Published in final edited form as:

Lasers Surg Med. 2014 August ; 46(6): 470–478. doi:10.1002/lsm.22254.

Multimodal optical imaging can reveal changes in microcirculation and tissue oxygenation during skin wound healing

Hequn Wang, PhD, Lei Shi, PhD, Jia Qin, MSc, Siavash Yousefi, MSc, Yuandong Li, BSc, and Ruikang K. Wang, PhD

Department of Bioengineering, University of Washington, Seattle, Washington, USA

Abstract

Background and Objective—Faster and better wound healing is a longstanding goal. Blood flow, angiogenesis, and tissue oxygenation are important parameters in evaluating the healing process. Optical microangiography (OMAG) allows 3D imaging of tissue vasculature and can provide quantitative blood flow information down to the capillary level of resolution. Dual wavelength laser speckle imaging (DW-LSI) can measure tissue oxygenation status.

Materials and Methods—Cutaneous wound healing of a mouse ear model using a multimodal imaging system that combines OMAG with DWLSI was studied.

Results—A complete microvasculature map of the ear *in vivo* was obtained. The imaging system revealed both hemodynamic and metabolic changes during acute stage wound healing. Blood flow velocity, blood flow direction, as well as changes in concentration of oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (Hb) were measured and quantified. In addition, capillary recruitment and angiogenesis were visualized during the chronic stage of repairing.

Conclusions—The combination of DW-LSI and OMAG imaging technique may be a powerful tool to visualize and understand microvascular, hemodynamic and metabolic changes during cutaneous wound healing.

Keywords

Laser speckle imaging (LSI); Microcirculation; Optical coherence tomography (OCT); Optical microangiography (OMAG); Wound healing

Introduction

Many people suffer from a variety of wounds every year [1–2]. Better wound healing may decrease suffering, shorten the time for wound closure, and reduce the overall healthcare cost. Blood vessels play a significant and complex role in wound healing. For example, they function to re-establish hemostasis and mediate cell migration during the inflammation stage

Correspondence: Ruikang Wang, Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle, Washington 98195, USA. wangrk@uw.edu.

Disclosure statement: The authors claim no relevant financial disclosure.

[3]. During the tissue granulation stage, blood vessels especially new capillaries are also involved, sprouting into the fibrin clot while epidermal cells resurface the wound [4]. Angiogenesis, the formation of new blood vessels, is necessary to sustain the newly formed granulation tissue [5]. One direct result of a wound is tissue hypoxia. Characterizing hypoxia is critical because hypoxia may cause tissue damage, and also stimulate the production of vascular endothelial growth factor (VEGF), an angiogenic growth factor that may promote wound closure [6].

Most previous studies trying to record the cutaneous neovascularization process have relied on traditional histological analysis [5], which is invasive and does not allow for continuous monitoring during wound healing. Other noninvasive techniques that have been used to study blood flow or oxygenation changes in healing include laser Doppler imaging (LDI) [7], laser Doppler flowmetry (LDF) [8], ultrasound imaging [9] and functional imaging modalities, such as PET [10] and MRI [11]. However, the limited resolution (both spatial and temporal) does not allow these techniques to image small blood vessels, or the detailed tissue oxygenation status within localized areas. Photoacoustic imaging (PA) is a newly developed technique, which has emerged in the past decade as a powerful hybrid modality to image optical-absorption contrast in turbid media [12]. Although PA imaging can provide blood vessel mapping and detect physiologically specific endogenous contrasts such as oxy-hemoglobin (HbO₂) and deoxy-hemoglobin (HbR) [13], the typical acquisition time for a large field of view can be as long as tens of minutes [14].

