# THE BIOSTIMULATION OF LOW LEVEL LASER IRRADIATION ON BLOOD PARAMETERS: *EX VIVO*

**by**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AP-1 | Activator protein-1 |
| APC | Allophycocyanin |
| ATP | Adenosine triphosphate |
| $Ca^{2+}$ | Calcium Ion |
| CBC | Complete Blood Count |
| CCO | Cytochrom c oxidase |
| CD | Clusters Differetiation |
| Cy | Cascade yellow |
| cAMP | Cyclic adenosine monophosphate |
| DNA | Deoxyribonucleic Acid |
| DPSS | Diode pumped solid state |
| EDTA | Ethylenediaminetetracidic acid |
| ERK | Extracellular signal–regulated kinase |
| Er:Yb:YCOB | Erbium, Yttrium, Yttrium Calcium Oxoborate |
| ESR | Erythrocyte sedimentation rate |
| FITC | Fluorescein isothiocyanate |
| Ga-As | Gallium- Arsenide |
| Ga-Al-As | Gallium Aluminum Arsenide |
| GRAN | Granulocyte |
| HCT | Hematocrit |
| He-Ne | Helium Neon |
| HGB | Hemoglobin |
| LED | Light emitting diode |

| | |
|---|---|
| MCH | Mean cell hemoglobin |
| MCHC | Mean cell hemoglobin concentration |
| MCV | Mean cell volume |
| MID | Mid-sized cells |
| MPV | Mean platelet volume |
| N | Number of samples |
| Nd:CaWO4 | Neodyminum Calcium tungstate |
| NADH | Nicotinamide Adenine Dinucleotide Phsphate Oxidase |
| Nd:YAG | Neodyminum Yttrium Aluminium Garnet |
| Nd:YVO4 | Neodyminum yttrium orthovanadate |
| NIR | Near infrared |
| NF-$_k$B | Nuclear factor kappa B |
| NK | Natural killer cells |
| RBC | Red blood cell |
| RPM | Revolutions per minute |
| SPSS | Statistical Package of Social Science |
| SD | Standard Deviation |
| LBI | Laser blood irradiation |
| LYMPH | Lymphocyte |
| LLL | Low-level laser |
| LLLI | Low-level laser irradiation |
| RBCs | Red Blood Cells |
| RDW | Red distribution width |
| RNA | RiboNucleicAcid |
| ROS | Reactive Oxygen Specie |

| | |
|---|---|
| PE | phycoerythrin |
| PerCP | Peridinin chlorophyll protein |
| PLT | Platelet |
| PDW | Platelet distribution width |
| TEM | Transverse Eletromagnetic Modes |
| WBC | White blood cell |

## LIST OF SYMBOLS AND UNITS

| | |
|---|---|
| $\lambda$ | Wavelength |
| $\theta$ | Diffraction angle |
| $^{\circ}C$ | Degree celsius |
| A | Area of the spot size |
| c | Speed of light in free space |
| E | Energy |
| Eg | Energy gravity |
| fL | Femtolitre |
| g/dL | Gram per deciliter |
| $h$ | Planck's constant |
| J/cm² | Joule per centimeter square |
| m | metre |
| min | minute |
| Pg | Picogramme |
| s | Second |
| T | Exposure time |
| W | Watt |

# BIOSTIMULASI KESINARAN LASER ARAS RENDAH TERHADAP PARAMETER DARAH: *EX VIVO*

## ABSTRAK

Pelbagai jarak gelombang laser aras rendah (LLL) telah digunakan untuk berbagai kegunaan klinikal kerana kemampuannya mengubah reologi darah dan meningkatkan mikroedaran. Tindak balas darah manusia terhadap kesinaran laser aras rendah (LLLI) memberikan maklumat penting tentang interaksi cahaya laser dengan tisu hidup. Kajian ini direkabentuk bagi mengkaji sama ada *in-vitro* LLLI boleh mengubah kadar sedimentasi eritrosit (ESR) dan indeks darah lainnya dari darah penuh. Sampel darah dikumpulkan melalui punktur vena ke dalam tiub mengandungi asid etilenediaminetetrasetik (EDTA). Setiap sampel dibahagikan kepada dua alikuot yang sama sebagai sampel kawalan (tanpa penyinaran) dan sampel yang disinarkan laser. Sampel yang disinarkan didedahkan kepada dos 36, 54, 72 dan 90 J/cm² pada jarak gelombang 405, 589 dan 780 nm. Menggunakan jarak gelombang laser 589 nm pada dos 72 J/cm², kiraan limfosit menunjukkan kenaikan maksimum yang signifikan (1.67%) berbanding dengan sampel yang tidak disinari laser. Peningkatan kiraan limfosit selepas kesinaran telah disahkan oleh sitometri aliran yang menunjukkan peningkatan signifikan dalam kiraan limfosit disebabkan peningkatan dalam kiraan sel pembunuh tabie (NK) (CD16 dan CD56). Tiada perubahan signifikan dalam kiraan jenis limfosit lain dicerap apabila dibandingkan dengan limfosit tanpa kesinaran. Keputusan ini dengan jelas menunjukkan bahawa kiraan sel NK terubah oleh LLLI yang akhirnya mempengaruhi secara signifikan kiraan jumlah limfosit. Pengurangan maksimum (0.44%) dalam min isipadu sel (MCV) dicerap menggunakan LLLI pada 405 nm dan dos kesinaran 72 J/cm².

Perubahan MCV yang disebabkan oleh LLLI dihasilkan semula dalam sel darah merah (RBC) yang terampai dalam larutan penimbal NaCl. Sebaliknya, pengurangan isipadu RBC yang disebabkan oleh laser terhenti sepenuhnya dengan menyingkirkan RBC dalam larutan yang mengandungi kepekatan EDTA yang lebih tinggi. Keputusan ini menunjukkan bahawa LLLI dapat mengurangkan isipadu RBC kemungkinannya dengan peningkatan dalam kepekatan $Ca^{2+}$ intrasel bebas, yang mengaktifkan sel membran $Ca^{2+}$ bersandar saluran $K^+$ dengan konsekuen $K^+$ ion efluks dan kecutan sel. Tambahan pula, tindak balas sel pada dos LLLI yang tetap dengan ketumpatan kuasa berbeza menunjukkan bahawa dos kesinaran laser 54 J/cm² pada jarak gelombang 405 nm dengan ketumpatan kuasa rendah menghasilkan penurunan yang lebih besar dalam MCV sel darah merah berbanding dengan ketumpatan kuasa yang lebih tinggi. Pemerhatian ini sangat menarik kerana ia menunjukkan bahawa ketumpatan kuasa lebih tinggi adalah kurang efektif dalam sistem *in vitro* yang digunakan. Berbanding dengan darah penuh tanpa kesinaran, pengurangan signifikan maksimum ESR (6.6%) dicerap dengan menggunakan gelombang 405 nm dan dos sinaran 72 J/cm². Pengurangan nilai ESR darah penuh didapati lebih kecil pada dos melebihi 72 J/cm² bagi semua jarak gelombang yang diuji. Tambahan pula, ESR sel darah merah yang diasingkan dan disingkirkan semula dalam plasma yang disinarkan laser adalah secara signifikannya lebih rendah (51%) berbanding ESR dalam sel darah merah yang disingkirkan semula dalam plasma tanpa disinari laser. Keputusan ini menunjukkan bahawa pengurangan ESR selepas LLLI adalah disebabkan terutamanya oleh kesan laser ke atas komposisi plasma, yang akhirnya mempengaruhi ESR darah penuh.

