



# Laser Nano-Neurosurgery from Gentle Manipulation to Nano-Incision of Neuronal Cells and Scaffolds: An Advanced Neurotechnology Tool

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Current optical approaches are progressing far beyond the scope of monitoring the structure and function of living matter, and they are becoming widely recognized as extremely precise, minimally-invasive, contact-free handling tools. Laser manipulation of living tissues, single cells, or even single-molecules is becoming a well-established methodology, thus founding the onset of new experimental paradigms and research fields. Indeed, a tightly focused pulsed laser source permits complex tasks such as developing engineered bioscaffolds, applying calibrated forces, transfecting, stimulating, or even ablating single cells with subcellular precision, and operating intracellular surgical protocols at the level of single organelles. In the present review, we report the state of the art of laser manipulation in neuroscience, to inspire future applications of light-assisted tools in nano-neurosurgery.

**Keywords:** laser nano-surgery, photo-polymerization, photo-stimulation, opto-transfection, intracellular surgery, laser dissection

## INTRODUCTION

The etymology of the word “surgery” is derived from the ancient Greek word *cheirurgike*, or the Latin expression *chirurgiae*, which refer to the “hand work”. Conventionally, surgery is performed through the physical contact of tools operated by the hands of a highly skilled and experienced surgeon. Currently, manipulation of living matter has entered a new era exploiting alternative approaches and devices that assist or even substitute the human hands through tele-operated devices and miniaturized tools. As a prominent example, recent advances of laser technology and optical systems pushed the central role of light not only to observe the living matter at greater resolution, but also to perform tissue manipulation through “hands of light”. The resolution of the light touch is so precise that light represents, at present, the only tool providing access to real micro- and even nano-scale surgery.

Light manipulation tools can be classified in two main categories: gentle and invasive. The former non-invasive approach concerns the exploitation of optical forces, as in optical tweezers, to manipulate viruses, cells, as well as molecules in living cells (Oddershede, 2012). The latter consists of exploiting the energy of high photon flux to overcome the break-down threshold of the sample, and induce local ablation.

Despite the presence of a numerous literature proposing the adoption of optical trapping in surgical operations (Waleed et al., 2013), *in vivo* application of such an approach is still limited by the maximum forces that optical tweezers can generate, even with state of the art optical fiber-based set-ups (Liberale et al., 2007). Indeed, forces in the pico-newton range are certainly suitable for *in vitro* studies of single molecules and/or single living cells, but they are too small to apply a significant strain to induce local deformations or to move small entities in the crowded and dense environment of a living tissue. On the other hand, laser ablation is a much more effective tool applicable either *in vitro* or *in vivo*. Moreover, an accurate choice of the working parameters (laser wavelength, pulse energy, pulse duration, spatial beam profile, pulse repetition rate, and irradiation time) allows tuning the interaction of light with biological matter, from reversible manipulation to irreversible hard cuts (Rudhall et al., 2012).

Laser tools are highly controllable through dedicated electronics, which could be integrated in robotic surgery systems, enabling precise task definition and repeatability. Although research in laser surgery is still in its infancy for broad clinical applications, it has given an important contribution to understand the physiology (Hayes et al., 2012) of distinct pathologies at single cell level (Tilve et al., 2015), and it has demonstrated the potentiality to directly focus the area of intervention in living tissues with minimal scar formation. Indeed, applications of lasers in eye surgery or in laparoscopic systems are becoming widespread approaches in clinic (Mattos and Caldwell, 2012).

Moreover, laser technology opens the avenue for new applications in the emerging field of nano-medicine. Light can be used to trigger the action of chemically engineered nanoparticles, designed to recognize molecular targets, and loaded with specialized photo-sensitizers. The action of light on such carriers can be multivalent: a caged compound can be delivered directly on the target tissue upon illumination (Yang et al., 2012), as well as a local enhancement of the light can induce a cell specific apoptotic effect (Pekkanen et al., 2014).

In the present review, prominent applications of laser nano-surgery will be highlighted, starting from the design of extracellular environment with micro- and nano-scale features, to the intracellular ablation of cellular compartments. All the reported examples, which are currently employed or could be exploited in the field of laser nano-neurosurgery, will provide an overview of the capability of laser manipulation.

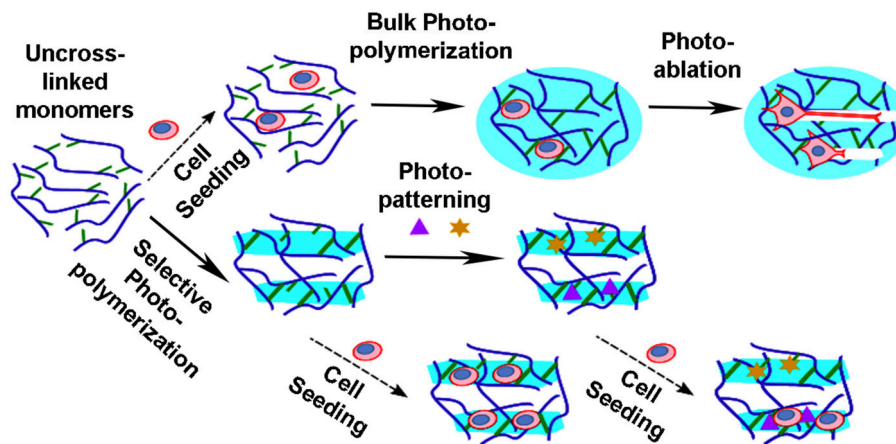
## LASER PROCESSING OF NEURONAL MICRO-ENVIRONMENT: ENGINEERING NEURON-SCAFFOLD INTERACTIONS

