



# Application of continuous-wave photoacoustic sensing to red blood cell morphology

Abhijeet Gorey<sup>1</sup> · Deblina Biswas<sup>1</sup> · Anshu Kumari<sup>2</sup> · Sharad Gupta<sup>2</sup> · Norman Sharma<sup>3</sup> · George C. K. Chen<sup>4</sup> · Srivathsan Vasudevan<sup>1,2</sup> 

Received: 2 February 2018 / Accepted: 15 August 2018 / Published online: 22 August 2018  
© Springer-Verlag London Ltd., part of Springer Nature 2018

## Abstract

The feasibility of continuous wave laser-based photoacoustic (CWPA) response technique in detecting the morphological changes in cells during the biological studies, through the features extracted from CWPA signal (i.e., amplitude) is demonstrated here. Various hematological disorders (e.g., sickle cell anemia, thalassemia) produce distinct changes at the cellular level morphologically. In order to explore the photoacoustic response technique to detect these morphological changes, we have applied CWPA technique onto the blood samples. Results of our preliminary study show a distinct change in the signal amplitude of photoacoustic (PA) signal due to a change in the concentration of blood, which signifies the sensitivity of the technique towards red blood cell (RBC) count (related to hematological disease like anemia). Further hypotonic and hypertonic solutions were induced in blood to produce morphological changes in RBCs (i.e., swollen and shrink, respectively) as compared to the normal RBCs. Experiments were performed using continuous wave laser-based photoacoustic response technique to verify the morphological changes in these RBCs. A distinct change in the PA signal amplitude was found for the distinct nature of RBCs (swollen, shrink, and normal). Thus, this can serve as a diagnostic signature for different biological studies based on morphological changes at cellular level. The experiments were also performed using conventional pulsed laser photoacoustic response technique which uses nano-second pulsed laser and the results obtained from both PA techniques were validated to produce identical changes. This demonstrates the utility of continuous wave laser-based photoacoustic technique for different biological studies related to morphological cellular disorders.

**Keywords** Continuous wave laser based photoacoustic (CWPA) · Red blood cell · Morphological cellular disorder · PA amplitude

## Introduction

Red blood cells (RBCs) play a crucial role in human health. The main function of RBC is to transport oxygen to different parts of the body through nerves. For a healthy person, RBCs are biconcave disk shaped having flexible structure [1]. It is well known that many diseases or genetic conditions can alter

the size and shape of RBCs. This reduces the ability of the cells to bend/deform, thereby restricting its ability of oxygen transportation. Usually the RBC of a healthy individual has a diameter of approximately 7.8  $\mu\text{m}$ , while its height is 1–2  $\mu\text{m}$  and volume is around 94  $\mu\text{m}^3$  [2, 3].

Automated clinical diagnosis of blood disorders use a set of indices to detect the health of the RBCs through techniques such as electrical impedance or light scattering. Patients having blood-related disorders would have the size, shape, and volume of RBCs altered. While the available automated techniques can determine the abnormality in RBC, but would fail to determine its shape and size directly due to their limitations [4]. Hence, detection of size and shape of any abnormal RBC would require more tests, thereby making it laborious and time consuming.

In this paper, we propose to use continuous-wave photoacoustic (CWPA) sensing technique to differentiate morphological changes in red blood cells. Although, conventionally

✉ Srivathsan Vasudevan  
svasudevan@iiti.ac.in

<sup>1</sup> Discipline of Electrical Engineering, Indian Institute of Technology Indore, Indore 453552, India

<sup>2</sup> Discipline of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Indore 453552, India

<sup>3</sup> Choithram Hospital and Research Center, Indore, India

<sup>4</sup> B. C. Photonics Ltd., Vancouver, Canada

photoacoustic sensing is performed using nano-second laser pulses, continuous-wave photoacoustic sensing has a significant advantage over the conventional photoacoustic sensing. Thus, we have indigenously developed the experimental setup for CWPA and characterized it with some standard sample. Further, the technique was applied onto red blood cells to differentiate their morphological variation. To ensure the accuracy of the results obtained through CWPA, they were also corroborated with the conventional photoacoustic sensing.

Briefly, photoacoustic (PA) imaging is a hybrid imaging modality that merges optical absorption contrast with ultrasound resolution. PA technique is mostly explored as an imaging technique in terms of tomography and microscopy. The basic principle of obtaining PA image is to irradiate the sample by a nano-second laser pulse. The sample absorbs the light (depending upon the absorption spectrum of sample), and this causes temperature excursion and subsequently undergoes thermal expansion. Between the time gap of two laser pulses, the expanded sample volume gets compressed thereby releasing a pressure wave, which would be acquired using ultrasound transducers. These time domain acoustic waves can be recorded using a digital oscilloscope. In order to obtain an image, an ultrasound array or a single sensor is scanned across the sample. The signals obtained from these sensor positions are used to form an image using various image reconstruction algorithms (such as time-reversal algorithm or delay and sum algorithm) [5].

