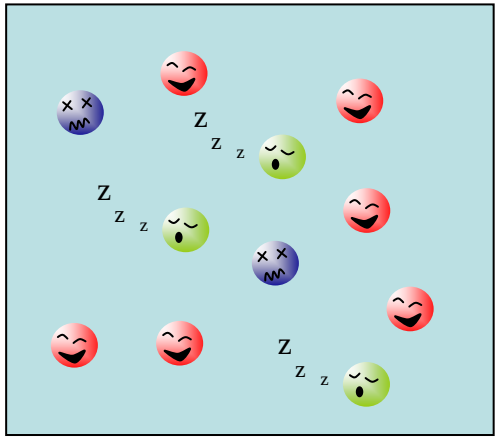
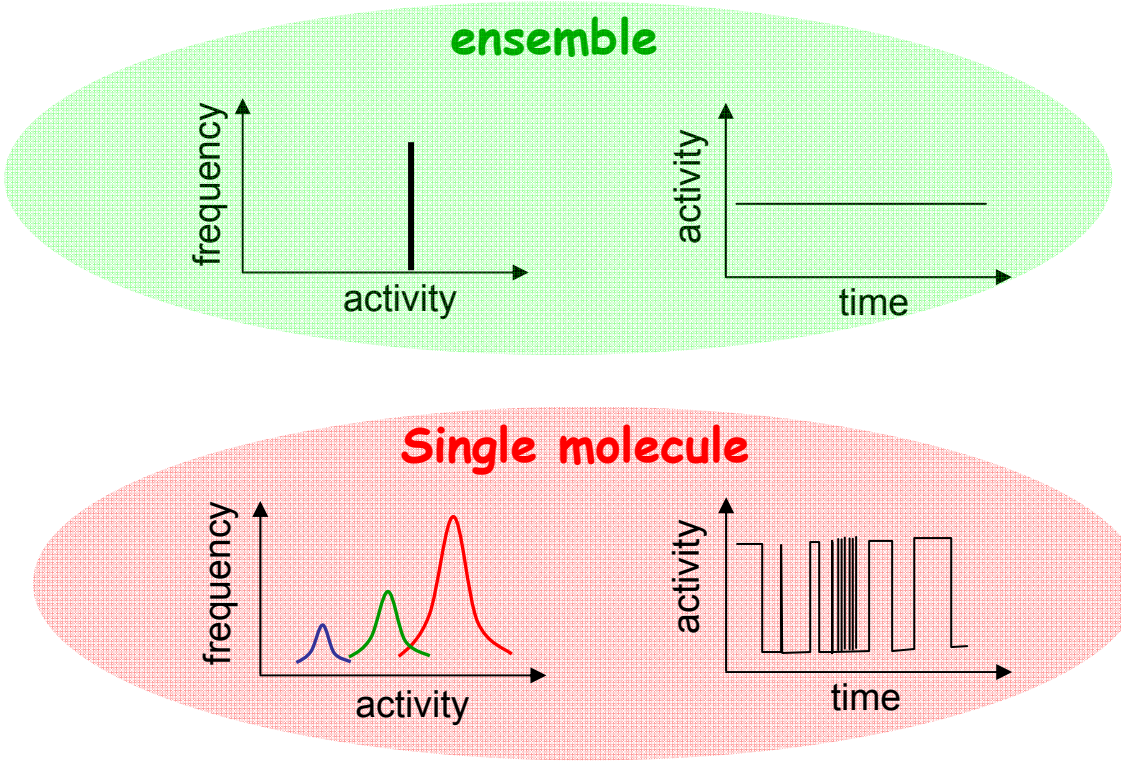
Tecniche sperimentali: **le optical tweezers**

Le tecniche di molecola singola rispetto a quelle di insieme





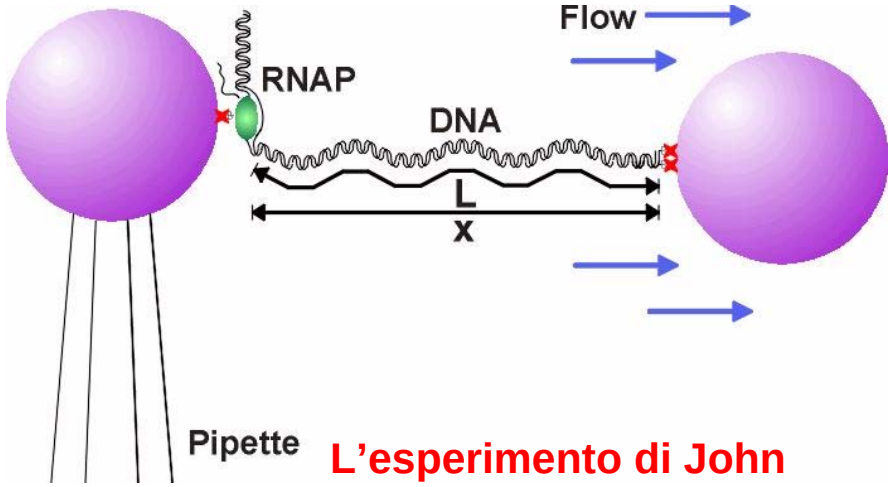
-Probability distribution function- frequency histogram of the actual distribution of values

-Important for systems which may show local heterogeneity

-May reveal unusual phenomena not observed in bulk measurements

Molti nuovi strumenti nascono dall'integrazione della microscopia ottica con altre tecniche.

