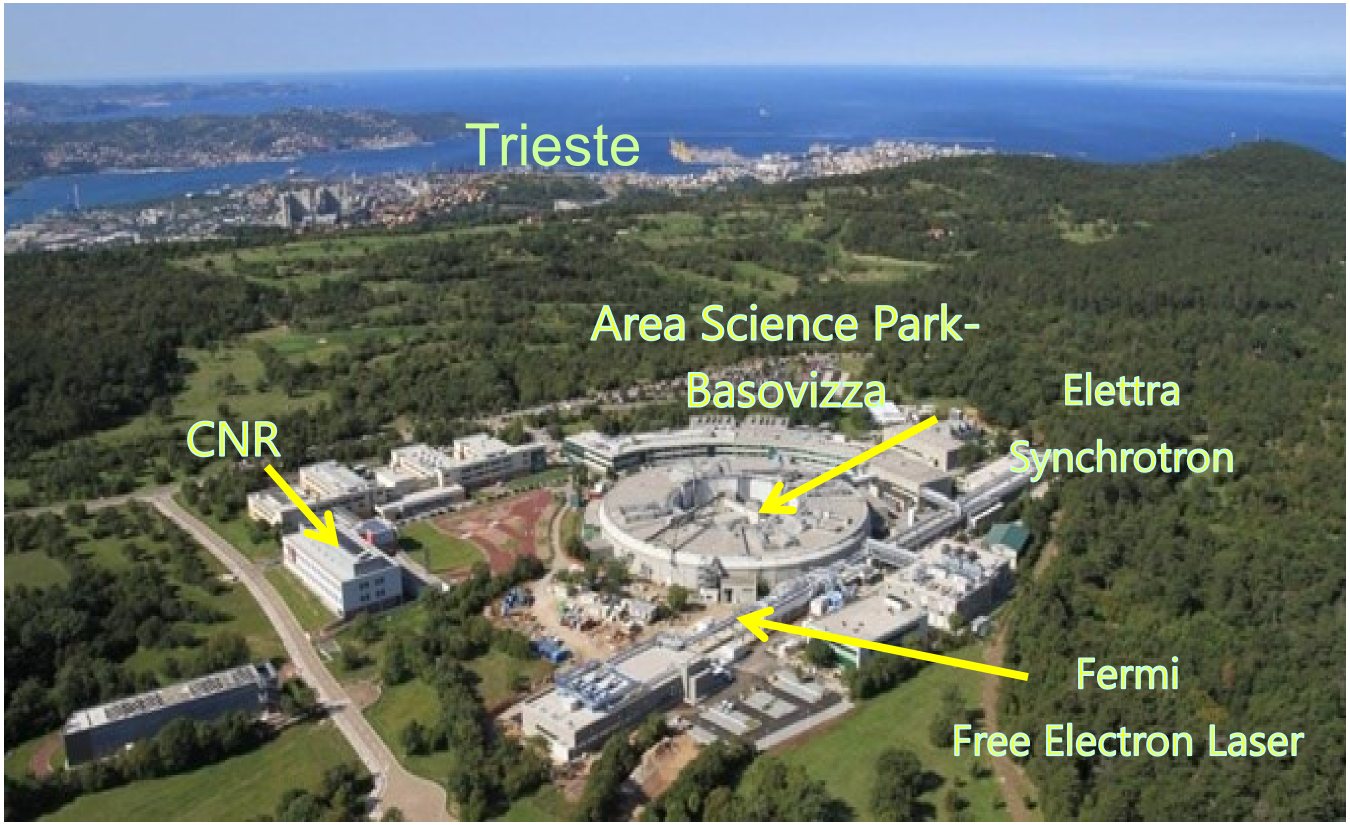
Optical Tweezers Microscopy
and some applications to biological systems

Dan COJOC



CNR National Research Council of Italy

Optical Tweezers: a Legacy of Arthur Ashkin

Arthur Ashkin
The Nobel Prize in Physics 2018

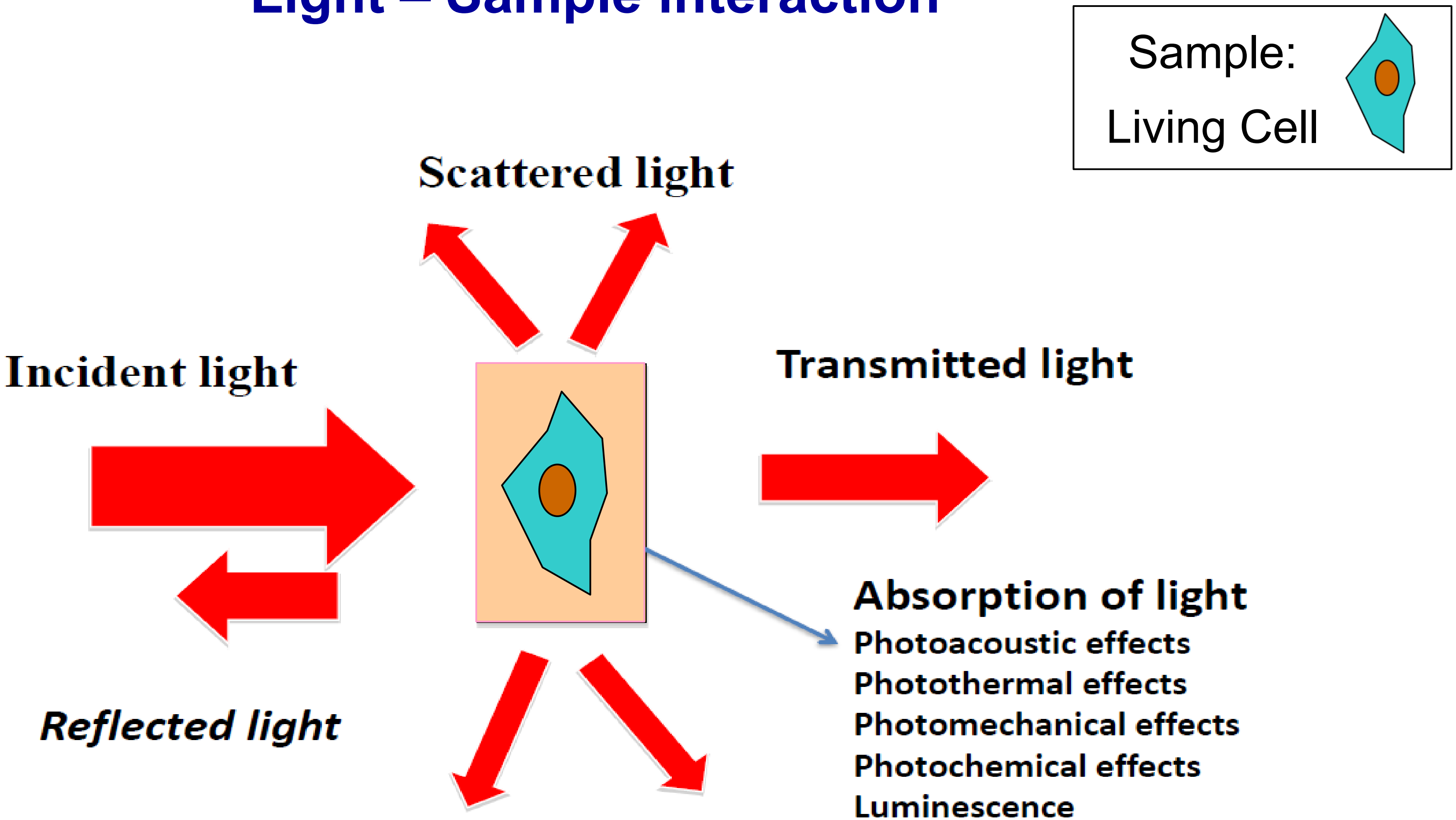
Born: 2 September 1922, New York, NY, USA

Affiliation at the time of the award: Bell Laboratories, Holmdel, NJ, USA

Prize motivation: "for the optical tweezers and their application to biological systems."

Prize share: 1/2

Light – Sample interaction



is governed by physics laws as

Energy and Momentum conservation

and includes **mechanical effects !**

Could Light exert Force on Objects ?

If Yes, How ?

Light is made by photons and a PHOTHON has MOMENTUM

(even if it does not have MASS):

$$p = \frac{h\nu}{c} = \frac{E_p}{c}$$

Momentum ***p*** and energy $\boldsymbol{E_p}$ of a photon

c - light velocity; ν – frequency;
λ = c / ν – wavelength.

How big is the photon momentum compared with the momentum of an object with mass m, moving at velocity v << c ?

Momentum and Energy of a single photon:

p ≈ 10^{-27} N s E ≈ 2 eV= 3.2 x 10^{-19} J

Momentum of a single E-coli bacteria swimming in liquid:

Mass: m= 1 pg = 10^{-15} Kg; Velocity: V= 100 nm/s = 10^{-7} m/s

Momentum: **P_{Ecb}= m V = 10^{-22} N s** (N s = kg m / s)

The momentum of a photon is very small !

Nevertheless, even a low power laser beam, has many photons.

Example: laser beam of power **W_{lb} = 1 mW** (energy E_{lb} = 1mJ)

The number of photons is: **N= E_{lb}/E ≈ 3 10^{15}** →

→ **Momentum** of the laser beam: **P_{lb}= 3 10^{-12} N s**

$$P_{lb} >> P_{Ecb}$$

Can the laser beam influence the motion of the bacteria ?

We need to consider / remember some laws of mechanics

Newton's three laws of motion:

L1. Every object in a state of uniform motion will remain in that state of motion unless an external force acts on it.

L2. Force equals mass times acceleration: **F= m a**

L3. For every action there is an equal and opposite reaction.

+ the laws of conservation of momentum and energy.

If we consider the second law: F= m a ,

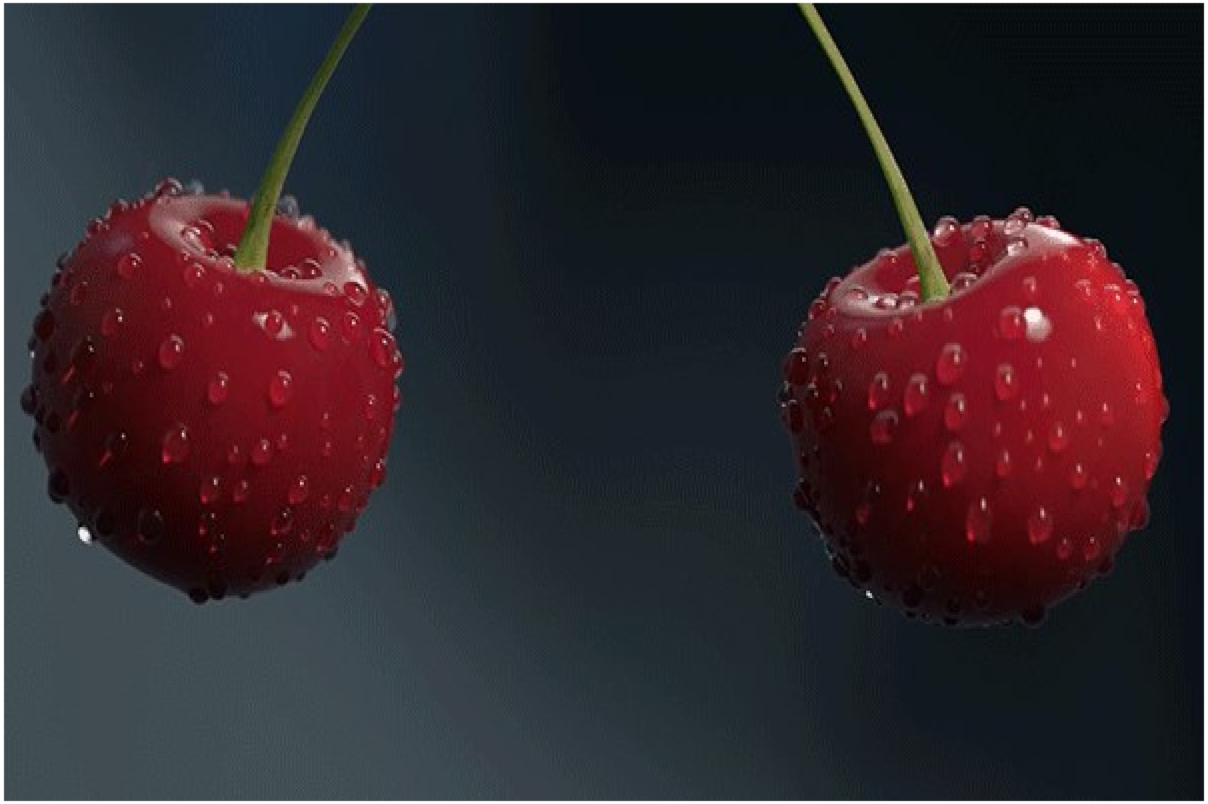
and express acceleration a, as a = ΔV / Δt , we get:

F = m ΔV / Δt = Δ (mV)/ Δt = **Δ P/ Δt,**

which means that:

the change of momentum Δ P in a given time Δt produces force F.

