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# New concepts in laser medicine: Towards a laser surgery with cellular precision

Gereon Hüttmann[a,*], Cuiping Yao[b,c], Elmar Endl[d]

[a]Institute of Biomedical Optics, University Lübeck, Peter-Monnik-Weg 4, D-23562 Lübeck, Germany
[b]Key Laboratory of Biomedical Information Engineering of Ministry of Education, PR China
[c]Institute of Biomedical Engineering, School of Life Science and Technology, Xi'an Jiaotong University, Xianning xi Road 28, Xi'an 710049, PR China
[d]Institute of Molecular Medicine and Experimental Immunology, University Bonn, Sigmund Freud Straße 25, D-53105 Bonn, Germany

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## Abstract

New concepts and instrumentation in laser medicine are driven by the progress in optical technology as well as by advances in the understanding of the interaction of optical irradiation with tissue, especially at a macromolecular scale, and by the changing needs in health care. Complexity and costs of laser sources will decrease due to the use of semiconductor and fiber lasers, and complex. Non-linear mechanisms by which the radiation effects tissues are better understood, especially when ultra-short laser pulses are used.

Especially femtosecond lasers and nanotechnology have the potential to treat diseases on a cellular level. Focused femtosecond irradiation was successfully used to manipulate tissues with subcellular precision. Laser-irradiated nanoparticles can selectively destroy individual cells.




## Introduction

Since their first use in medicine more than 40 years ago, lasers have found a certain place in therapy and diagnosis [1]. Especially in ophthalmology different types of laser are now used routinely for coagulation, photodynamic therapy (PDT) and imaging of the retina. The possibility to visualize and manipulate structure within the eye with very high resolution makes lasers in this field a unique tool.

In dermatology, lasers have found their place for the treatment of vascular malformations [2], the destruction of unwanted hair [3], and the so-called skin rejuvenation [4], the selective altering of epidermal and dermal structures, which shall improve the visual appearance of skin.

In surgery, lasers are used because of their ability to cut and/or coagulate tissue in a precise way. Although in this field there is a strong competition of electrosurgery, $CO_2$, Holmium, Nd:YAG and semiconductor lasers have also found application in this field [1].

The main disadvantages of laser technology are the high costs and complexity of the devices. Therefore, lasers can only compete with conventional technologies when they offer a real benefit for the therapy, which cannot be realized with other technologies or when the treatment is more cost effective. Today, the market for

*Corresponding author.
E-mail address: huettmann3@freenet.de (G. Hüttmann).

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medical laser systems, which amounts to about 2 Billion $, is mainly based on ophthalmologic laser systems, and on relatively few medical procedures like hair removal, skin rejuvenation, refractive surgery and therapy of benign prostate hyperplasia (BPH) with large number of patients [5].

As costs for laser devices go down, more medical applications will be found. Semiconductor lasers, which are rugged und comparably inexpensive, have already replaced solid-state laser in several applications, which require tissue coagulation or the excitation of a photodynamic active substance. However, semiconductor lasers are still limited to cw operation in the blue and the red/infrared spectral range. Recent advances in fiber technology offer the possibility to build fiber laser, which will operate at different wavelengths in a continuous or pulsed way. These lasers are compact, robust, efficient and offer an excellent beam profile. IR fiber lasers at 1.9 μm and at 2.7 μm were already tested for medical applications [6,7]. The advantages of fiber lasers compared to electrosurgery are contact-free application, predictable effects in a depth range, which depends on the wavelength used, and no interference with NMR, since metal parts or electric currents can be avoided. Miniaturized instruments, which combine diagnosis and therapy, can easily be built using lasers and other optical technologies.

The unique advantage of lasers and optical technologies are the possibility of a high precision, contact free manipulation of tissues and an easy combination with imaging and other diagnostic procedures. In the past, there was a steady trend for a higher precision of laser treatments. Starting with simple cutting and coagulation, today, pulsed irradiation below 200 nm or around 3 μm, which are both strongly absorbed by the tissue, is used clinically for a controlled removal of tissue layers with micrometer precision (Fig. 1). This microprocessing of tissue is an application for laser, which is without competition from other technologies. Future applications of lasers may lie in the manipulation of single cells in tissues, which would make lasers a unique instrument in the hands of the physician.

## Laser therapies with cellular and subcellular precision

Cellular and subcellular precisions can be achieved with a strongly focused laser beam or by using a selective absorption at the target structure (Fig. 2). If a femtosecond laser beam is focused to a micron-sized spot, high precision manipulation of cells is possible even within the tissue [8]. An extremely high irradiance can be reached in the focal spot, which destroys cells or subcellular structures by plasma formation or multiphoton absorption-induced photochemical reactions [9]. Modifications of intracellular structures were demonstrated in vitro with single cells. Perforation of membranes, cutting for filaments and destruction of cell organelles were possible in living tissue without side-effects [10]. Applications for these techniques are found today in biological research, where technical limitations of this approach, i.e. the limited depth of the accessible volume and the limited speed of processing, pose no severe restrictions. Clinical application is till to date restricted to the preparation of corneal flaps for refractive surgery, where focused femtosecond laser pulses are used for an intrastromal cutting of the cornea [11]. Modification of the lens by laser incisions with femtosecond irradiation is currently investigated in a preclinical stage for a treatment of presbyopia.

