Cell focal stimulation and probing by optical tweezers microscopy (2)

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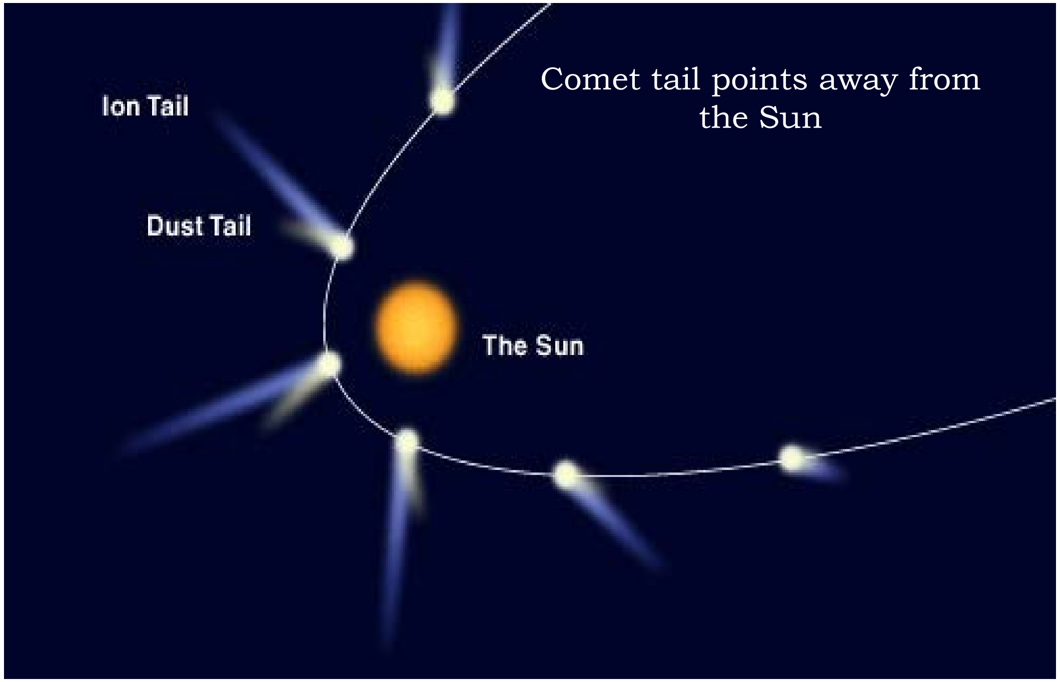
OUTLINE (continued from first lecture)

- Optical tweezers – working principle

- Biochemical local cell stimulation using optically manipulated vectors (e.g. beads, biodegradable micro-sources, liposomes, QDs)

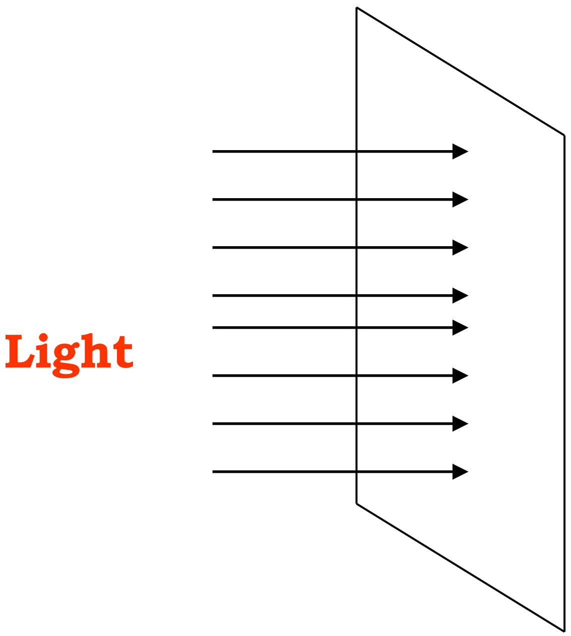
- **Biomechanical local cell stimulation and probing using optically manipulated beads** (e.g. mechanotransduction, force and viscoelasticity probing)

Answering to one comment to the first lecture:
Who was the first to observe sunlight radiation pressure effect ? c



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

Kepler 1619



Radiation pressure of Sunlight on Earth is in average 4.6 μPa

Comet Hale-Bopp (1995)

Radiation pressure and solar wind effects on the dust and gas tails are clearly seen. Whereas the ion tail (blue) is carried away by ‘solar wind’ of charged particles from Sun’s athmosphere, the dust tail (white) is pushed by radiation pressure of Sunlight. The momentum transfer in this second case is weaker than that in the first, resulting in the splitting of the tails.

Radiation pressure and light momnetum transfer to matter:

< 1900 Maxwell, 1900 Lebedev … and the subject is still very actual:

Momentum in an uncertain light

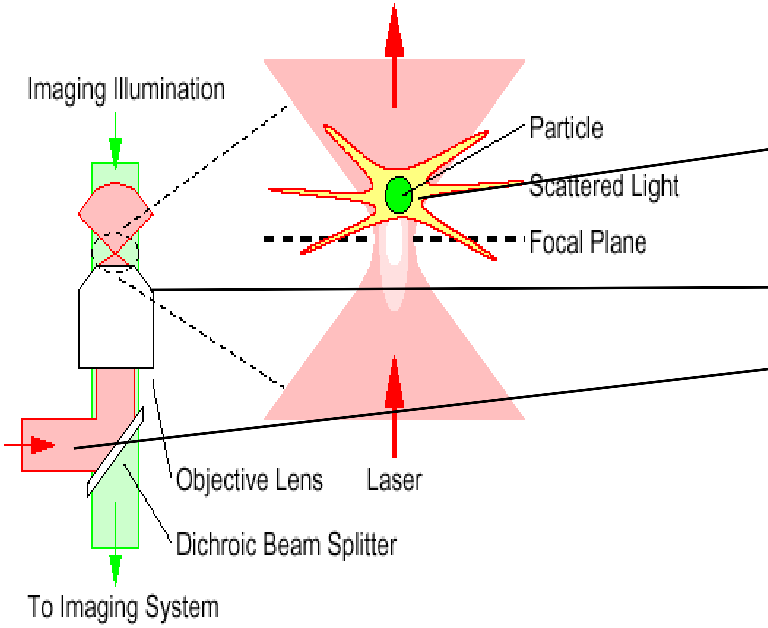
Ulf Leonhardt

How much momentum does light transfer to a material through which it passes? This is a surprisingly opaque matter, contested for almost a century, that is still the object of theory and experimentation.

Nature, 444, 823, 2006

Remember from the first lecture

A. Ashkin *et al*, *Opt.Lett.* 11 288 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

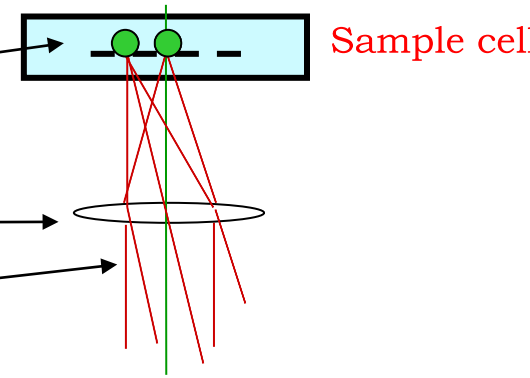
Q – dimensionless efficiency coefficient

W – power of the laser beam

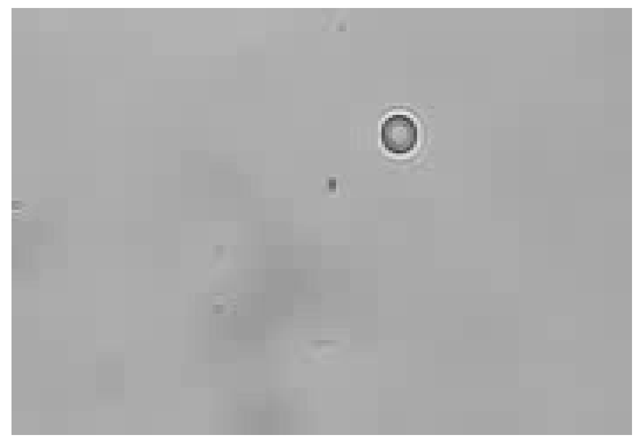
$\mathbf{n_m}$ – refractive index of the medium

c – light speed in vacuum

Beam focusing → 3D bead trapping



Beam deflection → bead displacement



Optical trapping and displacement of a silica bead (2 μm diam)

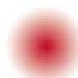
Optical Tweezers properties in a slide

Types of particles:

- **Material:**
  Dielectric (polystyrene, silica); Metallic (gold, silver, copper), Biological (cells, macro-molecules, intracellular structures, DNA filaments), Low index (ultrasound agent contrast); crystal or amorphous material.
- **Size:** 20 nm – 20 µm
- **Shape:** spherical, cylindrical, arbitrary

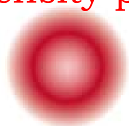
Types of laser beams:

- **Gaussian**



x-y intensity profile

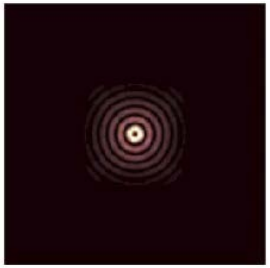
- **Laguerre-Gaussian**



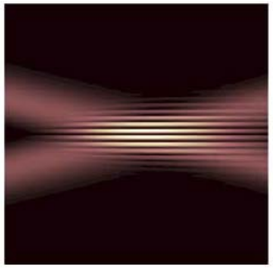
LG carries also orbital angular momentum that can be transferred to the trapped particles

