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# UV-Vis Spectrophotometry Observation to Find Appropriate Wavelength for Non-Invasive Blood Haemoglobin Level Measurement Optical Device

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**Abstract:** The wavelength convention for non-invasive blood hemoglobin measurement remains inconclusive. This experimental observation to find the appropriate wavelength candidate of LED for non-invasive blood hemoglobin level measurement optical device in 200 nm to 900 nm range. This observation ran in Prodia Kedoya and Biochemistry Lab, Universitas Krida Wacana, West Jakarta, in July 2019. The blood samples were obtained from 10 randomly selected consenting non blinded, healthy adult subjects between 18 and 60 years old. Each blood sample was diluted using double distilled water and measured absorbance using UV-Vis Spectrophotometry. Then it compared to blood hemoglobin level by standard gold measurement from Prodia Kedoya. The result shows functional group found in human Hb is C=O. Appropriate wavelengths were obtained based on the Pearson correlation, standard deviation, and human skin pigment, which are 525 nm, 550 nm, and 570 nm. Then the measurement of Hb levels is carried out at the selected wavelength and processed using a ZunZunSite3 to get the mapping data of Hb level from ten respondents. Root mean square error from the measurement. The error obtained is minimal, which indicates that the wavelength used is suitable for measuring the Hb level. Ethical Clearance: 076/IT3.KEPMSM-IPB/SK/2018.

**Keywords:** blood hemoglobin level; UV-Vis spectrophotometry; optical measurement; LED. wavelength.



## 1. Introduction

Hemoglobin (Hb) which is the most vital unit in human blood, is a protein in Red Blood Cell (RRBC) that responsible for carries oxygen to the tissues [1]. The healthy range of the haemoglobin concentration for an adult male between 20 and 80 years is from 143.6(g/l) to 154.3(g/l) and for an adult female is from 134.1(g/l) to 135.4(g/l) [2-4]. Hemoglobin levels that

are too high and too low can sign health problems such as anemia. Anemia is a medical condition when the concentration of Hb in the blood is below a specific threshold (70−130 g/L depending on age, sex, and pregnancy status and severity level) [5], resulting in the reduced oxygen-carrying capacity of red blood cells [6]. It is crucial to regularly measure Hb levels to maintain BHL within the normal range for monitoring health conditions [7]. So, it is necessary to know the appropriate LED wavelength to measure blood hemoglobin level (BHL). This study analyzes the best candidate wavelengths for non-invasive BHL measurement in the range of 200 - 900 nm. According to research by Jenie *et al.* [8], each wavelength between 250 nm to 900 nm has the potential to be used for BHL measurement.

The appropriate wavelength will be used for non-invasive BHL measurements. This non-invasive method is unlike conventional methods based on phlebotomy [9], which are hurting. This method does not require blood sampling, so there is no need for needles and minimizes the possibility of infection [10]. The observation of hemoglobin 'trends' can prove enormous clinical value in decision making regarding blood transfusions [11], produces data on the measurement of Hb concentration in real-time continuously [12,13]. Some non-invasive techniques are pulse oximetry, optoacoustic, diffuse reflectance spectroscopy, and photoplethysmograph (PPG). Pulse oximetry measures oxygen saturation by illuminating skin and measuring changes in light absorption of oxygenated (oxyhemoglobin) and deoxygenated blood (reduced hemoglobin) [11]. Optoacoustic technique with the utilization method of ultrasonic wave absorption (absorbance) [14,15]. Diffuse reflectance spectroscopy techniques by measuring the reflectance factor of the spectrum of light passing through the medium [16,17]. Photoplethysmograph (PPG) uses light as a source and a photodetector as a sensor to measure changes in the volume of pulsatile blood in the tissue [18-20] without pain and risk of infection [19].

Non-invasive hemoglobin concentration measurement can use light absorption at different wavelengths from UV-VIS spectrophotometry which has a range of 150-900 nm [8] and only requires a small amount of material for analysis. Hb produces the maximum absorption in the wavelength range of 415-476 nm, but its molar absorptivity is relatively low, with a value of about 3300 per M cm. Appropriate, high-quality methods for Hb measurement in clinical laboratories and field settings are necessary to ensure the accuracy of Hb measurements [21-23], so that the determination of the method needs to consider several factors, including the source of the blood sample, cost of the analysis, and reproducibility of the results [24-25]. The accuracy of BHL measurements using the selected wavelength is processed using ZunZunSite3. This study aims to find a suitable wavelength for measuring the Hb level device, determined from the analysis based on predetermined criteria and then tested to measure the error.

