OPTICAL STIMULATION OF NEURONAL GROWTH

R.A. CÂMPEANU, MARIA LUISA FLONTA

Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology,
University of Bucharest, 91–95, Splaiul Independenței, Bucharest 050095, Romania,
e-mail: flonta@biologie.kappa.ro

Abstract. Optically stimulating neuronal growth is a relatively new subject which could have numerous applications in different areas knowing that a control over neuronal growth is a fundamental objective in biology. In this short review we will achieve a synthesis of the results reported in the literature and of the main hypotheses proposed for the stimulation mechanisms.

Key words: neuron, optical tweezer, optical guidance, neuronal growth.

INTRODUCTION

In 1890 a Spanish neuroscientist named Santiago Ramón y Cajal first identified the growth cone and described it as being a highly motile sensory structure that leads an advancing neurite. Since then, many scientists have been trying to figure out the underlying mechanisms that determine neuronal growth, but many of the aspects have not yet been discovered despite their effort. Understanding the growth cone may reveal some insight into the development of the nervous system of organisms and could lead to future techniques of nerve regeneration and to the development of neuronal circuits *in vitro* [26].

During development, neurites through the growth cone are constantly probing the extracellular medium in search for cues with the help of filopodia and lamelipodium being controlled by guidance molecules (that are bound to cellular or extracellular surfaces) that orient migrating and growing cells. A guidance molecule can cause attraction, repulsion or both [19].

Due to the complexity of the chemical guidance signals, scientists have explored various other approaches to control neuronal growth. They tried artificial substrates such as silicon wafers where nerves have been directed by topographically structured surfaces [15] and also tried selectively patterning the substrate with adhesive materials for nerves [20, 33], however damaging tensions

Received: September 2013;
in final form September 2013.

ROMANIAN J. BIOPHYS., Vol. 23, No. 3, P. 159–170, BUCHAREST, 2013

ripped apart the neuronal structures formed on the substrate because of the axons tendency to straighten and stiffen in time. There also have been reports of guiding neurons using electrodes, but the specific impact of induced electrophoresis effects is not understood [17]. There have been attempts trying to identify ways of using nanotechnology in psychiatry, but without specific applications in practice. Synaptic modulation using nanoparticles may prove difficult [18]. Because of the clinical impact, prosthetic methods were developed using electrical evaluation of neural functioning [28]. Differences in complexity between the central nervous system and the peripheral nervous system create an opportunity for using the optical stimulation of neuronal growth technique first on the peripheral nervous system, mainly because of the probable high impact at the smart prostheses level.

Keeping in mind all of the failed attempts, there was a clear need of an alternative way to guide nerves. In 1991 Albrecht-Buehler observed a strange phenomenon involving 3T3 cells that extend pseudopodia towards a distant infrared laser light source [1], and in 2002 Ehrlicher succeeded in demonstrating that a near infrared laser light source placed on or near a growth cone of NG108 or PC12 cells can enhance the growth speed and turn the growth cone towards the direction of the laser [17]. This discovery is outstanding and yet not even today the mechanisms responsible for this outcome are fully understood although there are several proposed hypotheses.

The aim of this short review is to analyze the literature reports related to the application of optical tweezers in controlling the neuronal cone growth highlighting the main positive results. The most promising hypotheses regarding the mechanisms involved in the optical control of neuronal growth are discussed.

OPTICAL TWEEZERS

Arthur Ashkin first reported in 1970 the detection of optical scattering and gradient forces on micron sized particles [5]. In 1986 in his experiments, Ashkin described it using a high focused beam of light capable of holding microscopic particles stable in three dimensions [6]. What he reported back then is what now we call optical tweezers which are instruments that use a laser beam in order to manipulate particles of nano and micrometer size with a high refraction index (Fig. 1). The narrowest point of the laser beam (beam waist) contains a powerful electric field gradient, the region of maximum intensity being the center of the laser beam.

