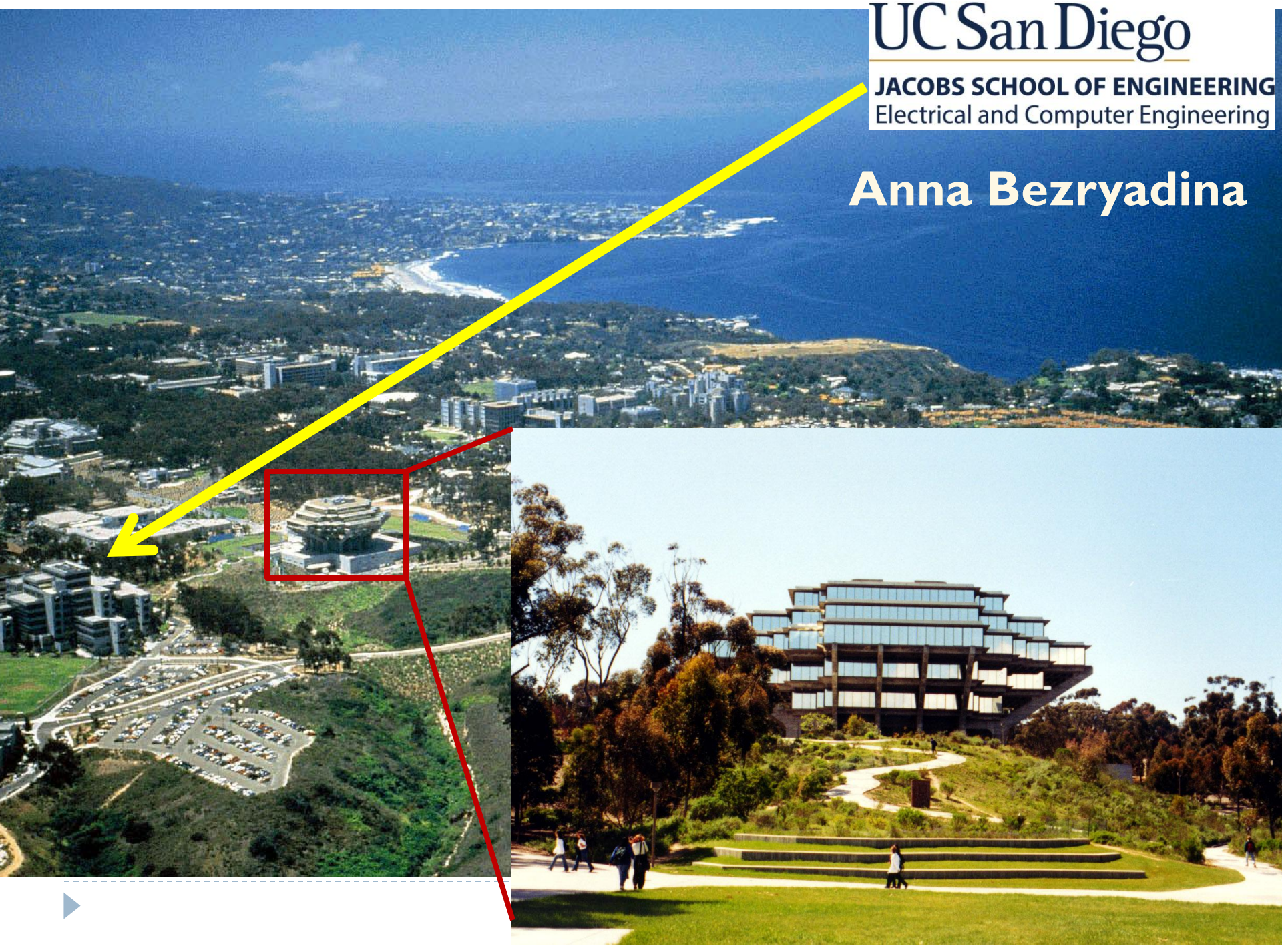

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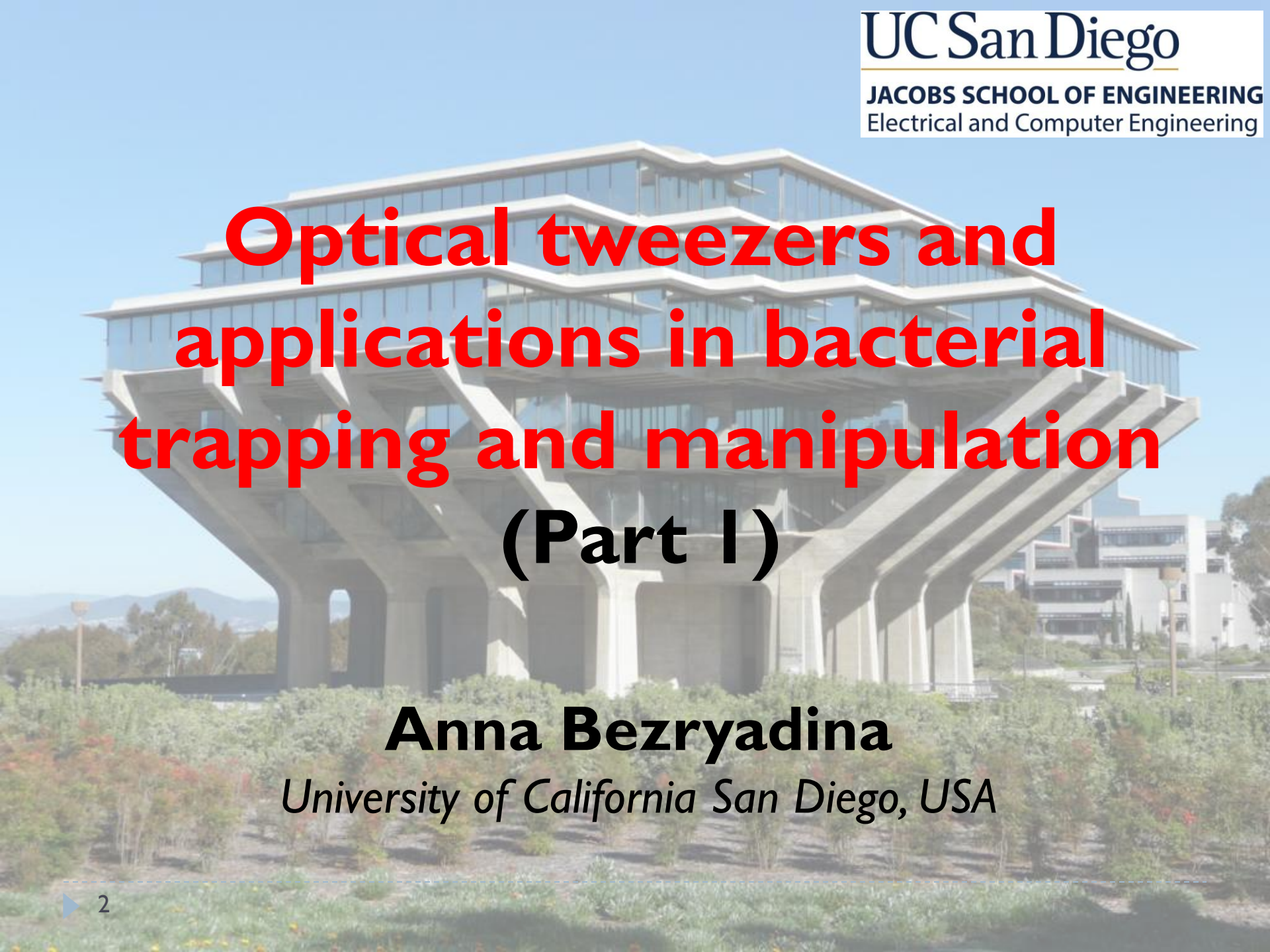
Anna Bezryadina



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Optical tweezers and applications in bacterial trapping and manipulation (Part I)

Anna Bezryadina

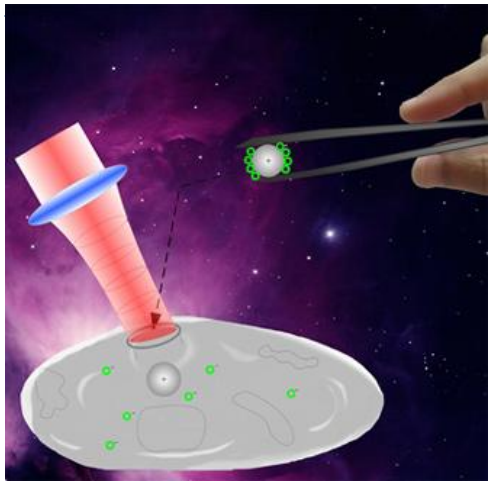
University of California San Diego, USA

Outline

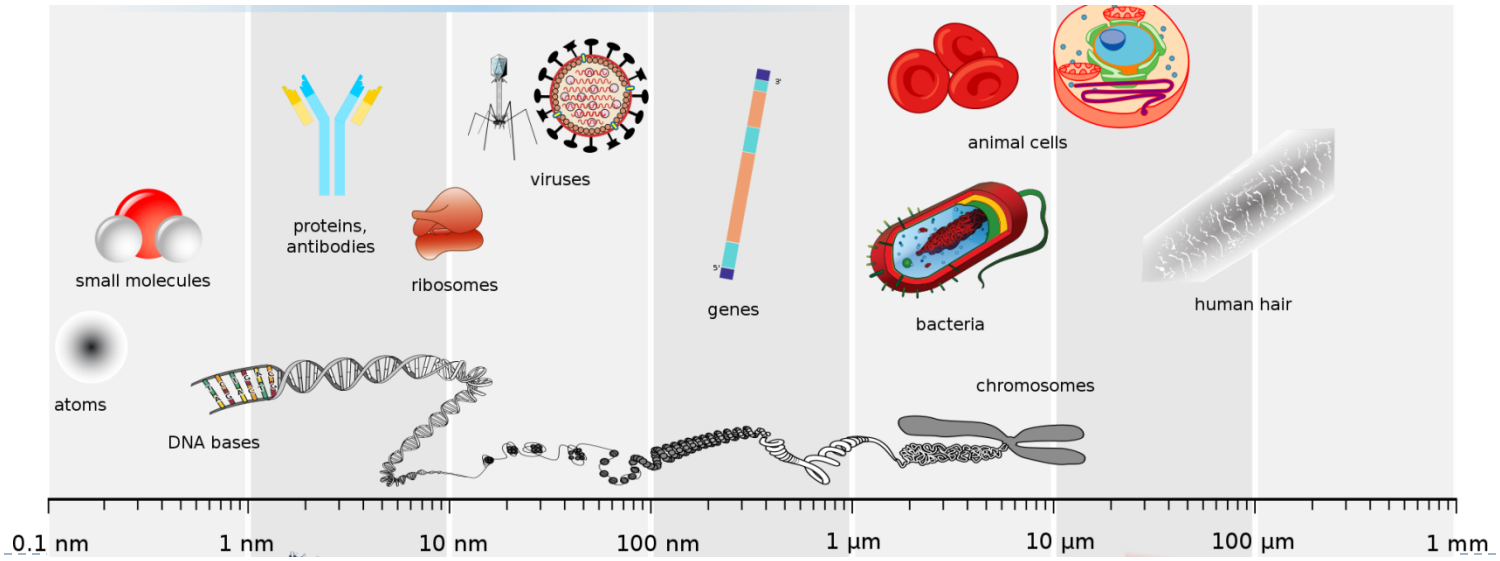
- **Optical Tweezers (part 1)**
  - **Basic concept**
  - **Force measurements**
  - **Holographic optical tweezers**
  - **Living organisms trapping and manipulation**
- **Application in medicine and biology (part 2)**

Optical tweezers

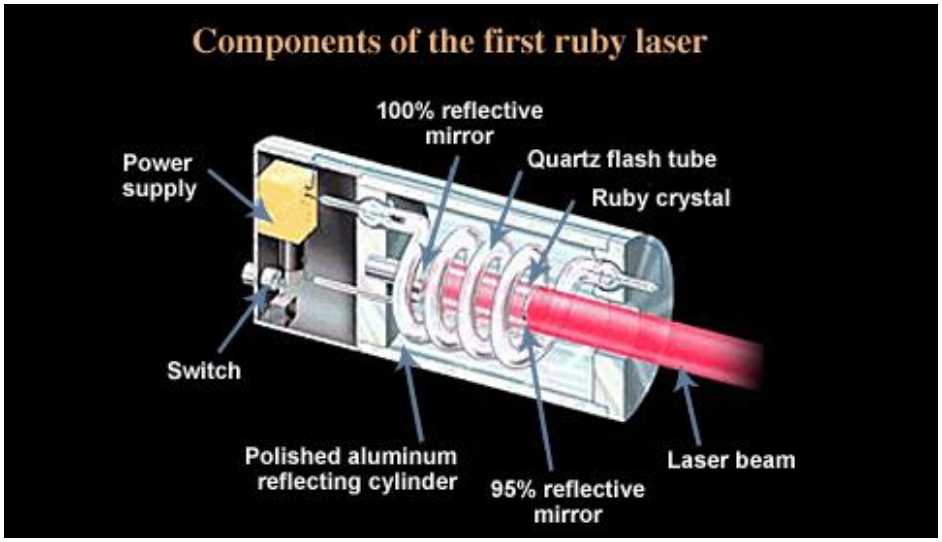
- Use focused light instead of physical equipment
- can trap and control objects: 10nm-10mm
- detect and apply forces: 0.1 – 1000pN
- measure displacements in nm range



Gwangju Institute of Science and Technology: Yong-Gu Lee group



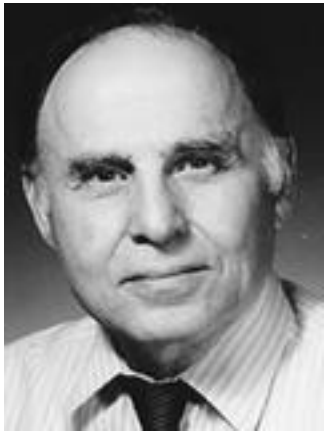
The birth of optical tweezers



The Laser

Theorised by Charles Towns (Bell labs)

Built by Theodor Maiman in 1960.



