



Is there a measure for low power laser dose?

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Abstract

Low power lasers have been used successfully for treatment of many diseases in soft and bone tissues. Basic and clinical researches have developed quickly being the scientific basis to therapeutic protocols based on these lasers. However, there are difficulties to compare experimental and clinical results obtained from different researchers because a complicated and intricate list of physical and biological parameters should be checked before the irradiation procedures as well as part of these parameters are omitted or inaccurately reported. This review focuses on the physical and biological parameters proposed to make experimental and clinical protocols accurate and reproducible as well as suggests dose parameters based on biological effects induced by low power lasers. A variety of parameters are reported by different authors and the number of parameter suggested could overcome three dozens. Thus, laser dose and laser dose equivalent are defined based on laser-induced biological effects and suggested as simplified dose parameters for low power lasers. These parameters could simplify and be useful to researchers and clinicians, permitting comparisons and decreasing mistakes and inaccuracies when laser-induced effects are evaluated and compared with those obtained in previous studies. The laser dose and laser dose equivalent could contribute significantly to improve accuracy, effectiveness, and safety of clinical protocols based on low power lasers.

Keywords Dose · Energy · Fluence · Power density · Laser · Spot size

Introduction

Low power lasers have been used successfully for treatment of many diseases in soft and bone tissues [1–6]. The so-called low power laser therapy (or low-level laser therapy), as a vibrant area, both to basic as to clinical research, has developed quickly and researchers have produced a number of scientific papers, which have appeared in topic-specific and devoted journals. In this large number of papers, important experimental and clinical results allowing the therapeutic protocols based on low power lasers to be improved, as well other therapeutic protocols be proposed, have been described. However, since its

beginning, the research on low power laser therapy presents difficulties to compare experimental and clinical results obtained from different researchers. This issue has been related, at least in part, to imprecision and omission of physical parameters of laser devices or irradiation procedures [7–11]. A lot of biological parameters have been claimed to be important for accurate and/or adequate description of the irradiation procedure, making things more complicated. Sometimes, both parameter groups (physical and biological) constitute a complicated and intricate list to be checked before starting an experimental or clinical procedure. The checking of all these parameters is considered necessary because radiations in the so-called therapeutic window (600–1100 nm) present complex interactions with biological tissues [12]. Thus, it would be interesting that, for experimental and mainly clinical research, simpler and quicker parameters could be used. These parameters (few parameters, if possible) could make comparison of researches easier and more available, as well as therapeutic protocols more accurate and reproducible. Before those, what are the necessary proposed irradiation parameters to ensure that laser therapy and experimental low power laser irradiation are well done?

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Physical and biological parameters

Since Endre Mester described for the first time the biostimulation effect (or photobiomodulation, as actually it is called) [13], researchers had in their minds that physical and biological irradiation parameters influence the laser-induced effects. Table 1 lists some studies dedicated to describe these parameters. In fact, in the 90s, researchers working low power therapeutic lasers proposed that the conditions of laser irradiation must be adjusted to permit obtained experimental and clinical results to be compared and evaluated by other researchers [7]. In those days, eight physical parameters were considered important to characterize the so-called laser exposure: (i) irradiance (or power density), (ii) radiant exposure (or fluence), (iii) irradiance diameter or effective area irradiated, (iv) exposure duration, (v) wavelength or wavelength band or radiation source, (vi) time between exposures for multiple exposures, (vii) number of exposures, and (viii) total cumulative radiant exposure. Also, it was suggested in the same study that, in most of cases of low power laser biostimulation, only three measurements need to be made: radiant energy incident on the tissue, effective area irradiated, and exposure duration.

Some years after, another study was carried out to verify the reasons to negative studies on low power laser therapy [8]. The authors cited wavelength, power, power at tissue, type of pulsing, pulse frequency, power density, dose, treatment technique, and treatment intervals as physical parameters that must be scrutinized strictly.

The lack or incomplete reporting of treatment parameters was considered as a problem in clinical trials based on low power lasers [14]. Irradiance (or power density), energy density (or fluence), duration of each treatment session, frequency (number of times treatment was done per week), as well as the cumulative dose given (individual doses multiplied by the number of treatment sessions) were suggested as necessary to permit an objective comparison between the studies [15].

In another paper, radiant power, radiant energy, power density, energy density, and wavelength were listed as the five basic physical parameters that influence the treatment. Beam spot size, size of the lesion, modes of treatment (contact, non-contact and scanning), total dose, frequency of treatment, and dose per treatment session were reported as factors that influence the dose, while pathology, etiology, optical density, nature of the target tissue, depth of the target tissue, skin pigmentation, and open versus closed lesion were pointed as patient-related factors [16].

Mean power and energy density were the physical parameters evaluated to characterize low power laser therapy and the physical therapy clinical procedures in physical therapy [18].

Other researchers proposed a reference document for non-physicist researchers conducting low power laser therapy, laboratory studies, and clinical trials to help design and report the beam and dose aspects of their trials [10]. In this paper, the

authors provided a checklist to help researchers understand and report all the necessary parameters for a repeatable scientific study involving low power therapeutic lasers, where the eight most important beam parameters to report would be wavelength, power, irradiation time, beam area, pulse parameters, anatomical location, number of treatments, and interval between treatments. The authors suggested also that coherence, application technique (contact, projection, scanning, pressure), beam profile, and spectral width may be important.

