International Journal of Electrical and Computer Engineering (IJECE)
Vol. 15, No. 5, October 2025, pp. 4569~4580
ISSN: 2088-8708, DOI: 10.11591/ijece.v15i5.pp4569-4580

A non-destructive approach for estimation of Hb, HCT and red blood cells using reflectance spectroscopic technique

P. Divyabharathi, Neelamegam Devarasu
Department of Electronics and Communication Engineering, Vel Tech Rangarajan Dr. Sagunthala R&D Institute of Science and Technology, Chennai, India

Article Info

Article history:

Received Sep 13, 2024
Revised May 29, 2025
Accepted Jun 30, 2025

Keywords:

Child friendly
Non-invasive
Paediatric haematology
Point of care
Reflectance spectroscopy

ABSTRACT

Paediatric haematology involves the use of non-invasive methods and technologies to evaluate haematological parameters in children. These techniques attempt to offer precise measurements of blood constituents without the necessity of intrusive procedures such as venipuncture or blood draws, which can be difficult and unpleasant for paediatric patients. The data gathered from the elbow will be given priority for further investigations to find haematological profiles. Estimates of haemoglobin, haematocrit, and red blood cell count were done and compared against the values obtained using conventional methods. This method achieves an accuracy of 75.56% with high precision and specificity which makes the method particularly beneficial for paediatric applications, potentially due to physiological differences or enhanced calibration for younger populations. The sensitivity varies with red blood cells (RBC) showing the lowest true positive detection rate. Future work could focus on improving the sensitivity of these parameters to enhance the accuracy. Conventional techniques cannot monitor continuously and remotely, which is crucial for a point-of-care screening device in the current era. The proposed non-destructive technique offers the benefits of infection control, pain reduction, and minimal operational cum maintenance expenses, all while being portable and child friendly.

Corresponding Author:

Neelamegam Devarasu
Department of Electronics and Communication Engineering, Vel Tech Rangarajan Dr. Sagunthala R&D Institute of Science and Technology
400 Feet Outer Ring Road, Avadi, Chennai, Tamil Nadu - 600 062, India
Email: drdneelamegam@veltech.edu.in

1. INTRODUCTION

Paediatric haematology is a specialised field of medicine that concentrates on the diagnosis, treatment, and management of blood abnormalities and hematologic disorders in children. Paediatric haematology is a crucial discipline focused on comprehending and managing blood diseases in children. It encompasses a thorough strategy that integrates sophisticated diagnostic methods, specific therapies, and comprehensive care to effectively manage and enhance the results of paediatric patients with hematologic disorders. Timely identification, continuous surveillance, and customised treatments are crucial to meet the distinct requirements of children and guarantee their well-being and standard of living [1]. Paediatric haematology necessitates an individualised approach to address the specific physiological and psychosocial requirements of children. Comprehending the significance of haematological parameters in the diagnosis and monitoring of paediatric illnesses, in addition to a meticulous and empathetic approach to collecting blood samples, is crucial for efficient paediatric healthcare. Thorough preparation, skilful technique, and careful

aftercare can reduce stress for both the kid and their parents, guaranteeing precise and dependable findings for medical evaluation and therapy [2].

Haematological indicators play a vital role in the diagnosis, monitoring, and treatment of a wide range of medical conditions. Blood tests offer crucial insights into an individual's blood condition, assisting healthcare professionals in making well-informed decisions regarding patient care and treatment options. Regular assessment of these factors leads to better health outcomes and effective management of different conditions. Crucial haematological metrics encompass the complete blood count (CBC), differential count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The red cell distribution width (RDW) is a measure used to assess the variation in the size of red blood cells. When RDW values are elevated, it suggests the possibility of anaemia or other haematological diseases [3].

Individuals who extract blood from toddlers and neonates must possess extensive training and experience in venepuncture methods. Implementing a standardised sample technique is crucial for minimising both physical discomfort and psychological distress. The selection of the site and method (venous site, finger-prick, or heel-prick - also known as “capillary sampling” or “skin puncture”) will be determined by the required blood volume for the procedure and the specific laboratory test to be conducted. Venepuncture is the preferred approach for obtaining blood samples from full-term infants [4], [5]; nevertheless, it necessitates the presence of a skilled and trained phlebotomist. In the absence of a qualified phlebotomist, the physician may be required to collect the specimen. Capillary blood specimens have a comparable oxygen content to arterial specimens, but they are only acceptable for a restricted range of assays due to their increased risk of contamination from skin bacteria and reduced overall volume. The choice between a finger-prick or a heel-prick will be determined by the child's age and weight. Venepuncture is the optimal technique for obtaining blood samples from full-term neonates and is associated with reduced pain compared to heel pricks [6]. After immobilising the infant or youngster, make a small hole in the skin around 3-5 mm distant from the vein. This will provide easy access to the vein without displacing it. If the needle is inserted adjacent to the vein instead of inside it, partially retract the needle without fully withdrawing it, then reposition it at an angle to enter the vessel. Extract blood gradually and consistently. Repeated venipuncture can cause significant distress for the patient, their parent, and the healthcare workers involved [7], [8]. It results in pain, worry, and a lack of adherence to medical treatment [9]. Children experience minimal bleeding due to the lesser size of their veins and the restricted dispersion of the vascular plexus. Multiple punctures and the application of restraints during bleeding procedures can result in bruising, nerve injury, and vasovagal reactions in youngsters [10]. This exacerbates the bleeding locations. To address the drawbacks of several venepunctures, certain facilities utilise a “blood cannula” which, however, has its downsides including reduced blood flow, sample clotting, patient upset and discomfort, and an elevated risk of infections. The presence of a cannula in both the hands and legs severely limits the child's ability to move and eat, while also causing significant fear and worry [11]. To address these issues, numerous studies have been conducted in the field of spectroscopic techniques [12].

