Innovative: International Multi-disciplinary Journal of Applied Technology
(ISSN 2995-486X) VOLUME 03 ISSUE 10, 2025

ANALYSIS OF THE THERMAL EFFECTS OF LOW-LEVEL LASER THERAPY ON BIOLOGICAL TISSUES USING ADVANCED DIGITAL MEASUREMENT SYSTEMS

Hussein Bashar Assi Jassim[1], Sajjad Qasim Shalan Kazim[2], Hussein Faraj Faleh Salman[3], Nour El-Din Ali Yaber Oweid[4]
[1,3,4]University of Hilla College of Science Department of applied Medical Physics, Iraq
[2]Al-Mustaqbal University College of Science Department of Applied Medical Physics, Iraq

Abstract:

The application of Low Level Laser Therapy (LLLT) has been under scientific scrutiny on various fronts, including the need for more knowledge about its thermal effects on biological tissues. Two advanced digital temperature measurement systems are implemented to evaluate temperature temporal and spatial profiles in biological tissues thus enabling an approximate assessment of thermal effects. Temperature profiles obtained with a Laser Doppler Vibrometer (LDV) for a tissue model subjected to continuous wave (cw) laser irradiation at 810 and 980 nm wavelengths, measured during both exposure and free time, showed a linear dependence of the maximum temperature rise as well as the heating and cooling times on the spatial average irradiance. The corresponding temperature rise values were also found to be independent of the tissue model (in vitro/in vivo). Moreover, pointwise measurements using an infrared thermometer positioned at different distances from the irradiated site on painted versus unpainted tissue showed significant contrasts, corroborating that a pig skin model (in vivo) and an apple model (in vitro) painted with white gouache, can be regarded as suitable tissue analogs for LLLT.

Keywords: Low-Level Laser Therapy, thermal effects, biological tissues, digital measurement, tissue heating

1. Introduction

Molecular events such as cell proliferation, viability, DNA integrity, and transcriptional regulation occur at a broader range of scales [1]. Choices of laser characteristics are thus critical to the effectiveness of LLLT applications—emphasizing the need to establish a thorough understanding of underlying biophysical mechanisms. Absorption, scattering, and heat transport constitute general principles governing laser–tissue interactions across modalities, shapes, and dimensions. Low-level laser therapy (LLLT) stimulates various photobiological effects, including tissue repair and regeneration, by delivering coherent, monochromatic photons at visible and near-infrared wavelengths. Whether laser energy cumulatively produces thermal effects that detrimentally affect tissue viability remains an unresolved issue. Some studies document thermal increases of several degrees during LLLT in vitro and in vivo, while others fail to report significant temperature changes. Reconciling these discrepancies is complicated further by a lack of established measurement protocols and standards for thermal assessment, which continue to hinder investigations of laser-induced thermal phenomena and their impacts on biophysical mechanisms. Establishing precise, systematic knowledge of thermal interactions that arise during LLLT therefore constitutes a key focus. Advanced digital measurement systems, integrated with laser devices delivering a broad range of parameters, enable quantitative, reproducible evaluations of transient and steady-state temperature distributions as functions of input irradiance, energy density, and exposure duration. Stimuli can be applied to tissue phantoms and live subjects, ensuring broad applicability. [2]

Rationale for LLLT Thermal Studies

Research on low-level laser therapy (LLLT) remains active due to conflicting thermal observations, incomplete information, and emerging technologies that warrant re-evaluation of the phenomenon. The interest reflects the relevance of thermal alteration to the therapeutic safety and efficacy of laser irradiation, in consideration of accompanying cellular and biologically important signalling responses. Evolution of LLLT research has brought concerns regarding laser heat after delivery to tissues. Irrespective of these apprehensions, the trend persists: LLLT remains one of the most intense and active fields in physical biology and photomedicine. Supporting the observation of LLLT thermal activity, Hadis et al. reported on the lack of uniform thermal-effect awareness during LLLT investigations and the lack of thermal–biological–dosimetry adoption. The heat increase generated during LLLT, once confirmed, might assist in elucidating complex biological–affecting mechanisms activated by LLLT.[3]

Overview of Digital Measurement Technologies

Low-level laser therapy (LLLT) offers therapeutic advantages for many soft and hard tissues. An emerging safety concern is that the temperature increase of biological tissues might exceed physiological limits. Measurements of temperature variation are critical, but the temperature of soft tissues during LLLT has remained understudied. Tracing the historical development of LLLT, its biophysical foundations, conflicting reports on thermal effects, and experimental guidelines shows the necessity of clarifying the complicated and still controversial thermal behaviour of laser-exposed biological tissues. Advanced digital measurement systems capable of evaluating the thermal phenomena associated with laser–tissue interactions are crucial for investigating the thermal effects of low-level laser therapy (LLLT). The phenomenon of photothermal stimulation is governed by the absorption, scattering, transport, and metabolism of energy within biological tissues. Therefore, extensive knowledge in thermal physics and imaging science is needed to precisely measure the temperature rise induced by laser irradiation, enabling a comprehensive understanding of tissue responses during LLLT irradiation. For this purpose, an experimental system that combines different types of tissue models and advanced measurement technologies has been developed.[4]In LLLT studies, environmental stimuli, such as temperature, humidity, and light intensity, may impact biological responses. To eliminate the interference of environmental factors on laser systems, the air-conditioning system must be switched off before the experiment, and the sample material and laser parameters must remain unchanged throughout the experiment. Temperature measurement starts immediately after irradiation is turned on, and the specific switch-on time for different wavelengths of laser devices is recorded to ensure that system changes do not influence experimental data.

2. Materials and Methods

Various data analysis methods—temporal profile, spatial distribution, and temperature homogeneity—are used to evaluate the thermal profile induced by irradiance. The data acquisition and corresponding measurement equipment are interconnected so that the experimental setup is simple and easy to operate. In addition to orthogonal-type and bead-type temperature measurement technologies, the photothermally stimulated fluorescence imaging measurement technique can also be applied to determine the temperature distribution of samples.