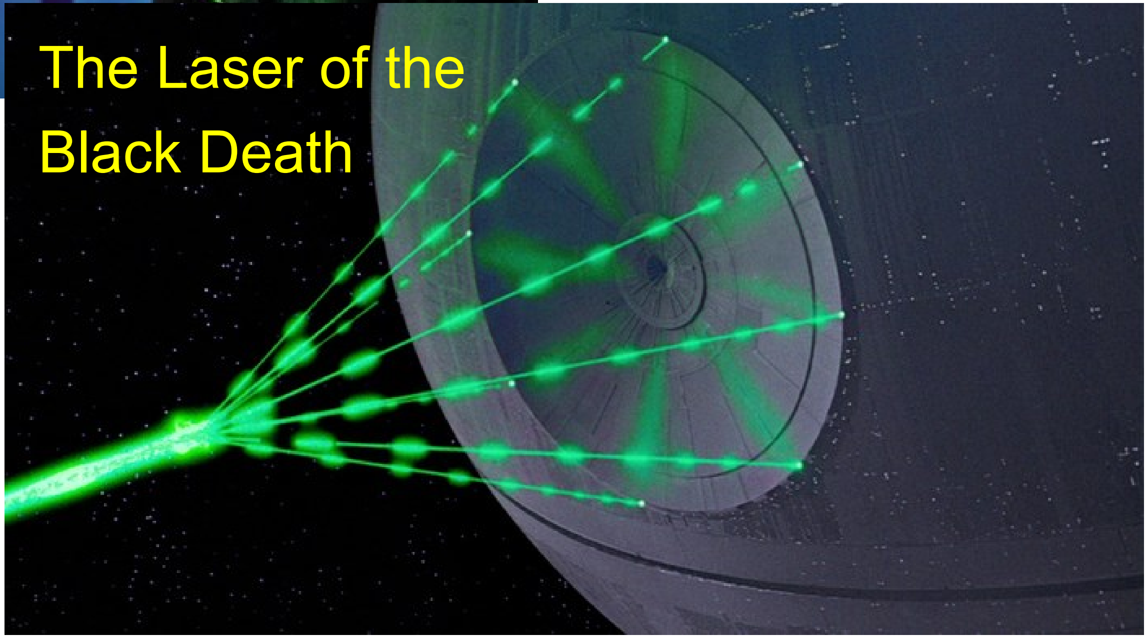
Example of interaction between two objects in motion



Another example (with ellastic and inellastic interaction).

Ellastic: Hammer – Tyre; Inellastic: Hammer-Tom.

Examples of interaction of “object(s)” without mass



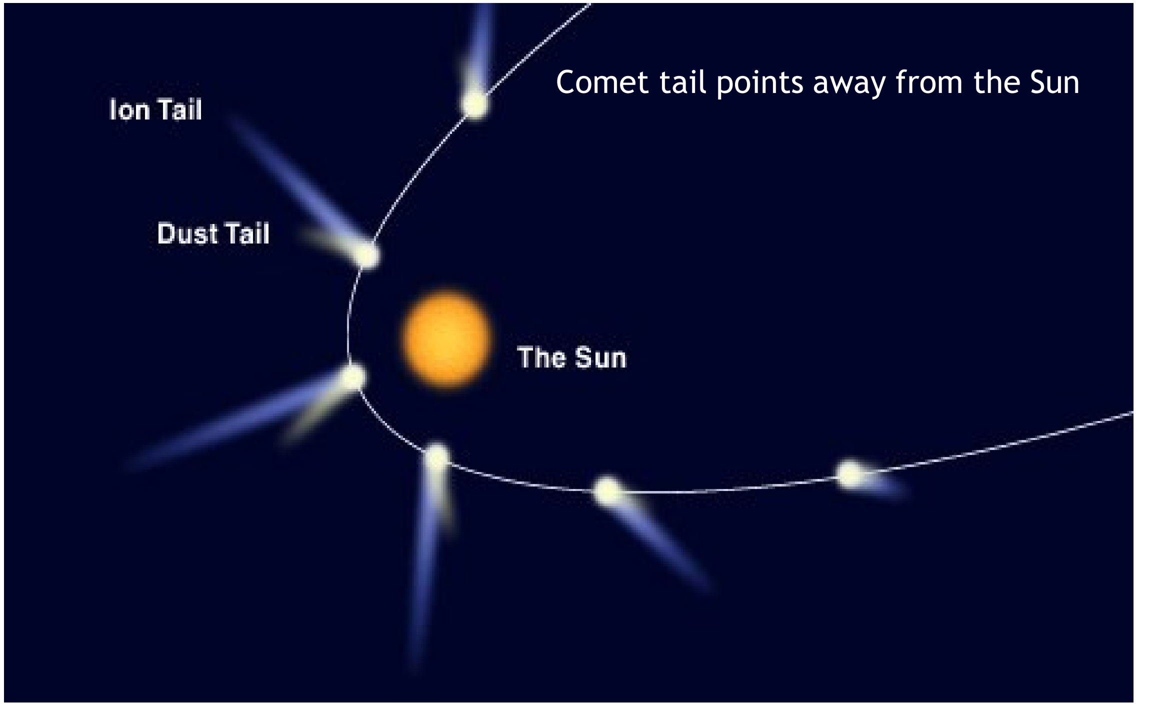
Science Fiction

Star Wars

Light has momentum and can generate force

1619 – Kepler :

Observation of the orientation of the comet tails → suggests that the Sun Light drives the orientation of the comets tail



1873 – Maxwell :

"In a medium in which waves are propagated, there is a pressure in the direction normal to the waves and numerically equal to the energy in unit volume"

1900-1901 Lebedev, Nichols, Hull:

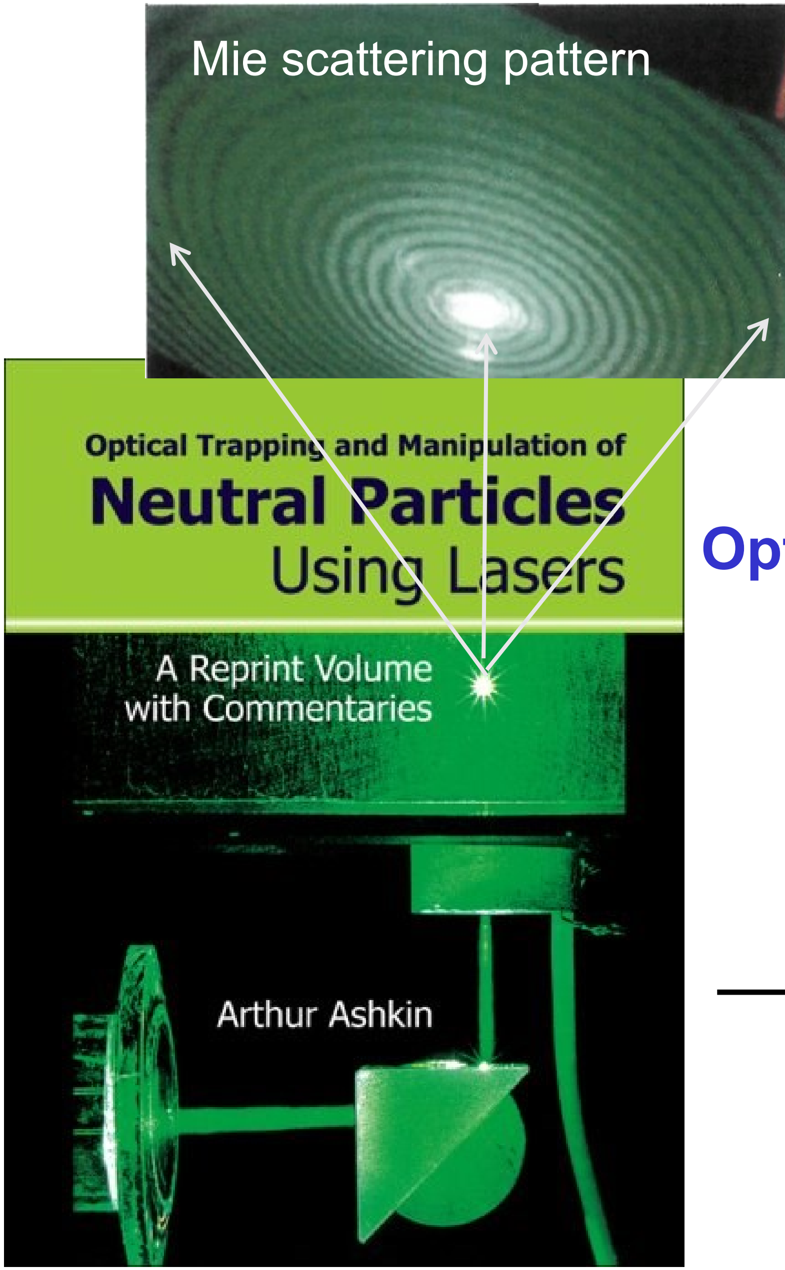
First measurement of the radiation pressure using a torsion balance

Forces generated by light on objects are in general very small and hence the effect is difficult to be detected

→ use LASER beam and small objects !

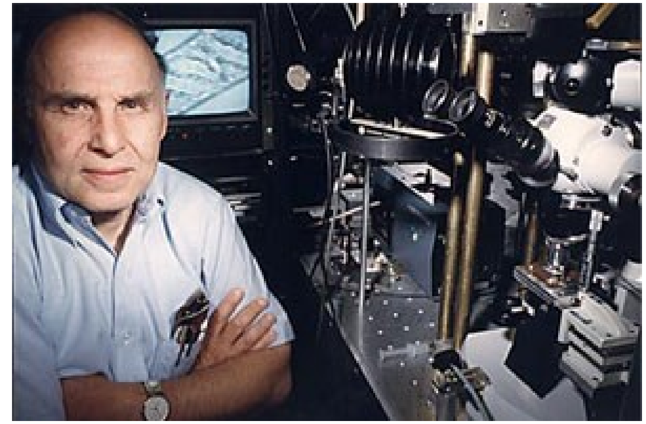
Newton's second law: $F = m a$ or $\mathbf{a = F / m}$

Even if the force F is smal, for small objects of small mass m, the effect (measured by acceleration a) can be considerable (detectable and measurable) !

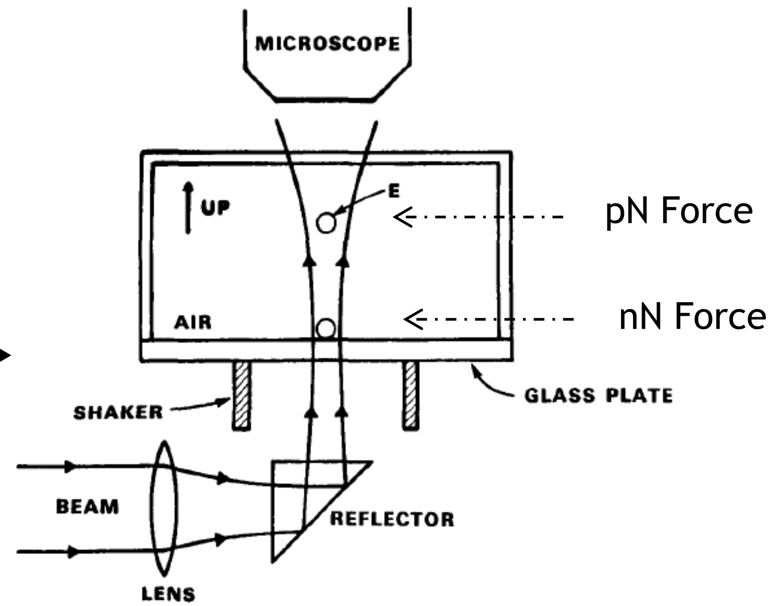


Scientific Publishing 2006

Arthur Ashkin, Bell Labs **(1986)**



Optical levitation of microparticles in air



(hollow silica,beads, diam 50-75 um)

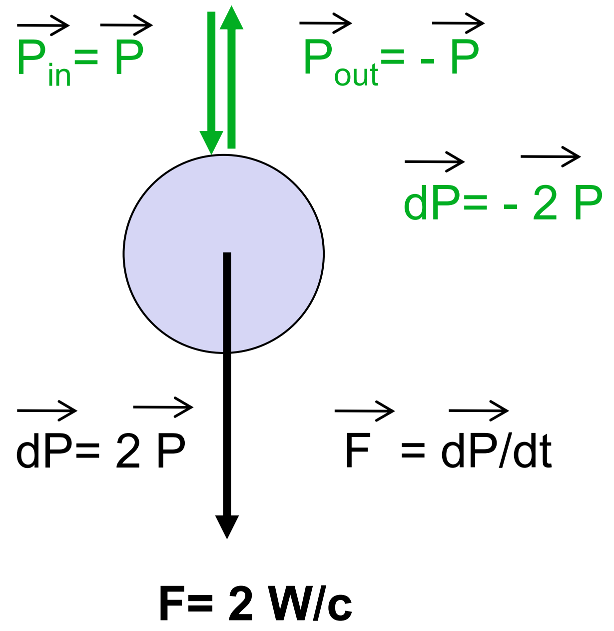
How big is the force exerted by a ray of light reflected perfectly by a microbead ?

Geometrical optics approximation --> light rays

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

The magnitude of the momentum associated to the ray of light composed by N photons:

$$P = E / c = N h \nu / c$$



The magnitude of the radiation pressure force exerted on the microbead

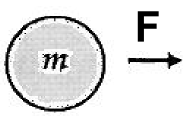
N= 1 photon, -> E≈ 2.5 eV, W≈ 4 x 10^{-19} W -> F≈ 2.7 x 10^{-27} N - very small

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

1 pN is the gravitational force of a particle with a mass of 0.1 ng (10^{-10} grams) !

Is the magnitude of this force significant ?

1)



Microbead in free space (vacuum) - no dumping:

F ≈ 2.7 pN - SMALL , but also the mass, m, of the microbead is small

m ≈ 8 pg → acceleration $a \approx F/m = 3.4 \times 10^2$ [m/s^2] = 34 g ,

which is very **BIG** !

