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The safe use of lasers in biomedicine: Principles of laser-matter interaction

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Abstract

Optical radiation sources, and in particular lasers, find an ever-increasing number of applications in the medical field. It is essential that personnel who are in the presence of an optical radiation source, whether operator, patient or researcher, know precisely the risks inherent in the exposure of the human body to radiation. In order to reduce the risk of biological damage, beyond the provisions of the law on safety regulations, the precise information and accurate preparation of personnel are the main guarantee for the correct use of these sources. In all the application fields, the possibility of a biological damage cannot be completely eliminated, assuming the connotation of occupational risks. In order to understand the risks and operate their effective mitigation, the basic knowledge of the fundamental concepts at the basis of laser-matter interaction will be presented and discussed, with a focus on the physical parameters needed to efficiently estimate and mitigate the related occupational risks, in both a laboratory and clinical context.

Keywords

Laser safety, laser-tissue interaction, light transport in tissues, scattering and absorption in tissues, laser-associated risks, photoinduced effects

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Significance statement

The use of artificial light sources and in particular lasers is growing rapidly in the healthcare environment for both diagnostic and therapeutic applications. Although light is considered a non-hazardous physical agent for organs other than the eye, it is very important to know how light interacts with biological tissues and to understand its undesirable effects. The precise information and accurate preparation of personnel are the main guarantee for the correct use of these sources.

Introduction

Management of safety issues[1] associated with the use of optical radiation in medicine has increasingly caught the attention of both the medical and physical counterparts, not to mention specific legislation in the field. In this respect, the scientific principles are to be found in the physics of light-matter interaction from two complementary sides: (i) how radiation exerts its therapeutic/diagnostic action; (ii) how the same radiation can contemporarily be a source of risk by interacting with both the operator and the patient. In this scheme, radiation interaction with both biological matter (the operator, the patient) and inanimate matter must be considered. In fact, it is clear that undesired laser reflection by the surgeon's scalpel may constitute a risk for the eyes and the skin, and the same could be invoked for reflected/diffused light by other specular and/or opaque surfaces such as the flooring, the wall, the surgeon's coat etc. The effects associated to undesired light-tissue interaction can relate for example, to excessive local heating, burns, temporary or permanent sight impairment, tissue ablation. Of course, light can interact with

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clothes, furnishings and furniture, and can constitute a possible fire hazard depending on the specific conditions.

Laser-matter interaction can be described by different approaches, ultimately relying on the interaction between photons and the atoms/molecules forming the specific encountered medium. Following a brief description of the relevant physical quantities and photon-matter interaction in the UV-visible and near infrared range, we will first consider the phenomena at the basis of laser-matter interaction (reflection, refraction, absorption, scattering, transmission), then illustrate the light-induced effects in terms of photo-thermal, photo-chemical and photo–mechanical effects. In both cases, the dependence of the considered phenomenon on both the light and matter properties will be considered.

In most phenomena related to laser-matter interaction in the biomedical field, extreme spectral purity (monochromaticity) is not a must-have property. This is associated with an increasing use of non-directional and/or non-coherent radiation whose spectrum can be relatively broad (e.g. FWHM of a few tens of nanometres). In a first instance and to a good approximation, those sources can be included in the following considerations by associating them with their peak wavelength.

Let us briefly illustrate some fundamental concepts at the basis of optical radiation interaction with the elementary constituents of organic matter. As is well known, the description of the structure of matter at the microscopic level is a very complex problem, which requires the concepts of quantum mechanics. It is possible to give a very qualitative picture of the physical phenomena affecting safety problems by using extremely simplified model situations which, however, maintain the salient elements of the characteristics of the processes in which we are concerned.

Primary constituents of matter are atoms and their aggregates, such as molecules, metals, semiconductors, etc. Quantum mechanics tells us that an isolated atom can only absorb precise discrete values of energy, differently from the classical mechanics interpretation where every system can take any possible amount of energy. These discrete energy levels define different atomic configurations, that is, different arrangement of electrons around the atomic nucleus. The separation between two consecutive energy levels is not constant but decreases as the energy of the atom increases. A similar description is also valid for molecules, while in the case of an atomic aggregate such as metals or semiconductors, the complex interaction among the various atoms compacts and groups the energy levels in a way to form bands. Inside each band, the energy levels are distributed continuously, but different bands can be spaced, thus defining an energy interval without allowed energy levels called ‘energy gap’.

To understand the light-induced effects, let us start by describing the fundamental optical properties needed to understand the propagation of optical radiation in tissues, as well as inanimate matter, such as absorption, transmission, reflection and diffusion.

Mechanisms of optical radiation-matter interaction

Biomedical optical applications rely on light sources that are generally based on lasers or on Light-Emitting Diodes (LEDs). Table 1 briefly resumes the definition of the most relevant physical quantities used to characterise the light sources and their interaction with matter, in the context of both clinical applications and for the management of the possible safety issues associated with their use.

Light can behave as a wave (i.e. electromagnetic radiation) or as a particle, called *photon*. Although these two descriptions are very different, they are consistent with each other. The electromagnetic radiation is classified according to the wavelength. The most relevant regions for biomedical applications are: the ultraviolet (UV-A: 315–400 nm, UV-B: 280–315 nm, UV-C: 100–280 nm), the visible (approx. 400–780 nm) and the near-infrared (780–2500 nm). If we consider a laser beam characterised by wavelength λ (or frequency $\nu = c/\lambda$), the energy associated to a single photon is $E_{ph} = hc/\lambda = h\nu$, where c is the light speed in vacuum and h is Planck’s constant ($h \sim 6.6 \cdot 10^{-34}$ J s). The energy is measured in Joules (J) and it only depends on the light wavelength. As a consequence, E_{ph} does not change with the laser power or intensity that instead takes into account the number of emitted photons N in a defined time span Δt. The emitted energy Q_e associated to the whole beam is therefore $Q_e = N \cdot E_{ph}$. Since the photons are emitted in the time span Δt, the laser radiant power is $P = Q_e / \Delta t$ and it is expressed in Watts (W): 1W=1J/1s. It is worth noticing that in photomedicine (as well as in photochemistry and photophysics), 1 mol of photons is also defined, corresponding to one Einstein (Ei) and referring to the photon being one of the reaction’s reagents (1Ei $\sim 6.0 \cdot 10^{23}$ photons). Relevant in medical applications is the irradiance, generally referred to by the symbol E, defined by the amount of energy received per unit time and unit area by a given surface, projected to be normal to the incident radiation. Sometimes this quantity is referred to as *intensity*, even if this term should be avoided in the context of radiometry, as it can be confused either with the luminous or radiant intensity, which refer to different quantities. In many practical applications, the term *beam intensity* refers to the beam energy crossing the unit surface per unit time at any given depth in the tissue, being therefore measured in Watt/m^2 or other homogeneous units. In this sense, the intensity corresponds to the energy fluence per unit surface at any given depth. It is worth remembering that in phototherapy applications both with visible and UV light[3,4] the term ‘light dose’ is used, corresponding to the product between the beam irradiance

Table 1. Definition of the most relevant physical quantities used to characterise the light sources and their interaction with matter.

