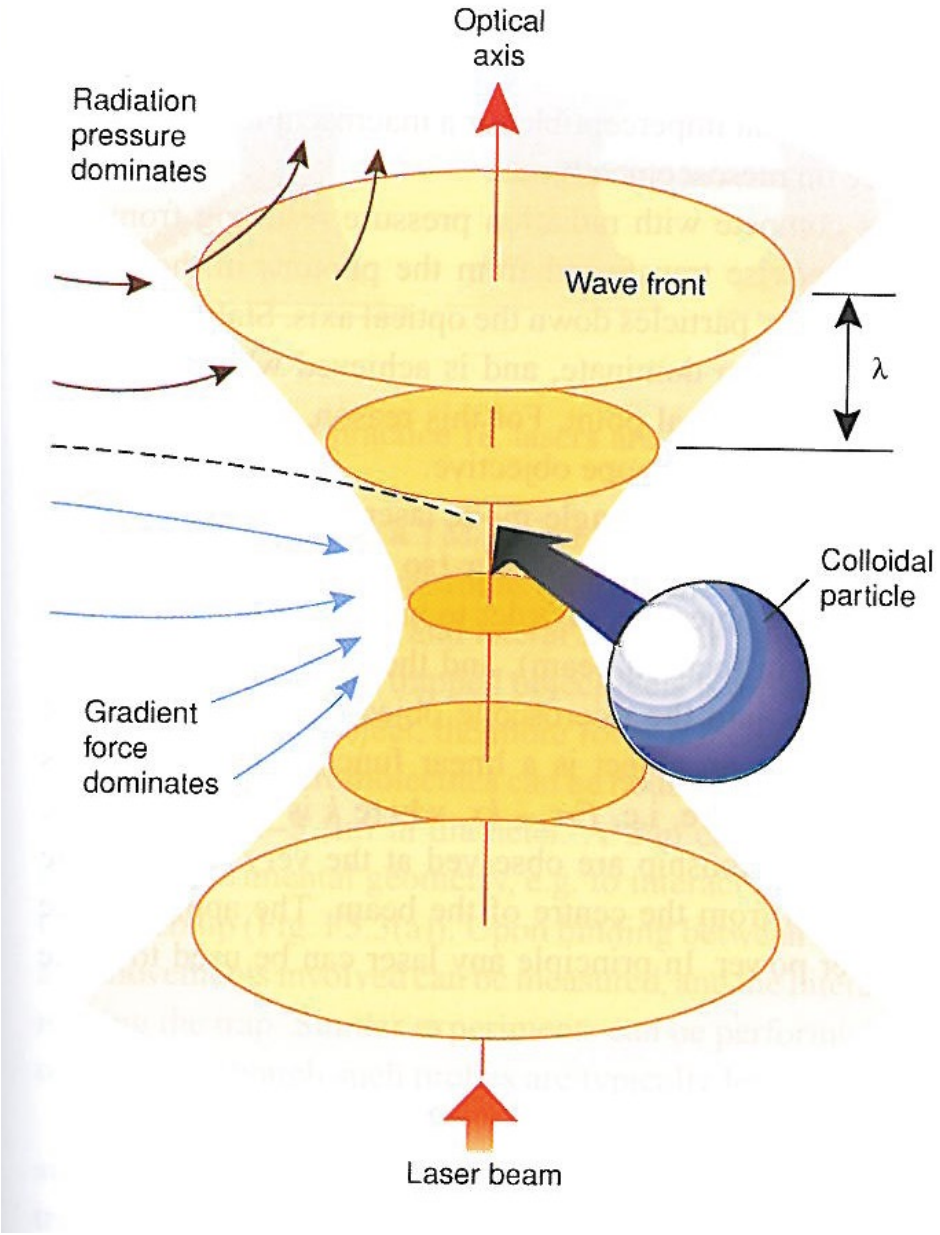
Optical tweezers

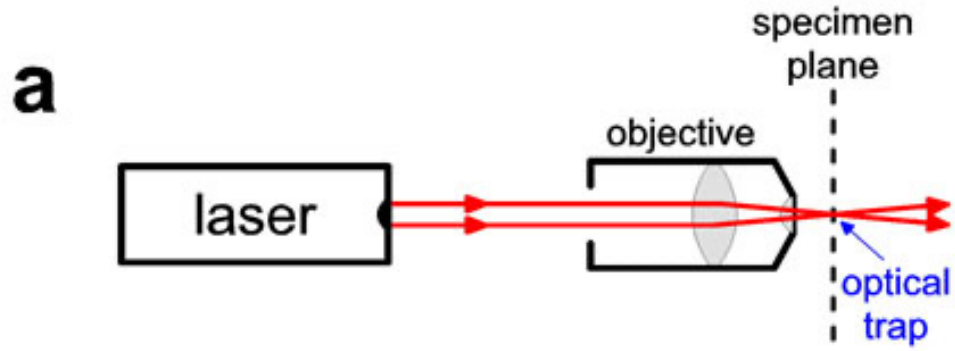
Light pressure is used to manipulate tiny objects.

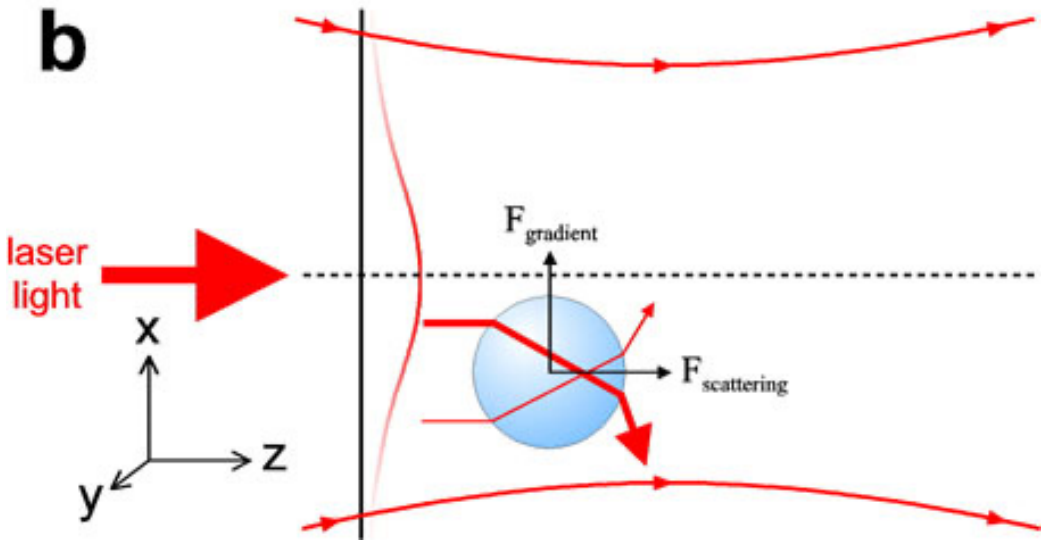
Particles with high refraction index (i.e.: lipid droplets, polystirene or silica beads) are attracted to the intense region of a laser beam field and can be permanently trapped at the focal point.

The force exerted by the laser beam on the bead is function of many parameters as:

•size and shape of the object
•difference in refraction index between the bead and the medium
•intensity gradient of the laser beam



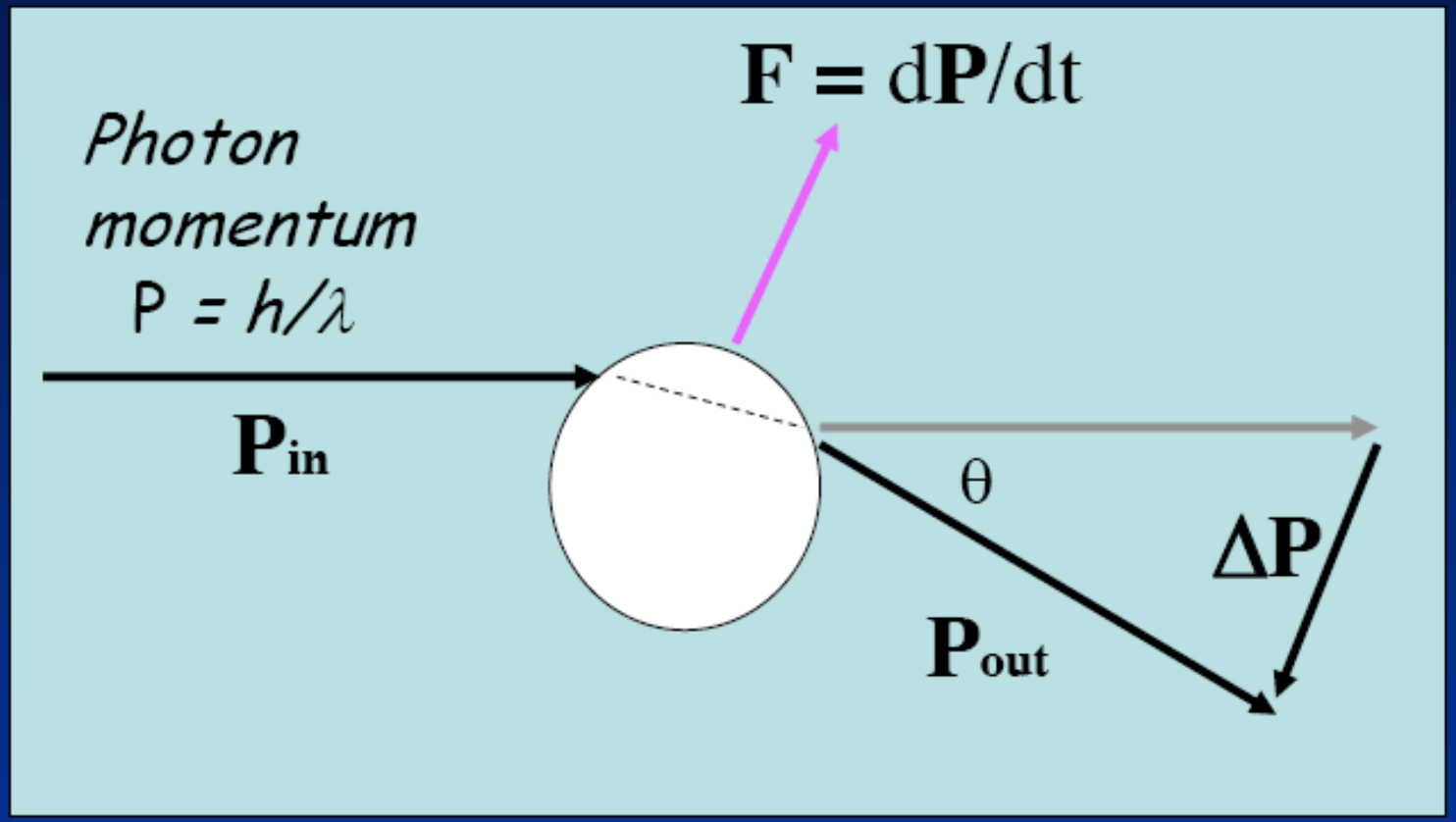




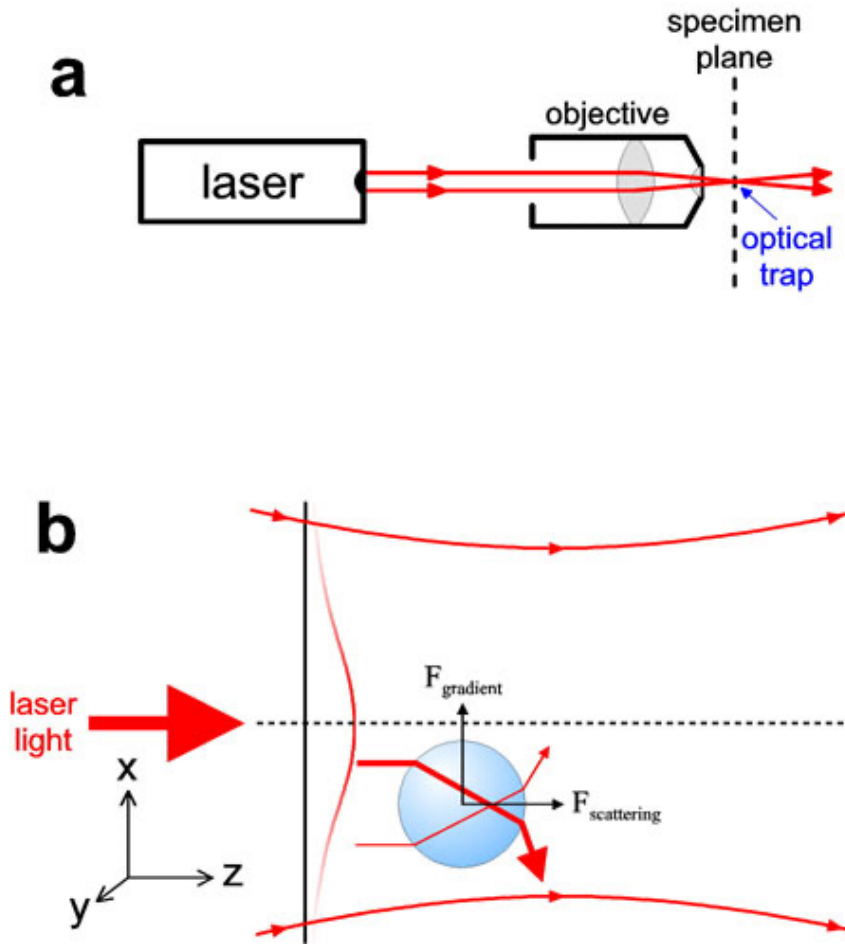
The basic principle behind optical tweezers is the **momentum transfer associated with bending light**. Light carries momentum that is proportional to its energy and in the direction of propagation. Any change in the direction of light, by reflection or refraction, will result in a change of the momentum of the light. If an object bends the light, changing its momentum, conservation of momentum requires that the object must undergo an equal and opposite momentum change. This gives rise to a force acting on the object.