Beside the necessity to report these parameters, it was suggested that is important to confirm the equipment specifications (not only by device guidelines), to measure the parameters accurately, to compare them across treatment groups when possible, and to report their individual effects on the outcome measure [17].

More recently, ten key parameters were suggested necessary to be reported in wavelength, power, irradiation time, beam area (at the skin or culture surface; this is not necessarily the same size as the aperture), radiant energy, radiant exposure (or fluence), pulse parameters, number of treatments, interval between treatments, and anatomical location [11].

Also recently, it was suggested that, due to the complexity of the light parameters other details could be included, as polarization, shape, profile, divergence of the beam, waveform, as well as frequency, pulse duration, and duty cycle (for switched/pulsed beams) [12].

It was pinpointed that the misunderstanding on physical parameters in studies based on low power therapeutic lasers leads researchers to neglect or misreport radiometric data, affecting the repeatability and reliability of these studies, as well efficacy of treatments [11]. This could be caused by researchers, who develop studies based on low power therapeutic lasers, who have no background in physics. Thus, including a physicist or an appropriately skilled engineer in the research team is necessary [11, 12].

How has laser dose been considered?

Low power laser dose in therapy and experimental research has been subject of controversy. There are studies where dose is presented as energy, fluence (or radiant exposure), as well as power density (or irradiance) (Table 2).

Also, laser dose is not presented as a parameter per se but a set of physical parameters, as power density, fluence, effective area irradiated, intensity profile of the beam, exposure duration, the wavelength or wavelength distribution, the total number of exposures, the time between exposures, and the total fluence [7]. In fact, in some studies, it has been suggested that a set of physical parameters should be made known to permit other researchers to repeat or to compare their results with the results from other researchers [10–12, 16, 17].

Table 1 Summary of studies about physical and biological parameters used to characterize low power therapeutic lasers

First author	Title	Physical	Biological	Total	Reference
Stuck BE, 1993 [7]	Measuring and reporting physical parameters in laser biomodulation research	Irradiance, radiant exposure, irradiance diameter or the effective area irradiated, exposure duration, wavelength or wavelength band or radiation source, time between exposures for multiple exposures, number of exposures, total cumulative radiant exposure	None	8	<i>Proc SPIE</i> . 1883, 21 (1993).
Tunér J, 1998 [8]	It's all in the parameters: a critical analysis of some well-known negative studies on low-level laser therapy	Wavelength, power, power at tissue, type of pulsing, pulse frequency, power density, dose, treatment technique, treatment intervals	Inclusion criteria, tissue condition	11	<i>J Clin Laser Med Surg</i> . 1998 16:245–248.
WALT, 2006 [14]	Standards for the design and conduct of systematic reviews with low-level laser therapy for musculoskeletal pain disorders	Dose, dose interval	Site of pathology, the nerve supplying the painful and/or paralyzed area, the acupuncture or trigger points, other sufficiently described locations	5	<i>Photomed Laser Surg</i> . 24, 759–760.
Enwemeka CS, 2008 [15]	Standard parameters in laser phototherapy	Power output, the wavelength, description of the source (solid state, gas, laser diode, light-emitting diode), shape, size, type of treatment applicator, irradiance or power density, dose in the form of energy density or fluence, duration of each treatment session, frequency of treatment, cumulative dose	None	11	<i>Photomed Laser Surg</i> . 26, 411.
Enwemeka CS, 2009 [16]	Intricacies of dose in laser phototherapy for tissue repair and pain relief	Radiant power, radiant energy, power density, energy density, wavelength, beam spot size, size of lesion, mode of treatment (contact, noncontact, or scanning), frequency of treatment, dose per treatment, cumulative dose, tissue optical density	Etiology, pathology, depth of target tissue, and skin pigmentation	16	<i>Photomed Laser Surg</i> . 2009 27:387–393.
Enwemeka CS, 2011 [17]	The relevance of accurate comprehensive treatment parameters in photobiomodulation	Wavelength, pulse frequency, power, power density, energy, energy density, total energy, total cumulative dose, number of treatments	None	9	<i>Photomed Laser Surg</i> . 29:783–784.
Jenkins PA, 2011 [10]	How to report low-level laser therapy (LLLT)/photomedicine dose and beam parameters in clinical and laboratory studies	Irradiation parameters: center wavelength, spectral bandwidth, operating mode, frequency, pulse on duration, pulse off duration or duty cycle, energy per pulse, peak radiant power, average radiant power, polarization, aperture diameter, irradiance at aperture, beam divergence, beam shape, beam profile Treatment parameters: beam spot size at target, irradiance at target, exposure duration, radiant exposure, radiant energy, number of points irradiated, area irradiated, application technique, number and frequency of treatment sessions, total radiant energy	Pathology, etiology, type of lesion, anatomical location, depth of the target tissue, skin pigmentation, overall condition of the patient	33	<i>Photomed Laser Surg</i> . 2011; 29:785–787.
Tunér J, 2016 [12]	Parameter reproducibility in photobiomodulation	Center wavelength, spectral bandwidth, operating mode, radiant power, aperture diameter, irradiance at aperture, beam spot size at target, irradiance at target, exposure duration, radiant exposure, radiant energy, number of points irradiated, area irradiated, application technique, number and frequency of treatment sessions, total radiant energy, polarization, shape, profile, divergence of the beam, frequency, pulse duration, duty cycle, waveform	None	23	<i>Photomed Laser Surg</i> . 2016 Mar;34(3):91–2.
Hadis MA, 2016 [11]	The dark art of light measurement: accurate radiometry for low-level light therapy	Light properties and irradiation: wavelength, irradiance, beam area, energy power, beam area, irradiance, pulse frequency, radiant energy, radiant exposure, Spectral quantities: peak wavelength, spectral irradiance, spectral half-width and absorption profiles	None	13 ^a	<i>Lasers Med Sci</i> . 2016 31:789–809.

