



*The Abdus Salam
International Centre for Theoretical Physics*



1932-2

Winter College on Micro and Nano Photonics for Life Sciences

11 - 22 February 2008

Light in action I: Optical tweezers principles

Dan COJOC
*CNR-INFN Laboratorio Nazionale TASC
Trieste, Italy*

Light in Action I

Optical Tweezers Principles

Dan Cojoc

CNR-Instituto Nazionale per la Fisica della Materia
Laboratorio Nazionale TASC Trieste, Italy



The interest for Optical Trapping and Manipulation is very high !

Even if OTM is a relatively young technique (20 years old)

05/02/2008 Key words search: **optical trapping manipulation**
result → **357000** web documents

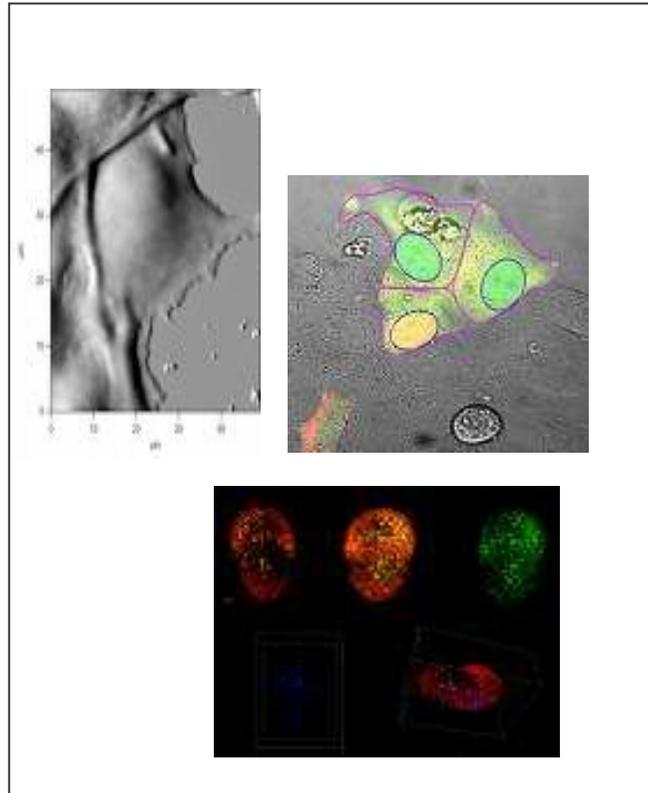


The screenshot shows a Google search interface. The search bar contains the text "optical trapping manipulation", which is circled in red. Below the search bar, the results are displayed as "Risultati 1 - 10 su circa 357.000 per optical tweezers manipulation laser trapping. (0,31 secondi)". A red arrow points from the search bar area down to a red-bordered box containing the title of a specific article.

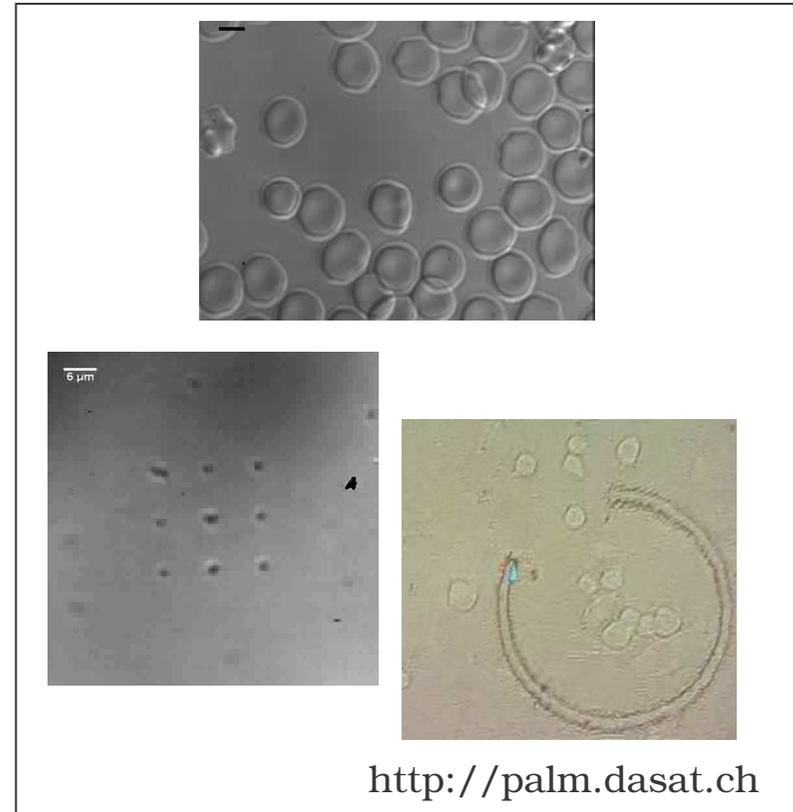
Optical trapping and manipulation of single cells using infrared laser beams

A. Ashkin J. M. Dziedzic & T. Yamane *Nature* **330**, 769 - 771 (1987).

**The goal of the lectures Light in Action I and II
is to show some arguments for this interest**



visualization



manipulation



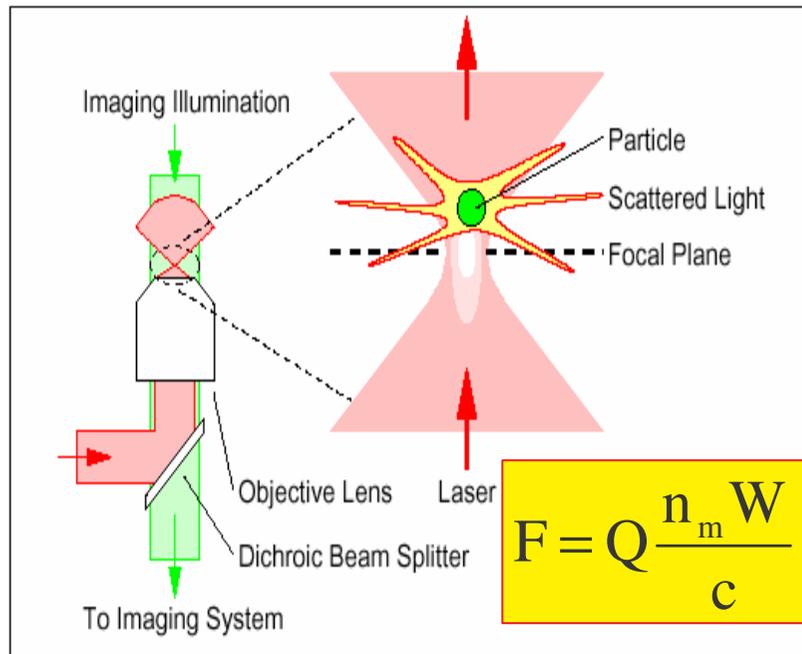
Optical Tweezers, principles: a tribute to Arthur Ashkin

OUTLINE

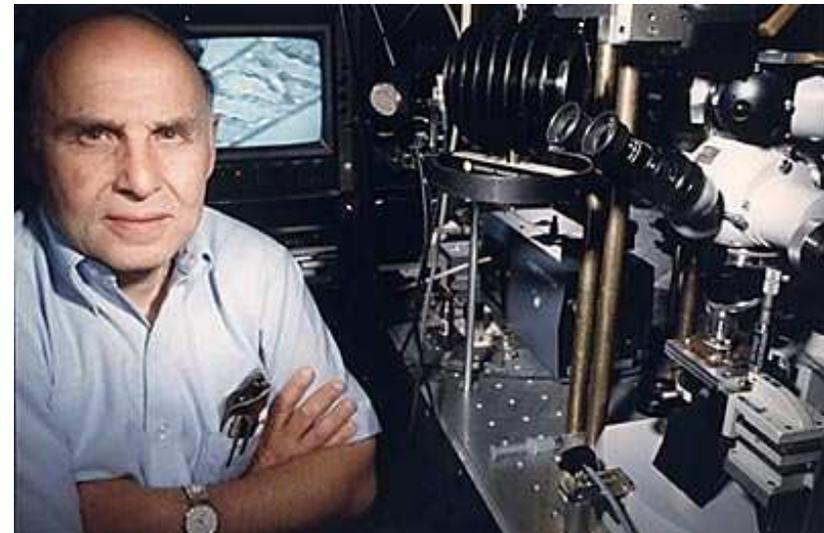
- **Optical trapping and manipulation**
 - first demonstration of 2d and 3D trapping of micro-particles using Gaussian beams through microscope objectives
 - some history about the work done by Ashkin and Chu at Bell Labs back in '80
- **Optical forces and trapping regimes**
- **Trapping/force calibration**
- **Characteristics of optical traps**
- **Optical manipulation examples**

What is an optical tweezers ?

A single-beam gradient force trap obtained by tightly focusing a cw laser beam through a high NA objective - **3D trap**



Arthur Ashkin 1986



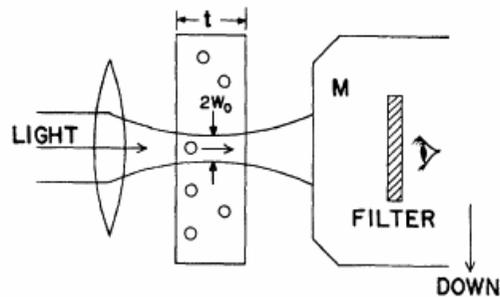
- F** – trapping force
- Q** – dimensionless efficiency coefficient
- W** – power of the laser beam
- n_m** – refractive index of the medium
- c** – light speed

www.bell-labs.com/user/feature/archives/ashkin/

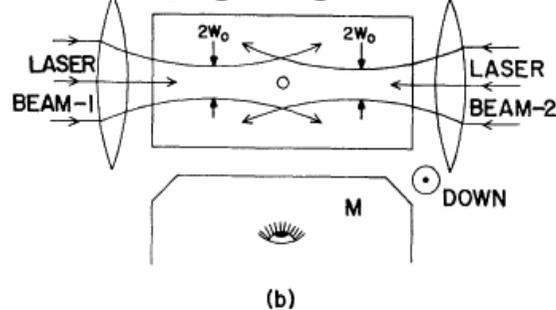
A. Ashkin, *et al Optics Letters* **11** 288 1986

Trapping with Gaussian laser beams

2D trapping with single beam

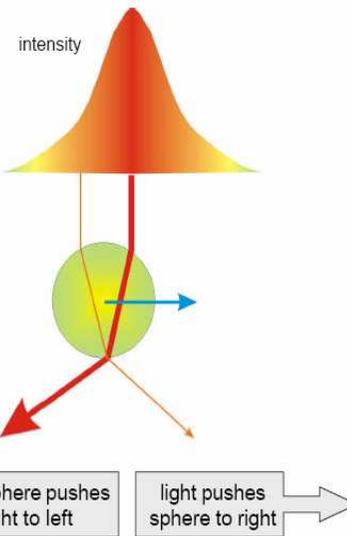


3D trapping with counter propagating beams



Low Numerical aperture ($NA < 0.8$)
 Long Working Distance ($WD > 1 \text{ mm}$)
 Limited resolution: $r = 1.2\lambda/NA > 1.5 \lambda$

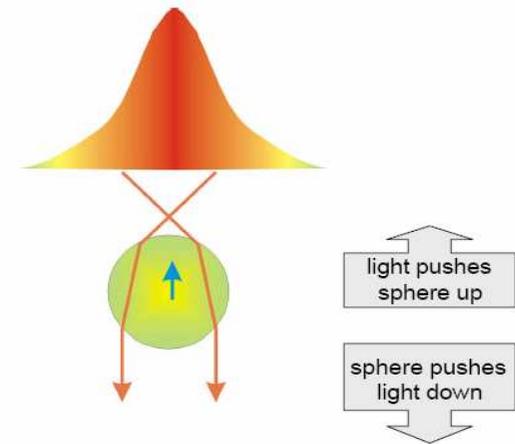
A. Ashkin *et al*, *PRL* **24** 156 **1970**



relative index n :

$$n = n_p / n_m > 1$$

3D trapping with single beam



Sphere moves along gradient of light intensity !

High NA ($NA > 1$)
 Short WD ($WD < 1 \text{ mm}$)
 High resolution: $r < \lambda$

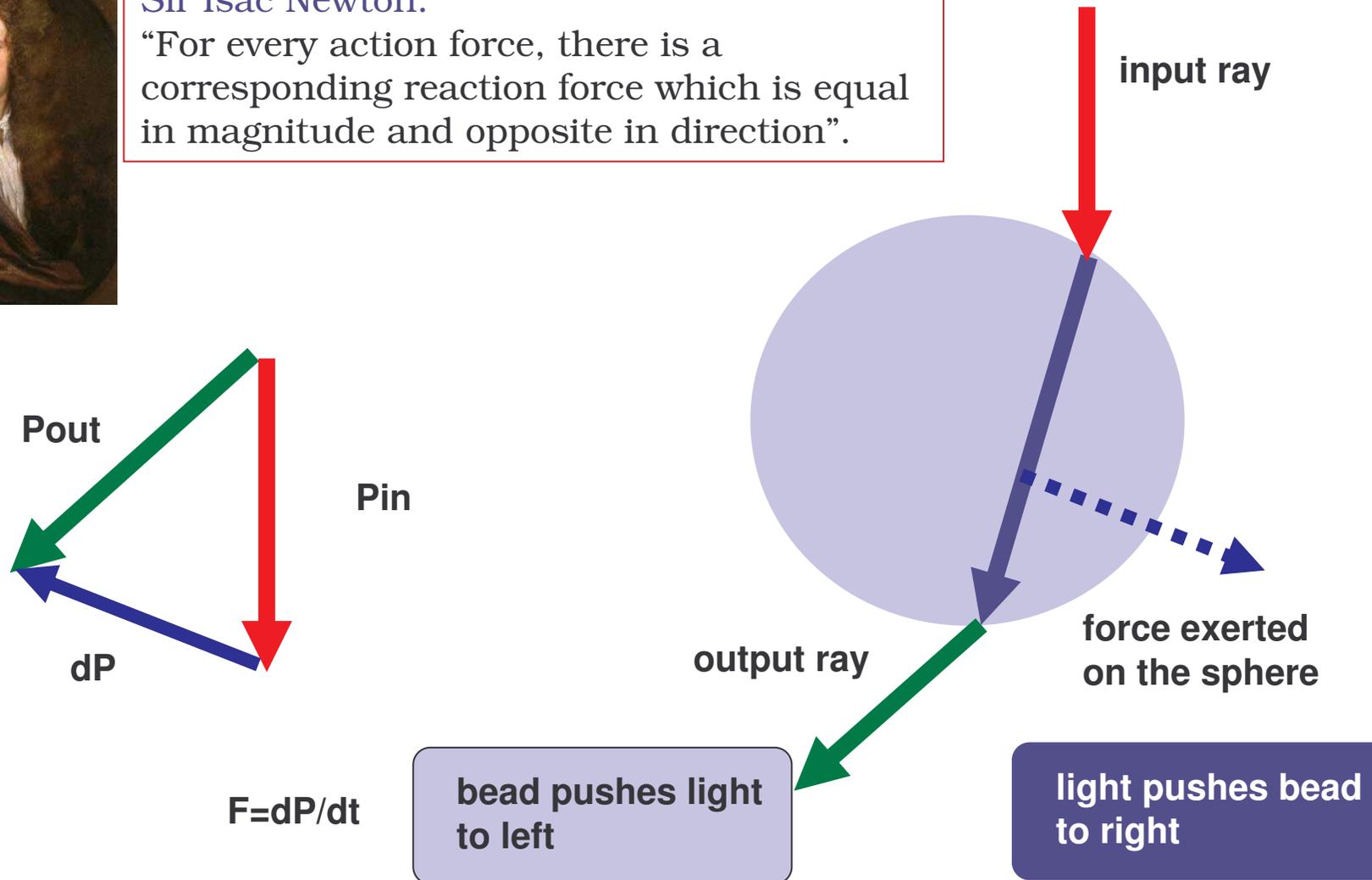
A. Ashkin *et al*, *Opt.Lett.* **11** 288 **1986**

