An Introduction to Optical Tweezers

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Optical tweezers

Single molecule applications

Optical tweezers

Optical tweezers use forces of laser radiation pressure to trap small objects

This technique is 20 years old, and used in biophysics the last 10 years

What Are Optical Tweezers?

- Matter can be influenced by light
- Wavelength selection
- Intensity of beam
- Optics
- Single beam tweezers
- Double beam tweezers
- Application in atom trapping
  UHV, low temperature (few °K)
- Biological applications in a liquid environment

Spectrum of Light

Approximate wavelength (in vacuum) and frequency ranges for the various colors

<table>
<thead>
<tr>
<th>Color</th>
<th>Wavelength (nm)</th>
<th>Frequency (THz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>780 - 622</td>
<td>384 - 482</td>
</tr>
<tr>
<td>Orange</td>
<td>622 - 597</td>
<td>482 - 503</td>
</tr>
<tr>
<td>Yellow</td>
<td>597 - 577</td>
<td>503 - 520</td>
</tr>
<tr>
<td>Green</td>
<td>577 - 492</td>
<td>520 - 610</td>
</tr>
<tr>
<td>Blue</td>
<td>492 - 455</td>
<td>610 - 659</td>
</tr>
<tr>
<td>Violet</td>
<td>455 - 390</td>
<td>659 - 769</td>
</tr>
</tbody>
</table>

1 terahertz (THz) = 10^12 Hz, 1 nm = 10^-9 m.

The white light is a mixture of the colors of the visible spectrum.
Can We Build an Optical Tweezers With a Diode Laser?

- Beam Shaping
- Laser Power
- Single mode vs. multimode Laser

http://www.mellesgriot.com/products/default.asp

Beam Shaping

Diode Laser
Single Mode Expander
\( \lambda = 830 \text{nm} \)
200mW

Optical Isolator Pinhole

Anamorphic Prisms
Collimated diode laser beam
After Anamorphic prisms
CCD image of laser beam

Lenses
Symmetric Bioconvex Lens

Identical Plano-convex Lenses

Beam After Isolator Before Lens and Pinhole
CCD image of laser beam
Identical Achromat Lenses
Spatial Filter

Spatial filters provide a convenient way to remove random fluctuations from the intensity profile of a laser beam. Laser beams pick up intensity variations from scattering by optical defects and particles in the air.

A pinhole centered on the axis can block the unwanted noise annulus while passing most of the laser’s energy. The fraction of power passed by a pinhole of diameter \( D \) is:

\[
\frac{P(D)}{\text{Total Power}} = 1 - e^{-\frac{\pi D^2}{2a^2}}
\]

where \( a \) is the radius of the pinhole.

\[D_{\text{opt}} = \frac{F \cdot \lambda}{a}\]

Spatial filters provide a convenient way to remove random fluctuations from the intensity profile of a laser beam. Laser beams pick up intensity variations from scattering by optical defects and particles in the air.

\[I(r) = I_0 e^{-\frac{r^2}{2\alpha^2}}\]

where \( I_0 = \frac{2P}{\pi a^2} \) and \( \alpha = \text{Radius of OPS Gaussian at } I(r) = I_0 e^{-\frac{r^2}{2\alpha^2}} \).

Spatial Filter

Spatial filters remember there is high energy of light at the pinhole.

Polarizing Beam Splitter

Variable Ratio Beamsplitter

If a polarizing cube is preceded by a half-wave retarder plate, the result is a variable ratio beamsplitter for linearly polarized, monochromatic input. The output beam intensity ratio can be continuously varied from below 1:49 to above 4:91, or fixed at any ratio of interest, by suitably rotating the half-wave retarder within its plane. A ratio of 1:1 is easily achieved. For unpolarized, monochromatic input at the intended wavelength, and without the retarder, these beamsplitters always achieve a very accurate 1:1 ratio regardless of beamsplitter orientation.

Quarter Wave Plate

Quarter-wave plates are used to turn plane-polarized light into circularly-polarized light and vice versa. To do this, we must orient the wave plate so that equal amounts of fast and slow waves are excited. We may do this by orienting an incident plane-polarized wave at 45° to the fast (or slow) axis.
Double Beam Laser Tweezers

Layout of the Core of the Instrument

The physics behind optical tweezers

Radiation pressure is the force per unit area on a object due to change in light momentum.

