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Quasi-Real Time Bio –Tissues Monitoring using Dynamic Laser Speckle Photography

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***Abstract*:** Joint development of a laser monitor for the real-time bio-tissue analysis is presented. The monitor is based on the digital dynamic laser speckle photography and deals with soft and hard bio-tissues. In soft tissues, the dynamic bio-speckles are formed in a scattered from a tissue laser light. An optically transparent model of hard bio-tissue was prepared and preliminary analysis of a stress field in the stressed model was performed using the dependence of the refractive index of transparent solids upon the state of stress and the double exposure speckle photography data. The refractive index of the stressed material was evaluated and the state of stress was reconstructed using the stress-optical law.

***Keywords*:** Visualization, Bio-tissue monitoring, Bio-speckle, Blood microcirculation.

1. Introduction

Monitoring of the blood microcirculation activity in living tissues is of considerable importance for many tissue diagnostic purposes. The use of laser for imaging through or into bio-tissue is currently an active area of research. Optical methods based on the analysis of the laser light scattered by the tissues are very attractive due to the possibility to use that in real- or quasi-real time mode. The image of living tissue illuminated with the light of a laser differs from an image taken under white light illumination by the extremely grainy structure (speckles) that is superimposed on the surface features of the tissue. Dynamic (moving) speckles are carriers, in addition, of the living tissue information and cross-correlation analysis of a sequence of the speckled images allows to determine, for instance, blood microcirculation parameters. Such technique becomes one of the principal direction in biological and medical application of laser monitoring. The technique is particularly attractive and promising because of non-contacting and non-disturbing character of measurement and the possibility of its implementation in vivo.

Similar techniques based on time-varying speckle were already applied to biomedical studies during the last two decades (Briers, 1975, Fercher, 1980, Briers and Fercher, 1982, 1982a, Briers,

1983, 1996). The first, who used laser speckle techniques to the blood flow measurement were Asakura et al. (Asakura and Takai, 1981, Iwai and Asakura, 1989, Okamoto and Asakura, 1990, 1992, 1995). They introduced the notion "bio-speckle" to describe the time-varying speckle produced by living organisms. The speckle technique was also used for the skin blood flow investigations by Fujii et al., who applied later this method to the retinal blood flow monitoring (Fujii et al., 1985, 1987, Tamaki et al., 1994, Konishi and Fujii, 1995). Ruth also used time-differentiated speckle to measure skin blood flow (Ruth, 1990, 1994, Ruth et al., 1993). Hinsch (1987) applied double-exposure speckle photography for monitoring the processes in various biological tissues.

During the last three decades, the underwater shock waves have been successively used in medical applications both for soft and for hard tissue treatment, see (Takayama, 1999). Real-time operation allow us to use the technique for tissue structure monitoring under tissues medical treatment, e.g. by shock wave. There are many problems for such bio-tissues monitoring due to multi-scattering of probing radiation in the tissues, and the present paper deals with analysis of these multi-scattering effects on the tissue monitoring by dynamic speckle photography.

2. Formation of Bio-Speckle and its Dynamics

2.1 Bio-speckles Formation

Coherent light scattered from diffuse object produces a random granular interference structure some distance away from the object, which is called speckle pattern. Such a pattern can also be observed when a laser light illuminates a living semi-transparent tissue. The visible laser light penetrates into the human skin on the deepness of about 200-1000 μm and is multiply scattered by the red blood cells, RBCs, flowing inside the smallest candelabra capillaries as well as by a surrounding tissue. So, an image of the tissue illuminated with the light of laser differs from an image taken under white light illumination by the speckle pattern that is superimposed on the surface features of the tissue. As the scatterers (RBCs) move, the speckles also move and change their shape. The dynamic (time-dependent) bio-speckle pattern is formed as a superposition of some moving speckles with different dynamics, including static speckles. These bio-speckles play a dual role: as a source of noise in tissue images, and as a carrier of useful information about biological or physiological activity of living tissues, such as subskin blood flow and general tissue-structure motility.

