A COMPARATIVE INTERACTION OF He-Ne AND CO_2 LASER IRRADIATION ON HUMAN BLOOD AND ITS COMPONENTS

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A COMPARATIVE INTERACTION OF He-Ne AND CO_2 LASER IRRADIATION ON HUMAN BLOOD AND ITS COMPONENTS

by

HEND A A HOUSSEIN

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Dedicated To

My father, and my mother for their prayers

My husband and my childrenfor their patient,

moral support and accepting the inconveniences

during the time of this work

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"All Praises and Thanks To Allah"

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LIST OF ABBREVIATIONS

BMI	Body mass index
CO_2	Carbon dioxide laser
CW	Continuous wave
2D	2-Dimensional
3D	3-Dimensional
eV	Electron volt
EFAS	Encircled flux analysis system
EDTA	Ethylenediaminetetraacetic acid
GRAN	Granulocyte
HCT	Hematocrit
HGB	Hemoglobin concentration
He-Ne	Helium Neon
LYM	Lymphocyte
MID	Monocyte
MCV	Mean cell volume
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MPV	Mean platelet volume
Nd:YAG	Neodymium yttrium aluminium garnet
PLT	Platelet
PDW	Platelet distribution width

PCT	Platelet crit
RBC	Red blood cell
RDW	Red blood cell distribution width
SPSS	Statistical packages for social science
TRT	Thermal relaxation time
UV	Ultra violet
UV-IR	Ultraviolet-infrared
WBC	White blood cell

LIST OF SYMBOLS

λ	Wavelength
A	Beam cross-sectional area
C	Speed of light in free space
e	Base of natural logarithms
E	Energy
E_f	Energy fluence
h	Planck's constant
P_{Out}	Power output
p_r	Power flowing through the circle
p_c	Power density at the center of the laser beam
r	Radius of the coaxial circle
t	Exposure time
w	Effective radius of the beam

LIST OF UNITS

Variable	Unit
λ	m
A	m^2
Age	yr
BMI	kg/m^2
C	m/s
E	J
E_f	J/m^2
Flux peak	counts
GRAN	%
h	J.s
HGB	g/dl
HCT	%
Height	m
LYM	%
MCV	fl
MCH	pg
MCHC	g/dl
MPV	fl
MID	%
P_{Out}	w
PLT	μl^{-1}
PCT	%
PDW	fl
RBC	μl^{-1}
RDW	%
t	s
Total flux	w
WBC	μl^{-1}
Weight	kg

PERBANDINGAN INTERAKSI KESINARAN LASER He-Ne DAN CO_2 KE ATAS DARAH MANUSIA DAN KOMPONENNYA

ABSTRAK

Di dalam kajian ini, subpopulasi parameter darah manusia yang terdiri daripada sel darah putih, sel darah merah, platelet dan komponennya ditentukan menggunakan pengukuran elektronik di Pusat Sejahtera, Universiti Sains Malaysia. Parameter ini dikorelasi dengan ciri manusia seperti umur, jantina, kaum, kumpulan darah, indeks jisim badan dan pelbagai sejarah perubatan, sebelum atau selepas penyinaran dengan laser 632.8 nm He-Ne pada ketumpatan tenaga 1.7×10^{-17} J/cm^2 dan juga dengan laser 10,600 nm CO_2 pada ketumpatan tenaga 1.9×10^{-19} J/cm^2. Korelasi tersebut didapati dengan mencari corak dalam perubahan parameter darah menggunakan ujian berpasangan dan tak bersandaran dan hubungan di antaranya dikenalpasti menggunakan analisis regressi. Analisis statistik dilakukan menggunakan pakej statistik SPSS versi 17. Objektif penyelidikan ini adalah untuk membandingkan antara penyinaran laser He-Ne dengan CO_2 ke atas parameter darah manusia, mengkaji korelasi antara parameter darah dengan ciri-ciri manusia sebelum dan selepas penyinaran dan mengkaji interaksi alur laser dengan darah melalui imej kontur-2D dan profil-3D. Penyinaran sampel darah menggunakan laser He-Ne dan CO_2 masing-masing, menunjukkan perubahan signifikan sel darah putih, sel darah merah, platelet dan komponennya. Perbezaan dalam perubahan parameter darah manusia bagi sesuatu ciri pesakit adalah bergantung kepada jenis laser yang digunakan. Hubungan di antara suatu perubahan dalam parameter darah manusia dengan ciri pesakit yang dilakukan dengan analisis regresi logistik mendapati bahawa dengan menggunakan laser He-Ne, peningkatan min sel darah putih adalah berhubungan

secara signifikan dengan umur, jantina dan jenis darah O. Peningkatan di dalam sel darah merah adalah berhubungan secara signifikan dengan jenis darah B, AB dan indeks jisim badan, dan penurunan min platelet berhubungan secara signifikan dengan responden jenis darah AB yang mempunyai satu penyakit kronik. Manakala, dengan penggunaan laser CO_2, peningkatan sel darah putih adalah berhubungan secara signifikan dengan umur, etnik Cina, dan satu penyakit kronik. Peningkatan min sel darah merah adalah berhubungan secara signifikan dengan etnik Cina, dan peningkatan min platelet adalah berhubungan secara signifikan dengan umur, dan satu penyakit kronik. Dapatan akhir penyelidikan ini menunjukkan bahawa peningkatan min puncak fluks adalah berhubungan secara signifikan dengan umur, indeks jisim badan, etnik India dan etnik lain. Peningkatan min jumlah puncak fluks adalah berhubungan secara signifikan dengan umur, jantina, etnik Cina dan India. Sebaliknya puncak fluks dan jumlah fluks adalah berkorelasi rapat dan menjadi indikator signifikan untuk analisis darah. Seterusnya, analisis fluks terkeliling menunjukkan prospek masa hadapan yang baik dalam penyekidikan darah, lantas menjadi penunjuk jalan sebagai alat diagnosis untuk menerangkan tentang penyakit berkaitan dengan sel darah.

