Announcements

HW: VMD (primarily) due Thursday Oct 29th.

From now till end of semester it will (more-or-less)
follow Fall 14 lecture schedule
Optical Traps, Fluorescence (FRET, Super-resolution), Electron microscopy, Diffusion, Nerves (Ion Channels), maybe Vision.

Optical Traps
Physics: use lasers and optical traps to control *individual* molecular motors.
Biology: can study how forces operate on these motors.
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.

Hold bead with some force, $F$.
Have the molecular motor pull against it.
How does motor act as a function of force? ATP? Mutation?
Biological scales

Force: 1-100 picoNewton (pN)
Distance: <1–10 nanometer (nm)
Motors include walkers (myosin, dynein, kinesin),
Twisters (like $F_1F_0$-ATPases)
RNA & DNA Polymerases
Viruses

Optical Tweezers is an ultrasensitive technique
Able to see an individual molecule at a time
--like magnetic trap, atomic force microscopy
--unlike x-rays diffraction

Single Molecules: Don’t need to have to synchronize a population to study kinetics. For example: a molecular motor which uses ATP.
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 $k_{\text{trap}}$ with laser intensity such that $k_{\text{trap}} \approx k_{\text{bio}}$ ($k \approx 0.1\text{pN/nm}$)

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

http://en.wikipedia.org/wiki/Optical_tweezers
How to generate force with an Optical Trap

Tightly focus beam (Diffraction says you can focus it to radius $\sim \lambda/2 \sim 500 \text{ nm}$)

Light generates 3 types of optical forces: absorbance, scattering (reflection), gradient.

Minimize scattering, absorbance
Water is clear –I.R. (1000 nm).

Gradient leads to radiation pressure, optical trap.

Trap strength depends on light intensity, gradient

At the power levels used:
Trap is harmonic: $F = -kx$ ; $k \sim 0.1 \text{pN/nm}$. This is good because $\sim$ motor strength. Why want this?

If opposing force = force of motor, see an effect of opposing force.
Can tell how much molecular motor pulls with.
IR traps and biomolecules are compatible

Neuman et al. Biophys J. 1999
Optical scattering forces – reflection

\[ P_i = \frac{h}{\lambda} \]

(Not of interest, here)

\[ F = \frac{\Delta P}{\Delta t} = \frac{(P_f - P_i)}{\Delta t} \]

Newton’s third law – for every action there is an equal and opposite reaction
Optical forces – Refraction

(leads to trapping)
Lateral gradient force

Bright ray

Dim ray

Object feels a force toward brighter light

(leads to trapping in x-y)
Axial gradient force: leads to trapping in z

How so?

Object feels a force toward focus

Force $\sim$ gradient intensity
Why does index of refraction of bead $n_{\text{bead}}$ > $n_{\text{water}}$ for trap to work?

$n_{\text{bead}} = n_{\text{water}}$  
Beam doesn’t change at all  
No scattering, no bending

$n_{\text{bead}} > n_{\text{water}}$  
Regular, stable trap.  
Force towards most intense light.  
Scattering, Bending.

$n_{\text{bead}} < n_{\text{water}}$  
Unstable trap.  
Force towards least intense light.  
Scattering, Bending, wrong way.

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Seeing a single molecular motor

Kinesin walks with 8 nm step!
Regular vs. Force-feedback

(Left) Stationary trapping of a polystyrene bead (blue) that is decorated with motor proteins in a focused laser beam (yellow). The optical trap exerts a force on the bead that can be approximated by Hooke’s Law \( F = k_c x \), where \( k_c \) is the spring constant of the laser trap and \( x \) is the displacement of the bead. The red curve illustrates “walking events” displayed by a single motor attached to a trapped bead. The number of such “walks” per time frame defines the SMWF of a motor and thus a measure for its motor activity. (Right) Force feed-backed trapping mode. The restoring force acting on the motor is clamped to a constant value \( F_0 \) by moving the piezo stage in the opposite direction to the motor’s movement. Contrary to the stationary modus, the piezo signal (light blue) gives information about the run length \( l \) of a motor and allows resolving discrete steps (step size \( d \), dwell time \( \tau \)) at limiting ATP concentrations.

Brunnbauer M et al. PNAS 2010;107:10460-10465
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.