Arthur Ashkin
(1922- Present)
(Bell labs)

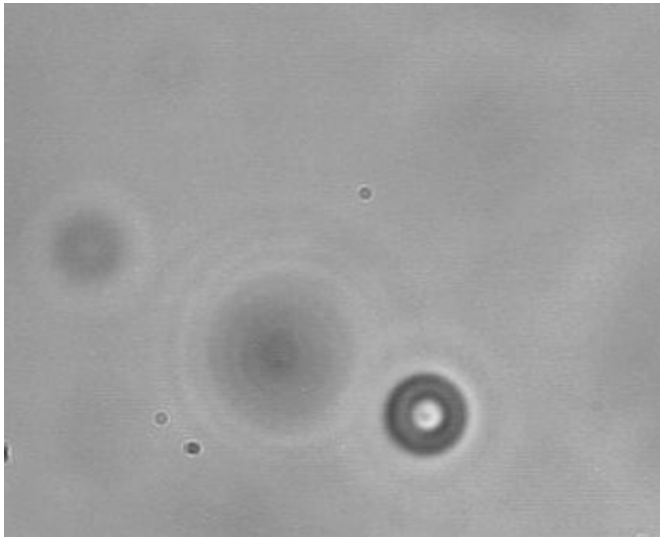
Coherent light can propagate losing little power.

Ashkin worked out that lasers could provide enough power to move small particles.

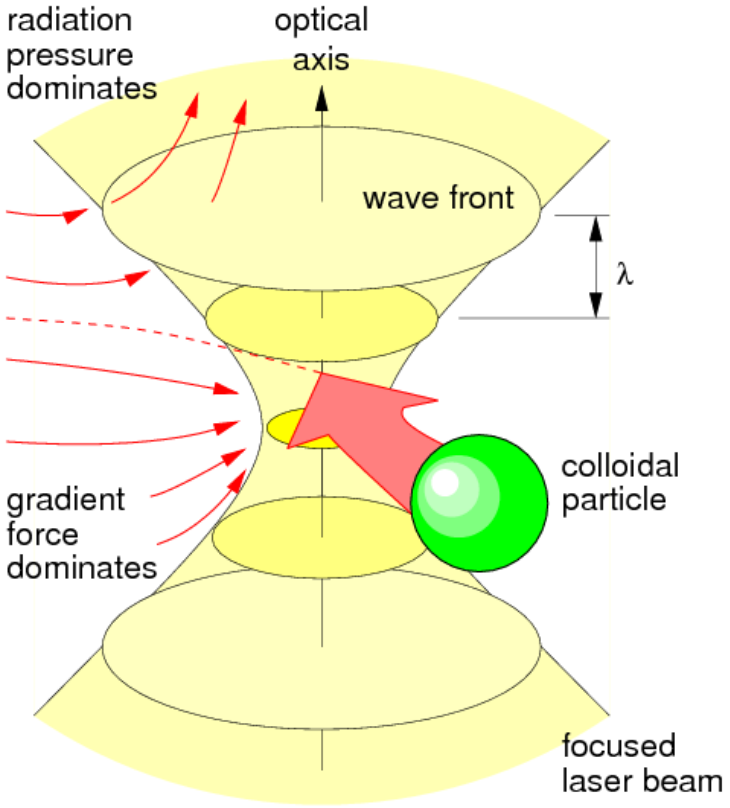
This would only work if light was focused into a tight spot.

Optical tweezers work

Optical Tweezers use radiation pressure from a focused laser beam to attract particle to to the the center of the beam (the highest intensity).



University of Cambridge: Joanne Gornall's group

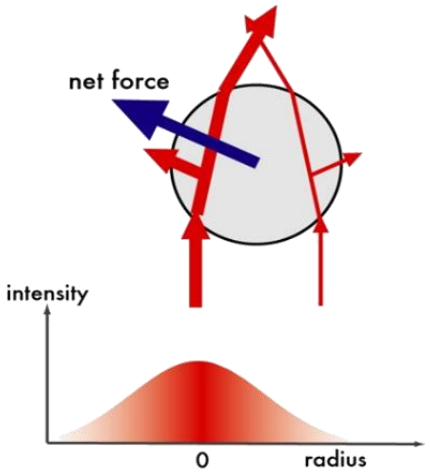


http://www.physics.nyu.edu/~dg86/figures/tweezer.png

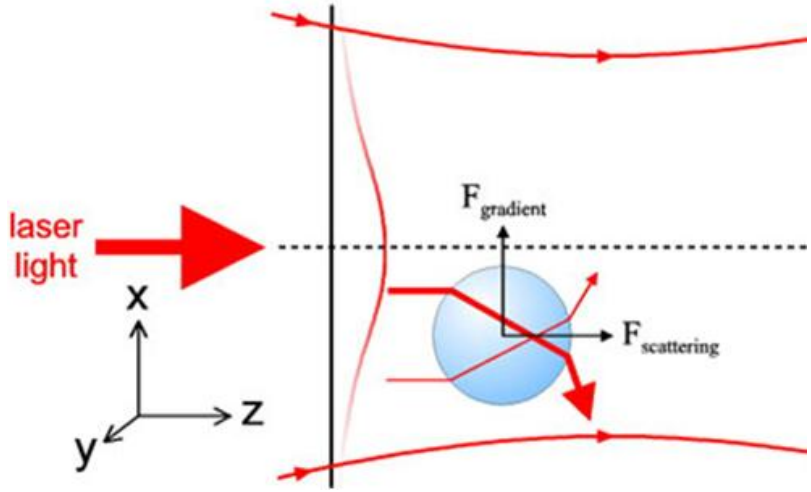
Optical forces

- **Gradient Force**
  - The intensity is greatest at the center of the beam, which pulls the particle towards the center of the beam

$$F_{grad} = \frac{\alpha}{4}\nabla I$$



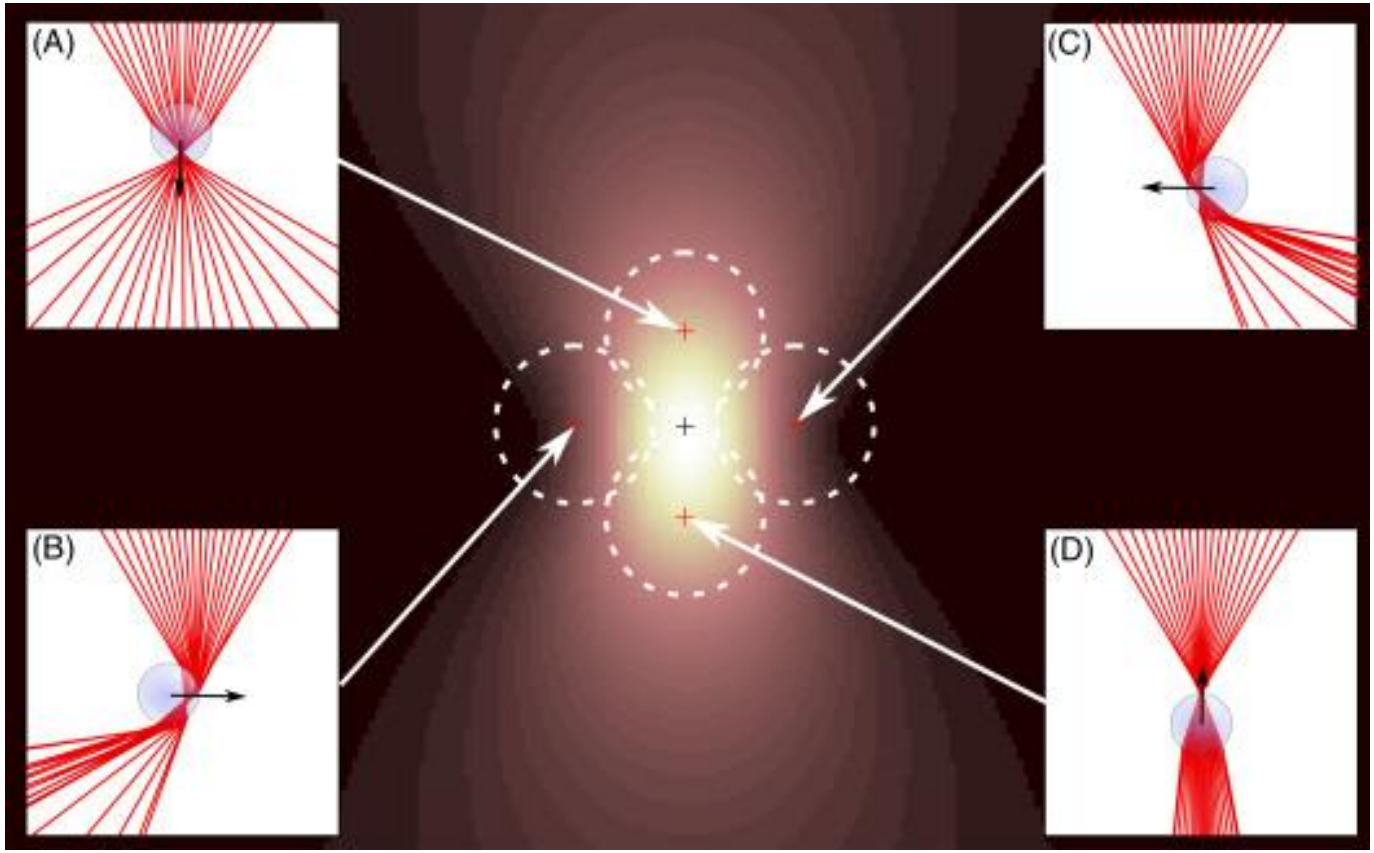
Particle polarizability: $\alpha = 3V_p\varepsilon_0 n_b^2\left(\frac{m^2-1}{m^2+2}\right)$ $\quad m = \frac{n_p}{n_b}$



http://www.stanford.edu/group/blocklab/Optical%20Tweezers%20Introduction.htm

- **Scattering Force**
  - created by light scattering of the surface of the particle
  - pushes the particle along in the propagation direction

Particle at different places in focused Gaussian laser beam



Conservation of momentum shows that a force must exist towards the focus of the beam.