Spectroscopic techniques are gaining significance in the measurement of haematological profiles in children because of their non-invasive nature, exceptional accuracy, and capability to offer real-time data [13]. These approaches provide notable benefits compared to conventional blood testing, rendering them especially beneficial in paediatric care. Conventional blood tests might cause upset in children since they involve the invasive procedure of drawing blood [14]. Spectroscopy, particularly methods such as near-infrared (NIR) and Raman spectroscopy, can frequently be conducted without causing harm or with minimal pain. Non-invasive techniques decrease the likelihood of infection and problems that are linked to blood extractions. Spectroscopic techniques offer the capability to analyse blood parameters in real time, allowing for prompt decision-making and intervention [15]. This is especially advantageous in critical care environments where prompt alterations in a child's state must be continuously monitored. Advanced spectroscopic techniques can accurately analyse a wide range of blood constituents, frequently identifying alterations at the molecular level. Nuclear magnetic resonance (NMR) spectroscopy techniques can offer comprehensive insights into the composition of blood metabolites and proteins [16].

NIR spectroscopy is capable of quantifying haemoglobin concentrations and distinguishing between oxy-haemoglobin and de-oxy haemoglobin, which can help diagnose anaemia and other haematological problems. Spectroscopy can also be used to identify aberrant cell shapes and compositions, aiding in the diagnosis of leukaemia and other hematopoietic malignancies [17]. NMR spectroscopy enables the analysis of metabolic alterations in blood, facilitating the identification of metabolic illnesses and the assessment of the efficacy of dietary therapies. Spectroscopic techniques can quantify indicators of inflammation, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and identify infections by detecting specific blood components or microorganisms. This is especially beneficial for paediatric children who have chronic inflammatory problems or frequently get infections [18]. Advancements in spectroscopic techniques

have resulted in the creation of compact and accessible instruments that can be utilised in many environments, such as domestic settings, medical facilities, and remote locations. These gadgets improve the accessibility of haematological monitoring, ensuring prompt identification and treatment of health issues in children. By integrating artificial intelligence (AI) and machine learning algorithms, the accuracy of diagnosis can be improved and outcomes can be predicted more effectively using spectroscopic data [19]. Artificial intelligence can assist in analysing intricate spectroscopic data, offering clinicians valuable information for tailored medical treatment [20].

The current study is dedicated to improving spectroscopic techniques to enhance their sensitivity and specificity for evaluating paediatric haematological conditions. Advancements in spectroscopy have the potential to result in the creation of novel diagnostic protocols that are less intrusive, more comfortable, and similarly dependable in comparison to conventional techniques. Spectroscopic research is crucial for advancing the determination of haematological profiles in children.

Technologies that are non-invasive, inexpensive, secure, and portable have become essential tools in patient management. These methods extract biomarkers generated from haemoglobin and can be used in real-time at the patient's bedside. Although pulse oximetry is widely used, the adoption of more advanced technologies that provide more comprehensive measurements is still limited [21]. However, several promising tools are being developed, including 3D volumetric imaging of vascular architecture and spatially resolved functional images of tissue oxygenation. Due to their lower cost and increased portability compared to traditional radiological imaging techniques, these approaches could significantly influence the treatment of various debilitating conditions, including rheumatoid and vascular diseases, neurological disorders, and cancer [22]–[24]. The measurement and imaging of haemoglobin oxygenation play a crucial role in detecting and diagnosing diseases. However, the equipment employed for this purpose differs significantly in terms of their imaging depth, spatiotemporal resolution, sensitivity, accuracy, complexity, physical size, and cost [25]. The extensive range of available instrumentation poses a challenge for end users in choosing suitable tools for their application and comprehending the relative constraints of various methods.

The use of spectroscopic techniques to estimate haemoglobin, haematocrit, and red blood cell, counts in a non-invasive way is a developing area with great potential, especially in the field of paediatric care. The infrared spectroscopy (IRS) employs near-infrared light with a wavelength range of 700-1000 nm to deeply penetrate tissue and quantify the absorption and scattering of light caused by blood components. Near-infrared spectroscopy (NIRS) can determine the levels of haemoglobin concentration and oxygen saturation by examining the light absorption properties of oxyhaemoglobin and de-oxy haemoglobin [26]. Raman spectroscopy is a technique that entails directing a laser onto the skin and quantifying the light that is dispersed. The changes in the wavelength of the scattered light yield insights into the molecular makeup of blood. Raman spectroscopy enables the identification of distinct molecular characteristics of haemoglobin and other constituents of blood, facilitating the determination of haemoglobin (Hb), haematocrit (Hct), and red blood cell (RBC) counts [27]. Computed tomography (CT) utilises low-coherence light to acquire high-resolution, cross-sectional pictures of tissues at the micrometre scale. The light that is reflected provides data regarding the amount of blood and the rate at which it is flowing [28]. Optical coherence tomography (OCT) can be utilised to quantify haematocrit levels and RBC counts by examining the optical properties and flow characteristics of blood within capillaries [29]. To achieve reliability, correct estimation necessitates thorough calibration against standard procedures such as routine blood testing. Spectroscopic measurements can be affected by variations in skin pigmentation, tissue composition, and external factors such as ambient light, which can impact their accuracy [30].

