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Light Scattering Analysis on Coagulation Detection with Magnetic Particles

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Clotting properties of human blood are important clinical information to monitor for patients with platelet and coagulation disorders. Most devices used to diagnose these disorders utilize blood plasma together with tissue factors and Ca^{++} additives. In some instruments, magnetic particles were mixed with blood samples and a rotating magnetic field was applied, resulting in the rotation of magnetic particles, which was probed by impinging light. The working principle seems obvious yet had not been investigated in depth. We modeled the collective behavior of light propagating through magnetic needles, aligned in the direction of the rotating external magnetic field, with scattering light analysis software. Simulation results indicated that the scattering pattern undergoes periodic undulations with respect to the slant angle of the magnetic needles. Also provided is a means of extracting meaningful information from the scattering measurement.

Keywords : Coagulation, Light scattering, Numerical simulation
OCIS codes : (290.2648) Stray light; (170.1470) Blood or tissue constituent monitoring; (170.4090) Modulation techniques

I. INTRODUCTION

To sustain a healthy cardiovascular system, the human body should guarantee smooth flow of blood through the entire cardio-vascular system. Normal circulation may be hampered by heart failure, blood leakage and vessel obstruction. Coagulation is a process to prevent further blood loss by forming a clot around the damaged tissue or vessel. When the vessel lining is disrupted, the extravascular compartment and the blood together produce a series of local coagulation responses to prevent further blood loss. Platelets which normally are in their inactive form turn active and adhere to the collagen layer next to the intrusion site. This together with the von Willebrand factor secreted from the interaction of blood plasma and the endothelial lining play the first critical role to form the initial platelet clot, reducing blood loss. After the formation of the platelet adhesion, two series of reactions proceed to form a fibrin mesh around the adhesion site: the extrinsic and the intrinsic pathways [1].

Since the formation of the fibrin mesh is a critical event that will eventually seal the wound, medical practitioners need to inspect the workings of these processes. Tests routinely performed for these purposes are Prothrombin Time (PT) and Partial Prothrombin Time (PPT). Each of these is designed to examine different coagulation mechanisms, i.e, the extrinsic and the intrinsic pathways. To run PT tests, blood serum is first obtained by the high-speed centrifugation and sodium citrate is added to prevent spontaneous coagulation. The sample is then treated with phosphor lipid and a special protein (Tissue Factor, TF) to get the clotting parameters ready for the start of the coagulation cascade. The addition of Ca^{++} initiates the coagulation process and the time it takes for the blood-clot to form is measured (PT). Measurement of PPT requires ground glass in place of TF [2].

Telling the time of the clot formation with the naked eye is prone to errors: one should discern the formation of the fibrin fiber in the serum sample and the fibrin fiber is rather difficult to observe in the early stage of clot formation. In practice, instruments are used to time this. Most instruments utilize the change of the physical properties

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of the sample upon clot formation: viscoelasticity [3], light scattering/transmission, dielectric constant or ultrasound velocities to name a few. Moreover, they require dedicated lab instruments in addition to the laboratory quality centrifuge which makes blood plasma. Since the monitoring of the coagulation time can be more effective if tested at a closer interval to adjust the intake of the medication, techniques were developed to measure the coagulation time with whole blood, eliminating the need of the centrifuge, thus enabling tests for home users.

A representative scheme [4] of such a device let the user apply a drop of finger blood to the reaction cartridge containing pre-dispensed biochemical reagents, where the blood is mixed with magnetic particles. This mixture is exposed to a time-varying magnetic field. As the coagulation proceeds, the viscosity of the sample increases and the (rotatory) motion of the magnetic particles is hindered. This motion is continuously monitored indirectly by the laser light probing the reaction chamber. The periodic variation of the external magnetic field produces reflected light signal that is closely correlated with the field direction. As the coagulation proceeds, the variation of the signal gradually decreases, and one can utilize this characteristic to tell the time of coagulation.

Despite its success in a commercial sector [5], the analysis of the process leading to the production of the signal is not readily available in the public domain. The original article [6] described the collective behavior of the transmitted light yet did not disclose the mechanism that provides explanation for the phenomenon. In this article, we provide a model that explains the signal generation with the help of scattered light analysis software.

