Cell Viability in Optical Tweezers: A Mini-Review

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Abstract. Optical tweezers are based on a transfer of momentum from laser photons to a transparent particle and are often applied to hold, move or manipulate single living cells. This paper discusses up to which light dose irradiance can be regarded as non-phototoxic, summarizes some possible applications, and describes experimental setups, which might fulfil these requirements. © 2022 Journal of Biomedical Photonics & Engineering.

Keywords: optical microscopy; cell viability; laser tweezers.

Paper #3521 received 6 Sep 2022; revised manuscript received 13 Dec 2022; accepted for publication 14 Dec 2022; published online 21 Dec 2022. doi: 10.18287/JBPE22.08.040510.

1 Introduction

Lasers have become indispensable tools in 3D microscopy, e.g. laser scanning microscopy [1, 2], multi-photon microscopy [3, 4], light sheet microscopy [5, 6], or super-resolution microscopy – including single molecule localization (SMLM) [7, 8], stimulated emission depletion (STED) [9] and structured illumination microscopy (SIM) [10, 11]. In addition, focused laser beams have been applied successfully for micromanipulation of cells or tissues via optical tweezers [12, 13], laser scissors [14], or laser-assisted optoporation using continuous-wave (cw) [15, 16] or pulsed light [17, 18]. Interaction is due to a sufficiently large number of photons each with an energy $W = h \times c_0/\lambda$ (with Planck's constant $h = 6.626 \times 10^{-34}$ Js and the velocity of light $c_0 = 3.00 \times 10^8$ m/s) as well as a momentum $p = h/\lambda$. In contrast to the photon energy of about 4×10^{-19} J (for $\lambda = 500$ nm), its momentum around 1.3×10^{-27} Ns is rather small. This should be considered for optical tweezers using a transfer of momentum from laser photons to a cell or tissue sample. Fig. 1 shows how a transparent particle, e.g. cell, can be moved towards the focus of a laser beam by deflection of incident photons, where it can be localized and further manipulated (in contrast, upon reflection or absorption of photons this cell would just be pushed into forward direction). Easy calculations show that for putting a pressure of 1 Pa on a surface of 1 µm² a local force of 1 pN (Pico-Newton) corresponding to about 10^{15} laser photons per second is needed, and the question arises whether the applied energy might be tolerable for living cells.

Previously we determined maximum non-phototoxic light doses, which can be applied to living cells upon whole cell illumination [19]. These doses increased with wavelength from about 25 J/cm² at 375 nm to 200 J/cm² at 633 nm (if cells were loaded with a dye or transfected with a fluorescent protein, the doses were ≤ 10 J/cm²). Corresponding photon numbers applied to native cells at $\lambda = 633$ nm were about 5×10^{17} per cm² or 5×10^9 per µm², so that the question arises again whether application of laser tweezers would be phototoxic to living cells.

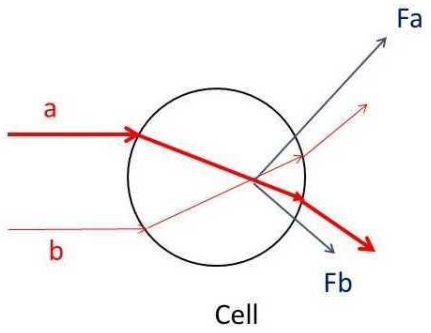

Fig. 1 Forces in an optical tweezer system. Photons a (from the center of a focused laser beam) and photons b (from a peripheral part of the laser beam) are deflected by a transparent particle, e.g. cell. If the number of photons b is smaller than that of photons a, the sum of repulsive forces F_a and F_b is directed towards the focus of the laser beam.

2 Dose and Wavelength Dependence

Applications of optical tweezers, e.g. for measuring motility forces of cells [20, 21], macromolecules [22, 23], or organelles [24], for micromanipulation of cells or chromosomes [25, 26] as well as for sperm insertion into oocytes through a previously drilled hole [27, 28] have been reported since the early 1990s. Red or near infrared laser light was considered to be most efficient for

application of a large number of laser photons without major cell damage. Cell damage was related to one-photon, but also to two-photon absorption by molecules of a cell with subsequent cytotoxic reactions at irradiances above 15–40 MW/cm² (750–1064 nm) [29]. In addition to photochemical reactions [29], photo-thermal reactions also appeared to be involved [30]. Liang et al. performed more detailed wavelength dependent studies of cell viability and found highest survival rates (percent of cells capable of clonal growth) at 800–850 nm and 950–1000 nm. Values were determined at an irradiance of 26 MW/cm² or 52 MW/cm² and an irradiation time of 3, 5, or 10 min, thus corresponding to light doses between 4.68 and 31.2 GJ/cm² [31]. Schneckenburger et al. specified light doses up to 1 GJ/cm² (8.3 MW/cm² applied within 120 s) applied around 670 nm (high power laser diode) as well as at 1064 nm (Nd:YAG laser), at which cell viability was maintained, i.e. the percentage of colony formation ("plating efficiency") was reduced by less than 10% in comparison with non-irradiated controls [32]. At these wavelengths one-photon absorption by water and most cellular pigments as well as two-photon absorption by the coenzymes nicotinamide adenine dinucleotide (NADH) and flavin mono- or dinucleotide were rather low. This proves that non-phototoxic light doses used for laser tweezers exceeded non-phototoxic light doses applied to whole cells [19] by several orders of magnitudes. Reasons for this may be an appropriate wavelength of irradiation as well as the fact that light doses applied to a tiny spot of about 1 µm diameter are much less phototoxic than the same light doses applied to whole cells.

