Phys 6715 - Biomedical Physics

Laser Tweezers and Other Advanced Physical Methods

Yong-qing Li, PhD

Department of Physics, East Carolina University
Greenville, NC 27858, USA

Email: liy@ecu.edu
Optical methods for single-cell studies: imaging, analysis and manipulation

- Hooke (1665) and Leeuwenhoek (1667) developed light microscopy: bacteria, cells, and nuclei found
- Zernicke (1935) phase contrast microscopy
- Fluorescence probes: molecular contrast for microscopy & flow cytometry
- Confocal microscopy: high contrast & 3D
- Scanning probe microscopy: AFM, SECM…
- Chemical-contrast microscopy without probes: Raman and CARS imaging
- Raman micro-spectroscopy: noninvasive analyses
- Optical tweezers Ashkin (1986) : manipulation
- Laser ablation, radiation damage/stress…

Part I. Optical trapping in liquid
Part II. Optical trapping in air

1. What is laser tweezers - history and development
2. Principles of optical trapping
   - General description
   - Optical forces - Ray optics and electric dipole approximation approaches
3. Experimental design, construction and operation
4. Biological/medical applications
I. What is an optical tweezers?

An optical tweezers is a scientific instrument that uses a focused laser beam to provide an attractive or repulsive force (typically on the order of pico-newtons), to physically hold and move microscopic dielectric objects.
Manipulation of single cells
Manipulation of internal organelles

Green Onion Root Tips
Why needs a trap?

- To confine thermal (Brownian) motion of single particles for a long observation time.

• How fast it walks?

\[ \frac{1}{2} m v^2 = K_B T \]

\[ K_B = 1.38 \times 10^{-23} \text{JK}^{-1} \]

• How far it walks?

\[ \langle x^2 \rangle = \frac{2 K_B T}{\beta} t \]

(Einstein)

\[ \beta = 6\pi \eta a \] (Stokes)
Brownian motion

• Gas: atoms in air, \( m_N = 2.32 \times 10^{-26} \text{ kg} \)

\[
\frac{1}{2m}v^2 = K_B T
\]

\( T = 27 \text{ C} = 300 \text{K}, \quad v = 2500 \text{ mi/hr} (1100 \text{ m/s}) \)
\( T = -270 \text{ C} = 3 \text{ K}, \quad v = 250 \text{ mi/hr} (110 \text{ m/s}) \)
\( T = 1.0 \mu \text{K}, \quad v = 25 \text{ cm/s} \)

• Solid in air or water at \( T = 300 \text{ K} \)

- cells \( d = 10 \mu \text{m}, \quad v = 45 \mu \text{m/s}, \quad x = 1.6 \mu \text{m/min} \)
- latex \( d = 1.0 \mu \text{m}, \quad v = 1.4 \text{ mm/s}, \quad x = 5.1 \mu \text{m} \)
- virus \( d = 100 \text{ nm}, \quad v = 4.5 \text{ cm/s}, \quad x = 16 \mu \text{m} \)
Optical Tweezers: 
Trap cells in liquids

Arthur Ashkin and S. Chu invented optical tweezers in 1986

Optical tweezers is a three-dimensional optical trap formed by a highly focused laser beam.

Biological particles (0.1 ~ 20 μm size) can be captured and manipulated by the focused laser beam for prolong observations.
II. Principles of Optical Trapping

Optical tweezers is a three-dimensional optical trap formed by a highly focused laser beam.

Harmonic approximation

\[ F_{trap} = -kx \]

\[ U(x) = \frac{1}{2} m \omega^2 x^2, \quad \kappa = m \omega^2 \]

Where \( k \sim 0.16 \text{pN/nm per 1W} \)
2.1 General description

\[ F = k_{trap} x \]

\[
F_{grad}(x) = -k_x x
\]

\[
F_{grad}(z) = -k_z z
\]
Optical Forces

Each photon carries energy of $hw$ and momentum of $hk$. Absorption, reflection or refraction of photons in the medium cause momentum change and produce optical forces.

