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# Investigation of Laser Induced Inhibition and Stimulation in Biological Samples

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## Abstract

In this research, some experimental measurements have been carried out to study the biological effects induced by laser irradiation on bacterial samples prepared by different ways and at different concentrations. Considering the induced samples, the effect of laser irradiation has been investigated through analyzing the properties of the transmitted and scattered laser beam for determining the stimulation or inhibition experienced by the investigated sample. In this study absorbance and scattering values have been used as indicators of sample response to the irradiation laser beam. Absorbance and scattering have been measured for different irradiation and sample parameters. Significant responses related to inhibition and stimulation effects of the investigated samples have been obtained. These results may significantly contribute to minimizing the effective utilization of the laser beam as a therapeutic tool for accelerating the wound healing of diabetic patients whom their response to anti-biotic is not appropriate. The simultaneous stimulation of samples with the use of anti-biotic shows significantly positive effect and fast response.

**Keywords:** Photobiology, Inhibition, Stimulation, Absorption, Laser Therapy

## 1. Introduction

Lasers as highly stable sources of coherent and monochromatic light, have been used extensively in technical applications and for medical therapy. Laser light can interact with tissue in four ways namely: transmission, reflection, scattering and absorption. Transmission refers to the passage of light through a tissue without having any effect on that tissue or on the properties of the light. The transmission of laser radiation in tissues is related to its wavelength. Reflection refers to the repelling of light off the surface of the tissue without entering the tissue. Scattering of light occurs after it has entered the tissue, whereby the beam of light is spread out within the tissue resulting in irradiation of a larger area than anticipated [1-3]. Absorption is a process by which a photon gives up energy to its surrounding medium. This energy is ul-

first used in the medical field as a focused laser beam with photothermal effects in which the tissue is vaporized by the intense heat. It was postulated that the laser power is highest at the center of the beam and falling off in a bell-shaped curve with the maximum power at the periphery of the beam diffusing into the surrounding undamaged tissues [1,6]. This phenomenon is known as the "alpha-phenomenon" [4]. Laser devices have been fabricated in which power densities and energy fluences of the laser were lowered to a point where no significant effects occurred but the photo-osmotic, photolytic, and photo-enzymatic effects were still operative. The applications of lasers are now widespread in various medical specialties, especially dermatology, ophthalmology and medical acupuncture [7].

The diverse tissue and cell types in the

timately responsible for photobiostimulation [4,5]. The effect of laser irradiation on biological objects depends on experimental conditions, such as the type of irradiated cells, wavelength and intensity of light, etc. Laser was

their own unique light absorption characteristics they will only absorb light at specific wavelengths, not at others. For example, skin layers, blood, with high blood and water content, absorb red light

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ily, while calcium and phosphorus absorb light of a different wavelength [8]. Once a photobiological response is observed, the next step should be to determine the optimum wavelength and dose of radiation to produce the effect, *i.e.*, an action spectrum. An action spectrum is a plot of the relative effectiveness of different wavelengths of light in causing a particular biological response, and under ideal conditions it should mimic the absorption spectrum of the molecule that is absorbing the light, and whose photochemical alteration causes the biological effect. Thus, an action spectrum not only identifies the wavelength(s) that will have the maximum effect with the least dose of radiation, but it also helps to identify the target of the radiation. For example, the action spectrum for killing bacteria mimics the absorption spectrum of deoxyribonucleic acid (DNA) [6,9-11]. This result is understandable in view of the unique importance of DNA to a cell.

Low-level laser Photobiology uses radiation both in the visible (400 nm - 700 nm) and in the near-infrared (700 nm - 1000 nm) regions of the spectrum. When a photon is absorbed by a molecule, the electrons of that molecule are raised to a higher energy state [7,8,12]. This excited molecule must lose its extra energy, and it can do this either by re-emitting a photon of longer wavelength (*i.e.*, lower energy than the absorbed photon) as fluorescence or phosphorescence, or it can lose energy by giving off heat, or it can lose energy by undergoing photochemical alteration.

Photobiological responses are the result of photo-physical and/or photochemical changes produced by the absorption of nonionizing radiation. Karu [6,13] has shown that visible and near-infrared radiation is absorbed in the respiratory chain molecules in the mitochondria (e.g., cytochrome), which results in increased metabolism, which leads to signal transduction to other parts of the cell, including cell membranes, and ultimately to the photoreponse (e.g., stimulation of growth) [8,12]. Laser irradiation as a phototherapeutic modality for the induction or acceleration of wound healing was first introduced by Mester *et al.* [4,14] in the 1970s but still is not an established therapy. This is mainly due to the fact that substantial amounts of research were originally done in

## 2. Experimental Methods

This research work has been initiated by the experimental set up illustrated in **Figure 1**. It consists of laser source, sample stage, optical circuit and magnetic stirrer.