The precise measurement of Hb, Hct, and RBC count is crucial in paediatric therapy, as these values indicate a child's overall health, capacity for oxygen transport, and potential blood-related issues. Recognising and managing anaemia, a prevalent condition that can hinder cognitive growth and development, relies on monitoring Hb levels in infants and young children. High levels may suggest polycythaemia, dehydration, or congenital heart issues, whereas low Hb levels might indicate iron deficiency anaemia, thalassaemia, or chronic illnesses. Anaemia of prematurity particularly impacts preterm infants, making diligent Hb monitoring crucial to prevent issues such as hypoxia and developmental abnormalities. Another important measure that reflects the proportion of red blood cells in the total blood volume is haematocrit (Hct). Unusual Hct levels may suggest blood loss, dehydration, or issues with bone marrow. Neonatal polycythaemia, indicated by elevated Hct levels in infants, can lead to increased blood viscosity and a higher risk of thrombosis or ischaemia. Conversely, low Hct levels may suggest haemolysis or bleeding, necessitating prompt clinical evaluation. The RBC count serves as a fundamental indicator of erythropoiesis and aids in the identification of haematological issues. Abnormal RBC counts in children may indicate disorders such as haemolytic anaemia, sickle cell disease, or bone marrow inhibition. A low RBC count may suggest chronic disease or various other conditions, whereas a high RBC count might indicate hypoxia or heightened activity in the bone marrow.

Especially in paediatric care, where real-time analysis and patient safety are rather crucial, spectroscopic techniques are far superior to conventional blood tests. Standard procedures including venipuncture and capillary sampling are intrusive and can cause discomfort and disturbance to small children and newborns. These operations, especially in babies whose veins are still fragile, involve risks including destruction of tissue, infection, or harm. Moreover, low blood volumes in infants limit the variety of tests accessible as well as the types of data that may be investigated and complicate frequent sampling. On the other hand, spectroscopic methods increase patient comfort as they are painless and free of invasive procedures. These methods use optical sensors and specific light colours to measure haemoglobin, haematocrit, and red blood cell absorption and reflection without blood samples. Constant monitoring helps this function, especially as it lessens the mental and physical stress experienced by young patients. Spectral tools allow one to view things in real-time, so they have numerous main benefits. Unlike conventional blood tests that must be handled in a lab, spectral approaches produce results right away; this is particularly vital in critical care settings where decisions must be taken quickly. For example, newborn intensive care unit (NICU) doctors may quickly spot and treat disorders like hypoxia or anaemia by continuously monitoring the haemoglobin and haematocrit levels. Portable spectroscopic tools also provide point-of-care testing in areas hard to access or lacking resources by their use. Faster diagnosis and treatment coming from this improves healthcare. This technique presents a beneficial substitute for conventional blood tests, especially for youngsters who are more vulnerable to being injured, since its accuracy, ease of use, and patient friendliness are enhanced.

2. METHOD

Data collection at N.M. Hospital, Erode, Tamil Nadu involves the use of reflectance spectroscopy in four distinct anatomical regions such as the ear lobe, elbow, wrist, and fingertip for the participant characteristics shown in Table 1. The inclusion requirements call for participants in all age ranges between 12-16 years. Exclusion criteria entail improving the data from non-cooperative children to match the measuring technique in the perfect supine position. By applying these criteria, finally, 45 participants' data have been taken into account for analysis. The participant traits reveal distinct differences in height, weight, and age between the groups of children and adults. The children's group showed a significantly lower average height (115.12 ± 14.01 cm) and weight (19.64 ± 6.07 kg) compared to the adults, who had averages of 151.00 ± 7.90 cm in height and 40.90 ± 7.83 kg in weight. Moreover, the younger group displays a wider age spectrum than the adult group, with an average age of 7.00 ± 5.00 years, in contrast to the adults who average 14.00 ± 2.00 years. 20 boys and 18 girls make up the group of children, while 5 men and 2 women make up the group of adults. Regarding size, the group of children (38 people) is much bigger than the group of adults (7 people). This creates a more substantial dataset for the paediatric study.

Table 1. Participant characteristics

Parameters	Children's	Adults
Height	115.12±14.01	151.00±7.90
Weight	19.64±6.07	40.90±7.83
Age	7.00±5.00	14.00± 2.0
Gender	Male: 20	Male: 05
	Female: 18	Female: 02

This study was performed in the presence of the child specialist in N.M. Hospital. The current research experimental setup is depicted in Figure 1. The non-invasive probe was placed on capillaries situated at the fingertip, ear lobes, wrist, and elbow of a child to acquire measurements. The experimental configuration utilised in this investigation comprised a Tungsten halogen light source, UV/VIS/NIR spectrometer and reflection probe. To guarantee stability and accurate placement of the probe, we employed a probe holder.

