

# Digital Camera-Based Spectrometry for the Development of Point-of-Care Anemia Detection on Ultra-Low Volume Whole Blood Sample

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**Abstract**—Objective of this paper: Early detection of anemia (low hemoglobin count) at point-of-care (PoC) in a low resource setting is challenging given the associated capital and recurring costs of the device. In this paper, we have developed a faster and reliable method to screen the hemoglobin level in the whole blood in a resource limited point of care setting. We have also investigated a simple scattering-based technique to predict the overall morphology of the red blood cells. **Methods:** In this paper, we have developed and validated a digital camera-based spectrometer for the early detection of anemia with the whole blood of 10- $\mu$ L volume in a low resource PoC setting. The developed device consists of two LEDs [Green (570 nm) and Red (631 nm)], a web camera, a fluid sensing chamber (cuvette holder), and associate electronics. Validation of Beer–Lambert law using the blood of various degree of dilution has been achieved in this *in vitro* experiment. **Major results:** A significant number of human subjects (300) having a wide range of hemoglobin counts in a hospital is tested and found to reproduce results from a gold standard automated hematology analyzer. Our developed microcuvette requires only 10- $\mu$ L unprocessed whole blood sample for the device. **Conclusions:** The device is expected to serve as a minimally invasive e-health care device for the anemia screening in any resource-limited point of care setting.

**Index Terms**—Anemia screening, digital camera-based low-cost device, e-health, point-of-care (PoC) screening and optics.

## I. INTRODUCTION

IRON deficiency has been assessed to be the most common cause of anemia globally. The World Health Organization (WHO) declares the prevalence of anemia strongly effects on maternal and infant health which in turn affects the socioeconomic development [1]–[3]. The hemoglobin concentration provides the information about the severity of iron deficiency in the blood [4], [5]. According to the global statistics of WHO, roughly 43% children, 38% pregnant women,

29% non-pregnant women and 29% of all women of reproductive age are affected by anemia [6]. Therefore, determination of hemoglobin concentration is considered as the integral part of healthcare service, particularly, early trace of anemia obviously a help to the blood transfusion management, which protects both donors' and recipients' health [7]. Three main reasons of anemia include blood loss, deficiency of red blood cell production and high rate of red blood cell destruction. Abnormalities in RBC morphology also indicates the anemia or abnormal cell development and are caused by numerous diseases like malaria, sickle cell anemia, megaloblastic and pernicious anemia [8].

The state-of-the-art of the available technologies for determining hemoglobin concentration and the average cell size varies from low-tech to high-tech depending on the target resource setting [9]. Hemoglobin measurement is usually carried out in well-equipped laboratory with sophisticated instruments based on either electrochemical impedance spectroscopy [10], [11] or Coulter principle (e.g. automated hematology analyzer) [12]. Although all these techniques are effective but involve complex and expensive experimental setup, operational technical skill and time consuming measurements for anemia detection. In this context HemoCue [13]–[15] provides semi-quantitative gravimetric copper sulfate method for hemoglobin determination in portable format but still cost ineffective. In addition many non-invasive methods are also developed for hemoglobin measurement [16]–[18] including the conventional Pulse CO-Oximeter, Pronto-7 monitor (version 2.1.9, Masimo Corporation, Irvine, USA), NBM-200MP monitor (Orsense, Nes Ziona, Israel) and spectrophotometer [19]. However, the precision and sensitivity level of these instruments depend on different physiological factors including arterial pulse, body movements, skin tones, venous pulsations. A survey on the aforesaid instruments reveals lack of an inexpensive, portable and accurate device for anemia diagnosis in low resource territories. In recent time smartphone based techniques are developed for blood analyses and hemoglobin detection. The variety of such devices has been reviewed in the literature [20]. One of such devices developed by Prof. Ozcan group from UCLA uses disposable plastic cuvette-based add-on for the hemoglobin detection [21] using processed whole blood. The whole blood sample used in the add-on was reported to be lysed and purified (chemical treatment followed by mechanical separation). The LED light source used in the add-on is  $\sim$ 430 nm, which essentially

