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Winter College on Micro and Nano Photonics for Life Sciences

11 - 22 February 2008

Light in action II: Optical tweezers, nano-bio applications

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Light in Action II
Optical tweezers, nano-bio applications

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Optical Tweezers is a valuable tool in Biophysics
Three examples/experiments

OUTLINE

- **Optical Trapping and Manipulation (OTM)**

  some related instrumentation developed at TASC

- **Three OTM examples:**

  1. OTM, ultrasound excitation and ultra high-speed imaging to study the dynamics of ultrasound contrast agent micro-bubbles near and far from interfaces
  2. OTM of individual micron-sized samples investigating at the same time their inner nanostructure by Synchrotron diffraction experiments
  3. OTM for force spectroscopy of growing neurons

Optical trapping and micro-manipulation (OTM)

some related instrumentation developed at TASC (2004-)

1. **OM – FSM (Optical Manipulation – Force Spectroscopy Microscope)**

2. **OM – MSXD (Optical Manipulation for Microfocused Synchrotron X-ray Diffraction experiments)**

3. **OM –UM (Optical Manipulation for the study of Ultrasound excited Microbbubles)**

Technical report (pdf)

www.tasc-infm.it

OM – FSM (Optical Manipulation – Force Spectroscopy Microscope)

- OTM particles in the range of 50nm-50μm, in a 3D volume, with sub micron resolution.
    - Types of particles: high index (e.g dielectric beads), low index (e.g. bubbles), living cells with variable shapes.
    - Lasers used for trapping: 514 nm (CW 150 mW), 632 nm (CW 50 mW), 830 nm (CW 150 mW), 970 nm (CW 300 mW), 1064 nm (CW 5W and 10W)
    - Multi-trapping capabilities based on diffractive optics projected to Spatial Light Modulators (SLM): two Phase Programmable Modulators - PPM Hamamatsu for IR 800-900 nm and 1000-1100 nm and one SLM Holoeye for visible light 400-700 nm).
    - Fluorescence microscopy allowed simultaneously with optical manipulation.
    - Force spectroscopy using back-focal interferometry in forward and back scattering configuration, stiffness and force range: 1-100 pN/μm, 0.1-150 pN, ms temporal resolution.

OM – MSXD (Optical Manipulation for Microfocused Synchrotron X-ray Diffraction experiments)

- The same optical manipulation characteristics (type and size of particles) as the setup 1, with IR laser (CW 1064 nm, 10W), and multiple trapping using the PPM-Hamamtsu.
- Sample cell is capillary to reduce at minimum the scattering of X-ray beam by the liquid in which the particles are immersed.

OM –UM (Optical Manipulation for the study of Ultrasound excited Microbbubles)

- Optical manipulation of microbubbles in the micro and submicrometric range using Laguerre Gaussian beams with IR laser (CW 1064 nm, 10W) and multiple trapping using the PPM-Hamamtsu.
- Fast imaging up to 25 MHz (allowed by the Brandaris camera, located at University of Twente)

Example 1

OTM, ultrasound excitation and ultra high-speed imaging to study the dynamics of ultrasound contrast agent micro-bubbles near and far from interfaces

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M. L. J. Overvelde, S. M. van der Meer, N. de Jong,c D. Lohse, and M. Versluis
University of Twente, Physics of Fluids, Enschede, The Netherlands

V. Garbin et al, *Appl. Phys. Lett.* **90** 114103 (2007)

Motivation and goal of our work

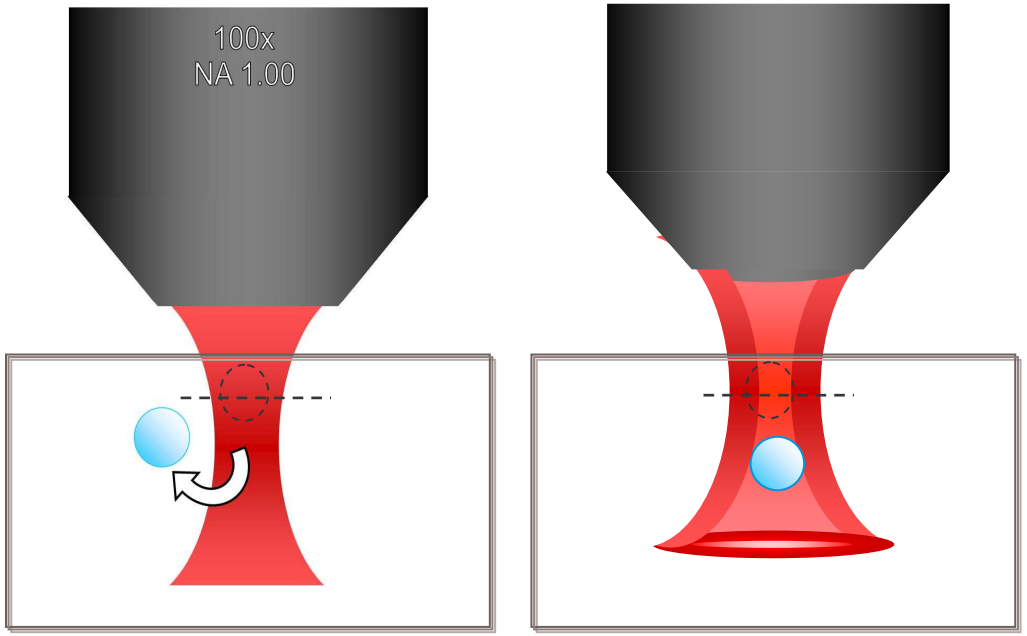
Micron-sized gas bubbles are used in medical ultrasound imaging for enhancing the blood pool visibility - Ultrasound Contrast Agents (UCA).

Phospholipid-coated microbubbles (BR14, Bracco) are trapped with a laser tweezer, a versatile tool for non-contact, non-destructive manipulation, capable of applying forces in the pN range.

Goal: study UCA micro – bubble dynamics near and far from interfaces

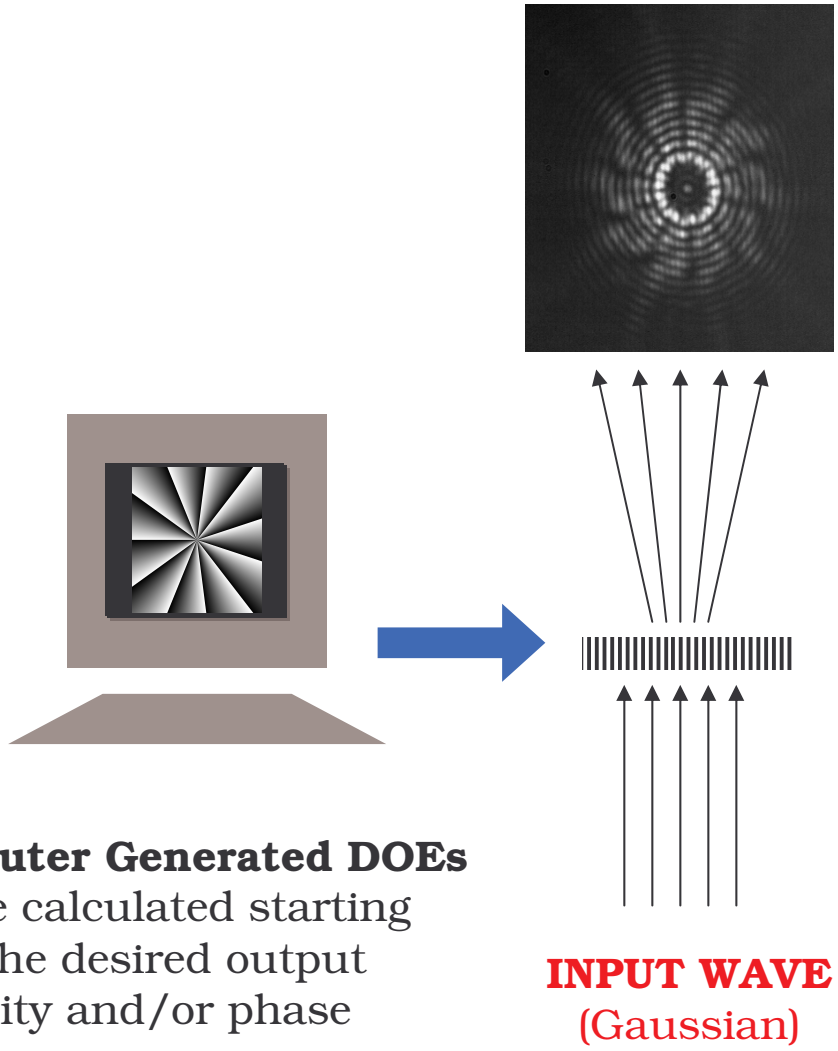
Laser trapping beam

- Gauss
- Laguerre- Gauss



Gauss Laguerre- Gauss

Laser beam shaping with DOEs



OUTPUT WAVE

(Laguerre Gaussian)

Observation plane :

the diffracted pattern is observed in the far-field

Diffractive Optical Element (DOE): it converts by DIFFRACTION the input wave into an output wave with a desired amplitude, phase or polarization distribution.

Computer Generated DOEs can be calculated starting from the desired output intensity and/or phase profile.

INPUT WAVE
(Gaussian)

Schematic of an experiment

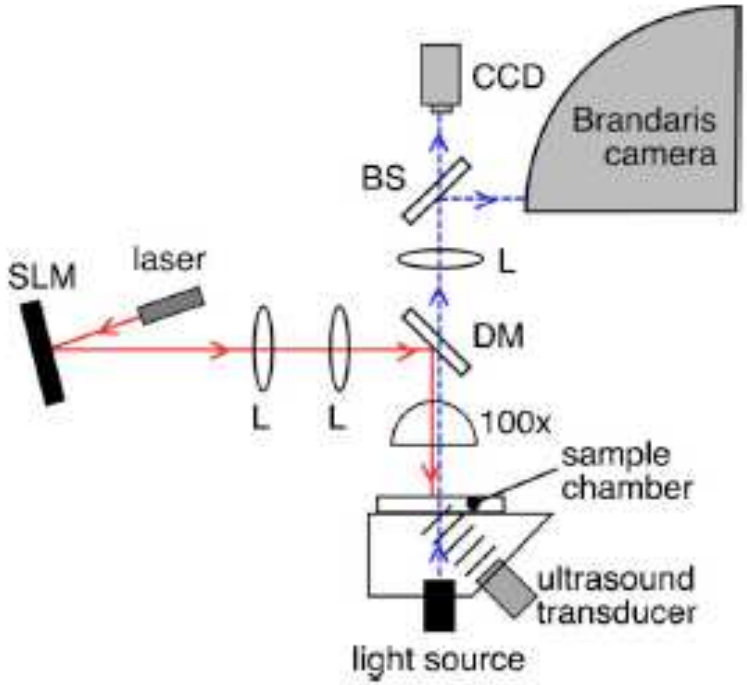
The laser beam is switched on to trap the selected microbubble. The trapped bubble is then positioned away from the chamber wall, by a prescribed distance

Before insonification the bubble is released by switching off the trap.

The laser shut down is detected by a photodiode, which triggers a burst of 10 ultrasound cycles and the recording.

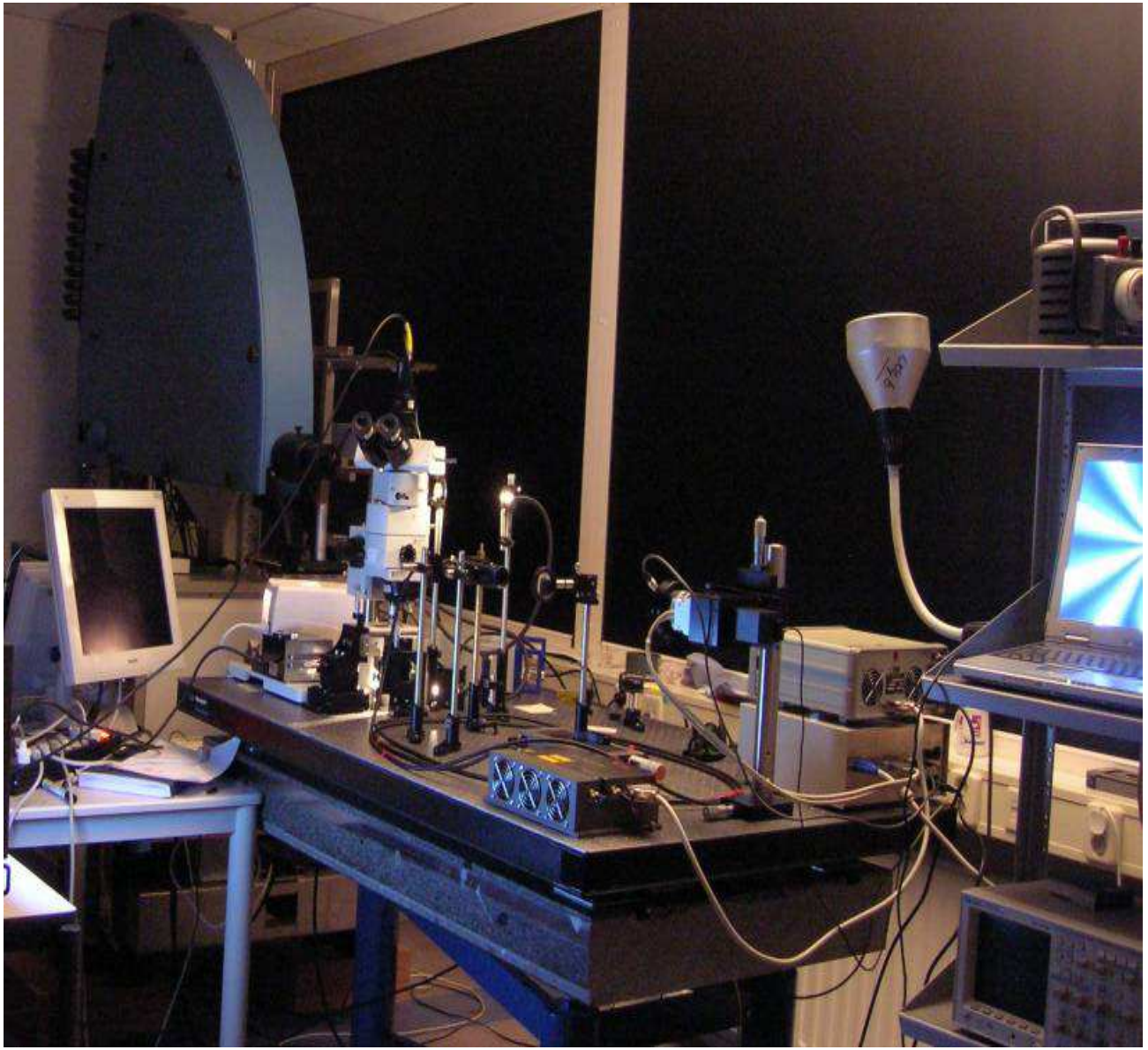
The oscillations are recorded after a delay corresponding to the time it takes to the ultrasound to travel from the transducer to the bubble (~20 ms)

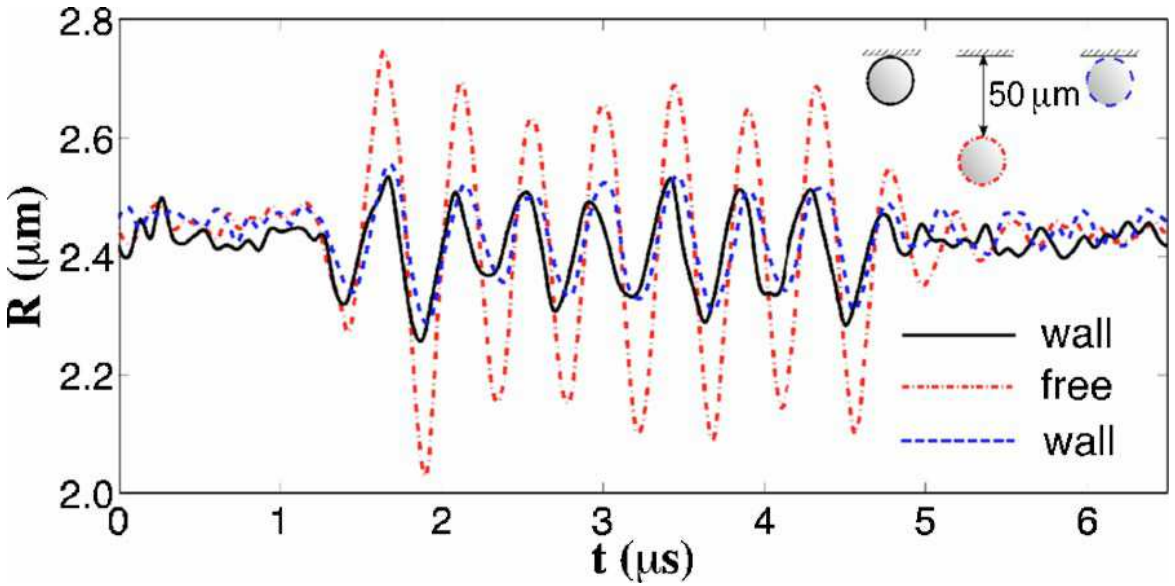
Setup for combined UCA microbubble trapping, acoustical driving, and ultrahigh speed optical recordings



The laser beam is converted by the spatial light modulator SLM into a Laguerre-Gaussian mode; upon reflection on a dichroic mirror DM, it enters the objective 100 and is focused into the sample volume. The ultrasound beam overlaps the optical focal volume. Beam splitter BS enables two imaging modes: monitor mode on a CCD camera T=20% and imaging mode on the ultrahigh speed camera Brandaris R=80%. L: lenses

