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The Effect of Green Laser on Human White Blood Cells

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Annotation: This graduation project investigates the effect of green laser light with a wavelength of 532 nanometers on the shape and size of human white blood cells. The study utilized samples of blood from adult male and female humans as models. Ten samples were prepared, consisting of 5 samples from males and 5 from females. Each sample was exposed to the laser for periods ranging from 0 to 20 minutes, with 5-minute intervals, resulting in varying doses received by the samples. The results showed that the external shape and size of white blood cells depend on the laser dose used, suggesting biological and chemical changes occurring in response to the applied laser doses.

Introduction

The laser first appeared in 1960, introduced by the American scientist Theodore Maiman, who successfully generated a laser using a ruby crystal. It didn’t take long before various materials were utilized to generate lasers, paving the way for extensive applications across many fields. Laser technology soon became the ideal solution for numerous technical and scientific challenges, leading to the famous statement that a laser is “a solution looking for a problem.” Today, it is difficult to find a modern, significant device that does not incorporate laser technology [1]. The term LASER is an acronym for "Light Amplification by Stimulated Emission of Radiation." Laser light is generated through specialized devices that produce coherent electromagnetic radiation, characterized by uniform wavelength, phase, and frequency [2]. The laser has four primary properties: high brightness, excellent directionality, good monochromaticity, and high coherence. These interrelated properties allow lasers to be adapted for various applications. In medical applications, the choice of laser depends on the nature of the tissue being treated, often involving animal experiments before human trials. For instance, it has been demonstrated that carbon dioxide lasers are the most effective for cutting tissues [1]. Concerning the effects of laser on human blood, numerous experimental studies have shown that lasers with wavelengths in the visible spectrum can significantly impact various blood components. These effects may alter their functions or cause damage, depending on the laser dose applied, without destroying or severely disrupting the irradiated cells [3, 4]. The Importance of Laser Applications in Medicine The advantages of using lasers in medical procedures can be summarized as follows [1]:

- ✓ Non-contact between instruments and the surgical site.
- ✓ Minimal bleeding during laser-based surgeries.
- ✓ Reduced pain during and after surgery.
- ✓ No need for extensive sterilization.
- ✓ Lower risk of bacterial infections.
- ✓ Minimal damage to surrounding tissues.
- ✓ Enhanced visibility during surgical procedures.
- ✓ Precise, localized cuts with high accuracy.
- ✓ The ability to target specific tissues using certain wavelengths.
- ✓ Non-invasive operations using optical fibers, such as treating bladder or lung tumors.
- ✓ Good wound healing.
- ✓ Shorter recovery periods, allowing patients to leave the hospital sooner.
- ✓ Precision control using computerized systems.
- ✓ Reduced need for local anesthesia.
- ✓ Lower patient anxiety due to minimal or no bleeding.
- ✓ Blood and Its Components

Blood is a vital connective tissue essential for many living organisms, including humans and animals, due to its role in transporting nutrients, oxygen, waste products, carbon dioxide, hormones, and other substances to tissues and cells throughout the body [5]. An average adult human has about 4 to 5 liters of blood, composed of plasma and cells circulating through the body. Blood consists of the following components [6]:

1. Plasma: A pale yellow fluid that suspends non-liquid components. It primarily consists of water, along with organic and inorganic substances, including salts and proteins. Plasma constitutes approximately 55% of blood volume.

2. Blood Cells:These make up about 45% of blood volume and include:

- ✓ Red blood cells (Erythrocytes):Derived from hemoglobin.
- ✓ White blood cells (Leukocytes).
- ✓ Platelets (Thrombocytes).

White blood cells are a type of blood cell produced in the bone marrow. Found in blood and lymphatic tissues, they are part of the immune system, helping the body combat infections and diseases. There are five types of white blood cells [7, 8]: - Neutrophils:Protect against infections by destroying bacteria, fungi, and foreign debris.

- ➢ Lymphocytes: Include T cells, B cells, and natural killer cells, which fight viral infections and produce antibodies. Eosinophils: Identify and destroy parasites and cancer cells and assist in allergic responses. Basophils: Trigger chemical signals, like histamine, to alert the immune system during infections and allergic reactions. Monocytes: Defend against infections by clearing damaged cells.

Figure 1. illustrates these five types of white blood cells [9].

