Fiber Optic Tweezers and Optical Stretcher: biophotonic tools for single cell studies

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Motivation

In molecular and cellular biology studies performed at a single cell level have revealed extremely heterogeneous behaviours and provided unpredictable information

The exploitation of optical forces, generated by the interaction between electromagnetic waves and the sample, represents an accurate, non-invasive and gentle manipulation technique for individual cell studies

Bio-photonics devices based on optical forces:

- can trap and move single cells without physical contact
- can be combined with diagnostic methods
- can be used for many applications including, cell sorting, bacterial adhesion forces and membrane interactions analysis and investigation of the viscoelastic properties

Aim: realization of miniaturized biophotonics tools through the use of optical fibers and integrated optics

Outline

- Biophotonic tools based on optical forces:
  Tweezers' working principle
- Biophotonic tools based on optical forces:
  Stretcher's working principle
- Optical Stretcher Capabilities and Experimental results
- Conclusion

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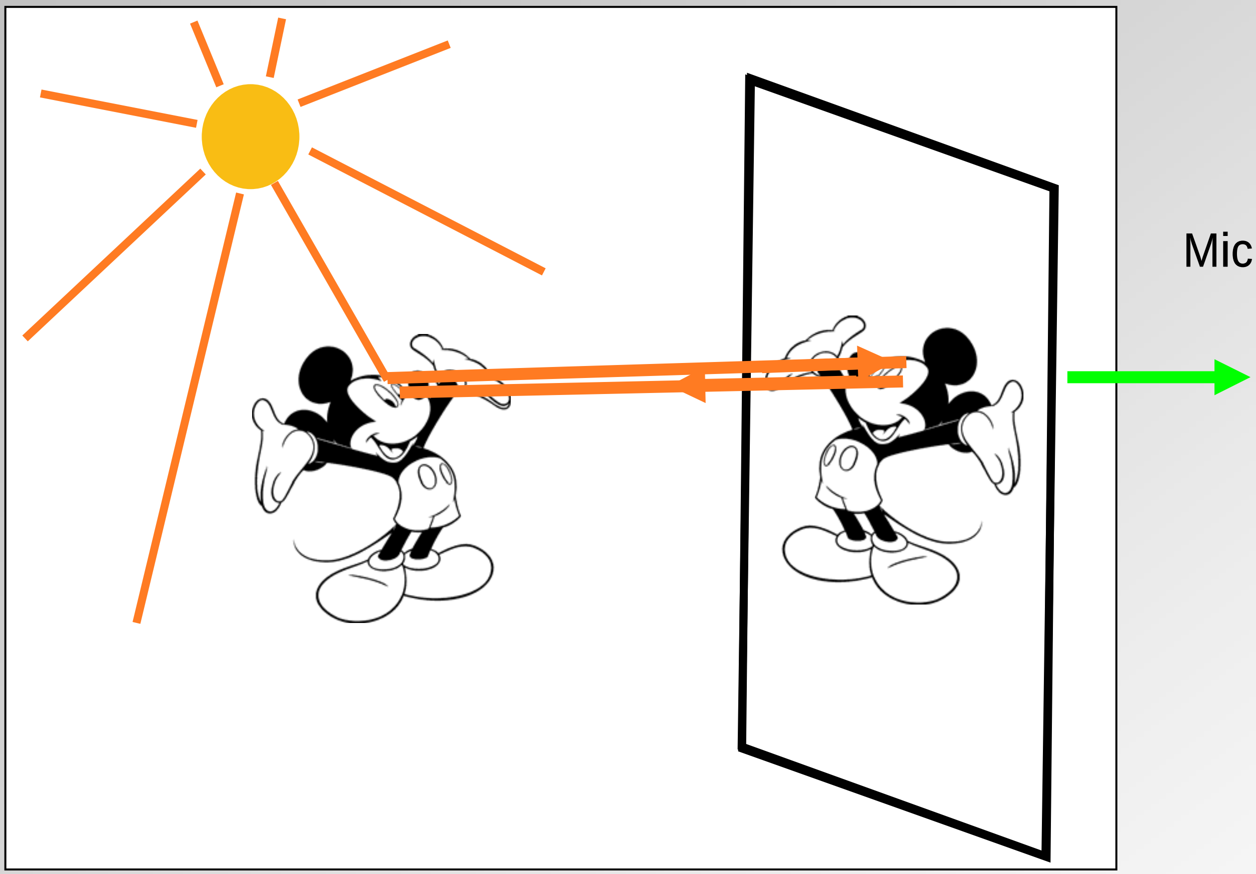
Light can move the objects!



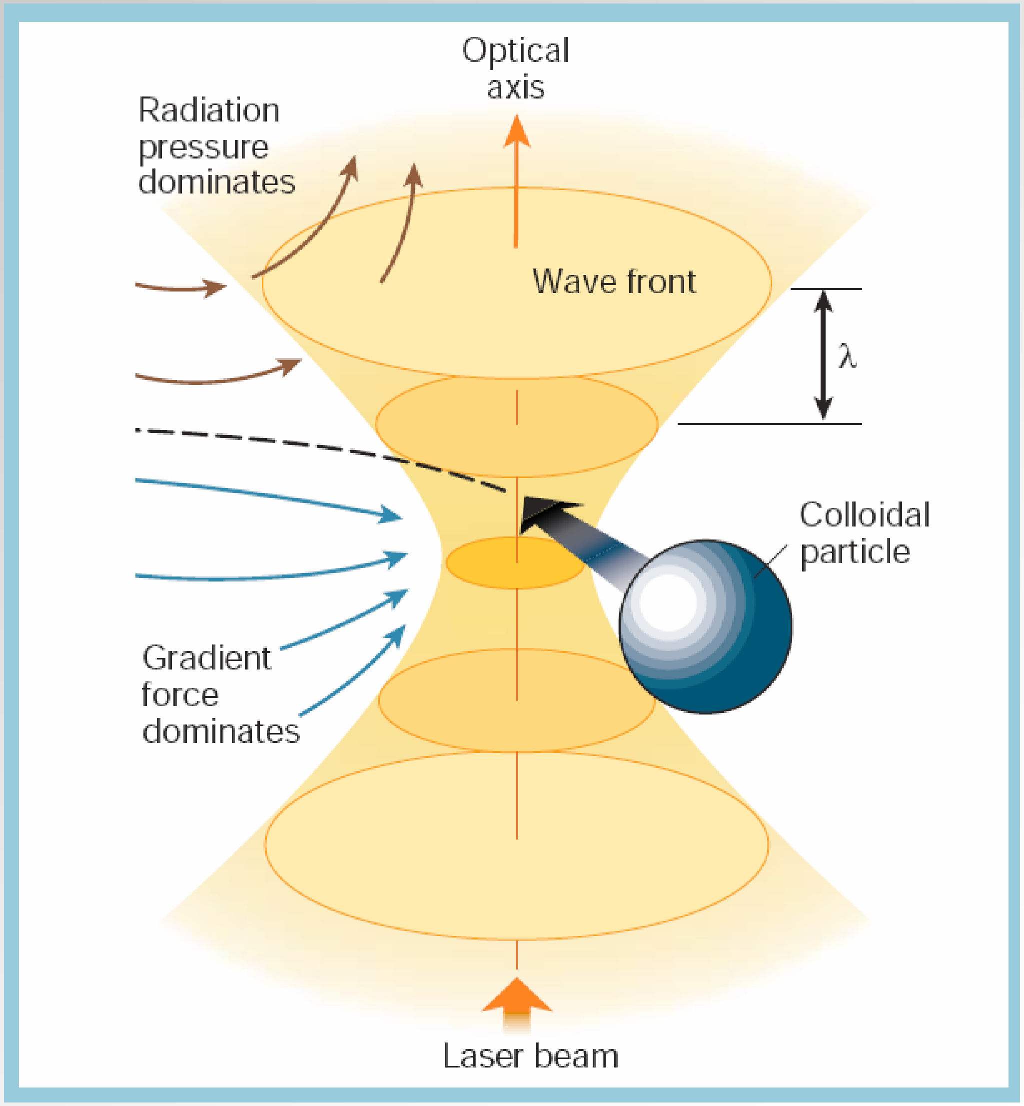
Micky mouse is pushing
the mirror

If light changes its momentum due to the scattering from an object,
at the same time it is applying a force

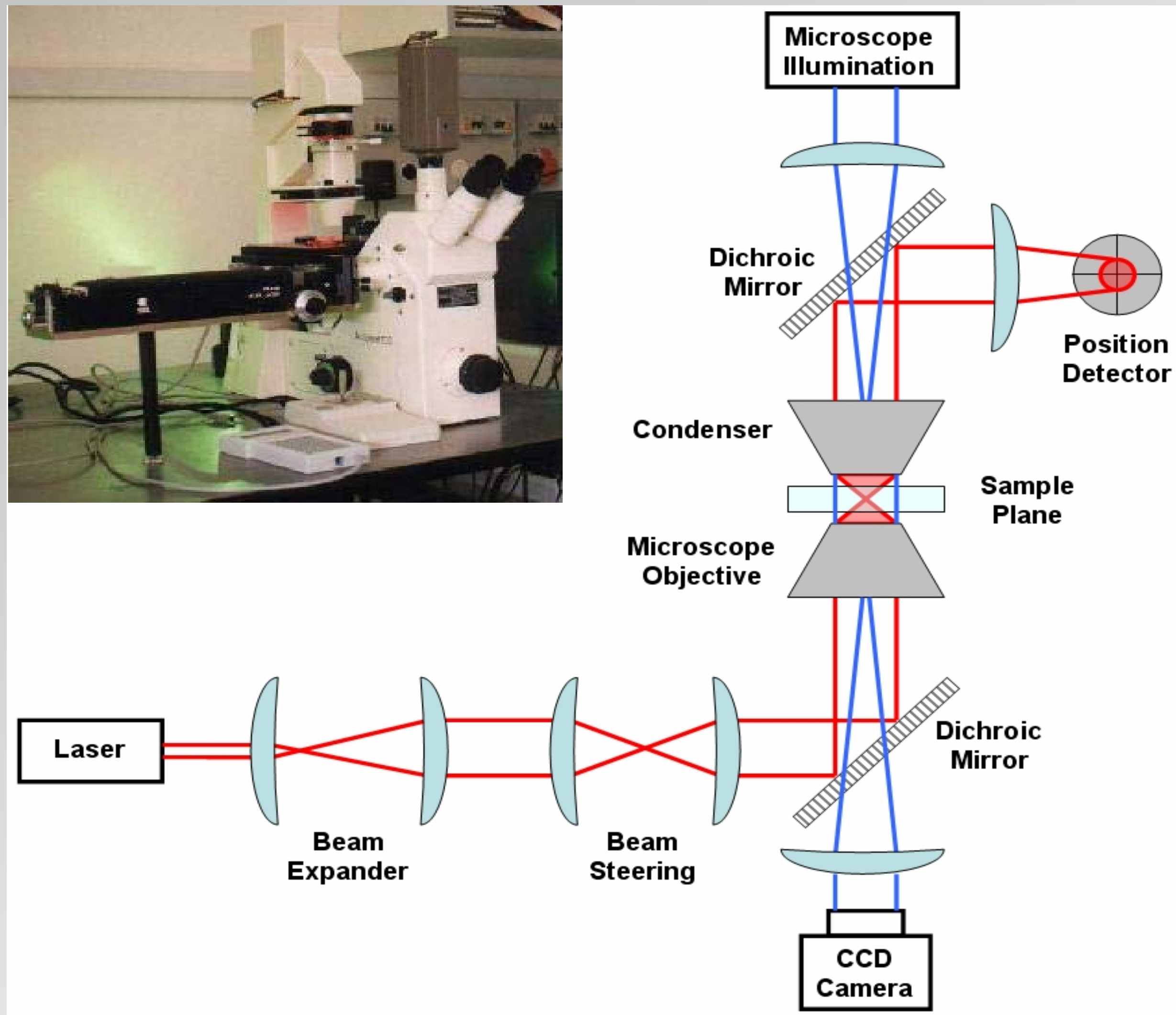
Single-beam Optical Trap

Optical trap: stable equilibrium point of optical forces acting on a particle

The trap is created by a single laser beam tightly focused: large intensity gradients in both the axial and transverse directions



Standard vs Fiber Optic Tweezers



Standard Optical Tweezers

Standard vs Fiber Optic Tweezers

NEWS & VIEWS

OPTICAL TWEEZERS

All-fibre design

Optical tweezers enable precise, controlled and non-contact manipulation of small biological specimens. Rather than using a bulky microscope, it is now possible to create optical tweezers at the end of a fibre probe.

