How to Cite:
Majeed, S. H., Hasan, J. A., & Khalil, E. G. (2022). Photoacoustic technique for diagnosis of blood clotting. *International Journal of Health Sciences*, *6*(S4), 12195–12205. https://doi.org/10.53730/ijhs.v6nS4.11834

Photoacoustic technique for diagnosis of blood clotting

Saman Hameed Majeed
Biomedical Engineering Department, College of Engineering, Al-Nahrain University, Baghdad, Iraq
Corresponding author email: samanhameedmajeed@gmail.com

Jamal A. Hasan
Biomedical Engineering Department, College of Engineering, Al-Nahrain University, Baghdad, Iraq
Email: jamal.a.hassan@nahrainuniv.edu.iq

Eman Ghadhban Khalil
Biomedical Engineering Department, College of Engineering, Al-Nahrain University, Baghdad, Iraq
Email: eman.g.khalil@nahrainuniv.edu.iq

***Abstract*---**The photoacoustic technique is considered a scientific renewal in the world of diagnosing blood diseases. The motivation of this study is to get the bio-information about RBCs aggregation by analyzing of the photoacoustic signals represented with microscopic images which linking with artificial intelligence technology. The photoacoustic technique uses the pulsed laser to get the power of photoacoustic signals for recognizing the non-aggregated and aggregated RBCs, as well as estimate the viscosity of blood before and after laser irradiation, that gives an indication to the RBCs aggregation level. On the other hand, microscopic imaging explains the shape, size and amount of RBCs clusters depending on the processing of microscopic images by threshold algorithms.The results of the photoacoustic technique are useful for detection, prediction and identification the potential RBCs clustering and formation of clots in arteries and veins, which occur in several diseases such as infection, inflammation and cardiovascular diseases.

***Keywords*---**RBCs aggregation detection, photoacoustic signal analysis, photoacoustic-artificial intelligence, photoacoustic image analysis.

Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

Introduction

Many human diseases are closely related to the blood, and cause red blood cells to accumulate due to the protein fibrinogen, which is activated in the presence of infections (Simmonds et al., 2013). The photoacoustic (also known as optoacoustic) effect is the phenomenon that includes ultrasound generation by laser induction. For clinical diagnosis, the blood sample is illuminated by laser light, which is absorbed by red blood cells within the blood. This energy is converted to acoustic waves, the transducer captured the acoustic waves at a certain range of frequency. The photoacoustic imaging is developed to obtain optimum technique related to the photoacoustic system, which is based on capturing the microscopic images to the RBCs aggregated and non-aggregated, then processing the images with algorithms of artificial intelligence.

Artificial intelligence plays a major role in accurately analyzing the data and images, as it has begun to exist in several medical fields as a sophisticated technology for deep and rapid analysis as well as obtaining better results for medical examinations (Hajdowska et al., 2022). Several studies have been introduced to detect the ESR value by estimate the RBCs aggregation in the blood by detect the power of photoacoustic signal (Kinnunen, 2015). Another study using the viscosity of blood to indicate the relation between the viscosity and blood irradiation that refer to the ESR value, when compared the viscosity before and after blood irradiation (Kim et al., 2015). In other hand, some studies have resorted to measure the ESR value and RBCs aggregation by analysis the microscopic images(Hajdowska et al., 2022; Jiang et al., 2022), or using an acousto-optic imaging technique (Mohammed et al., 2021). Current study, gathering more than technique to obtain accurate detection of RBCs aggregation which reflected the ESR value of blood viscosity by using PA system.

