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Application of whole blood coherent light scattering dynamics analysis

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When coherent light is incident upon an optically thick biological fluid having scattering centers (SC) in suspension, like whole blood, the backscattered light can be recorded, resulting an image speckle. 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 determined for blood samples from 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 different procedure to record the time series, using a detector and a data acquisition system was used as well and the autocorrelation time was calculated for the time series recorded using this procedure. The results of the work performed so far indicate that the two properties, the ESR and the autocorrelation time, appear to be correlated. A fast procedure for assessing the ESR and a screening method are suggested.

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1. Introduction

The Erythrocyte Sedimentation Rate, 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. 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 Red Blood Cells, RBCs hereafter). 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) [1].

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.

If 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. The speckled image appears as a result of the interference of the secondary wavelets scattered by the scattering centers, each wavelet having a different phase and amplitude in the interference field. The image changes in time as a consequence of the scattering centers (SC hereafter) 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” [2], [3].

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 [2]. The review paper [3] calls the two types of speckles far field speckle and image speckle. Speckle parameters like size, contrast, intensity and polarization carry information on the scattering media. Dynamical speckle analysis has become a current 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 [4 - 6] or by the LASCA technique (Laser Speckle Contrast Analysis) and the results are reported in articles like [7], [8]. In this work the time series corresponding to the fluctuations of the speckle image together with the far field interference in one location were recorded and 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 RBC sedimentation velocity, 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 [3].

2. Materials and method

The experiment was carried on in two different manners. The first consisted of recording the backscattered light using a CMOS camera with a fast framerate. 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 – vacutainer distance was 0.15 m and the CCD-vacutainer distance was 0.15 m; the θ angle was $25^0 30'$. 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.

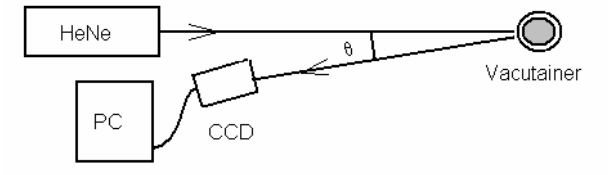

Fig. 1. The schematic of the experiment, view from above.

The backscattered configuration was chosen because the whole blood is an optically very thick sample, hence nontransparent. 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 [9], [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 prevent quality loose produced by compression algorithms. The color depth was 24 bits per pixel, in order to have a bigger 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 centered 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 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 desired time for the analysis, the time series is saved as a text file having the name of the AVI file plus the pixel location and radius and a different file extension. The radius is required to be one of the input parameters in order to make sure that the averaged area has the diameter of the average speckle size for that configuration. As the vacutainer – 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.

30 seconds AVI type movies were recorded for each sample. A time series extracted for one of the samples is presented in Fig. 2.

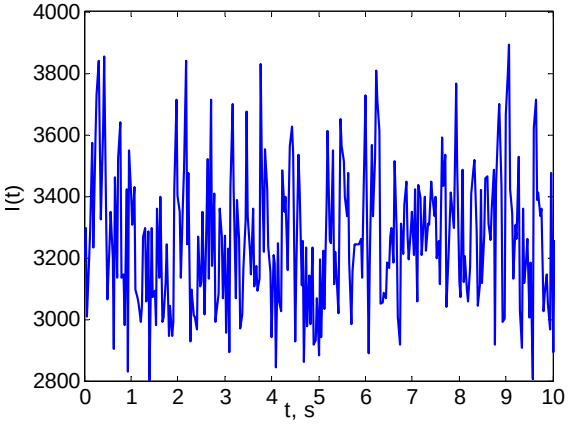

Fig. 2. A 10 seconds sequence from the time series of one of the samples.

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 average was extracted from each series, therefore the normalized autocorrelation function was calculated. The normalized autocorrelation function decreases from 1 and we can define the autocorrelation time (ACT) or τ_c 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.

The second manner to conduct the experiment was slightly different of the first manner. The first difference consists of using a sensitive photodetector and an external data acquisition system with a PC to acquire the time series for each sample. A bigger data acquisition rate was used, 100 per second, for an accurate fluctuation recording. The other main difference lays in conducting a transmission type of experiment rather than a backscattered light experiment. The schematic of the experiment is presented in Fig. 4.

The transmission type of experiment was employed to measure the autocorrelation time because the biological SCs scatter light primarily in the forward direction. RBC's

light scattering anisotropy is currently described with the Henyey–Greenstein phase function [11], [12]:

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

where μ = cos(θ) and g = <μ>.

