



Effect of Continuous Laser on The Number Lymphocyte Cells and its DNA

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Abstract:

The aim of this research is to evaluate the effects of a helium-neon laser on lymphocyte cells and DNA. Many different medical applications make extensive use of laser light. Like other lasers, the He-Ne laser's applicability in medicine is dependent on how laser light interacts with the biological system. The objective is to demonstrate the impact of 632.8 nm helium-neon (He-Ne) laser irradiation on human lymphocyte blood cells and their DNA. Techniques Eighty blood samples from individuals who seemed to be in good health were used in this investigation. The samples were split into two groups: thirty samples from the first group were processed solely for the purpose of separating lymphocyte blood cells, and fifty samples from the second group were used to assess the impact of He-Ne laser irradiation on the DNA extracted from the lymphocyte blood cells. Outcomes There was a significant difference ($P < 0.05$) in the survival percentage of lymphocyte cells (99.0%, 98.84%, 99.78%, and 98.78%) at the employed doses of He-Ne laser (19, 36, 53.5, and 70 J/m²) compared to those cells that were not exposed to He-Ne laser irradiation. The following doses (19, 36, and 70 J/m²) were applied to the extracted DNA immediately following the He-Ne laser irradiation alone. The DNA showed significant damage, with the fraction of DNA survival percentages being (85.5%, 88.5%, and 87.1%), respectively, and a significant difference ($P < 0.05$) between the DNA survival before and after the He-Ne laser irradiation. In summary with longer exposure times (3, 5, 7, and 9 s) and higher He-Ne laser doses, the fraction of lymphocytes that survive is declining. Independent of the irradiation dosages, He-Ne laser irradiation damages DNA to a considerable extent.

Keywords: Lymphocyte cells, He-Ne laser irradiation, DNA

Introduction:

Among the earliest civilizations to understand the therapeutic advantages of light were the Greeks and the Egyptians, who utilised laser beams in their medical practices for thousands of years. The ancient Greek god Apollo had ties to both the

physical and spiritual worlds, hence the idea that light could cure illness ran deep in mythology. Up until the invention of the laser some fifty years ago, light could only be used to a limited extent in medical applications. Laser technology outperforms natural light sources like sunshine in terms of accuracy and efficiency due to its unique

properties. One of the key advantages of lasers is that they create coherent light, meaning that the waves are in phase, and that this coherent light is produced within a very narrow and exact range of wavelengths. Additionally, lasers may generate intense light beams that, when focused precisely on a little area, can achieve a high power density. As a result of these characteristics, lasers have found widespread usage in modern medicine for diagnostic and therapeutic purposes. An exception to the rule among medical lasers is the Helium-Neon (HeNe) laser. The red part of the visible spectrum, around 632.8 nm, is where HeNe lasers, which are small gas lasers, mostly work. Research and industrial environments find numerous applications for these lasers. The consistent output levels of HeNe lasers, which can range from 0.5 to 35 mW, and their ability to generate basic Gaussian beams have made them famous. Depending on the type of laser, the output could be either linearly or randomly polarised. A HeNe laser's central component is a low-pressure sealed glass tube that holds a gain medium composed of helium and neon gases, typically in ratios ranging from 5:1 to 20:1. These lasers are excited by a high-voltage electrical discharge that happens between the glass tube's anode and cathode. The optical cavity of the HeNe laser has a flat, highly reflective mirror on one end and a concave output coupler mirror on the other; these two mirrors allow light transmission of approximately 1% when combined. These tiny lasers are famous for their cavity lengths that usually range from fifteen to fifty metres. By providing a precise, controllable, and effective light source for a wide variety of diagnostic and therapeutic purposes, the invention of the laser—and the HeNe laser in particular—has transformed healthcare. Medical treatments are now more targeted and efficient, and healthcare as a whole is better, all because of these innovations. Our understanding of the relationship between light and living things has also expanded.

HeNe Polarization:

The fact that helium-neon (HeNe) lasers are capable of producing linearly polarised light is well recognised. The polarisation of a HeNe laser

beam indicates the direction of the electric field in the light waves it generates. A linearly polarised HeNe laser operates with an electric field that oscillates continuously along a single plane.

