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Multi-channel deep tissue flowmetry based on temporal diffuse speckle contrast analysis

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Abstract: Recently, diffuse speckle contrast analysis (DSCA) was introduced as a competent modality for deep tissue blood flow measurement, where the speckle contrast is calculated over spatial domain on the CCD image of diffuse reflectance. In this paper, we introduce time-domain DSCA where temporal statistics are used for speckle contrast calculation and results in the same deep tissue flow measurement. This new modality is especially suitable for multi-channel real-time flowmetry, and we demonstrate its performance on human arm during cuff occlusion test. Independent component analysis (ICA) study on multi-channel data shows promising results about underlying physiology.

OCIS codes: (030.6140) Speckle; (290.1990) Diffusion; (120.3890) Medical optics instrumentation.

References and links
Blood perfusion, or microcirculation, plays a critical role in human health, because it is responsible for transport of oxygen and nutrients to tissue, as well as blood pressure regulation. Compromised blood perfusion can lead to various diseases, such as stroke and cerebral infarction. Existing methods for blood perfusion measurement being used in clinical environment include Laser Doppler Flowmetry (LDF), contrast-enhanced computational tomography (CT) and MRI based Arterial Spin Labeling (ASL), etc. However, each of them has its own limitation: LDF is mostly applied on superficial layer, CT and MRI suffer from lack of flexibility and high expense.

Recently, a novel method for deep tissue blood flow measurement was developed and named diffuse speckle contrast analysis (DSCA) [1], which has a list of advantages including its deep tissue sensitivity, portability, cost-effectiveness. It is based on the theoretical understanding that the laser speckle contrast of the diffuse reflected photons can serve as a BFI within the physiologically relevant range of blood flow rate. Its instrumentation resembles that of diffuse correlation spectroscopy (DCS), but its analysis resembles that of spatialtemporal quantification of cerebral bloodflow during functional activation in rat somatosensory cortex using laser-speckle flowmetry,” J. Cereb. Blood Flow Metab. 24(5), 518–525 (2004).


laser speckle contrast analysis (LSCA). As a background, DCS is a popular modality for deep tissue blood flow measurement [2–6], especially in brain study [7–13], although it requires expensive high sensitivity photon detector and complicated model fitting process to obtain blood flow index (BFI). LSCA, on the other hand, is a powerful vessel imaging tool that is widely used in microvascular blood flow imaging [14–17] and skin perfusion measurement [18–21]. Although it is only suitable for superficial layer imaging or requires transparent medium, because of the wide field illumination mode, its simple instrumentation and ease of analysis make it well accepted in neuroscience community. LSCA can be also performed in spatial frequency domain in order to minimize the effect of different optical properties [22, 23]. By combining the DCS geometry and the LSCA analysis, DSCA achieves deep tissue flow sensitivity without requiring expensive detectors.

Although DSCA has many advantages over existing modalities, it is not without disadvantages. For blood perfusion measurement, the CCD camera can’t be easily mounted on the subject’s skin. Furthermore, any movement of the subject can cause defocus which affects the result very much. Thus in real practice, the fiber based probe that can be fixed on the skin surface is preferred. One way to achieve this in DSCA is to incorporate temporal laser speckle contrast analysis (TLSCA) [18, 24] to make a multi-channel device out of one CCD camera. Here temporal laser speckle contrast $K_t$ is applied for deep tissue blood flow measurement rather than spatial laser speckle contrast $K_s$.

In this paper, a cost effective multi-channel deep tissue flowmetry based on the temporal domain diffuse speckle contrast analysis (tDSCA) method is introduced. Compared with spatial domain DSCA (sDSCA), tDSCA is more practical to serve as a bedside blood flow monitor, and multi-channel friendly. Because one CCD camera is inherently a multi-channel detector with millions of pixels, the tDSCA device can be expanded into multiple channels without loss of temporal resolution or additional cost. Both flow-controlled phantom experimental result and in-vivo result are demonstrated and discussed with respect to simulation results.

2. Theory

In LSCA, $K_s$ is defined as the ratio of the spatial standard deviation of the speckle intensity to the mean intensity [25–27],

$$K_s = \frac{\sigma_s}{\langle I \rangle} \tag{1}$$

where the subscript $s$ means spatial operation. Usually $7 \times 7$ adjacent pixels from one single image are used to calculate $K_s$ [18]. However in TLSCA, temporal standard deviation of the speckle intensity $\sigma_t$ is applied instead of $\sigma_s$, and $K_t$ is computed otherwise in the same way as $K_s$. Thus TLSCA requires multiple images of the same area, out of which $K_t$ is calculated for each pixel [24]. There exists trade-off between spatial and temporal resolutions, where the spatial analysis offers better temporal resolution while the temporal analysis offers better spatial resolution.