V. Garbin et al, *Appl. Phys. Lett.* **90** 114103 (2007)



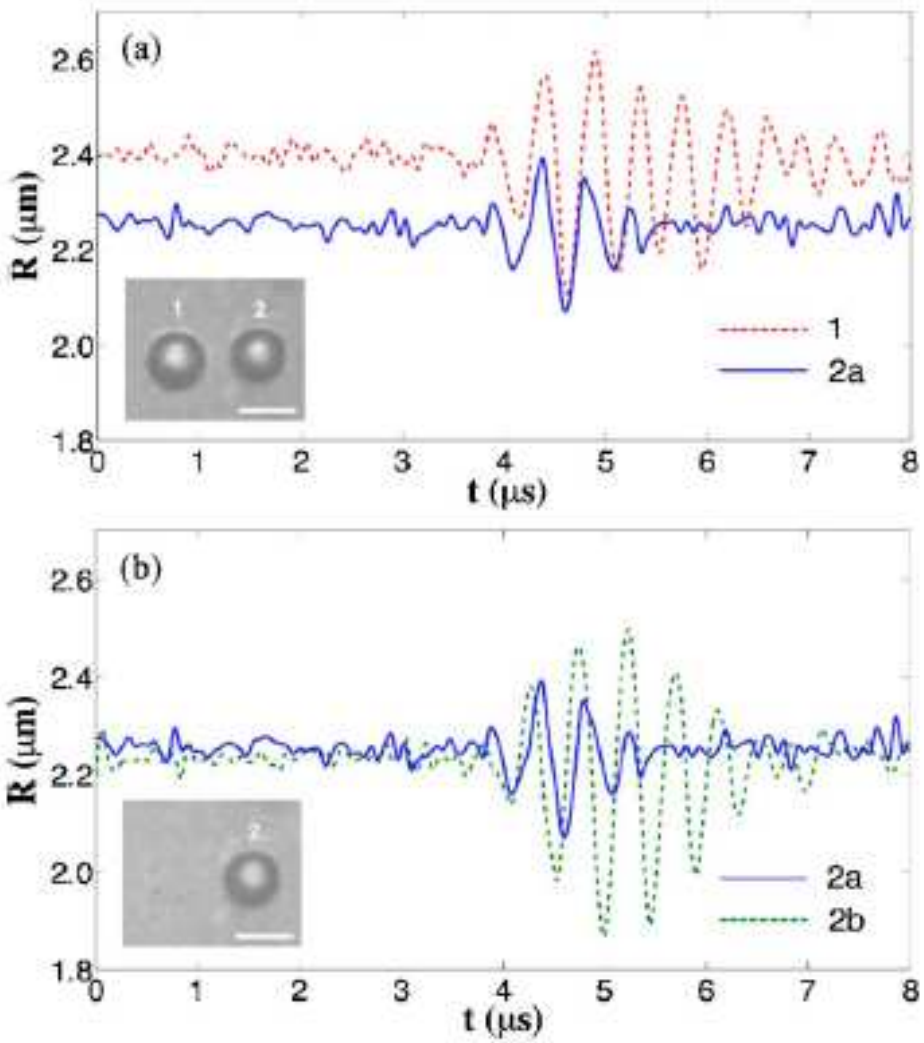


Three *R*-*t* curves of a single bubble with an initial radius *R*o=2.45 m, insonified with an eight-cycle ultrasound burst at 2.25 MHz with an applied pressure of 200 kPa. The frame rate is 15 Mfps.

The solid line represents the amplitude of oscillations at the wall; the dashdotted line is recorded with the bubble at a distance of 50 um from the wall.

The radius-time curve of the same bubble repeated at the wall identical dashed line is identical to the first one, showing that repeated insonations have not altered the initial bubble properties.

V. Garbin et al, *Appl. Phys. Lett.* **90** 114103 (2007)



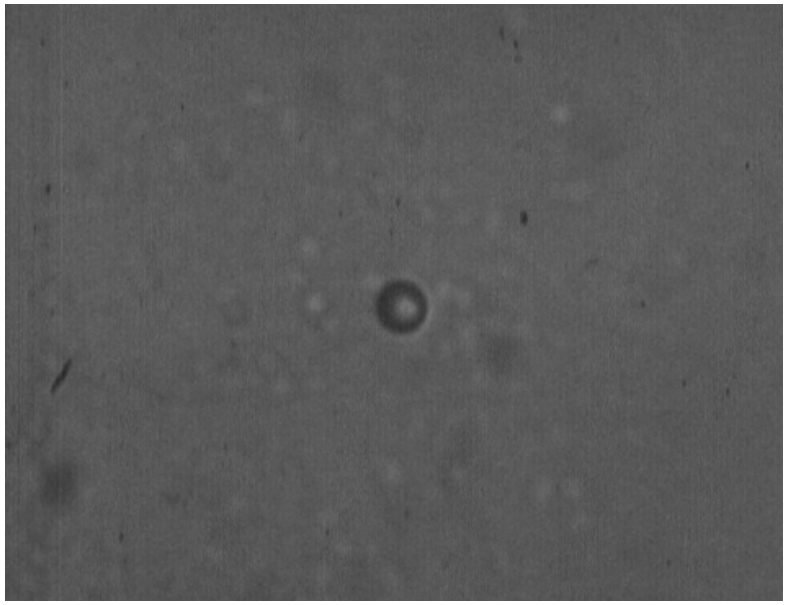
Radius-time curves taken at
15 Mfps for two interacting bubbles.

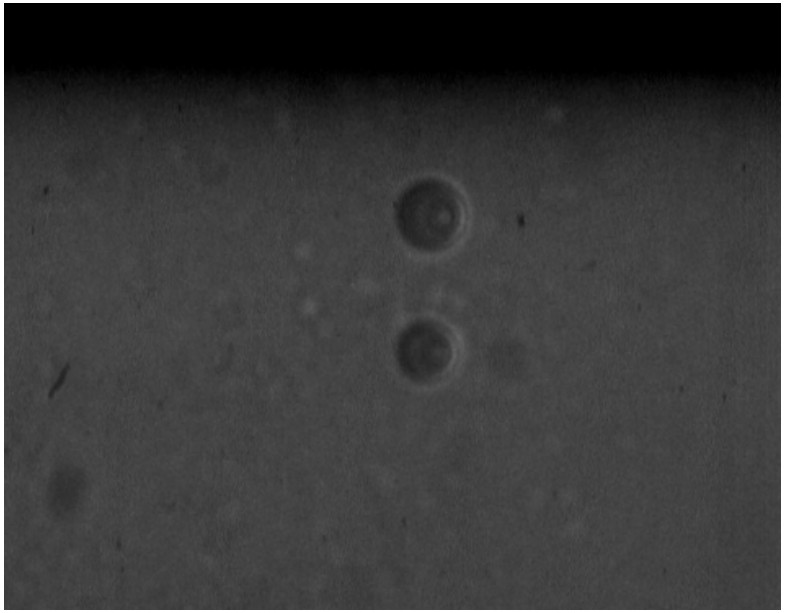
a) *R-t* curves of the two bubbles trapped at 8 m distance from each other and positioned 50 m away from the wall. The dashed curve 1 corresponds to bubble 1, the solid line 2a corresponds to bubble 2.

b) The dashed line 2b represents the *R-t* curve of bubble 2 oscillating after bubble 1 has been released. The *R-t* curve 2a is also plotted for comparison.

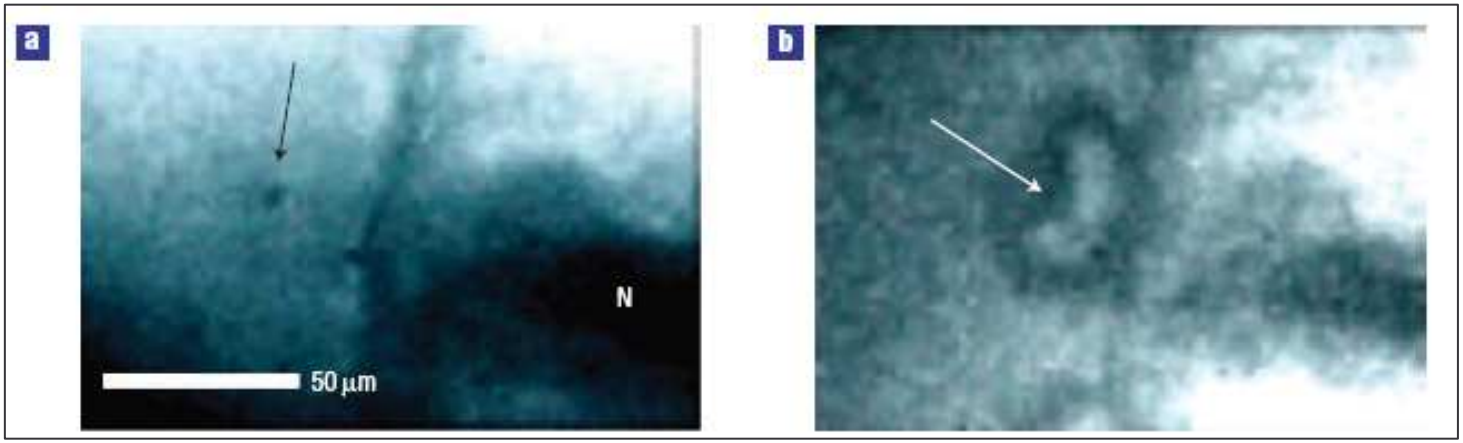
White scalebar in pictures: 5 um.

V. Garbin et al, *Appl. Phys. Lett.* **90** 114103 (2007)





Membrane disruption by optically controlled microbubble cavitation



Correlation of specific cavitation events with membrane damage.

a, A 163 µm×110 µm frame showing a quiescent 4-µm-diameter microbubble trapped 17 µm from a cell membrane.

b, At *t*=8 µs after cavitation inception, the microbubble has developed an involution (arrowed), which is in contact with the membrane over a region some 15 µm wide.

P. Prentice, A. Cuschieri, K. Dholakia, M. Prausnitz and P. Campbell, "Membrane disruption by optically controlled microbubble cavitation" Nature Physics **1** 107 (2005)

Example 2

OTM of individual micron-sized samples investigating at the same time their inner nanostructure by Synchrotron diffraction experiments (micro SAXS: Small Angle X-ray Scattering)

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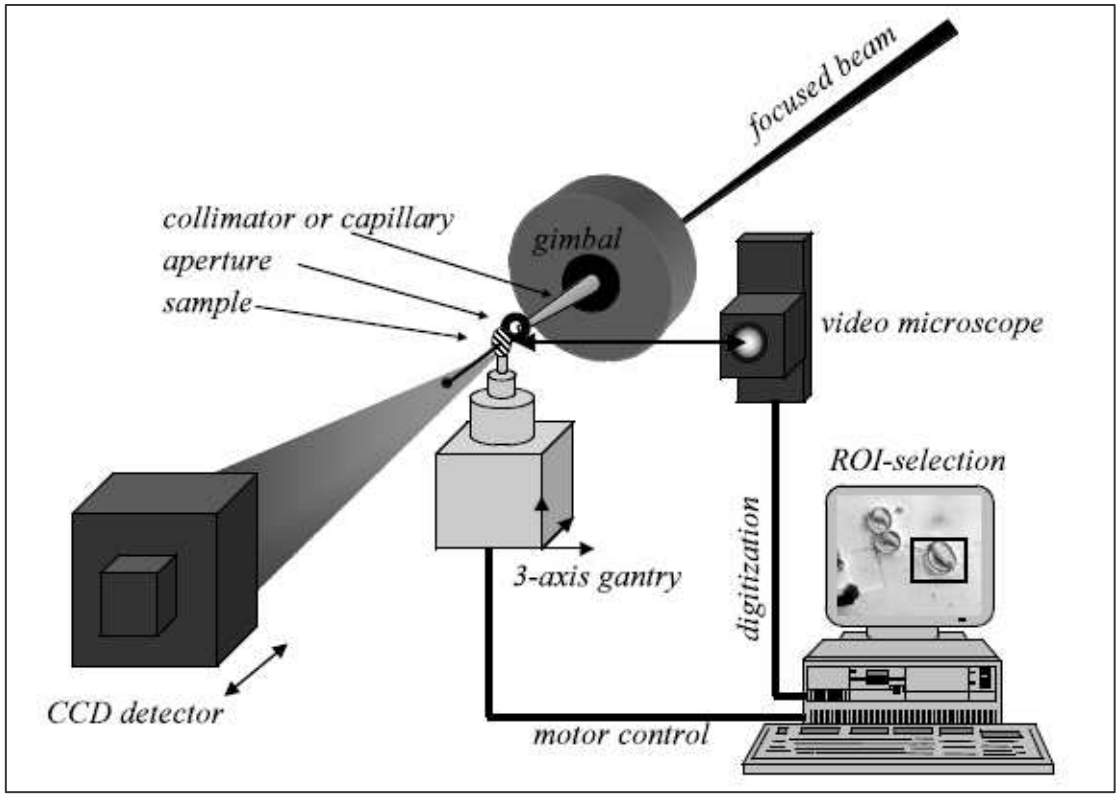
Austrian Academy of Sciences, IBN Graz, Austria

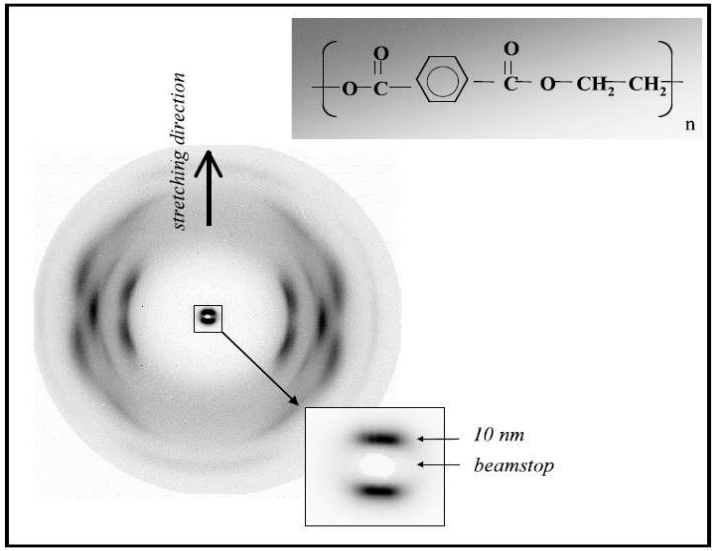
Christian Riekel, Manfred Burghammer
ESRF, ID13 Microfocus Beamline, Grenoble, France

D. Cojoc et al, *Appl. Phys. Lett.* **90** 234107 (2007)

Small Angle X-ray Scattering (SAXS)



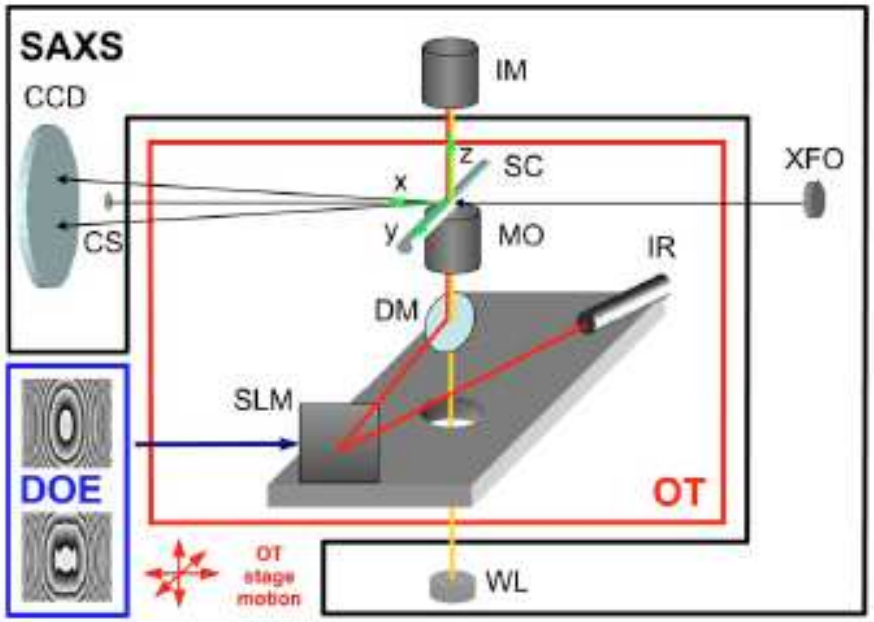
Schematic setup used for scanning microdiffraction at the microfocus BL. The 3-axis gantry allows translation of the sample between the optical microscope and the x-ray microbeam. A region of interest (ROI) defines the zone for a mesh scan.