A COMPARATIVE INTERACTION OF He-Ne AND CO_2 LASER IRRADIATION ON HUMAN BLOOD AND ITS COMPONENTS

ABSTRACT

In this study, the subpopulations of human blood parameters which consist of white blood cells, red blood cells, platelets, and its components were determined by electronic sizing in the Sejahtera Centre of Universiti Sains Malaysia. These parameters have been correlated with human characteristics such as age, gender, ethnicity, blood groups, body mass index, and various medical histories, before and after irradiation with 632.8 nm He-Ne laser at energy density of 1.7×10^{-17} J/cm^2, and with 10,600 nm CO_2 laser at energy density of 1.9×10^{-19} J/cm^2. Correlations were obtained by finding patterns in changes of blood parameters using paired and independent tests and the relationships were identified by using regression analysis. The statistical analyses were performed using SPSS version 17. The objectives of this research are to compare between He-Ne and CO_2 laser irradiation on human blood parameters, to study the correlations between blood parameters with human characteristics before and after irradiation and to study blood–laser beam interaction via 2D contour and 3D profile images. Irradiation of blood samples with He-Ne and CO_2 lasers respectively, showed significant changes in white blood cells, red blood cells, platelets, and its components. The difference in changes in human blood parameters of a patient's characteristics depend on the type of laser used. The relationship between a change in human blood parameters and a patient's characteristics conducted using a logistic regression analysis found that by using He-Ne laser an increase in mean white blood cells is significantly related with age, gender, and blood group O. An increase in mean red blood cells is

significantly related with blood group B, blood group AB, and body mass index, and a decrease in mean platelet is significantly related for respondents with blood group AB having one chronic disease. While by using CO_2 laser there are an increase in mean white blood cells is significantly related with age, Chinese ethnicity, and one chronic disease. An increase in mean red blood cells is significantly related with Chinese ethnicity, and an increase in mean platelets is significantly related with age, and one chronic disease. The last findings of this research show an increase in mean flux peak is significantly related with age, body mass index, Indian and other ethnicity. An increase in mean total flux is significantly related with age, gender, Chinese and Indian ethnicity. On the other hand, the flux peak and total flux were very much correlated and can become a significant indicator for blood analyses. Furthermore, the encircled flux analysis demonstrated a good future prospect in blood research, thus leading the way as a vibrant diagnosis tool to clarify diseases associated with blood cells.

CHAPTER ONE

INTRODUCTION

1.1 Background

Laser is a powerful beam of light that can produce intense heat when focused at close range (Maiman, 1960; Jensen and Brunetaud, 1990). Lasers are devices that generate or amplify coherent radiation at frequencies in the infrared, visible, or ultraviolet regions of the electromagnetic spectrum (Narducci and Abraham, 1988; Meschede, 2007). Many lasers are used in medicine in microsurgery, cauterization, for diagnostic purposes, etc, for example these lasers are employed in microsurgery to cut and to debulk, soft tissues (Kallard, 1977; Haken, 1984; Judy, 1995).

Interest in medical applications was intense, but the difficulty controlling the power output and delivery of laser energy, and the relatively poor absorption of these red and infrared wavelengths led to inconsistent and disappointing results in early experiments (Muller et al, 2006). The exception was the application of the Ruby laser in retinal surgery in the mid–60's. In 1964, the argon ion laser was developed. This continuous wave 488 nm blue-green gas laser was easy to control, and its high absorption by hemoglobin made it well suited to retinal surgery, and clinical systems for treatment of retinal diseases were soon available. In 1964, the Nd:YAG (Neodymium:Yttrium Aluminum Garnet) laser and CO_2 (Carbon Dioxide) laser were developed at Bell Laboratories. The CO_2 laser is a continuous wave gas laser, and emitted infrared light at 10,600 nm in an easily manipulated, focused beam that was well absorbed by water

(Duley, 1976). Since soft tissue consists mostly of water, researcher found that a CO_2 laser beam could cut tissue like a scalpel, but with minimal blood loss. The surgical uses of this laser were investigated extensively from 1967-1970 by pioneers such as Dr. Thomas Polanyi and Geza Jako, and in the early 70's, use of the CO_2 laser in ENT and gynecologic surgery became well established, but was limited to academic and teaching hospitals. Dye lasers became available in 1969, and noble gas-halide or Excimer lasers in 1975. Since then, many other different laser systems have become available for industrial scientific, telecommunication, as well as medical use.

In the early 1980's, smaller but more powerful lasers became available, and were soon appearing in community hospitals and even physician's offices. Most of these systems were CO_2 lasers used for cutting and vaporizing, and Argon lasers for ophthalmic use. Nd:YAG and KTP laser systems were used by larger hospitals for the new field of laparoscopic surgery. These second generation lasers were all continuous wave, or CW systems, which tend to cause non-selective heat injury, and proper use required a long learning curve and experienced laser surgeons (Barlow and Hruza, 2005).

The single most significant advance in the use of medical lasers was the concept of pulsing laser beam, which allowed selective destruction of abnormal or undesired tissue, while leaving surrounding normal tissue undisturbed. The first lasers to fully exploit this principal of selective thermolysis were the pulsed dye lasers introduced in the late 1980s for the treatment of port wine stains and strawberry marks in children, and shortly after, the first Q-switched lasers for the treatment of tattoos. Another major advance was the introduction of scanning devices in the early 1990s, enabling precision computerized control of laser beams. Scanned, pulsed lasers revolutionized the practice of plastic and

cosmetic surgery by making safe, consistent laser resurfacing possible, as well as increasing public awareness of laser medicine and surgery.

Medical lasers have made it possible to treat conditions which previously were untreatable, or difficult to treat. Patients benefit by improved results and less cost. In the last few years, the main focus of research and development of medical lasers has been on laser hair removal, the treatment of vascular lesions including leg veins, and vision correction. The thrust of current research is directed towards non-ablative laser resurfacing (laser skin toning), no-touch computerized vision correction, and improved photodynamic therapy for treatment of skin cancer and for hair removal.

1.2 Research problems statement

Nowadays, the low intensity laser irradiation is widely used in clinical practice. Lasers, suitable light sources for many medical applications, provide highly monochromatic, temporally and spatially coherent light with high stability of radiation.

There are widespread applications of low intensity laser radiation in various areas of the medical field, however, the mechanisms of its effect on human blood components still have not been sufficiently studied and remain a topic for discussion. The most important interest is in photoreactions initiated by intravenous laser irradiation of blood, which is acknowledged to be the most effective laser biostimulation method.

The low energy He–Ne and CO_2 lasers irradiation at wavelengths 632.8 nm 10,600 nm respectively is an interesting region of the electromagnetic spectrum with respect to its biological effects which renders it a beneficial clinical modality in enhancing the process of pain relief, tissue repair, wound healing, vascular restenosis and inflammatory

suppression in cases of rheumatic arthritis and Achilles tendinitis. These experimental medicine practices require details information on the mechanisms of their biological effects. Therefore, the study of the mechanism of interaction of low–intensity laser radiation with living organisms by various methods for the purpose of widening the field of its medical applications is undoubtedly a pressing problem. The solution of this problem will aid in further development of practical medicine. Despite the fact that the response of blood to the action of low intensity laser irradiation gives important information on the mechanism of interaction of laser irradiation with a living organism, only small numbers of works have been devoted to such investigation in living organism. Also, there is still lack of information concerning response of blood parameters with low laser light irradiation. Therefore more research need to be done to understand these effects.

