Phys 6715 - Biomedical Physics

Laser Tweezers and Other Advanced Physical Methods

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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…

Brehm-Stecher and Johnson, *Microbiol. Mol. Biol. Rev*. 68, 538 (2004)

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

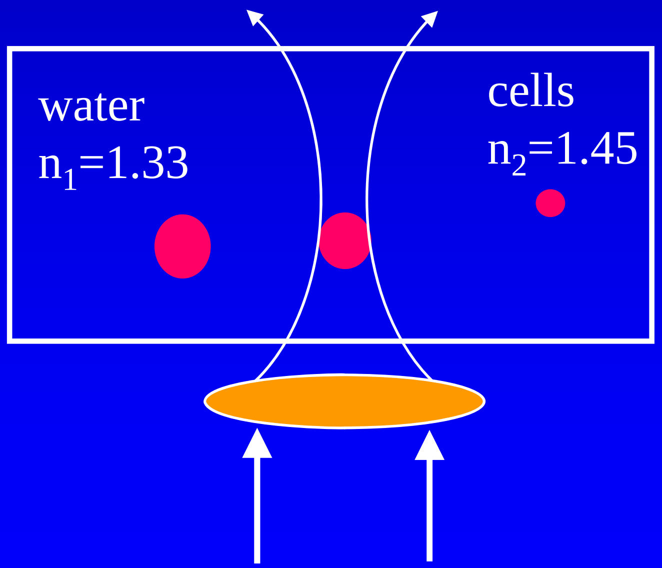
- Optical forces - Ray optics and electric dipole approximation approaches

3. Experimental design, construction and operation

4. Biological/medical applications

I. What is an optical tweezers?

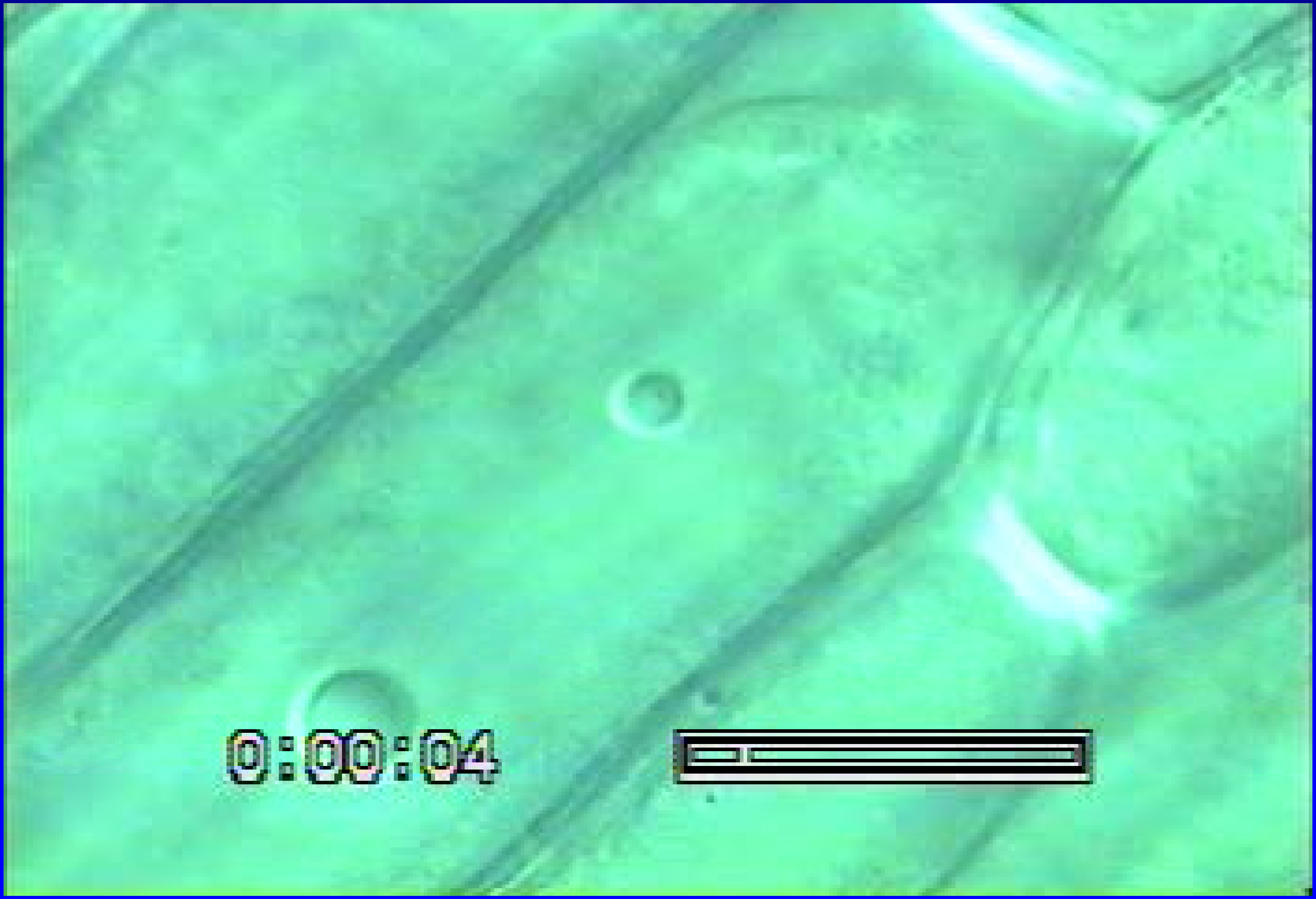
An **optical tweezers** is a scientific instrument that uses a focused laser beam to provide an attractive or repulsive force (typically on the order of pico-newtons), to physically hold and move microscopic dielectric objects.



Manipulation of single cells

Manipulation of internal organelles

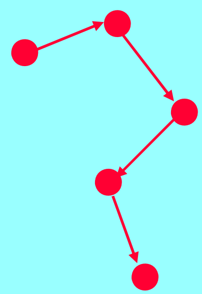
Green Onion Root Tips



Why needs a trap ?

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

Random "walk"



- How fast it walks ?

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

$K_B = 1.38 \times 10^{-23} JK^{-1}$

- How far it walks ?

$$< x^2 > = \frac{2 K_B T}{\beta} t$$

(Einstein)

$\beta = 6\pi\eta a$ (Stokes)

Brownian motion

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

- Gas: atoms in air, $m_N = 2.32 \times 10^{-26}$ kg

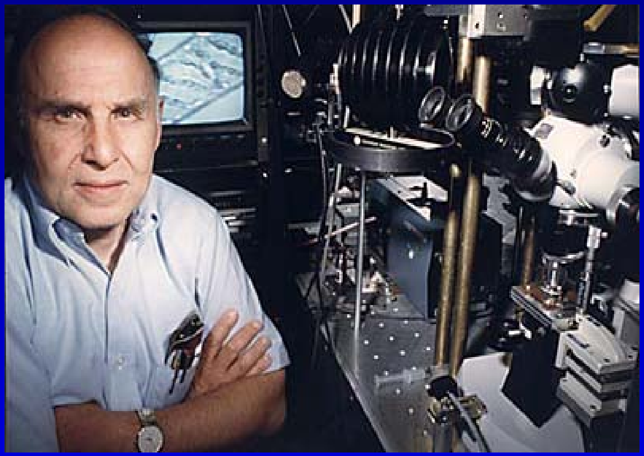
T= 27 C = 300K,	v = 2500 mi/hr (1100 m/s)
T= –270 C = 3 K,	v = 250 mi/hr (110 m/s)
T= 1.0 μK,	v = 25 cm/s