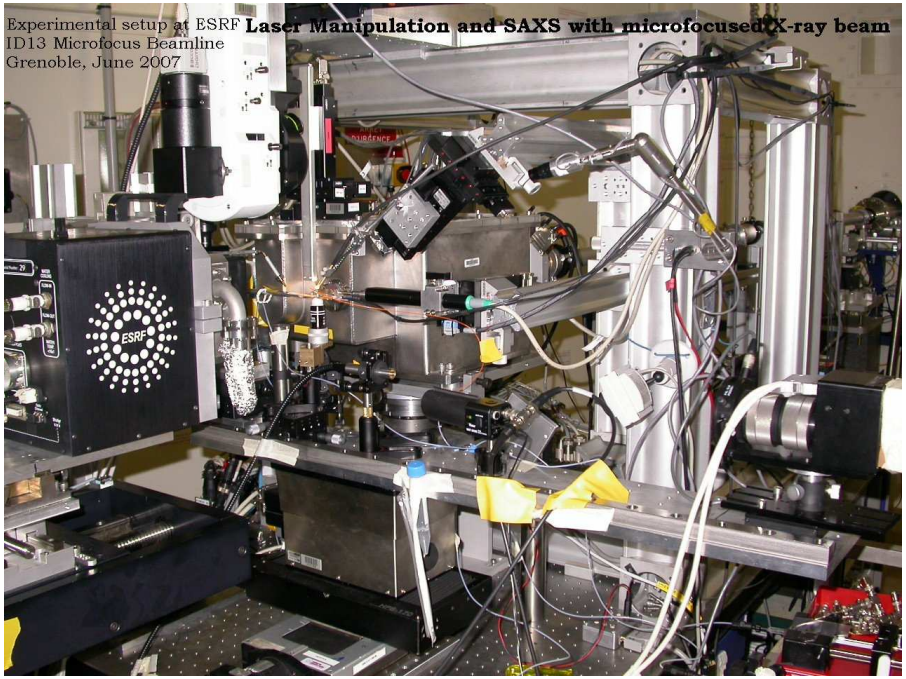


SAXS/WAXS pattern of an uniaxially stretched poly(ethyleneterephthalate) (PET) foil
- beam size: 10 um
- **wavelength ~ 1 Å**
- distance sample-to-detector 116 mm

C. Riekel, *Rep. Prog. Phys.* **63** 233 2000

Combined OTM and SAXS setup tested at ESRF

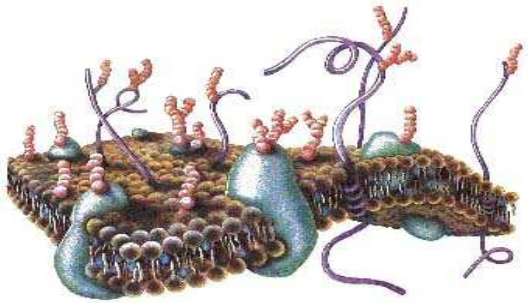




- The capillary SC, attached to the OT stage (red box), is illuminated by the WL source and observed by the imaging microscope IM.
- The x-ray beam is microfocused by x-ray focusing optics XFO inside the SC and the scattered light is recorded by the CCD.
- The IR laser beam is directed onto the SLM and shaped by the DOE, then reflected to the microscope objective MO and focused into the SC to form the trap.

D. Cojoc et al, *Appl. Phys. Lett.* **90** 234107 (2007)

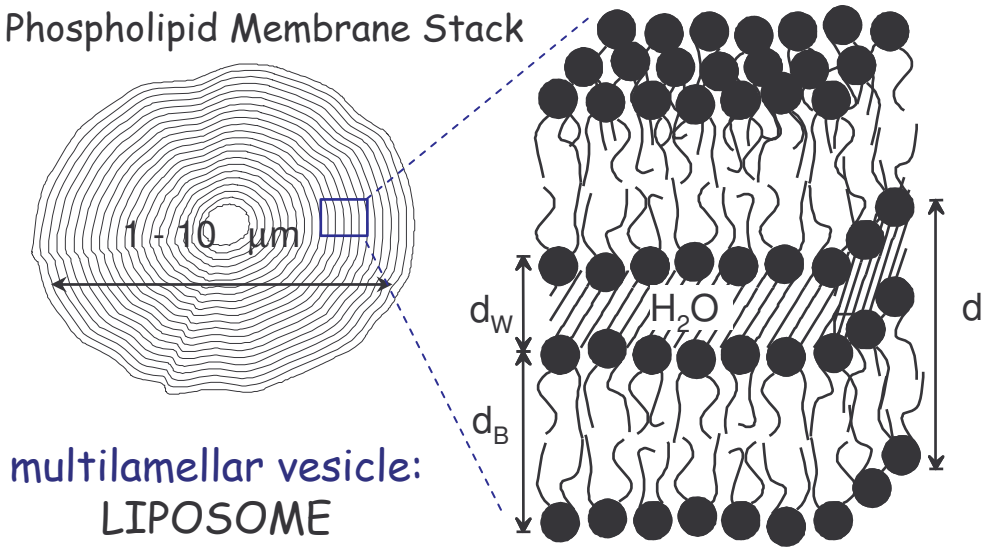
(one) Motivation to merge OT and SAXS



The boundaries of cells are formed by biological membranes, the barriers that define the inside and the outside of a cell.

Phospholipids are the major components of biological membranes that form the structural matrix into which proteins are imbedded.

In aqueous solution:
self assembly into, e.g.,
unilamellar vesicles



multilamellar vesicle:
LIPOSOME

LIPOSOMES are good models for the cell membrane

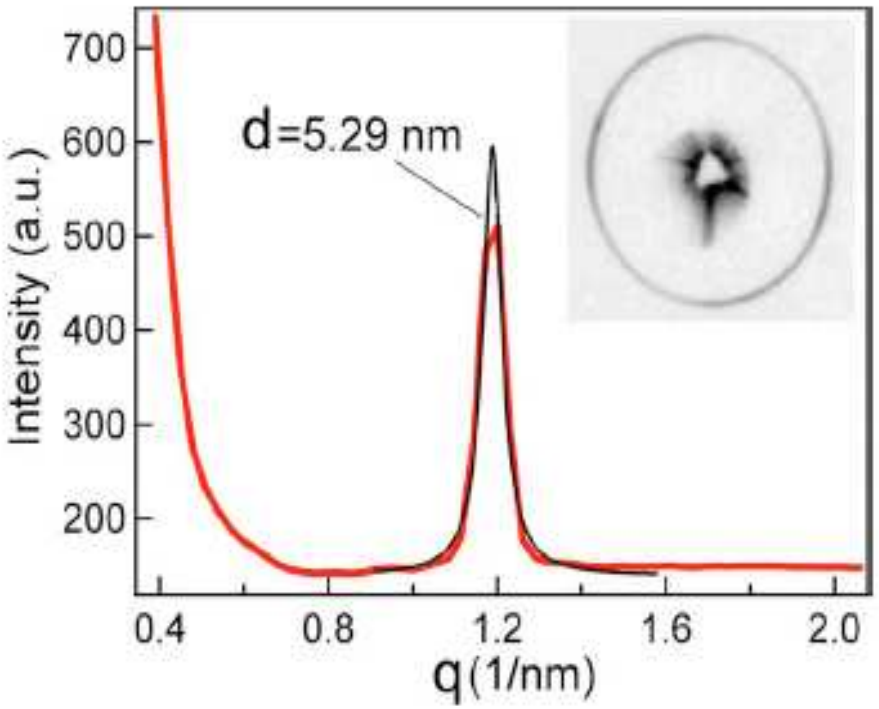
Liposomes OTM

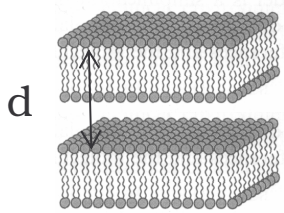
Multiple liposome trapping in capillary (80 um inner diameter)

POPE (Palmitoyl-Oleoyl-Phosphatidyl-Ethanolamine) multilamellar vesicle (1 wt%) in 1 mol $CaCl_2$, Cluster size: 8-10 μm

Liposome size: 1-2 μm, Phase: Liquid crystalline L_α

SAXS of optically trapped liposomes





POPE liposomes

(Palmitoyl-Oleoyl-Phosphatidyl-Ethanolamine)

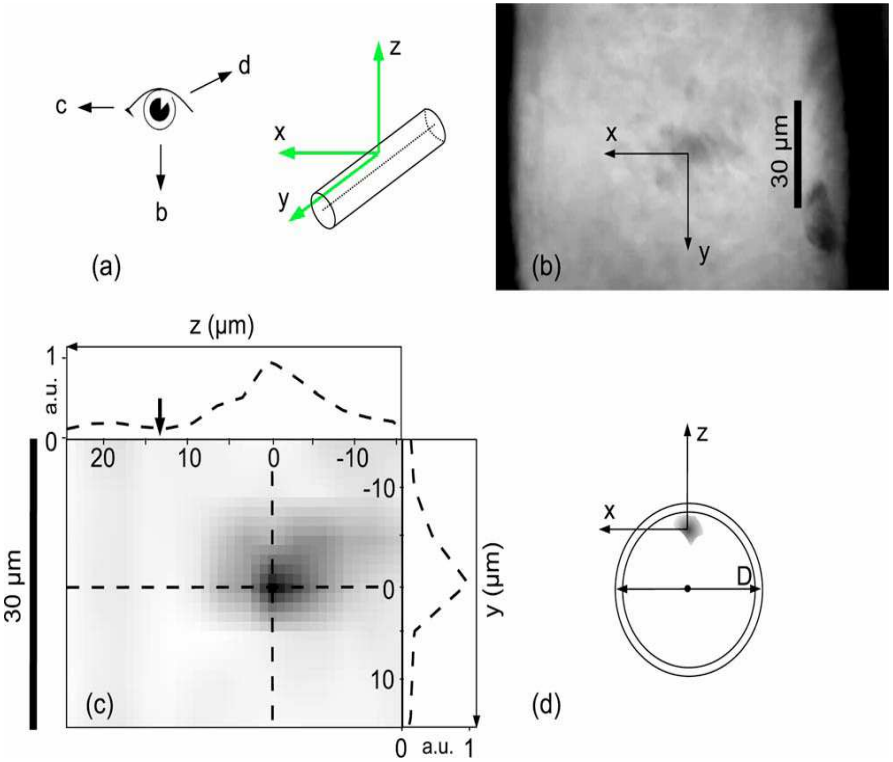
Liposome size: 1-2 μm

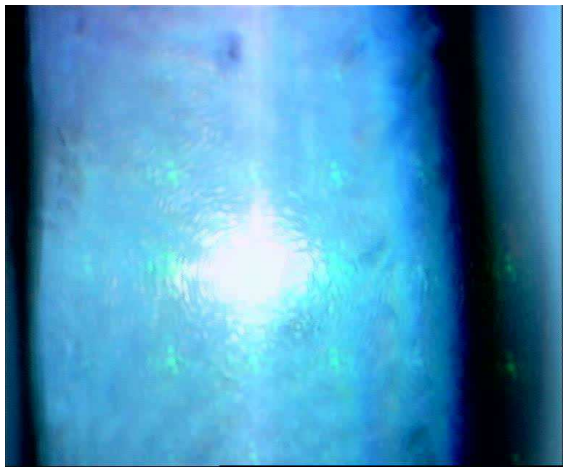
Diffraction image: exposure time 5 s

Plot of the azimuthally integrated intensity of a trapped POPE cluster (red line) and the Lorentzian function (black line) fitted to experimental data.

The inset shows the 2D diffraction pattern as recorded by the CCD detector.

D. Cojoc et al, *Appl. Phys. Lett.* **90** 234107 (2007)





Microcanning SAXS of an optically trapped POPE liposome cluster.

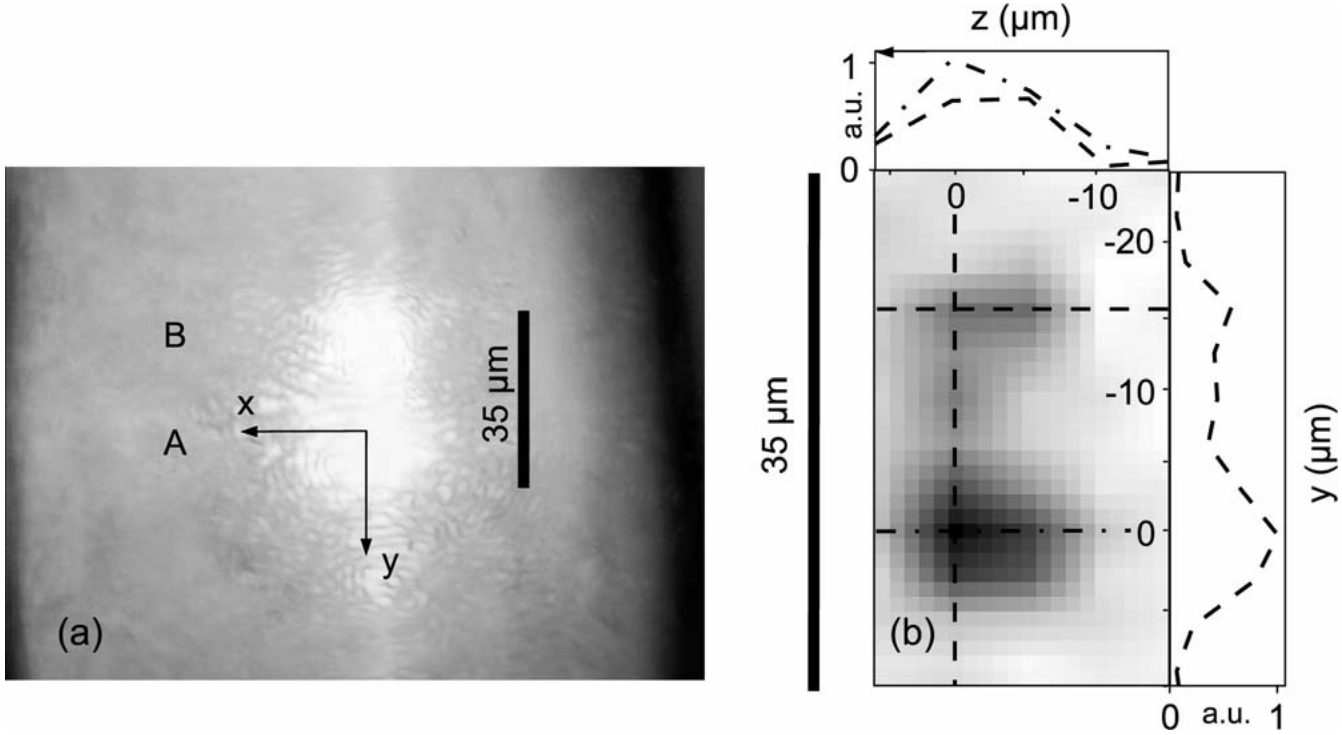
(a) The three view points for the trapping region in capillary.

(b) Optical image of the POPE cluster in the capillary.

(c) X-ray diffraction image of the POPE cluster the arrow indicates the capillary wall.

(d) Transversal section of the capillary with the position of the trapped POPE cluster.

D. Cojoc et al, *Appl. Phys. Lett.* **90** 234107 (2007)



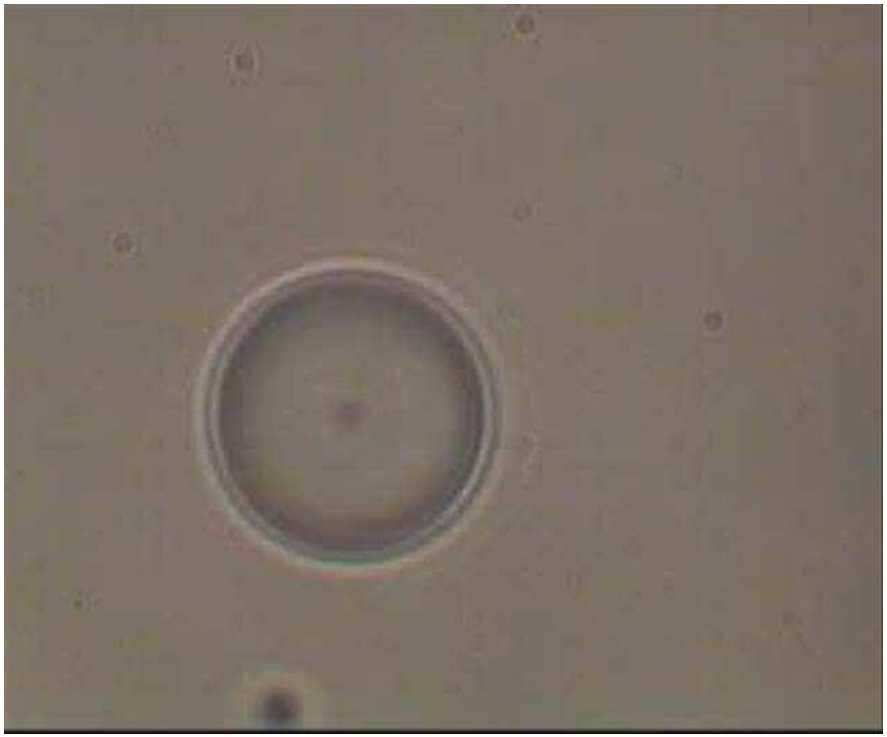
Multiple trapping of liposomes in two locations

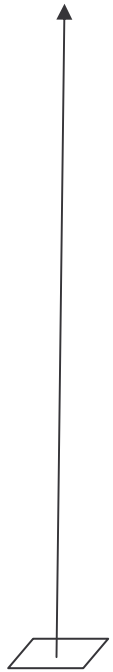
(a) Microscope image of the capillary with the IR-laser switched on, two laser traps are visible.

(b) X-ray diffraction image of the two clusters, viewed in *x* direction.