Despite high promising ability of the conventional PA imaging technique, it becomes very difficult for the conventional imaging technique to reach clinical benches. The cost and complexity involved in nano-second pulsed laser makes it difficult to shift the technique periodically for clinical trials. Therefore, research has moved towards utilizing compact lasers (solid state lasers and laser diodes). The intensity modulated continuous wave laser diode is explored in photoacoustic experiments and was found effective. In this technique, popularly known as CWPA, a compact laser diode would replace the bulky nano-second pulsed laser. It has found interesting applications in tomography where the technique has been applied to tissues [6]. The power of the laser diode (usually much lower than a peak power of nano-second pulsed laser) is one of the most important challenges faced in this field.

In a typical PA imaging technique, either array of sensors is used around the sample which increases the cost and complexity of the technique or single sensor is used which has to scan across the sample to get the PA information which causes repeated heating of the sample [7–9]. So as an alternative to PA imaging, the PA signal, known as PA response can also be utilized to form a quick diagnosis screening tool. In PA response technique, the features of time domain PA signal such as amplitude, rise time, and the fall time of the signal obtained from the ultrasound sensor, can provide more critical information of the biological sample [10, 11]. Thus, the extensive

array of sensors and signal processing algorithms for forming an image can be avoided which in turn can reduce the cost and complexity of the system.

In this study, we propose to utilize continuous-wave (CW) laser-based photoacoustic signal response to act as a screening tool to differentiate the structural properties of the red blood cells. Since, till now, the CW laser diodes are explored for photoacoustic imaging of tissues and this is the first attempt to utilize CWPA signal response for real biological samples, a comparative study with nano-second pulsed laser is performed and results are correlated. The diagnosis reported here would focus on the screening of red blood cells and differentiate them, based on the structural properties of the cells. This study would find applications in hematology particularly towards sickle cell anemia. Sickle cell anemia is an inherited blood disorder in which the population of red blood cells would reduce drastically as well as the shape of red blood cells would be affected.

In order to perform this study, the concentration of RBCs in whole blood sample was varied (signature of disease anemia) and subsequently change in the shape of RBCs (sickle cell anemia) was induced by preparing hypertonic and hypotonic solutions. Further, these set of blood samples were used in experiments and it was observed that the PA signal shows a distinct change in amplitude for different set of blood samples. Thus, the change in amplitude of PA signal can be used as a signature for the mentioned hematological diseases.

## Materials and methods

### Sample preparation

For this study, human blood from volunteer healthy donors was used as a standard sample (ethical clearance was obtained from bio-safety and bio-ethics committee, Indian Institute of Technology Indore) to perform experiments. To investigate the morphological changes in the shape of red blood cells (RBCs) some solution has to be added in the blood which can change its tonicity. In order to obtain different tonicity of the blood sample, three different salt (NaCl) solutions were prepared namely isotonic, hypertonic, and hypotonic solutions. Difference in the salt solutions lies in the different concentrations of NaCl added with distilled water. The proportion of distilled water and NaCl added for preparing the salt solutions is listed in the Table 1 below.

To prepare the solutions, NaCl was properly weighted and added in 5 ml of distilled water, stirred for around 10–15 min and was stored at room temperature. This solution is added in a specific amount to obtain different tonicity in the blood sample. Among these solutions, isotonic solution possesses similar tonicity to that of blood plasma [12]. Thus, addition of isotonic solution to the blood does not

**Table 1** Isotonic, hypotonic, and hypertonic salt preparation

Sample	Water (ml)	NaCl (g)
Isotonic	5	0.045
Hypotonic	5	0.001
Hypertonic	5	0.7

change the shape of its RBCs and it maintains the normal disc shape of RBC. It is also observed that by increasing the concentration of isotonic solution in the blood, the number of RBCs in the blood sample reduces. We have varied the proportion of isotonic solution in the pure blood sample to obtain four samples with 20%, 60%, 80%, and 100% of blood (rest isotonic solution). Further, experiments were performed with these set of blood samples in order to investigate the changes in amplitude of the photoacoustic signal with respect to the change in concentration of blood.

Now, in order to study the changes in the photoacoustic signal due to the morphological change in RBCs, hypertonic and hypotonic solutions were used with the human blood samples [13]. Table 1 indicates that hypertonic and hypotonic solutions contain more and less salt (NaCl) concentration respectively as compared to the isotonic solution. On mixing these salt solutions (hypertonic and hypotonic) with pure blood, it causes the change in the osmotic pressure of the blood, leading to shrinking or swallowing of the RBCs, respectively.

To perform experiments, these set of blood samples were classified under two categories namely, test and control. The sample which undergoes the morphological change is categorized as test samples (hypertonic and hypotonic samples) and one which does not undergo the morphological change (isotonic sample) is categorized as control sample [14]. To compare the PA signal response of normal disc-shape RBCs and morphologically changed RBCs, experiments were performed with test and control samples. To prepare the blood sample with different tonicity, the salt solution and blood were mixed in fixed proportion (depending upon the type of blood sample). After mixing, the sample was kept at room temperature for around 1 h so that the sample may undergo morphological change. Table 2 illustrates the proportion of blood and salt solution used for preparing the samples.