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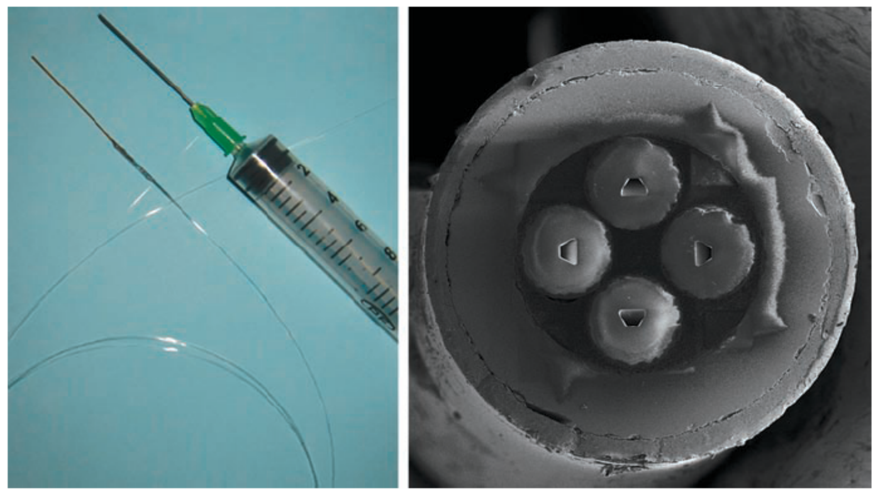
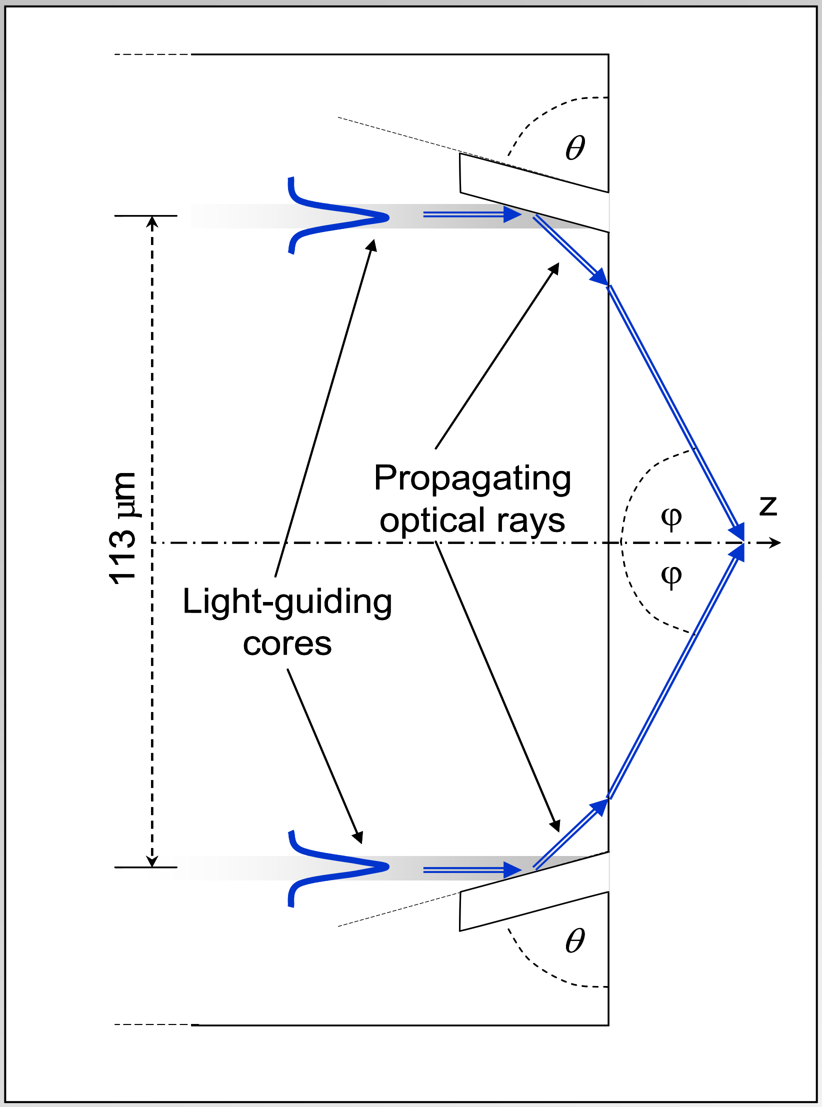
In the 20 years since they were pioneered by Arthur Ashkin[1] and his co-workers, optical tweezers have become a mainstream research tool in both the biological and physical sciences. Most optical tweezers rely on the force acting on a dielectric particle that arises whenever there is a large gradient in the intensity of the optical field. Usually this gradient is achieved by the strong focusing of a conventional laser beam using a high-magnification microscope objective. Indeed, most optical tweezers are themselves based around high-quality microscopes where the same objective lens is used for both focusing the trapping beam and viewing the trapped sample. Now, Carlo Liberale and colleagues[2] have come up with a different approach, one that enables tweezing using a single optical-fibre probe (page 723).



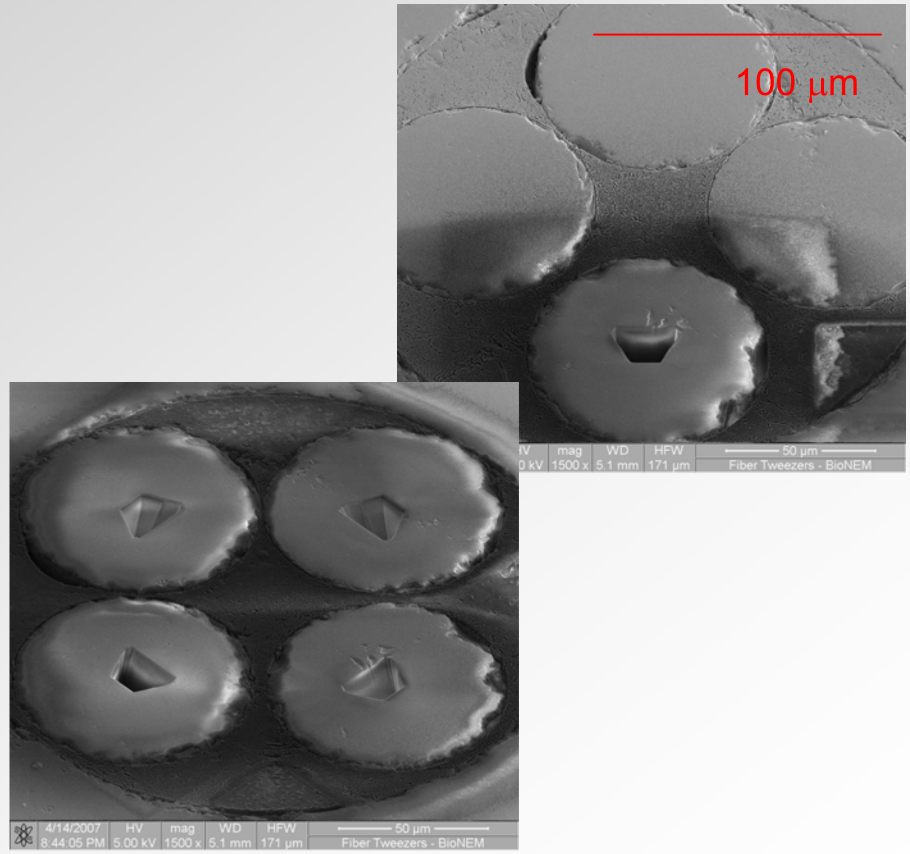
Images of the all-fibre tweezer[2]. The tweezer lying next to a syringe (left) and a scanning electron microscope image of the tweezer's end showing four internal fibre structures with specially shaped core regions (right). The diameter of the fibre probe is 360 μm.

Our proposal: Fiber Optic Tweezers

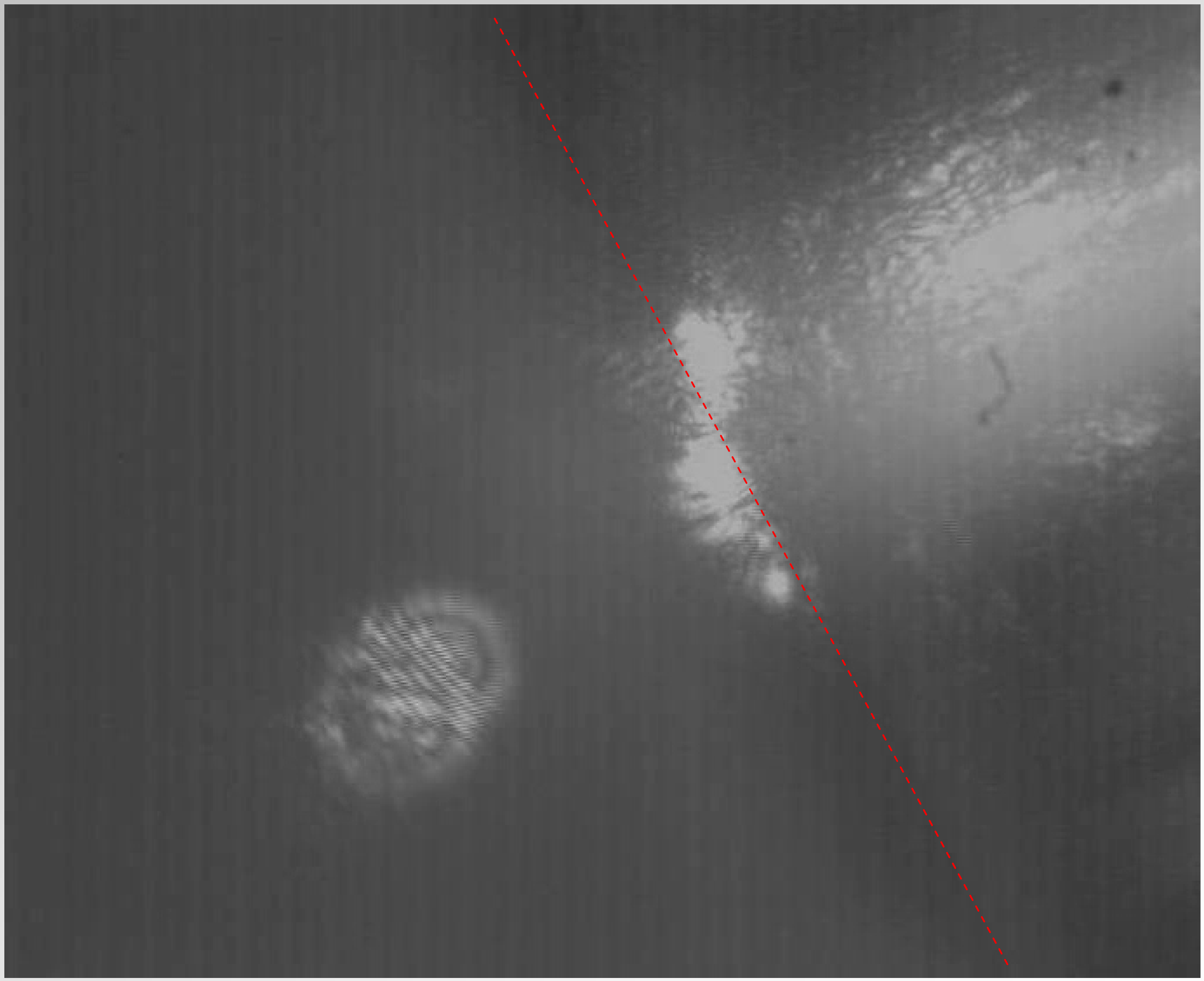
Fiber Optic Tweezers - Nanofabrication



With a Focused Ion Beam (FIB), we performed micro machining, digging only the core regions where the total internal reflection takes place.

