Analyzing Thermal and Mechanical Effects of Pulsed Laser Irradiation on Tissues

By

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“Analyzing Thermal and Mechanical Effects of Pulsed Laser Irradiation on Tissues”

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Abstract

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Pulsed lasers are known for their spatial and temporal specificity in delivering heat energy to the tissues. This is useful in laser ablation treatment mechanism where damage to the healthy tissues is highly undesired. However, the efficacy of the process is limited by the damage caused by the pulsed laser. A pulsed laser has both photothermal and photomechanical interaction with tissues. Photothermal interaction is caused by the rise in temperature due to the laser irradiation. This includes the denaturation of proteins, increased mitochondrial membrane permeability and ultimately vaporization. Photomechanical interaction causes the generation of pressure waves produced as a result of the pulse-laser interaction. This arises due to the thermoelastic expansion of the tissues due to heating. Photothermal and photomechanical interactions combined lead to damage in the tissues and are a potential threat to the
surrounding tissues. In this thesis, the effects of both the mechanisms are studied using finite element models of the skin. A three-layered model of the skin is considered which is irradiated upon by a focused Nd:YAG infrared laser beam. The finite-element solver COMSOL Multiphysics is used to simulate the thermal and mechanical interaction due to the laser irradiation. Thermal effects of the irradiation are evaluated using the Arrhenius damage integral and the equivalent thermal dose administered to the tissue. The results obtained are validated using the histology results when mouse tissues are irradiated with a focused beam Q-switched Nd:YAG laser which leads to temperature rise and tissue removal. The mechanical interaction is evaluated in terms of the stress generated in the tissue during the laser ablation damage. Results obtained here are useful in characterizing the parameters of laser ablation like the repetition rate, laser power and pulse width. This helps us in optimizing the laser ablation process for a more effective treatment with minimum damage to surrounding tissues.
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Chapter 1: Introduction

1.1 Background

Lasers began as “solution looking for a problem” [1] back in 1960, when its unique qualities- intense, narrow beam of light of single wavelength were a wonder not fully realized. It was invented in 1960 by Theodore Miaman at the Hughes Research Laboratory in California when he shone a high-power flash lamp on a ruby rod with silver coated surfaces. The findings of the experiment were published in *Nature* [2] and instantly received wide attention. Soon after that lasers began to find wide applications in various fields of science including biology and medicine. Today lasers are used almost in all aspects of science and technology from the research laboratories at the cutting edge of quantum physics to medical clinics, supermarket checkouts to the telephone network.

Use of lasers in medicine has increased over the years. Today lasers have begun to play an important role in medical systems and surgery. They are used in various areas of medicine like cancer diagnosis [3], cancer treatment [4], dermatology [5], ophthalmology (Lasik and laser photocoagulation), optical coherence tomography [6], and prostatectomy. Lasers also find use in cosmetic applications like laser hair removal and tattoo [5] removal. The various types
of lasers used in these processes are CO₂ lasers, diode lasers, dye lasers, excimer lasers, fiber lasers, gas lasers etc.

Lasers must be used in medicine with extreme caution. Use of continuous lasers leads to unwanted collateral damage on the human body which needs to be controlled or eliminated. This has prompted the use of pulsed wave lasers in medical imaging and therapy applications. Pulse lasers offer the advantage of targeted delivery of heat energy, thereby minimizing the spread of heat to surrounding healthy tissues.

This work deals with the comparison of the efficacy of continuous wave versus pulsed lasers in hyperthermia applications. The thermal and mechanical interaction of the pulsed laser is studied using computer models and subsequently visualized through histological studies. The results obtained through the models have been validated against experimental results collected in the past and during the course of this work. Parametric analyses have been done to show the significance of various parameters which influence the thermal dose to the targeted region. The following paragraph gives a brief explanation of the physics behind lasers and their use in medicine.
1.2 Laser Physics and Properties

A laser is a process of optical amplification of the energy produced by the electron when being transferred to a lower energy state from an excited state. The light produced by laser has three distinct features opposed to natural light. It is monochromatic that is, composed of photons of largely single wavelength and hence produce a light of intense color. This is unlike sunlight, which is composed of seven colors and breaks into its components when passing through a prism. This wavelength is also a measure of the laser’s energy. Laser light is coherent in nature which means all the laser light is in phase. This gives rise to interference patterns of light and dark bands when the rays travel different paths. This property is used in diagnostic medicine. Lastly, laser light is highly collimated which means that it doesn’t diverge. This gives rise to highly pointed and intense laser beams and can cause sufficient damage to our organs like eyes or skin than any other light. Even mili-watt lasers can cause significant damage to our retina. [7]. Hence extreme care is employed when dealing with lasers of higher power.

1.3 Laser Tissue Interaction

When a photon of laser light strikes a biological tissue, it may be absorbed by the tissue, scattered or may pass unaltered. Absorption of laser light by tissue leads to various kinds of interactions like photothermal,
photomechanical and photochemical [8]. These mechanisms can occur individually or in combination depending upon laser parameters. Most easily quantified and commonly seen is the photothermal effect. With high amounts of energy delivered to the tissue, a temperature rise is seen at the point of irradiation and the thermal energy spreads to surrounding tissues. Photochemical and photomechanical reactions also cause tissue damage. The release of free radicals due to the laser beam causes the photochemical reaction [8]. Vibration or expansion of gaseous products inside the tissue medium causes propagation of shock waves and high ablation rates. Photomechanical tissue destruction involves shock waves that propagate through tissue and can cause ablation at the surface of the tissue [9]. The manner in which these mechanisms contribute to a particular treatment can vary drastically.

1.3.1 Photothermal Laser-Tissue Interaction

Most of the treatments using lasers involve the localized or bulk heating of an affected area. This direct heat deposition to a specific region can destroy the cells at the site, causing damage to the cell walls and release of intercellular material, causing inflammation and swelling. This process is called necrosis of tissue and can be used to effectively kill diseased tissue. Ablation can be achieved using photothermal laser-tissue interaction through high energy deposition and vaporization of water in the tissue. This form of ablation raises the temperature of the targeted area and can cause thermal damage such as
necrosis and photocoagulation to the adjacent material. The extent of this
damage due to the distribution of thermal energy in a tissue depends on the
thermal relaxation time of the tissue. Heat energy derived from the laser beam
flows from the region of interest (target) to the neighboring healthy cells which
are at lower temperatures by conduction. This phenomenon is known as
thermal diffusion and the thermal diffusion time $\tau_{th}$ is defined as [8]:

$$
\tau_{th} = \frac{\delta^2}{4\alpha}
$$

where $\delta$ is the optical penetration depth of the laser light in the tissue. Optical
penetration is defined as the inverse of the absorption coefficient and $\alpha$ is the
thermal diffusivity of the tissue. If the time of irradiance of a laser pulse is
shorter than the thermal diffusion time, the distribution of laser thermal energy
is confined within the irradiated zone. Pulses longer than the thermal relaxation
time lead to diffusion of heat outside the affected zone and may cause damage
to the neighboring healthy tissues. For a pulsed laser beam with the same total
pulse energy and spot size, a longer pulse will result in lower peak power.
This longer pulse will induce a lower peak temperature rise within the optical
zone than a short or ultra-short pulse laser with the same pulse energy.
Continuous wave lasers which have infinite pulse width provide uncontrolled
thermal dose which leads to damage to the surrounding healthy tissues. This
has led to the increased interest in the use of short pulse lasers for its use in medicine. The thermal relaxation time and pulse duration will determine how the deposited thermal energy will propagate through the tissue. With short pulse durations, i.e. less than the thermal relaxation time, the thermal energy is deposited in a small region within the optical zone. High local temperatures are achieved due to the laser energy contributing to heating in this region, but surrounding tissues do not receive damaging heat deposition. Thus, with short pulse lasers, localized material removal can be achieved through vaporization with less thermal damage to surrounding structures.

Photothermal ablation is essentially the vaporization of water present in the tissue. Therefore, ablation can only occur if the laser is able to deliver high peak power and reach the latent heat of vaporization of water. If the threshold is not achieved, the laser energy will diffuse to tissue surrounding the optical zone and cause elevated temperatures in the medium. Although the heat is diffusing away from the optical zone, the bulk temperature of the tissue will increase as a result of the heat deposition.

Hyperthermia (HT) [10], Laser Induced Thermotherapy (LITT) [11,12] and Interstitial Laser Photocoagulation therapy (ILP) are three treatments that involve localized heating of cancerous or tumor tissue. This involves the use of photothermal mechanism of laser tissue interaction. This mechanism is used to damage the malignant tissue irreversibly as a means of treatment.
examples include cauterization of blood vessels, tissue welding, and destruction of cancerous tissue through necrosis [13].

