Smartphone-based Optical System for Blood Coagulation Self-monitoring

Ramji Sitaraman Lakshmanan and Vitaly Efremov
Biomedical Diagnostics Institute
Dublin City University
Dublin, Ireland
e-mail: ramji.lakshmanan@dcu.ie; vitaly.efremov@dcu.ie

Abstract— **Blood coagulation self-monitoring is vital in modern day healthcare, in particular supporting patients on anticoagulant therapy. Commercially-available coagulation self-monitoring devices are typically based on conventional end-point-based haemostatic tests and, measuring clotting time within a narrow range of values being connected to specific aspects of clot formation. In the present study, novel optical method combined with a smartphone was applied to measure the clotting time. The method was utilised for analysis of normal whole blood (WB) sample coagulation, activated by various tissue factor (TF) concentrations. The results demonstrated the ability of such a system to measure a wide range of clotting time values with appropriate level of accuracy and precision similar to standard thromboelastography (TEG). Significantly, the use of a smartphone as an optical detector and signal processor potentiates system miniaturisation, pertinent to high levels of functionality, cost-efficiency and user-friendliness.**

Keywords-blood coagulation; nephelometry; clotting time; light scattering; smartphone.

I. INTRODUCTION

Oral anticoagulation therapy is prescribed for both prophylactic and therapeutic use for patients at increased risk of thromboembolism including ones with mechanical heart valves [1]. In current practice, the efficacy of anticoagulation therapy is routinely determined by blood coagulation monitoring. This monitoring is typically based on a clotting time value measurement, specifically the time (in seconds) taken by method of blood sample activation to commence the coagulation process. Clotting time has a simple unambiguous interpretation. Its lower value corresponds to high blood coagulability and hence, increased risk of thromboembolism whereas higher clotting time values indicate increased bleeding risk. Furthermore, clotting time depends not only on patients' blood coagulability but also on the chosen method of coagulation activation. The oral anticoagulation drugs such as warfarin, heparin and many others became commercially available more than 60 years ago and their dosage monitoring was initially carried out in stationary clinical laboratories. More specifically, coagulation monitoring during a therapeutic regime is of critical importance for dosage control, since it provides key information about actual drug efficiency and reduces the risk of potential bleeding as a result of therapeutic overdose. In current practice, compact coagulometry devices for home-based self-monitoring are available [2]. The self-monitoring makes it possible to reduce the number of visits to clinics and optimise the resources of a local health-care system. Here, the patient collects a small volume of a blood sample with a finger-prick at the time agreed with by a clinical specialist, loads it on the device using an appropriate mechanism of transfer and records the clotting time value detected by the device, which is presented as a measurement output. Results are typically obtained in relatively short times, ultimately improving patient care and empowering patients as it allows them to take more responsibility for their own health [3].

There are several blood coagulation self-monitoring devices available on the market [2][4][5]. The absolute majority of these are designed to adapt some of the traditional laboratory assay of coagulation for use in self-monitoring. These conventional assays measure clotting time via the intrinsic and extrinsic pathways of the coagulation cascade, and selected methods of activation include prothrombin time (PT) and activated partial thromboplastin time (aPTT). Each assay measures a different part of the coagulation cascade. For example, PT uses TF as an activator of coagulation via the extrinsic pathway, and is sensitive to the effect of many widely-used anticoagulation drugs such as warfarin. Moreover, the PT is the only coagulation assay which to-date has been effectively standardised via the International Normalisation Ratio (INR), which explains why the PT assay is the most commonly-used in coagulation self-monitoring systems. In contrast, the aPTT assay uses negatively-charged contact activation material such as glass or kaolin in the presence of phospholipids as an activator of coagulation via the intrinsic pathway. This test is sensitive to the effect of anti-coagulants such as heparin. A panel of self-monitoring devices have been developed to essentially automate these assays through the integration of mechanical, optical, and electrochemical detection methodologies. One of the most recognisable device is the Roche CoaguCheck [2][4][5], which combines the PT/INR assay with microfluidics and a sample warming module. In the original device, a blood sample is applied to a disposable test strip containing activation reagents and iron filings. Next, an electromagnetic field moves the fillings, which is detected optically. As clot forms it prevents this movement, and the corresponding time where optical signal decays are returned to the end-user as the clotting time values. The Alere INRatio [5] also uses PT/INR assay and employs

 ISBN: 978-1-61208-470-1

electrochemical impedance measurements to determine clotting time. However, both systems are not compatible with mobile devices, such as smartphones.

