



**The Abdus Salam
International Centre for Theoretical Physics**



2037-18

Introduction to Optofluidics

1 - 5 June 2009

**High spatial resolution BDNF delivery by means of laser tweezers to stimulate
hippocampal neurons signalling**

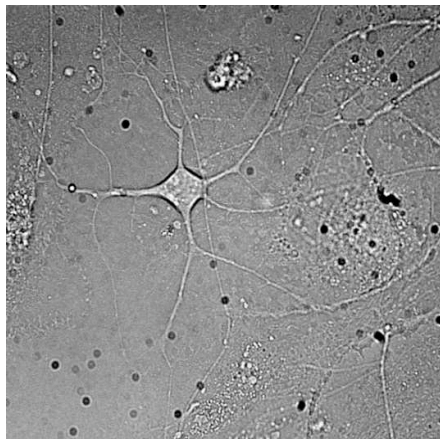
E. D'Este

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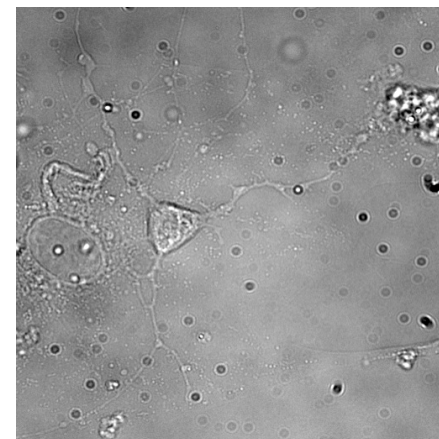


High spatial resolution BDNF delivery by means of laser tweezers to stimulate hippocampal neurons signalling

Elisa D'Este, Gabriele Baj, Paolo Beuzer, Enrico Ferrari, Federica Tavano, Enrico Tongiorgi, Dan Cojoc



Optofluidics
2 giugno 2009

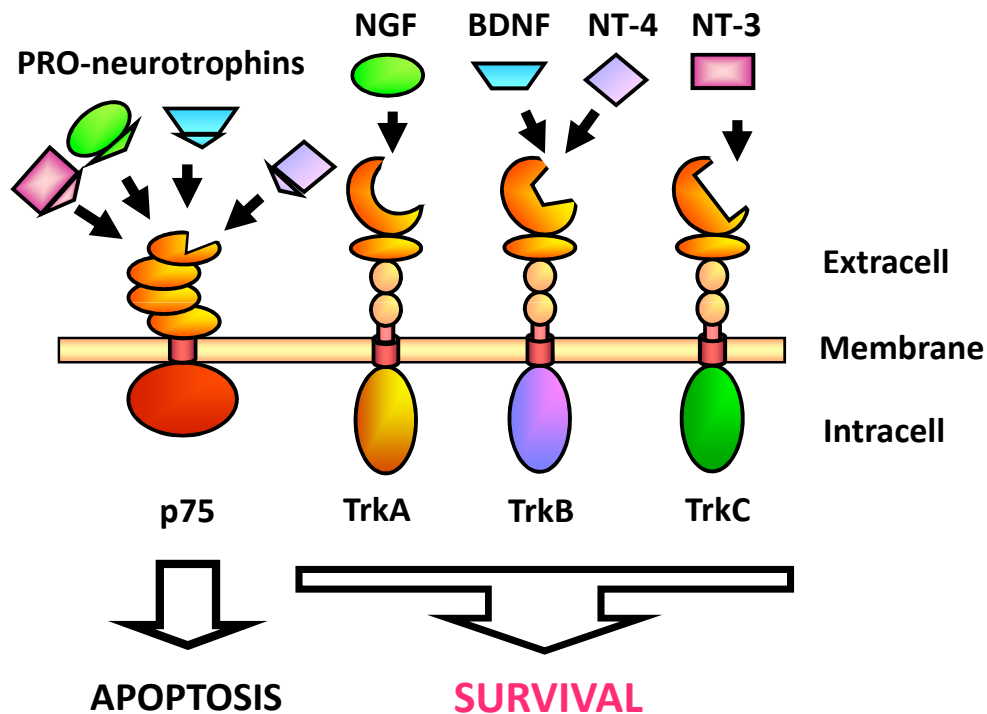


Outline

- The opposite faces of the signalling protein **Brain-Derived Neurotrophic Factor** and the importance of local stimulation
- How to reach a high spatial resolution delivery of BDNF attached to optically manipulated beads.
- Results