Laser-induced hyperthermia is a general title that encompasses the more specific treatments of Laser Induced Thermotherapy and Interstitial Laser Photocoagulation therapy. Hyperthermic treatment is often utilized in combination with other treatments such as chemotherapy in order to more effectively destroy tumor tissue than either treatment alone [10]. There have been comparisons made over the years between the efficacies of continuous wave laser versus pulsed lasers in hyperthermia treatment. Mathewson and group did a comparison [14] between the effect of continuous wave and pulsed lasers in laser-induced hyperthermia. They used a continuous wave laser and a microsecond pulsed laser at 10-40 Hz with same average power. They found no difference in the histological damage. They concluded that the pulsing rate does not influence the extent of damage in low power excitations. Panjehpour et. al. [15] compared the effect of continuous wave (CW) and pulsed laser light for photodynamic therapy. They irradiated canine esophagus with a CW and pulsed laser light and then performed histological examinations of the lesions developed. They couldn’t distinguish between the CW and the laser-induced injuries. Comparison done by Huang et al [16] proved conclusively that a single nanosecond pulse of laser is more efficient in inducing cell death than a CW laser. They used gold nanospheres with receptors which were
absorbed by the cells and hence increased the specificity of the laser irradiation. They studied cell death process by both the lasers and found out that the CW laser induces cell death via apoptosis whereas nanosecond pulsed laser induces cell death by cell necrosis.

The use of pulsed lasers in laser induced hyperthermia is prevalent because of the advantages mentioned in the earlier section of this chapter. Jaunich et. al. [11] used a focused beam Q-switched Nd:YAG and a diode short pulsed laser to study the rise in temperature in the dead mouse tissue samples and live anesthetized mouse. They also corroborated their results using a mathematical model which compared the difference between Fourier and non-Fourier heat conduction. Sajjadi [17] used an ultra-short pulsed 1552 nm laser focused beam to irradiate the surface of anesthetized mice. They used the histology results to show the efficacy of focused beam in produced precise ablation at the desired location with minimal collateral damage. Khan et. al. [12] used intradermal focused infrared laser pulses to produce controlled and spatially confined thermal effects in dermis layer of the skin. They observed that thermal effects of pulsed laser can be confined by focusing a low-power infrared laser into skin.
Nanotechnology has played an important role in improving the efficiency of the photothermal ablation procedure. In 1999, Lin et al. [18] first reported the selective PTT (Photothermal therapy) based on the use of light-absorbing microparticles that absorb light in the visible region. The development of nanotechnology in recent times has provided numerous nanostructures with unique optical properties that are useful in photothermal therapy using laser induced hyperthermia [19-21].

Laser energy may also be delivered into the tissue target using percutaneous optic probe in a procedure called Laser Induced Interstitial Thermotherapy (LITT). LITT has been studied for the treatment of head and neck cancers [22] and as well as liver cancer [23]. Feyh et al. [24] reported that tumor necrosis volume induced by LITT can reach up to approximately 4 cm³ around the tip of the fiberoptic delivery probe.

Interstitial laser photocoagulation is a therapeutic technique for the ablation of tumors using a low-powered laser for an extended duration, on the order of 8-10 minutes and was originally reported by Brown in 1983 [25]. Optical fibers are used for delivery of the laser light and enable patients to be treated without requiring a major surgical procedure. This treatment has been studied for treatment of many tumors, including those of the liver [26], urinary tract [27], breast [28] and prostate [29] with positive outcomes.
1.3.2 Photomechanical Laser Tissue Interaction

Photomechanical (or photoacoustical) tissue interactions are caused when a laser pulse with very high intensity produces plasma that causes shock waves to propagate within the tissue [30]. Plasma is an excited state of matter which is dense in ions and free electrons. As with the photothermal effect, a critical time constant exists for mechanical effects. When laser energy is deposited in tissue as heat, there is thermoplastic expansion of the tissue which yields stress, expressed in units of N/m² or Pascal. The stresses due to the tissue morphology will propagate as a wave into the surrounding tissue. Similar to heat dissipation within tissue, time is required for the stress wave to propagate deeper than the optical zone of laser deposition. This characteristic time is defined as [31]:

\[ t_0 \approx \frac{\delta}{v} = \frac{1}{\mu_a v} \quad (1.2) \]

where \( v \) is the velocity of sound in the medium (approx. 1500 m/s)

If the laser pulse is shorter than \( t_0 \), then the laser-induced stress accumulates before it can propagate away from the optical zone. If a pulse is very short (\( t = t_0/10 \)) the stresses are concentrated in a small region generating very high stresses which can propagate into the tissue more intensely and cause
physical cellular damage or spallation, which is the ejection of material fragments due to stress that removes surface layers of tissue.

If the laser pulse is longer than the characteristic time $t_0$, then the stress is distributed over a distance given by the pulse duration ($t_p$) times the speed of sound ($v$), $t_p v$. Because the stress is less concentrated, the stress gradients are lessened and therefore the effects of stress in the tissue are mitigated. The optics of the tissue and the laser pulse duration together determine the amount of stress generated by a laser within tissue. If the pulse is comparable to the critical time, $t_p = t_0$, then the stress is more distributed and the peak stress is much lower. Longer pulse durations further reduce the peak stress, and stress induced phenomena become less likely.

One of the most important biomedical applications utilizing the photomechanical interaction of pulsed lasers with skin tissue is the transdermal delivery of drugs inside the body. Haedersal and co. evaluated [32] the efficacy of drug delivery of topically applied into skin. They used millisecond laser pulses to administer a test drug into swine skin surface. They observed drug penetration upto a depth of almost 2mm below the skin surface. Another group used [33] the acoustic diffraction effects at the laser-pulse incidence interface for photomechanical drug delivery. They used pressure wave pulses from a Nd:YAG laser in a polystyrene well target and water system for drug delivery studies.
The photoacoustic waves generated by the pulsed lasers also give rise to an innovative method of imaging known as photoacoustic imaging. This makes use of acoustic waves instead of the photon transport. Hence it is not limited by the attenuation coefficient of the tissue medium. Ermilov et. al. [34] designed and fabricated a laser optoacoustic imaging system for breast cancer detection. They used pulsed NIR laser light to maximize light penetration depth in malignant breast tissues. Kircher and co. developed [35] a combined imaging technique comprising of magnetic resonance, photoacoustic effect and Raman imaging nanoparticle to detect tumors in the brain. Photomechanical interaction of pulsed lasers is also used in the stimulation of peripheral nerves and elicits action potentials from these neurons [36]. A detailed account of the advances made in this field in the past decade can be found in these proceedings [37-41].

1.3.3 Factors Affecting the Laser Ablation Process

There are many parameters that need to be taken care of when dealing with pulsed laser irradiation on tissues. All the parameters are required to be optimum to prevent maximum localized damage without damaging the surrounding healthy tissues. One usually should start the design of the irradiation experiment by selecting the wavelength of the laser. Further parameters include the pulse duration of the laser pulse, the size of the laser
beam, its spatial and temporal distribution, the frequency of the pulses (repetition rate) and the time of irradiance.

**Selection of wavelength**

The delivery of laser for ablation is effective only when it reaches the desired location and is not absorbed elsewhere causing unintended damage. This makes us select a laser that is strongly absorbed by the target tissues. The depth of the laser delivery is measured by the absorption coefficient which is the inverse of the penetration depth. A wavelength which is not well absorbed by the tissues leads to the deep penetration of the laser causing unnecessary damage. The penetration depth of the laser determines the volume of heat delivery and the energy required for ablation. There is strong absorption of optical energy in two regions of the spectrum- IR and the UV. Since water is the major component of the body tissues, the variation of absorption coefficient of water is especially essential. The maximum penetration depth occurs around 1 um (1000 nm) [42] which lies in a region of the electromagnetic spectrum called the near-infrared region. (NIR)
**Selecting Pulse Duration**

The pulse duration is very important parameter in the laser irradiation process. The pulse duration of the laser must be smaller than the thermal diffusion time of the tissue. In this way, the heat delivered by the laser is confined. Pulse duration also determines the peak power delivered to the region. Usage of small pulses in the order of picosecond and femtosecond leads to extremely high peak power delivered to the tissues. Pulse durations larger than thermal diffusion time leads to decrease in peak power resulting in an inefficient ablation process [43].

