Multiple Exposure Time Based Laser Speckle Contrast Analysis: Demonstration of Applicability in Skin Perfusion Measurements

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***Abstract*-The applicability of multiple exposure time based LASCA was demonstrated on skin blood perfusion monitoring. The method was compared to β-corrected and non-corrected single exposure time measurements while the data simultaneously recorded by a laser Doppler system were used as reference. The skin of the forearm was illuminated with the light of an 808 nm laser diode. Speckle images were recorded in the exposure time range of 1-100 ms and relative perfusion changes caused by ischemia were determined. The results showed an obvious nonlinear relationship between the data of the non-corrected single exposure time LASCA method and the laser Doppler system, while a good linearity was observed for the multiple exposure time method with a correlation factor of 0.83.**

Keywords-LASCA; Multiple Exposure Times; Skin Perfusion; Laser Doppler Flowmetry

I. INTRODUCTION

Since the study of Fercher and Briers [1], Laser Speckle Contrast Analysis (LASCA) has been a commonly used method for determination of blood flow due to the simplicity and relatively low cost of a LASCA imaging system. Theoretically, an accuracy near to that of a scanning Doppler system can be achieved during the real time monitoring of a relatively large area of interest, while the imaging of the same area with the scanning Doppler method can take minutes (with a sampling rate of ~30 pixel per second). The laser Doppler imaging technique based on high speed cameras (frame rate around 20 kHz) can result in a significantly decreased imaging time as compared to the scanning method (e.g. 5 frames/s for 128×128 pixel resolution [2]), however, due to the high costs of such cameras their wide-use is improbable in the near future.

The main application area of LASCA is the monitoring of cerebral ([3]-[7]) and ocular ([8]-[10]) perfusion, while its applicability for skin perfusion measurements is limited by the strong static scattering of the surface ([11]-[13]) and the motion of the cutaneous tissue.

During LASCA the studied surface is illuminated with a laser beam while a digital camera images the monitored surface and records the interference pattern (laser speckle) formed by the scattered light. The evaluation is based on the calculation of the *K* local spatial contrast of the laser speckle images, where *K* is defined as the ratio of the standard deviation and the average of the intensity values of the pixels. In most cases, the contrast is calculated on 5×5 or 7×7 pixel areas. Theoretically, for infinitely short exposure times the value of the contrast can reach 1, $K(0)=1$, while for infinitely long exposure times the contrast tends to 0, $K(\infty)=0$. Bandyopadhyay *et. al.* [14] modified the formula derived by Fercher and Briers [1] by introducing a triangular averaging factor resulting in the following relation:

$$K_1(T)=\left\{\frac{\tau^2}{2T^2}\left[\exp\left(\frac{-2T}{\tau}\right)-1+\frac{2T}{\tau}\right]\right\}^{1/2}, \qquad (1)$$

where T is the exposure time and τ is the autocorrelation decay time of the speckle intensity fluctuations. τ is also inversely proportional to the average blood velocity, so it is the key parameter for describing the intensity of the blood perfusion. In practice, due to multiple scattering, depolarization, etc. the contrast tends to a value less than 1 for short exposure time values. By including the β coherence factor introduced by Rojas *et al.* [15], Equation (1) changes to

$$K_2(T)=\left\{\beta\frac{\tau^2}{2T^2}\left[\exp\left(\frac{-2T}{\tau}\right)-1+\frac{2T}{\tau}\right]\right\}^{1/2}. \qquad (2)$$

The above formulas allow the determination of the correlation time based on the contrast analysis of images recorded with a single exposure time value. However, even Equation (2) cannot correctly describe the exposure time dependence of the contrast if static scattering is present on the sample (e.g. scattering from the surface of the skin). For a possible solution of this deficiency of the formula Smausz *et al.* [16], [17] proposed the use of a modified, simple semi-empirical formula accompanied with the recording of series of speckle images with different exposure times:

$$K_3(T)=P_1\left\{\frac{\tau^2}{2T^2}\left[\exp\left(\frac{-2T}{\tau}\right)-1+\frac{2T}{\tau}\right]+P_2\right\}^{1/2}, \qquad (3)$$

where P_1 plays a role similar to the β coherence factor, and P_2 characterizes the contribution of the static (time-independent) scattering to the speckle pattern. τ is determined by fitting Equation (3) to the local contrast data obtained at different exposure time values covering a range of 2-3 orders of magnitude. Since there are three fitting parameters (P1, P2 and τ) the calculation requires at least three different exposure time values. This method was compared to the non-corrected and the β-corrected evaluation methods during the measurement of cerebral blood flow of piglets [18].