- **Bessel**

x-y intensity profile



z axis propagation non diffracted beam

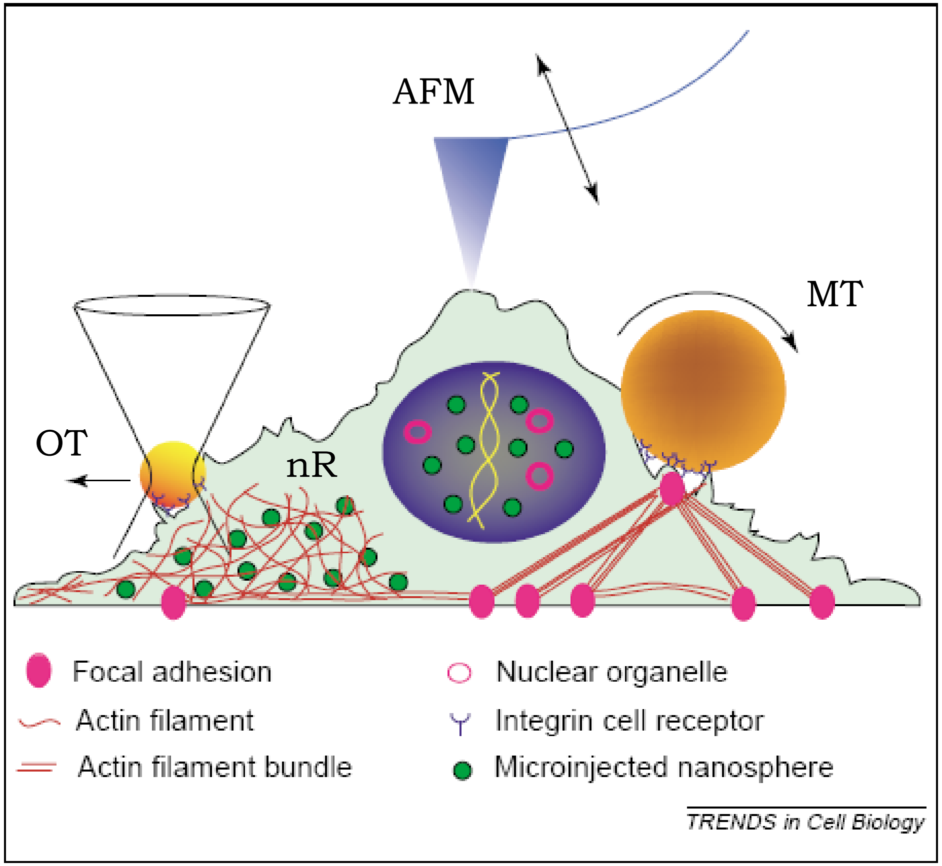


OT characteristics:

- Typical stiffness: 100 pN/µm
- Typical displacements: 1-500 nm
- Typical forces: 0.1-100 pN
- Measurable displacements < 1 nm @ 1 MHz sampling rate

Local mechanical stimulation

Illustration of different methods capable of making local and short time-scale mechanical stimulation/measurements on living cells.



AFM – Atomic Force Microscopy

OT – Optical Tweezers

MT – Magnetic Tweezers

nR – nano Rheology

S.R. Heidemann and D. Wirtz

Towards a regional approach to cell mechanics
TRENDS in Cell Biology Vol.14 No.4 April 2004

Multiple trapping

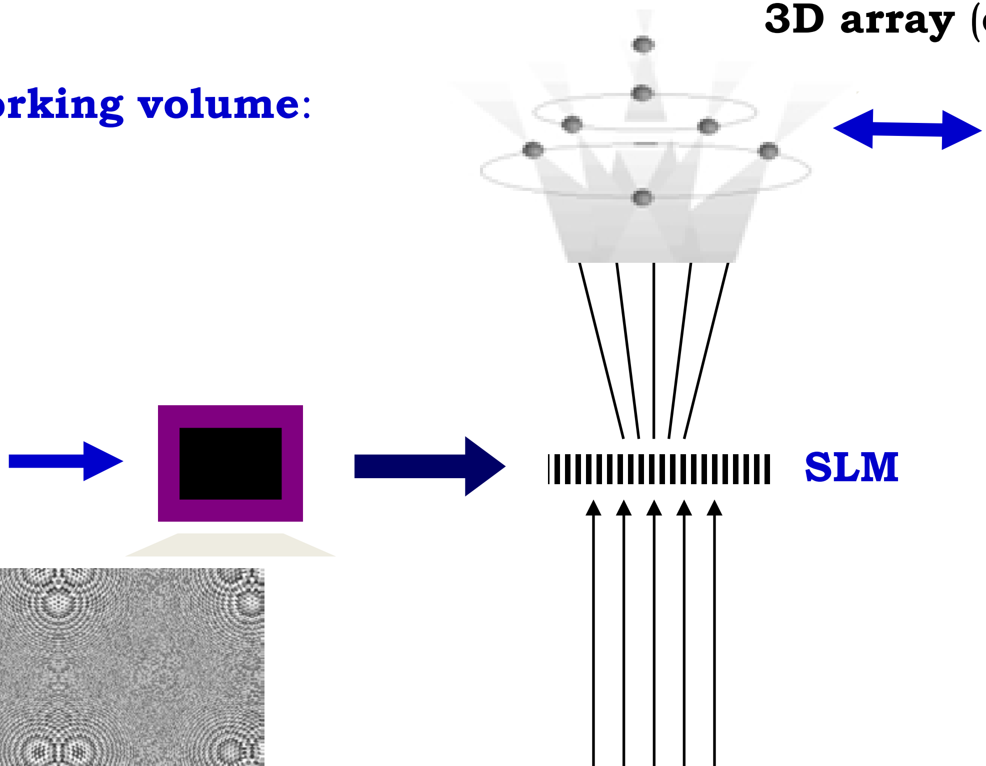
by means of Diffractive Optical Elements (DOE)
implemented on a Spatial Light Modulator (SLM)

Working volume:

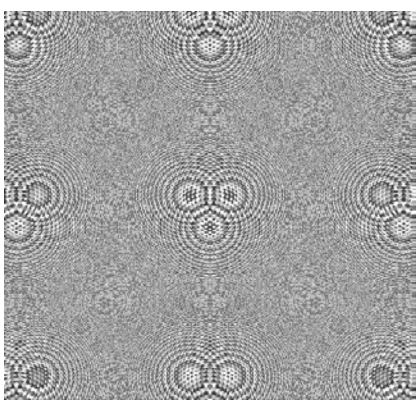
3D array (cage)

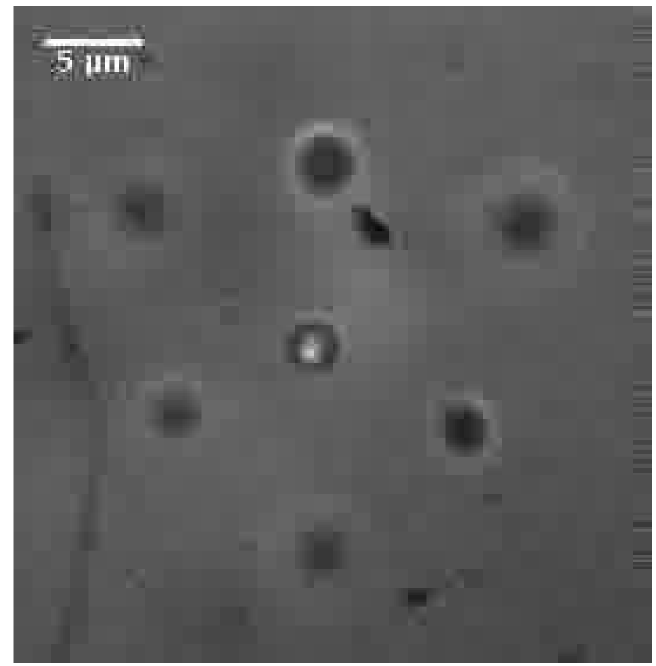
3D array

DOE



SLM

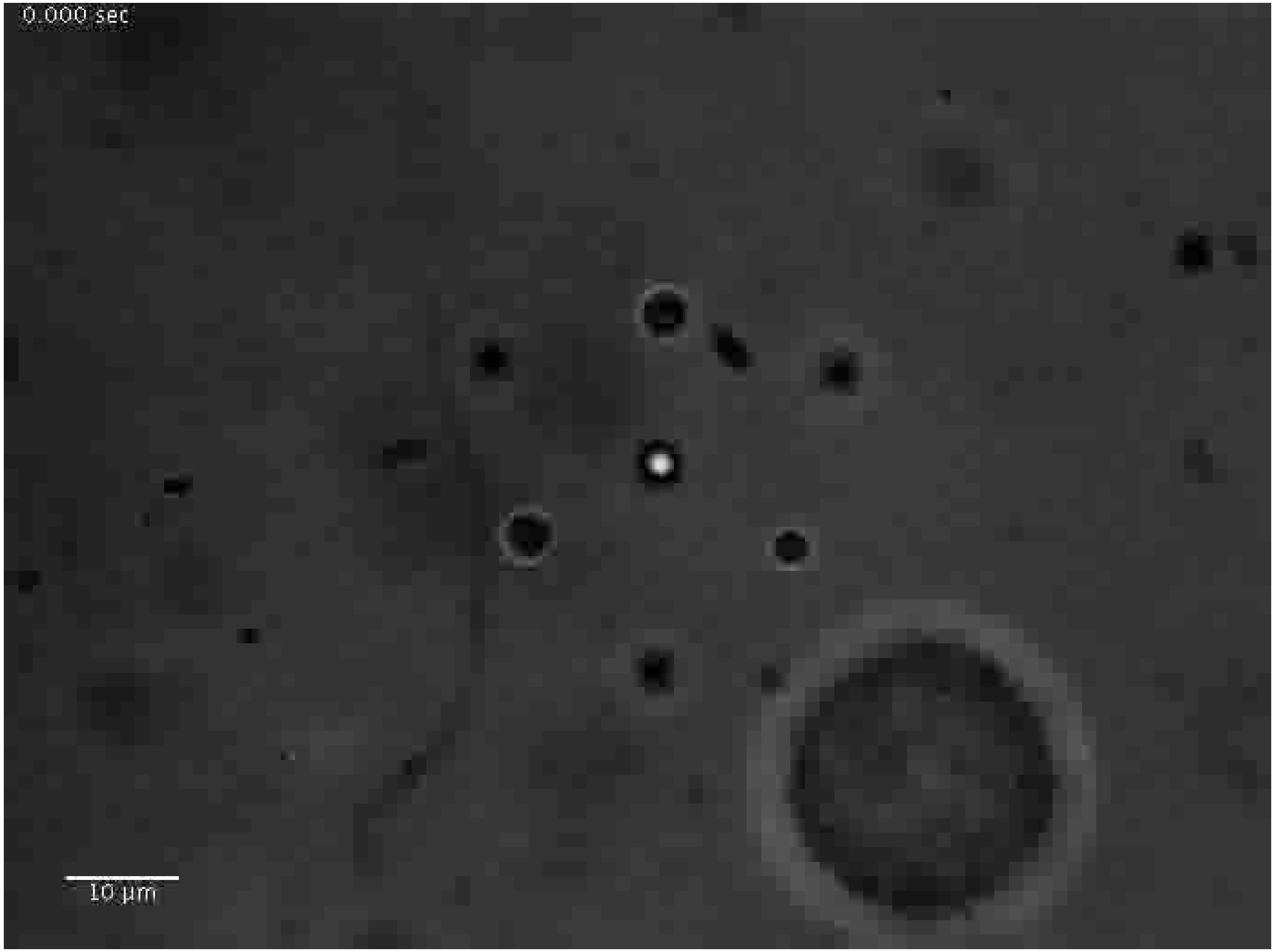




Input Wave
(Gaussian)