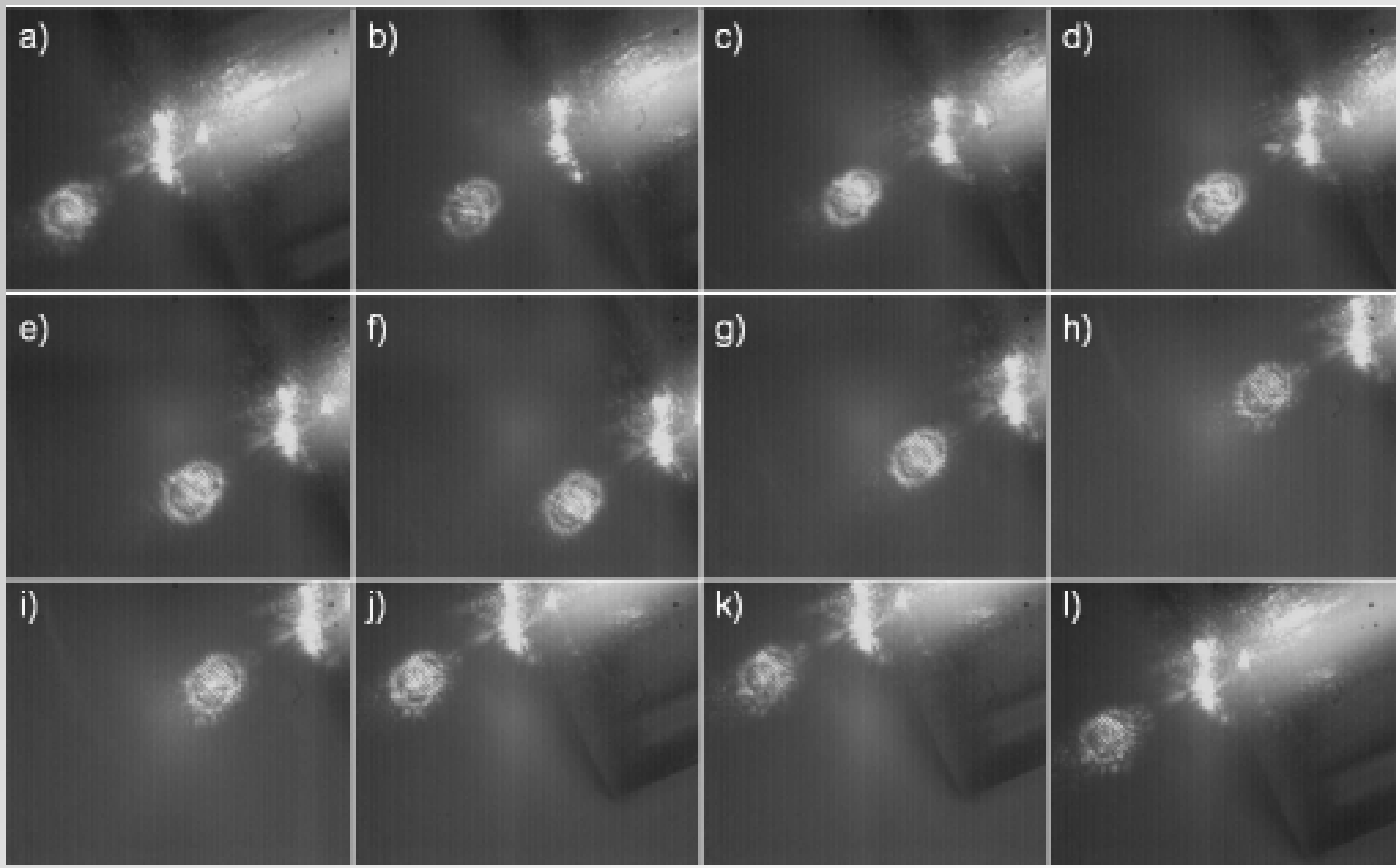


Fiber Optic Tweezers - Trapping

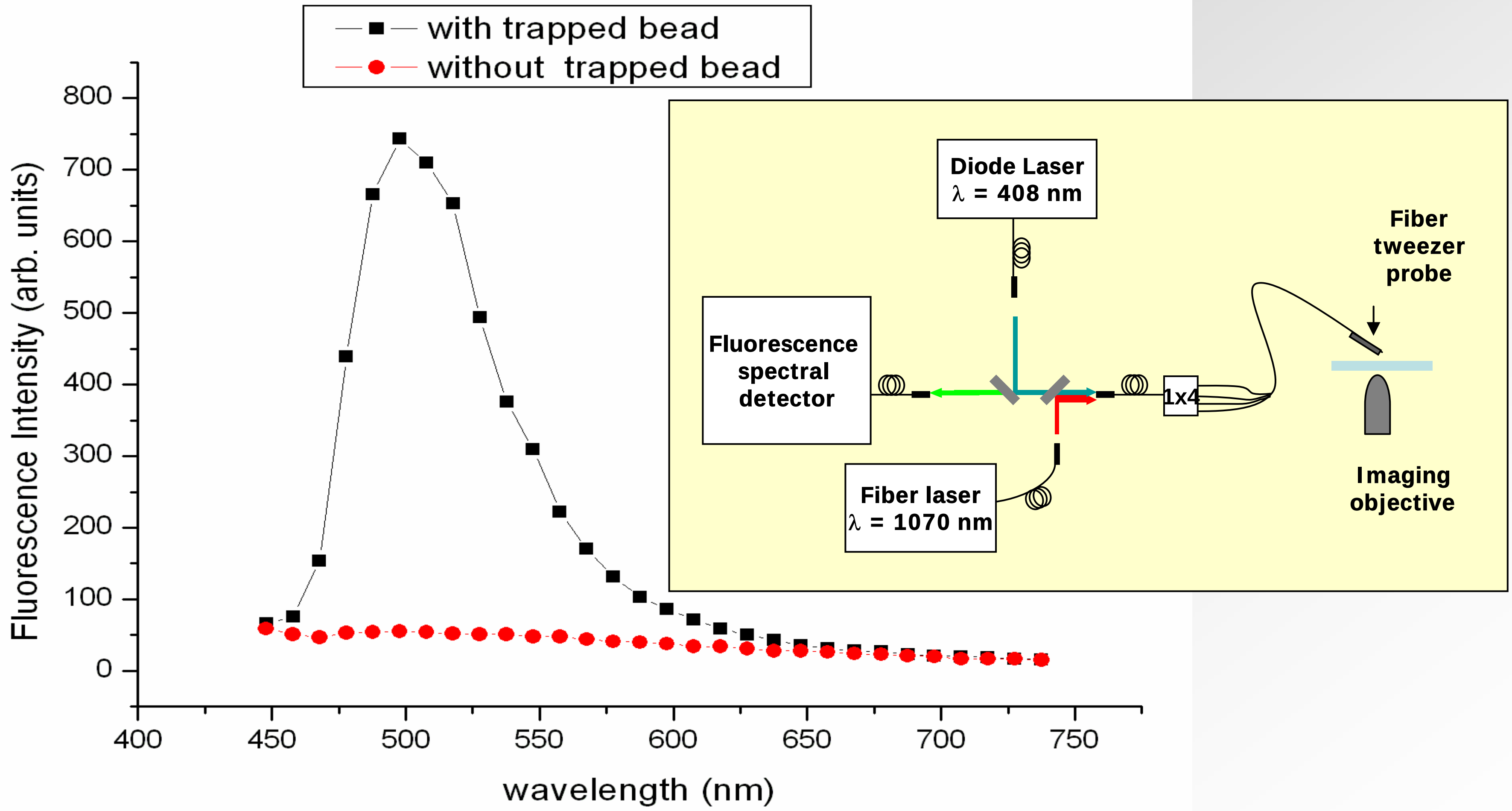


C. Liberale et al. "Miniaturized all-fibre probe for three-dimensional optical trapping and manipulation", Nature Photonics 1, 723, 2007

Fiber Optic Tweezers - Trapping



Fiber Optic Tweezers - Fluorescence collection



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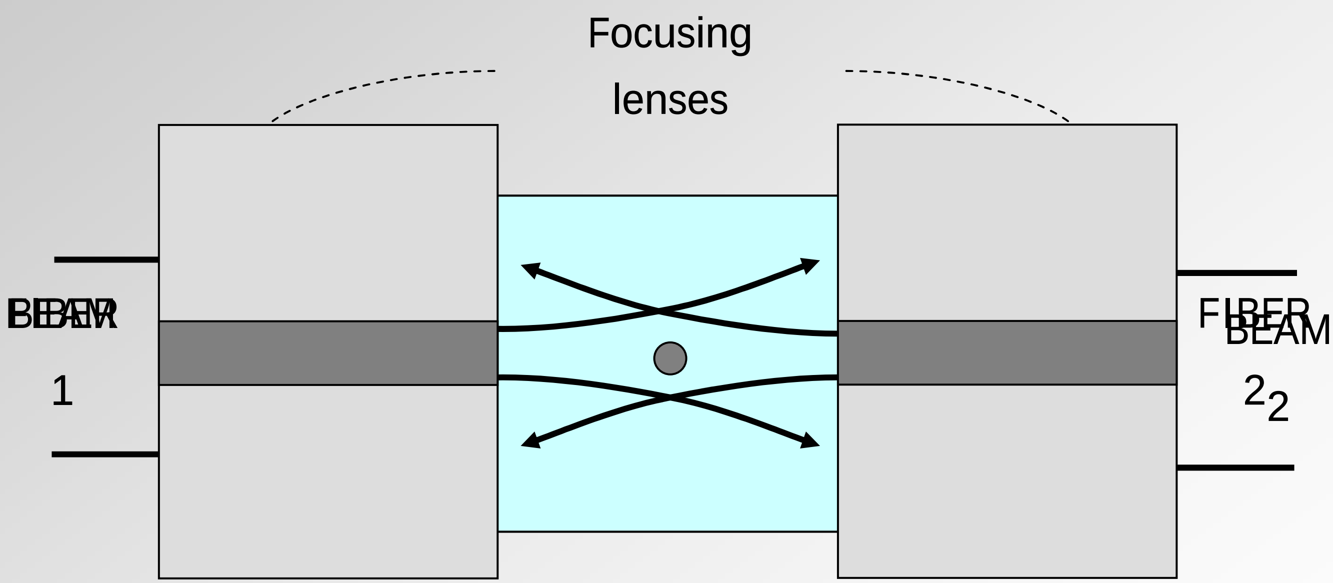
Dual-beam Optical Trap

Optical trap: stable equilibrium point of optical forces acting on a particle

The easiest way to realize a trap:
exploitation of two counterpropagating laser beams

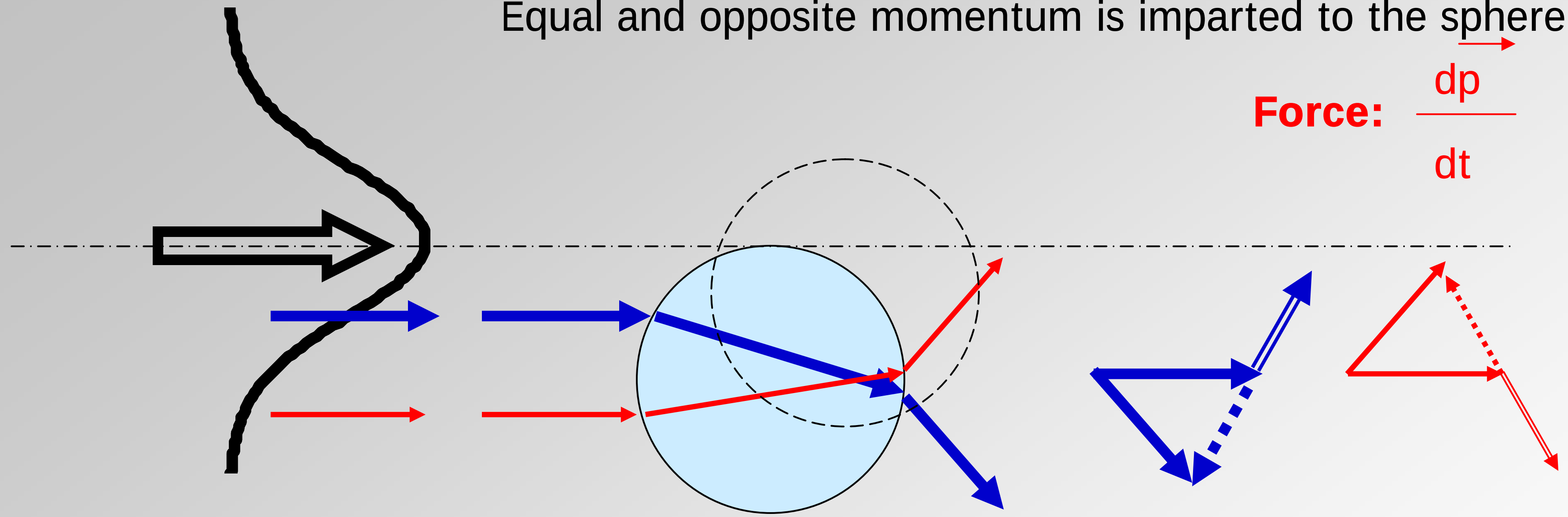


A. Ashkin "Acceleration and trapping of particles by radiation pressure" Phys. Rev. Lett. 24, 156, 1970

Optical Forces

Refraction of light: change in the carried momentum
Equal and opposite momentum is imparted to the sphere

