Introduction and applications

Magnetic tweezers

"MT"

1. Homework #3 will be assigned.
2. Quiz today
3. Bending & twisting rigidity of DNA with Magnetic Traps.

Many slides came from Laura Finzi at Emory University. Thanks!

Some came from Majid Minary-Jolandan, grad. student at UIUC. Thanks!

Quiz #2

1. What is the molecule that acts as the principle form of energy currency in a cell?

   adenosine triphosphate, ATP

2. The presence of this organelle is the most striking difference between prokaryotic and eukaryotic organisms.

   nucleus, membrane bound organelles

3. The ___mitochondria___ is the organelle most responsible for energy production in a eukaryotic cell.

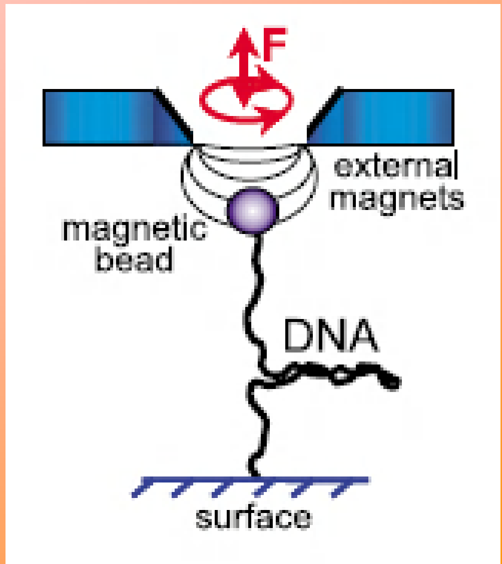
4. Name one of the two (or three) types of cytoskeletal filaments described from the reading.

   actin, microtubules, intermediate

5. Plants generate energy by a process known as ___photosynthesis___.

Magnetic Tweezers and DNA

Can be conveniently used to **stretch** and **twist** DNA.



With B-field horizontal, what direction is Force?

$F = qv \times B = \mu \times B \rightarrow$ vertical (z-axis)

- DNA tends to be stretched out if move magnet up.
- DNA also tends to twist if twist magnets (either mechanically, or electrically)

Forces ranging from a few fN to nearly 100 pN: Huge Range

Watch as a function of protein which interacts with DNA (polymerases, topoisomerases), as a function of chromatin: look for bending, twisting.

What can MT do for you.

MT is a **single molecule** biophysics tools.

(like Fluorescence, Optical Tweezers…)

As a s.m. technique, can resolve heterogeneity.

Examples?

Average vs. Individual:

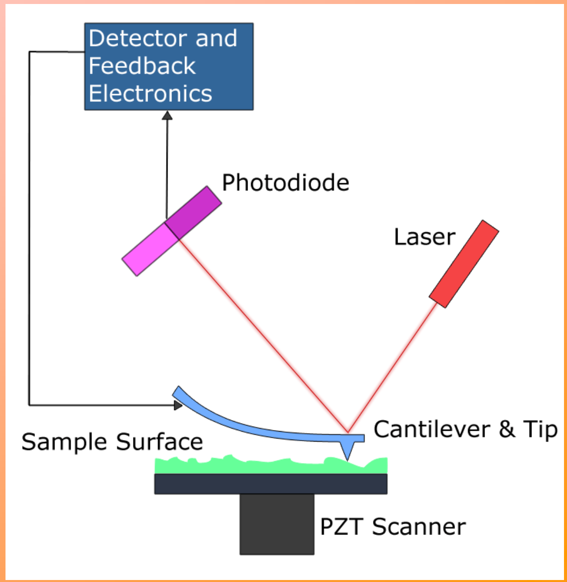
The average height 5’7” tall. In-fact, men 5’9”, women are 5’5”.

Average is ½ female features, ½ male features. In-fact: woman or a man.

Properties that are masked in bulk biochemical and biophysical measurements (measurements over the entire ensemble of molecules in the sample) can be revealed and studied.