Applications requiring exact control over the direction of the electric field of light are well-suited to HeNe lasers due to their linear polarisation. Spectroscopy, interferometry, telecommunications, and countless other optical and scientific fields rely on this property because of the critical role that polarised light plays in these fields' interactions with various materials and components.

To ensure their instruments and experiments are functioning correctly, engineers and scientists often use HeNe lasers with linear polarisation. In polarization-sensitive research and applications, HeNe lasers are advantageous due to their regulated polarisation state, which also reduces interference, simplifies experimental setups, and allows for accurate measurements and observations.

Randomly polarized beam:

The characteristic output of a Helium-Neon (HeNe) laser is a randomly oscillating linearly polarised beam. In essence, the laser can only emit light in one specific direction of oscillation, and this direction varies extremely quickly—usually in nanoseconds. Since no optical components depend on the polarisation of the light, randomly polarised HeNe lasers are perfect for applications where the polarisation state of the light beam is not crucial.

Remember that the polarisation orientation of these lasers can change quickly, which could lead to issues. These lasers might display discernible power variations depending on the use case and operating duration. Some situations may benefit from these variations, while others may encounter problems as a result. Each laser application must carefully examine the power output fluctuations caused by the ever-changing polarisation orientation.

A unique and adaptable light source, randomly polarised HeNe lasers are useful when controlling or even using power changes is not necessary and

the light's polarisation state is not critical either. There is a vast array of possible scientific and industrial applications for them, so long as the impact of the polarization's dynamic properties on power fluctuations is considered during experiment design and system implementation.

Polarized beam:

For polarization-sensitive applications, a polarised Helium-Neon (HeNe) laser beam's linear polarisation state offers an advantage. This indicates that there is a finite pattern to the direction changes made by the laser beams. A uniform and accurate electric field is essential in many optical and scientific applications, and linear polarisation is a common tool for this task.

For applications that are sensitive to polarisation, it is crucial to maintain a steady and known polarisation state. This is due to the fact that the orientation of the electric field produced by light is crucial to the functioning of specific materials, experiments, or optical apparatus. A linearly polarised HeNe laser beam provides a stable and controlled light source for these kinds of applications, since it ensures that the polarisation state remains constant across time.

The ability to precisely adjust polarisation is highly advantageous in fields such as optics, interferometry, spectroscopy, and telecommunications because it allows for more precise measurements, less interference, and the best operation of optical components. Researchers and engineers involved in polarization-sensitive projects find that the linear polarisation of HeNe lasers makes setup easier and increases the reliability of their systems or testing.

HeNe Linewidth:

In air, a red HeNe laser has a frequency of 632.816 nm, while this is much of the time expressed as 632 nm or 633 nm. A HeNe laser's frequency gain bend is truly made out of numerous longitudinal modes that differ inside the reach because of the cavity's warm development and other outer impacts.

A HeNe laser's linewidth differs relying upon the application. The quantity of modes, the free

ghastly reach (FSR), and the Doppler width characterize the hub mode construction of the HeNe laser (see picture beneath). Individual pivotal modes commonly have a tight linewidth (~kHz), which is generally impacted by outside factors and estimation time periods as opposed to fundamental laser qualities. For most interferometric applications, the rationality length — which is characterized by the hub modes that are farthest separated — is the main amount. The lucidness length of a red HeNe laser is around 30 cm.

The crash of electrons from the electrical release with the helium particles in the gas starts the laser cycle in a HeNe laser. Therefore, helium is invigorated from its ground state into a steady, enduring energized state. In the wake of crashing into the ground-state neon atoms, these energized helium molecules make invigorated neon particles. Neon molecules arrive at energized states in expanding numbers until populace reversal is accomplished. Alongside other emanation frequencies, unconstrained and incited outflow between the states delivers light with a frequency of 632.82 nm (see picture at right). The electrons quickly return to the ground state from these states. Since the neon lower-level changes directly with current, while the upper level immerses with expanded current, the HeNe laser's power yield is confined.