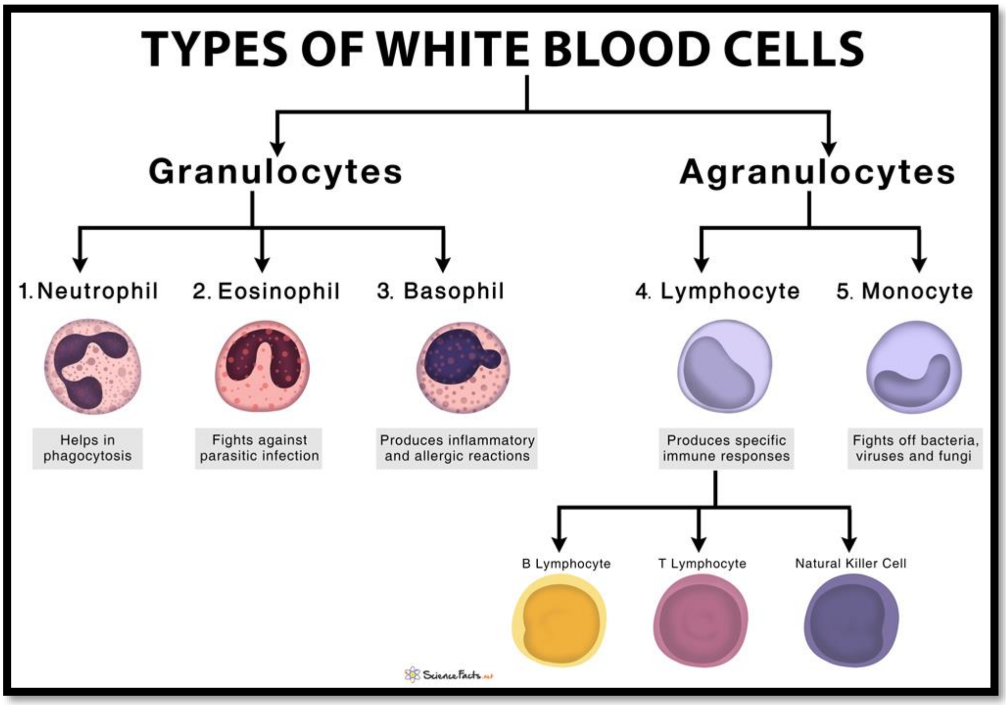


Figure 1: Types of White Blood Cells [9].

Objective

To conduct an experimental study on the effect of green laser light with a wavelength of 532 nm, emitted by semiconductor lasers, on the shape of white blood cells in adult humans (both male and female) under varying doses.

Low-Level Laser Therapy (LLLT)

The concept of low-level laser or low-power therapy, also known as low-level laser therapy (LLLT), cold laser therapy, or photobiomodulation, involves the use of low-power lasers typically under 500 milliwatts. It is a non-invasive, non-thermal, and painless technique [10-12]. This therapy involves applying low-power light from a laser or LED within the visible to near-infrared spectrum (400 nm to 1000 nm). This range is commonly used due to its ability to penetrate the skin and soft or hard tissues. Clinical trials have demonstrated its effectiveness in reducing pain, inflammation, and promoting tissue repair [10].

Mechanism of Low-Level Laser

Therapy LLLT, also referred to as low-light therapy or photobiomodulation (PBM), is a low-intensity phototherapy technique. Its effects are photochemical rather than thermal. The light induces biochemical changes within cells, akin to photosynthesis in plants, where cellular photoreceptors absorb photons, triggering chemical changes [10]. The use of low-level laser therapy has become increasingly prevalent across various medical fields due to experimental and clinical research providing conclusive evidence of its therapeutic efficacy without complications or adverse effects [13]. Clinical applications of cold laser therapy include alleviating acute and chronic pain, reducing inflammation, promoting tissue healing, and encouraging cellular regeneration. Results often show rapid pain relief and faster recovery periods, surpassing traditional methods [14-15].

Effects of Low-Level Laser on Blood

Numerous experimental studies suggest that all components of human blood are influenced by green laser light. These effects include changes in the number of red or white blood cells, as well as alterations in plasma, viscosity, and the size and shape of both red and white blood cells [16-19]. additionally, low-level lasers have an immunomodulatory effect by activating macrophages, a type of white blood cell found in infected tissues. These specialized white blood cells assist in clearing cellular debris and eliminating toxic waste from cells. Simply put, laser therapy enables patients to alleviate pain more quickly while simultaneously promoting healing [14].

Calculations and Results

This chapter discusses the procedural steps, related mathematical calculations, and practical results obtained in this study. Two blood samples were collected—one from an adult male and the other from an adult female. These samples were processed according to the following steps.