The problem statement of this research is: What are the effects of low power He–Ne and CO_2 lasers on human blood parameters? What is the relationship between these parameters and human characteristics (age, gender, ethnicity, blood group, body mass index, medical history, number of chronic disease, and type of chronic diseases) before and after irradiation? What are the differences that occurred after the laser interaction with blood via 2D contour and 3D profile images?

1.3 Motivation and objectives of research

This research was initiated after lengthy discussions with the Medical Physics group in the School of Physics, Universiti Sains Malaysia (USM) in early 2009. The initial aim of the research was to setup an experiment to study the human blood parameters using two

types of lasers namely He-Ne and CO_2 at USM Wellness Centre. As part of research requirements, the researcher attended two practical training workshops on SPSS software applications in the School of Mathematical Sciences, USM. Also, some practical training in using hematology analyzer device was provided by the diagnostic laboratory staff in the Wellness Centre. Permission was granted by the Director of the USM Wellness Centre and the head of the Diagnostic Laboratory before data collection.

This research comprised of three objectives. The first objective was to compare between Helium Neon and Carbon Dioxide laser irradiation on human blood parameters. The second one was to study the correlations between blood parameters with human age, gender, ethnic, blood group, body mass index, medical history, number of chronic diseases, and type of chronic diseases before and after irradiation. The third objective is to study blood–laser beam interaction via 2D contour and 3D profile images.

1.4 Scope of research

This research will be confined on two types of low power lasers, He–Ne and CO_2. These lasers will interact with fresh human blood samples taken from 480 patients. The characteristics of the patients will include the different age group, gender, ethnicity, blood types, body mass index, medical history, number of chronic diseases, and type of chronic diseases. All irradiated and un-irradiated (controlled samples) are analysed using Hematology analyser, and data analysis using SPSS software.

1.5 Outline of the thesis

The present chapter describes the objectives of this research and outlines its key aspects. Chapter One contains a background of the present work, research problems statement, motivation and objectives of research, scope of research, and outline of the thesis. Chapter Two will provide a comprehensive review of human blood, characteristics of laser light, theory of laser light, laser beam interaction, light propagation in tissue, and characteristics of laser beam and its interaction with blood. Chapter Three will describe the methodology which explains the equipments, the experimental setup, and the data acquisition of the project. Chapter Four will discuss the obtained results and statistical analysis techniques that used, and 2D countour, 3D profile images. In Chapter Five, conclusions of this research and suggestions for the possible future work will be presented.

CHAPTER TWO

THEORY AND LITERATURE REVIEW

2.1 Blood

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients and oxygen and transports waste products away from those same cells. Blood is a connective tissue, which is composed of a liquid extracellular matrix called blood plasma that dissolves and suspends various cells called blood cells and cell fragments. Blood is distributed throughout an organism by a circulatory system and there exist three types of circulatory systems (Schaller et al, 2008):

a) No circulatory system exists for instance in flat worms (platyhelminthes).
b) An open circulatory system is presented in many invertebrates like molluscs and arthropods. The circulatory fluid is called hemolymph and there is no distinction between blood and the interstitial fluid.
c) The closed circulatory system is presented in all vertebrates. The blood never leaves the blood vessels system, or cardiovascular system, which is composed of arteries, veins and capillaries.

2.2 Physical characteristics of blood

Blood is denser and more viscous than water and feels slightly sticky. Blood temperature is 38 ºC, which is about 1 ºC higher than oral or rectal body temperature, and it has a slightly alkaline pH ranging from 7.35 to 7.45. It constitutes about 20% of extracellular fluid, amounting to 8% of the total body mass. The blood volume is 5 to 6 liters in an average sized adult male and 4 to 5 liters in an average sized adult female. Several

hormones, regulated by negative feedback, ensure that blood volume and osmotic pressure remain relatively constant. Especially important are the hormones aldosterone, antidiuretic hormone, and atrial natriuretic peptide, which regulate how much water is excreted in the urine (Schneck, 1995).

2.3 Components of blood

Among all the body's systems, the blood is unique: it is the only tissue in the body that flows. This flowing tissue, endlessly making its course from the heart to the remotest parts of the body and returning, is a sea in which the body is bathed. Human blood consists of about 22% solids and 78% water and it has two components, blood plasma and blood cells.

2.3.1 Blood plasma

Plasma is the liquid part of the blood that makes up about 55% of total blood volume, it is a yellowish solution consisting of about 91% water and the other 9% is a host of substances indispensable to life. The role of plasma in the body is to help transport food and oxygen to the cells of the body and to carry wastes away from the cells. In addition, plasma plays a crucial role in maintaining the body's chemical balance, water content, and temperature at a safe level. That is, the plasma serves the body by helping to maintain homeostasis, or a stable internal environment in the body. In fact, essentially all the organs, tissues, and fluids of the body perform functions that help to maintain the body as a stable system (Titmuss, 1970).

2.3.2 Formed elements (Blood cells)

Blood cells are made in the bone marrow, which is the spongy material in the centre of the bones (Figure 2.1) that produces about 95% of the body's blood cells. The cellular portion of blood normally makes up about 45% of the blood volume and it consists primarily of three cellular components, that are the red blood cells (RBC), white blood cells (WBC), and platelets (PLT) (Bain et al., 2006).