Utilizing spectroscopic techniques in the above-mentioned areas can offer unique advantages and pose distinct challenges. The ear lobe's thin skin enables light to penetrate more easily, hence permitting precise spectroscopic readings. The ear lobe remains largely immobile, minimising any disturbances in motion during the measurement process. Conveniently available to both patients and healthcare practitioners. However, the ear lobe can exhibit sensitivity to variations in temperature, which can potentially impact blood circulation and the precision of measurements. The limited space can restrict the positioning of spectroscopic sensors, thus affecting the quality of data. The wrist provides a readily accessible and handy location for the placement of wearable electronics or portable spectroscopic sensors. Typically, it has a thinner dermis and noticeable blood vessels, which can aid in obtaining precise spectroscopic measurements. The radial artery is

readily detectable, enabling the concurrent assessment of both pulse and spectroscopic measures. Nevertheless, the repetitive motion of the wrist can cause distortions in spectroscopic measurements. The blood circulation in the wrist can fluctuate due to movement and location, which can potentially impact the accuracy of measurements. The fingertip possesses an abundant network of capillaries that guarantees efficient blood circulation, hence enabling accurate spectroscopic studies. The fingertip's thin epidermis enables more light penetration, hence enhancing the precision of measurements. Fingertips are readily available and commonly utilised in point-of-care devices. Yet, the sensitivity of fingertips might lead to discomfort when measures are taken frequently. The blood flow and accuracy of measurements might be affected by the movement or pressure exerted on the fingertip. The elbow has a superficial vein that runs close to the skin. Generous room for sensor positioning and potentially enhances reading stability, having visible veins at the elbow which can significantly improve the precision of spectroscopic measurements. Therefore, Reflectance spectroscopy is a valuable technique for visualising veins and estimating blood parameters. Valuable for evaluating blood flow and oxygenation in the area. According to the findings from the previous module of our research, it is evident that the elbow position outperforms the other positions. It shows the lowest error rate and the highest correlation coefficient, indicating superior performance. Therefore, current investigations regarding haematological profiles will prioritise the data obtained from the elbow.

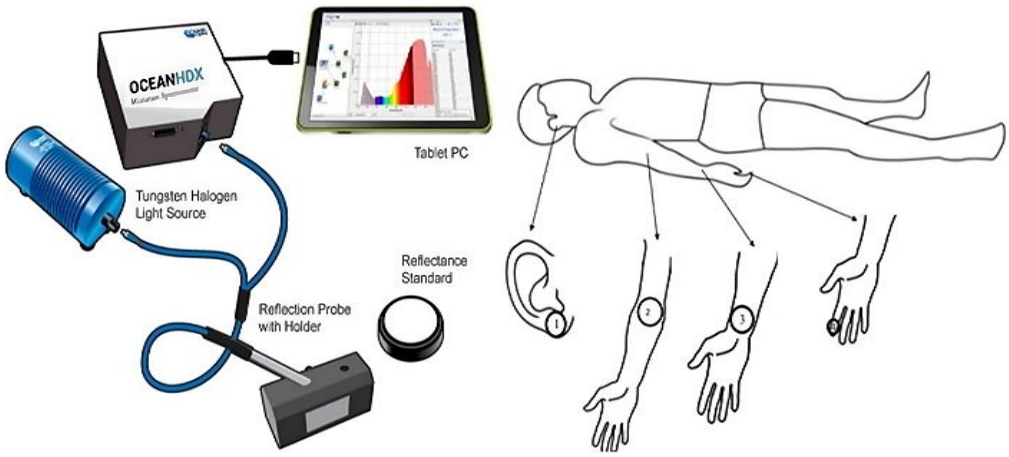

Figure 1. Measurement setup

2.1. Measurement protocol

The measurements and documentation encompassed anthropometric measurements initially, such as the subject's height, weight, age, and gender. The patient was directed to lie in a supine position for roughly 5 minutes, ensuring immobility and maintaining stillness of both their body and hands. Then perform the following steps to take reflection measurements using Spectra Suite:

a. Open ocean view 1.6.7 and switch to welcome screen and setup the spectroscopy application wizards, then click the Reflection icon to begin measuring reflectance.
b. Ensure that the entire signal is on scale, encompassing a wavelength spanning from 200 to 1100 nm.
c. Choose active acquisition as the acquisition mode (recommended) and set the acquisition parameter as,
   – Integration time: automatic
   – Scan to average: between 2 and 3
   – Boxcar width: 10
d. Take a reference spectrum with the WS-1 diffuse reflectance standard before measuring reflection. Click the store reference spectrum. This command merely stores a reference spectrum in memory.
e. Either block the light path to the spectrometer, uncheck the strobe/lamp enable box in the acquisition toolbar, or turn the light source off. Then, take a dark spectrum by clicking the store dark spectrum icon on the toolbar. This command merely stores a dark spectrum in memory.
f. Place the sample holder in targeted locations on the elbow, wrist, fingertip, and earlobe and ensure that the light path is clear. Then, take a reflection measurement by clicking on the reflection icon on the toolbar from the menu The reflectance measured from certain locations is shown in Figure 2.

The reflectance spectra observed at the elbow with wavelengths 660 and 940 nm for 45 patients are shown in Figures 3(a) and 3(b).

