BIOSPECKLE SIZE AND CONTRAST MEASUREMENT APPLICATION IN PARTICLE SIZING AND CONCENTRATION ASSESSMENT*

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A coherent light scattering experiment was done. The scattering centers were milk and urine. Different concentrations for each type of sample were used, digital images were taken, movies were recorded, the contrast and the average speckle size were calculated. We found that the speckle size decreases and the contrast decreases as the particle concentration increases.

Key words: coherent light scattering, biological scattering centers, contrast, speckle size.

1. INTRODUCTION

When coherent light crosses a medium having scattering centers an 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.


It was often pointed out in the literature that 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 movement. The motion of the speckle field was analyzed by correlometric methods [3–5] or by laser speckle contrast analysis [6, 7]. The speckle size can be used to measure the roughness of a surfaces [8–10] or to determine the thickness of semi-transparent thin slab like in [11]. Most of the above mentioned experiments use the backscattered speckle configuration. In papers like [12] a different optical set-up is used to measure the correlation function in the near field, and show the near-field speckle dependence on the particles size. The work reported in [13, 14] uses a transmission optical set-up to measure the far field parameters like contrast and speckle size. The transmission type of setup was used in the work reported in this paper, as well. The details are presented in the following section.

2. EXPERIMENTAL SETUP AND DATA PROCESSING PROCEDURE

This type of set-up was used in the work reported in this paper and a schematic is presented in Fig. 1.

![Fig. 1 – The schematic of the experiment.](image)

The He-Ne laser had 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 3.5 degrees from the beam axis using a CMOS camera and data acquisition was done on a PC using the USB port. The optical system of the camera was removed, therefore the recorded images are the direct interference on the CMOS detection matrix. Consequently the far field speckle was recorded, not the speckle image.

The average contrast of the image, either acquired as a bitmap or extracted from each of the frames of the movie, is calculated [1, 13, 14] as:

\[
C = \sqrt{\frac{\langle I(i,j) \rangle^2 - \langle I \rangle^2}{\langle I \rangle}}
\]  

(1)
where $I(i,j) = I(x_i, y_j)$ is the intensity recorded by the cell $(i, j)$ of the CCD, hence stored in the pixel $(i, j)$ of the array of pixels the image consists of. This is a space contrast and not a time contrast, as pointed out in [15]. In (1) the angular brackets stand for average over the entire $640 \times 480$ collection of intensity values for an image that is processed.

In [1], [13] and [14] the average speckle size is defined as the normalized autocovariance function of the intensity:

$$S_{ps} = \frac{R_s(x, y) - \langle I(x, y) \rangle^2}{\langle I(x, y)^2 \rangle - \langle I(x, y) \rangle^2}$$

where $R_s(x, y) = \langle I(x_i, y_j) \cdot I(0, 0) \rangle$ (2)

In this paper a different approach is proposed to calculate the speckle size. For each vertical profile the normalized autocorrelation function [16] is calculated. The speckle size for that profile is defined as the value of the x axis (pixel number or distance, if multiplied by the pixel size on the CMOS) where the autocorrelation function decreases to $1/e$. An average is calculated for each image and we define the speckle size as the average speckle size for that particular image. A vertical profile is presented in Fig. 2 and the autocorrelation function of that profile in Fig. 3.

3. RESULTS

First a preliminary experiment was conducted, by recording the far field speckle produced by light scattering on starch, wheat flour, orange juice and milk in deionized water at different dilutions, as targets. Whole milk in suspension was chosen to test the new procedure described in Section 2 for
calculating the speckle size and the contrast variation with the concentration of the scattering centers, because the concentration can be easily changed in a precise manner using a regular small volume syringe. When dissolving a powder in water clusters of particles having different sized occur, having a statistical distribution of the sizes, therefore the scattering centers concentration and size is not precisely known.

In the first part of the experiment a grayscale bitmap image, having a resolution of $640 \times 480$ pixels was recorded for different milk concentrations. Fig. 4 and 5 present the recorded bitmaps for milk samples having the volume ratios of 0.0012 and 0.0372.

Using a program written for this purpose the average intensity over the recorded area, the average contrast and the average speckle size, in pixels, were...
calculated. The variation of the average contrast and of the average speckle size with the concentration, expressed as volume ratio, for whole milk diluted in deionized water are presented in Fig. 6 and 7.

Examining Fig. 6 and Fig. 7 we notice a big spread of the data in a small concentration range between 0.06 and 0.08. This suggests that big differences between pictures taken for the same sample at different moments might occur, due to fluctuations. In order to verify this hypothesis, the experiment was repeated. An uncompressed, 24 bits grayscale AVI type movie was recorded for each concentration, using a framerate of 1 per second and the same resolution, 640 × 480 pixels.

A previous experiment using a data acquisition system revealed that the autocorrelation time for this type of samples is around 0.1 seconds therefore a framerate of 1 per second ensures that the images in consecutive frames are not correlated and that the average over the 60 frames for each sample does not depend on the specific time they were recorded at. Fig. 8 presents the variation of the average intensity.
Fig. 7 – The average speckle size variation with the concentration.

Fig. 8 – The average intensity variation with the concentration.

Fig. 9 presents the variation of the average contrast and Fig. 10 the variation of the average speckle size with the concentration. The error bars on

Fig. 9 – The average contrast variation with the concentration.
Particle sizing and concentration assessment

4. DISCUSSIONS AND CONCLUSIONS

Examining Fig. 8 we notice that at very small scattering centers (SC) concentration the average intensity increases with the SC concentration, presents a maximum and then decreases with the concentration. As the concentration increases in the range where multiple scattering is dominant, the average intensity remains constant.

The average contrast has a very fast increase with the SC concentration, then displays a plateau and a fast decrease. As the concentration increases the slope decreases in module and finally the contrast remains constant, as results from Fig. 6 and Fig. 9. This result is similar with the results reported in [13].

The average speckle size presents a constant decrease with the SC concentration and a slight increase in the big concentration range, as presented in Fig. 7 and 10. The linear decrease is similar with the results reported in [13], except for the big concentration range where data is not reported.

These results reveal that the speckle calculation algorithm presented in this paper can be used to calculate the average speckle size. They also reveal that the contrast speckle together with the average speckle size of the far field can be used as a fast procedure in assessing the scattering centers concentration in a biological fluid, in the small and medium concentration range.

\[ l = \frac{t \cdot s}{\sqrt{n}} \]  

where \( t \) is the parameter of the Student test, \( s \) is the standard deviation calculated as in section 2 and \( n \) is the number of frames, 60 for this experiment.
REFERENCES