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Literature Survey
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General Introduction

Biophotonics as a science and technology is growing very rapidly. Tissue optics, confocal and two photon microscopies, optical coherence tomography, lasers in medicine, photodynamic therapy, low level laser therapy, optical biomedical diagnostics are some of the important branches of Biophotonics research.

Due to variety of existing laser systems, diversity of their physical parameters the above branches got enriched with research. Diode lasers and free electron lasers have been important tools for advanced medical research, while pulsed lasers contributed more to laser surgery. Mathematical modeling of light transport in tissues and interaction with cells provided basics for all types of applications.

Infrared neural stimulation has been demonstrated in mammalian sciatic nerve, cochlea, brain, heart, vestibular system. Recent work is focused on dorsal roots, auditory cortex and individual nerve cells. Free electron lasers, Holonium YAG lasers are being used effectively in this domain of research.

Excitation of neuronal cells using infrared lasers has been reported, but the mechanism is not known. IR pulses get absorbed by water; produce local temperature changes which may alter membrane depolarization. Optogenetics is also now an important direction to study light neuron interaction.

Therefore, in the present work, considering the availability of laser techniques and resources certain objectives are defined to study laser nerve interaction on the basis of electrophysiological signals. Also, laser- cells (RBC) interaction based on FTIR spectroscopy are focused.
1.1 Laser tissue interactions

The biophysical modalities of laser tissue interactions are separated into thermal & non-thermal. Thermal mechanisms include vaporization & photocoagulation. Non-thermal mechanisms are photo ablation & photo-dry-etching of tissues. In photochemical interactions light induces reactions & chemical effects within the tissue. Biostimulation & photodynamic therapy are essentially based on photochemical interactions (Kulkarni, G.R. 1988). In low power laser to have any effect on living biological system, the photons must be absorbed by electronic absorption bands belonging to some molecular chromophores or photo acceptor (Sutherland, J. C. 2002). Optical properties of tissue determine the interaction of laser at certain wavelength, power & exposure time (Markolf, H. N. 1996). Different advanced photonics methods & laser stimulation techniques are effectively used in biomedical science & clinical studies (Tuchin, V.V. 2010). The interaction of light at the molecular level producing absorption, spontaneous emission & Raman scattering is described by P.N. Prasad along with various spectroscopic methods useful for biophotonics (Prasad, P. N. 2003). (Malton, C. et al., 2011), show that the fluorescent protein in cells are a viable gain medium for optical amplification, and report the first successful realization of biological cell lasers based on green fluorescent protein. Green fluorescent protein (GFP) purified from the jellyfish A. Victoria & has become a tool in biomedical science. When pumped at an appropriate wavelength, fluorescence proteins are expected to form quasi-four level laser systems. (Desmond, C.A. et al., 2007), Optical tomography enables micrometer-scale, subsurface imaging of biological tissue by measuring the magnitude and echo time delay of backscattered light. Endoscopic optical coherence tomography imaging inside the body can be performed using fiber-optic probes. The laser with a 160 nm tuning range at a wavelength $\lambda=1,315$ nm, can produce images with resolution of 5-7 $\mu$m in optical coherence tomography.

In photo dynamical therapy, a photosensitizing drug is used to cause the necrosis (cell death) and apoptosis ('programmed 'cell death). It is widely used in oncology to destroy the cancerous tumors. A new family of photodynamic therapy drugs designed for efficient two photon excitation were used. One of them is to
demonstrate selective closure of blood vessels through two photon excitation photodynamic therapy in vivo (Collins, H.A. et al., 2008). In photothermal interactions, the energy of the photons absorbed by any light absorbing molecules (chromophores) is converted into heat energy via molecular vibrations and collisions; it causes thermal effects from tissue coagulation to vaporization. In photoablation the higher energy ultraviolet (UV) photons are absorbed by tissues, raising them from a lower energy ‘binding’ orbital to a higher energy ‘non-bonding’ orbital & causing virtually immediate dissociation of the molecules are studied (Markolf, H. N. 1996). In plasma-induced photoablation, energetic electron collides with a molecule. Mechanical effects such as plasma generation, bubble formation, cavitations, jetting and shockwaves for photodisruption were studied (Ben, Cox. 2010).

1.2 Low power laser radiation effects on cells

The effects of laser irradiation with $\lambda=632.8$ and 532 nm and fluence rate 1.5 mW/mm$^2$ on rheological properties of blood were comparatively studied in vitro. Laser irradiation increased the electrophoretic mobility (EPM) of erythrocytes when the values of the sample's EPM were abnormally slow. This result suggests that membrane-bound hemoglobin (Hbm) might be the initial site of the interaction, since Hbm is the main cause of poor deformability when erythrocytes were treated with Ca$^{2+}$. In all experiments including ESR, blood viscosity, EPM and erythrocyte deformability, the 532 nm laser demonstrated more efficient effects on modulating rheological properties than 632.8 nm laser. This wavelength effect is consistent with the absorption spectrum of hemoglobin, reflecting that hemoglobin may be one of the action targets under laser irradiation (Mi, X. Q. et al., 2004).

He Ne laser ($\lambda=632.8$ nm, power 10 mW) with a spot of 5mm diameter was used to irradiate blood samples. The fluence was 1.5 mW /mm$^2$. The one minute irradiation applied energy was 0.6 J/ cm$^2$. Blood samples were irradiated at room temperature ($23\pm2$ °C). Low energy laser is capable of producing low energy density that all biologic effects are the results of direct irradiation and not of the thermal events. Erythrocytes demonstrated a rapid increase of oxygen saturation of hemoglobin and increase in oxygen tension of blood. Fluorescence excitation can result in the formation of reactive oxygen species and free radicals. In the case of
erythrocytes, reaction of these reactive oxygen species with membrane components caused morphological changes followed by cell hemolysis (Wasik, M. et al., 2007).

