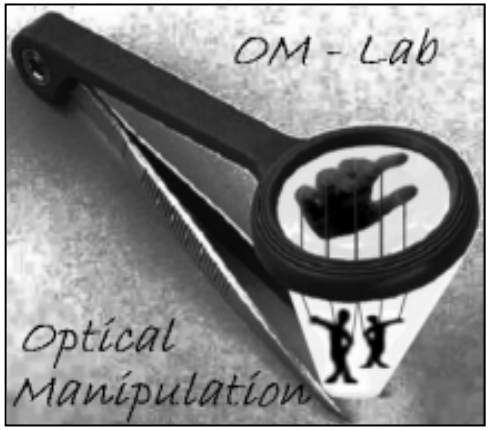
Cell focal stimulation and probing by optical tweezers microscopy

Dan COJOC

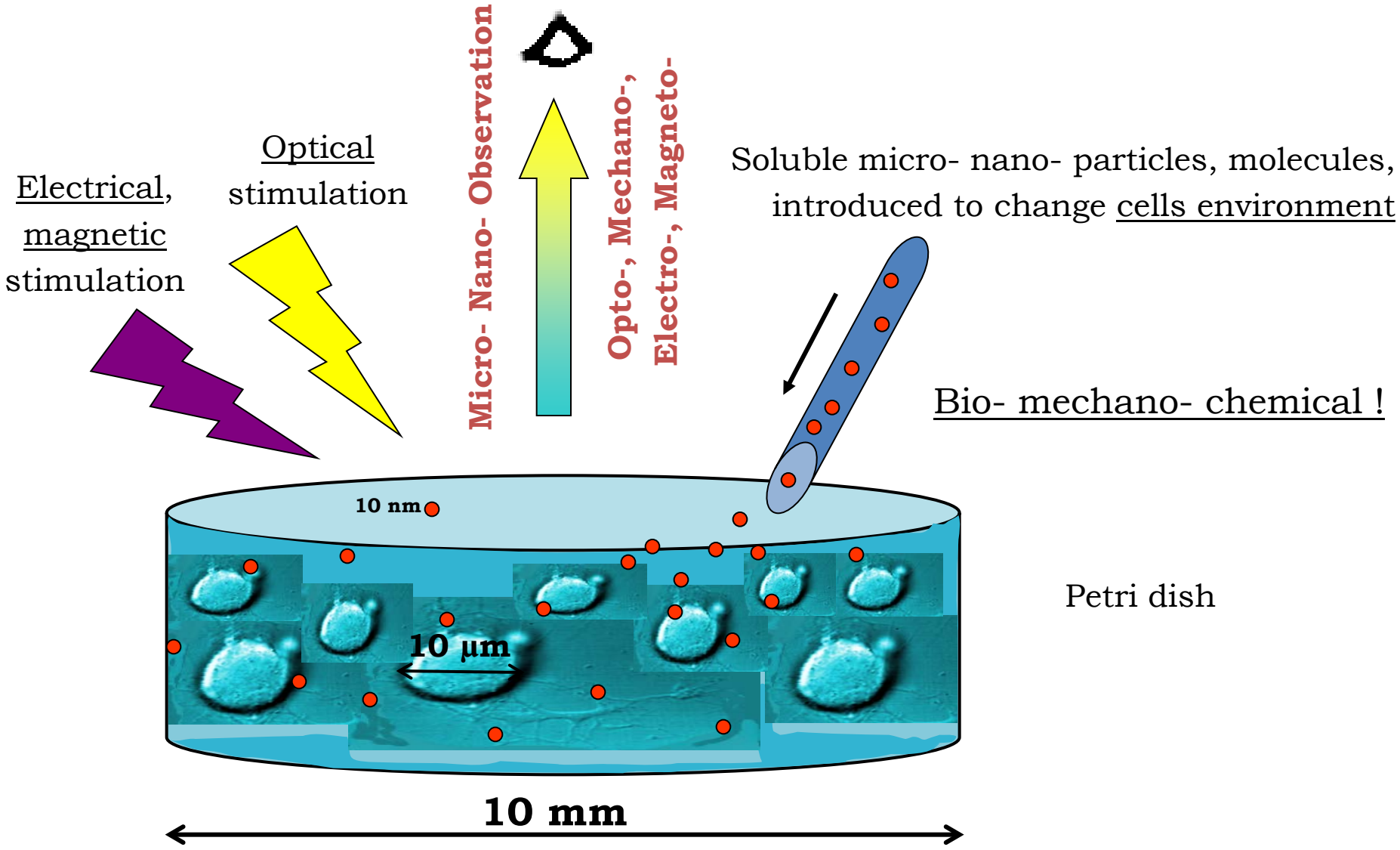
OM-Lab @ CNR-IOM, Trieste, Italy



cojoc@iom.cnr.it

Istituto Officina dei Materiali

Living cells - Typical experimental situation



OUTLINE

- **Optical tweezers – three dimension particle trapping: how this works**

- **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)

OUTLINE

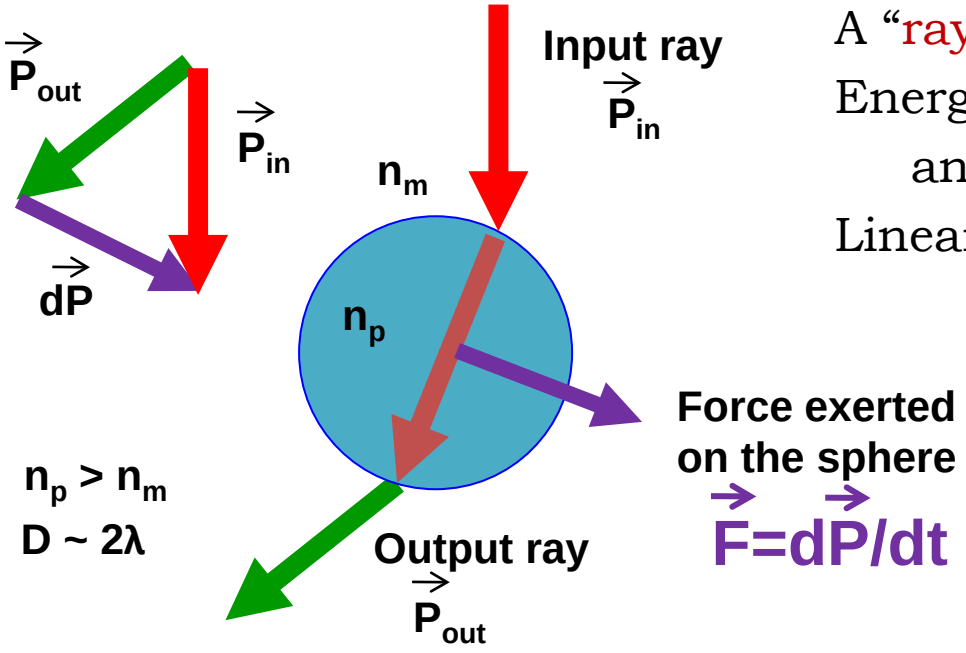
- **Optical tweezers – three dimension particle trapping: how this works**

- 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)

Force exerted by a Ray of Light on a micro-sphere of glass

A simple picture



A "ray of light" containing N photons has:

Energy: $E = Nh\nu$

and

Linear momentum: $|\vec{P}| = Nh\nu/c$

Arthur Ashkin, Bell Labs, 1970

n_m – refractive index of the medium

n_p – refractive index of the particle

D – diameter

$\vec{P}$ – Linear momentum

c – velocity of light in vacuum

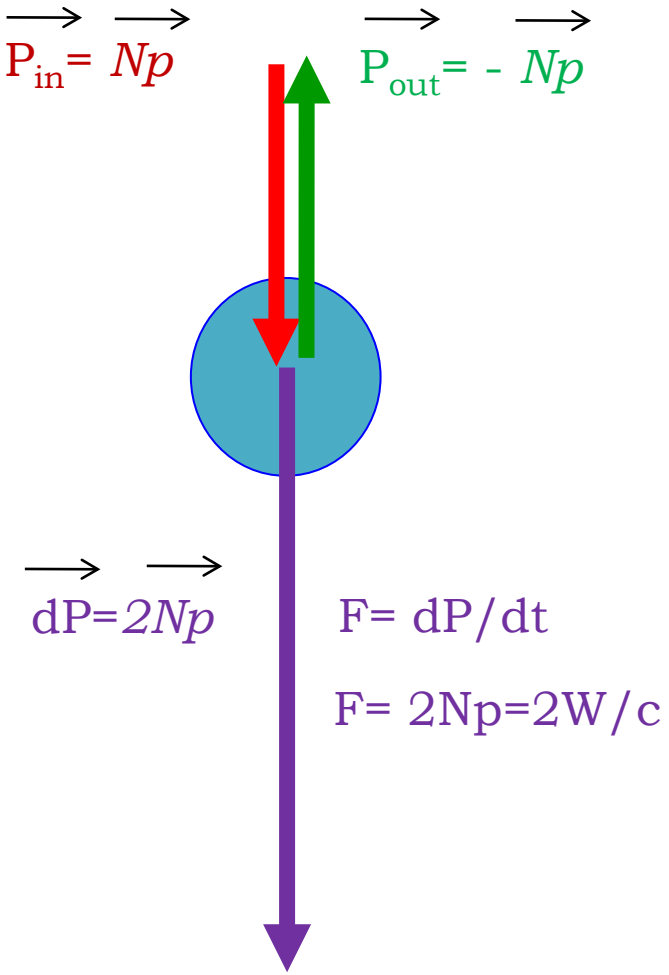
Excellent book by A. Ashkin:

Optical trapping and manipulation of neutral particles using lasers

World Scientific Publishing, 2006

Let us consider some numbers:

Given: W= 1mW, d=1um (m=1 pg), R=1; how big is **F** ?



Some crude calculation

N= W/hν - number of photons/second

p=hν/c - momentum of a photon

Force F, due to perfect reflection:

F=dP/dt=2Np=2W/c ~ 0.66 x10^{-11} N

F_{max} ~ 10 pN This is small !

But, acceleration, **a**, is:

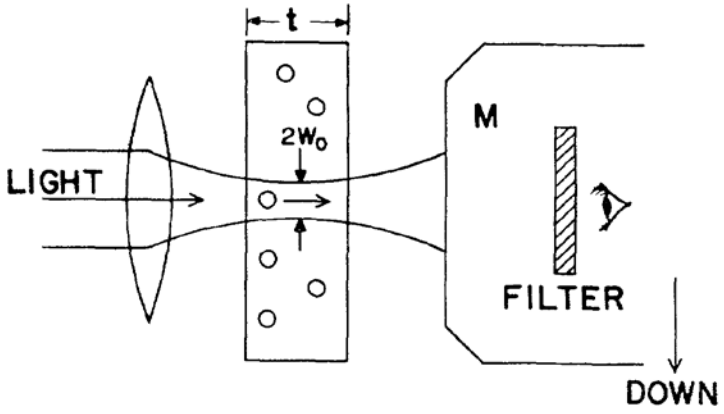
$\mathbf{a}_{max} = F_{max}/m = 10^4$ m/s^2

$\mathbf{a}_{max} = 10^3 g$ **This is big !**

actually F~0.1 Fmax and:

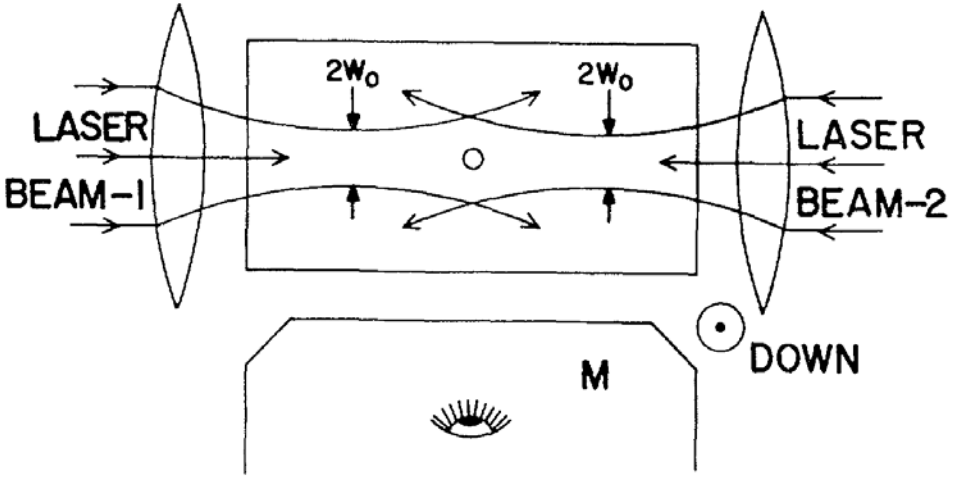
a ~10^2 g This is still big !