The light momentum of a single photo is:

$$|\vec{p}| = \frac{h}{\lambda}$$

The change in momentum can be calculated by the difference in momentum flux between entering and leaving a object.

$$\vec{F} = \frac{n}{c} \iint (\vec{S}_m - \vec{S}_{out}) dA$$

The physics behind optical tweezers

Applying this formula to a 100% reflecting mirror reflecting a 60W lamp gives a pressure of:

$$\vec{F} = 2\left(\frac{n}{c}\right) \iint (\vec{S}_m) dA$$

$$F = 2\left(\frac{n}{c}\right) W = 4 \times 10^{-7} \text{ N}$$

Gravity pulls on a 1 kg mirror with 9.8 N so the force of the photos is negligible.

However, if the same light is reflected by a object of 1 µg it can't be ignored!

Using a laser on a microscopic particle will realize this situation.
**A particle in a laser beam**

Force develops when rays of light act on objects with different index of refraction compared to the surrounding media. The particle gets pushed towards the highest light intensity.

However, there are also reflections pushing the bead forward.

But using a high NA objective, the refractive force can overcome the forces due to reflections.

**What is a high NA objective?**

\[ NA = n \sin(\theta_{\text{max}}) \]

Result: A particle can get sucked into the focus of a laser bundle and be stably trapped. Thus, highly focused laser beam acts as a three-dimensional potential minimum. Therefore, it takes force to dislodge a bead out of the laser focus.
A particle in a laser beam

Also the bead movement is measurable from the laser deflection.

**Why Can We Hold Particles by Light?**

(A) A refractile bead is drawn into the intense part of a light beam but is also propelled away from the light source.

(B) A converging laser beam, focused by a lens, holds the bead at a constant axial position.

Gradient force must be greater than scattering force to allow stable trapping along the optical axis.

-> Overfill back aperture of the microscope lens

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**Momentum of a Photon**

\[ P = h/\lambda \]

Reaction force on bead: \[ |F| = (W/c) n_b \sin \theta \]

Collector lens transforms exit angles into ray offsets (by Abbe sine conditions): \[ \chi_i = R_L n_b \sin \theta_i \]

Position sensitive light detector sums over all light rays to give a signal \[ S = \sum \chi_i W_i \]

External force is given by: \[ F = S/R_L c \]

**Force measurement using the momentum of the light**

Wavelength: 830 nm
Intensity: 200 mW (per Laserdiode)

Maximum force on a trap
\[ F_{\text{Max}} = (W/c)/(R_{\text{back-aperture}} - R_{\text{beam}})/R_L \]

This measurement scheme should not be used in a single-beam trap because such a narrow cone of light (as depicted in the above figures) will not efficiently trap an object. But if a high-NA beam were used instead, then the outermost exiting rays could not be collected by the analysis lens.
For an overdamped particle in liquid, thermal fluctuations of displacement reads as:

$$\Delta x_{\text{Noise}}^2 = \int_0^\infty S_x(f)df = \int_0^\infty \frac{k_B T}{\gamma^2} \left( \frac{f_c^2}{f^2} + f^2 \right) df$$

$$k_B T \quad 4.1 \text{ pN.nm @ RT}$$
$$\gamma \quad \text{viscous drag coefficient}$$
$$f_c \quad \text{corner frequency} : \text{Stiffness (K)/2\pi}$$

Noise (OT, AFM)

Using low-pass filtering @ <fc and sampling @ fs

$$\Delta x_{\text{Noise}}^2 = \int_0^f S_x(f)df = 4\gamma k_B T f_s$$

$$\Rightarrow \Delta F_{\text{Noise}}^2 \quad \text{Independent of K !!!!!}$$

Reducing Noise = reducing the size of the sensor

Optical Tweezers

OT have a small sensor, therefore a better force resolution

Fluid-Chamber

Molecule resp. receptor/ligand

Micropipette
**Calibration of optical tweezers**

**Virtual spring methods**

The optical trap is treated as though it were a linear spring pulling the bead toward the center of the trap.

1. Stokes law method (viscous drag force) \( F = 6\pi r \eta v \)

   trap stiffness: \( \kappa = F / \Delta x \)

2. Corner frequency method

3. Equipartition method

4. Pulling dsDNA

Using water immersion lenses with working distances of > 200 \( \mu m \) is enabling us to neglect surface effects.

**Correction of wall effect in fluid chambers**

**Corner Frequency Method**

Trap one bead

record its motion on Position Sensitive Detector (PSD)

\[ f_c = \frac{2\pi \kappa \gamma}{\kappa} \]

\[ \gamma = 6\pi r \eta \]

\( \kappa = \text{trap stiffness} \)  
\( \gamma = \text{damping coefficient} \)  
\( \eta = \text{viscosity} \)  
\( \eta_{H_2O} = 1 \times 10^{-9} \text{ pN} \cdot \text{s/nm}^2 \)

Frequency above which the bead doesn’t feel the potential of the light any more.

