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Results of light scattering dynamics analysis of biological fluids

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When coherent light passes through a transparent fluid having scattering centers (SC) the result of the far field interference is a "speckled" image. When the target is a biological sample or cells in suspension the image is called biospeckle. In a suspension the SCs have a complex movement of both sedimentation and the Brownian motion. As a result the biospeckle image is not static but presents time fluctuations. Human urine from both healthy patients and patients with different diseases was used as a target for the laser beam. The time variation of the light intensity in the far field speckle image was acquired. The autocorrelation function of the time series was calculated for each sample. The same samples were analyzed with the standard laboratory urine analysis procedure. We found that the autocorrelation time of the healthy patient urine is around 5 seconds. When albumin, pus, epithelial cells, red or white blood cells are present the autocorrelation time decreases to 2-3 seconds. When cells conglomerates, oxalate salt conglomerates or cylinders are present, the autocorrelation time increases. The technique can be used for a faster identification of the presence of individual cells or of conglomerates in urine and as a screening test.

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1. Introduction

When coherent light crosses a medium having scattering centers an un-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 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 review paper [2] classifies the two types of speckled images as far field speckle and image speckle. In this work the objective speckle, respectively far field speckle is considered.

The 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 [3,4,5] or by laser speckle contrast analysis [6,7]. In this work the time series corresponding to the fluctuations of the far field interference in one location was recorded and analyzed using the autocorrelation function. Details are presented in the next section.

2. Method

The schematic of the experiment is presented in Fig. 1. The He-Ne laser has a wavelength of 632 nm and a constant power of 2 mW. The active area of the glass cuvette was 12 mm thick. Measurements were done at small angles, 0.115 degrees. The time series corresponding to the interference field was recorded using a detector, a data acquisition system and a PC.

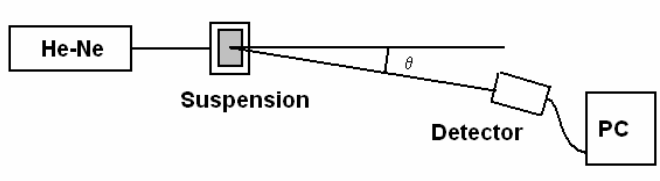

Fig. 1. The schematic of the experiment, view from above.

A time series resulted for each urine sample. Special care was taken for the turbulent motion of the fluid in the cuvette to be completely quenched. The turbulent flow produces pressure variations in the fluid, hence local variations of the refractive index of the fluid [3]. These

variations produce fluctuations of interference field. Moreover, the turbulent flow produced by filling the cuvette with the suspension causes bigger velocities of the SCs, therefore fluctuations with higher frequencies, typical for turbulent flow.

The turbulent flow quenches fast after filling the cuvette, but the temperature differences between the fluid and the cuvette glass walls can produce convection currents that make the SCs move with bigger velocities than in thermal equilibrium. The convections currents will be quenched when the temperature differences disappear. For these two reasons the calculations performed on time series recorded right after filling the cuvette, with the same sample, did not produce the same results. Reproducible results were obtained though when the recording started 10 minutes after the urine samples were introduced in the cuvette and this delay procedure was applied on all samples. A time series recorded for urine from a healthy patient is presented in Fig. 2.

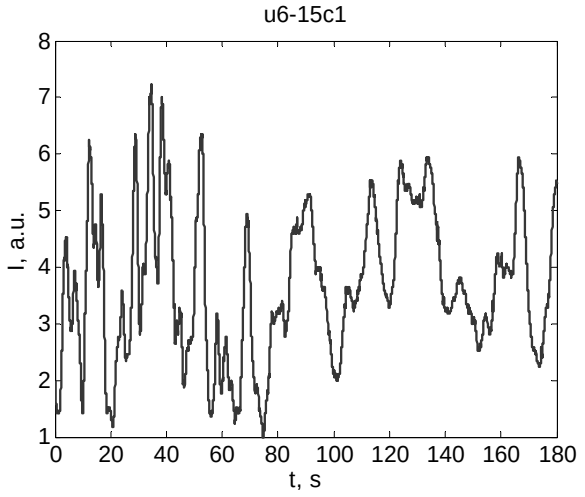

Fig. 2. The time series recorded for a healthy patient urine.

The autocorrelation function for each sample was calculated as:

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

where the angle brackets denote averages over time t, r represents the position of the detector, and τ is the correlation time.

The normalized autocorrelation function decreases from 1 and we can define the autocorrelation time (ACT) as the time when the autocorrelation function decreases to 1/e.

The correlation time has a variation with the velocity of the particle in suspension [1,8].

$$\tau=\frac{A}{k\cdot v} \tag{2}$$

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 has two main causes. First, a sediment motion of the SC is produced with a constant velocity, which is the consequence of the null resultant of three forces: gravity, Archimede's force and the viscous force in laminar flow regime. Considering the SC spherical, the velocity is given by equation (3):

$$v_s=\frac{2r^2g}{9\eta}\cdot(\rho-\rho_0) \tag{3}$$

where r is the radius of the sphere, ρ is the density of the scattering center, ρ_0 is the density of the fluid, η is the dynamic viscosity of the fluid.

