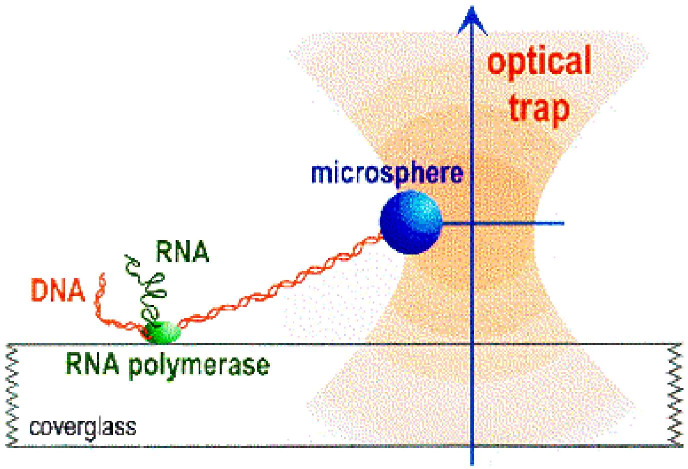
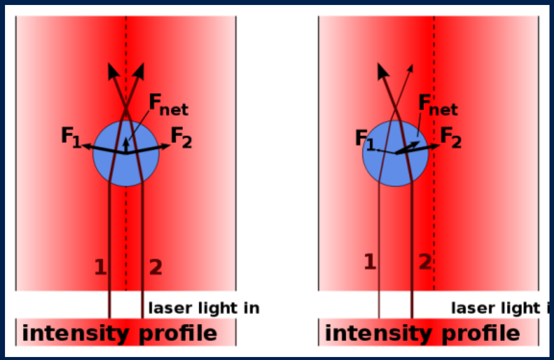
Optical Traps

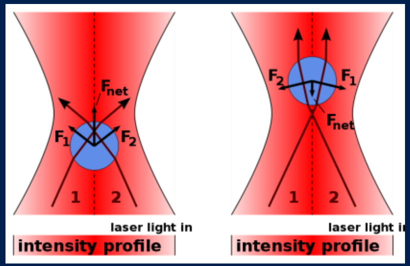
Optical Trap (Nobel Prize, 1997)

Bead is held by "optical force" in trap with effective spring constant k.

Can measure: "stall force" –max force motor can make.
displacement of bead with nm. resolution.



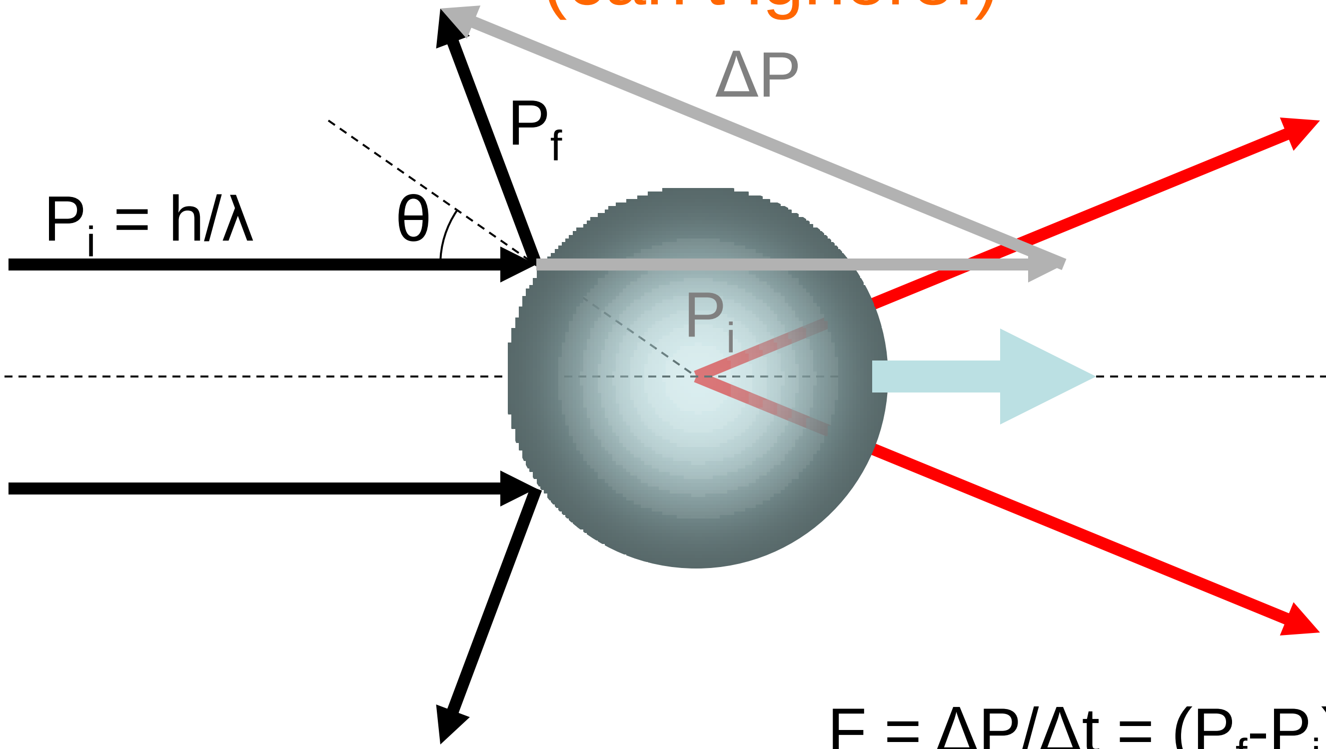




Optical Traps

Why does index of refraction of bead $n_{bead} > n_{water}$ for trap to work?

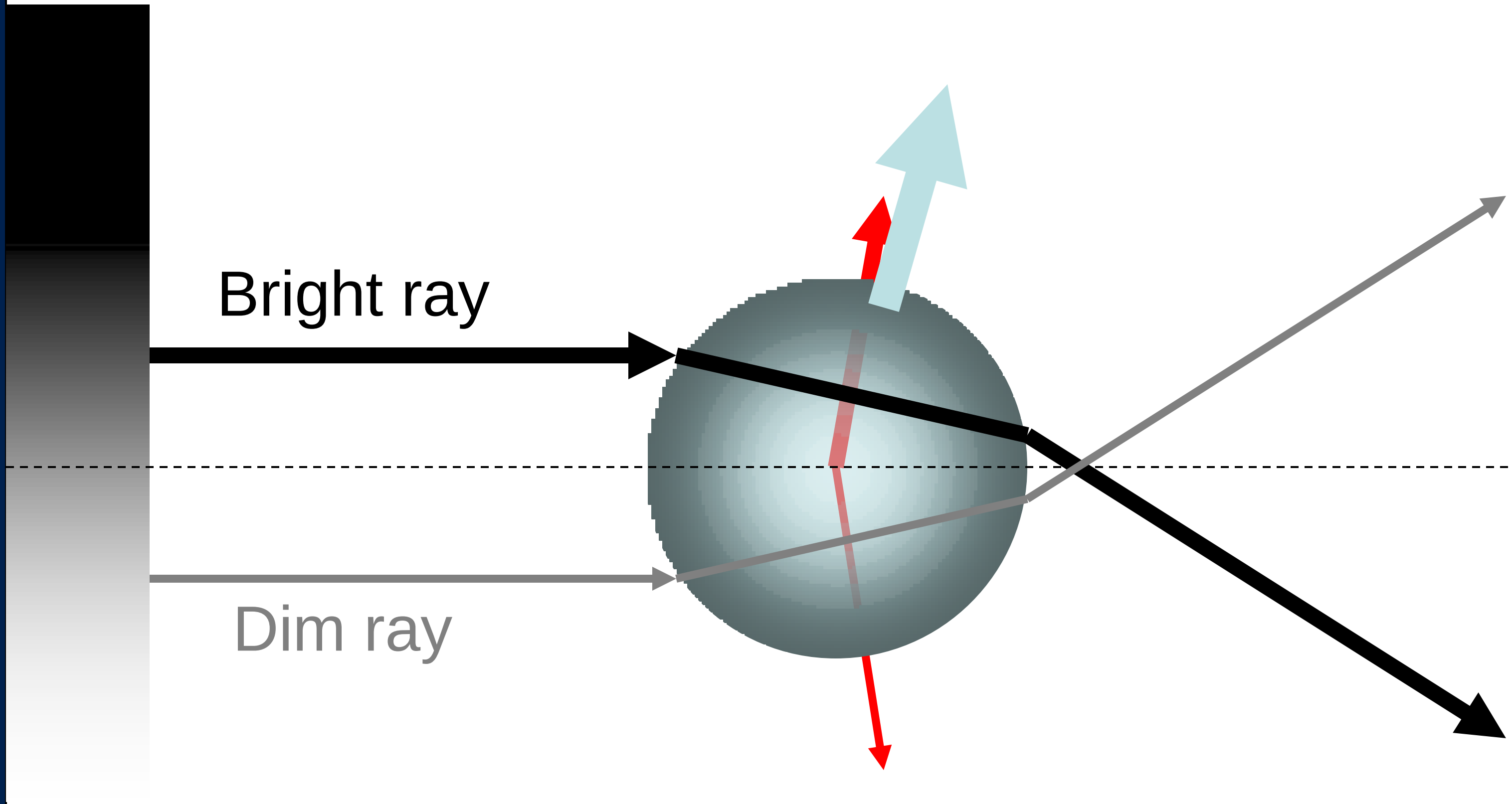
Optical scattering forces – reflection (can't ignore!)