The question arises whether cells loaded with specific pigments, e.g. hemoglobin, are more sensitive to laser treatment. This holds in particular for red blood cells, which are an object of numerous investigations with laser tweezers, e.g. in aging research [33, 34] or in studies of diseases [35–37]. So far, there is no evidence in the literature that blood cells might be more sensitive to laser irradiation than other cell species. This appears understandable, since absorption by hemoglobin is most pronounced in the blue and red spectral range, but is very low at wavelengths commonly used for laser tweezers (λ = 750–1100 nm). Furthermore, in contrast to free porphyrin molecules hemoglobin is not or only very little phototoxic.

3 Applications to Living Cells

If applications of laser tweezers to living cells are limited to a non-phototoxic light dose around 1 GJ/cm², this implies that the energy applied to a surface of 1 µm² should be restricted to 10 J, corresponding to 100 s illumination with 100 mW or 5×10^{17} photons per second (assuming a photon energy of 2×10^{-19} J at λ = 1000 nm). The photon momentum in this case is $h/\lambda = 6.5 \times 10^{-28}$ Ns, and a force $F = 5 \times 10^{17}\ s^{-1} \times 6.5 \times 10^{-28}$ Ns = 325 pN is put on the cell. Although only a minor part of this force (≤ 100 pN) can be used for light deflection in a tweezer system, the given parameters may correspond to a maximum for operation of optical tweezers under non-phototoxic conditions, e.g. for the applications summarized below. "Non-phototoxic conditions" assure survival of the majority of cells, but do not exclude any sub-lethal damage, e. g mechanical damage or delay of cell growth. In this context one should emphasize that high mechanical pressure is usually generated by ultra-short laser pulses, which are not further considered in this manuscript.

Applications of optical tweezers – partly in combination with laser scissors – include any type of micromanipulation of cells or chromosomes (for an overview see [34, 38]), measurement of adhesion forces [39–41] or deformability of cells, e.g. erythrocytes [42, 43]. Recent reviews on red blood cells describe applications in haemorheology, investigations of blood microcirculation, cell formation, maturation and erythropoiesis, often in connection with certain diseases [44, 45]. A further important issue is single cell sorting, which often occurs in combination with microfluidics or chip technologies [46–49]. An appropriate setup, where individual cells are deflected by optical tweezers, is shown in Fig. 2. For activation of cell sorting as well as for label-free analysis of single cells a combination of laser tweezers and Raman spectroscopy has been suggested [50–53]. Raman spectroscopy may be more specific than fluorescence spectroscopy [54], and an enhanced phototoxicity, which would occur upon loading of the cells with a fluorescent dye, can be excluded.

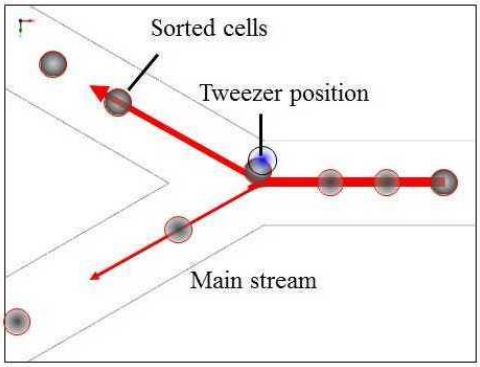

Fig. 2 Rapid cell sorting using a Nd:YAG laser at 1064 nm. Cells are deflected from a main stream by the force of an optical tweezer system (in relation to Ref. [49]).

4 Innovative Techniques

Application of optical tweezers with limited light doses needs innovative setups to perform experiments within a limited period of time. Beam deflection by galvano scanners [55], acousto-optic deflectors [56], or further kinds of spatial light modulators [57, 58] often exceeds the potential of manual operation. Holographic systems are now used increasingly for single or multiple laser

beam applications including micromanipulation, micro-patterning and cell analysis (see e. g. [59–61]).

5 Conclusion

Large photon numbers within a focused laser beam make it possible to use the small photon momenta for a transfer of forces from light to transparent particles, e.g. cells. It has been proven that living cells endure light exposures up to one or even a few Gigawatts per cm², if appropriate wavelengths in the red or near infrared spectral range are chosen. This gives us the possibility to hold, move or manipulate single living cells in various kinds of experiments. However, cell damage should always be kept in mind, and the present paper may stimulate authors to estimate possible phototoxicity during their own experiments.

Disclosures

The authors declare no conflict of interest.

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