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Examining the Impact of Sample Thickness Variations on the Hyperspectral Radiometric Responses of Flowing Blood

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***Abstract*— The hyperspectral reflectance and transmittance of flowing blood samples are employed in a wide range of biomedical applied research initiatives such as the detection and monitoring of hematological abnormalities. The success of these initiatives is tied to the correct interpretation of these radiometric quantities. This, in turn, requires a comprehensive understanding about their sensitivity to variations in the experimental conditions in which they have been obtained. In this paper, we aim to contribute to these efforts by systematically examining the effects of sample thickness variations on these quantities. More specifically, we employed controlled *in silico* experiments to assess these effects on samples with different biophysical characteristics, notably their hematocrit, hemolysis level and orientation of their constituent cells with respect to the flow direction. To ensure a high degree of fidelity in our experiments, we used a first-principles simulation framework supported by measured data. Our findings unveil distinct spectrally-dependent trends associated with reflectance and transmittance changes elicited by sample thickness variations.**

***Index Terms*— blood, flow, thickness, hemolysis, reflectance, transmittance, predictive simulations, *in silico* experiments.**

I. Introduction

The interactions of light with human blood result in radiometric responses that can be measured in terms of reflectance and transmittance. The correct interpretation of these responses, in turn, can provide valuable information about the composition and physiological parameters (*e.g.*, oxygenation and pH) of blood samples [1], [2], [3], [4].

Accordingly, considerable research efforts have been directed toward the understanding of blood optical properties, particularly under different rheological states [5], [6], [7] and hemolysis levels [8], [9]. While the former are associated with blood flow characteristics (*e.g.*, slow, moderate or fast) [5], [10], the latter are quantified in terms of the fraction of the red blood cells (RBCs) whose membrane rupture releases intracellular contents, notably hemoglobin, into the surrounding plasma [11], [12].

These studies are usually carried out in conjunction with radiometric measurements performed under *in vitro* conditions by placing blood samples in glass cuvettes. To prevent the introduction of undue biases in the interpretation of the resulting measured datasets, it is essential to be aware of their sensitivity to variations in the experimental conditions such as the light incidence geometry (*e.g.*, parallel or perpendicular to the blood flow) and the samples' thickness.

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To date, detailed information about the impact of sample's thickness variations on its radiometric responses is still scarce in the literature [13], [14]. Reports in this area are either based on the examination of blood data obtained through inversion procedures or overlook concomitant variations in samples' key characterization parameters such as their hemolysis level. It is worth noting that hemolysis is considered one of the most common causes of errors in the analysis of blood samples [15], and it has costly implications in medical practice as samples may need to be retaken [16].

In this paper, we aim to mitigate this knowledge gap by methodically assessing the impact of sample thickness variations on the hyperspectral radiometric responses of flowing blood subject to distinct hemolysis levels. We employ a *in silico* (computational [17]) investigation approach based on the use of a first-principles cell-based model of light interactions with human blood, known as CLBlood [10], [18], and supported by measured datasets [6]. These are employed as baseline references for our *in silico* experiments, which are perfomed in ultraviolet, visible and near-infrared domains (from 250 to 1000 nm).

The CLBlood model provides a hyperspectral simulation framework whose predictive capabilities have been extensively evaluated both qualitatively and quantitatively [18], [10], and used in a number of biomedical optics investigations involving blood optical properties (*e.g.*, [12], [19], [20], [21]). Within its algorithmic formulation, interactions of light (represented by discrete rays, each one traveling at a given wavelength λ) with plasma and RBCs are simulated as random walk processes. More specifically, as light traverses a blood sample, RBCs are generated probabilistically on the fly. Their generation is dependent on the distribution of their orientation and the percentage of the sample volume that they occupy, the sample's hematocrit (HCT). Light attenuation events (absorption and scattering) are stochastically accounted for using data driven procedures and wave optics resources when appropriate [10].

In samples with relatively high HCT, it has been noted that the orientation of the RBCs is predominantly random, rolling and aligned with the flow direction, at low, intermediate and high shear rates, respectively [22], [5]. Conversely, the alignment of RBCs with the flow becomes less pronounced for relatively low HCT. These aspects are also taken into account in our investigation since the CLBlood model has the capability of emulating different flow shear rates using an aggregate distribution of cell orientations (random, rolling and aligned) [10], [18], where the weight (%) of each distribution is a parameter for the model.

