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# Q-Switched UV Laser Interactions with the Human Blood in Vitro

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**Abstract**
A Q-switched UV laser beam emitting wavelength at 355 nm with different energy densities (fluence, J/cm$^2$) was used for shining sixty human blood samples in vitro. Absorption spectra of hemoglobin were measured for the first time for the samples that exposed to 500 laser pulses in comparison to control samples. It was found that the peak absorbance decreases as the laser fluence increases. This decrease in the peak absorption has been found to be due to the red blood cells deformation and aggregation resulting from the effect of the laser pulses. This phenomenon has been confirmed by examination of the blood cells slides after exposing to the laser radiation. Furthermore, the red blood cells (RBCs) counts were found to be decreased with increasing the laser fluence. The decrease in the RBCs after the irradiation explains the decrease in the hemoglobin absorbance and that represents additional evidence to the structural change according to the optical microscope images. The changes in hemoglobin absorbance are used in a clinically available optical oxymeters. The immediate hematology measurements of the blood samples after the exposure to the laser pulses indicate an increase in the white blood cells (WBCs) of the type basophiles. The measurements were obtained with a significant difference with the level of probability ($p \leq 0.05$) between the laser exposed samples and the control samples. This laser effect is also due to structural changes in the WBCs. The changes in mitochondria resulting in cells division are in a good agreement with many results in the literature for other types of laser irradiation and other types of the WBCs. The proliferation of the basophiles upon laser photolysis plays a vital role in the activation of the immune system and a consequent destruction of pathogens by these cells.

## Introduction 1.

A study of the blood optical and rheological parameters plays an important role in many medical routine diagnosis and therapeutic applications. Laser photodynamic therapy can be considered as the most important optical device for the therapeutic applications as it uses a selective treatment of the cancer cells without causing damage to the neighboring ones. This therapy has been shown to influence cells adhesion and consequently reducing the tumor metastasis process [Dong et al 2013]. For the optical diagnosis, many optical devices were in service today such as optical tomography, optical biopsy, optical oxymeters, etc. In addition to these optical applications, a new integral model for selective photothermolysis to aid laser treatment of Porte wine stains was presented [Salles et al 2014]. The mechanisms of the interactions between a laser beam and biological tissues including the blood vessels situated at the dermis layer were used in various medical areas, such as in surgery, dentistry, dermatology, etc.[ Commarate & Wautelet 1999]. Light interactions with the human blood cells receive a considerable interest both from the theoretical modeling [Sivaramakrishnan et al 2007 & Yim et al 2011] and experimental studies [Rathore & Ali 2014, Minkovich et al 2001, and Zheng et al 2011]. In a recent work, spectroscopic and histological study of $CO_2$ laser interactions with rats blood vessels were carried out [Mohammed et al 2014]. We observed a pronounced modification of hemoglobin absorption due to photothermal effects associated with the infrared laser radiation in comparison to the control samples. In this work, we make use of the UV laser wavelength (355 nm) to irradiate a human blood samples and make a comprehensive analysis of the changes in blood cells counts and absorption spectra of hemoglobin.

## Equipments and Methodology 2.

A Q-switched $Nd^{+3}$-YAG medical laser purchased from Diamond Beauty Co. was used for exposing a human blood samples in vitro. The wavelength of (355 nm) which represents the third harmonic generation of the main laser wavelength (1064 nm) was used. This laser delivers maximum energy of (300 mJ ) at the (355 nm)with variable pulse repetition frequencies of (1-6Hz). The diameter of the output laser beam on the sample is 4mm, and the pulse width is 10 ns. The laser parameters are digitally displayed at the CCD screen of a controller minicomputer.

Sixty healthy volunteers' blood samples were used. These samples were collected from Basrah republic hospital for males with ages from (20) to (35) years old and weights from (60) to (75)Kg. Every blood sample of volume (5ml) was divided into two equal parts: laser irradiated and control sample. Each sample is irradiated by 500 laser pulses and different laser fluencies. The experimental set-up of the Q-switched $Nd^{+3}$ – YAG laser exposure of the human blood samples is shown in the Fig. (1). The blood parameters for each blood sample such as RBCs, WBCs, etc. were analyzed before and after laser radiation by using automated hematology analyzer

system, Human Count 5. These parameters were measured after the sample collection from the hospital for the control and immediately measured after the laser exposure.