Ray optics explanation of optical trapping

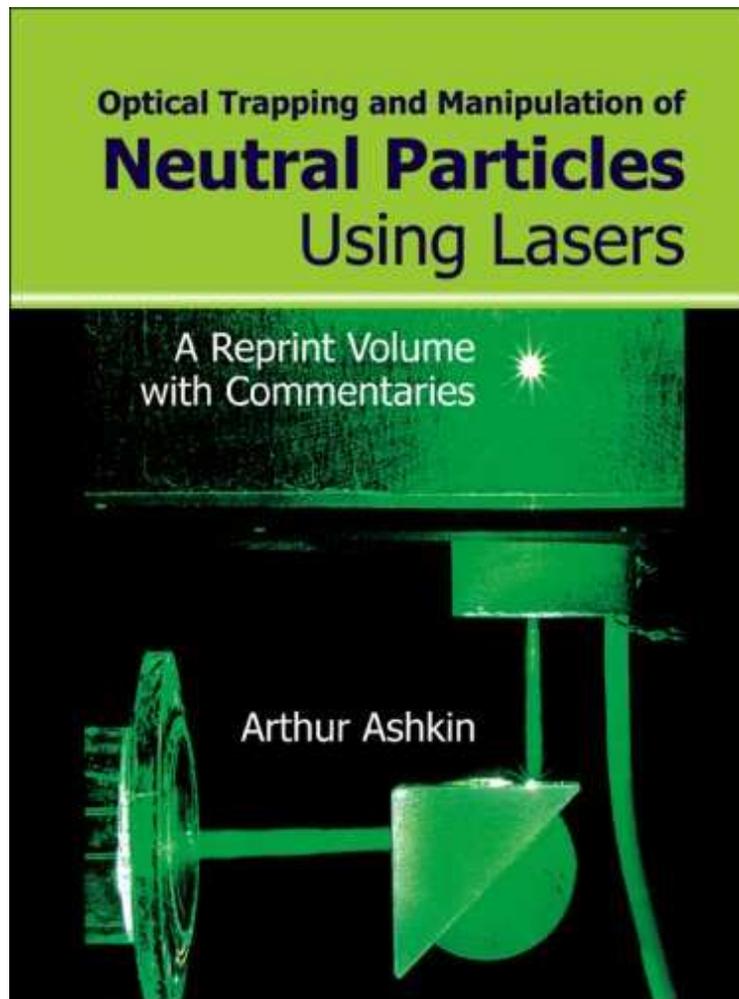


Sir Isac Newton:

“For every action force, there is a corresponding reaction force which is equal in magnitude and opposite in direction”.



Book by A . Ashkin, 2007



The Nobel Prize in Physics 1997

"for development of methods to cool and trap atoms with laser light"



Steven Chu

1/3 of the prize

USA

Stanford University
Stanford, CA, USA

b. 1948



**Claude
Cohen-Tannoudji**

1/3 of the prize

France

Collège de France; École
Normale Supérieure
Paris, France

b. 1933
(in Constantine, Algeria)



William D. Phillips

1/3 of the prize

USA

National Institute of
Standards and
Technology
Gaithersburg, MD, USA

b. 1948

<http://nobelprize.org>

S. Chu's Nobel Prize Lecture:
"The manipulation of neutral particles"

Interview to A. Ashkin

BLN: Tell me about how you started work on optical trapping, leading up to when you and Steve Chu and colleagues did the now-famous experiments?

Ashkin: I started in 1970 with my discovery that I could optically trap small latex spheres. And I went on to propose that we could do the same thing with atoms.

BLN: How did you start thinking about it in the first place?

Ashkin: Well, I had been interested in radiation pressure for a long time.

....

Then, I replaced the glass wall with another opposite beam to hold the particles in place with just light. I tried it and it worked. This was the first optical trap. It turned out to be a pretty important discovery. It led to Steve's Nobel Prize and, I believe, it will lead to two more Nobel Prizes.

.....

BLN: So that was the famous experiment with "optical molasses" and traps that you and Chu and colleagues did in 1985?

Ashkin: The initial plan was to combine slowing of atoms, cooling them and trapping them in a single experiment. Steve argued for a simpler first step. Steve was very enthusiastic. He wanted to first study the three-dimensional cooling scheme, using a technique proposed by T.W. Hansch and A. L. Schawlow in 1975. This is now referred to as "optical molasses." This was wise because molasses cooling succeeded so well that it affected our subsequent choice of traps. We used the now-famous optical tweezers trap. Steve was a very hands-on person, a great experimentalist. He did some absolutely brilliant experiments. I always thought in those days that he would one day get the Nobel Prize. I am extremely happy for him.

From the Bell Lab News **interview to A. Ashkin** in 1998 (probably)
www.bell-labs.com/user/feature/archives/ashkin/

Steven Chu's Nobel Prize lecture

Nobel Prize in Physics 1997

THE MANIPULATION OF NEUTRAL PARTICLES

Nobel Lecture, December 8, 1997

by

p. 122-158

STEVEN CHU

MOVING TO HOLMDEL AND WARMING UP TO LASER COOLING

TAKING THE PLUNGE INTO THE COLD

ON TO OPTICAL TRAPPING

OPTICAL MOLASSES REVISITED

APPLICATIONS OF LASER COOLING AND TRAPPING

OTHER APPLICATIONS IN ATOMIC PHYSICS

APPLICATIONS IN BIOLOGY AND POLYMER SCIENCE

CLOSING REMARKS

References (137)

http://nobelprize.org/nobel_prizes/physics/laureates/1997/chu-lecture.html

What might happen when the Intelligence joints the Enthusiasms

MOVING TO HOLMDEL AND WARMING UP TO LASER COOLING

My entry into the field of laser cooling and trapping was stimulated by my move from Murray Hill, New Jersey, to head the Quantum Electronics Research Department at the Holmdel branch in the fall of 1983. During conversations with Art Ashkin, an office neighbor at Holmdel, I began to learn about his dream to trap atoms with light. He found an increasingly attentive listener and began to feed me copies of his reprints.

From the Steven Chu's Noble Prize lecture
www.nobelprize.org/...

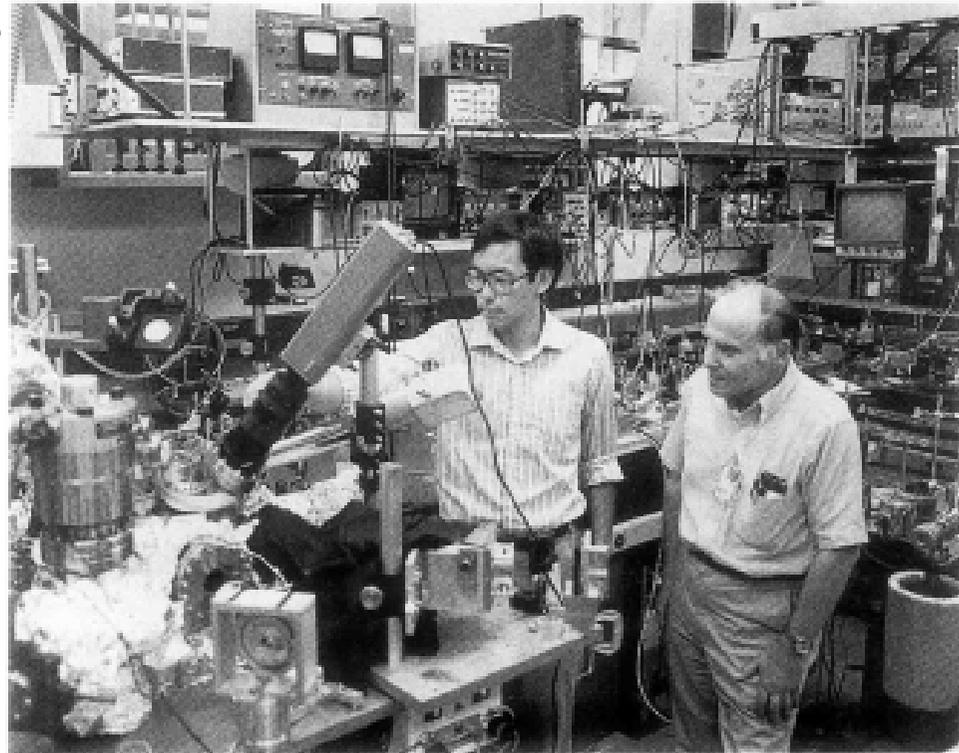
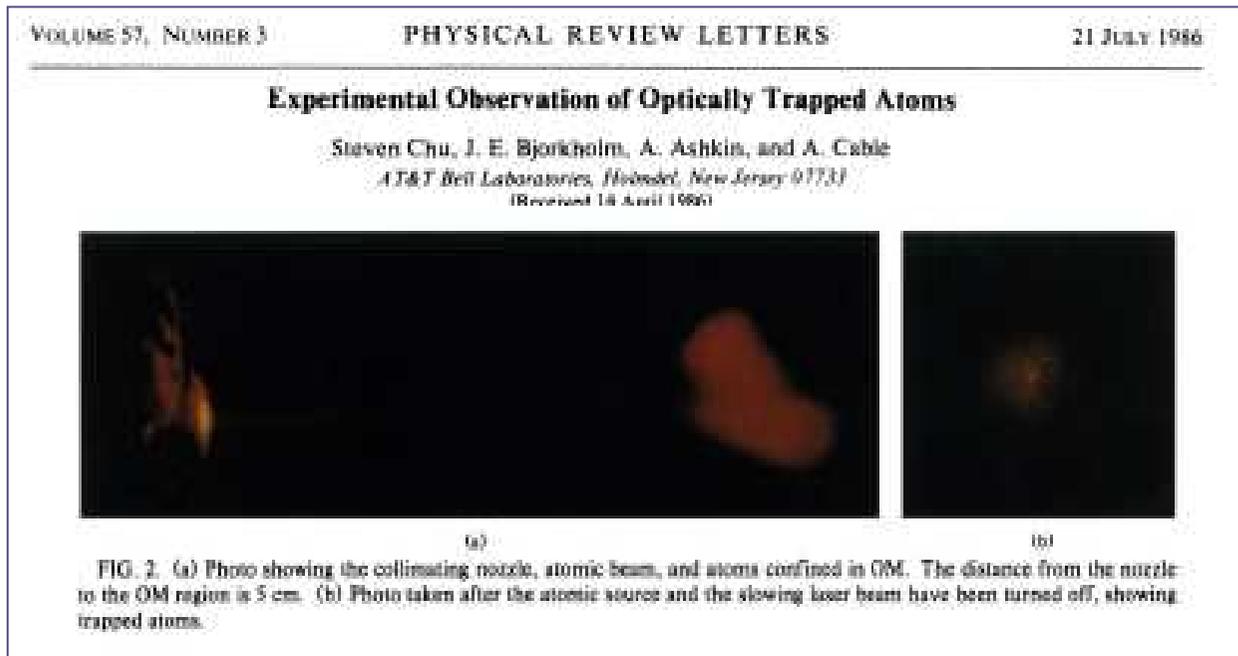


Figure 3 b. Art Ashkin and the author in front of the apparatus in 1986, shortly after the first optical trapping experiment was completed.

Optically trapped atoms



The first color
picture published
in PRL !

The trap worked. We could actually see the random walk loading with our own eyes. A tiny dot of light grew in brightness as more atoms fell into the trap. During the first days of trapping success, I ran up and down the halls, pulling people into our lab to share in the excitement. My director, Chuck Shank, showed polite enthusiasm, but I was not sure he actually picked out the signal from the reflections in the vacuum can windows and the surrounding fluorescence. Art Ashkin came down with the flu shortly after our initial success. He confessed to me later that he began to have doubts: as he lay in bed with a fever; he wasn't sure whether the fever caused him to imagine we had a working trap.

http://nobelprize.org/nobel_prizes/physics/laureates/1997/chu-lecture.html

A “Proof of principle”: Single-beam trapping of dielectric particles

As we began the atom trapping, Art decided to trap micron-sized particles of glass in a single focused beam as a “proof of principle” for the atom trap. Instead of an atom in optical molasses, he substituted a silica (glass) sphere embedded in water. A micron-sized sphere is far more polarizable than an atom and Ashkin felt that it could be trapped at room temperature if the intensity gradient in the axial direction that would draw the glass bead into the focus of the light could overcome the scattering force pushing the particle out of the trap. This more macroscopic version of the optical tweezers trap was demonstrated quickly and gave us more confidence that the atom trap might work.³⁸ At that time, none of us realized how this simple “toy experiment” was going to flower.