$F = \Delta P/\Delta t = (P_f - P_i)/\Delta t$

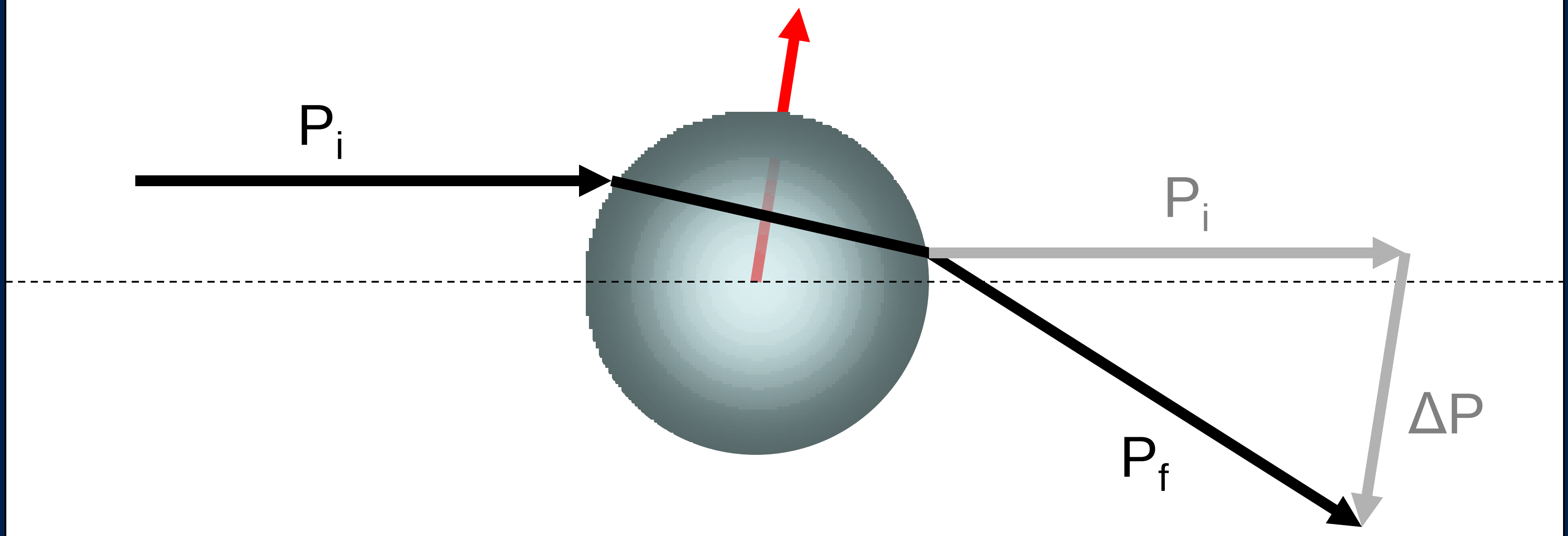
Newton's third law – for every action there is an equal and opposite reaction