The ANOVA software was utilized for the statistical calculations and data analysis. Absorption spectra of the blood samples were measured for the control and laser exposed samples by a double beam spectrophotometer model (UV-1800) supplied by Shimatzu Co. It covers the spectral range (190-1100 nm). An amount of 1µl from heparinized fresh blood was dissolved in 10 ml of NaCl isotonic solution for the spectroscopic analysis.

For the optical microscope imaging of the control and exposed samples, the samples were prepared by depositing one drop of heparinized blood on the microscope glass slide sandwiched by another similar glass slide, and then left for few seconds for drying. The coloration of the sample was done by using the lichen dye.

**Results and Discussion 3.**

Fig**.** (2) Shows that the number of the RBCs counts decreases as the UV laser dose increases. The change in the RBCs counts after the laser radiation didn't reach the statistical significance at probability ($p \leq 0.05$). However, the RBCs structural change confirms the change in the counts during the laser photolysis. A structural deformation and cells aggregation appeared through the optical microscope imaging gives an evidence of the effects of laser radiation on the number of the RBCs counts and on the structure. Fig. (3) shows the structural effects of the laser radiation in relative to the control samples. These slides clearly show the deformation of the cells, cells membrane damage and the cells aggregation resulting from the UV laser effects. The changes in the RBCs counts were detected by [Al Timimi et al 2012] using the green laser wavelength (532 nm) and fluencies of (0.5 to 5J/cm$^2$). They didn't mention however the statistical significance of their counts changes, and concluded that was due to ultra structural changes in the red blood cells cytoplasm.

The consequences of the UV laser interactions with the red blood cells and the decrease in the RBCs counts have also appeared in the absorption spectra of the laser exposed samples in relative to the control ones as shown in the Fig.(4). The primary peak situated at (410 nm) is pertained to deoxyhemoglobin (Hb), while the two secondary peaks at 539 and 572 nm are for the oxyhemoglobin ($HbO_2$) [Bosschaart et al 2013.].The absorbance of these peaks was found to be linearly decreased with the increase of the laser fluence(Fig. 5). This decrease in absorbance confirms the conformational changes of the heme groups. Vos et al 2008 have demonstrated that the conformational changes lead to slight variation in the index of refraction of the hemoglobin and to subsequent changes in the absorbance of the absorption spectrum. We have shown with another molecular system( $CO_2$ in $SF_6$ matrix) subjected to UV light that the decrease in the refractive index induces changes in the emission spectrum intensity of the oxygen singlet state $O(D^1)$ pertained to the $CO_2$ dissociation[ Mohammed 1990]. As the absorption spectrum is a mirror image of the corresponding emission spectrum, a change in the absorbance is expected to occur as a result of the change in the hemoglobin refractive index. Roggan et al 1999 have demonstrated that the laser beam might exerts a shear force when interacts with the RBCs in vitro producing a deformation and axial migration. We expect that the shear rate increases with an increase in the laser fluencies, and this mechanism along with corresponding RBCs deformation, might explain the decrease in the absorbance with increasing the laser energy densities. Mi et al 2004 have carried out a comparative study of 632.8 and 532 nm laser irradiation on some rheological factors in human blood in vitro. They have shown that the laser irradiation reduced the blood viscosities at different shear rates. This work gives an evidence for the change in blood refractive index through the viscosity change upon laser irradiation. Ma et al 2008 have found a significant increase in platelet aggregation by the excimer laser interactions with blood components in vitro. They demonstrated that higher laser energy had large aggregates.

These observations that are associated with the laser interactions with the human RBCs in vitro, confirm the interpretation of the decrease in the absorbance of blood hemoglobin with the UV laser fluencies in terms of the deformation and aggregation of the RBCs by the laser photolysis.