It has been proven that given certain exposure time, the speckle contrast $K$ is determined by the normalized electric field autocorrelation $g_i(\tau)$. The relationship is given by [25]

$$K^2(T) = V(T) = \frac{2\beta}{T} \int_0^\tau \left(1 - \frac{\tau}{T}\right) \left[g_i(\tau)\right]^2 d\tau \tag{2}$$

where $T$ is the exposure time, $V$ is the normalized variance, $\tau$ is delay time, $\beta$ is a constant that depends on the ratio of detector size to the speckle size and polarization. If the detector size is comparable to the speckle size, $\beta$ is known to be 0.5 for polarized source and unpolarized detector. Although $g_i(\tau)$ is mostly assumed as simple exponential form in LSCA where mostly singly scattered photons are detected, Eq. (2) holds for general cases, including semi-infinite reflection geometry of diffuse medium, where light diffusely propagates into
deep tissue where scatterers move (microcirculation) and back out to the surface before being detected.

In DCS, under the assumption of homogeneous semi-infinite boundary condition, BFI can be derived from unnormalized electric field autocorrelation function $G_l(\tau)$ which has the following expression [4, 6],

$$G_l(r, \tau) = \frac{3\mu_s}{4\pi} \left[ \frac{\exp(-k_d(r)\tau)}{r_1} - \frac{\exp(-k_d(r)\tau)}{r_2} \right].$$  \tag{3}

Here $k_d(r) = \sqrt{3\mu_s'\mu_s + \alpha\mu_s'k_s^2\langle r^2(\tau) \rangle}$, $\mu_s'$ is reduced scattering coefficient, $\mu_s$ is absorption coefficient, $\alpha$ is the fraction of dynamic photon scattering events in medium, $r_1 = \sqrt{r^2 + z_s^2}$, $r_2 = \sqrt{r^2 + (z_s + 2z)^2}$, and $r$ is source-detector separation, $z_s = 1/\mu_s'$, $z_s = 2(1-R_{\text{eff}})/3\mu_s(1+R_{\text{eff}})$, where $R_{\text{eff}}$ represents effective reflection coefficient. $\langle r^2(\tau) \rangle$ represents the mean square displacement of the moving scatterers after a delay time $\tau$.

After repeated observation that the real experimental data fitted better with Brownian motion model than with random flow model, $D_b\tau$ became widely accepted, where $D_b$ is the effective diffuse coefficient [4, 6]. Furthermore, several groups have shown that the fitted $aD_b$ correlates well with other blood flow measurement modalities, such as ASL-MRI and transcranial Doppler ultrasound [4, 28–30]. Thus $aD_b$ is accepted as BFI in DCS practice, despite the fact that its dimension is neither of flow rate nor of speed. In this paper we also follow this convention, using $aD_b$ as BFI.

Practically, what is measured in DCS is the temporal intensity fluctuation of the diffuse reflected laser light. Then the normalized intensity autocorrelation function $g_2(\tau)$ is computed by either hardware correlator or software correlator [6]. $g_1(\tau)$ and $g_2(\tau)$ are related by the Siegert relation,

$$g_2(\tau) = 1 + \beta|g_1(\tau)|^2$$ \tag{4}

where $\beta$ is the same constant as the one in Eq. (2).

In DSCA, Eqs. (2)-(3) are combined together to build the relationship between $K$ and $aD_b$.

Our previous work [1] has shown that $1/K^2$ is linearly proportional to $aD_b$ within the physiologically relevant range of blood flow. The numerical simulation result is demonstrated in Fig. 1.
Fig. 1. Theoretical calculation of $1/K^2$ as a function of $aD_b$ over a broad range. Red box region, which is magnified in the inlet, refers to the region where $aD_b$ is in the physiological range. A good linearity is observed between $1/K^2$ and $aD_b$ within the physiologically relevant region. In this simulation, $\mu_s' = 8 \text{ cm}^{-1}$, $\mu_a = 0.03 \text{ cm}^{-1}$, $\beta = 0.5$, and the exposure time of 0.2 ms was used.

We used the similar optical properties as human tissue, where $\mu_s' = 8 \text{ cm}^{-1}$ and $\mu_a = 0.03 \text{ cm}^{-1}$. From the simulation result, $1/K^2$ is shown to be an increasing function of $aD_b$, with a gradually decreasing slope. However, in the lower range of $aD_b$, the relation is close to linearity. Fortuitously, the reported physiologically relevant maximum values of in vivo $aD_b$ are located in the approximate linear range ($-1 \times 10^{-6} \text{ cm}^2/\text{s}$) [4, 7, 8, 10, 29]. This is the theoretical basis for DSCA that $1/K^2$ can be used as a substitute for the conventional BFI in DCS.

