Optical Tweezers

-working principles and applications

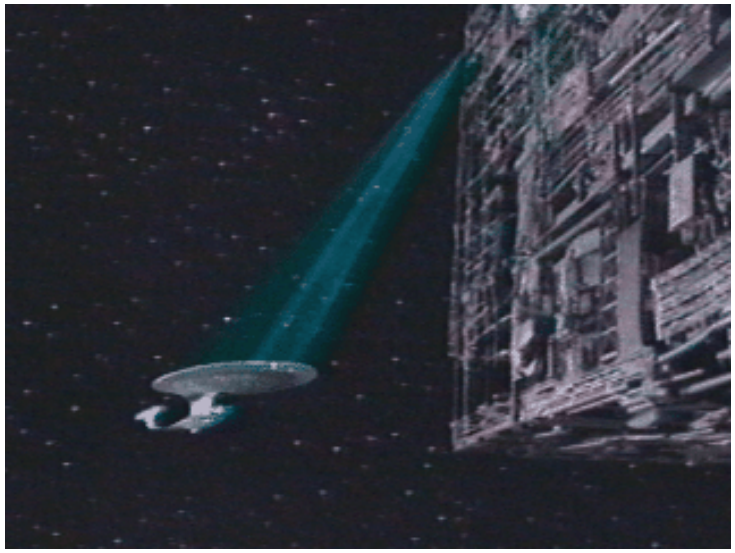


Photo taken from the WWW Star Trek Picture Page

Biophysics with optical tweezers

Optical tweezers use forces of laser radiation pressure to trap small objects

This technique is 20 years old, and used in biophysics the last 10 years

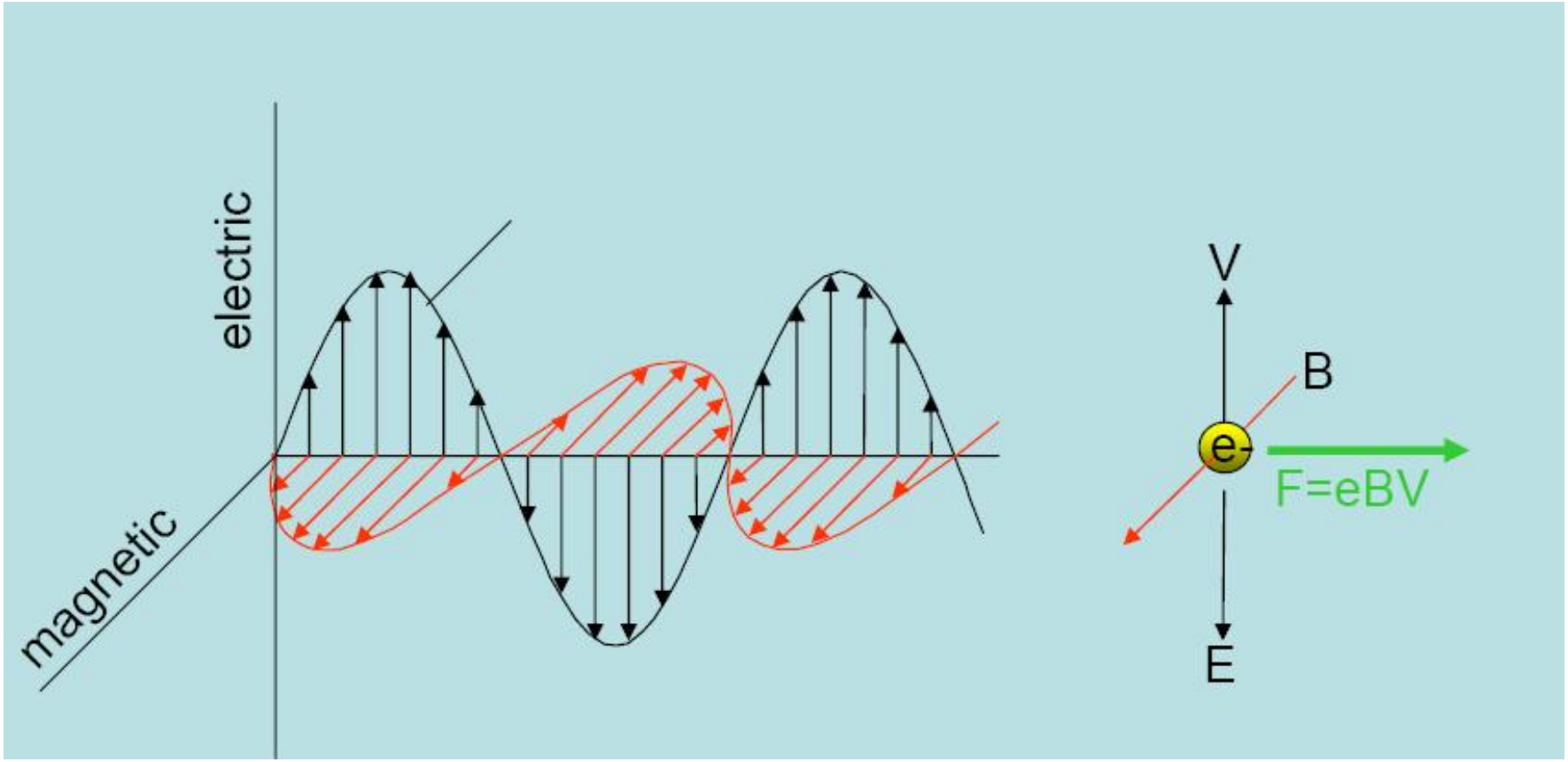
Outline of lecture:

- Optical tweezers physics (40 min)
- Technical issues (30 min)
- Characteristics of optical tweezers (15 min)
- How are they used (60 min)

Light exerts force on matter

- EM waves interact with electrons in matter

James Clerk Maxwell
1831-1879



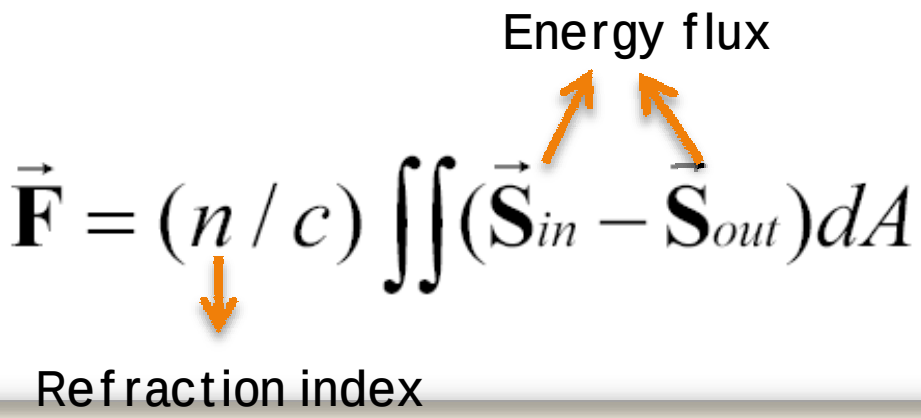
Radiation Pressure

Radiation pressure is the force per unit area on a object due to change in light momentum

The light momentum of a single photo is:

$$|\vec{p}| = \frac{h}{\lambda}$$

The change is momentum can be calculated by the difference is momentum flux between entering and leaving a object



How big is the optical pressure?

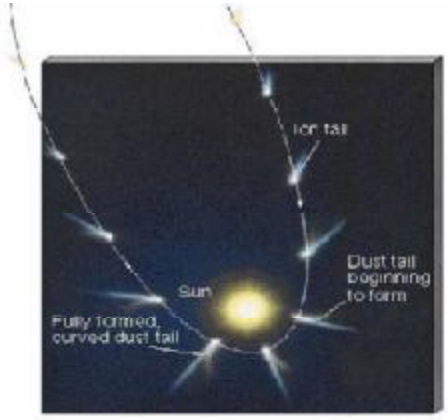
Applying this formula to a 100% reflecting mirror reflecting a 60W lamp gives a pressure of:

$$\vec{\mathbf{F}} = 2(n/c)\iint(\vec{\mathbf{S}}_{in})dA$$

$$F = 2(n/c)W = 4\text{x}10^{-7}\ \text{N}$$

Gravity pulls on a 1 kg mirror with 9.8 N so the force of the photos is negligible.

- Sunlight on earth 0.5 nN/cm2
- Laser pointer ~10 pN



However, if the same light is reflected by a object of 1 µg it can't be ignored! (1µg ~ a 100 µm droplet)

Using a laser on a microscopic particle will realize this situation.

Arthur Ashkin builds first optical trap (1970)

VOLUME 24, NUMBER 4 PHYSICAL REVIEW LETTERS 26 JANUARY 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

How the light passing through a transparent particle?

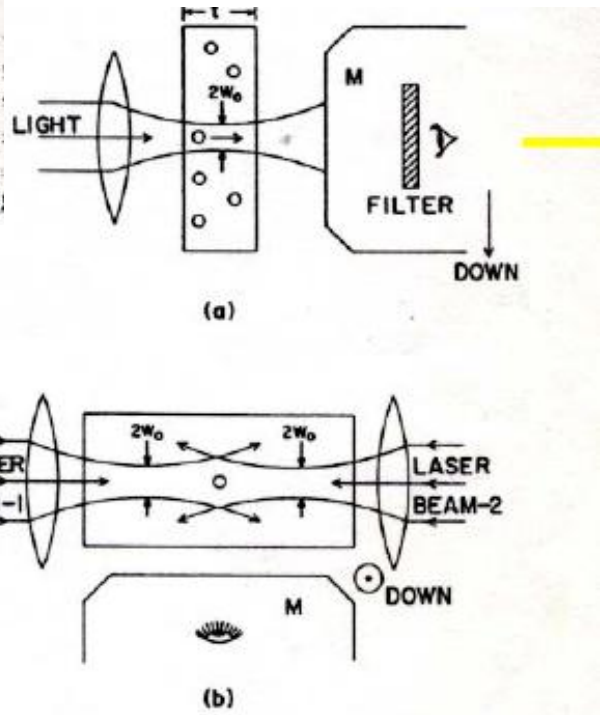
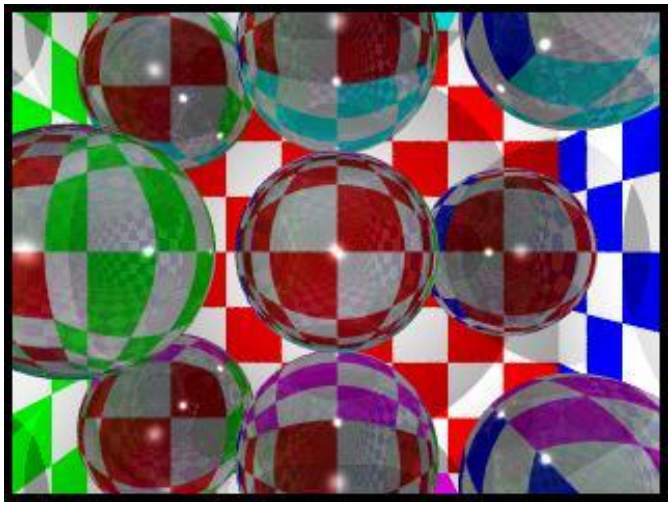


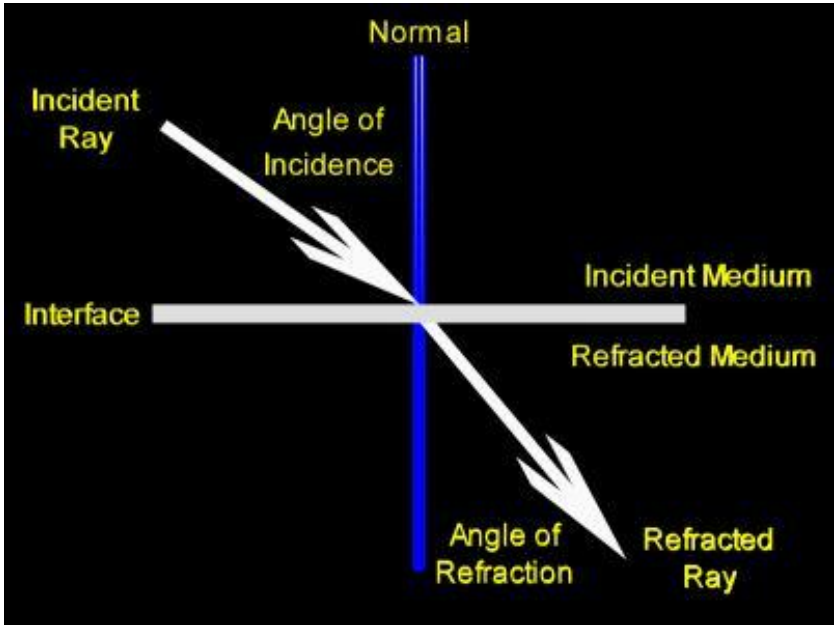
FIG. 1. (a) Geometry of glass cell, $t = 120\ \mu m$, for observing micron particle motions in a focused laser beam with a microscope M. (b) The trapping of a high index particle in a stable optical well. Note position of the TEM_{00}-mode beam waists.

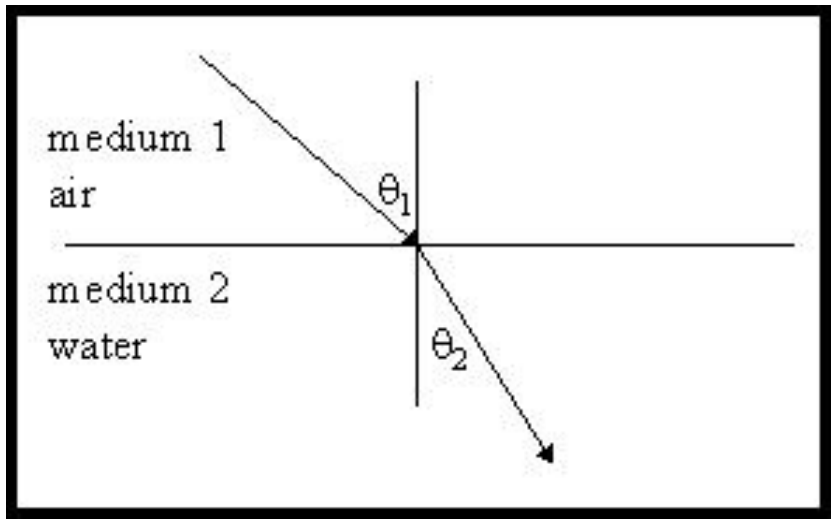
Basic Ray Optics



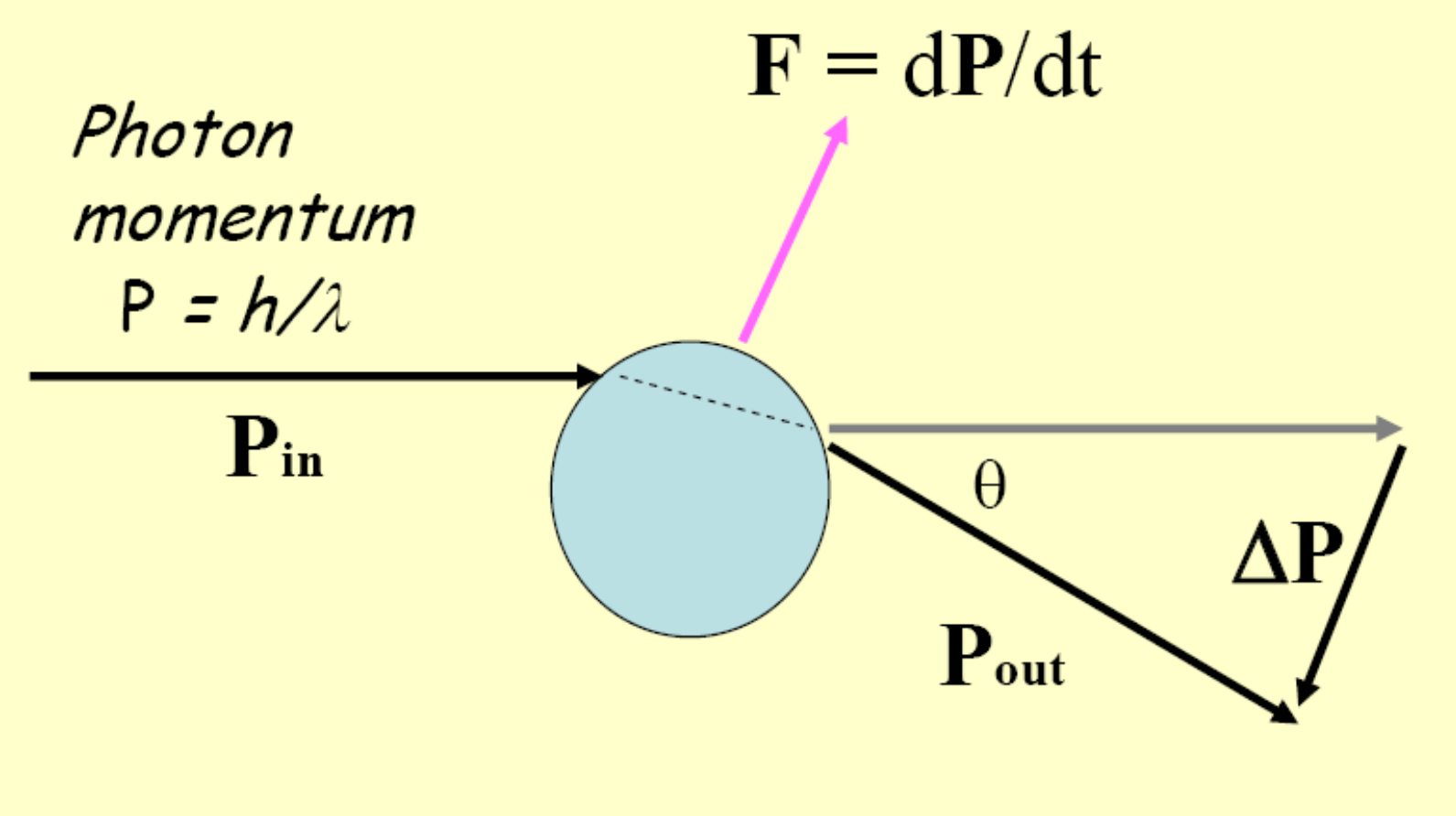
Light refraction
Light is bent by the glass sphere.