Engineering of neuronal scaffolds is gaining importance to reproduce neural circuits *in vitro* as well as *in vivo* to repair injuries, to locally deliver cells or molecules, and to promote regeneration. Mimicking the extracellular physiological milieu remains a major challenge, because it is extremely heterogeneous in terms of topographical/mechanical/biochemical features. In this context, the use of light for modeling optically transparent

three-dimensional (3D) hydrogels has been successfully applied to direct cell differentiation toward specific lineages or to promote and guide the outgrowth of neuronal processes. Design of topographical properties of such hydrogels can be accomplished by different strategies: photo-polymerization or photo-ablation (see **Figure 1**). Complex 3D structures can be photo-polymerized with a stereo-lithographic approach (Zorlutuna et al., 2011), or with ultrashort pulsed lasers exploiting the multi-photon absorption process within a femto-liter volume (Cumpston et al., 1999; Simitzi et al., 2015). Currently, super resolution techniques and development of new photo-resins are exploited to reduce the minimum size of features that can be generated (Scott et al., 2009; Gan et al., 2013). Indeed, there is growing evidence that the roughness of the surface could enhance cell differentiation, and direct the growth of neuronal projections (Bugnicourt et al., 2014; Marino et al., 2014). Otherwise, directional growth of neurons within a pre-existing hydrogel can be also obtained by post-processing of the scaffolds already containing the cells (Odawara et al., 2013). In this case, neurons are free to establish their hierarchical connectivity within the scaffold, and successive laser-mediated ablation of micro-channels opens the way for neurite sprouting (Sarig-Nadir et al., 2009). Otherwise, photo-cleavage of chemical moieties integrated in the hydrogel can locally modify hydrogel structures and allows the formation of *in vitro* neural networks with a specific geometry (McKinnon et al., 2014).

Mechanical properties of hydrogels can be also tuned by photo-degradation. Incorporating photo-sensitive moieties into the monomers that are assembled in the polymer backbone enables degradation of selected volumes by laser irradiation after the polymerization process. In particular, the spatial/temporal degradation of the hydrogel, leading to local modulation of the mechanical properties, can be finely controlled by the laser intensity (Kloxin et al., 2009), or by the use of a photo-mask (Lewis and Anseth, 2013). In this way the same scaffold could potentially mimic the mechanical properties of any tissue. Indeed, softening of a scaffold can be tuned to match the elastic properties of the nervous tissue to induce neural stem cell differentiation (Engler et al., 2006).

Biochemical functionalization of the extracellular environment through laser light can locally stimulate important cell functions. The first photo-chemical patterning of peptides in 3D hydrogels was performed by using photo-caged thiols, which upon irradiation with a focused laser, were freed to react with maleimide-modified peptides. The resulting biochemical channels promoted axonal sprouting (Luo and Shoichet, 2004). Further, photo-chemical immobilization of peptides or even whole proteins enables to pattern different growth factors in distinct volumes within the same 3D hydrogel (Wylie and Shoichet, 2011). Otherwise, two-photon laser scanning based lithography has been used in conjunction with photo-initiators to immobilize biomolecules during the photo-polymerization process at microscale resolution (Lee et al., 2008). Therefore, photo-patterning to generate biomolecular gradients (Owen et al., 2013), or concomitant functionalization with multiple proteins in 3D matrices (Wylie et al., 2011) allows achieving bioplatfroms with high degree of complexity and mimicry of the



**FIGURE 1 | Laser based approaches to produce bioscaffolds.** The photo-polymerization/ablation of polymers has permitted the production of complex polymeric structures which can be used as bioscaffolds. Hydrogels are produced from monomers (blue curves) by crosslinking of reactive groups (green lines). Neuronal cells can be seeded within or on the scaffolds prior or post-processing. Homogenous hydrogels (light blue circles) can be obtained by bulk photo-polymerization of the suspension. Then, physical channels (white rectangles) can be generated in the pre-formed gels by photo-ablation, in order to set out the path directing neurite elongation. Instead selected volumes (light blue rectangles) of the gel suspension can be photo-polymerized with the use of a focused laser or photo-masks. Biochemical properties can be further introduced by photo-patterning of peptides (purple triangles) and/or whole proteins (yellow stars), with function of grow factors, adhesion cues, enzymes, and so on.

native counterparts. Another possibility is to create hydrogels whose biochemical and physical properties can be reshaped by the cells themselves. For example, photo-polymerization of hydrogels containing enzymatically cleavable peptides enables the cell mediated remodeling of the scaffold by the expression of the cellular enzymes matrix metalloproteinases (Anderson et al., 2011).