- Solid in air or water at T = 300 K

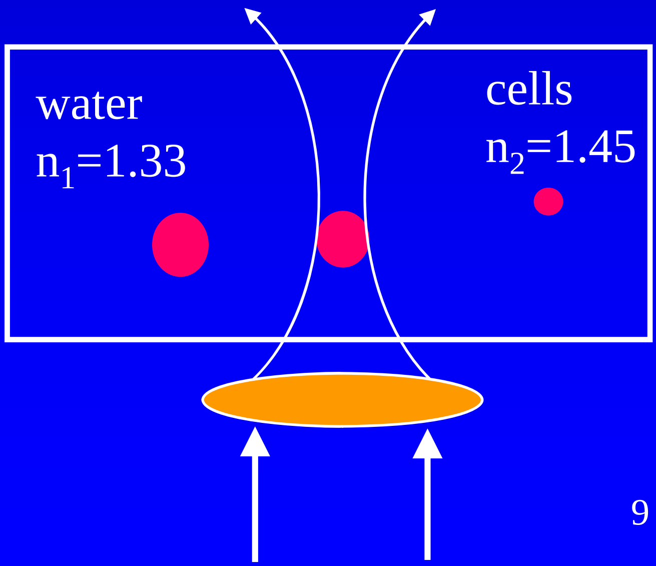
cells	d = 10 μm,	v = 45 μm/s,	x = 1.6 μm/min
latex	d = 1.0 μm,	v = 1.4 mm/s,	x = 5.1 μm
virus	d = 100 nm,	v = 4.5 cm/s,	x= 16 μm

Optical Tweezers:
Trap cells in liquids

Arthur Ashkin and S. Chu invented optical tweezers in 1986



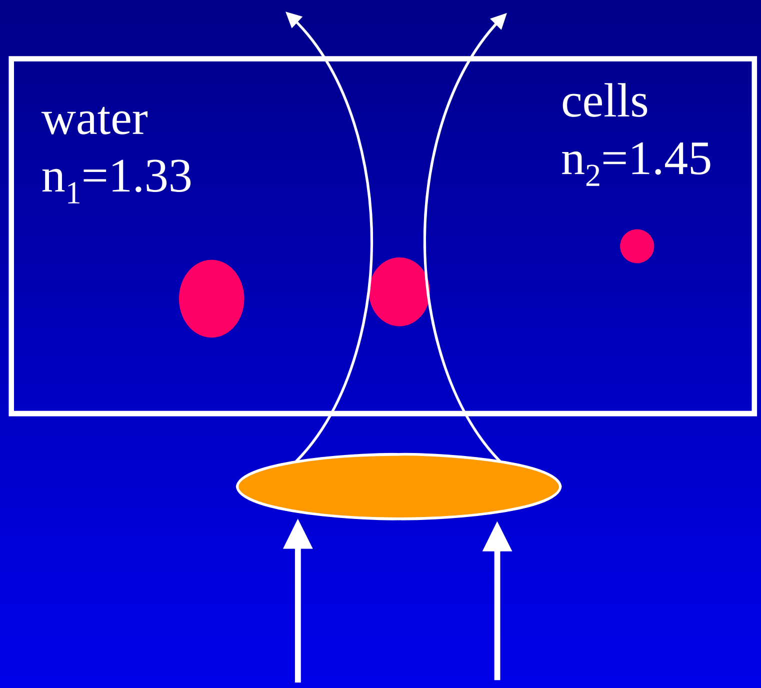
Optical tweezers is a three-dimensional optical trap formed by a highly focused laser beam.