**Size of the laser beam**

Size of the laser beam (spot size) determines the aspect ratio of the ablation crater. Larger beam diameters lead to large spot size which leads to a decrease in pulse energy per unit area of the tissue resulting in ineffective ablation. Smaller beam diameters lead to higher laser intensity and can make melt the tissues surrounding the ablation boundary [44]. Therefore it is extremely important to select the laser beam with optimum beam size.

**Effect of frequency**

The frequency defines the time interval between successive pulses of the laser beam. The pace of the arrival of the laser pulses can determine the
removal rate of the ablated tissues. A good estimate is that the next pulse should theoretically arrive after the thermal relaxation time since it is a measure of a time taken by the heated tissue to cool down. However the cooling is more complicated process and it typically takes around 10 times the thermal relaxation time to cool down which means, as a rough estimate the next pulse should arrive after around ten times the thermal relaxation time [45].

**Beam Profile**

A spatial beam profile can be either Gaussian or flat-top in shape. Flat-top shape gives cleaner ablation boundaries whereas Gaussian beam provides more depth to the ablation. Therefore Gaussian profile is more preferred in irradiation experiments [31]. The average radiant exposure at the center is around twice as high as the average radiant exposure over the area for a Gaussian beam.

**1.4 Analyzing the Laser-Tissue Interaction Mechanisms**

The effects of photothermal and photomechanical interactions of light can be quantified and analyzed in the form of damage they have caused to the tissues. The goal of the ablation process should be to confine the damage in any form to only the targeted region. Any kind of damage beyond the intended
region of interest is highly undesired and should be avoided. This calls for a careful selection of laser parameters and knowledge of damage parameters.

1.4.1 Analysis of Damage Due To Photothermal Interaction

Heat delivered by the laser ablation process is absorbed by the tissues. Heating of cells and tissues can produce reversible injury that can be repaired by innate cellular and host mechanisms. However, more severe, irreversible damage leads to death immediately (primary thermal effects) or after (delayed secondary effects) the heating event. Primary thermal effects refer to the structural and functional abnormalities in the tissues due to the direct physical interaction of the heat energy with the cellular and tissue proteins, lipoproteins and water [46]. These effects can be detected immediately or just after the tissue has undergone the heat treatment due to laser irradiation. One of the primary thermal effects in the tissues is the activation of increased mitochondrial membrane permeability of ions. This is followed by the thermal dissociation of phospholipid cellular membranes which comprise of the rupture of intracellular membranes, loss of membrane potentials and disruption of membrane associated enzymes and proteins [47]. The membrane rupture is followed by the denaturation of intracellular proteins which are vital for the cells survival and functioning like the receptor proteins, signal proteins, and other housekeeping proteins [48]. Further increase in temperature leads to the vaporization of water. This occurs when the temperature of the tissue medium
reaches 100°C at standard pressure. When generation of water vapor is faster than its rate of diffusion out of the tissue, the vapor can be trapped within the tissue, forming steam vacuoles. Pressure rapidly increases in the tissue due to the constant increase in temperature. As a result, the steam expands creating bigger vacuoles below the surface or explodes throwing tissue fragments of the vacuole wall from the surface ("popcorn effect") [31]. At even higher temperatures the cellular organic molecular in the tissue reduces to elemental carbon which forms a thin black membrane over the irradiated surface of the tissue. This phenomenon is called carmelization and usually occurs at temperatures below 200°C [30]. Any temperature above that leads to tissue ablation and involves the removal of tissue mass by explosive fragmentation. This is usually marked by a hole in the tissue.

Secondary thermal interaction is based on the delayed pathological cellular, tissue or host responses triggered by cellular and tissue injuries. This is usually marked by the formation of thermal vascular damage zone. This is followed by the traumatic cell and tissue death [49]. This may occur by lytic necrosis due to release of lytic enzymes from thermally damaged lysosomes [50]. Or necrosis may occur due to loss of energy production in thermally damaged mitochondria. Infarction may also result due to regional blood flow blockage. Release of local cytokines and other cytotoxic factors lead to the inflammatory response to the tissue death and necrosis. This activates the
overall immune response of the body. Mathematically, the photothermal interaction can be modeled as a first order rate process with two experimentally derived coefficients. This damage is exponentially dependent on temperature and linearly dependent on time of exposure. This was first postulated by Moritz and Henriques in a series of reviews [51-54]. They quantified damage using a single parameter $\Omega$ which is calculated using the Arrhenius equation.

$$\Omega = \int_0^\tau A e^{\left[\frac{-E_a}{RT(T)}\right]} dt \quad (1.3)$$

where $A$ is the frequency factor [s$^{-1}$], $\tau$ is the total heating time, $E$ is the activation energy barrier, $R$ is the universal gas constant (8.314 J.mole$^{-1}$.K$^{-1}$) and $T$ is the temperature of the tissue.

The basis for this model is the first order chemical reaction kinetics. In a typical reaction process, thermally active reactants jump over an energy barrier to form products, as illustrated in Fig. 1.1. Thermal damage in tissue is a unimolecular process. Tissue constituents transition from the native state to the damaged state. The rate of damage formation is then proportional to only those molecules that remain activated [55]. For a unimolecular process in the native state $C$, having an activated state, $C^*$, with velocity constants $k_a$, $k_b$ and $k_3$:

$$C + C \xrightleftharpoons{k_3} C + C^*$$
The term in parentheses in the equation suggests that the pre-exponential factor, $A$, is not constant but is in fact temperature dependent. However, the linear dependence of $A$ on temperature is extremely weak – when compared to the exponential dependence in the final term – and its effect is negligible, so that for all practical purposes $A$ may be treated as a constant over the temperature ranges of interest.

A more useful form of the Arrhenius damage may be obtained by recasting the result into a volume fraction model [46]. In this formulation, as above, $C$ signifies the remaining concentration of native state (undamaged) tissue constituent molecules. Therefore, the physical significance of the
traditional damage measure, $\Omega$, is the logarithm of the ratio of the original concentration of native tissue to the remaining native state tissue at time $\tau$:

$$\Omega(\tau) = \ln\left(\frac{C(0)}{C(\tau)}\right)$$

(1.4)

where the frequency factor, $A$, and energy barrier, $E_a$

The characteristic behavior of the kinetic damage model is that below a threshold temperature the rate of damage accumulation is negligible, and it increases rapidly when this value is exceeded. This behavior is to be expected from the exponential nature of the function. For purposes of discussion, it is useful to define the critical temperature, $T_{\text{crit}}$, as the temperature at which the damage accumulation rate, $d\Omega/dt$, is 1:

$$\frac{d\Omega}{dt} = 1 = Ae^{\frac{E_a}{RT_{\text{crit}}}}$$

(1.5)

$$T_{\text{crit}} = \frac{E_a}{R\ln(A)}$$

(1.6)

Thermal damage kinetic coefficients are usually determined from constant temperature exposures of relatively long duration. Threshold damage results are selected out of a set of damaged tissue samples for analysis from which estimates of $A$ and $E_a$ are obtained [51, 56].

There have been several attempts at quantifying the tissue damage during hyperthermia using the Arrhenius damage integral. The work by Dewhirst and co. [57] at Duke University is one of the highly regarded report in this field in
which they established hyperthermia guidelines and damage threshold in tissue using the damage caused. Arrhenius damage integral was used to quantify the damage in this case. Peters et. al [58]. at University of Toronto also used the Arrhenius method to evaluate biological damage in a canine model during a magnetic resonance image-guidance for interstitial thermal therapy. The Diller Group at University of Texas, Austin correlated [59] the expression of heat shock proteins in the tissues in response to heat treatment to the thermal damage in the tissues which was measured through Arrhenius analysis. Yaseen and co. identified the parameters of tissue damage of when the dextran coated mesocapsules containing Indocyanine Green is heated using lasers [60]. Mertyna and co. also used Arrhenius damage integral [61] to calculate the critical ablation margin when comparing the damage to the tissues by radiofrequency, microwave or laser-induced coagulation. Orgill et al. used Arrhenius integral [62] to predict damage in the porcine model when subjected the cutaneous burns.