D. Cojoc et al, *Appl. Phys. Lett.* **90** 234107 (2007)

Starch granules OTM

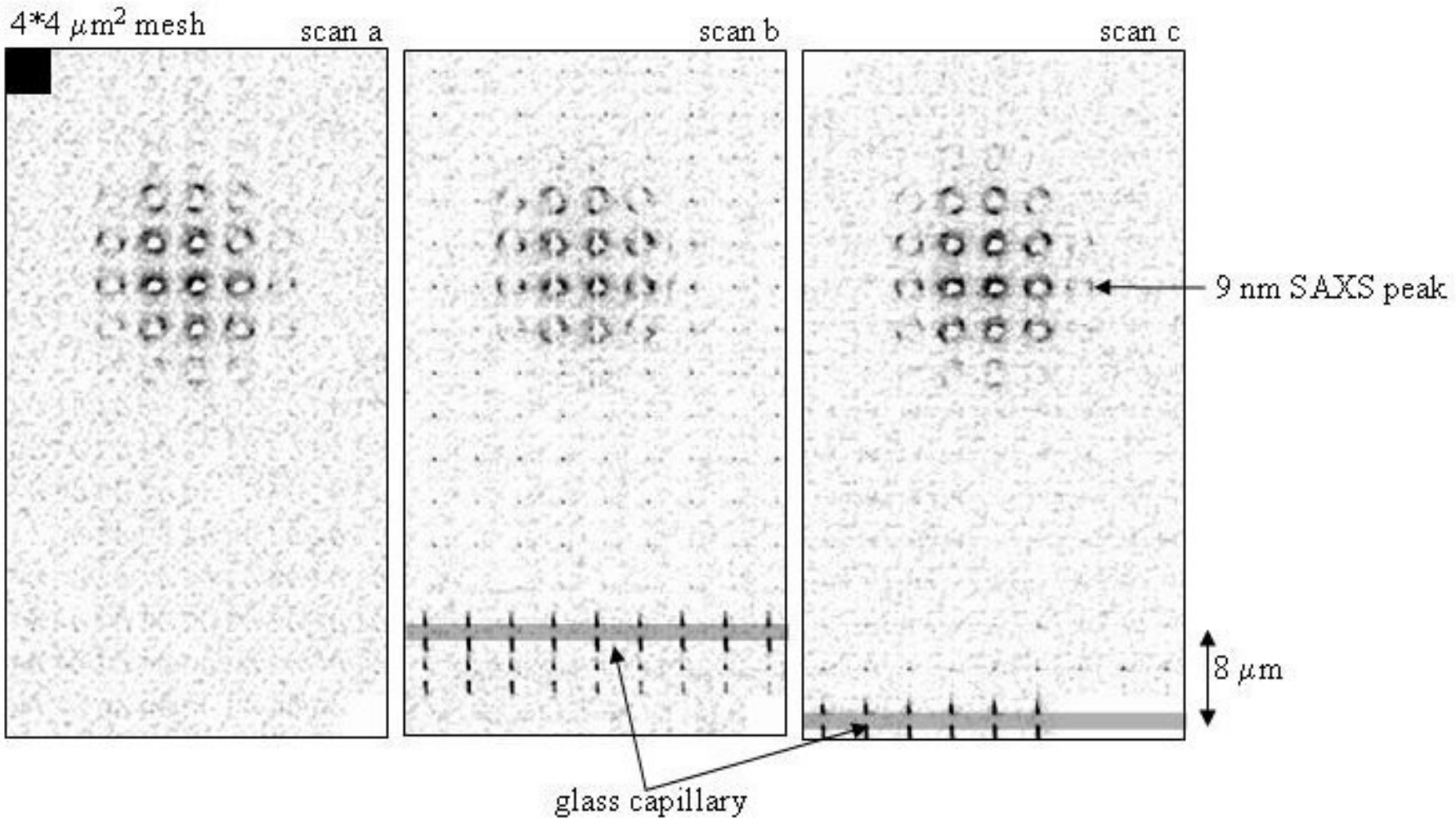




Starch granule trapped in square capillary (100 um inner size)

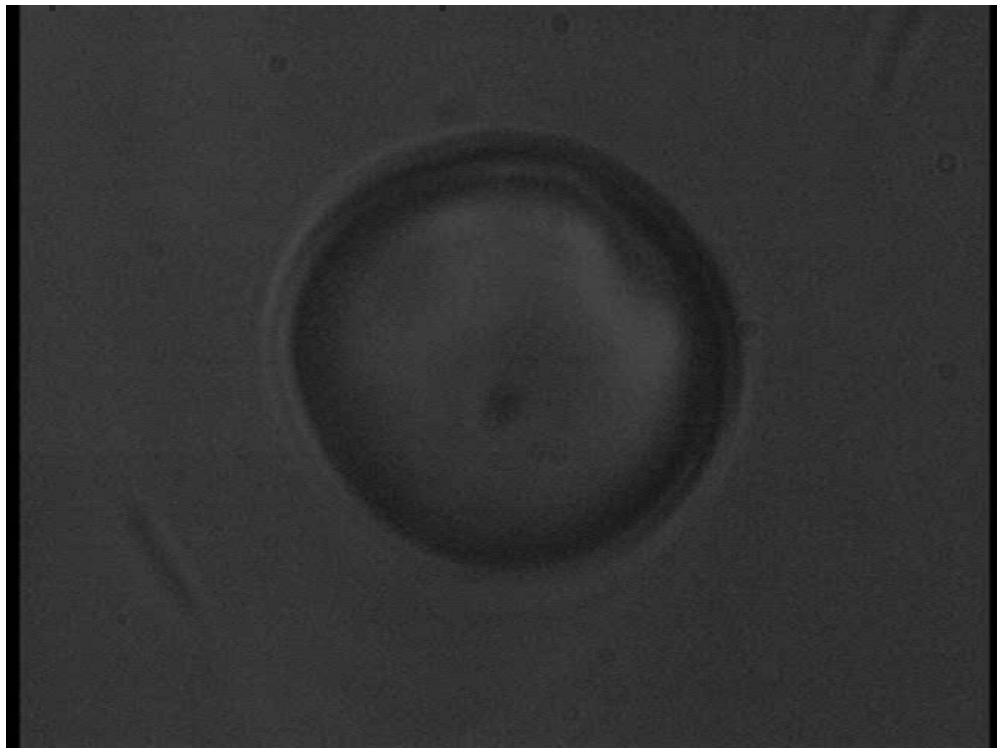
SAXS of optically trapped starch granules (from potatoe)

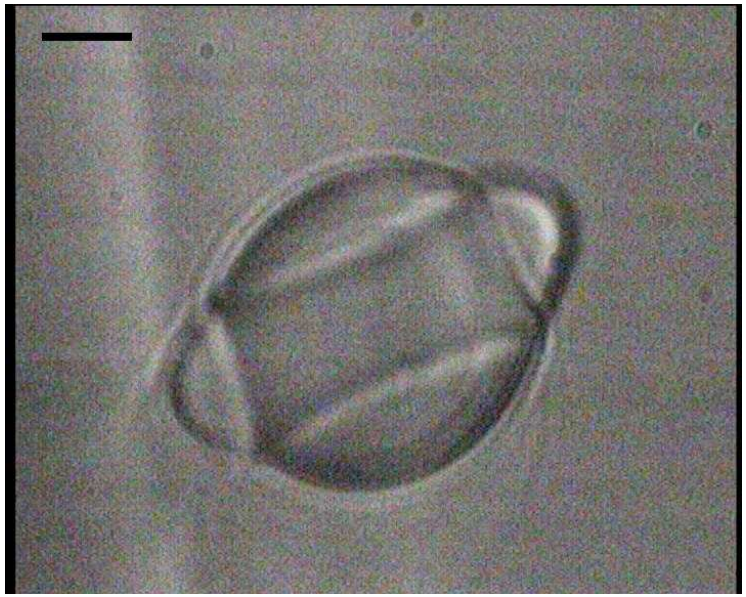
Exp 2007: scanning of a single granule



SAXS of optically trapped starch granules (from potatoe)

Exp 2007





Look at the radiation damage in Real time !!!!! ☺ ☹

Example 3

OTM for force spectroscopy of growing neurons

Dan Cojoc, Enrico Ferrari, Enzo Di Fabrizio
CNR-INFM, Laboratorio Nazionale TASC Trieste

Francesco Di Fato, Rajesh Shahapure, Jumi Laishram,
Massimo Righini, Vincent Torre
SISSA, Neurobiology sector, Trieste

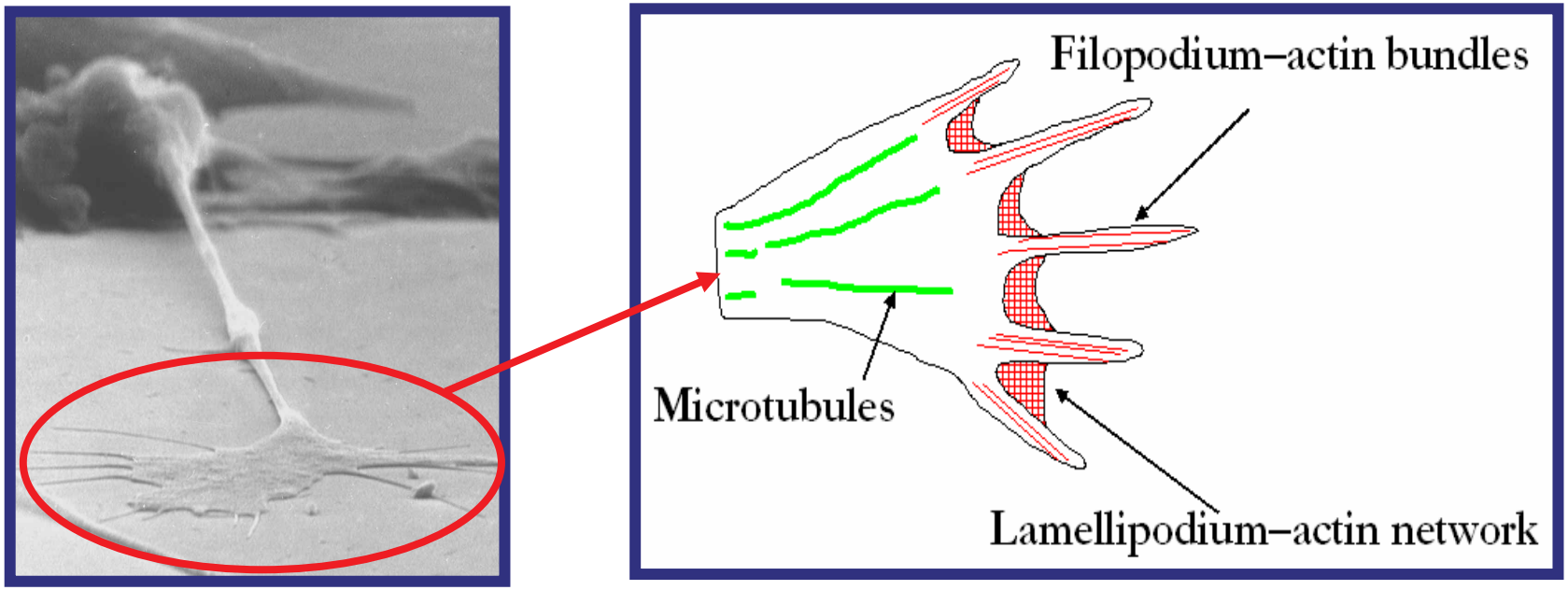
D. Cojoc, et al, *PLoS ONE* **2(10):** e1072. doi:10.1371/journal.pone.0001072 (2007)

Motivation and goal of our work

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

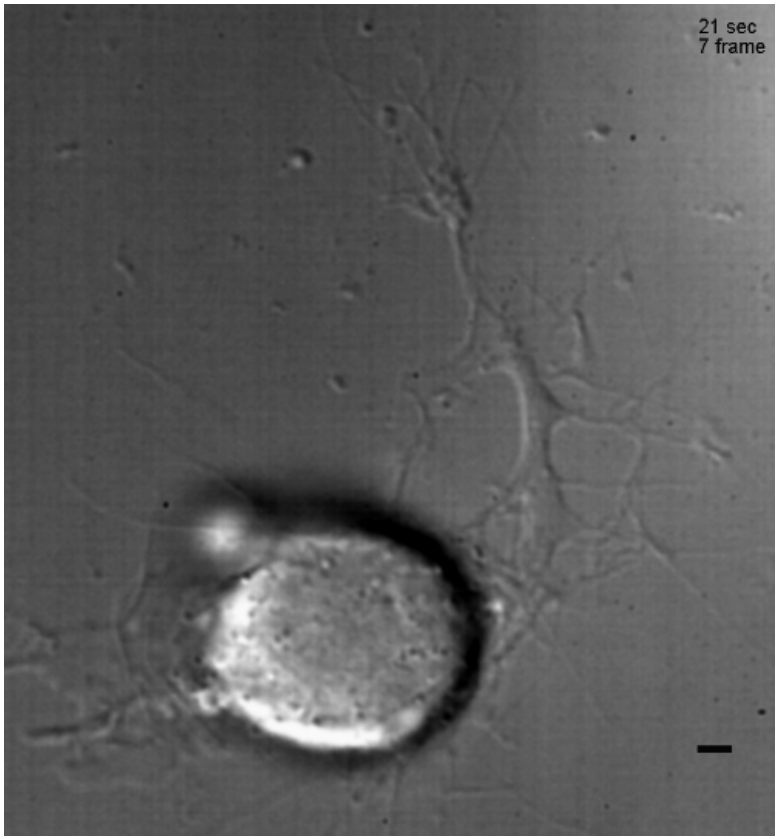
"They will adopt pre-determined directions and establish connections with defined neural or extra neural elements ... without deviations or errors, as if guided by an intelligent force ." *1890 RAMON Y CAJAL*

Structural elements of the growth cone

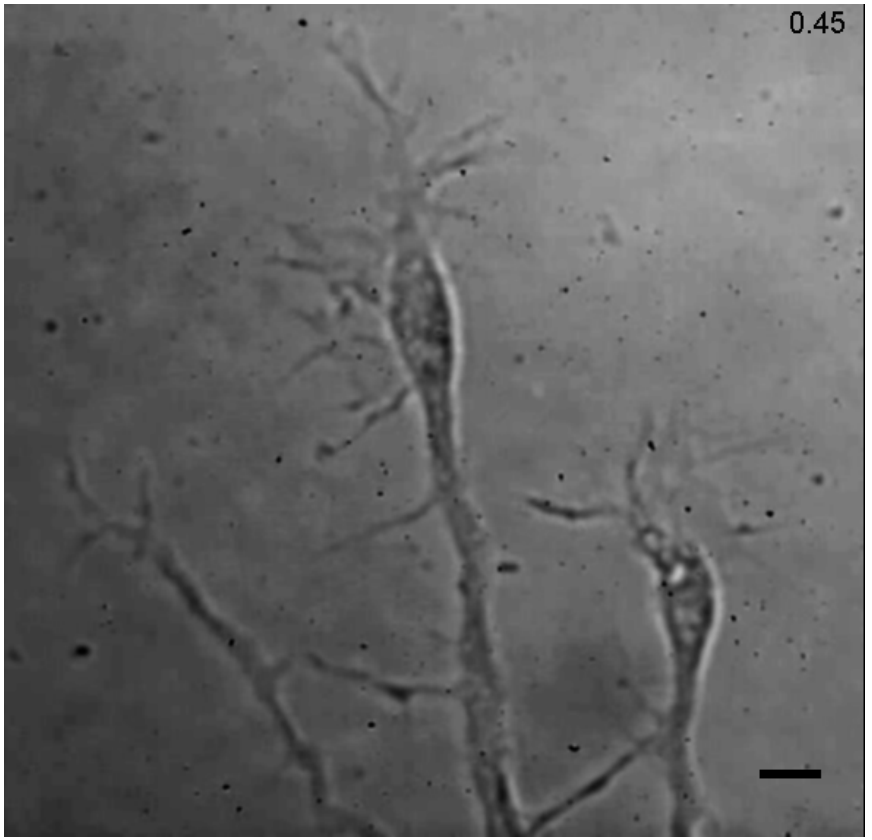


www.biology.lsa.umich.edu/research/labs/ktosney/

Growth cone dynamics

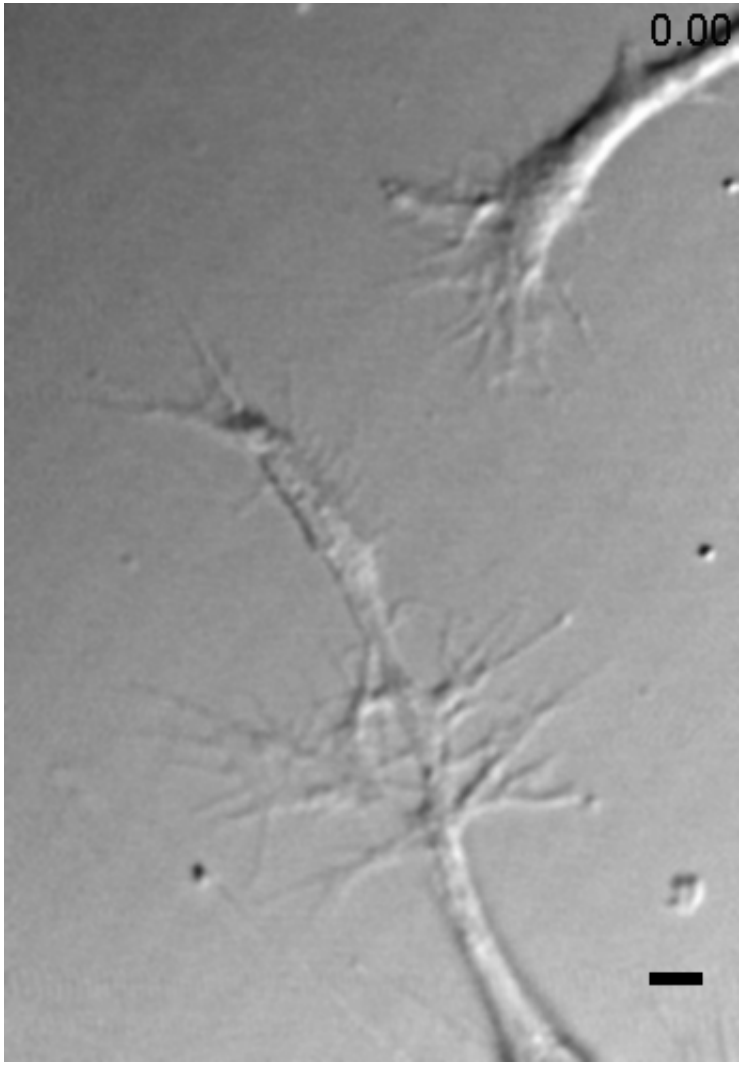


Scale bar = 2 μm;
Acquisition freq = 0.3Hz



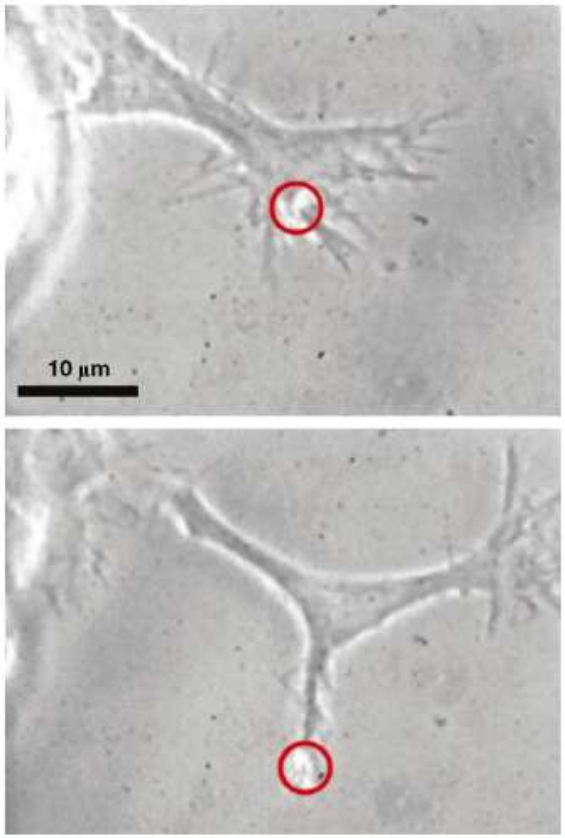
Scale Bar = 5μm
Acquisition freq= 0.2Hz
Time in min.sec