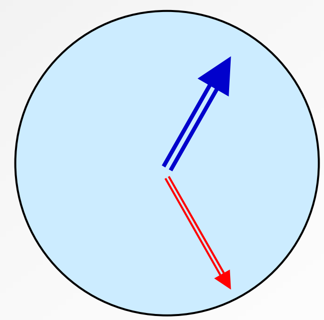
Force: $\frac{d\vec{p}}{dt}$



By illuminating a spherical sample through a gaussian beam two forces are produced:

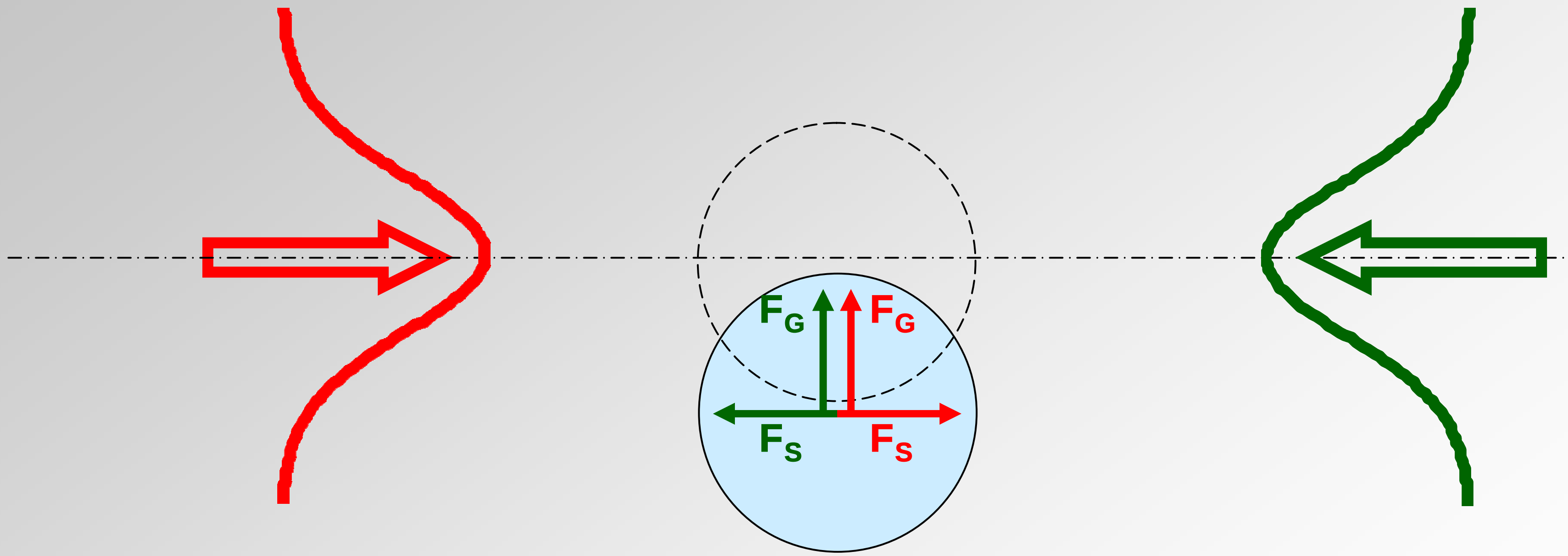
- scattering force that tends to push the particle

- gradient force that tends to pull the particle toward the beam axis



Optical Trapping: working principle

When two counterpropagating beams are present, the scattering forces are balanced whereas the gradient components are acting in the same direction



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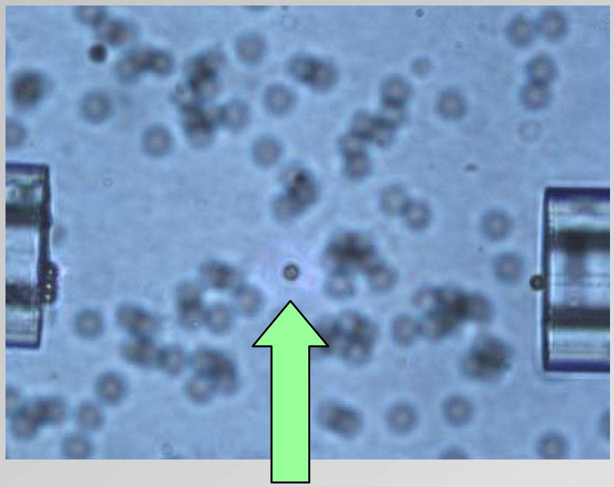
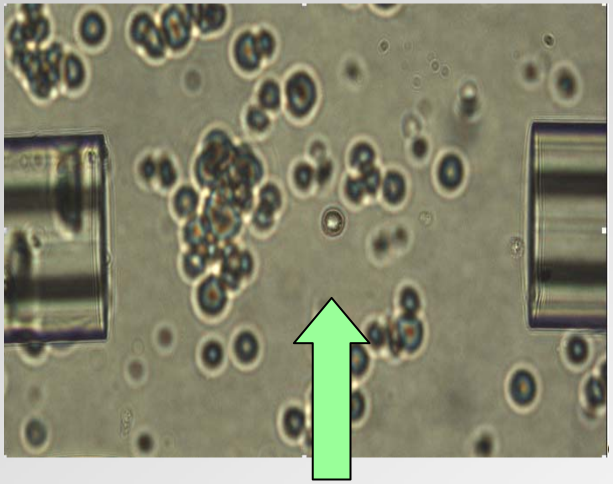
Trapping

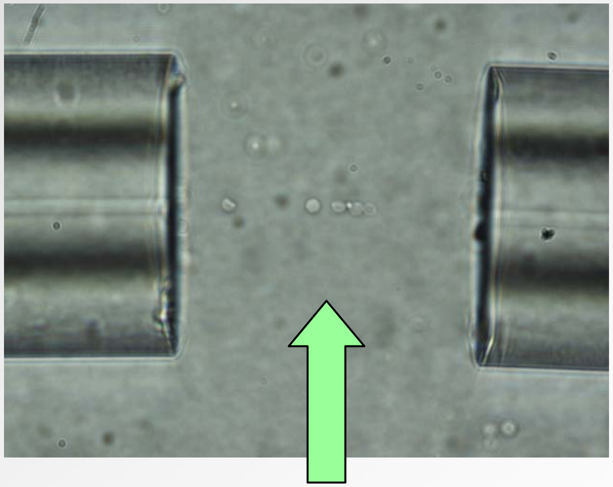
We efficiently trapped different dielectric and biological samples
(stem cells, yeast cells, red blood cells, white blood cells...)



• polystyrene bead
(d~10 μm)



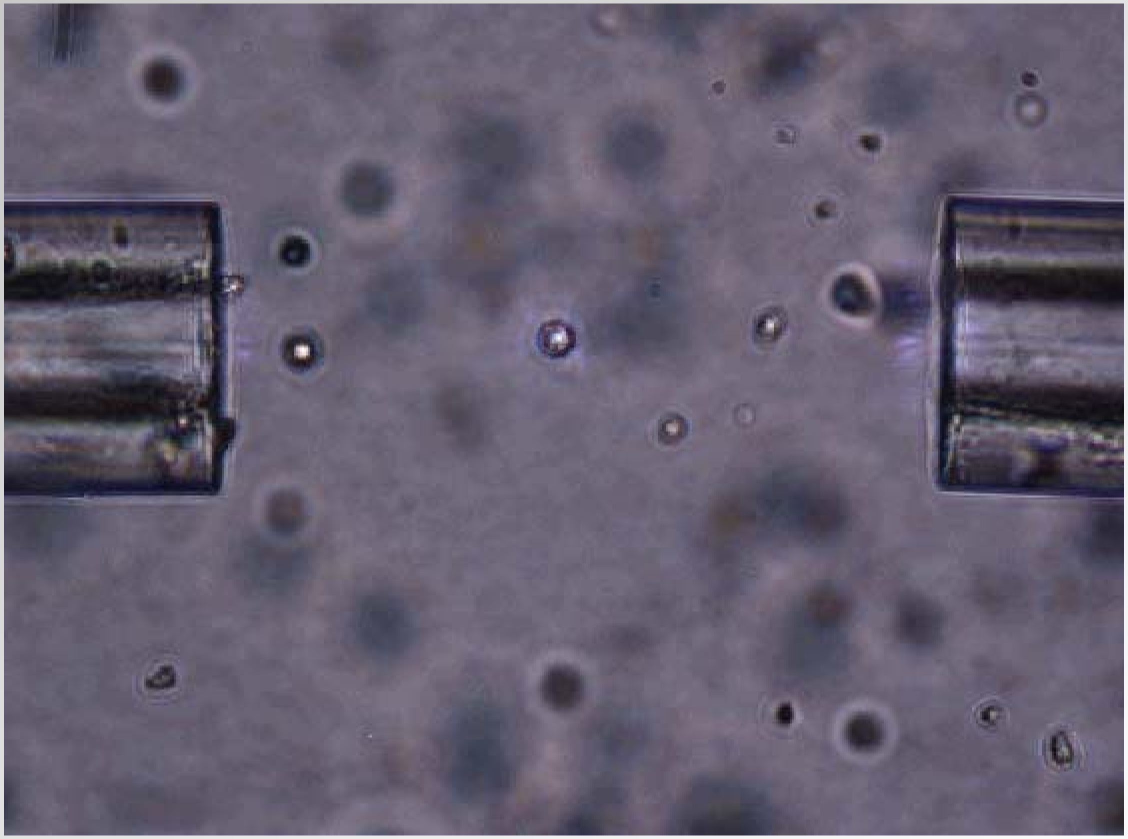
• stem cell
(d~10 μm)