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monitors the solet band of the hemoglobin having extremely high molar extinction coefficient ( $528600 \text{ cm}^{-1}/\text{M}$ ). A sample with very high extinction coefficient needs significant dilution, which may involve systematic error in the measurement. The add-on does not use any reference light source for the differential or ratiometric detection of hemoglobin. It is well known that differential or ratiometric type absorbance measurement offers better accuracy compared to that of absolute measurement. It has to be noted that use of smartphone with add-on for hemoglobin detection at very low resource settings including third world countries should be carried out with great care, as the smartphone may be misused rather in the detection of hemoglobin. Here, we report our development of an economic, reliable, minimally invasive portable device based on  $10\text{-}\mu\text{L}$  unprocessed human blood for hemoglobin estimation at point-of-care. Our innovation is based on the measurement of spectroscopic information obtained from a thin blood layer. The blood sample can be collected by standard medical procedures (finger prick) at reduced disposition time improving patient satisfaction compared to the conventional venipuncture based laboratory test [22]. Furthermore the device generated report could be transferred to the medical experts through different electronic communication protocols. Hence the proposed device has high potential to promote an e-healthcare for anemia detection at minimal resource community.

## II. MATERIALS AND METHODS

### A. Working Principle and Design of Light Source

The working principle of the device relies on Beer-Lambert law of molecular absorption of hemoglobin in blood samples [23] in the indigenously developed microcuvette (see latter). As shown in (1), the hemoglobin concentration ( $c$ ) in the in the microcuvette could be expressed as the function of the intensity of the light passing through the reference cell ( $I_0$ ) and the sample cell ( $I$ ) respectively [24].

$$c = \frac{\log_{10}\left(\frac{I_0}{I}\right)}{\varepsilon l} \quad (1)$$

here,  $\varepsilon$  is the molar absorptivity of the blood and  $l$  is the blood film thickness. The hemoglobin and the plasma govern the absorption of whole blood [25]–[27] both in the visible and near infrared region. The oxygenated hemoglobin ( $\text{HbO}_2$ ) significantly absorbs the light at the wavelength of about 420, 542 and 576 nm [28] and the absorption minimum of oxy and deoxy-hemoglobin lies at wavelength around 630 nm [29]–[31]. It has to be noted that the optical absorption at 570 nm is an isosbestic point [26] of oxy and deoxy-hemoglobin absorption spectra. Thus the absorption of whole blood at 570 nm absolutely measures the concentration of hemoglobin without any interference of oxygenation of the blood. The differential blood absorption (absorption<sup>570</sup> - absorption<sup>631</sup>) is used to calculate the hemoglobin concentration in order to minimize the interference of oxygenation of blood and baseline drift due to scattering of light in blood sample. In the present study, two LEDs [32] of wavelengths 631 nm (red) and 570 nm (green) are used in order to investigate differential absorption of hemoglobin in the whole blood

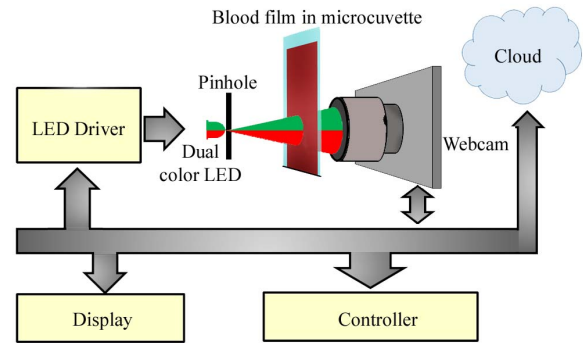


Fig. 1. A schematic representation of the developed whole device is shown. An indigenously developed dual color LED with wavelengths of 570 nm (green) and 631 nm (red) passes light through an indigenously developed micro-cuvette with blood under investigation to a CMOS sensor (webcam). The other peripheral devices including communication for the data transfer to the cloud are also shown.

sample. The light source in our study is made from two longitudinally cut commercially available LEDs (red and green) as shown in (Fig. 1). A pinhole arrangement is directly connected in front of the light source so that the central region of the microcuvette is illuminated avoiding unwanted light scattering.