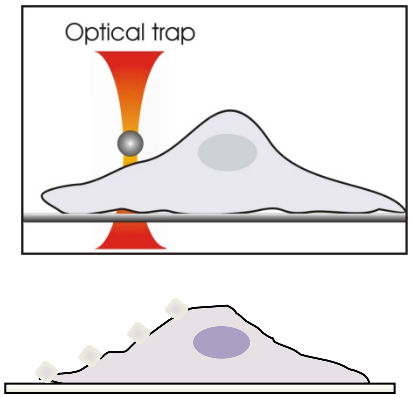
D. Cojoc *et al* 2004, 2005, 2006

Mechanically stressing the cells with pN forces



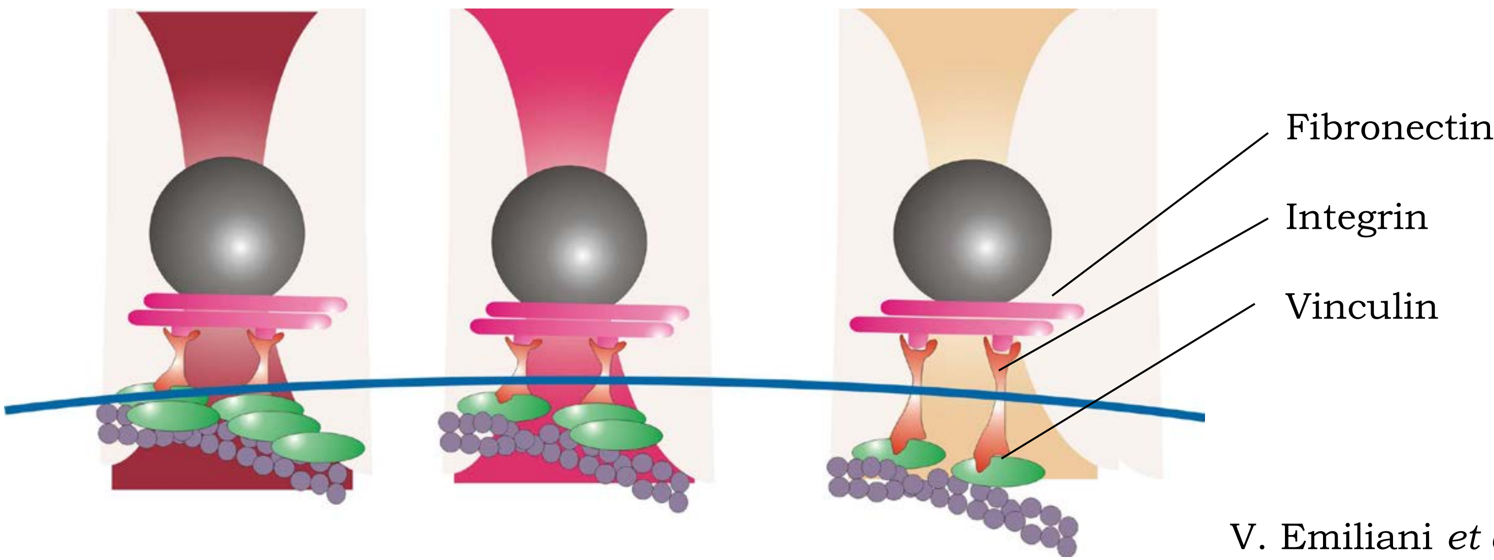
HeLa cell under the dynamic cage of beads

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



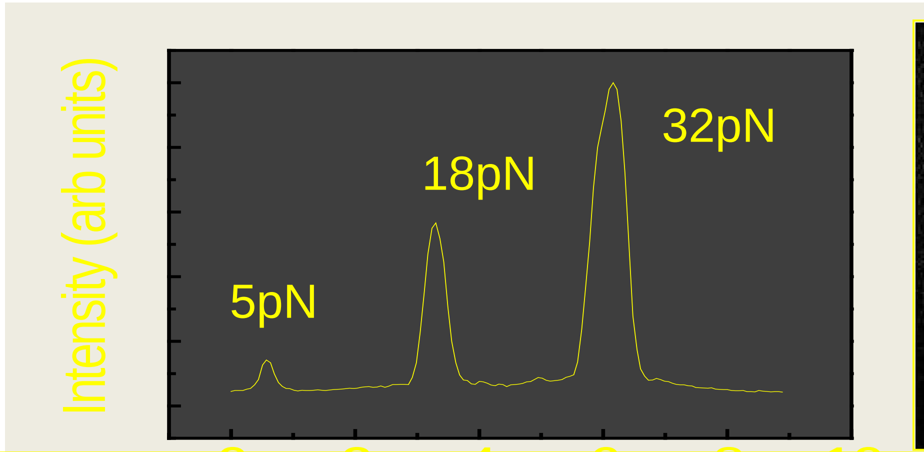
The multi force optical tweezers is combined with an epi-fluorescence microscope to monitor vinculin recruitment as a function of applied forces.

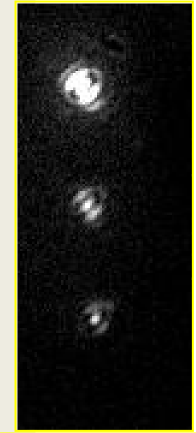
Fibronectin coated beads are manipulated on the dorsal surface of Vin-GFP transfected HeLa cell.



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

Trap strength



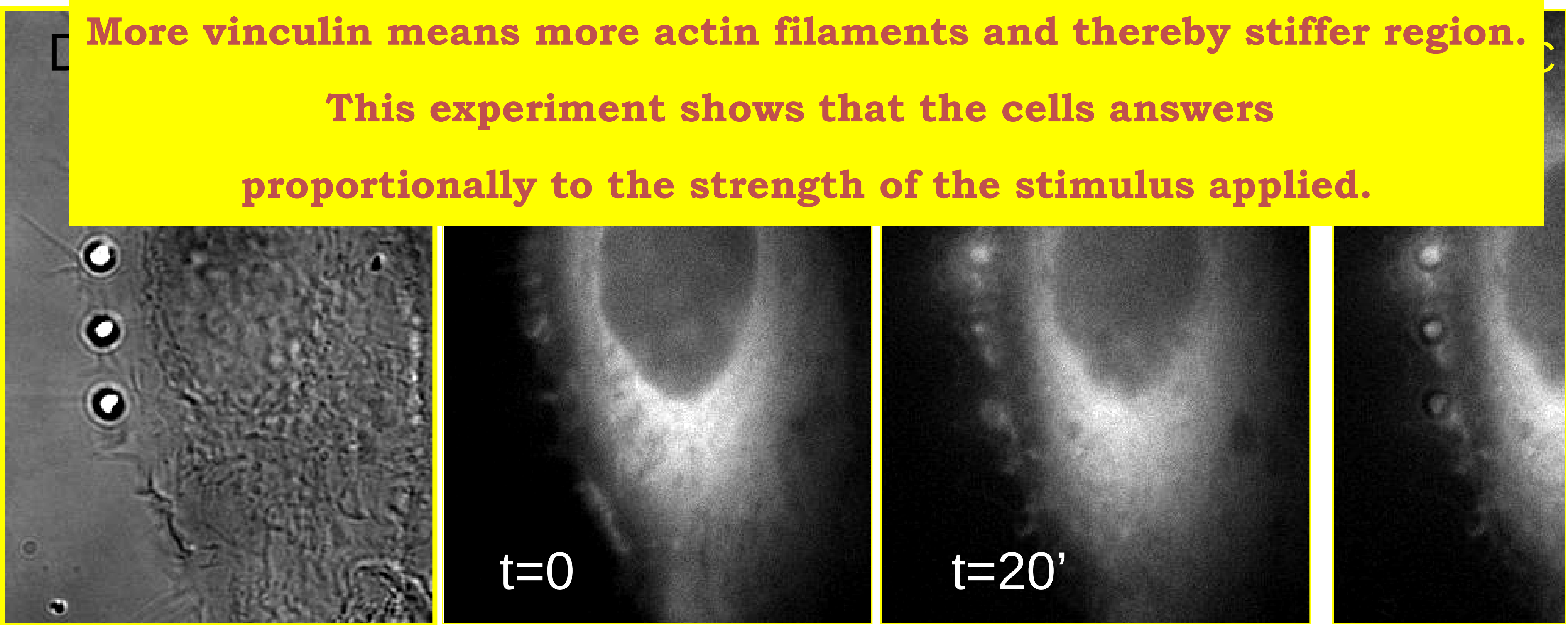


Vinculin recruitment

Vinculin indicates the accumulation of the actin filaments.

More vinculin means more actin filaments and thereby stiffer region.

This experiment shows that the cells answers proportionally to the strength of the stimulus applied.



Visualizing the mechanical activation of Src

letters to nature

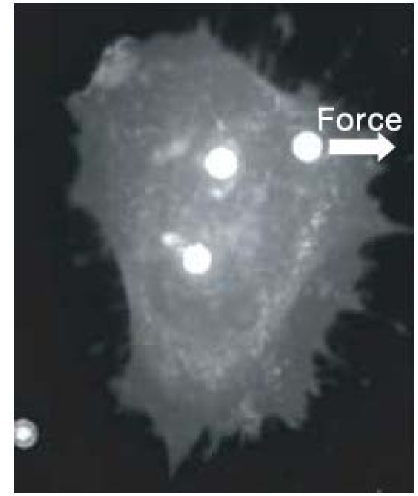
NATURE | VOL 434 | 21 APRIL 2005 | www.nature.com/nature

Yingxiao Wang[1], Elliot L. Botvinick[1,5], Yihua Zhao[1], Michael W. Berns[1,4,5], Shunichi Usami[1], Roger Y. Tsien[3] & Shu Chien[1,2]

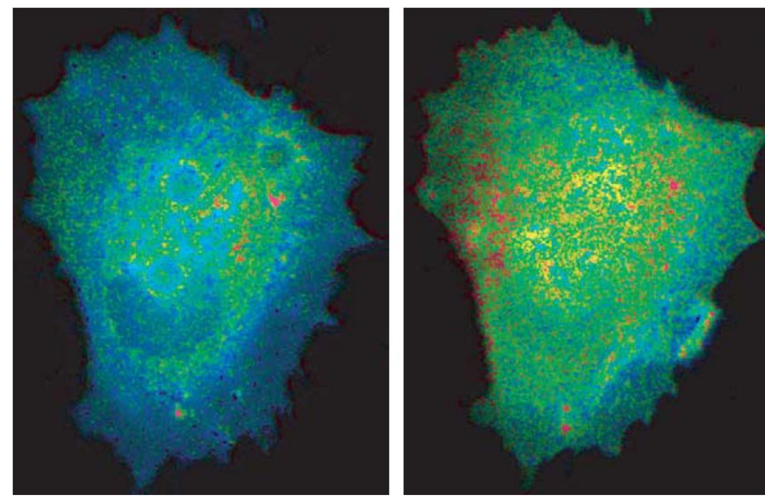
Using fluorescent resonance energy transfer (FRET), a genetically encoded SRC reporter that enables the imaging and quantification of spatio-temporal activation of SRC in live cells was developed.

