**Key words:** Laser-tissue interaction, thermal effects, PIF formula, Hiltherapy.

# Thermal effects of NIR laser radiation in biological tissue: a brief survey.

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## ABSTRACT

In this survey the laser-tissue interaction has been considered, with particular attention to thermal effects. Then Pulse Intensity Fluence formula for the hiltherapy pulse was retrieved. Thereafter PIF formula was applied with the lasers parameters used in some medical laser application to compare PIF values. In our opinion, PIF formula is easier to better understand HILT features and its differences with LLLT and Continuous Wave (CW) Power Lasers

Laser tissue-interaction has two main purposes: diagnosis and therapy. While no permanent change in tissue occurs in the diagnostic field, the ultimate objective of the therapeutic use of laser is to cause some sort of a modulated tissue damage that would be beneficial for the patient. The variety of interaction mechanisms that may occur when applying laser light to biological tissue is manifold. Specific tissue characteristics as well as laser parameters contribute to this diversity. Most important, among optical tissue properties, are the coefficients of reflection, absorption and scattering. Together, they determine the total transmission of the tissue at a certain wavelength. In addition, the following laser radiation parameters are equally important: wavelength, exposure time, applied energy, focal spot size, energy density and power density. Among these, the exposure time is a very crucial parameter when selecting a certain type of interaction.

Usually, laser–skin interaction can be categorized into five aspects [1], namely:

**1.** Photochemical interaction: this takes place at very low power densities (0.01 - 50 W/cm$^2$) and long exposure time ranging from seconds to continuous wave.

**2.** Thermal interaction: the radiation is absorbed by the tissue and transformed in internal energy that produces a temperature increment. The irradiance could be continuous-wave or pulsed, typical power densities are from 10 to $10^6$ W/cm$^2$; its temporal duration is from 1 μs to 1 min.

**3.** Photoablation: UV radiation is used because the high energy of the photons can break molecular links and ionize atoms. By this way only focalized atoms and molecules can be affected. The exposition times are very short, from 10 to 100 ns, and irradiance is from $10^7$ to $10^{10}$ W/cm$^2$.

**4.** Plasma-induced ablation: when power densities exceed $10^{11}$ W/cm$^2$ in solids and fluids, a phenomenon called "optical breakdown" occurs with plasma formation and shock wave generation. In this process the typical pulse durations are from 100 fs to 500 ps.

**5.** Photodisruption: if breakdown occurs inside soft tissue or fluids, cavitation and jet formation may also take place. In this process a very short exposition from 100 fs to 100 ns and high-power laser, focused by a lens into the treated tissue, are used in this way, irradiances in the magnitude of $10^{11}$ -$10^{16}$ W/cm$^2$ are obtained.

The majority of laser medical treatments, such as laser hyperthermia, coagulation, and surgery, involve thermal effects. To improve the treatment efficiency and for personal safety, accurate analysis of thermal transport and thermal damage is of paramount importance. In general, predicting thermal tissue damage due to laser irradiation involves three steps:

**1.** Modeling the propagation and distribution of light within the tissue: when laser strikes a tissue, or passes from one type of tissue to another, it can be reflected, transmitted, absorbed, scattered, internally reflected or some combination of these phenomena. Various methods are used when dealing with propagation of light. Ray tracing is the simplest method, while solving Maxwell's equations of electromagnetic provides the most complete albeit almost impossible

analysis. One common technique is to consider the laser light in terms of photon paths, this method is called "photon transport"; its application to laser tissue interaction has been well validated [2]. A different approach relies on the Monte Carlo technique [3] to create a probabilistic model of light propagation in the tissue [4].

**2.** Estimating the temperature rise and distribution in the tissue: heat is generated in the tissue when photons are absorbed. In most cases of laser-tissue interaction, a reasonable assumption is that convection, radiation, vaporization and metabolic heat effects are negligible. Heat transport is solely characterized by thermal tissue properties such as heat conductivity and heat capacity. If the rate of heat generation from the source term is known, the change in temperature can be calculated by using energy balance of the "bioheat equation" [1].

**3.** Predicting the thermal damage that would result: the thermal damage may be described mathematically by a rate process equation that defines a damage function [1; 5]. This damage function is expressed in terms of an Arrhenius integral.