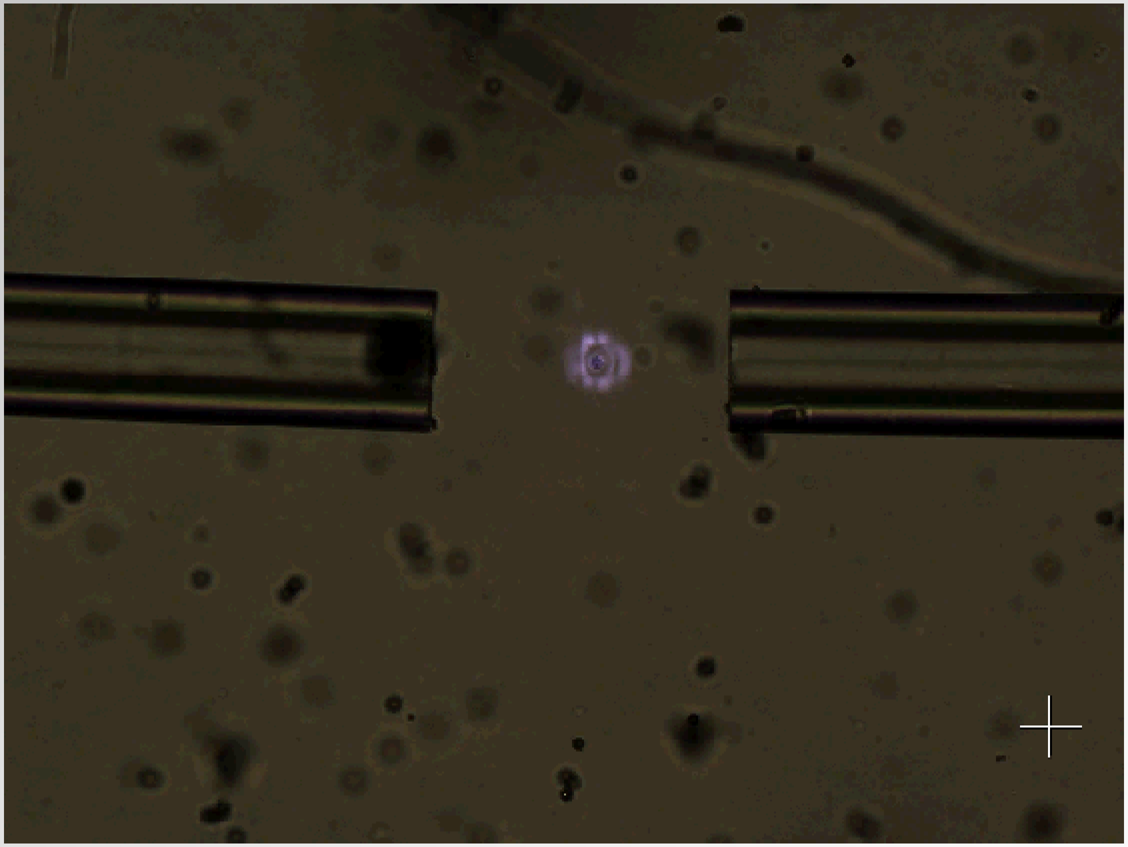
• red blood cell
(d~6-10 μm)

Collaborations with groups of the internal network of the project

Trapping

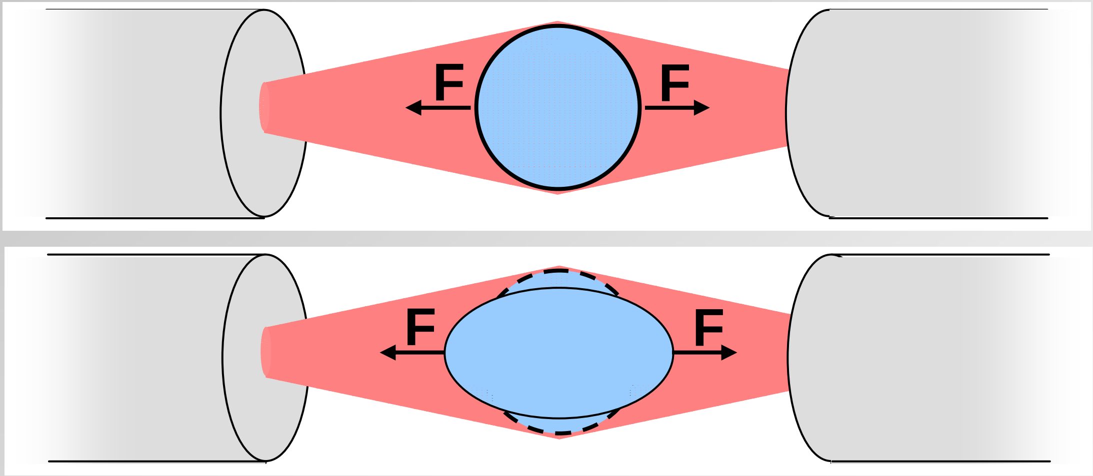


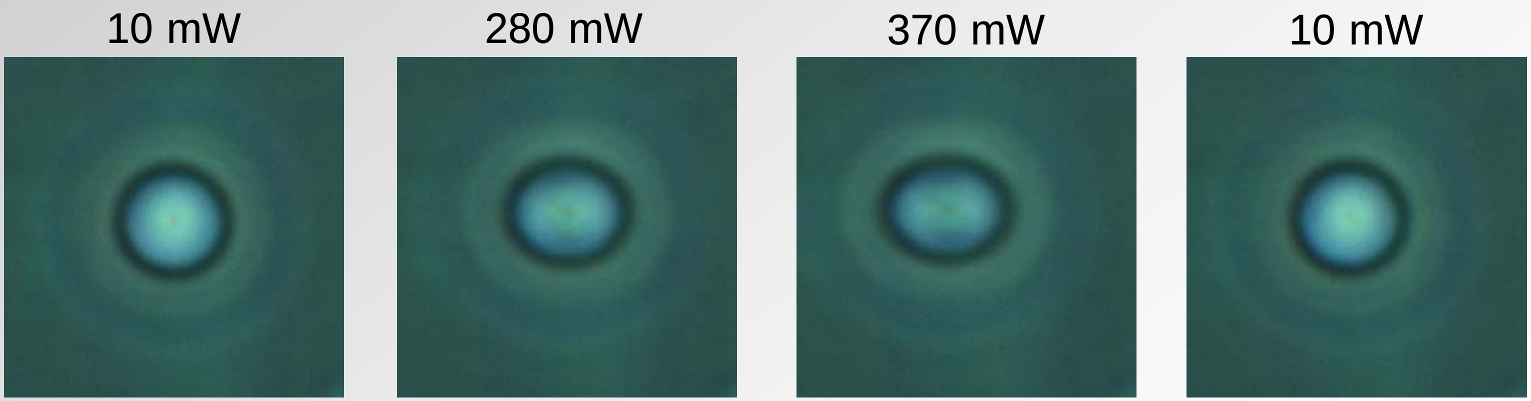
Moving



Dual-beam Optical Trap: Optical Stretcher

An optical stretcher is a device that can induce mechanical deformations on a sample without physical contact

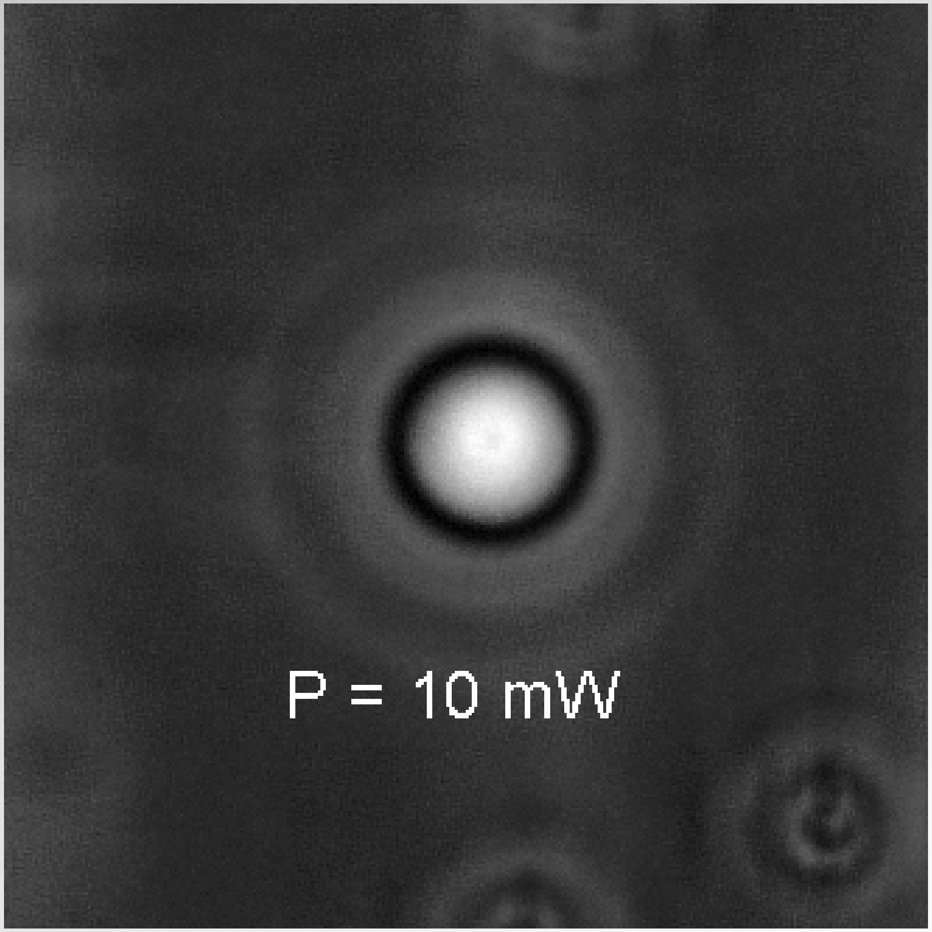




Red Blood Cells

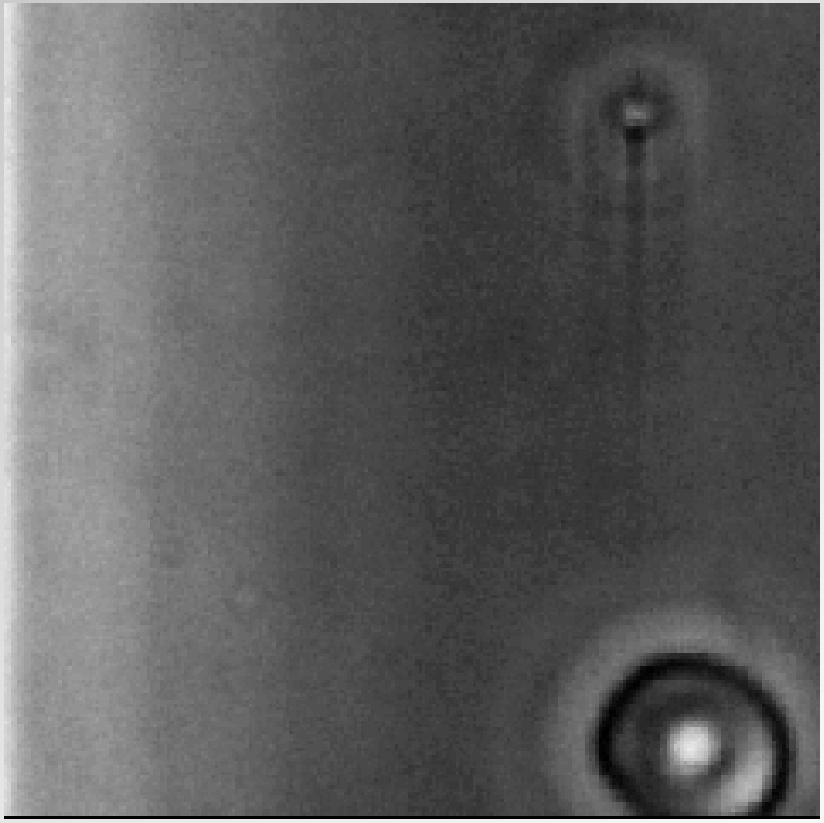
J. Guck et al. "The optical stretcher: a novel laser tool to micromanipulate cells" Biophys. J. 81,767, 2001