Essentially, the photons emitted by the laser are refracted as they enter and exit a particle, thus a photon will change direction while passing through a particle. Because light has a certain momentum attributed to it, this change in direction also means a change in the momentum, therefore due to Newton's third law there should be an equal and opposite momentum change on the particle resulting finally in a force exerted on the particle. Being constantly bombarded by photons from different angles, the particle is kept in the middle of the laser beam [27].

Optical tweezers have been used to manipulate objects for the past 30 years, being applied to single molecular and cellular studies. Many biological motors such as kinesis [4], cytoplasmatic dynein [30], myosin [23], nucleic acid-based enzymes [14, 32], flagellar motors [34] have been studied along with RNA and DNA mechanics [2, 8], protein conformation changes in folding/unfolding pathway [12], protein-protein binding/unbinding process [37] and DNA-protein interactions [23, 38].

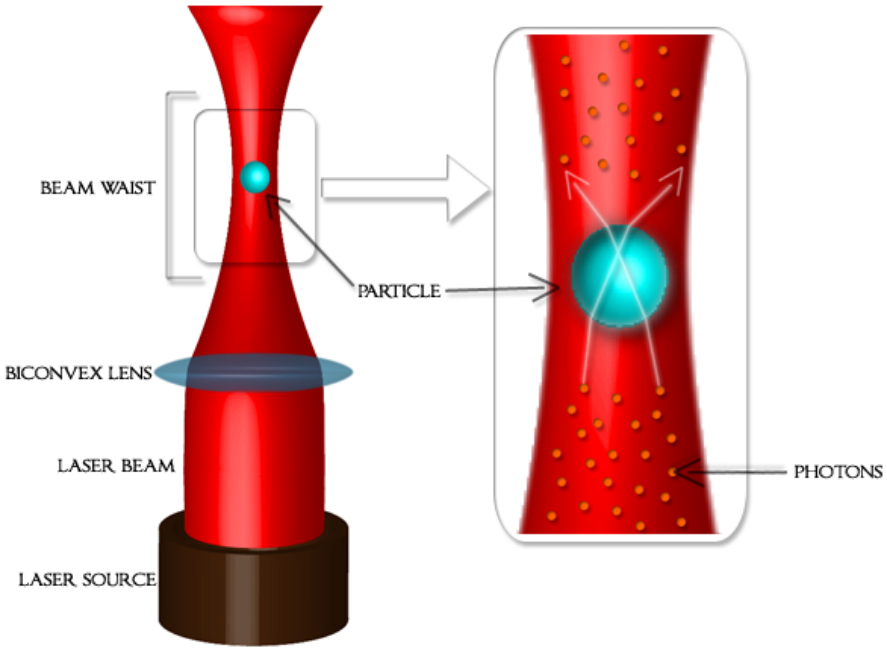

Fig. 1. On the left side there is a drawing of a laser beam being emitted by a laser source, passing through a microscope lens and manipulating a particle and on the right side there is an enhanced view of the laser beam presenting the photons that change direction while passing through a particle and keeping it in the middle of the laser beam.

GROWTH CONE

A growing axon searches for its synaptic target with the help of the growth cone (Fig. 2). This is composed of lamelipodium and filopodia. Filopodia are made up from actin filaments and resemble thin cylindrical extensions that can extend from the growth cone several micrometers. The lamelipodium is made up from a flat region that consists of dense actin meshwork [29].

Considering the cytoskeletal distribution, the growth cone can be divided into three domains (Fig. 3).

