# STUDY ON THE EFFECTS OF BLUE AND GREEN LASER BEAMS ON ANAEMIC BLOOD PARAMETERS

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# STUDY ON THE EFFECTS OF BLUE AND GREEN LASER BEAMS ON ANAEMIC BLOOD PARAMETERS

**by**

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# TABLES OF CONTENTS

**ACKNOWLEDGEMENTS** ..... ii
**TABLES OF CONTENTS** ..... iii
**LIST OF TABLES** ..... vi
**LIST OF FIGURES** ..... viii
**LIST OF ABBREVIATIONS** ..... viii
**ABSTRAK** ..... xiv
**ABSTRACT** ..... xvi

**CHAPTER 1** ..... 1
1.1 Background ..... 1
1.2 Problem Statement ..... 3
1.3 Objectives of the Research ..... 4
1.4 Scope of Study ..... 4
1.5 Outline of the Thesis ..... 5

**CHAPTER 2** ..... 6
2.1 Literature Review ..... 6
2.2 Laser Diode ..... 9
2.3 Diode Pumped Solid State Green Laser ..... 11
2.4 Human Blood ..... 13
2.5 Anaemias and other red cell disorders ..... 16
2.6 Laser-Tissue Interaction ..... 19
2.7 Adenosine triphosphate ..... 25

**CHAPTER 3** ..... 28
3.1 Instrument ..... 28
3.1.1 Laser Instrumentation ..... 28
3.1.2 Hematology Analyser ..... 29
3.1.3 Centrifuge Machine ..... 31
3.1.4 Cell-Titer Glo Luminescence Cell Viability Assay Kit ..... 31
3.1.5 TECAN Sunrise ELISA Reader ..... 32

3.1.6 Power Meter ..... 33
3.1.7 Inverted Microscope ..... 35
3.1.8 Chemical Materials ..... 35
3.1.9 Kits component for cell viability experiment ..... 38
3.2 Methodology ..... 40
3.2.1 Blood Sample Preparation ..... 41
3.2.2 Blood Irradiation ..... 43
3.2.3 Blood count ..... 45
3.2.4 Blood Smear Preparation ..... 46
3.2.5 Cell Viability Experiment ..... 48
3.2.6 Statistical Analysis ..... 53

**CHAPTER 4** ..... 54
4.1 Comparison of blood parameters before and after irradiation ..... 54
4.2 Effect of laser on cell viability ..... 85
4.3 Effect of laser on cell morphology ..... 94

**CHAPTER 5** ..... 107
5.1 Conclusion ..... 107
5.2 Recommendations ..... 109

**REFERENCES** ..... 111
**APPENDICES** ..... 117
Appendix A. Anaemic Male (<40 years) ..... 117
Appendix B. Anaemic Male (>40 years) ..... 119
Appendix C. Anaemic Female (<40) ..... 121
Appendix D. Anaemic Female (>40) ..... 123
Appendix E. Normal Male (<40 years) ..... 125
Appendix F. Normal Male (>40 years) ..... 127
Appendix G. Normal Female (<40 years) ..... 129
Appendix H. Normal Female (>40 years) ..... 131
Appendix I ..... 133
Appendix J. Optical Density of ATP measurement ..... 138

Appendix K. Percentage of RBC viability according to ATP measurement ..... 140
Appendix L ..... 141

**LIST OF PUBLICATION** ..... 142

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# LIST OF TABLES


| | | Page |
|---|---|---|
| 2.1 | Automated blood analyser readout | 19 |
| 2.2 | Thermal effects of laser irradiation | 24 |
| 3.1 | Hematological reference ranges for healthy Malaysian population | 42 |
| 4.1 | Paired test analysis of blood parameter before and after laser irradiation | 55 |
| 4.2 | The changes of blood parameters at different exposure time | 56 |
| 4.3 | Effect of blue laser irradiation on age group | 57 |
| 4.4 | Effect of blue laser irradiation among gender group | 57 |
| 4.5 | Paired test analysis of blood parameter before and after irradiation | 58 |
| 4.6 | The changes of blood parameters at different exposure time | 59 |
| 4.7 | Effect of green laser irradiation on age group | 60 |
| 4.8 | Effect of green laser irradiation on gender group | 60 |
| 4.9 | Effect of blue irradiation on blood category | 61 |
| 4.10 | Effect of green laser irradiation on blood category | 62 |
| 4.11 | Comparison of mean blood parameter between blue and green laser. | 83 |
| 4.12 | Comparison of mean blood parameter between blue and green laser among gender | 84 |
| 4.13 | Comparison of mean blood parameter between blue and green laser among gender | 84 |
| 4.14 | Percentage of cell viability for normal and anaemic red blood cells | 87 |
| 4.15 | Effect of blue laser irradiation on red blood cell viability among exposure time | 87 |

| | | |
|---|---|---|
| 4.16 | Effect of laser blue irradiation on red blood cell viability among blood category | 88 |
| 4.17 | Effect of green laser irradiation on red blood cell viability among exposure time | 89 |
| 4.18 | Effect of green laser irradiation on red blood cell viability among blood category | 89 |
| 4.19 | Comparison in change in cell viability among laser type | 91 |
| 4.20 | Comparison in cell viability among the two type of laser at different time exposure | 91 |

# LIST OF FIGURES


| | | Page |
|---|---|---|
| 2.1 | Schematic diagram of a laser diode. | 10 |
| 2.2 | Energy levels in a degenerate semiconductor | 11 |
| 2.3 | Atomic energy levels and light involved in the operation of a DPSS green laser | 12 |
| 2.4 | Major component of blood | 14 |
| 2.5 | Schematic assessment of haematocrit by centrifugation | 17 |
| 2.6 | Diagrammatic representation of different type of variation in red cell shape | 18 |
| 2.7 | Absorption spectra of tissue chromophores | 20 |
| 2.8 | Idealized biphasic dose response curve (often termed Arndt-Schulz curve) typically reported in LLLT studies | 21 |
| 2.9 | Geometry reflection, refraction, absorption and scattering | 22 |
| 2.10 | Map of laser-tissue interactions. | 23 |
| 2.11 | Flow chart with important parameters for modelling thermal interaction | 24 |
| 2.12 | Principles of photoablation | 25 |
| 2.13 | Process how ATP were produced from ADP and conversion cycle of ATP and ADP | 27 |
| 3.1 | Diode blue laser | 28 |
| 3.2 | DPSS green laser | 29 |
| 3.3 | Automated Hematology Analyzer | 30 |
| 3.4 | Universal 320R Benchtop Centrifuge machine | 31 |
| 3.5 | Cell-Titer Glo Luminescence Cell Viability Assay Kit | 32 |
| 3.6 | TECAN Sunrise ELISA Reader | 33 |
| 3.7 | Power meter | 34 |

| | | |
|---|---|---|
| 3.8 | S140A Integrating Sphere Sensor | 34 |
| 3.9 | Axiovert 25 Carl Zeiss Inverted Microscope | 35 |
| 3.10 | Trypan Blue Solution used in cell counting | 36 |
| 3.11 | Bovine Serum Albumin used in preparation of blood buffer | 36 |
| 3.12 | Phosphate Buffer Saline used in blood buffer preparation | 37 |
| 3.13 | Glucose used in blood buffer preparation | 37 |
| 3.14 | Pipette used in red blood cell viability experiment | 38 |
| 3.15 | Labnet Biofree Pipette tips used with pipette | 39 |
| 3.16 | Hemocytometer for red blood cell counting | 39 |
| 3.17 | Nunc 96 well plate for red blood cell seeding | 40 |
| 3.18 | Flow chart of study designed for this research | 41 |
| 3.19 | Blood sample collected from hospital | 43 |
| 3.20 | a) Experiment setup for blood irradiation using DPSS green laser.<br>b) Experiment setup for blood irradiation using diode blue laser | 44 |
| 3.21 | Power of laser was tested using power meter | 44 |
| 3.22 | Steps for blood smear preparation and evaluation | 47 |
| 3.23 | Final dilution of RBCs packed cell with blood buffer | 49 |
| 3.24 | Suspension was transferred to the edge of haemocytometer | 50 |
| 3.25 | Haemocytometer square grids to count | 51 |
| 3.26 | RBCs diluted were seeding into 96-well plate | 52 |
| 4.1 | Changes in white blood cell by blue laser irradiation at different exposure time for normal blood | 63 |
| 4.2 | Changes in white blood cell by blue laser irradiation at different exposure time for anaemic blood | 64 |
| 4.3 | Changes in white blood cell by green laser irradiation at different exposure time for normal blood | 65 |