Physical quantity	Symbol	Unit	Definition
Wavelength	λ	nm or μm	Electromagnetic radiation is a wave of electromagnetic field that propagates in space. The electromagnetic radiation is classified according to the wavelength.
Radiant power	P	W [J/s]	Energy per unit time emitted by a given source or impinging on a given surface
Emitted energy	Q_e	J	Energy emitted in a given time span Δt by a source. It can be obtained as a time integration of $P(t)$.
Radiant Exposure	H_e	J/m^2 or J/cm^2	Energy that directly reaches a surface of unit area (scattering or diffusion effects in the target environment are not considered).
Light dose	D	J/m^2 or J/cm^2	Used in therapeutic applications as a synonym of radiant exposure and corresponding to the product between the irradiance and the irradiation time
Irradiance or energy fluence	E	W/m^2 or W/cm^2	Amount of energy delivered per unit time and per unit area on a given surface projected to be normal to the incident radiation.
Beam intensity	I	W/m^2 or W/cm^2	Used as a synonym of irradiance or energy fluence in non-radiometric contexts (see text).
Beam diameter, radius and beam waist	D and ω	mm or μm	Used to measure the diameter of a laser or light beam at a given position. Since a beam typically does not have sharp edges, the diameter can be defined in many different ways. Starting from the distribution curve of the beam's intensity along a predefined axis passing through the beam's centre, which is also usually its point of maximum intensity, and perpendicular to its direction of propagation, the more used definitions of beam diameter (twice the radius) are: - ***FWHM*** (Full Width at Half Maximum): it corresponds to the distance between the two points closest to the peak that have 50% of the maximum irradiance or intensity; - ***I/e^2***: same as before but with the percentage value of 27% These two definitions are calculated from the intensity distribution along one predefined axis, neither of them considers the overall beam profile. For this reason, sometimes the beam diameter can be defined through: - ***D4σ*** (Second Moment Width): 4 times the standard deviation of the distribution of intensity along the beam major and minor axis (see reference 2 for a more accurate definition). This definition is recommended by the ISO international standard[2] for measuring beam diameter.

Each quantity is described and provided with its more widespread symbol and unit.

and time (J/m^2 or, mostly used, J/cm^2) at the tissue surface level.[5] If we suppose that a laser beam hits a tissue orthogonally to a surface of area S, the irradiance is $E = P / S$, usually expressed in W/cm^2. For example, let us consider a P =1,5 W laser emitting at λ = 1064 nm, focalised into a 5 mm diameter circular spot. The irradiance is about 7,6 W/cm^2. If the same power would be concentrated into a 1 mm diameter spot, the irradiance would increase to about 190 W/cm^2, as the spot area is now 25 times smaller than before. As described later, different photo-induced effects in a given tissue can be obtained by modulating both the irradiance and the exposure time.

Let us consider a typical situation, in which a beam of light impinges on a slice of matter (biological tissue, plastic sample, fabric, etc. . .), as depicted in Figure 1. There are essentially four effects that govern the light transport: reflection and refraction, scattering and absorption. The first three take place whenever the propagating radiation encounters a discontinuity in the index of refraction (defined as the ratio between the speed of light in vacuum and the speed of light in the medium). In biomedical laser application, reflection and refraction play a significant role only when very transparent media are irradiated, such as the corneal tissue. In opaque media, they are subtle effects while absorption and scattering are the dominant ones.

Absorption

Photon absorption processes are ultimately responsible for the therapeutic effects of light. The study of light absorption by biological media is also very important in the framework of safety issues (for both the operator and the patient),

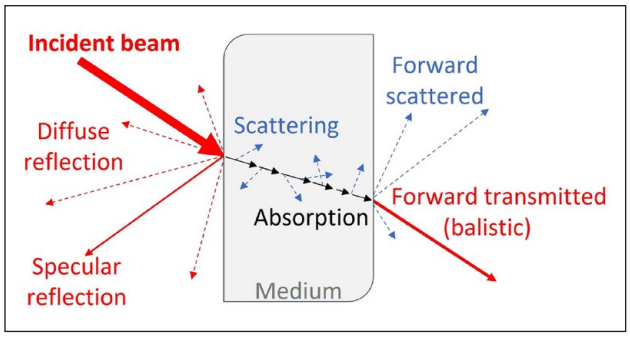

Figure 1. Scheme of the effects governing light transport in a medium.

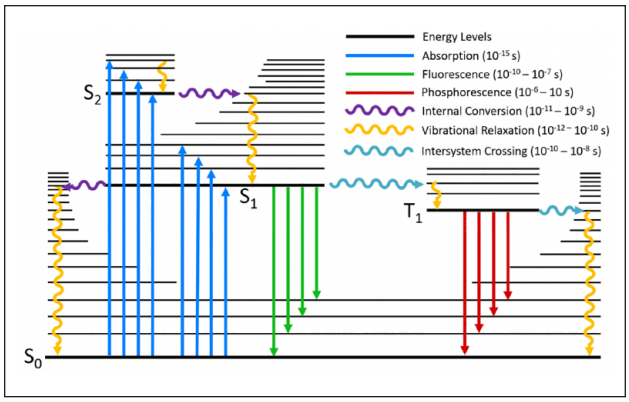

Figure 2. A typical Jablonski diagram showing the possible radiative and non-radiative transitions following a photo-excitation of a molecule (pointing-up arrows). Different energy levels of the molecules are depicted by the horizontal lines (electronic and vibrational states depicted with thick and thin lines respectively), while the arrows represent the various transitions that can happen between different layers: straight and wavy arrows indicate radiative and non-radiative transitions respectively. The label S_n and T_n stands for singlet and triplet state (referred to the basis of net spin quantum operator), while the subscript n indicates the n-th excited state (0 stands for ground state). For the different transitions, the typical timescale is also indicated. Source: Edinburgh Instruments www.edinst.com.

together with the knowledge of the scattering processes. In general, absorption can be characterised by micro- and macroscopic parameters, depending on the properties of both the impinging light and the encountered tissue.