Growth cones connection

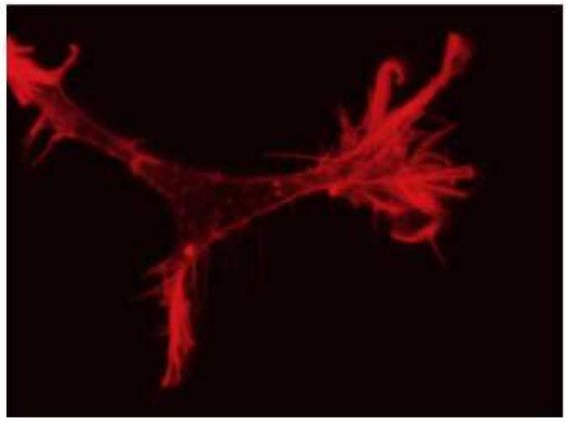


Scale Bar = 3 μm
Acquisition freq= 0.2Hz
Time in min.sec

Guiding neuronal growth with light



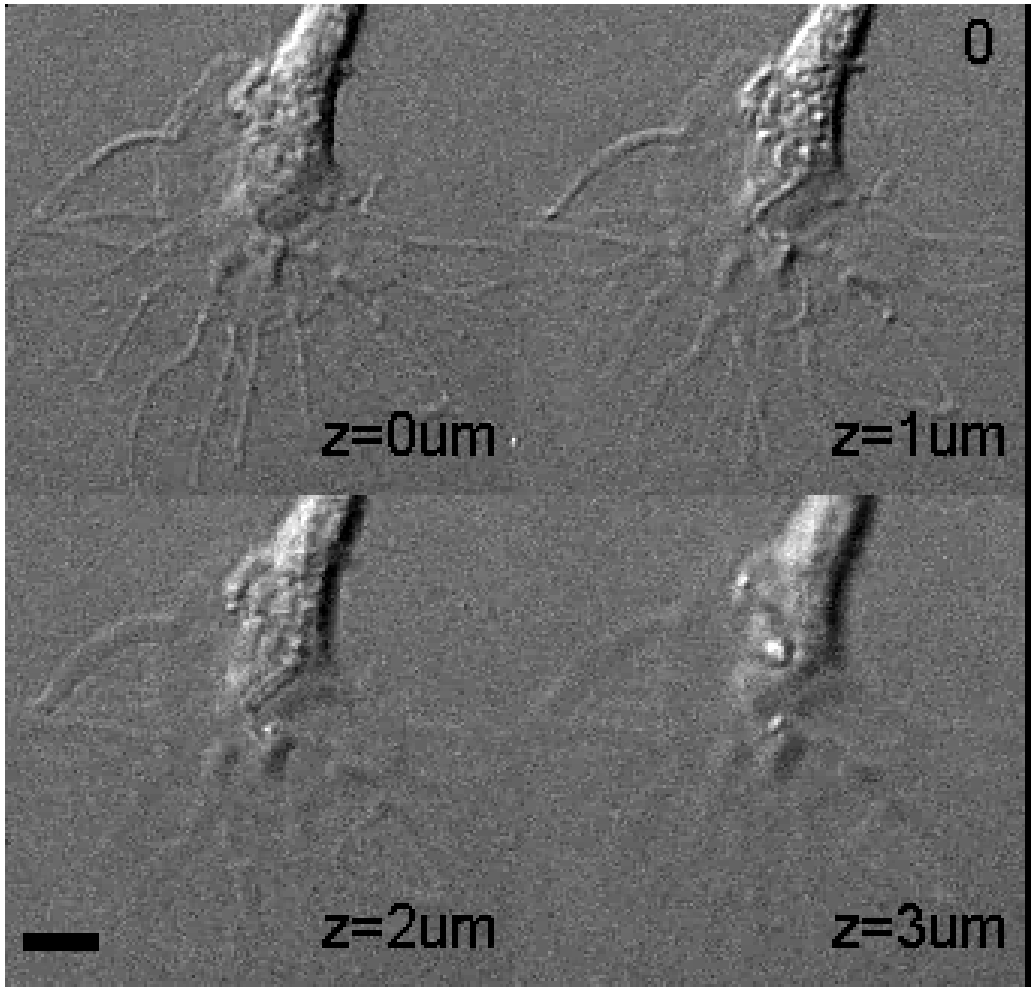
Optically induced bifurcation of a growth cone. A growth cone, which is growing to the upper right, sprouts off an extension to the lower right under the influence of the beam marked by a circle.



The distribution of actin filaments by rhodamine-phalloidin staining. Actin filaments are clearly accumulated at the areas of lamellipodia extension.

A. Ehrlicher et al, PNAS 99 16024 (2002)

Growth cone dynamics
confocal microscopy

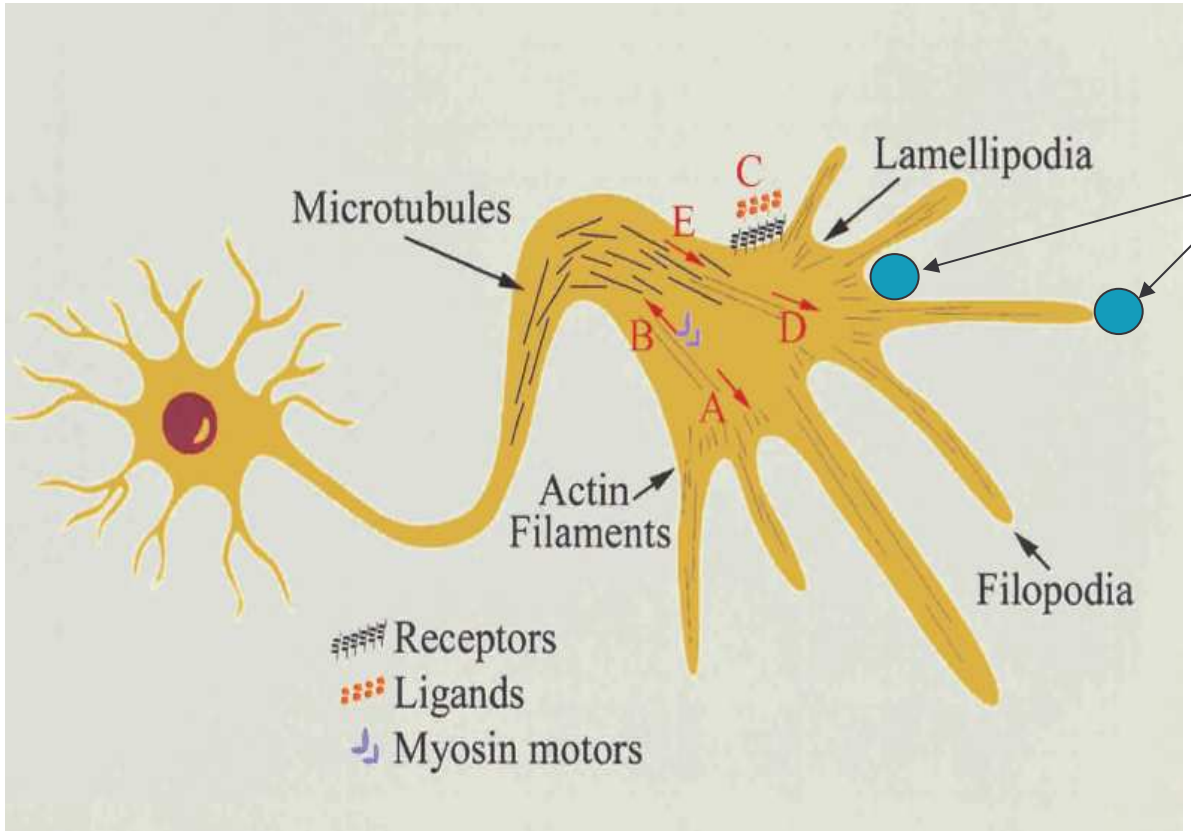


Scale Bar = 3 μm, Time in sec

Goal

measure the forces exerted by lamellipodia and filopodia

Experimental Approach

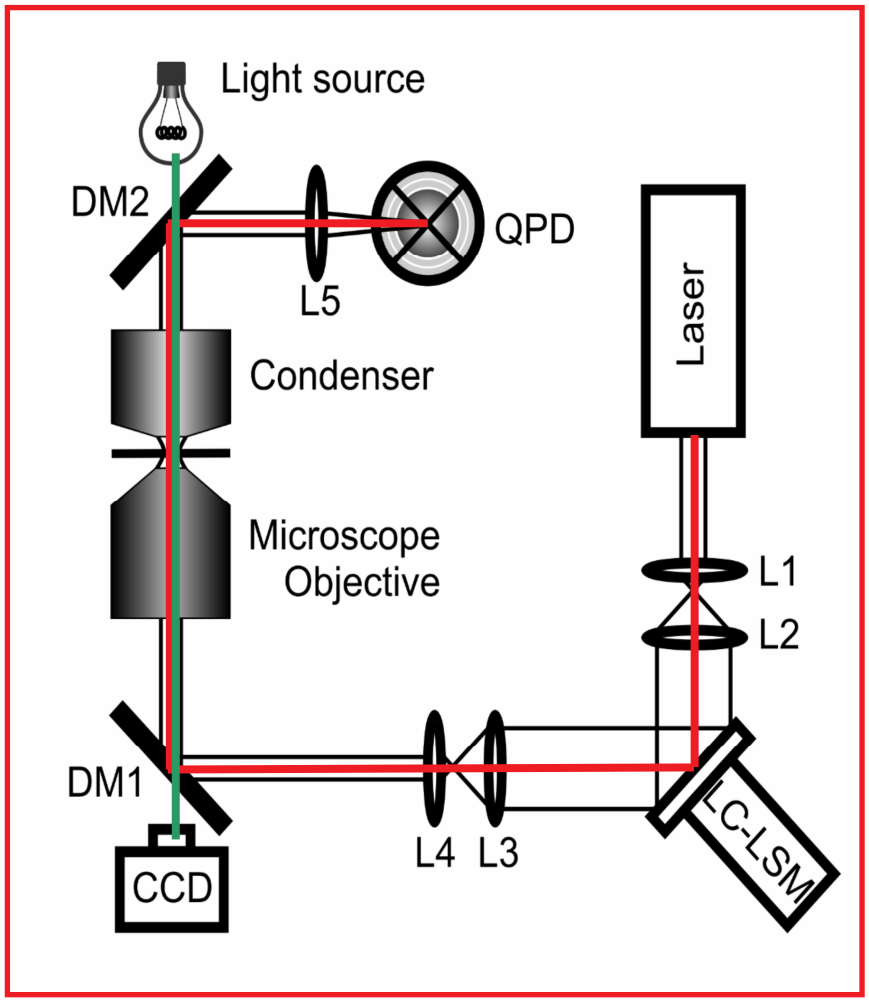


- Calibrate the trap
- Micro-beads trapped by IR laser and positioned in front of lamellipodia and/or filopodia
- Measure the fluctuations of the bead in the trap, due to its interaction with the motile structures, and convert them into forces.

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

Optical Tweezers setup

Includes Optical Manipulation and Force Spectroscopy



LC-LSM: Liquid Crystal Spatial Light Modulator

CCD: Charged Coupled Device

L: Lens

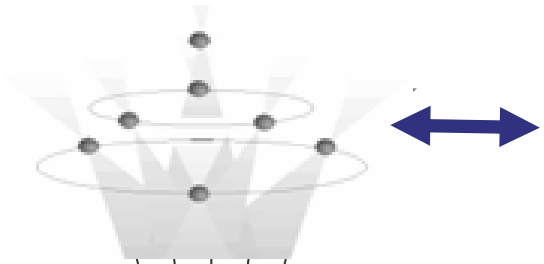
DM: Dichroic Mirror

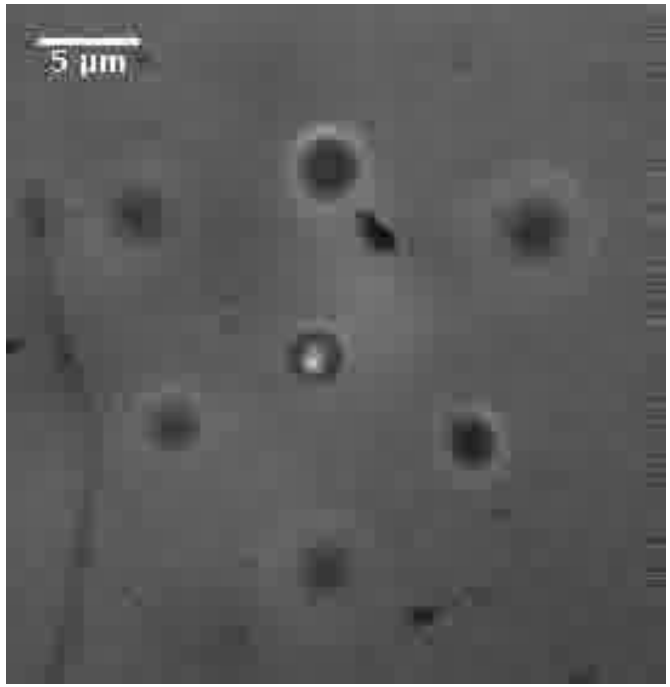
QPD: Quadrant Photo-Diode

Optical Manipulation

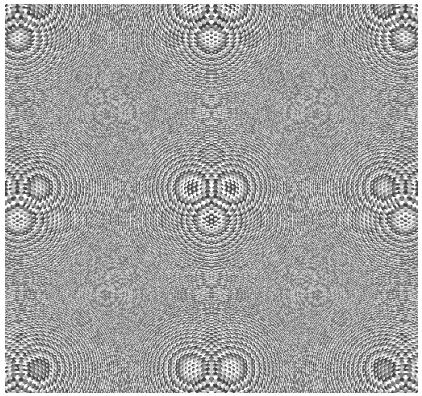
by means of diffractive optical elements (DOE) implemented on a Spatial Light Modulator (SLM)

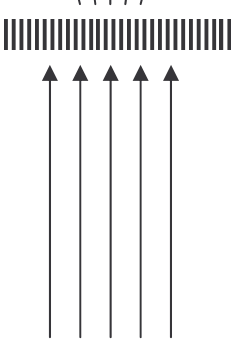
Working volume:





DOE





SLM

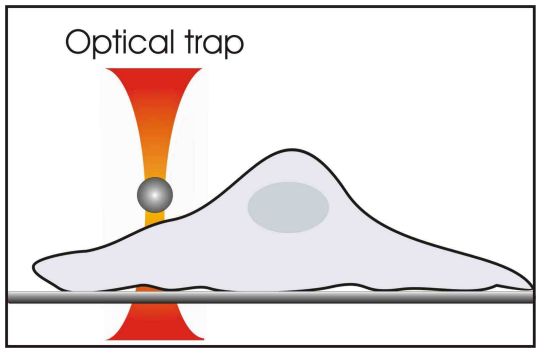
Input Wave
(Gaussian)

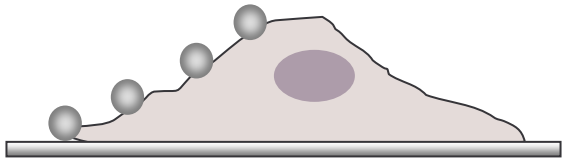
D. Cojoc *et al* Jap.J.Appl.Phys. ***43***(6), 3910 (2004)

E. Ferrari *et al* Microelectronic Engineering ***78-79***, 125 (2005)

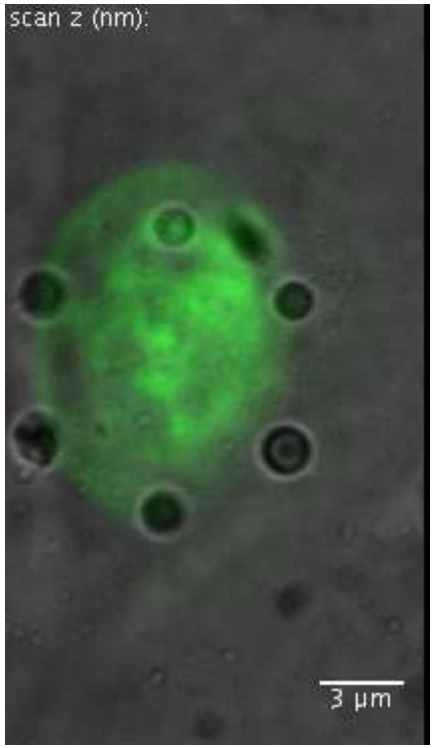
Mechanical stimulation of cells with pN forces