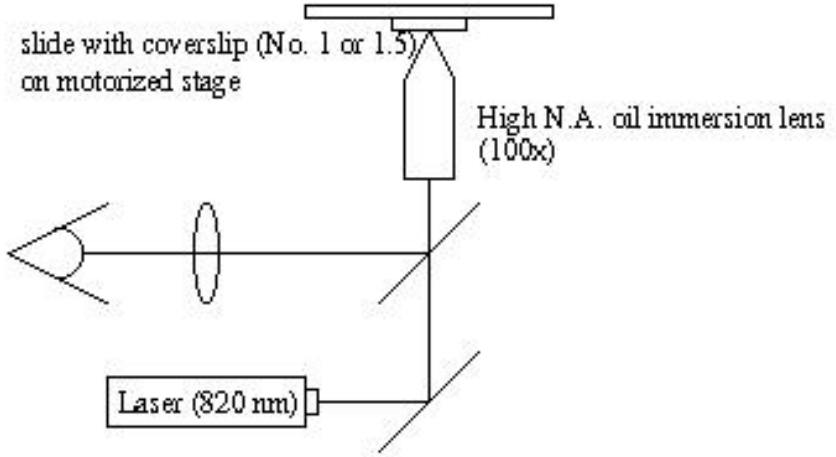
Negli ultimi anni sono stati progettati sistemi per studiare le forze di interazione tra le macromolecole biologiche. Integrando la video-microscopia ottica con le optical-tweezers e le micropipette, ad esempio, si possono studiare le forze applicate dai motori molecolari, come nel caso della RNA polimerasi



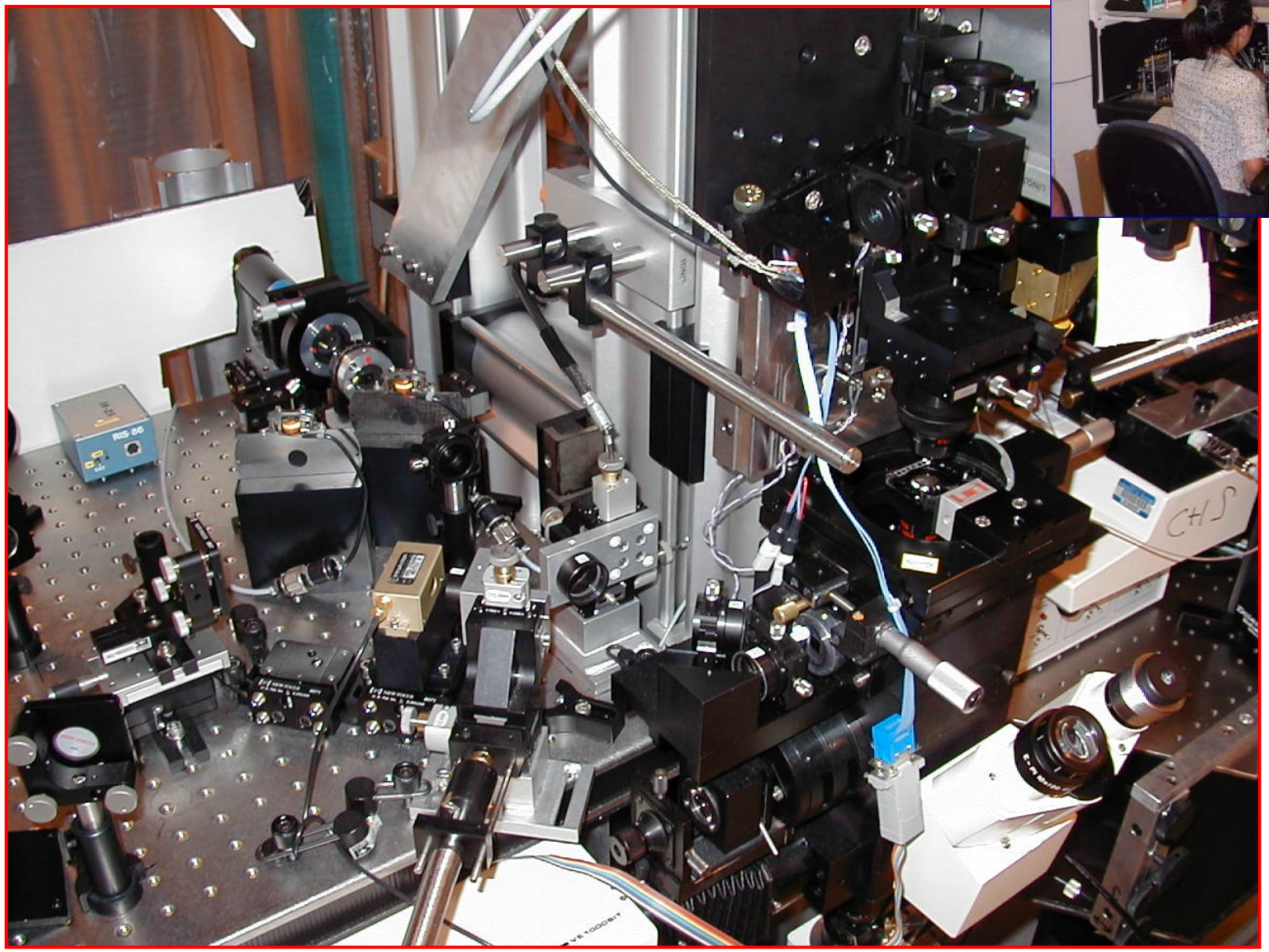
L'esperimento di John Davenport e collaboratori per la misura della forza applicata dalla RNA polimerasi e delle forze necessarie per bloccare la sintesi dell'RNA

