ON LIGHT SCATTERING ANISOTROPY OF BIOLOGICAL FLUIDS (URINE) CHARACTERIZATION★

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A light scattering experiment was conducted to assess the light scattering anisotropy parameter *g* of human urine. The light intensity at different angles was measured and the Henyey Greenstein phase function was fit on the data. The values of the *g* parameter are discussed in connection with the subject's disease.

Key words: light scattering anisotropy, human urine, diseases.

1. INTRODUCTION

If coherent light is incident on a medium with scattering centers a non-uniformly illuminated image is obtained, currently named speckled image, having a statistical distribution of the intensity over the interference field. The speckled image appears as a result of the interference of the wavelets scattered by the scattering centers, each wavelet having a different phase and amplitude in each location of 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 complex movement produces fluctuations of the image intensity in each location of the interference field, giving the aspect of "boiling speckles".

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 and it is named subjective speckle in [1]. The review paper [2] classifies the two types of speckled images as far field speckle and image speckle. In this work the objective speckle, respectively the far field speckle is considered.

Although light propagation through disperse systems such as biological cells in suspensions or grouped in tissues has been studied extensively, the

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scattering process modeling and characterization is not straightforward. Most of the biological cells have characteristic dimensions several times higher than the incident light wavelength. For all of them the scattering phase functions are strongly peaked in the forward direction. The light scattering anisotropy is conveniently described by the g parameter, which is currently defined as the mean cosine of the polar scattering angle θ, $g = \langle\cos(\theta)\rangle$. Consequently, for light scattering strongly peaked in the forward direction, the anisotropy parameter g is close to 1.

One of the biological systems frequently used to test the theoretical models is the Red Blood Cell (RBC) [3–7]. Numerous experiments reported using collimated laser beams and analyzing the far field speckle [4, 7, 8]. Several empirical phase functions are frequently used to fit the experimental data. The Henyey–Greenstein phase function as well as the two parameters Gegenbauer kernel phase function are the most commonly used [4, 7, 9]. A new approach replaces the g parameter with an effective g which is a function of the optical depth of the suspension, like in [10, 11]. In this work the well known, one parameter Henyey Greenstein phase function (1), where $\mu = \cos(\theta)$, was used to fit the experimental data and to find the light scattering anisotropy parameter g of different samples:

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

2. EXPERIMENTAL SETUP AND DATA PROCESSING

A simple experiment was set up. It consists of a He-Ne laser having the wavelength of 632 nm and a constant power of 2 mW, a cuvette, a sensitive detector, a data acquisition system and a computer. First the detection system was calibrated in order to provide the functional dependence of the recorded voltage with light intensity. A schematic of the experimental setup is presented in Fig. 1.

The cuvette-detector distance was $D = 2.5$ m and x was modified gradually, changing the angle accordingly. The detector was a phototransistor.

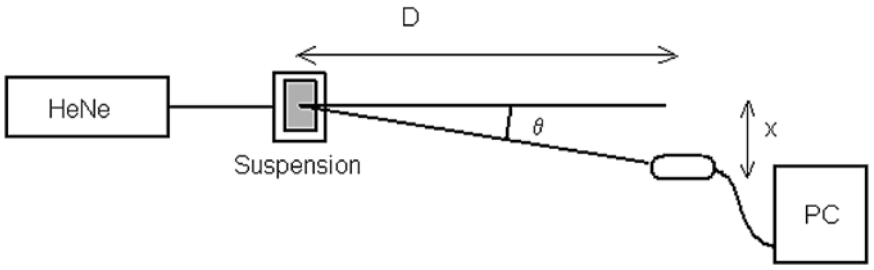

Fig. 1 – The schematic of the experimental setup, view from above.

The Henyey Greenstein phase function can not be measured directly. A detector has a finite transversal dimension d and can be seen from the active part of the cuvette as covering a certain solid angle, hence a polar angle interval $[\theta_1, \theta_2]$. Our detector had a $d = 4$ mm which makes an angular opening of 0.0016 radians or 0.0917 degrees.