2.2 Spatial-temporal Behavior of Bio-speckle Patterns

Dynamics of speckle patterns produced by a moving rough surface have been extensively studied for velocity measurements. However, the spatial-temporal properties of bio-speckle are essentially different from those of the speckle patterns formed by a moving rough surface due to the effect of the multiple scattering and different velocities of the scatterers. This effect is important for Laser Doppler measurements as well, but the description of the scattered light using speckles has the advantage of including multiple scattering, even if we consider the simplest case of multiple scattering from the "single" rough surface. For a single point measurements, the intensity fluctuations measured at the point are characterized by the time-correlation length defined by the time at which the normalized temporal autocorrelation function of intensity fluctuations falls to 1/e. This statistical quantity is inversely proportional to the fluctuating speed of the speckle intensity. Its reciprocal value measures the velocity of a diffuse object at least for the speckles scattered once.

Dynamic speckles have two fundamental motions of speckles. At the first type of the speckle motion called "translation" the speckles move as a whole, and their shape remains unchanged for considerable displacement. At the second type of speckle motion, speckles deform, disappear, and reappear without appreciable displacement of their positions. This type of speckle motion is called "boiling" of speckle. In both cases, the speckle behavior depends not only on the motion of the scatterers, but also on the parameters of optical scheme used for the speckle observation. In the most

cases, dynamic bio-speckle mode is mixed and speckles translate gradually changing the structure. Two parameters, the correlation time, τ_c, and the lapse time, τ_d, have been introduced by Asakura and Takai (1981) to describe the dynamic behavior of speckles composed of the boiling and the translation:

$$\gamma_{\Delta I}\left(\vec{r},\tau\right)=\exp\left(-\frac{\left|\vec{r}\right|^2}{r_c^2}\right)\exp\left[-\frac{\left(\tau-\tau_d\right)^2}{\tau_c^2}\right] \tag{1}$$

where $\vec{r}=\vec{r}_2-\vec{r}_1$, $\tau=t_2-t_1$, and the lapse time τ_d depends on $\vec{r}$.

2.3 Effects of Multiple Scattering on Bio-speckle Dynamics

The simplest model for accounting the multiple scattering effects on the bio-speckle dynamics is multiple (moving) screen models. The statistical properties of randomly scattered light by a random media using such models have been studied by Okamoto and Asakura (1990, 1992, 1995) and by Uschinski (1993). A thin diffuse model of refractive index fluctuations in turbulent media with a weak scattering has been used by Uschinski (1993) to describe the laser light propagation in a turbulent atmosphere. It is more appropriate to use the model of a set of "deep" random phase screens for bio-speckle. Since each deep phase screen produces a fully developed speckle pattern, a series of such phase screens becomes a model for the strong scattering regime in which the light that has passed through a scatterer has no specular or coherent components, see (Okamoto and Asakura, 1990, 1992).

2.3.1. Single phase screen model

For a single phase screen moving with a constant velocity $\vec{v}$, τ_c and, τ_d are expressed via parameters of the optical imaging scheme. Thus, for a single lens (L_2) in an arrangement shown in Fig. 1, they are:

$$\tau_c=\frac{1}{\left|\vec{v}\right|}\left[\frac{\sigma_i}{D^2}+\frac{d_i}{r_c^2}\left(\varepsilon\,\sigma_i-\frac{1}{d_0}\right)^2\right]^{\frac{1}{2}},\qquad \tau_d=\frac{\tau_c^2 d_i^2}{r_c^2}\left(\varepsilon\,\sigma_i-\frac{1}{d_0}\right)\vec{v}\quad\vec{r}\ , \tag{2}$$

where d_0 is the distance from the object plane to the imaging lens, d_i is the distance from the lens to the observation plane, $\varepsilon=1/d_0+1/d_i-1/r$ is a defocusing parameter, D is the imaging lens diameter, and $\sigma_i=1+d_0/p$. The dynamic speckle size, r_c', is related with a static speckle size, $r_c=\pi\lambda d_i/D$ and the translation distance, r_T:

$$r_c'=\frac{r_c^2+r_T^2}{1+\sin\Theta\cdot r_T^2/r_c^2},\qquad r_T=\frac{d_i D}{\sigma_i}\left(\varepsilon\quad\sigma_i-\frac{1}{d_0}\right) \tag{3}$$

with Θ being the angle between the vectors $\vec{v}$ and $\vec{r}$. Eq. (4) shows that the correlation time of the intensity fluctuations in speckle pattern is inversely proportional to the velocity of a phase screen that creates this pattern. This relation is widely used for the velocity measurement of rough surfaces by dynamic speckle methods; see (Asakura and Takai, 1981).

