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Coherent light scattering on RBCs - experimental results and possible biomedical application

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If coherent light is incident upon a whole blood sample, the backscattered light can be recorded, resulting a speckle image. A program was written to extract the time series from each pixel of the CDD conversion matrix. The autocorrelation time of the series was calculated and the autocorrelation time was measured for blood samples of different human subjects. The autocorrelation time was analyzed and compared with the erythrocyte sedimentation rate (ESR) measured during a standard laboratory test using the modified Westergren method. A fast procedure for assessing the ESR is suggested.

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1. Introduction

When coherent light is incident upon a medium having scattering centers, a speckled image, having a statistical distribution of the intensity over the interference field is obtained. It is the result of the interference of the secondary waves emitted by the by the scattering centers (SC hereafter), each wave having a different phase and amplitude in the interference field. The image changes in time as a consequence of the SCs complex movement of sedimentation and Brownian motion. This produces fluctuations of the image intensity in each location of the interference field. These fluctuations give the aspect of "boiling speckles" [1], [2].

The speckled image can be observed either in free space and is named objective speckle or on the image plane of a diffuse object illuminated by a coherent source; it is named subjective speckle in [1]. The two types of speckles are also named far field speckle and image speckle in [2]. Statistical speckle parameters (size, contrast, intensity and polarization) carry information on the scattering media.

In addition to the static image analysis, dynamical speckle analysis can be done as well. It became a currently used method to characterize the dynamic behavior of scattering medium such as flow, sediment and Brownian motion. The motion of the speckle field was analyzed by correlometric methods [3,4,5] or by the LASCA technique (laser speckle contrast analysis) and the results are reported in articles like [6,7]. In this work the time series corresponding to the fluctuations of the speckle image were analyzed using the autocorrelation function. Details on extracting the time series and on calculating the autocorrelation time are presented in the next section.

The samples that were used in this work were whole blood samples, in the vacutainer tube that was used for extracting them. The work described in this paper was carried on in order to test the hypothesis that the red blood cells (RBC) sedimentation velocity, (Erythrocyte Sedimentation Rate, ESR in medical terms) can be assessed using a speckle analysis technique related with the techniques used to measure the blood flow rate in arteries [2], [6], [7].

2. The Erythrocyte Sedimentation Rate, ESR

ESR is one of the traditional tests performed on whole blood in hematology laboratories. ESR measures the distance red blood cells sediment, or fall, in a vertical tube over a given period of time. The measurement of sedimentation is calculated as millimeters of sedimentation per hour and takes greater than one hour to complete a precise measurement. The principle behind ESR is that various "acute phase" inflammatory proteins can affect the behavior of red blood cells in a fluid medium (e.g., decrease the negative charge of RBCs). Inflammatory proteins, such as fibrinogen, will typically appear in the blood, or increase in concentration, during inflammatory processes, such as arthritis. The result is decreased negative charge (zeta-potential) of the erythrocytes that tends to keep them apart, and a more rapid fall of the cells in the analysis tube. The greater the fall of red blood cells in the vertical tube measured at a given period of time, the higher the ESR. A high (i.e., elevated) ESR is indicative of the presence of inflammatory proteins, (i.e., an active inflammatory processes, such as rheumatoid arthritis, chronic infections, collagen disease and neoplastic disease) [8], [9].

The process of collecting the blood specimen and the particular anticoagulant used are crucial in determining an accurate ESR. For example, in one well-known technique known as the Westergren method, blood is collected in the presence of the anticoagulant, sodium citrate, whereas in the modified Westergren procedure, EDTA is used as the anticoagulant. The modified Westergren procedure has become the standard for measuring ESR because it allows the ESR to be performed from the same tube of blood as is used for hematologic studies.

Essentially, ESR is a test that has been practiced for decades without much change in the procedure. The

modified Westergren procedure was used as reference procedure in the work described in this paper and the method we propose for assessing the ESR in a faster way is, therefore, compared with the above mentioned procedure.

3. Materials and method

The experiment consisted of recording the backscattered light using a CMOS camera with a fast framerate. The backscattered configuration was chosen because the whole blood is an optically very thick sample, hence nontransparent.

The schematic of the experiment is presented in Figure 1. The He-Ne laser has a wavelength of 632 nm and a constant power of 2 mW. The Laser – cuvette distance was 0.18 m and the CCD-cuvette distance was 0.15 m. The θ angle was $25^0 30'$.

Using a CCD camera is mandatory for this type of experiment because a CMOS conversion matrix is not sensitive enough to record the very low intensity backscattered light.