From the Steven Chu’s Noble Prize lecture, [www.nobelprize.org/...](http://www.nobelprize.org/)

288 OPTICS LETTERS / Vol. 11, No. 5 / May 1986

Observation of a single-beam gradient force optical trap for dielectric particles

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and Steven Chu

AT&T Bell Laboratories, Holmdel, New Jersey 07733

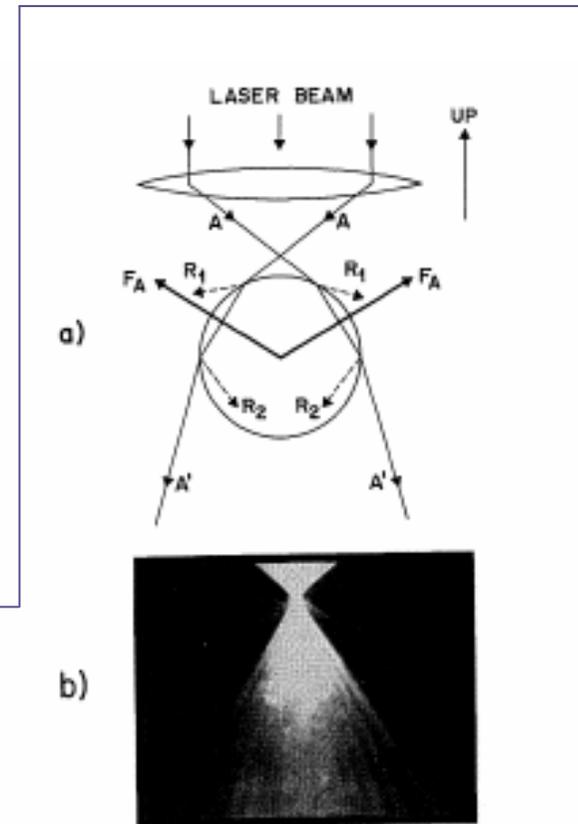


Fig. 1. a) Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single-beam gradient force trap. b) Photograph, taken in fluorescence, of a 10- μ m sphere trapped in water, showing the paths of the incident and scattered light rays.

Applications in biology

In 1986, the world was excited about atom trapping. During this time, Art Ashkin began to use optical tweezers to trap micron-sized particles. While experimenting with colloidal tobacco mosaic viruses,¹¹⁹ he noticed tiny, translucent objects in his sample. Rushing into my lab, he excitedly proclaimed that he had "discovered *Life*". I went into his lab, half thinking that the excitement of the last few years had finally gotten the better of him. In his lab was a microscope objective focusing an argon laser beam into a petri dish of water. Off to the side was an old Edmund Scientific microscope. Squinting into the microscope, I saw my eye lashes. Squinting harder, I occasionally saw some translucent objects. Many of these objects were "floaters", debris in my vitreous humor that could be moved by blinking my eyes. Art assured me that there were *other* objects there that would not move when I blinked my eyes. Sure enough, there were objects in the water that could be trapped and would swim away if the light were turned off. Art had discovered bugs in his apparatus, but these were *real* bugs, bacteria that had eventually grown in his sample beads and water.

His discovery was quickly followed by the demonstration that infrared light focused to megawatts/cm² could be used to trap live e-coli bacteria and yeast for hours without damage.¹²⁰ Other work included the internal cell manipulation of plant cells, protozoa, and stretching of viscoelastic cytoplasm.¹²¹ Steve

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

120. A. Ashkin, J.M. Dziedzic and T. Yamane, *Nature* 330 769 (1987)

121. A. Ashkin and J.M. Dziedzic, *Proc. Natl. Acad. Sci. USA* 86 7914 (1989)

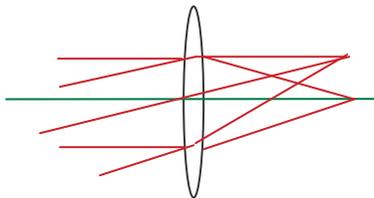
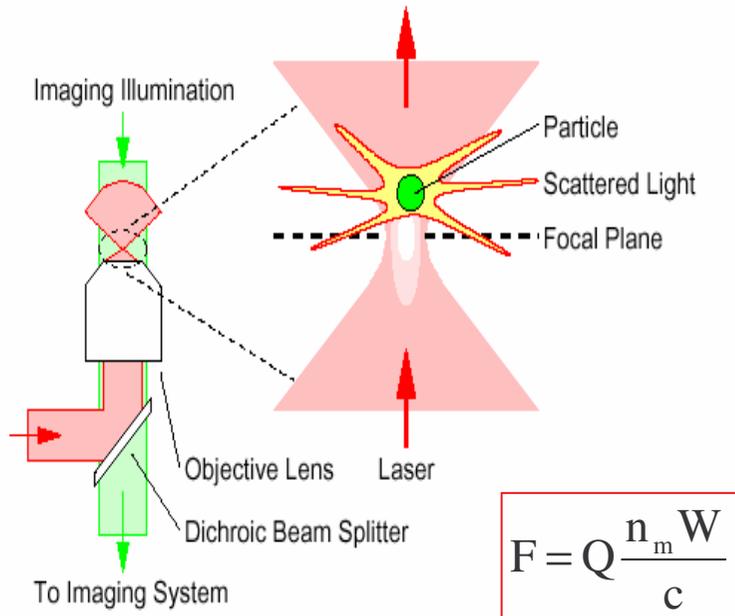
Block and Howard Berg soon adapted the optical tweezers technique to study the mechanical properties of the flagella motor.¹²² and Michael Burns and

122. S. Block, D.F. Blair and H.C. Berg, *Nature* 338 514 (1989)

From the Steven Chu's Noble Prize lecture, [www.nobelprize.org/...](http://www.nobelprize.org/)

Optical forces

Optical trapping and manipulation of a silica microbead (2 μm diam)



Optical forces

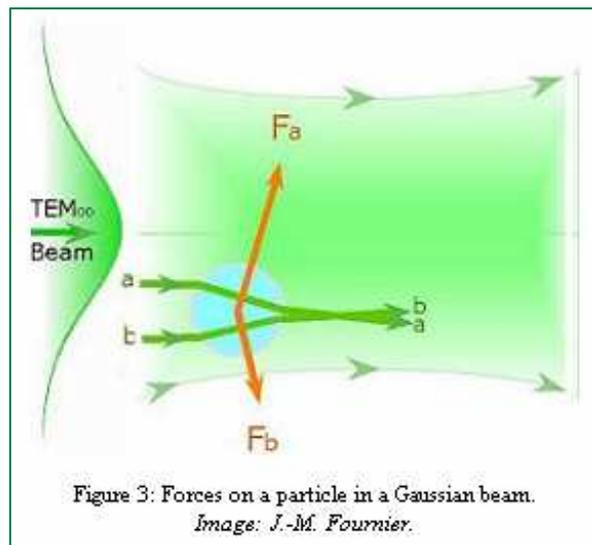
The e-m field governed by Maxwell's equations, exerts a force when impinging on objects.

This force can be either computed:

1. via a direct application of the Lorentz force and bound/free current/charges within the volume of changes
2. via the Maxwell stress tensor.

Advantage: computation efficiency since the electromagnetic fields need to be evaluated only on a surface enclosing the object, while method 1 needs the evaluation of the fields within the whole volume.

Disadvantage: the polarizability of the object within its volume is not computed



The **force** can be expressed as a sum of two terms:

1. the **scattering force**: a force that is parallel to the Poynting vector of the propagating wave, pushing or pulling the object in the same direction as the wave propagation.
2. the **gradient force**: a force due to the gradient of intensity of the electromagnetic radiation. Such gradient is typically obtained by laser beams or in optical lattices.

These forces depend on the property of the object: size, permittivity, permittivity contrast with the background, etc.

<http://web.mit.edu/~ceta/obt/fund-forces.html>

Optical forces

Usually there are more particles interacting with the laser beam →

3. The **binding force** represents the self-consistent interaction between the multiple particles and the incident wave

M. Burns, J-M. Fournier and J. Golovchenko, Optical Binding, Phys. Rev. Lett., 1989.

Optical forces calculation

Web docs/resources

<http://users.icfo.es/Dmitri.Petrov/teaching/lectures.htm>

Jean-Marc Fournier

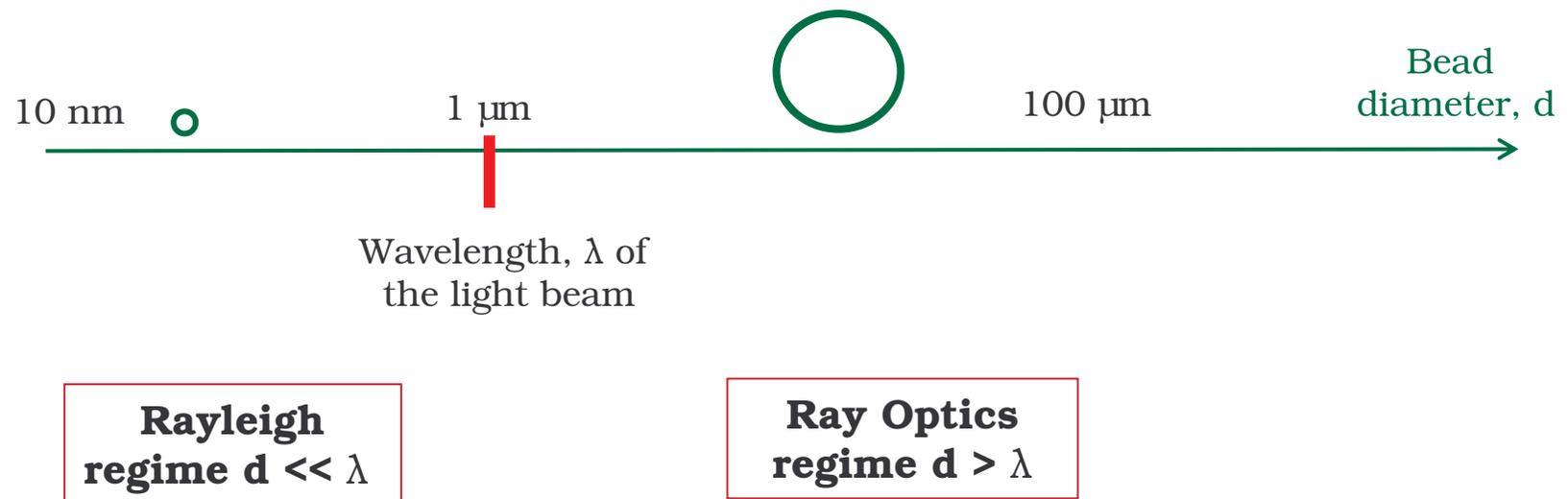
<http://web.mit.edu/~ceta/obt/fund-forces.html>

Optical forces calculation

Some (Few) references

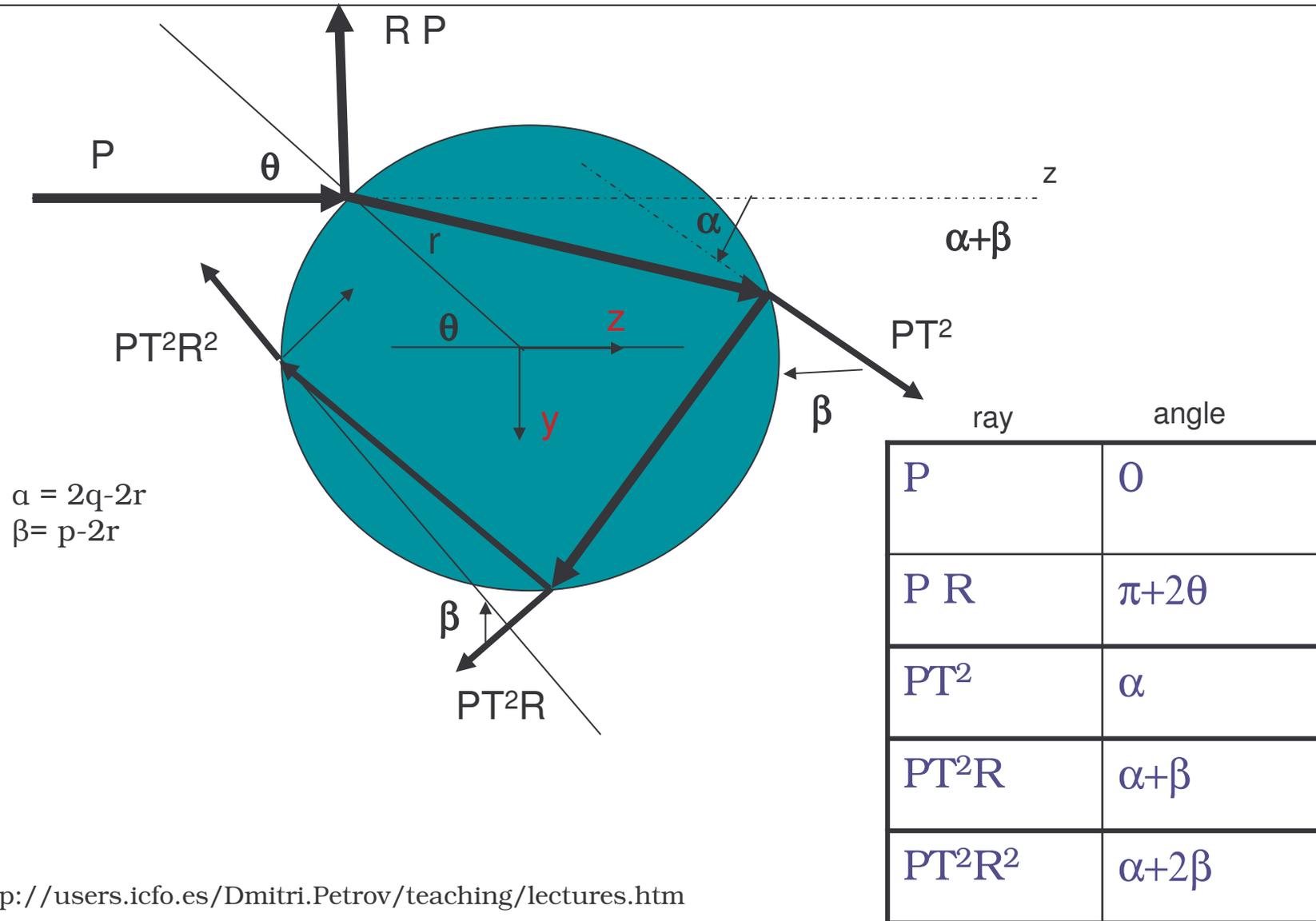
1. M. Mansuripur, "Radiation pressure and the linear momentum of the electromagnetic field," *Opt. Express* **12**, 5375-5401 (2004).
2. B.A. Kemp, T.M. Grzegorzczuk, and J.A. Kong, "*Ab initio study of the radiation pressure on dielectric and magnetic media*", *Optics Express*, 13, 23, 9280-9291 (2005)
3. J. P. Gordon, "Radiation forces and momenta in dielectric media," *Phys. Rev. A* **8**, 14-21 (1973).
4. R. Loudon, "Theory of the radiation pressure on dielectric surfaces," *J. Mod. Opt.* **49**, 821-838 (2002).
5. R. Loudon, "Theory of the forces exerted by Laguerre-Gaussian light beams on dielectrics," *Phys. Rev. A* **68**, 013806 (2003).
6. R. Loudon, "Radiation Pressure and Momentum in Dielectrics," De Martini lecture, *Fortschr. Phys.* **52**, 1134-1140 (2004).
7. Y. N. Obukhov and F. W. Hehl, "Electromagnetic energy-momentum and forces in matter," *Phys. Lett. A* **311**, 277-284 (2003).
8. 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**, 288-290 (1986).
9. A. Ashkin and J. M. Dziedzic, "Optical trapping and manipulation of viruses and bacteria," *Science* **235**, 1517-1520 (1987).
10. A. Rohrbach and E. Stelzer, "Trapping forces, force constants, and potential depths for dielectric spheres in the presence of spherical aberrations," *Appl. Opt.* **41**, 2494 (2002).
11. A. Ashkin, "Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime," *Biophys. J.* **61**, 569-582 (1992).

