The Abdus Salam
International Centre for Theoretical Physics

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Educational, Scientific and
Cultural Organization

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International Atomic Energy Agency

2037-15

Introduction to Optofluidics

1 - 5 June 2009

Optical manipulation of living cells in fluids

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The Abdus Salam International Centre for Theoretical Physics

Optical manipulation of living cells in fluids

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www.tasc.infm.it/research/**om**/scheda.php

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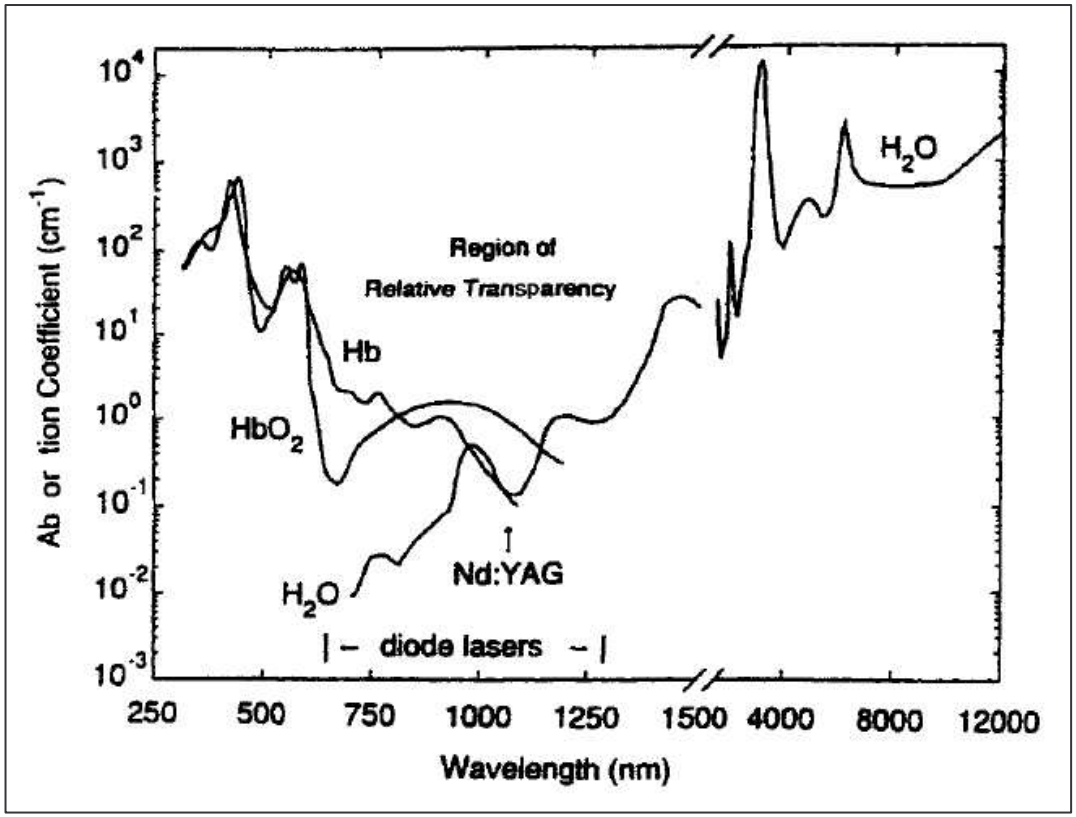
Consiglio Nazionale delle Ricerche - Istituto Nazionale per la Fisica della Materia

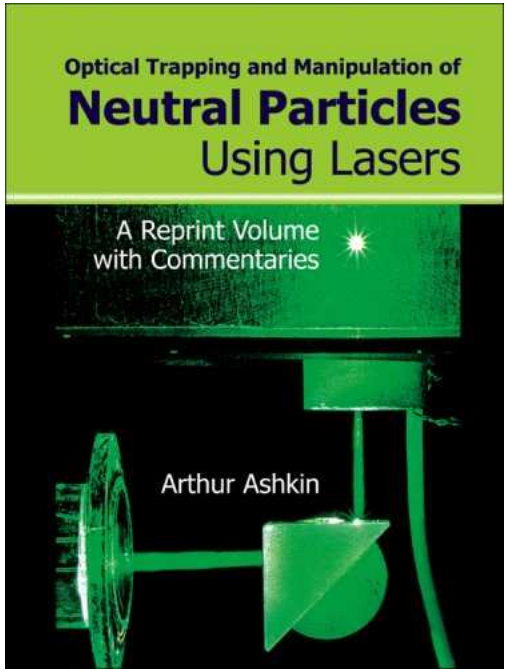
OUTLINE

- **Damage-free trapping of living cells**
- **Examples Cell mechanical stress: (neurons, HeLa, RBC)**
  1 apply pN forces
  2 measure pN forces measurements (neurons, HeLa, RBC)
- **Change the cell environment, drug delivery vectors (functionalized beads, liposomes) – short discussion**
- **Conclusions/Discussion**

There is more discussion on cell mechanics in the pdf file available to be downloaded from ICTP - I2O web site.

Does the laser trapping beam damage the cell ?



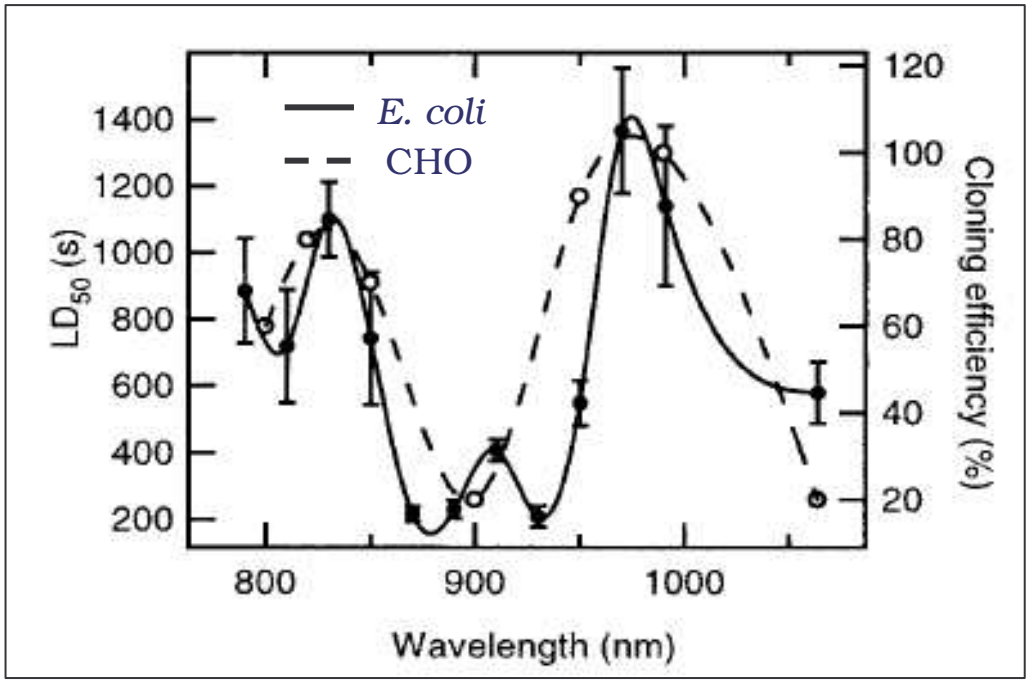


Plot of the optical absorption coefficients of hemoglobin (Hb), oxyhemoglobin (HbCh) and water versus the wavelength.

Minimum cell damage: Infrared lasers

A. Ashkin, J. M. Dziedzic and T. Yamane, Optical trapping and manipulation of single cells using infrared laser beams, *Nature* **330,** 769-771 (1987).

Quantitative evaluation of photodamage ?

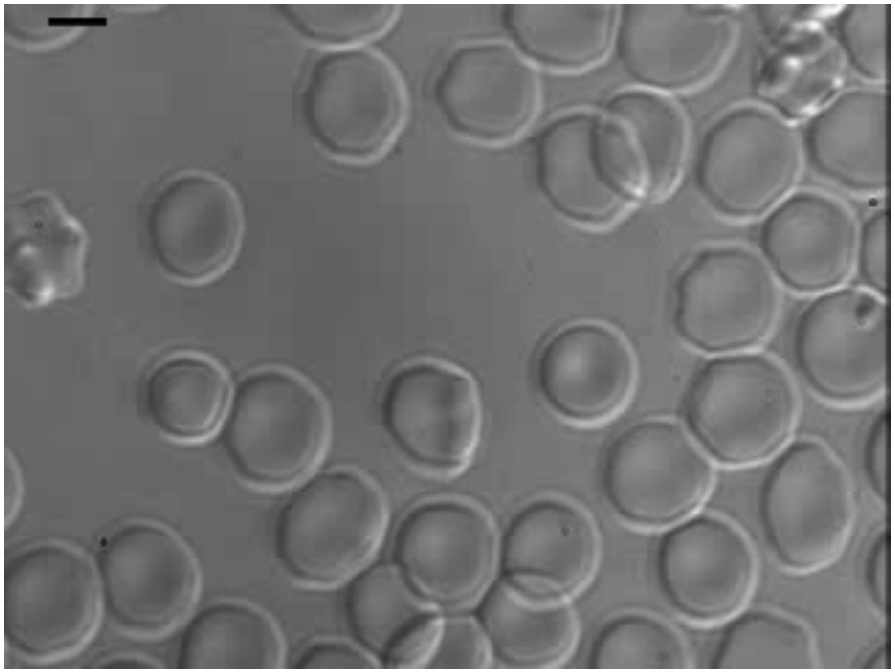


The wavelength dependence of photodamage in *E. coli* compared to Chinese Hamster Ovarian cells.

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

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

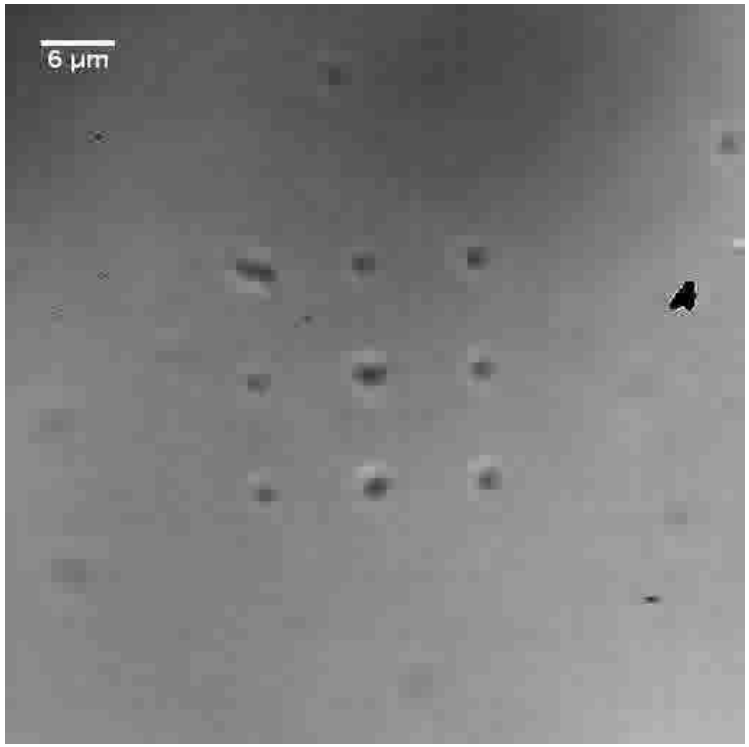
Single cell trapping



movie

Red Blood Cell RBC single cell

Cell array and sorting



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

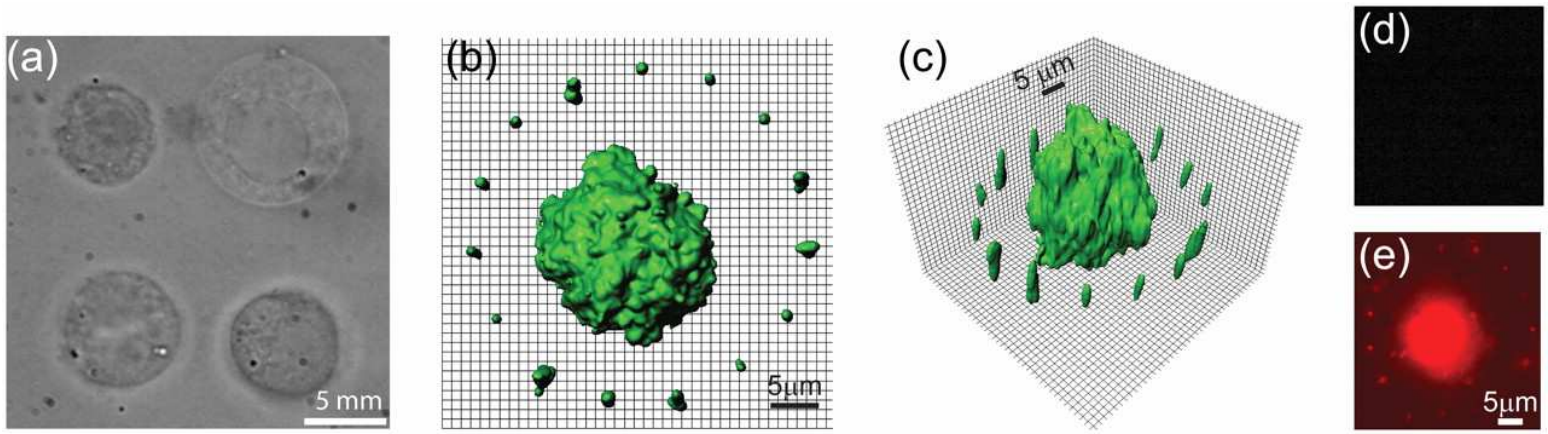
E. Di Fabrizio, D.Cojoc *et al*, Microscopy Research and Technique ***65***, 252 (2004)

Permanent assembly of 3D living cell microarrays

- The array is first configured by multiple traps created with AOD and SLM

- The position of the cells is fixed permanently using a photopolymerizable hydrogel

PEDGA = Polythylene glycol diacrylate



Heterotypic microarray of Swiss 3T3 mouse fibroblast and *P. aeruginosa* bacteria.
(a) Swiss 3T3 mouse fibroblasts trapped in a 2 x 2 2D array
(b,c) False-color isosurface reconstructions obtained from a confocal image of a Swiss 3T3 cell surrounded by a ring of 16 *P. aeruginosa.*
(d,e) Viability assay of the same heterotypic microarray showing an image obtained by exciting propidium iodide labels with 488 nm. The lack of red fluorescence in (d) indicates viability, but after killing the cells with ethanol the fluorescence is intensely red (*e*).