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{v_1}{v_2} = \frac{n_2}{n_1}$$ Snell's law





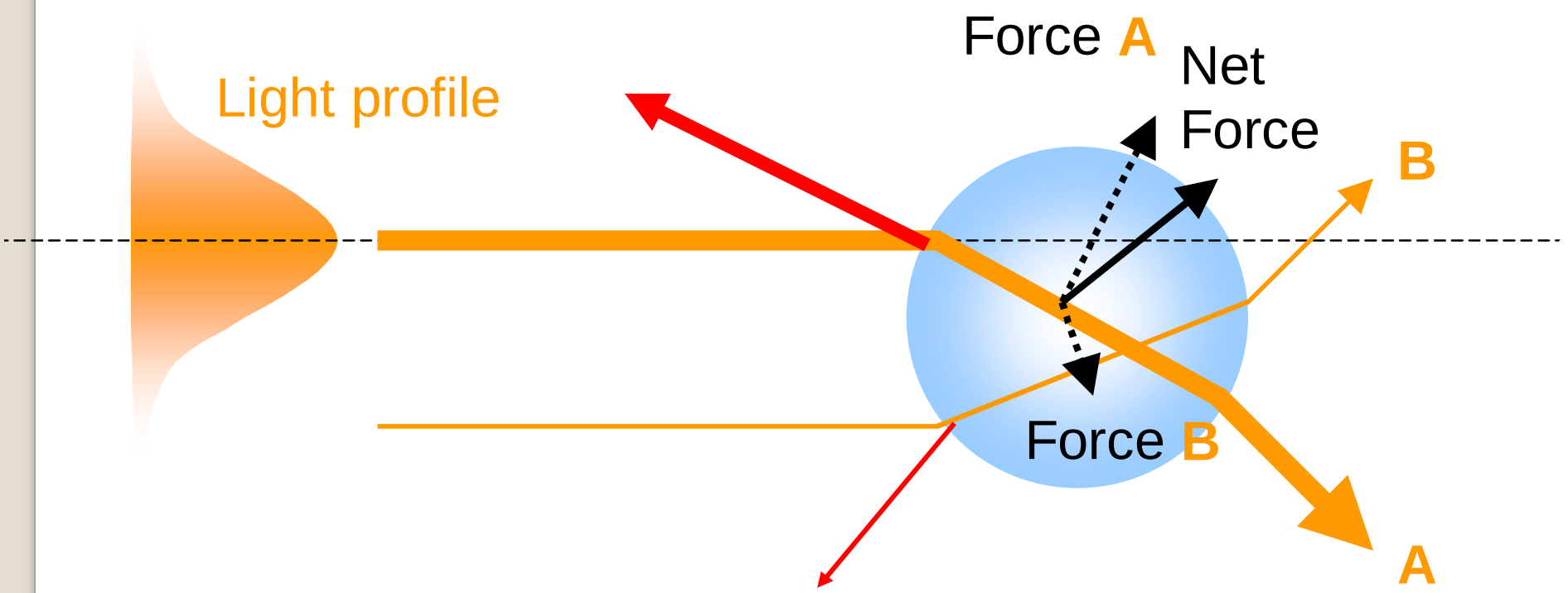
Photon meets refracting object



For every action there exists an equal but opposite reaction

~Sir Isaac Newton (3rd law of motion)

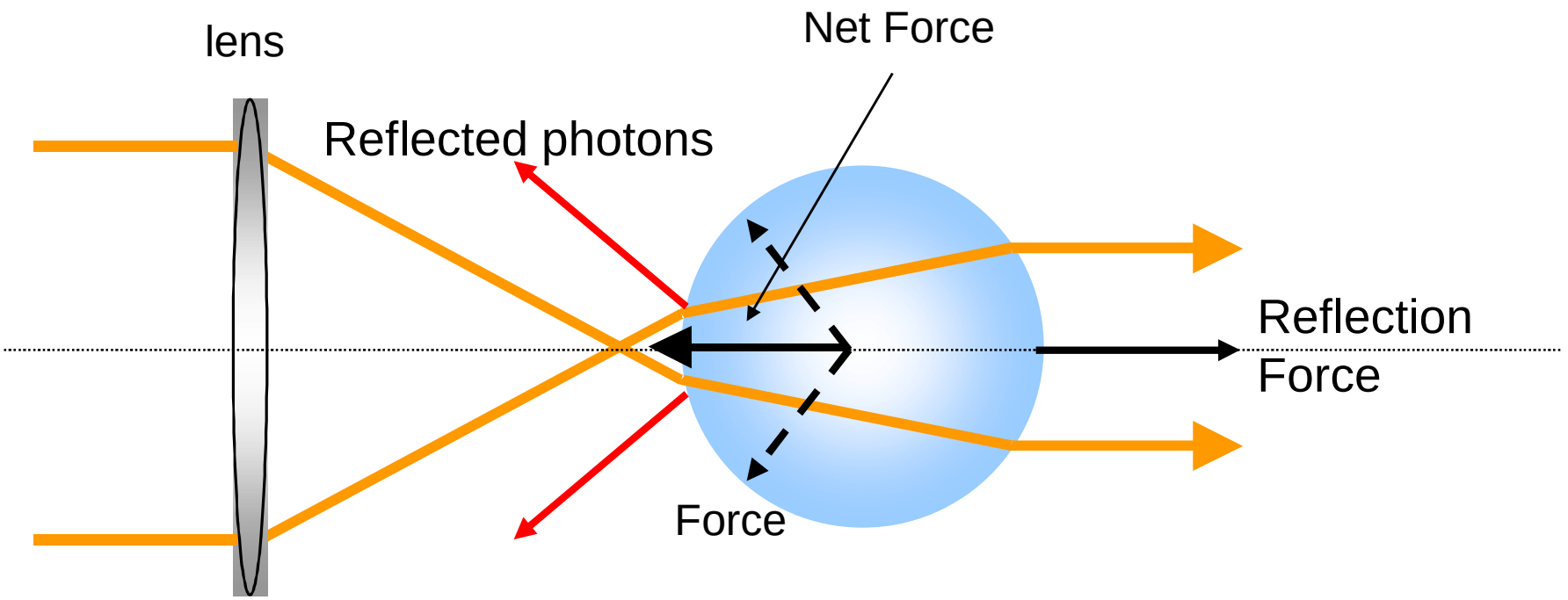
A particle in a beam (Transverse)



Force develops when rays of light act on objects with different index of refraction compared to the surrounding media.
The particle gets push towards the highest light intensity.

However there are also reflections pushing the bead forward

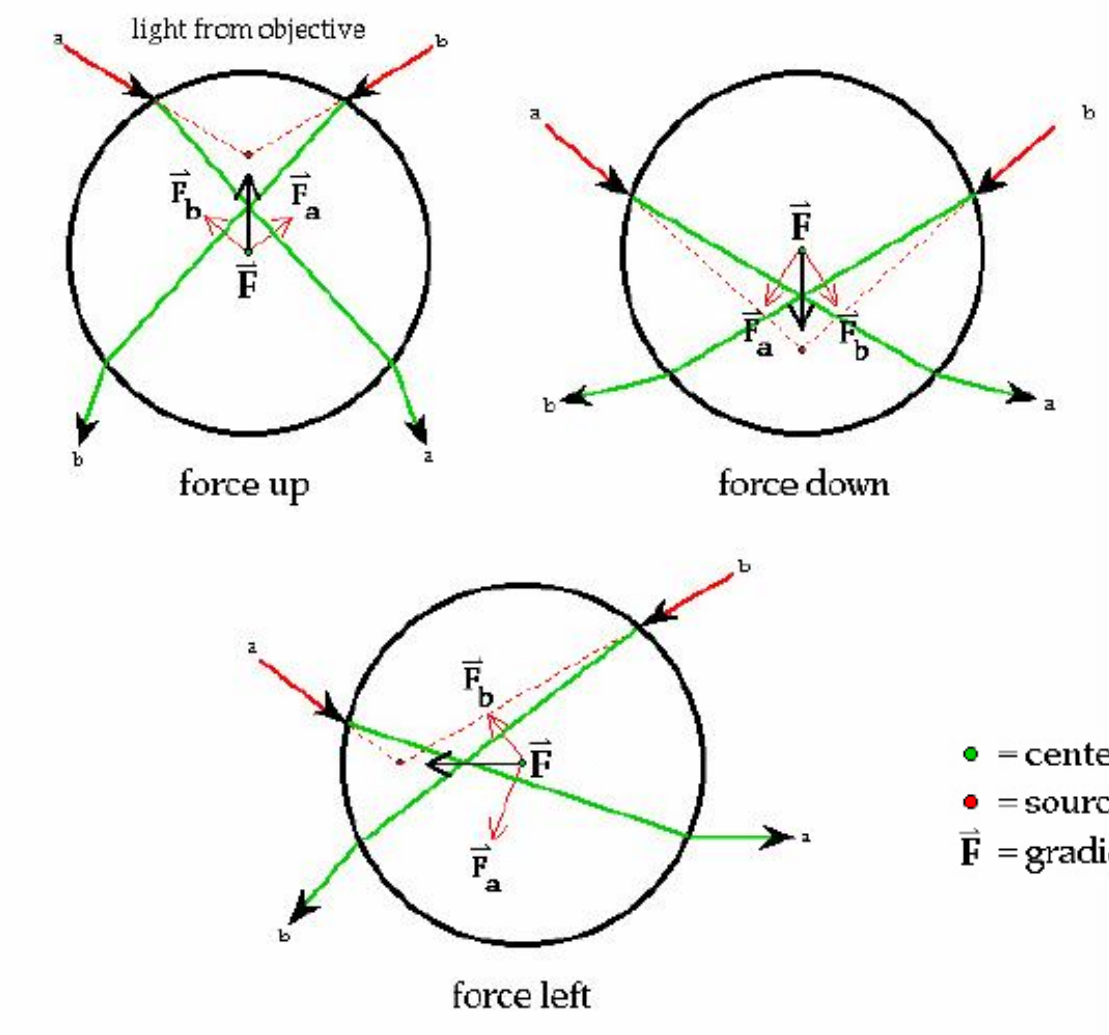
A particle in laser beam (Longitudinal)



Result: A particle can get sucked into the focus of a laser bundle and be stably trapped.

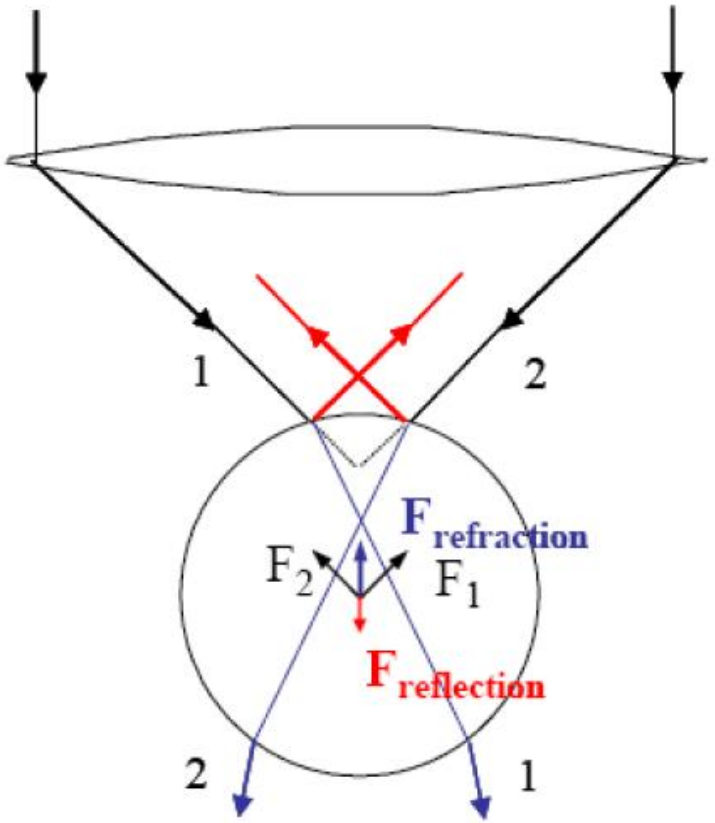
Thus highly focused laser beam acts as a three-dimensional potential minimum. Therefore it takes force the dislodge a bead out of the laser focus.

Sphere will be pushed toward focus



The Reflection force (can not be cancelled out)

- There will also be a force due to reflected light.
- This will push the sphere away from the focal point.
- The refractive force must overcome the reflection force to trap the sphere

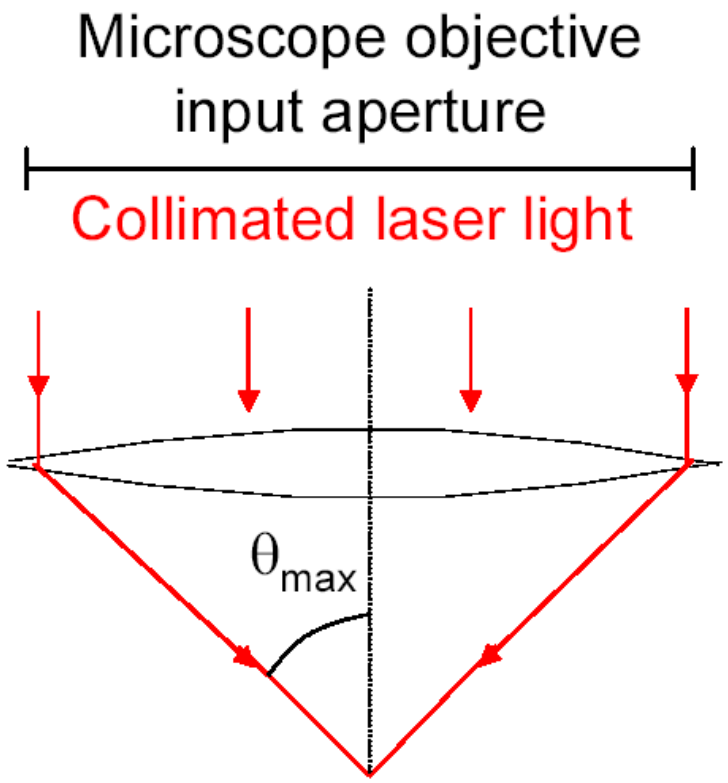


Solution 1: overcome it

But using a high NA objective, the refractive force can overcome the forces due to reflections.

What is a high NA objective?

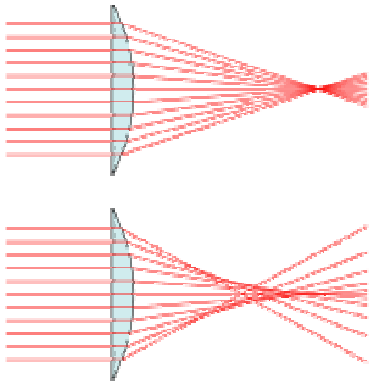
$$NA = n \sin(q_{\max})$$



Choosing microscope objective properly

- High numerical aperture objective (NA = 1.2 ~ 1.4)

- High NA through oil or water immersion
  - *Spherical aberration degrades performance*
  - *Water immersion objectives are better (less n mismatch; longer WD)*



- Transmission at trapping wavelength
  - *NIR transmission (we'll discuss why we need NIR later)*
  - *Dual-objective method to measure transmission*

288 OPTICS LETTERS / Vol. 11, No. 5 / May 1986

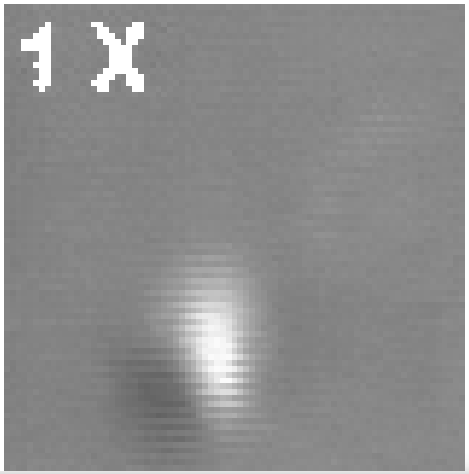
Observation of a single-beam gradient force optical trap for dielectric particles

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and Steven Chu

AT&T Bell Laboratories, Holmdel, New Jersey 07733

- a stable single-beam trap

Anti-scattering force:
Forward momentum is increased by lens-focusing effect.



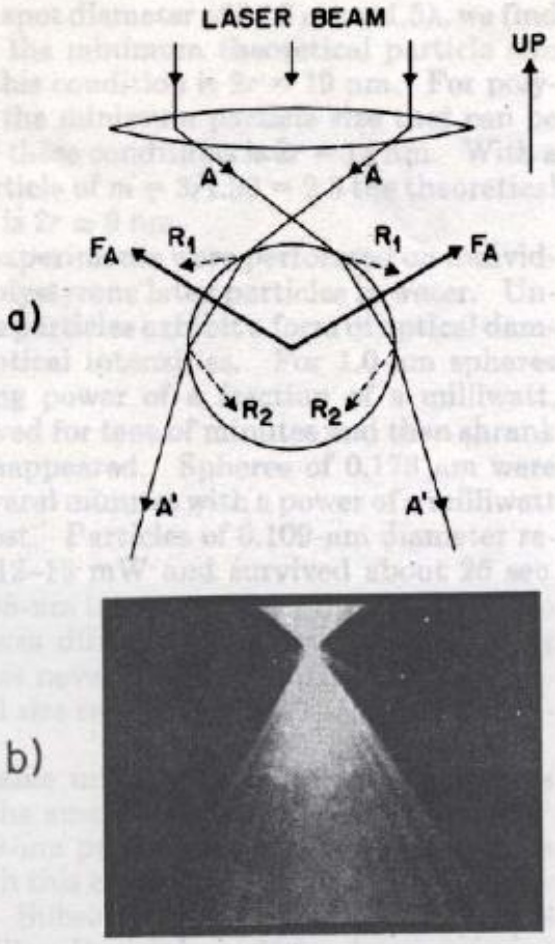
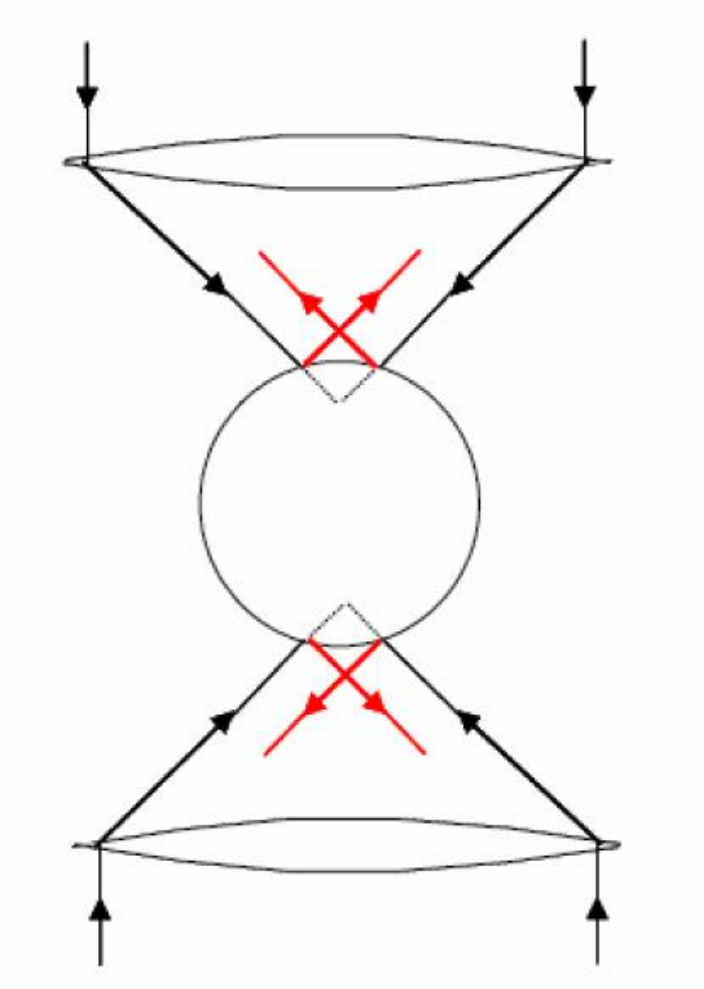


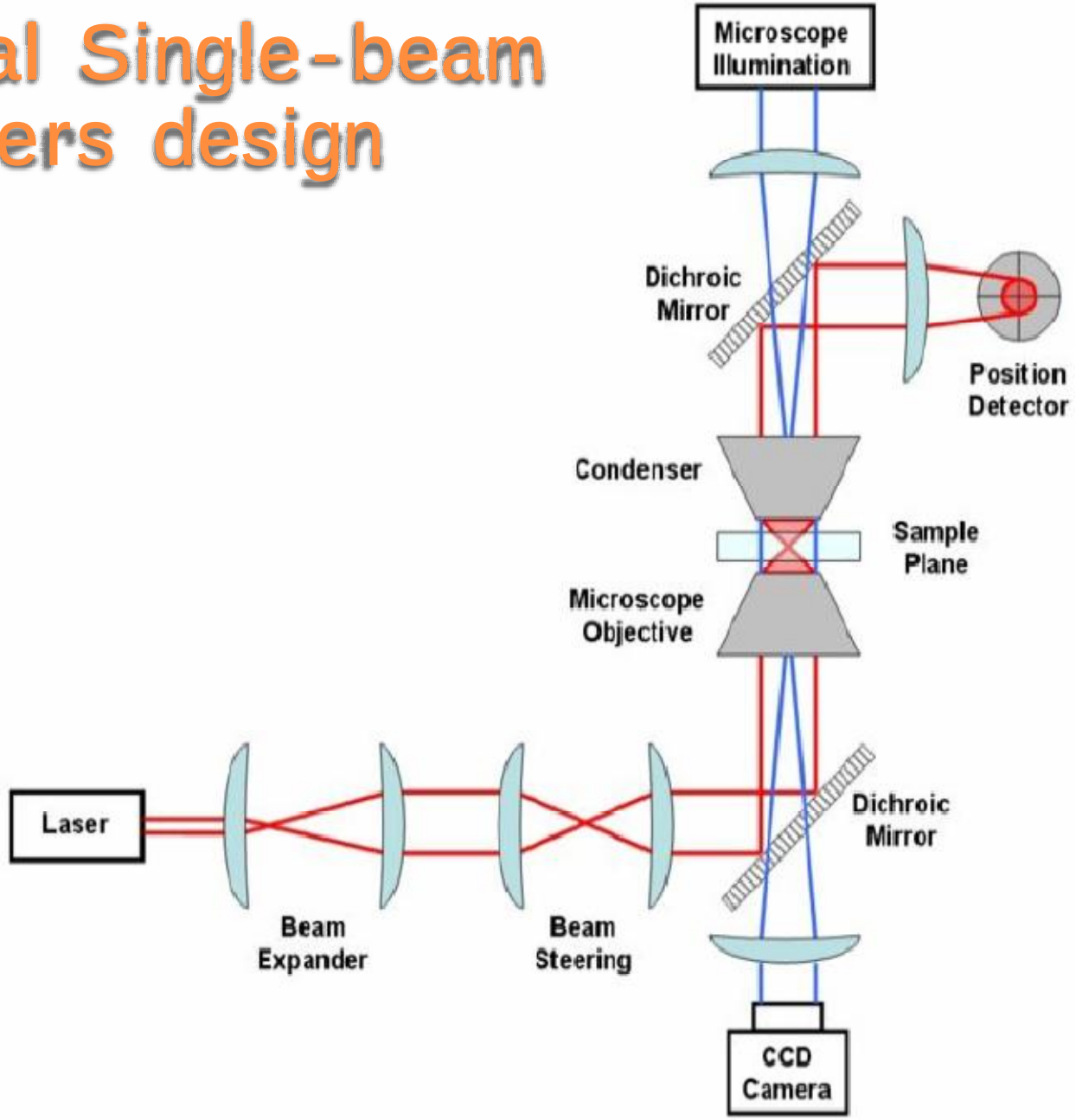
Fig. 1. a) Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single-beam gradient force trap. b) Photograph, taken in fluorescence, of a 10-μm sphere trapped in water, showing the paths of the incident and scattered light rays.