Trapping regimes



Ray optics regime: $d > \lambda$

Reflection/Refraction(Transmission) of a ray through the spherical microbead



<http://users.icfo.es/Dmitri.Petrov/teaching/lectures.htm>

Ray optics regime

The components F_z and F_y of the force exerted by the light ray the sphere:

$$F_z = \frac{n_m P}{c} \left\{ 1 + R \cos 2\theta - \frac{T^2 [\cos(2\theta - 2r) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2r} \right\} = F_{scat} = Q_{scat} \frac{n_m P}{c}$$

$$F_y = \frac{n_m P}{c} \left\{ R \sin 2\theta - \frac{T^2 [\cos(2\theta - 2r) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2r} \right\} = F_{grad} = Q_{grad} \frac{n_m P}{c}$$

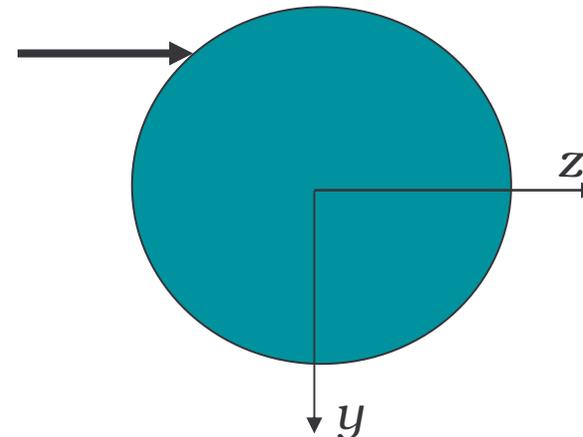
where R and T are the reflection and transmission Fresnel coefficients:

$$R = \left[\frac{n_a \cos \theta - n_p \cos r}{n_a \cos \theta + n_p \cos r} \right]^2$$

$$T = \left[\frac{2n_a \cos \theta}{n_a \cos \theta + n_p \cos r} \right]^2 \frac{n_p \cos r}{n_a \cos \theta}$$

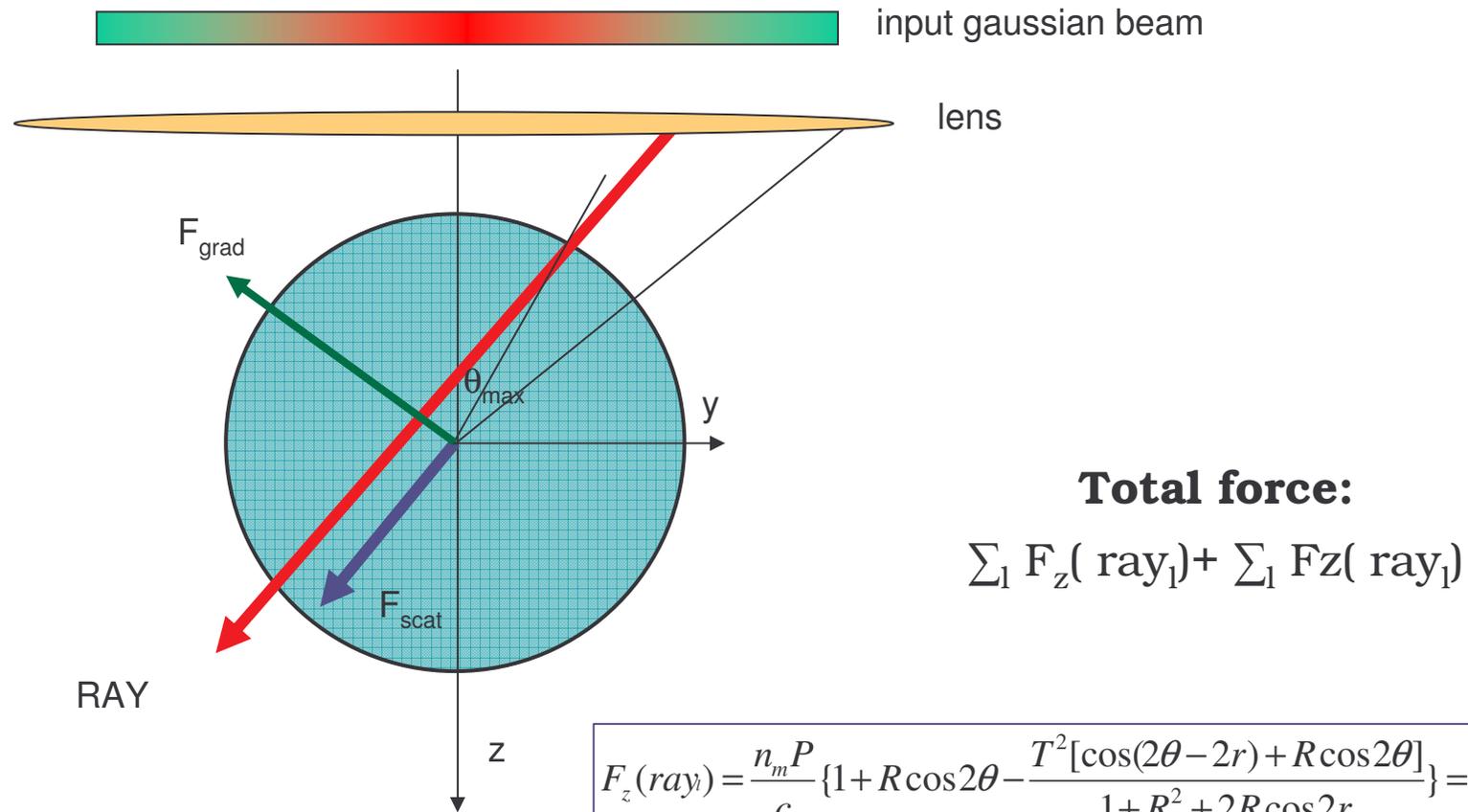
F_z - scattering force
is oriented along the direction of the incident ray

F_y - gradient force
is perpendicular to the direction the incident ray



Ray optics regime

The force produced by a focused beam



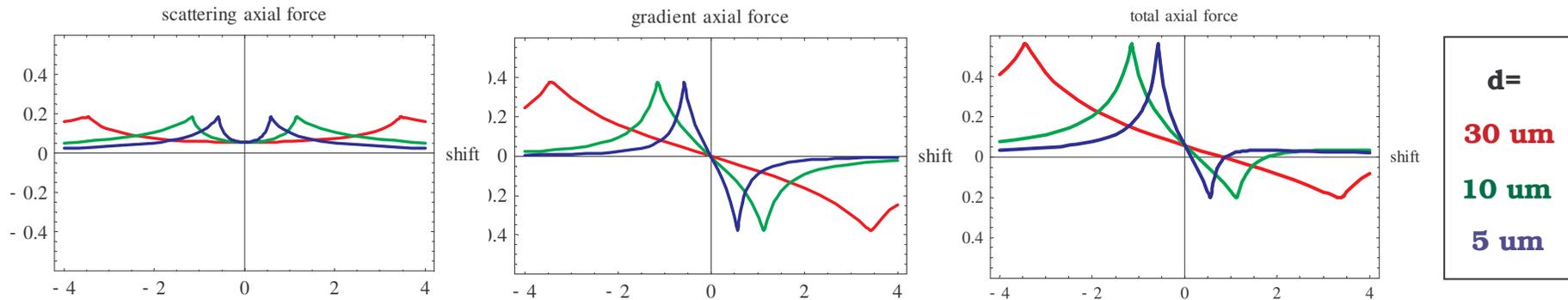
$$F_z(\text{ray}_i) = \frac{n_m P}{c} \left\{ 1 + R \cos 2\theta - \frac{T^2 [\cos(2\theta - 2r) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2r} \right\} = F_{\text{scat}} = Q_{\text{scat}} \frac{n_m P}{c}$$

$$F_y(\text{ray}_i) = \frac{n_m P}{c} \left\{ R \sin 2\theta - \frac{T^2 [\cos(2\theta - 2r) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2r} \right\} = F_{\text{grad}} = Q_{\text{grad}} \frac{n_m P}{c}$$

Ray optics regime

Some observations

$$F_z = \frac{n_m P}{c} \left\{ 1 + R \cos 2\theta - \frac{T^2 [\cos(2\theta - 2r) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2r} \right\} = F_{scat} = Q_{scat} \frac{n_m P}{c}$$
$$F_y = \frac{n_m P}{c} \left\{ R \sin 2\theta - \frac{T^2 [\cos(2\theta - 2r) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2r} \right\} = F_{grad} = Q_{grad} \frac{n_m P}{c}$$



- The maximum values of the scattering and gradient values do not depend of the diameter d of the microbead
- The trapp stiffness decreases with the diameter
- The equilibrium position moves from the objective lens for smaller beads maximal value of the gradient force

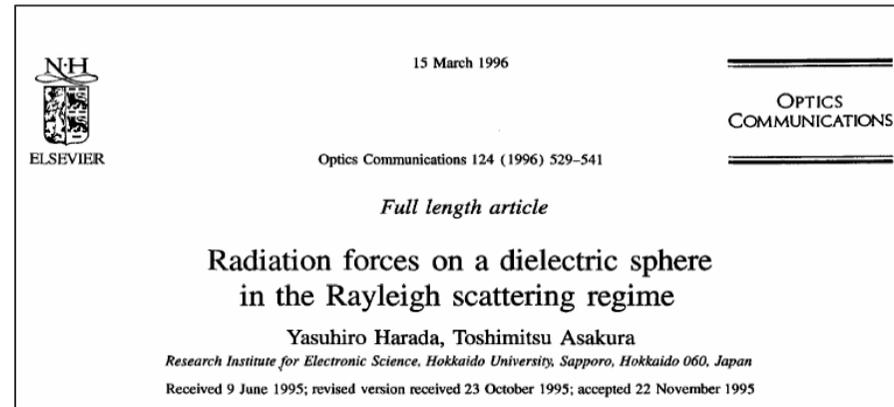
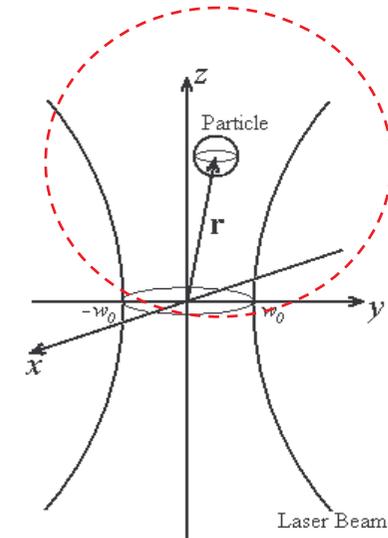
Rayleigh regime $d < \lambda/10$

the particle can be treated as a small dipole immersed in the optical field

$$\vec{F}_{scat}(\mathbf{r}) = \vec{z} \frac{n_a}{c} \left[\frac{8\pi}{3} (k_a d)^4 d^2 \left(\frac{\epsilon_p / \epsilon_a - 1}{\epsilon_p / \epsilon_a + 2} \right)^2 \right] \langle S(\mathbf{r}, t) \rangle$$

$$\vec{F}_{grad}(\mathbf{r}) = \left[\frac{2\pi n_a d^3}{c} \frac{\epsilon_p / \epsilon_a - 1}{\epsilon_p / \epsilon_a + 2} \right] \vec{\nabla} \langle S(\mathbf{r}, t) \rangle$$

$\langle S(\mathbf{r}, t) \rangle$ the time average Poynting vector (intensity)



K. Svoboda *et al* *Opt. Lett.* **19** 930 1994

Rayleigh regime $d < \lambda/10$

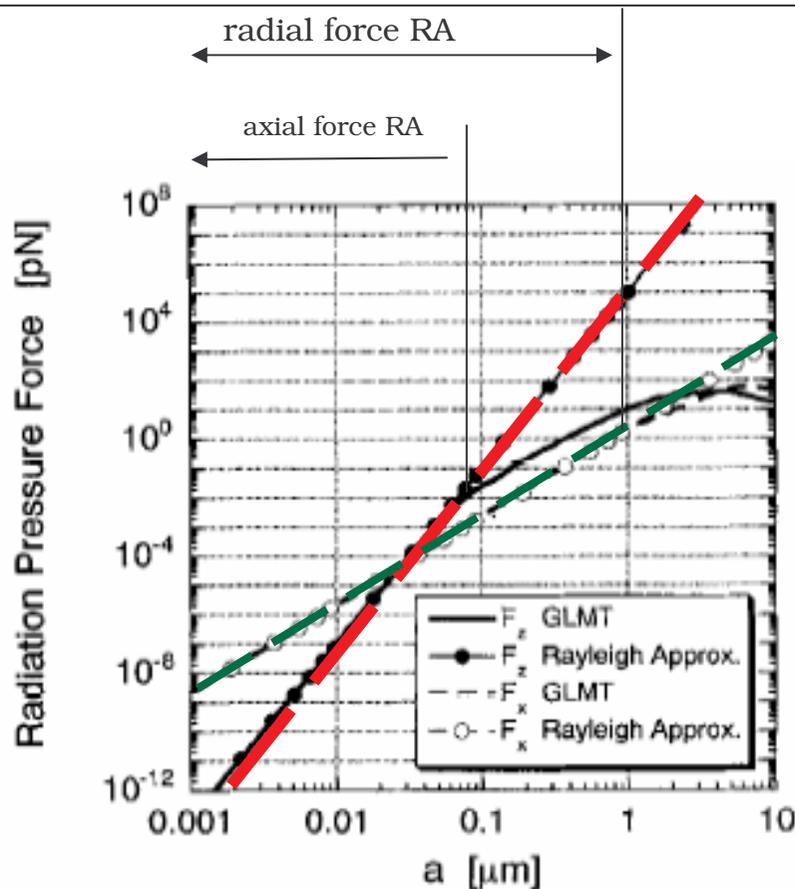
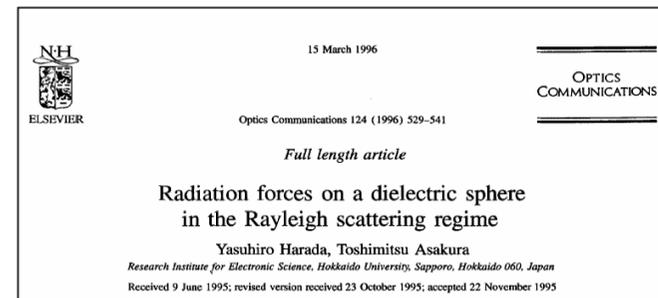


Fig. 5. Dependencies of the longitudinal component F_z and the transverse component F_x of the radiation force on the particle radius a with the results of the GLMT for a polystyrene particle in water: F_z and F_x represent components of the force exerted on a particle locating at $(x, y, z) = (0, 0, 0)$ and $(-w_0/2, 0, 0)$, respectively.

