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

Gladimir V. G. Baranoski and Spencer R. Van Leeuwen

Natural Phenomena Simulation Group

University of Waterloo, Canada

IEEE EMBC'22

Glasgow, Scotland, July 11-15, 2022

Outline

- Introduction
- *In Silico* Experimental Framework
- Results and Discussion
- Concluding Remarks

Introduction

Motivation

- The hyperspectral reflectance and transmittance of flowing blood are key elements in research initiatives aimed at a number of clinical applications
    - *e.g.*, detection and monitoring of hematological abnormalities

- The correct interpretation of these radiometric quantities is essential for achieving a high efficacy to cost ratio in these applications

- This requires a comprehensive understanding about these quantities' sensivity to variations in the experimental conditions in which they have been obtained

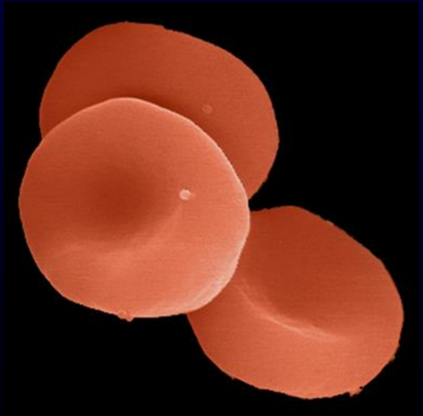
- Context
  - Information about the impact of thickness variations on the hyperspectral radiometric responses of blood samples is still scarce in the related literature


- Objective
  - Mitigate this knowledge gap by systematically examining the effects of sample thickness variations on reflectance and transmittance of flowing blood

- Approach

  - Controlled *in silico* experiments to assess the impact of thickness (t) variations on blood samples with distinct biophysical characteristics

    - Hemolysis level (h): percentage of the red blood cells (RBCs) whose membrane rupture releases intracellular contents (*e.g.*, hemoglobin) into surrounding plasma



RBCs (scanning electron microscope image, courtesy of Tina Carvalho)

- Hematocrit (HCT): percentage of a blood sample volume occupied by the formed elements, notably the RBCs

whole blood

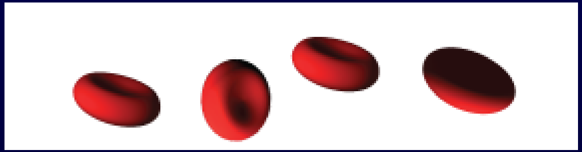
centrifuge

plasma

formed elements

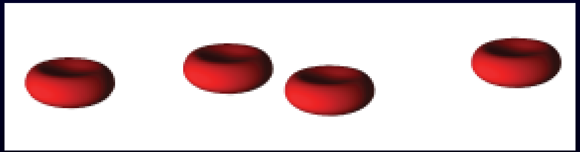
- <u>Orientation of the RBCs</u> with respect to the blood flow direction

→ flow direction



random (low shear rate flow)

rolling (intermediate shear rate flow)



aligned (high shear rate flow)

