In Silico Investigation of the Effects of Hemolysis on the Hyperspectral Absorptance of Blood in Motion

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***Abstract*— Measurement of the optical absorptance of blood can provide insight into its composition and behaviour. Accordingly, optical devices and sensors are commonly used in a clinical setting to measure the absorptance of blood, either directly or indirectly through measurement of skin spectral responses. These measurements enable the evaluation or constant monitoring of a patient's blood. In this paper, we perform predictive simulations to investigate the absorptance of blood and how it is affected by hemolysis. These simulations are performed using a cell-based light interaction model, known as CLBlood, which accounts for the orientation and distribution of red blood cells. This allows us to evaluate the effect of hemolysis under different flow conditions. Furthermore, we produce results in the ultraviolet, visible and infrared domains using CLBlood's hyperspectral capabilities. We then evaluate the sensitivity of the absorptance signature of blood to hemolysis in each of these domains under several experimental conditions. The observations in this paper enhance our understanding of the impact of hemolysis on the optical absorptance of blood, potentially leading to simplified and more accurate methods for its detection and monitoring.**

***Index Terms*— blood, absorptance, blood flow, sieve and detour effects, spectral sensitivity, predictive simulation.**

I. Introduction

Hemolysis is defined as the rupturing of red (blood) cells in a blood sample. This releases the hemoglobin solution contained within the red cells into the surrounding plasma. Any condition that applies excessive stress to the membrane of the red cells can cause hemolysis. For example, adding saline to a blood sample to create a hypotonic solution or spinning a blood sample in a centrifuge are methods used to induce hemolysis [1]. Diseases that weaken the red cell membrane, like spherocytosis [2] and beta thalassemia [3], increase a sample's vulnerability to hemolysis. Accordingly, clinical procedures, such as the osmotic fragility test and the autohemolysis test, can be used to detect excessive hemolysis and, thus, indicate the presence of such diseases [1]. In these procedures, the hemolysis is measured by comparing the sample's absorptance at a single wavelength before and after the hemolysis inducing event.

Hemolysis is also used to simplify measurement procedures and calculations performed in clinical tests [1]. The initial hemolysis of the sample eliminates the complex interactions of light with the individual red cells. While this approach simplifies the logistics of *in vitro* procedures, it is not practical to hemolyse a sample under *in vivo* conditions. Understanding how the absorptance signature for blood varies when subjected to hemolysis can help us evaluate the applicability of these procedures to whole blood. Additionally, this knowledge could allow us to improve tests aimed at detecting hemolysis and potentially design an optical sensor for the continuous monitoring of this phenomenon.

Some authors [4], [5] have used photon-diffusion theory to account for the distribution of red cells in blood when modelling the optical properties of whole blood. This requires the assumption that red cells are randomly oriented within the sample. However, under *in vivo* conditions, blood is in a constant steady or pulsatile movement which results in the red cells tumbling, rolling and aligning with the flow direction, changing its optical properties [6].

Recently, we introduced a cell-based light interaction model for human blood, known as CLBlood [7], [8], [9], which takes into account the orientation and distribution of red cells in blood. In this paper, we employ CLBlood to investigate how hemolysis in a blood sample affects its absorptance signature in the ultraviolet (UV), visible and infrared (IR) spectra. We also examine how the impact of hemolysis on the absorptance varies as we change the hematocrit, which is defined as the volume fraction of red cells in the sample. Additionally, we consider instances with incoming light entering the blood sample parallel or perpendicular to the flow since this can have a significant effect on absorption due to the alignment of the red cells.

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 interaction of light with individual red cells within a blood sample. Hemolysis is considered by reducing the hematocrit and combining the optical properties of the hemoglobin solution, released from the ruptured red cells, and the plasma [9]. The positioning of red cells in the sample is determined by the distribution of their orientation and the hematocrit of 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 [6]. We note that the rolling state in the model corresponds to what is called "tumbling" in blood rheology literature [10].

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We have deployed an online version of CLBlood [11] using our own model distribution system [12]. The online availability of the model allows readers to reproduce the *in silico* experiments presented in this paper and perform their own original experiments. The measured data used in our investigation can also be found on our website [13]. Furthermore, the quantitative and qualitative evaluation of the model can be found in our previous publications [7], [9].

