Experimental Techniques in Small Scale Manipulation of Biological Systems

Joshua W. Shaevitz
shaevitz@princeton.edu
www.princeton.edu/~shaevitz

Collaborators:
Steven Block (Stanford)
Elio Abbondanzieri (Stanford)
William Greenleaf (Stanford)
Michael Woodside (University of Alberta)
Daniel Fletcher (UC Berkeley)
Sapun Parekh (UC Berkeley)
Michael Rosenbluth (UC Berkeley)
Ovijit Chaudhuri (UC Berkeley) and more...
Tools for measuring dynamics and forces

“You can learn a lot by watching”

-Yogi Berra
Tools for measuring dynamics and forces

- Optical Trap
- Fluid flow
- Magnetic Force Microscopy
- Atomic Force Microscopy

and many more …
Force and position scales for different techniques

- Force Scale (pN)
- Length Scale (nm)

**AFM**

- Optical Trap
- Magnetic Trap
Light has momentum, but its small!

“A very short experience in attempting to measure these forces is sufficient to make one realize their extreme minuteness – a minuteness which appears to put them beyond consideration in terrestrial affairs…”

- J. H. Poynting ($\vec{S} = \vec{E} \times \vec{B}$) 1905
The force on a reflecting mirror

The momentum of a single photon is $h\nu/c$.

For a laser of power, $P$, there are $P/h\nu$ photons per second striking the mirror.

The total change in momentum of the light per second is $(2P/h\nu)(h\nu/c)=2P/c$.

By conservation of momentum the mirror feels an equal and opposite momentum change per second, which is a force!

e.g. if $P = 1$ watt $\rightarrow$ Force $= 10^{-11}$ Newtons $= 10$ nN!

My laser pointer is 1 mW $\rightarrow$ Force $= 10$ pN
Optical traps are 3D springs made of light.

Optical traps can . . .

- manipulate the position of micron-sized objects (like bacteria or glass beads)
- apply forces up to ~100 pN
- measure the motions produced by biological molecules with high 3D spatial (~1 Å) and temporal (<100 µs) resolutions
- readily combined with other optical microscopy techniques
At a focus there is a refractive restoring force

The trapping laser imparts a force onto the particle directed towards the laser focus.

The magnitude of this force is:

\[ F = \frac{Qn}{c} P \]

- \( P \) = laser power
- \( n \) = particle refractive index
- \( c \) = speed of light
- \( Q \) = trapping efficiency

One lens, and two rays is all you need
The restoring force acts in the axial direction too.
The optical trap used for the RNAP work
Basic ray optics

\[ \frac{1}{o} + \frac{1}{i} = \frac{1}{f} \]

---

**A**

- \( f \)

**B**

- \( o \)
- \( i \)
- \( f \)

**B**

- \( d \)
- \( 2d \)
- \( f \)
- \( 2f \)

**Divergent**

- \( f \)
- \(<f\)

**Convergent**

- \( f \)
- \( >f \)
Beam steering is achieved by rotations
Computer controlled steering technology

Piezo-driven mirror

Electro-optic deflector

\[ \Delta \theta \approx \lambda \frac{\Delta f}{V_{\text{sound}}} \]

\[ \theta = K \frac{LV}{a^2} \]
Bead position detection
Techniques for making two traps

**AOD splitting: simultaneous multiple frequencies**

Two frequencies are fed into an acousto-optic crystal at the same time creating two first-order diffracted beams.

Pros: Easy to implement; More than two traps can be created
Cons: Beam intensities fluctuate as position changes; Traps can be moved independently in only one dimension; “Ghost” traps created

**AOD splitting: time shared multiple frequencies**

AOD rapidly alternates between two different frequencies (beam positions).

Pros: Traps can be moved independently in two dimensions; More than two traps can be created; Traps intensities are independent of each other
Cons: Requires a fast computer or RF capable electronics; Non-linear and harmonic effects distort trap; “Ghost” traps created

**Polarization splitting**

Beam is split into two orthogonal polarizations

Pros: No non-linear AOD effects; Traps can be steered independently in two dimensions; Traps intensities are independent of each other
Cons: Requires more table space and optics; Difficult to add additional traps; Requires two sets of AOD crystals and associated electronics.
Two traps are better than one

By taking into account the correlations in bead motion for a bead-DNA-bead dumbbell you can increase the signal-to-noise, especially at large DNA stiffnesses (i.e. large stretching forces).