❖ alignment becomes less pronounced for relatively low HCT

In Silico Experimental Framework

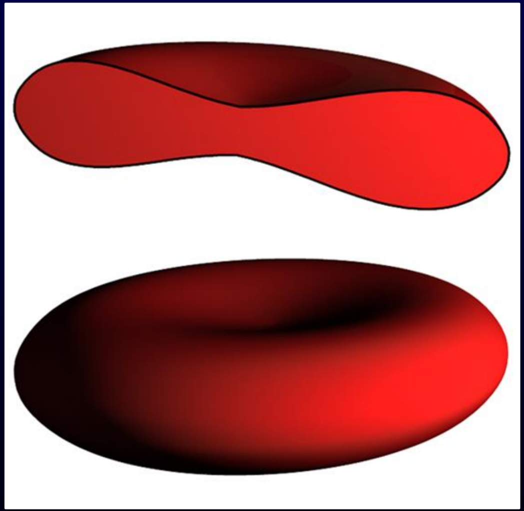
- Materials

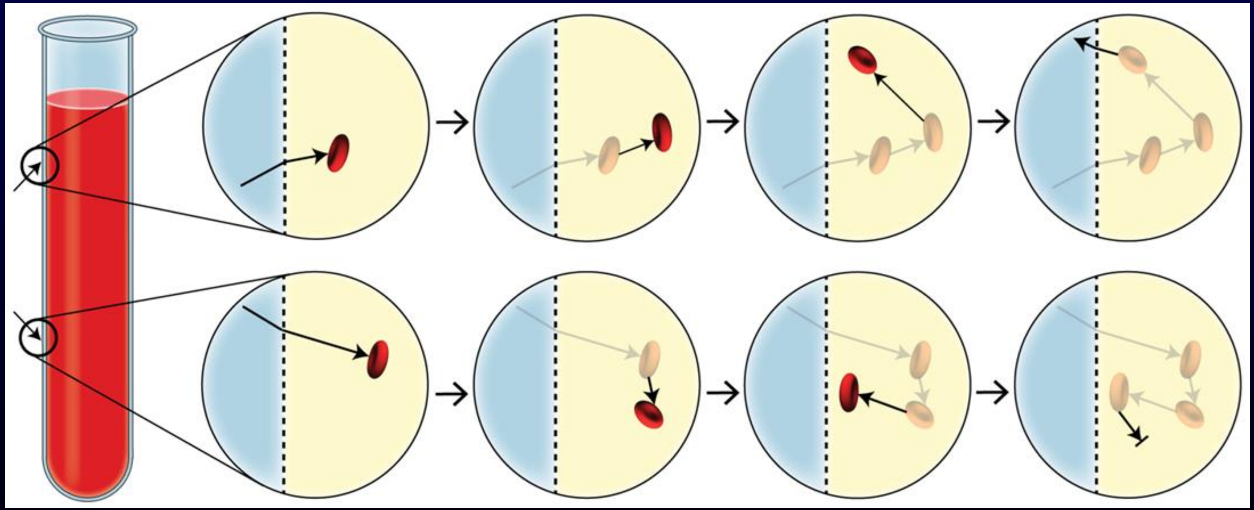
  - We considered two blood samples, LH (lower HCT) and HH (higher HCT), based on samples employed in actual radiometric experiments (Meinke *et al.*, 2005)

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

Methods

- Reflectance and transmittance curves obtained using a first-principles cell-based model of light interactions with human blood: CLBlood (Yim & Baranoski, 2012)

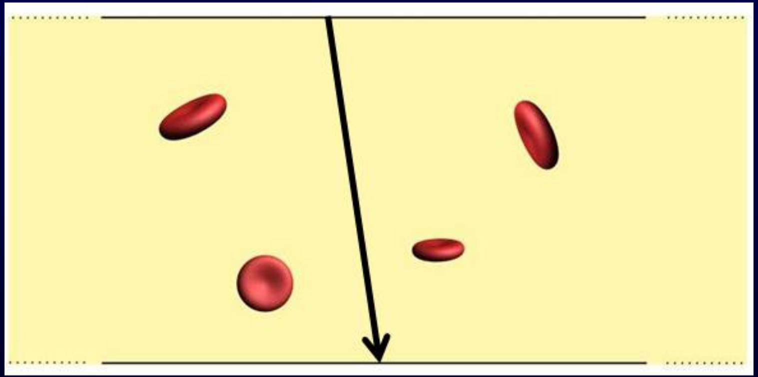




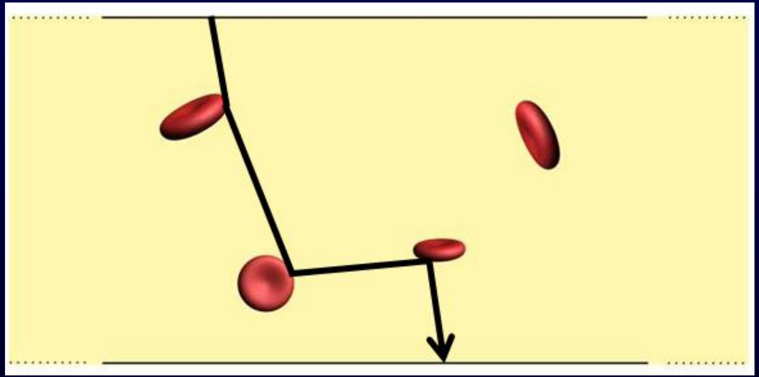
http://www.npsg.uwaterloo.ca/resources/videos/clblood.php

