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Optical Diagnostic of Dengue Virus Infected Human Blood using Raman, Polarimetric and Fluorescence Spectroscopy

Shamaraz Firdous and Shahzad Anwar

Additional information is available at the end of the chapter

Abstract

In this chapter, we present the optical diagnosis of normal and dengue viral-infected human blood using Raman, Polarimetric, Transmission, and Fluorescence Spectroscopic techniques. The possibility of using light in diagnosis and treating illness has been known for thousands of years. The properties of light and lasers provided many modern applications at home, in industry, and in the field of medicine. Laser use in the field of medicine is large and steadily growing. This growth is based on the versatility of laser light. Efficient and accurate diagnosis of dengue is of primary importance for clinical care. A range of laboratory diagnostic methods has been developed to support patient management and disease control. The choice of diagnostic method depends on the purpose for which the testing is done, the type of laboratory facilities and technical expertise available, costs, and the time of sample collection. The dengue viral infection is mostly diagnosed through laboratory tests; these tests include detection of the virus, virus antigen, anti-dengue virus antibody, complement fixation test, neutralization tests, and detection of virus nucleic acid. As dengue infection most rapidly increases in different regions, early diagnostic confirmation of dengue infection in patients allows for timely clinical intervention, etiological investigation, and disease control. Hence, diagnosis of dengue disease during the acute phase should be a priority and is a public health concern. Lasers and optics have many applications in medical sciences; diagnosis and treatment of diseases with lasers and light are latest and noninvasive techniques. Development of light-based apparatus has evolved into tools for improved diagnosis and treatment modalities in medical sciences. The methods of the laser spectroscopy make it possible to obtain direct information regarding the structure and dynamics of the functional groups of biomolecules. Development of new light sources, optics, and diode laser of different wavelengths makes them attractive for spectroscopy of biological molecules. In our study, more than 600 dengue viral-infected blood or blood sera samples and 25 non-dengue healthy blood samples were analyzed using four different optical methodologies. In the first study, Raman spectrum peaks for normal samples observed at 1527, 1170, and 1021 cm^{-1} show the presence of different biological materials, including lipids, carbohydrates, skeletal C–C stretch of acyl chains, and

guanine. Raman peaks at 1467, 1316, 1083, and 860 cm^{-1} were observed in dengue-infected patients, representing CH2/CH3 deformation of lipids and collagen, guanine, lipids, and protein peaks using 532 nm laser sources. In our second study, an optical diagnosis of dengue virus infection in the whole blood is presented utilizing Mueller matrix polarimetry. Mueller matrices were extracted using light source from 500 to 700 nm with scanning step of 10 nm. Polar decomposition of the Mueller matrices for all the blood samples was performed that yielded polarization properties including depolarization, diattenuation, degree of polarization, retardance and optical activity, out of which, depolarization index clusters up the diseased and healthy into different groups. The average depolarized light in the case of dengue infection in the whole blood decreases, whereas for the healthy blood samples it increased. This suggests that the depolarization index of the polarized light was at wavelengths 500–700 nm; in this case, we find that depolarization index values are higher for dengue viral infection when compared to normal samples. This technique can effectively be used for the characterization of the dengue virus infected at an early stage of the disease. In the third experiment, the transmission absorption spectra of dengue-infected whole blood samples were observed in ultraviolet to near infrared range (400–800 nm) of about 30 conformed infected patients and were compared to normal blood samples. Transmitted spectra of dengue-infected blood showed two strong spectrum peaks at 540 and 580 nm wavelength of illuminating light, whereas in case of normal blood below 600 nm total attenuation was observed. The two strong absorption peaks from 500 to 600 nm are characteristic of cell damage and dengue virus antibodies IgG and IgM produced against dengue antigen. In the last study, we report an optical diagnosis of dengue-infected whole blood and controlled samples with Laser Scanning Confocal Microscopy (LSCM) over a laser excitation of 488, 543, and 633 nm wavelength. Based on our findings, the system has potential applications in the detection and quantification of dengue virus-infected cells, antigen, and antibodies in blood in vitro.

Keywords: optical diagnosis, dengue viral infection, transmission Raman spectroscopy, polarimetry, confocal laser scanning microscopy (CLSM)

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1. Introduction

Dengue is an endemic viral disease affecting tropical and subtropical regions around the world and is a viral infection transmitted by the bite of an infected female Aedes mosquito. There are four distinct serotypes of the dengue virus (DEN 1, DEN 2, DEN 3, and DEN 4). Symptoms appear in the first 15 days after the infection. Dengue virus affects humans from newborn baby to aged persons, mostly children and young ones. Dengue fever has no treatment yet, but precautions, early diagnoses, and proper care can protect patients from severity [1–4]. Recently, most of dengue viral infection cases are from Asia, Europe, Africa, and even America. Last year, majority of cases were from Asia and Africa. In 2013, indigenous transmission of dengue was also reported in America and Europe. Dengue spread in America and Europe is mostly by the movement of infected persons and goods; the lack of healthy environment and preventive arrangements also contribute to the global increase of dengue [5–7].