It is feasible to make the laser hole with the right mirrors and length to empower the emanation of laser light at various frequencies. Notwithstanding a range of noticeable changes, like a green (543.365 nm), yellow (593.932 nm), yellow-orange (604.613 nm), and orange (611.802 nm) progress, there are infrared changes at 3.39 μm and 1.15 μm frequencies (see picture underneath). When contrasted with different frequencies, such as the 1.15 μm and 3.39 μm lines, the typical red 632.8 nm frequency result of a HeNe laser has a significantly lower gain.

Lymphocytes:

One subset of white platelets are lymphocytes. They are critical to the working of your safe framework, which supports the body's guard

against disease and contamination. The intricate organization of invulnerable cells, lymph hubs, lymph tissue, and lymphatic organs that makes up your insusceptible framework. Among the immunological cell types are lymphocytes. Lymphocytes come in two essential assortments:

- **T lymphocytes, or T cells:** T cells directly target and destroy tumour and infected cells while also regulating your body's immune response.

- **B cells, or B lymphocytes:** B cells produce antibodies. Proteins called antibodies battle against microorganisms, infections, and other unfamiliar trespassers.

Function:

The body's safe framework utilizes lymphocytes to battle malignant growth and unfamiliar microorganisms and infections, or "antigens." Your resistant framework needs lymphocytes to assist it with recalling each antigen it experiences. Certain lymphocytes form into memory cells after a collaboration. Memory cells can recognize and respond quickly when they come into contact with an antigen once more. You don't get sicknesses like the chickenpox or measles at least a few times along these lines. It's additionally the explanation immunizations can fight against specific sicknesses.

The Lymphocytes control your body's immunological reaction to unfamiliar substances and help in the annihilation of contaminated cells. To get actuated, most of your Lymphocytes need help from another insusceptible cell. Your Immune system microorganisms multiply and separate into a few White blood cell types when they are enacted. Among these sorts are:

- **Cytotoxic T cells**, frequently known as executioner Immune system microorganisms, stick to antigens on infected or atypical cells. From that point onward, they cut the films of the contaminated cells and acquaint catalysts with annihilate the cells.

- **Helper T cells:** These cells support other safe cells in your body. Certain partner Immune system microorganisms help B cells in creating antibodies to battle unfamiliar trespassers. Others aid

cytotoxic Immune system microorganism actuation.

- **Regulatory (suppressor) T cells:** These cells produce chemicals that aid in stopping the immune system's reaction to an assault. They may sometimes stop negative reactions from happening.

Antigens grip to receptors on the outer layer of B cells. B cells foster the capacity to recognize a few antigens and produce fitted antibodies to battle everyone. There are two different ways that B lymphocytes respond to antigens:

- **Primary immunological response:** Your B cells are initiated when an antigen ties to a receptor. A piece of B cells forms into memory cells. Extra B cells form into plasma cells. An immunizer well defined for the specific antigen that set off it is delivered by plasma cells. A few days might pass before enough of that specific immunizer is created.

- **Secondary immunological response:** Your B cells will remember and proliferate if they come into contact with that antigen again. They transform into plasma cells and start making the appropriate antibodies right away.

Conditions and Disorders:

Different factors such as age, race, sex, altitude, and lifestyle affect lymphocyte counts.

Adults typically have 1,000–4,800 lymphocytes per microliter of blood, depending on their age. Within the usual range, there should be between 3,000 and 9,500 lymphocytes per microliter of blood in youngsters. Lymphocytes make about 20% to 40% of your white blood cells.

Lymphocytosis refers to an excess of lymphocytes in the blood. The most common cause of lymphocytosis is an infection or disease. Your body sometimes creates more lymphocytes to aid in the defence against diseases and infections. However, a higher risk illness such as the following may also result in a high lymphocyte count.

Lymphocytopenia, also known as lymphopenia, is the low concentration of lymphocytes in the

blood. Lymphoma may be brought on by the flu or other minor diseases, but it can also be brought on by a more severe illness.

This work includes a thorough analysis of the effects of Helium-Neon (HeNe) laser irradiation on lymphocyte cells and their DNA genetic material.