Local mechanical stimulation to human umbilical vein endothelial cells (HUVECs) by applying laser-tweezer traction on fibronectin-coated beads adhering to the cells.

Rapid distal SRC activation and a slower directional wave propagation of Src activation along the plasma membrane.

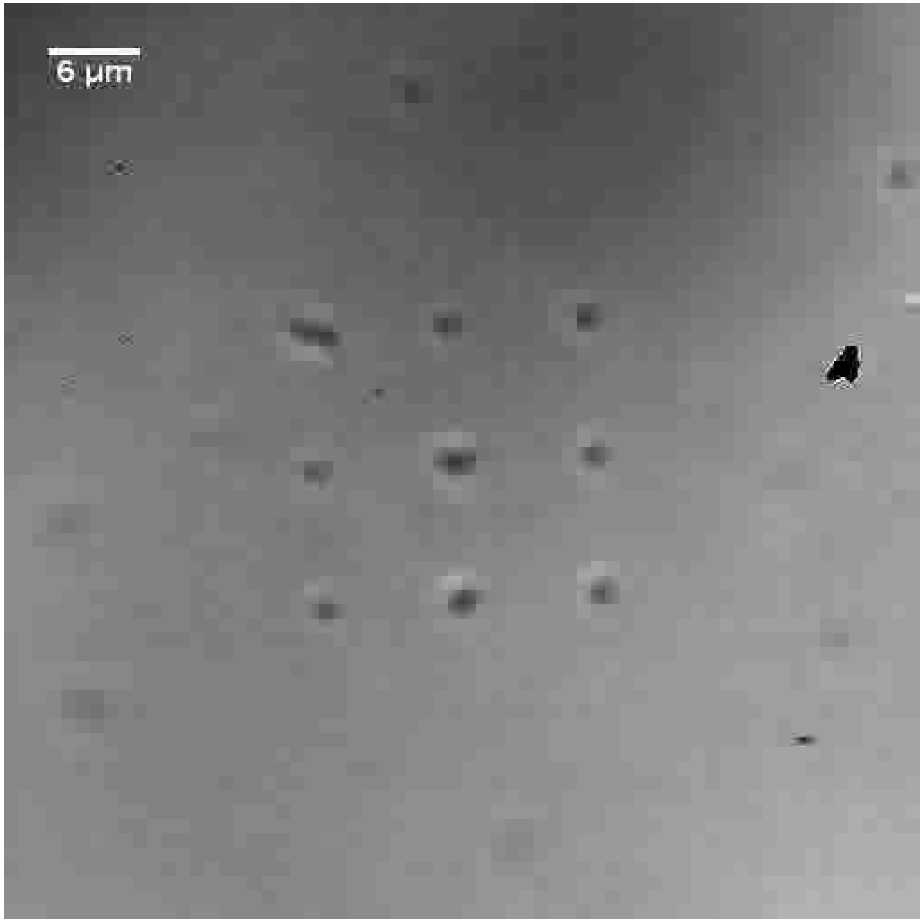


0 15 min



OT traction induced directional and long-range propagation of FRET responses of the membrane-targeted Src reporter in HUVECs

Cell array and sorting



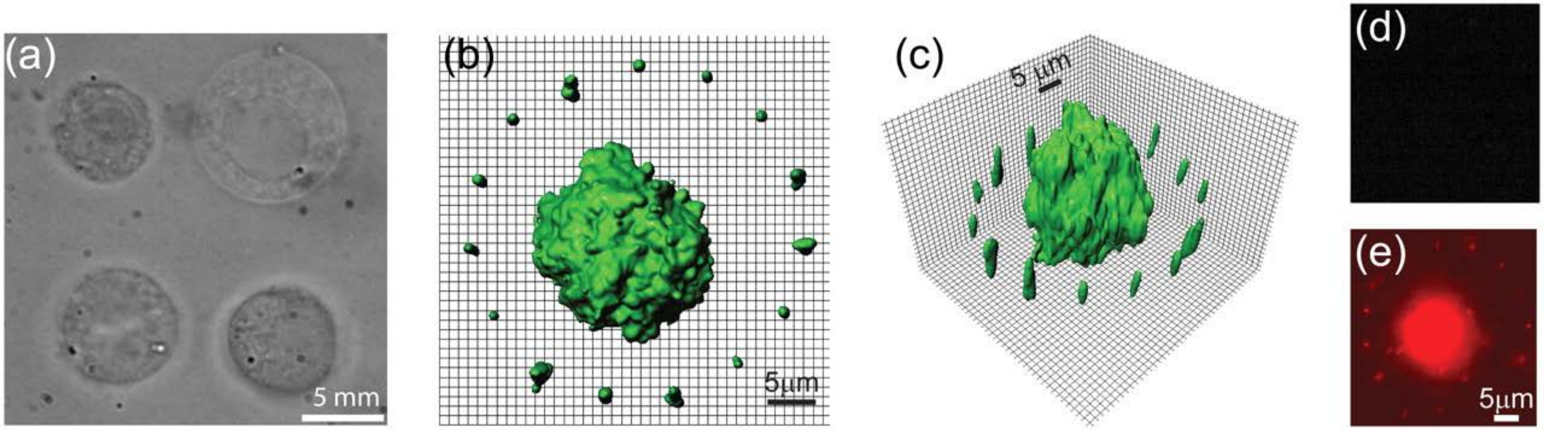
E. colli cell array and sorting

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**

AOD = Acusto Optic Modulator ; SLM = Spatial Light Modulator ; 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.*

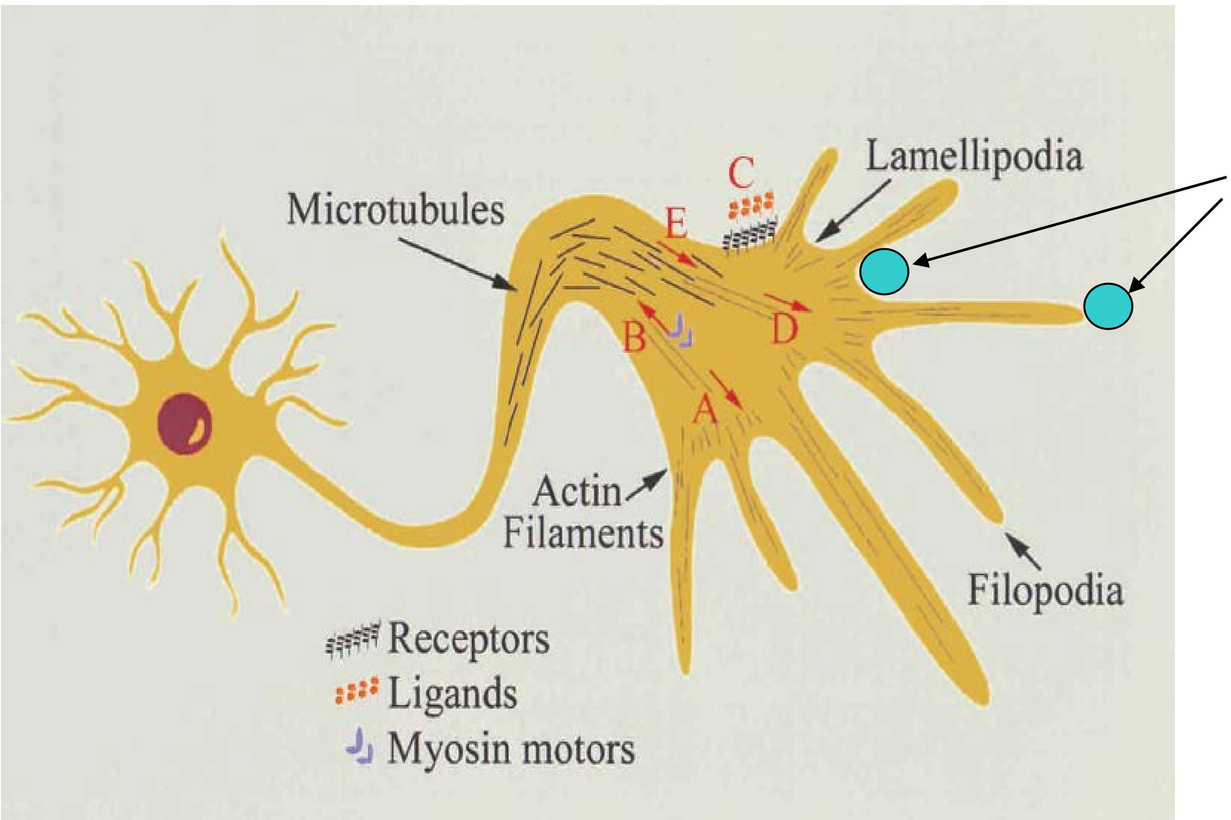
(d,e) Viability assay of the same heterotypic microarray showing an image obtained by exciting propidium iodide labels with 488 nm. The lack of red fluorescence in (d) indicates viability, but after killing the cells with ethanol the fluorescence is intensely red (*e*).

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

Using the trapped bead as a probe

Goal measure the forces exerted by lamellipodia and filopodia

Experimental Approach

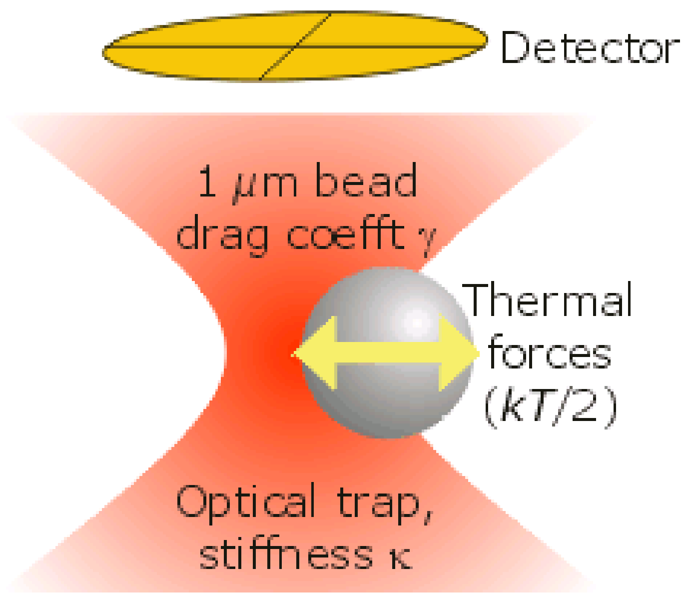


J.L. Goldberg, Genes and Dev. ***17*** 941 (2003)

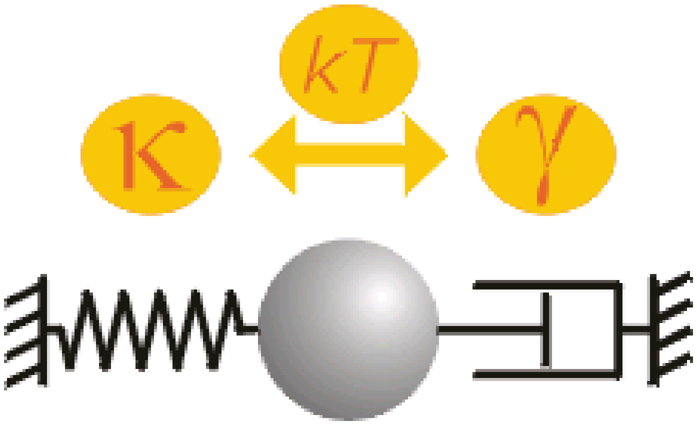
- Calibrate the trap
- Micro-beads trapped by IR laser and positioned in front of lamellipodia and/or filopodia
- Measure the fluctuations of the bead in the trap, due to its interaction with the motile structures, and convert them into forces.