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

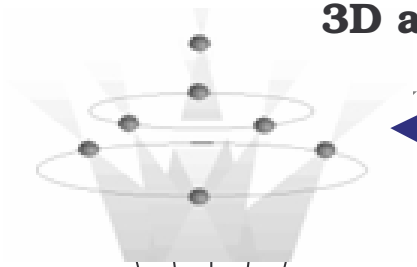
Multiple trapping

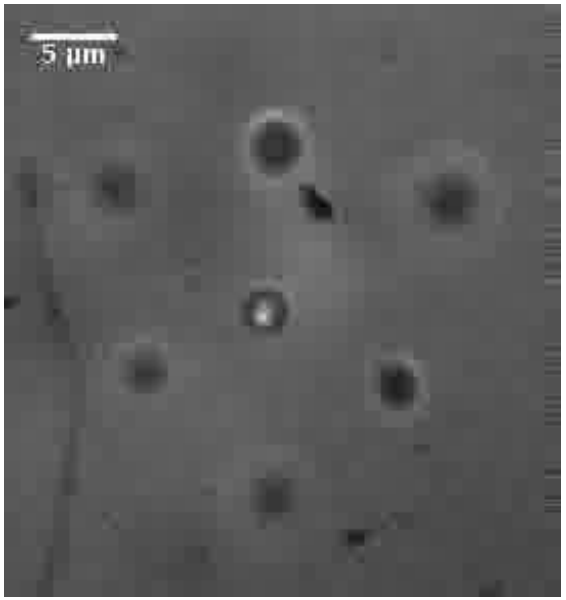
by means of Diffractive Optical Elements (DOE)
implemented on a Spatial Light Modulator (SLM)

Working volume:

3D array (cage)

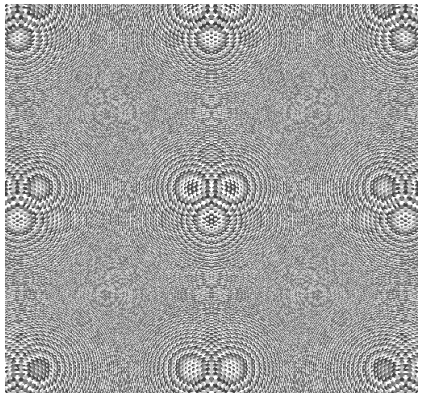
3D array

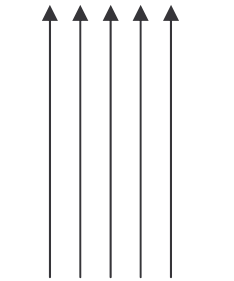




movie

DOE

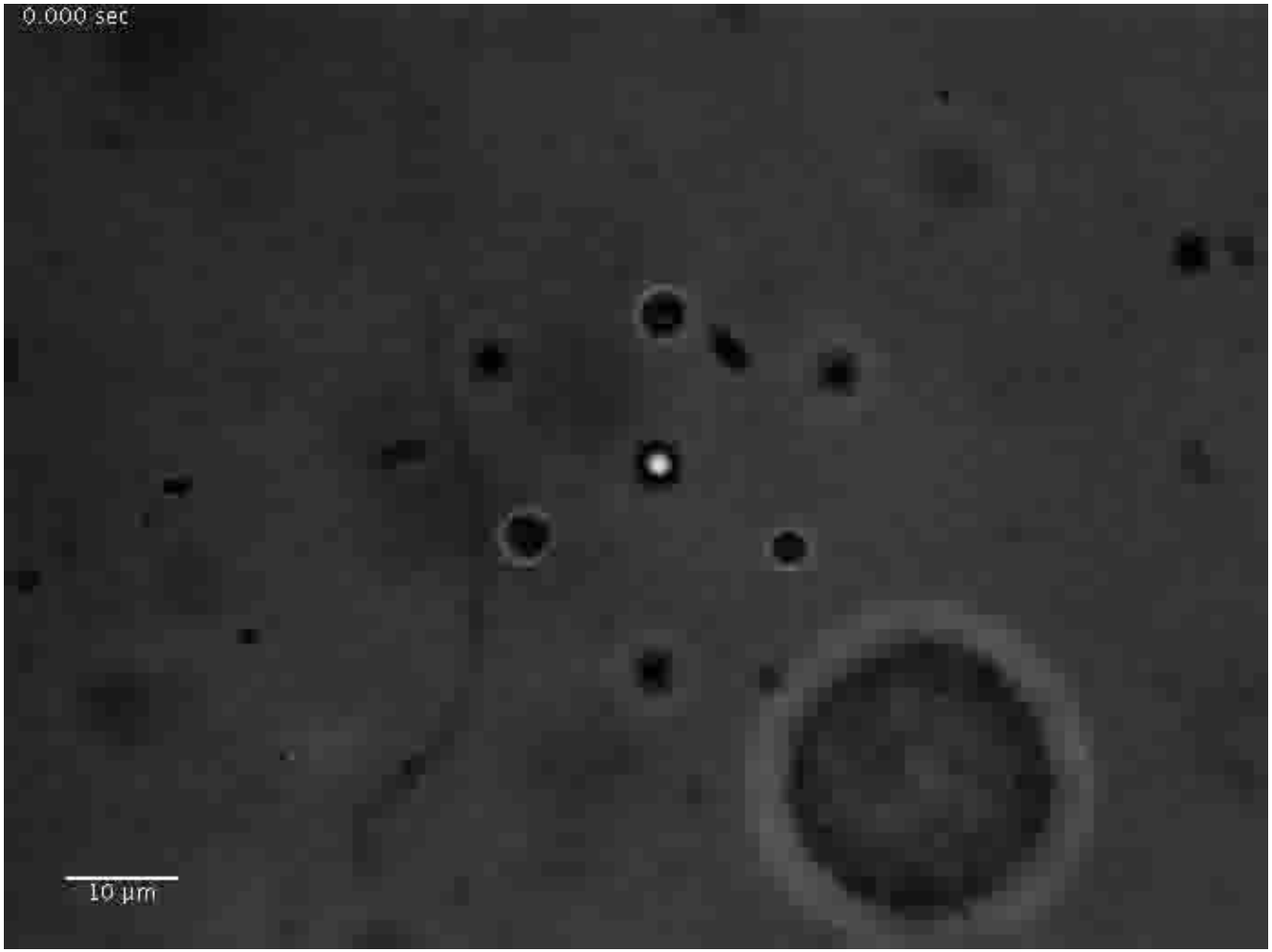




SLM

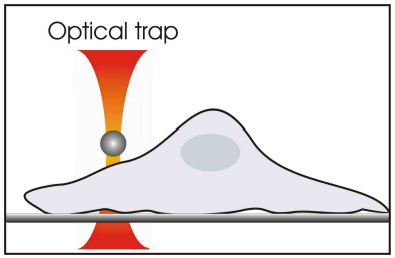
Input Wave
(Gaussian)

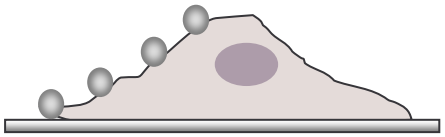
Mechanical stimulation of cells with pN forces



HeLa cell under the dynamic cage **movie**

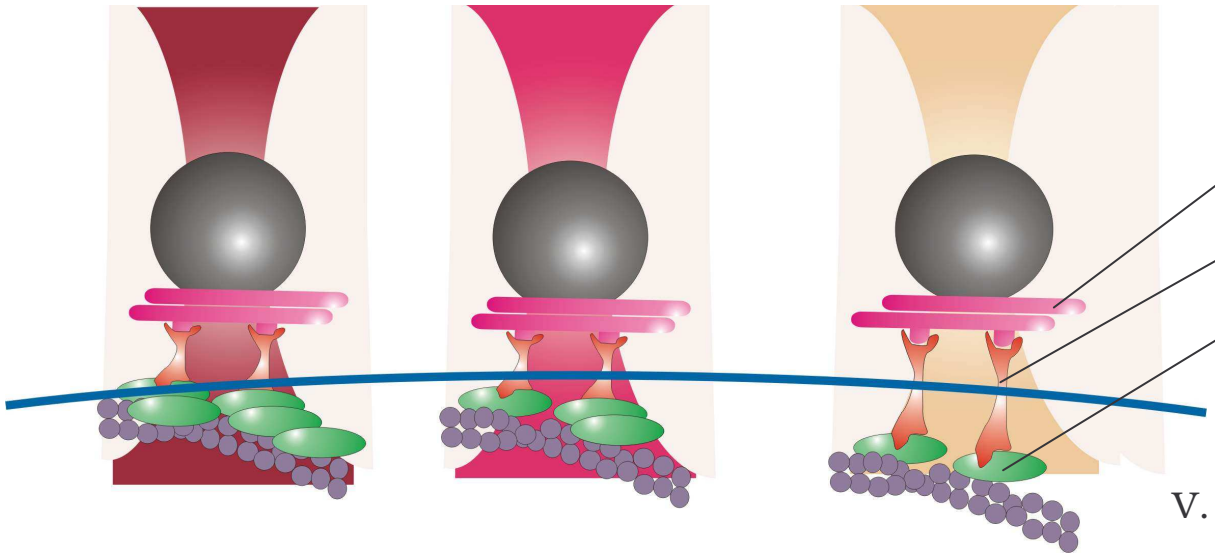
With the optical tweezers technique one can control very precisely the mechanical stimulation at the level of single or multiple adhesion sites





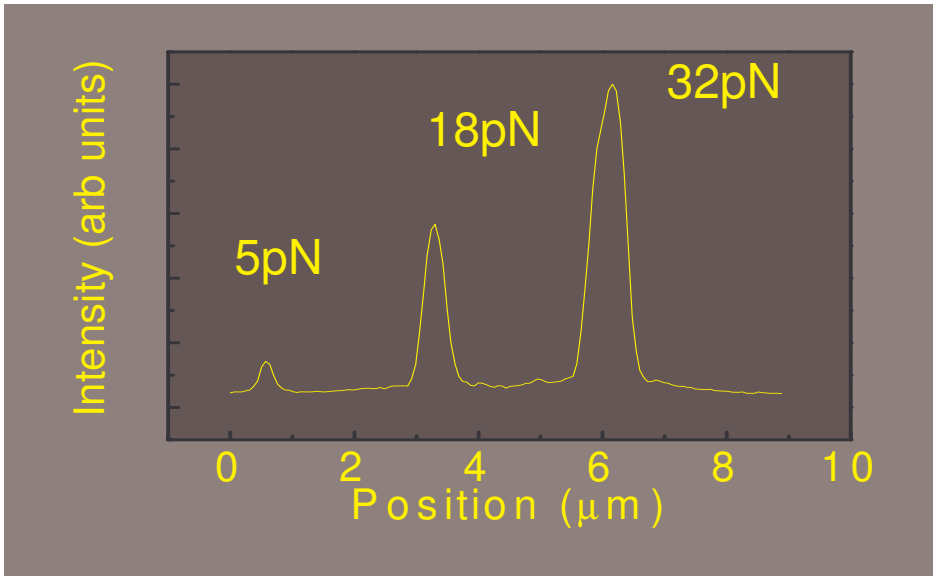
The multi force optical tweezers is combined with an epi-fluorescence microscope to monitor vinculin recruitment as a function of applied forces.

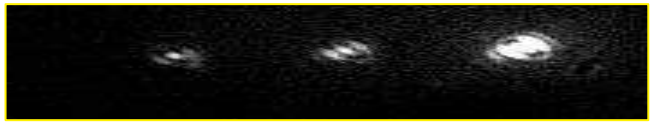
Fibronectin coated beads are manipulated on the dorsal surface of Vin-GFP transfected HeLa cell.