This experimental setup has been designed for different types of measurements, *i.e.* transmission and absorption measurements. It consists of a laser, a sample stage, a magnetic stirrer (to provide a homogeneous environment of the investigated sample), a photodetector and a display system (Multimeter and digital oscilloscope).

The samples were irradiated with (Iridium Ion Solid State Laser) DPSS of output power 150 mW and wavelength of 532 nm (green). The investigated samples were Catalase Enzyme and *Staphylococcus aureus* coccus bacteria that are prepared with different concentrations using the common biological methods. The experimental results were based on the measurement of the transmitted and/or scattered laser beam at different angles.

## 3. Results and Discussions

### 3.1. Results for Catalase Enzyme

Catalase Enzymes samples are prepared with different concentrations from yeast in cuvette with magnetic stirrer and irradiated for different irradiation times. The irradiation process has been performed using the experimental setup. The obtained results revealed that significant effects occurred as shown in **Figure 2**. The reaction time is significantly decreasing with the irradiation time and increasing with the sample concentration. After a specific concentration the reaction time is increasing with the irradiation time. In this experiment, at a concentration of 0.6 g/mL the reaction time is increasing with irradiation times and drastically decreasing



East European countries and published in non-peer-reviewed journals. Moreover, there has often been a lack of accuracy in the documentation of exact irradiation protocols and the incorporation of appropriate controls in the past. Additionally, the variety of laser systems and experimental conditions utilized made comparison of results difficult. Since more well-controlled studies have been performed and since the Food and Drug Administration (FDA) has initiated research in the field of low intensity laser therapy [1], this phototherapy is gaining increasing interest.

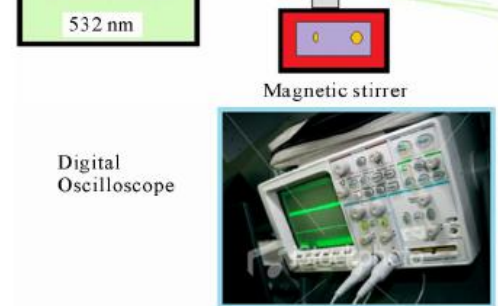


Figure 1. Experimental setup

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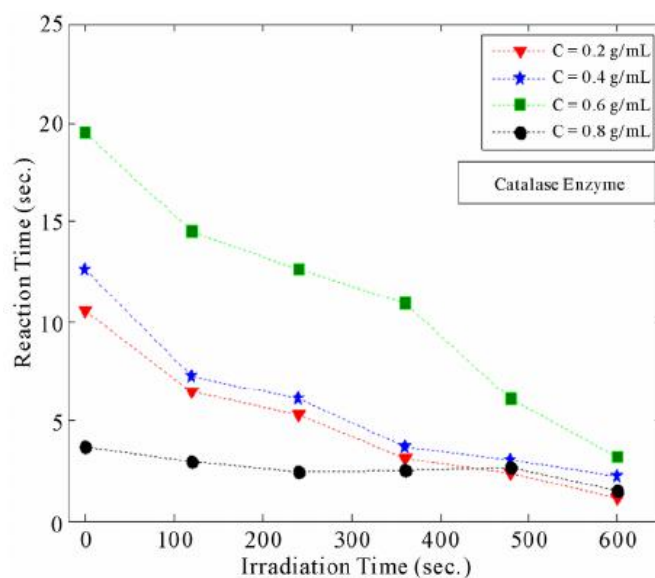


Figure 2. Variation of reaction time with the irradiation time for different sample concentration.

creasing of irradiation time for higher concentration as it is clear for concentration of 0.8 g/mL which is depicted in Figure 3. These results assured the applicability of laser irradiation to stimulate Enzymes reaction concentration. It also reveals that there will be an optimum concentration that that exhibit significant response to the laser irradiation. Moreover, the stimulation effect is enhanced with the increasing of irradiation time.

### 3.2. Bacteria Sample (Staphylococcus)

Samples (Staphylococcus injected into normal saline solution) have been clinically collected and prepared in different unit cell of formation (ucf). The samples were put on a stirrer at 15 cm away from the laser source (laser spot diameter 0.2 cm). The samples have been arranged in two different forms, *i.e.* on plates and/or in