$W(\frac{1}{3}) \quad hk' \quad (reflection) \rightarrow \textbf{Scattering Force}$

Water ($n=1.3$)

Cell surface ($n=1.45$)

$hk'' \quad (refraction) \rightarrow \textbf{Gradient Force}$
Scattering and Gradient Forces

- Scattering force: from reflection/absorption
- Gradient force: from refraction

For transparent and biological media, $F_{\text{grad}} > F_{\text{scatt}}$. 
2.2 The ray optics approach

Ray optics explanation. When the bead is displaced from the beam center, as in (a), the larger momentum change of the more intense rays cause a net force to be applied back toward the center of the trap. When the bead is laterally centered on the beam, as in (b), the net force points toward the beam waist.
2.3* The electric dipole approximation

The particle can be treated as a point dipole in an inhomogenous electromagnetic field. The force applied on a single charge in an electromagnetic field is known as the Lorentz force.

\[
F_1 = q \left( E_1 + \frac{dx_1}{dt} \times B \right)
\]

The polarization of a dipole is where is the distance between the two charges \( p = qd \), where \( d = x_1 - x_2 \)

\[
F = q \left( E_1 (x, y, z) - E_2 (x, y, z) + \frac{d(x_1 - x_2)}{dt} \times B \right)
\]

\[
= q \left( E_1 (x, y, z) + ((x_1 - x_2) \cdot \nabla) E - E_1 (x, y, z) + \frac{d(x_1 - x_2)}{dt} \times B \right)
\]

\[
F = (p \cdot \nabla) E + \frac{dp}{dt} \times B
\]

\[
= \alpha \left[ (E \cdot \nabla) E + \frac{dE}{dt} \times B \right],
\]

Where \( p = \alpha E \)
The electric dipole approximation

Use

\[ (\mathbf{E} \cdot \nabla) \mathbf{E} = \nabla \left( \frac{1}{2} E^2 \right) - \mathbf{E} \times (\nabla \times \mathbf{E}) \]

and

\[ \nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \]

The result indicates that the force on the dielectric particle, when treated as a point dipole, is proportional to the gradient along the intensity of the beam.

\[ \mathbf{F} = \alpha \left[ \frac{1}{2} \nabla E^2 - \mathbf{E} \times (\nabla \times \mathbf{E}) + \frac{d\mathbf{E}}{dt} \times \mathbf{B} \right] \]

\[ = \alpha \left[ \frac{1}{2} \nabla E^2 - \mathbf{E} \times \left( -\frac{d\mathbf{B}}{dt} \right) + \frac{d\mathbf{E}}{dt} \times \mathbf{B} \right] \]

\[ = \alpha \left[ \frac{1}{2} \nabla E^2 + \frac{d}{dt} (\mathbf{E} \times \mathbf{B}) \right]. \]
Scattering force and gradient force

- Scattering force is due to the absorption and reradiation of the light by the dipole. For a sphere of radius \( a \),

\[
F_{\text{scat}} = \frac{I_0 \sigma n_m}{c},
\]

\[
\sigma = \frac{128 \pi^5 a^6 (m^2 - 1)^2}{3 \lambda^4 (m^2 + 2)},
\]

\( n_m \) – index of refraction of the medium
\( n_p \) – index of refraction of the particle
\( m = n_p / n_m \)

\[
F_{\text{scat}} = n_b P_{\text{scat}} / c, \quad \text{where } P_{\text{scat}} \text{ is the power scattered}
\]

- Gradient force is due to interaction of the dipole with inhomogeneous field,

\[
F_{\text{grad}} = \frac{2 \pi \alpha}{cn_m^2} \nabla I_0,
\]

where

\[
\alpha = n_m^2 a^3 \left( \frac{m^2 - 1}{m^2 + 2} \right)
\]
Catch transparent cells by gradient force

\[ F_{\text{grad}} \propto \nabla I(x,y,z) \]

Input focused Guassian beam:

\[ I(x, y, z, t) = I_0 \exp \left( -\frac{2(x^2 + y^2)}{\omega(z)^2} \right) \]

Transverse and axial force for small displacements

\[
\begin{align*}
F_{\text{grad}}(x) &= -k_x x \\
F_{\text{grad}}(z) &= -k_z z
\end{align*}
\]

Where \( k \sim 0.16 \text{pN/nm per 1W} \)
Transparent and non-transparent particles