The Brownian motion velocity can be estimated using the thermal equilibrium velocity (4):

$$v_s=\sqrt{\frac{3kT}{m}} \tag{4}$$

In (4) k is Boltzman's constant, T is the absolute temperature and m is the mass of the particle in thermal equilibrium with the environment.

For a red blood cell (RBC), having a typical radius of 4 μm [9], [10] in isotonic saline aqueous suspension the sediment motion velocity at room temperature, calculated with (3) is around $3.5*10^{-6}$ m/s and the Brownian motion velocity, calculated with (4) is 3.5×10^{-4} m/s. We notice that the fluctuations are produced primarily by the Brownian motion of the microparticles in suspension. This suggests that the bigger the mass of the particles in suspension is, the smaller the velocity is and therefore the bigger the autocorrelation time is. The samples that were analyzed were urine samples. Urine can contain albumin, pus, epithelial cells, red or white blood in suspension, albumin cells conglomerates or oxalate salt conglomerates and the hypothesis we investigated is that the autocorrelation time will differ from one urine sample to another accordingly to the type and amount of cells and particles in suspension, therefore urine samples from different patients were analyzed using the procedure described above.

3. Results

31 urine samples were used and for each sample a time series was recorded. The autocorrelation function was calculated and the autocorrelation time (ACT hereafter) was determined for each sample. The same samples were

analyzed in a medical laboratory using the standard procedure and digital photos were taken using a Nikon NIKON E8400, 8.1 Mpixel camera, 3264x2448 resolution, 24 bits color depth and the microscope objective was 40X.

Deionized water was used as reference, having ACT=4.90 s and urine having no suspensions, taken from healthy patients has an ACT around 5 s.

Fig. 3 presents the optical microscope photo of a urine sample taken from a patient having albumin particles in suspension only. The autocorrelation function for this sample is presented in Fig. 4.

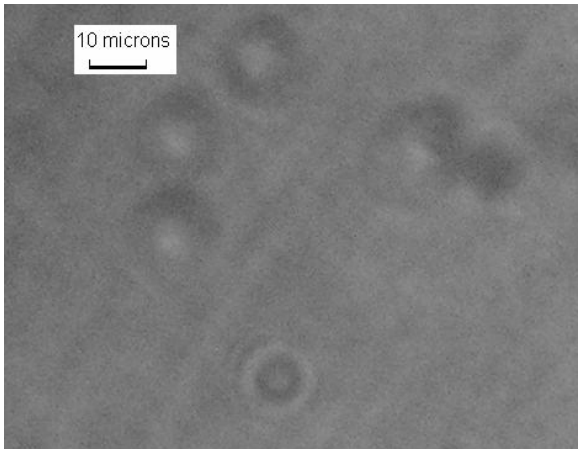

Fig. 3. Small albumin particles in sample 24, microscope photo.

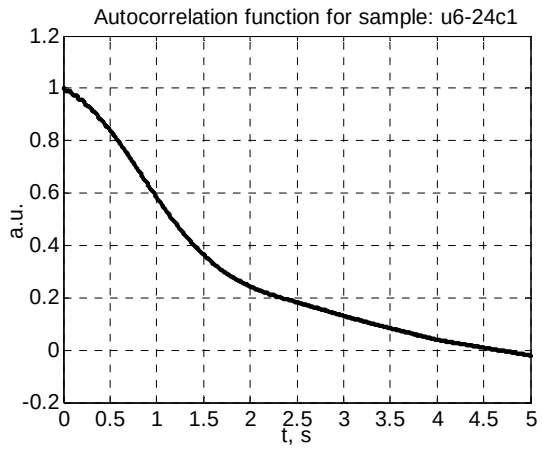

Fig. 4. The autocorrelation function for sample 24.

For this sample, number 24, the autocorrelation time ACT=1.49 s is smaller than for deionized water. The decrease is caused by individual cells (albumin, pus, epithelial, white blood cells) in bigger amount that were identified in the standard urine laboratory test. The decrease of the autocorrelation time was noticed when individual cells, no matter of the type, were present in the urine samples.

The autocorrelation time is considerably modified by the presence of conglomerates, which can be albumin, oxalate salt conglomerate, oxalate salt cylinders. Sample 26 presented a normal amount of individual cells but a considerable amount of oxalate crystals. Fig. 5 presents the microscope image of sample 26.

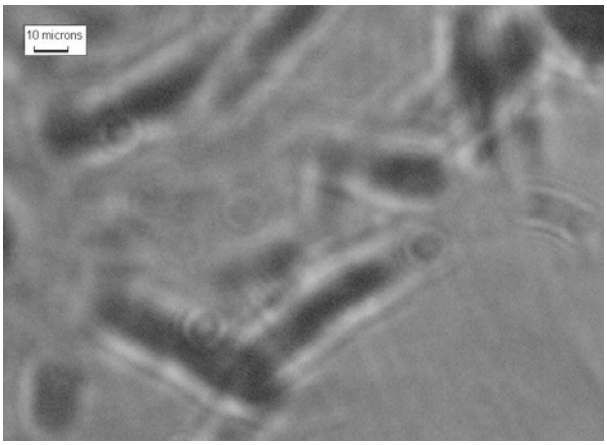

Fig. 5. Oxalate crystals present in sample 26.

