Three-Wavelength Method for the Optical Differentiation of Methemoglobin and Sulfhemoglobin in Oxygenated Blood

Spencer R. Van Leeuwen[1] and Gladimir V. G. Baranoski[1], *Senior Member*, *IEEE*, and Bradley W. Kimmel[1]

***Abstract*— Methemoglobinemia and sulfhemoglobinemia are rare, but potentially life threatening, diseases that refer to an abnormal amount of methemoglobin or sulfhemoglobin in the blood, respectively. Unfortunately, blood samples containing abnormal quantities of methemoglobin or sulfhemoglobin have similar spectral characteristics. This makes it difficult to optically differentiate them and, hence, difficult to diagnose a patient with either disease. However, performing treatments for one of the diseases without a correct diagnosis can introduce increased risk to the patient. In this paper, we propose a method for differentiating the presence of methemoglobin and sulfhemoglobin in blood, under several conditions, using reflectance values measured at three wavelengths. In order to validate our method, we perform *in silico* experiments considering various levels of methemoglobin and sulfhemoglobin. These experiments employ a cell-based light interaction model, known as CLBlood, which accounts for the orientation and distribution of red blood cells. We then discuss the reflectance curves produced by the experiments and evaluate the efficacy of our method. In particular, we consider various experimental conditions by modifying the flow rate, hemolysis level and incident light direction.**

***Index Terms*— blood, reflectance, dysfunctional hemoglobins, predictive simulation.**

I. Introduction

Hemoglobin in blood is responsible for the circulation of oxygen throughout the body. This process relies on the reversible binding of oxygen to hemoglobin so that it can alternate between its functional forms, oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb). However, some compounds can irreversibly bind to hemoglobin. In particular, their bond will not be reversed under normal physiological conditions. The binding of these compounds with hemoglobin results in so-called dysfunctional hemoglobins, such as methemoglobin (MetHb), sulfhemoglobin (SHb) and carboxyhemoglobin (COHb). Under normal physiological conditions, only small traces of COHb and MetHb ($< 2\%$) are present in blood [1], [2] and SHb is completely absent [3]. However, various environmental and genetic factors can cause an increase of dysfunctional hemoglobins in the blood. Accordingly, a patient with abnormal levels of MetHb or SHb in the blood is said to have methemoglobinemia or sulfhemoglobinemia, respectively.

Methemoglobinemia and sulfhemoglobinemia are rare, but life threatening, diseases. In particular, high quantities of MetHb and SulfHb in the blood (70% [2] and 60% [4], respectively) can result in end-organ damage and a high mortality rate. Additionally, the correct detection of a dysfunctional hemoglobin in the blood is necessary to treat a patient. While it is possible to reverse the oxidization of hemoglobin resulting in MetHb by prescribing methylene blue [2], there is no known method to reverse the sulfur bond in SHb. Therefore, it is only possible to treat sulfhemoglobinemia through blood transfusions, introducing additional risk to the patient [4]. Furthermore, administering methylene blue to a patient with sulfhemoglobinemia can lead to renal failure [5]. For these reasons, it is important to have a reliable method to differentiate methemoglobinemia and sulfhemoglobinemia.

While it is relatively straightforward to visually distinguish the presence of COHb or MetHb in blood with a simple screening test [2], it is difficult to differentiate samples containing MetHb or SHb due to their similar spectral characteristics [4]. Additionally, most drugs that cause methemoglobinemia also cause sulfhemoglobinemia [4], [5] which makes it unlikely that a diagnosis can be formulated based on a patient's history. However, there are various existing methods for distinguishing the presence of MetHb or SHb in blood. Co-oxymeters use four, or more, wavelengths of monochromatic light to measure the spectral signature of whole blood in order to differentiate hemoglobins [2], [6]. However, co-oxymeters vary considerably and can falsely detect SHb as MetHb [4], [6]. Gas chromatography is capable of giving more accurate results [7] at the expense of requiring highly specialized equipment and a high degree of expertise [4]. Baranoski *et al.* [8] introduced a method for differentiating the presence of MetHb or SHb in the dermal tissues through non-invasive measurements of skin spectral signatures. However, their method employs a differentiation formula that was not devised to be applied directly to blood samples.

Recently, we introduced a cell-based light interaction model for human blood, known as CLBlood [9], [10], [11], which accounts for the orientation and distribution of red (blood) cells in a sample. The quantitative and qualitative evaluation of the model can be found in our previous publications [9], [11]. In this paper, we employ CLBlood to examine the differences between the spectral signature of a blood sample containing abnormal levels MetHb or SHb. We then introduce a three-point central-difference formula capable of differentiating the presence of MetHb or SHb. In order to explore the efficacy of our method under several conditions, we vary the experimental conditions by modifying the flow rate, hemolysis level and incident light direction.