II. In Silico Experimental Framework

In this investigation, we considered two blood samples with distinct HCTs. The characterization parameter values (presented in Table I) for these samples, henceforth referred to as LH (lower HCT) and HH (higher HCT), were chosen based on the description of the actual samples employed in the laboratory experiments [6], [18] that provide the baseline references for our simulations. In those experiments [6], measured reflectance datasets were obtained by placing fully oxygenated blood samples (under steady flow and high shear rate conditions) inside a glass cuvette. Those measured datasets are used here as a fidelity standard for our modeled (baseline) reflectance datasets.

TABLE I: Parameters employed in characterization of the selected blood samples LH and HH.

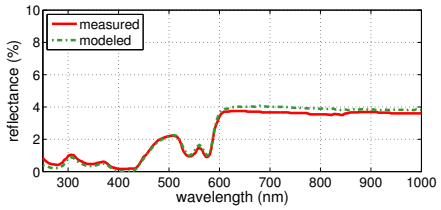
Parameter	Value (LH)	Value (HH)
HCT (%)	8.4	33
Rolling RBCs (%)	90	40
Aligned RBCs (%)	10	60
Mean cell hemoglobin content (g/L)	330	330

Our *in silico* experiments consisted in the computation of modeled directional-hemispherical reflectance and transmittance curves for the selected samples using the CLBlood model. For consistency, we adopted a spectral resolution of 5 nm in all modeled curves presented in this work, which were obtained using a virtual spectrophotometer [23] casting 10^6 sample rays per λ. Moreover, we strived to reproduce the measurement conditions adopted in the aforementioned laboratory experiments [6] as faithfully as possible. These include an angle of incidence of $8°$, incident light parallel to the flow, and a cuvette made of fused silica [18].

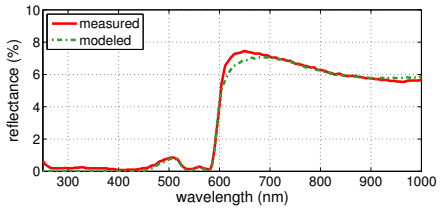
For the baseline reflectance computations, we considered a sample thickness (t) equal to 116 μm and a fraction of hemolyzed RBCs (h) equal to 2%. These figures correspond to those employed in the actual laboratory experiments [6]. As it can be observed in Fig. 1, the parameter values (Table I) chosen to characterize the selected samples (according to their described biophysical and rheological traits [6], [18]) resulted in a close agreement between the measured and modeled reflectance curves. This, in turn, indicated an appropriate degree of reliability associated with the chosen sample characterization datasets.

In order to assess the impact of thickness variations on the samples' hyperspectral radiometric responses, we computed their reflectance and transmittance curves considering a 50% and 100% increase in their thickness, which correspond to t equal to 174 and 232 μm, respectively. Furthermore, to expand our scope of observations, we have also considered higher hemolysis fractions (h equal to 50% and 100%), and repeated the experiments accordingly.

Lastly, to enable the reproducibility of our *in silico* experimental results, we have made CLBlood available for online use [18], [24] through our model distribution system [25]. We have also provided access to supporting biophysical datasets (*e.g.*, refractive index and extinction coefficient curves) [26] employed in our investigation.



(a) LH sample.



(b) HH sample.

Fig. 1: Comparison of measured and modeled (baseline) reflectance curves obtained for the selected blood samples.

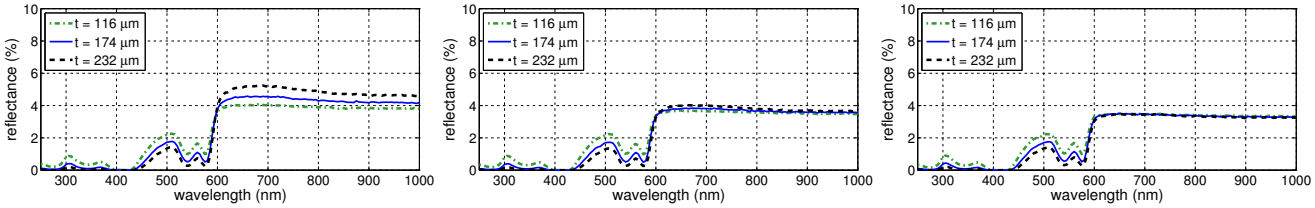
III. Results and Discussion

The outcomes of our *in silico* experiments have revealed distinct qualitative and quantitative trends in the regions between 250 and 600 nm, and between 600 and 1000 nm. It has been noted that blood hyperspectral radiometric responses tend to be more affected by light absorption events in the former, and by light scattering events in the latter [10], [6]. For conciseness, we will refer to these regions as $\mathcal{A}$ and $\mathcal{S}$, respectively, in the remainder of this paper.

