Published in final edited form as:

J Neurosci Methods. 2010 December 15; 194(1): 108–115. doi:10.1016/j.jneumeth.2010.09.021.

Label-free *in vivo* optical imaging of functional microcirculations within meninges and cortex in mice

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Abstract

Abnormal microcirculation within meninges is common in many neurological diseases. There is a need for an imaging method that is capable of monitoring dynamic meningeal microcirculations, preferably decoupled from cortical blood flow. Optical microangiography (OMAG) is a recently developed label-free imaging method capable of producing 3D images of dynamic blood perfusion within micro-circulatory tissue beds at an imaging depth up to ~2 mm, with an unprecedented imaging sensitivity to blood flow at ~4 $\mu\text{m/s}$. In this paper, we demonstrate the utility of OMAG in imaging the detailed blood flow distributions, at a capillary level resolution, within the meninges and cortex in mice with the cranium left intact. Using a thrombotic mouse model, we show that the OMAG can yield longitudinal measurements of meningeal vascular responses to the insult and can decouple these responses from those in the cortex, giving valuable information regarding the localized hemodynamics along with the dynamic formation of thrombotic event. The results indicate that OMAG can be a useful tool to study therapeutic strategies in preclinical animal models in order to mitigate various pathologies that are mainly related to the meningeal circulations.

Keywords

Optical microangiography; microcirculation; blood flow; meningeal blood vessel network; thrombosis

1. Introduction

Meninges are the system of membranes enclosing central nervous system (CNS), which consists of three histological layers, i.e., the dura, the arachnoid and the pia. The dura is traditionally considered as an inert, fibro-elastic membrane, serving largely the mechanical and periosteal functions (Lipowsky, 2005; Rowbotham and Little, 1965a). Review of the anatomy reveals that the dura is not a simple fibrous tissue covering, but a complex structure innervated with dense micro-blood vessels (Kerber and Newton, 1973). Recently, a complete description of dural vascularity is made possible by use of the radiography, microdissection, clearing and histology techniques (Roland et al., 1987). The postmodern studies show the presence of a fine anastomotic network emerging from middle meningeal arteries, located close to the bone. Despite the fact that the branches of arterioles in the dural membrane supply the metabolic needs of the cranium, it was speculated that this dural

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Conflict of Interest

The authors declare no conflict of interests.

plexus is likely a source of collection of blood or fluid in subdural space caused by traumatic or nontraumatic subdural hemorrhage (SDH) and may be responsible for the causes of migraine and meningiomata (Rowbotham and Little, 1965b). A more thorough understanding of this dural plexus in terms of its hemodynamic response to the internal or external stimulus and its interaction with the pial or cortical microvessels may help to reveal mechanisms behind a number of pathological and physiological conditions that involve meningeal blood circulation, for example migraine.

In the past, the functional magnetic resonance imaging (fMRI) (Lindner et al., 1996) provides an unsurpassed tool to image non-invasively the hemodynamic response of meningeal vessels in pre-clinical as well as clinical settings. However, interpretation of fMRI data is often confounded by relatively poor specificity to vascular events because current fMRI, even at its highest imaging resolution, cannot resolve the detailed dural vascular plexus and its functional blood flow. Therefore, there has been a constant search for a noninvasive/minimally-invasive optical imaging technique that can be applied in preclinical animal models as a guiding tool and as an alternative to standard angiography requiring contrast agents or excisional biopsy. Optical imaging methods such as laser speckle imaging (Bolay et al., 2002) have shown the ability to visualize dural vessels with spatial resolution of $\sim 50\mu\text{m}$, as well as to delineate hemodynamic response based on the Doppler effect induced by moving blood cells. Despite these advantages, this 2D imaging modality is not capable of providing the depth-resolved imaging of microcirculation. Thus, its measurement of vascular responses in the dura would most likely be contaminated by those from the cortex. Two-photon microscopy (Bartholomaeus et al., 2009), a widely used laboratory imaging technique capable of ultrahigh resolution ($\sim 1\mu\text{m}$) imaging, has been used to provide specific and accurate morphological information of blood vessels in the dura. In doing so, however, it requires removal of intervening bone to access the dura and cortex since its imaging depth is only up to $200\mu\text{m}$, which is not desirable for repeated and long-term imaging studies. In addition, it requires injection of fluorescent tissue markers, which may complicate the interpretation of the results (Misgeld and Kerschensteiner, 2006).