- Optical behaviour of whole blood differs from that of a homogeneous solution with the same concentration of hemoglobin due to sieve and detour effects

sieve effect



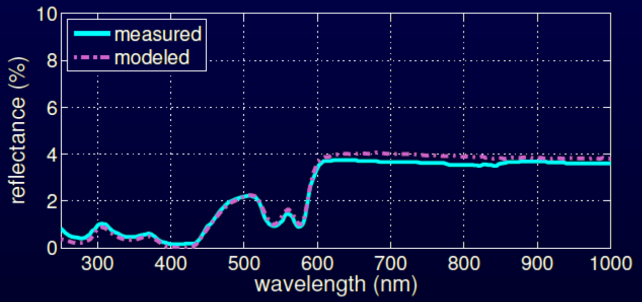
detour effect



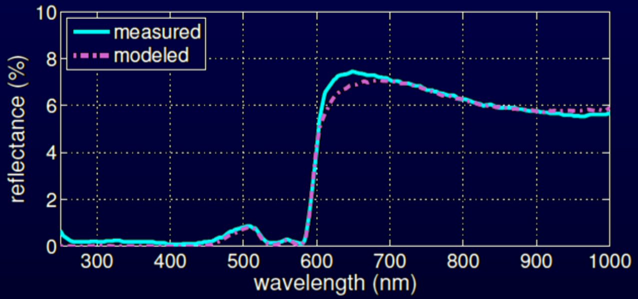
- sieve effects reduce absorption, notably in high absorptance regions
- detour effects increase absorption, notably in low absorptance regions

- We strived to reproduce the actual measurement conditions as faithfully as possible: angle of incidence of $8°$ and samples inside a glass cuvette

baseline *in silico* experiments



LH sample



HH sample

- To enable the reproduction of our *in silico* experimental results, we provide the employed biophysical data and made CLBlood available for online use

CLBlood

Cell-Based Light Interaction Model for Human Blood

(Spectrometric Mode*)

CLBlood model uses a Monte Carlo formulation to simulate light interaction with blood. Red blood cells are created and discarded on the fly as the light propagates through the blood plasma. The light rays can be scattered or absorbed by these blood cells as well as by the plasma medium. For more details about this model, please read our related publications (2012 and 2017).

In this version of the CLBlood online model, we use a virtual spectrophotometer to obtain directional-hemispherical reflectance values by integrating the reflected and transmitted light (rays) over the incident and collection hemisphere, respectively.

Some properties of the red blood cells are precomputed for optimization considering a red blood cell with a hemoglobin content equal to 29.5 pg, a volume of 83 μm^3, and a mean cell hemoglobin content (MCHC) of 330.0 g/L. These precomputed parameters are listed in the item labeled "Red cell parameters", which displays values for oxyhemoglobin %, deoxyhemoglobin %, sulfhemoglobin %, methemoglobin %, carboxyhemoglobin % and MCHC, respectively.

In the future, we intend to make it possible for users to perform simulations with other precomputed values. The spectral input data files for the materials considered by the online CLBlood model are available here. To access the goniometric mode of the CLBlood online model, click here.

Additional Notes

- We have observed that variations of albumin, globulin and fibrinogen in the plasma within a normal range do not have an overall effect on the results of our simulations. Therefore, we note that in our experiments, average values of protein concentrations (albumin 4.6 g/dL, globulin 2.6 g/dL and fibrinogen 0.38 g/dL) are used for Rayleigh scattering and the calculation of the refractive index of plasma which, as previously mentioned, can be found here.
- For a cuvette configuration, we consider the refractive index of air equal to 1.00 and the refractive index of the cuvette, equal to the refractive index of fused silica, can be found here.
- For a vein configuration, we consider average values for the refractive indices of the reticular dermis tissue (1.41) and the vein wall (1.39).