The absorption process is the phenomenon in which atoms and molecules, interacting with an electromagnetic radiation, absorb some energy from the field and convert it into internal energy, which can be described in terms of energy levels. If we consider a molecule in a well-defined energy level E_i, it can increase its internal energy by absorbing a photon of energy $h\nu$ from the electromagnetic field and reach a higher energy level E_f with energy $E_f = E_i + h\nu$, associated with an excited state of the molecule.

Excited molecules tend to release the absorbed energy and return to the initial state, via a so-called *relaxation process*. The relaxation process may be accompanied with radiation emission (fluorescence or phosphorescence) or by non-radiative relaxation processes. These processes can occur with direct transition from the excited state towards a lower energy level, or with a series of intermediate transitions in which the released energy is transferred more gradually to the surrounding medium, generally in the form of heat. The excited molecule can also use its energy to rearrange its bond orbitals, resulting in chemical reactions that can end up in new stable molecular species. In this case we speak of a photochemical channel. A typical structure of energy levels for polyatomic molecules is indicated in Figure 2, where the most important relaxation processes are depicted.

At the microscopic level, absorption is described by the absorption cross section σ_a [cm^2], that is related to the macroscopic quantity represented by the absorption coefficient μ_a [cm^{-1}] through the formula:

$$\mu_a = N\sigma_a \tag{1}$$

where N [cm^{-3}] is the number of absorbent molecules per unit volume. Therefore, μ_a describes the probability of absorption per unit pathlength. In the simple case of a single absorbing molecular species with molar concentration C [mol/l], we have:

$$\mu_a = C\epsilon \tag{2}$$

where ϵ is called the molar extinction coefficient [cm^2/mol] and represents the intrinsic absorbing properties of a given species. In a biological tissue, characterised by a large variety of molecular species, the total μ_a^{tot} contains all the contributions from the different absorbing molecules ($\mu_{a,i}$) being it a weighted mean of the extinction coefficients of the various absorbers over their respective concentrations:

$$\mu_a^{tot} = \sum_i \mu_{a,i} = \sum_i C_i \varepsilon_i \tag{3}$$

The absorption coefficient is a fundamental parameter to describe the optical properties of a material because it is wavelength dependent, thus very useful when comparing absorption from different lasers.

In reference to Figure 3, let us consider a collimated radiation field of intensity I_0 impinging orthogonally onto a purely absorbing medium of absorption coefficient μ_a. For any layer of infinitesimal thickness dx of encountered material, located at coordinate x and illuminated with an intensity $I(x)$, the change in intensity dI of the beam exiting the layer is:

$$dI = -\mu_a I(x)\, dx \tag{4}$$

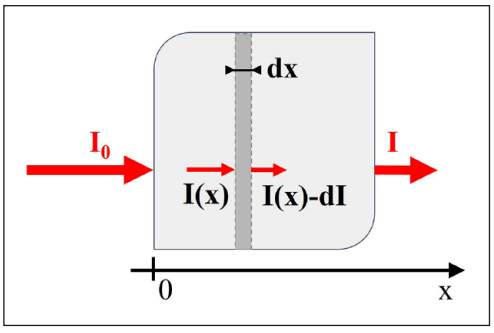

Figure 3. Illustration of light absorption by a purely absorption medium (in grey). I_0 is the incoming beam intensity, I is the (forward) transmitted intensity.

Integrating the previous equation from the surface of the material, assumed to be at $x = 0$, to a general position x, the well-known Beer-Lambert's law is obtained:

$$I(x) = I_0 exp(-\mu_a x) \quad \text{where } I_0 = I(x = 0) \tag{5}$$

describing the exponential attenuation of a light beam propagating inside a purely absorbing medium. A very useful quantity used in the biomedical and chemical field is the absorbance A, defined as follow:

$$A = log_{10}(I_0 / I) = \epsilon CL \tag{6}$$

where L is the thickness of the specific medium considered. For practical purposes (including e.g. the correct choice of laser protection glasses), it is useful to remember the correspondence between the A value and the percentage for both the absorbed and transmitted intensities (Table 2). The quantity defined in equation (6) is also found as A_{10}. Sometimes the absorbance can be found in terms of natural logarithm defined as $A_e = log_e(I_0 / I) = ln(I_0 / I)$. In the case of a purely absorbing medium of thickness L and absorption coefficient μ_a, it can easily be found that:

$$A_{10} = ln10 \cdot A_e = ln10 \cdot \epsilon CL = ln10 \cdot \mu_a L \tag{7}$$

For the sake of completeness, when the number of photons per unit time and volume investing the medium exceeds a given threshold, nonlinear phenomena can arise, such as two-photon absorption or upconversion processes, that are not considered in equation (5). These multiphoton processes are only possible with the use of very powerful and short laser pulses, with increasing applications in the field of bioimaging and nanomedicine,[6,7] especially associated with the use of externally-delivered nanoparticles.

Scattering

Scattering in tissues is the result of light interacting with random variations in refractive index (whose average in tissue lies in the range 1.36–1.40, and 1.46 in adipose tissues). Considering a biological sample, the membrane, organelles and the other intracellular structures are characterised by a larger index of refraction if compared to the intracellular liquid embedding them. A similar relation is also valid for the extracellular structures and proteins with respect to the extracellular fluids. The overall result of this heterogeneity ends up in the scattering effect.

From a physical point of view, scattering can be studied using a simple model where scattering particles have a characteristic dimension represented by a radius R, as depicted in Figure 4.

On encountering one scattering particle within a homogeneous medium, photons travelling in a direction s are scattered into a new direction s'. The angle between them is called scattering angle θ. A more interesting parameter is represented by the anisotropy factor g, defined as the average scattering angle $g = < cos\theta >$. Let us consider two limiting cases: (i) $R << \lambda$: for very small particles the value of g approaches 0, indicating a nearly isotropic scattering; (ii) $R >> \lambda$: g tends to 1 and the scattering is peaked in the forward direction. Most tissue have a high g value in the range 0.7–0.97, meaning that the scattering is very forward-peaked. Moreover, in sufficiently thick samples (i.e. over 10–100 µm in most tissues), multiple scattering effects become significant, as we will see later in the text.

Table 2. Relations between the absorbance A_{10} and A_e value and the correspective absorbed and transmitted intensity expressed in percentage of the incident laser intensity.