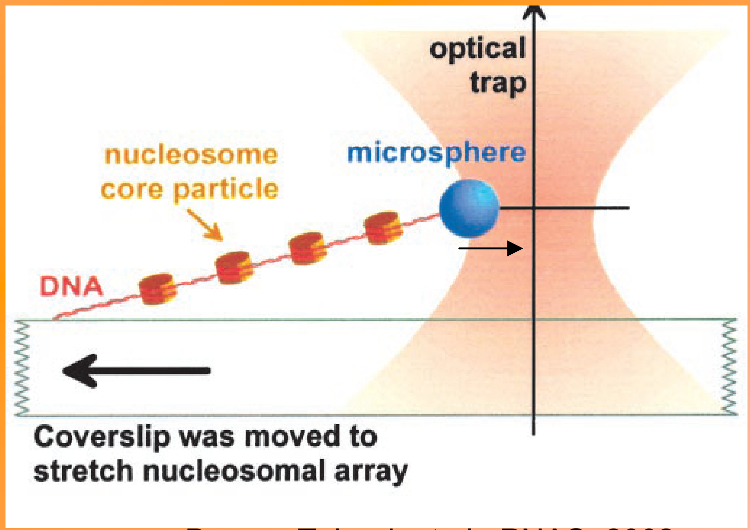
Two types of Single Molecule Methods

1. **Mechanical force transducers** — the AFM, microneedles and optical fibers — forces are applied or sensed through bendable beams.



http://en.wikipedia.org/wiki/Atomic_force_microscope

Laser traps handle is a polystyrene bead

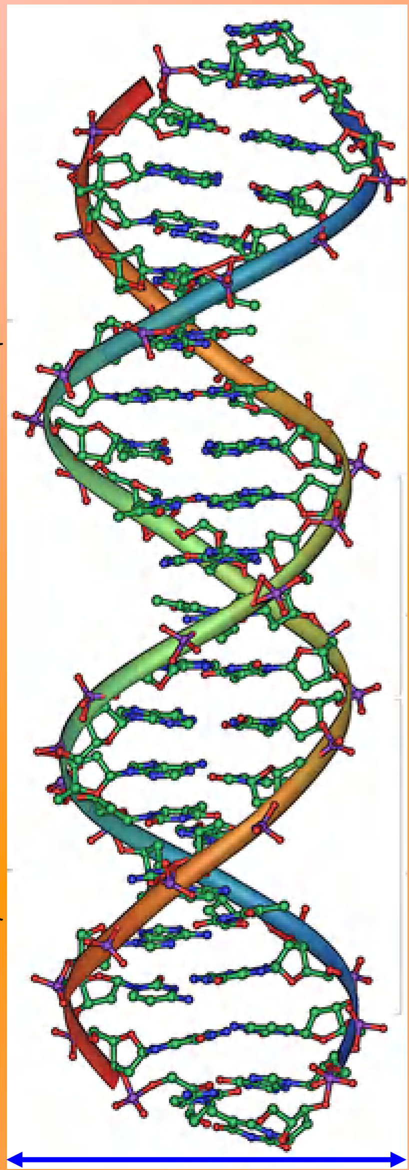


Brower-Toland, et al., PNAS, 2002

2. **External field manipulators** — optical tweezers (OT), magnetic tweezers (MT), and flow fields — the molecule is acted upon from a distance, by application of external fields (photonic, magnetic, or hydrodynamic) either to the molecule itself or to an appropriate handle to which the molecule is attached.