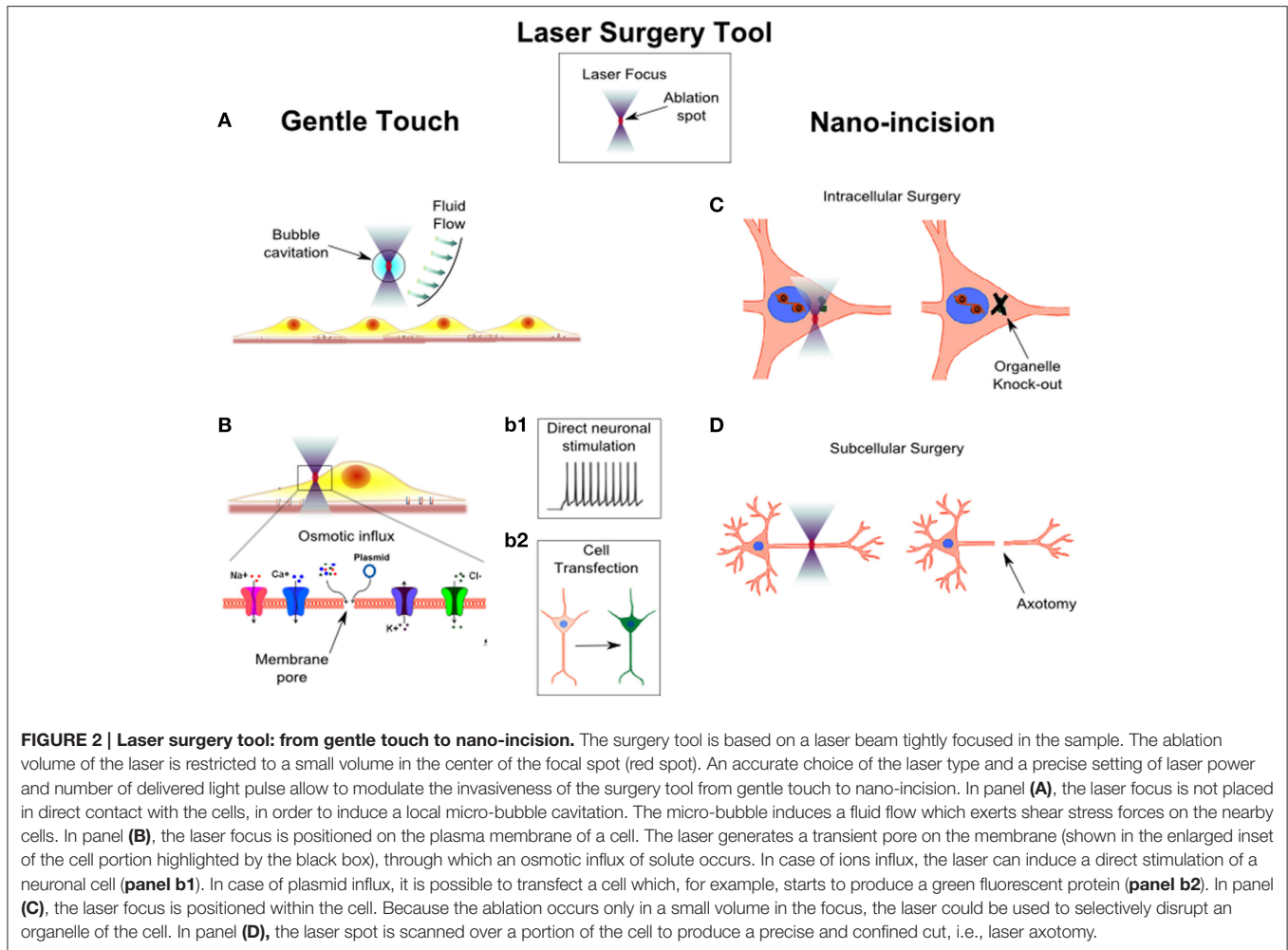
Overall, the use of laser-based technologies within optically transparent biomaterials offers highly versatile tools for the processing and imaging of multifunctional scaffolding biosystems (Lewis and Anseth, 2013) that are suitable for neural tissue engineering.

## GENTLE LASER MANIPULATION OF NEURONAL CELLS

Probing and manipulating the complex structure and function of a neural circuit, without prompting any permanent morphological change, allows understanding the functional connectivity of neural circuits, and treating their pathological activities non-invasively. The interaction of light with living tissues induces focal perturbations (see the laser surgery tool in **Figure 2**), that can be transiently applied to perform cell stimulation. As an example, the possibility to locally generate shockwaves through microplasma-cavitation effects in water has been proposed as a high-throughput approach to exert mechanical stimulation of cells (Compton et al., 2014; **Figure 2A**). The ability to precisely control the mechanical environment of brain tissue is raising enormous attention especially after the discovery that the brain is one of the most mechanosensitive organs (Tyler, 2012). Inducing mechanical alterations would therefore allow to understand

the mechanobiology of neuronal migration and development, providing important insights into the design of efficient neuronal scaffolds (Palazzolo et al., 2015). In other studies, a high-throughput mechanical stimulation of cells has been engaged to understand how abrupt stimulation of integrins could produce mild-traumatic brain injury (Hemphill et al., 2011; Grevesse et al., 2015).

