Accessing to arteriovenous blood flow dynamics response using combined laser speckle contrast imaging and skin optical clearing

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Abstract: Laser speckle contrast imaging (LSCI) shows a great potential for monitoring blood flow, but the spatial resolution suffers from the scattering of tissue. Here, we demonstrate the capability of a combination method of LSCI and skin optical clearing to describe in detail the dynamic response of cutaneous vasculature to vasoactive noradrenaline injection. Moreover, the superior resolution, contrast and sensitivity make it possible to rebuild arteries-veins separation and quantitatively assess the blood flow dynamical changes in terms of flow velocity and vascular diameter at single artery or vein level.

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OCIS codes: (120.6150) Speckle imaging; (290.0290) Scattering; (170.1470) Blood or tissue constituent monitoring; (150.1135) Algorithms.

References and links

1. L. A. Holowatz, C. S. Thompson-Torgerson, and W. L. Kenney, “The human cutaneous circulation as a model of generalized microvascular function,” J. Appl. Physiol. 105(1), 370–372 (2008).
2. H. A. Struijker-Boudier, A. E. Rosei, P. Bruneval, P. G. Camici, F. Christ, D. Henrion, B. I. Lévy, A. Pries, and J. L. Vanoverschelde, “Evaluation of the microcirculation in hypertension and cardiovascular disease,” Eur. Heart J. 28(23), 2834–2840 (2007).
3. F. Quondamatteo, “Skin and diabetes mellitus: what do we know?” Cell Tissue Res. 355(1), 1–21 (2014).
4. M. Rossi, A. Carpi, F. Galetta, F. Franzoni, and G. Santoro, “The investigation of skin blood flow motion: a new approach to study the microcirculatory impairment in vascular diseases?” Biomed. Pharmacother. 60(8), 437–442 (2006).
5. G. J. Hodges and A. T. Del Pozi, “Noninvasive examination of endothelial, sympathetic, and myogenic contributions to regional differences in the human cutaneous microcirculation,” Microvasc. Res. 93, 87–91 (2014).
6. J. Henricson, A. Nilsson, E. Tесселаар, G. Nilsson, and F. Sjöberg, “Tissue viability imaging: microvascular response to vasoactive drugs induced by iontophoresis,” Microvasc. Res. 78(2), 199–205 (2009).
7. G. M. Palmer, A. N. Fontanella, S. Shan, G. Hanna, G. Zhang, C. L. Fraser, and M. W. Dewhirst, “In vivo optical molecular imaging and analysis in mice using dorsal window chamber models applied to hypoxia, vasculature and fluorescent reporters,” Nat. Protoc. 6(9), 1355–1366 (2011).
8. R. K. Jain, L. L. Munn, and D. Fukumura, Transparent Window Models and Intravital Microscopy: Imaging Gene Expression, Physiological Function and Therapeutic Effects in Tumors, Tumor Model in Cancer research, 641–679, Humana Press (2011)
9. S. Yousefi, J. Qin, S. Dzieniss, and R. K. Wang, “Assessment of microcirculation dynamics during cutaneous wound healing phases in vivo using optical microangiography,” J. Biomed. Opt. 19(7), 076015 (2014).
10. Y. Zhou, J. Yao, and L. V. Wang, “Optical clearing-aided photoacoustic microscopy with enhanced resolution and imaging depth,” Opt. Lett. 38(14), 2592–2595 (2013).
11. Y. Boulafliji, L. Lamrani, M. C. Rouzaud, S. Loyau, M. Jandrot-Perrus, M. C. Bouton, and B. Ho-Tin-Noé, “The mouse dorsal skinfold chamber as a model for the study of thrombolysis by intravital microscopy,” Thromb. Haemost. 107(5), 962–971 (2012).
12. M. W. Laschke, B. Vollmar, and M. D. Menger, “The dorsal skinfold chamber: window into the dynamic interaction of biomaterials with their surrounding host tissue,” Eur. Cell. Mater. 22, 147–167 (2011).
13. H. Wang, L. Shi, J. Qin, S. Yousefi, Y. Li, and R. K. Wang, “Multimodal optical imaging can reveal changes in microcirculation and tissue oxygenation during skin wound healing,” Lasers Surg. Med. 46(6), 470–478 (2014).
14. L. An, J. Qin, and R. K. Wang, “Ultrahigh sensitive optical microangiography for in vivo imaging of microcirculations within human skin tissue beds,” Opt. Express 18(8), 8220–8228 (2010).
15. S. Hu, K. Maslov, and L. V. Wang, “Second-generation optical-resolution photoacoustic microscopy with improved sensitivity and speed,” Opt. Lett. 36(7), 1134–1136 (2011).
16. M. Rousiti and J. L. Cracowski, “Non-invasive assessment of skin microvascular function in humans: an insight into methods,” Microcirculation 19(1), 47–64 (2012).
17. J. Qin, R. Reif, Z. Zhi, S. Dzienni, and R. Wang, “Hemodynamic and morphological vasculature response to a burn monitored using a combined dual-wavelength laser speckle and optical microangiography imaging system,” Biomed. Opt. Express 3(3), 455–466 (2012).
18. G. Mahé, A. Humeau-Heurtier, S. Durand, G. Leftheriotis, and P. Abraham, “Assessment of skin microvascular function and dysfunction with laser speckle contrast imaging,” Circ Cardiovasc Imaging 5(1), 155–163 (2012).