The World Health Organization estimates that worldwide each year, there may be approximately 100 million cases of dengue virus infections [8, 9]. Dengue spread is mostly related

with special kind of mosquito bites; however, some normal mosquitoes are infected through dengue virus and then become the source of infection. Dengue virus spreads from person to person but not directly. In fact, it can never transfer from one person to another directly [10, 11].

An evaluation of commercial capture immunosorbent assay for detection of immunoglobulin M and G antibodies produced during dengue infection and Pan Bio kit was evaluated with paired serum specimens from patients. They proved that ELISA should be useful in the clinical diagnosis of dengue infection. Similarly, an evaluation of a commercial ELISA kit for the detection of IgM during dengue infection revealed that primary dengue infection was detected positive for anti-dengue antibodies. Study of Pan Bio duo ELISA and MRL dengue fever IgM capture ELISA for the diagnosis of dengue virus infection in Southeast Asia was done, and comparing the specificity and sensitivity of the tests at different cutoff values revealed that similarly in distinguishing dengue virus from non-flaviviruses showed significantly better distinction between dengue virus and other viruses [12]. Dengue-infected person generates antibodies against dengue virus, which cannot be observed in normal blood or blood sera. The optical changes observed are reflection of biochemical changes in blood. This is the basic principle of dengue fever diagnosis based on optical spectroscopic techniques [13].

Medical tools and sensors based on lasers and optics are used in many applications. Optical diagnostic apparatus are very effective as compared to conventional disease detection methods. Optical techniques are noninvasive, direct, cost-effective, and easy to use with high specificity, sensitivity, and small size. Optical diseases diagnostic research and development has been useful to healthcare, environmental applications, biotechnology industry, and medical sciences [14, 15]. For the development of new optical equipment to be used in medical sciences and devices for practical applications, all the experimental findings and practical aspects, such as robustness, reproducibility, simplicity, and shelf life, should be carefully considered. In these experimental results and optical detection of different diseases using light allows construction of sensitive, simple, and cheap analytical devices with a wide variety of possible applications in screening and monitoring of diseases for use in personalized medicine, remote areas or in developing countries where the availability of inexpensive diagnostic tools are not accessible [16, 17].

2. Raman spectroscopy

Raman spectroscopy was carried out using high-resolution Raman spectroscopy system. The main elements of setup are laser sources 532nm, samples slides, and chamber light collection optics detection system. The target material, blood serum of normal, and dengue samples on glass substrate at room temperature (300±2 K) are used as optimized parameters. The sample was excited by He-Cd laser of 532 nm wavelength and 80 mw power, and output intensity signal detected with objective lens and air-cooled charge-coupled device (CCD) detector. The Raman spectra collected in Raman shift of 600–1800 cm^{-1}. We used the accumulate acquisition mode to reduce noise and thermal fluctuation and improve signal to noise ratio [18–20].

For normal blood sera, the Raman shifts are observed at 1527, 1170, and 1023 cm^{-1} with intensity level of 7200–9500 pixels, showing a compound that occurs in guano and fish scales and is one of the four constituent bases of nucleic acids. A pure derivative, it is paired with cytosine in double-stranded DNA (guanine). Adenine is used in forming nucleotides of the nucleic acids. Adenine can be found in DNA and RNA, in first with two hydrogen bonds for the nucleic acid structures stabilization and in second for protein synthesis. Tryptophan is α-amino acid that is used in the biosynthesis of proteins TRP (protein) carbohydrates peak for solids. Carbon skeletons are the backbones of organic molecules. They are composed of carbon-carbon atoms that form chains to make an organic compound. Length, shape, location, and amount of double bonds are characteristics of carbon skeletons (skeletal C-C), and Cardiolipins are a subclass of glycerophospholipids containing four acyl chains and three glycerol groups that are particularly abundant in the inner mitochondrial membrane. They are believed to activate enzymes involved with oxidative phosphorylation (stretch of lipids acyl chains) as shown in **Figure 1A**. The Raman shifts observed in Raman spectrum of a dengue-infected blood serum are at 1467, 1316, 1083, and 860 cm^{-1} with intensity level of 6000–7500 pixels attributed to the bands of stretching and deformation vibrations of CH_2, C-CH_3, and OCH_3 groups in the infrared and Raman spectra (CH_2/CH_3) deformation of lipids and collagen, guanine, lipids, and protein bands as shown in **Figure 1B**.

