Dynamics of the temperature field in biological tissues under local laser heating

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Received May 5, 2025
Revised June 20, 2025
Accepted June 20, 2025

A series of experiments on skin modification with a laser beam with a wavelength of 1560 nm and a diameter of 200 and 600 μm were conducted. Threshold values of laser radiation power, which causes the denaturation of skin collagen, were established. Using an integrating sphere and a thermal imager the optical and thermophysical parameters of the tested samples used were determined. These parameters were used to calculate both the light and temperature fields. It was shown that it is necessary to take into account the light scattering coefficient and the scattering anisotropy factor when calculating the dynamics of the temperature field in the skin.

Keywords: temperature field, fractional photothermolysis, Monte Carlo methods, laser radiation, skin.

DOI: 10.61011/TPL.2025.09.61827.7990

Laser irradiation has become an indispensable technique for targeted modification of biological tissues in regenerative medicine. It enables precise local alteration of tissue structure and composition, stimulating regeneration of the affected region and activating regenerative processes in surrounding tissues [1]. In cosmetology, local treatments facilitate rejuvenation of aging skin and significantly reduce scarring caused by trauma or acne [2]. Typical tissue alterations involve heating to the coagulation temperature within a small region with a diameter of 200−300 μm and a depth up to 500 μm. Visible improvements occur shortly after healing begins, without leaving noticeable marks from the laser treatment itself. To ensure effectiveness and safety of the procedure, it is crucial to select optimal laser exposure parameters — such as radiation wavelength and power, duration, and laser beam diameter [3]. Estimating the dimensions of the coagulation zone requires understanding the dynamic behavior of the induced temperature field, which strongly relies on the optical and thermophysical properties of the tissue [4]. The primary objective of this study was to determine the optical and thermophysical parameters of the skin, then considering the aforementioned parameters and light scattering effects simulate the transient light and temperature fields induced by a narrow laser beam during fractional laser thermolysis and validate these findings against experimental observations regarding the threshold power required for collagen denaturation of the skin.

An LSK 1.56 laser scalpel(IRE-Polus, Russia) with a radiation wavelength of 1560 nm and adjustable power levels up to 5 W was used in experiments. Quartz fibers of 200 and 600 μm in diameter (numerical aperture 0.22) delivered the radiation to the test samples. Samples of skin tissue from the inner part of the rabbit's auricle were examined. Fresh ears from adult rabbits were obtained from a rabbitry no later than 5 h after slaughter. After mechanical hair removal, the skin was separated and divided into segments measuring roughly 20×20 mm each. A total of 40 samples were prepared. The thickness of the skin ranged from 300 to 500 μm across the auricular surface, and sections of intermediate thickness (approximately 350−400 μm) were selected for the experiments, the error was ± 30 μm. The water content of skin samples was measured via freeze drying and thermogravimetric analysis, yielding value of $c_w = 74 \pm 2\%$. Since water is the main absorber of radiation at 1560 nm, the absorption coefficient (μ_a) was approximated as proportional to the water faction ($\mu_a = \mu_w c_w$), assuming $\mu_w = 10.5\,\text{cm}^{-1}$ [5]. Consequently, the modeled absorption coefficient adopted a value of $\mu_a \approx 7.8\,\text{cm}^{-1}$.

An integrating sphere was used to determine scattering coefficient μ_s and scattering anisotropy factor g of the skin. Transmitted (T_d) and reflected (R_d) fractions of incident laser beam energy were measured. During experiments, the output end of the optical fiber was positioned at a distance of several millimeters from the sample surface. Additionally, the energy propagating through a diaphragm placed behind the sample (aligned with the beam's entry trajectory) was measured (T_c). The diameters of diaphragms (260 and 648 μm) were close to the diameters of optical fibers used to transport laser radiation. Experimental R_d, T_d, and T_c values were subsequently correlated with theoretical predictions derived from iterative adjustments of μ_s and g. Least-squares minimization techniques (Levenberg–Marquardt algorithm) were implemented to optimize agreement between empirical and computational outcomes.