A_{10}	A_e	% absorbed intensity	% transmitted intensity
0	0	0	100%
0.05	~0.02	10%	90%
0.3	~0.13	50%	50%
1	~0.43	90%	10%
2	~0.86	99%	1%
3	~1.30	99.9%	0.1%

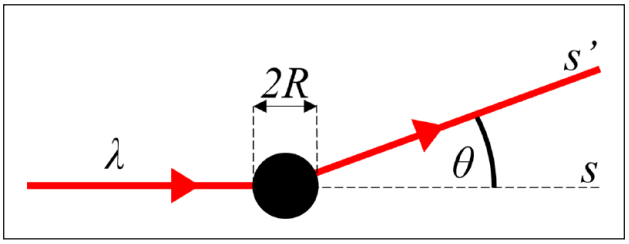

Figure 4. A scattering event occurs when an incident photon, travelling in the direction s, hits a scattering particle that deviate the photon trajectory into a new direction s'. In the figure we have indicated the photon wavelength λ, the characteristic dimension of the particle with its radius R and the scattering angle θ.

Laser penetration in tissues

The behaviour of a light beam propagating inside a tissue mainly depends on λ and on the specific tissue type, being described in terms of absorption and scattering. We now want to merge these two effects to define a single model describing light penetration.

Let us consider a collimated laser beam impinging on a tissue surface in the case of a pure absorbing medium. This hypothesis of absence of scattering may look like a brutal simplification for biological tissues, even if it will be possible to include the scattering effect by a proper modification of this simple model. This assumption allows us to start from the Beer-Lambert law derived in the previous section (see equation (5)), that describes the light intensity at various depths in the medium. If we introduce a new parameter called 'penetration depth' or 'extinction length' L_a (usually measured in mm or um) defined as:

$$L_a = 1/\mu_a \tag{8}$$

Equation (5) can be written as:

$$I(x) = I_0 exp(-x/L_a) \tag{9}$$

The penetration depth L_a indicates the depth increase (in the tissue or medium) where the light intensity is reduced by a factor e (or at about 36%) with respect to any depth x. This is notably applicable for light penetration with respect to the intensity at the skin level, if external in-air irradiation is performed.

It is important to note that the definition of L_a is independent both from the laser intensity I_0 and laser power P_0, being it dependent only on λ via the wavelength dependence of μ_a. This is valid both for continuous and pulsed lasers (exception made for the presence of nonlinear processes) since the Beer-Lambert law does not depend on the laser pulse duration. In a practical application, in order to calculate L_a, we simply need the proper value of μ_a at the specific laser wavelength λ for the specific tissue type.

It is worth noticing that L_a is often misinterpreted as the maximum penetration length of light in a given material or tissue, wrongly assuming that $I = 0$ for $x > L_a$. This could be possibly considered as a rough 'rule of thumb' when comparing the effects of different laser sources, but nothing more than that.

Up to now we have considered purely absorbing media, but for a comprehensive description of the light-tissue interaction, we need to merge absorption with scattering. If we now add scattering properties to the medium, both absorption and scattering will contribute to deplete the beam of photons. We can calculate the amount of unscattered light intensity I_{ns} (also called coherent component, meaning photons that have not experienced any scattering event) at any position x inside the medium as:

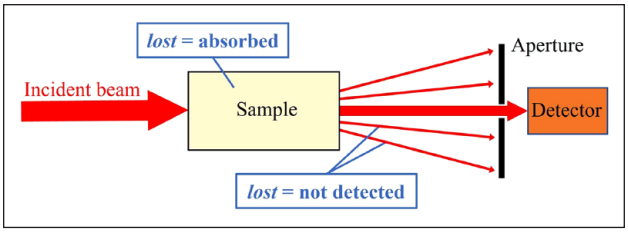

Figure 5. Example of a simple setup used to measure the unscattered light intensity, also called coherent or ballistic component, that arise from the propagation of a collimated light beam through an absorbing and weakly scattering media. The usage of an aperture allows for the detection of only the unscattered light.

$$I_{ns}(x) \quad I_0 e^{-(\mu_a+\mu_s)x} = I_0 e^{-\mu_t x} \tag{10}$$

where $\mu_t = \mu_a + \mu_s$ is called the 'total attenuation coefficient'. A practical way to measure I_{ns} is to illuminate a slab of tissue with collimated light and use an aperture to shield the scattered light, thus measuring only the unscattered one. In such a setup, knowing the slab thickness and μ_a, it is possible to estimate the scattering coefficient μ_s, as shown in Figure 5. In many practical cases, light intensity measurements intrinsically select the forward component without the use of any aperture (e.g. due to the limited acceptance angle of the detector and /or the associated optical elements), in accordance with equation (10) and the setup shown in Figure 5.

The situation becomes more complicated if we want to determine the overall intensity of light at a given depth inside a medium, considering both the unscattered and the scattered light, besides the absorbed one. Media where the scattered light component is non-negligible respect to the unscattered one are called turbid media. The complexity in turbid media is introduced by the presence of multiple scattering events. The medium can be considered as a group of absorbing and scattering centres whose random interactions with the photons are governed by quantitative coefficients. The Boltzmann transport model establishes the right physical approach to solve a similar problem. Without entering mathematical details, we consider only two biologically relevant limiting cases: dominant absorption and dominant scattering.

In the absorption-dominant limit $\mu_a >> \mu_s$ (see Figure 6(a)), the unscattered light field is dominant with respect to the scattered one, resulting in a much lower penetration depth. In this case the forward-directed diffusive light intensity follows an equation similar to equation (10), thus confirming the physical meaning of the total attenuation coefficient. The equation describing the light scattered intensity towards different directions (not forward) has a more complex structure that can be found in specific text-books.[8,9]

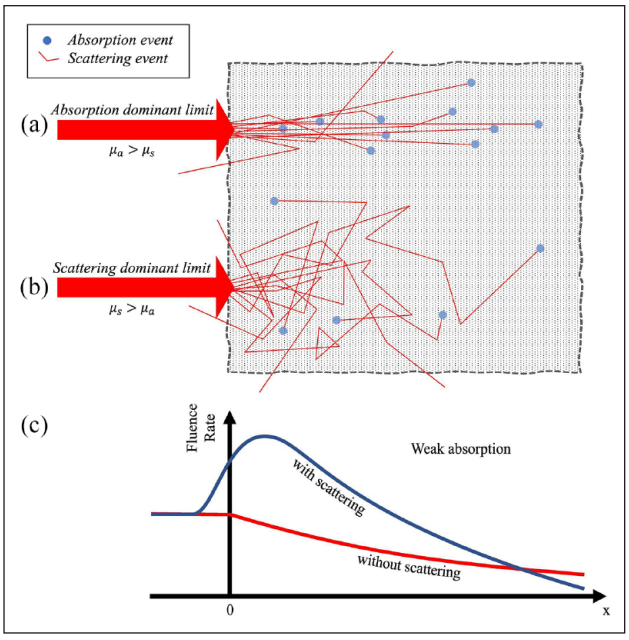

Figure 6. Schematic illustration of a laser-tissue interaction in the absorption (a) and scattering (b) dominant limit. The big arrow represents the laser that impinges on a turbid medium (dot-filled grey space). The thin lines represent the possible photon paths where the single scattering or absorption events are illustrated. (c) Represents the fluence rate, in case of weak absorption, showing the difference between scattering and not scattering medium.

