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ASSESSMENT OF OPTICAL CLEARING INDUCED IMPROVEMENT OF LASER SPECKLE CONTRAST IMAGING

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Laser Speckle Contrast Imaging (LSCI) plays an important role in studying blood flow, but suffers from limited penetration depth of light in turbid tissue. The strong scattering of tissue obviously reduces the image contrast which decreases the sensitivity to flow velocity. Some image processing or optical clearing methods have been proposed to lessen the deficiency, but quantitative assessment of improvement is seldom given. In this study, LSCI was applied to monitor the blood flow through a capillary embedded within various tissue phantoms at depths of 0.25, 0.45, 0.65, 0.85 and 1.05 mm, and the flow velocity in capillary was controllable from 0 to 4 mm/s. Here, glycerol, a common optical clearing agent, was mixed with Intralipid at different volume ratio to make the reduced scattering coefficient of tissue phantom decrease from 13.00 to 0.50 cm^{-1}. The quantitative analysis demonstrates that the optical clearing method can obviously enhance the image contrast, imaging depth, and sensitivity to blood flow velocity. Comparing the Laser Speckle Contrast Analysis methods and the optical clearing method, we find that for typical turbid tissue, the sensitivity to velocity estimated by the Laser Speckle Temporal Contrast Analysis (LSTCA) is twice of that by the Laser Speckle Spatial Contrast Analysis (LSSCA); while the sensitivity to velocity estimated by using the two analysis methods has a 10-fold increase, respectively, if addition of glycerol makes the reduced scattering coefficient of tissue phantom decrease by 30%. Combining the LSTCA and the optical clearing method, the sensitivity to flow velocity will be further enhanced.

Keywords: Glycerol; laser speckle contrast imaging; the sensitivity to flow velocity estimation; optical clearing; scattering; tissue phantom.

1. Introduction

Blood flow and perfusion dynamics is tightly coupled with health status of biological tissues. It is very important to monitor the dynamics of microvascular structure and function for clinical diagnosis, curative effect and basic research. Several techniques have been developed,[1–9] including Magnetic Resonance Imaging (MRI),[1] Computed Tomography (CT),[2] Angiography,[3] laser Doppler flowmetry (LDF),[4] and Laser Speckle Contrast Imaging (LSCI),[5,6] etc. However, some of the techniques lack in spatio-temporal resolution (MRI, CT, LDF), and Angiography is an invasive procedure since it requires some exogenous marker. Moreover,

most of the techniques cannot provide both the structural and functional information simultaneously. In contrast, LSCI, a full-field optical imaging technique proposed by the group of Briers and Fercher,[5,6] can provide a two-dimensional map of blood flow with high spatio-temporal resolution, which has played an important role in studying cerebral blood flow,[10,11] mesentery microcirculation,[12] etc. Unfortunately, LSCI suffers from the limited penetration depth of light in turbid tissues, so the previous investigations were mainly restricted within the imaging of blood flow in transparent tissues.

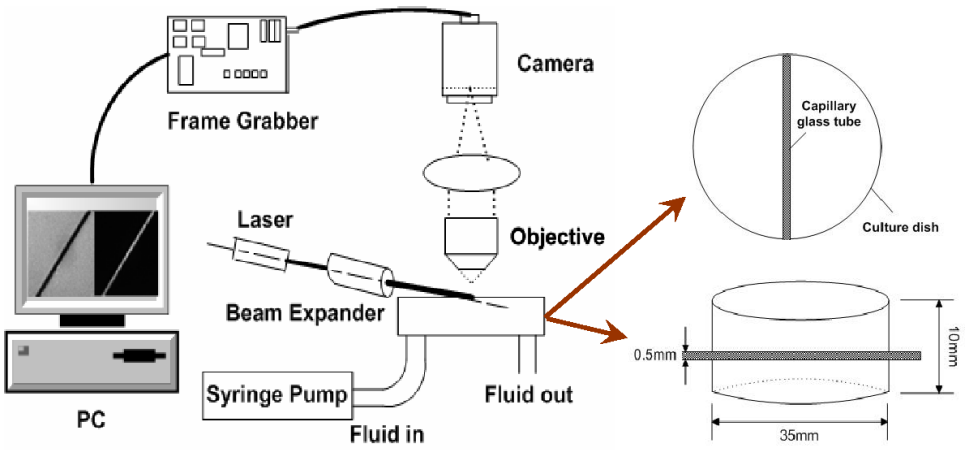
In order to improve the image contrast of LSCI in turbid tissue, various image processing methods have been proposed,[10,13] and some positive results have been attained. The Laser Speckle Temporal Contrast Analysis (LSTCA) method,[10] which could suppress the influence of static speckle, compared with the Laser Speckle Spatial Contrast Analysis (LSSCA) method, was proved to be able to access the cerebral blood flow velocity and vessel structure through an intact rat skull. The dynamic Laser Speckle Imaging (dLSI) also obtained activity maps through an intact and thinned rat skull with laser speckle imaging.[13] In reality, optical clearing method is efficacious for reducing the scattering of tissue.[14–18] Recently, we attained the dermal blood flow information with LSCI by topical application of a mixture of PEG400 and Thiazone onto the intact rat skin.[18] The above methods are significant to develop biomedical applications of LSCI, but previous investigations mainly show the improvement in visualization of image because LSCI is only valid for measuring relative velocity. Therefore, through *in vivo* experiment, it is difficult to quantitatively evaluate a developed method for improving the image contrast, imaging depth and sensitivity to blood flow velocity.