Threshold values of laser radiation power inducing collagen denaturation were determined in a separate experiment. The skin samples with a thickness of 350 ± 30 μm were subjected to contact pulsed irradiation with a pulse duration of 70 ms using optical fibers with a diameter of 200

and 600 μm. The radiation power was varied from 0.5 W to 2 W with an increment of 0.5 W for a 200 μm beam and from 1 to 3 W for a 600 μm beam. A UP12-H power meter (Gentec Electro-Optics, Canada) was used to monitor the laser radiation power. Following laser irradiation, the skin samples were held in a trypsin solution with a concentration of 1.5 mg/ml at a temperature of 37 °C for 24 h. The tissue was then removed from the solution and rinsed with a 0.15 M NaCl solution. It is known that all proteins except native collagen undergo proteolysis under the influence of trypsin [6,7]. Trypsin treatment of skin samples with completely denatured collagen resulted in a complete dissolution of the matrix in the region of laser exposure and the formation of a hole that matched the shape and size of the laser spot. When tissues with a partial denaturation were subjected to trypsinization, tissue thinning was the only effect observed. The presence of holes in the tissue samples was determined using a GS-23 Digital Industrial Microscope (Fig. 1).

The dynamics of the temperature field on the surface of samples subjected to laser heating was monitored using a FLIR A655sc thermal imager with an additional FOL25 lens (FLIR Systems Inc., United States). The maximum frame rate was 200 Hz, and the spatial resolution was ~ 10 px/mm. The temperature measurement error and the temperature sensitivity were ± 2 °C and < 30 mK, respectively. The FLIR Research IR Max software was used to process the obtained thermograms. It allowed the determination of the dynamics of the maximum temperature on the surface, the average temperature in the irradiation region, and the temperature profile along a given line.

The Monte Carlo method was used to calculate the light field. The geometric parameters of the samples were simulated by a two-dimensional matrix. This was done by dividing a cylindrical sample into n_z layers of equal thickness and n_r circular segments with a certain radial step. An equivalent two-dimensional grid A with a size of $n_z \times n_r$ was thus formed. The generated distribution of coordinates of incoming beams on the sample surface was either uniform or Gaussian. In the latter case, the beam diameter was set at a level of $1/e$ of the maximum value. The beams were directed perpendicular to the surface. Rough calculations have demonstrated that, since the sample is thin, the influence of beam divergence is negligible in comparison with the scattering effect. In further modeling, the absorption coefficient and the refraction index were fixed at $\mu_a = 7.8\,\mathrm{cm}^{-1}$ and $n = 1.45$, respectively. Scattering coefficient μ_s and anisotropy factor g were the only variable parameters. The beams free path length was chosen as $l = -\lg(\mathrm{RAND})/(\mu_a + \mu_s)$. In the case of an absorption event, the energy value in the corresponding cell of matrix A was increased by the beam energy. In the case of a scattering event, the beam deflection was determined using the Henyey–Greenstein formula. Upon reaching the upper or lower boundary of the sample, the beam either was reflected with a probability specified by the Fresnel formula (and continued its propagation in the medium) or escaped from the sample. When leaving the sample, the beam energy complemented the R_d or T_d components (depending on which boundary was reached). Upon reaching the side wall, the beam ceased to exist, contributing to the T_{lost} term. The following inequality holds in most cases: $T_{lost} \ll T_d, R_d$.

Figure 1. Holes in the skin of a rabbit ear after laser irradiation and trypsinization.

The calculation of the three-dimensional temperature field induced by infrared laser radiation was based on the heat conduction equation in cylindrical coordinates:

$$\frac{\partial T}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(\chi(r,z)r\frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(\chi(r,z)\frac{\partial T}{\partial z}\right) + f(r,z,t),$$

where $\chi(r,z)$ is the thermal diffusivity [cm^2/s], $T(r,z,t)$ is the temperature at a point with coordinates (r,z) at time t, and $f(r,z,t)$ is the heat source function specified by absorption of laser radiation [K/s]. The temperature distribution over the sample at the initial time is uniform and corresponds to ambient temperature T_0. Conditions of the third kind (established by air convection) were set at the sample boundaries. They were characterized in more detail in [8]. Elements of the heat source matrix were calculated in accordance with the following expression: $f(i,j,t) = P(t)A(i,j)/NC_pV(i,j,t)$, where P is the laser beam power [W], N is the total number of incoming beams, C_p is the heat capacity of the sample material [J/K · cm^3], and $V(i,j)$ is the physical volume of cell (i,j) of matrix A [cm^3].