Run CLBlood Online

Enter your email address:
(used to send the results)

Model Parameter	Value
Number of samples	100000
Wavelength range	250-1000 nm
Relationship of incoming light to flow	Parallel
Angle of incidence	8 degrees
Rotate angle of incidence	☐
Hematocrit	0.33 fraction
Thickness of the blood sample	116.0 um
Red cells rolling in the flow direction	0.40 fraction
Red cells aligned with the flow direction	0.60 fraction
Red cells oriented randomly	0 fraction
Hemolysed red cells	0.02 fraction
Experiment configuration	Cuvette
Red blood cell parameters	100 / 0 / 0 / 0 / 0 / 330.0

Submit

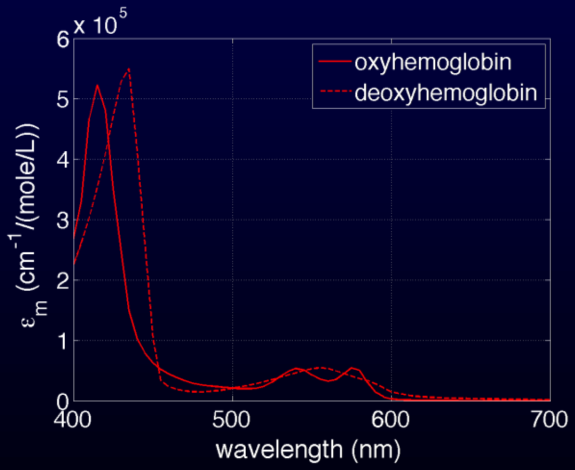
http://www.npsg.uwaterloo.ca/models/clblood.php

Results and Discussion

➢ Hyperspectral radiometric (*in silico*) experiments

- Distinct qualitative and quantitative trends have been observed in two spectral regions with distinct sensitivity to light attenuation events

    - *A* (250 to 600 *nm*): blood responses are more affected by <u>absorption</u> events

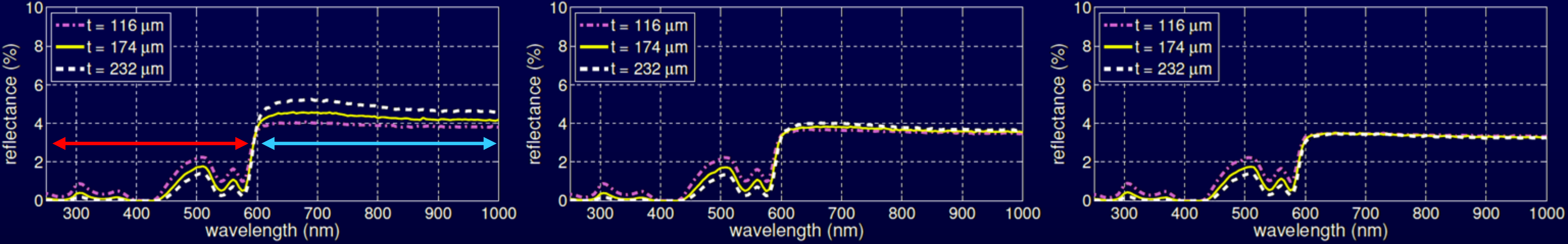


extinction coefficients of functional hemoglobins

    - *S* (600 to 1000 *nm*): blood responses are more affected by <u>scattering</u> events