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

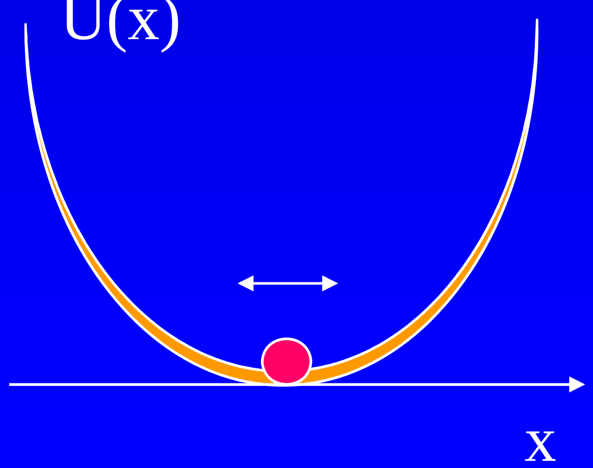
II. Principles of Optical Trapping

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



Harmonic approximation

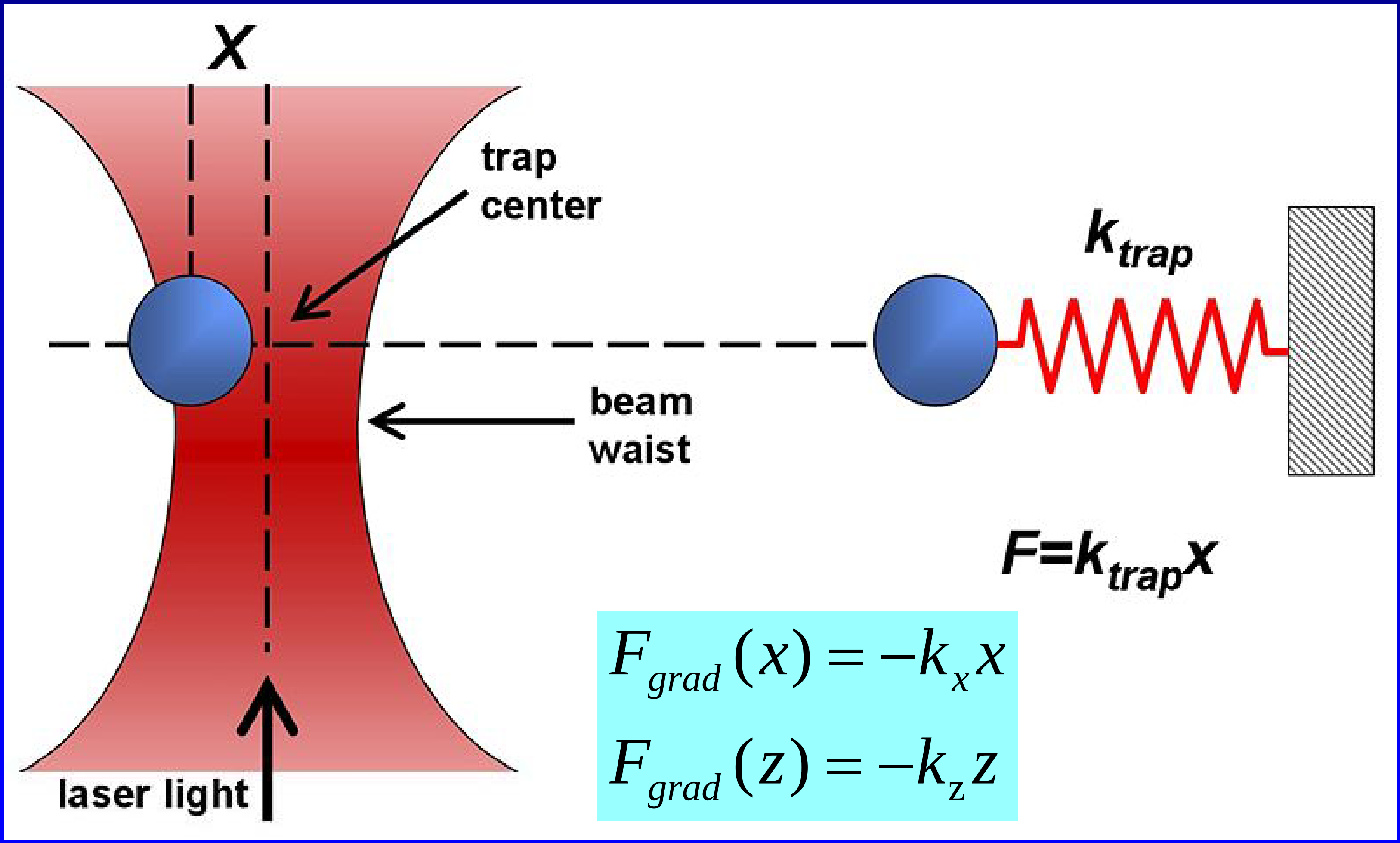
$F_{trap} = - k\,x$



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

Where k ~ 0.16pN/nm per 1W

2.1 General description

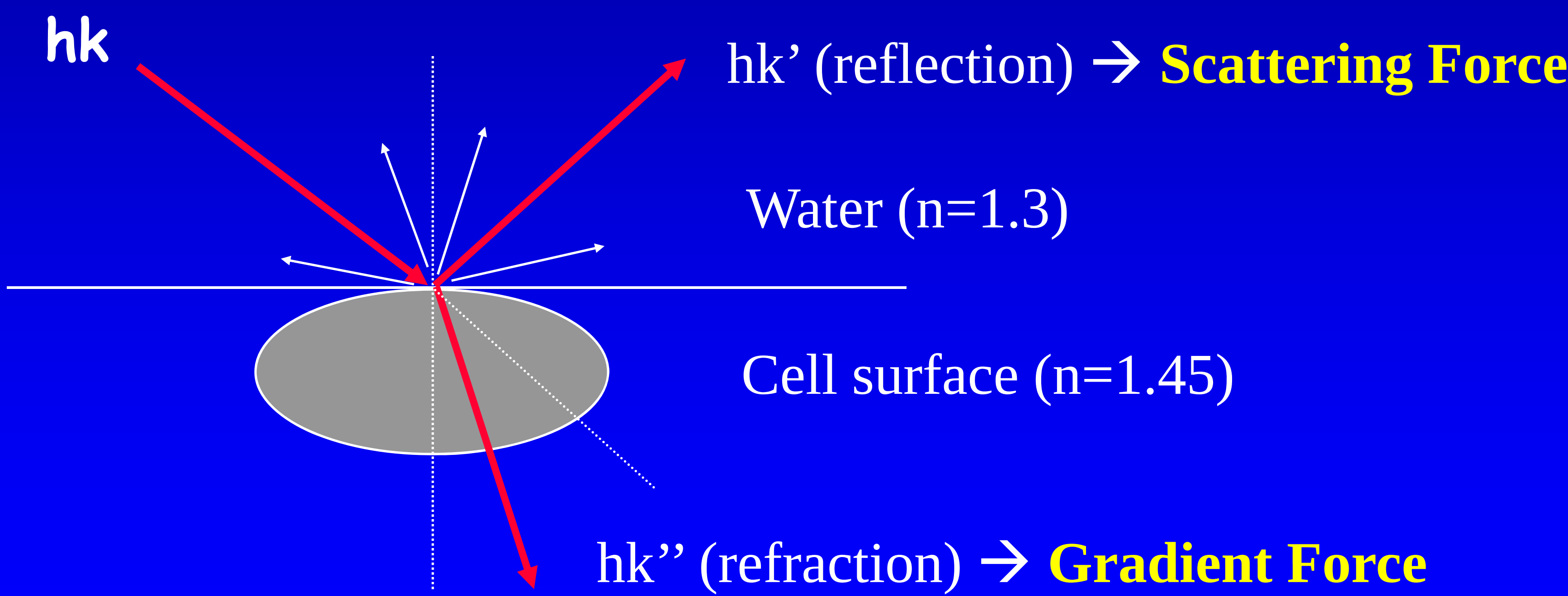


$$F_{grad}(x) = -k_x x$$

$$F_{grad}(z) = -k_z z$$

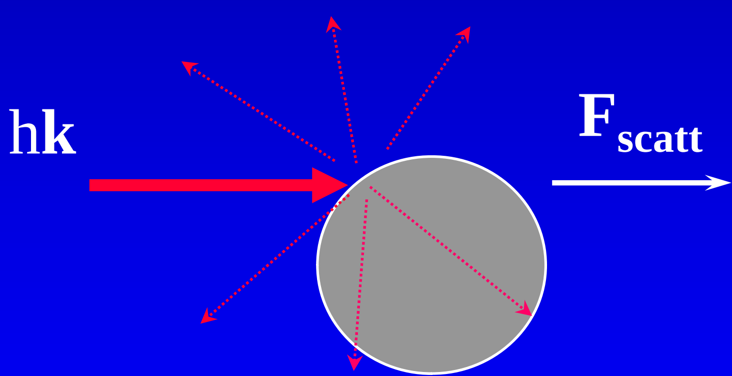
Optical Forces

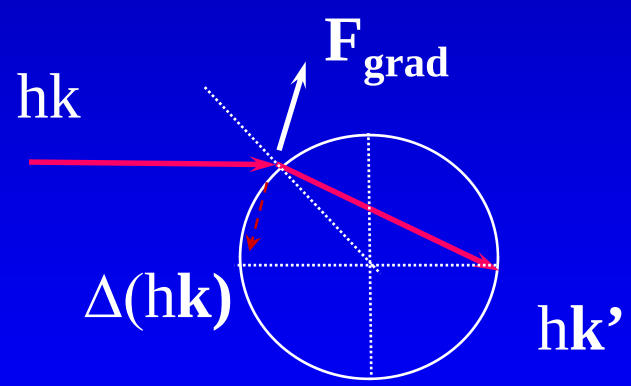
Each photon carries energy of hw and momentum of hk. Absorption, reflection or refraction of photons in the medium cause momentum change and produce optical forces.



Scattering and Gradient Forces

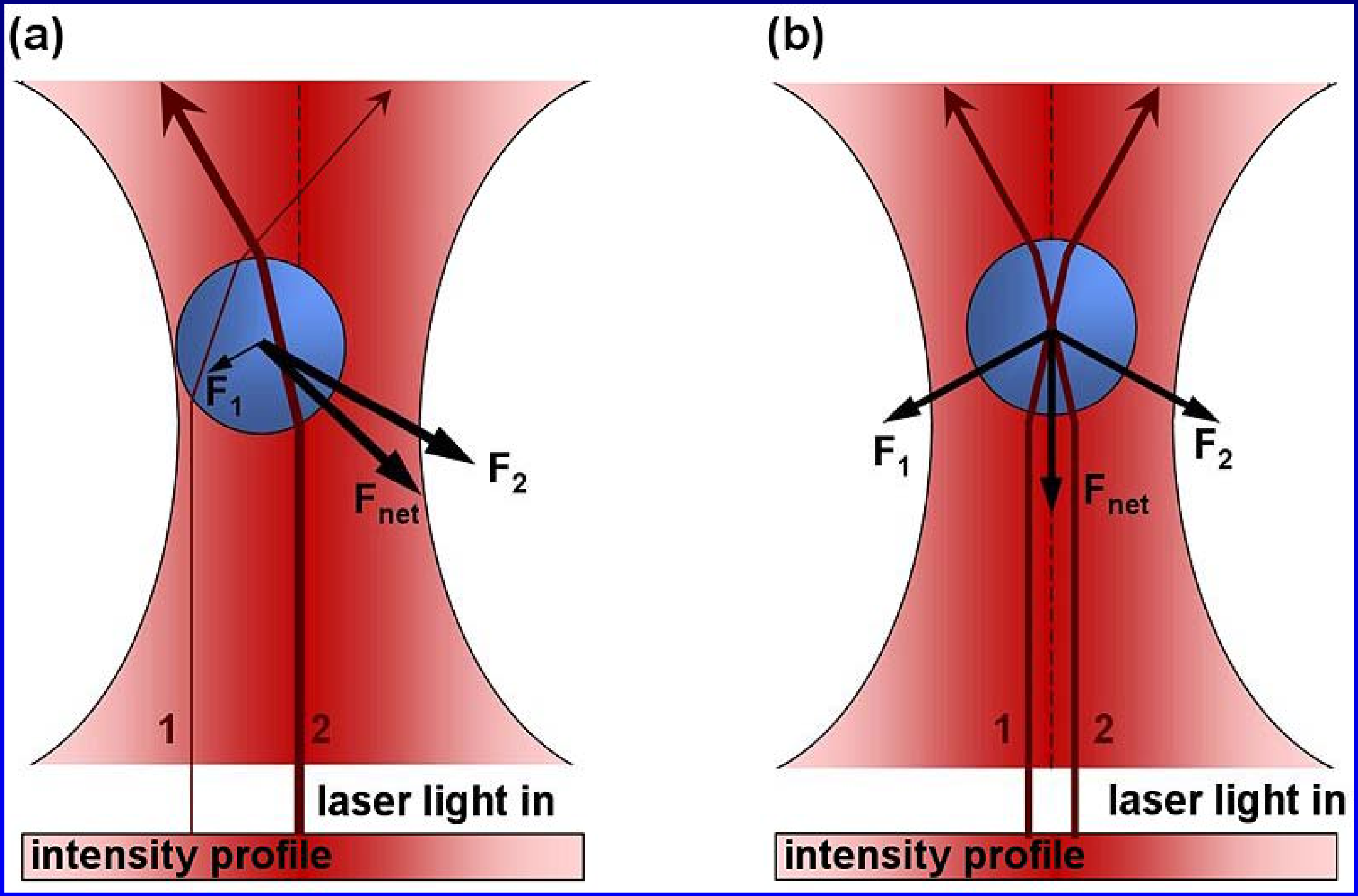
- **<u>Scattering force</u>: from reflection/absorption**
- **<u>Gradient force</u>: from refraction**





For transparent and biological media, $F_{grad} > F_{scatt}$.