# THE BIOSTIMULATION OF LOW LEVEL LASER IRRADIATION ON BLOOD PARAMETERS: *EX VIVO*

## ABSTRACT

Various low-level laser wavelengths have been used for a variety of clinical applications because of their ability to modulate blood rheology and improve microcirculation. The response of human blood to low-level laser irradiation (LLLI) provides important information about the interactions of laser light with living tissues. This study was designed to investigate whether *in vitro* LLLI changes the erythrocyte sedimentation rate (ESR) and other blood indices of whole blood. Blood samples were collected by venipuncture into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Each sample was divided into two equal aliquots as a control (non-irradiated) and irradiated samples. The irradiated sample was subjected to LLLI doses of 36, 54, 72 and 90 J/cm² at wavelengths of 405, 589 and 780 nm. At 72 J/cm² with a laser wavelength of 589 nm, lymphocyte counts showed a significant maximum increase (1.67%) compared with non-irradiated samples. The increase in the lymphocyte count after irradiation was confirmed by flow cytometry that showed a significant increase in lymphocytes due to an increase in natural killer (NK) (CD16 and CD56) cells. No significant changes in the counts of other types of lymphocytes were observed compared with their non-irradiated counterparts. These results clearly demonstrated that the NK cell count was altered by LLLI, which ultimately affected the total lymphocyte count significantly. The maximum reduction (0.44%) in the mean cell volume (MCV) was observed with LLLI at 405 nm and 72 J/cm². MCV changes induced by LLLI were reproduced in red blood cells (RBCs) suspended in a buffered NaCl solution. In contrast, the laser-induced RBC volume reduction was

completely abolished by suspending RBCs in a solution containing a higher concentration of EDTA. This result suggested that LLLI reduced the RBC volume possibly by an increase in free intracellular $Ca^{2+}$ concentrations, which activates cell membrane $Ca^{2+}$ dependent $K^+$ channels with consequent $K^+$ ion efflux and cell shrinkage. Furthermore, the cell response at constant LLLI doses with different power densities showed that LLLI of 54 J/cm² at 405 nm with a low power density produced a larger decrease in the MCV of RBCs compared with higher power densities. This observation is of considerable interest because it suggests that higher power densities were less effective in the current *in vitro* system. Compared with non-irradiated whole blood, the maximum significant reduction of ESR (6.6%) was observed using a wavelength of 405 nm and radiation dose of 72 J/cm². The reduction in the whole blood ESR value was smaller at doses beyond 72 J/cm² for all tested wavelengths. Furthermore, the ESR of RBCs that had been separated and re-suspended in irradiated plasma was significantly lower (51%) than that of RBCs that had been re-suspended in non-irradiated plasma. These results indicate that the ESR reduction after LLLI is mainly due to the effect of the laser on the composition of plasma, which ultimately affects the whole blood ESR.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Lasers are widely used in modern medicine. Laser beams of various wavelengths, powers, and optical properties are used in cosmetology, diagnostics, surgery, and various other medical procedures (Shapshay, 1987; Simunovic, 2000; Fritsch, 2003; Simunovic, 2003). The treatment of certain disorders by laser light is considered to be controversial. A therapeutic method is commonly known as low level laser irradiation (LLLI) therapy. In LLLI, beams of low power are applied for long exposure periods. The total amount of laser energies delivered can be large with no adverse heat generation to the target tissue. As temperature is not increased the healing process was observed to be due to photochemical interactions. A large amount of literature occurs on the use of LLLI in various experimental biological models (Gulsoy et al., 2006; Albertini et al., 2007; Buravlev et al., 2013). LLLI has various degrees of clinical significance in medicine depending on its application (Karu et al., 2001). Different powers, wavelengths and exposure times of LLLI have been utilised in various clinical applications regarding blood therapy and the ability of LLLI to improve microcirculation and blood rheology (Korolevich et al., 2000; Siposan & Lukacs, 2000).

Phototherapy with various light sources (i.e., sunlight, ultraviolet radiation, laser, and light-emitting diodes) has been used for wound healing. Sunlight was employed to treat various skin disorders by the ancient Greeks. Over the past 40 years, LLLI therapy administered with low power lasers or light-emitting diodes in the red to near-infrared range (630 – 1000 nm) has been applied to the treatment of

soft tissue injuries and shown to promote tissue regeneration by increasing biological effects *in vivo* and *in vitro* (Schindl et al., 1997; Karu 2003; Anders et al., 2004; Eells et al., 2004; Wong-Riley et al., 2005). Phototherapy is based on the light energy effects on cellular metabolism. Biological responses of cells to visible and near-infrared laser radiation occur because of physical and/or chemical changes in photoacceptor molecules, components of respiratory chains (Karu 1999; Simunovic, 2000). Some major changes induced by lasers in irradiated cells are the acceleration of electron transfer and redox properties, photodynamic actions and biochemical activity changes, superoxide generation and the release of nitric oxide from catalytic centre cytochrome c oxidase (CCO). As well as changes induced by local transient heating to biochemical activity (Ailioaie et al., 2005).

The biological effects of light vary according to the wavelength, duration of light exposure, delivered light intensity, and energy density (dose or fluence). For light-induced biological effects to occur, light must be absorbed by photoacceptor molecules in the cell. Whereas high power lasers ablate tissue, low power lasers have been proposed to stimulate tissue and encourage cells to function. LLLI is integrated with mainstream medicine with ongoing research to determine where there is a demonstrable effect (Karu, 2003).

One theory states that the mechanism of action of LLLI illustrates that the laser has a capability to influence photoreceptors in cells. This mechanism is known as biostimulation or photobiology.

The biostimulating effect of LLLI results in an increase in microcirculation and higher production rates of adenosine triphosphate (ATP), deoxyribo nucleic acid (DNA), and ribo nucleic acid (RNA), which shows an improvement in nutrition,

regeneration and cellular oxygenation (Takac & Stojanović, 1997), and enhancing the mitochondrial electron transport system (Yu et al., 1997). Photons enter the cell and become readily absorbed by biological chromophores located in either mitochondria or the cell membrane. These chromophores strongly interact with the laser radiation. The photonic energy is converted to chemical energy within the cell in the form of ATP, which enhances cellular functions and proliferation rates. Cell membrane permeability is altered, followed by physiological changes in target cells. The magnitude of the laser biostimulation effect depends on the wavelength and the physiological state of the cell at the moment of irradiation (Pinheiro et al., 2002).

Widespread applications of laser radiation in the practice of medicine causes the necessity to conduct investigations of blood and other biosubstrates thereby avoiding effects caused by laser therapy that are deemed to be undesirable. Several positive observations, including anti-inflammatory effects, immune stimulation, bactericidal effects, and normalization of blood rheology, have been recorded following blood irradiation (Karu & Kolyakov, 2005). The effects of autotransfusion of irradiated blood and the wide spectrum of its therapeutic action have also been studied (Karandashov et al., 1989).

The effects of laser irradiation can also be attributed to the improvement of microcirculation and modulation of the rheological properties of blood in terms of the erythrocyte sedimentation rate (ESR). The ESR is one of the most important rheological parameters of human blood (Mi et al., 2006).