Lateral gradient force: Refraction

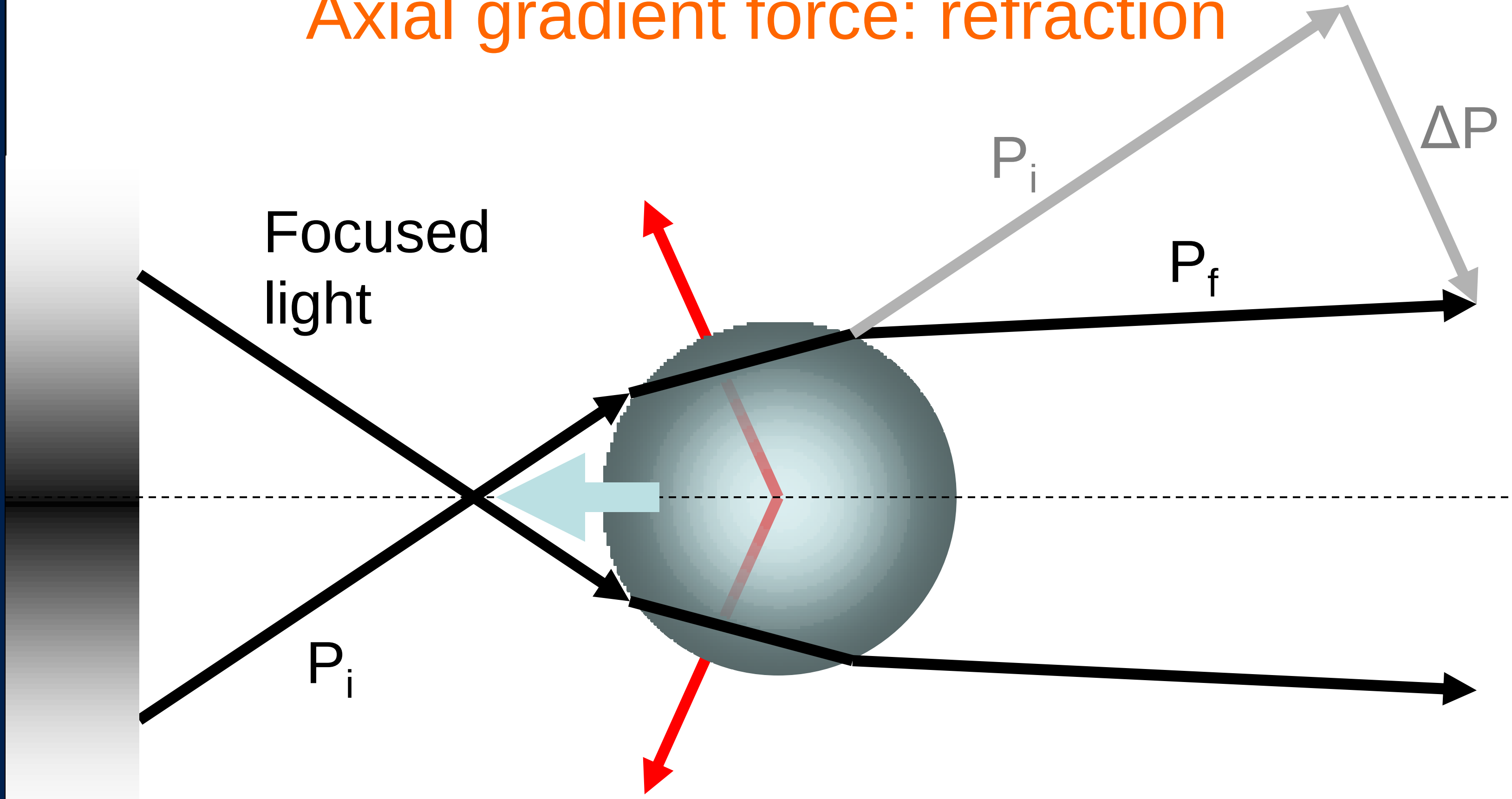


Object feels a force toward brighter light

Optical forces – Refraction



Axial gradient force: refraction

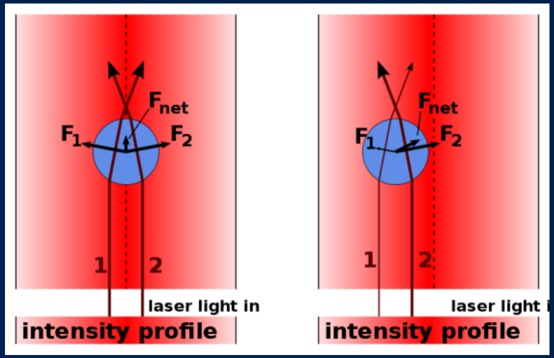


Object feels a force toward focus

(Not at focus because of scattering forces)

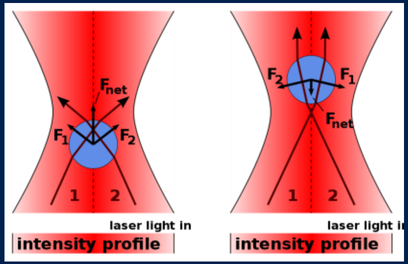
Force ~ gradient intensity

Why does index of refraction of bead $n_{bead} > n_{water}$ for trap to work?



$n_{bead} = n_{water}$

Beam doesn't change at all
No scattering, no bending



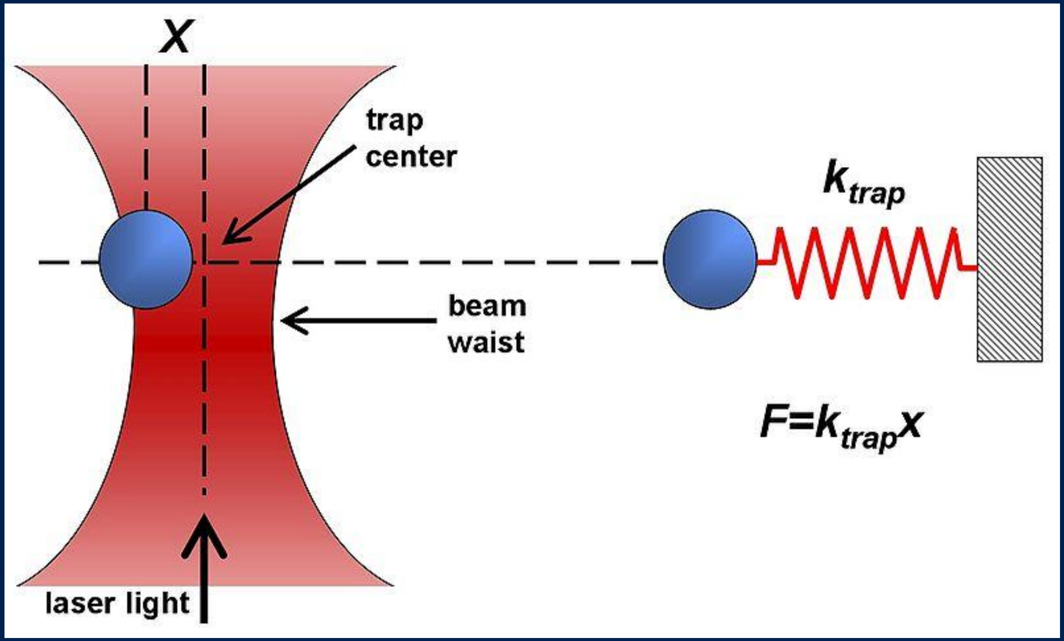
$n_{bead} > n_{water}$

Regular, stable trap.
Force towards most intense light.
Scattering, Bending.

$n_{bead} < n_{water}$

Unstable trap.
Force towards least intense light.
Scattering, Bending, wrong way.

Optical Tweezer = Optical Trap



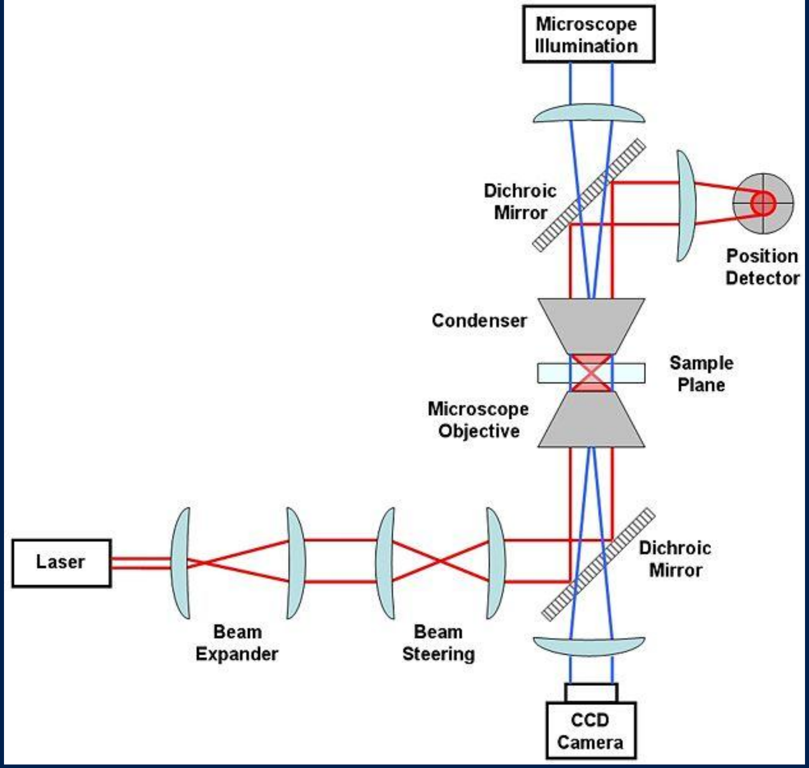
Dielectric objects are attracted to the center of the beam, slightly above the beam waist. This depends on the difference of index of refraction between the bead and the solvent (water).

Vary k_{trap} with laser intensity such that $k_{trap} \approx k_{bio}$ ($k \approx 0.1$pN/nm)

Can measure pN forces and (sub-) nm steps!