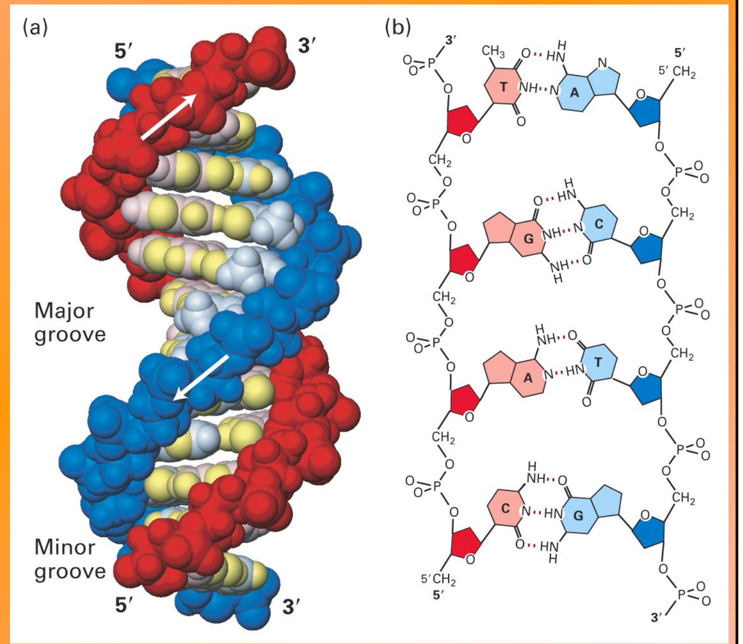
DNA Structure

- Right-hand helix
- One turn: 3.4 nm, ~10.5 bp
- Twist angle between bps θ=36

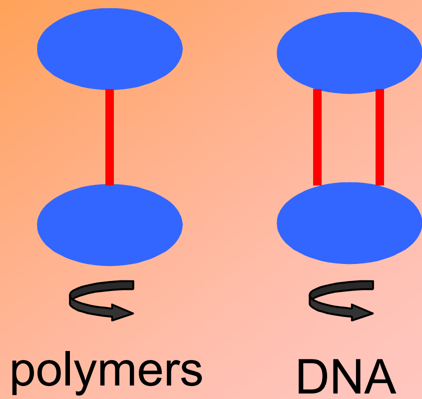


Wikipedia

2nm

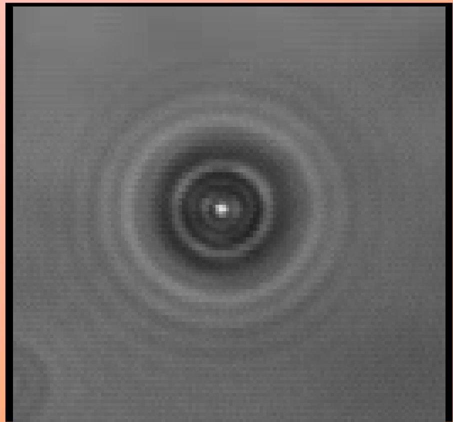


Molecular Cell Biology, Lodish

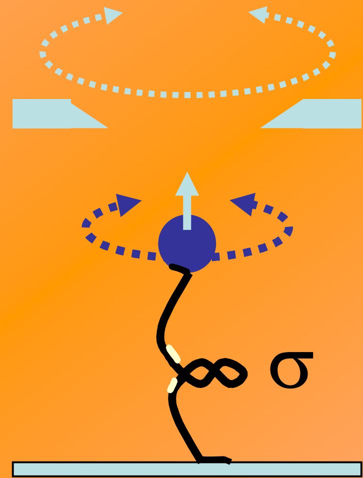


DNA will resist twisting