Optical microangiography (OMAG) (Wang et al., 2007) is a novel extension of optical coherence tomography technology (Fercher et al., 2003; Tomlins and Wang, 2005), which is capable of producing 3D images of dynamic blood perfusion within micro-circulatory tissue beds at an imaging depth up to approximately one transport-mean-free-path below the surface ($\sim 2\text{ mm}$ in brain). OMAG produces imaging contrasts via endogenous light scattering from flowing blood cells within functional blood vessels; thus, it is a label free imaging technology. Previously, we have demonstrated the feasibility of OMAG in imaging cerebral microcirculation in mice with the cranium left intact (Jia et al., 2009). Due to its depth-resolved imaging capability, studies have also shown that OMAG was able to separate the blood circulations within the meninges from those in the cortex (Wang, 2008; Wang, 2010). In these studies, the rich capillary loops within the meninges were not imaged, even though large vessels such as the meningeal arterioles and venules were clearly imaged. This failure is attributed to that the previous reported OMAG system was only capable of imaging minimal blood flow at $\sim 160\mu\text{m/s}$, limited by the optical heterogeneous property of the cranium and brain tissue (Wang, 2010; Wang and An, 2009). However, the blood flow in the meningeal vessels, particularly in the capillaries, is very slow, ranging from tens of microns per second (in capillaries) to millimeters per second (in large vessels) (Lipowsky, 2005). In order to visualize the capillary plexus in dura mater, the sensitivity of OMAG to the blood flow must be improved.

Most recently based on OMAG, we have further developed an ultrahigh sensitive OMAG (UHS-OMAG) system (An et al., 2010), capable of imaging blood flows within patent vessels with an unprecedented sensitivity to the blood flow, ranging from $4\mu\text{m/s}$ to 22.2

mm/s. With such high sensitivity to the flow, imaging of the true capillary blood flows within human skin (An et al., 2010) and human retina (Wang et al., 2010) were successfully demonstrated. The primary objective of this paper is to demonstrate the utility of the UHS-OMAG to image the detailed meningeal and cortical vasculatures at capillary level resolution. The secondary objective is to evaluate the capability of UHS-OMAG for *in vivo* chronic imaging of dural vessel morphology and blood flow changes in mice under both healthy and pathologic conditions. To achieve this, we adopted a well-established, ferric chloride-induced thrombosis model (Wang and Xu, 2005). Upon the topical application of ferric chloride on the cranium surface of a mouse, we used the UHS-OMAG system to image the dynamic thrombosis formation in meningeal and cortical blood vessels. The results from this dynamic study on induced microvascular thrombosis and occlusions within the meninges show that UHS-OMAG may provide valuable and useful information in the meningeal thrombosis research and the preclinical drug development.

2. Materials and Methods

All experimental animal procedures were performed in conformity with the guidelines of the US National Institutes of Health. The laboratory animal protocol for this work was approved by the Animal Care and Use Committee of Oregon Health & Science University (Portland, OR, USA).