In the scattering-dominant limit (also known as diffusion limit) photons undergo many scattering events before being absorbed, which results in a much greater path before being absorbed (see Figure 6(b)). In such a scenario it is convenient to introduce the reduced scattering coefficient μ_s' defined as:

$$\mu_s' = \mu_s(1-g) \tag{11}$$

Its physical meaning is clear considering its inverse $1/\mu_s'$ that represents the distance over which the photon loses information about its original direction of propagation. The scattering-dominant limit can be identified with $\mu_s' >> \mu_a$. Under this condition, the transport equation can be simplified to the diffusion equation allowing the intensity of the diffused field to be written as

$$I_{diff}(x) \quad I_0 e^{-\mu_{eff} x} \; with \;\; \mu_{eff} = \sqrt{3\mu_a(\mu_s' + \mu_a)} \tag{12}$$

where x is the coordinate along the incident beam direction. The parameter μ_{eff} represents the 'effective attenuation coefficient' and it is a key parameter in biomedical applications, where the condition $\mu_s' >> \mu_a$ is often verified. Through μ_{eff} is defined the optical penetration depth $L_D = 1/\mu_{eff}$ (or diffusion depth) that indicates the length scale over which the optical energy attenuates in tissue. Table 3 reports the L_D parameter in human skin for the most broadly employed biomedical lasers, while Figure 7 reports it for the case of human mucosa and skin in the 400–2000 nm range. For a more complete list of the optical properties of most relevant tissue, such as scattering coefficients and refractive index, please refer to the literature.[8–10]

It is clear that an increase in μ_s leads to an increase in μ_{eff} and a decrease in L_D. This is only a part of the story. As, in fact, scattering is characterised by a change in propagation direction (see Figures 4 and 6(b)), it will also contribute to enlarging the beam dimensions while the beam penetrates in the tissue. As a consequence, the mean beam energy fluence per unit time across a given section rapidly decreases (see Figure 6(c)). This is accompanied by an increase in the volume of tissue affected by the radiation and, in general, a decrease in the light power transferred per unit volume. These effects must be carefully evaluated when considering the risks associated with undesired laser interaction or when planning a laser-based clinical application in order to provide an effective treatment.

Even if it is clear that the penetration depth does not change with laser intensity, it is worth remembering that a higher impinging intensity results in a higher energy fluence at any depth in the tissue, once the irradiation time (i.e. the treatment) is fixed. Vice versa, a higher energy fluence can be obtained in deep tissue layers by increasing the interaction or treatment time, once the laser intensity (at the first tissue layer) is fixed. These are non-trivial considerations, as many light-matter interaction phenomena depend upon the absorbed energy, which in turn is a function of the energy fluence arriving at a given depth. For example, in the case of photo-chemically induced production of cyto-toxic species (like ROS, Reactive Oxygen Species), a threshold can be defined and associated with irreversible effects, leading to cell death like in the case of photodynamic therapy. This threshold can be expressed in terms of total number of produced ROS per cell; alternatively, it can be expressed in terms of number of absorbed photons and ultimately of light dose. Therefore, it is clear that any deep target can in principle be reached by an over-the-threshold dose. In this scheme many drawbacks can be present, related to possible tissue overheating in the more superficial layers or other undesired effects.

Photo-induced effects

When laser light interacts with biological tissues, a variety of mechanisms take place that depend on both the specific characteristics of the tissue and the parameters of the laser emission. In the following, we will focus on non-radiative processes, discarding radiative processes due to their negligible importance with regard to laser safety, being of interest mainly for diagnostic purposes.

Non-radiative processes can be classified according to a chart (Figure 8) which relates them to the laser irradiance E at the tissue surface level and the interaction/exposure time τ_{laser}. E can be either calculated from the laser

Table 3. Penetration depth L_D values for light of different wavelengths in human skin for the most broadly employed biomedical lasers.[11,12]

Laser	λ [nm]	L_D [mm]	Laser	λ [nm]	L_D [mm]
Excimer ArF	193	1E-6	He:Ne	632	1.704
Excimer XeCl	308	4.0E-5	Ti:Sapphire	650	1.754
Nitrogen	337	1.5E-4	Ruby	694	1.987
Argon	488	0.8	Alexandrite	754	2.170
	514	0.949	Diode	808	2.326
KTP	532	0.915	Diode	820	2.334
Nd YAG 2x	534	0.915	Diode	904	2.520
PDL	576	1.140	Diode	980	2.532
Gold	628	1.655	Nd YAG	1064	3.390
He:Ne	632	1.704	Ho YAG	2100	0.400
PDL	576	1.140	Erbium	2940	(1–3)E-6
Gold	628	1.655	CO_2	10600	(20–30)E-6

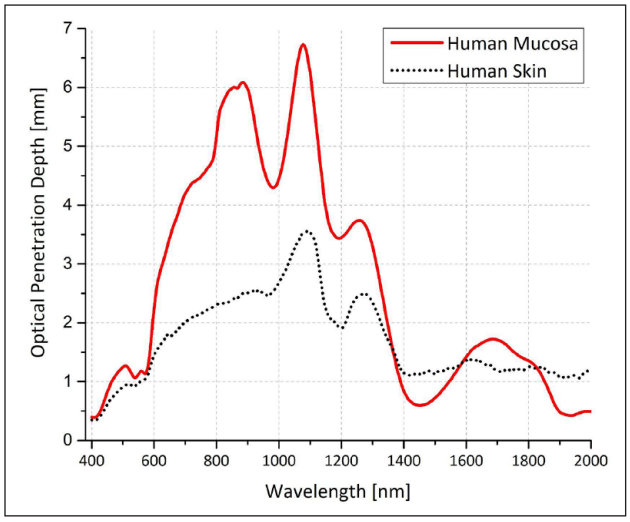

Figure 7. Optical penetration depth of light into human mucosa and skin over the wavelength range from 400 to 2000 nm. Data adapted from.[13]