^a Repeated parameters were counted one time only

What are the concerns with these doses?

Energy (Eq. 1) and fluence (radiant exposure, Eq. 2) could not be available physical parameters because the equal energies and fluences can be obtained at different powers, meaning that time of irradiations were different (Figs. 1 and 2). Also, equal fluences can be achieved with laser beams with different spot sizes. From these considerations, power density (irradiance, Eq. 3) was considered the most critical physical parameter in an experimental procedure or treatment based on low power lasers [16] (Fig. 3).

$$E = \sum_{i=1}^{i=n} E_i \quad (1)$$

$$F = \frac{E}{A} \quad (2)$$

$$P_d = \frac{E}{\Delta t \times A} = \frac{P}{A} \quad (3)$$

On the other hand, the energy considered as laser dose is the output energy, which means the energy transported by laser beam. Despite the laser source is just on the biological system (tissue or cell culture), part of the laser beam will not interact with the molecules into this biological system because part of the laser beam will be reflected at the surface and it will not reach the biological target, as well as part of the laser beam energy could not be absorbed (when irradiating a cell suspension or a cell monolayer, for example), being transmitted through the biological system. Also, part of the laser beam will be scattered along of the laser beam pathway and not necessarily absorbed. Thus, the laser beam energy will be different of the energy absorbed by the biological system and, more strictly, laser beam energy will be numerically different of the energy absorbed or transferred to photoacceptors, which are the specific molecules (or part of molecules) that absorb laser energy and involved in the laser-induced effects (photobiomodulation effect, for example).

If fluence is used to represent the laser dose, again the laser beam energy would be considered as the energy absorbed by the photoacceptors involved in the laser-induced biological effects. Also, calculus of fluence considers the energy delivered, which is not equal to the energy absorbed by the irradiated area due to light reflection. Another issue is that the biological effect does not occur at the tissue surface only. In fact, in most situations, laser-induced effects are expected to occur into tissues, below the skin surface, mainly for infrared lasers.

The issue continues if power density is considered to express the laser dose because this parameter is defined by laser beam energy, time of irradiation, and beam spot size.

In fact, considering these parameters, laser dose is not defined strictly, since the energy absorbed by photoacceptors is not quantified or not strictly specified. It is expected that, by

the first law of photobiology, light must be absorbed for photochemistry (or photophysics) to occur [47]. This laser dose, here considered, is related to laser-induced biological effect. For example, if wound healing is the expected laser-induced effect, it is expected that this effect comes from the energy absorbed by specific photoacceptors (cytochrome c oxidase, for example).

What are the photoacceptors for low power lasers?

A chromophore is a molecule or part of this molecule that is able to absorb the energy of an incident radiation photon. For radiations emitted by low power lasers, photoacceptors were proposed to differentiate from photoreceptors, which is normally related to molecules sensitive to light in retina, bacteria, and plant cells, while photoacceptor is a molecule that takes part in a metabolic reaction, which is not related to response to radiation absorption [48].

After Endre Mester reports the biostimulatory effect induced by low power lasers, researches were interested on identification the photoacceptor (or photoacceptors) involved in this effect. Despite hemoglobin, melanin, porphyrin, and some flavoproteins are capable to absorb radiations in the therapeutic window for low power lasers; these molecules are not considered as responsible for the photobiomodulation effect. Some studies suggested that the photoacceptor is part of mitochondria structure, mainly these are related to ATP synthesis [19, 49–52].

Cytochrome c oxidase is an enzyme that belongs to the respiratory chain and considered as the main photoacceptor for radiations in low power lasers [53] (Fig. 4). This protein is the terminal enzyme of the respiratory chain in eukaryotic cells responsible to mediate the transference of electrons from cytochrome c to molecular oxygen [54]. Studies demonstrated that this enzyme presents different oxidation states, which makes it able to absorb both red and infrared radiations, with absorption peaks at 620, 680, 760, and 820 nm [48]. Some of these absorption peaks coincide with the action spectra for DNA and RNA synthesis in HeLa cells [20], as well as for ATP synthesis [52] and proliferation of HeLa cells [21]. These effects are considered the basic molecular mechanisms by which radiations emitted by low power lasers act on biological tissues to promote wound healing, pain relief, and anti-inflammatory effect. Thus, interaction of these laser radiations with cytochrome c oxidase could be an interesting approach to define a physical parameter relating directly the biological effect with the absorbed energy from the laser radiation.