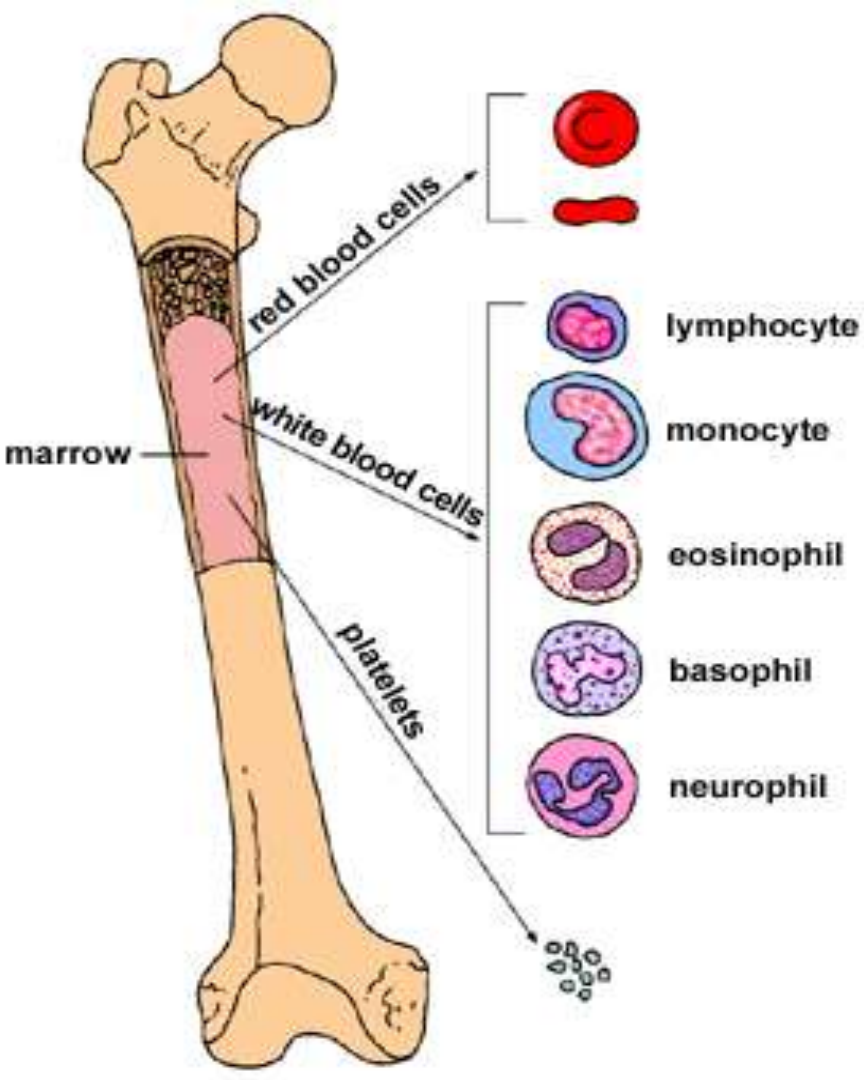

Figure 2.1: Blood and blood components

2.3.2.1 Red blood cells (Erythrocytes)

The red blood cell (RBC) is also known as erythrocytes. The RBC carries oxygen from the lungs to the rest of the body. The count is simply the total number of RBC; the hemoglobin (HGB) concentration is the concentration of RBC taken from the blood. Hematocrit (HCT) is the proportion of blood volume occupied by RBC. The mean cell volume (MCV), is a measure of the average red blood cell volume. The mean cell hemoglobin (MCH), is the average mass of hemoglobin per RBC in a sample of blood. The mean corpuscular hemoglobin concentration (MCHC) is derived from the concentration of hemoglobin, RBC and MCV. The RBC distribution width (RDW), the variation in size of RBC is indicated by the red cell distribution width.

2.3.2.2 White blood cells (leukocytes)

The chemical name for white blood cells (WBC) is leukocytes. The WBC consists of several types of cells which include lymphocytes (LYM), monocytes (MID), granulocytes (GRAN). Granulocytes are further divided into three cells which are eosinophils, basophils and neutrophils. The WBC count is the total number of lymphocytes, monocytes, and the granulocytes. The WBC helps heal wounds not only by fighting infection but also by ingesting matter such as dead cells, tissue debris and old RBC. Also, the WBC protected from foreign bodies that enter the blood stream, such as allergens and are involved in the protection against mutated cells, such as cancer.

2.3.2.3 Platelets (Thrombocytes)

Scientific term for platelets is thrombocytes. The count is simply the total number of platelet. Mean platelet volume (MPV) is a measurement of the average size of platelets

found in blood platelet distribution width (PDW). PDW is a measure of the variation of the platelet.While the platelet crit (PCT) is the relative volume of platelets in a blood sample. The platelets help in blood clotting.

2.4 Functions of blood

Blood is a liquid connective tissue, which is maintaining the constancy of the internal environment through its functions of transportation, regulation, and protection. Therefore, blood performs important functions operate as follows (Ferguson et al, 1965; Cohen and Hull 2009):

a) Supply of oxygen to cells / tissues.
b) Supply of nutrients such as glucose, amino acids and fatty acids.
c) Removal of waste such as carbon dioxide, urea and lactic acid.
d) Making body immune by circulation of white cells, and detection of foreign material by antibodies.
e) Regulation of core body temperature.
f) Hydraulic functions.
g) Aiding body's self-repair mechanism by coagulation.
h) Messenger functions like transportation of hormones and the signaling of tissue damage.
i) Regulation of body pH (the normal pH of blood range between 7.35 and 7.45).

2.4.1 Functions of blood cells

The primary function of RBC, or erythrocytes, is to carry oxygen and carbon dioxide. Hemoglobin (HGB) is an important protein in the RBC that carries oxygen from the lungs to all parts of our body.

The primary function of WBC, or leukocytes, is to fight infection. There are several types of WBC and each has its own role in fighting bacterial, viral, fungi, and parasitic infections. Types of white blood cells that are most important for helping protect the body from infection and foreign cells include neutrophils, eosinophils, lymphocytes, monocytes, and granulocytes.

The primary function of platelets, or thrombocytes, is blood clotting. Platelets are much smaller in size than the other blood cells. They group together to form clumps, or a plug, in the hole of a vessel to stop bleeding (Farhi, 2009).

2.5 Blood groups and blood types

The surfaces of erythrocytes contain a genetically determined assortment of antigens composed of glycoproteins and glycolipids. These antigens, called agglutinogens, occur in characteristic combinations. Based on the presence or absence of various antigens, blood is categorized into different blood groups (Lawler et al., 1971).

2.5.1 ABO Blood group

The ABO blood group is based on two glycolipid antigens called A and B (Figure 2.2). People whose ABO display only antigen A have type A blood. Those who have only

antigen B are type B. Individuals who have both A and B antigens are type AB; those who have neither antigen A nor B are type O (Daniels and Bromilow, 2007; Mourantet al, 1976; Prokop and Uhlenbruck, 1969).

Blood plasma usually contains antibodies called agglutinogens that react with the A or B antigens if the two are mixed. These are the anti-A antibody, which reacts with antigen A, and the anti-B antibody, which reacts with antigen B. The antibodies present in each of the four blood types are shown in Figure 2.2.

