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Low-cost Non-invasive Diagnosis of Malaria Infected Red Blood Cells

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0. Abstract

Malaria is one of the most serious diseases and is currently diagnosed microscopically by detecting malaria infected red blood cells in samples. The available approaches are inefficient in that they require trained workers, blood samples, complex logistic system and laboratory infrastructure. This research aims to find an innovative, low-cost, and non-invasive method through the signal from backward elastic scattering of red blood cells. Currently, forward and backward elastic light scattering are used to establish a clear difference between the red blood cells with malaria and red blood cells in solution of different salt concentrations. Corresponding differences in intensity and blood sample concentrations with blood sampled covered with Polyethylene film were able to be detected and a consistent, strong backward scattering through the fingertips was also observed. This will enable the use of elastic light scattering as a promising and non-invasive tool to detect and catalogue malaria infected red blood cells.

I. Introduction

Malaria is a serious and potentially fatal blood borne disease that causes a reported 350 to 500 million cases and nearly one million deaths annually, mostly occurring in Africa. The current system of diagnosis for diseases that affect red blood cells generally requires highly trained professionals with extensive lab equipment and fresh blood samples acquired from the patients invasively. While treatment may be available, the costly, invasive diagnosis of blood-based diseases hinders its implementation. Many victims of these diseases live without access to the medical resources needed to properly and efficiently diagnose and treat the conditions. An effective system of non-invasive diagnosis would be much easier to implement in these impoverished regions, allowing for disease to be caught early on and treated more effectively, thus helping prevent the unnecessary loss of life.

In elastic light scattering, the spatial distribution of a particle's scattering spectrum is not random but forms a complex spatial pattern that is dependent on the properties of the particle. This is due to the complicated encounter of an incident electromagnetic wave perturbing the electron orbits of the molecules and atoms in a particle, which induces oscillating dipole moments that gives off scattered light as a source of electromagnetic radiation. Most of the scattered light is emitted at the same frequency as the incident light and therefore is elastic. The pattern that forms when a particle scatters light upon illumination is dependent on the size, shape, refraction index and chemical composition of the particle. Malaria is a blood borne disease, therefore the red blood cells invaded by malaria parasite experience significant modification in the structural, mechanical and biochemical properties such as morphological deviation from the biconcave shape of healthy red blood cells, the reduction of membrane fluctuations and elasticity, and decrease of hemoglobin concentration. The objective of this research is to utilize forward and backward elastic light scattering to detect the changes in red blood cells altered by dilution in

different saline concentration and ultimately approach a low-cost and non-invasive diagnosis of blood borne diseases.

II. Materials and Method

A. Equipment Setup

The optical setup in Fig. 1 is consists of a lamp, a spectrometer, lens, optic fibers, a motorized rotation unit and a variable aperture. This setup is a depiction of forward elastic light scattering noting that the placement of the collecting fiber is on the opposite of the lens, with the blood sample and slide in between.

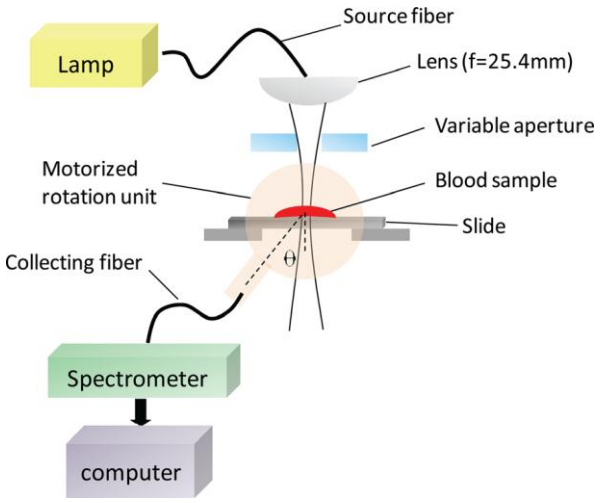


Fig. 1 Schematic Setup of forward elastic light scattering measurement.

The optic components are arranged vertically, allowing its light to pass through the blood sample on a slide, which is placed horizontally. The distance between the lens and the blood is approximately 70mm. This setup allows a broadband light source to be delivered through a 400µm optic fiber and focused on a small test spot by a Plano-convex lens with a focus length of f = 25.4mm. The collecting fiber is attached to a computer controlled motorized rotation stage that allows precise control and orientation change of the scattering relative to the incident beam, enabling the scattered light to be measured at various angles, θ. The scattering in the collected fiber is then passed to a spectrometer connected to a computer for analysis. Lastly, the variable aperture is used to adjust the diameter and intensity of the illuminated light passing through the sample as well as keeping a separation between the light and the collecting fiber to avoid interference of the incident beam to the scattered light.

B. Procedure

The blood samples are diluted in 5 different concentrations of saline concentration in order to alter the properties of the blood cell particles and to reduce multiple scattering events. The

isotonic solution for blood cells is 0.9% saline solution. In order to create isotonic, hypertonic and hypotonic solutions, dilutions are made at 0.5%, 0.9%, 1.5%, 3.0% and 5.0% saline concentrations. To ensure consistent single scattering events, two drops of blood sample are diluted in 0.25mL saline concentration.