The main dengue viral infection sera peaks appear at 1467 and 860 cm^{-1}. The intensity of these peaks, as well as those at 1316 and 1081 cm^{-1}, is plotted against the intensity level in **Figure 1B**. In our previous chapter [6], we reported transmission spectra of dengue-infected whole blood samples irradiated with light of 400–800 nm wavelength. We collected data of 30 conformed infected patients and compared them to normal blood samples. Transmission spectra of dengue-infected blood show strong and prominent peaks at 540 and 580 nm. In case of normal blood, total absorption has been observed from 400 to 600 nm of wavelength. In case of dengue, the peaks indicate damage and dengue virus antibodies immunoglobulin G (IgG) and immunoglobulin M (IgM) produced against dengue antigen. In another chapter, we determined that normal whole blood and serum characteristic peaks were excited at 442 and 532 nm. In dengue-infected whole blood and serum, two peaks at 1614 and 1750 cm^{-1} are observed, which are due to the presence of immunoglobulin antibodies IgG and IgM.
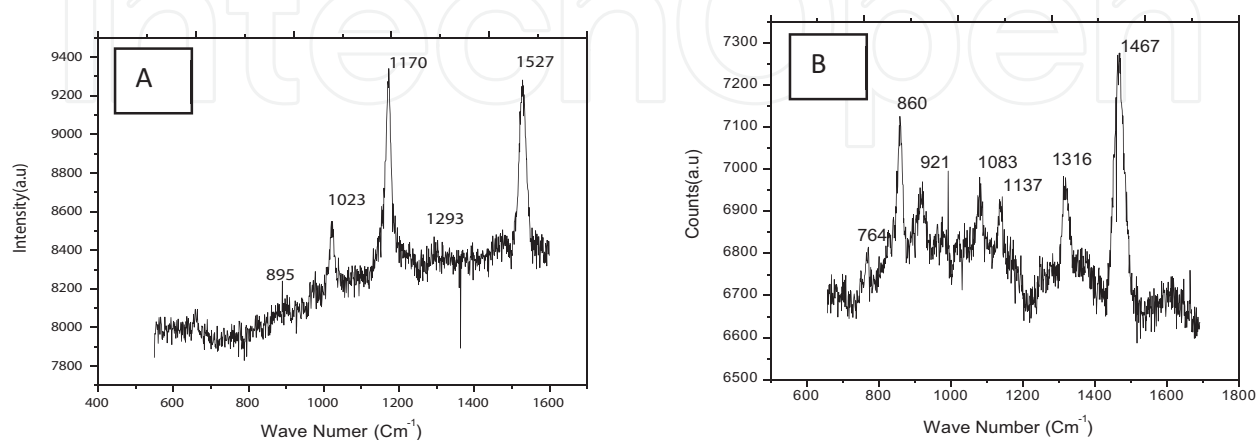

Figure 1. Raman spectrum of (A) normal blood and (B) dengue blood at 532nm wavelength of light.

Raman spectroscopy has proven to be an effective analytical approach in geology, semiconductor, materials, and polymer science fields. The application of Raman spectroscopy and microscopy within biology is rapidly increasing because it can provide chemical and compositional information. Raman spectroscopic technique based on protein and lipid changes due to antibodies and antigen reactions, while the Protein and lipids concentration totally changes in dengue viral sample as compared to normal ones [21].

3. Mueller matrix polarimetry

Polarimeters are optical instruments used for determining the polarization properties of light beams and samples. To perform accurate polarimetry, all the issues necessary for careful and accurate radiometry must be considered together with many additional polarization issues. Mueller matrix polarimetry yields four optical parameters, namely depolarization coefficient, linear retardance, optical activity, and diattenuation, which can be used to characterize the tissues or turbid media [22–34]. We represented very interesting results from the dengue-infected whole blood by exploiting the depolarization nature of polarized light in whole blood; this can be used for the discrimination between the healthy and viral infection diseased blood samples [5].