Two dimensional (2D) and three dimensional (3D) particle trapping



2D trapping

With single laser beam focused through a lens with low NA



3D trapping

With two counter propagating laser beams

Ashkin, Phys. Rev. Lett. **24** 156 **1970**

3D particle trapping using a single laser beam

Laser beam focused through a high NA lens
OPTICAL TWEEZERS

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

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

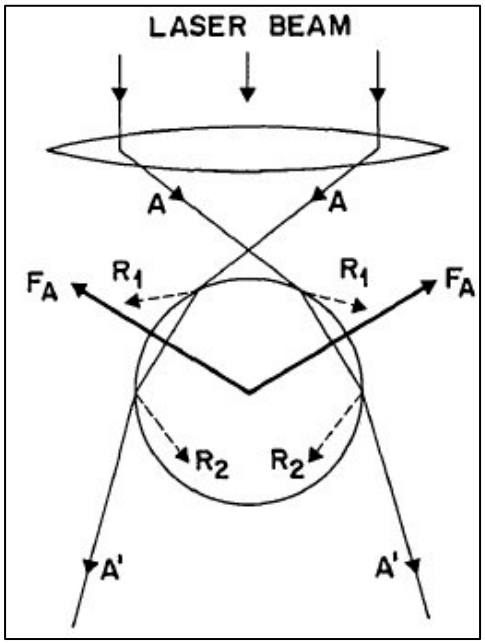
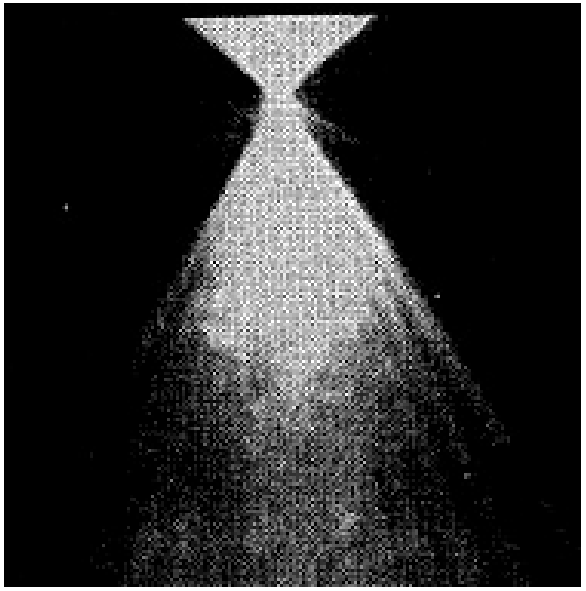
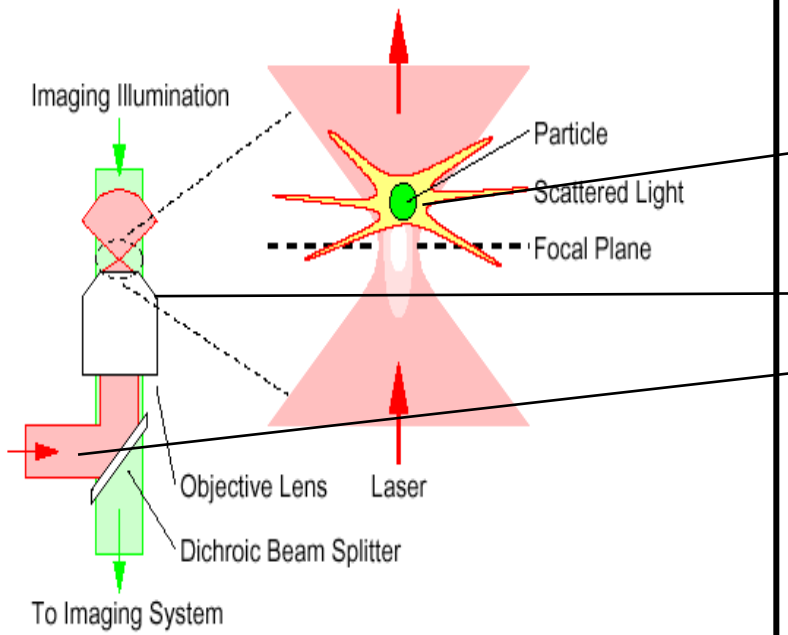


Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single-beam gradient force trap.



Photograph taken in red (water fluorescence), of a 10 um sphere trapped with an argon laser beam, showing the paths of the incident and scattered light rays.

A. Ashkin *et al*, *Opt.Lett.* **11** 288 **1986**

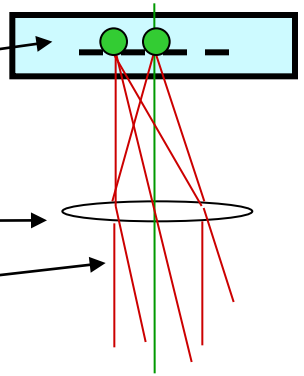


$$F = Q\frac{n_m W}{c}$$

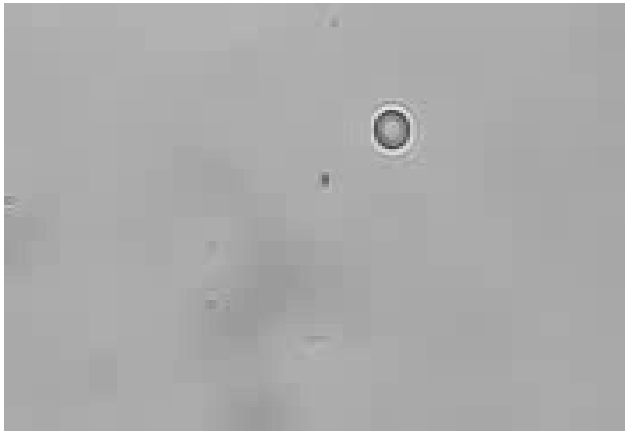
Ex: **F= 1.33 pN** for
W= 1 mW, n_m=1.33, Q=0.3

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)

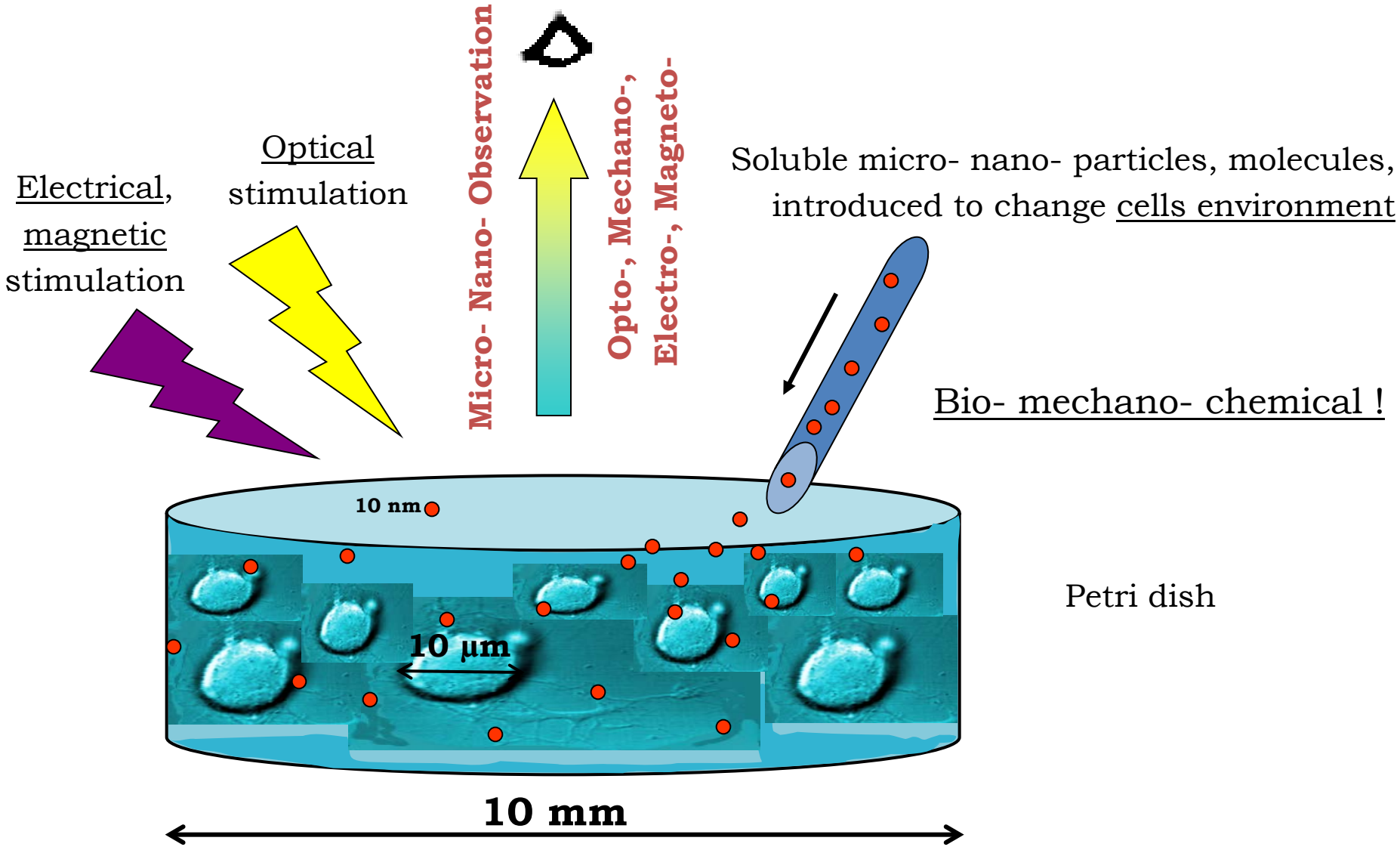
OUTLINE

- Optical tweezers – three dimension particle trapping: how this works

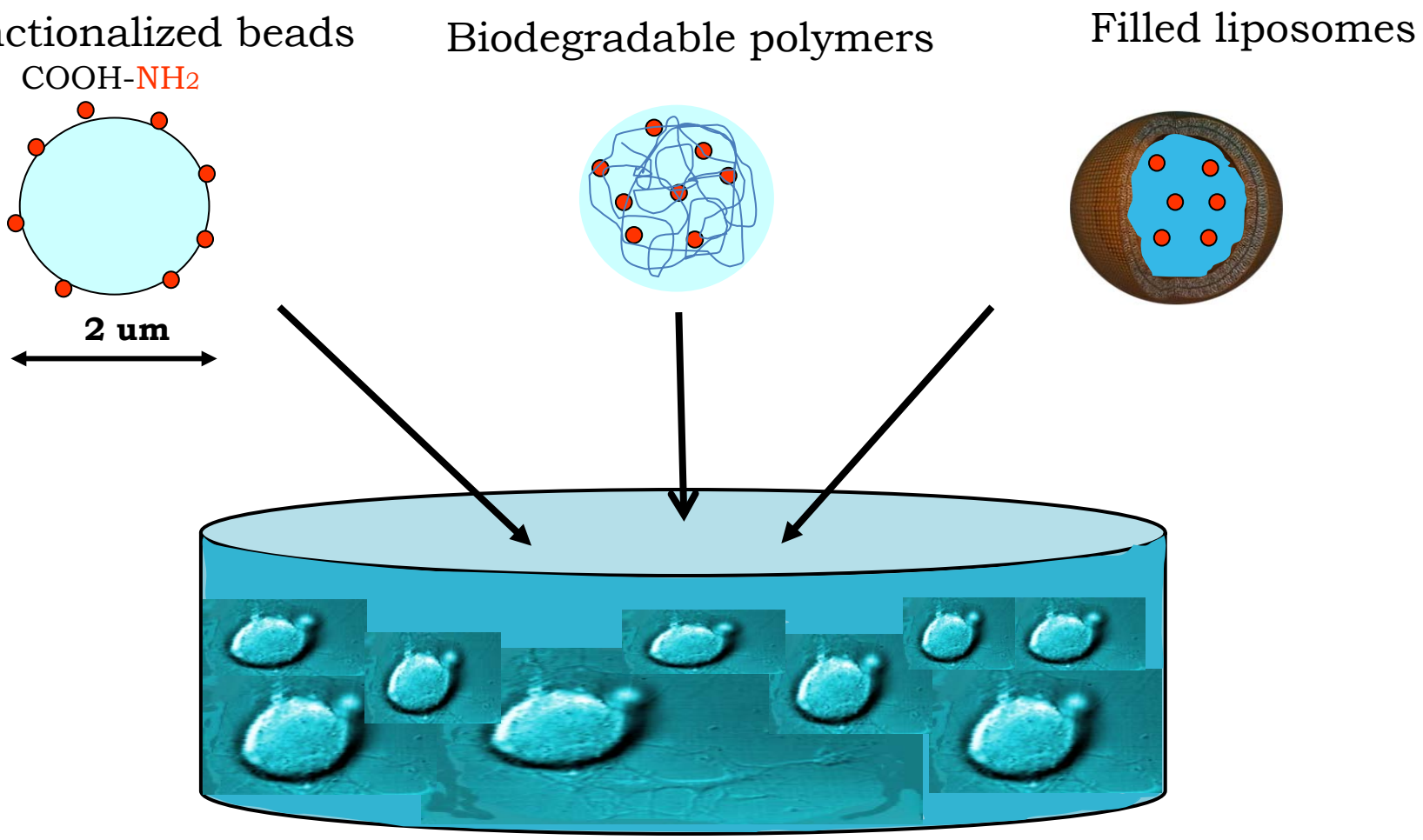
- **Biochemical local cell stimulation using optically manipulated vectors** (e.g. beads, biodegradable micro-sources, liposomes, QDs)