## 2. Materials and Methods

### *2.1. Data retrieval.*

Blood samples were taken from ten respondents with the criteria of men and women, 18-60 years old, not afraid of needles, and women who were neither pregnant nor breastfeeding. This observation ran in Prodia Kedoya and Biochemistry Lab, Universitas Krida Wacana, West Jakarta, in July 2019. The blood samples were diluted 400x using distilled water with the amount of solute compared to solvent ten µl: 3990 µl. Then the samples were homogenized using the Wiggens vortex3000 tool. The dissolved samples were inserted into a 3-4 ml cuvette

to measure the absorbance using a UV-Vis spectrophotometer. Each sample was observed three times. Ethical Clearance: 076/IT3.KEPMSM-IPB/SK/2018.

*2.2 Data processing.*

The absorbance values were processed using Microsoft Excel to produce the required wavelength candidates. After that, the wavelength candidates were analyzed using ZunZunSite3. The first and second repetitions as training data were used to obtain the equation and distribution data of BHL from ten respondents. Meanwhile, the third repetition test data is processed to obtain root mean square error (RMSE) values. The wavelengths are said to be suitable if the RMSE value is minimal.

## 3. Results and Discussion

Characterization of the sample using a UV-Vis spectrophotometer resulted in peak absorption to identify functional groups of a molecule. The Hb pattern in the UV-Vis spectrum (Figure 1) shows five absorbance peaks at 275 nm, 345 nm, 416 nm, 542 nm, and 577 nm. The absorption peaks show the same functional group bond, namely C = O, because according to Creswell *et al.* [26], the absorption of the C = O functional group is in the wavelength range of 200-900 nm.

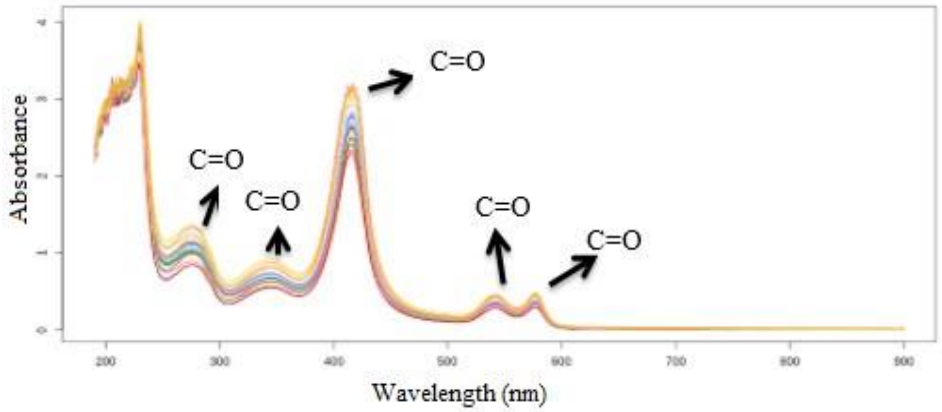


**Figure 1.** UV-Vis spectrum pattern of BHL from ten respondents.

Figure 2 shows the standard deviation value of 10 samples. Standard deviation analysis determines the sample distribution from the sample and how close the individual data is to the sample mean. A more considerable standard deviation value indicates that the personal data points are far from the mean value [27], vice versa. Pearson correlation analysis was used to determine the level of closeness of the relationship between two variables [28]. The high correlation indicates a relationship between the UV-Vis absorbance data and the blood hemoglobin concentration from Prodia shown in the range 300-600 in Figure 3. Determining suitable wavelength candidates for non-invasive BHL measurements should have a small standard deviation value, high person correlation, taking into account the body tissue that absorbs wavelength regions in the UV-Vis range. Based on these criteria, the graph of Pearson correlation, standard deviation, and absorbance are put together in figure 4 to make it easier to determine the wavelength that fits these criteria.