II. THE LIGHT SCATTERING MODEL

The standard procedure for coagulation testing demands that blood plasma be used in observing the formation of the fibrin. If one should desire to test the coagulation time away from the lab setting such as at patients' bedsides or at home, preparing a blood plasma sample would be impractical. The cited article [4] suggested fine-grade magnetic particles be imbedded in the mixture of blood with appropriate combination of bio-chemical elements. The test sample prepared as such is now exposed to a time-varying external magnetic field. The magnetic field forces magnetic particles to line up in a needle like formation. These "needles" align themselves parallel to the applied local magnetic field (Fig. 1) and undergo directional changes as the external magnetic field rotates in time. Light transmission through or reflection from the sample should reflect the collective directional information of these needle particles.

At the beginning of the test, these magnetic needles are relatively free to rotate with a certain magnitude of drag, originating mostly from uncoagulated blood itself, under the time-varying external magnetic field. As the coagulation proceeds, fibrin fibers are formed and they cross-mesh red blood cells together with magnetic needles, increasing overall rotation-resistance of the sample mixture. The rotation of magnetic needles is hindered as the drag from the sample mixture grows till it eventually stops moving. Light transmission through or reflection from this sample mixture gets modulated with the change of magnetic needles and the modulation amplitude decreases as the coagulation cascade evolves. This steady decrease of the modulation would render information on the progress of the coagulation. The experimental investigation for this is presented in Lee *et al*. [7].

To simulate the light scattering by these magnetic particles rotating in the blood sample under the influence of the rotating external magnetic field, we set up a model of these particles to work with LightTools™. Magnetic needles in practice are formed randomly distributed in the test cell. They were modeled as rectangular rods, 0.05 mm × 0.05 mm × 1.00 mm in size, distributed in a checkerboard pattern with the grid size of 0.05 mm. This checkerboard pattern was adopted because such a distribution was a feature implemented internally: the software does not have the function of distributing objects in a random manner. The test cell size was 6 mm × 5 mm × 1 mm, with 1 mm

FIG. 1. Magnetic needles made of clustered paramagnetic particles aligned along the local line of applied magnetic field. Paramagnetic particles were ~1 mm long.

being the depth of the sample holder. These needles were assigned material properties of iron. Top and side walls were modeled as Lambertian reflectors, with the reflectance of 0.5. These properties were derived from the observation of photographic images revealing the rough surface structure, originating from accumulation of finer iron particles on side walls and ends of the magnetic "needles". Also, we assumed these particles were immersed in water 1 mm in depth so that they would rotate suspended in water. Of course, one would expect further scattering by blood-borne particulates in real applications, further making the scattering pattern smoother that observed with water medium. The refractive index of the needle was given that of the bulk material of 2.360 as available in open literature sources [7].

Throughout simulation runs, the direction of magnetic needles was set at one angle and the scattered light intensity at the detector location was computed. This process was repeated for differing angles of magnetic needles and the results were tallied with respect to angles. Results obtained from these simulation runs were compared with the experimentally observed ones as described by Lee *et al*. [8]

Figure 2 illustrates the behavior of scattered light inside and around samples aligned at two different angles. Of 500,000 rays used for simulation runs on each angle, only a few representative ones are shown to render the general behavior of scattered rays. One can discern that, upon close examination of Fig. 2, a fraction of light proceeds into the space among needles and wanders off. One can also notice the trend of scattered light veering off in the direction of "specular reflection" of light off of those magnetic particles. For example, with particles tilted at 60 degrees and with light impinging vertically downward, one finds more light rays proceeding in the direction that the light would have reflected into if magnetic needles were facets of reflecting mirrors. The similar trend is observable with magnetic needles tilted at 150 degrees, where more scattered light is observed in the general direction dissecting the first quadrant diagonally than into any other directions.

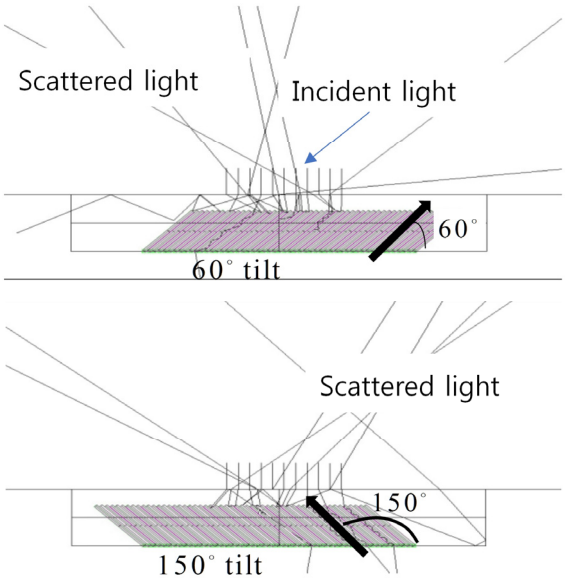

FIG. 2. Light scattering patterns by needles at 60 (top) and 150 degrees (bottom) each with normally incident light.