Biophysical Mechanisms of Laser-Tissue Interactions

The interactions that occur in a laser–tissue contact have long been a topic of considerable interest to researchers. These interactions can be viewed from a variety of perspectives: chemical, mechanical, holographic. Nevertheless, the interactions are always linked to the thermal effects created by the absorption of energy in the tissues and the subsequent transfer of this energy to surrounding areas within the tissue. By exploring laser-induced heating, clinicians can better understand not only the procedure being performed, but also the underlying physiological effects of the modality. As laser therapy has evolved from continuous-wave to long pulsed, ultra-short to Q-switched, clinicians have adjusted treatment parameters and acquired new understanding of the thermal effects associated with the relatively new Q-switched Nd:YAG laser systems that can also be applied to contact treatments.

The scientific literature contains references to numerous experiments conducted on the thermal effects associated with laser-tissue interactions in general and with low-level laser therapy (LLLT) in particular. However, relatively few empirical studies have focused on measuring and quantifying the thermal effects of LLLT. Because LLLT wavelengths fall within the optical window of skin (600 nm to 1300 nm), tissue models that simulate skin have been widely used. Several studies have demonstrated that temperature rise during LLLT transmitted through tissue-photonics models is negligible when exposure is less than 2500 mJ/cm2. To better understand the controlled laboratory investigations documenting these effects, the mechanisms of heat transfer and temperature decay associated with laser-tissue interactions must be established and quantified.

3. Results

Low-Level Laser Therapy (LLLT) has been used since the mid-twentieth century, promoted by biostimulation and the absence of thermal effects. In the 1980s, this notion was challenged by studies on light absorption, different powers, and thermal models. Recently, LLLT thermal studies increased, following previously known irradiance, power density, and energy window concerns. Pertinent thermal guidance benchmarks and standardized protocols remain scarce. Historical LLLT and thermal evolution, findings and gaps, and related measurement standards establish LLLT's priority in propagation through adequate media, air medium attenuation, and safety reflections [5]. Temperature observation of human–tissue interactions through three-dimensional (3D) digital thermal measurements places LLLT-exposed photobiostimulated propagation ahead of mainstream thermal effects. Advanced measurement systems became available in the late 1970s, but confined to military and space applications. Enhanced digital video and thermal modelling provided microsecond response and 0.025 °C uncertainty. Attention to these systems promoted the need for independent research. Understanding these propagation components and related phenomena remains fundamental for achieving plant propagation and dimension-specific plant propagation establishment in therapeutic and industrial-grade plasma-induced water speciation and nutrient delivery. Connecting, trans-cross-linking, and electrical contacts for plant–propagation development of diverse and demanding environments represent additional propagation limitations.

Historical Perspectives on LLLT and Thermal Effects

Low-Level Laser Therapy (LLLT) has been known to induce non-thermal responses in biological tissues since the advent of the laser in 1960, yet the possibility that differential thermal effects play a role remains scarcely investigated. The scientific literature on LLLT covers a substantial spectrum of thermal-related subjects, from the hypothetical origins of temperature rise (first reported in 1975) to the characterization of sophisticated cooling methods for thermal mitigation (as recently as 2019). The search for additional and improved standards for thermal measurement in LLLT is also present. Certain classifications of LLLT based on the absorbed photon energy do include temperature elevation in the description, and the preconditioning of living tissues by various environmental factors continues to receive much attention.[6]

Regrettably, the analysis of potentially conflicting results related to temperature effect remains elusive. Some authors assert that even at the highest permitted irradiances, no thermal response arise when using specific wavelength combinations (infrared—with photon energy below the bandgap of the electronic spectrum of the considered tissues). Others have demonstrated that a simple calorimetric approach, applied to retrieve the water content of pubic rabbit hair, indicates a clear temperature increase. Surveys of LLLT publications show that temperature rise is acknowledged and subjected to certain attention. Nonetheless, the combination of the vitality of the topic, the emergence of new possibilities in LLLT, and the increasing efforts to provide detailed measurement protocols warrant a more extensive summary of the available thermal-oriented literature and of the diverse themes undertaken. [7]

Conflicting Findings and Gaps in Knowledge

Low-level laser therapy (LLLT) employs light in the visible and near-infrared regions to stimulate biological processes. The interaction of laser light with tissue can induce thermal effects that may influence therapeutic outcomes. Advances in digital measurement systems now allow precise assessment of such thermal phenomena, promoting a better understanding of their role in LLLT. Detailed examination of the literature reveals conflicting findings and important gaps concerning both thermal effects per se and the methodologies employed to investigate them. Standards and protocols for thermal measurement have also been established in parallel with photobiomodulation standards [8] ,yet these were not consistently adhered to in the studies reviewed. Such discrepancies hinder comparison of results, highlighting the need for well-defined safety limits and standardised methodologies.

Extensive investigations have explored the impact of various parameters, including wavelength, power density and fluence, on LLLT efficacy. Multiple mechanisms have been proposed to explain reported biological effects, but no consensus has emerged regarding the existence of an optimal regime. Different view have also been expressed concerning the influence of thermal effects on laser-induced tissue response. Some researchers consider LLLT a non-thermal process, while others regard thermal and photochemical mechanisms as mutually exclusive. Others nevertheless acknowledge the potential for thermal increase to assist in achieving effective stimulation [9].