Laser irradiation has been found to have a lot of applications in the biomedical field, such as wound healing, tissue repair and vascular restenosis. The biological mechanisms of laser irradiations were also extensively studied. At the cellular level, it has been found that the oxidative metabolism increased and cytochrome C became more oxidized due to irradiation. The intravenous low power laser irradiation has recently been applied clinically in aid of treatment of acute cerebral infarction, psoriasis and rheumatoid arthritis. The effects of laser irradiation can be attributed to the improvement of microcirculation as well as the modulation of the rheological properties of blood. Laser irradiation improved the erythrocyte deformability, which might be one of the main reasons of improvement of blood circulation. The binding of Hbm is mainly attributed to the weak bond interaction between Hbm and the erythrocyte membrane. When the laser beam irradiates the erythrocytes, the Hbm molecules on the membrane can absorb the incident photons obtaining energy as well as momentum, and thus get separated from the membrane. Erythrocyte ghosts and intact erythrocytes were used to study the effect of laser irradiation on the separation of Hbm from the erythrocyte membrane. The property and functions of pig’s erythrocytes are similar to those of human erythrocytes (Mi, X.Q. et al., 2006).

The effects of three lasers namely Argon (λ= 514 nm), Nd: YAG (λ=1.6 μm) and Argon-pumped Dye (λ= 630 nm) on three types of platelet preparations were reported. In platelet rich plasma, a fall in counts was noted for the Argon and Nd: YAG laser (at high energy only). An artifactual increase in “Platelet counts” the appearance of spherocytes (red blood cell fragments), and an increase in plasma Hb indicated RBC injury. The Nd: YAG laser did not cause a change in platelet number unless extremely high levels of energy were used. The Argon pumped dye laser caused a slight artifactual increase in platelet count, due to fragmentation of white blood cells. The final series of studies were undertaken to vary the power levels (2, 5 and 10 watt) at each of the constant total energy levels (20, 60 and 100 Joules) in both PRP (plate rich plasma) and whole blood (Phyllis, T. D. et al., 1985).

In a series of articles and letters to the editor Singh and Vatsala (1979), Singh (1979) and Periasamy (1984) reported that red blood cells exposed to low levels of HeNe laser light showed changes in shape. Thus use of laser light in various medical
diagnostic methods increased to evaluate the potential effects of low-level laser light on biological tissues.

Venous blood samples were drawn into heparinsed tubes from two healthy persons and divided into three test tubes per person. The first tube was used to measure blood hemoglobin content. The blood in the second tube was exposed to HeNe laser light of 6.2 mW for 1 min, where as third tube was left as an unexposed control. The HeNe laser with wavelength $\lambda = 555$ nm & power $= 2$ mW was used. The hemoglobin contents of plasma of exposed and unexposed samples remain unchanged. The cells were focused in the microscope and photo-micrographs were taken before and after a 60 sec exposure with 2.7 mW. No visible change in cell shape was found due to the exposure. A few cells were found with spike club formation before exposure and remained with this shape after exposure (Wilander, L. et al., 1986).

Laser light irradiation has biostimulating effect on various types of cells. The effect of a green Nd: YAG laser (532nm, 30mW) irradiation on platelet activation and the expression of activated glycoprotein complex (fibrinogen receptor) of whole blood platelets was reported. The low and medium laser light energies (18 and 54 Joule) increased platelet activation, the irradiation with a high-energy laser light (108 Joule) resulted in depressed platelet reactivity and attenuated platelet response to activators (Gresner, P. et al., 2005). It has been shown that low-energy laser light can stimulate the mitosis and protein synthesis in cells, while the cells can be damaged or destroyed when high energy laser light is applied. There are a number of studies dealing with the effect of UV,red and rarely green laser light irradiation on blood platelet,damage activation, adhesion, aggregation and response to various platelet activators as well as on the production of superoxide anion radicals, peroxidation of platelets, plasma membrane lipids.

Nanosecond laser photolysis studies of hemoglobin in red blood cells (RBCs) were performed to elucidate the effects of cell membrane on the oxygen release mechanism of RBCs. For comparison with oxygen, the CO-binding reactions of deoxy RBCs and deoxyhemoglobin molecules in aqueous solutions were studied by laser flash photolysis. Hemoglobin in red blood cells plays an essential role in transportation of oxygen in vivo. The X-ray analysis studies of crystalline hemoglobin demonstrate that the structure of oxyhemoglobin differs in the protein conformation from that of deoxyhemoglobin. This finding indicates that the oxygen uptake of
hemoglobin accompanies the conformation changes of the protein. The decay process in the time range $10^{-13} \text{ to } 10^{-3}$ sec are composed of the geminate recombination between oxygen and deoxyhemoglobin. Fresh whole blood of sheep in Krebs-Ringer phosphate buffer solutions (pH=7.4) was centrifuged five times to remove serum components. The absorption spectrum of deoxy RBCs exhibits the maxima at 430 and 555 nm. With an increase in the oxygen pressure the spectrum gradually changes to that of oxy RBCs, which shows absorption peaks at 416,540 and 575 nm. The ratio (deoxy RBCs/ oxy RBCs) is unity at the oxygen pressure of 35 Torr. The absorption peaks of RBCs in the Q-band region are almost identical with those of hemoglobin. The Soret band of RBCs is much broader than that of hemoglobin molecules in the aqueous solution (Mikio, H.S. et al., 2001).