Angstrom precision aided by helium

**b**

![Graph showing noise density and integrated noise vs. frequency.](image)

**c**

![Plot showing position vs. time in angstroms.](image)

**d**

![Plot showing position vs. time in angstroms.](image)
A passive force clamp increases bandwidth

(a) $F \sim \text{constant}$

(b) Force (pN) vs. Displacement from center (nm)
Using an optical trap as a heater

\[ S_{yy}(f) = \frac{k_B T}{6\pi^3 \eta(T) r} \left[ \left( \frac{\alpha}{12\pi^2 \eta(T) r} \right)^2 + f^2 \right] \]

Decay distance ~10-20 microns

Gold particles heat up alot more:
~250 degC / Watt
3D tracking sheds light on a brownian ratchet

![Graph showing velocity vs. inverse viscosity](image)

- **Velocity (nm/s)**
  - 30
  - 40
  - 50
  - 60
  - 70

- **η⁻¹ (Pa s)⁻¹**
  - 160
  - 200
  - 240

- **Equation**
  - \( k_\text{on} \)
  - \( k_\text{detach} (F) \)
  - \( k_\text{attach} \)
  - \( \alpha_{\text{spring}} \)
  - \( k_B T \)
Trajectory shapes yield details of ratchet motion

- **Graphs:**
  - Top left: Velocity (nm/s) vs. Time (s)
  - Bottom left: Y Position (µm) vs. X Position (µm)
  - Top right: Step Size (nm) vs. Probability
  - Bottom right: Normalized Curvature vs. A_curvature [µm²] vs. X Position [µm]

- **Equation:**
  
  \[ P(k) = \frac{2k}{k_{RMS}}e^{-\frac{1}{2}(k/k_{RMS})^2} \]
Measuring 4D PSF

XY  XZ  YZ

0 μm

1 μm

2 μm

3 μm

Normalized Intensity

Normalized Intensity

Normalized Intensity

FWMH (μm)

Depth (μm)
Holographic optical traps

Wavefront Modification $\exp(i\varphi(\beta))$

Optically Trapped Particles

Wavfront Phase $\varphi(\beta)$

Optical Traps $I(r)$

(c) 2003 Grier Group
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
- **Probe Tip**
  - Senses surface properties and causes cantilever to deflect
- **Sample**
- **Sensor Output, \( \delta c, F_c \)**
- **Feedback Loop**
  - Controls z-sample position
- **Computer**
  - Controls system
  - Performs data acquisition, display, and analysis
- **Piezoelectric Scanner**
  - Positions sample \((x, y, z)\) with Å accuracy

Ortiz Lab @ MIT
Imaging and perturbing microtubules

AFM used for stretching proteins

Julio Fernandez’s Lab
Can study unfolding and folding kinetics

Folding is slowed by force

$k_0 = 100 \text{ s}^{-1}$

$\Delta x_f = 8.2 \text{ Å}$
Measuring the FV curve of a growing actin network

**Diagram:**
- **a**
  - i. AFM cantilever in air
  - ii. Cell extract immersion
  - iii. Actin growth deflects cantilever
- **b**
  - i. Network length vs. time
  - ii. Network length vs. force
  - iii. Network length vs. normalized velocity

**Graphs:**
- Network Length (µm) vs. Time (min)
- Force (nN) vs. Network Length (µm)
- Normalized velocity vs. Normalized force

**Description:**
- The diagram illustrates the measurement of the FV curve of a growing actin network using an AFM cantilever.
- The network length changes over time, and the force exerted is measured.
- Normalized velocity is also calculated.
Loading history determines growth velocity

1. Grow actin network under a constant force.
2. Allow network to increase the applied force as it grows.
3. Return network to the original constant force.
4. Quantify network growth rates.

Growth velocity before and after loading is different

Remodeling of network in response to load

Side view allows you to view actin density

Desired sideview image:

Does the network get denser with increasing force?
AFM as a local rheology probe

-measure cantilever

-drive surface

\[ E'(f) + iE''(f) = \frac{\tilde{\sigma}(f)}{\tilde{\varepsilon}(f)} \]
A model of actin network elasticity

1. linear elastic
2. stress stiffening
3. critical stress
4. stress softening due to buckling

Both entropic & enthalpic contributions play a role
Measuring cell stiffness with an AFM

Rosenbluth et al., Biophys J (2006)

Lam et al., Blood (2007)
Mechanical coupling on short distance scales

Documented in Speidel et al., *Optics Letters* 2003