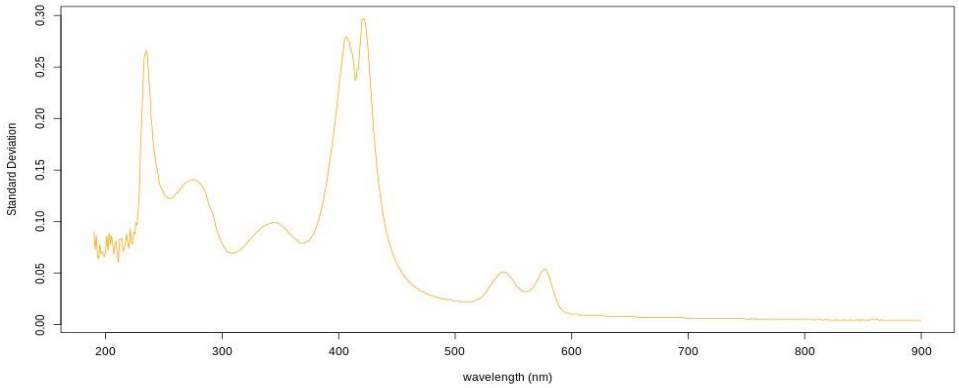


**Figure 2.** Standard deviation value of all samples absorbance at a wavelength of 200-900 nm.

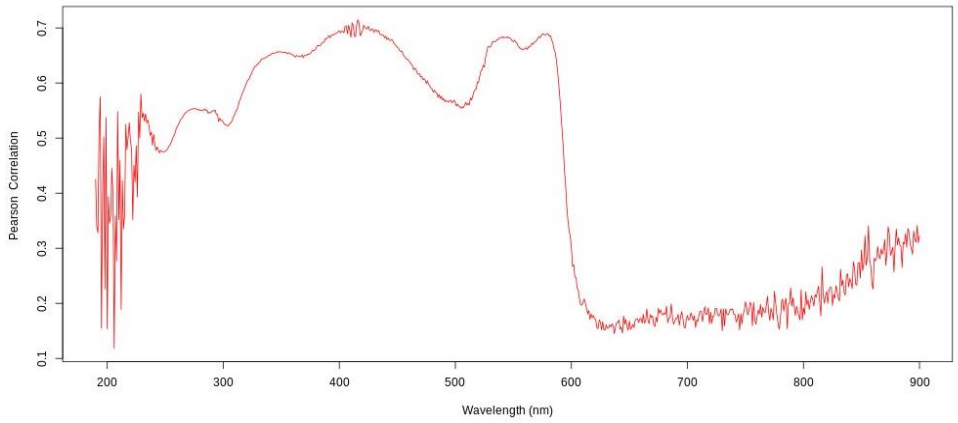


**Figure 3.** Pearson correlation value between absorbance and Hb level at a wavelength of 200-900 nm.

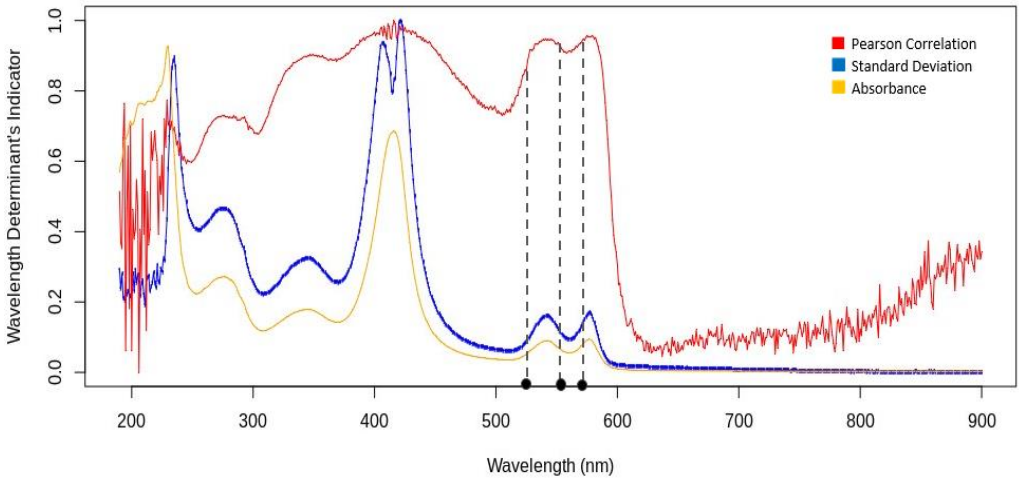


**Figure 4.** A graph to determine the appropriate wavelength for BHL measurement.

The fingers will be inserted into the hemoglobin probe equipped with the LED that has been selected. Human skin has a body pigment called melanin which can absorb UV-VIS wavelengths with the highest absorption range of 300 nm - 500 nm [29] and low absorption in 500-800 nm [28]. Apart from melanin, the human body also contains water. Water has the highest absorption in the wavelength range of 600 nm - 1000 nm [8]. Considering the presence of melanin and water, LEDs with wavelengths below 500 nm and above 600 nm are not used to measure the hemoglobin level. So, the candidate wavelength ranges of LEDs that fit these criteria have a small standard deviation value, high person correlation (Figure 4), and the absorption range of water and melanin are 525 nm, 555 nm, and 570 nm. According to Irzaman [30], wavelength above 500 nm has low absorbance. However, the correlation to blood