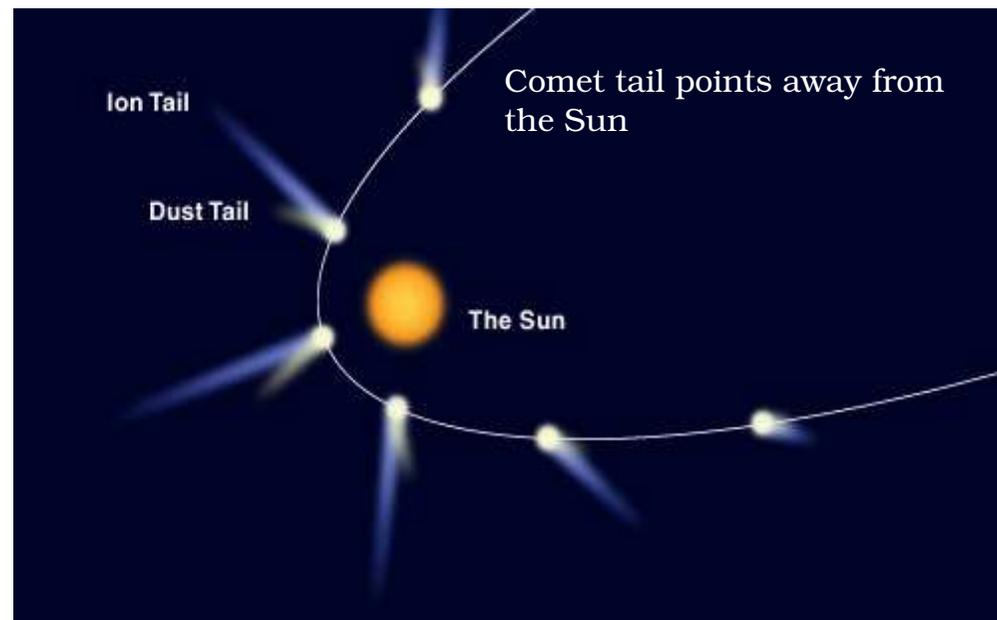
$$F_{scat}(r) = \vec{z} \frac{n_a}{c} \left[\frac{8\pi}{3} (k_a d)^4 d^2 \left(\frac{m^2 - 1}{m^2 + 2} \right)^2 \right] \langle S(r, t) \rangle$$

$$F_{grad}(r) = \left[\frac{2\pi n_a d^3}{c} \frac{m^2 - 1}{m^2 + 2} \right] \vec{\nabla} \langle S(r, t) \rangle$$



Optical forces – radiation pressure of light

Johannes Kepler noticed that tails of comets always point away from the Sun which suggested that the Sun-light was exerting a sort of radiation pressure.
(1609)



Momentum in an uncertain light

Ulf Leonhardt

Nature, 444, 823, 2006

How much momentum does light transfer to a material through which it passes? This is a surprisingly opaque matter, contested for almost a century, that is still the object of theory and experimentation.



Figure 1 | Light, momentum, action. The comet Hale-Bopp, seen here over the Joshua Tree National Park in southern California on the evening of 28 March 1997, has both a blue ion tail and a white dust tail. Whereas the ion tail is carried away by the 'solar wind' of charged particles from the Sun's atmosphere, the dust tail is pushed by the radiation pressure of the sunlight. The momentum transfer in this second case is weaker than that in the first, resulting in the splitting of the tails.

Box 1 | Theoretical routes to light's momentum

The reasoning behind both the Minkowski and the Abraham formulae for the momentum of light is best explained with a little support from two pillars of modern physics: wave-particle duality and Einstein's relativity.

Wave-particle duality is a fundamental tenet of quantum mechanics, and states that light, like everything else in the quantum world, simultaneously has characteristics of both a particle and a wave. Light acting as a wave oscillates

with period τ and wavelength λ . During each period, the wave advances by an amount determined by its speed in the medium that contains it: thus, $\lambda = (c/n)\tau$, where c is the speed of light *in vacuo* and n is the refractive index of the medium. Light acting as particles consists of photons, each of which carries an amount of energy $E = h/\tau$, where h is Planck's constant. Combining these two expressions, and after a little rearrangement, you obtain Minkowski's prediction² for

light's momentum, $p = nE/c$, if you require $p = h/\lambda$. This fundamental relationship between momentum and wavelength now bears the name of the de Broglie relation.

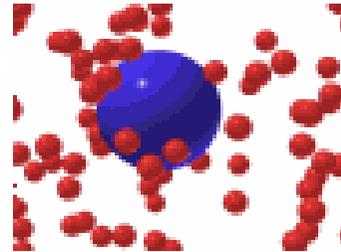
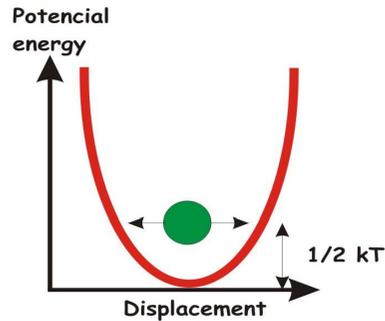
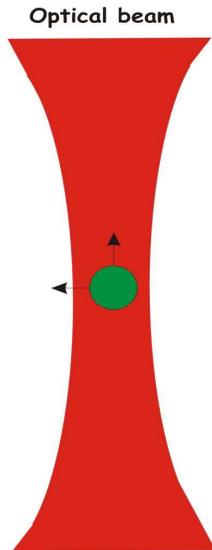
Alternatively, however, you can start from Einstein's energy-mass equivalence formula $E = mc^2$. This implies that light has mass, and mass multiplied by velocity c/n gives momentum. The expression that emerges in this case is $p = E/(nc)$, Abraham's momentum³.

U.L.

References

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2. Minkowski, H. *Nachr. Ges. Wiss. Göttn Math.-Phys. Kl.* 53 (1908).
3. Abraham, M. *Rend. Circ. Matem. Palermo* 28, 1 (1909).
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7. Campbell, G. K. *et al. Phys. Rev. Lett.* 94, 170403 (2005).
8. Loudon, R. *J. Mod. Optics* 49, 821–838 (2002).
9. Gordon, W. *Ann. Phys. (Leipz.)* 72, 421–438 (1923).
10. Leonhardt, U. *Phys. Rev. A* 73, 032108 (2006).

**Stable trap condition:
Optical potential \gg the kinetic energy of the Brownian motion**



Brownian motion

Stable trap condition

$$\exp\left(-\frac{U_{opt}}{k_B T}\right) \ll 1$$

$$\frac{2\pi n_a a^3}{c} \left(\frac{m^2 - 1}{m^2 + 2} \right) \frac{2P}{\pi w_0^2} \geq 10k_B T$$

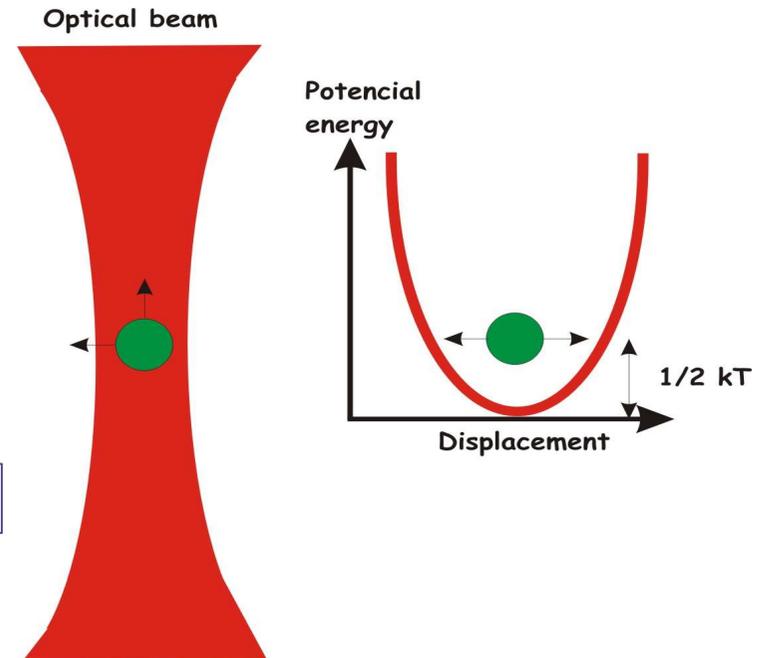
$$U_{opt} = \frac{2\pi n_a a^3}{c} \left(\frac{m^2 - 1}{m^2 + 2} \right) \frac{2P}{\pi w_0^2}$$

Trapping/force calibration

Why ?

Whenever a bead leaves the center of the optical trap the restoring force pulls it back to the center.

$$m\ddot{x}(t) + \gamma\dot{x}(t) = \text{OpticalForce} + \text{RandomForce}(t)$$



$$F_{\text{optical}} = -k \Delta x$$

k - the stiffness of the OT

Trapping/force calibration

Drag method

Stokes' Law the drag force on a sphere of *radius* a moving through a fluid of *viscosity* η at speed v is given by:

$$\vec{F}_{drag} = -6\pi a \eta \vec{v}$$

Viscosity is a measure of the resistance of a fluid to deformation under shear stress.

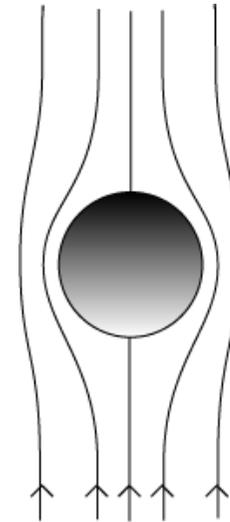
The SI physical unit of dynamic viscosity is Pa·s , which is identical to 1 Ns/m²

$$\vec{F}_{drag} = F_{trap}$$

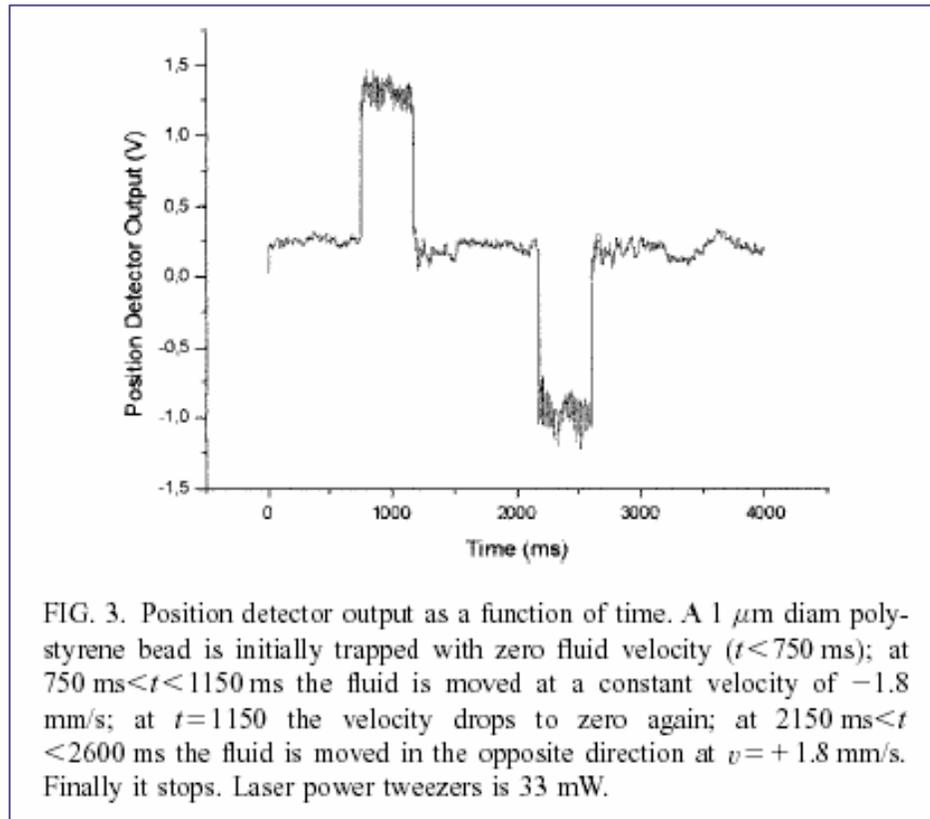
The velocity of the fluid V for which the bead escapes from the trap $\rightarrow F_{trap}$
Or the velocity is kept fixed and the power of the trapping laser changed.

Example:

Velocity of the liquid is $V=1.8$ $\mu\text{m/s}$. The bead, of radius $r= 2.6$ μm “escapes” at 10 mW trapping intensity, $F_{trap}= 2$ pN.