It is reported that the red blood cells are the primary light scattering particles in the whole blood [33]. The light scattering properties of the whole blood predict the average morphology of the blood cell. The well-known static light scattering (SLS) and dynamic light scattering (DLS) [34] rely on the principle of Rayleigh scattering. The techniques are commonly used to determine the RBC size and to distinguish the healthy cells from the infected cells [35], [36]. The diameter of the normal RBCs ranges from 7 to  $8\text{-}\mu\text{m}$  [37] and their population is higher than the other cells in the whole blood. For  $20\text{-}\mu\text{m}$  blood film thickness used in our study, the heterogeneity of tissues becomes negligible. Therefore, a little change in RBC diameter causes a significant change in the scattered light intensity in order of sixth power of the change in the diameter. The change in scattered light intensity can be detected in a launch coupling technique [38] in order to monitor the change in the morphology of the RBCs. Light scattering is a well-established technique to find the average size of the RBCs [39]. Here, the change of light coupling between the glasses of the developed microcuvette (see below) at 631 nm was also collected by the CMOS sensor and investigated [40] for qualitative information on the average diameter of the RBCs in the whole blood under investigation.

### B. Design of Disposable Microcuvette

Validation of Beer-Lambert law needs a constant optical sample length. To meet the requirement, glasses of size about  $35 \text{ mm} \times 12 \text{ mm} \times 1 \text{ mm}$  were cut carefully from  $75 \text{ mm} \times 25 \text{ mm}$  commercially available microscope slide (Fig. 2). A pair of cut slides were cleaned by deionized water, alcohol and air dried. Next, the glass slides were stacked and fixed with a both-side sticking tape of thickness 0.02 mm (Fig. 2a). The optical path length of the microcuvette were estimated using crystal violet solution with known concentration and extinction coefficient [26], and found to be  $(20 \pm 1) \mu\text{m}$ . A volume

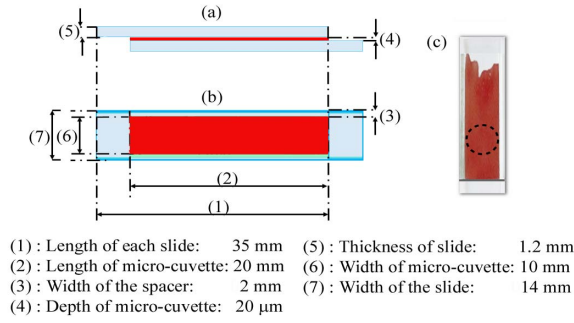


Fig. 2. A schematic of the developed micro-cuvette is shown. (a) and (b) Side and top views of the micro-cuvette. A photograph of the developed micro-cuvette with the whole blood is shown in (c). The dotted circle in the photograph is the region of investigation through which the dual color LED passes light to the CMOS sensor.

of 10- $\mu$ L blood is enough to fill the space between the glasses uniformly due to capillary action [41]. The uniformity of film thickness is explained in Section III.

### C. Experimental Setup

An electronic circuit is prepared to control the LED's luminosity to assure that light beam is able to pass through the thin blood film of the microcuvette at highest hemoglobin concentration (16.6 g/dL). The device as shown in Fig. 1 consists of one raspberry pi, a debit card-sized computer which provides all the expected features of a palm-top computer. A programme is written in Python language to control the LED's duty cycle, the web-cam functionality, image processing, test result evaluation and data communication. A low cost Quantum QHM495LM 25MP Web Camera is used in the present set up. The camera has focal length of 39 mm with 58° angle of view. Accordingly the microcuvette holder is mounted  $\sim$  3.9 cm away from the web-cam. The CMOS sensor of the web-cam is used to record the transmitted photons through the microcuvette in the form of a circular image. The RGB pixels values of the CMOS sensor converted into grey scale are proportional to the brightness of the image itself. The grey values of the pixels are calculated to get total photon counts in terms of the area under the curve (*AUC*). To nullify the artifacts introduced by the microcuvette material and the aqueous medium of the blood, initially a microcuvette filled with plasma was tested in the system. The corresponding *AUC* is considered as the reference value (*AUC*<sub>0</sub>). To minimize the effect of ambient light the same procedure is followed for individual experiment before turning the light source on. The corresponding calculated area under the curve is treated as ambient value (*AUC*<sub>A</sub>). Hence, the working expression for the concentration (*c*) of whole blood can be obtained from (2–4).