Although blood coagulation self-monitoring devices take a quite strong position on the market, there are at least two critical aspects that need to be addressed. Firstly, current devices typically measure clotting time only within narrow range of values since they are specifically adapted to be used with certain conventional coagulation assays. This is a quite serious limitation for the monitoring of the effect of specific and/or atypical anticoagulation drugs. For instance, a PT/INR-based device is usually tuned to provide effective resolution of clotting time values not exceeding 60 seconds, whereas many current therapeutic strategies require the rate of coagulation monitoring to be decreased to facilitate measurement (see, e.g., [6] where diluted TF activation is used and clotting time exceeds normally 4-5 minutes). Moreover, Siemens Healthcare has recently published a report where the applicability of the standard laboratory assays for the monitoring of the effect of novel drugs is analyzed [7]. The results set forth in the report show that PT, APTT, and other conventional coagulation assays are less sensitive or not sensitive to a number of novel effective anticoagulants such as thrombin inhibitors (e.g., dabigatran, argatroban) and direct factor FXa inhibitors (e.g., rivaroxaban). These novel drugs offer greater advantages over the traditional anticoagulants such as warfarin or unfractionated heparin which present the aforementioned well-documented drawbacks. Therefore, the extension of the dynamic range of measured clotting time values seems to be crucial for the ongoing evolution of self-monitoring devices. In an ideal case scenario, the device should have almost unlimited dynamic range, such as that afforded through thromboelastograph (TEG). TEG offers assays similar to the conventional assays that investigate the intrinsic and the extrinsic pathway of coagulation [8] and is notwithstanding its deficits, is considered as one of the gold standard in coagulation monitoring in clinical laboratories. TEG is not a self-monitor since it is too expensive, is not compact and has a level of operational complexity. Hence, reaching the TEG's level of performance and functionality but in the form of simple and compact device is an actual challenge for the biomedical diagnostic industry in developing innovative assays monitoring the onset of coagulation.

Other imperfections of the current coagulation self-monitoring devices include their poor compatibility with the modern telecommunication technologies. A simple connection to a smartphone with a specific application (App) on-board may deliver a new level of functionality, communicability and user-friendliness.

A novel optical method for blood coagulation monitoring is presented in this work. The method is based on recording of an optical signal acquired from a whole blood (WB) sample. The physical principle is optical nephelometry [9] where intensity of the light scattered by red blood cells (RBC) during their sedimentation is recorded. The time at which the fibrin clot becomes strong enough to stop the RBC sedimentation process is returned as the clotting time. The method has a wide dynamic range of measured clotting time values and is successfully implemented in the form of a compact device where a smartphone is used as the optical detector and the signal processor.

The paper is organized in four sections. In Section 2, the assay methodology, optical system operation principle and software algorithm are presented. In Section 3, the results are presented and compared to TEG. Finally, in Section 4, the conclusions are evidenced.

II. MATERIALS AND METHODS

A. *Preparation of blood samples*

Normal healthy controls were recruited at Dublin City University and patients with cardiovascular disease or individuals taking anti-platelet medication (e.g. Aspirin) were excluded from the study. Venous blood was collected from the antecubital vein with minimum stasis, and was citrated to provide a final sodium citrate concentration of 10.5mmol/L. Blood was stored at 37^0C for a period no longer than 60 min post-collection. Coagulation was activated by diluted tissue factor reagent (Dade Innovin®) which was obtained from Sysmex. Three types of TF reagent dilution (0.29, 2.9 or 29.0 pM) were used. A 10uL aliquot of TF and 10uL of saline were added to a vial containing 100 uL of whole blood (WB) sample, maintained at 37°C, and incubated for 5 minutes. Pre-warmed (37°C) 10uL of $CaCl_2$ (100 mM) was added to this vial and 20uL of this mixture was introduced into measurement cuvette and immediately transferred to the measurement chamber of the optical system.

B. *Optical System*

The system's optical layout is shown on Figure 1. The measurement chamber consists of a rectangular hole in a black nylon panel body sealed with a transparent plastic window from below. The polycarbonate rectangular cuvette containing the WB sample is loaded vertically to the measurement chamber. The black nylon panel body also contains a rectangular-shape adit with a mirror on its distal end to collect the light scattered by the sample via one of the four cuvette walls. The proximal end of the adit is sealed by a transparent light collection window being in direct optical contact with the lower half of measurement chamber volume. The system also comprises a source of collimated light, a warming module and an optical focusing module. The source of collimated light is a diode laser module with focusing elements (wavelength:650nm, power: 3mW, spot diameter: ~1mm) located directly above the measurement chamber. The focusing module comprises a mirror, apertures and lenses to deliver the light from the adit's mirror to an optical detector pupil. Finally, a Samsung Galaxy S3 smartphone was used as the optical signal processor with its main camera being used as the optical detector.