The influence of LLLI on an organism has biological effects and several clinical including immunostimulatory, neurotrophic, antiedemic, anti-inflammatory, analgesic, bactericidal desensitization, blood rheology normalization, and

hemodynamics effects, depending on the pathology and the patient's condition (Mostafa et al., 2013). Accordingly, LLLI can be applied to the therapy of several pathological conditions in among various branches of medicine. The current study was undertaken to assess the effects of various wavelengths of laser irradiation on human blood *in vitro*, how modifications of some rheological constants of blood are induced, and the significance of these modifications. This study has effectively demonstrated that blood irradiated at low power densities and doses results in subsequent effects without any blood cell damage being caused. Generally, the wavelength of the laser used in LLLI therapy is not fixed. Therefore, it is worth studying which wavelength of LLLI has better therapeutic efficacy and its underlying principles.

## 1.2 Motivation and Problem Statement

There are widespread applications of LLLI in various areas of medicine (Posten et al., 2005). However, the mechanisms of its effect on human blood components have not been characterized sufficiently, and it is still a topic of discussion. The most important point of interest is the photoreactions initiated by intravenous laser irradiation of blood, which is generally accepted to be the most effective method of laser biostimulation. This method was originally developed for cardiovascular disease treatment (Yinghua & Wenming, 2002). The mechanisms of the interactions between LLLI and blood are unclear and undoubtedly a pressing issue of great interest because of the complications due to the numerous reactions that occur simultaneously during LLLI therapy of blood (Mi et al., 2004b). Therefore, the study of LLLI effects on human blood is crucial to understand better the mechanisms involved in the interactions of laser irradiation with tissues. The

resolution of this issue will aid in future development of practical medicine. Blood response to the action of LLLI yields information that is important to the mechanisms of laser irradiation with tissues (Zalesskaya & Sambor, 2005). In addition, there is still a lack of information regarding the response of blood parameters, such as white blood cells (WBCs), red blood cells (RBCs), platelets (PLTs) and their components, to LLLI. Despite the fact that the blood response to the action of LLLI provides important information on the mechanisms of the interactions with cells (Piasecka et al., 2000). The possibility of treatments with different laser light wavelengths (blue, green, red, infrared, and yellow) and the settings of various laser frequencies provide more strategies for treatment and a new field of research that is not yet assessable. Moreover, the safe range of irradiation levels and their duration in terms of various human blood parameters have not been clearly defined (Reddy, 2003). Furthermore, why laser irradiation improves microcirculation is not clear, although the interactions of blood with laser irradiation have been studied in a few instances (Siposan & Lukacs, 2000). Improvement in the rheological properties of blood and microcirculation, and a reduction in the infarction area have been demonstrated previously (Zalesskaya & Sambor, 2005). Further research is required to gain an understanding regarding the response of these parameters to LLLI. This study will clarify whether laser irradiation at various wavelengths (405, 589, and 780 nm) can improve the parameters of blood cells, which may improve microcirculation.

## 1.3 Objectives of the study

The aim of this study was to provide evidence of LLLI effects on rheological factors of healthy human blood *in vitro.* The specific objectives of the research are:

1. To clarify whether different low-level laser doses delivered at various wavelengths of 405, 589, and 780 nm modify blood indices and the ESR of human blood *in vitro,* and discuss the significance of these modification.
2. To determine the optimal dose that affects *in vitro* blood cell counts and ESR.
3. To determine the relationship between laser exposure time and power density at the same dose of LLLI on the measured blood parameters.

## 1.4 Scope of the study

This research focuses on the effects of different doses of a low-level laser DPSS delivered at various wavelengths of 405, 589, and 780 nm on healthy human blood *in vitro*. The study is *in vitro*, in which LLLI was applied to healthy human blood samples. The characteristics of the healthy human includes different age groups ranging from 18 to 60 years and of both genders (male and female) with no medical history of major illnesses or history of taking medication for major diseases. The blood samples were collected by venipuncture into ethylenediaminetetraacetic acid (EDTA; anticoagulant)-containing tubes. Each blood samples were divided into two equal aliquots as a non-irradiated sample (control) and irradiated sample for comparison. Cell counts and the ESR were measured before (control samples) and after irradiation. Statistical analysis and calculations were performed using Statistical Package of Social Science (SPSS) software.

## 1.5 Thesis organization

This thesis consists of five chapters. The present chapter highlights the key points of the research. This chapter describes the research background, motivation and problem statement, objectives of the research, scope of the research, and organization of the thesis. Chapter 2 presents a literature review of the studies performed in the fields related to this thesis. Chapter 3 describes the sampling of the research, the equipment used, experimental setup, and methodology of the data analysis. Chapter 4 discusses the results and procedures of this study, together with the statistical analysis. Finally, chapter 5 discusses the conclusions of this research and provides suggestions for possible future work.

# CHAPTER 2

# THEORY AND LITERATURE REVIEW

## 2.1 Introduction

The applications of LLLI to the treatment of various pathological conditions such as wound healing and the control of inflammation and pain was introduced by the work of Mester and colleagues, who noted an improvement in wound healing by the application of a low energy (1 J/cm$^2$) ruby laser (Mester et al., 1971; Mester et al., 1975; Mester et al., 1985). In addition, it has been reported by many investigators that *in vitro* biostimulation depends on many factors, including laser irradiation parameters such as wavelength, laser output power, dose, beam area, irradiance and polarization (Almeida-lopes & Za, 2001), as well as the cell type being irradiated (Moore et al., 2005).

The phenomenon of biostimulation was published originally in 1967 by Endre Mester of Semmelweis University (Budapest, Hungary), a few years after the invention of the first working laser (Mester et al., 1967). Mester conducted an experiment to test whether laser radiation causes cancer in mice. Hair was shaved off their backs, the mice were divided into two groups, and irradiated one with a low power ruby laser and wavelength of 694 nm. The treatment group did not develop cancer, and to his surprise, their hair grew back more quickly than the untreated group. This is called laser biostimulation. Tissue biostimulation is only possible when irradiated cells possess molecular photoacceptors to absorb the light and enter into a state of excitation that triggers an intracellular cascade of signals that lead to a biological effect that is measurable (Siposan & Lukacs, 2000; Vladimirov et al., 2004). The biostimulating effect of laser light on cells can be derived at present,

because numerous studies have shown that the biological effect of such irradiation depends on the type of cell as well as the laser type and applied energy doses (Dörtbudak et al., 2000; Piasecka et al., 2000; Pereira et al., 2002; Yu et al., 2003). The emitted laser light is coherent and polarized and, can be absorbed by various tissues (Vladimirov et al., 2004).

According to Posten et al. (2005), the properties of LLLI refer to the use power output of lasers being 1 - 100 mW, power density of 0.1 - 10 W/cm² and energy density of 0.1 to 100 J/ cm². Other significant parameters include laser wavelength between 300 and 10,600 nm, pulse duration of 1 to 500 milliseconds and a pulse rate of 0 (continuous) to 5,000 Hz. Parameter changes have been used in several studies that complicate the issue of making significant comparisons.