Solution 2: cancel it out

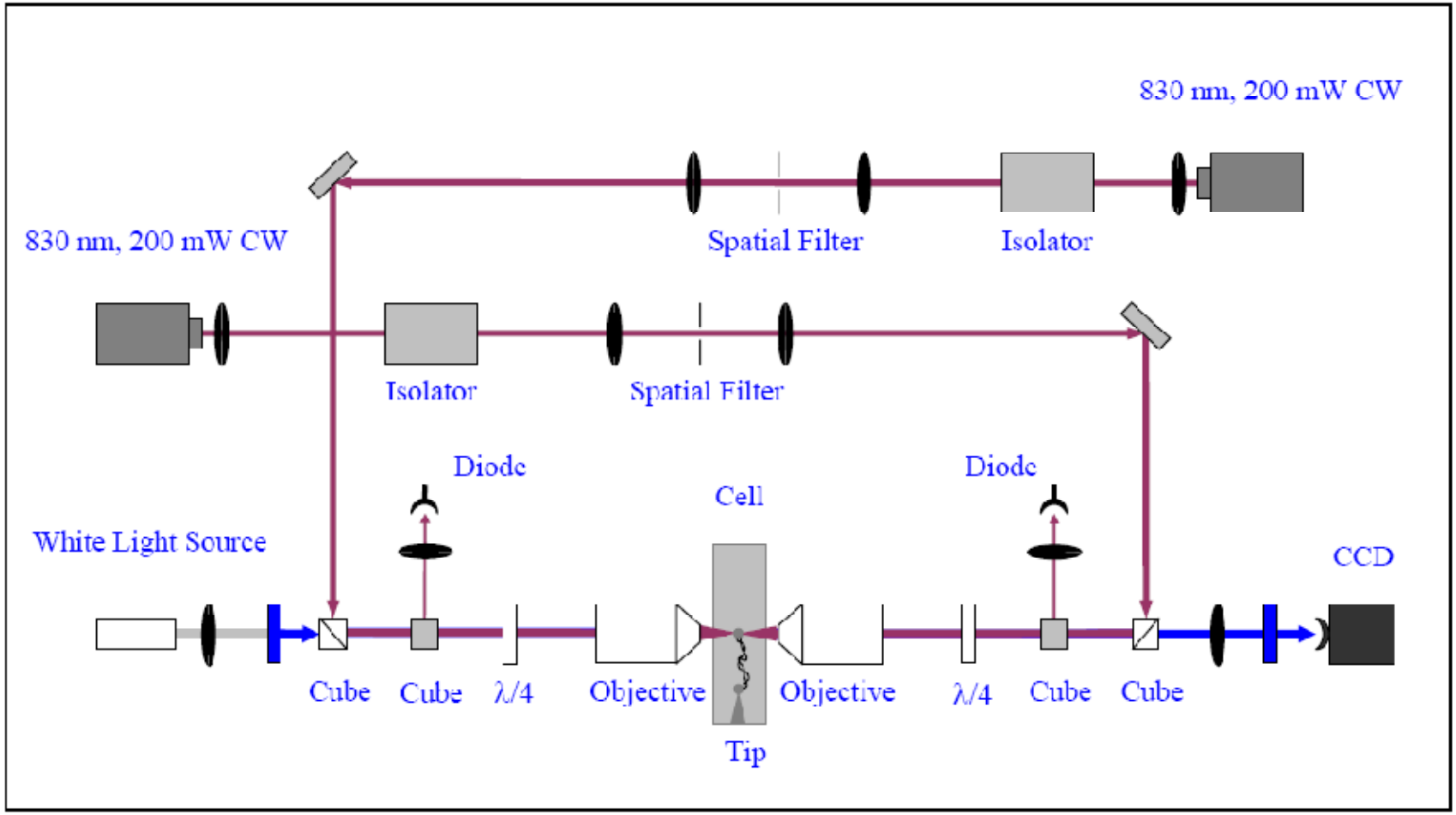
- We can also use two laser beams to trap the sphere
- Reflective forces cancel
- Low NA objectives
- The design is complex and difficult to keep both lasers aligned (at least twice the equipment investment)



Typical Single-beam tweezers design

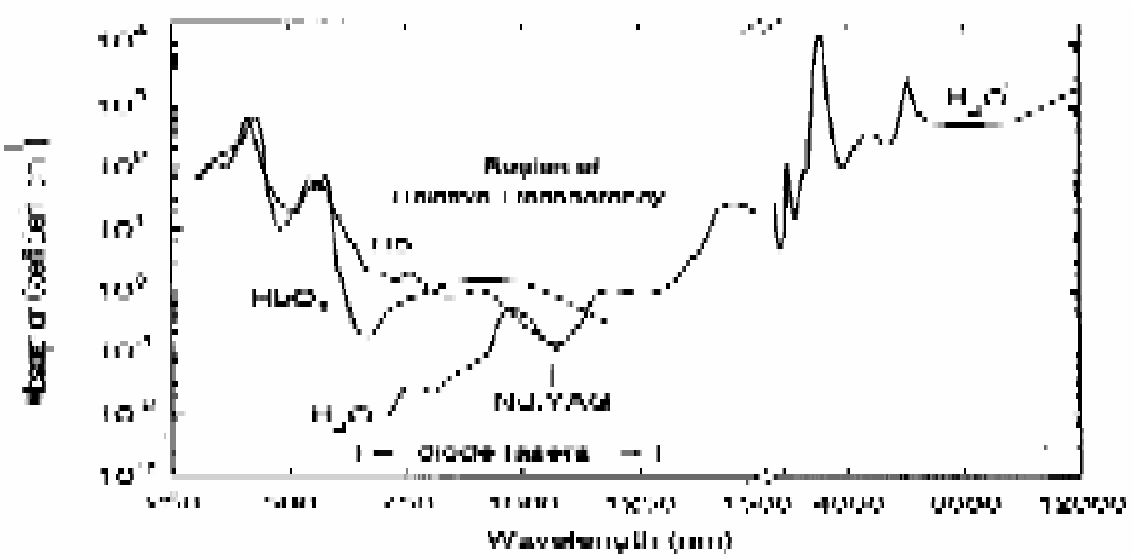


Typical Dual beam tweezers design



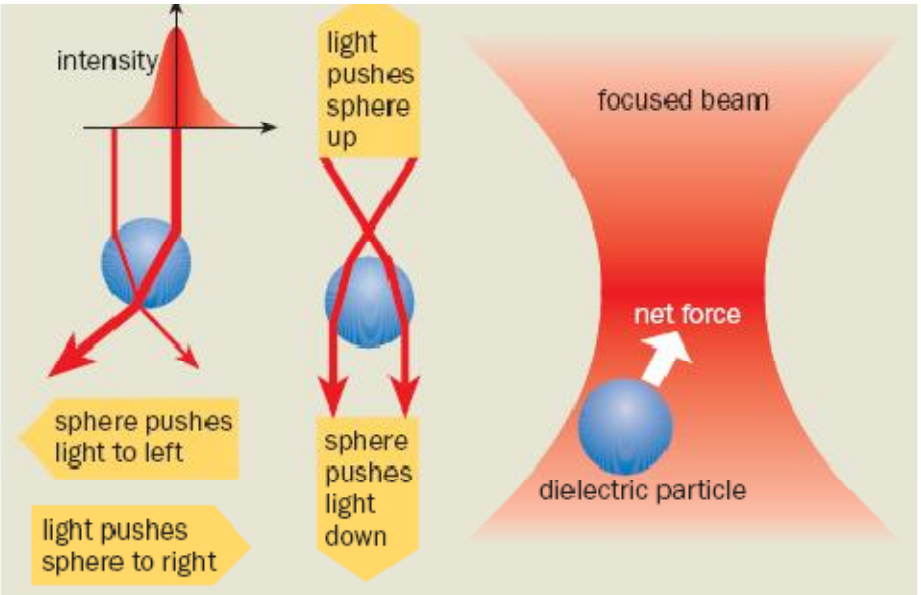
Optical damage

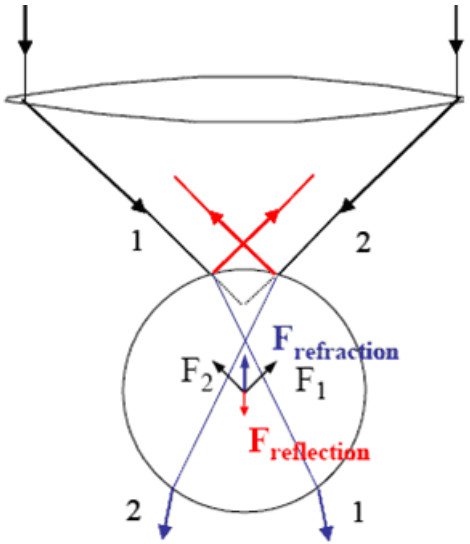
- Biological specimens are relatively transparent in the near infrared (750 – 1200 nm)
- Damage minimum 830 and 970 nm



Basic Physics Summary

. Index refraction mismatch deflect the light
. Momentum variation push sphere towards high intensity region
. High NA or dual beam overcome the reflection



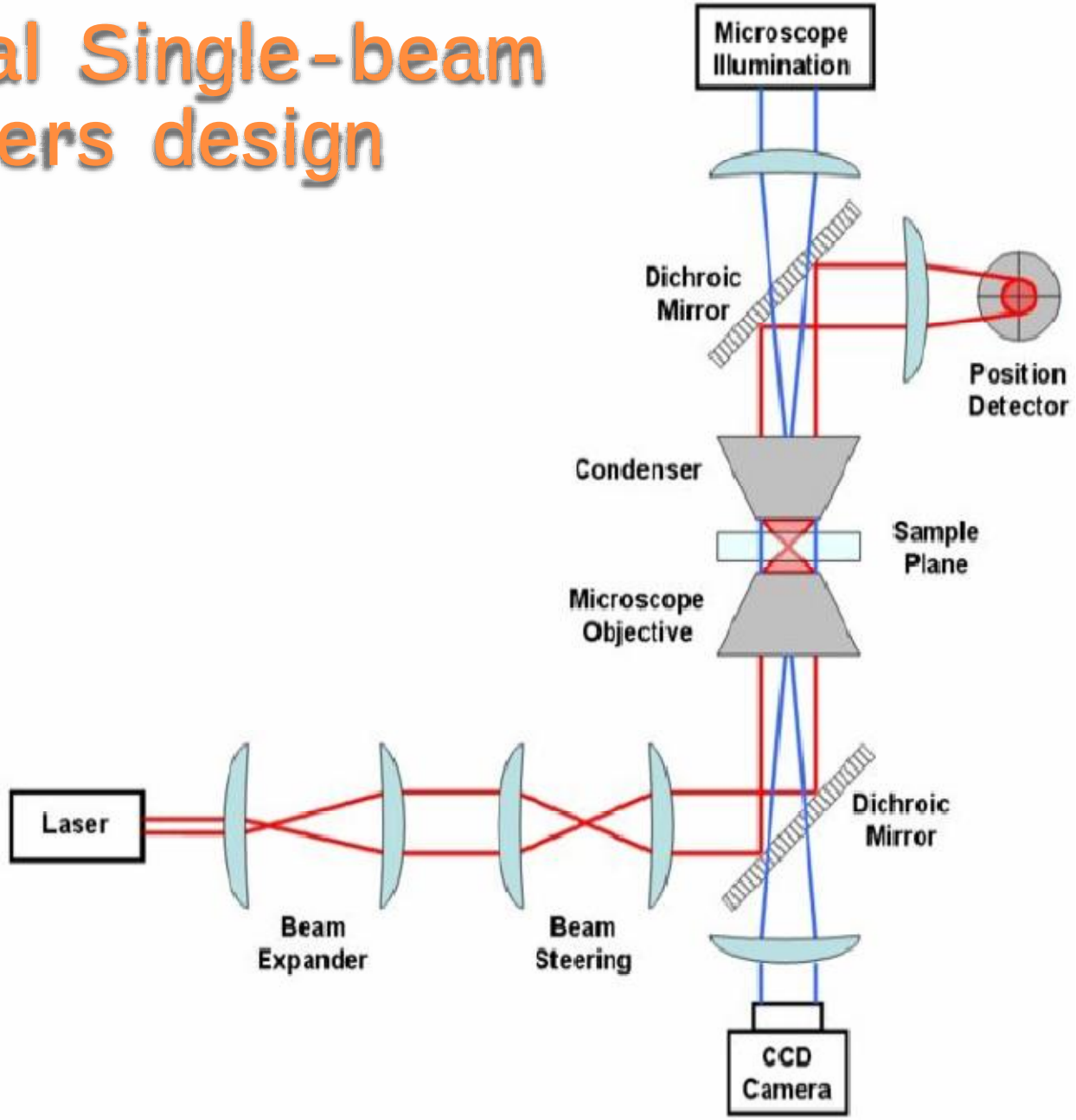


Some Technical issues

1. Trap manipulation
2. Position detection
3. Force & trap stiffness calibration
4. Apply to biophysics studies

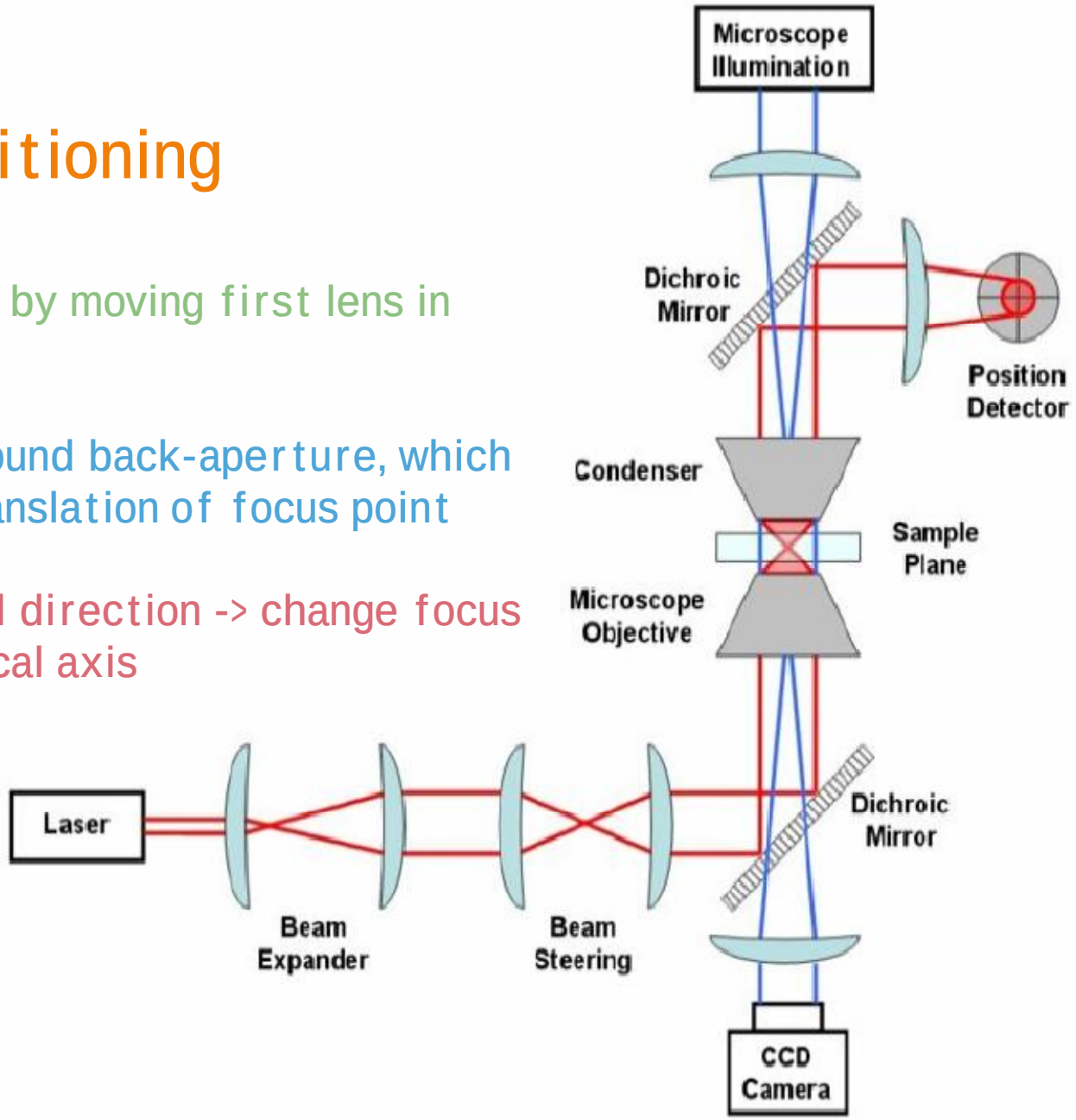
Laser pointer (mW)~10 pN

Typical Single-beam tweezers design



3D trap positioning

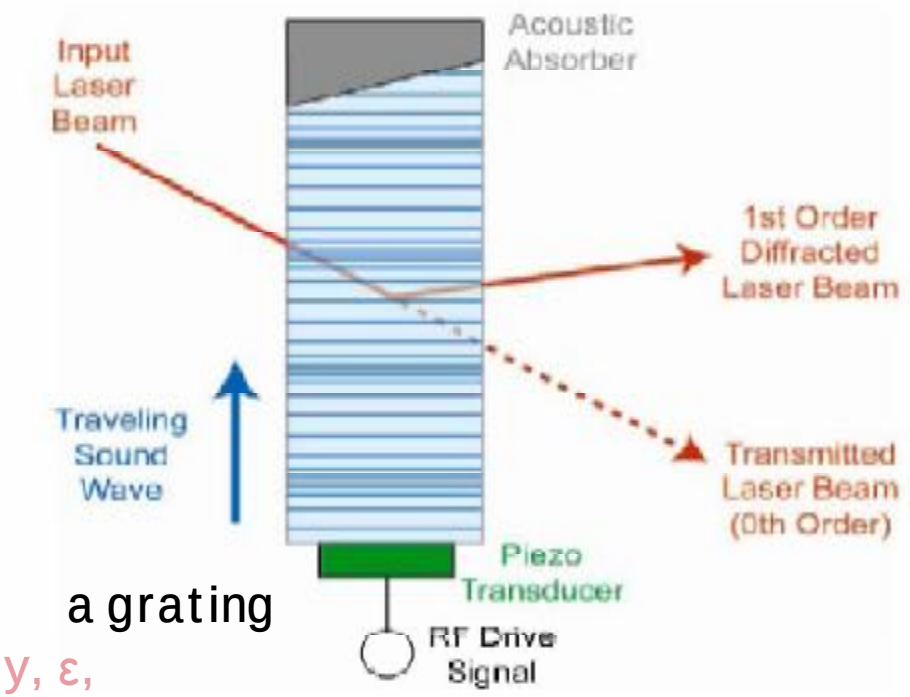
- Move laser focus by moving first lens in telescope
- Beam rotates around back-aperture, which corresponds to translation of focus point
- Move lens in axial direction -> change focus position along optical axis



Dynamic position control

- **Scanning mirror** (Disco Pub)
  - Low losses
  - Large range
  - Slow (1-2 kHz)
  - Lower resolution