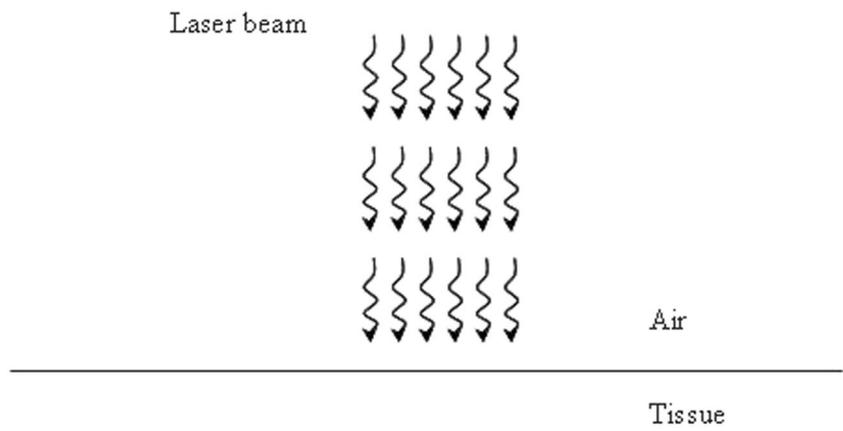
Table 2 Summary of some studies expressing dose of low power lasers

First author	Title	Dose parameter	Reference
Passarella S, 1984 [19]	Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by He-Ne laser	Radiant exposure	<i>FEBS Lett.</i> 1984 175:95–99.
Karu TI, 1984 [20]	Bio-stimulation of HeLa cells by low-intensity visible light III—stimulation of nucleic-acid synthesis in plateau phase cells	Radiant exposure	<i>Il Nuovo Cimento.</i> 1984; 3: 310–324.
Karu TI, 1987 [21]	Bio-stimulation of HeLa cells by low-intensity visible light V—stimulation of cell proliferation in vitro by He-Ne laser irradiation	Radiant exposure	<i>Il Nuovo Cimento.</i> 1987; 9: 1485–1494.
Ryden H, 1994 [22]	Effect of low-level energy laser irradiation on gingival inflammation	Energy	<i>Swed Dent J.</i> 1994; 18: 35–41.
Tunér J, 1998 [8]	It's all in the parameters: a critical analysis of some well-known negative studies on low-level laser therapy	Radiant exposure	<i>J Clin Laser Med Surg.</i> 1998 16:245–248.
Stadler I, 2000 [23]	In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes	Radiant exposure	<i>Lasers Surg Med.</i> 2000; 27:255–261.
Coombe AR, 2001 [24]	The effects of low-level laser irradiation on osteoblastic cells	Energy	<i>Clin Orthod Res.</i> 2001 4: 3–14.
Barbos Pinheiro AL, 2003 [25]	Effect of 830-nm laser light on the repair of bone defects grafted with inorganic bovine bone and decalcified cortical osseous membrane	Radiant exposure	<i>Clin Laser Med Surg.</i> 2003 21: 383–388.
Kujawa J, 2003 [26]	Low-intensity near-infrared laser radiation-induced changes of acetylcholinesterase activity of human erythrocytes	Energy	<i>J Clin Laser Med Surg.</i> 2003 21: 351–355.
Pinheiro AL, 2005 [27]	Polarized light (400–2000 nm) and non-ablative laser (685 nm): a description of the wound healing process using immunohistochemical analysis	Radiant exposure	<i>Photomed Laser Surg.</i> 2005 23: 485–492.
Ilic S, 2006 [28]	Effects of power densities, continuous and pulse frequencies, and number of sessions of low-level laser therapy on intact rat brain	Energy	<i>Photomed Laser Surg.</i> 2006 24: 458–466.
Albertini R, 2007 [29]	Anti-inflammatory effects of low-level laser therapy (LLLT) with two different red wavelengths (660 nm and 684 nm) in carrageenan-induced rat paw edema	Radiant exposure	<i>J Photochem Photobiol B.</i> 2007 89: 50–55.
Lam LK, 2007 [30]	Effects of 904-nm low-level laser therapy in the management of lateral epicondylitis: a randomized controlled trial	Energy	<i>Photomed Laser Surg.</i> 2007 25: 65–71.
Boschi ES, 2008 [31]	Anti-Inflammatory effects of low-level laser therapy (660 nm) in the early phase in carrageenan-induced pleurisy in rat	Energy	<i>Lasers Surg Med.</i> 2008 40: 500–508.
Abdi S, 2009 [32]	The effects of helium-neon light therapy on healing of partial osteotomy of the tibia in streptozotocin-induced diabetic rats	Radiant exposure	<i>Photomed Laser Surg.</i> 2009 27: 907–912.
Peplow PV, 2010 [33]	Laser photobiomodulation of wound healing: a review of experimental studies in mouse and rat animal models	Radiant exposure	<i>Photomed Laser Surg.</i> 2010 28:291–325.
de Souza TO, 2011 [34]	Phototherapy with low-level laser affects the remodeling of types I and III collagen in skeletal muscle repair	Energy	<i>Lasers Med Sci.</i> 2011 26: 803–814.
Ibuki FK, 2013 [35]	Laser irradiation affects enzymatic antioxidant system of streptozotocin-induced diabetic rats	Radiant exposure	<i>Lasers Med Sci.</i> 2013 28: 911–918.
Naghdi S, 2013 [36]	A pilot study into the effect of low-level laser therapy in patients with chronic rhinosinusitis	Energy	<i>Physiother Theory Pract.</i> 2013 29: 596–603.
Acauan MD, 2015 [37]	Effect of low-level laser therapy on irradiated parotid glands—study in mice	Radiant exposure	<i>J Biomed Opt.</i> 2015 20: 108002.
	Can low-level laser therapy when associated to exercise decrease adipocyte area?	Energy	<i>J Photochem Photobiol B.</i> 2015 149: 21–26.