19. J. L. Cracowski, F. Gaillard-Bigot, C. Cracowski, M. Roustit, and C. Miller, “Skin microdialysis coupled with laser speckle contrast imaging to assess microvascular reactivity,” Microvasc. Res. 82(3), 333–338 (2011).
20. M. Rousiti and J. L. Cracowski, “Assessment of endothelial and neurovascular function in human skin microcirculation,” Trends Pharmacol. Sci. 34(7), 373–384 (2013).
21. D. Ringuette, I. Sigal, R. Gad, and O. Levi, “Reducing misfocus-related motion artefacts in laser speckle contrast imaging,” Biomed. Opt. Express 6(1), 266–276 (2015).
22. S. M. S. Kazmi, S. Balial, and A. K. Dunn, “Optimization of camera exposure durations for multi-exposure speckle imaging of the microcirculation,” Biomed. Opt. Express 7(5), 2157–2171 (2014).
23. S. Bricq, J. D. Letourneur, F. Chapeau-Blondeau, J. R. Varela, and P. Abraham, “Assessing spatial resolution versus sensitivity from laser speckle contrast imaging: application to frequency analysis,” Med. Biol. Eng. Comput. 50(10), 1017–1023 (2012).
24. V. V. Tuchin, I. L. Maksimova, D. A. Zimnyakov, I. L. Kon, A. H. Mavlyutov, and A. A. Mishin, “Light propagation in tissues with controlled optical properties,” J. Biomed. Opt. 2(4), 401–417 (1997).
25. E. A. Genina, A. N. Bashkatov, and V. V. Tuchin, “Tissue optical immersion clearing,” Expert Rev. Med. Devices 7(6), 825–842 (2010).
26. V. V. Tuchin, Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis, 3rd ed. (SPIE Press, Bellingham, WA 2015) pp. 419–590.
27. J. Wang, D. Zhu, M. Chen, and X. Liu, “Assessment of Optical Clearing Induced Improvement of Laser Speckle Contrast Imaging,” J. Innov. Opt. Heal. Sci. 03(03), 159–167 (2010).
28. R. Samatham, K. G. Phillips, and S. L. Jacques, “Assessment of Optical Clearing Agents Using Reflectance-Mode Confocal Scanning Laser Microscopy,” J. Innov. Opt. Heal. Sci. 03(03), 183–188 (2010).
29. Y. Liu, X. Yang, D. Zhu, R. Shi, and Q. Luo, “Optical clearing agents improve photoacoustic imaging in the optical diffusive regime,” Opt. Lett. 38(20), 4236–4239 (2013).
30. R. Cicchi, D. Sampson, D. Massi, and F. Pavone, “Contrast and depth enhancement in two-photon microscopy of human skin ex vivo by use of optical clearing agents,” Opt. Express 13(7), 2337–2344 (2005).
31. A. Ertürk, K. Becker, N. Jährling, C. P. Mauch, C. D. Hojer, J. G. Egen, F. Hellal, F. Bradke, M. Sheng, and H. U. Dött, “Three-dimensional imaging of solvent-cleared organs using 3DISCO,” Nat. Protoc. 7(11), 1983–1995 (2012).
32. K. V. Larin, M. G. Ghosn, A. N. Bashkatov, E. A. Genina, N. A. Trunina, and V. V. Tuchin, “Optical Clearing for OCT Image Enhancement and In-Depth Monitoring of Molecular Diffusion,” J. Vac. Sci. Technol. B 30(5), 1244–1259 (2012).
33. B. Yang, J. B. Treweek, R. P. Kulkarni, B. E. Deverman, C. K. Chen, E. Lubeck, S. Shah, L. Cai, and V. Gradinaru, “Single-Cell Phenotyping within Transparent Intact Tissue through Whole-Body Clearing,” Cell 158(7), 945–958 (2014).
34. Y. Ding, J. Wang, Z. Fan, D. Wei, R. Shi, Q. Luo, D. Zhu, and X. Wei, “Signal and depth enhancement for in vivo flow cytometer measurement of ear skin by optical clearing agents,” Biomed. Opt. Express 4(11), 2518–2526 (2013).
35. Y. A. Menyav, D. A. Nedosekin, M. Sarimollaoglu, M. A. Juratli, E. I. Galanzha, V. V. Tuchin, and V. P. Zharov, “Optical clearing in photoacoustic flow cytometry,” Biomed. Opt. Express 4(12), 3030–3041 (2013).
36. J. W. Wilson, S. Degani, W. S. Warren, and M. C. Fischer, “Optical clearing of archive-compatible paraffin embedded tissue for multiphoton microscopy,” Biomed. Opt. Express 3(11), 2752–2760 (2012).
37. J. Wang, Y. Zhang, P. Li, Q. Luo, and D. Zhu, “Review: tissue optical clearing window for blood flow monitoring,” IEEE J. Sel. Top. Quantum Electron. 20(2), 680112 (2014).
38. D. Zhu, K. V. Larin, Q. Luo, and V. V. Tuchin, “Recent progress in tissue optical clearing,” Laser Photon. Rev. 7(5), 732–757 (2013).
39. J. Wang, R. Shi, and D. Zhu, “Switchable skin window induced by optical clearing method for dermal blood flow imaging,” J. Biomed. Opt. 18(6), 061209 (2013).
40. D. Zhu, J. Wang, Z. Zhi, X. Wen, and Q. Luo, “Imaging dermal blood flow through the intact rat skin with an optical clearing method,” J. Biomed. Opt. 15(2), 026008 (2010).
41. J. Wang, N. Ma, R. Shi, Y. Zhang, T. Yu, and D. Zhu, “Sugars Induced Skin Optical Clearing From Molecular Dynamics Simulation to Experimental Demonstration,” IEEE J. Sel. Top. Quantum Electron. 20(2), 710107 (2014).
42. N. E. Cameron and M. A. Cotter, “The relationship of vascular changes to metabolic factors in diabetes mellitus and their role in the development of peripheral nerve complications,” Diabetes Metab. Rev. 10(3), 189–224 (1994).