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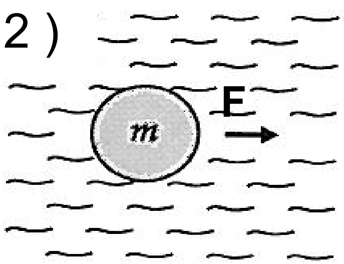
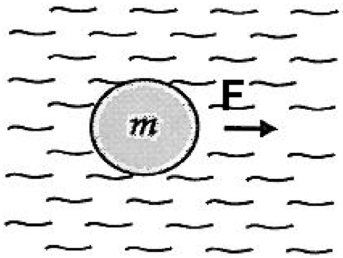
2)



Microbead in liquid - dumping:

F≈ 3.6 pN

refractive index (water) n_m = 1.33; force by light : F= 2 $\mathbf{n_m}$ W/c ;

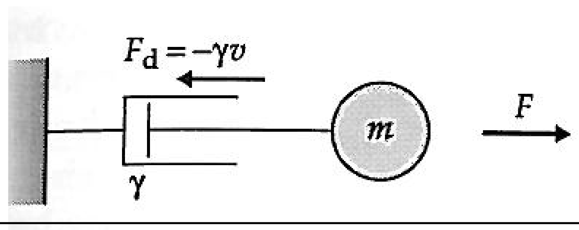


Microbead in liquid - dumping:

F≈ 3.6 pN

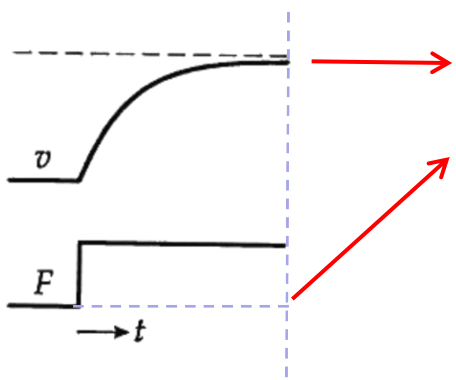
refractive index (water) n_m = 1.33; force by light : F= 2 $\mathbf{n_m}$ W/c ;

mass + dashpot model



$$m\frac{dv}{dt} = F - \gamma v$$

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



max velocity $v_t = \dfrac{F}{\gamma}$ **= 360 μm / s**

time constant $\tau = \dfrac{m}{\gamma}$ **= 0.8 μs**

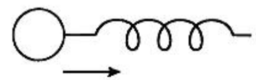
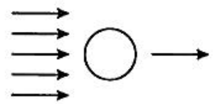
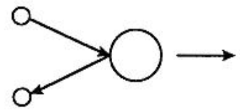
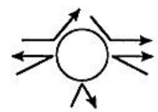
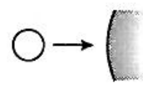
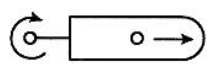
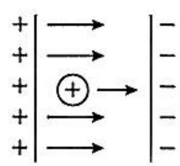
- the max velocity is reached very fast and maintained until the force F is applied.
- When the force is cancelled the particle stops very fast.

For a small particle dumping is dominant over inertia because: *m -> d*^{3} , *γ-> d*

Example from biology: movement of a bacterium in water. The bacterial motor must be able to generate a 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

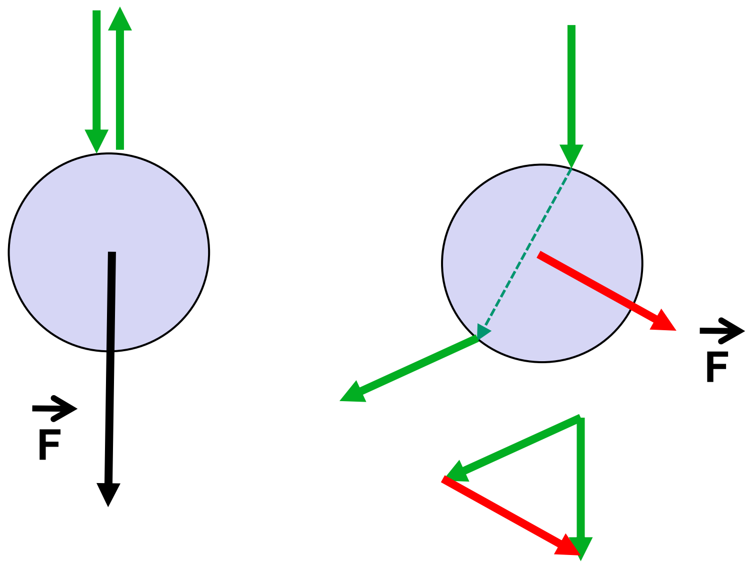
pN

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; $\mathbf{n_b > n_m}$



The magnitude of the force:

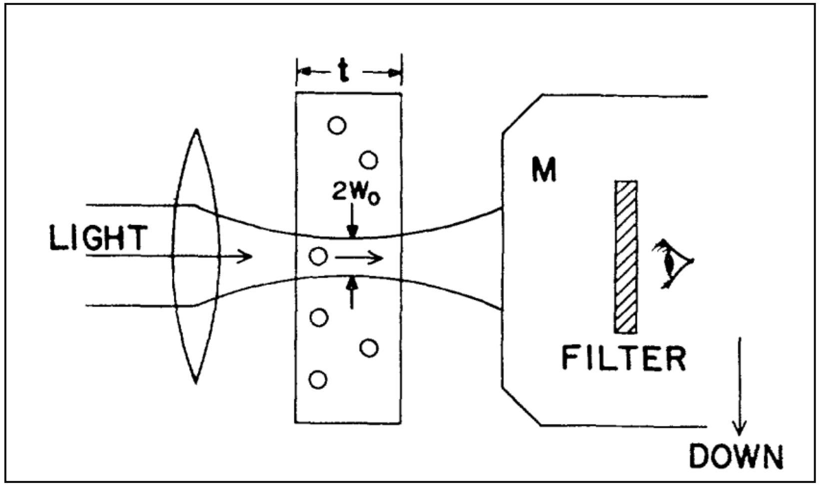
$$\mathbf{F = Q\, n_m W_{in}/c}$$

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

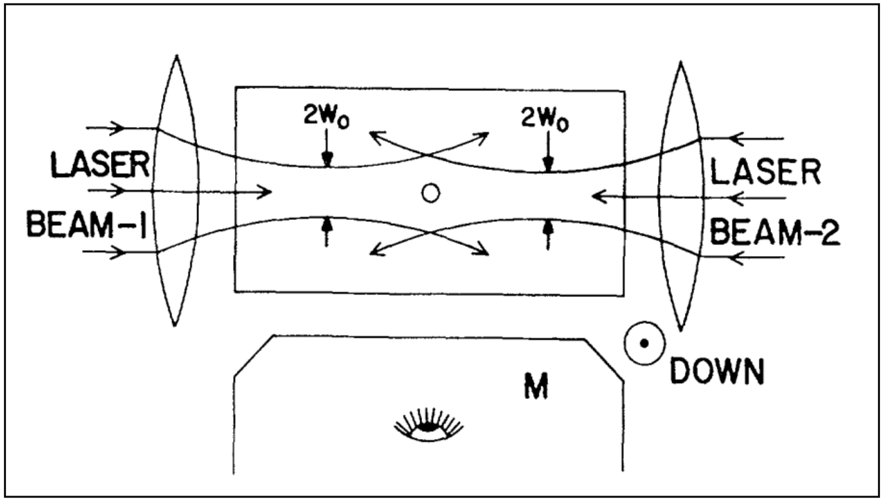
Q - dimensionless factor, Q ≤ 2
Q - function of shape, material

- If the beam of light is not focused or midly focused, the force always pushes the object forward.
- However, if the beam is tightly focused, there is a force component attracting the object toward the focus → 3D trapping

2D and 3D optical trapping



ONE focused laser beam
2D trapping



TWO focused laser beams,
(counter propagating)
3D trapping

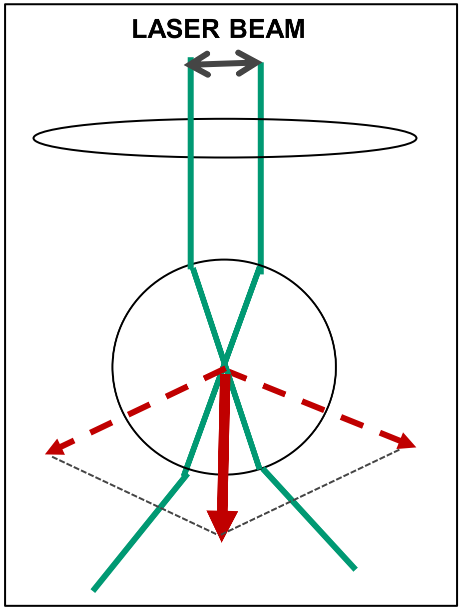
NOTE: focusing through relatively low NA lenses

Acceleration and trapping of particles by radiation pressure
A. Ashkin, *Phys. Rev. Lett.* 24, 156 (1970) >5000 citations

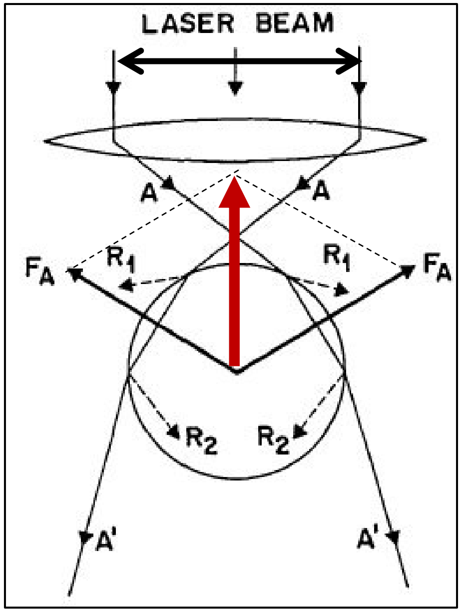
Observation of a single-beam gradient force optical trap for dielectric particles

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

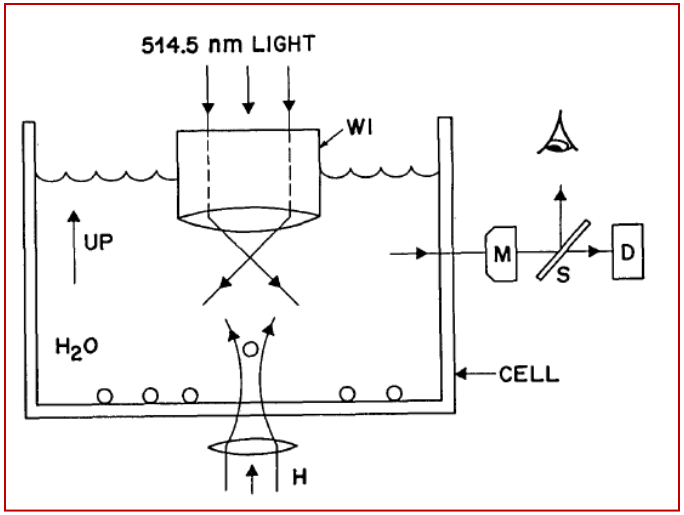
> 6000 citations



Force generated by a **midly** focused laser beam on a transparent microparticle in water.



Force generated by a **tightly** focused laser beam.



Sketch of the basic apparatus.

Size of particles :

10 um (Mie) to 25 nm (Rayleigh)

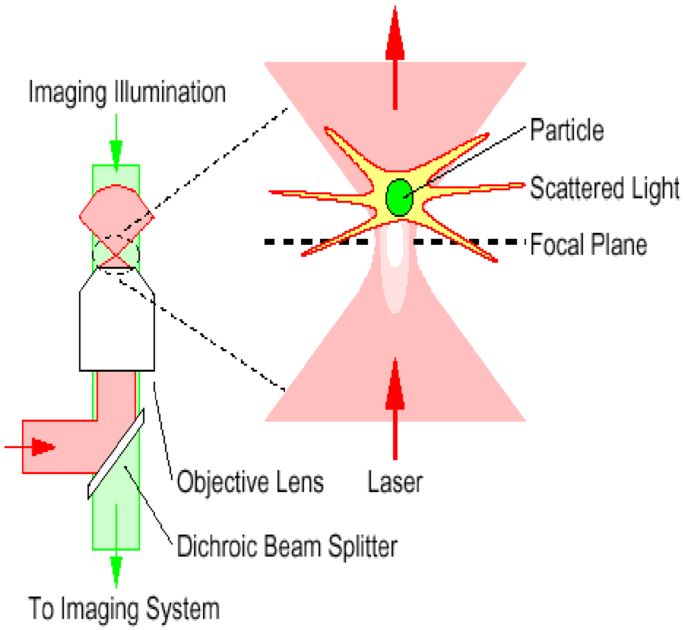
Acceleration and trapping of particles by radiation pressure

A. Ashkin, *Phys. Rev. Lett.* 24, 156 (1970) >5000 citations

What is an Optical / Laser Tweezers ?