| | | |
|---|---|---|
| 4.4 | Changes in white blood cell by green laser irradiation at different exposure time for anaemic blood | 65 |
| 4.5 | Changes in red blood cell by blue laser irradiation at different exposure time for normal blood | 66 |
| 4.6 | Changes in red blood cell by blue laser irradiation at different exposure time for anaemic blood | 66 |
| 4.7 | Changes in red blood cell by green laser irradiation at different exposure time for normal blood | 68 |
| 4.8 | Changes in red blood cell by green laser irradiation at different exposure time for anaemic blood | 69 |
| 4.9 | Changes in haematocrit by blue laser irradiation at different exposure time for normal blood | 70 |
| 4.10 | Changes in haematocrit by blue laser irradiation at different exposure time for anaemic blood | 70 |
| 4.11 | Changes in haematocrit by green laser irradiation at different exposure time for normal blood | 72 |
| 4.12 | Changes in haematocrit by green laser irradiation at different exposure time for anaemic blood | 72 |
| 4.13 | Changes in mean cell volume by blue laser irradiation at different exposure time for normal blood | 73 |
| 4.14 | Changes in mean cell volume by blue laser irradiation at different exposure time for anaemic blood | 74 |
| 4.15 | Changes in mean cell volume by green laser irradiation at different exposure time for normal blood | 75 |
| 4.16 | Changes in mean cell volume by green laser irradiation at different exposure time for anaemic blood | 76 |
| 4.17 | Changes in mean corpuscular haemoglobin by blue laser irradiation at different exposure time for normal blood | 77 |
| 4.18 | Changes in mean corpuscular haemoglobin by blue laser irradiation at different exposure time for anaemic blood | 77 |
| 4.19 | Changes in mean corpuscular haemoglobin by green laser irradiation at different exposure time for normal blood | 78 |

| | | |
|---|---|---|
| 4.20 | Changes in mean corpuscular haemoglobin by green laser irradiation at different exposure time for anaemic blood | 79 |
| 4.21 | Changes in platelet by blue laser irradiation at different exposure time for normal blood | 80 |
| 4.22 | Changes in platelet by blue laser irradiation at different exposure time for anaemic blood | 81 |
| 4.23 | Changes in platelet by green laser irradiation at different exposure time for normal blood | 81 |
| 4.24 | Changes in platelet by green laser irradiation at different exposure time for anaemic blood | 82 |
| 4.25 | Optical density of ATP measurement at different exposure time for normal blood sample | 86 |
| 4.26 | Optical density of ATP measurement at different exposure time for anaemic blood sample | 86 |
| 4.27 | Percentage of viability after blue laser irradiation among blood category | 89 |
| 4.28 | Percentage of viability after green laser irradiation among blood category | 90 |
| 4.29 | Percentage of cell viability between blue and green lasers for normal blood samples | 92 |
| 4.30 | Percentage of cell viability between blue and green lasers for anaemic blood samples | 92 |
| 4.31 | Percentage of normal red blood cell shape for normal blood sample | 95 |
| 4.32 | Blood smear control at 30 s for normal blood sample | 97 |
| 4.33 | Blood smear after blue irradiation at 30 s for normal blood sample | 97 |
| 4.34 | Blood smear after green irradiation at 30 s for normal blood sample | 98 |
| 4.35 | Blood smear control at 50 s for normal blood sample | 98 |
| 4.36 | Blood smear after blue irradiation at 50 s for normal blood sample | 99 |
| 4.37 | Blood smear after green irradiation at 50 s for normal blood sample | 99 |

| | | |
|---|---|---|
| 4.38 | Blood smear control at 90 s for normal blood sample | 100 |
| 4.39 | Blood smear after blue irradiation at 90 s for normal blood sample | 100 |
| 4.40 | Blood smear after green irradiation at 90 s for normal blood sample | 101 |
| 4.41 | Percentage of normal red blood cell shape for anaemic blood sample | 102 |
| 4.42 | Blood smear control at 60 s for anaemic blood sample | 103 |
| 4.43 | Blood smear after blue irradiation at 60 s for anaemic blood sample | 103 |
| 4.44 | Blood smear after green irradiation at 60 s for anaemic blood sample | 104 |
| 4.45 | Blood smear control at 80 s for anaemic blood sample. | 104 |
| 4.46 | Blood smear after blue irradiation at 80 s for anaemic blood sample. | 105 |
| 4.47 | Blood smear after green irradiation at 80 s for anaemic blood sample | 105 |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AC | Absorption coefficient |
| ANOVA | Analysis of Variance |
| ADP | Adenosine diphosphate |
| ATP | Adenosine triphosphate |
| CBC | Complete Blood Count |
| DPSS | Diode pumped solid state |
| EDTA | Ethylenediaminetetracidic acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| FBC | Full blood count |
| HCT | Haematocrit |
| MCH | Mean corpuscular haemoglobin |
| MCV | Mean cell volume |
| OD | Optical density |
| PLT | Platelet |
| PBS | Phosphate Buffer Saline |
| RBC | Red blood cell |
| WBC | White blood cell |

# KAJIAN KESAN ALUR LASER BIRU DAN HIJAU TERHADAP PARAMETER DARAH ANEMIA

## ABSTRAK

Untuk beberapa dekad yang lalu, banyak eksperimen telah menunjukkan tindak balas positif kepada rangsangan-bio selepas sinaran laser ke atas sel hidup seperti penyembuhan luka, pembaikan tisu, dan rawatan terapeutik. Dalam kajian ini, parameter darah manusia daripada responden normal dan anemia yang terdiri daripada sel darah putih, sel darah merah, hematokrit, jumlah min sel, min korpuskel hemoglobin dan platelet dianalisis tentang kesan penyinaran laser berkeamatan rendah menggunakan DPSS laser hijau (532 nm) dan diode laser biru (460 nm). Objektif kajian ini adalah untuk mengkaji tindak balas biologi laser keamatan rendah kepada sel darah merah yang normal dan anemia sebelum dan selepas disinari dengan laser, untuk mengkaji kemandirian sel atau daya hidup sel darah sebelum dan selepas penyinaran, untuk mengkaji sifat-sifat morfologi sel darah berdasarkan parameter laser yang berbeza dan menilai peranan laser berkeamatan rendah panjang dalam menambah baik dan meningkatkan anemia sel darah merah. Penyinaran sampel darah dengan laser hijau dan biru menunjukkan perubahan bermakna dalam sel darah merah, hematokrit, jumlah min sel dan platelet tanpa kaitan dengan jantina dan umur kecuali platelet. Kategori darah telah menunjukkan perubahan signifikan dalam hematokrit selepas penyinaran dengan kedua-dua laser biru dan hijau. Apabila membandingkan kesan kedua-dua laser pada parameter darah, sel darah putih dan platelet menunjukkan perbezaan yang signifikan antara jenis laser. Perbezaan parameter darah antara kedua-dua laser tidak bergantung kepada jantina responden tetapi perbezaan dalam platelet bergantung kepada umur responden. Seterusnya, siasatan ke atas daya hidup sel darah merah untuk sampel normal dan anemia

menunjukkan perbezaan yang bermakna terhadap daya hidup sel darah merah antara masa pendedahan selepas penyinaran laser. Dari analisis ini, ia telah menunjukkan bahawa terdapat perbezaan yang ketara antara daya hidup sel di kalangan jenis laser. Penyinaran laser biru memberi lebih kesan ke atas daya hidup sel berbanding penyinaran laser hijau. Akhir sekali, kajian morfologi sel darah merah sebelum dan selepas penyinaran laser menyebabkan deformasi kepada sel darah merah. Penyinaran laser hijau memberi lebih kesan kepada bentuk sel darah merah berbanding laser biru. Morfologi sampel darah anemia yang paling banyak terkesan terhadap penyinaran laser berbanding dengan sampel darah yang normal dan laser hijau memberi lebih kesan morfologi kepada sel darah merah berbanding laser biru. Dari semua calitan darah yang disediakan, sebahagian besar daripada sel tidak normal yang diperhatikan adalah sel krenasi berbanding lain variasi sel darah merah yang lain. Kesimpulannya, untuk mendapatkan kesan terapi yang dikehendaki menggunakan laser berkeamatan rendah ke atas pesakit anemia, laser biru dengan masa dedahan antara 30 - 60 saat dianggap selamat kerana kurang kesan kerosakan diperhatikan berbanding laser hijau. Selepas 60 saat, lebih kesan yang merosakkan parameter sel darah merah diperhatikan. Oleh itu, 60 saat harus dijadikan had masa dedahan sekiranya menggunakan laser biru.