43. F. Hansen-Smith, A. S. Greene, A. W. Cowley, Jr., and J. H. Lombard, “Structural changes during microvascular rarefaction in chronic hypertension,” Hypertension 15(6), 922–928 (1990).
44. D. M. McDonald and P. L. Cheyke, “Imaging of angiogenesis: from microscope to clinic,” Nat. Med. 9(6), 713–725 (2003).
45. Y. Liu, X. Yang, H. Gong, B. Jiang, H. Wang, G. Xu, and Y. Deng, “Assessing the effects of norepinephrine on single cerebral microvessels using optical-resolution photoacoustic microscope,” J. Biomed. Opt. 18(7), 076007 (2013).
46. A. N. Pavlov, O. V. Semyachkina-Glashkovskaya, Y. Zhang, O. A. Bibikova, O. N. Pavlova, Q. Huang, D. Zhu, P. Li, V. V. Tuchin, and Q. Luo, “Multiresolution analysis of pathological changes in cerebral venous dynamics in newborn mice with intracranial hemorrhage: adrenorelated vasorelaxation,” Physiol. Meas. 35(10), 1983–1999 (2014).
47. H. Narasimha-Iyer, J. M. Beach, B. Khoobeh, and B. Roysam, “Automatic identification of retinal arteries and veins from dual-wavelength images using structural and functional features,” IEEE Trans. Biomed. Eng. 54(8), 1427–1435 (2007).
48. I. Schiessl, W. Wang, and N. McLaughlin, “Independent components of the haemodynamic response in intrinsic optical imaging,” Neuroimage 39(2), 634–646 (2008).
49. I. Vanzetta, R. Hildesheim, and A. Grinvald, “Compartment-resolved imaging of activity-dependent dynamics of cortical blood volume and oxygen extraction,” J. Neurosci. 25(9), 2233–2244 (2005).
50. Z. Luo, Z. Yuan, Y. Pan, and C. Du, “Simultaneous imaging of cortical hemodynamics and blood oxygenation change during cerebral ischemia using dual-wavelength laser speckle contrast imaging,” Opt. Lett. 34(9), 1480–1482 (2009).
51. H. F. Zhang, K. Maslov, M. Sivaramakrishnan, G. Stoica, and L. V. Wang, “Imaging of hemoglobin oxygen saturation variations in single vessels in vivo using photoacoustic microscopy,” Appl. Phys. Lett. 90(5), 053901 (2007).
52. P. Miao, M. Li, N. Li, A. Rege, Y. Zhu, N. Thakor, and S. Tong, “Detecting of cerebral arteries and veins: from large to small,” J. Innov. Opt. Heal. Sci. 3(1), 61–67 (2010).
53. N. Feng, J. Qiu, P. Li, X. Sun, C. Yin, W. Luo, S. Chen, and Q. Luo, “Simultaneous automatic arteries-veins separation and cerebral blood flow imaging with single-wavelength laser speckle imaging,” Opt. Express 19(17), 15777–15791 (2011).
54. J. D. Briers and S. Webster, “Laser speckle contrast analysis (LASCA): A nonscanning, full-field technique for monitoring capillary blood flow,” J. Biomed. Opt. 1(2), 174–179 (1996).
55. A. B. Parthasarathy, W. J. Tom, A. Gopal, X. Zhang, and A. K. Dunn, “Robust flow measurement with multi-exposure speckle imaging,” Opt. Express 16(3), 1975–1989 (2008).
56. P. Li, S. Ni, L. Zhang, S. Zeng, and Q. Luo, “Imaging cerebral blood flow through the intact rat skull with temporal laser speckle imaging,” Opt. Lett. 31(12), 1824–1826 (2006).
57. M. Draijer, E. Hondebrink, T. van Leeuwen, and W. Steenbergen, “Review of laser speckle contrast techniques for visualizing tissue perfusion,” Lasers Med. Sci. 24(4), 639–651 (2009).
58. K. Basak, M. Manjunatha, and P. K. Dutta, “Review of laser speckle-based analysis in medical imaging,” Med. Biol. Eng. Comput. 50(6), 547–558 (2012).
59. J. Senarathna, A. Rege, N. Li and N. V. Thakor, “Laser speckle contrast imaging theory, instrumentation and applications,” IEEE Rev. Biomed. Eng. 6, 99–110 (2013)
60. J. Wang, Y. Zhang, T. Xu, Q. Luo, and D. Zhu, “An innovative transparent cranial window based on skull optical clearing,” Laser Phys. Lett. 9(6), 469–473 (2012)
61. A. Izquierdo-Román, W. C. Vogt, L. Hyacinth, and C. G. Rylander, “Mechanical tissue optical clearing technique increases imaging resolution and contrast through ex vivo porcine skin,” Lasers Surg. Med. 43(8), 814–823 (2011).
62. K. Murari, N. Li, A. Rege, X. Jia, A. All, and N. Thakor, “Contrast-enhanced imaging of cerebral vasculature with laser speckle,” Appl. Opt. 46(22), 5340–5346 (2007).
63. V. V. Tuchin, Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis, 3rd ed. 661–710, SPIE Press, Bellingham, WA (2015)
64. S. G. Proskurin and I. V. Meglinski, “Optical coherence tomography imaging depth enhancement by superficial skin optical clearing,” Laser Phys. Lett. 4(11), 824–826 (2007).
65. M. Bonesi, S. Matcher, and I. Meglinski, “Doppler optical coherence tomography in cardiovascular applications,” Laser Phys. 20(6), 1491–1499 (2010).
66. M. Bonesi, S. Proskurin, and I. Meglinski, “Imaging of subcutaneous blood vessels and flow velocity profiles by optical coherence tomography,” Laser Phys. 20(4), 891–899 (2010).
67. X. Wen, Z. Mao, Z. Han, V. V. Tuchin, and D. Zhu, “In vivo skin optical clearing by glycerol solutions: mechanism,” J. Biophotonics 3(1-2), 44–52 (2010).
1. Introduction