Brain-derived neurotrophic factor

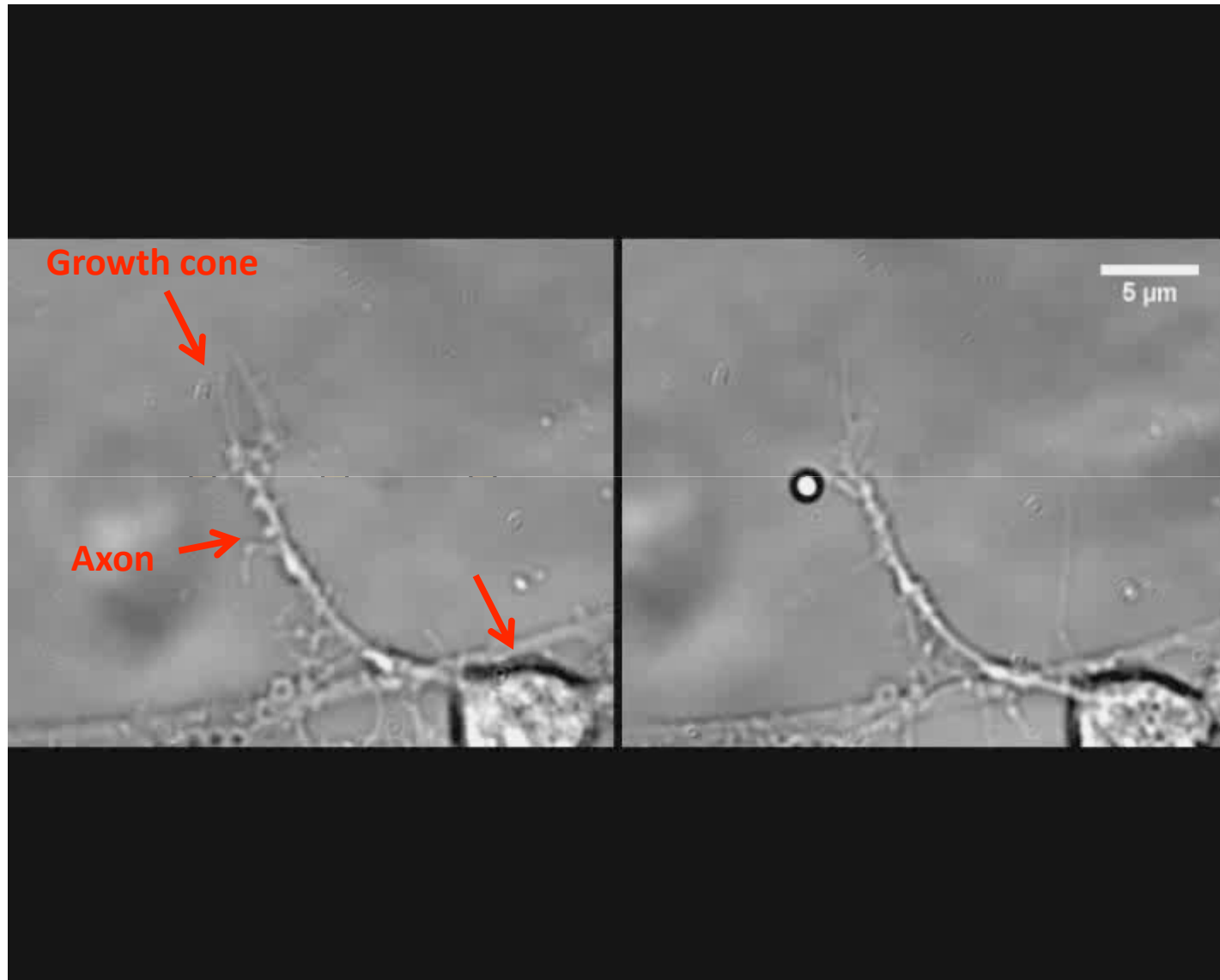
BDNF is a potent morphoregulatory molecule



BDNF is involved in:

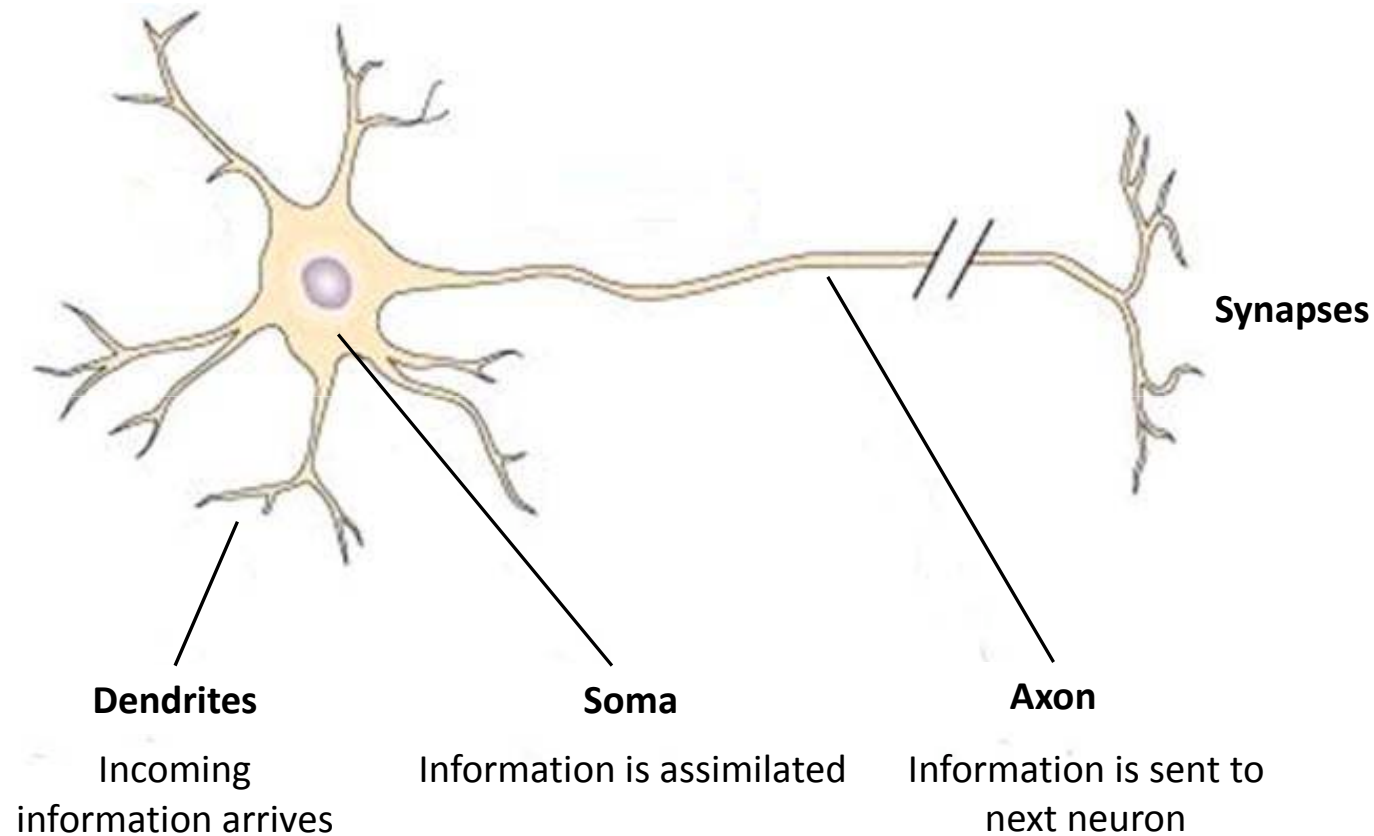
- Cell proliferation
- Cell survival/cell death
- Dendritogenesis and axonogenesis
- Spines formation
- Synaptic plasticity
(Long Term Potentiation & Long Term Depression)

