Optical Tweezers: basics and applications

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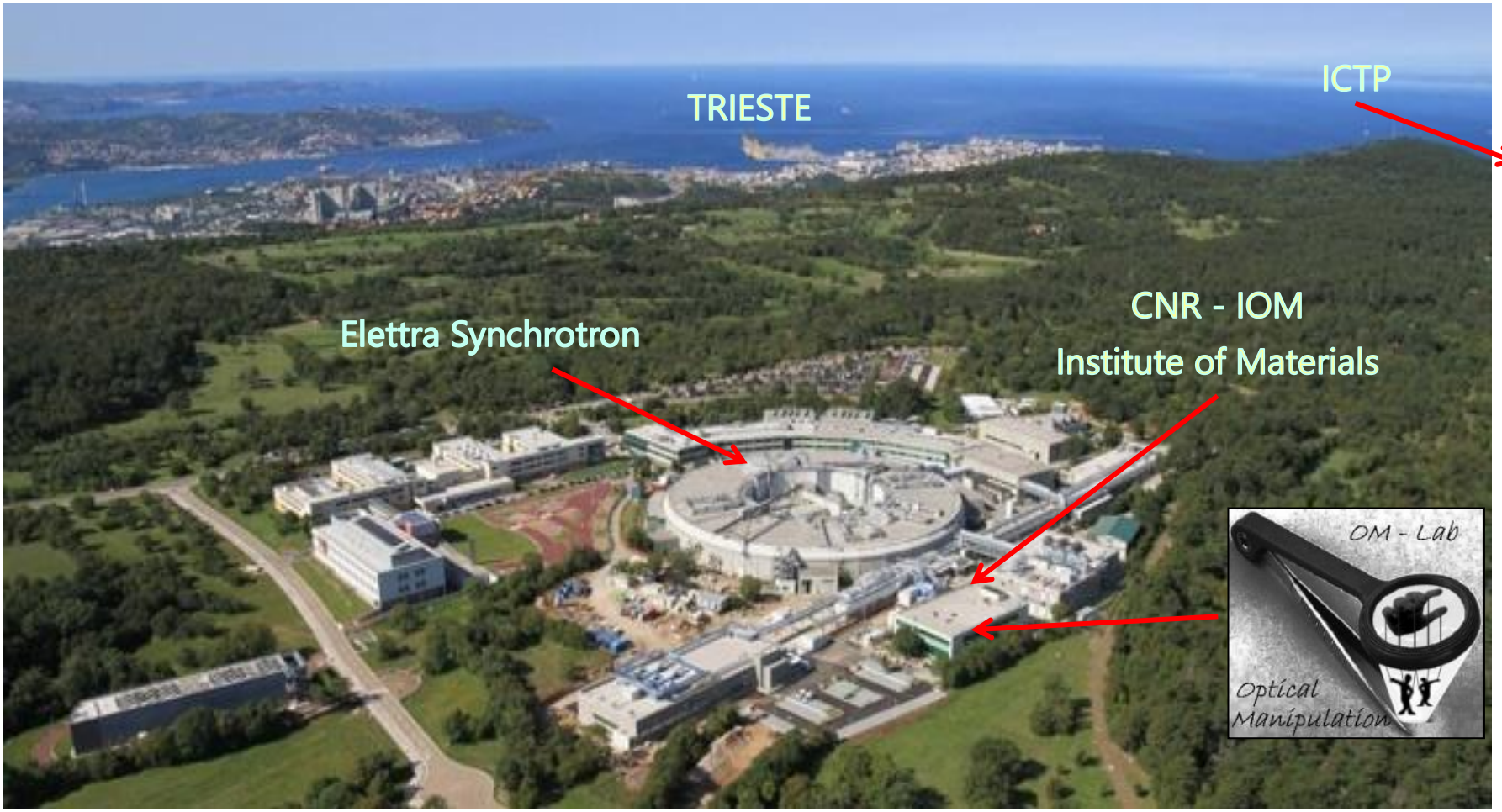


Winter College on Optics: Advanced Optical Techniques for Bio-imaging
13 - 24 Feb 2017, ICTP - Trieste

OUTLINE

- **Optical Tweezers (OT) – single-beam gradient force 3D optical trap: how this works, optical manipulation of microparticles**

- **Measuring picoNewton forces with OT: direct and indirect methods**

- **Applications of OT in living cell studies:**

  - **probing forces expressed by developing neurons**
  - **probing the stiffness of cancer cells**
  - **mechanotransduction - conversion of the mechanical stimulus into a biochemical signal by the cell**
  - **biochemical local cell stimulation using optically manipulated vectors** (coated beads, biodegradable micro-sources, liposomes)

Radiation pressure of light

A photon has the energy: $E = h\nu$ **and carries momentum :** $p = E / c$

- Momentum can be transfered to an object by interaction (e.g. reflection)
- **Radiation pressure:** the pressure exerted by light on the object surface

- For a light wave beam, carrying a momentum flux: $$d\left(\frac{d\vec{P}}{dt}\right) = \vec{S}\, dS$$

$\vec{S}$ - the Poynting vector, dS - element of area normal to $\vec{S}$

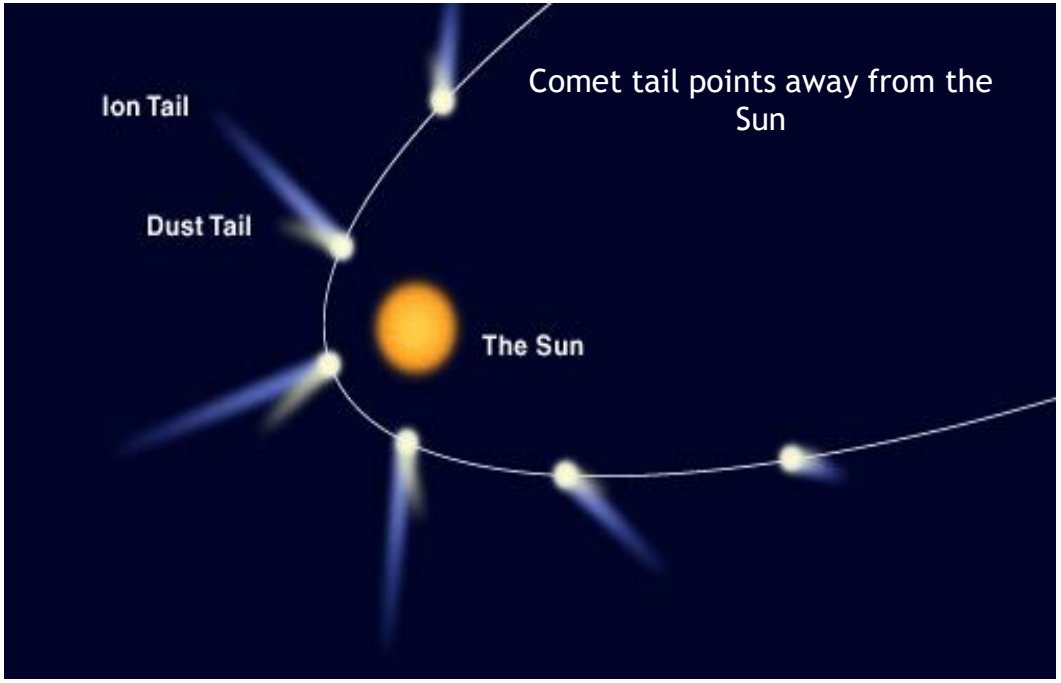
the radiation pressure is: $P_R = \langle S \rangle / c$ measured in [N/m^2]

Radiation Pressure = The momentum transferred per second per unit area =

= Energy deposited per second per unit area / c

- Forces generated by radiation pressure of light are in general very small and hence difficult to be detected --> use of laser light (LASER) which is able to generate high optical intensities and high optical intensity gradients.

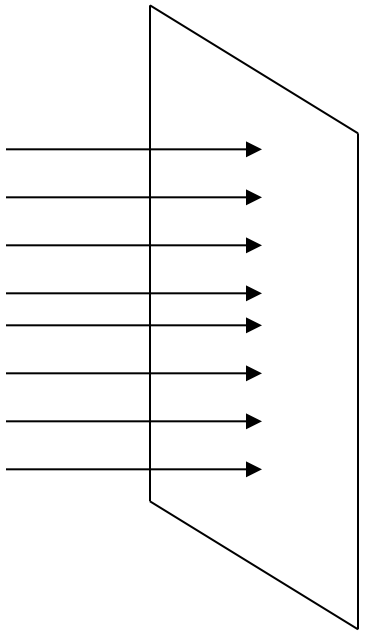
Radiation Pressure of Light



The comet tail is always pointing away from the Sun due to radiation pressure

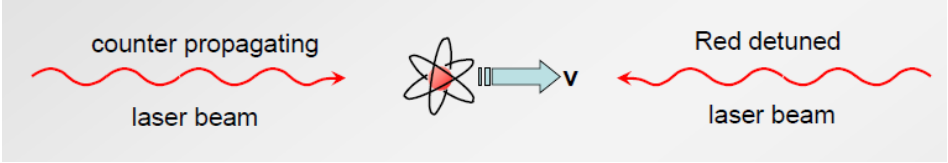
Kepler 1619

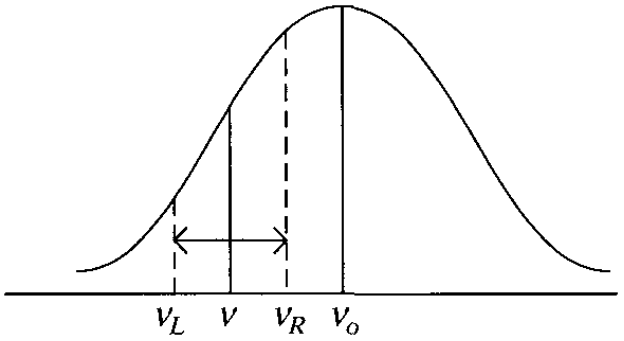
Light



Radiation pressure of Sunlight on the Earth is in average 4.6 [μPa]

Doppler cooling/damping atomic motion -- > optical molasses



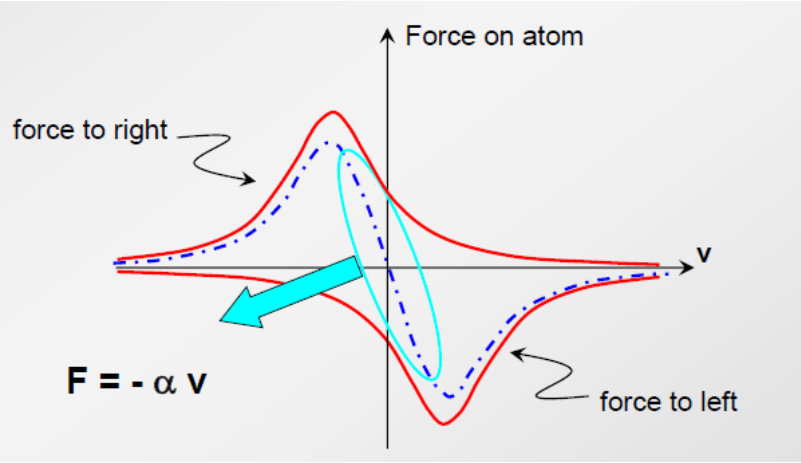


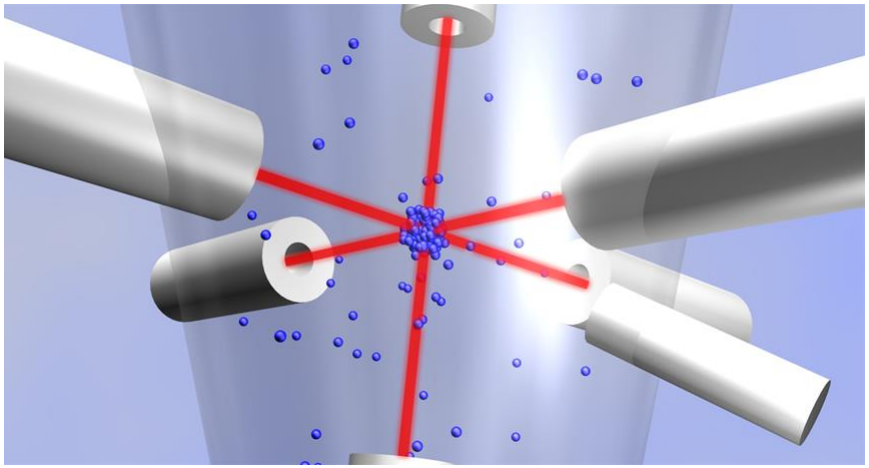
- laser beams tuned slightly below resonance

- atoms will absorb more photons if they move towards the light source, due to the Doppler effect

$\nu < \nu_o$ $\qquad F = -\gamma v$

in three dimensions $\longrightarrow$ optical molasses





Hänsch, T. W. & Schawlow, A. L. Cooling of gases by laser radiation. Opt. Commun. 13, 68-69

How big is the force exerted by a ray of light on a microbead ?

Geometrical optics approximation --> light rays

- (bead diam) d > λ (light wavelength)
- reflection coefficient R= 1
- d = 2 [μm], λ= 0.5 [μm]

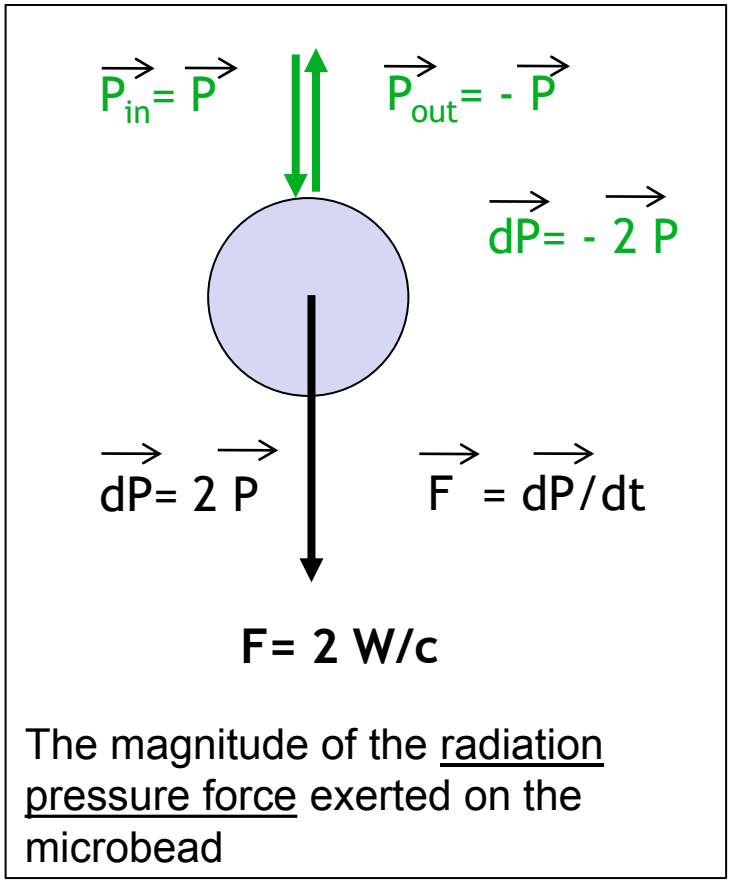
The magnitude of the momentum associated to the ray of light:

$$P = E / c \;\; ; \;\; E = N h v$$

P - momentum; E- energy;

c - light velocity in vacuum ; h - Plank constant;

v - light frequency; W - power of the light ray W= dE/dt



The magnitude of the radiation pressure force exerted on the microbead

N= 1 photon, -> E≈ 2.5 eV, W≈ 4 x 10^{-19} W -> F≈ 2.7 x 10^{-27} N - very small

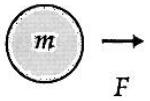
N= 10^{15} photons, W ≈ 0.4 mW, F≈ 2.7 x 10^{-12} N = 2.7 pN - **SMALL**

Is the magnitude of this force significant ?

Microbead in free space (vacuum)):

F≈ 2.7 x 10^{-12} N = 2.7 pN - SMALL , but also the mass of the microbead is small,

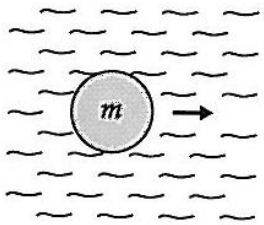
m ≈ 8 pg --> acceleration **a**≈ F/m = 3.4 x 10^{2} [m/s^2] = **34 g** , which is very **BIG** !



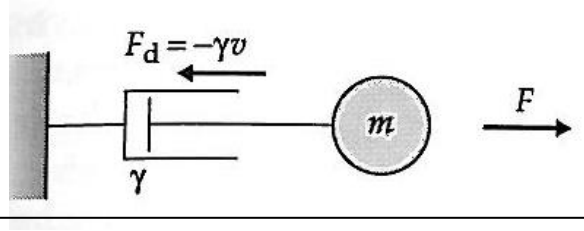
Microbead in water:

refractive index : n_m = 1.33; drag coefficient : γ= 10 nN s/m;

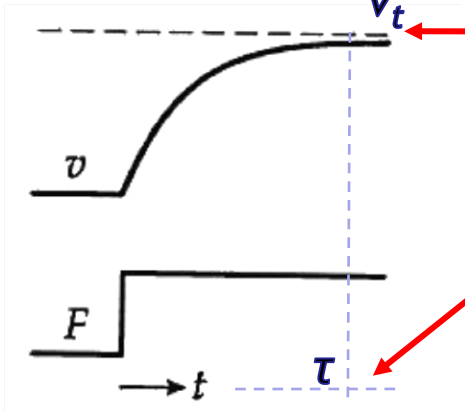
force by light : F= 2 n_mW/c ; **F≈ 3.6 pN**



mass + dashpot model



$$m\frac{dv}{dt} = F - \gamma v \qquad v(t) = \frac{F}{\gamma}\left[1 - \exp\left(-\frac{t}{\tau}\right)\right]$$



terminal velocity, v_t: $v_t = \frac{F}{\gamma}$ **= 360 [µm/s]**

time constant, τ: $\tau = \frac{m}{\gamma}$ **= 0.8 [µs]**

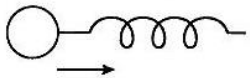
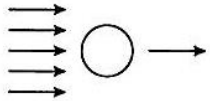
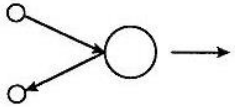
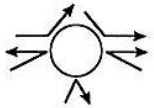
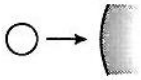
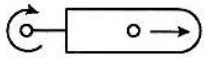
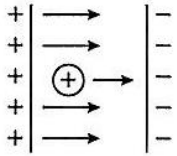
for a small size particle dumping is dominant over inertia

because: *m* -> d^3 , *γ*-> *d*

An example from biology: the movement of a bacterium in water. The bacterial motor must be able to generate force > 0.5 pN to swim through water and stops immediately when motor stops.

Physical forces and their magnitudes at the single molecule level

Table 2.1 Examples of forces acting on molecules

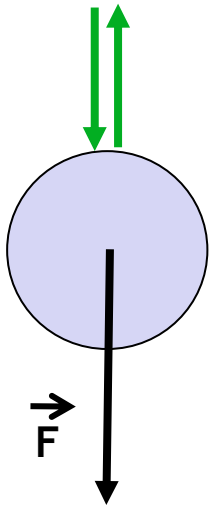
Type of force	Diagram	Approximate magnitude
Elastic		1–100 pN
Covalent		10,000 pN
Viscous		1–1000 pN
Collisional		10^{-12} to 10^{-9} pN for 1 collision/s
Thermal		100–1000 pN
Gravity		10^{-9} pN
Centrifugal		$< 10^{-3}$ pN
Electrostatic and van der Waals		1–1000 pN
Magnetic		$<< 10^{-6}$ pN

pN

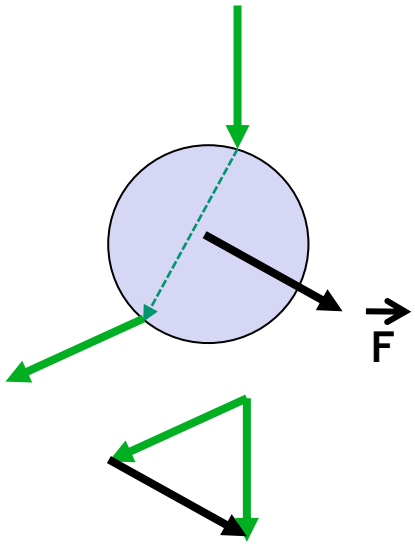
J. Howard, Mechanics of motor protein and the cytoskeleton, Sinauer Associates Inc. , 2001

Force induced by a ray of light by refraction on a bead in water

reflection only
R=1



refraction only,
R=0; $n_b > n_m$



The magnitude of the force:

$$F = Q\, n_m W_{in}/c$$

the incident momentum /s of a ray of power W_{in}

Q - dimensionless factor, $Q \leq 2$
Q - function of shape, material

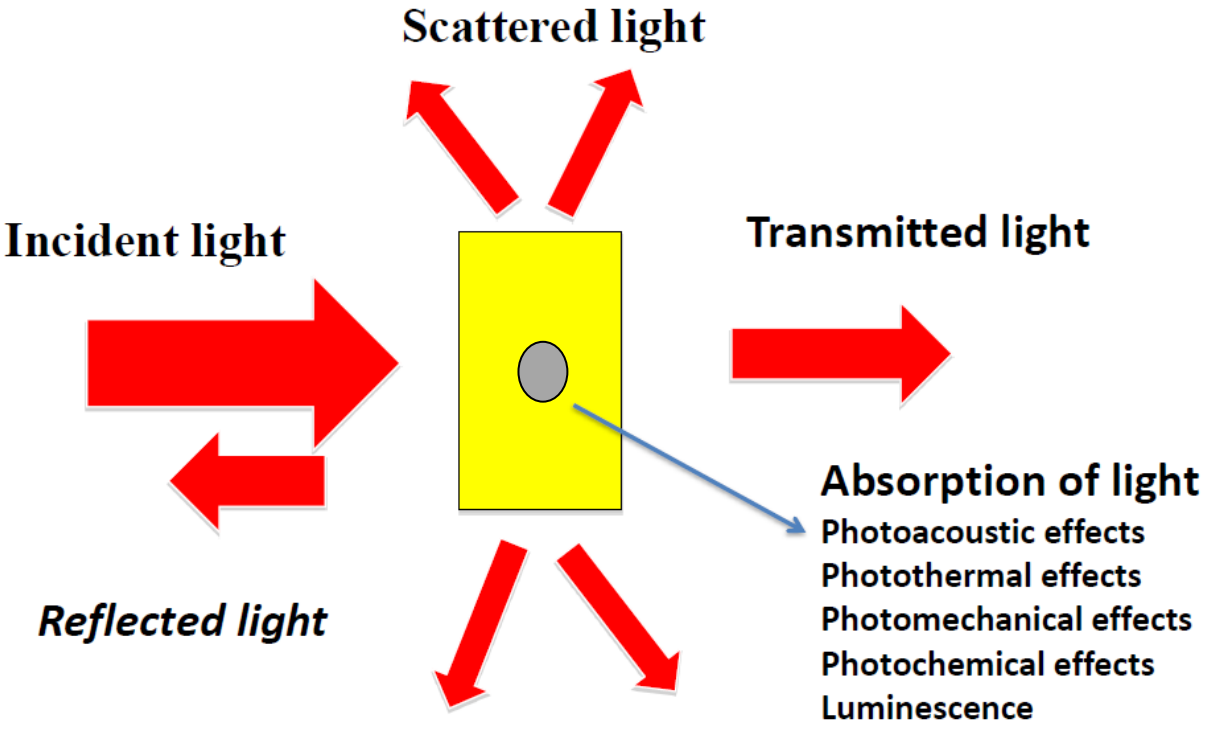
The total force on a particle interacting with an incident light beam (reflection, scattering, refraction, absorption, emission) is given by the difference between the momentum flux entering the object and the one leaving it:

$$\vec{F} = \frac{n_m}{c} \int_S \left(\vec{S}_{in} - \vec{S}_{out} \right) dS$$

In principle it is possible to directly calculate / measure the force on a particle using the light momentum flux through it.

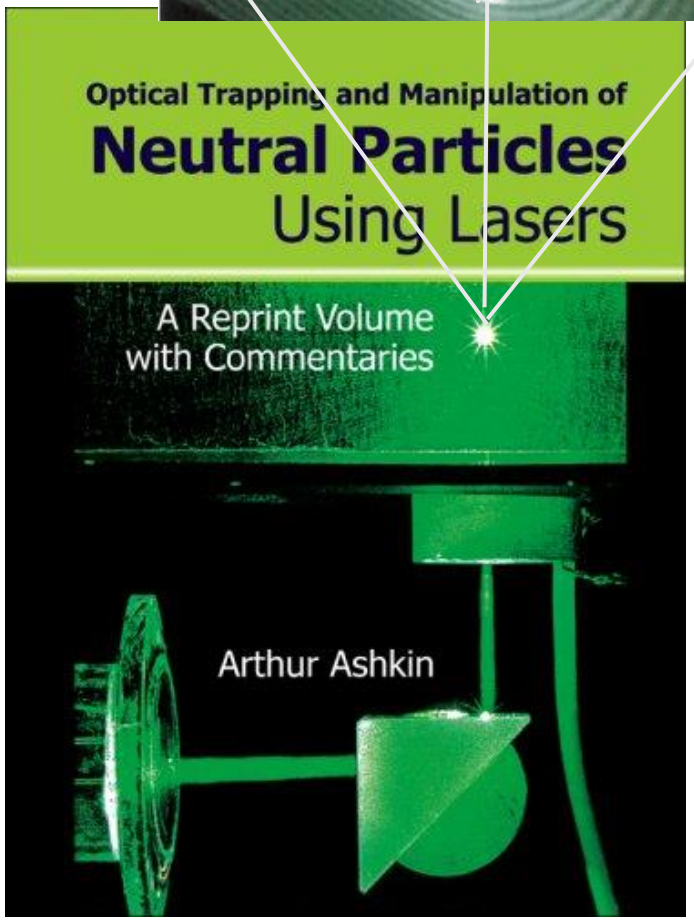
Sample interacting with light --> effects

Energy conservation, momentum conservation



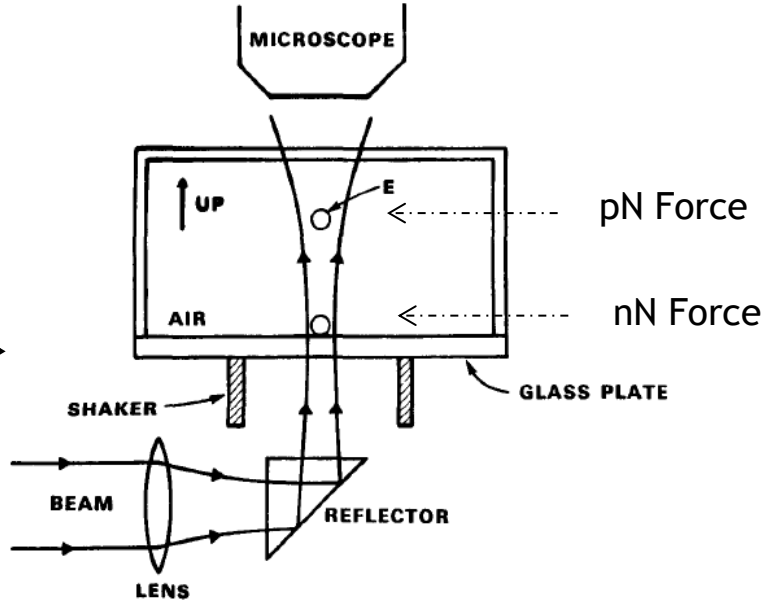
adapted from slide of the lecture on PhotoThermal Lens by Prof. Aristides Marcano

Mie scattering pattern



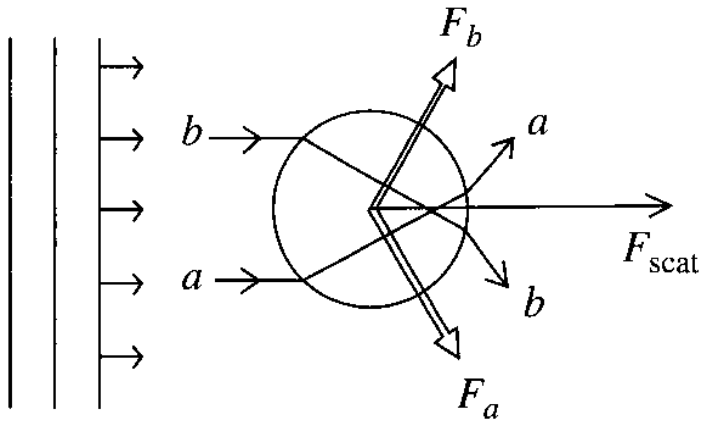
Warmly recommended book

Arthur Ashkin - Scientific Publishing 2006



Optical levitation of microparticles in air (hollow,diam 50-75 um)

Simplified ray optics diagrams of the scattering force and gradient force components of the radiation force on a dielectric Mie particle ($d > \lambda$)



Plane wave

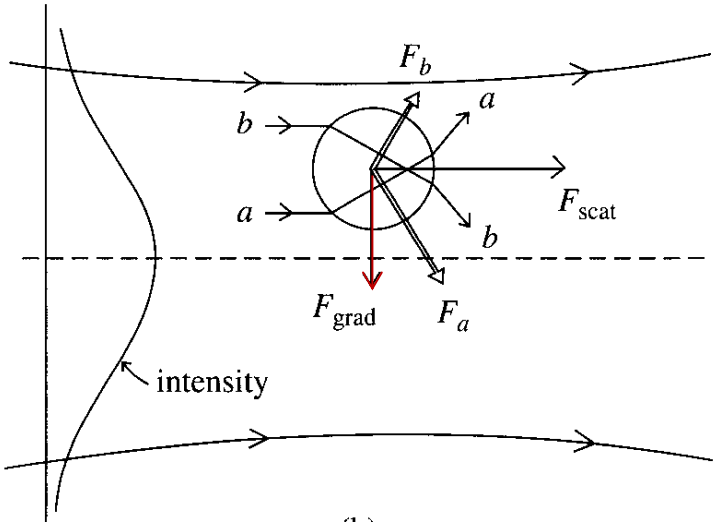
high index particle $n_p > n_m$

Origin of the scatterring force - $\mathbf{F_{scat}}$

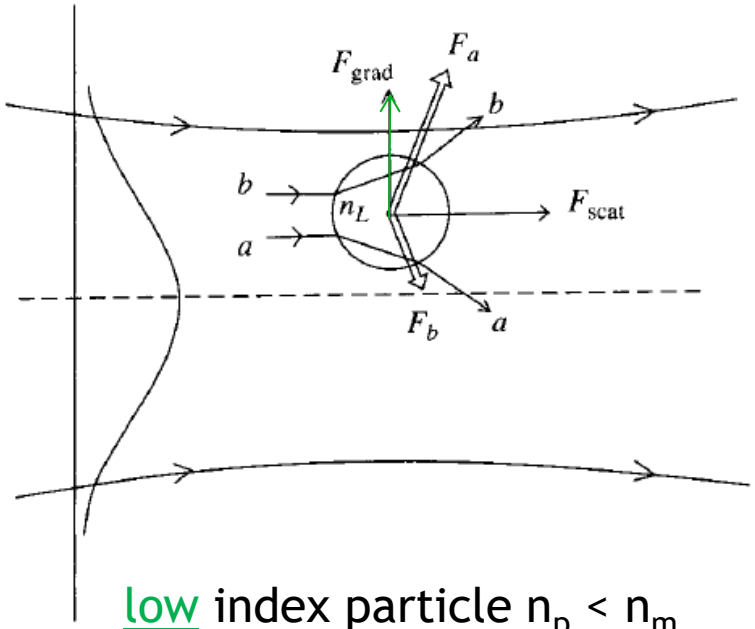
in the direction of the intensity of the incident plane wave beam

Ashkin 1970 -> Ashkin Book (2006)

Scattering and gradient forces



high index particle $n_p > n_m$



low index particle $n_p < n_m$

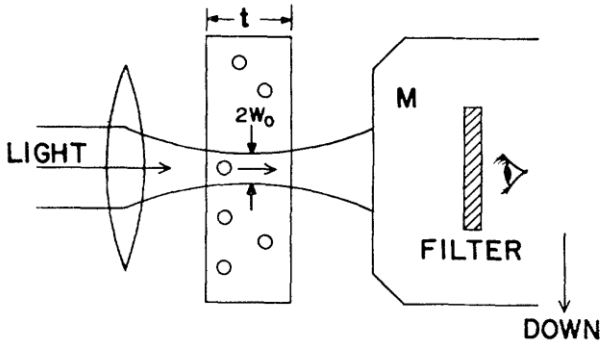
mildly focused Gaussian wave beam

Origin of the transverse gradient force component - $\mathbf{F_{grad}}$

for a particle located off-axis

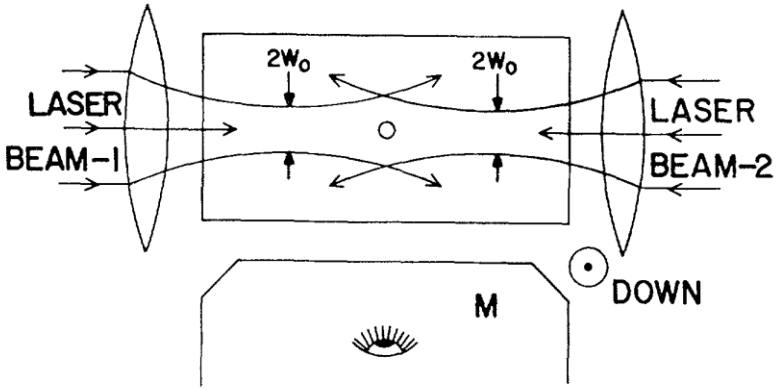
A. Askin, Acceleration and trapping of particles by radiation pressure

Phys. Rev. Lett. **24** 156 **1970**



2D trapping

Single laser beam focused through a lens with low NA



3D trapping

Counter propagating laser beams

Experimental results: dielectric microparticles in water and water droplets in air

It is hypothesized that similar acceleration and trapping are possible with atoms and molecules using light tuned to specific transitions.

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

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, *Opt.Lett.* 11, 288 (1986)

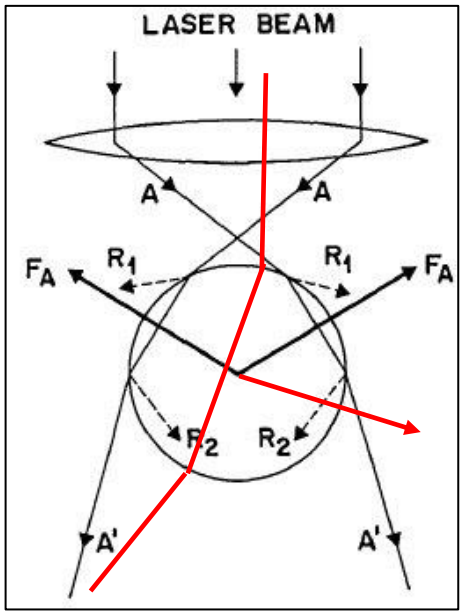
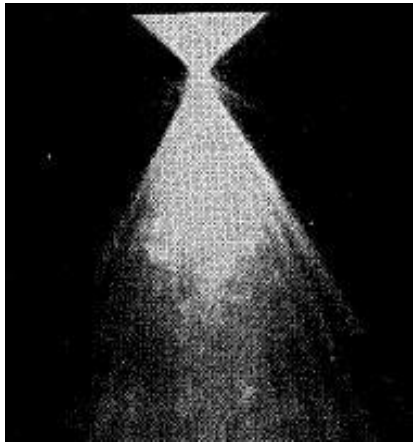
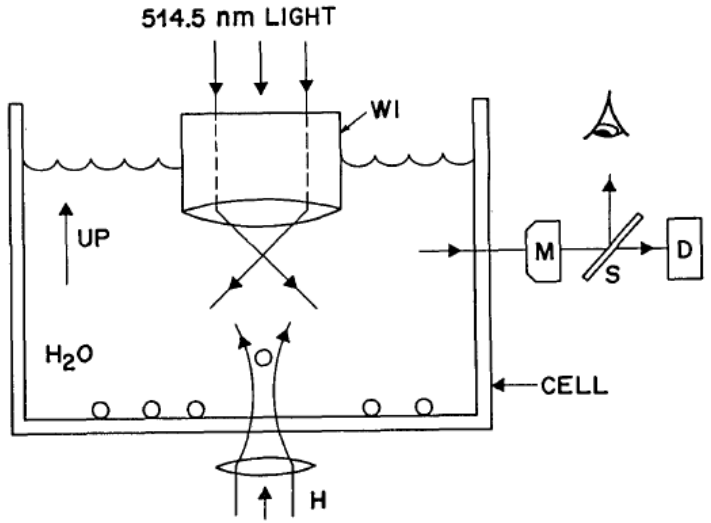


Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single-beam gradient force trap.