http://stanford.edu/group/blocklab/

Photon meets refracting object



For every action there exists an equal but opposite reaction
Sir Isaac Newton



The incoming light comes from a laser which has a "Gaussian intensity profile". Basically, the light at the center of the beam is brighter than the light at the edges. When this light interacts with a bead, the light rays are bent according the laws of reflection and refraction (two example rays are shown in Fig 1b). The sum of the forces from all such rays can be split into two components: $F_{scattering}$, the scattering force, pointing in the direction of the incident light (z, see axes in Fig 1b), and $F_{gradient}$, the gradient force, arising from the gradient of the Gaussian intensity profile and pointing in x-y plane towards the center of the beam (dotted line). The gradient force is a restoring force that pulls the bead into the center. If the contribution to $F_{scattering}$ of the refracted rays is larger than that of the reflected rays then a restoring force is also created along the z-axis, and a stable trap will exist. Incidentally, the image of the bead can be projected onto a quadrant photodiode to measure nm-scale displacements

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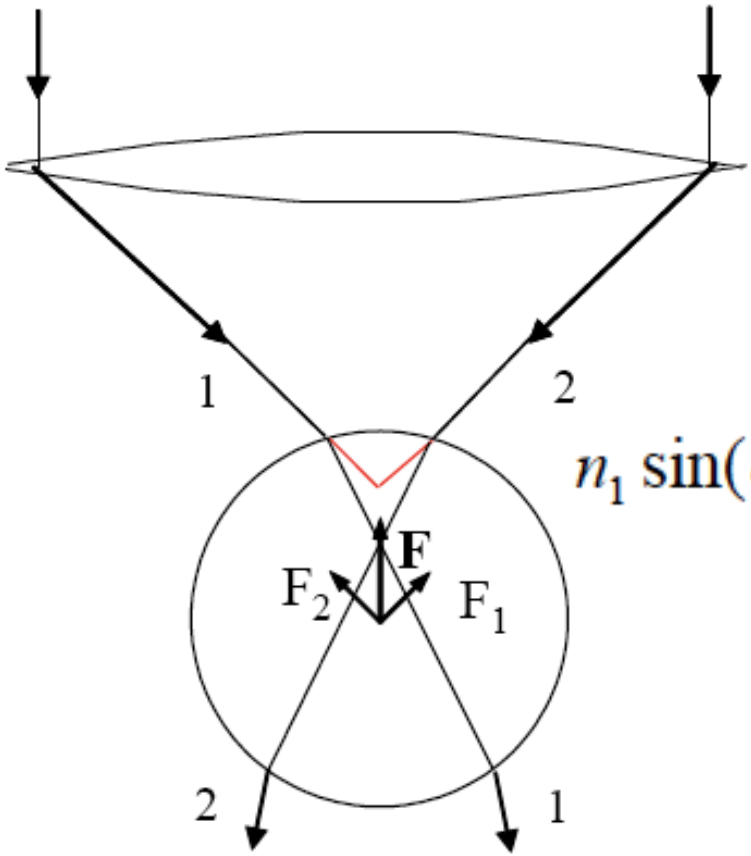
To efficiently trap an object in the beam propagating direction, the force due to refraction should compensate the ones due to reflection



$$n_1 \sin(\theta_1) = n_2 \sin(\theta_2)$$

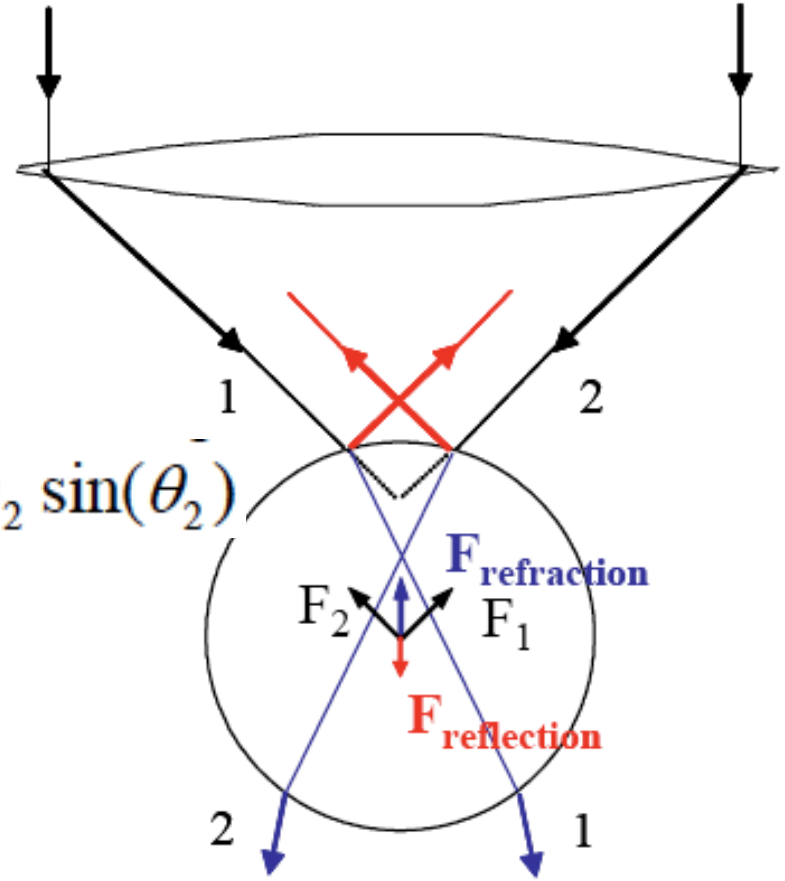


Figure 1. Schematic diagram showing the force on a dielectric sphere due to refraction of two rays of light, 1 and 2. The resultant force on the bead due to refraction is towards the focus.

Figure 2. Schematic diagram showing the force on a dielectric sphere due to both reflection and refraction of two rays of light.

To overcome the loss mechanisms, the optical trap must be designed to have a **high trapping force**. Gaussian laser beams should be focused by a **high NA objective**

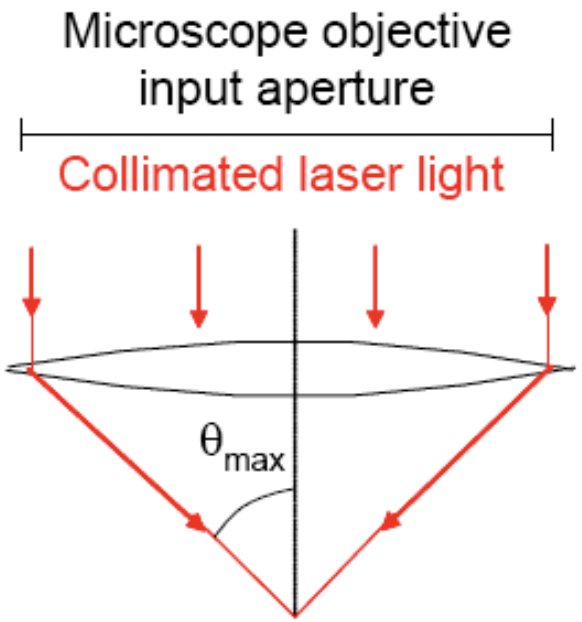
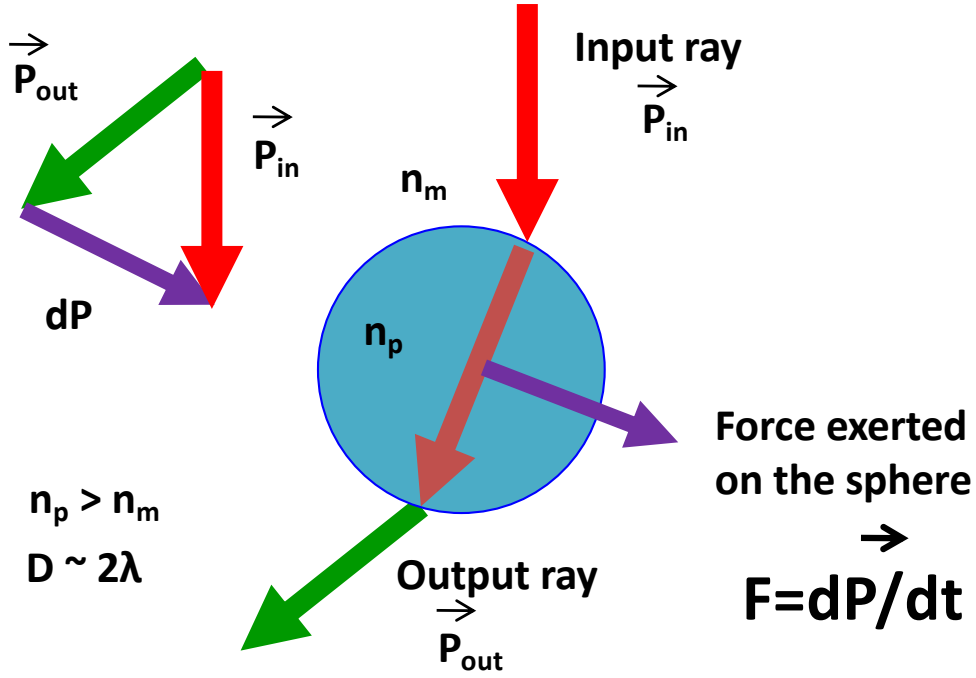


Figure 3. Collimated light focused by a microscope objective (indicated schematically as a lens). If laser light completely fills the objective input aperture, the maximum angle of light that can be focused is θ_{max}. The numerical aperture (NA) of the objective is $n\sin(\theta_{max})$, where n is the index of refraction of the medium below the objective.

The light coming from the edges of the objective lens contributes the most to the trapping force. In practice, this means that a microscope objective with a high numerical aperture (NA) must be used to generate the greatest trapping force. The maximum numerical aperture is **n** , where **n** is the index of refraction of the medium immediately following the objective. For air, this is one. Therefore, oil immersion objectives, which are designed to have oil between the microscope objective and sample, are often used in optical tweezers instruments. These can have numerical apertures of up to 1.4.
The use of high power lasers also maximizes the trapping force.

Force exerted by a Ray of Light on a glass micro-sphere

A Simple Explanation



A **Ray of Light** containing **N photons** has Energy, **E** and Linear Momentum, $\vec{P}$