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

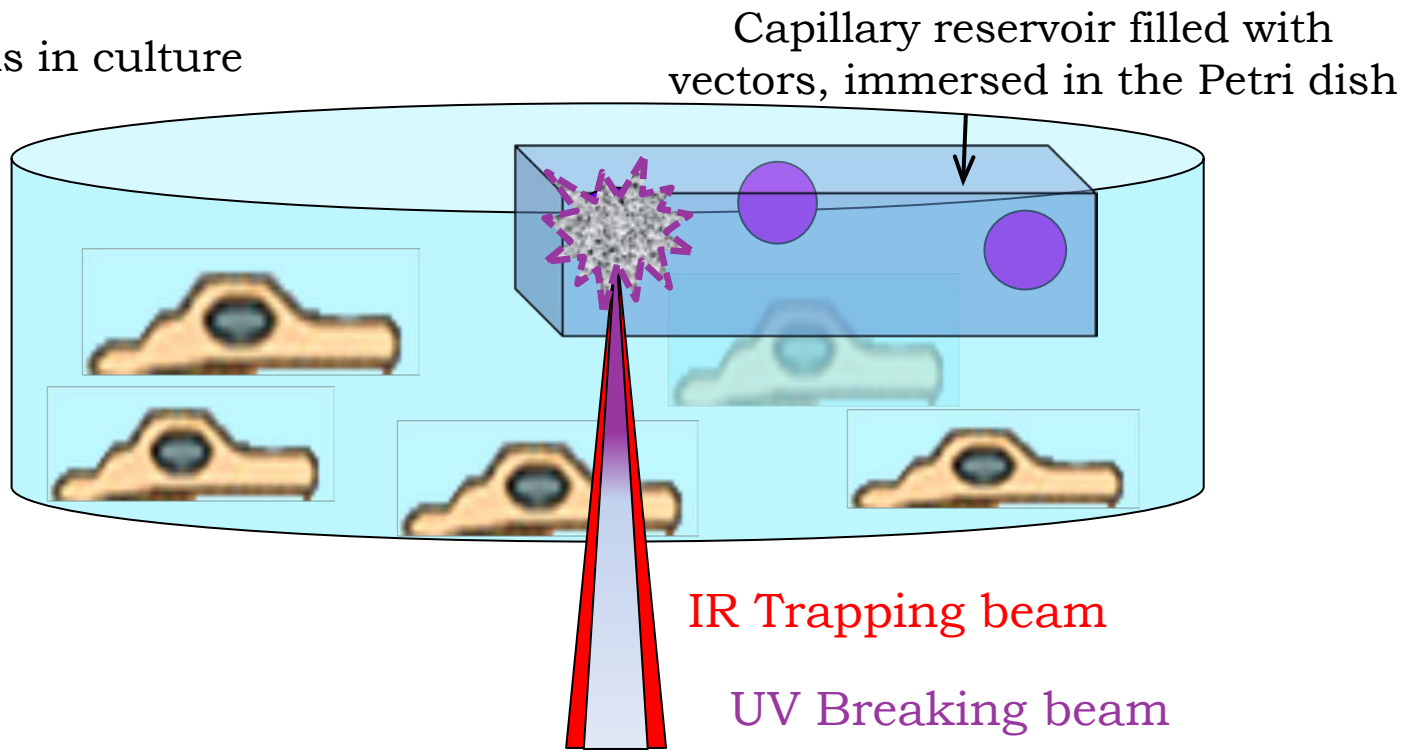
Living cells - Typical experimental situation



micro & nano Vectors



The vectors are driven to defined location by optical tweezers



and delivered by:

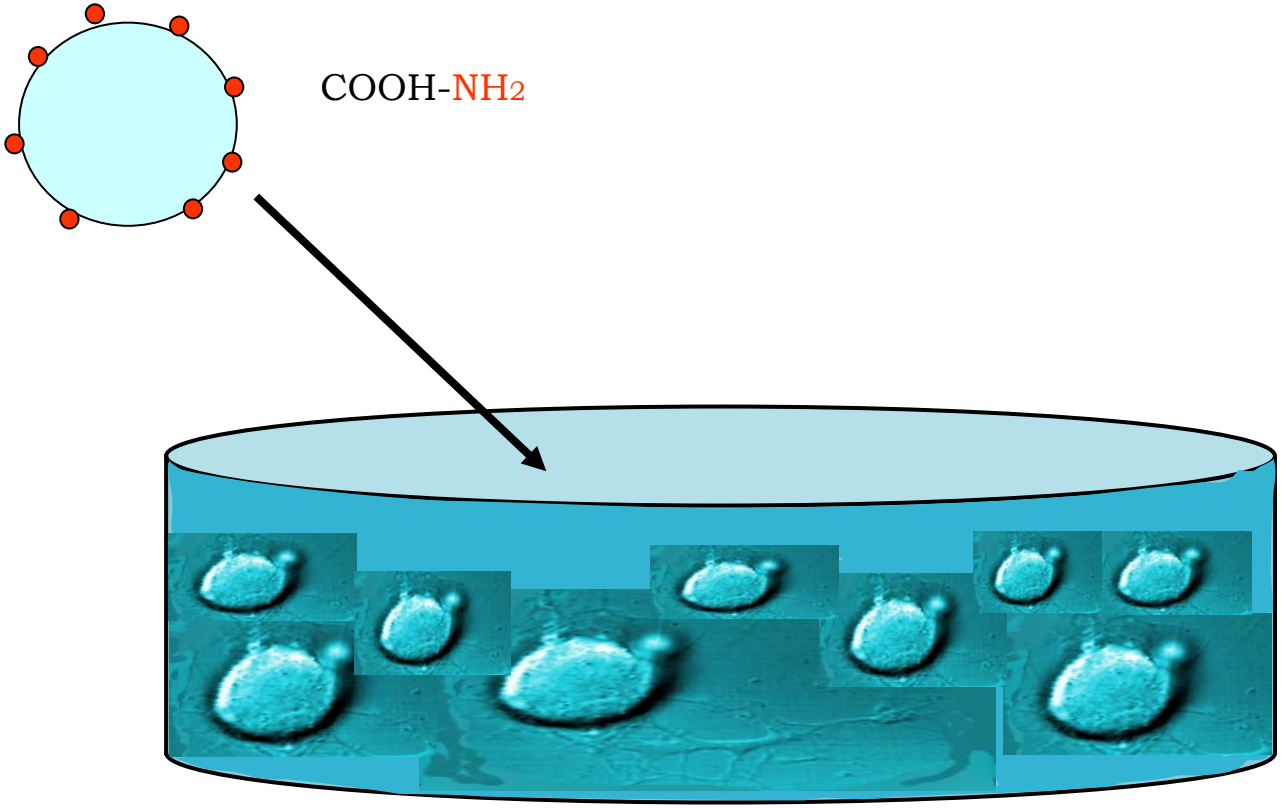
- contact (beads or liposomes) – D'Este *et al* (2011) Integrative Biology

-or by breaking the liposome with UV laser pulses (liposomes)
Sun and Chiu (2003) JACS

Why local delivery / stimulation ?

- ideal for single (selected) cell experiments
- different compartments of the cell can have different behaviors under the same stimulus
- scale down the stimulation study at the level of very few molecules
- stimulate different compartments of the cell with different molecules
- high spatial and temporal resolution
- ?????????? Open discussion

Functionalized/coated beads (silica, polystirene, QDs ?)

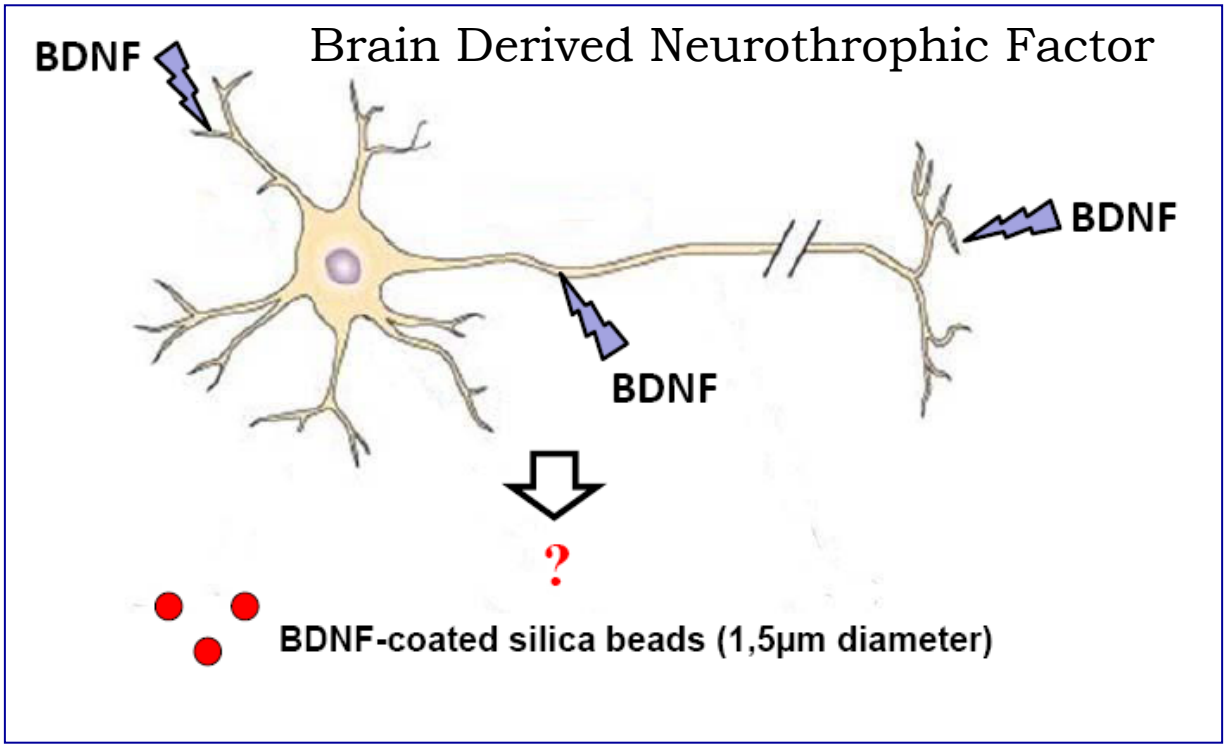


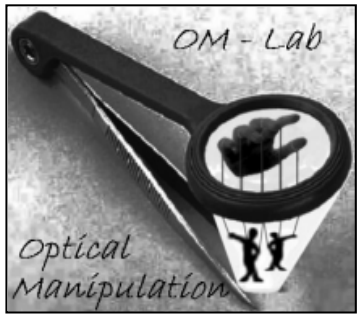
Cite this: *Integr. Biol.*, 2011, **3**, 568–577

www.rsc.org/ibiology **Integrative Biology** **PAPER**

Use of optical tweezers technology for long-term, focal stimulation of specific subcellular neuronal compartments†

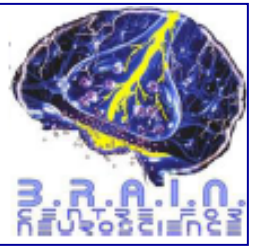
Elisa D'Este,‡[a] Gabriele Baj,[b] Paolo Beuzer,§[a] Enrico Ferrari,¶[a] Giulietta Pinato,[a] Enrico Tongiorgi[b] and Dan Cojoc*[a]



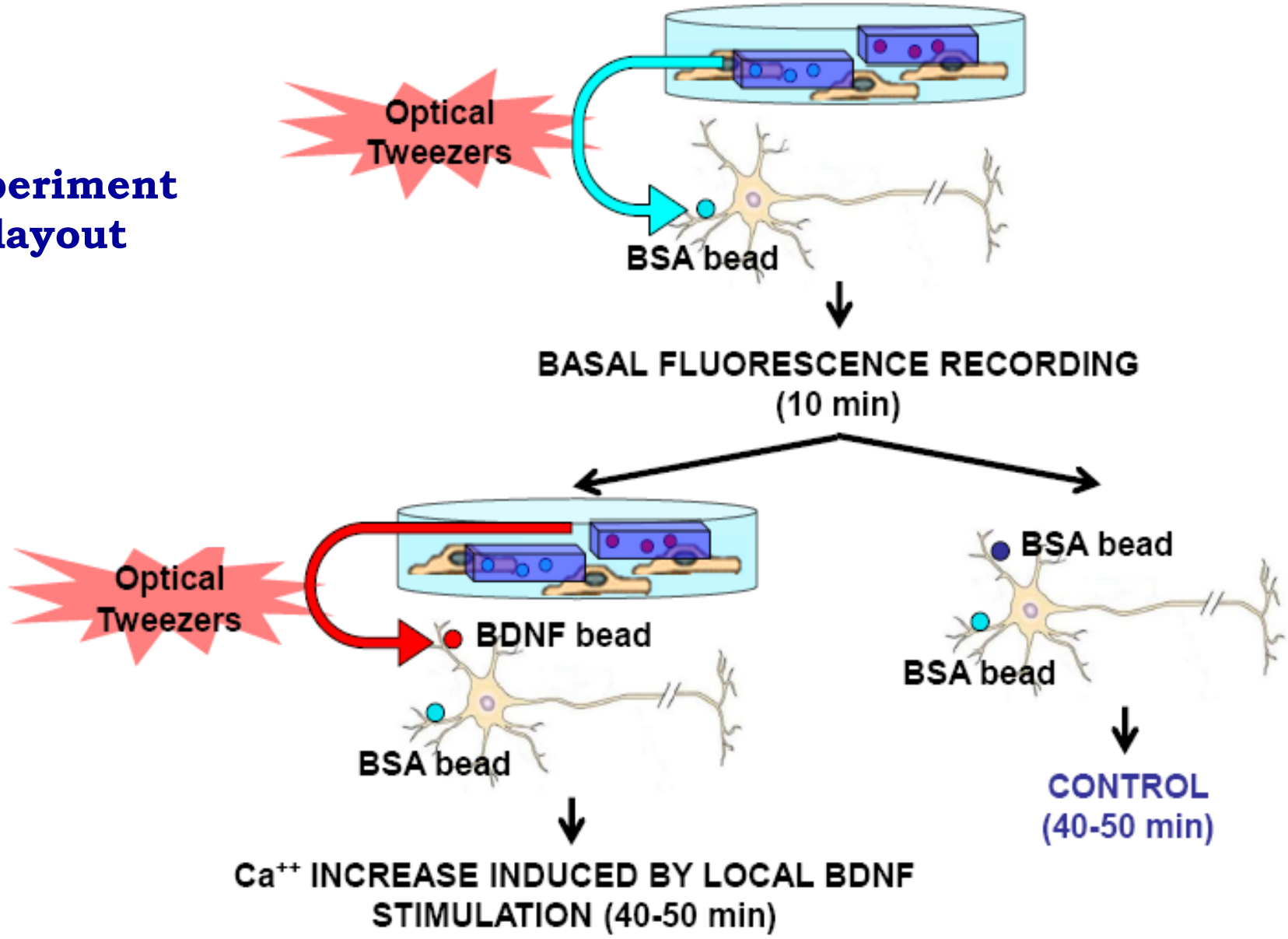


in collaboration with:

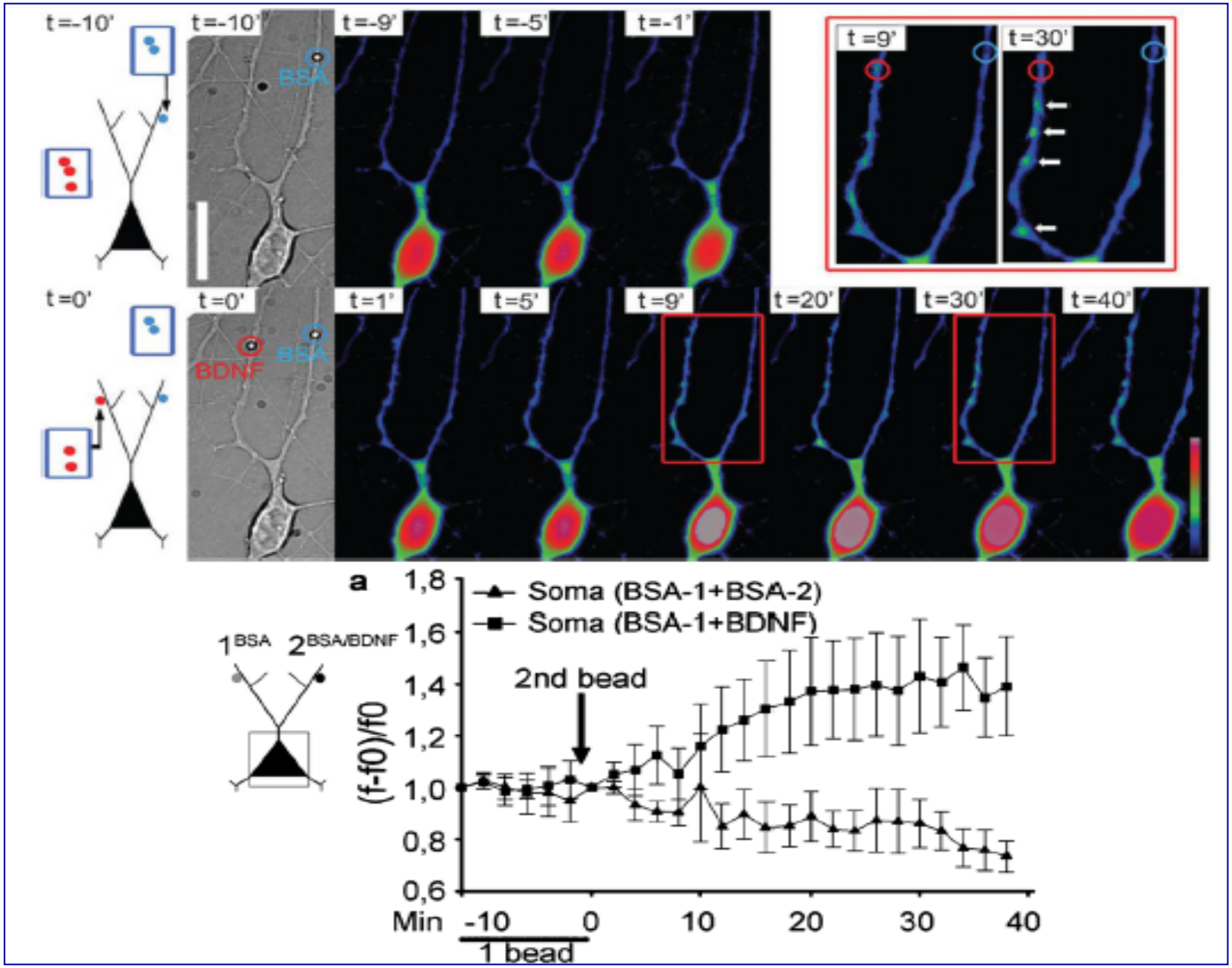
Lab Prof. Tongiorgi

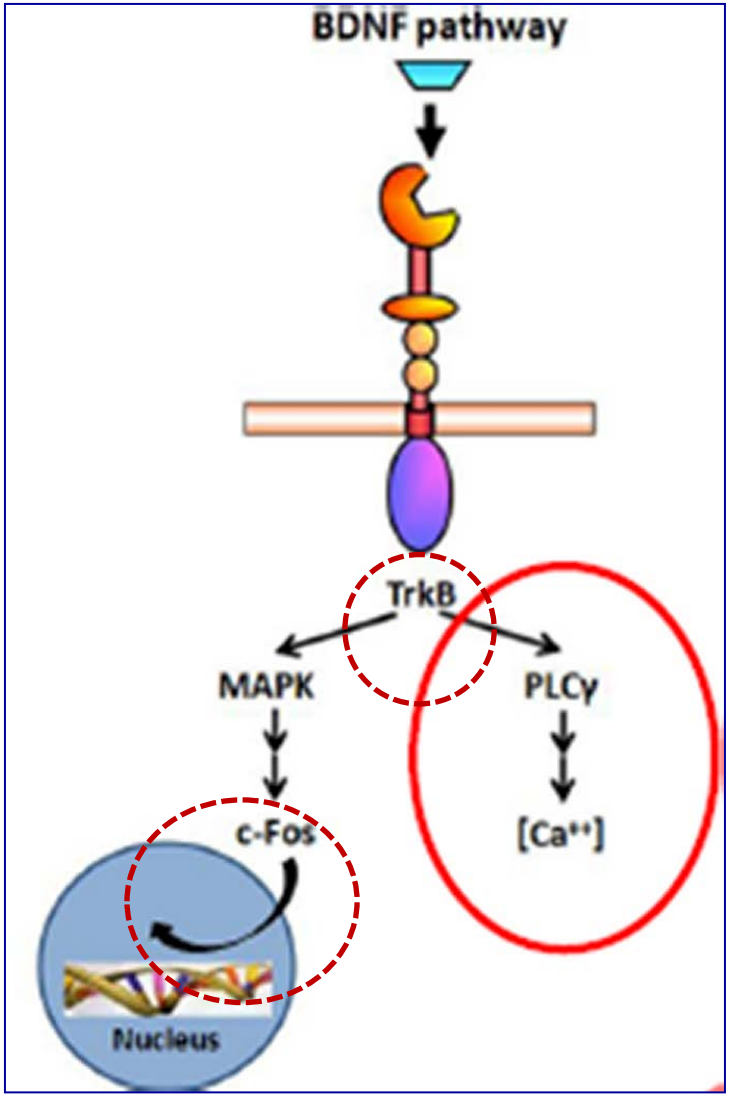


Experiment layout



Increase of Ca^{++} level in cell body and stimulated dendrite, induced by BDNF local stimulation





Beside Ca^{++} we have also proved:

- the BDNF bead activates the TrkB receptor (phosphorilation)

- c-Fos translocaton in nucleus

- BDNF bead guides and stimulates dendrite /growth cone motility

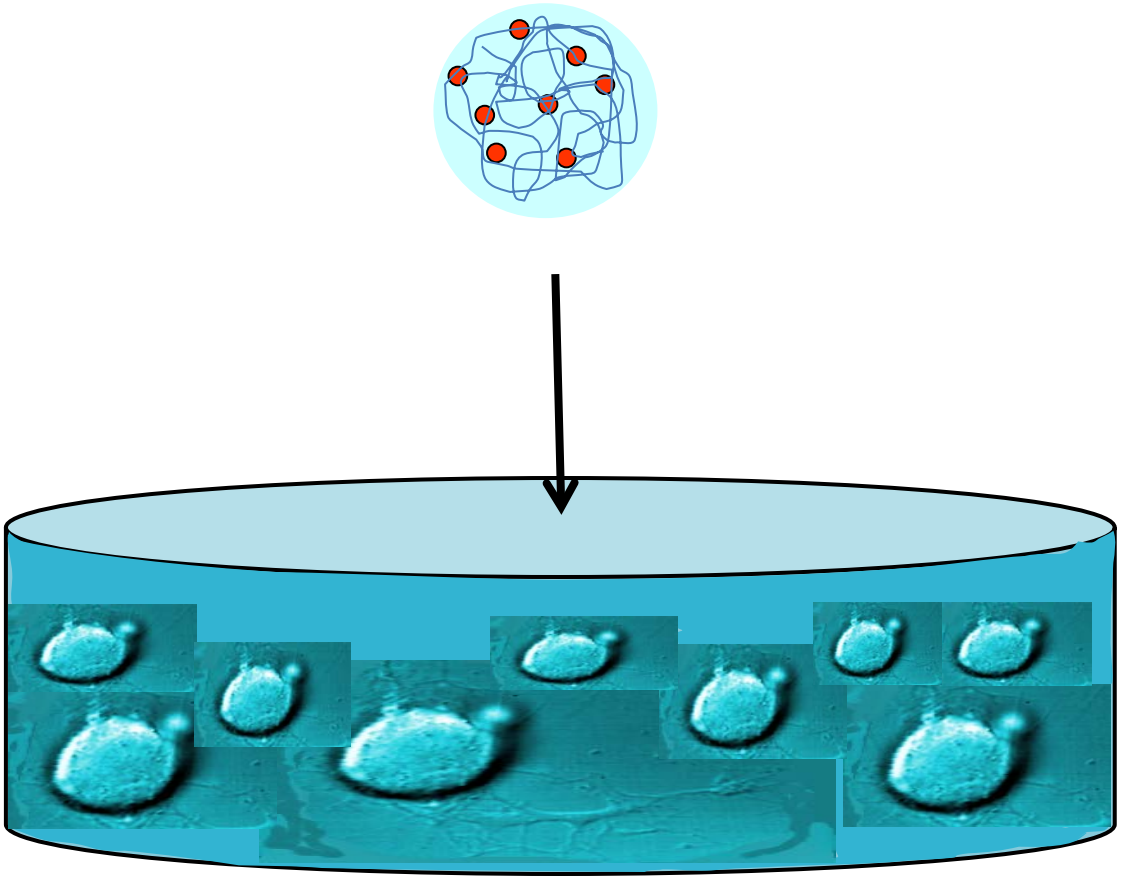
What we learned ?

• A single BDNF coated bead is enough to activate the biological pathways in neurons.

• BDNF do not necessarily need to be endocyted in order to trigger the receptor TrkB pathway (since the molecules are covalently cross-linked to the bead surface).

• However, it would be interesting to study what happens when these molecules or other molecules are released in the vicinity of the cell and are endocyted.

Microsources:
beads biodegradable polymers



Two facts

Molecular gradients (spatial and temporal) are very important for various biological processes including cell polarization.

Biodegradable polymer μ-sources/beads, which steadily release molecules, enable the control of molecular concentrations over length scales down to about 1 μm and time scales from 0.1 s to 1 h.

An example

PolyLactic-co-Glycolic Acid (PLGA) μ-sources/beads loaded with chemo attractant and respectively chemo repellant molecules are optically manipulated to study single human neutrophils (HL-60 cells) polarization and motility.