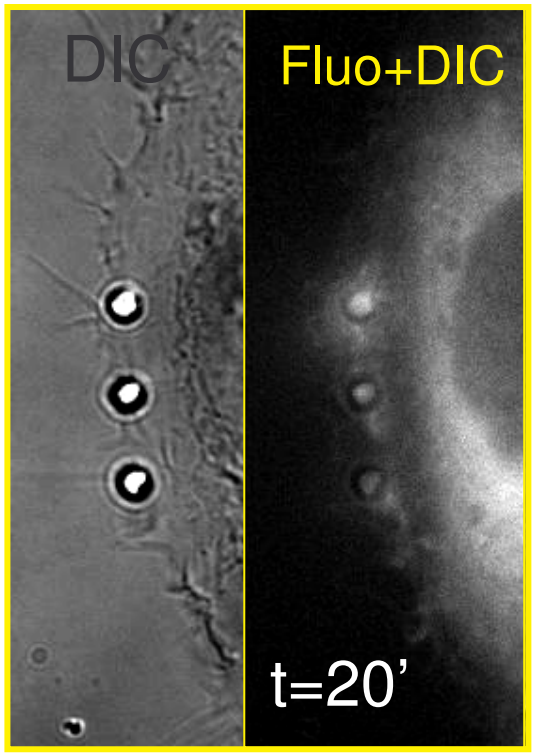
V. Emiliani *et al*, SPIE (2006)

Trap strength



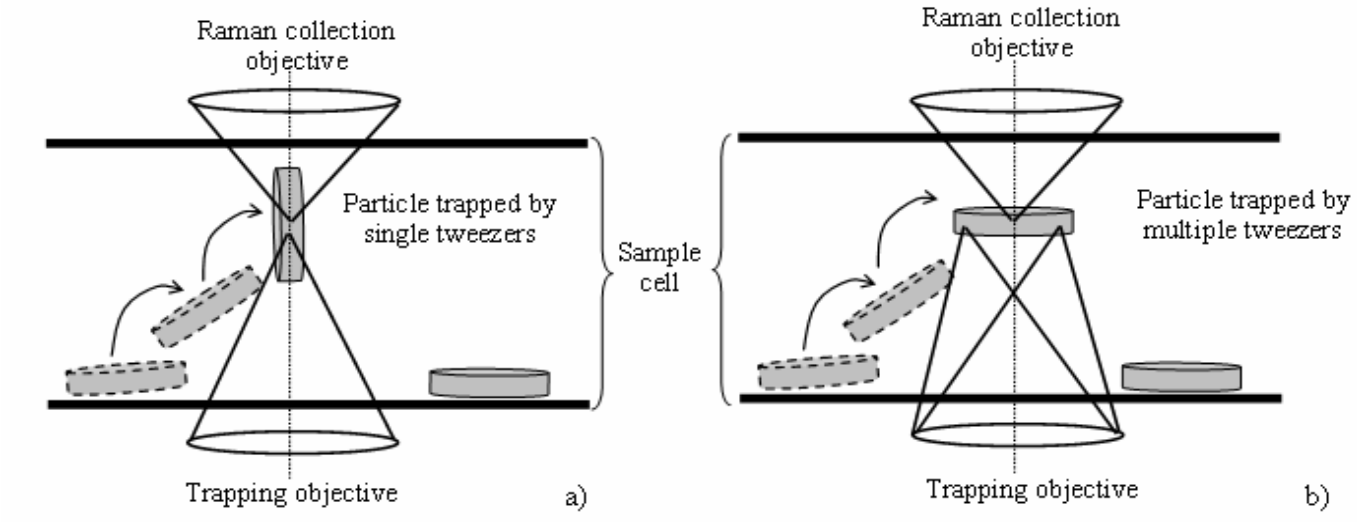


Vinculin recruitment

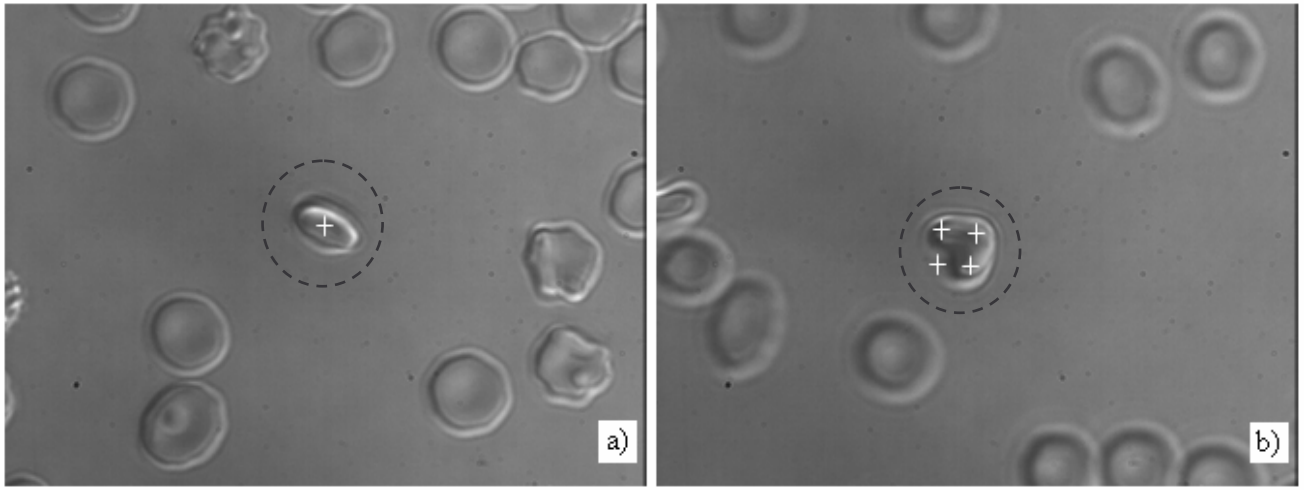


V. Emiliani *et al*, SPIE (2006)

Changing the orientation of a RBC for microRaman mapping the cell



Orientation of a RBC trapped by:
single optical tweezers a) and **four** optical tweezers b)



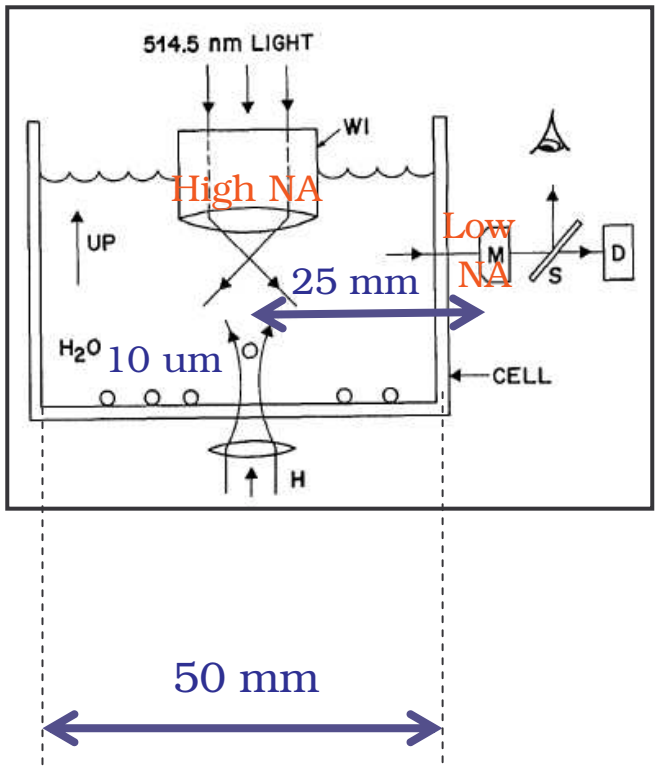
Raman imaging

→ **(confocal microscope + cell scanning)**

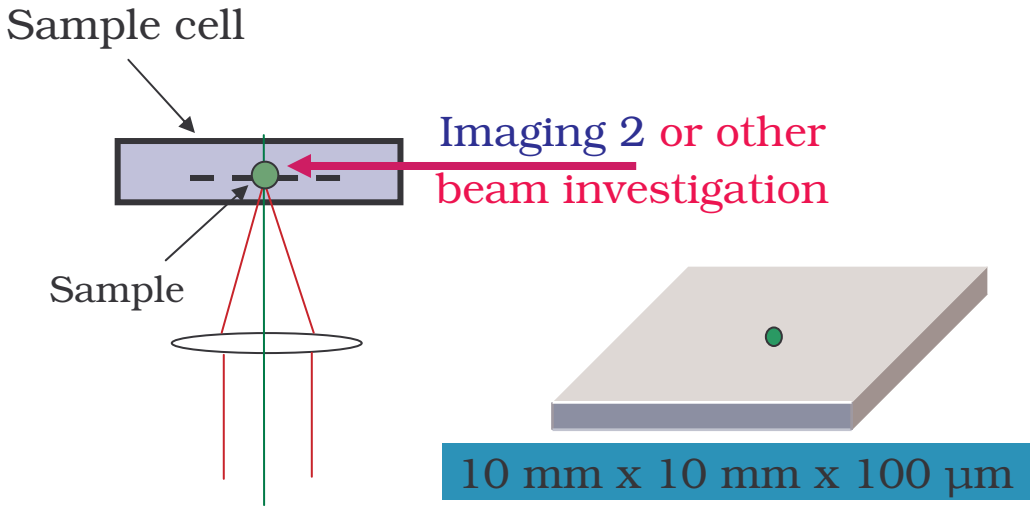
D. Cojoc *et al*, *SPIE* **5930**, 64, (2005)