Trapping/force calibration Drag method

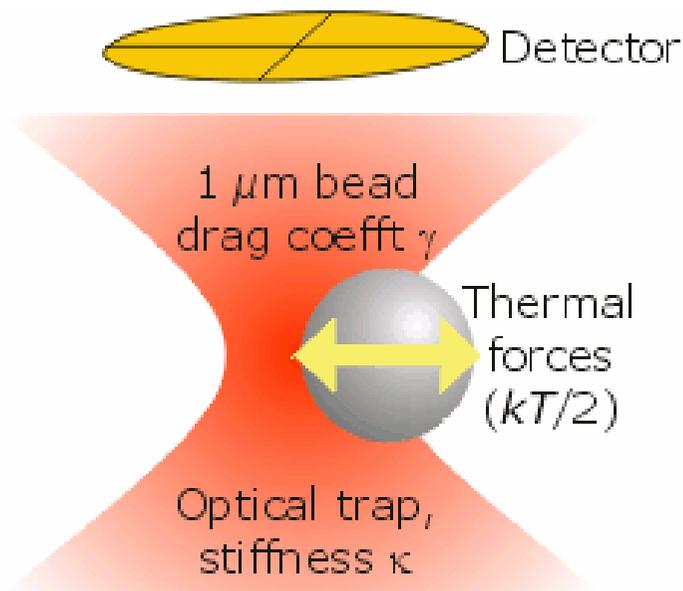


Disadvantage:

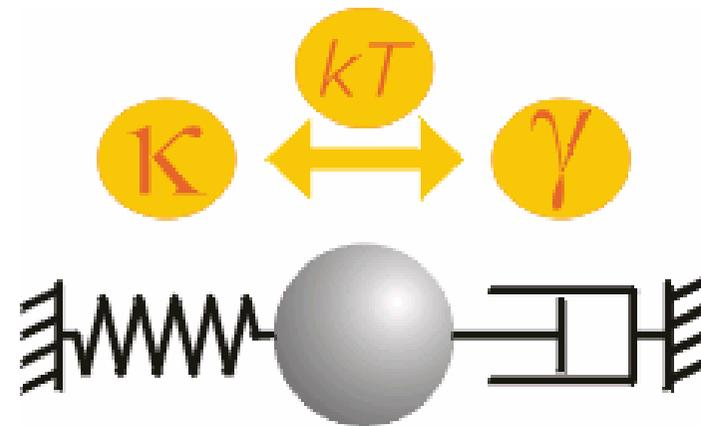
viscosity in a small chamber differs from the bulk value

Brownian motion and Back Focal Plane Interferometry

Schematic of a μm bead
diffusing in an optical trap



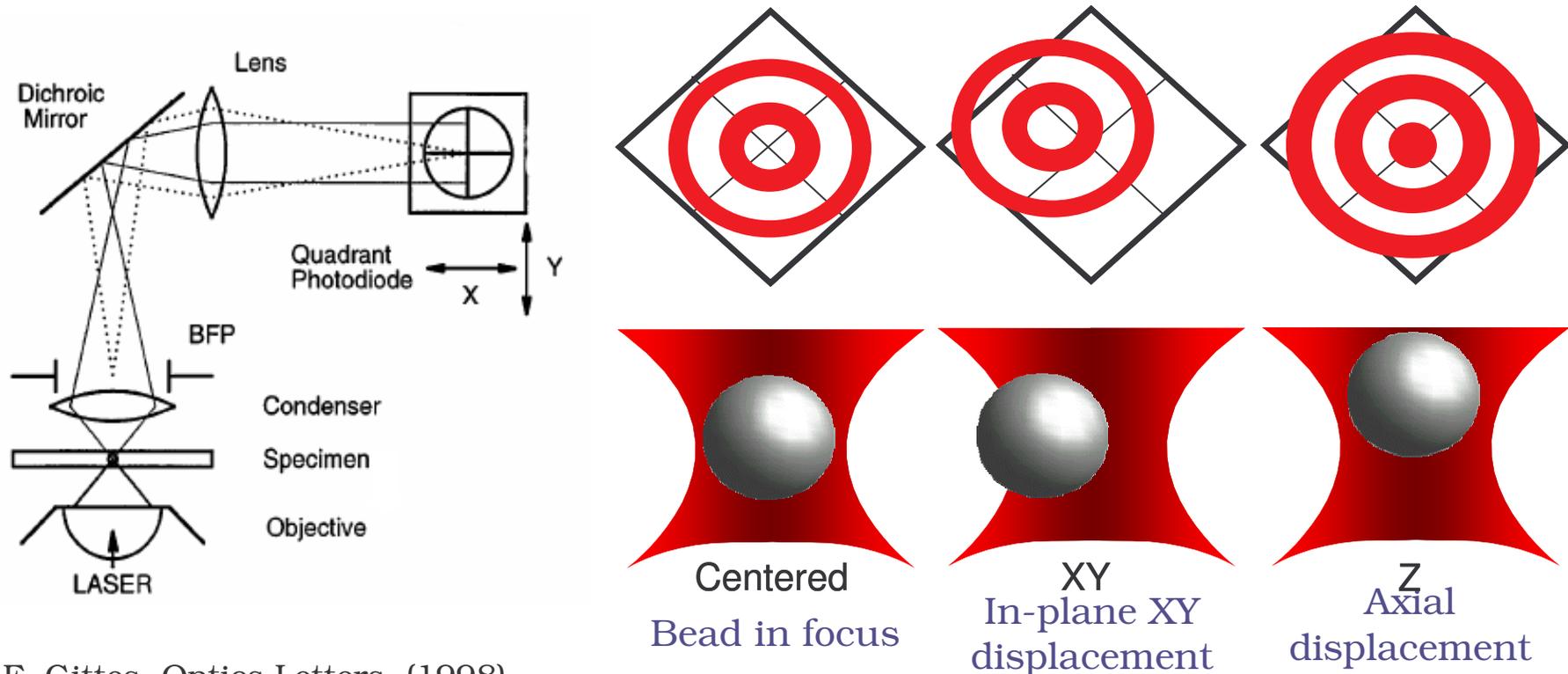
Mechanical model of the
forces acting on the bead



The power spectrum density $S(f)$ of these fluctuations near the center of an optical trap is approximately Lorentzian (Svoboda and Block, 1994; Gittes and Schmidt, 1997)

Bead position is determined by back focal plane (BFP) detection (BFP is imaged onto a QPD)

Interference pattern obtained by superposition of scattered and not-scattered light by the bead



F. Gittes, Optics Letters, (1998)

$$\mathbf{F} = \mathbf{K} \Delta$$

\mathbf{F} = Force

$\mathbf{K} = (K_x, K_y, K_z)$ stiffness of the trap

$\Delta = (\Delta x, \Delta y, \Delta z)$ Displacement

Trap stiffness and detector sensitivity

$S_v(f)$ - measured power spectrum
 $S(f)$ - density Lorentzian fit

$$S(f) = \frac{S_0 f_0^2}{f_0^2 + f^2}, \quad f_0 = \kappa / 2\pi\gamma$$

f_0 - corner frequency

k - trap stiffness

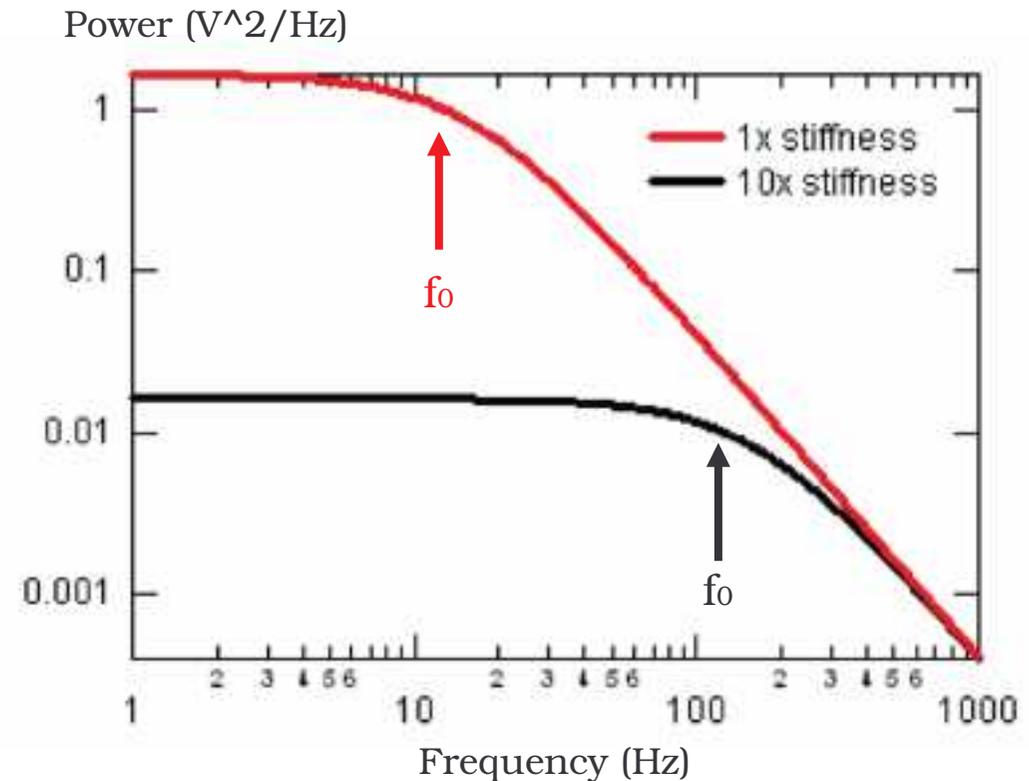
γ - Stokes drag coefficient

β - detector sensitivity

S_0 - trap stiffness

PV - plateau of $f^2 S^v(f)$

$$P^v = \beta^2 S_0 f_0^2$$



The power spectrum for two different stiffnesses

(**black** is 10 times stiffer than **red**)

Equipartition theorem

For a particle confined in a three-dimensional potential

$$\frac{1}{2}k_B T = \frac{1}{2}\kappa_i \langle x_i^2 \rangle \quad \text{or} \quad \kappa_i = \frac{k_B T}{\langle x_i^2 \rangle}$$

$$\langle x_i^2 \rangle = \lim_{T \rightarrow \infty} \left(\frac{1}{T} \sum_{i=1}^{\infty} (x_i - \bar{x})^2 \Delta t \right)$$

with T being the total observation time, Δt being the time interval between observations, and \bar{x} being the sample mean. Thus, if we can accurately estimate the statistical **variance** of the particle's x-coordinate, and if we know the temperature, we can deduce the spring constant κ !

$$\langle x_i^2 \rangle \propto \langle V \rangle$$

can be measured using the signal from the photodetector

TABLE I. A comparison of the calibration methods performed on 1 μm diam polystyrene beads.

	Viscous drag method	Power spectrum method	Equipartition theorem method	Time of flight method
Validity range	± 250 nm along x and y , ± 100 nm along z	± 250 nm along x and y	± 250 nm along x and y	All the optical potential well, along x or y
Calibration Parameters	k_x, k_y, k_z and $\beta_x, \beta_y, \beta_z$	$k_x \approx k_y$ and β_x	$k_x \approx k_y$ or β_x	$k_x \approx k_y$
Typical errors	$\delta k/k \approx 2\%$ $\delta \beta/\beta \approx 2\%$	$\delta k/k \approx 5\%$ $\delta \beta/\beta \approx 3\%$	$\delta k/k \approx 5\%$ $\delta \beta/\beta \approx 3\%$	$\delta k/k \approx 6\%$
Advantages	It allows measurement of k and β along all the three spatial directions.	It is fast (about 1 min). No particular solution cleanliness is needed.	It is fast (about 1 min). No particular solution cleanliness is needed.	It is the only calibration method that allows a complete reconstruction of the potential well. It is fast when measuring.
Disadvantages	Long calibration time is needed, beads attached to the cover-slip surface and extreme solution cleanliness are needed.	It does not allow us to determine all three components of k and β .	It allows us to determine only one calibration parameter at a time. It does not allow us to determine all three components of k and β .	A $V(x)$ measurement (like the one shown in Fig. 2) is needed. A long time is needed to analyze the experimental data.

Characteristics of optical traps

Types of particles:

- **Material:** Dielectric (polystyrene, silica); Metallic (gold, silver, copper), Biological (cells, macro-molecules, intracellular structures, DNA filaments), Low index (ultrasound agent contrast)
 - **Size:** 5 nm – 20 μm
 - **Shape:** spherical, cylindrical, arbitrary
-

Types of laser beams:

- **Gaussian**

x-y intensity profile

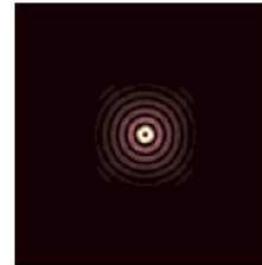
- **Laguerre-Gaussian**



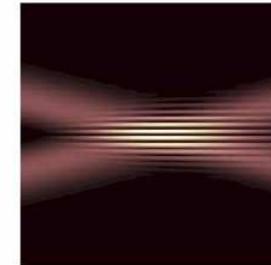
LG carries also orbital angular momentum that can be transferred to the trapped particles

- **Bessel**

x-y intensity profile



z axis propagation
non diffracted beam



OT characteristics:

- Typical stiffness: 100 pN/ μm
 - Typical displacements: 1-500 nm
 - Typical forces: 0.1-100 pN
 - Measurable displacements < 1 nm @ 1 MHz sampling rate
-

Characteristics of optical traps

Comparison of forces with other techniques and biological processes:

Optical traps	0.1 - 100 pN
Electric fields (electrophoresis)	0-1 pN
AFM	10 - 10000 pN
Kinesin step	3-5 pN
RNA polymerase stalling	15-30 pN
Virus motor stalling	~50 pN
DNA conformational change	~65 pN
Biotin-streptavidin binding	300-400 pN

Courtesy Prof. D. Petrov, ICFO, Barcelona, Spain
<http://users.icfo.es/Dmitri.Petrov/Teaching/lectures.htm>

Some properties of the LG beams (optical vortices)