n_m – refractive index of the medium

$\vec{n}_p$ – refractive index of the particle

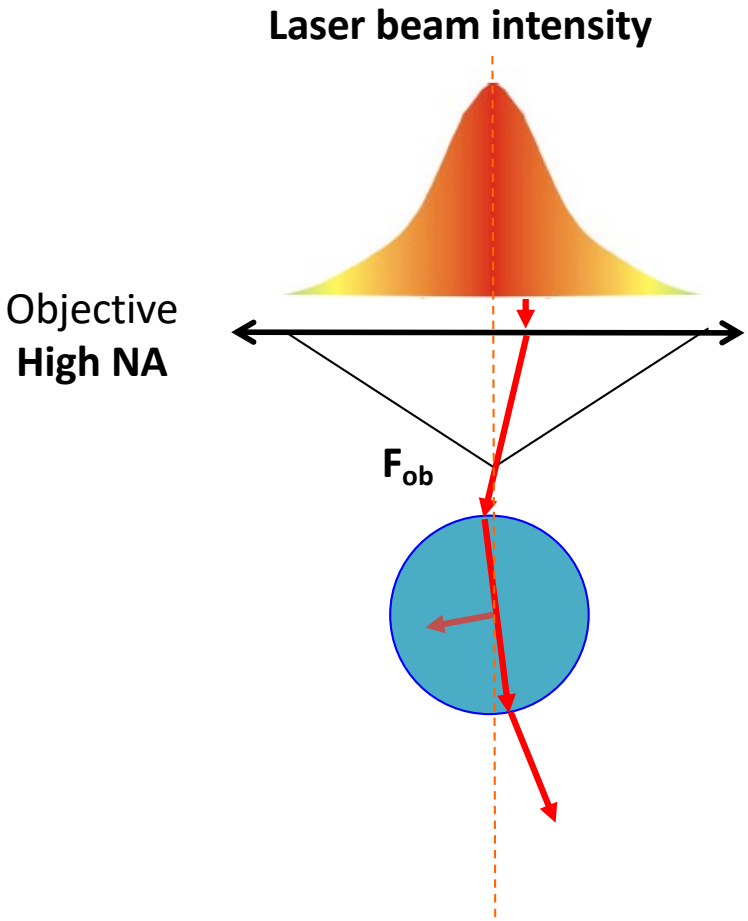
D – diameter

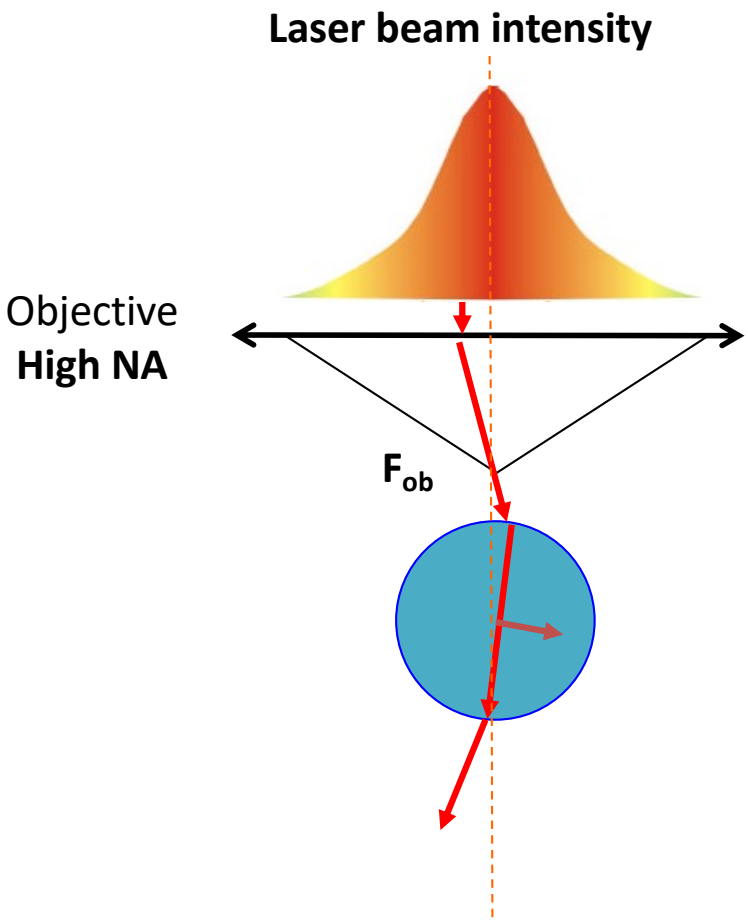
P – Linear momentum

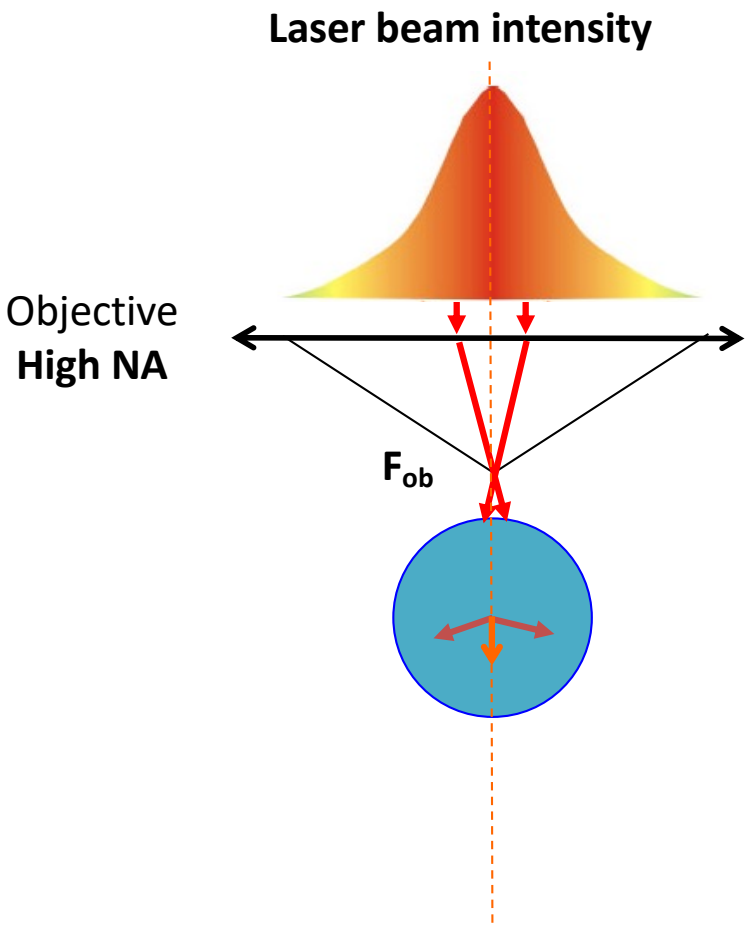
$$E = Nh\nu$$

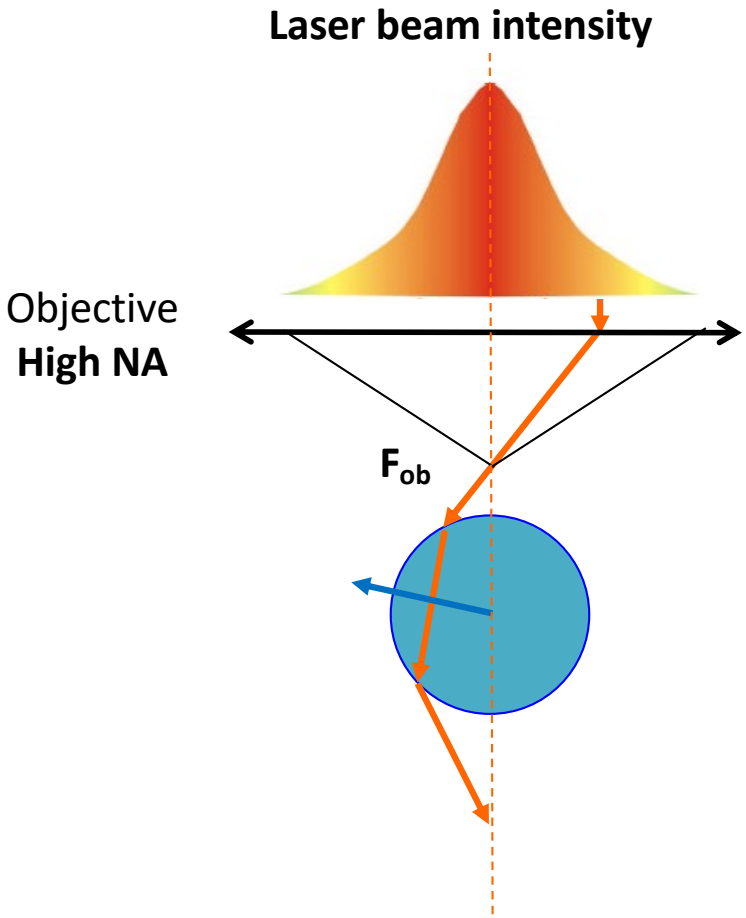
$$\left|\vec{P}\right| = E \times n_{m(p)}/c$$

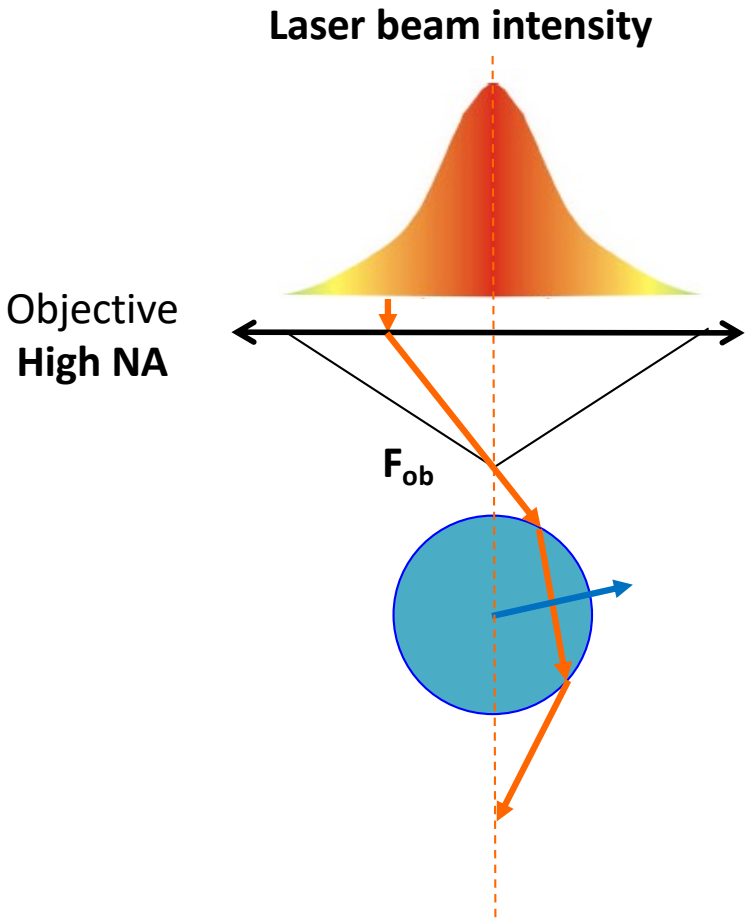
Ashkin, 1986 Optics Letters, Vol 11, N° 5

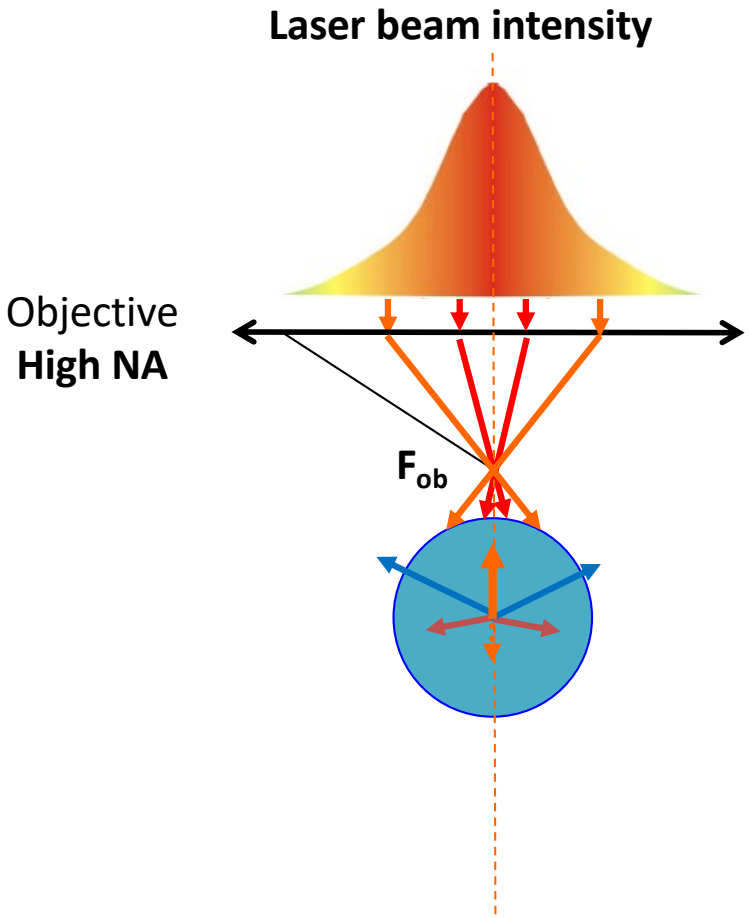

Laser beam intensity
Objective
High NA
F_{ob}

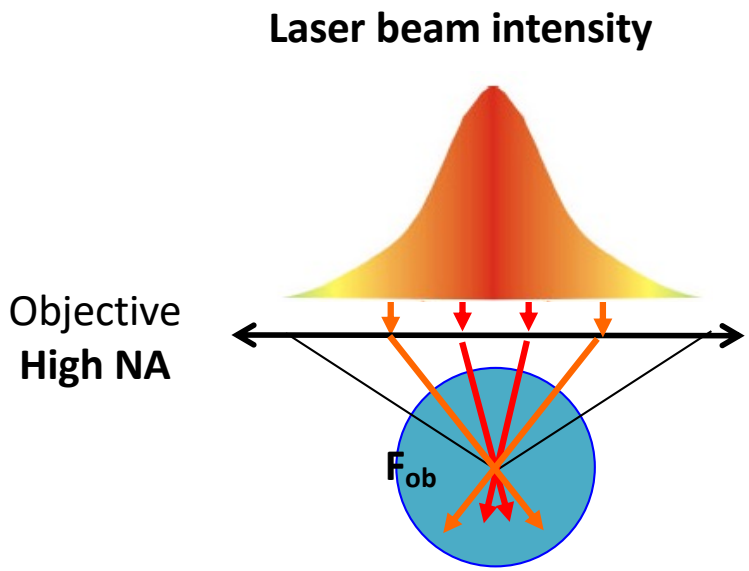

Laser beam intensity
Objective
High NA
F_{ob}


Laser beam intensity
Objective
High NA
F_{ob}


Laser beam intensity
Objective
High NA
F_{ob}


Laser beam intensity
Objective
High NA
F_{ob}


Laser beam intensity
Objective
High NA
F_{ob}



3D trapping

Arthur Ashkin builds first optical trap

VOLUME 24, NUMBER 4 PHYSICAL REVIEW LETTERS 26 JANUARY 1970

1970

ACCELERATION AND TRAPPING OF PARTICLES BY RADIATION PRESSURE

A. Ashkin
Bell Telephone Laboratories, Holmdel, New Jersey 07733
(Received 3 December 1969)

Micron-sized particles have been accelerated and trapped in stable optical potential wells using only the force of radiation pressure from a continuous laser. It is hypothesized that similar accelerations and trapping are possible with atoms and molecules using laser light tuned to specific optical transitions. The implications for isotope separation and other applications of physical interest are discussed.

Single-beam trap

Dual-beam trap

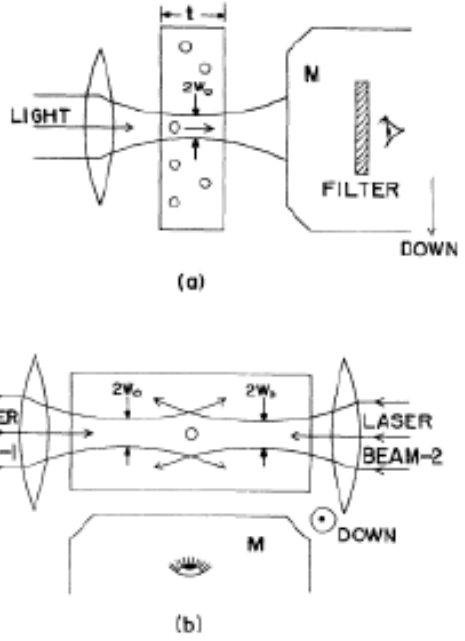


FIG. 1. (a) Geometry of glass cell, $t = 120\ \mu m$, for observing micron particle motions in a focused laser beam with a microscope M. (b) The trapping of a high-index particle in a stable optical well. Note position of the TEM_{00}-mode beam waists.

Axial escape

Infrared trap supports life !

Trap live bacteria

Sort living cells

Manipulate organelles inside cells

Nature, 1987

Optical trapping and manipulation of single cells using infrared laser beams

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† AT&T Bell Laboratories, Murray Hill, New Jersey 07974, USA

Use of optical traps for the manipulation of biological particles was recently proposed, and initial observations of laser trapping of bacteria and viruses with visible argon-laser light were reported[1]. We report here the use of infrared (IR) light to make much improved laser traps with significantly less optical damage to a variety of living cells. Using IR light we have observed the reproduction of *Escherichia coli* within optical traps at power levels sufficient to give manipulation at velocities up to ~500 μm s^{-1}. Reproduction of yeast cells by budding was also achieved in IR traps capable of manipulating individual cells and clumps of cells at velocities of ~100 μm s^{-1}. Damage-free trapping and manipulation of suspensions of red blood cells of humans and of organelles located within individual living cells of spirogyra was also achieved, largely as a result of the reduced absorption of haemoglobin and chlorophyll in the IR. Trapping of many types of small protozoa and manipulation of organelles within protozoa is also possible. The manipulative capabilities of optical techniques were exploited in experiments showing separation of individual bacteria from one sample and their introduction into another sample. Optical orientation of individual bacterial cells in space was also achieved using a pair of laser-beam traps. These new manipulative techniques using IR light are capable of producing large forces under damage-free conditions and improve the prospects for wider use of optical manipulation techniques in microbiology.