Mueller matrices from all the 30 ultra-thin smeared blood slides were acquired using Mueller Matrix Polarimeter AxoScan™ (Axometrics, USA). AxoScan™ system employs dual rotating retarder as discussed in our previous study [5]. A tunable light source ranging from 400 to 800 nm, coupled with 200 nm multimode fiber optic probe, is used to shine the sample through Polarization State Generator (PSG). Light source consists of a low-noise 150 W Xenon arc lamp and a scanning diffraction-grating monochromatic light. PSG consists of a fixed linear polarizer along with a rotating linear retarder. As the retarder rotates, a wide variety of polarization states, including linear (horizontal, vertical), elliptical (−45°/+45°), and circular (left/right),is generated. These six polarization states are steered to pass through the smeared blood slides. The rotating retarder in PSG is rotated five times faster than the retarder in the generator for different combinations of the rotation angles of retarders in PSG and Polarization State Analyzer (PSA); light coming out from PSA is collected by a very sensitive detector. The resulting combination of polarization states at different rotation angles of retarders in PSG and PSA is adequate for determining the Mueller matrix of the sample.

In the present study, Mueller matrices from all the blood samples were measured from 500 to 800 nm, and their polar decomposition yields the polarization parameters for each wavelength, out of which depolarization coefficients showed distinct differentiation. **Figures 2** and **3** displayed the diattenuation and retardance of the dengue-infected and non-dengue healthy blood samples as a function of wavelength. We find that in the case of dengue viral infection, diattenuation and retardance values are lower when compared to normal samples. The diattenuation and retardance of both types of samples can be used for the characterization of the early stage detection of dengue infection in the human blood. The Mueller matrix is generally a diagonal matrix having $m_{00} = 1$ and $m_{11} = m_{22}$. The $m_{11} = m_{22}$ is due to axial symmetry of the material.
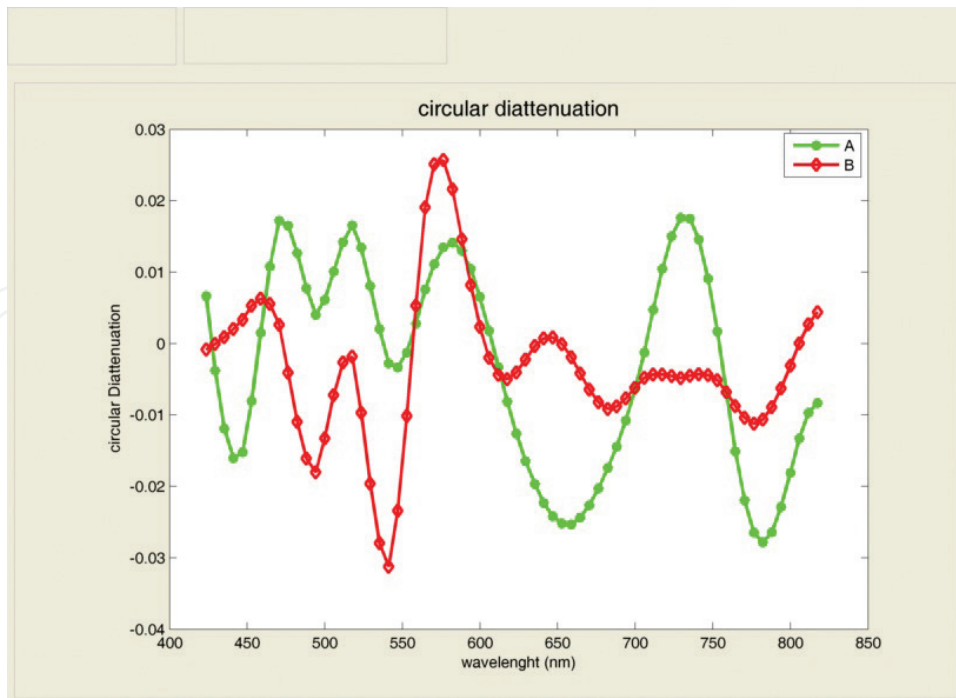
Figure 2. Polarization properties in terms of Mueller matrix, diattenuation of normal (━●━), and of dengue-infected blood (━◆━) at 400–800nm wavelength.
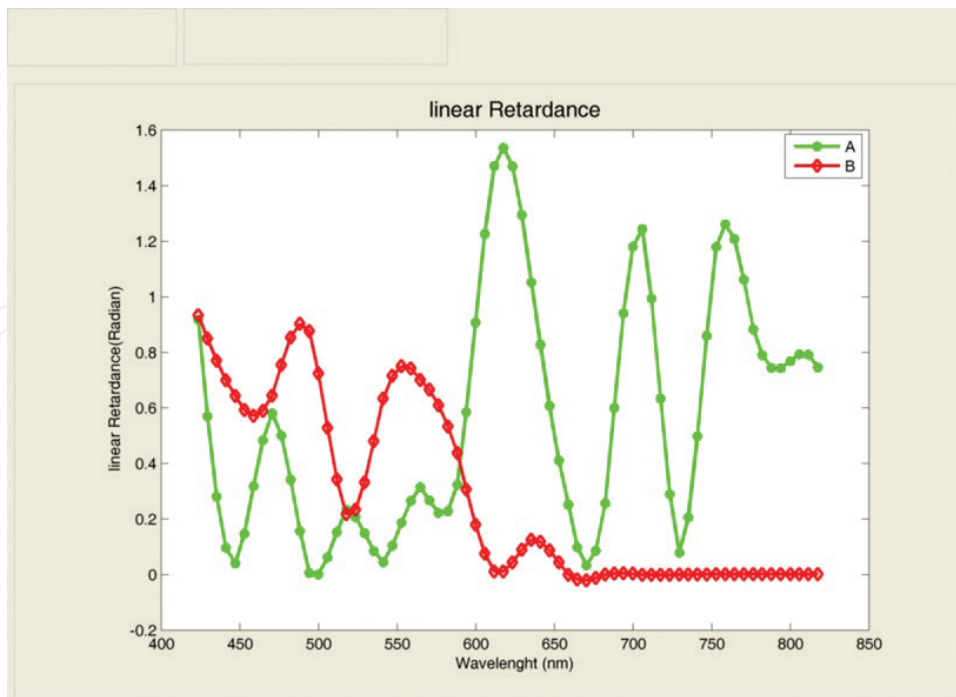