Material and Method

Prepare the samples

From sixty subjects blood samples were collected in EDTA K3 tubes at 20 C ° temperature. The erythrocyte sedimentation rate (ESR) test refers to the level of RBCs aggregation in the blood, therefore the manual ESR test must be done to all the blood samples before applying the photoacoustic system. The centrifugation at 2000 rpm for 6 minutes was done for all the blood samples and the precipitated cells washed two times with phosphate-buffered saline (PBS, Germany) PH 7.4 to remove the plasma and the other contents of blood. The RBCs aggregated by Dextran-70($C_6H_{10}O_5$,7000kDa molecular weight, Sigma, assay 99.9%) which is synthetic polymer has the same action as fibrinogen protein. The dextran concentrations (1%, 3% and 8% weight/volume) were used for suspend the RBCs at (0%, 10%, 20% and 40%) hematocrit, in order to induce various levels of aggregation.

Photoacoustic make effect on the blood sample

Several medical information can be obtained from blood due to PA effect when was irradiated with pulsed laser and generate the ultrasound. When the blood absorbed the radiation energy, convert this energy to vibrations, which generate the acoustic (ultrasound) waves, can be captured by piezoelectric transducer. When these signals amplified by amplifier circuit, to be analyzed and recorded by the oscilloscope and laptop. The analysis of power photoacoustic signals is useful to detect the RBCs aggregation, also estimation viscosity of the blood before and after irradiation were used to explain the difference in the absorbed energy depending on the RBCs aggregation levels, the Schematic photoacoustic system shown in Fig (1)

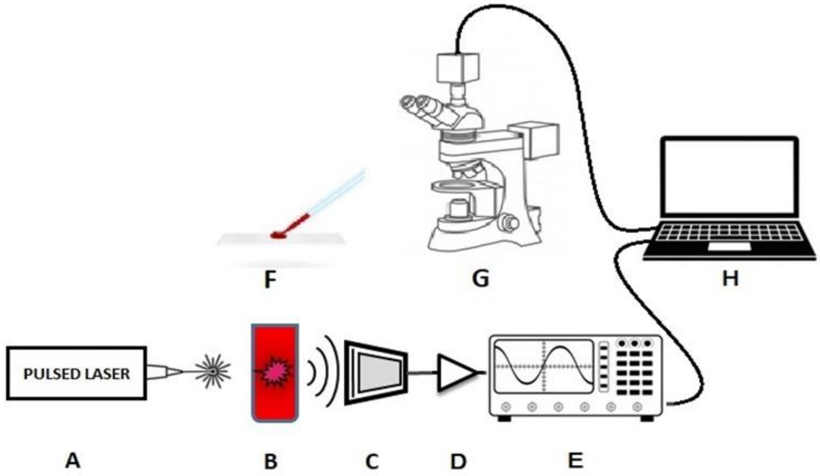


Figure 1. Schematic photoacoustic system: A) Nd:YAG laser 1064nm, B) Cubic quartz cuvette, C) Transducer, D) Amplifier circuit, E) Oscilloscope, F)Glass slide, G) Optical microscope with CCD camera, H) laptop.

Microscopic images with PA technique

The radiated blood samples were examined by optical microscope (GENEX – OPTIK 20), which provides by a CCD camera (Genex Ltd. - 5mm) and the magnification was 40X. The blood smears on the slides were collected from all the irradiated blood samples to get the RBCs images. The microscopic images of RBCs aggregation are analyzed by algorithms of artificial intelligent technique to detect the aggregation level, also watching the morphology of RBCs clusters and evaluate the hemoglobin concentration of blood. The threshold algorithms act as common and fast in image processing, there are two types were used for analyzing the microscopic images:

1- Global threshold algorithm:
   This algorithm depends on using only one threshold value to segment each image (Rogowska, 2009).
2- Local (multi) threshold algorithm:
   The image segmentation, that contains more than two modes of regions, it is possible to segment it by using a multi-thresholding technique (Gonzalez, n.d.).