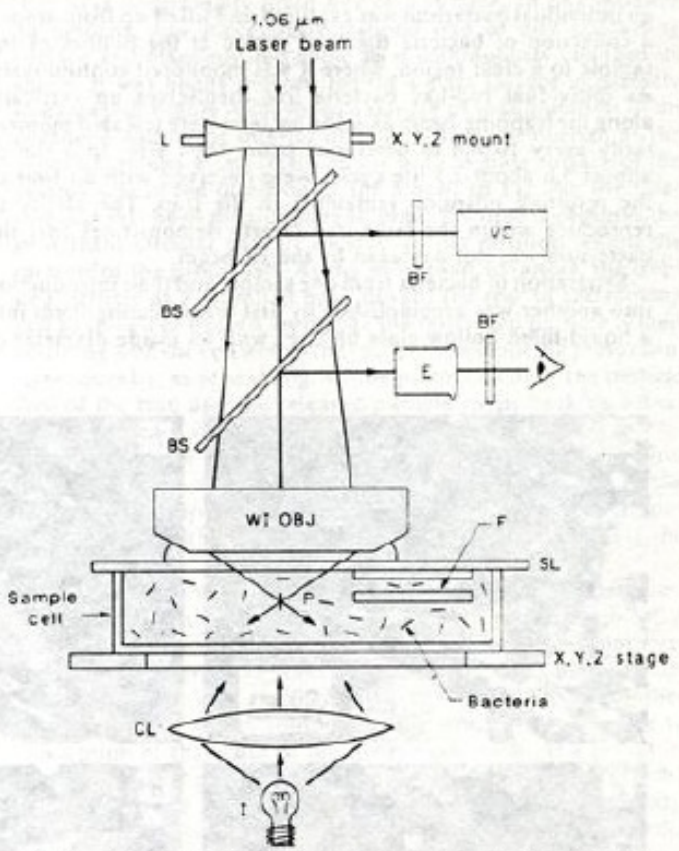
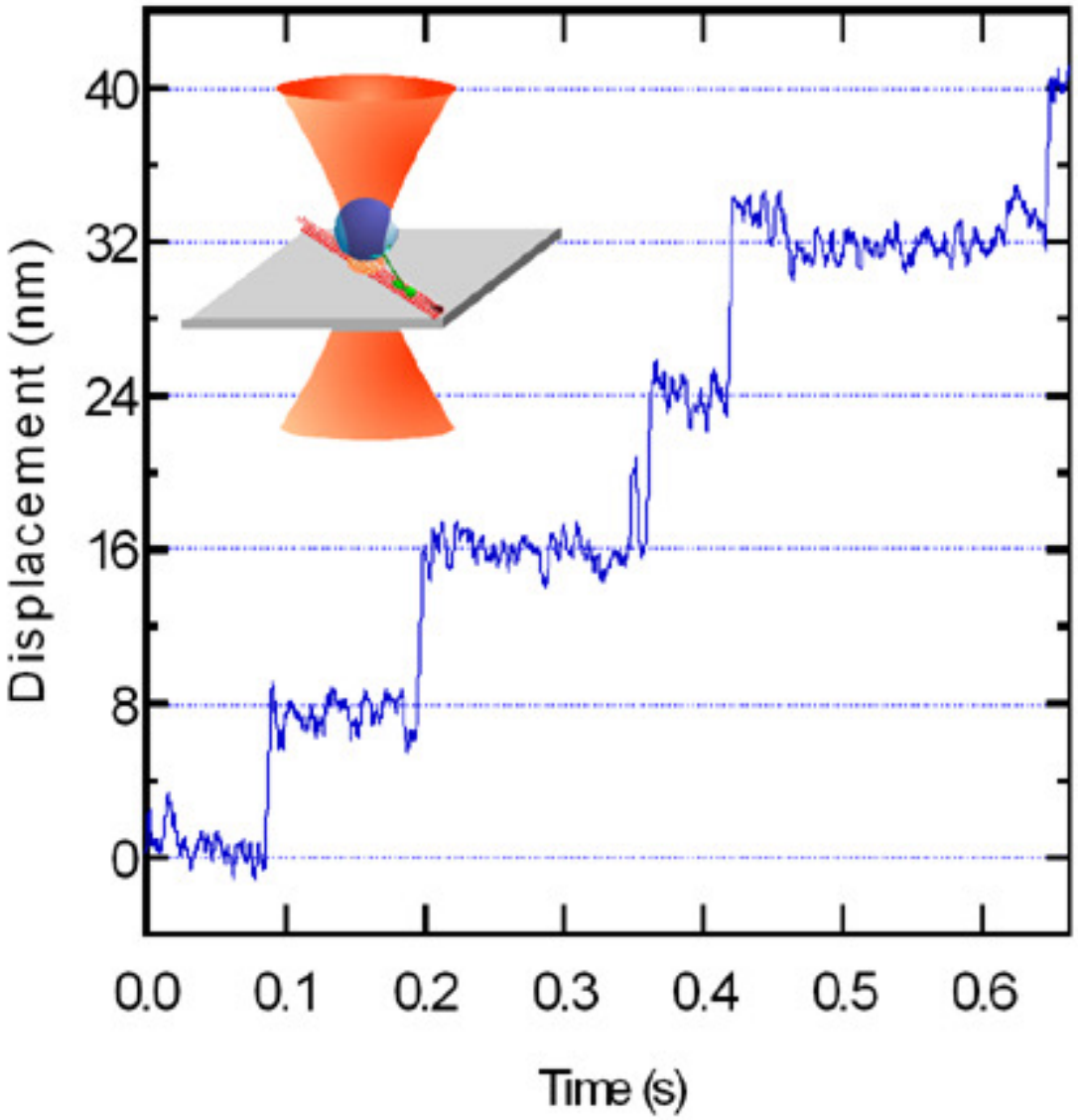


Fig. 1 Combined high-resolution optical microscope and 1.06 μm infra-red laser trap for observing, manipulating and separating bacteria and other organisms.



When the bead is displaced from the center of the trap, what force does it feel? The restoring force of the optical trap works like an optical spring: the force is proportional to the displacement out of the trap. In practice, the bead is constantly moving with Brownian motion. But whenever it leaves the center of the optical trap the restoring force pulls it back to the center. If some external object, like a molecular motor, were to pull the bead away from the center of the trap, a restoring force would be imparted to the bead and thus to the motor. An example trace of a single kinesin motor taking 8 nm steps against a 5-pN force is shown in Fig 2.

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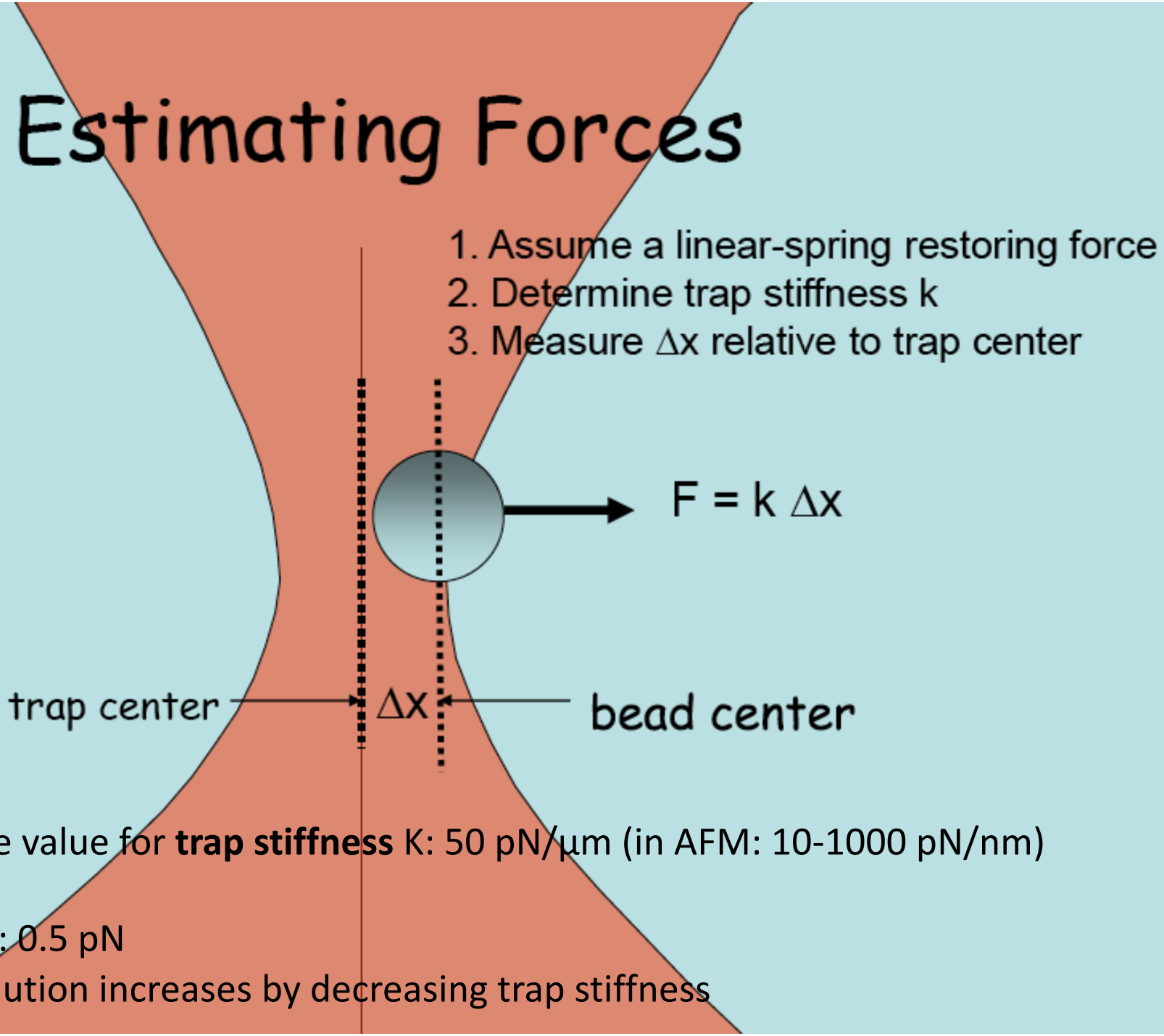
Estimating Forces

1. Assume a linear-spring restoring force
2. Determine trap stiffness k
3. Measure Δx relative to trap center



Reasonable value for **trap stiffness** K: 50 pN/µm (in AFM: 10-1000 pN/nm)

Resolution: 0.5 pN

Force resolution increases by decreasing trap stiffness

CALIBRATING TRAP STIFFNESS

Equipartition Theorem (simple but not very precise)

1. Track bead position (**x,y,z)** in the optical trap for 1-2 s with a detector with bandwidth > 5 kHz
2. Calculate the Variance **V (Vx, Vy, Vz)**, determine stiffness:

$$\mathbf{K^{OT}_x = K_BT / Vx} \qquad \mathbf{K^{OT}_y = K_BT / Vy} \qquad \mathbf{K^{OT}_z = K_BT / Vz}$$

Typical values for OT : $\mathbf{K_{OT}}$ = **0.001 – 1 pN/nm**

Compared to AFM: K_{AFM} = 10 – 1000 pN/nm

Alternative methods for calibration of an OT:

- Stokes drag force $(F=6\pi\eta r v)$
- Power Spectrum Density

Some useful references:

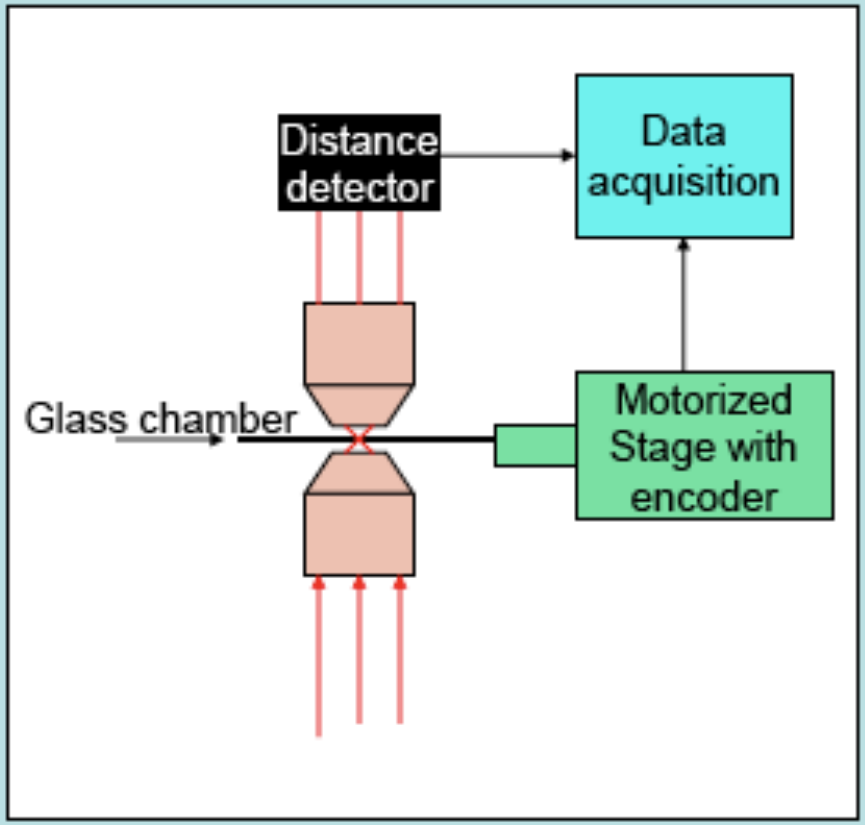
Svoboda and Block, 1994; Gittes and Schmidt, 1997,