Cutaneous microcirculation, as an accessible and representative vascular bed [1], has been widely applied to assess microcirculatory function and dysfunction, pathology-induced vascular dysfunction, including hypertension [2], diabetes [3] and some other peripheral vascular diseases [4]. Thus, various imaging techniques have been widely developed, e.g. laser Doppler flowmetry (LDF) [5] and tissue viability imaging (TiVi) [6], for studying the cutaneous microcirculation. However, LDF cannot simultaneously provide microvascular structural and functional information because of lacking adequate spatiotemporal resolution; TiVi is insensitive to the velocity of red blood cells [6]. Furthermore, some other high-resolution optical imaging modalities, such as intravital microscopy (IVM) [7, 8], optical coherence tomography (OCT) [9] and photoacoustic microscopy (PAM) [10], have also been applied to image the cutaneous microcirculation. Unfortunately, IVM usually depends on the dorsal skinfold chamber, which may accompany with bleeding and changes in the physiological environment [11, 12]; Due to lack of sufficient temporal resolution, it is difficult for OCT and PAM to monitor a large field of view of the cutaneous blood flow dynamical response [13–15].

By contrast, Laser speckle contrast imaging (LSCI), as a real-time, full-field imaging modality, allows for real-time monitoring the microcirculatory structural and functional information [16–18]. It has been becoming a fascinating tool to assess the cutaneous blood flow dynamics [18–22]. However, the turbid skin-induced static speckles conceals the dynamic information of the blood flow and reduces the imaging resolution and contrast, that makes it difficult to sensitively monitor the cutaneous blood flow dynamical response [23]. Fortunately, the tissue optical clearing technique [24–26] has shown a great potential to improve the performance of many optical imaging modalities [27–36]. Especially, some in vivo skin optical clearing (SOC) methods [37, 38] can make LSCI image the cutaneous microcirculation with superior resolution and contrast [39–41]. Nevertheless, it has received little attention whether it is sensitive to assess the cutaneous blood flow dynamical response with high spatial resolution.

In addition, identifying arteries and veins is extreme important to clinical diagnostics, such as evaluating the changes in vascular structure caused by some diseases [42, 43], studying the blood supply [44] and assessing the blood flow dynamical response during vascular reactivity test [45, 46]. Besides the proposed methods based on multi-wavelength [47–51], Miao et al. distinguished arteries and veins by vascular anatomical features and optical intensity profiles in single-wavelength LSCI (SW-LSCI) image, but artery and vein may be misclassified because of similar absorption to the cerebral cortex [52]. Feng et al. proposed an automatic arteries-veins separation method based on imaging the exposed cerebral cortex using SW-LSCI, whereas the absorption and scattering of underlying parenchyma would also influence the accuracy of the arteries-veins separation [53].
The aim of this work is to demonstrate the capability of the combination method of LSCI and SOC for monitoring blood flow dynamical response of cutaneous vasculature in detail, especially, quantitatively distinguish the changes in blood flow and vascular diameter at single artery or vein level.

2. Materials and methods

2.1 Chemical agents and animal preparations

In this study, the skin OCA was based on our previous study [39, 40], which was a mixture of two biocompatible agents, PEG-400 (Kemiou Reagent Development Corporation, Tianjing, China) and thiazone (Heming Trading Company Limited, Guangzhou, China) at a volume ratio of 9:1. Noradrenaline (NA), as a common drug for vascular reactivity test [18, 45], was used to induce the blood flow dynamical changes in cutaneous microvasculature, which was purchased from Affiliated Hospital of Huazhong University of Science and Technology (Wuhan, China).