Another model which is commonly used to quantify the thermal interaction of heat with tissues is the *equivalent minutes* model. It was first proposed by Sarapeto and Dewey [63]. It is also called the thermal isoeffective dose. In this method, the temperature-time data is used to calculate the equivalent time for which the tissue remained at 43°C. This is also called the thermal dose administered to the tissue during the laser irradiation process. An arbitrary
value of 43°C is chosen as a result of the knowledge of the temperature during treatment as a function of time combined with a mathematical description of the time-temperature relationship for thermal inactivation or damage. This technique is helpful in determining a quantifiable dose accumulated in real time. For example, in cases where the temperatures during the treatment process exceed the proposed temperature, the treatment time can be shortened to compensate for the extraneous damage incurred during the process. According to the model, the thermal dose $t_{43}$ can be calculated using the formula as following

$$ t_{43} = \sum_{t=0}^{t=final} R^{(43-T)} \Delta t $$

where $T$ is the temperature in °C, and the value of $R$ (empirically obtained from hyperthermia experiments with living tissues) is 0.5 when the temperature $T$ is above 43°C and 0.25 when the temperature is below 43°C. This method extricates us from the determination of tissue parameters like activation energy or the frequency factor as is required when calculating the Arrhenius damage. This method is based on the principle that the majority of biological systems display similar transient temperature relationship for a given thermal interaction.

Researchers at the Washington University of Medicine have also used the equivalent minutes method to evaluate the thermal damage during the
treatment of short duration heat shocks to inhibit the repair of DNA damage [64]. Feng and Fuentes [65] used the thermal dose to optimize the delivery of pulsed laser treatment in thermal therapy. However review by Pearce [66] suggests that the Arrhenius formulation is more useful than the thermal dose model in laser heating at higher temperatures. Thermal dose has been used to optimize dose in cases of hyperthermia caused by other source of heating [67-69]. These examples illustrate the efficacy of the method in determining the thermal damage in not only model cases but also in clinical settings.

1.4.2 Analysis of Damage Due To Photomechanical Interaction

The heat absorbed by the tissues during the laser irradiation is largely converted to rise in temperature of the tissues. The temperature rise in the tissues $\Delta T$ is related to the local volumetric heat energy density $W(r)$ [70] as

$$\Delta T = \frac{W(r)}{\rho_c v}$$  \hspace{1cm} (1.8)

where $\rho$[kg/m$^3$] is the tissue density and $c_p$[J·kg$^{-1}$·K$^{-1}$] the specific heat capacity at constant volume. The damage due to the photoacoustic interaction of the laser irradiation process can be evaluated by the maximum stress generated in the vicinity of the laser affected area. The photoacoustic wave
generation and propagation in an inviscid medium is described by the following general photoacoustic equation [70]:

\[
\left( \Delta^2 - \frac{1}{v^2} \frac{\partial^2}{\partial t^2} \right) p(r, t) = \frac{-\beta}{\kappa v^2} \frac{\partial^2 T(r,t)}{\partial t^2}
\]  (1.9)

where \( p(r, t) \) denotes the acoustic pressure at location \( r \) and time \( t \), and \( T \) denotes the temperature, \( v \) the velocity of sound in the medium, \( \beta \) is the coefficient of isothermal compressibility and \( \kappa \) denotes the thermal coefficient of thermal expansion of the tissue. Left-hand side of this equation describes the wave propagation, whereas the right-hand side represents the source term.

Using Fourier’s law of heat conduction, the following equation is obtained

\[
\left( \Delta^2 - \frac{1}{v^2} \frac{\partial^2}{\partial t^2} \right) p(r, t) = \frac{-\beta}{\kappa c_p} \frac{\partial S(r,t)}{\partial t}
\]  (1.10)

The presence of the first derivative of the laser source term in the right hand side of the equation implies that a time variant heat source like a pulse source can only lead to a pressure wave. Time invariant or constant heat source does not result in the generation of acoustic wave.

There have been few examples where it has been attempted to evaluate the structural damage caused by short-pulse lasers on tissues in terms of the stress generated due to thermal expansion. Alex Humphries and group used [71] finite element analysis to model the acoustic process during the laser tattoo
removal process. Finite element modeling using COMSOL has been also used to model the photoacoustic behavior of gold nanoparticles subject to RF heating [72]. The model was used to study the photoacoustic behavior of plasmon nanoparticles and their effect on local environment. The results of the model were also validated against an analytical model. The efficacy of the finite element modeling has been studied by comparing with other known simulation models such as Monte Carlo method and heat-pressure model [73]. Ko and group studied the generation of acoustic waves by the irradiation of nanosecond pulsed laser beam. They used Schlieren photography to visualize the transient interaction of the plane acoustic wave in various internal channel structures [74]. Laser induced stress has been used to photomechanical drug delivery [33]. Researchers used Nd:YAG laser as a photothermal source to generate photomechanical pressure pulse in water, the fluid of choice in many cell membrane permeabilization studies. Apart from tissues, the generation of acoustic waves has also been studied in other mediums like metals and liquids. Audible acoustic waves generated during laser irradiation with aluminum surface have been studied to monitor the interaction and to analyze the nature of interactions such as laser ablation and surface cleaning [75]. Kim and group at University of California, Berkeley examined the mechanisms of material ablation and acoustic pulse generation in the ablation of aqueous solutions by a short-pulse lasers [76].
1.4.3 Histological Study

Histology is the study of microscopic anatomy of cells. It is commonly performed by examining cells and tissues by staining and sectioning, followed by examination under a microscope. The detailed step by step procedure and the chemicals used in the present histology work performed are mentioned in Chapter 2 of the thesis. There have been several studies which have examined histology following treatment with the Nd:YAG laser. Anderson and Parish [77] demonstrated specific laser induced damage to blood vessels both clinically and histologically. They exposed human skin to a flash lamp-pumped dye laser operating at 577 nm with a pulse width of 0.3 µs. This study demonstrated the use of laser induced selective vascular damage as a treatment modality for portwine stain and other vascular lesions. Laser ablated zone produced by nanosecond and picosecond focused beam has also been showed by histological studies by Januich et al. [11]. Histological studies by Sajjadi et al. [17] show that focused pulsed laser can also be used to ablate subsurface tumors.
1.5 Numerical Modeling of Laser Tissue Interactions

Software programs such as COMSOL and ANSYS are capable of utilizing the FEM method on complex engineering problems which require solutions in two or more dimensions. Modern computer processors enable these programs to solve these problems by discretizing the geometry into incredibly small FEs. The following discussion will give a brief overview of how COMSOL has contributed to the modeling and understanding of laser tissue interactions.

1.5.1 Modeling Pulse Laser Irradiation with COMSOL

COMSOL offers an excellent tool to model fluid and mass transport in materials including body tissues. It has very visual and user friendly interfaces which make solving of computational fluid dynamics very easy even for beginners. COMSOL has some formulas already built in which govern the various physics like heat transfer or sound propagation. It also offers the advantage of defining our own governing equations in case if it’s not already present in the package.

Solving pulsed lasers in COMSOL poses the difficulty of resolving the laser pulses. This is difficult because the time steps need be in the order of the pulse width of the laser pulse to account for the pulse width. Usually pulse widths in the duration of nanosecond or shorter require the solver to select time
steps in the range of the pulse width. Hence only a few pulses can be studied at a time. In this work, a novel approach of using staggered time steps has been used. Time steps of 25 ns has been used for the time period for which the laser is on (200 ns) or in short the “positive duty cycle”. For the time period in between successive pulses (negative duty cycle), longer time periods of one tenth of the inverse of repetition rate is used. This optimizes the working of the COMSOL solver. However this is not a very effective solution and only limits the solver’s capability to compute to only about 500 pulses. For studying pulsed laser irradiation for longer periods of time, the laser beam is considered to be continuous in nature of same average power. This is been shown and validated by experiments in the past [78]. In this work, the results of a pulsed laser irradiation experiments on a live anesthetized mice were validated by a COMSOL model which was solved using the approximate method and the results were in acceptable conformity. The group of Sun and Mikula [79] have also modeled the temperature increase in the human cadaver retina during direct illumination by 150 kHz femtosecond laser lasers. A clinical setting 150 kHz femtosecond laser over a 52 second interval procedure was performed and simulated using a COMSOL model. The results obtained through modeling and clinical trials were in good agreement. Eze [80] simulated the interaction of focused laser beam with skin using COMSOL. The effects of pulsed lasers and CW lasers on the skin were considered and feasible model of fractional
photothermolysis was developed. Shafirstein et. al. [81] used finite element modeling using COMSOL to apply the diffusion approximation theory for selective photothermolysis modeling and its implication in laser treatment of port-wine stains. They studied the effect of upto 3 laser pulses each in the millisecond range to calculate the depth of the heat flux in the tissue layer.

Pulsed laser irradiation using COMSOL has been studied not only on tissues but also on other materials. Darif [82] simulated the temperature and phase change in material irradiated by KrF pulsed beam which is confined to the silicon’s surface. The pulse width is 27 ns and effects were studied at various time intervals. Parametric studies were done by varying the laser fluence rates. Jouvard et. al. [83] modeled the modification of metal surface by an ablation of matter due to laser irradiation. An Nd:YAG laser pulse of 180 ns was modeled on a metal surface which led to the melting of the metal at the surface. After the laser is switched off, the solidification time is also found out.