Brain-derived neurotrophic factor



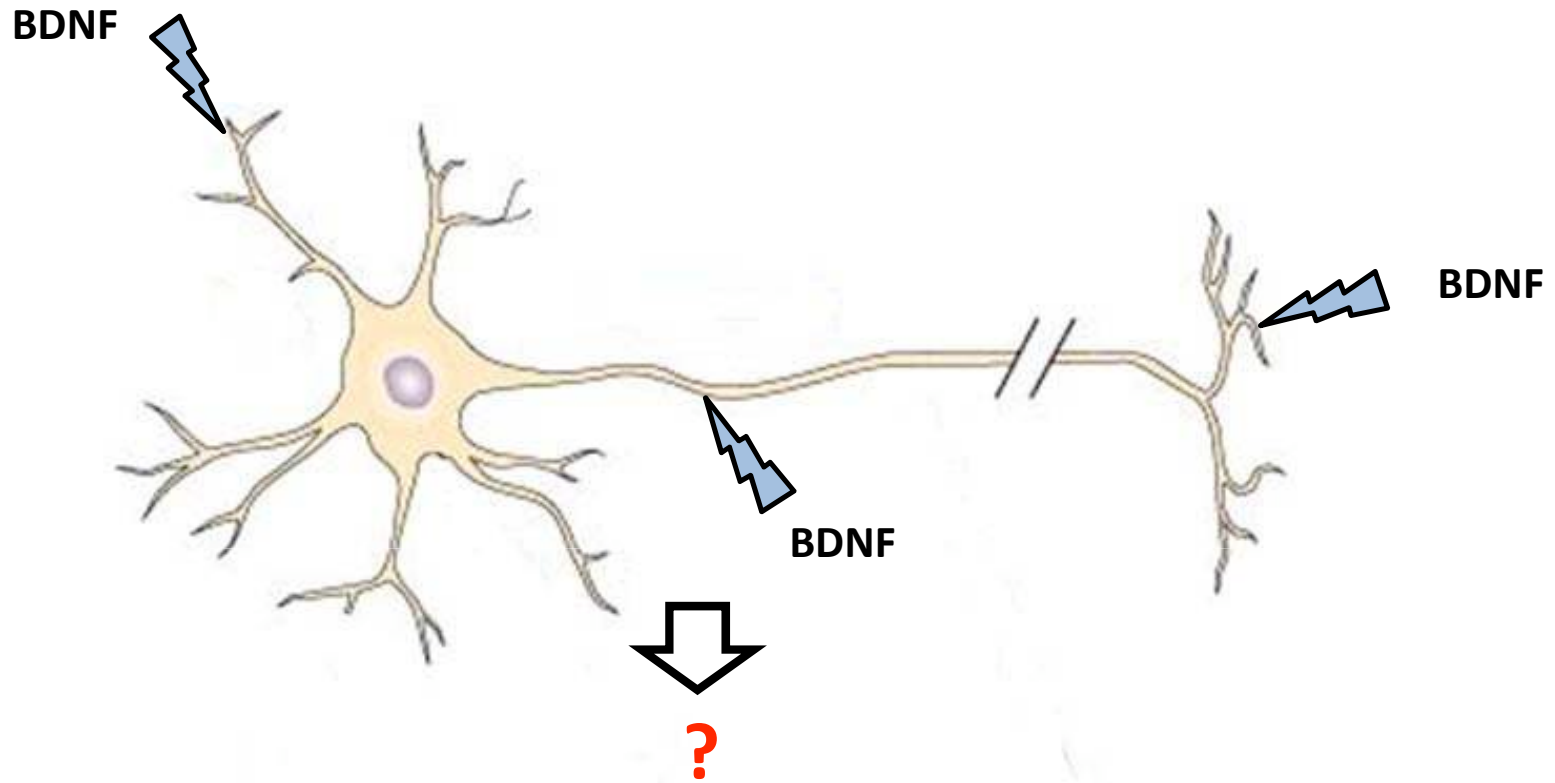
Neurons

Neurons have a functional polarity



Apical and basal dendrites of the same cells respond differently to the same neurotrophin.

Aim of the study



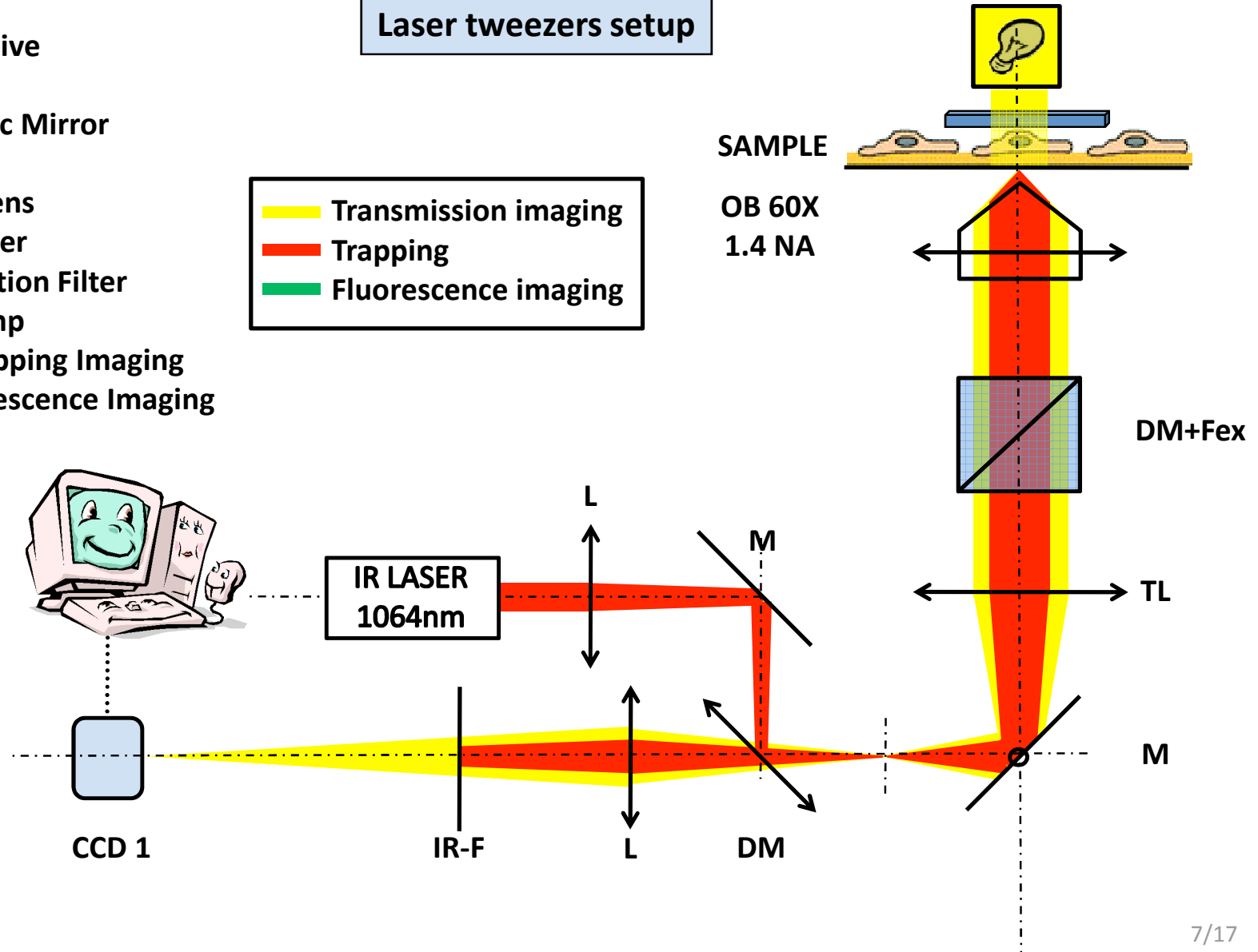
- BDNF-coated silica beads
- 1,5 μ m diameter