Review Optical Tweezers: Neuman and Block, Rev. Sci. Instr. 2004

Calibrating trap stiffness

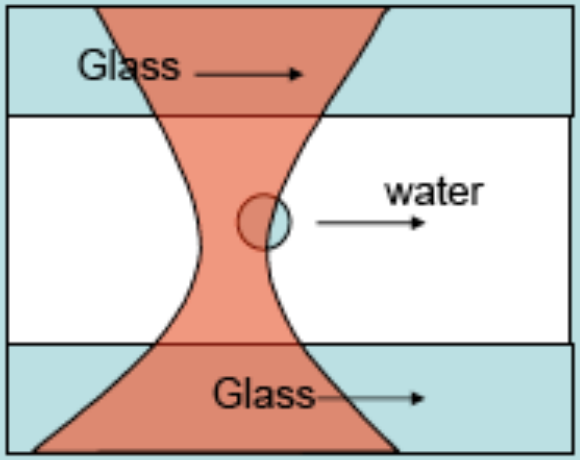
Fluid drag test force

Stokes' law $F = 6\pi r\eta V$ but corrected for proximity to walls

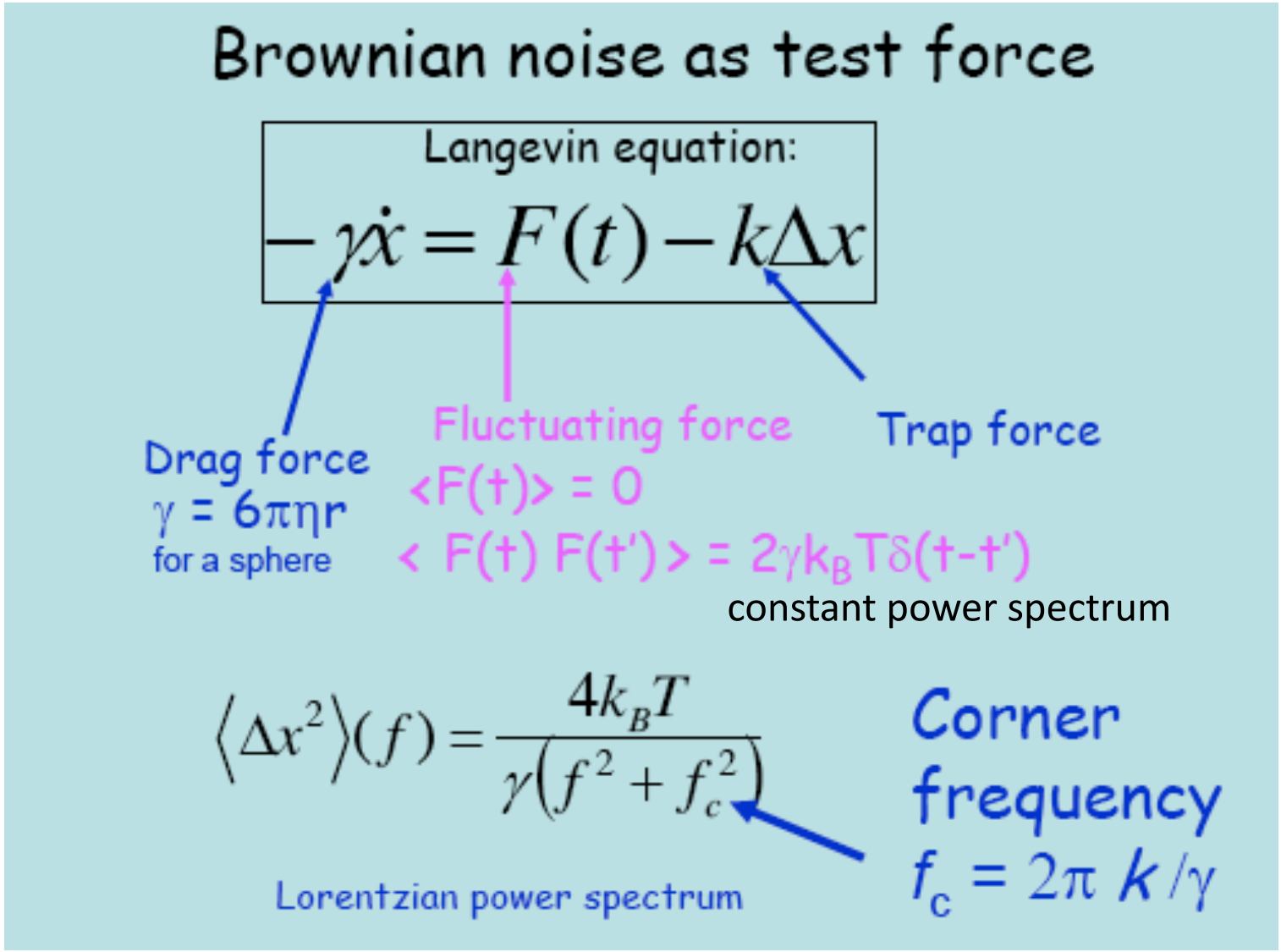


sphere of radius r with velocity v

η = viscous coeff.



By measuring the velocity of the liquid, V, we know the viscous drag force



An isolated bead in an optical trap experiences random forces due to thermal fluctuactions: analysis of power spectrum density (FT of autocorrelation function)

Brownian motion of a particle in a fluid, with deterministic (trap) and random forces: Overdamped Langevin equation (inertia negligible with respect to friction, small mass)

Particle is experiencing:

- Optical force (spring-like) −kx
- Viscous Force $-\gamma\dot{x}$
- Thermal noise F(t)

$$\gamma \frac{dx}{dt} + kx = F(t)$$

$$\left|F(f)\right|^2 = 4\gamma k_B T \,. \qquad (12)$$

The solution to Eq. 11 can be obtained by taking the Fourier transform of both sides, which yields

$$2\pi\gamma\left(f_c - if\right)X(f) = F(f)\,, \qquad (13)$$

where $f_c = \left(k/2\pi\gamma\right)$. Taking the modulus of Eq. 13 and using Eq. 12 gives

$$\left|X(f)\right|^2 = \frac{k_B T}{\gamma\left(f_c + f\right)^2}\,. \qquad (14)$$

This gives the frequency dependence of fluctuations, or the power spectrum, of the position of a bead in an optical trap.

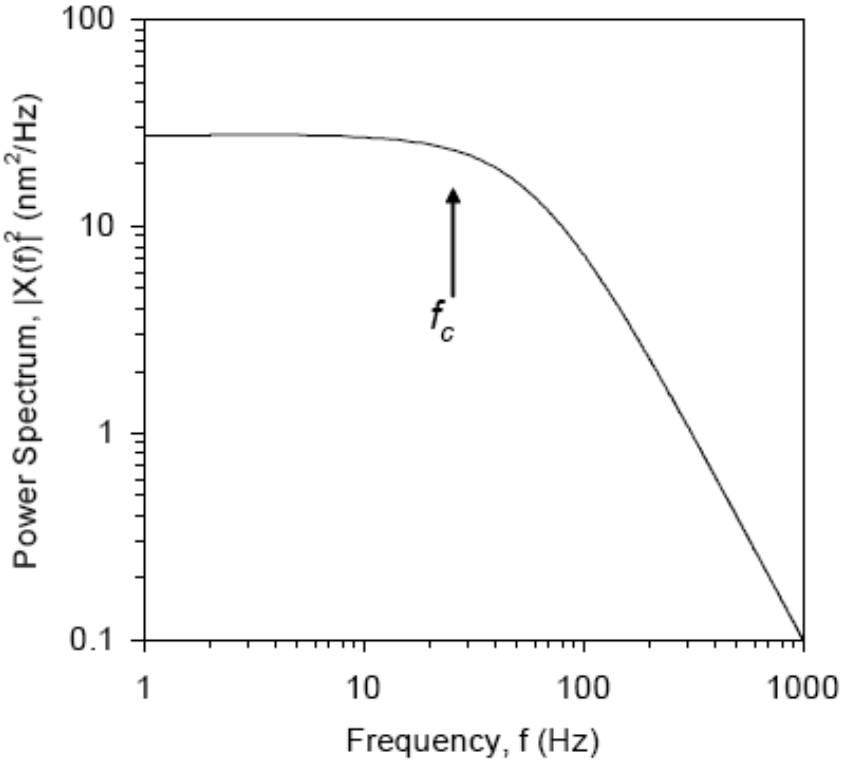
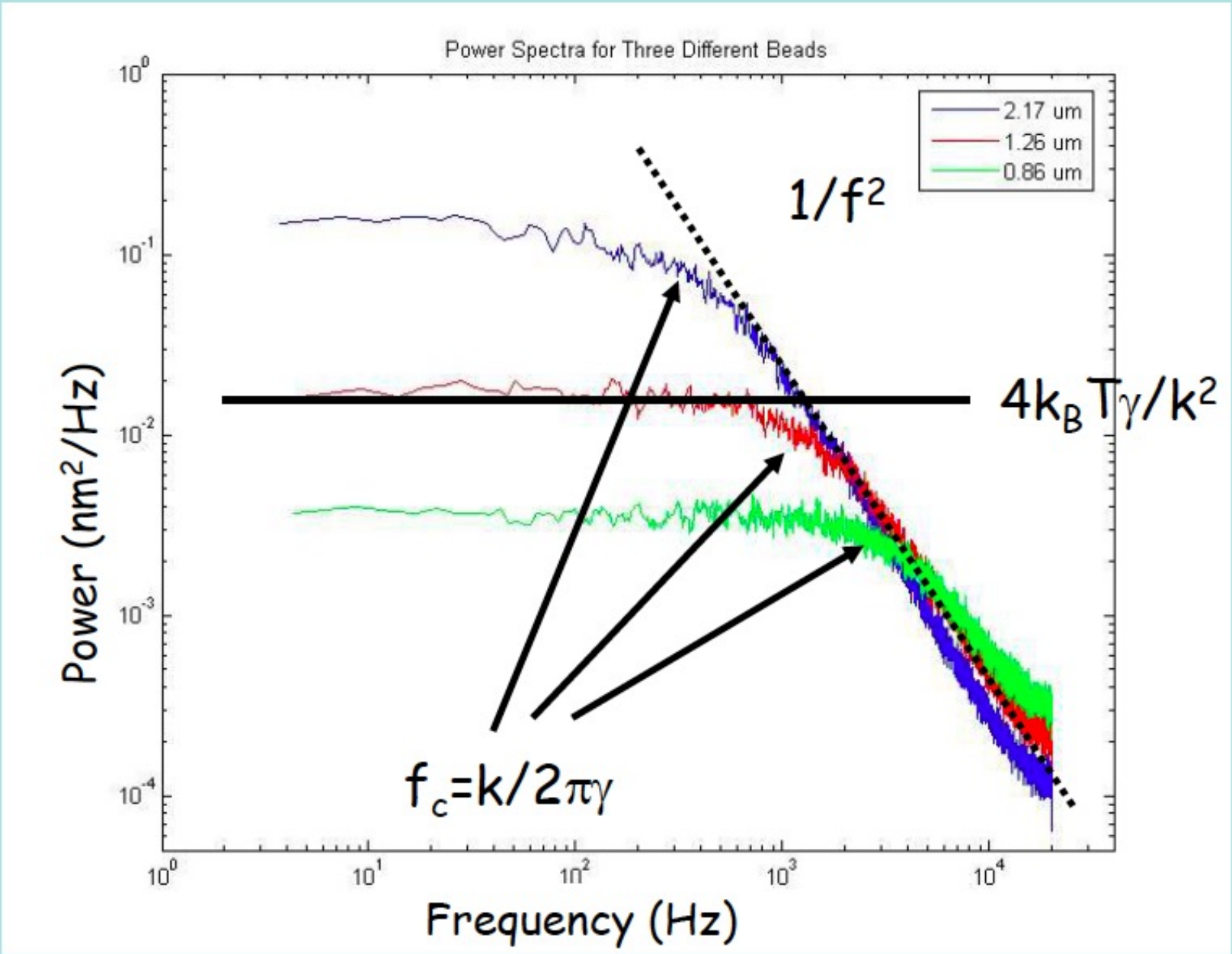
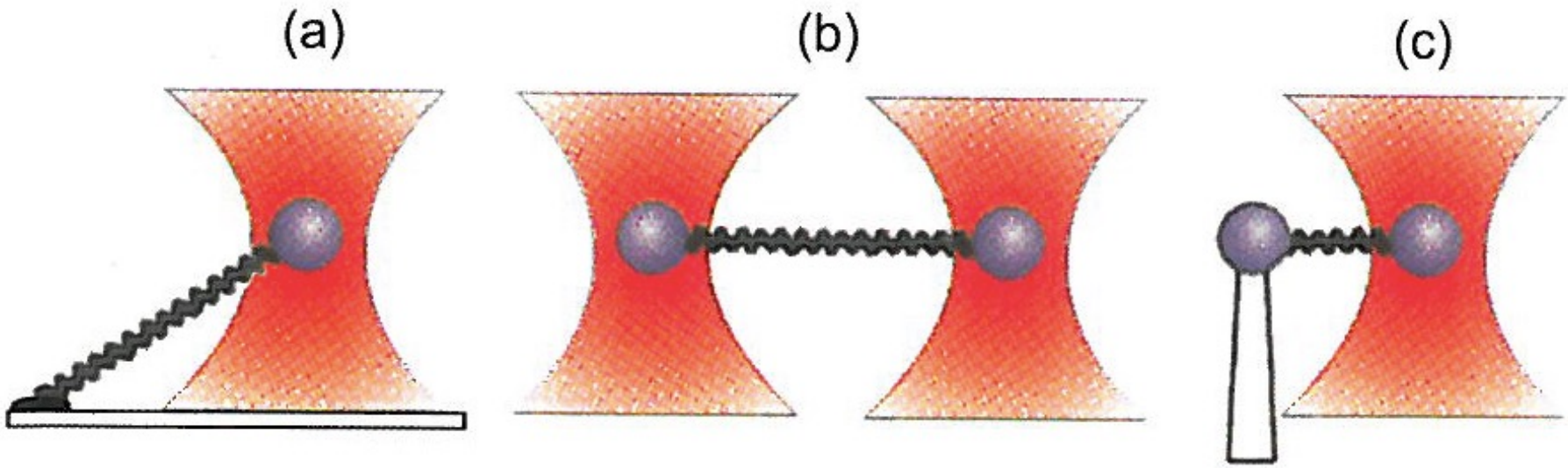


Figure 8. Ideal power spectrum of a bead in an optical trap. The power spectrum can be used to determine the stiffness of the trap.