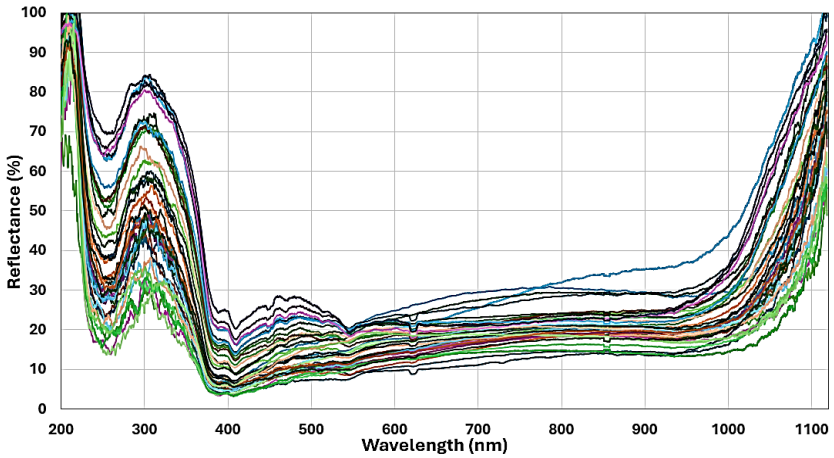

Figure 2. Reflectance measured from the elbow

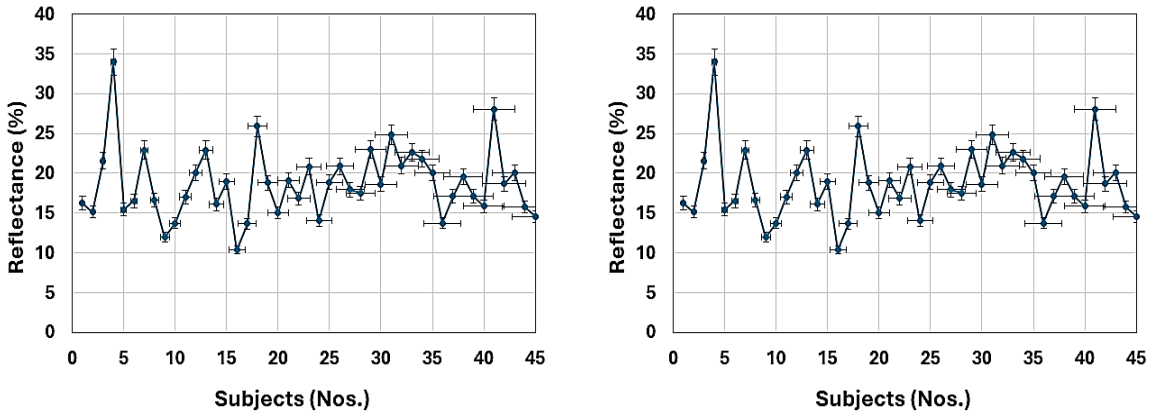

Figure 3. Reflectance Spectra taken at the elbow with (a) 660 and (b) 940 nm

3. MATHEMATICAL IMPLEMENTATION

3.1. Quantification of haemoglobin

Haemoglobin is a biomolecule found in red blood cells that transports oxygen to cells in tissues. Haemoglobin consists of four heme groups and a protein component called a globin. Beer-Lambert's law is employed in Spectro-photometric experiments to express light absorption as a function of haemoglobin content. This is done by developing the notation of absorbance, as provided in (1).

$$OD = log\left(\frac{I_o}{I}\right) = \epsilon cl \quad (1)$$

where OD is optical density, I_o is intensity of incident light, I is intensity of transmitted light, ϵ is extinction coefficient of the absorbing species at wavelength λ, c is concentration of the absorbing species; l is optical path length

The variable represents the extinction coefficient of haemoglobin, which is a measure of how strongly haemoglobin absorbs light. Since it affects the amount of light absorbed by haemoglobin molecules at a particular wavelength, estimating haemoglobin concentration mostly depends on the extinction coefficient. Every form of haemoglobin—oxy-haemoglobin (Hb), deoxy-haemoglobin (Hb), and methaemoglobin (MetHb)—has a varied extinction coefficient depending on their various optical properties. Haemoglobin absorbs light most strongly in the visible and near-infrared (NIR) bands; peak absorptions for both oxy- and deoxy-haemoglobin are 415 nm. These extinction coefficients define sensitivity of spectroscopic measurement. Variations in extinction coefficients allow difference and measurement of Hb oxygenation levels, therefore enabling exact calculation of total haemoglobin content and oxygen saturation (SpO2) in the blood. Important

for non-invasive diagnostic instruments, the precision of the extinction coefficients applied in the Beer-Lambert model determines the accuracy of Hb estimate. Accurate Hb concentration estimation using spectroscopic methods helps to real-time monitor anaemia, hypoxia, and blood oxygenation in paediatric haematology, hence lowering the demand for intrusive blood sample. When the measured sample contains a combination of oxygenated and deoxygenated haemoglobin, equation (1) can be enlarged further as in (2).

$$I^{\lambda} = \{\epsilon^{\lambda}_{HbH}[HbH] + \epsilon^{\lambda}_{HbO}[HbO]\}p \quad (2)$$

where, ϵ is molecular concentration of haemoglobin, $OD\lambda$ is the optical density at λ, HbH and HbO were the extinction coefficients of deoxygenated and oxygenated haemoglobins respectively. Given the condition of the light path p=1cm, the extinction coefficients are calculated by (3) and (4) respectively.

$$[HbO] = \frac{\epsilon^{\lambda 2}_{\mathrm{HbH}} I^{\lambda 1} - \epsilon^{\lambda 1}_{\mathrm{HbH}} I^{\lambda 2}}{\mathrm{p}(\epsilon^{\lambda 2}_{\mathrm{HbH}}\epsilon^{\lambda 1}_{\mathrm{HbO}} - \epsilon^{\lambda 1}_{\mathrm{HbH}}\epsilon^{\lambda 2}_{\mathrm{HbO}})} \quad (3)$$

$$[HbH] = \frac{\epsilon^{\lambda 2}_{HbO} I^{\lambda 1} - \epsilon^{\lambda 1}_{HbO} I^{\lambda 2}}{p(\epsilon^{\lambda 1}_{HbH}\epsilon^{\lambda 2}_{HbO} - \epsilon^{\lambda 2}_{HbH}\epsilon^{\lambda 1}_{HbO})} \quad (4)$$

