Study Effect of Different Low-Level Laser Intensity on Absorbances and Fluorescence Properties of Blood Components

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Abstract
Low-level laser intensity (LLLT) had been employed to induce and diagnose several biological activities within tissues and blood. The project study was designed to explain the effects of LLLT on absorbance and fluorescence features of different blood components including (plasma, serum, serum without proteins, and precipitated serum proteins. LLLT (437nm, 532nm, 632nm) was used to irradiate of blood drive component for 10 minutes with power (20mw, 28mw, 40mw). Results of non-radiated components showed there was a high peak (absorbance) of precipitated protein flowed by serum, plasma, and serum without proteins. The irradiated plasma samples showed increased absorbance with blue laser in comparison with other components whereas irradiated serum samples indicated high absorbance with red laser compared to other components precogitated serum protein recorded increase opted density with green lases with lower than control. Samples of serum without proteins pointed out a significant increase of absorption in green and blue laser compared to control and other laser radiation. Regarding fluorescent properties of blood components, plasma samples showed a higher peak with red laser compared to other laser radiated samples. On the other hand, serum radiated samples confirmed a remarkable peak with green laser compared to other samples. However, precipitated radiated serum protein was a significantly lower fluorescence peak when matched with those control samples. Also, samples serum without proteins and irradiated with different laser wavelengths showed lowering fluorescence peaks in matching with control (non-irradiated samples). In conclusion, the results which are mentioned above can be attributed to different densities of components of blood because isolation of proteins (clotting and serum proteins), as well as the photo-biochemical modulation or changes induced by laser radiation, can affect absorption and fluorescence peaks of that component.
Keywords: Laser radiation, Human blood radiation, Fluorescent, and absorption of proteins.

1. Introduction

Laser beam radiation is extensively employed in a range of domains, including physics, chemistry, biology, and medicine [1]. Because of its specific features, the laser beam is advantageous in several domains. A powerful, coherent, and monochromatic beam is emitted by the laser. In addition, the laser beam is easy to control, and its large collimated nature allows it to be concentrated into a tiny region for maximum incidence intensity [2]. The impact of laser radiation on biological systems are being investigated. Blood and Tissues are of significant interest and have gotten a lot of studies [3].

Laser beam irradiation has been demonstrated to be most beneficial in biomedical applications and may be utilized efficiently for medical therapies and biological [4]. When interacting with biological tissues and human blood, laser beams lately it has been discovered and may create chemical effects and certain optical. The optical properties of various human blood, and biological tissues, such as transmittance, absorbance, reflectance, refractive and index absorption coefficient, would be significantly altered as a result of these actions [5].

2. Design of Experiment

An adequate venous blood sample was collected from the anti-cubital vein of healthy adult subjects, the blood samples were divided into two parts, and the first part was put in tubes containing anticoagulant (EDTA-Tubes) to ensure obtaining plasma after centrifugation at 3000 rpm for 5 minutes. The second part of the blood was transferred into gel tubes for separation of serum after centrifugation at 3000 rpm for 5 minutes. Some of the serum samples had been treated with trichloro acidic acid solution for precipitation of serum proteins and the remaining fluid of serum after protein precipitation was collected by other plain tubes. Concerning precipitated proteins of serum had been dissolved by the addition of high NaCl concentrations.

All yield components (plasma, serum, precipitated proteins, and serum without proteins) were diluted with distilled water: 1 (3ml final volume).

Furthermore, all components were irradiated with low laser intensity (473mm, 532, and 632nm respectively) with power (20, and 28mw, 40mw, respectively) for 10 minutes. Then after, the absorbance and emission spectra had been measured for all treated samples and control. As

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shown in Figure (1).

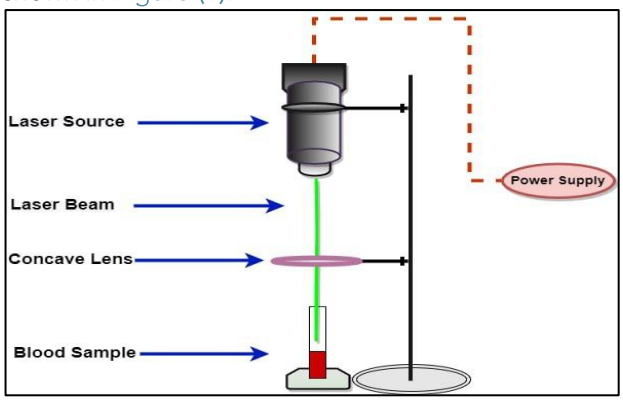

Figure (1): Set up of laser source and blood sample

The spectrophotometer (Cecil CE-72000) was employed to measure emitted spectra of irradiated and non-irradiated samples that have a wavelength ranging between 190-1100nm. Laser-induced fluorescence (LTF) was included to record the fluorescent emitted light.