Table 2 (continued)

First author	Title	Dose parameter	Reference
Aquino AE Jr., 2015 [38] Luo GY, 2015 [39]	The effects of low-intensity He-Ne laser irradiation on erythrocyte metabolism	Irradiance	<i>Lasers Med Sci.</i> 2015 30: 2313–2318.
Zaichkina SI, 2016 [40] Aver Vanin A, 2016 [41]	Combined effect of low-intensity helium-neon laser and X-ray radiation on in vivo cellular response of the whole blood and lymphoid organs in mice Pre-exercise infrared low-level laser therapy (810 nm) in skeletal muscle performance and postexercise recovery in humans, what is the optimal dose? A randomized, double-blind, placebo-controlled clinical trial	Radiant exposure Energy	<i>Bull Exp Biol Med.</i> 2016 Sep;161(5):679–682. <i>Photomed Laser Surg.</i> 2016 34:473–482.
Alessi Pissulin CN, 2017 [42] Djavid GE, 2017 [43]	Low-level laser therapy (LLLT) accelerates the sternomastoid muscle regeneration process after myonecrosis due to bupivacaine Photobiomodulation leads to enhanced radiosensitivity through induction of apoptosis and autophagy in human cervical cancer cells	Radiant exposure Radiant exposure	<i>J Photochem Photobiol B.</i> 2017 Mar;168:30–39. <i>J Biophotonics.</i> 2017.
Santos MTBR, 2017 [44] Buchaim DV, 2017 [45] Oliveira FA, 2017 [46]	Efficacy of photobiomodulation therapy on masseter thickness and oral health-related quality of life in children with spastic cerebral palsy Efficacy of laser photobiomodulation on morphological and functional repair of the facial nerve Low-level laser therapy modulates viability, alkaline phosphatase and matrix metalloproteinase-2 activities of osteoblasts	Radiant exposure Energy Energy	<i>Lasers Med Sci.</i> 2017. <i>Photomed Laser Surg.</i> 2017. <i>J Photochem Photobiol B.</i> 2017 169:35–40.

Fig. 1 Schematic representation for energy (E) of a laser beam. A , area; Δt , time interval



Energy (E):
$$E = \sum_{i=1}^{i=n} E_i$$

$A = ?$

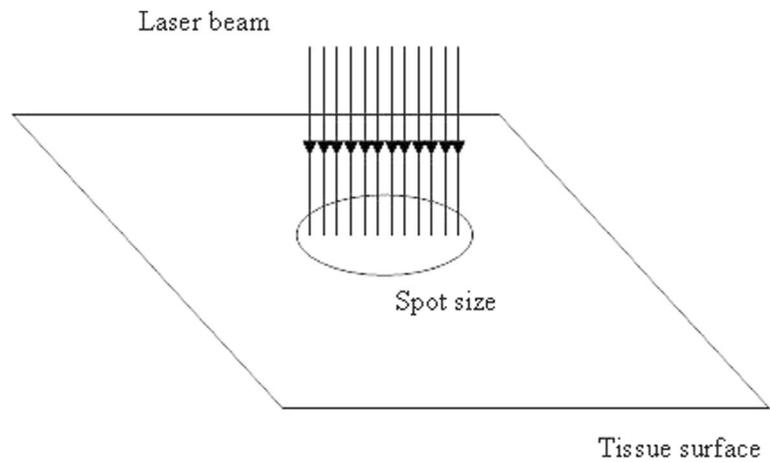
$\Delta t = ?$

Could this parameter be defined?