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 .

$$\mathbf{F_1} = q\left(\mathbf{E_1} + \frac{d\mathbf{x_1}}{dt} \times \mathbf{B}\right)$$

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

$$\begin{aligned}\mathbf{F} &= q\left(\mathbf{E_1}(x,y,z) - \mathbf{E_2}(x,y,z) + \frac{d(\mathbf{x}_1 - \mathbf{x}_2)}{dt} \times \mathbf{B}\right) \\ &= q\left(\mathbf{E_1}(x,y,z) + ((\mathbf{x}_1 - \mathbf{x}_2)\cdot\nabla)\mathbf{E} - \mathbf{E_1}(x,y,z) + \frac{d(\mathbf{x}_1 - \mathbf{x}_2)}{dt} \times \mathbf{B}\right)\end{aligned}$$

$$\begin{aligned}\mathbf{F} &= (\mathbf{p}\cdot\nabla)\mathbf{E} + \frac{d\mathbf{p}}{dt} \times \mathbf{B} \\ &= \alpha\left[(\mathbf{E}\cdot\nabla)\mathbf{E} + \frac{d\mathbf{E}}{dt} \times \mathbf{B}\right],\end{aligned}$$

Where $p=\alpha E$

The electric dipole approximation

Use

1. $(\mathbf{E}\cdot\nabla)\,\mathbf{E} = \nabla\left(\frac{1}{2}E^2\right) - \mathbf{E}\times(\nabla\times\mathbf{E})$
2. $\nabla\times\mathbf{E} = -\frac{\partial\mathbf{B}}{\partial t}$

and

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

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.

$$\mathbf{F} = \frac{1}{2}\alpha\nabla E^2.$$

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,

$$F_{\text{scatt}} = \frac{I_0 \sigma n_m}{c},$$

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

n_m – index of refraction of the medium

n_p – index of refraction of the particle

$m=n_p/n_m$

$F_{scat} = n_b P_{scat}/c$, where P_{scat} is the power scattered

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

$$F_{\text{grad}} = \frac{2\pi\alpha}{c n_m^2}\nabla I_0,$$

where

$$\alpha = n_m^2 a^3\left(\frac{m^2-1}{m^2+2}\right)$$

Catch transparent cells by gradient force

$$F_{grad} \propto \nabla I(x,y,z)$$

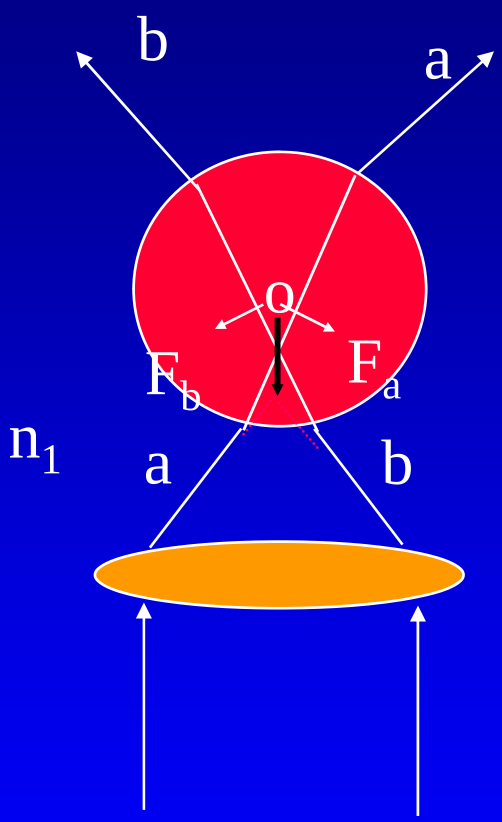
Input focused Guassian beam:

$$I(x,y,z,t) = I_0 \exp\left(-2\left(x^2+y^2\right)/\omega(z)^2\right)$$

Transverse and axial force for small displacements

$$F_{grad}(x) = -k_x x$$
$$F_{grad}(z) = -k_z z$$

Where k ~ 0.16pN/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 ~ 1.6), n_{water} =1.33.

$F_{grad} > F_{scatt}$

Non-transparent:

- Metal particles;
- Color dusts, black paints;
- Semiconductor powders;

- Large reflectivity;
- Large absorption;
- High index of refraction (n=1.7 ~ 4.0).

$F_{grad} < F_{scatt}$

Scattering Force in Laser Tweezers

For absorbing particles, the scattering force dominant.

Assume that a laser beam of P=10 mW at λ=1.06 μm is absorbed or reflected by a gold sphere of 1.0 μm size.

$h\omega \sim 1.24$ eV, $h\mathbf{k} = h/\lambda = 6.62 \times 10^{-28}$ N s

scattered **photon number**: $N = P/h\omega = 5\times10^{16}$ photons/s

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

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

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

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

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

F_{scatt} ~ 170 times gravity force !

Scattering force on metallic particles

APPLIED PHYSICS LETTERS VOLUME 81, NUMBER 6 5 AUGUST 2002

Raman spectra and optical trapping of highly refractive and nontransparent particles

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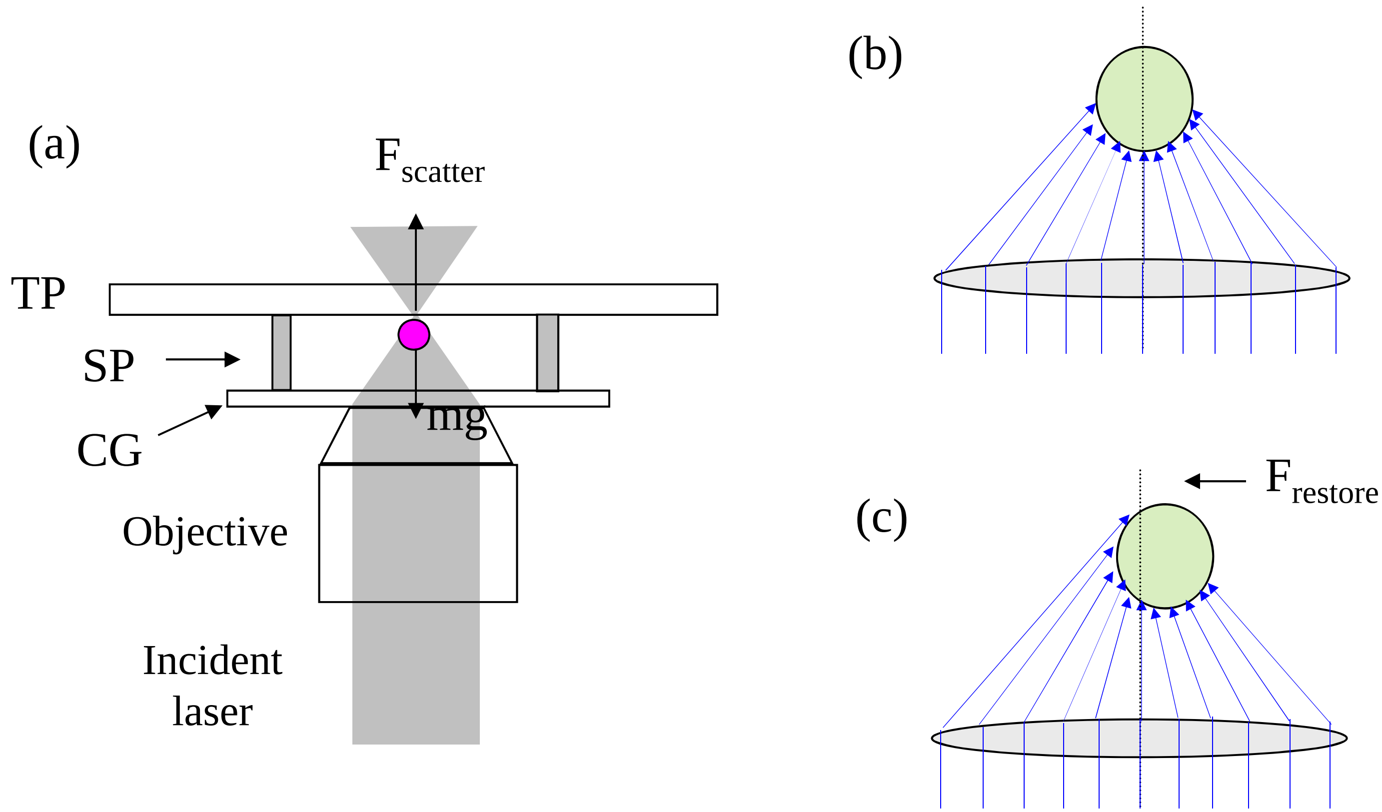
For non-transparent and metal particles, $F_{scatt} > F_{grad}$.