C. Data Collection

The spectrum of scattered light is collected within a wavelength range of 300 to 1500nm at various angles. For comparison, we show the range of wavelength that demonstrates the most evident differences. The intensities of scattered light by the particles are calculated taking into account the presence of the sample, the background and the reference light intensities. Calculating using $I_{particle}(\theta) = [I_{sample}(\theta)-I_{background}(\theta)]/I_{reference}(\theta)$ with $I_{sample}(\theta)$ to be the intensity of the blood sample, $I_{reference}(\theta)$ to be the intensity of the light source where the collecting fiber is directly below the lens and θ is 0 degrees which eliminates the noise of the source light, $I_{background}(\theta)$ to be the intensity collected at angle θ of an empty glass slide with no sample on it which eliminates the effect of scattering caused by the glass slide. Lastly, in order to remove the effect of the number of red blood cells in the test spot, the calculated intensity is normalized by the area under the scattering curve.

Five measuring angles ranging from 10 to 20 degrees for forward elastic light scattering were tested to determine the optimal scattering angle for data collection. As the magnitude of the angle increased, the scattering intensity decreased. In order to ensure significant differences in intensity for visible comparison, angles 10 and 12 degrees were used to measure and compare all the scattering light intensities. The strongest scattered light intensity for backward scattering is when the collecting fiber is closest to the incident beam line. Due to the constraint of the setup, it is impossible to collect back scattering very close to the incident light direction without the collecting fiber to block the incident light. The closest angle and strongest scattering used to collect the backward elastic light scattering measurements is $\theta = 163$ degrees (or 17 degrees from the opposite incident beam direction).

The optimal time for the blood sample to completely adjust to each concentration saline solution dilution is approximately 15 minutes. Refer to Fig. 3 for three comparisons of two forward scattering concentrations at three different times. It is evident that at 15 minutes the difference between the two concentrations is the most evident. As a result, all diluted blood samples are tested 15 minutes after they have been prepared in order to achieve the best results.

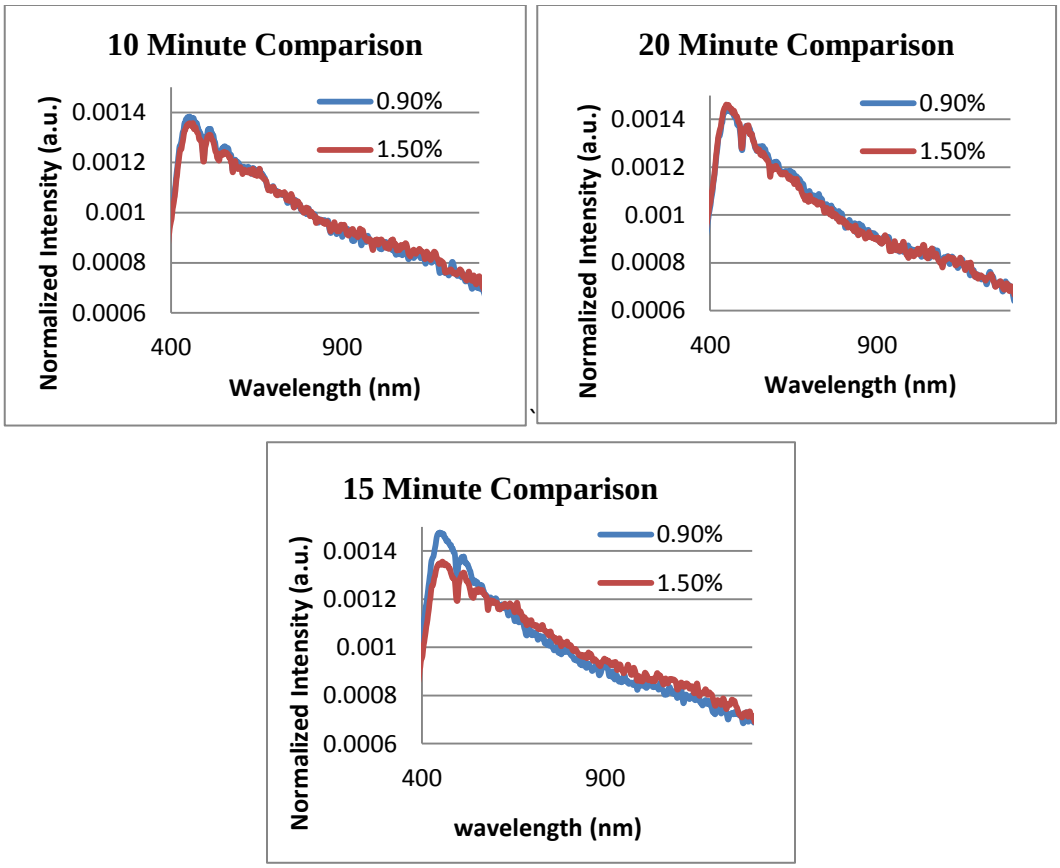


Fig. 3 Forward elastic light scattering comparison of two concentrations of blood samples at 10, 15 and 20 minutes.

D. Setup Validation

To validate the optical operation system that is used in this experiment, results obtained from the scattering of polystyrene microspheres were compared with the results from the Mie theory as shown below in Fig. 2.