The light intensity, which can be measured using a detector, is proportional with the integral of the phase function over the polar angle interval $[\theta_1, \theta_2]$, which is:

$$F(\mu_1, \mu_2) = \int_{\mu_1}^{\mu_2} f(\mu)\mathrm{d}\mu = \frac{C(1-g^2)}{g}\left[\frac{1}{\sqrt{1+g^2-2g\mu_1}} - \frac{1}{\sqrt{1+g^2-2g\mu_2}}\right] \tag{2}$$

where $\mu_1 = \cos(\theta_1)$ and $\mu_2 = \cos(\theta_2)$ and C is a constant that must be determined simultaneously with the anisotropy parameter g.

Light scattering intensity at angles between 0 and 2.5° was measured using the detector and the data acquisition system. Due to the "boiling speckle" aspect of the far interference field one measurement at one angle does not provide a realistic value. A 60 seconds recording time series for each angle was stored on the hard disk and processed later on. The average light intensity for each angle was used as the input for a program that fits F in (2) on the experimental data. The program written for this purpose minimizes χ^2 per point, defined as (3) and provides the optimum values of the C and g parameters and the errors in finding the parameters, in respect to the fit.

$$\chi^2 = \frac{1}{n}\sum_{i=1}^{n}\left[F(\mu - \Delta\mu, \mu + \Delta\mu) - F_{\exp}(\theta)\right]^2 \tag{3}$$

where F is calculated with (2) for each data pair, $\Delta\mu$ is the half of the polar angle covered by the detector, as seen from the active area of the cuvette, and $F_{\exp}$ is the value of the scattered light intensity measured and averaged as described above.

3. RESULTS

Human urine from both healthy patients and from patients presenting health problems was used as a sample. For each sample the light intensity was measured at different polar angles, as described before. The results of the fit, that is the values of the C and g, the anisotropy parameter together with the errors in determining them, in respect to the fit, are presented in Table 1 for some representative samples. The samples were subject to a standard summary medical laboratory urine analysis and the results are stated in the last column of Table 1.

Table 1

The results of the light scattering anisotropy measurement and of the summary urine analysis

No.	Sample	C [a.u.]	ΔC [a.u.]	g	Δg	Comment
1	U6-20	2.1327	0.010204	0.99773	3.0303e–005	Proteins present
2	U6-21	0.64694	0.0020408	0.99783	5.0505e–005	Urobilinogen
3	U6-22	1.0646	0.0010101	0.99279	5.0505e–006	Puss present
4	U6-23	1.303	0.0010101	0.9988	1.0101e–005	Urine from healthy patient

Fig. 2 presents the plot of the experimental data and of the F function for sample u6-29, Fig. 3 for sample u6-22 and Fig. 4 for sample u6-23.

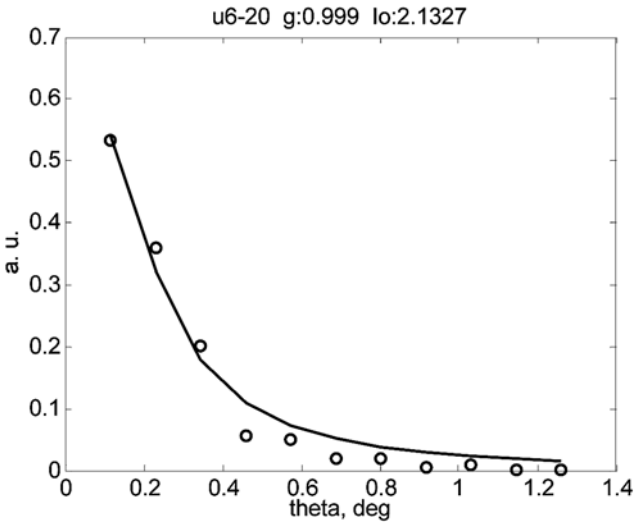

Fig. 2 – The plot of the experimental data and of the F function for sample u6-20.

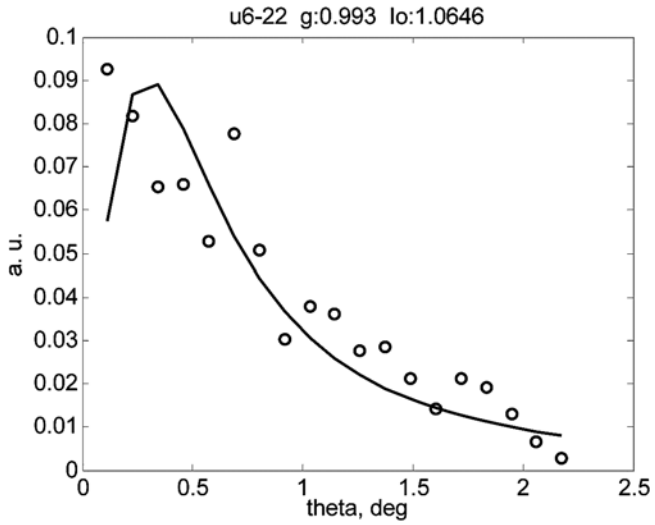

Fig. 3 – The plot of the experimental data and of the F function for sample u6-21.