What are Optical Tweezers?

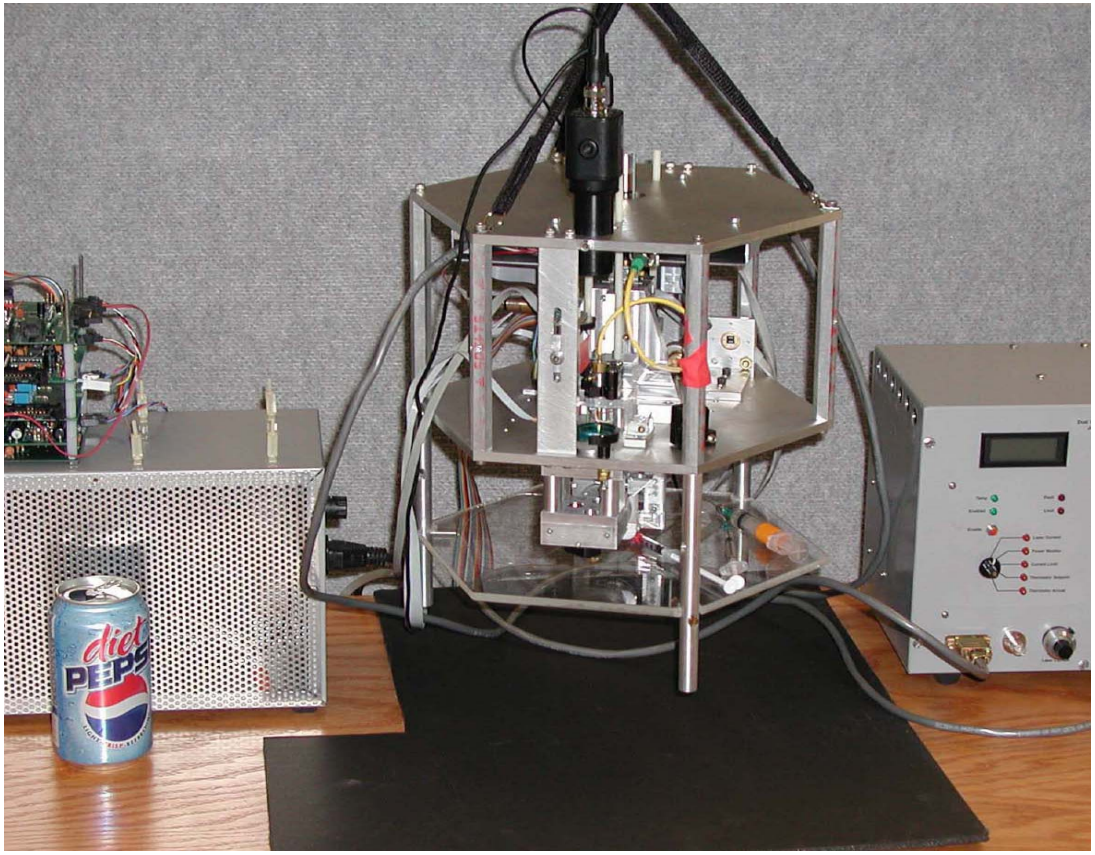
- A low power, continuous wave laser that is focused through a high N.A. objective can trap particles of diameter ~10 μm.
- Can move the trapped particle by moving the laser or stage, hence the laser acts as a “tweezer” by picking up and moving an individual particle.



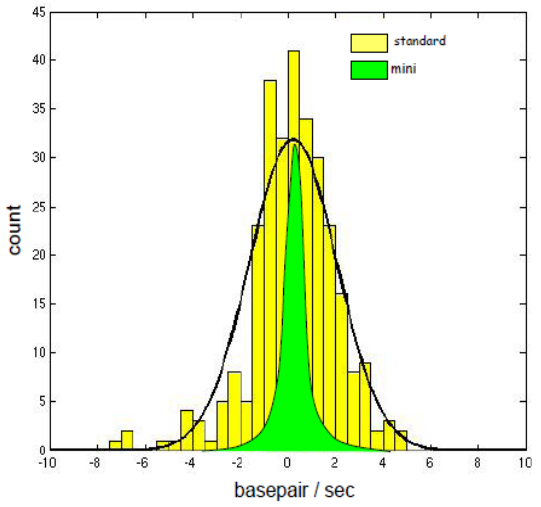
…and what they look like



…and what they are beginning to look like



Mini-tweezers by Steve Smith (UC Berkeley)