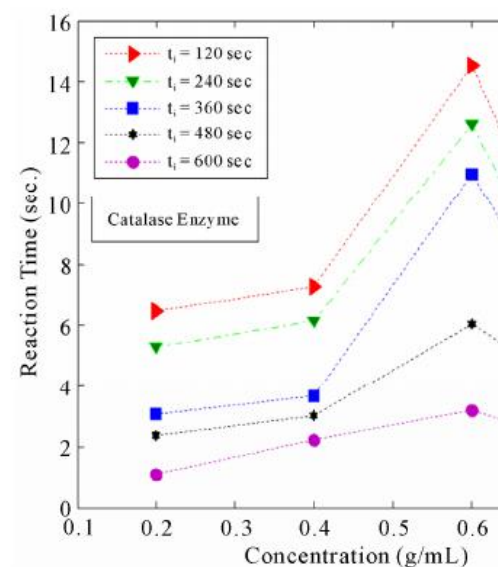
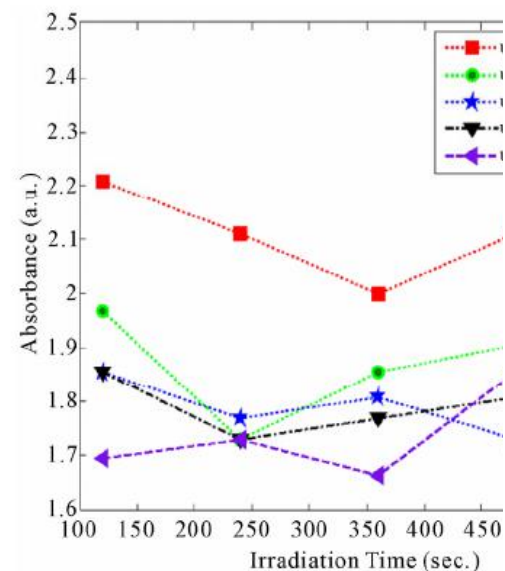


Figure 3. Variation of reaction time with the tration for different irradiation times.



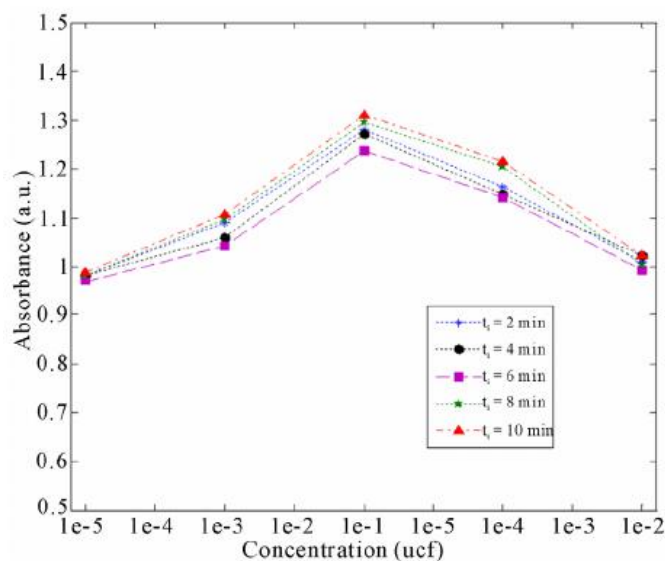
cuvettes. The effects of irradiation with laser of 150 mW, 50 mW and wavelength of 532 nm on reaction time and absorbance of the prepared samples have been studied for different sample concentration. The obtained results revealed that significant effect have been occurred. The response was also investigated after each experimental procedure using the convenient microbiological analysis and inspections. It has been found that the change in absorbance and/or scattered laser intensity was due to variation of one or more of the characters of bacterial samples, (such as viability or produced enzymes). It is therefore, quite amenable to take the variation in laser absorbance or scattered intensity as indicators to the sample response to the laser irradiation. As shown in **Figure 4**, the absorbance of the laser beam is found to be strongly dependent on the concentration of the sample and time of irradiation. The inhibition effect was observed with in-

**Figure 4.** Variation of absorbance with the concentration for different irradiation time.

creasing irradiation time until certain time (depending on the sample concentration) after which the effect is starting increasing with the increase of irradiation time. Moreover, there is an optimum irradiation time that exhibit best response as it is further discussed in **Figure 5**.

In both figures the variation of absorbance with irradiation time for different concentration of absorbance with concentration for different irradiation time, it can be clearly noticed that there is a variation for each case until certain specific irradiation time and/or concentration at which the trend is reversed. Meaning that by optimizing the irradiation parameters, it is possible to control the required