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

Basic Optical Trap set-up



Requirements for a *quantitative* optical trap:

1) Manipulation – intense light (laser), large gradient (high NA objective), moveable stage (piezo stage) or trap (piezo mirror, AOD, …) [AcoustOptic Device- moveable laser pointer]

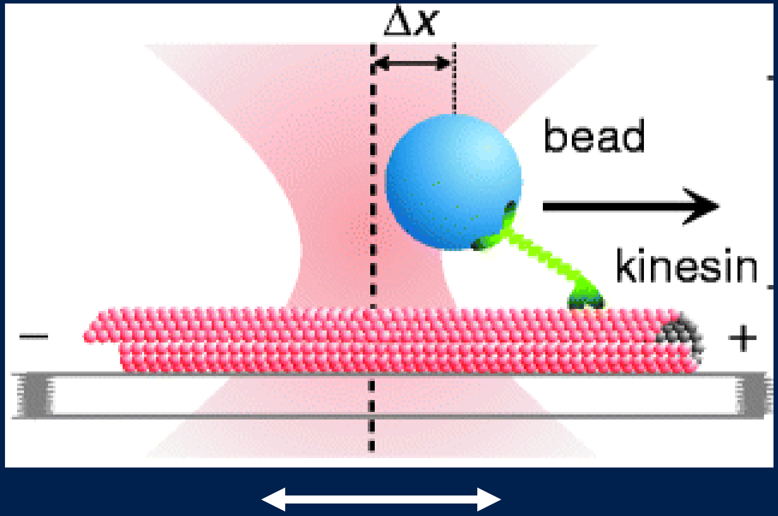
2) Measurement – collection and detection optics (BFP interferometry)

3) Calibration – convert raw data into forces (pN), displacements (nm)

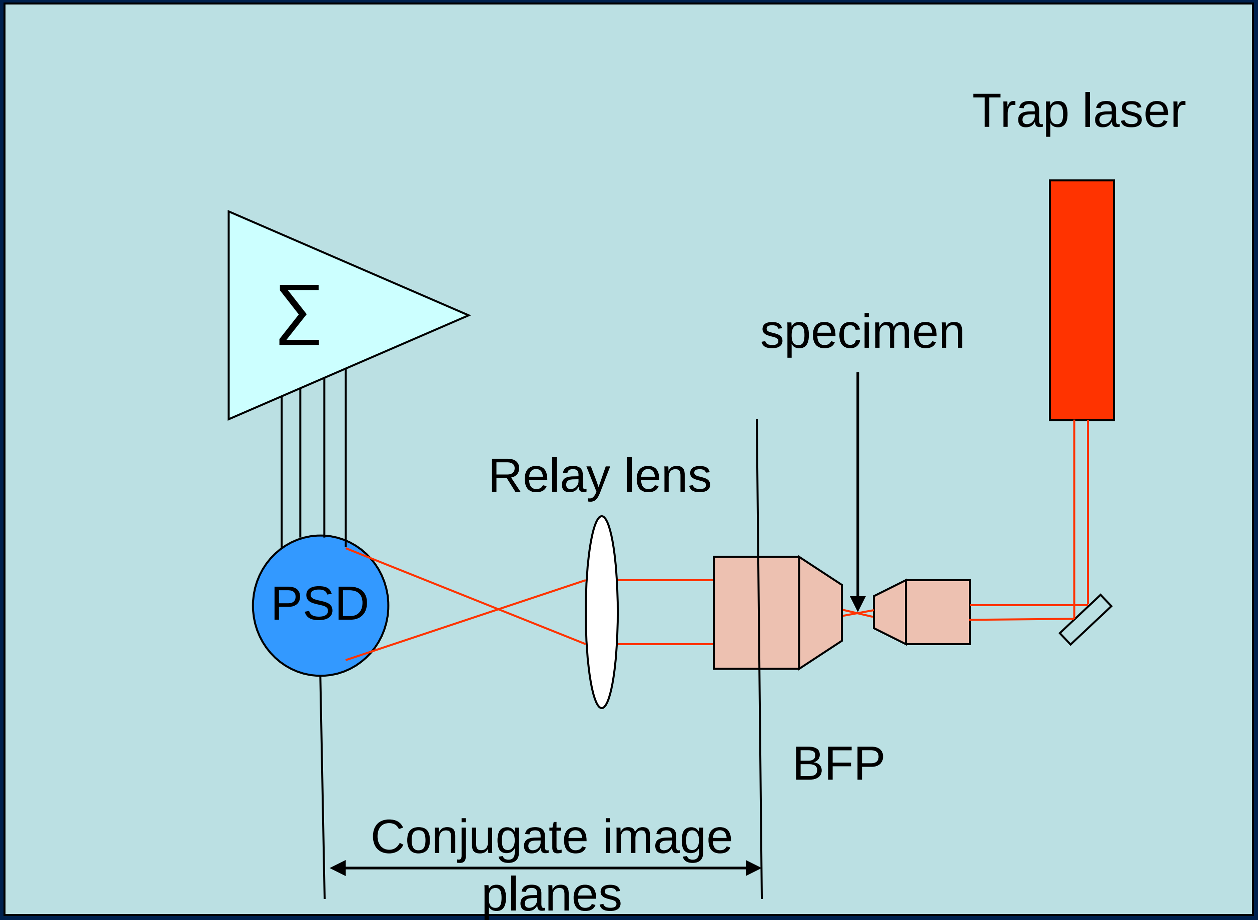
1) Manipulation

Want to apply forces – need ability to move stage or trap (piezo stage, steerable mirror, AOD…)

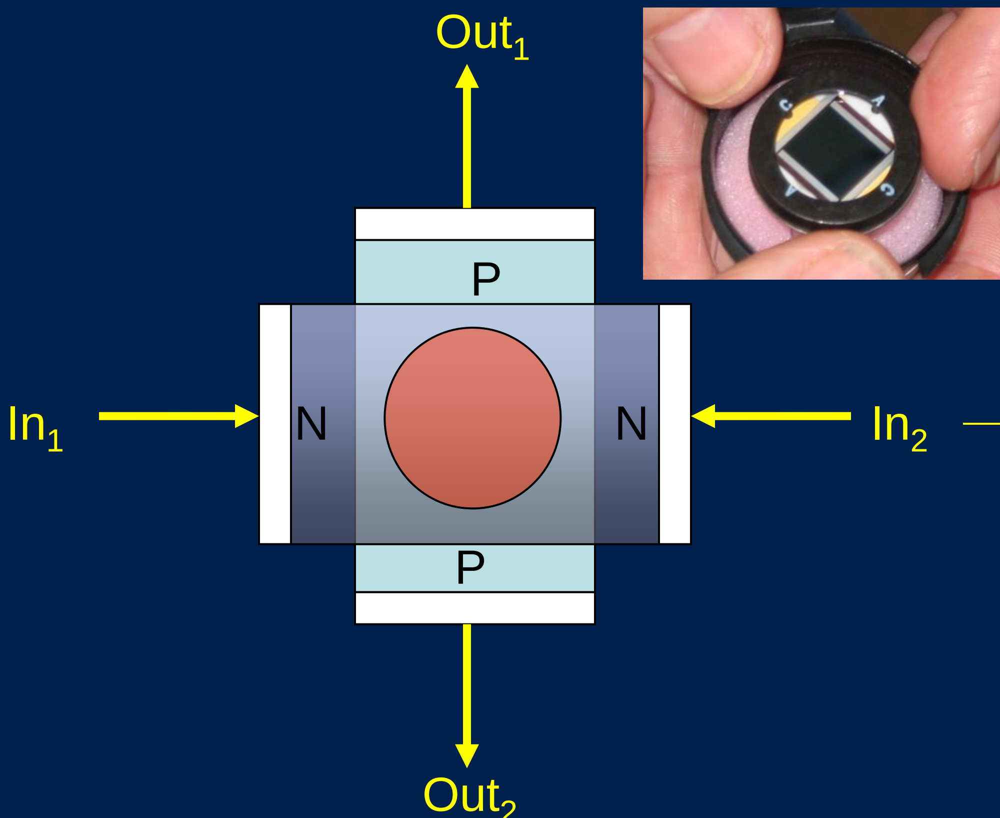
(Acouto Optic Device: variable placement of laser)