$$c^{570} = A_0 \log_{10} \left( \frac{AUC_0^{570} - AUC_A}{AUC^{570} - AUC_A} \right) \quad (2)$$

$$c^{631} = A_0 \log_{10} \left( \frac{AUC_0^{631} - AUC_A}{AUC^{631} - AUC_A} \right) \quad (3)$$

$$c = A_0 \left( c^{570} - c^{631} \right) \quad (4)$$

where,  $A_0$  is constant and equal to  $1/\epsilon\lambda$ . The difference in the concentrations at two different wavelengths is considered as the instrument index value of our developed device.

### D. Blood Sample Collection

The study included 300 whole blood samples (including 130 females; 50 of whom were children) with age group ranging from 5 to 72 years. All the samples examined in our proposed system were obtained from the outpatient department in Nil Ratan Sircar (NRS) Medical College and Hospital, Kolkata: 700014, India from October to December 2016, following the standard clinical guideline approved by the institutional ethical committee (No/NMC/437, Dated 25/01/2016). The hospital serves the low socio-economic population of all regions of the state of West Bengal, India. All the patients, who essentially came for their regular routine blood test, were invited for contributing their blood for testing in our developed system. A duly signed consent form was received from the patients or their legal guardians stating their acceptance, involvement and full understanding of the study. The study was executed in three phases. In the first phase the device was calibrated. In the second phase of the study the accuracy of the system was examined and compared with that of the gold standard technique (Automated Hematology Analyser). The device reproducibility was tested in the last phase. Following the blood collection with the standard medical procedure for the gold standard Automated Hematology Analyser (KX-21NTM, Sysmex, America), a 10- $\mu$ L of residual unprocessed whole blood from the used syringe was immediately tested in our developed system. Both the data were later used for calibration and comparison study.

## III. RESULT AND DISCUSSION

### A. Uniformity of Blood Film

In order to get the similar information from the same whole blood sample, integrity of the blood film thickness is unavoidable. A 10- $\mu$ L blood of known hemoglobin concentration (15.2 g/dL) is taken to justify the integrity of the blood film thickness in the microcuvette (Fig. 2c). In order to measure the optical density of the blood film thickness in the microcuvette we have used a home-made spectrophotometer consisting of a light source (LSS-LED-3650, Ocean Optics, Florida, USA) with wavelength range of 400 nm to 700 nm and a spectrograph (HR 4000, Ocean Optics, Florida, USA) having optical resolution approximately equal to 0.02-8.4 nm full width half maxima. We have measured optical density of eleven blood films in the microcuvette from the test blood of fixed concentration (15.2 g/dL). Those eleven spectral responses are normalized and plotted (Fig. 3a) to investigate the uniformity of the blood film during different sampling events. Inset of Fig. 3a shows absorption spectra [26] oxy and deoxy-hemoglobin samples revealing an isosbestic point at 570 nm. As shown in Fig. 3b the used green LED source in our study offers irradiance maximum at 570 nm. Thus the differential absorption as described earlier should be independent of the oxygenation of the blood used investigation.

### B. System Calibration

Fig. 4 shows repeatability of absorption measurement (Fig. 4a) and consistency of the blood film (Fig. 4b) in the developed microcuvette. It is evident that ten consecutive