# STUDY ON THE EFFECTS OF BLUE AND GREEN LASER BEAMS ON ANAEMIC BLOOD PARAMETERS

## ABSTRACT

For the past few decades, a lot of experimental has shown positive response on bio-stimulation after laser irradiation on living bodies such as wound healing, tissue repair, and therapeutic treatment. In this study, human blood parameter from normal and anaemic respondents which consists of white blood cell, red blood cell, haematocrit, mean cell volume, mean corpuscular haemoglobin and platelet were analysed on the effects of low level laser irradiation using DPSS green laser (532 nm) and diode blue laser (460 nm). Objectives of this research are to study biological response of low intensity laser on normal and anaemic red blood cell before and after irradiate with laser, to study cell survival or viability of the blood cell before and after irradiation, to study morphological properties of blood based on different laser parameters and assessing the role of low intensity laser wavelength in improving and enhancing anaemic red blood cell. Irradiation of blood samples with green and blue lasers showed significant changes in red blood cell, haematocrit, mean cell volume and platelet with no significant changes correlated with gender and age except platelet. Blood category has showed significant changes in haematocrit after irradiation with both blue and green laser. When comparing the effect of both lasers on blood parameter, white blood cell and platelet parameter show significant difference among laser types. The difference in blood parameter between both lasers does not depend on respondent's gender but difference in platelet does depend on respondent's age. Further, investigation on red blood cell viability for normal and anaemic sample shows significant differences on red blood cell viability among exposure time after laser irradiation. From the analysis, it was revealed that there is

significant difference between cell viability among laser type. Blue laser irradiation affected most on cell viability compared to green laser irradiation. Finally, study of morphology of red blood cells before and after laser irradiation caused deformability to red blood cell. Green laser irradiation affected the shape of red blood cell more compared to blue laser. Anaemic blood samples morphology affected most by laser irradiation compared to normal blood samples and green laser affected red blood cell morphology more compared to blue laser. From all the blood smears prepared, most of the abnormal cell observed is echinocyte which is crenated cell compared to other red blood cell variations. As a conclusion, for desired therapeutic effect on anaemic patient using low level intensity laser, blue laser considered safer at 30 – 60 s as less damaging effect were observed compared to green laser. After 60 s, more damaging effect was observed on red blood cell parameter. Thus, 60 s should be a cut point if using blue laser.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Lasers emit a beam of intense electromagnetic radiation that is essentially monochromatic which typically has minimal divergent and easily focused into external optical systems (Judy, 2000) From a medical point of view, lasers are a convenient but sophisticated source of light in the visible, ultraviolet, and infrared parts of the spectrum. The light beam are easy to control, can be focused to a small spot and in many cases can be transmitted via flexible fibres, making internal delivery of light feasible (Bown, 1998).

All biomedical laser applications are based on the interaction of laser light with biological systems. The most important parameter in the way light affects the tissue is the laser line wavelength, but power and time exposure are also important (Rodrigues, 2004). Laser interaction causes a broad spectrum of effects which can be divided into three groups. First, low-intensity laser light is absorbed, reflected, or reradiated (as fluorescence) by the substance so that no changes occur within it. Second, low intensity UV and visible radiation can excite electronic states in molecules, and specific photobiological effects occur due to excitation of chromophores in cells (endogeneous or exogenous). The third class of effects involves high intensity laser radiation which causes damage to tissues by thermal or hydrodynamical destruction (Karu, 1991).

For the past few decades, a lot of experimental has shown positive response on bio-stimulation on living bodies such as wound healing, tissue repair, and therapeutic treatment (Maksimova, 2007). The effects of laser irradiation also can be attributed to the improvement of microcirculation as well as the modulation of the rheological properties of blood in terms of erythrocyte sedimentation rate, viscosity and deformability of erythrocytes from blood samples (Mi, 2004, ; 2006).

Anaemia is a multi-symptom syndrome involving both physical and emotional problems that can be evaluated for their impact on quality of life (Cella, 1998). Anaemia describes the condition in which the number of red blood cells in the blood is low. Anaemia is one of the most prevalent diseases in the general population and is a very frequently found condition in medical and surgical patients in all medical specialties. A good evaluation of its clinical impact and its therapeutic possibilities is essential (Madrazo-González, 2011).

Anaemia is one of the most prevalent human pathologies, especially in elderly people (17%-63%), and occurs very frequently (prevalence of 40%) in medical and surgical patients (Madrazo-González, 2010). Anaemia is a common finding in critically ill patients. There are often multiple causes. Obvious causes include surgical bleeding and gastrointestinal haemorrhage but many patients have no overt bleeding episodes (McLellan, 2003). Anaemia is also viewed as a negative prognostic factor in the elderly population; its independent impact on survival is unclear (Zakai, 2005).

Two main factors contribute to anaemia in the critically ill are insufficient production of red blood cells and blood loss (Thomas et al., 2010). Type of treatment

received depends on the cause of decreased red blood cell. Blood transfusions are often used as a solution to replace blood loss due to injuries and during certain surgeries. They are also commonly used to treat severely anaemic patients who have thalassemia, sickle cell disease, myelodysplastic syndromes, or other types of anaemia. Some patients require frequent blood transfusions. Iron overload can be a side effect for these frequent blood transfusions and it often damages the liver, heart, pancreas, thyroid, and other endocrine glands (Shander et al., 2009).

## 1.2 Problem Statement

Laser-biostimulation has led to new ways of dealing with anaemia disease. Low intensity laser irradiation has primarily been shown useful in medical application and widely used in clinical practice. Such widespread interest in the laser largely resulted from the fact that the laser's creation was an interdisciplinary enterprise.

Laser therapy treatment has becoming popular phenomenal especially in cosmetic industry because of its positive effect. Using visible light which considered safe has attracted many to use this treatment including those who have anaemia thinking it will not give any effect to them. However, when talking about laser irradiation, light photon will be absorbed by a system and will be excited to another state and will release its extra energy to achieve stability. Thus, there will still be effect or interaction occurred unless it is not absorbed in the system at all. This research also aimed to study biological response of low intensity laser on blood taken from anaemia patient and healthy patient, then compare between lasers with different wavelength and observe their cell survival properties. From this research, it will be useful for anaemia patient whose need regular

blood transfusion if the laser can enhance red blood cell upon the survival of cell after irradiated with laser. However, the safety and usage of laser is not fully explored by researcher especially in finding solution for anaemia patient. Therefore, the study of the mechanism of interaction of low intensity laser radiation with tissue by various methods is undoubtedly needs to ensure new method can be discovered for treatment of various illness.

### 1.3 Objectives of the Research

There are several objectives in this research. The objectives of this research are:

a) To determine biological response of low intensity laser wavelength on anaemic red blood cell before and after laser irradiation.
b) To identify cell survival or viability of the red blood cell before and after laser irradiation.
c) To characterize morphological properties of anaemic red blood cell based on different laser wavelengths.

### 1.4 Scope of Study

This research will focus on the effect of human blood irradiation on normal and anaemic patient. Only 6 parameters will be considered which is white blood cell, red blood cell, haematocrit, mean cell volume, mean corpuscular haemoglobin and platelet. Samples criteria will be chosed base on haemoglobin content obtained from full blood count of the patient. Only patient with haemoglobin reading range from 7.0 to 9.0 were selected and others were excluded. Characteristic of patients will include age group

range from 18 years old to 40 years old and above 40 years old and gender. Samples were taken from different patient for each exposure time due to limitation of blood samples taken from each patient. Blood irradiation will be done using two types of laser, diode blue laser (460 nm) and DPSS green laser (532 nm) with output power of 100 mW for each laser.