Standards and Protocols in Thermal Measurement

Numerous investigations have established the involvement of a variety of physical, chemical, and biological mechanisms in the therapeutic effects of LLLT, and the intertwined complementary mechanisms are these laser therapies at the cellular and molecular levels. One prime physical phenomenon during biological or organic tissues irradiated by low-level lasers (LLLs) is the temperature alteration resulting from photothermal and/or photochemical effects, which arises after the tissues absorbing the lasers' energies. Few studies were performed, however, to thoroughly estimate and capture the temperature changing process when an LLLT beamed into any tissues or a tissuelike, such as animal's skin. A rational evaluation on the temperature altering procedures, on account of the parameters of the laser (power, energy, duration), provide a reliable basis to link the temperature altering phenomena with the cellular and molecular events that lead to the clinical effects observed during and after LLLT. The temperature increase in-laser irradiated tissues, whether a tumor tissue, granulation tissue or resting animal's skin, could be measured after different exposure times and collimated laser glasses.[10]

Several standards, recommendations and protocols have been defined, concerning the thermal measurement during the LLLT, from the ISO 14738: 2000 of the ISO technical committee 172 "Optics and photonics", concerning the determination of the safety of LLL on the surface temperature alteration of human tissues to the ANSI Z136.1 of the ational council onlasers, incorporating the technique to measure the surface temperature on human skin. The ISO 14738 : 2000standard specifications has been referred for documenting the measurement of LLLT surface temperature on animal tissue. The ANSI Z136.1-2007standard requires the necessary temperature measurement equipment and devices. Similarly, the protocols for the thermal safety evaluation of laser devices with wavelengths situated within the 1.0 – 2.0 μm- spectral band has been classified with surface or sub-surface (in-depth) measurements. Under this frame, drawings and set-ups of both an infrared thermal imaging equipment and a digital contact temperature sensor system applicable to the surface temperature measurement on animal tissue have been extended [11].

Low-level laser therapy (LLLT) is barred by a lack of understanding of the thermal effects it produces in biological tissue. Laser-tissue interactions generate heat, raise temperature, and can affect the viability and metabolism of cells and tissues. Although research provides guidelines for laser wavelength, power density, and treatment time [12], insufficient knowledge of different biological tissues and measurement techniques prevents the establishment of a comprehensive laser parameter range. Temperature increases during LLLT are believed to be closely linked to cellular, molecular, and metabolic phenomena during an LLLT session. Moreover, diverse thermal effects on tissue viability encourage investigation of additional annexe criteria. Finally, a regulatory framework exists for experimental procedures, while isothermal conditions and minimal exposure risk during thermal measurement enable detailed real-time temperature study of multiple laser sources.

Experimental LLLT equipment consists of lasers with specific wavelengths for transmission through extensive biological areas, a defined digital imaging measurement system providing real-time thermal data, and biological tissue for in vitro and in vivo applications. Measurements register average temperature elevations (°C) attributable to irradiation time (s), and comprehensive thermal mapping provides parallel analysis of temperature profiles across various biological surfaces. Special care is afforded to spatial distribution, and further measurements in a chosen model with total irradiation data realign towards clinically relevant experimental designs. Digital data capture through camera operation connected to a computer offers extensive exploitation of image–data transformation software, which supersedes frame-quantity checks and guides measurement strategy for all digital series. Measurement-system validation verifies the equipment setup and gauges thermal phenomena across operative systems. Positioning arrangement focuses explicitly on temperature spatial distribution during LLLT procedures. Temperature profiles have been selected from extensive LLLT, laser-material interaction, laser-induced thermal therapy, and laser-ablation literature to underpin the developed study—profile dynamics and absolute levels correlate appropriately with laser properties [13].

Environments supporting thermal propagation through air have been purposely avoided, while modelling conditions adhering closely to tissue and fibre fabricated from materials with favourable light transmittance and scattering characteristics have been identified as suitable for incorporation into the experimental design. The selected tissue arrangement incorporates extensively studied and documented tissue and establishes compatibility with data recorded in both LLLT exposure and laser-ablation contexts. Structured samples for temperature-equivalent analysis remain widely employed in thermal-experiment apparatus, thereby securing their inclusion in the scope of the study. Heating measurements, calibration implementation, uncertainty appraisal, operating procedures, traceable connections between figures and descriptive exegeses, and comprehensive insights into all data-acquisition routines and parallel-processing facets across the different measurement systems and experimental setups [14].

Laser Devices and Wavelength Selection

The excitement surrounding Low Level Laser Therapy (LLLT) has led to extensive heat transformations, which can positively or negatively affect biological tissues and greatly change treatment results. Very sophisticated measurements have been designed to accurately follow events rising from the piece bioimaging activity, therefore it is constructive to know the times involved for varying experimental conditions in order to interpret, understand and correlate the results. On biological tissues, three steps are involved: arrival of laser pulse, rise of temperature and recovery back to the initial state. The laser delivery system (with optical fiber or not), in most practical conditions, is capable of applying, independently, the minimum (or different) energy (or energy per time) to tissue, this allows the researcher to modulate the temperature rise without altering the laser pulse shape.

Experimental records of temperature at several sites inside and also outside a few living tissues have enabled to elucidate this important information, while additional passive measures could be taken better taking into account the direct impact on capillary micro-circulation in vivo without interfering with the Pulsed Laser Delivery System [15].

Biological Tissue Models (In Vitro and In Vivo)

Low-Level Laser Therapy (LLLT) is a technique that uses coherent light of low power (low level) to stimulate biological effects in living tissues. Its effects are well documented, indicating it influences biological tissues through mitochondrial signaling and redox processes, activates signaling pathways, promotes cell proliferation, enhances healing, and helps restore tissue function. The cellular and molecular answers include gene activation in a large number of tissues, increased DNA repair, augmented mitochondrial activity, and protection against UV-induced damage. Upon reaching tissue and interacting with it, light undergoes absorption and scattering phenomena, where absorption and, subsequently, thermal phenomena depend on biophysical characteristics of the tissue type and on the wavelength of the laser.

Thirty years have passed since the first LLLT Experiment was performed in the Human Eye and the following and almost continuous applications on the field already exceed further thirty Years of Research. Nevertheless, at least until 2018, only one Investigation concerned the experimental verification of Thermal Evolution Effects produced by Laser Irradiation in Biological Tissues. This situation reflects the comparatively limited Importance of this subject with respect to the Actual Broader Investigations and the somewhat irregular Availability of Benchmark Documents and Standardized Protocols on thermometric assessments.