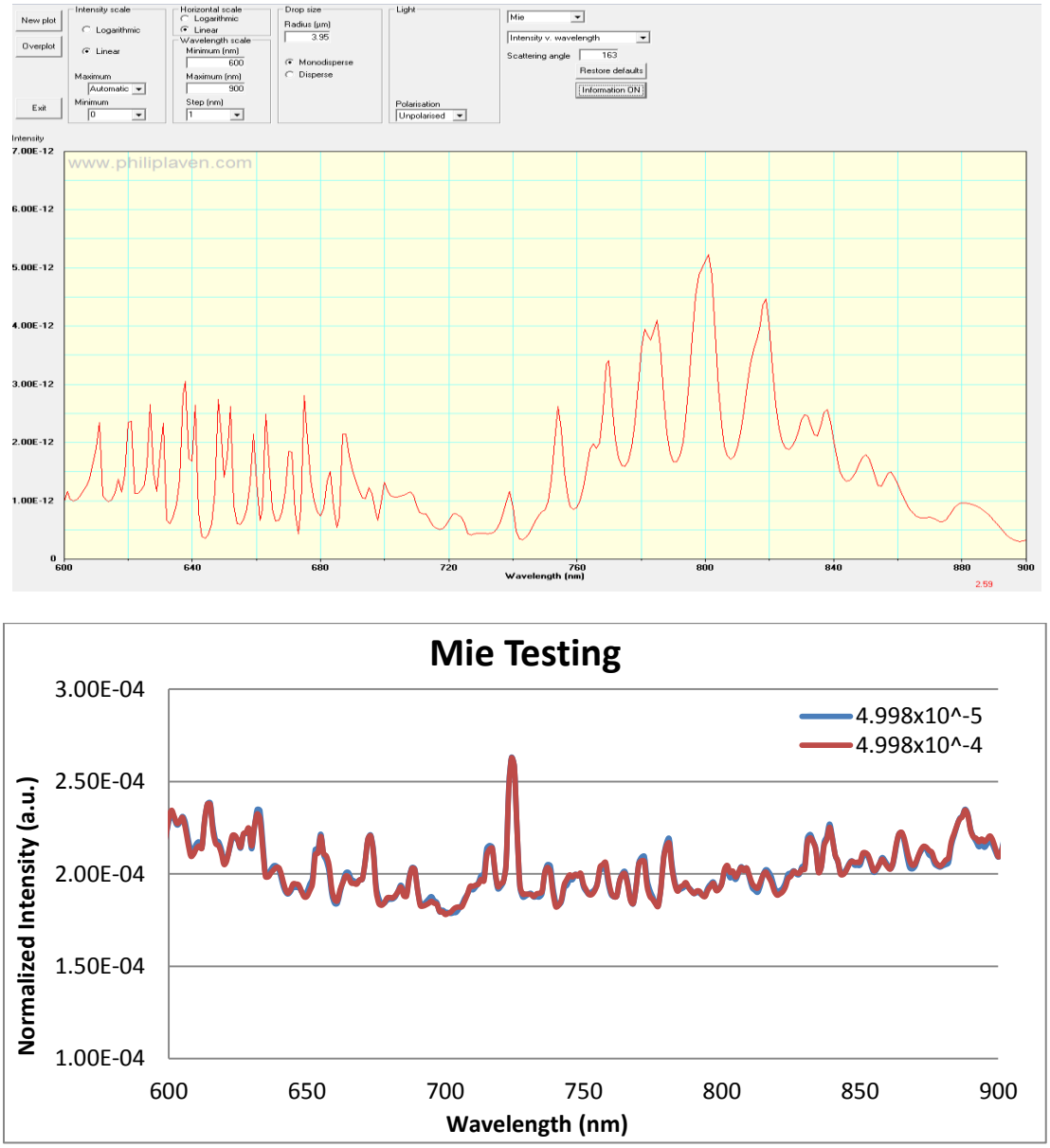


Fig. 2 Comparison of Mie theory calculations and experimental measurement of polystyrene microspheres with a mean diameter of 7.9 µm at a backward scattering angle of 163 degrees.

Microspheres were used to validate the experiment because the Mie theory assumes only spherical shape and single-scattering condition, therefore not possible for red blood cells. In order to closely resemble that of red blood cells, microspheres of diameter of 7.9 µm were used. In previous research, the forward scattering setup has already been verified. This scattering data was collected at a backward angle of 163 degrees to validate the theory for backward scattering. The microsphere samples were diluted to concentrations of $4.998 * 10^{-3}\%$ and $4.998 * 10^{-2}\%$ in order to ensure single-scattering events depicted by the Mie theory. Based on the graphs, a

visible valley appears at 700nm, a negative slope at 900nm and repetitive waves from 600 to 700nm appear in both cases. The two graphs were roughly compared because Mie theory represents ideal scattering conditions that disagree with the conditions of experimental measurements, for instance the difference in light source characteristics and real particle size distribution. The results are approximately similar and therefore show that the system is capable of capturing and detecting scattered light from micro particles and can be used to distinguish differences in the properties of multiple particles.

III. Results

A. *Forward Elastic Light Scattering*

Forward elastic light scattering was first measured because it has a stronger scattering intensity than backward elastic light scattering and therefore results in a bigger distinction among different concentrations. After dilution of each blood sample in five concentrations(0.5%, 0.9%, 1.5%, 2.0% and 5.0% saline solution), the data have been calculated and graphed as shown in Fig. 4 below.