Male Balb/c mice (n = 30, 25-30 g, 8 weeks old) were supplied by Wuhan University Center for Animal Experiment (Wuhan, China). The animal care and experimental procedures were approved by the Experimental Animal Management Ordinance of Hubei Province, P. R. China. The mice were randomly divided into two groups: 10 mice were used to assess the effect of OCA on cutaneous microvasculature by saline injection (as saline group); and other 20 mice were used to investigate the cutaneous arteriovenous blood flow dynamical response to NA before or after OCA-treatment (as drug group). After mice were anesthetized with a mixture of 10% urethane and 2% α-chloralose (0.2mL/25g) via intraperitoneal injection, the dorsal hair was shaved and the residual hair was removed. Then, mice were placed on the experimental platform.

2.2 LSCI for monitoring the cutaneous blood flow dynamics

In this study, LSCI was used to monitor the cutaneous blood flow dynamical response. Figure 1 shows the schematic of the LSCI system. Briefly, a He-Ne laser beam (λ = 632.8 nm, 3 mW) expanded by a collimating lens was used to illuminate the interested cutaneous areas. A sequence of white-light images and raw speckle images can be recorded by a CCD camera (Pixelfly USB, PCO Computer, Germany) mounted on a stereo microscopy (SZ61TR, Olympus, Japan) by switching the light source. Images were acquired by Camware software (PCO, Germany) with 14 Hz.

For the saline group, the cutaneous vasculature through the turbid skin was monitored for the first 5 min by LSCI. Thereafter, OCA was topically applied for 5 min to make dorsal skin transparent, and then the raw speckle images were recorded. Then 100μl of saline was injected via tail-vein, LSCI was used to monitor the cutaneous blood flow information for another 30 min at 1-min interval.

For the drug group, the cutaneous vasculature through the turbid skin was monitored; and then 100μl of NA (0.2μg/μl) was injected via tail-vein, the blood flow dynamical response was monitored for 30 min at 1-min interval. In the next day, the following experiments were performed. Firstly, the cutaneous vasculature in the same area was monitored; Secondly, OCA was topically applied for 5 min to make dorsal skin transparent, and then the microcirculation was imaged for 5 min; Finally, the same dosage of NA was injected, and the raw speckle images were sampled for another 30 min at 1-min interval. During NA injection, all the conditions were kept the same, including the light intensity of laser, time-interval of imaging, sampling frequency and the extent of mice anesthesia, etc.
2.3 Rebuilding arteries-veins separation based on SW-LSCI

Consideration of the importance and value of the arteries-veins separation in basic researches, clinical diagnostics and treatments, we rebuilt an arteries-veins segmentation on the basis of the methods proposed by Miao et al. [52] and Feng et al. [53]. The primary steps were briefly described as follows:

Step 1: Reconstructing the speckle contrast image using laser speckle temporal contrast analysis (LSTCA)

Laser speckles are produced in the form of complex interference pattern at laser beam scattering from the illuminated rough surface, and a time-varying speckle pattern is generated when the scattering particles are moving. Blood flow distribution can be obtained by analyzing the blurring of the speckle pattern, which can be represented by speckle contrast $C_{\text{spec}}$ as follows [54]:

$$C_{\text{spec}} = \frac{\sigma_s}{\langle I \rangle},$$  \hspace{1cm} (1)

where $\sigma_s$ is the standard deviation of the speckle intensity, $\langle I \rangle$ is the mean intensity, and $C_{\text{spec}}$ is the speckle contrast, which has been completely defined by Parthasarathy et al. [55]. In order to suppress the influence of static speckles and non-uniform light distribution, laser speckle temporal contrast analysis method (LSTCA) was used to produce the temporal speckle contrast images [56]. Since the LSTCA is based on the temporal statistics analysis of 40 successive time sequence raw speckle images, so this method can effectively restrain the influence of the pulsatility on arterial flow velocity analysis by averaging in time. It was also demonstrated that LSCI would have an optimal contrast-to-noise ratio (CNR) and a low noise when the exposure time of the CCD camera $T$ is set to 5 ms [57]. Under the widely-used assumption of a random Lorentzian velocity distribution, the speckle contrast $C_{\text{spec}}$ can be simplified as follows [54, 58, 59]:

$$C_{\text{spec}} = \frac{\sigma_s}{\langle I \rangle} = \left( \frac{\tau_c}{2T} \left[1 - \exp(-2T/\tau_c)\right] \right)^{-\frac{1}{2}},$$  \hspace{1cm} (2)

where $\tau_c$ is the decorrelation time. The simplest approach after ignoring many inessential factors leads to a characteristic velocity $u_c$ defined as follows [54, 59]:

$$u_c = \frac{\lambda}{2\pi \tau_c},$$  \hspace{1cm} (3)
Based on the obtained speckle contrast images by LSTCA, the corresponding relative blood flow velocity maps can be deduced using the Eqs. (2) and (3).