The experimental results in this paper are obtained by measuring the directional-hemispherical reflectance and transmittance of an *in silico* blood sample using a virtual spectrophotometer [14]. The absorptance is then calculated by subtracting the measured reflectance and transmittance from 100%. The parameters that remain fixed across the various experiments are presented in Table I. Note that the results presented in this paper consider deoxygenated venous blood because it is more commonly used when performing blood tests [1]. However, as we point out in the following section, the trends observed in this paper are also present in arterial blood.

In our experiments, we demonstrate the effects of hemolysis on samples with a low hematocrit level (10%) and a normal hematocrit level (45%). Furthermore, 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, the alignment of red cells with the direction of the flow at a given shear rate is reduced for lower hematocrit levels [6], [15]. Due to the lack of research precisely quantifying the alignment of red cells under varying conditions, we set the alignment in the flowing case at a constant value of 75%, with 25% of the red cells rolling. However, we acknowledge that it may be necessary, under *in vitro* conditions, for the sample with 10% hematocrit to have a higher shear rate than the 45% hematocrit sample in order to reproduce our results. 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. 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, 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 discourage red cell aggregation [1], or the sample is flowing with a low shear rate [6].

To evaluate the effect of hemolysis on absorptance, we perform a parameter differential sensitivity analysis [16], [17] for the ultraviolet ($250-400\ nm$), visible ($400-700\ nm$) and infrared ($700-1000\ nm$) spectra. The analysis consists in calculating a sensitivity index which provides the relative change in the absorptance of the sample as the hemolysis varies and the other parameters remain fixed. An index of 1.0 indicates maximum sensitivity, and an index below 0.01 indicates that the absorptance is insensitive [18]. Accordingly, we calculate the mean sensitivity index (MSI) for a given region as follows:

$$MSI = \frac{1}{N}\sum_{i=1}^{N}\frac{|\rho_0(\lambda_i) - \rho_h(\lambda_i)|}{\max\{\rho_0(\lambda_i), \rho_h(\lambda_i)\}}, \qquad (1)$$

where ρ_0 is the baseline absorptance curve with a hemolysis value of $h = 0$ and ρ_h represents the absorptance curve with a hemolysis value of h. The parameter N corresponds to the total number of wavelengths in the given region considering with a spectral resolution of $5\ nm$.

TABLE I
CLBLOOD PARAMETERS [11] USED TO GENERATE THE PLOTS IN FIG. 1.

CLBlood Parameter	Value
Number of samples (per wavelength)	1000000
Wavelength range (nm)	250-1000
Angle of incidence ($^\circ$)	0
Rotate angle of incidence	False (unchecked)
Sample thickness (μm)	116.0
Red cell parameters (Oxy-Hb (%) / Deoxy-Hb (%) / Sulf-Hb (%) / Met-Hb (%) / Carboxy-Hb (%) / $MCHC$ (g/L))*	70 / 30 / 0 / 0 / 0 / 330.0

* x-Hb represents the percentage of the hemoglobin in the sample classified as $x$hemoglobin.

III. RESULTS AND DISCUSSION

The absorptance curves resulting from our experiments are presented in Fig. 1. First, let us consider the parallel-flow case (Fig. 1 (left)). We can see that at 10% hematocrit (top), hemolysis has a large effect on the absorptance in the UV and visible ranges. However, this effect is significantly decreased at 45% hematocrit (bottom). Conversely, in the perpendicular-flow case (center), it is the 10% hematocrit sample (top) that are minimally impacted by hemolysis. Moreover, the 45% hematocrit sample (bottom) is significantly affected by hemolysis in the visible and IR regions. We note that although the provided results only consider samples with 10% and 45% hematocrit, we also performed experiments with intermediate hematocrit levels and noticed that the trend is consistent with the expected behaviour. For example, in the parallel-flow experiments, the variance of the curves in the UV range gradually decreased as the hematocrit increased.