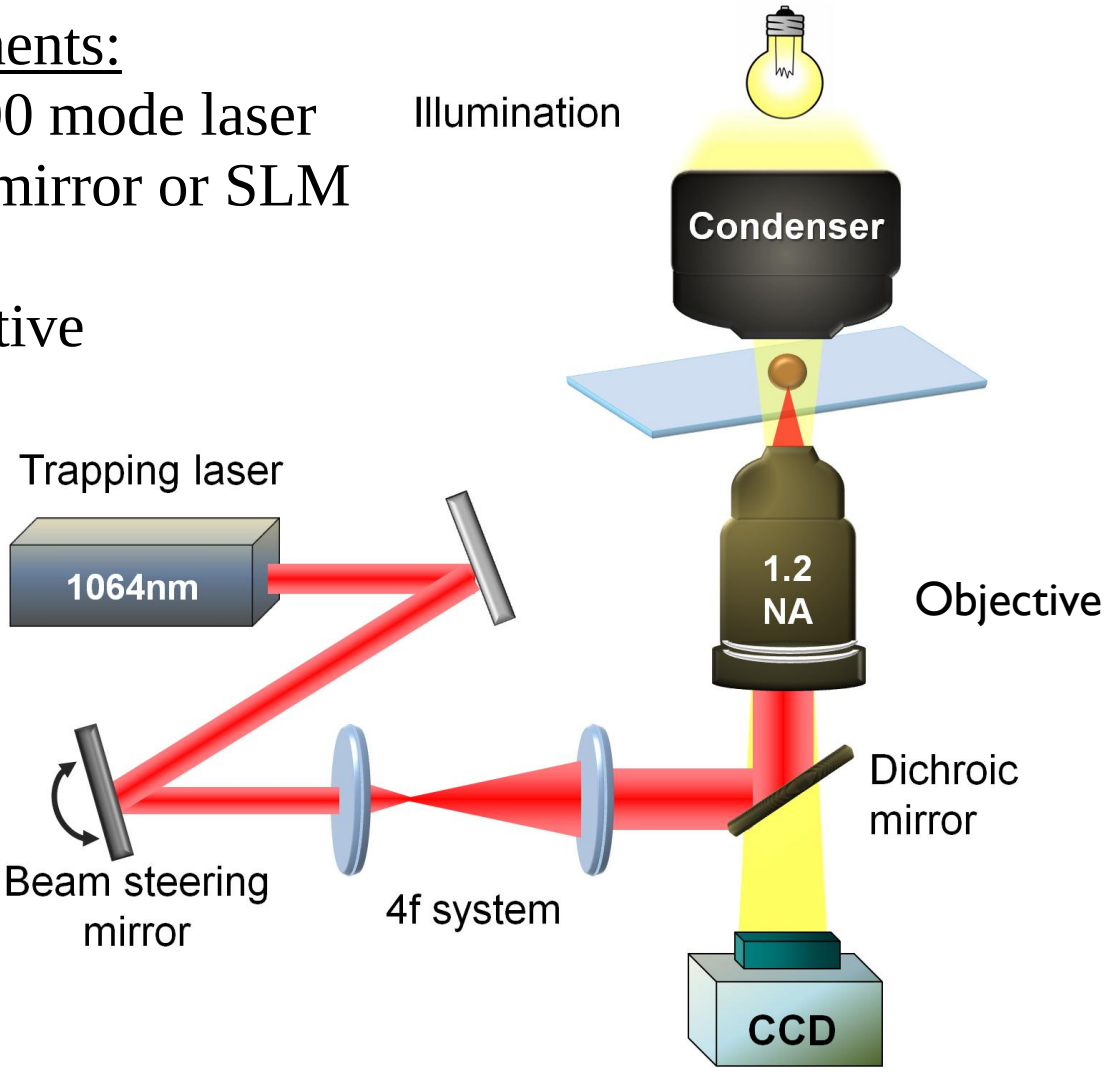
D. Preece, Elsevier, 2017, Light Robotics: Structure-Mediated Nanobiophotonics

Conventional Optical Tweezers

Typical requirements:

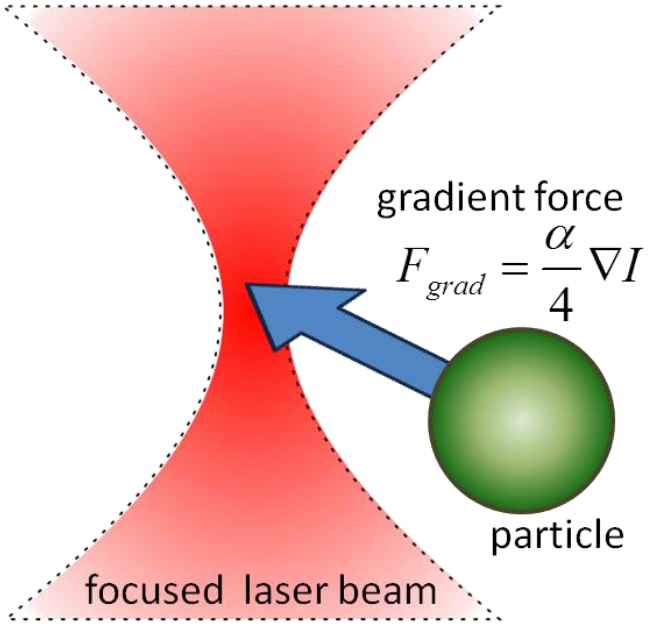
-Gaussian TEM00 mode laser

- Beam steering mirror or SLM
- 4f system
- High NA objective

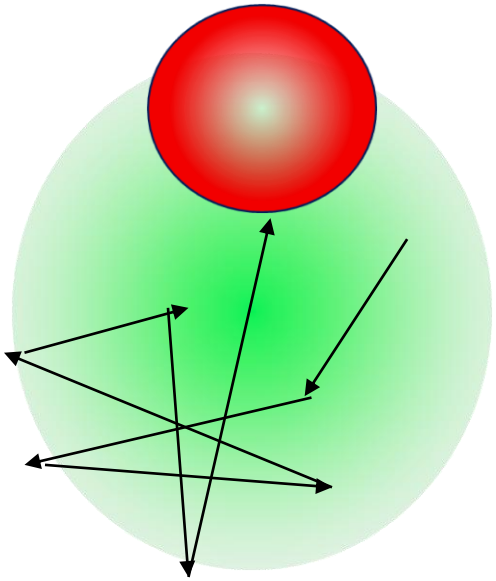


Optical trapping of a particle

Done by Dr. Daryl Preece



Particles also have other forces acting on them….



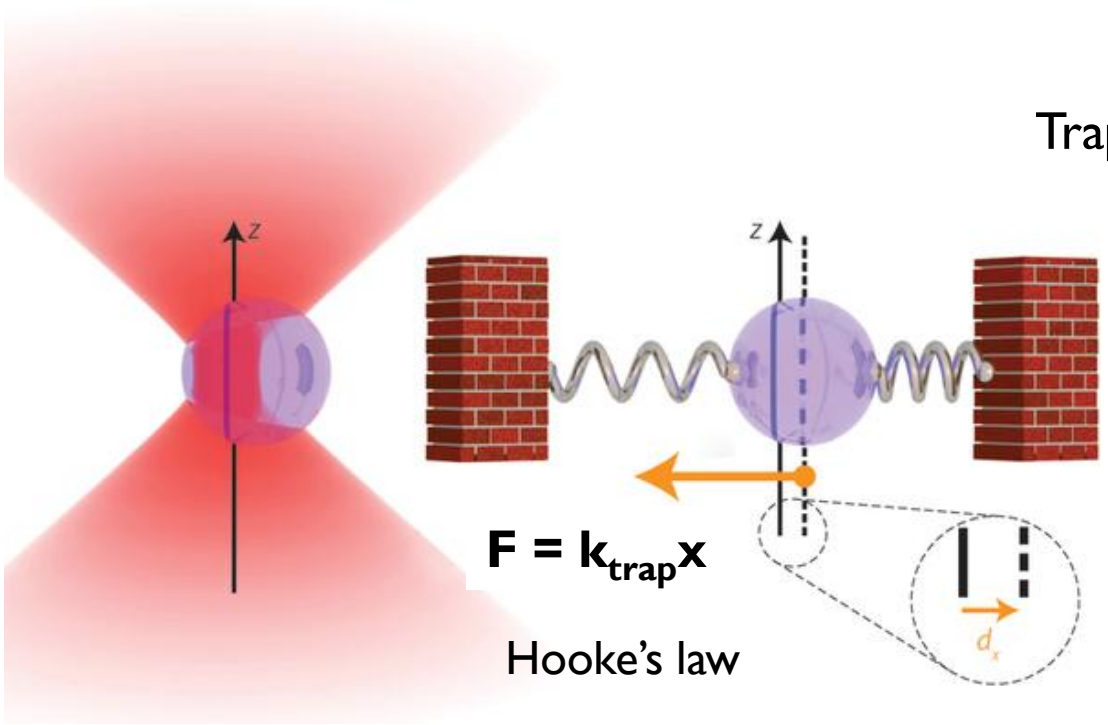
Thermal forces:
Brownian motion jolts small particles around in side the trap. (Proportional to temperature)

Viscous (Stokes) drag
Viscous forces slow the movement of particle. (proportional to viscosity and particle velocity)

$$F_{drag} = \gamma_0 v \text{ where } \gamma_0 = 6\pi a\eta$$

Inertia
Inertia is normally negligible in microscopic systems, but it can have an effect in some circumstances.

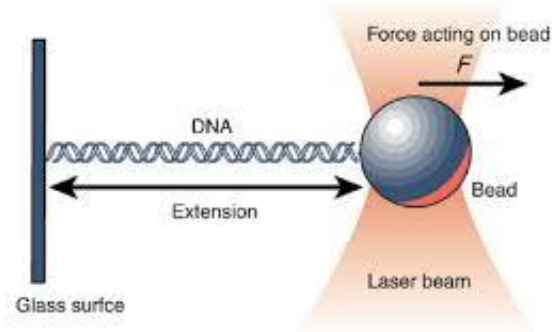
How can we detect forces and tiny displacements?



K. Dholakia and T. Cizmar, *Nature Photonics* **5**, 335–342 (2011)

Trap stiffness (k_{trap}) depends on

- laser power
- size of the trap
- media
- particle size and material



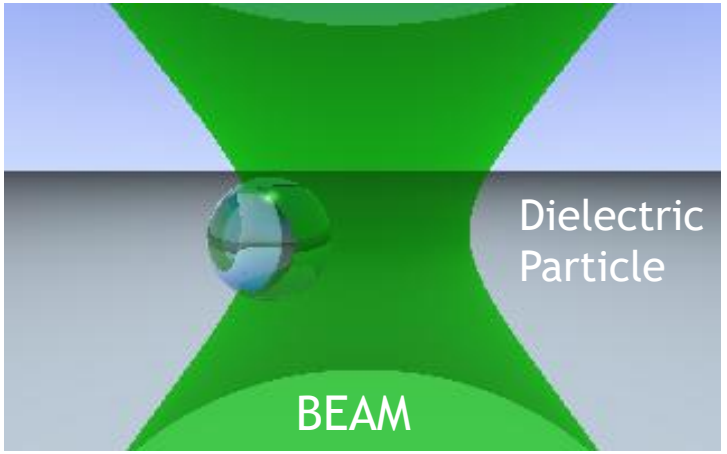
http://umdberg.pbworks.com/

If we know k_{trap}, we can measure precisely small forces.
It can detect forces in the range of 0.1 – 100pN.