In Fig. 2, we present the modeled reflectance curves obtained for the LH sample. As it can be observed in Fig. 2(a), the increases in the sample's thickness under a low hemolysis level resulted in decreases in its reflectance in region $\mathcal{A}$, and increases in region $\mathcal{S}$. As depicted in the graphs presented in Fig. 2(b) and (c), similar thickness-driven reflectance decreases in region $\mathcal{A}$ are observed when the sample's hemolysis fraction is increased. However, the thickness-driven reflectance increases in region $\mathcal{S}$ become less noticeable following an increase in the hemolysis level.

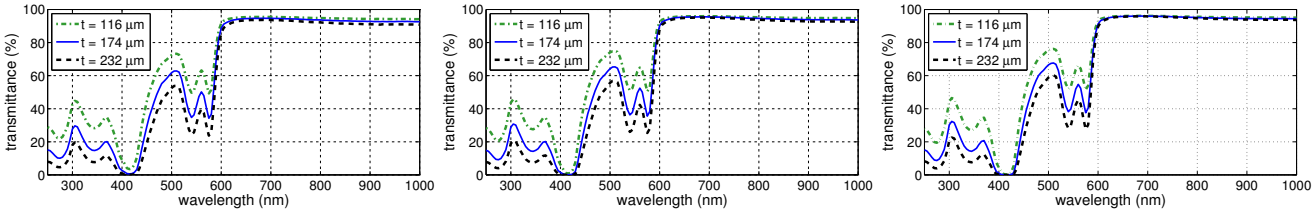
In Fig. 3, we present the modeled transmittance curves obtained for the LH sample. In contrast with the observations regarding its reflectance changes, the increases in its thickness under a low hemolysis level resulted in transmittance decreases in both spectral regions. However, the magnitude of the thickness-driven transmittance decreases in region $\mathcal{S}$ becomes practically negligible as the sample's hemolysis fraction is increased (Fig. 3(b) and (c)).

In Fig. 4, we present the modeled reflectance curves obtained for the HH sample. As it can be observed in Fig 4(a), the increases in the sample's thickness lead to decreases in its reflectance in region $\mathcal{A}$, around 500 nm, and increases in region $\mathcal{S}$. Like it was observed for the LH sample, similar thickness-driven reflectance decreases in region $\mathcal{A}$ are observed when the sample's hemolysis fraction is increased (Figs. 4(b) and (c)), while the thickness-driven reflectance increases in region $\mathcal{S}$ become markedly less pronounced.



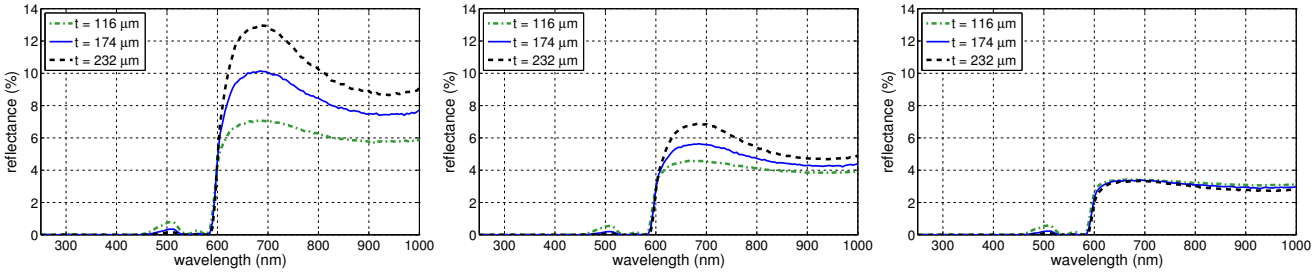
(a) $h = 2\%$. (b) $h = 50\%$. (c) $h = 100\%$.

Fig. 2: Reflectance curves obtained for the LH sample considering distinct thicknesses (t) and hemolysis fractions (h).