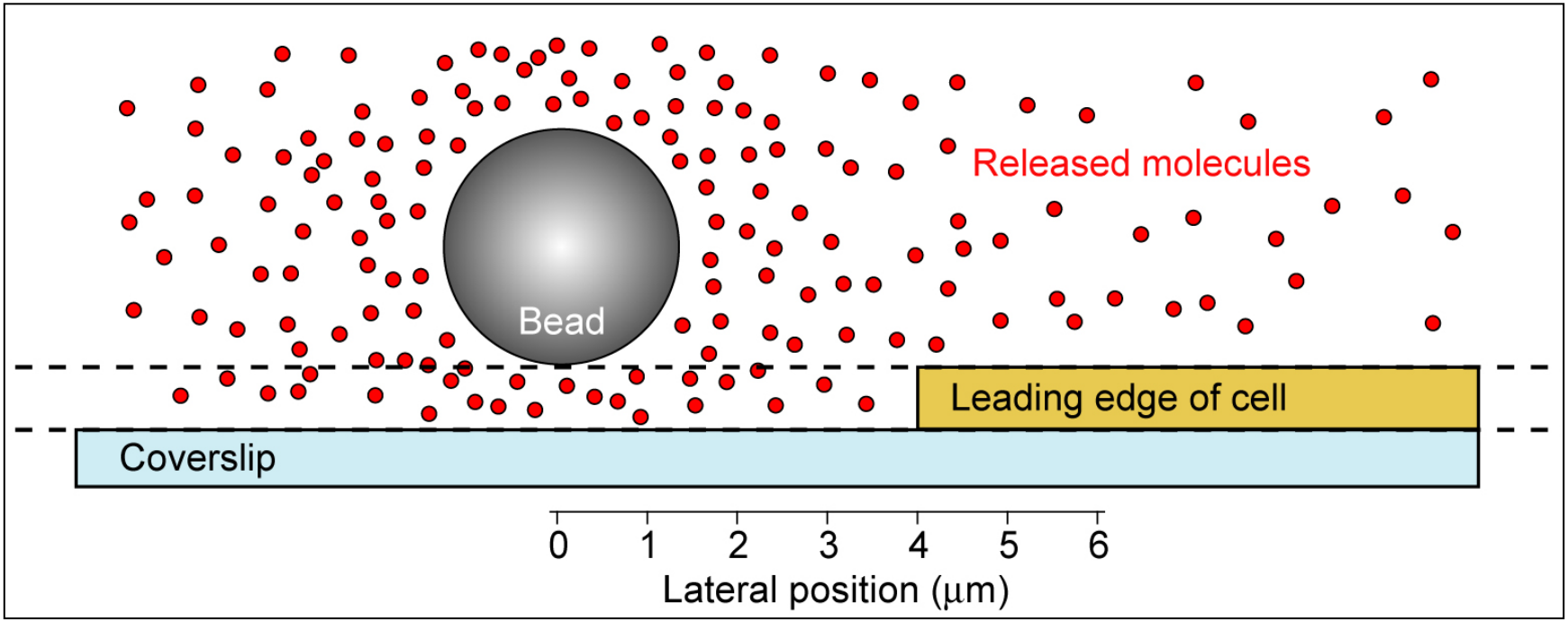
Chemoattractant: formyl-methionine-leucine-phenylalanine

Chemo repellant: cytochalasin D.

ARTICLE

Nature Methods **6**, 905 - 909 (2009)
Published online: 15 November 2009 | doi:10.1038/nmeth.1400

Cell stimulation with optically manipulated microsources



Holger Kress[1], Jin-Gyu Park[1], Cecile O Mejean[1], Jason D Forster[1], Jason Park[2], Spencer S Walse[3,8], Yong Zhang[4], Dianqing Wu[4], Orion D Weiner[5], Tarek M Fahmy[2,3] & Eric R Dufresne[1,3,6,7]

A chemo attractant-loaded particle induces cell motility and change of the direction of migration of a neutrophil.

Movie – download supplementary material

ARTICLE

Nature Methods **6**, 905 - 909 (2009)
Published online: 15 November 2009 | doi:10.1038/nmeth.1400

Cell stimulation with optically manipulated microsources

Holger Kress[1], Jin-Gyu Park[1], Cecile O Mejean[1], Jason D Forster[1], Jason Park[2], Spencer S Walse[3,8], Yong Zhang[4], Dianqing Wu[4], Orion D Weiner[5], Tarek M Fahmy[2,3] & Eric R Dufresne[1,3,6,7]

Migrating cell squeezes between two optically manipulated microsources of cytochalasin D (chemo repellant)

Movie – download supplementary material

ARTICLE

Nature Methods **6**, 905 - 909 (2009)
Published online: 15 November 2009 | doi:10.1038/nmeth.1400

Cell stimulation with optically manipulated microsources

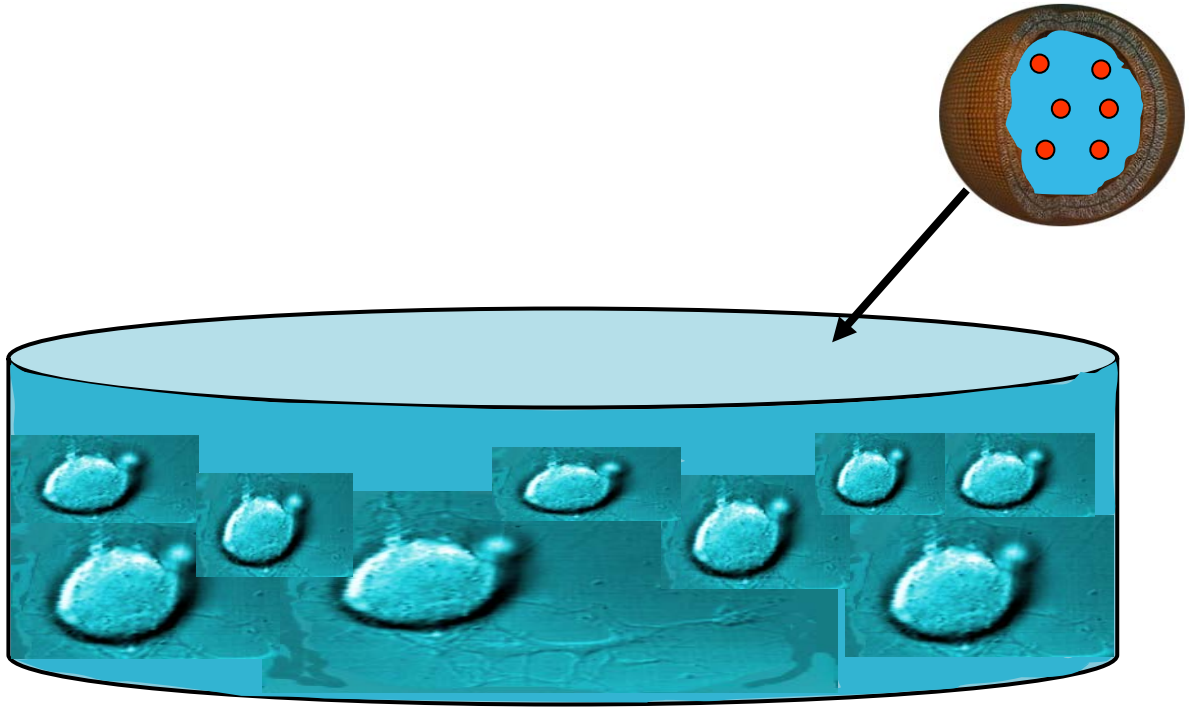
Holger Kress[1], Jin-Gyu Park[1], Cecile O Mejean[1], Jason D Forster[1], Jason Park[2], Spencer S Walse[3,8], Yong Zhang[4], Dianqing Wu[4], Orion D Weiner[5], Tarek M Fahmy[2,3] & Eric R Dufresne[1,3,6,7]

Take home learned things

•Loaded microbeads allow to create spatial and temporal gradients of concentration inducing polarization and migration of the cell

• However, the release of active molecules can be not triggered with high temporal resolution

Filled liposomes

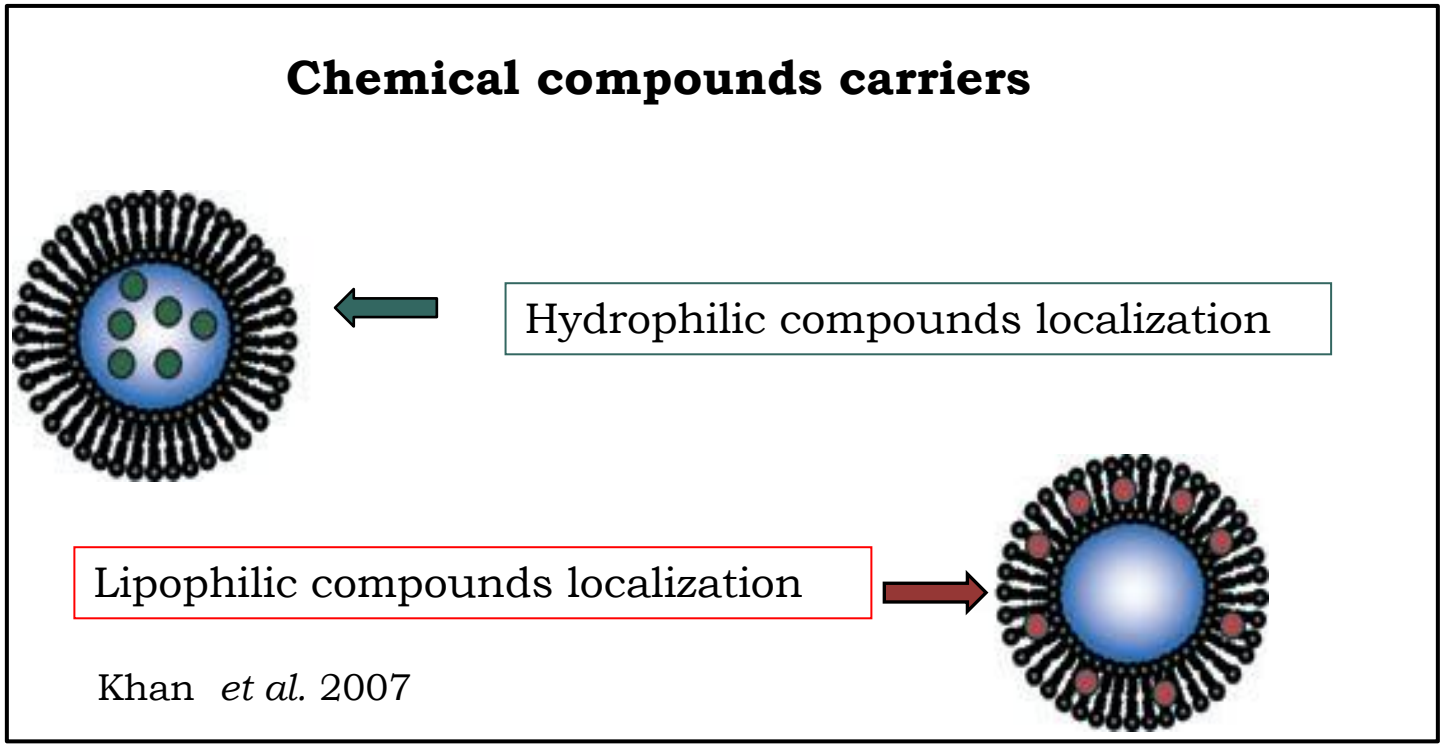


Numerical example:

1 nM concentration, **1 µm** liposome → **1 molecule** (mean value) **!!!**

Liposomes

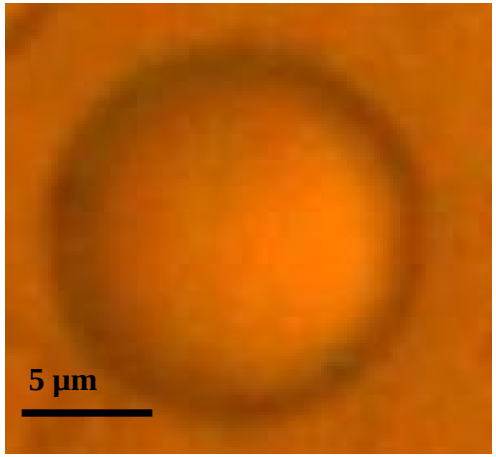
- ✓ Spherical vesicles from 50 nm to 50 µm
- ✓ Phospholipid bilayer membrane
- ✓ Aqueous core