Depending on the duration and peak value of the tissue temperature achieved, different effects like coagulation, vaporization, carbonization and melting may be distinguished. The spatial extent and degree of tissue damage primarily depend on magnitude, exposure time and placement of deposited heat inside the tissue. The absorption by water molecules plays a significant role in thermal interaction, and the absorption coefficient strongly depends on the wavelength of the incident laser radiation. Assuming a body temperature of 37°C, no measurable effects are observed for the next 5°C above this. The first mechanism by which the tissue is thermally affected can be attributed to conformational changes of molecules. These effects, accompanied by bond destruction and membrane alterations, are summarized in the single term "hyperthermia" ranging from approximately 42°-45°C. If such a hyperthermia lasts for several minutes, a significant percentage of the tissue will already undergo necrosis as described by Arrhenius' equation [1]. Beyond 50°C, a measurable reduction in enzyme activity is observed, resulting in a reduced energy transfer within the cell and immobility of the cell itself. Furthermore, certain repair mechanisms of the cell are disabled. At 60°C, denaturation of proteins and collagen occurs which leads to coagulation of tissue and necrosis of cells. At 100°C, the water molecules contained in most tissues start to vaporize. Due to the large increase in volume during this phase transition, gas bubbles are formed inducing mechanical ruptures and thermal decomposition of tissue fragments. Only if all water molecules have been vaporized, and laser exposure still continues, does the increase in temperature proceed. Above 150°C, carbonization takes place, which is observable by the blackening of adjacent tissue and the emission of smoke. Finally, beyond 300°C, melting can occur, depending on the target material.

However, not only the temperature achieved, but also the temporal duration of this temperature plays a significant role for the induction of irreversible damage. It is important to notice that many of the thermal effects described above can take place simultaneously in different areas of the irradiated volume, as a result of different temperatures locally developed because of laser radiation penetration in the tissue. When using laser pulse durations above microseconds, it is important to control the exposure time in relation to the tissue characteristic thermal relaxation time, in order to mitigate the effects of laser radiation in the target tissue volume. It should be taken into account that if the laser irradiation time is longer than the thermal relaxation time of the tissue, the heat can diffuse within the tissue beyond the typical optical penetration depth.

Many studies and models are presented in literature to study the thermal process in laser irradiated tissue; most of these studies are very specific and aimed at investigating thermal processes in certain organs and tissues of the body and under limited physical conditions, some are conducted to calculate temperatures profiles and thermal damage, while others develop models for calculating the tissue thermal response to laser irradiation [6-29].

In their work D. Fortuna and L. Masotti [30] synthesize in one formula, the "pulse intensity fluence" (PIF) formula, the Hiltherapy pulse features. Correlating their clinical and experimental data with the biological effects of Hiltherapy they defined the Hiltherapy domains and indicated a range of acceptable PIF values for tissue regeneration: from just under 0.2 $[J/cm^3]^2$ to just under 1.0 $[J/cm^3]^2$. Below 0.1 $[J/cm^3]^2$, there may be just an anti-inflammatory effect and not a regenerative effect; whereas for PIF values exceeding 1.0 $[J/cm^3]^2$ there may be a histo-toxic effect.

In this paper we applied the PIF formula to the laser configurations used in different applications to evaluate the relationship between the biological effects with the PIF values.

The formula is:

$$\mathbf{PIF\ [J/cm^3]^2 = I_p \tau_{on} \frac{E_p}{10{,}07 r_{sp}^3} \alpha \frac{\tau_{off}}{\tau_{on}+\tau_{off}}}$$

were $I_p$ is the peak intensity ($W_p/cm^2$) that is the peak power ($W_p$) divided by the surface area of the spot ($cm^2$); $\tau_{off}$

is the pulse duration; $E_p$ is the energy per pulse; $r_{sp}$ is the spot size ray; $\alpha$ is the water absorption coefficient that varies in relation to $\lambda$; and $\tau_{off}$ is the turned off phase.
It should be noticed that, if the radiation is a continue wave, $\tau_{off}$ is almost zero so that the PIF value is well below the acceptable PIF values for tissue regeneration, therefore only the pulsed laser radiation can be considered.