### 1.5 Outline of the Thesis

Chapter One describes background of this study, problem statement, objectives of the study, scope and importance of study and outline of the thesis. This chapter highlight the key point of the research. Chapter Two provides a comprehensive literature review on past studies related to this research and brief review on laser, type of laser, human blood, anaemia and other red cell disorders, laser-tissue interaction and cell viability. Chapter Three describes materials and equipment used in this research as well as methodology including experimental setup, protocol and data acquisition. Chapter Four discuss the data and analysis obtained from the research with statistical analysis. Lastly, chapter Five, conclusions and recommendation for further research were presented.

# CHAPTER 2

# THEORY AND LITERATURE REVIEW

## 2.1 Literature Review

Laser is another type of light source which make use of processes that amplifies light and produces a highly directional and high-intensity beam. Laser produced intense beams of light which are monochromatic (light at one specific wavelength), coherent (all waves are in a certain phase relationship to each other), and collimated. Laser beam parameter such as beam spot size, power required, efficiency, stability, and reliability described physical state of the light coming from a laser (Csele, 2004; George, 2009; Ready, 2001).

Laser has been used widely in medical field because of its biostimulating effect in various cell types. There are many research has been done to investigate effect of laser irradiation on cells. However, no general conclusion can be derived concerning the effects of laser light irradiation on cell because of difficulty in understanding the molecular mechanism of various changes occurring at cellular level (Gresner et al., 2005).

Most lasers used in bio-stimulation are low level laser therapy (LLLT) power range of 1 – 500 mW. Studies have revealed that LLLT has positive effects of bio-stimulation on living bodies. After first working laser was invented, on 1967, Endre Mester published his experiment work to test if laser irradiation might cause cancer in mice. Back hair of the mice were shaved and divided into two groups. One of the groups was irradiated with a low powered ruby laser (694 nm). The result is, the

treatment group did not get cancer but the hair grew back more quickly than the untreated group (Huang et al., 2009).

The study of human blood by laser has been widely investigated because of its physiological importance. Previous studies has shown positive therapeutic effect concerning improving blood microcirculation and perfusion, cell proliferation, improving rheological properties, inhibit the progress of inflammatory and influence local cytokines production. However, the molecular mechanism associated with the stimulatory effects of LLLT has not been fully clarified. One fundamental mechanism involved is that the laser energy is absorbed by intracellular chromophores and converted to metabolic energy, since cellular ATP levels increase after He–Ne laser irradiation (Alghamdi & Kumar, 2012; Ferreira et al., 2006; Mi et al., 2004; Mittermayr et al., 2007).

The response of Na+/K+-ATPase of human erythrocytes to green laser irradiation has been investigated by Kassak, (2006). Effects of green laser light were studied with isolated erythrocyte membranes were irradiated by Nd:YAG laser (532 nm, 30 mW). Activity of ATPase was determined colorimetrically by measuring the colored reaction product of liberated inorganic phosphate and malachite green with absorbance values were estimated spectrophotometrically at 640 nm. The experiments has shown a positive biostimulation of human erythrocyte membrane Na+/K+- ATPase activity by green light irradiation within the fluences in the range of 9-63 $J.cm^{-2}$ (Kassák et al., 2006).

LLLT also has been reported to improved blood microcirculation and was confirmed by in vivo measurement with time-dependent T1-weighted contrast-enhanced magnetic resonance imaging (MRI). Mi, (2004), has done a research to

clarify experimentally whether laser irradiation can improve the rheological parameters of blood, which may be resulting in the improvements of microcirculation. In this research, several rheological factors such as blood viscosity, erythrocyte sedimentation rate, erythrocyte deformability and the electrophoretic mobility of erythrocytes were investigated comparatively with two lasers of 632.8 and 532 nm. From the result obtained, it was found that laser irradiation can modulate the properties of hemorheology, including blood viscosity, erythrocyte sedimentation rate, erythrocyte deformability and electrophoretic mobility of erythrocytes (Mi, 2004).

A study by Zhang (2008), suggested that reactive oxygen species (ROS) are considered to be the key secondary messengers which induce photochemical reaction and activate several intracellular signaling pathways. Zhang (2008) studied the signaling pathway mediated by ROS upon the stimulation of LLT irradiation by using Src tyrosine kinases as targets to activate oxidative events. Cellular viability assay revealed laser irradiation of low doses promoted cell viability while high doses impaired (Zhang et al., 2008).

From the study by Ferreira et al (2006), it can be suggested that laser photo-stimulation was efficient in minimizing fungal infection dermal effects caused by a direct or indirect fungi- static/fungicidal light. Laser treatment enhances phagocytic activity of macrophages, neutrophils and cytokine synthesis. In this work, author also observed the inhibition of growth of fungi collected from laser-treated animals. It is possible that HeNe laser modifies, or even destroyed fungic structures that are used by the pathogen as resistance mechanisms and is able to activate monocytes to increase intracellular killing of the fungus (Ferreira et al., 2006).

A research conducted by Giacomo (2011), was done to evaluate the maximum thickness of power density could still produce a reparative cellular effect by measuring transmitted laser radiation through dead biological tissues of various animals (chicken, adult and young bovine, pig). From the transmission results, it suggested that even with tissue thicknesses of several centimeters the power density is still sufficient to produce a cell reparative effect (Di et al., 2013).

## 2.2 Laser Diode

Laser diodes have much in common with LEDs from which they were developed and have become one of the most important types of laser in use today. Laser diodes belong to the most important opto-electronic devices replacing the traditional crystal lasers in many application areas because they allow the direct transformation of electrical current into (coherent) light (Meschede, 2004; Szweda, 2001).

Diode lasers or semiconductor lasers are unique when compared to other types of lasers because they are small, operate with relatively low power input, and very efficient .They are distinguished from all other types of solid-state lasers by stimulation principle. While other solid-state laser media have to be pumped optically, semiconductor lasers are directly pumped by supplying an electrical current. The direct electrical pumping makes use of the specific properties of direct semiconductors such as gallium arsenide (GaAs) or indium phosphide (InP) in conjunction with different dopings (n-type and p-type). The n-type doping is material that has an excess of electrons and the other material (p-type) has a deficit of electrons or an excess of holes (missing electrons). Figure 2.1 shows schematic diagram of laser diode (Poprawe, 2011).

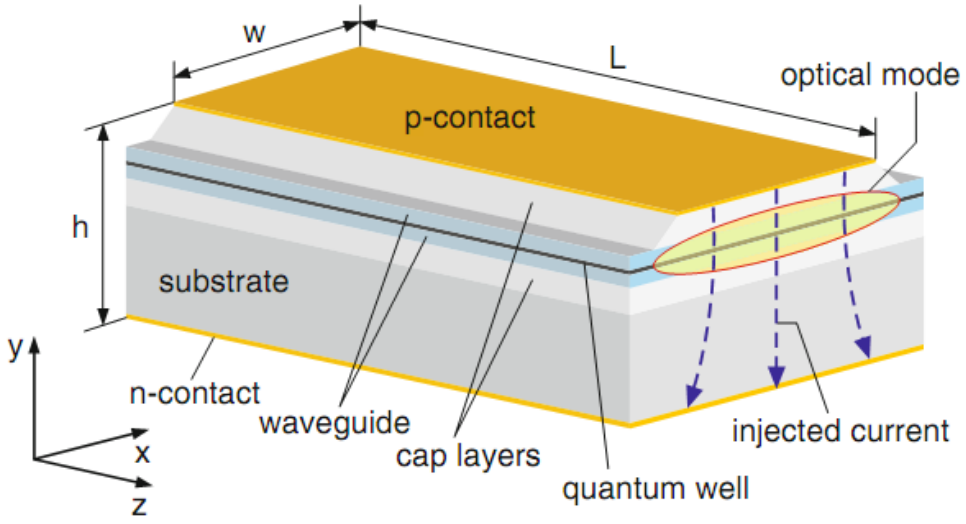


Figure 2.1 Schematic diagram of a laser diode. In this example, a broad laser diode is shown. Typical dimensions are L = 1 mm, W = 200 µm, h = 110 µm (Poprawe, 2011).

From these two difference material, it generate radiating transitions between different energy bands (valence band and conduction band). It designated as the bandgap of the material: the amount of energy that is released when the recombination radiative process occurs. Different material combinations have different bandgaps and thus emit different wavelengths. Figure 2.2 shows difference of energy level in degenerate semiconductor (Csele, 2004; Poprawe, 2011; Silfvast, 1996).