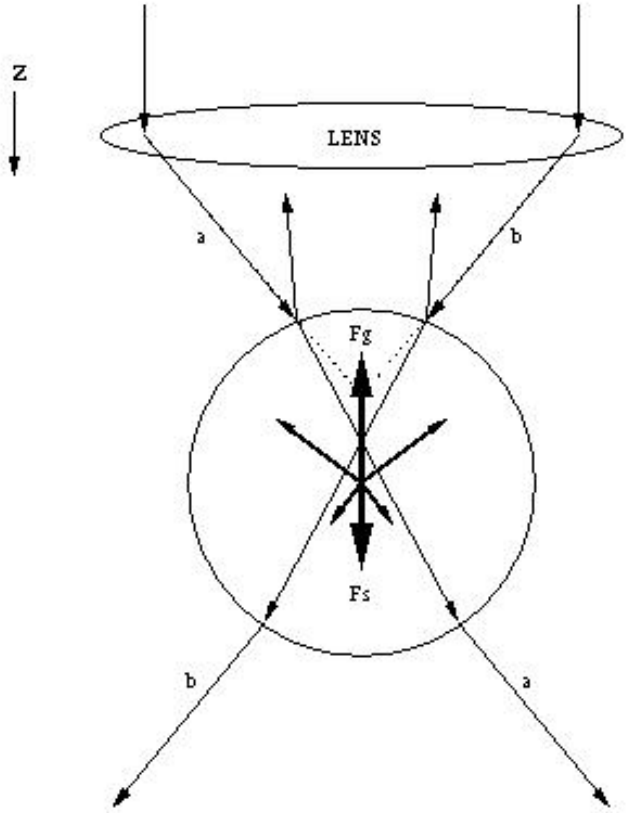


Notes on Optical Tweezers

- Most of the early work in this field was done by Arthur Ashkin of Bell Labs.
  - 1978: two opposing laser beams were used to trap and cool atoms.
  - 1986: a single laser focused through a microscope was used to trap polystyrene balls with diameters 10 μm to 25 nm.
  - 1987: bacteria and protozoa were trapped, first with a 514.5 nm Ar laser, followed by a 1064 nm Nd:YAG laser.
- First experiments using a 514.5 nm Ar laser killed bacteria at power levels of 100mW.
- When a 1064 nm Nd:YAG laser was used, there was no noticeable damage. In fact, cells that were trapped reproduced, and the offspring remained in the trap.
- Liu et. al used the membrane probe Laurdan to monitor cellular temperature changes during trapping.
  - For motile cells (ex. human sperm), ΔTemp = 0.93 °C/100 mW
  - For immotile cells (ex. CHO), ΔTemp = 1.1 °C/100 mW
- In addition, Liu et al. found that continuous wave trapping had no effect on intracellular pH or DNA.
- Optical Tweezers have been used to…
  - manipulate organelles
  - measure forces associated with transport and adhesion
  - study the swimming forces of sperm
  - study kinesin motors
  - stretch DNA molecules to their full length

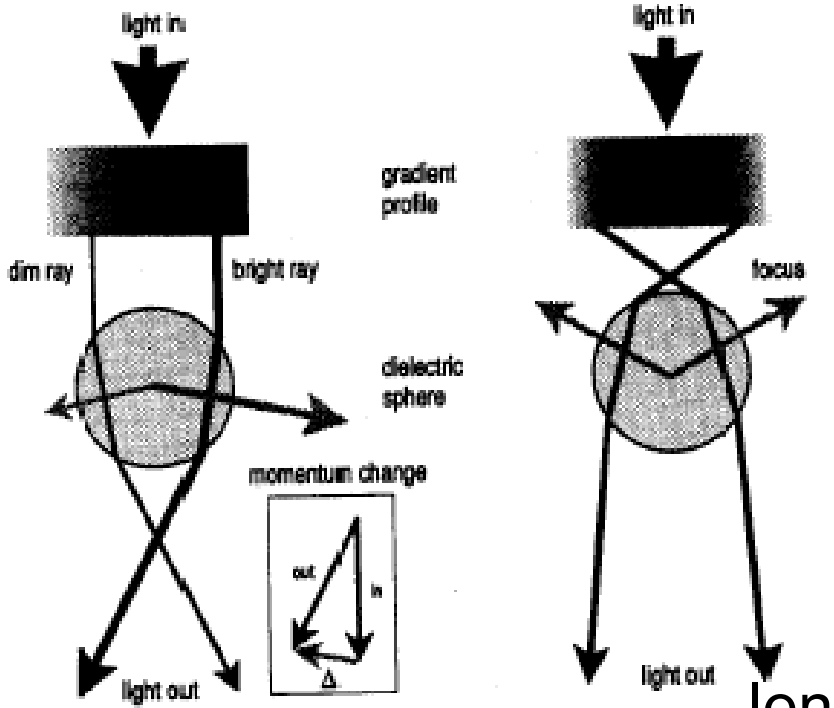
How do Optical Tweezers work?