Power spectra



We have shown that optical tweezers instruments can be used to precisely measure piconewton forces and nanometer position changes of polystyrene spheres. Is this useful? It turns out that this capability can be very useful when combined with a little biochemistry. We can now precisely manipulate micron-sized polystyrene beads, so if we can find a way to attach single molecules to these beads we can study the forces acting on the molecules under various conditions and use these results to model their physical behavior. This gives us two major advantages. First, we can isolate a single molecule and simplify the system that we are studying significantly in the absence of interactions between these molecules that might complicate the analysis. Second, we can measure the properties of a single molecule, thus avoiding the configurational averaging that always takes place in a bulk experiment,



Molecular motors: DNA/RNA polymerase

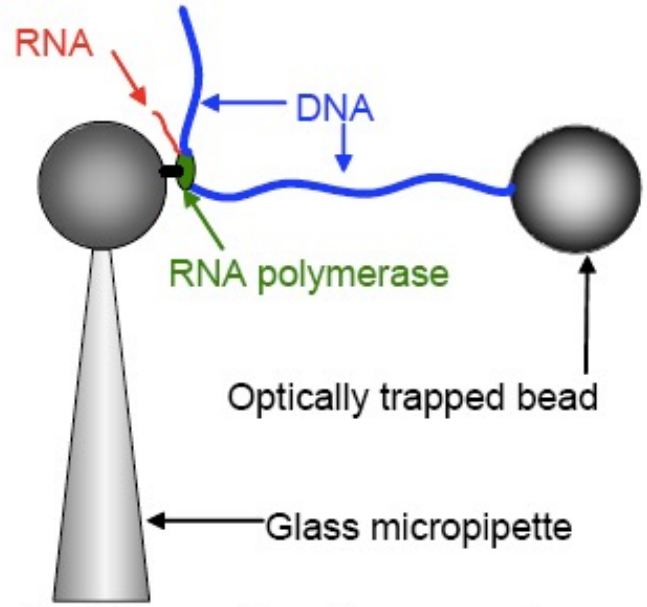


Figure 9. Schematic diagram of an optical tweezers experiment to measure the transcription forces generated by E. coli RNA polymerase. Based on the experiments described in (Davenport et al. 2000).

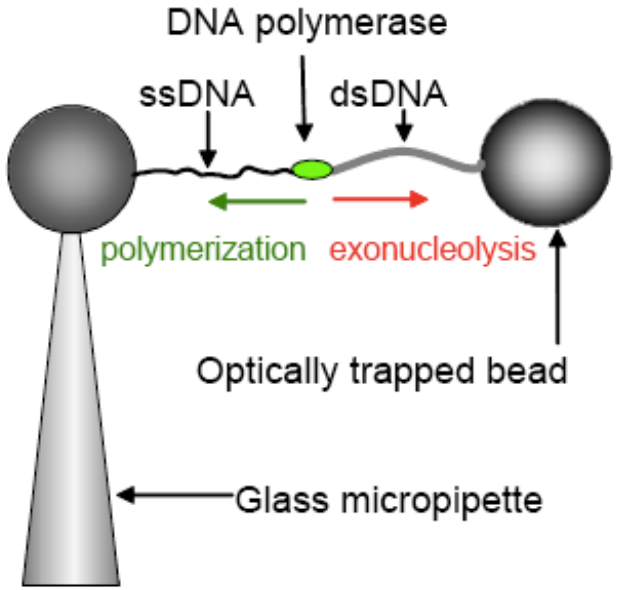


Figure 10. Schematic diagram of an optical tweezers experiment to measure the polymerization forces generated by T7 DNA polymerase. Based on the experiments described in (Wuite *et al.* 2000).

E. coli RNA polymerase incorporates NTP into the nascent mRNA molecules, and used the released energy to move along DNA. The average polymerization velocity was independent of tension up to 25 pN, when transcription was stalled.

T7 DNA polymerase velocity was very sensitive to tension, and stalled at 34 pN.

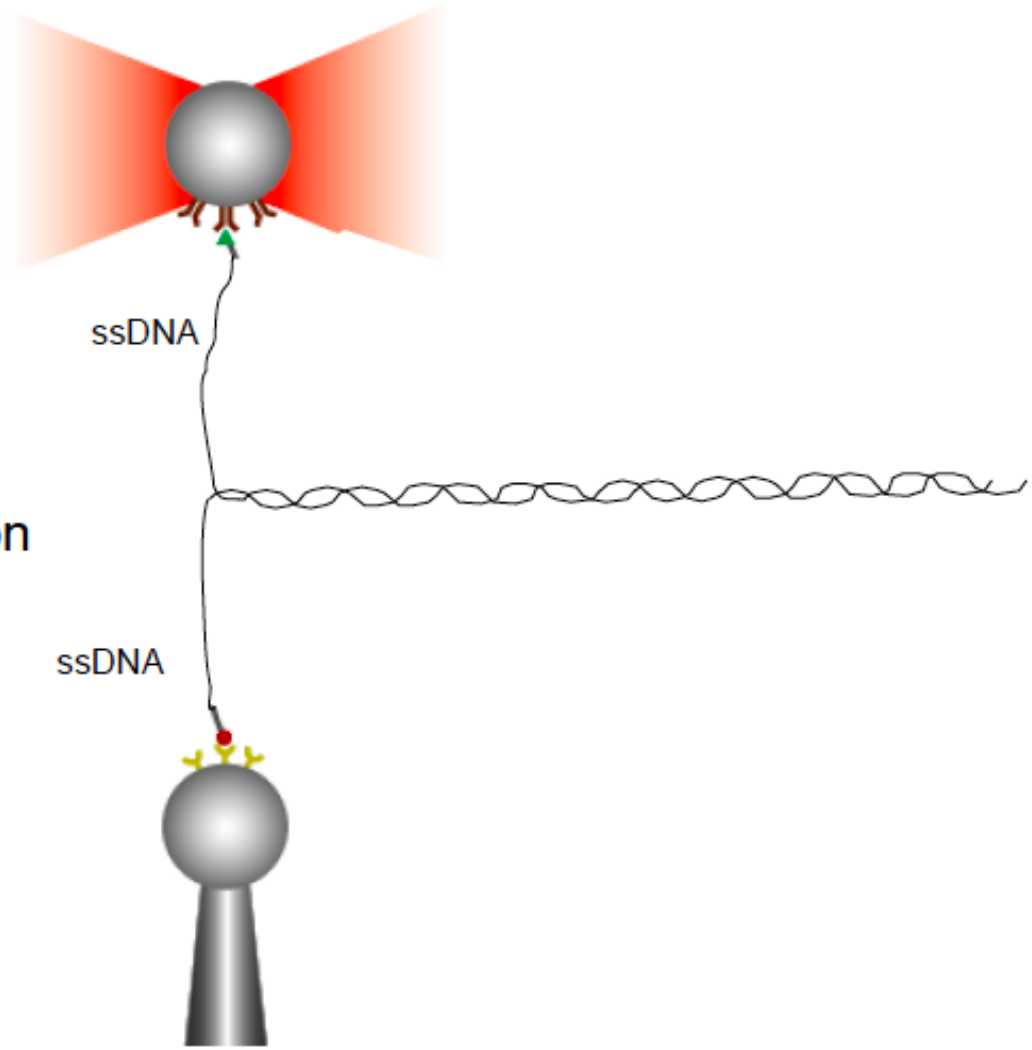
In order to stretch the DNA molecule, thus exerting a force opposing transcription, the glass micropipette is moved to a specific position or is moved until a specific force on the bead in the optical trap is observed.

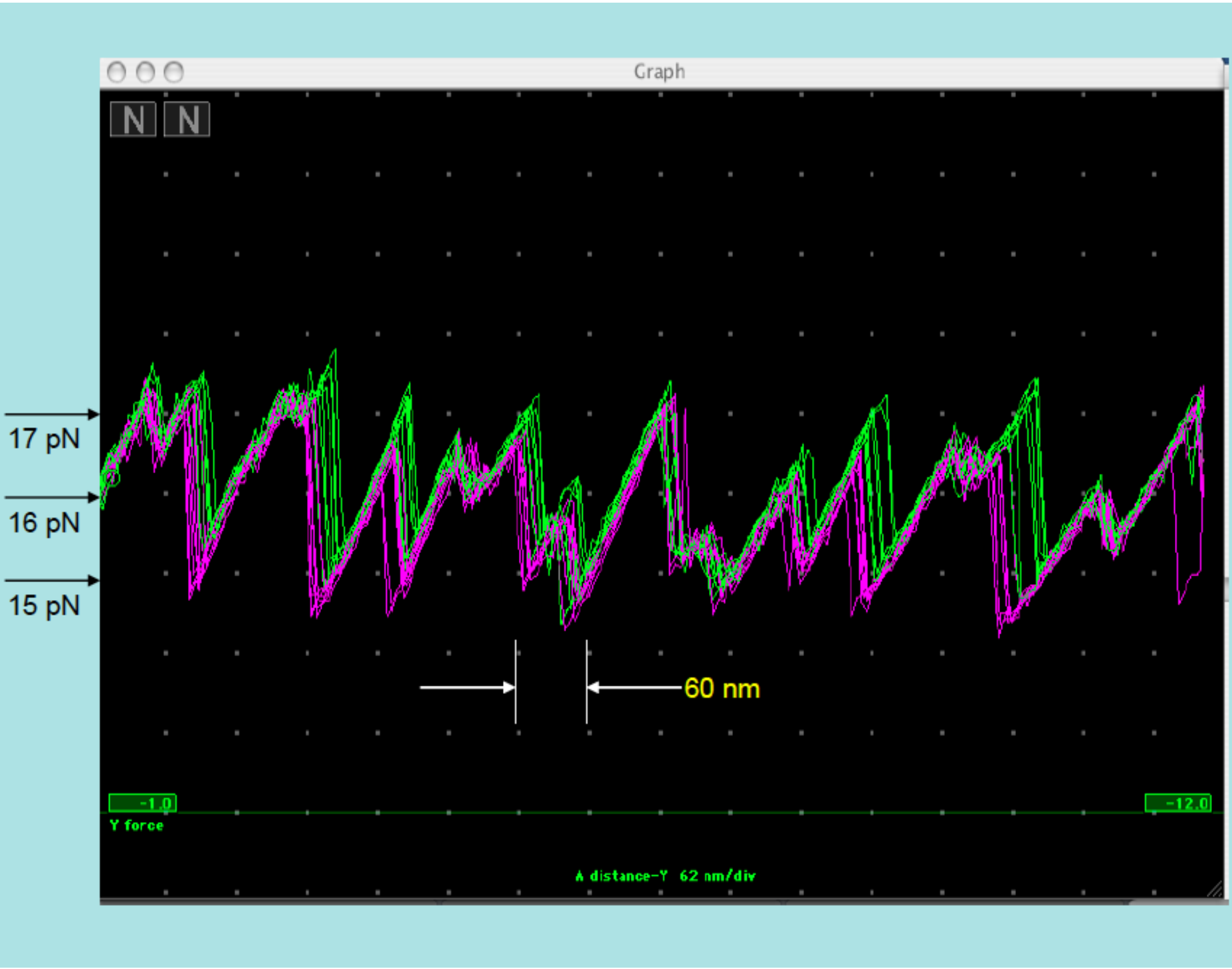
Unzipping dsDNA

Bockelmann, Heslot, 2002

S. Koch, M. Wang, 2003

Felix Ritort et al., in preparation




Graph
N
N
17 pN
16 pN
15 pN
60 nm
-1.0
Y force
-12.0
A distance-Y 62 nm/div

Biophysical properties of nucleic acids

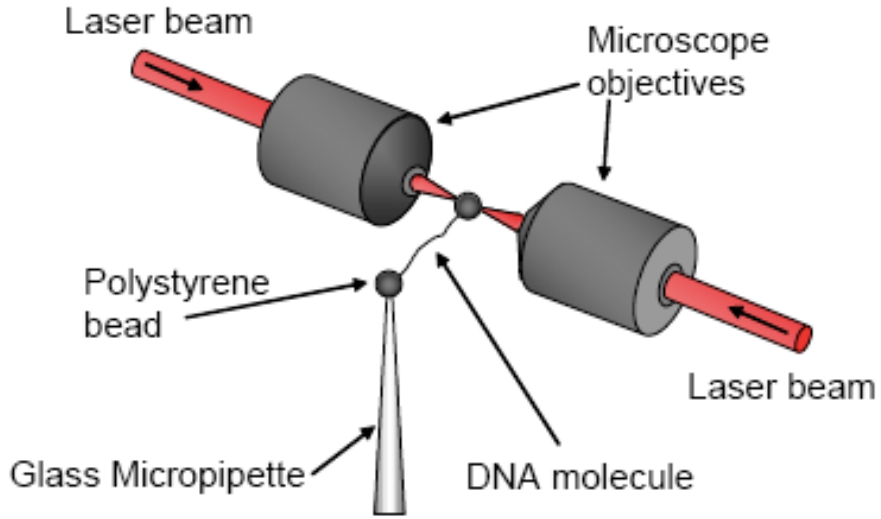


Figure 11. Schematic diagram of an experiment to stretch single DNA molecules in a dual-beam optical tweezers instrument.

To understand DNA replication and repair or RNA transcription/translation it is important to understand the behavior of nucleic acids under the wide variety of conditions present in the cell.

To understand DNA replication it is crucial to understand the transition from dsDNA to ssDNA, or the “helix-coil transition”.

Biophysical properties of nucleic acids

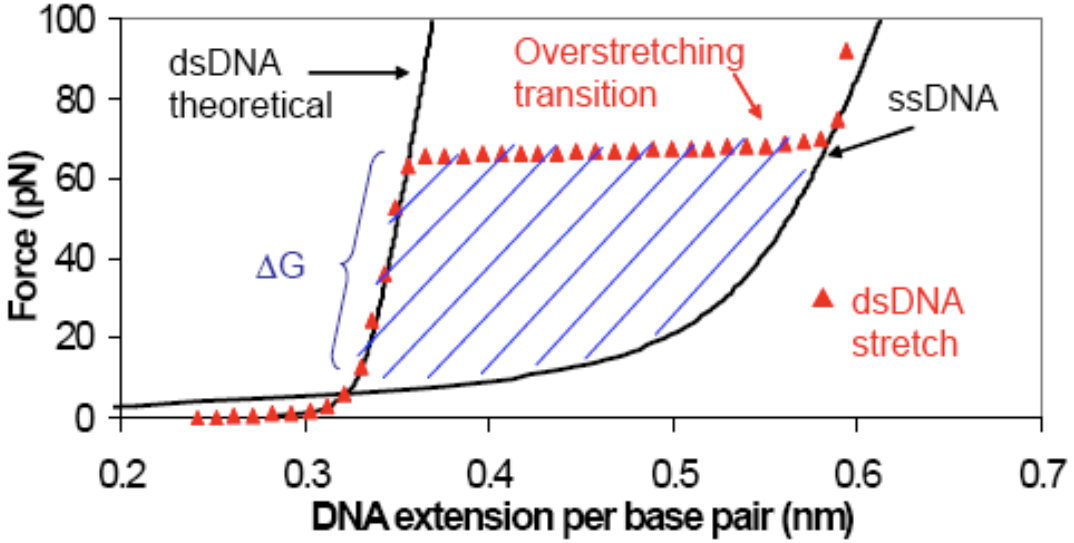


Figure 12. Force-extension curves for single molecules of dsDNA (red triangles) and ssDNA (right black line). A theoretical curve for dsDNA is shown as the left black line. The overstretching transition appears to be a transition from dsDNA to ssDNA.