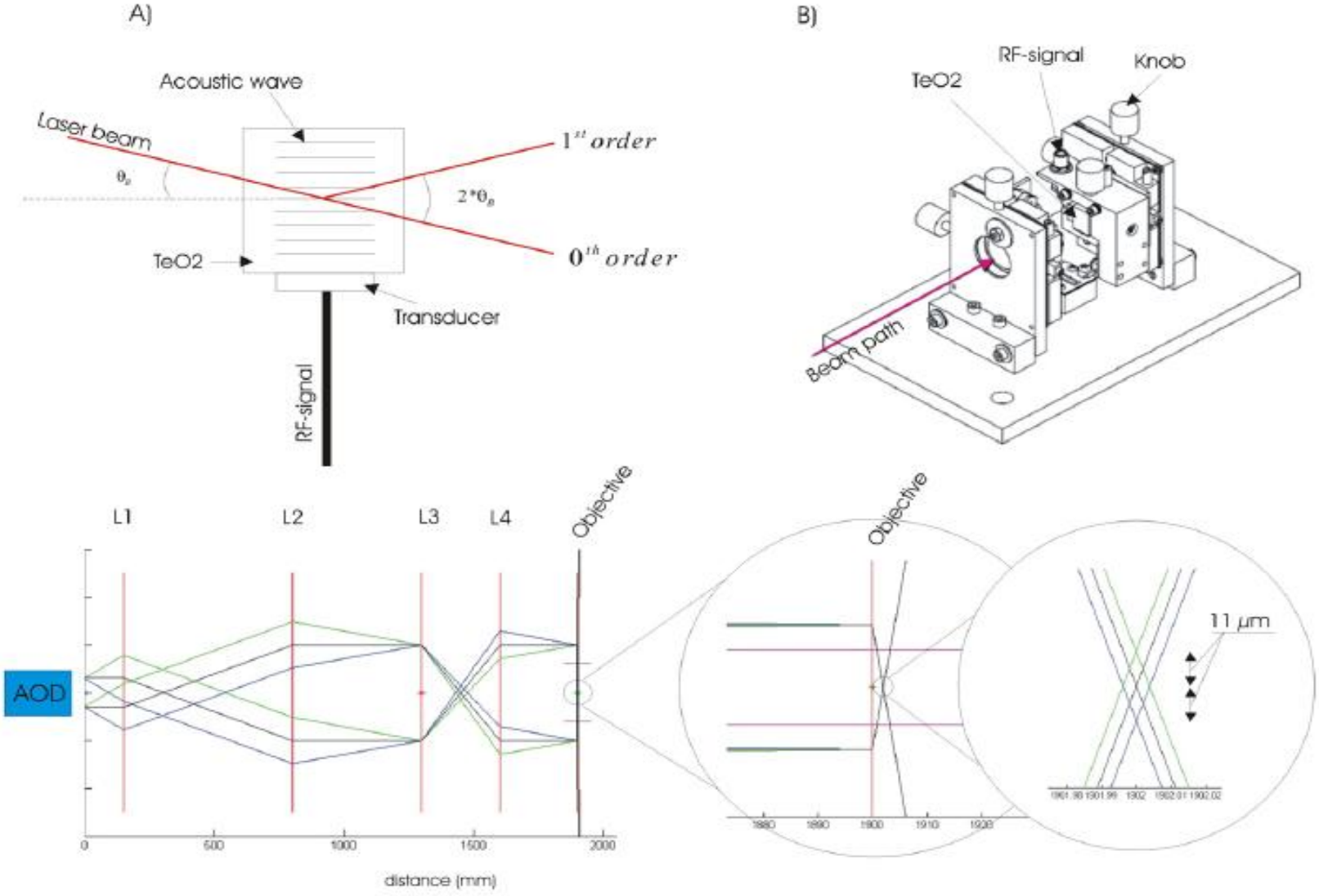
- **Acousto-Optical Deflection (AOD)**
  - Fast (100 kHz)
  - High losses
  - Non-uniform diffraction
  - High-resolution



a grating

a change of a materials permittivity, ε, due to a mechanical strain

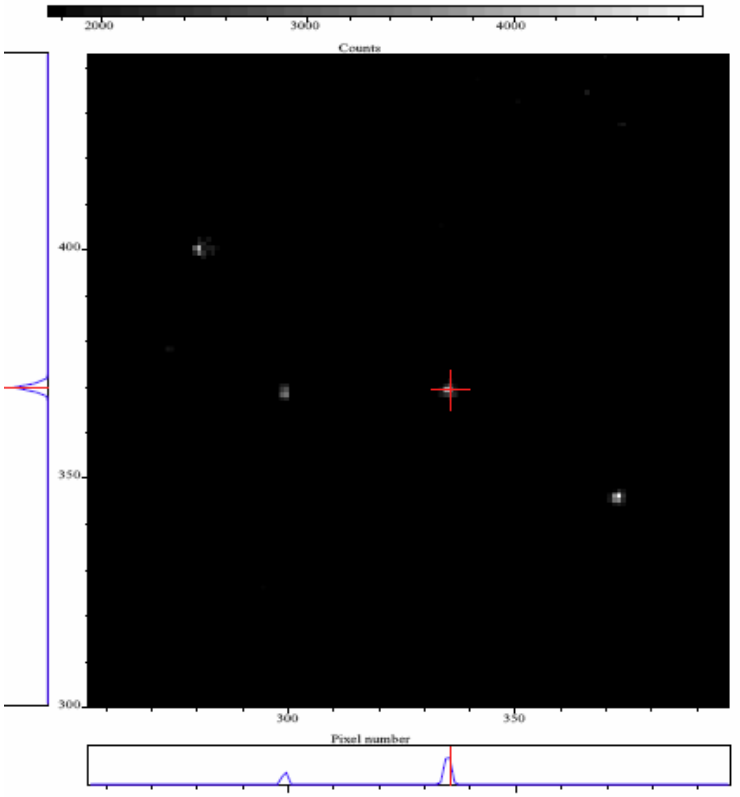
AOD for Trap steering



Position detection

- Video tracking
  - Slow

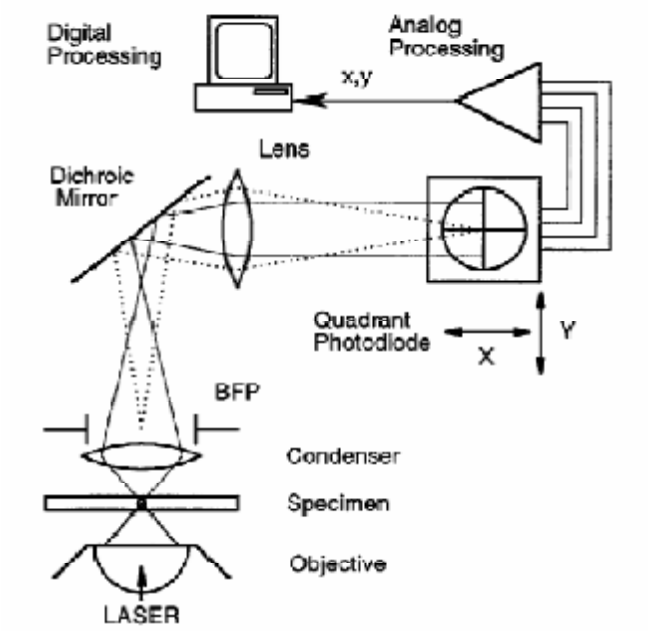
  Data bandwith needed for a 1kx1k CCD
  = 1000x1000x8bit + processing
  (30-120Hz limited by video rate)
  - Absolute position with
    ~10 nm position

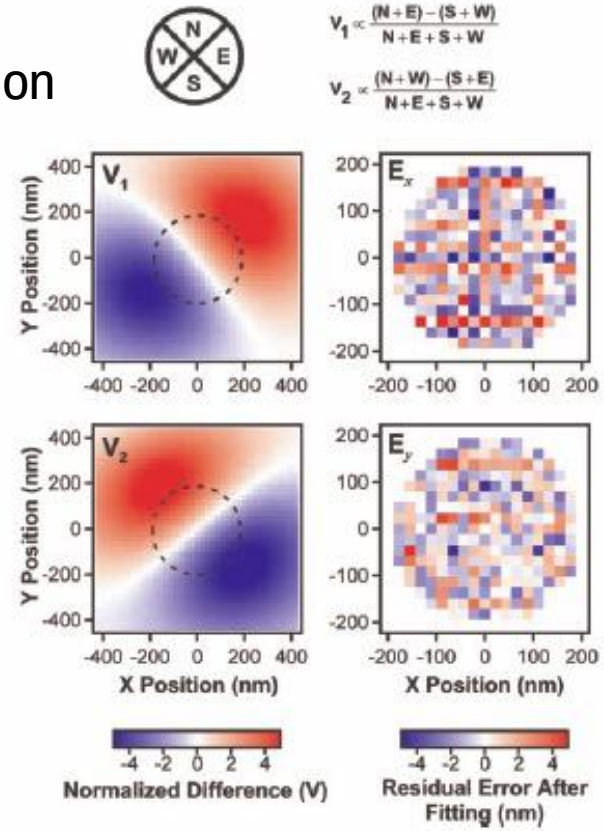


- Laser based Back-focal-plane detection
  - Fast (100 kHz)
  - Relative position (bead - focus)
  - 1nm or better resolution

Laser based Back-focal-plane detection

- Focus a laser on the bead
- Collect light on condensor side.
- Detect interference between unscattered and scattered light
- Image back-focal plane onto a position sensitive detector.





Precise position measurement è force measurement

Force Calibration: Theoretical Power Spectrum

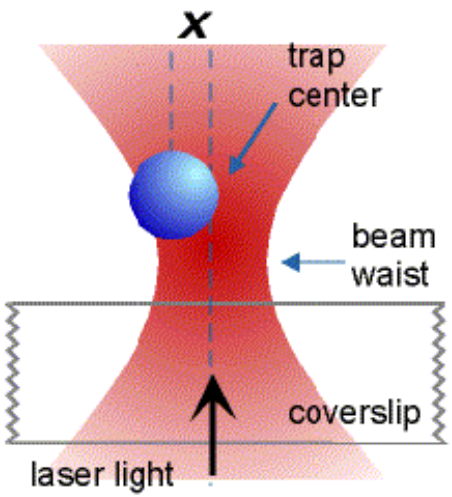
- Eq. Of motion for a Brownian particle in a harmonic potential:

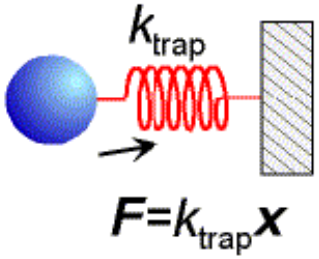
$$\gamma\dot{x} + kx = F(t)$$

$$\gamma = 6\pi r\eta = \text{Stokes drag}$$

k = trap stiffness

$F(t)$ = random thermal force



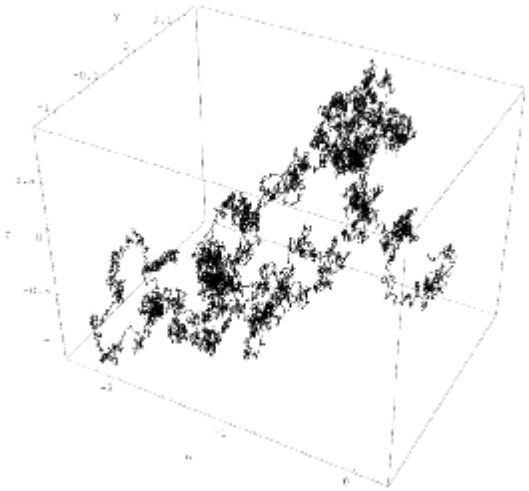


Fourier transform gives Power Spectrum :

$$S_{xx}(f) = \frac{k_B T}{2\pi^3 \gamma (f_0^2 + f^2)}$$

$$f_0 = \frac{k}{2\pi\gamma} = \text{corner frequency}$$

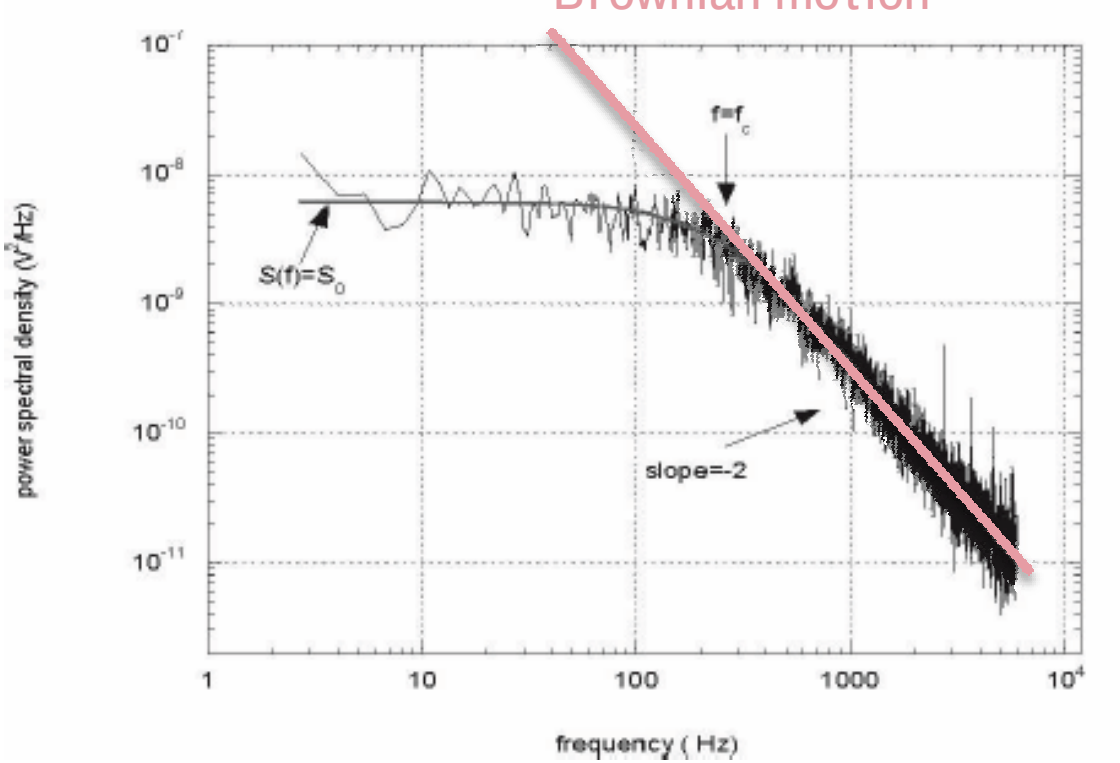
How does the Power Spectrum of a trapped bead look like?



3d Brownian motion

$S_x(f)$ falls off as $\frac{1}{f^2}$

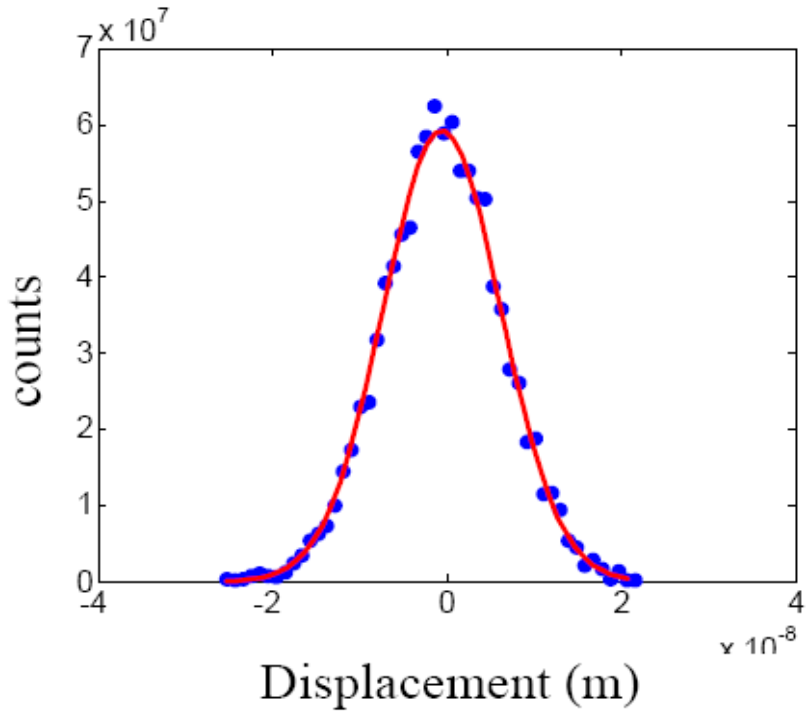
Brownian motion



Force Calibration: Equipartiton Theorem

In any physical system in thermal equilibrium, every particle has exactly the same average kinetic energy, $(3/2)k_BT$. *The* E_k is shared equally among all of its independent parts, *on the average*, once the system has reached thermal equilibrium.

- Equipartition Theorem: $< x^2 >= \frac{k_B T}{k}$



Other technical issues

- Temperature gradients

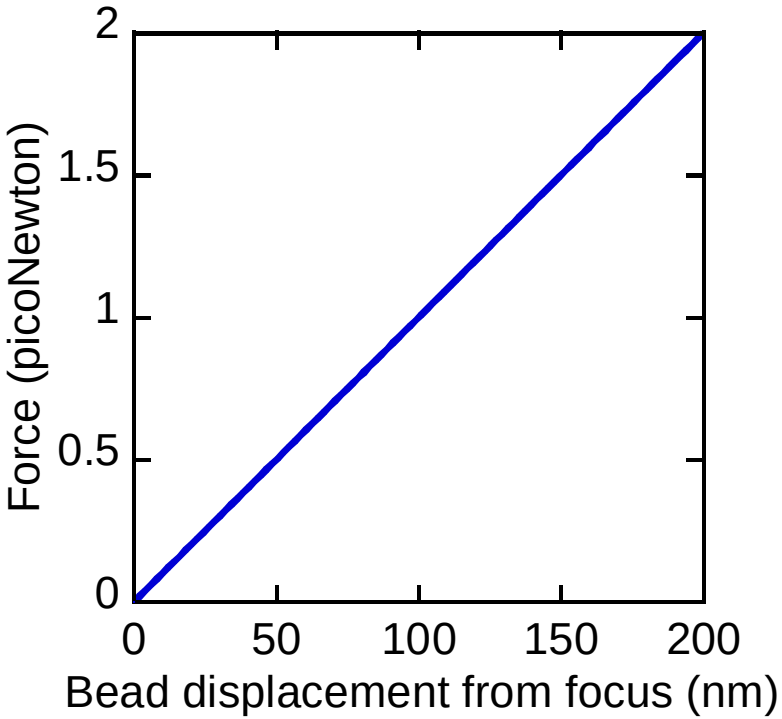
- Acoustic vibration
  - *Powersupplies etc. outside room*
  - *Music and voices easily coupled to trap*

- Mechanical vibration
  - *Short optical path*
  - *Damped table*

- Air currents

Setting up "good enough" optical tweezers is not that trivial

Characteristics of optical traps



- Forces are linearly related to the object displacement.
- The slope of the force-displacement curve is called the stiffness of the optical trap (in N/m).
- The stiffness dependence on the bead size and shape and the laser power.

Characteristics of optical traps

Micrometer sized glass or polystyrene beads are commonly used as attachment handles of the materials under investigation.

The advantage of this approach is the clear and uniform interaction between the beads and the laser beam.