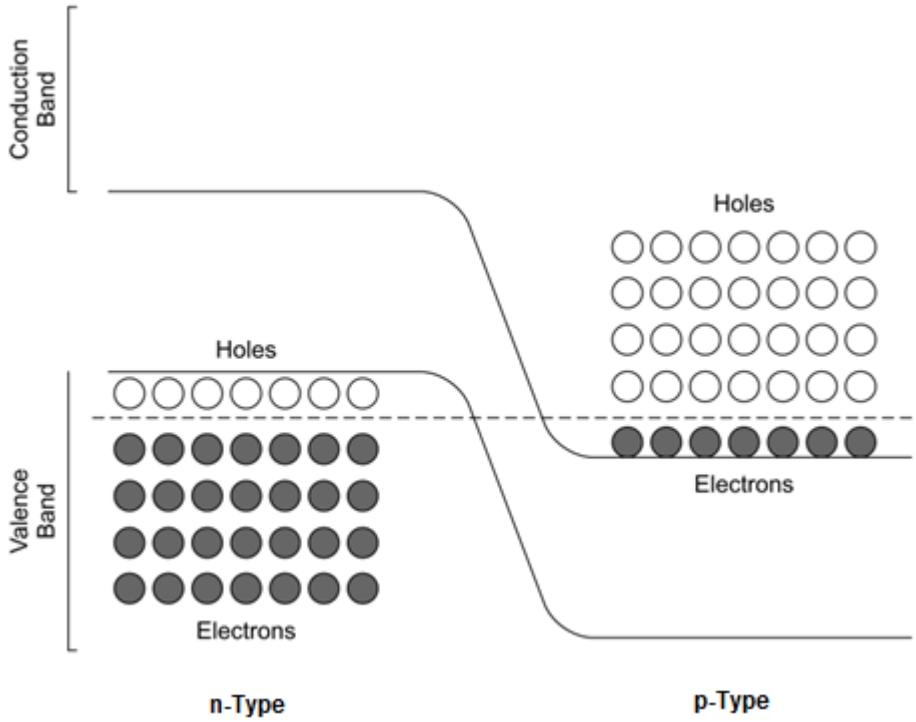


Figure 2.2 Energy levels in a degenerate semiconductor (Csele, 2004).

### 2.3 Diode Pumped Solid State Green Laser

Diode-pumped solid state (DPSS) lasers are solid state lasers made by pumping a solid gain medium with a laser diode to obtain desired wavelength. DPSS has broad application in industry, scientific research, medical and military fields. It has advantages of high efficiency, long lifetime, good beam quality and high reliability that superior to conventional lamp-pumped units. DPSS lasers are now available in the blue–green range (473– 561 nm), with the DPSS 488 nm variant replacing traditional ion lasers for many instrument applications (Davarcioglu, 2010; Ion, 2005; Kapoor et al., 2008; Wang, 2012).

This laser exhibits three essential elements of operation, a semiconductor diode laser, GaAlAs operating at infrared range wavelength of 808 nm pump a neodymium doped Nd:YAG ion oscillator at a wavelength of 1064 nm and a

frequency-doubling crystal that generates light of half of that wavelength, which is 532 nm, green colour.

The atomic energy level diagram of the DPSS green laser pointer is shown in Figure 2.3. Triply-charged ions of the neodymium atoms, $Nd^{3+}$, are present as dopants in a crystal of yttrium orthovanadate (Nd:$YVO^4$). A semiconductor diode laser with an infrared wavelength of 808 nm excites the lowest $^4I$ state to an electronically excited 4F state. The $Nd^{3+}$ ion emits infrared radiation, at a wavelength of 1064 nm, as it drops from the excited state into a different $^4I$ state. This radiation is directed into a "frequency doubling" crystal of potassium titanyl phosphate, which emits light of half the wavelength: 532 nm (Davarcioglu, 2010; Galang, 2010).

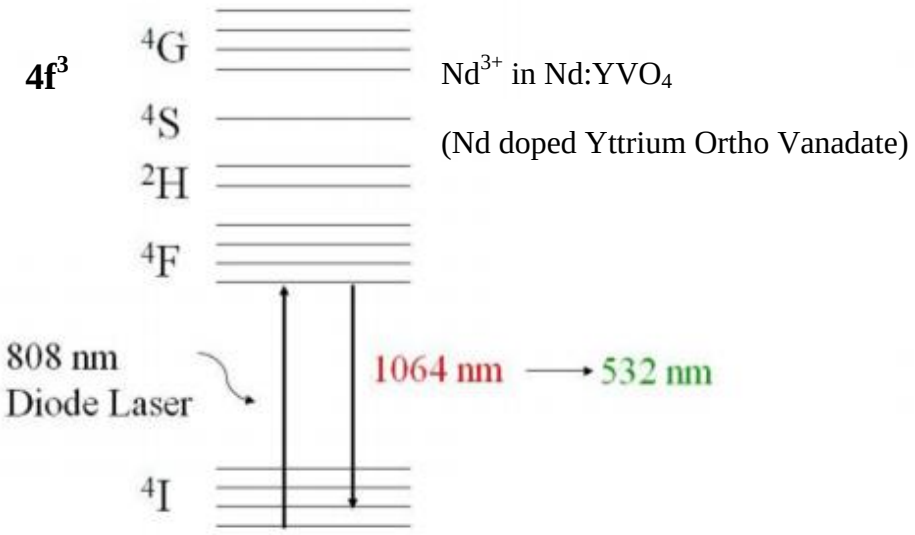


Figure 2.3 Atomic energy levels and light involved in the operation of a DPSS green laser (Galang, 2010).

## 2.4 Human Blood

Blood is a connective tissue that provides communication between the cells of different parts of the body and the external environment. It functions related to maintaining and balancing homeostasis of the body pumped by heart and carried by blood vessels throughout the body. Primarily, to transport oxygen, nutrients, chemical and cell wastes to certain targeted area (Rizzo, 2001; Waugh & Grant, 2001).

Blood makes up about 7% of body depending on his or her size. Colour of the blood does vary for example, arterial blood is bright red because it contains high levels of oxygen but venous blood (most is deoxygenated) has a darker, dull red colour. The normal pH range of blood is 7.35 to 7.45, which is slightly alkaline. Blood is about three to five times thicker than water. Viscosity is increased by the presence of blood cells and the plasma proteins, and this thickness contributes to normal blood pressure (Rizzo, 2001; Scanlon, 2007).

### 2.4.1 Components of blood

The blood consists of cells and cell fragments, called formed elements, and water with dissolved molecules, called blood plasma. Figure 2.4 shows major component of blood. Plasma constitutes about 55% and cells about 45% of blood volume.