Fifteen blood samples in each category were prepared for experiments. Once the sample was prepared, it was investigated for the PA signal amplitude using CWPA response

**Table 2** Isotonic, hypotonic, and hypertonic sample preparation

Blood sample	Blood (ml)	Solution (ml)
Isotonic sample	0.3 ml	0.2 ml isotonic solution
Hypotonic sample	0.3 ml	0.2 ml hypotonic solution
Hypertonic sample	0.3 ml	0.2 ml hypertonic solution

technique and conventional ns-pulsed laser PA technique. After each experiment the shape of RBCs was also investigated through microscope. Thus, the change in PA signal amplitude with respect to the change in shape and size of RBCs were investigated.

### PA experimental setup

This study illustrates the comparative study between pulsed and continuous wave laser based PA signal response technique. Thus the PA signal response for each blood sample was measured consecutively using CWPA and pulsed laser PA technique separately as shown in Figs. 1 and 2, respectively.

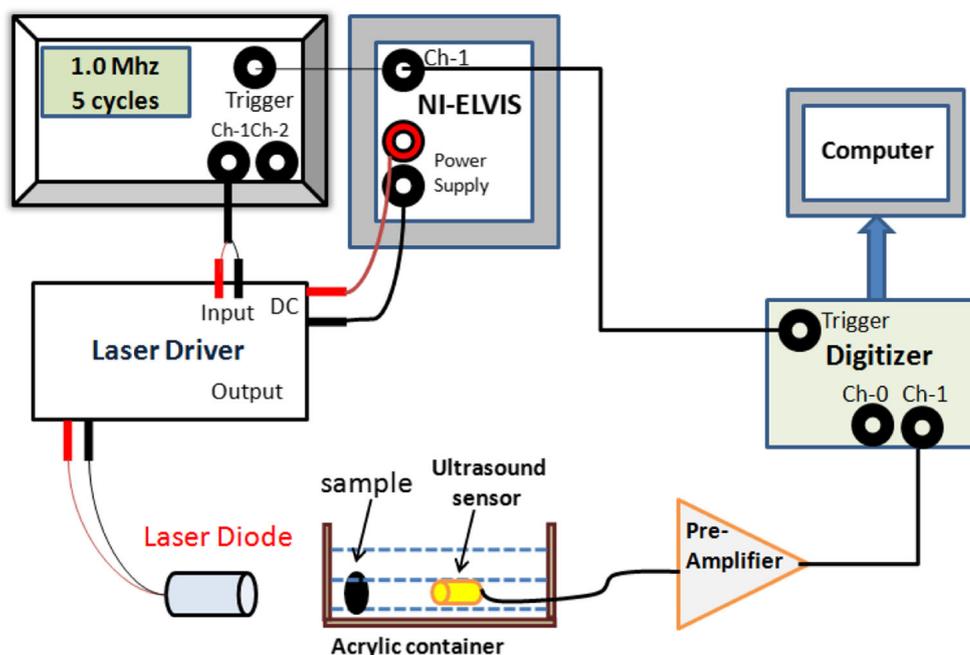
### Continuous-wave laser excited PA experimental setup

The CW PA system mainly involves excitation and the data acquisition systems as shown in Fig. 1. The compact and durable laser diode from Osram having center wavelength 460 nm and 1.6 W maximum power is used for experiments whose intensity was modulated and controlled by a custom made laser driver circuit. The laser driver circuit can deliver the maximum average current of 1 A and can be modulated till 5 MHz. The modulation signal to the laser driver is given through function generator from Rigol (DG-1022A). The CW laser beam is modulated through 5 cycles at 1 MHz to achieve the power depth of 25% (100–500 mW laser power for each cycle). The modulated laser beam (having spot size of 4mm<sup>2</sup>) was used to irradiate the blood sample placed in the water tank. The single element ultrasound (US) sensor (panametricV-383-SU) having center frequency 3.5 MHz, bandwidth 3.8 MHz and diameter of 16 mm was placed at a distance of 25 mm, axially to the laser beam on other side of the sample container in the water tank. Upon irradiation, the acoustic waves generated from the sample are acquired by the ultrasound sensor. The output of US sensor (5 cycles of acoustic waves at 1 MHz corresponding to 5 cycles of laser irradiation) is passed through two stages of preamplifications (having 40 and 28 dB gain, respectively). The amplified output is subsequently given to a NI digitizer (PXI-5124) in which sampling rate is set to 100MSPS. The signal from digitizer was processed in LABVIEW-2010 and was averaged for 200 times to improve the signal to noise ratio (SNR) of the signal. Further, the final averaged signal was retrieved in the computer. The modulation signal and data acquisition signals were synchronized by the 3.3 V, 50 KHz TTL.