Substitute $\lambda 1 = 660$ nm and $\lambda 2 = 940$ nm in (3) and (4). Figures 4 and 5 illustrate the graph depicting the molecular concentration of the haemoglobin at different wavelengths. The total haemoglobin concentration is estimated through (5) where the oxygenated and deoxygenated haemoglobins are summed.

$$[Hb]_{total} = [HbH] + [HbO] \quad (5)$$

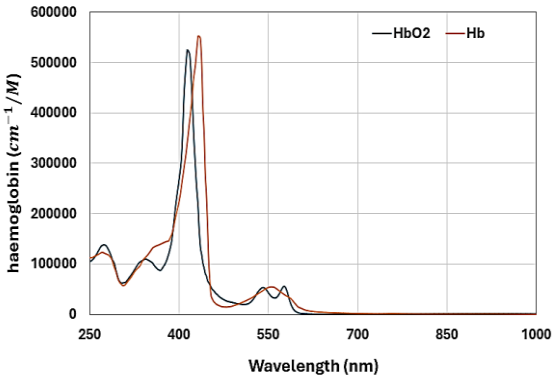

Figure 4. Molecular concentration of haemoglobin at different wavelengths

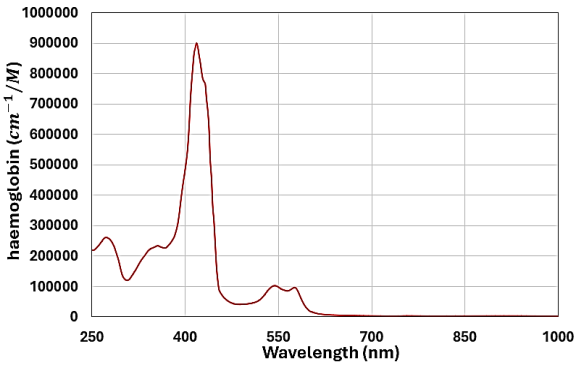

Figure 5. Molecular concentration of total haemoglobin at different wavelengths

3.2. Estimation of Hct from Hb

Estimating haematocrit from haemoglobin is a prevalent technique in clinical haematology due to the firmly established correlation between these two variables. Haemoglobin concentration is a measure of the quantity of haemoglobin in the blood, whereas haematocrit indicates the proportion of the blood volume made up of red blood cells. The correlation between haemoglobin and haematocrit can be utilised to approximate one value based on the other using a relatively straightforward mathematical expression shown in (6).

$$[Hct] = 3 \times Hb \quad (6)$$

Equation for estimating haematocrit from haemoglobin relies on the assumption that a specific percentage of haematocrit corresponds to each gram of haemoglobin per decilitre of blood. Haemoglobin and haematocrit are intimately linked as haemoglobin is enclosed within red blood cells, which contribute to the overall haematocrit measurement. Under normal physiological conditions, there is usually a stable ratio between the levels of haemoglobin and haematocrit. This ratio is frequently employed in clinical practice to approximate one parameter when the other is already known. Though it is an approximation and can be erroneous in some circumstances, the Hct-Hb connection is a good estimate tool.

3.3. Estimation of RBC from Hb

Haemoglobin is located within erythrocytes, and the red blood cell count indicates the quantity of erythrocytes in a specific blood volume. The correlation between Hb and RBC can be affected by variables such as the average haemoglobin content of individual red blood cells, commonly referred to as the MCH. Estimating the RBC counts based on the concentration of haemoglobin can be beneficial in clinical settings for rapid evaluations, particularly when direct measurements of RBCs are unavailable. The correlation between haemoglobin and red blood cell count is more intricate compared to the correlation between Hb and HCT. However, a rough approximation can be obtained by utilising average data as shown in (7).

$$[\text{RBC}]\left(\text{in}\frac{\text{millions}}{\text{microliter}}\right)=\frac{[\text{Hb}]\left(\frac{\text{g}}{\text{dL}}\right)\times 10}{\text{Hb content}\left(\frac{\text{g}}{\text{RBC}}\right)} \quad (7)$$

Utilising the correlation between haemoglobin content in grams per RBC and haemoglobin concentration is a valuable and efficient approach for estimating RBC count in clinical evaluations. Although this estimation may be useful, it is crucial to take into account individual variability and the clinical situation to accurately interpret it. The technique for estimating RBC count relies on the assumption of a constant MCH value, which may not be suitable in various clinical situations such as anaemia, haemolytic diseases, dehydration, or in infants. In increasingly complex clinical scenarios, the precision of these calculations can be enhanced by utilising advanced methodologies and thorough haematology profiles.

4. RESULT AND DISCUSSION

In order to assess the effectiveness of estimated values for Hb, Hct, and RBC levels, it is essential to compare the real Hb values obtained from laboratory testing with those estimated using non-invasive or indirect approaches, such as reflectance spectroscopy. The estimated values obtained from the samples are taken from the elbow area specifically to improve the accuracy and other parameters and the corresponding plots are shown in Figures 6 to 8.