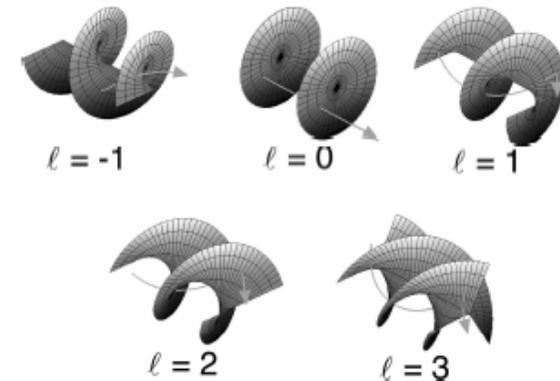
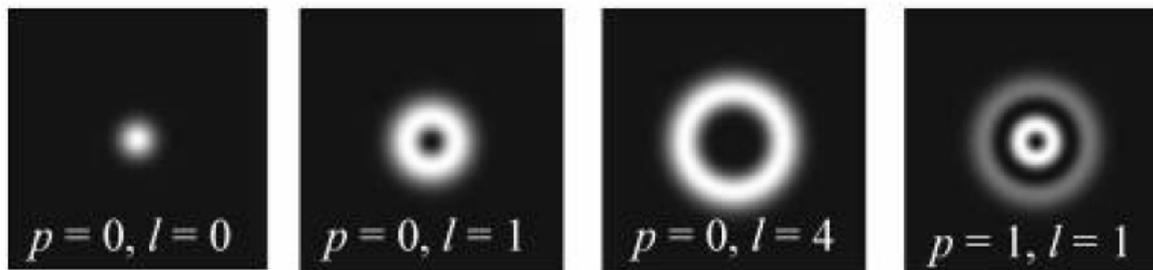
The full mode description of a LG:

$$E(LG_p^l) \propto \exp\left[\frac{-ikr^2z}{2(z_R^2 + z^2)}\right] \exp\left[\frac{-r^2}{\omega(z)^2}\right] \exp\left[-i(2p + |l| + 1)\arctan\left(\frac{z}{z_R}\right)\right] \times$$

$$\exp[-il\phi] \times \left(\frac{r\sqrt{2}}{\omega(z)}\right)^{|l|} L_p^{|l|}\left(\frac{2r^2}{\omega(z)^2}\right)$$

LG carries orbital angular momentum:
 $l\hbar$ per photon

- l is the azimuthal index and refers to the number of complete (2π) phase cycles around the circumference of the mode,
- $p + 1$ gives the number of radial nodes in the mode profile



M.W. Beijersbergen *et al*, *Opt. Comm.* **96** 123 1993

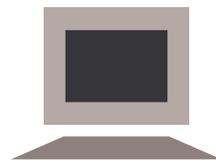
L. Allen, S. Barnett, M.J. Padgett, *Optical Angular Momentum*, IoP Publishing Ltd 2003

Generating LG beams with Diffractive Optical Elements

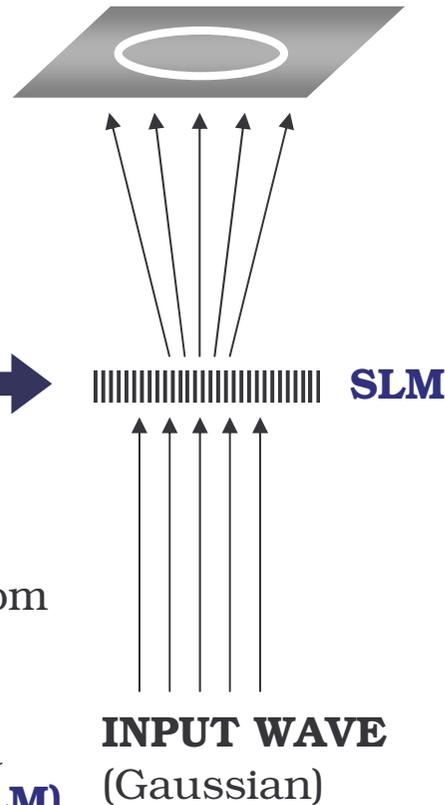
LG beams can be generated converting a Gaussian beam by means of DOE

Working plane:

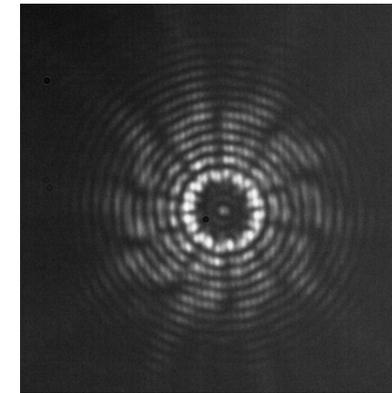
the desired pattern is obtained after the propagation of the diffracted beam



Computer Generated DOE can be calculated starting from the desired output intensity and/or phase profile and addressed electronically on a **Spatial Light Modulator (SLM)**

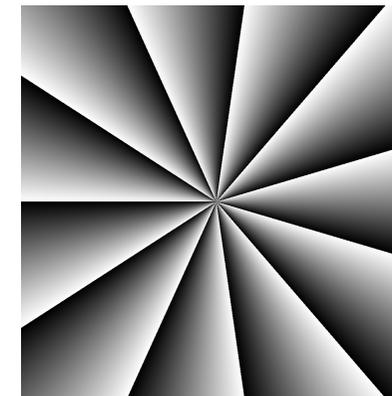


Generated intensity pattern



5 μm

phase DOE $l = 11$

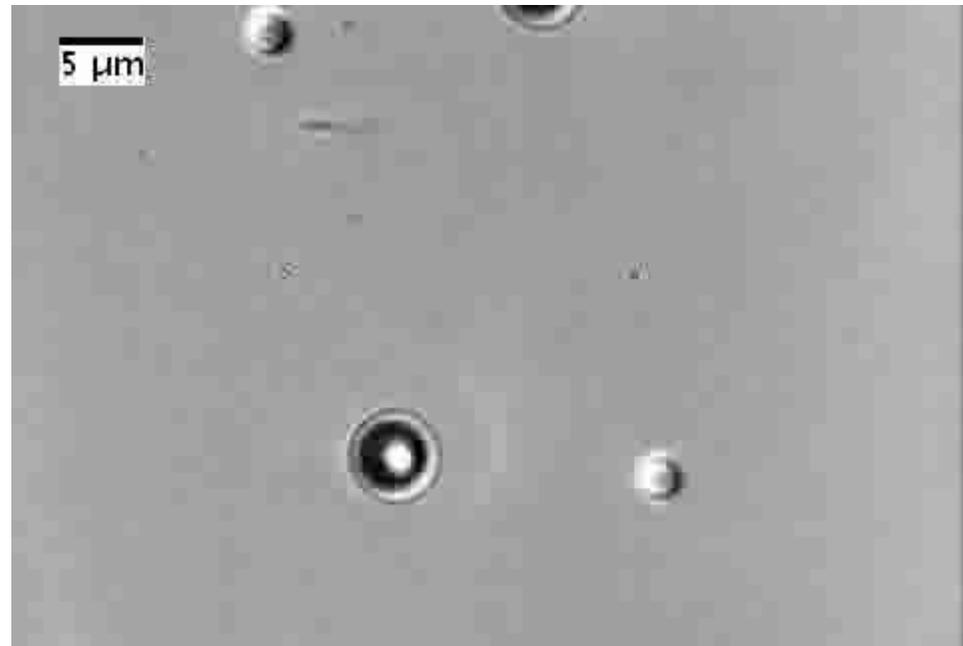
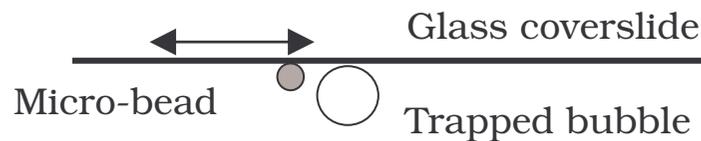


Trapping low index particles with LG beam

A low index particle is repelled by a Gaussian beam, since its refractive index is lower than the refractive index of the surrounding medium, but can be trapped inside the 'doughnut' of a LG beam

Example of low index particles: Ultrasound Contrast Agents (UCA)
phospholipid shelled micro-bubbles

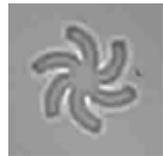
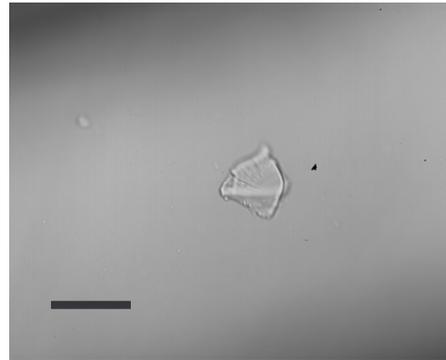
Trapping strength is tested
against the silica micro-bead
stacked on the glass coverslide



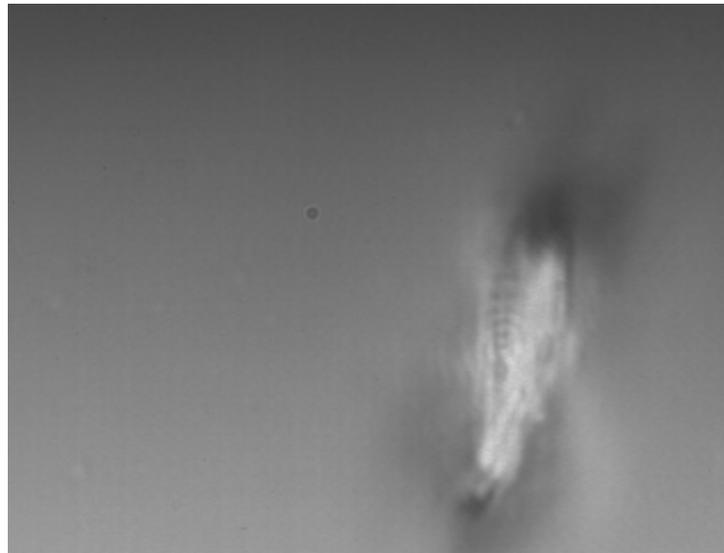
Optically driven micro-pumps

Glass micro-palette pump driven by the optical tweezers

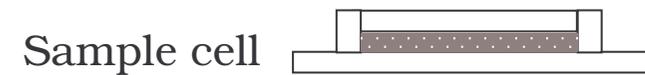
Alternate Gauss-LG beams



Micro -
fabricated
turbine

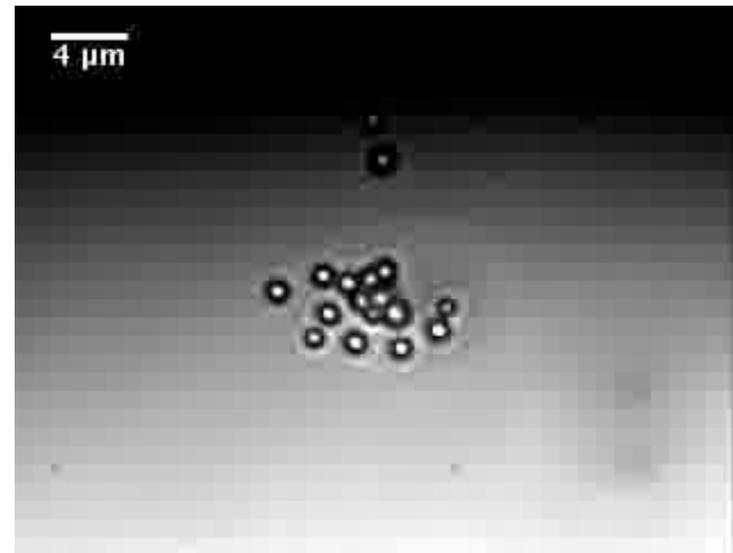


Optical angular momentum transfer using LG beam



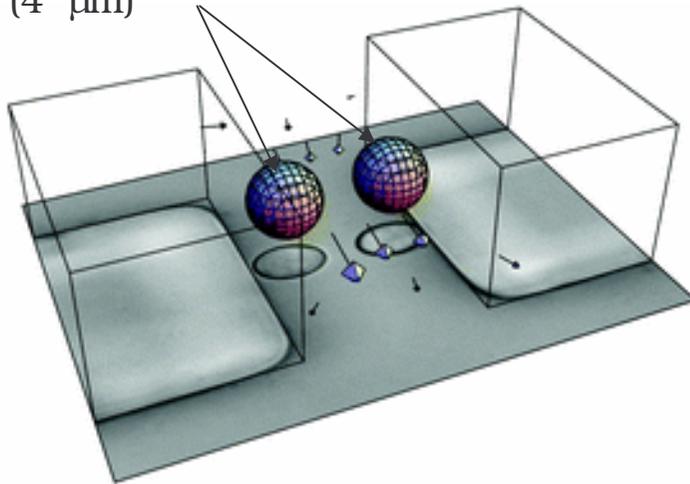
Microspheres: silica 1 μm

LG beam with topological charge $l=11$



Optical driven pumping and optical sensing flow measurement

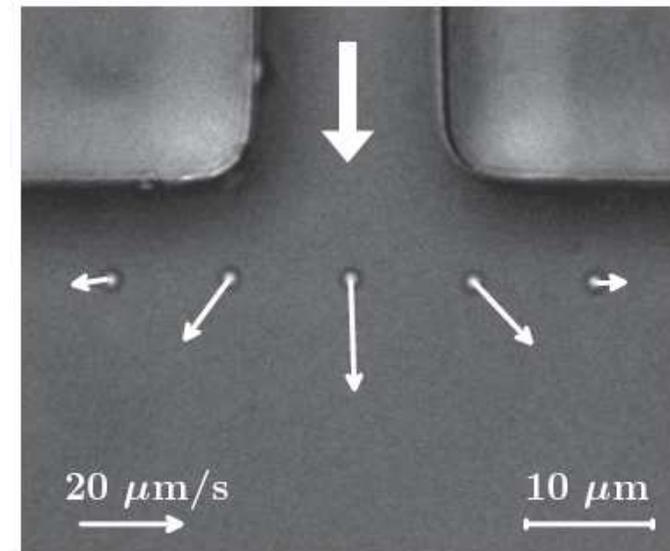
Birefringent
vaterite microspheres
(4 μm)



The transfer of spin angular momentum from a circularly polarised laser beam rotates the particles at up to 10 Hz.

Flow rates of up to $200 \mu\text{m}^3 \text{ s}^{-1}$ (200 fL s^{-1}).