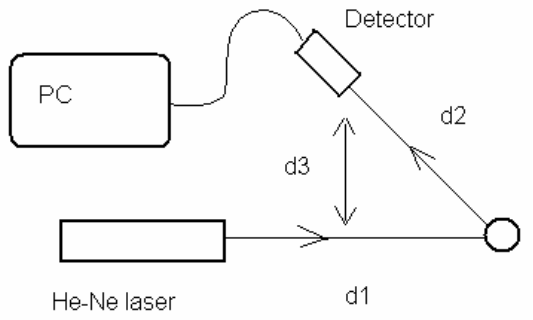

Fig. 1. The schematic of the experiment, view from above.

Previous work of measuring the speckle size in coherent light scattering experiments revealed that the speckle size is big enough to use a small resolution recording [10]. The resolution used in this work was 160x120, the framerate was 60 frames/s. The format used for recording was an uncompressed avi, in order to avoid quality loose as a consequence of using image compression algorithms. The color depth was 24 bits per pixel, in order to have a big variation of the recorded values in the time series.

A computer program was written and used to extract the time series for a given pixel of the CCD conversion matrix. The program first reads the entire movie frame by frame, extracts the recorded intensity of the pixels inside a circle centerd on the (x,y) pixel having the radius r, (the file name, x,y and r are input parameters), calculates the average of the intensity inside the circle on that current frame and saves the average intensity value as an element in the one dimension array. After repeating the procedure for each frame of the movie or of the part of the movie lasting the time desired for the analysis, the time series is saved on the PC hard disk as a text file having the name of the avi file plus the pixel location and radius and a different file extension. More details on the program that was written for this purpose are presented in [10].

The radius is necessary to be adjusted in order to make sure that the averaged area has the diameter of the average speckle size for that configuration. As the cuvette – CCD distance is very small, a radius of one pixel was chosen to extract the time series I(t) for the experiment conducted in this manner.

Recordings of 30 seconds avi movies were done for each sample. A time series extracted for one of the samples is presented in Fig. 2.

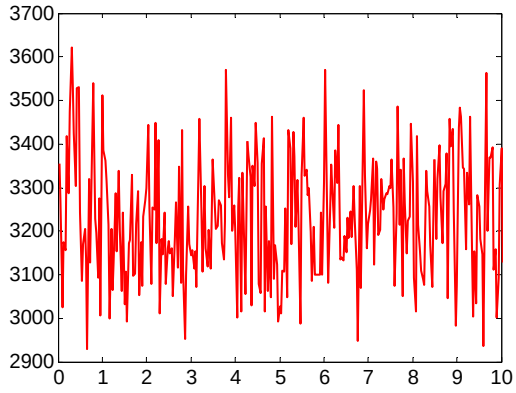

Fig. 2. A sequence of 10 seconds from the time series of one of the samples.

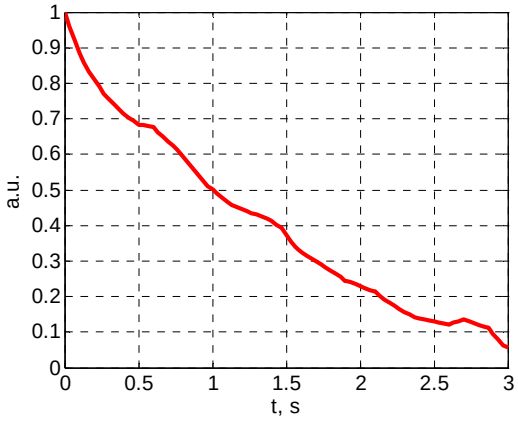

Fig. 3. The autocorrelation function of the time series of one of the samples.

The autocorrelation function for each sample was calculated as:

$$A(\tau) = \frac{\langle I(\vec{r},t) * I(\vec{r},t+\tau) \rangle}{\langle I(\vec{r},t) * I(\vec{r},t) \rangle} \quad (1)$$

where the angle brackets denote averages over time t, r represents the position of the CCD conversion matrix, and τ is the correlation time. The normalized autocorrelation function decreases from 1 and we can define the autocorrelation time τ as the time when the autocorrelation

function decreases to 1/e. The autocorrelation function of the time series of one of the samples is presented in Fig. 3.