**White Light Illumination for CCD?**

Choose the right filters to transmit the laser and reflect your illumination source.

Due to combination of optical elements within the OT measure the spectra of your combined optical components!
Manipulation of Micron Sized Beads by Optical Tweezers

Bead placement on Pipette and in 1st trap

Bead held by suction on movable pipette

3 µm

Bead in 3D movable trap

Bead in the double-trap

Bead in the movable trap

Optical Tweezers @ IfP

Bead manipulation with 2nd trap

fixed in 3D

molecule with affinity to DNA binding protein

3 µm

pipette movable in 3D

Beam steering

Tilting the optical plane within the back-aperture

No loss of light (energy) within the trap

Loss of light (energy) within the trap asymmetric trapping

Kinematic mirror

Gimbal mirror

L7

L6

L5

Beam expander 6X

Height adjustment

DM

Shutter

Diode laser (1064 nm)

Power of the laser used

• Remember that the laser light may affect the molecule of investigation.
• If the focal spot is within a fluid environment then the heat of high intensity light is dissipating into the liquid quickly.
• Loss of power from the source till the focal spot of the trap ~ 50% due to beam shaping...
• Remember that the wavelength should be in the NIR to avoid damage of biology.
• With a power of ~ 80 mW @ trap we have a heating of ~1°C locally.
Wave length of choice for OT

From Characterization of Photodamage to Escherichia coli in Optical Traps

Newer paper on that topic:
Stress Response in Caenorhabditis elegans Caused by Optical Tweezers: Wavelength, Power, and Time Dependence
G. Leitz, E. Fällman, S. Tuck and O. Axner

Fluid delivery

Optical Tweezers Without / With Fluid Chamber

Microscope Lens:
Olympus N.A. 1.2; 60x
Water Immersion
Working Distance 280 µm

Fluid-Chamber:
Two Cover-slides with Parafilm
Channel for Micropipette
Combining two powerful techniques for single-molecule studies

**Optical tweezers:** nanoscale manipulation & sub-piconewton sensitivity

**Single-molecule fluorescence:** measuring nanoscale structural changes

**Combination:** correlating structural changes & biomechanical transitions

The complete chamber is placed on a closed-loop piezoelectric element such that nanometer displacements can be applied. Also shown are donor and acceptor dyes.

Current development of the instrument

**Motivation:**
- Biosensor with sub pN sensitivity for biological investigations on single molecules in buffer solutions
- Optical path allows combination with light spectroscopy

Simultaneous Single Molecule Mechanics and Fluorescent Resonance Energy Transfer (FRET) Measured by Optical Tweezers

Visualization of Local Motion e.g. on RNAP or DNAP on DNA Applying an External Load

The Optical Tweezers Setup (Combining Static (Counter-propagating) Double Beam Trap With steerable Micropipette and steerable Second Trap)

Inter or intra protein rearrangement upon ligand interaction within filament protein-complexes (Actin; Intermediate filaments)
New single photon detection setup

- Instrument today
- Epi-luminescent in-coupling
- iCCD + 2 APD detectors

Pulling Single DNA Fragments With Optical Tweezers

Elasticity of single DNA fragments

- Overstretch Transition of dsDNA

The Fields of Application

- Optical Tweezers ($\Delta F \sim 200 \text{ fN}$)
- Mechanical Properties
- Molecular Motors
- Unfolding of single molecules

Mechanical Properties of a single dsDNA

- Scanning Probe ($\Delta F \sim 10 \text{ pN}$)
- Molecular Recognition
- Mechanical Deformation
- Unfolding of single molecules

Lebrun A et al. NAR (1996) 24, 2260

Biomechanics on DNA with FRET detection
DNA pulling with AFM

Rief et al.,
Sequence-dependent mechanics of single DNA molecules
Nature Struct. Biol. 6
346 – 349 (1999)

Essevaz-Roulet, B. et al
Mechanical separation of the complementary strands of DNA Proc.
Natl. Acad. Sci. USA 94
11935 – 11940 (1997)

Is there a DNA in-between the beads?

FORCE: 6pN

Data Fitting to the Worm-Like Chain

Manipulation of a single dsDNA molecule

Detection of a single fluorophore label

Combining optical tweezers & single-molecule fluorescence

Single Qdot on lambda DNA
Attachment of organelles to polystyrene spheres for future molecular motor experiments

GFP labeled Mitochondria trapped in steerable OT

Aldehyde activated Polystyrene sphere Ø 3 µm

In collaboration with W. Voos Uni Freiburg

Optical tweezers references


http://www.nbi.dk/~tweezer/introduction.html
http://www.lightforce.harvard.edu/~tweezer/index.html
http://www.phys.umu.se/laser/