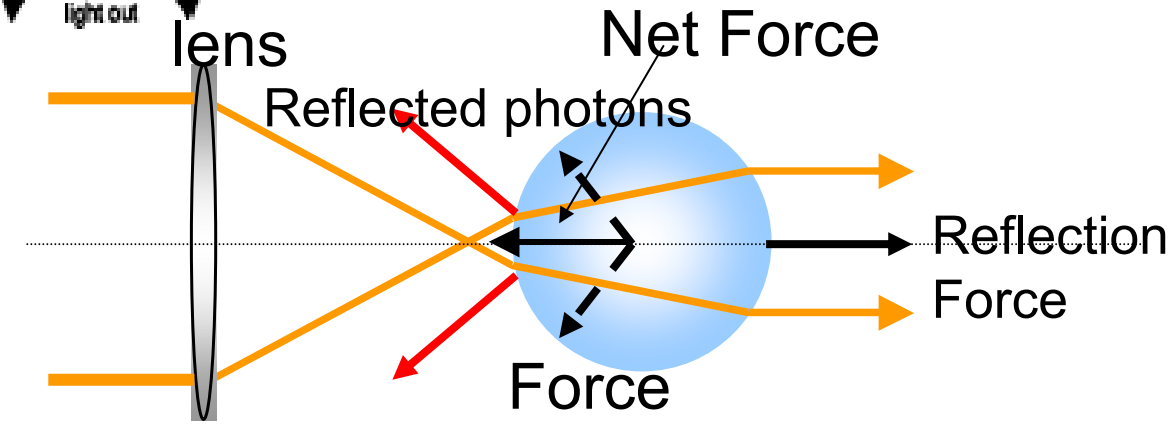
- Two regimes of operation:
  - Rayleigh regime (diameter of particle << λ)
  - Mie regime (diameter of particle >> λ)
- Ray optics used for simple explanation in Mie regime



- Two main forces
  - Scattering force
  - Gradient force
- Scattering force caused by reflection of incident beam
- Gradient force caused by the deflection (transmission) of incident beam
- Gradient force dominates scattering force

Applied force depends on the laser power; equilibrium point is offset from the center of the particle by 0.06*radius towards the beam





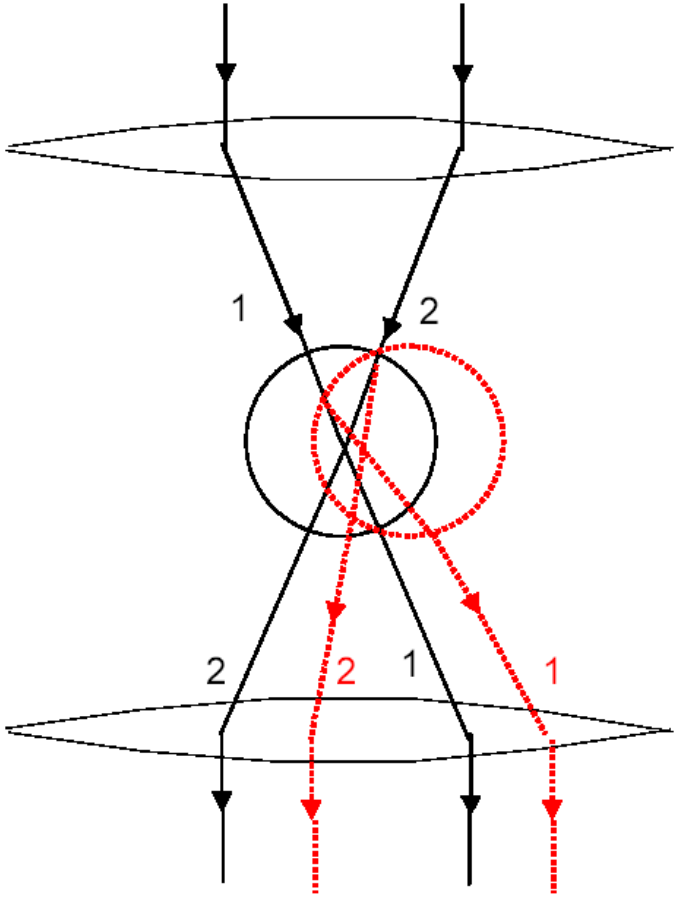
Result: A particle can get sucked into the focus of a laser bundle and be stably trapped.
Thus highly focused laser beam acts as a three-dimensional potential minimum.
Therefore it takes force the dislodge a bead out of the laser focus.