Trap calibration from the fluctuations of the bead

Schematic of a μm bead diffusing in an optical trap



Mechanical model of the forces acting on the bead

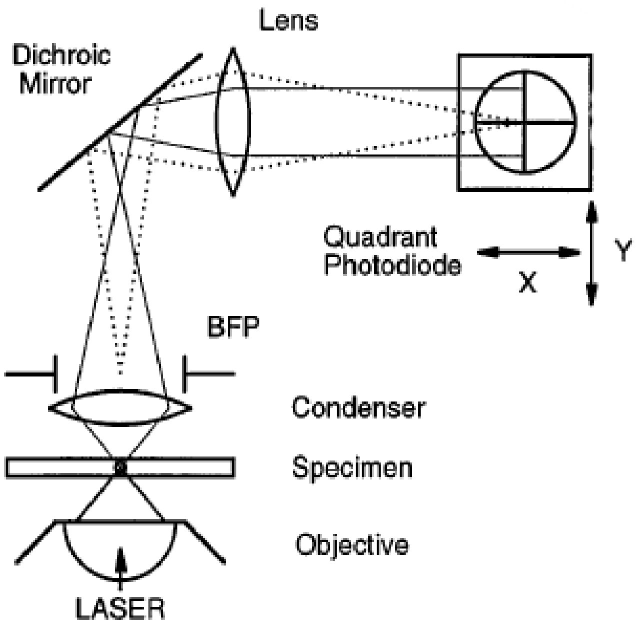


The power spectrum density $S(f)$ of these fluctuations near the center of an optical trap is approximately Lorentzian

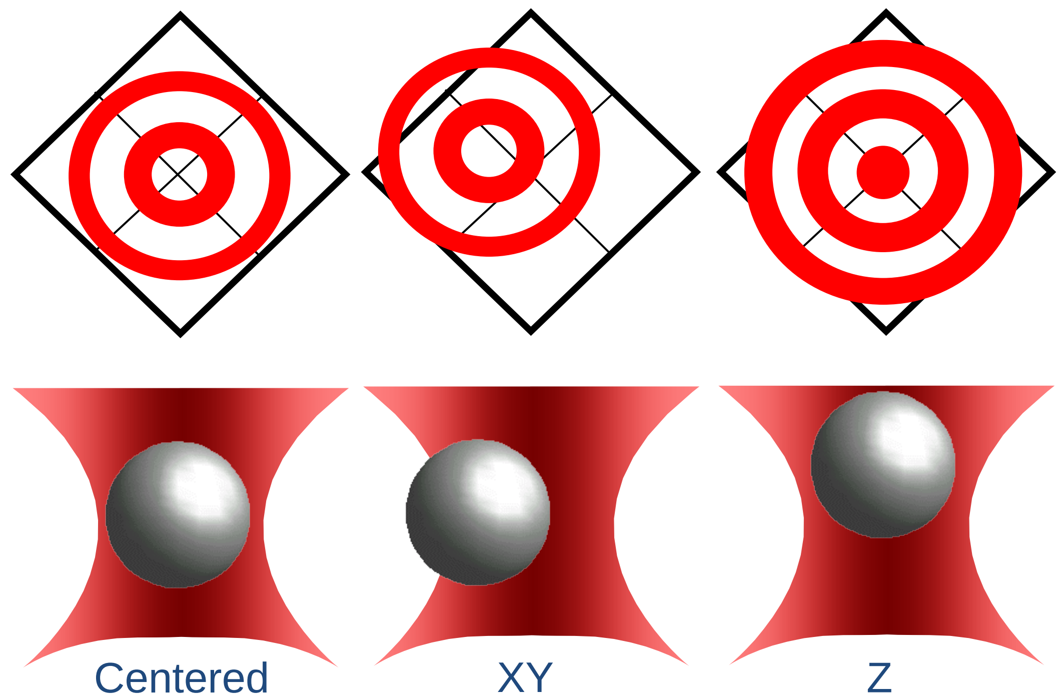
(Svoboda and Block, 1994; Gittes and Schmidt, 1997)

Back focal plane interferometry

detect the thermal fluctuations of the bead with



F. Gittes, Optics Letters, (1998)



Displacement from the focus

Voltage change on the detector

Trap stiffness and detector sensivity

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

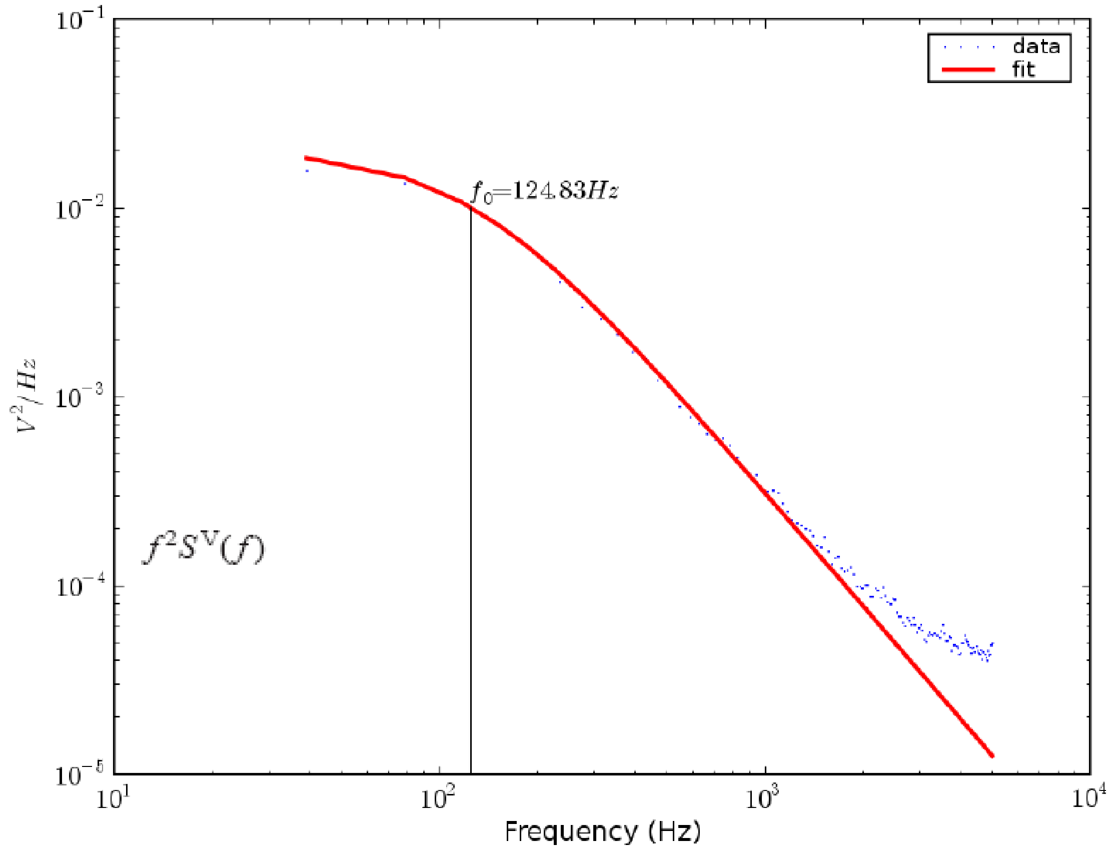
$$S(f) = \frac{S_0 f_0^2}{f_0^2 + f^2},$$

f_0 – corner frequency
k – trap stifness
γ – Stokes drag coefficient of the bead

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

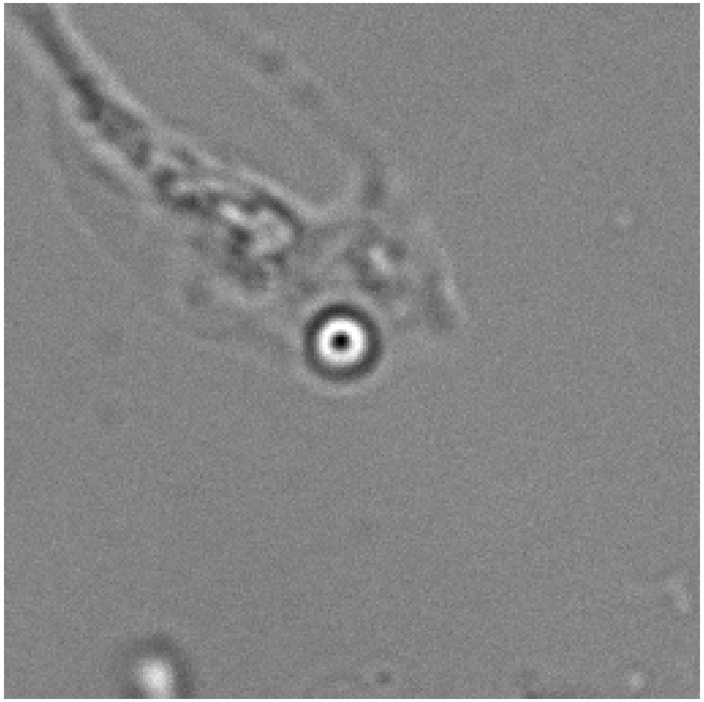
β – detector sensivity
S_0 – trap stifness
PV – plateu of $f^2 S^V(f)$

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

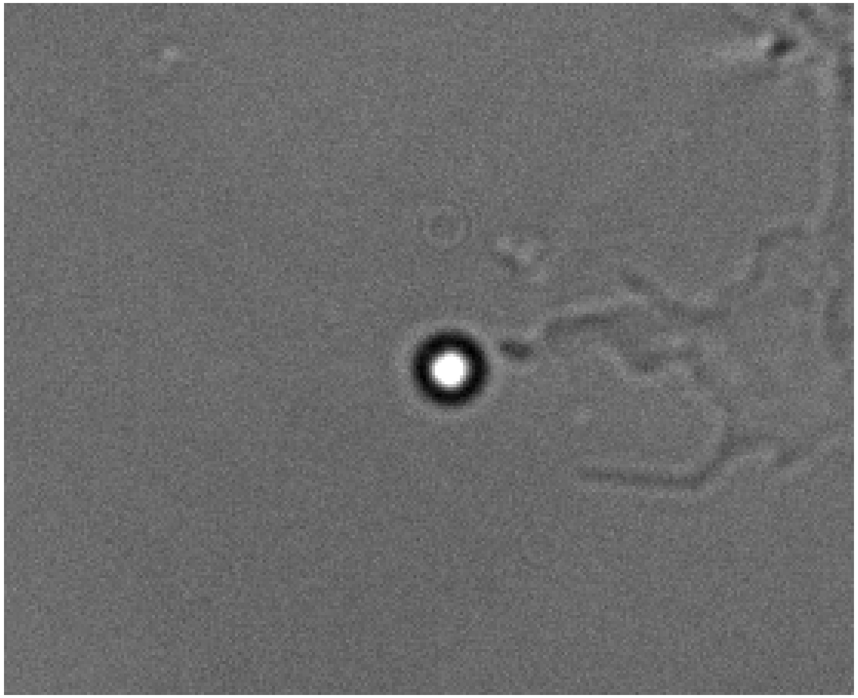


The power spectrum (dotted line) of a trapped 1 μm silica bead acquired at 10 KHz and fitted to a Lorentzian (solid line).