3. Results and Discussion

This paragraph will show multiple explanations, including

1-absorbance spectra

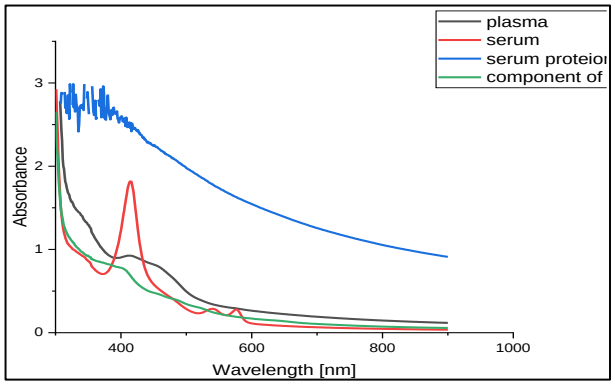

Figure (2): Absorbance Spectra sample for blood component before radiation (plasma, serum, serum protein, and component of serum without protein).

Also, from these observations, it appears clearly that precipitated serum proteins were recorded with high levels of absorbance compared to other samples these results indicated that pure and high concentrations of proteins lead to a heightening of absorbance because of high levels of density of protein molecules. Subsequently, serum recorded the second stage after precipitated proteins in the level of absorbance and these results indicated that serum has proteins molecules diluted with fluids of serum so that low absorbance in comparison with those precipitated proteins because of fluid of serum diluted of proteins molecules and decrease its absorbance.

From the finding depicted in Figure 2, it was found clearly that density was a prominent factor that led to an increased absorbance of the sample. Plasma samples are denser than other samples because they have all proteins of blood especially clotting proteins and other proteins which elevate the optical density (absorbance of plasma). On another hand, samples of serum-free protein showed lower levels of absorbance, and these findings confirm that proteins in blood have an effective factor in increasing the absorbance of light and also, they represent a major factor in the density of blood components. When laser light is beamed into the tissue, a tiny percentage of the light is reflected, as shown in Figure (1), however, most of the laser light gets into the tissue and is either dispersed by the molecules or absorbed. Water has two high absorption areas, one in the UV and one in the IR, Aromatic rings of proteins and nucleic acids have an absorption peak in the UV area between 260 and 280 nm. As a consequence, laser light in the UV range is extensively absorbed by proteins and water in the tissue, resulting in low light penetration. The same thing is the infrared area, which starts at 1.3 microns. The light can be absorbed by Blood in a wide wavelength range increasing to red light (630 nm), and absorption is available over 600 nm. Melanin can absorb light in the UV to the closer to IR range. The absorption coefficient of tissue molecules is minimal between 600 nm and 1.3 microns, resulting in an intriguing optical window for laser light penetration into tissue [6]. In three collections the common sample gets exposed to the laser with three wavelengths of the laser power (473,632, and 532nm) for various output energy (40, 20, and 28mw) at fixed exposure for 10 minutes for each collection. The blood component for one sample was not radiated and used as a control sample. Figure (3-) depicts the absorbance of samples following irradiation with (473nm, 532nm, 650 nm) at output power (20nw, 28nw, 40nw) for a 10-minute exposure duration. Because of the interaction between laser and molecules of material (blood component samples), the absorbance of healthy blood component samples increased after irradiation with different wavelengths. This means that the temperature of the medium increased due to the transformation of energy from incident photons to molecules of material, and then increases in the vibrational energy of material molecules. The excitation state in medium molecules occurs when they absorb the energy of light photons, causing the medium temperature to rise. As a result, this process is significant in determining several physical properties, such as thermal conductivity.