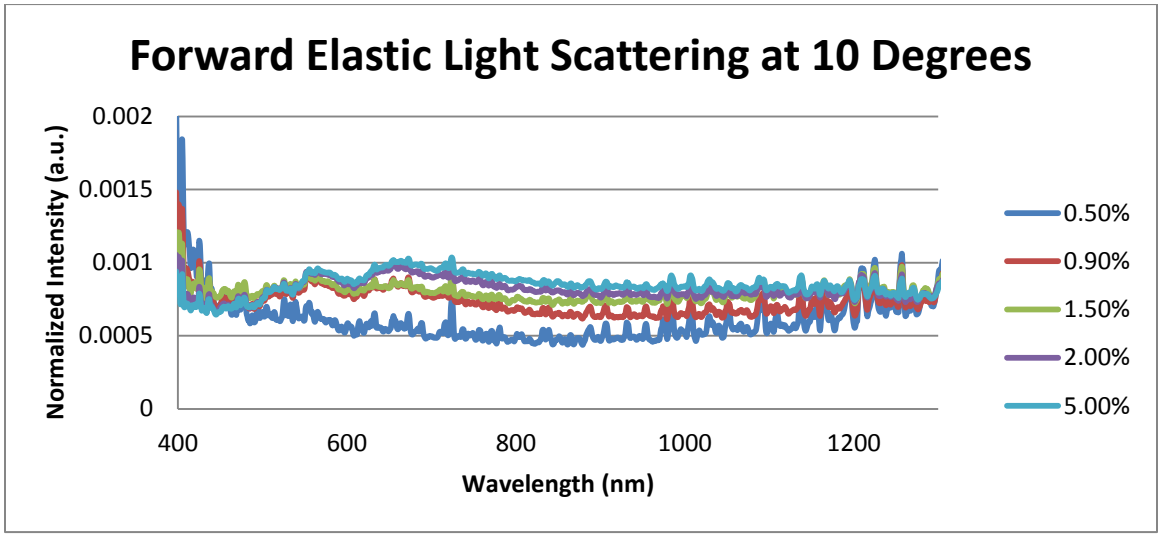


Fig. 4 Normalized plot of forward elastic light scattering through glass at a deflection of 10 degrees and recorded 15 minutes after dilution.

We have found a significant and consistent difference in the light spectrum that correlates to differences in the saline concentration of blood samples. It is clearly shown in Fig. 4 that among all the concentrations, the locations of key features such as valleys and peaks are all consistent with one another, which indicates that the experimental conditions are similar. The intensity difference increases as the wavelength deviates higher from 600nm with its most significant difference at wavelengths between 700 and 900nm. Also note that the intensity of the scattering

increases as the saline concentration of blood sample decreases, creating an inverse relationship between the two. The results of forward light scattering indicates that it is important to observe the characteristics of valleys and peaks as those are key features of the spectrum. Therefore the best wavelength window to better observe light intensity from backward light scattering would be 600 to 900nm.

B. *Backward Elastic Light Scattering*

i. *Blood Sample*

Backward elastic light scattering is more useful than forward light scattering because the collecting fiber is on the same side of the blood sample as the source of the incident light. This makes practical application of light scattering on blood vessels near the surface of the skin or the finger tips where the scattering is collected on the same side of the skin rather than through the skin.

Blood samples in different concentrations of saline solution were tested without a polymer film on top to initially figure out if backward scattering can successfully distinguish each diluted concentration of blood samples. Fig. 5 shows the calculated graphical results for the different samples.

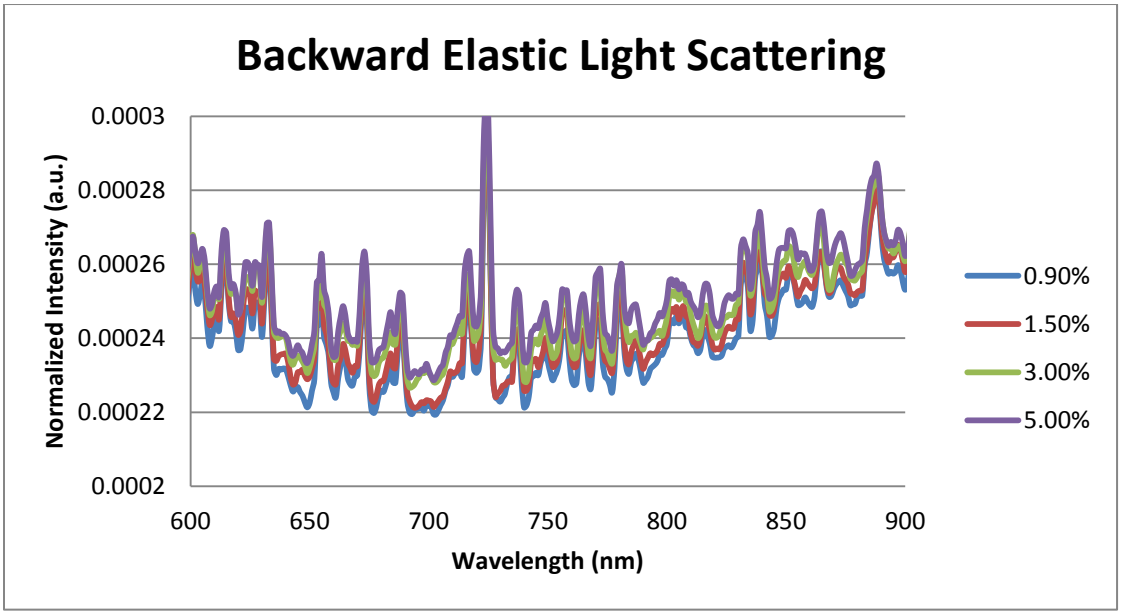


Fig. 5 Normalized plot of backward elastic light scattering through glass at a deflection angle of 163 degrees and recorded 15 minutes after dilution.