Optical coherence tomography (OCT) is another noninvasive imaging technique that can provide millimeter scale cross-sectional morphological views of tissue microstructures *in vivo* with a micron-level image resolution [15]. As one significant extension of the traditional OCT technique, optical microangiography (OMAG) is a more recently developed method to image volumetric vasculature and blood perfusion map down to capillary level *in vivo* [16–17]. Combining with the Doppler principle, Doppler optical microangiography (DOMAG) has been developed and utilized to quantitatively imaging of blood flow velocity and the direction of flow *in vivo* [18–19]. Dual wavelength laser speckle imaging (DW-LSI) is a novel technique, which integrates laser Doppler flowmetry with near infrared (NIR) oxymetry for simultaneous imaging of blood flow and hemoglobin oxygenation changes *in vivo* [20]. In the present study, we propose to use an ultrahigh-sensitive OMAG (UHS-OMAG) system integrated with DW-LSI system to study cutaneous wound healing process on a mouse ear model. This model is chosen because mouse ear is easy to access with relatively simple microvasculature network. We demonstrate the capability of the proposed imaging system in monitoring both the hemodynamic and the metabolic changes during acute stage of wound healing. Important parameters such as blood flow velocity, blood flow direction, as well as changes in concentration of oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (Hb) are measured and quantified. In addition, we show that the system is capable of visualizing and identifying critical events during wound healing, such as capillary recruitment and angiogenesis.

Materials & Methods

Animal preparation and procedure

In total, five hairless SKH-1E mice (22 to 26 g) (Charles River, Hollister, CA) were tested and imaged for this study. During imaging, the mice were anesthetized with isoflurane mixed with 0.2 L/min oxygen and 0.8 L/min air by a face mask. The mouse ear was fixed with double-sided tape with the dorsal side facing upwards onto a stage to minimize motion caused by breathing and heart beats. A baseline image of the whole mouse ear, which consists of 25 2 mm×2 mm sub-images, was first acquired before inducing the wound using the UHS-OMAG imaging system. The targeted punch area along with its surrounding tissue was then selected as region-of-interest (ROI). Baseline images of the ROI were also acquired using the combined DW-LSI and DOMAG imaging system. To generate the wound in the ear, a 1 mm size biopsy punch used to punch a round hole through the skin. To monitor the acute stage responses from both blood vessels and the surrounding tissues caused by the injury, a series of DOMAG images were obtained on the same area at 10 min, 25 min, 40 min and 55 min after the wound induction. The dual-wavelength laser speckle system was used to continuously acquiring data from the onset of the wound till 1 hour after. To record the chronic stage wound healing process, UHS-OMAG images of the whole mouse ear were obtained at 1, 3, 7, 14, and 30 days after the induction of the wound. All the procedures were performed with approval from the Institutional Animal Care and Use Committee at the University of Washington (protocol #4262-01).

System setup and parameters

The details of the UHS-OMAG imaging system can be found elsewhere [17]. Briefly, a superluminescent diode (SLD) with the center wavelength of 1310 nm and bandwidth of 65 nm, delivering an axial resolution of ~12μm in the air. An optical circulator was used to couple the light from the SLD into a fiber-based Michelson interferometer. A microscope objective lens with a 18 mm focal length was used to achieve ~5.8 μm lateral resolution. Since the wavelength of the light source is in near-infrared range which is invisible to human eyes, a 633nm laser diode was used as guiding beam to locate the imaging position. The light output from the interferometer was then directed to a home-made high-speed spectrometer, which contains a high-speed camera with a line scan rate of 47 kHz. The 3D imaging was achieved using an X-Y galvanometer scanner. The light power exposed at the sample plane was ~3 mW.