Experimental Procedures

The experimental steps were carried out as follows:

1. The blood was placed in two EDTA (Ethylenediaminetetraacetic acid) tubes to prevent coagulation.
2. The blood samples were used within 24 hours.
3. Each sample was divided into four parts, each containing approximately 0.3 ml, in addition to a standard control sample for comparison, which was not irradiated. This resulted in a total of ten blood samples.
4. The blood samples were irradiated with laser light for varying durations: 5 minutes, 10 minutes, 15 minutes, and 20 minutes.
5. A continuous-wave semiconductor laser was used with a wavelength of 532 nm and an output power of 200 mW.
6. After laser irradiation, a smear was prepared for each sample, and Leishman stain was applied to highlight the cells, focusing on the white blood cells targeted in this study.
7. Finally, a microscopic image of each smear was captured using a light microscope at a magnification of 400x.

In total, ten blood samples were analyzed: eight irradiated samples and two standard control samples (with zero irradiation time and zero dose). The experimental setup, including the configuration for laser irradiation of the samples, is shown in Figure 2.

Figure 2: The experimental setup used in the study for irradiating the samples with green semiconductor laser.

Mathematical Calculations

The type of laser used in this study is a semiconductor laser with a wavelength of **532 nm**, emitting in the green range of the electromagnetic spectrum.

- **Laser Power (P)**: P=200 mW
- **Area of the sample irradiated by the laser (A)**:

$A=\pi r2=227\times(0.4)^2=1.25$ cm2

where **r** is the radius of the laser beam on the sample's surface, which was calculated as:

r=0.4 cm

- **Relationship between power and energy (E)**:

P=E/t

where **t** is the irradiation time for each sample.

- **Dose (D)** received by the sample from the laser is given by:

D=E/A

Based on these calculations, the doses for each sample can be computed and presented in **Table 1**.

Table 1: Irradiation Times and Corresponding Doses for Blood Samples

Sample ID	Irradiation Time (s)	Dose (J/cm^2)
M1 (Control) / F1 (Control)	0	0
M2 / F2	. 300	48
M3 / F3	600	96
M4 / F4	900	144
M5 / F5	1200	192

Practical Results

White Blood Cell Morphology Observations

Male Samples (M):

- **Control Sample (M1):**

The blood smear for the male control sample (irradiation time = 0, dose = 0 J/cm^2) was stained with **Leishman stain**. The white blood cells in this sample exhibit a **regular, spherical shape** with no visible alterations, as shown in **Figure 3**.

- **Irradiated Samples (M2–M5):**

✓ After irradiation with the green laser at different times (5, 10, 15, and 20 minutes), changes were observed in the morphology and size of the white blood cells.

✓ In **Figure 4**, the irradiated samples reveal that white blood cells began to lose their regular spherical shape and displayed an **increased size**, suggesting **biological and chemical changes** due to the low-level laser effects.

✓ The most significant change was observed in sample **M5**, irradiated for **20 minutes** (dose = 192 J/cm^2), where the white blood cells appeared larger and irregular, indicating the potential onset of damage due to high laser doses.

Female Samples (F):

- **Control Sample (F1):**

The female control sample smear (irradiation time = 0, dose = 0 J/cm^2) showed white blood cells with a **spherical, regular shape**, as depicted in **Figure 5**.

- **Irradiated Samples (F2–F5):**

✓ Similar to the male samples, the white blood cells in irradiated female samples lost their regular shape and showed an **increase in size** as the laser dose increased.

- ✓ In **Figure 6**, sample **F5**, irradiated for **20 minutes** (dose = 192 J/cm²), displayed the most pronounced changes, where cells began to lose their structural integrity, potentially transitioning from the effects of low-level laser therapy to **thermal effects** due to the higher dose.