A laser beam **tightly** focused through a high Numerical Aperture (NA) objective

A. Ashkin *et al* ***Opt. Lett.*** **1986**



$$F = Q\frac{n_m W}{c}$$

Ex: **F= 1.33 pN** for W= 1 mW, n_m=1.33, Q=0.3

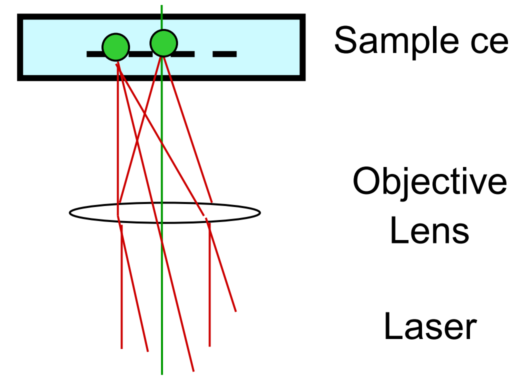
F – trapping force

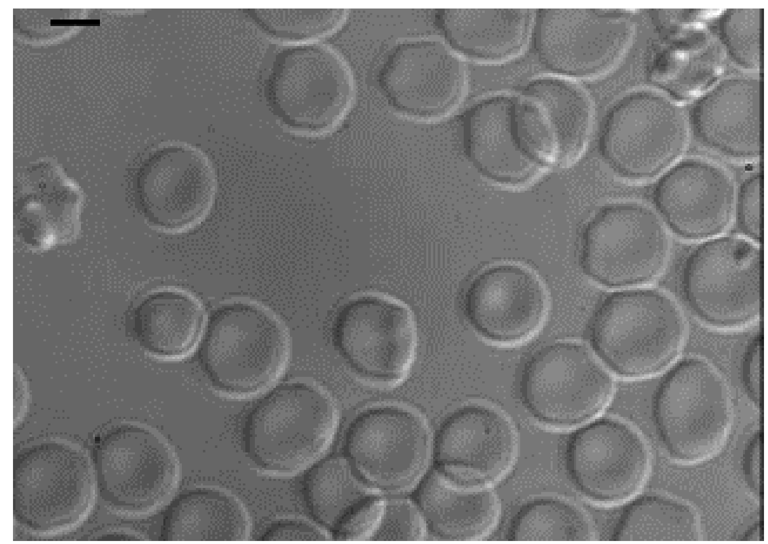
Q – dimensionless efficiency coefficient

W – power of the laser beam

$\mathbf{n_m}$ – refractive index of the medium

c – light speed in vacuum

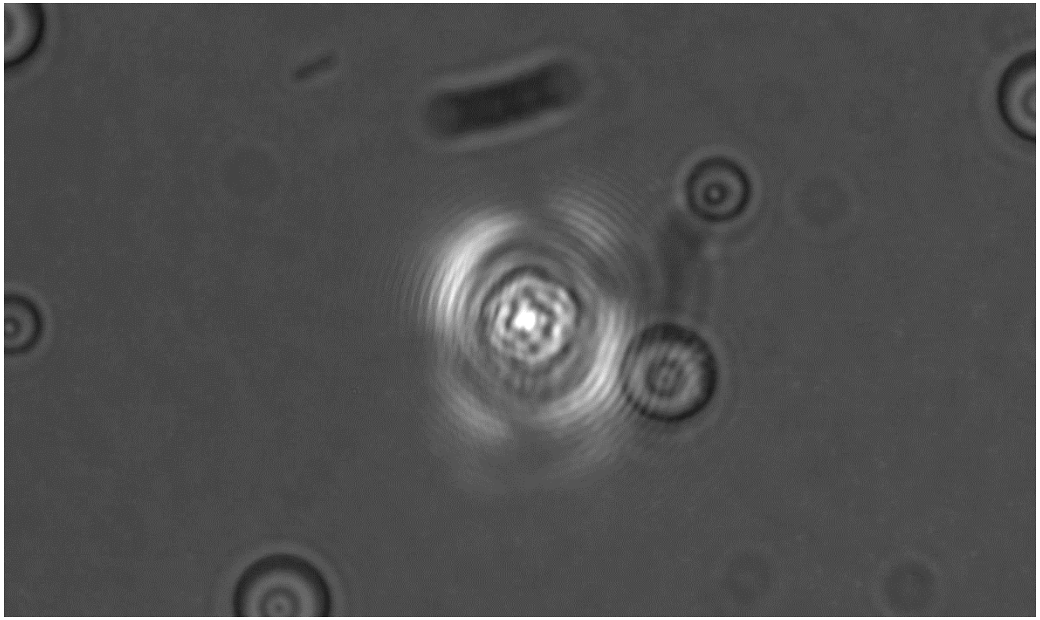




Example of human erythrocyte trapping

2004 - OM Lab

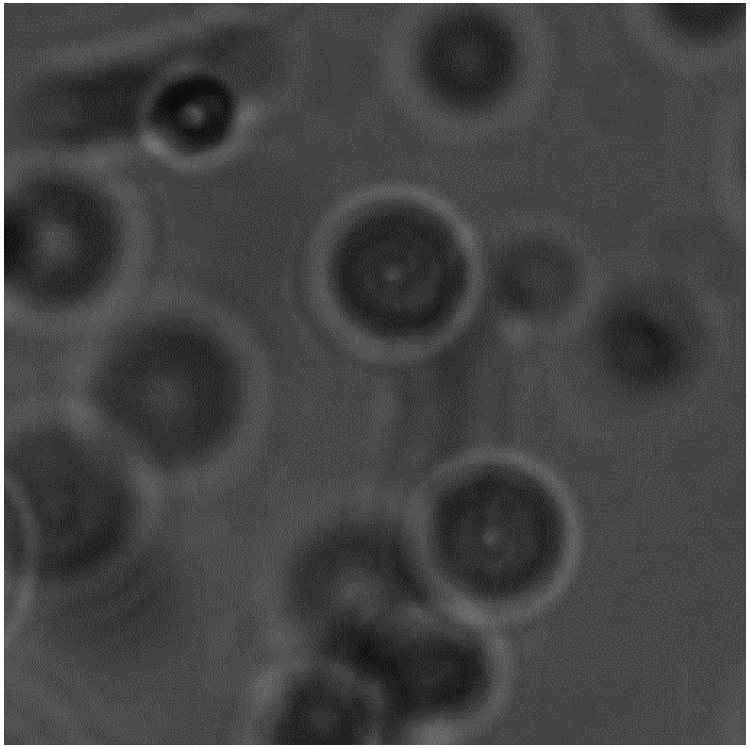
Some examples of trapping from OM Lab



silica microbeads, laser 970 nm,

power at the sample about

P= 5 mW



Optical trap behaves as an

attractor of particles

P= 120 mW

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.

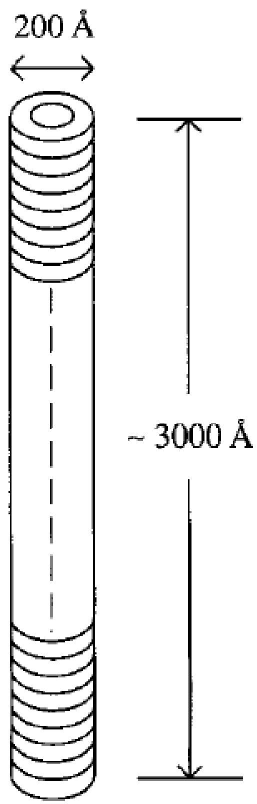
Does this mismatch prevent trapping ?

First optical trapping of a biological sample

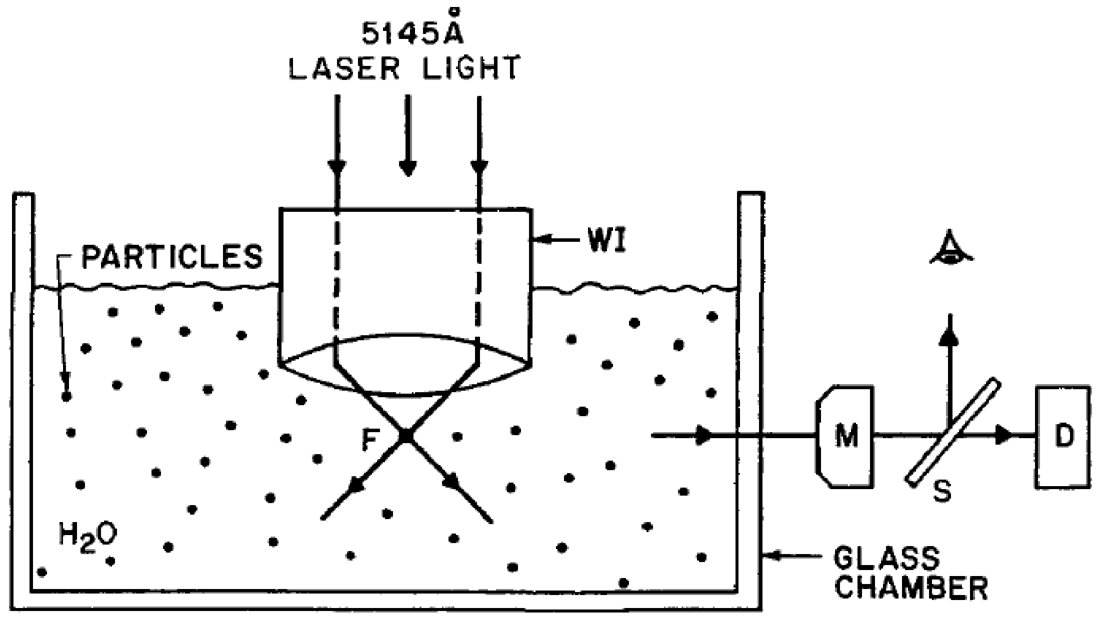
Tobacco Mosaic Virus (TMV)

TMV

shape & size



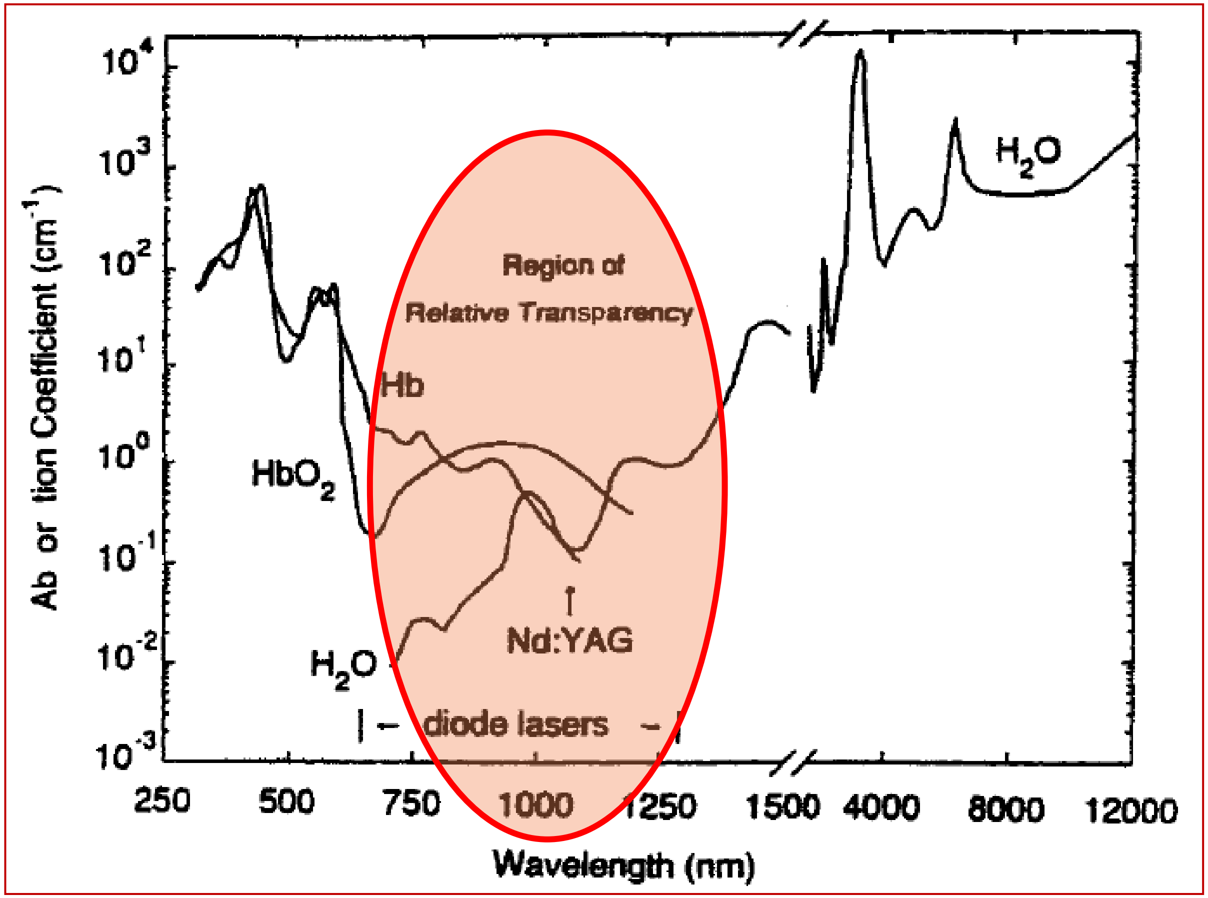
Apparatus used for optical trapping of TMV particles and mobile bacteria



Bacteria (which are slightly larger than Rayleigh particles) trapping was accidentally observed and then rigorously characterized for *E. Coli* in a closed sample cell.

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

Damage – free trapping of living cells with infrared light



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

Damage – free trapping of living cells

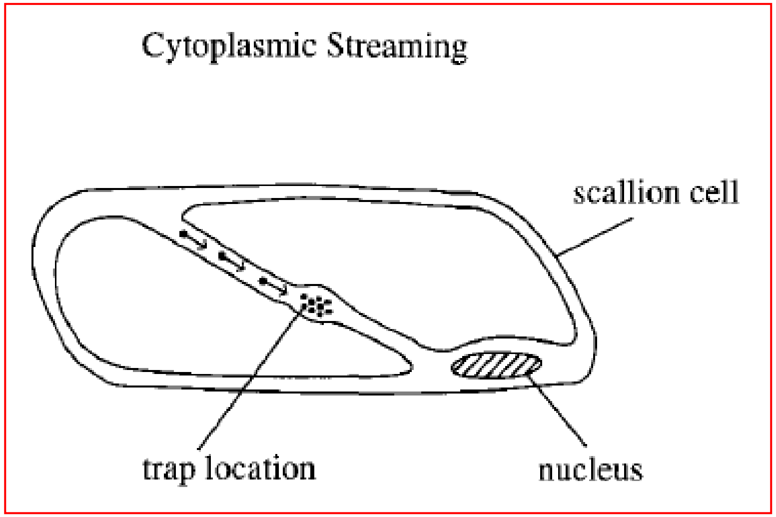
A. Ashkin, J.M. Dziedzic, T. Yamane, "Optical trapping and manipulation of single cells using infrared laser beams", *Nature* 330, 769 (1987)

Ashkin: "We tried red blood cells, plant cells, and the huge number of different types of protozoa, diatoms, and single cells of algae one can find in pond water." **One can trap almost any type of cells with IR beam without, or with limited damage.**

Not only were the cell types quite varied, but also their sizes and shapes. Shape and optical properties of particles are crucial to the trapping process.

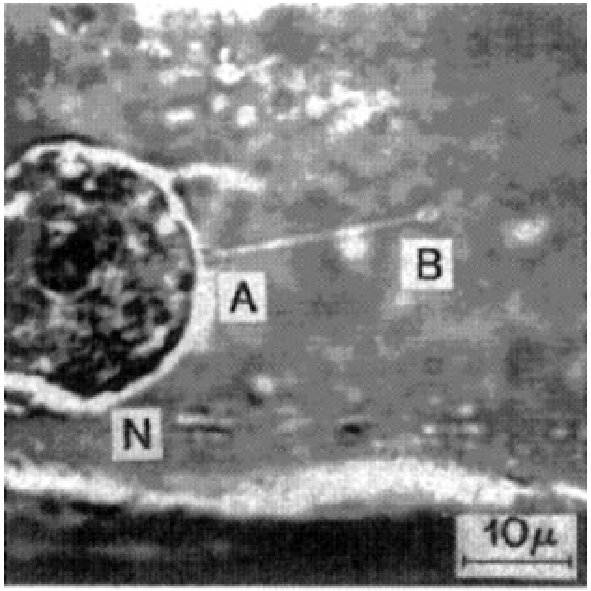
Optical tweezer-type traps are very tolerant of shape particle variation .