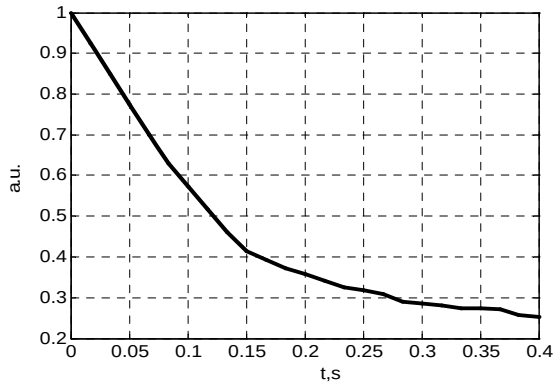

Fig. 3. The normalized autocorrelation function of the time series of one of the samples.

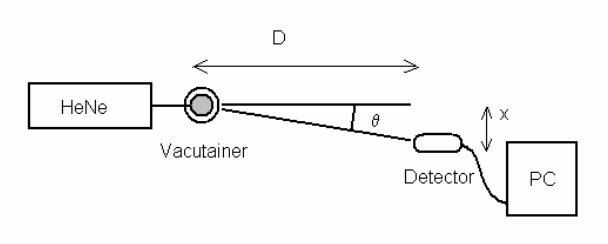

Fig. 4 The schematic of the transmission experiment, view from above.

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 null value for g indicates isotropic scattering and a value near 1 indicates a strong forward directed scattering. Different values from 0.95 to 0.98 are currently used to describe light scattering on RBCs [11], [12] 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, therefore the second experiment was conducted in this manner. In Fig. 4 D is 0.68 m and x is 0.05 m, therefore θ is 4^0 45'.

Special care must be taken though, because the whole blood sample is optically opaque for transmission, therefore this type of experiment requires a preparation technique. First the sample is kept vertical till the RBCs settles on the bottom of the sampling tube (vacutainer). Using a syringe equipped with a long needle a small amount of RBCs was extracted from the bottom of the tube. The syringe was slowly raised and the RBCs were slowly released on the upper part of the sample. In this way a small amount of RBCs were left again to sediment, thus maintaining the transparency of the sample.

The time series were recorded for another batch of samples using the data acquisition system and the PC. A sequence of 10 seconds from a time series recorded in this manner is presented in Fig. 5 and the autocorrelation function in Fig. 6. The autocorrelation time τ_c has a variation with the velocity of the particle in suspension described by equation (4) [2,13]:

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

where k is the wave number and A is a constant depending on the scattering properties of the sample. The velocity of the SC in suspension, in this experimental setup, is the ESR measured in the standard laboratory test, as previously described.

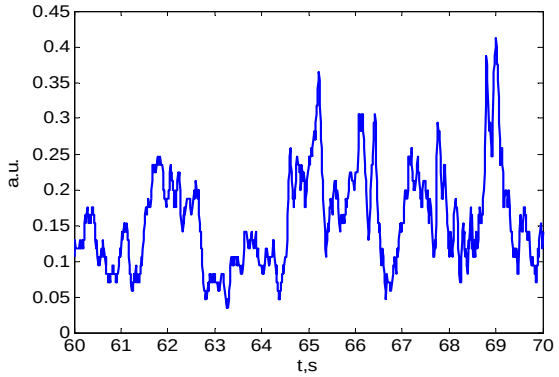

Fig. 5. A sequence of 10 seconds from a time series recorded using a photodetector.

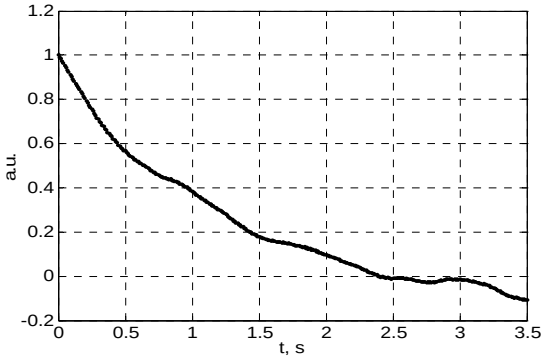

Fig. 6. The autocorrelation function for the time series in Fig. 5

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. 30 minutes after, the tube was rotated upwards down and back several times to homogenize back the sample and than was analyzed using one of the procedures described above. 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.

3. Results

The experiment was conducted in the first manner described in the previous section of this paper on 20 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. Data was sorted by the measured ESR. The data in Table 1 is presented in Fig. 7. The triangles are the experimental values, the middle line is the linear fit, the upper and lower lines are the 50% confidence bounds.

Table 1. The ESR and the autocorrelation time for the backscattered type experiment. Samples are sorted by the measured ESR value.