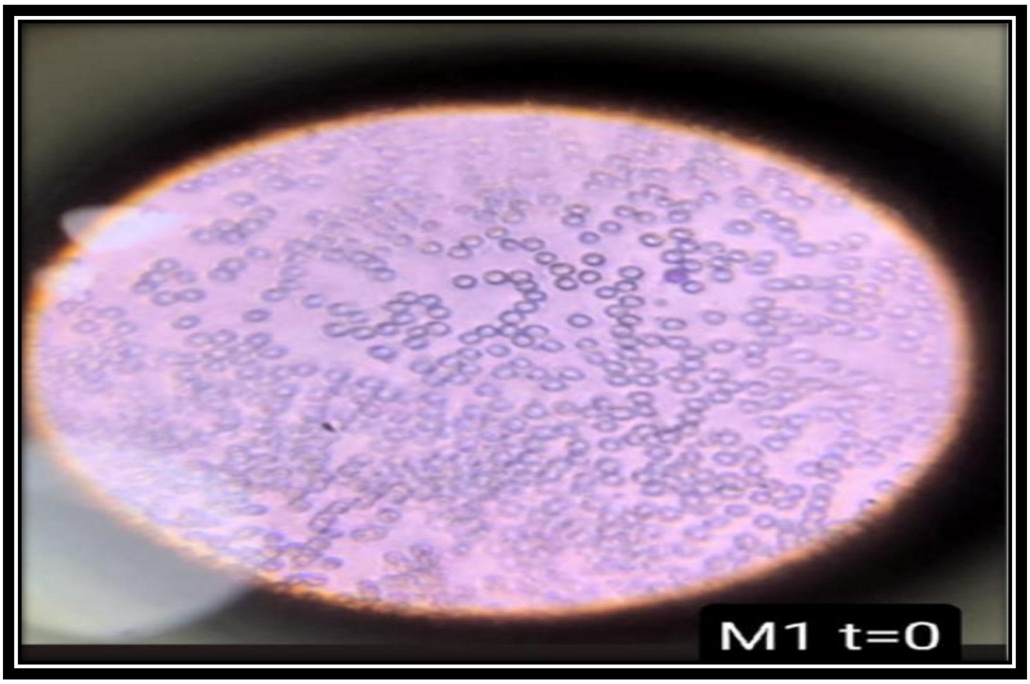
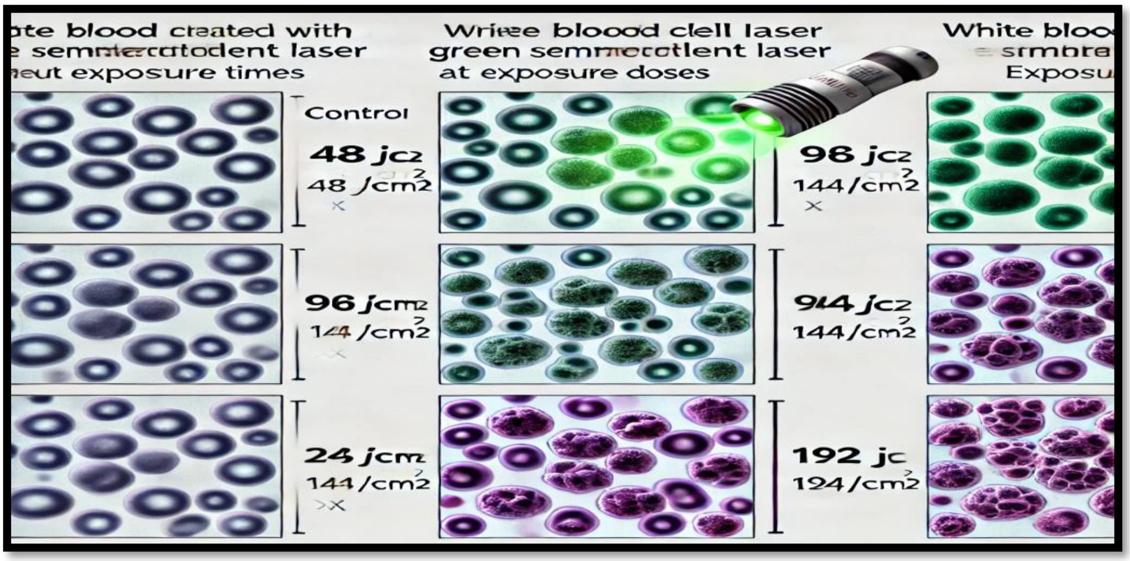


Figure [3]: A blood smear for the male control sample, i.e., without laser exposure.



Here is the illustration showing the experimental results of blood smears treated with a green semiconductor laser, highlighting the changes in white blood cell shapes and sizes at various irradiation doses.