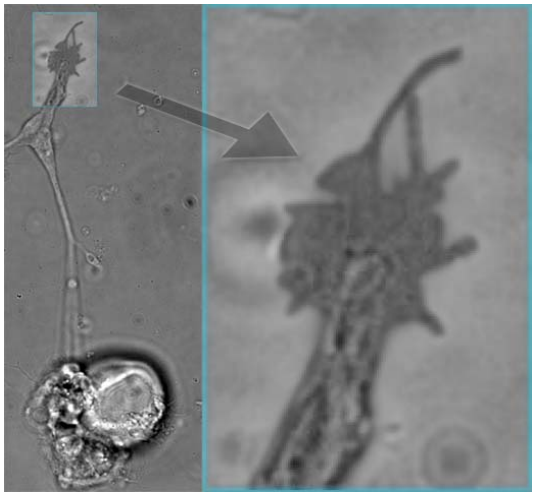

Fig. 2. The growth cone (personal data) – On the left side there is a picture of a neuron captured using a 100x objective and on the right side there is a magnified view of the growth cone.

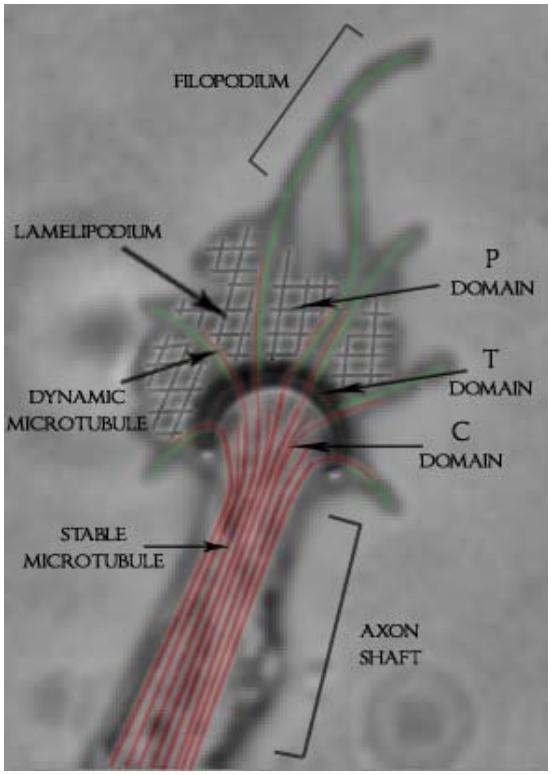

Fig. 3. The growth cone – detailed sketch presenting the three domains and all the elements that make up the growth cone.

The peripheral domain (P) consists of long beams of actin filaments (F-actin bundles) which form filopodia and the dense network of F-actin which gives the lamelipodium its structure. Additionally, individual dynamic microtubules explore the region along with the F-actin beams.

The central domain (C) is made up of stable microtubule beams which penetrate into the growth cone through the axonal shaft among numerous organelles, vesicles and the central beams of actin.

The transitional domain (T) is located between domain P and domain C where contractile actomyozin structures lie perpendicular to the F-actin bundles and form a hemicircumferential ring [29].

OPTICAL STIMULATION OF THE GROWTH CONE

A typical experiment of optical stimulation of the growth cone consists in exposing a viable growth cone to a laser beam by placing approximately half of the laser beam on the growth cone and stimulating at a given frequency (e.g. for using a 0.1 Hz frequency, the laser needs to be active 1 second and inactive 9 seconds) and having the laser beam focused on the focal plane. The laser beam's position is constantly adjusted as the growth cone expands.

All experiments demonstrating optical stimulation of neuronal growth have been done on NG108 and PC12 cells and were conducted using various inverted microscopes that had attached to them an optical tweezer (Fig. 4) using a wavelength between 800 nm and 1200 nm with a spot diameter between 2 μm and 16 μm which was achieved by optically defocusing the beam. The laser power was measured directly after the microscope objective to be between 4 mW and 200 mW. Some experiments used a thermostatic microscope stage that maintained a constant temperature for the cell culture (37 ^{o}C) [16].

There are two beam shapes used in these experiments with different success rates:

- Gaussian beam profile – 50–60% successful guidance
- Line trap beam profile – 20–25% successful guidance [9]

All of the parameters used in the literature for optical stimulation of neuronal growth are presented in the table below (Table 1).