A home-made combined dual wavelength laser speckle imaging (DW-LSI) and Doppler optical microangiography (DOMAG) system was used to measure the blood flow velocity and flow direction inside the vessels as well as the changes in the oxygenation status. The detailed information of the combined system can be found elsewhere [19, 21]. A brief systematic diagram is included in the supplementary material. The DW-LSI system contains two collimated laser diodes with a wavelength of $\lambda_1 = 780$ and $\lambda_2 = 825$ nm. The lasers were combined by a dichroic mirror. Both lasers were expanded through a lens and uniformly illuminated over the sample at an incident angle of ~60° from the tissue normal direction. The backscattered light was transmitted to a zoom lens and to another dichroic mirror. The light from the two wavelengths was collected by two cameras. Both cameras were acquiring

images at frame rate of 96 frames per second. The exposure time of the camera was set to 10 ms, which is optimized to achieve high contrast for speckle flow imaging [20]. The DW-LSI system has a field of view of 8 mm $\times$ 8 mm with a lateral resolution of 70 μ m. The DOMAG module employed a superluminescent diode (SLD) light source with a central wavelength 1310 nm and a bandwidth 56 nm, which generated an axial resolution around $\sim$ 13 μ m. A pair of galvanometer scanners was used to scan the illumination beam. A lens with a 30 mm focal length was utilized to focus the beam, which provided a lateral resolution of $\sim$ 10 μ m. The light reflected from the sample and from the reference arm are recombined and transmitted to a home-made spectrometer system, which contains a grating, a lens and a camera for the detection of optical spectral interference signals. In this study, the OMAG and DOMAG images were acquired using separate systems.

Results

Complete microvasculature mapping of whole mouse ear

A baseline image of a whole mouse ear *in vivo* was first taken using our ultra-high sensitive OMAG (UHS-OMAG) system (Figure 1). Three main arterial and venous pairs labeled as 1, 2 and 3 are uniformly distributed across the whole mouse ear. The arteries are smaller in diameter and straighter, while the veins have larger diameters and are more tortuous. Small arterioles and venues, as well as the functional capillaries of the mouse ear were also well visualized. The capability of acquiring the complete microvasculature network of the whole mouse ear serves as the basis for monitoring the wound healing process.

Hemodynamic changes during acute wound healing

In order to study the hemodynamic changes during acute wound healing, an imaging system that combined DW-LSI with DOMAG was used to simultaneously image the mouse ear. Before introducing the injury, baseline images from the two imaging channels were first acquired. A biopsy punch with a 1 mm diameter was used to generate a round, full-thickness wound targeting the vessel pair 2 (labeled in Figure 1). The punched area is labeled as a red circle with a cross inside (Figure 2(b)–(f)). No obvious bleeding was observed during the experiment. Immediately after the punch, a reduction of blood flow of the first level branches of the affected arteries and veins were observed from laser speckle images. This can be seen in Figure 2(b). Ten seconds after wounding, as marked with an orange circle in Figure 2(b), there were changes of blood flow in anastomotic vessels. During the period between 5 to 25 min after wounding, no significant microvascular changes were observed, within the limited imaging depth and relatively low image resolution of laser speckle imaging. However, at 55 min after the punch, reappearance of the right branch of the affected vein was observed as shown in the orange circle in Figure 2 (f).

Detailed changes in the axial blood flow velocity can be found in the DOMAG images shown in Figure 3, where the images are given by maximal projection views of the microvasculature of the mouse ear. Compared to baseline image, the two venous branches and their downstream of the affected vessel disappeared from the DOMAG image obtained at 10 min after the punch (white circle in Figure 3(b)). In the meantime, an increase of blood flow at the left collateral vein was clearly seen, indicated by a white arrow in Figure 3(b).

On the right side, the two venous anastomoses labeled in a blue circle in Figure 3(a) disappeared at 10 min after the punch, re-appeared at 25 min and their flow strengthened at 55 min after the punch. Another anastomosis (blue arrow in Figure 3(b)) was not visible at baseline, became very obvious at 10 min time point, and was stable till 55 min after the punch. In arterioles, the blood flow for some segments of the two affected branches reversed direction, presumably because the supplies to these vessels have changed from the original injured artery to its two main collateral arteries on the left and right, which are the two arteries of vessel pair 1 and 3 labeled in Figure 1. The reestablished arterial flow directions remained unchanged till 55 min after the punch. Although some segments of these two arteries were immediately rescued from their collaterals, the downstream arterioles of these two arteries were no longer visible from 10 min till 55 min after the punch. In addition, a reconnection between the right collateral artery to the left branch of the affected artery was observed (yellow arrow in Figure 3(b)).