In this study, we employed LSCI to monitor the blood flow through a capillary embedded within various tissue phantoms at depths of 0.25, 0.45, 0.65, 0.85 and 1.05 mm. Glycerol, a common optical clearing agent, was mixed with Intralipid at different volume ratio to change the reduced scattering coefficient of tissue phantom. The optical clearing induced improvements of LSCI, including the image contrast, imaging depth and sensitivity to flow velocity, were examined. In order to quantitatively evaluate the enhancement in sensitivity to flow velocity induced by different methods, both the laser speckle temporal contrast and the laser speckle spatial contrast were calculated for different cases.

2. Materials and Methods

2.1. *Laser Speckle Contrast Imaging System*

Figure 1(a) shows the LSCI system used in this work. A He-Ne laser (632.8 nm, 17 mW, Melles Griot, 25 LHP 925-23, USA) beam illuminates the area of interest evenly at 45° incidence through a beam expander. The illuminated area is imaged through a zoom microscope (SZ6045TR, Olympus, Japan) onto a CCD camera (CoolSNAPes, Roperscientific, Tuscon, U.S.A) with 640 × 480 pixels. The exposure time of CCD was set as 20 ms.



(a) (b)

Fig. 1. Schematic of (a) Laser speckle contrast imaging system and (b) sample box.

A series of laser speckle images reflected from the area of interest were then acquired by using PVCAM software (Roper Scientific, Tuscon) at 40 Hz, connected to a PC.

In order to simulate skin blood flow, a glass capillary with an inner diameter of 500 μm (outer diameter of 700 μm) across a plastic box (35 mm diameter by 10 mm high) is connected with a syringe through a plastic hose [see Fig. 1(b)]. Whole blood from a healthy volunteer is injected into the capillary by a syringe pump, and the blood flow velocity could be controlled between 0 and 4 mm/s.

Intralipid (Sichuan Kelun Pharmaceutical Co. Ltd.), a common tissue phantom,[19,20] is applied to simulate the turbid skin. Previous studies show that the reduced scattering coefficients of *in vivo* skin range between 0.53 and 3.2 mm^{-1} at 633 nm,[21] which is closed to that of 1% Intralipid. In this work, we used the 1% Intralipid solution to simulate the native skin, and the mixtures of glycerol and 1% Intralipid at different volume ratios to imitate the skin after application of optical clearing agent (OCA). And pure water was also used as a transparent medium. When the solution just covers the capillary, we define the depth of capillary as 0.25 mm. The depth of capillary in the turbid solution can be changed with increasing volume of solution in the box.

2.2. *Principle of laser speckle contrast imaging*

Laser speckle is an interference pattern produced by light reflected or scattered from different parts of the illuminated area. If the illuminated object moves, a time-varying speckle pattern will be generated at each pixel in the image. The spatial and the temporal intensity variations of the pattern contain information on the movement of scatterers. By analyzing the spatial blurring of speckle images obtained by a CCD, a 2D blood flow distribution will be obtained. The blurring can be represented by speckle contrast (C),[5,6]

$$C = \sigma_s / \langle I \rangle, \tag{1}$$

where the mean intensity $\langle I \rangle$, and the standard deviation of the intensity variationσ_s, are calculated along square windows of $N \times N$ ($N = 5$) slid around the blurred image using LSSCA method. The contrast values can then be used to produce a map of speckle spatial contrast. Recently, the LSTCA method was more often used since it could suppress the influence of static speckle patterns.[10] The speckle temporal contrast TC is calculated using the following equation[10,22]:

$$TC(i,j) = \sigma_{i,j} / \langle I_{i,j} \rangle = \sqrt{\left\{ \sum_{n=1}^{N} [I_{i,j}(n) - \langle I_{i,j} \rangle]^2 \right\} \Big/ N - 1} \Big/ \langle I_{i,j} \rangle, \tag{2}$$

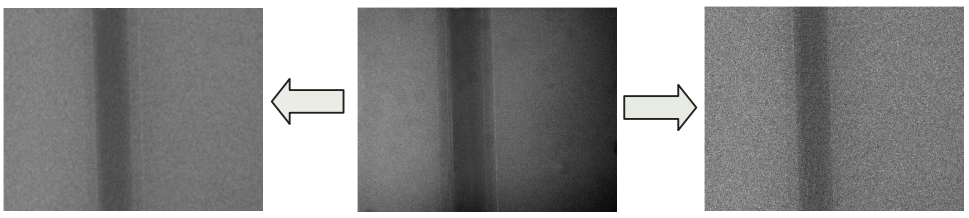
where $I_{i,j}(n)$ is the CCD counts at pixel (i, j) in the nth raw speckle map, N is the number of maps acquired, and $\langle I_{i,j} \rangle$ is the mean value of CCD counts at pixel (i, j) over the N maps. Figure 2 shows typical raw speckle map (b) and corresponding spatial contrast map (a) and temporal contrast map (c) of blood flow in tissue phantom.