Ehrlicher demonstrated that optical stimulation and guidance of neuronal growth was possible using a wavelength of 800 nm for the laser and a variety of laser powers (20 mW, 60 mW, and 100 mW). He was able to estimate the optical stimulated growth rate at 37.5±22.5 μm/h compared to a control growth rate of 7±3 μm/h [17]. The only problem with these growth rates is that they were calculated from one optically guided growth cone and one normal growing growth cone being fair to say that these growth rates cannot be taken into account.

Koch also successfully achieved laser induced optical control of neuronal growth for PC12 and NG108 cells in 2004 by using a wavelength of 800 nm for the laser and a wide range of experimentally explored beam powers (20 mW – 200 mW) [26].

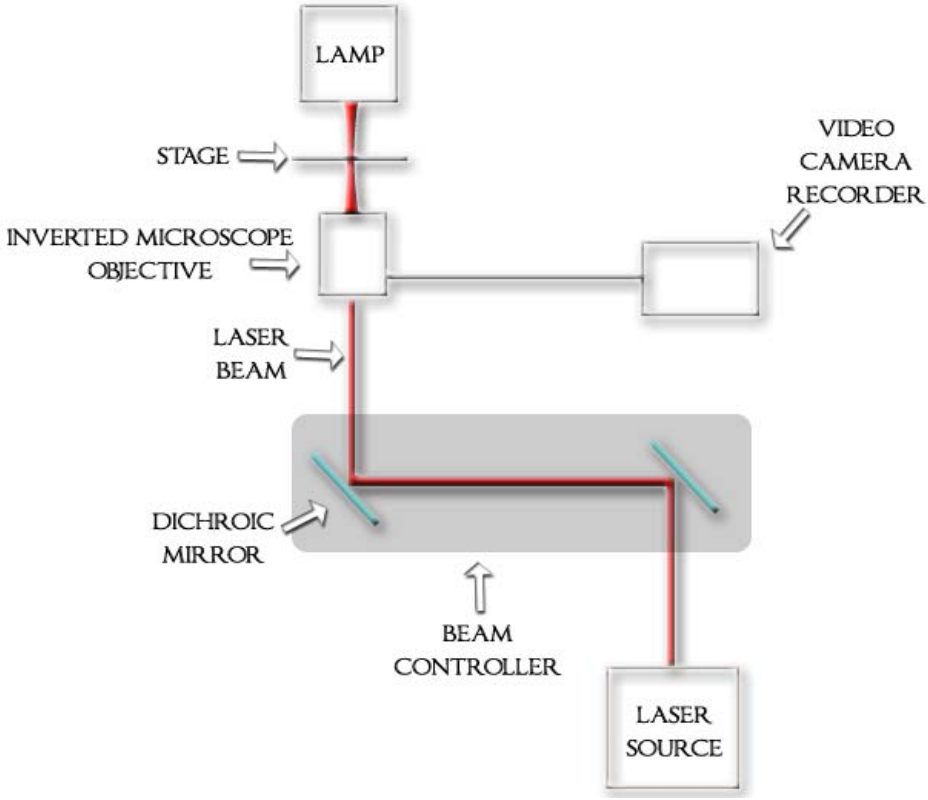

Fig. 4. General diagram presenting a typical optical setup used for optical tweezers.

Mohanty in 2005 was the first one to use a Line Trap as a beam shape in order to achieve optical stimulation of neuronal growth. He used a wavelength of 1064 nm for the laser and a beam power ranging between 120 mW and 200 mW [31].

Stevenson reported the first direct comparison for optical stimulation of neuronal growth using only one cell type at two near infra-red wavelengths (780 nm and 1064 nm) with the same beam shape and keeping the laser power low (9–25 mW) [35].

Carnegie using a wavelength of 1064 nm, a laser power of 35–70 mW, and a line trap as the beam shape concluded that an asymmetric intensity profile of the optical line trap is as effective as a symmetric intensity profile [10].