Figure 3. Polarization properties in terms of Mueller matrix, retardance of normal (━●━), and of dengue-infected blood (━◆━) at 400–800nm wavelength.

The retardance matrix is also a diagonal matrix, where all diagonal elements are equal as shown in **Figure 4**. The optical characteristics of dengue-infected samples at 540 nm of light showing depolarization index values were higher as compared to normal samples. The diattenuation is less than polarization as seen in the first row and first column of the Mueller matrix. In the retardance matrix, image m_{11} and m_{22} are same. m_{33} depends on the size of scatterers.

The color intensity of the elements m_{11}, m_{22}, and m_{33} increases when compared to the retardance image of **Figure 4** of dengue-infected ones. The depolarization image is showing a diagonal matrix with maximum intensity at diagonal elements. It is clear that depolarization coefficient decreases as a function of wavelength for both types of samples, as well as the difference of depolarization coefficients reduces for both types of the samples. The hematocrit of the dengue virus-infected blood increases due to decreasing platelets; this increasing of volume fraction of red blood cells may be responsible for the more depolarization coefficient in comparison with the non-dengue healthy blood samples as red blood cells play an important role in scattering that causes the depolarization of polarized light.

In the dengue virus-infected human blood, antibodies IgM and IgG appeared against the infection by the defensive system. These antibodies are like protein structures that must also be responsible for producing more depolarization effects in the dengue-infected blood samples.
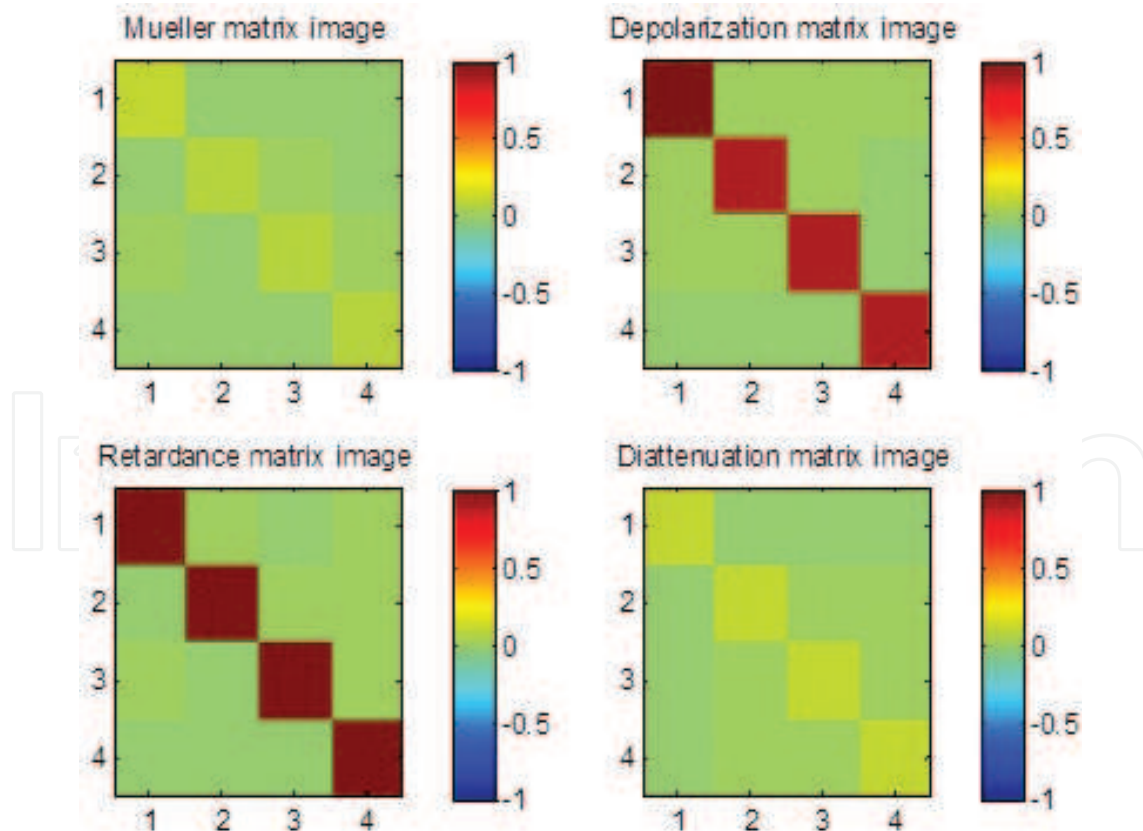