Another feature worth noting is the apparent ratio of scattered photons to the incoming ones as can be observed in Fig. 2. It turned out that this ratio would not render meaningful information: the software only generates a small number of rays for Fig. 2 for graphical clarity. Of the 500,000 rays used for the main analysis, only a few of them are displayed and hence counting the number of rays in Fig. 2 may introduce an aberrant conclusion different from that with all of 500,000 rays counted together.

III. SIMULATIONS AND COMPARISON WITH MEASUREMENTS

At the beginning of simulations runs, magnetic needles were assumed lying flat at the bottom. 500,000 rays were introduced vertically from top onto the needles. The receiver, located at 56.8 degrees from the axis normal to the sample container, collected and counted the total number of rays directed into that direction (Fig. 3). This number of rays should be correlated to the optical signal measured at that angle of the needles. With the counting operation done, needles were remodeled to be slanted at 5 degrees counterclockwise and the whole operation was repeated. This procedure was applied for needle angles in the range of 0~360 degrees. It was expected that the scattering pattern would repeat every 180 degrees: needles at 0 degree should be identical to those at 180 degrees. Figure 4 shows the summary of the simulation. The vertical axis represents the scattering intensity as measured by the number of photons collected at the modeled detector plane. One can easily discern the periodic nature of the scattering pattern repeating every 180 degrees as described above.

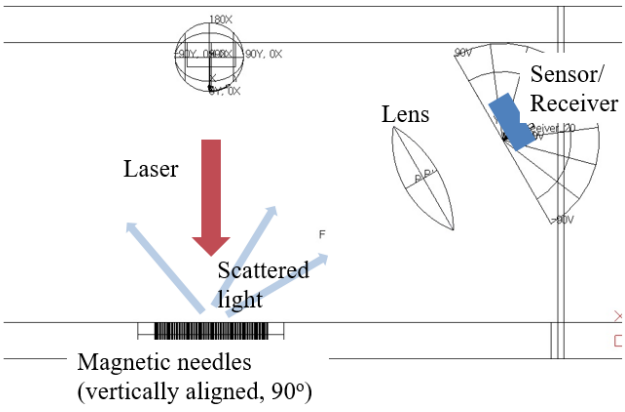

FIG. 3. Simulation arrangement. Normal incident light is scattered by magnetic needles. The sensor/receiver collects and counts the photons scattered into that direction.

The scattering with respect to needle slant angle demands further understanding. The wall of the magnetic needle is assumed to be a Lambertian reflector. This should imply that the reflected energy, not the brightness, is highest in the surface normal direction and decreases in a fashion described by the cosine law (Fig. 5). With such an arrangement, one would expect the scattering level to reach the highest level at the slant angle of ~123 (90.0 + 33.2) degrees. However Fig. 4 shows that not to be the case. Upon close examination of the scattering process as shown in Fig. 6, one can tell that a considerable portion of photons get trapped in the space among needles. Compared to the scattering pattern with needles lying flat (0 degrees, Fig. 6(a)), the number of photons scattered into the direction of the detector is considerably less at 120 degrees. As a result, the scattering level at 0 or 180 degrees is highest since no photon gets lost into the inter-needle space, which is well illustrated in Fig. 4. As such, one could validate the scattering model as a justifiable representation of the real sample.