Neon-Helium Laser: is a gas laser type that generates coherent light; it is also known as the Neon-Helium laser. This proves that the synchronisation and phase-matching of the light waves cause the production of a single, undulating colour. The red part of the visible light spectrum, with a wavelength of 632.8 nanometers (nm), is what HeNe lasers typically emit. The unique characteristics and pinpoint accuracy of this wavelength make it valuable in many fields of science and medicine.

- **Cells of Lymphocytes:** Lymphocytes are white blood cells that play a crucial role in the immune system. Their primary function is to detect and eliminate pathogens, viruses, and other foreign substances from the body. Among their numerous functions, T cells in the immune system can eliminate contaminated cells; B cells, on the other hand, produce antibodies. For progress in immunology and medicine, it is essential to understand how lymphocytes react to external stimuli like laser irradiation.

- **DNA:** A molecule known as deoxyribonucleic acid (DNA) contains the genetic instructions necessary for the creation, development, and functioning of every known living entity. The molecular structure of DNA is a double helix formed by two long chains of nucleotides. Because mutations and abnormal cell function can result from DNA damage or alterations, any alteration to DNA has the potential to have far-reaching consequences. To determine any genetic injury, it is necessary to examine how laser irradiation impacts DNA in lymphocyte cells.

- **The appearance and actions of lymphocyte cells:** Important areas of study include lymphocyte cell survival, behaviour, and responses to HeNe laser light. To find out whether the cells undergo any discernible functional alterations, changes in

their development patterns, or how well they tolerate laser irradiation, researchers may conduct tests. If the laser raises cell numbers, that's great news; but, if it kills cells or makes them less effective, that could be cause for concern.

- **Possible Damage to DNA:** An essential part of our research is determining if lymphocyte cells are damaged by DNA due to HeNe laser irradiation. Some symptoms of DNA damage include mutations, structural abnormalities, fractures in the DNA molecule, and other forms of damage. Understanding the potential risks and benefits of employing lasers in biological environments can be better understood by discovering this type of harm.

- **Bio photonics and Medical Applications:** Bio photonics is an interdisciplinary field that studies how light interacts with biological processes. It encompasses a wide range of applications, from imaging and diagnostics to medications and treatments. A better knowledge of the effects of HeNe lasers on DNA and lymphocyte cells enhances bio photonics, which might lead to the development of more effective and safer medical tools and procedures.

- **Benefits and Risks:** The primary goal of the study is to identify the benefits and drawbacks of using HeNe lasers in connection to lymphocyte cells and their DNA. It has been demonstrated that laser irradiation can enhance cell viability and induce beneficial biological responses without significantly damaging DNA; thus, it has the potential to be utilised in medical therapies and diagnostics. In contrast, prior to utilising lasers in medical procedures, it is essential to identify and mitigate any risks that the laser may pose to cell health or genetic integrity.

Finally, this study delves into the intricate web of relationships that HeNe laser irradiation forms with lymphocyte cells and their DNA. A comprehensive examination of each aspect is required to offer comprehensive understanding regarding the impacts and potential applications of HeNe lasers in a biological or medical setting.

Dataset description:

The photo acceptor particle, cytochrome c oxidase, is invigorated when photons of a The examination for this study was directed from November 2010 to June 2011 at the Division of Physiology and Clinical Physical science, School of Medication, Al-Nahrain College.

Eighty blood tests, forty male and forty female, ages going from 20 to 47 years of age, with a mean period of 33.5 ± 8.21 , were attracted from solid workers request to assess the effect of He-Ne laser on the DNA of human blood lymphocyte cells. Two arrangements of 80 blood tests were made. The main clump of 30 examples was handled exclusively for fringe blood lymphocyte cell detachment (PBL) involving the Boyum method to survey the effect of the He-Ne laser on lymphocyte cells. Fifty blood tests from the subsequent gathering were recovered involving He-Ne pre-illumination to survey the effect on the DNA recuperated from the lymphocyte cells. How much lymphocyte cells harmed by the He-Ne laser and the grouping of DNA were resolved utilizing the spectrophotometer and hemacytometer, individually.