Photograph taken in red (water fluorescence), of a 10 um sphere trapped with an argon laser beam, showing the paths of the incident and scattered light rays.



Sketch of the basic apparatus used for optical trapping.

Size of particles (polystyrene beads) experimentally trapped:

10 um (Mie) to 25 nm (Rayleigh)

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

A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, *Opt.Lett.* 11, 288 (1986)

Forces on submicrometric Rayleigh particles:

Gradient Force

$$\boldsymbol{F_{grad}} = (\boldsymbol{P}\cdot\boldsymbol{\nabla})\cdot E = \frac{\mathbf{1}}{\mathbf{2}}\boldsymbol{\alpha}\ \boldsymbol{\nabla E^2}$$

$$F_{\text{grad}} = -\frac{n_b}{2}\alpha\nabla E^2 = -\frac{n_b^3 r^3}{2}\left(\frac{m^2-1}{m^2-2}\right)\nabla E^2$$

P - polarization vector,
α - polarizability
E - optical electric field

Scattering Force

$$\boldsymbol{F_{scat}} = \boldsymbol{P_{scat}/c}$$

$$F_{\text{scat}} = \frac{I_0}{c}\frac{128\pi^5 r^6}{3\lambda^4}\left(\frac{m^2-1}{m^2+2}\right)^2 n_b$$

Io – incident beam intensity
r – particle radius

Conditions for trapping stability

axial stability

$$R = \frac{F_{\text{grad}}}{F_{\text{scat}}} = \frac{3\sqrt{3}}{64\pi^5}\frac{n_b^2}{\left(\frac{m^2-1}{m^2+2}\right)}\frac{\lambda^5}{r^3 w_0^2} \geq 1$$

Size that can be trapped (polystirene latex):
14 nm (theory)
25 nm (experimental)

transverse stabiltiy

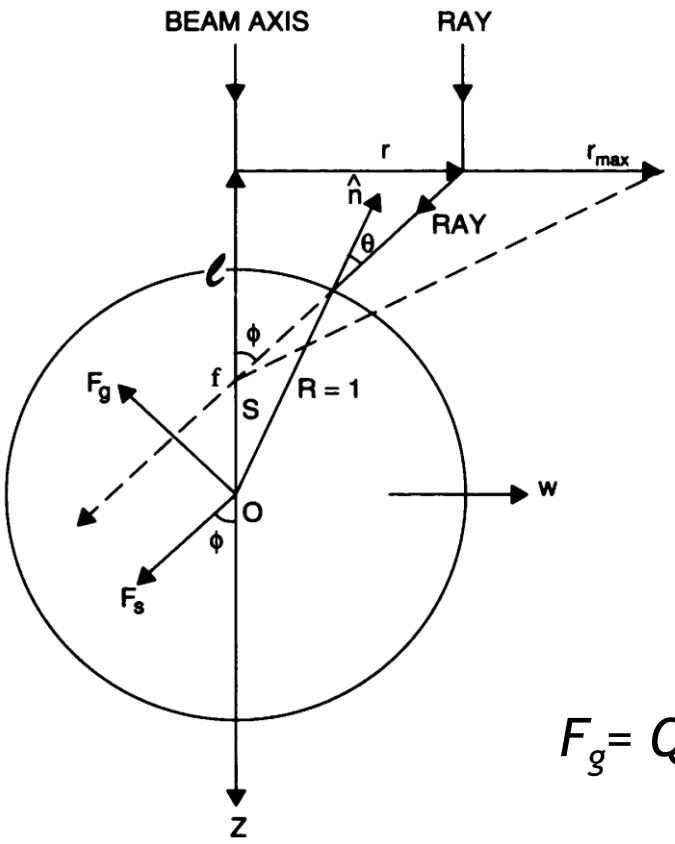
exp(- U/kT) << 1 --> U> 10kT,

where $U = n_b \alpha E^2/2$ is the potential of the gradient force

the time to pull a particle into the trap should be less than

the time for the particle to diffuse out of the trap by Brownian motion

Geometry of an incident ray giving rise to gradient and scattering force contributions F_g and F_s



$$F_s = \frac{n_1 P}{c} \times \underbrace{\left\{1 + R\cos 2\theta - \frac{T^2[\cos(2\theta - 2r) + R\cos 2\theta]}{1 + R^2 + 2R\cos 2r}\right\}}_{Q_s}$$

$$F_g = \frac{n_1 P}{c} \times \underbrace{\left\{R\sin 2\theta - \frac{T^2[\sin(2\theta - 2r) + R\sin 2\theta]}{1 + R^2 + 2R\cos 2r}\right\}}_{Q_g}$$

$$F_g = Q_g\, n_1 P/c$$

$$F_s = Q_g\, n_1 P/c$$

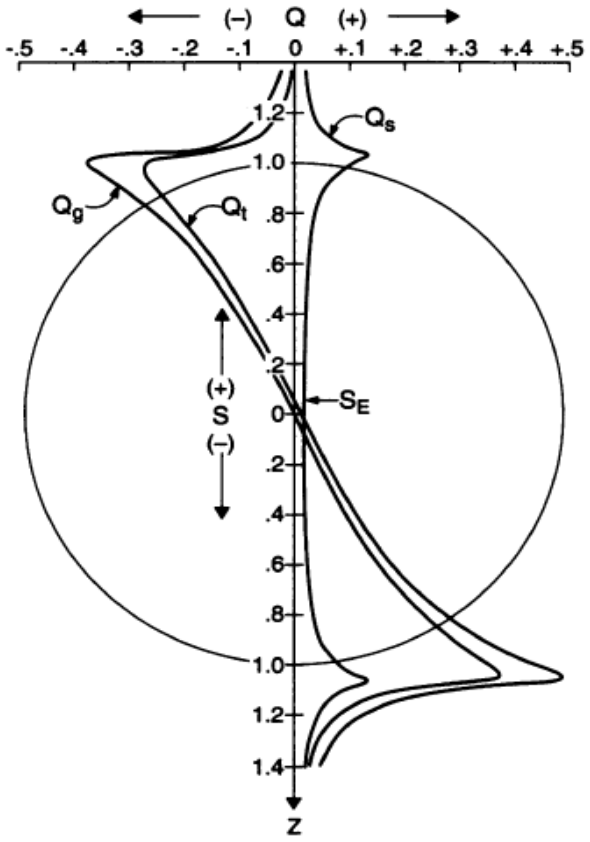
$$F = F_s + F_g = Q\, n_1 P/c$$

$$Q = \sqrt{\left(Q_g^2 + Q_s^2\right)}$$

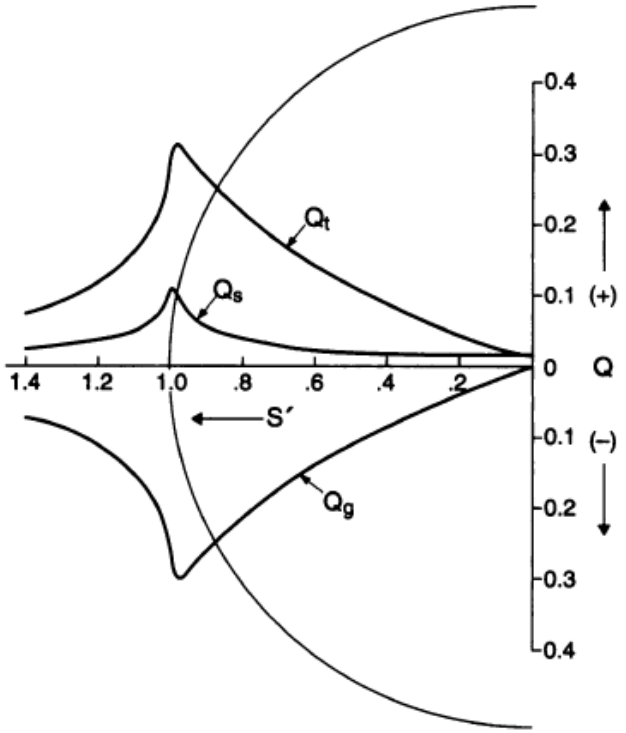
A. Ashkin, *Biophys. J.* 611, 569 (1992)

Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime

Which is the magnitude of the forces experienced by a particle close to the trap ?



axial



transverse

A. Ashkin, *Biophys. J.* 611, 569 (1992)

Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime

Why the way to the single-beam gradient force optical trap took 16 years ? (1970 - 1986)

Ashkin's dream was to trap atoms

A. Askin, Trapping of atoms by resonance radiation pressure
Phys. Rev. Lett. 40, 729 (1978)

A method of stably trapping, cooling, and manipulating atoms on a continuous-wave basis is proposed using resonance radiation pressure forces. Use of highly focused laser beams and atomic beam injection should give a very deep trap for confining single atoms or gases at temperatures $\sim 10^{-6}$ °K. An analysis of the saturation properties of radiation pressure forces is given.

Steven Chu, J. E. Bjorkholm, A. Ashkin, and A. Cable
Experimental Observation of Optically Trapped Atoms
Phys. Rev. Lett. 57, 314 , (1986)

We report the first observation of optically trapped atoms. Sodium atoms cooled below 10^{-3} K in "optical molasses" are captured by a dipole-force optical trap created by a single, strongly focused, Gaussian laser beam tuned several hundred gigahertz below the D_1 resonance transition. We estimate that about 500 atoms are confined in a volume of about 10^3 μm^3 at a density of 10^{11}–10^{12} cm^{-3}. Trap lifetimes are limited by background pressure to several seconds. The observed trapping behavior is in good quantitative agreement with theoretical expectations.

Optical trapping contributed to:

1997 Nobel Prize in Physics

Steven Chu, Claude Cohen-Tannoudji and William D. Phillips

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

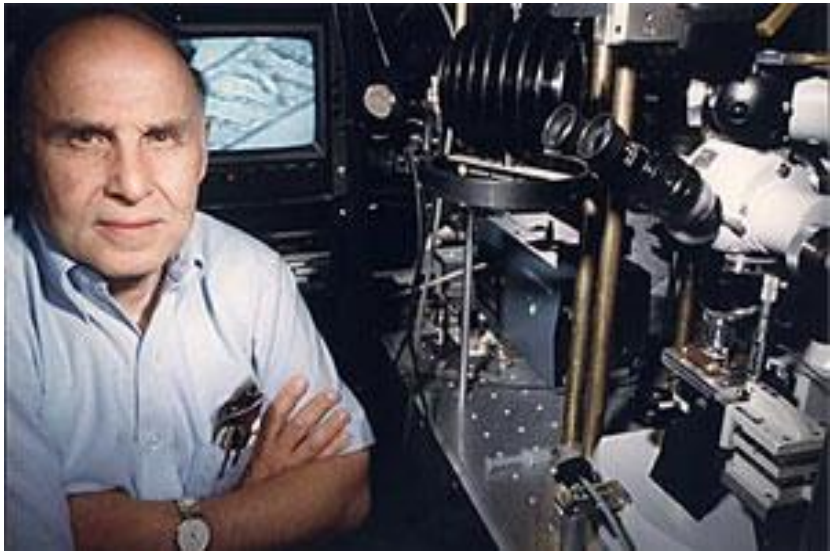
2001 Nobel Prize in Physics

Eric A. Cornell, Wolfgang Ketterle and Carl E. Wieman

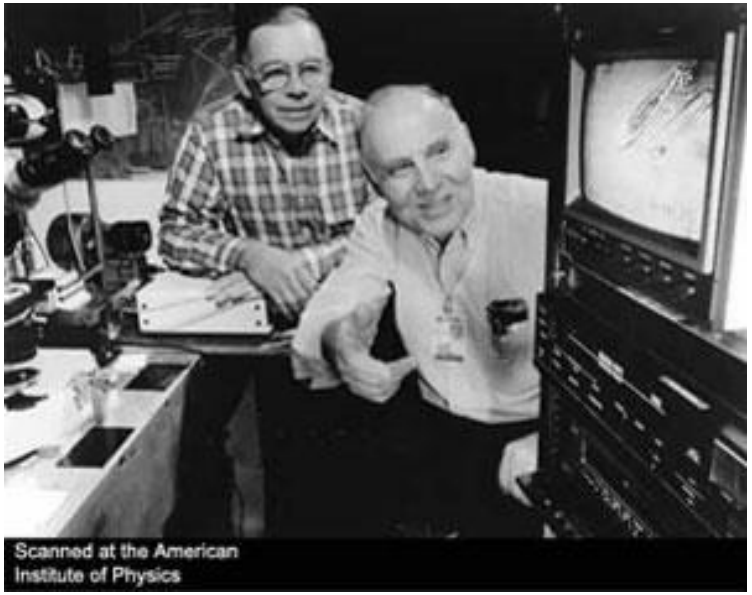
"for the achievement of Bose-Einstein condensation in dilute gases of alkali atoms, and for early fundamental studies of the properties of the condensates"

Ashkin, meanwhile, focused on using optical tweezers to trap and study various living things, including the tobacco mosaic virus, various bacteria, red blood cells, and algea, without damaging them. He went on to probe the internal cell structure, using his tweezers to manipulate the cell's cytoplasm and organelles in what he describes as "a form of internal cell surgery."

Arthur Ashkin at Bell Labs (1986)



Ashkin and Dziedzic



http://laserfest.org/lasers/pioneers/ashkin.cfm

Optical Tweezers - properties in a slide

Types of particle:

- **Material:**

Dielectric (polystyrene, silica); Metallic (gold, silver, copper), Biological (cells, macro-molecules, intracellular structures, DNA filaments), Low index (ultrasound agent contrast); crystal or amorphous material.

- **Size:** 20 nm – 20 μm
- **Shape:** spherical, cylindrical, arbitrary

Types of laser beam:

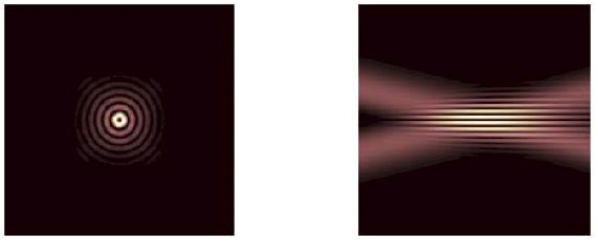
- Gaussian

- Laguerre-Gaussian

LG carries also orbital angular momentum that can be transferred to the trapped particles and make move on the ring and spin around their axis.

- Bessel

(non diffracting beam)



x-y beam intensity z

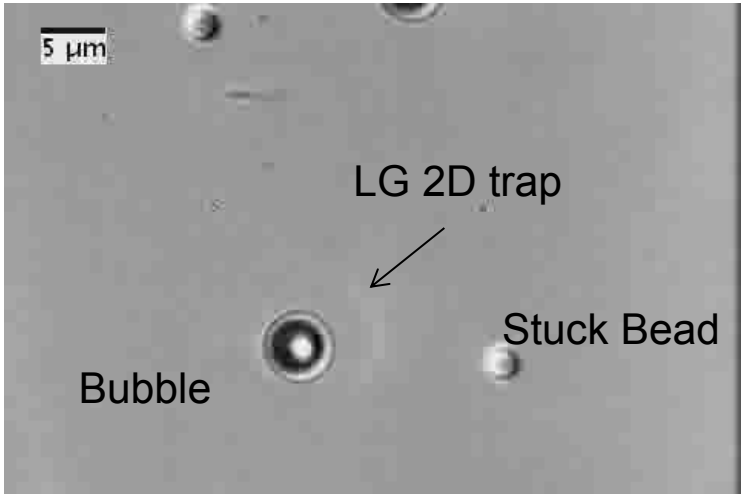
Particles can be trapped in a bottle since the beam reconstructs itself

Range of forces that can be applied and measured : 0.1 - 300 pN

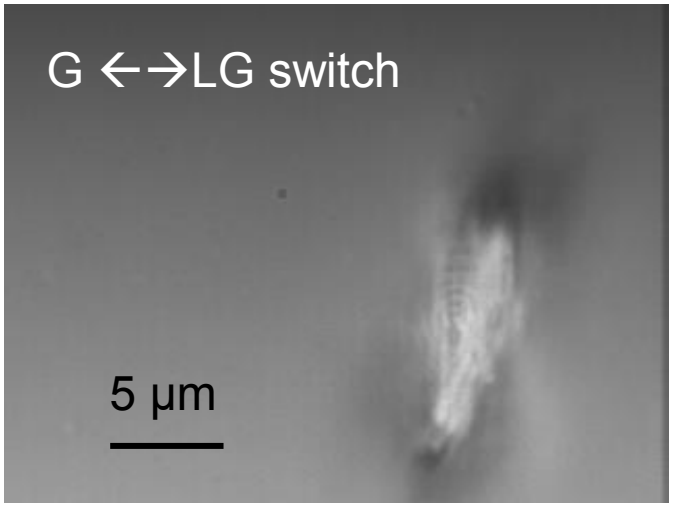
Reviews: Svoboda and Block, Annu. Rev. Biophys. 1992; Neuman and Block, Rev. Sci. Instr. 2004;
Grier, Nature 2003; Moffit, Chemla, Smith and Bustamante, Annu. Rev. Biochem. 2008; Neuman and Nagy, Nat. Meth. 2008; Bendix, Jauffred, Norregaard and Oddershede, IEEE J. Sel. Topics in Quantum Electronics 2014

Some examples from OM Lab

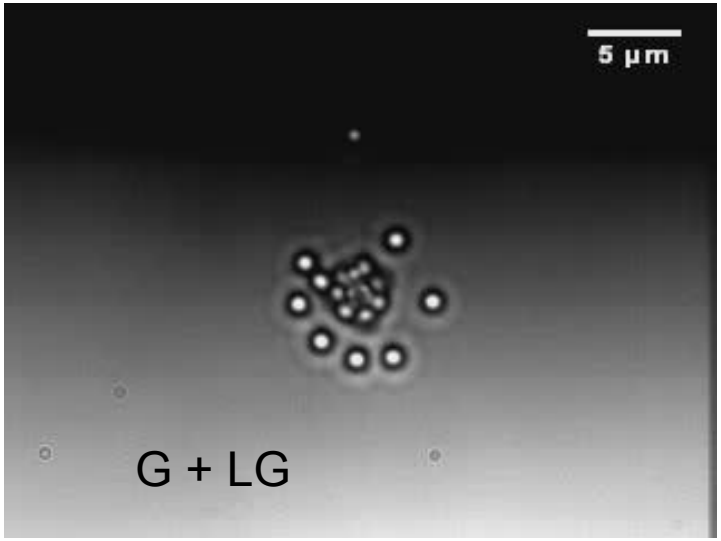
Ultrasound Contrast Bubble – LG 2D trap



Very simple rotor - piece of glass



LG OAM transfer to silica bead



OAM = Optical Angular Momentum

Garbin et al New J Phys 2009

Garbin et al, Appl Phys Lett 2007

Cojoc et al Microel. Eng. 2005

Optical trapping and manipulation of bioparticles / living cells

Are there sensitive issues when using optical tweezers to trap biological particles ?

1. The intensity at the trapping position (focal plane) is very high !
   Absorption of light by different components of a biological sample is wavelength dependent !

   Is the laser beam damaging the sample ?

   If yes, which is the level of damage ?

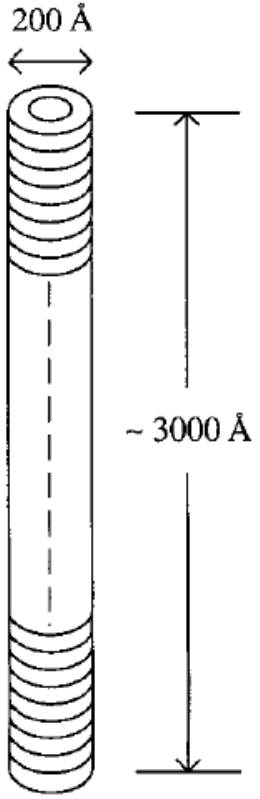
2. Biological samples (e.g. viruses, bacteria, cells) have arbitrary shapes while the laser beam is symmetric.

   Does this mismatch prevent trapping ?

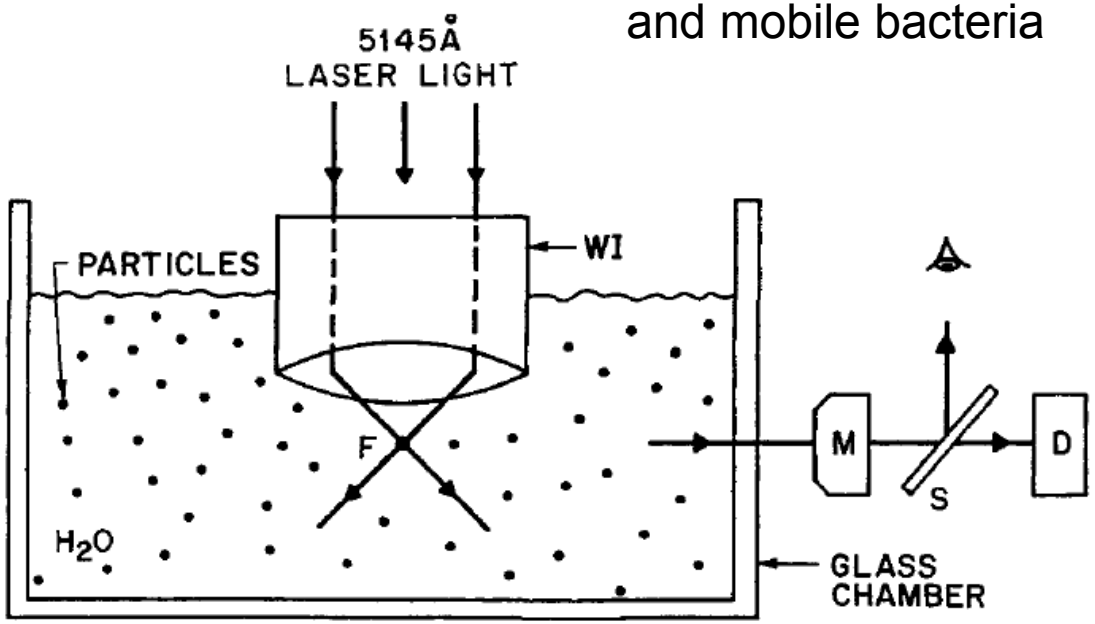
First optical trapping of a biological sample

Tobacco Mosaic Virus (TMV)

TMV
shape & size



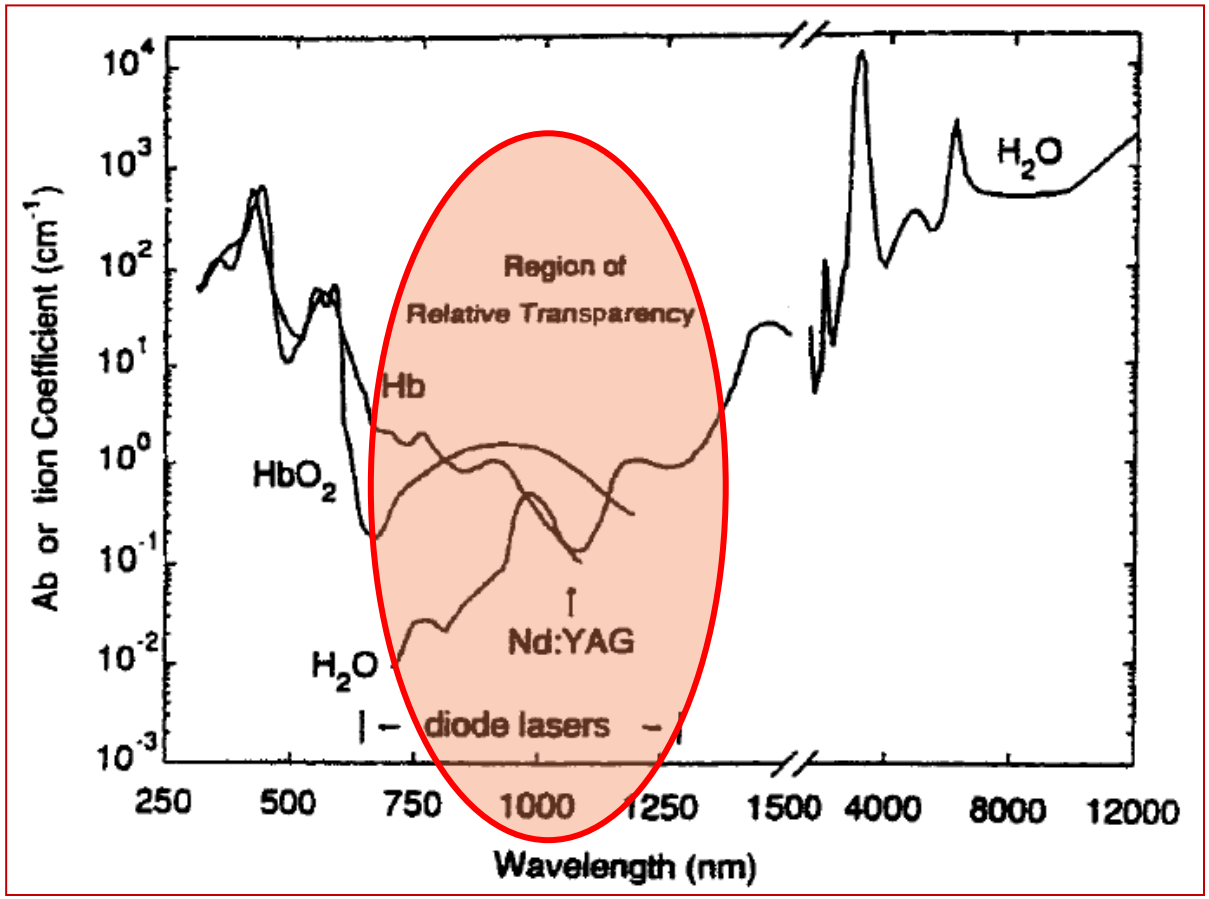
Apparatus used for optical trapping of TMV particles and mobile bacteria



- **Laser light scattering → detect the particles and their orientation with respect to the optical field orientation**
- **Laser power modulation + scattering → study damaging**

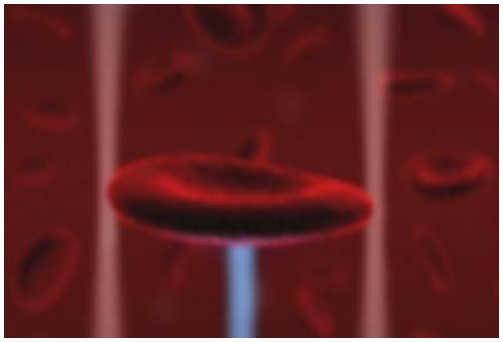
A. Ashkin and J.M. Dziedzic, "Optical trapping and manipulation of viruses and bacteria", *Science* 235, 1517 (1987)

Damaging free OTM of living cells → Infrared Laser

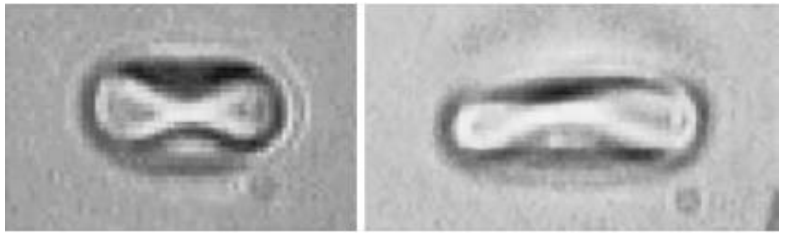


Plot of the optical absorption coefficients of hemoglobin (Hb), oxyhemoglobin (HbO2) and water versus the wavelength.

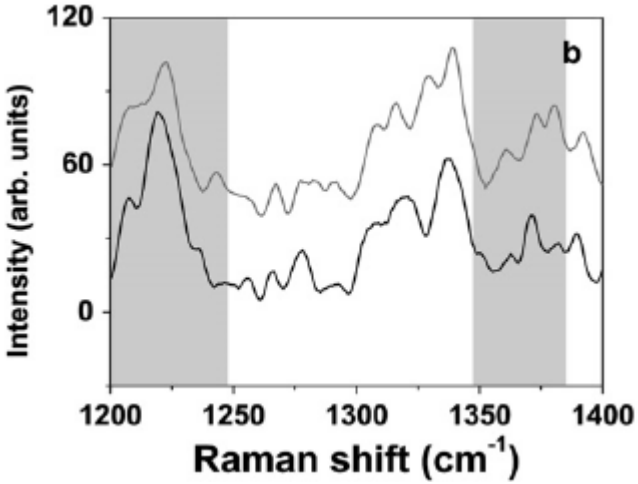
Example: Red Blood Cell (RBC) + OTM stretching + micro Raman



Cover



RBC trapped by two beams in the equilibrium (left) and stretched (right) conditions.



equilibrium (top)
stretched (bottom spectra)

The overall result reveals a bidirectional relationship between chemical binding and mechanical force in the oxygenation cycle of the Hb structure.

RBC with a significant oxygen concentration were pushed to a deoxy state when stretched with optical tweezers

S. Rao *et al*, Biophys. J. 96 (2009) 209

How can we get multiple optical traps / tweezers?

1. **time-sharing a single beam among several different locations**

   using galvano mirrors (GM), acousto-optic deflectors (AOD)

   - Allow to obtain: 2D arrays of dynamic traps; modulate the strength of the traps individually
   - GM are relatively cheap but have a lower frequency (kHz) and hence only few traps can be generated; AOD are more expensive but have a high frequency (MHz) and hence even tens of traps can be generated and controlled.

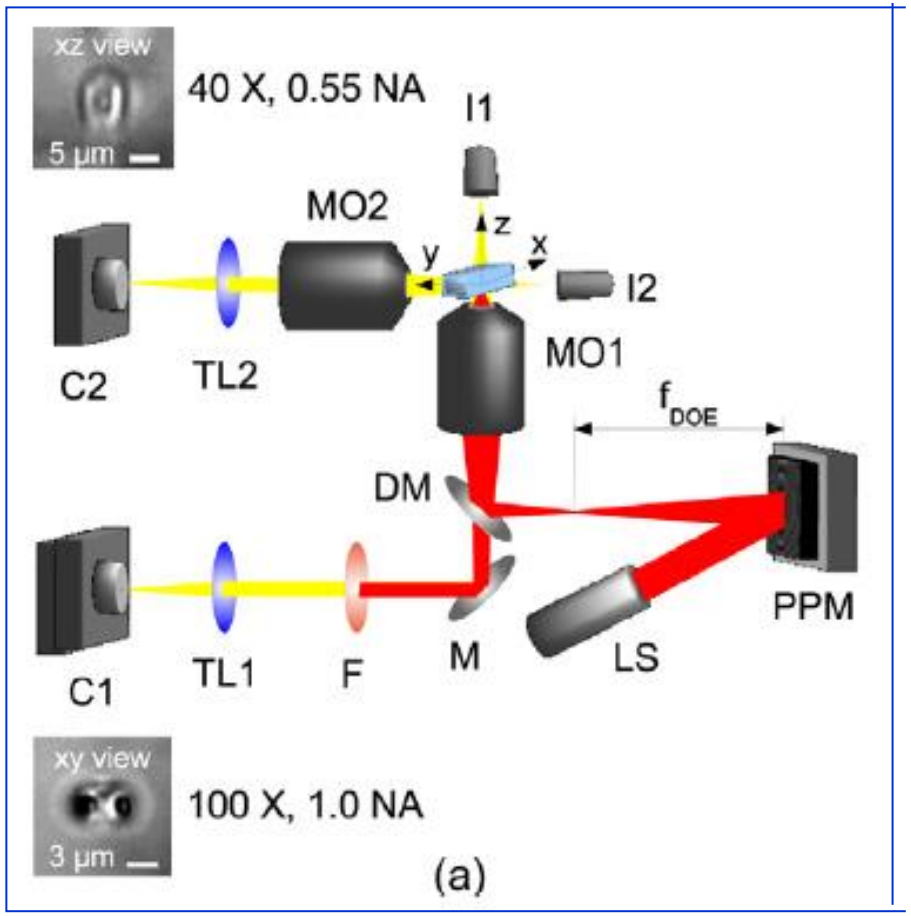
2. **split the beam into multiple beams**

   using beam-splitter (BS) or spatial light modulators (SLM)

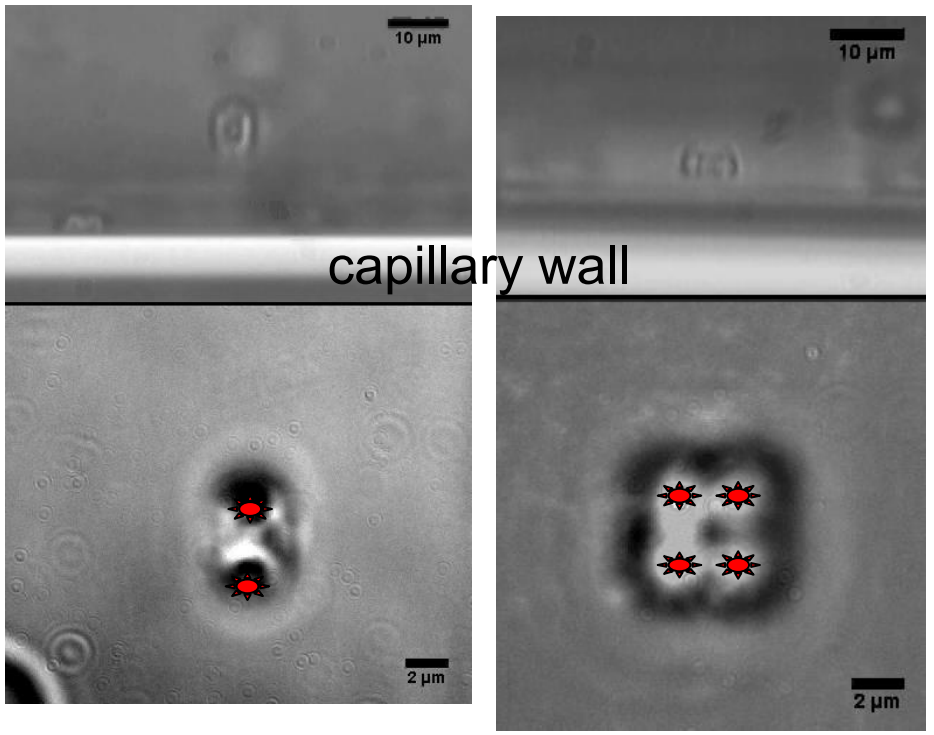
   - BS allow to obtain 2 fixed traps with fixed strengths;
   - SLM allows to obtain: 2D and 3D arrays of dynamic traps; modulate the strength of each trap individually; convert Gaussian beams to Laguerre-Gauss beams (to get helical-vortex beams) or Bessel beams

Single RBC - multiple traps – multiple view imaging

Optical setup



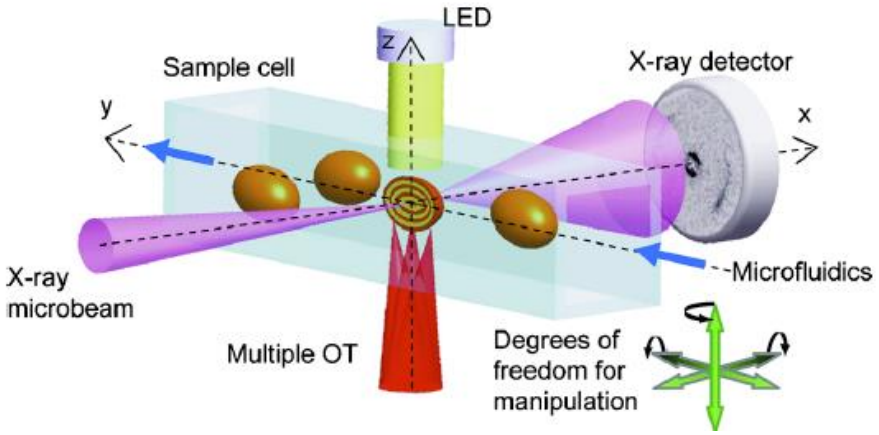
40X lateral view

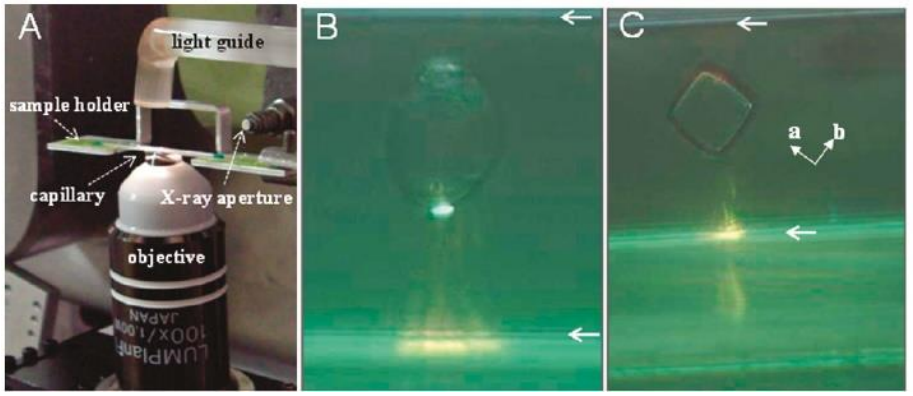


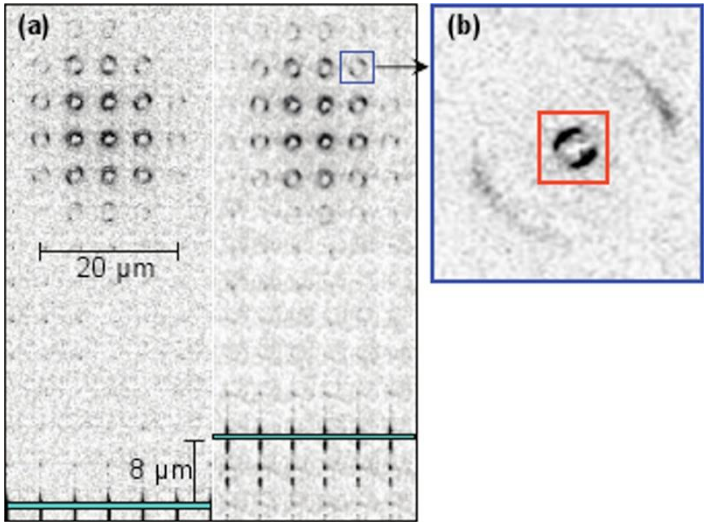
100X axial view

L. Selvaggi, A Moradi *et al*, J. Optics 12 (2010) 035303

Optical Manipulation of biological micro-objects for Synchrotron radiation probing