### Pulsed laser PA experimental setup

For pulsed PA setup, the sample was illuminated by a Q-switched neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (Spectra Physics, USA) with the pulses of

Fig. 1 CWPA experimental setup



532 nm wavelength. The duration of the pulses was 5 ns to ensure pulsed heating of the sample and the repetition rate was 10 Hz. The sample was exposed to maximum 1.5 mJ with the spot size of 4 mm<sup>2</sup> laser beam. The sample absorbs the energy of the laser and generates acoustic signals which are acquired by a single element ultrasound (US) sensor (Panametric, V383-SU, center frequency 3.5 MHz, bandwidth 3.8 MHz, diameter 16 mm). During all the experiments, the sensor was fixed at a particular position and distance between the sample and the sensor was kept constant. The output of the US sensor was connected to National Instruments digitizer PXI 5124 to digitize and store the time domain PA signal. The signal from digitizer was processed in LABVIEW-2010 and was averaged for 100 times to improve the signal to noise ratio (SNR) of the signal. Data was sampled at a rate of 200 MSPS to avoid aliasing and the final averaged signal was stored in PC using LABVIEW 2010 software. The schematic representation of the experimental setup is illustrated in Fig. 2.

## Results

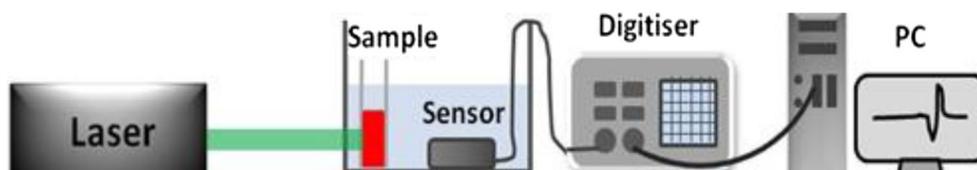
Before performing experiments with biology it is important to characterize the signals obtained from both pulsed and CWPA technique for their consistency and repeatability. For this

experiment, a uniform circular black rubber is used as a sample and the photoacoustic signals were recorded using both the PA techniques. Since in this study, both the PA techniques exhibits different wavelengths (532 nm and 460 nm) thus, the sample having almost identical absorption spectra for both these wavelengths is chosen to characterize the techniques. The PA amplitudes obtained for different trials are consistent and repeatable with both the experimental techniques although they possess different wavelengths. With conventional and CWPA, the amplitude were found to be  $3.9 \pm 0.8$  and  $33.7 \pm 0.55$ , respectively. The consistency in amplitude of a PA signal ensures that if the sample remains uniform then PA signal amplitude remains almost constant. These set of experiments confirm that the change in amplitude obtained in our studies are due to the biology of the sample and not due to any experimental errors.

As hemoglobin is one of the major constituents in RBC and also its optical absorption is identical for 532 nm and 460 nm as shown in Fig. 3 [15], thus usage of these two different wavelengths would not alter the PA signals from RBCs. Figure 3 represents the molar extinction coefficient of oxyhemoglobin (which is the measure of absorbance) for different wavelengths.

The experiments were mainly performed in two steps. In the first step, PA response technique (amplitude of PA signal)

Fig. 2 Pulsed laser PA experimental setup



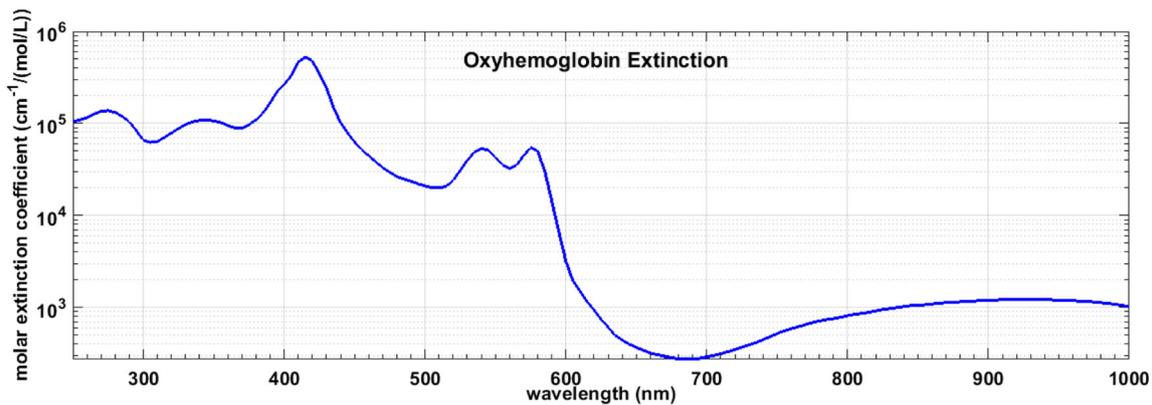


Fig. 3 Absorption spectra of hemoglobin [15]