To better quantify the changes in blood flow, we characterized the blood flow direction and calculated the absolute blood flow of major arteries and arterioles before and after the injury. An integration method of the flow velocities in the projection plane was employed [22–23]. The skeletonized arterial flow maps of baseline and at 10 min after punch are shown in Figure 3(e) and (f). The blood vessel segments that show significant change in blood flow are named as 1–6 with their flow velocities denoted. As shown in Figure 3(f), at 10 min after the punch, arterial branches B and C, which are the direct downstream branches, show significant reduction in blood flow. To provide immediate rescue to the occluded vessels, flow direction in segment 1 was reversed with ~4 times increase in absolute blood flow. In the meantime, segment 2 showing a slightly increase in blood flow, merged with segment 1 at segment point 3. However, branch B was not successfully saved till 55 min after the punch as shown in Figure 3(d). To provide supply to branch C, segment 5 showed reversal in direction with an increased blood flow. Segment 6 also showed almost 4 times increase in blood flow. With these immediate responses, branch C started to flow in the opposite direction but with a much slower absolute blood flow compared to baseline.

Metabolic responses during acute wound healing

The DW-LSI system was utilized to obtain changes of the tissue oxygenation status after the wound induction. As shown in Figure 4 (a), a slight increase in the concentration of oxygenated hemoglobin (HbO) were seen at tissue surrounding the first level branches of the affected vessel, while a significant decrease at tissue surrounding the far-end downstream vessels was observed. The changes in deoxygenated hemoglobin (Hb) had an opposite trend compared to the changes in HbO, where an increase in Hb was noticed at tissue surrounding the far-end downstream vessels. The overall variations for both HbO and Hb tend to go back to baseline within 40 min after the punch as illustrated in Figure 4(c) and (f).

Microvasculature changes during chronic wound healing

In order to study the long-term repair process, the UHS-OMAG system was employed to serially monitor the same mouse ear until 30 days after the wound induction. Figure 5 shows a serial of projection views of the UHS-OMAG images taken at before punch, day 1, 3, 7, 14, and 30 during the healing process. At 1 day after the injury, the wounded area appeared

to be completely dark, indicating either a significant loss of blood flow, or sufficient edema thickening of the ear to preclude detailed imaging. The downstream of the affected veins disappeared while the downstream of the affected arteries became less obvious, which is consistent with the laser speckle and DOMAG findings. We also observed a higher density of capillaries around the injured area, which represents the capillary recruitment activities. By day 3, the overall blood flow seemed to be strengthened with a much more clear appearance of the anastomoses compared to day 0. Interestingly, we found several bright spots at the downstream of the injured vessels. Moreover, the size of the wound seemed to be smaller with more capillaries tending to invade into the wounded area compared to day 1. At day 7, the size of the wound was decreased with numerous capillaries surrounding it. Those bright spots noted on day 3 became less obvious. Moreover, the two directly affected vessel pairs including the two veins seemed almost recovered. At day 14, the size of the wound decreased significantly compared to day 7. By day 30, the wound is almost closed with a diameter ~0.5 mm.

Discussion

Vascular responses such as blood coagulation, hemostasis, cytokine release, angiogenesis, as well as hypoxia rescue are important activities for wound repair [24]. Continuously monitoring the microvasculature network and tissue microstructure till the closure of the wound would aid in the investigation of the healing process. We have shown that using Doppler OMAG and dual wavelength laser speckle imaging can simultaneously provide both hemodynamic and metabolic information during acute cutaneous wound healing. The vascular anatomy, blood flow velocity and hemoglobin saturation changes have been simultaneously acquired. In addition, we demonstrated collateral circulation changes due to anastomoses, which may provide immediate rescue when there is a blockage in one of the main arteries or veins. Moreover, UHS-OMAG has been successfully utilized to monitor the chronic healing process. In general, although each animal has its unique microvasculature network with slightly different responses to the injury, we found that the overall trend for changes in microcirculation during skin wound healing is very similar to the presented results.

