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Abstract. We designed a miniature laser speckle imager that weighs ~20 g and is 3.1-cm high for full-field high-resolution imaging of cerebral blood flow (CBF) in freely moving animals. Coherent laser light illuminates the cortex through a multimode optical fiber bundle fixed onto the supporting frame of the imager. The reflected lights are then collected by a miniature macrolens system and imaged by a high-resolution CMOS camera at a high frame rate (50 fps). Using this miniature imager, we achieve high spatiotemporal resolution laser speckle contrast imaging of CBF in freely moving animals in real time. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3625231]

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As a full-field high-resolution optical imaging technique,^{1,2} laser speckle contrast imaging (LSCI) has gained increasing attention in the neuroscience community.³ It is used for monitoring cerebral blood flow (CBF) under different physiological^{4,5} or pathological^{6–8} conditions. Until recently, the use of almost all CBF monitoring devices equipped with LSCI has been restricted to anesthetized animals.⁹ Anesthesia always introduces various types of physiological changes such as local blood flow changes¹⁰ and suppression of CBF response,¹¹ which may result in biased experimental results. LSCI has shown its considerable potential in the study of the cerebral blood flow;^{3,7,8,12} therefore, the head-mounted imager for small animals facilitates the study of cerebral blood flow changes without anesthesia.

Two major challenges are encountered in designing a laser speckle imager for small fully conscious animals. First, the imaging system, which includes an optical fiber bundle, macrolens system, and imaging sensor and circuits, should be sufficiently small and lightweight. Second, severe motion artifacts must be corrected. These problems occur only to a minimal extent in anesthetized animals, but are exacerbated under free motion. To overcome the above-mentioned problems, we designed a miniature head-mounted laser speckle imager (20 g)

that can monitor the real-time neurovascular changes in freely moving small animals, such as rats.

The imager system consists of five major components. i. A cylinder base (height, 4.20 mm; radius, 5.50 mm; thickness, 0.5 mm) is fixed onto the scalp over the imaging area with dental cement. The body of the imager is attached to the cylinder base through a screw connection during imaging. ii. A multimode optical fiber bundle (50 fibers; diameter, 0.25 mm; 0.45 NA; SMOIF, Shanghai, China) is used for laser light delivery. Coherent light from a laser diode (780 nm; 10 mW; L780P010, Thorlabs, U.S.A.) powered by a driver module (LDC220C, Thorlabs, U.S.A.) is coupled into the fiber bundle using a collimating lens ($f = 8$ mm; C240TM, Thorlabs, U.S.A.). The end of the fiber bundle is enclosed in an aluminum tube (length, 7.5 mm; radius, 1.54 mm) that is fixed onto the supporting frame as the light source of LSCI. iii. The system also has a printed circuit board (PCB; 3.6×3.6 cm; Thorlabs, U.S.A.) with a CMOS sensor (1024×1396 pixels; 8-bit precision; Thorlabs, U.S.A.), as well as image acquisition and transferring circuits, which are connected to a computer via USB cable. iv. A macrolens system (radius, 3.44 mm; focal length, 5.05 mm; minimum focus distance, 20 mm; 0.011 NA; custom-made to our design by GiantTec, Shanghai, China) is located within a plastic tube fixed onto the PCB, whose parameters were carefully designed to be adapted to the illumination tube (length, 7.5 mm), CMOS sensor (size, 2/3 in.) in order to obtain good imaging quality. v. A supporting frame is used to fix the PCB, the macrolens system, and fiber bundle. Four springs are added in the screw connections between the PCB and the supporting frame to reduce the influence of motion artifacts. The connection screws are also adjustable for focusing the macrolens onto the imaging plane. The entire imager weighs ~20 g and is 3.1 cm high. Figure 1(a) illustrates the design of the miniature head-mounted imager, and Fig. 1(b) is a snapshot of a fully conscious rat wearing the imager during the experiment.

Before LSCI, the imager is fixed onto the cylinder base. Laser light (e.g., 780 nm in this study) reflected by the cortex is collected by the macrolens system and then imaged by the CMOS sensor. The imager can be configured by software through a USB interface (pixel clock of 35 MHz, frame rate of 50 fps, exposure time of 5 ms, area of interest of 640×640 pixels). A custom-developed software program reads the raw images (25 frames/stack) through a USB cable for further CBF analysis on the computer. To eliminate motion artifacts, the speckle images in each stack (i.e., 25 frames) are aligned to the first frame by registered laser speckle contrast analysis (rLASCA).¹³ The raw speckle images were preprocessed with a 3×3 convolution kernel, and then registered by normalized correlation metric and finally resampled with cubic B -spline interpolator.¹³ After the registration, raw speckle images were processed by the random process estimator (RPE) method, utilizing the Gaussian property of estimation noise in integrated intensity random process, to obtain the contrast image with high signal-to-noise ratio.¹⁴ According to theory of laser speckle contrast imaging,^{1,9,15} blood flow speed v is related to contrast value K [Eq. (1)].⁹

$$K^2 = \beta \left[\frac{\tau_c}{T} + \frac{\tau_c^2}{2T^2} (e^{-2T/\tau_c} - 1) \right]. \quad (1)$$

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Fig. 1 (a) Design of the miniature head-mounted laser speckle imager. The system includes a i. cylinder base, ii. multimode optical fiber bundle, iii. CMOS sensor on a PCB, iv. macrolens system, and v. supporting frame. (b) A snapshot of the imager mounted onto the head of a conscious rat during the experiment.

where the correlation time τ_c is assumed to be inversely proportional to the blood flow speed v . $\beta = 1/N$, where N is the number of speckle in each pixel area.¹⁶ T is the exposure time.