Using the wavelength window suggested by the forward elastic light scattering results, it is shown in Fig. 5 that the intensities of backward scattering of different concentration blood

samples are weaker. Even though the weaker scattering intensities created smaller differences between the blood sample saline concentrations, they are still significant enough to be compared, especially in the valleys of the spectrum. Similarly to that of forward light scattering, there is an inverse relationship between the intensity of the samples and the saline concentrations of the blood. Although with less significant differences, there are spectrum differences, especially in valleys, that suggest backward scattering is a possible approach to detect changing properties in red blood cells.

ii. Polyvinylidene Chloride Cover

To approach the task of mimicking the skin, a material that is thin and clear was initially tested. Polyvinylidene Chloride can be commonly found in plastic saran wraps that was easy to obtain and use. A small rectangular piece of Polyvinylidene Chloride cover was cut out and placed directly on top of the blood sample, touching the sample. Note that due to the elastic property of the cover, the blood sample is unevenly distributed under the cover. Fig. 6 below shows the results of backward scattering of Polyvinylidene Chloride cover.

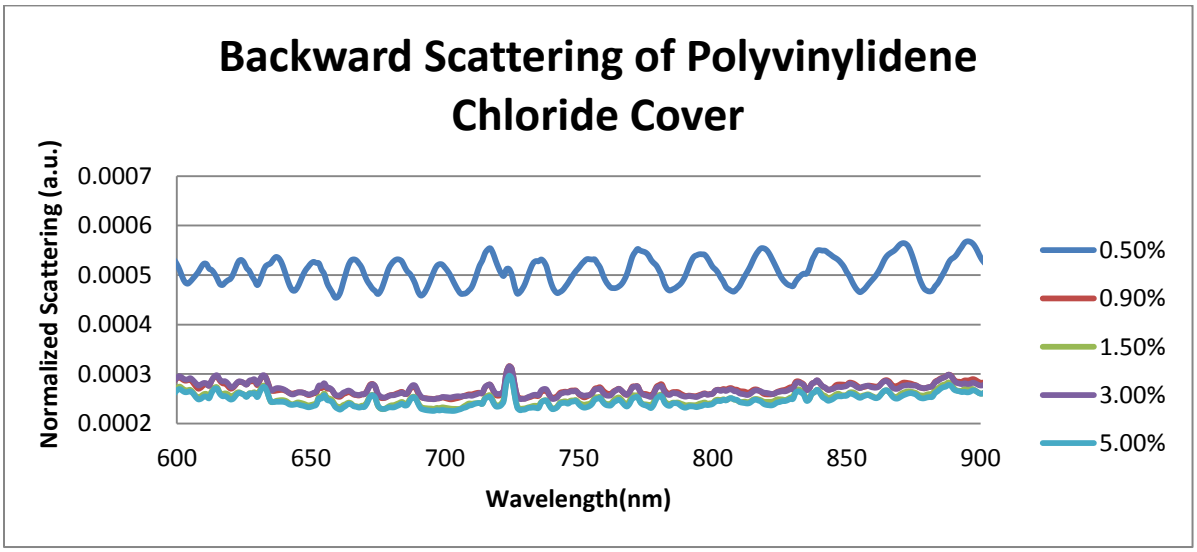


Fig. 6 Normalized plot of backward elastic light scattering through glass and Polyvinylidene Chloride Cover at a deflection angle of 163 degrees and recorded 15 minutes after dilution.

The spectrum difference in Fig. 6 suggests that Polyvinylidene Chloride is greatly affecting the scattering of blood samples that causes random scattering. Some possible explanations could be the uneven distribution of blood samples caused by the presence of the cover, the transparency of the film and the interference of the reflection of the cover with the scattered light.

Firstly, in order to correct the distribution of the blood sample under the cover, a microscopic cover glass was placed between the film and the blood. The cover glass evenly distributed the blood sample. The cover glass's scattering effect was eliminated by subtracting the intensity of it from the intensity of the sample in the calculations. The final result was still unsuccessful. Secondly, the cover film is very thin; the incident light was able to pass through and on to the blood sample. Lastly, in order to determine the effect of reflection of the film itself, we collected and compared the light scattering of two different locations on the same sample. Fig. 7 shows the difference in the backward scattering of the two locations.