DNA that **rotates freely** is overstretching at 65 pN: a force-induced melting.
The molecule unwind with the base-pairs holding breaks.
Poly(dG-dC) have an overstretching transition 30 pN higher then poly (dA-dT), consistent with the difference in melting T between the two molecules
(GC free energy = **5.2** k_BT, **AT** free energy = **3.2** k_BT)
The area between the stretching curves gives the helix-coil transition free energy.

Biophysical properties of nucleic acids

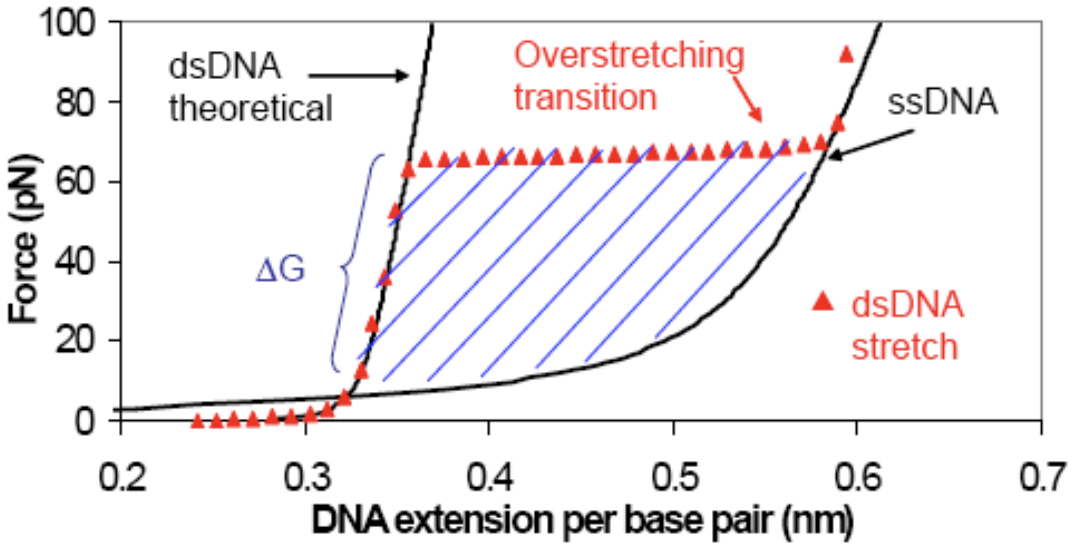
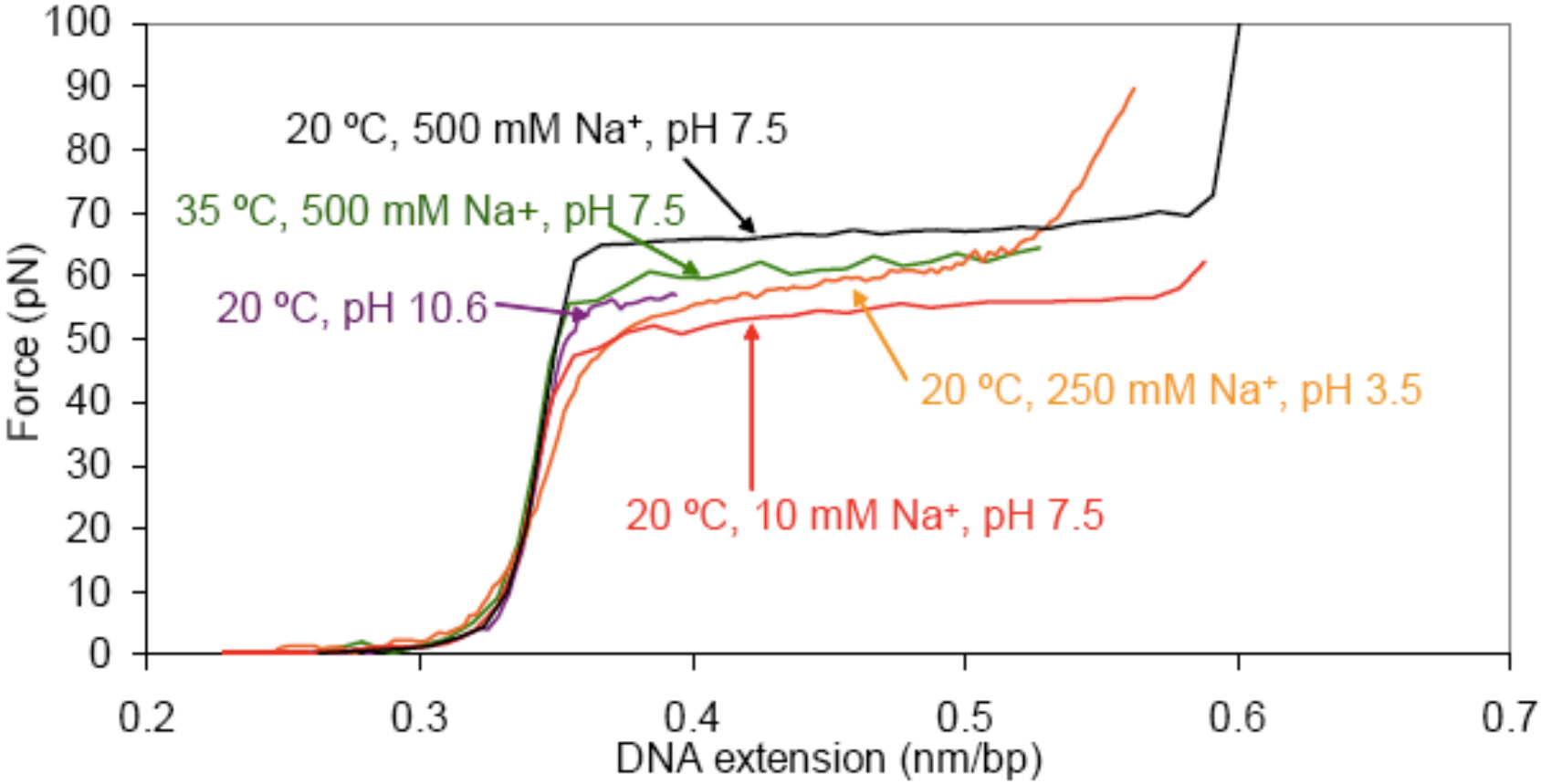


Figure 12. Force-extension curves for single molecules of dsDNA (red triangles) and ssDNA (right black line). A theoretical curve for dsDNA is shown as the left black line. The overstretching transition appears to be a transition from dsDNA to ssDNA.

In a later work (Clausen- Schaumann et al. 2000), they showed a strand separation force depended on the rate at which the dsDNA was stretched, while the overstretching transition did not depend on the pulling rate. In the force-induced melting theory, the overstretching transition is an equilibrium melting transition, while the second transition at higher force is a nonequilibrium strand separation transition, during which the last base pairs holding the two strands together are irreversibly broken. A rate-dependent force is expected when single bonds are irreversibly broken (Evans and Ritchie 1997)

Biophysical properties of nucleic acids



To test the force-induced melting model, Williams et al. measured DNA overstretching as a function of pH and T (Williams et al. 2001b).

Since extremely high and low pH lower the melting temperature of dsDNA, the overstretching force should also decrease if melting occurs during the transition. This decrease in the overstretching force was demonstrated, and the fitted value of the change in entropy of DNA upon melting was in agreement with calorimetric measurements.

Biophysical properties of nucleic acids

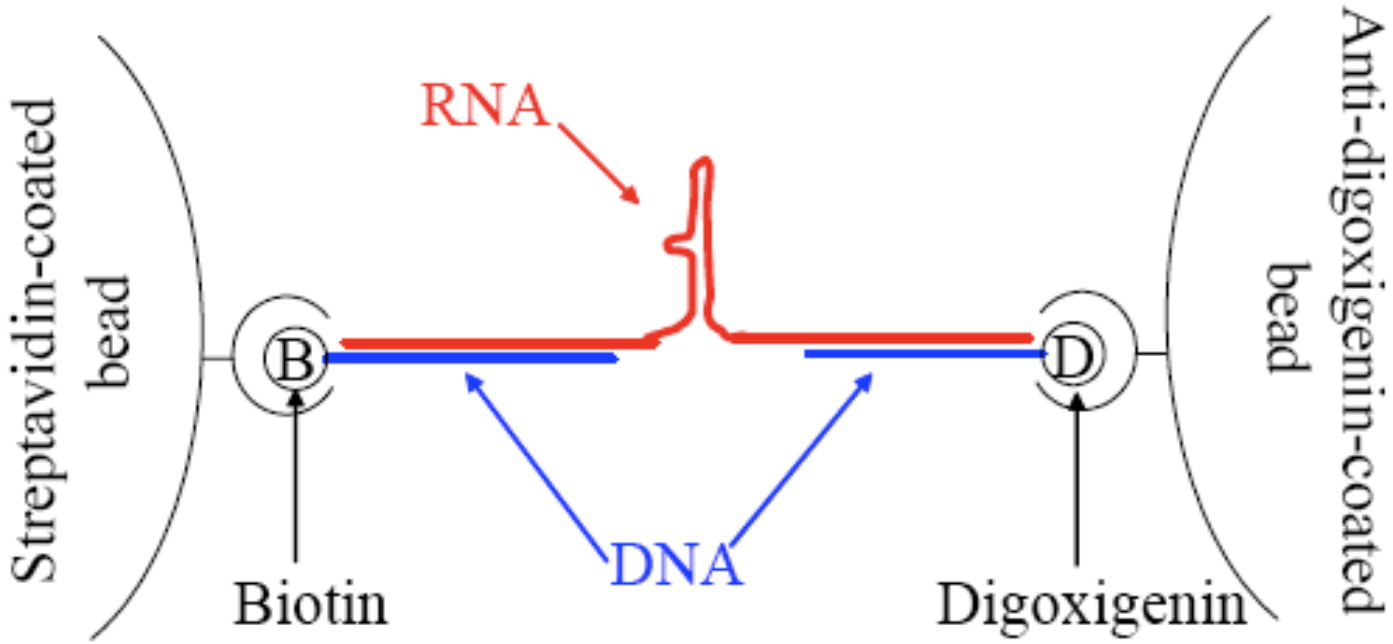
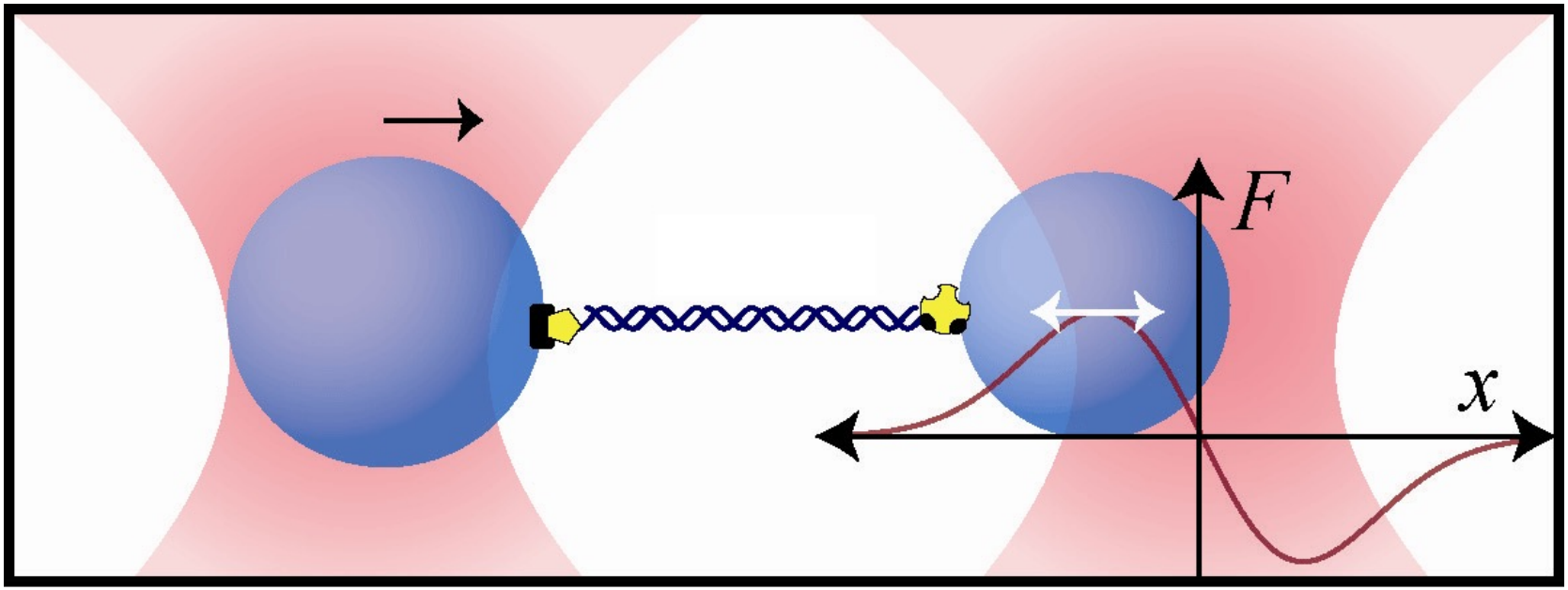
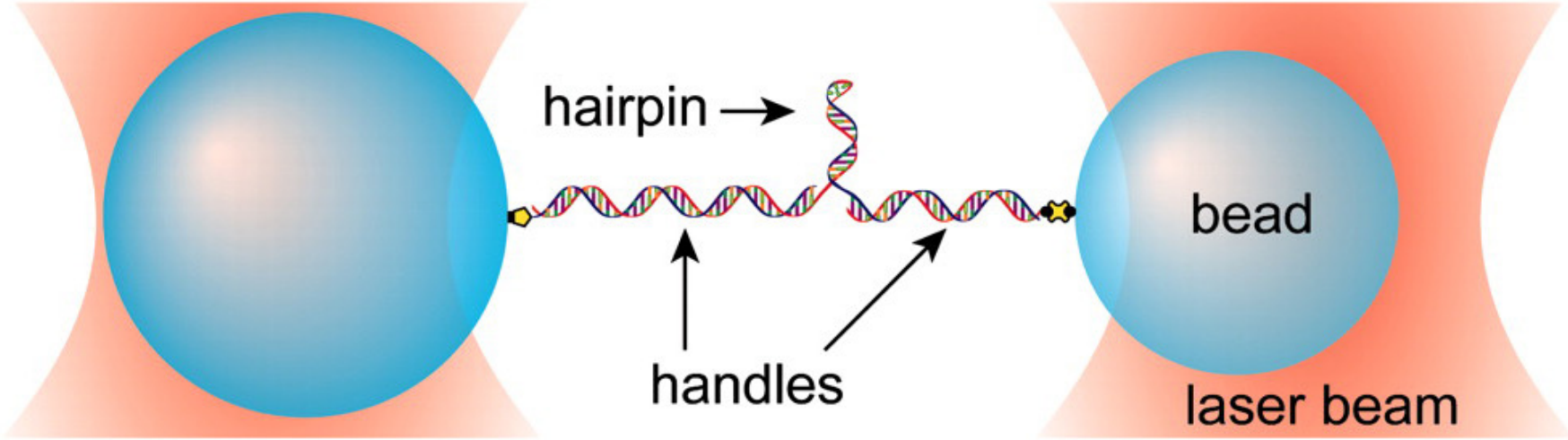
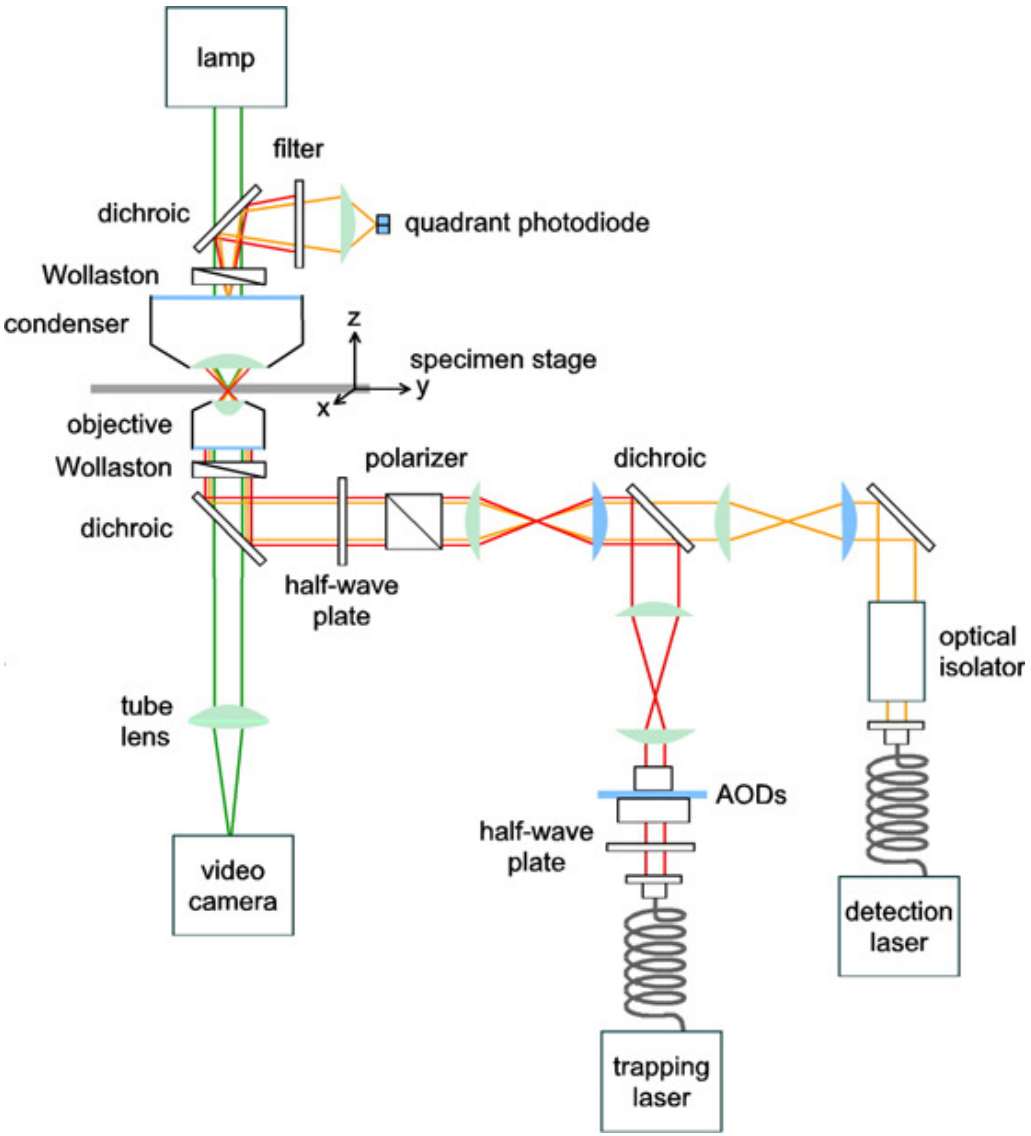