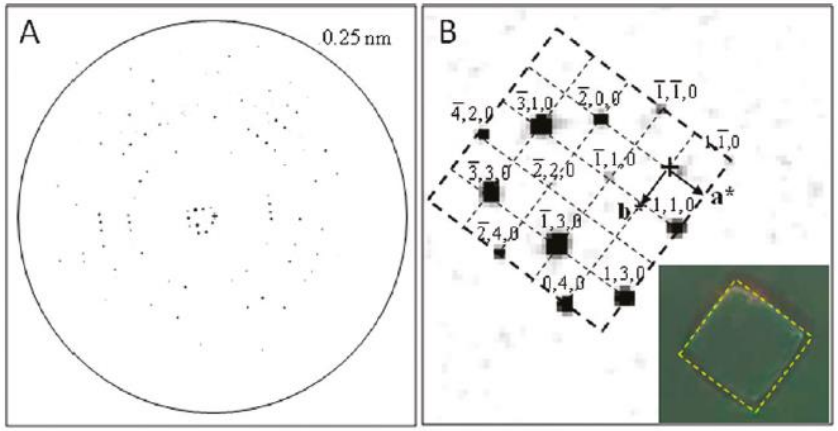






X-Ray diffraction pattern @ mutiple points 4 a starch granule OTM

D. Cojoc et al Appl. Phys. Lett. 2007 + 2010



X-Ray diffraction pattern of an insulin microcrystal OTM

S. Santucci et al, Anal. Chem. 2011

OUTLINE

- Optical Tweezers (OT) – single-beam gradient force 3D optical trap: how this works, optical manipulation of microparticles

- **Measuring picoNewton forces with OT : direct and indirect methods**

- Applications of OT in living cell studies:

  - probing forces expressed by developing neurons
  - probing the stiffness of cancer cells
  - mechanotransduction - conversion of the mechanical stimulus into a biochemical signal by the cell
  - biochemical local cell stimulation using optically manipulated vectors (coated beads, biodegradable micro-sources, liposomes)

Measuring picoNewton forces with OT : direct and indirect methods

Direct methods:

the force is measured by detecting light momentum changes

$$F = \alpha S$$

- α - conversion factor [N/V]
- *S* - electrical signal [V] $S_x = \psi \iint \frac{x}{R_D} I(x,y)\mathrm{d}x\mathrm{d}y$, *I* - radiant power

Indirect methods:

the force is measured by detecting bead position changes

$$F = k \cdot x$$

- k - trap stiffness [N/m]
- x - displacement of the bead in the trap [m]

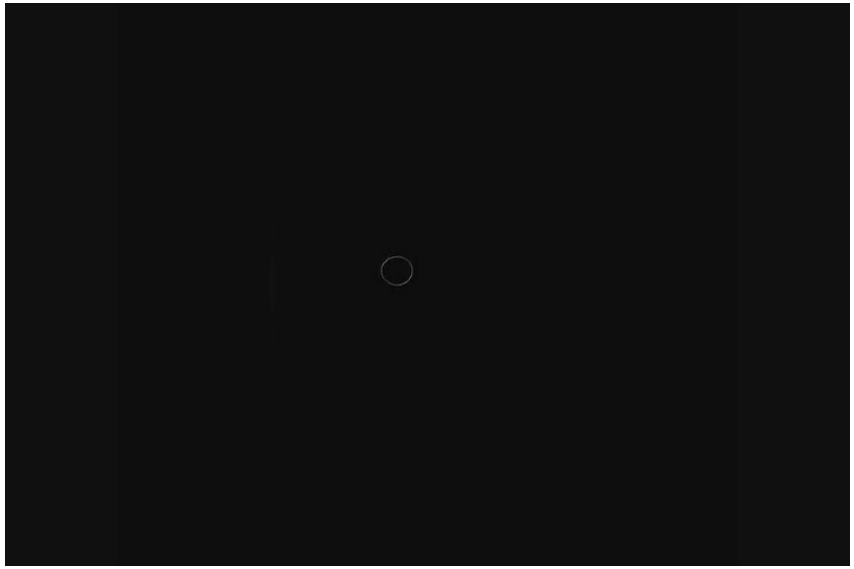
Direct method: the total force on the particle in the trap is given by the difference between the momentum flux entering the object (*in*) and the one leaving it (*out*):

$$\vec{F} = \frac{d\vec{P}_{in}}{dt} - \frac{d\vec{P}_{out}}{dt} = \frac{n_m}{c}\int_S \left(\vec{S}_{in} - \vec{S}_{out}\right) dS$$

It requires to detect all the light before and after interaction with the particle.

Measuring all the back and forward scattered light after interaction is unfeasible.

Backward scattererd light is a small fraction of the emitted light.



Light scattered by a bead in trap

1 μm polystyrene bead in water;

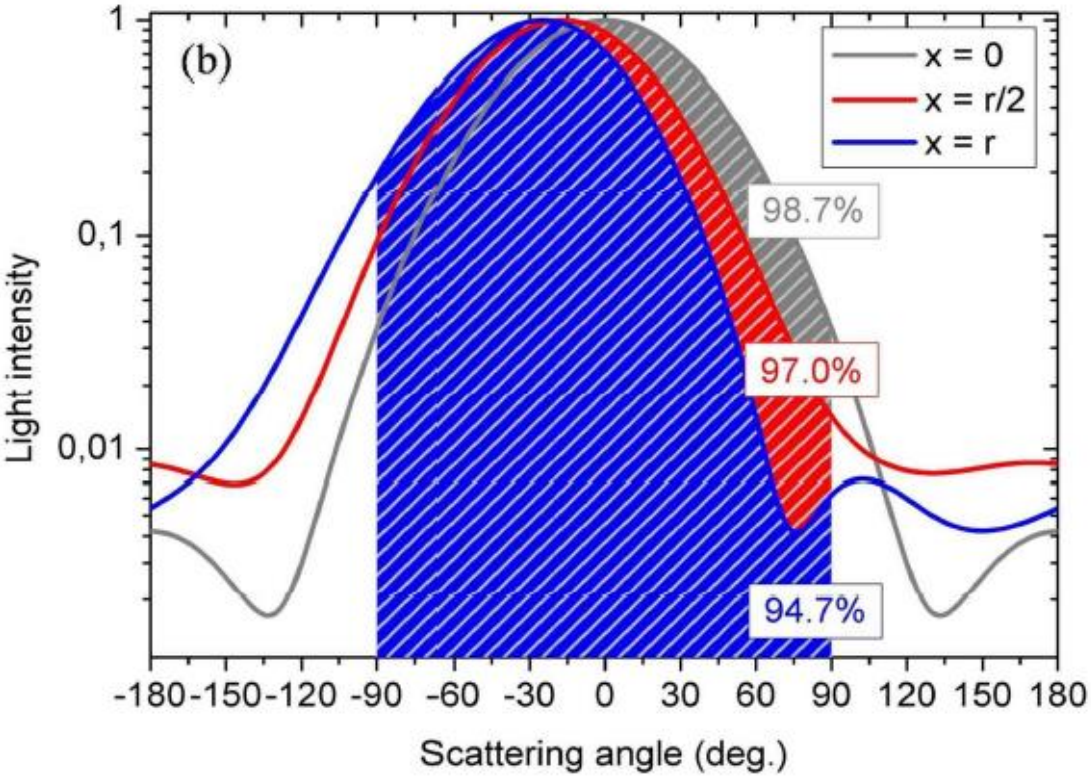
λ = 1064 nm; focusing lens NA= 1.3 ;

Simulation for the E_x ocomponent of the electric field.

Most of the light is scattered forward

Farré and Montes-Usategui, Optics Express 18, 11955 (2010)

Forward-scattered light is dominant

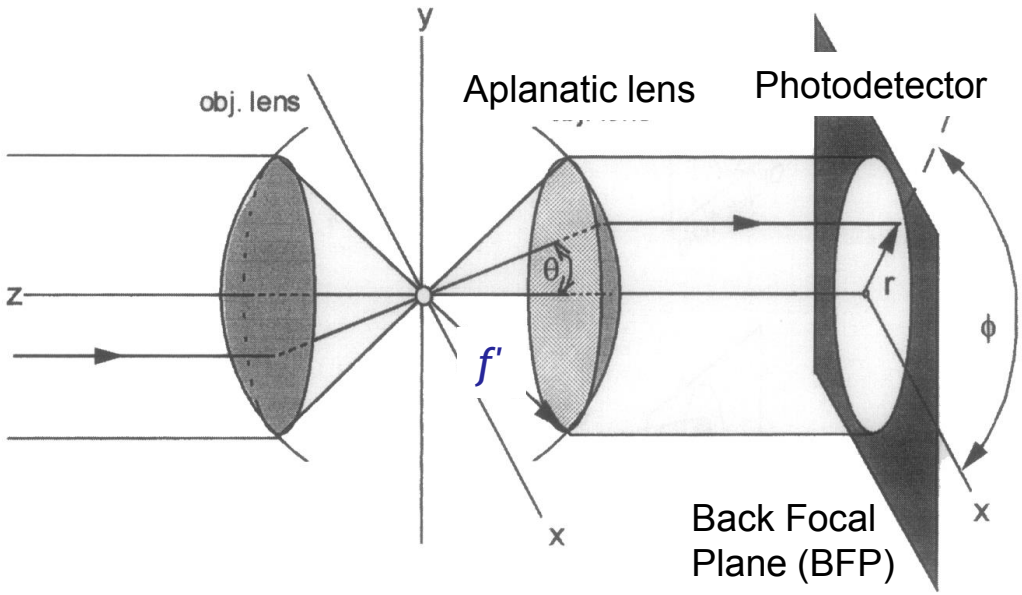


Angular intensity distribution of the light scattered by the bead.

The amount of forward-scattered light contained between [−90° : 90°] is > 95 %.

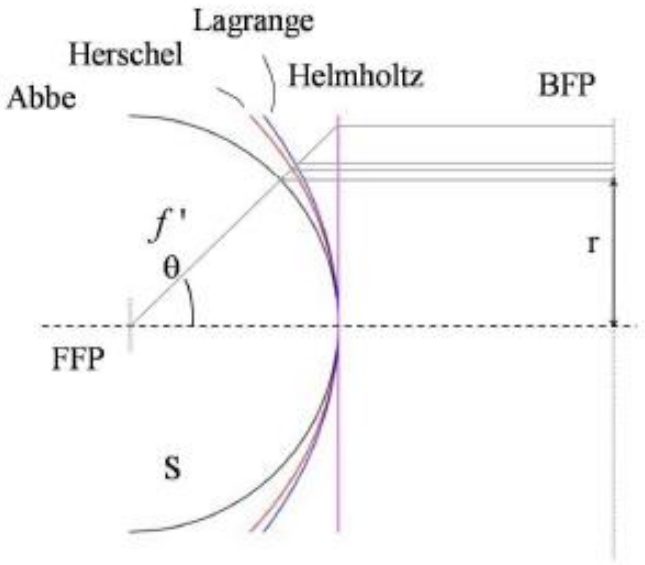
To collect this light one needs a lens with high NA, which fulfills the Abbe sine condition and a photodetector placed in a plane conjugated with the back focal plane of the lens.

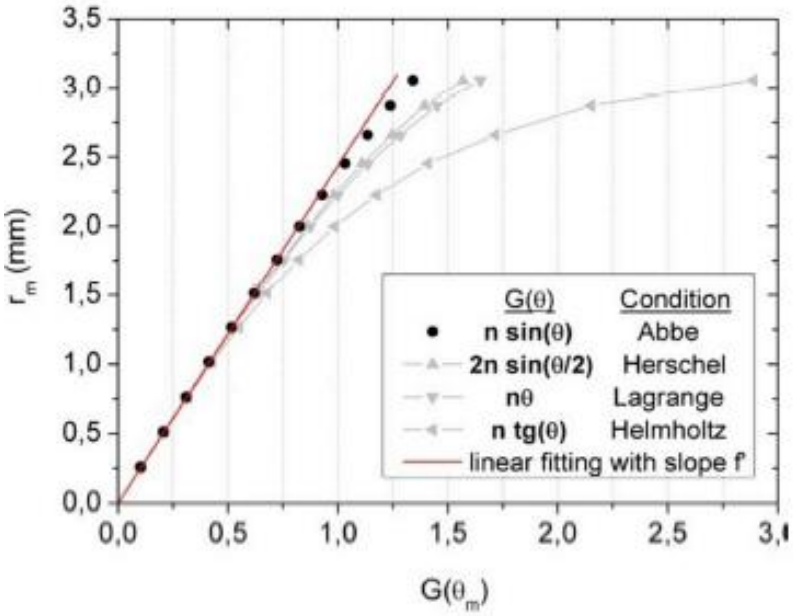
Farré and Montes-Usategui, Optics Express 18, 11955 (2010)



Abbe's sine condition:

$$\frac{r}{n_1 \sin\theta_1} = const = f'$$





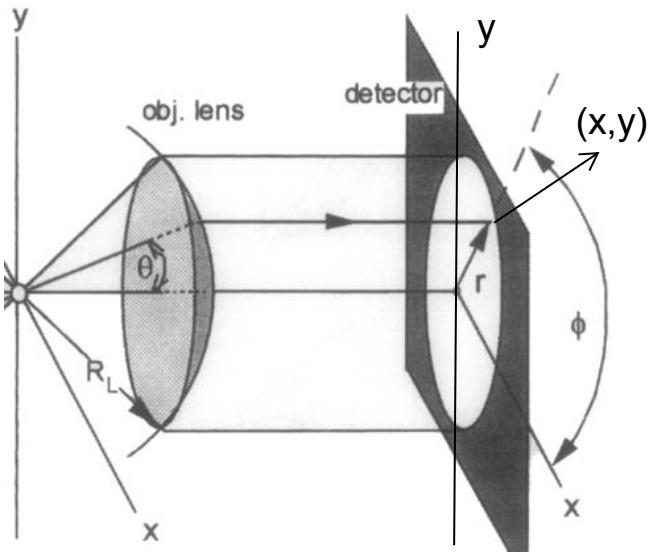
Smith et al, Methods in Enzymology, vol 361, 134 (2003)

Farré and Montes-Usategui, Opt. Express 18, 11955 (2010); Sheppard and Gu, J. Mod. Opt. 40, 1631-1651 (1993)

Condition for the photodetector position: in Back Focal Plane (BFP) of the lens

Why: the intensity pattern in the BFP does not depend on position of the focus, which means the signal on the photodetector does not change with the position of the trapped bead.

BFP can be seen as the Fourier plane of the lens, and hence the shift invariance of the Fourier transform applies.



The plane wave with momentum p_r

$$p_r = p_0 n_1 \sin\theta_1$$

focuses in BFP at r:

$$r = f' n_1 \sin\theta_1 = f' \frac{p_r}{p_0} = f' \frac{k_r}{k_0}$$

coordinates represent the transverse components of light momenta in a proper scale

where: p_0 - light momentum in vaccum

The intensity pattern *I(x,y)* projected onto the PSD which produces an electric signal:

$$S_x = \psi \iint \frac{x}{R_D} I(x, y) dx dy$$

I(x,y) is the radiant power at point *(x,y)*, proportional to the number of photons per time having momentum (p_x, p_y); R_D and ψ are the size and the efficiency of the detector.

The *x* component of the force *Fx*

$$F_x = \frac{1}{f'c} \iint x I(x, y) dx dy = \frac{R_D}{\psi\ f'\mathrm{c}} S_x = \alpha\ S_x$$

- **the direct method is independent of the shape, size and refractive index of the particle**
- **this method is also insensitive to changes to the trap shape**
- this method requires a high NA (1.4) condenser lens --> low WD (2-300 μm) and hence small height for the sample chamber limitating some applications

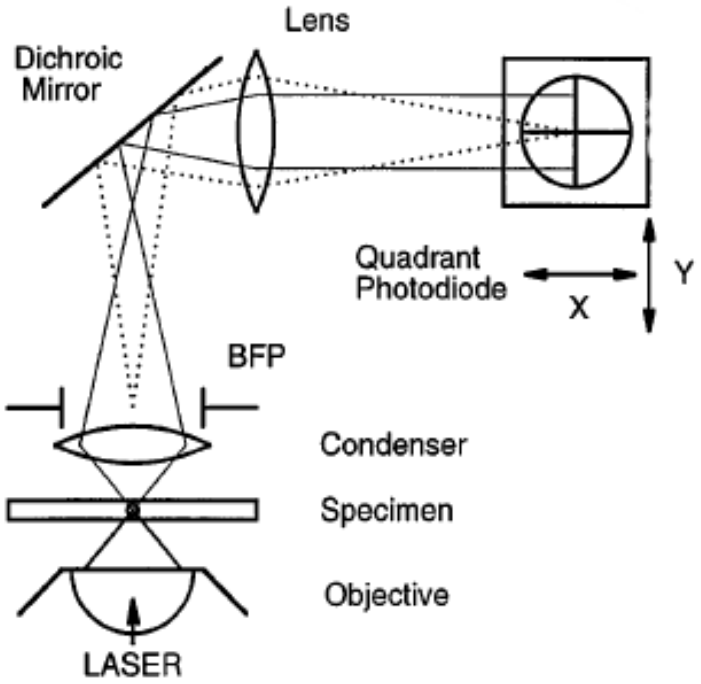
For positions close to the center of the trap the force is linear with the position:

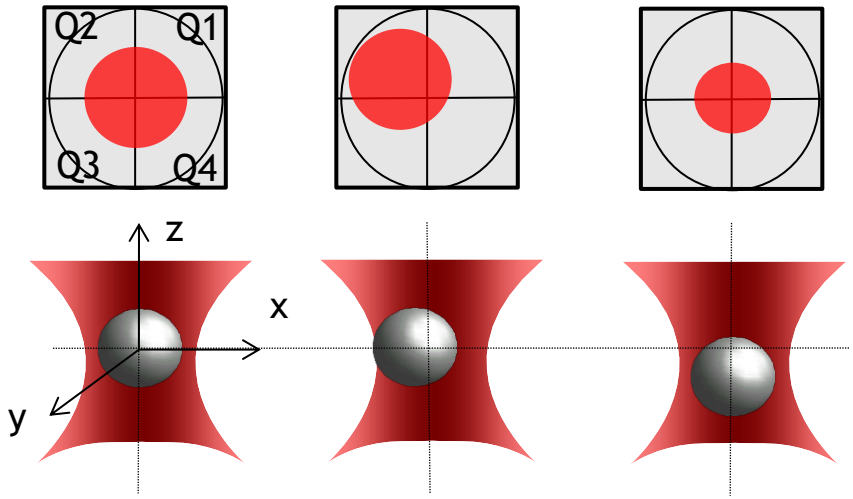
$$F_x = \alpha\ S_x = k\ \mathrm{x}$$

Farré et al , Optics Express 20, 12270 (2012)

Back Focal Plane (BFP) interferometry

Intensity shifts were indentified as first-order far-field interference between the outgoing laser beam and scattered light from the trapped particle. This interference also reflects momentum transfer to the particle, giving the spring constant of the trap.





$$X = \frac{(Q2+Q3)-(Q1+Q4)}{(Q1+Q2+Q3+Q4)} = \frac{X_{Diff}}{SUM} \qquad Y = \frac{(Q1+Q2)-(Q3+Q4)}{(Q1+Q2+Q3+Q4)} = \frac{Y_{Diff}}{SUM}$$

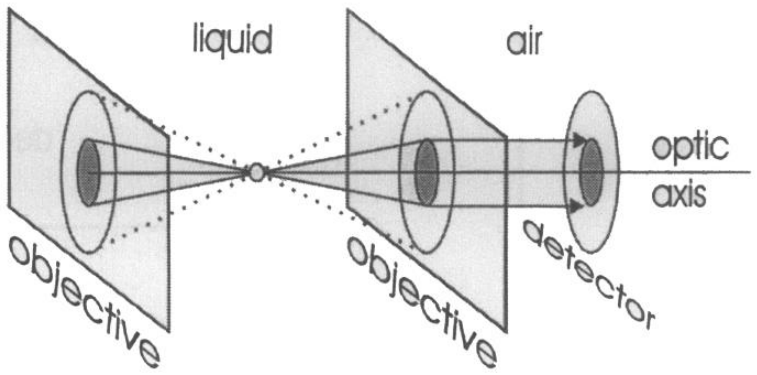
$$Z = (Q1+Q2+Q3+Q4) = SUM$$

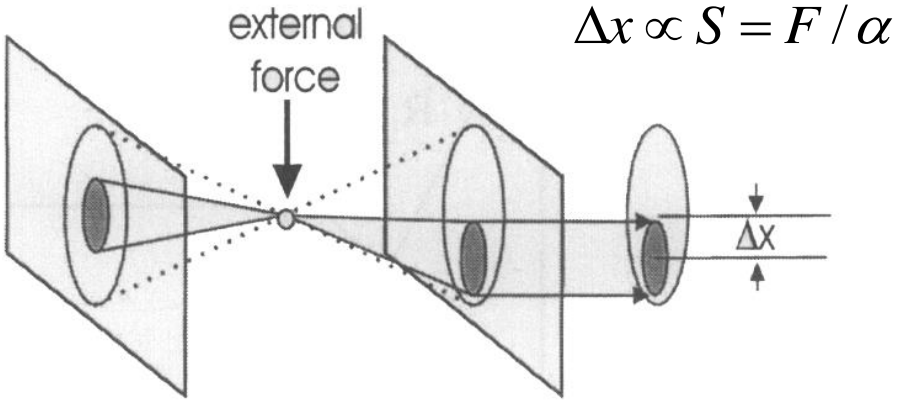
The intensity shift is determined with a Quadrant Photo Diode (QPD) or a Position Sensing Detector (PSD). For small displacements of the bead:

$$F_x = k\,\mathrm{x} = \alpha\, S_x$$

Gittes and Schmidt, Optics Letters 23, 7 (1998)

Light momentum force transducer with low NA lenses





$$\Delta x \propto S = F / \alpha$$

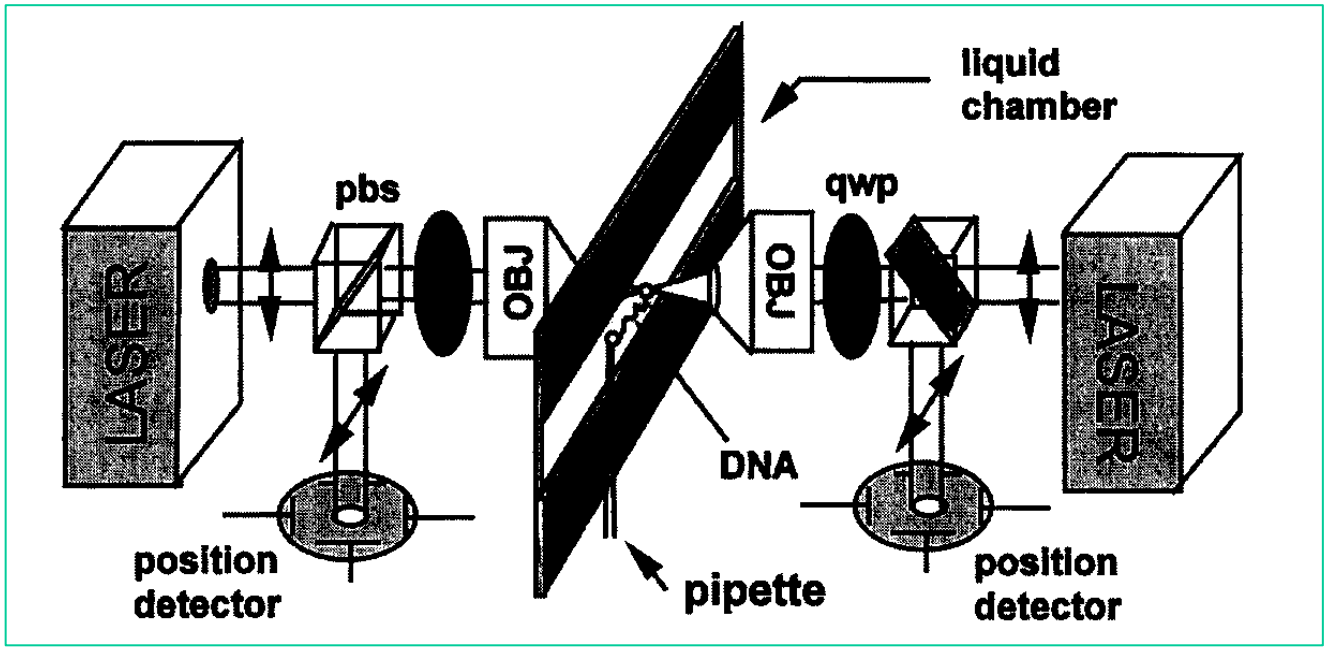
The second objective should collect all the forward scattered light

If the diameter of the incident laser beam is small and the first lens has a relatively low NA then also the collecting lens can have a relatively low NA.

Note: Dashed lines define the lens NA

Smith *et al*, Methods in Enzymology, vol 361, 134 (2003)

Dual Beam Laser Tweezers (DBLT) + light momentum force transducer

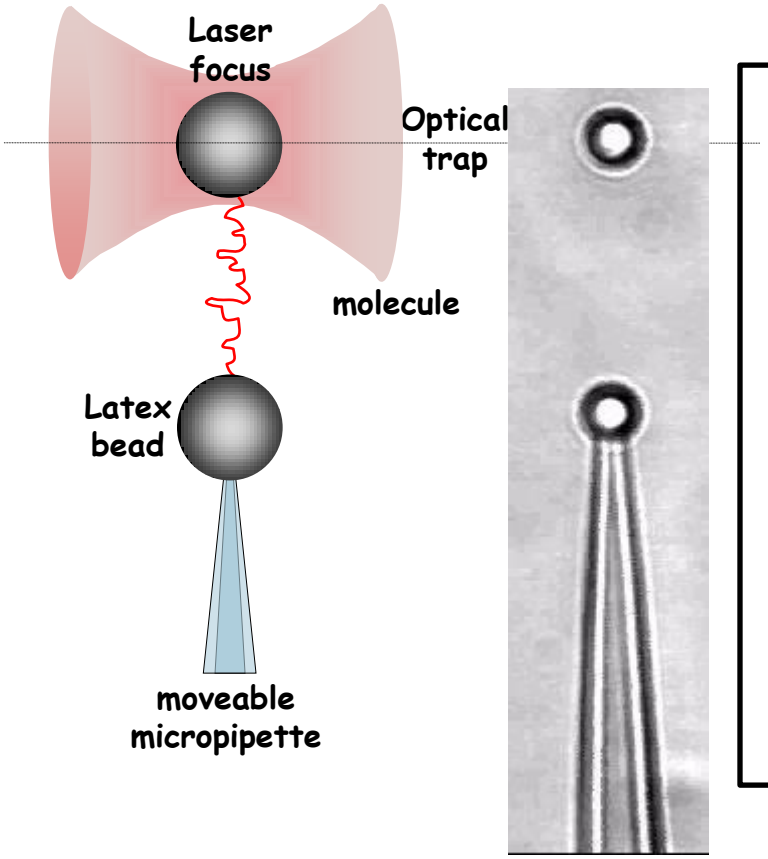


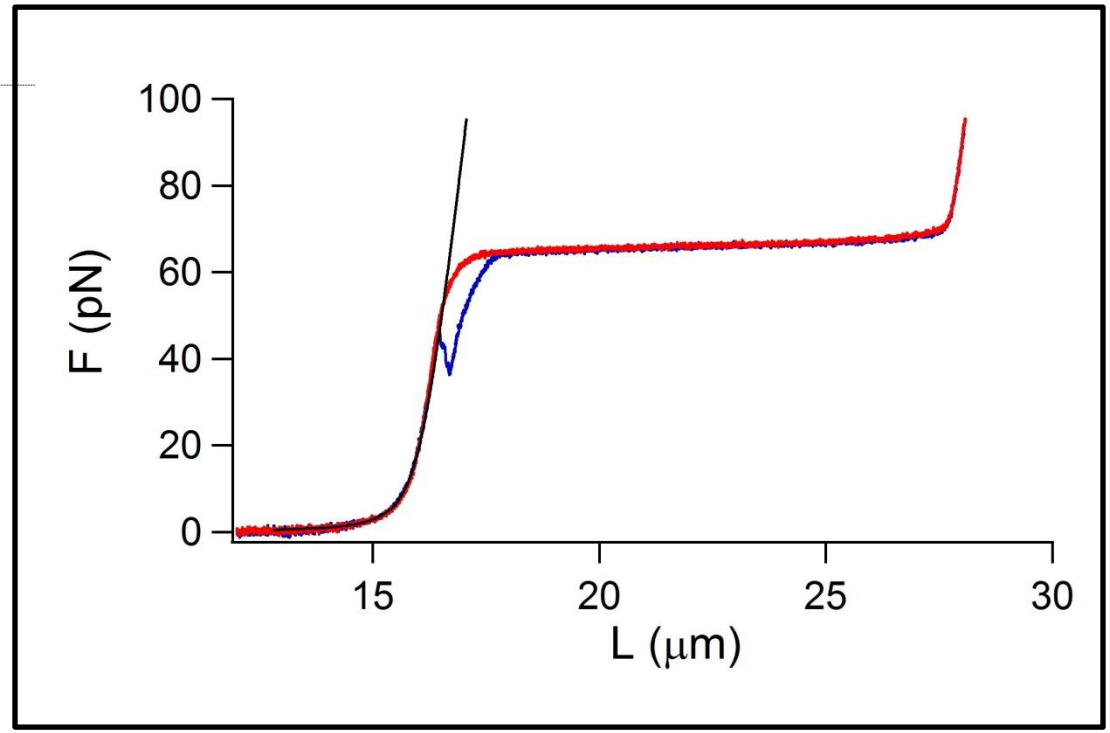
Why a DBLT (counter propagating beams) tweezers has been chosen

and not a single beam optical tweezers?

Smith et al, Methods in Enzymology, vol 361, 134 (2003)

Example:
Force and length range tested by recording the overstretching transition in λ-phage DNA

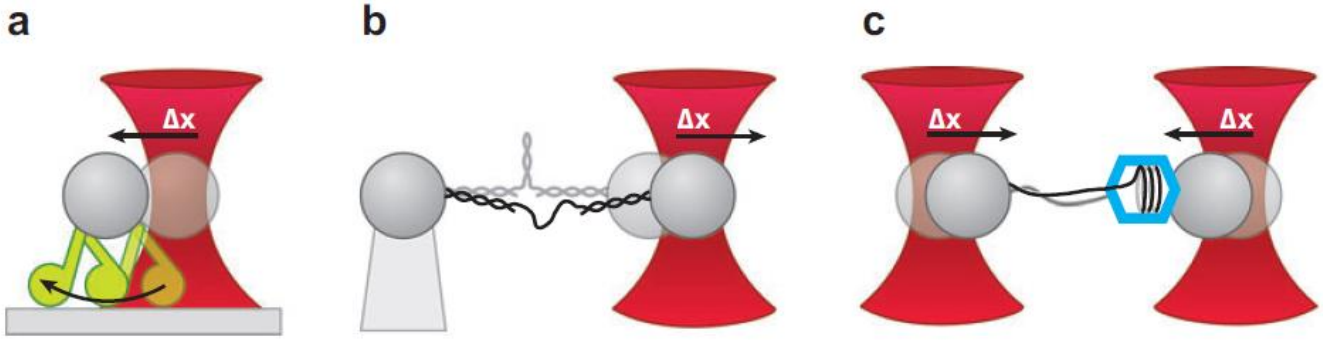




The molecule undergoes a highly cooperative structural change at ~65 pN that implies 70% elongation and is likely involved in the modulation of the access to genetic information

(Univ of Florence Physiology Lab prof V. Lombardi, dr P. Bianco)

Different experimental geometries for single molecule optical tweezers force experiments



(a) Processive cytoskeletal motors, such as kinesin, the motor is typically attached directly to a polystyrene bead held in an optical trap, and the filament is attached to the surface of a sample chamber. Motions of the motor are revealed by motions of the trapped bead.

(b) It is also possible to attach one end of the biological system to a second polystyrene bead suctioned onto the end of a micropipette. The motion of the biological system, such as the unfolding of a RNA hairpin, is again revealed in the motion of the trapped bead.

(c) The second bead can also be held in a second optical trap. In this case, changes in the length of the tethered DNA by the action of a bacteriophage portal motor are revealed in the motions of both beads. The relative motion of each bead depends on the relative stiffness of the two optical traps.