[1] Spencer R. Van Leeuwen, Gladimir V. G. Baranoski and Bradley W. Kimmel are with the Natural Phenomena Simulation Group, David R. Cheriton School of Computer Science, University of Waterloo, 200 University Avenue, Waterloo, Ontario, Canada N2L 3G1. gvgbaran@cs.uwaterloo.ca

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II. Investigation Framework

In this paper, we perform *in silico* experiments with the CLBlood model which simulates light attenuation processes in a blood sample using measured optical properties of its components. Additionally, CLBlood accounts for the distribution and interaction of light with individual red cells within a blood sample. The positioning of the red cells in the sample is determined by the distribution of their orientation and the hematocrit (i.e., the volume fraction of the red cells in the sample). The former is given to the model as an input parameter represented by a combination of three orientation states, namely random, rolling and aligned with the flow direction. When blood is flowing with a low, intermediate or high shear rate, we expect the dominant state to be random, rolling or aligned, respectively [12]. We note that the rolling state in the model corresponds to what is called "tumbling" in blood rheology literature [13].

The experimental results in this paper are obtained by measuring the directional-hemispherical reflectance of an *in silico* blood sample using a virtual spectrophotometer [14] with a spectral resolution of 5 nm. The reflectance of a blood sample can vary greatly under different conditions. Therefore, we consider four distinct cases in our experiments. First, we consider two different possibilities for the distribution of the red cells' orientation: flowing and randomly oriented.

It has been observed that when the blood is flowing, high shear rates result in the red cells aligning with the direction of the flow [12]. Due to the lack of research precisely quantifying the alignment of red cells under varying conditions, we set the alignment in the flowing case to 75%, with 25% of the red cells rolling. Additionally, we remark that changing the entry point of the light affects the absorptance of the sample, due to the red cells aligning with the flow [12]. Therefore, for flowing blood, we consider two experimental cases associated with the incidence direction of the incoming light: parallel and perpendicular to the flow. For simplicity, we call these the parallel-flow case and the perpendicular-flow case, respectively.

In the experiments with randomly oriented red cells, or the random case, 100% of the cells are randomly oriented which corresponds to two possible *in vitro* conditions. Either the blood is stationary and anti-coagulant has been added to the sample to discourage red cell aggregation [15], or the sample is flowing with a low shear rate [12].

We also perform experiments with ruptured, or hemolysed, red cells. We refer to experiments with this condition as the hemolysed case. Whole blood is commonly hemolysed in a clinical setting before performing optical measurements of the concentration of MetHb and SHb. Therefore, we consider hemolysis in our experiments by reducing the hematocrit to 0% and combining the optical properties of the hemoglobin solution, released from the ruptured red cells, and the plasma [11].

In our baseline experiments, we use the parameters presented in Tables I and II. Note that we consider the expected trace amounts of COHb and MetHb ($< 2\%$) [1], [2] and the absence of SHb [3]. In addition to performing baseline curve experiments, we also examine samples with an abnormal level of MetHb or SHb. In these experiments, only the quantity of the dysfunctional hemoglobin of interest is altered. The other dysfunctional hemoglobin values remain fixed. Additionally, we retain a fixed ratio for the functional hemoglobins, $O_2Hb : HHB = 97 : 3$, as the quantity of a dysfunctional hemoglobin is increased. This ratio corresponds to the oxygenation saturation for arterial blood. We remark that a constant oxygen saturation as the amount of dysfunctional hemoglobin increases corresponds to the observed *in vivo* behaviour of blood [2].

TABLE I

CLBlood parameters [16] for the baseline experiments.

CLBlood Parameters	Value
Number of samples (per wavelength)	1000000
Wavelength range (nm)	250-1000
Angle of incidence ($^\circ$)	0
Rotate angle of incidence	False (unchecked)
Hematocrit (%)	45
Sample thickness (μm)	116.0

TABLE II

Red cell parameters used to precompute the cell-light interactions for the baseline experiments.

Red Cell Parameters	Value
O_2Hb (%)	94.09
HHb (%)	2.91
SHb (%)	0
MetHb (%)	1.5
COHb (%)	1.5
Mean cell hemoglobin content (g/L)	330.0

It was demonstrated by Baranoski *et al.* [8] that it may be possible to non-invasively differentiate the presence of MetHb or SHb in the dermal tissues by measuring the skin's spectral signature and calculating the second derivative at 615 nm. The second derivative was calculated using a three-point central-difference formula [17], considering the wavelengths 600, 615 and 630 nm.