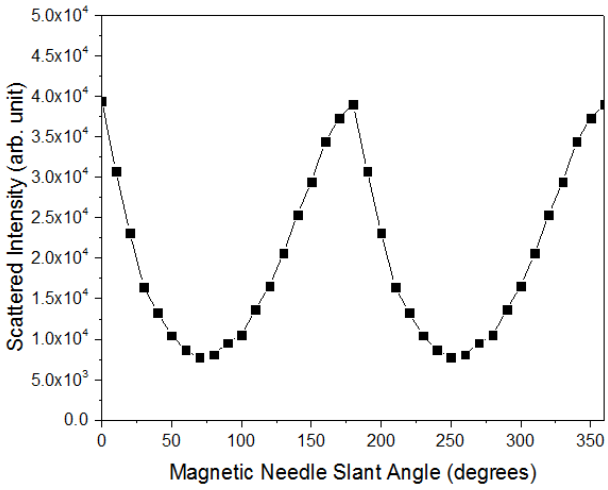

FIG. 4. Light scattering by magnetic needles. The pattern repeats itself every 180 degrees.

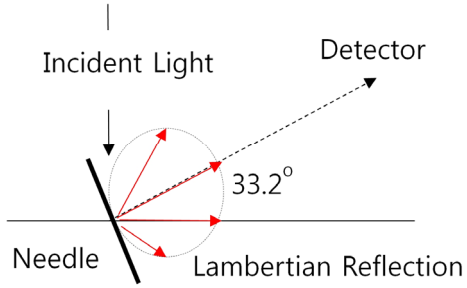

FIG. 5. Consideration for the Lambertian nature of the reflection off magnetic needles.

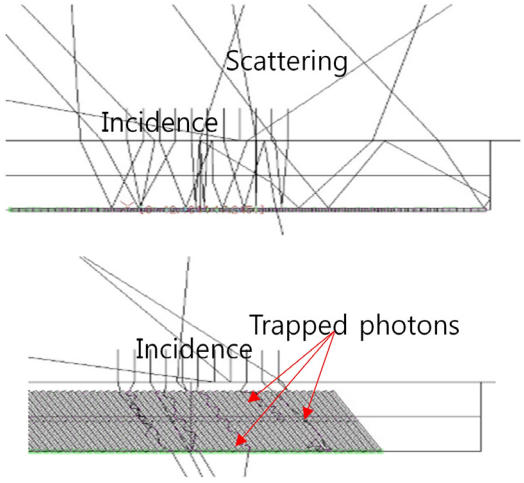

FIG. 6. Process of photon trapping. (a) Top: Normal incidence (b) Bottom: Oblique incidence.

IV. COMPARISON WITH EXPERIMENTS AND DISCUSSION

Figure 7 shows the result of actual measurement (shown with solid circles), taken from the set-up described in Ref 8. Magnetic particles, 1 mm long, (Fe_3O_4 powder clusters) were immersed in fresh, whole blood from a volunteer. Particles were prepared with the concentration of 1.0×10^3 kg/m^3 and the sample holder was subjected to a rotating magnetic field while a 633 nm laser from a laser pointer was directed to the holder at normal angle from top. An external magnet was mounted on a motor to provide a time-varying magnetic field. One can observe the distinct periodic nature in Fig. 7, as predicted in Fig. 4. The scattering undergoes a periodic variation: in Fig. 4, the decreasing of the modulation amplitude was not considered because the software could not accommodate the coagulation mechanism. One could only prove that the light scattering pattern would oscillate as magnetic needles rotate with the external magnetic field. Meanwhile, the result of experimental observation in Fig. 7 involving human blood clearly displays the characteristic decaying oscillatory pattern as the coagulation proceeds upon addition of thromboplastin. Also noted is the migration of the base line (bias), the behavior of which is to be interpreted in further studies.

Figure 4 represents the expected scattering pattern (simulated) for magnetic needles in distilled water, hence does not reflect the case when the medium surrounding them was human blood undergoing a coagulation process. With blood coagulation in progresses, fibrin fibers form around red blood cells and magnetic needles, the number of needles undergoing rotation with the rotating magnetic field should decrease continuously, with time dependence yet to be identified. As time goes on and with the rotation of the external magnetic field, the scattering signal displays a periodic pattern. The amplitude decrease could be approximated by an exponentially decreasing function, whose characteristics could be influenced by practical parameters such as particle concentration, dimensions of

the needles and blood composition. This behavior is well demonstrated in Fig. 7. On top of the decreasing peak signals, the bias or the base signal also undergoes a slow drift, upward or downward, until the AC component of the periodic variation eventually disappears.

One can imagine from Fig. 7 that fewer and fewer magnetic particles are actively moving along the magnetic field. Yet it requires another data processing procedure to derive a piece of information that is clinically meaningful. Since we are interested in the speed (or time) of coagulation, the decrease of the oscillation amplitude in Fig. 7 must correlate with the coagulation process. We derived this value by computing the modulation value defined as $Modulation = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$.