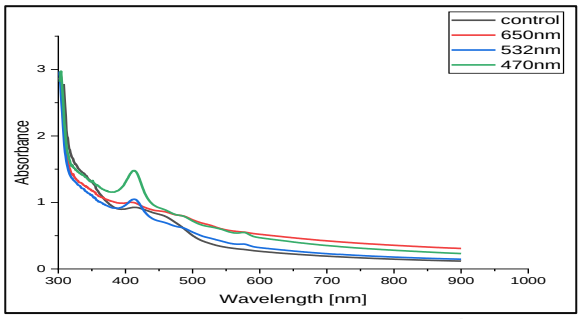

Figure (3): Absorbance Spectra sample for Plasma component before and radiation for (632 nm, 532nm, and 470nm) at (10) min

Figure (3) showed an absorbance spectrum of

plasma samples irradiated with different LLLT (473nm blue, 532nm green, and 632nm red respectively). From data obtained from this figure, it appears to increase the absorbance (optical density) of all irradiated plasma samples in matching with no-irradiated samples (control), these findings can be attributed that laser radiation gave the ability to affect organic molecules of plasma other proteins. Also, there was found that the increase in absorbance is inversely proportionate to the wavelength of the laser beam. It appears that blue absorbance and then green 532nm and finally red 632nm, this is maybe attributed to plasma absorbance because of conformational changes of molecules and increase their aggregation within the sample.

Regarding the effects of laser radiation on blood plasma, blood plasma was prepared and plasma samples were exposed to (650nm, 532nm, and 473nm) laser beams for ten minutes. It was noted that there was a difference in the peaks of the irradiated samples when compared with the non-irradiated ones. At the same time, it was noted that there was a difference in the peaks of (peaks) of samples irradiated with 532nm laser when compared with samples irradiated with 650nm laser and 473nm. Treating the control blood sample and plasma sample that have absorption spectra with a laser beam with wavelength (20 nw, 473 nm) to give the laser beam a large increase in the output power, during the exposure duration of (10 minutes) the amount of absorption will be increased significantly in the irradiated sample. The irradiated blood sample and control that irradiated with wavelength (532 nm, 28mW) of the laser beam and the exposure time (10) minutes, increase the peak of absorbance spectra at (415nm) but these values increase gradually. Water absorption dominates plasma absorption in the near-infrared. Plasma absorbs a lot of light in the UV to the visible range because of the chromophores found in proteins and other substances. Individual diversity in plasma absorption is caused by variable protein concentrations, nutritive substances, or medications, such as contraceptives. Such as visual examination of plasma components from various granters reveals a spectrum of hues spanning from yellow then to green then to orange then to brown, as well as their transmission. Plasma has a 450 nm absorption peak, which can be linked to bilirubin absorption. The absorption peak at 415 nm is frequently the most conspicuous, and it is caused by free hemoglobin from plasma formation or leftover RBCs. Therefore, spread particles like lipids can cause dense plasma samples, which they normally ruled out during standard plasma quality checks.

Rayleigh scattering of protein molecules describes the scattering properties of pure plasma, resulting in a scattering cross-section that decreases with increasing wavelength. Plasma samples contain a variety of components, including molecules, and aggregations of molecules. The optical characteristics of blood are determined by measuring the plasma components, the absorption, and the scattering of blood cells. Data on blood's visual characteristics are crucial. Data on blood's optical characteristics are crucial. It's used for different therapeutic applications in laser medicine and diagnoses, not just for normal medical diagnoses. They are used in fluorescence diagnostics, optical tomography, photodynamic treatment, diaphanoscopic, and thermotherapy laser-induced to count the percentage of light distribution in the blood tissue components. The optical parameters can be used to explain light dispersion according to the transport theory. Coefficient of absorption.

Plasma makes up roughly 55% of normal human whole blood. 90% of the time, it's water, and 10% of the time, it's proteins[7]. Therefore, they believe the enzymes of lipid metabolism are affected by the low-level laser irradiation and improve the balance of cholesterol in the plasma according to the amount of absorption peak size change after a low-intensity laser session for the plasma sample.

By comparing Fig (3), it can see that the irradiated sample of the absorbance is rising at the same level due to output power convergence, which results in a boost in the same values of absorbance amount. It was found that irradiation with low-level lasers such as 630.8 nm results in the formation of a single state of oxygen that enhances the production of reactive oxygen species (ROS) including H2O2, hydroxyl radical, and super peroxide. ROS presented in blood serum proteins leads to damage and aggregation of serum proteins and increases their density[8].