Effect of low intensity near infrared laser radiation on red blood cell ATPase & membrane structure was investigated. Laser radiation of 810nm, radiant exposure 3.75-25 J/cm$^2$ at 37°C on the structure of protein and lipid components of red blood cell membranes and it functional properties was reported. The role of membrane ATPase as possible targets of laser irradiation was analyzed. In vivo and vitro studies showed significant influence of laser irradiation on cell functional state. Some different explanations based on the light absorption by primary endogenous chromophores (mitochondrial enzymes, cytochromes, flavins, porphyrins) have been proposed to describe biological effects of laser light. The structural and functional changes of cell membranes and the activities of Na, K, and Mg ions ATPases, fluorescence of membrane proteins and fluorescence of Pyrene incorporated into membrane lipid bilayer were reported. It was found that near-infrared low intensity laser radiation changes the ATPase activities of the membrane ion pumps and is dose &fluence rate dependent. At the same time no changes of such integral parameters as cell stability, membrane lipid peroxidation level, intracellular reduced glutathione or oxyhaemoglobin level were observed. Near infrared laser light radiation (810nm) induced long term conformational transitions of red blood cell membrane which were related to the changes in the structural states of both erythrocytes membrane proteins and lipid bilayer. This resulted in the modulation of membrane functional properties changes in the activity of membrane ion pumps and thus changes in membrane ion flow (Kujawa, J.et al., 2002).

Freshly collected human blood (acid-citrate-dextrose (ACD) was used as anticoagulant from apparently healthy donors. He-Ne laser (p= 3 mW) beam diameter
2 mm was used to irradiate the blood at room temperature. This result shows there are no modifications in the hematocrit, the RBC count, the mean cellular volume or mean cellular hemoglobin concentration within the sensitivity limits. Therefore, erythrocyte membrane does not contain any proteins absorbing in visible red region, and the erythrocytes are perfectly transparent after washing. The data presented above suggest that He-Ne laser irradiation currently used as an aiming beam in laser surgery does cause any important change to human red blood cell membranes (Mersini, M. et al., 1995).

The time-domain optical properties of blood undergoing photocoagulation in vitro using two newly developed time-resolved techniques and asymptotic effect of laser photocoagulation on the chemical and structural properties of the components of the blood matrix were studied. He observed that 590 nm light actually has a lower penetration depth than 585 nm light in pig skin, despite having lower hemoglobin absorption. The laser photothermo coagulation of blood in vitro is a complicated process involving time-and temperature dependent changes in both optical and structural properties. A three stage mechanism for coagulation involving a heating phase, a primary coagulation phase involving mainly the cytoplasm of the erythrocytes and a secondary coagulation phase involving a more long range intercellular coagulation is suggested (Black, J. F. et al., 2004).

Whole blood was exposed to Nd: YAG laser radiation of varying powers (10, 20 and 30 watts) and duration 1, 2.5 and 5 seconds. Compared to control samples which were not subjected to laser light no significant decrease in hematocrit was seen. The platelet rich plasma subjected to the laser of 30 watts for five seconds failed to demonstrate the presence of membrane denaturation of blood components. Nd: YAG laser can be used intravascularly without fear of hemolysis or debris “microemboilization” up to a power of 30 watts for five seconds was shown in this work. The emission peak wavelength of the Nd: YAG was 1064 nm, as opposed to the argon laser, with emission peak wavelengths of 488 and 514nm, well within the major absorption range of hemoglobin (400 to 600 nm). The effects and the mechanisms of action of Nd: YAG laser radiation on blood constituents (erythrocytes, leucocytes and plasma) were evaluated in vitro (Edgar, B. et al., 1988).

A cw mode Nd: YAG laser (λ=1064nm) along with 100 cm long, 400 μm optical silica fiber was used to irradiate whole blood. Nd: YAG laser radiation produced no decrease in hematocrit, hemoglobin, and no increase in free hemoglobin,
which indicates a lack of hemolysis. These results were predictable because the Nd:YAG laser emission peak wavelength of 1064 nm is not within the major absorption range of hemoglobin (400 to 600 nm). The effects of argon laser radiation on red blood cells are more "cell damaging" because the argon laser has emission peak wavelengths of 488 and 514 nm, well within the absorption range of hemoglobin (Edgar, B. et al., 1988).

Blood cell damage was observed after irradiation of fresh whole blood with 630nm laser light in vitro. Fluence rates of laser light were used with and without cooling of blood. Damage to blood was assessed by blood cell counts and osmotic fragility measurements. Exposure of a 1 mm blood layer to 630nm laser light without cooling led to change the blood counts first detected at fluence rates of 130 mW/cm² and osmotic fragility changes at 210 mW/cm². Increasing fluence rates resulted in increasing cell damage. A dye laser pumped by an Ar⁺ laser was used as the irradiation source. The dye laser was adjusted to the desired wavelength maximum of (630 ±1) nm with a width of (3±1) nm. This laser light was coupled into a microlens fiber which was adjusted to a flow-through suprasil cell containing blood.

The results of blood cell counts, osmotic fragility measurements and blood films after 630 nm laser irradiation with and without cooling of the blood lead to the assumption that the observed blood damage is caused by irradiation due to heating. The effect of heating on blood is known to cause changes in red blood cell membrane. Since the bulk temperature of the irradiated blood even without cooling did not exceed physiologic values (T= 37 °C), there seems to be an additional damaging effect of the laser irradiation on red blood cells. Cooling of blood prevents blood damage of red blood cells after irradiation in the fluence rate intervals (E= 0-348 mW/cm²), (Fischer, F. et al., 1998).