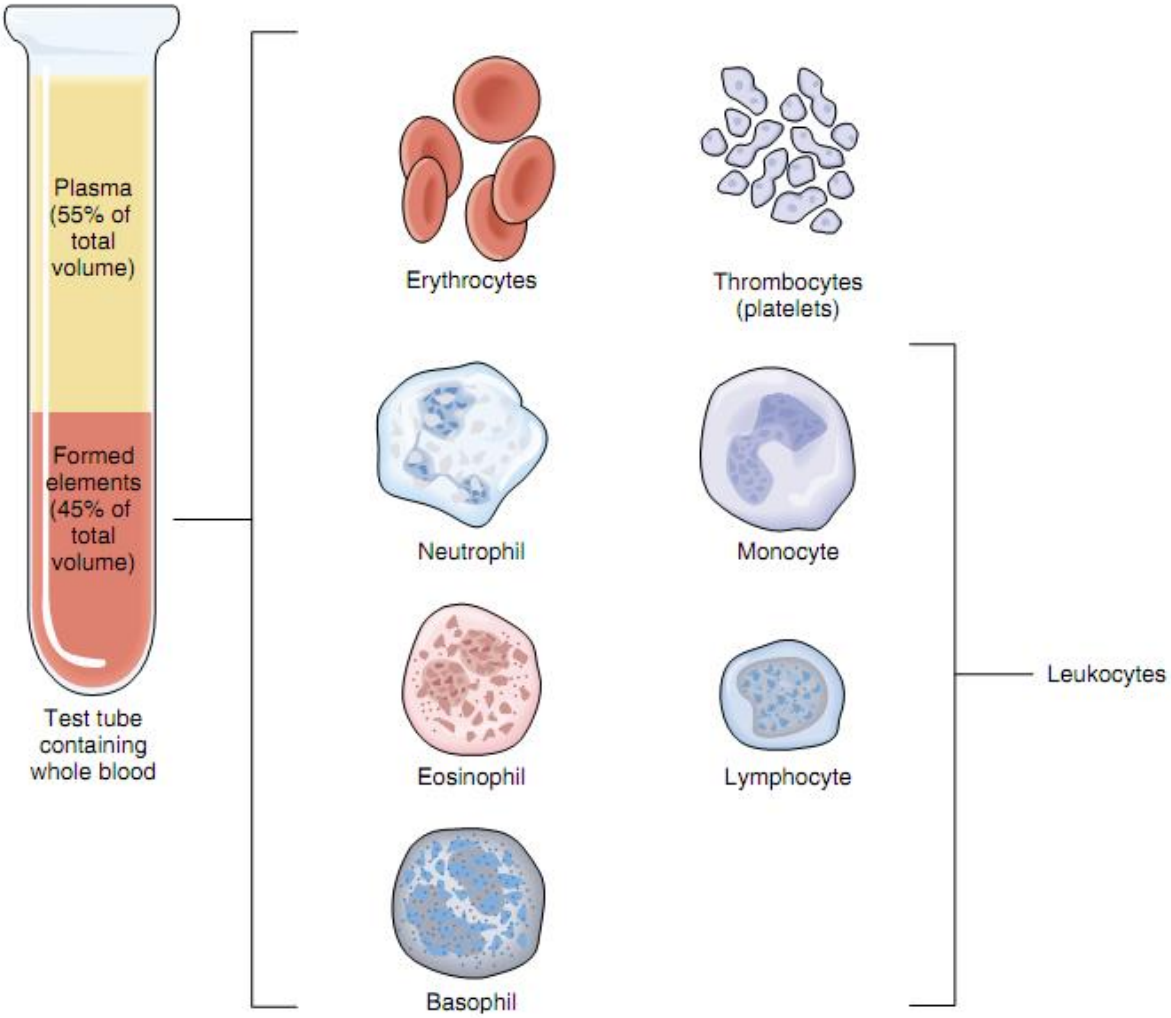


Figure 2.4 Major component of blood (Rizzo, 2001).

### 2.4.1.1 Blood Formed Elements

There are three kinds of blood elements which are red blood cells (RBC), white blood cells (WBC) and platelets. Red blood cells are also called erythrocytes, has shape of biconcave disc with a diameter about 7 microns. There are several routines clinical assessment for RBC namely erythrocyte count, packed cell volume or haematocrit, mean cell volume (MCV), haemoglobin and mean cell haemoglobin (MCH). The life span of erythrocyte is about 120 days, and their haemolysis is carried out by phagocytic reticuloendothelial cells (Scanlon, 2007; Waugh & Grant, 2001).

Erythrocytes contain protein called haemoglobin (Hb), to which both oxygen, ($O_2$) and Carbon Dioxide ($CO_2$) attach. Each erythrocyte contains about 280 million molecules of haemoglobin. Haemoglobin is a complex protein, consisting of globin and an iron-containing substance called haem. The primary function of erythrocytes is to combine with oxygen in the lungs and to transport it to the various tissues of the body. It then combines with carbon dioxide in tissues and transports it to the lungs for expulsion from the body (Pack, 2001; Scanlon, 2007; Waugh & Grant, 2001).

White Blood Cells also called leukocytes are classified into two groups, granular and agranular. The granular are neutrophils, eosinophils, and basophils and the agranular leukocytes are lymphocytes and monocytes. A normal WBC count (part of a complete blood count, CBC) is 5,000 to 10,000 per µL. Leukocytes play an important role in defending the body against microbes and other foreign materials. Neutrophils and monocytes are capable of the phagocytosis of pathogens. Eosinophils are believed to detoxify foreign proteins and will phagocytize anything labeled with antibodies. Basophils release heparin that helps to prevent abnormal clotting within blood cells and released as part of the inflammation process and make capillaries more permeable. Lymphocytes have two major kind, B cells and T cells which produced antibodies and destroy specific target cells (Scanlon, 2007).

Platelets or thrombocytes, are small cellular fragments that originate in the bone marrow from cytoplasm of megakaryocytes. Platelets are small, disc-shaped with size range from 2 to 4 micrometers in diameter which may last for 5 to 9 days, if not utilized before that. Platelets conglutinate to damaged blood vessel walls and release enzymes that activate hemostasis, to stop bleeding (Scanlon, 2007; Waugh & Grant, 2001).

#### 2.4.1.2 Plasma

Blood plasma is about 91.5 % water and 8.5 % solvents and most of them are proteins. Plasma protein includes albumins, globulins and fibrinogen. Important function of these proteins is to maintain proper blood osmotic pressure, which important in the exchange of fluids across capillary wall. Among the solvents are electrolytes, nutrients, gases, regulatory substance and waste product. Electrolyte and nutrients, involved in cell formation, contraction of muscles, transmission of nerve impulses, formation of secretions and maintenance of the balance between acids and alkalis (Tortora, 2009).

### 2.5 Anaemias and other red cell disorders

Anaemia is a reduction of haemoglobin within the red blood cells usually accompanied by a reduction in haematocrit. Figure 2.5 shows schematic assessment of haematocrit by centrifugation. Anaemia caused weakness and loss of breath, especially after physical exertion, dizziness or fainting, confusion, insomnia and increased pallor depending on severity of the red cell deficiency (Bain, 2004; Powers, 1989).

There are many different types of anaemia in red blood cells. Some of the types are sickle anaemia, iron-deficiency anaemia, haemolytic anaemia and thalassemia. Sickle cell anemia is a hereditary disease of haemoglobin, which causes erythrocytes to sickle, clog capillaries, and rupture with abnormal haemoglobin. Iron-deficiency anemia results from nutritional deficiencies or from excessive iron loss to form sufficient hemoglobin. A person with this type of anemia may have a normal RBC and haematocrit count and but low haemoglobin level.

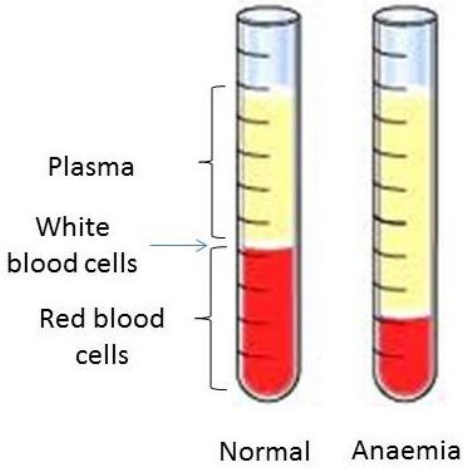


Figure 2.5 Schematic assessment of haematocrit by centrifugation (Bridges, 2008)**.**

Hemolytic anemia is a disorder that causes in erythrocytes rupture faster rate than normal life span. It can be caused by drugs, autoimmune diseases, or snake venom. Examples of haemolytic anaemia are sickle-cell anaemia, Rh disease of the new- born and malaria in which a protozoan parasite reproduces in RBCs and destroys them. Thalassemia is an inherited disorder of haemoglobin in the blood. There is reduced globin synthesis with resultant reduced haemoglobin production leading to early haemolysis. Severe cases may cause death in infants or young children (Rizzo, 2001; Scanlon, 2007; Waugh & Grant, 2001).

### 2.5.1 Assessing Red Blood Cell

Red blood cell can be assessed by its number, shape, size, colour and count. Individual cells of abnormal share are referred to as poikilocytes. The variations of red blood cell shapes are illustrated in Figure 2.6.