The high resolution imaging achieved by the UHS-OMAG enabled us to visualize morphological details of the perfused microcirculation network. In this study, we chose to use hairless mouse ear model because (1) hairless mice do not require hair removal which, if so doing, may be associated with inflammation and obscure the healing response; (2) mouse ear has relatively thin skin, which allows almost full skin thickness imaging; (3) mouse ear has a rich blood vessels with relatively simple connections. Despite these advantages, one potential limitation with this animal model is that there is very little muscle underneath the skin, which may not be able to provide full support for healing. Although the SKH-1 mice should have no hairs, we observed several fine hairs in some areas of the ear, which cause some discontinuities of the blood vessels in Figure 1. Laser speckle imaging can well visualize the blood vessel response at large scale [25]; therefore, provide a quick overview during the experiment. However, it fails to image small capillaries and vessels in the deeper part of the skin. DOMAG, on the other hand, can provide detailed information on blood flow velocity and direction at micron-level scale with a deeper imaging depth because of the

longer wavelength used in the system. Therefore, the two systems working together can be very useful to generate complementary information in studying acute blood flow responses after injury. Immediately after the punch, we observed significant local vasodilation along the collateral main vessels with an example vessel labeled by a white arrow in Figure 3(b). There are two possible explanations for this phenomenon. One is that the collaterals may start to function more in order to provide necessary supply to the injured region. The second possible explanation is that right after the punch injury, the local dilation of the collaterals along with blood/fluid extravasation and blockage of lymphatic drainage can result in inflammation [24]. As shown in Figure 3, we observed the strengthened anastomoses, reversal of arterial blood flow, and newly formed connection between different branches, which testifies the homeostasis response after the injury. Figure 3(a)–(d) are projection views which are optimized to display the morphology and flow direction, but may not show the connection of each vessel properly. Therefore, we validated the flow direction and vessel connection in 3D in order to generate the plots in Figure 3(e)–(f).

Ischemia and hypoxia are characteristic of tissue damage. We have visualized these tissue conditions immediately after the punch induction through both DOMAG and DW-LSI systems. Insufficient vascular supply and the resultant reduction in tissue oxygenation often lead to capillary recruitment and neovascularization in order to meet the needs of the living tissue [26]. Also, compensatory development of collateral blood vessels in ischemic tissues associated with healing of hypoxic wounds have been reported previously [27]. These events were visualized and confirmed in our study as demonstrated in Figures 2, 3 and 4. The relationship between tissue hypoxia and angiogenesis is complicated but cooperative. On one hand, hypoxia induces the expression of VEGF through hypoxia inducible factors (HIF) [28–29], promoting new vessel formation. On the other hand, angiogenesis restores tissue perfusion, reestablishes microcirculation, and increases tissue oxygenation [30]. We observed a gradient of tissue hypoxia from the near-end of the injured area to the far-end downstream as illustrated in Figure 4. Indeed, a gradient of VEGF expression is usually established that parallels the hypoxic gradient, and endothelial cells subsequently migrate towards the most hypoxic areas [31]. Moreover, hypoxic tissue gradient is mandatory for wound healing-related angiogenesis, and previous work has indicated that removal of the hypoxic gradient inhibits capillary growth [32]. From the technical point of view, most of the previous work trying to measure hemoglobin concentration has been using wavelengths in the visible range because of their higher sensitivity when compared to the near-infrared wavelengths. Based on the current availability of laser sources in our lab, 780 nm and 825 nm were chosen for this study. Future plans include replacing these two laser sources with shorter wavelengths, which may provide higher sensitivity in quantifying blood oxygenation responses.