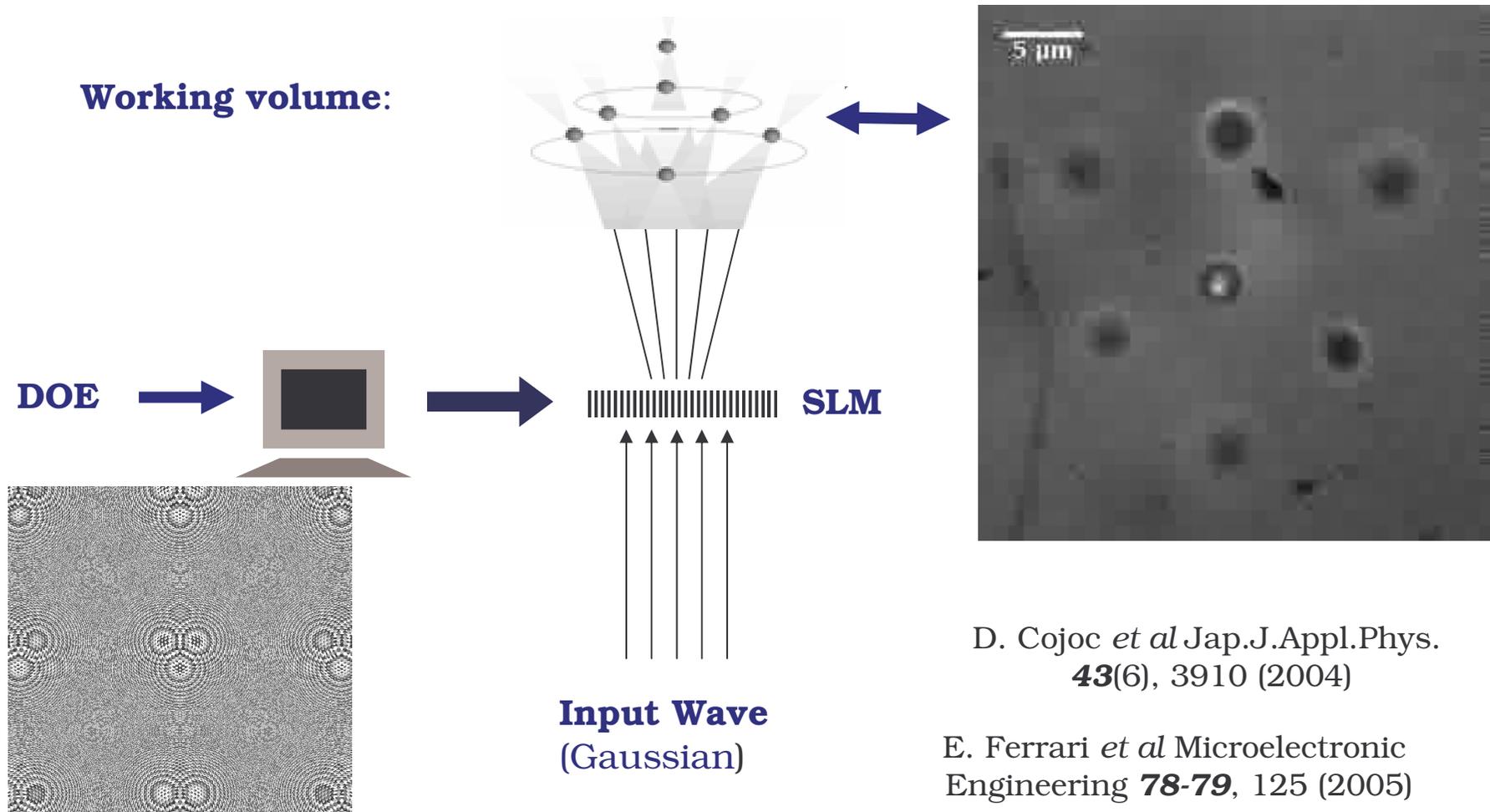
J. Leach *et al* Lab on a Chip, 2006, 6, 735



R. Di Leonardo, PRL **96**, 134502 (2006)

Multiple trapping with Diffractive Optical Elements on SLM

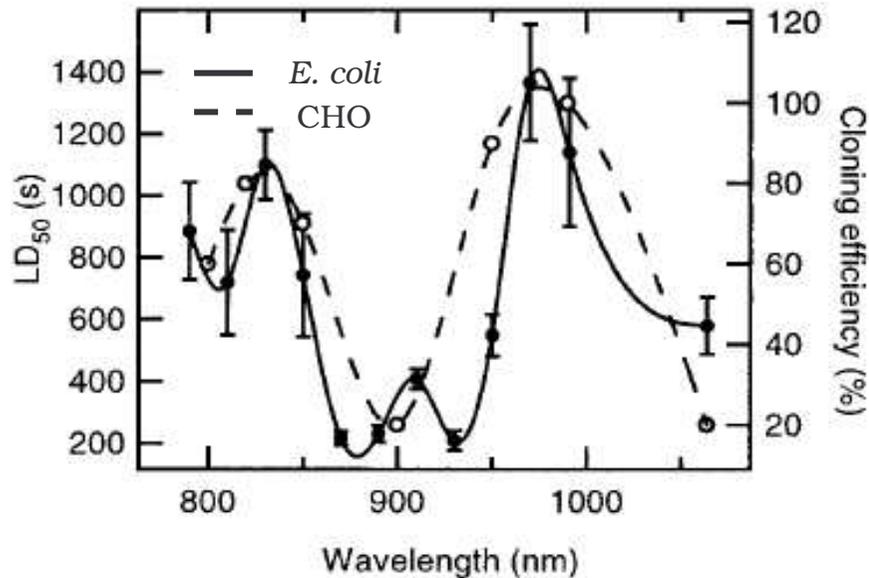
2D or 3D arrays of traps can be generated



Cell manipulation

Minimum cell damage

IR lasers

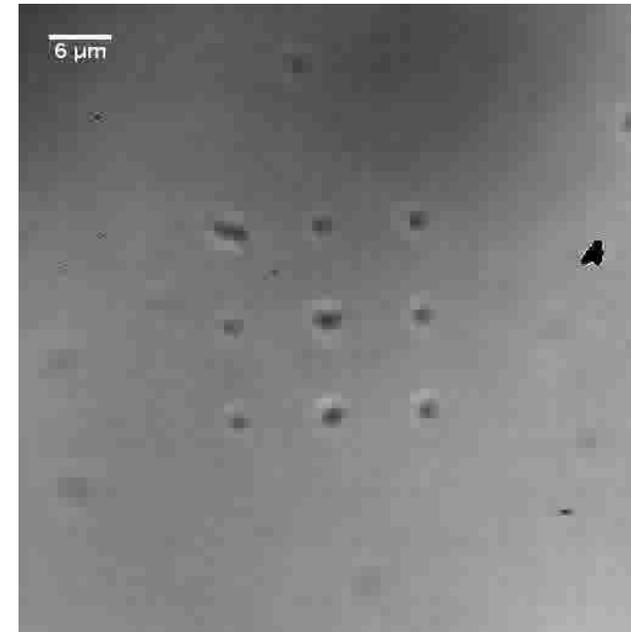


The wavelength dependence of photodamage in *E. coli* compared to Chinese Hamster Ovarian cells.

Liang *et al*, Biophys. J. **70**,1529 (1996)

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

Cell array and sorting



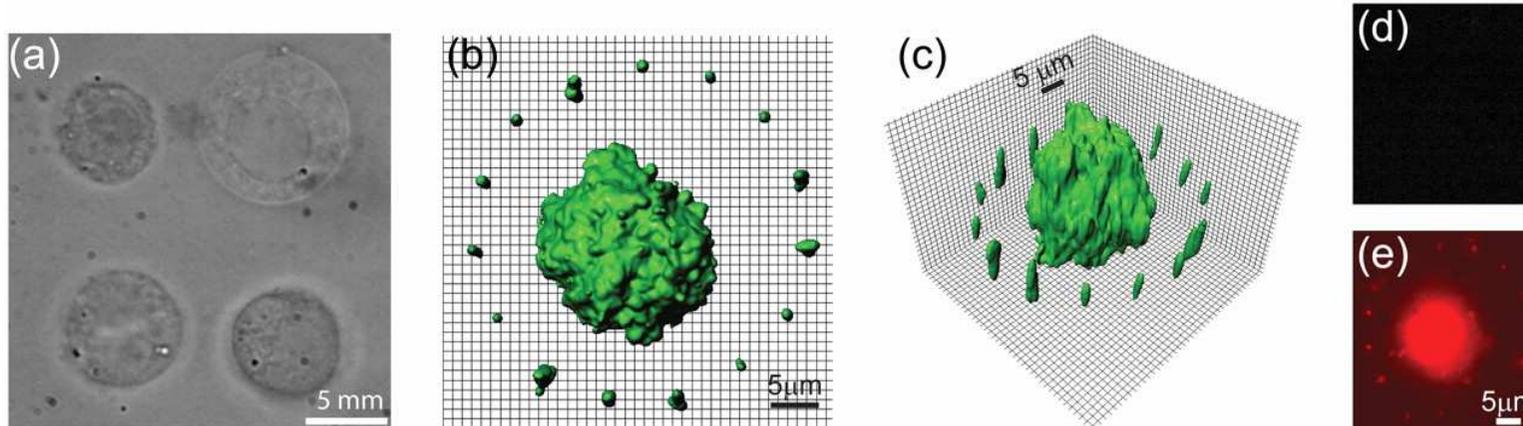
E. coli cells are trapped in a 3x3 array and the position of two cells is then interchanged

E. Di Fabrizio *et al*, Microscopy Research and Technique **65**, 252 (2004)

Permanent assembly of 3D living cell microarrays

- The array is first configured by multiple traps created with AOD and SLM
- The position of the cells is fixed permanently using a photopolymerizable hydrogel

PEDGA = Polyethylene glycol diacrylate



Heterotypic microarray of Swiss 3T3 mouse fibroblast and *P. aeruginosa* bacteria.

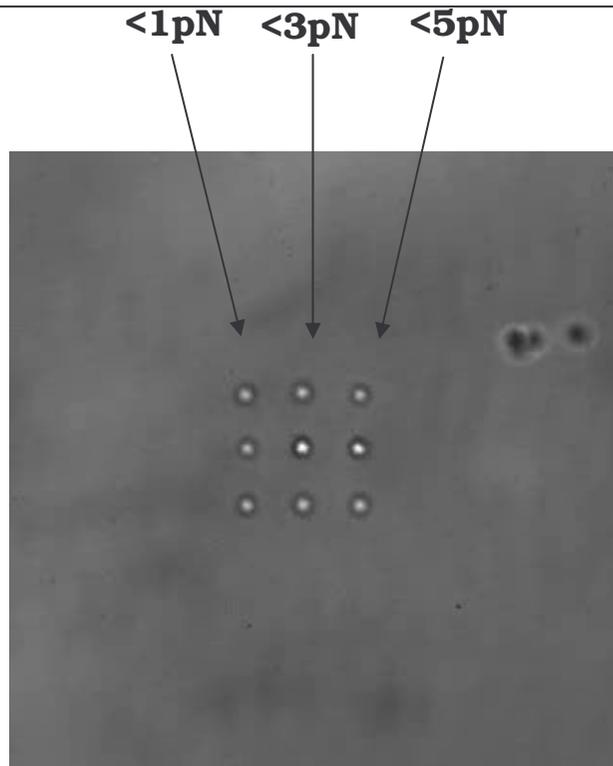
(a) Swiss 3T3 mouse fibroblasts trapped in a 2 x 2 2D array

(b,c) False-color isosurface reconstructions obtained from a confocal image of a Swiss 3T3 cell surrounded by a ring of 16 *P. aeruginosa*.

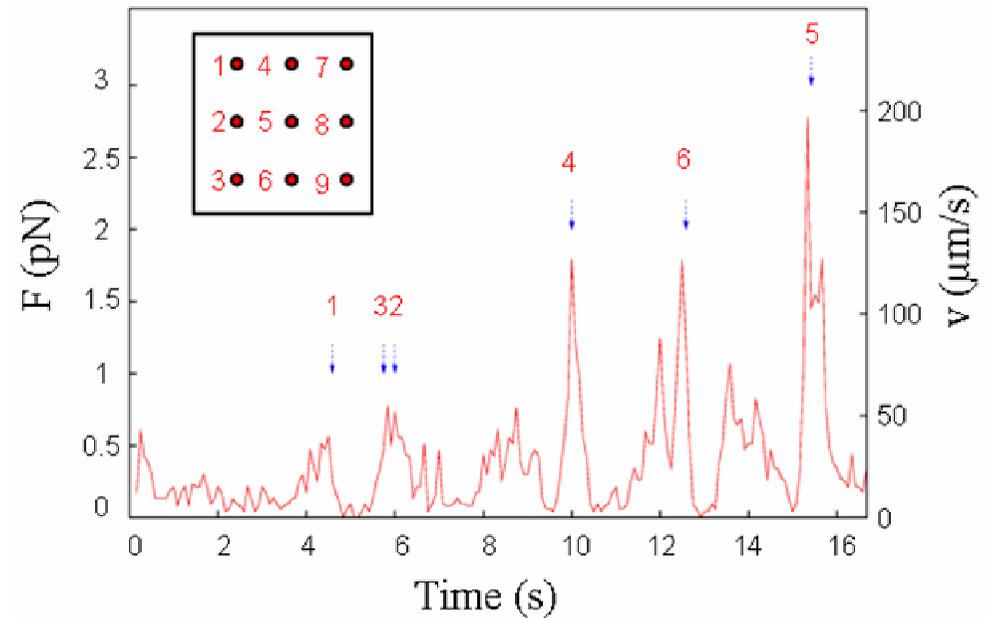
(d,e) Viability assay of the same heterotypic microarray showing an image obtained by exciting propidium iodide labels with 488 nm. The lack of red fluorescence in (d) indicates viability, but after killing the cells with ethanol the fluorescence is intensely red (e).

G.M. Akselrod *et al* Biophys J 91, 3465 (2006)

Strength control of the trapping forces



Array of traps with different Strength of the trapping force



Trapping force and relative velocity bead-water for the array of 3x3 traps.

A. R. Moradi *et al*, JOAM, (2007), accepted

OPTICAL TRAP

SELECT AND MOVE A GIVEN CELL

REMOTELY APPLY CONTROLLED FORCES
ON LIVING CELLS, INTERNAL PARTS OF CELLS,
AND LARGE BIOLOGICAL MOLECULES WITHOUT
OPTICAL DAMAGE

MEASURE MECHANICAL PROPERTIES
(FOR INSTANCE, ELASTICITY) OF DIFFERENT
PARTS OF CELLS

MEASURE THE FORCES GENERATED BY SINGLE
MOTOR MOLECULES IN THE PICONEWTON RANGE

USING OPTICAL SPECTROSCOPY TO MEASURE

TEMPERATURE, DNA STRUCTURE

CELL VIABILITY, INTRACELLULAR pH
OF A GIVEN SINGLE CELL

Acknowledgements

- **Organizers of the Winter College for inviting me to give this lecture**
- **Enrico Ferrari, Valeria Garbin, Enzo Di Fabrizio, LILIT @TASC**
- **Prof. D. Petrov from ICFO Barcelona, for permission to use his lectures/slides**
- **To all the people not mentioned here, whose material I could find and use**
- **Special thanks to ... Arthur Ashkin of course ! 😊**

I hope that you agree with David Hilbert (1930):

“We should know and we will know”

(with Optical Trapping and Manipulation in mind 😊)

Thank You for Your Patience !