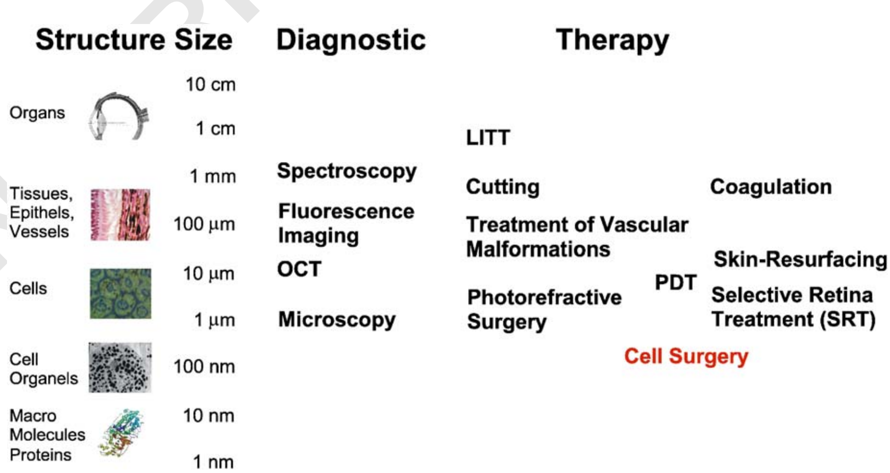


**Fig. 1.** Precision and resolution of different laser procedures for treatment or diagnosis. Today lasers can span the range from centimeters down to a few micrometers. With femtosecond laser and nanoparticles submicrometer precision for the modification of biological structures was demonstrated in vitro.

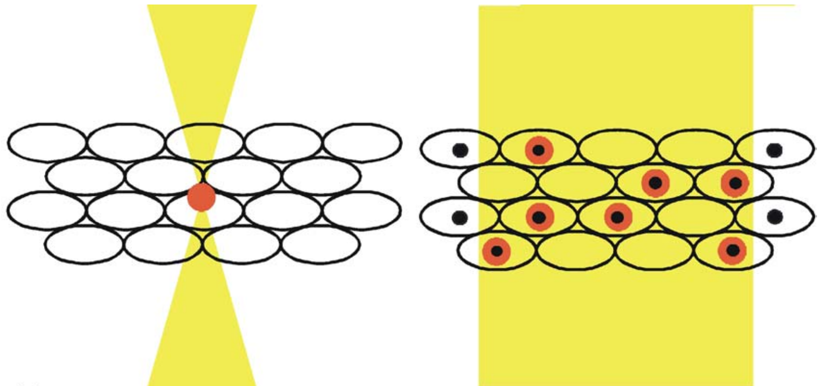


**Fig. 2.** The two approaches for cell surgery with submicrometer precision: (a) focused irradiation with ultrashort pulses and (b) laser irradiated micro- or nanoabsorber.

A selective absorption at target sites, which is necessary for the second approach of cell surgery, may be caused be endogenous chromophores (e.g. melanin), or by exogenous chromophores (dyes, nanoparticles). Photodynamic therapy relies on this principle using photochemical active dyes. Nanoparticles offer a significantly higher absorption than dye molecules, which can lead to very high temperatures in the vicinity of the particles. The absorbing nanoparticles can be brought to the target structure by antibodies or other selectively binding biomolecules [12]. When the pulse width of the irradiation is short enough, thermal and thermomechanical effects on the tissue can be restricted to the vicinity of the particles. With nano- or picosecond pulses the principle of selective thermolysis [13], which is already used clinically to destroy selectively blood vessels, can be extended to a submicrometer scale.

In preclinical work, the selective destruction of cells and proteins as well as a selective permeabilization of the plasma membrane by laser-irradiated gold nanoparticles was shown [14,15]. For example, lymphoma cells with gold nanoparticles bound to their plasma membrane were selectively destroyed in mixed cell cultures (Fig. 3). Possible application in research and biotechnology are selective protein inactivation as an alternative to chromophore assisted laser inactivation (CALI) of proteins or a negative depletion of in cell or tissue culture. A selective targeting of immune or tumor cells in the blood stream seems to be feasible. In preclinical work, laser-irradiated gold nanoparticles were used for targeting lymphoma [16] and breast tumor cells. Irradiating strongly red absorbing nanoparticles with cw irradiation was successfully used to destroy macroscopic tumors in animals although a real cellular precision is not possible due to heat diffusion [17].

Clinical trials are underway for a laser therapy with cellular selectivity, which uses the high absorption of endogenous melanin in pigmented cells of the eye. By irradiation of the retina with microsecond pulses at a wavelength around 500 nm the cells of the retinal pigment epithelium (RPE) can be destroyed without damage to the photoreceptor layer or the underlying choroidia. The melanin granules in the RPE cell selectively absorb the irradiation, and due to the short pulse width the conduction of heat can be restricted to the vicinity of the cells. This selective retinal treatment (SRT) with a cellular precision SRT is expected to improve diseases, which are based on a malfunction of the PRE cells. In ongoing clinical studies SRT is used to treat soft drusen, retinopathia centralis serosa (RCS), macular edema, and branch vein occlusion.