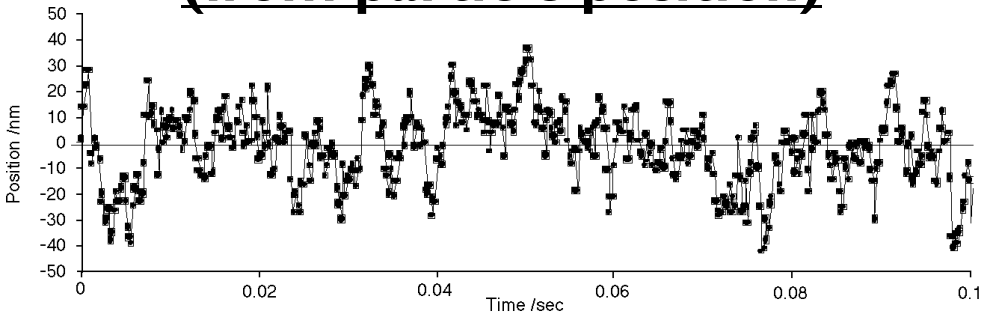
Measuring Trap Stiffness

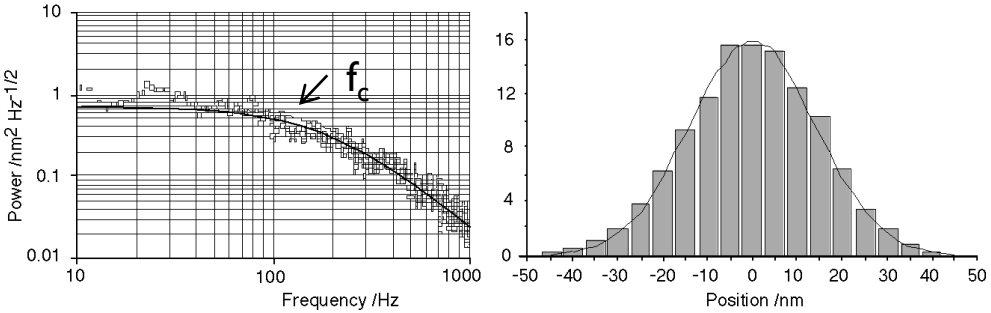
$$m\ddot{x}(t) + \gamma_0 v(t) + \kappa x(t) = \sqrt{2k_B T\gamma_0}\xi(t)$$

Stokes drag force — Optical force



1) Power spectrum method (from particle position)





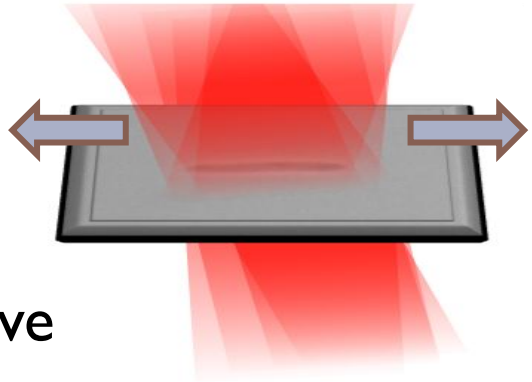
$$PSD(x) = |\tilde{x}(f)|^2 = \frac{k_B T}{\pi^2 \gamma (f_c^2 + f^2)}$$

Trap Stiffness: $k_{trap} = 2\pi\gamma f_c$, where f_c is a corner frequency and $\gamma = 6\pi\eta r$ is a hydrodynamic drag coefficient

Measuring Trap Stiffness

$$m\ddot{x}(t) + \gamma_0 v(t) + \kappa x(t) = \sqrt{2k_B T\gamma_0}\,\xi(t)$$

Stokes drag force — Optical force



2) Drag force method

Stage with sample oscillates in a triangular wave pattern with amplitude A_0 and frequency *f*.

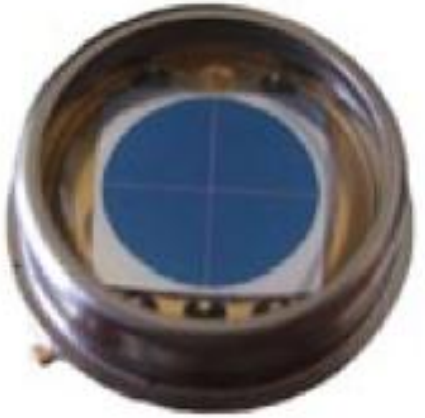
The particle trajectory is $x(t) = \frac{\gamma A_0 f}{2k_{trap}}\left[1 - \exp(-\frac{k_{trap}}{\gamma}t)\right]$

From asymptotic value can estimate $\boldsymbol{k_{trap}}$

Quadrant photo-diodes

Pros.

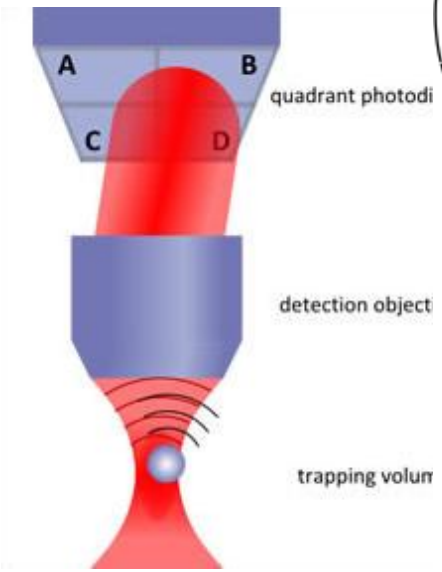
- 10s of MHz bandwidth
- Particle position can be measured to nm resolution



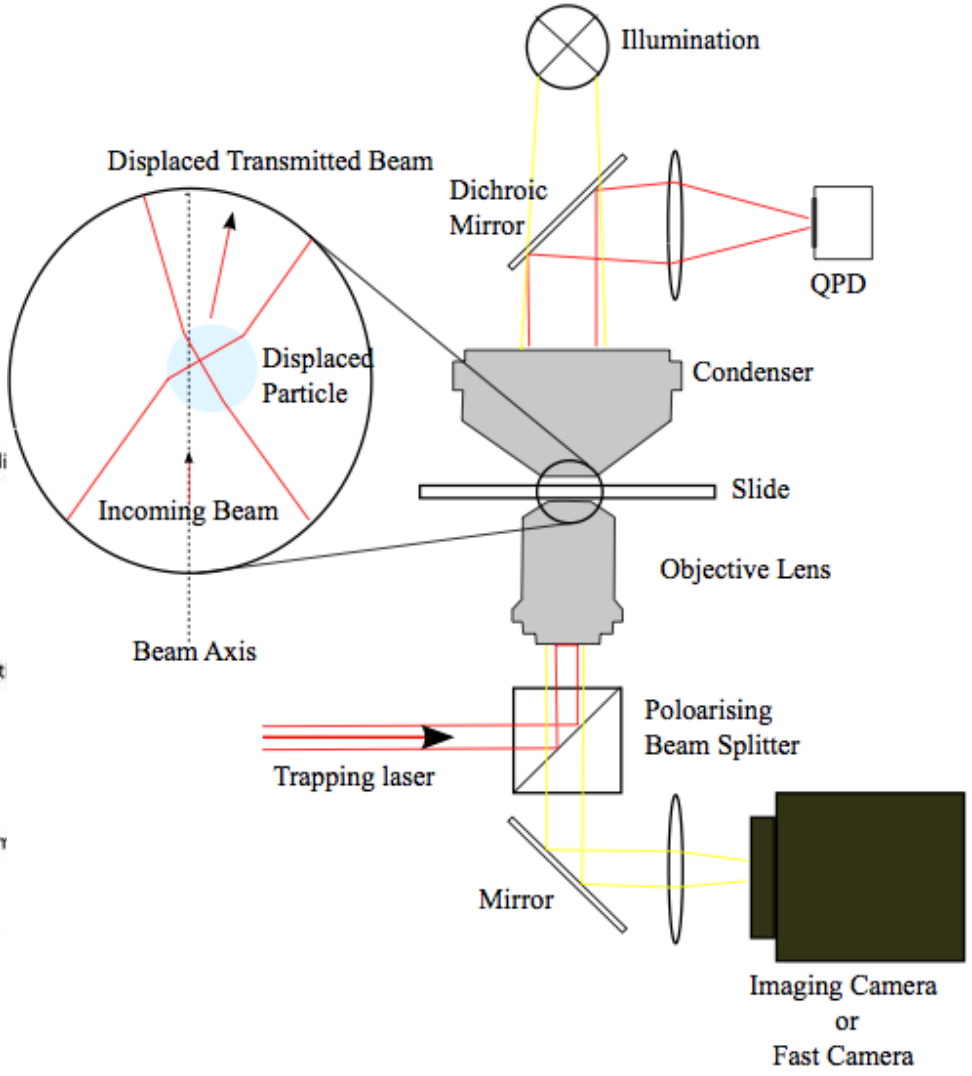
Cons.

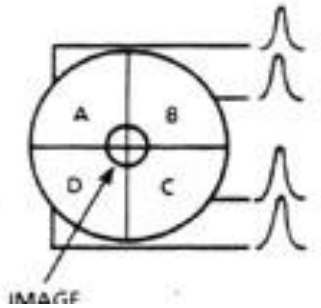
- Particles cannot move far
- Difficult to track more than one particle
- Only sensitive to Gaussian or symmetric shapes
- Hard to get depth information except by calibration

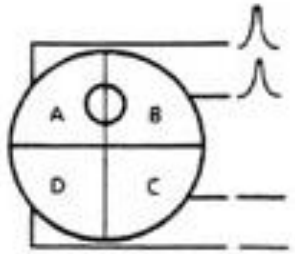
QPD Tracking

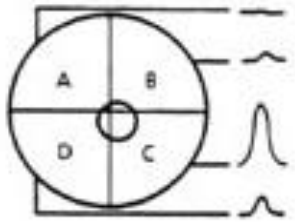


http://usa.jpk.com/force-sensing-optical-tweezers.424.us.html







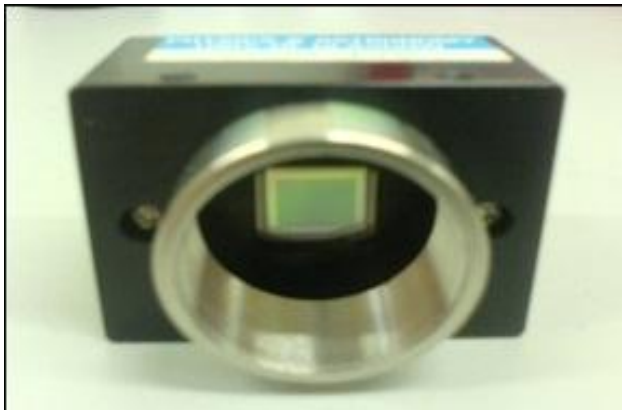


http://pe2bz.philpem.me.uk

CCD sensors (video camera)

Pros.

- Can track many particles
- Can track many shapes
- Can track rotation
- Wide field of view



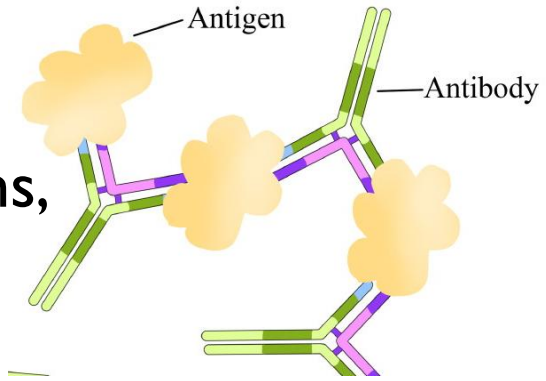
Cons.

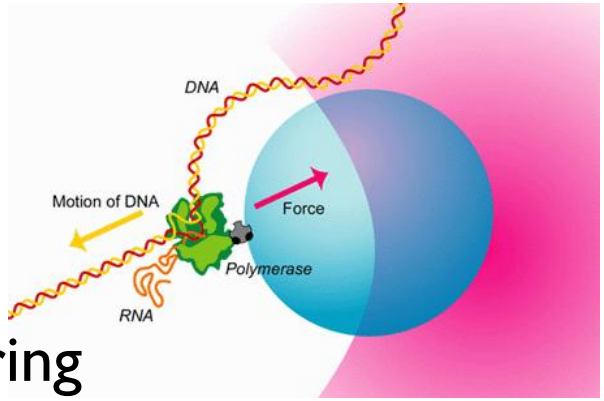
- In general frame rate 10s of KHz (At best MHz)
- Image processing required
- Often significant processing to achieve sub-pixel accuracy (use centroid-finding algorithms)

S. Keen, J. Leach, G. M. Gibson, and M. J. Padgett, *Journal of Optics A* **9**, S264 (2007).

Once calibrated, optical traps can resolve 0.1 to 500pN forces!