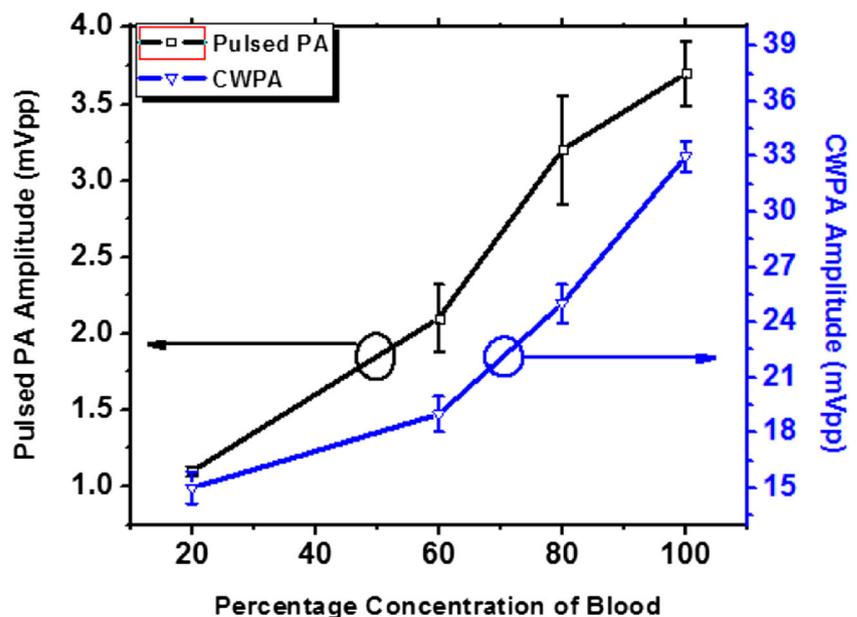
was investigated with respect to the change in concentration of RBCs in the sample. Here, the isotonic blood sample with different volume concentrations of blood was prepared and experimented immediately using both the PA techniques. With the increase in concentration of blood, there is an increase in amplitude of PA signal. For the same set of blood samples, Fig. 4 shows that in case of CWPA, amplitude has increased from 15.17 to 33 mV and for conventional PA an increase of 1.1 to 3.6 mV is observed, when the concentration of RBCs has changed from 20 to 100% (5 samples for each set of concentration were used to verify its consistency).

In the second step hypertonic, hypotonic samples (test samples) and isotonic samples (control sample) were investigated for their change in PA signal's amplitude due to the change in shape of RBCs. The experimental results show a prominent change in amplitude of PA signals for these set of blood samples. Further, the change in CWPA and pulsed PA signal's amplitude for hypertonic and hypotonic blood samples is

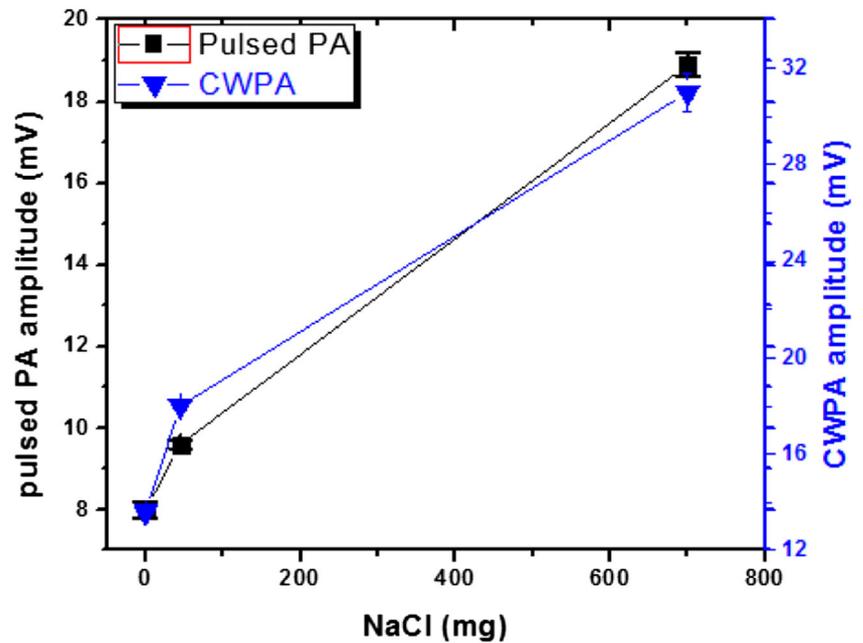
compared with the PA signal's amplitude for isotonic blood sample. As compared to isotonic sample, Fig. 5 reveals that CWPA signal's amplitude for hypertonic sample, increases by 13 mV and conventional PA signal's amplitude increases by 9.3 mV. Also for hypotonic samples CWPA signal's amplitude reduces by approx 4.5 mV and a reduction of around 1.6 mV in case of conventional PA signal's amplitude. Also, Table 3 below gives the change in CWPA signal response and pulsed PA signal response for different set of blood samples.