Method:

A continuous 642.9 nm wavelength and 1 mm diameter He-Ne laser beam was used (Griffin and George, Britain). The laser had a 10 mW maximum output power. Using a converging lens, the He-Ne laser beam diameter was increased to a spot of 1.5 cm, which matched the 1.4 cm diameter of the sample tube, in order to guarantee uniform lighting on the sample. Different exposure periods (3, 5, 7, and 9 s) were used to irradiate the laser, resulting in energy doses of 19.3, 38.7, 60.2, and 76 J/m², respectively. Approximately 9% will be lost at each of the two lens surfaces because approximately 5% of the intensity reflected back at each lens surface. As a result, the final energy dosages were 19, 36, 53.5, and 70 J/m².

Thirty blood samples are separated into their constituent lymphocytes. Each suspension sample of lymphocyte cells was separated into about five equal portions, one of which was utilized as a standard and wasn't treated. A hemacytometer was used to determine the number of viable lymphocytes in the untreated sample portion using the trypan blue exclusion test. The counts were reported as viable cells/mm³, and the remaining four sample sections were each treated to a single dosage of the He-Ne laser beam (19, 36, 53.5, and 70 J/m²). The percentage of cells that survived each irradiation was calculated in relation to the viability of untreated (standard) cells.

To investigate the impact of He-Ne laser irradiation on DNA, fifty blood samples were utilized. The phenol chloroform technique was used to extract DNA from human blood lymphocyte cells. To assess the quality of the extracted DNA, a portion of each sample was used. As a benchmark, the optical density (OD) of DNA for the untreated sample portion was determined using a spectrophotometer set to 262 nm UV wavelength. The second component was exposed to a He-Ne laser beam (635.2 nm) for a first exposure before being incubated for 50 minutes at room temperature. Each time DNA was exposed to radiation, its OD was measured. There were three distinct He-Ne laser exposure times used: 3, 5, 7, and 9 seconds. As a result, this group's 50 samples were split up into three smaller groups. There are fifteen samples in each sub-group, and each sub-group was exposed to a He-Ne laser beam for either five, ten, or twenty-five seconds (19, 36, 53.5, or 70 J/m²).

Results:

Utilizing the Microsoft Succeed application, the mean and standard deviation for each gathering's information were determined. The outcomes for both when UV light illumination was looked at utilizing a matched example test. Any distinction with a P worth of under 0.05 was considered measurably critical.

Table 1: The average viability percentage of lymphocyte blood cells following varying durations of exposure and different doses of He-Ne laser irradiation.

Time exposure (s)	Dose (J/m ²)	Mean SD	P value
0	0	*99.9+0.07	
3	19	99.0+0.15	< 0.001
5	36	98.84+0.16	< 0.001
7	53.5	98.78+0.18	< 0.001
9	70	98.78+0.19	< 0.001