The physics behind optical tweezers

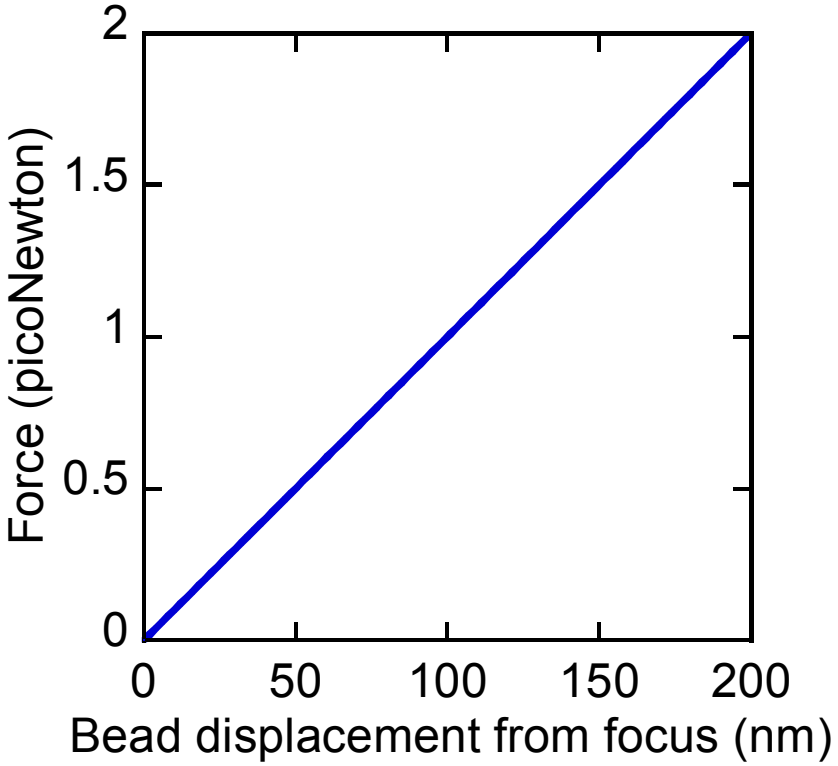
Radiation pressure is the force per unit area on a object due to change in light momentum.
A 100% reflecting mirror reflecting a 60W lamp gives a force of 10^{-7} N: gravity pulls on a 1 kg mirror with 9.8 N so the force of the light is here negligible.
However, if the same light is reflected by a object of 1 μg it can't be ignored! Using a laser on a microscopic particle will realize this situation.

A particle in a laser beam

Also the bead movement is measurable from the laser deflection.



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

Characteristics of optical traps

Comparison of forces with other techniques and biological processes:

Optical traps	10^{-13}-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

Pro's and Con's optical traps for Biophysics

Pro's:

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 (but they will soon be)

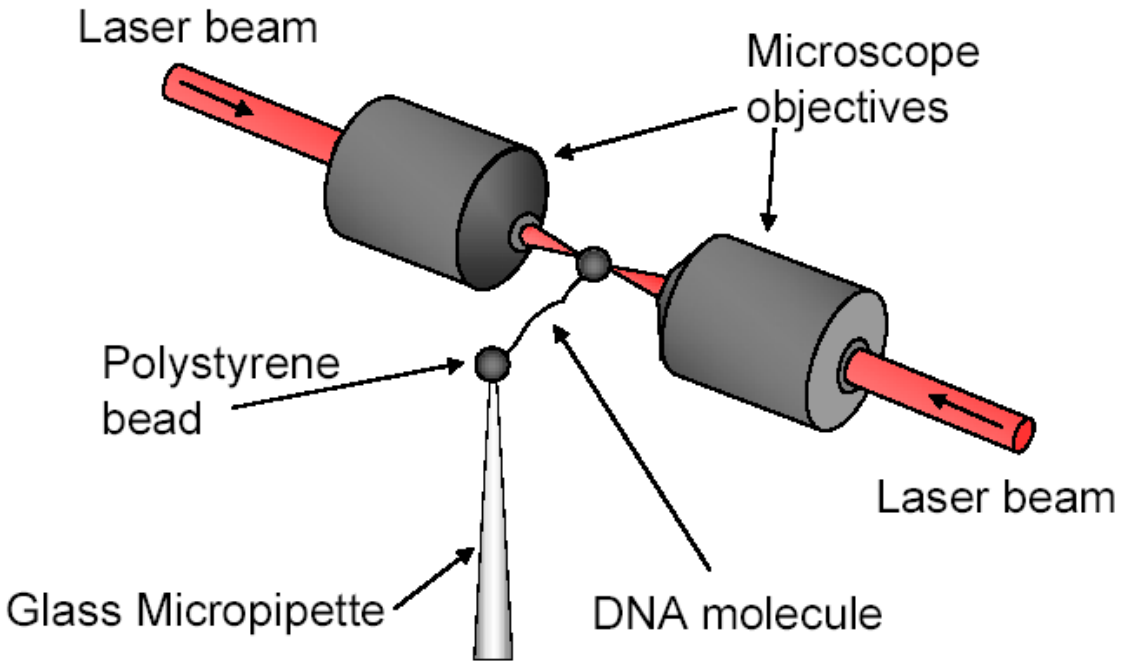
What can we do with optical tweezers?