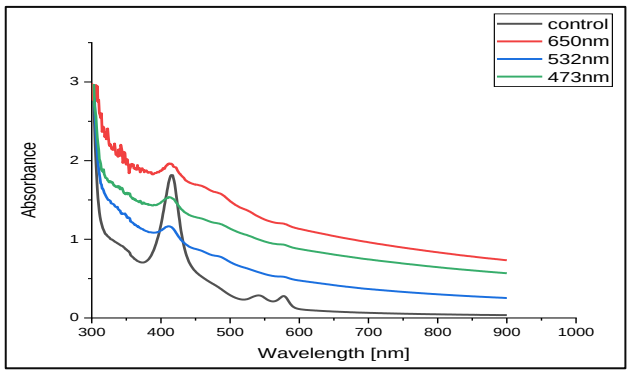

Figure (4): Absorbance Spectra sample for serum blood before radiation for (532nm, 473 nm, 650 nm) at (10) min

Treating the serum sample and absorption spectra of a control serum sample with a laser beam of wavelength (632 nm, 28 mw) resulted in a big increase in the output power of the laser beam, and the absorption value of the irradiated sample increases significantly during the exposure duration (10 minutes). It has a higher absorption rate compared to other lasers.

Regarding the effects of laser rays on blood proteins, the blood serum was prepared after the coagulation process took place. The serum samples were

exposed to the laser rays (650nm, 532nm, 473) for ten minutes. A difference in the peaks of the irradiated proteins was observed when compared with the non-irradiated ones at the same Time There is a difference in peaks of samples irradiated with 532nm laser when compared with samples irradiated with 650nm and 473nm lasers.

We noticed the appearance of the highest peak in the absorbance of the sample irradiated with the red laser, which is higher than the rest of the lasers because the red light penetrates deeper into the thick samples.

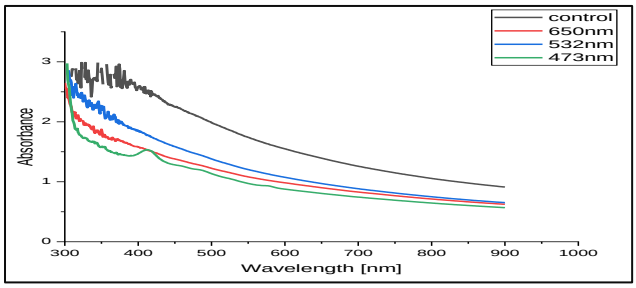

Figure (5): Absorbance Spectra sample for serum protein before radiation for (650 nm, 532 nm, 473 nm) at (10) min

The observation noted in Figure (5), revealed that all laser irradiation causes a decrease in absorbance spectra of precipitated serum proteins in a comparison with control samples. in addition, the laser showed a variety of effects on the proteins, it showed that the green laser recorded a high peak of absorbance and then the red and blue respectively[9]. These data suggested that the effects of laser on pure proteins are different from those on plasma and serum proteins which are mixed with other molecules, and these effects may result because laser radiation exerts damage and break down of proteins also there are inter and intramolecular changes occurring in pure serum protein that led to decrease their absorbances compared to control.

A difference in the peaks of the irradiated proteins was observed when compared with the non-irradiated ones at the same Time There is a difference in peaks of samples irradiated with 532nm laser when compared with samples irradiated with 650nm and 473nm lasers. It noticed the appearance of an absorption peak at the blue laser (473) nm, and the reason is that it is the closest to the absorption spectrum of proteins.

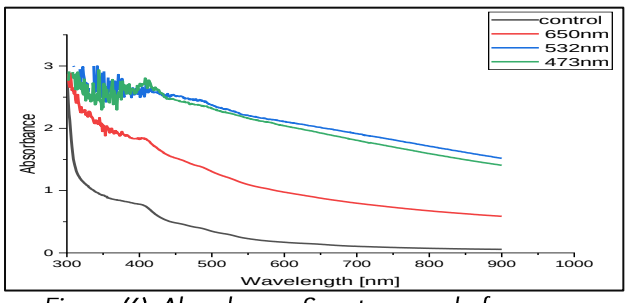

Figure (6): Absorbance Spectra sample for serum without protein before radiation for (650 nm, 532 nm, 473 nm) at (10) min

Figure 6 showed the different results of the absorbance of serum-free protein. It was revealed that together green and blue laser pointed out a high absorbance level at the same time these effects were similar, on the other hand, red laser showed low absorbance when compared with blue and green laser and a higher absorbance level in compared to control samples

Serum without proteins has a low density in matching with that plasma, serum, and precipitated serum proteins, but serum without protein has other organic biomolecules such as lipid, vitamins, carbohydrates as well as minerals, and ions. these components may be affected by laser radiation (green, blue, and red) because either high water contents and vitamines absorb light and also increase the heat of these molecules causing molecular interactions among described molecules and these changes were dependent inversely on a wavelength of light. It markedly increases absorbance with a low wavelength.