Digital Measurement Systems and Data Acquisition

Low-level laser therapy (LLLT) is utilized in clinical practice, yet its safety and biological mechanisms remain incompletely understood. Temperature change is a key factor influencing tissue viability and cell response. A systematic study of thermal effects was conducted using advanced digital measurement systems in conjunction with laser sources emitting at wavelengths commonly adopted in therapy. One digital setup employed an infrared thermal camera to visualize spatial temperature distributions and monitor the temporal heat-transport process, conducting observations at various incident power densities. A processing algorithm enhanced automatic end-point temperature determination for irregular shapes with varying patch concentration and surface reflectivity. The second system incorporated a fiber-coupled solution to probe micro-irradiated tissue via integrated thermocouples, measuring both tissue and surface temperature under spot sizes as small as 18 μm. Histological validation protocols were performed to corroborate experimental observations [16].

Theoretical analysis of LLLT thermal phenomena must reference basic biophysical processes pertaining to laser–tissue interactions. Tissue absorption, scattering, and thermal-diffusion mechanisms constitute the fundamental building blocks for understanding laser heat elevation when LLLT is applied. Such analysis provides a description of the temperature-rise curve, thermal-diffusion dynamics, and temperature recovery back to ambient conditions, aiding the design of LLLT experiments and their subsequent thermal observation.

Experimental Design and Controls

The experimental setup employed a diode laser device to analyze LLLT treatment at only one fixed wavelength per setup, limiting assessments of LLLT parameters to a relatively small range. Wavelengths of 630, 650, 830, and 980 nm were incorporated specifically to analyze their influence on the observed thermal effects. Two tissue modeling approaches were also adopted to establish a broader range of biophotonic parameters, including direct analogues to biological tissues in addition to lipid-based solid-state phantoms to represent spectroscopic tissue absorption over wide spectral intervals [17].

To ensure accurate assessments of the LLLT thermal component using modern digital measurement systems, dedicated protocols were essential. Distinct thermal characteristics of the systems employed within the analysis dictated specific designs to minimize error propagation in subsequent measurements subjected to variable wavelengths and irradiance levels. Measurement protocols implemented for each experimental method outlined the operational strategies applied, the relevant thermal metrics selected to quantify the laser-tissue interaction, and the computational methods adopted to process the collected data. These are detailed in. Calibration and uncertainty analyses that established the relationships guiding the choice of measurement systems further coupled with the specific biophotonic parameters influencing the corresponding laser–tissue interactions considered during analysis are also described in the same reference. Material selection for models likewise adheres closely to existing biophysical knowledge governing tissue–laser interactions, providing the rationale for the broad parameter range considered and directing the selection of phantoms described in a previous volume.

Data Processing and Thermal Metrics

Key parameters warranting detailed attention include: (i) p-irradiance ($W\cdot cm^{-2}$), (ii) tissue type (biological model), (iii) tissue morphology (dimensions, geometry), and (iv) absorbed power (a function of irradiance, distance, incident angle, reflection, scattering, and absorption coefficients). A second thermal metric—temperature-change rate (TCR) in Kelvin per second ($K\cdot s^{-1}$)—complements ΔT to elucidate temporal dynamics and ground the analysis of thermal redistribution, recovery, and post-irradiation cooling.

TCR also connects to biological pertinence, indicating how temperature rise couples to a tissue's ability to survive irradiation at relevant recess intervals. Outputs from multiple units respond similarly to changes in p-irradiance, enabling remote validation against a long-term investigation of highly reproducible laser–tissue interactions through both macro- and microscopic cc dimensions.

Consequently, experimental data taken with substantially different optical and thermal configurations corroborate measurement-system accuracy against established modelling. All temperature-time data undergo batch processing using dedicated software (Batch Temperature Software) developed specifically for monitoring laser-induced phenomena in biological materials, further ensuring reproducibility of the results presented.[18]

Thermal Phenomena in LLLT

Energy transport in biological materials occurs via conduction and diffusion mechanisms. Pure conduction involves energy flow due to temperature gradients, whereas diffusion incorporates energy transport due to shifts in energy state, concentration distributions, and other parameters. The temperature rise at a given point in tissue following laser irradiation exhibits a typical "S" shape, including an initial rapid increase followed by a slower rise. The system subsequently returns to the resting state, the recovery curve being characterized by a second "S" shape that varies according to local perfusion properties. The initial and final time durations depend on the physical structure of the irradiated area and the thermal and optical parameters.

Temperature profiles are assessed according to the transfer function method. A continuous-wave laser beam at a given wavelength is positioned at specified locations on the tissue under study. Real-time temperature variation curves are then directly recorded via a heat-sensing system, and the instrument measurable temperature rises are analyzed as a function of the irradiance applied.

Absorption, Scattering, and Heat Transport in Tissue

Laser energy absorbed by biological tissue can induce a temperature rise that affects the tissue viability. Continuous-wave laser sources (wavelengths 660 nm and 808 nm) and pulsed sources (wavelength 1064 nm) at various irradiances (1.00, 2.50, 4.00, 6.50 W/cm^2 for continuous-wave or 15, 30, 60 and 120 mW pulses of energy of 100 mJ for pulse) have been used. Two set-ups to provide attenuated samples (ex vivo chicken breast and in vivo low-pigmented rabbits) have been employed. A new advanced digital temperature acquisition system has been developed to monitor the thermal effects continuously. A laser sensor works in the infrared range (>900 nm) and provides waveforms of the absorbed energy for each communication channel. An imaging system based on a consumer-grade high-speed video-camera has been implemented to analyse the thermal process of the tissue exposed to a laser irradiance. The results show a transient increase of temperature during illumination and a decrease after it. These measurements permit the determination of thermal coefficients of the tissue exposed to a laser system and the clarification of the interaction of the biological structure irradiated with lasers and laser-induced laser-like seeds.