- Effects of thickness increase on the LH sample's spectral responses

reflectance curves

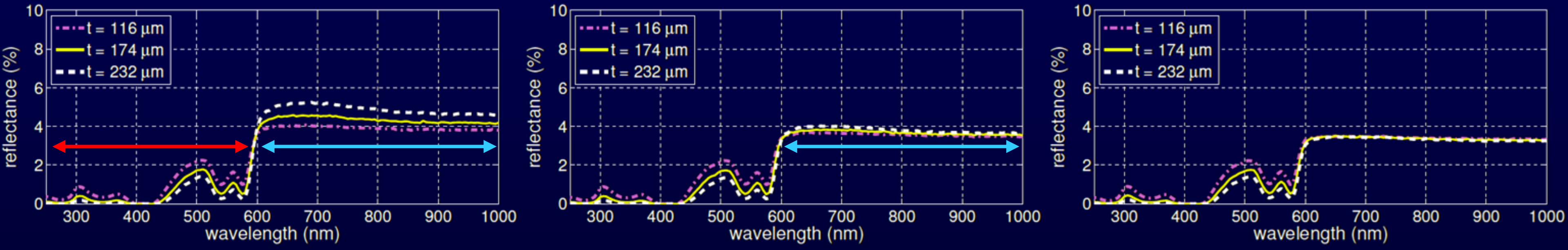


h = 2% h = 50% h = 100%

- reflectance decreases in region A, and increases in region S

- Effects of thickness increase on the <u>LH</u> sample's spectral responses

reflectance curves

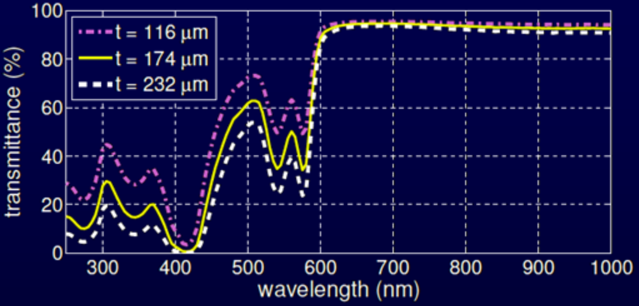


h = 2% h = 50% h = 100%

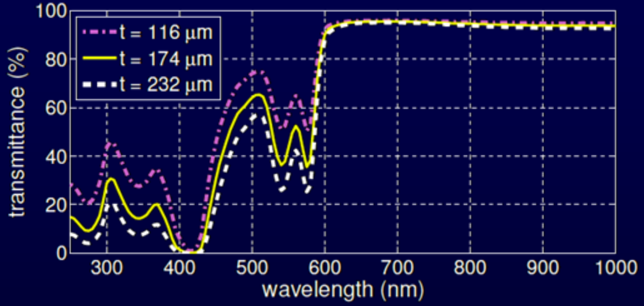
- reflectance decreases in region A, and increases in region S
- reflectance differences in region S become less noticeable for larger h