In this paper, we use a similar approach to differentiate the presence of MetHb or SHb in blood. However, calculating the second derivative at 620 nm yields the best results for oxygenated blood samples. In particular, we employ the following central-difference formula:

$$\rho''(620) = \frac{\rho(610) - 2\rho(620) + \rho(630)}{100} \qquad (1)$$

where $\rho(\lambda)$ corresponds to the measured reflectance at wavelength λ.

To facilitate the reproduction of our experimental results, we have deployed an online version of CLBlood [16] using our own model distribution system [18]. The measured data used in our investigation can also be found on our website [19].

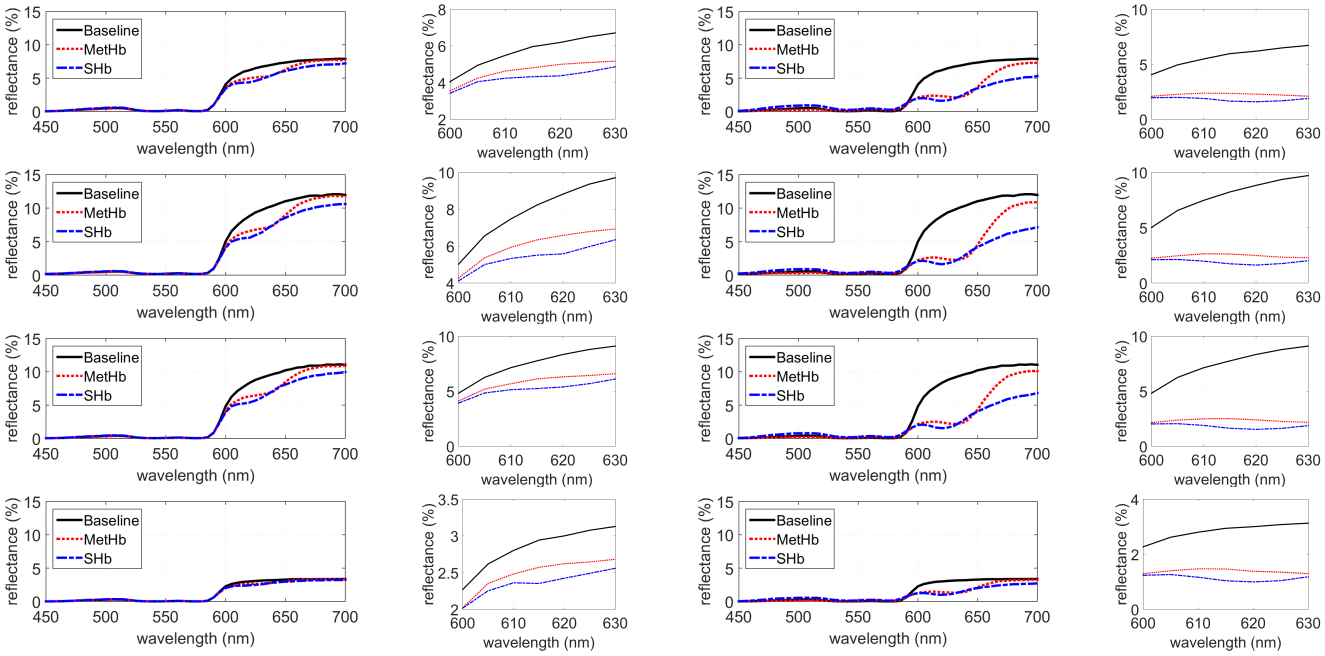

Fig. 1. Comparison of the modeled reflectance curves corresponding to the baseline sample and the samples with their respective dysfunctional hemoglobin (MetHb or SHb) making up 10% (1st and 2nd columns) and 50% (3rd and 4th columns) of the total hemoglobin. The experimental cases, from top to bottom, are as follows: the blood is flowing with the light entering parallel to the flow; the blood is flowing with the light entering perpendicular to the flow; the red cells in the sample are randomly oriented; and the blood sample is hemolysed. To the right of each plot considering the full visible spectrum (1st and 3rd columns), we provide a zoom-in of the 600-630 nm range (2nd and 4th columns).

III. Results and Discussion

In Fig. 1, we present the experimental results with each plot comparing the baseline reflectance against the reflectance of a sample with MetHb or SHb making up 10% (left) or 50% (right) of the total hemoglobin. From top to bottom, we provide the experimental results for the parallel-flow, perpendicular-flow, random and hemolysed case, respectively. We can observe that there are two common relationships between the MetHb and SHb curves in each of the plots. First, the reflectance of a sample containing SHb has a lower reflectance at 700 nm than a sample containing MetHb. Second, in the 600-630 nm range, the samples containing MetHb or SHb have a curvature in directions opposite to each other.