Studies indicate that LLLI refers to the use of lasers with output powers of 1 – 500 mW and low energy densities (0.04 – 50 J/cm²) directed at a monolayer of cells or target tissue. LLLI can prevent apoptosis and improve cell proliferation, migration, and adhesion at low levels of visible light illumination. LLLI is not thermal or an ablative mechanism, but rather a photochemical effect similar to photosynthesis in plants that means the light is absorbed and caused a chemical change (Huang et al., 2009; AlGhamdi et al, 2012). In the literature, studies comparing the effects of lasers using visible and near-infrared wavelengths show differences in most of them, indicating that optimal results are obtained using a visible wavelength (Young et al., 1989; Lubart et al., 1992; Loevschall, 1994).

Xu et al. (2015) studied the absorption spectra blood and hemoglobin by effects of LLLI at different wavelengths on hemoglobin. These results showed that lasers with the peak wavelengths of 200, 240, 275, and 342 nm in the absorption

spectra curve of whole blood are easy to destroy protein molecules and then lead to lose biological activity of hemoglobin. Whereas laser with wavelengths longer than 800 nm will decrease the oxygen carrying capacity of blood, only visible lasers with wavelengths between 630 and 670 nm have the best efficiency.

The most important factor is the photoreaction initiated by the intravenous laser irradiation of blood, which is acknowledged to be the most effective laser biostimulation method (Yinghua & Wenming, 2002). This may partly be due to the fact that laser irradiation can improve the rheological properties of blood. Further, it has been reported that the power density of the laser irradiation is more important than the total energy dose for photobiostimulation (Cen & Chen, 2004). Improvements in the blood rheological properties and microcirculation, as well as a reduction in the area of infarction, have been demonstrated (Zalesskaya & Sambor, 2005). Some studies have reported the effects of LLLI on human blood parameters, especially RBCs and WBCs (Dörtbudak & Mallath-Pokorny, 2000; Mi et al., 2004a; Zalesskaya et al., 2006; Suardi et al., 2016).

## 2.2 Diode lasers

In 1963, a solid state laser was pumped using a semiconductor source, i.e. an NdCaWO4 laser pumped with a 880 nm Ga-As light-emitting diode (Newman, 1963). Since then, the development of diode-pumped solid state (DPSS) lasers has grown alongside the development of diode lasers (Byer, 2009).

Light is emitted from a semiconductor material when a pn junction is forward biased so that holes and electrons can recombine. The photonic energy of the emitted light is determined by the energy gap between the conduction and valance bands of the pn junction. It therefore follows from the relation $E=hv$ that the wavelength of

the photons emitted is $\lambda = \frac{hc}{E}$, and if λ is expressed in $\mu$m and the energy gap ($Eg$) is in electron volts, then the wavelength of the light emitted by the semiconductor material is

$$\lambda = \frac{1.42}{E_g}$$

Such a device can emit laser radiation with a narrow spectral bandwidth when operated with sufficient injection current to establish a population inversion, and when the cleaved facets of the material produce optical feedback (Sinclair & Dunn, 1994). A very basic schematic of a laser diode is shown in Figure 2.1.

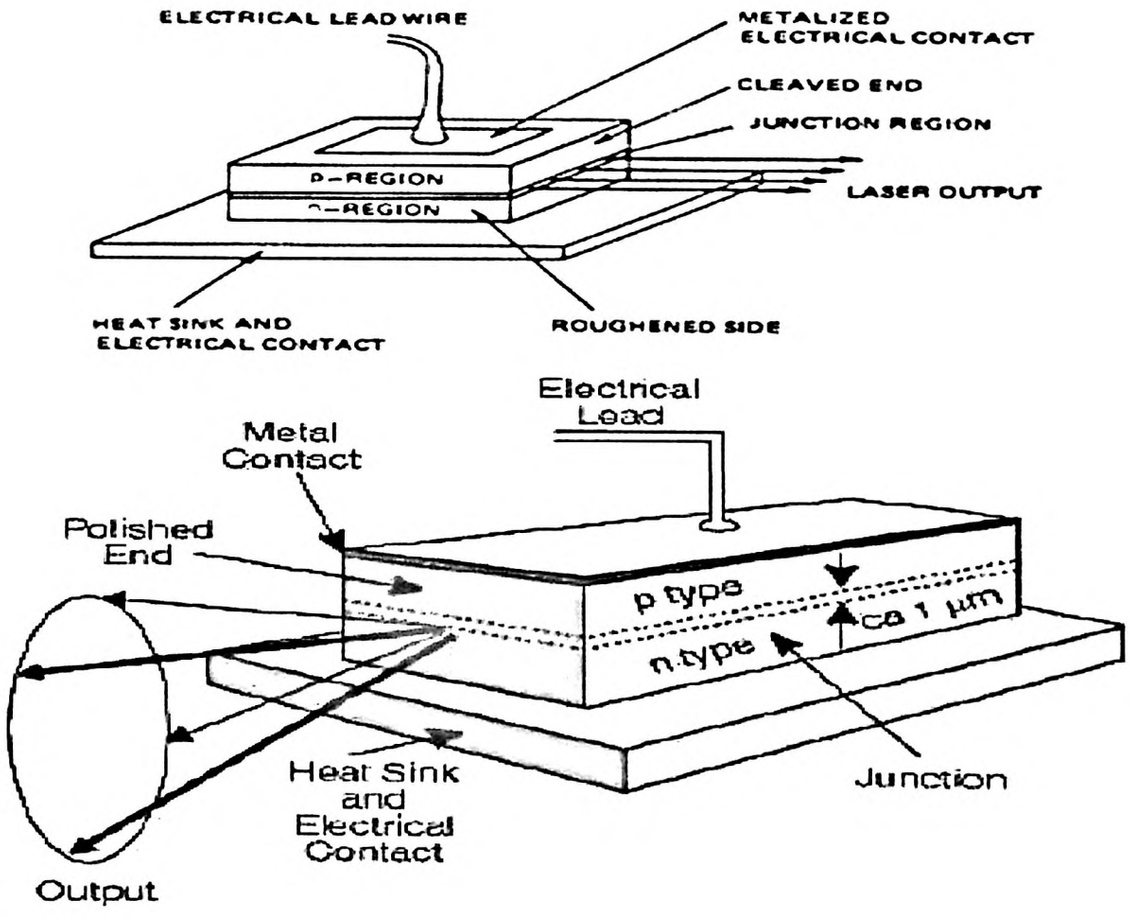


Figure 2.1 Basic construction of a laser diode (Thompson, 1980).

In a typical laser diode, the crystal of semiconductor is made into a shape like a piece of paper: very thin in one direction and rectangular in both the others. The bottom of the crystal is p-doped, and the top is n-doped, resulting in a large, flat pn junction. The two ends of the crystal are hewn in to a form that is perfectly smooth and has parallel edges. The two reflective parallel edges are called a Fabry-Perot cavity. Photons emitted in exactly the right direction will be reflected several times from each end face before emission. Each time the photon pass through the cavity and the light is amplified by stimulated emission. Therefore, if there is more amplification than the loss, the diode begins to laser (Csele, 2011).

There are ranges of wavelengths produced by diode lasers based on the large number of semiconductor materials (Thompson, 1980). Diverse procedures have been developed to couple the diode lasers radiation into solid-state laser media. The procedure for coupling the radiation directly into the medium without any intervening optics, which makes a laser system very compact, is especially suitable for microchip solid state lasers (Byer, 2009).