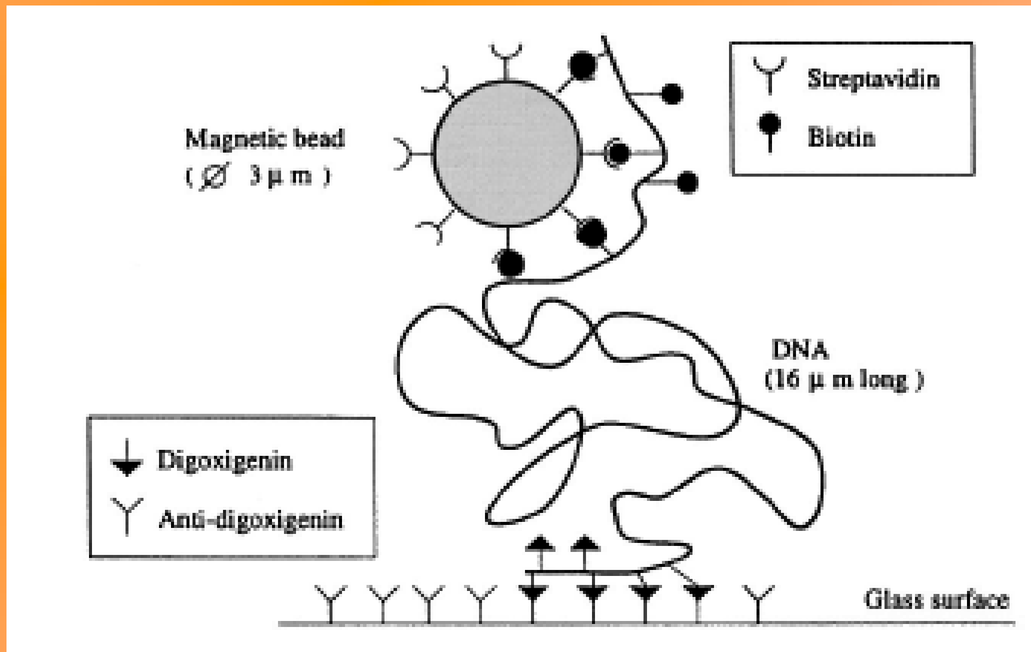
Magnetic Traps: Measuring twist



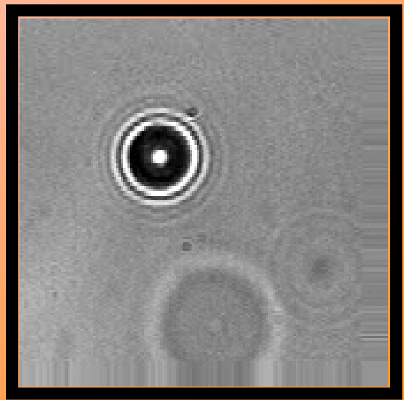
DNA twisting



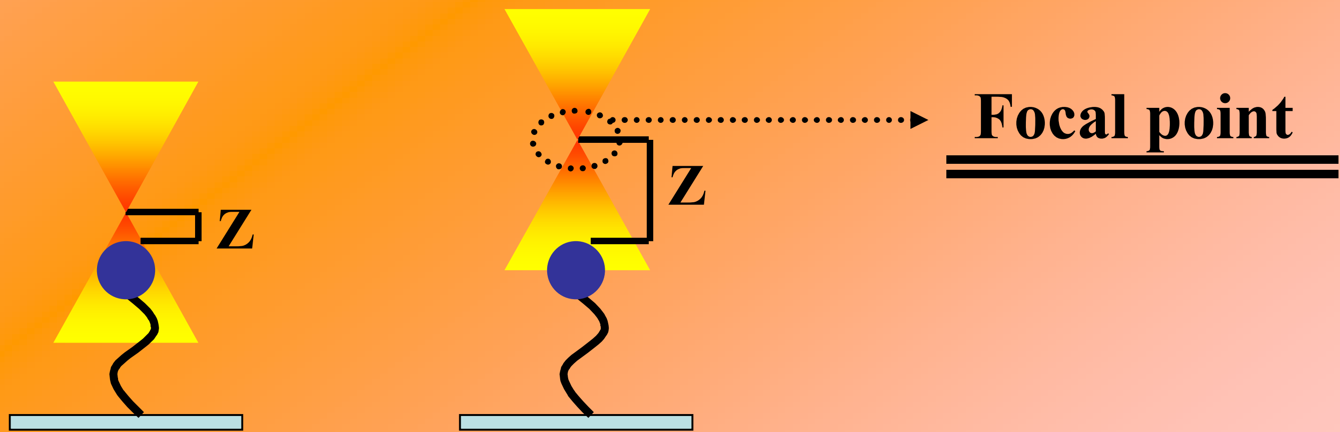
Twisting leads to motion in x-y plane



Magnetic Traps: Measuring DNA extension

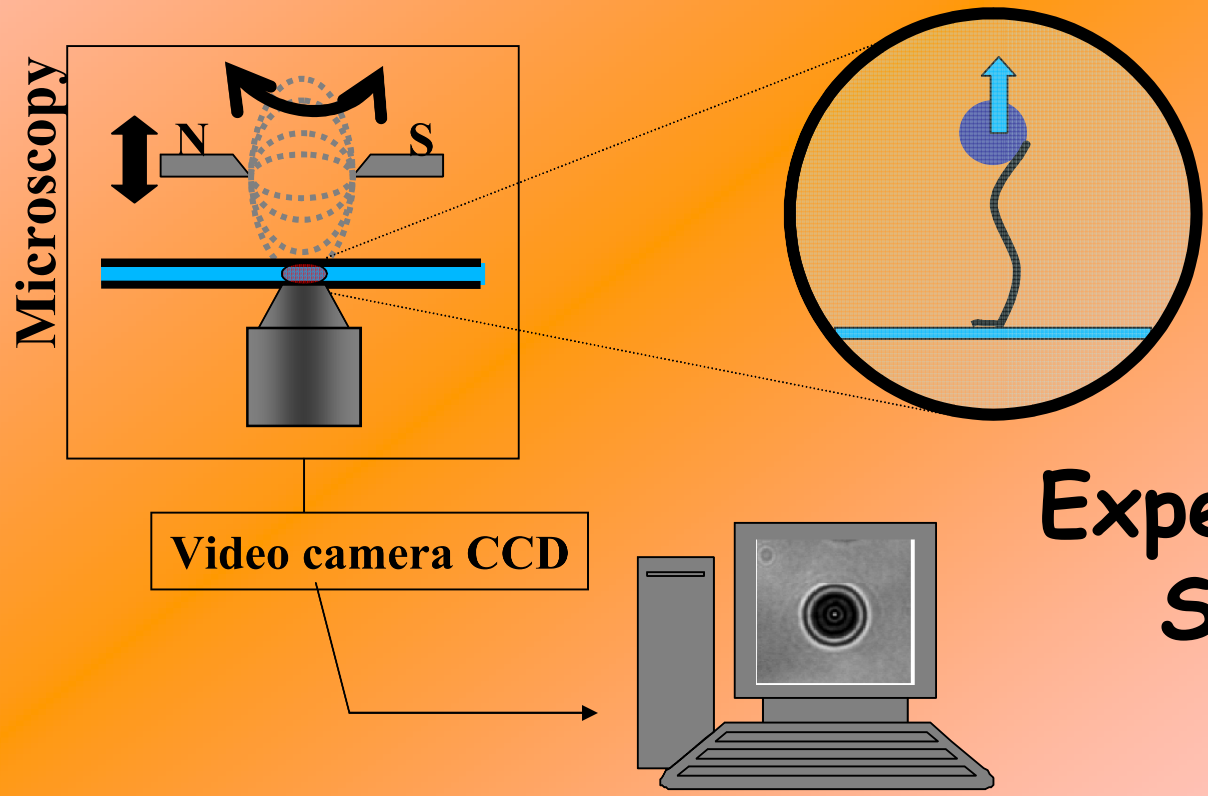


Diffraction rings



Magnetic Trap movie (ADN.SWF)