- Effects of thickness increase on the LH sample's spectral responses

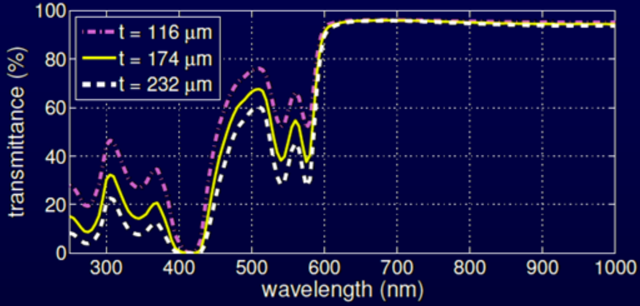
transmittance curves



h = 2%



h = 50%

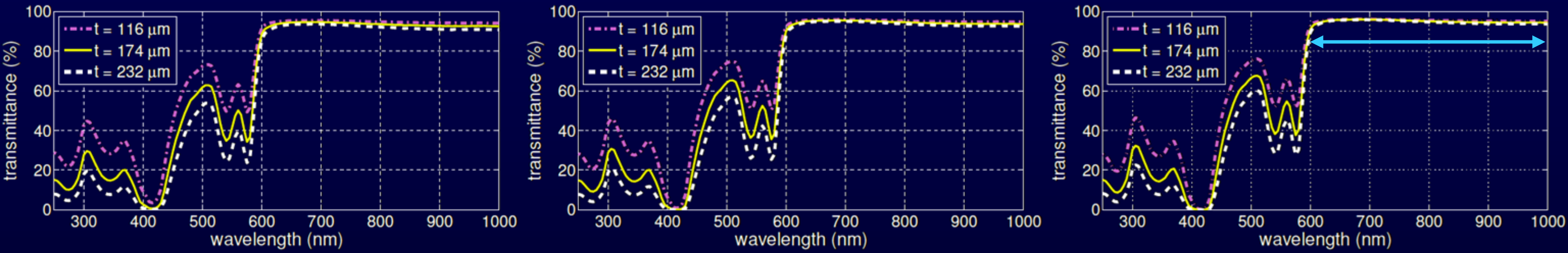


h = 100%

- transmittance decreases in both regions *A* and *S*

- Effects of thickness increase on the LH sample's spectral responses

transmittance curves

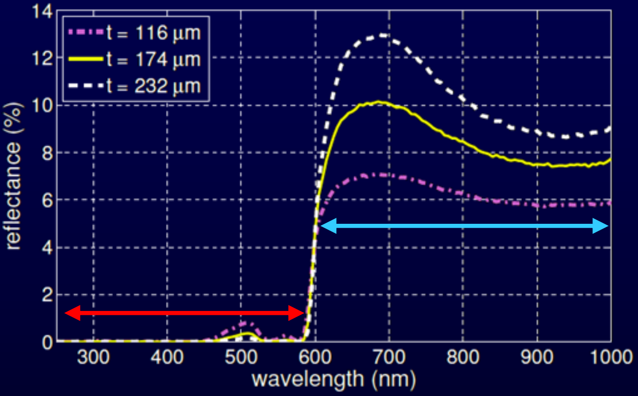


h = 2% h = 50% h = 100%

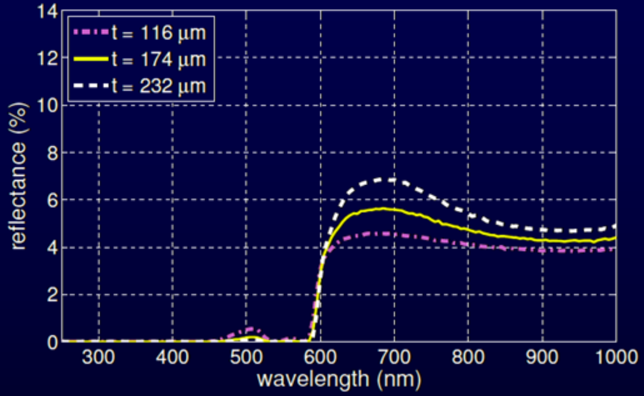
- transmittance decreases in both regions A and S
- transmittance reductions in region S become negligible for larger h