In another paper the IR absorption spectra of blood, plasma, and packed erythrocytes to study the effect of in vivo exposure of blood to low intensity emission from a He-Ne laser (wavelength λ=632.8nm, 4 mW/cm²) on the molecular components of blood are reported. The IR spectra of blood samples and blood components before and after irradiation show the changes in the amide I, amide II and amide III absorption regions and in the absorption bands of methylene and phosphate groups. The effect of HeNe laser irradiation on blood shows the decrease in the contribution of the α-helix conformation in the secondary structure of the blood proteins. The emission of a He–Ne laser is absorbed by the heme group of
hemoglobin, but is not absorbed by other proteins and lipoproteins included in composition of blood.

The greatest changes are seen in the IR bands of the blood, while in the spectra of plasma, changes are insignificant. In the irradiated blood, the conformational transitions may be induced for several reasons: cleavage of ligands in hemoglobin complexes with ligands, breaking of salt, disulfide, and hydrogen bonds on reaction with free radicals formed in secondary reactions. It was shown that in hemoglobin solutions, detachment of oxygen is apparent as decrease in the intensity and an increase in the frequency \( v_{\text{max}} \) of the absorption bands of groups participating in hydrogen bond formation, in particular bands for \( \text{C=O} \) stretching vibrations of carboxyhemoglobin with \( v_{\text{max}} = 1760 \text{ cm}^{-1} \) (Zalesskaya, G.A. et al., 2009).

1.3 Spectral analysis of laser effects on different biological systems

The absorption spectra of whole blood, erythrocytes, and plasma to study photochemical reactions initiated by exposure of blood in vivo to UV radiation has been reported (Zalesskaya*, G.A. et al., 2008). When blood is exposed to the therapeutic doses of UV radiation (0.5 J/cm\(^2\)), the absorption of blood proteins decreases. In this paper changes in the position and relative intensities of the IR absorption bands of the peptide groups (stretching and bending vibrations of NH, CN, and CO bonds) are reported. They may be due to conformational transitions in the blood protein macromolecules, induced with a change in the intermolecular hydrogen bonds on absorption of UV radiation by blood.

The spectra for blood from donors was similar; the shape and position of the Soret bands were somewhat different, and the Soret band intensity varied with the amount of hemoglobin (Hb) in the given sample. For the UV irradiation dose used, in the blood samples absorption changed both in the UV band for proteins at 200-250 nm and in the Hb bands with \( \lambda_{\text{max}} = 415-417 \text{ nm and 541, 577 nm} \). Typical features for all the irradiated blood samples are a small low-frequency shift and broadening of the IR bands for NH stretching vibrations and the amide I and amide II bands. A number of blood components absorb in this region, aliphatic amino acid residues of proteins, free aliphatic amino acids in blood plasma, membrane lipids, blood plasma
lipids, polysaccharides, and other nonprotein organic components of plasma. In the 250–300 nm region, the effect of UV radiation on proteins is mainly due to absorption of aromatic amino acid residues: tryptophan, tyrosine, phenylalanine, and also absorption by free amino acids in blood plasma, hydroxyl groups and their free radicals, playing an important role in the initiation of oxidation chain branching when aqueous media are exposed to UV radiation. The photochemically active UV radiation with $\lambda = 254$ nm can lead to damage in nucleic acids, proteins, and lipids.

IR absorption bands for stretching vibrations of NH, amide I, and amide II for blood samples before UV irradiation, and after one UV irradiation were compared. Furthermore, when blood is irradiated for therapeutic purposes, interest is more on the effect of radiation on the processes occurring in the body with participation of the irradiated blood, rather than the effect of radiation on the individual blood components. The decrease in the intensity of 200-250 nm band observed in blood and erythrocyte samples may be due to degradation of proteins that are part of the structure of erythrocytes when they are exposed to UV radiation. The changes in the Hb absorption bands may be due to both the direct effect of light on the photosensitive sections of the heme of the Hb macromolecule, and indirectly through transfer of the energy absorbed by its protein component. The low-frequency shift of the Soret band and the decrease in the dip between the 541 nm and 577 nm bands, are typical of an increase in the concentration of deoxyhemoglobin, which has a lower-frequency Soret band and a broad absorption band with maximum close to 554 nm (Zalesskaya, G.A. et al., 2008).

Fourier transform infrared (FT-IR) spectra of fixed single erythrocytes infected with plasmodium falciparum at different stages of the intraerythrocytic cycle were studied. Bands assigned to the hemozoin moiety at 1712, 1664 and 1209 cm$^{-1}$ were observed in FTIR difference spectra between uninfected erythrocytes and infected trophozoites. All stages of the intraerythrocytic lifecycle of the malarial parasite, including the ring and schizont stage, were differentiated by visual inspection of the C-H stretching region (3100-2800 cm$^{-1}$). Fourier transform infrared spectroscopy was used to identify vibrational modes specifically associated with the propionate groups that link the heme groups together in the heme dimeric array (Webster, G. T. et al., 2009).

FTIR spectroscopy has been widely applied in biology and recently also in medicine. Biophysicists apply FTIR to characterize the structure and conformation of
biomolecules such as proteins, nucleic acids & lipids. FTIR Micro spectroscopy (FTIR-MC) has been used to characterize various types of malignancies and other disorders. Besides the application of FTIR to the tissue diagnostics, its role in the diagnostic aspects involving body fluids is gaining importance. A successful diagnosis of arthritis based on NIR (Near Infrared) analysis of synovial fluid obtained from the patients was reported (Mordehai\textsuperscript{1}, J. et al., 1976).