The experimentally measured values of total diffuse reflection R_d and transmission T_d coefficients and fraction of the output beam T_c passing through the diaphragm are presented in Table 1. The values of scattering coefficient $\mu_s = 85 \pm 5\,\mathrm{cm}^{-1}$ and anisotropy factor $g = 0.65 \pm 0.05$ were determined by minimizing the $\sum\left(1 - R_{exp}/R_{calc}(\mu_s, g)\right)^2$ expression through the variation of μ_s and g. The summation was performed based on the totality of all experimental points. It is worth noting that the main contribution to the error in determining these parameters was produced by the skin sample thickness uncertainty ($h = 0.35 \pm 0.03$ mm), which is attributable to the non-uniformity of samples and the varying degree of compression in thickness when measured with a micrometer. Table 1 also lists the corresponding values obtained via Monte Carlo simulations of the light field.

The minimum power (P) and energy density (E) of laser radiation inducing complete denaturation of collagen

Table 1. Comparison of experimental results with model calculations

R_d		T_d		T_c		Diameter, mm (fiber/diaphragm)
Experiment	Model	Experiment	Model	Experiment	Model	
0.18 ± 0.02	0.16	0.31 ± 0.03	0.31	0.80 ± 0.02 0.48 ± 0.02	0.81 0.48	0.6/0.64 0.2/0.248

Table 2. Minimum power and energy density of laser radiation inducing complete denaturation of collagen in skin samples

Spot diameter, μm	P, W	E, J/mm^2	$T_{\max}$, °C
200	1.0 ± 0.2	2.23 ± 0.45	72 ± 3
600	2.0 ± 0.2	0.49 ± 0.05	74 ± 3

in skin samples during the experiments are presented in Table 2. It also lists the values of the maximum temperature ($T_{\max}$) values measured on the back side of the samples with a thermal imager. Denaturation temperature T_{den} of collagen in the skin subjected to quasi-equilibrium heating in a calorimeter furnace is 65 °C [9]. However, T_{den} is not a fundamental characteristic of collagen. Its value may vary from 55 to 90 °C depending on external heating conditions (applied load, duration [10,11], and uniformity [9]). The temperature threshold of collagen denaturation characterizes the tissue stability under specific conditions. It turned out that denaturation throughout the entire depth of the sample in a volume sufficient for visual detection via trypsinization occurred in our experiment when a point temperature $T_{\max} \sim 75$ °C on the back surface was reached.

Figure 2 presents the temperature dynamics at the center of the laser beam at different depths within the skin sample. It can be seen that when the maximum temperature $T_{\max}$ of the lower sample layer reaches the denaturation threshold (~ 75 °C), the upper layer facing the laser beam is overheated significantly.

In fact, our simulation provides an opportunity to determine the profile of the temperature field boundary that has reached a given value. The boundaries of the region where the sample temperature was above 75 °C are shown in Fig. 3.

Note that under pulsed heating, these boundaries are outside the limits of the laser beam; the smaller the beam diameter is, the more profound is this difference. This fact is naturally attributable to the transfer of heat from the overheated central region to adjacent colder areas.

The results of experiments aimed at targeted modification of the skin by localized pulsed laser radiation with a wavelength of 1560 nm were presented. Measurements of beam transmission through a skin sample and a diaphragm revealed the necessity of taking into consideration the contribution of light scattering. An integrating sphere was used to determine the scattering coefficients and the anisotropy factor, which were then applied in Monte Carlo calculations of the light field and used to solve a nonstationary thermal problem with distributed heat sources. The values of threshold powers inducing coagulation of the sample throughout its entire thickness were estimated. The geometric boundaries of the areas in which tissue coagulation occurs for the laser beam with a diameter of 200 and 600 μm were calculated. It was demonstrated that the coagulation region may be significantly larger than the laser spot. The model and experimental data agreed closely. The obtained results may be used to plan laser treatment procedures similar to fractional thermolysis.