2.3.2 Double screen configuration

Several attempts have been made to investigate the influence of two different sorts of scatterers on the dynamics of the bio-speckle pattern. In one model, the laser light passes through the first stationary transparent diffuser representing the skin. Then, the laser light is scattered back by the second moving diffuser, which represents the moving blood, and finally, it passes again the stationary first diffuser. In a more general form, the statistics of dynamic speckles for the double screen configuration when both screens are moving was studied by Okamoto and Asakura (1995). To include into consideration the effect of the multi-scattering in this model, a moving diffuse object is illuminated by the light that has been scattered by another diffuser in motion. In this case, the correlation time, τ_c , is expressed as

$$\tau_c = \sqrt{a_{11} \cdot \left|\vec{v}_1\right|^2 - 2a_{12} \cdot \vec{v}_1 \vec{v}_2 + a_{22} \cdot \left|\vec{v}_2\right|^2} \,, \tag{4}$$

where $\vec{v}_1$ and $\vec{v}_2$ are velocities of diffusers, and a_{11}, a_{12} and a_{22} are constants, which depend on optical configuration,

2.3.3 Multiple screen configuration

Figure 1 shows the schematic diagram of the optical arrangement used for evaluating the space-time correlation function of multiply scattered speckle pattern in the image plane. Random phase screens are spaced in parallel with each other and translate at constant velocities in their own plane. By illuminating these diffusers with a coherent light, a dynamic speckle pattern resulting from the multiple scattering is produced at the observation plane located behind an imaging lens.

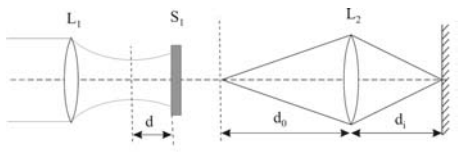


Fig. 1. Models for multiple scattering in bio-speckle formation.

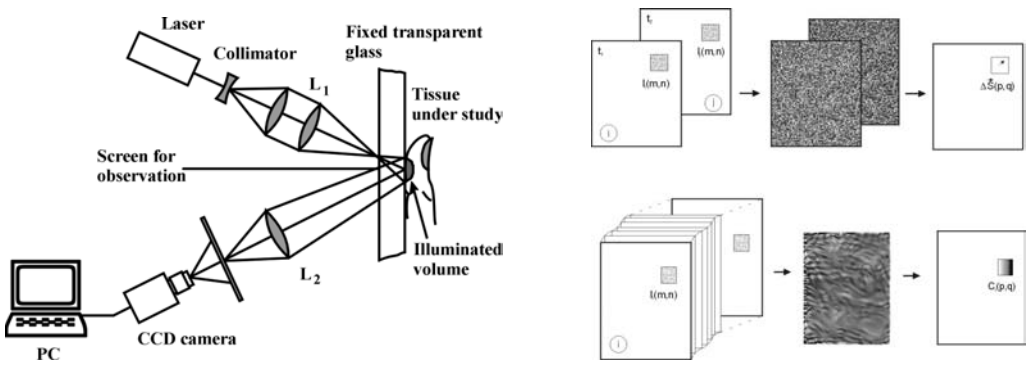
As it was already shown, in the case of double scattering screens, the velocity of each phase screen can be detected separately by focusing an imaging system on the screen to be measured. In that case, the condition is required that the size of the point spread at the front focal plane of the lens must be smaller than the size of the speckles illumination at the second diffuser. Numerical simulation of Okamoto and Asakura (1995) for multi-screen configuration shows that it is more difficult to measure the velocity of each screen separately from the time correlation length of the dynamic speckle pattern, because it is affected not only by the motion of the phase screen located at the focal position, but also by the motion of the other screens. Nevertheless, even for five screens, the time correlation length is more strongly affected by the change in the velocity of the phase screen on which the lens is focused.