When considering clinical effects, as those which have as basis the photochemical effects (photobiomodulation effect, for example), the dose of therapeutic agent could be specified in order to control and to be more accurate, as well safe to patients and professionals. On the other hand, when considering experimental procedures, the dose of a physical or a chemical agent

should be specified in order to have accuracy of the measurements and to enable the researcher to compare these experimental measurements with those obtained from other researchers. In fact, the authors reported difficulty to compare and replicate experimental data from different researchers because there are parameter pitfalls [8], missing data [14], incorrect reporting of dosages [16, 17], beam measurements that are not standardized [10], lack of proper reporting of the involved

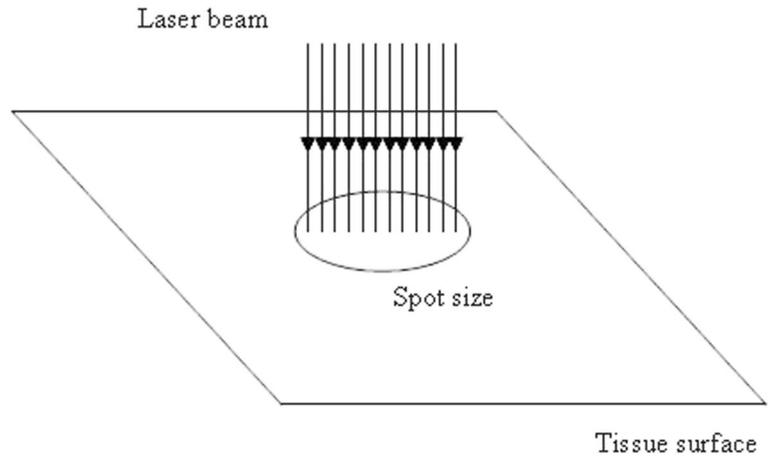
Fig. 2 Schematic representation for fluence (F) of a laser beam. E , energy; A , area



Radiant exposure or fluence (F):
$$F = \frac{E}{A}$$

$\Delta t = ?$

Fig. 3 Schematic representation for power density (Pd) of a laser beam. *E*, energy; *A*, area; Δt , time interval; *P*, power output



Irradiance or power density (P_d):
$$P_d = \frac{E}{\Delta t \times A} = \frac{P}{A}$$

However, $A \neq V$

parameters or incomplete reporting of the parameters used [12], as well as incomplete, inaccurate, and unverified irradiation parameters and miscalculation of “dose” [11].

It is possible that there is, at least, no consensus about an adequate or available definition for dose when beneficial or adverse effects are considered in clinical practice or experimental procedure based on low power lasers. Then, a definition of dose, related to photochemical effects induced by these lasers, seems necessary.

Considering an incident laser beam on a biological tissue (Fig. 5), a volume element (with infinitesimal volume, dV , and infinitesimal mass, dm) absorbs part of the energy of this radiation, $d\varepsilon$. The absorbed radiation dose related to photochemical effects is:

$$D_A = \frac{d\varepsilon}{dm} \tag{4}$$

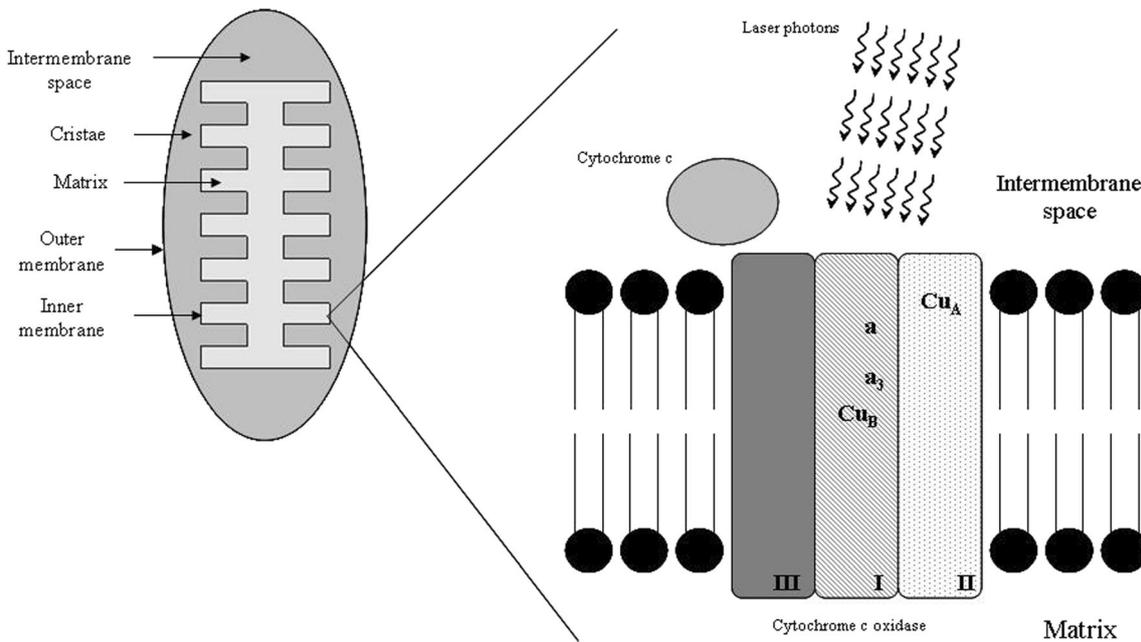
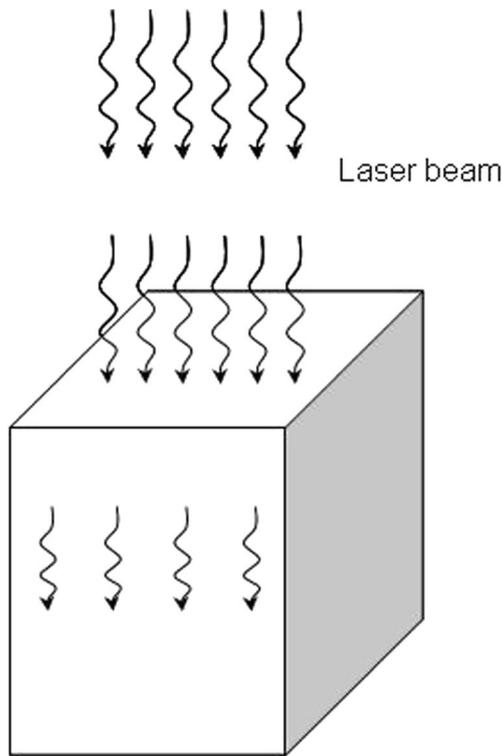