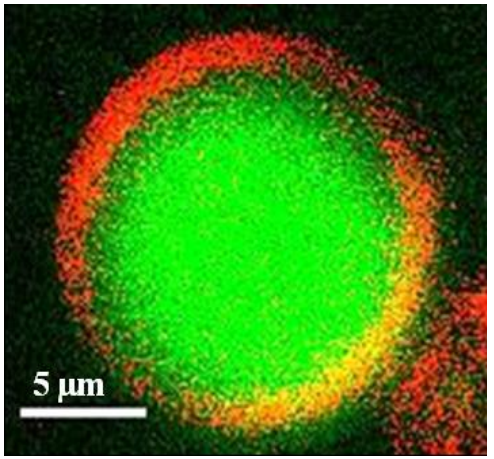
Lipid films hydration

MAIN STEPS

1. Deposition of lipid film
2. Hydration with aqueous phase
3. Purification in glucose gradient



100X bright field



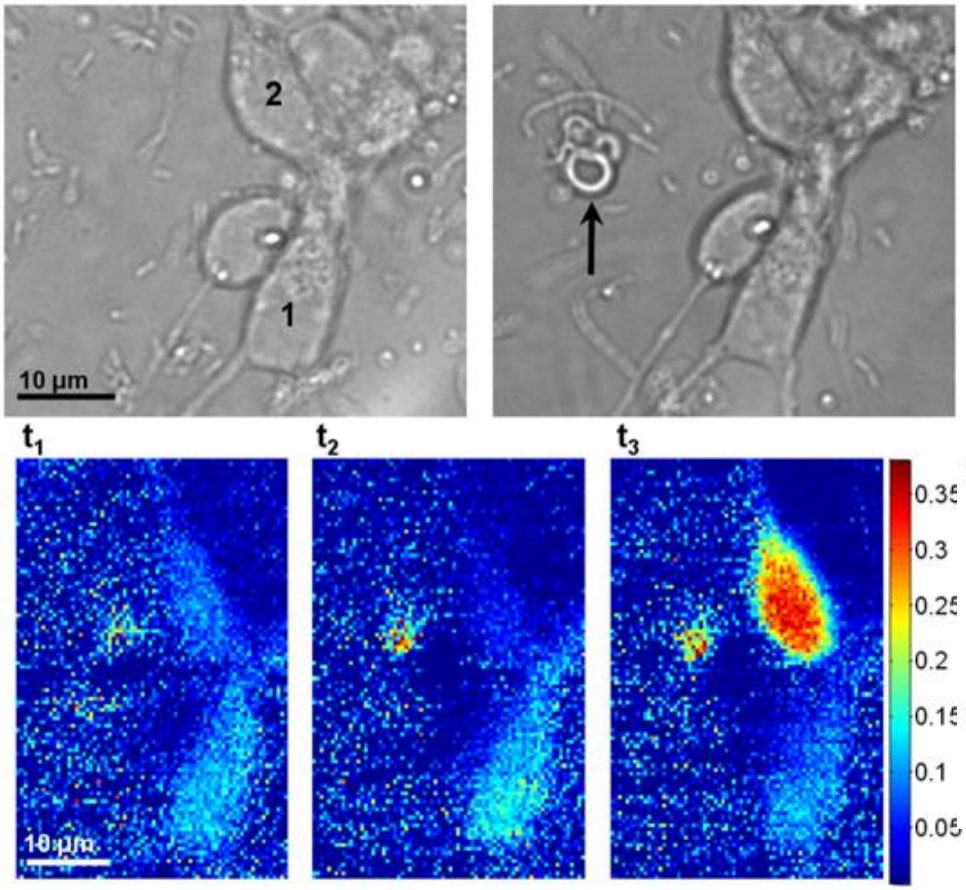
Confocal stack

ADVANTAGES

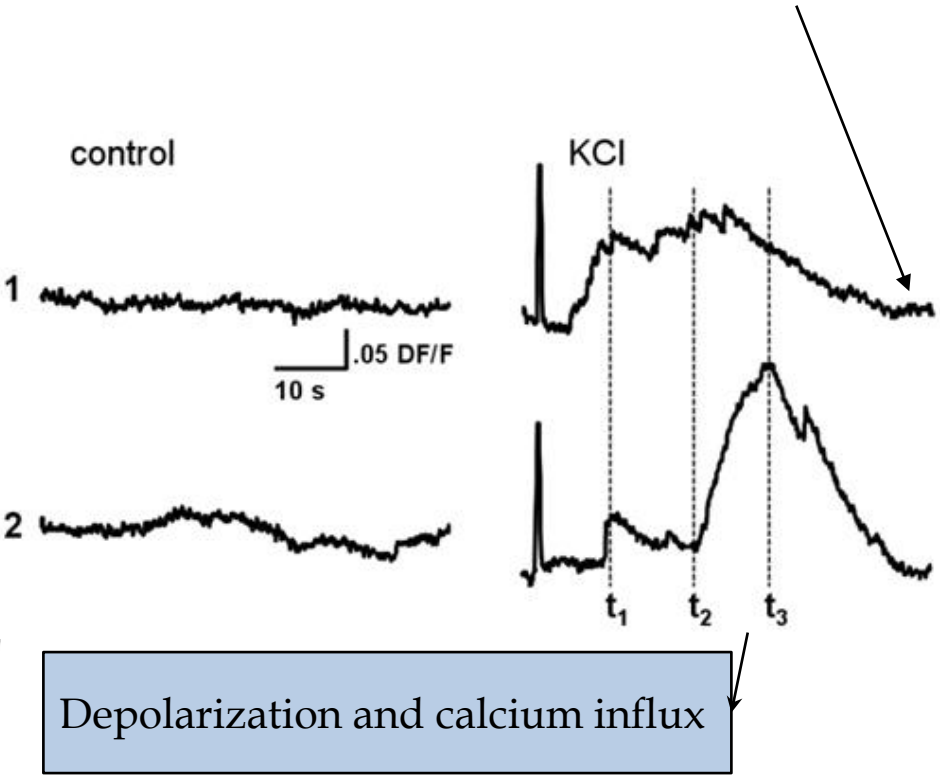
- ✓Very simple and high reproducible
- ✓Unilamellar liposomes 1-10 micron
- ✓Encapsulation of large spectrum of molecules
- ✓Low cost equipment required

Hub *et al*, (1982)

Technique Validation: Hippocampal neurons stimulation with KCl



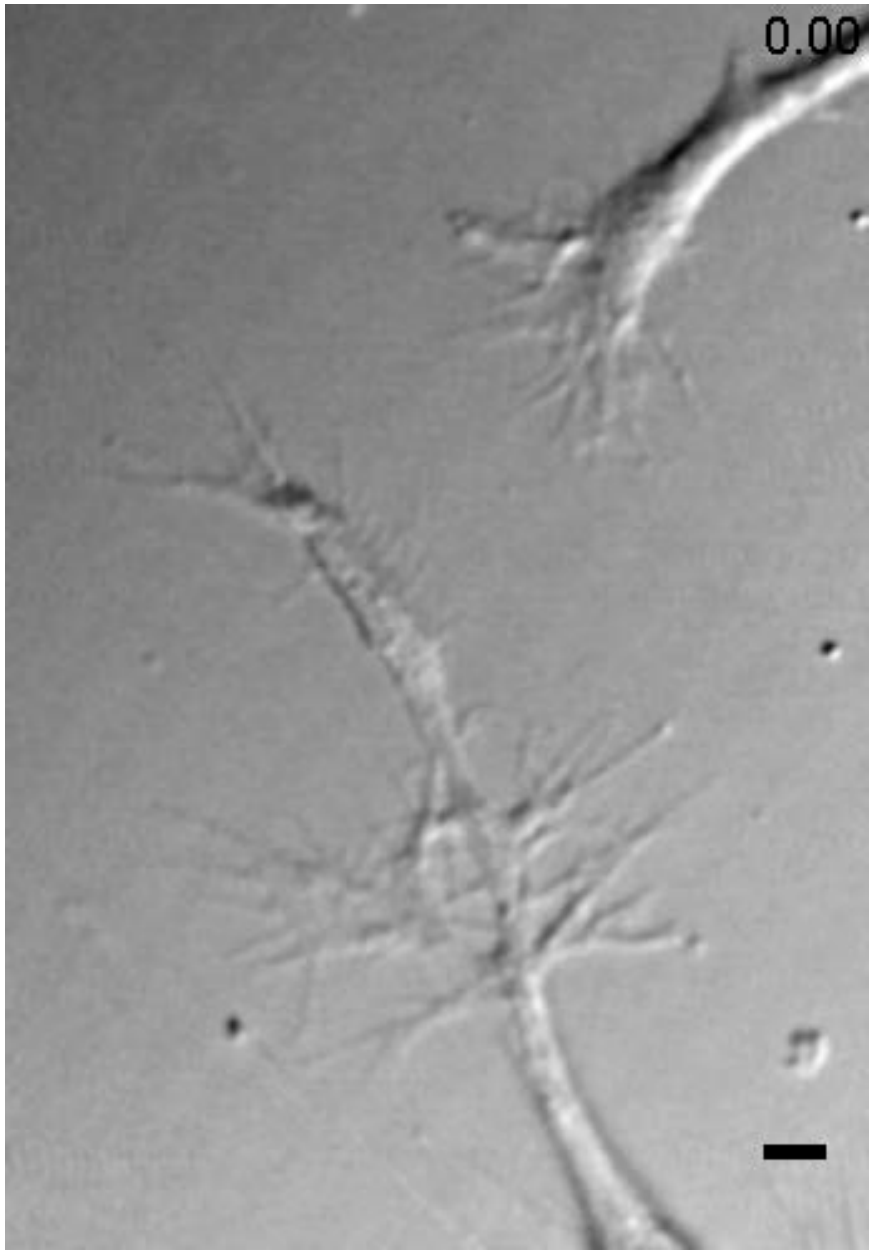
Cells stained with OGB-488-BAPTA-1-AM



Return on basal levels

Depolarization and calcium influx

G. Pinato, T. Raffaelli, E. D'Este, F. Tavano and D. Cojoc Optical delivery of liposome encapsulated chemical stimuli to neuronal cells, J. Biomed. Optics 16, 2011.



Growth cones ‘flirt’

Neurons release cues which are interpreted by nearby neurons.

We mimic the release of one type of cue molecules and study the morphological answer (growth cone dynamics) of the neuron .

Scale Bar = 2 µm
Acquisition freq= 0.2Hz
Time in min.sec
Total time length ~ 2 h

Study case:
Diffusion of the PrPc molecules from a photolysed liposome

Motivation:

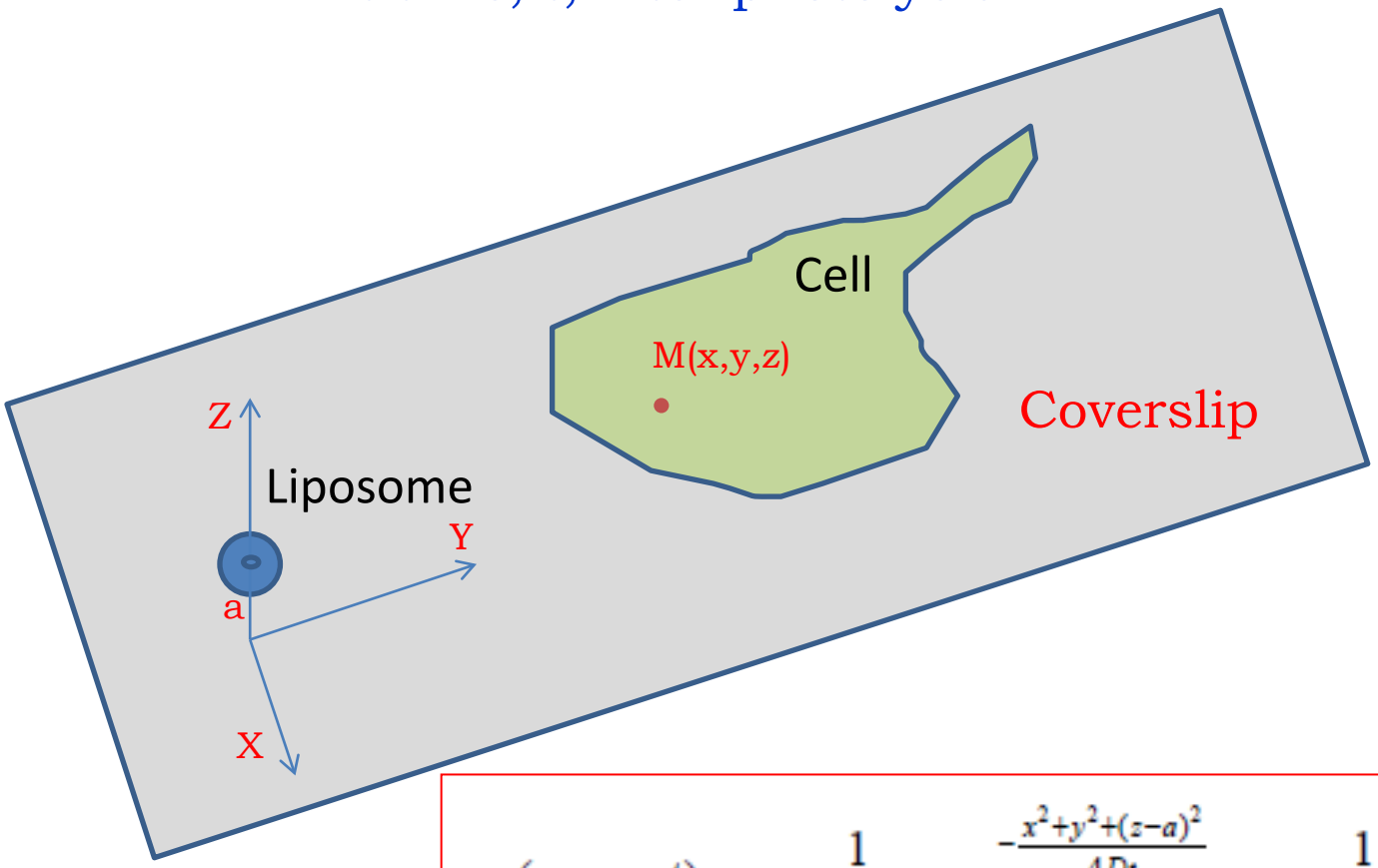
PrPc - > Prion → neurodegenerative disease not understood

PrPc was studied only in bulk and shown to have chemo attractant effect after 48 hours

PrPC functions are mostly unknown

Collaboration with Dr. Ladan Amin and
Prof. Giuseppe Legname SISSA – Prion Lab

Which is the concentration, c, in a point M(x,y,z) at time, t, after photolysis ?



$$c(x,y,z,t) = \frac{1}{(4\pi Dt)^{3/2}} e^{-\frac{x^2+y^2+(z-a)^2}{4Dt}} + \frac{1}{(4\pi Dt)^{3/2}} e^{-\frac{x^2+y^2+(z+a)^2}{4Dt}}$$

The liposome is approximated with a point source, unitary concentration,
Coverslip surface is reflecting (second term)
D – diffusion coefficient, a – liposome position (center) in z

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
BIOLOGICAL MOLECULES
DEVELOPMENT
NEUROANATOMY
NANOBIOTECHNOLOGY

Received
15 May 2012

Accepted
3 September 2012

Published
20 September 2012

Correspondence and requests for materials should be addressed to G.P. (pinato@iom.cnr.it) or D.C. (cojoc@iom.cnr.it) or V.T. (torre@sissa.it)

*These authors contributed equally.