There are further fundamental advantages for using diode lasers to pump solid state lasers including (Lupei et al., 2003):

i. The high brightness output of a diode laser lets the pump light be focused with high optical density, leading to a high gain in the laser medium.
ii. The good spectral overlap between absorption spectrum of a laser medium and the diode radiation leads to a reduction in thermal load of the medium, which can result in better beam quality.
iii. The high coupling efficiency can lead to the development of lasers, which are not feasible as lamp pumped systems, such as three level lasers and

lasers operating on small gain transitions.

iv. Laser diodes are quiet pump sources with suitable amplitude and great spectral stability, allowing the stable operation of solid-state lasers.

## 2.3 DPSS lasers

The large amount progress, which has taken place in solid-state laser technology over the past few years, is due to the completion of a number of technical innovations that have covered a wide range of disciplines. The process by which atoms are raised from a low level to an upper level is called pumping. Diode pumped lasers are becoming increasingly important in the machining of laser. DPSS lasers are solid-state lasers constructed by pumping a solid gain medium, such as a ruby or a neodymium doped yttrium aluminum garnet (Nd-YAG) crystal with a laser diode. DPSS lasers have the advantages of compactness and efficiency over the other laser types. High power of DPSS lasers have replaced ion lasers, and flash lamp pumped lasers in many scientific applications, and are now commonly appearing in green and other colored laser pointers (Davarcioglu, 2010).

The energy level scheme of the $Nd^{3+}$ ion is shown in Figure 2.2 with possible pumping routes depicted by red arrows and laser transitions by purple arrows. The neodymium-doped yttrium orthovanadate (Nd-YVO4) laser is most commonly operated at 1064 nm because this transition has the greatest emission cross section. Diode pumping at 808 nm results in a four level laser system in which excitation enters the upper laser level and leaves the lower laser level through fast non-radiative transitions. Pumping promotes ions from the ground state ($^4I_{9/2}$) to an excited state, $^4F_{5/2}$. Non-radiative transitions then depopulate this level and result in excitation in

the 1064 nm transition upper laser level ($^4F_{3/2}$). Lasing at 1064 nm occurs between this level and the $^4I_{11/2}$ level, which is rapidly emptied through non-radiative decay to the ground state (Lu et al., 2002).

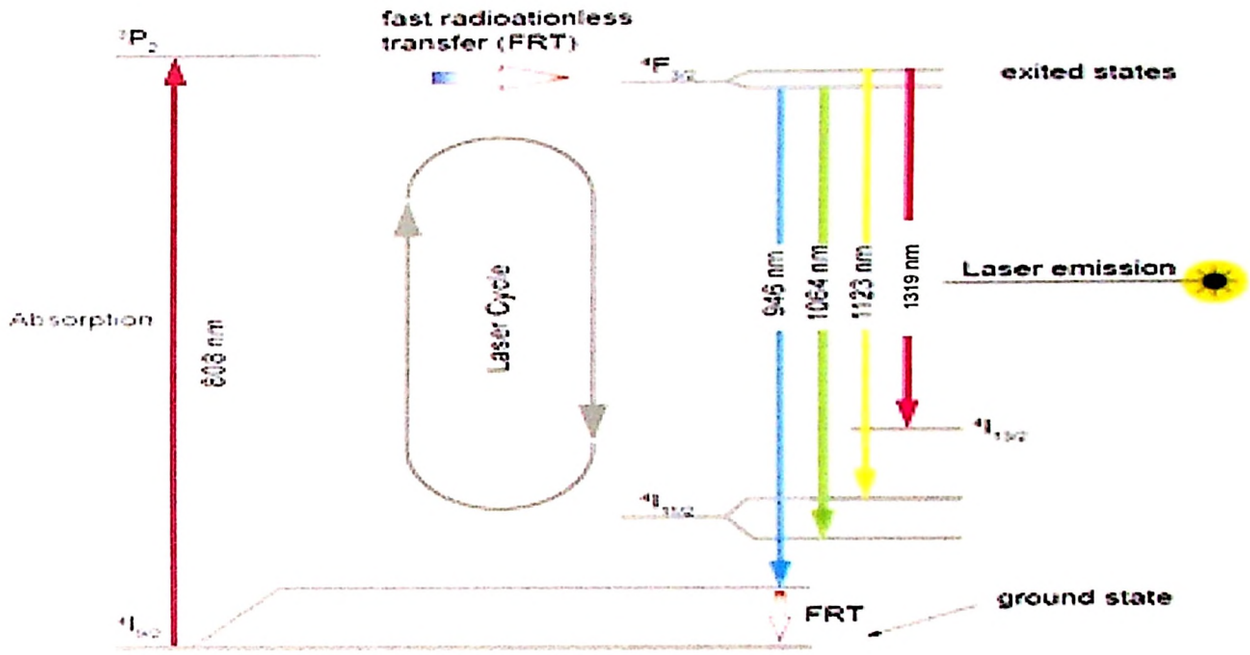


Figure 2.2 Energy level diagram of the $Nd^{3+}$ ion. Red arrows indicate pump lines. Purple arrows indicate common laser transitions in Nd-YAG (Lu et al., 2002).

Violet-DPSS lasers at 405 nm have been produced by direct frequency double technology starting from 1 W/808 nm gallium-aluminum-arsenide (Ga-Al-As) pump infrared diodes without a longer wave Nd laser interposed between the diode laser and doubler crystal (Doualan, 1989).

Yellow-DPSS lasers use an even more complicated process. An 808 nm wavelength infrared Ga-Al-As laser diode pumps an Nd-YAG or Nd-YVO4 crystal producing 1064 and 1319 nm light wavelengths that are summed together with a

nonlinear crystal to become 589 nm laser (1/1319 +1/1064 = 1/589), as shown Figure 2.3. This process is more efficient with about (3%) of the pump-diode power converted to yellow light (Saito et al., 2006).