How?

Laser tweezers setup

- OB = Objective
- M = Mirror
- DM = Dicroic Mirror
- L = Lens
- TL = Tube Lens
- IR-F = IR Filter
- Fex = Excitation Filter
- Hg = Hg Lamp
- CCD 1 = Trapping Imaging
- CC2 = Fluorescence Imaging

- Transmission imaging
- Trapping
- Fluorescence imaging

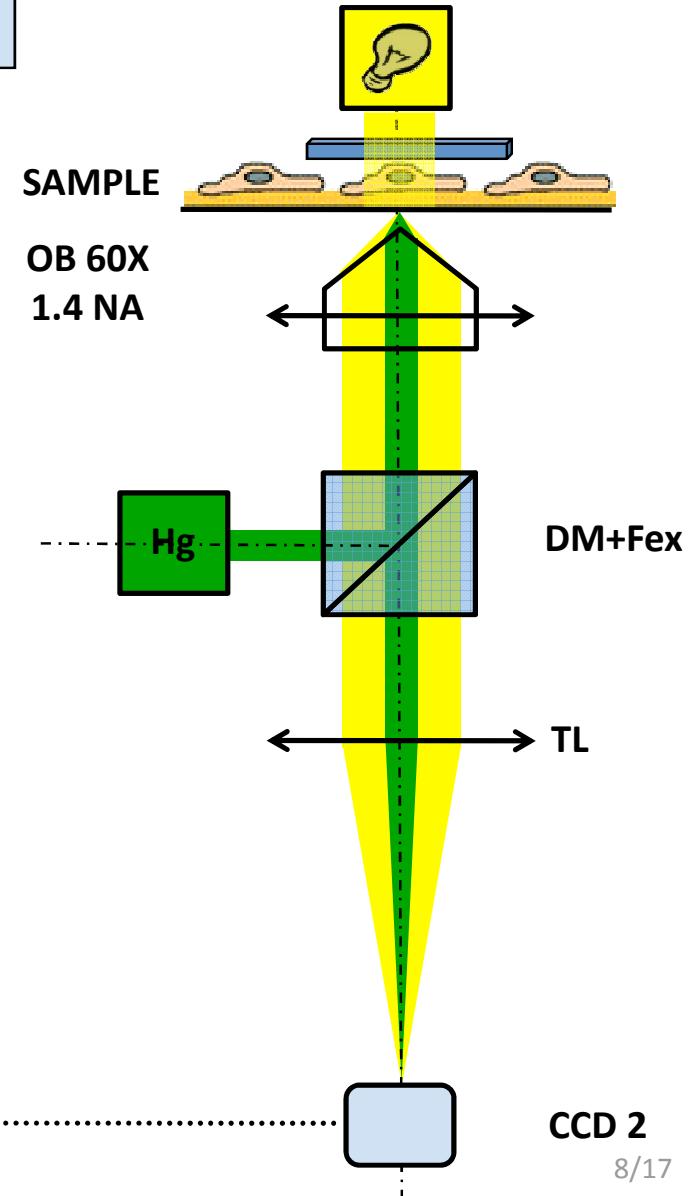
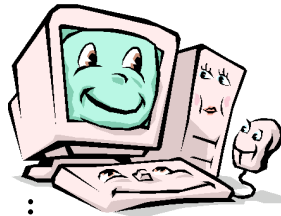


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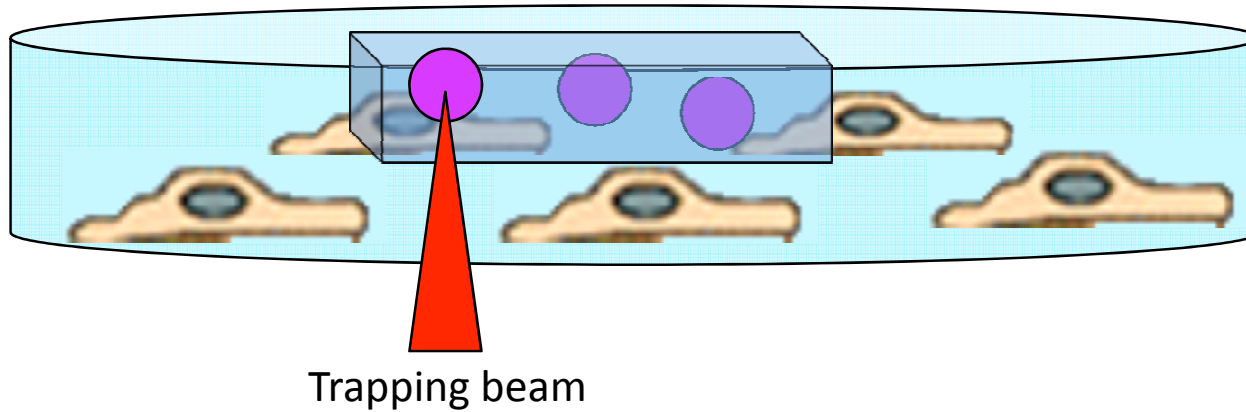
Laser tweezers setup

