Single Molecule Force Microscopy

Optical Trap
Magnetic Tweezers
Atomic Force Microscopy
Optical Trapping

For every action there exists an equal but opposite reaction

Sir Isaac Newton

A small dielectric sphere (with high index of refraction) of radius $r \gg \lambda$ acts like a lens. The momentum transfers with the deflection of the laser beam.
Trapping a Glass Bead

(Right) When the bead is in the center of the focused laser beam, forces that push the bead to right and left cancel out each other.

(Left) If the sphere moves off the trap, the refracted light exerts radial forces to push the sphere back on the axis.

The diameters of the bead usually range from 200 nm to 5 µm, to achieve trapping potential larger than kT.
For a Gaussian beam, the intensity gradient is almost linear near the beam center. Therefore, trap acts as a Hookean spring ($F = -k \Delta x$).

- Assume a linear-spring restoring force
- Determine trap stiffness $k$
- Measure $\Delta x$ relative to trap center

\[ F = k \Delta x \]
Design of Optical Traps

Trapping wavelength is usually between 800-1100 nm due to:
• High beam powers available
• Transparency of cells and proteins
• Low water absorption

The laser beam can be steered with acousto or electro optic deflectors or piezoelectric mirrors.

Bead position can be detected by a quadrant photodiode.

Trap stiffness can be calibrated by the variance of the position of the bead

Detector surface is calibrated by moving the trap in predetermined distances.
Combination of Trap and Fluorescence
Technical Capabilities

Single biological molecules are too small to be trapped. They need to be attached to a large bead to achieve pN levels of forces.

Time resolution is on the order of 100 µsec. (limited by the roll off frequency of the bead molecule system)
Traps can achieve 1 Å precision in bead position. (limited by vibrational, thermal noise and laser beam pointing stability)
Single motor protein movement along surface immobilized microtubules
Trapping geometries-2

DNA packaging motor bound to a dual beam trap
Three Bead Optical Trapping for Nonprocessive Motors: Actin Filament is attached to a dual beam and myosin II motors are on an immobilized bead.
Elastic Properties of DNA
Studies of DNA Packaging by Single \( \phi 29 \) Bacteriophage Particles Using Optical Tweezers
Optical Tweezers Set-up

- **Constant force feed-back**
- **No feed-back**

**Diagram Elements:**
- Optical trap
- Bead
- Straptavidin biotin
- Stalled DNA-capsid complex
- Antibody protein G
- Pipette

**Notes:**
- "Constant force feed-back" and "No feed-back" indicate different conditions or states in the optical tweezers setup.
Constant Force (5 pN) Experiments

- Initial packaging rates ~ 100 bp/sec
- Pauses are observed
- Occur more often at higher fillings
Evidence of Internal Pressure Build up from Constant Force Experiments

- Rate decreases to zero as head fills up
- Up to 105% of the φ29 genome is packaged before stalling
- An *internal pressure* must be building up due to DNA confinement.

External force = 5 pN

A single complex

8 complexes averaged & smoothed
Calibrating the Force Constant

1. Equipartition

- Position of the trapped bead will fluctuate by thermal motion.
- Energy in each axis is equal to \( \frac{1}{2} k_B T \)
- Energy stored in trap is \( \frac{1}{2} \kappa (\Delta x)^2 \)

\[
\frac{1}{2} \kappa <\Delta x>^2 = \frac{1}{2} k_B T \quad \kappa = \frac{k_B T}{<\Delta x>^2}
\]

- You must have an accurate measure of bead position at high frequencies to monitor the Brownian motion of the bead.
- External noise factors (drift, acoustic noise, mechanical vibration) will overestimate the variance of bead position
2. Drag Force

- The sample is moved at constant velocity while trap position is being fixed.
- The bead moves at constant velocity in opposite direction with respect to water.
- Stoke’s drag of water exerts force on the bead and moves it away from the trap center.
- Drag forces are equilibrated by the trap force.

\[
\gamma = 6\pi r \eta \quad F_d = F_t \quad \Rightarrow \quad \gamma V = \kappa \Delta x \\
\kappa = \frac{\gamma V}{\Delta x}
\]

Stokes’ law \( F_d = 6\pi r \eta V \) but corrected for proximity to walls
3. Corner Frequency

Langevin equation:

\(- \gamma \dot{x} = F(t) - k \Delta x\)

Drag force
\[ \gamma = 6 \pi \eta r \]
for a sphere

Fluctuating force
\[ \langle F(t) \rangle = 0 \]
\[ \langle F(t) F(t') \rangle = 2 \gamma k_B T \delta(t-t') \]