The operational principle of the system is very similar to nephelometry. Here, a laser light beam passes through the liquid sample introduced into the measurement chamber. If the chamber is empty or the sample is transparent, the light passes through the chamber with no scattering and minimal loss of intensity. However, when the analytical sample is

 ISBN: 978-1-61208-470-1

turbid, this effects light scattering in a wide range of spatial angles. In this analysis, the scattering indicatrix depends on sample thickness, chamber geometry, and the concentration and shape of the light-scattering particles in the sample of interest. There are numerous examples of technologies with diagnostic applications which are based on interactions of red light with blood cells, and one may find typical scattering indicatrices elsewhere (see, e.g., [10]). The part of the scattered light leaving the measurement chamber through the light collection window is delivered to the smartphone camera pupil. The advanced design of the system could also incorporate a panel of microfluidic channels and additional chambers for blood sample preparation, incubation, mixing with specific reagents. However, the current design does not integrate these features.

The customized software (Android App) driving the camera, recording and processing the optical signal was written in Processing© 2 Java-style language with the use of Android® Development Tools and Ketai© library. The App takes the 640x480 pixel image every 0.25 sec and crops it in order to operate with its central region (100x100 pixel) where the light spot is located. The red colour channel is used only. When 3 images are taken and cropped, the camera signal value, *CS*, is calculated as follows:

$$CS = \{\Sigma RPI^{i}_{1} + \Sigma RPI^{i}_{2} + \Sigma RPI^{i}_{3}\}/7650000 \qquad (1)$$

where $RPI^{i}_{1,2,3}$ is the red-channel pixel intensity in a format of 8-bit number (0-255 scale) for the first, second and third images taken, respectively. Summation is set for the entire 100x100 pixel array, i.e., for a total of 10,000 pixels. The result of summation is then divided by 7650000=255x3x100x100, i.e., by a product of maximal single pixel intensity value, the number of images taken and the size of the pixel array.

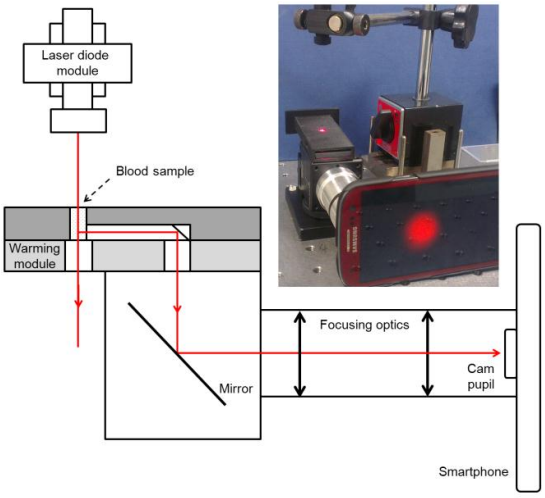

Figure 1. Optical schematics of the blood coagulation monitoring system and its actual implementation based on Samsung Galaxy S3 smartphone.

Thus, *CS* is proposed to be a normalised value characterising a luminosity level in the range from 0 to 1, i.e., from absolute darkness to a camera saturation level respectively. The App generates *CS* values every 1.0 sec and stores it in a phone memory. Further, the signal time-course, *CS = CS(t)*, alongside with the key diagnostic indices characterising coagulation can be directly seen on the smartphone screen or transferred to a PC via a USB port connection. The App also comprises an interface for the camera adjustment procedure, which is recommended to be completed before every signal recording session in order to provide reproducible results and maximise camera sensitivity. The user-friendly adjustment routine involves firstly switching the laser module off, and subsequently adjusting the camera to its "auto-settings" mode with the focus being set to "infinity". Auto-adaptation of the camera to absolute darkness makes its sensitivity intensely high, and close to the limit afforded by Android hardware drivers. Next, the "auto-settings" mode is locked following which the laser module can be switched on. At this stage, the camera is ready for the sensitive measurement and reproducible signal recording.