Opposite to surgery lasers, which have high power and are capable of tissue destruction, those used in physical medicine and rehabilitation have low power (1-20 mW) and have no thermal effect. They are capable of cell photobiostimulation and healing. This photobiostimulation effect is believed to promote tissue healing and repair while the bioinhibitory effect promotes pain management and relief. The clinical effects of laser light include marked improvements in wound healing, nerve repair, musculoskeletal pain and various inflammatory processes. However, one of the most confusing aspects of light therapy is explicated by dozens of published reports, which fail to find any effect from low laser therapy.
Regarding the safe use of lasers, there are several documents and guidelines dealing with the damage they can cause mainly to eyes (organs at higher risk) and secondarily to the skin. The ICNIRP (International Commission on Non-Ionizing Radiation Protection) published the guidelines on limits of exposure to laser radiation of wavelengths between 180 nm and 1400 nm to establish the basic principles of protection against optical radiation emitted by lasers and that are considered to be adequate for the general population as well as for occupational exposure [31-33].
The limits presented in these guidelines were considered in the European normative in the directive 2006/25/EC [34] as: "Adherence to the exposure limit values should provide a high level of protection as regards to the health effects that may result from exposure to optical radiation."
The same directive was implemented in the italian legislature with the D.lgs 81/2008 [35]. If we consider the limits for the skin, for a wavelength of 1064 nm, an exposure time of 150 μs and a spot size of 3.5 mm, the energy density limits is 0.6 J/cm$^2$. Applying these values for wavelength, spot size and exposure time in the PIF formula we obtain a PIF value of 0.06 [J/cm$^3$]$^2$, just below the lower limit for the Hiltherapy. We have to consider that the safety limits are much lower than the pain thresholds as adopted by the standard DIN 33403 [33; 36].
To understand the characteristic of the pulse in the Hiltherapy we can apply the PIF formula to the characteristic variables of some laser applications in medicine. Many papers found in literature are missing an important factor for the formula therefore, the PIF values cannot be achieved. Those works where all terms of the PIF formula are present, can be divided considering the exposure time: ultrashort pulse, from fs to ns, are used for tissue ablation, for treatment of cutaneous lesions with a selective photothermolysis or for very particular applications such as the DNA transfection; short pulse, from few μs to few hundred μs, can be used in Hiltherapy but also in both ablative and nonablative treatments; from ms to s pulse, papers can be found about temperature and thermal effects in different tissue, laser welding of connective tissue, laser biostimulation, permanent damage of blood vessel and collagen synthesis. In the following we report some of these works calculating for each one the PIF values.
For instance, the work of M. Leandri et al. [37], studied skin temperature during and immediately after irradiation with pulses by Nd:YAP laser. They found that Nd:YAP pulses yielded temperatures that were correlated with pulse energy, but not with pulse duration; much higher temperatures were obtained irradiating blackened skin than white skin (ranges 100-194°C vs 35-46°C). Temperature decay was extremely slow in white skin, reaching its basal value in more than 30s. Pulse duration was 12 ms with a spot of 6 mm diameter. Energy was increased from a minimum of 0.5 (energy density 18 mJ/mm$^2$) to a maximum of 4.5 J (energy density 159 mJ/mm$^2$). Considering the absorption coefficient at the Nd:YAP wavelength (1340 nm), the following PIF values were obtained: PIF = 650 [J/cm$^3$]$^2$ for the maximum energy of 4.5 J;
PIF = 72 [J/cm$^3$]$^2$ at energy 1.5 J; PIF = 30 [J/cm$^3$]$^2$ at 1 J and
PIF = 8 [J/cm$^3$]$^2$ at 0.5 J.
With the lower pulse the temperature increases of about 3°C without warm perception. A. da Costa Ribeiro et al. [38] investigated the thermal effects and the morphological changes after diode laser irradiation (810 nm) of root canals. Samples were irradiated in pulsed mode, with a duty cycle of 50% at 1.25 W (mean power), 10 Hz, Ø = 400 μm, 994 W/cm$^2$. The maximum temperature variations at the apical region were analyzed and ranged from 1.2° to 3.3°C (group 3). From these parameters PIF = 146 [J/cm$^3$]$^2$ despite this, thermal damage is absent, but it must be remembered that in this case the laser interacts directly with dentine and the mechanism of heat conversion depends directly on the tissue constituents and the irradiation wavelength. The dentin absorption coefficient is low for the wavelength used in this work (808 ± 5 nm), so scattering is predominant against absorption [1].
In a report of a study on the application of laser-activated nanoparticles in the direct welding of connective tissues F. Ratto et al. [39] achieved the local

denaturation of the endogenous collagen filaments, which reveals that the treated area reached temperatures above 50°C, with an 810 nm diode laser pulses of 40 msec and approximately 100–140 J/cm$^2$ emitted by a 300-μm core fiber. Also for these parameter the PIF value is high $4 \div 8 \cdot 10^3$[J/cm$^3$]$^2$.