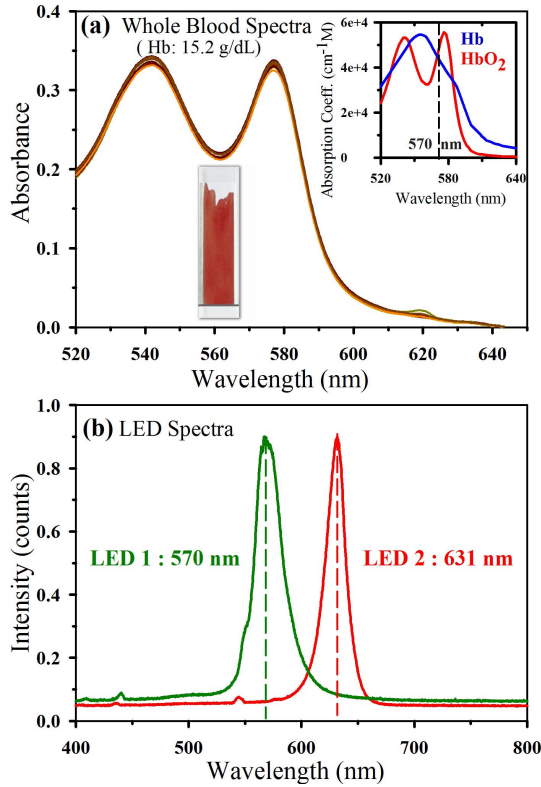


Fig. 3. (a) UV-VIS absorption spectra of the same blood sample from a human subject in a number of developed micro-cuvette using a commercial spectrometer is shown. Note the repeatability of the measurement. The inset shows standard blood spectra of oxygenated and deoxygenated blood with one of the isosbestic points at 570 nm. (b) The wavelengths of the developed dual color LED used in our developed device is shown. Note that the wavelength of green LED (570 nm) matches with the isosbestic point in the blood spectra.

experiments on a blood film by ten different users find results with  $\leq 4\%$  error (Fig. 4a). The consistency of the blood film found to be at least up to 15 minutes (Fig. 4b) with less than 2% error. Randomly selected 120 human subjects (including 58 female) of all age groups having hemoglobin count ranging from 4.4 g/dL to 16.6 g/dL were employed for the system calibration. The light intensities of dual-color LED were acquired and the AUCs were calculated (Fig. 5). The linear dependency of instrument index values (i.e. the differential absorbance at 570 and 631 nm) with the Automated Hematology Analyzer (gold standard) count is shown in (Fig. 6). The figure also reveals linear regression curve with 95% confidence interval (CI) and 95% prediction interval. The Pearson's coefficient,  $r = 0.96$ , provides a satisfactorily strong correlation [42]–[44] between the instrument index values and the hemoglobin counts from gold standard technique. The slope and the intercept from the linear regression model (5) were incorporated to optimize the operating equation for the developed system in order to measure hemoglobin count in g/dL using our device.

$$y_i = (14.81 \pm 0.34)c_i + (4.85 \pm 0.15) \quad (5)$$

where,  $y_i$  and  $c_i$  represent the optimized hemoglobin value and the instrument index value respectively.

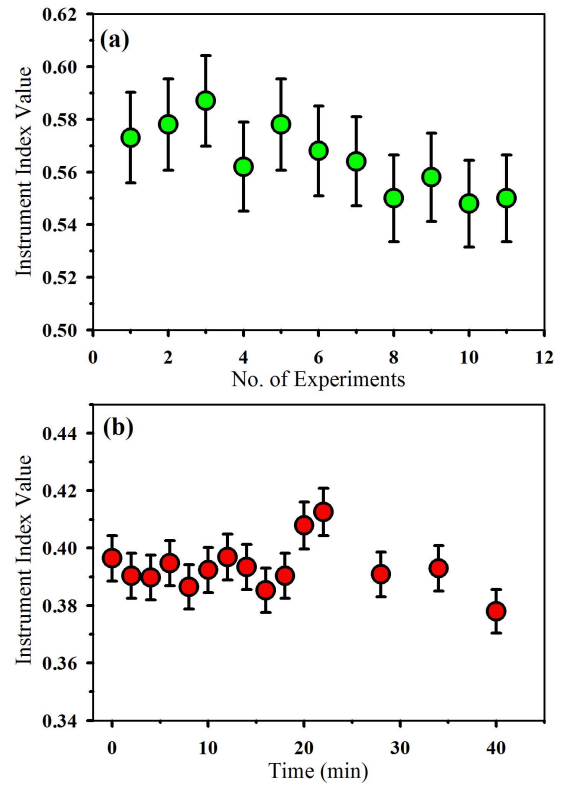


Fig. 4. (a) The repeatability of absorbance measurement for 11 consecutive experiments on a unprocessed blood film in the microcuvette. (b) The consistency of the blood film up to 40 minutes is shown. Note the consistency up to 15 minutes is within  $\leq 2\%$  error bar.