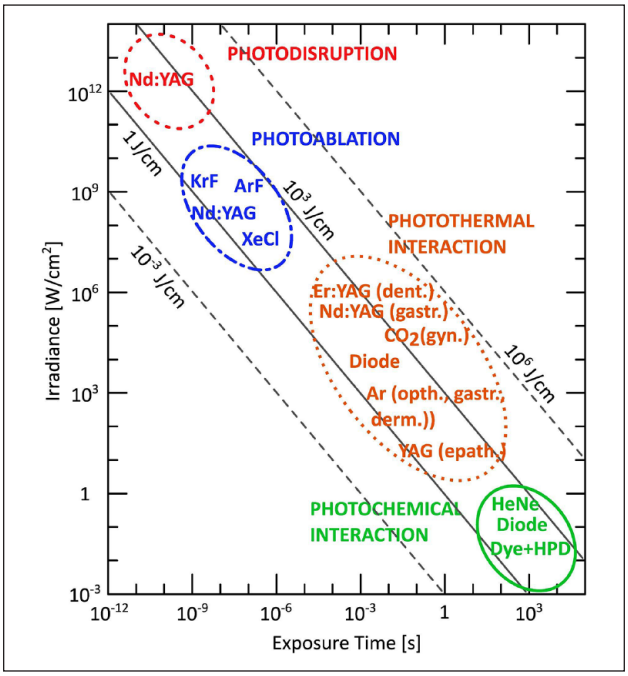

Figure 8. Map of photo-induced effects according to exposure time and light source irradiance.

emission characteristics, possibly modified by reflections, beam expansion in air or the presence of other tissues, or measured directly with a power metre. It must be noted that a correct measurement of E needs a method for the estimation of the spot area, which in turn may need the use of appropriate tools. Among them, apart from simple solutions like calibres or rulers, or more advanced methods such as the Knife-edge techniques,[14] light-sensitive films are worth considering. Among them, photochromic films like Gafchromic™, can be used also in the UV-visible field, with up to about 50 µm resolution.[15] Unfortunately, new models (e.g. EBT3) are poorly sensitive to the visible spectrum, still maintaining a good response in some UV regions.[16] Concerning τ_{laser}, operator reasonable estimation could be sufficient to locate the main expected effect(s) according to Figure 8.

Once E and τ_{laser} are known, their product can be calculated, corresponding to the energy fluence [J/m^2], which is constant along the lines in the E - τ_{laser} graph, once log scales are used (Figure 8).

According to Figure 8, there are three main photo-induced effects: photochemical, photothermal and photomechanical, according to increasing values for the energy fluence.

Photochemical effects

In general, photochemical processes occur for much lower power and longer excitation time than those needed to obtain photothermal and photomechanical effects (see chart in Figure 8).

Photochemical effects can be modelled by chemical reactions where the photon is one of the reagents, leading to one or more products upon photon absorption. These reactions represent the first step in a series of complex processes, eventually leading to a biological response and accompanied by possible damage in the exposed tissue(s). In fact, the photoproducts formed upon photon absorption by the target chromophore(s) elicit reactions which can directly or indirectly alter a cellular or enzymatic or biochemical function, such as in the case associated with the photo-induced production of reactive oxygen species (ROS). For example, solar erythema (sunburns) is a common and well-known photo-induced effect in the case of solar UV exposure of the skin, based on photochemical processes. Drug-induced photosensitivity (photoallergy and phototoxicity) are other undesired effects associated with photochemical processes elicited by the presence of both light and light-absorbing drugs, especially if systemically administered. As reported in *Kowalska et al.*,[17] 'photoexcitation and photoconversion of drugs trigger multidirectional biological reactions, including oxidative stress, inflammation, and changes in melanin synthesis'.

Among the various photochemical processes (see Figure 9), the ones of greatest interest for safety issues are molecular photodissociation, photo isomerisation, inter- and intramolecular energy transfer. Examples of photochemical effects are UV-induced synthesis of vitamins or DNA and cellular damage. In the visible range, the eye (and in particular the retina) can be damaged starting from reasonably low laser irradiance, with a threshold which can be far lower than the one causing skin damage. A different damage induced by visible laser radiation can occur on the skin of a patient who is being treated with exogenous photosensitising products. In fact, a patient undergoing a photo-dermatological treatment for a skin pathology, may be administered drugs which increase the photosensitivity of the region to be treated prior to irradiation. This is the case of the Photodynamic Therapy (PDT) approach,[18] where exogenous light absorbers (called 'photosensitisers' – PS) are delivered in situ and irradiated with a laser whose emission matches the PS absorption spectrum. In these cases, undesired and possible PS distribution inside the healthy tissues can occur and its activation must be properly limited by avoiding irradiation outside the area under treatment.

The skin is the target organ for various visible and UV light phototherapy treatments having a strongly wavelength-dependent effect that generally causes inflammation.[19,20] During this process, the DNA is one of the most important targets of UV light, having an absorption maximum at about 260 nm. Wavelengths in the 240–300 nm range interact mainly with nucleic acids, amino acids, urocanic acid and melanin, which is the chromophore acting as a shield for radiation between 300 and 400 nm. The amount and location of chromophores in the different skin layers, as well as variations in the thickness of the epidermis and stratum corneum, determine the degree to which UV radiation is absorbed.

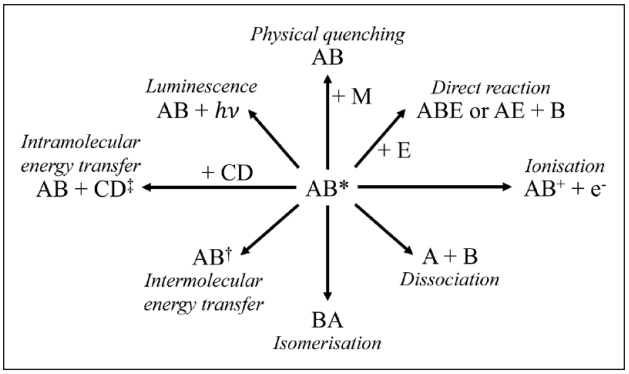

Figure 9. Chart of the photochemical reactions following photon absorption by the molecular species AB, being AB* the excited species.