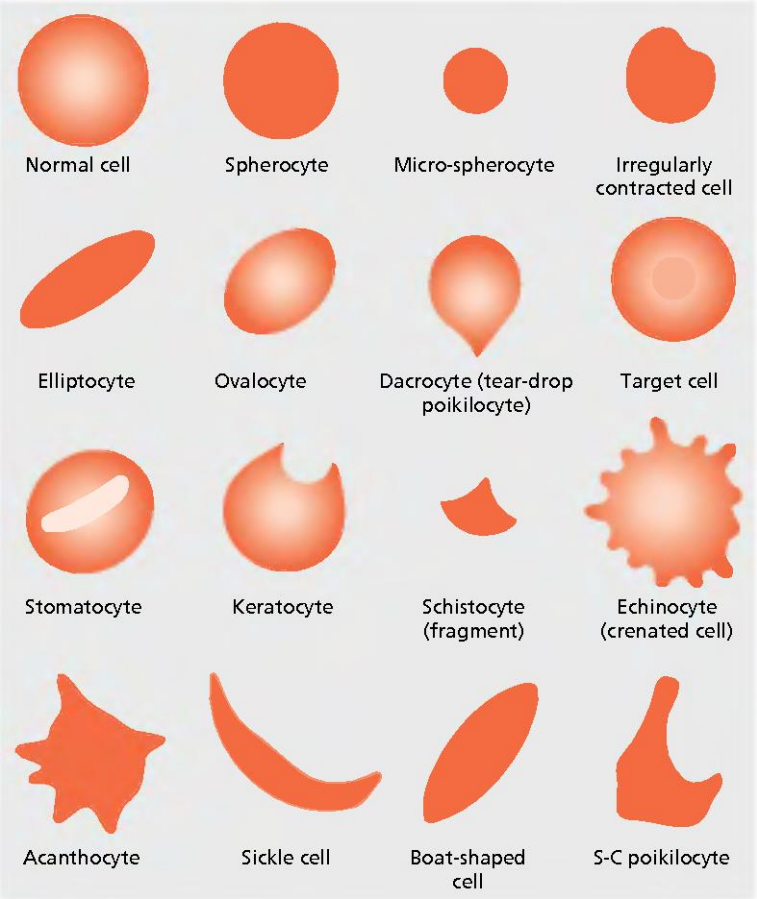


Figure 2.6 Diagrammatic representation of different type of variation in red cell shape (Bain, 2004).

### 2.5.2 Procedure of detecting anaemia

In detecting anaemia, automated blood analyzer, provide CBC or "complete blood count," which contains a wealth of information pertinent to the nature of the anemia as shown in Table 2.1. Valuable information provided, either the count of cell below or above normal average from this device help to guide subsequent evaluation of anaemia.

The peripheral blood smear is another step in detecting anaemia. It gives valuable insight into the nature and possible causes of anaemia through the morphology of the cell. The standard Wright-Giemsa stain highlights many

characteristics of circulating erythrocytes, some of which are obvious while others are diaphanous (Bridges, 2008).

Table 2.1 Automated blood analyser readout.

| Variable | Readout | Interpretation |
|---|---|---|
| Red Cell number | Millions/µL | The number of red cells per volume of blood |
| MCV | Femtoliters | The mean volume of a single red cell |
| Hb | g/dL | Quantity of haemoglobin in a volume of blood |
| MCH | Picograms | The mean haemoglobin content of a single red cell |
| MCHC | Percentage | Haemoglobin concentration within individual red cells |
| RDW | Percentage | Coefficient of size variation of red cells in a sample = (Standard of deviation of red cell volume mean cell volume) x 100. A larger value indicates greater size variation. |
| HCT | Percentage | Mathematical deviation of red cell fraction of the total blood volume. |
| WBC | Thousands/µL | Number of white cells per unit of blood |
| PLT | Hundreds of thousands per µL | Number of platelets per unit of blood |

## 2.6 Laser-Tissue Interaction

The absorption of light by an object is directly correlated to the incident radiation and the absorption coefficient (AC) of the object. Objects or materials with a high coefficient of absorption will absorb a large amount of light if the wavelength of the incident light corresponds to the absorption band of the material. Conversely,

light can propagate along distance without much attenuation in a material with a low AC. In this research, blue and green laser has been chosen as a radiation tool as according to absorption spectra (Figure 2.7), haemoglobin which is chromophore in blood cell is at a peak (400 nm – 600 nm) in this range of wavelengths. Therefore, incident radiation in this range will be highly absorbed by blood. Various biomolecules contained within a volume of tissue irradiated using laser treatment will differ from one another in their absorption spectra, thus various interactions can occur in this same tissue. (Baxter, 1994)

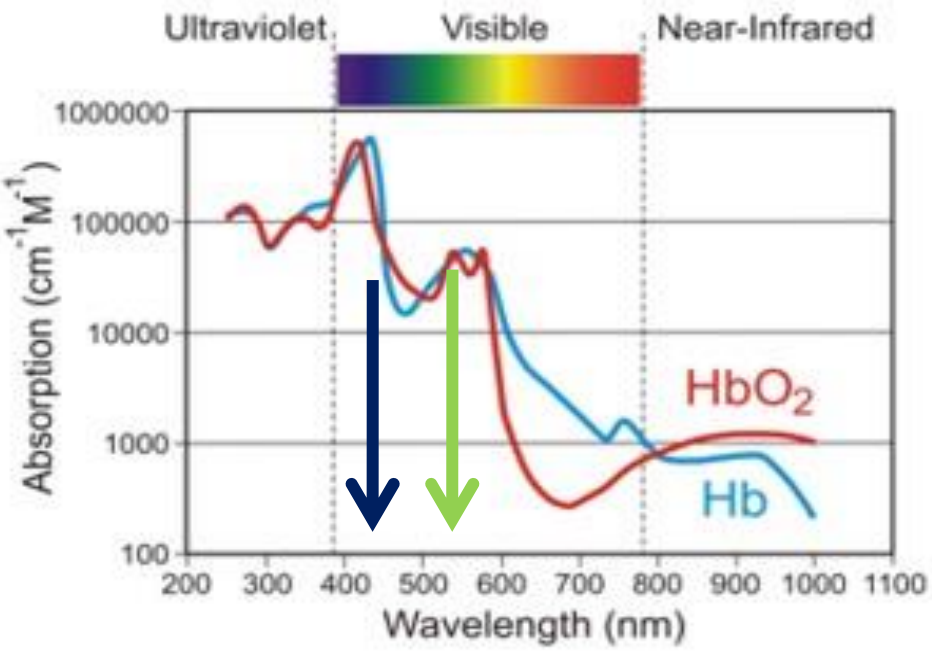


Figure 2.7 Absorption spectra of tissue chromophores

Applying therapeutic laser irradiation often related to the concept of minimum dose which can be verified by Arndt-Schultz law that small doses stimulate, medium doses suppress and large doses kill. Therefore, once therapeutic effect wavelength has been found, one need to determine optimum dose for the beneficial effect (Chung et al., 2012; Huang et al., 2009; Smith, 1991).

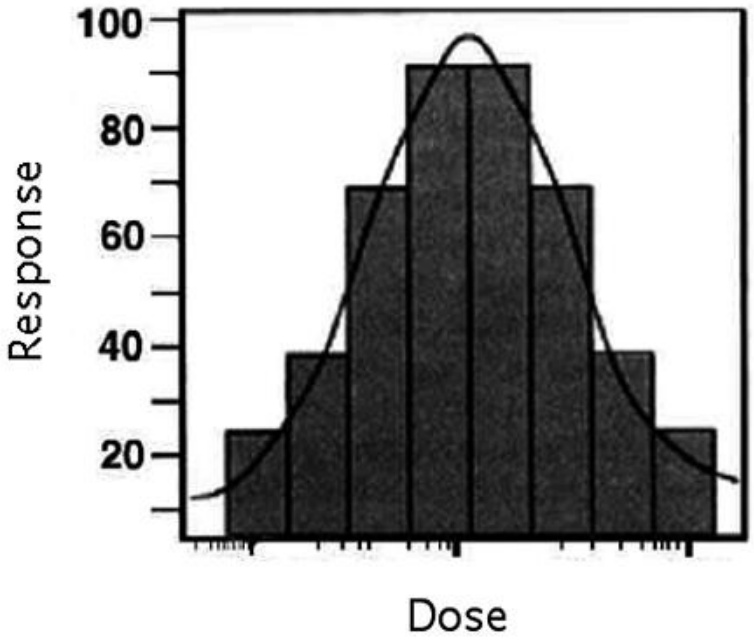


Figure 2.8 Idealized biphasic dose response curve (often termed Arndt-Schulz curve) typically reported in LLLT studies (Huang et al., 2009).

### 2.6.1 Laser effects on tissues

Laser Light can interact with tissue in four ways, by transmission, reflection, scattering, and absorption. Transmission refers to the passage of light transmitted through a tissue without having any effect on that tissue. Reflection refers to the repelling of light off the surface of the tissue without an entry into the tissue. Scattering of light occurs after light has entered the tissue due to heterogenous structure of tissue and different index of refraction between parts of tissue. Absorption is due to conversion of light energy into heat motion or certain vibrations of molecules of the tissue. The absorption of the photons of laser light is responsible for the effects on the tissue. Figure 2.9 shows geometry reflection, refraction, absorption and scattering (Carroll & Humphreys, 2006).