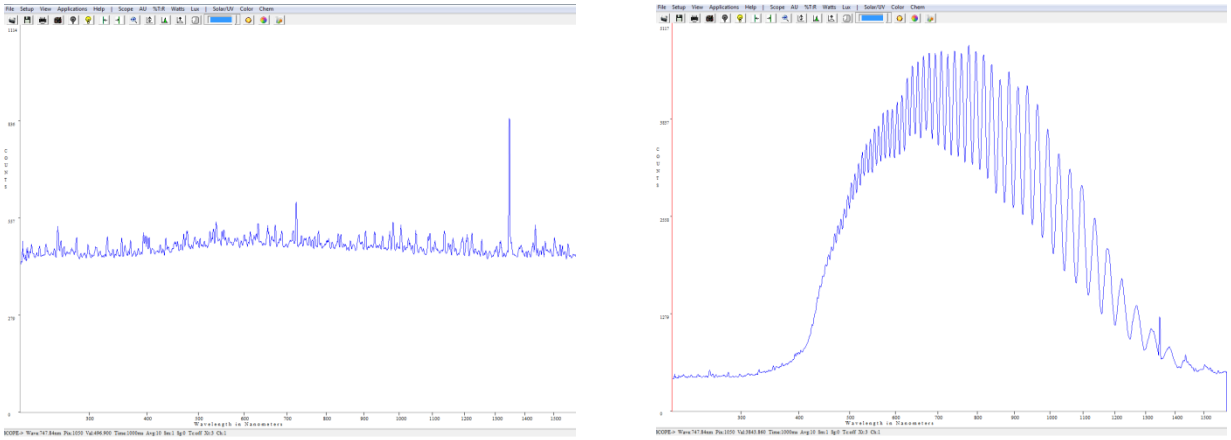
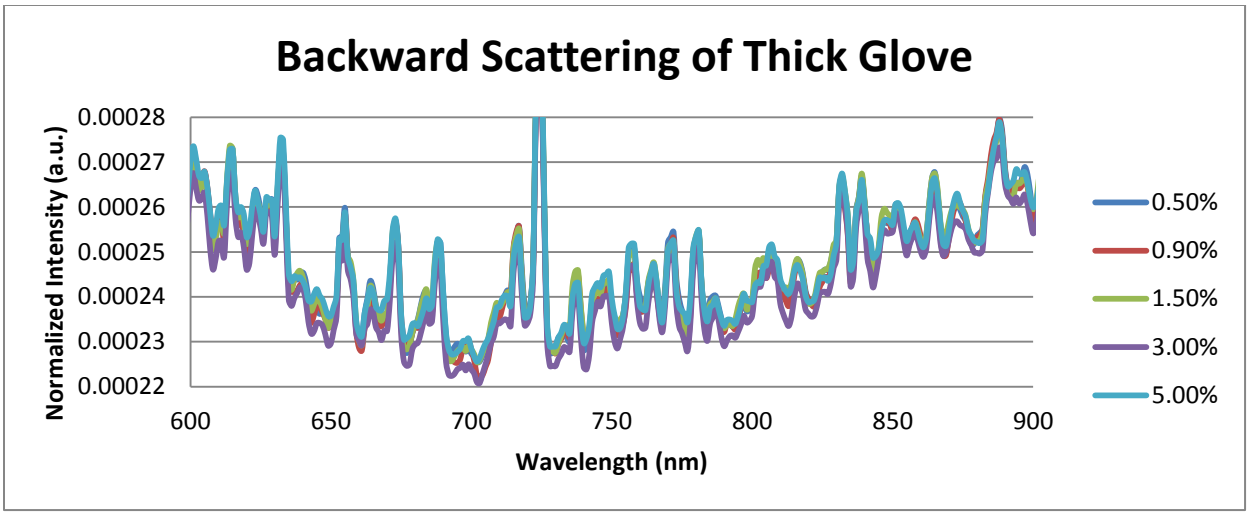


Fig.7 Backward =light scattering comparison of two different locations on 1.5% blood sample.

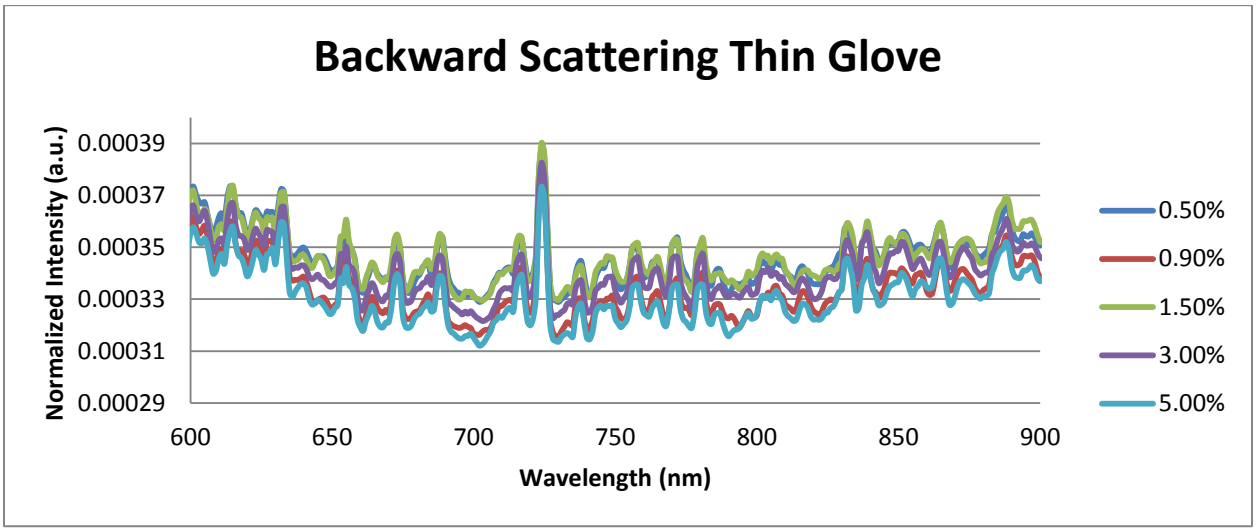
The comparison in Fig. 7 shows that a blood sample of a single concentration resulted in random scatterings of drastically different intensities. This indicates that the surface of the Polyvinylidene Chloride film is shiny and therefore reflecting light that interferes with the scattering light collected by the computer. The Polyvinylidene Chloride film therefore is not an accurate mimic of the skin or fingernail and cannot be used to verify that backward elastic light scattering can be used to detect properties of red blood cells through a barrier.