Stretching - 1



Hypotonic buffer: Swollen Red Blood Cell

Stretching - 2



Isotonic buffer: Red Blood Cell

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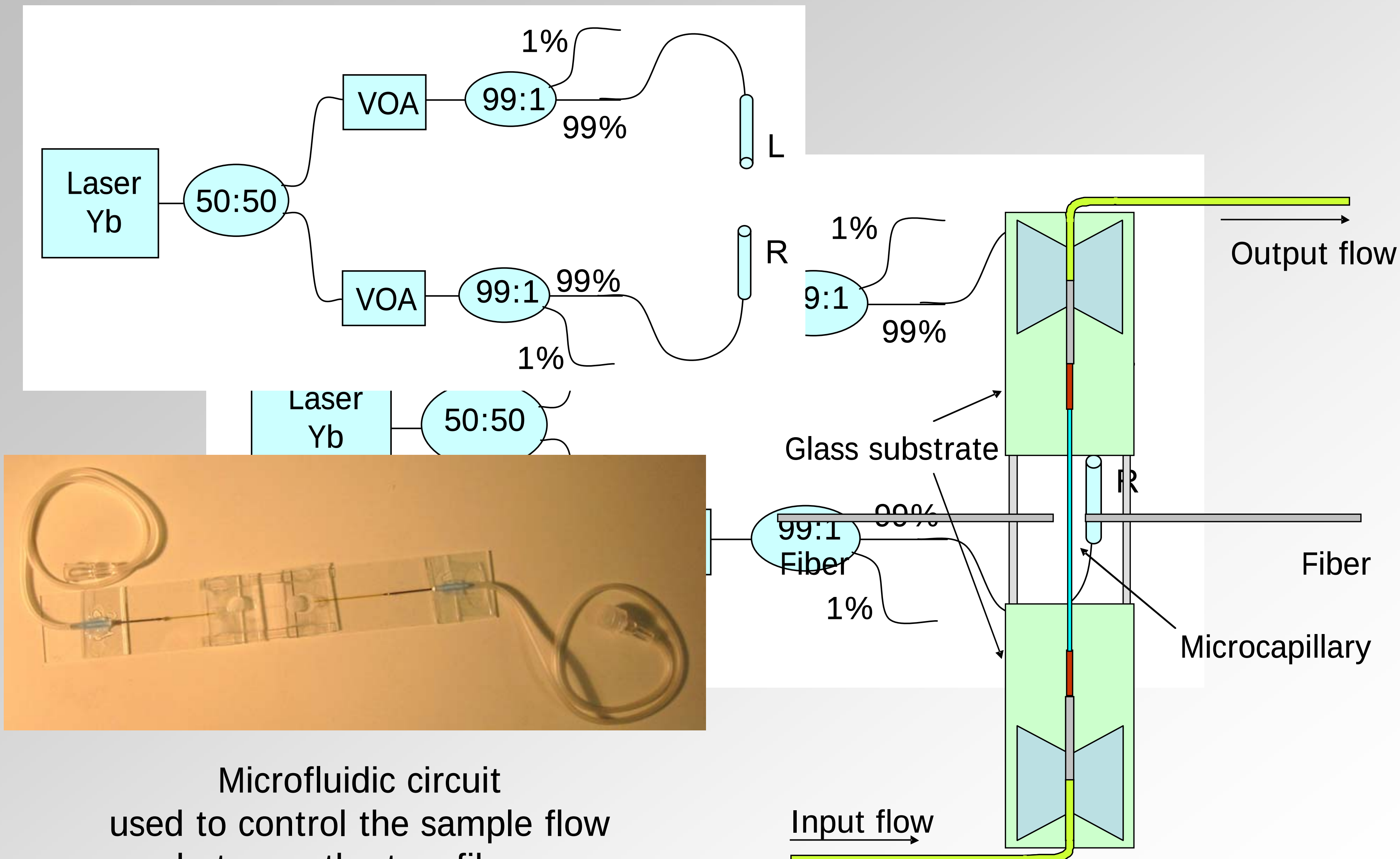
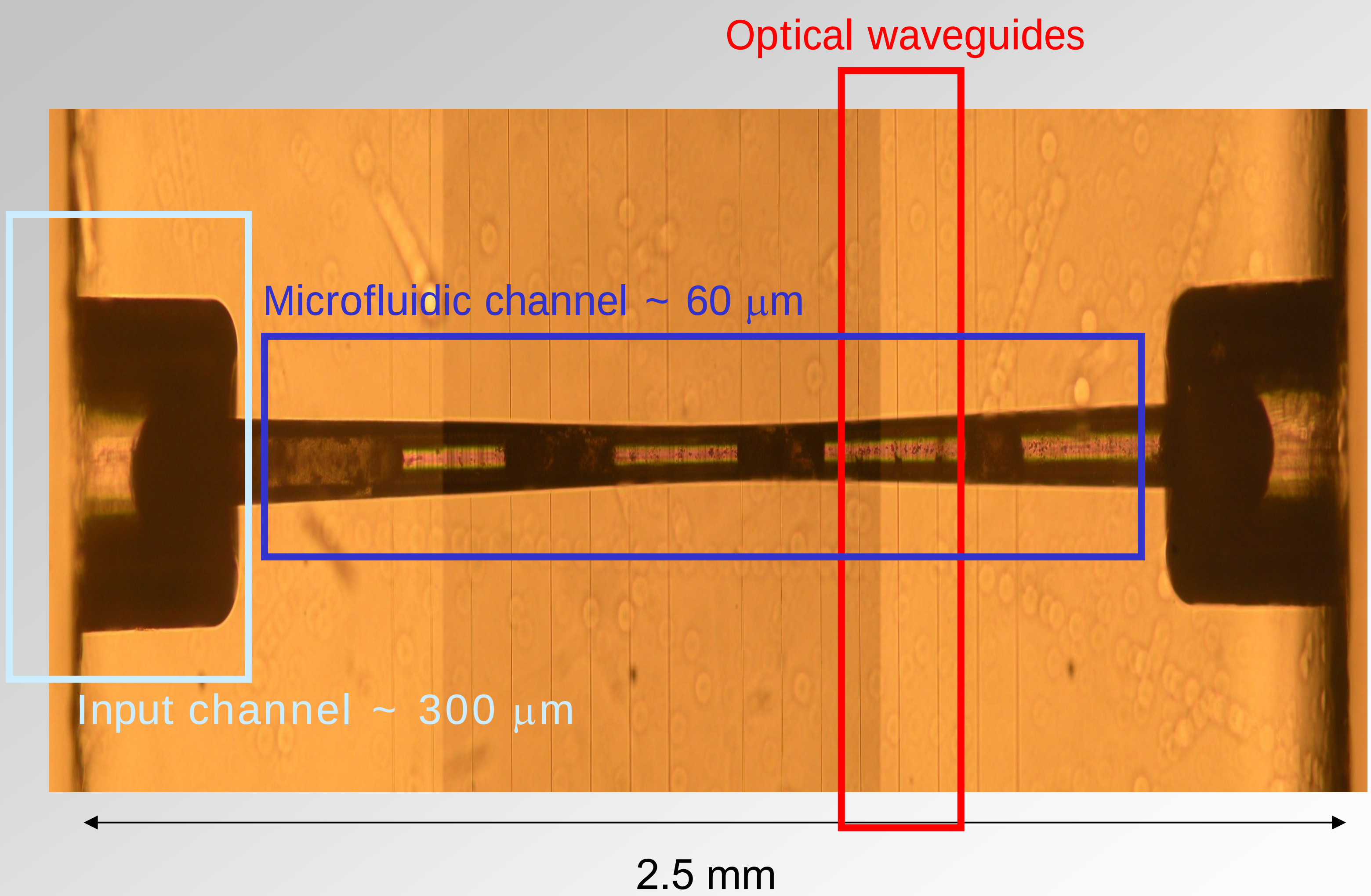
All-fiber experimental setup



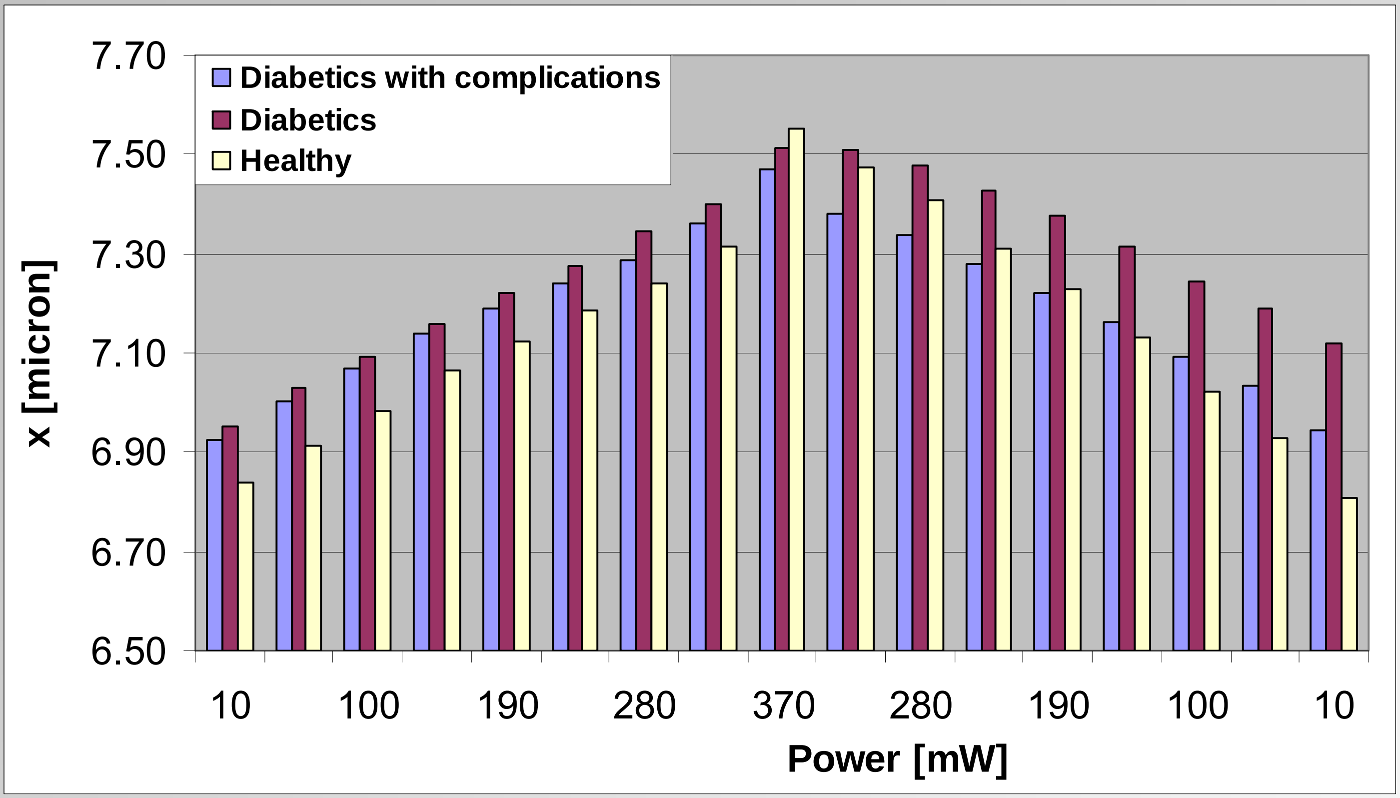
Microfluidic circuit
used to control the sample flow
between the two fibers

Integrated experimental setup



In collaboration with Politecnico di Milano - CNR

Experimental results - Diabetes



Diabetic patients with complications: 7 samples (50 RBCs each)

Diabetic patients: 4 samples (50 RBCs each)