FTIR measurements were conducted in transmission mode using the FTIR microscope IRscope II with sensitive MCT detector (BRUKER EQUINOX model 55/S OPUS software). The wavenumber range of the measured spectra was 600-4000 cm\textsuperscript{-1}. Such area contains enough quantity of sample to obtain good quality spectra with high signal to noise ratio. It is important to study the blood components in terms of biomolecular changes using FTIR-Microspectroscopy. All the spectra were normalized to amide I peak at 1643 cm\textsuperscript{-1}. There were significant spectral differences between the three blood components Symmetric and asymmetric stretching vibrations from the phosphate group were seen in the FTIR spectra in the region between 950-1250 cm\textsuperscript{-1}. Apart from phospholipids, energy metabolites and phosphorylated proteins, nucleic acids are the main contributors of these bands in the cells. FTIR spectra of WBC showed clear bands at 965, 1080 and 1245 cm\textsuperscript{-1}, which were absent in RBC and plasma. The FTIR spectra of plasma showed a new peak at 1404 and around 1585 cm\textsuperscript{-1} which were absent in WBC and RBC components. Asymmetric stretching vibrations of CH\textsubscript{2} and CH\textsubscript{3} from proteins, nucleic acids and phospholipids mainly contribute to this region of the spectrum. (Mordehai\textsuperscript{1}, J. et al., 1976).

FTIR has been used in the diagnosis of cancer of human breast (Webster, G. T. et al., 2009). They have reported that their method of analysis results in nearly 100% diagnostic accuracy of carcinomal tissues from normal ones. The chronic lymphocytic leukemia could be well characterized by FTIR based on lipid and DNA content and the overall spectral characters. Also the diagnosis of lung cancer was done using FTIR by measuring the ratio of the peak intensities of the 1030 cm\textsuperscript{-1} and 1080 cm\textsuperscript{-1} bands (originated mainly in glycogen and phosphodiester groups of nucleic acids) which differs greatly between normal and lung cancer samples.

FTIR spectra of lymphocytes isolated from blood of two children having B and T-type ALL respectively were recorded. The spectra were recorded along with age-matched four healthy controls. In the case of patients, the spectra were recorded
before and during the chemotherapy treatment. The spectra obtained for the average of four controls and before the treatment were similar with minor changes in the absorbance of symmetric (1000-1100) and asymmetric (1200-1245) regions of the phosphate group and also in the amide II region arising from the proteins. The chemotherapy treatment caused drastic molecular changes in the cells, which could be observed in the spectra. It shows differences in the absorbance of phosphate bands corresponding to the nucleic acids. Also the band at 965 cm\(^{-1}\) accounting for the symmetric stretching vibration of the phosphodiester bonds in nucleic acids showed marked changes in the intensity before and after the treatment. Spectral pattern changes were observed in the phosphate bands in the region between 1400-1600 cm\(^{-1}\). Decrease in protein concentration was evident from the lower intensity for amide II band. The protein content remained constant during the chemotherapy treatment. The absorbance changes in the higher wavenumber region (2500-3500) followed a trend similar to the 800-1800 cm\(^{-1}\) region. Amide I normalization has been applied to all the spectra. The reduction in DNA content was confirmed by two different methods of analysis such as amide I/II absorbance ratio and monitoring the absorbance at 1084 cm\(^{-1}\), which corresponds to phosphate group of the nucleic acids (Mordehai, S. et al., 2001).

Preliminary studies have reported success in the diagnosis of different types of cancer such as lung, colon, breast, and cervix using FTIR-MSP. Measurements of the concentrations of urea, glucose, protein, and ketone in human urine have been performed using NIR spectroscopy.

FTIR measurements in the 600–4000 cm\(^{-1}\) region were performed in transmission mode using an FTIR microscopeIR scope II. Symmetric and anti-asymmetric stretching vibrations from the phosphate group are seen in the FTIR spectra in the region between 950 and 1280 cm\(^{-1}\). The FTIR spectra of WBCs showed clear bands at 965, 1080, and 1245 cm\(^{-1}\), which were absent in RBCs and plasma. The absorbance of multiple bands between 2800 and 3000 cm\(^{-1}\) was higher for WBCs than RBCs and plasma. Antisymmetric stretching vibrations of CH2 and CH3 from fatty acids in phospholipids mainly contribute to the absorbance. The ratio of bands I and II, represent the absorbance due to Asymmetric CH3 and antisymmetric CH2 vibrations (Mordehai, J. et al., 2004).
(Zaleskaya, G.A. et al., 2009), shows the most intense bands in the IR spectra of blood are due to absorption by polypeptides with a large number of amino acid residues. The IR bands of the peptide groups, assigned to stretching and bending vibrational bands of the NH, CN, and C=O bonds, contain information about the conformational structure of the globular protein molecules. When blood is exposed to low therapeutic doses of light, the spectral changes in the regions of the IR spectrum where the absorption bands of peptide groups appear, are more sensitive to breaking of weak intermolecular bonds.

The fluorescence spectra of the whole blood and the RBC have much similarities in the intensity, the emission peaks and the emitting region, and abundant peaks. For hemoglobin, fluorescence is found in the wavelength range of 650-850 nm, the fluorescence spectra were emitted by the new fluorophores generated by the breakdown of some weak bonds on the RBC membrane such as the C-C bond and the C-N bond. In the wavelength range of 590-650nm, the fluorescence spectra are mainly emitted by the hemoglobin.

Excitation source used in this experimental study was a continuous wave (cw) argon laser with wavelength 457.9 nm and power density 25.7 mW/cm² and irradiated spot of 0.7 cm². All of the samples are excited by 457.9nm light from a low intensity CW, Ar+ laser. The fluorescence spectra in the wavelength range of 550-850 nm were measured.

The fluorescence spectra of the whole blood and the RBC were very similar in spectral intensity, emission peaks and emitting region, and there were abundant and strong peaks at 616, 666, 691, 708,739, 752,766, 800, 812 and 844 nm. But in fluorescence spectrum of the hemoglobin there were only two weak peaks at 629 and 666nm respectively, and spectral intensity is zero in the wavelength range of 650-850nm.