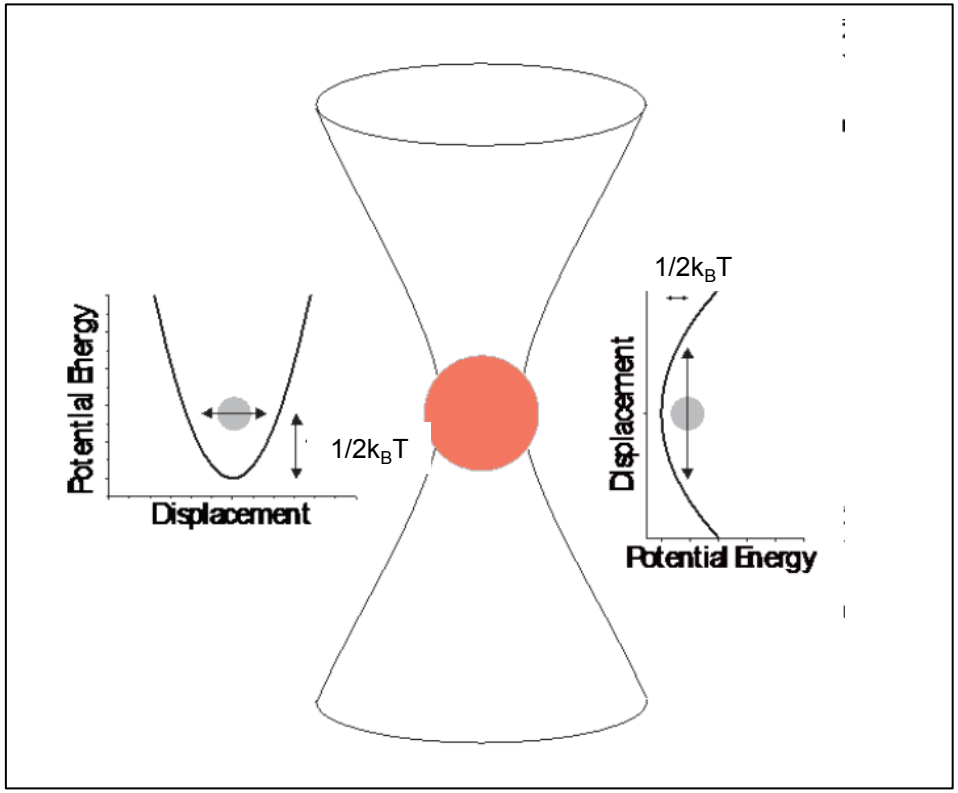
Moffit et al Annu. Rev. Biochem 77, 205 (2008)

Indirect method :

the force is measured by detecting bead position changes

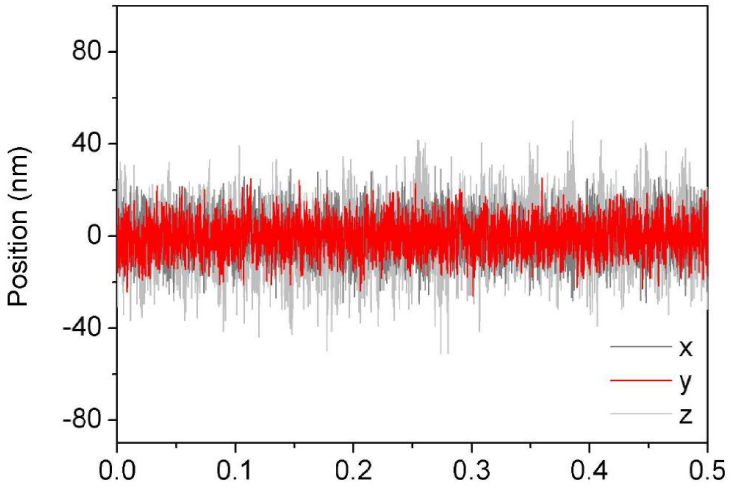
$$F = k \cdot x$$

- k - trap stiffness [N/m]
- x - displacement of the bead in the trap [m]



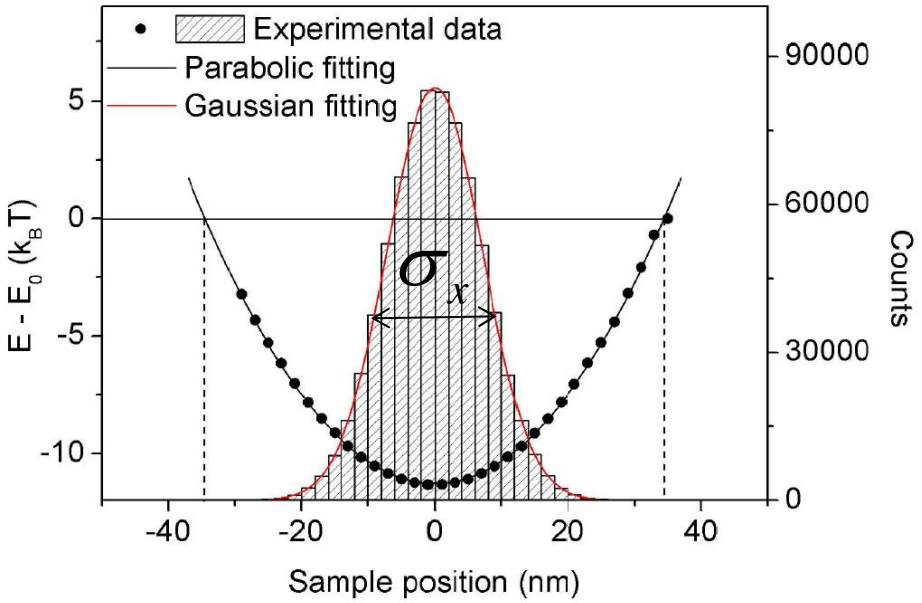
The trap stiffness k should be determined first

Tracking the displacement of the bead in the optical trap with high sampling frequency (> 5 kHz)



X, Y, Z - bead in trap

Position histogram, potential energy



Probability density of the bead position (Botzmann statistics)

$$\rho(x,y) = C \exp\left(\frac{-U(x,y)}{k_B T}\right)$$

$$\rho(x,y) = C\, e^{\frac{-k_x x^2}{2 k_B T}}\, e^{\frac{-k_x y^2}{2 k_B T}}$$

$$k_x = \frac{k_B T}{\sigma_x^2} \qquad k_y = \frac{k_B T}{\sigma_y^2}$$

Problem with gaussian noise --> underestimated trap stiffnness

Power Spectrum Analysis

The power spectrum Sv(f) of the signal sv(x) is: $S_v(f) = |F(s)|^2$

F- Fourier transform

Sv(f) - measured power spectrum

S(f) - density Lorentzian fit

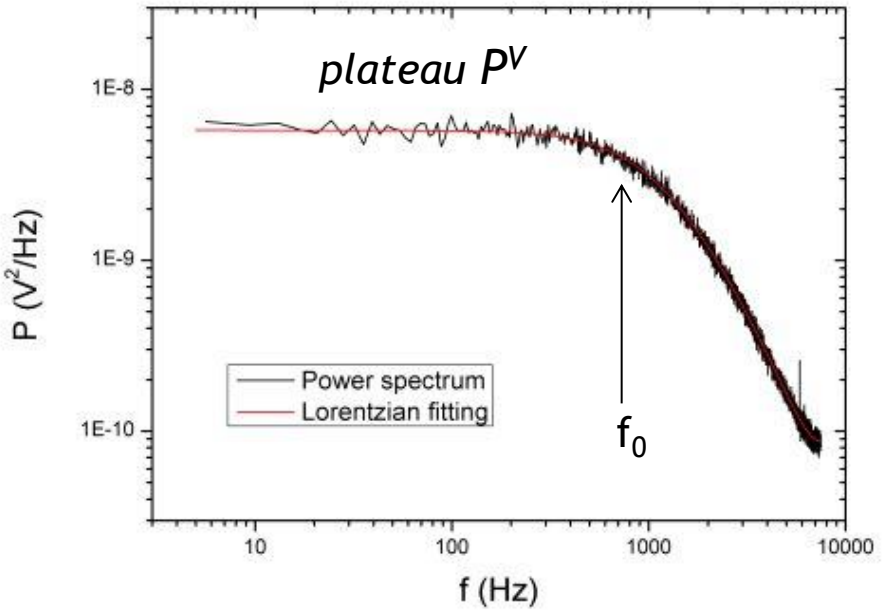
$$S(f) = \frac{S_0 f_0^2}{f_0^2 + f^2},$$

f_0 – corner frequency

$$f_0 = \kappa / 2\pi\gamma$$

k – trap stifness

γ – Stokes drag coefficient



The power spectrum (black) of a trapped 1 μm silica bead acquired at 10 KHz and fitted to a Lorentzian (red).

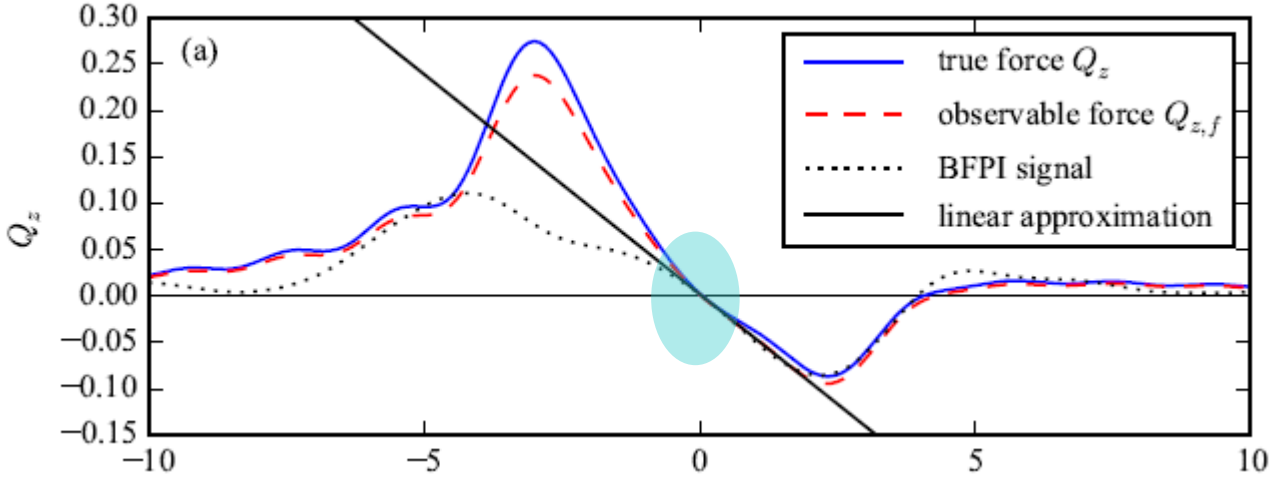
Neuman and Block, Rev Sci Instr 2004

Tolic-Norrelykke et al, Rev Sci Instr 2006

Axial force - comparison between simulation and measurement

$$F = \alpha S = k\,x$$

$$F = Q\, n_1 P / c$$



Thalhammer *et al* Optics Express 23, 6112 (2015)

Fig. 3. Calculated axial force profile for a 3 µm diameter polystyrene bead. (a) True axial force Q_z (solid blue line) and estimate $Q_{z,f}$ from forward scattered light only (red dashed line). Also shown (black dotted line) is the axial BFPI signal (for a condenser with NA = 0.8), scaled and shifted such that value and slope coincides with the true force at the zero crossing. (b) Difference ΔQ of the estimated and the true force (red dashed line), and

---

Typical values for **OT : K_{OT} = 0.001 – 10 pN/nm**

Typical values for **AFM: K_{AFM} = 10 – 1000 pN/nm**

OT and AFM are complementary Techniques

OUTLINE

- Optical Tweezers (OT) – single-beam gradient force 3D optical trap: how this works, optical manipulation of microparticles

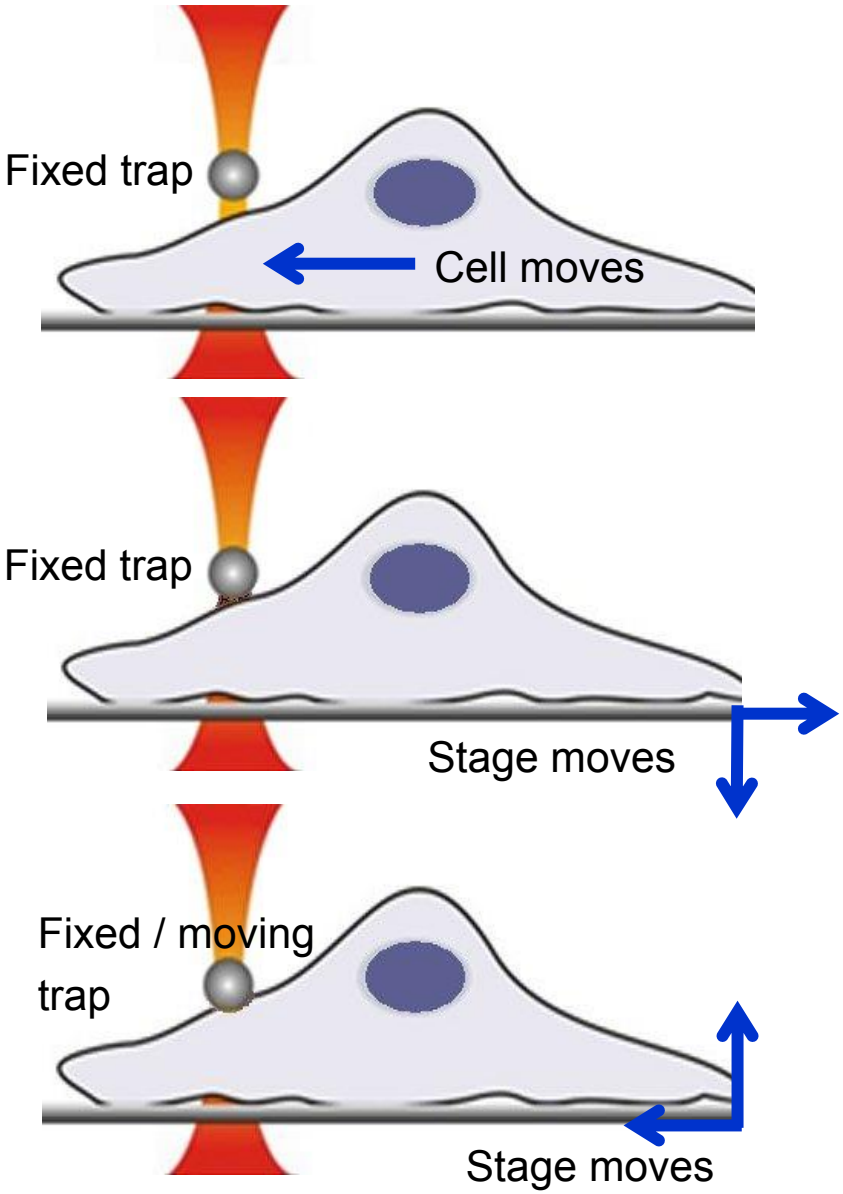
- Measuring picoNewton forces with OT : direct and indirect methods

- **Applications of OT in living cell studies:**

  - **probing forces expressed by developing neurons**
  - **probing the stiffness of cancer cells**
  - **mechanotransduction - conversion of the mechanical stimulus into a biochemical signal by the cell**
  - **biochemical local cell stimulation using optically manipulated vectors (coated beads, biodegradable micro-sources, liposomes)**

OT local probing living cells

(touch - pull - push approaches)



Touch / intercept

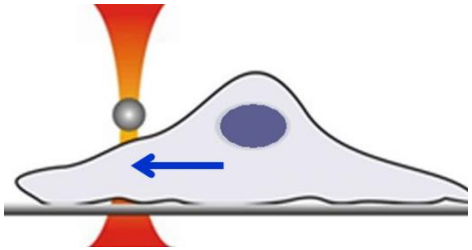
Measure forces when full cell or part of the cell move

Pull (Coated beads)

Local adhesion / binding
Local viscoleasticity (tether membrane)

Push

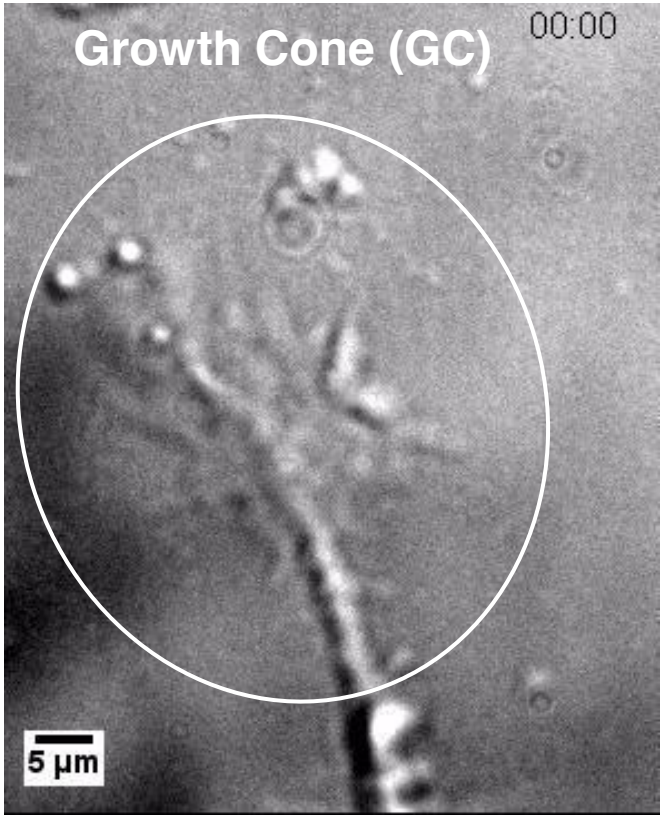
Local viscoelastic properties
Local cell stressing



TOUCH Example:

measuring the forces expressed by lamellipodia and filopodia of the Growth Cone

min:sec

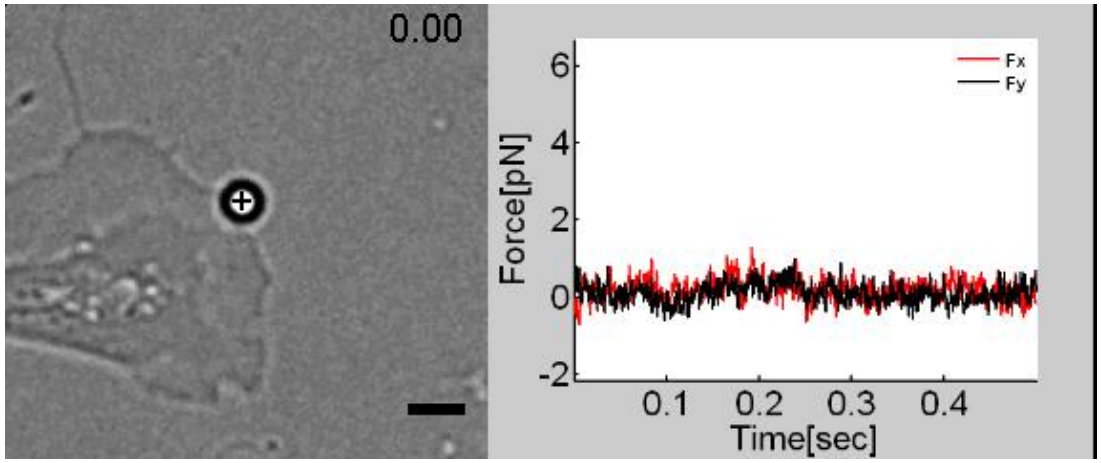


2 DIV hippocampal neuron from mouse stimulated with BDNF at t= 5 min.

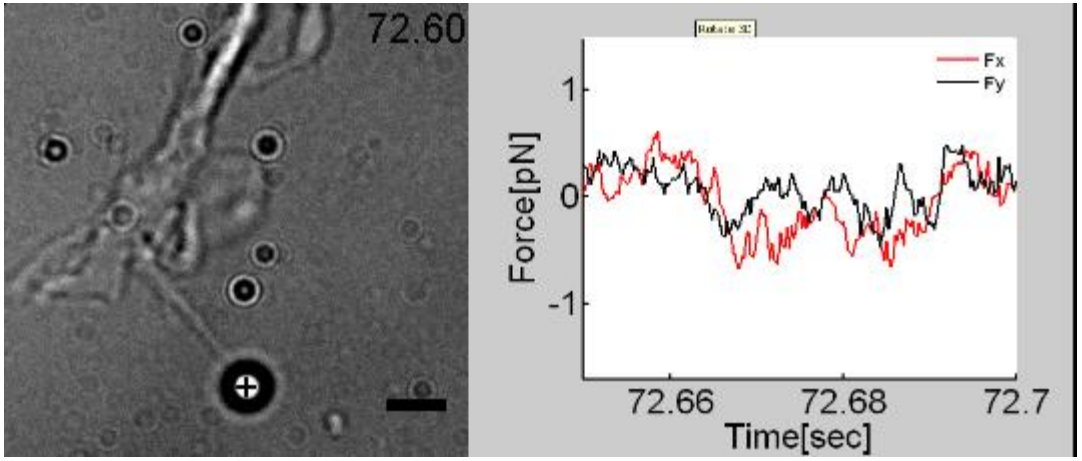
Cojoc, D, … & Torre, V, PLoS One 2 (10), e1072 (2007)

Difato, F, Pinato, G & Cojoc, D, *Int. J. Mol. Sci.* **14**, 8963 (2013) - REVIEW

Force exerted by Lamellipodia

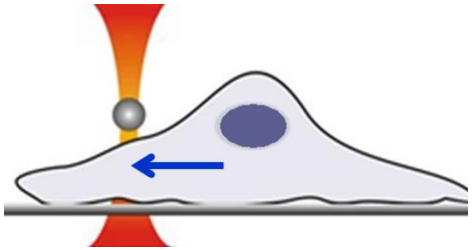


Force exerted by Filopodia - Protrusion



Acquisition rate: 20Hz; Scale Bar = 2μm; Time in seconds

Acquisition rate : 4KHz, Subsampeled at : 2KHz

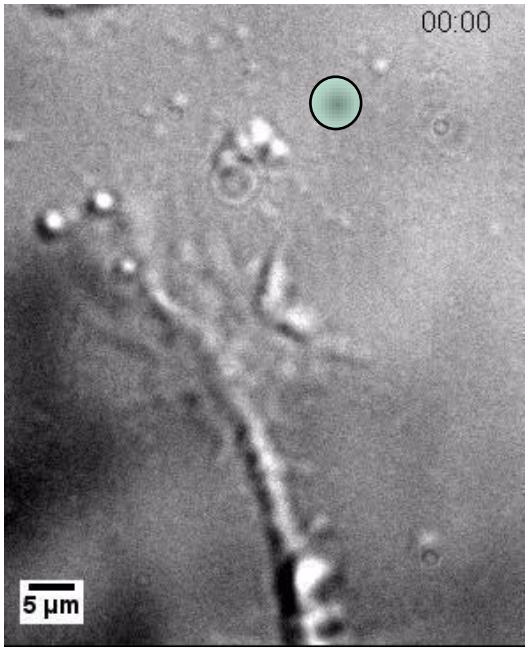


TOUCH Example:

measuring the forces expressed by lamellipodia and filopodia of the Growth Cone

We found that:

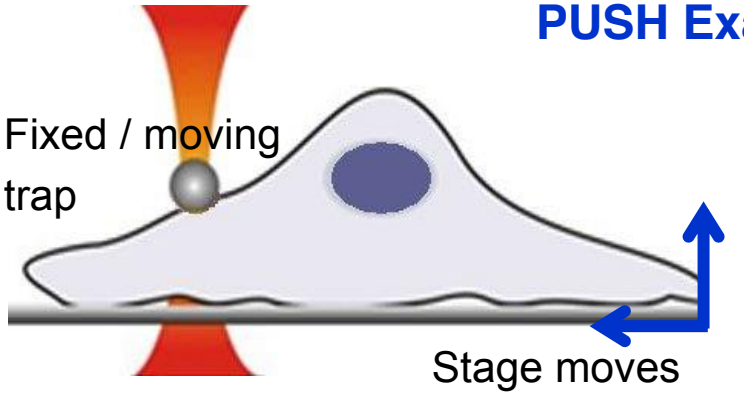
- **Forces exerted** by filopodia were < 3 pN and by lamellipodia were < 20 pN
- **Forces** were discontinue (max frequency about 200 Hz)
- **Inhibitors** of **myosin** light chain kinase (ML-7) or of **microtubule** polymerization drastically reduced the force exerted by lamellipodia, while filopodia continued to exert forces up to 3 pN.
- **Inhibitor** of **actin** polymerization blocked the GC from expressing any force



Cojoc, D, … & Torre, V, PLoS One 2 (10), e1072 (2007)

Difato, F, Pinato, G & Cojoc, D, *Int. J. Mol. Sci.* **14**, 8963 (2013) - REVIEW

PUSH Example



Cell membrane identation by OT

to measure cells elasticity

(same type of experiment as with AFM, but with much smaller loading rate)

Why measuring the elasticity of cancer cells ?

- Different cells have different mechanical properties
- Cancer cells change their mechanical properties during their cancer journey
- Elasticity might be a label free bio-marker
- Investigating cell mechanics helps to understand cell alterations

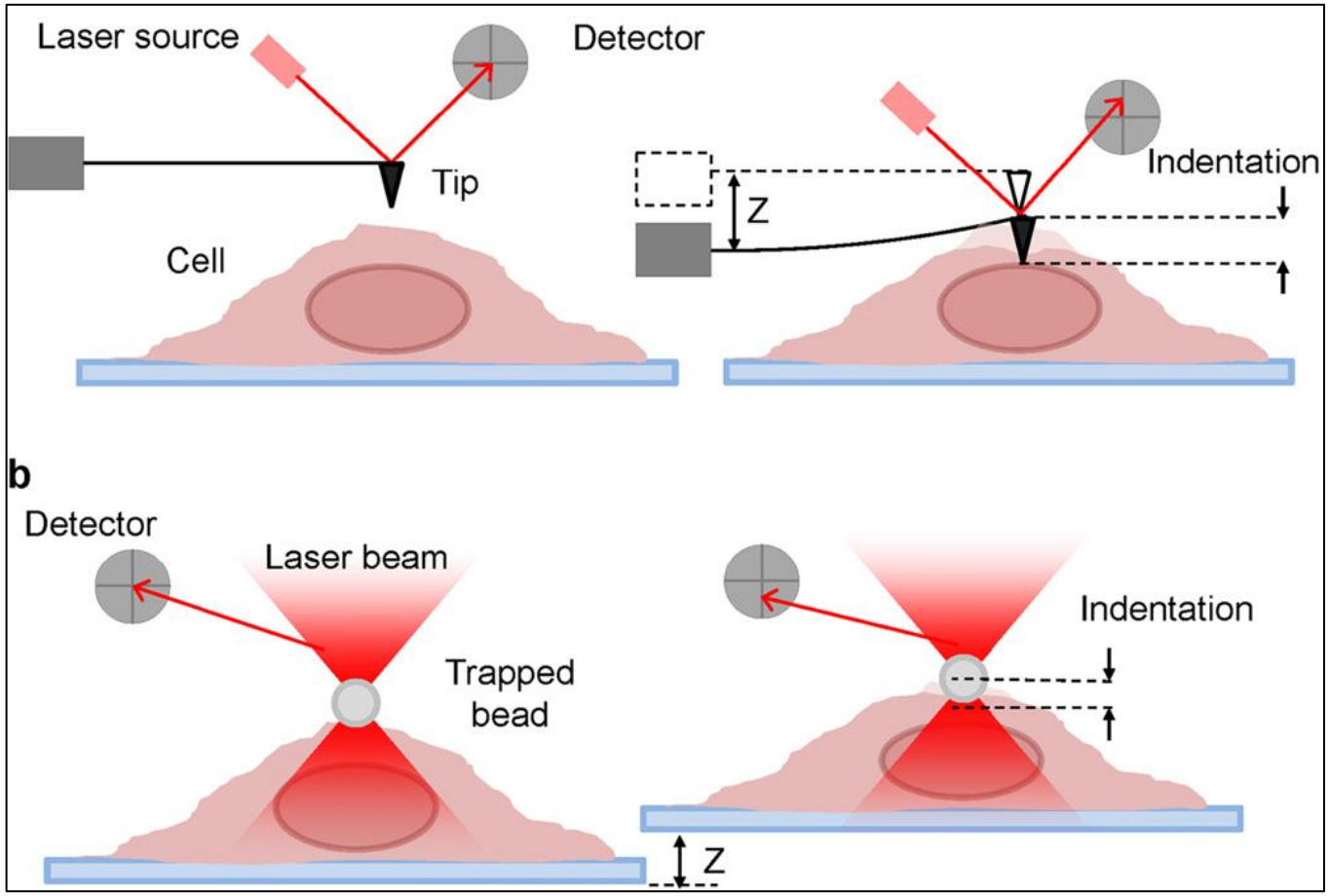
It is generally accepted that cancer cells are softer than the non-neoplastic cells.

Is it always true ?

Cell vertical indentation: AFM vs OT

AFM

OT



	AFM	OT
Force	10 - 10^3 pN	10^{-1} – 10^2 pN
Stiffness	> 10 pN/nm	< 10 pN/nm

Yousafzai *et. al.* 2015, *Opt. Lasers Eng.*

Coceano *et. al.* 2016, *Nanotechnology*

Nawaz S, et al. 2012, *PLoS One*

Comparing cell stiffness of cells from 3 human breast cancer cell lines

Normal myoepithelial	Luminal breast cancer	Basal breast cancer cells
Non neoplastic	Low metastastic potential	High metastatic potential
HBL-100	**MCF-7**	**MDA-MB-231**

by using 2 complementary techniques

OT: k = 0.015 pN/nm, A= 1um, f= 0.2 Hz, F= 10 pN

AFM: k = 150 pN/nm, A_{PF}= 1um, f_{PF}= 200 Hz, F_{SP}= 1 nN

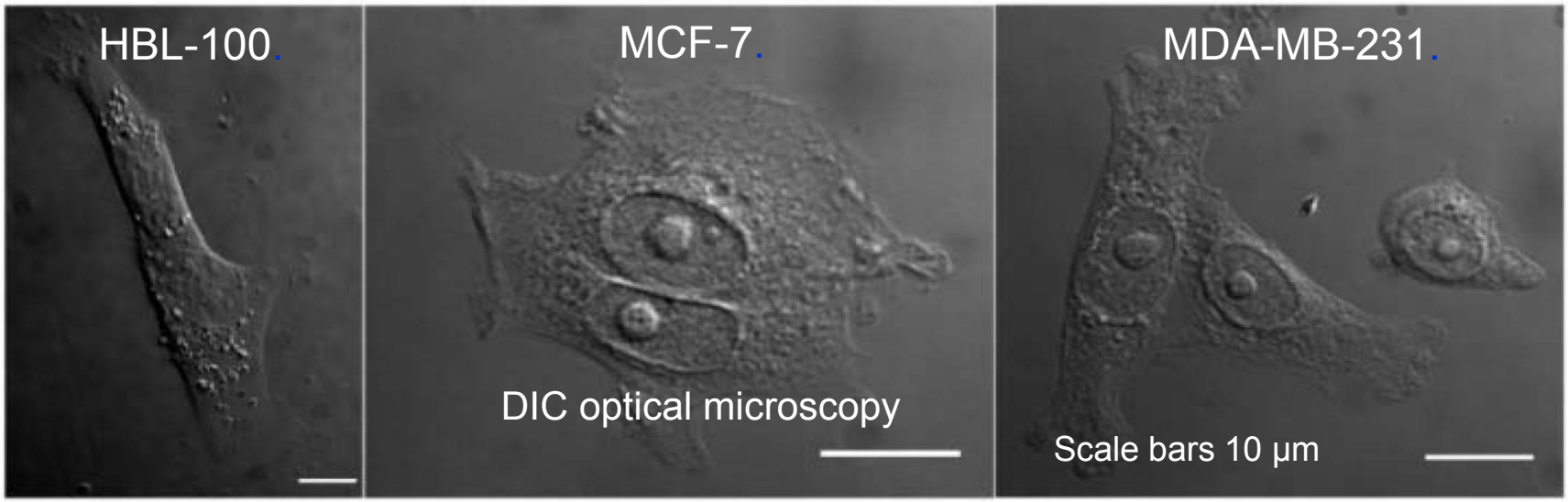
Some questions:

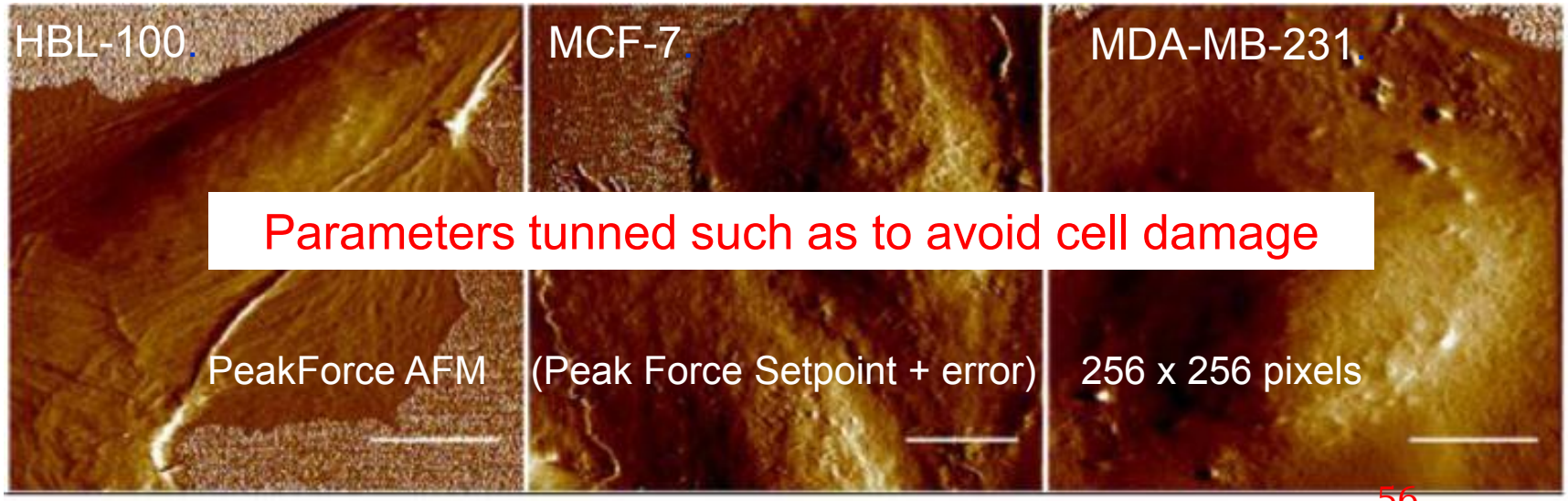
- do we damage the cells by laser radiation (OT) or mechanical ineraction (AFM) ?
- where should we measure ? on top of the nuclear region, near the leading edge ?
- are the results obtained for cell stiffness by using OT and AFM comparable ?

Coceano, Yousafzai, *et al*, Nanotechnology , 2016

Cell morphology – DIC optical microscopy + AFM

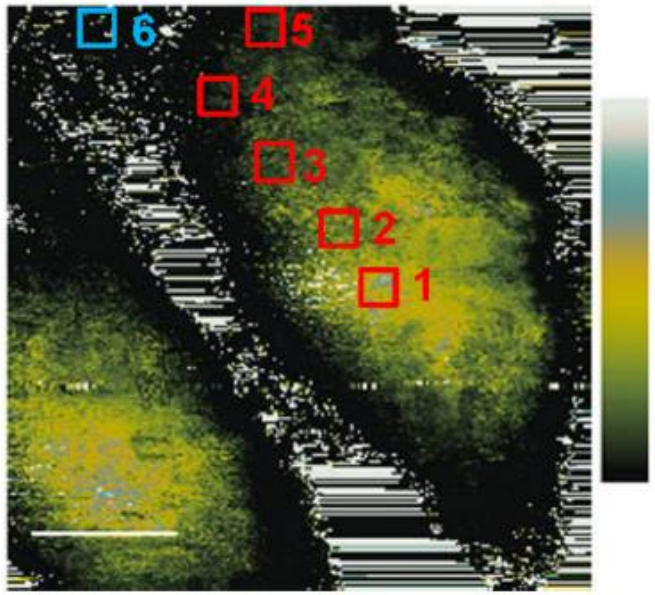
Normal myoepithelial	Luminal breast cancer	Basal breast cancer cells
Non neoplastic	Low metastastic potential	High metastatic potential

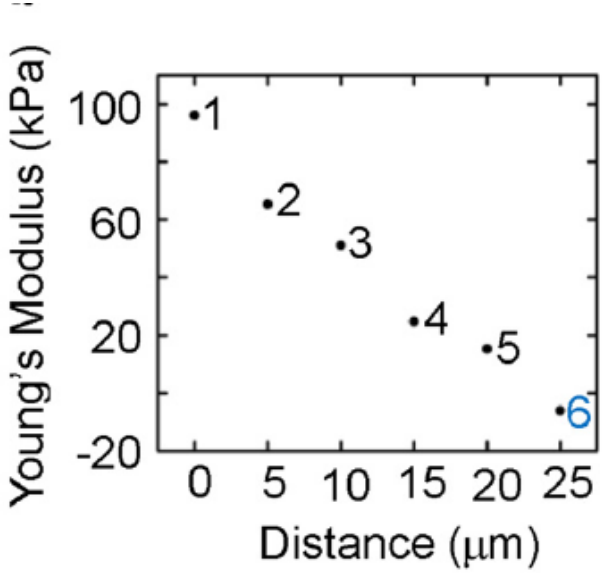




Coceano, Yousafzai, *et al*, Nanotechnology , 2016

Where to measure ?





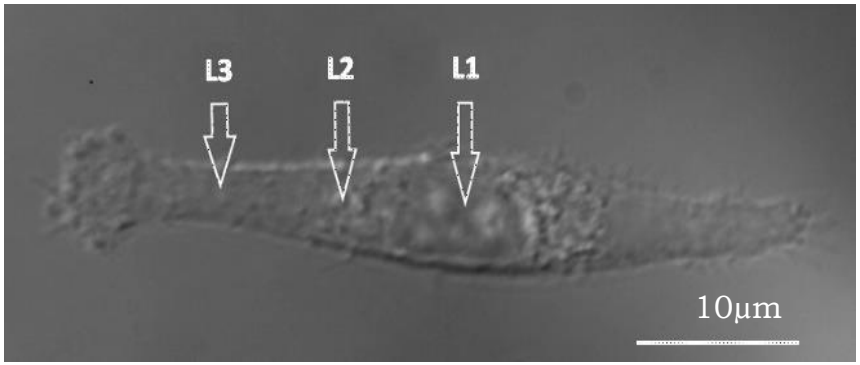
HBL-100 cell stiffness map by Peak Force AFM

Local cell stiffness is calculated averaging the values inside a 2.5 x 2.5 um square.
6 squares, positioned at different distances from the nuclear region 1 are considered.
The nuclear region 1 is chosen from topography of the cell as the highest feature in the height channel. Square 6 (blue) is on the substrate and hence the value is irrelevant.
Scale bar 10 um. Color bar : 0- 300 kPa

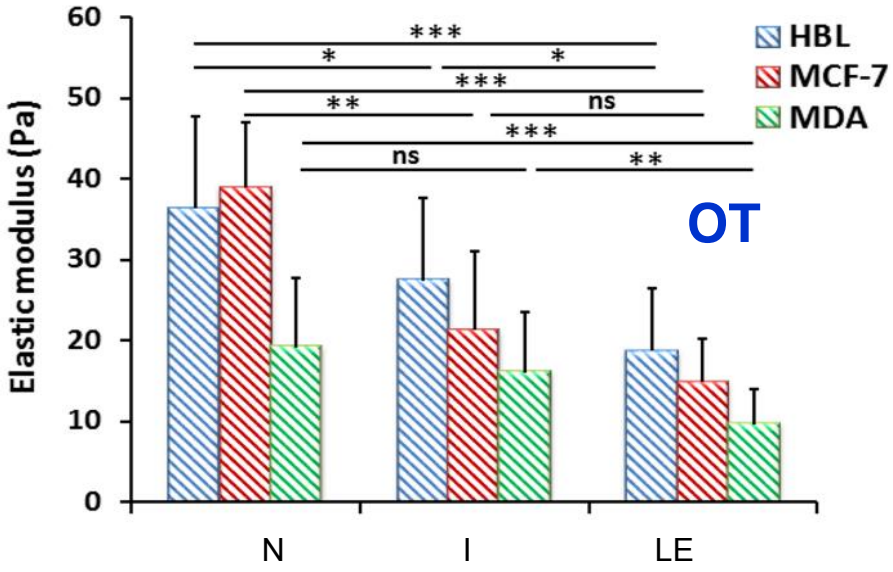
- Cell stiffness decreases from the nuclear region (centre) to the leading edge
- The nuclear region is the most reliable region to measure since it is well defined by topography.