The model developed by Zakharov *et al.* [19] also takes the static scattering into account, while still using single exposure time image capturing. Their method utilizes a white paper to determine the value of the β coherence factor, while the static scattering is calculated by cross-correlating consecutive image pairs. The applicability of this method was demonstrated in cerebral blood flow monitoring. The cross-correlating process of images requires a well-positioned area which is readily obtained when the scull is fixed in stereotactic frame. However, that stability can not be easily realized in case of skin perfusion measurements when the finest movements (e.g. from tremor of the monitored forearm) cause a variation in both of the static and dynamic component of the speckle pattern. Further multiple exposure time speckle contrast analysis methods were elaborated by Parthasarathy *et al.* [20] and Rege *et. al.* [21], who tested their methods on synthetic samples and cerebral tissue, respectively. Thompson *et. al.* compared multiple exposure laser speckle imaging and laser Doppler flowmetry by performing computer based simulations and real-time measurements on milk and skin [22]. They found that the power spectral density of the intensity fluctuations computed from the autocorrelation function (derived from the spatial speckle contrast) and the laser Doppler method show similar characteristics. This indicates that LASCA may be just as quantitative as the laser Doppler method, however, they have not performed quantitative blood flow monitoring. Their method does not require any assumptions on the velocity distribution, and they also showed that the Lorentzian velocity distribution might not be the most suitable in every case for the determination of the correlation time.

The aim of the present study is to prove the effectiveness of the multiple exposure time based measurement method when monitoring the blood perfusion of skin where the dynamics of the speckle pattern is strongly influenced by static light scattering. As reference, simultaneous measurements were performed with laser Doppler flowmetry, which is widely used for skin perfusion monitoring.

II. EXPERIMENTAL SETUP

The blood perfusion measurements were performed on the skin of the forearm. The skin surface was illuminated by the polarised light of a near infrared diode laser (808 nm wavelength, 200 mW maximum output power) in an area of ~1 cm^2 with approx. 15° inclination as compared to the normal of the surface. To avoid any changes of the properties of the light beam, the temperature and the current of the laser diode needed to be precisely set to a constant value. The speckle images were recorded by a Pixelink PL-B771F monochrome camera (1280×1024 pixels) equipped with a colour filter and a polarizer. The polarizer was set as to minimize the direct reflection from the illuminated skin surface (Fig. 1). 25 images were recorded for each applied exposure time values (1, 2, 5, 10, 20, 50 and 100 ms, respectively) at a frame rate of 6 to 10 fps depending on the actual exposure time. Since several exposure time values were applied, the average greyscale intensity had to be assured by a computer controlled variable neutral density filter. The recording and data processing were realised with a home-made software running in LabVIEW environment.

Simultaneously to the speckle measurements, blood perfusion was measured with a two channel Periflux 4000 laser Doppler system operating at 635 nm wavelength. The flow rate was obtained in perfusion units, which is further referred in the present paper as Laser Doppler Perfusion Unit (LDPU). Since the simultaneous measurement of the same area with both the LASCA and laser Doppler system was not possible, the Doppler probes were placed around the area monitored by the speckle system, as shown in Fig. 1 (the diameter of the probe holders was 35 mm, core diameter 0.125 mm, 0.25 mm fibre separation). The studied surfaces were chosen as to avoid large, visible blood vessels.

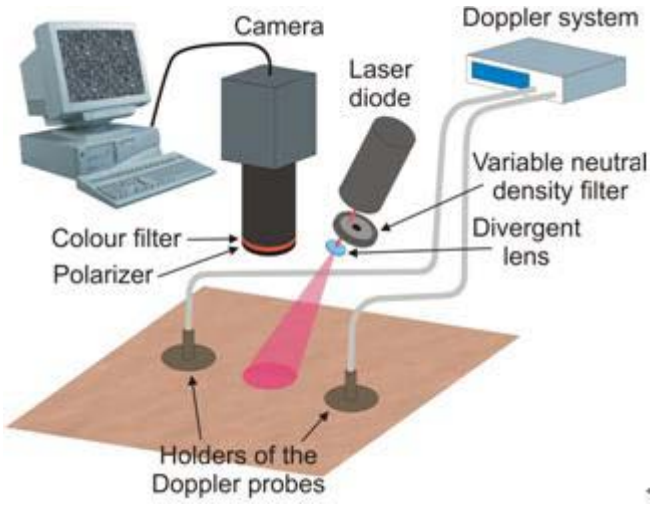


Fig. 1 The experimental setup consisting of a laser speckle and a commercial laser Doppler system

III. IMAGE PROCESSING

The contrast maps were obtained by the calculation of the local contrast on 5×5 pixel areas. For further calculations, the contrast was averaged over an area corresponding to ~2mm×2mm skin surface for every image.