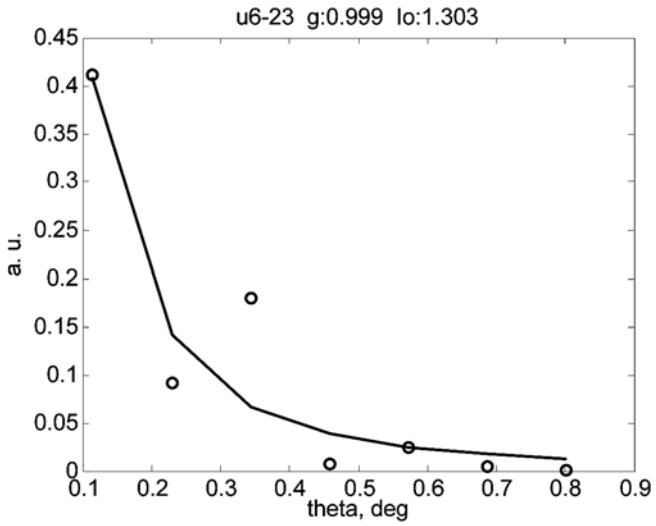

Fig. 4 – The plot of the experimental data and of the F function for sample u6-23.

4. DISCUSSION

Examining Table 1 we notice that the values found for the *g* parameter are consistent with the anisotropy parameter reported in the literature [3–11] for human cells, that is higher than 0.98. The *C* parameter describes the scattering properties of the medium and is connected both with the individual cell scattering properties and with the cell concentration while the *g* parameter shows how close to the forward scattering is the average scattering angle and for low concentration is a characteristic of the scattering centre. A value of 0 means perfectly isotropic scattering while a value of 1 means perfect forward scattering.

Sample u6-23 is urine from a patient that was found healthy; $C = 1.303$ and $g = 0.9988$. The urine in sample u6-20 contains proteins; for this sample $C = 2.1327$ and $g = 0.99773$. The value of the *C* constant is higher than for normal urine, which is a clear indication that the protein containing urine has increased light scattering properties. The value of the *g* parameter is smaller, which indicates that light scattering is less peaked in the forward direction. The presence of proteinuria (abnormally high presence of proteins in urine) is significant for glomerular renal disease.

Sample u6-21 was found to have $C = 0.64694$ and $g = 0.99783$. The *g* parameter was comparable with the *g* value for a sample taken from a healthy patient but the *C* parameter is much smaller. The summary lab examination of the same urine sample revealed the abnormal presence of urobilinogen (metabolism product of haemoglobin, during red blood cell turnover), which is significant for hepatic cells damage.

Sample u6-22 has a smaller value for *C*, $C = 0.99279$ and a considerably smaller value of the *g* parameter, $g = 0.99279$. The summary laboratory exami-

nation revealed the presence of puss (pyuria). This is significant for urinary tract infections or genital infections.

After examining many plots, the changes from normal can be easily noticed even by watching the maximum values on the *Y* axis, which are higher for an increased value of the *C* parameter and by the presence of the maximum at low angles for a decreased value of the *g* parameter.

5. CONCLUSION

The light scattering anisotropy measurements and scattering parameters assessment we performed on a number of 23 urine samples revealed that a small value for the anisotropy *g* parameter is connected with the presence of puss, indicating a urinary or genital infection. A big value of the *C* parameter was related with the presence of proteins, indicating glomerular renal disease. A small value of the *C* parameter can be associated with urobilinogen, which is significant for hepatic cells damage.

The results of this study reveal that the light scattering parameters of urine can be measured using this fast procedure. They also indicate that the changes of the *C* and *g* parameters can be used to identify urinary infections, hepatic cells damage or glomerular renal disease.

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