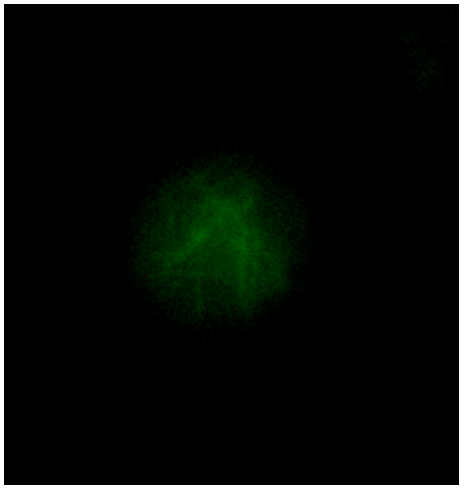
With the optical tweezers technique one can control very precisely the mechanical stimulation at the level of single or multiple adhesion sites





HeLa cell surrounded by a 2D array of beads

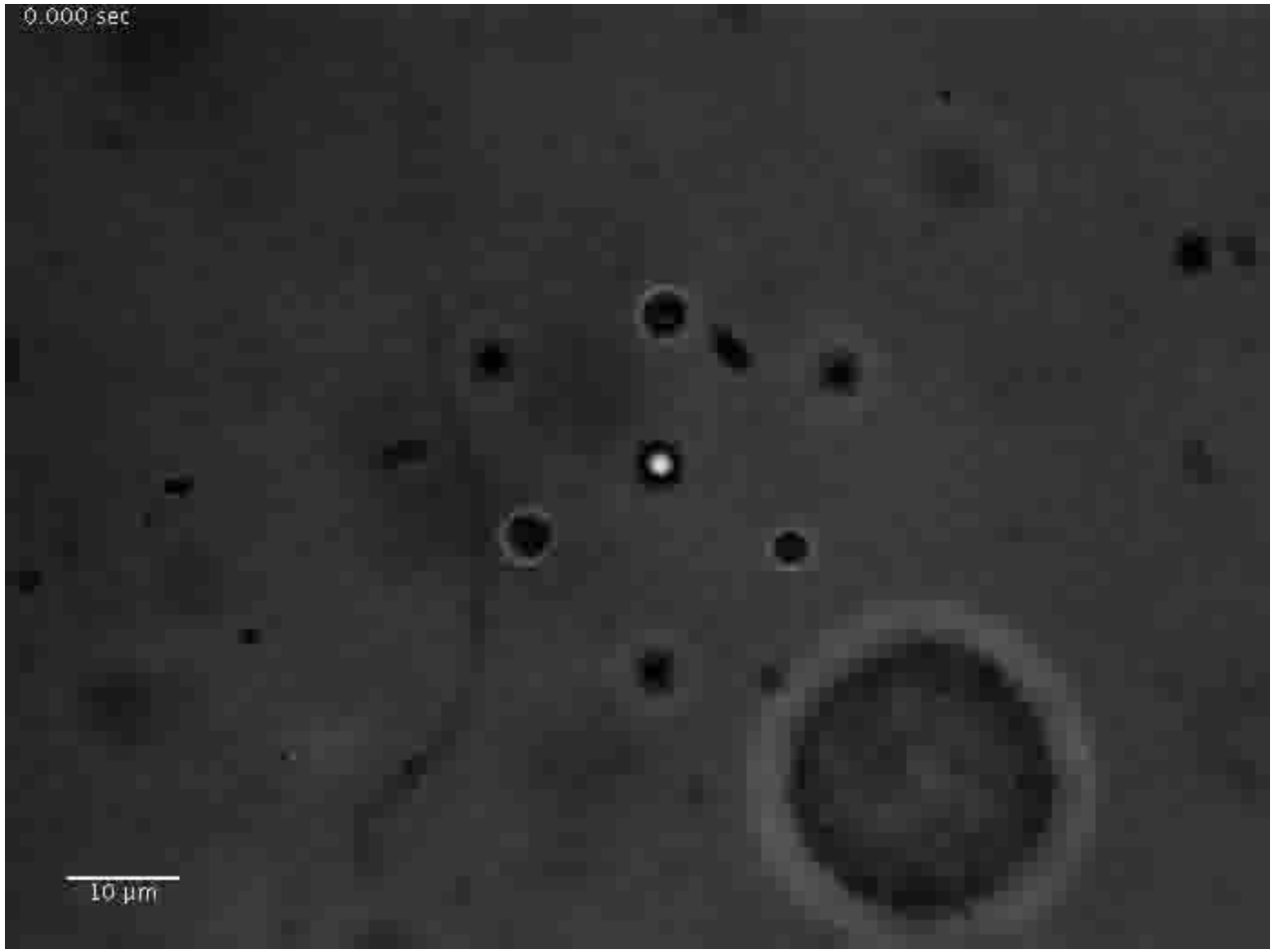




In collaboration with Institute Jacques Monod, Paris

V. Emiliani *et al*, Optics Express, ***13*** 1395 (2005)

HeLa cell surrounded by a 3D array of beads, get stressed

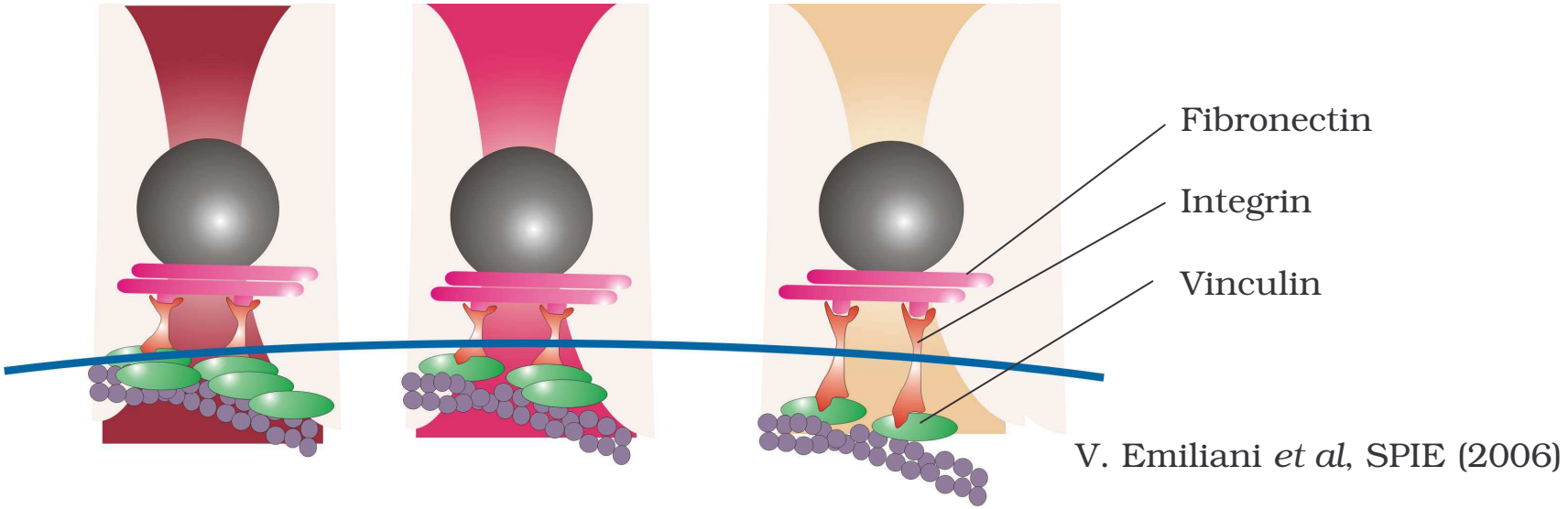


Force dependent Vinculin recruitment

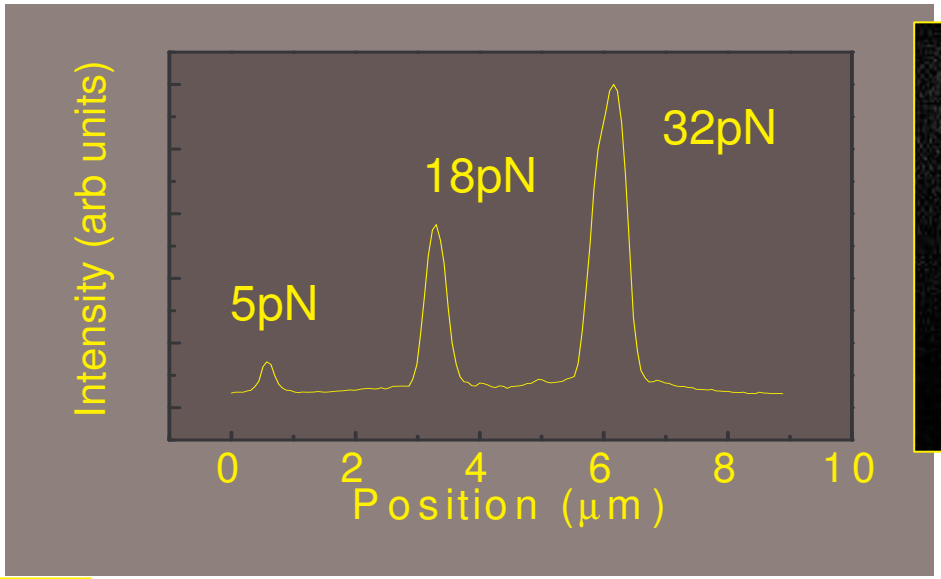
The multi force optical tweezers is combined with an epi-fluorescence microscope.

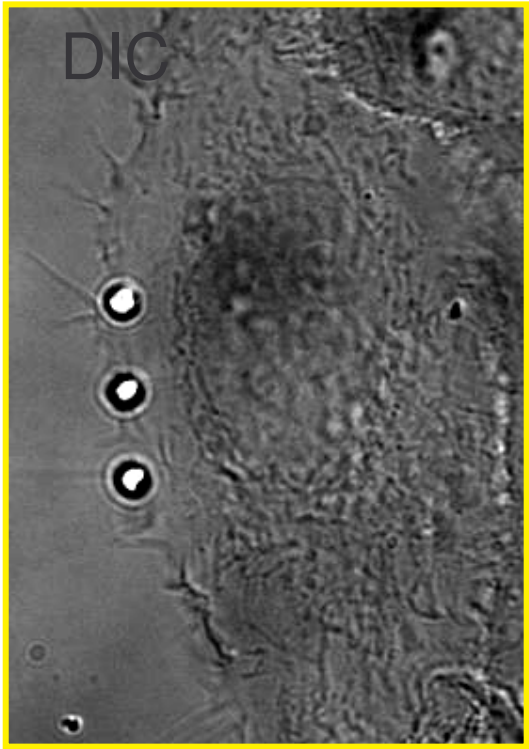
Monitor vinculin recruitment as a function of applied forces

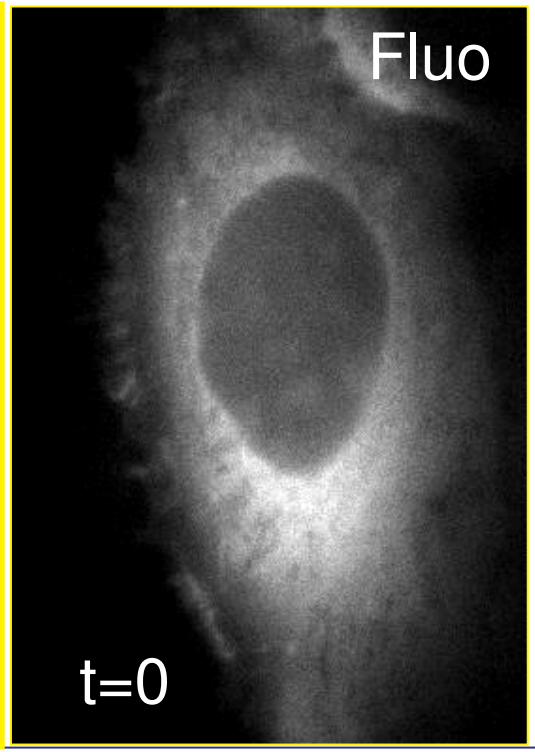
by attaching and maintaining Fibronectin coated beads on the dorsal surface of Vin-GFP transfected HeLa cell.

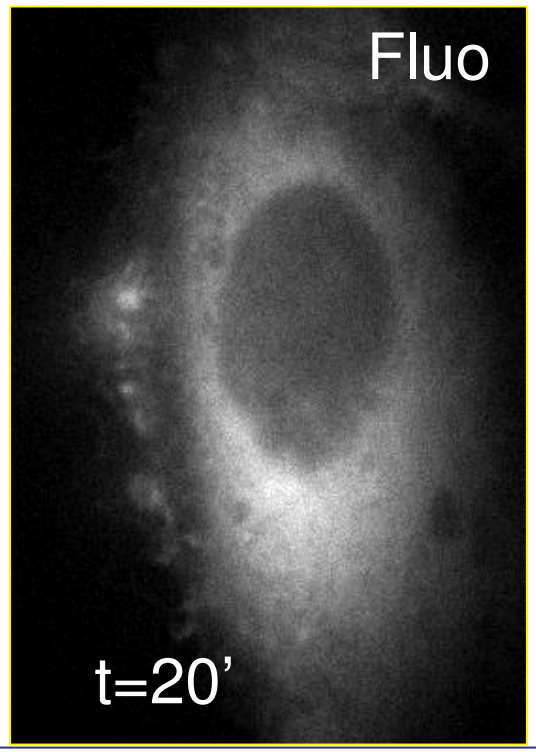


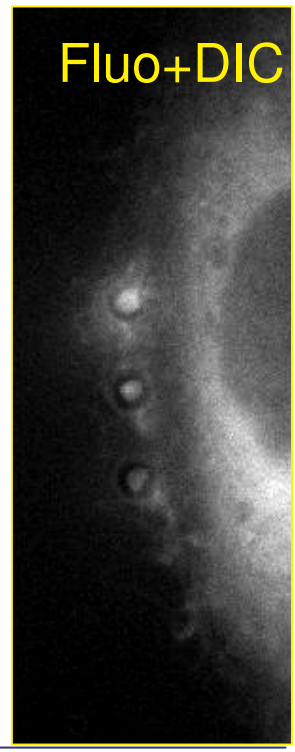
V. Emiliani *et al*, SPIE (2006)


Intensity (arb units)
5pN
18pN
32pN
0
2
4
6
8
10
Position (µm)

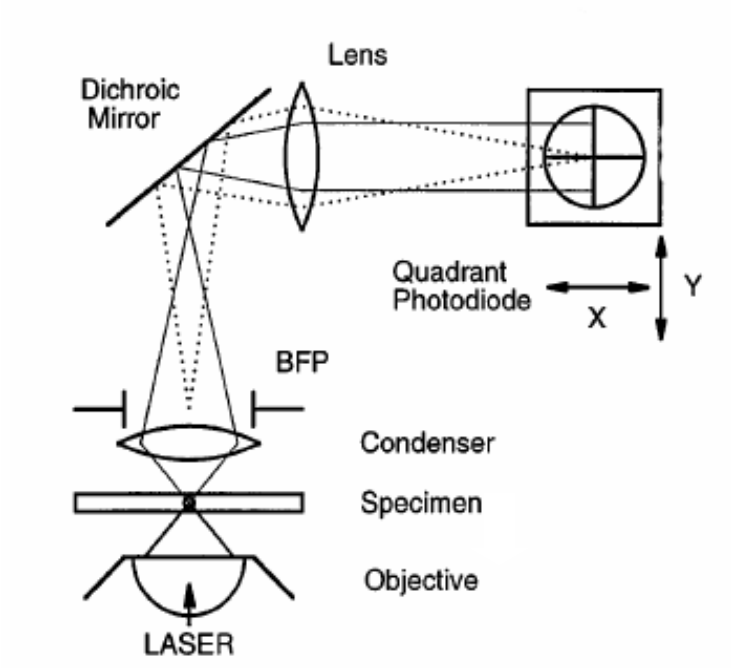

DIC


Fluo
t=0


Fluo
t=20’