1) Pull or displace microscopic particles

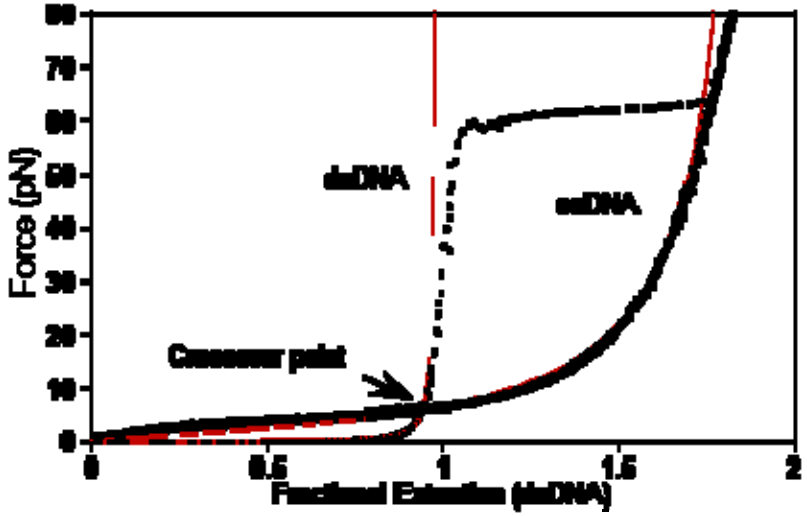
2) Measure microscopically small forces like:

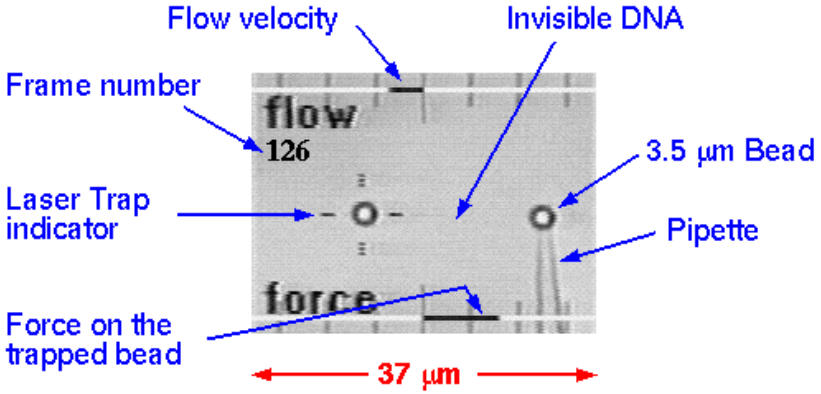
   - Studying the strength of biological materials such as cells, membranes, proteins or DNA.
   - Detection of force generation in molecular motors such as kinesin (the protein responsible for pulling apart chromosomes during cell division) or RNA polymerase.
   - Elucidation of the microscopic properties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

Optical tweezers can be used together with other force transducers / positioners, such as micropipettes, magnetic traps, controlled hydrodynamic flows.