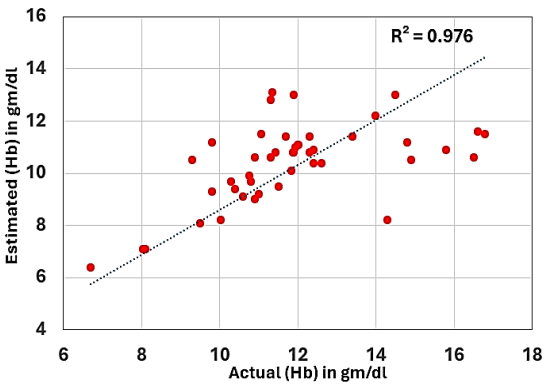

Figure 6. Comparison between the actual Hb Vs. estimated Hb

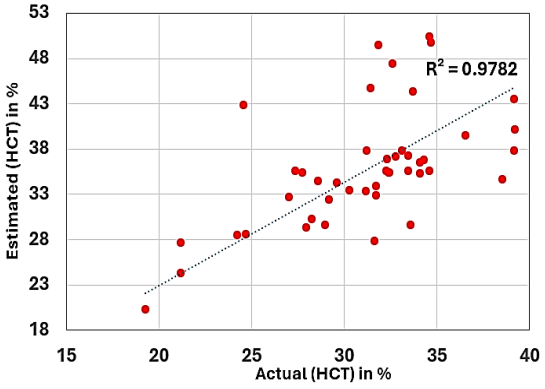

Figure 7. Comparison between the actual HCT Vs. estimated HCT

Performance metrics for regression analysis are crucial to evaluate how well a model predicts the continuous outcomes. The coefficient of determination R2 for estimation of Hb is found to be 0.976 with mean absolute error of 0.018 and root mean squared error of 0.023. The coefficient of determination R2 for estimation of HCT is found to be 0.9782 with mean absolute error of 0.052 and root mean squared error of 0.070. The coefficient of determination R2 for estimation of RBC is found to be 0.972 with mean absolute error of 0.072 and root mean squared error of 0.094. When assessing the accuracy and precision of a diagnostic test in classifying the normal and abnormal group, it is crucial to comprehend the significance of true positive, true negative, false positive, and false negative. These performance measures are essential for evaluating the test's performance. Data enhancements are necessary to decrease the occurrence of incorrect results which also improves the overall precision of diagnosis.

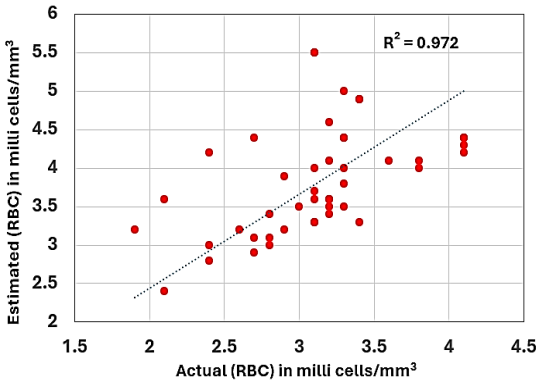

Figure 8. Comparison between the actual RBC Vs. estimated RBC

The given data in Table 2, presents the performance parameters for the estimation of performance metrics such as accuracy, precision, sensitivity and specificity in identifying haemoglobin, haematocrit, and red blood cell count using a non-invasive diagnostic approach for normal and abnormal subjects and same has been given in Table 3.

Table 2. Confusion matrix obtained for normal and abnormal subjects

Parameters	Total no. of Children		
	Hb	HcT	RBC
True Positive (TP)	24	17	09
True Negative (TN)	10	14	23
False Positive (FP)	02	01	00
False Negative (FN)	09	13	13

The statistical analysis shown in Figures 9 to 11 indicates that the proposed model effectively identifies abnormal category participants from normal category, with a high degree of accuracy. There are only a few instances where the model incorrectly predicts normal participants as abnormal, slightly impacting overall accuracy. This accuracy can be further enhanced by removing outliers using preprocessing procedures. However, the model's robust capability to accurately classify abnormal participants underscores its potential as a valuable pre-screening tool for disease identification that relies on blood tests.

Table 3. Performance metrics for Hb, Hct and RBC

Haematological Parameters	Performance Metrics			
	Accuracy	Precision	Sensitivity	Specificity
Hb	75.56 %	92.30 %	72.72 %	83.33 %
HcT	68.89 %	94.44 %	56.66 %	93.33 %
RBC	71.77 %	100.00%	40.90 %	100.00%

The I_N (Hb) denotes the invasive normal category of Hb in gm/dl, and its level is higher than the invasive abnormal category I_A(Hb). Also, the non-invasive results of normal NI_N(Hb) and abnormal NI_A(Hb) predicted using the proposed technique is highly correlated with the invasive measurements as shown in Figure 9. The I_N (HCT) denotes the invasive normal category of haematocrit in percentage, and its level is higher than the invasive abnormal category of haematocrit I_A(HCT). Also, the non-invasive results of normal NI_N(HCT) and abnormal NI_A(HCT) predicted using the proposed technique is highly correlated with the invasive measurements as shown in Figure 10. The I_N (RBC) denotes the invasive normal category of red blood cells in milli cells per millimetre cube, and its level is higher than the invasive abnormal category of haematocrit I_A(RBC). Also, the non-invasive results of normal NI_N(RBC) and abnormal NI_A(RBC) predicted using the proposed technique is highly correlated with the invasive measurements as shown in Figure 11. From Figures 9 to 11, It has been observed that the overall performance of prediction is high for children under the age of 12, and the same can be used for prognosis of haematological profile which requires for continuous monitoring in case of dengue, anaemia and other diseases without any blood loss.