1.6 Research Objectives

The objective of this research work is the study the heat affected and ablated zone during pulsed laser irradiation of tissues. Two major interaction mechanisms, the photothermal and photomechanical interactions are studied.
A three layered tissue structure is modeled using the software package COMSOL Multiphysics. The irradiation of the laser beam is modeled as the heat source term in the “Bioheat Transfer” module of the software. The software provides various options to vary the average power, frequency, pulse width and the radius of the laser beam.

The efficacy of thermal interaction is quantified using the extent of heat spread caused by the laser. The ablated zone is measured using the Arrhenius damage integral. This takes into account the temperature history of the tissue and also requires the tissue activation energy and the frequency factor. The thermal dose administered to the tissue is also measured by calculating the effective heating time of the tissue at 43°C under the laser irradiation process. The extent of ablation zone is compared with representative histological studies. Excised mice samples were subject to Q-switched Nd:YAG laser irradiation. The laser affected tissue region were isolated and stained. The laser induced ablation zone were observed under the microscope. The affected region observed in the histological studies were compared with that predicted by the COMSOL models. Along with the photothermal interaction, photomechanical interaction of the pulse laser tissue irradiation process is modeled using the Acoustic Module of the software package COMSOL Multiphysics. The choice of module used is due to the nature of the stress waves produced due to the irradiation of pulsed laser in the tissue.
Chapter 2: Materials and Models

2.1 Major Equipment

The Laser Optics and Instrumentation Laboratory and Department of Biomedical Engineering at Florida Institute of Technology provided the majority of equipment and software used in experimentation. The details and specifications of these items are described below.

2.1.1 Q-switched Nd:YAG Laser, 1064 nm

An Nd:YAG (neodymium-doped yttrium aluminum garnet) laser with 1064 nm wavelength and 200 ns pulse width is used in this study. The Nd:YAG laser is a very functional system and consequently very common. This type of laser is optically pumped using Krypton flashlamps, xenon lamps, or laser diodes. The particular system used in experimentation utilizes light flashes produced by a Krypton lamp to excite the Nd:YAG crystal. The lamp and rod are mounted in a parallel configuration.

This laser operates in the near-IR range. The system used for these tests (Lasermetrics, series 9500) operates in pulsed mode. The user can control the current, which therefore allows for a desired variation in time-averaged power. The current can vary from 11 A to 21 A and the power ranges from 100 mW to 10 W. The laser has a power stability of 2-3% over one hour.
A Q-Switch driver (MVM Electronics, Incorporated) was required in order to produce a pulsed laser beam. The Q-switch incorporates an optical switch which is housed within the optical resonator of the laser. If the Q-switch is off, the laser will operate in CW mode. However, when the Q-switch is operating, pulsed mode is achieved. Lasing cannot start when light escapes the gain medium (the YAG crystal). The Q-switch will first prevent lasing by preventing feedback of the light leaving the gain medium. The decrease in amplitude as light leaves the medium will relate to the decrease in what is known as the quality factor (Q-factor) of the optical resonator. A low Q-factor will create a population inversion in the system, where the majority of molecules are in a high-energy or excited state. Lasing can still not begin because feedback is still being prevented by the Q-switch. However, the medium will continue to be pumped, which will cause an energy buildup in the medium. Eventually it will reach its point of maximum energy, which is known as a gain-saturated state. Once this occurs, the Q-switch will move from the current position of low Q-factor to a higher Q-factor, allowing feedback and corresponding to low attenuation levels. Since much energy is built up during the low-Q state in the gain medium, the light intensity builds up quickly and then is quickly released, resulting in a short pulse with high peak intensity.
The Q-switch used for this research is a variable attenuator that is not incorporated within the laser itself but as a supplemental, external component. An acousto-optic modulating device is used which delivers pulses of 200 ns duration. The user can vary the repetition rates from 10 Hz to 100 kHz.

2.1.2 Thermal Imaging Camera

An infrared camera operates in a different range from that of a normal camera. It can capture energy in the infrared (above 750 nm) which is actually heat energy. The camera converts the energy into a signal corresponding to the temperature at the specific location. This information is compiled to create a thermal image, where pixel values can be related to temperature values which will depend on the user-defined settings and the range of temperatures expected. In this way, heat-affected zones of the sample can be studied.

A thermal imaging camera (Agema Thermovision 450) is utilized to measure surface temperature of samples during irradiation testing. During testing, the camera can capture images at a user-defined interval of time (1 second interval for this research). The images are recorded using a data acquisition system and are then processed with National Instruments IMAQ Vision Builder image-processing software. The camera provides a measurement range of -4°F to 932°F with a sensitivity of ±0.18°F and
accuracy of ±3.6°F (2%). The camera has a spectral response rated at 2 to 5 μm.

2.1.3 Excised mouse tissue samples

Laboratory mice are chosen for experimentation due to the similarity between the optical properties of their skin and those of human tissue. Tissue is removed from euthanized mice, which were killed by CO₂ inhalation. All animal use procedures have been approved by the Institutional Animal Care and Use Committee of Florida Institute of Technology. Skin tissue samples of 1.5 mm are used for the majority of experiments due to the relative ease in mounting these specimens and the ability to prepare samples which have fairly uniform thickness. These thin samples included the epidermal and dermal layers of skin.

2.1.4 Laser Calibration

Before testing begins, a power calibration must be performed. Time averaged power is a critical parameter in achieving desired results in tissue samples during laser irradiation. For the Q-switched Nd:YAG laser, the output power can be adjusted by varying the current from 10 A to 21 A as well as the repetition rate from 40 Hz to 100 kHz. The power can be correlated to the current; however, due to instability in the laser, this relationship does not
always remain constant. Because of this, the time-averaged output power is monitored during experimentation.

Due to the high front-end power of the Nd:YAG, a HeNe laser is used for alignment and calibration. The front-end power of the collimated HeNe beam is measured, as is the power after reflection of the beam using a 3% glass crystal. After achieving the correct 3% power reading of the reflected HeNe beam, proper alignment has been ensured. The Nd:YAG laser is then calibrated. The final step requires the user record the back-end power for each current setting. In this way, the back-end power is monitored during experiments and is correlated with the front-end power.

<table>
<thead>
<tr>
<th>Current (A)</th>
<th>Frequency (kHz)</th>
<th>Back End Power (mW)</th>
<th>Attenuation Factor</th>
<th>Front End Power (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1</td>
<td>3</td>
<td>441</td>
<td>1.32</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>10.2</td>
<td>441</td>
<td>4.49</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>2.7</td>
<td>441</td>
<td>1.19</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>11.5</td>
<td>441</td>
<td>5.07</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>3.6</td>
<td>441</td>
<td>1.58</td>
</tr>
</tbody>
</table>

Table 2.1: Calibration Table for Nd:YAG Laser

35
2.2 Experimental Setup

The setup for tissue irradiation using the Nd:YAG is shown in Figure 2.1. The beam is then focused to a 1 mm spot size by a converging lens with a 21 cm focal length.

![Diagram of laser setup](image)

**Figure 2: Setup for mouse tissue laser irradiation experiment**

The laser is calibrated at the beginning of each experiment so that the power incident on the sample is known for each current setting (from 11 A to 21 A). This is correlated with the back-end power which is then monitored throughout testing using a power meter. Samples are placed on the three-axis translation stage and positioned appropriately. The stage is adjusted using the three micrometers so that the target area of the sample is brought within the focal distance of the laser-optics setup. A Thermovision Agema thermal imaging camera records the surface temperature of the samples during testing using a computerized data acquisition system. Images are processed with
National Instruments IMAQ Vision Builder Image software to convert pixel values into temperature readings. Thermocouples are inserted at specific subsurface locations and the temperature is recorded using a computerized data acquisition system and LabView Software.