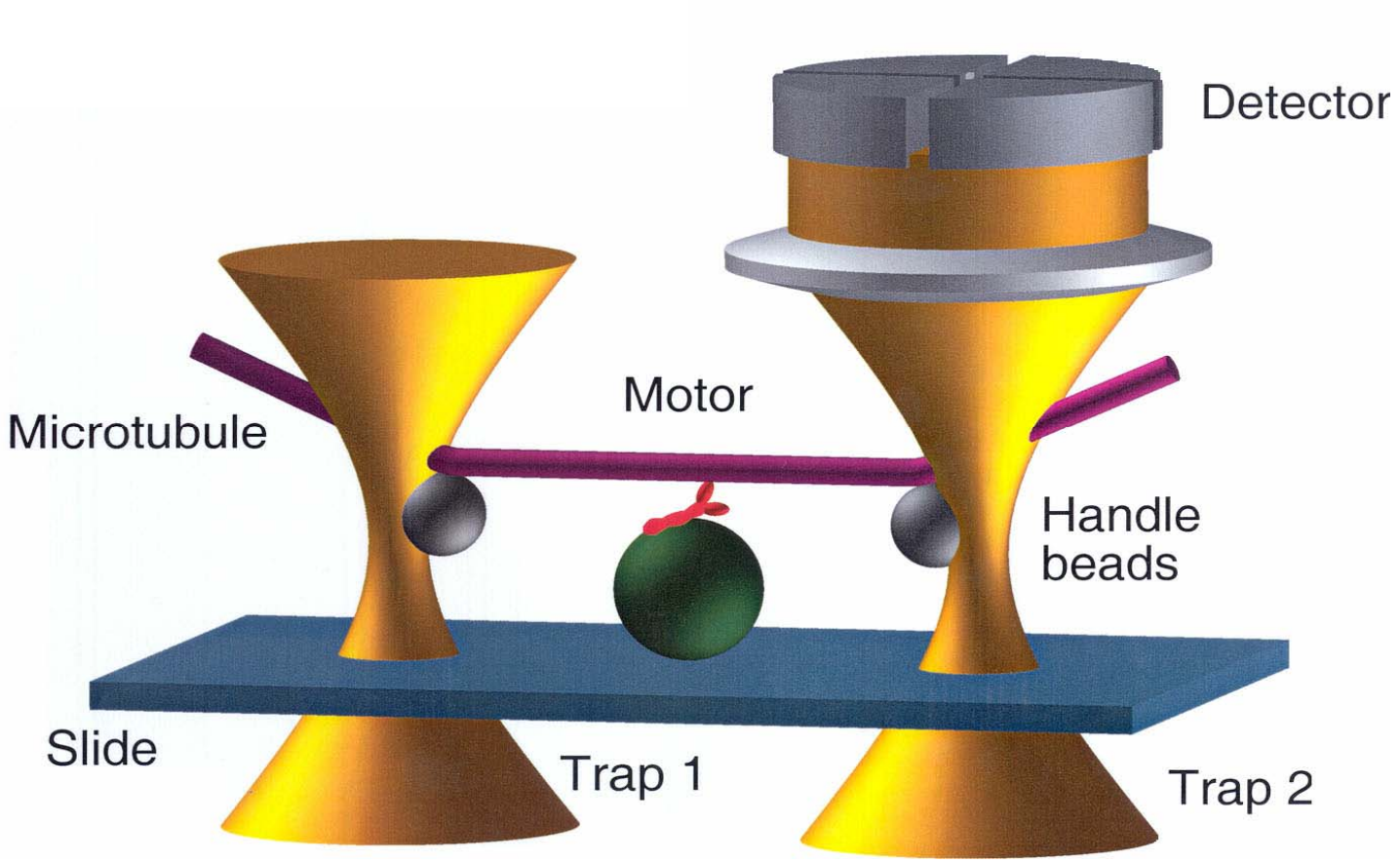
The DNA elasticity





Data from the Bustamante lab

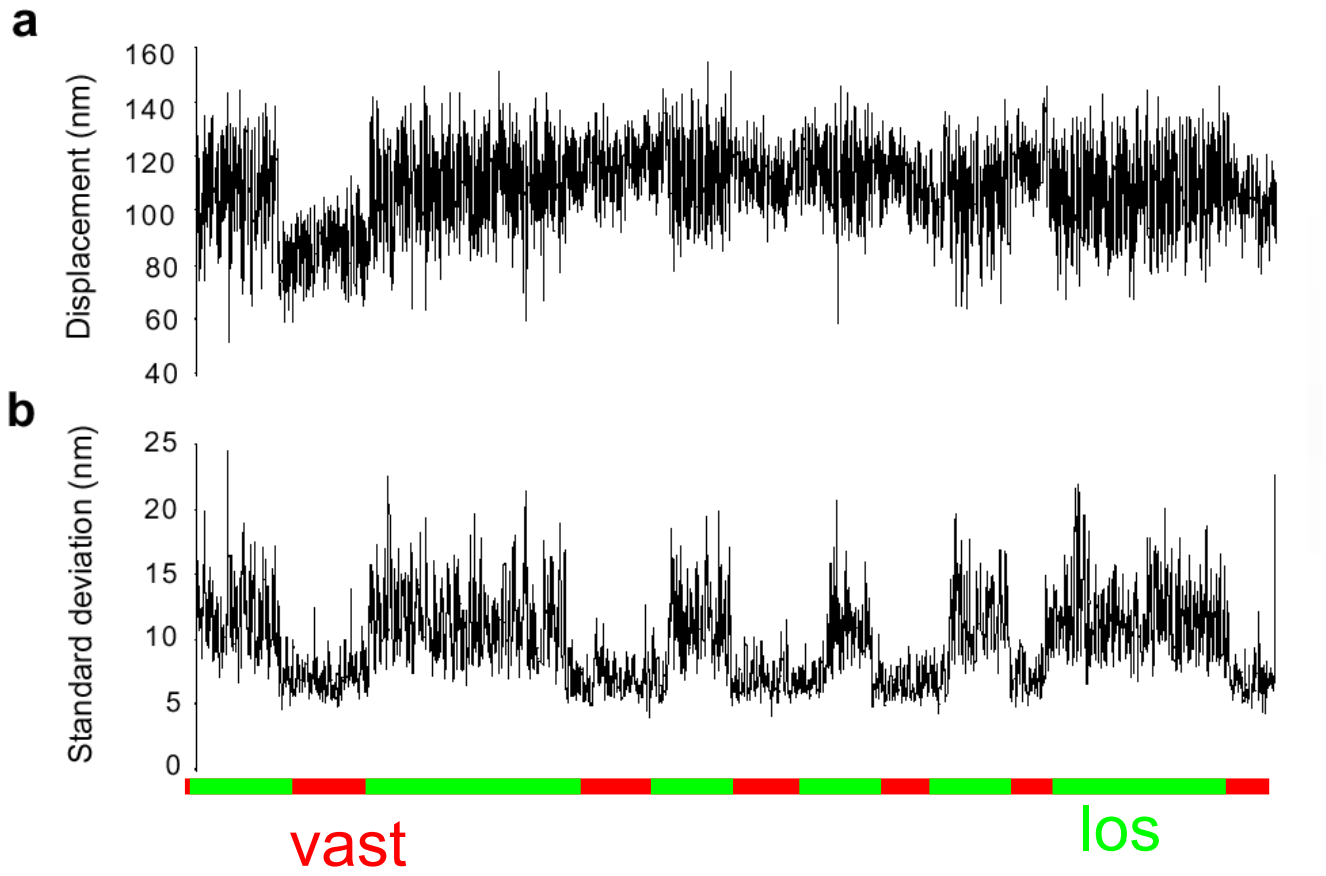
Power strokes from the motor protein NCD



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

Example from the Wuite lab (Amsterdam, NL)

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.