2.3. *Experimental procedure*

2.3.1. *Preparation of tissue phantom*

In this work, we used 1% Intralipid solution to simulate the scattering property of native skin, and the mixture of glycerol and 1% Intralipid at a volume ratio of 15:85, 30:70, 50:50 and 75:25, respectively, to simulate more and more transparent tissue. In order to know glycerol-induced optical clearing, the reduced scattering coefficient of tissue phantom was measured as follows.

The prepared solution (1% Intralipid or mixture) was filled into a specially designed sample cell with a size of 50 × 50 mm and a thickness of



(a) (b) (c)

Fig. 2. (a) Spatial contrast map, (b) raw speckle map, and (c) temporal contrast map.

Table 1. Reduced scattering coefficient (μ_s') of various tissue phantom at 633 nm.

	Volume ratio of glycerol and 1% Intralipid				
	0:1	15:85	30:70	50:50	75:25
μ_s' (cm^{-1})	13.00 ± 0.01	9.30 ± 0.17	6.10 ± 0.01	3.20 ± 0.08	0.50 ± 0.01

1 mm. A commercially available spectrophotometer (Lambda-950, PerkinElmer, Waltham, Massachusetts) with a 150-mm integrating sphere was used for measuring the diffuse reflectance and transmittance of the samples at 633 nm. The sphere has an entrance-port and an exit-port of 24 mm in diameter. The reduced scattering coefficient was calculated with the IAD algorithm.[23] A total of six experiments were performed for each solution.

Table 1 shows the reduced scattering coefficient of tissue phantoms used in this work at 633 nm. It can be seen that the reduced scattering coefficient decreases obviously with the volume ratio of glycerol increasing which is consistent with the results available in literature.[20]

2.3.2. *Laser speckle contrast imaging for blood flow in tissue phantom*

Prior to experiments of laser speckle contrast imaging, we put one tissue phantom (1% Intralipid) into the special plastic box, and controlled the volume strictly so as to keep the capillary embedded within the phantom at the depth of 0.25 mm. The syringe pump was opened and a value of velocity was set. A series of raw speckle maps were then recorded after the velocity of blood flow became stable.

After that, we increase the value of velocity at 0.5 mm/s intervals, and recorded the raw speckle images again. The blood flow velocity used in this study was between 0 and 4 mm/s. Then, the depth of capillary was increased at 0.2 mm intervals and the above experiment was repeated; the maximal depth was 1.05 mm. And then the whole experiment was repeated when the capillary was embedded within the prepared mixture as shown in Table 1 or pure water.

3. Results

3.1. *OCA-induced improvement of LSCI*

Since the LSTCA is proved to be more valid than the LSSCA to eliminate the influence of static speckle, we constructed the corresponding temporal contrast images based on original raw speckle maps. Figure 3(a) shows typical temporal contrast images

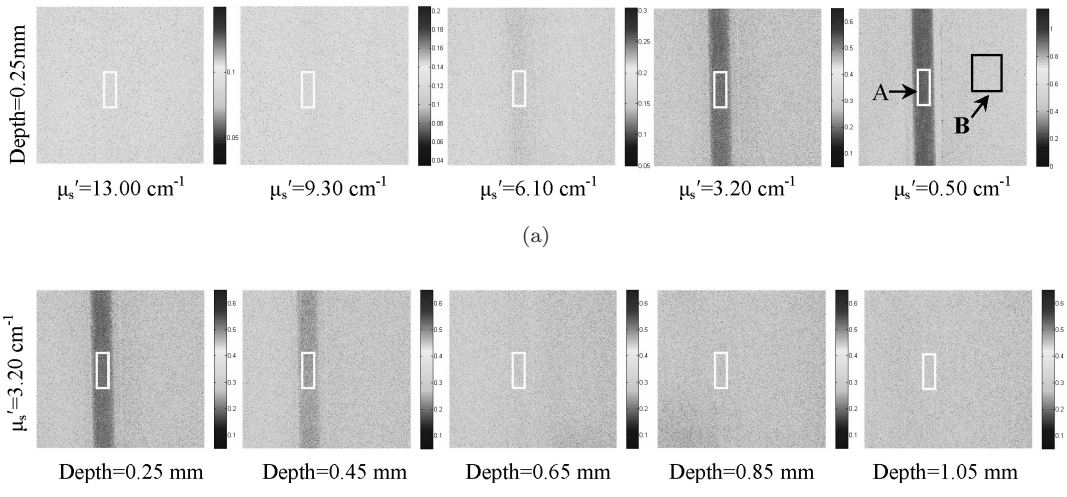


Fig. 3. Typical temporal contrast images when the blood flows with velocity of 1 mm/s through the capillary. (a) The capillary is embedded within tissue phantoms with reduced scattering coefficients between 13.00 and 0.50 cm^{-1} at a depth of 0.25 mm. (b) The capillary is embedded within a tissue phantom with the reduced scattering coefficient of 3.20 cm^{-1} at depths of 0.25, 045, 0.65, 0.85, and 1.05 mm. The rectangle A and B indicate the area of interest of blood flow or tissue phantom.