The analysis of the WBCs type basophiles after the UV laser irradiation of the blood samples showed a significant increase in the counts with ($p \leq 0.05$) with respect to the control samples (Table1). Fig. (6) shows the basophile cells counts as a function of laser fluence. The curve indicates a natural feature of particles growth. This increase in basophile WBCs is due to alterations in mitochondria leaded to fission of such mononuclear cells owing to the penetration of the UV laser inside the cells. Such a significant increase was detected by (Houssaen et al 2012), but for lymphocytes type WBCs using infrared $CO_2$ laser. The stimulating effect of the UV laser light on basophiles is the result of initiation of primary ,free radical reactions inducing activation of cells. Other authors [Wasik et al 2007] have also measured stimulating effects of low level He-Ne laser (632.8 nm) on human WBCs type lymphocytes. They concluded that the changes in mitochondria indicate the preparation of the cells for division.

The results of the interactions of the infrared and visible lasers with the human WBCs: lymphocytes can be extended for the inclusion of the UV laser WBCs stimulation and the basophiles cells division.

An immediate biological consequence of prestimulation of basophiles and lymphocytes by the laser

radiation of blood is an activation of phagocytosis and proliferation leading to destruction of pathogens by these cells. These events are the basis for the therapeutic action of the laser therapy.

## 4. Conclusions

We have established the mechanisms that responsible for the alterations of the RBCs, WBCs and the hemoglobin by the UV laser photolysis. The increase in the basophiles WBCs activates the immune system for the pathogens destruction. The decrease in the RBCs along with the decrease in hemoglobin absorption is associated with the deformation and aggregation of the RBCs. Based on the correlation between specific spectral changes and oxygen pressure saturation, it might possible to develop and optimize a clinical oxymeters. The sequence of events followed the UV laser irradiation of blood are the basis for many diagnosis and therapeutic applications.

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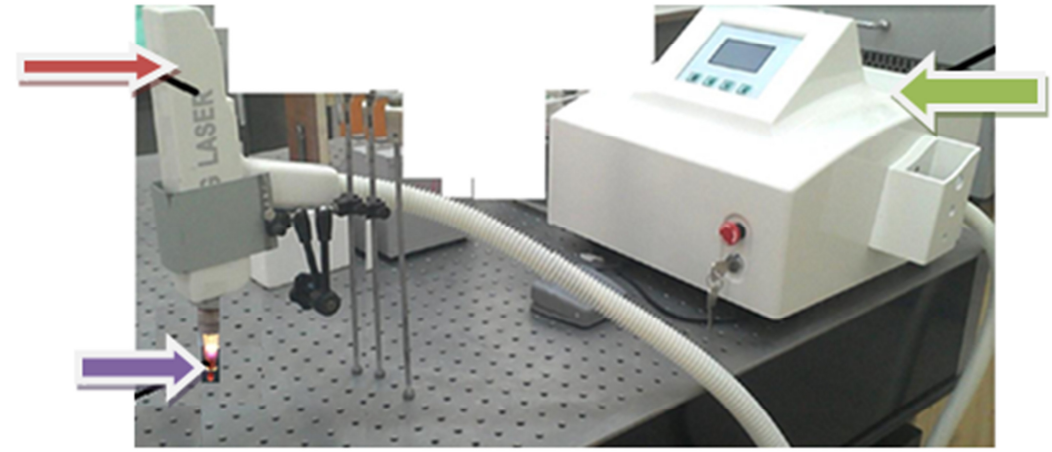