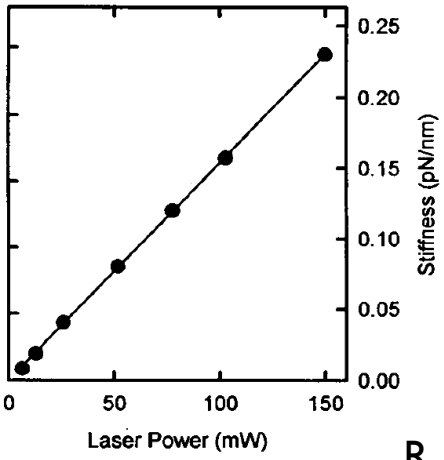
- 1000pN (1nN) – break a covalent bond, pull apart 2 mammalian cells
- 150pN – disrupt antibody-antigen interactions, break an actin filament
- 60pN – unravel the DNA double helix, distort biological membrane
- 30pN – hold DNA process enzymes (helicases, polymersases), overcome push by the bacterial flagellar motors
- 10pN – stop cytoskeletal motor proteins (myosin, kinesin, …), which are powering cell motility and muscle contraction

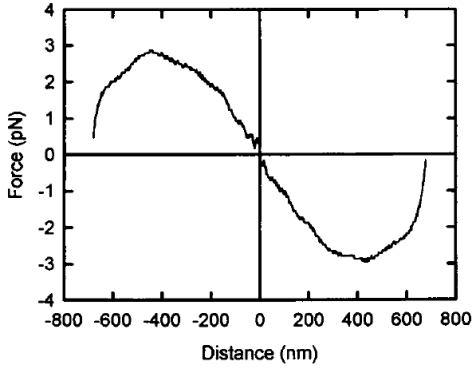


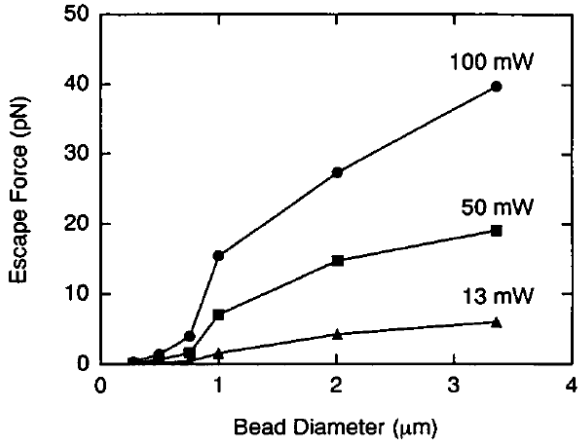


Optical Forces

- The maximum force and stiffness (restoring force per unit displacement from equilibrium) of optical tweezers depend on laser power and particle size.







R. M. Simmons, J. T. Finer, S. Chu, J. A. Spudich, *Biophys. J.* **70,** 1813 (1996).

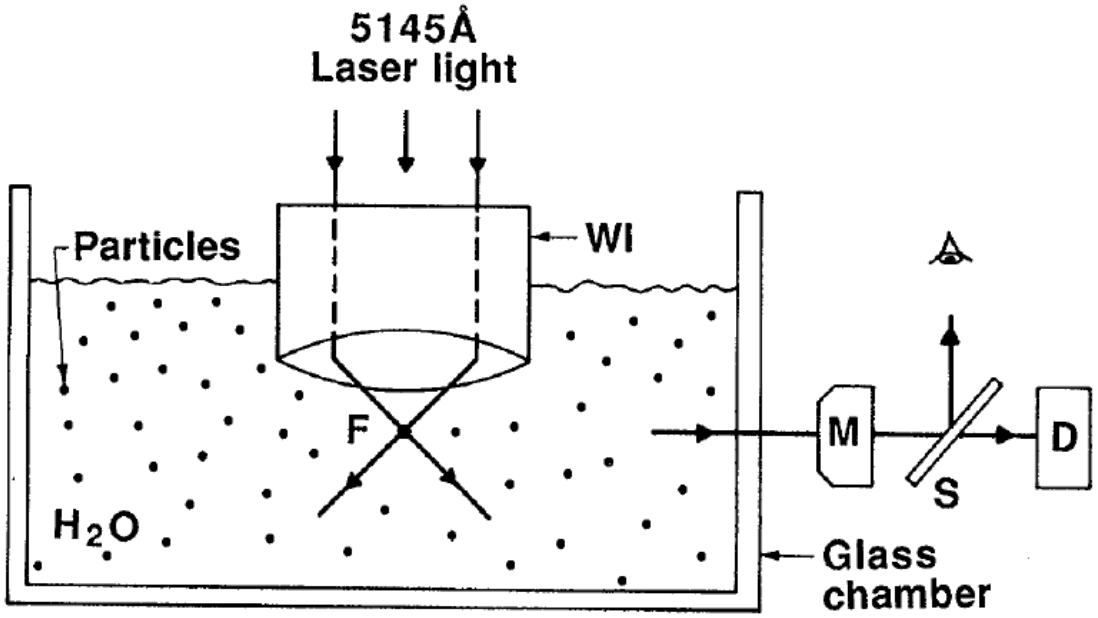
- Trapping forces are on the order of 1-2 pN per 10 mW of laser power in the specimen plane (for ~1μm bead).
- 100mW gives optical trap stiffness of ~0.08 pN/nm and max force ~20pN. (sufficient to manipulate cells, push/pull single molecules)

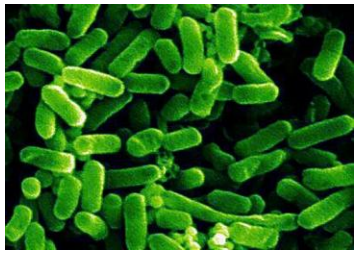
K.C. Neuman & S.M. Block, *Rev. Sci. Instrum.* **75**, 2787 (2004)

First biological trapping

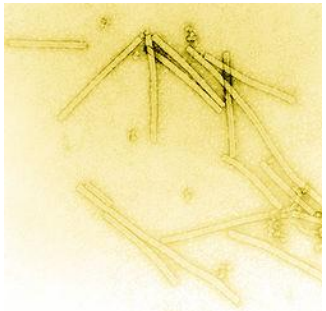
- 1987 - A. Ashkin optically trapped and manipulate *E.Coli* bacteria (~2μm) and tobacco mosaic virus (~300nm long) with 120mW argon laser.

A. Ashkin & J. M. Dziedzic, *Science* **235**, 1517 (1987)





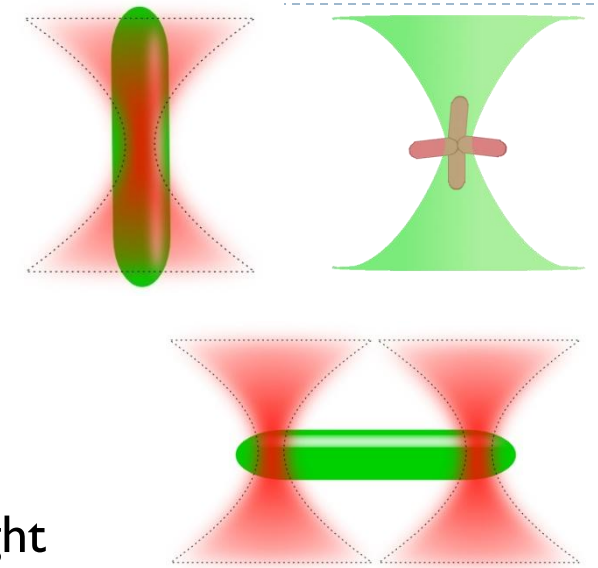
E. Coli



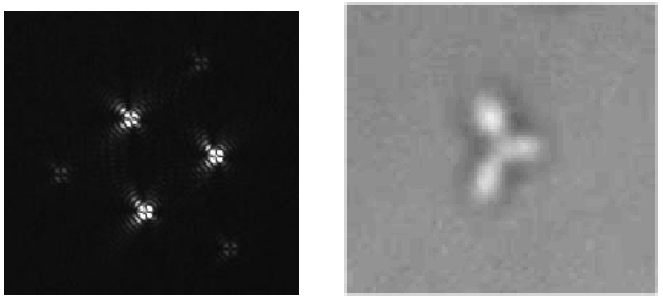
tobacco mosaic virus

Multiple traps to fit complex shape

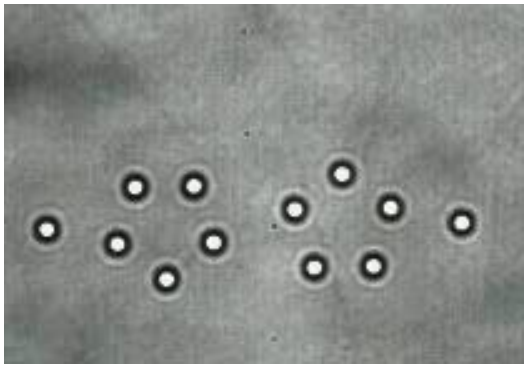
- Bacteria prefer to be aligned along the axis of a trapping beam.
- Dual optical tweezers (a beam splits in two and focuses into plane)
- Time- shared optical tweezers (a beam quickly ran over multiple points)
- Holographic optical tweezers (use spatial light modulator to create multiple "clusters" of traps)



3 spots applied to a mutant *S.Meliloti*



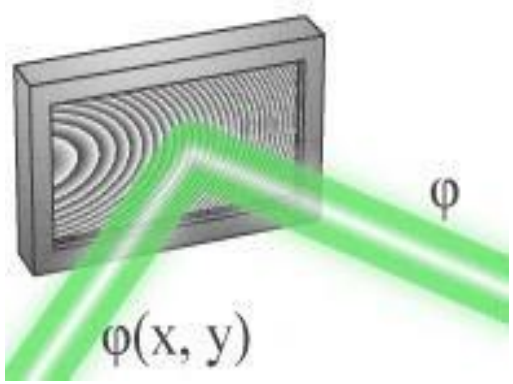
Dancing beads



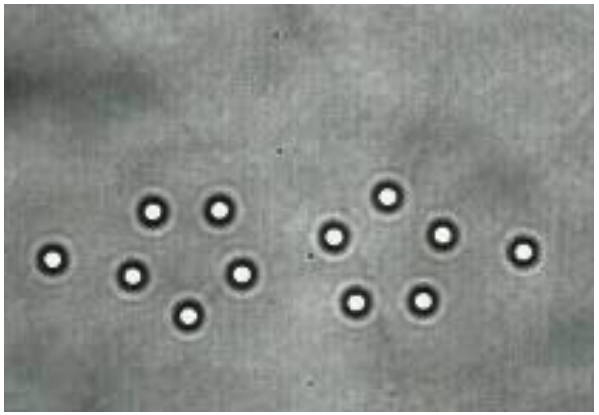
Y. Roichman and D. G. Grier, 2005

Spatial light modulator (SLM)

- When light hits and reflects from SLM, it's phase changes.



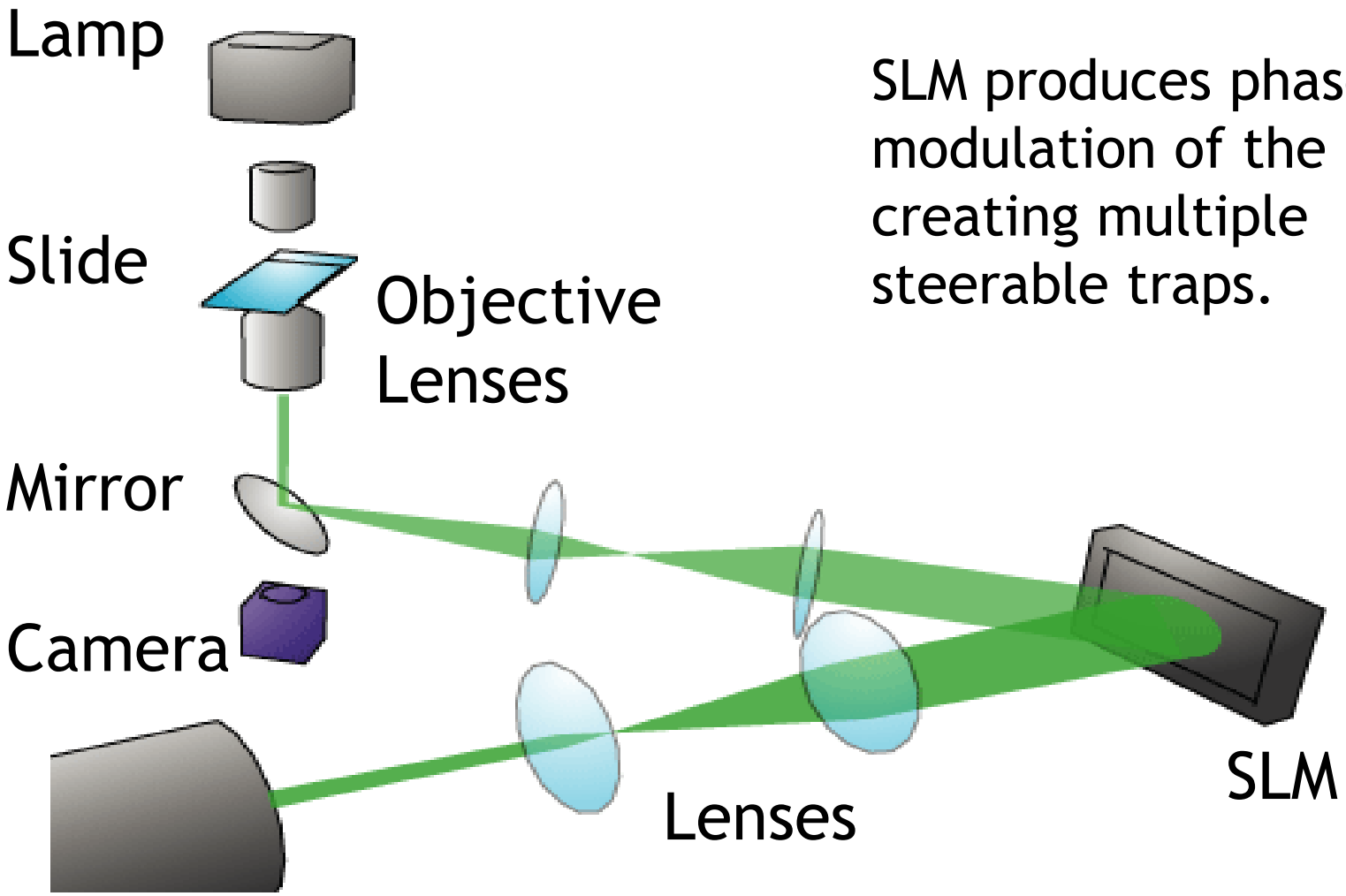
- Using computer generated holograms, we can alter the phase of the light in real time, splitting one beam (i.e. one trap) into many beams (many traps!)