Figure 4. Polarization properties in terms of Mueller matrix, depolarization, retardance, and diattenuation matrix of dengue-infected blood at 540 nm of light.

Mueller matrix polarimetry has been exploited successfully for the optical diagnosis of dengue virus infection in human blood. Depolarization coefficient of the dengue-infected and non-dengue human blood samples is clearly distinct in light spectra for the characterization of both types of samples. In addition, diattenuation, retardance, and depolarization coefficients may be helpful for monitoring the disease from time to time and to monitor very minute structural changes in the tissue of blood chemistry.

4. Transmission spectroscopy

In this study, we provide transmission spectroscopy of dengue viral infection analysis. The cell culture method is still considered the standard for viral diagnosis as it has the advantages of detecting infectious viral particles and the ability to achieve low detection limits. This new method has the potential to be extended for the detection of other viruses and adapted into a portable, automated system for detection of viruses from environmental samples. The presented results are very effective for initial screening of dengue-infected patients and to minimize the diagnostic cost [6].

We used Avaspec. Spectrometer (Avantes Inc, Netherlands) of wavelength range 400–1000 nm and Avaspec.software to record data. About 50 µl drop of whole blood excited with light as shown in **Figure 5**. Two prominent absorption peaks at 540 and 580 nm of blood spectrum, as measured within a set of about 30 essentially conformed dengue-infected individuals, are observed as shown in **Figure 6**. The change represented by these peaks in the protein and blood cells within the human body is a manifestation of significant biochemical changes due to the antibodies IgG activated after 2–3 days of dengue virus infection and IgM produced after one week. We have recorded transmission spectra of other viral-infected blood samples, like hepatitis and malaria, besides dengue infection, but there is no transmission in the range of 400–600 nm. The spectrum peaks at 540 and 580 nm are only in dengue-infected samples.
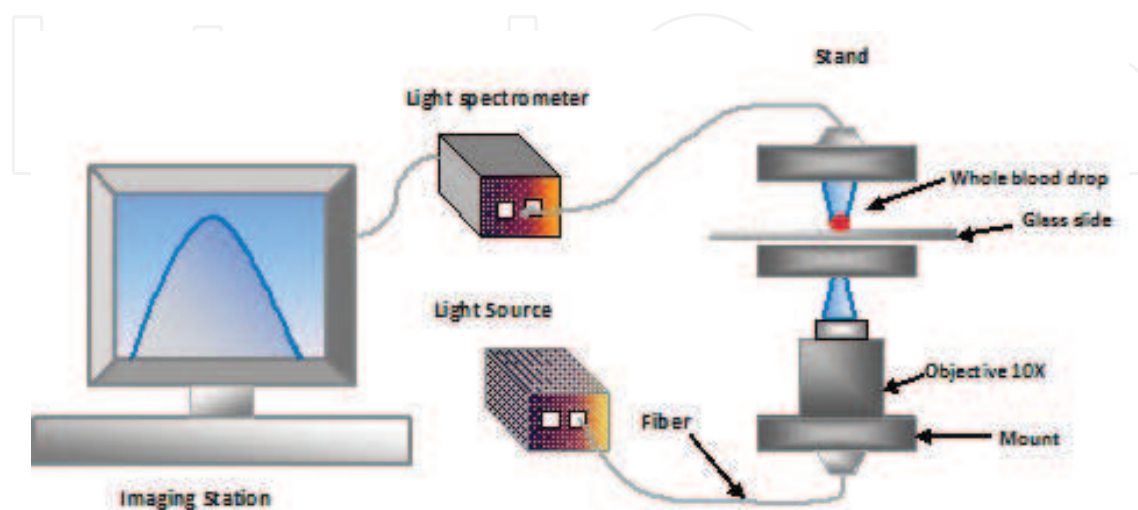