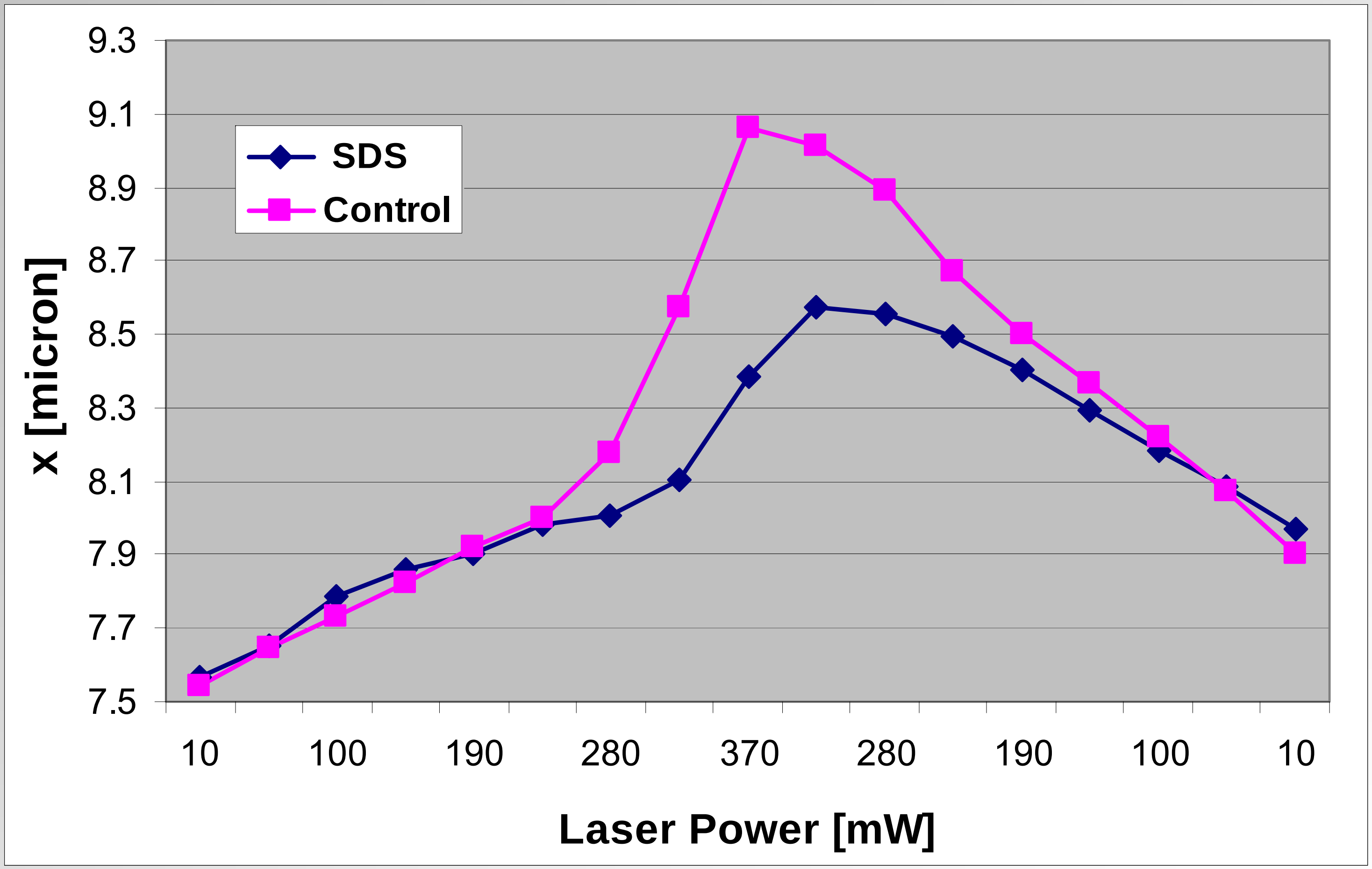
Healthy patients: 4 samples (50 RBCs each)

Slope of interpolating curves

	Increasing Power	Decreasing Power
Healthy	0.00115861	0.0013566
Diabetics	0.00096231	0.00077057
Diabetics with comp.	0.00091648	0.00088093

In collaboration with ASP IDR S. Margherita - Prof B. Solerte and coworkers

Experimental results – Rare Genetic Disease



In collaboration with Dipartimento di Patologia Umana ed Ereditaria - Prof C. Danesino and coworkers

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Conclusions

- Optical forces are a powerful tool for non-invasive cell manipulation
- A Fiber Optic Tweezers capable of trapping and manipulating single cells has been realized
- An Optical Stretcher apparatus capable of trapping, manipulating and stretching particles has been realized
- Very preliminar results on different diseases shows a slightly reduced deformability of patients' red blood cells with respect to healthy controls
- Optical stretching can be combined to optical diagnostic techniques for cell sorting and analysis
- We believe that integrated optics approach could provide reliable and handy tools for real applications

Thanks for your attention!

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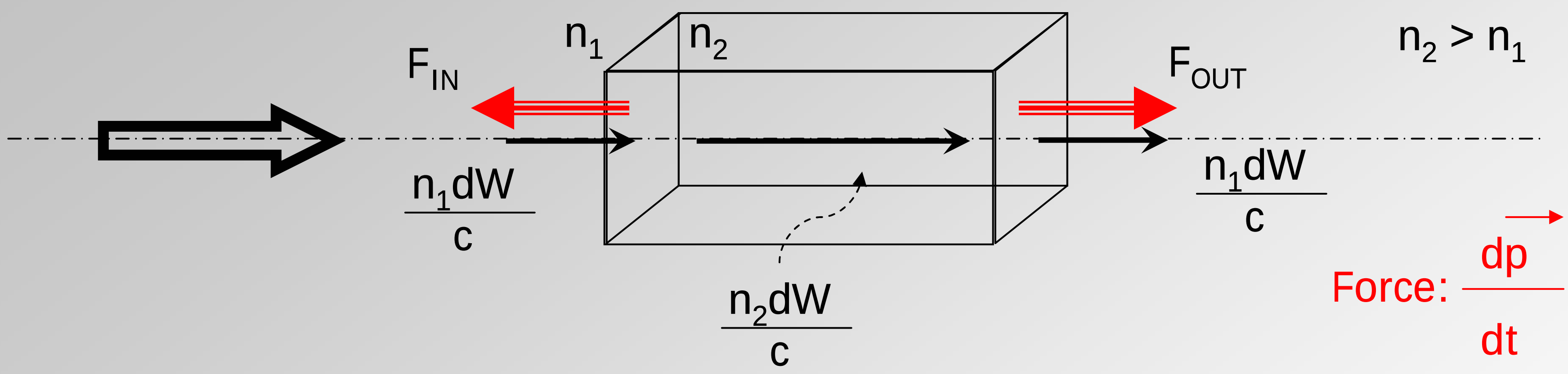
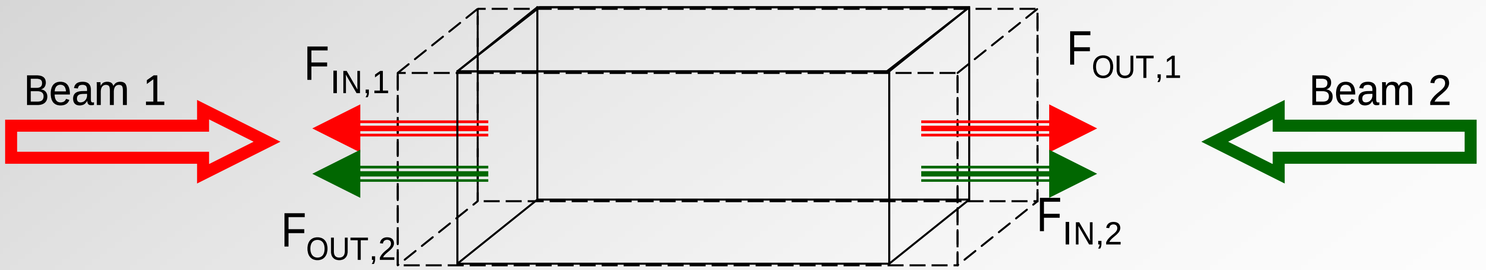
Optical Stretching: working principle