Typical stiffness: 100 pN/micrometer

Typical displacements: 1-500 nm

Typical forces: 0.1-100 pN

Measurable speeds: ~1 kHz

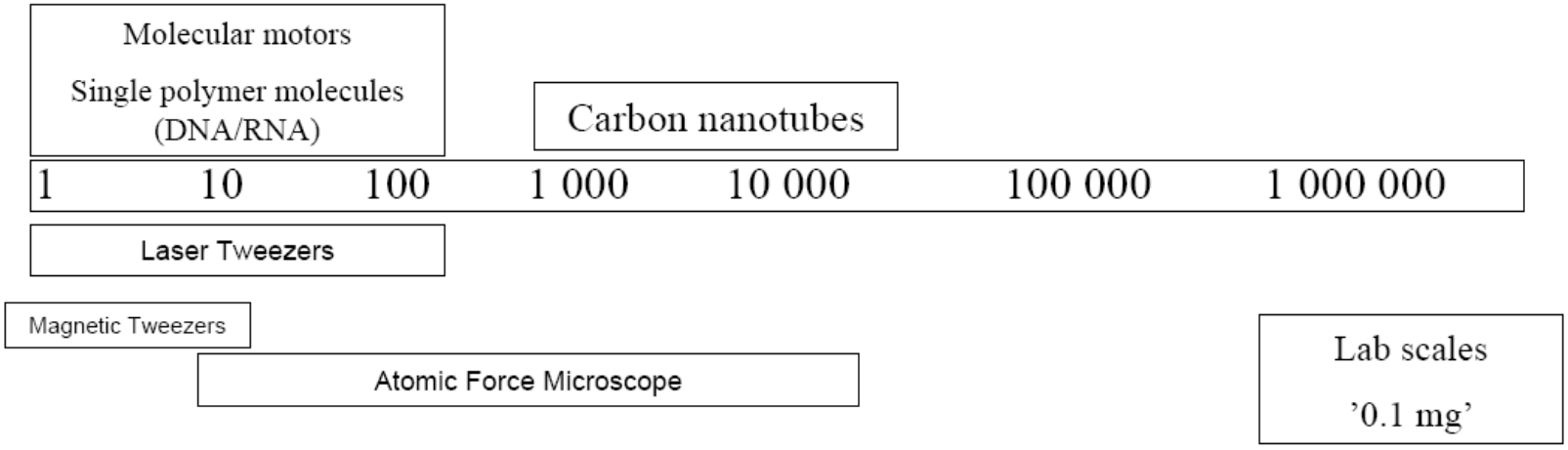
- 1 picoNewton (10^{-12} N) is roughly equal to…

– …the gravitational attraction between you and a book at arms length

– …the radiation pressure on a penny from a flashlight 1 yard away

– …1 millionth the weight of a grain of salt

Optical Trap typical forces: 0.1-100 pN



Comparison of forces with other techniques and biological processes:

Optical traps	10^{-14}-10^{-10} N
Electric fields (electrophoresis)	0-10^{-12} N
AFM	10^{-11}-10^{-7} N
Kinesin step	3-5 pN
RNA polymerase stalling	15-30 pN
Virus motor stalling	~50 pN
DNA conformational change	~65 pN
Biotin-streptavidin binding	300-400 pN a good linker

Table 1 | **Overview of single-molecule manipulation methods**

Methods	$F_{min-max}$ (N)[a]	X_{min} (m)[a]	Stiffness (N m^{-1})	Applications	Practical advantages
Cantilevers*	10^{-11}–10^{-7}	10^{-10}	0.001–100	Protein/polysaccharides[5,64] Bond strength[65,66]	High spatial resolution Commercially available
Microneedles*	10^{-12}–10^{-10}	10^{-9}	10^{-6}–1	Myosin motor force[12] DNA/titin strength[26,28]	Good operator control Soft spring constant
Flow field‡	10^{-13}–10^{-9}	10^{-8}	n.a.	DNA dynamics[38] RNA polymerase[35]	Rapid buffer exchange Simplicity of design
Magnetic field‡	10^{-14}–10^{-11}	10^{-8}	n.a.	DNA entropic elasticity[6] Topoisomerase activity[41]	Specificity to magnets Ability to induce torque
Photon field‡	10^{-13}–10^{-10}	10^{-9}	10^{-10}–10^{-3}	Protein motors[13,14] Protein unfolding[52]	Specific manipulation High force resolution

*Mechanical transducers: probes are bendable beams; spatial location is by beam deflection. ‡External field manipulators: probes are microscopic beads; spatial location is by bead displacement. [a]These numbers represent only empirical, not absolute limits. ($F_{min-max}$, force range; X_{min}, minimum displacement.)

What can we do with optical tweezers?

1) Pull or displace microscopic particles

2) Measure microscopically small forces like:

- Studying the strength of biological materials such as cells, membranes, proteins or DNA.
- Detection of force generation in molecular motors such as kinesin (the protein responsible for pulling apart chromosomes during cell division) or RNA polymerase.
- Elucidation of the microscopic properties of complex solutions (for example: polymer solution).

Optical Tweezers in biology (Review)

- Virus, Bacteria, cell trapping
- Mechanical properties of DNA
- Nucleic acid enzymes
- Studying molecular motors
- Disruption of nucleosomes

Optical Trapping and Manipulation of Viruses and Bacteria

A. Ashkin and J. M. Dziedzic

Science 235, 1517 (1987)

Optical trapping and manipulation of viruses and bacteria by laser radiation pressure were demonstrated with single-beam gradient traps. Individual tobacco mosaic viruses and dense oriented arrays of viruses were trapped in aqueous solution with no apparent damage using ~120 milliwatts of argon laser power. Trapping and manipulation of single live motile bacteria and *Escherichia coli* bacteria were also demonstrated in a high-resolution microscope at powers of a few milliwatts.

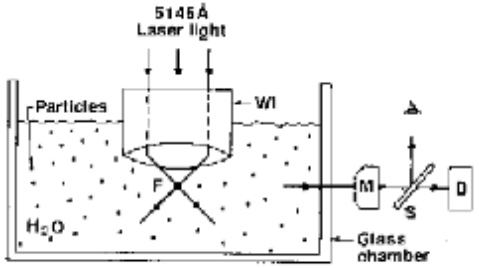


Fig. 1. Apparatus used for optical trapping of TMV particles and mobile bacteria. Spatially filtered argon laser light at 5145 Å is focused to a spot diameter of about 0.6 μm in the water-filled chamber by the high numerical aperture (1.25) water-immersion microscope objective (WI) forming a single-beam gradient trap near the beam focus (F). The 90° scattering from trapped particles can be viewed visually through a beam splitter (S) with a microscope (M) or recorded using a photodetector (D).

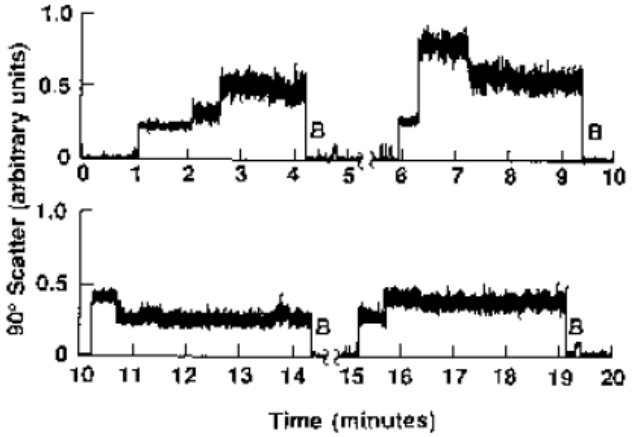


Fig. 2. Scattered light observed at 90° as successive TMV viruses enter the optical trap. At times labeled "B" the trapping beam is momentarily blocked, releasing the viruses. The trap subsequently refills with new virus particles.

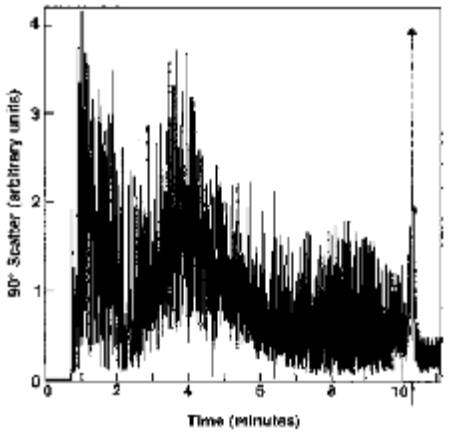
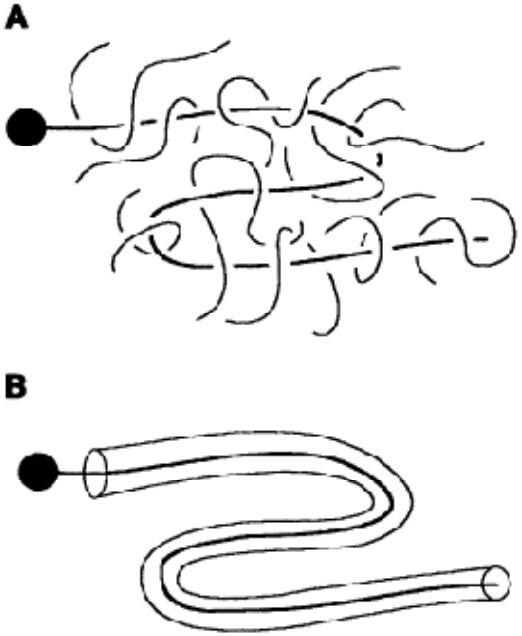
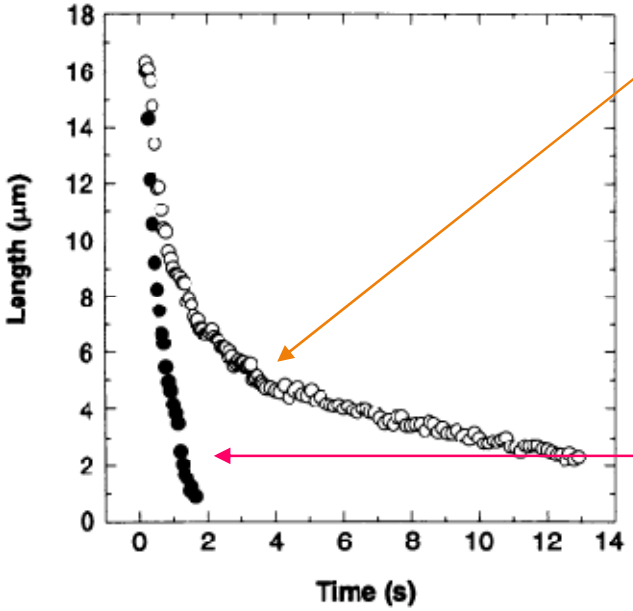


Fig. 3. Scattered light at 90° from a live bacterium trapped by ~5 mW of laser power. At about 10.3 minutes (indicated by arrow) the power was increased to 100 mW. The bacterium was killed and apparently loses much of its cell contents.

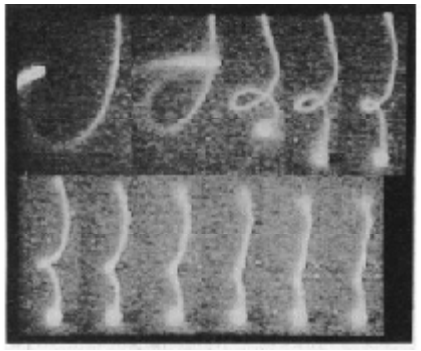
Direct Observation of Tube-Like Motion of a Single Polymer Chain

Thomas T. Perkins, Douglas E. Smith, Steven Chu*



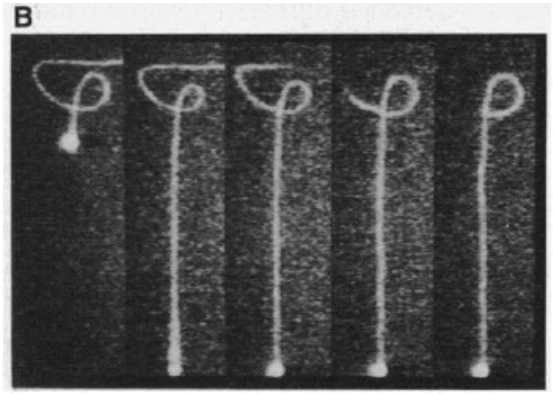


12 DNA/ um^3

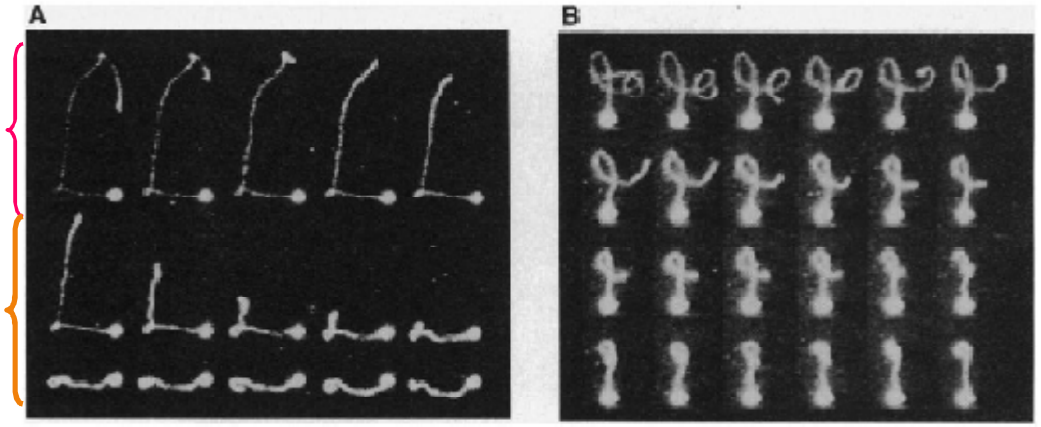


0.9 cp solvent

C= 7 DNA/um^3
dt= 1.5s

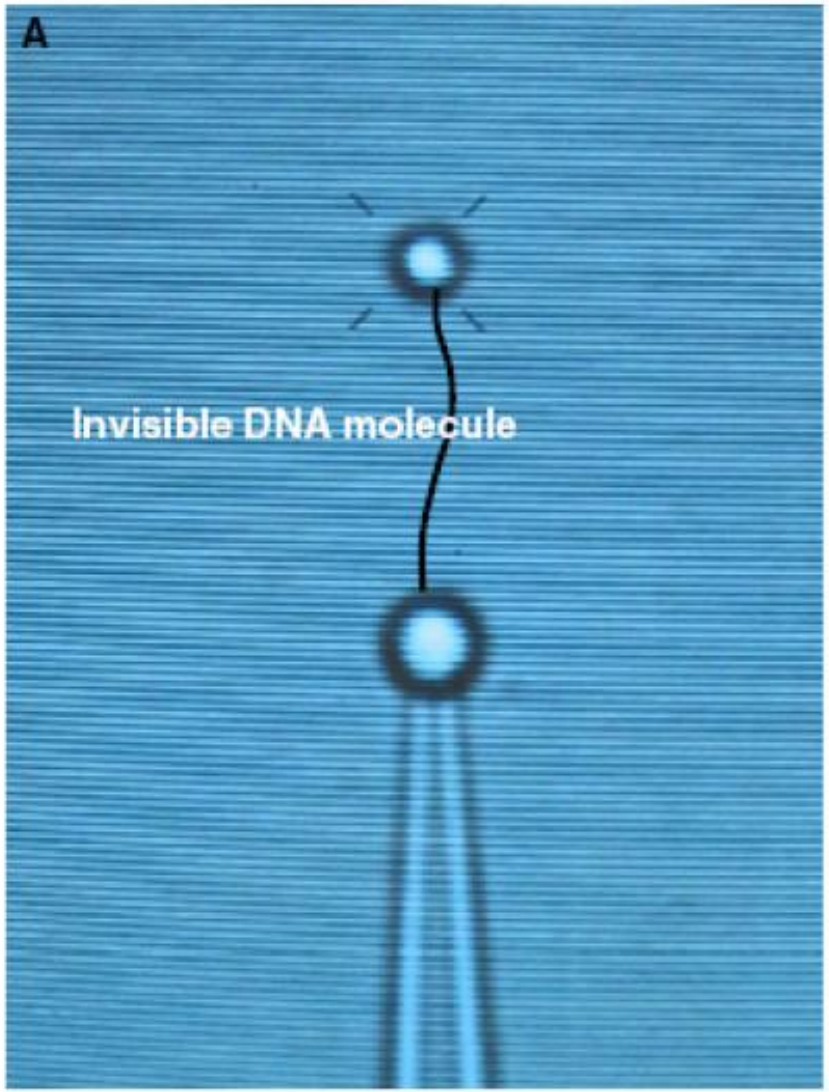


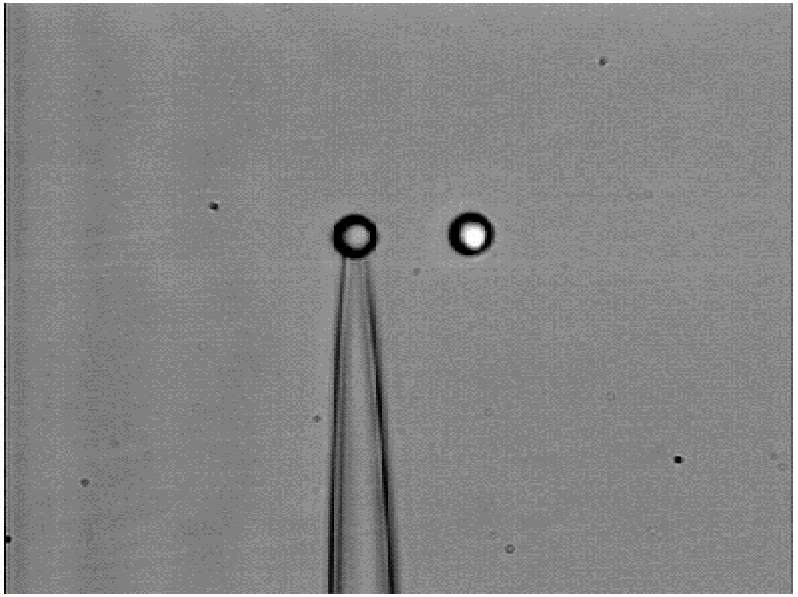
C= 12 DNA/ um^3



dt~ 2.3s & 13s

Science 264, 819 (1994)


A
Invisible DNA molecule



Thermodynamics of DNA Interactions from Single Molecule Stretching Experiments

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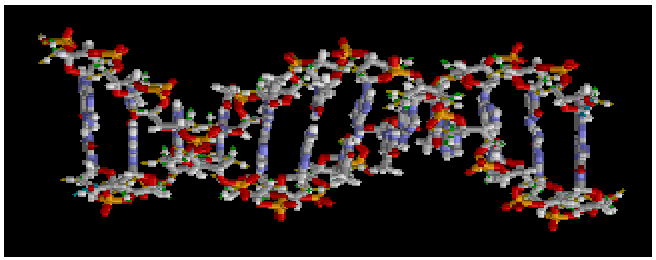
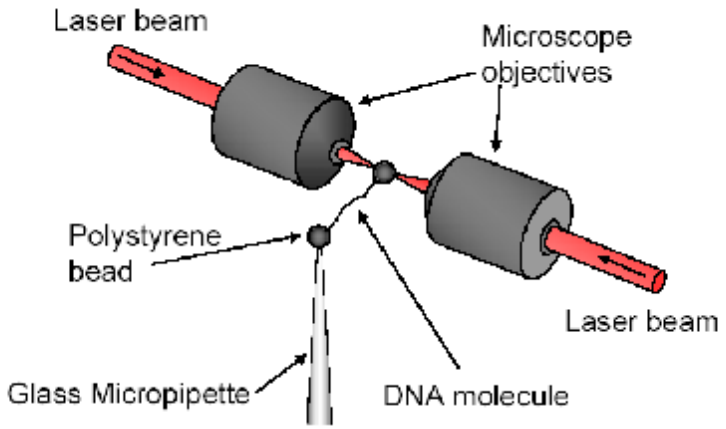
ABSTRACT

On the basis of our analysis of detailed measurements of the dependence of the overstretching transition of double-stranded DNA (dsDNA) on temperature, pH, and ionic strength, we have demonstrated that a model of force-induced melting accurately describes the thermodynamics of DNA overstretching. Measurements of this transition allow us to determine the stability of dsDNA and obtain information similar to that obtained in thermal melting studies. This single-molecule technique has the advantage that it can be used to measure DNA stability at any temperature. We discuss the use of this technique to study the nucleic acid chaperone activity of the HIV-1 nucleocapsid protein.