J. Liesener, et al., *Opt. Commun.*, *185*, 77 (2000)

Y. Roichman and D. G. Grier, 2005

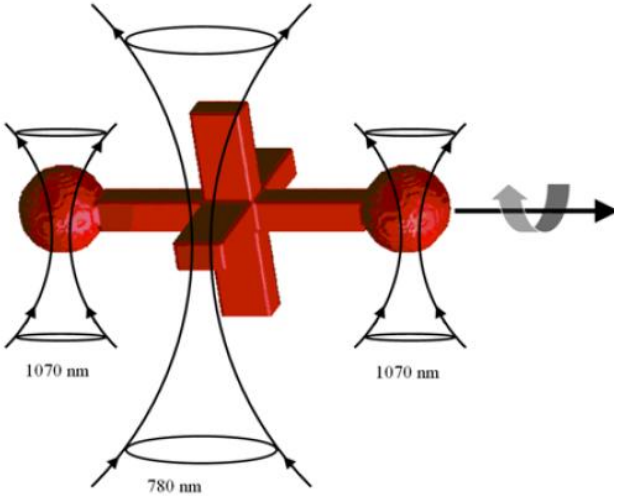
Holographic Optical Tweezers Setup

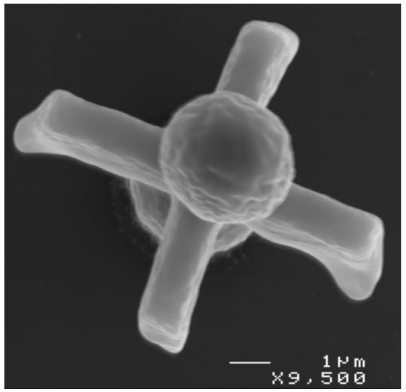


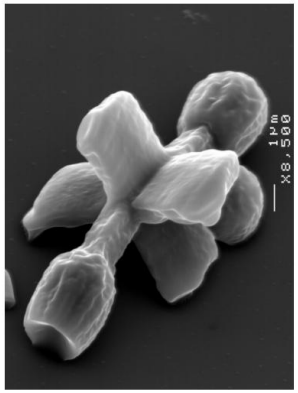
SLM produces phase modulation of the beam creating multiple steerable traps.

Control of micro-structure

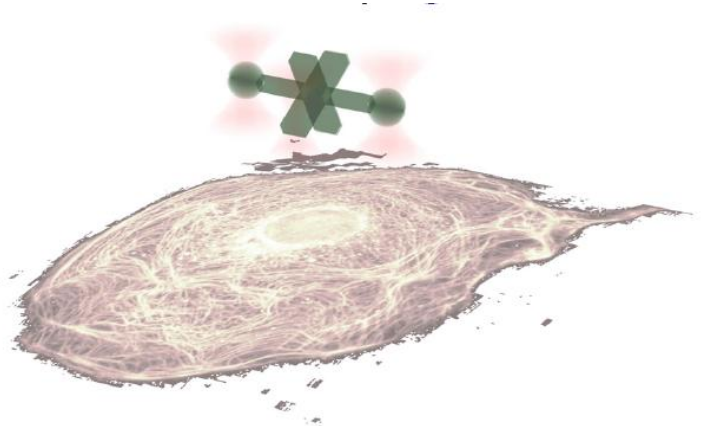
- Optically driven 5um paddle wheel







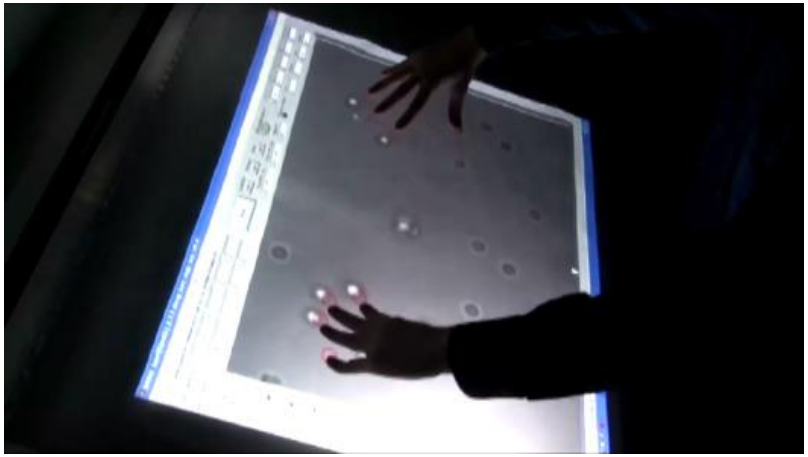
- This probe was used control fluid flow over cells.
- Cellular responses were measured in florescence microscopy.



T. Asavei, et al., *New J. Phys.* ***15,*** *063016* (2013)

Remote optical tweezers: Multi-touch console

- Multi-touch interface enables the independent but simultaneous interactive control of numerous optical traps by multiple users.



J.A. Grieve, *et al.* Opt Express, 17, 3595 (2009)

The Multi-touch console in action: Users move SiO_2 spheres

Remote optical tweezers: Multi-touch interface

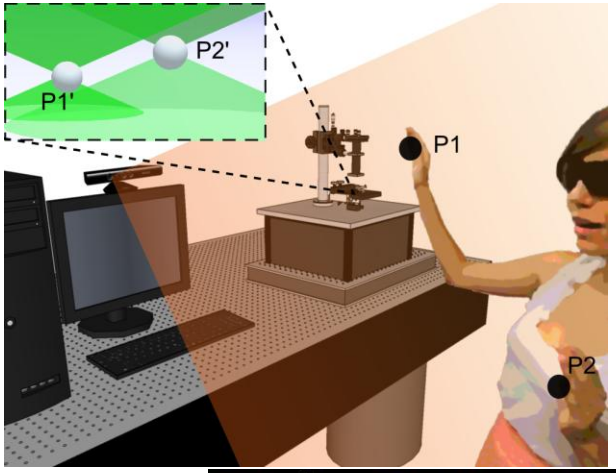
- Multi-touch interface implemented on an Apple iPad for multi-particle manipulation.

- This interface connects to the tweezers system hardware over a wireless network, allowing it to function as a remote monitor and control device.

R.W. Bowman, J Opt, 13, 44002 (2011)

iTweezers: optical micromanipulation in action

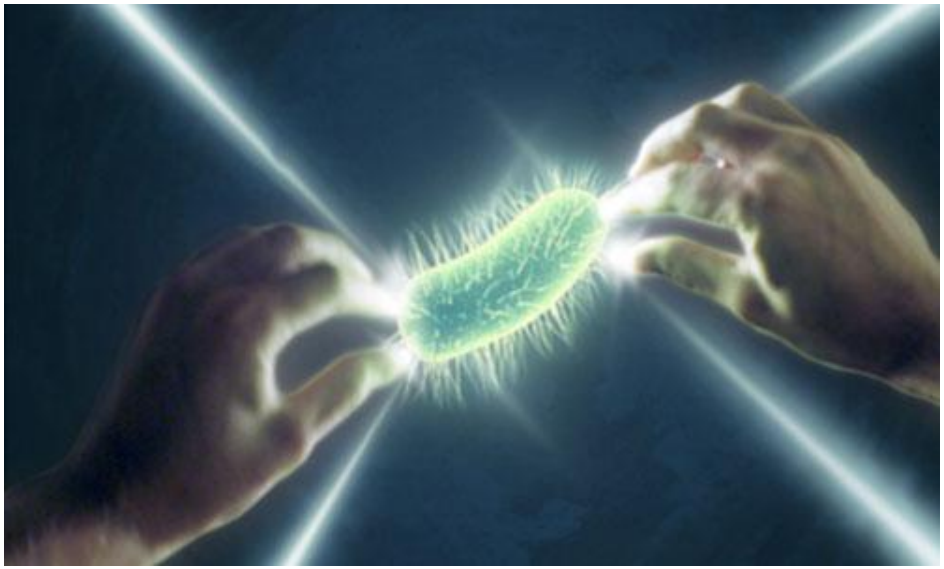
Remote optical tweezers: Kinect optical tweezers



- Utilize gaming technology (Microsoft Kinect sensor bar) to facilitate user interaction
- Use to generate arbitrary optical force fields and control optically trapped particles

L. Shaw, D. Preece, H. Rubinsztein-Dunlop, J Opt, 15, 75303 (2013)

What do we need to know to trap microorganisms?

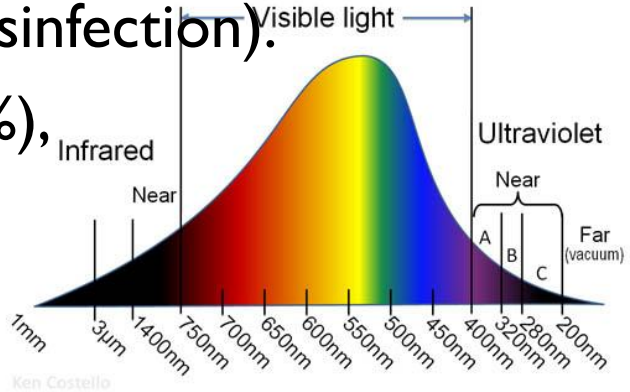


University of Glasgow: Miles Padgett group

Phototoxicity and photodamge

- Very high flux of laser power (10^7 W/cm^2) might cause cell damage or death of live cells ("opticution").
- High laser can break covalent bond, cause photochemical reaction, heating.
- Biological molecules are easily damaged by heating above 45C.

- Infrared light cause damage to bacteria and cultured mammalian cells from oxygen-dependent photochemical reactions.
- Ultraviolet light damages DNA (used for disinfection).
- If the material is transparent (absorbs <10%), then little heating occurs.