2.3 Mathematical Modeling

2.3.1 Modeling the Laser Source Term

To calculate the source term $Q_L$ which is the heat generated due to the irradiation, the laser beam is assumed to be Gaussian in both the spatial and temporal domains and it is expressed as follows:

$$Q_L = \frac{\mu_a}{4\pi} I_c (\mu^c \mu + \eta^c \eta + \xi^c \xi)$$

Where the unit vector of $(\mu^c, \eta^c, \xi^c)$ represents the collimated laser incident direction and $\mu_a$ is the absorption coefficient [m$^{-1}$]. The collimated intensity is given by

$$I_c = L_0 \exp \left\{ -4\ln 2 \times \left[ \frac{(t - \frac{Z}{c})}{\tau_p} - 1.5 \right]^2 \right\} \times \exp \left( \frac{-2r^2}{\sigma^2} \right) \exp (-z \mu_e)$$
where \( t \) is the time, \( t_p \) is the laser pulse width, \( c \) is the velocity of light in the tissue medium, \( r \) is the spatial variable denoting radial distance from the center of the laser beam [m], \( z \) is the spatial variable denoting depth from the surface of the tissue [m], \( \mu_e \) is the extinction coefficient [m\(^{-1}\)]. \( L_0 \) is the peak intensity of laser beam at the sample surface, \( \sigma(z) \) is the beam radius varying with \( z \) for which the peak intensity drops to the \( 1/e^2 \) value. \( L_0 \) is given as:

\[
L_0 = \frac{P}{\pi r_0^2} \tag{2.3}
\]

where \( P \) is the power of the laser [W] and \( r_0 \) is the laser beam radius at the tissue surface [m]. For the case of the focused laser beam, \( \sigma(z) \) is given by [11]:

\[
\sigma(z) = \sigma(0) \left( \frac{-(r_0-r_d)}{r_0} \right) \left( \frac{z}{F_d} + 1 \right) \quad 0 \leq z \leq F_d \tag{2.4}
\]

\[
\sigma(z) = \sigma(0) \left( \frac{-(r_0-r_d)}{r_0} \right) \left( \frac{z}{F_d} - \frac{(r_0-2r_d)}{r_0} \right) \quad z > F_d \tag{2.5}
\]

where \( F_d \) is the focal depth [m], \( r_d \) is the beam radius at the focal depth [m], and \( \sigma(0) \) is the beam radius at the tissue surface for which the peak intensity drops to the \( 1/e^2 \) value [m].

The various laser parameters used in the model are summarized in Table 2.2.
2.3.2 Continuum Model Development

The geometry of the continuum model is shown in Figure 2.2. The geometry simulates the three layers of the skin: the epidermis, dermis, and hypodermis. The tissue block is 6 mm square, with a thickness of 6.5 mm. The

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$F_d$ (m)</th>
<th>$r_d$ (m)</th>
<th>$\sigma(0)$ (m)</th>
<th>$r_0$ (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.002</td>
<td>100E-6</td>
<td>76E-6</td>
<td>20E-6</td>
</tr>
</tbody>
</table>

Table 2.2: Laser beam geometric properties

2.3.2.1 Thermo-physical properties of tissue components

The thermo-physical properties and the perfusion rates of the three tissue layers [18] and blood are obtained from literature and are presented in Table 2.3.

<table>
<thead>
<tr>
<th>Components</th>
<th>$k$ (W m$^{-1}$ K$^{-1}$)</th>
<th>$C$ (J kg$^{-1}$ K$^{-1}$)</th>
<th>$\rho$ (kg m$^{-3}$)</th>
<th>$w_{01}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>0.25</td>
<td>3600</td>
<td>1200</td>
<td>0</td>
</tr>
<tr>
<td>Dermis</td>
<td>0.45</td>
<td>3300</td>
<td>1200</td>
<td>0.0004</td>
</tr>
<tr>
<td>Hypodermis</td>
<td>0.20</td>
<td>3000</td>
<td>1000</td>
<td>0.0026</td>
</tr>
<tr>
<td>Blood</td>
<td>1.3</td>
<td>3600</td>
<td>1000</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2.3: Thermo-physical properties of tissue components

epidermis is 0.5 mm thick, while the dermis and hypodermis are each 3 mm thick.
Figure 3: Continuum model geometry

The geometry mesh consists of 82,985 elements with maximum and minimum element sizes of 6.5E-4 m and 1.17E-4 m, respectively. Figure 2.2 shows the meshed geometry.

Figure 4: Meshed continuum model geometry
In the entire domain, the temperature distribution is obtained by solving the bio-heat transfer equation (BHTE) given by [11]:

$$\rho c_t \frac{\partial T_t}{\partial t} = \nabla \cdot (k_t \nabla T_t) + Q_{bl} + Q_{met} + Q_L \quad (2.6)$$

The heat sink term due to blood perfusion is given by

$$Q_{bl} = \rho_{bl} w_{bl} c_{bl} (T_a - T_t) \quad (2.7)$$

where $k$ is the thermal conductivity [W m$^{-1}$ K$^{-1}$], $\rho$ is density [kg m$^{-3}$], $c$ is specific heat [J kg$^{-1}$ K$^{-1}$], $Q_{met}$ is the volumetric metabolic heat generation [W m$^{-3}$], $w_{bl}$ is the blood perfusion rate (m$^3$blood/s/m$^3$ tissue), and $T_t$ is temperature of the tissue [K]. $T_{bl}$ is the temperature of blood and $T_a$ for arterial temperature. Due to the relatively high magnitudes of the induced laser energy, and the relatively short times constants associated with the thermal energy, it is assumed that metabolic heat generation $Q_{met}$ may be safely neglected. The arterial blood temperature, $T_a$, is set to the physiological temperature of 310.15 K (37 °C). The specific heat of blood $c_{bl}$ is obtained from Sassaroli et al. [84] as 4000 J kg$^{-1}$ K$^{-1}$. $w_{bl}$, the blood perfusion term, is given in units of (m$^3$blood/s/m$^3$ tissue) and multiplied by the blood density (1000 kg m$^{-3}$) to obtain the proper units of kg m$^{-3}$ s$^{-1}$. The optical properties, $\mu_s$ (scattering coefficient) and $\mu_a$ (absorption coefficient), are detailed for each tissue layer and blood in Table 2.4 and the values are derived from Jacques [8].
<table>
<thead>
<tr>
<th>Components</th>
<th>$\mu_s$ (1/m)</th>
<th>$\mu_a$ (1/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>8000</td>
<td>355</td>
</tr>
<tr>
<td>Dermis</td>
<td>8000</td>
<td>49</td>
</tr>
<tr>
<td>Hypodermis</td>
<td>7000</td>
<td>50</td>
</tr>
<tr>
<td>Blood</td>
<td>1000</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2.4: Optical properties of tissue components**

For the boundary conditions, the top layer of the epidermis is subjected to convective cooling. The convective heat transfer coefficient ($h$) is set to 10 W (m$^2$K)$^{-1}$ and the ambient temperature ($T_{amb}$) is assumed to be 298.15 K (25 °C). The contribution of convective cooling to the thermal load is given by:

$$q_{conv}'' = h(T_{amb} - T)$$ (2.8)

All other external boundaries of the geometry are maintained at the physiological temperature of 310.15 K (37 °C).

### 2.2.3 Software Package- COMSOL Multiphysics

COMSOL Multiphysics is a FEM solver designed with engineering applications in mind. COMSOL is especially useful for coupling multiple scientific models together. COMSOL features a large number of inbuilt physics modules such as computational fluid dynamics (CFD), acoustics, corrosion, electrochemistry, heat transfer, microfluidics, structural mechanics,
optics, and more, including sub-modules for many of these. COMSOL enables users to control all steps of the FE modeling process, as discussed in section 1.4.3.

COMSOL does have some inbuilt CAD capabilities, however; users will generally wish to create geometry in true CAD software such as SolidWorks, and import the file into COMSOL. To aid in this, COMSOL has the capability to live-link to SolidWorks. Thus users can instantly import their SolidWorks model into COMSOL and any changes made via SolidWorks are updated real time in COMSOL. Additionally, there is a preset material property library in COMSOL, but users can also create their own material and input their own data. COMSOL’s functionality for engineering problems makes it favorable software to use for parametric studies and experimental comparison.

**2.4 Histological Study**

After irradiating samples, a histological study is performed to assess the extent of laser-induced damages. Samples are inspected for thermal damages including swelling, dehydration, and photocoagulation of the tissue. In samples where tissue removal has been achieved, the size of ablated zone is considered as well as the quality of surrounding, non-targeted tissue. When treating unhealthy tissue such as a tumor with therapeutic laser systems, it is
important to limit the extent of collateral damage to the surrounding healthy tissue.

To prepare the tissue samples, excised tissue is sectioned, stained, and mounted for observation using an optical microscope. Steps involved in histological studies are described below.

2.4.1 Frozen Sectioning Technique

Excised tissue samples are cut into small sections approximately 5 mm in diameter that include the irradiated area as well as surrounding healthy tissue. These segments are embedded in molds filled with optimal cutting temperature (OCT) compound. The molds are placed in a cryostat which is kept at -30°C and allows the OCT to solidify. The frozen samples are cut into 20 μm-thick sections in a direction perpendicular to the surface of the skin using the cryostat and placed on microscope slides. A few drops glycerol-based mounting medium is placed on the slides followed by slide covers.