Both experimental and theoretical investigations of Aizu and Asakura (1991, 1996) show that equations similar to a single screen model can be used for double and triple scattering from the moving rough object. Numerical investigation of Okamoto and Asakura (1992, 1995) shows that the value of τ_c near to linearly increases with the increase of the averaged scatterers velocity in the case of multiple scattering as well. Thus, the value of $1/\tau_c$ is seen to be proportional to the velocity of scatterers even in more complicated cases, including multiple scattering. However, the relation similar to Eq. (4) for such case must include many factors like the density of scatterers, diameters of capillaries, etc., so the measurement of the absolute value of the scatterers velocities for such cases needs additional experimental calibration.

Thus, the evolution of the dynamic bio-speckle pattern has more complicated character as compared with PIV technique, where the particle image displacement is always directly proportional to the particle displacement in the flow studied (Kompenhans et al., 1998, Fomin, 1998a).

3. Dynamic Laser Speckle Photography

3.1 Experimental Set-up



(a) (b)

Fig. 2. Experimental installation for sub-skin blood flux monitoring (a) and block diagram of image correlation analysis (b). Upper part corresponds to double (multiple) exposure speckle photography with subsequent cross-correlation analysis of the obtained speckle pattern. The lower part corresponds to single (prolonged) exposure speckle photography with the exposure time comparable with the characteristic time of the process studied.

The experimental arrangement used in this study is shown in Fig. 2,a. A low power He-Ne laser is used as a light source. The collimated laser beam is focused onto the tissue under study through a thin transparent glass used to prevent a tissue from mechanical movement. A light scattered by moving erythrocytes in the illuminated volume is collected by the lens L2 onto the screen, where the speckle pattern is formed. As erythrocytes are moving, the speckles are moving as well thus forming dynamic speckle field. The bio-speckle patterns are recorded using a standard digital CCD camera (768 x 494 pixels) with a frame rate of 25 frames/ second. The exposure time varied from 10 μs (for cross-correlation analysis of subsequent frames) to 1/60 s (for a single exposure mode). Speckle patterns are recorded as a distribution of gray values I(m,n) in digital form for each pixel (m,n) of the CCD matrix.

In real-time operation the image analysis is performed during the time interval between subsequent (two or more) frames. Three different mathematical approaches are used.

3.2 Decorrelation Analysis

The most simple is decorrelation analysis. Many parameters are introduced into practice of measurements to quantitatively characterize such speckle patterns variations. Konishi and Fujii (1995) while measuring the retinal blood flow use the average rate of change of speckles called AD - "the average derivative", and the reciprocal value, BR - "the blur rate" of the speckle intensity variations. Oulamara et al. (1989) while studying biological activity of botanical specimen by bio-speckle patterns decorrelation analysis introduce a parameter defined as the decorrelation mean speed (DMS) of the temporal speckle signals. The parameter has been computed as an averaged

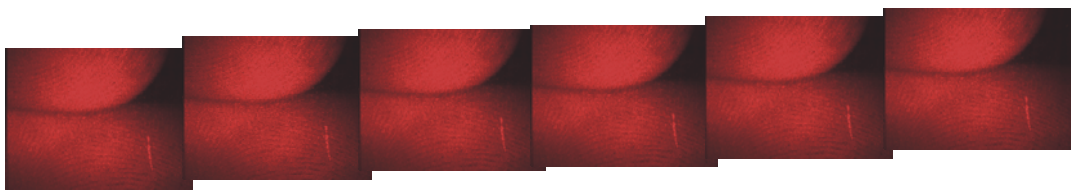


Fig. 3. The sequence of dynamic bio-speckle patterns produced in the laser light scattered by a living tissue. Time interval between subsequent frames is 40 ms.