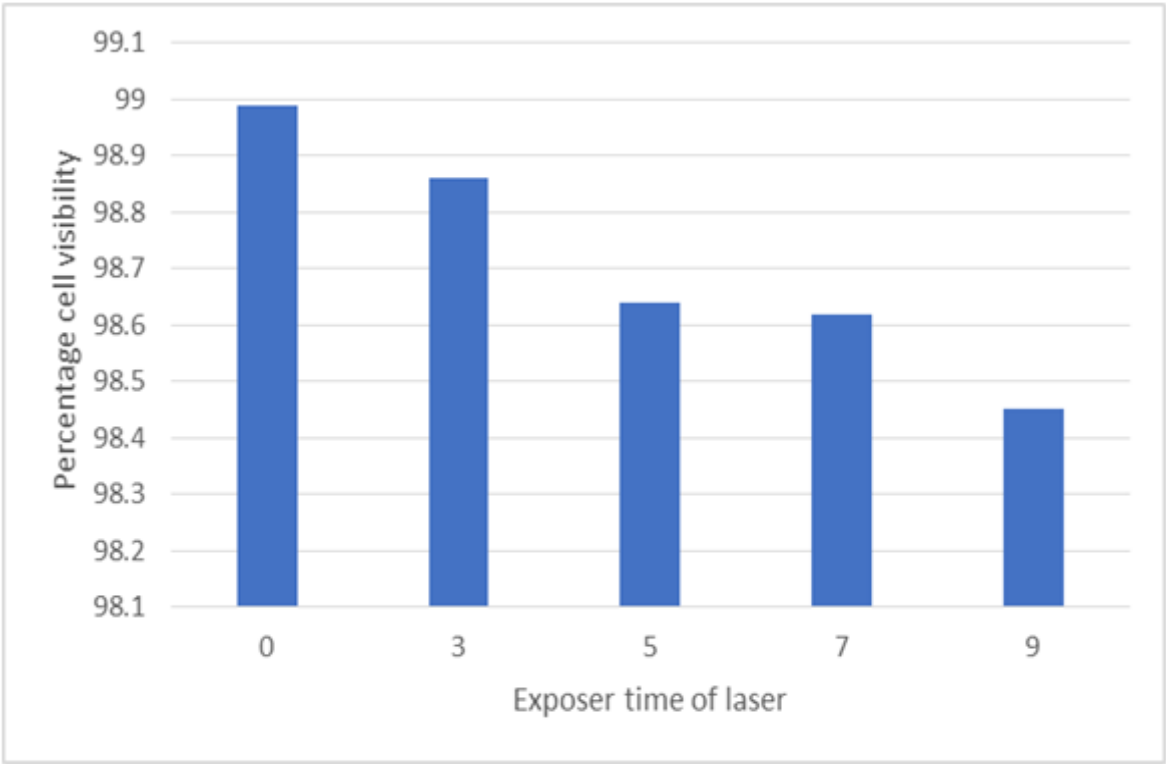


Fig 1 The Percentage of cells viability because of effect of laser.

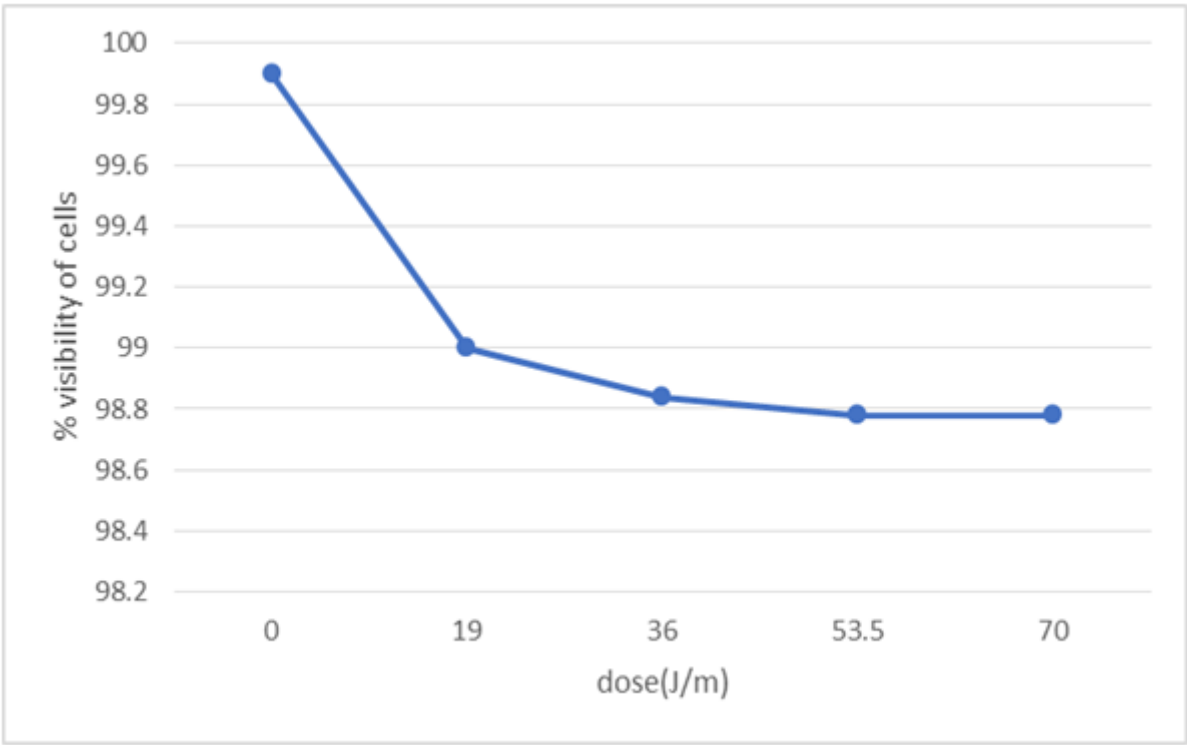


Fig 2: the percentage of lymphocyte cells and the dosage of laser beam energy.