The change in amplitude of test and control blood samples was further confirmed by determining the absorption spectrum of hypertonic, hypotonic, and isotonic samples through spectrophotometer as shown in Fig. 6(a). It clearly indicates that the absorption of hypertonic sample is greater than isotonic sample at 532 nm as well as at 460 nm due to which there is an increase in PA signal amplitude. Also, Fig. 6(b) illustrates that the absorption of water and NaCl is one order less than blood for the same wavelength range. The change in

Fig. 4 PA signal amplitude vs percentage concentration in blood



**Fig. 5** PA signal vs isotonic, hypotonic, and hypertonic sample plot



shape of RBCs in case of the hypertonic, hypotonic and isotonic blood sample was also confirmed using the microscopic images as shown in Fig. 6(c). The microscopic images were captured using the  $\times 40$  lens and at a scale of 100  $\mu\text{m}$ .

## Discussion

The discussion section is divided into two parts. The first part discusses the photoacoustic results presented in the previous section. The second part relates the importance of the results with respect to some of the blood related diseases such as sickle cell anemia, thalassemia.

A typical PA signal for one of the blood sample, obtained from both the techniques is shown in Figs. 7a, b. In a conventional PA ('N'-shaped) signal, peak to peak amplitude is dependent on the optical absorption of the sample and rise and fall time depends on its density or elasticity [16]. In the present case, the time period of conventional PA signal is approximately 1.5–1.8  $\mu\text{s}$  and for CWPA, the time period is mainly decided by the number of cycles and frequency of each cycle used in burst mode (in this study it is 5  $\mu\text{s}$ ) as shown in Fig. 7a.

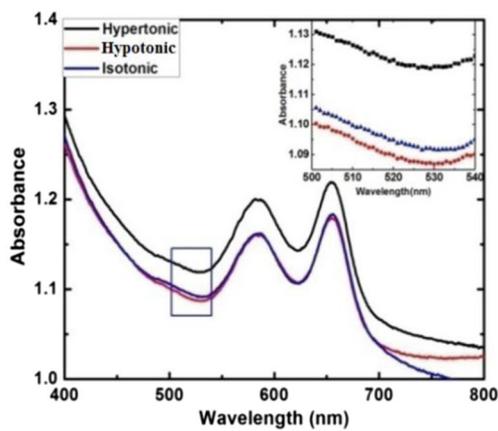
**Table 3** Comparison of CWPA and pulsed PA response for different sets of blood sample

Blood sample	Pulsed laser PA response (mV)	CWPA response (mV)
Isotonic sample	$9.6 \pm 1.5$	$18 \pm 2$
Hypertonic sample	$18.9 \pm 2$	$31 \pm 3.2$
Hypotonic sample	$8.0 \pm 0.8$	$13.6 \pm 1$

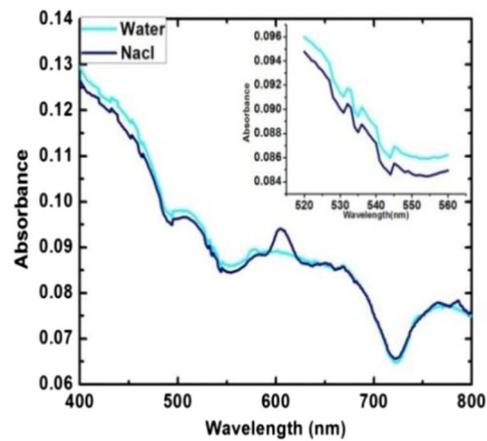
Results, presented in this study, aim to detect the morphological changes in blood through continuous photoacoustic signal response technique. The peak-peak amplitude of the photoacoustic signal obtained is used as a parameter to distinguish the different RBC shapes. Shape changes in RBCs are known to be present in various hematological disorders like sickle cell anemia. Sickle cell anemia is a type of hemolytic anemia where some RBC's aggregates resembling the bunch of grapes [17]. This paper uses the peak to peak amplitude of the photoacoustic signal as a signature to detect the morphological change in RBCs.

In the first study, the hypothesis that concentration of blood is proportional to the peak amplitude of the PA signal is verified. Since the laser beam size is constant, while there is an increase in concentration, the number of cells under the beam would increase, thereby increasing the peak amplitude of the PA signal. This proves that the PA signal amplitude is directly proportional to the number of absorbers. In the second study, the experiments were performed to ascertain the sensitivity of PA signal's amplitude towards the morphological changes in the blood samples. As shown in Fig. 5, the PA signal amplitude increases for hypertonic sample and reduces for hypotonic sample, as compared to isotonic sample.