Experimental work

The photoacoustic system is based on the radiation by Nd:YAG laser 1064nm at the pulsed rate 6Hz and the radiation energy of 20 mJ/cm^2 per pulse, which irradiate the blood sample that represents as RBCs suspended in PBS solution [40%] at different dextran concentrations [1%, 3%, and 8%] at various aggregation levels. The RBCs sample was put in the cubic quartz cuvette (1x1x4 cm^3), to achieve the condition of equal access of the laser to all levels of the cuvette equally without any refraction in the spot area. The flat transducer contains 6 channels array of piezoelectric crystals (5MHz) used to capture the ultrasound waves from large area. The power of photoacoustic signals represents the state of RBCs, whether it was aggregated or not, these signals analyzed by the oscilloscope (ATTEN 1022C) after amplified by a circuit composed from double MOSFET amplifiers (CA3140A) can be handled with high frequencies, the components of photoacoustic system shown in Fig (2).

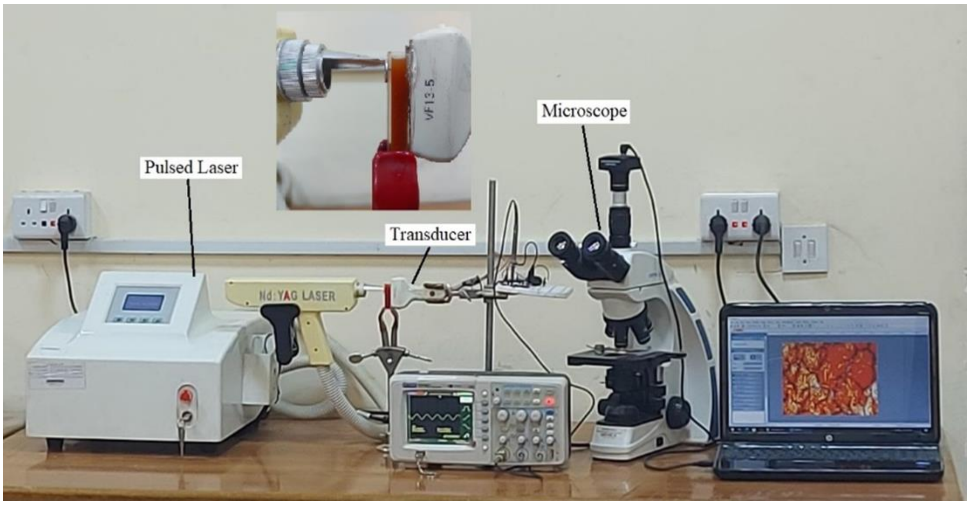


Figure 2. Photoacoustic components: Nd:YAG laser, cubic cuvette, transducer, amplifier circuit, oscilloscope, optical microscope and laptop.

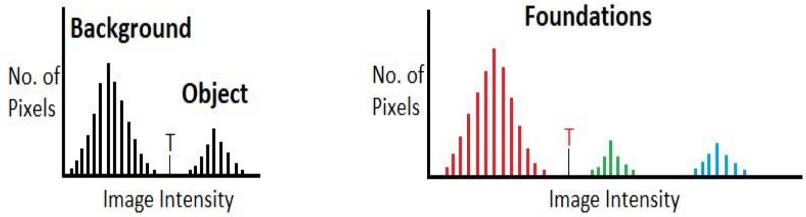
Signals and images analysis

The envelope of the out shape of signals can be used to compute the power of photoacoustic signals and take the Fast Fourier Series (FFT) that converts the time domain to the frequency domain (McCallen, 2022). The viscosity of all samples measured before and after irradiation according to Marwa J. Yahia, in 2020 (Yahia et al., 2020), Then the power of PA signal and viscosity were calculated with mean value and plotted for all samples with the statistical significance of the $p\text{-value} < 0.05$ was computed by using the MATLAB software.

The microscopic digital images analyzed by segmentation algorithm, it is considered the common and modern method to detect the clusters and the amount of RBCs aggregation. Threshold algorithm considered the simple and the fast technique in the segmentation method, based on the pixels of the image, these pixels represent the objects and background. There are two algorithms used to separate or distinguish the pixels of object from pixels of background:

1- Global threshold: This algorithm used a single threshold value to separate the pixels of each image.
2- Local threshold or multi threshold: which is based on conversion the color image into binary image mode.