2.1 Ultrahigh sensitive optical microangiography (UHS-OMAG) system setup

The system setup used to achieve UHS-OMAG is shown in Fig. 1A, similar to the one used in our previous work (An et al., 2010; Jia et al., 2009). Briefly, the setup used a broadband infrared superluminescent diode (SLD) as the light source, which has a central wavelength of 1310 nm and bandwidth of 65 nm that provided a $\sim 12 \mu\text{m}$ axial resolution in air ($\sim 8.9 \mu\text{m}$ in biological tissue). The light from the SLD was coupled into a fiber-based Michelson interferometer. The light emerging at the output of interferometer was sent to a custom-built high-speed spectrometer, which had a designed spectral resolution of $\sim 0.141 \text{ nm}$ that provided a detectable depth range of $\sim 3.0 \text{ mm}$ in air (2.22 mm in biological tissue). To match the lateral resolution with the axial resolution while keeping sufficient depth of focus for imaging the meningeal vessels through the intact cranium, we improved the sampling arm optics by having the beam diameter of $\sim 3 \text{ mm}$ and using an objective lens with a focal length of 30 mm (Fig. 1B). With this modification, the system provided a measured lateral resolution of $\sim 9 \mu\text{m}$ and a depth of focus of $\sim 400 \mu\text{m}$. Because the average size of capillary vessel is about $10 \mu\text{m}$, we expect that the system with designed spatial resolution of $\sim 10 \mu\text{m}$ in x-y-z would be able to image the capillaries in the intracranial dura mater. The system sensitivity was $\sim 105 \text{ dB}$ measured at the focal spot of the sampling beam with $\sim 3.0 \text{ mW}$ power of light incident on the object and an exposure time of $\sim 21.0 \mu\text{s}$.

When imaging, the sample beam was rapidly scanned over the sample with a x-y scanner, while the line-scan camera in the spectrometer was continuously recording the OMAG signals formed between the reference and the sampling light. The camera was running at 47,000 Hz, meaning that the imaging speed was 47,000 depth-scans (A-scan) per second. With this imaging speed, we acquired 256 A-scans in the x-direction (B-scan) of 2.56 mm to form one cross-sectional image (B frame). This configuration determined the system frame rate at 180 frames per second (fps). In the y-direction, we captured a high density C-scan (i.e. y-direction scan), consisting of 1500 B-scans over 2.56 mm. Accordingly, it required $\sim 8 \text{ sec}$ to acquire a complete 3D data volume of $2.56 \times 2.56 \times 2.22 \text{ mm}^3$. Then, a UHS-OMAG algorithm (An et al., 2010) was applied to this 3D data set to obtain morphological and microcirculatory images of the scanned tissue volume. With this setup, our OAMG system is capable of imaging blood flow ranging from $\sim 4 \mu\text{m/s}$ to $\sim 22.2 \text{ mm/s}$ (An et al., 2010), which is sufficient to image the meningeal blood circulation.

2.2 In vivo imaging

To test the performance of the UHS-OMAG imaging of the dural as well as cortical vascular networks *in vivo*, three-month-old C57 BL/6 mice were used in this study. The mice, each weighing 23 to 28 g, were prepared for OMAG one day before the experiments by shaving the heads followed by a depilatory cream to remove all remaining hair from the top of the scalp. During the imaging, the animal was immobilized in a stereotaxic stage and was lightly anesthetized with isoflurane (0.2 L/min O₂, 0.8 L/min air). The body temperature was kept between 35.5–36.5 °C by use of a warming blanket, and monitored by a rectal thermal probe throughout the experiment. An incision of ~1 cm was made along the sagittal suture and the frontal parietal and interparietal bones were exposed by pulling the skin to the sides. The animal was then positioned under the scanning optical probe, with the meninges carefully placed within the depth of focus of the probe beam (see Fig. 1B) as monitored by real time UHS-OMAG/OCT structural images displayed on the computer screen on the fly.

To better show the results, a volume segmentation algorithm (Wang and Hurst, 2007) was then applied to each 3D UHS-OMAG image to isolate the blood flow signals in the meninges from those in the cortex. The segmentation resulted in two volumetric flow images, one for the meninges and the other for the cortex. The maximum amplitude projection was applied to each segmented meningeal and cortical volumes, resulting in the blood flow distribution maps. To evaluate the feasibility of using UHS-OMAG as a useful tool in characterizing meningeal blood flow over a larger area of dural mater, we collected the multiple 3D images over the different regions on the entire brain. The UHS-OMAG images from 16 different regions were then combined as a mosaic, covering an area of ~10×10 mm² over the entire mouse head.