Table 1

The experimental data used in the literature

AUTHOR	CELL LINE	LASER WAVELENGTH (nm)	LASER POWER (mW)	SPOT SIZE (μm)	BEAM SHAPE	FREQUENCY (Hz)	RATE OF SUCCESS* (%)	OPTICALLY STIMULATED GROWTH RATE (μm/h)	GROWTH CONE ANGLE CHANGE
Ehrlicher (2002)	PC12 and NG108	800	20–120	2–16	Gaussian Spot	0.1	79.50	37.5 ± 22.5	30–90°
Koch (2004)	PC12 and NG108	800	20–200	unknown	Gaussian Spot	unknown	85	unknown	unknown
Mohanty (2005)	unknown	1064	120–200	2.5–20	Line Trap	unknown	unknown	unknown	unknown
Stevenson (2006)	NG108	780 and 1064	9–25	2.5–20	Gaussian Spot	unknown	50	unknown	30°
Carnegie (2008)	NG108	1064	35–70	1 x 45	Line Trap	unknown	unknown	111 ± 11	~ 20°
Graves (2009)	PC12	1064	50–90	1	Gaussian Spot	0.1	unknown	44.4 ± 6	50°
Forrest Jesse (2013)	PC12	800	4–45	unknown	Tapered Optical Fiber	unknown	unknown	unknown	45°

*percent of successful neuronal growths using optical tweezers out of all the experiments conducted.

Graves achieved optical stimulation of neuronal growth in a 3D collagen matrix using a wavelength of 1064 nm and a laser power of 50–90 mW [21].

Jesse in 2013 demonstrated that optical stimulation of neuronal growth can be accomplished by controlling the position and direction in 3D of a tapered optical fiber through which the light is projected and controlling the position, angle and power of the laser beam in order to promote neuronal growth [24].

MECHANISMS PROPOSED FOR EXPLAINING OPTICAL STIMULATION

OPTICAL FORCES MECHANISM

Albrecht-Buehler's results in 1991 reported that the extension of pseudopodia towards a distant infrared light source could not be attributed to temperature effects because the infrared radiation produced by the laser only raised the local temperature by 0.00001 ^{o}C. Cells contain approximately 85% water which has a 30 times larger absorption coefficient at 1200 nm than at 900 nm; in consequence, the water in the cytoplasm of the irradiated cell would absorb 30 times more infrared energy at 1200 nm than at 900 nm, but the results obtained using a wavelength of 1200 nm had a weaker response than using a wavelength of 900 nm [1].

Ehrlicher in 2002 and Koch in 2004 discussed that using the laser at a low power does not detach the growth cone from the substrate and therefore the optical tweezer is not used in a conventional manner, concluding that the laser spot influences the general actin based processes, basically the optical forces can only impact the small oligomeric structures and the globular proteins found in the growth cone. By placing the laser beam on the growth cone, it creates an intensity gradient for the globular proteins of the cytoplasm such as actin monomers, concentrating them at the leading edge of the growth cone; a higher concentration of G-actin in a growth cone resulting in a faster growing rate [17, 26].

In 2006 Stevenson *et al*. conducted an experiment in which he compared two different wavelengths 780 nm and 1064 nm using exactly the same experimental conditions and concluded that both wavelengths are equally effective in optically stimulating neuronal growth, meaning that the light detection mechanism within the cell is not due to a single protein with a defined activity wavelength as occurs with the photoreceptor opsin proteins in the mammalian eye [35].

Cojoc and his colleagues, using an optical tweezers, measured in 2007 the forces exerted by lamelipodium and filopodia during neuronal differentiation finding values ranging from pNs(single filopodium) to tens of pNs(lamelipodium) [13]. They showed that the presence of these forces is conditioned by the actin and tubulin polymerization, no forces being detected in the absence of cytoskeleton proteins polymerization. These findings may suggest a possible mechanism of light guided cone growth by modulation of the local polymerization rate at the level of neural cytoskeleton.