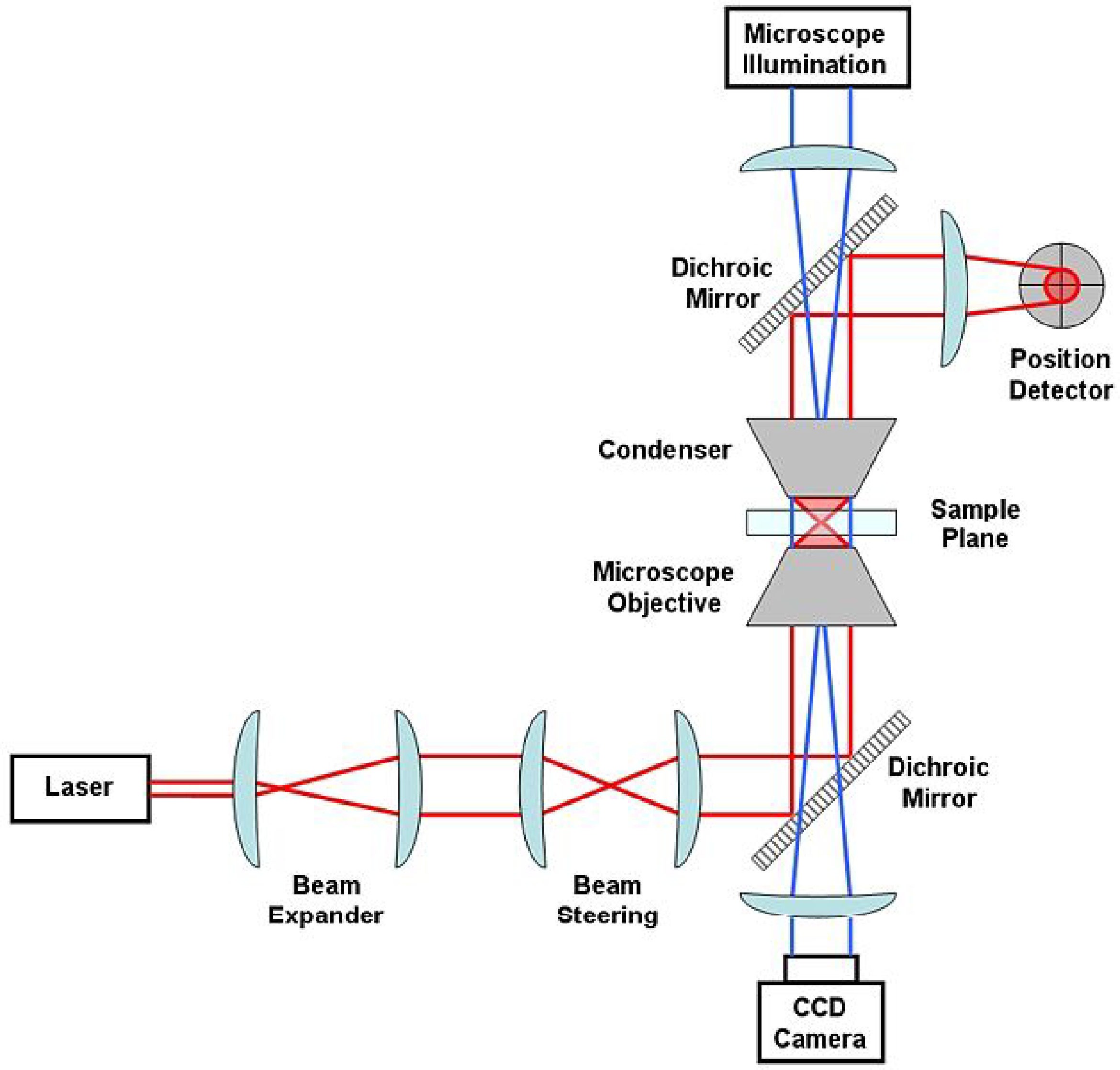


Xie & Li, Appl. Phys. Lett. 81, 951 (2002)

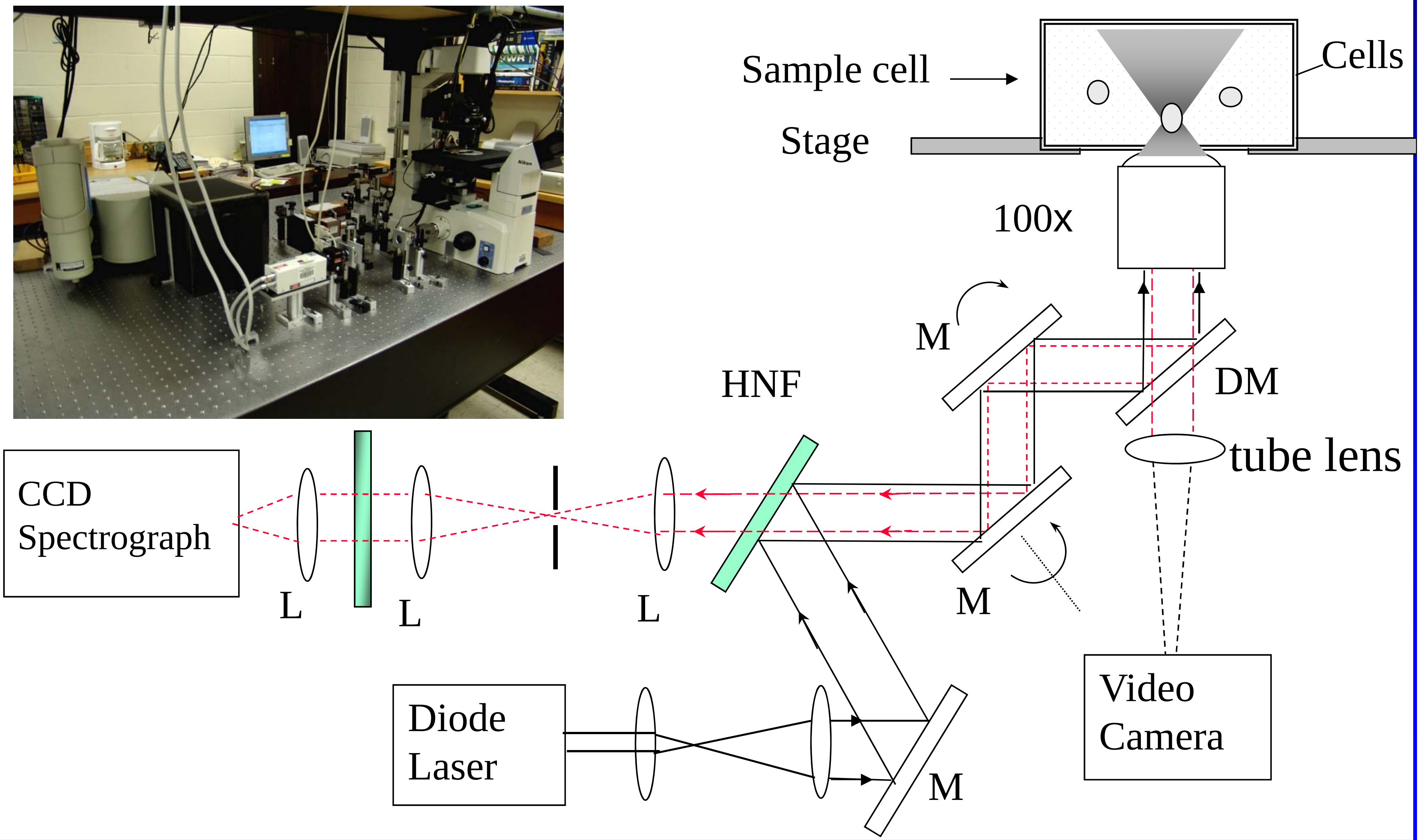
3. Experimental design, construction and operation