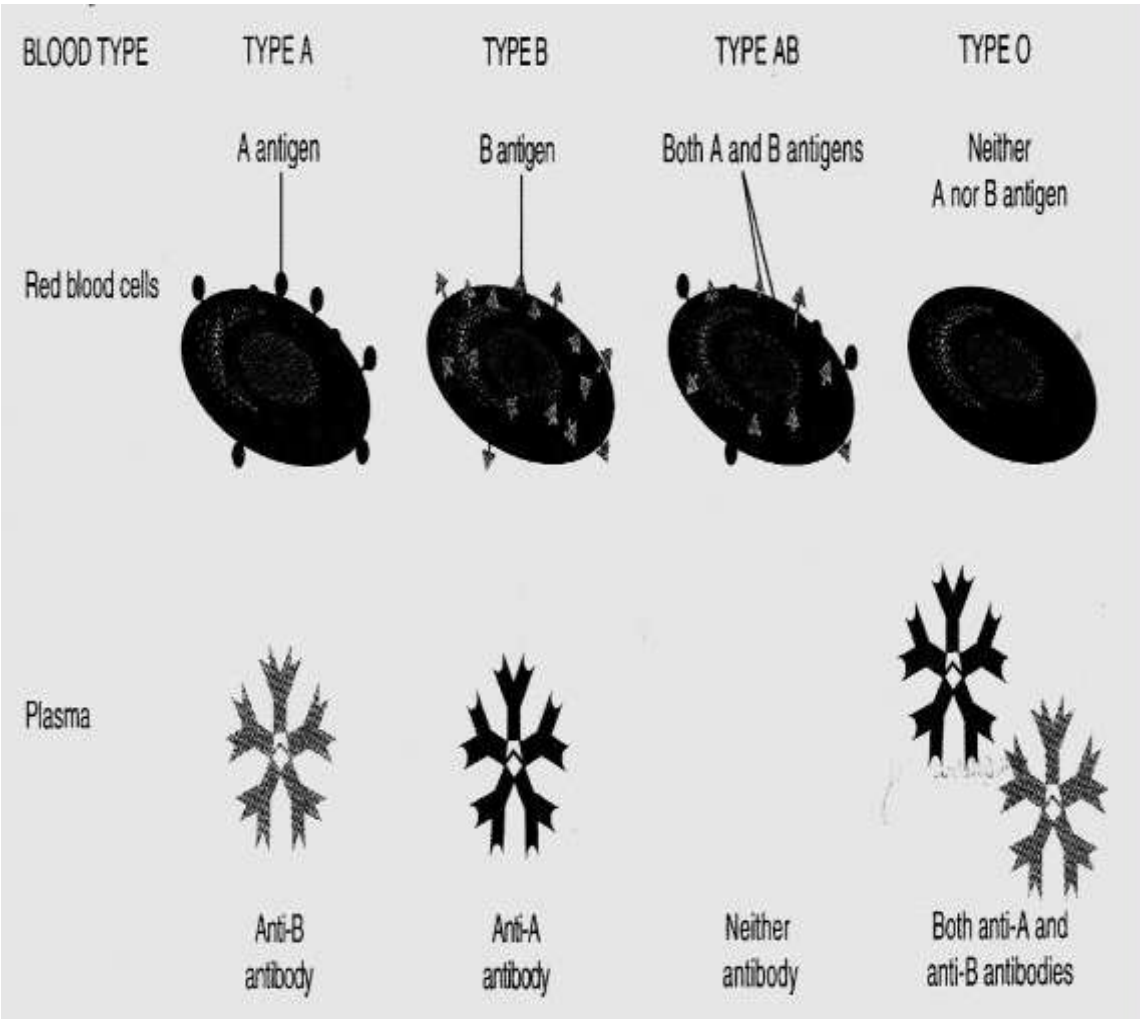

Figure 2.2: Antigens and antibodies of the ABO blood types (Tortora and Derrickson., 2006)

2.5.2 Rh Blood group

The Rhesus system is much more complex than the ABO system and has become known specifically for its role in haemolytic anaemia of newborns (Sibinga, 1995).

The alleles of three genes may code for the Rh antigen. People whose RBCs have Rh antigens are designated Rh^+ (Rh positive) those who lack Rh antigens are designated Rh^- (Rh negative) (Tortora and Derrickson., 2006).

2.6 Characteristics of laser light

A typical laser have three basic components: a laser (active) medium, an energy source (pumping system), and a resonant optical cavity. Lenses, mirrors, shutters, saturable absorbers, and other accessories may be added to the system to obtain greater power, shorter pulses, or special beam shapes, but only the mentioned three basic components are necessary for laser action (United Nations Environment Programme, 1982).

Lasers have three primary properties that have been utilized by the medical community, which is, monochromatic emission, coherence, and collimation. No real laser produced light having these characteristics absolutely. The power density profile in any cross-section has the characteristic bell-shaped Gaussian curve when power density at point within that section is plotted versus the radial distance of that point from the axis of the beam. This profile is the same when measured across any diameter of the beam; it would have the shape of a three-dimensional bell if it could be seen by an observer. Figure 2.3. shows a perspective view of a Gaussian laser beam, with one quadrant of the beam cut away to reveal the radial profile of power density (Shapshay., 1987).