Emission spectra

Fluorescence is a type of luminescence (emission of photons or light energy). It is known that when a molecule absorbs a photon (light), the acquired energy promotes the passage of the molecule itself from the ground state to an excited state. Conversely, when a molecule emits light, the energy of the molecule decreases by an amount equal to the energy of the released photon. The releaser fluorescent photon usually exhibits a lower frequency and a longer wavelength than the thrilling photon is absorbed Because some energy is lost in the process [10].

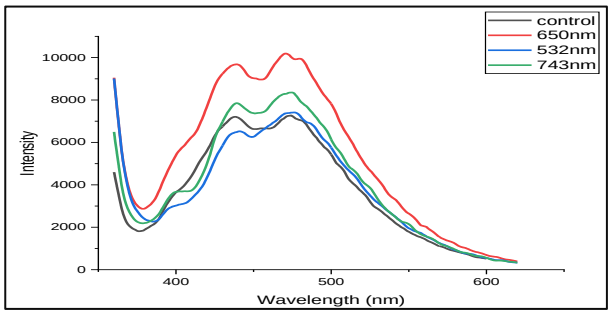

Figure (7): Emission Spectra sample for plasma before radiation for (650 nm, 532 nm, 473 nm) at (10) min

It was well noted that results which were adopted in this figure, plasma samples which exposed to red laser showed high peak level of fluorescent intensity and there flowed by blue laser and finally green laser. these results were consistent with the facts indicated that red laser (high wavelength) can be absorbed by components of plasma molecules in particular proteins that have fluorophores amino acids (tyrosine, tryptophan) and absorption of the red laser by water molecules increase heat content of plasma and laser maybe ender of biomolecules more excited.

The revelation of pathological processes in humans using the Native fluorescence of bioliquids and tissues is widely used for organisms. This tactic depends on the estimate of native molecular

fluorophores revival, which is based on pathological conditions and the metabolism of the organism. In addition, many works are staunch to cancer detection using tissue and (less frequently) blood serum fluorescence.

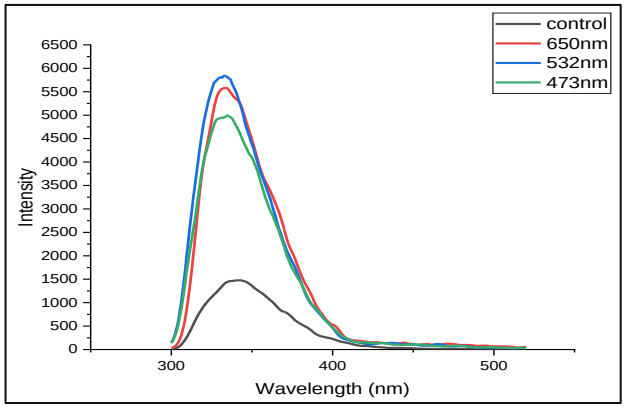

Figure (8): Emission Spectra sample for serum before radiation for (650 nm, 532 nm, 473 nm) at (10) min

Because of their similar biosynthetic origin, involvement in the same processes, and presence as significant extracellular components of the circulatory system, serum proteins form a dynamic system with varied biological activities. The intrinsic fluorescence intensity at a wavelength of excitation corresponding to tryptophan or tyrosine fluorescence, as well as surface hydrophobicity, were used to investigate the unfolding of human serum proteins (HSP). Human serum albumin (HSA) and human serum globulin (HSG) have maximum emission wavelengths (max) of 336.0 and 337.0 nm, respectively. A reduction in fluorescence intensity, a shift in emission maxima, and a rise in surface hydrophobicity, all of which indicated protein unfolding. Disruption of protein structure is blamed for differences in fluorescence behavior [11].

Protein crystals are routinely imaged using their inherent protein fluorescence. Tryptophan fluorescence accounts for the majority of this. We should use very strong UV light power and highly sensitive cameras because protein fluorescence is so low. Therefore, the period of exposure time is very important to get essential data because the UV light sources may harm the protein.