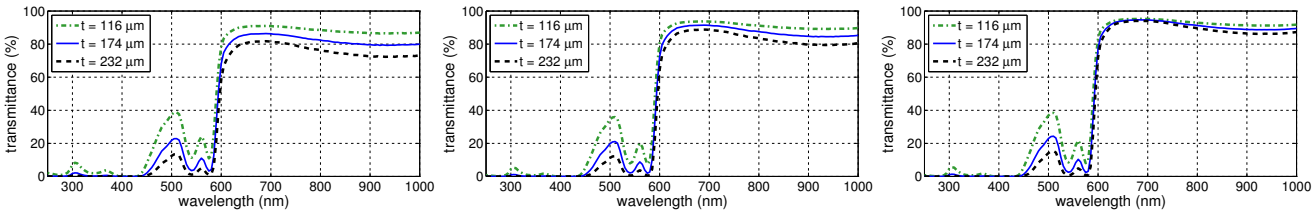
(a) $h = 2\%$. (b) $h = 50\%$. (c) $h = 100\%$.

Fig. 3: Transmittance curves obtained for the LH sample considering distinct thicknesses (t) and hemolysis fractions (h).



(a) $h = 2\%$. (b) $h = 50\%$. (c) $h = 100\%$.

Fig. 4: Reflectance curves computed for the HH sample considering distinct thicknesses (t) and hemolysis fractions (h).



(a) $h = 2\%$. (b) $h = 50\%$. (c) $h = 100\%$.

Fig. 5: Transmittance curves computed for the HH sample considering distinct thicknesses (t) and hemolysis fractions (h).

Finally, in Fig. 5, we present the modeled transmittance curves obtained for the HH sample. Again, like it was observed for sample LH, the increases in the HH sample's thickness under a low hemolysis level resulted in transmittance decreases in both regions. Also, the magnitude of the thickness-driven transmittance decreases in the region $\mathcal{S}$ becomes practically negligible as the sample's hemolysis fraction is increased (Fig. 5(b) and (c)).

In short, our *in silico* experimental results indicate that the impact of sample thickness variations on blood samples' hyperspectral radiometric responses in region $\mathcal{A}$ tends to be quantitatively dependent of their HCT and independent of their hemolysis fraction. However, in region $\mathcal{S}$, it tends to be quantitatively dependent on both their HCT and hemolysis fraction. Moreover, in this region, the trends are qualitatively reverse for reflectance and transmittance curves, with the former being increased following a thickness increase, and the latter being reduced.

It is important to highlight the fact that the observations reported above were made with respect to the selected samples under the described flow conditions. Taking that into account, we can then elaborate on the light attenuation mechanisms behind the observed changes in the samples' hyperspectral radiometric responses.

As a starting point, one can assume that an increase in the sample's thickness raises the probability of light attenuation events (absorption and scattering) to take place. In region $\mathcal{A}$, more absorption events lead to absorptance increases followed by reflectance and transmittance reductions. On the other hand, in region $\mathcal{S}$, more scattering events, particularly

backwardly oriented, lead to reflectance increases and transmittance reductions.

Regarding the implications of an increase in the samples' hemolysis level, it has been noted that it reduces the occurrence of light detour effects [10], [12]. These effects are associated with an increase in the light optical path length in a whole blood sample in comparison with a hemoglobin solution [27]. The reduced occurrence of detour effects, in turn, leads to a decrease in the probability of absorption and scattering events directly elicited by the presence of RBCs. These ramifications of the detour effects are often more noticeable in spectral regions characterized by a relatively low absorptance [10], [12], like region $\mathcal{S}$.

Our findings suggest that, when it comes to the probability of scattering events within region $\mathcal{S}$, the impact of an increase in the samples' thickness may be progressively counterbalanced by the impact of a reduction in the detour effects associated with higher hemolysis levels. As a consequence, the reflectance and transmittance curves within this region tend to converge to low and high plateau curves, respectively. The low plateau curve is likely to be associated with a decrease in backward scattering, and the high plateau curve with a decrease in absorption, which is also connected to the reduction in the detour effects.

IV. Concluding Remarks

In this paper, we have examined the impact of thickness variations on the reflectance and transmittance of flowing blood samples with distinct HCTs and subject to different hemolysis levels. Our *in silico* findings, albeit still subject to *in vitro* verification, indicate that such an impact may be significant. Furthermore, they also suggest that the effects of thickness variations on reflectances responses may differ considerably from those on the samples' transmittances, particularly in spectral regions in which light attenuation is dominated by scattering events. Accordingly, we believe these changes should be carefully accounted for in the study and interpretation of blood samples' hyperspectral radiometric responses, especially when these are employed in protocols for the detection and monitoring of medical conditions associated with blood disorders.

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