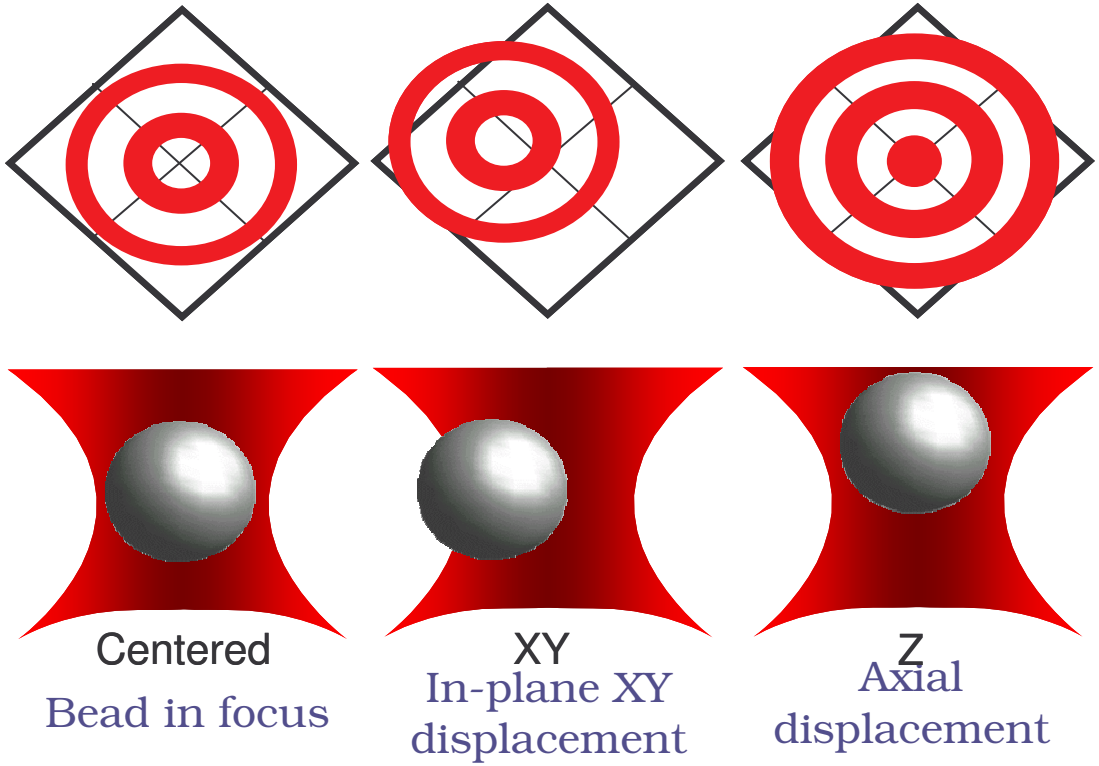

Fluo+DIC

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



F. Gittes, Optics Letters, (1998)

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



$$F = K \Delta$$

F= Force

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

Δ = (Δx, Δy, Δz)Displacement

Trap stiffness and detector sensivity

Sv(f) - measured power spectrum
S(f) - density Lorentzian fit

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

f_0 – corner frequency

k – trap stifness

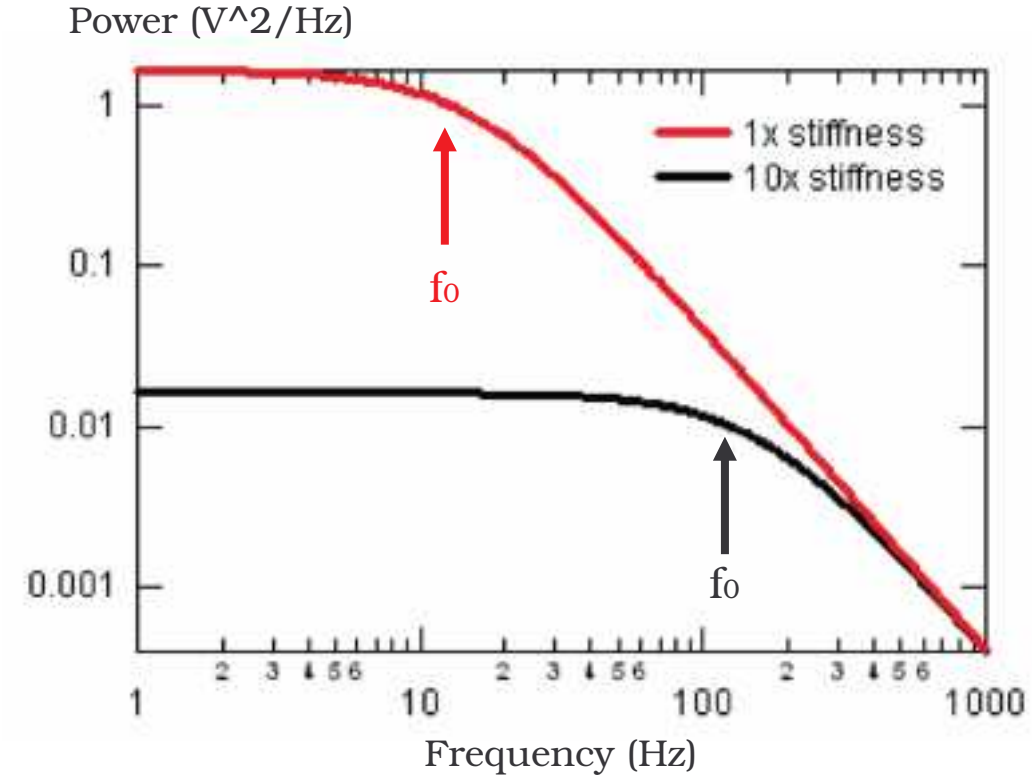
γ – Stokes drag coefficient

β – detector sensivity

S_0 – trap stifness

PV – plateu of $f^2 S^V(f)$

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



The power spectrum for two different stiffnesses

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

Force measurements - 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:

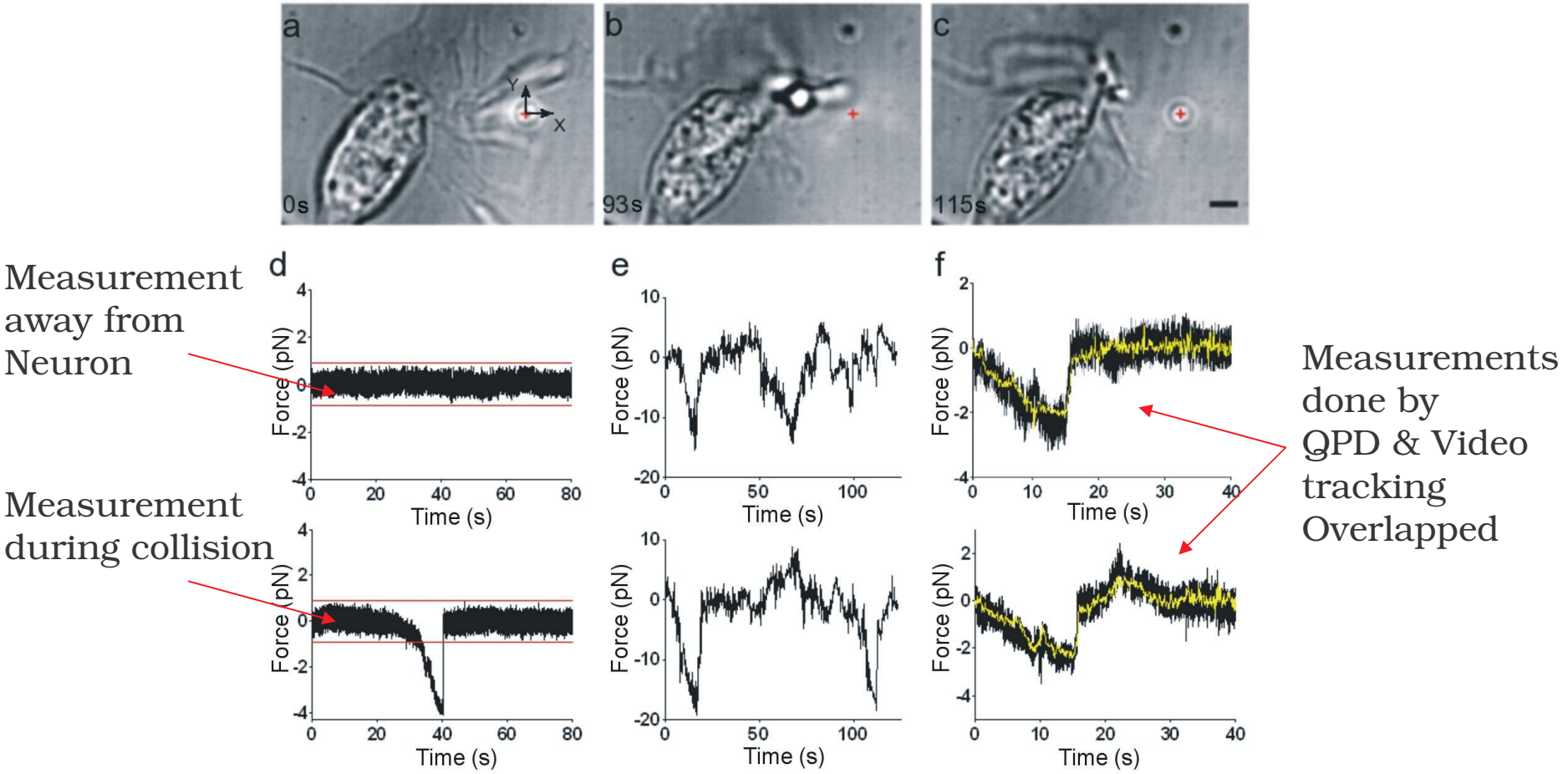
Stuck beads to the substrate

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

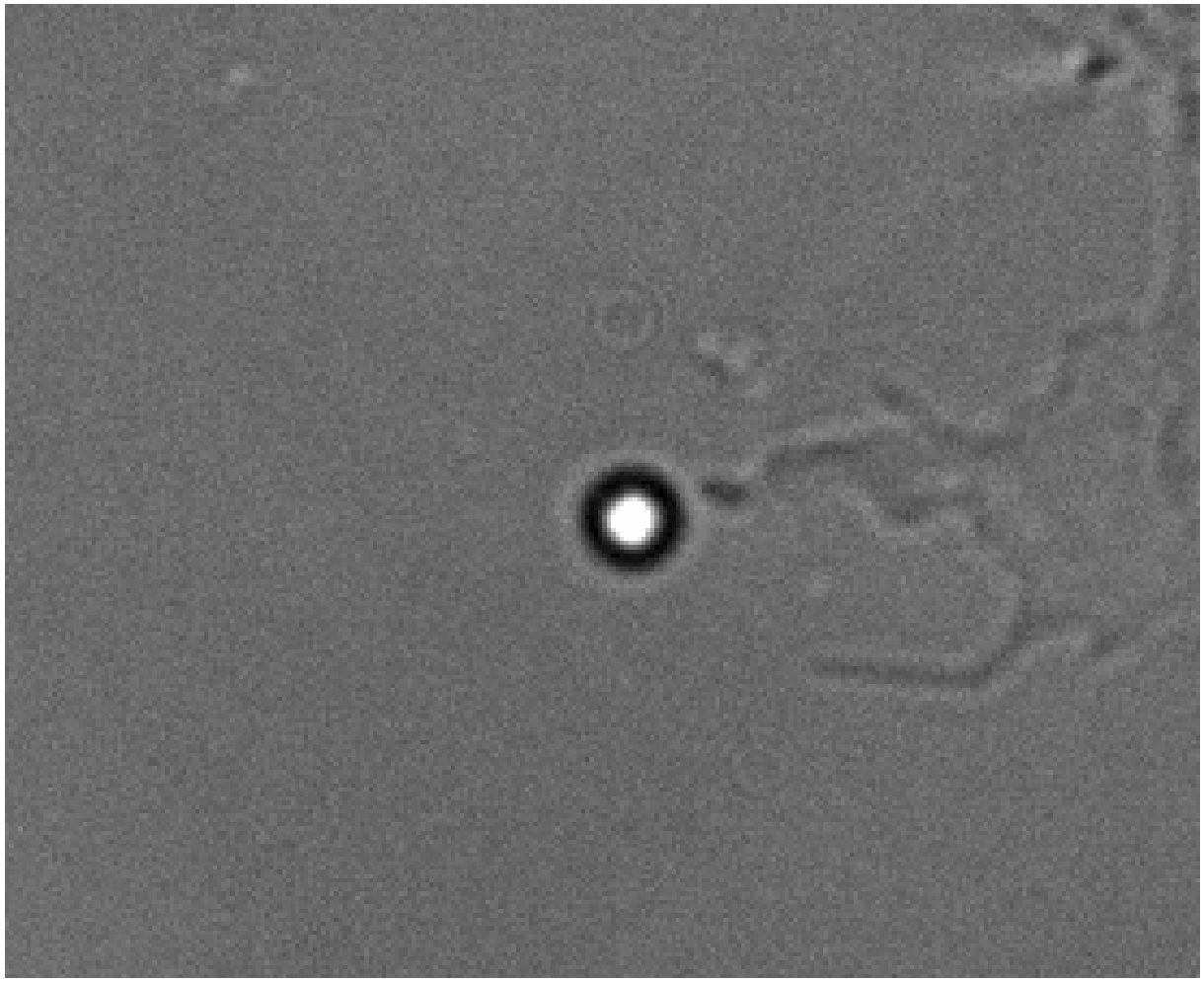
Influence of floating particles on the interference pattern