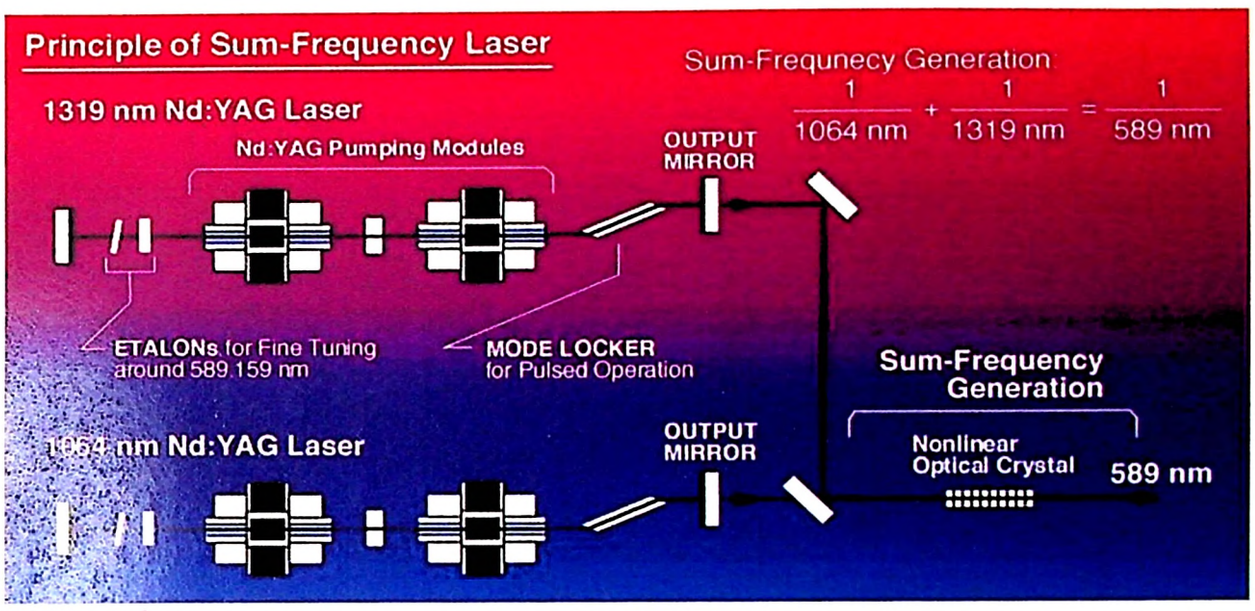


Figure 2.3 The sum-frequency Nd:YAG laser developed to generate a powerful 589 nm laser beam (Saito et al., 2006).

YCOB is a nonlinear optical crystal thus, nonlinear frequency conversion can be used to obtain a wavelength, such as 780 nm (self-frequency doubling), within the Er-Yb-YCOB crystal and pumped by a 976 nm diode at room temperature. Effective energy transfer (over 90%) from Yb to Er is possible, and Er-Yb-YCOB crystals have the potential for 1546 – 1570 nm (Wang et al., 2002). The diode-pumped laser setup is shown in Figure 2.4.

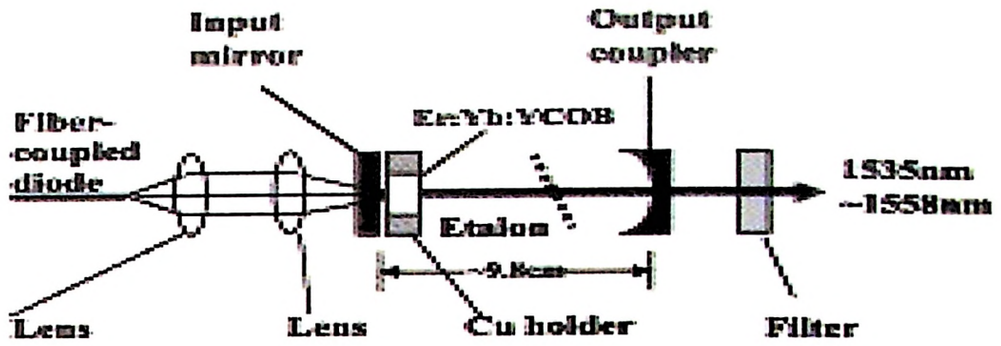


Figure 2.4 Experimental setup of a diode-pumped Er-Yb-YCOB laser (Wang et al., 2002).

## 2.4 Components of human blood

Blood is a body fluid that is vital to maintain life (McKenzie, 1996). On average, a normal adult has 6 – 7 L of blood in total. Approximately 45% of blood consists of cellular elements, and the remaining 55% is the fluid portion termed plasma, as shown in Figure 2.5.

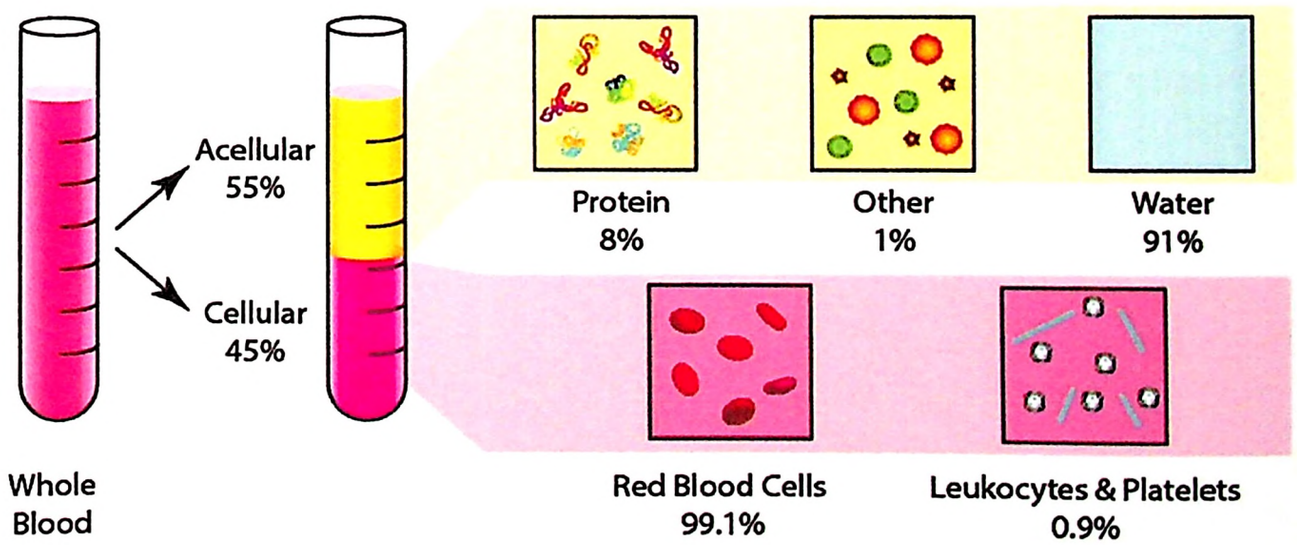


Figure 2.5 Composition of whole blood (Lazarovits et al., 2014).

Remarkably, blood constantly circulates throughout the body and carries out a wide variety of important functions (Brown et al., 1993). For example, it transports oxygen and nutrients to various organs and transfers substances such as hormones to other tissues. In addition, metabolic waste products from tissues are carried away by blood circulation. It is appreciated in modern hematology that many diseases cause changes in the composition of blood. Therefore, the analysis of blood is important for clinical diagnosis (Williams, 1990; Bain, 2015). The cellular components of blood consist mainly of three types of cells, erythrocytes (RBCs), leukocytes (WBCs), and thrombocytes (PLTs) (McKenzie, 1996). RBCs contain the protein hemoglobin that is responsible for the transportation of oxygen and carbon dioxide. WBCs play key roles in the immune system, defending the body against foreign pathogens such as viruses, bacteria, and parasites. PLTs are primarily responsible for stopping blood loss. In addition, WBCs can be further classified into three major types: lymphocytes, granulocytes (GRANs; neutrophils, eosinophils, and basophils), and monocytes. B and T cells are two types of lymphocyte. T cells themselves have two subtypes, cluster of differentiation CD3 and CD4 (Dean, 2005), and each subtype performs distinct functions (Brown, 1993; McKenzie, 1996). The various types of WBCs are shown in Figure 2.6.