Trap force

\[ |F(f)|^2 = 4 \gamma kT \]
(Brownian force is independent of frequency at low f)

\[ \langle \Delta x^2 \rangle (f) = \frac{kT}{\gamma \pi^2 (f_c^2 + f^2)^2} \]
(Power spectrum of bead is Lorentzian)

\[ f_c = \frac{\kappa}{2 \pi \gamma} \]
Typical trap stiffness: 0.1 pN/nm

\[ f_c = 3 \text{ kHz for 500 nm bead.} \]

\[ \frac{\Delta x^2}{\gamma k} < \frac{4\gamma kT}{\kappa^2} \] at low frequencies
Force-noise spectral density is proportional to bead size

\[ \langle \Delta F^2 \rangle = 4k_B T \gamma \]

at low frequencies
Balancing Signal, Noise and Time Resolution

Note that a sort of “Uncertainty Thermal Relation” applies for such systems, since the product of the root mean square fluctuations in position and force is equal to the thermal energy:

$$\langle \Delta F^2 \rangle^{1/2} \cdot \langle \Delta x^2 \rangle^{1/2} = kT$$

Thus, the stiffer the mechanical transducer, the smaller its position noise and vice-versa.

Fluctuations are not spread uniformly over all frequencies, however. The spectrum of fluctuations is determined by the proportionality that exists between the ability of the linear system to absorb thermal energy and its ability to dissipate it by friction.
Balancing Signal, Noise and Time Resolution

Thus, a transducer with higher corner frequency makes it possible to take more data in the same amount of time. Moreover, since:

\[
\langle \Delta x^2 \rangle = \int_0^\infty \langle \Delta x^2(\omega) \rangle d\omega = \int_0^\infty \frac{4kT}{\gamma(\omega_c^2 + \omega^2)} d\omega = \frac{kT}{\kappa}
\]

Thus, for the same \( \kappa \), the total area under the power spectrum is the same.
Balancing Signal, Noise and Time Resolution

Measurements are often performed in a narrow band (bandwidth B) around the frequency of the signal. Suppose that the signal to be measured is a force developed by a molecular motor. The signal-to-noise in that measurement, for $B \ll \omega_c$ is:

$$\frac{S}{N} = \frac{F}{\sqrt{4\gamma kT B}}$$

Thus for the same $\kappa$ and the same $B$, the signal-noise ratio of the measurement will be higher for the transducer having the higher corner frequency.
Balancing Signal, Noise and Time Resolution

that is, for the one having the smaller friction coefficient, or, for the one possessing the **smallest dimensions**. This is the rationale for the development of mini-cantilevers, whose stiffness are comparable to regular cantilevers, but whose \( \gamma \)'s are significantly smaller and their corner frequencies, correspondingly larger.

\[
\frac{S}{N} = \frac{F}{\sqrt{4\gamma kTB}}
\]

Notice also that the S/N ratio is independent of the stiffness of the transducer: as \( \kappa \) decreases, the noise increases exactly as fast as the signal. Thus a softer transducer does not provide higher S/N than a stiffer one.
Balancing Signal, Noise and Time Resolution

Finally, the S/N can be increased by reducing the bandwidth and therefore the time-resolution of the measurement, but this approach is ultimately limited by the frequency of the biological process.

\[
\frac{S}{N} = \frac{F}{\sqrt{4\gamma kTB}}
\]
Springs in parallel for thermal noise
Thermal noise

\[ \Delta x_{\text{therm}} = \frac{\Delta F_{\text{therm}}}{(k_1 + k_2)} \]

\[ = 2 \frac{(k_B T \gamma B)^{1/2}}{(k_1 + k_2)} \]

where B is bandwidth in Hz

Signal to noise ratio

\[ \text{SNR} > 1 \text{ when } \Delta x_{\text{sig}} > \Delta x_{\text{therm}} \]

\[ \Delta x_s \frac{k_1}{(k_1 + k_2)} > 2 \frac{(k_B T \gamma B)^{1/2}}{(k_1 + k_2)} \]

\[ \Delta x_{\text{step}} > 2 \frac{(k_B T \gamma B)^{1/2}}{k_1} \]

Tether stiffness
Thermal limit to step detection

$$\Delta x_{\text{step}} > 2(k_B T \gamma B)^{1/2} / k_1$$

Resolution depends only on tether stiffness, not trap stiffness.

Resolution degrades as $(\text{drag})^{1/2}$

Comparing AFM to laser tweezers, the force noise scales as $\sqrt{\text{cantilever length} / \text{bead diameter}}$. Therefore a 100um cantilever has 10x more force noise than a 1 um bead, and 10x bigger distance noise for fixed $k_1$.