The threshold value (T)is very sensitive to separate color or gray modes intensity of the image that is graduated between 0 to 255 color brightness as shown in Fig (3).



A B

Figure 3. The histogram of threshold algorithms: A) global, B) multi-threshold

The difference between the colored or gray pixels is explained as histogram plots that represent the peak values of pixels. The ratio of red pixels to other color pixels gives an indicator to the RBCs clusters in the blood smear, while the ratio of dark-red pixels to white-red pixels detects the hemoglobin concentrations.

Results and Discussion

The power of photoacoustic signal calculated from 60 subjects, the average power or signal amplitude of the RBCs suspensions with dextran concentrations (1%, 3% and 8% wt/vol) increase from [1%] to reach the maximum at [3%] when the frequency 5MHz and decrease the curve to the minimum at the [8%] as shown in Fig (4). The peak value of the average power of the photoacoustic signal at [3%] refers to occurrence the maximum absorption of laser by RBCs aggregation, that give high peak value of power compared with the other dextran concentrations (1% and 8% wt/vol), the minimum and maximum power of photoacoustic signal ranging from 1.1 and 22 dB at a different of hematocrit levels.

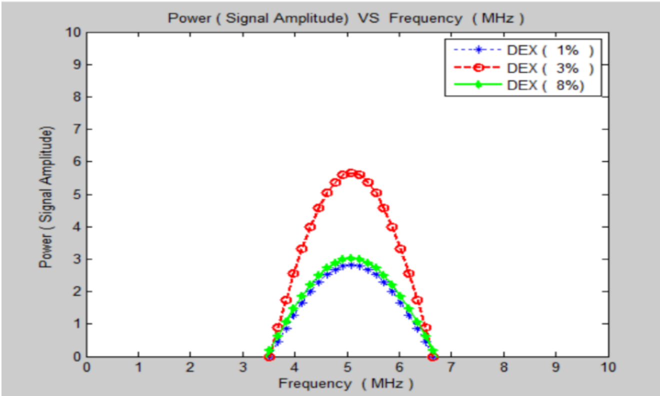


Figure 4. The power (signal amplitude) at variant dextran concentrations (1% 3% 8% w/vol) with the center frequency 5 MHz

The viscosity also estimated for each blood sample before and after laser irradiation, the results noticed the inverse relation between the time of radiation and the viscosity of sample as well as the viscosity decreased after irradiation as shown in Table (1).

Table 1. The mean of samples viscosities for 60 subjects before and after laser irradiation

T(min)	Mean samples viscosities(cP) (Before Irradiation)			Mean samples Viscosities(cP) (After Irradiation)		
	DEX/PBS 1% (wt /vol)	DEX/PS 3% (wt /vol)	DEX/PS 8% (wt /vol)	DEX/PS 1% (wt /vol)	DEX/PBS 3% (wt /vol)	DEX/PS 8% (wt /vol)
1	49	72	47	46	66	44
2	46	66	44	43	65	40
3	43	65	40	40	58	37
4	40	58	37	39	54	35
5	39	54	35	38	45	34
6	38	45	34	37	43	35
7	37	43	35	36	38	33
8	36	38	33	35	36	31
9	35	36	31	31	33	30
10	31	33	30	30	31	30

Also, the relation showed a high decline in the viscosity at the RBCs sample with dextran [3%] compared with the other concentrations of dextran [1% and 8%], that is an indicator to the strong effect of the irradiation on the RBCs aggregation as shown in Fig (5).