2.3 Thrombosis experiments

To evaluate the capability of UHS-OMAG for *in vivo* longitudinal imaging of dural vessel morphology and blood flow changes. We adopted a well-established, ferric chloride-induced thrombosis mouse model (Wang and Xu, 2005). With this model, vascular injury and thrombus formation were artificially induced. For UHS-OMAG imaging, an imaging region [~2.5mm(X)×~1.3mm(Y)] was selected next to the posterior coronal suture (PCS) as shown in Fig. 4A. Following Nagai et al (Nagai et al., 2010), we induced thrombus formation via topical application of 10% FeCl₃-soaked paper (1×3 mm) placed over the exposed posterior coronal suture along the dotted outline shown in Fig.4A. The piece of paper was then removed after 5 min and the administration region was washed twice with warm saline. The microvascular occlusion and thrombus formation was monitored in real time by the optical imaging system. 3D optical data set were acquired once every five minutes for total 35 min since the application of FeCl₃.

2.4 Blood flow quantification

To quantify the meningeal blood flow, we applied the phase-resolved technique (Zhao et al., 2002) to the UHS-OMAG flow signals, which measures the phase changes in optical signal due to flowing erythrocytes (Wang and An, 2009). Because the phase changes linearly relates to the velocity of moving particles, we calculated the phases from adjacent B-scans collected at the same position, and then the absolute velocity was obtained by converting the calculated phases to the velocities scaled by the Doppler angle. The Doppler angle was obtained from the 3-D blood vessel network because the angle of the incident beam was known *a priori*. Five repeated B-scans at each location were collected at each x-y position for phase estimation. This method has previously been validated in flow phantoms (An et al., 2010). Cross sectional flow profiles across the vessels of interest were fit to a second-order polynomial and corrected for the angle of incident beam to provide the velocity in millimeters per second, from which the mean blood velocity (V_{mean}) and the vessel inner

diameter (D) were determined. The flow rate in the vessel was calculated as a product between the average velocity and the cross sectional area of the vessel $[V_{mean} * \pi * (\frac{D}{2})^2]$. The blood volume in the imaging region in each time point was evaluated from the UHS-OMAG 3D flow data, which is defined as the total volume of blood flow in the scanned tissue volume, and then normalized by the baseline.

3. Results

3.1 Imaging of meningeal and cortical microvascularization

The depth-resolved imaging results using UHS-OMAG for blood perfusion within an area of $\sim 2.5 \times 2.5 \text{ mm}^2$ are shown in Fig. 2. Fig. 2A gives one typical cross-sectional image (B-scan) within the UHS-OMAG structural volume, which is identical to the conventional OCT image where typical morphological features, such as cranium and cortex, are visualized. Fig. 2B gives the corresponding blood flow image obtained from UHS-OMAG, where the micro-blood flows (e.g., pointed by arrows) originating from the dura mater as well as the rich cortical blood flows are clearly identified. Considering that the dural plexus is a vascular network exclusively above the cortical plexus, the flow signals above the cortical layer were simply treated as signals from the dural vessels, which were usually at the depths from 100 to 200 μm below the cranium surface. With this as the reference, we applied the segmentation algorithm (Wang and Hurst, 2007) to separate the blood flows within the meninges from those in cortical layers. This segmentation resulted in the blood flow distribution maps shown in Figs. 2C (meninges) and 2D (cortex), respectively. From Fig. 2C, the dural blood vessels are seen to be tortuous. Further, the large vessels do not greatly diminish in size, in spite of giving off many side branches. More importantly, the UHS-OMAG is capable of visualizing the rich anastomotic, as well as the splitting vessels (e.g., pointed by the arrows) in the meninges. These appearances of the dural vessels are directly resulted from characteristic slow blood circulation in the intracranial dura mater. These findings from the UHS-OMAG correspond well to what is called the primary or secondary anastomotic network in standard dural histopathology (Kerber and Newton, 1973). Because of its capability of imaging much slower blood flows, it is not surprising that UHS-OMAG gave an image of greater vascular density for the cortex (Fig. 2D).