The correlation times were calculated by using three different methods and the $1/\tau$ is referred in the present paper as Speckle Contrast measured Perfusion Unit (SCPU):

i) Conventional single exposure: Only images recorded with 10 ms exposure time were considered and the correlation time was calculated from equation (1), which supposes an ideal case when the contrast values are in the range of [0, 1] depending on the exposure time and actual correlation time ($K_1(0)=1$, $K_1(\infty)=0$).

ii) β corrected single exposure: Only images recorded with 10 ms exposure time were considered and the correlation time was calculated from equation (2) which takes the β coherence factor into account, however it still neglects the contribution of the static scattering ($K_2(0)=\sqrt{\beta}<1$, $K_2(\infty)=0$). The β was determined from the speckle contrast of images recorded from a white paper sheet placed at the object plane of the camera as proposed in the literature.

iii) Multi-exposure: The images recorded with all the applied exposure times were taken into account and the correlation time is calculated by fitting of the equation (3) to the measured contrast values.

IV. RESULTS AND DISCUSSION

The absolute perfusion value in the skin (and also the correlation time in LASCA measurements) strongly depends on the actually investigated area and differs from person to person. Since the Doppler probes could not be directly placed on the area monitored by the LASCA system, therefore relative

perfusion changes were measured instead of the use of the absolute perfusion unit values. Three series of multiple exposure time images were captured within every set of measurements (a baseline, one after applying ischemia and one after the reperfusion) while Doppler signal was continuously recorded. The first image series was recorded on the resting forearm, and the measured LDPU and SCPU values were regarded as 100%. Then ischemia was applied by pumping the cuff of a sphygmomanometer 30 mmHg above the systolic pressure. When the Doppler measurement indicated a stabilized perfusion value, the second series of speckle images were recorded. The third series was captured after the cuff was released (reperfusion) and the blood perfusion value stabilized. The relative perfusion values after ischemia and reperfusion were determined from speckle images with all three calculation methods and compared to the data of laser Doppler measurements. Although the wavelength used in LASCA and Doppler measurements were different, a study of Abbot *et al.* [23] indicated that results of Doppler imaging using red and infrared light, respectively, are similar on normal (healthy) skin of dorsal hand, if there are no larger vessels under the skin that could influence the results given by the deeper penetrating near-infrared light.

Measurements were performed on seven healthy volunteers aged between 20 and 35. On the same day two measurements were performed on the same person with 20 minutes resting time between them. A part of the measurements were unusable due to the significant displacement of the arm during the measurement.

Figure 2 shows an example for contrast values calculated for different exposure times. The contrast values which were determined for each image captured at the same exposure time, have a fluctuation due to the statistical noise and the fine random movements of the skin. The movements obviously lead to a slight decrease of the measured contrast (blurring), especially in case of higher exposure times. The graph also shows the curves obtained with the three evaluation methods fitted on the contrast data. It is clearly visible that the $K_1(T)$ curve corresponding to the single exposure time method (*i*) is strongly deviated from the experimental contrast values, except for the 10 ms data, since determination of the correlation time is based on this exposure time. The use of β correction factor (*ii*) results in a better agreement between the theoretical $K_2(T)$ curve and experimental data. The best agreement can be achieved by fitting equation (3) to the contrast values measured for all the exposure times. In this case, the $K_3(T)$ fits to the data well within the uncertainty of the measured values.

Figure 3 shows the relative perfusion values measured by the three LASCA evaluation methods as compared to the relative perfusion measured with the Doppler flowmeter. A line with a slope value of 1 is also shown on the figures to make the agreement/disagreement between the measured perfusion values more evident. The strong deviation of the experimental data can be the result of two main factors: the natural differences between the samples, and the non-uniform response of the different areas of the tissue. In most cases, when measurements are performed on biological tissues, the natural differences between the samples lead to a relatively high standard deviation. In the case of an evident tendency, this can be accepted if several data points are present. There is an evident discrepancy between the LASCA and laser Doppler measurements in the case of using method (*i*) (Figure 3.a, single exposure time without correction). In the same time, the best agreement can be reached by the use of multiple exposure time analysis (*iii*), which can be seen in Figure 3.c.