Intra-cellular trapping



Internal cell manipulation. Collection of particles and a blob of cytoplasm trapped within a streaming channel of cytoplasm inside a living scallion cell. When released, they simply move on.

A. Ashkin and J. M. Dziedzic, Internal cell manipulation using infrared laser traps, *Proc. Natl. Acad. Sci. USA* ***86,*** 7914 (1989).



Nobel Prize in Physics 2018

Arthur Ashkin invented optical tweezers that grab particles, atoms, viruses and other living cells with their laser beam fingers.

This new tool allowed Ashkin to realise an old dream of science fiction – using the radiation pressure of light to move physical objects.

He succeeded in getting laser light to push small particles towards the centre of the beam and to hold them there. Optical tweezers had been invented.

A major breakthrough came in 1987, when Ashkin used the tweezers to capture living bacteria without harming them. He immediately began studying biological systems and optical tweezers are now widely used to investigate the machinery of life.

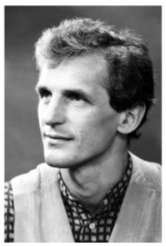
Prize motivation for Arthur Ashkin:

"for the optical tweezers and their application to biological systems."

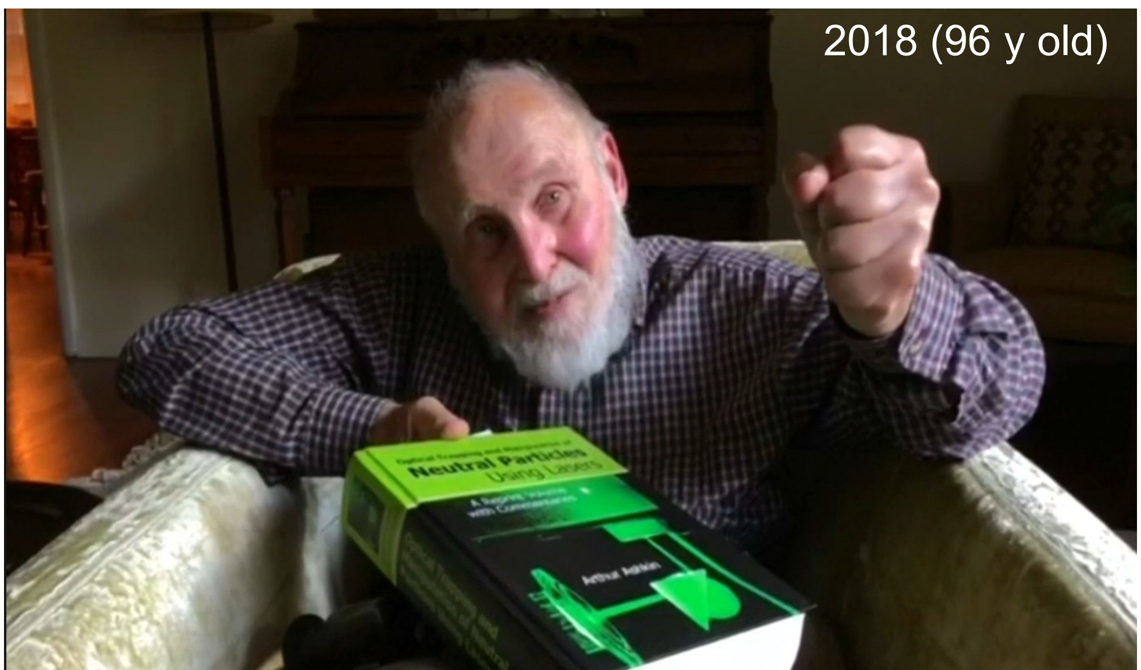
Other Two Nobel Prizes in Physics – related to optical trapping



The Nobel Prize in Physics 1997 was awarded jointly to Steven **Chu**, Claude **Cohen-Tannoudji** and William D. **Phillips** "for development of methods to cool and trap atoms with laser light."


The Nobel Prize in Physics 2001 was awarded jointly to 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."



Arthur Ashkin

Noble Prize in Physics

2018

Optical Tweezers – some properties

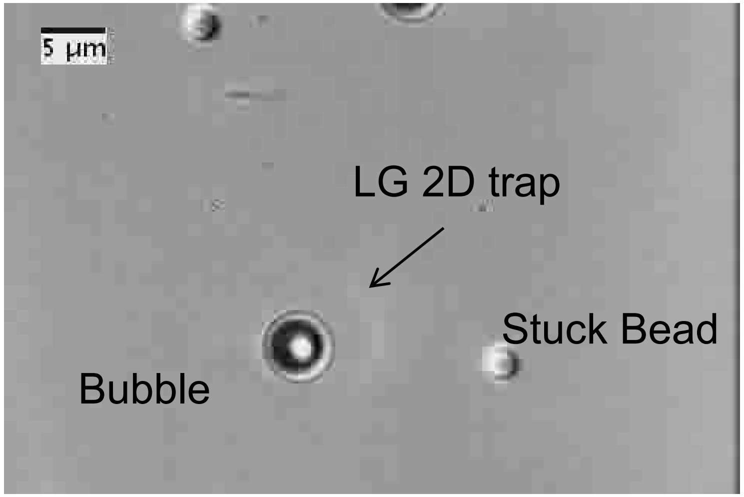
What type of particles can be trapped ?

- **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.

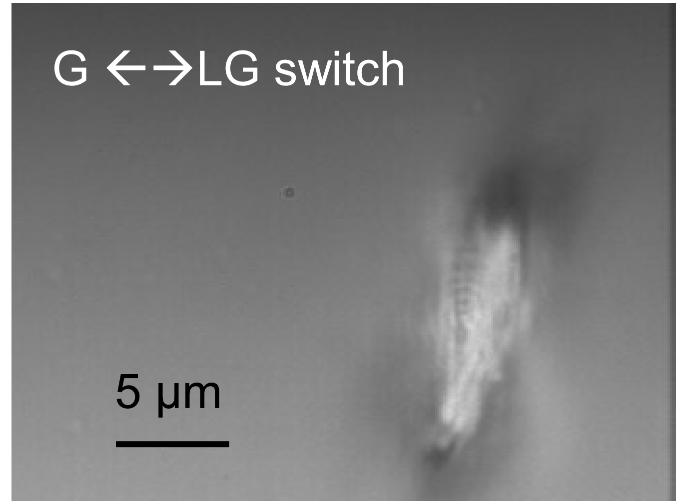
Range of forces that can be applied and measured : **0.1 – 100 pN**

Some examples of optical manipulation from OM Lab

Ultrasound Contrast Bubble – LG 2D trap



Very simple rotor - piece of glass

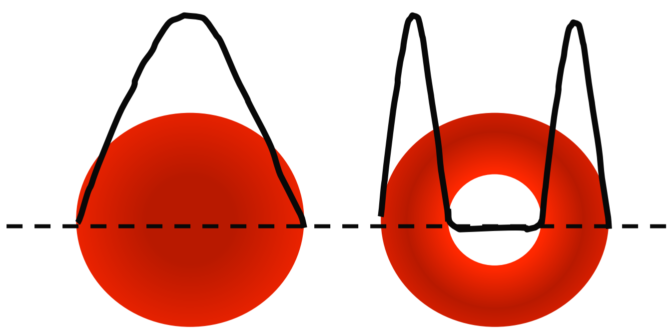


Gaussian

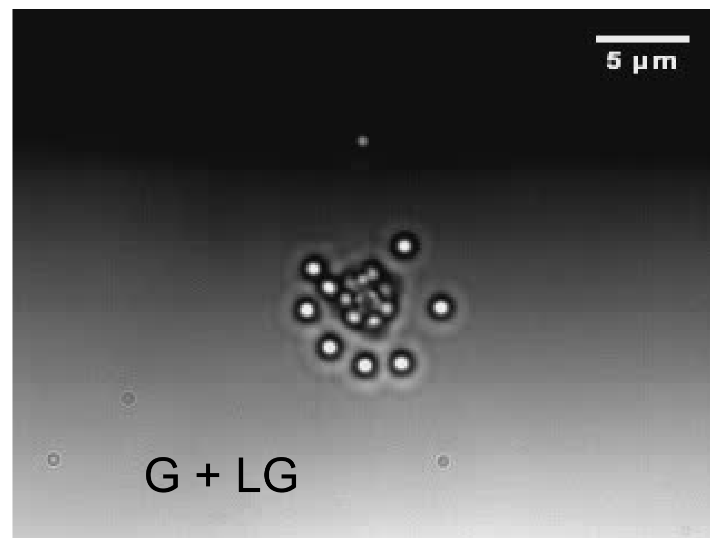
TEM 00

Laguerre Gaussian

TEM 01

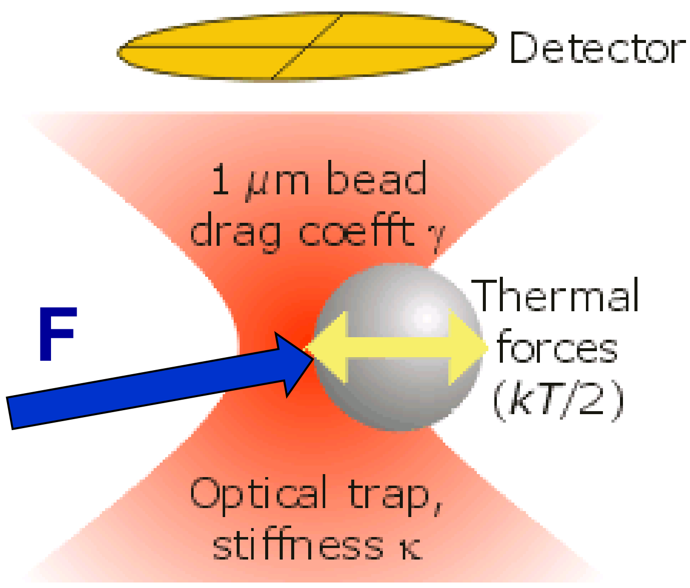


LG OAM transfer to silica bead



OAM = Optical Angular Momentum

Using the trapped bead to probe external forces



Measuring the displacement Δ of the particle and knowing the stiffness of the trap K we get F:

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

F = (Fx, Fy, Fz) Force

K = (Kx,Ky,Kz) stiffness of the trap

Δ = (Δx, Δy, Δz) Displacement

OT allows measuring forces in 3D !

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

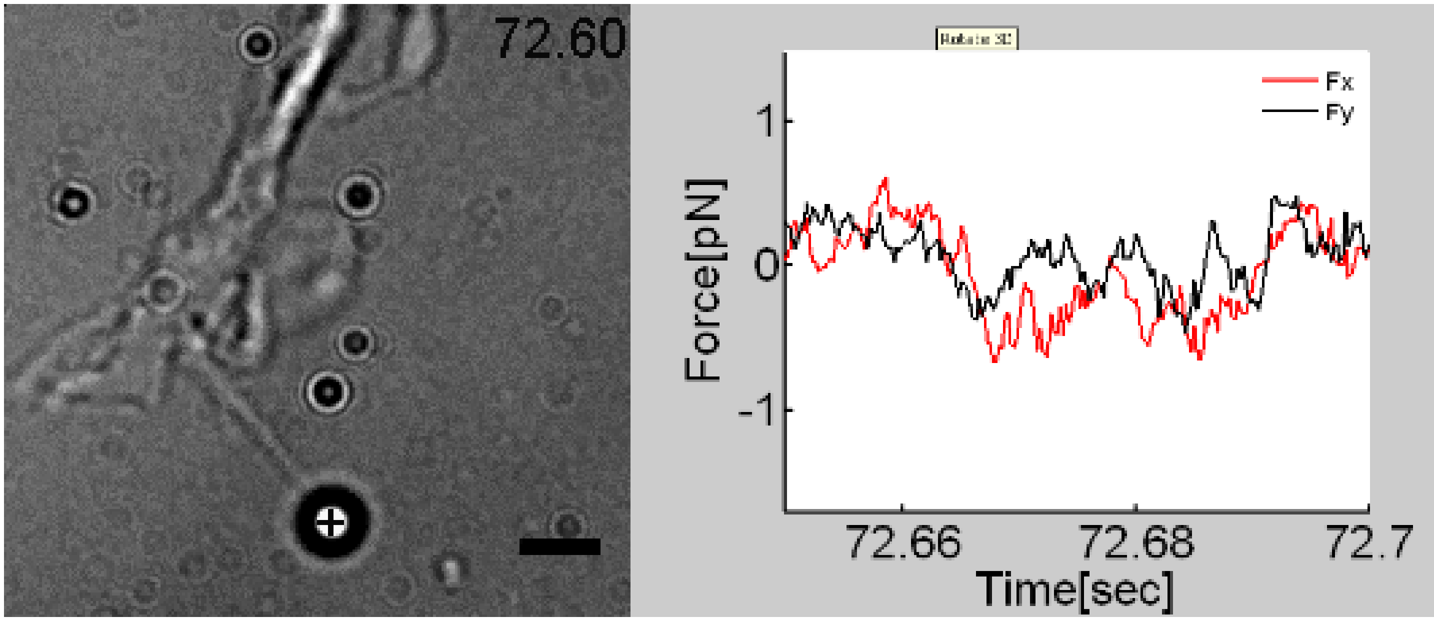
Typical values for **AFM: K_{AFM} = 10 – 1000 pN/nm**