Fig. 3 shows whole-brain mosaic flow images from both the dura (Fig. 3A) and cortex (Fig. 3B), providing volumetric measurements of detailed microcirculation through the vascular tree down to the capillary level with the skull left intact, without a need for dye injections, contrast agents, or surgical craniotomy. The dural vessels are seen to distribute unevenly as they course over the dural surface. The fine vascular network is concentrated on three typical sutures, ie, sagittal suture (SS), anterior coronal suture (ACS) and posterior coronal suture (PCS), and in the non-suture area cross-linked blood vessels are directed more towards the posterior than the anterior segment (Fig. 3A). These features from dural vessels do not resemble those of cortical vessel distribution (Fig. 3B). Such appearances observed from UHS-OMAG are consistent with the vascular convolution described in the well-known references (Roland et al., 1987; Rowbotham and Little, 1965a), demonstrating potential power of the UHS-OMAG in the investigations of neurological diseases and complications that may involve the meningeal microcirculations.

3.2 Dynamic imaging of thrombosis within meninges and cortex

Meningeal or cortical blood vessel occlusions due to the thrombus formation can occur in diseases and pathophysiological processes. An ability to non-invasively image microvasculature in preclinical models is useful for testing and evaluating new thrombolytic drugs capable of dissolving thrombi or preventing thrombus formation.

Figure 4 shows dural microvascular network of a region in a mouse during thrombus formation initiated by the topical application of FeCl_3 . Progress of the occlusion and the subsequent effect on the functional vessels were observed for 35 minutes with the UHS-OMAG data sets acquired every five minutes. Figure 4B shows the time serial images of the region outlined by the small box in Fig. 4A, which shows the gradual change in functional vessel density as thrombi formed over time. Vascular occlusion gradually propagated over approximately 35 min, resulting in decreased functional vessel density due to restricted and occluded blood flow. To examine the blood flow dynamics in more detail, relative flow magnitude were assessed in each single vascular branch in these serial optical reflectance maps. According to the location of the applied FeCl_3 solution and the occlusion sequences from the optical images, four main side-branches could be identified in the imaged region as shown in Fig. 4C where different vessels are differently colored and the numbers denote their occlusion sequence. It was interesting to note that in some vessels when thrombosis was formed in the distal end of vessels, the blood flow was ceased gradually in a direction to their main branch; and when thrombosis was formed in the main branch of vessels, the blood flow in the lower branches was ceased rapidly. For example, in vessel 2, the microvascular occlusion was firstly developed in the distal end (“D” in Fig. 4C) because the FeCl_3 solution is more easily absorbed by capillary with one-cell thickness vascular wall, and then the main branch (“M” in Fig. 4C) was occluded probably due to its position priority. This was followed by a rapid drop in the flow in the remaining part of the same vessel. Therefore, this vessel was an arteriole, flowing from the main branch to its distal end. These sequential events also inform that the thrombotic plugs were dynamic structures that expanded during the observation period of 35 min and grew from the distal end. Moreover, within the region we observed, complete cessation of the blood flow only occurred in the vessel 1; whereas the vessels 2 and 3 did not even though their flow was dropped dramatically. Another interesting phenomenon is that the blood flow in some branches was decreased initially, but later was compensated by those from the branches connected with the functional neighboring vessels.

Besides the dynamic imaging of the meningeal vascular flow, the flow dynamics in the cortical layer can also be visualized by OMAG. As shown in Fig. 5, the dark shadows starting from the left bottom corner indicate a gradual propagation of the thrombosis in the vicinity of FeCl_3 solution, resulting in an expansion of the occlusion front. This occlusion front advanced about $35\mu\text{m}/\text{min}$ during the observation period. Compared with the occluding speed within first 15 minutes, it seems that the occlusion front moved slower during the remaining 20 min of the observation period. In addition, from the growth of the occlusion in the cortex, it can be seen that the thrombosis mainly occurred on the side branches of the main vessel.