Double view imaging

Ashkin's sample cell



Sample cell modification

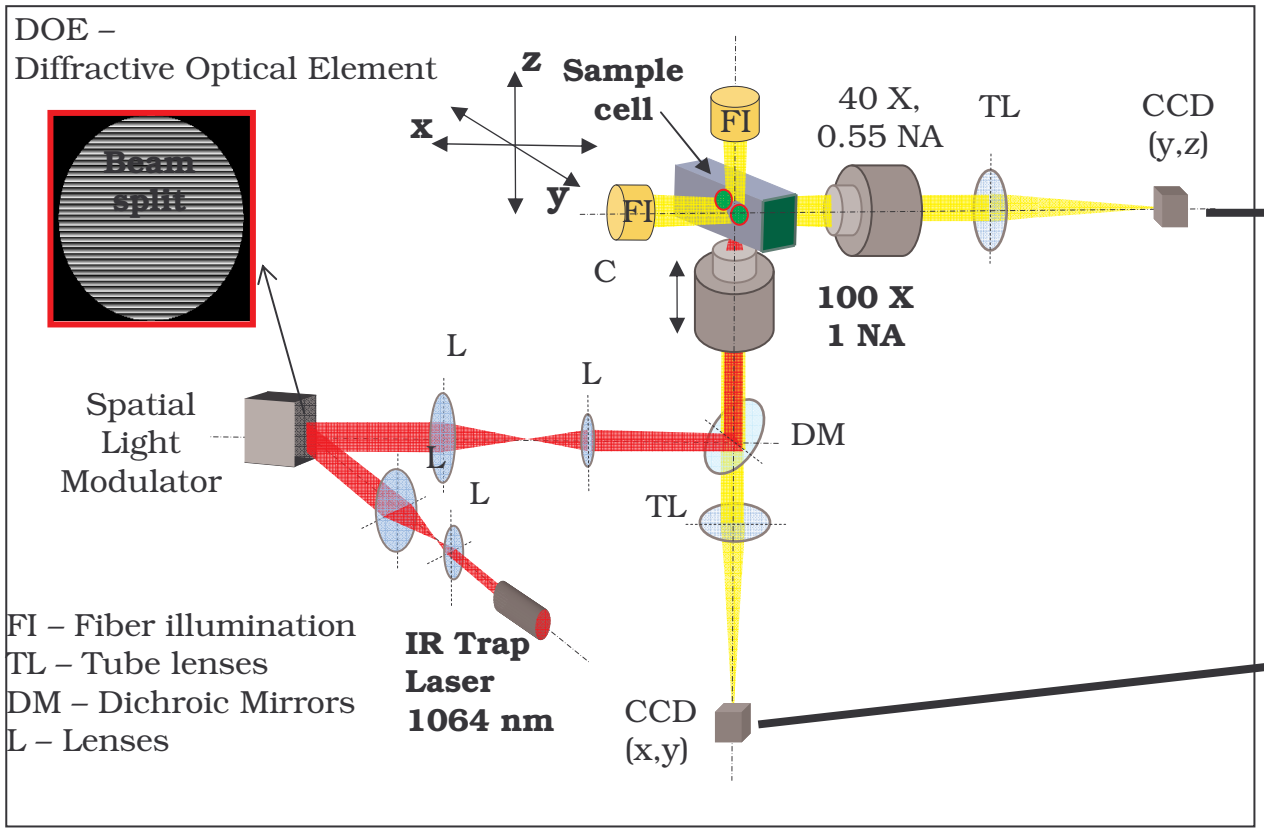


Optical tweezers + Imaging 1

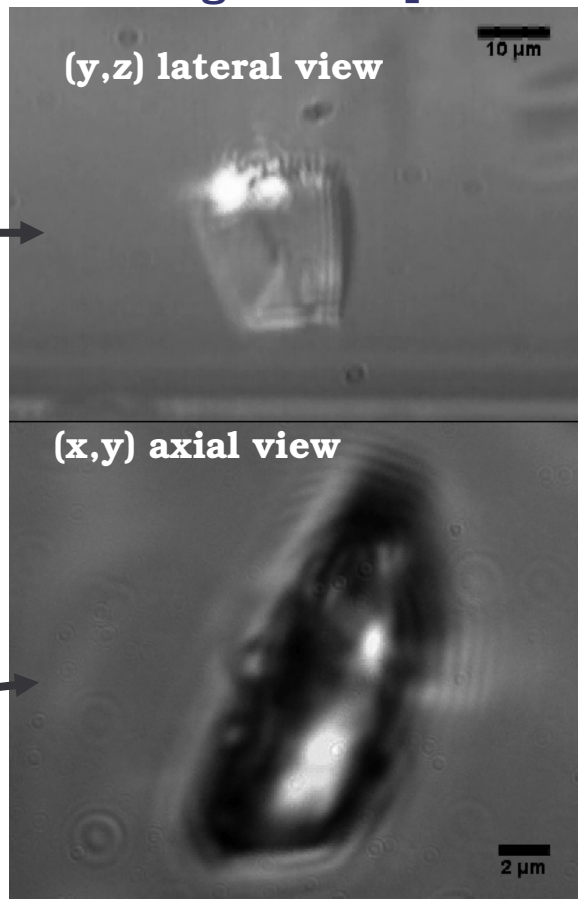
Capillaries as sample cells

Inner diameter <100 µm

Double view setup



Piece of glass with irregular shape



movie

Notice the different scale bar values

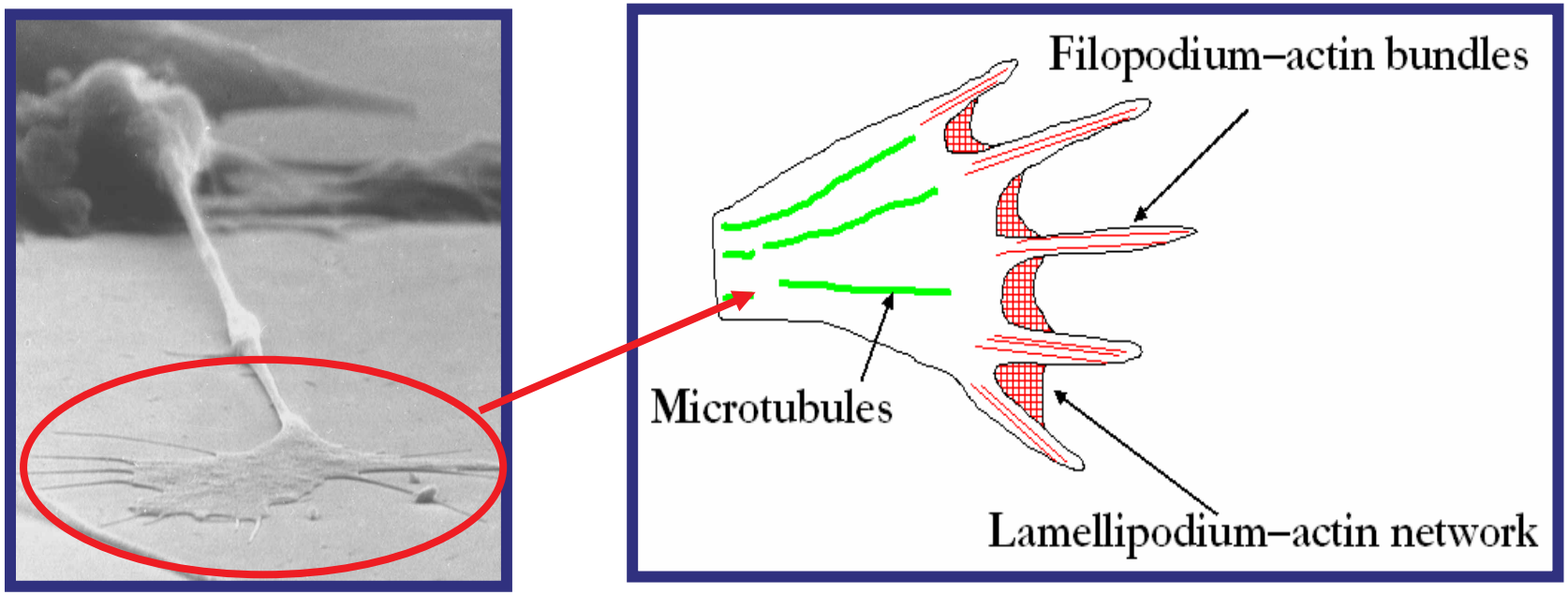
Force measurements

Motivation, goal, approaches

***Key determinant of axonal growth is* the growth cone:**

"They will adopt pre-determined directions and establish connections with defined neural or extra neural elements ... without deviations or errors, as if guided by an intelligent force ." *1890 RAMON Y CAJAL*

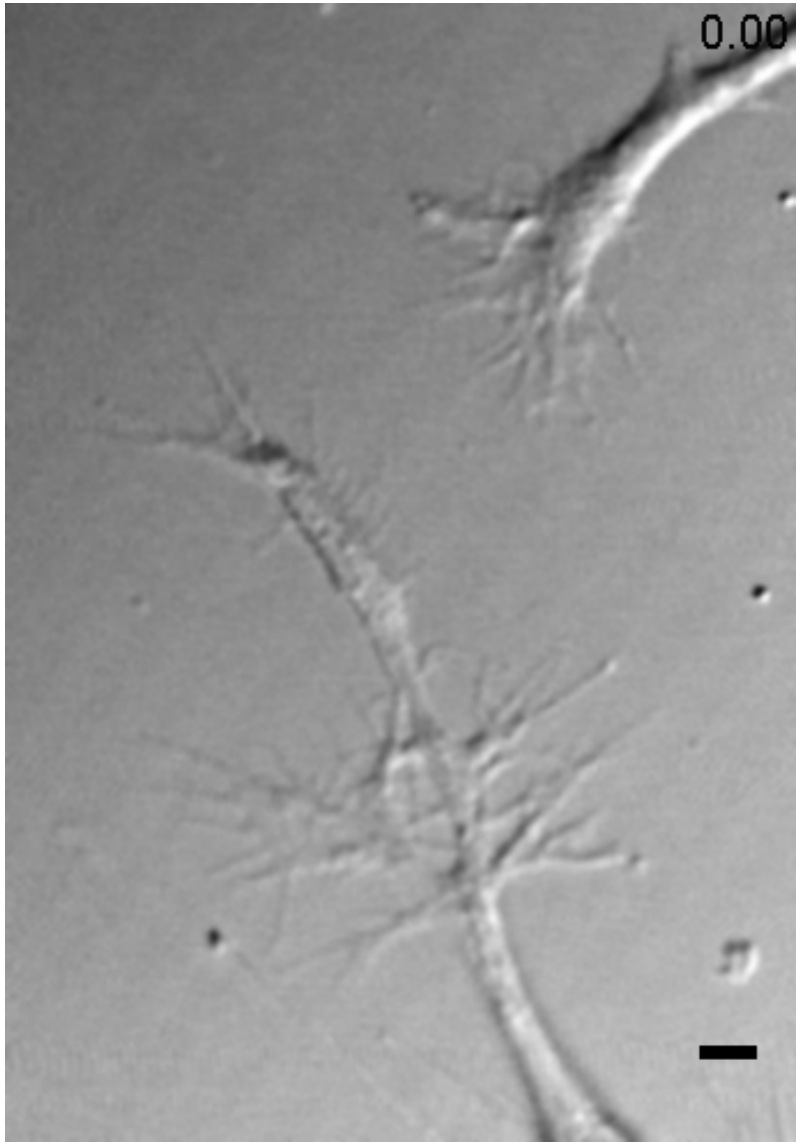
Structural elements of the growth cone



www.biology.lsa.umich.edu/research/labs/ktosney/

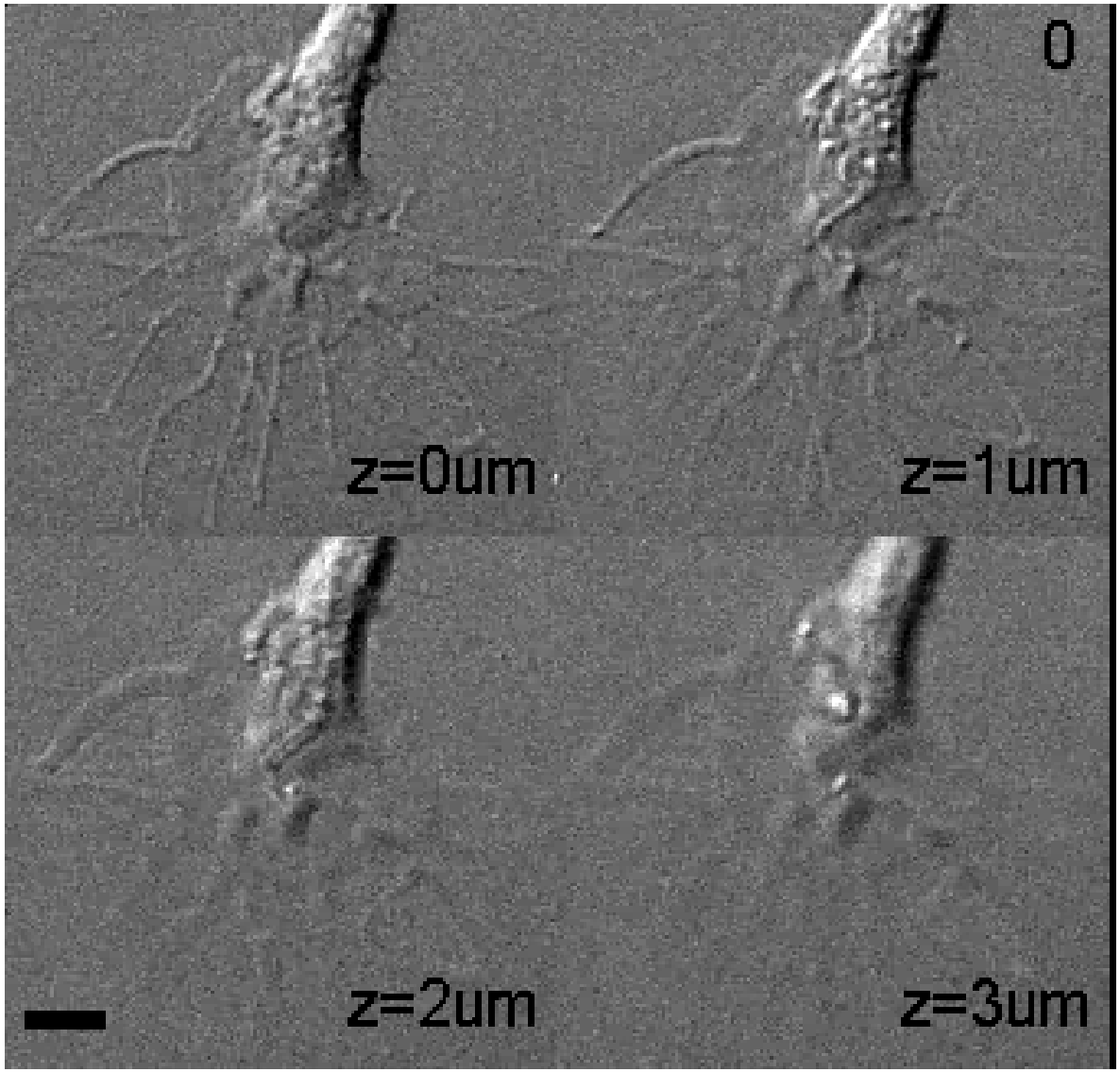
Collaboration with SISSA Trieste, Neurobiology Sector, Prof. V. Torre