According to the Grotthus-Draper law, only radiation absorbed may cause a chemical change. The energy supplied by the absorbed photon excites the electronic

structure of a molecule, its relaxation subsequently proceeding by emission of heat or light (fluorescence or phosphorescence), by breaking of chemical bonds (photolysis), by undergoing a chemical change within the molecule or by transfer of energy to another atom or molecule (Carroll & Humphreys, 2006; Kolar et al., 2000).

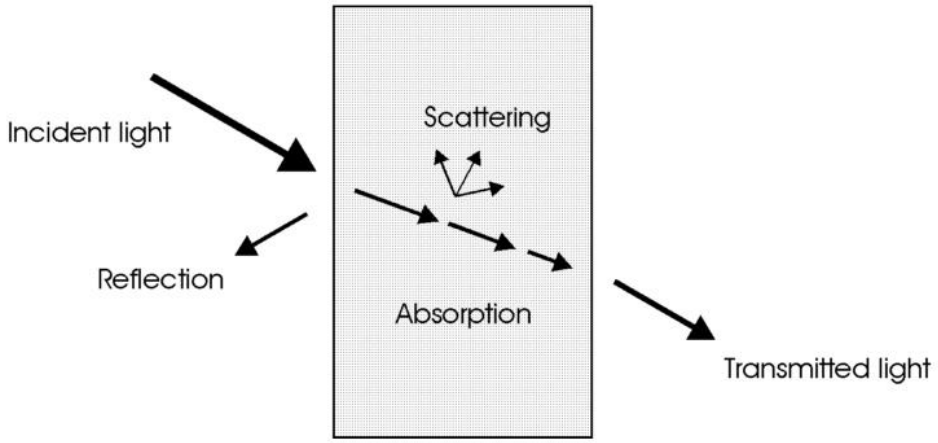


Figure 2.9 Geometry reflection, refraction, absorption and scattering (Niemz, 2007).

### 2.6.2 Types of interaction

A variety of interaction mechanisms may occur when applying laser light on biological tissue. There are mainly three main categories of interaction which are photochemical interactions, thermal interactions and photoablation. Figure 2.10 shows map of laser-tissue interaction.

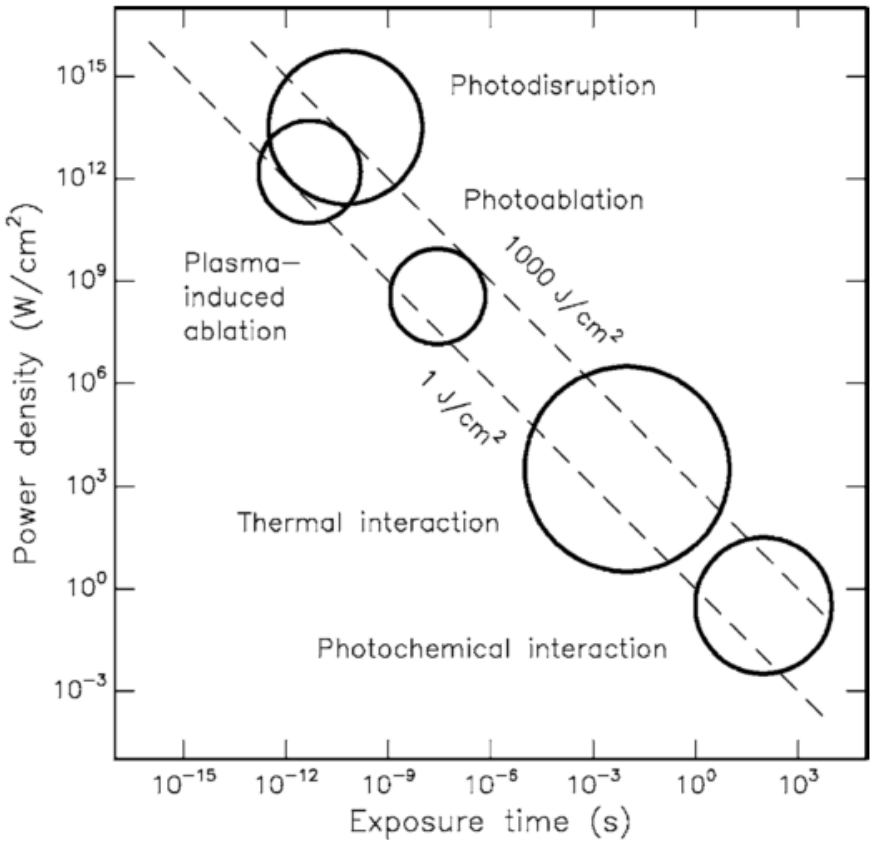


Figure 2.10 Map of laser-tissue interactions. The circles give only a rough estimate of the associated parameters (Niemz, 2007).

Photochemical interaction is which light initiates a chemical reaction in the tissue. It takes place at very low power densities (typically 1W/cm$^2$) and long exposure times ranging from seconds to continuous wave. Photochemical interaction mechanisms play a significant role during photodynamic therapy (PDT). PDT is defined as the administration of a non-toxic drug or dye known as a photosensitizer and the most important aspect in PDT is the process of light absorption and energy transfer (Niemz, 2007; Robertson et al., 2009).

In photothermal interaction, thermal effects can be induced by either CW or pulsed laser radiation. In photothermal interaction, light absorbed from the laser will be converted into heat energy. However, different effects may be achieved depends on range of thermal value of tissue interaction like coagulation,

vaporization, carbonization, and melting. Table 2.2 describes different thermal effects of laser irradiation (Niemz, 2007).

Table 2.2 Thermal effects of laser irradiation.

| Temperature | Thermal effect |
|---|---|
| 37◦C | Normal |
| 45◦C | Hyperthermia |
| 50◦C | Reduction in enzyme activity, cell immobility |
| 60◦C | Denaturation of proteins and collagen, coagulation |
| 80◦C | Permeabilization of membranes |
| 100◦C | Vaporization, thermal decomposition (ablation) |
| > 100◦C | Carbonization |
| > 300◦C | Melting |

Heat generation is determined by laser parameters and optical tissue properties such as irradiance, exposure time, and the absorption coefficient. Heat transport is solely characterized by thermal tissue properties such as heat conductivity and heat capacity. Heat effects, finally, depend on the type of tissue and the temperature achieved inside the tissue. They are summarized in a flow chart as shown in Figure 2.11 (Niemz, 2007).

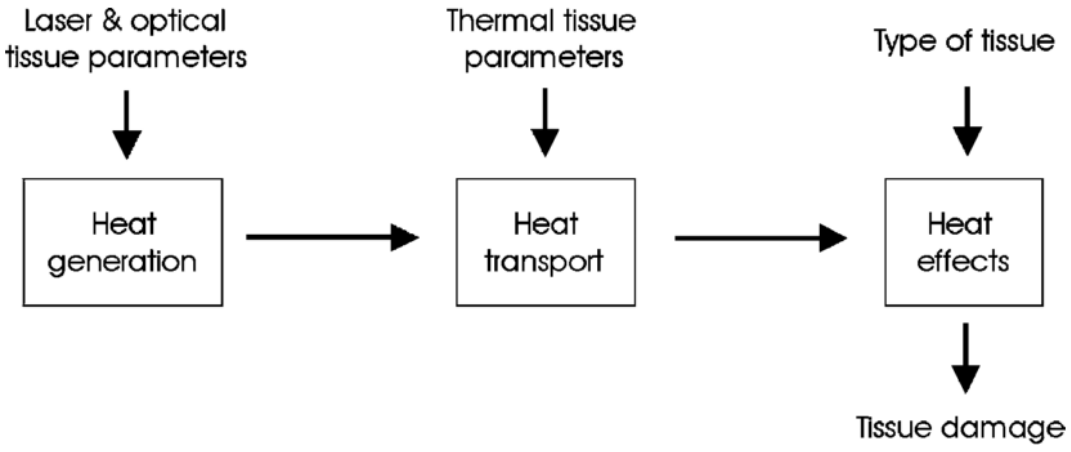


Figure 2.11 Flow chart with important parameters for modelling thermal interaction (Niemz, 2007).