Less than 5 Netrin-1 molecules initiate attraction but 200 Sema3A molecules are necessary for repulsion

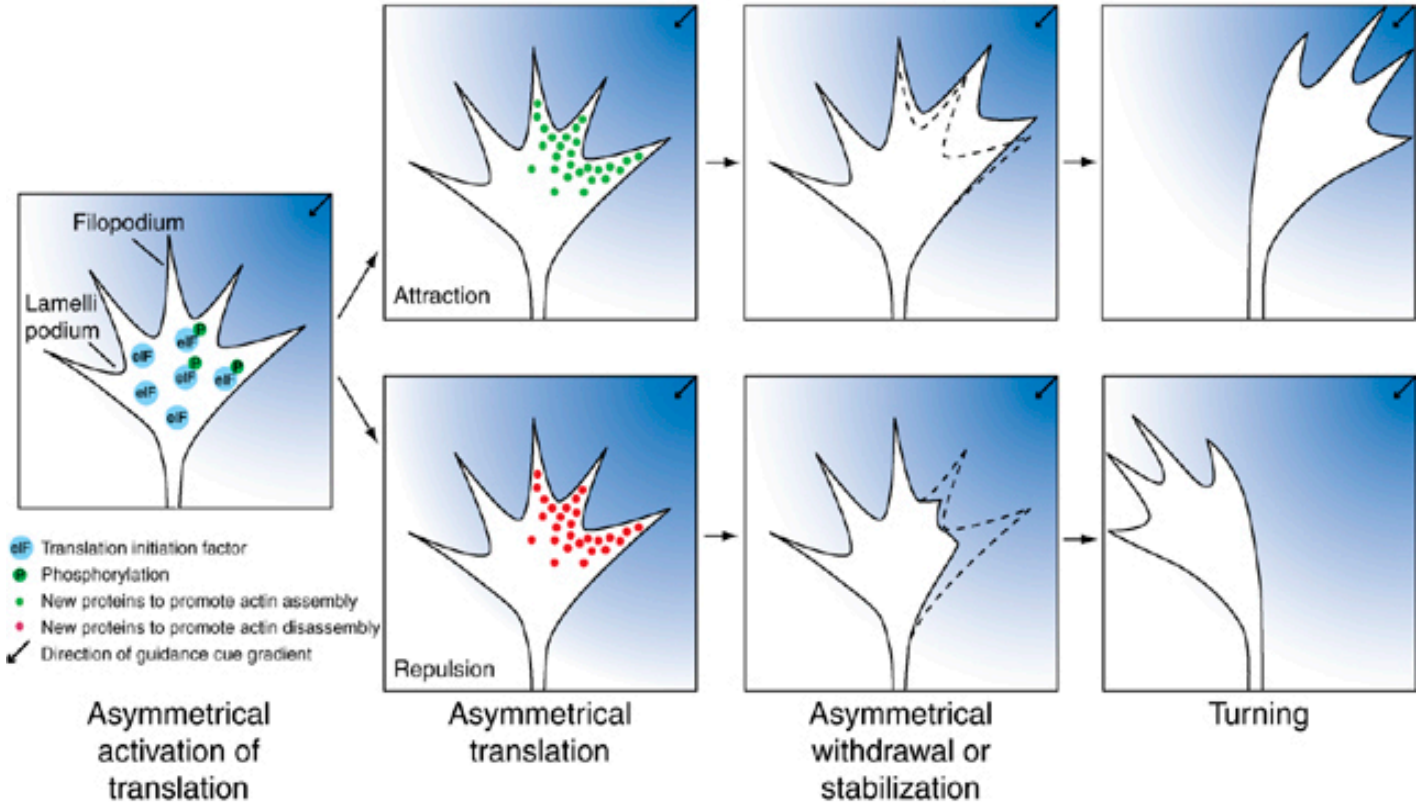
Giulietta Pinato[1]*, Dan Cojoc[1]*, Linh Thuy Lien[2], Alessio Ansuini[2], Jelena Ban[2], Elisa D'Este[1] & Vincent Torre[2,3]

[1]Istituto Officina dei Materiali (IOM-CNR), Area Science Park, Basovizza, S.S. 14, Km 163.5, 34149 Trieste, Italy, [2]Neurobiology Sector, International School for Advanced Studies (SISSA), via Bonomea 265, 34136 Trieste, Italy, [3]IIT, Italian Institute of Technology, SISSA-Unit, Trieste, Italy.

Guidance molecules, such as Sema3A or Netrin-1, induce growth cone (GC) repulsion or attraction. In order to determine the speed of action and efficiency of these guidance cues we developed an experimental procedure to deliver controlled amounts of these molecules. Lipid vesicles encapsulating 10–10^4 molecules of Sema3A or Netrin-1 were manipulated with high spatial and temporal resolution by optical tweezers and their photolysis triggered by laser pulses. Guidance molecules released from the vesicles diffused and reached the GC membrane in a few seconds. Following their arrival, GCs retracted or grew in 20–120 s. By determining the number of guidance molecules trapped inside vesicles and estimating the fraction of guidance molecules reaching the GC, we show that the arrival of less than 5 Netrin-1 molecules on the GC membrane is sufficient to induce growth. In contrast, the arrival of about 200 Sema3A molecules is necessary to induce filopodia repulsion.

Collaboration with
Prof. Vincent Torre - Lab
SISSA – Neurobiology Sector

Growth cones guidance



Netrin – 1

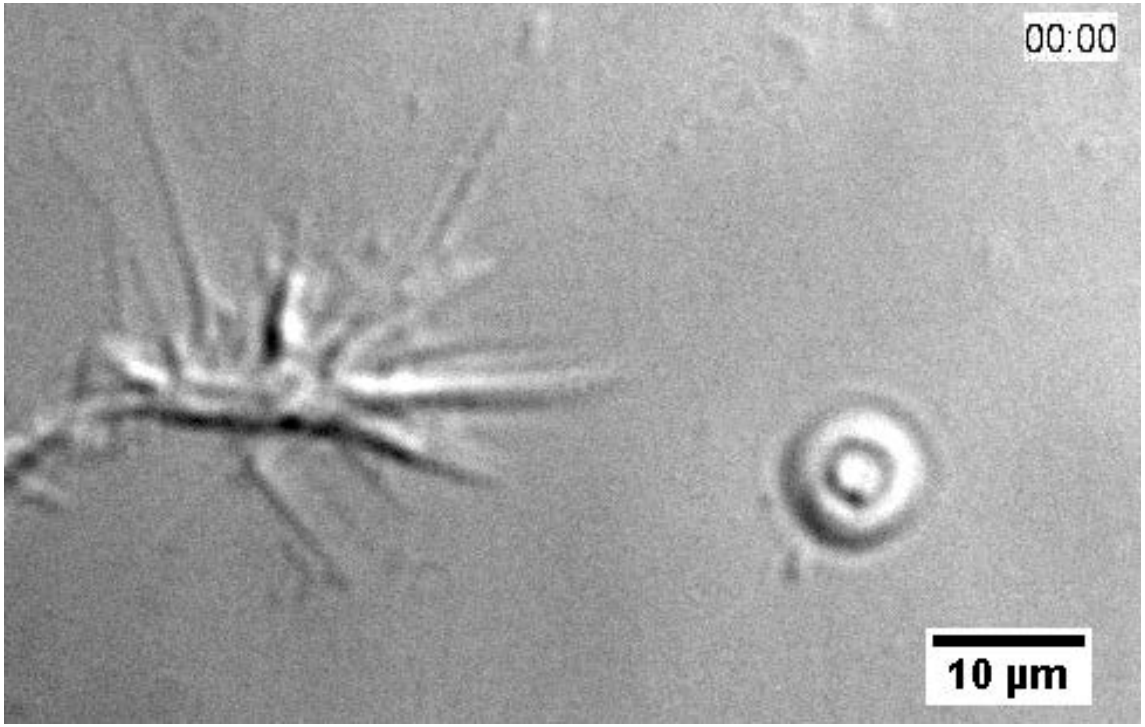
(attractant / reppealent)

Semaphorin 3A

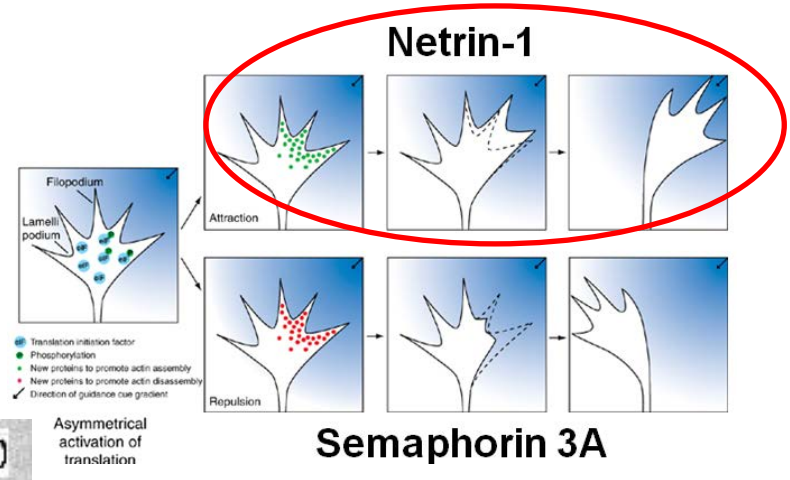
repellant

Netrin-1 release

Primary hippocampal neuron 1 DIV



1 frame every 5 sec



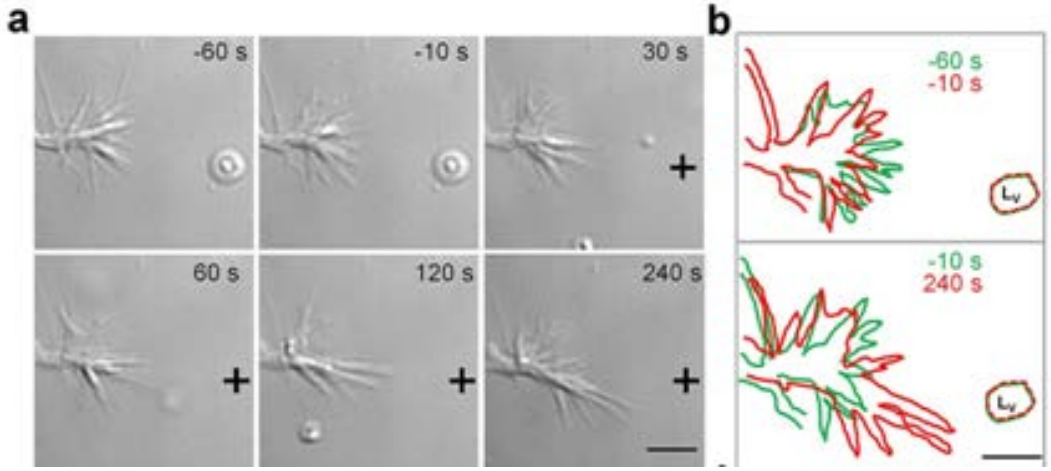
Before After

(2:00) (6:00)

min

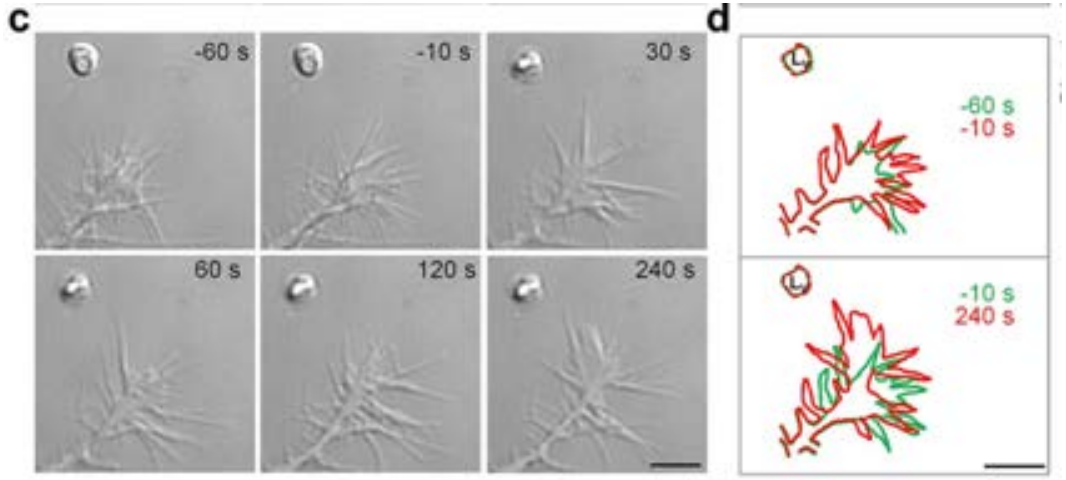
Netrin-1 release

(different number of molecules)