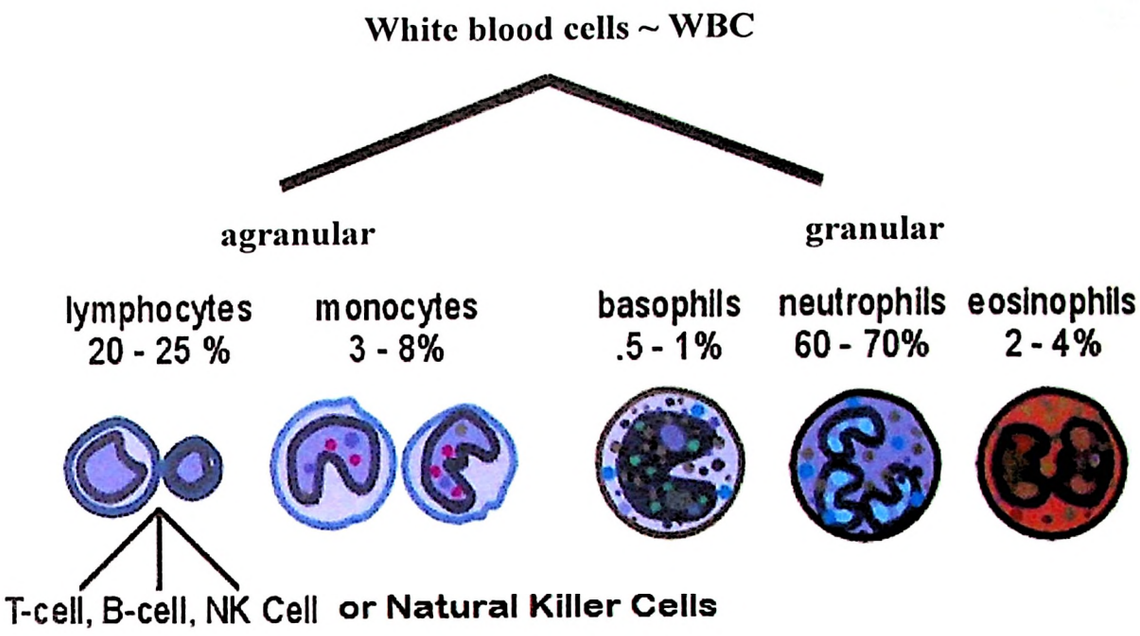


Figure 2.6 WBC classifications (Theera-Umpon & Dhompongsa, 2007).

Numerous tests have been developed for blood cell analysis. One of the most widely used tests in clinical medicine is the complete blood count (CBC). The CBC test determines the numbers of blood cells that are present in blood, including WBCs, RBCs, and PLTs. In particular, the enumeration of WBCs consists of the WBC count (the total number of WBCs per volume of blood) and the WBC differential count (the absolute numbers or percentages of different types of WBCs). The measurement of RBCs consists of the RBC count (total number of RBCs per volume of blood), hemoglobin (total amount of hemoglobin protein per volume of blood), hematocrit (HCT; volume ratio of RBCs in blood), mean cell volume (MCV; a measure of the average RBC volume), mean cell hemoglobin (MCH; average mass of hemoglobin per RBC in a sample of blood), mean cell hemoglobin concentration (MCHC; derived from the concentrations of hemoglobin, RBCs, and MCV), and RBC distribution width (RDW; the variation in size of RBCs indicated by the RBC distribution width). The measurement of PLTs consists of the PLT count (total

number of PLTs), mean PLT volume (MPV; a measurement of the average size of PLTs), and PLT distribution width (PDW; a measure of the variation of PLTs) (Williams, 1990; Bain, 2015).

Modern instruments for CBC tests can measure more than 20 parameters. Tables 2.1 and 2.2 summarize the most common parameters in the CBC test and the recommended normal ranges (George et al., 2003; Bain, 2015; Kenzie & Williams, 2015). Commercial instruments dedicated to automatic CBC testing are normally referred to as hematology analyzers.

Table 2.1 Test parameters of the CBC test and their normal ranges.

| Blood Parameters | Male | Female |
|---|---|---|
| WBC Count ( $\times 10^3/\mu l$ ) | 3.7 – 9.5 | 3.9 – 11.1 |
| RBC ($\times 10^6/\mu l$) | 4.3 – 5.7 | 3.9 – 5.0 |
| MCV (fL) | 81.2 – 94.0 | 78.5 – 96.4 |
| MCHC (mg/fL) | 32.5 – 36.7 | 31.8 – 35.9 |
| Hemoglobin ( g/dl ) | 13.3 – 18.0 | 11.8 – 16.5 |
| Hematocrit (%) | 39 – 51 | 36 – 48 |
| Platelet Count ($\times 10^3/\mu l$) | 140 – 330 | 140 – 350 |

Table 2.2 WBC differential count and the normal ranges

| WBC Type | Absolute ($\times 10^3/\mu l$) | Percentage (%) |
|---|---|---|
| Total WBC | 3.7 – 11.1 | 100 |
| Granulocyte | | |
| Neutrophil | 1.7 – 7.5 | 40 – 80 |
| Eosinophil | 0.03 – 0.46 | 0 – 7 |
| Basophil | 0.02 – 0.09 | 0 – 1 |
| Nongranulocyte | | |
| Lymphocyte | 1.0 – 3.2 | 15 – 40 |
| Monocyte | 0.2 – 0.6 | 3 – 12 |

The clinical utility of the CBC test has been extensively addressed elsewhere (Dixon, 1997; Sandhaus & Meyer, 2002; George-Gay & Parker, 2003; Kenzie & Williams, 2015). The test results of the blood parameters are very useful to diagnose the presence and severity of many diseases including infections (viral, parasitic, bacterial, and fungal), leukemia, various allergies, inflammation, and immunodeficiency (Dixon, 1997; George-Gay & Parker, 2003). In addition, it is used to reflect the body's ability to fight disease and used to monitor the adverse effects of medicines such as chemotherapeutics (Shapiro & Greenfield, 1987, Crawford et al., 2008). From a technology perspective, the tasks of counting WBCs, RBCs, and PLTs, and identifying all RBC, WBC, and PLT types with high accuracy are still among the most challenging issues of the modern CBC test (Buttarello & Plebani, 2008).

## 2.5 Laser-tissue interactions

When laser light strikes a tissue, some of it is reflected, while some are absorbed, scattered, or transmitted. A change, in the air and tissue (refractive index) produces this phenomenon. Thus obeying reflection the law of Snellius (Figure 2.7) that states:

$$\frac{\text{Sin}\,\theta_1}{Sin\,\theta_2} = \frac{n_1}{n_2}$$

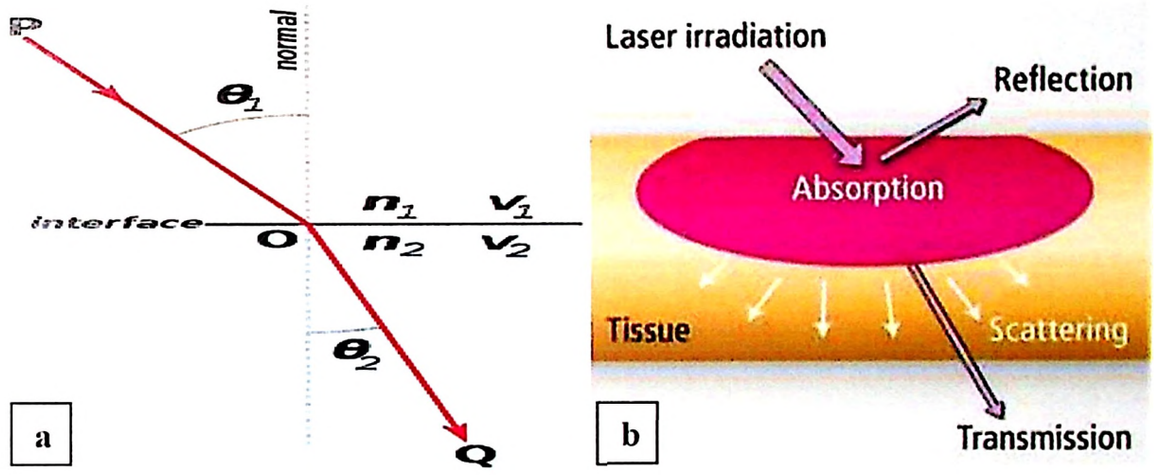


Figure 2.7 Laser light striking a tissue. a) Snellius' law of reflection. b) Laser light behavior in a tissue (Niemz, 2013).

where $\Theta_1$ is the angle between surface normal in air and the light, $\Theta_2$ is the angle between surface normal in the tissue and the ray, $n_1$ is the refraction index of air, and $n_2$ is the refraction index of the tissue.