III. RESULTS AND DISCUSSION

Typical time-courses of the camera signal recorded by the system for normal WB samples are presented in Figure 2. For these initial measurements, coagulation was not activated and the observed monotonous increase in camera signal can be explained by the continuous sedimentation of RBCs onto the sensor surface, with haematocrit concentrations directly influencing red light scattering efficiency. During the initial stages of signal recording, WB samples can be identified as a suspension of homogenously-distributed cells in plasma. As RBCs sediment, there is a proportional increase in the concentration of cells at the bottom of the chamber, and an inverse decrease in cell concentration at the top of the chamber.

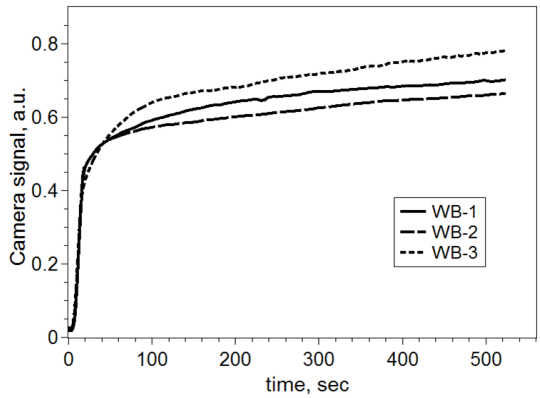

Figure 2. Camera signal recorded for not coagulating blood samples collected from 3 normal healthy individuals.

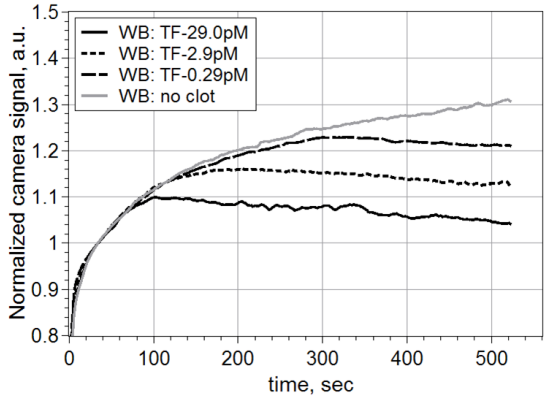

Figure 3. Camera signal recorded for coagulating blood sample collected from 1 normal healthy individual and activated by different TF concentrations.

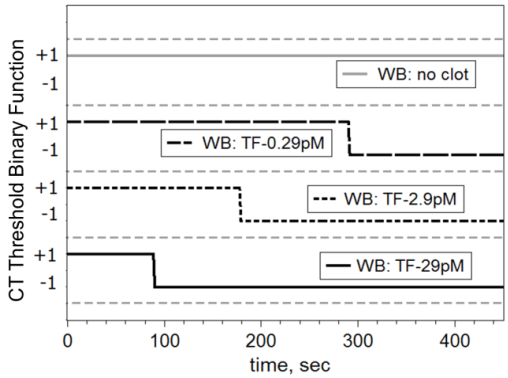

Figure 4. Clotting time determination by the threshold binary function. The results correspond to the camera signal time courses presented on Figure 3.

Concomitantly, the camera signal also increases since the light-receiving window is located at the lower half of the measurement chamber volume, and is in proximity to the sedimenting RBCs. The suspension of cells tends to reach its final state where the lower part of the volume is occupied by RBC mass, with the almost transparent plasma layer settling on top of this. It is important to note that the camera signal doesn't stop increasing during at least 1000 sec of the test when non-coagulating blood samples are selected for analysis. RBC count and sedimentation rate are the physiological parameters which can directly affect the scattered light intensity. The variable normal range is one contributing factor to the differences observed in the signal. A second contributing the factor the observed differences in the signal is due to the fact that the liquid sample is housed in the open-top cuvette and, hence, its upper layer forms a meniscus playing the role of a lens with optical characteristics which are challenging to predict. In order to perform an adequate comparative analysis, it seems reasonable to normalise time-course by the signal value corresponding to some point in the beginning of the reading.

Figure 3 represents the camera signal time-courses in the normalised form recorded for a WB sample collected from the same healthy individual and activated by 4 different concentrations of TF, namely 29pM, 2.9pM, 0,29pM and 0pM (non-coagulating). Every time-course is normalized by its value corresponding to 40sec after start of the test. One can see that the profile of the RBC sedimentation curve has deviated (at approximately 80s) from its normal course during coagulation. This point of deviation can be interpreted as the state of a sample where the fibrin clot becomes strong enough to prevent the RBC sedimentation process, and the corresponding time can be measured as the clotting time. Moreover, if TF concentration is higher the deviation is observed earlier, strongly suggesting that it is indeed indicative of the start of the coagulation process. Moreover, the time-course tends to decrease after the break-point since the growing fibrin network influences the scattering characteristics of the sample.