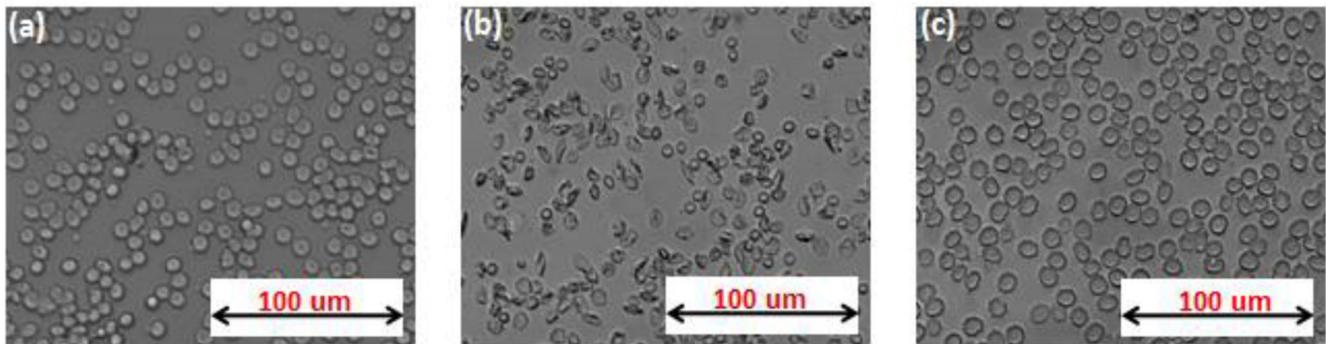
In the case of hypertonic sample, the cell shrinks due to excessive solute concentration. Thus, the decrease in size brings in more cells under the beam, thereby increasing the peak-peak PA amplitude. On the contrary, RBCs in the hypotonic solution allows diffusion of water from the surrounding medium (due to lower concentration of solute) which in turn swells the cells. Subsequently, the swollen cells also lyse, thereby decreasing the PA signal amplitude.



(a) Absorbance of blood samples



(b) Absorbance of water and NaCl

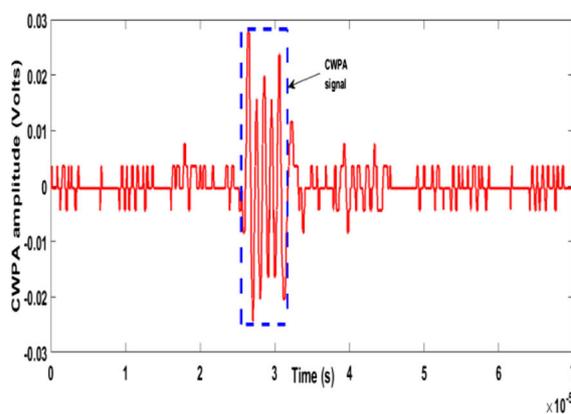


(c) Microscopy images of (a) isotonic (b) hypertonic (c) hypotonic samples

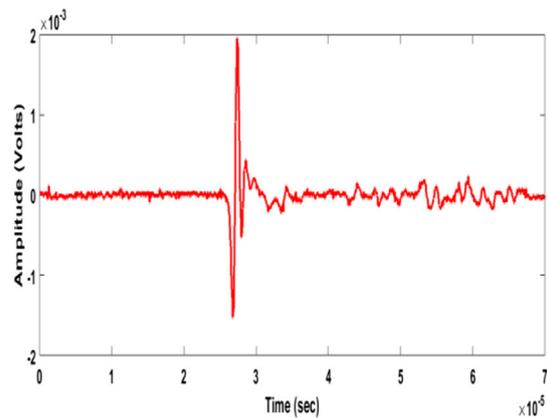
**Fig. 6** (a) Absorbance of blood samples. (b) Absorbance of water and NaCl. (c) Microscopy images of (a) isotonic, (b) hypertonic, and (c) hypotonic samples

To corroborate the results obtained from photoacoustics, spectrum obtained through a spectrophotometer, as shown in Fig. 6(a), (b), proves that blood is the major absorber. In addition, hypertonic samples show greater absorption compared to isotonic samples followed by hypotonic sample. Microscopic images shown in Fig. 6(c) validate the structural changes in RBCs of these three types of samples.

Morphological changes in RBCs play a very important role in diseases like sickle cell anemia and thalassemia. For example, in sickle cell anemia, the RBCs shrink and form bunches due to the rheologic and morphologic changes [18]. Many abnormalities such as cellular dehydration and abnormalities in cell membrane can contribute to this process of cell aggregation [19]. The shrinkage in



(a) CWPA signal



(b) Conventional PA signal

**Fig. 7** a CWPA signal. b: Conventional PA signal

cell size resembles with the hypertonic blood sample, wherein the density of cells increases [20], which causes the increase in PA signal amplitude.

Similarly, in the case of thalassemia, the blood cells are characterized by reduced (or absent) beta globin chain synthesis, resulting in reduced level of hemoglobin in red blood cells (RBC) [21]. Since hemoglobin is a major light absorber in RBCs, its overall reduction will cause the amplitude of PA signal to reduce considerably. Therefore, there is a direct relationship between the PA signal amplitude and the shape of the RBCs (number of light absorbers in illuminated region), which are very important in the diagnosis of diseases such as sickle cell anemia and thalassemia.