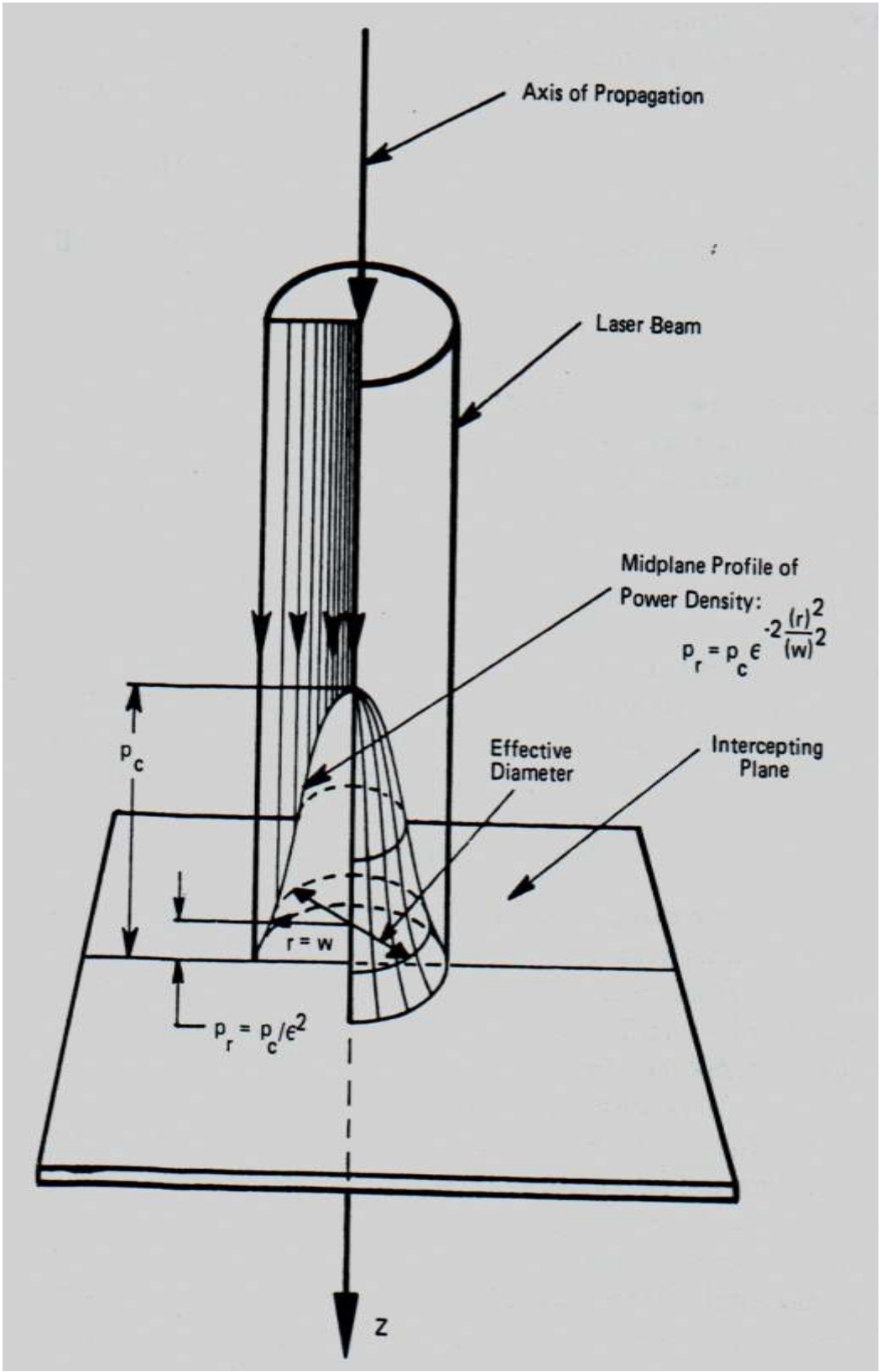

Figure 2.3: Gaussian laser beam (Shapshay., 1987)

2.6.1 Monochromatic emission

Laser light consists of essentially one wavelength, having its origin in stimulated emission from one set of atomic energy levels. The identity of the atom or molecule which is being excited determines the wavelength of the radiation produced. More precisely, this is a narrow band in a Gaussian distribution around the characteristic wavelength of the laser. The argon laser is unusual in that it emits light of two wavelengths (488 and 514 nm), a consequence of there being intermediate orbits between the excited and resting states. Quite often, lasers with visible wavelengths are described in terms of their colour: the argon laser beam is blue or blue–green; the He–Ne laser beam is red, and so on.

2.6.2 Coherence

Different parts of the laser beam are related to each other in phase. These phase relationships are maintained over long enough time so that interference effects may be seen or recorded photographically. Light can be considered as a sine wave. The light emitted by a laser has the distinction of being both temporally and spatially coherent, i.e. the waves are in phase in time and space.

2.6.3 Collimation

This is a direct consequence of coherence and refers to the non divergent and energy conserving properties of light in which the waves are parallel. It means that the diameter of the beam changes only minimally over distance, unless it is focused by a lens (Figure 2.4). Laser beam bounced back between mirrored ends of a laser cavity, those paths which sustain amplification must pass between the mirrors many times and be very

nearly perpendicular to the mirrors. As a result, laser beams are very narrow and do not spread very much. Both forms may be useful, such as in CO_2 laser surgery where a focused beam is required for excisional applications and where a scanner is used with a focused or collimated beam for resurfacing (Barlow et al, 2005; Shapshay, 1987; Siegman, 1986).

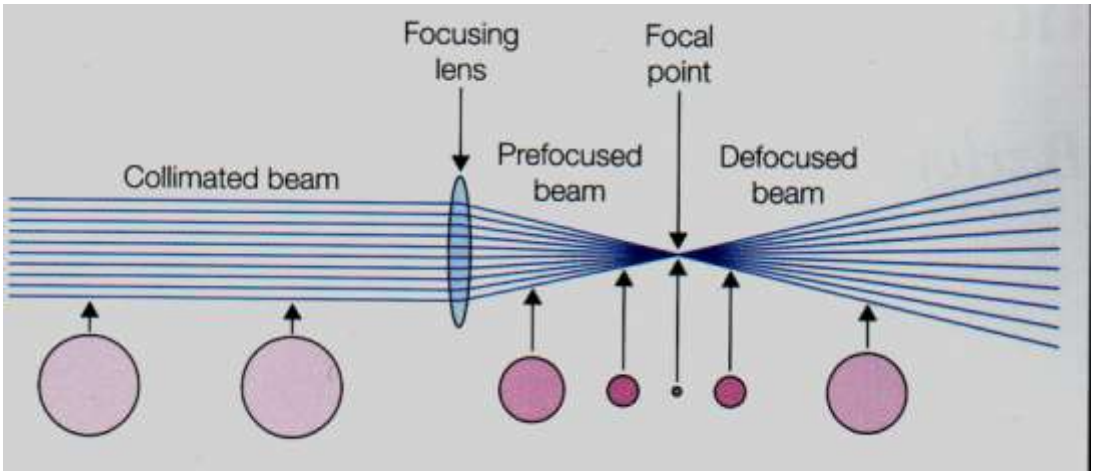

Figure 2.4: Representation of a collimated beam of light (Goldberg, 2005)

2.7 Theory of laser light

A laser is an optical source that emits photons in a coherent beam, which consists of a single wavelength or hue. Laser light is typically near-monochromatic, and emitted in a narrow beam. There is an inverse relationship between the energy of a photon (E) and the wavelength of the light (λ) given by an equation:

$$E = \frac{hc}{\lambda} \tag{2.1}$$

where, h is Planck's constant and c is the speed of light.

The above inverse relationship means that light consisting of high energy photons (such as blue light) has a short wavelength. Light consisting of low energy photons

(such as red light) has a long wavelength. When dealing with particles such as photons or electrons, a commonly used unit of energy is the electron-volt (eV) rather than the joule (J). An electron volt is the energy required to raise an electron through 1 volt, thus 1 eV = 1.602 x 10^{-19} J.

By expressing the equation for photon energy in terms of eV and μm we arrive at a commonly used expression which relates the energy (E), and wavelength (λ) of a photon, as shown in Equation 2.2

$$E = \frac{1.24}{\lambda} \tag{2.2}$$

In order to develop an adequate understanding of the blood response to the laser radiation, it is necessary to note three characteristics of the laser application that is, irradiance or fluency rate, energy fluence, and exposure time.

Irradiance or fluency rate is simply the power density or power per unit area incident on the blood during a single pulse and is given by (Arndt et al., 1983):

$$\text{Irradiance} = \frac{P_{out}}{A} \tag{2.3}$$

where, P_{out} is the laser power output expressed in unit of W, and A is the laser beam cross-sectional area in unit of cm^2.

The fluence (E_f) is energy density or the energy per unit area incident on the blood and given by (Arndt et al., 1983; White and Klein., 1991):

$$E_f = \frac{E}{A} \tag{2.4}$$

The energy contained within light is expressed in Joules (J) and its fluence or energy density per unit area in J/cm^2.