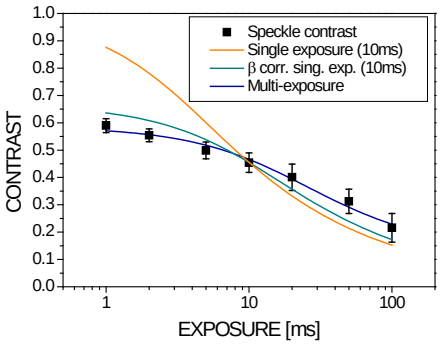
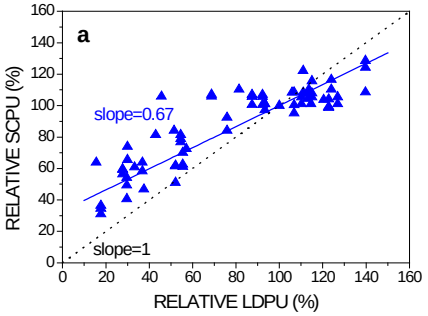
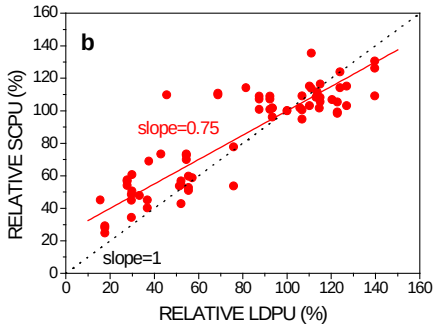


Fig. 2 Speckle contrast measured on forearm skin and curve fittings corresponding to the three evaluation methods as the function of the exposure time. The single exposure (K1(T)) and β corrected single exposure (K2(T)) analysis is based on the data measured at 10 ms exposure time, while the multi-exposure (K3(T)) evaluation method takes the data at every exposure times into account.

In order to get a quantitative value for the correlation between the different LASCA evaluations and the LD method, a straight line crossing the (100%, 100%) coordinates was fitted on the results. The slope of the line is a linear correlation factor, which gives the average ratio of the perfusion changes ($\Delta SCPU/\Delta LDPU$) measured with the LASCA and the laser Doppler system. The correlation factors were 0.67, 0.75 and 0.83 for the non-corrected single exposure, β corrected single exposure and multi-exposure methods, respectively. This indicates that there is an underestimation of the changes indicated by the Doppler measurements for all the three speckle evaluation methods. By taking a closer look onto the position of the data points along the fitted lines, it can be seen well that in the case of the multi-exposure method the points are uniformly distributed along the two sides of the line (Figure 3.c). In the same time there is an obvious non-linear behaviour in the case of (non corrected) single exposure (Figure 3.a): in the LDPU range of ~80-140% there is a relatively small change in the SCPU values, while in the case of lower perfusion values the $\Delta SCPU/\Delta LDPU$ is much higher. Therefore the linear correlation factor of 0.67 obtained for the whole studied range can be only used as a rough estimation for the correlation between the single exposure LASCA and LD data. The application of the β correction eliminates the non-linear behaviour (Figure 3.b). Roustit *et. al.* [24] compared Doppler data with speckle analysis on forearm skin using a commercial LASCA setup only utilizing β corrected single exposure time analysis. Although there was a good reproducibility for the speckle results, they also found that there is a nonlinear relationship between the real tissue perfusion and the results of the β corrected single exposure time based speckle analysis.





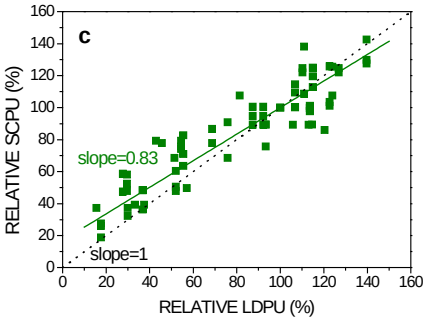


Fig. 3 Correlation between the LDPU and SCPU values for single exposure measurements (a), β corrected single exposure (b) and multiple exposure time method (c). Solid lines are linear fittings to crossing the (100%, 100%) point. Dotted lines correspond to the perfect match between the LASCA and LD measurements.

The results indicate that the contribution of the static scattering from the skin surface cannot be disregarded during laser speckle contrast analysis, which is a deficiency of conventional single exposure time methods. This is the reason of the relatively good agreement of the results given by the multiple exposure time analysis with the laser Doppler data. Though a ~17% underestimation of the measured perfusion change was observed as compared to the Doppler data, it may be attributed to the fine movements of the arm during the measurements which cause slight decreases of the speckle image contrast. These fine movements did not influence the Doppler signal since the probes were fixed on the surface of the skin.