A peak with strong intensity was found in the spectra of the RBC and the whole blood because the energies of normal C-C bond and C-N bond are only 2.55 and 2.13 eV, respectively. Some macromolecules containing such low energy bond on RBC membrane easily absorb photons when excited by polarized and coherent laser, causing optical breakdown and generating unpaired electrons.. Low level laser blood interaction is the process of cytochrome absorbing photons to make mitochondrion from ADP into ATP. In intravascular LLLT (low level laser therapy)
indicated that low level laser can not only act on cytochromes but also interact on molecules with chromophore in cells. (Shumei, G. et al., 2003).

The structures of laser generated coagula were investigated using a scanning electron microscope (SEM). Coagula were prepared by placing undiluted blood in a dish to a depth of about 4mm and irradiating the exposed blood with 10 slightly overlapping shots from the 532 nm laser (10ms pulse width, 10 J/cm², 3 mm spot size), (Shumei, G. et al., 2003).

The SEM pictures of a laser-generated macroscopic coagulum are interesting with respect to the discussion of the secondary phase. The top surface of the coagulum appears smooth consistent with protein “melting” at the high temperature anticipated at this level. Several RBCs were seen on the top surface of the coagulum. The bottom surface of the coagulum had a different appearance, with “intact” RBCs and spherocytes fused together or entrained in a denaturated protein meshwork. The peak temperature at the bottom of the coagulum is lower than at the top. This view shows many of the intermediate steps in coagulum formation. The cells range in shape from distorted biconcave discs to spheroids to wrinkled fragments. The heated RBCs change shape from biconcave to spheroid, causing increase in backscatter, then rupture causing a decrease in scattering. For longer heating times the RBC membranes in these clusters would burst (Black, J. F. et al., 2004).

The red blood cells showed no morphological changes with HeNe laser (λ= 630 nm) and medium fluence rates (mW/cm²). With a further increase in fluence rates more and more red blood cells exhibited crenation, and rouleaux were seen. At higher fluence rates the number of crenated red blood cells increased and some red blood cells showed auto-agglutination. Finally, with a further increase in fluence rates all red blood cells were visibly damaged and few white blood cells that remained also showed a jagged outer membrane and a fuzzy nuclear structure. To demonstrate the described morphological changes untreated blood is compared with heavily damaged blood for wavelength λ=630 nm, fluence rate E= 296 mW/ cm², for SEM of blood (Fischer, F. et al., 1998).
1.4 Laser radiation effects on neuronal systems

(Austin, R.D., Anita, Mahadevan-Jansen, et al., 2009), showed that low-intensity, pulsed infrared light provides a novel nerve stimulation modality that avoids the limitations of traditional electrical methods. Infrared neural stimulation (INS) is limited by a 2:1 ratio of threshold radiant exposures for damage to that for electrical stimulation. It shows nonlinear relationship between the subthreshold depolarizing electrical stimulus and additional optical energy required to reach stimulation threshold. The change in optical threshold decreases linearly as the delay between the electrical and optical pulses is increased.

Male Sprague-Dawley rats were anesthetized and the sciatic nerve was exposed to laser from the pelvic cavity to the knee. A pulsed infrared diode laser $\lambda=1.875\ \mu$m (Lockheed Martin Aculight Capella) was coupled to a 400$\mu$m diameter optical fiber (Ocean Optics). The laser spot size on the nerve was 0.3584 mm$^2$. The optical penetration depth in tissue at the selected wavelength was approximately 400 $\mu$m, which has been shown to allow selective recruitment of a single 50-200$\mu$m-diameter fascicle positioned below the 200$\mu$m-thick perineurium.

The pulse duration for both electrical and optical stimulation was 2 ms, which was dictated by the minimum pulse duration needed to obtain sufficient pulse energy to optically stimulate the nerve. Pulses were delivered at a repetition rate of 2 Hz for all experiments. Monophasic electrical stimulation was used with stimulation threshold voltages averaging 0.86 $\pm$ 0.30 V. Threshold radiant exposures for infrared neural stimulation (INS) alone averaged 1.69 $\pm$ 0.30 J/cm$^2$. Combined with a subthreshold electrical stimulus, radiant exposures were reduced to 1.49 $\pm$ 0.22 J/cm$^2$ at 60% of electrical threshold and 0.60 $\pm$ 0.29 J/cm$^2$ at 95% of electrical threshold. No visible indication of thermal damage was present at the radiant exposures used in this study.

The recovery of the crushed sciatic nerve of rats after low-power laser irradiation applied to the corresponding segments of the spinal cord is reported. After a crush injury to the sciatic nerve in rats, low-power laser irradiation (He Ne laser, $\lambda=632$ nm, Power= 16 mW) was applied transcutaneously to corresponding segments of the spinal cord. The laser treatment was repeated for 30 minutes daily for 21 consecutive days. Low-power laser irradiation applied directly to the spinal cord improved recovery of the corresponding injured peripheral nerve. Measurement of the
compound muscle action potentials (CMAP) was achieved in all rats by stimulating the nerve proximally to the crush and recording from the corresponding gastrocnemius muscle. When laser irradiation was applied to the injured area of the sciatic nerve, significant improvement of CMAPs occurred. The laser stimulation increased CA$^{2+}$ release from the mitochondria to the cytoplasm, together with increased ATP production in the mitochondria. This ATP also became available to surrounding cells, both directly and indirectly, by altered chemical and enzyme levels as a consequence of the increased cellular activity. The increase in ATP synthesis was found after low power laser irradiation (LPLI) (Semion, Rochkind, et al., 2001).