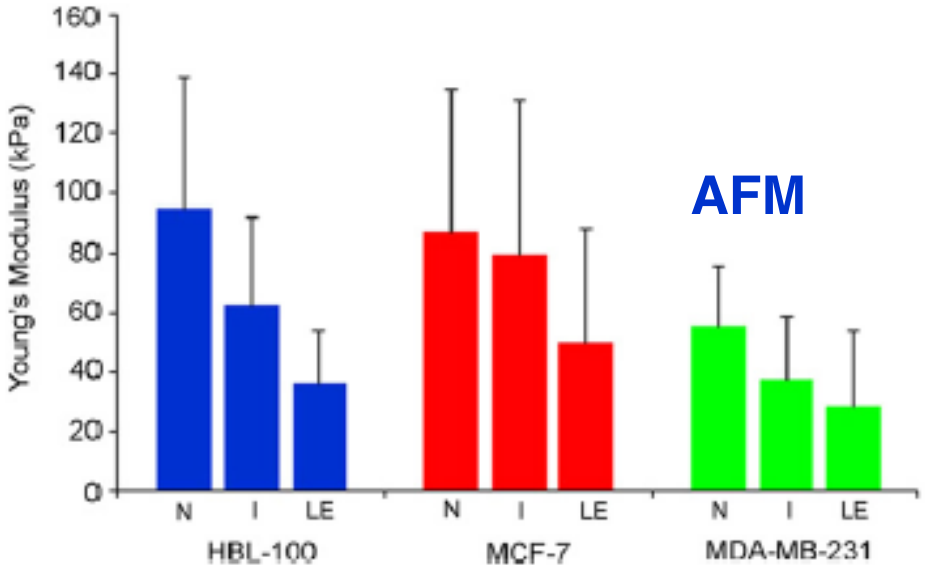
Coceano, Yousafzai, *et al*, Nanotechnology , 2016

Cell stiffness variation over the cell : AFM vs OT



OT: Each cell was indented at three different locations:
N – Nuclear region
I - Intermediate
LE -Leading Cedge



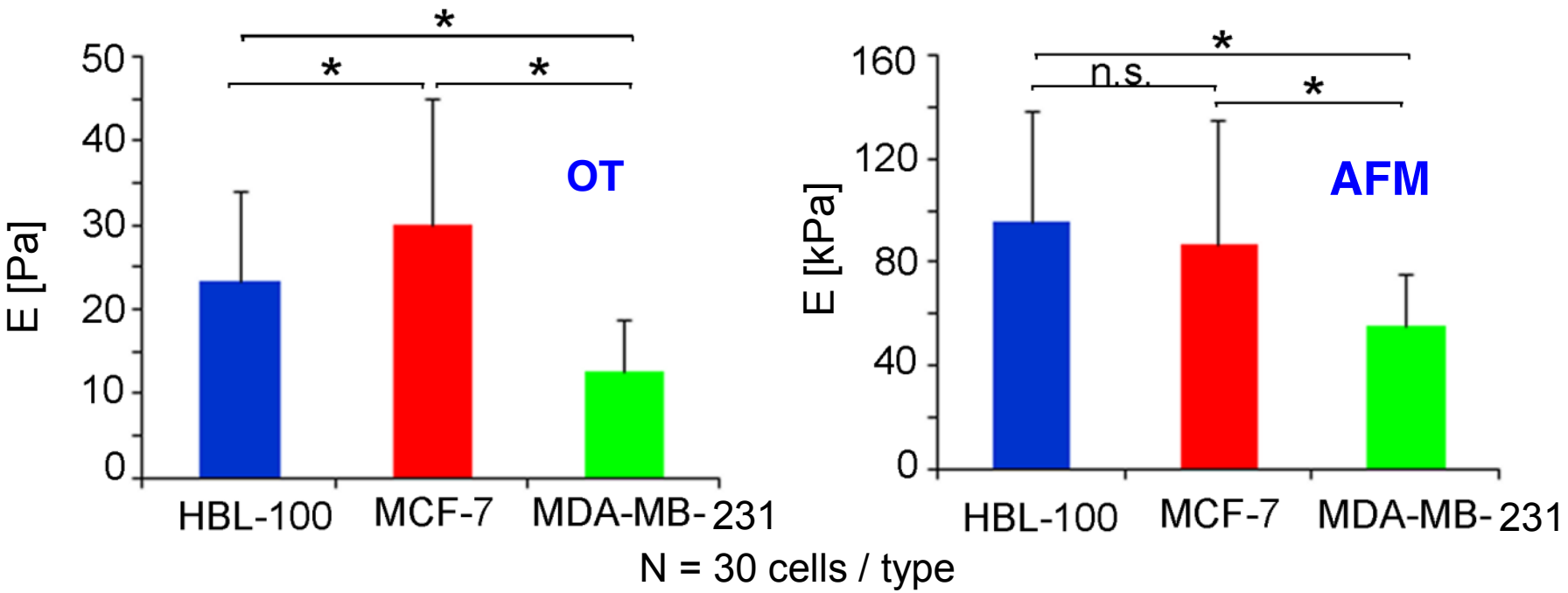


- Cells are stiffer at the center (for all the cell lines).
- The trend was confirmed by both OT and AFM measurements.

Yousafzai *et al*, J. of Biomed. Optics 2016

Coceano *et al*, Nanotechnology , 2016

Cell stiffness measured above the nuclear region

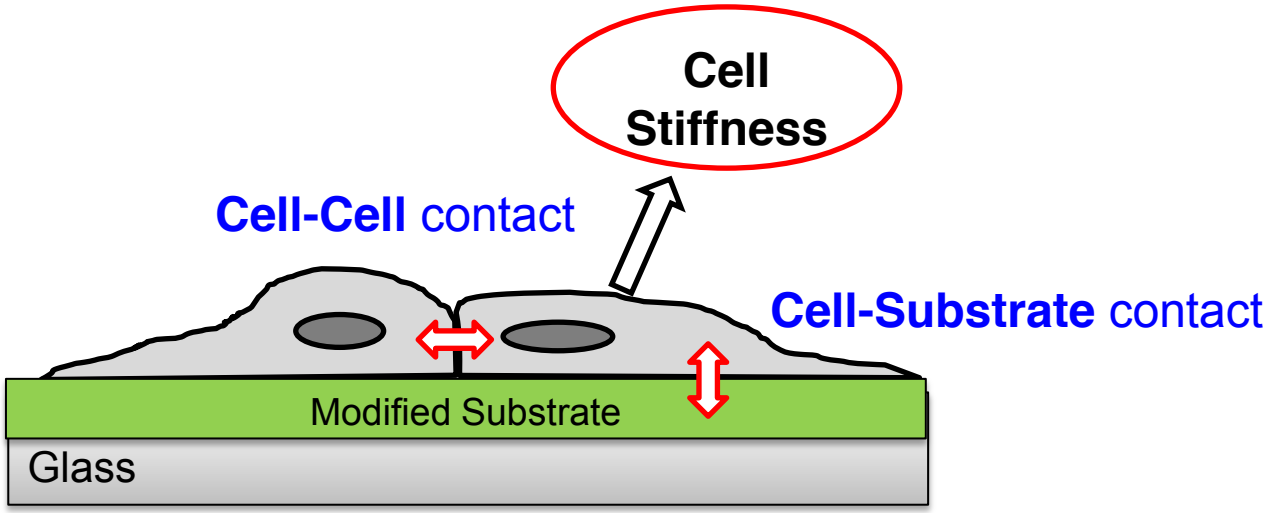


- MDA – MB- 231 cells (**high metastatic potential**) are significantly **softer** than the other two cell types
- this **result is confirmed both by OT and AFM** techniques
- the absolute values obtained for E are different because the force range and the loading rate are different for OT and AFM
- OT reveals a significant difference between HBL and MCF cells

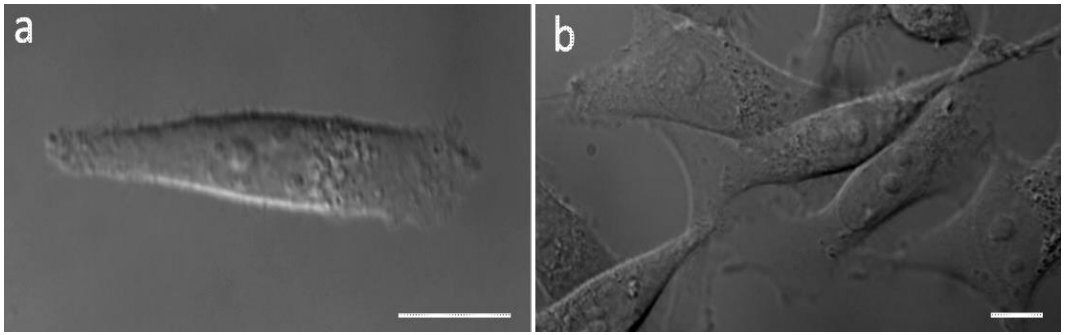
Coceano *et al*, Nanotechnology 2016

Calzado-Martín *et al* ACS-Nano 2016

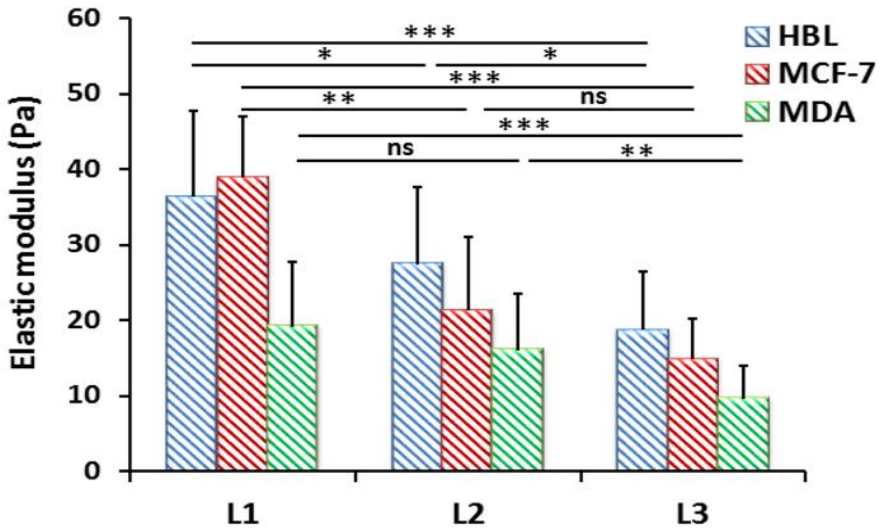
Is the cell stiffness influenced by cell's microenvironment ?

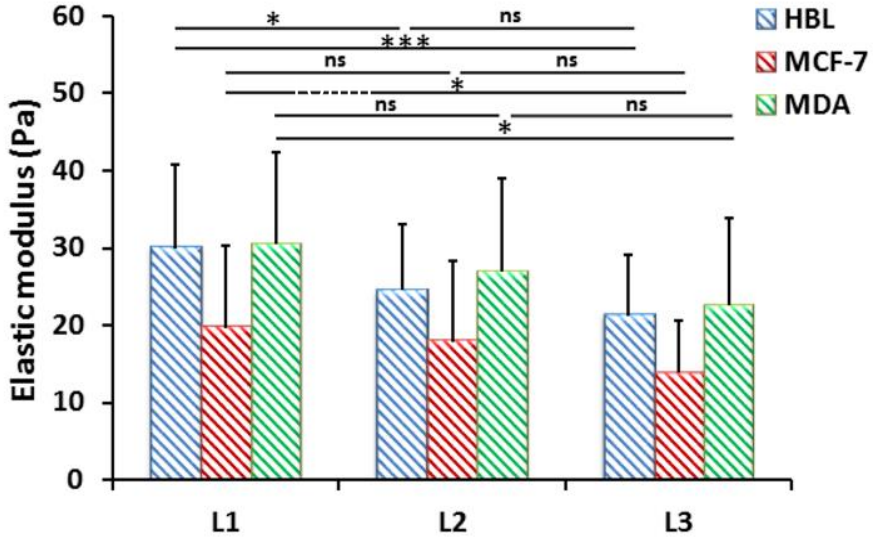


OT only: Cell –Cell contact



Each cell was indented at three different locations:
L1 - Center (nucleus)
L2 - Nucleus edge
L3 -Leading edge





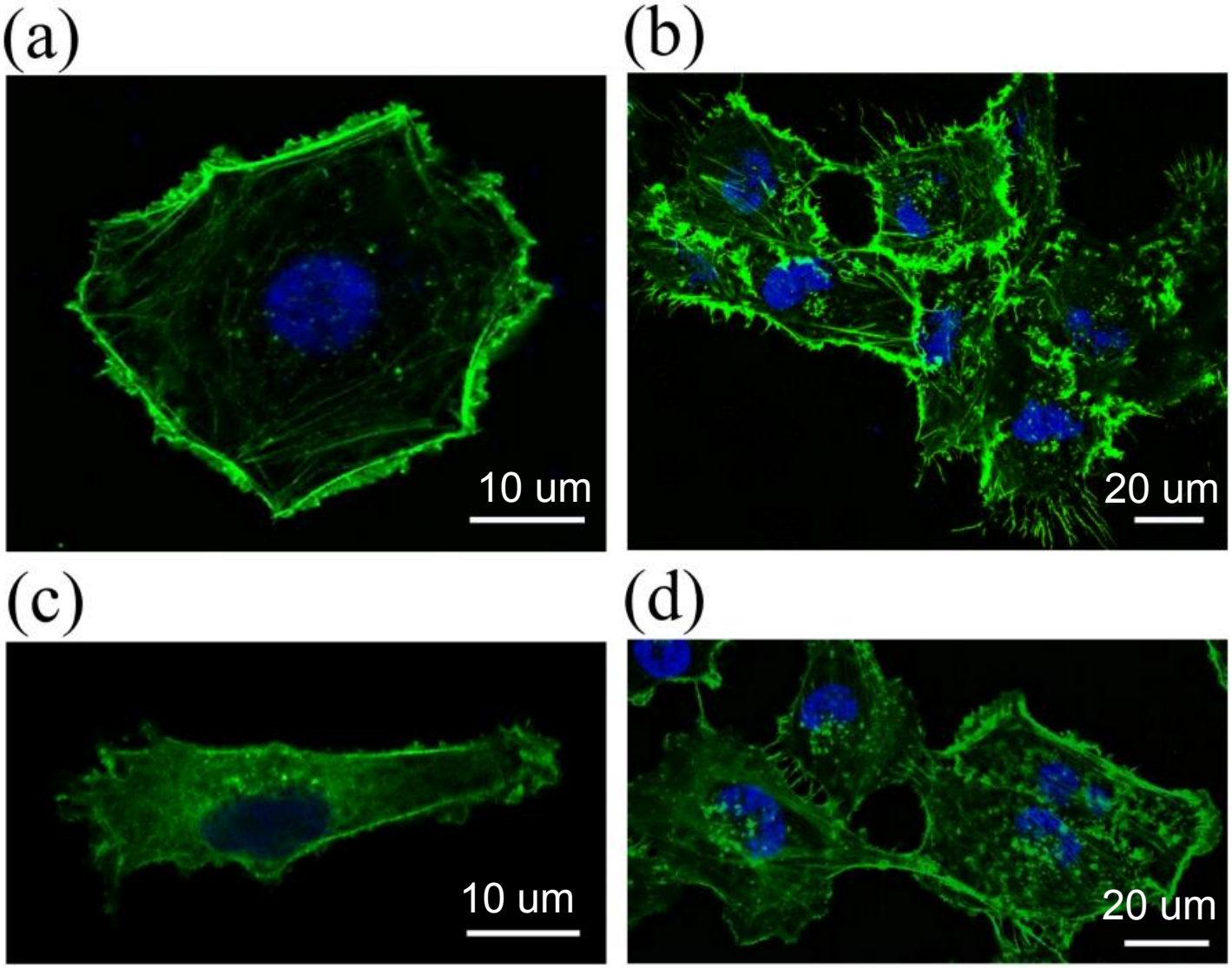
- **MDA cells get stiffer when in contact, being similar to HBL and MCF**
- MCF and HBL become softer.

Yousafzai *et al*, J. Biomed. Opt. 2016

Confocal Images of actin (green) + nucleus DAPI (blue)

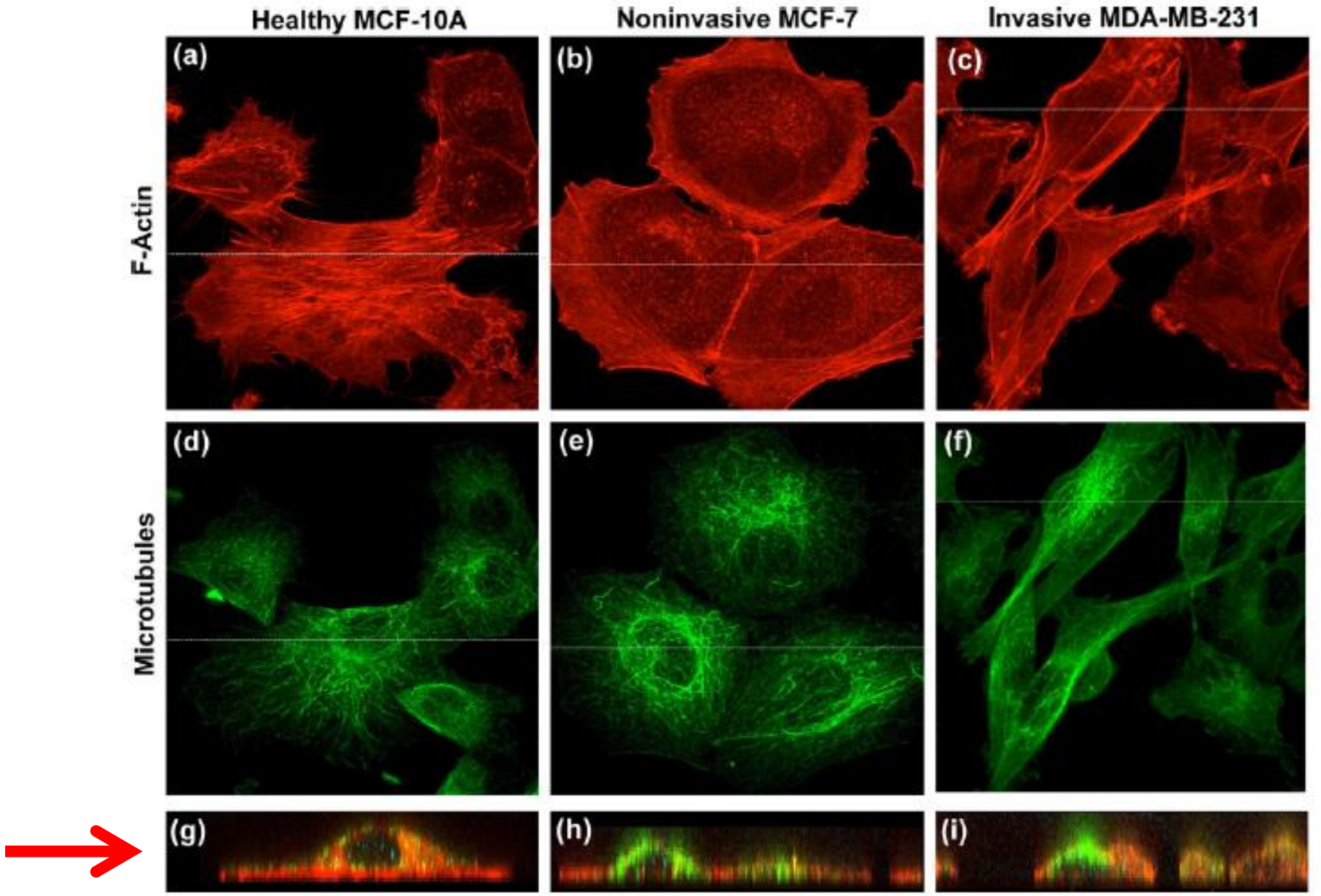
HBL – 100

MDA-MB-231



Yousafzai *et al*, J. Biomed. Opt. 2016

Confocal Images of actin (red) + microtubules (green)



Calzado-Martín *et al* ACS-Nano 2016

OUTLINE

- Optical Tweezers (OT) – single-beam gradient force 3D optical trap: how this works, optical manipulation of microparticles

- Measuring picoNewton forces with OT : direct and indirect methods

- Applications of OT in living cell studies:

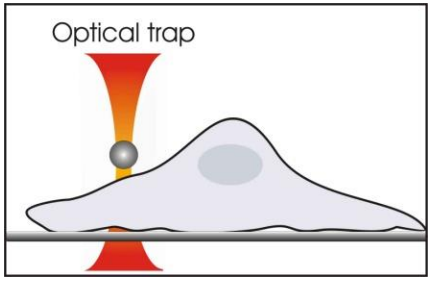
  - probing forces expressed by developing neurons
  - probing the stiffness of cancer cells
  - **mechanotransduction - conversion of the mechanical stimulus into a biochemical signal by the cell**
  - **biochemical local cell stimulation using optically manipulated vectors (coated beads, biodegradable micro-sources, liposomes)**

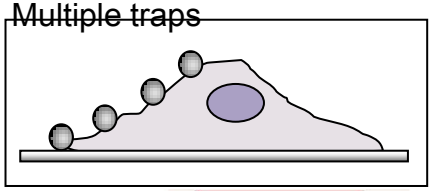
Focal Mechanical Stimulation (Examples)

Mechanical stimulation is induced by trapped beads, moving either the beads or the cell.

The effect of the mecanical force applied by the beads on the cell is monitored by optical microscopy techniques on the same platform.

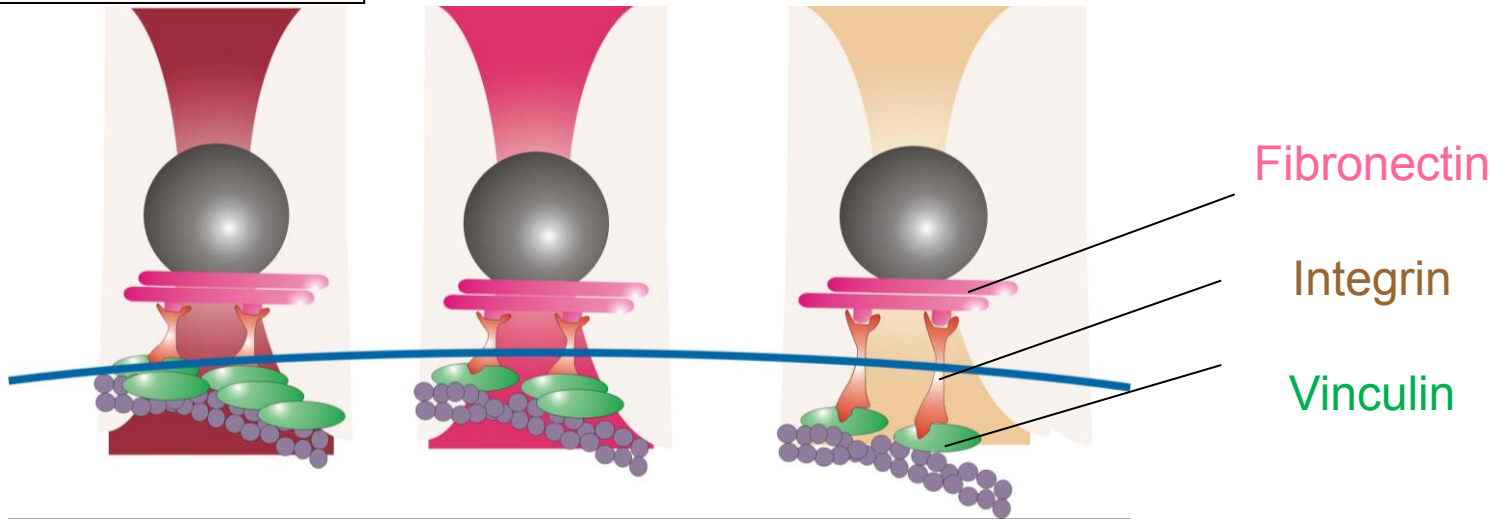
Example 1: Cell mechanical stimulation at multiple adhesion sites, with force modulation





Multiple optical trapping is combined with epi-fluorescence to monitor vinculin recruitment as a function of the trap strength.

Fn coated beads are manipulated on the dorsal surface of Vin-GFP transfected HeLa cell.

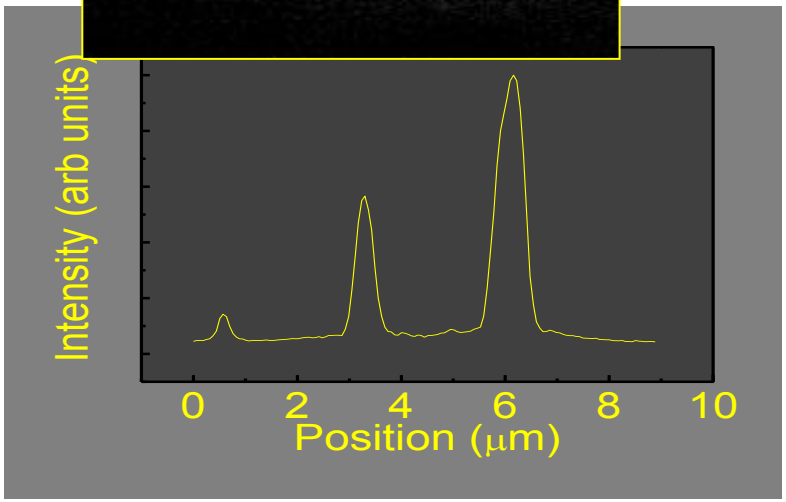


V. Emiliani *et al*, SPIE (2006)

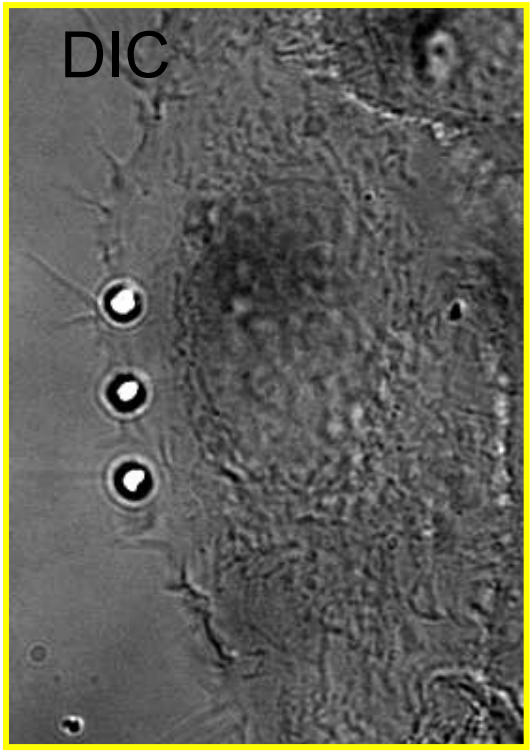
Vinculin recruitment

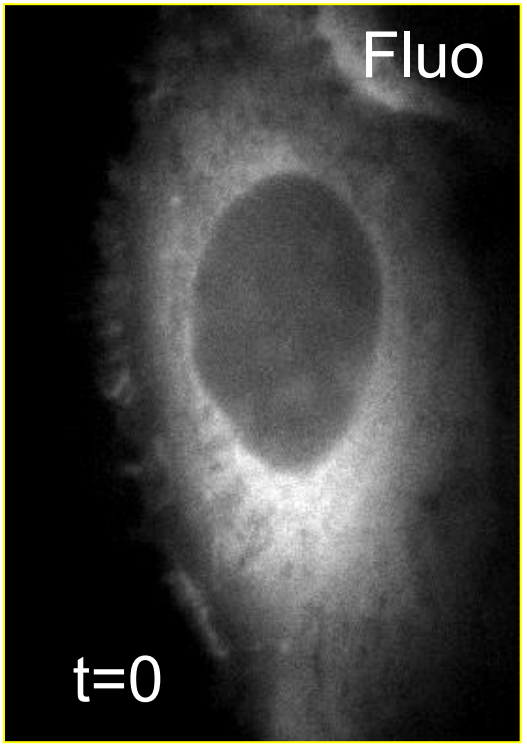
The strength of the traps is modulated in 3 steps

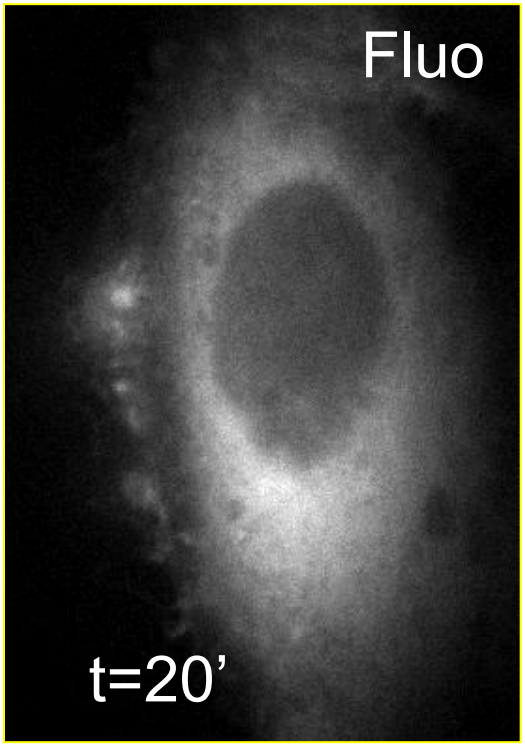
changing the power of the laser



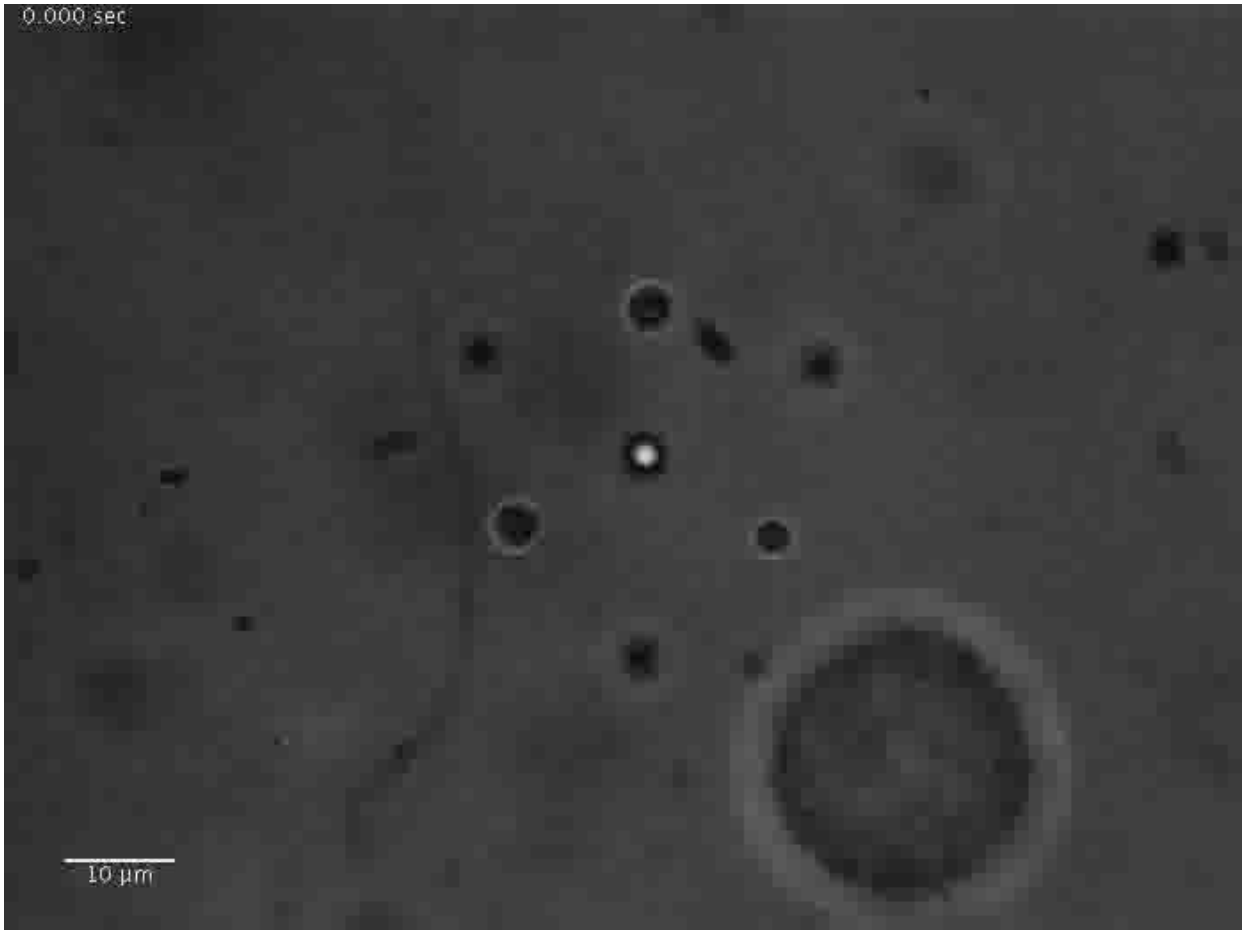
Vinculin recruitment increases with the strength of the trap, Showing a selective response of the cell to the mechanical stimulus.