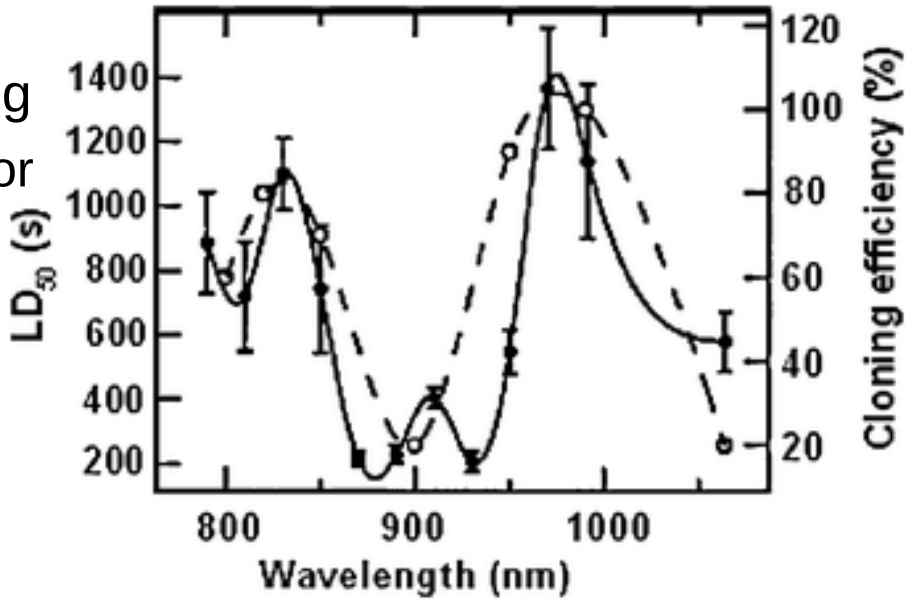
Laser wavelength for biological samples

- For trapping living microorganisms need to use 750-1200nm IR laser light to minimize damage & heating

  (due to absorption by either protein or water in cell)

- The most harmful wavelength are 850-950nm, safest are on a side

- Most common trapping wavelengths: 1064nm and 830nm



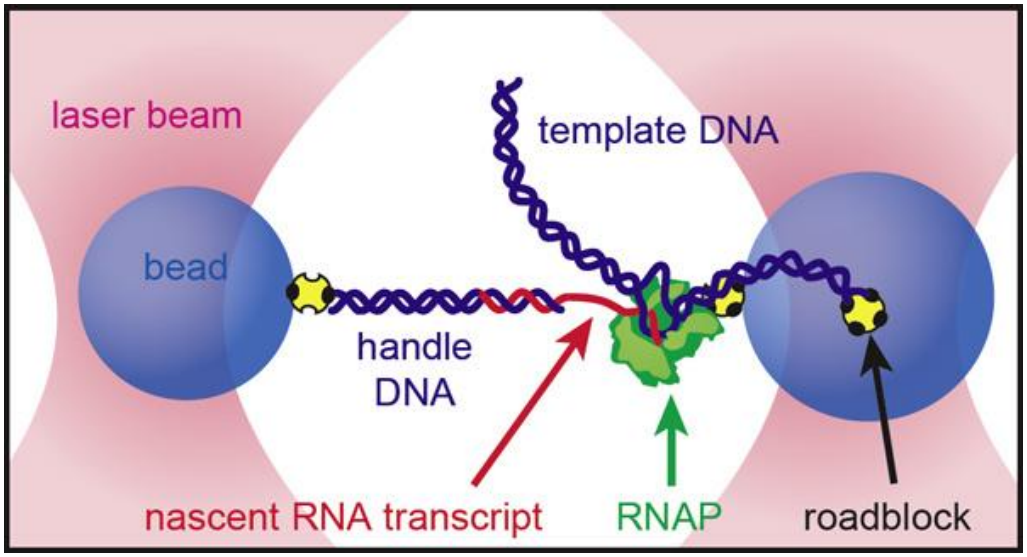
Relationship between wavelength and cell photo damage for *E. coli* (solid line and left axis) and chinese hamster ovary cells (dashed line and right axis). The higher the LD_{50} and % cloning the less damage the laser causes for a given wave number.

K. Neuman, *Biophys. J.* **77**, 2856 (1999)

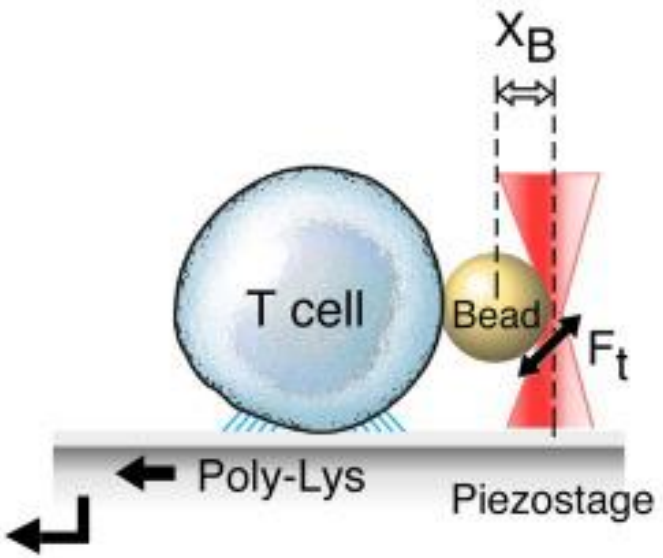
Many biological samples cannot be directly trapped due to size, shape, and adherent properties.

Beads to trap biological samples

Attach a micro or nano-particle onto the sample to use as a trapping "anchor" to hold onto the sample with



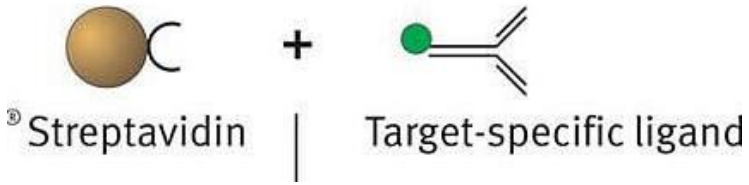
S. Block website, Stanford University

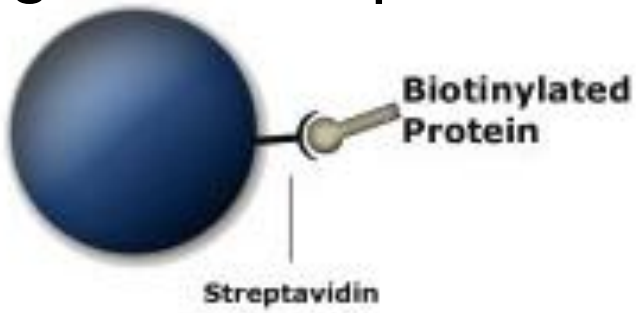


S.T. Kim, *Front. Immunol.* 3(76), 1(2012)

Attaching sample to anchor bead

- DNA, proteins, and lipid membranes are smaller than 25nm in diameter.
- How to guarantee specific sample attachment to bead?
- The bead surface treated by specific coupling chemistries or by nonspecific adsorption so binding occur for specific type of bond (target-specific ligand).

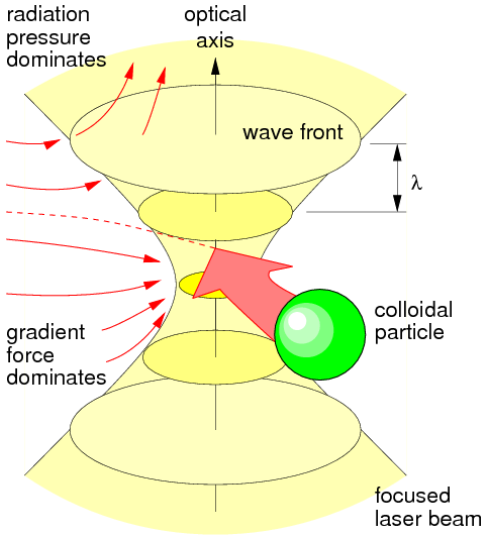




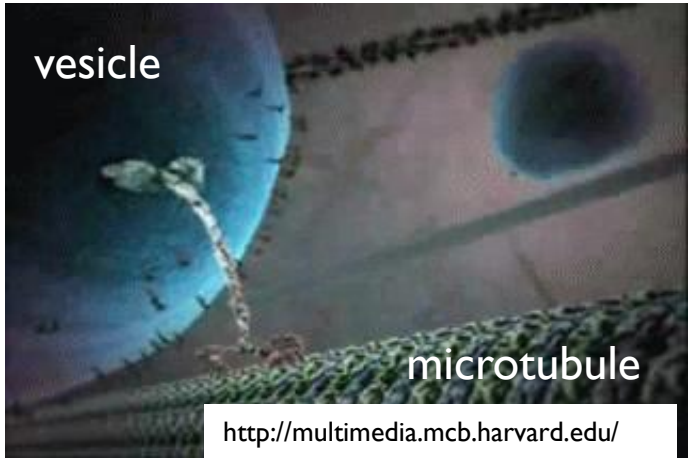
- Usually choose 1-10 molecules per bead to achieve individual sample response per bead. The incident of binding events is governed by Poissonian statistics.

Optical Tweezers Summary:

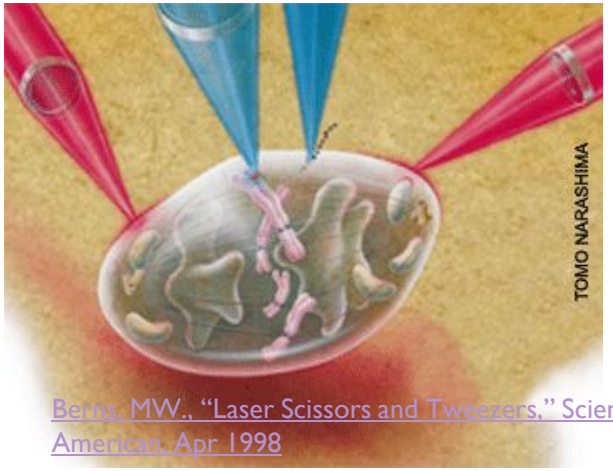
- Optical Tweezers use radiation pressure from a focused laser beam to manipulate microscopic objects as small as a single atom.
- It can trap and control objects in size 10nm-100mm, detect and apply forces in the range of 0.1 – 100pN, measure displacements in nm range.
- Widely used in biology and medicine to understand physical properties and responds of microorganisms.