Max and Min values were extracted from each period of measurements and one representative modulation value was assigned for that period. Figure 8 shows the variation of these modulation values. The result in Fig. 8 clearly indicates that this modulation does decrease in time, in a fashion that one might model as an exponentially decaying function: one could fit a curve to find a function. The coagulation time for the sample blood was tested in the clinical laboratory to produce a PT of 12.5 sec, which in this case corresponds to about 1/2 of the initial modulation. One may claim, if supported with further works with enough repetition, that the PT can be established at the time when the modulation value reaches 1/2 of the initial modulation.

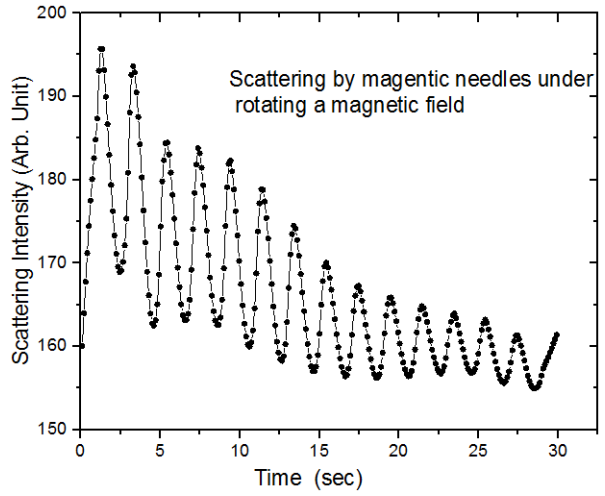

FIG. 7. Light scattering by magnetic particles in human blood undergoing coagulation.

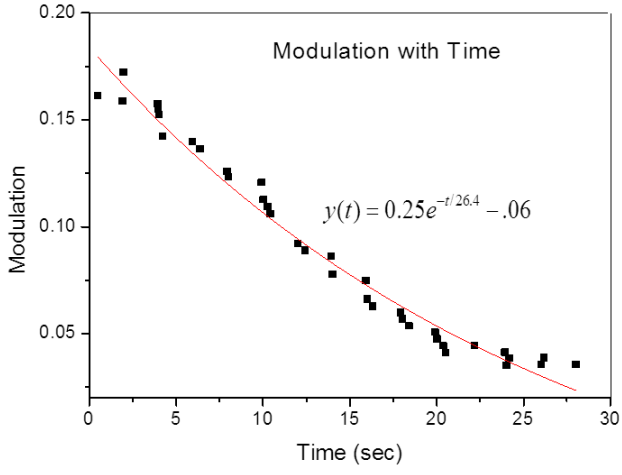

FIG. 8. Modulation values for results in Fig. 7.

V. CONCLUSION

Analyzing light scattering behavior from a collection of aligned magnetic needle-line structures made of clustered ferromagnetic particles would be an impossible task if observed from the strict boundary value aspect: boundaries cannot be readily defined. Only the statistical approach involving so many Lambertian reflections form the collection of participating magnetic needles could provide some insight into the collective behavior of such a system. This work proved such collective scattering characteristics can be simulated by modeling the embedded ferromagnetic particles in the liquid to form magnetic needles with Lambertian outer surfaces. These magnetic needles change their aligned directions as dictated by the external magnetic field. Light scattering from the collection of single magnetic needles was tallied at discrete scattering angles for comparison with experimental measurements. The simulation with the software Lightools™ resulted in a periodic behavior with respect to slant angles. The period was 180 degrees as could be expected since the magnetic needle at 0 degree is undistinguishable from the one at 180 degrees and the angular dependence of the scattering repeats again for angles from 180 to 360 degrees and so on.

The periodic nature of the simulation was confirmed by an experimental observation made with magnetic particles in human blood. Not predicted by the simulation by the software was the decreasing oscillation amplitude: the SW did not support any means of modeling the increasing viscosity of the medium in which magnetic particles are immersed. Yet the rate of oscillation amplitude could be studied by obtaining the modulation values for each cycle of the oscillatory behavior in the light scattering pattern. For the test sample blood used in the measurement, one could claim that the Prothrombin Time could be the time it takes for the modulation to reach 50% of its initial value.

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