Also,

$$E_f = \text{Irradiance x t} \tag{2.5}$$

where, *t* is the exposure time.

$$\therefore E_f = \frac{P_{out} \text{ x t}}{A} \tag{2.6}$$

2.8 Laser–tissue interaction

There are three important factors that lead to the expanding biomedical use of laser technology, particularly in surgery. These factors are: (1) the increasing understanding of the wavelength selective interaction and associated effects of ultraviolet-infrared (UV-IR) radiation with biologic tissues, including those of acute damage and long-term healing, (2) The rapidly increasing availability of lasers emitting (essentially monochromatically) at those wavelengths that are strongly absorbed by molecular species within tissues, (3) The availability of both optical fibre and lens technologies as well as of endoscopic technologies for delivery of the laser radiation to the often remote internal treatment site. Fusion of these factors has led to the development of currently available biomedical laser systems (Judy, 1995). The following reactions will occur when the laser light interacts with tissue (Figure 2.5): photostimulation, photodynamic reactions, and photothermolytic and photomechanical reactions.

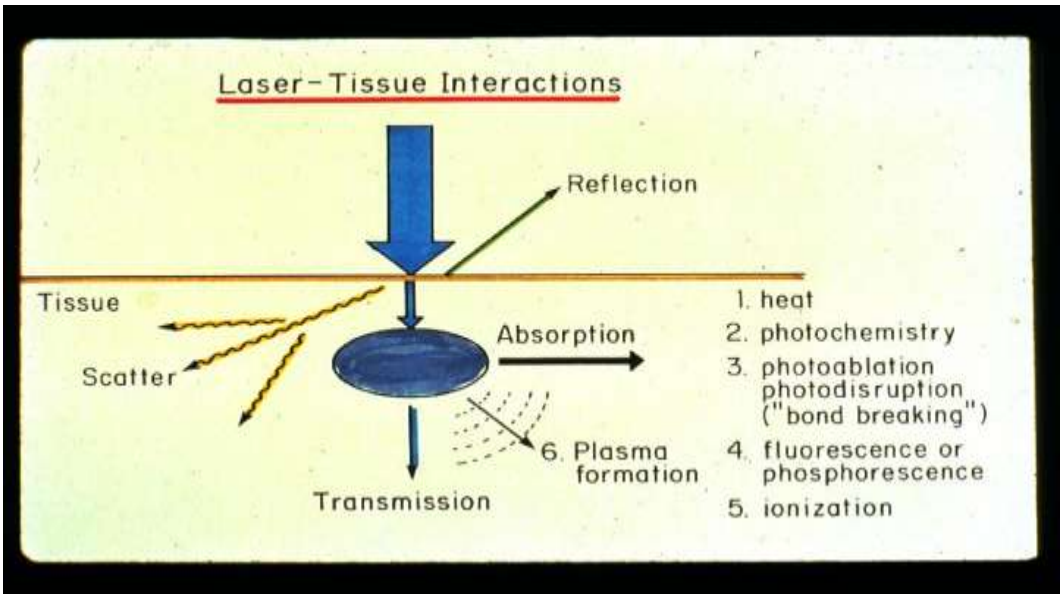

Figure 2.5: Laser–tissue interactions.

2.8.1 Photostimulation

Photostimulation is the use of light to artificially activate biological compounds, cells, or even whole organisms. Photostimulation can be used to noninvasively probe the causal relationships between different biological processes, using only light. In the long run, photostimulation may be useful as a therapy, using light to adjust the biological state of human patients. There is equivocal evidence to suggest that low energy lasers expedite wound healing. The mechanism for this is unclear.

2.8.2 Photodynamic reactions

This forms the basis of photodynamic therapy and involves the topical or systemic administration of a photosensitizer or precursor thereof. Subsequent irradiation with an appropriate light source elicits two types of photo-oxidative reaction and an immediate cytotoxic effect. Photodynamic therapy can also use endogenous chromophores such as

those found in pityrosporum acnes where the acnes organisms are killed with blue light irradiation with subsequent clinical improvement of acne.

2.8.3 Photothermolytic and photomechanical reactions

Photothermal, in these reactions the laser light is absorbed by tissue chromophores and is converted to heat; this process is accompanied by a local temperature increase, and the heat is conducted to cooler regions. Photochemical, in these reactions very low–power irradiation inactivates cell function by means of induced toxic chemical processes: temperature increases in discernible. The theory of selective photothermolysis has been applied to the removal of superficial vascular malformations, exogenous tattoos, certain benign pigmented lesions, and hair. It postulates that light can be used to selectively damage or destroy a target chromophore if its wavelength is selected so that there is as big a difference as possible between the absorption coefficient of the target and the surrounding tissue, the energy fluence is sufficiently high to damage the target, and the pulse duration is less than or equal to the thermal relaxation time (TRT). The TRT is the time taken for the target to dissipate about 63% of the incident thermal energy. These factors are considered in more detail below.

2.8.3.1 Wavelength

The absorption spectrum of important tissue chromophores as shown in Figure 2.6 in relation to the wavelengths of the lasers is widely used in dermatology. Hemoglobin has a number of different absorption peaks whereas absorption by melanin gradually diminishes with longer wavelengths of incident light. Consideration must also be given to the depth of the target structure, as scattering in the dermis is strongly influenced by wavelength, making a longer wavelength, which may be relatively poorly absorbed,

often preferable, to a short wavelength with the opposite characteristics. In some situations, particularly in relation to melanin, a single wavelength may not be necessary and it may even be preferable to use flashlamps because of their broad emission spectrum (500-1200 nm). These are cheaper to manufacture than lasers and can be used with light filters (515-755 nm) to allow a potentially wide range of applications. It is possible to vary their pulse durations from 0.5 to 88.5 ms and to introduce intervals between pulses of 1-300 ms. At present they cannot substitute for lasers where focused, high energy beams are required (Goldberg, 2005).