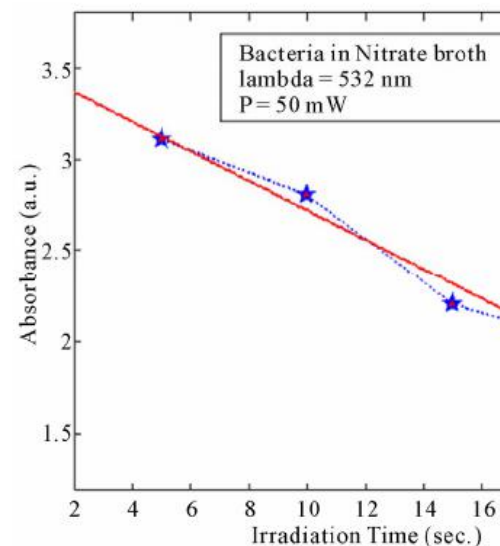
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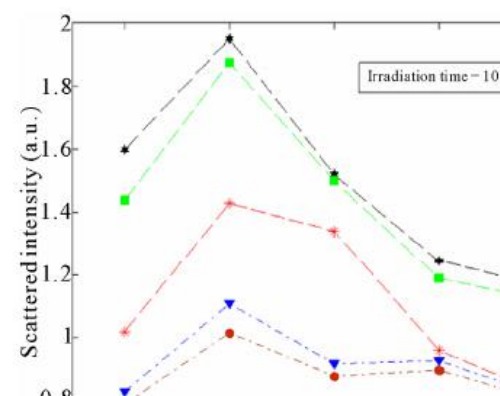
**Figure 5.** Variation of absorbance with the irradiation time for different concentration.

A linear variation of absorbance with the irradiation time was obtained when a laser power of 50 mw was employed as shown in **Figure 6**. This means that there was a response resulted in an inhibition only without any stimulation which is manifested by the decrease of absorbance with the irradiation time. This suggests that low laser power can work better for wound healing in diabetes patients.

On the other hand, the scattered laser intensity from the irradiated sample has been also measured at the op-



**Figure 6.** Variation of absorbance with the irradiation time for a 50 mW laser power.



timum irradiation time for different scattering angles as depicted in **Figure 7**.

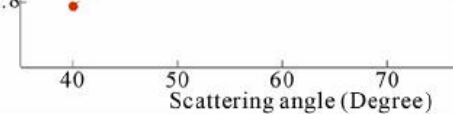
There is an optimum scattering angle (50 degree) at which the scattered intensity is maximum and decreasing below and above that angle. The scattered intensity is also greatly affected by sample concentration and irradiation time (here the effect was best observed for irradiation time of 10 min.). Moreover, the possibility of incorporating laser irradiation simultaneously with antibiotics as a therapeutic method for diabetic patients whose response to antibiotic is very slow was investigated with three different types of antibiotics, *i.e.*, QB, CB and ZX and the result is illustrated in **Figure 8**. It is obvious that this procedure increases the impact effectiveness of the antibiotics on the investigated samples which is represented by the effective diameter range of the implanted samples.

All those results mean that good selection and optimization of the process parameters such as laser wavelength, intensity and the time of irradiation with the type of antibiotic is an important process to determine the effectiveness of the medical treatment utilizing these methods.

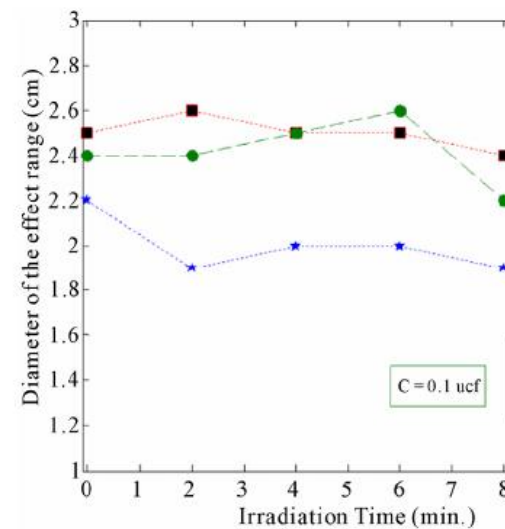
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#### 4. Conclusions

Enzyme catalase and Staphylococcus Bacteria samples have been prepared in various concentrations and different conditions. They were irradiated for different irradiation times with different laser beam characteristics. From the obtained results, the photobiological stimulation and inhibition was clearly demonstrated for both Enzymes and Bacterial samples. That was clear from the absorbance and scattering trends. However, for the case of low laser power irradiation, it has been found that the irradiated bacterial samples experienced only inhibition effect which was obvious from the decreasing of absorbance with the irradiation time. Moreover, the simultaneous irradiation along with the anti-biotec incorporation shows that the effectiveness of the anti-biotec was significantly enhanced with the laser irradiation. The process of laser irradiation as well as optimization of both laser beam characteristics and samples conditions led to a conclusion of the effectiveness of laser irradiation on the investigated samples which means that this method with the required optimization can eventually be an effective therapeutic tool for many diseases especially for wound



**Figure 7.** Variation of scattered intensity with angle for different concentrations.



**Figure 8.** Variation of diameter of the effect range with irradiation time.

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healing of diabetic patients. Moreover, further development of this technique can end up with an efficient tool for cancerous and malignant diseases.

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