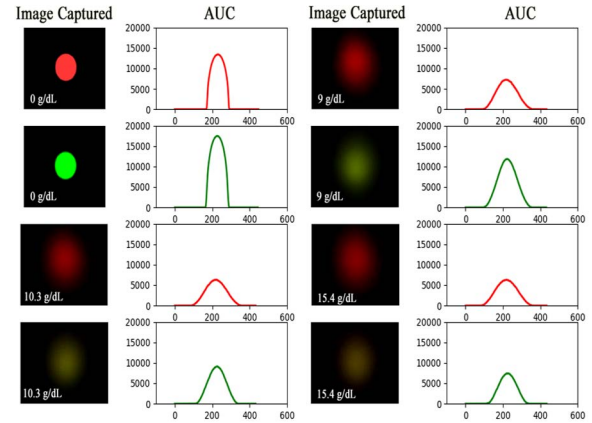


Fig. 5. The images of the green (570 nm) and red (631 nm) through the developed micro-cuvette with an aqueous buffer solution is shown in upper left side. The images with various concentrations of human blood samples are also shown. The intensities of light through the samples in terms of grey scale values are also shown.

### C. System Validation

Introducing a new biomedical measuring device required to be compared with an established technique to justify the consistency in the output of the new to replace the old one. Bland-Altman method [45] is a tool to investigate the convergence in the output of the new method towards the old one. The statistical interpretation including the correlation and regression analysis [46], [47] are used to justify the appropriateness of the proposed e-health care unit. To compare our

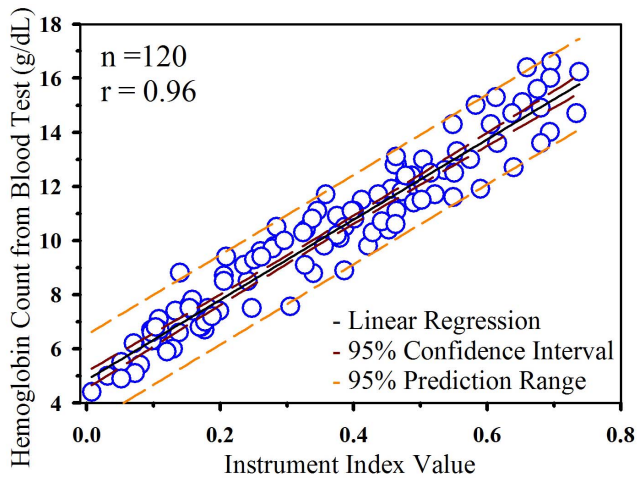


Fig. 6. The device generated index value with the hemoglobin count measured with gold standard method (cell counter) for a number of human subjects (120) is shown.

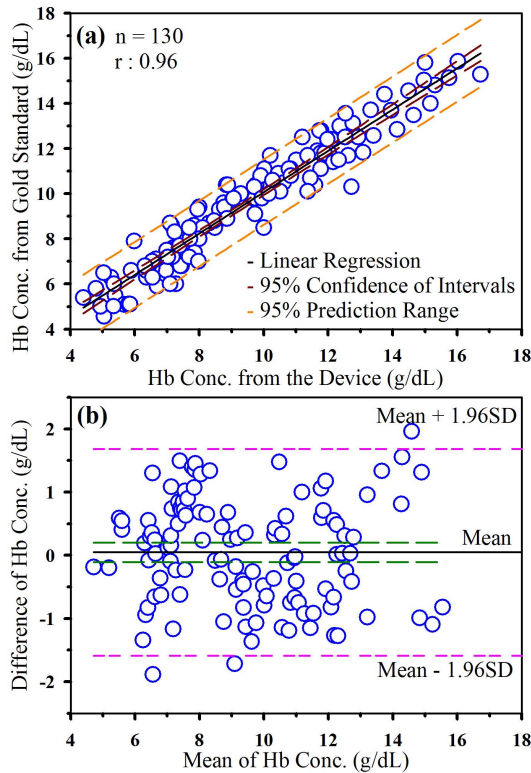


Fig. 7. The linear regression plot of the data from various human subjects (130) is shown in (a). Bland-Altman plot is shown in (b) (see text).