Step 2: Extracting the vasculature network from the blood flow velocity image

For LSCI, intensity fluctuation caused by the motion of particles will blur the image and reduce the speckle contrast to an extent that is related to the speed, such as moving RBCs [57]. Thus, the vasculature network can be extracted from the blood flow velocity images according to the difference in flow velocity between the vasculature and tissue. Then, the vasculature network was marked by 1, the non-vascular region was marked by 0.

Step 3: Weighting the temporal minimum intensity image by multiplying the white light image

The temporal minimum intensity image was obtained by the approach proposed by Feng et al. [53]. To perform accuracy arteries-veins separation, we weighted the temporal minimum intensity image by multiplying the white-light image. Thereafter, the vasculature network was further multiplied to eliminate the influence of the tissue. Thus, the vasculature network obtained the weighted temporal minimum intensity values, whereas the non-vascular region was 0.

Step 4: Separating the artery and vein

When imaging with light of 600-635nm, the oxy-hemoglobin absorption is negligible compared with that of deoxy-hemoglobin. Ideally, the relations for the relative concentration \( C \) of deoxy-hemoglobin in artery, tissue, and vein satisfy \( C_{\text{artery}} < C_{\text{tissue}} < C_{\text{vein}} \) [52]. Therefore area with higher level of deoxy-hemoglobin \( C \) (e.g. the veins) leads to more absorption and thus makes the corresponding area darker, i.e. smaller intensity values in the images, and then we can realize the arteries-veins separation based on the distinct intensity by the multiple threshold method. In case of a misclassification, it was re-corrected according to the pre-existing arteries-veins separation image and the connectivity of blood vessels.

2.4 Quantitative analysis of the improved resolution, contrast and sensitivity

To quantitatively evaluate the optical clearing efficacy of mice skin \textit{in vivo} after treatment with different OCAs, the minimum resolution diameter of blood vessels was calculated by analyzing the laser speckle contrast image [60]. According to Rayleigh’s criterion [61], the speckle contrast value of vessel area is less than or equal to \( 2/\pi \) times that of the non-vessel area, the vessel can be distinguished. The smaller the distinguished diameter of blood vessel is, the higher the resolution of images.

In addition, the ability of LSCI for distinguishing vessels from the tissue was assessed according to the CNR, which could characterize the image contrast and be calculated as follows [62]:

\[
\text{CNR} = \frac{|C_{\text{back}} - C_{\text{vessel}}|}{\sqrt{f_{\text{vessel}} \sigma_{\text{vessel}}^2 + f_{\text{back}} \sigma_{\text{back}}^2}},
\]

where \( C_{\text{back}} \) and \( C_{\text{vessel}} \) refer to the mean speckle contrast values constituting the background and vessels in the speckle contrast images. The \( f_{\text{back}} \) and \( f_{\text{vessel}} \) are the fractions of pixels classified as the background and vessels in all the selected pixels. The \( \sigma_{\text{back}}^2 \) and \( \sigma_{\text{vessel}}^2 \) refer to the variance of the speckle contrast values constituting the background and vessels, respectively.

To calculate the changes in vascular diameter, we counted the number of pixels in the interested vessels. Then the relative diameter was expressed as the ratio of the measured diameter under condition of injection to that of the initial. Similarly, the flow velocity in each
vessel can be obtained from the blood flow velocity maps, and the relative flow velocity was calculated as the ratio of the measured flow velocity under condition of injection to that of the initial.

As for monitoring the blood flow dynamical response, the sensitivity is another key index. If the change in vascular diameter or flow velocity caused by drug is \( \Delta C_{vd} \), and the standard deviation of the changes is \( C_{sd} \), the signal-to-noise ratio (SNR) can be defined as follows [23, 27]:

\[
SNR = \frac{\Delta C_{vd}}{C_{sd}}.
\]

If \( SNR \geq 1 \), the variation of vascular diameter or flow velocity is detectable; otherwise, it could not be detected. Hence, when the \( SNR \) is equal to 1, \( \Delta C_{vd} \) corresponds to the sensitivity of monitoring the blood flow dynamical response.

3. Results

3.1 Monitoring saline injection-induced arteriovenous blood flow dynamics using combined LSCI and SOC

Figure 2 demonstrates the typical white-light images, blood flow velocity maps and profiles of the flow velocity values along the horizontal white line. It can be found that neither the cutaneous microvasculature nor the blood flow information is visible through the turbid skin. After OCA has been topically applied to skin for 5 min, the cutaneous microvasculature is visible to naked eyes, and smaller microvessels can be easily detected with superior contrast. Saline injection does not induce any observable changes in the cutaneous blood flow and microvasculature in the follow 30 min.