(Robert Orchardson, et al., 1997), has studied the effects of laser on nerve conduction in isolated nerves. He used a pulsed Nd:YAG laser. Experiments were carried out on 36 spinal nerve preparations dissected from rats. A pulsed Nd: YAG laser with 320 mm diameter optical fiber was used in all experiments. Pulsed laser radiation was applied continuously for 1 minute at pulse energies of 30–150 mJ at 10–20 pulses per second (pulse width = 150 μs) to a central portion of the nerve bundle immersed in Krebs’ solution and isolated by silicone grease seals from the terminal parts of the nerve bathed in mineral oil. The tip of the fiber optic cable was placed either in gentle contact with the surface of the nerve or held at a distance of 2 mm from the surface of the nerve bundle. The laser radiation was applied for one minute, and the Compound action potential recovery was monitored for a further seven minutes. Five action potential recording made over a period of two minutes to establish the baseline (control) response. Lasing was applied for one minute at 2.0 W and repeated three times at seven minute intervals. Repeated lasing caused equivalent percentage reductions in the CAP.

(Anita, Mahadevan –Jansen, et al., 1997), reported that rat sciatic nerve preparation was stimulated in vivo with HO:YAG laser with wavelength ($\lambda$= 2120 nm) Alexandrite laser ($\lambda$= 750nm), free electron laser ($\lambda$=2100nm) and solid state laser nerve stimulator ($\lambda$=1870 nm) were used. The results support the hypothesis that direct neural activation with pulsed laser light is induced by a thermal transient. This interaction is a photothermal effect from, moderate, transient tissue heating direct and indirect activation of transmembrane ion channels causing action potential generation. The stimulation threshold (0.3-0.4 J/cm²) at optical wavelengths in the infrared (2100, 1870 nm) is ~ 2.5 times less than threshold and tissue damage occurs at (0.8-1.0 J/cm²). Photothermal effects result from the transformation of absorbed light energy
to heat, which may lead to hyperthermia, coagulation, or ablation of the tissue. Photomechanical effects are secondary to rapid heating with short laser pulses (< 1μs) that produce mechanical forces such as laser induced pressure waves able to disturb cells and tissue. Thermomechanical process depends on the parameters of lasers such as wavelength, pulse duration and laser radiance exposure or irradiance. Biophysical mechanism responsible for pulsed laser stimulation of the nerve tissue is thermally mediated leading to direct activation of action potentials and stimulation parameters.

(Mark Merris, et al., 1994), used Helium-Neon laser energy (HeNe λ= 632.8 nm at 3.5 mW) which was delivered via fiber optic material directly to the point of contact with the nerve cord. He used glass microelectrode individually placed within each motor neuron. He demonstrated data with statistically significant changes; p< 0.05, in motor neuron membrane potentials. Laser energy was generated using a Spectra Physics HeNe laser (model 120s) producing 17 mW of power. Laser energy was then transmitted to a multimode optical fiber. Extracellular and intracellular recording were recorded for control and experimental treatments. As per statistical analysis is concerned, changes in spontaneous action potentials and excitatory presynaptic potentials were also evaluated.

(Anita-Mahadevan-Jansen, E. Duco Jansen, Jonathan M. C., et al., 2010), showed that infrared neural stimulation (INS) of peripheral nerve stimulation with high spatial precision and without causing the typical electrical stimulation artifact in the recording electrodes. Neural stimulation is the process of using an external source to activate ion channels causing depolarization of the neural membrane and evoking an action potential to propagate down the axon of a stimulated neuron. They used infrared light at wavelengths between 1.8 and 5.0 μm to selectively excite muscles by the sciatic nerve in vivo. Resultant electrical recordings show no stimulation artifact and histological studies confirm no damage was caused by the irradiation of the nerve with infrared light. The brain of Spraque-Dawley rats was used for experimental purpose. The brain was rapidly dissected from the cranial cavity and the cerebellum was removed. Electrical signal recordings were made with glass patch electrodes (3-6 MΩ) pulled from borosilicate glass capillaries.

The free electron laser is a tunable pulsed laser source with wavelength range between 2.51 and 10 μm. The beam generated by the FEL consists of a macro pulse width that is 5 μs ( FWHM) which is a sum of a train of 1PS pulses of laser light at 2.85 GHz. Different wavelength such as 2.51, 3.65, 4.00, 4.40 and 5.30 μm were
used and radiant energy per pulse was varied to determine threshold at each wavelength. The brain slice methodology results in significant damage to the outer layer of tissue with thickness ranging from 50 to 200 μm.

As spot size decreases, the resulting electrical signal magnitude is smaller since the number of neurons contributing to the signal is decreased making it more difficult to detect the signal with a recording electrode. To stimulate the brain slice, infrared light has to penetrate through the damaged layer of tissue (Anita-Mahadevan-Jansen, E. Duco Jansen, Jonathan M. C., et al., 2010).

(E. Duco Jansen, Agnella, D. Izzo, MS, et al., 2006), used electric current to stimulate the neurons in vivo. Experiments using gerbils were conducted and evoked action potentials from the cochlea were recorded. Stimulation threshold was measured as 0.018 ± 0.003 J/cm². All measurements were made in vivo using adult gerbils (Meriones unguiculatus). CAPs were measured between 50 and 2 KHz, with resolution of six steps/octave. The optical source was a Holonium: YAG laser with a wavelength of 2.12 μm, pulse duration of 250 microseconds operating at 2 Hz. The laser output was coupled to a low-OH 100 μm diameter. The fiber was heated to 36°C with a heating wire coil to prevent hearing loss upon cooling the cochlea. The radiant exposure was between 0.01 and 0.1 J/cm² which generated 50-100 μV CAP.