- Transmission imaging
- Trapping
- Fluorescence imaging



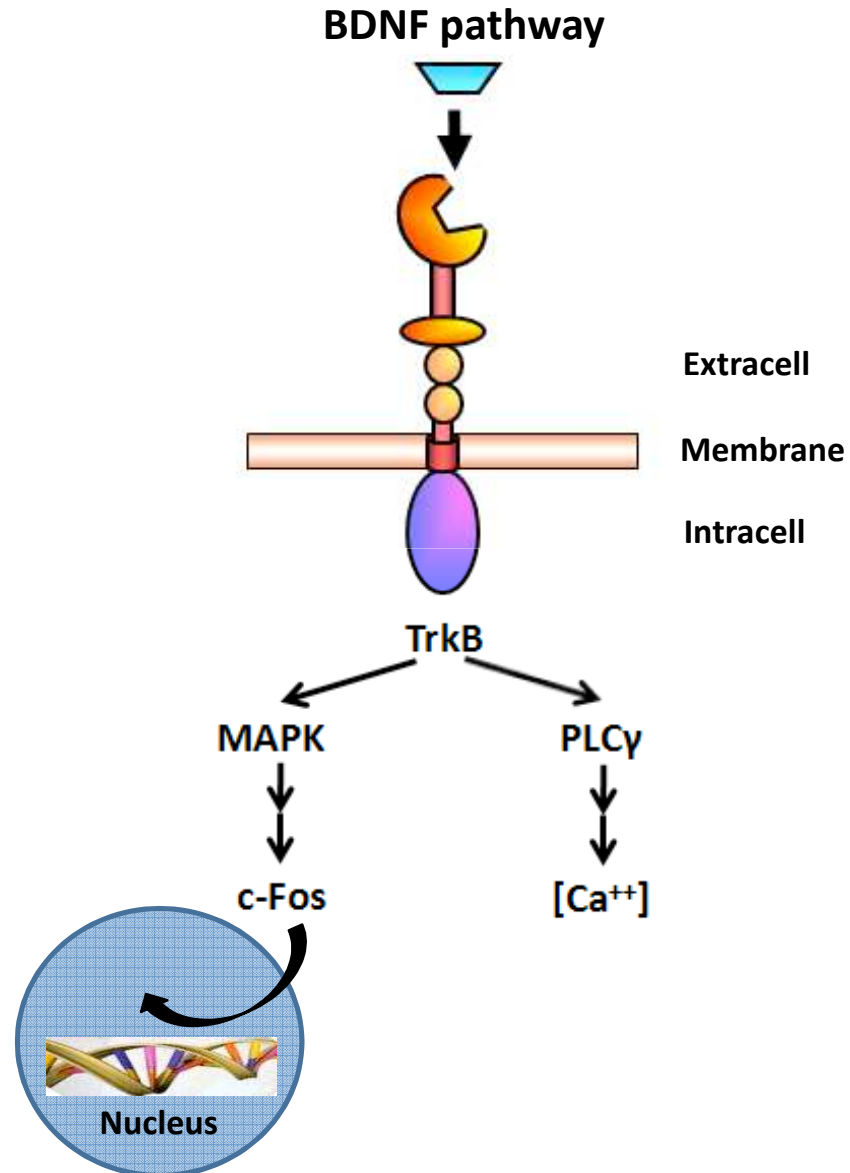
How?

Single vector manipulation

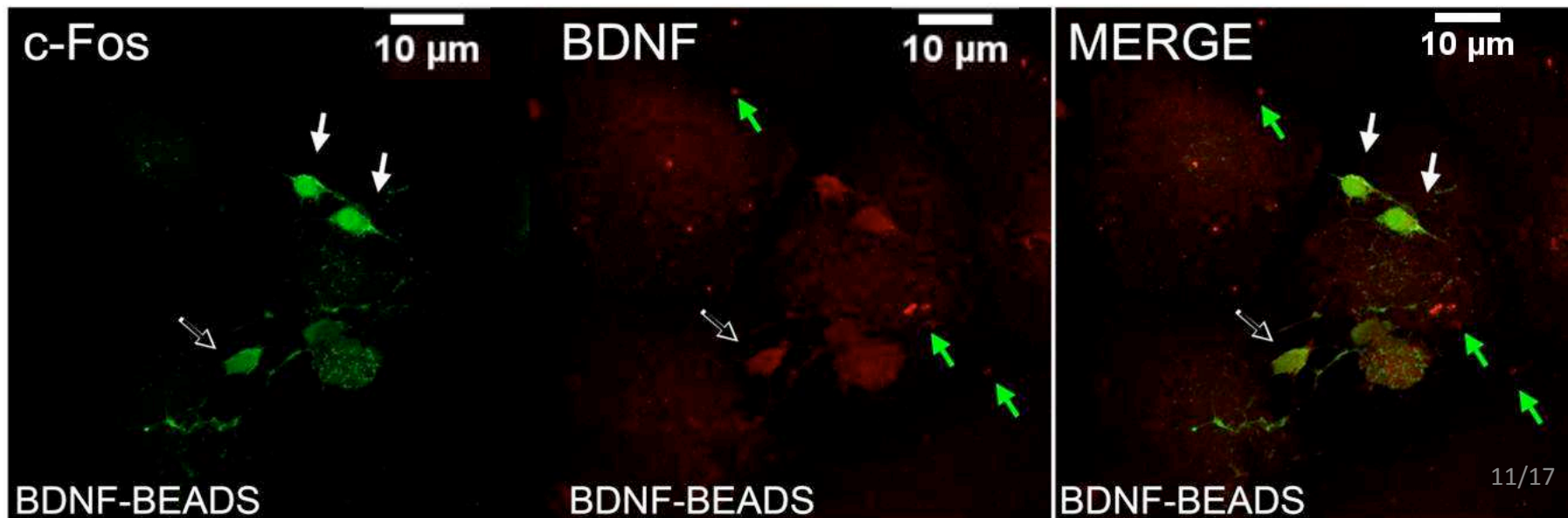
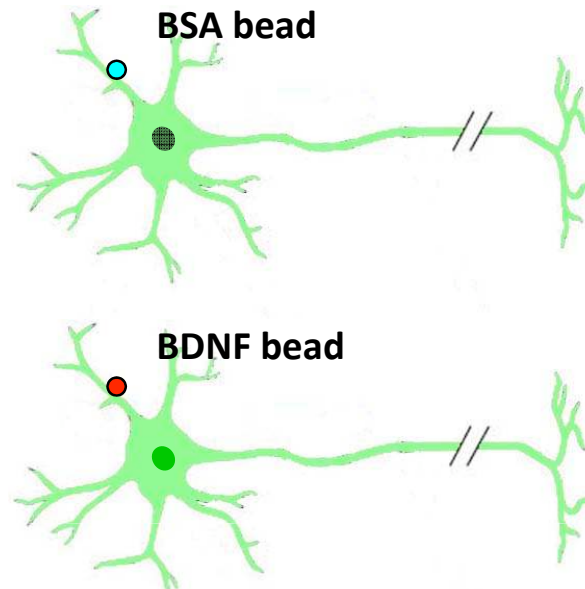
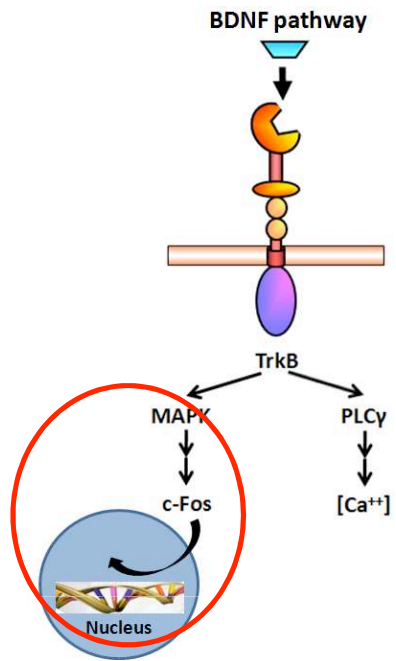