2.4.2 Staining Method

The samples are stained in order to more easily distinguish among the different tissue structures. Hematoxylin and Eosin (H&E) is a rapid staining method that is commonly used due to its simplicity. This progressive stain creates
stained sections which have deep blue nuclei with a pink or rose-colored background of other cytoplasmic structures.

Eosin stain is prepared by dissolving 1 gm of eosin Y powder in 70% ethyl alcohol. Glacial acetic acid (5 mL) is added to the eosin-ethyl solution.

Once the mixtures have been prepared, the following procedure is followed

**Staining Method:**

1. Place slide in alcoholic formalin for 15 seconds.
2. Dip slides 10 times in 70% alcohol.
3. Rinse in distilled water
4. Stain in hematoxylin for 45 seconds to 1 minute.
5. Rinse again in tap water.
6. Counterstain by placing in eosin for 30 seconds.
7. Place in empty beaker to dehydrate.
8. Drop a small amount of buffered glycerol on the stained slide and place cover slip to protect the sections.

**2.4.3 Observation and Damage Evaluation**

Once the sample preparation is complete, slides are viewed under an optical microscope using 40x magnification. Tissue morphology is considered by
evaluating the extent of the heat-affected zone of the samples. Damage is evident in several ways. For example, heat deposition in tissue causes it to swell. Extensive thermal damage causes dehydration to the tissue which can lead to shrinkage and photoagulation of the tissue. For some therapeutic laser treatments, this is desirable as thermal damage can lead to necrosis of unhealthy tissue, such as tumors or cancerous cells.

Ablation depth and width can be measured using Amscope image software in order to correlate ablation volume to laser parameters. The software is calibrated to provide a measurement in millimeters corresponding to the magnification at which an image is was captured. Maximum depth and width of each ablated volume is measured, along with precise quantification of irregular or non-uniform cross-sectional areas using a free-hand drawing tool. The extent of the damage is associated with the laser parameters which are used to irradiate the tissue sample. In this way, laser-induced damages can be better predicted and controlled for a particular set of laser parameters.
Chapter 3: Results

The goal of this research is to model the interaction of infrared pulsed lasers on skin tissues and also to model the damage caused by the process. The interaction of the laser is observed in terms of photothermal and photomechanical interaction of laser light with tissues. Temperature distribution inside the tissue is modeled using the bio-heat transfer equation to determine the transient temperature function required to calculate the photothermal and photomechanical interaction subsequently. Photothermal interaction observed is measured in terms of effective thermal dose and Arrhenius damage integral. Both of these are proven and widely used concepts for measuring thermal damage in biological systems. Photomechanical interaction observed is measured in terms of the acoustic pressure generated as a result of the pulsed laser irradiation. Magnitude of damage is reported in terms of the peak stresses generated in the tissue in the early moments of the process. All the computational operations have been performed using finite element analysis in the software package – COMSOL Multiphysics. Representative histological results have also been included to depict the damage observed to provide a more visual representation.
3.1 Transient temperature distribution

In order to perform the damage analysis, we would require the temperature distribution in the tissue as a function of time. This is evaluated by the use of the bio-heat transfer equation which governs the transfer of heat energy inside the tissue medium.

The following figures show the temporal profile of the Gaussian laser beam and also temperature rise variation at the surface of the tissue geometry as a function of time for the first 10, 100 and 500 pulses respectively for a pulse width (FWHM) of 200ns and repetition rate of 1000 Hz. The average laser power is 1.3 W in all the cases.

![Figure 5: Gaussian pulse beam shape](image)
Figure 6: Temperature distribution at the surface after 10 pulses

Figure 7: Temperature distribution at the surface after 100 pulses
In our earlier work [78], we had used average power of the laser beam to compute the temperature distribution inside the tissue due to pulse laser irradiation method. However, in this work, we have resolved the pulse exactly using staggered time stepping and optimizing the COMSOL study steps. This method is explained in detail in the section 1.5.4 of the thesis. However, we can only produce results up to the first 500 pulses in this work. We have concluded that the using the average power approximation is valid when analyzing temperature distribution over longer time intervals in the range of seconds whereas the individual pulses are resolved when analyzing effects in the microscopic time intervals in the order of a few pulses of the beam (fraction of a time). This is very useful when analyzing the mechanical effects of the pulsed laser irradiation which last for only a few pulses worth of time.

The following figure displays the temperature distribution of the mid-plane YZ slice at the end of 500 pulses. The depth of penetration of the laser heat can be gauged by the diagram.
Figure 8: YZ slice temperature distribution after 500 pulses

The accuracy of computer model generated has been validated against experimental measurements in the following figure where the surface temperature of the tissue predicted by the computer model is compared with the experimental measurements.
3.2 Thermal damage

3.2.1 Comparison with CW lasers

The thermal dose of the pulsed laser irradiation was measured by the Sarapeto and Dewey method [63] as discussed in chapter section 1.4.1.
The thermal dose was calculated for a time averaged power of 1.3 W for both CW and pulsed laser. The results in Figure 3.8 have been computed only till 0.1 seconds (100 pulses) because of the computational complexity required to resolve a nanosecond pulse as sparse as 1 millisecond. It is clearly visible that for the first 100 pulses, the damage caused by the pulsed in higher in magnitude than the damage caused by the CW laser for similar time averaged power. This might be counterintuitive in nature. However as we can see, for the CW case, the damage plot is exponential in nature as compared to that for the pulsed laser which is nearly directly proportional to the time period.

Figure 10: Thermal dose comparison of CW laser and pulsed laser for 100 pulses with average power of 1.3 W
Comparison of damage was also done using the Arrhenius integral method and is presented in the following figure. As expected, the variation observed is very similar to the one for thermal dose calculated using Dewey method.

Figure 11: Comparison of Arrhenius damage of CW laser and pulsed beam for 100 pulses with average power of 1.3 W

3.2.2 Parametric Analysis of Thermal Dose

The following figure shows the variation of thermal damage as a function of the pulse width of the laser beam at the point on the surface where the beam is focused for the first 100 pulses of the laser irradiation. The damage data has
been shown on a log scale to account for the exponential change in the damage with the variation of the pulse width of the laser.

![Graph showing variation of thermal dose with pulse width of pulsed laser](image)

**Figure 12: Variation of thermal dose with pulse width of pulsed laser**

A sharp decline of thermal dose is seen as pulse width increases and the trend continues with further increase in pulse width. This is because as pulse width increases, keeping other factors constant, the peak power of each pulse subsequently decreases. This leads to less thermal stress generated and less temperatures per pulse.
The following shows the damage caused by the laser as a function of the average laser power which is directly correlated to the irradiance of the laser. Damage is shown in log scale.

![Graph showing the variation of thermal dose with laser pulse power for first 100 pulses.](image)

**Figure 13: Variation of thermal dose with laser pulse power for first 100 pulses**

There is an exponential rise in the dose with the increase in laser pulse power. This is because of the increase in the energy per pulse delivered to the tissue during the irradiation. This directly contributes to the thermal dose administered to the tissues.
Figure 14: Variation of thermal dose at the surface with the frequency of pulsed laser beam

With the increase in frequency, keeping other factors constant, leads to a decrease in the peak power delivered. This directly affects the thermal dose administered to the tissue. This trend is very similar to the variation of the thermal dose with respect to the pulse width. The exponential nature of the plot is because of the exponential term in the expression used to calculate the thermal dose which is mentioned in detail in section 1.4.1 of the thesis.
3.2.3 Parametric Analysis of Arrhenius Damage Integral

The trends observed for the change in damage with respect to change in frequency, pulse width and average power are very similar to that observed for the thermal dose. This establishes the fact that there is a strong correlation in the two damage models which is inherent in the formulation of their expressions.

The following figure shows the variation of Arrhenius damage with the change in laser pulse power of the pulsed laser beam at the point on the surface where the beam is focused for the first 100 pulses of the laser irradiation. Damage is shown in log scale.
Figure 15: Variation of Arrhenius damage with laser pulse power for first 100 pulses

The following figure shows the variation of Arrhenius damage with the change in pulsed width of the pulsed laser beam at the point on the surface where the beam is focused for the first 100 pulses of the laser irradiation.
The following figure shows the variation of Arrhenius damage with the change in the frequency of the pulsed laser beam at the point on the surface where the beam is focused for the first 100 pulses of the laser irradiation. Damage is shown in log scale.