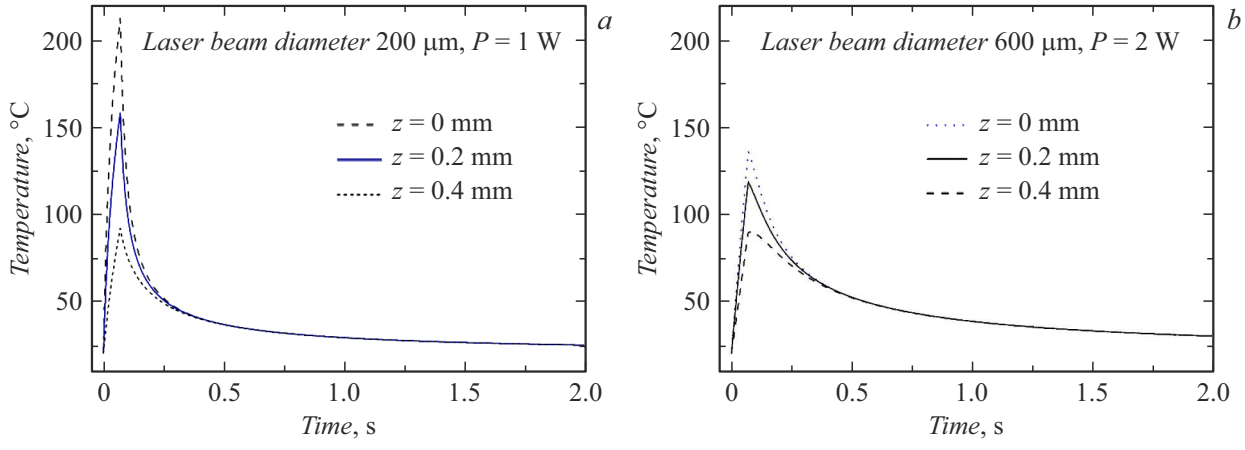

Figure 2. Dynamics of temperature at the center of the laser spot with a diameter of 200 (a) and 600 μm (b) at different depths (z) within the 0.4-mm-thick skin sample corresponding to the threshold power for denaturation over the entire thickness.

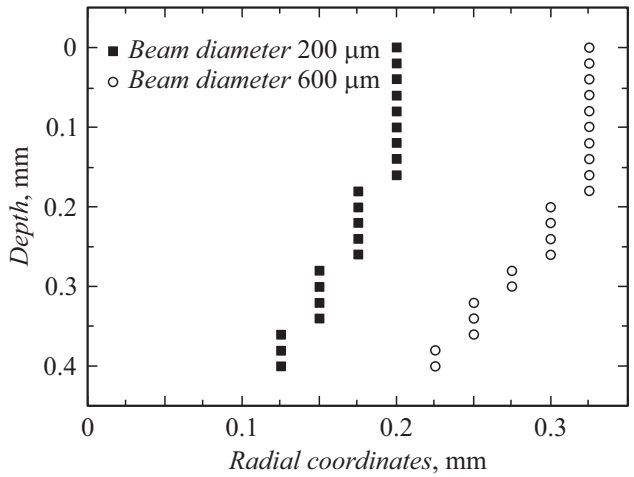

Figure 3. Coordinates of points where the temperature exceeded 75 °C during pulsed heating under the same conditions as in Fig. 2.

Funding

Optical measurements and mathematical modeling were performed as part of the state assignment of the National Research Center „Kurchatov Institute," while measurements of the volume of coagulated tissue were carried out as part of the state assignment AAAA-A21-121011990019-4.

Compliance with ethical standards

This article does not contain any studies involving animals performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by D.Safin