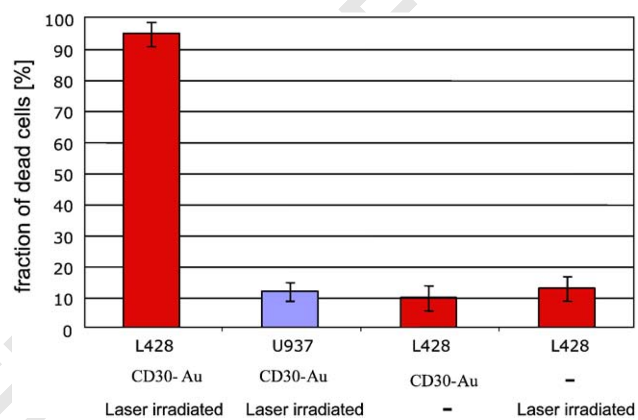


**Fig. 3.** Selective destruction of cells by laser-irradiated nanoparticles, which were bound to the plasma membrane by antibodies. A mixed cell culture of CD30 positive cells (L428) and CD30 negative cells (U937) was incubation with anti-CD30 gold conjugates and irradiated with nanosecond laser pulses at 532 nm. Only the L428 cells were affected by the irradiation.

Focused femtosecond irradiation and irradiated nanoparticles, have their specific advantages and disadvantages. Cell surgery with focused pulses needs to steer the beam to the target structure, which has to be known for it exact position. In scattering tissues the depth of the effects is limited to a few hundred micrometers, because the irradiation in the focus decreases rapidly with depth. Also, it is technologically challenging and time consuming to treat larger areas, in which the focused beam has to be scanned with a high precision. Nanoparticle cell surgery enables a simultaneous treatment at different sites without an individual aiming to the target structure and is, therefore, especially useful for cell surgery in tissues. Additionally the spatial position of the target is not known. A drawback is that the particles have to be functionalized with selectively binding molecules and the particles have to reach the target structure by active or passive transport. Therefore the lack of selectively binding biochemical structures and diffusion barriers can impede a selective targeting. Toxicity of nanoparticles may also limit in vivo applications.

## Conclusion and outlook

After more than 40 years, lasers have found their place in medical diagnosis and therapy. As optical technology evolves, lasers will find many more applications. In future, smart instruments, which combine different functions, can be realized with the help of lasers and modern optics. Optical diagnosis by imaging, in vivo microscopy and spectroscopy can be combined with laser procedures. The same fibers and optics can be used for diagnosis and treatment. For example, a smart laser scalpel could detect blood vessels or different types of tissues.

Recent developments in optical technologies, especially femtosecond lasers, and nanotechnology open a new door for therapies with cellular and subcellular selectivity. Lasers may be used to influence cell function. In this field, lasers will compete more with pharmaceuticals and drugs than with classical medical technology. In contrast to drugs, laser-based cell surgery allows one to control the treatment area spatially and temporally with very high precision.

One drawback for cell surgery with focused irradiation is the complex and expensive technology. But this may change in future. Femtosecond lasers, which are needed for cell surgery, can now be built in fiber technology. Microstructured fibers were developed which can transmit the high peak power of femtosecond pulses. All these developments were made mainly for information technology, telecommunication and laser material processing. Optics for scanning volumes in three dimensions with a focused beam is being developed for optical data storage. Laser cell surgery will profit in future from developments in these areas and as the market for these devices grows prices will come down.

The advances in nanotechnology will provide nanoparticles with tailored optical and biological properties. Nanoshells [18] and quantum dots [19] are examples how absorption and emission of particles can be chosen according to special requirement.

The aging population in the industrialized countries will pose new challenges for medicine. The importance of minimal invasive therapies will increase. Especially degenerative and tumor diseases will increase. Early diagnosis of cancer will confront the physician with more patients with tumors in early stages. Hence selective minimal invasive microtherapies will increasingly be used in future. Laser and optical technologies are expected to play an important role in the development of medical technology for microtherapies.

## Zusammenfassung

Neue Konzepte und Geräte im Bereich der Lasermedizin werden durch die Fortschritte in den optischen Technologien, dem zunehmenden Verständnis der Wirkung von Licht auf Gewebe, besonders auch in makromolekularen Dimensionen, und den sich verändernden Anforderungen an die Medizin entstehen. Komplexität und Kosten von Lasergeräten werden sinken, vor allem auch durch die Fortschritte bei Halbleiterlasern und Lichtleitern. Das Verständnis komplexer nichtlineare Wirkungen von Licht auf Gewebe, speziell bei ultrakurzen Pulsen, wächst beständig.

Zelluläre Therapien basierend auf Femosekundenlaser und Nanotechnologie haben die Chance völlig neue diagnostische und therapeutische Verfahren auf zellulärer Ebene zu ermöglichen.


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