A transmission type of experiment appears to be more appropriate to measure the autocorrelation time because the biological SCs scatter light primarily in the forward direction. RBC's light scattering anisotropy is modeled with the currently used Henyey–Greenstein phase function [12], [13]:

$$f(\mu)=\frac{1}{2}\frac{1-g^2}{\left(1-2\mu g+g^2\right)^{\frac{3}{2}}} \tag{2}$$

where μ = cos(θ) and g = <μ> is the anisotropy parameter. Starting from (2) we can derive the θ probability distribution:

$$p(\theta)=\frac{1}{2}\frac{1-g^2}{\left(1-2g\cos(\theta)+g^2\right)^{\frac{3}{2}}}\sin(\theta) \tag{3}$$

A 0 value for g indicates isotropic scattering and a value near 1 indicates strong forward directed scattering. Different values from 0.95 to 0.98 are currently used [12], [13] indicating a strongly forward peaked anisotropy, which explains the low backscattered recorded intensity.

A transmission experiment at small angles provides a bigger signal to noise ratio, but the backscattering setup was chosen because the whole blood sample is optically opaque for transmission. As this work was carried on to investigate the possibility of assessing the ESR by coherent light scattering, one of the requirements was to keep the biological sample, which carries biological contamination risk, sealed in the tube. The original vacutainter tube used for extracting the sample was used for the backscattering experiments, therefore

For drifting SCs, the autocorrelation time has a variation with the velocity of the particle in suspension [1].

$$\tau=\frac{A}{k\cdot v} \tag{4}$$

where k is the wave number and A is a constant depending on the scattering properties of the sample. This experimental configuration we used is in good agreement with the conditions used in [1] for deriving equation (4), that is particles in suspension that are flowing with the fluid having a constant velocity v, on a steady direction. The velocity of the SC in suspension, in this experimental setup, is not the velocity of the RBCs in veins, arteries or capillaries but the velocity of the sedimentation motion, ESR in medical terms, measured in the standard laboratory test, as previously described.

Each sample was first analyzed using a standard blood laboratory test and the ESR was measured using the modified Westergren method, as described in the introductive section. The ESR is measured in mm/h and the first of the two standard values, that is the value measured in 20 minutes, is used in the work reported here. The results are presented in the next section.

4. Results

The experiment described in the previous section was conducted on 13 samples randomly selected. The time series were extracted from the uncompressed movie recorded for each sample after the standard laboratory ESR measurement, as described above and the autocorrelation time was calculated. Table 1 presents the ESR in mm/h measured using the modified Westergren method and the autocorrelation time, calculated as described in the previous section. The data in Table 1 is presented in Fig. 4.

Table 1. The sample number, ESR and the autocorrelation time for the backscattering experiment.

Sample number	ESR, mm/h	Autocorrelation time, s
1	2.00	0.87
2	4.00	0.72
3	15.00	0.57
4	15.00	0.77
5	16.00	0.73
6	28.00	0.68
7	30.00	0.57
8	30.00	0.75
9	37.00	0.60
10	52.00	0.50
11	56.00	0.48
12	70.00	0.50
13	78.00	0.55

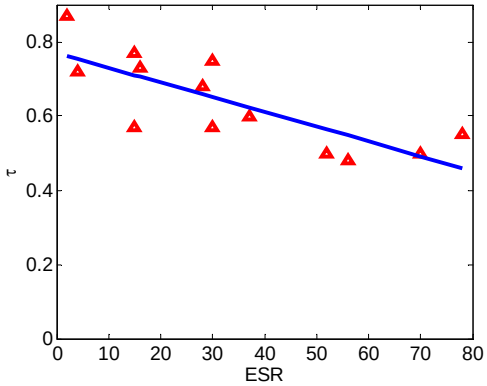

Fig. 4. The Autocorrelation time versus ESR for the time series extracted from uncompressed movies.

A the linear regression equation for the data in Fig. 4 is:

$$ESR = -0.003969\cdot\tau + 0.769877 \tag{5}$$

Examining Fig. 4 we notice a slight decrease of the calculated autocorrelation time with the increase of the measured ESR, as expected from theory. We notice that

there does exist a correlation of the calculated autocorrelation time with the measured ESR. The correlation coefficient of the ESR with the τ is 0.78, which proves that the correlation in not strong.

5. Conclusions and discussions

A simple experiments of recording the speckle fluctuations produced by coherent light incident on a vacutainter tube with whole human blood during erythrocyte sedimentation were performed. The backscattered light is recorded with a CCD, a time series is extracted for each sample and the autocorrelation time is calculated. The existence of a possible correlation of the ESR measured with a standard method (modified Westergren) with the autocorrelation time was investigated and was found to exist, although it is not very strong.

The correlation found in the preliminary backscattered type experiment is scheduled to be verified on a larger number of samples to increase the statistic significance. Weak as it appears, if confirmed on a larger number of cases, it suggests a simple and very fast procedure (less than two minutes) of assessing the ESR, although not as precise as measuring the sedimentation velocity of the upper part of the RBC area, as the standard ESR procedures do. Work is in progress on the subject.

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