It is also important to monitor the long-term vascular changes after an injury in order to understand the critical events during the chronic healing process. OMAG technique allows us to track detailed morphological and functional changes of the 3D microvasculature with micron-level resolution, therefore, is a perfect candidate for studying long-term wound healing. As shown in Figure 5, complete microvascular network can be imaged at various time points after the injury. From the OMAG images, we can see that the angiogenesis process peaks at 3–7 days after the punch, which matches well with the previous finding

where the authors have shown that maximal VEGF mRNA is found between 3 and 7 days after wound induction during the period of granulation tissue formation [33].

The interesting bright spots lining up at areas close to the downstream vessels of the affected area on the OMAG image at day 3, may be caused by extra fluid of tissue edema, or by accumulation of macrophages. It is known that during inflammation stage, tissues tend to be swollen with an increased amount of extracellular fluid [34]. This may contribute to a higher degree of scattering which causes the increased intensity of local regions within the OMAG image. During the first stage of healing, cytokines are released that trigger the recruitment of neutrophils and monocytes. These cells usually arrive at the wounded region within minutes of the injury [35]. When neutrophil infiltration ceases at a few days after the injury, macrophages start to phagocytose the expended neutrophils. Therefore, at day 3, it is highly possible that there is an increased amount of macrophages accumulated at the downstream of the injured area to phagocytose the remaining pathogenic organisms and cell/matrix debris. Further experiments to determine the cause of these bright spots will be performed in conjunction with histological staining. After day 7, the angiogenesis process became less active, and OMAG image showed a weaker blood flow compared to day 3, and 7. This angiogenesis activity and blood vessel decline when endothelial cells undergo apoptosis during the latter stage of healing.

There are a number of on-going efforts to better understand the complicated healing process, and many novel strategies have been proposed for better wound healing outcomes [36]. For examples, VEGF, transforming growth factor- β (TGF- β) and other growth factors such as fibroblast growth factor (FGF) are attracting increasing interest to promote the healing process [33, 37]. Our imaging method could provide direct visualization and quantification for the vasculature changes induced by applying or blocking these growth factors. Moreover, it could be applied to studies of topical drugs, stem cell therapies, and related strategies to improve wound healing. Our imaging method can reveal not only the blood flow information but also changes in tissue morphology, therefore, is potentially useful for studies of scar formation. In summary, our work has established new ways to directly visualize tissue and blood vessel responses, and has provided new potentials to study cutaneous wound healing and its related diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported by research grants from the National Institutes of Health (RO1EB009682) (RO1HL093140). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Brigham PA, McLoughlin E. Burn incidence and medical care use in the United States: estimates, trends, and data sources. *J Burn Care Res.* 1996; 17:95–107.