3.3 Quantitative measurement of meningeal and cortical blood flow during thrombosis

In order to be more informative about the thrombus growth and microvessel occlusion, blood flow on the microvessel level was quantified via UHS-OMAG. The velocity profiles across the dural vessels 1 and 2 (Fig. 4C, pointed by black arrows) are shown in Fig. 6 along with the second-order polynomial fit and the R-squared value of the fit at the time points as shown [0–15min (vessel 1), 15–30min (vessel 2)]. The shape and peak of the velocity profile changes with time, indicating the decrease in vessel diameter and flow velocity over the 35-min time course.

Figure 7 shows quantitative measurements of the mean velocity (Fig. 7A), the vessel inner diameter (Fig. 7B), and the flow rate (Fig. 7C) over the 35-min time course for four individual feeding vessels (numbered in Fig. 4C). These plots indicate that all variables change with time. Each vessel appears to vary differently with time, especially in terms of the velocity (Fig. 7A) and flow rate (Fig. 7C) parameters. For example, the vessel 1 reacted

more rapidly than other three vessels, due to its position priority and the early thrombosis formed in its parent branch. In vessel 2, the three parameters were slightly over the baseline during a period from 5 minutes to 20 minutes after the topical administration of FeCl₃ and then decreased dramatically. In vessel 4, the blood flow was down to its extreme low point at 20 minutes and then slowly reperused by its proximal bypass connected to another vessel. Despite discrepancy between them, the velocity and blood flow in all regions at 25 minutes declined to at least 50 percent of their baseline. Comparing the baseline with the endpoint, the vessel diameter did not diminish greatly except the vessel 1. After 35-minute reaction, the diameter of vessel 1 diminishes to zero during thrombosis process. There may be two reasons for this to happen: 1) the vessel is fully closed, and 2) the blood flow in vessel is lower than the detection limit of 4 $\mu\text{m/s}$, or simply the blood is static.

With respect to the flow dynamics in cortex, the time evolution of blood volume is shown in Fig. 7D. There was a significant decrease from the baseline to 25 minutes and a slight decline during the remaining 10 minutes. This is in agreement with the results observed from the meningeal vessels that indicated that 25 minutes is a critical transition point.

4. Discussion

The optical imaging method presented in this article provides an attractive alternative to other methods because of its ability to accurately image the meningeal and cortical blood flow responses in living animals over an area of a few millimeters with the cranium left intact. This imaging modality is non-invasive, relatively inexpensive, and fast. These attributes are well suited for long-term repeated measurement of blood flow *in vivo*. Through comparison of the time-lapse 3D images, the dynamic alterations of microcirculation associated with the cerebral pathophysiologic events, such as thrombosis, can be observed. More importantly, due to the depth-resolved feature of the UHS-OMAG, the dynamic responses of the microcirculation within the meninges can be decoupled from those in the cortex.

In addition to its capability of visualizing the detailed functional microcirculation (e.g. Figs. 2 and 3) and the relative flow distribution along the whole pathological event (e.g. Figs. 4 and 5), UHS-OMAG is able to quantify the flow-related parameters, such as velocity, vessel diameter and flow rate of the individual vessels of interest, which are critical in investigating the cerebral flow responses to a certain insult. With a sensitivity to the flow as low as 4 $\mu\text{m/s}$, we believe that the reported UHS-OMAG imaging technique is the most precise method to date for quantifying localized blood flow and visualizing the detailed microcirculations within the scanned tissue beds *in vivo*.

Any cerebral blood flow is tightly coupled to the underlying neuronal response. The meningeal blood flow has no exception. It is crucial to monitor the spatial and temporal extent of the meningeal blood flow changes in the studies of functional activation and pathophysiologic conditions accompanied by uncoupling, such as the thrombosis. Optical imaging of the functional microcirculations using UHS-OMAG showed the gradual formation of the chemically induced thrombi and the effects of the thrombi on the microcirculation within the meninges and cortex. With the help of the flow quantification algorithm on the serial 3D datasets, OMAG can provide the dynamic flow parameters due to thrombosis formation and micro-vessel occlusion. The results may give additional useful information, if together with the systemic physiological measurements, to assess local micro-vessel physiology during a thrombotic event. Real-time imaging of the thrombotic event as well as its associated changes in microflow parameters is also useful in the development of new therapeutic agents designed to treat the pathological thrombi, because

OMAG makes it possible to observe subtle differences in the timing and the magnitude of drug action on the microvessel physiological response and thrombus formation.