The mean proportion of viable lymphocytes in the 30 standard blood samples that were left untreated was 99.9 ± 0.07 . The impact of various laser exposure periods (3, 5, 7, and 9 s), which translate into the corresponding energy doses (19, 36, 53.5, and 70 J/m²), on the percentage of viable lymphocytes is shown in Table 1. The vitality of

the cells after He-Ne laser irradiation differs little from that of the cells before to irradiation (untreated) ($P < 0.001$). The data clearly demonstrate that, as figure 1 illustrates, lymphocyte cell viability is highest at the shortest exposure duration (3 s).

Table 2: The measurement of DNA optical density (OD) using a spectrophotometer both before and after exposure to He-Ne laser irradiation.

dose (J/m ²)	OD before laser	OD after laser	P value	DNA survival %
19	4.21+0.40	3.21+0.45	< 0.001	85.5+6.7
36	4.31+0.39	2.89+0.44	0.001	88.5+9.5
70	3.56+0.19	2.1+0.30	< 0.001	87.1+6

The assimilation optical thickness (OD) of DNA both when He-Ne laser illumination is displayed in Table (2). That's what these discoveries exhibit, when contrasted with standard DNA (OD) results (untreated example), HeNe (632.8 nm) light alone at the accompanying three dosages (19, 36, and 70 J/m²) brought about a decline in DNA retention (OD), and that implies a decrease in DNA focus endurance. Following the three laser-illumination medicines, the extent of DNA that endure was 85.5%, 88.5%, and 87.1%. These discoveries show that significant DNA harm happens just after laser illumination and that this harm is portion autonomous for He-Ne lasers.

Discussions:

The photobiological reaction of the cells to the He-Ne laser specifically and to light overall not entirely set in stone by how much retained measurements. A photobiological response to He-Ne laser light (632.8 nm) creates different outcomes, including raised temperature and electronic excitation of the photo acceptor particles. As the temperature climb in the illuminated tissue is restricted to under 0.2→0.6°C, the electro-energized state as opposed to an expansion in photoceptor atom temperature is the significant reason for the effect of the low measurements of He-Ne laser used in this review.

This study researched the impacts of prompt low-energy He-Ne laser (632.8 nm) illumination on lymphocytes at different openness terms (3, 5, 7, and 9 s), yielding energy levels of (19, 36, 53.5, and 70 J/m²), individually. Since the mean feasibility level of lymphocyte cells is (99.0%, 98.84%, 99.78%, and 98.78%), separately, these various energies or portions of He-Ne laser cause a little level of cell demise or sub-deadly harm (Table 1), while figure (2) exhibited that the lymphocyte mean practicality with dosages of

laser lighted is profoundly corresponded ($r=0.99$). Since there was no brooding period after the radiation, there was no cell security distinguished.

Since the phone answers flags that commit it to implosion, He-Ne lasers can by and large animate intracellular or extracellular impacts, which pass in the underlying responsibility stage. This demonstrates that the illumination of mononuclear cells with He-Ne lasers can invigorate momentary responses and that the lighted cells didn't enter the S period of the cell cycle. The creation of singlet oxygen in numerous cell types is an illustration of an intracellular activity that might advance redox command over cell homeostasis. Proteins, enacted calcium channels, and receptor ligands are instances of extracellular signs.

In conclusion, longer exposure times and higher He-Ne laser doses result in a declining proportion of lymphocyte survival. Independent of the irradiation dosages, He-Ne laser irradiation damages DNA to a considerable extent.