Another way to achieve neuronal stimulation by laser irradiation is based on photo-thermal effects occurring in the laser focus. The earliest report of direct laser stimulation on neuronal cells was described by Fork on Aplysia ganglion neurons (Fork, 1971). The development of ultrashort pulsed sources incorporated in advanced two-photon microscopy setups permitted the exploitation of the non-linear excitation in the focal volume to directly stimulate neurons without using any caged compounds or fluorescent molecules (Hirase et al., 2002). Recently, microparticles or light sensitive conjugated polymers have been used to produce patterned photo-stimulation of neuronal circuits (Farah et al., 2013) or to develop new retinal prosthetic devices (Ghezzi et al., 2013). The main mechanism producing direct neuronal stimulation is photo-thermal but it does not require temperature-sensitive channels. Indeed, it was shown that thermal stimulation of cells was related to the rate of temperature increase (instead of the maximum temperature reached), which modified the capacitive properties of the cell membrane (Shapiro et al., 2012). However, depending on the light intensity delivered to the cells, other mechanisms could be involved in the direct stimulation of neurons (Beier et al., 2014), as seen in transient plasma membrane nano-poration (**Figure 2Bb1**). Although membrane opto-poration provides single cell stimulation, it cannot be exploited as an efficient protocol when repetitive cycles of stimulation are required. However, transient disruption of the cell membrane represents



an intriguing approach to inject foreign molecules intracellularly, which in the specific case of nucleic acids injection, it can be used to achieve single cell opto-transfection (Figure 2b2). Tirlapur et al. (Tirlapur and König, 2002) reported the first evidence of optical-assisted delivery of foreign DNA into cells *in vitro*, thus showing the possibility to inject non membrane-permeable molecules. In such a way, it is possible to experimentally observe the consequences of delivering molecules into specific subcellular regions. For example, the introduction of specific mRNA in the dendrites or in the soma of living neurons highlighted the importance of subcellular localization of transcription factors in distinct cellular compartments (Barrett et al., 2006). A variety of lasers have been used to perform single cell opto-poration/transfection (Paterson et al., 2005; Marchington et al., 2010). Engineered beam shapes have been exploited to raise the throughput of opto-transfection (Rendall et al., 2012; Breunig et al., 2014). Recently, the application of extremely ultrashort femtosecond laser decreased the power necessary to nano-process cells (Uchugonova et al., 2008) by more than one order of magnitude. Therefore, optical re-programming of human cells into induced human pluripotent stem cells is becoming a safe and efficient approach (Breunig et al., 2015). Furthermore,

opto-transfection of single neurons has been proposed in combination with optogenetic (Antkowiak et al., 2013), and technical efforts have been spent to apply the technique in an *in vivo* scenario (Ma et al., 2011). Optical transfection of single cells to induce expression of optogenetic probes could circumvent the need for viral particles or vectors to target specific cells, and it could provide a way to apply complex optical stimulation patterns with single cell resolution also on awake animals (Antkowiak et al., 2013).

## SURGERY AT THE NANO-SCALE: FROM INTRACELLULAR ABLATION TO SUBCELLULAR DISSECTION *IN VIVO*

Nano-fabrication has a tremendous impact in pharmaceutical and medical fields. Nano-scale knife (Kruskal et al., 2015), and implants are discovering new aspects in biology at the molecular scale (Betancourt and Brannon-Peppas, 2006). Proper control and manipulation of these nano-tools burden the development of robotic devices and automated control to operate them in living matter (Chang et al., 2010). On the contrary, the massless