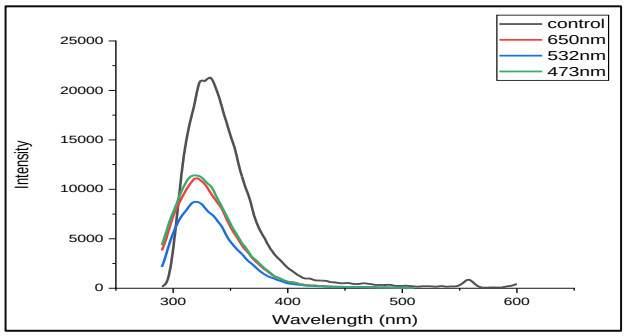

Fig (9): Emission Spectra sample for serum protein before radiation for (650 nm, 532 nm, 473 nm) at (10) min

There are special characteristics of laser radiation having unpleasant and its usefulness in several applications one of them is based on the excitation of chromones that absorb radiation visible or infrared wavelength spectra, in biological fluids that are exposed to the infrared laser, molecules of water are excited and raise to maximum levels of vibrational state [12]. Also, there is evidence that the proposed infrared laser can evoke photo-chemical interactions [13]. The precipitated serum protein had low fluorescent peaks compared to that non-irradiated these data can be produced as a result of the impact of laser radiation on conformational frame of proteins and these changes occurs at a level of molecules of intra-molecules can attenuate the level of fluorescence compared with control.

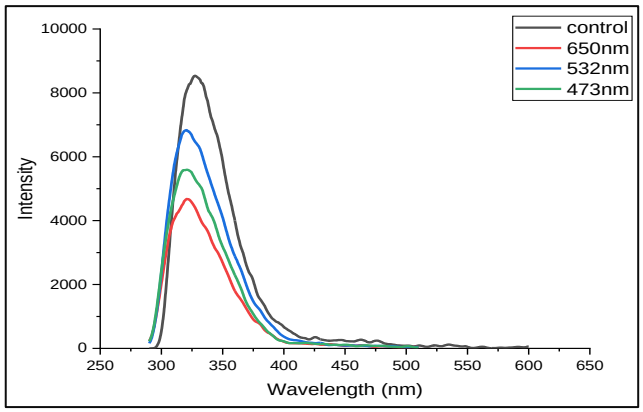

Figure (10): Emission spectra non-radiated and irradiated component of serum without protein sample for (650nm, 532 nm, and 473nm) at (10) min.

Also, from these presentations appear that the levels of the fluorescent peak were low in irradiated samples of serum without proteins compared with control, and these results were attributed to molecular changes of organic and inorganic molecules remaining in serum after protein precipitation and laser light can affect these molecules and changes the structures of the inflorescent molecules. Most, if not all tissues of body organs and fluids have many various fluorescent molecules other than protein in particular NADPH, Porphyrin, and Pyridoxal acid--. On the bases of the results documented in Figure (10), it may be laser radiation affects fluorophores and maybe prevent the excitation of fluorophores molecules remaining in the serum without proteins.

4. Conclusions

This thesis studied the influence of laser radiation on the fluorescence of healthy blood samples and their absorption. Three continuous waves (CW) with different wavelengths (650 nm, 532 nm, and 473nm) nm and different output power (40, 20, and 28) mW and the exposure time was (10) minutes for every wavelength were used in this thesis. The results provide substantial information about irradiated normal healthy blood samples and emission spectra between normal healthy blood samples (control) and the change in absorption spectra. There is a pure

variation between the emission spectra of all wavelengths and the output power in the exposure time (10 minutes) and absorption depending on the increase in the power output of the laser radiation that is used, as shown in Tables (1 and 2).

Table (1): Peak of Absorbance						
Output power (mW)	Wavelength (nm)	Time of exposure (min)	Peak of absorbance			
			plasma	serum	serum proteins	serum without protein
40	632	10	0.9	1.9	1.5	1.8
20	473	10	1.4	1.5	1.4	2.5
28	532	10	1.01	1.1	1.8	2.5

Table (2): Peak of Fluorescence						
Output power (mW)	Wavelength (nm)	Time of exposure (min)	The peak of fluorescence (Intensity)			
			plasma	serum	serum proteins	serum without protein
40	632	10	10056	5495	11056	4649
20	473	10	8341	4958	11396	5565
28	532	10	7383	5793	8418	6757

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