Sample number	ESR, mm/h	τ_c, s
1	2.00	0.87
2	4.00	0.72
3	8.00	0.75
4	15.00	0.61
5	15.00	0.77
6	16.00	0.73
7	20.00	0.70
8	28.00	0.68
9	30.00	0.57
10	30.00	0.75
11	37.00	0.60
12	40.00	0.68
13	45.00	0.63
14	52.00	0.50
15	53.00	0.62
16	56.00	0.48
17	64.00	0.56
18	70.00	0.50
19	75.00	0.52
20	78.00	0.55

Examining Table 1 and Fig. 7 we notice the decreasing trend of the calculated autocorrelation time with the increase of the measured ESR, as expected from theory. A linear fit on the data was done and the regression coefficients are presented in equation (5).

$$\tau_c = -0.0036806 \cdot ESR + 0.77531 \qquad (5)$$

The equations of the upper and lower lines, which are the error bounds that contain at least 50% of the predictions, are obtained from equation (5) by adding or subtracting 0.065. We notice that there does exist a reasonably good correlation of the calculated autocorrelation time with the measured ESR, as the correlation coefficient is 0.684 (a value of 1 means perfect correlation).

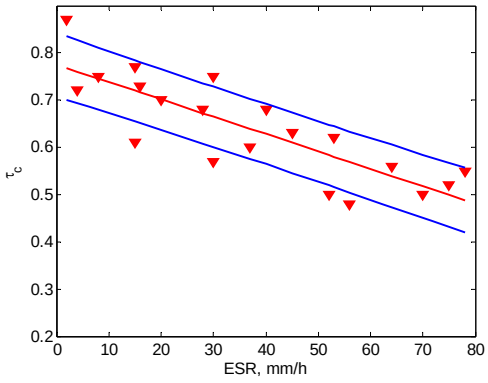

Fig. 7. The Autocorrelation time versus ESR for the time series extracted from uncompressed movies.

We also notice that the ESR values are elevated as compared with the values reported in the literature [14 – 16]. The studies reported in [14 – 16] were conducted on healthy adult patients while the study reported here was conducted on patients who requested medical examination, thus being subject to different laboratory tests conducted with the purpose of establishing a diagnosis.

Many studies like [15] present results of the ESR measurement on healthy population and indicate an upper normal value. Other papers like [16] highlight that average ESR values differ from a human race to another. [15] also reveals average ESR variation with age and gender. It also states that only 5 % of the healthy subjects presented a value above 9 mm/h. No matter which rule or standard is applied, an upper border line must be established by the physician.

Another batch of 20 samples was used in measurements conducted in the second manner described in the previous section, in a transmission type experiment. The time series were extracted from the file recorded from the data acquisition system for each sample after the standard laboratory ESR measurement, as described above and the autocorrelation time was calculated. The data in this type of experiment presents a bigger spread, therefore the plot and the table are not presented here. Nevertheless, the data presents a decreasing trend of the calculated autocorrelation time with the increase of the measured ESR. Although the signal to ratio rate is bigger for this

type of time series, the correlation is weaker, as revealed by the correlation coefficient, which is 0.206 in this case, as compared with 0.684 for the backscattered type of experiment. An explanation for the bigger data spread can be found in the recipe used for sample preparation. The procedure involves extraction of a small amount of RBCs from the bottom of the vacutainer tube and dissolving it back in the upper part. In this manner the RBCs concentration can vary from one sample to another. ESR is slightly affected by the RBC concentration, as presented in [14] and this can be an explanation for the bigger data spread in this type of experiment. Another possible explanation might be the size of the detector, 3 mm in diameter, which covered an area bigger than the average speckle size, thus not recording accurately the speckle fluctuations.

As the correlation is weak, this procedure does not appear to be suitable for developing a fast screening procedure to identify the samples which have abnormally big ESR value, as described in next section.

4. Conclusions and discussions

Two simple experiments of recording the speckle fluctuations produced by coherent light incident on a tube (vacutainer) with whole human blood during erythrocyte sedimentation were performed. For the first experiment the backscattered light was recorded with a CCD, a time series was extracted for each sample and the autocorrelation time was calculated.

The existence of a fairly good correlation of the ESR measured with a standard method (modified Westergren) with the calculated autocorrelation time was investigated and was found to exist, although it is not very strong. This correlation suggests a faster procedure to assess the ESR, following the steps described for the first type of experiment. Once τ_c is calculated, the ESR can be found by reverting equation (5).

The whole procedure might last less than 3 - 4 minutes, way faster that the standard procedure, which is the modified Westergren procedure that lasts for 40 minutes. The method appears to be less precise than the standard method but much faster. For this reason it can be used as a fast screening method and the samples which appear to present an ESR value bigger than a borderline value can be subject to the standard method for an accurate ESR measurement.

Moreover, the procedure suggested in this paper has the same advantage as the standard method. The standard vacutainer tube is used for the experiment described here and the tube is not opened at any time, thus reducing the possible contamination risk.

The correlation found in the preliminary backscattered type experiment is scheduled to be verified on a bigger number of samples to increase the statistic significance. Work is in progress on the subject.

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