**Figure 16: Variation of Arrhenius damage with pulse width of pulsed laser beam for first 100 pulses**
Figure 17: Variation of Arrhenius damage with frequency of pulsed laser beam

3.3 Parametric Analysis of Photomechanical Interaction

As discussed earlier, the photomechanical interaction of the laser tissue interaction was measured using the peak stress generated as a result of the pulsed laser irradiation on tissues. The mechanism of the generation of the stress waves have been explained in the section 1.4.2 of the thesis.

The stress distribution due to nanosecond pulsed laser irradiation after the first 10 pulses (200ns, 1.8 W average power) on the tissue surface is shown in the following figure.
It is visible that the stress reaches a few microns away from the point where the laser is focused at the surface. After it reaches the peak, the stress slowly decreases as we move radially away from the point of irradiance. A better visualization of how the stress varies with the radial distance is shown in the following figure.

**Figure 18: von Mises stress distribution seen at the tissue surface at the end of 10 laser pulse irradiation**
Figure 19: Variation of von Mises stress along the surface at the end of 10 laser pulse irradiation

There is a sharp increase of stress as we move away from the point of irradiance. This reaches a peak stress value and then decreases as we move further. The sharp stress gradient produced as a result of pulse laser irradiance is injurious to the tissue. This takes place in the early stages of the irradiation process when the damage due to the thermal interaction has not taken place in the order of a few pulses. A parametric analysis of how the maximum stress at the surface varies as a result of pulse width of the laser beam and laser pulse power is shown below in the figures.
It is evident that the maximum stress at the surface increase in an almost a direct proportion to the average power of the laser beam. The average laser power is also in direct proportion with the laser fluence which is the average energy per unit area. This plot shows us the maximum stress increase almost linearly with the increase in laser fluence or the average laser power.

The variation of maximum stress generated at the surface with the pulse width of the pulsed beam is shown in the following figure.
Figure 21: Variation of maximum von-Mises stress at the surface with pulse width of the laser irradiation with average power 1.3 W

As with the case of the thermal damage, the maximum stress decreases steadily with the increase in pulsed width of the laser beam. This can be attributed to the decrease in the temperature gradient of the laser pulse when the pulse width is increased. Also, the peak power is decreased with the increase in the pulse width. This trend is also consistent with thermal damage results we saw where there was an exponential decrease with the increase in the pulse width of the laser.
3.4 Evaluation of Damage Zone

One of the major objectives of the work was to identify the damage caused by the pulsed laser irradiation. Thermal damage has been calculated in terms of the thermal dose and the Arrhenius damage function. In this section direct comparison is made between the damage results obtained by the laser irradiation results and the damage zone observed in the histological results.

Below is the histology result showing damage caused due to a particular case of laser irradiation of high power and long irradiation time [85]. The targeted tissue as well as the area surrounding the ablated volume has been photocoagulated due to heat deposition in situ. The tissue at the margin appears darker and has distorted which can occur as a result of dehydration [86]. Dehydration of tissue also leads to formation of vacuoles (white bubble-like formations) and tissue at the surface is ablated. Because collateral thermal damage is significant in this sample as shown by tissue shrinkage and vacuole formation, it is concluded that the photothermal mechanism was contributing to the laser-induced damages. Photomechanical effects may be present, but it is difficult to uncouple them from the thermal damages observed.
Figure 22: Excised mouse skin tissue after 20 second irradiation by Nd:YAG laser focused at $z = 0$ mm at 1.8 W time average power

According to various sources in literature [87] [68] [88], a dose threshold of 240 minutes is adequate to coagulate all tissue and thus can be used as an indicator for identifying the damage zone caused due to the photothermal interaction of the laser beam with the tissue. The results obtained when the thermal dose is calculated for the same conditions are shown below. The zone circled in the figure is the region of extreme damage where the thermal dose is many times the damage threshold which is enough to cause the type of damage seen in the histology sample in the following figure.
Figure 23: Evaluation of ablation depth for a case of laser irradiation of 20 seconds by Nd:YAG laser focused at $z = 0$ mm at 1.8 W time average power

The damage zone is observed clearly in the figure above. A rough estimation of the ablation depth can be seen as the depth of the tissue which exceeds the damage threshold as mentioned. The size of the damage zone according to the software is measured to be around 500 µm which approximately correlates to the damage observed in the experimental sample.

We also irradiated some excised mouse samples with Nd:YAG laser under a different frequency with varying current and observed the damage. A slight increase in damage depth is observed as the power of the pulsed laser beam is increased.
Figure 24: Excised mouse tissue sample exposed to Nd:YAG laser. Average Power 4.5 W, Frequency 5 kHz

Figure 25: Excised mouse tissue sample exposed to Nd:YAG laser. Average Power 5.1 W, Frequency 5 kHz
Chapter 4: Conclusions and Future Work

In this thesis, the photothermal and photomechanical effects of short pulsed laser irradiation on tissues are studied. The heat affected zone in tissues as a result of laser irradiation is studied for a range of laser parameters. These parameters are the pulse width, pulse power and the frequency of the laser beam. The pulse width is varied from 50 to 500 nanoseconds, peak pulse power from 5000 to 25000 W and frequency from 500 to 5000 Hz. Numerical simulation was performed using a finite element model developed in COMSOL Multiphysics software. Histological studies of excised mouse tissue samples were performed to measure the size of heat affected zone as a result of laser irradiation. The temperature distribution due to laser irradiation was obtained using the Pennes’ bioheat transfer equation. The accuracy of the model developed was validated with the experimentally determined surface temperature in excised mouse tissue samples after irradiating them for 20 seconds with different average laser powers. After validation, the effect of individual pulses was taken into account instead of a long term average effect as found in prevalent models. Short pulses of laser beam deliver very high power to the target location in a short period of time and hence restrict the heat affected zone to a localized region. This is in contrast to CW lasers where the heat energy spreads to nearby tissues leading to an increase in the heat affected zone. The resolution of individual pulses of laser beam enabled the analysis of
the thermal and mechanical effects in the microscopic time interval in the order of the laser pulse width which was 200 nanoseconds in this study. The final temperatures observed by numerical simulations at the end of 0.5 seconds considering individual laser pulses and average laser power were found to be in close agreement.

The photothermal effect is the most dominant effect in laser tissue interactions. This was analyzed using the two prevalent models. The Arrhenius integral which gives a measure of the fraction of tissues damaged and the thermal iso-effective dose model which gives the time period for which the tissue is subjected to temperature above its damage threshold. Understanding the variation of the photothermal interaction is required for limiting the heat affected zone during the laser irradiation process. The parametric study involving frequency, pulse width and pulse power was considered. The size of the heat affected zone observed as a result of pulsed laser irradiation on excised mouse tissues during histological studies was close to what was predicted by the COMSOL thermal dose model.

The coupling feature of COMSOL Multiphysics was utilized for simulating the photomechanical interaction due to the nanosecond laser beam. The “Heat Transfer” module was coupled with the “Acoustic-Structure” module. The stress distribution reaches a peak and gradually decreases as the distance from the point of irradiation increases. The variation of the acoustic
stress wave generated as result of nanosecond laser beam was analyzed with respect to change in pulse width and pulse power of the laser.

The current pulsed laser irradiation model has overcome previous shortcomings by incorporating the effect of individual pulses in obtaining the temperature distribution due to pulsed laser irradiation. However the number of pulses is still limited to a few hundred pulses. The solver of the model has to be optimized to be able to incorporate a few thousand pulses. This will enable the study of the effects of pulsed lasers on tissues on longer time scales in the order of a few seconds. The influence of the presence of blood vessels in the variation of observed irradiation effects needs to be considered. The photomechanical model developed can be used to model stresses in other laser based applications like tattoo removal or transdermal drug delivery involving the use of picosecond and shorter pulses. With the successful inclusion of these features, the photochemical and other complex laser-tissue interactions can be studied.
Bibliography


[42] G. Hale and M. Querry, "Optical constants of water in the 200nm to 200

[43] Walsh et. al, "Pulse CO2 laser ablation: Effect of tissue type and pulse

[44] J. Cummings and J. Walsh, "Tissue tearing caused by pulsed laser

[45] Brugmans et al , "Temperature Respnse of biological materials to pulsed

[46] S. Thomsen and J. Pearce, "Thermal Damage and Rate Processes in
Biological Tissues," in Optical-Thermal Response of Laser Irradiated

[47] Alberts et. al. , Molecular biology of the cell, New York: Garland

[48] H. Yoshimura , J. Viator and Jacques SL, "Relationship between
damaged fraction and reflected spectra of denaturing tissues," Lasers

[49] S. Thomsen, "Non-thermal effects in thermal treatment applications of