hemoglobin level is relatively high, making them easy to utilize for measurement. Our team has observed BHL measurements using wavelengths of 660 and 940 nm and obtained an accuracy of about 94.2% [30].

The wavelengths used to measure non-invasive BHL are 525 nm and 570 nm. The wavelength of 555 nm is not used because it is too close to the other two wavelengths, potentially leading to an inaccurate reading. Mapping of BHL data at wavelengths 525 nm and 570 nm of 10 respondents shown in Figure 5. The absorbance values at 525 nm and 570 nm wavelengths and the Hb level values were processed using ZunZunSite3 to obtain the measurement equation (eq 1).

$$\frac{a+(b\,x\ln(x))+(c\,x\exp(y))}{1+dx+fy} \quad (1)$$

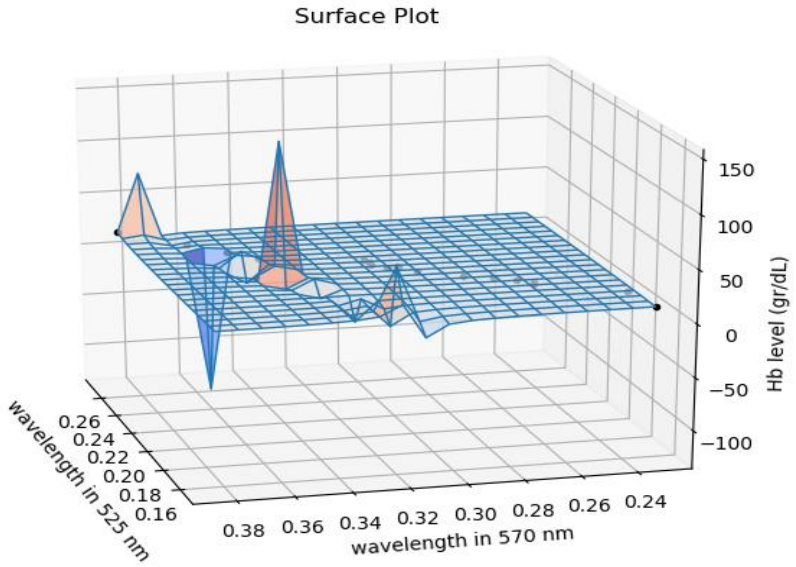


**Figure 5.** Plot surface mapping of Hb level of training data from ten respondents.

Measurement of BHL in ten respondents was carried out three times. The first and second repetitions were training data, the third repetition was test data. The third repetition data is processed using the equation obtained from ZunZunSite3 (eq 1) to obtain the BHL value. This BHL value will be compared with the BHL value of Prodia Kedoya. The RMSE value was measured from the difference between two BHL values (Table 1). The RMSE value used to evaluate the accuracy of the results and degree of error measuring LED on concentration [31]. The absolute mapping error of BHL measurement is shown in Figure 6.