developed e-health care device for hemoglobin detection with a widely accepted conventional technique, total 130 whole blood samples (including 50 females) of all age groups with hemoglobin counts ranging from 4 g/dL to 18 g/dL were randomly tested. (Fig. 7a) presents the linear regression analysis with the 95% CI and 95% prediction interval. The Pearson coefficient,  $r = 0.96$  and the statistical parameter,  $P$  value  $< 0.0001$  indicate a strong correlation between the two methods. To measure the agreement between the two methods of hemoglobin detection the Bland & Altman method was used (Fig. 7b). A direct comparison between the two methods yielded a bias of 0.05 g/dL and standard deviation (SD)

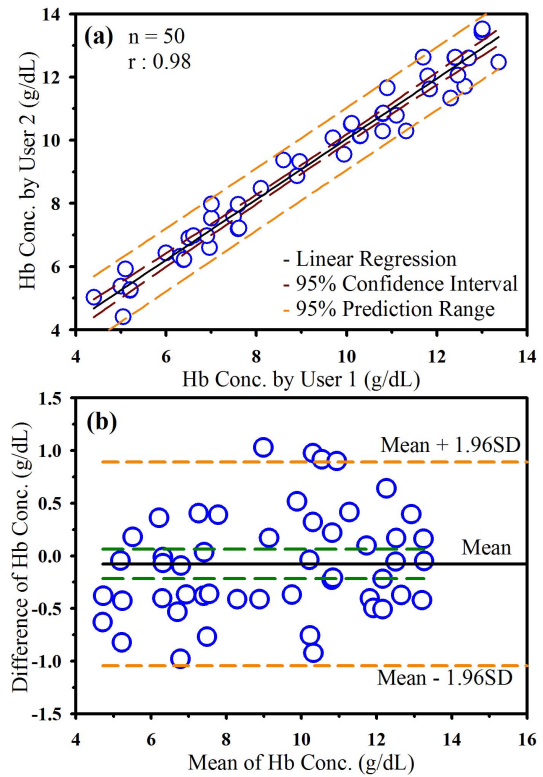


Fig. 8. The reproducibility test of the developed device by two different users is shown in (a). Bland-Altman representation of the same test is shown in (b).

of 0.845. The 95% CI for the bias was from -0.10433 to 0.20004 and the 95% limits of agreement ( $\text{Mean} \pm 2\text{SD}$ ) were  $-1.5$  to  $1.6$  g/dL. The accuracy of the proposed technique with respect to the reference method was calculated using the accuracy root mean square [48]. For the proposed technique the accuracy root mean square was 0.9 g/dL throughout the hemoglobin range. These results exhibit a strong agreement between the conventional method as well as the developed method of anemia detection at high accuracy.

*D. System Reproducibility Test*

The reproducibility of the device was obtained by measuring a clinically significant 50 no. of whole blood samples of the same test twice by two different individuals. The statistical analysis of the linear regression curve (Fig. 8a) uttered the concordance coefficient [44] between the two methods as 0.98. The Pearson coefficient (0.98) with  $P$  value  $< 0.0001$  indicate a strong match between the tests. The reproducibility was further justified with Bland & Altman method (Fig. 8b). The study yielded a bias of  $-0.076$  g/dL and SD of 0.49. The above results indicate that the two measurements exhibit a close agreement and the high reproducibility to trace the hemoglobin concentration.