Using multiple OT to stimulate the cell

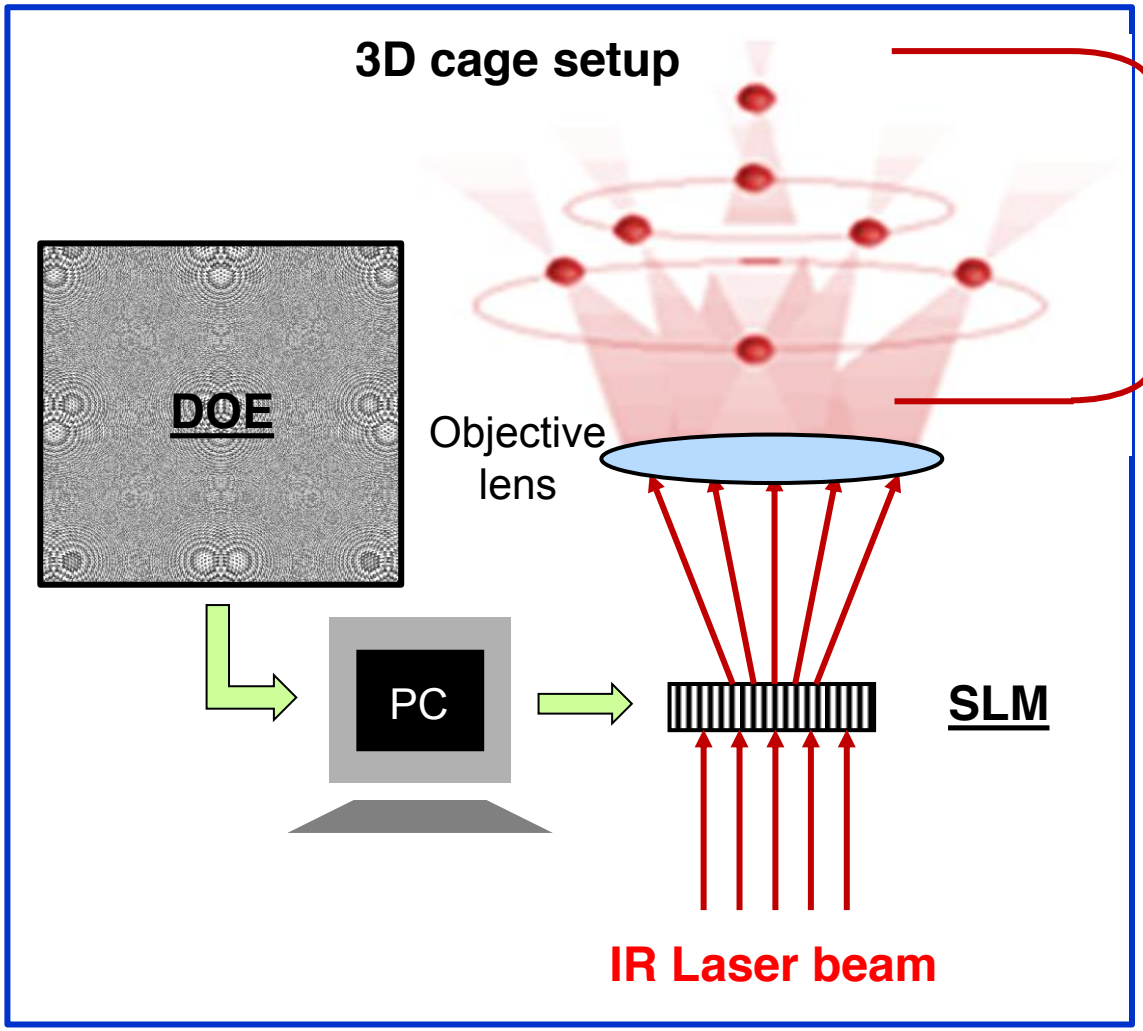


HeLa cell goes under the cage (configured by OT)

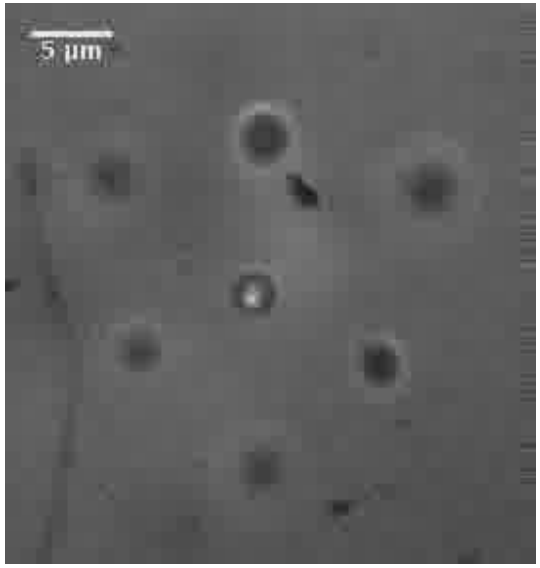
2004-2006, E. Ferrari OM-Lab collalboration with Dr. V. Emiliani from Pierre and Marie Curie University (Paris VI)

Building the cage

by means of **D**iffractive **O**ptical **E**lements implemented on a **S**patial **L**ight **M**odulator



3D cage (top view)



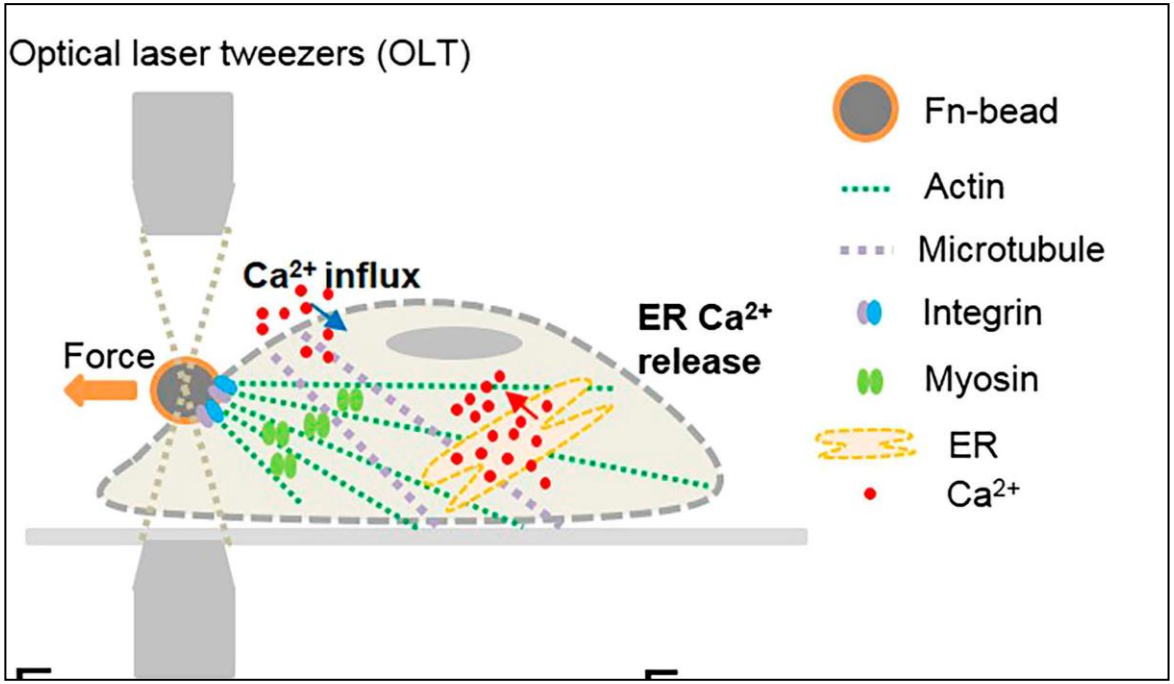
D. Cojoc *et al* 2004-2006

OM-Lab

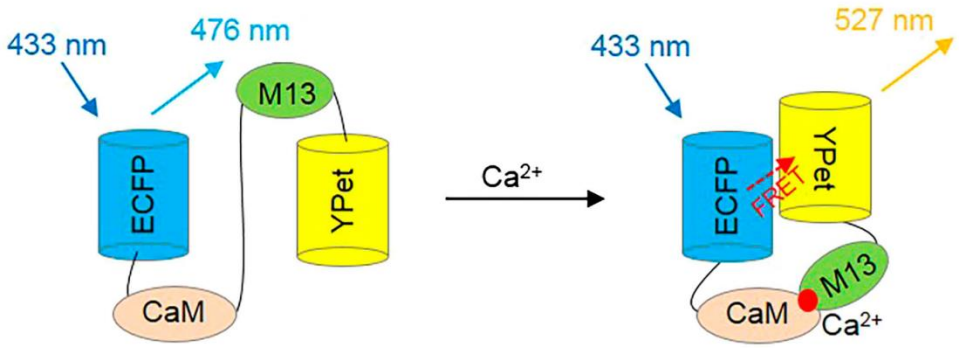
Distinct mechanisms regulating mechanical force-induced Ca^{2+} signals at the plasma membrane and the ER in human MSCs

Tae-Jin Kim *et al*, eLife 2015;4:e04876. DOI: 10.7554/eLife.04876

Investigate how mechanical forces are transmitted in a human mesenchimal stem cell using optical tweezers for mechanical stimulation and a FRET probe for Ca^{2+} to CaM protein binding measurement.



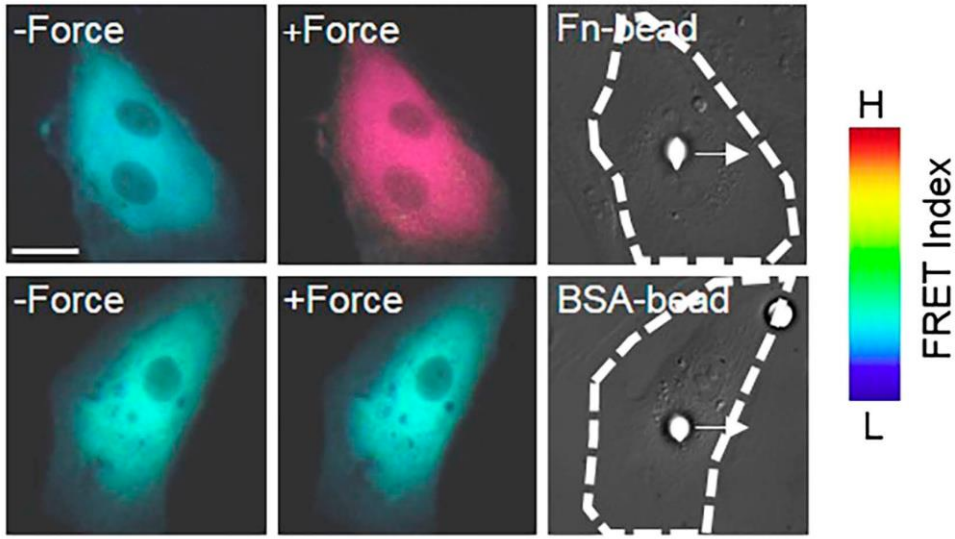
Schematic drawing of the activation mechanism of the Ca2+ FRET biosensor.

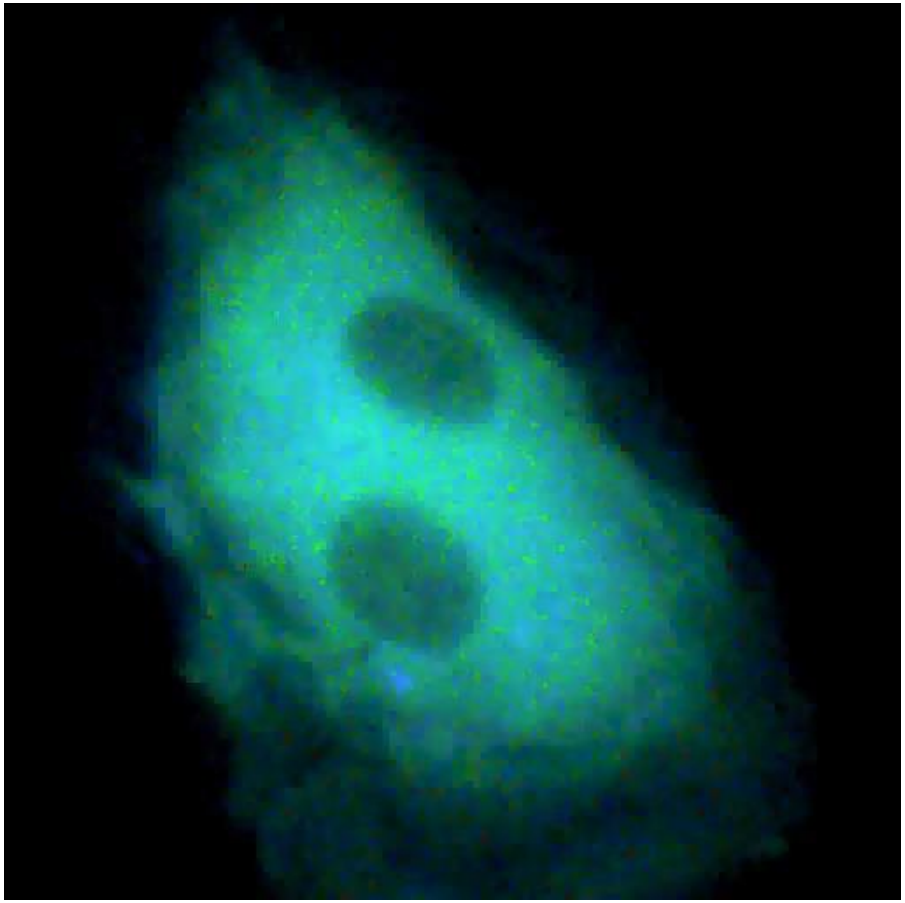


Color images represent the YPet/ECFP emission ratio of the cytoplasmic Ca2+ biosensor.

The color scale bars represent the range of emission ratio, with cold and hot colors indicating low and high levels of Ca2+ concentration, respectively.

Notice the high ratio when the force is applied by the Fn bead.





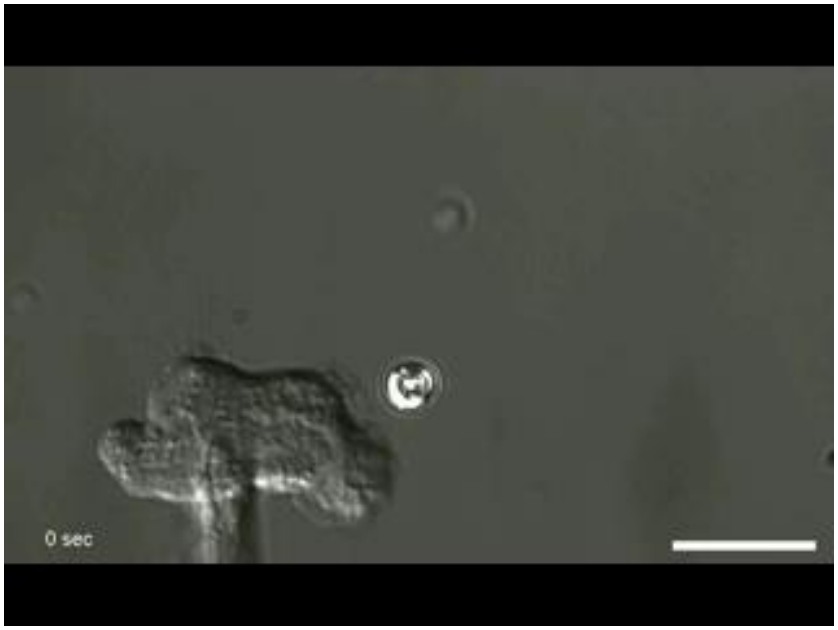
A HMSC transfected with cytosolic Ca2+ biosensors before and after mechanical force application by optical laser tweezers on a Fn-coated bead attached to the cell (Duration of Video: 2700 s).

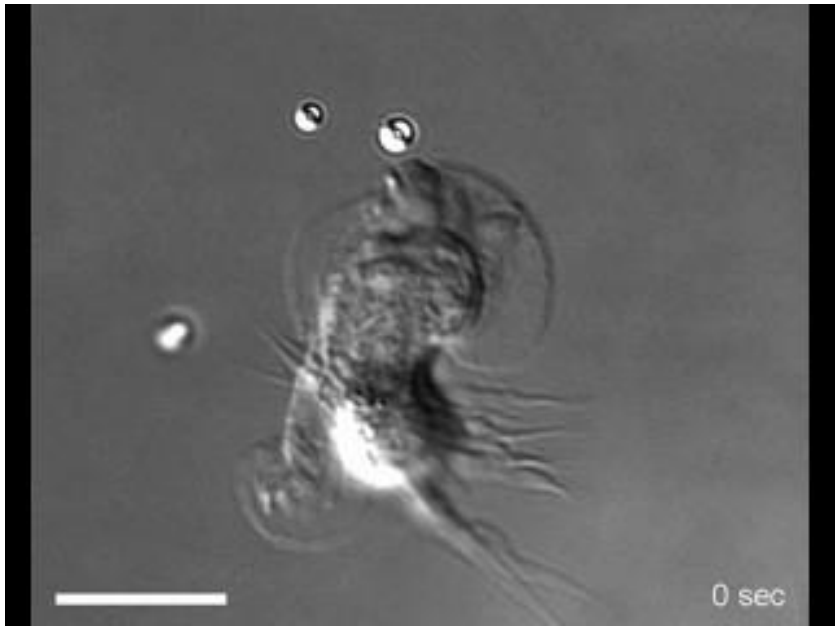
Biochemical Stimulation

Biomechanical stimulation is induced by coated beads
optically manipulated in contact to the cell
or
filled liposomes opticaally manipulated
in the vicinity of the cell and potholysed

The effect on the cell is observed by
optical microscopy techniques on the same platform

Cell stimulation with biodegradable micro sources





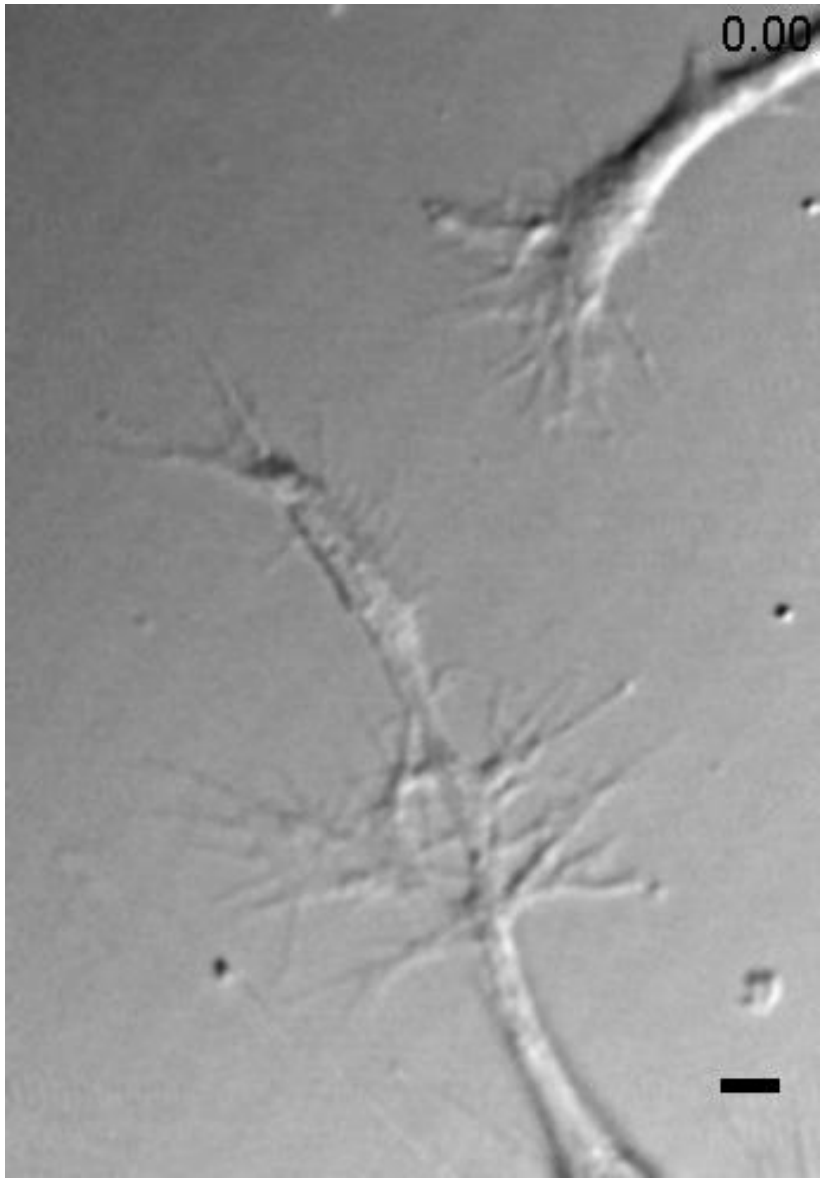
Chemoattractant

Chemorepellent

Human Neutrophil Cells, scale bar 10 um

Kress *et al*, Nat. Methods, 2009

Neuronal development



Neurons release biochemical cues which are intercepted and interpreted by their nearby neurons.

- The **Growth Cone (GC)** searches and detects molecular signposts that are displayed by the nearby developing neuron and the environment.

- **GC** responds to these signs by advancing, pausing and turning until it reaches its proper destination

Scale Bar = 2 μm
Acquisition freq= 1 frame every 5 s

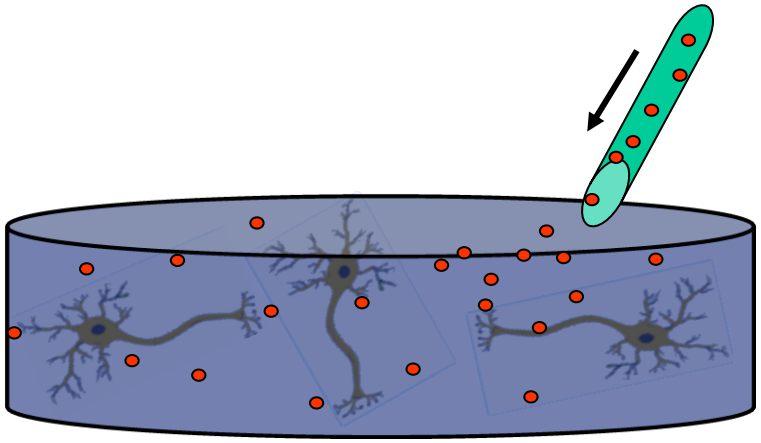
F. Difato *et al* (2006) OM-Lab & SISSA

GOAL:

Create physiological inspired experimental conditions !

E.g. **mimic one of the two neurons in the previous example by using functionalized microvectors carrying the stimuli and manipulate them to stimulate the neuron at specific sites !**

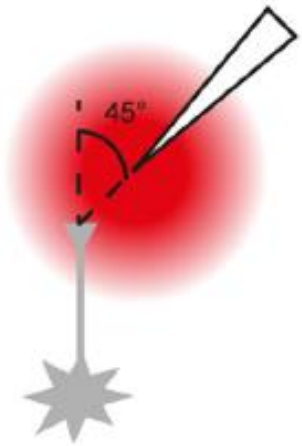
Classical bath administration of molecules rarely reflects the physiological conditions in which molecules are locally released at low concentrations, creating spatial and temporal gradients.



Assays for Localized Sources of Guidance Cues

I. Dupin *et al* (2013) J. Neurosci., 33: 17647

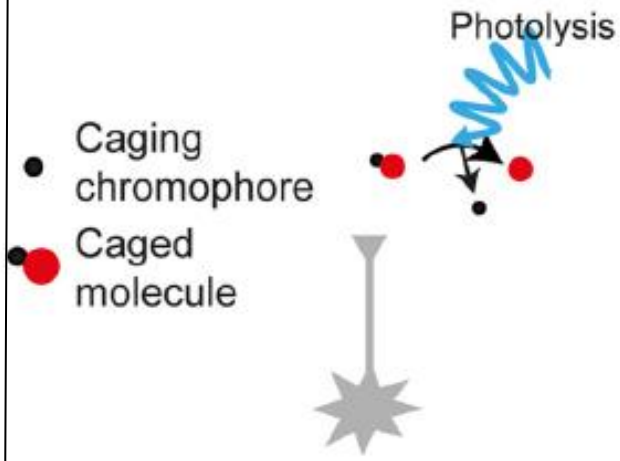
Micro-pipette based assay



Pujic Z *et al*, (2008)
J Neurosci Methods 170:220

Gundersen RW, Barrett JN
(1979) Science 206:1079

Optical uncaging of caged molecule



Ellis-Davies GC (2007)
Nat Methods 4:619

Gomez TM, Spitzer NC
(1999) Nature 397:350

Photorelease of encapsulated molecules

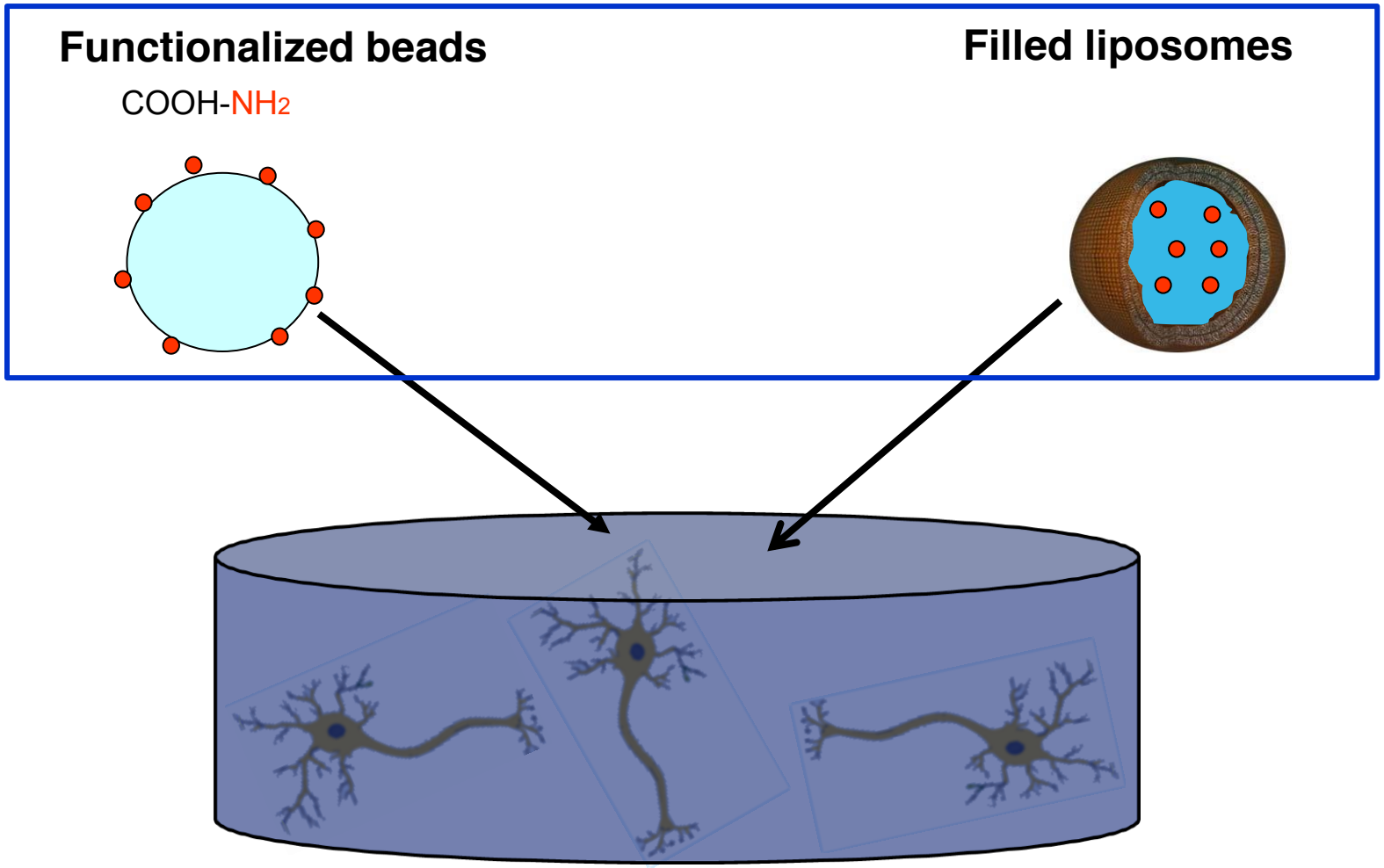
Pinato G *et al*, (2012)
Sci Rep 2:675

Pinato G *et al*, (2011)
J Biomed Opt 16:095001

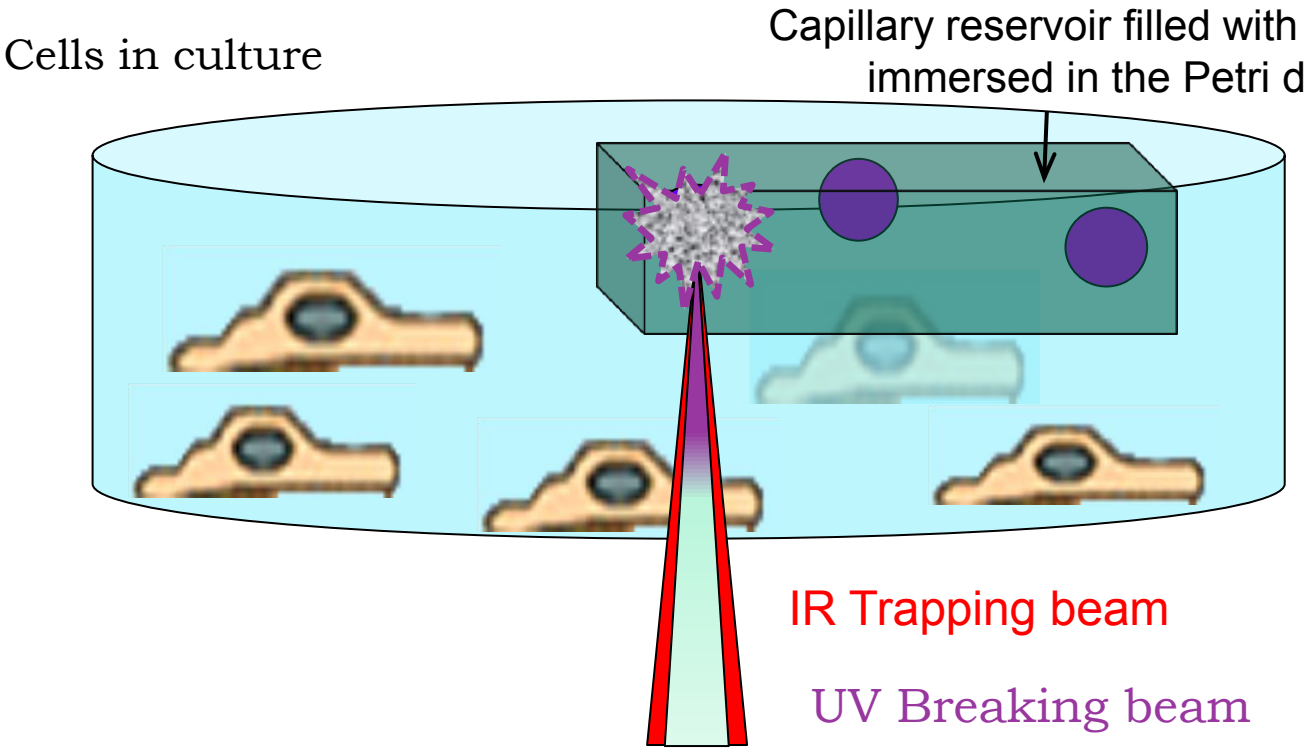
Sun B, Chiu DT (2003)
J Am Chem Soc 125:3702

Local stimulation using micro/nano vectors

Active molecules (e.g. guidance cues) are cross-linked to the surface of **microbeads** or encapsulated in **liposomes** (lipid vesicles)



Vector - Cell Positioning by Optical Manipulation



and delivered by:

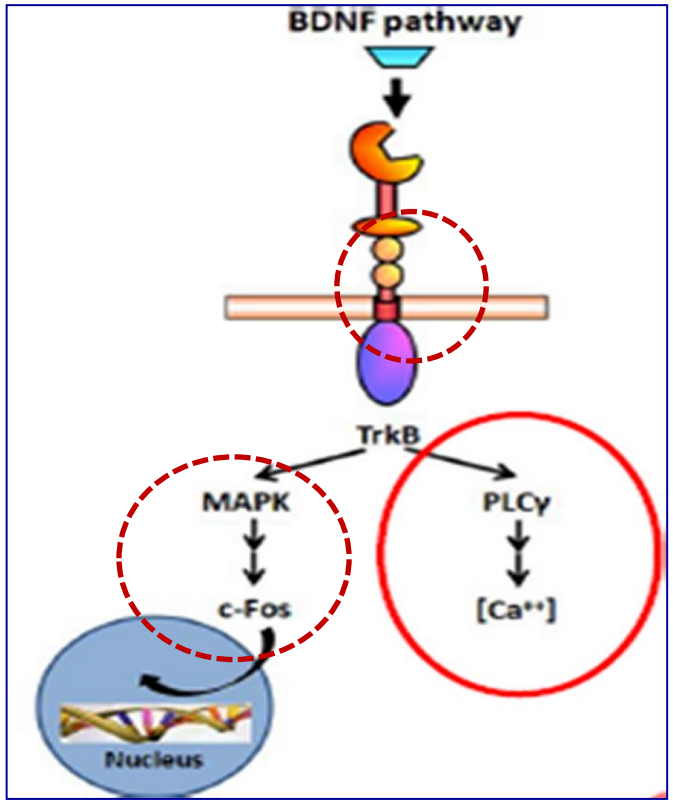
- contact (beads or microsources) – D'Este *et al* Integrative Biology (2011)

- photolysis of liposomes Sun B, Chiu DT , J ACS (2003)

Example 1

Focal stimulation of specific neuronal compartments by optically manipulated microbeads coated with BDNF

Silica beads functionalized with COOH allow cross-linking of any type of proteins on bead surface (beads and kit are commercially available)



A single microbead positioned at about 30 μm from the cell body is enough to:

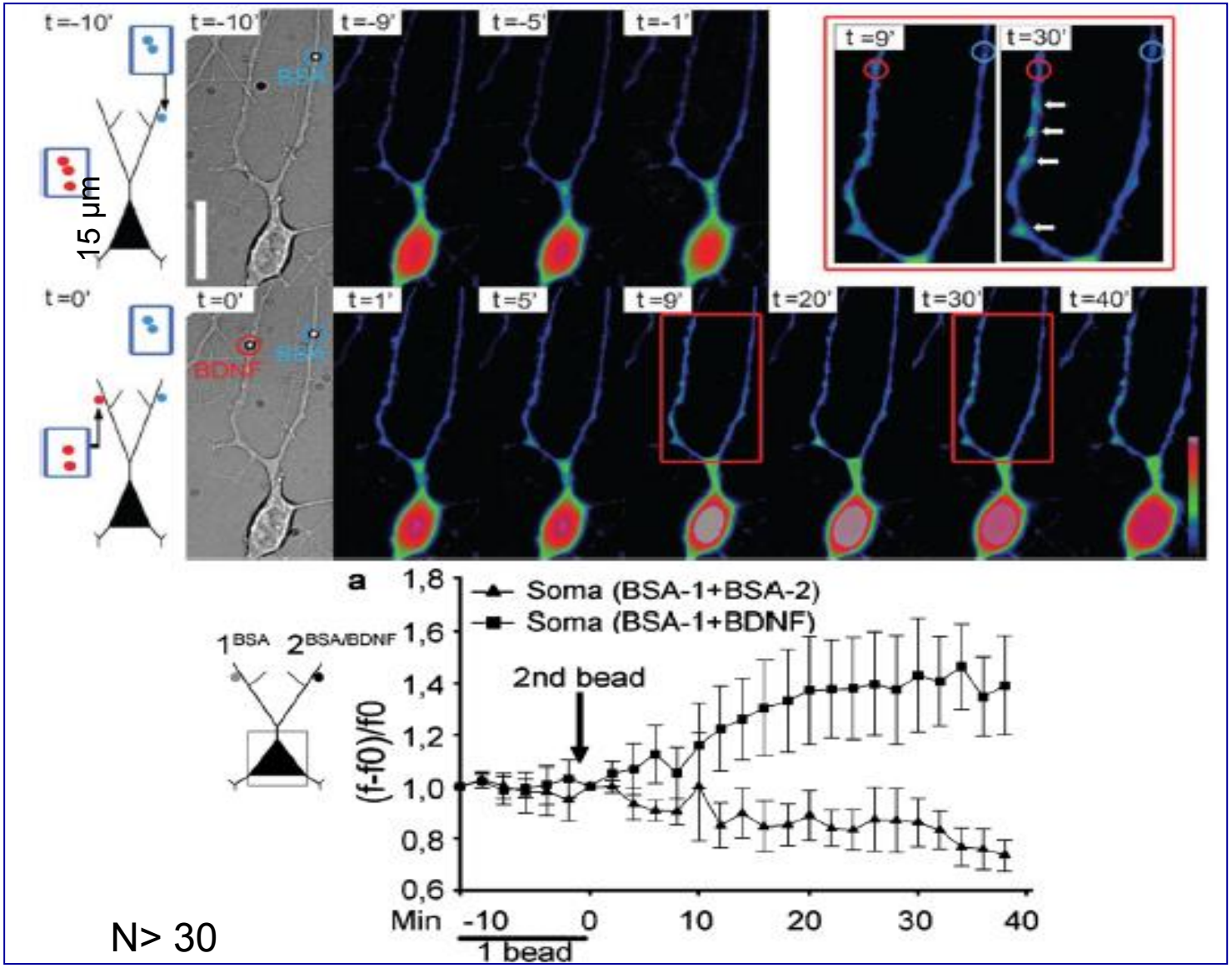
- increase Ca++ in the cell body and stimulated dendrite
- activate the BDNF receptor TrkB
- Induce c-Fos translocation in nucleus
- increase neurite motility