How to attach DNA: to glass; to paramagnetic bead
Set-up of Experimental system
Detect nanometer displacements with visible light



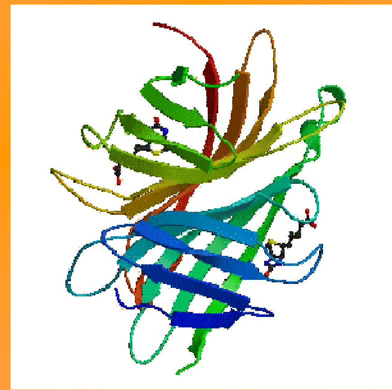
Experimental Set-up

Streptavidin (egg white) -Biotin

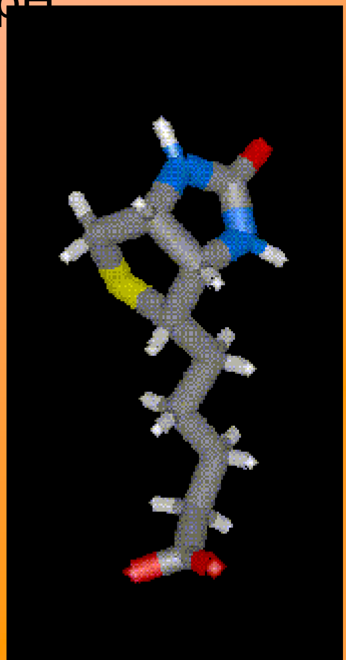
The tightest non-covalent bond known roughly ~100kT