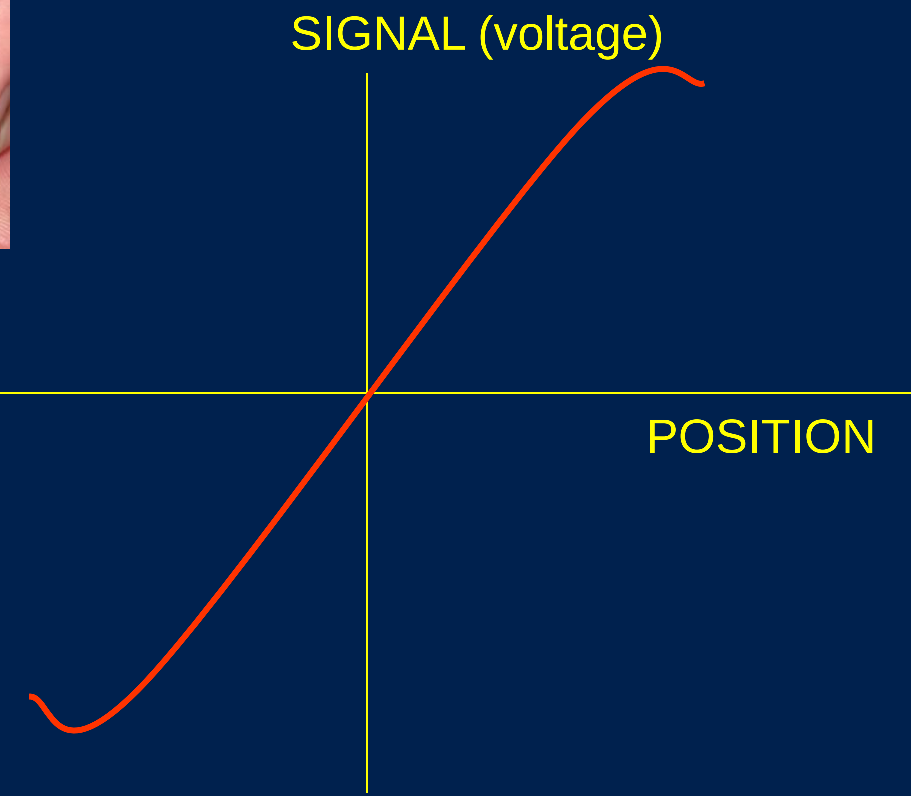


2) Measurement: BFP imaged onto detector



Multiple rays add their currents linearly to the electrodes, where each ray's power adds W_i current to the total sum.





$\Delta X \sim (In_1 - In_2) / (In_1 + In_2)$

$\Delta Y \sim (Out_1 - Out_2) / (Out_1 + Out_2)$

3) Calibration

Want to measure forces, displaces – measure voltages from PSD – need calibration

$$\Delta x = \alpha \, \Delta V$$
$$F = k\Delta x = \alpha \, k\Delta V$$

α allows you to go from the photodetector voltage to distance in nm, k gets you from distance to force in pN

Need to measure α, k.

Brownian motion as test force

Langevin equation:

$$\underbrace{m\ddot{x}}_{\approx 0} + \gamma\dot{x} + kx = F(t)$$

Inertia term (ma)

Drag force $\gamma = 6\pi\eta r$

Trap force

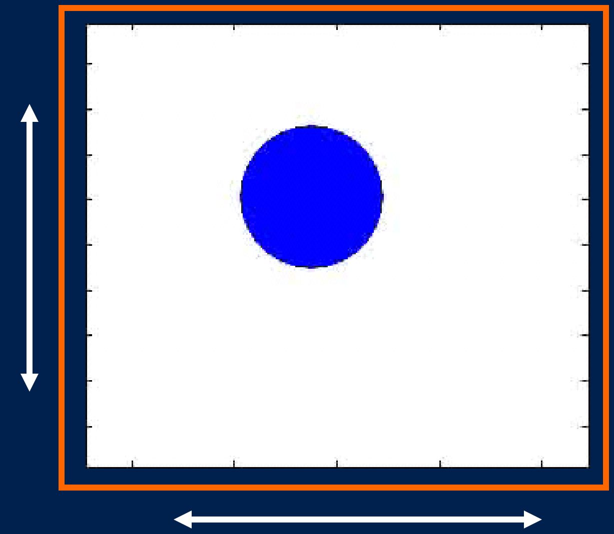
Fluctuating Brownian force

$$<F(t)> = 0$$

$$<F(t)F(t')> = 2k_BT\gamma\delta(t-t')$$

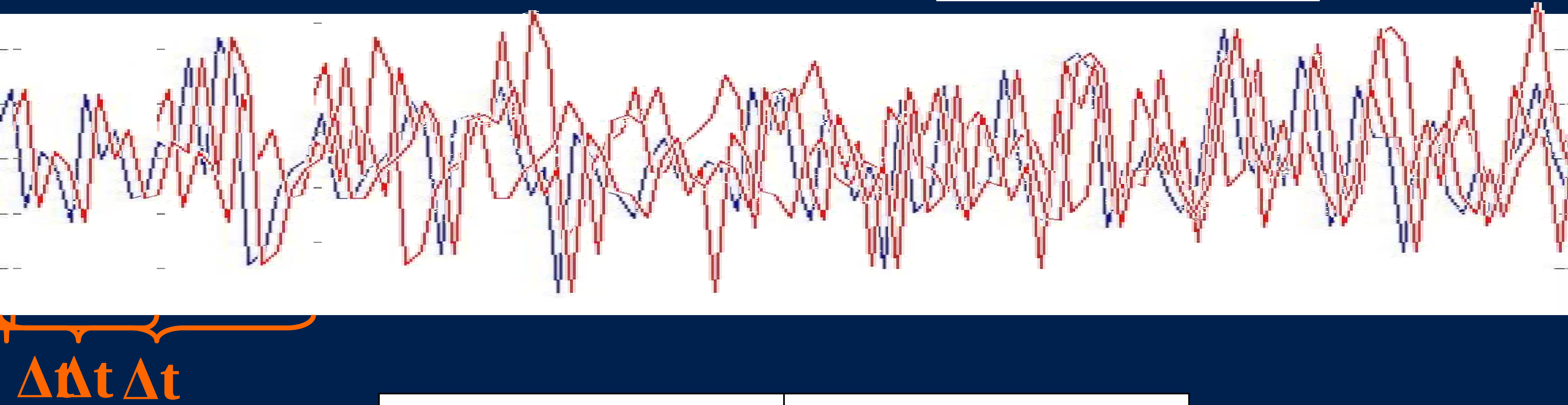
Inertia term for um-sized objects is always small (…for bacteria)