when the blood flows with velocity of 1 mm/s through the capillary within different tissue phantoms at a depth of 0.25 mm. The blood flow is almost concealed by strong noise when the reduced scattering coefficient of tissue phantom is larger than 9.30 cm^{-1}, and can be barely distinguishable till the reduced scattering coefficient of tissue phantom decreases to 6.10 cm^{-1}. The lower the scattering of tissue phantom, the more noticeable the image contrast.

Figure 3(b) demonstrates temporal contrast images when the capillary is embedded within a tissue phantom ($\mu_s' = 3.20\,cm^{-1}$) at depths of 0.25, 0.45, 0.65, 0.85, and 1.05 mm; here the blood flow velocity is 1 mm/s. As can be seen in the Fig. 3(b), the visualization of speckle contrast image is very well when the depth of capillary is less than 0.45 mm. In contrast, the visualization decreases when the depth of capillary is more than 0.65 mm, and the deeper the capillary stays, the worse the image contrast becomes.

Furthermore, the change of temporal contrast in the flow area (area A as shown in Fig. 3) with blood flow velocity is shown in Fig. 4. Here, Figs. 4(a)–4(e) represent the groups of the capillary at the depths of 0.25, 0.45, 0.65, 0.85 and 1.05 mm, respectively. Each curve corresponds to different tissue phantoms, including 1% Intralipid, and mixtures with different ratio of glycerol and 1% Intralipid, which have reduced scattering coefficient of 13.00, 9.30, 6.10, 3.20, 0.50 cm^{-1}. In order to compare the dependence of temporal contrast on the blood flow velocity for transparent and turbid medium, the measurements in pure water are shown in Fig. 4(a). The results show that temporal contrast decreases with increasing blood flow velocity, but the rate of change depends on the scattering of tissue phantom and the depth of capillary. For a depth, the lower the scattering, the larger the rate, and the largest rate is from the results of pure water; while the deeper the capillary is embedded, the less the rate. Comparing two extreme conditions, i.e., curve 5 in Fig. 4(a) and curve 1 in Fig. 4(e), the former represents temporal contrast when the capillary is embedded at the subsurface of a typical turbid phantom