With optical tweezers we can do …

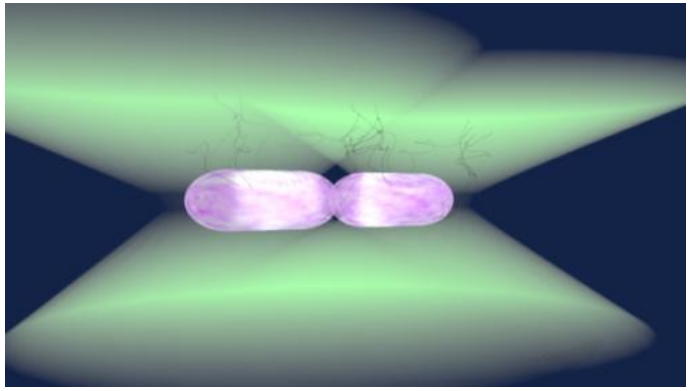


Force estimation for Kinesin motors and other molecular motors

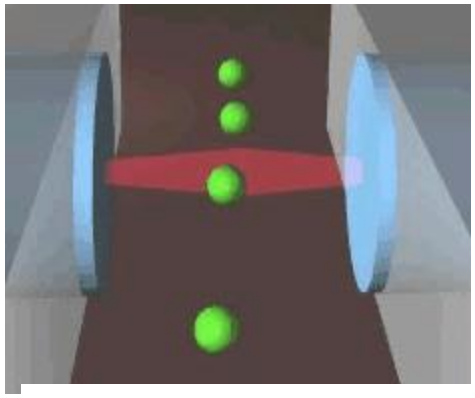


Berns, MW., "Laser Scissors and Tweezers," Scientific American, Apr 1998

Microsurgery and manipulation of cells
DNA injection and/or incorporation



Physical properties of microorganism
Bacteria- drug interaction

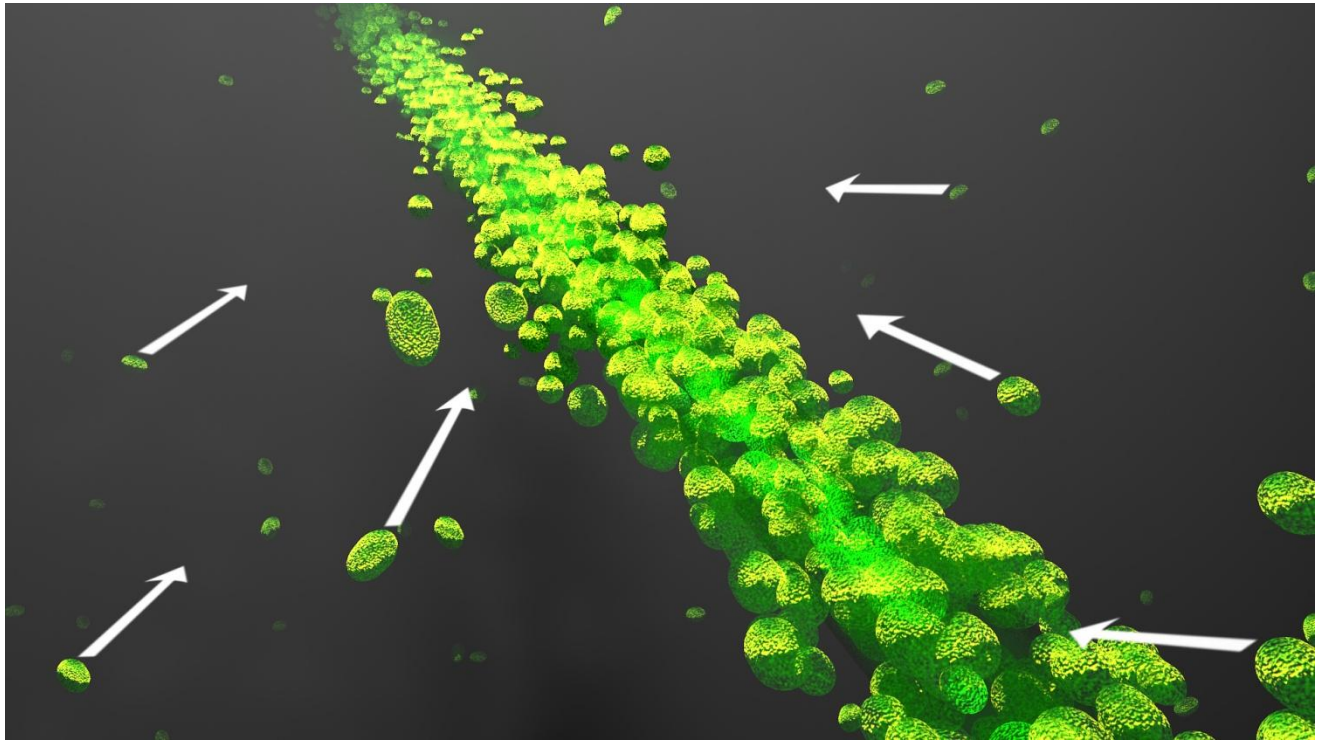


J. Guck et al, *Biophys. J.* 88(5):3689(2005)

Detect cancer cell by stretching

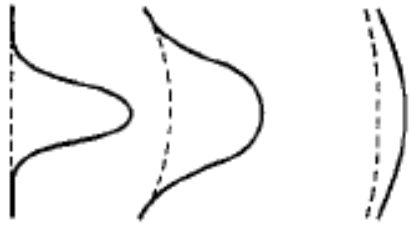
Light-induced bio-fiber

Bacteria are drawn inward and forward by a light beam, creating a waveguide



A. Bezryadina, T. Hansson, R. Gautam, B. Wetzel, G. Siggins, A. Kalmbach, J. Lamstein, D. Gallardo, E. J. Carpenter, A. Ichimura, R. Morandotti & Z. Chen, *Phys. Rev. Lett.* **119**, 058101 (2017) "Nonlinear self-action of light through biological suspensions"

Soliton (self-trapped beam)

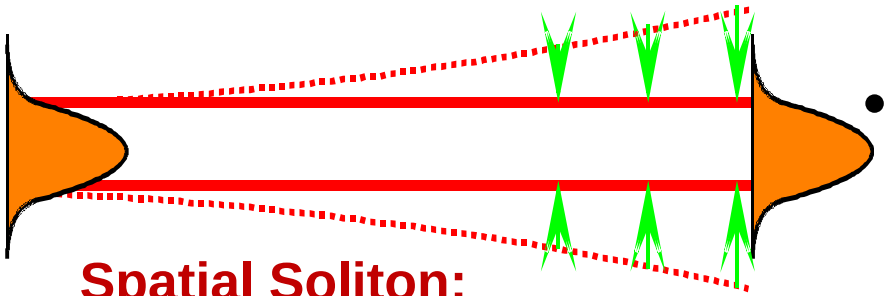


Diffraction

In a linear system

Self-focusing

Due to nonlinear effects



Spatial Soliton:

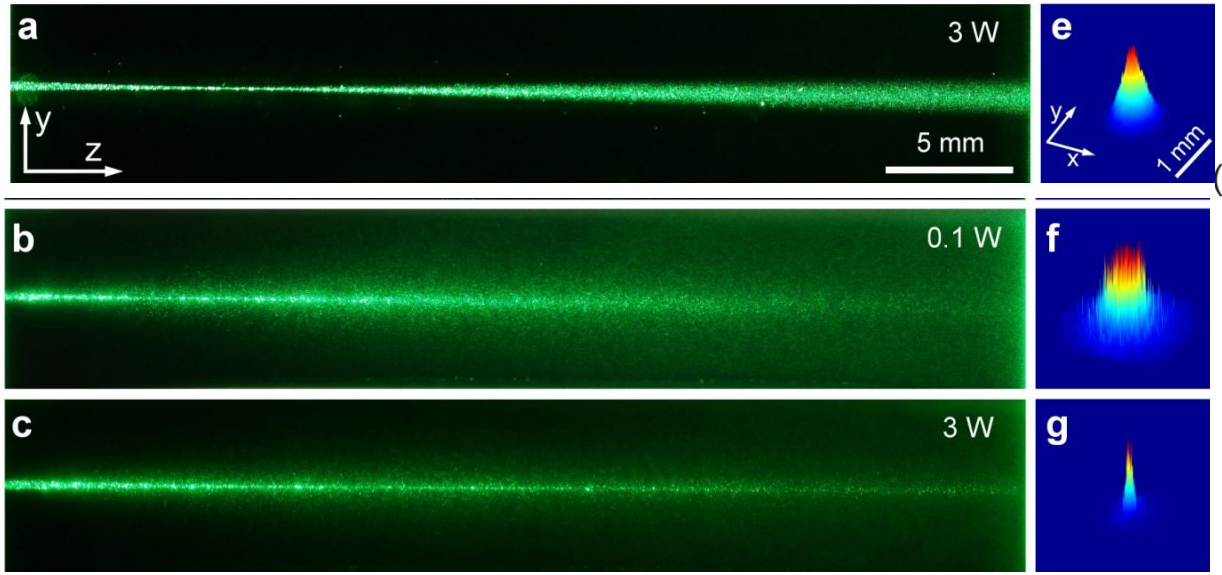
- Normally light diffracts in linear regime
- At nonlinear regime self focusing can occur
- When they balance a "soliton" is formed

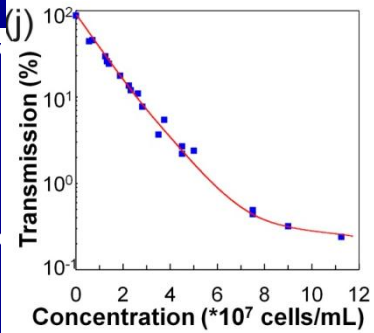
Nonlinear self-focusing in bacterial suspension

Synechococcus sp. - Small ovoid immotile unicellular cyanobacteria (2 μm), very common marine bacteria

ASN media (no bacteria)

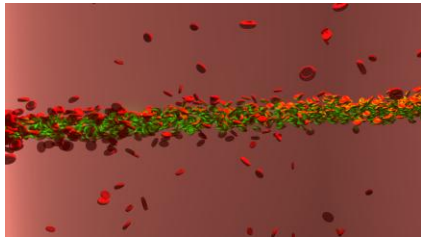
Synechococcus in media





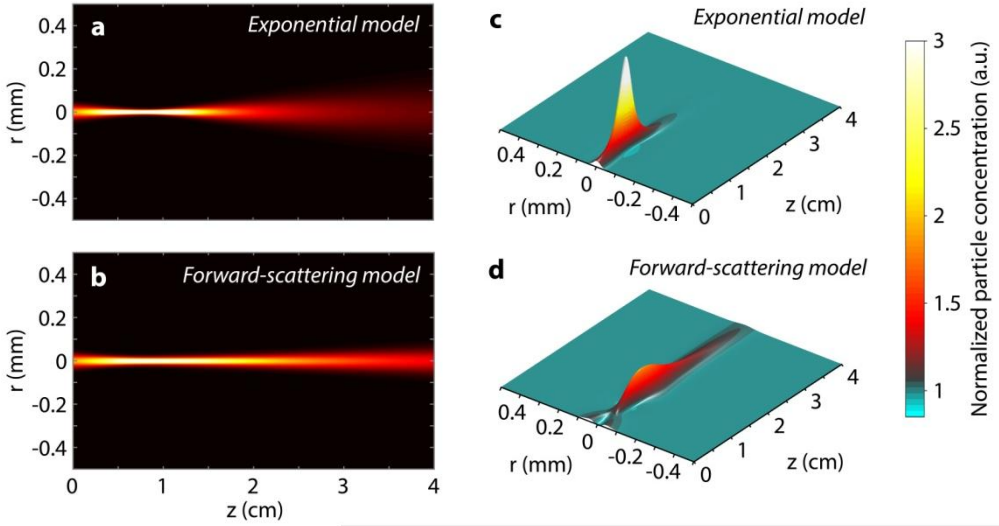
Viability assessment tests show great health of cells (the mortality rate in the light-exposed groups of bacteria was only 0.1% greater than in a control group)

Similar self-focusing effect happens in other bacteria and in blood cells



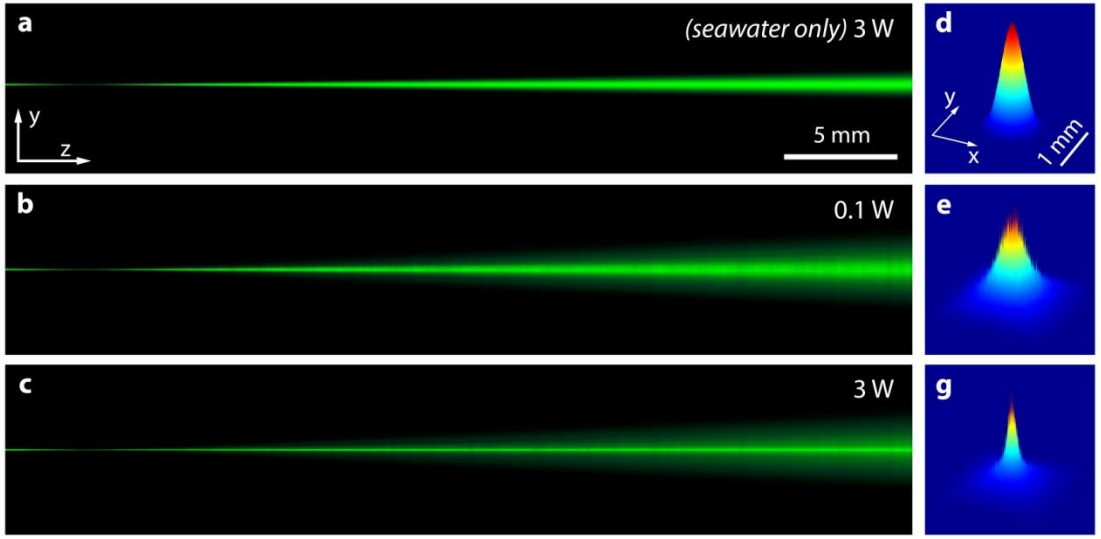
Theoretical models:

Nonlinear beam dynamics in biological suspensions

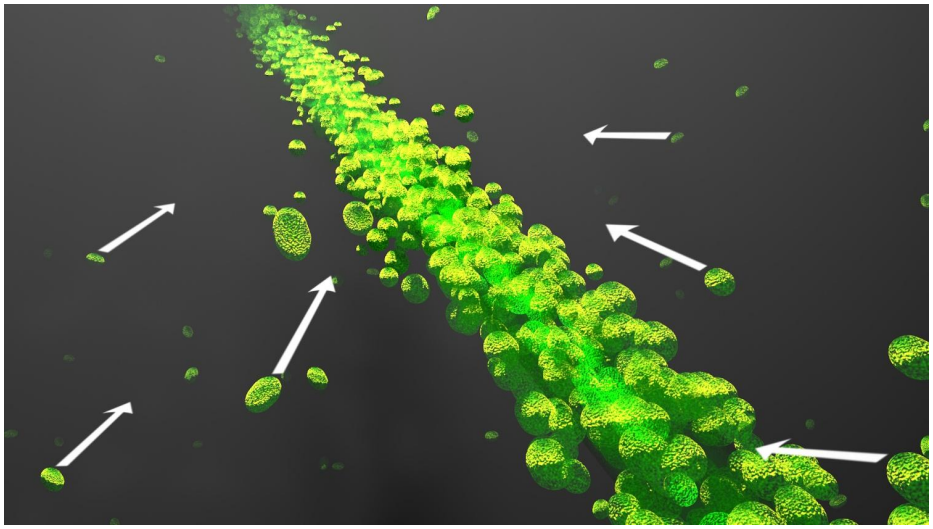


Exponential model :
an optical gradient force only

Forward-scattering model:
optical gradient along with a forward-scattering force



Biological waveguides -future



- Construct optical conduits in biological suspensions, which can transmit energy or information
- Could be used for imaging through biological fluids (such as blood, amniotic fluid, etc) or for non-invasive medical diagnostics
- Create biochips: grow and fix optical waveguides and biological microchips based on our recent studies.

Thank you!

OSA Foundation

CENTRO DE INVESTIGACIONES EN OPTICA, A.C.