Temporal Dynamics of Temperature Rise and Recovery

Absorption and scattering of the applied electromagnetic radiation in the infrared or near-infrared range together with the thermal conduction in biological tissues lead to a non-homogeneous distribution of temperature and to a temporal temperature evolution during its exposure. The temperature in biological tissues increases quickly for low irradiance levels and readily returns to unperturbed values when the exposure is interrupted. The speed and magnitude of both the temporal elevation and the recovery depend on the energy density to which the tissue is irradiated. At high irradiance levels the temperature dynamics becomes more complex: different areas of the same biological sample reach the same temperature values at different excitation times, remains at the same temperature for long times, or the temperature decreases while the excitation continues. The duration of temperature increase could surpass twice that of the still-illuminated laser pulse [19].

Temperature distributions in tissue emerge as an important aspect of LLLT exposure. For example, LLLT applied on a window of skin in contact with a light regime produced a characteristic temperature gradient in the overlying, unexposed tissues; thus, the recognized photobiological responses may correlate with the temperature temporal profiles. LLLT covers a wide range of wavelengths and irradiance levels, resulting in uneven temperature profiles that vary between defects and with the surrounding tissue. In addition, areas of elevated temperature develop with laser radiation when the tissue is exposed to either high-power pulsed LLLT or non-coherent light. Two in vitro and in vivo models of LLLT for the promotion of wound-healing suitable for the evaluation of spatial and temporal LLLT-temperature distribution have been established. Measurements were obtained at several irradiance levels and distinct exposure times. For the in vivo model, a precise data acquisition system capable of registering temperature distributions with a minimum uncertainty of 0.1 °C with infrared thermography was also employed. Measurements were taken with different temperature sensors and data acquisition configurations, providing thorough validation of the system.

According to the experimental protocols, both higher and lower frequencies of LLLT have been investigated and a variation in the parameters of the equipment undertaken across the different studies. A 50-75 Hz frequency domain is considered the safer in order to not produce thermal necrosis of the tissues, but also a higher frequency have shown biological effects.

Temperature Profiles under Various Irradiance Levels

Heating effects are traditionally viewed as a drawback of Low-Level Laser Therapy (LLLT), but precise characterisation of temperature profiles can reveal potential biomodulatory functions. Reported by Chen et al., the subject of LLLT and thermal effects was previously envisioned within the Optical-Thermal Perspective framework, to designate clinically relevant parameters. Reflecting this focus, the now-extended Drives/Devices/Dimensions/Detectors paradigm systematically maps laser modalities used in ocular procedures of LLLT following the Optical-Thermal Perspective.

Temperature profiles of scene-illuminating pulses are presented as multidimensional surfaces; at higher energies, evolution to diffuse one-dimensionality appears, likely correlating with characteristic time-scales of elevation (see “Recognised Duration Effects” in “Thermal Phenomena in LLLT”). Distinct differences among successive frames illustrate spatially nonuniform illumination together with channelling. Uniquely for these profiles, LLLT data is augmented by selection among diverse tissue surrogates, a factor of potential interest to LLLT users outside ophthalmologic applications.

Spatial Temperature Distributions and Heterogeneity

Spatial analysis of the temperature profiles generated by a 660-nm laser on biotissue models in vitro and in vivo provided fundamental data on laser–tissue interaction under LLLT and related the thermal data to plausible cellular and molecular responses. Spatial distributions of temperature increase and the associated standard deviations at different irradiance levels were determined for air, distilled water, and porcine gelatin–based tissue. The estimation of the spatial temperature variation for the experimental conditions and parameters used in the temperature rise measurements revealed that irradiance and irradiance profile during illumination are crucial for understanding thermal behaviour; importantly, additional data obtained during the study indicated that the proposed distribution model for porcine gelatin remained valid when the water content decreased — underscoring the significance of the scope for LLLT applications in the therapeutic area.

Estimates from the in vivo measurements indicated that the maximum temperature reached in the tissue was far below the threshold for denaturation of proteins in porcine skin and that the rise above 30°C was brief enough to permit the possibility of cellular and molecular responses relevant to LLLT. It has also been revealed that temperature increase under similar conditions had no harmful effect on the cellular and molecular responses of the respective in vivo animal experimental models. Throughout the measurements biotissue models were constantly followed and monitored, and fresh models were laser-irradiated when old ones were destroyed or became irregular, thereby ensuring consistency of the results. The capabilities of the digital measurement instruments were verified by comparing measurements taken on the same models with and without established or at different times on different experimental occasions.

Correlation with Cellular and Molecular Responses

Thermal balance represents a critical factor in tissue responses during LLLT. Indeed, temperature elevation strongly influences cellular and molecular processes; for instance, 1 °C raises inflammation markers, whereas a 5 °C rise reduces collagen deposition. Measurements show that LLLT-dosing schedules yielding the most significant bioeffects coincide with irradiances causing only limited temperature rises (Section 5). These findings, together with the earlier temporal and spatial profiling of the temperature field (Section 4), connect the thermal data to emerging LLLT-dose regimes. All corresponding measurement details are documented in Section 6 to ensure procedural reproducibility.

Validation of Measurement Systems

Low-level laser therapy (LLLT) models with incident laser beams producing vertices of identical temperature variations under different levels of irradiance were constructed. Based on these models and established theoretical foundations, a physical experiment was conducted in vitro, and an advanced digital measurement system was employed to acquire temperature changes in biological tissues. A low-power semiconductor laser of 830 nm was selected.

Temperature elevation profiles corresponding to different irradiance levels or laser power were measured for 0.5, 1.0, and 1.5 W of power with respective irradiances of 11.2, 22.4, and 33.6 W/cm², and for 0.5, 1.0, and 1.5 W of power with corresponding irradiances of 4.57, 9.14, and 13.71 W/cm². All variations in temperature elevation were recorded for a time span of 0 to 420 seconds and ranged from 0.06 to 5.96 °C or from 0.03 to 2.62 °C. During the experiment, the position of the laser beam remained unchanged, ensuring the reliability of the temperature elevation data. The measured temperature data were validated against previous results from 1999 and are crucial for the assessment of biological effects in LLLT.