Primary rat hippocampal neurons

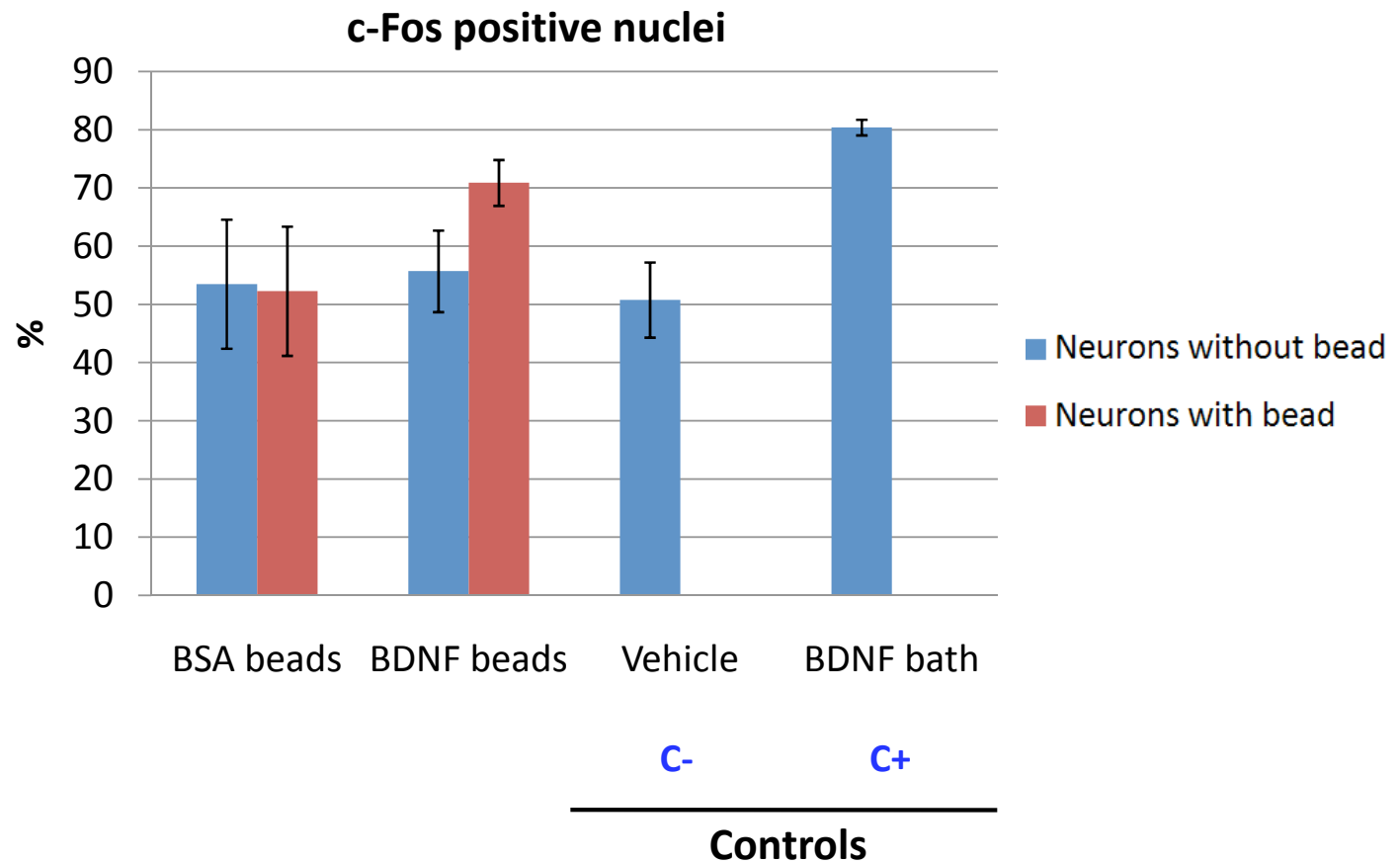
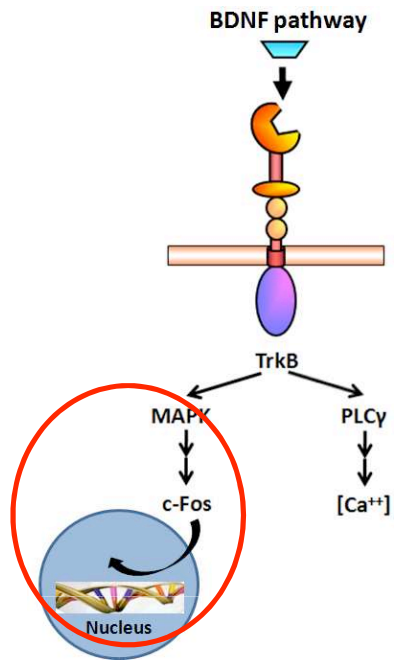
Is BDNF bound to the beads biologically active?