iii. Latex Cover

A non-reflecting surface of a latex film cover is used to detect the different concentrations of red blood cell samples through backward light scattering. The latex films are cut into small rectangular pieces and placed directly above the blood samples. After testing with two different latex films (one thin and one thick), there were stronger backward elastic scatterings. However, as Fig. 8 shows, the saline concentrations of the blood samples are inconsistent with the intensities of the light scatterings.



(a)



(b)

Fig. 8 Backward scattering of samples with (a) thick latex glove cover. (b) Thin latex cover.

The scattering spectrum is displaying inconsistent intensities for both the thin and thick latex gloves for different trials. To ensure similar conditions, same piece of latex cover placed on top of the cover glass to be reused for all samples. However, the results are still inaccurate and inconsistent for different trials without any significant pattern between the different outcomes. It was later discovered that the incident light was unable to go through the materials of both types of films and therefore causing the computer's collecting fiber to be collecting only the backward scattering of the film surfaces instead of the blood samples. Therefore, a polymer

film that is thinner and non-reflective was needed in order to let incident light pass through without its reflecting light interfere with the incident light.

iv. Polyethylene Film

The new polymer Polyethylene Film is relatively thin and non-reflective. The polymer film was first tested under the incident light and confirmed that it was thin enough for a strong light to pass through. The surface is not shiny and non-reflective and to verify, backward scatterings were collected of the polymer film at different locations and all results are consistent. The experiment was performed 3 times for verification and all offered the same result with one result provided below.

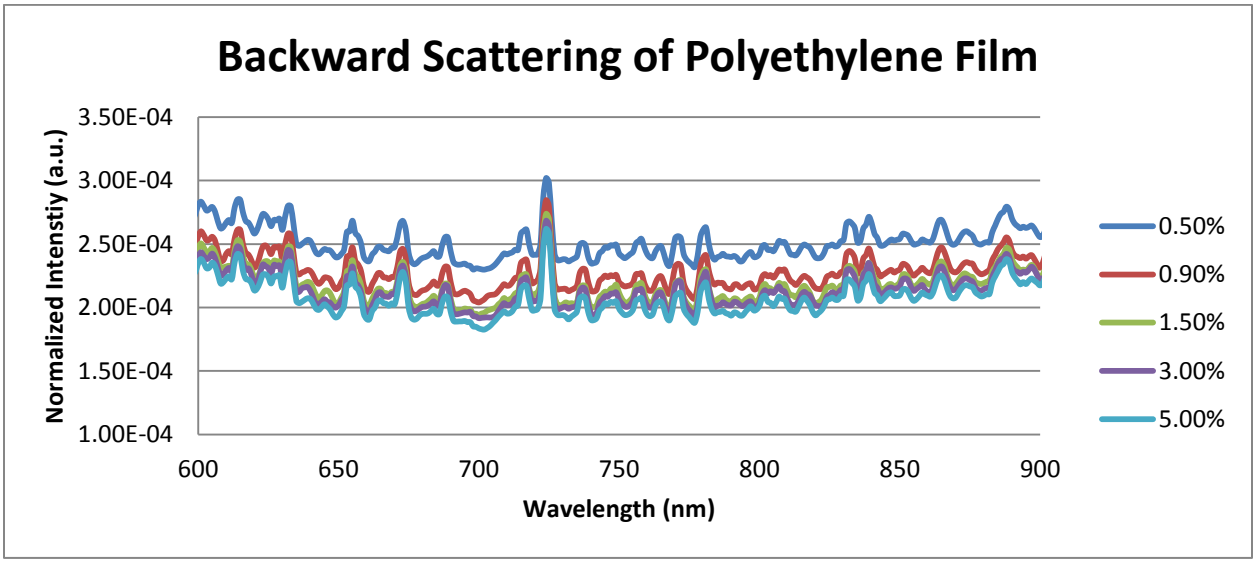


Fig. 9 Normalized backward scattering of Polyethylene Film and blood sample at a deflection angle of 163 degrees and recorded 15 minutes after dilution.

As shown in Fig. 9, the saline concentrations of the blood samples are consistent with the intensities of the backward elastic light scattering. The valleys and peaks of the curve are nearly identical and therefore suggest that the experimental conditions for the five different samples are nearly identical.