During the entire set of experiments, the beam size of laser and its energy, power was kept constant. Thus, the PA amplitude would only depend on the number of absorbers irradiated by the laser beam. Since, in case of hypertonic sample, the cells shrinks and form bunches, thus the number of absorbers in given illuminated region increases, which in turn increases PA amplitude. In contrast, in isotonic sample, the number of absorbers in given illuminated region is less, thus its corresponding PA amplitude will be lesser. Hence it reveals that for a fixed laser beam, change in PA signal amplitude is directly dependent on the size, shape or volume of the RBC.

## Conclusion

In this study, we have demonstrated the application of photoacoustic response technique in detecting the quantitative changes as well as the morphological changes in RBCs. It is verified that amplitude of photoacoustic signal changes significantly for changes in shape of the RBCs or if the concentration of RBCs (absorbers) is varied. Therefore, the PA response technique can be used as a signature tool to diagnose different hematological diseases like sickle cell anemia. It is evident from Table 3 that CWPA response technique can be used as an alternative to pulsed laser PA technique to detect hematological diseases. As the CWPA technique is compact, efficient and cost-effective it can be easily shifted to the hospitals for clinical trials.

**Funding** This work is financially supported by Department of Biotechnology, India (Grant No. BT/PR/5879/MED/32/241/2012).

## Compliance with ethical standards

**Conflict of interest** The authors declares that they have no conflict of interest.

**Ethical approval** Since in this study, human blood from volunteer healthy donors was used as a standard sample (ethical clearance was obtained from bio-safety and bio-ethics committee, Indian Institute of Technology Indore) to perform experiments.

## References

- Xu M, Wang LV (2006) Photoacoustic imaging in biomedicine. *Rev Sci Instrum* 77:–041101, 22
- Li C, Wang LV (2009) Photoacoustic tomography and sensing in biomedicine. *Phys Med Biol* 54:R59
- Beard P (2011) Biomedical photoacoustic imaging. *Interface Focus* 1:602–631. <https://doi.org/10.1098/rsfs.2011.0028>
- Wang X, Chamberland DL, Jamadar DA (2007) Noninvasive photoacoustic tomography of human peripheral joints toward diagnosis of inflammatory arthritis. *Optics Letters* 32(20):3002–3004
- Manohar S et al (2005) The twente photoacoustic mammoscope: system overview and performance. *Phys Med Biol* 50:2543
- Maslov K, Wang LV (2008) Photoacoustic imaging of biological tissue with intensity- modulated continuous-wave laser. *J Biomed Opt* 13(2):024006
- Xu M, Wang LV (2005) Universal back-projection algorithm for photoacoustic computed tomography. *Phys Rev E* 71:016706–016707
- Xu Y, Wang LV, Ambartsoumian G, Kuchment P (April 2004) Reconstruction in limited view thermoacoustic tomography. *Med Phys* 31(4):724–733
- Kolkman RGM et al (2004) Photoacoustic determination of blood vessel diameter. *Phys Med Biol* 49:4745–4756
- Marion A et al (2011) A quantitative study to design an experimental setup for photoacoustic imaging. In: *Engineering in Medicine and Biology Society. EMBC, 2011 Annual International Conference of the IEEE*, pp 7211–7214
- Andreev VG et al (2003) Detection of ultrawide-band ultrasound pulses in optoacoustic tomography. *IEEE Trans Ultrason Ferroelectr Freq Control* 50:1383–1390
- An R et al (2014) Spatially variant red blood cell crenation in altering current non-uniform. *Biomicrofluidics* 8:421–425
- Huber SM et al (2001) Chloride conductance and volume-regulatory nonselective cation conductance in human red blood cell ghosts. *Pflugers Arch* 441:551–558
- Glaser R (1979) The shape of red blood cells as a function of membrane potential and temperature. *J Membr Biol* 51:217–228
- Prahl S (1999) Optical absorption of haemoglobin. *Tech Rep*, Oregon Medical Laser Centre, Portland, Oregon
- Biswas D et al (2017) Quantitative characterization of blood clot in blood: a mechanobiological assessment through spectral information. *Rev Sci Instrum* 88(2):024301
- Ford J (2013) Red blood cell morphology. *Int J Lab Haematol* 35: 351–357
- Westerman MP et al (1983) Red blood cell morphology in sickle cell anaemia as determined by image processing analysis: the relation to painful crises. *Am J Clin Pathol* 79:667–672
- Glader BE et al (1978) Cation permeability alterations during sickling: relationship to cation composition and cellular hydration of irreversibly sickled cells. *Blood* 51:983–989
- Hebble RP (1991) Beyond Haemoglobin Polymerization: the red blood cell membrane and sickle disease pathophysiology. *Blood* 77: 214–237
- Galanello R et al (2010) Beta thalassemia. *Orphanet J Rare Dis* 5:11