BDNF = Brain Derived Neurotrophic Factor

collaboration with the group of prof. **Enrico Tongiorgi**
BRAIN Centre, University of Trieste

http://www2.units.it/brain/

Ca^{++} increases in soma and stimulated dendrite

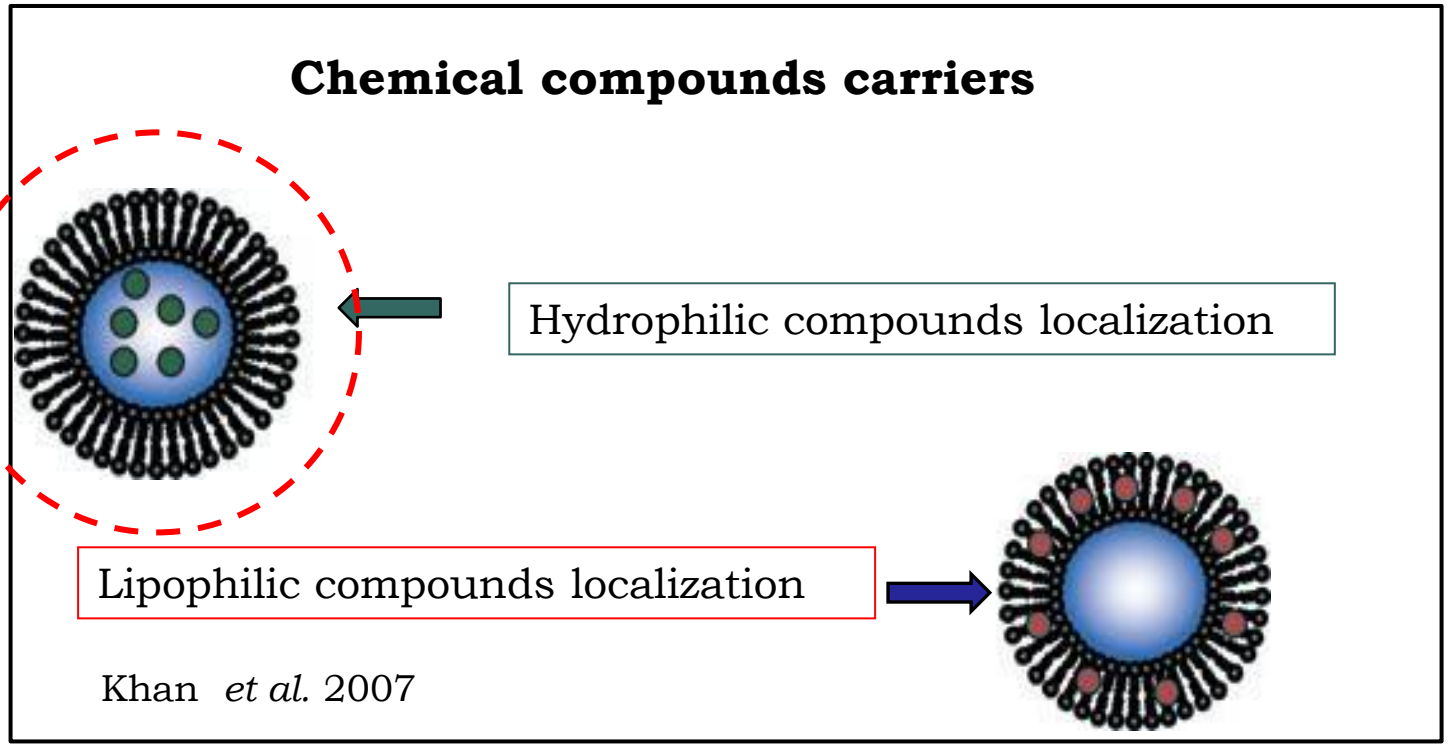


Hippocampal neurons P0-P1 – 1-2 DIV from rats

E. D'Este *et al* , Integr. Biol. **3**, 568 (2011)

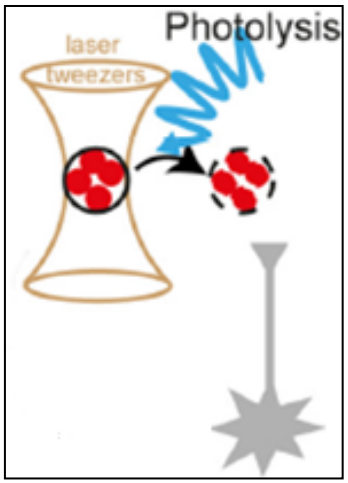
Using Liposomes as Vectors carrying active molecules

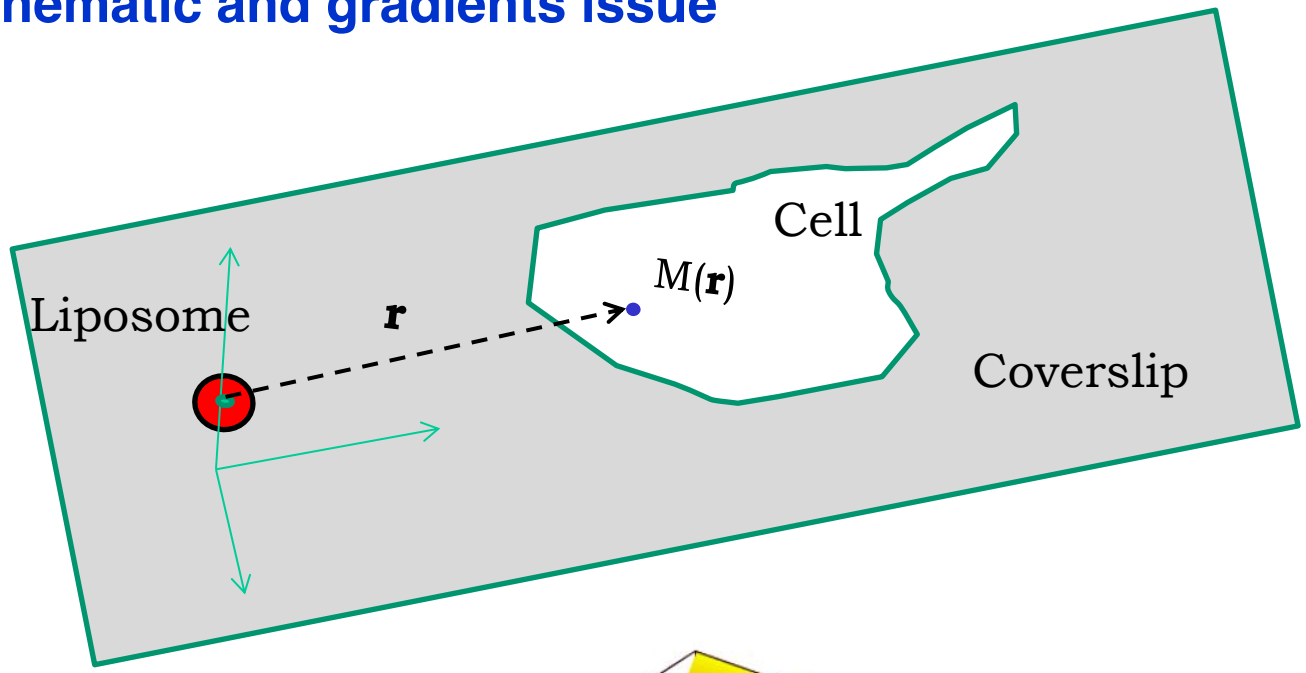
- ✓ Spherical vesicles from 50 nm to 50 µm
- ✓ Phospholipid bilayer membrane
- ✓ Aqueous core

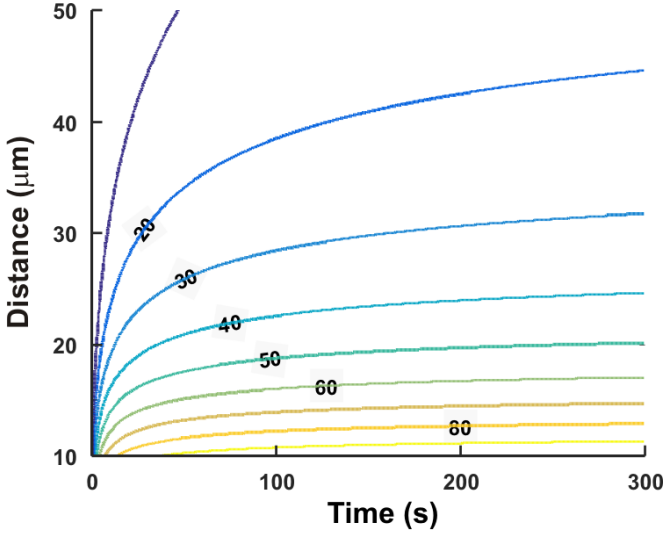


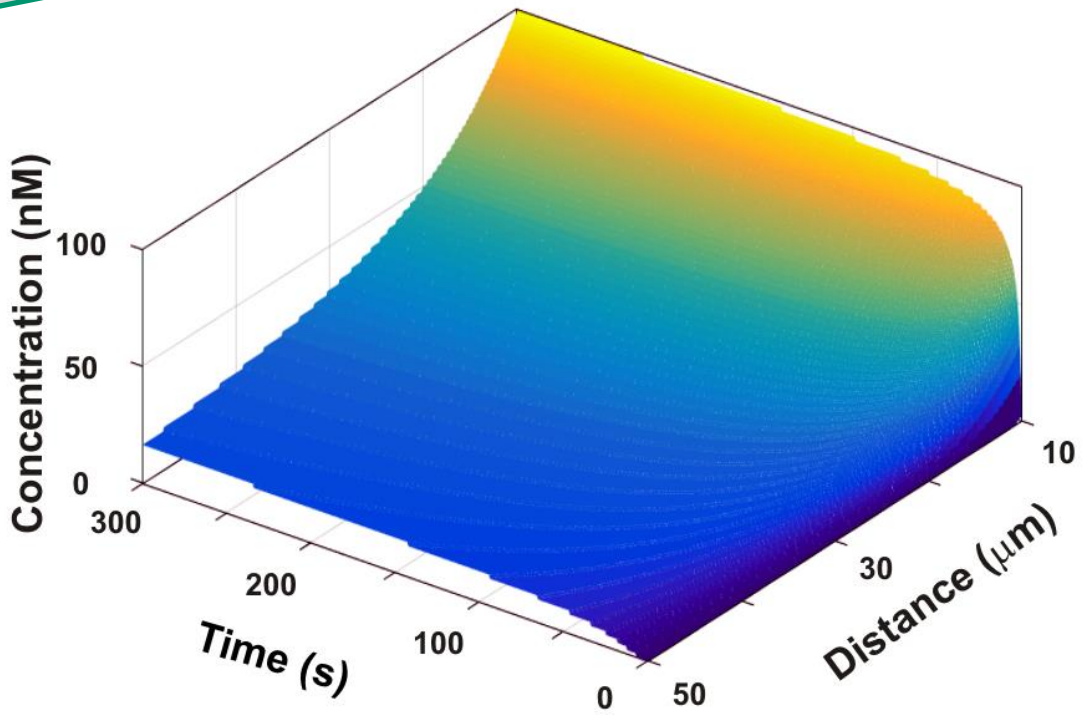
A liposome of 1 µm diameter, filled with 1 nM solution contains 1 MOLECULE (mean value) !!!!!!!!!

Release Schematic and gradients issue









Example 2
Focal stimulation of hippocampal neurons by PrP^C

The **cellular prion protein (PrP^C)** is present in all cells, particularly in neurons. PrP^c has been associated with many cellular processes, including the **regulation of ion transport, neuritogenesis, cell survival, cell-to-cell interactions, cell signaling and synaptic transmission** (Linden *et al.* 2008).

Characterization of prion protein function by focal neurite stimulation

Ladan Amin[1], Xuan T. A. Nguyen[1], Irene Giulia Rolle[1], Elisa D'Este[2], Gabriele Giachin[1,*], Thanh Hoa Tran[1], Vladka Čurin Šerbec[3], Dan Cojoc[4,‡] and Giuseppe Legname[1,‡]

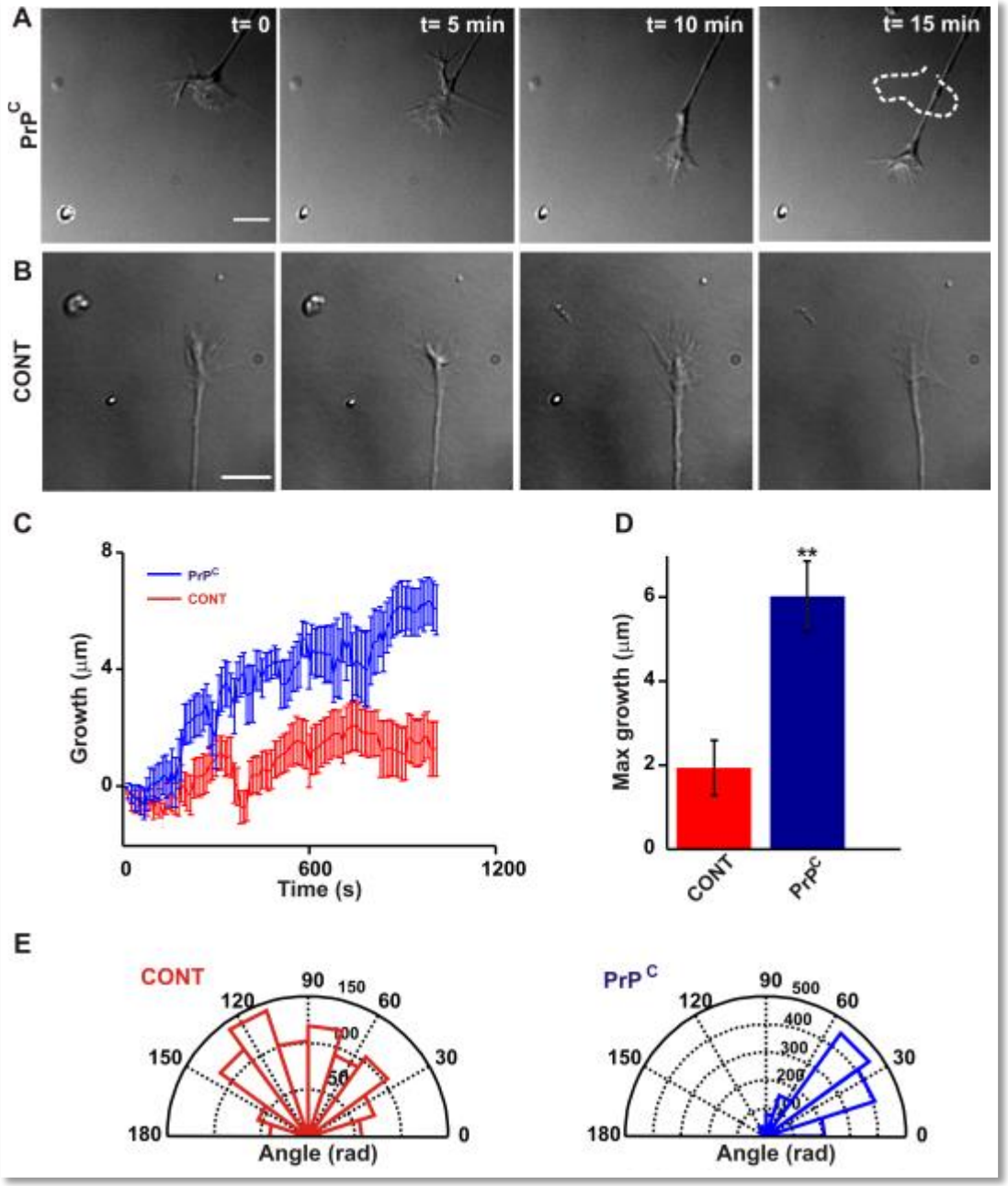
Journal of Cell Science (2016) 129, 3878-3891 doi:10.1242/jcs.183137

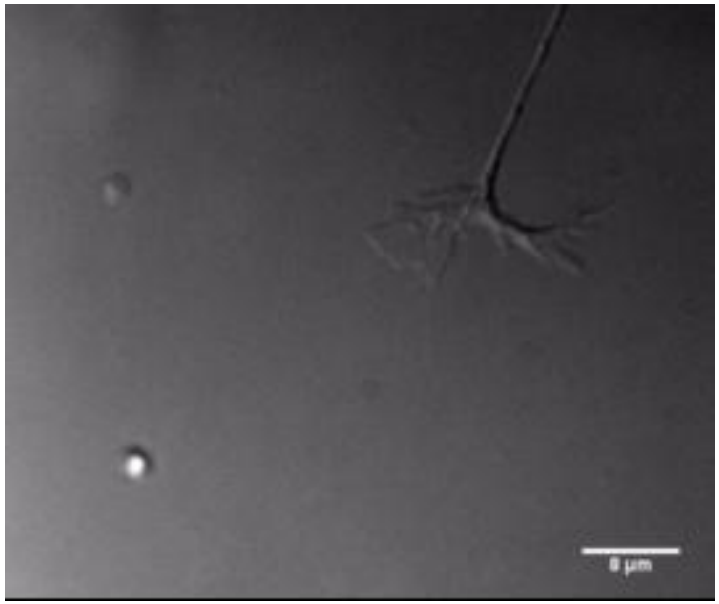
PrP^C encapsulated in lipid microvesicles or cross-linked to the surface of microbeads

We found:

- recPrP^C works as a guidance molecule
- membrane PrP^C is required for the extracellular PrP^C to bind (PrP^C might be the receptor of itself)
- full length PrP^C is required to have the guidance function
- concentration modulates the GC growth

Local delivery of controlled amount of MoPrP^{c} to neurons





Neurite growth is observed in 15 min after local stimulation.

Stimulation by bath administration induced this effect **after 24** h incubation. (Kanaani 2005).

Control liposomes (BSA) do not induce growth or turning. .

PrpC KO neurons do not respond to the stimulation with PrPc

Hippocampal neurons frome mouse P0-1, 1-2 DIV

Amin *et al*, J, Cell Science 2016

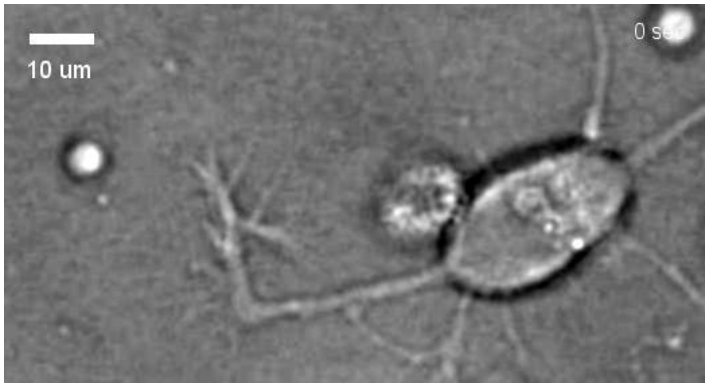
Example 3 Focal stimulation of hippocampal neurons by guidance cues encapsulated in liposomes

Netrin-1

Growth Cone (GC) growing + turning

Proof of concept

Pinato G, *et al* J. Eur. Opt. Soc. – Rap. Comm. 6, 11042, (2011)

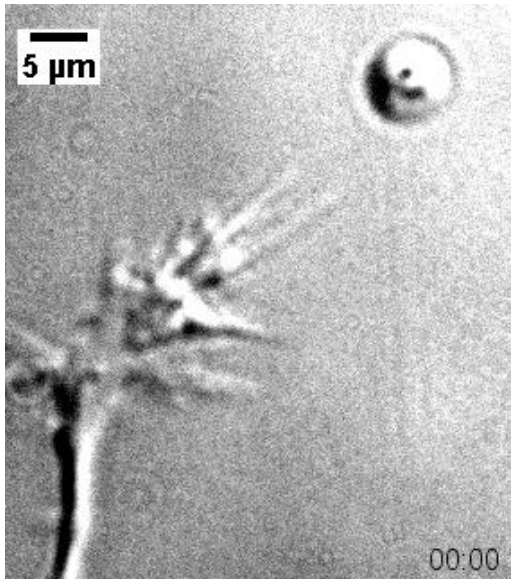


SemA3 – GC repealing and collapse

A more quantitative study:

Less than 5 Netrin-1 molecules initiate attraction but 200 Sema3A molecules are necessary for repulsion

Pinato G *et al* Sci. Rep. 2, 675 (2012)



Collaboration with the group of prof. **Vincent Torre,** Neurobiology Sector**, SISSA, Trieste**

Example 4
Signal transduction dynamics
Local stimulation + FRET microscopy

Stimulating the GC with coated beads and liposomes filled with **Sem3A.**

- Signal transduction is a very complex mechanism, regulated by many "players" among which the GTPases: Rac1, RhoA and Cdc42, which act together to control cytoskeleton dynamics. [Machacek, M, …& Danuser, G, Nature 461, 99 (2009)].
- **Goal: vizualize the RhoA and Cdc42 activation and their dynamics upon local stimulation with Sem3A**
- **Study case:** Ng 108-15 neuroblastoma cells

Project in collaboration with the group of prof. **Vincent Torre**
Neurobiology Sector, **SISSA, Trieste**

Sem3A = Semaphorin 3A
is a guidance (repellant) molecule released by neurons during their differentiation

GTPase = hydrolyse enzymes that can bind and hydrolyze guanosine triphosphate (GTP)

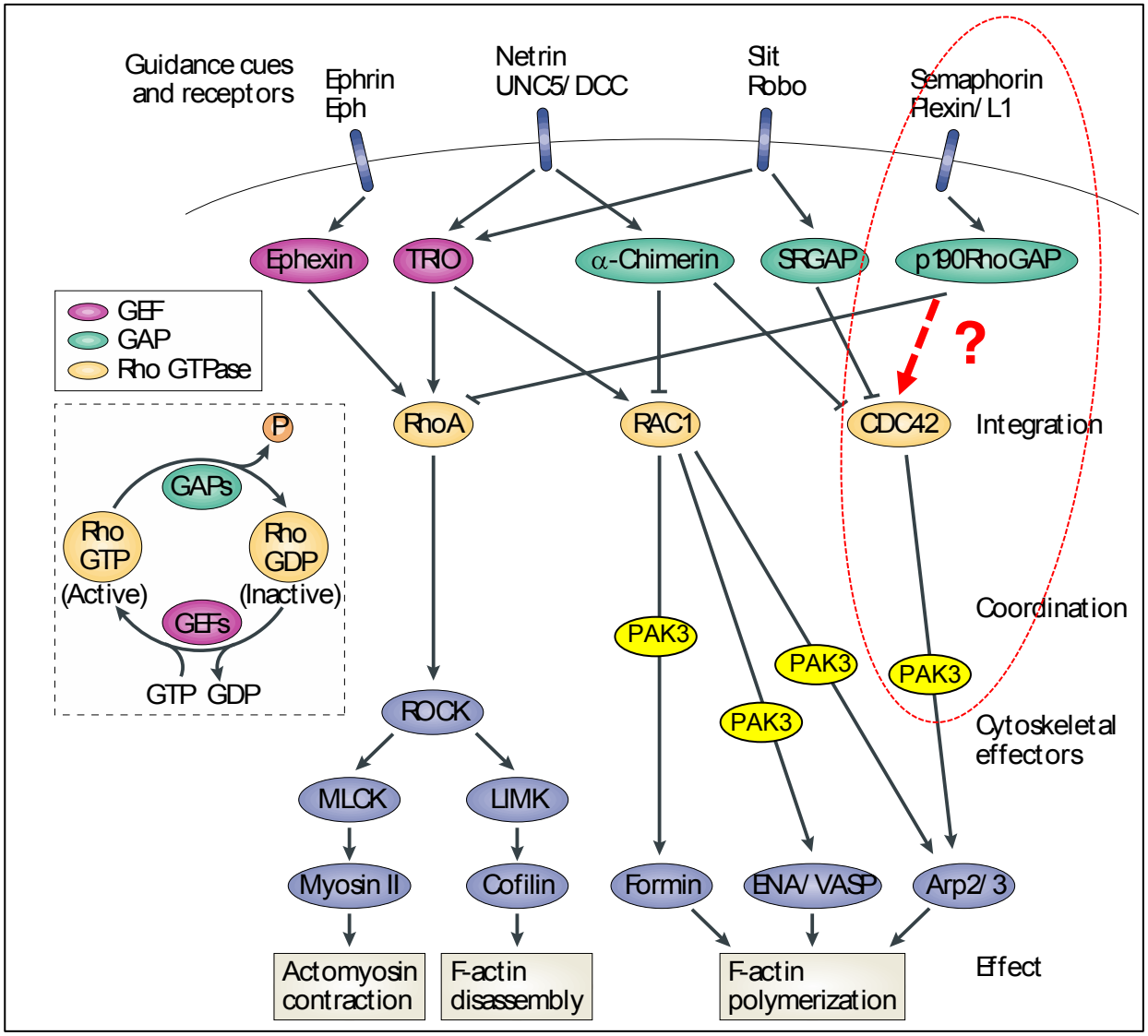
RhoGTPases are signalling nodes that couple upstream directional cues and downstream cytoskeletal rearrangements to either enhance actin polymerization for protrusion or promote disassembly and actomyosin contraction for retraction.

GTPases are a large family of hydrolase enzymes that can bind and hydrolyze guanosine triphosphate (GTP).

PAK proteins are critical effectors that link Rho GTPases to cytoskeleton reorganization and nuclear signaling.

They serve as targets for the small GTP binding proteins Cdc42 and RAC

Guidance cues signaling pathways

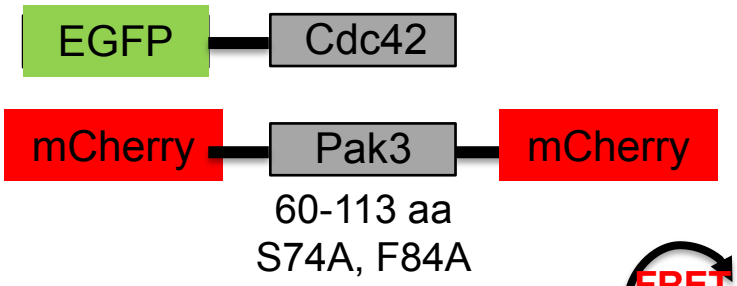


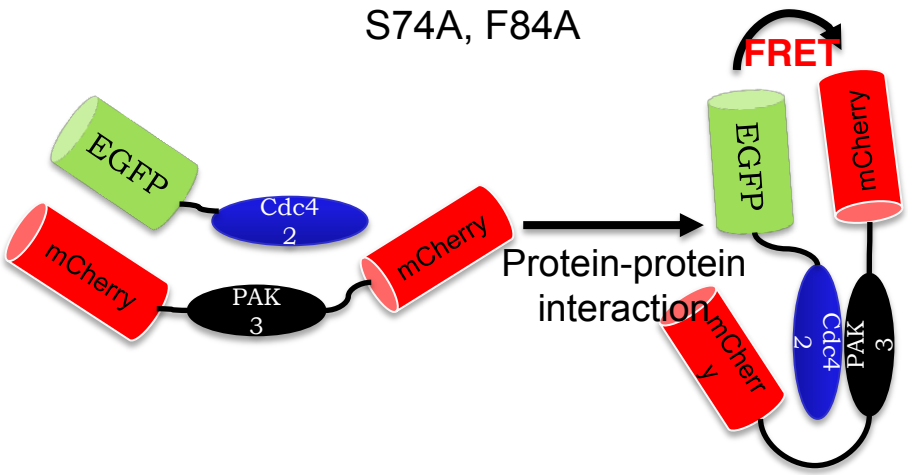
Lawery L.A. Van Vactor D. *Nature Rev-Mol Cell Biol (2009)*

FRET probes

Inter - Molecular

Cdc42 FRET sensor

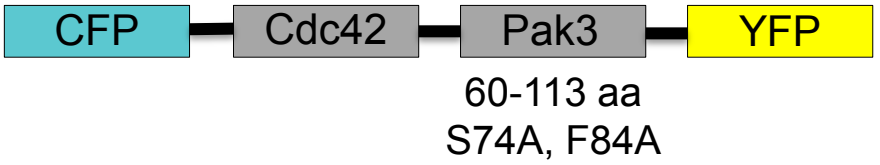


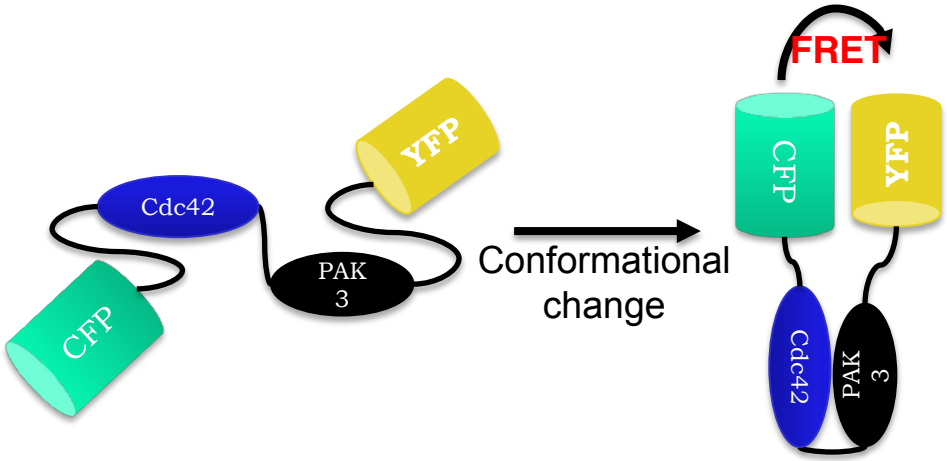


- Suitable for Protein-Protein interaction studies;
- Fluorophore Stoichiometry uncertain.
- Sensitized FRET.

Intra - Molecular

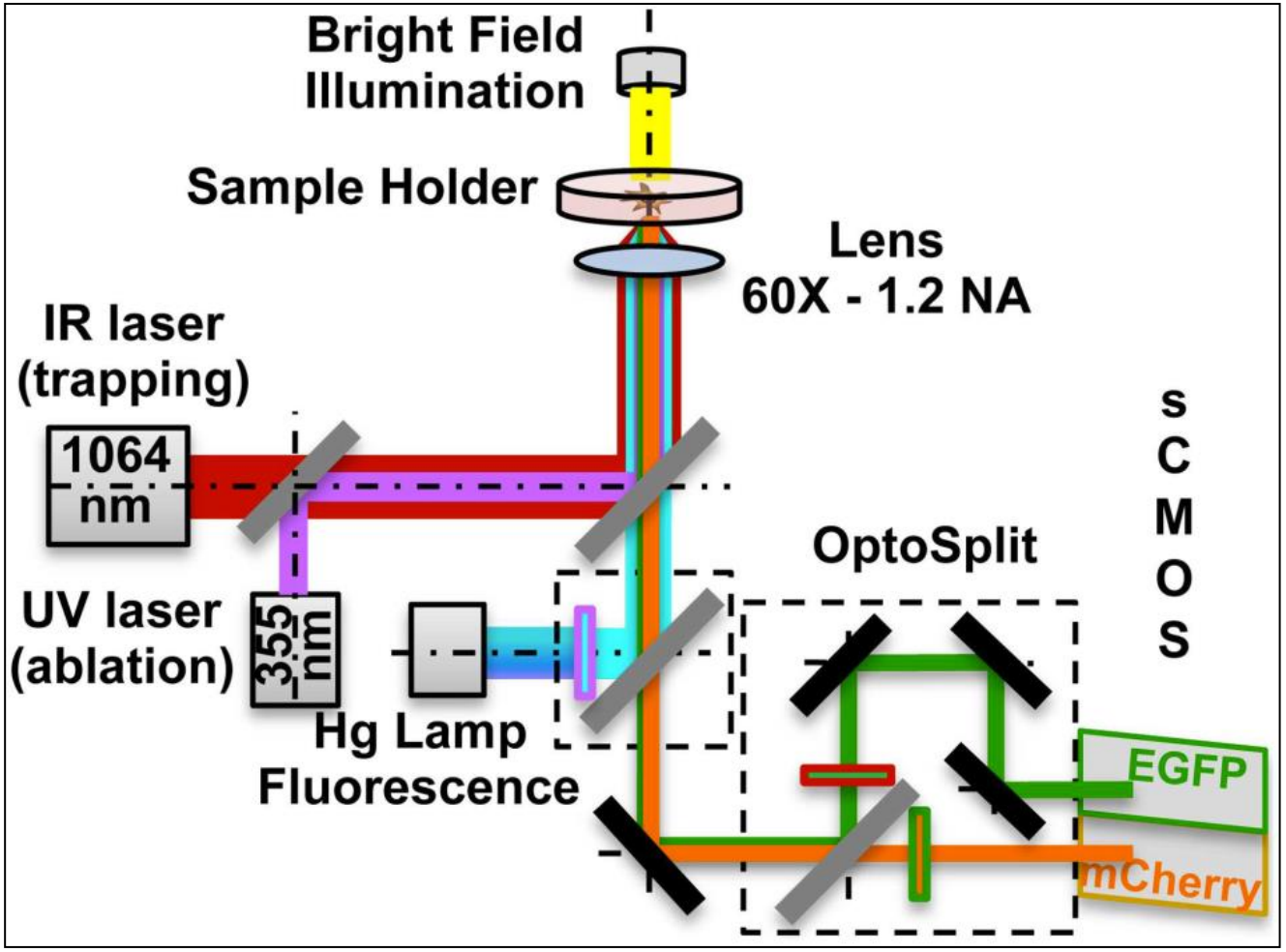
"Raichu" Cdc42 FRET sensor





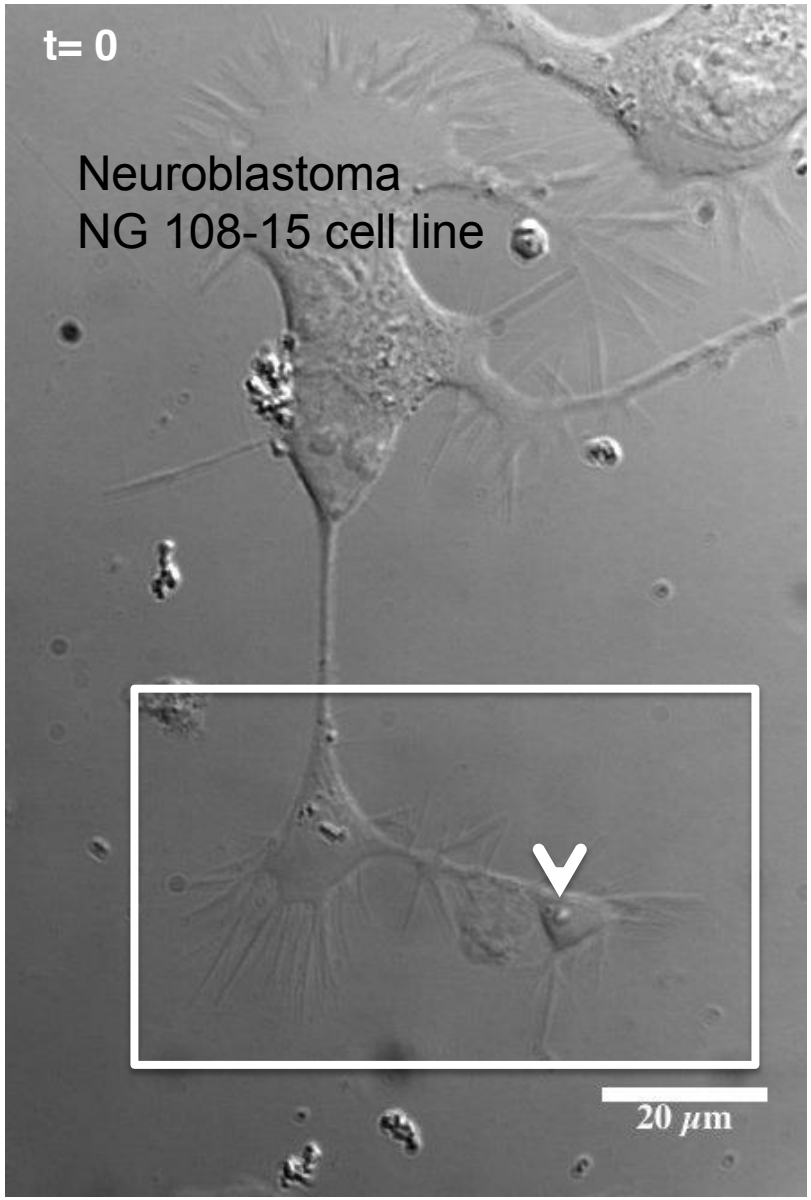
- Suitable for Protein activation studies;
- Fluorophore Stoichiometry 1:1;
- Ratiometric FRET

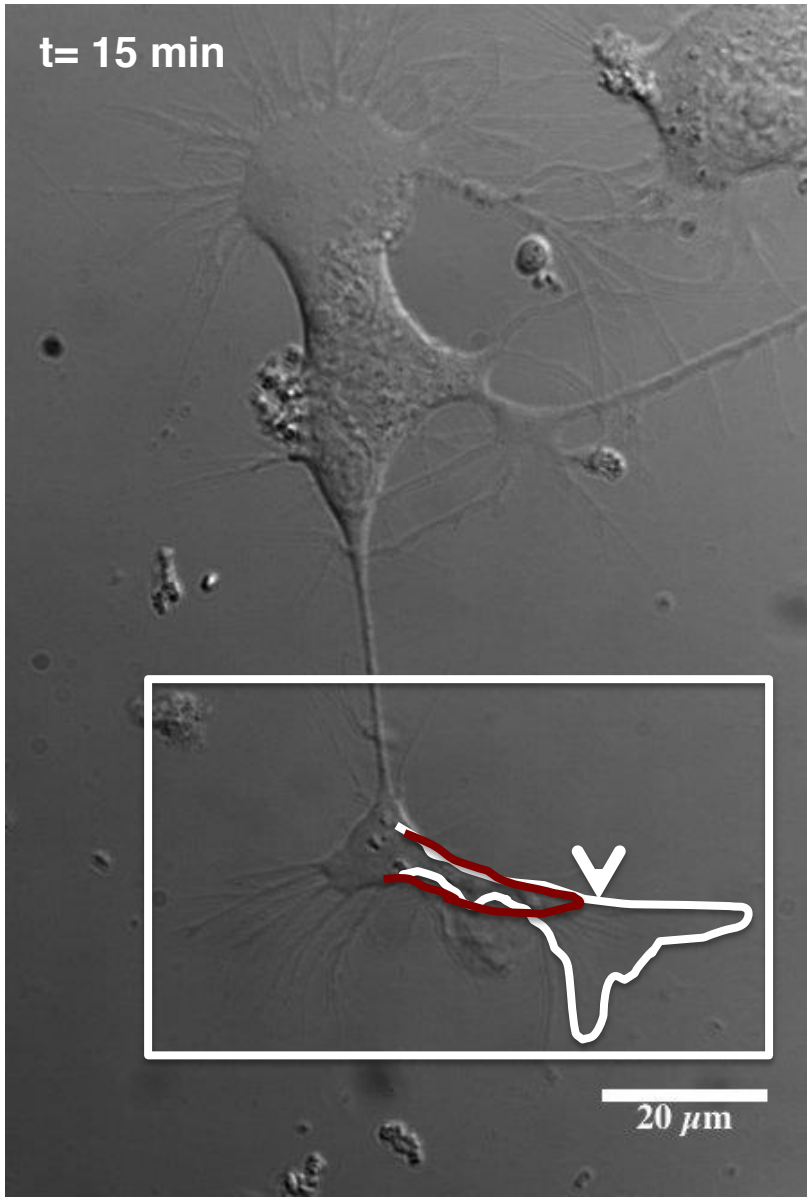
OT local stimulation – FRET imaging setup



Iseppon F *et al* Frontiers Cell. Neuroscience, 2015
Iseppon F *et a*l J. Biol. Methods, 2017

Local stimulation: SemA3 bead positioned on the GC and kept in contact for 30 s



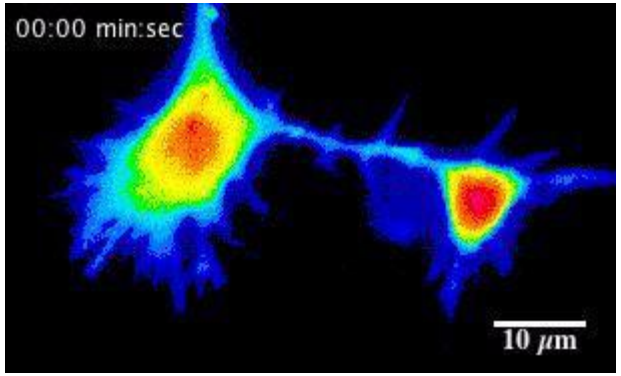


After 30 s the trap is switched off and the bead released.