Recently, partly as a result of the COVID 19 pandemic, there has been much increased use of UV radiation below 300 nm for sterilisation of airborne pathogens, with particular interest for $\lambda < 240$ nm . This radiation does not occur naturally at the ground level due to the filtering effect of the atmosphere. Artificial sources such as excimer lamps and newly designed LEDs are currently used. UVC radiation has recently been rehabilitated from a safety point of view, mainly due to its high absorption by the keratin present in the stratum corneum of the skin and its very small penetration length (≈a few μm) both in the skin and the cornea.[21]

Photothermal interaction

The conversion of optical energy into thermal energy is at the basis of many laser applications, both in the biomedical and industrial fields. In this case, the photon energy is deposited into the target tissue via energy transfer towards rotational and vibration modes of the target molecules. This increases the molecular mean kinetic energy with the results of a local temperature increase.

The photothermal reaction responsible for temperature increase in a biological tissue can be described according to the following two-stage process:

1. $A + h\nu \rightarrow A^*$: the incident photon is absorbed by the target molecule A, promoted to an excited state A*
2. inelastic collision of A* with a molecule M of the medium transfers the excess energy ΔE of A* towards M

To analyse the photothermal effects we must consider three steps: heat generation, heat transport and heat effects.

Heat generation is determined by the laser parameters (mainly irradiance and exposure time) and the tissue

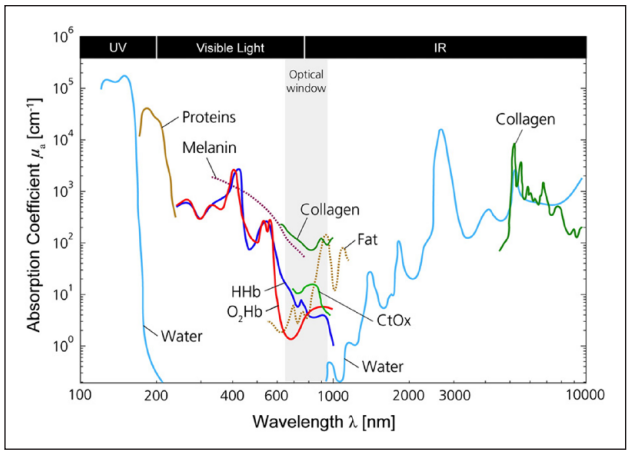

Figure 10. Absorption spectra of the main body pigments and water in the 100 nm – 10 μm range. Image from.[22]

optical parameters, such as the absorption coefficient, the thermal conductivity, the mass density and the specific heat. The magnitude of the biological effects of heat are largely controlled by the target molecule(s) absorption coefficient as depicted in Figure 10: water, emo proteins, pigments (such as melanin, carotenoids, flavin, bilirubin etc.), other macromolecules (e.g. nucleic acids and aromatic molecules) and possibly nanoparticles, whose de-excitation path(s) may be optimised to perform localised photothermal therapy.

From the theory of heat diffusion,[23] in a time t heat travels over a distance L obtained by the formula:

$$L = \sqrt{4\alpha t} \tag{13}$$

where α represents the thermal diffusivity of the material [cm^2/s], dependent in turn on thermal conductivity, specific heat and density. The reader is reminded that the factor 4 is valid for a 2D geometry (as in the case of thermal diffusion from axial heating along a collimated laser beam), while for the 3D case a factor 6 must be used. For liquid water $\alpha = 1.43 \times 10^{-3}$ cm^2/s: in a second heat diffuses for a length of 0.8 mm. Similarly, the diffusion time associated with a blood vessel of 10 μm is of the order of 180 μs, while for a vessel of 100 μm it rises to about 18 ms. If we equal the value of L to the light penetration depth in the tissue ($1/\mu_a$), a parameter called *time of thermal relaxation* $\tau_{th.rel.}$ is defined:

$$\tau_{th.rel.} = 1/(4\alpha\mu_a{}^2) \tag{14}$$

corresponding to the time needed for heat to propagate along a distance equal to the light penetration length. Equivalently, $\tau_{th.rel.}$ is the time during which the heat remains confined in a thickness equal to the light penetration length. The subsequent cooling (thermal relaxation) is influenced by the thermal coefficients of the tissue, the properties of the surrounding tissue or fluids, and the differential temperature between the irradiated and unirradiated tissue. At different laser intensities and pulse duration we will experience different effects such as coagulation, carbonisation, vaporisation and melting of the tissue. Two main cases can be distinguished:

$$(1)\ \tau_{laser} < \tau_{th.rel.}$$

This corresponds to the heat confinement regime: heat remains confined into a volume $V = SL$ where S is the area of the irradiated surface and L the diffusion length. The sharp increase in temperature and the relative thermal damage will be localised in this volume, while the surrounding tissues will undergo a much more modest heating as a result of the subsequent thermal diffusion. This result is exploited for example in dermatology to produce well-localised damage to pigmented structures without appreciably altering the surrounding healthy tissues (selective photo thermolysis).

$$(2)\ \tau_{laser} > \tau_{th.rel.}$$

In this case, heat has the opportunity to diffuse in the tissue over lengths greater than the optical penetration. Thermal damage of the tissue adjacent to the volume affected by the radiation is therefore possible.

These parameters are very important for both therapeutic applications and safety issues, as they can give an estimate of the tissue volume damaged by a light-induced temperature increase. See Table 4 for a list of thermal relaxation times for some biological tissue relative to the more widespread biomedical-laser sources.

The heating of the biological tissue in the range 37°C–42°C has no measurable effects. The hyperthermic regimen (42°C–50°C) is accompanied by changes in macromolecular conformation, destruction of molecular bonds and alterations of the cell membrane with consequent tissue retraction. Cell mortality via apoptosis is also observed, in particular for cells of oncological type. In the coagulation regimen (50°C–60°C), enzymatic activities are modified and reduced, while macromolecule denaturation begins; this is the basis of the coagulation process. Of particular interest is heat-induced denaturation of collagen. This transition is accompanied by a noticeable structural contraction of collagen fibres and by a visible change in the absorption and scattering properties, such as for thermal denaturation of the egg white. Vaporisation starts at around 100 °C, mainly due to the heating of the water contained in the tissues. The transformation of water into steam produces a great increase in volume, accompanied by cell wall explosive rupture and escape of steam. The high vaporisation heat of water (about 2300 J/g) is responsible for excess heat dissipation by vapour formation and escape, preventing further temperature increase. After

Table 4. Thermal relaxation time (s) for different biological media and laser wavelengths.