Conclusion:

Numerous important conclusions emerged from the investigation of how He-Ne laser irradiation affected lymphocyte cells. The effects of low-energy He-Ne laser irradiation, at a wavelength of 632.8 nm, on lymphocytes were dose-dependent, with varied energy values resulting from different exposure durations. With high mean viability percentages and just a little amount of cell death or sub-lethal damage, it was shown that this laser irradiation had a protective impact on lymphocyte cells. It was also shown, nevertheless, that substantial DNA damage was caused by He-Ne laser irradiation, regardless of the radiation dosage. This shows that lymphocyte cells respond to He-Ne laser irradiation in a complicated and dose-dependent manner that may entail a number of different biological processes and signaling

pathways. To completely comprehend these impacts and any possible ramifications for cell health and function, further study is required.

References:

1. Avci, P., A. Gupta, M. Sadasivam, D. Vecchio, Z. Pam, N. Pam and M. R. Hamblin (2013). "Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring." *Semin Cutan Med Surg* 32(1): 41-52.
2. Ayuk, S. M., N. N. Houreld and H. Abrahamse (2012). "Collagen Production in Diabetic Wounded Fibroblasts in Response to Low-Intensity Laser Irradiation at 660 nm." *Diabetes Technol Ther* 14(12): 1110-1117.
3. Ballas, C. B. and J. M. Davidson (2001). "Delayed wound healing in aged rats is associated with increased collagen gel remodeling and contraction by skin fibroblasts, not with differences in apoptotic or myofibroblast cell populations." *Wound Repair Regen* 9(3): 223-237.
4. Belperio, J. A., M. P. Keane, D. A. Arenberg, C. L. Addison, J. E. Ehlert, M. D. Burdick and R. M. Strieter (2000). "CXC chemokines in angiogenesis." *J Leukoc Biol* 68(1): 1-8. Bisht, D., S
5. Branski, L. K., G. G. Gauglitz, D. N. Herndon and M. G. Jeschke (2009). "A review of gene and stem cell therapy in cutaneous wound healing." *Burns* 35(2): 171-180
6. Brown, D. C. and K. C. Gatter (2002). "Ki67 protein: the immaculate deception?" *Histopathology* 40(1): 2-11.
7. Busnardo, V. L. and M. L. Biondo-Simoes (2010). "[Effects of low-level helium-neon laser on induced wound healing in rats]." *Rev Bras Fisioter* 14(1): 45-51.
8. Byrnes, K. R., L. Barna, V. M. Chenault, R. W. Waynant, I. K. Ilev, L. Longo, C. Miracco, B. Johnson and J. J. Anders (2004). "Photobiomodulation improves cutaneous wound healing in an animal model of type II diabetes." *Photomed Laser Surg* 22(4): 281-290.
9. Carvalho, P. T., N. Mazzer, F. A. dos Reis, A. C. Belchior and I. S. Silva (2006). "Analysis of the influence of low-power HeNe laser on the healing of skin wounds in diabetic and non-diabetic rats." *Acta Cir Bras* 21(3): 177-183.
10. Castilla, C., P. McDonough, G. Tumer, P. C. Lambert and W. C. Lambert (2012). "Sometimes it takes darkness to see the light: pitfalls in the interpretation of cell proliferation markers (Ki-67 and PCNA)." *Skinmed* 10(2): 90-92.
11. Chung, H., T. Dai, S. K. Sharma, Y. Y. Huang, J. D. Carroll and M. R. Hamblin (2012). "The nuts and bolts of low-level laser (light) therapy." *Ann Biomed Eng* 40(2): 516- 533.
12. Huang, Y. Y., A. C. Chen, J. D. Carroll and M. R. Hamblin (2009). "Biphasic dose response in low level light therapy." *Dose Response* 7(4): 358-383.
13. Peng, Q., A. Juzeniene, J. Chen, L. O. Svaasand, T. Warloe, K.-E. Giercksky and J. Moan (2008). "Lasers in medicine." *Rep Prog Phy* 71(5): 056701.
14. Peplow, P., T. Y. Chung and G. D. Baxter (2010). "Application of low level laser technologies for pain relief and wound healing: overview of scientific bases." *Phys Ther Rev* 15(4): 253-285
15. Sommer, A. P., A. L. Pinheiro, A. R. Mester, R. P. Franke and H. T. Whelan (2001). "Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system." *J Clin Laser Med Surg* 19(1): 29-33.