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



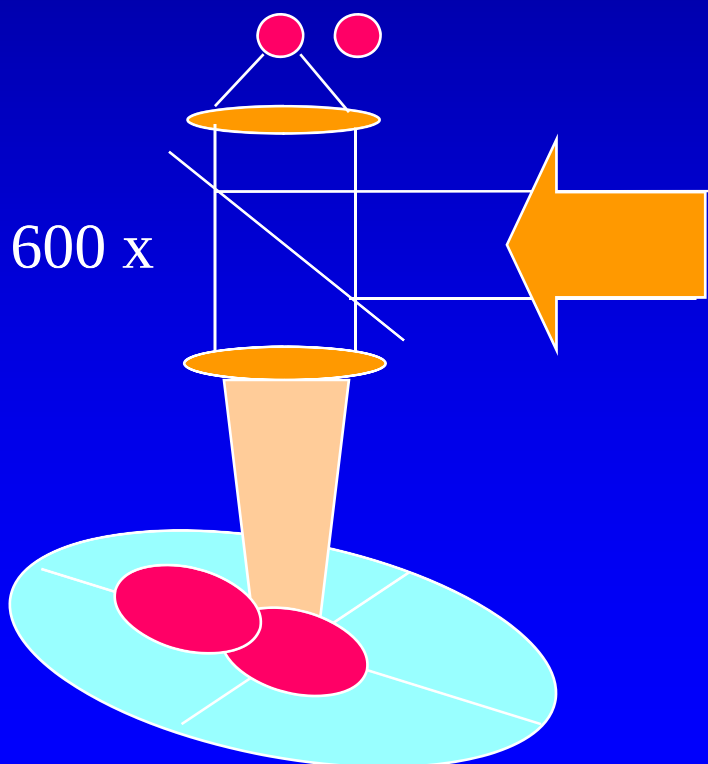
Optical tweezers with infinity-corrected microscope



Tracking of Bead Brownian motion

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

- Monitoring Z movement of trapped particle;



Quadrant detectors

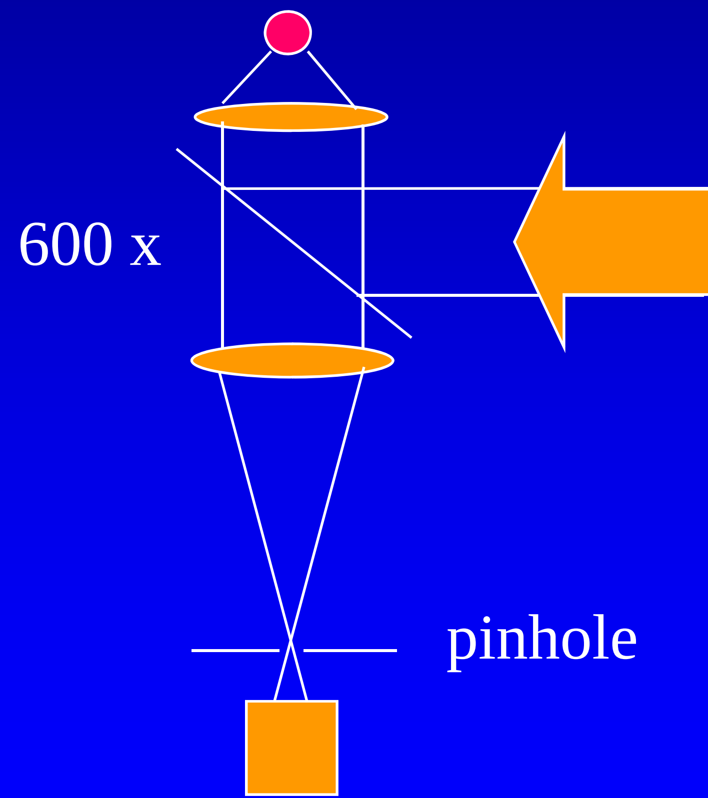


Photo-detector

Pulsed optical tweezers for levitate stuck particles

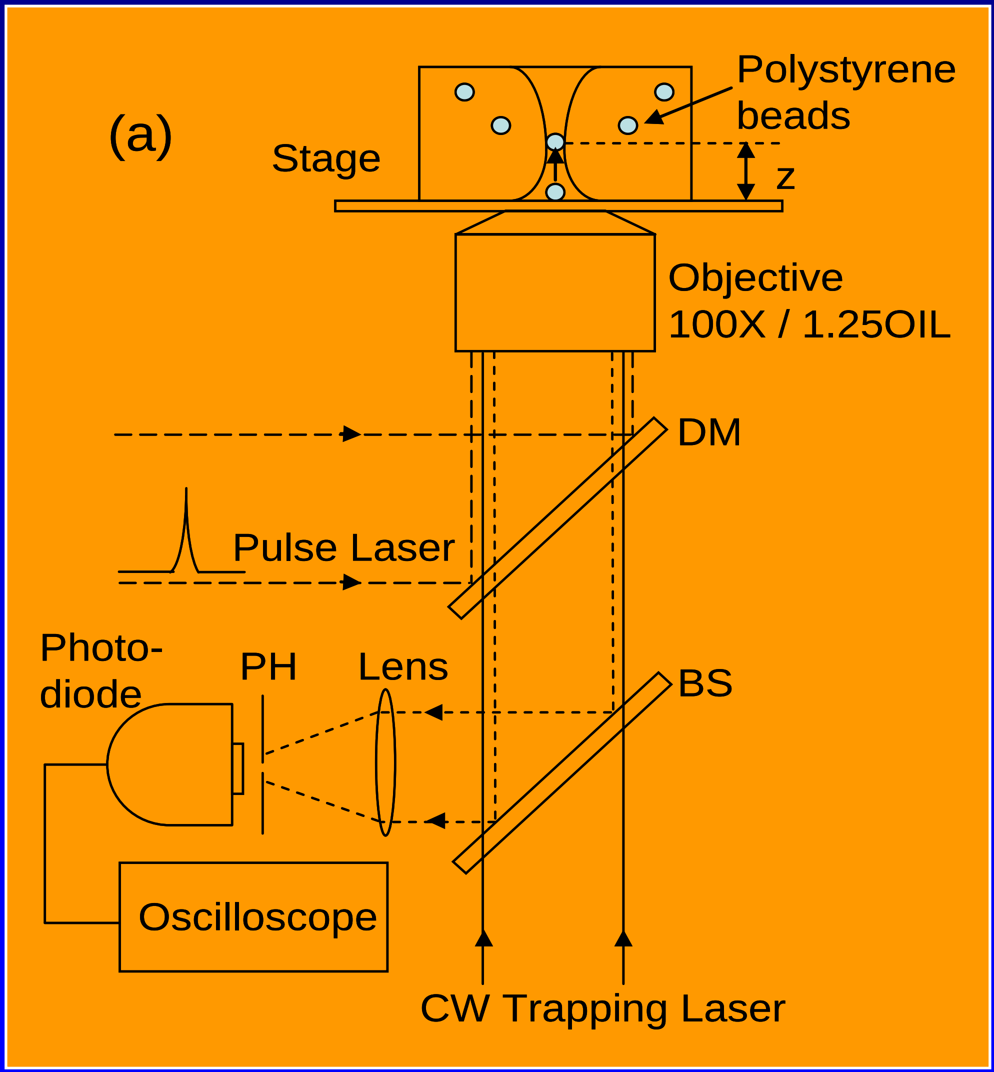
Cw laser: F ~ 1-10 pN
for manipulation (<100mW)