## REFERENCES

- Allegra Mascaro, A. L., Cesare, P., Sacconi, L., Grasselli, G., Mandolesi, G., Maco, B., et al. (2013). *In vivo* single branch axotomy induces GAP-43-dependent sprouting and synaptic remodeling in cerebellar cortex. *Proc. Natl. Acad. Sci. U.S.A.* 110, 10824–10829. doi: 10.1073/pnas.1219256110
- Anderson, S., Lina, C., Kuntzler, D., and Anseth, K. (2011). The performance of human mesenchymal stem cells encapsulated in cell-degradable polymer-peptide hydrogels SB. *Biomaterials* 32, 3564–3574. doi: 10.1016/j.biomaterials.2011.01.064
- Antkowiak, M., Torres-Mapa, M. L., Witts, E. C., Miles, G. B., Dholakia, K., and Gunn-Moore, F. J. (2013). Fast targeted gene transfection and optogenetic modification of single neurons using femtosecond laser irradiation. *Sci. Rep.* 3, 1–8. doi: 10.1038/srep03281
- Barrett, L. E., Sul, J. Y., Takano, H., Bockstaele, E. J., van Haydon, P. G., Eberwine, J. H., et al. (2006). Region-directed phototransfection reveals the functional significance of a dendritically synthesized transcription factor. *Nat. Methods* 3, 455–460. doi: 10.1038/nmeth885
- Beier, H. T., Tolstykh, G. P., Musick, J. D., Thomas, R. J., and Ibey, B. L. (2014). Plasma membrane nanoporation as a possible mechanism behind infrared excitation of cells. *J. Neural Eng.* 11:066006. doi: 10.1088/1741-2560/11/6/066006
- Bergeron, E., Boutopoulos, C., Martel, R., Torres, A., Rodriguez, C., Niskanen, J., et al. (2015). Cell-specific optoporation with near-infrared ultrafast laser and functionalized gold nanoparticles. *Nanoscale* 7, 17836–17847. doi: 10.1039/C5NR05650K
- Betancourt, T., and Brannon-Peppas, L. (2006). Micro- and nanofabrication methods in nanotechnological medical and pharmaceutical devices. *Int. J. Nanomed.* 1, 483–495. doi: 10.2147/nano.2006.1.4.483
- Bonifazi, P., Difato, F., Massobrio, P., Breschi, G. L., Pasquale, V., Levi, T., et al. (2013). *In vitro* large-scale experimental and theoretical studies for the realization of bi-directional brain-prostheses. *Front. Neural Circuits* 7:40. doi: 10.3389/fncir.2013.00040
- Bradke, F., Fawcett, J. W., and Spira, M. E. (2012). Assembly of a new growth cone after axotomy: the precursor to axon regeneration. *Nat. Rev. Neurosci.* 13, 183–193. doi: 10.1038/nrn3176
- Breunig, H. G., Uchugonova, A., Batista, A., and König, K. (2014). High-throughput continuous flow femtosecond laser-assisted cell optoporation and transfection. *Microsc. Res. Tech.* 77, 974–979. doi: 10.1002/jemt.22423
- Breunig, H. G., Uchugonova, A., Batista, A., and König, K. (2015). Optical reprogramming of human cells in an ultrashort femtosecond laser microfluidic transfection platform. *J. Biophotonics*. doi: 10.1002/jbio.201500240. [Epub ahead of print].
- Bugnicourt, G., Brocard, J., Nicolas, A., and Villard, C. (2014). Nanoscale surface topography reshapes neuronal growth in culture. *Langmuir* 30, 4441–4449. doi: 10.1021/la5001683
- Canty, A. J., Huang, L., Jackson, J. S., Little, G. E., Knott, G., Maco, B., et al. (2013). *In-vivo* single neuron axotomy triggers axon regeneration to restore synaptic density in specific cortical circuits. *Nat. Commun.* 4, 2038. doi: 10.1038/ncomms3038
- Chang, W. C., Hawkes, E., Keller, C. G., and Sretavan, D. W. (2010). Axon repair?: surgical application at a subcellular scale. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2, 151–161. doi: 10.1002/wnan.76
- Colombelli, J., Reynaud, E. G., Rietdorf, J., Pepperkok, R., and Stelzer, E. H. K. (2005). *In vivo* selective cytoskeleton dynamics quantification in interphase cells induced by pulsed ultraviolet laser nanosurgery. *Traffic* 6, 1093–1102. doi: 10.1111/j.1600-0854.2005.00334.x
- Compton, J. L., Luo, J. C., Ma, H., Botvinick, E., and Venugopalan, V. (2014). High-throughput optical screening of cellular mechanotransduction. *Nat. Photonics* 8, 710–715. doi: 10.1038/nphoton.2014.165
- Cumpston, B. H., Ananthal, S. P., Barlow, S., Dyer, D. L., Ehrlich, J. E., Erskine, L. L., et al. (1999). Two-photon polymerization initiators for three-dimensional optical data storage and microfabrication. *Nature* 398, 51–54. doi: 10.1038/17989
- Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 2, 752–758. doi: 10.1038/nn1472