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

Criteria to define a collision

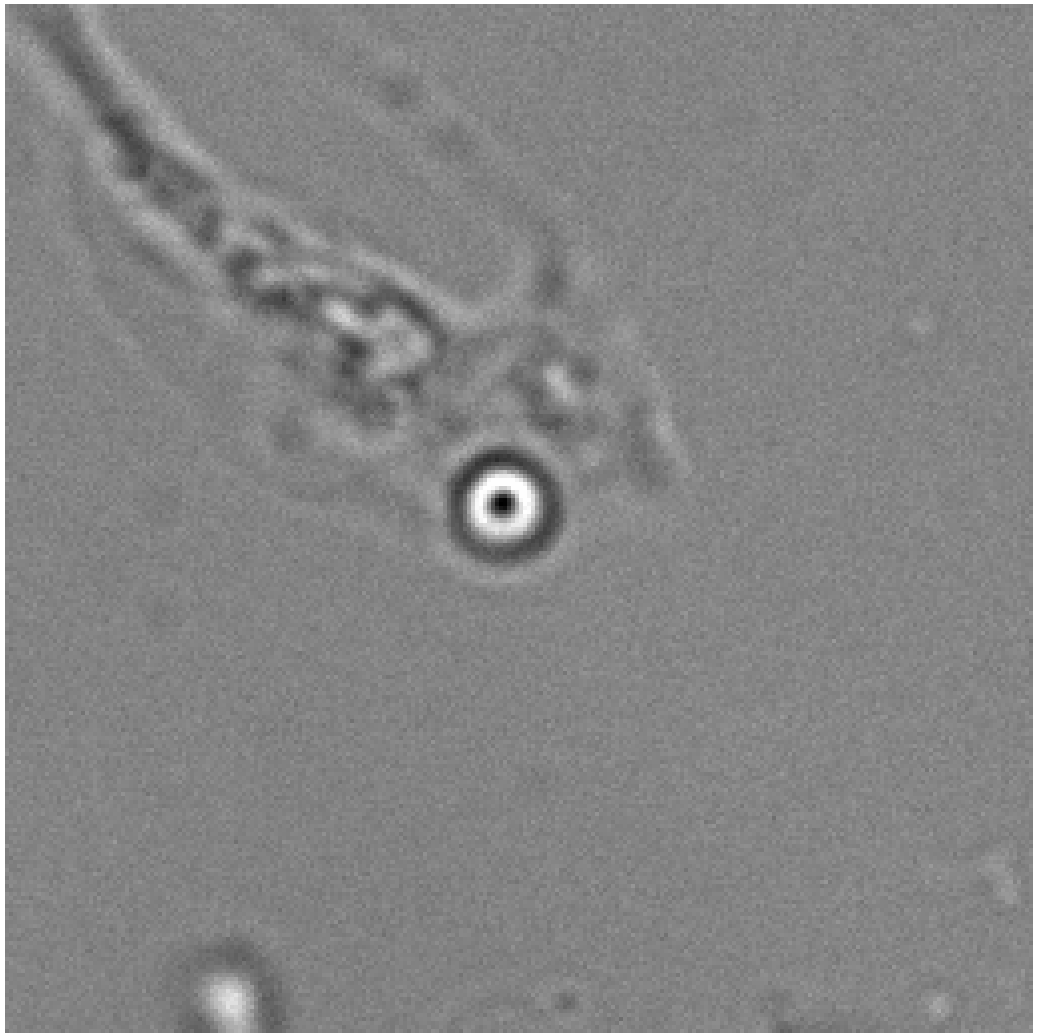


Filopodia 2 minutes event, Fmax= 3 pN

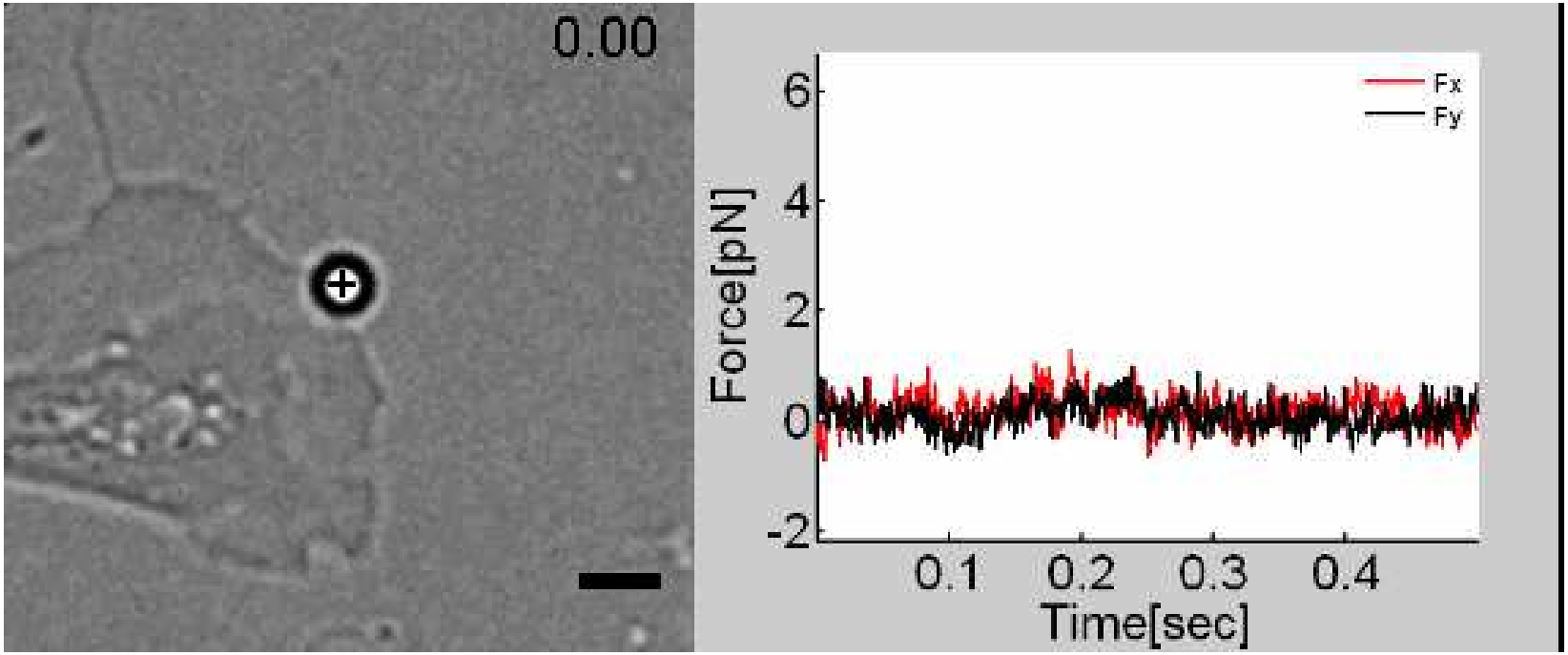


Lamellipodia 2 minutes event,

Fmax measured = 20pN



Force exerted by Lamellipodia



Aquisition rate: 20Hz

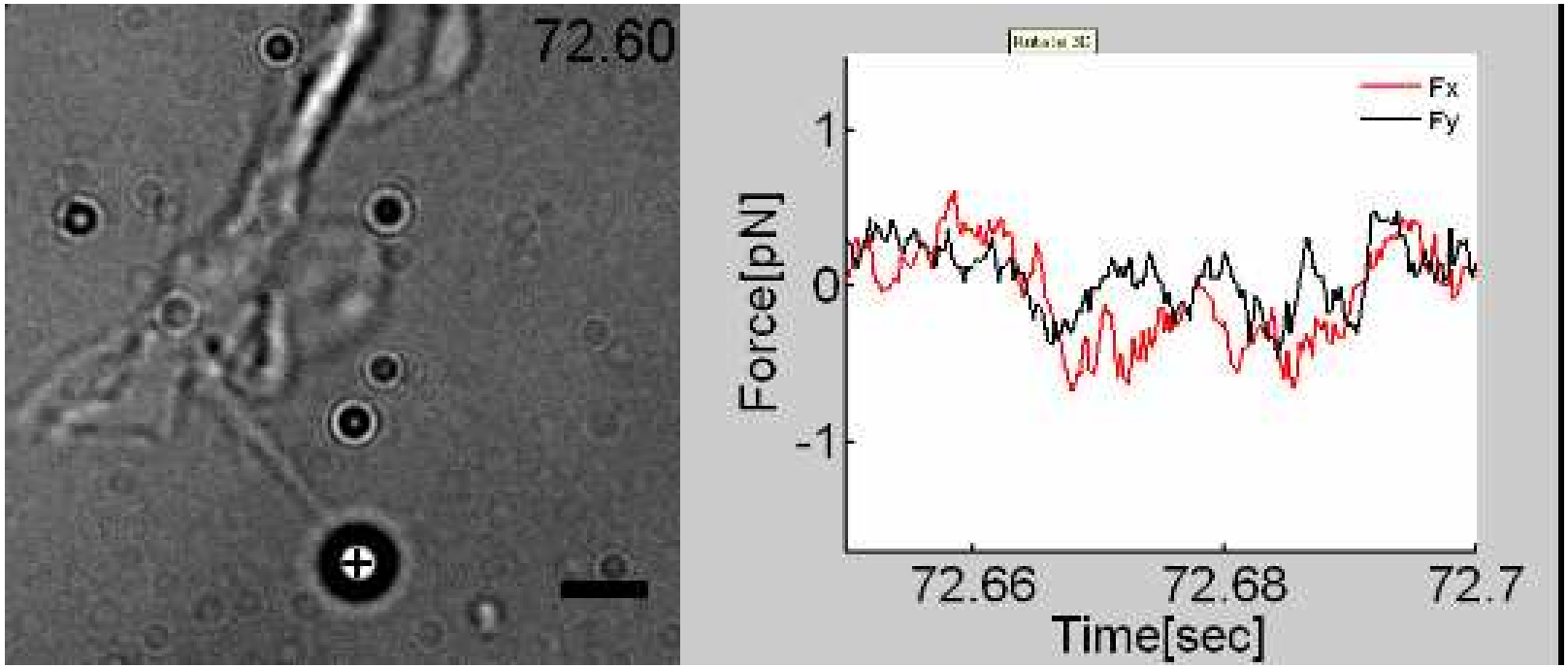
Scale Bar = 2μm

Time in seconds

Aquisition rate : 4KHz

Subsampled at : 2KHz

Force exerted by Filopodia - Protrusion



Acquisition rate: 20Hz

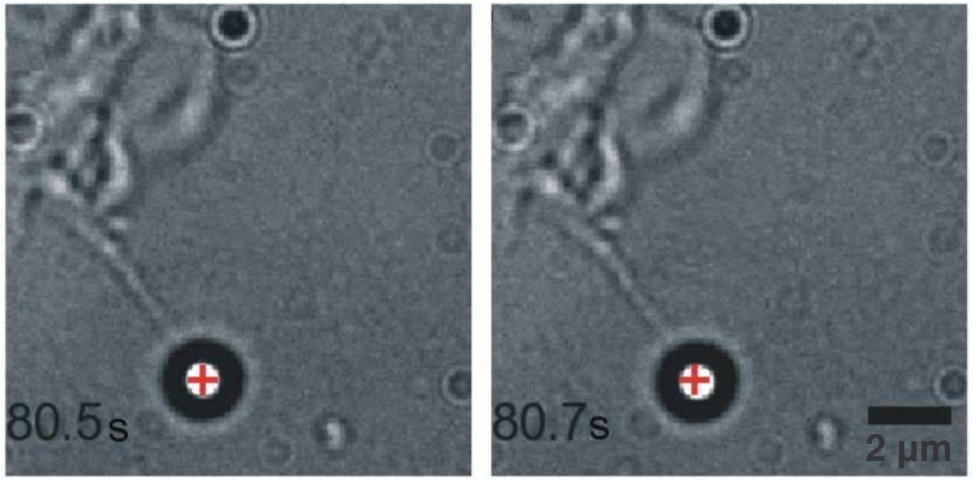
Scale Bar = 2µm

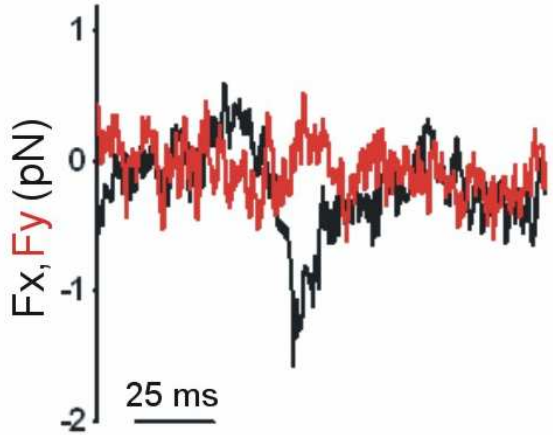
Time in seconds

Acquisition rate : 4KHz

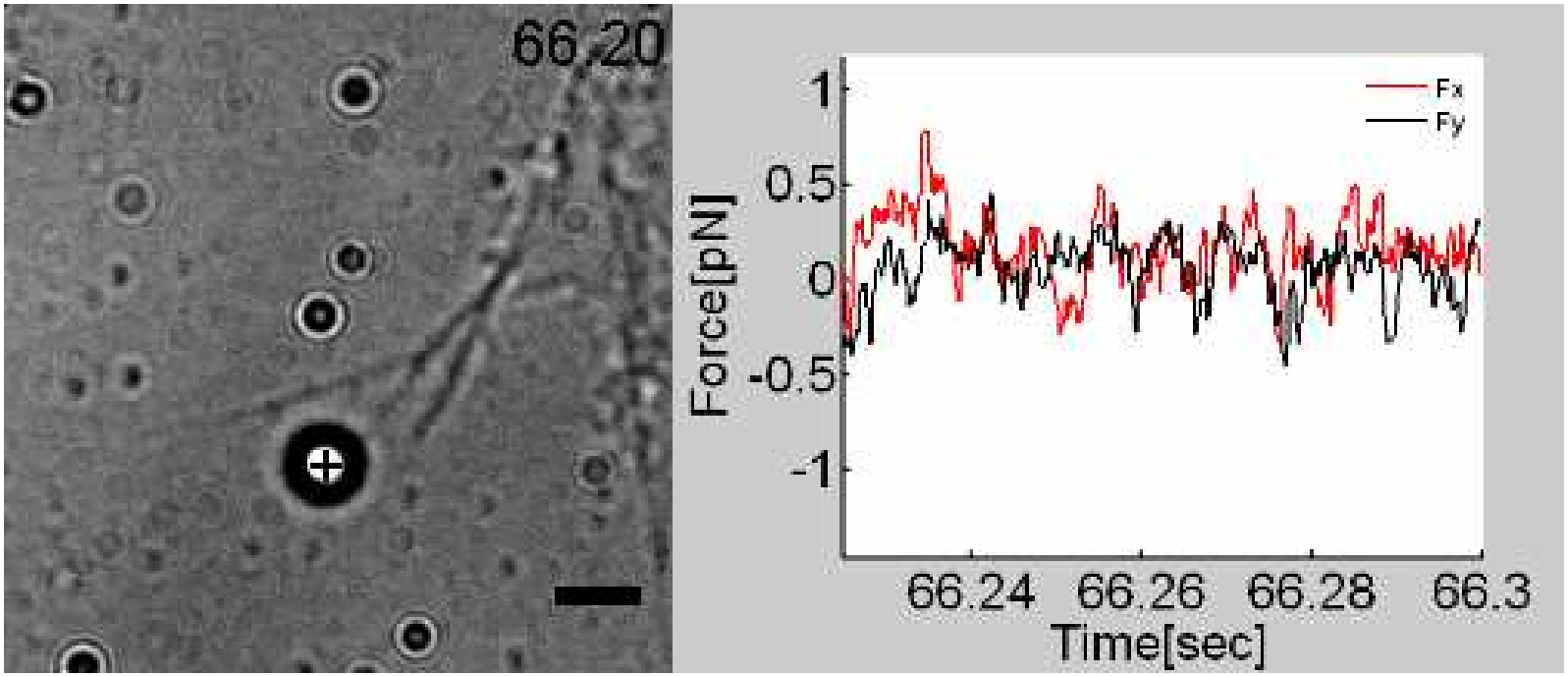
Subsampeled at : 2KHz

Force exerted by Filopodia - Protrusion





Force exerted by Filopodia - Lateral collision



Acquisition rate: 20Hz

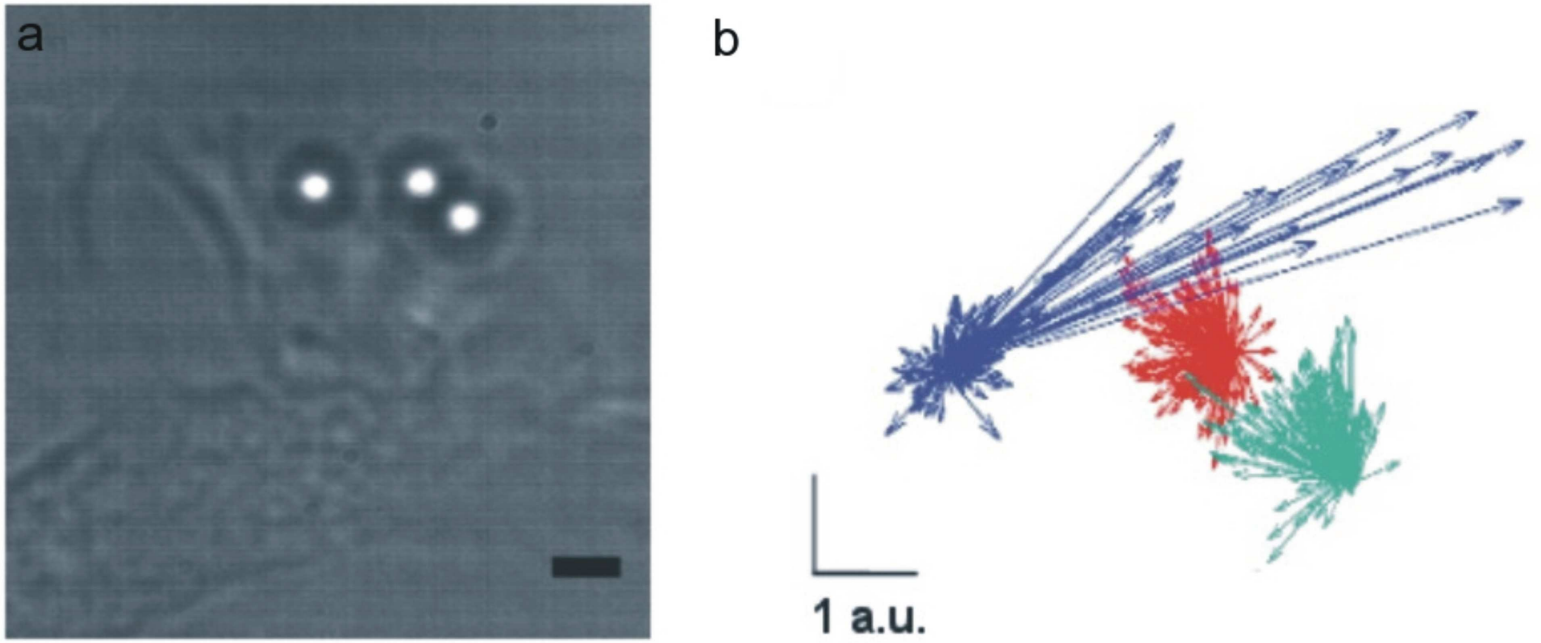
Scale Bar = 2μm

Numbers indicate time in seconds

Acquisition rate : 4KHz

Subsampeled at : 2KHz

Multiple beads trapped near Lamellipodia using DOE to split the laser beam



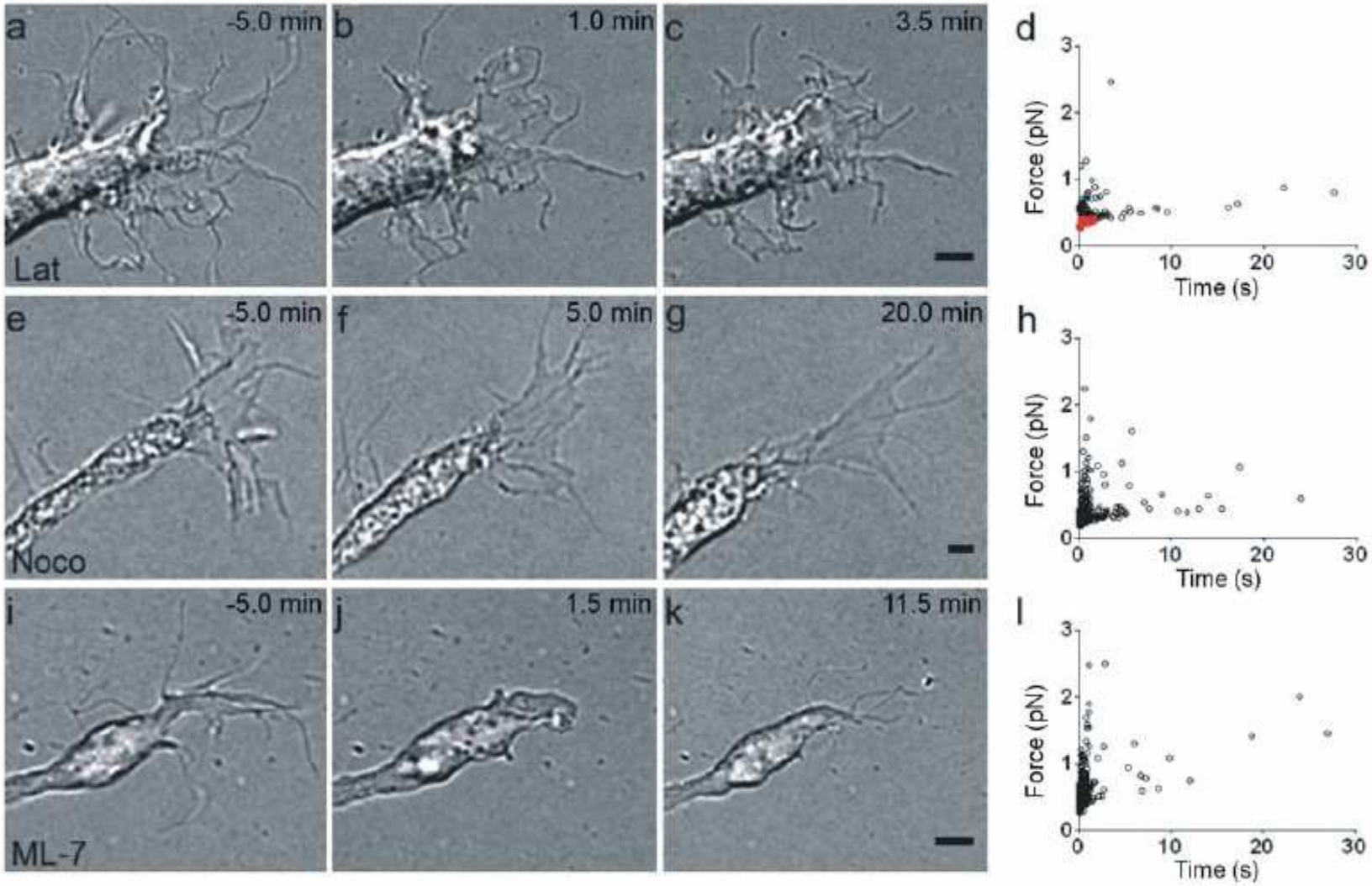


Figure 6. Effect of inhibitors on force exertion. A growth cone before (**a**) and after (**b–c**) application of 100 nM latrunculin A. No motion was observed after 3.5 min of exposure. (**d**) Scatterplot of force duration for collisions after application of 50 nM (black symbols) and 100 nM (red symbols) latrunculin A. A growth cone is shown before (**e**) and after (**f–g**) application of 50 nM nocodazole. The growth cone retracted, but filopodia continued to move for at least 30 min after drug exposure. (**h**) Scatterplot of force duration for collisions after application of 50 nM nocodazole. A growth cone is shown before (**i**) and after (**j–k**) application of 4 μM ML-7. Filopodia quickly retracted but then regrew and moved for at least 20 min after drug application. (**l**) Scatterplot of force duration for collisions after application of 4 μM ML-7. Scale bars, 2 μm. Drugs were added at time 0.
doi:10.1371/journal.pone.0001072.g006

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Francesco Difato, Jummi Laishram

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

"We should know and we will know"

(with Optical Trapping and Manipulation as

a useful tool for Biophysics in mind ☺)

Thank You for Your Patience !