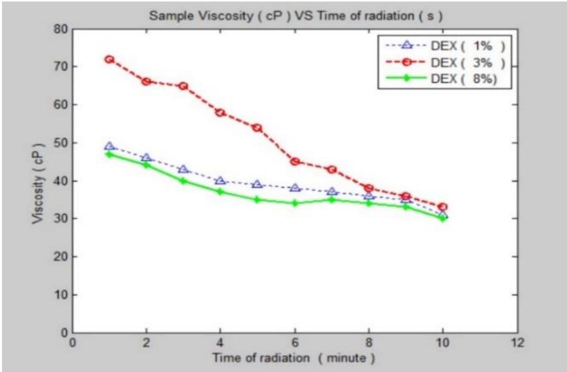


Figure 5. The inverse relation between the viscosity (cP) of the samples and the periods of illumination (minute)

The analysis of the photoacoustic microscopic images by threshold techniques showed another method to detect RBCs aggregation while gave an indication to ESR value. The global algorithm separates the RBCs aggregation of black pixels from the white pixels of the background by computed the ratio of gray level of the pixels image as shown in Fig (6).

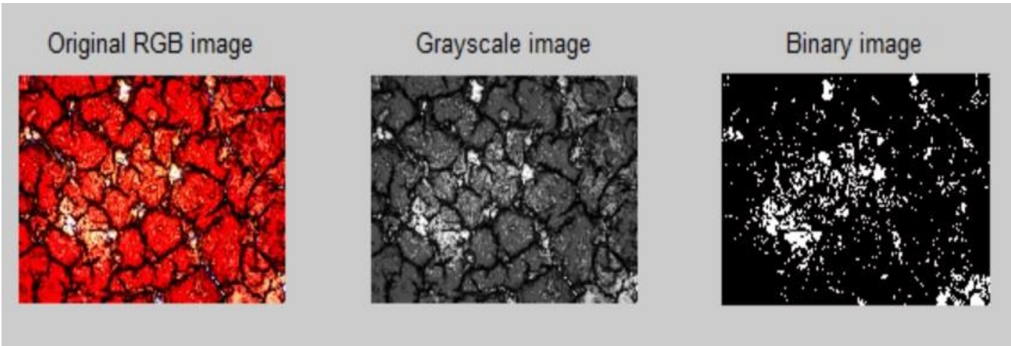


Figure 6. RBCs aggregation detected by global threshold algorithm

The global threshold can be represented as a histogram to explain the different values of pixels between the object and the background as shown in Fig (7)

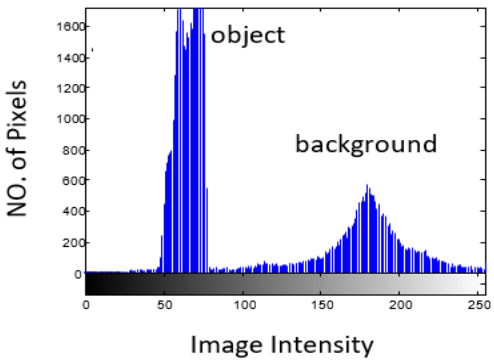
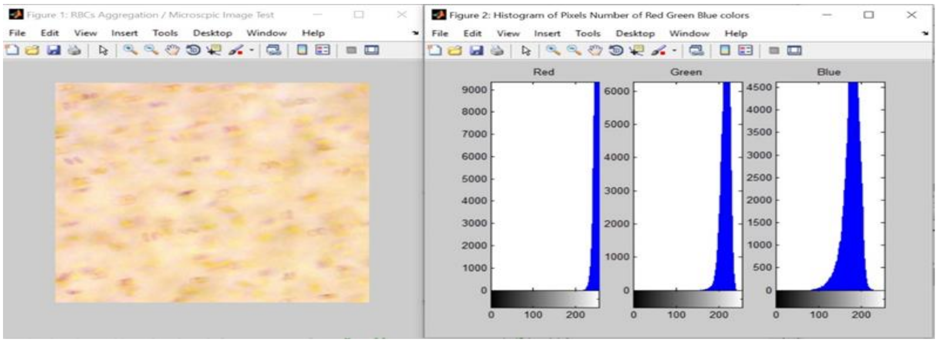
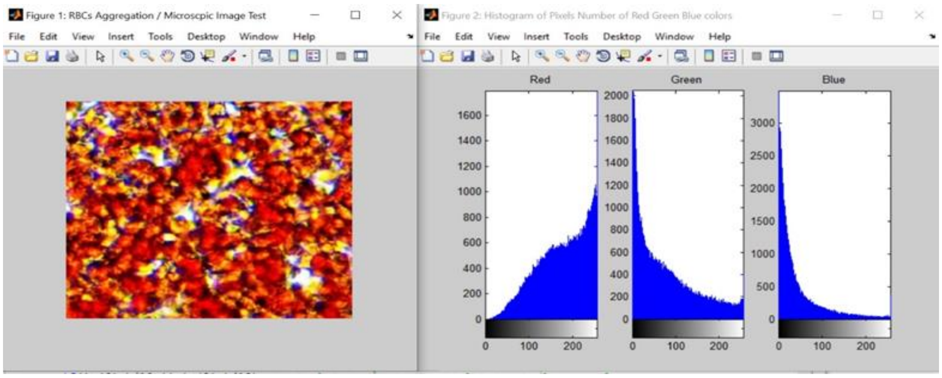