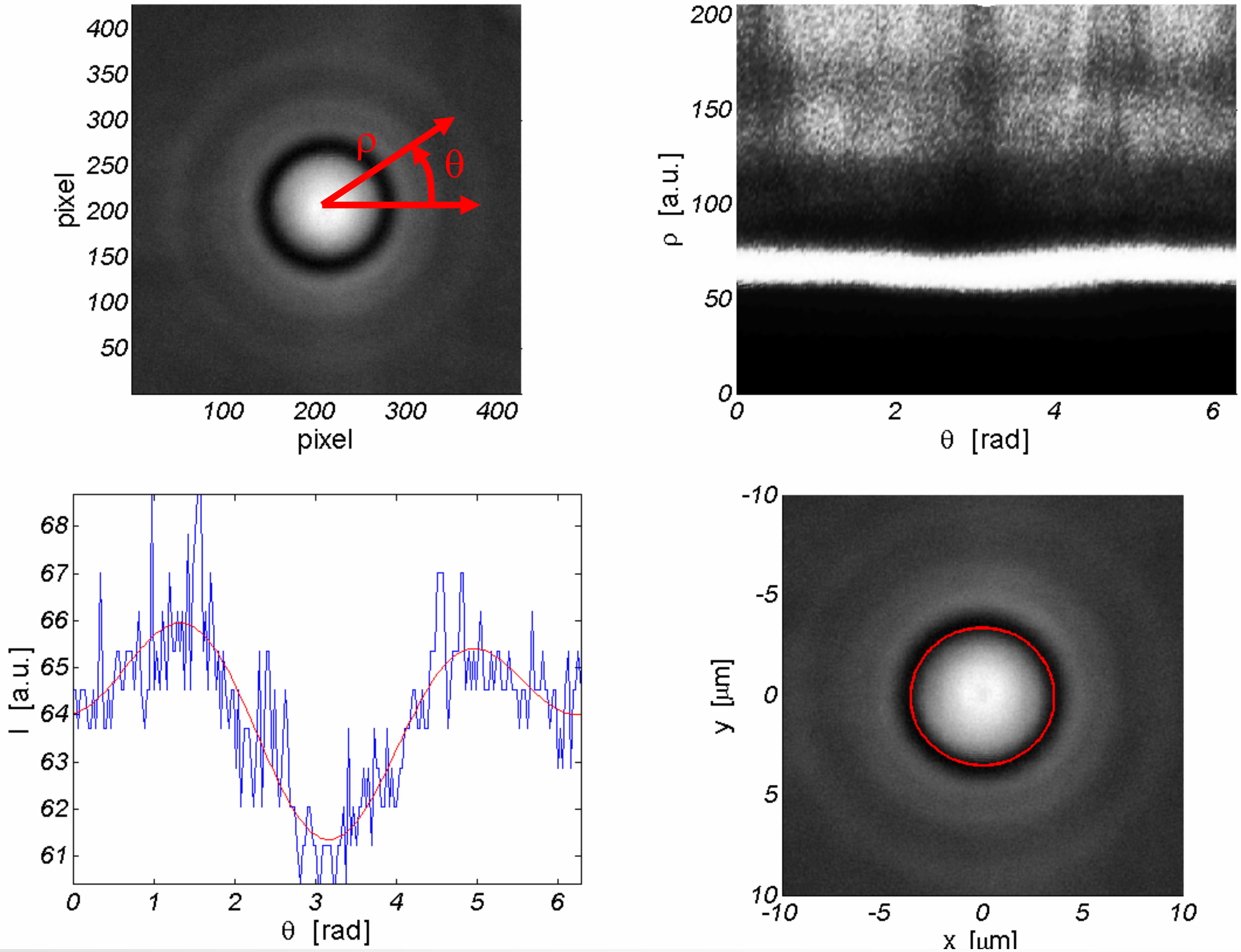
Single ray impinging on a particle



Two counterpropagating beams

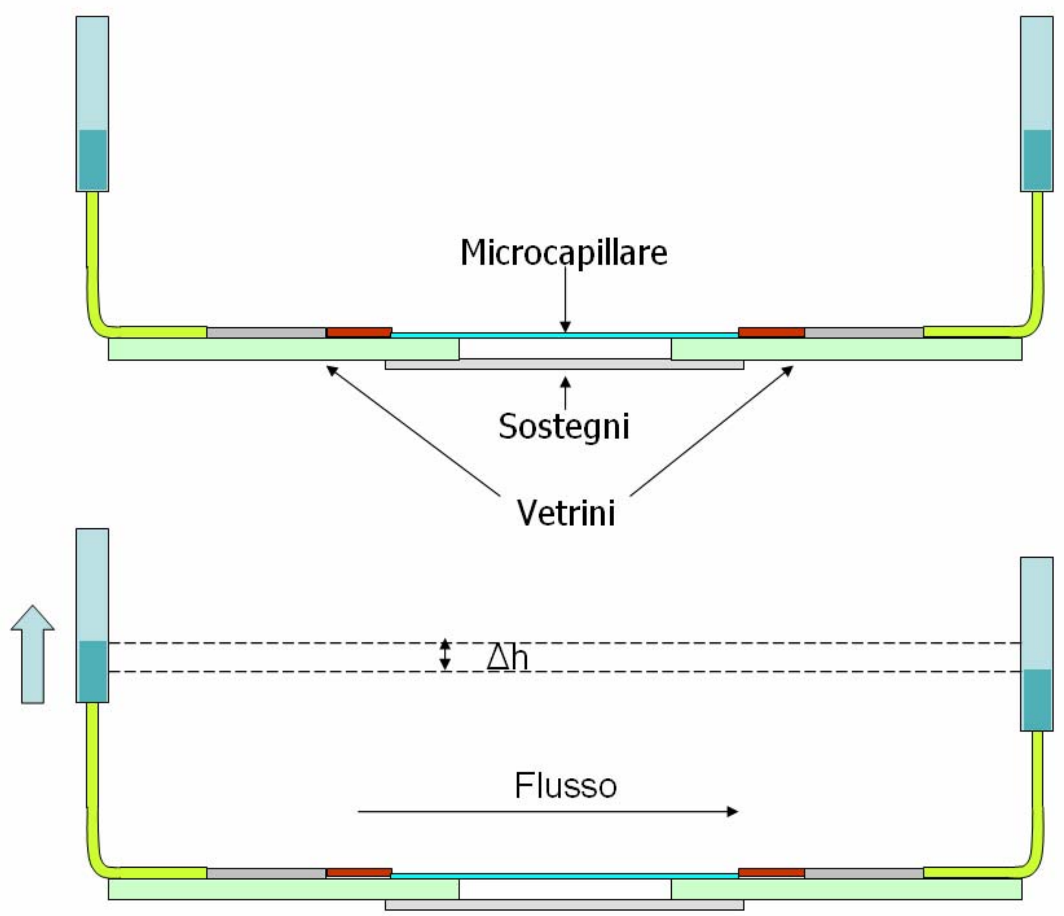


Deformation analysis



REALIZZAZIONE DELL'APPARATO

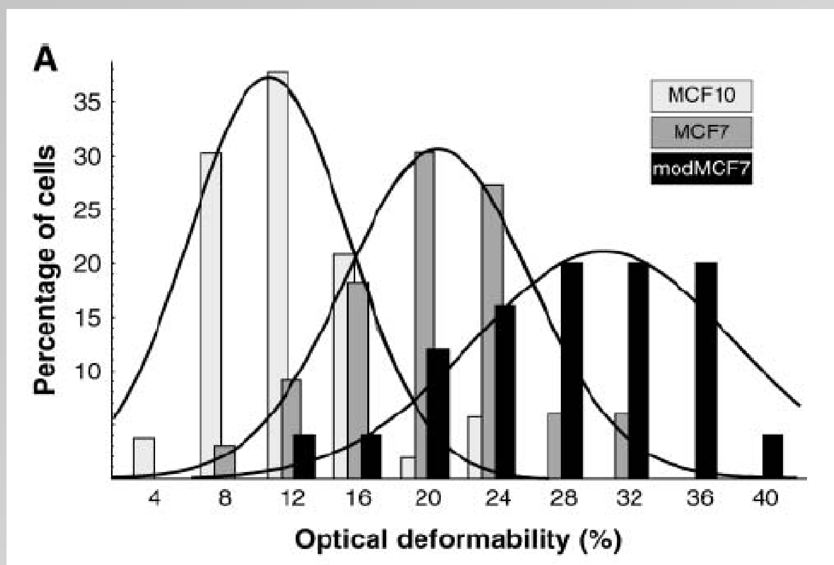
Tecnica per il controllo del flusso



Alzando, con un traslatore micrometrico, di Δh uno dei due tubicini si produce il flusso di una quantità di soluzione pari a $S\Delta h$, dove S è la sezione del tubicino

Application in biology

Investigation of the viscoelastic properties through the application of intense optical forces, able to cause a significant deformation of the cytoskeleton whose degree of deformability is altered by many diseases and provides a marker of the cell status



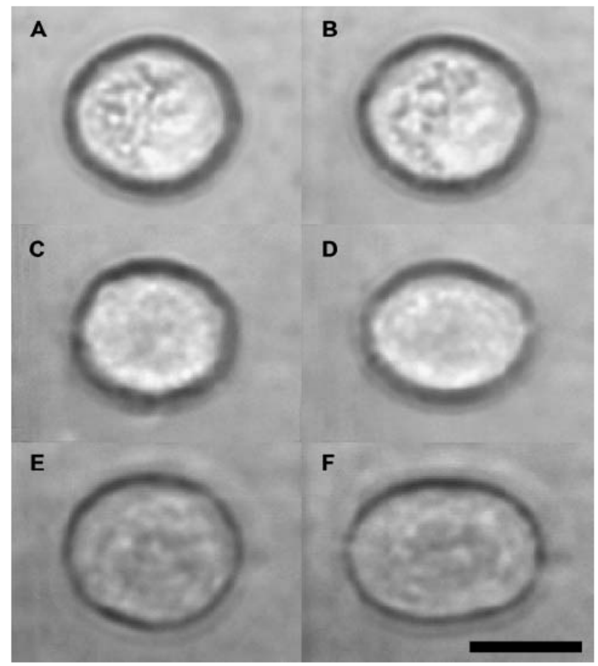
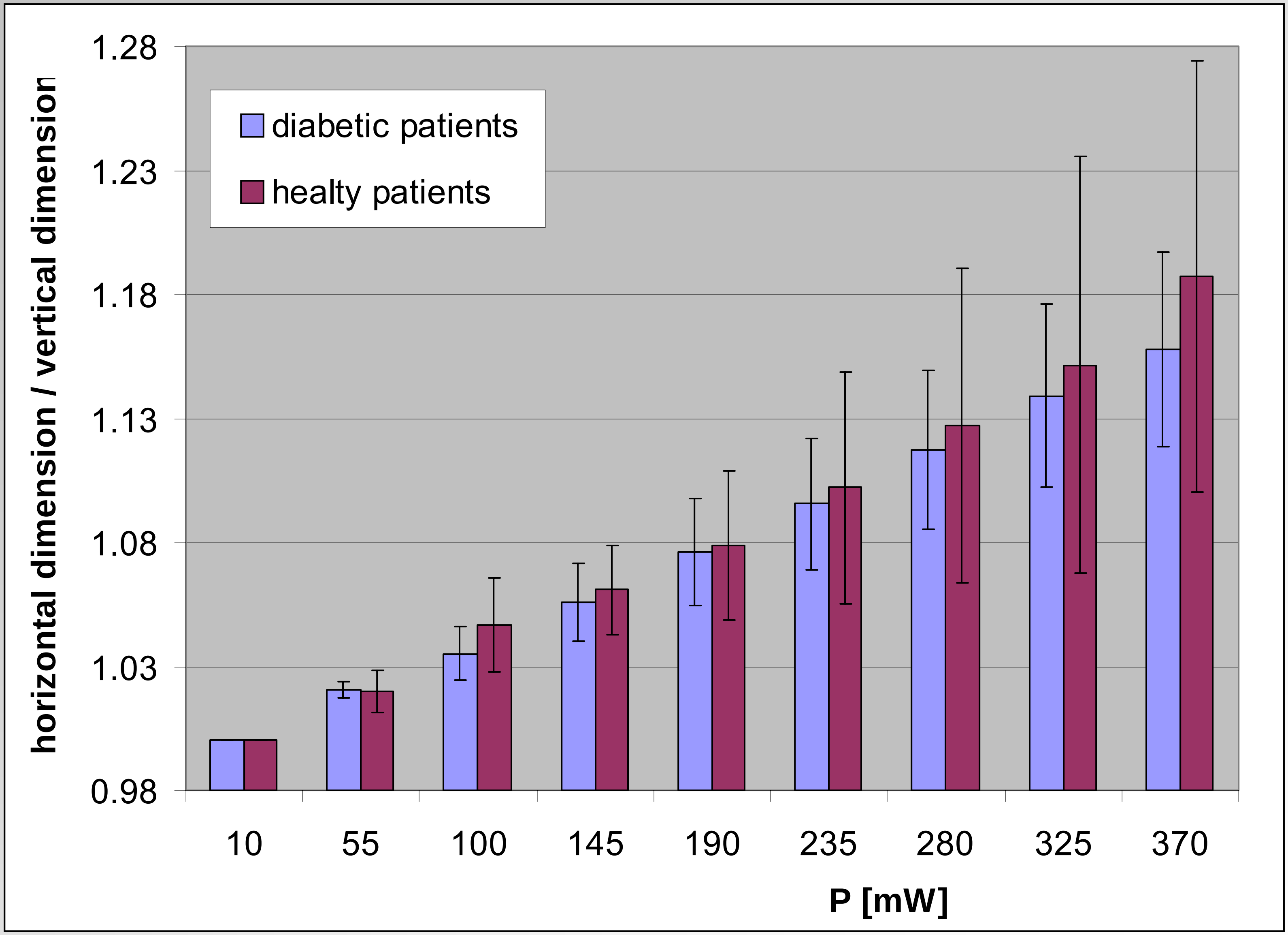
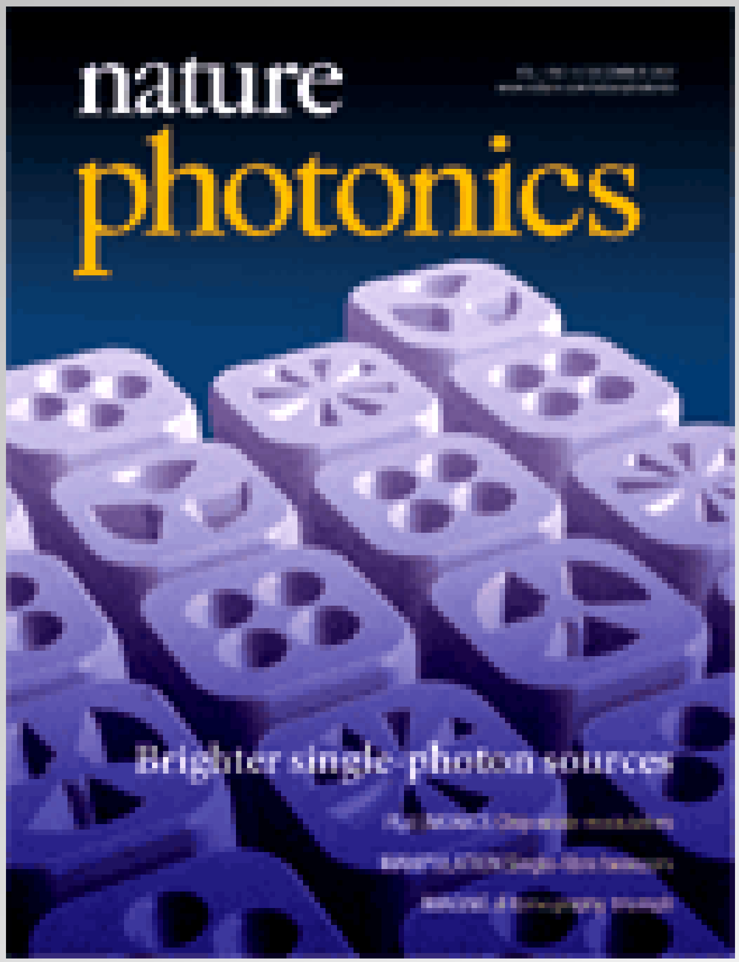


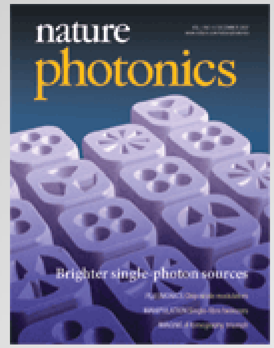
FIGURE 6 Typical examples of the stretching of breast epithelial cells. The images in the left column are taken at an incident light power of 100 mW in each beam, which is sufficient for the trapping of the cells. At an incident light power of 600 mW (*right column*), the cancerous MCF-7 cells (*C* and *D*) deform more than the nonmalignant MCF-10 cells (*A* and *B*). The metastatic modMCF-7 cells (*E* and *F*) deform the most. The scale bar is 10 μm.

Deformation study on human breast epithelial cells:
- MCF-10 non tumorigenic epithelial cells
- MCF-7 human breast cancer cell non-methastatic
- Mod-MCF-7 methastatic cell

- J. Guck et al "Optical Deformability as Inherent Cell Marker for Malignant Transformation and Metastatic Competence" Biophys. J. 88, 3689, 2005.


diabetic patients
healty patients
horizontal dimension / vertical dimension
1.28
1.23
1.18
1.13
1.08
1.03
0.98
10
55
100
145
190
235
280
325
370
P [mW]


nature
photonics
Brighter single-photon sources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Brighter single-photon sources