- Effects of thickness increase on the HH sample's spectral responses

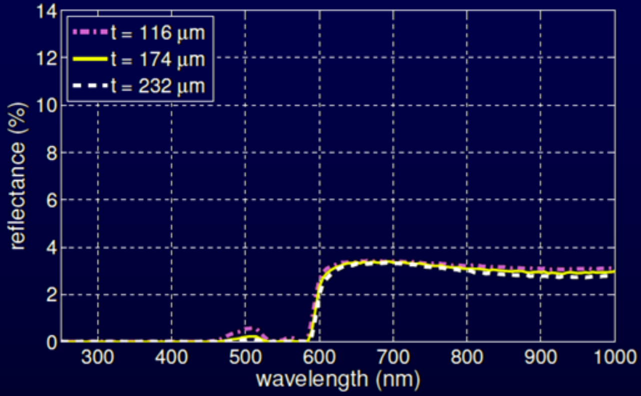
reflectance curves



h = 2%



h = 50%

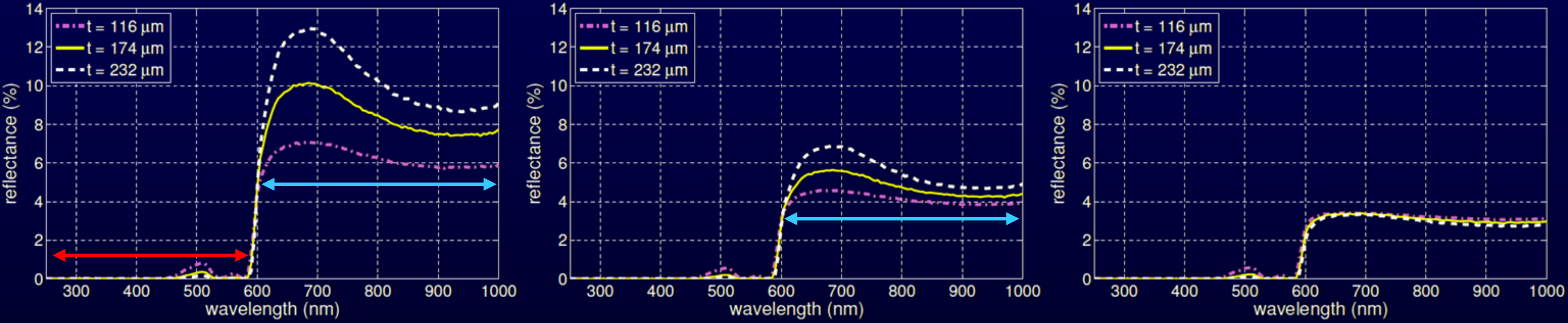


h = 100%

- reflectance decreases in region A (around 500 *nm*), and increases in S

- Effects of thickness increase on the HH sample's spectral responses

reflectance curves

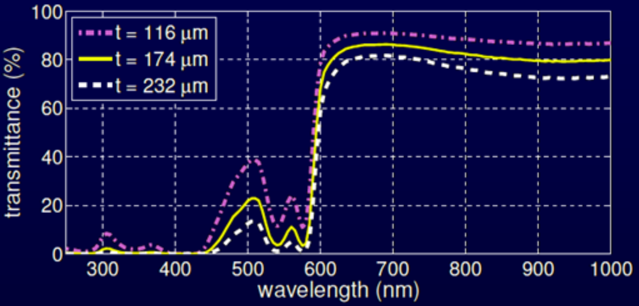


h = 2% *h* = 50% *h* = 100%

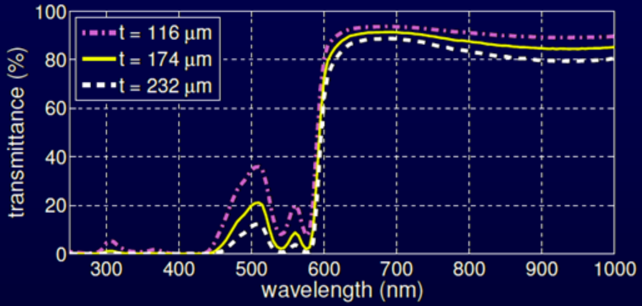
- reflectance decreases in region *A* (around 500 *nm*), and <u>increases</u> in *S*
- reflectance differences in region *S* become markedly smaller for larger *h*