Figure 3(a) indicates the primary steps of the arteries-veins separation of the transparent microvasculature induced by SOC, and Fig. 3(b)-3(e) show that saline injection-induced relative changes in the vascular diameter and flow velocity in artery (marked by A) and vein (marked by V). We observe that the vein is parallel with the artery, and saline injection hardly changes vascular diameter or flow velocity in both artery and vein for the next 30 min.
3.2 Assessing arteriovenous blood flow dynamical response to NA through OCA-treated skin

Thereafter, the same dose of NA was injected and the blood flow dynamical response to NA was monitored by the combination method of LSCI and SOC method. Figure 4 shows the typical white-light images, blood flow velocity maps and profiles of flow velocity values along the horizontal white line at status of turbid skin; OCA-treatment for 5 min; and 5, 10, 15, 25, 30 min after NA injection through the OCA-treated skin, respectively. The results demonstrate that the cutaneous microvasculature and blood flow can be detected by LSCI with superior resolution and contrast after topically treatment of OCA to skin for 5 min. From the profiles of the flow velocity, we can found that NA injection firstly induces a significant increase in the flow velocity, which attains to the maximum response amplitude in about 10 min; then gradually decreases; and finally recovers to the base line in about 25 min.
To assess the blood flow dynamical response to NA between artery and vein, we realized arteries-veins separation and calculated NA injection-induced relative dynamic changes in vascular diameter and flow velocity in artery and diverse veins. Figure 5(a)-5(h) show the results. It can be found that arteries and veins have the different vasoconstrictive responses to NA. For instance, NA-induced decrease in vascular diameter and increase in flow velocity in artery (marked by A1) are smaller than those in the parallel vein (marked by V2). For the artery (A1), the maximum flow velocity increases by $27.5 \pm 4.7\%$ after NA injection, and the vascular diameter decreases by $16.9 \pm 4.3\%$. Whereas for the parallel vein (V2), the relative changes in flow velocity and vascular diameter are $54.3 \pm 5.2\%$ and $25.5 \pm 3.1\%$, respectively. As similar to the dynamical response amplitude, A1 and V2 also have the different response time to NA. For instance, it takes about 8 min to reach up to extreme value for A1; while it takes 12 min for V2.

To comparably understand the blood flow dynamical response to vasoactive drug between arteries and veins, we quantified the blood flow dynamical response in another vein (marked by V1) with similar size to the artery A1 mentioned above. It can be found that the relative changes in flow velocity and vascular diameter are $11 \pm 4.1\%$ and $9.7 \pm 3.9\%$, respectively, which is smaller than that in the artery A1 showed above. And it needs about 15 min to reach the extreme value. In addition, the recovery of vascular diameter is more quickly than that of the flow velocity for the artery A1. Whereas the recovery of the vascular diameter is slower than that of the flow velocity for both veins V1 and V2; and in about 25-30 min after NA injection, the vascular diameter and flow velocity in all the vessels mentioned above can recover to the initial state.

Fig. 5. Speckle contrast image (a); arteries-veins separation image (b); NA injection-induced relative changes in vascular diameter and flow velocity in artery A1 (c, d); vein V1 (e, f); and vein V2 (g, h) accompanied with OCA-treatment. The injection time is set to be 0, which is consistent with the arrow shown in (c-h). Bar = 500 μm.

3.3 OCA-induced improvement of LSCI for monitoring blood flow dynamical response to NA

To quantitatively assess the improvement of the combination method of LSCI and SOC in monitoring cutaneous blood flow dynamical response to NA, the same experiment was performed in case of without OCA-treatment on one day prior to OCA-treatment. Figure 6 shows NA injection-induced blood flow dynamical response in the same area through the turbid skin, including the typical white-light images, blood flow velocity maps and profiles of the flow velocity values along the horizontal white line before and after NA injection. It can be found that only the blood flow information and vascular structure of the large vessels can be distinguished hazily, and the smaller vasculature are invisible. The image resolution and contrast are too poor to separate the arteries and veins. In addition, neither the white-light image nor the velocity image shows any detectable changes in cutaneous blood flow...
dynamics caused by NA. The statistical analysis from the flow velocity profiles does not demonstrate apparent differences between the base line and those after NA injection.

We further analyzed the improvement in CNR, and found that at 10 min after NA injection, the mean CNR is increased by 450.4 ± 22.7% through the OCA-treated skin compared with that through the turbid skin. Meanwhile, the minimum resolution diameter (MRD) was also used to assess the capacity of SOC in optimizing the LSCI imaging resolution, the results show that the MRD in Fig. 4 is 144.3 ± 16.2 μm and 30.5 ± 3.7 μm through the turbid skin and 5 min-OCA-treatment skin, respectively. To further quantitatively assess the improvement in the sensitivity of LSCI combined with SOC method for monitoring the cutaneous blood flow dynamical response to NA, the minimum detectable change in vascular diameter or flow velocity was calculated in the large vessels since it was completely undetectable for the small ones through the turbid skin. The results show that the sensitivity of the cutaneous blood flow dynamical response to NA can be tremendously enhanced through the OCA-treated skin compared with that through the turbid skin; which increases by 303.3 ± 20.4% and 258.7 ± 25.8% in detecting the blood flow dynamical response in terms of the vascular diameter and flow velocity, respectively.

4. Discussion

There is α-receptor on the vascular smooth muscle cells, NA can effectively interact with the α-receptor and cause vasoconstriction. In this study, NA was used to study the enhancement in the capacity of LSCI combined with SOC method for monitoring the cutaneous arteriovenous blood flow dynamical response to vasoactive drug.

Through the turbid skin, it is difficult for LSCI to detect the blood flow dynamical response to NA, except for that some large vessels could be visual hazily. That is due to the dynamic speckles derived from the moving red blood cells are concealed by the strong static speckles produced from the turbid skin [63]. The resolution, contrast and sensitivity of LSCI are not enough to image the dynamical blood flow caused by vasoactive drug.