The laser biostimulation applied by P. Vescovi et al. [40] that used Nd:YAG laser (power 1.25 W, frequency 15 Hz, fiber 320 μmin diameter) defocalised at 1–2 mm from the tissue (theoretical PD 1555 W/cm$^2$, theoretical Fluence/ min 167.94 J/cm$^2$) leads, assuming a 2 mm size spot, to a PIF = 7.4 [J/cm$^3$]$^2$. In a study of J. K. Barton et al. [41], the probability of permanent damage to a given type and size of blood vessel was determined as a function of fluence at the top (superficial edge) of the vessel lumen. A 532 nm wavelength, 10 ms pulse duration, 3 mm spot size and radiant exposure 2-20 J/cm2 laser was used resulting in PIF values from 0.003 to 0.31 [J/cm$^3$]$^2$. Visible clearance (indicating permanent blood vessel damage) has been obtained at radiant exposures of 9.5-12 J/cm$^2$.

Nonablative methods may produce collagen synthesis in sun-damaged skin. V. G. Prieto et al. [42] studied sun-damaged skin treated with a 1064 nm Nd:YAG laser (130 J/cm$^2$, triple pulse, 7.0/7.0/7.0-millisecond pulse duration, 75 - millisecond delay, 6 mm spot size and application of a thin coat of water-based cooling gel). The end point of therapy was mild erythema, but in the settings employed, there is no obvious morphologic damage to the epithelial and mesenchymal structures in the skin. Their data indicate that treatment with Nd:YAG may result in increased collagen deposition in the papillary and upper reticular dermis. The PIF in this configuration is [J/cm$^3$].
In an optical-thermal-damage model of the skin under laser irradiation, developed by using finite-element modeling software by B. Chen et al. [43; 44], the predictions are compared to experimental measurements for a 2000-nm laser irradiation. The model enables the authors to verify the suitability of the American National Standards Institute (ANSI) maximum permissible exposure (MPE) standard for a wavelength of 2100 nm with exposure duration from 0.1 to 1 s and 3.5-mm beam diameter. The total exposure energy at the ED50 damage threshold is reported for three beam diameters (4.83 mm, 9.65 mm and 14.65 mm) and four exposure durations (0.25 s, 0.5 s, 1s and 2.5s).

Replacing the values in the PIF formula for the two smaller exposure duration we obtain the following values:

- $\tau_{on}$ = 0.25 s, Ø = 0.48 cm
  $E_{th}$ = 0.66 J PIF = 360 [J/cm$^3$]$^2$.
- $\tau_{on}$ = 0.5 s, Ø = 0.48 cm
  $E_{th}$ = 0.75 J PIF = 300 [J/cm$^3$]$^2$.
- $\tau_{on}$ = 0.25 s, Ø = 0.965 cm
  $E_{th}$ = 2.12 J PIF = 120 [J/cm$^3$]$^2$.
- $\tau_{on}$ = 0.5 s, Ø = 0.965 cm
  $E_{th}$ = 2.47 J PIF = 106 [J/cm$^3$]$^2$.
- $\tau_{on}$ = 0.25 s, Ø = 1.465 cm
  $E_{th}$ = 4.02 J PIF = 51 [J/cm$^3$]$^2$.
- $\tau_{on}$ = 0.5 s, Ø = 1.465 cm
  $E_{th}$ = 4.23 J PIF = 38 [J/cm$^3$]$^2$.