The performance of the imager was tested using a standard USAF 1951 resolution test chart, which resulted in a 16 lp/mm resolution and <2% distortion as the best spatial resolution. In the recorded image, each pixel corresponded to a $12.5 \mu\text{m} \times 12.5 \mu\text{m}$ area in the imaging plane.

Subsequently, the performances of the imager in the cortical neurovascular imaging of freely moving rats (male, Sprague-Dawley, 280 g) were tested. The experimental protocols used in this study were approved by the Animal Care and Use Committee of Med-X Research Institute of Shanghai Jiao Tong University. To create the cranial imaging window and fix the aluminum cylinder base, the rat was anesthetized via intraperitoneal injection of 70 mg/ml chloral hydrate (5 ml/kg). The animal was then placed in a stereotactic frame (Benchmark Deluxe™, MyNeuroLab.com, St. Louis, Missouri) during the surgical procedure. The scalp was shaved and disinfected with 70% ethanol and povidone iodine solution. All procedures were performed under standard sterile precautions. After a midline scalp incision, the

Fig. 2 A typical laser speckle image from the miniature head-mounted imager (a) and the corresponding contrast image (b) of the freely moving rat.

Fig. 3 (a) and (b) Zoomed-in images of the white window area in Fig. 2(b) for the (a) anesthetized and (b) freely moving rats. (c) Center position of the vessel cross-section in the white window area at different imaging times. (d) Diameter of the superior sagittal sinus along the white line in Fig. 2(b) at different imaging times.

galea and periosteum bilaterally overlying the parietal bone were removed. A $7 \text{ mm} \times 7 \text{ mm}$ area centered at 3.5 mm posterior to the bregma over the entire cortex was thinned using a high-speed dental drill (Fine Science Tools Inc., North Vancouver, Canada) until the cortical vessels were clearly visible. During the experiment, rectal temperature was maintained at 37°C using a heating pad and dc control module (FHC Inc., Bowdoinham, Maine). The cylinder base was fixed onto the skull enclosing the thinned area using reinforced glass ionomer cements (Dental Materials Factory of Shanghai Medical Instruments Co., Shanghai, China). After cement hardening, the animal can be imaged at any time by screwing the imager onto the cylinder base.

Figure 2(a) shows a frame of the typical speckle images of the freely walking rat. In the corresponding contrast image [Fig. 2(b)], no obvious motion artifacts were observed. To further test the resolution and spatiotemporal stability of the imager for freely moving animals, we analyzed the vasculature within the selected area [Fig. 2(b)] in either the anesthetized [Fig. 3(a)] or freely moving [Fig. 3(b)] animal. While the rat was freely moving, we could still observe that the selected cortex contained one of the smallest recognizable blood vessels [white arrow in Fig. 3(b)], whose diameter was only 4 pixels ($50 \mu\text{m}$) determined by averaging 10 human observers' manual segmentation. Such a spatial resolution is comparable to the laser speckle imaging systems in literature³⁻⁵ and suitable for studying the CBF of

the major vessels under different physiological or pathological conditions.

The laser speckle images of the i. anesthetized rat and ii. the conscious and freely moving rat were continuously compared for 20 min. For free motion, the first 25 frames of the speckle images at each minute were used for rLASCA and RPE analysis. Two crossing vessels (Fig. 3) were purposely selected to test whether the coordinates of their crossing center, at which point the pixel's contrast value is a local minimum [see embedded image in Fig. 3(c)], would change in a moving animal. Figure 3(c) shows that rLASCA successfully eliminated the motion artifacts and resulted in images with highly stable contrast. These images were comparable to those of the anesthetized rat.

Finally, to test the stability of the optical focusing of the imager, we analyzed the diameter of the superior sagittal sinus along the white line in Fig. 2(b) based on the manual segmentations.¹³ The vessel diameter from 10 volunteers' segmentations showed very low variance (Mean $\pm$ Std: 56.6 $\pm$ 0.05 pixels), indicating high stability of the imager in optical focusing. This high stability is attributed to several special considerations in the design: i. the fast frame rate of the CMOS sensor (50 fps); ii. springs around the connection screws between the supporting frame and PCB; and iii. efficient registration of the rLASCA algorithm.

In summary, we designed a miniature head-mounted laser speckle imager for full-field high resolution imaging of CBF in freely moving rats. The new laser speckle imager provides the possibility of studying the structural and functional CBF of small conscious and freely moving animals.

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