In contrast, after building a model of a filopodium in 2008 Carnegie calculated the optical forces and torques for several positions and orientations of the filopodium with respect to the laser beam and concluded that there is only one attractive equilibrium angular position for the Gaussian beam and two for the line trap and also their experiments show that the filopodia feel a small torque that would reorientate them in the direction of the laser beam and thus promoting neuronal growth. In the same paper they presented the results from their experiments using an asymmetrical line trap in the forward bias configuration and in the reverse bias configuration and resulted in similar growth rate efficiency suggesting that the actin flow in not being affected by the laser [10].

THERMAL MECHANISM

Ebbesen and Bruus suggested in 2012 that the effect is due to a biochemical signaling cascade initiated by the laser beam heating the cell membrane and, although previous reports had shown that the temperature increase is negligible, they reported a more detailed simulation showing that the temperature increase is actually in the range of 1 ^{o}C/100 mW of laser power. Taking into account that the neuronal transient receptor potential channels (TRP) can be activated by an increase in temperature [11, 36] or by depolarization of the cell membrane which is also sensitive to small variations in temperature and also pressure [3, 7, 39, 40] and knowing that the growth cones speed can be mediated by an asymmetric influx of calcium ions [22] they proposed that the underlying mechanism of optical guidance of neuronal growth is the influx of Ca^{2+} ions due to heat-induced activation of neuronal TRP channels [16].

CONCLUSIONS

The optical stimulation of the highly motile structure that leads an advancing neurite called the growth cone has been clearly demonstrated over a broad range of laser wavelengths, spot sizes, spot intensities beam shapes and beam modulation. However, the exact underlying mechanisms that lead to these results have not yet been pinpointed even if several mechanisms responsible for this outcome have been suggested. Keeping in mind that none of the proposed mechanisms can sustain the outcome on their own it is only fair to conclude that there is not a singular mechanism that leads to the enhanced growth rate of a neuron while being stimulated optically because they all play an important part in this. It is possible that once an optical stimulation is started the filopodia starts aligning into the direction of the laser beam feeling a small torque and while getting closer to the laser beam, the growth cone would start sensing the increase in temperature and therefore activating the TRP channels which would in turn start a biochemical signaling cascade that would promote neuronal growth in the direction of the laser beam.

Although optical stimulation of neuronal growth is a rather new subject for the science world, being able to produce such results is amazing and the focus of this subject should be applying this technique in nerve regeneration and figuring out how this could be achieved rather than racing to find the exact thing that produces these results.

Acknowledgements. This paper was supported by the grant: CAPACITATI 158/2008.