Indeed, single fibrin fibres can be presented as thin and long cylinders with a diameter from 50 to 200nm [11], i.e., comparable with the quarter of the light wavelength. Hence, the fibrin network is a quite effective scattering object preventing straight light propagation through the sample and reducing its intensity at the lower half of the measurement chamber where the light collection window is located.

In order to measure actual clotting time based on the above measurement outputs, a simple data processing algorithm was developed. Here,*TBF(t)* is a threshold binary function taking two possible values of -1 or +1 indicating two possible states of a blood sample, namely "coagulating" or "non-coagulating" respectively. The *CS* value is being updated every 1.0 sec during the test. Let's consider the time series, $CS^{50}(t)$, containing 50 *CS* values following the current *CS=CS(t)* value. Let *A(t)* and $SE_A(t)$ be the first coefficient (slope) of linear regression for $CS^{50}(t)$ and its standard error respectively.

$$TBF(t) = sign(A(t) — 2SE_A(t)). \quad (2)$$

One can see that the *TBF(t)* function indicates "non-coagulating state" (*TFB(t)=+1*) only where the slope of the camera signal time-course takes the statistically significant positive value. If the slope cannot be determined by linear regression or its value is negative the *TBF(t)* function indicates "coagulating state" (*TFB(t)=-1*). Thus, the time where threshold binary function changes its value can be returned as the clotting time (see Figure 4). One obvious weakness of the algorithm is that it requires > 50 seconds to make a decision since the clotting time is detected retrospectively. On the contrary, a strong point of the algorithm is the low risk of false clot detection insofar as *TFB(t)* function value is always equal to +1 for a non-coagulating blood sample. Other strong point is that *TFB(t)* function changes its sign only once and there are no multiple shifts for all three coagulating samples. Therefore, the algorithm provides unambiguous clotting time determination.

 ISBN: 978-1-61208-470-1

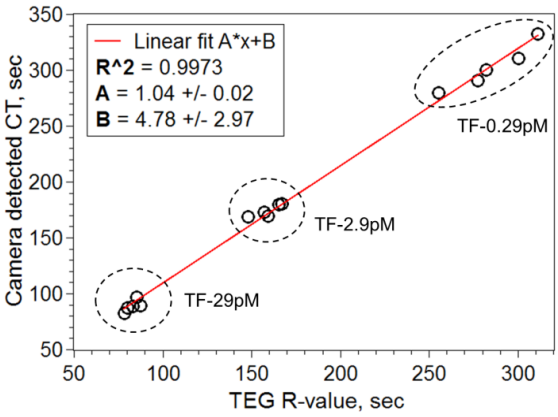

Figure 5. Comparison of clotting time values measured by the presented optical system and TEG for 5 healthy individuals and 3 TF levels.

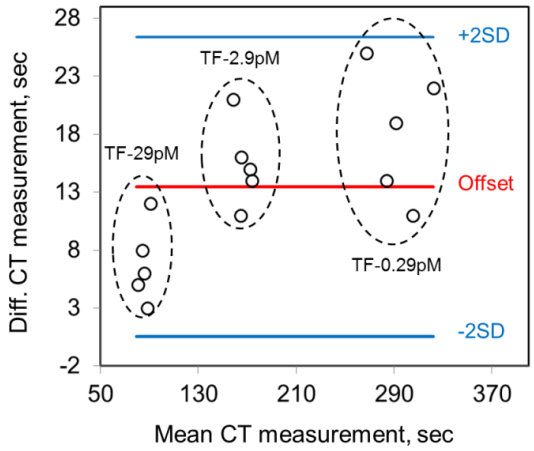

Figure 6. Bland-Altman plot for the presented optical system and TEG for 5 healthy individuals and 3 different TF levels.

As previously mentioned, clotting time depends not only on the blood sample coagulability but also on concentration and type of activation reagent. Coagulation self-monitoring device is supposed to use some standardized assay. If activation assay is based on TF, its concentration should be fixed in order to capture and recognise actual coagulability status of a sample.