These experiments showed that it is possible to stimulate the auditory nerve with optical radiation. No neural damage could be detected even for hours of continual stimulation. Optical radiation stimulates outer or inner hair cells. If hair cells were involved in optical stimulation of auditory neurons, the optical CAP amplitude should have decreased with increase in acoustic thresholds. At a wavelength of 2.12 μm, there is little light scattering in tissue and the light tissue interaction is the absorption of the light by water in the tissue. During absorption, this optical energy is transferred to thermal energy and results in heating of the target area.

(Pavel Balaban, et al., 1992), used the neurons of 50 to 100 μ diameter of subesophageal ganglia of Helix pomatia and irradiated via a 125 mm fiber probe with a 10 mW He-Ne laser (λ = 632.8 nm). The rate of membrane depolarization, duration of latent period were measured. When the spontaneously active neurons generating spikes every 7-10 min. were irradiated in between their spontaneous spikes, the depolarization of membrane and generation of action potentials occurred as a function of light intensity. The irradiation with a 4 mW HeNe laser was found to decrease the duration of synaptic depression after the spike. Resting potentials of silent neurons
varied from $-65 \text{ mV}$ to $-74 \text{ mV}$, and no significant correlation between these changes and irradiation was observed. These results show that the silent neurons were not responsive to laser irradiation. After increase of irradiation intensity up to $0.96 \text{ W/cm}^2$ it gives response with a great number of spikes. In case of Aplysia neurons the laser light is absorbed by cytoplasmic lipochondria. In this paper the effects of the He-Ne laser irradiation with intensity range from $0.05$ to $4.0 \text{ W/cm}^2$ were investigated. It was found that only spontaneously active cells of the snail nervous system were sensitive to the laser stimulation.

(Fork, R. L. 1971), reported that laser radiation at $488 \text{ nm}$ selectively stimulates neurons in the abdominal ganglion of the marine mollusk Aplysia californica. The laser stimulation of cells with blue or green light produced firing with the light pulse on in some cases & light pulse off in others. The irradiation caused an initial depolarizing component which gradually returned to near rest potential with the light pulse off the membrane potential dropped momentarily and then moved above the critical firing threshold, causing spiking.

(Kobayashi, K, et al., 2009), reported that radiant heat laser pulses evoke potentials (LEP) that can be recorded from the scalp or intracranial electrodes. The response of thalamic neurons to a cutaneous laser stimulus occurs at latencies predicted by the conduction delay between the periphery and the thalamus. Thalamic neurons generated low threshold spike bursts of action potentials due to laser stimulation. The results show that many neurons respond to the laser with early and/or late latency peaks of activity cutaneous heat stimulation was delivered by thulium YAG laser with laser pulses of 300-500mJ. In this paper neuronal response to laser stimulation and statistical characteristics of spike trains are described with every detail.

(Richter C.P., et al., 2011), reported the spatial selectivity of infrared neural stimulation in the acutely damaged cochlea of guinea pigs and compared it to stimulation with acoustic tone pips in normal hearing animals. The radiation was delivered via a 200 $\mu$m diameter optical fiber, which was inserted through cochleostomy into the scala tympani of the basal cochlea. The stimulated section along the cochlear spiral ganglion was estimated from the neural responses recorded from the central nucleus of the inferior colliculus. Optical stimulation was done using diode laser (Lockheed Martin Aculight) ($\lambda=1.86 \mu$m, pulse duration 100 $\mu$sec, radiant energy 0-127 $\mu$J/pulse, penetration depth at 1.86 $\mu$m is about 700 $\mu$m). Signal
recording were made while cochlea was acoustically & optically stimulated using multichannel electrode array

In the forthcoming SPIE-BIOS conference in January 2012 a special conference on Neurons and photons is organized. This includes many new papers related to laser stimulation of neurons and nervous system.
1.5 Objectives of the present thesis

- To study the effect of HeNe laser ($\lambda = 632\text{nm}$, power=$2\text{mW}$) on red blood cells (RBC) in vitro using uv-visible spectroscopy & Fourier transform infrared spectra (FTIR) spectroscopy & Fluorescence spectroscopy.
- To study the effect of cw mode Nd:YAG laser (wavelength $\lambda = 1064\text{nm}$, power=$150\text{mW}$) irradiation on red blood cells (RBC) in vitro using uv-visible FTIR & Fluorescence spectroscopy. Effect of variation in laser power of HeNe & Nd:YAG laser, exposure time on RBC using FTIR spectroscopy are also to be studied.
- To study the morphological changes before and after HeNe ($\lambda = 632\text{nm}$, power=$2\text{mW}$) & Nd:YAG (wavelength $\lambda = 1064\text{nm}$, power=$150\text{mW}$) laser radiation using scanning electron microscopy.
- To study the effect of cw mode Nd:YAG($\lambda = 1064\text{nm}$, power=$150\text{mW}$) & HeNe (wavelength $\lambda = 632\text{nm}$, power=$2\text{mW}$) laser on the chitin and nervous tissue from cockroach leg using FTIR spectroscopy.
- To study the effect of HeNe ($\lambda = 632\text{nm}$, power=$2\text{mW}$) & Nd:YAG (wavelength $\lambda = 1064\text{nm}$, power=$150\text{mW}$) laser irradiation on peripheral nerve from cockroach leg.
- To record the action potentials from the cockroach leg nerve fibers using metal microelectrodes, amplifiers and data acquisition system.
- To analyse the train action potentials (spike potentials) recorded from cockroach leg nerves using statistical parameters such as variance, standard deviation & information of entropy.
- Thus in the present thesis interaction of HeNe & Nd:YAG laser on RBC & cockroach leg nerve fiber is aimed and effects are to be studied using spectroscopic and electrophysiological techniques.
1.6 References


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