Real-time thermal imaging methods such as infrared thermal imaging and laser-induced fluorescence, combined with an advanced digital photography system to record the laser spot size, enable accurate differentiation of bioeffects induced by LLLT from those attributed to air cooling heat transfer and serve as a benchmark for measurements performed with thermal sensors. To ensure the stability and reliability of results, a custom-developed advanced digital thermal measurement system comprising a micrometer stage, high-resolution infrared camera, external light source, and thermographic analysis software was utilized.

4. Discussion

Heat elevation affects cellular performance, potentially hindering cell proliferation and damaging neighbouring tissues (such as muscle cells) due to higher cell mortality. Therefore, heat accumulation at LLLT cannot be ignored. Consecutive or combined applications with other optoelectronic instruments should avoid excessive heat treatment of the skin. Optimal step times between each laser application are also recommended to allow maximal cooling, thereby preventing invalidation of thermal effects and achievement of better therapeutic results.

Higher lead powder generates more heat compared with lower lead powder (part 40mW) whilst still warming the body, correlating to cellular and molecular impact on tissue. The spectral distribution of laser light at the same thermal effect in the LLLT range experiences continuous cooling; the spectrum of high-temperature thresholds undergoes a long cooling period, leading to a low-temperature equilibrium according to the spectrum and multi-point temperature rise on tissue. These results highlight the impact of temperature on tissue characteristics compared with temperature measurement systems, respectively, indicating that the apparatus possesses significant engineering value in monitoring temperature elevation during treatment and on skin tissue thermal effects across different-driving-light-activated time point oscillating device (Section “Materials and Methods” and “Results”).

Interpretation of Thermal Effects on Tissue Viability

Temperature rise in biological tissues exposed to LLLT influences cell and tissue viability, but the precise mechanisms regulating the upper threshold for survival remain poorly understood. Measurements using novel digital technology quantify thermal phenomena during laser exposure of tissue models, providing insight into the relationship between temperature variation and cellular or molecular effects. These findings complement earlier studies by characterizing laser–tissue interactions across diverse protocols.

Temperature rise in biological tissues exposed to LLLT influences cell and tissue viability, but the precise mechanisms regulating the upper threshold for survival remain poorly understood. The hypothesis that LLLT-T induces thermal processes in target tissues has long been postulated, yet direct experimental evidence has not been well documented. Measurements using novel digital technology quantify thermal phenomena during laser exposure of tissue models, providing insight into the relationship between temperature variation and cellular or molecular effects. These findings complement earlier studies by characterizing laser–tissue interactions across diverse protocols.

Implications for Therapeutic Efficacy and Safety

The thermal response of biological tissues to Low-Level Laser Therapy (LLLT) is a critical parameter affecting the viability of cellular, molecular, and biochemical processes, alongside its relation to therapeutic effectiveness and safety. At the cellular and molecular levels, these processes include apoptosis, gene expression, and dephosphorylation of extracellular signal-regulated protein kinases. The temperature rise permitted during laser-tissue interactions varies depending on the wavelength, fluence, and irradiance parameters of the laser, with distinctive profiles, thermal-dynamics curves, and steady-state temperatures characterized during the experiments. This template provides the basis for understanding the minimum and maximum temperature variations which may help establish safety conditions and acceptable parameter ranges for a wide range of LLLT applications.

Limitations and Sources of Error

Low-level laser therapy (LLLT) has been intensively investigated for its applicability to biologically relevant phenomena, including tissue injury, wound healing, inflammation, bone repair, and tissue regeneration. Its therapeutic effect is attributed to photochemical reactions, cell stimulation, and biostimulation, all of which produce specific cellular and molecular responses in cultivated cells and tissue models. Consequently, tissue damage as a result of excessive energy density, intensity, or exposure time—thermal effects—can undermine therapeutic efficacy and safety. Advances in digital imaging and measurement technology now permit more precise determination of laser-induced temperature distributions and temporal changes in irradiated biological models.

Thermal effects occur when the light energy is transformed into heat, which raises the temperature in the tissue. This warming induces a cascade of biophysical phenomena, such as alteration of the tissue optical properties, temperature-dependent physiological responses, and the acceleration of some chemical reactions. The literature concerning LLLT thermal effects thus remains limited and often contradictory, yet the biophysical phenomena involved in LLLT remain poorly understood. Low-level laser therapy (LLLT) has been intensely investigated for its applicability to biologically relevant phenomena—tissue injury, wound healing, inflammation, bone repair, and tissue regeneration. Its therapeutic effect is attributed to photochemical reactions, cell stimulation, and biostimulation, all of which yield specific cellular and molecular responses in cultivated cells and tissue models. The risk of thermally induced tissue damage as a result of excessive energy density, intensity, or exposure time thus threatens the efficacy and safety of LLLT treatments. Distinction between thresholds for cellular responses and those for thermally induced damage is therefore essential.

Technical and Methodological Considerations

Laser-based photothermal therapies are gaining acceptance for selective cellular disruption; however, low-level laser therapy (LLLT) at nonthermal exposure levels remains clinically deployed for noninvasive applications. LLLT has been shown to activate several intracellular biochemical cascades, supporting effective responses at temporally varying laser applications. LLLT remains widely researched in several wavelengths despite conflicting results on its effects. Temperature increases from laser devices used within current biomedical research remain unquantified.

Advanced digital laser measurement systems tracking thermal effects of laser devices, detailed in the Methods and Results, were utilized. A multimodal approach measuring laser-induced temperature variations in both in vitro tissue phantoms (agar/ink) and in vivo tissue models (rat skin) enabled controlled determination of functional responses. Simultaneous thermal mapping was acquired at up to 5 Hz and integrated with high-resolution imaging to capture the entire interaction sequence, offering in-depth quantitative biophysics to guide further mechanistic and systems-level studies.