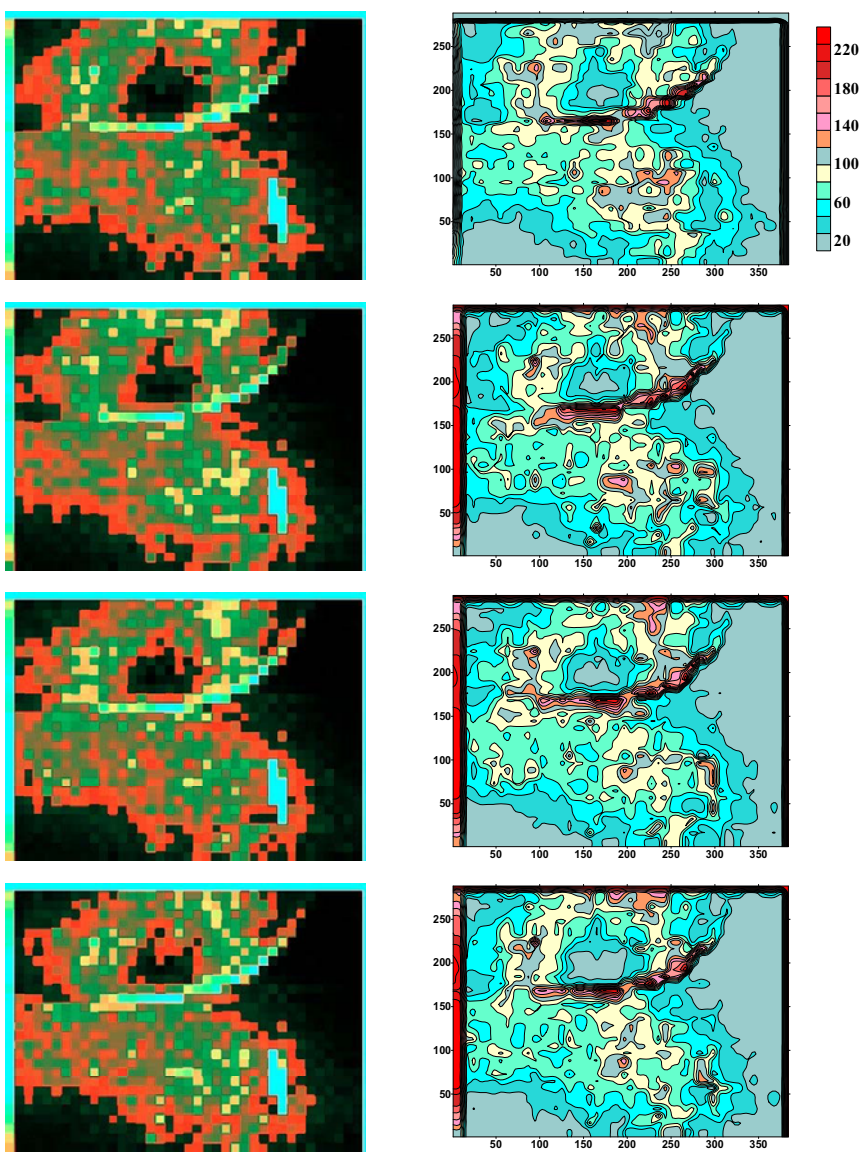


Fig. 4. Real time maps showing the intensity of the subskin blood flux of a hand finger reconstructed by the contrast variation in single (prolonged) exposure speckle photography (scheme left) and isolines of these maps (right). The illuminated surface was 2 x 2 mm.

value of the squared difference between the speckle signals, the first being taken as a reference The same parameter is used in our approach (Fomin et al. 1998b, 2001).

To evaluate this function two sequential image maps, I_k and I_n, are subsampled. The total illuminated area is about 2 - 5 mm in diameter, thus the size of interrogation window is from 100 x

100 μm to 0.5 x 0.5 mm. The typical speckle patterns for a living tissue are shown in Fig. 3. The obtained values of the decorrelation function is proportional to the blood flow velocity, but due to the complicated processes of the light scattering in a living tissue as was shown in Section 2 the direct experimental calibration is necessary for evaluation of the absolute values of the velocity.

3.3 Auto-correlation Analysis

The second method is auto-correlation analysis. In this case speckle contrast is evaluated. Each image is interrogated with a window of varying size from 8 x 8 to 50 x 50. Windows can overlap. Window size $M \times N$ and the overlapping degree were optimized for each particular optical scheme of the experiment. Contrast is calculated in each window k. The contrast of the integrated by time speckle field may characterize the mean speckle displacement during the exposure time, being 1-1000 μs. As shown by Briers (1996) this contrast is inversely proportional to the velocity of scatteres in the interrogation area.