Figure 5. Experimental setup for the whole blood dengue infection diagnosis with light spectroscopy.
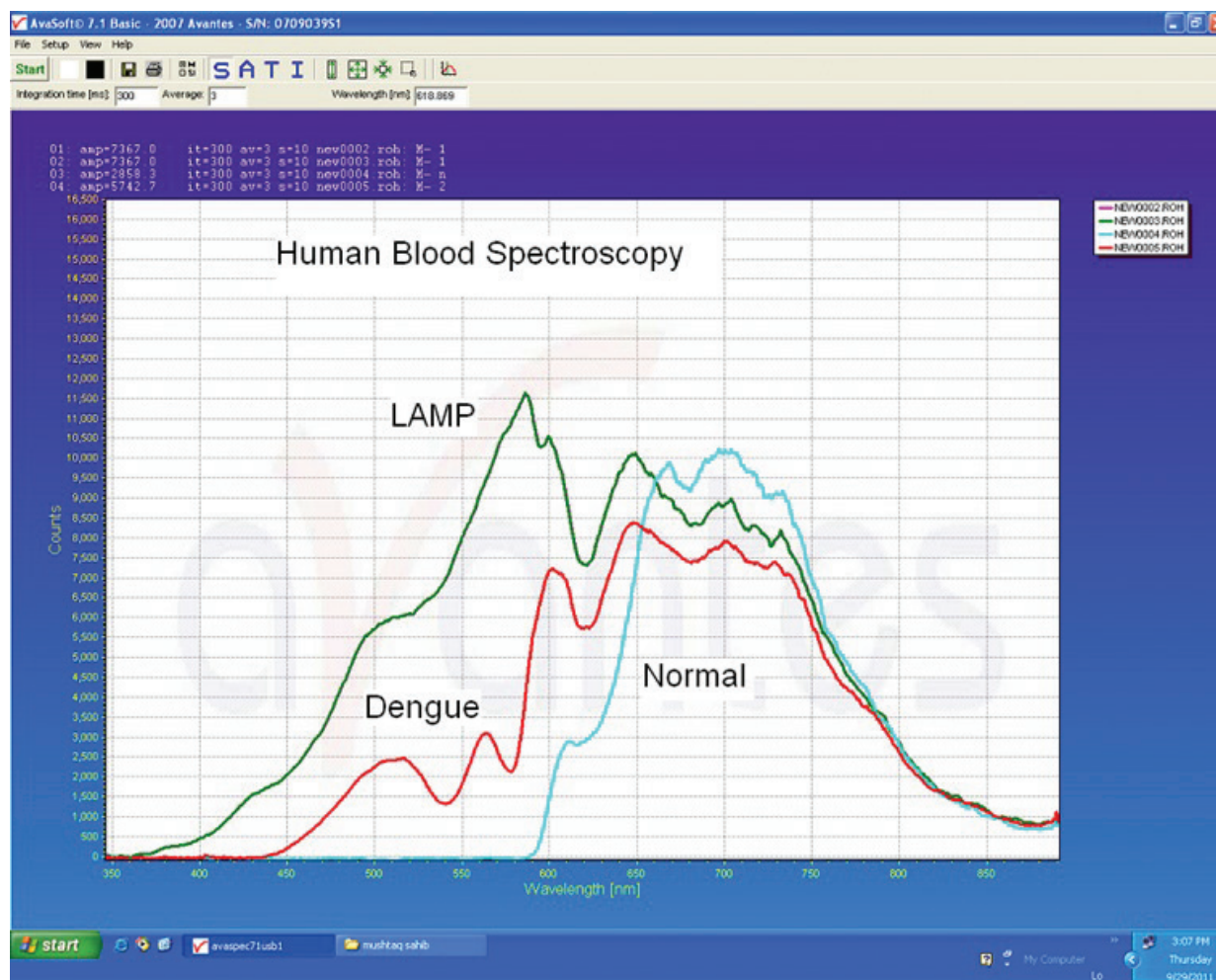
Figure 6. The transmission spectroscopy of normal and dengue-infected whole blood spectrum at 400–1000 nm wavelength.