The tissue absorbs most of the light, and the energy states of the molecules become quantized. Therefore, photon absorption arises only when its energy becomes equal to the energy difference between such quantized states. This absorption phenomenon creates the desired effects in the tissue. The coefficient $\mu_a$ ($cm^{-1}$) characterizes the absorption. The inverse, *l*a, defines the penetration depth

(mean free path) into the absorbing medium. A tissue's scattering behavior is important because it determines the volume of light intensity distribution within the tissue. This is the first phase of the tissue interaction, which is followed by absorption. Scattering of a photon is associated with a propagation direction change without loss of energy. Similar to absorption, scattering is expressed by the scattering coefficient $\mu_S$ ($cm^{-1}$). The inverse parameter, $1/\mu_S$ (cm), is the mean free path length until the next scattering event occurs (Chung et al., 2012).

Laser-tissue interactions depend on the interplay of irradiation parameters (Carroll & Humphreys, 2006):

i. Wavelength or wavelength band of a laser source

ii. The physical properties of the irradiated tissue by a particular wavelength or wavelength band Irradiance or pulse energy

iii. Continuous or pulsed laser light wave

iv. Beam size of the laser on the tissue

v. Repetition rate, laser pulse length and irradiation duration, or indeed, any physical property changes in the tissue due to laser irradiation within specific parameters.

At low energies and/or irradiances, laser tissue interactions are either purely optical or a combination of optical and photobiostimulative or photochemical. As laser power or pulse energy increases, photothermal interactions begin to dominate. Eventually, photomechanical (sometimes referred to as photoacoustic) effects become apparent when repetitive very short laser pulses are conveyed to the tissue.

Thus, there are five interaction mechanisms associated with the use of lasers in biomedicine (Reza & Katayoun, 2011):

i. Purely optical, such as, fluorescence spectroscopy for cancer screening, and high-resolution imaging for optical coherence tomography.

ii. Photochemical (target cells undergo light-induced chemical reactions) such as photodynamic therapy, and photobiostimulative such as laser acupuncture.

iii. Photoablative that causes photodissociation such as molecular bond breaking in tissue.

iv. Photothermal that converts light energy into heat energy, which causes vapourising and heating of a tissue. For example, laser assisted refraction correction by ablation of parts of the cornea as well as for tattoo removal.

v. Photomechanical (photo-acoustic) that induces dielectric breakdown in tissue that is caused by shock wave plasma expanding that results in a localized mechanical rupture, such as laser lithotripsy.

A comparative plot of the activity of laser-tissue interactions because of exposure time and irradiance is shown in Figure 2.8.

Niemz (2013) emphasized that all laser tissue interaction mechanisms share a common datum: characteristic energy density varies typically between 1 and 1000 J/cm$^2$. As a result, the duration of the exposure to laser light is the parameter used to resolve the mechanisms of laser tissue interactions (Carroll et al., 2006).

Niemz (2013) also showed that every effect of near-infrared laser wavelengths of 1 microsecond or greater are in nature thermal. Six factors need to be considered regarding the production of these laser effects: optical penetration laser depth, wavelength, the exposed tissue's characteristics, temporal mode (continuous, or pulsed), time of exposure, and the irradiance of the laser beam.

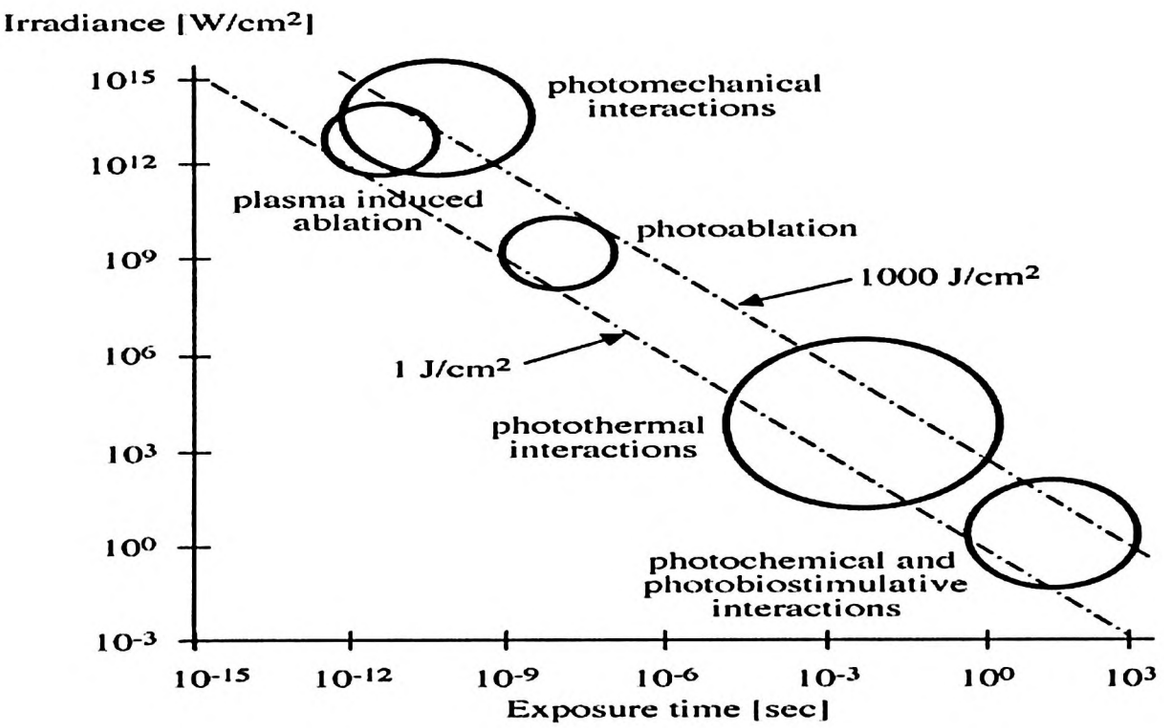


Figure 2.8 Representation of laser-tissue interactions in terms of exposure time and irradiance (Niemz, 2013).

## 2.6 Mechanisms of the action of LLLI in tissue

The first law of photobiology states that for low power visible light to have any effect on a biological system, photons have to be absorbed by electronic absorption bands belonging to a photoacceptor or a molecular chromophore (Sutherland, 2002). One way to identify chromophores is to carry out action spectra.