Growth cones sensing and connecting



Scale Bar = 3 μm
Acquisition freq= 0.2Hz
Time in min.sec

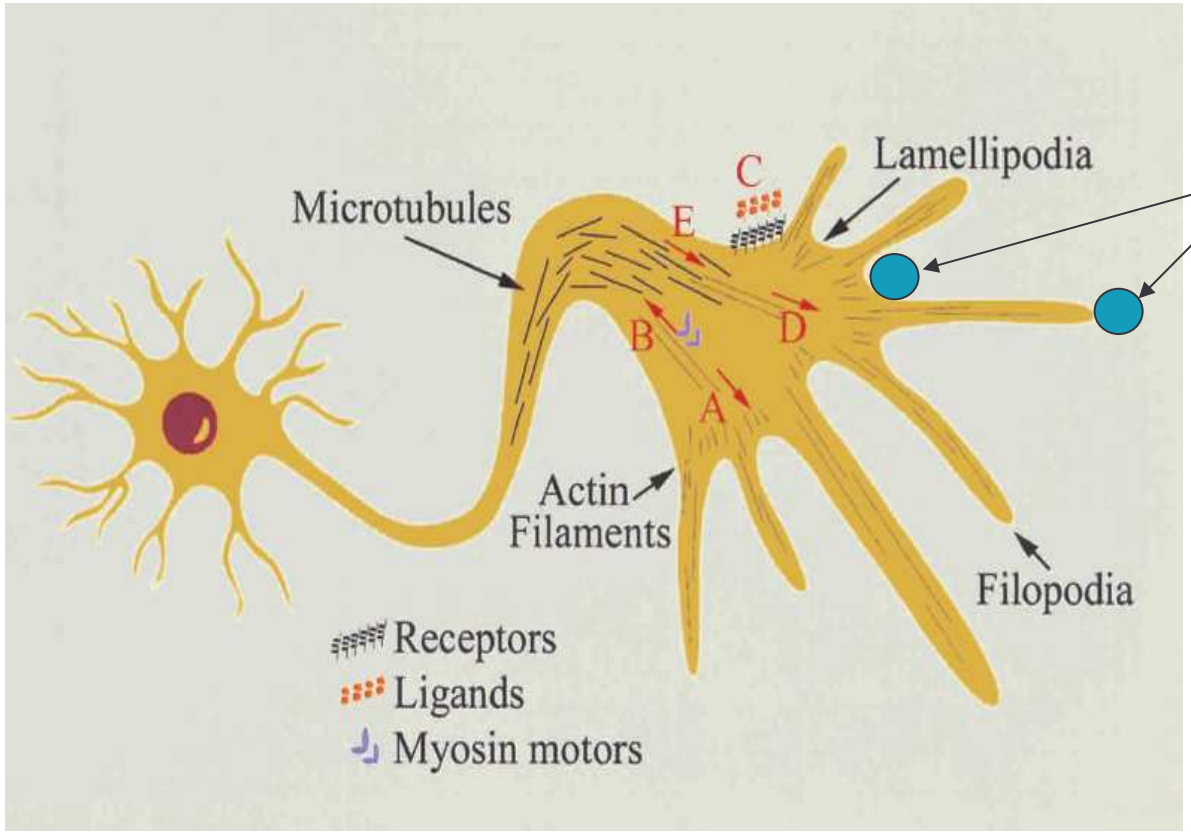
Growth cone confocal microscopy



Goal

measure the forces exerted by lamellipodia and filopodia

Experimental Approach



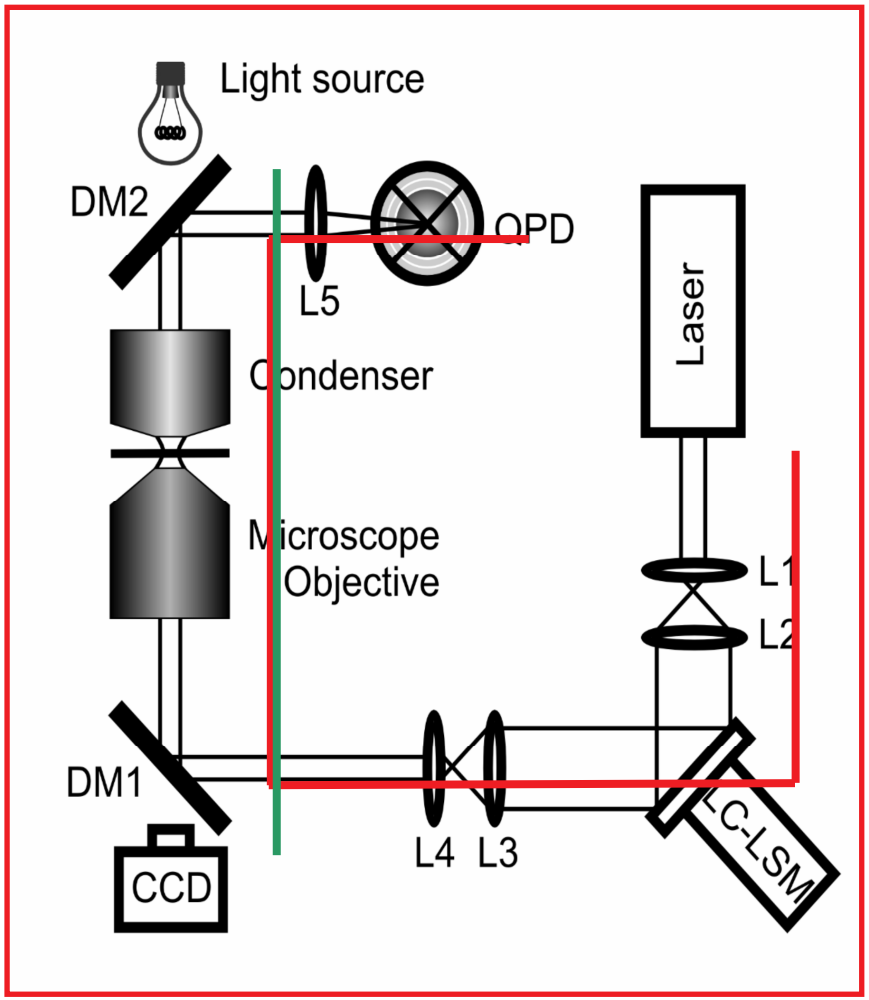
- Calibrate the trap
- Micro-beads trapped by IR laser and positioned in front of lamellipodia and/or filopodia
- Measure the fluctuations of the bead in the trap, due to its interaction with the motile structures, and convert them into forces.

J.L. Goldberg, Genes and Dev. ***17*** 941 (2003)

D. Cojoc *et al. PlosOne* 2007

Optical Tweezers setup

Includes Optical Manipulation and Force Spectroscopy



$$F = K \Delta x$$

K = stiffness of the trap (spring constant)

Δx = Displacement

LC-LSM: Liquid Crystal Spatial Light Modulator

CCD: Charged Coupled Device

L n: Lens 1,2,3,4,5

DM: Dichroic Mirror

QPD: Quadrant Photo-Diode

Bead position was determined by Back Focal plane (BFP) detection:

BFP of the condenser was imaged onto a QPD

D. Cojoc *et al. PlosOne* 2007

Experimental results

Neurons obtained from dorsal root ganglia (DRG), isolated from P0-12 rats and plated on poly-L-lysine-coated glass dishes. 48 hours after incubation in 50 ng/ml of nerve growth factor (NGF).

Features of our setup

Trap stiffness: 5-100 pN/µm

Resolution: ~10nm (1 nm)

Force range: 1-25 pN

Errors are about 10%

(Some) Problems encountered:

Stuck beads to the substrate

Trapping and calibration close to the substrate (<2 µm) and at T=37 C

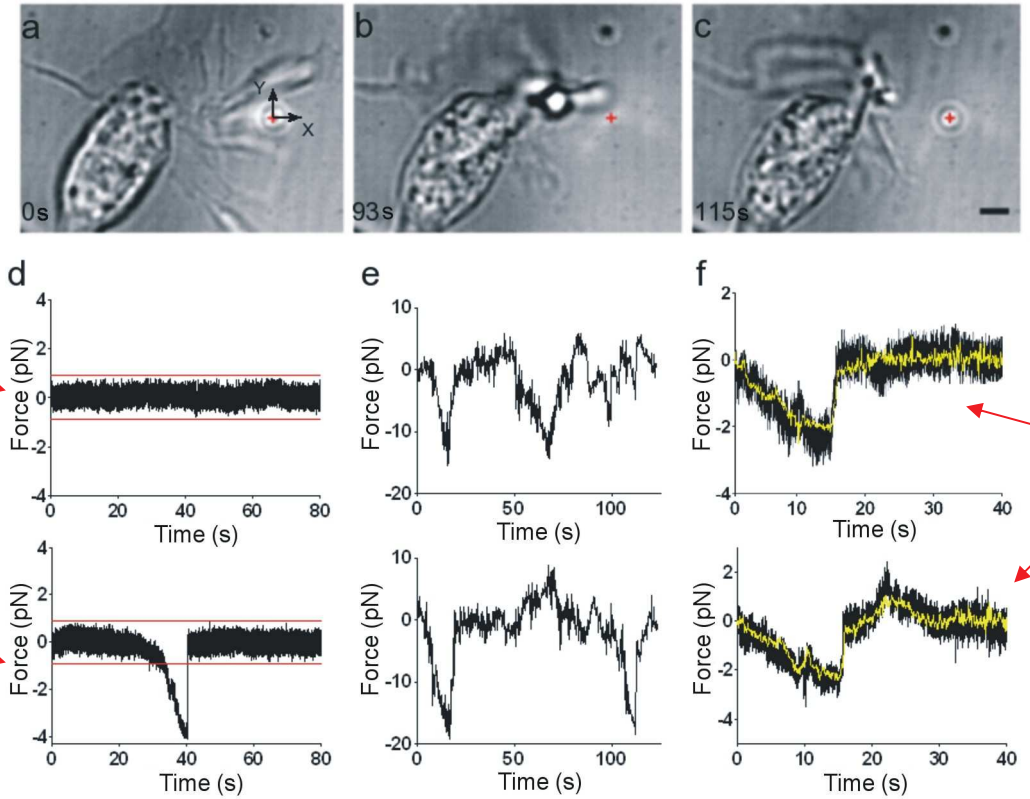
Influence of floating particles on the interference pattern

Filopodia collisions reveal lower forces than expected ? Tam-Tam !