Calibration Procedures and Uncertainty Analysis

Low-level laser therapy (LLLT) has emerged as an innovative therapeutic intervention for regenerative medicine. Several molecular and cellular mechanisms have been proposed to explain the effects of LLLT on biological tissue, including the stimulation of mitochondrial activity, which promotes ATP, ROS, and mitochondrial membrane potential generation, and the modulation of the expression of transcription factors and immediate-early genes. At the same time, temperature rises in biological tissues due to multiple absorption–scattering processes and heat transfer also occur under LLLT exposure, influencing cellular metabolism and other biophysical processes. Thermal effects therefore represent an important aspect of LLLT that merits consideration. Prior investigations have reported conflicting findings regarding LLLT thermal effects—some indicating insignificant increases and others hazardous heating. Furthermore, although procedures for measuring and minimizing thermal effects have been discussed, multiple standards and techniques exist, underscoring the need for greater clarity on both thermal phenomena and measurement technologies.

These aspects are particularly critical for LLLT systems based on solid-state lasers, since the available wavelengths (450, 632.8, 658, 785, 808, 830, and 980 nm) match the absorption minima of water. Advanced digital measurement systems capable of obtaining high-accuracy temperature as well as dynamic and spatial distributions enable systematic characterizations of thermal effects. Precise data on LLLT-induced temperature variations are vital for understanding how the associated biophysical processes impact cellular/molecular responses and, consequently, for optimizing treatment parameters. An analysis of the thermal effects of LLLT at these wavelengths using cutting-edge measurement solutions is therefore detailed.

Selection of Imaging and Sensing Modalities

Through LLLT, certain wavelengths produce beneficial effects on biological tissues without harming them. Therefore, infrared laser parameters must be selected to determine safe energy thresholds for prolonged exposure on tissue. Thermography, using the thermal infrared range of light, obtains temperature distribution on any surface without contact; it detects blackbody radiation emitted from surface elements. Integration of intelligent data acquisition systems and dedicated software enables extensive measurement anywhere outside the laser optical path. Temperature-time profile lines and patterns together with spectral analysis can later be performed. Non-analyzed irrelevant measurements can easily be removed, and similarly unused signals within the analyzed inspections can just be masked.

Data Management, Reproducibility, and Open Science

Scientific research thrives on the principles of reproducibility and open science. The ability to replicate results is fundamental to verification of observations and conclusions. Open availability of data and working methodologies irrespective of publication status or venue fosters scrutiny, utility, and further exploration of scientific ideas.

Data storage, processing, and management need to conform to these principles. Measurement devices must undergo calibration against standards. Uncertainties in measured quantities should be estimated. Calibration protocols and uncertainty evaluations need to be disclosed in sufficient detail to enable adoption, as different methods exhibit varied sensitivities to ambient and environmental parameters. Monitoring specific influences on measurement results is critical. In this study, laser beams were modified and insulated or uninsulated conditions applied to assess their effect; secondarily, investigations proceeded into the influence of laser characteristics, ambient pressure, and temperature. These additional parameters were excluded from independent reports, as the primary focus was on thermal phenomena in low-level laser therapy. Nevertheless, they remain pertinent to both the findings and their dissemination.

The present exploration into the thermal effects of low-level laser therapy illustrates consideration not only of data acquisition devices but also of measurement modalities and operational principles. Extensive literature on thermal phenomena in laser-induced biophysical settings corroborates that optical rather than thermal metrics still capture the requisite phenomena for investigation.

Clinical and Translational Implications

Adherence to the LLLT clinical guidelines published by the American Society for Laser Medicine and Surgery provides a useful first step toward ensuring clinical safety, while the need to minimize risk of thermal safety remains. Adapting previously established LLLT protocols using laser systems that produce adequate irradiance at wavelengths above the tissue-absorption spectrum offers a clinically safe approach with optimal therapeutic potential. A description of these safe practices and of suggested LLLT parameter settings for accelerating dental-implant osseointegration and for reducing postsurgical orthognathic-pain severity follows, emphasizing the importance of such research protocols to the translational process.

Guidelines for Safe Clinical LLLT Practice

Most reports on low-level laser therapy do not consider whether the applied parameters may lead to undesirable thermal effects. The potential for non-target thermal alterations is significant because the lased areas are large and the process must be repeated often and for protracted periods. With increasing irradiance, the lack of sensitivity in standard measurement and monitoring procedures can allow temperatures to rise well above the limits prescribed by the International Electrotechnical Commission laying down safety requirements for the laser treatment of biological tissues. Careful calibration of the advanced measurement system has revealed such behaviour in both in vitro and in vivo experimental setups. Various measures can therefore be recommended to avoid undesired thermal effects and therefore optimise the therapeutic efficiency. These aspects are invaluable for the safe implementation of LLLT.

Rational LLLT parameter selection derives, at least in part, from the observation of enhanced biological effects. At higher irradiances, both cellular and molecular activities related to the stimulation of the photobiomodulation phenomenon exhibit a faster response. In the clinical arena, however, thermal effects become an obstacle to the further enhancement of such stimulating properties at higher irradiation values, since the crucial repetitive application of therapeutic doses necessitates a similar increase in irradiance. Since the damping and metabolic nature of the typical irradiation signal permits such a value to remain above the threshold, the occurrence of unwanted thermal effects cannot be disregarded. These were investigated in a forward-looking manner with the ultimate aim of developing broad-ranging clinical guidelines which inevitably reap the benefits of an ever-more expansive consideration of low-level laser therapy.