When comparing the two flowing cases with each other, we can make several observations. First of all, when the light enters the sample parallel to the flow (Fig. 1 (left)), the absorptance of the sample with 0% hemolysis is lower than the sample with 100% hemolysis. This is related to the fact that red cells have a biconcave shape, so they have a smaller cross-sectional surface area when viewed from the side than the top. In this case, the majority of the red cells are aligned with the flow so their sides are facing the light source. This allows the light to pass in

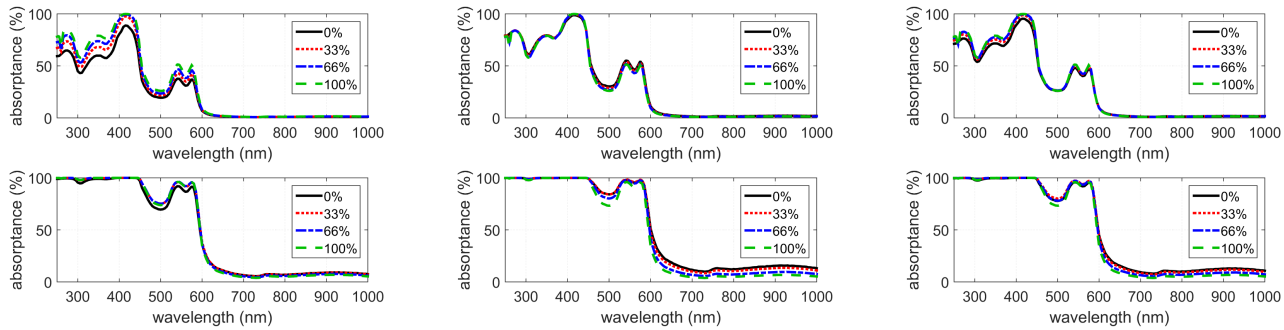

Fig. 1. Modeled absorptance curves for samples with 10% hematocrit (top) and 45% hematocrit (bottom) highlighting the variation in absorptance caused by hemolysis. Left: The blood is flowing and the incident light is parallel to the flow. Center: The blood is flowing and the incident light is perpendicular to the flow. Right: The red cells are randomly oriented.

between the red cells which reduces the absorptance of the sample. This is known as the sieve effect [19]. However, when the light enters the sample perpendicular to the flow (Fig. 1 (center)), many of the red cells have their top facing the incident light, minimizing the chance for light to pass between two red cells. Since the concentration of hemoglobin within the red cells is significantly higher than its overall concentration in a hemolysed sample, the higher probability of light hitting a cell increases the overall absorptance of the sample. Accordingly, we can observe that the absorptance curve for the sample with 0% hemolysis is higher than the sample with 100% hemolysis.

Examining the randomly oriented case (Fig. 1 (right)), we can observe a combination of the trends in the two flowing cases. In the 10% hematocrit plot (top), there is a slight variance in the UV and visible ranges which is similar to the parallel-flow case (left). In the 45% hematocrit plot (bottom), there is a slight variance in the visible and IR regions, which is similar to the perpendicular-flow case (center). This behaviour can be explained by the fact that the red cells are randomly oriented, so the major axis of each red cell is somewhere between parallel and perpendicular to the incoming light.

Although the plots presented in Fig. 1 are useful for examining some qualitative trends, their scale makes it difficult to observe small variations that may impact measurements performed by an optical device. For this reason, we also provide MSI values for the UV, visible and IR ranges for each experiment in Fig. 2. In particular, the MSI plots depict the sensitivity of the absorptance with respect to the change in hemolysis, where the baseline absorptance corresponds to the sample with 0% hemolysis. It is important to note that the sensitivity index is relative to the magnitude of the absorptance. Therefore, in areas where both curves have low values, a minor difference between the curves can result in a significant sensitivity.

First, let us examine the parallel-flow case (Fig. 2 (left)). At 10% hematocrit (top) we notice that, the absorptance is sensitive to hemolysis in all regions and hemolysis levels. Given our previous observations regarding Fig. 1, we may not have expected sensitivity in the IR range. However, this behaviour is a consequence of the sensitivity index being relative to the magnitude of the absorptance. These results indicate that the influence of hemolysis on the absorptance of a 10% hematocrit blood sample can be reliably assessed in all three ranges even with relatively low hemolysis levels. In the 45% hematocrit sample (bottom), the sample has low sensitivity in all regions when 33% of the red cells are hemolysed. As the hemolysis level increases, however, the sample becomes more sensitive in the visible and IR ranges. When examining the perpendicular-flow case (Fig. 2 (center)), we see that for both hematocrit levels, the absorptance is sensitive in the visible and IR range, even in the case with the lowest hemolysis.