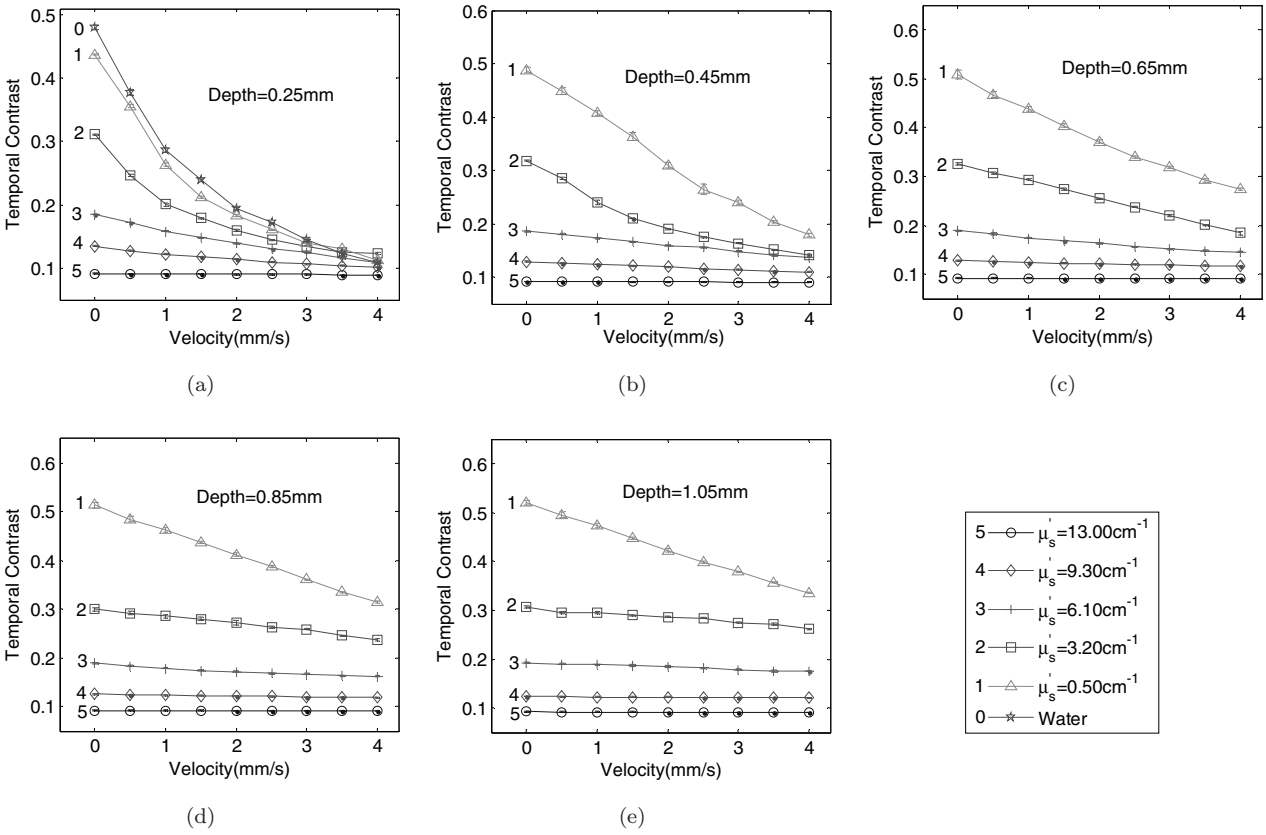


Fig. 4. Change of temporal contrast with blood flow velocity. (a)–(e) represent groups of the capillary at the depths of 0.25, 0.45, 0.65, 0.85 and 1.05 mm, respectively. Curves No. 1–5 reflect the data of tissue phantom with the reduced scattering coefficients of 0.50–13.00 cm^{-1}, and the curve No. 0 corresponds to the data of pure water.

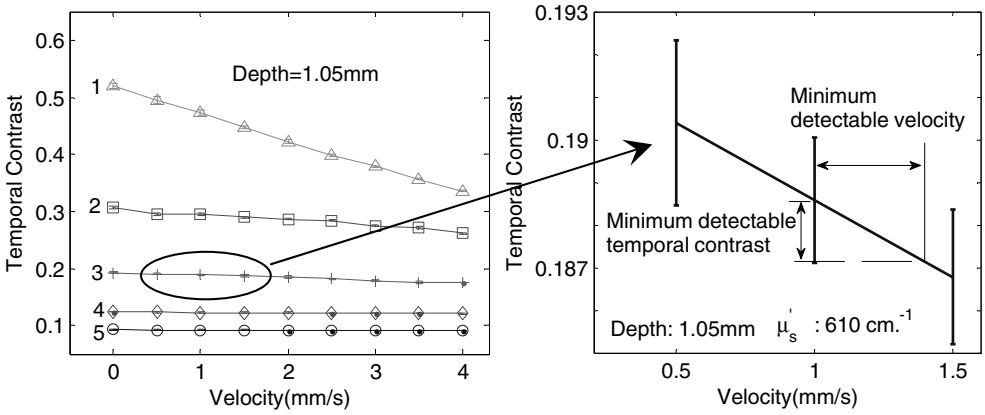


Fig. 5. Magnified curve for the calculation of minimum detectable flow velocity.

($\mu_s' = 13.00\,\mathrm{cm}^{-1}$), and the latter indicates that when the capillary is embedded within transparent tissue phantom ($\mu_s' = 0.50\,\mathrm{cm}^{-1}$) at the depth of 1.05 mm, we can find that the slope of the former is much smaller than that of the latter. Therefore, the imaging depth will be enhanced with the optical clearing of tissue phantom.

3.2. *Optical clearing enhances sensitivity to velocity*

The sensitivity of the LSCI system to flow velocity depends on its minimum detectable speckle contrast. In order to evaluate optical clearing–induced enhancement of sensitivity, Fig. 4(e) was redrawn [see Fig. 5(a)], and a curve was magnified [see Fig. 5(b)]. All data are shown in the format of Mean ± SD (standard deviation). For a LSCI system, when its parameters are set up, the deviation of measurement is unavoidable. The deviation decides the sensitivity of a system, but some additional factors may reduce the sensitivity to measurements. If the standard deviation of speckle contrast is C_{SD}, and the variation of speckle contrast caused by change in blood flow velocity isΔC_{SC}, we define the signal-to-noise ratio as follows:

$$SNR = \Delta\, C_{SC}/C_{SD}. \tag{3}$$

If $SNR \geq 1$, the variation of speckle contrast is detectable; otherwise, the variation cannot be detected. Hence, when the signal-to-noise ratio is equal to 1, the variation of speckle contrast corresponds a minimum detectable flow velocity which reflects the sensitivity of the system to flow velocity.