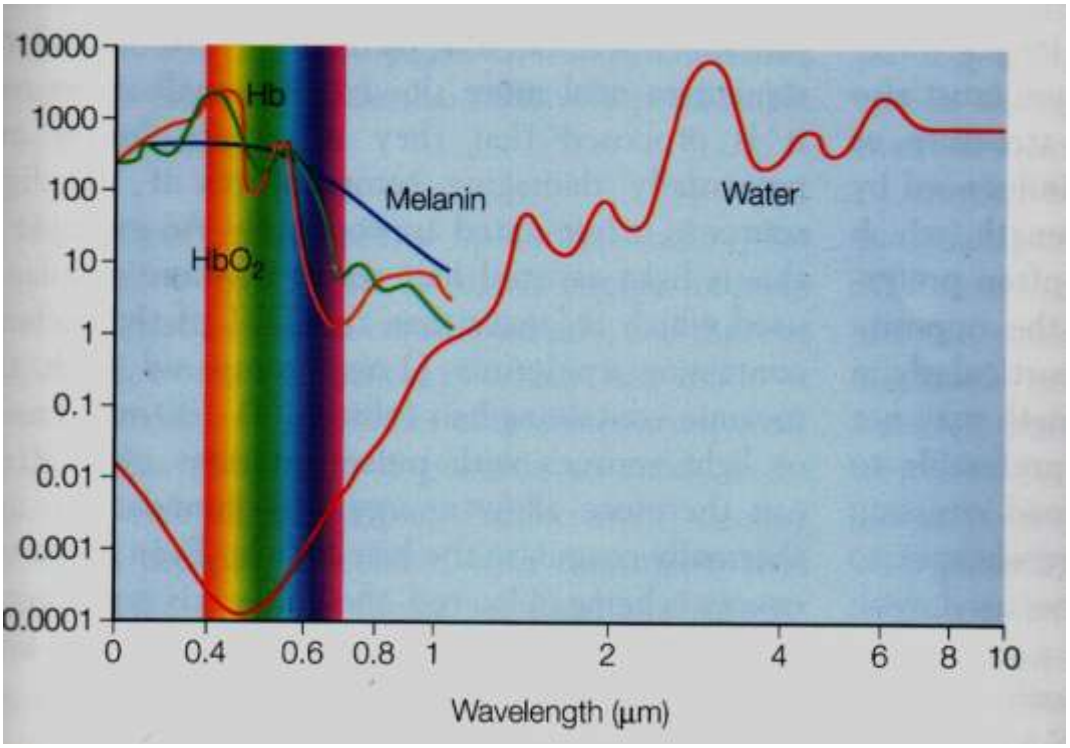

Figure 2.6: Absorption spectra of principal tissue chromophores (Goldberg, 2005).

2.8.3.2 Energy fluence

The energy fluence is sufficiently high to damage the target, and the pulse duration is less than or equal to the thermal relaxation time (TRT). The TRT is the time taken for the target to dissipate about 63% of the incident thermal energy.

2.8.3.3 Thermal relaxation time (TRT)

The TRT is related only to the size of the target chromophore, being proportional to the square of the target diameter squared, and varies from a few ns (tattoo particles) through several hundred ms or more (leg venules) (Goldberg, 2005).

Light impinging on tissue is subjected to the normal laws of physics. Some of the light is reflected and some is transmitted through the air-tissue barrier and passes into the tissue where it is scattered or absorbed. Obviously the extent to which each process dominates is dependent upon the physical properties of the light and the tissue. The theory of light-tissue interaction is not as well developed as that of ionizing radiation (Wall et al, 1988) but enough is known to explain the macroeffects upon which most laser treatments depend. Figure 2.7 shows the effect of changing the focal length of the focusing lens while maintaining the lens to tissue distance constant.

At very low-energy density levels (power density × time), say below 4 J/cm^2, a stimulating effect on cells has been observed but above this level the effect is reversed and suppression occurs (Mester et al., 1968). As the energy density rises to 40 J/cm^2, indirect cell damage can take place if any sensitizing agents present become activated (e.g. haematoporphyrin derivative). Direct tissue damage does not take place until about 400 J/cm^2 when the first thermal effects appear and photocoagulation occurs. Another

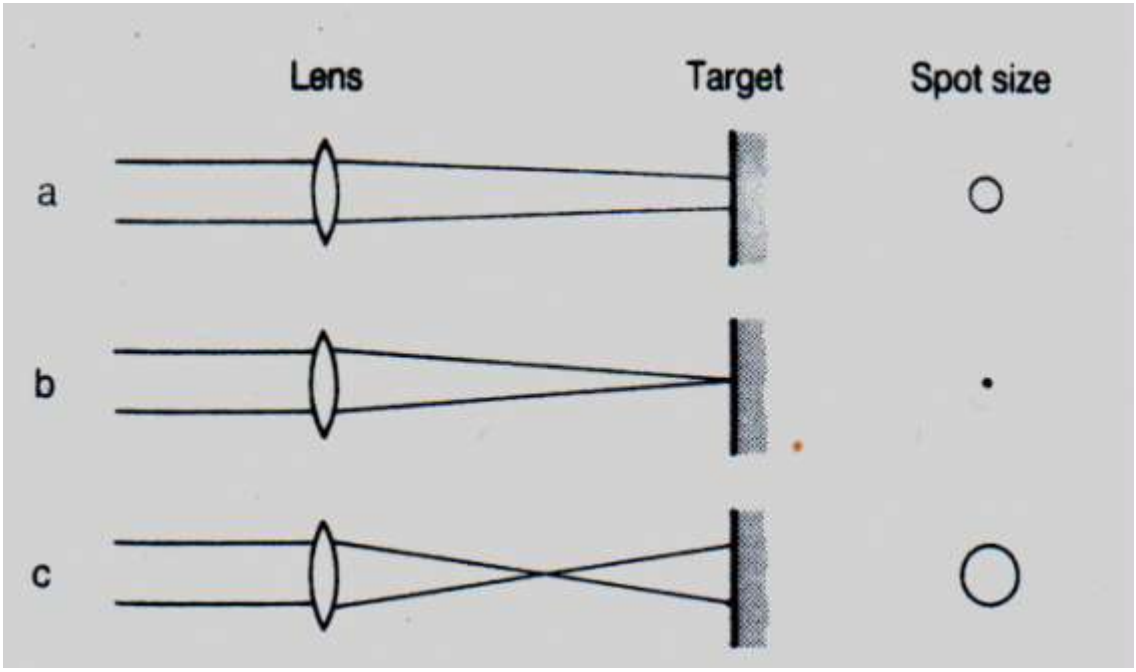

Figure 2.7: Changing the focal length of the focusing lens while keeping the lens-to-tissue distance constant alters the spot size, the diameter of the beam at the point of contact with the tissue. a. The focal length of the lens is greater than the lens –to-tissue distance; b. the focal length of the lens is the same as the lens –to-tissue distance, i.e. the laser is focused on the tissue; c. the focal length of the lens is less than the lens –to-tissue distance (Shaw et al, 2003).

10-fold increase in energy density results in complete tissue destruction as it is sufficient to raise the cell temperature rapidly to 100ºC, causing tissue vaporization. Obviously these are general observations and other properties of the incident beam will have an influence but the general 10-fold relationship alluded to above holds even though other parameters may be varied. Amongst the most important of these are the wavelength and the pulsatile nature of the radiation involved.

The wavelength absorption characteristics of various body tissues are reasonably well understood, qualitatively if not quantitatively. Figure 2.8 shows the absorption curves for water, melanin and hemoglobin which, to a large extent, will determine the curves for tissue as a whole. In the ultraviolet and the middle-to-far infrared spectrum,