- Effects of thickness increase on the HH sample's spectral responses

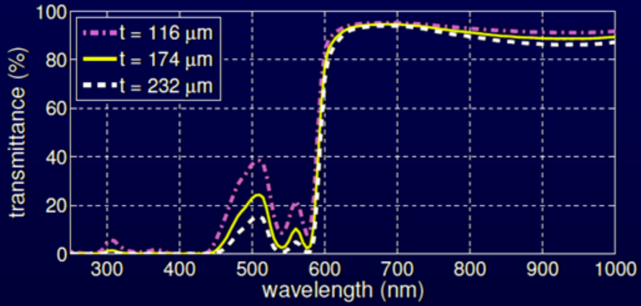
transmittance curves



h = 2%



h = 50%

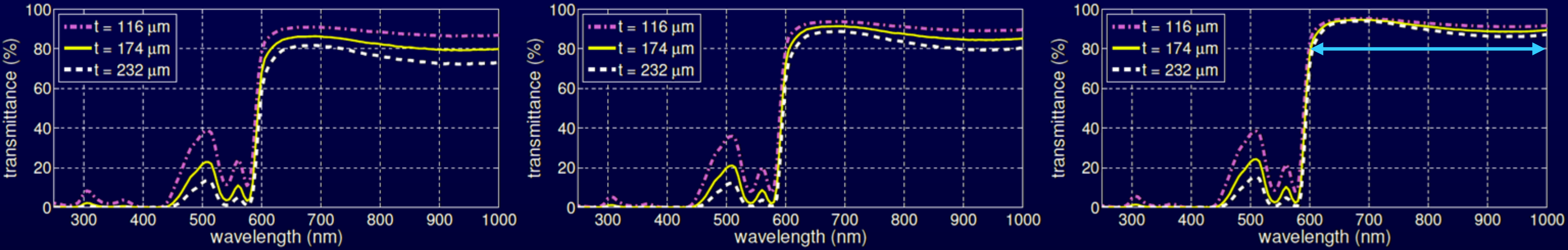


h = 100%

- transmittance decreases in both regions *A* and *S*

- Effects of thickness increase on the HH sample's spectral responses

transmittance curves



h = 2% *h* = 50% *h* = 100%

- transmittance decreases in both regions *A* and *S*
- transmittance differences in region *S* become less noticeable for larger *h*

- The impact of thickness variations on blood samples' responses tends to be:
  - quantitatively <u>dependent</u> on their HCT and <u>independent</u> of their h in region A
  - quantitatively <u>independent</u> on their HCT and h in region S

- Premises of the light attenuation mechanisms behind these observations
  - thickeness increase raises the probability of light attenuation events
  - hemolysis increase reduces the probability of light detour effects

- In region S:
  - the effects of thickness and hemolysis increases on the probability of scattering events tend to balance each other
  - reflectance curves converge to a low plateau, which is likely to be associated with a decrease in RBC-elicited backward scattering
  - transmittance curves converge to a high plateau, which is likely to associated with a decrease in absorption due to reduced detour effects

Concluding Remarks

- The reported *in silico* experiments were performed on two blood samples under specific flow conditions: the resulting observations have a preliminary character

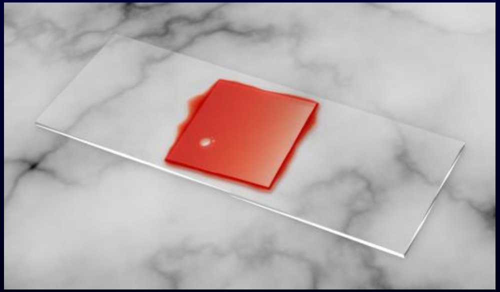
- Our findings, albeit still subject to *"wet lab"* verification, show that the impact of thickness variations is not monotonic across the 250 to 1000 *nm* spectral domain

- They also indicate that thickness-driven reflectance changes can differ considerably from thickness-driven transmittance changes

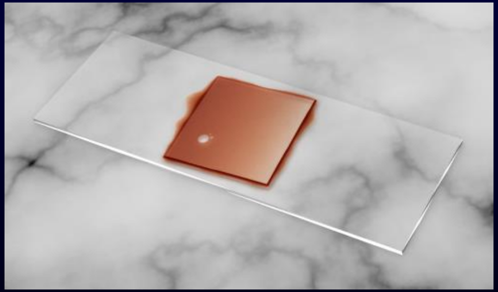
    - Particularly in regions in which light attenuation is dominated by scattering

- These changes should be carefully accounted for in the study and interpretation of blood samples' hyperspectral responses
    - Especially when these are employed in protocols for the detection and monitoring of medical conditions associated with blood related disorders

methemoglobinemia



normal arterial blood with
1.5% of methemoglobin



abormal arterial blood with
70% of methemoglobin

Thank you!