In the group of laser applications with a short pulse (from 1 to few hundred μs) there is the effect of laser radiation on meniscal tissue as examined by M. Bernard et al. [45]. They observed that laser systems caused greater damage to meniscal tissue and a more extensive healing reaction than cuts with mechanical instruments. Using the Nd:YAG parameters: 1.44 mm wavelength, 4.9 W power , 980 mJ energy per pulse, pulse duration 650 μs, repetition rate 5 Hz and 800 μm fiber, we obtain PIF = $9 \cdot 10^6$ [J/cm$^3$]$^2$.

K. Hayashi et al. [46] evaluated the effect of laser nonablative energy on the ultrastructure of joint capsular collagen. Laser energy was applied using a holmium: YAG laser and was delivered at 10 pulses/sec with a 250 μsec pulse duration and a 400 μm fiber diameter. Transmission electron microscopy showed significant ultrastructural alterations in collagenous architecture for all laser treatment groups, with increased fibril cross-sectional diameter for each of the treated groups. For this reason we expected a PIF value greater than 1 [J/ cm$^3$]$^2$, indeed we obtained [J/cm$^3$]$^2$.

A. D. Izzo et al. [47] have pioneered a novel method of stimulating cochlear neurons, using pulsed infrared radiation, based on the hypothesis that optical radiation can provide more spatially selective stimulation of the cochlea than electric current. They found evidence that water absorption of optical radiation is a significant factor in optical stimulation. A diode laser was used for the optical stimulation with infrared radiation approximately between 1.92 and 1.94 μm and pulse durations were selected between 5 and 300 ms and the repetition rate of the laser was 2 Hz; the diameter of the fiber coupled was 200 μm. They estimate that the instantaneous temperature rise, associated with cochlear nerve stimulation after a laser pulse using a radiant exposure of 5 mJ/cm$^2$ ,would be 0.15°C at the distal tip of the optical fiber and 0.08°C at the neurons.
The PIF value in this case is

$$0.08 \cdot \alpha \,|\, \lambda = 1{,}9\text{amm} = 9 \text{ [J/cm}^3\text{]}^2$$

due to the high absorption of the water in the tissue at these light wavelengths.

The application of the ultrashort pulses is for example with the Q-switch Nd:YAG laser. The parameters for the treatment of cutaneous lesions (48) are 2 mm spot size and a high fluence of 12 J/cm$^2$ with pulse of the order 100 ns, so the PIF value is 2000 [J/cm$^3$]$^2$. The high PIF value accounts for the fact that Q-switching works on the basis of selective photothermolysis.
Applying PIF formula for the ultrashort laser pulse ablation that removes material with low-energy fluence required and minimal collateral damage proposed by M.D. Feit et al. [49] we obtain a value of 18 [J/cm$^3$]$^2$ due to 2 J/cm$^2$ 0.5 ps pulses with a repetition rate of 1 kHz and a laser spot diameter of 200 μm, whereas PIF = 3•10$^3$ [J/cm$^3$]$^2$ to 25 J/cm$^2$ 5 ns.

A specific research was reported by S.-W. D Tsen et al. [50] where a very low power, near-infrared (1043nm) femtosecond laser technique was employed to enhance the transfection efficiency of intradermally and intratumorally administered DNA plasmid. They found that femtosecond laser treatment can significantly enhance the delivery of DNA into the skin and into established tumors in mice. In particular they found that mice receiving laser treatment with a laser that provides 500 fs duration pulses at 1043 nm wavelength with repetition rate 200 kHz, at a laser power density of 0.04 GW/cm$^2$, generated the highest transfection efficiency compared to mice treated at different laser power densities. This laser power density corresponds to a laser energy of 2.6 μJ/pulse with a spot size of 4 mm. The PIF value in this case is very small, approximately 6•10$^{-13}$ [J/cm$^3$]$^2$.

In this work, consequences of thermal effects of laser radiation interaction with biological tissues have been considered. Moreover, the formula proposed by D. Fortuna and L. Masotti [30] to characterize the fluence intensity of the hiltherapy pulse was also considered. By inserting in the same formula the parameters used in other clinical applications of lasers, it is possible to obtain relative PIF values. These values do not ever fall in the range of effectiveness of hiltherapy. They are indeed either below the minimum threshold, as in the case of the limits for laser safety as contained in the ICNIRP guidelines and in the european and italian legislation (which is very conservative concerning workers), or beyond the maximum threshold because the laser radiation is used in this cases to cause selective damage to selected region of specific tissues.

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