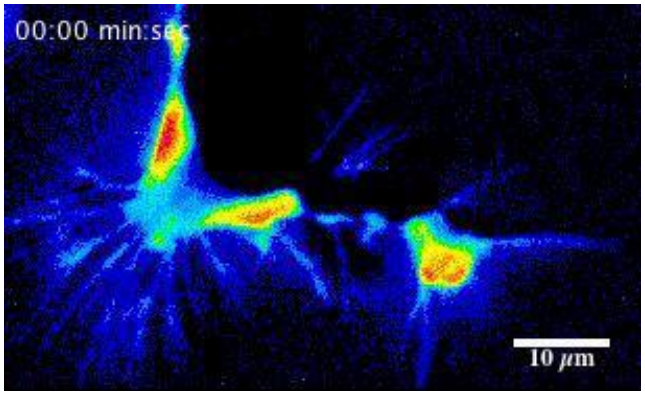
The GC retracts about 15 um after t= 15 min

Dynamics of the Cdc42 activation

using a Cdc42 FRET probe based on mEGFP and mCherry

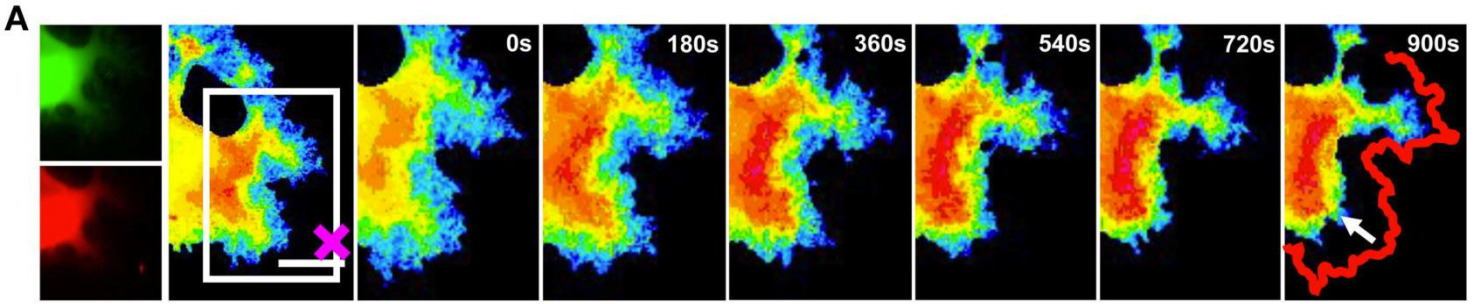


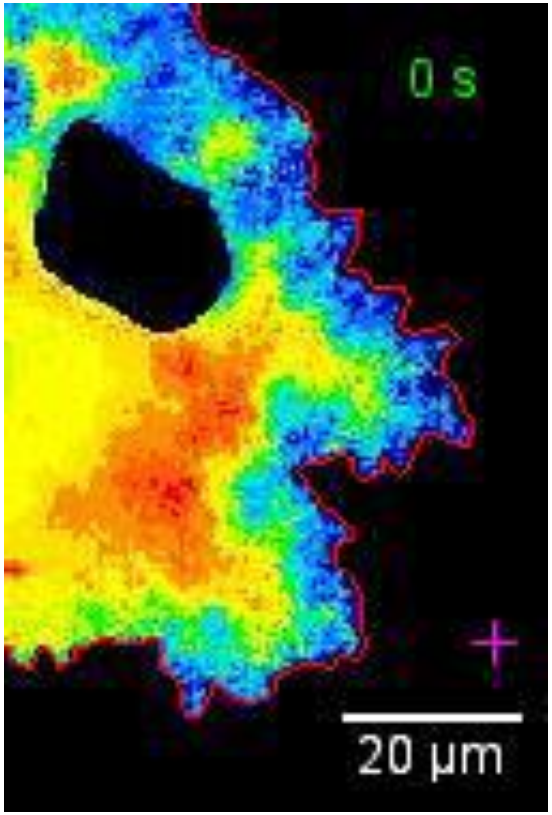
Spontaneous FRET before stimulation (Control)

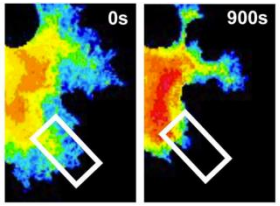


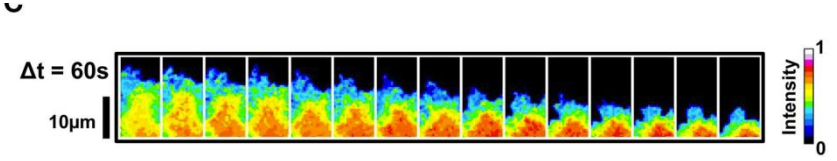
FRET after stimulation with SemA3 bead

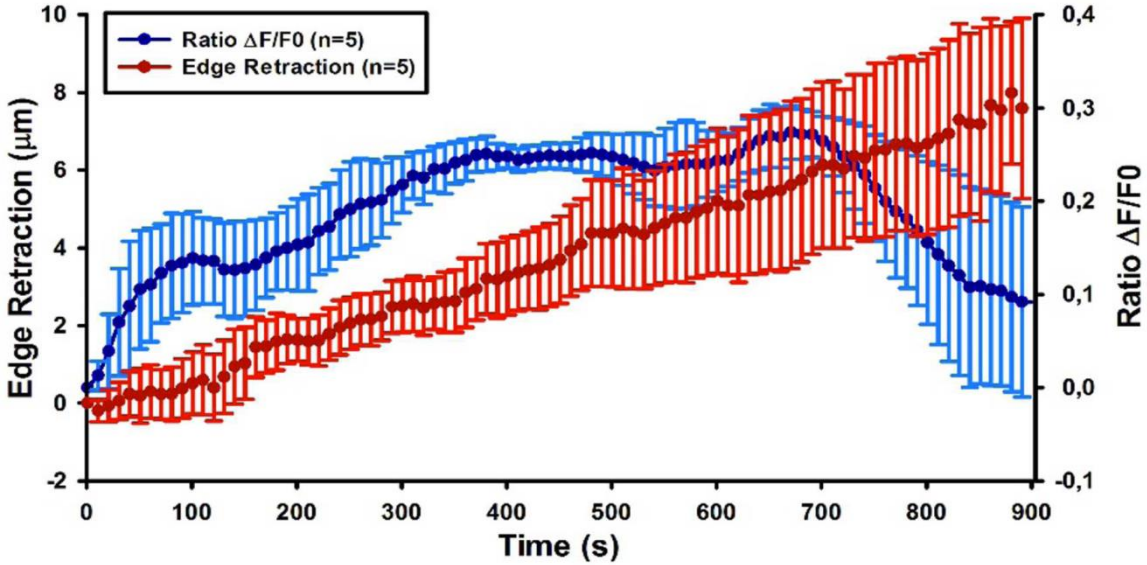
RhoA dynamics upon local delivery of Sema3A from liposome





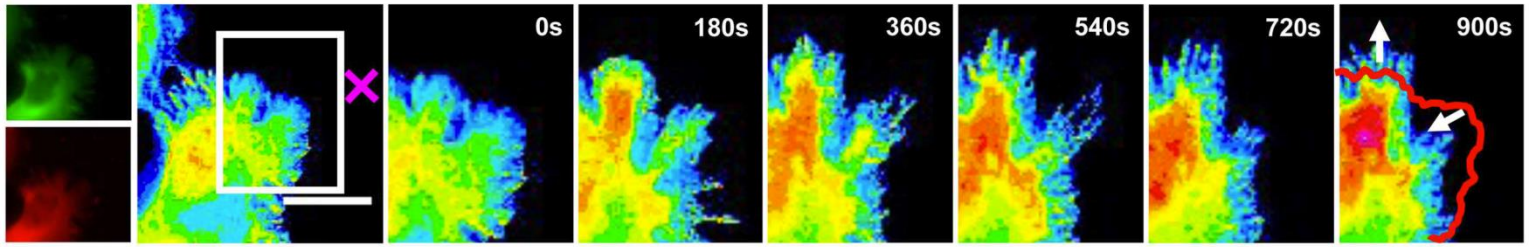


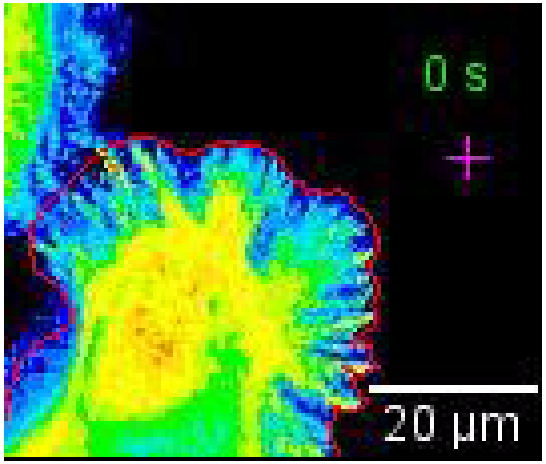


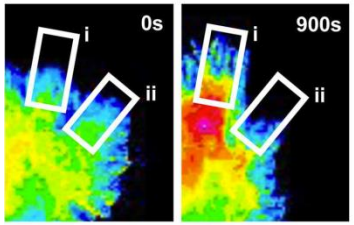


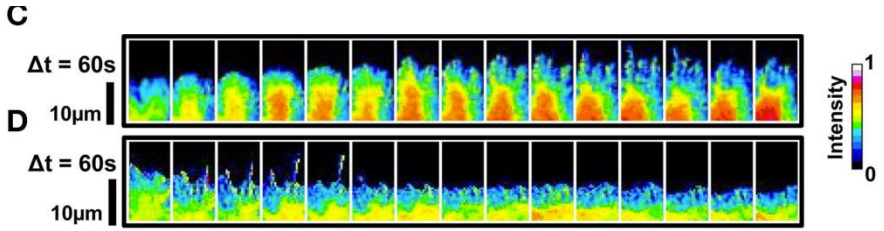
Iseppon F *et al* Frontiers Cell. Neuroscience, 2015

CdC42 dynamics upon local delivery of Sema3A from liposome

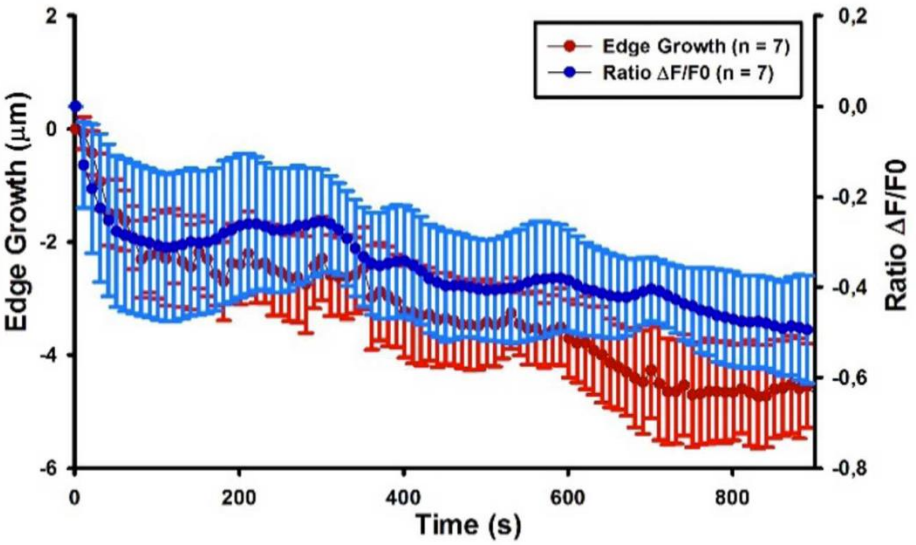








Iseppon F *et al* Frontiers Cell. Neuroscience, 2015



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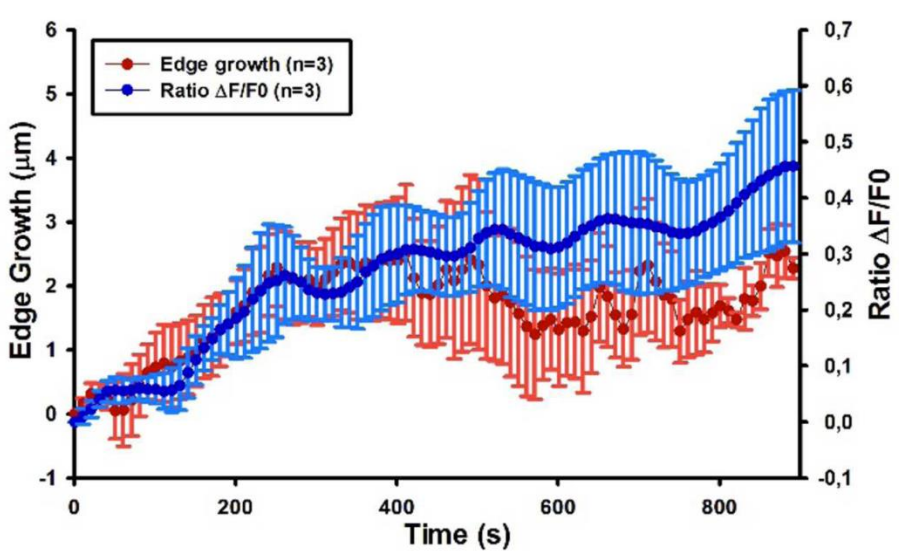
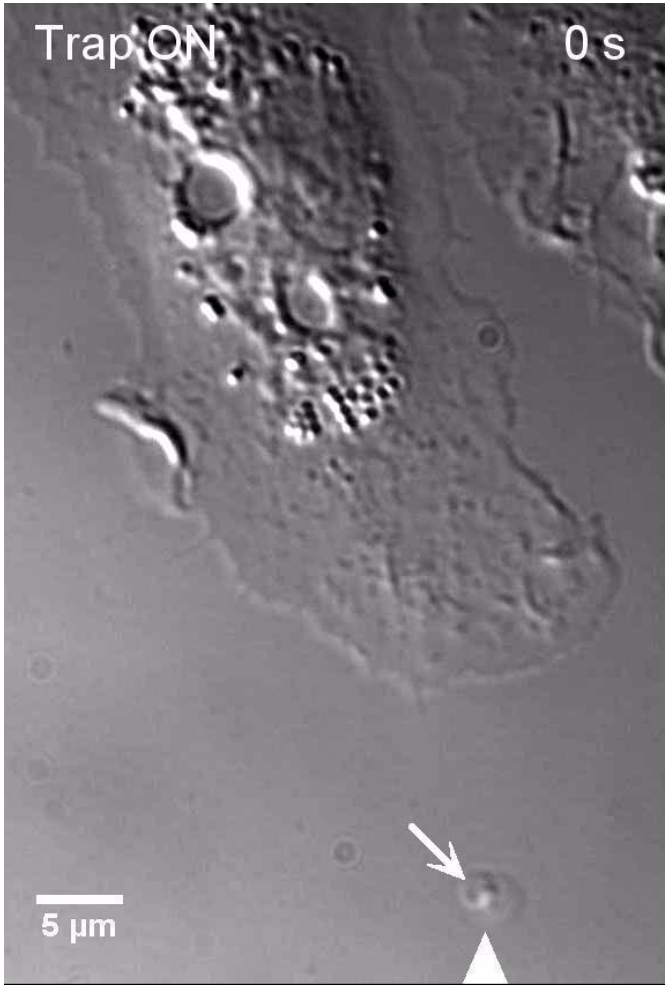


Example 5

Extracellular Vesicles (EV)



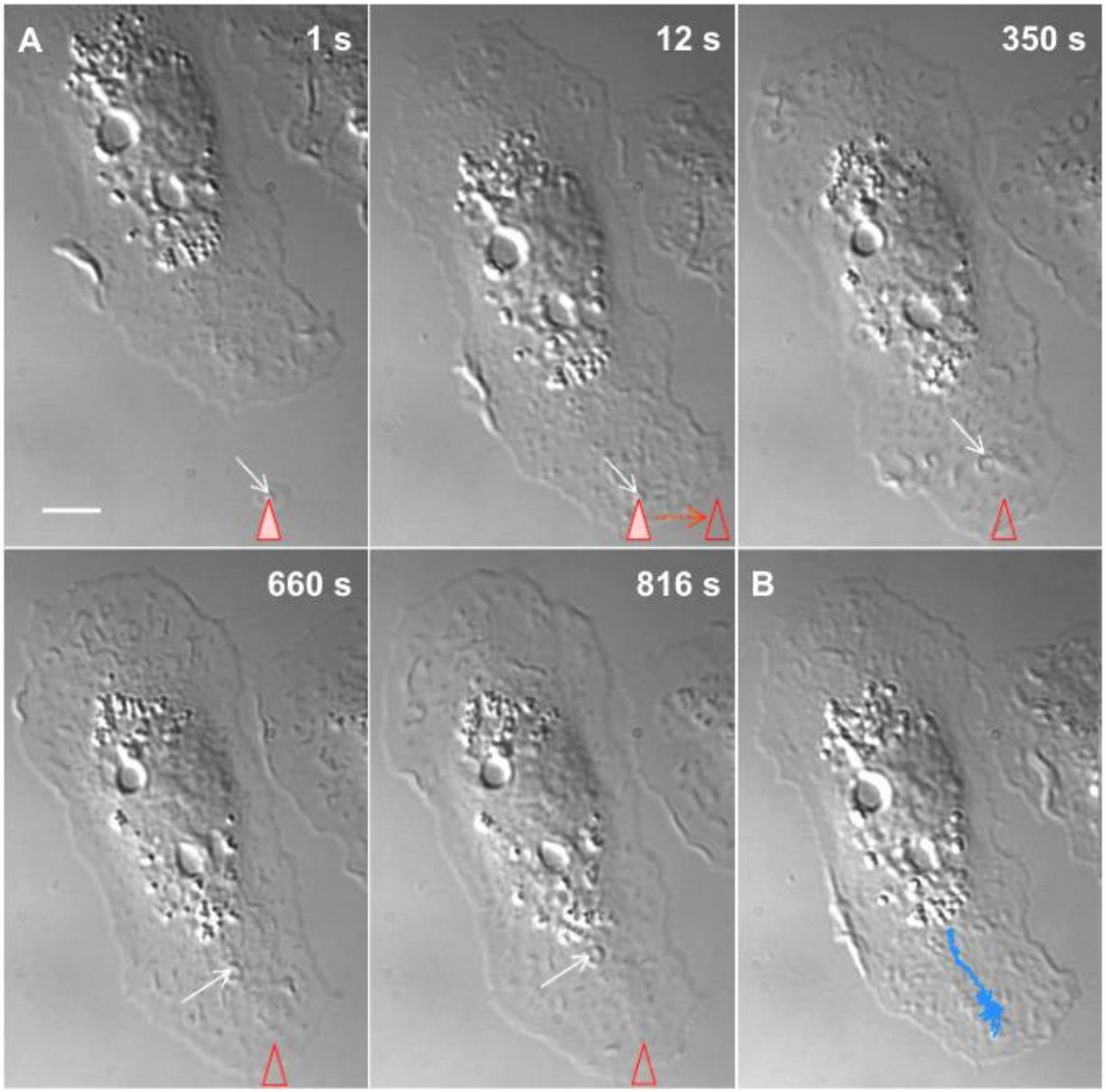
EV from microglial cells on a microglia cell.

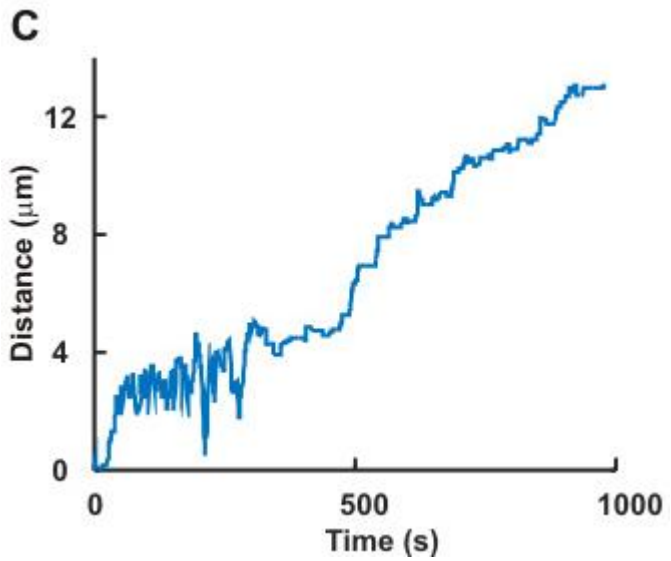
- EV are circular membrane structures released by most cells which represent highly conserved mediators of intercellular communication.
- EV carry proteins, lipids and genetic materials and transfer these cellular components between cells by different mechanisms, such as endocytosis, macropinocytosis or fusion.
- Temporal and spatial dynamics of vesicle-cell interaction still remain largely unexplored

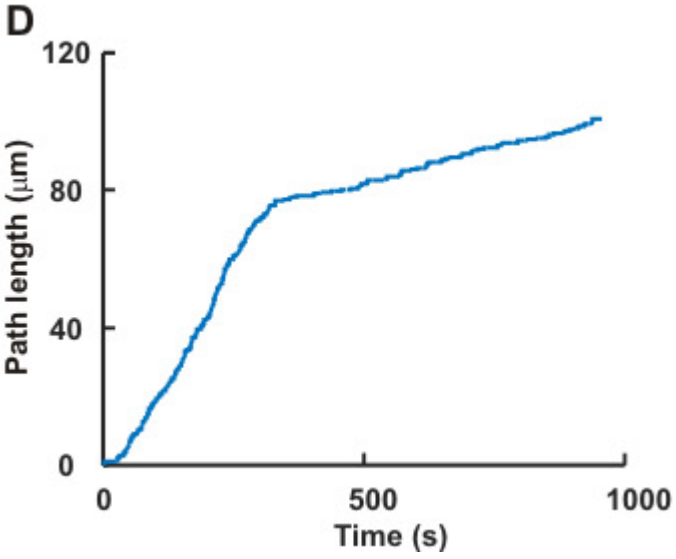
Collaboration:
Claudia Verderio - CNR-Institute of Neuroscience Milan
Roberto Furlan – San Raffaelle, Milan
Giuseppe Legname – SISSA, Trieste

Prada I *et al* BioTehniques, 2016

Interaction between single microglial EVs and microglia: adhesion and transport







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Conclusions

Optical Tweezers Manipulation (OTM) technology allows to :

- **measure forces exerted by cells**
- **apply forces to cells and measure stiffness**
- **handle vectors carrying active molecules to stimulate locally cells**

  - local stimulation by OM coated beads is simple and extremely flexible; any type of protein can be cross-linked on surface

  - filled liposomes are flexible as well and the released molecules can interact freely with the cell

OMT is compatible with Optical microscopy imaging –

See what you manipulate and manipulate what you see !

F. Difato, G. Pinato, D. Cojoc, "Cell signaling experiments driven by optical manipulation", *Int. J. Mol. Sci. 14, 8963 (2013)* Review

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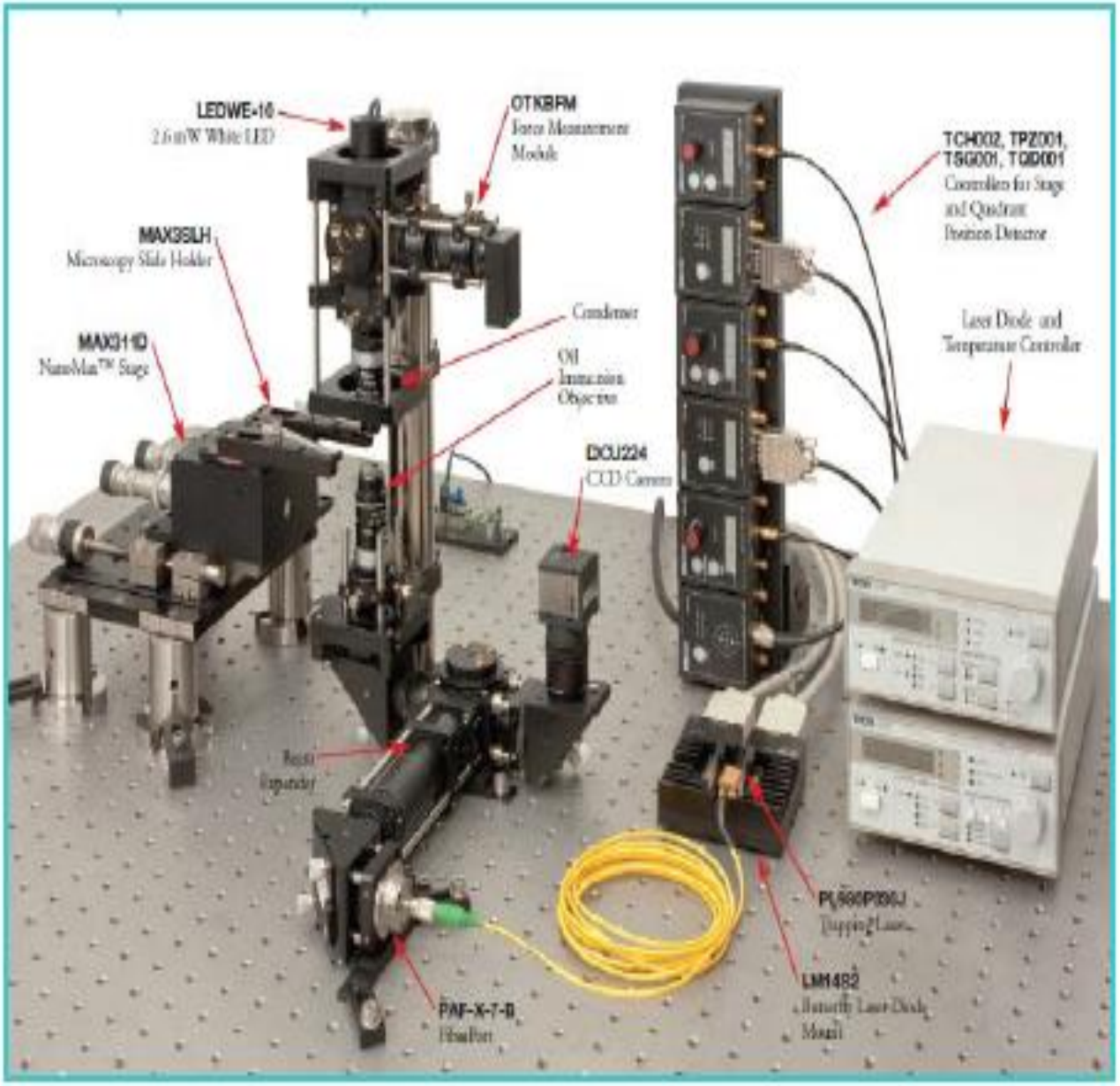
www.iom.cnr.it/optical-manipulation-laboratory

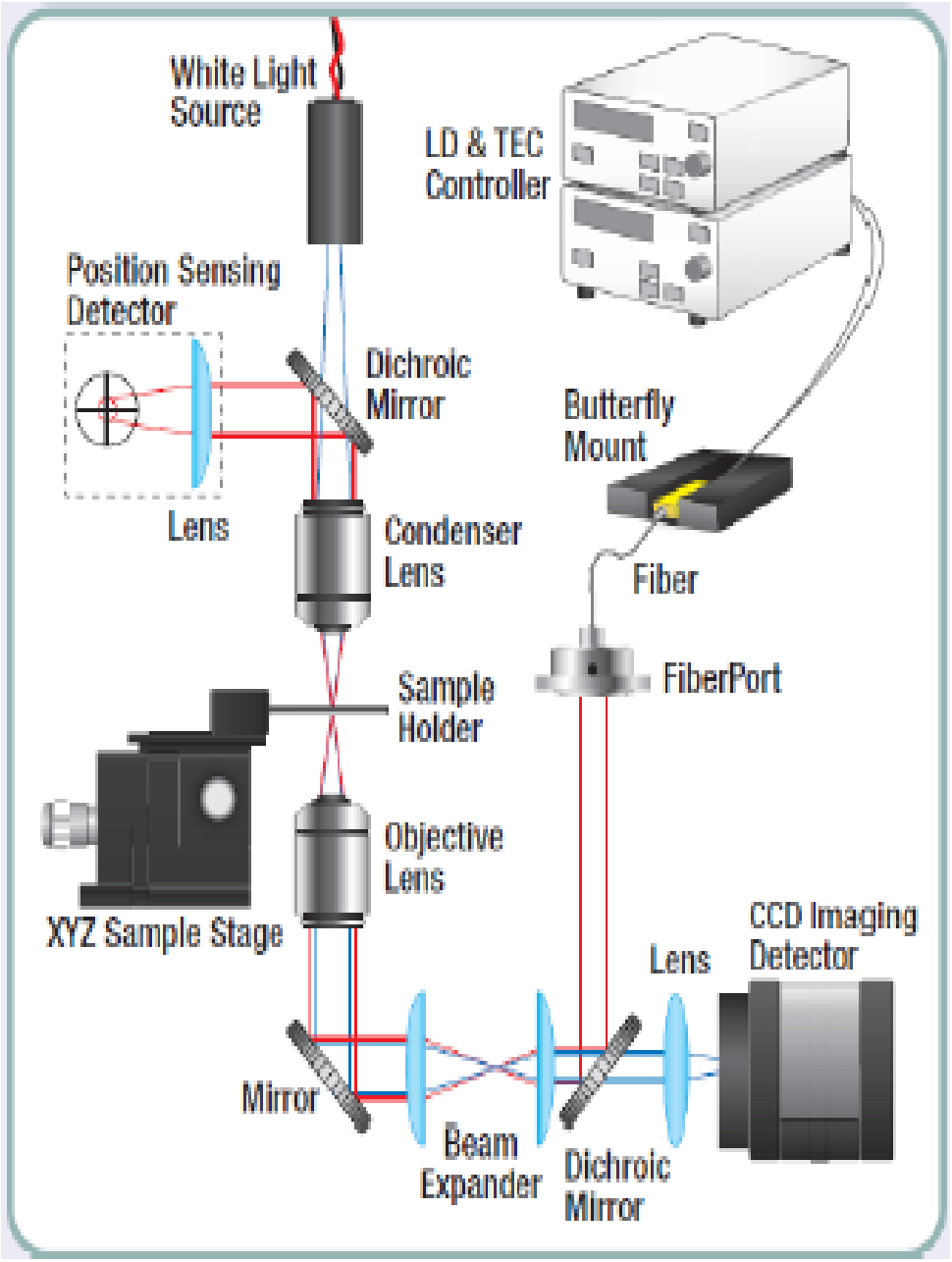
dancojoc.wix.com/om-lab

"Progress in science depends on new techniques, new discoveries, and new ideas, probably in that order", Sydney Brenner (Nobel Prize in Physiology or Medicine 2002)

Experiments Optical Tweeers Hands on - modular setup Thorlabs [1]



[1] www.thorlabs.com



two modules:

1. trapping and manipulation module
2. position detection and force measurement module

Important feature of this kit : modular design --> implement easily additional modules as fluorescence imaging, Raman spectroscopy, laser dissection, and laser beam steering.

This system is a result of the design and development work of the prof. M. Lang group at the Massachusetts Institute of Technology (MIT), Boston/USA. A more detailed description of the setup and experiments that can be performed with it can be found in reference [2].

[2] Appleyard et al, “Optical tweezers for undergraduates”, Am. J. of Physics (2007), http://www.vanderbilt.edu/langlab/Publications/Appleyard-etal(2007).pdf

Trapping module:

- 975 nm trapping laser source (stabilized single mode laser diode), 330 mW Power (Max), power at optical trap is about 40 % of Fiber Output
- trapping objective Nikon 100 X, Numerical Aperture NA 1.25, oil immersion, depth of focus 1 µm, spot size 0.6 µm (min), Working Distance WD 0.23 mm, transmission 380-1100 nm, recommended cover glass thickness 0.17 mm
- condenser objective Nikon 10X, NA 0.25, WD 7 mm, transmission 380 – 1100 nm - XYZ sample stage: 4 mm of manual travel in combination with 20 µm of piezo actuation and a resolution of 20 nm; using the internal strain gauges for positional feedback, 5 nm resolution can be achieved; the stage is mounted on a single-axis, long-travel translation stage, which allows scanning over a range of 50 mm, facilitating loading/uploading of the sample cel.

Position detection and force measurement module:

- position detection based on interference pattern in the back focal plane of the condenser, interference formed by trapping laser beam scattered by the trapped bead (probe); Quadrant Position Detector (QPD) detects the pattern displacement sampling it at high frequency rate (100 kHz) - position calibration capability with 5 nm resolution; trap stiffness calibration using different methods as: Power Spectral Density (PSD), Stokes drag and Equipartition theorem (for details of these methods see for instance references [2], [3]); determining the trap stiffness and knowing that the bead probe near the equilibrium position of the trap, behaves as in a Hooke potential well (linear spring with stiffness k), force measurement of the probe interacting with a sample (e.g. cell) can be calculed measuring the displacement x of the bead: $F=kx$; the trap stiffness depends of the power of the trapping laser and of the material and geometry of the trapped probe; the stiffness range is 10^{-4}–1 pN/nm, which allows to measure forces in pN range with resolution of tens of fN; the stiffness is 2-3 order of magnitudes smaller than that of the cantilever stiffness in AFM
- MATLAB-based graphical user interface (GUI) – open access code available from Thorlabs-MIT.

[3] Neuman and Block, “Optical trapping–review”, Rev. Sci. Instr. (2004)

ACKNOWLEDGMENTS : ICTP and SPIE for kindly funding the Oprical Tweezers Kit setup including trapping, position detection and force measurement, and laser beam steering modules.

Some hystorical notes

2003 - OT lab at INFM-Syncrotron Trieste, ICTP-STEP PhD program

2005- many requests to visist and work with the only OT setup we had

2009 - with Joe Niemela we planned to build a simple setup transportable for demo

2010 - Francesco Difato - IIT Genova - OT tweezers in a box --> workshop Ghana

2012 - thanks to ICTP and SPIE --> OT Thorlabs setup

2013 - two demos: ICTP Trieste, Univ. N. Gorica Vipava Slovenia

2013 - PhD students, master, visitors worked on it, mainly within ICTP programs

Fatou NDOYE, Senegal (ICTP- STEP program, defended 2017)

Muhammad Sulaiman YUSAFZAI, Pakistan (Univ Trieste PhD Nanoechnology, ICTP - TRIL 2016-2017)

Jose J. SUAREZ-VARGAS, Venezuela (ICTP- associate)

Humberto CABRERA, Venezuela (ICTP- associate)

Ali Reza MORADI , Iran (ICTP associate)

and of course Joe Niemela