Another outcome of the experiments is the proof of significant alteration of the *K(0)* short exposure- and *K(∞)* long exposure speckle contrast when the skin perfusion varies. As Figure 4 shows, the application of ischemia shifted the exposure time – contrast curve towards higher contrast values as compared to the initial state, while the reperfusion caused a decrease of the contrast. If the scattering properties would remain unchanged, the variation of the correlation time would only shift the exposure time - contrast curve parallel to the time axis. The explanation of the observed change of the fitting parameters is that the red blood cells and the static components have different scattering properties, and the alteration of the perfusion influences the ratio of the light originating from the two types of scatterers. Therefore the use of a single predetermined β value can lead to significant measurement errors when a wide range dynamics of blood perfusion is monitored. The application of multi-exposure method eliminates this deficiency by the continuous reconstruction of the actual *K(T)* curve.

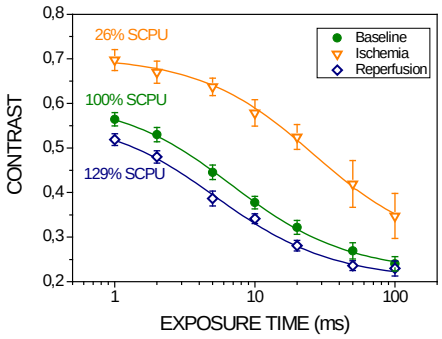


Fig. 4 Dependence of the speckle contrast on the exposure time for the same skin area for different perfusion states. Lines are fittings to the measured data. The relative perfusion values obtained with multi-exposure evaluation method are also indicated.

Another advantage of the multiple exposure time method is the flexibility of the system. Any variation in the optical settings of the imaging system (change of working distance and aperture) leads to a change in the local speckle contrast. Therefore in such cases the use of single exposure time with the β correction requires the recalibration (determination of the new β value) for the whole field of view, while such a calibration is not needed in case of the analysis of multiple exposure time images.

Since this multi-exposure method requires the recording of images with different exposure times, the temporal resolution is obviously lower than in case of single exposure time measurements. The aim of the present study was to demonstrate the viability of the multiple exposure time evaluation method, our setup was not optimized for increased temporal resolution. The optimisation for both the repetition rate and the elimination of the effects of the statistical noise requires further experiments. The $K_3(T)$ function contains three parameters (P_1, P_2 and τ), therefore the fitting requires the use of at least three different and well separated exposure times: a short one, an exposure time from the steeper part of the *K(T)* curve and a longer exposure time (e.g. 1, 10 and 100 ms, respectively). As a first approach, with the continuous alteration of the exposure time from frame to frame and the recording of a set of 3 images (1, 10 and 100 ms, respectively) can be easily realised with a period time of ~200 ms. This technique would offer an acceptable lateral resolution and the determination of the correlation time with the repetition rate of 5 fps.

V. CONCLUSIONS

Our earlier developed multiple exposure time based laser speckle contrast analysis method was compared to single exposure time evaluation procedures generally used for perfusion measurements. It is well known that the conventional single exposure time analysis methods can give only qualitative information on the perfusion changes when measurements are performed on skin, where the scattering from the stationary cutaneous tissue – in addition to the light scattered from the moving red blood cells – strongly contributes to the formation of the detected speckle pattern. Measurements were performed on forearm skin where relative perfusion changes caused by the application of ischemia were measured. Laser Doppler flowmetry was used to obtain reference data. The measured relative changes show an obvious nonlinear response for the non-corrected single exposure time evaluation when compared

to the Doppler data. The use of β correction significantly improves the accuracy. However, the best agreement with a linear correlation factor of 0.83 between the speckle and Doppler data was obtained by the use of multiple exposure times. The method was able to take the contribution of the static scattering into consideration and to handle the actual influence of the perfusion to the forming speckle pattern, which heavily affect the scattering properties of the sample. The existing discrepancy between the SCPU and LDPU data may be attributed to the fine movements of the arm during the measurements.

Concerning the speed of the different procedures, while for single exposure methods a contrast map can be obtained from every captured speckle image, our technique requires the recording of at least three images with different exposure times which presumably still can allow a contrast map repetition rate of approximately 5 fps depending on the applied exposure time values.

The linear relationship between the results of multiple exposure speckle method and the laser Doppler measurements obtained for a wide perfusion range demonstrate the flexibility and efficiency of our procedure.

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