Usually, the velocity of capillary blood flow is about 1 mm/s, so we list the sensitivity to flow velocity varying with scattering of tissue phantom and depth of capillary when the blood flows with velocity of 1 mm/s (see Table 2). As can be seen in Table 2, the sensitivity of LSCI to flow velocity is almost the same when the capillary is embedded within tissue phantom with reduced scattering coefficient of 9.30 cm^{-1} at the depth of 0.25 mm, tissue phantom with $\mu_s' = 6.10$ cm^{-1} at the depth of 0.45 mm, tissue phantom with $\mu_s' = 3.20$ cm^{-1} at the depth of 0.65 mm, or tissue phantom with $\mu_s' = 0.50$ cm^{-1} at the depth of 0.85 mm. Therefore, we can find that the sensitivity to blood flow velocity is enhanced with the decreasing scattering

Table 2. Sensitivity to flow velocity for different tissue phantoms or capillary depths.

	Sensitivity to velocity (mm/s)				
Capillary Depth	$\mu_s' = 13.00\,\mathrm{cm}^{-1}$	$\mu_s' = 9.30\,\mathrm{cm}^{-1}$	$\mu_s' = 6.10\,\mathrm{cm}^{-1}$	$\mu_s' = 3.20\,\mathrm{cm}^{-1}$	$\mu_s' = 0.5\,\mathrm{cm}^{-1}$
0.25 mm	0.87	0.09	0.08	0.07	0.05
0.45 mm	1.06	0.13	0.09	0.08	0.07
0.65 mm	1.34	0.23	0.10	0.09	0.08
0.85 mm	1.47	0.30	0.12	0.10	0.09
1.05 mm	1.67	1.20	0.43	0.25	0.15

of tissue phantom when the capillary is embedded at a depth, and it becomes difficult for LSCI method to detect the change in flow velocity with the increasing depth of capillary for a tissue phantom.

Since the thickness of rat skin is between 0.4–0.5 mm,[18] here we paid more attention to results when the capillary is embedded within different phantoms at the depth of 0.45 mm. For typical skin ($\mu_s' = 13.00\ \text{cm}^{-1}$), the LSCI system can only detect the variation of velocity with 1.06 mm/s, which means that the variation of blood flow is undetectable except that the velocity increases more than twice. When the reduced scattering coefficient of tissue phantom decreases to 9.30 cm^{-1}, the change in velocity with 0.13 mm/s can be detected, which means that the sensitivity to flow velocity is enhanced by eight times comparing to typical turbid tissue.

3.3. *Comparison of improvement of sensitivity to velocity caused by contrast analysis and optical clearing methods*

The above results indicate that there are obvious enhancements of image contrast, imaging depth and sensitivity to velocity when the addition of glycerol decreases the scattering of tissue phantom. In order to further compare the improvement caused by the optical clearing and contrast analysis methods, the LSSCA and the LSTCA are used to calculate the sensitivity to flow velocity when the blood flows with velocity of 1 mm/s through the capillary within different tissue phantoms at depth of 0.25 mmand 0.45 mm. Table 3 summarizes the data.

As shown in Table 3, we can find that the sensitivity to velocity estimated by the LSTCA method is almost twice as great as that by the LSSCA method if the reduced scattering coefficient of tissue phantom is larger than 6.10 cm^{-1}. Nevertheless, if the reduced scattering coefficient of tissue phantom is less than 3.20 cm^{-1}, the LSTCA method can only make the sensitivity to velocity increase by 10–15% comparing with the LSSCA method.

Table 3. Sensitivity to flow velocity (at 1 mm/s).

Scattering of the tissue phantom (cm^{-1})	Sensitivity to velocity (mm/s)			
	Capillary Depth			
	0.25 mm		0.45 mm	
	LSSCA	LSTCA	LSSCA	LSTCA
13.00	1.91	0.87	1.97	1.06
9.30	0.19	0.09	0.23	0.13
6.10	0.15	0.08	0.17	0.10
3.20	0.08	0.07	0.09	0.08
0.50	0.06	0.05	0.08	0.07

Considering the effect of optical clearing, we can observe that the sensitivity to flow velocity almost increases by 10 fold at the depth of 0.25 mm and 8 fold at the depth of 0.45 mm whatever contrast analysis methods are adopted when the reduced scattering coefficient of tissue phantom decreases by 30%, i.e., from 13.00 to 9.30 cm^{-1} caused by optical clearing method. When the tissue becomes further transparent, i.e., the reduced scattering coefficient decreases by 53%, 75% or 96%, the LSTCA method can make the sensitivity to velocity increase by 13-, 24-, and 32-fold at the capillary depth of 0.25 mm, and by 12-, 22-, and 25-fold at the capillary depth of 0.45 mm, respectively. In contrast, the further enhancement of the sensitivity to flow velocity by using the LSSCA method is less than that by using the LSTCA for the same conditions. Combining the LSTCA and the optical clearing method, the sensitivity to flow velocity will be further enhanced.