*E. Qualitative Morphology of Whole Blood*

The strategy for the detection of the corpuscular volume of blood cells is shown schematically (Fig. 9a). A red LED (631 nm) was used as scattering light source. The intensity of light is detected by the CMOS camera (A low cost Quantum

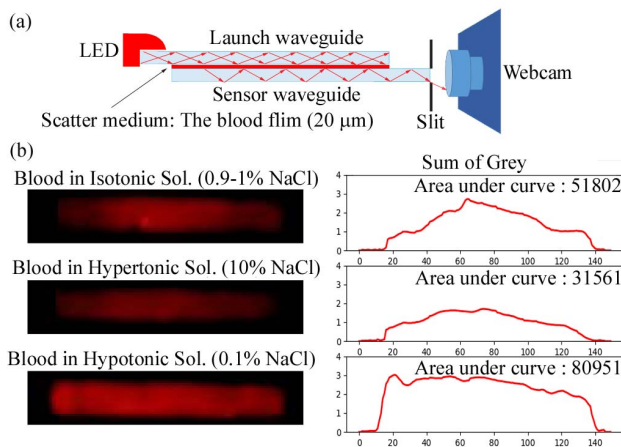


Fig. 9. The strategy for the detection of the corpuscular volume of blood cells is shown schematically in (a). A red LED (631 nm) is used as scattering source. (b) The intensity of light detected by the camera at the edge of the micro-cuvette with whole blood in isotonic, hypertonic and hypotonic solutions (from top to bottom) are shown. The corresponding intensity profiles are also shown.

QHM495LM 25MP Web Camera. The camera has focal length of 39 mm with  $58^\circ$  angle of view.) at the edge of the microcuvette with whole blood in isotonic, hyper-tonic and hypo-tonic solutions (from top to bottom) as shown in the (Fig. 9b). The corresponding intensity profiles are also described in Fig. 9b. We have also measured the average RBC diameter at isotonic ( $4.5 \mu\text{m}$ ), hyper-tonic ( $2.5 \mu\text{m}$ ) and hypo-tonic ( $6 \mu\text{m}$ ) solutions using DLS technique (data not shown). Thus, it is expected that the light scattering leading to light coupling between glasses in the microcuvette would score higher in hypotonic solution compared to those in hypertonic as well as isotonic solutions. The grey scale count for the hypo-tonic solution was found to be significantly higher in comparison to those in hypertonic and isotonic solutions as shown in Fig. 9b. Although the exact calculation of the mean corpuscular volume of the RBC under investigation from the experiment is progressing in our group, our observation clearly highlights a promise for the detection of average morphology of RBC in unprocessed whole blood.

#### IV. CONCLUSION

In this paper, we have designed and tested a minimally invasive low-cost device for hemoglobin detection in low resource point of care setting. A disposable microcuvette for uniform film preparation without any technical skill utilizing ultra-low volume of unprocessed blood has also been developed. The proposed device is found to be appropriate for detection of hemoglobin concentration in most clinically significant region (4 to 14 g/dL) with good accuracy. Moreover, the developed self-powered device would be able to instantaneously transfer the generated report to the medical expert through email, text messaging. Thus the developed device has a potential to establish an e-health center at any place at any time. We have also proposed and validated a scattering based strategy for qualitative estimation of RBC morphology (size). The overall architecture of our developed device for direct measurement of hemoglobin concentration and estimation of average RBC size is shown schematically (Fig. 10). The other

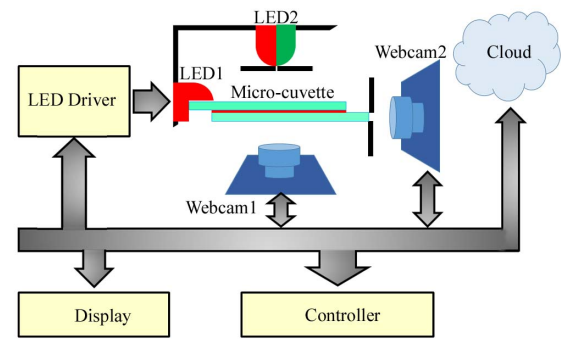


Fig. 10. The overall architecture of the developed device is schematically shown. The device is capable of detecting concentration as well as the average morphology of blood samples (see text).

features of the developed device including data saving and transeiving through cloud are also shown in the schematic.

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