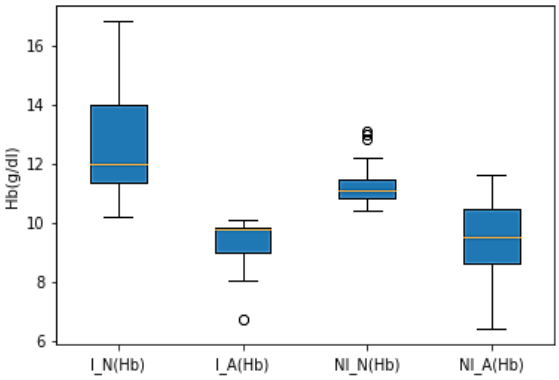

Figure 9. Comparison between normal and abnormal category of predicted Hb

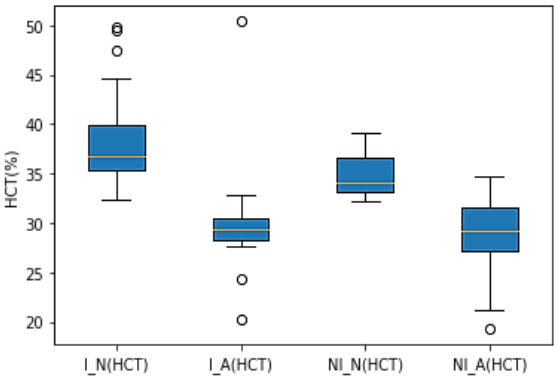

Figure 10. Comparison between normal and abnormal category of predicted HcT

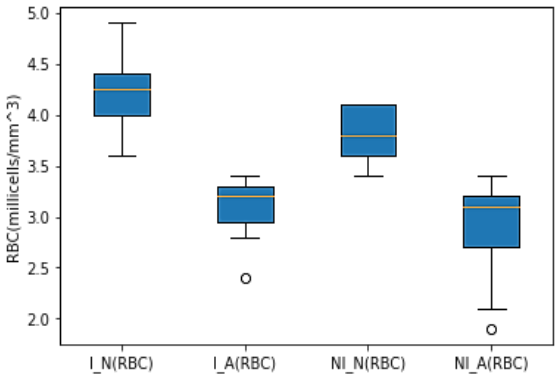

Figure 11. Comparison between normal and abnormal category of predicted RBC

5. CONCLUSION

The conventional method of conducting blood tests using needles might induce anxiety, particularly in youngsters who have a fear of needles. Infections seldom occur at blood extraction sites. Failure to apply pressure after removing the needle might lead to substantial bleeding. To overcome these restrictions, the non-invasive reflectance spectroscopic technique is more effective in estimating haematological parameters such as Hb HCT and RBC in patients. The coefficient of determination in identifying haematological parameters is 0.976 in estimating Hb, 0. 9782 in estimating HCT and 0.972 in estimating RBC. The statistical analysis demonstrates superior accuracy, precision, sensitivity, and specificity and showcases the potential advancements in the proposed non-destructive approach. The non-invasive reflectance spectroscopy technique is more successful in establishing haematological parameters (Hb, Hct, and RBC) in children rather than adults. Children demonstrate with respect to all three criteria superior accuracy, precision, sensitivity, and specificity. This implies that the technique may be particularly useful for paediatric applications may be due to physiological variations or better calibration for younger persons.

6. LIMITATION AND FUTURE SCOPE

This study is sensitive to the measurement protocol, which makes it essential to strictly follow the experimental setup and measurement protocol. The slightly inaccurate calibration of dark and light references may reduce the overall accuracy. Using a small or homogeneous sample limits this research, hence the performance of the model could not be very generalizable to more varied or bigger populations. Haematological profiles change with age, ethnicity, and underlying medical disorders; so, more data are needed for more general therapeutic relevance. Future research might investigate a more complete patient evaluation combining non-invasive haematology with other indicators such as oxygen saturation, pulse rate, hydration markers, lipid profiles, electrolytes and so on.

ACKNOWLEDGEMENTS

We extend our gratitude to Dr. N. Murugan, M.B.B.S., D.C.H., a dedicated child specialist, for his invaluable contributions. Additionally, we would like to acknowledge and appreciate the Director and staff of NM Hospital for their collaborative spirit and unwavering support.

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BIOGRAPHIES OF AUTHORS

P. Divyabharathi was born on April 8, 1993, in Salem, Tamil Nadu. She holds a B.E. in electronics and communication engineering and an M.E. in applied electronics. She is currently pursuing her Ph.D. at Veltech Rangarajan Dr. Sagunthala R&D Institute of Science and Technology. With over 9 years of academic experience as an assistant professor, she has contributed significantly to research through 8 Scopus-indexed journal publications and 12 presentations at international conferences. Her areas of expertise include biomedical signal processing, wireless communication, and VLSI design. She is an active member of several professional bodies, including the International Association of Engineers (IAENG), the Institute of Research Engineers and Doctors (IRED), and the society of digital information and wireless communications (SDIWC). She can be contacted at email: divyabharathi0804@gmail.com.

Neelamegam Devarasu was born on April 6, 1988, in Villupuram, Tamil Nadu, India. He is currently serving as a professor in the Department of Electronics and Communication Engineering at Vel Tech Rangarajan Dr. Sagunthala R&D Institute of Science and Technology. He received his B.Tech, M.Tech, and Ph.D. degrees from Pondicherry University. With over 14 years of experience in teaching and research, Dr. Neelamegam has published more than 25 papers in reputed international journals indexed in SCI and Scopus. He is the inventor of 2 published and 2 granted patents and has authored 4 book chapters with international publishers. He is currently leading two ongoing research projects funded by the Department of Biotechnology (DBT), Government of India. His research interests include UV/VIS/NIR/THz spectroscopic techniques and bioelectrical impedance spectroscopy. Dr. Neelamegam is a member of professional societies such as IEEE, IAENG, and InSc. He can be contacted at email: drdneelamegam@veltech.edu.in.