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Supercontinuum light source enables in vivo optical microangiography of capillary vessels within tissue beds

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Abstract

This letter reports on the use of supercontinuum light source to achieve ultra-high resolution and ultra-high sensitive optical microangiography (OMAG) imaging of microcirculations within tissue beds in vivo. After passing through a special designed optical filter with a pass-band of 120 nm centered on 800 nm, the light source is coupled into an optic-fiber based OMAG system that provides a measured axial resolution of $\sim 3 \mu\text{m}$ over a ranging distance of 2 mm. Within this ranging distance, the system gives an averaged signal to noise ratio of 87 dB and a sensitivity roll-off of 7 dB at an A-scan rate of 70 kHz. We demonstrate the capability of the system to visualize detailed microvascular perfusion map, including the single red blood cells within the capillaries, by imaging the mouse ear flap *in vivo*.

Optical coherence tomography (OCT) [1] is a noninvasive and noncontact interferometric technology that enables depth-resolved imaging of biological tissues at micrometer scale resolution. In general, the axial resolution is determined by the bandwidth of the light source used, which is given by $\delta_z = 2(\ln 2) \lambda^2 / (\pi \Delta \lambda)$ where λ is the central wavelength and $\Delta \lambda$ is the spectral bandwidth. The wider the bandwidth, the more resolving power the OCT system is to differentiate the fine microstructures within tissue. The compact and cost effective superluminescent diodes (SLD), emitting in the spectral region centered around 800 nm or 1300 nm, are commonly used to implement the OCT systems. Unfortunately, the spectral bandwidth of the available SLDs is usually limited, which determines the achievable axial resolution at 7–10 μm for 800 nm and 10–20 μm for 1300 nm band light sources. In order to enhance the axial resolution, light source with broader spectral bandwidth has to be applied.

Researchers attempted to integrate a number of SLDs with non-overlapping central wavelengths into one single broadband source for resolution improvement [2], but the improvement is suboptimal in terms of emitting wavelength of the multiplexed SLD that is typically centered at $>900 \text{ nm}$, overlapping the water absorption at 980 nm that may affect the imaging depth. A number of reports [e.g., 3, 4] have demonstrated the use of state-of-the-art femtosecond lasers to achieve a 2–3 μm axial resolution, but with the disadvantage of being expensive and bulky, limiting its uses to a few of research laboratories. Being spectrally very broad and visually very bright, supercontinuum (SC) light source has been attracted recently as an excellent alternative for OCT imaging to achieve sub-cellular axial resolution [5]. The feasibility of Fianium SC source for time-domain and spectral-domain OCT (SDOCT) has been demonstrated by Kurz group [6, 7]. Recently, Koch group [8] has successfully used a commercial available Koheras SC source to realize a dual-band SDOCT system for flexible imaging of tissue microstructures at either 800 nm or 1300 nm

wavelength bands. However, all these previous works are focused on using the SC light source to improve the system power to resolve the fine microstructures within tissue. No report has been so far demonstrated the feasibility of utilizing a SC light source to image tissue microcirculations.

As a variation of Fourier domain OCT, optical microangiography (OMAG) [9] is a new imaging modality capable of generating 3D images of dynamic blood perfusion distribution within microcirculatory tissue beds. An unprecedented sensitivity to the blood flow down to $4\mu\text{m/s}$ was reported with the recent development of an ultrahigh sensitive OMAG (UHS-OMAG) [10]. To achieve ultrahigh resolution and ultrahigh sensitive imaging of tissue microcirculations, here, we report the engineering of a SC source into an optic-fiber based UHS-OMAG system that provides a useable light source centered at 800 nm with a bandwidth of 120 nm. By applying the UHS-OMAG algorithm to extract the flow signal out of the static tissue background, we successfully achieved ultrahigh resolution imaging of not only the tissue microstructures, but more importantly the functional microvasculature. To the best of our knowledge, this is the first study that reports the utilization of the SC light source to achieve ultrahigh resolution imaging of microcirculations.

The schematic of the system is shown in Fig.1a, which is an optic-fiber based SDOCT setup. The system layout is similar to the one published in [11], but with the design consideration and the use of components that can handle the high intensity and broad bandwidth of SC light source. The light source applied was a SuperK Versa Supercontinuum Source (Koheras A/S, Denmark) pumped by a Nd:YAG laser, which provides an output spectrum from 460 nm to 2400 nm and an total output power of 1.5 W. The output light from the SC source was passed through an optical filter that was specially designed to filter and re-shape the incoming SC light into the light that centers at 800 nm with a bandwidth of 120 nm. The filtered light was then collimated and coupled into an optic-fiber (single mode) based SDOCT system via an optical circulator (AC Photonics, USA). All the optic-fiber components used were specified at 800 nm with a spectral bandwidth of at least 120 nm. The output power from the single-mode fiber in the sample arm was measured at $\sim 12\text{ mW}$. The spectral interferograms formed by the lights between the reference and sample arms were routed via the optical circulator into a home-built high-speed spectrometer. The spectrometer consisted of a collimating lens, a volume transmission grating and a CMOS line-scan camera (Basler SPL 4096-70 KM, Germany). The camera had 4096 pixels and a maximum line scan rate of 70 kHz. The designed spectral resolution of the system was 0.044 nm that provides a ranging distance of 4 mm, implying that each pixel in the final OCT image practically represents $\sim 1.9\mu\text{m}$. With these designs, the system sensitivity had a measured maximal roll-off of 20dB at the depth of 4 mm (Fig.1b).