**Table 1.** Hb level comparison of test data from Prodia and ZunZunSite3.

| **Absorbance in wavelength** | | **Hb level (gr/dL)** | | **Different in Hb level** |
|---|---|---|---|---|
| **525 nm** | **570 nm** | **Prodia** | **ZunZunSite3** | |
| 0,254 | 0,357 | 13,2 | 13,2603931 | 0,06039311 |
| 0,256 | 0,374 | 12,9 | 13,0195863 | 0,11958632 |
| 0,157 | 0,23 | 12,1 | 11,2614429 | -0,8385571 |
| 0,181 | 0,268 | 14,5 | 12,1055761 | -2,3944239 |
| 0,231 | 0,343 | 15,8 | 14,4352938 | -1,3647062 |
| 0,177 | 0,264 | 11,3 | 11,9732511 | 0,67325109 |
| 0,213 | 0,3 | 13,6 | 12,934629 | -0,665371 |
| 0,197 | 0,278 | 12,5 | 12,5136423 | 0,01364227 |
| 0,197 | 0,278 | 12,2 | 12,5136423 | 0,31364227 |
| 0,197 | 0,288 | 12,8 | 12,6182407 | -0,1817593 |
| RMSE | | | | 0,9666641 |

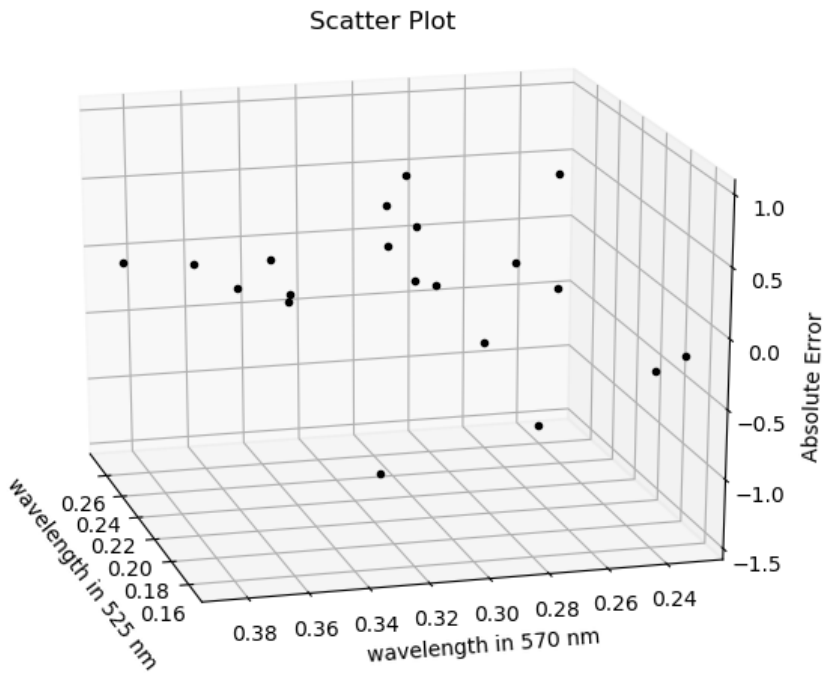


**Figure 6.** Scatter plot mapping of an absolute error on Hb level measurement.

The value of BHL obtained from Prodia and ZunZunSite3 has a slight difference. The RMSE value obtained was very small, around 0.966. So it can be said that the Hb level measurement using an LED light source with a wavelength of 525 nm and 570 nm is accurate. The finding of 525 nm and 570 nm wavelengths is expected to help develop non-invasive BHL measurement devices. In addition to the high Pearson correlation and low standard deviation, the two wavelengths have also considered good absorption areas for melanin and water in the body so that Hb can adequately absorb the light without having to remove a blood sample from the body. The error level of both wavelengths has also been tested by comparing the data measured using these wavelengths with the actual data from Prodia. The data obtained is only a difference 0.01-1 gr/dL, so it can be said that the selected wavelength is suitable for BHL measurements.

## 4. Conclusions

Electron transition analysis based on the appearance of absorption peaks on hemoglobin indicates the presence of a functional group C = O. Candidate wavelength LEDs are obtained based on small standard deviation values, high person correlations and considering body tissues that absorb UV-Vis wavelengths. The LED wavelengths that meet these criteria are 525 nm, 555 nm and 570 nm. 525 nm and 570 nm are the wavelengths used to measure BHL using the equation from ZunZunSite3. Then the data is compared with the BHL data from Prodia and found to have an RMS error of about 0.966. The small error indicates that the Hb level measurement using an LED light source with a 525 nm and 570 nm wavelength is appropriate.

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## Conflicts of Interest

The authors declare no conflict of interest.

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