Figure 7. Histogram of global threshold algorithm

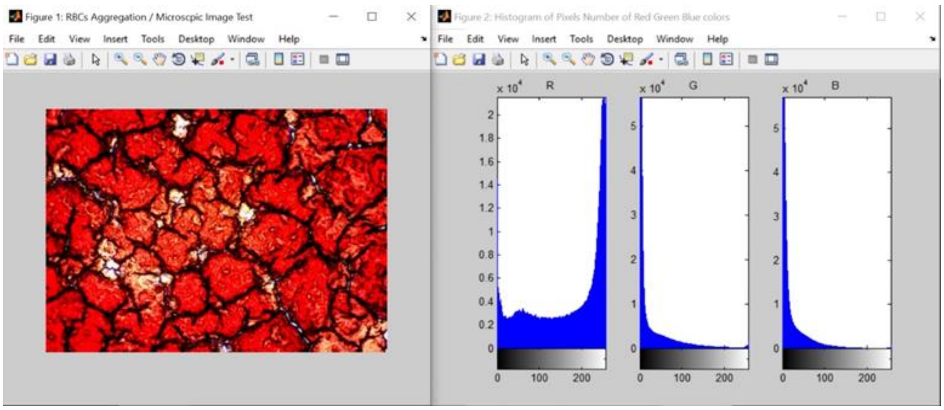
The local multi-threshold algorithm was more flexible for detecting the red color pixels when the different red levels were found by the multi-threshold value levels Red – Green – Blue colors (RGB) for each image range from 0 to 255 color brightness as shown in Fig (8).



A



B



C

Figure 8. Detect the colors level of microscopic image by using threshold algorithm in a histogram A) Low red color density in the image (483x466 Pixels), B) Increase red color density in the image (362x317 Pixels), C) High red color density scale in the image (1280x916 Pixels).

The alteration hemoglobin (Hb) concentration increased in the RBCs (erythrocytes), is used clinically to determine the presence of diseases or smokers, the degree color intensity of high hemoglobin concentration was red-dark while the color was faint or whitish red at low [Hb] concentration (Article, 2018), therefore the ratio of dark color was more in the high [Hb] image than low [Hb] image, thus more white color in the image with low [Hb]. The red color graduation was (Hb = 10g/dl, HCT= 31), (Hb = 14g/dl, HCT =29), (Hb= 16g/dl, HCT=33), which manifested a direct relation between the [Hb] and RBCs count with other color pixels as shown in Fig (9).