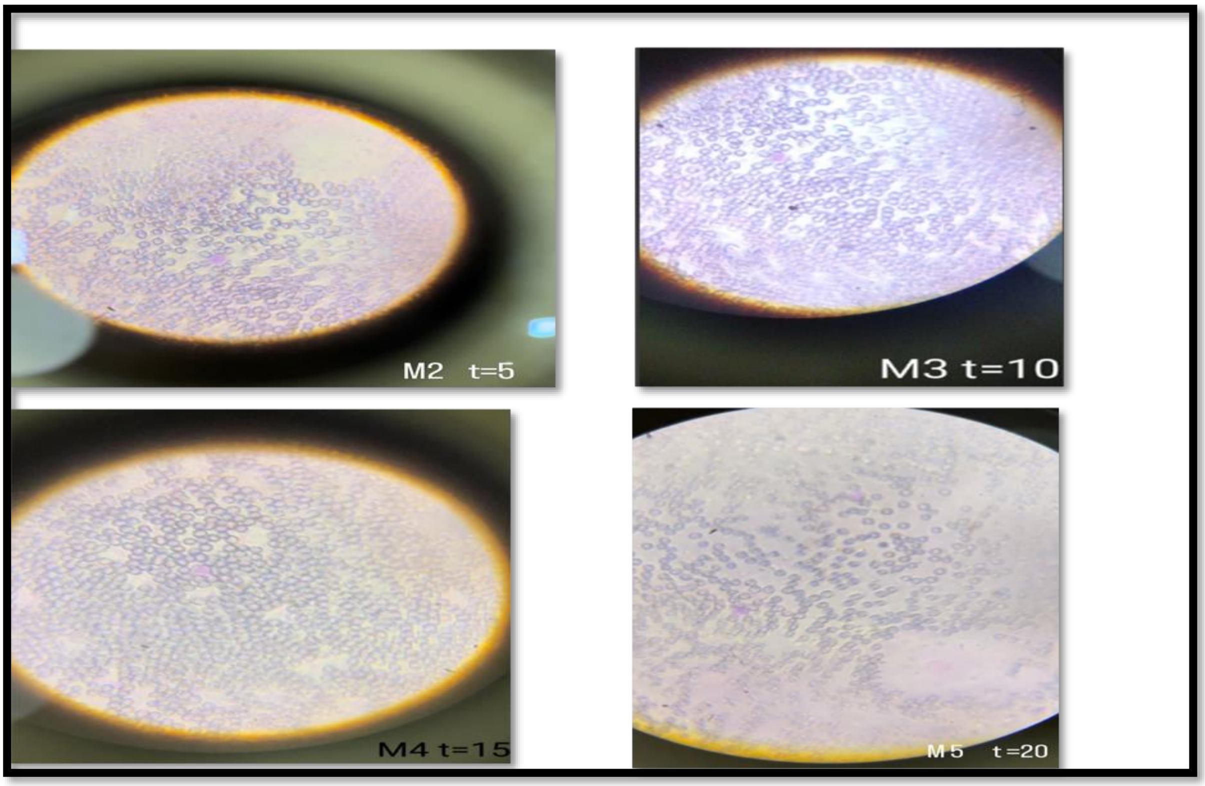


Figure [4]: Images of blood smears for the male subject at different green laser irradiation times: 5, 10, 15, and 20 minutes.

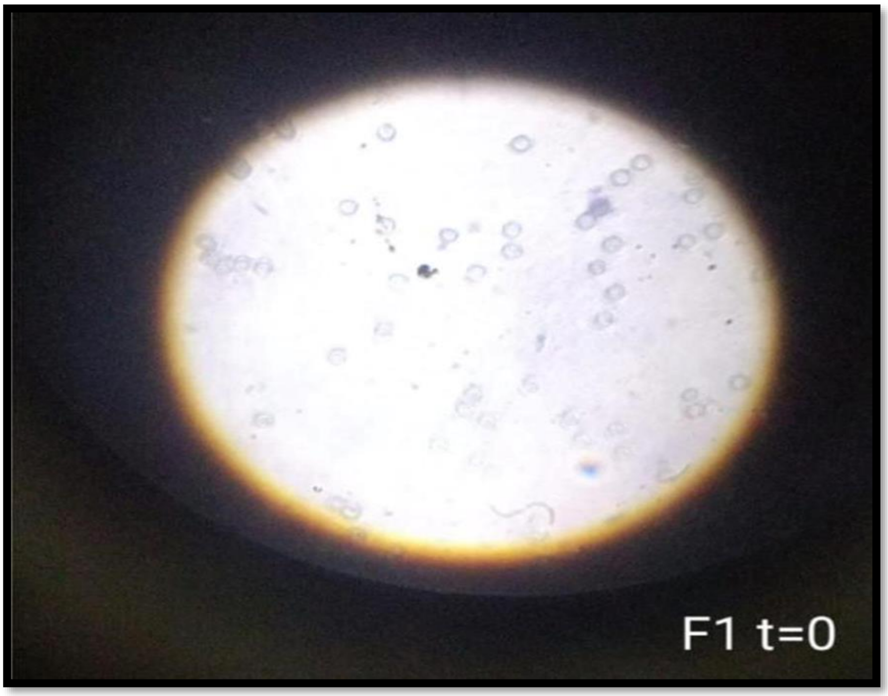


Figure 5 A blood smear for the female control sample, i.e., without laser exposure.