A stiff linkage (large $k_1$) gives an AFM very good resolution when it pushes against a hard sample. To make a DNA tether stiff requires some tension in the tether.
Averaging reduces bandwidth, suppresses noise
For example:

Bead is 2 \text{um} diameter, immersed in water.

Tether is 10 kbp of dsDNA and tether tension is either 2 pN or 20 pN.

Signal of interest is at 1 Hz, so that much bandwidth is required.

Tether stiffness $k_1 = \frac{dF}{dx}$ for WLC at either tension (assume P$\sim$50nm).

at 2 pN tension, $k_1 = 12 \text{ pN/um}$

at 20 pN tension, $k_1 = 170 \text{ pN/um}$

Then smallest resolvable step \[ \Delta x_s = \frac{2(k_B T \gamma B)^{1/2}}{k_1} \]

\[ \Delta x_s = 1.5 \text{ nm @ 2 pN tension} \]

\[ \Delta x_s = 0.1 \text{ nm @ 20 pN tension} \quad \text{(in 1 Hz bandwidth)} \]
Magnetic Tweezers

Magnetic tweezers are a unique tool to manipulate, i.e. stretch and twist, single biomolecules and to read out their response in the form of length changes.

To apply force and torque, these biomolecules are attached to small superparamagnetic particles, which can be moved in magnetic field gradients.

Typical spatial resolution is between 2 and 10 nm and time resolution is around 1 msec
Calibration profile can be obtained to determine z position of the bead with 10 nm precision.
Force on a magnet is calculated from mean square displacement.
DNA extension/looping

Worm-Like Chain Model

\[ F(x) = \frac{k_B T}{l_p} \left[ \frac{1}{4 \left(1 - \frac{x}{l_c}\right)} - 0.25 + \frac{x}{l_c} \right] \]

WLC:
\[ p = 51 \pm 5 \text{ nm} \]
\[ L = 3.27 \pm 0.05 \text{ \(\mu\text{m}\)} \]
DNA Supercoiling

Replication creates supercoiling

Topoisomerases relax supercoils
Dynamics of Supercoiled DNA

Sudden force switch

DNA nicking enzyme
RNA Polymerase

RNAP unwinding adds positive supercoils to the DNA.

If the DNA already contains positive supercoils RNAP activity reduces the distance between the bead and the surface.

If the DNA contains negative supercoils, bead goes up.
Atomic Force Microscopy (AFM): General Components and Their Functions

- **laser diode**
- **mirror**
- **cantilever**
  - spring which deflects as probe tip scans sample surface
- **position sensitive photodetector**
  - measures deflection of cantilever
- **sample**
- **probe tip**
  - senses surface properties and causes cantilever to deflect
- **sensor output, δc, Fc**
- **computer**
  - controls system
  - performs data acquisition, display, and analysis
- **piezoelectric scanner**
  - positions sample (x, y, z) with Å accuracy
- **δc**
- **ERROR = actual signal - set point**
- **feedback**
  - controls z-position
Position Detection

Recording of the force at every pixel is used to reconstruct the surface topography.

Constant Force Mode: Force on AFM is kept constant by feedback loop. The image detects the height change for each pixel and reflects the sample topography.

Oscillation Mode: AFM head is oscillated sinusoidally and feedback loop ensures the probe touches the surface at the minima of the downward movement. This interaction changes the amplitude, which is reconstructed to a topograph.

AFM can achieve ~0.5 nm precision in xy imaging. Vertical resolution is in Angstrom range.

Measures short range interactions between a scanning probe and object to contour conducting and nonconducting surfaces.
a-d) Fo-F1 ATPase, e) the mixture of light harvesting complex, small rings with ~50A diameter, and a reaction center, f-g) Rho dimers on a cell membrane
Folding Studies

AFM tip can also be used as a single molecule spectroscope.
Membrane Protein Unfolding

Controlled unfolding of one bacteriorhodopsin (trimers) from native purple membrane.
Unlike water soluble proteins that unfold at one step, membrane proteins unfold in a stepwise manner, each step representing an unfolding intermediate.
Advantages

- Biological sample can be investigated in buffer at RT.
- Bypasses the labeling procedures.
- Studies proteins in their native environment.
- Offers outstanding SNR.
- can be used as single molecule force spectrocope.
- is well suited to study surfaces.
Future Directions

AFM is particularly well suited to study membrane proteins.

AFM head is 'fishing' for molecular sites recognized by an antibody tethered to the probe by a fine polymer thread.

AFM can be performed at a high speed to study protein dynamics.