Figure 1: The experimental set-up of the UV laser photlysis: the controller

The laser head

The blood sample

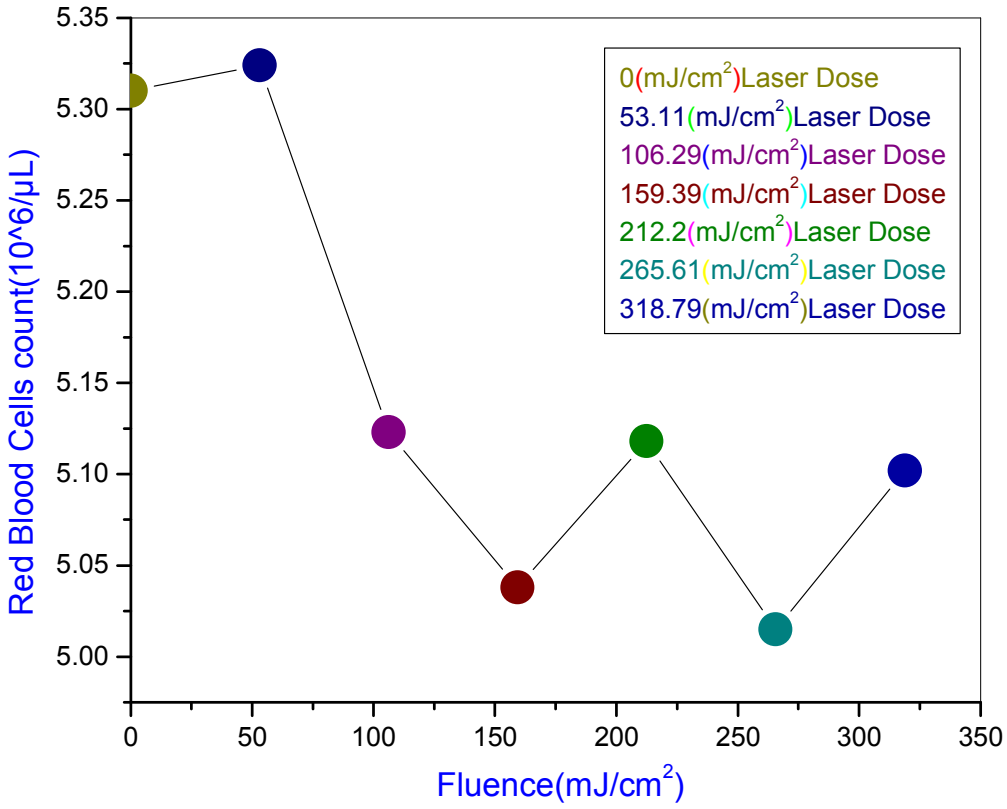
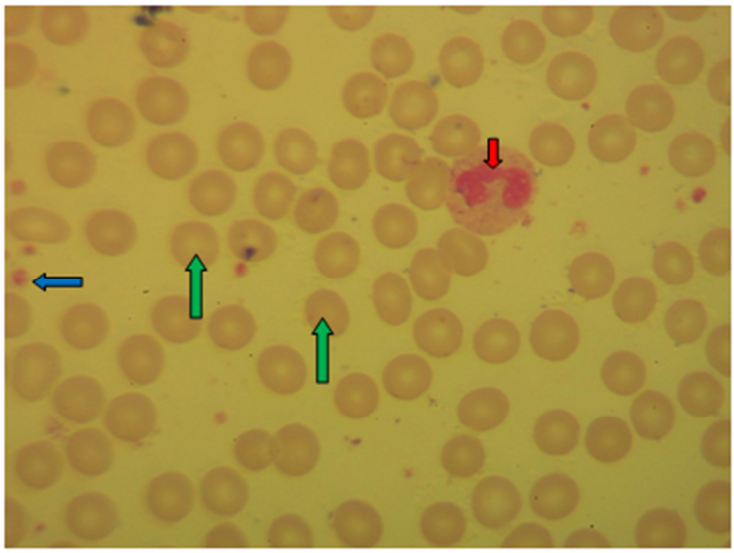
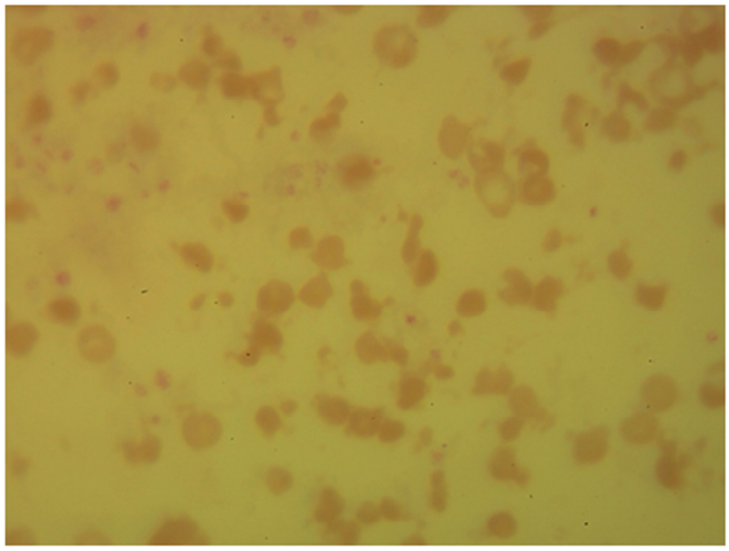