4. Discussion

Recently, the hot topics of LSCI technique mainly focus on two aspects, one is to expand various applications of LSCI in transparent tissues[24,25]; another is to develop new methods to improve the image quality of LSCI in turbid tissue, such as the LSTCA[10] and the dLSI.[13] However, monitoring blood flow of turbid tissue, such as skin, needs an advanced imaging technique. Through skin, relative basic studies can be made, i.e., the influence of various vascular drugs on blood flow, estimation of hemodynamic response and functional activation can be obtained. It will be very significant for clinical diagnosis and treatment about peripheral vascular disease. In addition, the previous investigations just show the improvement in image contrast instead of quantitative assessment. Because LSCI is only valid for measuring relative velocity, it is very difficult to quantitatively evaluate a developed method for improving the image contrast, imaging depth and sensitivity to blood flow velocity based on *in vivo* experiment.

This study not only proves that the optical clearing method can decrease the scattering of

tissue and make LSCI for monitoring blood flow in turbid tissue possible, but also quantitatively assess the image contrast, imaging depth, and the sensitivity to blood flow velocity based on the experiments of blood flow in tissue phantom. Firstly, the quantitative evaluation indicates that it is indeed difficult for LSCI to detect the change in flow velocity of capillary embedded at the subsurface of typical turbid tissue. Secondly, our results support that the LSTCA method could enhance the sensitivity to velocity for high scattering media compared with the LSSCA method. Nevertheless, only doubled improvement caused by LSTCA is still not enough to detect the blood flow of capillary embedded under the subsurface of typical turbid phantom. In contrast, the optical clearing method for enhancing the sensitivity to velocity is more effective than the LSTCA method because after the decrease of 30% in scattering of tissue phantom after application of glycerol, the sensitivity to flow velocity at depth of 0.25, 0.45 mm can increase by ten or eight times when the LSTCA or the LSSCA methods are used, respectively. Moreover, the improvement of sensitivity to velocity will be further enhanced by combining the LSTCA with optical clearing method.

We admit that it is much easier to make tissue phantom transparent than to make skin transparent because of the barrier of the epidermis. However, it is possible to make the decrease of 30% in the scattering of skin by topical application of OCAs with advanced physical or chemical methods.[18,26] With the development of optical clearing method, LSCI technique will be able to be applied to monitor blood flow of turbid tissue, such as blood flow of skin. In this study, some quantitative evaluation has been given based on experiments of tissue phantom, but there may be some differences between the experiments performed in this work and in *in vivo* condition. We know that living tissues or organisms are very complex, and some side effects of OCAs on the structure and function of the vascular tissue may occur. Our previous investigations show that the flow velocity would be reduced and the blood vessels were even blocked when glycerol or glucose with high concentrations were directly applied on blood vessels of chick chorioallantoic membrane; the side effects were reduced with the decrease of concentration of glycerol or glucose, and meanwhile the application of two OCAs showed different short-term and long-term effects.[25] Therefore, the different response of vascular to OCAs may depend on the doses and kinds of OCAs that penetrated into blood vessels. Actually, the dose of OCA that penetrated into blood vessels with topical application will be much less than that with direct application; hence, the side effects should be less. Recently, topical application of a mixture of PEG400 and Thiazone on rat skin *in vivo* could make the local skin transparent and dermal blood flow could be assessed by using LSCI, and the flow velocity almost kept constant during the whole clearing period.[18] Future experiments are warranted to study biocompatibility of various OCAs in more detail.

5. Conclusions

In this work, LSCI was applied to monitor the blood flow in capillary embedded within various tissue phantoms at different depths. The enhancement of image contrast, imaging depth and sensitivity to flow velocity was quantitatively analyzed. Comparing the Laser Speckle Contrast Analysis methods and the optical clearing method, we find that for typical turbid tissue, the sensitivity to velocity estimated by the LSTCA is twice of that by the LSSCA; while the sensitivity to velocity estimated by the two analysis methods has several times increase respectively if addition of glycerol makes the reduced scattering coefficient of tissue phantom decrease. Combining the LSTCA and the optical clearing method, the imaging depth and the sensitivity to velocity will be further enhanced. It may be possible for LSCI to be applied to optical diagnosis of skin microcirculation and assessment of healing efficacy of related diseases. In summary, this study provides a quantitative analysis approach to evaluate a developed method for improving LSCI.