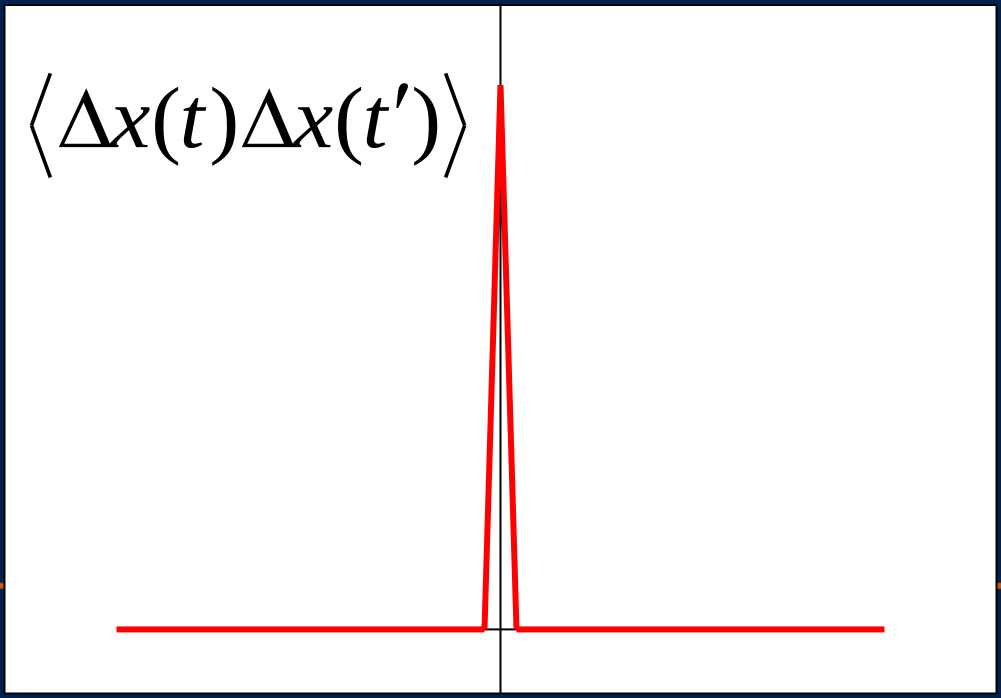
k_BT



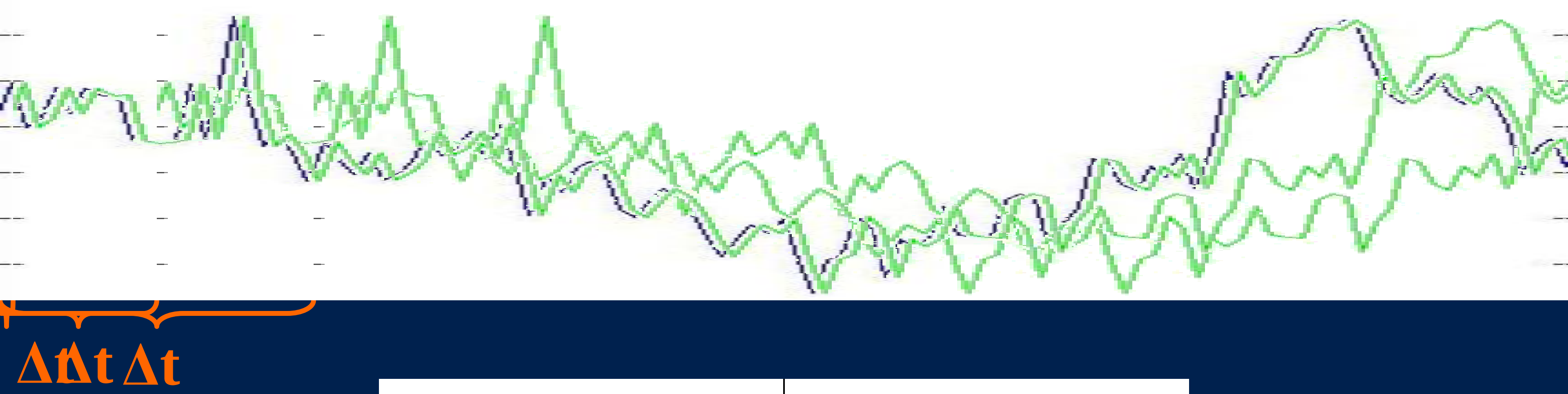
k_BT= 4.14pN-nm

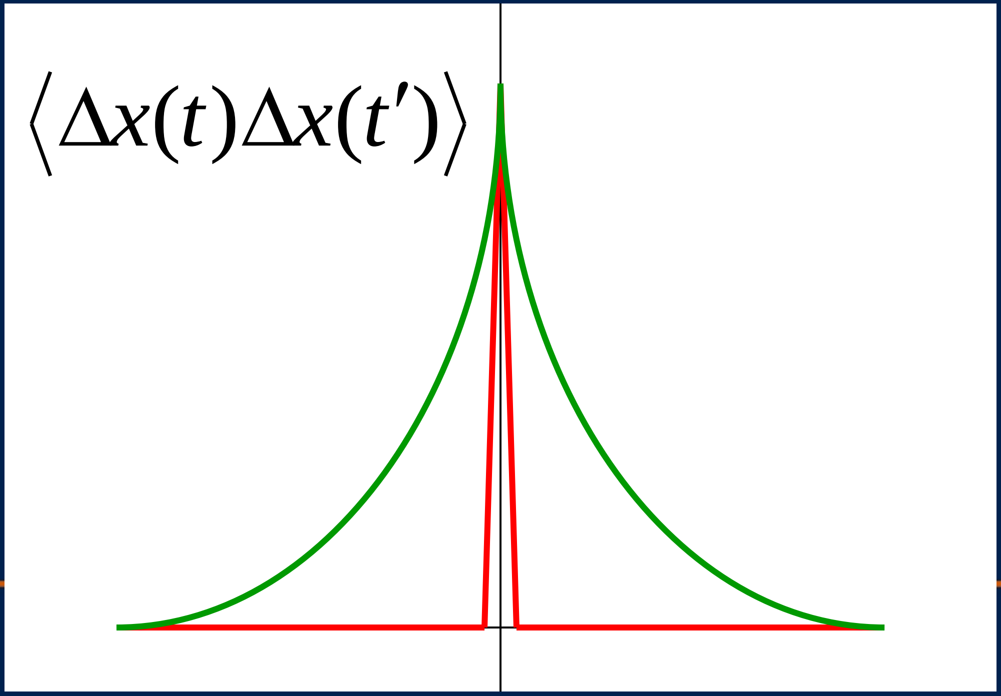
Autocorrelation function $\langle \Delta x(t) \Delta x(t') \rangle$





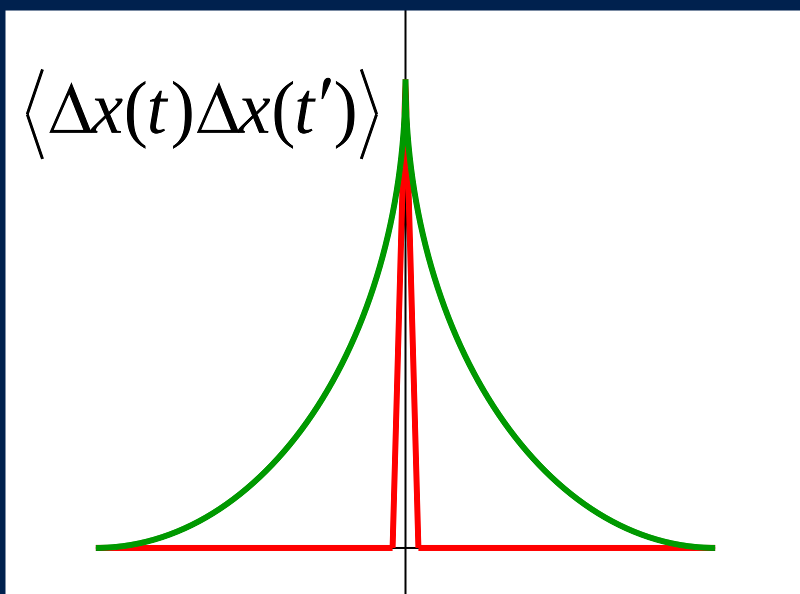
Autocorrelation function $\langle \Delta x(t)\Delta x(t')\rangle$





Why does tail become wider?

Answer: If it's headed in one direction, it tends to keep going in for a finite period of time.
It doesn't forget about where it is instantaneously.
It has memory.



$$\langle F(t)\rangle = 0$$

$$\langle F(t)F(t')\rangle = 2k_B T\gamma\delta(t-t')$$

This says it has no memory.
Not quite correct.