3.4 Cross-correlation Analysis

The most general approach in image analysis is cross-correlation analysis. The image is interrogated with small windows like in the approaches above. And then a correlation coefficient between the corresponding windows of two subsequent frames is calculated. In addition to the common cross-correlation analysis, not only the peak position, but also the value of the cross-correlation function at the peak was evaluated. The last value characterizes the decorrelation rate discussed above. Full cross-correlation analysis requires relatively large interrogation time, and only quasi-real-time operation with a frequency rate of about 5 maps/s was realized with present hardware.

Figure 4 represents the intensity of the subskin blood flux reconstructed in a real time by the contrast variation and isolines of these maps. The information obtained with cross-correlation analysis seems to be a little excessive to the present task as contains the direction of the averaged bio-speckle displacement. For such random fields as subskin blood flux it seems that decorrelation or auto-correlation analysis is sufficient to extract only the value of the averaged blood flux intensity.

Similar approach can be applied to hard tissues as well. An optically transparent model of hard bio-tissue was prepared and analysis of a stress field in the stressed model was performed using the dependence of the refractive index of transparent solids on the state of stress and the double exposure speckle photography data. Two exposures of speckle fields were made, one in the state before stressing the specimen, and another - during the stressing. The refractive index of the stressed material was evaluated and the state of stress was reconstructed using Maxwell-Neumann stress-optical law. Examples of the preliminary results are shown in Fig. 5.

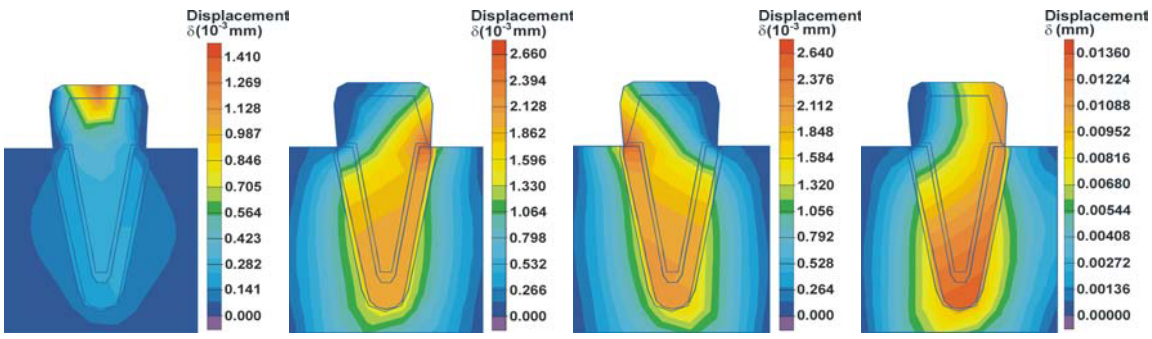


Fig. 5. Displacement fields in a model of strained-deformed state of tooth root restored by cast stump pin inserted at different stressed states.

4. Conclusion

Dynamic speckle photography methods were developed and employed for soft tissue microcirculation monitoring. Preliminary results for hard tissue structure analysis are presented. Detailed analysis of multiple scattering on bio-speckle formation and its dynamics shows that the time-space cross-correlation analysis of the temporal evaluation of the bio-speckle patterns is an effective means of real time flow and stress visualization of a living tissue. Digital processing of bio-speckle patterns records yields 2D maps exhibiting the blood flow temporal and spatial variations.

Three methods of the dynamic speckle patterns evaluation were tested. Both decorrelation and auto-correlation analysis were realized in a near-to-real time mode, when all digital specklegram treatment was performed during the time interval between subsequent frames (40 ms), and results in the form of 2D maps of subskin blood flux were visualized on the PC monitor with frequencies being 10-25 Hz. The full cross-correlation analysis of the dynamic bio-speckle pattern needs a little more PC time and only quasi-real time operation with present hardware was achieved at a frequency of about 5 maps/s. The information obtained with cross-correlation analysis seems to be a little excessive for the present task as contains the direction of the averaged bio-speckle displacement. For such random fields as subskin blood flux it seems that decorrelation and/or auto-correlation analysis is faster and sufficient to extract only the value of the averaged blood flux intensity.

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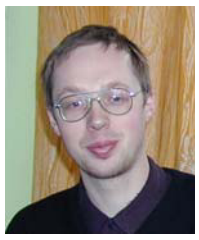

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