R E F E R E N C E S

1. ALBRECHT-BUEHLER, G., Surface extensions of 3T3 cells towards distant infrared light sources, *J. Cell Biol.*, 1991, **114**, 493–502.
2. ALLEMAND, J., D. BENSIMON, V. CROQUETTE, Stretching DNA and RNA to probe their interactions with proteins, *Curr. Opin. Struct. Biol*., 2003, **13**, 266–274.
3. ANDERSEN, S.S., A.D. JACKSON, T. HEIMBURG, Towards a thermodynamic theory of nerve pulse propagation, *Prog. Neurobiol.*, 2009, **88**, 104–113.
4. ASBURY, C., A. FEHR, S. BLOCK, Kenesin moves by an asymmetric hand-over-hand mechanism, *Science*, 2003, **302**, 2130–2134.
5. ASHKIN, A., Acceleration and trapping of particles by radiation pressure, *Phys. Rev. Lett*., 1970, **24**, 156–159.
6. ASHKIN, A., J.M. DZIEDZIC, J.E. BJORKHOLM, S. CHU, Observation of a single-beam gradient force optical trap for dielectric particles, *Opt. Lett.*, 1986, **11**, 288–290.
7. BLICHER, A., K. WODZINSKA, M. FIDORRA, M. WINTERHALTER, T. HEIMBURG, The temperature dependence of lipid membrane permeability, its quantized nature, and the influence of anesthetics, *Biophys. J.*, 2009, **96**, 4581–4591.
8. BUSTAMANTE, C., Z. BRVANT, S. SMITH, Ten years of tension: single-molecule DNA mechanics, *Nature*, 2003, **421**, 423–427.
9. CARNEGIE, D.J., T. CIZMAR, J. BAUMGARTL, F.J. GUNN-MOORE, K. DHOLAKIA, Automated laser guidance of neuronal growth cones using a spatial light modulator, *J. Biophotonics*, 2009, **2**, 682–692.
10. CARNEGIE, D.J., D.J. STEVENSON, M. MAZILU, F. GUNN-MOORE, K. DHOLAKIA, Guided neuronal growth using optical line traps, *Opt. Express*, 2008, **16**, 10507–10517.
11. CATERINA, M.J., M.A. SCHUMACHER, M. TOMINAGA, T.A. ROSEN, J.D. LEVINE, D. JULIUS, The capsaicin receptor: a heat-activated ion channel in the pain pathway, *Nature*, 1997, **389**, 816–824.
12. CECCONI, C., E. SHANK, C. BUSTAMANTE, S. MARQUSEE, Direct observation of the three-state folding of a single protein molecule, *Science*, 2005, **309**, 2057–2060.
13. COJOC, D, F. DIFATO, E. FERRARI, R.B. SHAHAPURE, J. LAISHRAM, M. RIGHI, E.M. DI FABRIZIO, V. TORRE, Properties of the force exerted by filopodia and lamellipodia and the involvement of cytoskeletal components, *PLoS ONE*, 2007, **2**, article number 1072.
14. DUMONT, S., W. CHENG, V. SEREBROV, R.K. BERAN, I.Jr. TINOCO, A.M. PYLE, C. BUSTAMANTE, RNA translocation and unwinding mechanisms of HCV N53 helicase and its coordination by ATP, *Nature*, 2006, **439**, 105–108.
15. DUNCAN, A.C., F. WEISBUCH, F. ROUAIS, S. LAZARE, Ch. BAQUEY, Laser microfabricated model surfaces for controlled cell growth, *Biosens. Bioelectron*., 2002, **17**, 413–426.