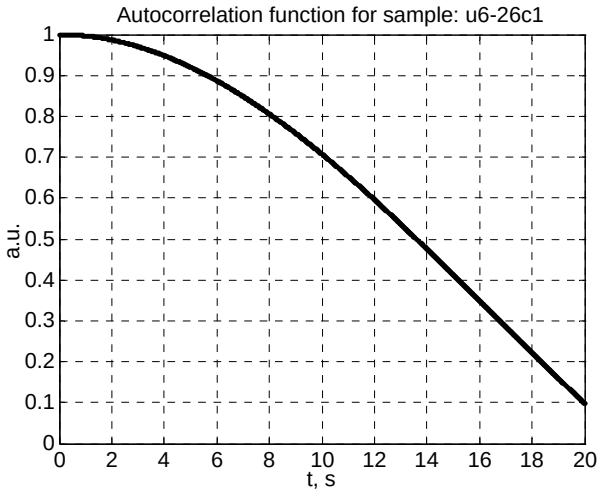

Fig. 6. The autocorrelation function for sample 26.

Fig. 6 presents the autocorrelation function for sample 26. The autocorrelation time ACT=15.59 s and the increase is caused by the smaller velocity (4) caused by the bigger mass of the particles. The smaller velocity increased the autocorrelation time (2).

Urine can present other type of conglomerates, cell conglomerates or cells on exalate crystals being frequent. Fig. 7 presents the optical microscope image of the urine sample 27. The conglomerates that can be observed were identified by the standard urine laboratory test as albumin conglomerates. Fig. 8 presents the autocorrelation function for the time series recorded on sample 27. For this sample the autocorrelation time ACT=14.82 s is bigger than for healthy patient urine. The increase is caused by the albumin conglomerates that were identified in the standard urine laboratory test The increase has the same cause, smaller velocitiy hence bigger ACT.

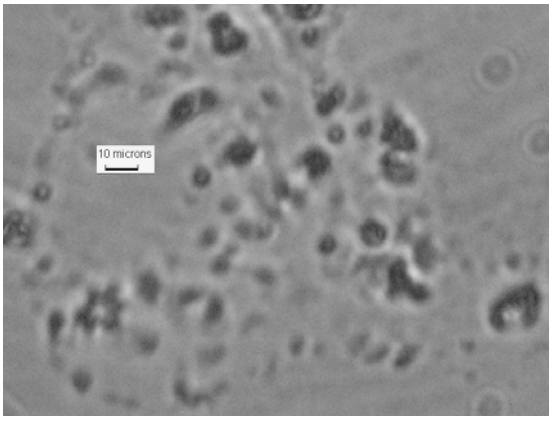

Fig. 7. Albumin conglomerate present in sample 27.

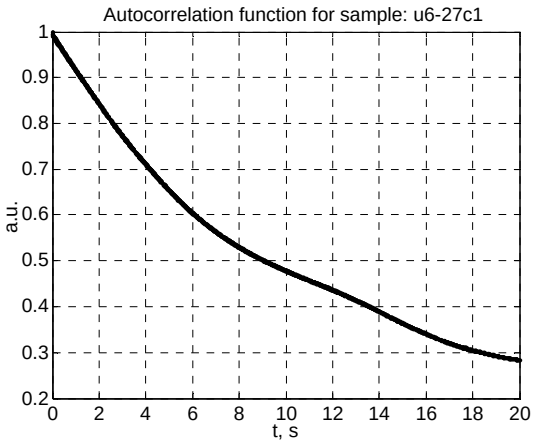

Fig. 8. The autocorrelation function for sample 27.

4. Conclusions

The technique we developed records the far field interference fluctuations produced by the SC motion in human urine and calculates the autocorrelation time. We found so far that the autocorrelation time of a healthy patient urine is around 5 seconds. When individual cells like albumin, pus, epithelial, red or white blood cells are present, the ACT time decreases. The presence of individual cells in urine samples in bigger concentration is clinically significant for the existence of urinary tract lesions, therefore can be used for diagnosis.

When oxalate crystals, cell conglomerates, cell cylinders are present the ACT time increases up to 15 seconds. This indicates that a specific urine analysis (microscopy) is required for the identification of pathological elements with their clinical significance. The presence of crystals in urine is suggestive for urinary lithyasis while cell conglomerates or cell cylinders are suggestive for a large variety of renal and urinary tract diseases like severe urinary tract infections.

These results suggest a procedure that is faster than the standard microscope observation, to identify the presence of big size conglomerates or of individual cells, but can not be used to clearly distinguish between the individual presence of proteins, pus, urobilin, red or white blood cells. Therefore this technique might be used as a fast and low cost screening method to identify the pathological urine samples that must undergo a subsequent specific laboratory examination including microscopy and biochemical analysis for an accurate diagnosis.

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