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References

1. R. A. Dashner, D. W. Chakeres, A. Kangarlu, P. Schmalbrock, G. A. Christoforidis, R. M.

DePhilip, "MR imaging visualization of the cerebral microvasculature: A comparison of live and postmortem studies at 8 T," *Am. J. Neuroradiol.* **24**, 1881–1884 (2003).
2. R. von Kummer, J. Weber, "Brain and vascular imaging in acute ischemic stroke: The potential of computed tomography," *Neurology* **49**, 52–55 (1997).
3. M. Schumacher, "Microangiographic study of the normal anatomy of the cerebral venous system in rats," *Neuroradiology* **26**, 137–140 (1984).
4. S. A. Sheth, M. Nemoto, M. W. Guiou, M. A. Walker, A. W. Toga, "Spatiotemporal evolution of functional hemodynamic changes and their relationship to neuronal activity", *J. Cereb. Blood Flow Metab.* **25**, 830-841 (2005).
5. J. D. Briers, S. Webster, "Laser speckle contrast analysis (LASCA): A non-scanning, full-field technique for monitoring capillary blood flow," *J. Biomed. Opt.* **1**, 174–179 (1996).
6. J. D. Briers, "Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging," *Physiol. Meas.* **22**, R35–R66 (2001).
7. S. S. Ulyanov, "A new type of manifestation of Doppler effect: An application to blood and lymph flow measurements," *Opt. Eng.* **34**, 2850–2855 (1995).
8. S. S. Ulyanov, V. V. Tuchin, A. A. Bednov, G. E. Brill, E. I. Zakharova, "Speckle- interferometric method in application to the blood and lymph flow monitoring in microvessels," *Lasers Med. Sci.* **12**, 31–41 (1997).
9. S. S. Ulyanov, "Speckled speckles statistics with a small number of scatterers: An implication for blood flow measurements," *J. Biomed. Opt.* **3**, 237–245 (1998).
10. P. Li, S. Ni, L. Zhang, S. Zeng, Q. Luo, "Imaging cerebral blood flow through the intact rat skull with temporal laser speckle imaging," *Opt. Lett.* **31**, 1824–1826 (2006).
11. H. Cheng, Q. Luo, S. Zeng, S. Chen, W. Luo, H. Gong, "Hyperosmotic chemical agent's effect on *in vivo* cerebral blood flow revealed by laser speckle," *Appl. Opt.* **43**, 5772–5777 (2004).
12. D. Zhu, W. Lu, Y. Weng, H. Cui, Q. Luo, "Monitoring thermal induced changes in tumor blood flow and micro-vessels with laser speckle contrast imaging," *Appl. Opt.* **46**, 1911–1917 (2007).
13. A. K. Dunn, H. Bolay, M. A. Moskowitz, D. A. Boas, "Dynamic imaging of cerebral blood flow using laser speckle," *J. Cereb. Blood Flow Metab.* **21**, 195–201 (2001).
14. V. V. Tuchin, I. L. Maksimova, D. A. Zimnyakov, I. L. Kon, A. H. Mavlutov, A. A. Mishin, "Light propagation in tissues with controlled optical properties," *J. Biomed. Opt.* **2**, 401–417 (1997).
15. V. V. Tuchin, "Optical immersion as a new tool for controlling the optical properties of tissues and blood," *Laser Phys.* **15**, 1109–1136 (2005).
16. Z. Zhi, Z. Han, Q. Luo, D. Zhu, "Improve optical clearing of skin *in vitro* with propylene glycol as a penetration enhancer," *J. Innovat. Opt. Health Sci.* **2**, 269–278 (2009).
17. Z. Mao, D. Zhu, Y. Hu, X. Wen, Z. Han, "Influence of alcohols on the optical clearing effect of skin *in vitro*," *J. Biomed. Opt.* **13**, 021104 (2008).
18. D. Zhu, J. Wang, Z. Zhi, X. Wen, Q. Luo, "Imaging dermal blood flow through the intact rat skin with an optical clearing method," *J. Biomed. Opt.* **15**, 026008 (2010).
19. H. J. van Staveren, C. J. M. Moes, J. van Marie, S. A. Prahl, M. J. C. van Gemert, "Light scattering in Intralipid-10% in the wavelength range of 400–1100 nm," *Appl. Opt.* **30**, 4507–4514 (1991).
20. X. Wen, V. V. Tuchin, Q. Luo, D. Zhu, "Controling the scattering of Intralipid by using optical clearing agents," *Phys. Med. Biol.* **54**, 6917–6930 (2009).
21. T. V. Dinh, *Biomedical Photonics Handbook*, CRC Press, Florida (2003).
22. H. Cheng, Q. Luo, S. Zeng, S. Cheng, J. Cen, H. Gong, "Modified laser speckle imaging method with improved spatial resolution," *J. Biom. Opt.* **8**, 559–564 (2003).
23. S. Prahl, M. van Gemert, A. Welch, "Determining the optical properties of turbid media by using the adding doubling method," *Appl. Opt.* **32**, 559–568 (1993).
24. B. Choi, N. M. Kang, J. S. Nelson, "Laser speckle imaging for monitoring blood flow dynamics in the *in vivo* rodent dorsal skin fold model," *Microvasc. Res.* **68**, 143–146 (2004).
25. D. Zhu, J. Zhang, H. Cui, Z. Mao, P. Li, Q. Luo, "Short-term and long-term effects of optical clearing agents on blood vessels in chick chorioallantoic membrane," *J. Bio. Opt.* **13**, 021106 (2008).
26. C. Liu, Z. Zhi, V. V. Tuchin, Q. Luo, D. Zhu, "Enhancement of skin optical clearing efficacy using photo-irradiation," *Lasers Surg. Med.* **42**, 132–140 (2010).