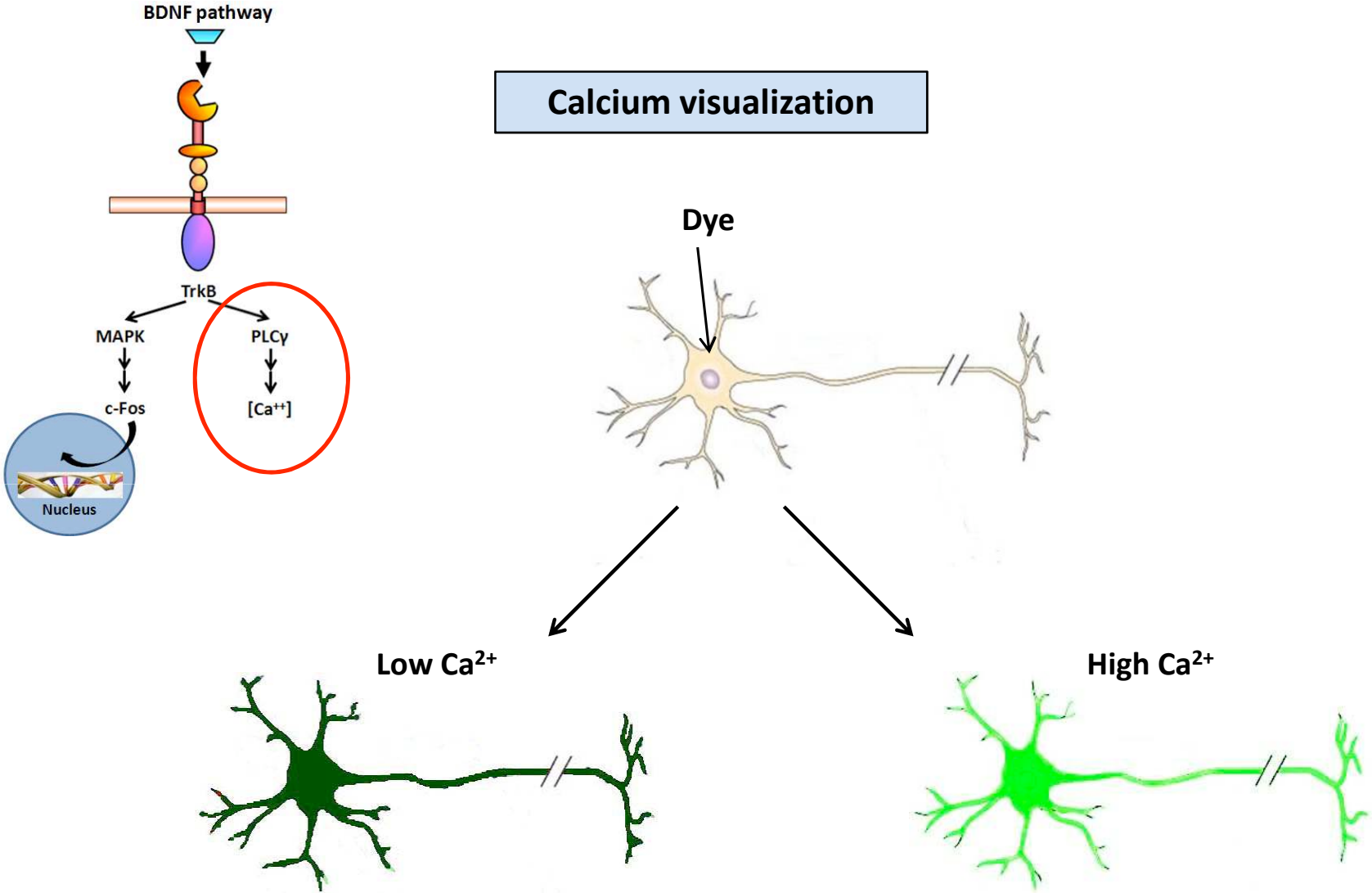
c-Fos translocation to the nucleus



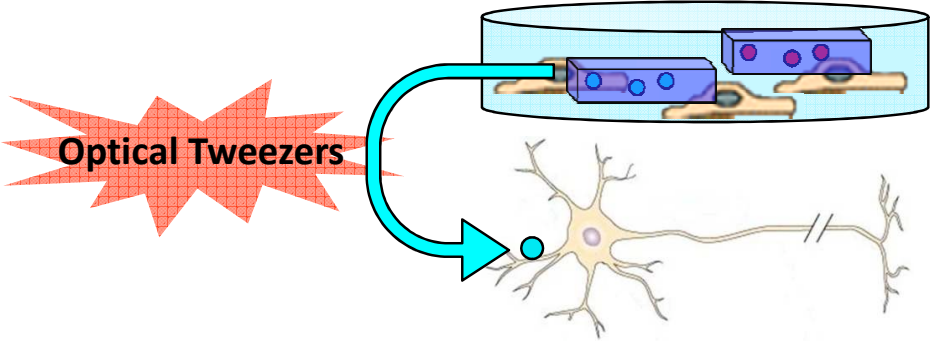
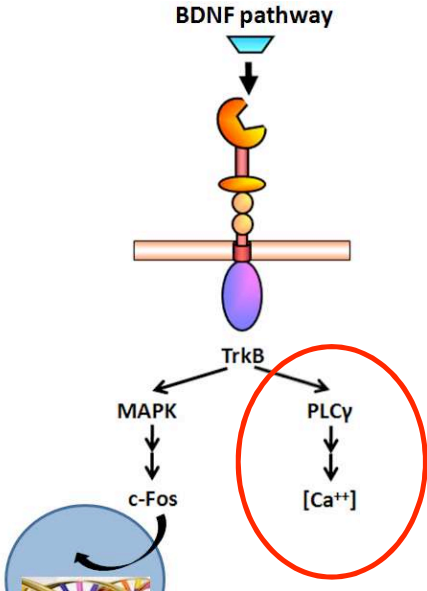
c-Fos translocation to the nucleus