	KrF (248 nm)	Ar (514 nm)	He-Ne (633 nm)	Nd:YAG (1064 nm)	CO_2 (10.6 μm)
Water				2×10^{3}	12×10^{-5}
Human dermis (in vitro)		0.12	0.2	0.78	
Blood thrombus (clot)	0.2×10^{-3}	12×10^{-3}	1.7	2.4	4.3×10^{-3}
Fibrous plaque		0.17	3.4	21	4.3×10^{-3}
Vascular wall		0.48	6.7	30	

Table 5. Photothermal effects of laser-tissue interaction as function of the tissue temperature.

42°C –45°C	Protein structural changes, hydrogen bond breaking, retraction
45°C –50°C	Enzyme inactivation, changes in membrane permeabilisation, oedema
50°C –60°C	Coagulation, protein denaturation
~ 80°C	Collagen denaturation
80°C –100°C	Dehydration
>100 °C	Boiling, steaming
100°C –300°C	Vaporisation, tissue ablation
>300 °C	Carbonisation

Table 6. The commonly used medical continuous wave (cw) and pulsed lasers.

cw laser type and emitted power		pulsed laser type and pulse duration	
HeNe @ 633 nm	100 mW	Alexandrite @ 720–800 nm	100 μs
KTP/Nd: YAG @ 532 nm	1–10 W	KTP/Nd: YAG @ 532 nm	100 ns–250 μs
Nd:YAG @ 1064 nm	100 W	ND:YAG @ 1064 nm	30–100 ps
CO_2 @ 10.6 μm	100 W	CO_2 @ 10.6 μm	100 ns–1 ms

complete water evaporation, the residual tissue fragments undergo a rapid increase in temperature until reaching 300–400 ° C, when the tissue blackens and carbonises producing gas and smoke. If the temperature exceeds 500 °C, the tissue burns and evaporates. All these thermic regimens are summarised in Table 5.

Photomechanical interaction

When the duration of the laser pulse is less than microseconds, in general, photomechanical effects develop alongside processes of a purely thermal nature. These manifest themselves as pressure pulses which propagate both in the area in front of the irradiated surface and in the tissue itself. When the pulses are of short duration of the order of a nanosecond or even better than a picosecond, the mechanical shock waves that are generated can damage the very structure of the medium in which the wave propagates, especially if this is biological matter.[24,25] This phenomenon can also be used for medical purposes, such as laser lithotripsy,[26] intravascular treatment[27] such as laser angioplasty and endovenous laser ablation, dermatological applications such as skin rejuvenation[28] or even cosmetic purposes such as the removal of tattoos.

In some of these applications, laser light is delivered intra-operatively while in others the laser beam is propagating in the air before reaching the target organ (e.g. the skin). This should be considered when analysing safety issues, being it clear that the basic safety principles in the use of optical radiation sources must be followed in any case.

Practical considerations

To further clarify the most relevant photo-induced effects and connect them with biomedicine applications, let us consider Table 6. This table reports some of the most employed continuous (cw) and pulsed biomedical lasers, together with some important characteristics such as the radiant power for the cw lasers and the pulse duration for pulsed ones. According to Figure 8, a proper combination of the irradiance and exposure-time allow to initiate a specific photo-induced effect on the target tissue. Once the laser operation regime has been chosen between cw or pulsed, as well as its power, the irradiance can be modified by a proper optical system (e.g. optical lenses) that modifies the laser beam diameter, further expanding or focalising it. We recall that the irradiance is increased by a factor 10 if the same power is delivered onto a circular surface with one-third smaller diameter. In this respect, an often underestimated risk consists in the potential production of hazardous airborne contaminants obtained during the laser-tissue interaction. Laser induced pyrolytic products of biological samples can contain toxic by-products among which known carcinogens.[29] The operating environment must be properly ventilated to reduce worker and patient exposition risk.

Conclusions

All the light-matter interaction phenomena are intrinsically dependent on both λ and the tissue optical properties. Therefore, very different physical and biological effects

can be obtained by different lasers irradiating the same tissue type or, vice versa, by the same laser irradiating different tissue types.

Light interaction with biological matter can be described with a sequence of distinct events, which can be grouped into two main phases: damage induction and damage response. The first is completed in a time span of the order of seconds and includes the processes described above. In general, the ray propagation model can be invoked for a first understanding of the possible sources and/or conditions of danger while using laser beams in a biomedical environment. This can apply for example to the identification of reflective surfaces to be kept far from the laser beam, or estimate the beam dimensions at any distance from the source from its divergence. At the same time, the laws describing laser absorption and scattering inside a given tissue or medium drive the knowledge of the main effect(s) associated to their interaction (photochemical, photothermal, photomechanical). For example, in the absence of suitable chromophores able to selectively increase the extinction coefficient of the biological material (e.g. tattoo pigments, melanin), the effects of thermal increase have poor specificity, as in the case of infrared light absorption by water. On the contrary, processes based on photochemical reactions are highly specific and strongly dependent on the radiation wavelength.

Out of the knowledge of laser – biological matter interaction principles, a list of questions may arise and be applied to the specific working conditions, for analysis of the possible risks and the relative countermeasures: (i) which are the laser emission characteristics and the relevant tissue type(s)? (ii) which is the interaction geometry? are non-negligible reflections to be expected (which is the surface(s) type(s) and quality?) (iii) are there known absorbers? Which is their depth into the tissue? How is this related to the laser penetration depth and the expected photo-induced effects? (iv) Which emission and/or geometry parameters can be controlled/changed to maximise treatment efficacy and safety?

Together with the increasing therapeutic use of laser radiation, new and more complex issues arise regarding their safety of use, not only for the operator but also for the patient. For example, in intraoperative use of lasers[30,31] internal organs become direct light targets, being the surrounding tissues/organs part of the possible indirect/undesired targets. In the last years, an increasing number of solutions have been developed to reach 'difficult' districts by light, such as the bladder, prostate, stomach, lungs, throat etc. mainly in the development of alternative and/or coadjuvant antitumoral therapies. At the same time, new ways to deliver light are being developed and commercialised, consisting in for example, diffusive optical fibres, modified gastroscopes or bronchoscopes, intracutaneous needles, ingestible or even inhalable sources.[32]

Together with the research advancement in terms of light sources, treatment planning is rapidly evolving in the optical field, including sophisticated modelling of the interaction between light and the target organ(s) to predict light distribution in space and time, together with the analysis of the relevant photo-induced effects. This makes this field exciting but introduces new challenges from the safety point of view. For these reasons, the knowledge of light-matter interaction principles will be more and more necessary in the years to come.

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