A characteristic of the Polyethylene film results that is different from previous results for only the blood samples without cover is that there is a direct relationship between the concentrations of the blood samples and the intensity of the backward scattering. As the saline concentration of the blood sample dilutions increase, the scattering intensities also increase. This is most likely due to the effect and backward scattering of the Polyethylene film, but it cannot be verified until further research is done on the Polyethylene film separately from the blood sample.

v. Finger Tips

In addition to detecting the red blood cells through the skin, the blood cells under the finger tips are also a good place to test for scattering because fingernails are relatively transparent and non-reflective. The scattering spectrum was collected on two different locations of the five fingers on the left hand. One location is in the middle of the finger while the other is on the tip. Note that the room was completely dark during data collection to ensure that no normal light interferes with the scattered light. The results are shown in the Fig. 10 below.

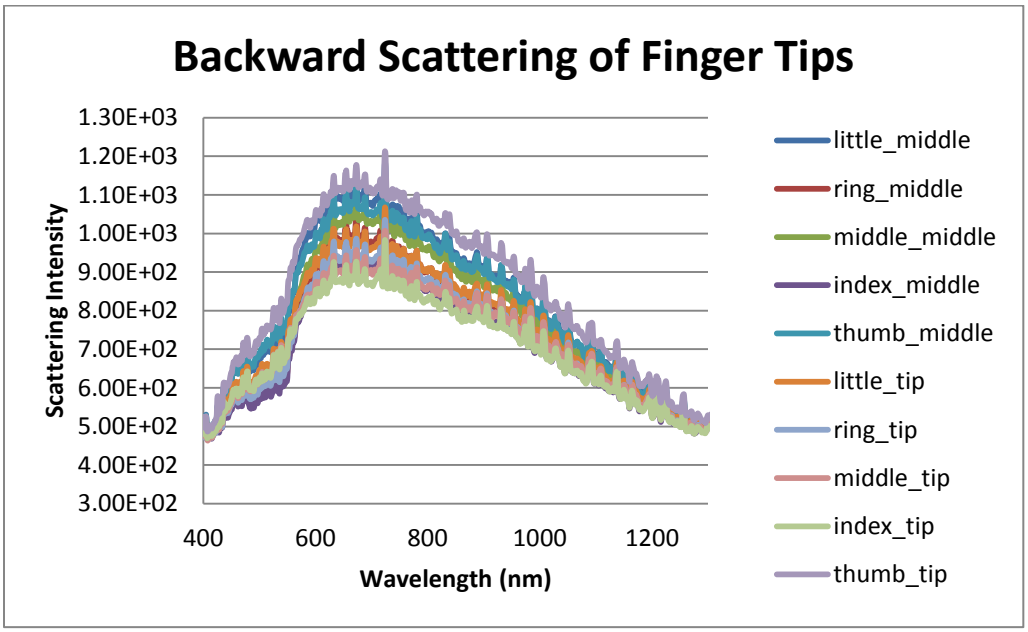


Fig. 10 Backward elastic scattering of different locations on different fingertips.

Fig. 10 clearly shows that there is a strong, relatively uniformed backward elastic light scattering from different location on the fingertips. The strong scattering is an advantage when it comes to comparison because stronger scattering result in bigger differences for different blood cell properties. The uniform curve and magnitude of the scattering spectrum shows that the fingernails are not reflective at different positions and will most likely give consistent results. The differences in magnitudes have no obvious pattern and will need further studies with altered red blood cells to determine. The spectrum unity and strength during backward elastic light scattering suggests that it is a very promising approach to diagnose changing properties in red blood cells through the fingernails on the fingertips.

IV. Future Work

Future experiments will be carried out to further verify the accuracy of backward elastic light scattering in determining the properties of red blood cells covered by a polymer film and

determine the effect the polymer film has on the backward scattering of the blood samples. Tests will be carried out using other materials of polymer film that mimic skin. Once it is confirmed that backward scattering can accurately detect properties of particles through a film, experiments will be performed on malaria infected cells and find a signature spectrum for not only malaria but also other blood-borne diseases such as sickle cell anemia. Lastly, a device will be created by programming the signature scattering spectrums of different diseases for practical use.

V. Conclusion

In conclusion, the experimental setup has been validated with the Mie theory using polystyrene microspheres in detecting physical and chemical properties differences in various particles. Elastic light scattering is effective and accurate in detecting the difference in red blood cells diluted in different saline concentrations as reported in the research.

Testing with different polymer films have shown that backward elastic light scattering is unable to detect accurate scattering of blood samples when the films covering them are reflective or too thick for light to pass through. So far, Polyethylene film is a mimic of skin for incident light to penetrate and create a successful scattering spectrum that represents the corresponding saline concentration of red blood cell dilutions. Another possible location to measure scattering intensity is through the fingernails on the fingertips. Results have shown that this approach offers relative consistent and strong backward scattering which is beneficial when distinguishing between the properties of the red blood cells under the fingernails.

The data compiled in the report will establish the foundation for further research into the actual signatures found in backward elastic scattering of malaria or other diseases infected red blood cells. This approach could ultimately lead to the development of a non-invasive device that detects the different signals of red blood cells and diagnosing different blood borne diseases such as Malaria.

VI. Acknowledgement

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VII. References

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