Lamellipodia 2 minutes event, Fmax measured - 20pN

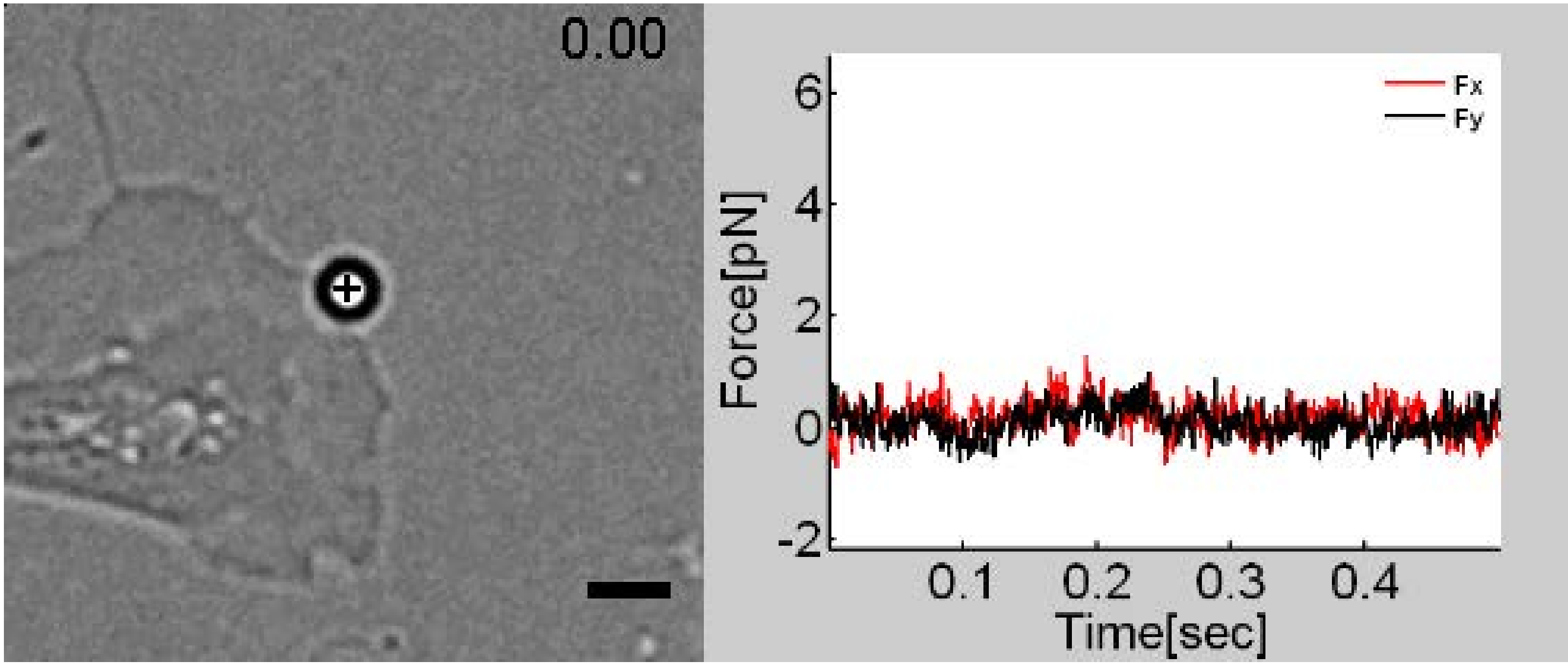


Filopodia 2 minutes event, Fmax= 2-3 pN



D. Cojoc *et al* PlosOne 2007.

Force exerted by Lamellipodia



Acquisition rate: 20Hz

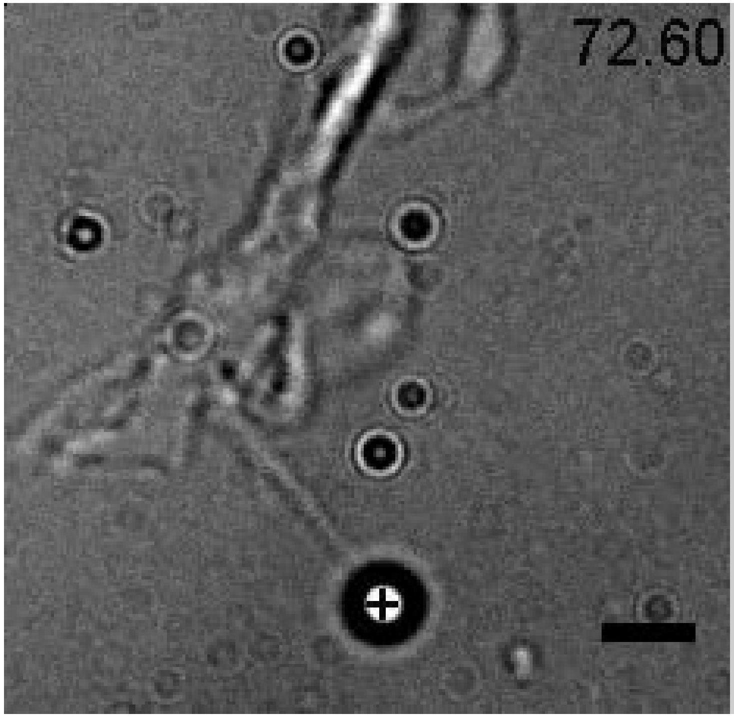
Scale Bar = 2µm

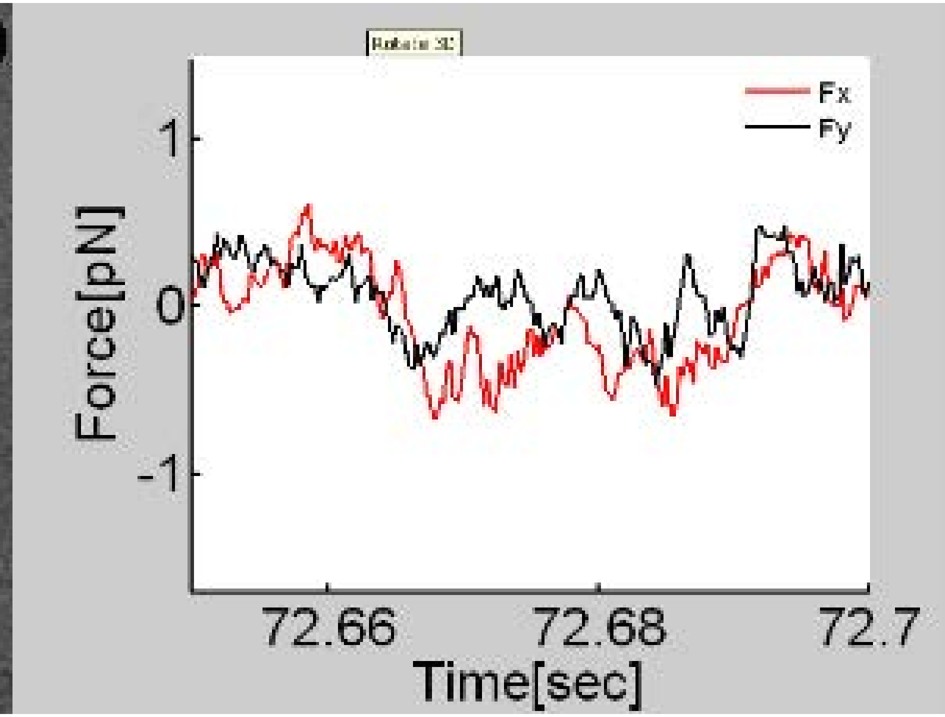
Time in seconds

Acquisition rate : 4KHz

Subsampeled at : 2KHz

Force exerted by Filopodia - Protrusion





Acquisition rate: 20Hz

Scale Bar = 2μm

Time in seconds

Acquisition rate : 4KHz

Subsampeled at : 2KHz

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 ?