Brownian motion as test force

Langevin equation:

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

Exponential autocorrelation function

$$\left\langle \Delta x(t) \Delta x(t') \right\rangle = \frac{k_B T}{k} e^{-k|t-t'|/\gamma}$$

Notice that this follows the Equilibrium Theorem

$$\left\langle \Delta x^2 \right\rangle = \frac{k_B T}{k}$$

FT → Lorentzian power spectrum

$$S_x(f) = \frac{4 k_B T \gamma}{k^2} \frac{1}{1 + (f/f_c)^2}$$

Corner frequency $f_c = k/2\pi\gamma$

$$S_x(f) = \frac{4k_B T g}{k^2} \frac{1}{1+\left(f/f_c\right)^2}$$

As f→ 0, then

$$S_x(f) = \frac{4k_B T g}{k^2}$$

As f→ f_c, then

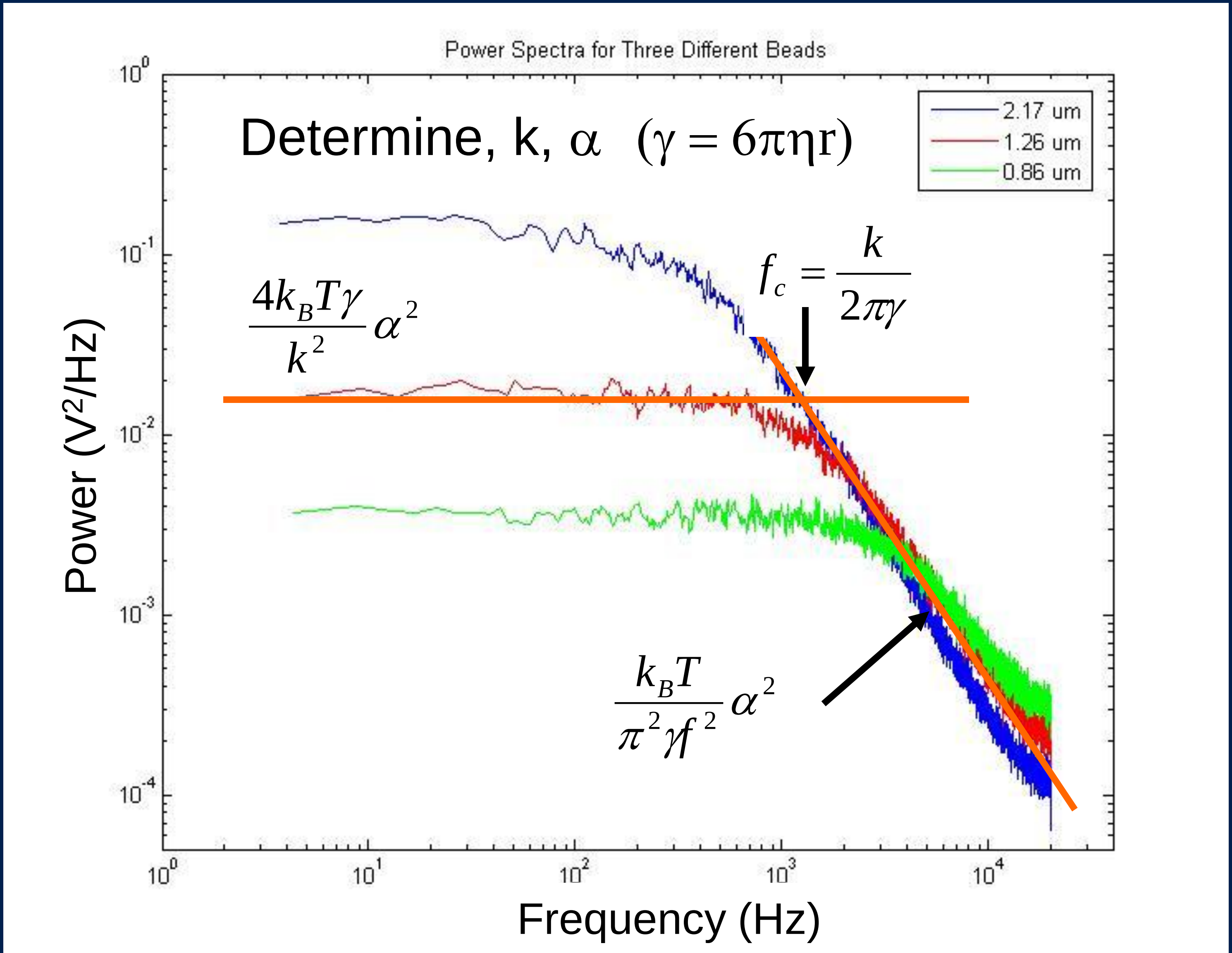
$$S_x(f) = \frac{4k_B T g}{k^2} \frac{1}{2}$$

As f >> f_c, then

$$S_x(f) \rightarrow 0$$

$$S_x(f) = \frac{k_B T}{p^2 f^2}$$

1. Voltages vs. time from detectors.
2. Take FT.
3. Square it to get Power spectrum.
4. Power spectrum = α^2 * $S_x(f)$.



What is noise in measurement?

The noise in position using equipartition theorem

→ you calculate for noise at all frequencies (infinite bandwidth).

For a typical value of stiffness (k) = 0.1 pN/nm.

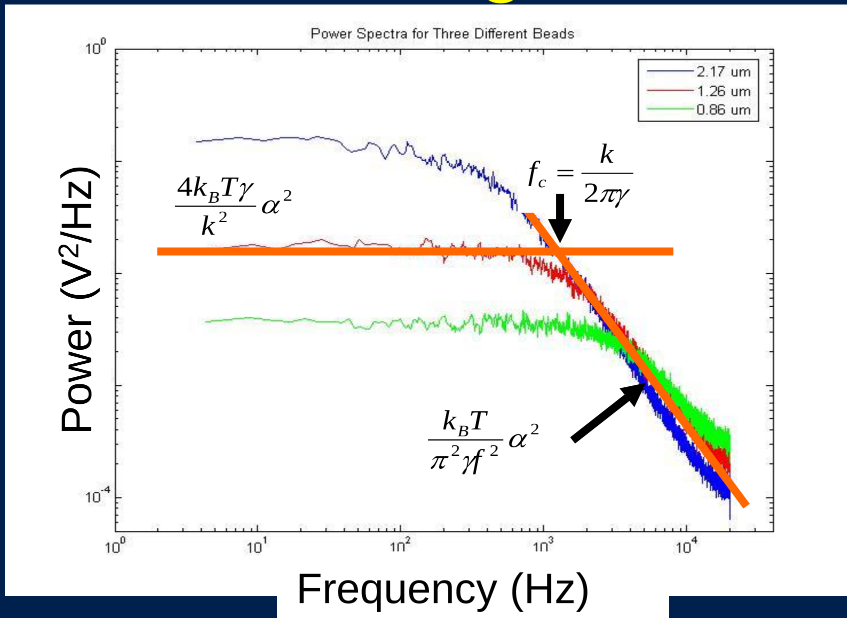
$<x^2>^{1/2} = (k_B T/k)^{1/2} = (4.14/0.1)^{1/2} = (41.4)^{1/2} \sim 6.4$ nm

6.4 nm is a pretty large number.

[Kinesin moves every 8.3 nm; 1 base-pair = 3.4 Å]

How to decrease noise?

Reducing bandwidth reduces noise.



If instead you collect data out to a lower bandwidth BW (100 Hz), you get a much smaller noise.