Criteria to define a collision

Measurement away from Neuron

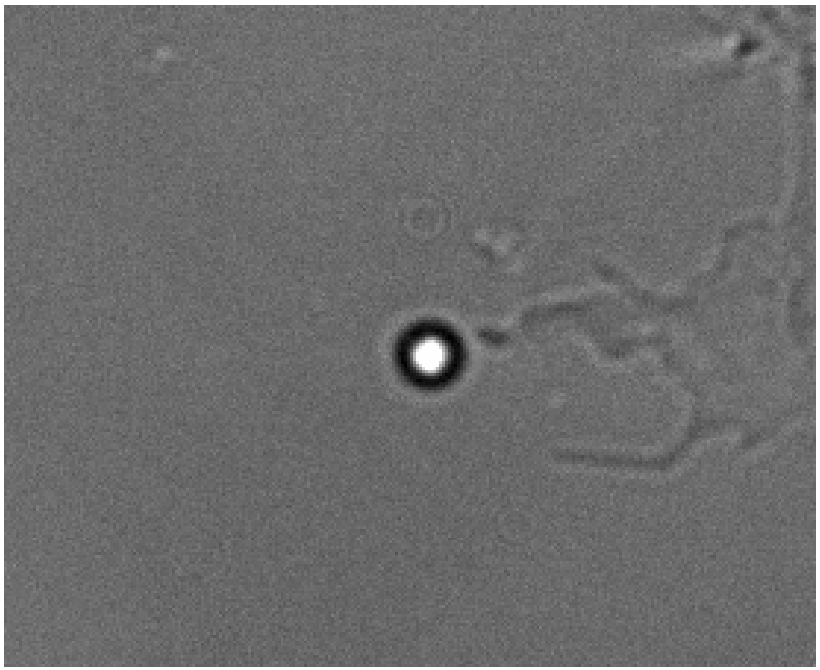
Measurement during collision



Measurements done by QPD & Video tracking Overlapped

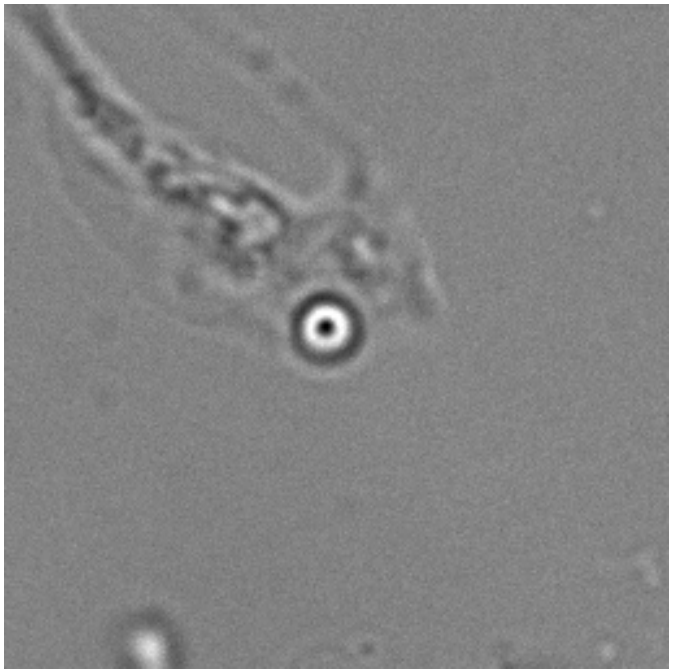
D. Cojoc *et al. PlosOne* 2007

Results



Filopodia 2 minutes event

Fmax= 2pN



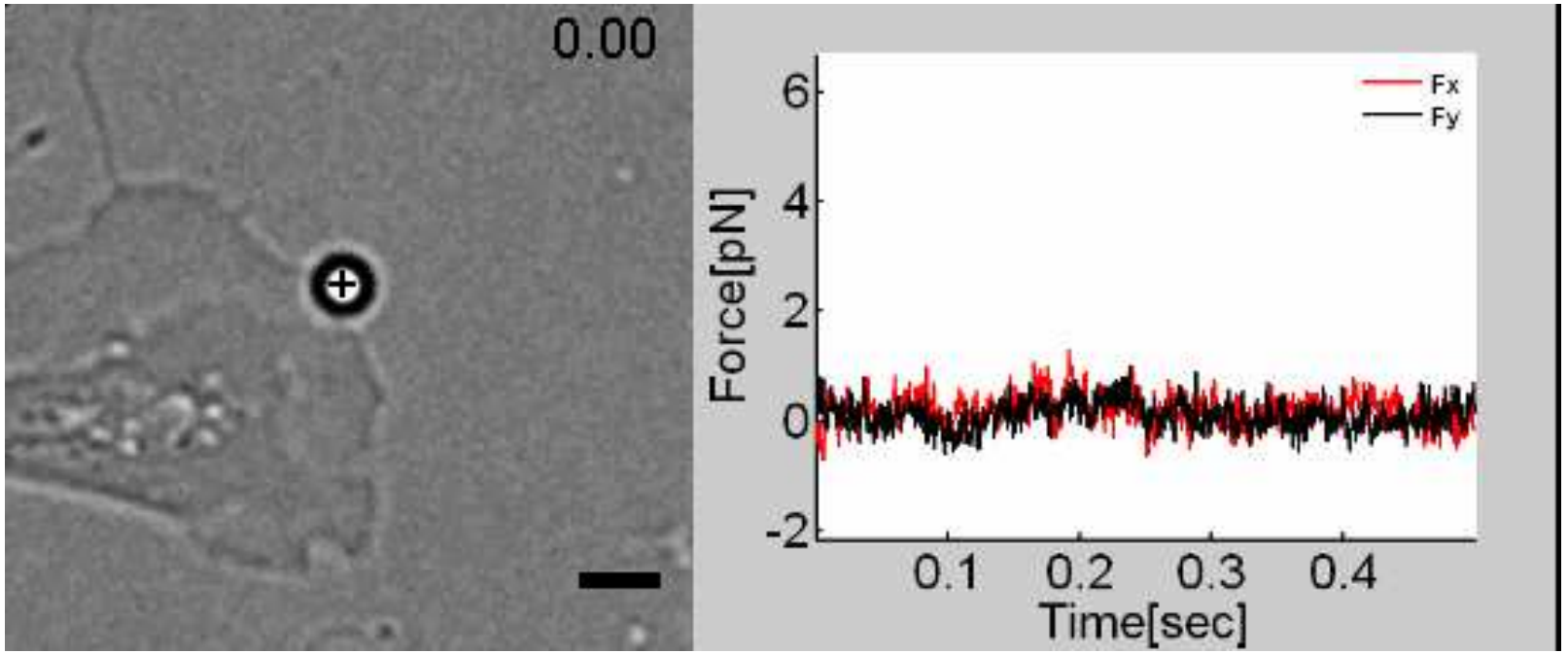
Lamellipodia 2 minutes event

Fmax measured = 20pN

> 20 pN possible

D. Cojoc *et al. PlosOne* 2007

Force exerted by Lamellipodia



Acquisition rate: 20Hz

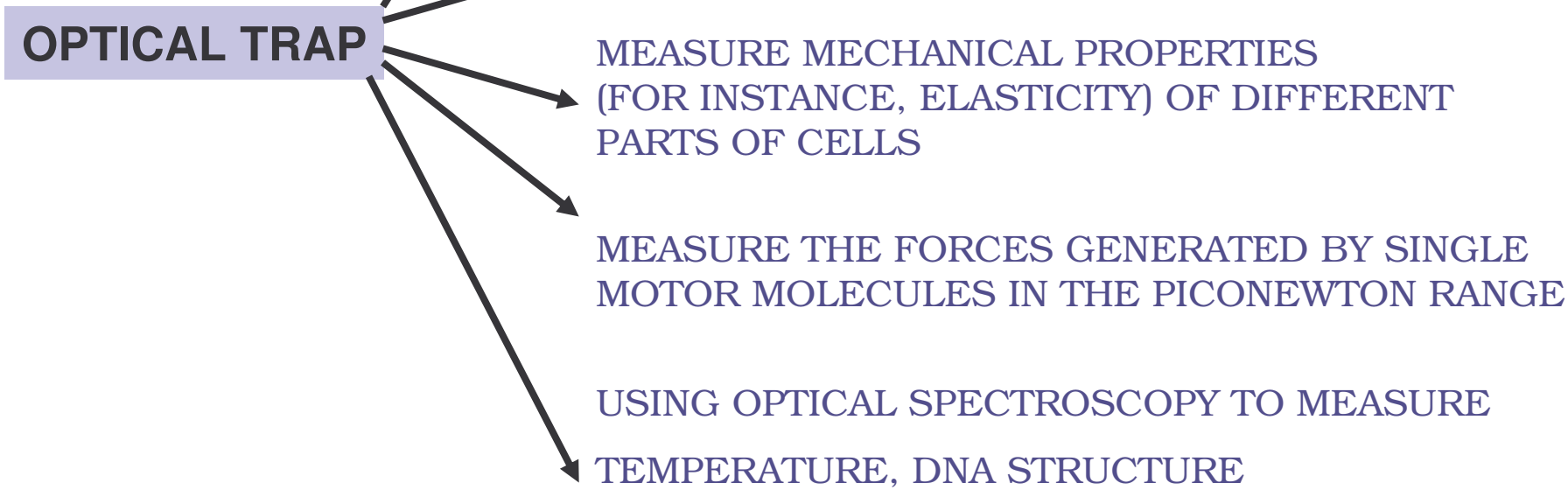
Scale Bar = 2μm

Time in seconds

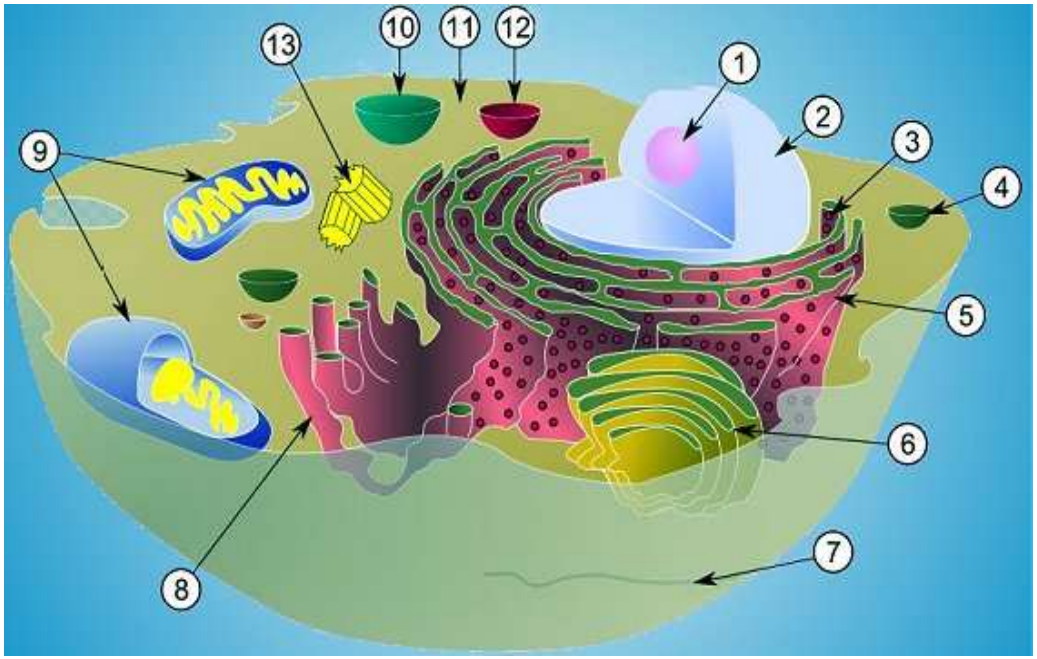
Acquisition rate : 4KHz

Subsampeled at : 2KHz

D. Cojoc *et al. PlosOne* 2007


OPTICAL TRAP
SELECT AND MOVE A GIVEN CELL
REMOTELY APPLY CONTROLLED FORCES
ON LIVING CELLS, INTERNAL PARTS OF CELLS,
AND LARGE BIOLOGICAL MOLECULES WITHOUT
OPTICAL DAMAGE
MEASURE MECHANICAL PROPERTIES
(FOR INSTANCE, ELASTICITY) OF DIFFERENT
PARTS OF CELLS
MEASURE THE FORCES GENERATED BY SINGLE
MOTOR MOLECULES IN THE PICONEWTON RANGE
USING OPTICAL SPECTROSCOPY TO MEASURE
TEMPERATURE, DNA STRUCTURE
CELL VIABILITY, INTRACELLULAR pH
OF A GIVEN SINGLE CELL

The cell



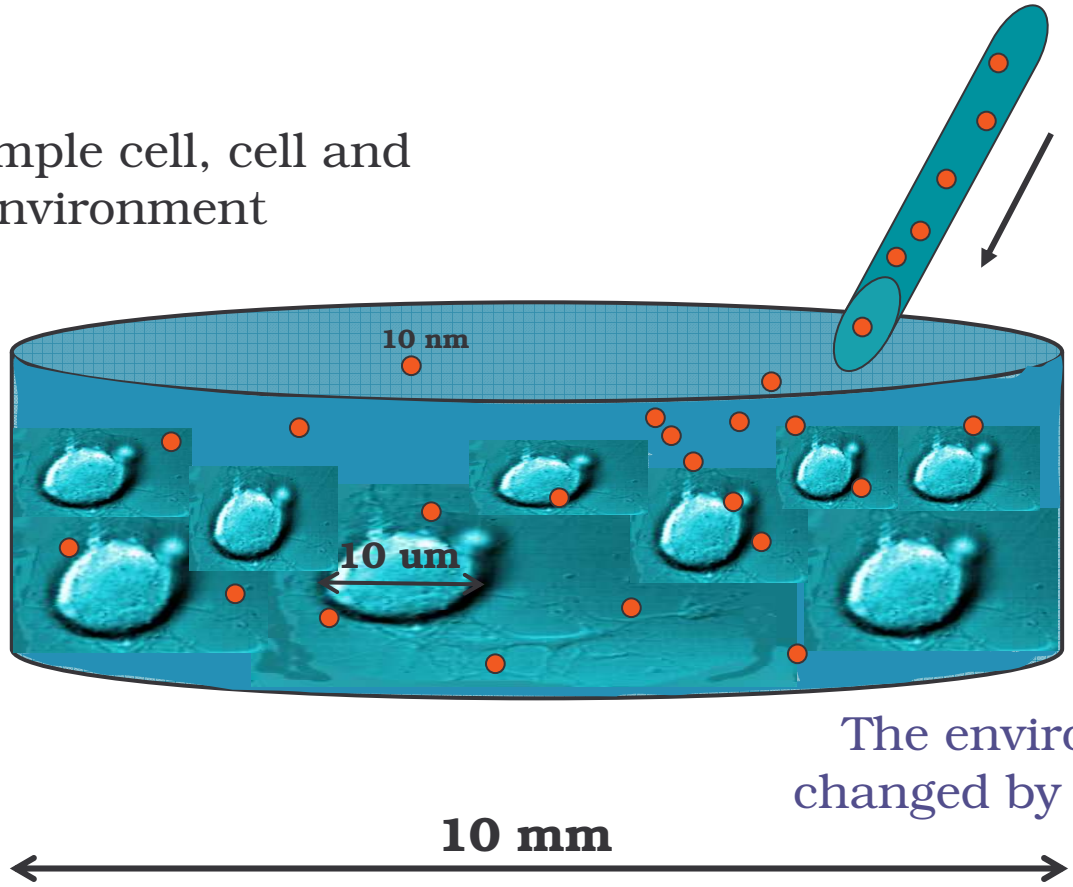
1) Nucleolus
2) Nucleus
3) Ribosome
4) Vesicle
5) Rough endoplasmic reticulum
6) Golgi apparatus (or "Golgi body")
7) Cytoskeleton
8) Smooth endoplasmic reticulum
9) Mitochondrion
10) Vacuole
11) Cytosol
12) Lysosome
13) Centriole

Cell mechanics skipped see the pdf file

- **Why and how to change the environment of a cell ?**
- **Optically driven micro-pumps**
- **Optically driven vectors**

  functionalized beads and filled liposomes

Typical sample cell, cell and its environment



The environment is usually changed by micro-nano fluidics

- In general, the entire environment of the sample cell is changed

- For a localized delivery the cell position should be adapted to the micro-fluidic structure

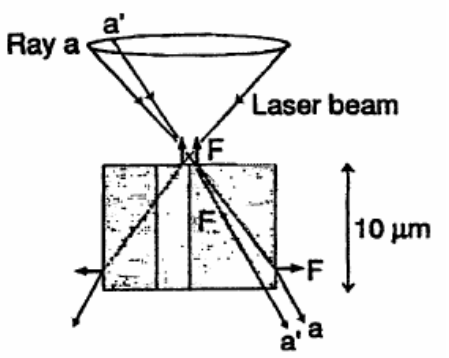
- Why and how to change the environment of a cell ?

- **Optically driven micro-pumps**

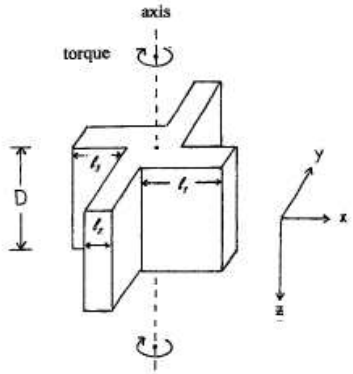
- Optically driven vectors

  functionalized beads and filled liposomes

Micro-fabricated rotors



Side view



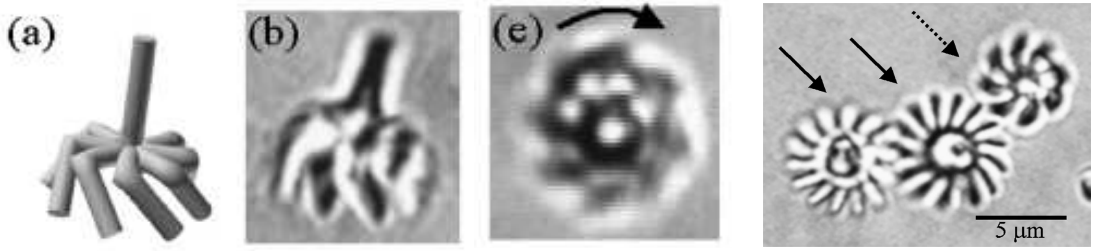
Perspective diagram

SiO2

22 rpm/80 mW

Optical lithography
+ RIE

E. Higurashi *et al*,
Appl. Phys. Lett. **64**, 2209 (1994)

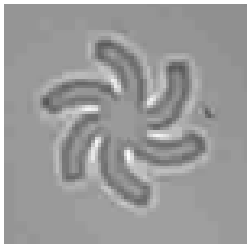


Norland resin

Two-photon lithography

20 rpm /80 mW

P. Galajda and P. Ormos,
Appl. Phys. Lett. **78**, 249 (2001).



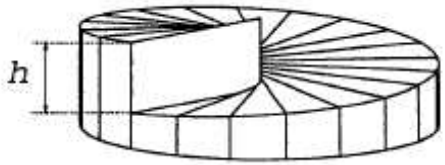
5 µm

PMMA
E-Beam + X-ray lithography

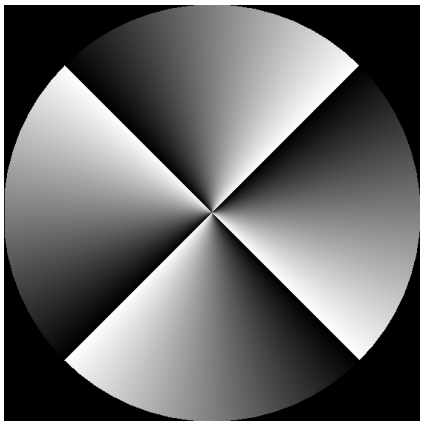
3 rpm/10mW

L. Businaro et al, TASC 2005

Laguerre-Gauss beams and Optical angular momentum transfer

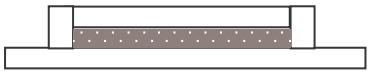


Spiral phase plate to convert a TEMoo mode into a LG (Laguerre-Gauss) mode With topological charge m=1.