**Transparent:**
- Biological cells;
- Latex or glass beads;
- Low absorption;
- Low relative index of refraction \( (n=1.4 \sim 1.6), \)
  \( n_{\text{water}} = 1.33. \)

**Non-transparent:**
- Metal particles;
- Color dusts, black paints;
- Semiconductor powders;
- Large reflectivity;
- Large absorption;
- High index of refraction \( (n=1.7 \sim 4.0). \)

\[ F_{\text{grad}} > F_{\text{scatt}} \]

\[ F_{\text{grad}} < F_{\text{scatt}} \]
Scattering Force in Laser Tweezers

For absorbing particles, the scattering force dominant.

Assume that a laser beam of $P=10 \text{ mW}$ at $\lambda=1.06 \mu\text{m}$ is absorbed or reflected by a gold sphere of 1.0 $\mu\text{m}$ size.

$h\omega \sim 1.24 \text{ eV}$, \hspace{1cm} $hk = h/\lambda = 6.62 \times 10^{-28} \text{ N s}$

scattered photon number: $N = P/h\omega = 5 \times 10^{16}$ photons/s

$$F_{\text{scatt}} = N \ hk = 33 \times 10^{-12} \ N = 33 \ pN$$

density of gold particle: $\rho = 19.3 \ g/\text{cm}^3$

volume: $V \sim r^3 = 10^{-12} \ \text{cm}^3$

mass: $m = \rho \ V = 1.93 \times 10^{-11} \ g$

acceleration: $a = F_{\text{scatt}}/m = 1.7 \times 10^3 \ \text{m/s}^2$

$F_{\text{scatt}} \sim 170 \times \text{gravity force}$!
Scattering force on metallic particles
Raman spectra and optical trapping of highly refractive and nontransparent particles

Changan Xie and Yong-qing Li
Department of Physics, East Carolina University, Greenville, North Carolina 27858-4353

For non-transparent and metal particles, $F_{\text{scatt}} > F_{\text{grad}}$.

3. Experimental design, construction and operation

3.1 General design

- Trapping laser
- Beam expansion
- Beam steering
  - scanning mirrors
  - AO or EO or PZT
- Dichroic mirrors
- Microscope
- Objective
- Position detector
  - lateral position, quadrant photodiode (QPD)
  - axial position
- Optical table
Optical tweezers with infinity-corrected microscope
Tracking of Bead Brownian motion

- Monitoring X-Y movement of trapped particle;
- Monitoring Z movement of trapped particle;
- <10 nm resolution;

600 x pinhole

600 x

Quadrant detectors

Photo-detector
Pulsed optical tweezers for levitate stuck particles

Cw laser: \( F \sim 1-10 \text{ pN} \)
for manipulation (<100mW)

Pulsed laser:
for levitation

- Nd:YAG laser, 1064 nm
- 50μs pulse width
- 300-500 μJ/pulse

Peak power \( \sim 10\text{W} \)
\( F_{\text{grad}} \sim 1000 \text{ pN} \)

Levitation and manipulation of stuck particles
Levitation and manipulation of stuck biological particles

(a) focused
(b) defocused
(c) Pulse fired
(d)
(e)
(f)
4. Applications of Optical Tweezers: Biomechanics

- Measurements of mechanical properties (elasticity, stiffness, rigidity and torque) of cells
- Biomechanics of protein-protein unbinding, protein unfolding, and DNA stretching
- Biological motors: Kinesin, Myosin, Nucleic acid-based enzymes
- Manipulation of intracellular materials (organelles and chromosomes)
Single-molecule experiment with tweezers

- Stall and monitor motor protein;
- Stretch DNA;

![Diagram](image-url)

- Tracking bead

protein
Chromosomes are made up of a complex combination of DNA and histone proteins organized into chromatin.

Questions:
How to obtain Raman spectral patterns for chromosomes number 1, 2, and 3, potentially 24 human chromosomes?

Microscopic image of unstained chromosomes of leukemia cells.

Optics Express, 14, 5385-5393 (2006)
Experimental procedures

- capture an unknown chromosome
- Raman acquisition
- manipulation
- fixation
- G-banding verification

Sample reservoir  Buffer  Fixed slide
Further references for optical tweezers in solution