As shown in this study, one of the advantages of the OMAG technique over other methods, such as laser speckle, is that it provides sufficient depth resolution to differentiate the contributions of the meningeal microcirculations from those of the cortical vessels. This ability would make the UHS-OMAG a useful and attractive tool in the investigations of the meningeal hemodynamic responses to other functional activation or neurovascular disorders, such as migraine headache, in which the changes in blood flow within dural vessels play an important role in the etiology, pathogenesis, prognosis, and responses to treatment.

Acknowledgments

Source of Funding

This work was supported in part by research grants from the National Heart, Lung, and Blood Institute (R01 HL093140); National Institute of Biomedical Imaging and Bioengineering (R01 EB009682); National Institute of Deafness and other Communication Disorders (R01 DC010201); and the American Heart Association (0855733G).

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Fig. 2.

Typical *in vivo* UHS-OMAG images of the cerebral microcirculation in mice. Images were taken with the skull left intact. (A) is one typical UHS-OMAG cross-sectional image (B-scan) of microstructures showing morphological features, such as cranium and cortex, and (B) is the corresponding blood flow image where the dural microvessels (e.g., pointed by arrows) are distinguishable from the vessels in cortex. The projection maps of functional blood vessel network within (C) the meninges and (D) the cortex, respectively, obtained from one 3D scan. White bar = 500µm.

Fig. 3.

The functional blood flow networks of the entire brain in mice were imaged by UHS-OMAG *in vivo*. Because of its depth-resolved imaging capability, OMAG is capable of providing simultaneous imaging of microcirculations within (A) the meninges and (B) the cortex of a mouse. The whole imaging area is $\sim 10 \times 10 \text{ mm}^2$. SS, sagittal suture; ACS, anterior coronal suture; PCS, posterior coronal.

Fig. 4. Representative meningeal microvascular network during thrombus formation induced by FeCl_3 . (A) is a sketch of the mouse skull; the red box indicates the region imaged ($\sim 2.5\text{mm} \times \sim 1.3\text{mm}$) by UHS-OMAG and the dotted line indicates the place where the FeCl_3 was topically applied. (B) shows serial OMAG images of microvascular occlusion and blood flow changes, indicating development of thrombus for the region shown in (A). Numbers in the bottom left corner of each panel are minutes after application of FeCl_3 . (C) is a vascular sketch based on the vascular occlusion sequence in (B), where four vessels are indicated in different colors, the numbers indicate their occlusion sequence and the arrows show four regions of interest in which blood flow will be quantified.

Fig. 5.
Representative cortical microvascular network during thrombus formation in the region (~2.5mm×~1.3mm) shown in Fig. 4A. Numbers on the bottom left corner are minutes after application of FeCl_3 . The dark shadows starting from 5 mins indicate a gradual propagation of the thrombosis in the vicinity of FeCl_3 solution, resulting in expansion of the occlusion fronts shown by the dotted outlines.

Fig. 6. Plots of the velocity profiles across the dural vessels 1 and 2 pointed by arrows in Fig.4C, respectively, along with the second-order polynomial fit and the R-squared value of the fit at the time point as shown [0–15min (vessel 1), 15–30min (vessels 2)]. The time of imaging in minutes is given in the upper right corner of each plot.

Fig. 7. Quantitative measures of the vessel mean velocity (A), vessel inner diameter (B), and flow rate (C) over the 35-min time course in four individual feeding vessels (see Fig. 4C), following the induction of thrombus. The time evolution of blood volume changes in cortex for the imaged region is shown in (D).