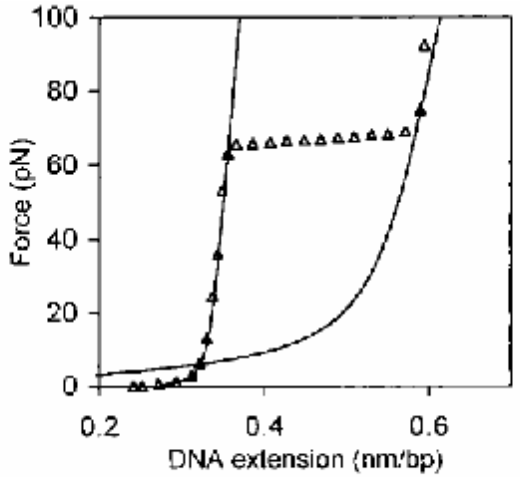
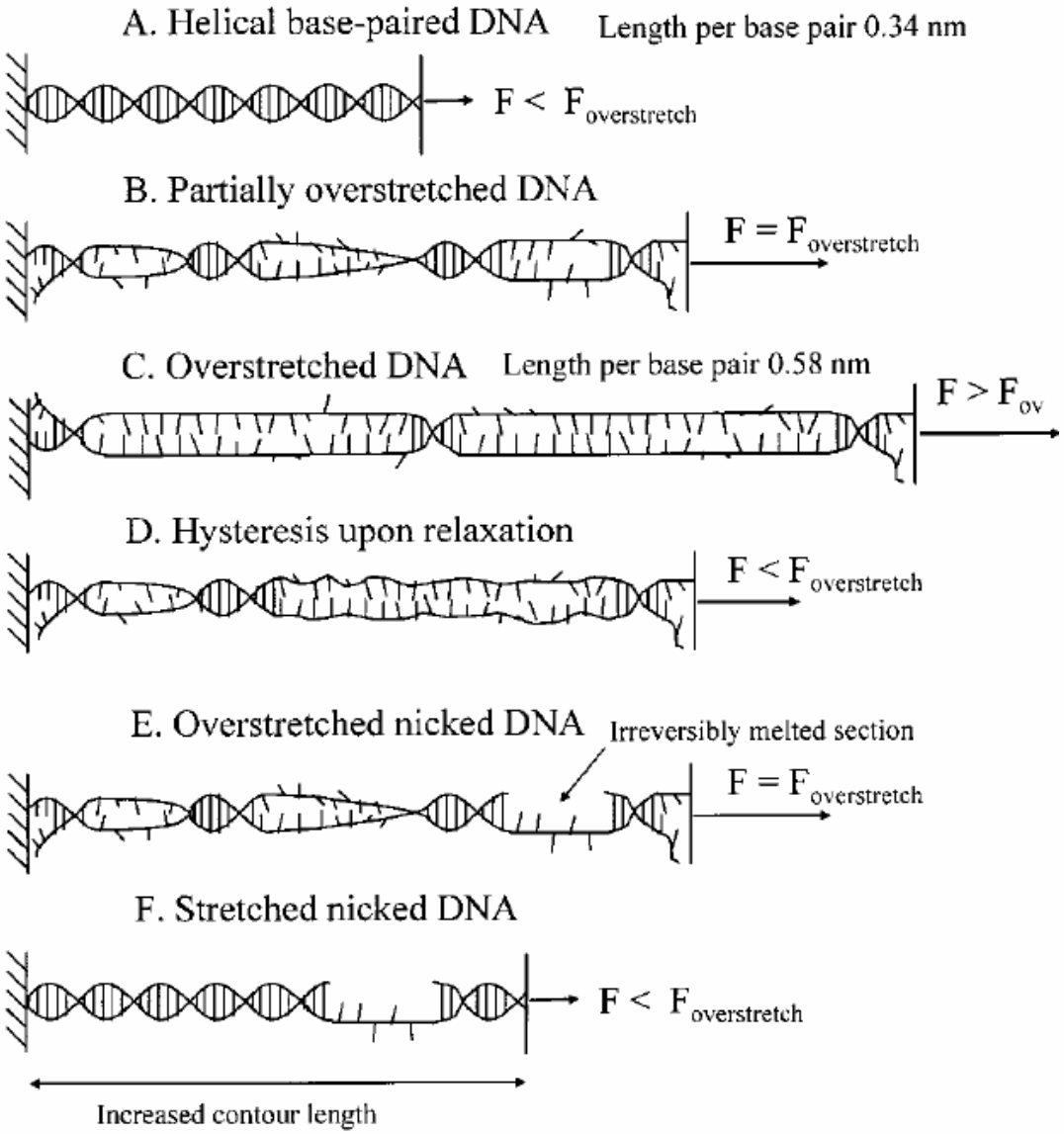


FIGURE 1. Typical DNA stretching curve in high-salt (250 mM NaCl) buffer (△). Lines representing the WLC model (eq 1) for the elasticity of dsDNA with P_{ds} = 50 nm and K_{ds} = 1200 pN (left) and the measured elasticity of ssDNA[2] (right) are also shown. The DNA stretching curve appears to be a transition from dsDNA to ssDNA.



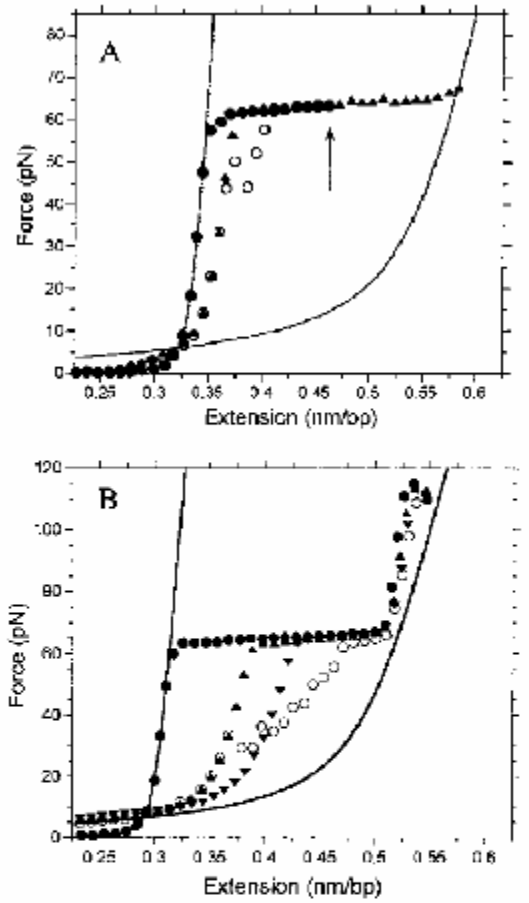


FIGURE 3. Hysteresis seen in DNA overstretching is consistent with force-induced melting. (A) A single dsDNA molecule that has been tethered and stretched to 0.46 nm/bp (arrow) in 250 mM buffer (●) displays partial ssDNA character during the relaxation curve (○) and subsequent stretch (▲). This is due to irreversible melting, as described in Figure 2E,F. (B) Multiple stretches to high extensions produce partially ssDNA. A dsDNA molecule that has been tethered in 250 mM buffer and stretched to near the maximum force achievable with this instrument (●) displays large hysteresis and partial ssDNA character during relaxation (○). Second (▲) and third (▼) stretches increase ssDNA character (from ref 23)

Processive translocation and DNA unwinding by individual RecBCD enzyme molecules

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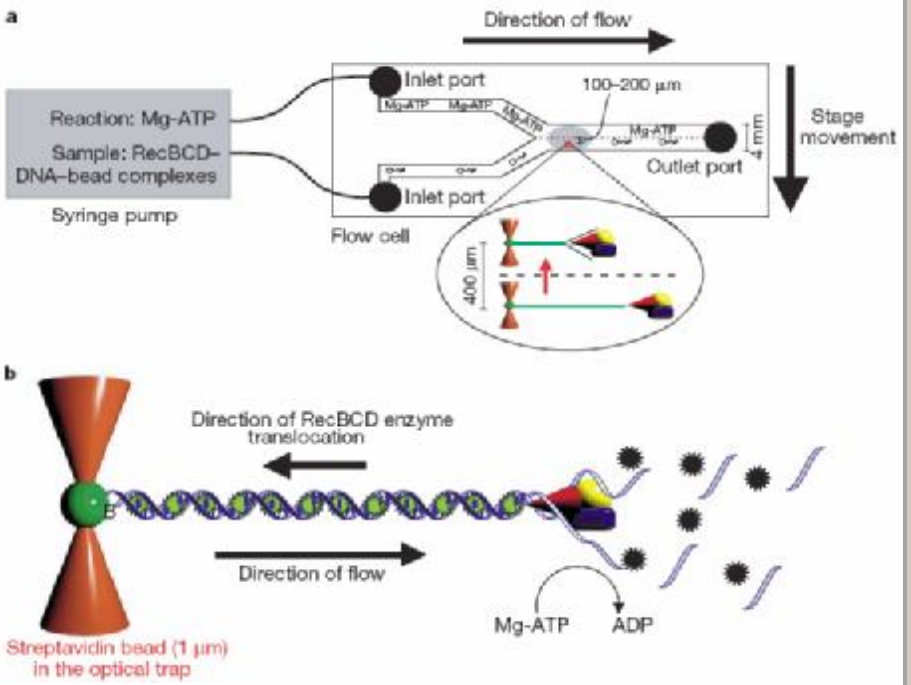
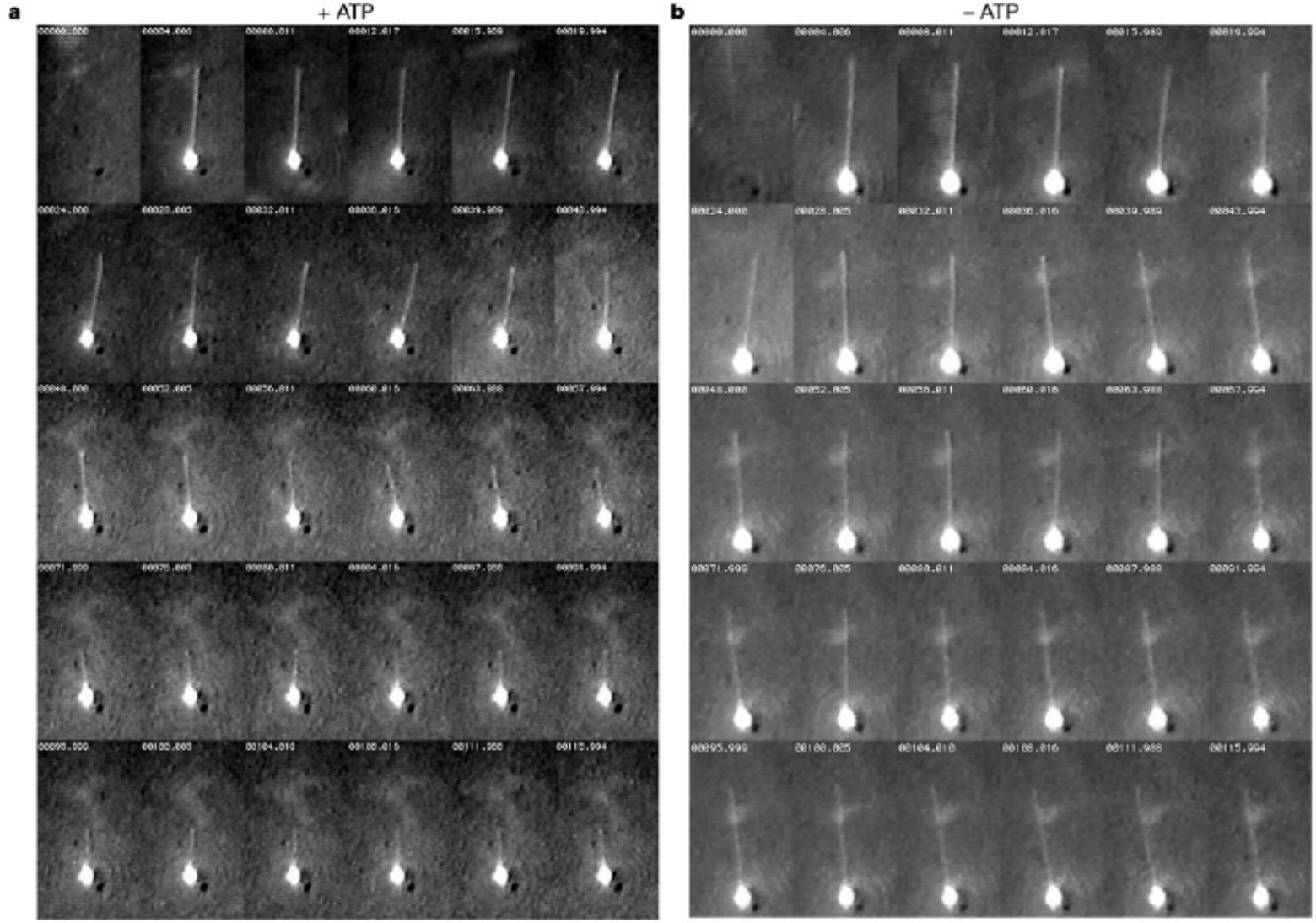
‡ Electronics Engineering Technologies Division, and § Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California 94550, USA

RecBCD enzyme is a processive DNA helicase[1] and nuclease[2] that participates in the repair of chromosomal DNA through homologous recombination[3,4]. We have visualized directly the movement of individual RecBCD enzymes on single molecules of double-stranded DNA (dsDNA). Detection involves the optical trapping of solitary, fluorescently tagged dsDNA molecules that are attached to polystyrene beads, and their visualization by fluorescence microscopy[5,6]. Both helicase translocation and DNA unwinding are monitored by the displacement of fluorescent dye from the DNA by the enzyme[7]. Here we show that unwinding is both continuous and processive, occurring at a maximum rate of 972 ± 172 base pairs per second (0.30 μm s^{-1}), with as many as 42,300 base pairs of dsDNA unwound by a single RecBCD enzyme molecule. The mean behaviour of the individual RecBCD enzyme molecules corresponds to that observed in bulk solution.



RecBCDis a protein of the E. coli bacterium that initiates recombinational repair from DNA double strand breaks which are a common result of ionizing radiation, replication errors, endonucleases, oxidative damage and a host of other factors. It is both a helicase that unwinds, or separates the strands of, DNA and a nuclease that makes single-stranded nicks in DNA.


a
+ ATP
b
– ATP

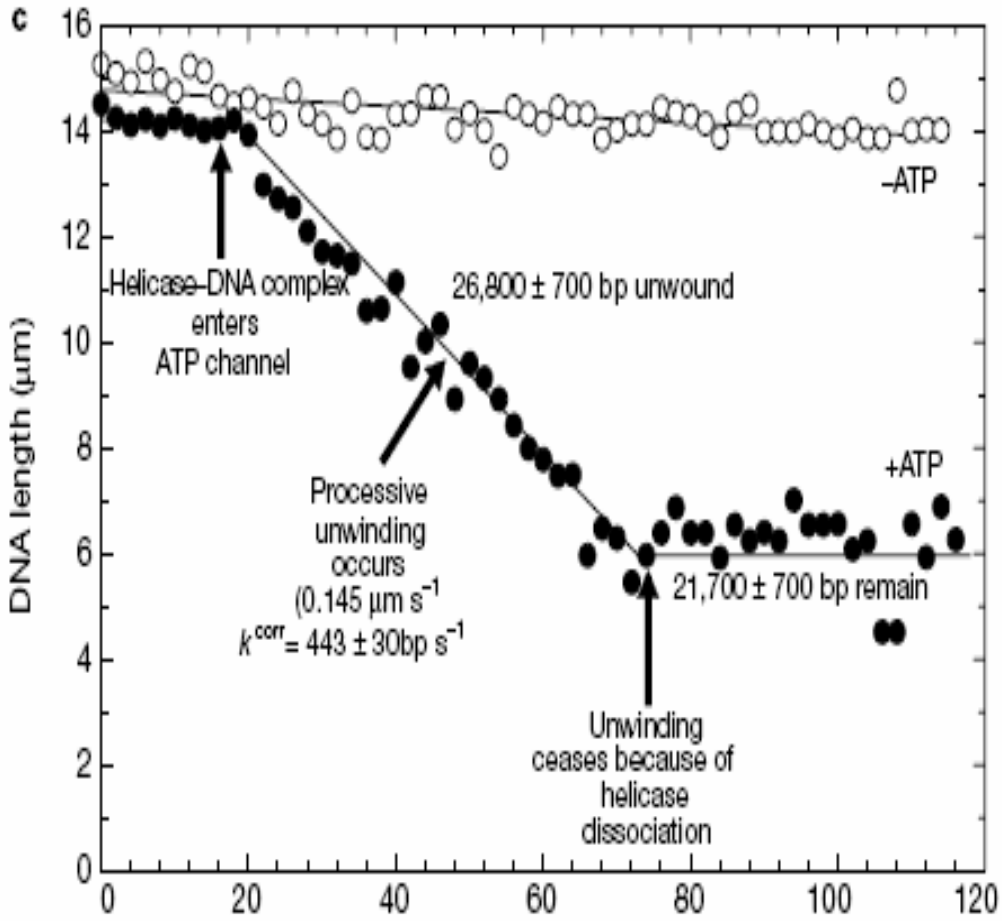
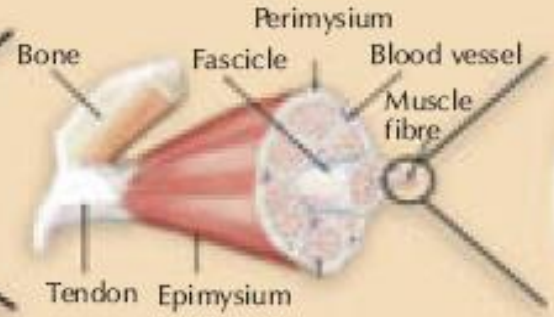
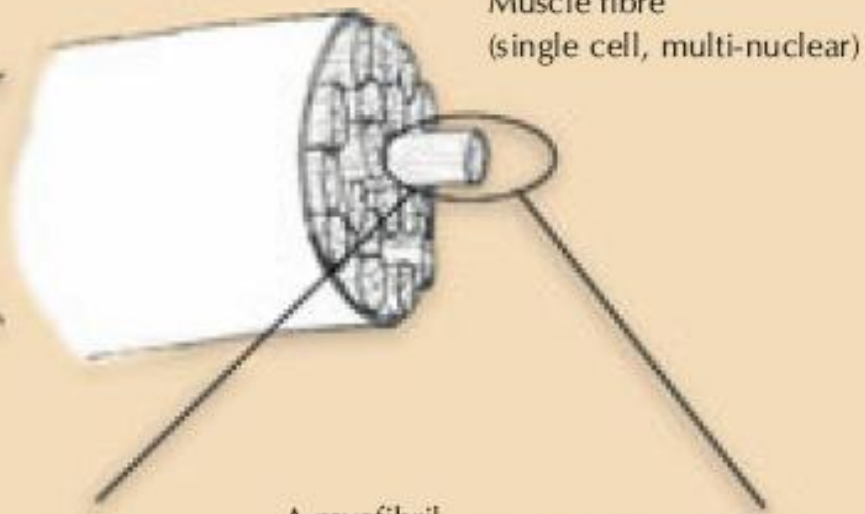
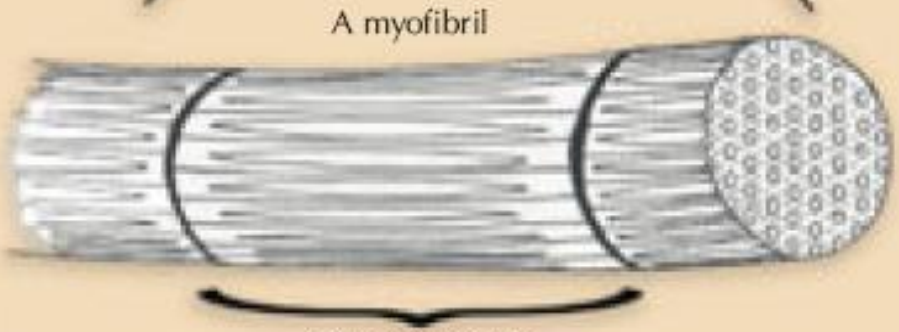
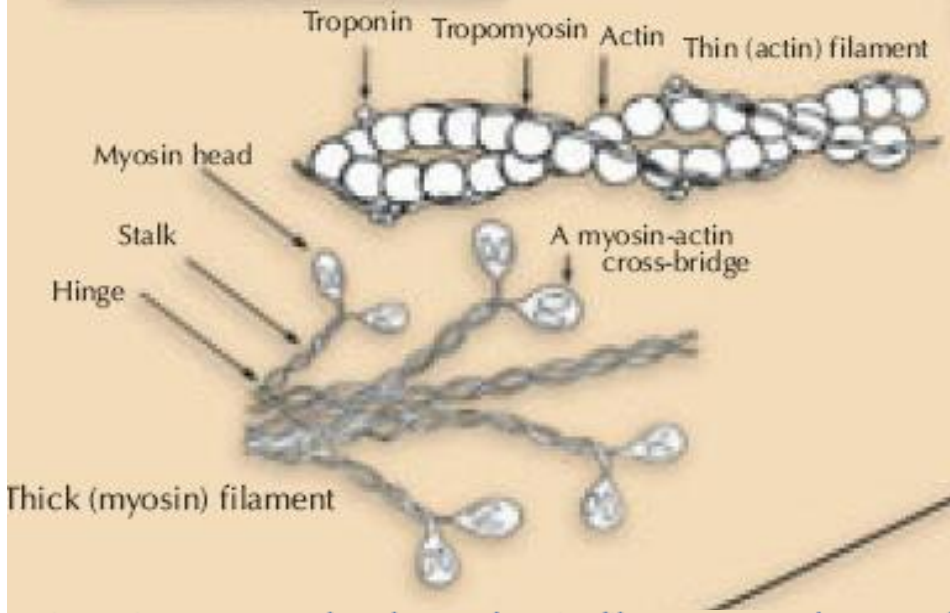


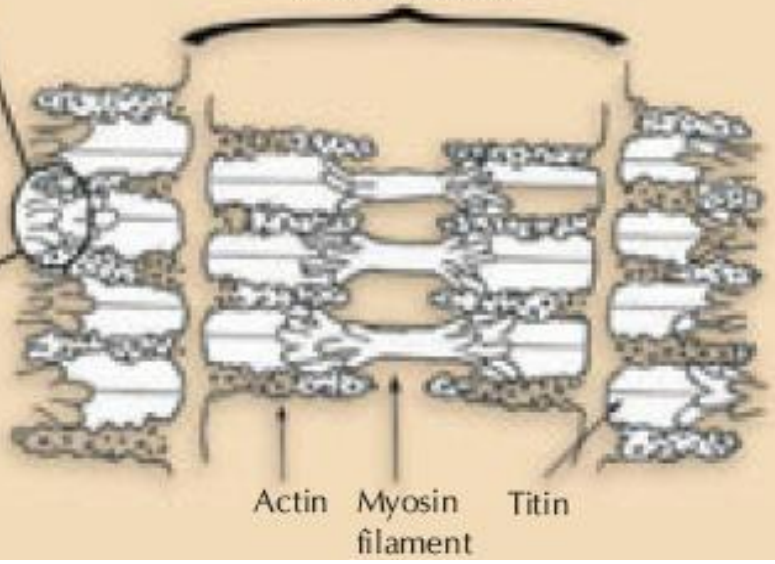
Figure 2 Unwinding of a DNA molecule by RecBCD enzyme. **a**, **b**, Selected, sequential frames from a video recording of reactions either in the presence (**a**) or absence (**b**) of ATP (1 mM). The direction of translocation and DNA unwinding by RecBCD enzyme is from the DNA end opposite the bead, towards the bead (that is, from the top of each frame towards the bottom). Numbers at the top of each frame indicate elapsed time. **c**, Analysis of the time courses in **a** and **b**.