OT and AFM are Complementary Techniques

Measuring the forces exerted by neuronal cells during development

Force exerted by Filopodia - Protrusion

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



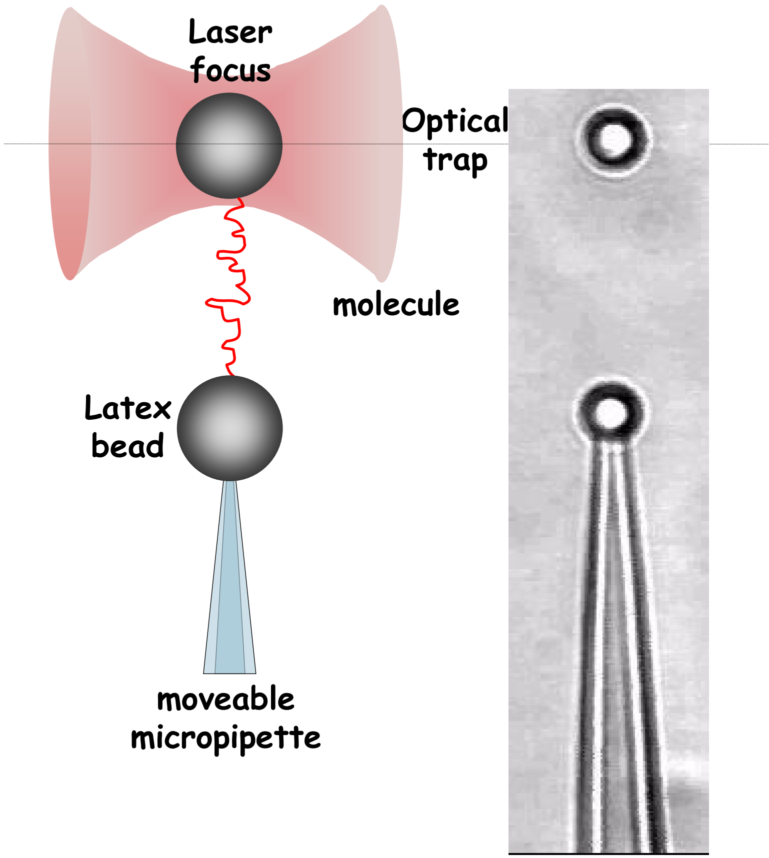
2 Days In Vitro hippocampal neuron from mouse

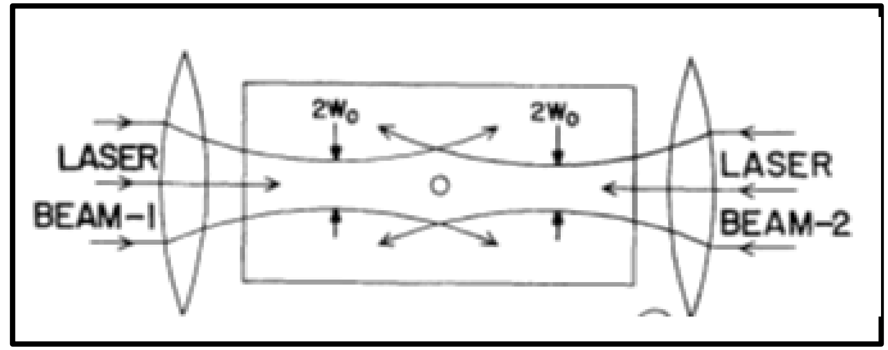
The force and protrusion due to actin polymerization of the bundle of actin filaments in the filopodia is observed.

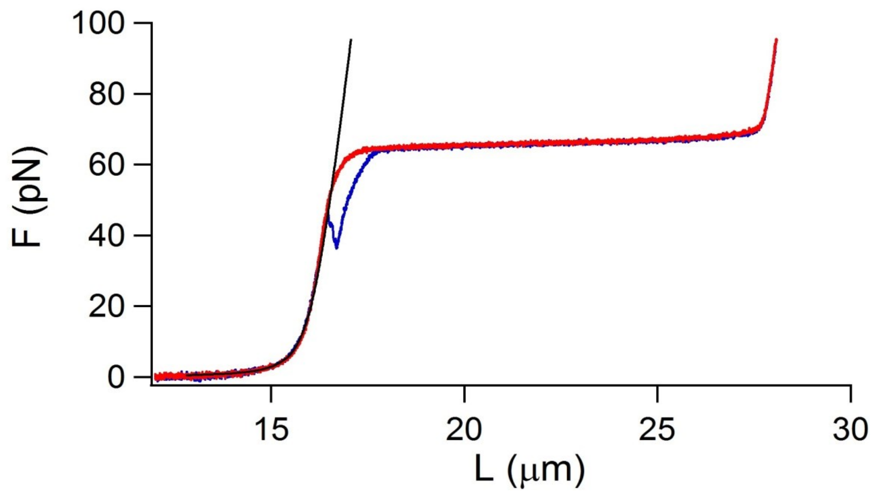
Cojoc, D, … & Torre, V, PLoS One 2 (10), e1072 (2007)

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

Measuring force and length range when stretching λ-phage DNA



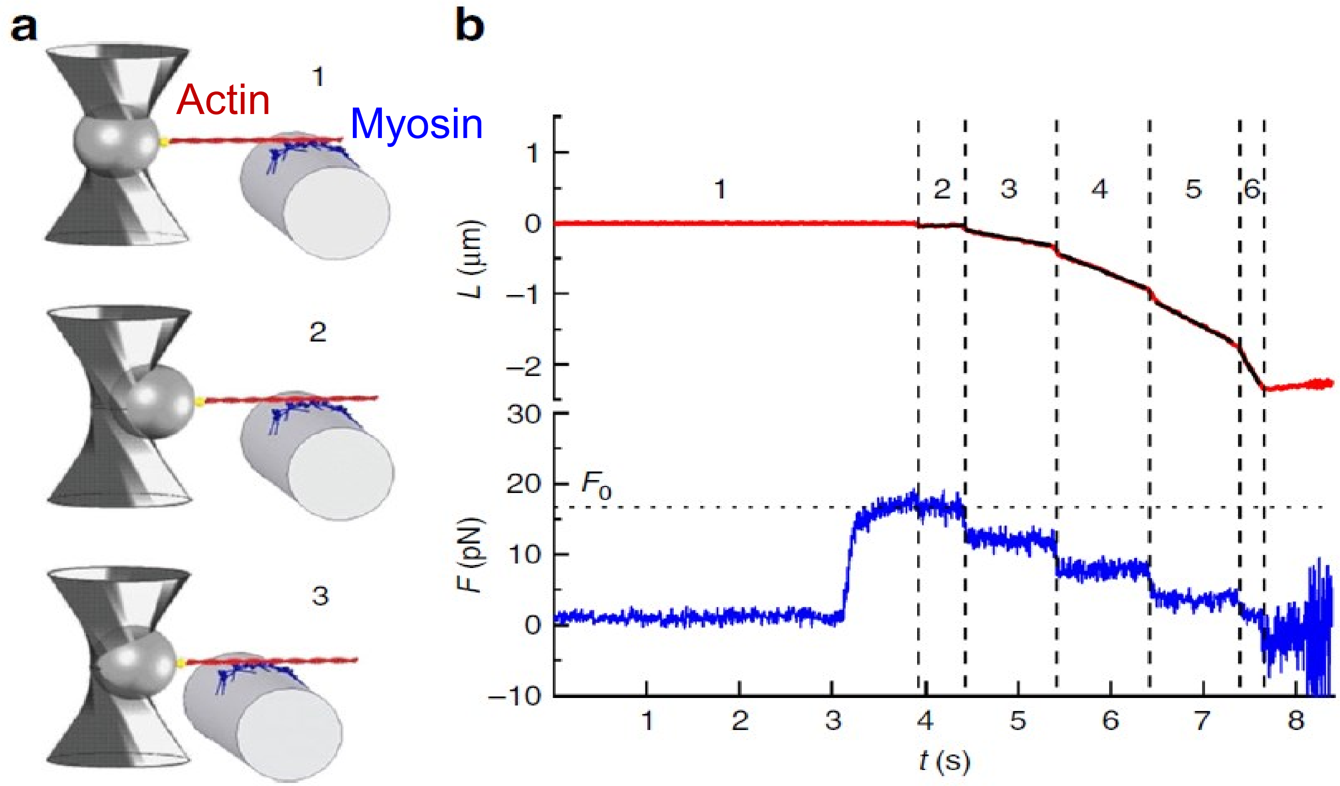




The molecule undergoes a highly cooperative structural change at ~65 pN that implies 70% elongation and is likely involved in the modulation of the access to genetic information .

collab V. Lombardi, P. Bianco, Florence Univ.

A myosin II nanomachine mimicking the striated muscle,

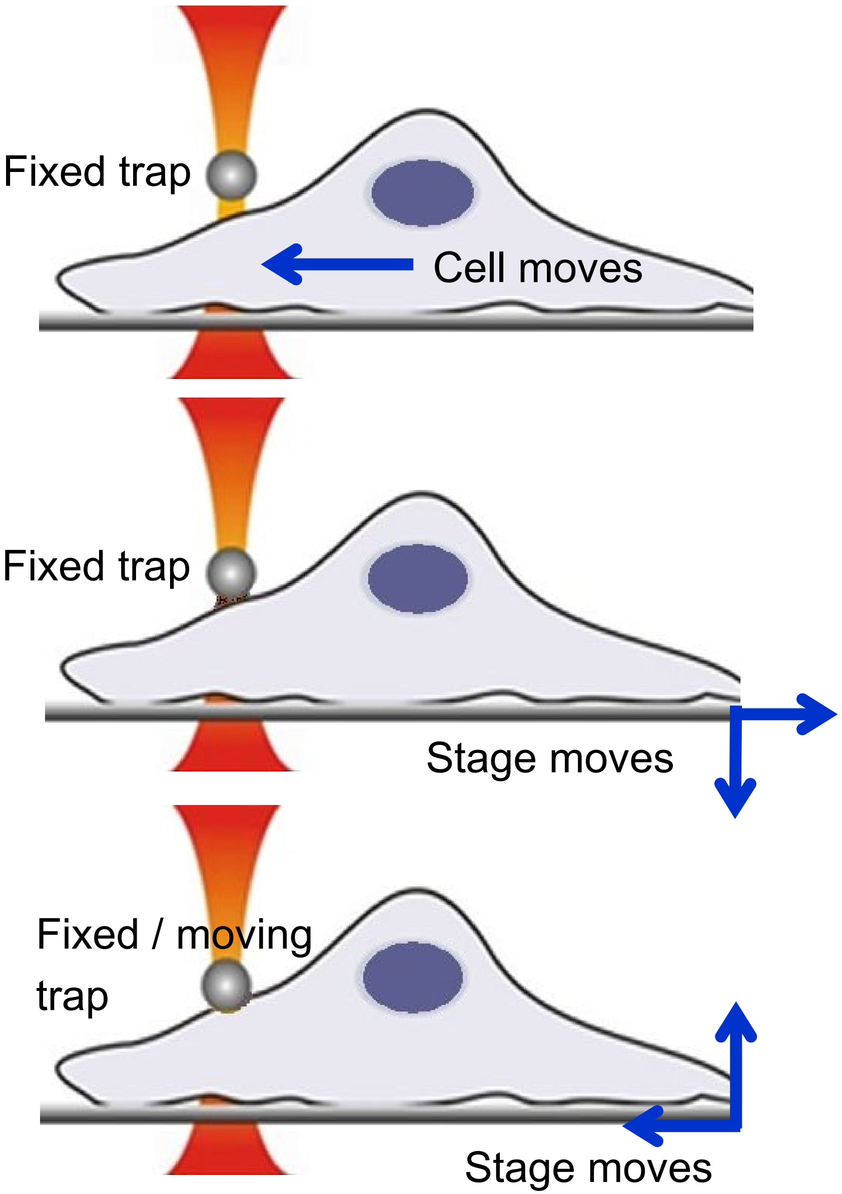


a. Schematic representation of three snapshots during the phases of the interaction between the actin filament and the motors.
b. Recording of the relative sliding (red) and force (blue) during interaction. Phase 1, following the formation of the first bonds between the actin filament and myosin motors, the force rises in position feedback to the maximum isometric value ~17 pN.

Pertici et al., *Nature Communications*, (2018) 9:3532.