- Difato, F., Dal Maschio, M., Beltramo, R., Blau, A., Benfenati, F., and Fellin, T. (2012). “Spatial light modulators for complex spatio-temporal illumination of neuronal networks,” in *Neuronal Network Analysis: Concepts and Experimental Approaches*, eds T. Fellin and M. M. Halassa (New York, NY: Springer), 61–81.
- Difato, F., Dal Maschio, M., Marconi, E., Ronzitti, G., Maccione, A., Fellin, T., et al. (2011a). Combined optical tweezers and laser dissector for controlled ablation of functional connections in neural networks. *J. Biomed. Opt.* 16, 051306. doi: 10.1117/1.3560268
- Difato, F., Tsushima, H., Pesce, M., Benfenati, F., Blau, A., and Chierigatti, E. (2011b). The formation of actin waves during regeneration after axonal lesion is enhanced by BDNF. *Sci. Rep.* 1, 183. doi: 10.1038/srep00183
- Drummond, E. S., Nayak, S., Ueberheide, B., and Wisniewski, T. (2015). Proteomic analysis of neurons microdissected from formalin-fixed, paraffin-embedded Alzheimer's disease brain tissue. *Sci. Rep.* 5, 15456. doi: 10.1038/srep15456
- Engler, A. J., Sen, S., Sweeney, H. L., and Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell* 126, 677–689. doi: 10.1016/j.cell.2006.06.044
- Farah, N., Zoubi, A., Matar, S., Golan, L., Marom, A., Butson, C. R., et al. (2013). Holographically patterned activation using photo-absorber induced neural-thermal stimulation. *J. Neural Eng.* 10, 056004. doi: 10.1088/1741-2560/10/5/056004
- Fork, R. L. (1971). Laser stimulation of nerve cells in aphasia. *Science* 171, 907–908. doi: 10.1126/science.171.3974.907
- Gan, Z., Cao, Y., Evans, R. A., and Gu, M. (2013). Three-dimensional deep sub-diffraction optical beam lithography with 9 nm feature size. *Nat. Commun.* 4, 2061–2066. doi: 10.1038/ncomms3061
- Ghezzi, D., Antognazza, M. R., Maccarone, R., Bellani, S., Lanzarini, E., Martino, N., et al. (2013). A polymer optoelectronic interface restores light sensitivity in blind rat retinas. *Nat. Photonics* 7, 400–406. doi: 10.1038/nphoton.2013.34
- Grevesse, T., Dabiri, B. E., Parker, K. K., and Gabriele, S. (2015). Opposite rheological properties of neuronal microcompartments predict axonal vulnerability in brain injury. *Sci. Rep.* 5, 9475. doi: 10.1038/srep09475
- Habibey, R., Golabchi, A., Latifi, S., Difato, F., and Blau, A. (2015). Microchannel device for selective laser dissection, long-term microelectrode array electrophysiology and imaging of confined axonal projections. *Lab Chip* 15, 4578–4590. doi: 10.1039/C5LC01027F
- Hayes, J. A., Wang, X., and Del Negro, C. A. (2012). Cumulative lesioning of respiratory interneurons disrupts and precludes motor rhythms *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* 109, 8286–8291. doi: 10.1073/pnas.1200912109
- He, J., Wang, Y., Missinato, M. A., Onuoha, E., Perkins, L. A., Watkins, S. C., et al. (2016). A genetically targetable near-infrared photosensitizer. *Nat. Methods* 13, 263–268. doi: 10.1038/nmeth.3735
- Hemphill, M. A., Dabiri, B. E., Gabriele, S., Kerscher, L., Franck, C., Goss, J. A., et al. (2011). A possible role for integrin signaling in diffuse axonal injury. *PLoS ONE* 6:e22899. doi: 10.1371/journal.pone.0022899
- Hirase, H., Nikolenko, V., and Yuste, R. (2002). Multiphoton stimulation of neurons and spines. *Dev. Neurobiol.* 51, 237–247. doi: 10.1002/neu.10056
- Kim, Y., Karthikeyan, K., Chirvi, S., and Davé, D. P. (2009). Neuro-optical microfluidic platform to study injury and regeneration of single axons. *Lab Chip* 9, 2409–2612. doi: 10.1039/b903720a
- Kloxin, A. M., Kasko, A. M., Salinas, C. N., and Anseth, K. S. (2009). Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* 324, 59–63. doi: 10.1126/science.1169494
- König, K., Andersen, P., Le, T., and Breunig, H. G. (2015). Multiphoton imaging with a novel compact diode-pumped Ti:sapphire oscillator. *Microsc. Res. Tech.* 78, 1154–1158. doi: 10.1002/jemt.22599
- König, K., Riemann, I., and Fritzsche, W. (2001). Nanodissection of human chromosomes with near-infrared femtosecond laser pulses. *Opt. Lett.* 26, 819–821. doi: 10.1364/OL.26.000819
- Kruskal, P. B., Jiang, Z., Gao, T., and Lieber, C. M. (2015). Beyond the patch clamp: nanotechnologies for intracellular recording. *Neuron* 86, 21–24. doi: 10.1016/j.neuron.2015.01.004