Biotin + SA ↔ Biotin-SA

K_{assoc} = [B-SA]/ [B][SA] = $10^{14}M^{-1}$

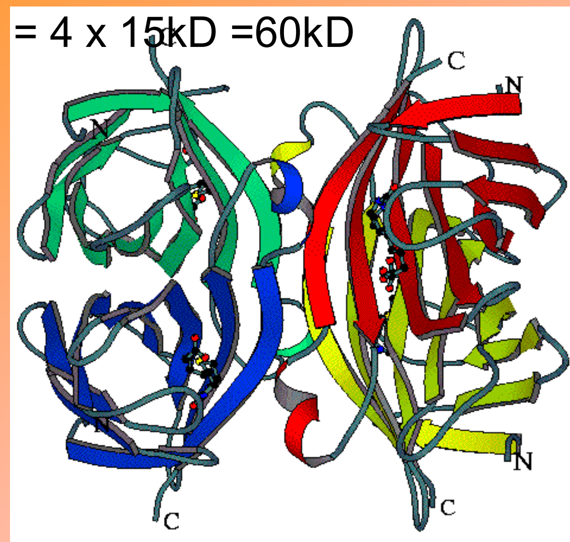


The complex is also extremely stable over a wide range of temperature and pH



254 AA = 46 x 93 x 104 Å

= 4 x 15kD =60kD



http://faculty.washington.edu/stenkamp/stefanieweb/abstract.html

http://ambermd.org/tutorial/streptavidin/index.html

Quick calculation about K_{eq}

Biotin + SA $\leftrightarrow$ Biotin-SA

K_{assoc} = [B-SA]/ [B][SA] = $10^{14}M^{-1}$

Let's say you start with:

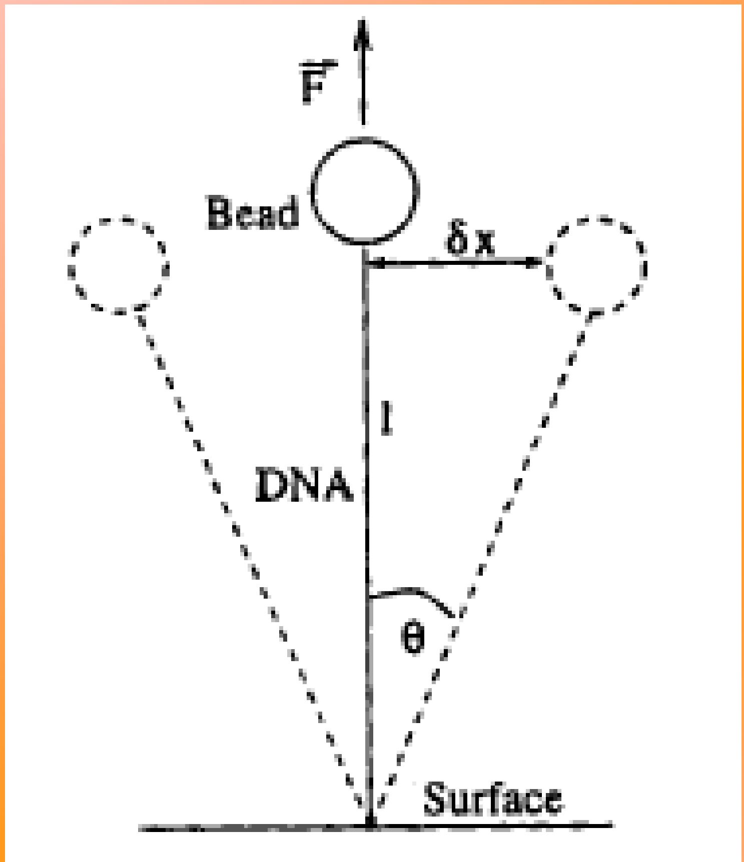
10^{-6}M of [B]=[SA]. How much B-SA do you end up with?

10^{-9}M of [B]=[SA]. How much B-SA do you end up with?

Force measurement- Magnetic Pendulum

The DNA-bead system behaves like a small pendulum pulled to the vertical of its anchoring point & subjected to Brownian fluctuations

Do not need to characterize the magnetic field nor the bead susceptibility, just use Brownian motion



T. Strick et al., J. Stat. Phys., **93**, 648-672, 1998

Equipartition theorem

Each degree of freedom goes as x^2 or v^2 has $\frac{1}{2}k_BT$ of energy.