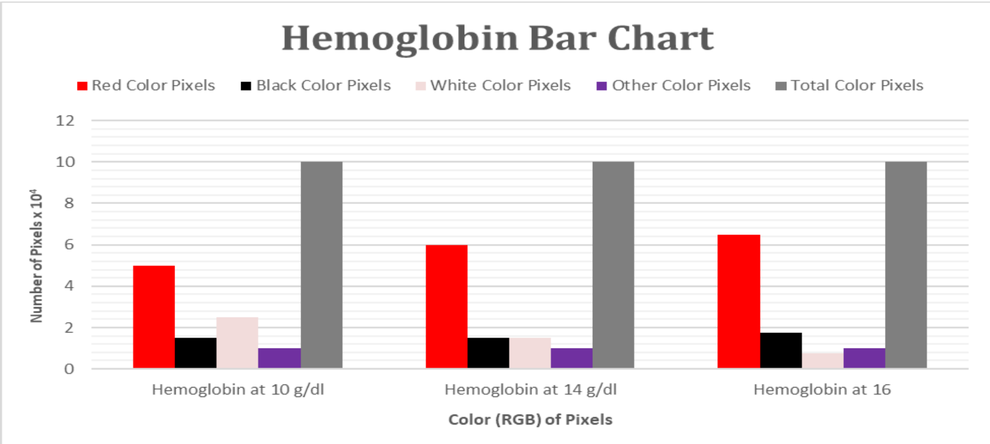


Figure 9. Relation between hemoglobin (Hb) and color pixels of image 1) Low Hb(g/dl) level, 2) Middle Hb(g/dl) level, 3) High Hb (g/dl) level

The mean of pixel's number of red color was significantly high in the (RGB) image, thus when compared with the other colors pixels through the ratio of red pixels color to other colors in (RGB) image which indicates to high RBCs aggregation thus this means an increasing ESR value, but a decrease in the number of red pixels means reducing the ESR value, as shown in Fig (10).

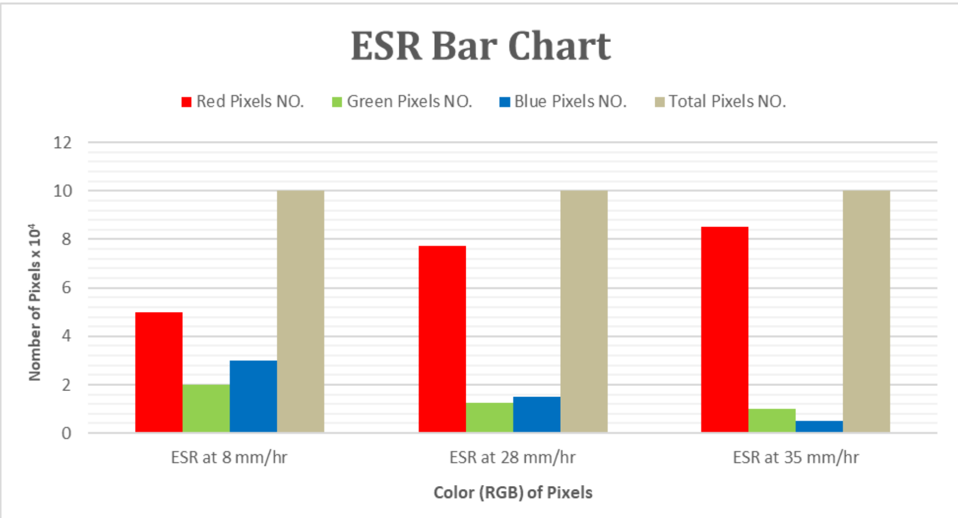


Figure 10. Relation between ESR values and colors pixels numbers of image

Conclusion

The photoacoustic system explains a new method to detect the RBCs aggregation, as well as [Hb] level. The changing power of photoacoustic signals associated with the energy absorbed by RBCs, which released as a power of photoacoustic signals ranging from 1.1 to 22 dB at the minimum and maximum aggregation of different RBCs suspension with dextran concentrations 1%, 3% and 8%, the maximum power of PA signal at [3%] which reveals minimum aggregation, while the minimum power of PA signal at [1% and 8%] revealing minimum aggregation.The RBCs aggregation and viscosity not significantly decreased with the present of laser, but there is inverse relation between the viscosity and radiation time determined by high decline curve at RBCs-dextran [3%] while less decline curve at RBCs- dextran [1% and 8%].