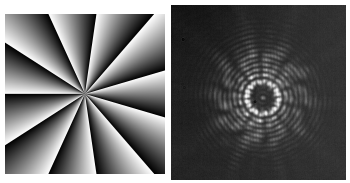
Phase diffractive optical element to convert a TEMoo mode into a LG mode with topological charge m=4

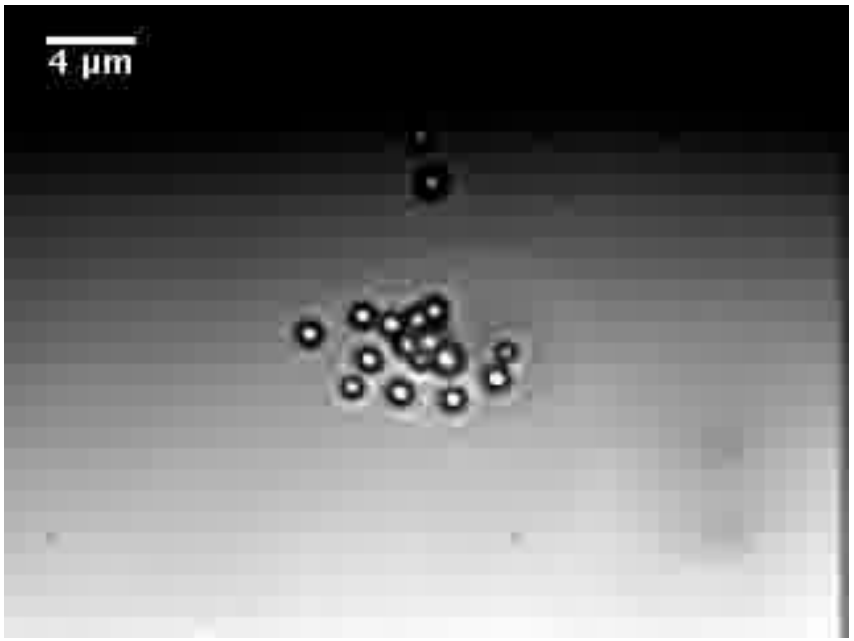
Sample cell



Micro-spheres: silica 1 μm

LG topological charge m= 11





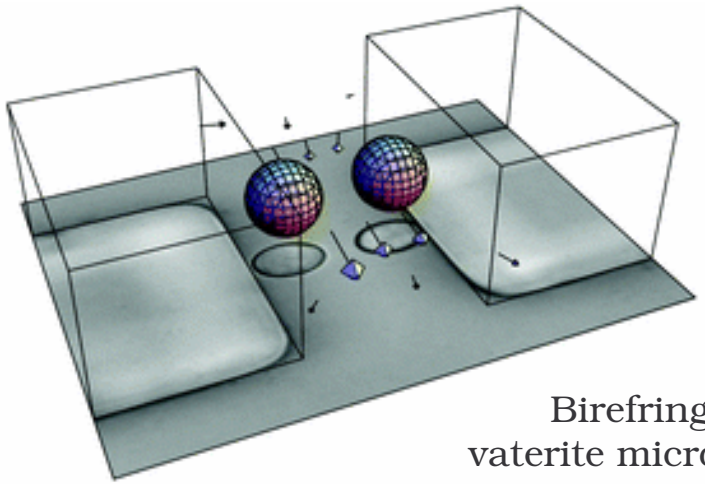
Polarized light on birefringent particles

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

Crystal structure birefringence

CaCo3 crystal trapped, aligned and rotated with a linear polarized laser beam H.Rubisntein-Dunlop et al, Nature 1999

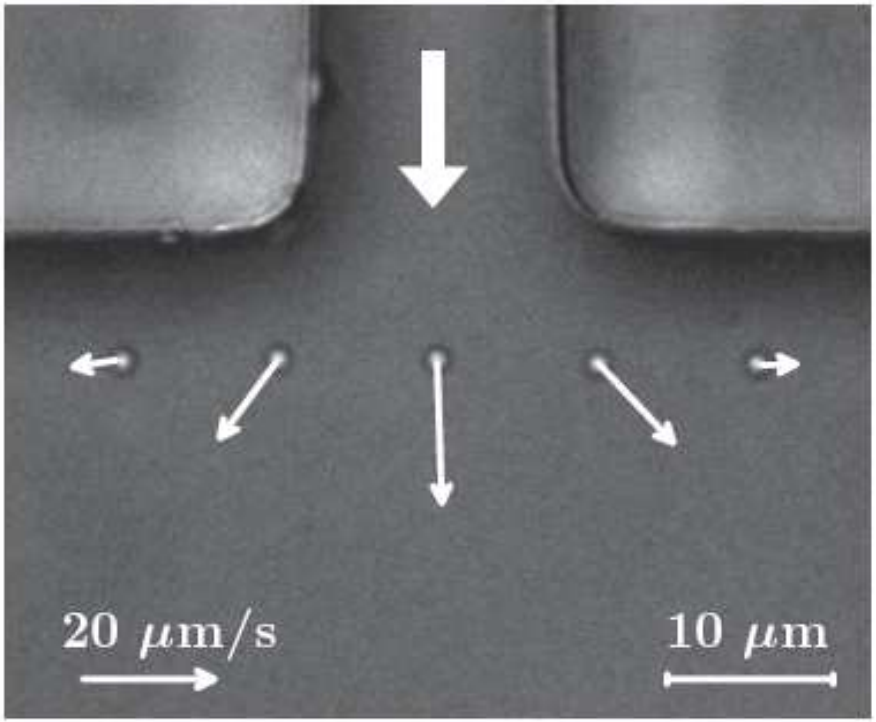
Shape birefringence



Birefringent vaterite microspheres (4 μm)

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

Measuring the flow



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

- Why and how to change the environment of a cell ?

- Optically driven micro-pumps

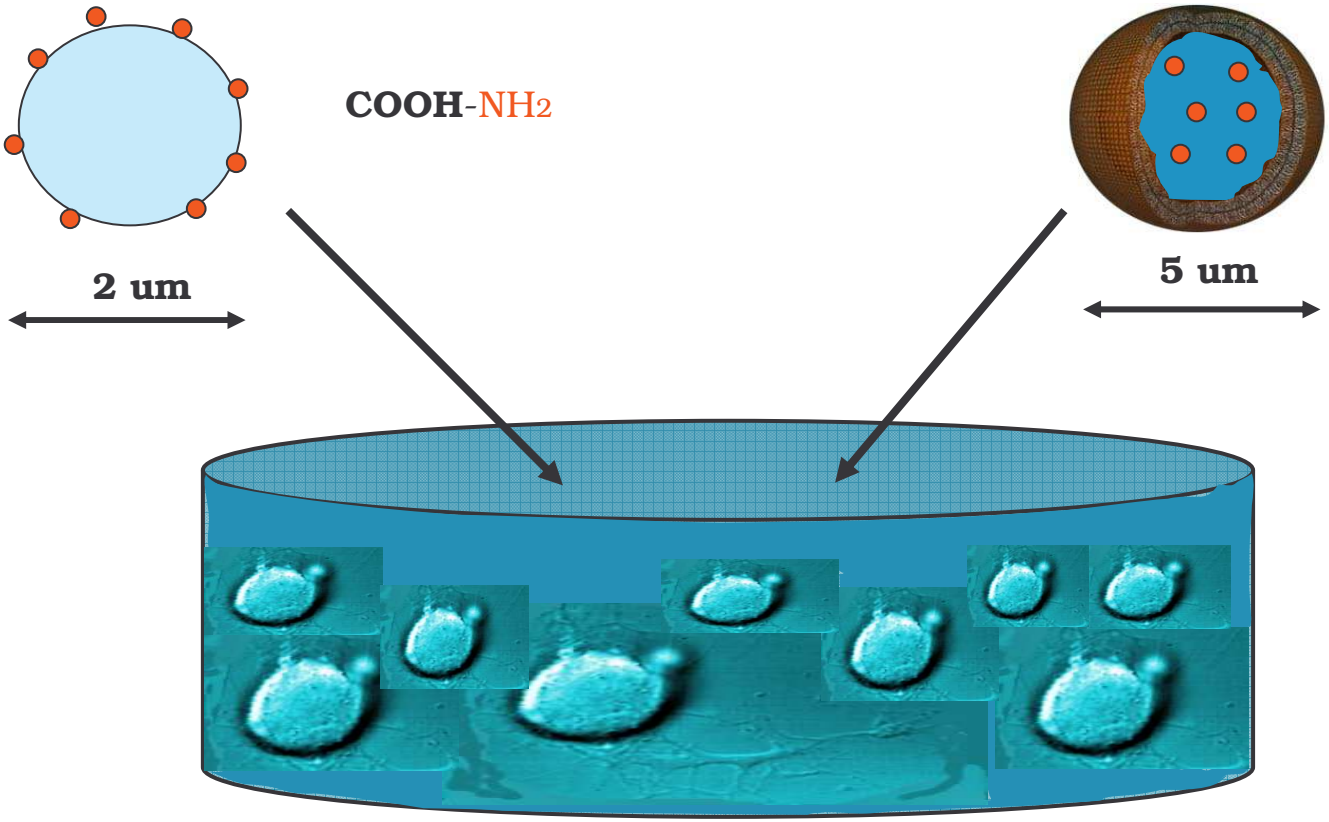
  a simple solution and the importance of the lateral view

- **Optically driven vectors**

  functionalized beads and filled liposomes

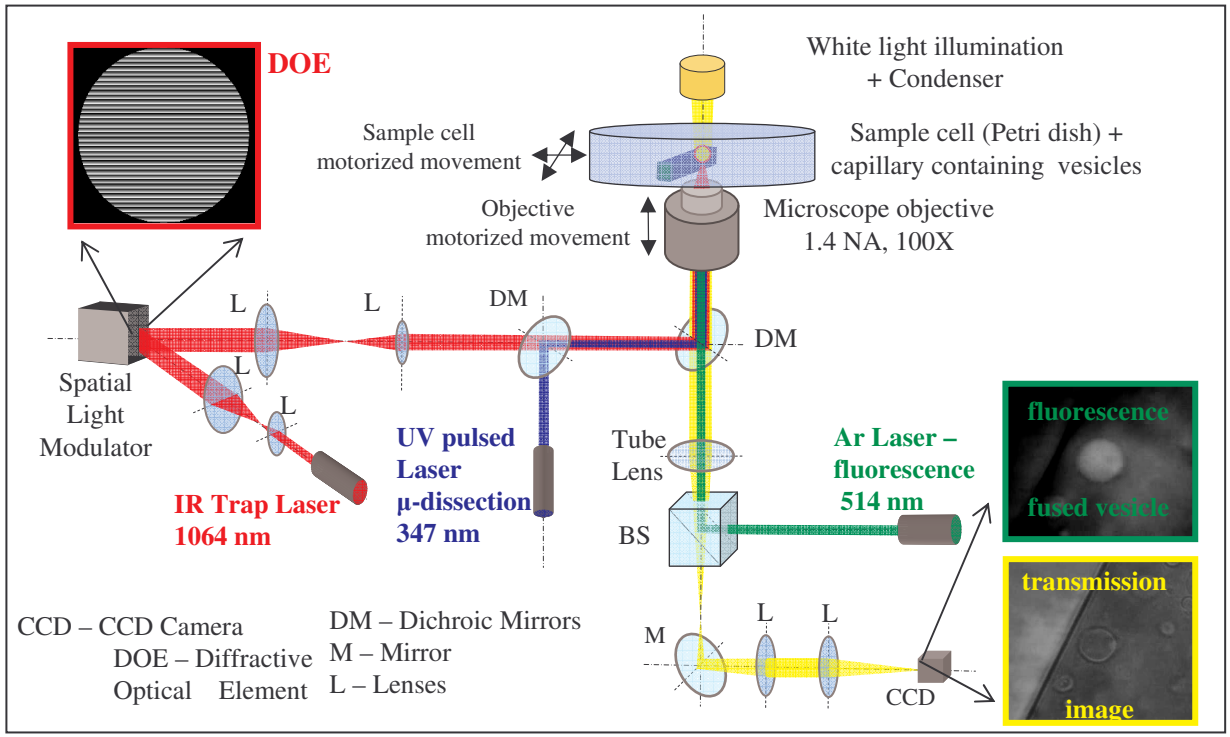
Functionalized silica beads

Filled liposomes



- The vectors are optically driven to precise defined location
- Delivery by contact (beads) or breaking the liposome

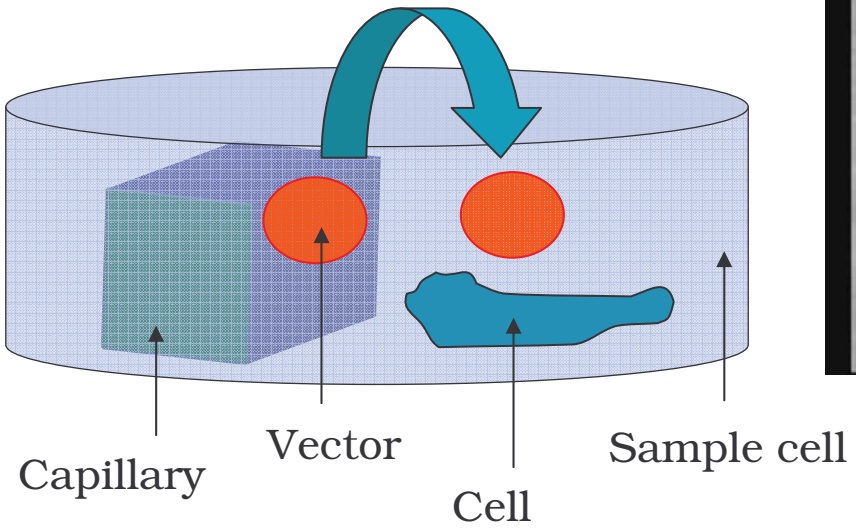
LCM-CA (Laser Cell Manipulation – Chemical Analysis) Optical setup

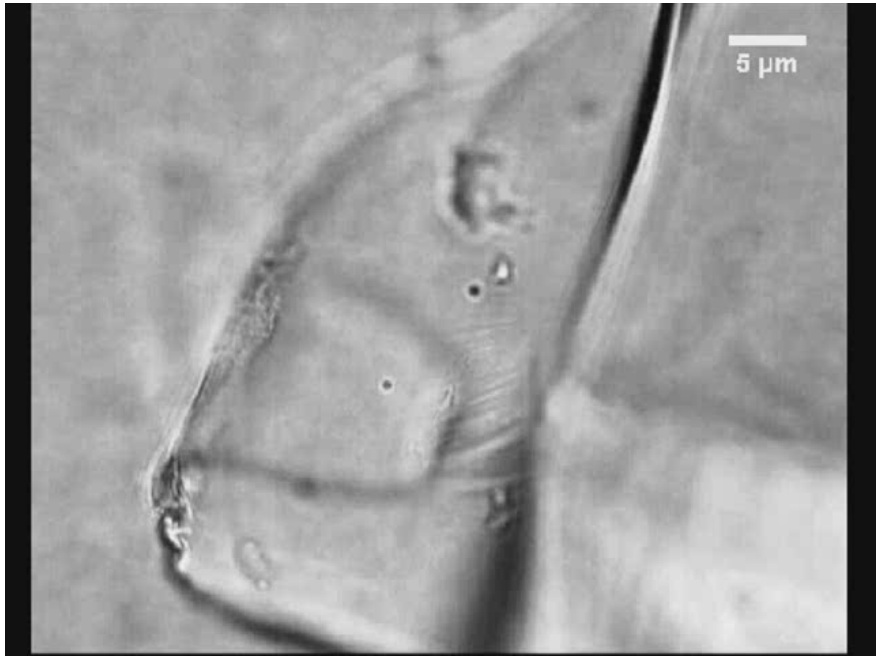


The setup has been developed starting from an inverted Olympus microscope equipped with a PALM micro-dissection. CBM-TASC

Functions: cell and vectors manipulation, fluorescence imaging, micro-dissection and micro-Raman spectroscopy (to come).