- Lee, S.-H., Moon, J. J., and West, J. L. (2008). Three-dimensional micropatterning of bioactive hydrogels via two-photon laser scanning photolithography for guided 3D cell migration. *Biomaterials* 29, 2962–2968. doi: 10.1016/j.biomaterials.2008.04.004
- Lewis, K. J. R., and Anseth, K. S. (2013). Hydrogel scaffolds to study cell biology in four dimensions. *MRS Bull.* 38, 260–268. doi: 10.1557/mrs.2013.54
- Liberale, C., Minzioni, P., Bragheri, F., De Angelis, F., Di Fabrizio, E., and Cristiani, I. (2007). Miniaturized all-fibre probe for three-dimensional optical trapping and manipulation. *Nat. Photonics* 1, 723 – 727. doi: 10.1038/nphoton.2007.230
- Luo, Y., and Shoichet, M. S. (2004). A photolabile hydrogel for guided three-dimensional cell growth and migration. *Nat. Mater.* 3, 249–253. doi: 10.1038/nmat1092
- Ma, N., Gunn-Moore, F., and Dholakia, K. (2011). Optical transfection using an endoscope-like system. *J. Biomed. Opt.* 16, 028002. doi: 10.1117/1.3541781
- Marchington, R. F., Arita, Y., Tsampoula, X., Gunn-Moore, F. J., and Dholakia, K. (2010). Optical injection of mammalian cells using a microfluidic platform. *Biomed. Opt. Express* 1, 527–536. doi: 10.1364/BOE.1.000527
- Marino, A., Desii, A., Pellegrino, M., Pellegrini, M., Filippeschi, C., Mazzolai, B., et al. (2014). Nanostructured brownian surfaces prepared through two-photon polymerization?: investigation of stem cell response. *ACS Nano* 8, 11869–11882. doi: 10.1021/nn5052426
- Mattos, L. S., and Caldwell, D. G. (2012). “Safe teleoperation based on flexible intraoperative planning for robot-assisted laser microsurgery,” in *Annual International Conference of the IEEE Engineering in Medicine and Biology Society* (San Diego, CA), 174–178.
- McKinnon, D. D., Brown, T. E., Kyburz, K. A., Kiyotake, E., and Anseth, K. S. (2014). Design and characterization of a synthetically accessible, photodegradable hydrogel for user-directed formation of neural networks. *Biomacromolecules* 15, 2808–2816. doi: 10.1021/bm500731b
- Medvid, R., Ruiz, A., Komotar, R. J., Jagid, J. R., Ivan, M. E., Quencer, R. M., et al. (2015). Current applications of MRI-guided laser interstitial thermal therapy in the treatment of brain neoplasms and epilepsy: a radiologic and neurosurgical overview. *Am. J. Neuroradiol.* 36, 1998–2006. doi: 10.3174/ajnr.A4362
- Nishimura, N., Schaffer, C. B., Friedman, B., Tsai, P. S., Lyden, P. D., and Kleinfeld, D. (2006). Targeted insult to subsurface cortical blood vessels using ultrashort laser pulses: three models of stroke. *Nat. Methods* 3, 99–108. doi: 10.1038/nmeth844
- Odawara, A., Gotoh, M., and Suzuki, I. (2013). Control of neural network patterning using collagen gel photothermal etching. *Lab Chip* 13, 1981–2168. doi: 10.1039/c3lc00036b
- Oddershede, L. B. (2012). Force probing of individual molecules inside the living cell is now a reality. *Nat. Chem. Biol.* 8, 879–886. doi: 10.1038/nchembio.1082
- Owen, S. C., Fisher, S. A., Tam, R. Y., Nimmo, C. M., and Shoichet, M. S. (2013). Hyaluronic acid click hydrogels emulate the extracellular matrix. *Langmuir* 29, 7393–7400. doi: 10.1021/la305000w
- Palazzolo, G., Broguiere, N., Cenciarelli, O., Dermutz, H., and Zenobi-Wong, M. (2015). Ultrasoft alginate hydrogels support long-term three-dimensional functional neuronal networks. *Tissue Eng. A* 21, 2177–2185. doi: 10.1089/ten.tea.2014.0518
- Papagiakoumou, E., Bègue, A., Leshem, B., Schwartz, O., Stell, B. M., Bradley, J., et al. (2013). Functional patterned multiphoton excitation deep inside scattering tissue. *Nat. Photonics* 7, 274–278. doi: 10.1038/nphoton.2013.9
- Patel, G. N., Cymbalyuk, G. S., Calabrese, R. L., and DeWeerth, S. P. (2012). Bifurcation analysis of a silicon neuron. *Artif. life Robot.* 17, 731–737. doi: 10.1007/s10015-012-0016-6
- Paterson, L., Agate, B., Comrie, M., Ferguson, R., Lake, T., Morris, J., et al. (2005). Photoporation and cell transfection using a violet diode laser. *Opt. Express* 13, 595–600. doi: 10.1364/OPEX.13.000595
- Pekkanen, A. M., Dewitt, M. R., and Rylander, M. N. (2014). Nanoparticle enhanced optical imaging and phototherapy of cancer. *J. Biomed. Nanotechnol.* 10, 1677–1712. doi: 10.1166/jbn.2014.1988
- Rendall, H. A., Marchington, R. F., Praveen, B. B., Bergmann, G., Arita, Y., Heisterkamp, A., et al. (2012). High-throughput optical injection of mammalian cells using a Bessel light beam. *Lab Chip* 12, 4816–4820. doi: 10.1039/c2lc40708f
- Rudhall, A. P., Antkowiak, M., Tsampoula, X., Mazilu, M., Metzger, N. K., Gunn-Moore, F., et al. (2012). Exploring the ultrashort pulse laser parameter space for membrane permeabilisation in mammalian cells. *Sci. Rep.* 2, 858. doi: 10.1038/srep00858