OT local probing living cells

(touch - pull - push approaches)



Touch / intercept

Measure forces when full cell or part of the cell moves

Pull (Coated beads)

Local adhesion / binding

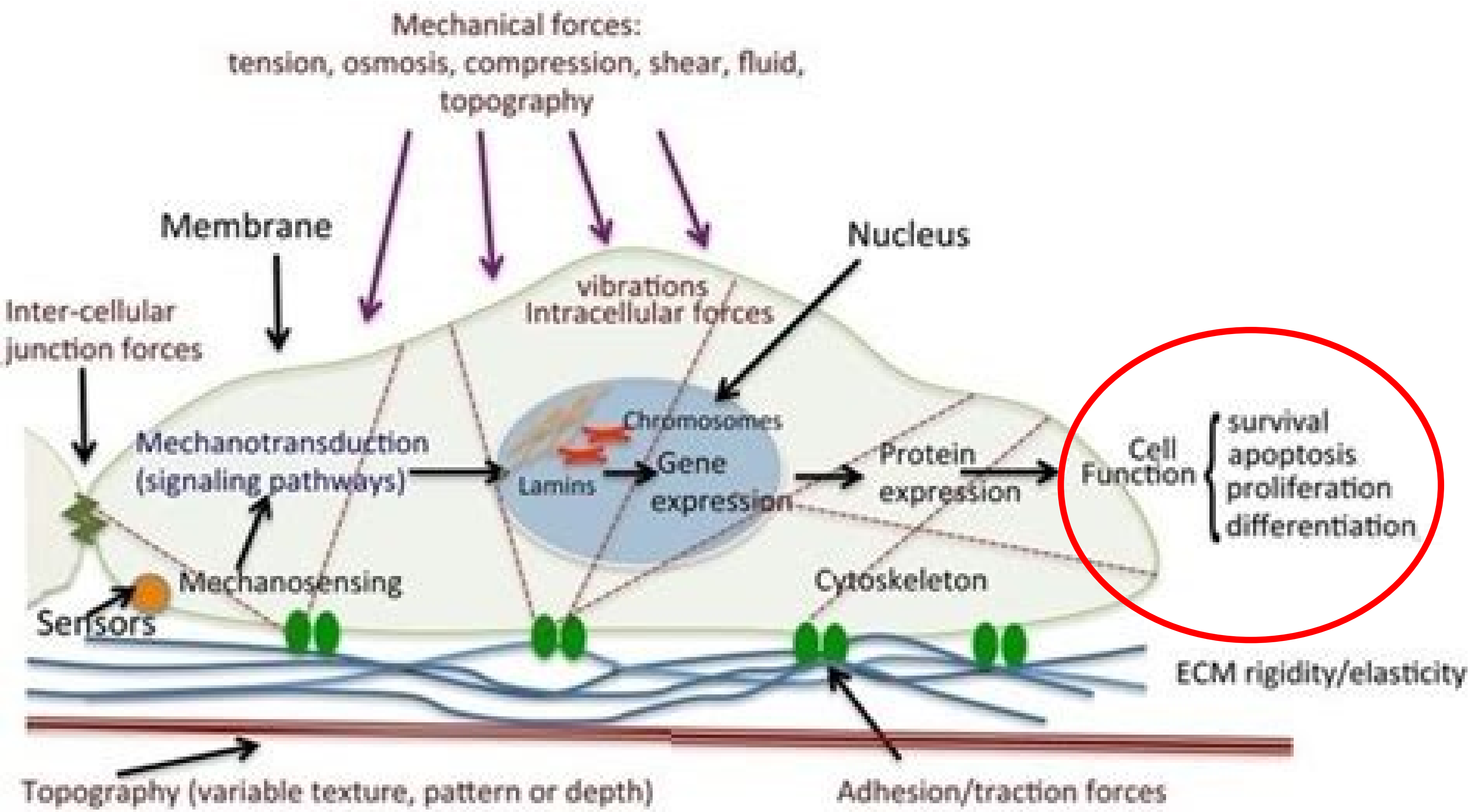
Local viscoleasticity (tether membrane)

Push

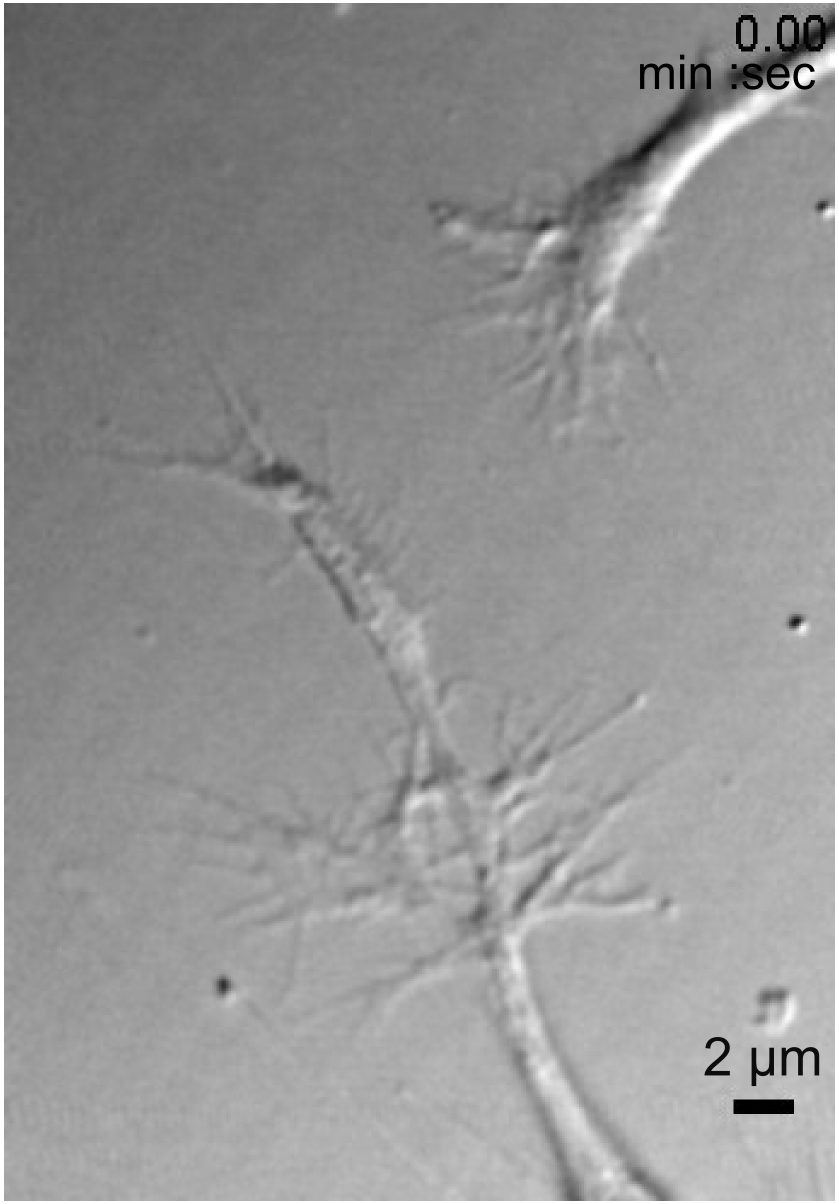
Local viscoelastic properties

Local cell stressing

Cell mechanotransduction – cell function



Neuronal development (pre and post natal)



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

they interact also mechanically

- 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

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

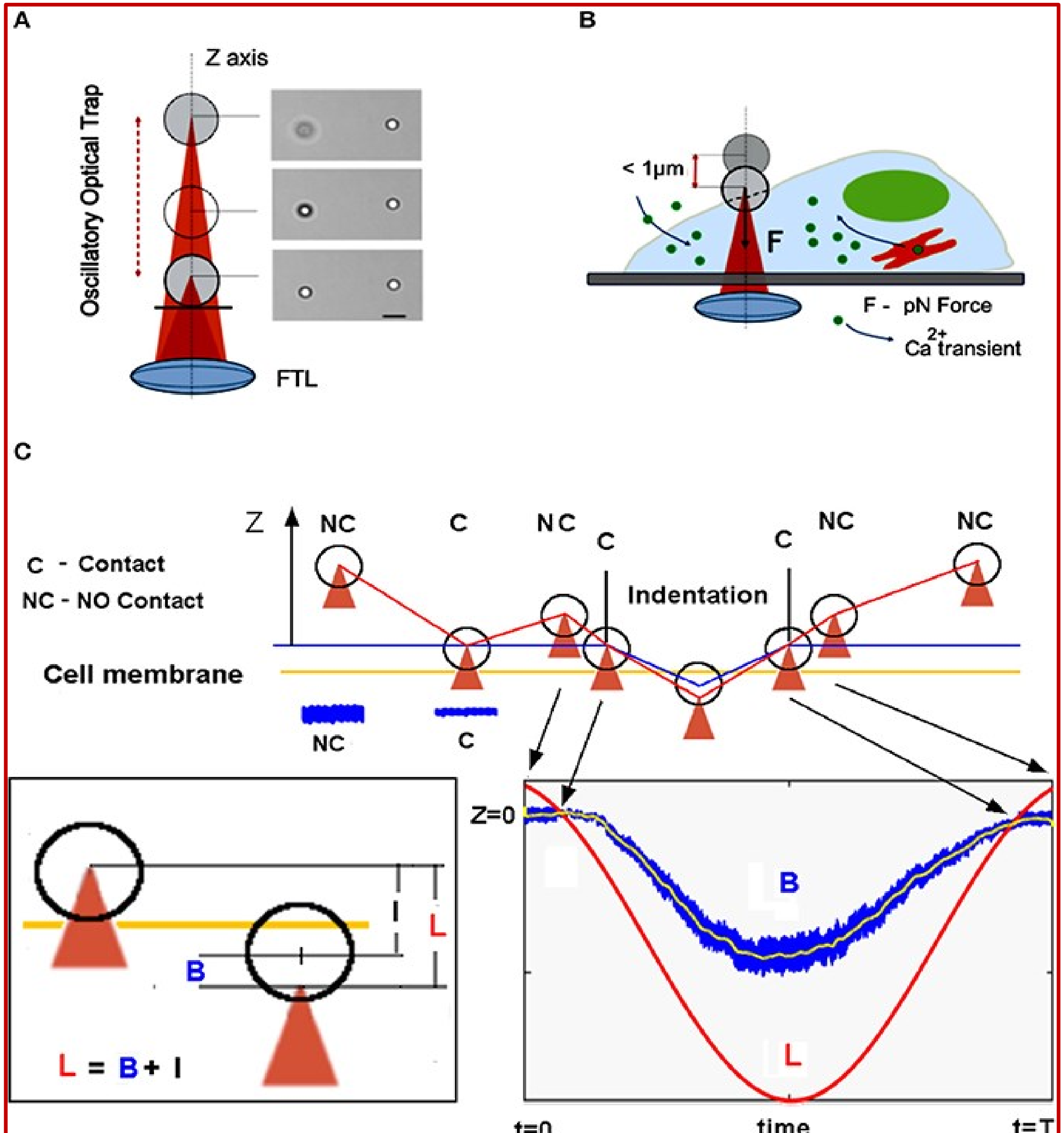
Mechanotransduction studies how cells sense physical forces and the cellular signal transduction in response to mechanical stimuli.

Piconewton forces, characteristic for OT, are in the range of forces expressed by neurons during development, cell-cell and cell ECM interaction.

Transduction of the mecanical stimulus applied by OT to the cell can be investigated on the same optical microscopy platform (e.g. Calcium signaling).

We demonstrate cell mechanotransduction in neurons, using very small (piconewton forces), applied with unprecedent high spatial and temporal resolution.