16. EBBESEN, C.L., H. BRUUS, Analysis of laser-induced heating in optical neuronal guidance, *J Neurosci Methods*, 2012, **209**, 168–177.
17. EHRLICHER, A., T. BETZ, B. STUHRMANN, D. KOCH, V. MILNER, M.G. RAIZEN, J. KAS, Guiding neuronal growth with light, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 16024–16028.
18. FOND, G., A. MACGREGOR, S. MIOT, Neuropsychiatry – The potential role of nanotechnologies in the future of psychiatry: A systematic review. *Eur. Neuropsychopharm*., 2012, http://dx.doi.org/10.1016/j.euroneuro.2012.10.016.
19. FRANZE, K., J. GUCK, The biophysics of neuronal growth, *Rep. Prog. Phys*., 2010, **73**(9), 094601.
20. FROMHERZ, P., H. SCHADEN, T. VETTER, Guided outgrowth of leech neurons in culture, *Neurosci. Lett.*, 1991, **129**, 77–80.
21. GRAVES, CATHERINE E., R.G. MCALLISTER, W.J. ROSOFF, J.S. URBACH, Optical neuronal guidance in three-dimensional matrices, *J. Neurosci. Methods*, 2009, **179**, 278–283.
22. HENLEY, J., M.M. POO, Guiding neuronal growth cones using Ca^{2}+ signals, *Trends Cell Biol.*, 2004, **14**, 320–330.
23. HUISSTEDE, J., V. SUBRAMANIAM, M.L. BENNINK, Combining optical tweezers and scanning probe microscopy to study DNA–protein interactions, *Microsc. Res. Tech.*, 2007, **70**, 26–33.
24. JESSE, F., Z. MIAO, L. ZHAO, Y. CHEN, Y.Y. LV, Optical fiber light source directs neurite growth, *Biomed. Opt. Express*, 2013, **4**, 614–618.
25. KNIGHT, A., C. VEIGEL, C. CHAMBERS, J.E. MOLLOY, Analysis of single-molecule mechanical recordings: application to acto-myosin interactions, *Prog. Biophys. Mol. Biol.*, 2001, **77**, 45–72.
26. KOCH, D., T. BETZ, A. EHRLICHER, M. GOGLER, B. STUHRMANN, J. KAS, Optical control of neuronal growth, *Proc. SPIE – Int. Soc. Opt. Eng.*, 2004, **5514**, 428–436.
27. KUO, S.C., Optical Tweezers: A Practical Guide, *Microsc. Microanal.*, 1995, **1**, 65–74.
28. LI, Y., L.H. SMITH, L.J. HARGROVE, D.J. WEBER, G.E. LOEB, Sparse optimal motor estimation (SOME) for extracting commands for prosthetic limbs, *IEEE Trans. Neural Syst. Rehabil. Eng.*, 2013, **21**, 104–111.
29. LOWERY, L.A., D.V. VACTOR, The trip of the tip: understanding the growth cone machinery, *Nat. Rev. Mol. Cell Biol*., 2009, **10**, 332–343.
30. MALLIK, R., B.C. CARTER, S.A. LEX, S.J. KING, S.P. GROSS, Cytoplasmic dynein functions as a gear in response to load, *Nature*, 2004, **427**, 649–652.
31. MOHANTY S., M. SHARMA, M. PANICKER, P. GUPTA, Controlled induction, enhancement, and guidance of neuronal growth cones by use of line optical tweezers, *Opt. Lett.*, 2005, **30**, 2596–2598.
32. PEASE, P.J., O. LEVY, G.J. COST, J. GORE, J.L. PTACIN, D. SHERRATT, C. BUSTAMANTE, N.R. COZZARELLI, Sequence-directed DNA transcription by purified FtsK, *Science*, 2005, **307**, 586–590.
33. PRINZ, A.A., P. FROMHERZ, Electrical synapses by guided growth of cultured neurons from the snail Lymnaea stagnalis, *Biol. Cybern.*, 2000, **82**, L1–5.
34. RYU, W., R.M. BERRY, H.C. BERG, Torque-generating units of the flagellar motor of Escherichia coli have a high duty ratio, *Nature*, 2000, **403**, 444–447.
35. STEVENSON, D.J., T.K. LAKE, B. AGATE, V. GARCES-CHAVEZ, K. DHOLAKIA, F. GUNN-MOORE, Optically guided neuronal growth at near infrared wavelengths, *Opt. Express.*, 2006, **14**, 9786–9793.
36. TALAVERA, K., B. NILIUS, T. VOETS, Neuronal TRP channels: thermometers, pathfinders and life-savers, *Trends Neurosci.*, 2008, **31**, 287–295.

37. THOUMINE, O., P. KOCIAN, A. KOTTELAT, J.J. MEISTER, Short-term binding of fibroblasts to fibronectin: optical tweezers experiments and probabilistic analysis, *Eur. Biophys. J.*, 2000, **29**, 398–408.
38. WILLIAMS, M.C., I. ROUZINA, J.R. WENNER, R.J. GORELICK, K. MUSIER-FORSYTH, V.A. BLOOMFIELD, Mechanism for nucleic acid chaperone activity of HIV-1 nucleocapsid protein revealed by single molecule stretching, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 6121–6126.
39. WODZINSKA, K., A. BLICHER, T. HEIMBURG, The thermodynamics of lipid ion channel formation in the absence and presence of anesthetics, *Soft Matter*, 2009, **5**, 3319–3330.
40. WUNDERLICH, B., C. LEIRER, A.L. IDZKO, U.F. KEYSER, A. WIXFORTH, V.M. MYLES, T. HEIMBURG, M.F. SCHNEIDER, Phase-state dependent current fluctuations in pure lipid membranes, *Biophys. J.*, 2009, **96**, 4592–4597.