We have shown the spatial speckle contrast $K_s$ can be used for rBF measurement in our previous work [1]. Since there is no fundamental difference between $K_s$ and $K_t$, we build our fiber based multi-channel rDSCA device on the same theoretical basis.

3. Instrument and method

In our 3-channel rDSCA setup, the light source is a continuous-wave laser with a long coherence length (>10 meter) operating at 785 nm (DL785-100-S, ~100mW, CrystaLaser, Reno, Nevada, USA). A high quality VGA camera (Stingray F033, Allied Vision Technology) is used as detector. As shown in Fig. 2(a), the laser source is divided equally into four multi-mode fibers with three cascading 50:50 fiber splitters, where three of them are used as source fibers in the probes, and the other one is used for source power monitoring. Three single mode fibers are used as detector fibers in the probes, with the other ends touching onto the CCD chip directly. The CCD camera is controlled by a laptop, with LabVIEW program (National Instrument).

We set the frame rate at 60 fps, and calculate $1/K_t^2$ every 1 second with 60 data points. Thus the update rate for rDSCA system is 1 Hz. The moving average of $1/K_t^2$ with a window size of 4 is applied for further data smoothing.

Fig. 2. Schematic of (a) rDSCA setup and (b) the phantom experiment. S1, S2 and S3 are 50:50 fiber splitters, PD is photon detector for power monitoring. Small glass beads are filled inside the hollow tube which is embedded in the solid phantom to provide randomized interstitial space for the flow.
To test the 3-channel tDSCA device, we performed a flow-controlled phantom experiment. The optical properties of the solid phantom were $\mu_s' = 8 \text{ cm}^{-1}$ and $\mu_a = 0.03 \text{ cm}^{-1}$, as described in our previous work [6]. As shown in Fig. 2(b), a hollow tube was embedded inside the solid body, and was tightly filled up with small glass beads to provide randomized interstitial space for the flow. To mimic the blood, we diluted Lipofundin N 20% (B.Braun Melsungen AG, Germany) into concentration of 0.6%. A peristaltic pump was used to control the flow rate inside the tube.

Before the tDSCA test, we performed DCS measurement on the upper surface of the phantom by our software correlator-based DCS system [6], with a source-detector separation of 3 cm. The flow rate was controlled from 0 to 0.35 ml/s, in steps of 0.05 ml/s.

Then we located each tDSCA probe in the same position as that of DCS probe, and tested them one by one with the same flow-control protocol. These three tests can be regarded as independent. The exposure time was 0.2 ms. The results will be shown in the next section.

4. Result

![Figure 3](image.png)

**Fig. 3.** (a) Relationship between $aD_b$ and flow rate measured by DCS on the flow phantom. (b) Dependence of $1/K_2$ on BFI, measured on three different tDSCA channels. Note the x-axis is $aD_b$ not flow rate, as we converted flow rate into $aD_b$ using the linear relationship shown in Fig. 3(a). The simulation result from Eqs. (2)-(3) is also plotted in (b) for comparison.

Figure 3(a) shows the result from DCS measurement on flow phantom. Under Brownian motion assumption, the fitted $aD_b$ is very linear to the flow rate with a small offset. This offset value observed for zero flow is attributable to both innate Brownian motion of the particles inside the phantom and the inherent source fluctuation. We also observed very similar result in our previous work [6] with the same flow phantom, where we also confirmed the Brownian motion model gives better fitting than the random flow model. Therefore, the Fig. 3(a) can be regarded as a calibration curve between the flow rate and $D_b$ in our particular flow phantom, and it enables us to make comparison between the simulation result and the experimental tDSCA result. In Fig. 3(b), experimental results on three different channels are shown in different colors, and they almost overlap with each other showing very good consistency. Simulation result using Eqs. (2)-(3) is also shown for comparison, and both experimental and simulation results are in the same order of magnitudes and have similar slopes.

After the validation study using flow phantom, we performed in vivo experiment on human forearm and palm using cuff occlusion protocol, with exposure time of 0.2 ms. Two probes were placed on the surface of the inside of forearm, and one more probe was placed on the surface of the hypothenar area, with the same source-detector separation of 1.5 cm. During the experiment, we measured the baseline for 100 s. Then a cuff occlusion was applied on upper arm with a pressure of 200 mmHg for about 75 s, which would lead to a
dramatic decrease of blood flow in the forearm and palm. Finally the pressure was released, and the recovery period was also recorded.