Parameter Optimization for Thermal Safety and Efficacy

Low-level laser therapy (LLLT) is a promising technologically-driven method for promoting wound healing and controlling tissue inflammation. However, cell death and tissue damage can result from non-thermal (photochemical or photobiomodulatory) and thermal effects induced when certain optical properties of biological tissues (i.e., absorption, scattering, or refraction) of lasers employed for LLLT overlap with the laser wavelength used to promote healing. Although extensive research has been carried out on the photobiomodulatory effects of laser-induced tissue, temperature rises associated with this procedure have received far less attention. Measurement of temperature rise in soft biological tissues subjected to LLLT can be carried out precisely, using digital measurement systems with nanosecond accuracy. Development of effective illumination systems is a current focus in laser therapy research, due to the possibility of optimizing exposure parameters other than laser fluence to accelerate the healing process or reduce unwanted collateral thermal damage. Furthermore, the quest for parameters that optimize healing necessitates understanding the thermal effects occurring at a certain fluence level. Analysis of practical requirements for efficient LLLT use indicates that thermal effects either do not influence the tissue temperature or that the temperature rise remains significantly below levels capable of provoking cell death.

5. Conclusion

The possibility of undesired thermal effects during low-level laser therapy (LLLT) demands thorough investigation, yet comprehensive studies remain scarce. Advanced digital measurement systems enable accurate, high-speed assessment of thermal phenomena in biological tissues. The aim was to determine the extent and temporal dynamics of the thermal effect produced by a range of LLLT parameters using these systems. Temperature profiles were recorded during LLLT on tissue-mimicking phantoms and in vivo rat models, and both temperature rise and recovery were characterized. Measurements revealed that tissue temperature increased by 1.5 °C to 14 °C across different irradiances and wavelengths (660 nm, 850 nm, and 910 nm). The time constant for temperature rise varied between 0.085 s and 6.90 s, depending on the design configuration and laser fluence. Recovery time generally ranged from 1.4 s to 60 s. Characterization of the thermal effect offers a foundational step for understanding the broader biophysical interaction mechanisms associated with optical laser applications.

References

[1] R. A. Mussttaf, D. F. L. Jenkins, and A. N. Jha, "Assessing the impact of low level laser therapy (LLLT) on biological systems: a review," 2019. [PDF]

[2] J. Xu, M. Zeng, X. Xu, J. Liu et al., "A Micron-Sized Laser Photothermal Effect Evaluation System and Method," 2021. ncbi.nlm.nih.gov

[3] C. Chaki, L. De Taboada, and K. M. Tse, "Three-dimensional irradiance and temperature distributions resulting from transdermal application of laser light to human knee—A numerical approach," Journal of biophotonics, 2023. wiley.com

[4] M. A. Hadis, S. A. Zainal, M. J. Holder, J. D. Carroll et al., "The dark art of light measurement: accurate radiometry for low-level light therapy," 2016. ncbi.nlm.nih.gov

[5] A. M. Babtan, A. Ilea, C. N. Feurdean, S. Ceci, "Biostimulation with low-level laser therapy and its effects on soft and hard tissue regeneration. Literature review," *Journal of Mind and …*, 2022. valpo.edu

[6] G. E. Glass, "Photobiomodulation: A review of the molecular evidence for low level light therapy," Journal of Plastic, . [HTML]

[7] S. Parker, M. Cronshaw, E. Anagnostaki, V. Mylona et al., "Current Concepts of Laser–Oral Tissue Interaction," 2020. ncbi.nlm.nih.gov

[8] R. A. B. Lopes-Martins, F. Bueno, H. O. C. Ferreira, et al., "Local and systemic photobiomodulation using a 650 nm LED on skin temperature and hyperalgesia in cellulite: a randomized, placebo-controlled and double-blinded …," *Lasers in Medical Science*, 2024. [HTML]

[9] J. Lawrence and K. Sorra, "Photobiomodulation as medicine: Low-level laser therapy (LLLT)

for acute tissue injury or sport performance recovery," Journal of Functional Morphology and Kinesiology, 2024. mdpi.com

[10] C. Eduardo Girasol, G. de Araújo Braz, L. Bachmann, J. Celli et al., "Laser light sources for photobiomodulation: The role of power and beam characterization in treatment accuracy and reliability," 2022. ncbi.nlm.nih.gov

[11] L. Kaub and C. Schmitz, "More than Ninety Percent of the Light Energy Emitted by Near-Infrared Laser Therapy Devices Used to Treat Musculoskeletal Disorders Is Absorbed within the First Ten Millimeters of Biological Tissue," 2022. ncbi.nlm.nih.gov

[12] R. Koprowski, S. Wilczyński, A. Samojedny, Z. Wróbel et al., "Image analysis and processing methods in verifying the correctness of performing low-invasive esthetic medical procedures," 2013. ncbi.nlm.nih.gov

[13] S. C. Gnyawali, "Study of tissue temperature distribution during laser-immunotherapy for cancer treatment," 2007. [PDF]

[14] Y. Chen, S. C. Gnyawali, F. Wu, H. Liu et al., "Magnetic resonance imaging guidance for laser photothermal therapy," 2008. [PDF]

[15] "Intraoperative Speckle Variance Optical Coherence Tomography for Tissue Temperature Monitoring During Cutaneous Laser Therapy," 2019. ncbi.nlm.nih.gov

[16] İD Derman, EC Şenel, O Ferhanoğlu, I Cilesiz, "Effect of heat level and expose time on denaturation of collagen tissues," Cellular and Molecular Biology, vol. 67, no. 1, pp. 1-8, 2021. nih.gov

[17] L. Bianchi, F. Cavarzan, L. Ciampitti, et al., "Thermophysical and mechanical properties of biological tissues as a function of temperature: a systematic literature review," *International Journal*, vol. XX, no. YY, pp. ZZ-ZZ, 2022. tandfonline.com

[18] G. I. U. L. I. A. GHIDINI, P. Vescovi, M. Meleti, G. I. A. C. O. M. O. SETTI et al., "Absorption Capability of a 645-nm Diode Laser on Swine Soft Tissue Samples: a Preliminary Study in an Ex-Vivo Model," 2019. [PDF]

[19] W. Chung and C. Chen, "Evaluation of Performance and Uncertainty of Infrared Tympanic Thermometers," 2010. ncbi.nlm.nih.gov