Pulsed laser:
for levitation

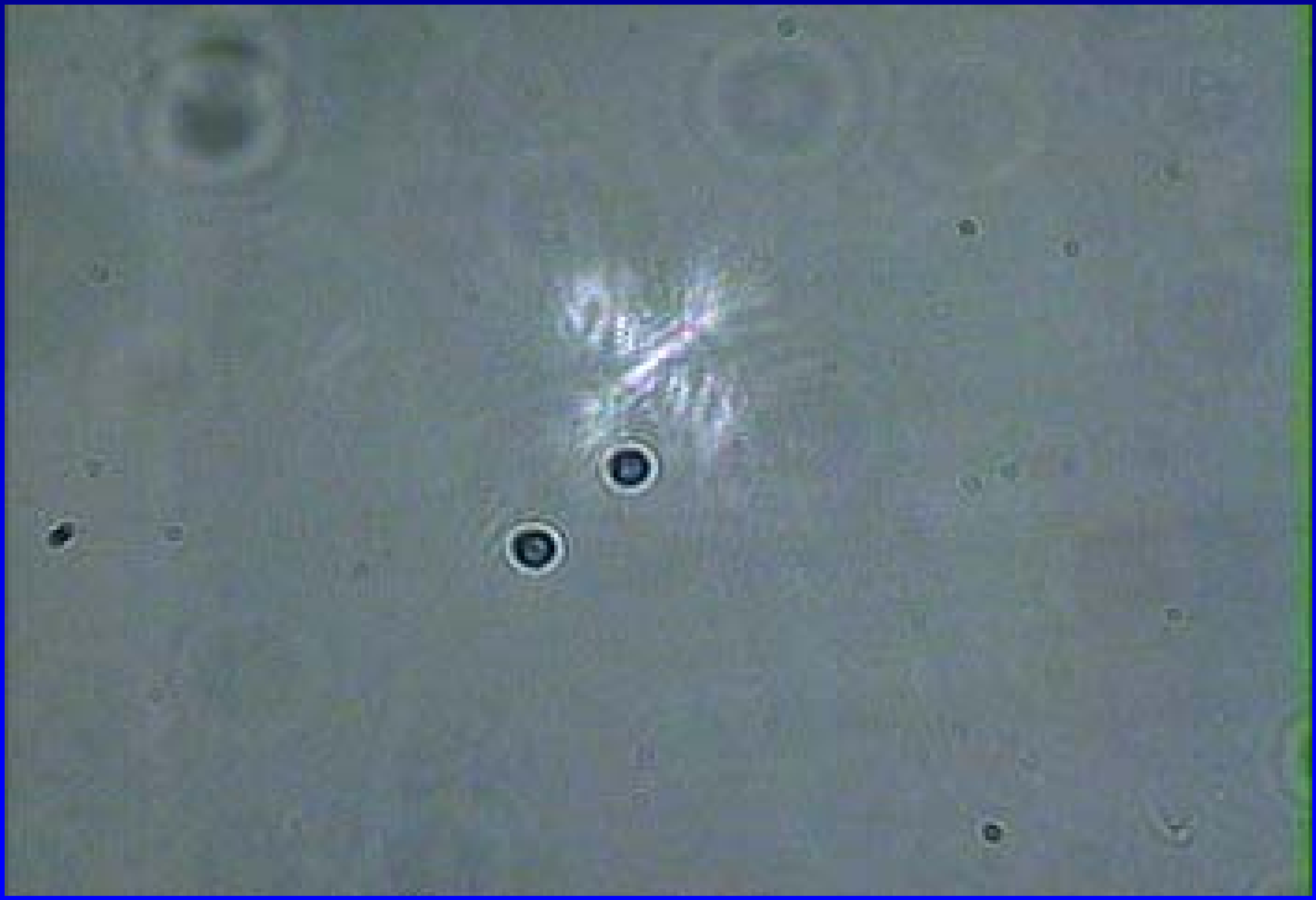
- Nd:YAG laser, 1064 nm
- 50μs pulse width
- 300-500 μJ/pulse

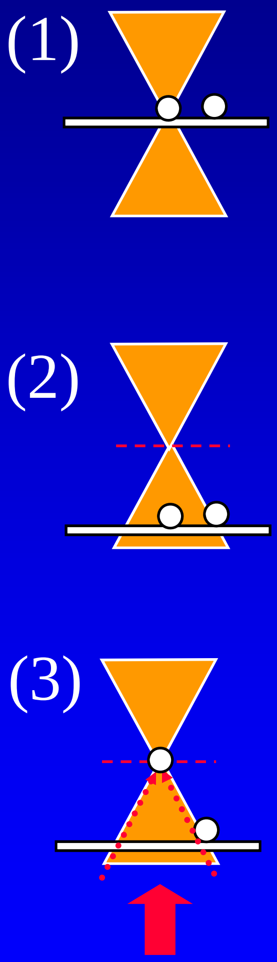
Peak power ~ 10W
F_{grad} ~ 1000 pN

Opt. Lett. 30, 1797 (2005)