Figure 2. The red blood cell counts as a function of laser fluence.



(a)



(b)

Figure 3. (a) The control blood sample: RBCs ↑

WBCs ↓

Platelets ←

(b) The blood sample exposed to 318.79 mJ/ $cm^2$.

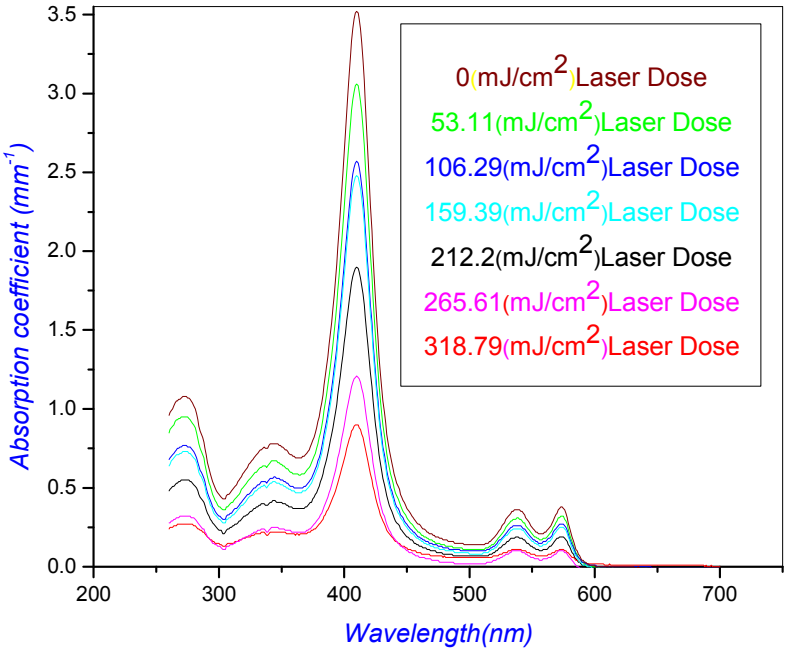



Figure 4. Absorption spectrum of the control blood sample and samples exposed to different laser fluence.