In the present study, the real patients with abnormal coagulability and/or taking anticoagulation drugs were not available for examination. Thus, the inverse methodology was applied where 3 different TF concentrations were used for coagulation activation for conventionally normal blood coagulability status. In order to approve the method relevance and estimate the dynamic range of detectable clotting time values 5 normal healthy individuals were examined. The results then were compared to TEG. Scatter-plots for the clotting times evaluated by the presented system versus TEG R-values (clotting time equivalent in terms of TEG) are presented in Figure 5. Total number of data points is equal to 3x5=15, and we demonstrate a strong correlation between the two methods.

The fitting line intercept parameter equal to approximately 5 sec indicates the existence of an offset in the clotting time values measured by the two methods. Figure 6 represents the offset value along the measured clotting times. One may note that the system accuracy tends to be lower for the slower blood coagulation process. The delay in clot detection can be explained in terms of the optical system measurement principles. Here, a clot is detected only when the fibrin network is strong (or mature) enough to stop the RBC sedimentation, i.e., at some time after the actual physical appearance of the first fibrin fibres in the sample volume.

Nonetheless, the agreement between the system presented herein and the gold standard TEG is appropriate, and the offset can be corrected by more advanced data processing algorithms in the future system.

IV. CONCLUSION

Here, a novel optical coagulation monitoring method was developed and implemented in a form of compact system where a commercially-available smartphone is used as the optical detector and the signal processor. The customized Android App was developed to calibrate and drive the smartphone camera, record the optical signal during the test and process the data in order to return clotting time value being the main output parameter of the monitoring. The data processing algorithm provides low risk of false clot detection and precise clotting time measurement.

The method was approved by the series of clotting time measurements with tissue factor activation assay for 5 normal healthy individuals and 3 tissue factor concentrations. The results correlate well with the gold standard thromboelastography method, and demonstrate the ability of the method to measure a wide range of clotting time values with high levels of precision. The use of a smartphone as a part of future self-monitoring system can provide compatibility with modern telecommunication technologies, high level of functionality, cost-efficiency and user-friendliness.

ACKNOWLEDGMENT

The work was supported by the Science Foundation Ireland under Grant No. 14/TIDA/2443.

REFERENCES

[1] T. Christensen, “Self-management of oral anticoagulation therapy - Methodological and clinical aspects,” Dannish Med. Bull., 58(5), B4284, 2011.

[2] L. Harris, V. Castro-Lopez, and A. Killard, “Coagulation monitoring devices: Past, present, and future at the point of care,” Trends Anal. Chem., 50, 2013, pp. 85–95.

[3] M. Bissell and F. Sanfilippo, “Empowering patients with point-of-care testing,” Trends Biotechnol., 20, 2002, pp.269–270.

 ISBN: 978-1-61208-470-1

[4] A. van den Besselaar and M. Hoekstra, "Long-term stability and reproducibility of CoaguChek test strips," Thromb. Haemost., 93(6), 2005, pp. 1189-1192.

[5] A. van den Besselaar, F. van der Meer, C. Abdoel, and E. Witteveen, "Analytical accuracy and precision of two novel Point-of-Care systems for INR determination," Thrombosis Research, 135(3), 2015, pp. 526-531.

[6] S. Butenas, C. van't Veer, and K. Mann, ""Normal" Thrombin Generation," Blood, 94(7), 1999, pp. 2169-2178.

[7] D. Funk, " Coagulation assays and anticoagulant monitoring," Hematol. Am. Soc. Hematol. Educ. Program. 2012, pp. 460–465.

[8] M. Thakur and A. Ahmed, "A Review of Thromboelastography," Int. J. Perioperative Ultrasound and Appl. Techn., 1(1), 2012, pp. 25-29.

[9] H. Oishi, Y. Hatayama, H. Shiraishi, Y. Miyashita, and Y. Sakata, "Development of a multifunctional laser nephelometer using backward light scattering detection for clinical diagnosis of blood coagulation and fibrinolysis," Industrial Metrology, 1(1), 1990, pp. 55-58.

[10] M. Yurkin et al., "Experimental and theoretical study of light scattering by individual mature red blood cells by use of scanning flow cytometry and a discrete dipole approximation," Appl. Opt., 44(25), 2005, pp. 5249-5256.

[11] E. Ryan, L. Mockros, J. Weisel, and L. Lorand, "Structural Origins of Fibrin Clot Rheology," Biophys. J., 77, 1999, pp. 2813-2826.

 ISBN: 978-1-61208-470-1