Fig. 4. Results from 3-channel tDSCA system during in vivo experiment with arm-cuff protocol. The positions of the three probes are indicated by corresponding colors on the arm.

The responses from three channels are plotted in Fig. 4. The two channels on forearm show very similar response, while the other channel on palm shows much higher blood flow reading during baseline. The palm channel also shows quite different reactive hyperemia pattern than the other two after the release of the cuff. However, the results from all channels are very similar in amplitude during cuff occlusion period, during which the microcirculation becomes minimal.

The higher baseline BFI in palm can be explained by the presence of many well-perfused small muscles in palm, which makes the palm look red. During arterial cuff occlusion, however, the blood flow underneath the skin is very low in the whole upper limb equally, because the blood supply is blocked. Thus there is little difference of BFI among all channels during cuff occlusion.

Further analysis can be done on the simultaneous 3-channel data described above. As the three time series data are acquired concurrently, it can be assumed that they are in fact linear combinations of more fundamental modes. We indeed observe similar behaviors among the three channels in the baseline measurement, and performed independent component analysis (ICA) on the first 100s data shown in Fig. 4 assuming there are only two independent components (ICs). ICA is a well-known iterative method to find out a set of source time
series that are most independent of each other, typically used to solve blind source separation problems in audio signals or EEG signals. The results are shown in Fig. 5, where the two ICs are shown in Fig. 5(a), and the original data with common-mode removed data are shown in Fig. 5(b). The common mode that is removed is IC1 which shows big oscillations with frequencies below 0.1 Hz, that is believed to be the low-frequency oscillation (LFO) related to the waves in vasodilatation. This common-mode removal results in much flatter baseline behavior as shown in Fig. 5(b) as dotted lines, proving that ICA is beneficial when dealing with multi-channel concurrent data, as it enables one to separate out different physiologies.

5. Discussion

Compared with our previous work using sDSCA [1], the result from tDSCA system corresponds much better with the simulation. This is because the single mode fiber has a very small diameter (~4.5 μm), which is comparable to the speckle size. Thus β is close to 0.5. In the three independent tests, it is difficult to guarantee the three probes are exactly in the same position, which may cause the slight difference in slopes in Fig. 3(b).

In DSCA, we can take reference from LSCI on the optimal exposure time issue. It is suggested that the exposure time T should be around $\tau_c$ where $g_s(\tau) = 1/e$, to obtain the best sensitivity to particle dynamics [31]. Thus $T$ should be about 0.1~1 ms in typical DSCA measurement. We used exposure time of 0.2 ms in our tDSCA system, and it worked properly.

From our simulation result in Fig. 1, there is one-to-one correspondence between $aD_\nu$ and $1/K^2$, when optical properties, β and exposure time are given. Thus the simulation result can serve as the calibration curve from $1/K^2$ to the BFI in DCS. Because of the approximate linear relationship in physiologically relevant range of $aD_\nu$, it is straightforward to use $1/K^2$ to indicate rBF as a function of time for specific site.

The noise-like fluctuation in the baseline of our in vivo result is due to the low frequency oscillation (LFO) in normal blood flow regulation, not the instrument noise. The main frequency components (0.06 Hz in palm and 0.07 Hz in arm) correspond well with in vivo LFO frequency band [32, 33]. Moreover, during cuff occlusion, the readings are much smoother without obvious fluctuation. This confirms the stability of the tDSCA device and certifies that the fluctuation in the baseline originates from blood flow regulation.

We further attempted to separate out the LFO signal from the fluctuating baseline signal using ICA, and the result is promising as shown in Fig. 5. Although one of the positions (palm) is quite far from the other two (arm 1 and arm 2), we were able to see common behaviors among different channels as same brachial artery is responsible for perfusing whole lower arm. We anticipate that a systematic study with more number of channels that cover broader area on human body will result in interesting physiological assessment which is impossible using a single channel perfusion monitoring.

6. Conclusion

In conclusion, tDSCA is reported for the first time for deep tissue blood flow measurement, and a cost effective multi-channel tDSCA flowmetry device based on single CCD camera is demonstrated. tDSCA is clearly a more practical choice for bedside blood flow monitoring on multiple sites than sDSCA is. Taking the advantages of simple instrumentation, easy analysis and low cost into account, tDSCA can be regarded as a good alternative for DCS.

In the future, high sensitivity and high frame rate CCD can greatly increase the time resolution of tDSCA, which will enable observation of fast responses on multiple positions at the same time.

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