Fig. 4 Schematic representation for mitochondrion and cytochrome c oxidase. CuA, CuB, and hemes a and a3 are metal sites



$$\text{Absorbed dose } (D_A): D_A = \frac{d\varepsilon}{dm}$$

Fig. 5 Schematic representation for laser dose. $d\varepsilon$, infinitesimal energy; dm , infinitesimal mass

where $d\varepsilon$ is the infinitesimal energy deposited into an infinitesimal mass of biological tissue and able to induce photochemical effects. This energy is proportional to a specific photochemical effect (variation of ATP synthesis or cytochrome c oxidase activity, for example). However, direct measurement of this energy could be difficult by a physical dosimeter because this dosimeter must absorb the laser radiation as a biological photoacceptor.

Thus, for practical purposes, this laser dose could be considered as the energy absorbed by cytochrome c oxidase, which leads to the increasing ATP concentration (or amount of cytochrome c oxidized) into mitochondria being reported by the amount (or variation) of ATP (or cytochrome c oxidized) per unity of volume. Thus, laser dose (D_L) could be considered as:

$$D_L = \frac{\Delta[\text{ATP}]}{dV} \quad (5)$$

or

$$D_L = \frac{\Delta[\text{cytochrome c oxidized}]}{dV} \quad (6)$$

where $\Delta[\text{ATP}]$ is the variation of ATP concentration ([ATP] after laser irradiation minus [ATP] before laser irradiation) and $\Delta[\text{cytochrome c oxidized}]$ is the variation of cytochrome c oxidized ([cytochrome c oxidized] after laser irradiation minus [cytochrome c oxidized] before laser irradiation). A little but measurable mass could be considered ($dV = 1 \mu\text{L}$, for example) for practical purposes.

Could laser dose be measured?

To measure the laser dose as function of this laser-induced biological effect, a suspension of isolated mitochondria (containing a number of mitochondria similar to this into cells) could be exposed to a standard low power laser beam and the production of ATP, or cytochrome c oxidase activity, be evaluated. Isolated mitochondria are obtained from biological tissues or cell cultures by differential centrifugation [55] technique or, more recently, by magnetic beads coated with antibodies [56, 57]. Measurement of ATP synthesis in mitochondria is done by colorimetric/fluorometric assay [58, 59] as well as cytochrome c oxidase activity is evaluated by absorbance at 550 nm [60].

Could laser dose from different lasers be compared?

However, the amount of this specific photochemical effect could depend on physical laser parameters, as radiation wavelength, and this could be evaluated comparing these quantities at different laser doses. In fact, laser-induced effects are wavelength-dependent, either experimental [48] or therapeutic effects [1]. Also, laser-induced biological effects are dependent on emission mode (continuous or pulsed wave) [61, 62].

A laser dose equivalent (D_E) could be defined as:

$$D_E = D_L \times F_{phys} \quad (7)$$

F_{phys} is a physical proportionality factor that permits to convert a D_L from a laser beam into a D_L from a standard laser beam. Thus, for example, if a 660-nm continuous wave laser beam with a specified irradiance is considered as the standard laser beam, an 808-nm continuous D_L would have a specific F_{phys} value to convert its D_L value to D_L from the standard low power laser beam. Similarly, specific F_{phys} values could be obtained to pulsed wave lasers or to lasers at different irradiances. These F_{phys} values could be presented into devices or guides or automatically adjusted in new laser devices. Thus, results from procedures using different physical laser parameters could be compared. However, it is necessary to define what would be the standard low power laser beam, but this is not a hardwork to physicists dedicated to laser optics or dosimetry.