However, since the penetration depth of NIR light is usually limited to within 2 mm in turbid medium, the meaningful system performance should only be considered for the ranging distance of 0–2mm. This is shown in Fig.1c, where the averaged signal to noise ratio is rated at 87 dB, and importantly, the roll-off of system sensitivity is only $\sim 7\text{dB}$ at the depth of 2 mm, which would no doubt improve the overall system performance of imaging the microcirculations within tissue beds *in vivo*. Meanwhile, we also evaluated the free space axial resolution along this depth range by using the full width at half maximum of the FFT amplitude signal as shown in Fig. 1d. The achieved axial resolution is $\sim 3.2\mu\text{m}$, slightly worse than the theoretical coherence gate $2.3\mu\text{m}$, probably due to the residual optical dispersion in the system (after numerical dispersion compensation).

It is known that the capillary vessels have an average diameter between 6 – 8 μm . To resolve them well, the system design also needs to consider the lateral resolution besides the high axial resolution. Here, we adopted a microscopy objective lens with an effective focal length

of 18 mm to deliver the probing light onto the sample. The beam spot size before the objective lens was measured at ~ 3.0 mm, giving a theoretical lateral resolution of ~ 5.8 μm . For UHS-OMAG imaging of microcirculations, a specially designed scanning protocol was used. In the fast-scan direction, 256 A-lines were captured to compose one B-scan cross sectional image ($\sim 80\%$ duty cycle). By setting a ~ 4.6 μm spatial interval between adjacent A-lines, each B-scan image covered ~ 1.2 mm range. In the slow-scan direction, 1000 B-frames were captured to cover ~ 1.2 mm range. The system imaging speed was set at 70,000 A-lines per second (the maximum camera speed). Due to the effective duty cycle of the fast scan and the data-transfer between the camera and the computer, the practical achievable frame rate of the system was 220 frames per second (fps). With this frame rate, it took only 4.5 seconds to finish one 3D scan that is short enough to minimize motion artifacts for *in vivo* imaging.

To demonstrate the capability of the designed UHS-OMAG system powered by the SC light source, we conducted experiments to image the capillary vessel networks lying within the mouse pinna (ear) *in vivo*. The scanning protocol described above was applied to capture a 3D data cube that covered $\sim 1.2 \times 1.2$ mm^2 , onto which we applied UHS-OMAG algorithm to obtain both the structural and blood flow images. Fig.2a shows a representative B-scan image of microstructure, where we can clearly identify the typical, layered microstructural features of the mouse pinna, e.g., epidermis, dermis and auricular cartilage. In addition, the ultrahigh resolution of the system also enabled us to distinguish some of the other particular features, such as the hair follicle and fatty subcutaneous tissue. Fig. 2b gives the corresponding B-scan blood flow image where the abundant capillary blood vessels (pointed by arrows) are seen. To provide a clearer demonstration of the system capability to resolve single capillary vessels within tissue beds, Fig. 2c gives the 2D maximum-intensity projection view of a 3D OMAG image of microcirculation obtained from a field of view of 0.5×0.5 mm^2 . Owing to its ultrahigh spatial resolution, the system was able to clearly identify the looped structures of the capillary network (open-head arrows), a common feature in the skin microcirculation. The capillaries pointed by the thick arrows have the measured diameter of ~ 6 μm , close to the lateral resolution of the system. Even the individual red blood cells (thin arrows) moving in the capillaries can be identified in the OMAG flow image. Fig. 2d illustrates a 3D view rendered by merging microstructural image with the blood vessel image, where the capillary vessels (white arrows) on the top of big vessel could be seen more clearly.

The UHS-OMAG system has a broad detectable velocity range, from ~ 4 $\mu\text{m/s}$ to ~ 15 mm/s [10], which enables us to image not only the big arteries and veins, but also the detailed capillary bed within tissue. By scanning multiple small regions and combining them together afterwards, Fig.3 shows the detailed and impressive vascular perfusion map of the whole ear, where the inserted is a photograph of the mouse ear imaged. Since the densely patterned capillary beds and plexus (red circles) can be imaged and resolved clearly, this image demonstrates the power of the OMAG imaging of microcirculations within tissue beds *in vivo*.

In summary, we have constructed an UHS-OMAG system with ultra-high resolution and ultra-high sensitivity for blood flow imaging by utilizing a broadband SC light source. The system provides an axial resolution of ~ 3 μm and a lateral resolution of ~ 6 μm in air. Within the depth range of 2mm, the system delivered an averaged SNR of 87 dB and a sensitivity falling-off of 7dB. With the use of the CMOS camera capable of 70 kHz A-scan rate, it has been shown that the system took less than 5 seconds to capture a 3D image for a field of view of 1.2×1.2 mm^2 . The performance of the system for microvasculature imaging was demonstrated by showing high quality and impressive blood vessel network obtained from a mouse ear flap *in vivo*, promising future applications of this high-resolution OMAG system

for investigating microvascular involvements in various disease models, either for humans or small animals.

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Fig. 1. Schematics of the system and characterization of its performance. (a) System setup, SD: spectral filter. (b) Sensitivity falling off curve along the whole designed imaging depth and (c) the falling off curve within the meaningful depth range of 2mm (i.e, zoomed from the region square-marked in (b)). (d) The evaluated free space axial resolution. Horizontal dash line is the mean value.

Fig. 2.

(a) Cross-sectional structural image of mouse pinna *in vivo*, A: auricular cartilage, E: epidermis, H: hair follicle, F: fatty subcutaneous tissue; and (b) corresponding blood flow image. (c) 2D projection view of a 3D OMAG image of blood vessels within a $\sim 500 \times 500 \mu\text{m}^2$ area and (d) a 3D view rendered by merging structure with blood vessels.

Fig. 3. Whole-ear vascular network of a living mouse imaged by SC light source coupled UHS-OMAG system. Inserted is the picture of the mouse ear.