Figure 14. Unzipping a single RNA molecule.

Force induced unfolding/refolding of small RNA hairpins.
Same force for unfolding/refolding at low pulling rates (thermodynamic reversibility).


F
x


hairpin
handles
bead
laser beam



Modern Optical Tweezers: In practice, optical tweezers are very expensive, custom-built instruments. These instruments usually start with a commercial optical microscope but add extensive modifications. In addition, the capability to couple multiple lasers into the microscope poses another challenge. High power infrared laser beams are often used to achieve high trapping stiffness with minimal photo-damage to biological samples. Precise steering of the optical trap is accomplished with lenses, mirrors, and acousto/electro-optical devices that can be controlled via computer. Figure 3 is meant to give an idea of the number of elements in such a system. In short, these are very complicated instruments that require a working knowledge of microscopy, optics, and laser techniques.

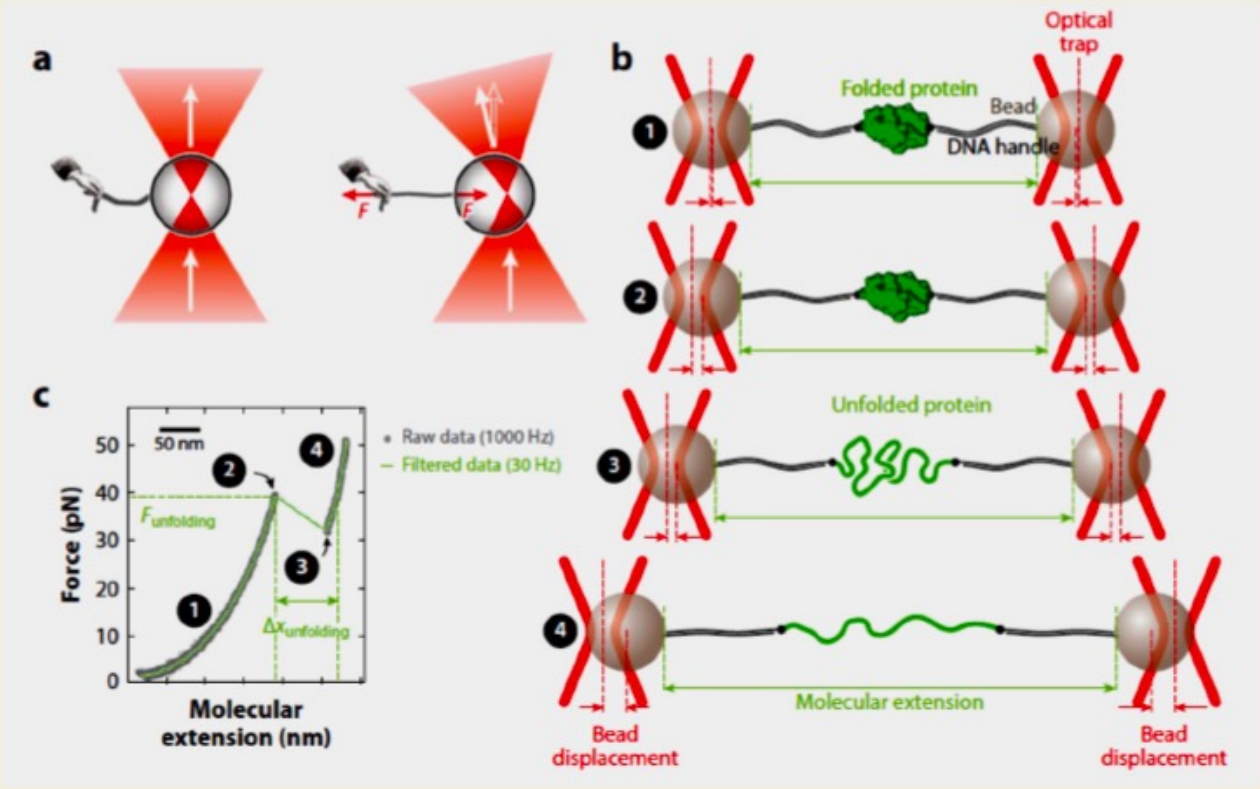
http://stanford.edu/group/blocklab/

e F5.1. *Comparison of atomic force microscopy, the laser trap, the magnetic trap and the gl microneedle*

	Atomic force microscopy	Laser trap	Magnetic trap	Glass microne
ion	Bending of cantilever obeys Hooke's law	Intensity gradient from photons from laser source	Supermagnetic bead in magnetic field	Bending of microneedle o Hooke's law
c range	20 pN–20 nN	0.1–400 pN	A few fN–100 pN	1 pN and as la desired
onstant	0.05 N m^{-1} (typically)	0.0002 N m^{-1}	$\sim 10^{-7}$ N m^{-1}	2×10^{-6} N m^{-} (typically)

lapted from Wang *et al.* (2001).

Manipulation of single molecules using dual OT.



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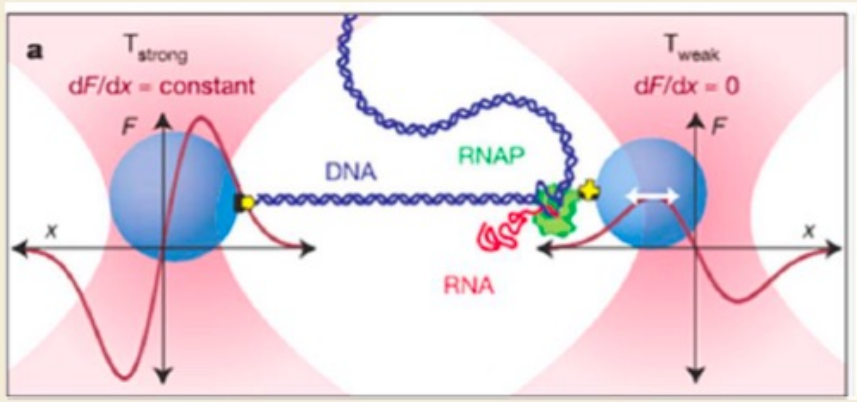
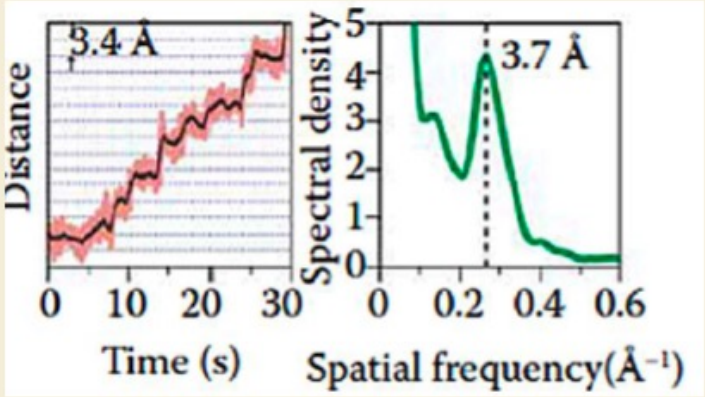
Single-Molecule Studies of Protein Folding with Optical Tweezers
Carlos Bustamante et all Review

Double-trap assay.



Stiff trap

Weak trap



Example: Dynamics of DNA-processing enzymes.

Single, transcriptionally active molecule of RNA polymerase (RNAP, green) attached to a bead held in a trap and tethered via the upstream DNA to another trapped bead. During elongation, the DNA tether lengthens and the beads move apart.

A representative record for a single RNAP molecule transcribing under 18 pN of assisting load. Horizontal lines (dotted) are spaced at 3.4 Å intervals (distance between base sets)

The power spectrum of the average autocorrelation function derived from position histograms shows a peak at the spatial frequency corresponding to the inverse of the fundamental step size, 3.7 ± 0.6 Å.

Abbondanzieri, E.A., et al., Nature, 438, 460–465, 2005

OT for single molecule spectroscopy; spatial and temporal resolution.

- measure conformational changes and displacements produced by single biological molecules.

Such movements range from several nanometers (molecular motors) down to one base pair (0.35 nm, for DNA and RNA processing enzymes) -- > **high spatial resolution** detection based on Interferometry (see next section).

Actually, position detectors do not set a limit on spatial and temporal resolution of OT.

Thermal noise sets instead fundamental limits on displacement and force measurements with single molecules.

Temporal resolution limit due to relaxation time for bead position.

When a single bead trapped in optical tweezers is perturbed from equilibrium, for example, by protein conformational changes or by trap displacements, it moves exponentially to a new equilibrium position with a time constant (relaxation time) $\tau = \gamma/k$, where γ is the viscous drag coefficient and k the stiffness of the system. Therefore, systems with higher stiffness attached to small probes exhibit faster responses to perturbations. If the perturbation develops faster than τ, the bead moves with the same relaxation time τ, filtering out all the movements that occur on shorter time scales.

AFM : k = 1 pN /nm → τ – microseconds , OT : k= 0.001 pN/nm → τ - milliseconds