Measuring viscoelastic properties of cancer cells

Motivation:

Find new markers for cancer prognosis and understand specific mechanisms at single cell level

Local invasion and metastasis → **Primary cause of death in cancer patients**

Increase replication

Increase plasticity

Loss of cell polarity

Increase motility

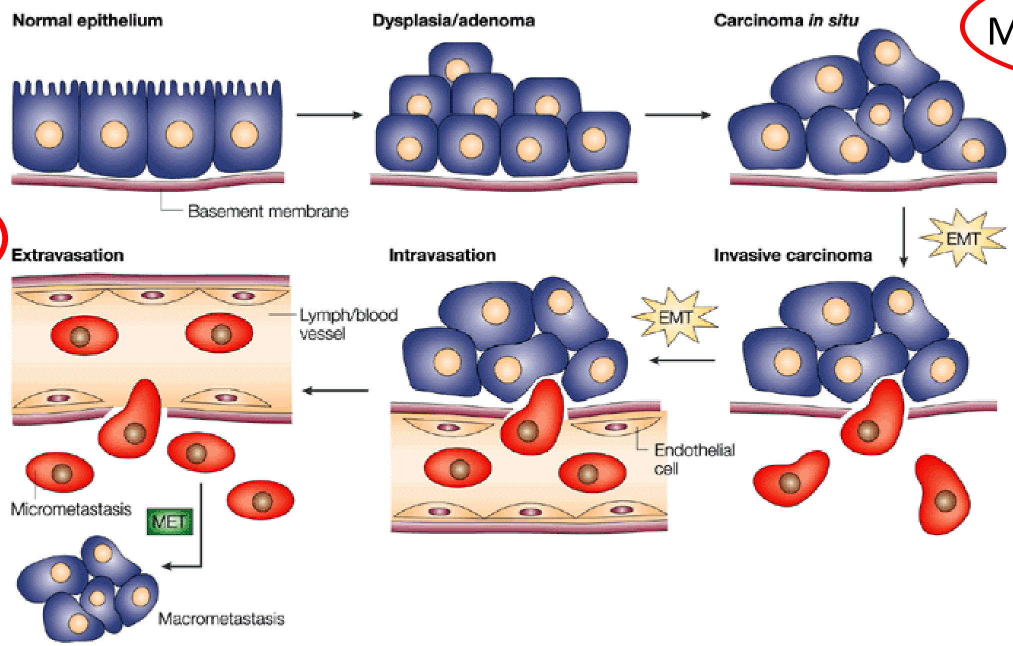
Membrane modification

Alteration in cytoskeleton organization

Loss of adhesion

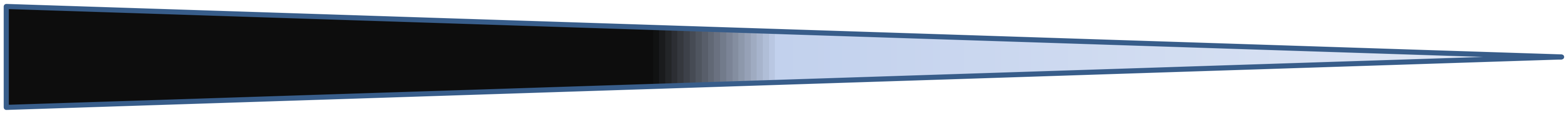
More deformable

Softer



Experimental Plan

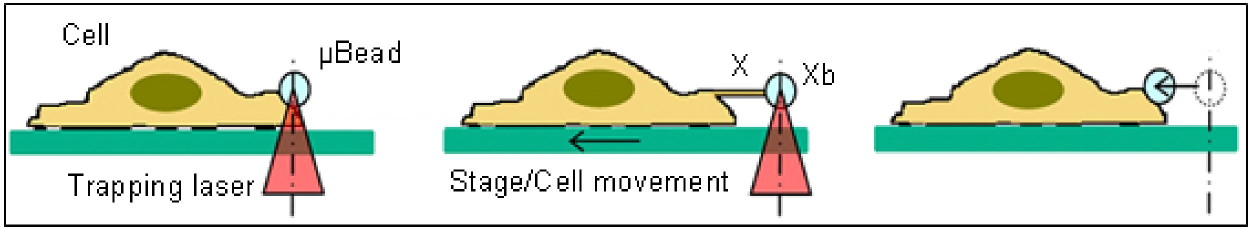
AGGRESSIVENESS LEVEL



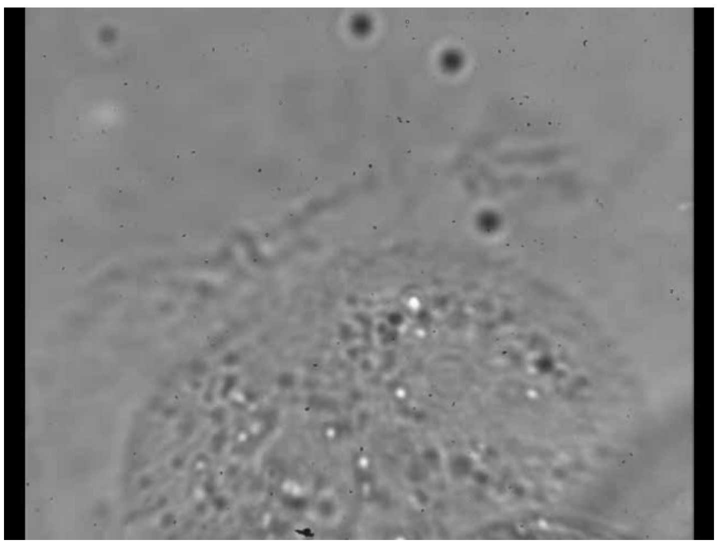
MDA-MB-231 High metastatic potential	**MCF-7** Low metastatic potential	**HBL-100** Non neoplastic
Poorly differentiated. Tumorigenic. Basal type. Associated to poor prognosis	Morfology of differentiated mammary epithelium. Luminal A type. Associated to better prognosis	Epithelial cell line derived from milk of a nursing mother. No evidence of a breast lesion in the milk donor

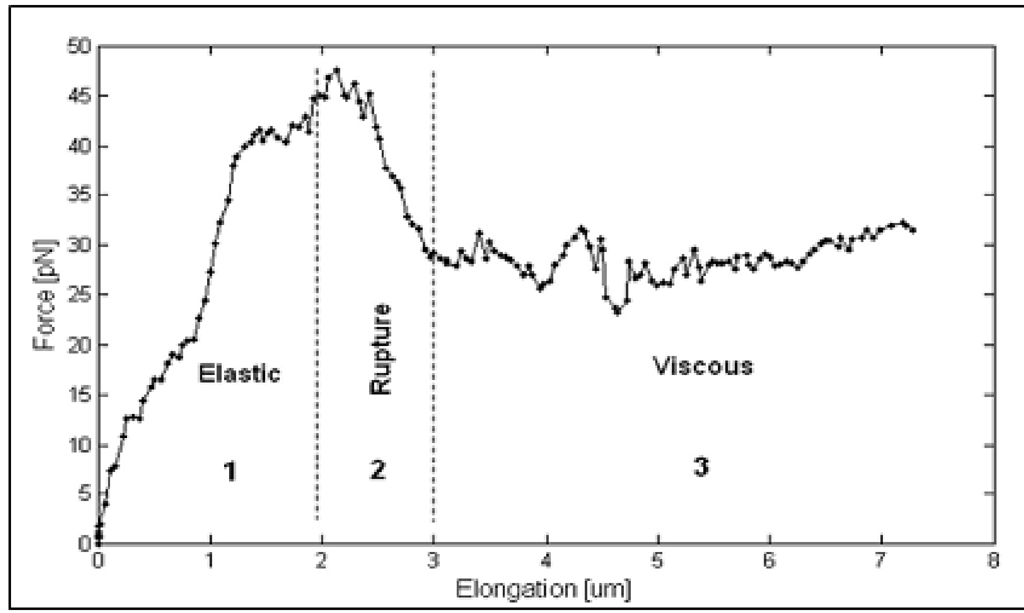
- **Optical Tweezers**
- **Speckle Sensing Microscopy**

Membrane tether pulling by optical tweezers



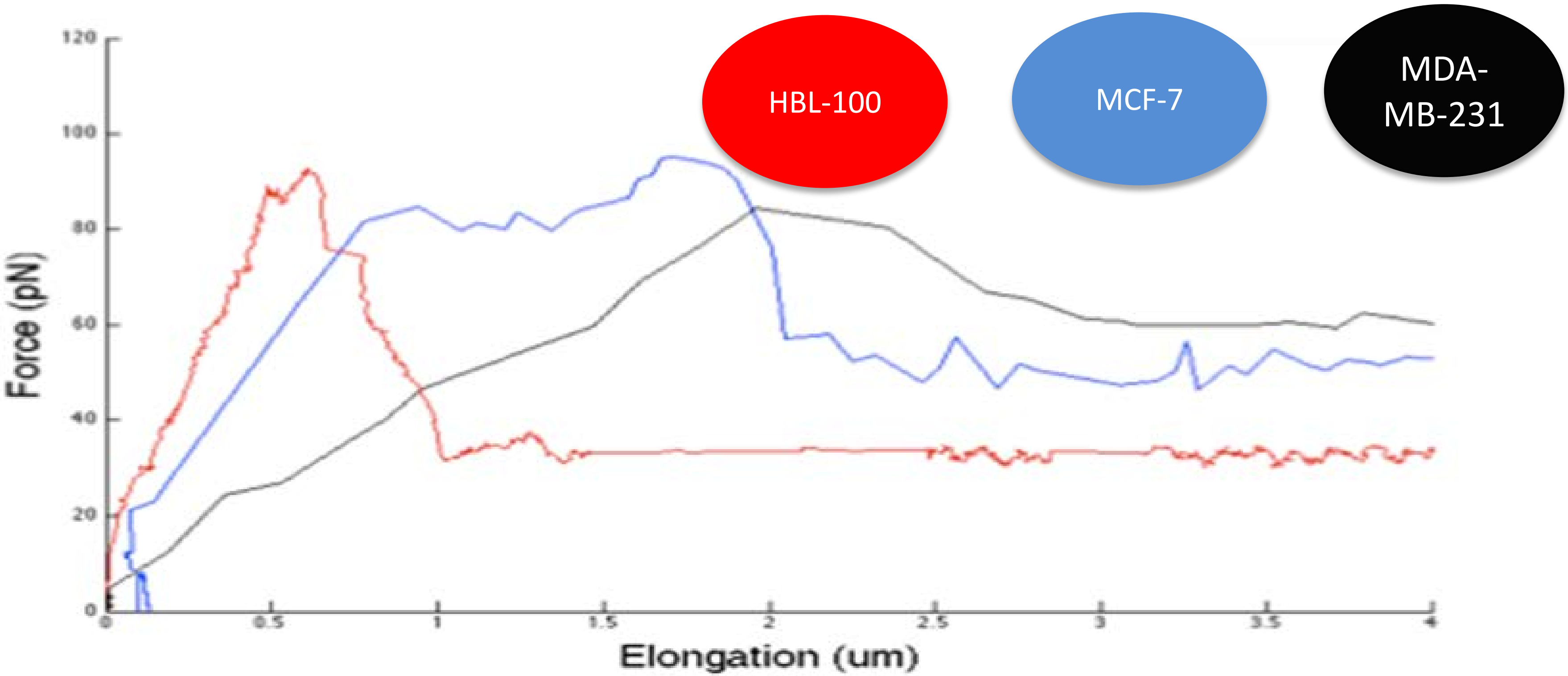
Force – Elongation curve





F. Tavano, S. Bonin, G. Pinato, G. Stanta and D. Cojoc, 2011, Int. J. Optomech. 5(3): 231-246.

Results



OT: tether pulling	MDA-MB-231	MCF-7	HBL-100
Tether stiffness (pN / µm)	**40**	**54**	**142**
Tether Force / Tether lenght (pN / µm)	**42**	**58**	**158**
Viscosity (pN s / µm)	**120**	**100**	**80**