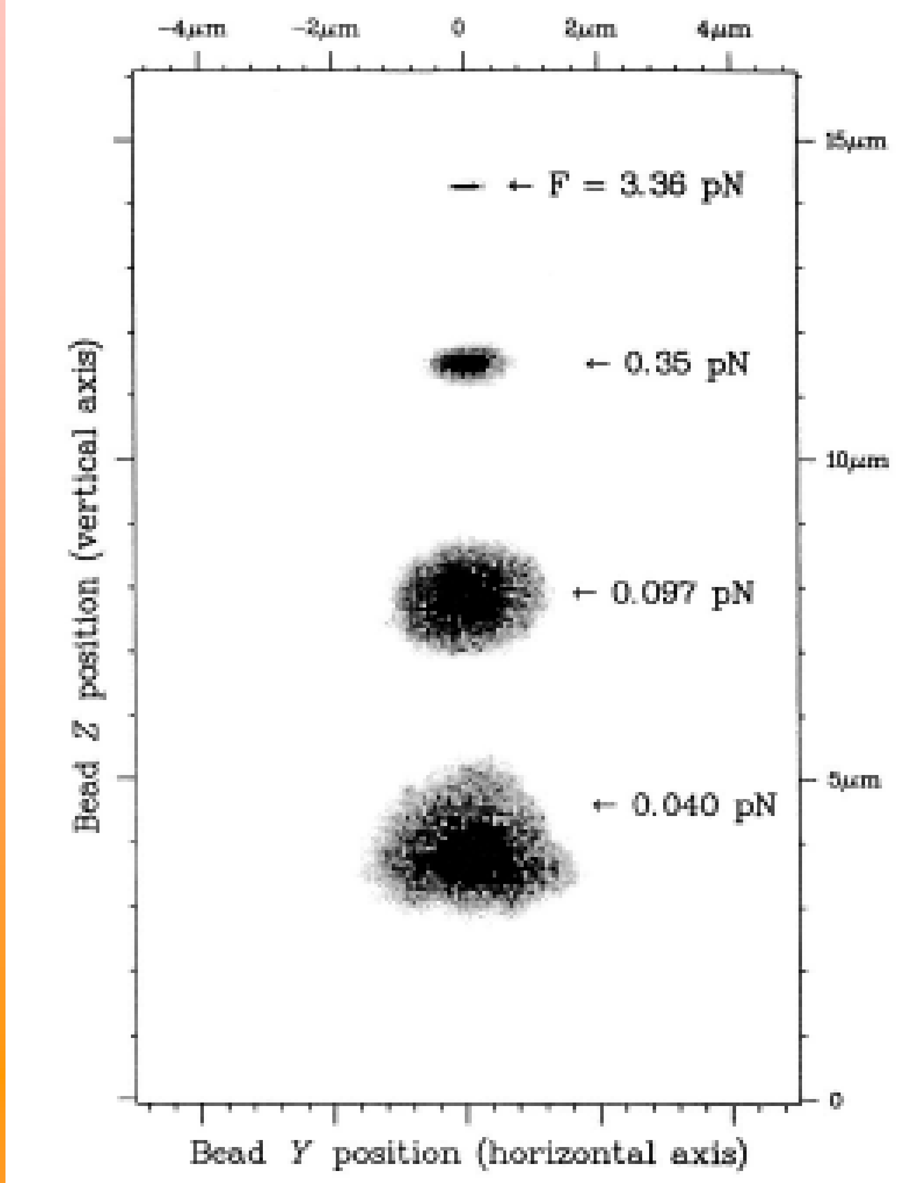
$$\frac{1}{2} k \langle \delta x^2 \rangle = \frac{1}{2} k_B T$$

$$F = k\, l$$

$$\frac{1}{2} (F/l) \langle \delta x^2 \rangle = \frac{1}{2} k_B T$$

$$F = \frac{k_B T\, l}{\langle \delta x^2 \rangle}$$

Force measurements- raw data



$Z = l$

Measure $< \delta x^2 >$, l and have F!

$$F = \frac{k_B T l}{< \delta x^2 >}$$

(4.04 pN-nm)(7800nm)/ 577^2 nm = 0.097 pN

T. Strick et al., J. Stat. Phys., **93**, 648-672, 1998

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.