The reflectance at 700 nm is not necessarily useful for differentiation because the properties of blood have a wide range of values that could be considered normal. Therefore, to differentiate dysfunctional hemoglobins based solely on reflectance at a single wavelength, it would either be necessary to have a healthy sample of a specific patient's blood or the reflectance would have to be exceptionally low in order to draw any conclusions.

The curvature in the 600-630 nm range, on the other hand, is potentially useful. In particular, it indicates that we can differentiate between the two samples by calculating the second derivative of the curve. We can see that as the amount of dysfunctional hemoglobin decreases, the curves for the respective samples approach the baseline curve. Furthermore, the prominence of the curves' distinct features is diminished in the hemolysed sample (Fig. 1, bottom row). Accordingly, both of these conditions, low dysfunctional hemoglobin and hemolysed red cells, could potentially impede our ability to differentiate between MetHb and SHb.

Using Equation (1), we calculated the second derivative of the reflectance at 620 nm for samples containing 10-60% MetHb or SHb considering each of the four cases. The signs of these second derivatives are presented in Table III. In particular, a $+$ or $-$ sign indicates that the function is concave up or concave down, respectively. We can see that our method enables us to successfully differentiate between MetHb and SHb in samples with quantities as low as 10% of the total hemoglobin for all whole blood samples. We remark that this is below the percentage that the first symptom of methemoglobinemia, asymptomatic cyanosis (i.e., a purple or bluish skin coloration), begins to present itself [2].

Unfortunately, we are not able to consistently differentiate the two dysfunctional hemoglobins in the hemolysed case. This limitation can be attributed to the similar spectral characteristics of the samples (Fig. 1, bottom row). This observation indicates that there may be a universal limitation for the optical differentiation of MetHb and SulfHb in hemolysed blood samples.

We note that in addition to the experimental results presented in this paper, we considered several additional conditions. In particular, we considered samples with lower amounts of dysfunctional hemoglobin and reduced oxygen saturation. When examining the efficacy of our method for lower dysfunctional hemoglobin levels, we found that we

TABLE III

SECOND DERIVATIVE SIGNS AT 620 nm FOR THE MODELED REFLECTANCE CURVES CONSIDERING VARIOUS LEVELS OF A GIVEN DYSFUNCTIONAL HEMOGLOBIN (METHB OR SHB) AND EACH OF THE FOUR EXPERIMENTAL CASES.

	MetHb				SHb			
	Parallel-Flow	Perpendicular-Flow	Random	Hemolysed	Parallel-Flow	Perpendicular-Flow	Random	Hemolysed
10%	−	−	−	−	+	+	+	+
20%	−	−	−	−	+	+	+	+
30%	−	−	−	−	+	+	+	+
40%	−	−	−	−	+	+	+	+
50%	−	−	−	+	+	+	+	+
60%	−	−	−	−	+	+	+	+

could not consistently differentiate between MetHb and SHb. Similarly, our experiments considering an oxygen saturation of 70% provided poor results. We detected inaccuracies similar to the hemolysed case, which indicates that our method is less effective considering higher quantities of HHb. However, it is possible that another formula could be more effective for differentiating MetHb and SHb in deoxygenated blood.

As mentioned previously, the oxygen saturation employed in our experiments corresponds to arterial blood. However, variation of a few percent in oxygen saturation would have minimal impact on the reflectance. Therefore, the trends observed in this paper should apply to any sort of oxygenated blood. In particular, various methods could be used to oxygenate venous blood (e.g. equilibration [20]) in order to leverage our differentiation method.

IV. CONCLUSION

In this paper, we presented a method for differentiating the presence of MetHb or SHb in a blood sample given the measured reflectance at three wavelengths. Since our method is accurate when considering whole arterial blood, it can be potentially employed in the design of a, low-cost, point-of-care optical device.

In the future, there are many ways in which we could expand this work. As we mentioned, the proposed method does not directly apply to deoxygenated blood. However, since the opposite curvatures are still present in the 600-630 nm range, we could explore the possibility of using different wavelengths in the central-difference formula. We also mentioned that many drugs that cause sulfhemoglobinemia also cause methemoglobinemia. In theory, this means that abnormal levels of both dysfunctional hemoglobins could be present in the blood simultaneously. Accordingly, we could design a method that detects SHb assuming the presence of MetHb, or vice-versa. Additionally, it would be useful to test the applicability of our method to capillary blood. This could lead to more convenient point-of-care technologies that utilize blood extracted by pricking a fingertip.

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