- Saquilabon Cruz, G. M., Kong, X., Silva, B. A., Khatibzadeh, N., Thai, R., Berns, M. W., et al. (2015). Femtosecond near-infrared laser microirradiation reveals a crucial role for PARP signaling on factor assemblies at DNA damage sites. *Nucleic Acids Res.* 1, gkv976. doi: 10.1093/nar/gkv976
- Sarig-Nadir, O., Livnat, N., Zajdman, R., Shoham, S., and Seliktar, D. (2009). Laser photoablation of guidance microchannels into hydrogels directs cell growth in three dimensions. *Biophys. J.* 96, 4743–4752. doi: 10.1016/j.bpj.2009.03.019
- Scott, T. F., Kowalski, B. A., Sullivan, A. C., Bowman, C. N., and McLeod, R. R. (2009). Two-color single-photon photoinitiation and photoinhibition for subdiffraction photolithography. *Science* 324, 913–917. doi: 10.1126/science.1167610
- Shapiro, M. G., Homma, K., Villarreal, S., Richter, C.-P., and Bezanilla, F. (2012). Infrared light excites cells by changing their electrical capacitance. *Nat. Commun.* 3, 736. doi: 10.1038/ncomms1742
- Shen, N., Datta, D., Schaffer, C. B., LeDuc, P., Ingber, D. E., and Mazur, E. (2005). Ablation of cytoskeletal filaments and mitochondria in live cells using a femtosecond laser nanoscissor. *Mech. Chem. Biosyst.* 2, 17–25.
- Simitzi, C., Efstathopoulos, P., Kourgiantaki, A., Ranella, A., Charalampopoulos, I., Fotakis, C., et al. (2015). Laser fabricated discontinuous anisotropic microconical substrates as a new model scaffold to control the directionality of neuronal network outgrowth. *Biomaterials* 67, 115–128. doi: 10.1016/j.biomaterials.2015.07.008
- Stiess, M., Maghelli, N., Kapitein, L. C., Gomis-Rüth, S., Wilsch-Brauninger, M., Hoogenraad, C. C., et al. (2010). Axon extension occurs independently of centrosomal microtubule nucleation. *Science* 327, 704–707. doi: 10.1126/science.1182179
- Taratula, O., Schumann, C., Duong, T., Taylor, K. L., and Taratula, O. (2015). Dendrimer-encapsulated naphthalocyanine as a single agent-based theranostic nanoplatfrom for near-infrared fluorescence imaging and combinatorial anticancer phototherapy. *Nanoscale* 7, 3888–3902. doi: 10.1039/C4NR06050D
- Tilve, S., Difato, F., and Chierregatti, E. (2015). Cofilin 1 activation prevents the defects in axon elongation and guidance induced by extracellular alpha-synuclein. *Sci. Rep.* 5, 1–13. doi: 10.1038/srep16524
- Tirlapur, U. K., and König, K. (2002). Targeted transfection by femtosecond laser. *Nature* 418, 290–291. doi: 10.1038/418290a
- Tyler, W. J. (2012). The mechanobiology of brain function. *Nat. Rev. Neurosci.* 13, 867–878. doi: 10.1038/nrn3383
- Uchugonova, A., Breunig, H. G., Ana BaKönig, K., Bueckle, R., Isemann, A., Tempea, G., et al. (2008). Targeted transfection of stem cells with sub-20 femtosecond laser pulses. *Opt. Express* 16, 9357–9364. doi: 10.1364/OE.16.009357
- Waleed, M., Hwang, S. U., Kim, J. D., Shabbir, I., Shin, S. M., and Lee, Y. G. (2013). Single-cell optoporation and transfection using femtosecond laser and optical tweezers. *Biomed. Opt. Express* 4, 1533–1547. doi: 10.1364/BOE.4.01533
- Wang, K., Milkie, D. E., Saxena, A., Engerer, P., Misgeld, T., Bronner, M. E., et al. (2014). Rapid adaptive optical recovery of optimal resolution over large volumes. *Nat. Methods* 11, 625–628. doi: 10.1038/nmeth.2925
- Williams, D. C., El Bejjani, R., Ramirez, P. M., Coakley, S., Kim, S. A., Lee, H., et al. (2013). Rapid and permanent neuronal inactivation *in vivo* via subcellular generation of reactive oxygen with the use of KillerRed. *Cell Rep.* 5, 553–563. doi: 10.1016/j.celrep.2013.09.023
- Wylie, R. G., Ahsan, S., Aizawa, Y., Maxwell, K. L., Morshead, C. M., and Shoichet, M. S. (2011). Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat. Mater.* 10, 799–806. doi: 10.1038/nmat3101

- Wylie, R. G., and Shoichet, M. S. (2011). Three-dimensional spatial patterning of proteins in hydrogels. *Biomacromolecules* 12, 3789–3796. doi: 10.1021/bm201037j
- Yang, X., Rode, D. L., Peterka, D. S., Yuste, R., and Rothman, S. M. (2012). Optical control of focal epilepsy *in vivo* with caged  $\gamma$ -aminobutyric acid. *Ann. Neurol.* 71, 6875. doi: 10.1002/ana.22596
- Yanik, M. F., Cinar, H., Cinar, H. N., Chisholm, A. D., Jin, Y., and Ben-Yakar, A. (2004). Neurosurgery: functional regeneration after laser axotomy. *Nature* 432, 822. doi: 10.1038/432822a
- Zorlutuna, P., Jeong, J. H., Kong, H., and Bashir, R. (2011). Stereolithography-based hydrogel microenvironments to examine cellular interactions. *Adv. Funct. Mater.* 21, 3642–3651. doi: 10.1002/adfm.201101023

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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