The vectors (beads or liposomes) are first placed in a capillary which is introduced in the sample cell and then the vectors optically transported

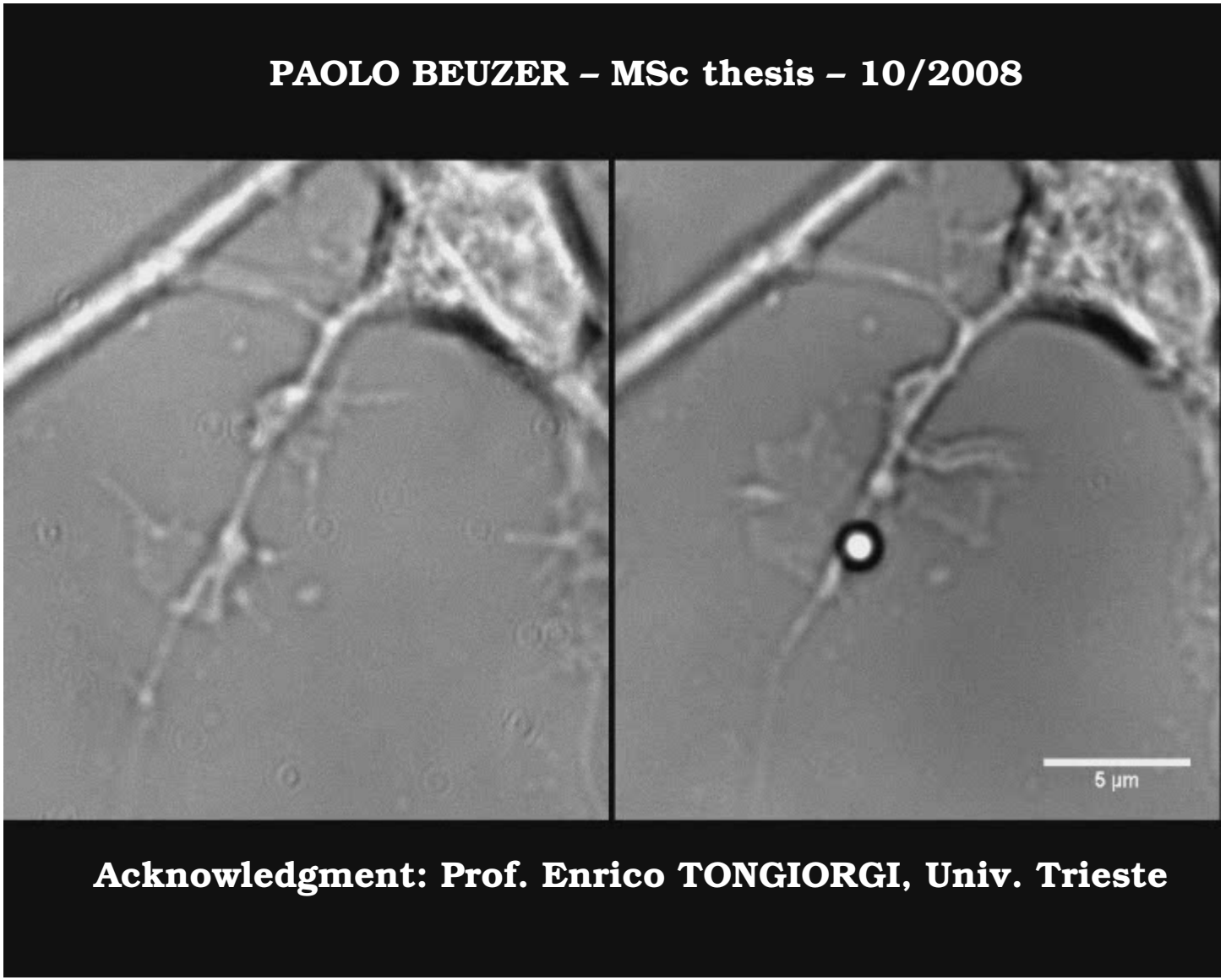




LOCALIZED DELIVERY OF BDNF BY MEANS OF OPTICAL TWEEZERS

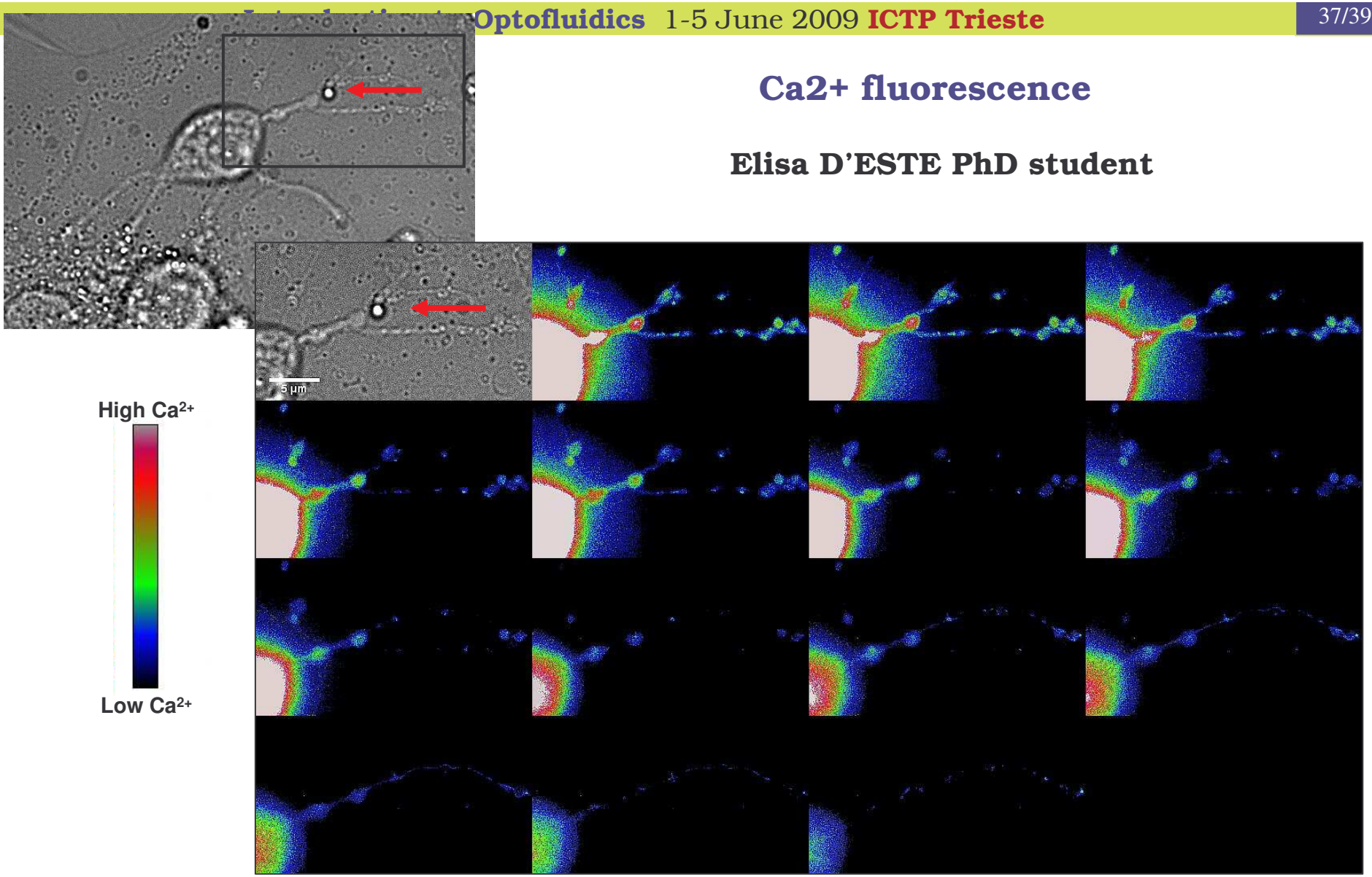
BDNF functionalized bead

BDNF = Brain Derived Neurotrophic Factor

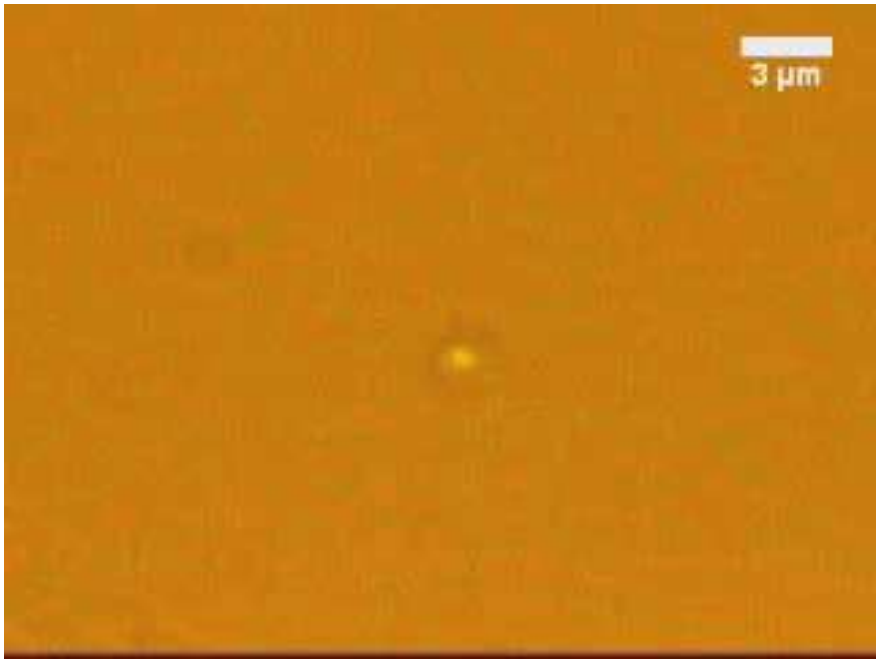


Ca2+ fluorescence

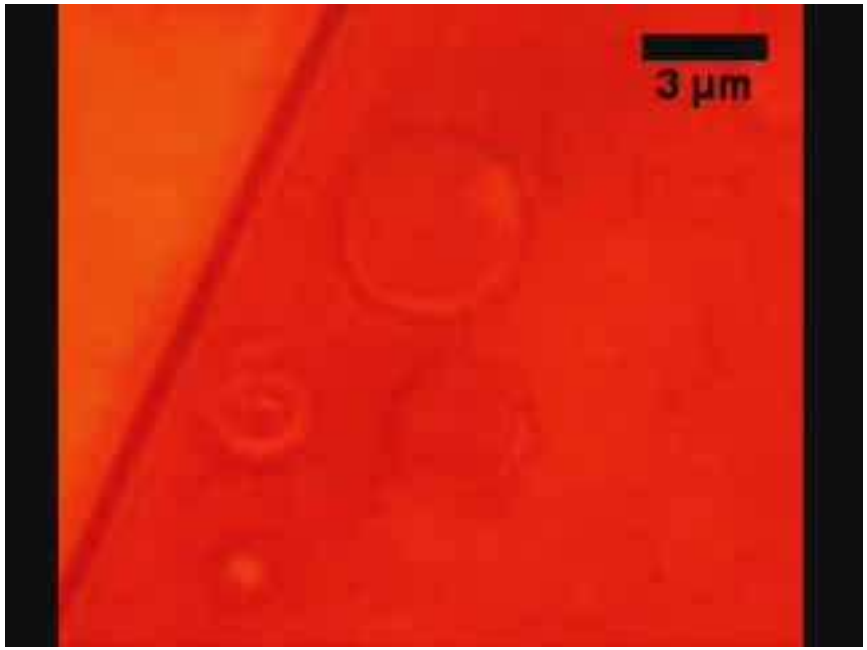
Elisa D'ESTE PhD student



Filled liposomes



Liposome positioned on an axon
Fluorescence imaging



Liposome fusion

This is a proof of concept, preliminary results.

In both cases, liposmes are filled with a fluorophor.

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