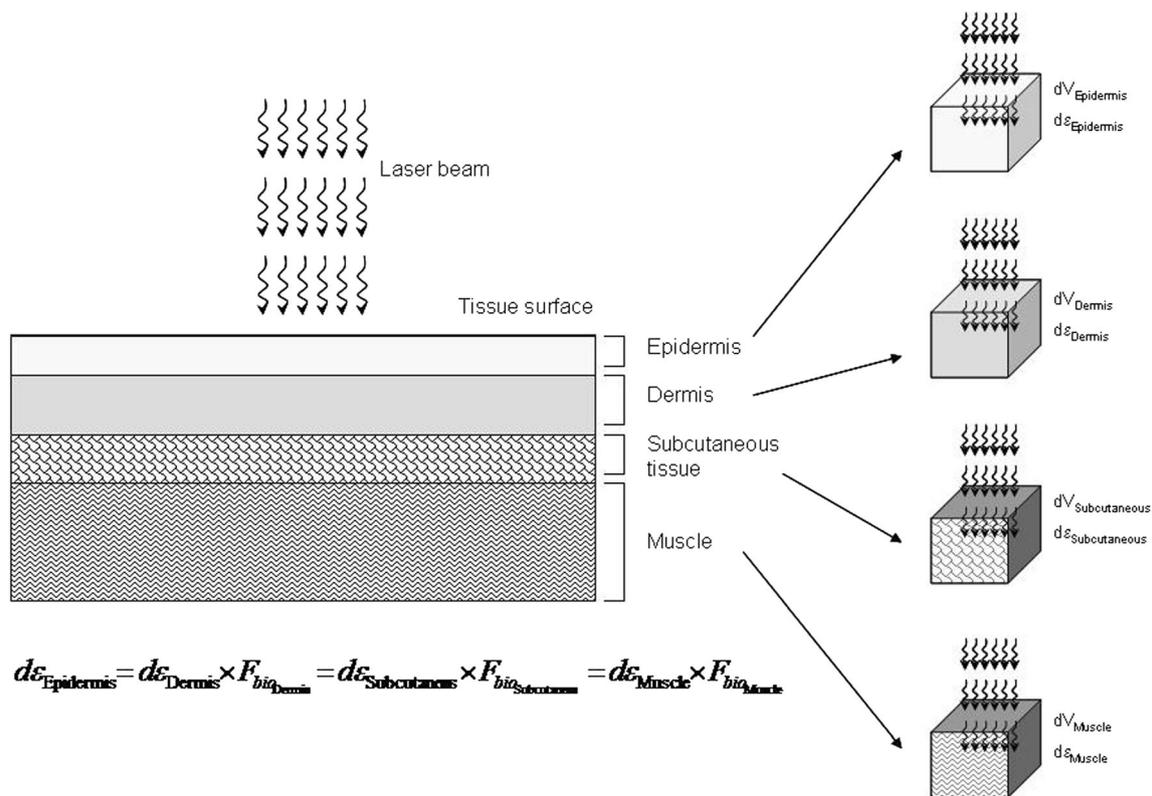


Fig. 6 Schematic representation for relationship among different layer and tissues. $d\varepsilon$, infinitesimal energy; dV , infinitesimal volume; $d\varepsilon$, infinitesimal energy deposited into an infinitesimal mass of biological tissue and able to induce photochemical effects; F_{bio} , biological proportionality factor

Could be compared a laser-induced effect in different biological tissues?

Each biological tissue has its own optical properties and each biological tissue contains its own photoacceptor concentration. If the laser-induced photochemical effects are dependent on a specific photoacceptor (cytochrome c oxidase, for example), which is in tissues targeted by low power lasers, the D_L could be compared between different two tissues. Thus, considering infinitesimal volumes in these two tissues (dV_1 and dV_2):

$$d\varepsilon_1 = d\varepsilon_2 \times F_{\text{bio}} \quad (8)$$

This leads us to:

$$D_{L_1} = D_{L_2} \times F_{\text{bio}} \quad (9)$$

And:

$$D_{E_1} = D_{E_2} \times F_{\text{bio}} \quad (10)$$

where F_{bio} is the biological proportionality factor for D_L between two biological tissues (among different skin layers or between epithelial and muscle tissues, for example) (Fig. 6). Similarly to F_{phys} , F_{bio} must be determined for the different biological tissues that are targeted by low power laser to

obtain a photobiomodulation effect. F_{bio} values could be obtained by ATP production (or cytochrome c oxidase activity) in isolated mitochondria from a reference biological tissue (epithelial, for example) irradiated with a standard low power laser beam. Thus, the laser-induced photobiomodulation in different tissues could be compared to laser-induced photobiomodulation in the reference tissue.

Conclusions

Simplified laser dose parameters for low power lasers could be useful to researchers and clinicians. Also, these parameters (laser dose, D_L , and laser dose equivalent, D_E) could permit comparisons and decrease the mistakes and inaccuracies when a laser-induced biological effect is evaluated and compared with these from previous studies. The calculus of the proportionality factors (F_{phys} and F_{bio}) for different laser wavelengths, emission modes, irradiances, and biological tissues could be performed by biologists and physicists; meanwhile, the determination of a standard laser beam could be not hard for physicists working in an institution dedicated to measurements and setting standards. Also, it could be not hard for biologists to set the standard conditions to measure a specific

laser-induced biological effect (ATP production or cytochrome c oxidase activity). These could be new and challenging proposals for multidisciplinary researches interested on study of the biological effects induced by these lasers, which can contribute significantly to improve accuracy, effectiveness, and safety of clinical protocols based on low power lasers.

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