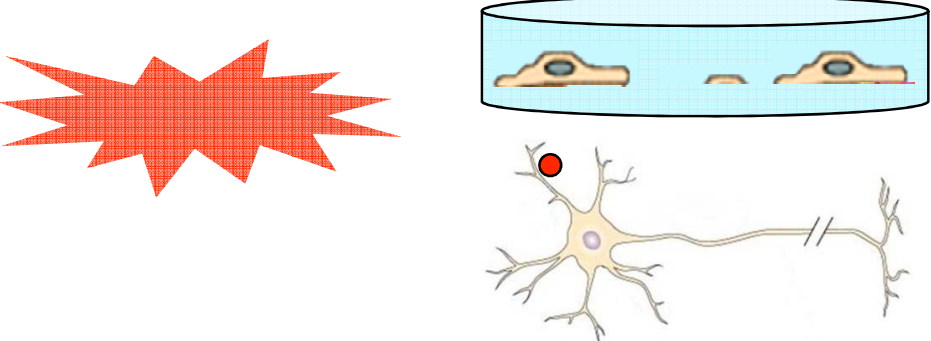
Increase in Calcium levels



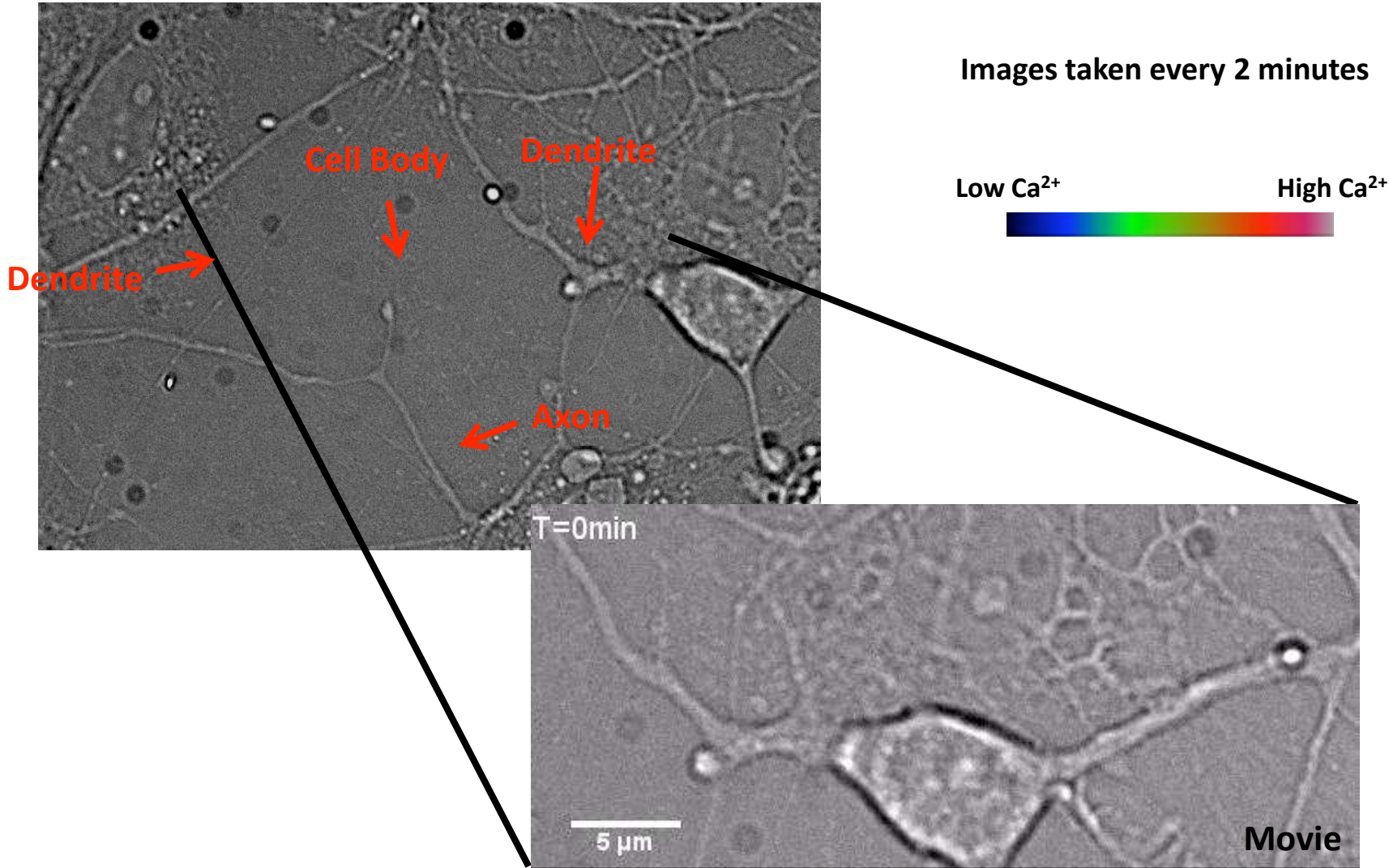
Increase in Calcium levels



10 min fluorescence recording

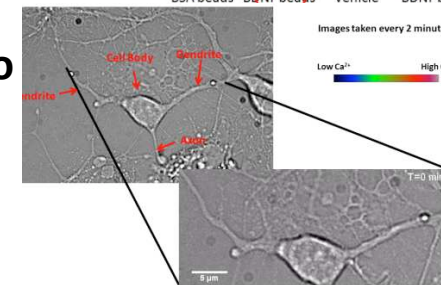
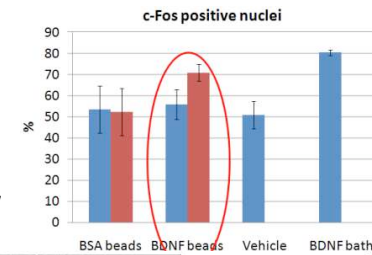


Increase in Calcium levels



Conclusions

- BDNF covalently bound to silica beads maintains its biological activity
- We are able to reach a high spatial resolution delivery of BDNF to neuronal cells by means of optical tweezers



Future Perspectives

- To stimulate neurons with BDNF-coated beads and analyze different biological processes
- To use other neurotrophins
- To develop other vectors

Aknowledgments



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Silvia Santucci

Federica Tavano

Enrico Ferrari

Paolo Beuzer

Federico Salvador



Enrico Tongiorgi

Gabriele Baj