5. Confocal microscopic analysis

We have used light spectroscopy, Raman and absorption spectroscopy, and Laser scanning confocal microscopy (LSCM) techniques for repaid screening of dengue infection. LSCM has become an invaluable tool for a wide range of investigations in the biological and medical sciences for imaging thin optical sections in living and fixed specimens. To image the dengue-infected blood, a drop of 50μl whole blood was placed on quartz glass slide and seen through (63X, 100X) water and oil emulsions objectives of LSCM (LSM-510, Carl Zeiss, Micro Imaging Inc., Germany) equipped with 451, 471, 488, and 517nm line of Argon ion, 553 and 633 nm He-Ne lasers, 10 to 100X water and oil emulsions objectives and three Photo Multiplier Tubes (PMTs). Fluorescence light from the sample was collected by the objective, directed to an analyzer, and spatially filtered by the confocal pinhole in the detection path. Reflected light that passed through the pinhole was spectrally separated by dichroic filters and directed to three PMTs to detect light at each wavelength. Images were acquired as 8-bit TIFF files (512 × 512 pixel frame) and processed using Zen software (Carl Zeiss, Micro Imaging Inc., Germany).

We demonstrated the dengue-infected human blood in in vitro using LSCM. The confocal images of normal and dengue-infected whole blood samples are analyzed with LSCM and fluorescence is detected. The dengue cells are different in looks compared to the normal ones, and the fluorescence from dengue infected is prominent than normal as shown in **Figure 7**. The whole blood analyzed on day 10 and day 13 shows antibodies IgG and IgM structure over blood cells. We suggest that as platelets decrease, fluorescence increase due to complex interactions between the virus and host cells, leading to the activation of transcription factors, cytokines, and enzymatic factors. The simple blood images taken at day 3 of infection are shown in **Figure 7** and fluorescence of cells at day 10 is shown in **Figure 8**. These interactions may induce not only pathologic prion flammatory responses that influence the severity and progression of the disease but also virus clearance and apoptotic pathways that could be controlling infection by anti-viral mechanisms. The present study may, therefore, contribute to a better understanding of the cell activation mechanisms triggered during dengue infections occurring in human target cells. The viral infection images at day 10 and 13 after infection are shown in **Figure 8**.

Blood sample analysis with LSCM provides a way for diagnosis of infection. The results obtained can be used in the development of new methods and optimization of existing ones for dengue-infected patients. The presented techniques can be used for new light-based diagnostic apparatus for the quantities analysis of dengue patients. In our previous research findings,
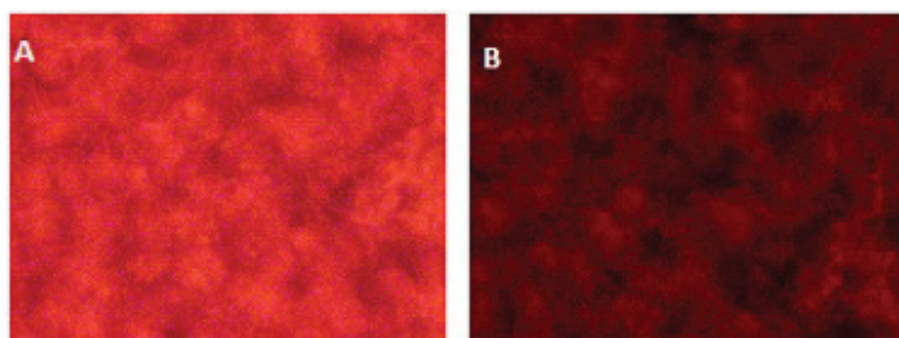

Figure 7. An early comparison of dengue-infected blood samples with a confocal microscope (63×). (A) normal blood drop on glass slide and (B) dengue-infected blood at day 4 after infection.
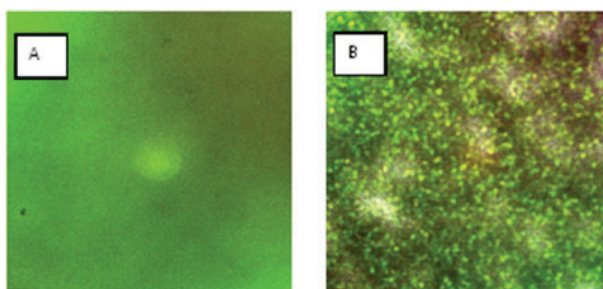

Figure 8. An early comparison of dengue-infected blood samples with a confocal microscope (63×). (A) normal blood drop on glass slide and (B) dengue-infected blood at day 10 after infection.

we have characterized tissues and biomaterials for optical imaging to diagnose diseases and develop optical detection equipments [35–53].

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