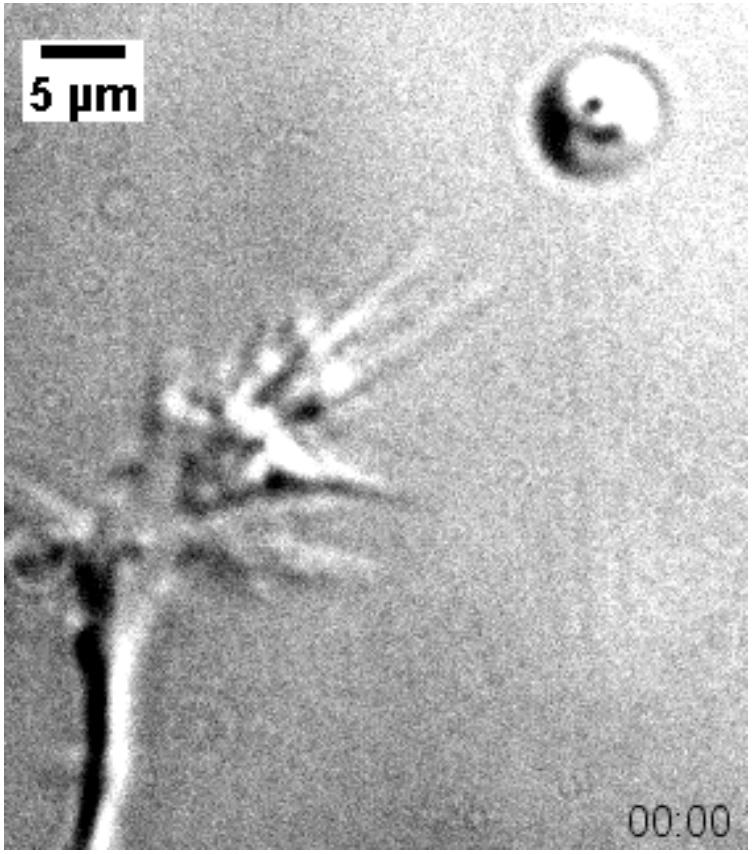
Number of molecules encapsulated in the vesicle (mean), N= 1800

Scale bar 10 um

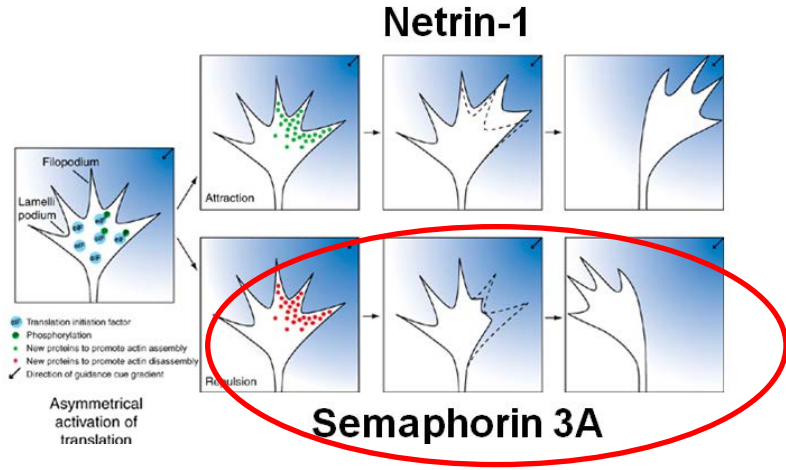


Number of molecules encapsulated in the vesicle, N= 900

Semaphorin-3A release



1 frame every 5 sec



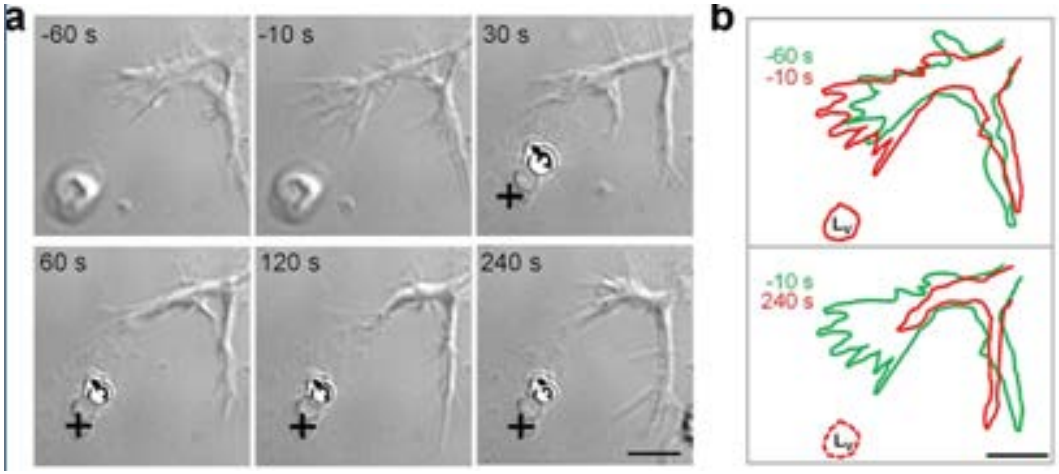
Before After

(2:00) (6:00)

min

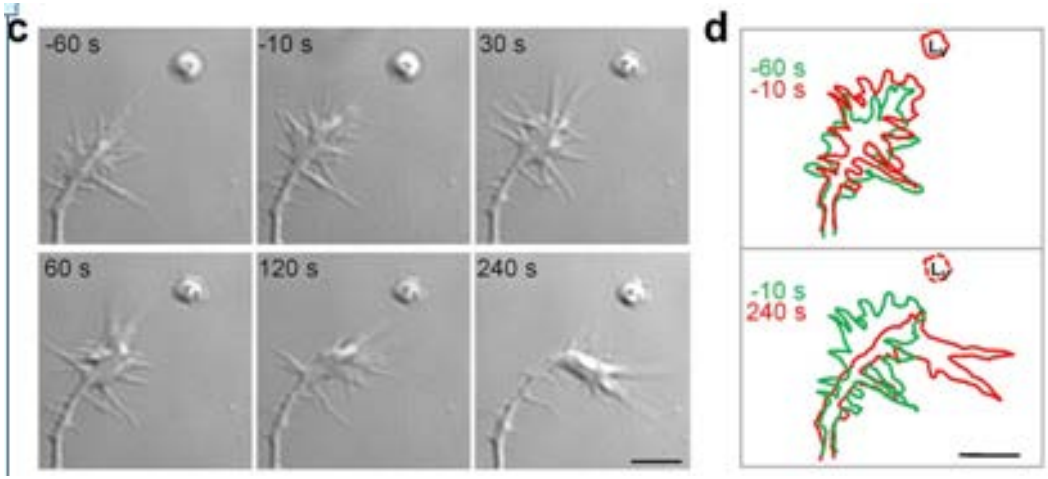
Semaphorin-3A release

(different number of molecules)



Number of molecules encapsulated in the vesicle N= 2500

Scale bar 10 um



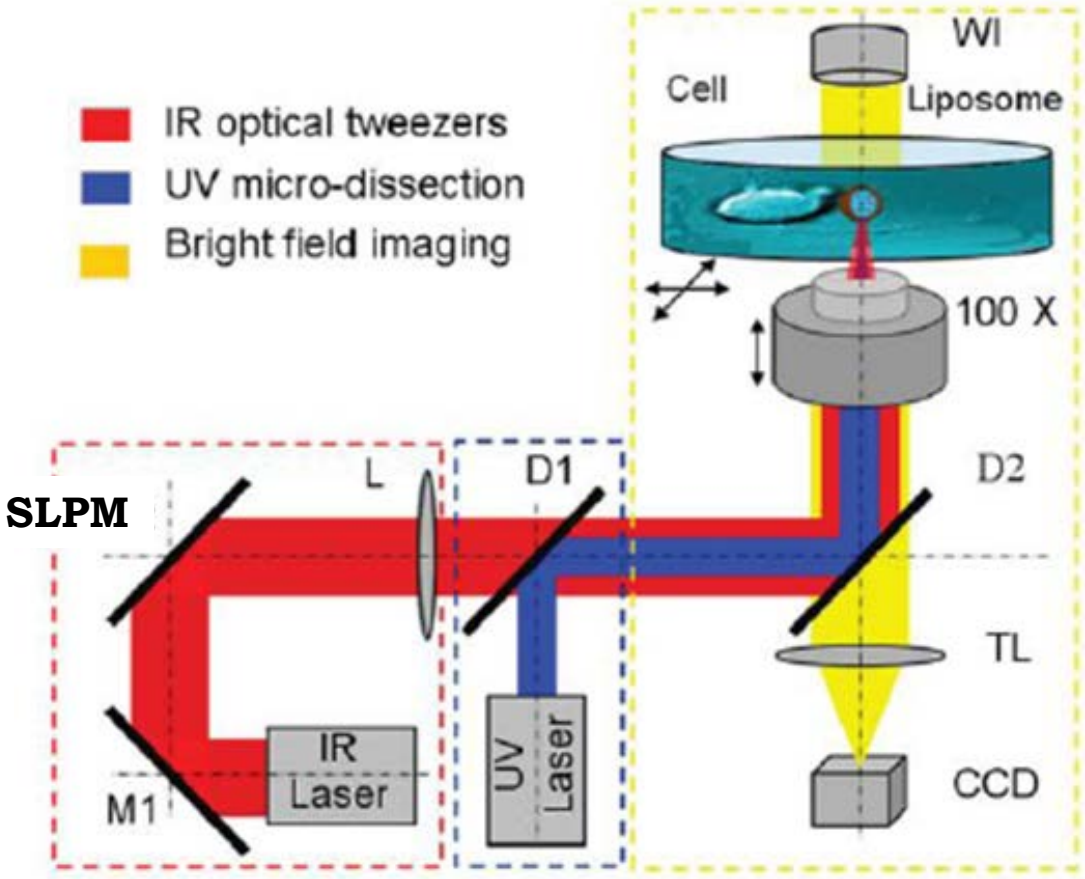
Number of molecules encapsulated in the vesicle N= 1100

So what ? Are there here some facts ?

- We introduce an optical manipulation technique to deliver with high spatial and temporal resolution active molecules for neurons stimulation
- We scaled down to hundreds the number of molecules encapsulated by a liposome showing that less than 5 Netrin-1 molecules initiate attraction but 200 Sema3A molecules are necessary for repulsion of the neuron's growth cone
- Vectors as beads, microsources and liposomes can be used depending on application for local cell chemical stimulation

Setup:

- custom built IR optical tweezers;
- commercial UV micro-dissection system (MMI-CellCut Plus, MMI, Zurich);
- inverted microscope Nikon Eclipse TE-2000-E
- **multiple tweezers with Spatial Light Phase Modulator - Hamamatsu**



Allows to implement all the schemes discussed before

Recent review:

F. Difato, G. Pinato, D. Cojoc,

“Cell signaling experiments driven by optical manipulation”,

Int. J. Mol. Sci. 2013, 14(5), 8963-8984; DOI:10.3390/ijms14058963

Online (OPEN ACCESS):

http://www.mdpi.com/1422-0067/14/5/8963

Acknowledgments

- **Giulietta Pinato, Elisa D'Este, Enrico Ferrari, Paolo Beuzer**

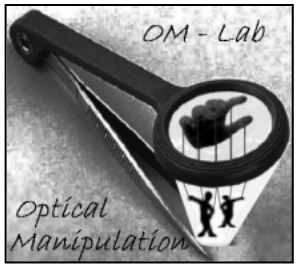
cojoc@iom.cnr.it

- **Vincent Torre, Giuseppe Legname, Ladan Amin, Linh Thuy Lien, Alessio Ansuini, Jelena Ban**, International School for Advanced Studies (SISSA), Trieste, Italy

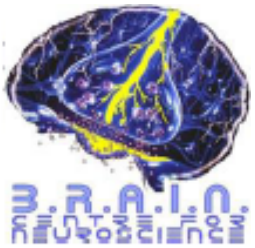
torre@sissa.it
legname@sissa.it

- **Enrico Tongiorgi, Gabrielle Baj**, BRAIN Centre, University of Trieste

tongi@units.it



Lab Prof.
Tongiorgi



Optical Manipulation OM-Lab

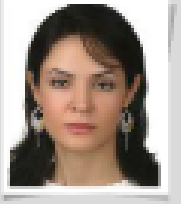
http://dancojoc.wix.com/om-lab

WHO WE ARE | PROJECTS | PEOPLE | PUBLICATIONS | COLLABORATIONS | WHERE WE ARE

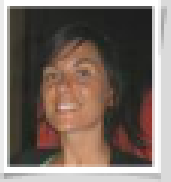
People (click photo for short bio)

Dan COJOC - coordinator
Senior Scientist
e-mail: cojoc<at>iom.cnr.it
tel: +39 040 375-8772

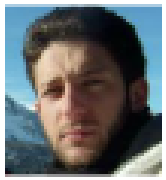
Giulietta PINATO
Associate Researcher
e-mail: pinato<at>iom.cnr.it
tel: +39 040 375-8417



Ladan AMIN
PostDoc SISSA
e-mail: amin<at>sissa.it
tel: +39 040 375-8417

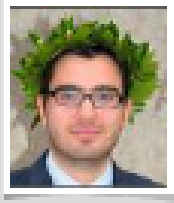


Giovanna COCEANO
PhD Student
coceano<at>iom.cnr.it
tel: +39 040 375-8769



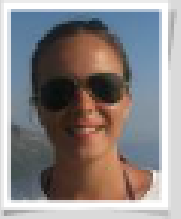
Leonardo VENTURELLI
PhD Student
venturelli<at>iom.cnr.it
tel: +39 040 375-6438

Fatou NDOYE
PhD Student
fndoye<at>ictp.it
tel: +39 040 375-8769



Federico ISEPPON
PhD Student SISSA
fiseppon<at>sissa.it
tel: +39 040 375-8417

Muhammad Sulaiman YOUSAFZAI
PhD Student
yousafzai<at>iom.cnr.it
tel: +39 040 375 6438



Elena GOI
Master Degree Student
elena.goi1988<at>gmail.com
tel: +39 040 375-8469

iom
Istituto Officina dei Materiali

F. Nietzsche: *There are no facts , only interpretations !*