Similarly to the absorptance curves, we notice that the MSI values calculated for the randomly oriented case (Fig. 2 (right)) seem to be a combination of the other two cases. As with the parallel-flow case (left), the 10% hematocrit sample (top) is sensitive in all ranges and the 45% hematocrit case (bottom) is sensitive in the visible and UV range. However, the MSI values associated with the IR region are higher than those calculated for the parallel-flow case (left), which resemble the results obtained for the perpendicular-flow case (center).

We note that in all experiments, the sensitivity in the IR range is higher in the experiments with 45% hematocrit (bottom). This may be attributed to the fact that hemoglobin has lower absorptance in the IR region [13], [20], which means that more light in the IR range is scattered by red cells instead of absorbed. Accordingly, a higher quantity of intact red cells causes more scattering, which increases the path length in the sample. This results in a higher probability of absorption, a phenomenon known as the detour effect [21]. We remark that a sample with higher hematocrit has a larger change in red cell count given the same change in hemolysis percentage. Accordingly, the reduction of scattering due to hemolysis is greater for high hematocrit samples, which causes a higher sensitivity in the IR range.

As observed with respect to Fig. 2, the absorptance in the IR range is sensitive to hemolysis in each of the experiments. This suggests that it would be beneficial to consider the IR range when designing an all-purpose model or device for

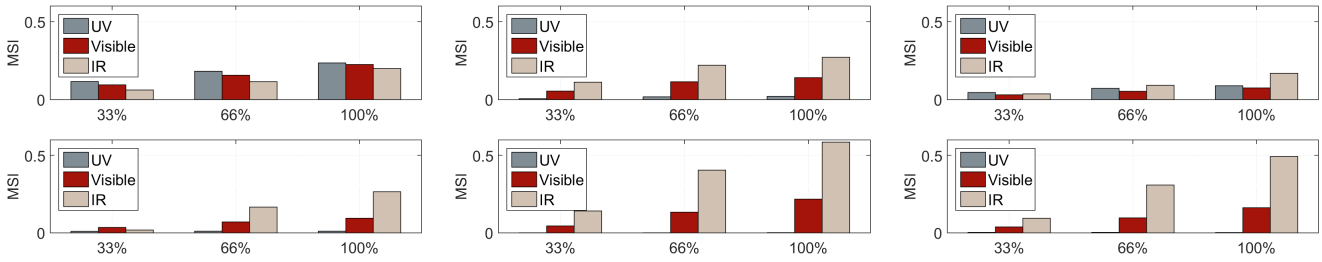

Fig. 2. Mean sensitivity index (MSI) values computed for samples with 10% hematocrit (top) and 45% hematocrit (bottom) demonstrating the variation in absorptance caused by hemolysis in the ultraviolet (UV, $250 - 400\ nm$), visible ($400 - 700\ nm$) and infrared (IR, $700 - 1000\ nm$) ranges. Left: The blood is flowing and the incident light is parallel to the flow. Center: The blood is flowing and the incident light is perpendicular to the flow. Right: The red cells are randomly oriented.

detecting hemolysis. We can also say that in regions with low sensitivity (e.g., the UV region of the perpendicular-flow case), the measurements obtained are unaffected by hemolysis. This means that in these regions, we can detect changes in other parameters without considering the effects of hemolysis.

Finally, we would like to make a couple of remarks about our results. First, although we performed our experiments on a sample with 70% oxygenation to simulate the conditions of venous blood, we also performed experiments with 100% oxygenation and noticed the same trends in absorptance and MSI. This indicates that the observations in this paper also apply to arterial blood. Second, although we provide absorptance curves in this paper, these curves directly relate to the transmittance and reflectance of a blood sample (see Section II) and should be considered accordingly.

IV. Conclusion

In this paper, we employed CLBlood to evaluate the impact of hemolysis on the optical absorptance of blood. We demonstrated that biophysical factors, such as flow rate and the direction of incoming light, can considerably impact the effect of hemolysis on absorption. Furthermore, we observed that variations in the absorptance signature of a sample are strongly dependent on its hematocrit level. These observations indicate that the behaviour of red cells in flow significantly affects the optical properties of blood.

We believe that, while necessary for understanding qualitative trends, *in vivo* and *in vitro* experiments are not sufficient in quantifying the complex behaviour of blood flow and how it affects other properties of blood, such as its ability to absorb light. In the future, it will be necessary for experimental and computational scientists to work together in order to completely understand blood in motion and its optical properties.

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