2. Branski LK, Gauglitz GG, Herndon DN, et al. A review of gene and stem cell therapy in cutaneous wound healing. *Burns*. 2009; 35:171–180. [PubMed: 18603379]
3. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med*. 1999; 341:738–746. [PubMed: 10471461]
4. Hunt, TK., editor. Wound healing and wound infection: theory and surgical practice. Appleton-Century-Crofts; 1980.
5. Clark, RA., editor. The molecular and cellular biology of wound repair. Springer; 1996.
6. Bao P, Kodra A, Tomic-Canic M, et al. The role of vascular endothelial growth factor in wound healing. *J Surg Res*. 1999; 153:347–358. [PubMed: 19027922]
7. Clark S, Campbell F, Moore T, et al. Laser Doppler imaging—a new technique for quantifying microcirculatory flow in patients with primary Raynaud's phenomenon and systemic sclerosis. *Microvasc Res*. 1999; 57:284–291. [PubMed: 10329254]
8. Debbabi H, Bonnin P, Ducluzeau PH, et al. Noninvasive assessment of endothelial function in the skin microcirculation. *Am J Hypertens*. 2010; 23:541–546. [PubMed: 20168305]
9. Lockwood GR, Turnball DH, Christopher DA, et al. Beyond 30 MHz [applications of high-frequency ultrasound imaging]. *Eng in Med Biol Mag IEEE*. 1996; 15:60–71.
10. Anderson H, Price P. Clinical measurement of blood flow in tumours using positron emission tomography: a review. *Nucl Med Commun*. 2002; 23:131–138. [PubMed: 11891466]
11. Helbich TH, Roberts TP, Rollins MD, et al. Noninvasive assessment of wound-healing angiogenesis with contrast-enhanced MRI. *Acad Radiol*. 2002; 9:S145–S147. [PubMed: 12019853]
12. Beard P. Biomedical photoacoustic imaging. *Interface Focus*. 2011; 1:602–631. [PubMed: 22866233]
13. Hu S, Maslov K, Wang LV. Noninvasive label-free imaging of microhemodynamics by optical-resolution photoacoustic microscopy. *Opt Express*. 2009; 17:7688–7693. [PubMed: 19399148]
14. Hu S, Maslov K, Wang LV. Second-generation optical-resolution photoacoustic microscopy with improved sensitivity and speed. *Opt Lett*. 2011; 36:1134–1136. [PubMed: 21479007]
15. Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Sci*. 1991; 254:1178–1181.
16. Wang RK, Jacques SL, Ma Z, et al. Three dimensional optical angiography. *Opt Express*. 2007; 15:4083–4097. [PubMed: 19532651]
17. An L, Qin J, Wang RK. Ultrahigh sensitive optical microangiography for in vivo imaging of microcirculations within human skin tissue beds. *Opt Express*. 2010; 18:8220–8228. [PubMed: 20588668]
18. Wang RK, An L. Doppler optical micro-angiography for volumetric imaging of vascular perfusion in vivo. *Opt Express*. 2009; 17:8926–8940. [PubMed: 19466142]
19. Shi L, Qin J, Reif R, et al. Wide velocity range Doppler optical microangiography using optimized step-scanning protocol with phase variance mask. *J Biomed Opt*. 2013; 18:106015. [PubMed: 24165741]
20. Luo Z, Yuan Z, Pan Y, et al. Simultaneous imaging of cortical hemodynamics and blood oxygenation change during cerebral ischemia using dual-wavelength laser speckle contrast imaging. *Opt Lett*. 2009; 34:1480–1482. [PubMed: 19412312]
21. Reif R, Qin J, Shi L, et al. Monitoring Hypoxia Induced Changes in Cochlear Blood Flow and Hemoglobin Concentration Using a Combined Dual-Wavelength Laser Speckle Contrast Imaging and Doppler Optical Microangiography System. *PloS One*. 2012; 7:e52041. [PubMed: 23272205]
22. Srinivasan VJ, Sakadžić S, Gorczynska I, et al. Quantitative cerebral blood flow with optical coherence tomography. *Opt Express*. 2010; 18:2477–2494. [PubMed: 20174075]
23. Zhi Z, Yin X, Dziennis S, et al. Optical microangiography of retina and choroid and measurement of total retinal blood flow in mice. *Biomed Opt Express*. 2012; 3:2976–2986. [PubMed: 23162733]
24. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin Dermatol*. 2007; 25:9–18. [PubMed: 17276196]