Cell membrane indentation and cellular calcium transients



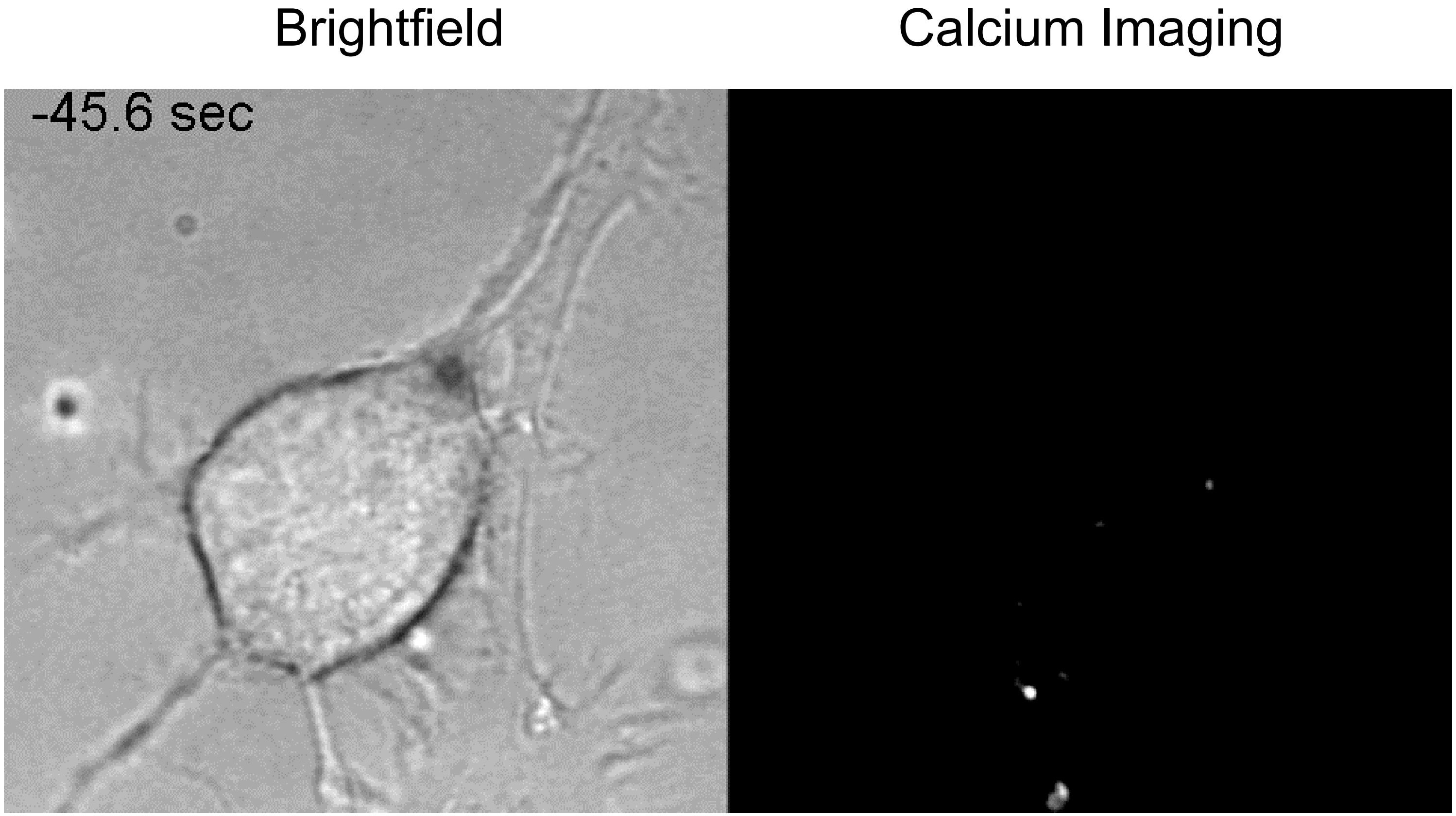
Overview of the

mechanical stimulation

Dynamic Optical Trap

F. Falleroni *et al*,
Frontiers Cell Neurosci, 2018

Ca^{2+} transients evoked by calibrated mechanical stimulations

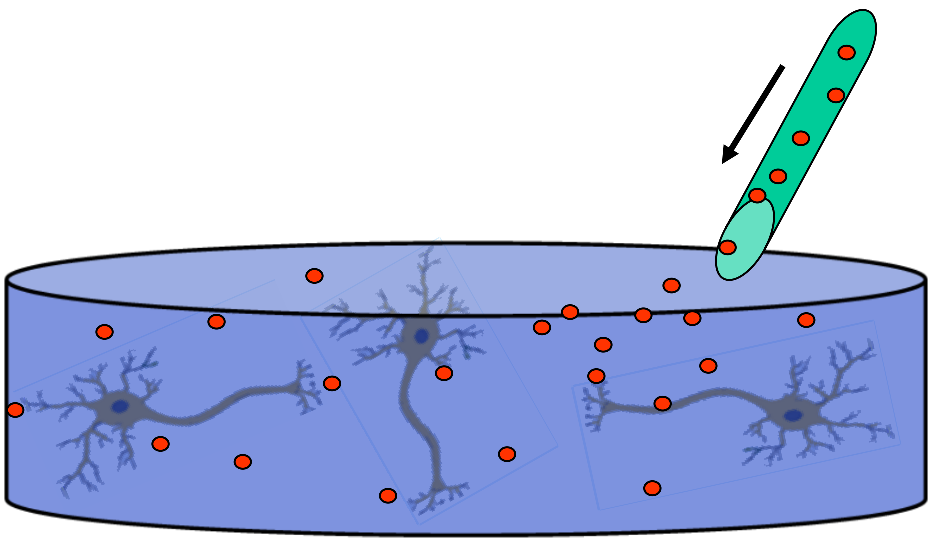


mouse neuroblastoma NG108-15

F. Falleroni *et al*, Frontiers Cell Neurosci, 2018

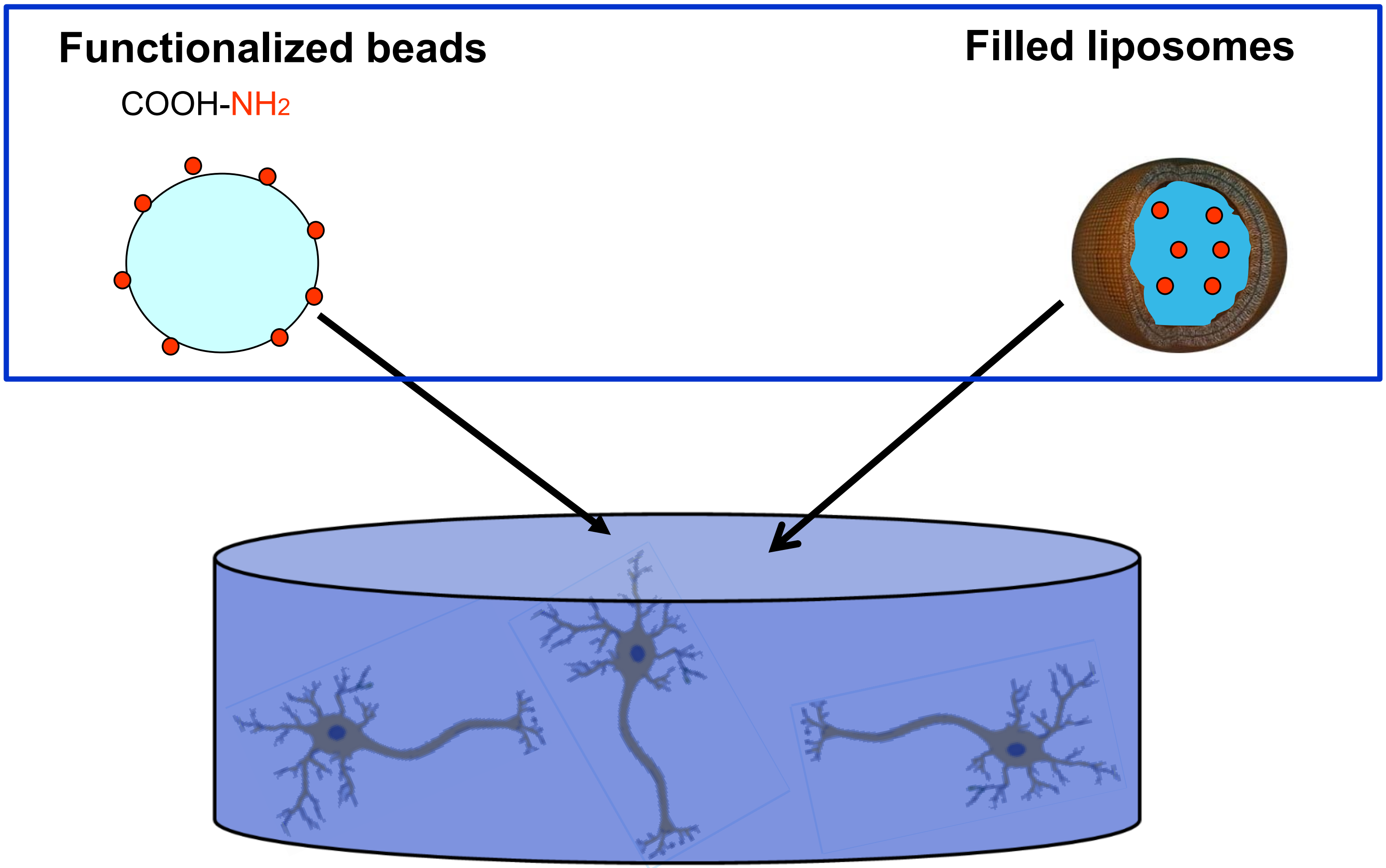
Create physiological inspired experimental conditions !

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

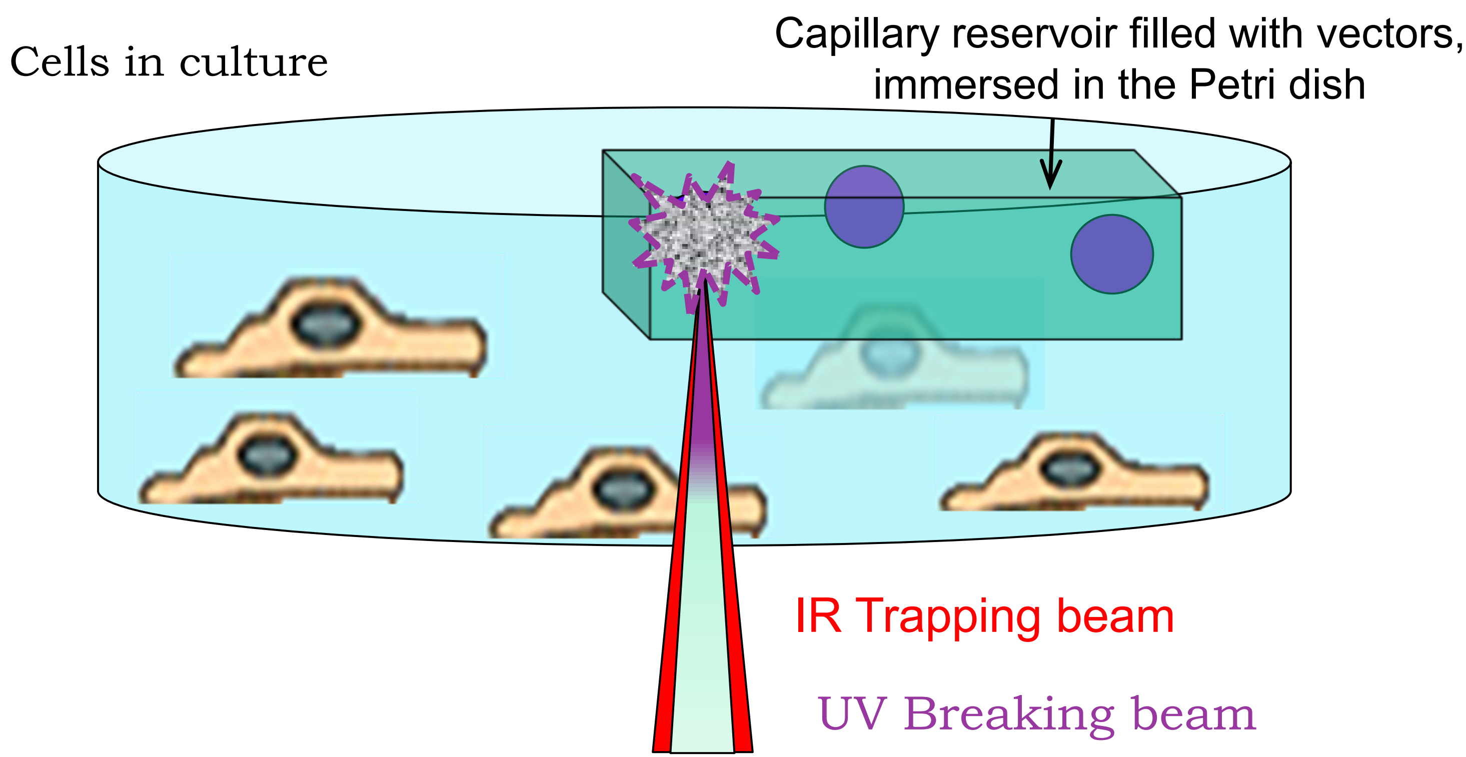


Local stimulation using micro/nano vectors

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

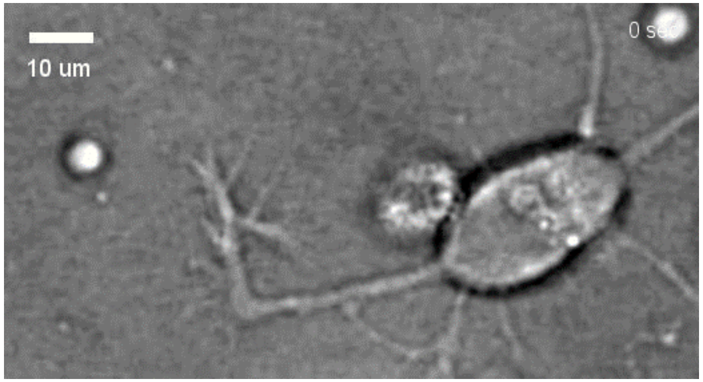


and delivered by:

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

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

Focal stimulation of hippocampal neurons by guidance cues encapsulated in liposomes

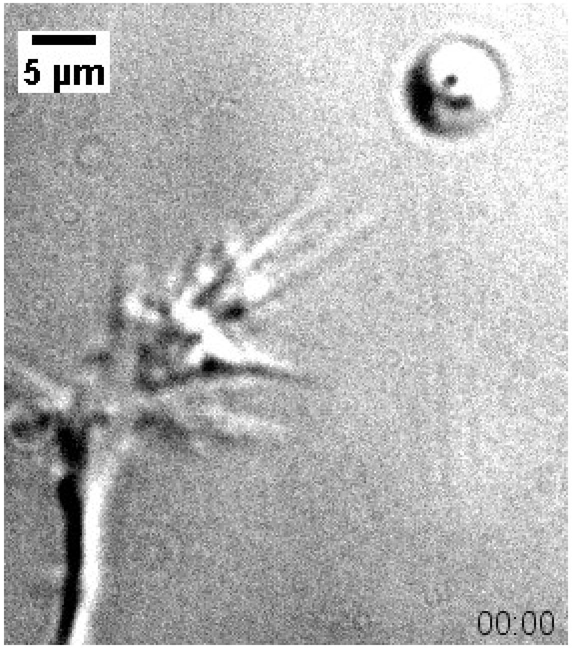


Netrin-1

Growth Cone turning

Proof of concept

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



SemA3

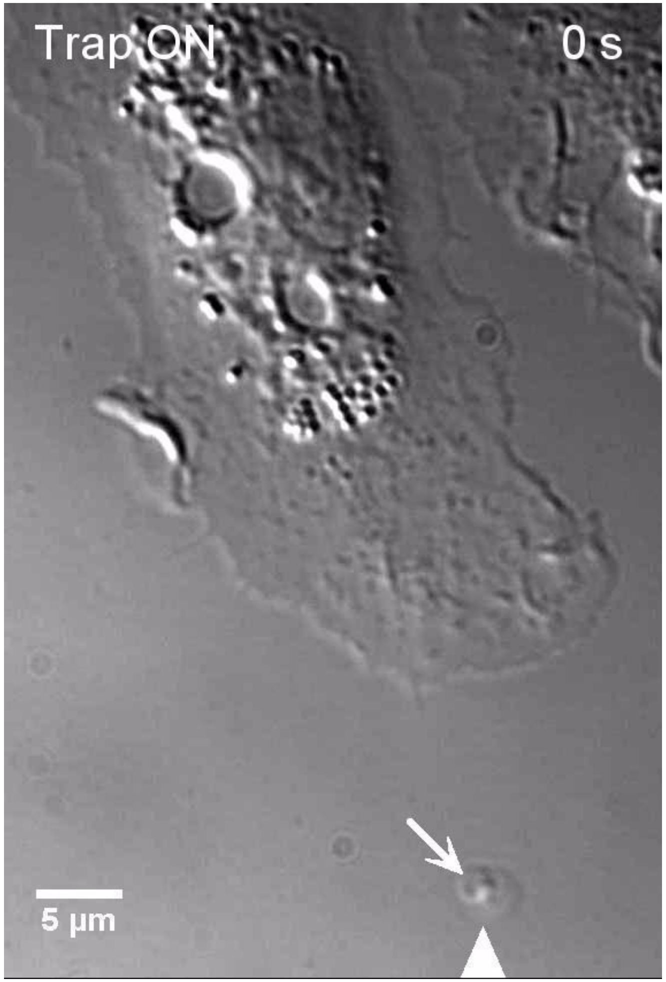
A more quantitative study:

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**

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, (January 2016)

Optical Tweezers Microscopy

- Light has momentum, change of momentum generates force
- Optical Tweezers (OT): Laser beam tightly focused on micro/nano objects in liquid
- Forces applied and measured by OT: 1 - 200 pN
- OT with IR laser can be applied to living cells and biomolecules without damaging them
- OT is implemented on a microscope platform → trap and manipulate what you see and see what you manipulate (ex. Mechanotransduction)

Thank you Arthur Ashkin !