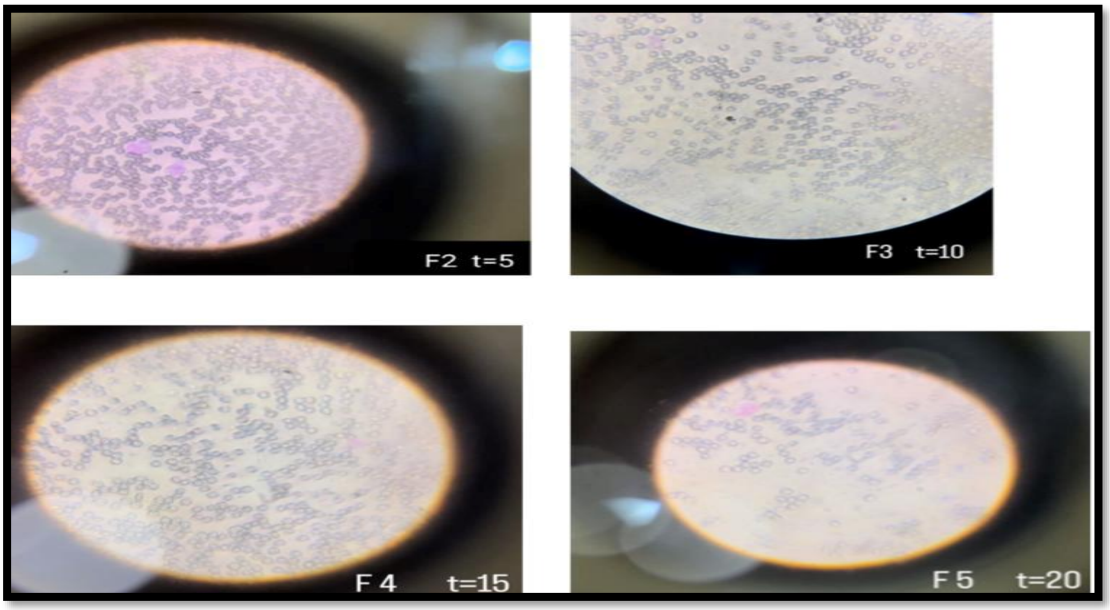


Figure [6]:Images of blood smears for the female subject at different green laser irradiation times: 5, 10, 15, and 20 minutes.

Discussion and Recommendations

Discussion

Visible lasers have a significant impact on human white blood cells. Based on the results in Chapter 3, it can be discussed that the semiconductor laser with a wavelength of 532 nm and low energy, which falls within the green light range of the electromagnetic spectrum, has a profound effect on human white blood cells. This wavelength can either alter their shape or leave it unchanged, depending on the dose used. In general, external deformations in white blood cells were observed with increasing laser irradiation doses, which is supported by the clear deformations in surrounding red blood cells in all samples. It was also noted that the size of white blood cells increased with laser irradiation, indicating the occurrence of chemical and biological changes inside the cell. Since white blood cells contain a nucleus, appropriate laser doses (not necessarily high) could lead to their division, whereas higher doses could destroy them. However, these effects can only be confirmed through more comprehensive testing. The visual and superficial observations of the samples, which show changes in the shape and size of white blood cells, indicate such alterations.
It is expected that low-power laser therapy (LLLT) has an effect on the blood of individuals, whether male or female, healthy or diseased. This effect can be confirmed on any component or factor of the blood through conducting a Complete Blood Count (CBC) test.

Recommendations

For the continuity and development of research in this field, we recommend the following:

1. Study the effect of other lasers with different visible wavelengths on human white blood cells.
2. Study the effect of various visible lasers on white blood cells of individuals with leukemia.
3. Conduct a study on the effect of green laser on white blood cells of females in different age groups, both pre- and post-puberty, as well as before and after pregnancy.
4. Research the effect of green laser on white blood cells of adult smokers and compare them with the reference values of non-smokers.
5. Conduct practical studies on the effect of green laser on healthy adult individuals and analyze the results through Complete Blood Count (CBC) tests.

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