This photoacoustic microscopic imaging is a hybrid modality in medical clinical diagnosis of RBCs morphology, clustering density and hemoglobin concentration.
The link between photoacoustic microscopic image and artificial intelligence techniques creates a complete system to analyze the functional and structural image depending on the intensity of image color pixels to detect the RBCs aggregation, hemoglobin concentration in the blood sample and predict the cardiovascular disorders.

References

Article, O. (2018). *World Health Organization Hemoglobin Color Scale: A Useful Point-of-care Test to Detect Anemia*. 133–136. https://doi.org/10.4103/MJDRDYPU.MJDRDYPU

Gonzalez, R. C. (n.d.). *Digital Image Processing Third Edition*.

Guerrero, J. A. A., Moreira, J. A. M., Zambrano, M. J. Z., Rivas, F. E. C., & Pilligua, M. L. B. (2021). Project-based learning and its contribution in virtual education. *International Research Journal of Management, IT and Social Sciences*, *9*(1), 10-18. https://doi.org/10.21744/irjmis.v9n1.1973

Hajdowska, K., Student, S., & Borys, D. (2022). Graph based method for cell segmentation and detection in live-cell fluorescence microscope imaging. *Biomedical Signal Processing and Control*, *71*(PA), 103071. https://doi.org/10.1016/j.bspc.2021.103071

Jiang, X., Chen, Z., & Xing, D. (2022). Super-resolution photoacoustic imaging based on saturation difference of transient absorption. *Optics and Lasers in Engineering*, *150*, 106877. https://doi.org/10.1016/j.optlaseng.2021.106877

Kim, J., Lee, H., & Shin, S. (2015). Advances in the measurement of red blood cell deformability: A brief review. *Journal of Cellular Biotechnology*, *1*(1), 63–79. https://doi.org/10.3233/jcb-15007

Kinnunen, M. (2015). A pulsed photoacoustic technique for studying red blood cell sedimentation. *Journal of Biomedical Photonics & Engineering*, *1*(1), 81–89. https://doi.org/10.18287/jbpe-2015-1-1-81

McCallen. (2022). *Power spectrum calculations using the fast Fourier transform*. https://doi.org/10.2172/188886

Mohammed, F., Hasan, J. A., & Ghadhban, E. (2021). *Development of the determination of ABO and Rh systems of blood group typing using acousto-optical technique Development RI the Determination of ABO and Rh Systems of*

Blood Group Typing Using Acousto-Optical Technique. *030002*(November).
Rogowska, J. (2009). Chapter 5 - Overview and Fundamentals of Medical Image Segmentation. In I. N. BANKMAN (Ed.), *Handbook of Medical Image Processing and Analysis (Second Edition)* (Second Edi, pp. 73–90). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012373904-9.50013-1
Sawali, L. (2018). Drills forehand training strategy on the stroke of forehand drive ability in tennis. *International Journal of Physical Sciences and Engineering*, *2*(2), 11–20. https://doi.org/10.29332/ijpse.v2n2.133
Simmonds, M. J., Meiselman, H. J., & Baskurt, O. K. (2013). Blood rheology and aging. In *Journal of Geriatric Cardiology* (Vol. 10, Issue 3, pp. 291–301). https://doi.org/10.3969/j.issn.1671-5411.2013.03.010
Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
Yahia, M. J., Hasan, J. A., & Musawi, M. S. A. (2020). Biostimulation effect of DPSS laser irradiation with different power densities and radiation times on blood viscosity in vitro. *AIP Conference Proceedings*, *2213*. https://doi.org/10.1063/5.0000438