However, topically OCA-treatment on the skin can make the skin transparent and reduce the scattering of skin. Thus, dynamic speckle signals can be detected easily through the transparent skin. Previous investigation indicated that increasing transparency of skin could
significantly improves depth of imaging, image contrast and spatial resolution [64]. Finally, the LSCI can be applied to obtain better image quality for monitoring the cutaneous microcirculation. However, the similar improvement in blood vessels imaging using OCT could be not found at application of glycerol as an OCA [64–66]. The main reasons may be from the two aspects: firstly, thiazone, as a chemical penetration agent, was used in our work, could significantly enhance the skin optical clearing efficacy [39–41], as well as the penetration of glycerol into skin sample is relatively limited [29, 67, 68]. In addition, the thickness of mice skin in our study is thinner than human skin. Therefore, it is unavoidable for the difference between this work and those work [29, 67, 68].

As we know that the main role of vasculature system is to delivery oxygen, nutrition, fluid and signaling molecules required by living tissue, and the lymphatic system usually developed in parallel to the blood vessels in the skin [69]. Kalchenko et al. showed that the combined application of LSCI and fluorescence intravital microscopy approaches provided synchronous \textit{in vivo} images of blood vessels and lymph vessels [70]. They also realized simultaneous imaging of the blood vessels and lymph vessels using a long-exposure time LSCI approach [71]. However, the long exposure time would significantly decrease the \textit{CNR} [57], which suffer from semi-transparent tissue (\textit{e.g.} ear skin). Therefore, it will be a prospective technique for visualization of the blood vessels and lymph vessels in dorsal skin simultaneously with a superior resolution and contrast using the combined SOC and LSCI. What’s more, the transparent cutaneous microvasculature makes it possible to accurately separate the artery and vein in skin using the rebuilt arteries-veins separation method. It can be seen that the artery parallels with the vein in Fig. 3 and Fig. 5, which agrees with the microvascular anatomical characteristic [20]. The studies about the effect of OCA on cutaneous microcirculation also indicate that there is no obvious changes in the cutaneous microvasculature and blood flow after OCA-induced skin transparent, which was consistent with previous studies [37, 39].

In addition, according to the research on the fate of NA [72], NA will disappear in a short time without a further injection, relaxing of vascular smooth muscle cells will lead to a recovery of the microvasculature. We can monitor the whole blood flow dynamical response process with superior resolution, contrast and sensitivity. Meanwhile, we found that the vasoconstriction of the cutaneous microvasculature would induce a promotion of the flow velocity, which was consistent with the studies of S. Jhanji \textit{et al.} [73] and A. C. Sharma \textit{et al.} [74], who demonstrated that the cutaneous blood flow velocity would be improved after a high-dosage of NA injection.

Furthermore, by combining Figs. 4 and 5, we find that the artery has a slight slower flow velocity than its accompanying vein before or after drug injection, which could be due to big differences in the vascular kind and vessel diameter, Feng \textit{et al.} demonstrated that there may be similar blood flow velocity in small arteries as larger veins based on the study of the cerebral blood flow [53]. We also detected that there were some different vasoconstrictive responses to NA between arteries and veins on the basis of the rebuilt arteries-veins separation method. The much stronger vasoconstriction in vein V2 would induce a higher elevation of the flow velocity than that in the parallel artery A1. Blood flow dynamical response to NA in this artery is a little weaker than its accompanying vein may be relative to the anatomical feature of distinct percentage of vascular smooth muscle cells in different kind and size of blood vessels. Nevertheless, this artery had a stronger blood flow dynamical response in vascular diameter and flow velocity than that in another vein V1 with the similar size. The ratio of the increased flow velocity to the decreased vascular diameter in artery (A1) and vein (V1) were 1.62 and 1.13, respectively. It indicates that NA increases mean artery pressure in clinical treatment. It can help explain why the recovery of vascular diameter is more quickly than that of the flow velocity for the artery. The blood flow dynamical response and this kind of difference between arteries and veins could not be detected through the turbid dorsal skin, because of inadequate resolution and contrast, let alone sensitively evaluate the
distinct blood flow dynamical response to NA between arteries and veins in the turbid skin by
the rebuilt arteries-veins-separation. It means that SOC cannot only considerably improve the
performance of LSCI for monitoring the cutaneous vascular structure and function with
higher resolution, contrast and sensitivity, but also has a prospective application in studying
the arteriovenous blood flow dynamical response and some vascular diseases when combined
with the arteries-veins-separation method, e.g. diabetes, tumor, arteriosclerosis and venous leg
ulceration, etc.

5. Conclusion

In this study, we demonstrated that SOC could improve the performance of LSCI for
monitoring the cutaneous vascular structure and function, including the imaging resolution,
contrast and sensitivity of blood flow dynamical response to vasoactive drug. In addition,
based on the rebuilt arteries-veins separation method, the blood flow dynamical response time
and amplitude, as well as recovery process in arteries and veins can be quantitatively assessed
and compared. Thus, combination of SOC and LSCI provides a valuable way to monitor and
evaluate diagnosis, the cutaneous arteriovenous pharmacological intervention and treatment
of some vascular diseases, such as diabetes, tumor, arteriosclerosis and venous leg ulceration.

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