25. Luo, Q.; Jiang, C.; Li, P., et al. Laser speckle imaging of cerebral blood flow. In: Tuchin, VV., editor. *Coherent-Domain Optical Methods: Biomedical Diagnostics, Environmental Monitoring and Material Science*. New York: Springer-Verlag; 2012. p. 167-211.
26. Adair TH, Gay WJ, Montani JP. Growth regulation of the vascular system: evidence for a metabolic hypothesis. *Am J Physiol-Reg I*. 1990; 259:R393–R404.
27. Knighton DR, Hunt TK, Scheuenstuhl H, et al. Oxygen tension regulates the expression of angiogenesis factor by macrophages. *Sci*. 1983; 221:1283–1285.
28. Elson DA, Ryan HE, Snow JW, et al. Regular Articles-Tumor Biology-Coordinate Up-Regulation of Hypoxia Inducible Factor (HIF)-1 α and HIF-1 Target Genes during Multi-Stage Epidermal Carcinogenesis and Wound Healing. *Cancer Res*. 2000; 60:6189–6195. [PubMed: 11085544]
29. Mace KA, Yu DH, Paydar KZ, et al. Sustained expression of Hif-1 α in the diabetic environment promotes angiogenesis and cutaneous wound repair. *Wound Repair Regen*. 2007; 15:636–645. [PubMed: 17971009]
30. Silver IA. The measurement of oxygen tension in healing tissue. *Progr Resp Res*. 1969; 3:124–135.
31. Talks KL, Turley H, Gatter KC, et al. The expression and distribution of the hypoxia-inducible factors HIF-1 α and HIF-2 α in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol*. 2000; 157:411–421. [PubMed: 10934146]
32. Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. *Surgery*. 1981; 90:262–270. [PubMed: 6166996]
33. Frank S, Hübner G, Breier G, et al. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. *J Biol Chem*. 1995; 270:12607–12613. [PubMed: 7759509]
34. Stamatas GN, Southall M, Kollias N. In vivo monitoring of cutaneous edema using spectral imaging in the visible and near infrared. *J Invest Dermatol*. 2006; 126:1753–1760. [PubMed: 16675964]
35. Hübner G, Brauchle M, Smola H, et al. Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine*. 1996; 8:548–556. [PubMed: 8891436]
36. Menke NB, Ward KR, Witten TM, et al. Impaired wound healing. *Clin Dermatol*. 2007; 25:19–25. [PubMed: 17276197]
37. Broadley KN, Aquino AM, Woodward SC, et al. Monospecific antibodies implicate basic fibroblast growth factor in normal wound repair. *Lab Invest*. 1989; 61:571–575. [PubMed: 2811305]

Fig. 1.
A complete microvasculature network of whole mouse ear acquired by ultra-high sensitive optical microangiography imaging system. Scale bar: 1 mm.

Fig. 2.

Images acquired from the laser speckle imaging system showing the large-scale blood flow changes of the mouse ear following a punch biopsy. (a) baseline image of the mouse ear before punch; (b) – (f) are images taken at 10 s, 5 min, 10 min, 25 min, and 55 min after punch, respectively. Scale bar: 1 mm.

Fig. 3.

Changes in blood flow velocity and direction of the mouse ear following a punch biopsy. (a) Baseline DOMAG image of the mouse ear; (b), (c) and (d) are DOMAG images taken at before punch, 10 min, 25 min and 55 min after the punch, respectively. Red represents flow against (arterial) and green represents flow towards (venous) the incident beam with a velocity range of ± 0.7 mm/s; (e) and (f) are skeletonized arterial network at baseline and at 10 min after the punch, respectively. Absolute blood flow is labeled for the vessel segments that show significant responses after punch. 1–6 denote the blood vessel segments that have shown significant blood flow changes, A–E denote the five main arterial branches. Scale bar: 1 mm.

Fig. 4.

Tissue oxygenation maps of the mouse ear following a punch biopsy. (a), (b) and (c) represent changes of oxygenated hemoglobin (HbO) of the mouse ear at 10 s, 5 min, and 40 min after the punch, respectively. (d), (e) and (f) show changes of deoxygenated hemoglobin (Hb) of the mouse ear at 10 s, 5 min, and 40 min after the punch, respectively. M = molar. Scale bar: 1 mm.

Fig. 5. Long-term monitoring of microvasculature changes during chronic stage of wound healing using UHSOMAG imaging system. (a) – (f) show maximal projection views taken at baseline, 1, 3, 7, 14, and 30 days after the punch, respectively. Scale bar: 1 mm.