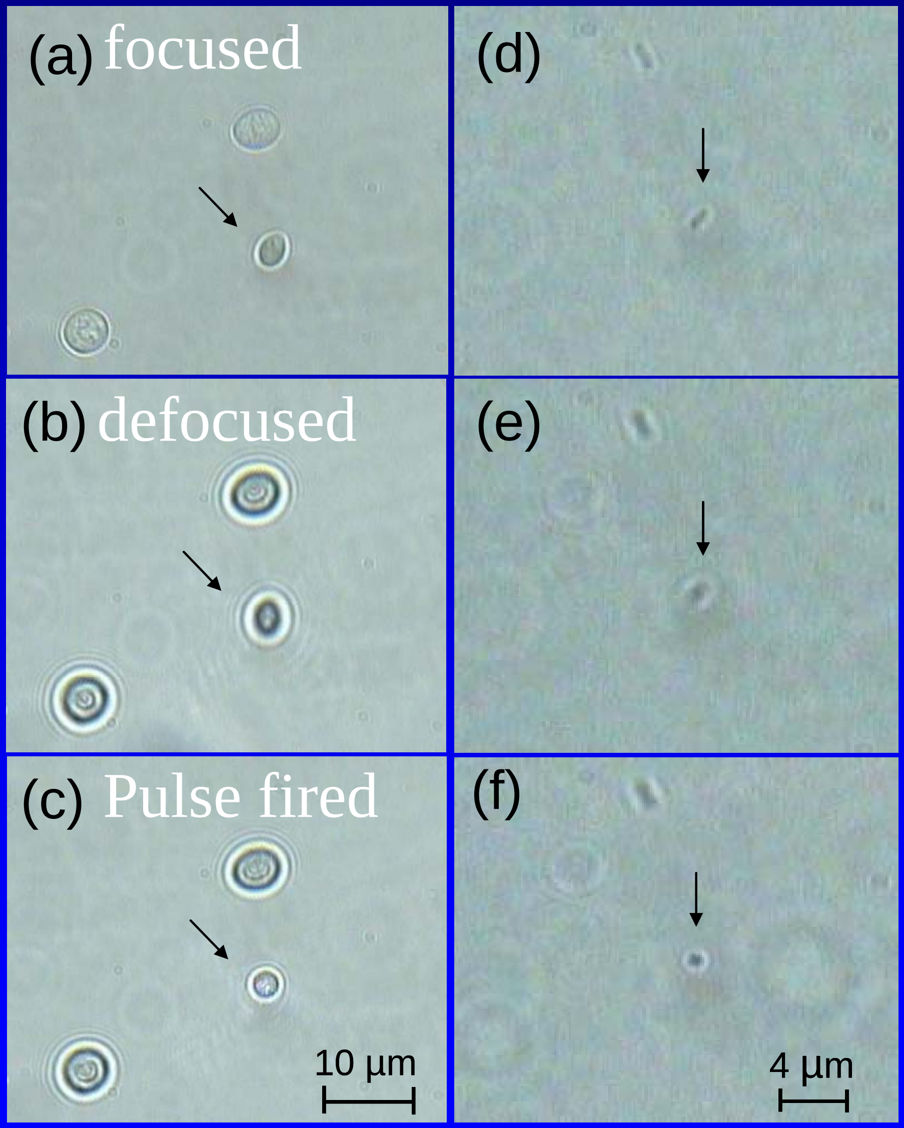


Levitation and manipulation of stuck particles





Levitation and manipulation of stuck biological particles

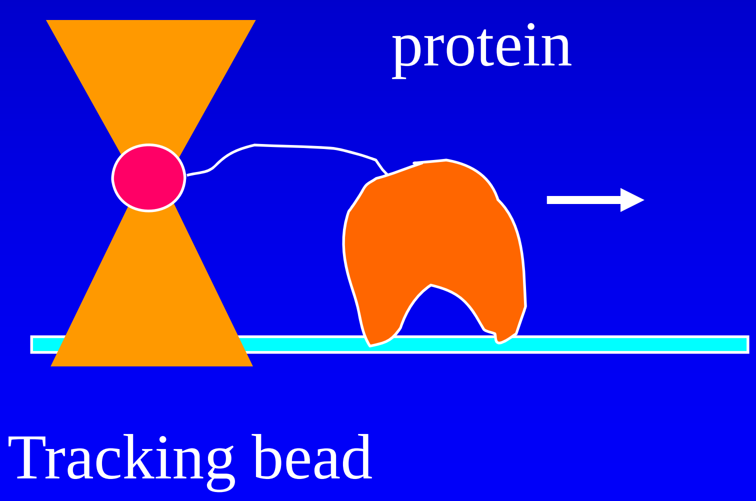


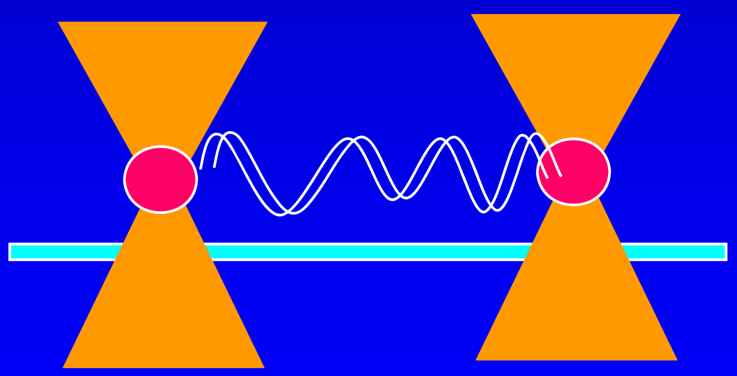
4. Applications of Optical Tweezers: Biomechanics

- 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;**

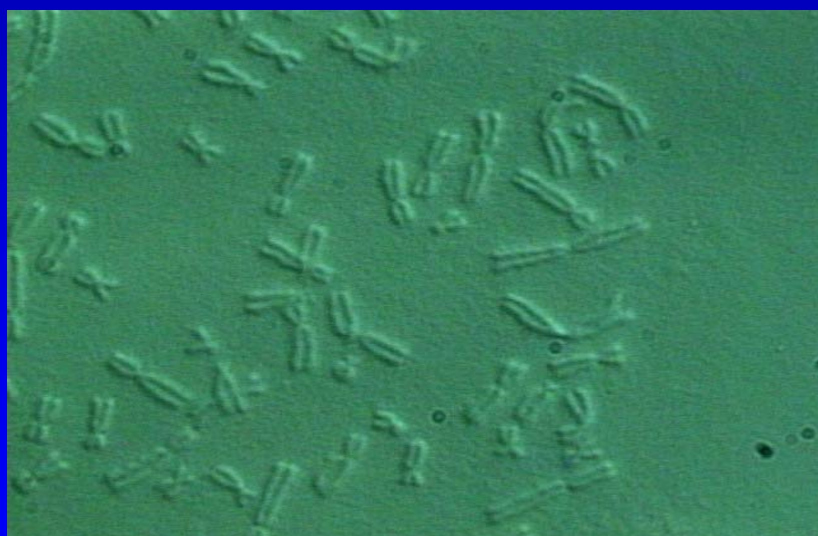




Chromosomal analysis and identification based on optical tweezers and Raman spectroscopy

Jenifer F. Ojeda[1], Changan Xie[2], Yong-Qing Li[2], Fred E. Bertrand[3], John Wiley[4], and Thomas J. McConnell[1]

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



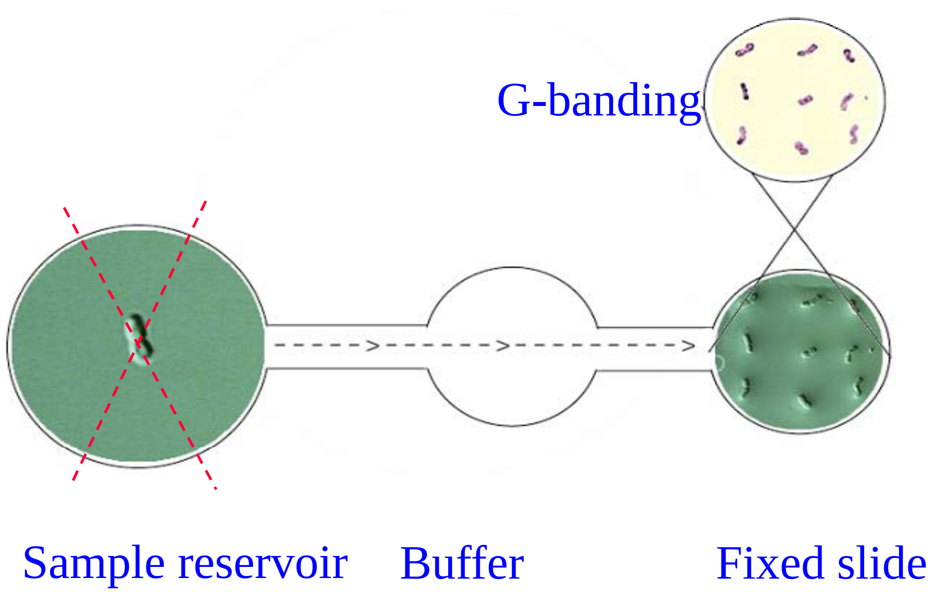
Microscopic image of unstained chromosomes of leukemia cells.

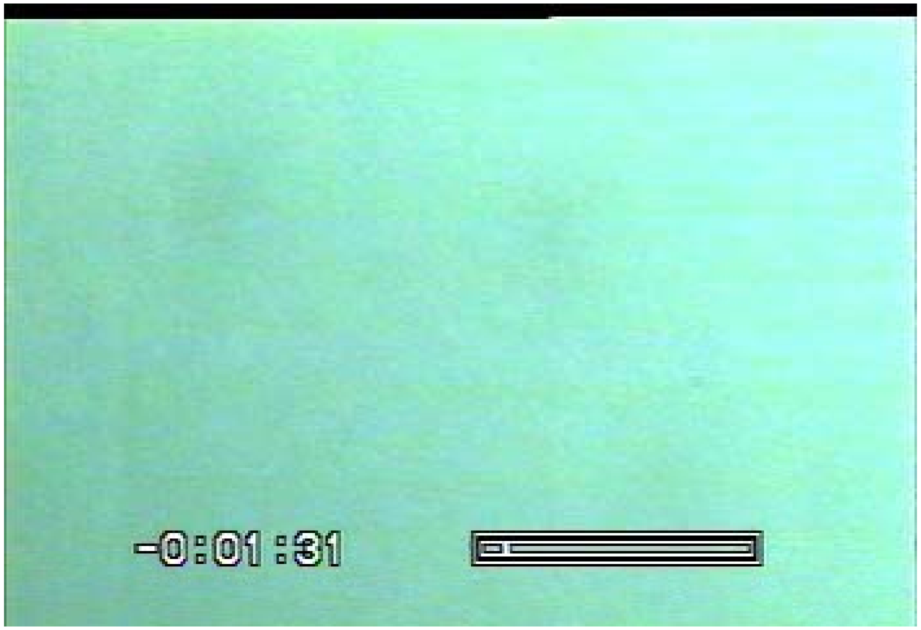
Questions:
How to obtain Raman spectral patterns for chromosomes number 1, 2, and 3, potentially 24 human chromosomes?

Optics Express, 14, 5385-5393 (2006)

Experimental procedures

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





Further references for optical tweezers in solution

1. A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, and S. Chu, “Observation of a single-beam gradient-force optical trap for dielectric particles”, *Opt. Lett*. 11, 288 (1986).
2. A. Ashkin, K. M. Dziedzic, T. Yamane, “Optical trapping and manipulation of single cells using inferred laser beams”, *Nature*, 330, 769 (1987).
3. K.C. Neuman, S.M. Block, “Optical trapping”, *Review Scientific Instruments*, 75, 2787-2809 (2004). – review article.
4. A.A. Ambardekar, Y.Q. Li, “Optical Levitation and manipulation of stuck particles with pulsed optical tweezers”, *Opt. Lett.*, <u>30</u>, 1797-1799 (2005).
5. J.Joykutty et al, “Direct measurement of the oscillation frequency in an optical tweezers…”, PRL,95, 193902 (2005).
6. Y. Roichaman, “Optical force arising from phase gradient”, PRL, 100, 013602 (2008).