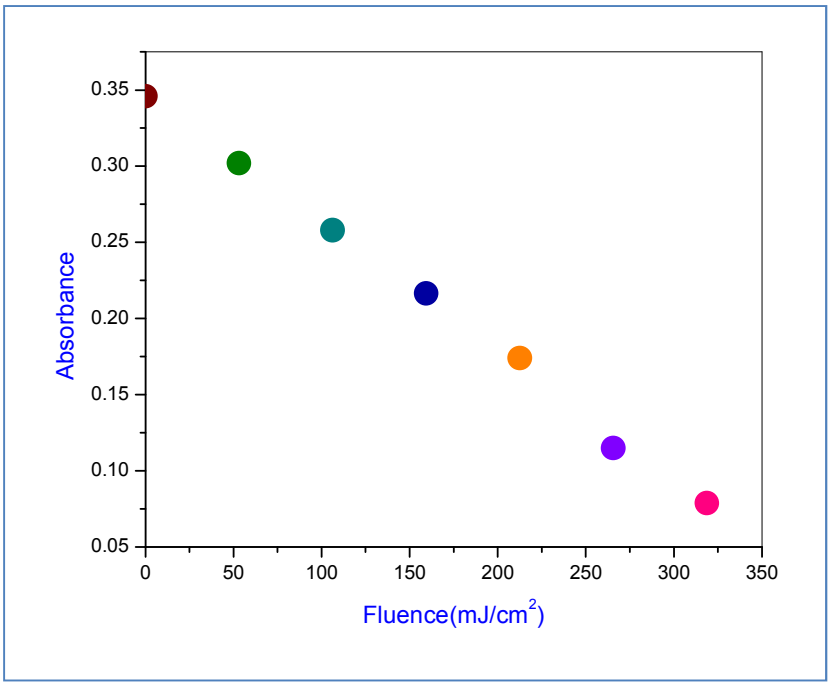



Figure 5.The hemoglobin absorbance as a function of the laser fluence.

Table 1. The mean number of counts of the basophiles WBCs as a function of the laser fluence.

| Fluencies (mJ /cm2) | (Mean×$10^3$ /μL)±SD |
|---|---|
| 0 | 0.033±0.312B |
| 53.11 | 0.031±0.0288B |
| 106.29 | 0.039±0.039B |
| 159.39 | 0.063±0.076BC |
| 212.5 | 0.24±0.367AC |
| 265.61 | 0.297±0.334A |
| 318.79 | 0.302±0.274A |

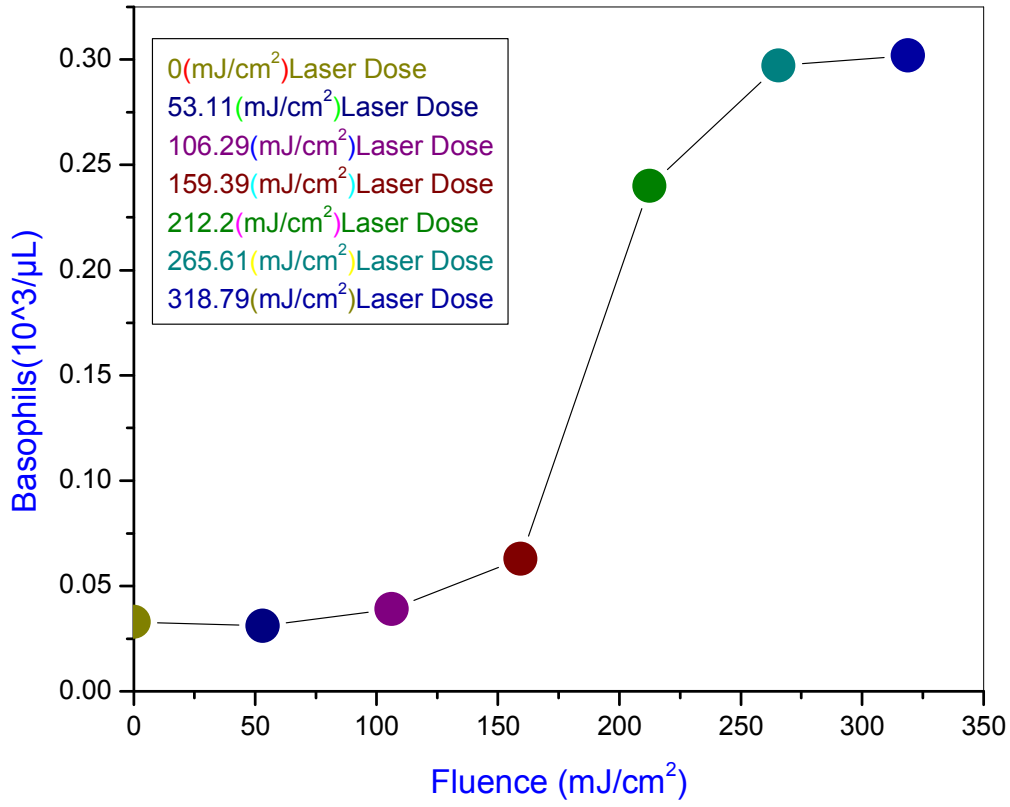



Figure 6. The basophiles counts as a function of the laser fluence.

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