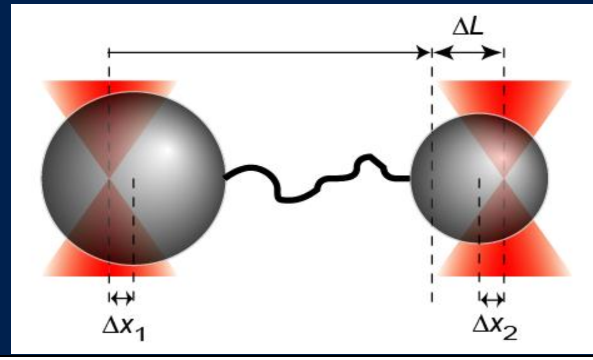
Noise = integrate power spectrum over frequency.
If BW < f_c then it's simple integration because power spectrum is constant, with amplitude = $4k_BT\gamma/k^2$

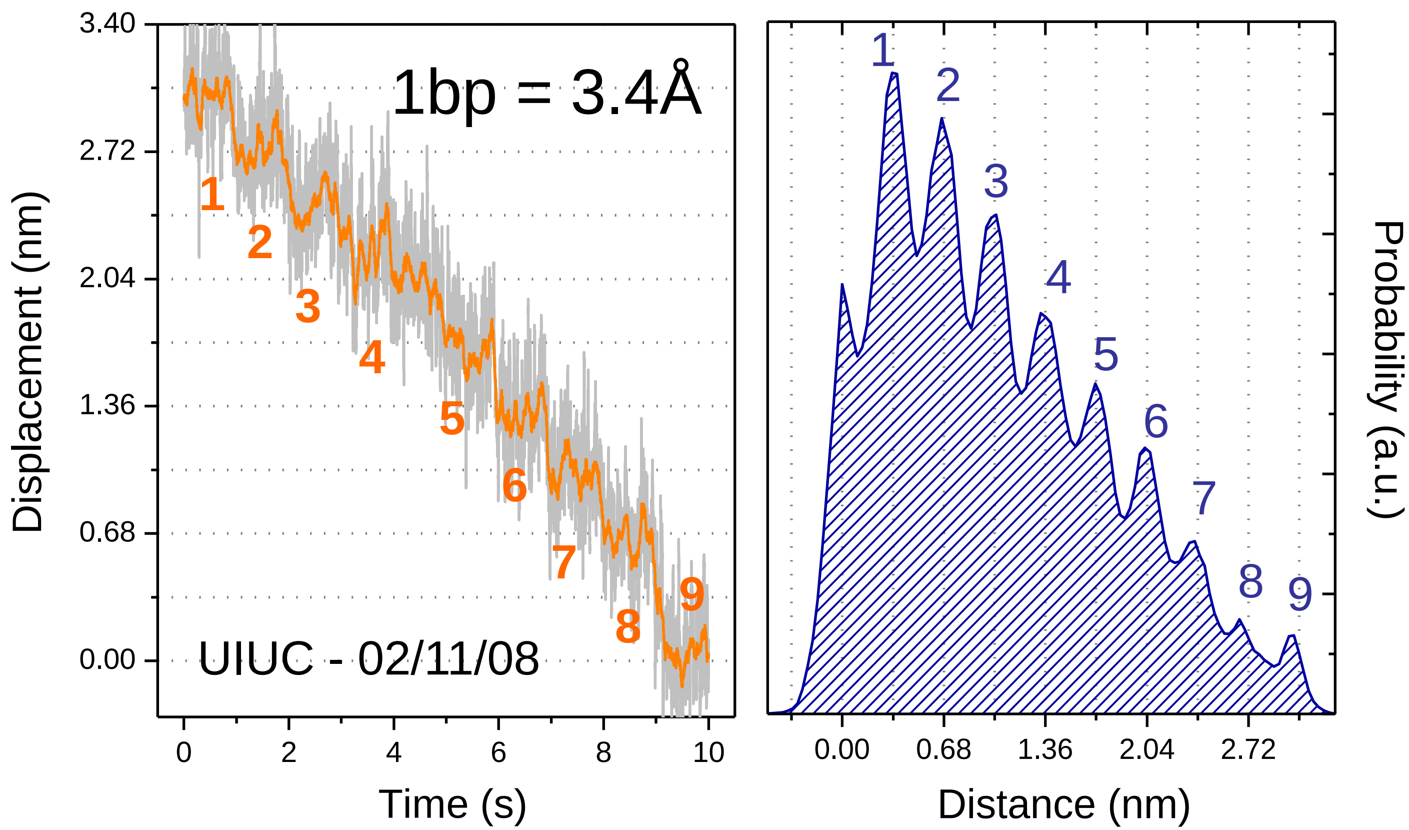
Let's say BW = 100 Hz: typical value of γ (10^{-6} for ~1 μm bead in water).

But $(\langle x^2 \rangle_{BW})^{1/2}$ = $[\int \text{const}^*(BW)dk]^{1/2}$= $[(4k_BT\gamma 100)/k]^{1/2}$ =
$[4^*4.14^*10^{-6*}100/0.1]^{1/2}$
~ 0.4 nm = 4 Angstrom!!

1867

Basepair Resolution—Yann Chemla @ UIUC





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3.4 kb DNA

F ~ 20 pN

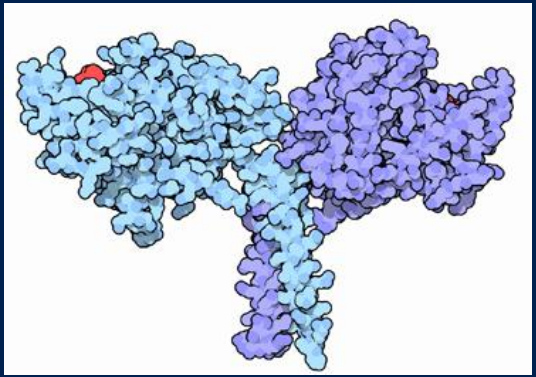
f = 100Hz, 10Hz

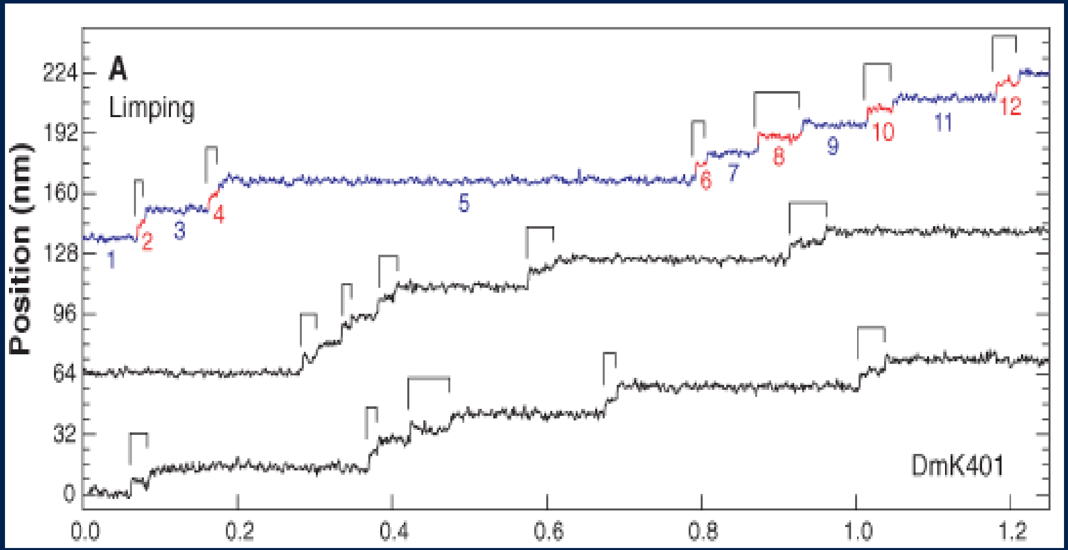
Observing individual steps

Motors move in discrete steps

Detailed statistics on kinetics of stepping & coordination

Kinesin





Step size: 8nm

Asbury, et al. *Science* (2003)