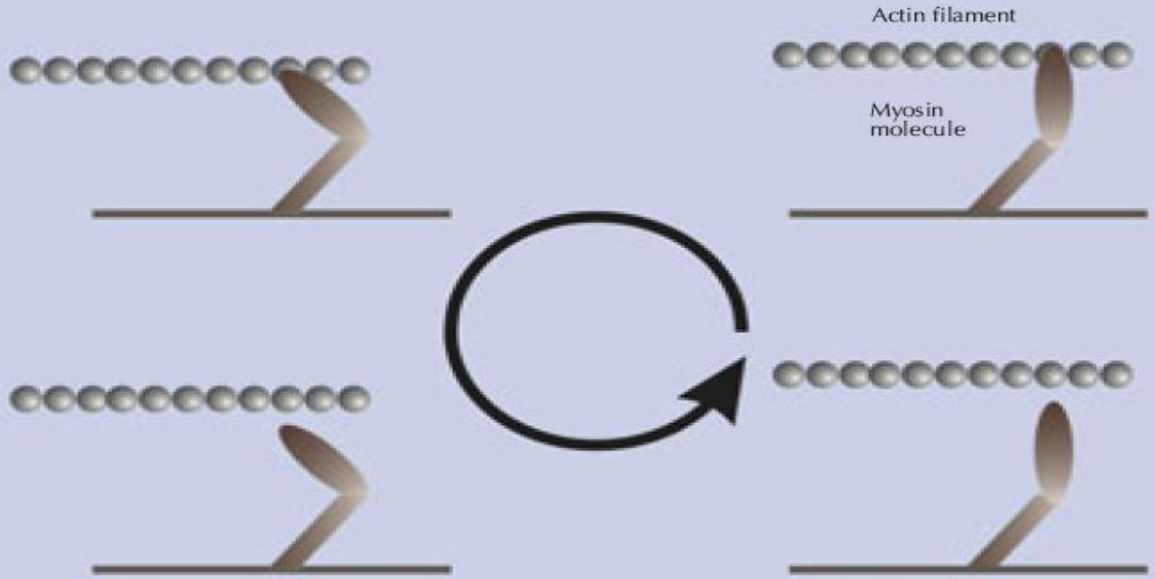




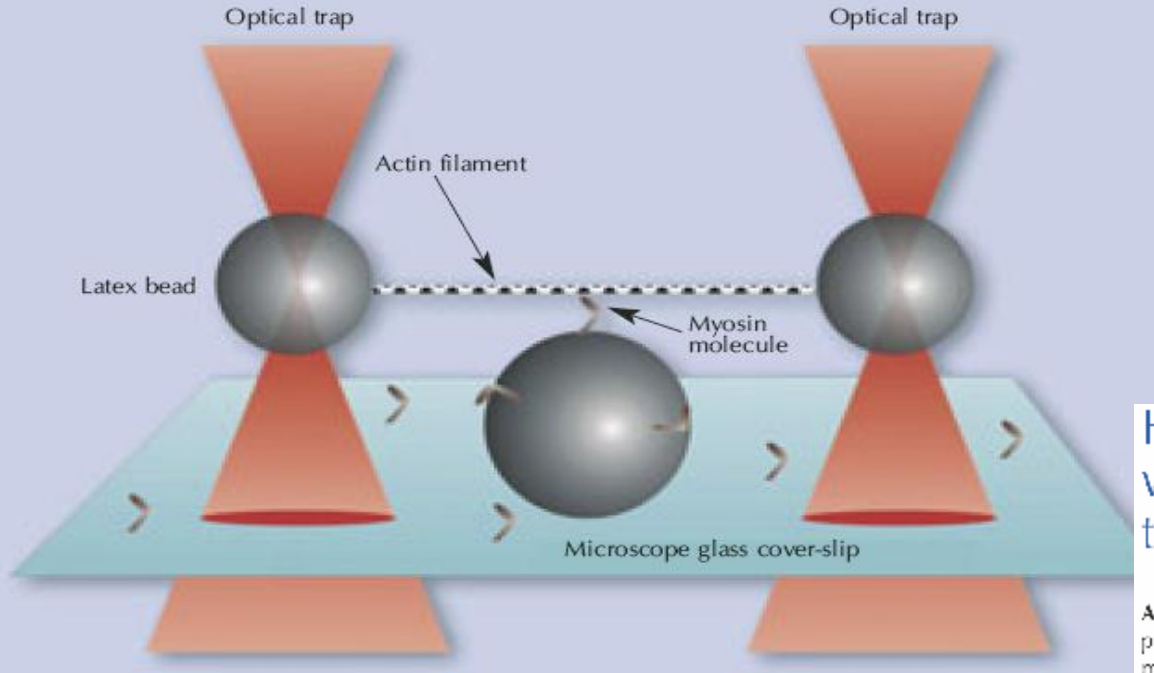




Myosin motor molecules and actin filaments are the most basic constituents of muscle tissue. They form overlapping filaments in the cells that slide past one another to make the muscle contract



The myosin motor produces mechanical work by pulling on the actin filament in a cyclical manner: (1) binding to the filament, (2) pulling on the filament, (3) releasing the filament, and (4) refuelling (one molecule of ATP)



The 'dumbbell' experiment. A single filament of actin is stretched between two plastic beads held in optical tweezers. The myosin molecule can then bind to the filament, and its working stroke is detectable from the positions of the two trapped beads

How do muscles produce work? Using optical tweezers to study molecular machines

Alexandre Lewalle from King's College, London, UK, pushes back the frontiers of our knowledge of motors at the molecular level.

Working strokes by single molecules of the kinesin-related microtubule motor ncd

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‡Department of Biophysics & Physics of Complex Systems, Division of Physics, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands
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§e-mail: cfs@nat.vu.nl
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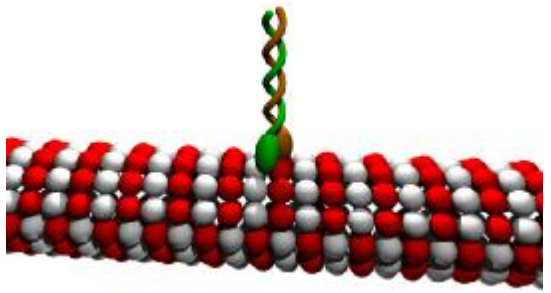
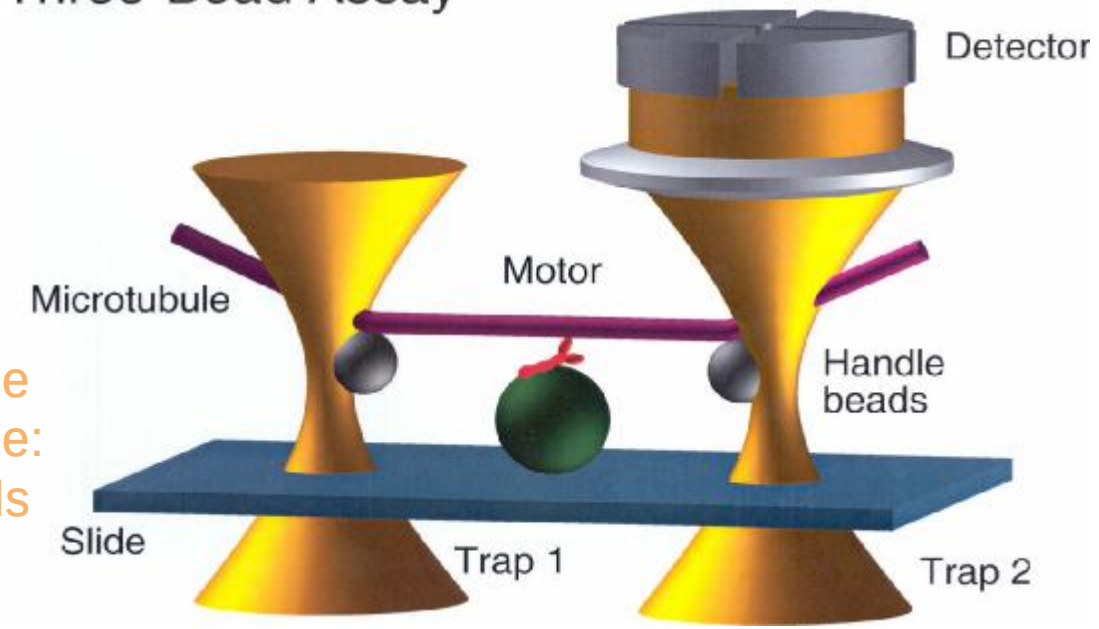
The ncd protein is a dimeric, ATP-powered motor that belongs to the kinesin family of microtubule motor proteins. Here we resolve single mechanochemical cycles of recombinant, dimeric, full-length ncd, using optical-tweezers-based instrumentation and a three-bead, suspended-microtubule assay. Under conditions of limiting ATP, isolated and transient microtubule-binding events exhibit exponentially distributed and ATP-concentration-dependent life-times. These events do not involve consecutive steps along the microtubule, quantitatively confirming that ncd is non-processive. At low loads, a single motor molecule produces ATP-triggered working strokes of about 9 nm, which occur at the ends of binding events.



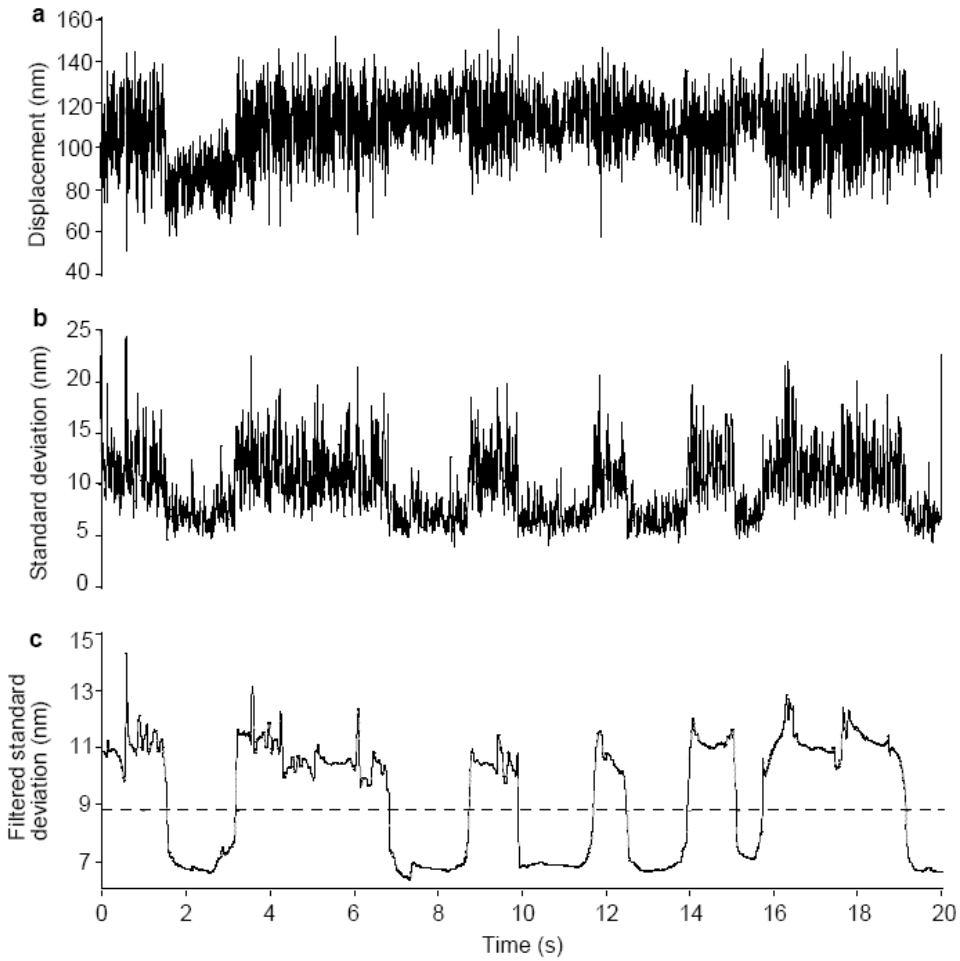
The kinesin dimer attaches to, and moves along, microtubules

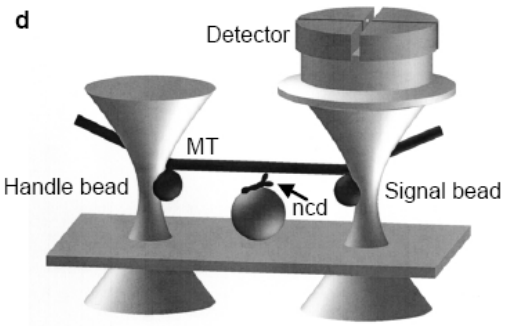
Three-Bead Assay

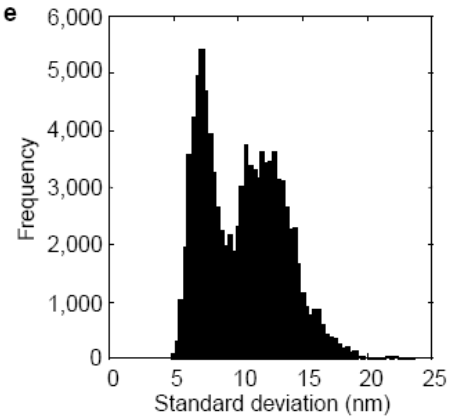


NCD acts just like the muscle-motor protein myosine: it binds the microtubule, pulls it and lets go.

Power strokes from the motor protein NCD







The cyclical reaction of binding and unbinding of NCD are clearly visible it this graph. These kind of measurements reveal many details of these kind of processes.

Mechanical disruption of individual nucleosomes reveals a reversible multistage release of DNA

Brent D. Brower-Toland*, Corey L. Smith†, Richard C. Yeh*, John T. Lis‡, Craig L. Peterson†, and Michelle D. Wang*§

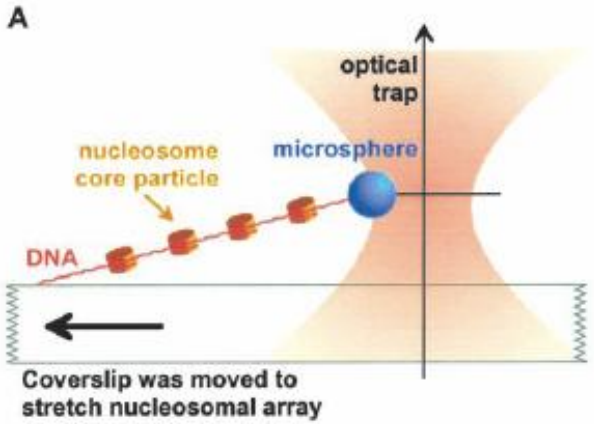
*Department of Physics, Laboratory of Atomic and Solid State Physics, and †Department of Molecular Biology and Genetics, Cornell, University, Ithaca, NY 14853; and ‡Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605

R_{ncp} ~ 5.5nm

R_{n-n} ~200 bp

NCP 147bp DNA

1.65 round



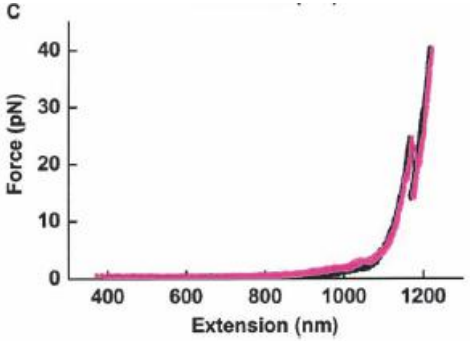
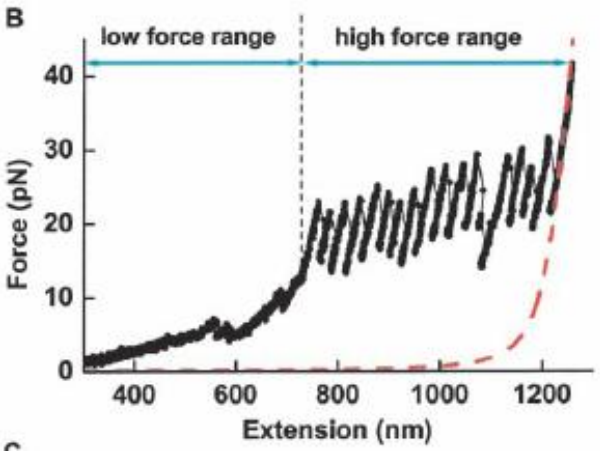
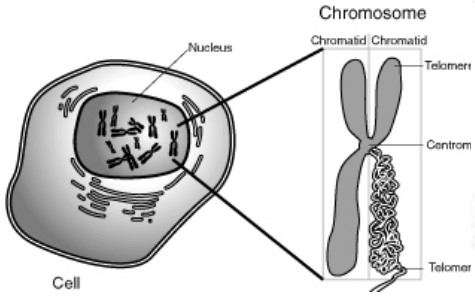
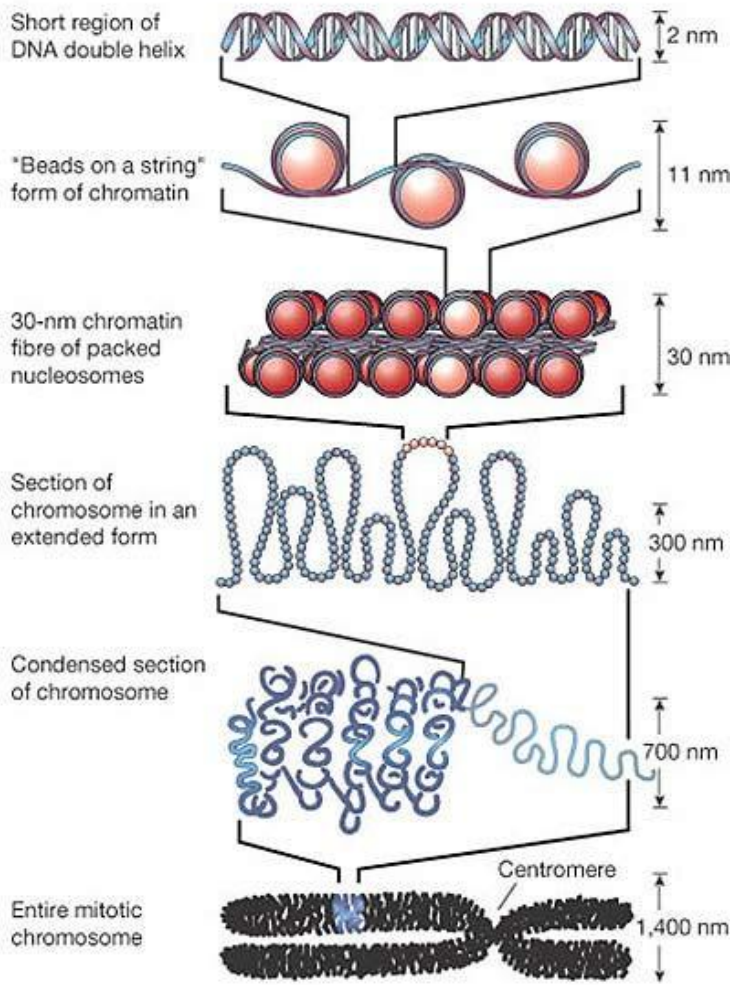