- **Optical trapping and manipulation – working principle**

- **Biochemical local cell stimulation using optically manipulated vectors** (e.g. beads, biodegradable micro-sources, liposomes, QDs)

- **Biomechanical local cell stimulation and probing using optically manipulated beads** (e.g. mechanotransduction, force and viscoelasticity probing)

ICTP /IOM-CNR collaborative programme

Optical trapping and manipulation

Coordinated by: Joseph Niemela / Dan Cojoc

Participants 2013, through ICTP:

Fatou Ndoye (Senegal), Alireza Moradi (Iran), Jose J. Vargas Suarez (Venezuela), Humberto Cabrera (Venezuela)

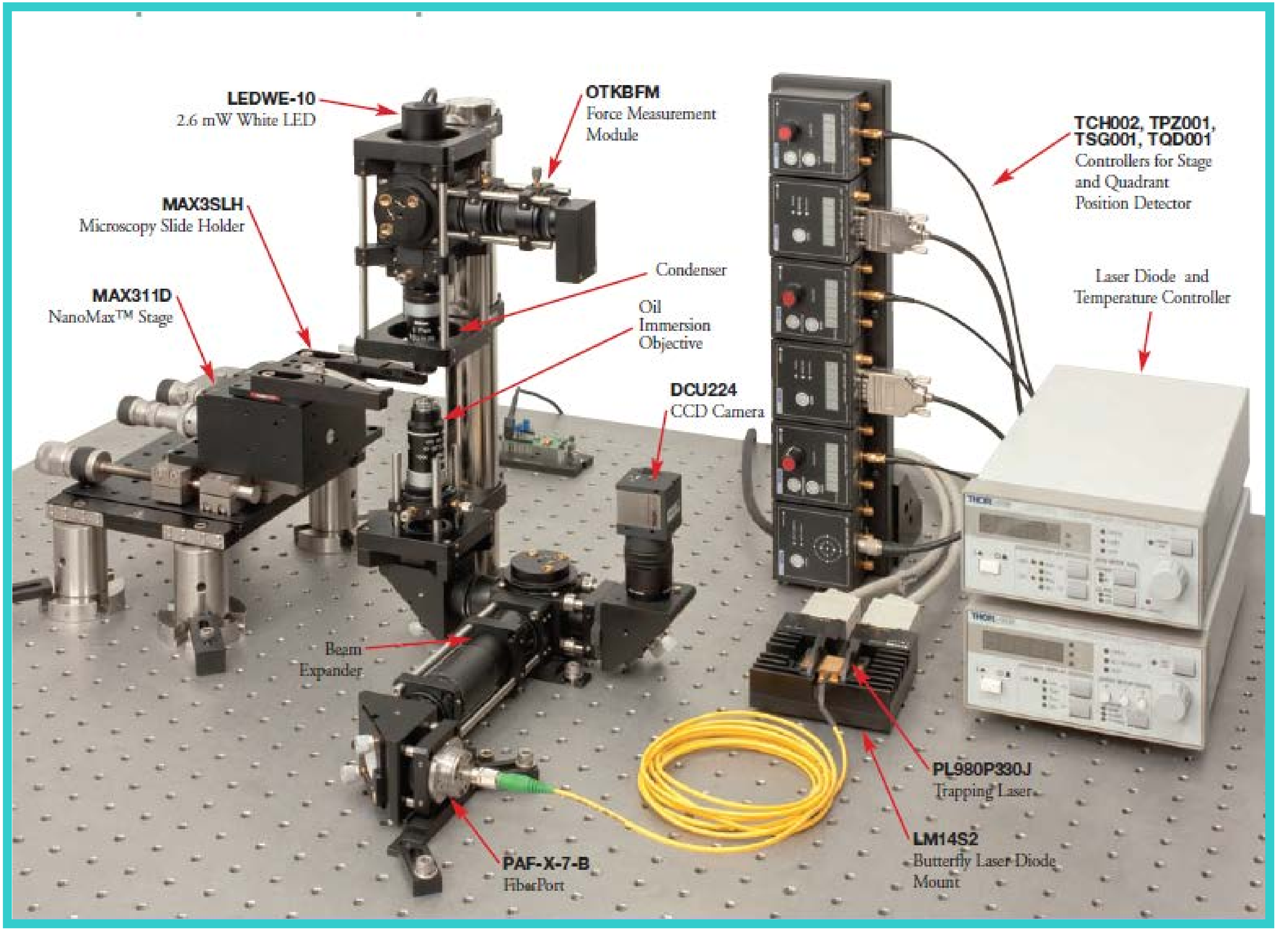
Participant from IOM-CNR/Univ. of Trieste:

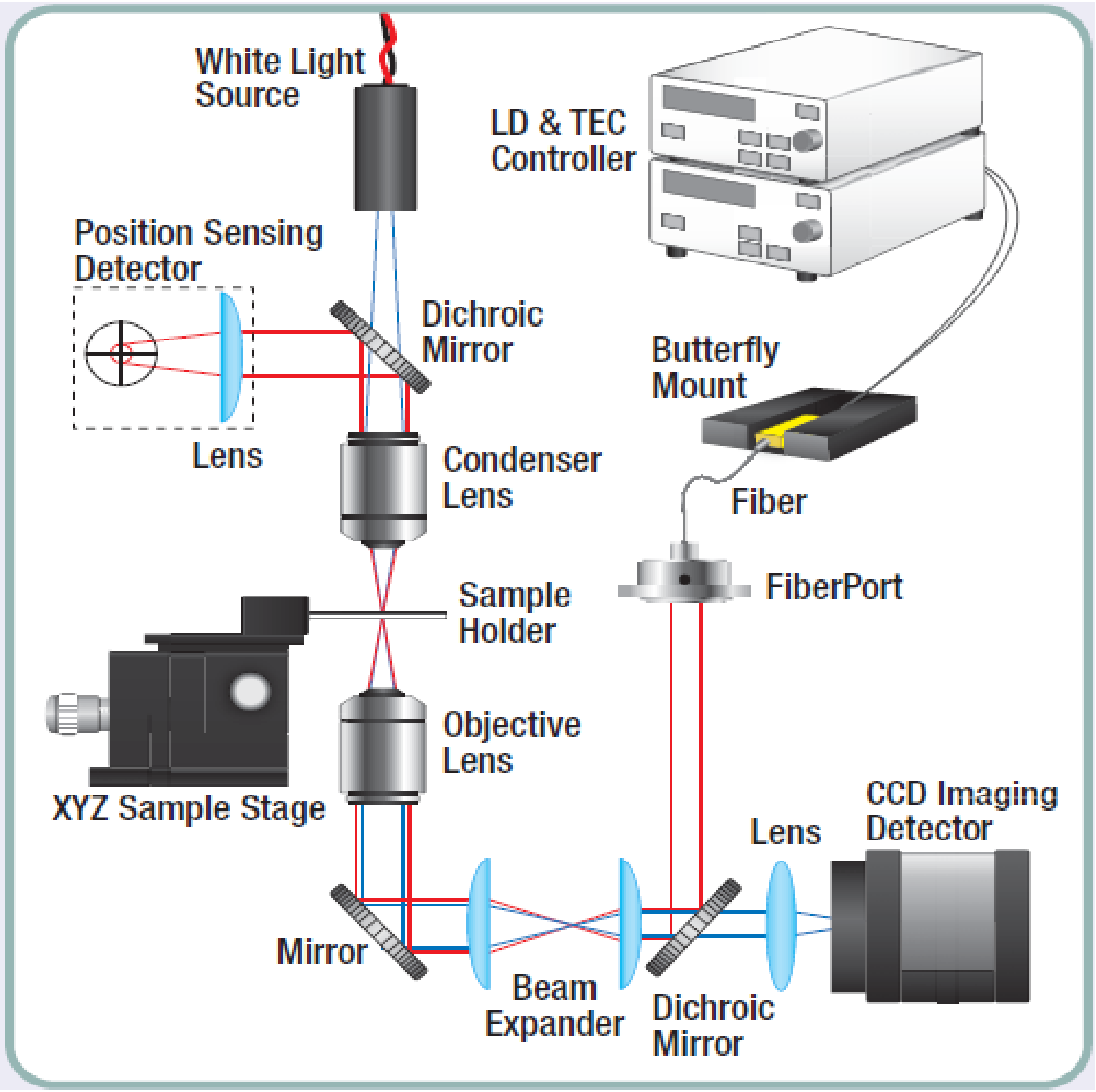
Sulaiman M. Yousafzai

Modular optical tweezers kit from Thorlabs

Funded by: ICTP and SPIE

modular optical tweezers kit from Thorlabs




White Light Source
LD & TEC Controller
Position Sensing Detector
Dichroic Mirror
Butterfly Mount
Lens
Condenser Lens
Fiber
Sample Holder
FiberPort
Objective Lens
XYZ Sample Stage
CCD Imaging Detector
Lens
Mirror
Beam Expander
Dichroic Mirror

Main characteristics:

Trapping module:

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

scanning over a range of 50 mm, facilitating loading/uploading of the sample cel.

Position detection and force measurement module:

- position detection based on interference pattern in the back focal plane of the condenser, interference formed by trapping laser beam scattered by the trapped bead (probe); Quadrant Position Detector (QPD) detects the pattern displacement sampling it at high frequency rate (100 kHz)

Main characteristics (2):

- position calibration capability with 5 nm resolution; trap stiffness calibration using different methods as: Power Spectral Density (PSD), Stokes
drag and Equipartition theorem (for details of these methods see for instance references [2], [3]); determining the trap stiffness and knowing that the bead probe near the equilibrium position of the trap, behaves as in a Hooke potential well (linear spring with stiffness k), force measurement of the probe interacting with a sample (e.g. cell) can be calculed measuring the displacement x of the bead: $F=kx$; the trap stiffness depends of the power of the trapping laser and of the material and geometry of the trapped probe; the stiffness range is 10^{-4}–1 pN/nm, which allows to measure forces in pN range with resolution of tens of fN; the stiffness is 2-3 order of magnitudes smaller than that of the cantilever stiffness in AFM

Laser beam steering

- X-Y beam steering with galvano mirrors; max bandwith 1kHz for angular amplitude 0.4°, step response 300 µs ; full scale bandwidth 100 Hz square wave, 250 Hz sinewave; applications: precisely move the trapped particle in X-Y, create two stable traps by properly switching the beam from a trap to another.

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- **Serena Bonin, Giorgio Stanta**, Cattinara Hospital – University of Trieste

- **Giacinto Scoles**, University of Udine

Optical Manipulation OM-Lab

http://dancojoc.wix.com/om-lab

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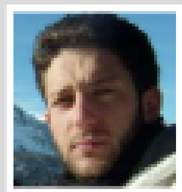

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OM - Lab Optical Manipulation

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F. Nietzsche: There are no facts , only interpretations !
Miramare Castle,
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THANK YOU !