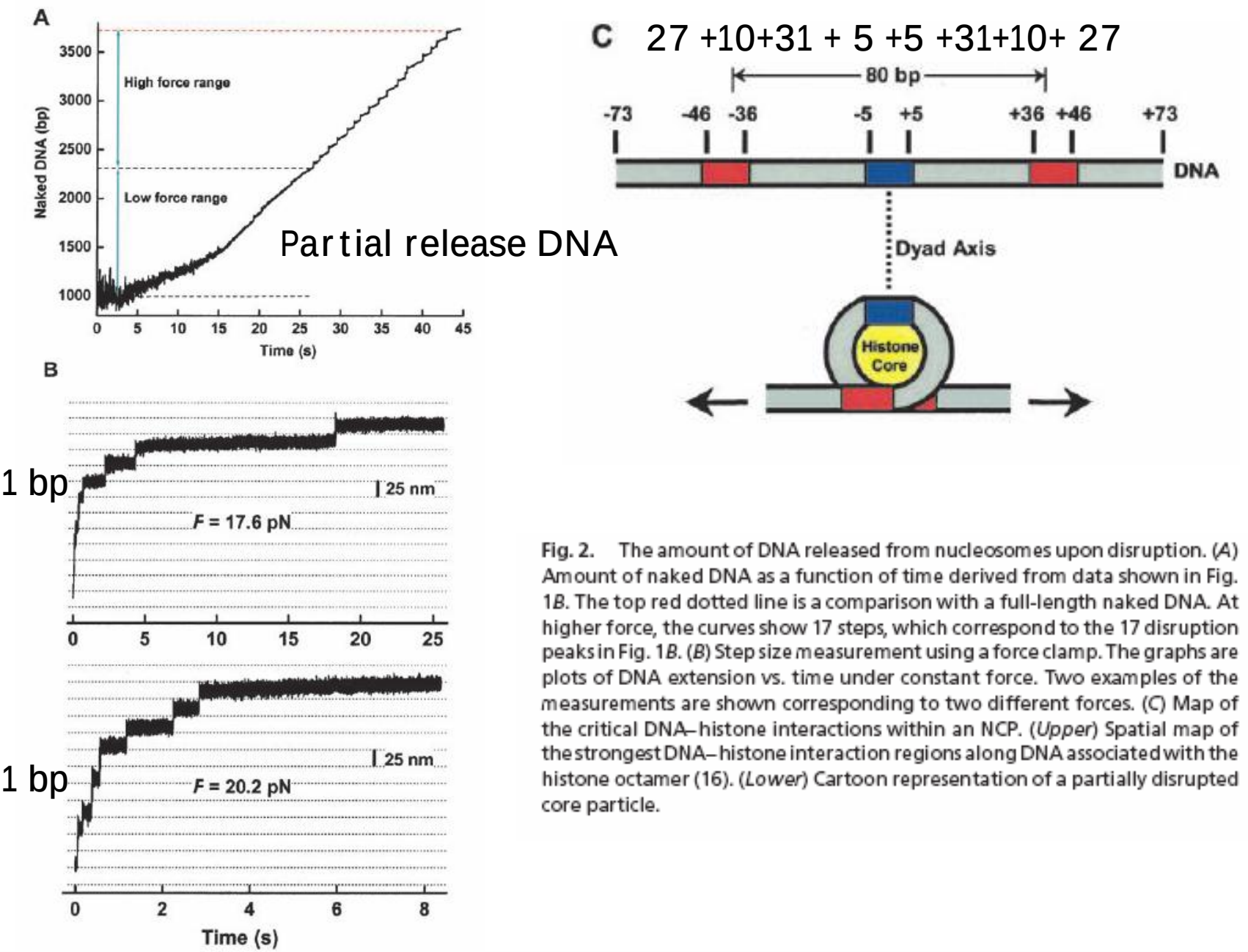
Fig. 1. Disruption of individual nucleosomes. (*A*) Experimental configuration (not to scale). Under feedback control, a nucleosomal array was stretched between the surface of a microscope coverslip and an optically trapped microsphere. Fig. 1 *B* and *C* were obtained with a velocity clamp at 28 nm/s. (*B*) Force-extension curve of a fully saturated nucleosomal array. At higher force (>15 pN), a sawtooth pattern containing 17 disruption peaks was observed. Force-extension characteristics of a full-length naked DNA (red dotted line) is shown for comparison. (*C*) Force extension curve of nucleosomal arrays containing minimal number of nucleosomes. Overlaid curves are shown for both native (black) and cross-linked (magenta) histone octamers.



3684 bp DNA with 17 tandem repeat







Partial release DNA

80+/-1 bp

78+/-1 bp

Fig. 2. The amount of DNA released from nucleosomes upon disruption. (*A*) Amount of naked DNA as a function of time derived from data shown in Fig. 1*B*. The top red dotted line is a comparison with a full-length naked DNA. At higher force, the curves show 17 steps, which correspond to the 17 disruption peaks in Fig. 1*B*. (*B*) Step size measurement using a force clamp. The graphs are plots of DNA extension vs. time under constant force. Two examples of the measurements are shown corresponding to two different forces. (*C*) Map of the critical DNA–histone interactions within an NCP. (*Upper*) Spatial map of the strongest DNA–histone interaction regions along DNA associated with the histone octamer (16). (*Lower*) Cartoon representation of a partially disrupted core particle.

10s relaxation after each stretch

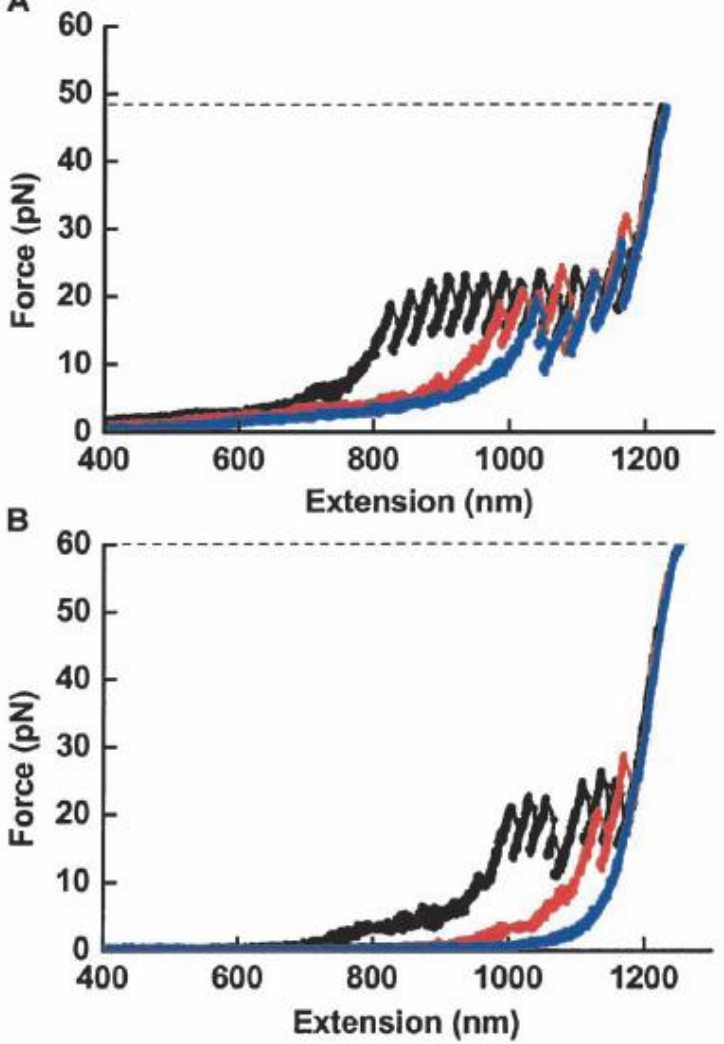


Fig. 3. Nucleosome reassembly after disruption by repetitive stretching with a velocity clamp. Nucleosomes were repetitively stretched with a 10-s relaxation period after each stretch. (*A*) Maximum force at ~50 pN. Force-extension curves of a nucleosomal array repetitively stretched three times with maximum force at ~50 pN: first stretch (black), second stretch (red), and third stretch (blue). (*B*) Maximum force at ~60 pN. A nucleosomal array first was stretched to a maximum force of 50 pN (black). In subsequent stretches (red then blue), the maximum force was increased to ~60 pN.

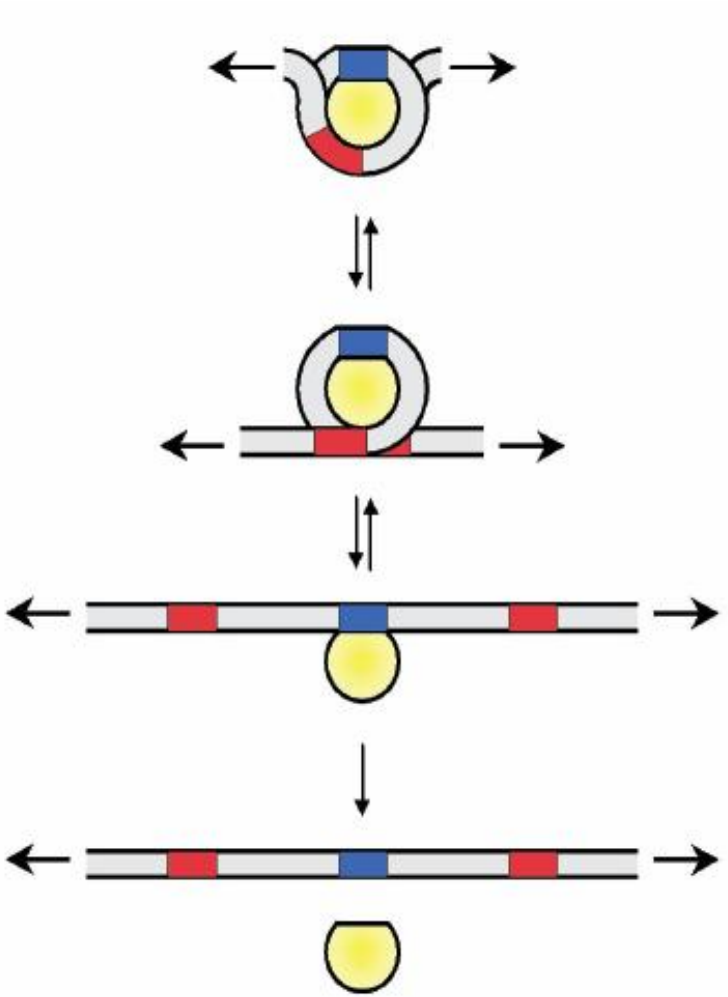
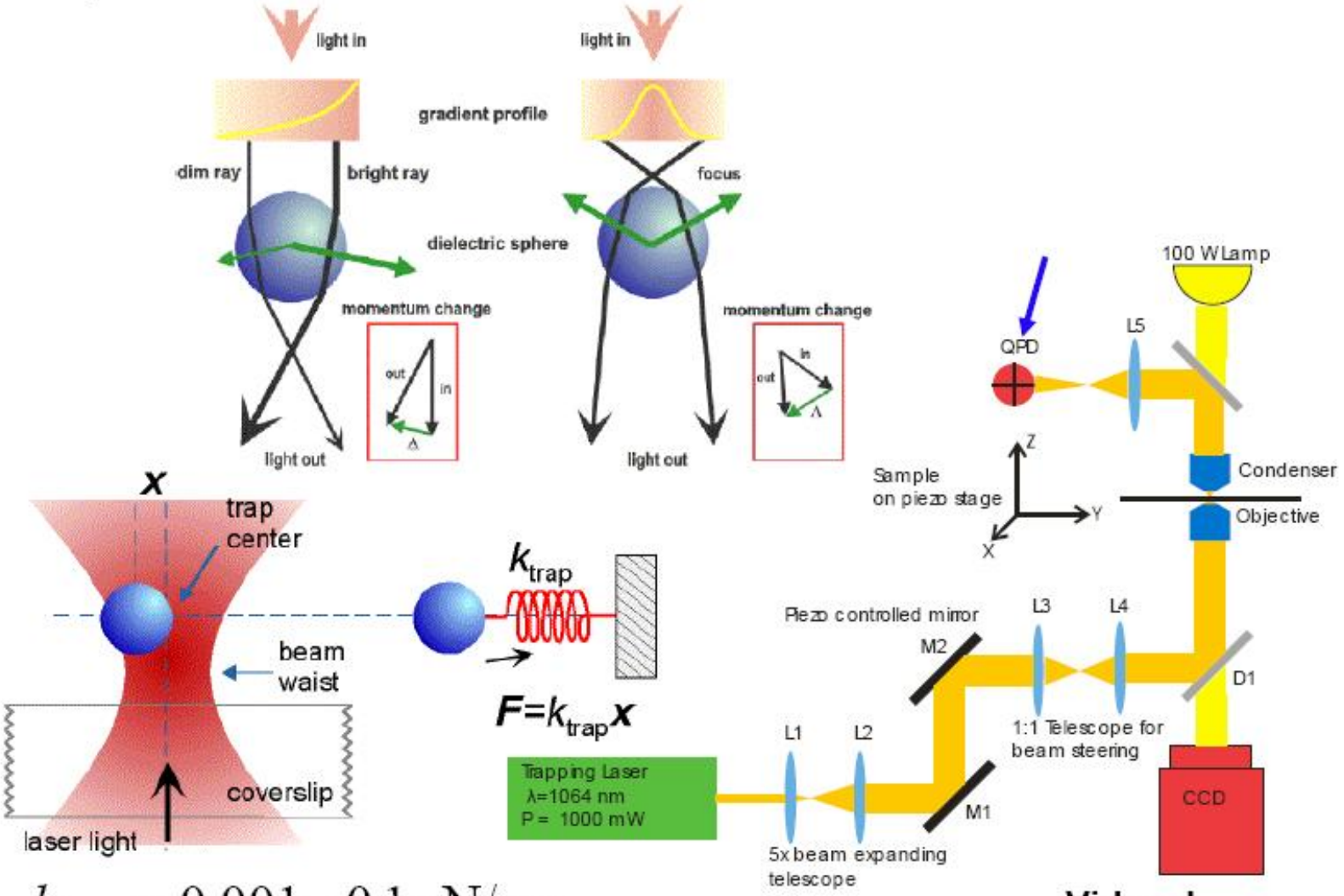


Fig. 5. A three-stage model for the mechanical disruption of the NCP. See text for a description.

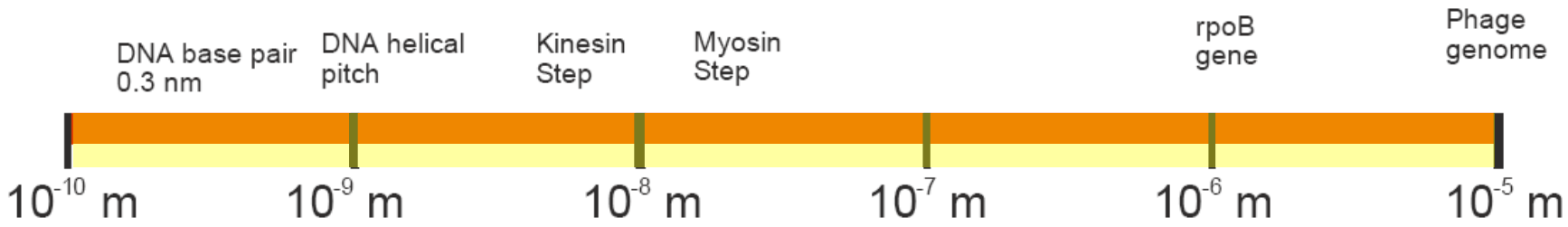
Optical Tweezers – How it works



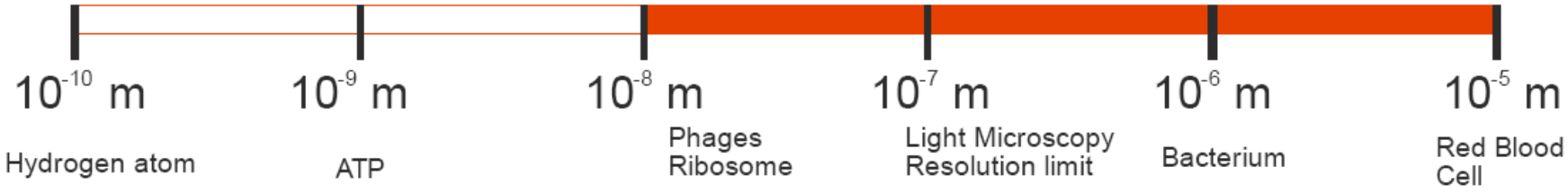
$$k_{\mathrm{Trap}} \approx 0{,}001 - 0{,}1\,\mathrm{pN/nm}$$

Length scales

Biological length scales

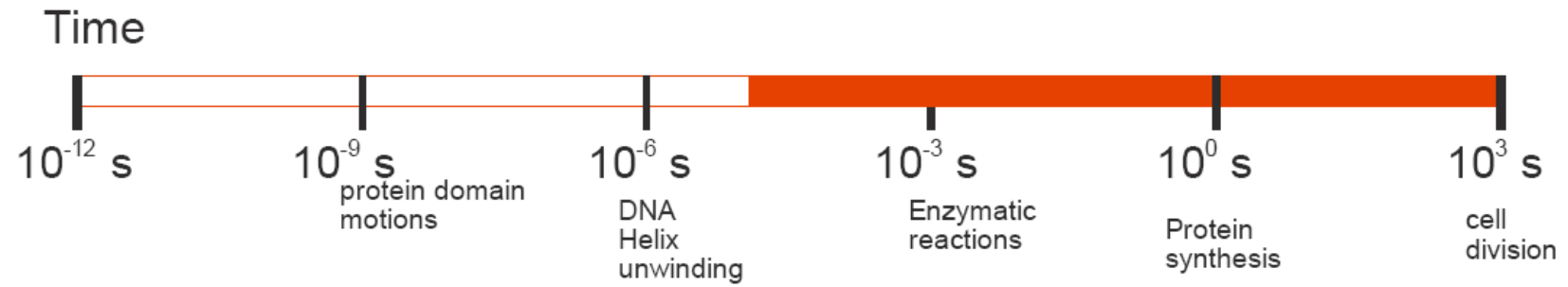


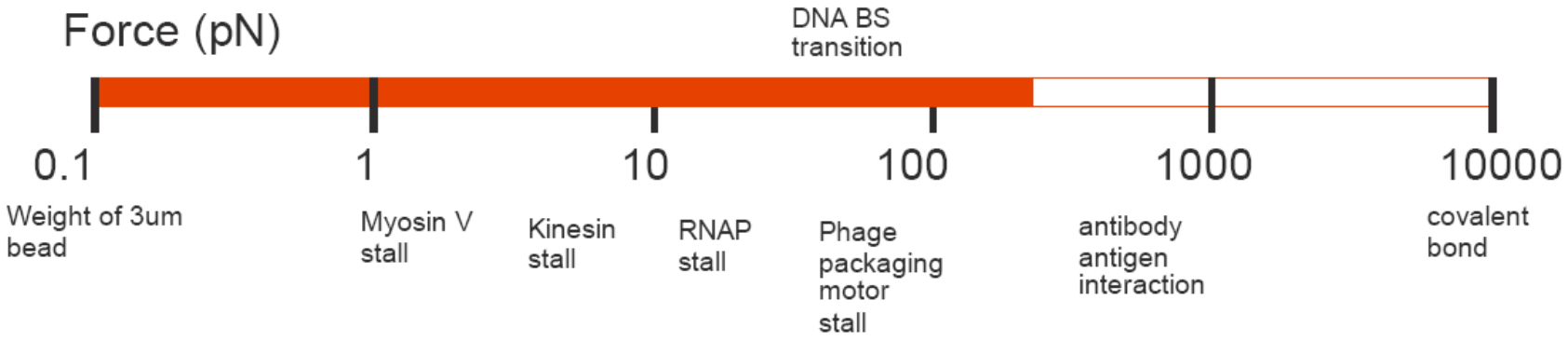
Trapped particles



Red bar: OT working range

Time and force scales





Optical traps for Biophysics

Pro's:

Remote manipulation of biomolecules

Measurable forces and distances are well suited for enzyme dynamics and molecular motors

They work in normal buffer conditions

Con's:

Radiation damages of samples

Slow throughput

Not commercially available (*)